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Identification, diagnosis and management of persistent Hepatitis E virus infection

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Statement of originality

This body of work is all my own that I have instigated, co-ordinated and completed. However, there are a number of people who assisted me in various aspects all detailed and acknowledged.

Specifically, I co-ordinated all three prevalence studies and undertook HEV RNA testing, HEV serology and HEV antigen detection for all prevalence studies. I performed HEV cell culture for all HEV antigen neutralisation work. I co-ordinated and assisted in clinical data collection for all studies and performed all the data analysis for the prevalence studies.

I performed all the statistical analysis and cost-effectiveness analysis with close collaboration and guidance. Whole genome sequencing was performed at the CVR, but I undertook all bioinformatics analysis.

In the descriptive cases series diagnostics including phylogenetic analysis were performed through the routine clinical diagnostics service. I undertook the phylogenetic analysis for the write-up.

Copyright declaration

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Abstract

Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis in the UK and leads to persistent HEV infection in immunosuppressed individuals. The prevalence and clinical outcomes of persistent HEV are unknown in the UK. It is hypothesized that persistent HEV is an under-recognised disease in the UK, that screening of high-risk immunocompromised patients will be cost-effective and enhanced surveillance of persistent HEV cases will identify pragmatic parameters for clinical monitoring.

Within this study, the prevalence of HEV infection was investigated in three distinct immunocompromised cohorts. A commercial assay for detecting HEV antigen (HEV-Ag) was explored for use as a screening assay and monitoring tool. A cost-effectiveness analysis modelled the impact of annual HEV screening in solid organ transplant (SOT) recipients. The diagnostic findings and clinical outcomes were reported on a case series of persistent HEV infections across England and Wales and whole genome sequencing (WGS) was utilized to explore viral mutations with and without antiviral pressure.

This work demonstrates that persistent HEV infections are under-recognised in transplant recipients, with biochemical abnormalities often attributed to other causes by clinicians. Viraemia rates were similar to other European studies among SOT recipients. HEV-Ag had both high sensitivity and specificity as a screening assay for persistent HEV infections. The annual screening of SOT recipients either by RNA or HEV-Ag testing is projected to be cost-effective for the NHS. The case series showed that a broad range of immunosuppressed patients are at risk of

persistent infection, however the magnitude of risk in antibody-deficient patients and those with a haematological malignancy were lower than in SOT. Finally, WGS revealed the emergence of mutations in the RNA-dependent RNA polymerase region associated with clinical phenotypic resistance to ribavirin. However, further optimization of HEV sequencing is required to investigate samples with lower HEV viral loads.

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1 General Introduction

1.1 Hepatitis E virus as an emerging viral infection

1.1.1 The changing epidemiology of HEV infection

Hepatitis E virus (HEV) is a positive-sense RNA virus in the widespread *Hepeviridae* family [1]. HEV strains infecting humans are found within species A of the *Orthohepevirus* genus which consists of eight recognised genotypes [1, 2]. Of these genotypes four are recognised to commonly infect humans (Table 1.1). The anthropotropic genotypes (G1 and G2) are obligate human pathogens and do not infect other hosts. They are predominantly acquired through the consumption of food or water contaminated with human faecal material shed from an acutely infected person. This can lead to large outbreaks in areas of poor sanitation such as refugee camps. In contrast, the enzootic genotypes (G3 and G4) have a wider host range of mammals and lead to human infection through the consumption of food containing virus, though in this case it is through eating meat from an animal that is viraemic at the time of slaughter. A single case of genotype 7 HEV human infection has been reported, but the extent to which these other genotypes (G5-8) can infect humans is largely unknown [3, 4]. Species B and C of the *Orthohepevirus* genus were not known to lead to human infection, however highly divergent strains of HEV within species C commonly infecting rats have been recently recognised to cause human infections and may be underrecognised [5-7].

The virus was first isolated during an outbreak in a soviet military camp in Afghanistan in the early 1980s. A member of the research team confirmed the presence of an infective agent in an unorthodox manner by ingestion of pooled faecal extract from the affected soldiers [8]. This novel virus, later cloned and named HEV, was seen by electron microscopic examination of his stool after he fell ill with jaundice [9]. Prior to this time a large waterborne outbreak of acute non-A non-B hepatitis and jaundice in Kashmir, India in 1978 had been suspected to be caused by a novel virus [10]. Retrospective serological testing of clinical samples from an outbreak in Delhi, India in the 1950s confirmed the presence of circulating HEV at that time [10]. Since its discovery large epidemics of HEV affecting thousands of people have continued to occur periodically in the developing world, particularly in parts of Africa, Asia and Mexico [11].

HEV was considered a disease of poor sanitation in certain developing countries and only occurring in developed countries as a travel-associated infection until the late 1990s. At this time anti-HEV seroprevalence rates of 1% to 4% in developed countries considered non-endemic for HEV suggested local acquisition was occurring [12]. This was subsequently confirmed and at a similar time novel related viruses, designated swine HEV, were identified circulating amongst pigs [13]. HEV is now considered endemic amongst swine in Europe and are an important transmission source leading to zoonotic infections.

The World Health Organization (WHO) estimate that 20 million HEV infections occur annually leading to over 3 million symptomatic cases globally leading to 44 000 HEV-related deaths [14]. These figures are approximated from the

epidemiology in endemic regions particularly developing countries in Africa and Asia where large waterborne outbreaks of G1 and G2 HEV occur. In Europe where G3 HEV is the dominant genotype, reported hepatitis E case numbers have been increasing year on year particularly in Western European countries [15]. Such cases are typically sporadic but outbreaks linked to commercially catered events are documented [16, 17].

1.1.2 HEV in the UK

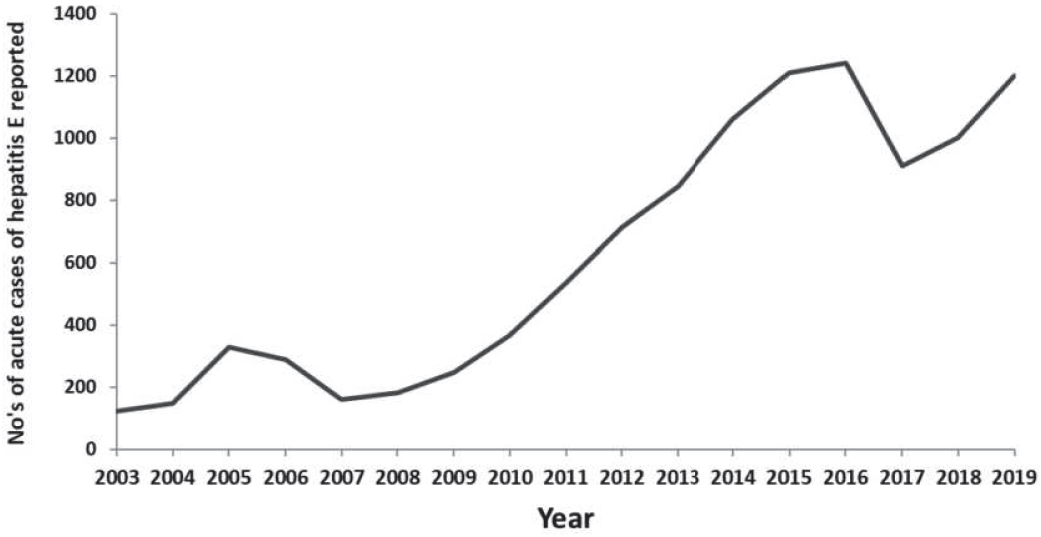
Across England and Wales specifically, similar to other Western European countries, the reported clinical cases of acute hepatitis E have followed an upward trend since 2010 peaking at 1243 cases in 2016 (Figure 1.1) [15, 18-21]. If one extrapolates from blood donors presenting for donation in South-East England with HEV viraemia between 80 000 and 100 000 HEV infections occur in England annually, most of which are asymptomatic [22]. The majority of these HEV infections in humans in the UK are not acquired from UK pigs and are likely to arise from the importation of continental pigmeat of animals viraemic at the time of slaughter [23]

Table 1.1 HEV genotypes that commonly affect humans

	Genotype 1	Genotype 2	Genotype 3	Genotype 4
Species infected	Humans only		Primarily pigs but many other mammals including humans	
Route of infection	Waterborne/Faeco-oral Direct person-to-person spread very limited		Foodborne Direct person-to-person spread not proven	
Epidemiological pattern	Sporadic cases in between large epidemics		Sporadic cases	
Clinical features	Acute hepatitis can be severe Severe disease seen in pregnancy		Symptomatic hepatitis common in elderly males Chronic in immunocompromised	
Geographical distribution	Asia & Africa	Mexico & Africa	Worldwide	Asia, recent spread to Europe

Comparison of the epidemiology and clinical features of the four main HEV genotypes causing disease in humans.

Figure 1.1 Acute hepatitis E in England and Wales



Reported cases of acute HEV infection reported across England and Wales. Cases include those virologically diagnosed at the Public Health England's (PHE) bloodborne virus unit (BBVU) at Colindale and Birmingham public health laboratory and those reported to the second generation surveillance system (SGSS) by diagnostic laboratories across England and Wales [21].

1.1.3 Seroprevalence of HEV

Anti-HEV IgG seroprevalence data, reflecting prior HEV infection from various countries, demonstrate that the areas of highest seroprevalence are observed in geographical areas considered HEV-endemic such as Nepal (47%) and Bangladesh (50%), with much lower seroprevalence figures found in Argentina (11%), Uruguay (10%), USA (9.5%), Brazil (6%), New Zealand (4%), Australia (6%), Canada (6%), and Scotland (5%) [24-33]. However, many European countries such as France (25-56%) and the Netherlands (21-27%) also demonstrate high seroprevalence figures [34-36].

In Europe HEV seroprevalence figures range from 0.6% to 53% [37]. Some of the variation observed has been blamed on the variable performance of different anti-HEV assays [38]. When analysis is restricted to comparison of studies using the Wantai IgG ELISA, regarded as the most sensitive ELISA, there remains a wide variation of seroprevalence between countries (Table 1.2) [39]. Within countries there are also large regional variations, most marked in France, with areas including the southwest and southeast of France considered hyperendemic for HEV [40]. The consumption of regional delicacies such as the raw liver sausage figatellu and fitone may partly explain these variations but obvious regional dietary delicacies are not evident in other countries [3]. Such high seroprevalence figures in industrialized European countries seem surprising when relatively few clinically symptomatic cases are identified but can be understood in the context of blood donor studies where asymptomatic infections are the norm [41].

Table 1.2 Seroprevalence in blood donors and the general population

	Country	Anti-HEV IgG in blood donors	Anti-HEV IgG in general population	Year of sampling
Europe	Austria	14%		2013/2014 [42]
	Denmark	20%		2013 [43]
	England	12%		2010 [44]
	France	56%		2017/2018 [36]
		25%		2012/2013 [29]
			34%	2010/2011 [35]
		53%		2003/2004 [35]
	Germany		30%	2010 [45]
	Ireland	5%		2012 [46]
	Italy	9%		2015/2016 [47]
	Netherlands	27%		2011/2012 [34]
		21%		2011 [48]
	Scotland	5%		2004-08 [27]
	Spain	20%		2013 [50]
	Switzerland	20%		2014-2016 [51]
Rest of the world	South Africa	43%		2014-2015 [52]
	New Zealand	10%		2014/2015 [53]
	USA	16%		2012 [54]
	Canada	6%		2013 [26]
	Hong Kong	16%		2015 [55]

Prevalence of anti-HEV IgG in blood donors and the general population using the Wantai IgG assay (Fortress Diagnostics Ltd). All percentages rounded up to whole numbers.

1.1.4 Transmission routes of HEV

In broad terms transmission of HEV to humans can be considered as either enteric or parenteral. G1 and G2 HEV are transmitted faeco-orally when water sources become contaminated with faecal material. In contrast, the most significant transmission route of G3 and G4 is through consumption of meat from an infected animal. However, other routes of transmission remain possible.

1.1.4.1 HEV as a foodborne zoonosis

G3 HEV is of particular importance because it is found worldwide in a wide variety of animals including pigs, wild boar, deer, rabbits, cattle, sheep, horses and mongooses [56-61]. Seropositivity has also been found in primates, cats and dogs [62-65].

Pork meat, in particular that which contains liver, is the predominant food source for G3 and G4 HEV but infection from venison and wild boar is also recognised [3]. Multiple questionnaire-based case-control studies indicate the consumption of pork products as a risk for HEV infection and molecular phylogeny has proven direct transmission from raw pig liver sausages [40, 66, 67]. Bacon, pigs' liver and cured pork meats were strongly associated with HEV infection in English blood donors [68]. Porcine blood as a distinct ingredient in other meat products is also considered high risk for HEV acquisition [69]. HEV is highly transmissible amongst pigs and there is evidence of HEV infection in more than 80% of pig herds in the US, Canada and the UK [23, 70, 71]. Furthermore, HEV viraemia is common at

the time of pig slaughter and infectious virus has been detected at every step of the food chain in several European countries [23, 72-76]. Meat from rabbits may also lead to human infection [77]. A distinct subtype of G3 was identified in rabbits in France and human infections with such subtypes do occur, albeit rarely [78-80]. Other food products including shellfish, soft fruits and vegetables may be contaminated by pig effluent or irrigation water and lead to HEV infection [81-83]. An outbreak of HEV on a cruise ship was linked to shellfish consumption, but overall there is scarce evidence that these foodstuffs contribute significantly to HEV transmission [83].

1.1.4.2 HEV transmission from substances of human origin (SOHO)

Where acutely infected viraemic individuals occur in the human population from which blood donors are drawn, blood and its components will also infect those who receive such substances. Initially transfusion transmission of HEV was described in HEV-endemic countries where G1 is dominant [84]. More recently transmission of G3 HEV by transfusion has been reported in European countries including France, the UK and Germany [22, 85-87]. In Europe prevalence rates of G3 viraemia in blood donors varies significantly between countries but also within countries over time. Of particular note was the significant increase in the prevalence of G3 viraemia in Scottish blood donors from 1 in 14520 in 2012 to 1 in 2841 in 2016 [49]. Similar rises have been seen in other countries including the Netherlands (Table 1.3 and Table 1.4) [88].

Transmission is described for red blood cells, platelet preparations, pooled granulocytes and fresh frozen plasma including pathogen-inactivated plasma [89, 90]. The risk of transmission is influenced by the presence of antibody, the viral load in the donor and the volume of plasma transfused in the final blood component [22]. Overall, the risk of a viraemic donation leading to infection in the recipient is estimated to be 40-50% [22]. Extrapolating figures from a transmission study in the UK, the lowest total virus inoculum of G3 known to have led to infection in the recipient is 2×10^4 international units (IU) [22].

Transmission of HEV can also occur through the transplantation of both hepatic and non-hepatic infected solid organ grafts [91-94]. The receipt of a liver transplant containing HEV from a donor who was aviraemic at the time of donation led to cirrhosis in the recipient [91]. Two further kidney transplant recipients were infected with HEV by kidney grafts from a single donor [92]. To date there are no published cases of stem cell donation leading to infection in the recipient, but a potential stem cell donor was identified as having HEV viraemia prior to donation and a second donor in Ireland was found to be viraemic on the day of stem cell harvest [95, 96]. To mitigate the risk of HEV acquisition from substances of human origin, eight countries in the European Union have introduced blood donation screening for HEV RNA since 2012 [97, 98]. Of these eight countries, three have a universal screening strategy (UK, Ireland and the Netherlands) and the remaining five (France, Austria, Luxembourg, Spain and Germany) have a selective screening strategy for blood donations [98, 99]. Testing varies by country; either individual donation testing or the testing of mini-pools for HEV RNA [98]. Despite mini-pool

testing there remains a residual risk of transfusion-transmitted HEV infection [100, 101]. In addition, stem cell donors and solid organ transplant donors are screened for HEV RNA in a number of countries. However, even in countries where screening is not performed, the dietary risk of HEV acquisition is far greater than unscreened substances of human origin [102, 103].

Table 1.3 Prevalence of HEV viraemia in blood donors in Europe

	Country	Blood donors HEV RNA positive	Year of sampling
Europe	Austria	1:5369	2016/2017 [98]
		1:8416	2013/2014 [42]
	Denmark	1:2331	2015 [101]
	England	1:4736	2017 [98]
		1:2848	2012/2013 [22]
	France	1:2218	2012/2013 [29]
		1:591	2011 [40]
	Germany	1:1294	2015-2017 [104]
		1:1240	2011 [105]
		1:4525	2011 [106]
	Ireland	1:4997	2012 [46]
	Italy	0:10 011	2015/2016 [47]
	Luxembourg	0:914	2017 [98]
	Netherlands	1:2179	2017/2018 [98]
		1:762	2013/2014 [88]
		1:2671	2011/2012 [34]
	Poland	1:1266	2014/2015 [107]
	Scotland	1:14 520	2012 [27]
		1:2841	2016 [49]
	Spain	1:3846	2017-2018 [98]
		1:3333	2013 [50]
	Sweden	1:7986	2011 [106]

Table 1.4 Prevalence of HEV viraemia in blood donors outside Europe

	Country	Blood donors HEV RNA positive	Year of sampling
North America	USA	1:42 673	2015 [108]
		1:9500	2013 [31]
		0:1939	2011 [106]
	Canada	0:5000	2013 [26]
		0:13 993	2013 [109]
South America	Uruguay	1:133	2017/2018 [30]
Asia	Cambodia	1:301	2014 [110]
	China	1:486	2013/2014 [111]
	Thailand	1:1158	2015 [112]
	Hong Kong	1:5000	2015 [55]
	India	1:1864	2017 [113]
	Japan	1:15 075	2004-2014 [114]
Australasia	Australia	1:74 131	2016 [115]
		1:14 799	2014 [116]
	New Zealand	0:5000	2014/2015 [53]
Africa	Ghana	0:239	Not stated [117]
	South Africa	1:10 000	2014/2015 [52]

1.1.4.3 Other routes of transmission

The frequent finding of higher anti-HEV seroprevalence in people with exposure to pigs and other livestock via their occupation including veterinarians, pig handlers, forestry workers, slaughterhouse workers and butchers is suggestive that direct animal contact may also lead to HEV infection [118-122].

Direct human to human transmission of G1 HEV also occurs in outbreaks leading to prolonged epidemics, but is considered extremely rare the zoonotic genotypes of HEV including G3 [123, 124].

The evidence for sexual transmission is mixed and inconclusive [125-128]. A recent study did not find any increased anti-HEV seroprevalence in a high-risk cohort among men taking pre-exposure prophylaxis (PrEP) against HIV [129].

Mother to child transmission occurs commonly with G1 HEV infection in pregnancy with reported ranges of 33-100%, particularly if HEV is acquired in the third trimester, but it is not known whether transmission occurs *in utero*, through labour, breastfeeding or close contact post-delivery [130].

In addition to liver tissue, plasma and stool, HEV RNA has been detected in cerebrospinal fluid, bone marrow, urine and breast milk, but the infectivity and thus the likelihood of transmission from these bodily fluids is unknown [131-136].

1.2 The Virology of HEV

1.2.1 Genomic structure and replication of HEV

HEV is a small (27-34nm), non-enveloped, positive sense single-stranded RNA virus. Both plasma-derived virus and cell culture-derived virus exists in a quasi-enveloped form in which virions are surrounded by a lipid coat derived from host membranes [137].

The ~7.2 Kb genome contains multiple, separately transcribed genes with three partially overlapping open reading frames (ORFs). ORF1 encodes a polyprotein with multiple putative functional domains vital for RNA replication (Figure 1.2) [138]. These include a methyltransferase, Y, papain-like cysteine protease, X, helicase, and RNA-dependent RNA polymerase domains. ORF2 immediately follows ORF1 and is the source of the single capsid protein essential for virion assembly, host cell interaction and immunogenicity [138]. The immunogenicity of the ORF2 product has been exploited to generate a vaccine by expressing high levels of the antigen *in vitro* [139]. It has four defined domains, namely the N terminus, the middle (m), protruding (p) and shell (s). Monoclonal antibodies directed against the m and p domains have demonstrated neutralising capacity therefore are considered important regions for cell binding and entry [140]. ORF2 is also the source of the recently recognised secreted form of ORF2 which is non-virion associated but whose biological function remains unclear [141]. Both products of ORF2 are thought to share the major neutralising epitopes [141]. ORF3 codes for a small phosphoprotein which is considered vital for virion assembly and

release [142]. In G1 only, a fourth ORF has been recently recognized, the product of which controls the activity of the viral polymerase [143].

Figure 1.2 Genomic structure of HEV

[a] ORF4 found only in G1 HEV.

Abbreviations: C, cap; CP, capsid protein; CRE, cis-reactive elements; Hel, helicase; HVR, hypervariable region; JR, junction region; MFP, multifunctional protein; Met, methyltransferase; ORF, open reading frame; PCP, papain-like cysteine protease; RdRp, RNA-dependent RNA-polymerase; SL, stem loop structure; UTR, untranslated region; X, macro domain; Y, Y domain. Adapted from van Tong et al [144].

1.2.2 Genotypic diversity of HEV

There is considerable sequence diversity found across the genome within Orthohepevirus A. The large sequence diversity led to the classification of eight distinct genotypes and numerous subtypes. G1 HEV has six subtypes (1a to 1f), G2 has two subtypes (2a and 2b), G3 currently has ten subtypes (3a to 3j) in addition to rabbit G3 (3ra) and G4 nine subtypes (4a to 4i) [2, 145]. There are also unassigned subtypes within G3 [146]. However, the subtypes remain controversial since the criteria for assigning subtypes has been inconsistent between genotypes partly due to varying methodologies and the inadequate numbers of reference strains available [145]. Proposed reference sequences of whole genomes have been put forward by the International Committee on Taxonomy of Viruses and proposals for defining subtypes published [145, 147, 148]. As exemplified by the numbers of subtypes, the greatest variation is seen with G3 and G4 viruses.

The sequence variation across the HEV genome is relatively constant with a strong selection pressure against non-synonymous mutations, however there are notable exceptions. The region of overlap between ORF2 and ORF3 is highly conserved, whereas between the papain-like cysteine protease and X domains lies a region termed the hypervariable region (HVR). This HVR contains a highly disordered region rich in proline and serine residues, which may have an important, but as yet undefined, structural role [149]. Cell culture studies have identified human coding sequences which have been incorporated in-frame into this region which appear to enhance the replication efficiency *in vitro* [150, 151].

The biological significance of different genotypes is evident from the contrasting epidemiological patterns and clinical presentations as described. However, the biological significance of subtypes remains unclear. Among blood donors with G3 infection, those infected 3efg subtypes had higher viral loads and more severe symptoms than those infected with subtype 3abchij in one study [41]. Several other reports have found associations between specific variants and severe outcomes; in particular progression to fulminant hepatitis [152-154]. However, a re-analysis of such reported associations concluded that sampling bias may have led to the incorrect attribution of viral variants with the development of fulminant hepatitis and that host-specific factors are more likely responsible [155].

1.3 Clinical manifestations

1.3.1 Acute hepatitis E

Many HEV infections will be completely asymptomatic, more so in the case of the zoonotic G3 and G4 infections than in G1. Where symptoms arise they do so with a prodrome of non-specific malaise late in the incubation period followed by onset of acute jaundice. This clinical symptomatology is indistinguishable from other causes of acute viral hepatitis, including hepatitis A [156].

The overall mortality from G1 infections ranges from 0.5 to 3.0%, but is significantly lower with G3 infections. Severe courses of infection with G1 may occur in pregnancy, particularly in the third trimester, with an excess mortality rate of approximately 25% [157, 158]. By contrast in developed countries G3 infections do not express this increased pathogenicity in pregnant women [159, 160].

Acute G1 infections in the context of chronic liver disease can lead to decompensation with excess mortality [161].

1.3.2 Extra-hepatic manifestations of HEV infection

Infection has been associated with a wide range of extra-hepatic disease processes including neurological, immune complex and haematological complications [162, 163].

Neurological symptoms: Neurological symptoms have been observed during G1 and up to 5% of both acute and chronic G3 cases [164]. Symptoms may be more commonly seen in younger males and with only modestly deranged liver function

tests which can mislead clinicians [164]. Manifestations include Guillain-Barré syndrome, neuralgic amyotrophy, Bell's palsy, acute transverse myelitis and acute meningoencephalitis. Bilateral neuralgic amyotrophy is a specific variant clinical phenotype associated with HEV [165].

Non-neurological: Glomerulonephritis, myocarditis, polyarthritis and haematological complications such as autoimmune haemolytic anaemia, severe thrombocytopenia, macrophage activation syndrome and cryoglobulinaemia have all been associated with acute HEV infection [166]. Acute pancreatitis, including necrotizing forms, is also described with G1 infections within one to three weeks of hepatitis onset [167].

1.3.3 Persistent HEV infection

Persistent HEV infections were first described in France in solid organ transplant recipients in 2008 and since then have been increasingly recognised [168]. They are also reported in other immunosuppressed patients including haematopoietic stem cell transplantation (HSCT) recipients, those co-infected with HIV, those receiving immunosuppressive therapy for chronic inflammatory diseases and patients undergoing chemotherapy [169-171]. Immunosuppression appears to be a pre-requisite for development of persistent HEV infection. Of the cases of persistent HEV infection described to date in patients without iatrogenic immunosuppression all have invariably had some level of occult immunosuppression from underlying medical disorders [172-176].

Nearly all described cases have been caused by G3, however persistent G4 cases are increasingly recognised in Asia [177, 178]. Single cases of G7 HEV acquired from a camel and of a highly divergent strain of HEV found in rats have evolved to persistence in liver transplant recipients [4, 7]. G1 and G2 HEV were considered not to cause persistent infections until case reports in recent years have challenged that concept [179-182]. Such reports have been challenged due to significant limitations and where systematic studies have attempted to look for persistent infections in G1-endemic areas they have not found it [183].

Currently, persistent HEV infection is defined as “ongoing viraemia in excess of three months duration” [184]. It is likely in an immunosuppressed patient that the detection of viraemia at a steady level over a period of a month or longer is a more timely working definition of persistence. However, this definition may not hold true in patients with fluctuating levels of immunosuppression in particular HSCT recipients and is not universally accepted.

1.3.4 Prevalence of HEV infection in immunocompromised cohorts

The prevalence of persistent HEV infection in a specific patient cohort varies by a number of factors influenced by the individual HEV exposure risk including country of residence and dietary habits, but also their predisposition to developing a persistent infection largely influenced by the patients’ net immunosuppression (Table 1.5).

Solid organ transplant (SOT) recipients represent a uniquely high-risk cohort for persistent HEV infection likely reflecting the duration and intensity of

immunosuppression. The prevalence of persistent HEV infection in cohorts of SOT patients in Western Europe typically range between 0.7% and 1.5%, but smaller studies have found higher prevalence figures of up to 4.4% [185-193]. A retrospective study of 328 allogeneic HSCT patients in the Netherlands found a prevalence of HEV viraemia of 2.4% (8/328) of whom 63% developed persistent infection [194].

Several studies across Europe have systematically tested for HEV in HIV cohorts and found six cases of active viraemia amongst over 3000 patients tested. Of these six infections, two patients had preserved CD4 counts but follow up was insufficient to confirm persistent infection [171]. Studies in Iran, China and West Africa found no evidence of persistent HEV infection amongst HIV cohorts [195-198]. One case of persistent HEV infection was found amongst nearly 3000 HIV infected patients in the US [197].

The extent to which immunosuppressed patients beyond transplantation and advanced HIV can support persistent HEV is not known. Cases of persistent HEV leading to rapidly progressive liver fibrosis have been reported in patients treated with rituximab-based chemotherapy for underlying haematological malignancies [170]. In one study of 14 HEV viraemic patients with underlying haematological malignancy (one case with recent allogeneic HSCT), 5 (36%) developed persistent infection [199].

Studies following the outcome of 23 cases of acute HEV in patients with inflammatory arthritidies on a variety of immunosuppressants did not identify any chronic cases suggesting that the risk is not high in this cohort [200]. However,

another European study in a similar cohort of internal medicine/rheumatology patients found seven cases of acute HEV infection (33%) developed into persistent HEV infection amongst 21 patients [201]. Early manipulation of patients' immunosuppression and the use of ribavirin in both studies make it difficult to determine the natural history of HEV infection in such patients [200, 201]. Large studies investigating the prevalence of HEV infection amongst immunocompromised patients in the UK have not been undertaken.

Table 1.5 Prevalence of HEV infection in immunosuppressed cohorts

Patient group	Patient category	Cohort size	Country	Prevalence of persistent infection	Reference
HSCT	Allogeneic-HSCT	328	The Netherlands	1.52%	[194]
SOT	Heart	274	Germany	1.5%	[185]
	Kidney	1469	Germany	1.09%	[186]
	Kidney/Liver	871	France	0.7%	[187]
	Liver	206	France	1.5%	[193]
	Liver	226	Germany	0.9%	[188]
	Liver	80	Canada	1.3%	[202]
	Liver	76	Greece	0%	[189]
	Kidney - paediatric	90	Germany	4.4%	[190]
	Kidney	316	Brazil	0%	[203]
	Lung	95	Germany	3.2%	[191]
	Heart/Lung/Liver/Kidney	1200	The Netherlands	0.9%	[192]
HIV	HIV	123	Germany	0%	[204]
	HIV	115	Spain	0.87%	[205]
	HIV	86	Iran	0%	[195]
	HIV	770	China	0%	[196]
	HIV	1544	Ghana/Cameroon	0%	[198]
	HIV	2919	USA	0.03%	[197]

Abbreviations: HIV, human immunodeficiency virus; HSCT, haematopoietic stem cell transplant; SOT, solid organ transplant.

1.3.5 Risk factors for persistent HEV infection

An estimated 60% of SOT patients who acquire HEV infection will develop persistent infection [168]. Amongst SOT recipients risk factors for the development of a persistent infection include a shorter time from the transplant to diagnosis, a recent episode of acute rejection and low CD4 and CD8 T cell counts [206]. Multivariable regression analysis in one study demonstrated the use of the calcineurin inhibitor immunosuppressant, tacrolimus, as opposed to ciclosporin A and low platelet counts as independent risk factor for persistent infection in this cohort [207].

There has been significant interest in which immunosuppressants pose the greatest risk of chronic HEV infection. *In vitro* studies demonstrate increased HEV replication in cell culture in the presence of both mammalian target of rapamycin (mTOR) inhibitors and calcineurin inhibitors (tacrolimus and ciclosporin), but mycophenolic acid has an inhibitory effect in these models [208, 209]. Analysis of small numbers of cases in heart transplant recipients suggest that mycophenolate mofetil is associated with spontaneous clearance of HEV [185]. In contrast once persistent HEV is established the response to antiviral treatment does not appear to be influenced by the specific drug regime, but by the net level of immunosuppression [210].

Outside of transplantation the risk factors for persistent infection are poorly defined. The severity of immunosuppression and co-morbidities including graft versus host disease (GvHD) affect the chance of HEV clearance. Persistent HEV

infection is usually observed only with advanced HIV with CD4 counts below 200mm^3 [171].

1.3.6 Natural history of persistent HEV infection

Persistent infections are frequently pauci-symptomatic and the transaminitis usually mild, if the infection persists for a long time chronic hepatitis, fibrosis and rapidly progressive cirrhosis may follow, which can occur within two years [168]. Up to 15% of SOT recipients with persistent HEV infection will develop cirrhosis [207]. This has led to retransplantation in select cases of persistent HEV infection in liver graft recipients [207].

HEV infections are considered clinically indistinguishable from other viral causes of chronic hepatitis and may be misdiagnosed as other conditions including drug-induced liver injury and graft-versus host disease (GvHD) [211-213]. Histological analysis may also show the typical, but non-specific, changes seen in chronic hepatitis, however some patients may exhibit a florid cholangitis with a significant infiltrate of polymorphonuclear leucocytes [214].

Hepatocellular carcinoma may be a rare complication of HEV-induced cirrhosis but the incidence and mechanism is unknown [215].

1.3.7 Diagnosis of persistent HEV infection

An imputable diagnosis of acute hepatitis E in jaundiced immunocompetent patients is by the detection of HEV RNA in plasma with coincident IgM anti HEV

and IgG seroconversion. In the absence of RNA testing a diagnosis can usually be made by the interpretation of titres or reactivity of anti-HEV IgG and IgM results. In contrast, diagnosis of HEV in many persistently infected hosts relies solely on HEV RNA because of a delayed or non-existent serological response [216]. RNA assays were historically of limited availability, usually restricted to reference laboratories and are inherently expensive. As a result there has been some interest in the utility of assays for plasma HEV antigen (HEV-Ag) to detect infection. These relatively simple and inexpensive tests are less sensitive than RNA testing so are unlikely to be recommended for the diagnosis of acute HEV. Since the viral load in persistent infection is high it is possible such assays may find a niche in identifying persistent infections and perhaps in the monitoring of response to treatment with antivirals in chronic infection [217].

1.3.8 Clinical management of persistent HEV infection

It is considered best practice to attempt HEV clearance in patients presenting with persistent HEV in order to prevent liver disease and rarer neurological and renal complications which can develop during the course of persistent infection. Some patients will tolerate persistent infection with minimal disease progression, however currently there are no reliable tools to predict those patients who will progress to severe liver disease. Clearance can be achieved by reducing iatrogenic immunosuppression or by the use of antivirals.

1.3.8.1 Reduction of iatrogenic immunosuppression

Up to one third of SOT patients may clear HEV following reduction of immunosuppression and this should be considered first in those whose graft will tolerate this [218]. This however may be considered an overestimate due to publication bias [219]. Drugs that specifically target T cells should be tapered preferentially since it is known that HEV-specific T-cell responses are decreased in SOT patients with chronic HEV [220-222].

1.3.8.2 Antiviral therapy

1.3.8.2.1 Ribavirin therapy

If the reduction of immunosuppression is not possible, is contraindicated or unsuccessful then ribavirin should be considered. Ribavirin has become the treatment of choice for persistent HEV because of its high efficacy, relative good safety profile and low cost, however no placebo-controlled trial data are available to support its use [223]. The mechanism of action of ribavirin against HEV is unknown, but it is effective against a broad range of viruses and multiple different mechanisms of antiviral activity have been proposed [224].

1.3.8.2.2 Evidence for clinical efficacy of ribavirin

The best available evidence comes from a multicentre retrospective study of 59 SOT patients that found 78% of patients achieved a sustained virological response

(SVR), defined as an undetectable serum HEV RNA level at least six months after cessation of ribavirin therapy, following treatment for a median of three months (range 1-18) [223]. The SVR rates were lower in studies which took a more standardised treatment protocol of three months of ribavirin for all patients [210]. A recent meta-analysis including 395 patients taking ribavirin therapy estimated a pooled SVR rate of 78% [219].

There are few clinical predictors of successful therapy. A higher lymphocyte count at ribavirin initiation was positively correlated with the chance of achieving a SVR [223]. An on-treatment fall of greater than 0.5 log copies/ml drop at day seven of ribavirin therapy had positive predictive values of greater than 88% with three months of therapy in one study [210]. Interestingly, there does not appear to be an association with ribavirin plasma levels and SVR rates [210]. However, the optimal duration and dosing of ribavirin is undefined and should be tailored to individual patients by monitoring HEV RNA in both plasma and stool. Therapy should only be discontinued once clearance from both plasma and stool has been achieved. Persistence of RNA in the stool indicates that the infection has not been cleared and is predictive of relapse if ribavirin is stopped [225].

Not all patients will tolerate ribavirin due to frequently occurring side effects, particularly anaemia, which can be significant but rarely life threatening. In the inaugural paper on the use of ribavirin in persistent HEV infection, 29% of patients required dose reduction of ribavirin, 54% required supportive erythropoietin and 12% required blood transfusion [223]. Therapeutic drug monitoring may assist in some cases to balance the efficacy and toxicity of ribavirin [226].

Both HIV infected patients and HSCT recipients present unique treatment decisions and there is sparse evidence on which to base treatment decisions. HIV infected patients have cleared persistent HEV with immune reconstitution following the introduction of antiretroviral therapy and this should be considered first [227]. However, others have been successfully treated with interferon with or without ribavirin [135, 228].

Likewise, haemato-oncology or HSCT patients may spontaneously clear HEV as immunosuppression varies. However, this is highly unpredictable and treatment for HEV is often given to these patients to enable them to complete the therapeutic schedule for their primary haematological disorder. Ribavirin is effective in this cohort but decisions on timing of treatment are more complex in the context of a recent HSCT and a fragile bone marrow [199].

1.3.8.2.3 Alternative treatment options

Few alternative antiviral strategies to ribavirin currently exist; pegylated interferon α (PEG-IFN-α) has been used with some success as monotherapy and in combination with ribavirin with which it shows synergy *in vitro* [229, 230]. Therefore it remains an option for some liver transplant recipients and perhaps HSCT recipients, but interferon has no role in the treatment of HEV in in heart, lung and renal transplant patients because of the high risk of acute rejection [230].

Sofosbuvir, an NS5B polymerase inhibitor which was developed against Hepatitis C virus (HCV), shows activity against HEV *in vitro* and has a favourable safety profile in transplant patients [231]. Virological responses with falling levels of

viraemia are seen when using sofosbuvir *in vivo*, but no patients treated to date either as monotherapy or in combination with ribavirin or daclatasvir have achieved a sustained virological response [232-238]. Zinc and silvesterol showing inhibition of HEV replication *in vitro* but have not been studied in humans [239, 240].

Finally, the use of high-titre convalescent plasma is a potential therapeutic approach. Passive immunoprophylaxis has been used successfully to prevent cynomolgus monkeys from developing hepatitis following an intravenous challenge if HEV, however whether convalescent plasma would be successful in eradicating an established hepatic infection in an immunocompromised patient is not known [241, 242].

1.3.8.2.4 Relapses and treatment failures

Relapses occur when infection rebounds following cessation of ribavirin therapy and in general may be predicted by detectable HEV RNA in the stool of patients displaying plasma clearance [225]. The majority of patients who relapse will clear HEV with a further six months of ribavirin if this can be tolerated [210]. Whether these relapses constitute ongoing biliary excretion of hepatic-derived virus in the absence of plasma spillover or an as-yet unidentified extrahepatic site of replication is unresolved. Animal models in pigs and rabbits suggest there may be important extrahepatic sites of HEV replication [243]. Intestinal cells are susceptible to HEV infection and may be the predominant site of extrahepatic replication [244].

A minority of patients are unable to clear HEV infection with ribavirin alone. Studies in these treatment failures have revealed that under drug pressure of ribavirin a number of mutations emerge in the viral polymerase region which may contribute to drug resistance (G1634R, K1383N, D1384G, V1479I, Y1587F and Y1320H) [245-247]. However, the association is not straightforward since G1634R variants have been detected before therapy in patients achieving a SVR as well as those not achieving a SVR [245]. Furthermore i*n vitro* studies demonstrate that some mutations such as G1634R leads to increased viral replicative capacity rather than drug resistance *per* se, yet other mutations such as K1383N lead to reduced replicative capacity, so there is much still to learn regarding the clinical relevance of these mutations [247].

1.4 Research hypotheses

Persistent hepatitis E virus infection is an under-recognised emerging disease in the UK.

It is predicted that due to a number of factors including the non-specific and pauci-symptomatic clinical presentation of HEV infection and a lack of awareness of among clinicians that HEV infections will not have been clinically identified in immunosuppressed cohorts

Screening of high-risk immunocompromised patient cohorts will be cost-effective.

If the expectation of significant numbers of undiagnosed cases of persistent HEV infection in immunosuppressed cohorts holds true, screening of such cohorts for may be indicated. It is predicted that screening for HEV infection in a systematic manner using relatively inexpensive diagnostic assays will be justified by the excess healthcare costs and morbidity associated with the complications of persistent HEV infection.

Enhanced monitoring of persistent HEV infection will identify vital clinical monitoring parameters.

Given the limited clinical experience of persistent HEV infection in single clinical centres, it is predicted that by describing a national cohort of patients being

monitored and treated for HEV infection, the data generated will identify key parameters important for monitoring the efficacy of treatment.

1.5 Objectives

The hypotheses will be tested by

1. defining appropriate strategies for the identification of persistent HEV infection.
2. identifying high-risk cohorts for persistent HEV infection.
3. undertaking a cost-effectiveness analysis of HEV screening of identified high risk cohorts.
4. describing the demographics, virology, serological responses and clinical outcomes of persistent HEV infections across England and Wales.
5. characterising viral quasi-species evolution in both treated and untreated patients.

2 Materials and Methods

2.1 Clinical studies methods

2.1.1 Study sites and patient samples

Patients were recruited from three sites for the three separate prevalence studies. Transplant recipients (solid organ transplant and haematopoietic stem cell transplant recipients) undergoing therapeutic drug monitoring were recruited from the Queen Elizabeth Hospital, University Hospitals Birmingham. Patients with haematological malignancy were recruited from University College London Hospitals (UCLH) Haematology department. Antibody-deficient patients on immunoglobulin replacement therapy were recruited from the Royal Free Hospital Immunology department, London.

Archived, residual samples that were sent to the National reference Laboratory for routine diagnostics work were used for assay development work. Anonymised blood donor and convalescent blood donor samples from a previous HEV donor-transmission study were donated by NHS Blood and Transplant (NHSBT) [22].

2.1.2 Study approvals

The prevalence study in transplant recipients at the Queen Elizabeth Hospital, Birmingham did not require approval through the centralised NHS research ethics

committee process. The protocol was approved by University Hospital Birmingham Clinical Audit Department (registration no. CARMS-12238).

The prevalence study in antibody-deficient patients at the Royal Free Hospital was approved under a research biobank approval (National Health Service [NHS] Research Ethics Committee reference 04/Q0501/119). All patients provided written informed consent.

The prevalence study in haematological malignancies was approved as a service improvement project by the haematology department at UCLH. Patients were informed of HEV testing by patient information leaflets and were given the choice of opting out of the testing service. Telephone or direct face-to-face support by a clinical nurse specialist was offered to any patient with specific queries about the study.

An enhanced surveillance system for persistent HEV across England and Wales was approved by Public Health England (PHE) to collect clinical data on persistent HEV infections identified by the national infection service (NIS). The collection of patient identifiable information was approved under the current PHE permissions for public health surveillance under Section 251 of the NHS Act 2006 and the Health Service (Control of Patient Information) Regulations 2002 ('section 251 support').

All other experiments on clinical samples were performed in accordance with the 'Guidance on Conducting Research in Public Health England' (Version 3, October 2015; Document code RD001A). Experiments used archived, residual samples that were sent to the National Reference Laboratory for routine diagnostics work

with consent for residual sample to be used in other assays. The use of anonymised blood donor samples was approved by the Blood Supply Clinical Audit, Risk and Effectiveness Committee at NHSBT.

2.1.3 Communication of results and management of HEV-infected patients

All positive HEV RNA results were communicated to a dedicated study lead onsite for each of the three prevalence studies. Results were discussed with the relevant clinical team and HEV viraemic patients were informed of the result. As part of the ongoing clinical management of a patient, a confirmatory test was taken and expert clinical advice offered, including a Hepatology assessment in accordance with standard clinical practice.

2.1.4 Clinical data collection

To characterise patients tested in the prevalence studies of HEV viraemia, clinical information was collected in line with each study protocol. For transplant patients from Birmingham, the demographic data (age, gender, postcode), transplant details (type and date of procedure), immunosuppressive medication and blood results (ALT, bilirubin, ciclosporin/tacrolimus/everolimus/sirolimus level) were triangulated from the local pathology results system and the electronic prescribing system. Test results and prescription details were recorded for the date of the HEV plasma sample.

For haemato-oncology patients from UCLH, patient demographics (age and gender), underlying primary haematological disease, disease status (classified as no remission, partial remission, complete remission, progressive disease), lines of treatment, previous use of small molecule inhibitors, monoclonal antibodies or history of autologous stem cell transplantation, specific immunosuppressive medication in the preceding six months and blood results (total white cell count, lymphocyte count, neutrophil count, ALT and bilirubin values) at the time of HEV RNA testing were collected directly from patient records. The numbers of transfused blood components given to each patient in the preceding five years before enrolment was taken from the blood transfusion laboratory information management system (Bank Manager, Sussex Biologicals, UK). Transfusions were only considered in the five years prior to HEV RNA testing for each individual patient and any transfusions given after 10th April 2017 were excluded, as this was the implementation date of universal screening of blood donors in England for HEV RNA.

For antibody-deficient patients, data on demographics (age and gender), underlying diagnosis, immunoglobulin product infused and date of most recent infusion, iatrogenic immunosuppression and blood results (ALT, bilirubin, total lymphocytes, counts of $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$ and $CD16^+56^+$ cells) were recorded directly from patient records.

The following clinical data were collected for cases of persistent HEV infection diagnosed through the NIS: underlying diagnosis, co-morbidities, immunosuppressive medication at the time of HEV diagnosis, liver assessment,

treatment and clinical outcomes. Clinical data were collected primarily by completion of a questionnaire via an online encrypted questionnaire (Select Survey https://surveys.phe.org.uk/) or paper form (available in Appendix A1.1) and supplemented with discussions with the primary clinician (or local infection specialist). In the final analysis, additional steps were taken to ensure this dataset was available for relapse patients and present the data collected on the case series as a whole.

2.2 Laboratory Materials and Methods

2.2.1 Sample preparation

2.2.1.1 Processing of faecal samples

Faecal suspensions for HEV-Ag testing and HEV RNA testing were made using a 10µl disposable loop half-filled with raw faeces resuspended in 1000µl of stool transport and recovery buffer (S.T.A.R. buffer, Roche Diagnostics) which was vortexed and centrifuged for 5 minutes at 18500 x g for 5 minutes. A further 1:10 dilution was made in the same buffer.

For cell culture infection a 10% weight/volume suspension was prepared in 10mM Tris hydrochloride using a faecal sample from a patient with persistent HEV infection (quantified at 1.0 x 10^8 IU/ml) and vortexed. This faecal suspension was centrifuged at 1600 x *g* at 4°C for 30 minutes, the supernatant was recovered and centrifuged again at 6200 x *g* at 4°C for 10 minutes [248]. The supernatant obtained was diluted 1:5 in Dulbecco's phosphate buffered saline ((PBS), D8537, Sigma-Aldrich) and filtered through a 0.45µm and 0.22µm filter (Sartorius). Aliquots of 500µl were labelled and stored at -80°C.

2.2.1.2 Minipooling of plasma samples

Minipools were made using 16 plasma samples of 100µl each by the JANUS Automated Workstation with Varispan (Perkin Elmer).

2.2.1.3 Preparation of normal human plasma

Pooled normal human plasma (NHP) used for controls and dilutions were a kind gift from the clinical services unit (CSU), PHE, Colindale. Citrate plasma packs from NHS Blood & Transplant which had been screened for markers including HEV RNA, anti-HEV IgG and IgM were treated with preservative (Bronidox) and filtered prior to storage at -30˚C.

2.2.1.4 HEV quantitative standards

HEV quantitative standards were prepared from a faecal sample harbouring a high titre of HEV which was diluted in NHP and assayed to quantify it against the WHO standard. Dilutions of a stock sample quantified at 1 x 10^8 IU/ml were made in NHP to generate standards of 1 x 10^7, 1 x 10^6, 1 x 10^5, 1 x 10^4, 1 x 10^3 and 1 x 10^2 IU/ml.

2.2.1.5 Generation of tissue culture-derived HEV-Ag

HEV-Ag from tissue-culture was generated to provide a stock of HEV-Ag used as internal quality control material for HEV-Ag testing and neutralization.

Cell line: HepG2/C3a cells were obtained from ATCC® (CRL-10741™ lot 61777384) and upon receipt were kept frozen in liquid nitrogen vapour phase [249].

Resuscitation of cells: A cryovial of frozen cells was removed from the liquid nitrogen storage facility and transferred to the clean tissue culture room. The

cryovial surface was decontaminated with a 70% alcohol wipe. The cap was turned a ¼ to release any residual trapped liquid nitrogen and gently warmed in a shallow water bath at 37°C. Prior to completely thawing, 1ml of pre-warmed Dulbecco's modified Eagle's medium (DMEM, D86429, Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (FBS, 10500064, Invitrogen) and 1% penicillin-streptomycin (11568876, Invitrogen) was added to the vial of cells, gently mixed and transferred into a pre-labelled 25cm^2 flask containing 5ml of medium. Cells were incubated at 37°C in a humidified 5% CO_2 atmosphere (standard conditions).

Cell culture: After 24 hours the complete cell culture medium was replaced with fresh medium using a sterile 10ml pipette. Cells were inspected daily by phase contrast microscopy and passaged at 80% confluency on day two by complete removal of culture medium, followed by a single wash of the adherent monolayer with 5ml of PBS. Approximately 1ml of 0.25% trypsin-EDTA solution (T4049, Sigma-Aldrich) was added to cover the cell monolayer which was incubated for five minutes. The flask was gently tapped to remove any adherent cells and the 1ml of solution was transferred into 11ml of culture medium in a pre-labelled 75cm^2 flask. Cells were passaged 1:3 following a further 48 hours of incubation under standard conditions and the flask transferred to CL3 conditions. Once the monolayer of cells was confluent, the culture medium was removed and the cells were washed once with 5ml of PBS and 1.5ml of 0.25% Trypsin-EDTA was added prior to incubation under standard conditions for five minutes. Approximately 10.5ml of medium was added and the flask tapped gently to remove any adherent

cells. Cells were counted manually in a haemocytometer by addition of 15µl of cells to 15µl 0.4% Trypan Blue solution (93595, Sigma-Aldrich). Six-well microplates were prepared with 2ml of standard culture media with the addition of 1% dimethyl sulfoxide (DMSO, D4540 Sigma). Cells were seeded at a concentration of 5 x 10^5 cells per well.

Cell line infection and maintenance: Virus inoculation and work with infected cells was carried out under CL3 conditions. Aliquots of filtered faecal suspensions were thawed and diluted 1:10 in PBS. Confluent monolayers of cells were washed once with 1.5ml of PBS and 200µl of diluted filtered faecal suspension was inoculated on to the cells in each well. The 6-well plates were gently rocked for 30 minutes. Into each well 2ml of standard medium supplemented with 1% DMSO was added. Plates were incubated at 35.5°C from this point on. On day 1 post-infection, the culture medium was totally removed and the cells washed ten times with 1ml of PBS before replenishing the culture medium. Every 2-3 days 1ml of cell culture supernatant fluid was harvested and replenished with an equal volume of medium. Supernatant harvests were stored at -80°C. Viral culture was monitored by RT-PCR of 100µl of harvested supernatant which was added to 100µl of lysis buffer (MagNA Pure 96 External Lysis Buffer, Roche).

Derogation from CL3: To enable HEV-Ag work to proceed in CL2 conditions permission was given to remove inactivated HEV cell culture supernatant from CL3 conditions. A sample on day 45 post-inoculation with a known titre of 1.11 x 10^7 IU/ml was removed from the -80°C freezer and thawed in the microbiological safety cabinet. A 1:10 dilution of the culture material was made in NHP to lower the viral

genome concentration to the level found in clinical specimens. The exterior of the vial was decontaminated with chlorine wipes and placed in a sealed plastic container prior to removal from the CL3 suite.

2.2.1.6 Preparation of internal quality control materials

For HEV-Ag detection, internal quality control (IQC) material was prepared using the derogated sample described above. The sample was further diluted 1:100 (high IQC) and 1:1000 (low IQC) in NHP and aliquoted into 250µl volumes prior to storage at -20°C.

For anti-HEV IgG, IQC material was prepared from the plasma packs of blood donors in convalescence from HEV infection and aliquoted into 50µl volumes prior to storage at -20°C.

2.2.1.7 Generation of HEV virus-like particles

G1 and G3 HEV virus-like particles (VLP) were a kind gift from Becky Haywood, BBVU, NIS, PHE, Colindale (see Appendix A1.2-A1.4 for details of generation and purification).

2.2.1.8 Production of a putative HEV-Ag containing pool

Plasma samples from five patients with HEV viraemia persisting for more than two months with a high HEV-Ag binding ratio (S/CO >18.0) were pooled to generate a

standard antigen. This pooled plasma was titrated in half $\log_{10}$ dilutions in NHP and tested in the HEV-Ag assay.

2.2.1.9 Production of a putative HEV-Ag neutralising reagent

Ten plasma samples from convalescent blood donors previously HEV viraemic with high IgG binding ratios (Anti-HEV IgG S/CO >20.0) and HEV-Ag non-reactive (S/CO <1.0) were selected and screened for neutralising activity (Methods 2.2.2.7). Four of the ten convalescent donor plasma samples which demonstrated potent neutralising activity were used to make an equivolumetric pool of neutralising reagent.

2.2.2 RNA extraction, PCR and serological assays

2.2.2.1 Nucleic acid extraction

For the purposes of HEV RNA detection from individual samples, nucleic acid was extracted from 200µl of each primary sample (plasma or faecal suspension) on the MagNA Pure 96 (Roche Diagnostics Ltd. Burgess Hill, UK; virus-specific cell-free protocol) and eluted into 50µl of MP96 elution buffer (Roche). In the case of minipooled samples of 1.2ml total volume, nucleic acid was extracted on the QiaSymphony platform (Qiagen, Crawley, UK; virus-specific cell-free protocol) and eluted into 60µl of buffer. Samples were spiked with the bacteriophage MS2 (whole virus) prior to extraction which acted as an internal control. Extracts were stored at -80 ºC if not being tested within 24 hours.

For whole genome sequencing, samples were extracted using the NucliSENS easyMAG extraction platform (BioMérieux, Basingstoke, UK; generic 2.0.1 protocol) from 200µl of each primary plasma sample without the addition of an internal control and eluted into 40µl of EasyMag elution buffer (BioMérieux).

2.2.2.2 Preparation of HEV and MS2 TaqMan primer and probe mix

Prior to use, batches of primer and probe mixes were made for HEV and MS2. For HEV, 120µl of 100pmol/µl stocks of forward primer, reverse primer and probe were mixed and aliquoted into single-use quantities of 36µl prior to freezing at -20°C. For MS2, 30µl of 100pmol/µl stocks of forward primer, reverse primer and probe

were mixed with 510µl of nuclease-free water and aliquoted into single-use quantities of 24µl.

2.2.2.3 Real-time PCR for HEV RNA detection

HEV RNA was detected and quantified using a validated 'in-house' quantitative reverse transcription Taqman HEV PCR targeting a highly conserved region of HEV ORF 3 [250, 251]. A real-time PCR mastermix was prepared using Qiagen Quantitect probe RT-PCR kit (Qiagen, Crawley, UK) for n+1 reactions. Each HEV reaction contained 12.5µl of Quantitect probe RT-PCR mastermix, 0.3µl HEV primer & probe mix, 0.25µl of Quantitect RT enzyme and 1.95µl of nuclease-free water. Each MS2 reaction contained 12.5µl of Quantitect probe RT-PCR mastermix, 0.4µl MS2 primer & probe mix, 0.25µl of Quantitect RT enzyme and 1.85µl of nuclease-free water.

15µl of reaction mixture was dispensed into each well of a microAmp™ optical 96-well reaction plate and 10µl of nucleic acid extract was added to each well prior to thermocycling as follows; 1 cycle at 50°C for 30 minutes, 1 cycle at 95°C for 15 minutes followed by 45 cycles of 15 seconds at 95°C then 60°C for 60 seconds.

The HEV and MS2 are assayed in parallel (dual well) with one well for HEV RNA detection and a second for the MS2 internal control. HEV and MS2 are detected in real-time by differently labelled TaqMan probes by an ABI 7500 real-time PCR system (Applied Biosystems).

Quantification is achieved by the generating a standard curve from the results of five quantitative standards calibrated to the WHO standard and the result is expressed in international units per ml (limit of detection 22 IU/ml). Results were plotted on Levey-Jennings charts in Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). The Levey-Jennings charts were analysed using Westgard rules, a multirule quality control system, to monitor performance variation of the assay to detect factors which may affect the quantitative value assigned to a positive sample [252]. Assay runs were repeated if the Westgard rules were broken.

Table 2.1 Primers and probes for detecting of HEV and MS2 bacteriophage

Name	Direction	Residue co-ordinate[a, b]	Sequence
HEV P1	Forward	5261-5278	5'-GGTGGTTTCTGGGGTGAC-3'
HEV P2	Reverse	5284-5301	5'-AGGGGTTGGTTGGATGAA-3'
HEV Probe	-	5313-5330	5'-FAM- TGATTCTCAGCCCTTCGC-MGB-3'
MS2 P1	Forward	289-314	5'-TGGCACTACCCCTCTCCGTATTCACG-3'
MS2 P2	Reverse	366-387	5'-GTACGGGCGACCCCACGATGAC-3'
MS2 Probe	-	330-358	5'-JOE-ACATCGATAGATCAAGGTGCCTACAAGCBHQ1-3'

Forward and reverse oligonucleotide primers and probes for the detection of HEV and MS2 bacteriophage internal control. All primers and probes were used at a stock concentration of 100pmol/µl.

[a] For HEV based on GenBank accession no. M73218.

[b] For MS2 based on GenBank accession no. EF204940.

Abbreviations: FAM, 6-Carboxyfluorescein fluorophore; MGB, minor grove binder quencher; JOE, 4,5-dichloro-dimethoxy-fluorescein fluorophore; BHQ1, black hole quencher; TAMRA.

2.2.2.4 HEV PCR and sequencing of ORF2 for phylogenetics

The first viraemic sample for a given patient underwent sequence and phylogenetic analysis of a 1.3kb region of the ORF2 of HEV to ascribe genotype and subtype [20]. Where viral load was sufficient this was also undertaken in cases of virological relapse following cessation of therapy. The primers used for ORF2 PCR are shown in Table 2.2.

First strand cDNA synthesis

A 10µl aliquot of nucleic acid extract was heated to 98°C for 5 minutes in a thermocycler, spun in a picofuge then snap frozen for 3 minutes. To each sample, 1µl of R30 primer (10pmol/µl) and 1µl of dNTP mix (10mM) were added, briefly centrifuged and heated to 65°C for 5 minutes before snap-freezing for 3 minutes. Subsequently, 4µl of 5X first strand buffer (Invitrogen 18064014), 2µl of 0.1M DTT (Invitrogen 18064014) and 1µl of RNaseOUT (Invitrogen 10777019) were added, centrifuged and incubated for 2 minutes at 42°C. Finally, 200 units (1µl) of SuperScript II (Invitrogen 18064014) were added to each sample, mixed and centrifuged prior to incubation at 42°C for 50 minutes, then 70°C for 15 minutes.

First round PCR

A first-round PCR mastermix was prepared for n+1 reactions. Each mastermix reaction contained 25.0µl of 2x MyFi Mix (Bioline BIO-25050), 18.0µl of nuclease-free water, 1.0µl of primer R30 (10pmol/µl) and 1.0µl of primer ORF2FWD1 (10pmol/µl). A 5µl aliquot of cDNA was added to 45µl of PCR mastermix and thermocycled as follows; at 92°C for 2 minutes, followed by 35 cycles of 95°C for

15 seconds, 52°C for 30 seconds and 2 minutes at 72°C. The reaction was subsequently kept at 72°C for 10 minutes.

Second round PCR

A second-round PCR mastermix was prepared for n+1 reactions. Each mastermix reaction contained 25.0µl of 2x MyFi Mix (Bioline BIO-25050), 18.0µl of nuclease-free water, 1.0µl of primer R30 (10pmol/µl) and 1.0µl of Primer ORF2-G3 (10pmol/µl). A 5µl aliquot of first-round product was added to 45µl of PCR mastermix and thermocycled as follows: at 92°C for 2 minutes, followed by 35 cycles of 95°C for15 seconds, 52°C for 30 seconds and 2 minutes at 72°C. The reaction was subsequently kept at 72°C for 10 minutes.

Gel electrophoresis and purification

A 1.5% agarose gel was prepared with the addition of Red Safe Nucleic Acid Staining Solution (iNtRON IB-21141). A volume of 2µl of PCR product was mixed with 6µl of water and 2µl of gel loading dye (Invitrogen 10816015). The agarose gel was poured into the electrophoresis tank ensuring 1X Tris/Borate/EDTA (TBE) buffer covered the gel. A volume of 10µl of the mixture containing the PCR product were loaded into the wells alongside a mixture containing a 1Kb DNA ladder (Invitrogen 10787018). The gel was run for 20 minutes at 130V, visualised using a Biorad UV transilluminator and photographed.

Second-round PCR products were purified prior to sequencing using the Illustra GFX PCR DNA and gel band purification kit (Illustra 28903470). A GFX column was placed into a labelled collection tube and 500µl of capture buffer were added to each

column. A volume of 40µl of PCR product was added to the column and mixed by pipetting. Columns were centrifuged at 18 500 *x* g for 1 minute, the eluate discarded and 500µl of wash buffer added. Columns were then centrifuged again at 18 500 *x* g for 1 minute and the eluate discarded. The column was transferred to a clean labelled collection tube and 40µl of nuclease free water added to the column matrix. This was incubated at room temperature for 1 minute and centrifuged a further time at 18 500 *x* g for 1 minute. The columns were then discarded.

Sequencing

A sequencing plate was set up with a total of six reactions for each sample each containing a different primer (A-F Table 2.2). Each reaction mix consisted of 1µl purified PCR product, 3µl nuclease-free water and 2µl of sequencing primer A-F. The plate was submitted to the Genomic Services and Development Unit for Sanger sequencing on Applied Biosystems' Capillary platforms.

Sequence analysis

A 'contig' was built for each sample from the six sequences A-F using DNASTAR software and checked for mixed bases or gaps and edited as appropriate. Consensus sequences were aligned with HEV reference sequences and ascribed a genotype and subtype based on maximum-likelihood phylogenetic trees drawn in MEGA 7.0 (Version 7.0.26) [145].

Table 2.2 Primers for ORF2 HEV PCR for phylogenetics

Primer label	Name	Direction	ORF2 residue co-ordinate	Sequence
-	ORF2-FWD1	Forward	258-280	5'-TTGGCGTGACCAGKCCCAGCGCC
A	ORF2-G3	Forward	538-560	5'-TCYAAYTAYGCYCAGTAYCGGGT
B	MENG-F1	Forward	826-846	5'-GTYATGYTYTGCATACATGGCT
C	MENG-R1-FWD	Forward	1152-1172	5'-GACAGAATTGATTTCGTCGGC
D	MENG-R1	Reverse	1152-1172	5'-AGCCGACGAAATYAATTGTGTC
E	MENG-R0	Reverse	1249-1271	5'-CCCTTATCCTGCTGAGCATTCTC
F	R3O	Reverse	1951-1980	5'-AGACTCCCGGGTTTTACCTACCTT CATTTT

Forward and reverse oligonucleotide primers to generate ORF2 PCR product for phylogenetic analysis. All primers kept at stock concentration of 100pmol/µl
Abbreviations: ORF2, open reading frame 2.

2.2.2.5 HEV serology

HEV antibodies were detected using the Wantai IgM and IgG detection assays (Fortress Diagnostics, Antrim, Northern Ireland, UK). The IgG assay is an indirect ELISA in which HEV recombinant antigen is bound to the solid phase and the conjugate comprises horseradish peroxidase (HRP) bound to anti-human IgG. The IgM assay is a capture ELISA with anti-μ chain antibodies bound on the solid phase and a conjugate comprising recombinant ORF2 antigen bound to HRP. The procedure for both assays is the same.

Briefly, 10μl of sample are added to 100μl of specimen diluent in each well and incubated at 37°C for 30 minutes. The wells were washed five times using the supplied buffer and tapped dry. One hundred microliters of horseradish peroxidase-conjugate were added to each well and the plate incubated for a further 30 minutes at 37°C. The wells were washed again five times using the supplied buffer, then chromogen added. Following a third incubation in the dark for 15 minutes at 37°C, the reaction was stopped using 'Stop' solution. Optical densities (OD) were measured immediately with an ELISA plate reader (EL808™ Absorbance Microplate Reader, BioTek; $OD_{450/630}$). The assay cut-off value (CO), the OD threshold above which determines the positive status of a sample (S), was calculated using the mean OD absorbance value of the three negative controls plus 0.16 (or 0.26 for anti-HEV IgM assay) in accordance with the manufacturer's criteria. Samples with sample/cut-off (S/CO) ratios $\geq$1.1 were considered reactive.

In addition to fulfilling the manufacturer's quality control criteria for the OD absorbance values of blank wells, negative controls and positive controls, further internal quality control reagents were added to each run. The OD absorbance values of the internal control reagents were plotted on a Levey-Jennings chart and analysed using Westgard rules. If the internal quality control results failed Westgard rules, the assay was repeated.

2.2.2.6 HEV-Ag detection

HEV-Ag was detected using a commercial sandwich ELISA (HEV-Ag ELISA, Fortress Diagnostics, Antrim, Northern Ireland, UK) according to the manufacturer's recommendations. The assay is a sandwich ELISA with polyclonal antibodies directed against the ORF2 product on the solid phase with enzyme-linked monoclonal antibodies in the detection system. Briefly, 50µL of sample were added to the ELISA plate and incubated for 1 hour at 37°C. Horseradish peroxidase–conjugated monoclonal anti-HEV ORF2 antibody was added, followed by a further incubation for 30 minutes at 37°C. The wells were washed five times using the supplied buffer and then chromogen added. Following a third incubation in the dark for 15 minutes at 37°C, the reaction was stopped using 'Stop' solution. Optical densities (OD) were measured immediately with an ELISA plate reader (EL808™ Absorbance Microplate Reader, BioTek; OD450/630). The assay cut-off value (CO), the OD threshold above which determines the positive status of a sample (S), was calculated using the mean OD absorbance value of the three negative controls plus 0.16. Any sample with a S/CO ratio >1.0 on initial testing

was labelled reactive (IR) and if on repeat testing a S/CO >1.0 as repeat reactive (RR).

2.2.2.7 Measurement of HEV-Ag neutralising activity

Neutralising activity was determined as a percentage of the reduction in binding in the HEV-Ag assay of the test incubation mixture (antigen sample plus neutralising reagent) when compared to a non-neutralising control mixture (antigen sample plus NHP) using the following formula:

$$\% \text{ neutralisation} = \frac{(OD_{\text{sample+neutralising reagent}} - OD_{NHP})}{(OD_{\text{sample+NHP}} - OD_{NHP})} \times 100$$

For stool samples the OD of STAR buffer was subtracted from the test OD rather than OD of NHP.

2.2.2.8 Illumina whole genome sequencing of HEV

Nucleic acid extracts were sent to the Centre for Virus Research Glasgow for whole genome sequencing.

2.2.2.8.1 Design of target enrichment probes

A custom set of SeqCap EZ HEV probes (Roche) were used for target enrichment of HEV in clinical samples. The probes were designed by Roche following submission of 241 full genome sequences of HEV recovered from human

infections. Briefly, the submitted sequences were split into 50-mers and all exact duplicates removed to minimise biasing the probe pool towards highly represented sequences. An overlapping probe set was designed to cover the whole genome and was subsequently screened against human and porcine sequences to reduce the risk of enriching host sequences.

2.2.2.8.2 NGS library preparation

RNA libraries were prepared using the KAPA stranded RNA-Seq protocol (KAPA Biosystems). An aliquot of 10µl of extracted RNA was mixed thoroughly with 10µl of 2X fragment, prime and elution buffer prior to fragmentation by incubation for 2 minutes at 85°C.

The first strand of cDNA was synthesised by adding 10µl of first strand mastermix (containing KAPA reverse transcriptase and random primers) to 20µl of fragmented, primed sample and incubated at 25°C for 10 minutes to allow primer extension, then 42°C for 15 minutes for first strand synthesis, and finally at 70°C for 15 minutes to inactivate the enzymes.

The second strand of cDNA was synthesised by adding 30µl of second strand mastermix (containing enzyme and dUTP to mark the 2nd strand) to 30µl of the sample, mixing thoroughly and incubated at 16°C for 60 minutes to allow second strand synthesis and marking.

The sample was purified by adding 1.8X volume of AMPure XP beads (Beckman Coulter) to each sample and incubated at room temperature for 5-15 minutes before placing on a magnetic rack until the beads and solution had fully separated.

The supernatant was discarded and the beads were washed twice with 200µl of 80% ethanol before air drying for 3-5 minutes.

A polyadenylation tail was added by suspending the beads in 30µl of A-tailing mastermix (containing A-tail buffer, A-tail enzyme and nuclease-free water) and incubated at 30°C for 45 minutes for A-tailing and then at 60°C for 30 minutes to inactivate the enzyme.

The adapters, containing illumina sequences and the indexes for multiplexing, were ligated to the cDNA by adding 35µl of adapter ligation mastermix (containing ligation buffer and DNA ligase) to 5µl of 10nM diluted adapter stock and 30µl of the beads with A-tailed DNA and subsequently incubated at 20°C for 60 minutes.

The samples were then subjected to two post-ligation clean-up steps. Initially an equal volume of solid-phase reversible immobilisation solution (SPRI: 20% Polyethylene glycol 8000; 2.5M NaCl; 10 mM Tris-HCl pH 8.0) was added to each sample and incubated at room temperature for 5-15 minutes. The beads were then separated from the solution by placing on a magnetic rack until they had fully separated and the supernatant discarded. The beads were washed twice with 200µl of 80% ethanol before air drying for 3-5 minutes and eluting in 50µl TrisCl pH 8.0. A further 50µl of SPRI (1.0X SPRI:sample) was added to each sample and then incubated at room temperature for 2 minutes, the beads separated on a magnetic rack and washed twice with 200µl of 80% ethanol. The beads were then air dried for 3-5 minutes and eluted in 22µl 10mM TrisCl pH 8.0.

The library was amplified by adding 30µl of library amplification mastermix (containing KAPA HF HotStart ReadyMix and 10X Library Amplification primers

targeting the ligated illumina adapters) to each sample and incubated at 98°C for 45 seconds for the initial denaturation. This was followed by 18 cycles at 98°C for 15 seconds to denature, 60°C for 30 seconds to anneal and 72°C for 30 seconds to allow extension to take place, before the final extension at 72°C for 5 minutes. The sample was subjected to a final purification step by adding 45µl of AMPure XP beads to each sample (0.9X AMPure:sample) of amplified library DNA, incubated at room temperature for 5-15 minutes and separated on a magnetic rack. The beads were washed twice with 200µl of 80% ethanol and air dried for 3-5 minutes prior to elution into 22µl of 10mM TrisCl pH 8.0.

2.2.2.8.3 Target enrichment

Libraries were quality checked and quantified using the 2200 Tapestation (Agilent) and Qubit 3.0 fluorometer (Invitrogen) and pooled to ensure viral loads were in equivalent proportions within each pool to generate a multiplexed library (max 16 samples per pool, range 4-16). The library was then enriched using NimbleGen SeqCap EZ library SR Kit (Roche, UK), according to the manufacturer's instructions.

Approximately 1µg of multiplexed library was added to 5µl of COT DNA and 2µl of xGen Universal Blocking Oligos TS mix (IDT). To this, 2X volume of AMPure XP beads were added, thoroughly mixed and incubated at room temperature for 10 minutes, prior to separation of the beads on a magnetic rack. The supernatant was discarded and the beads were washed once in 80% ethanol and air-dried for 3-5 minutes. The DNA was eluted by adding 10.5µl of mastermix (containing 2X

hybridisation buffer and hybridisation component A), mixing thoroughly and incubating at room temperature for 2 minutes. The tubes were placed on a magnetic rack and the entire volume was eluted into a new PCR tube containing 4.5 µl SeqCap EZ HEV probe pool (Methods 2.2.8.1). This was mixed thoroughly and incubated at 95^{o}C for 5 minutes, then at 47^{o}C for 72 hours.

The SeqCap EZ Pure Capture Bead Kit and streptavidin capture beads were equilibriated to room temperature for 30 mins prior to use. The streptavidin beads were vortexed for 15 seconds and 100µl of beads was transferred to a 1.5 ml tube for each capture. Each tube was placed on a magnet and the excess liquid discarded. The beads were washed twice in 1 x bead wash buffer by adding 200µl of 1 x bead wash buffer to each tube, vortexing for 10 seconds, placing back on the magnet and the excess liquid discarded. After washing, the beads were then resuspended in 200µl of 1x bead wash buffer by vortexing and 100µl of resuspended beads was added to a 0.2 ml PCR tube for each capture. The tube was placed on a magnet and the liquid discarded. Next, 15µl of the sample which had been incubated for 72 hours with the probe pool was added and thoroughly mixed and incubated for 45 mins at 47°C, mixing every 15 minutes. A volume of 100µl of pre-warmed 1x wash buffer 1 was added and vortexed for 10 seconds, placed on a magnet and the excess liquid discarded. The beads were washed twice in pre-warmed 1x Stringent Wash Buffer (pre-warmed to 47°C) by placing the tube on a magnet, discarding the excess liquid, adding 200µl of 1x Stringent Wash Buffer, mixing thoroughly and then incubated at 47°C for 5 mins. The mixture was then transferred to a 1.5 ml DNA LoBind tube (Eppendorf), placed on a magnet

and the excess liquid discarded. A volume of 200µl of 1x Wash Buffer 1 at room temperature was added and vigorously vortexed. The tube was then placed on a magnet and the excess liquid discarded. This was repeated with 200µl of 1x Wash Buffer 2 and subsequently 1x Wash Buffer 3. After the excess liquid was discarded, the beads were eluted into 20µl of water.

2.2.2.8.4 Amplifying captured multiplex DNA using LM-PCR

A volume of 15µl of prepared mastermix (containing KAPA HiFi HotStart ReadyMix and post-LM-PCR Oligos 1+2) was aliquoted into two reaction tubes per capture. The bead-bound captured DNA was vortexed briefly and 10µl were added to each reaction tube and subjected to PCR as follows: 95°C for 3 minutes then 14 cycles of 98°C for 20 seconds, 65°C for 15 seconds, 72°C for 30 seconds. This was followed by a final incubation at 72°C for 2 minutes, before the reaction was held at 4°C.

The two 25µl PCR reactions were then pooled to make a total of 50µl. To this 0.9X AMPure XP beads was added and mixed thoroughly before incubation at room temperature for 5 minutes, then placed on a magnetic rack until the beads had separated from the solution. The excess liquid was discarded and the beads washed twice with 200µl 80% ethanol prior to air drying for 5 minutes and finally eluting in 15µl of 10 mM Tris pH 8.0. Final libraries were quality checked and quantified using the 2200 Tapestation (Agilent) and Qubit 3.0 fluorometer (Invitrogen) prior to sequencing.

2.2.2.8.5 Double target enrichment

Sixteen of the prepared libraries were selected for double enrichment due to low HEV genome coverage (<70%) of the consensus sequence identified during sequence analysis. In this case target enrichment proceeded as described except for the following amendments: in the first enrichment the SeqCap EZ HEV probe pool was used at a 1:2 dilution in nuclease-free water and the PCR was cycled for four cycles. The resulting 50µl of PCR reaction were subjected to a second enrichment using the SeqCap EZ HEV probe pool at a 1:2 dilution and the PCR was cycled for 14 cycles.

2.2.2.8.6 Illumina whole genome sequencing

Deep sequencing was carried out on samples in two separate batches using the NextSeq 550 with a NextSeq 500/550 Mid output v2 kit (Illumina) following the manufacturers protocol for paired-end sequencing (loading a final concentration of 1.6 pM with the addition of 1% phiX).

2.2.3 Bioinformatics analysis of Illumina sequences

2.2.3.1 Reference mapping

FASTQ files generated from short-read Illumina sequencing underwent quality control screening (Trim Galore version 0.4.0, Cutadapt version 1.16, Phred score cut-off 30 using ASCII+33 quality encoding). Trimmed files were subsequently used to generate SAM files by reference mapping against the proposed HEV reference sequences using Tanoti in-house assembler and consensus sequences produced (http://bioinformatics.cvr.ac.uk/tanoti.php) [145]. In-house 'Sameer' reports were generated providing analysis of reference mapping details, HEV genome coverage, sequence depth across the genome and the full nucleotide sequence. Samples with sequence data covering 70% or more of the HEV genome were accepted for further analysis.

2.2.3.2 Data analysis

Sequences were aligned using the MAFFT sequence alignment program (https://mafft.cbrc.jp/alignment/software/). Aligned consensus sequences were viewed in Bioedit (Version 7.2.5) for amino acid switches over time. HEV GLUE offline (Version 0.1.152, Copyright (C) 2018 The University of Glasgow) was used to analyse SAM files for amino acid polymorphisms at specific loci identified by Bioedit analysis [253]. Amino acid variations were only called when the depth of reads at the loci exceeded 10 and frequencies only called when they exceeded

5% of the viral population. Phylogenetic trees were constructed in MEGA 7.0 (Version 7.0.26).

The full linux commands used for whole genome sequence analysis are available in Appendix A1.8.

2.2.3.3 Analysis of published sequences

To determine the frequency of specific HEV mutations amongst circulating viruses, as determined by the frequency in published HEV sequences available in GenBank, HEV GLUE offline was used. Sequences available covering the region of interest for the HEV subtype of interest were downloaded, aligned and analysed to determine the consensus amino acid at the locus of interest and the frequency in the downloaded sequences.

2.2.3.4 Diversity analysis

The diversity of the HEV quasispecies was investigated at the amino acid level by means of sorted BAM files in DiversiTools (http://josephhughes.github.io/DiversiTools/). Using in-frame sequence alignment of coding regions of the HEV genome, the depth of reads and the frequency of amino acid variations were calculated.

2.2.3.5 Statistical analyses

Continuous data were compared using the Wilcoxon two-sample signed-rank test, while categorical data were compared using the Fisher's exact test or Chi-squared. Paired data were compared using the Wilcoxon matched pairs test. Confidence intervals (CIs) for measures of prevalence were calculated using the Wilson method. Pearson's correlation determined correlation coefficients between continuous variables. Multivariable logistic regression models were built in a stepwise fashion to ensure the best fit. Missing data are summarized in the relevant sections. All statistical analyses were performed in STATA 13.1.

2.3 Health economic analyses

2.3.1 Development and modification of Markov model

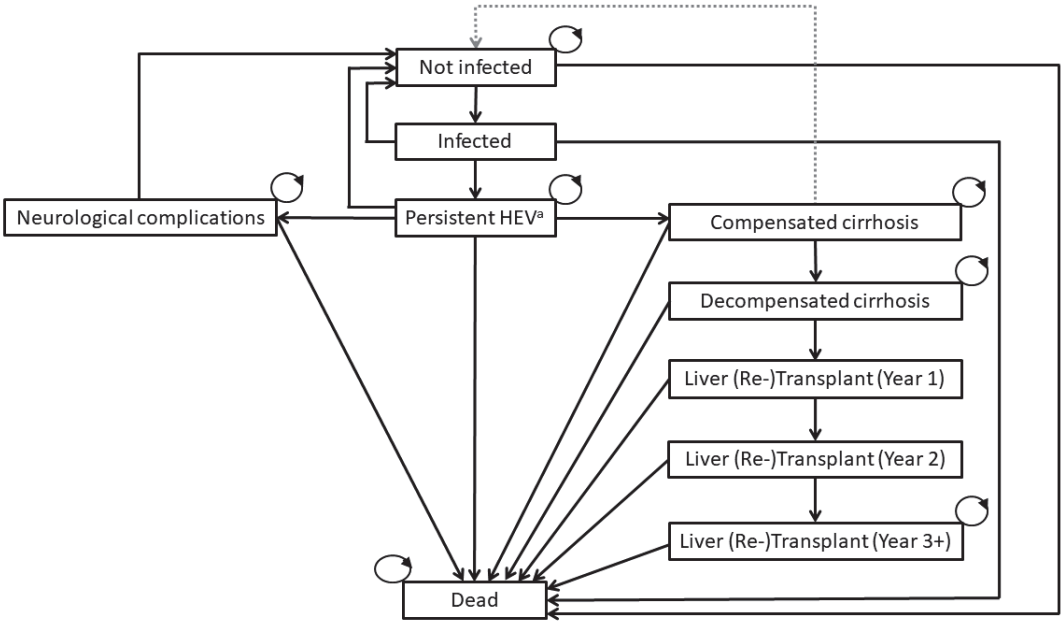
A Markov model for HEV screening of blood donors was available to us. This had been previously utilised by the Advisory Committee on the Safety of Blood, Tissues and Organs *(SaBTO, UK*) HEV working party (Siobhain McKeigue, John Cairns; Pers. Comm.) and was extensively updated and modified for this analysis to suit solid organ transplant patients and follow the NICE reference case. The model was developed in Microsoft Excel 2010 (Microsoft, Redmond, WA, USA), using the inbuilt Visual Basic for Applications for the uncertainty analyses.

The model follows five hypothetical cohorts. The baseline cohort is the group of individuals to which no screening is offered and HEV diagnosis, and thus treatment, relies on clinically prompted testing. We then compared in separate cohorts annual HEV screening for all patients by PCR (strategy A) or HEV-Ag (strategy B) with a selective screening programme using an abnormal alanine aminotransferase (ALT) value as an indicator for HEV testing by PCR (strategy C) or HEV-Ag (strategy D).

The Markov model allows the modelling of transitions of a patient through different health states over time. The model was populated with parameters for costs, outcomes and the probability of each outcome occurring to simulate the impact of screening compared with not screening recipients of solid organ transplants. Outcomes were measured in quality-adjusted life years (QALYs). Patients enter the model and transition from one state to the next at yearly intervals with a half-

cycle transition point; at the end of each yearly cycle the accumulated QALYs and costs are totalled. A schematic overview of the model is shown in Figure 2.1.

Figure 2.1 Markov Model for HEV Infection in SOT recipients



Markov model showing transitions between the main health states during the natural history of HEV infection in solid organ transplant recipients.

[a] Screening (strategies A-D) was modelled to identify patients in the persistent HEV infection health state. The transition from persistent HEV to uninfected was determined by the success of treatment (reduction of immunosuppression and up to two courses of ribavirin therapy).

A separate structural scenario analysis explored a transition from compensated cirrhosis to uninfected (dotted line), assuming the same parameters as for the transition from persistent HEV to uninfected.

Abbreviations: HEV, hepatitis E virus.

In the model, all SOT patients start as uninfected and exposed at an annual constant rate to HEV. Subsequently, infected patients may develop a persistent HEV infection; in this state they may be diagnosed and treated and return to the uninfected state. However, a proportion will develop neurological complications, or liver cirrhosis at which point there is a risk of decompensation and requirement for liver (re-)transplantation. Patients with compensated cirrhosis were also allowed to be diagnosed and treated and return to uninfected in a structural scenario analysis.

The results of the model are reported as an incremental cost-effective ratio (ICER), where the difference between the mean costs of the screening programme (C_1) and no screening (C_0) are divided by the difference in the mean effects of the screening programme (E_1) and no screening (E_0):

$$ICER = \frac{(C_1 - C_0)}{(E_1 - E_0)}$$

Results are presented in a fully incremental fashion by comparing strategies to the next-best strategy after removing (extendedly) dominated strategies.

2.3.2 Model assumptions

The model considered a hypothetical cohort of 1,000 SOT recipients. The base-case analysis was initiated with recipients aged 48 years, which is the weighted mean age of transplant recipients in the UK in 2016/17 [254]. The model used cycle lengths of one year until all living recipients reached the expected end-of-life based on a composite mortality rate incorporating a background rate and a

transplant-specific rate, or to a maximum age of 100; whichever came first (see Table 2.6). Half-cycle corrections were applied [255]. The risk of HEV acquisition was considered as a constant annual dietary risk. All patients were considered equally susceptible to HEV infection and once cleared of HEV infection were considered equally susceptible again [256]. In contrast to established models for hepatitis C virus (HCV), a health state of hepatocellular carcinoma (HCC) was not included in the model [254, 257]. Discounting was applied at 3.5% to both QALYs and costs as recommended by NICE [258].

2.3.3 HEV screening algorithms and modelling the impact of screening

The different testing strategies considered are detailed in Table 2.3; the baseline scenario represents the current UK situation whereby patients are only tested for HEV infection when the diagnosis is suspected clinically. The two main HEV screening assays compared were HEV RNA detection by PCR (strategies A and C) and HEV-Ag detection by ELISA with confirmation by PCR (strategies B and D). We considered an annual convenience screening strategy whereby SOT patients are tested each year following transplantation during one of their regular healthcare visits.

The parameters that differed in each scenario were the costs of screening and the probability of HEV being diagnosed owing to the different sensitivity and specificity of the screening options, informed by results reported in Chapter 3. The probability of being diagnosed informed the numbers of patients proceeding to treatment, incurring treatment costs and the different utility values of each health state. For

example, the proportion of patients diagnosed in the absence of routine screening was considered as 10% in the base-case analysis. Thus only 10% proceed to treatment and incur treatment costs and the lower utility state associated with treatment-related adverse events.

Table 2.3 Screening strategies for HEV infection in SOT recipients

Strategy	Patients to be tested for HEV infection	Initial screening assay	Confirmatory assay
baseline	Only when HEV suspected by clinician[a]	PCR	none
A	All UK SOT patients	PCR	none
B		HEV-Ag ELISA	PCR
C	UK SOT patients with abnormal ALT[b]	PCR	none
D		HEV-Ag ELISA	PCR

The four screening strategies (and confirmatory testing) for HEV infection in SOT recipients are detailed that were compared in the cost-effective analysis.

[a] Represents the current scenario in the UK of no screening.

[b] Abnormal ALT considered as >41 IU/L.

Abbreviations: Ag, hepatitis E virus antigen; ALT; alanine aminotransferase; ELISA, enzyme linked immunosorbent assay; HEV, hepatitis E virus; PCR, polymerase chain reaction; SOT, solid organ transplant; UK, United Kingdom.

2.3.4 Input parameters of model

The input parameters of the model consisted of the annual transition probabilities, healthcare costs, and the health-related impact on patients' quality of life as measured with utility scores. We assumed conventional gamma distributions for costs, beta distributions for utilities and transition probabilities, and a truncated normal distribution for the observed range of years of age at transplantation [254]. The full set of model parameters are presented in Table 2.4. Unit healthcare costs informing the final healthcare states are detailed in Table 2.5. Where costs, probabilities or quality-of-life scores were not available for direct input into the model, they were calculated from available figures and are detailed in Table 2.6.

Table 2.4 Input parameters for cost-effectiveness model

Variable	Base Case Value	Lower Limit used in PSA[a]	Upper Limit used in PSA[a]	Distribution of data for PSA	Reference
Healthcare costs (annual):					
Cost of HEV PCR test[b]	£56.28	£28.14	£84.42	Gamma	expert opinion
Cost of HEV-Ag test[b]	£11.49	£5.75	£17.24	Gamma	expert opinion
Treatment/monitoring persistent HEV infection[c]	£3 696	£2 838	£6 183	Gamma	calculated [221, 259, 260]
Compensated Cirrhosis	£1 412	£1 130	£1 694	Gamma	[261]
Decompensated Cirrhosis	£11 317	£9 053	£13 580	Gamma	[261]
Neurological Complications[d]	£23 516	£2 363	£73 066	Gamma	[262]
Liver Transplant Y1	£65 203	£52 162	£78 244	Gamma	[263]
Liver Transplant Y2	£13 262	£10 609	£15 914	Gamma	[263]
Liver Transplant Y3+	£5 526	£4 421	£6 631	Gamma	[263]
Utilities (HRQoL):					
SOT recipient in uninfected & infected state[e]	0.763	0.721	0.837	Beta	calculated [257, 264]
SOT recipient in persistent HEV state[f]	0.736	0.704	0.791	Beta	calculated [257, 261, 264]
Compensated cirrhosis	0.550	0.440	0.660	Beta	[261]
Decompensated cirrhosis	0.450	0.360	0.540	Beta	[261]
Neurological complications[g]	0.588	0.471	0.706	Beta	calculated [265]
Liver Transplant Y1/Y2/Y3+[h]	0.763	0.721	0.837	Beta	calculated [257, 264]

Variable	Base Case Value	Lower Limit used in PSA[a]	Upper Limit used in PSA[a]	Distribution of data for PSA	Reference
Annual transition probabilities:					
Annual risk of HEV acquisition	0.23%	0.04%	1.06%	Beta	[22, 27, 102, 266]
Persistent infection from infected	65.90%	57.80%	78.80%	Beta	[207]
Neurological complication from persistent HEV[i]	1.13%	0.90%	1.35%	Beta	[164]
Compensated cirrhosis from persistent HEV[j]	5.82%	4.65%	6.98%	Beta	[207]
SVR from persistent HEV (overall)[f]	88.70%	75.30%	98.60%	Beta	composite [223]
SVR with reduction of immunosuppression	32.14%	25.71%	38.57%	Beta	[207]
SVR with up to 2 courses RBV	85.00%	68.00%	100.00%	Beta	[223]
SVR from neurological complication	75.00%	60.00%	90.00%	Beta	[164]
Decompensation from compensated cirrhosis	10.00%	4.00%	12.00%	Beta	[207, 261, 267]
Death from compensated cirrhosis[k]	5.50%[k]	4.40%	6.60%	Beta	[267]
Death from decompensated cirrhosis[k]	30.50%[k]	24.40%	36.60%	Beta	[267]
Liver transplant from decompensated cirrhosis	20.00%	5.00%	50.00%	Beta	[207]
Death from liver (re)transplant[k]	3.96%[k]	3.17%	4.75%	Beta	[254]
Death from solid organ transplant[k,l]	2.64%[k]	2.11%	3.17%	Beta	calculated [254]
Age-related background mortality[k]	Variable	fixed	fixed	fixed	[268]
Diagnostic strategy performance:					
Probability of HEV infected SOT recipient with ALT > ULN	92.9%	68.5%	98.7%	Beta	Ch. 4
Sensitivity of HEV PCR [specificity] (Y)	99.7% [100%]	79.2% [fixed]	100% [fixed]	Beta	[250, 251]
Sensitivity of HEV-Ag detection [specificity] (Z)	94.7% [97.9%]	75.8% [96.0]	100% [99.0]	Beta	Ch. 4
Sensitivity of ALT then HEV PCR[m]	X x Y	73.5%	100%	Beta	assumption
Sensitivity of ALT then HEV-Ag[m]	X x Z	70.3%	100%	Beta	assumption
Probability of HEV diagnosis in absence of screening	10%	8.0%	50.0%	Beta	Ch. 4
Probability of SOT recipient with ALT >ULN in year 1/yea	33.6%/10.5%	29.7%/9.2%	37.7%/12.0%	Beta	reworked from Ch. 4

Variable	Base Case Value	Lower Limit used in PSA[a]	Upper Limit used in PSA[a]	Distribution of data for PSA	Reference
Other Parameters:					
Initial age[f]	48	16	81	truncated normal[n]	calculated [254]
Utilities discounting rate	3.5%	fixed	fixed	fixed	[258]
Cost discounting rate	3.5%	fixed	fixed	fixed	[258]
Cohort size	1000	fixed	fixed	fixed	convention

Legend for Table 2.4:

[a] Where specific figures were not available to inform extremes, +/- 20% of base-case value was used as for the DSA.

[b] Representative of reference laboratory testing price (Steve Harbour, VRD, PHE, Pers. Comm.). Total annual screening costs varied in the selective screening strategies (C and D) and included confirmatory testing for the HEV-Ag assay (see Table 2.6).

[c] Composite score (see Table 2.6).

[d] Conversion to UK-specific value using consumer price index and purchasing power parity conversion [269, 270].

[e] Mean HRQoL of different SOT categories weighted by prevalence of the transplant category in UK.

[f] Composite score (see Table 2.6).

[g] Calculated as 20% reduction of HRQoL of case of diagnosed persistent HEV infection [265].

[h] Assumed same as initial underlying transplant.

[l] 6% of cases developed a neurological complication over 5.33 years (0.0113) [164].

[j] Eight cases of 56 developed cirrhosis during a follow up of 29.5 months [207].

Abbreviations: HEV-Ag, hepatitis e virus antigen; ALT, alanine aminotransferase; Ch.; Chapter; CI, confidence interval; DSA, deterministic sensitivity analysis; HEV, hepatitis E virus; HRQoL, health-related quality of life; NA, not available; PCR, polymerase chain reaction; PHE: Public Health England; PSA, probabilistic sensitivity analysis; SD, standard deviation; SOT, solid organ transplant; SVR, sustained virological response; UK, United Kingdom; ULN, upper limit of normal; VRD, Virus Reference Department; Y, year.

Table 2.5 Healthcare costs to treat persistent HEV Infection

Investigation/Intervention	No. in typical treatment pathway[a]	% receiving investigation/ intervention	Unit cost	Reference
Diagnostic work-up/initial management:				
Initial diagnostic clinic appointment	1	100	£ 219.26	[260]
Clinic appointments	2	100	£ 167.47[b]	[260]
Phlebotomy	7	100	£ 3.00	[260]
HEV PCR in blood and stool	6	100	£ 56.28	Expert Opinion[c]
Other blood tests at diagnosis[d]	1	100	£ 108.00	[260]
Other monitoring blood tests	2	100	£ 28.00	[260]
Imaging: Liver USS	1	100	£ 51.78	[260]
Imaging: Fibroscan	1	100	£ 113.18	[260]
Attendance for imaging	1	100	£ 18.71	[260]
Liver biopsy & histopathology as day case	1	10e	£ 247.84	[260]
Drug cost saving[f]	1	100	-£ 450.49	[259]
Total cost for average patient			**£ 715.67 (X)**	
1st course of ribavirin:				
3 month course of ribavirin 800mg/day	1	100	£ 729.73[g]	[259]
Clinic appointments[h]	7	100	£ 167.47[b]	[260]
Phlebotomy	7	100	£ 3.00	[260]
HEV PCR in blood and stool	14	100	£ 56.28	Expert Opinion[c]
Other blood tests	7	100	£ 28.00	[260]
Extra clinic appointments for side effects	2	10[d]	£ 167.47[b]	[260]
EPO treatment of 3m for side effects	1	10[d]	£ 1,194.66	[259]
RBC transfusion for side effects	4	10[d]	£ 496.00	[260]
Total cost for average patient			**£ 3268.39 (Y)**	
2nd course of ribavirin:				
6m course of ribavirin 800mg/d	1	100	£ 1,502.11[g]	[259]
Clinic appointments[i]	10	100	£ 167.47[b]	[260]
Phlebotomy	10	100	£ 3.00	[260]
HEV PCR in blood and stool	20	100	£ 56.28	Expert Opinion[c]
Other blood tests	10	100	£ 28.00	[260]
Extra clinic appointments for side effects	2	10	£ 167.47[b]	[260]
EPO treatment of 6m for side effects	1	10	£ 2,389.32	[259]
RBC transfusion for side effects	4	10	£ 496.00	[260]
Total cost for average patient			**£ 5097.60 (Z)**	
Weighted cost[j] for average patient			**£ 3695.68**	

Legend for Table 2.5:

Unit costs to calculate healthcare costs of treatment for persistent HEV infection

[a] Typical pathway informed by British and European Guidelines [220, 221].

[b] Weighted cost of 50% clinics seen in consultant led (£219.26), 50% seen in non-consultant led clinics (£115.69).

[c] Representative of reference laboratory testing price (Steve Harbour, VRD, PHE, Pers. Comm.)

[d] Includes Full Blood Count, Urea and Electrolytes, Liver Function Tests, International normalised ratio, Hepatitis B & C serology, PCR testing for EBV and CMV and therapeutic drug monitoring for tacrolimus or ciclosporin levels.

[e] Expert opinion reflecting clinical experience of transplant hepatologist managing HEV in a wide range of transplant groups (Ahmed Elsharkawy, Chairman of British Viral Hepatitis Group, Pers. Comm.)

[f] Reduction of immunosuppression leading to savings in drug costs; assumed reduction of tacrolimus of 2mg/day for 3 months.

[g] Average of two common brands (Copegus and Rebetol).

[h] Outpatient appointment at baseline, week 1, 4, 8, 12 and 12 and 24 weeks post EOT.

[l] Outpatient appointment baseline, week 1, 4, 8, 12, 16, 20, 24 and 12 and 24 weeks post EOT.

[j] (probability of being diagnosed x X) + (probability of being treated with RBV x Y) + (probability of requiring 2nd course RBV x Z)

Abbreviations: BNF, british national formulary; BTS, british transplant society; CMV, cytomegalovirus; d, day; EBV, Epstein barr virus; ELISA, enzyme-linked immunosorbent assay; EOT, end of treatment; EPO, erythropoietin; m, months; mg, milligrams; Pers. Comm., personal communication; RBC, red blood cells; USS, ultrasound scan.

Table 2.6 Calculations for Markov Model parameters

Parameter name	Base-case value	Calculation	References
Annual transition probabilities:			
Probability of SVR from persistent HEV state	88.70%	pSVR with reduction of immunosuppression (0.321) + ((1 - pSVR with reduction of immunosuppression (0.321)) x pSVR with up to 2 courses RBV (0.85))) – pNeurological complication (0.0113)	[164, 207, 271]
Mortality rate from solid organ transplantation	2.6%	(Average annual mortality rate for kidney SOT recipient[b] (0.0197)) x proportion in UK transplant population (0.726)) + (Average annual mortality rate for liver SOT recipient[b] (0.0396) x proportion in UK transplant population (0.197)) + (Average annual mortality rate for cardiothoracic SOT recipient[b] (0.0558) x proportion in UK transplant population (0.077))	[254]
Mortality rate from liver transplantation	3.96%	(Average annual mortality rate for DCD liver recipient[b] (0.036)) x proportion in UK transplant population (0.777) + (Average annual mortality rate for DBD liver recipient[b] (0.052)) x proportion in UK transplant population (0.223)	[254]
Healthcare costs:			
Annual screening costs for HEV-Ag (strategy B)[a]	Variable (X)	No. of uninfected patients x cost of HEV-Ag (£11.49) + (cost of HEV PCR (£56.28) x (1 – specificity of HEV-Ag (0.979)) + no. of infected patients x cost of HEV-Ag (£11.49) + cost of HEV PCR (£56.28)	Ch. 4

Parameter name	Base-case value	Calculation	References
Annual screening cost (per patient) for selective screening by HEV PCR (strategy C)	£19.14 year 1/ £5.63 year 2+	assay costs (HEV PCR (£56.28) x % patients with abnormal ALT (0.34 in first year post transplant, 0.10 in year 2 onwards).	Ch. 4
Annual screening cost (per patient) for selective screening by HEV-Ag (strategy D)	Variable	assay costs (HEV-Ag (X)) x % patients with abnormal ALT (0.34 in first year post transplant, 0.10 in year 2 onwards).	Ch. 4
Utilities (HRQoL):			
HRQoL of SOT recipient in uninfected and infected state	0.763	(HRQoL of kidney SOT recipient (0.760) x proportion in UK transplant population (0.726)) + (HRQoL of liver SOT recipient (0.780) x proportion in UK transplant population (0.197)) + (HRQoL of cardiothoracic SOT recipient (0.750) x proportion in UK transplant population (0.077))	[254, 257]
HRQoL of SOT recipient in persistent HEV state	0.736	HRQoL of SOT recipient (0.763) x pSVR from reducing immunosuppression (0.321)) + ((HRQoL of patient on treatment (0.650) x 0.25 + (HRQoL of SOT recipient (0.763) x 0.75) x pRequiring 3 months of RBV (0.529)) + ((HRQoL of patient on treatment (0.650) x 0.75) + (HRQoL of SOT recipient (0.763) x 0.25) x pRequiring 9 months RBV (0.150)	[207, 223, 261]

Parameter name	Base-case value	Calculation	References
Other Parameters:			
Initial age at transplantation	48yrs	(Average age of deceased donor kidney recipient (50)) x proportion in UK transplant population (0.495) + (Average age of living donor kidney recipient (45)) x proportion in UK transplant population (0.231)) + (Average age of deceased donor liver recipient (47) x proportion in UK transplant population (0.197)) + (Average age of deceased donor cardiothoracic SOT recipient (43) x proportion in UK transplant population (0.077))	[254]

Legend for Table 2.6:

[a] HEV-Ag screening costs includes all the confirmatory PCR testing required for a reactive HEV-Ag result.

[b] Average calculated using 10 year survival data or 5 year survival data where 10 year was not available (Liver DCD and Lung DCD) and weighted for by type i.e. for kidney the proportions were 0.40 for DBD, 0.28 for DCD and 0.32 for living donor kidneys; for liver as above.

Abbreviations: Ch.; Chapter; DBD, donation after brainstem death; DCD, donation after circulatory death; HEV-Ag, hepatitis E virus antigen; PCR, polymerase chain reaction; RBV, ribavirin; SVR, sustained virological response; SOT, solid organ transplantation; HRQoL, health-related quality of life score.

2.3.5 Uncertainty analyses

A wide range of exploratory uncertainty analyses assessed the sensitivity of the results to the assumptions and parameters. Given that we evaluated multiple options in this analysis and in light of the national policy objective of the NHS of maximising health outcomes with limited resources, we calculated the net health benefit (NHB) for each of the options in the uncertainty analyses as follows [272-274]:

NHB = QALYs – costs / willingness-to-pay per QALY threshold

This conversion of the outcome in terms of net QALYs gained simplifies the (visual) interpretation of results by aiming to find the option that maximises the NHB. We used the pre-determined willingness-to-pay value of £20,000 per QALY, which is recommended for the reference case by NICE [258].

Deterministic sensitivity analysis (DSA) was used on all parameters, by changing them in isolation to 80% and 120% of the base-case value, to identify the potential importance of specific parameters on the NHB. Scenario analysis was then undertaken on parameters of specific interest and including those identified in the DSA as being influential. These parameters were varied in isolation between ranges of two extreme but plausible values to explore the impact on the NHB. We also explored the impact of setting all parameter values simultaneously to the worst level.

Probabilistic sensitivity analysis (PSA) was used to explore the combined impact of parameter uncertainty. The model was run in 5,000 Monte Carlo simulations

where each parameter value was chosen randomly from a population distribution limited by the pre-determined upper and lower extremes (see Table 2.4) [275]. Based on the PSA, cost-effectiveness acceptability curves (CEAC) were generated to determine the probability of each option being cost-effective at a given willingness-to-pay threshold [276], ranging from £0-50,000/QALY gained. For each simulation the screening options were ranked and the probability that an option is cost-effective was calculated as the proportion of times it generated the highest mean NHB at a given willingness-to-pay threshold. The full ranking of the cumulative probability of being cost-effective was plotted in rankograms at a threshold of £20,000/QALY [277]. Given that the intervention with the highest probability of being cost-effective does not need to be the one with the highest mean NHB, a cost-effectiveness acceptability frontier (CEAF) was plotted to facilitate decision making on the optimal screening option [276].

3 HEV-Ag detection as a screening modality

3.1 Introduction

Systemic viral infections are often associated with circulating non-virion associated viral antigens in plasma, such as p24 antigen in HIV infection, p22 antigen in HCV infection and hepatitis B surface antigen (HBsAg) subviral particles in HBV infection [278, 279]. The detection of circulating antigenaemia typically is less sensitive than the detection of nucleic acid by PCR, particularly in acute infections, however it is commonly more cost-effective than genome-based assays [217, 280, 281]. In some circumstances the detection of viral antigens can provide enhanced diagnostic information. For example the loss of core antigen can predict viral clearance during therapy for HCV infection and HBsAg titres can be used to guide the cessation of interferon therapy for HBV infection by predicting when a virological response is unlikely [282, 283].

In hepatitis E virus infection circulating antigen (HEV-Ag), transcribed from ORF2, can be detected in both acute and persistent infections [217, 284]. This antigen forms the nucleocapsid protein but relatively little is known about the biology of antigen production and dissemination in acute and persistent HEV infections. HEV-Ag has been detected weeks after HEV RNA clearance in a subset of patients treated with ribavirin for persistent G3 infection, and in the urine post-treatment for G4 infection [131, 217]. However, the clinical relevance of ongoing antigen detection is not known.

A commercial assay for the detection of the viral ORF2 antigen using a solid-phase enzyme linked immunoassay (HEV-Ag ELISA, Fortress Diagnostics,

Antrim, Northern Ireland, UK) has increased the opportunity to utilise this as a screening assay and to study the dissemination of antigen by detection in other bodily fluids, such as urine, faeces and CSF. However, no studies have specifically assessed the performance of this assay in screening immunocompromised patients. Nor is there is a test protocol for confirming the specificity of a reactive HEV-Ag result, which is crucial particularly in the absence of detectable HEV RNA [285]. The necessity for a neutralisation confirmatory step in antigen detection has been demonstrated for HBsAg where misdiagnoses have historically occurred [285]. The aims of this chapter are:

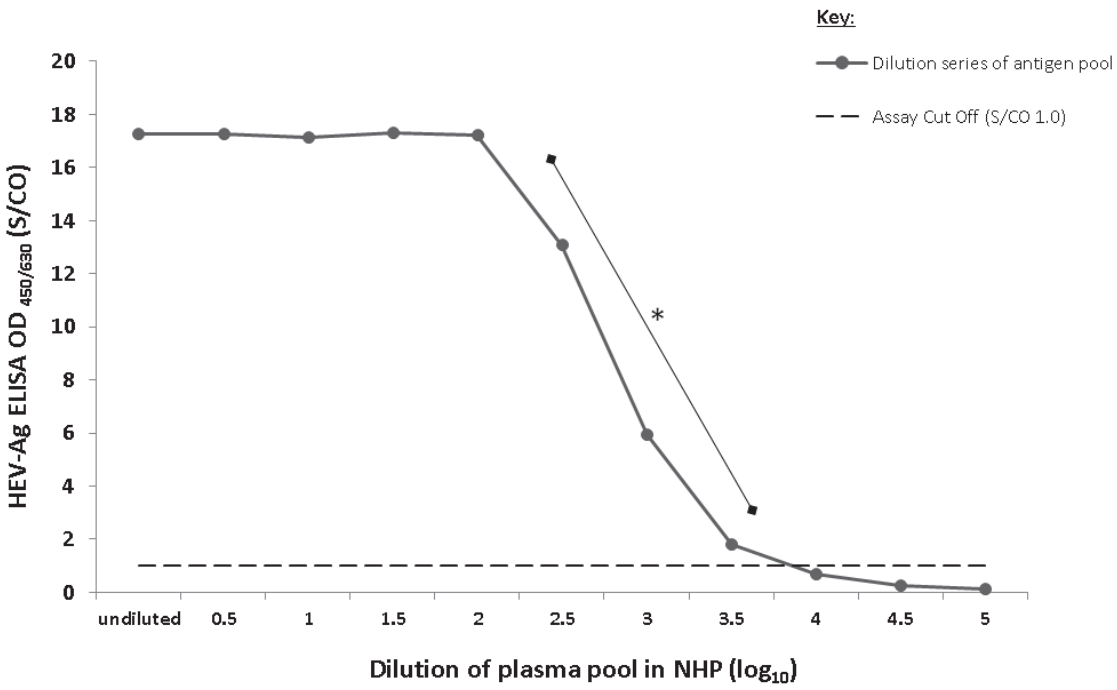
1. to develop a protocol for confirming the specificity of plasma, stool and urine samples reactive for HEV-Ag.
2. to determine the performance of the commercial HEV-Ag assay as a screening assay for active HEV infection in immunocompromised patients.

3.2 Results

3.2.1 Selection of reagents and validation of HEV-Ag neutralisation

HEV-Ag from the antigen pool derived from five viraemic patients was detectable in the ELISA up to a dilution of 3.5 $\log_{10}$ (S/CO 1.81, Figure 3.1). The dilutions of 2.5 $\log_{10}$ and 3 $\log_{10}$ which gave S/CO values of 13.08 and 5.94, respectively were chosen for further work to ensure the antigen was sufficiently dilute to prevent saturation of the putative neutralising reagent.

Figure 3.1 HEV-Ag assay reactivity in pooled samples



HEV-Ag assay reactivity in a dilution series of pooled samples from five patients with persistent HEV infection. HEV-Ag is detectable up to a dilution of 3.5 $\log_{10}$ in normal human plasma. The line marked with an asterix (*) represents the S/CO ratio range at which it is desirable to perform neutralisation.

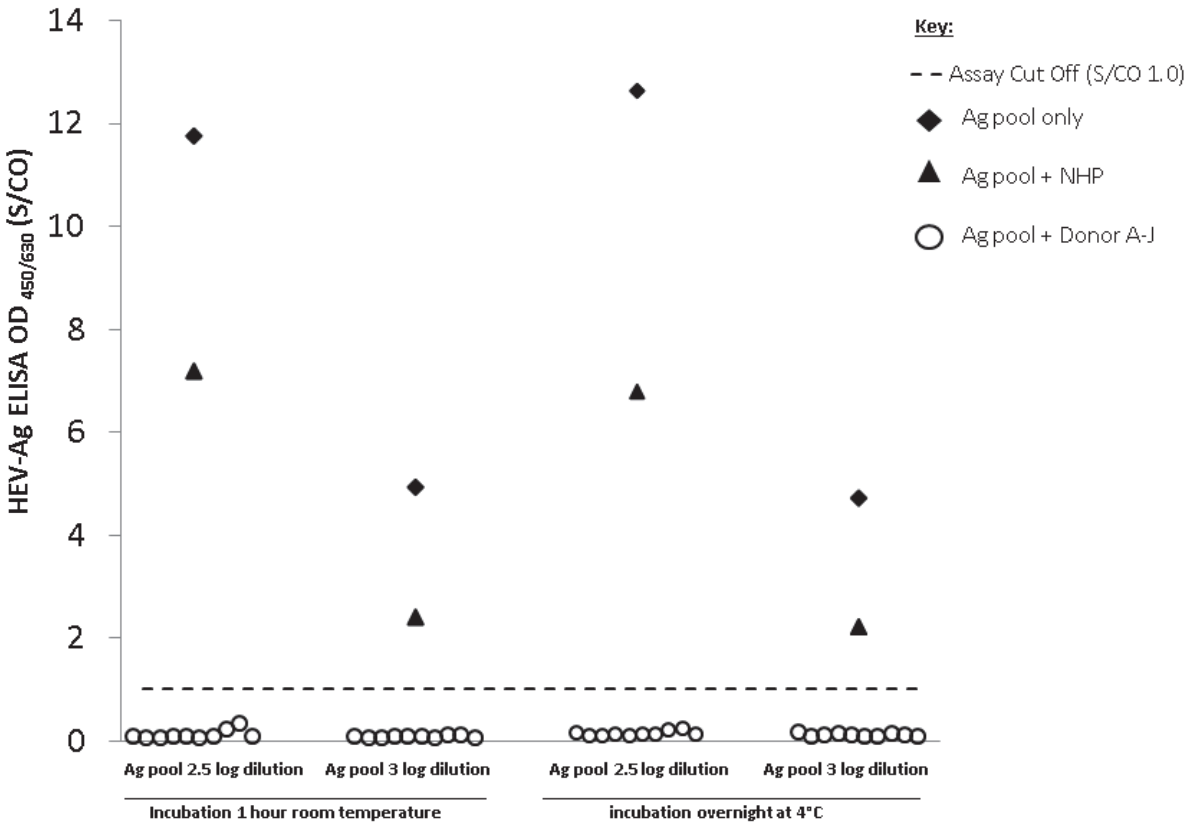
Abbreviations: NHP, normal human plasma; S/CO, sample over cut-off of optical density values.

3.2.2 Demonstration of neutralising activity in various incubation conditions

In order to identify potent convalescent samples to make a putative neutralising reagent, ten plasma samples from convalescent donors were screened for neutralising activity by pre-incubation of each sample with the two dilutions of the antigen pool at a 1:1 ratio (30µl of each). Incubation was allowed to proceed for either one hour at room temperature or overnight at 4°C in a round-bottomed microtitre plate prior to 50µl of the incubation mixture being assayed for HEV-Ag under normal conditions.

All ten donor samples demonstrated significant neutralising activity when incubated undiluted with the diluted antigen pool at 2.5 $\log_{10}$ and 3 $\log_{10}$ dilutions. There was no demonstrable difference between the two incubation conditions (Figure 3.2). Six of the ten samples were able to neutralise the antigen pool to below the assay cut-off when used at a pre-dilution of 1 $\log_{10}$ in NHP. No significant neutralising activity was seen in any samples at dilutions of 2 $\log_{10}$ and 3 $\log_{10}$ (Figure 3.3). The four samples demonstrating highest neutralising activity at a 1 $\log_{10}$ dilution were selected to make an equivolumetric pool of putative neutralising reagent.

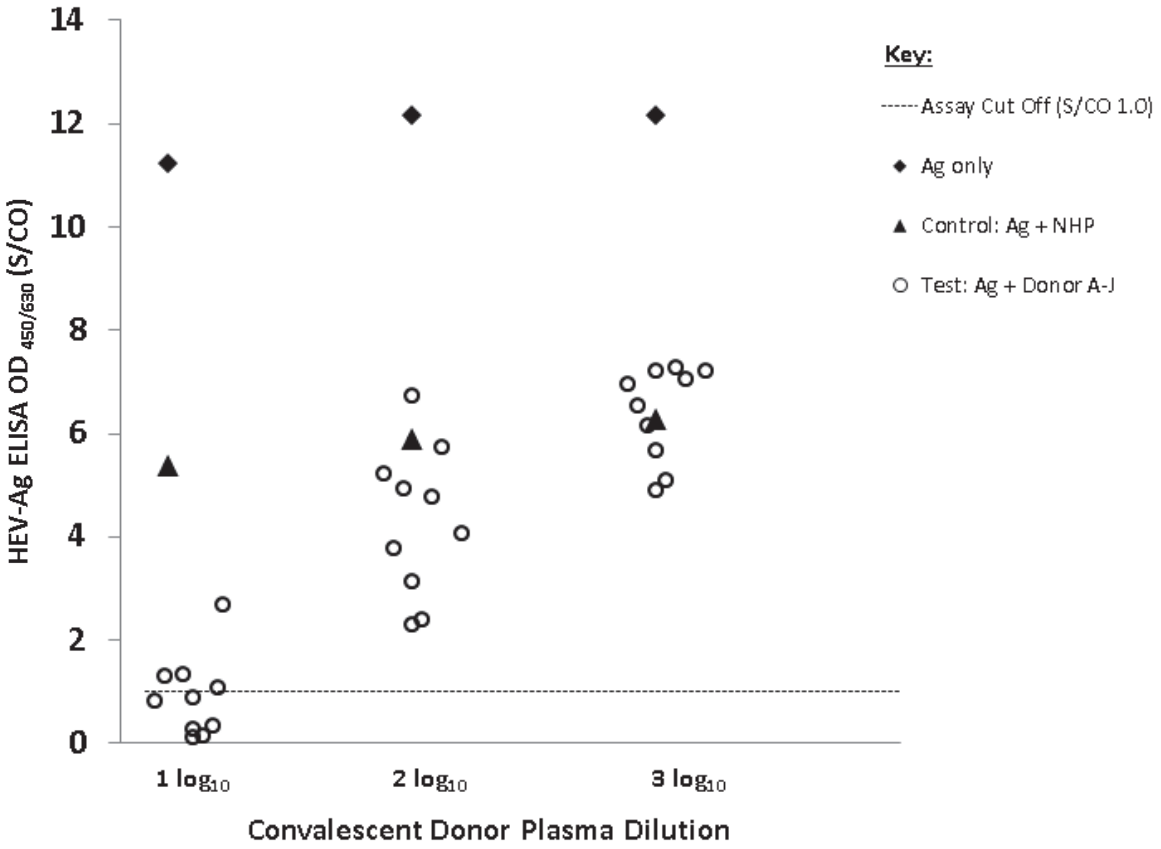
Figure 3.2 Trial of HEV-Ag neutralisation in blood donors



Initial trial of HEV-Ag neutralisation using blood donor samples in convalescence from acute HEV infection. HEV-Ag assay reactivity of diluted HEV-Ag pool is shown following incubation with NHP (control) or one of the convalescent donor samples A-J (test) in two separate incubation conditions. All donor samples neutralised the dilute HEV-Ag pool when incubated in a 1:1 ratio in either of the two incubation conditions.

Abbreviations: NHP, normal human plasma; S/CO, sample over cut-off of optical density values.

Figure 3.3 Neutralisation of HEV-Ag pool by convalescent donor plasma



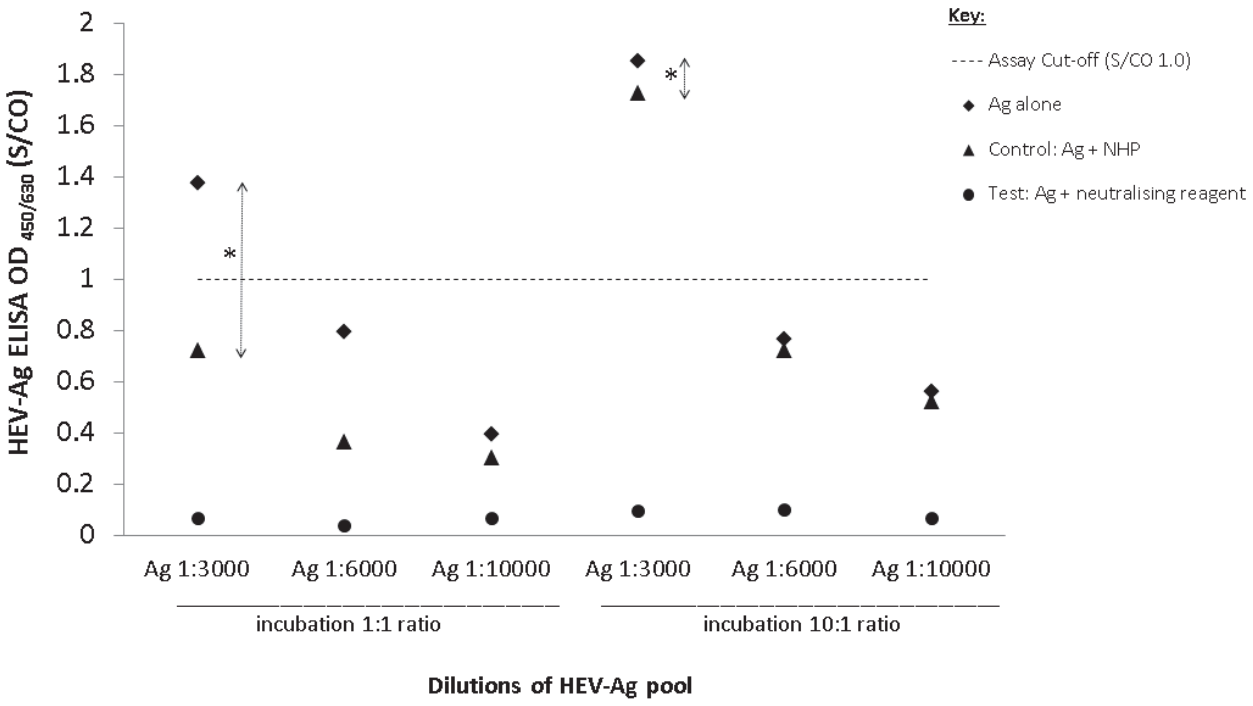
HEV-Ag assay reactivity of diluted HEV-Ag pool (2.5 $\log_{10}$) following incubation for one hour at room temperature with NHP (control) or one of the convalescent donor samples A-J (test) diluted in NHP (3 $\log_{10}$, 2 $\log_{10}$ or 1 $\log_{10}$).

Abbreviations: NHP, normal human plasma; S/CO, sample over cut-off of optical density values.

3.2.3 Dilution effect reduced by using low volume neutralising reagent

In an attempt to reduce the dilution effect of adding a non-neutralising NHP reagent (non-neutralising control) to the antigen pool when used in a 1:1 volumetric ratio, the ratio of neutralising reagent to antigen pool was altered. We elected to use a ratio of 10:1 (50µl antigen sample: 5µl neutralising reagent) having previously shown that the individual convalescent donor samples all demonstrated potent neutralisation at a 1 $\log_{10}$ dilution in a 1:1 ratio. Using a 10:1 ratio reduced the dilution effect and this is observed even below the assay manufacturer's cut-off (Figure 3.4). This optimised assay using the undiluted neutralising reagent in a 10:1 ratio was used for further neutralisation experiments.

Figure 3.4 Optimisation of antigen and antibody concentrations for neutralisation



Neutralisation of dilutions of HEV-Ag pool by neutralising reagent in varying ratios. HEV-Ag assay reactivity of diluted HEV-Ag pool (1:3000, 1:6000 or 1:10000) following incubation for one hour at room temperature with NHP (control) or the neutralising reagent in a 1:1 ratio (reagent used at 1 $\log_{10}$) or in a 10:1 ratio (reagent used undiluted). The dilution effect (arrows marked with asterix *) is the change of S/CO ratio before (♦) and after (▲) the addition of NHP.

Abbreviations: Ag, hepatitis E virus antigen

3.2.4 Neutralisation of tissue culture antigen, virus-like particles and the HEV-Ag ELISA kit positive control

The neutralising reagent demonstrated neutralising activity against antigens presented as virions from HEV tissue culture (day 45 post–inoculation of Hep G2/C3a cell line using G3 faecal sample), virus-like particles (VLPs) and the HEV-Ag ELISA positive control (Table 3.1).

To determine suitability as a control for neutralisation the stability of reactivity of purified genotype 3 VLPs was assessed. The VLPs were diluted in HRP conjugate stabiliser buffer (Clin-Tech, Guildford, UK) and dilutions were stored at 37°C, 4°C or at room temperature and tested at baseline, day 10 and day 21 for HEV-Ag. Reactivity in the HEV-Ag assay was consistent over 21 days in the three conditions tested (Figure 3.5).

Table 3.1 Neutralisation of HEV-Ag from variety of sources

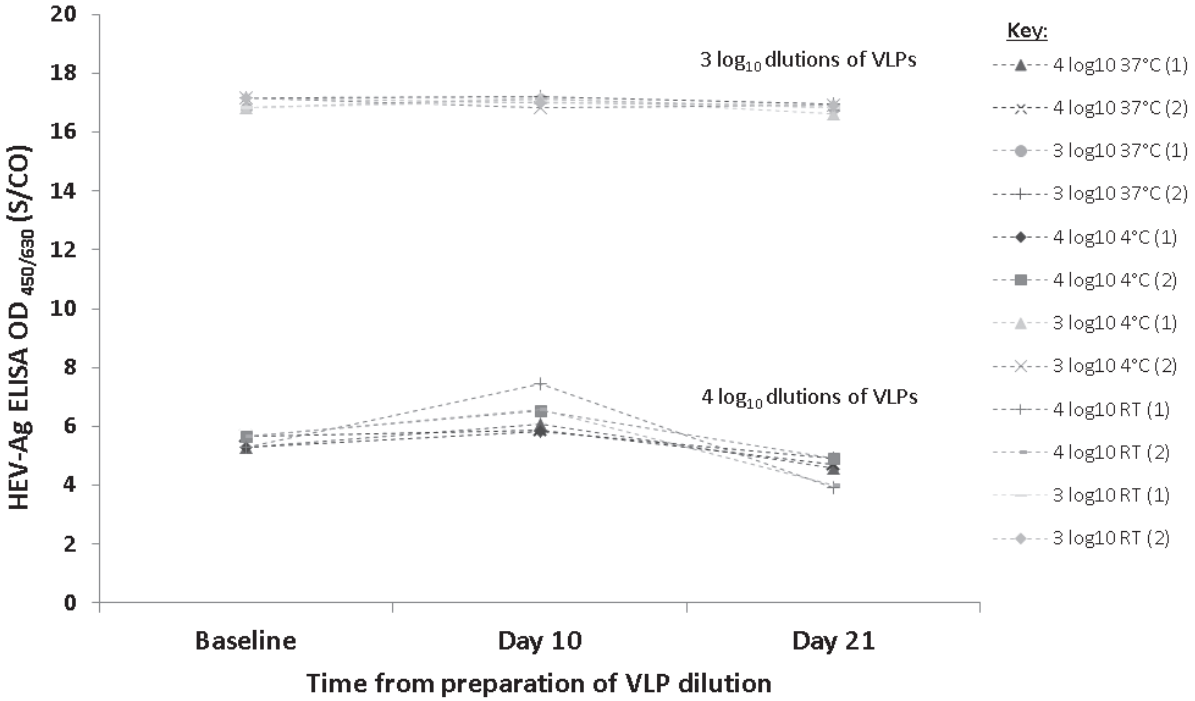
Sample type	Dilution (log_{10})	Sample only (OD[a])	Control: Sample + NHP (OD)	Test: Sample + neutralising reagent (OD)	% Neutralisation
HEV-Ag pool	1	3.590	3.630	3.440	5.24
	1.5	3.651	3.595	0.356	90.28
	2	3.232	3.189	0.007	100.00
	2.5	1.708	1.514	0.001	100.41
	3	0.645	0.606	0.002	100.86
	3.5	0.179	0.185	0.001	103.45
	4	0.054	0.052	0.000	115.92
G3 VLP	4.5	2.107	1.344	0.037	98.49
	5	0.749	0.637	0.028	98.23
G1 VLP	4	1.817	1.559	0.073	97.73
	4.5	0.658	0.589	0.030	96.37
HEV culture supernatant	2	3.299	3.007	0.022	99.83
	2.5	1.401	1.013	0.025	99.20
	3	0.527	0.443	0.020	99.30
HEV-Ag ELISA positive kit control	0.5	3.580	3.770	1.720	54.75
	1	3.520	3.450	0.600	83.24
	1.5	1.770	2.000	0.230	89.67
	2	0.670	0.740	0.090	91.04
	2.5	0.250	0.260	0.040	94.02

HEV-Ag assay reactivity (raw OD450/630 values) of HEV-Ag pool, VLPs, HEV culture supernatant and the HEV-Ag ELISA positive kit control following incubation with either NHP (control) or the neutralising reagent (used undiluted in a 10:1 ratio). The percentage of neutralisation was calculated as a percentage of the reduction in binding (Methods 2.2.2.7).

[a] Cut-off for reactivity varied between plates, but ranged from OD450/630 0.210-0.236.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV-Ag, Hepatitis E virus antigen; OD, optical density; VLP, virus-like particle.

Figure 3.5 Stability of HEV-Ag ELISA reactivity of G3 VLPs



HEV-Ag assay reactivity of G3 VLPs diluted in HRP conjugate stabiliser buffer in three different storage conditions (37°C, room temperature or 4°C) over 21 days.

Abbreviations: G3, genotype 3; OD, optical density; RT, room temperature; S/CO, sample over cut-off ratio; VLP; virus-like particle.

3.2.5 Neutralisation of HEV-Ag in analytes other than plasma

Stool samples from three patients with persistent HEV infection and urine from one patient with persistent HEV infection were tested and found reactive in the HEV-Ag assay. All three stools were HEV RNA positive (HEV RNA quantification ranging 1.64 x 10^6 IU/ml to 3.8 x 10 IU/ml). The urine sample was collected from a male patient with persistent HEV infection during ribavirin therapy whilst the patient remained HEV viraemic; HEV RNA was not detected in the urine sample. HEV-Ag was detected in all three stool samples and the urine sample. Reactivity was neutralised by at least 90% in all four samples confirming the specificity of the reactivity (Table 3.2).

Table 3.2 Neutralisation of HEV-Ag in non-plasma analytes

Sample type		Dilution (log_{10})	Sample only (OD [a])	Control: Sample + NHP (OD)	Test: Sample + neutralising reagent (OD)	% Neutralisation
Urine [b]		undiluted	0.274	0.227	0.028	99.00
Stool [c]	1	1	1.210	1.070	0.050	98.06
	2	1	0.620	0.560	0.060	95.97
	3	undiluted	1.603	1.014	0.072	96.12

Neutralisation of HEV-Ag detected in urine and stool of patients with persistent HEV infection. Data presented are raw $OD_{450/630}$ values following incubation with either NHP (control) or the neutralising reagent (used undiluted in a 10:1 ratio). The percentage of neutralisation was calculated as a percentage of the reduction in binding (Methods 2.2.2.7).

[a] Cut-off varied between plates, but ranged from $OD_{450/630}$ 0.21-0.236.

[b] Urine from a male patient with persistent HEV infection during ribavirin therapy whilst the patient remained HEV viraemic; the urine sample was HEV RNA-negative.

[c] Stool samples from three patients with persistent HEV infection. All three stools were HEV RNA positive (HEV RNA quantification ranging 1.64 x 10^6 IU/ml to 3.8 x 10 IU/ml).

Abbreviations: OD, optical density; NHP, normal human plasma.

3.2.6 HEV-Ag detection and neutralisation for screening immunocompromised patients for HEV infection

To determine the performance of the HEV-Ag assay as a screening assay 439 plasma samples from transplant recipients and 1591 plasma samples from patients with underlying haematological malignancy and 176 anonymised blood donors were tested. The samples included 21 samples identified as viraemic by HEV PCR.

3.2.7 Sensitivity of HEV-Ag detection

Nineteen of the 21 samples (90.5%, 95% CI 71.1- 97.4%) from viraemic patients were reactive in the HEV-Ag assay (S/CO range, 6.51-19.08). A significant correlation was seen between $\log_{10}$ HEV RNA level (IU/mL) and HEV-Ag S/CO ratio (Figure 3.7) (Pearson's correlation 0.8184, $p < 0.001$). The two patients (R & U) whose samples tested negative in the HEV-Ag assay (Figure 3.7) harboured low viral loads of 9.60×10^1 IU/ml and 3.52×10^2 IU/ml, respectively. Follow-up testing in both patients revealed a rising viral load with a reactive HEV-Ag result. In patient R, an allogeneic stem cell transplant recipient, follow-up testing at 10 weeks demonstrated a viral load of 3.00×10^5 IU/mL and a reactive HEV-Ag result (S/CO, 19.38). In patient U, with underlying multiple myeloma, follow-up testing three weeks later showed a viral load of 3.90×10^5 IU/ml and detectable HEV-Ag by ELISA (S/CO 17.31). When analysis was restricted to samples from patients

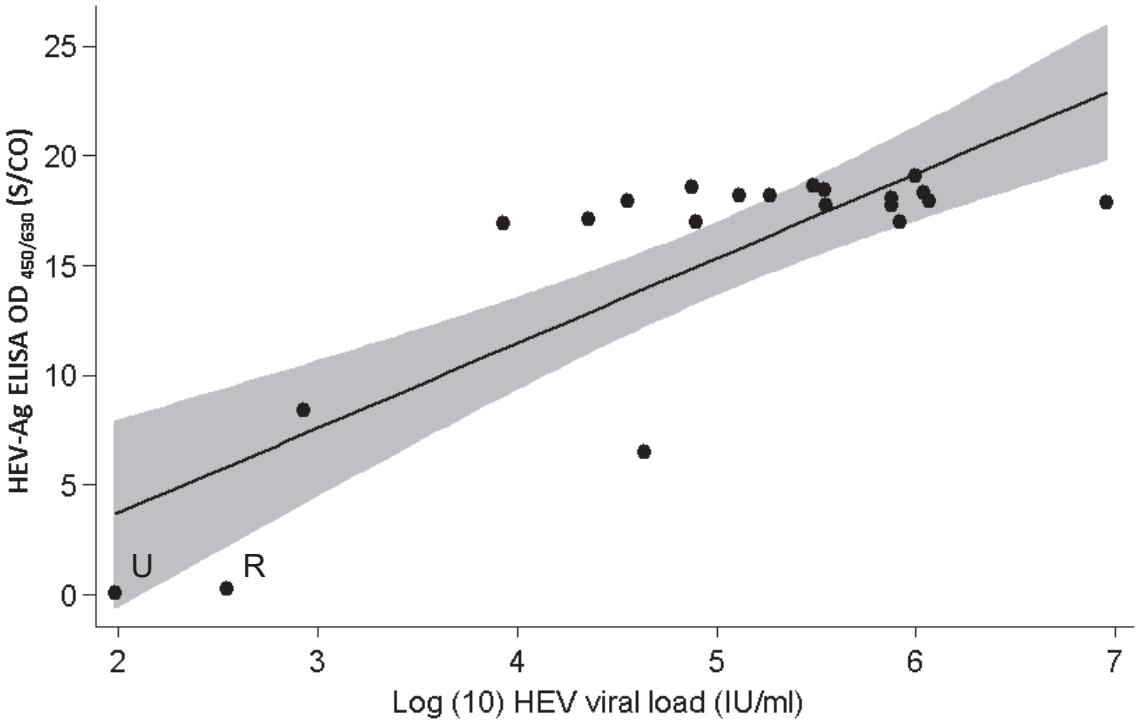
with established HEV infection (viral load stable for period of >4 weeks) sensitivity was 100% (18/18, 95% CI 82.4-100%).

3.2.8 Specificity of HEV-Ag reactivity

The samples with very high S/CO ratios were diluted prior to neutralisation. Thus, prediluted to be off the plateau, all 19 HEV RNA positive/HEV-Ag reactive samples were neutralised by greater than 95% (Table 3.3).

In addition to samples from viraemic patients being reactive in the HEV-Ag ELISA, eighteen samples from aviraemic immunocompromised patients (0.9%) were repeat reactive by HEV-Ag ELISA. The S/CO ratios of all the RNA-positive/antigen reactive samples were significantly higher than the RNA-negative/antigen reactive samples (median, 17.90; IQR, 17.00-18.26 vs median, 2.98; IQR, 1.79-8.34; P = <0.0001) (Figure 3.7). In contrast, none of the 176 plasmas from anonymized blood donors were reactive. Of the 18 samples which were RNA negative and repeat reactive by HEV-Ag ELISA, only 1 could be neutralised (sample 6, Table 3.4), confirming 17 to be non-specific, false-positive results. Further testing revealed sample 6 to be reactive for anti-HEV IgM (S/CO 8.61) and IgG (S/CO 16.44), suggesting a recently cleared HEV viraemia with ongoing antigenaemia. Thus, the overall specificity amongst the immunocompromised cohort was 99.15% (95% CI, 98.65-99.51) and 100% in immunocompetent blood donors (Table 3.5).

Figure 3.6 Correlation between HEV viral load and reactivity by HEV-Ag ELISA in HEV viraemic patients



Pearson's correlation between HEV viral load and HEV-Ag ELISA S/CO was 0.8184 ($p < 0.001$). Samples R and U represent those non-reactive in the HEV-Ag ELISA.

Abbreviations: Log (10), logarithm to base 10; IU, international units; ml, millilitres; OD, optical density; S/CO, sample over cut-off ratio

Table 3.3 Neutralisation of HEV RNA-positive/HEV-Ag reactive samples

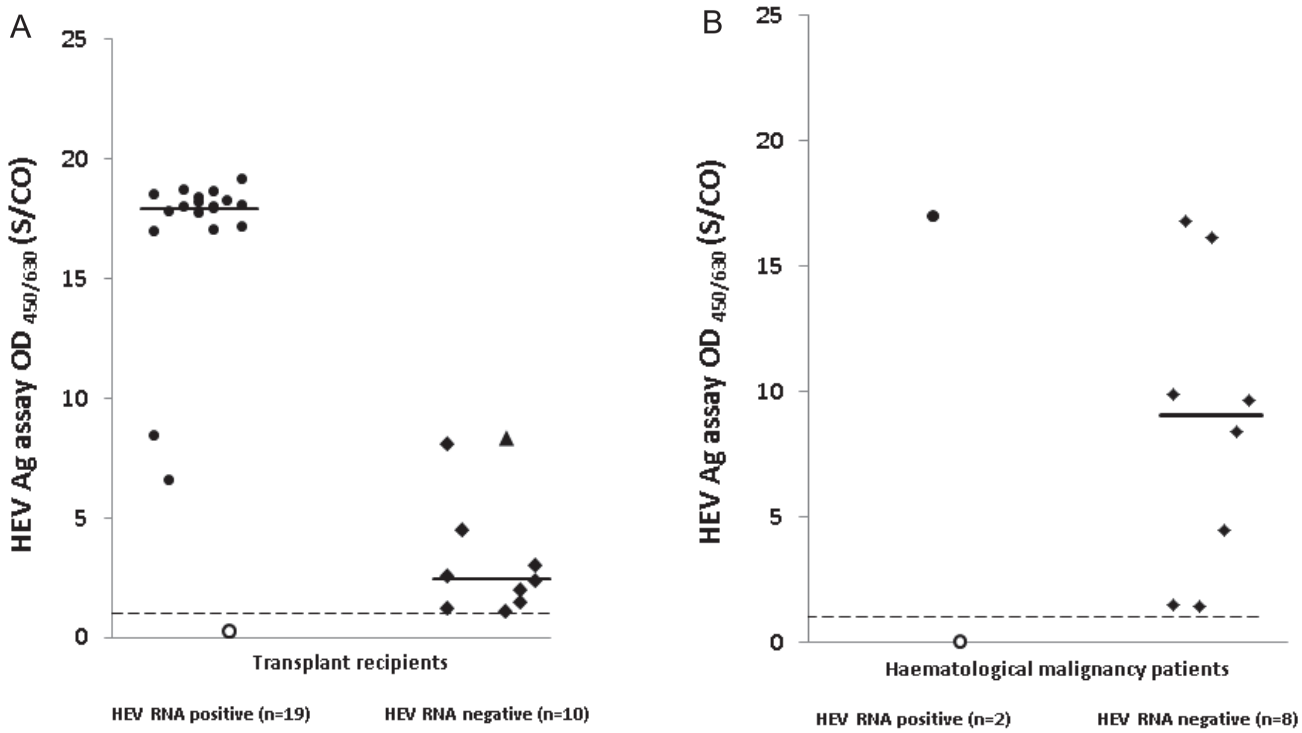
Sample	Dilution (log_{10})	Sample only (OD[a])	Control	Test	% Neutralisation
			Sample + NHP (OD)	Sample + neutralising reagent (OD)	
A	undiluted	1.943	1.576	0.015	103.08
B	2.5	3.540	3.548	0.103	98.81
C	2.5	3.520	3.703	0.084	99.38
D	2.5	0.900	1.038	0.022	104.05
E	2.5	2.610	2.238	0.030	101.45
F	2.5	3.382	3.238	0.047	100.46
G	2.5	2.260	1.733	0.044	101.05
H	2.5	3.740	3.826	0.190	96.59
I	2.5	3.616	3.663	0.100	98.93
J	2.5	0.807	1.287	0.022	103.23
K	2.5	1.010	1.048	0.047	101.48
L	1	0.755	0.764	0.015	106.63
M	2.5	3.496	3.667	0.073	99.68
N	2.5	0.400	0.352	0.021	113.97
O	1	0.380	0.350	0.060	100.55
P	2.5	3.650	3.722	0.104	98.84
Q	2.5	3.700	3.764	0.234	95.34
R	2.5	0.440	0.701	0.030	104.94
Z	2.5	3.552	3.525	0.059	98.33

Samples with very high OD values were diluted in NHP to prevent saturation of the neutralising reagent. Data presented are raw $OD_{450/630}$ values following incubation with either NHP (control) or the neutralising reagent (used undiluted in a 10:1 ratio). The percentage of neutralisation was calculated as a percentage of the reduction in binding (Methods 2.2.2.7).

[a] Cut-off for reactivity varied between plates, but ranged from $OD_{450/630}$ 0.210-0.227.

Abbreviations: OD, optical density; NHP, normal human plasma.

Figure 3.7 HEV-Ag ELISA reactivity of viraemic and aviraemic patients



Plotted values of the HEV-Ag ELISA S/CO values in HEV RNA positive and HEV RNA negative samples from transplant patients and patients with haematological malignancy. Filled circles represent HEV viraemic patients reactive by HEV-Ag ELISA Open circles represent HEV viraemic patients non-reactive by HEV-Ag ELISA. Diamonds represent aviraemic patients reactive by HEV-Ag ELISA. The triangle represents a sample which harboured HEV-Ag (neutralised) but no detectable RNA (see Discussion).

Abbreviations: Log (10), logarithm to base 10; IU, international units; ml, millilitres; OD, optical density; S/CO, sample over cut-off ratio.

Table 3.4 Neutralisation of samples HEV RNA-negative but HEV-Ag reactive

Screening Results				Neutralisation results		
No.	Initial result/ repeat result (OD[a])	Mean (OD)	Sample only (OD)	Control Sample + NHP (OD)	Test Sample + neutralising reagent (OD)	% Neutralisation
1	0.382/0.410	0.396	0.551	0.439	0.402	8.71
2	1.654/0.238	0.946	0.213	0.207	0.200	3.63
3	0.266/0.253	0.260	0.188	0.158	0.149	6.25
4	0.633/0.401	0.517	0.400	0.374	0.373	0.28
5	0.298/0.231	0.265	0.162	0.167	0.164	1.96
6	1.672/1.695	1.684	1.335	1.016	0.053	96.11
7	0.675/0.591	0.633	0.790	0.527	0.623	-18.71
8	0.600/1.297	0.949	0.615	0.450	0.516	-15.14
9	1.210/2.200	1.705	1.584	1.112	1.391	-25.41
10	0.337/0.309	0.323	-	0.142	0.141	0.78
11	2.549/2.050	2.300	1.208	1.448	1.024	29.92
12	0.633/1.245	0.939	1.081	0.993	1.100	-11.12
13	3.285/3.492	3.389	0.595	0.475	0.477	-0.42
14	3.514/3.537	3.526	2.755	2.380	2.694	-13.37
15	0.297/0.252	0.275	0.108	0.092	0.080	19.66
16	2.589/0.942	1.766	0.794	0.564	0.321	45.59
17	0.469/3.595	2.032	2.022	2.272	2.230	1.87
18	0.375/0.262	0.319	0.232	0.227	0.225	1.02

Data presented are $OD_{450/630}$ values following incubation with either NHP (control) or the neutralising reagent (used undiluted in a 10:1 ratio). Neutralisation was calculated as a percentage of reduction in binding (Methods 2.2.2.7).

Abbreviations: NHP, normal human plasma; OD, optical density.

Table 3.5 Specificity of HEV-Ag assay

	Anonymised Blood donors	Transplant recipients	Haemato-oncology patients	p value
HEV-Ag repeat reactive samples	0	9	8	0.029
HEV-Ag non-reactive samples	176	411[a]	1582[b]	
Specificity	100% (one-sided 97.5% CI 97.93-100.0)	97.86% (95% CI 95.97-99.02)	99.5% (95% CI 99.01-99.78)	

Assay specificity was determined by confirmatory testing of HEV-Ag reactive samples by HEV RNA and by antigen neutralisation. Specificity was higher in blood donors than the immunocompromised patients (p = 0.029).

[a] Includes three samples reactive on initial testing (S/CO >1.0) but not on repeat testing.

[b] Includes 13 samples reactive on initial testing (S/CO >1.0) but not on repeat testing.

3.2.9 HEV-Ag detection throughout the course of HEV infection

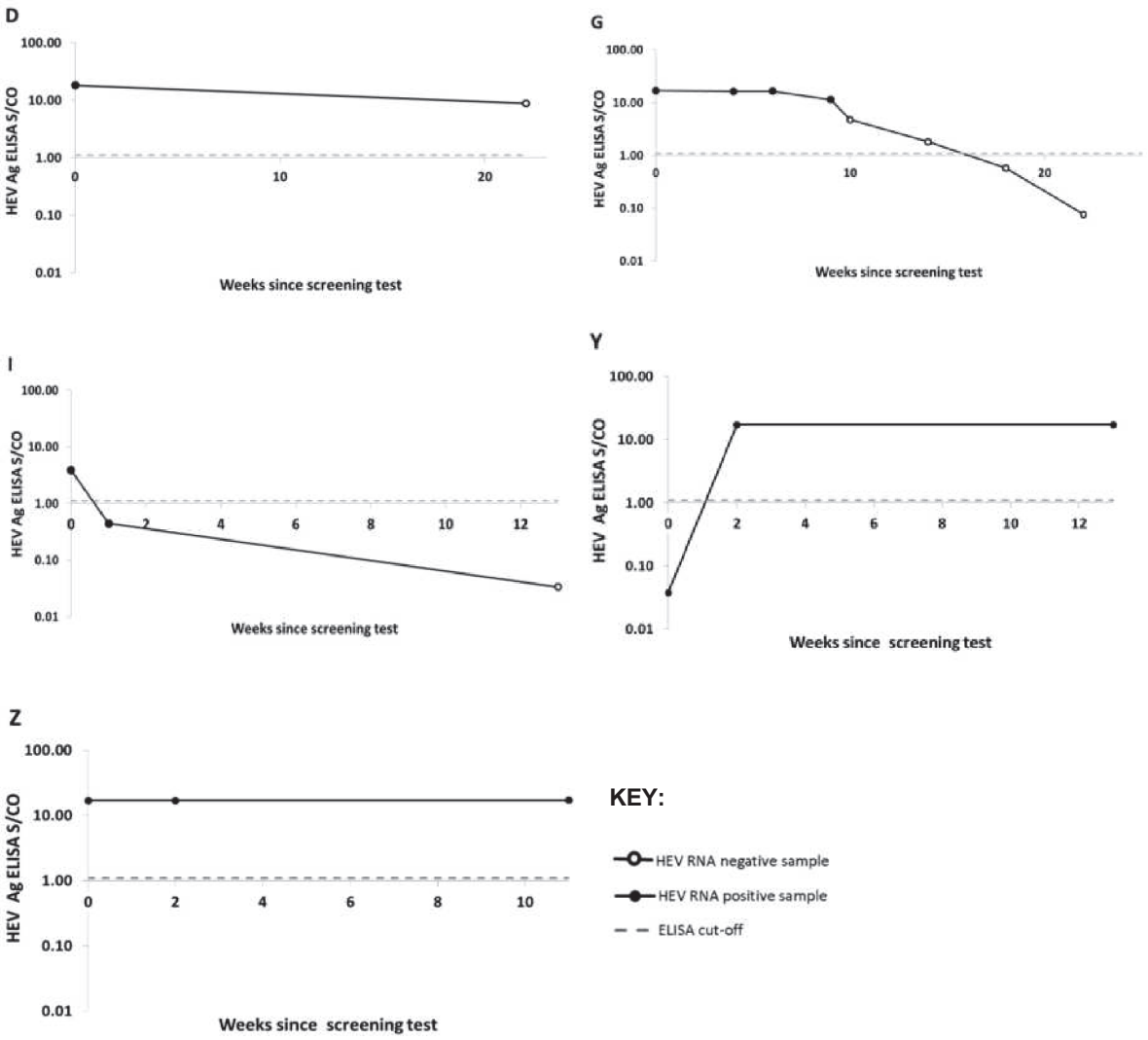
Five patients with active HEV infection who were untreated and eight patients with persistent HEV infection who had been treated were selected who had stored follow-up samples available for testing.

Amongst the five untreated patients with detectable HEV viraemia, four of the five patients (80%) had detectable HEV antigen in plasma in the baseline sample. A plasma sample taken two weeks later in the patient with undetectable HEV-Ag in the baseline sample (Patient Y, Figure 3.8) was reactive for HEV-Ag. Of these five patients, three cleared HEV viraemia spontaneously during follow-up. In one case (Patient I, Figure 3.8) HEV-Ag became undetectable in plasma prior to HEV RNA clearance. In the remaining two patients (Patients D and G, Figure 3.8) plasma HEV-Ag was detectable beyond HEV RNA clearance; over three weeks in the case of patient G.

Amongst the eight patients with persistent HEV infection who received ribavirin therapy, in five patients HEV-Ag was detectable in plasma for a prolonged period after HEV RNA became undetectable in plasma (Patients A, F, K, R and S, Figure 3.9). HEV-Ag persisted for between 8.0 weeks up to 32.0 weeks in plasma beyond HEV RNA detection. Two patients experienced virological rebound (Patient A and E, Figure 3.9). In patient A, HEV-Ag was detectable in all follow up samples over 19.4 weeks despite undetectable HEV RNA prior to the subsequent rebound of detectable HEV RNA. In patient E, HEV RNA was detectable at very low levels (4.2×10^1 IU/ml) in plasma prior to the detection of HEV-Ag.

Where stool samples were available for HEV testing amongst the antiviral treated patients the relationship between HEV RNA detection and HEV-Ag detection in stool was less clear. There was not a strong relationship between HEV RNA quantitation and HEV-Ag OD values (Pearson's correlation, 0.4467). The OD values of the HEV-Ag ELISA fell over time as HEV RNA was cleared from stool. In only two stool samples from two separate patients was HEV-Ag detected in the absence of detectable HEV RNA (open circles, patient A and R, Figure 3.10). In four stool samples HEV-Ag was not detected in the presence of detectable HEV RNA; in these samples the quantitation of HEV RNA ranged from 4.00×10^1 IU/ml to 1.36×10^4 IU/ml. In no patients was HEV-Ag detectable in stool beyond HEV-Ag detection in plasma.

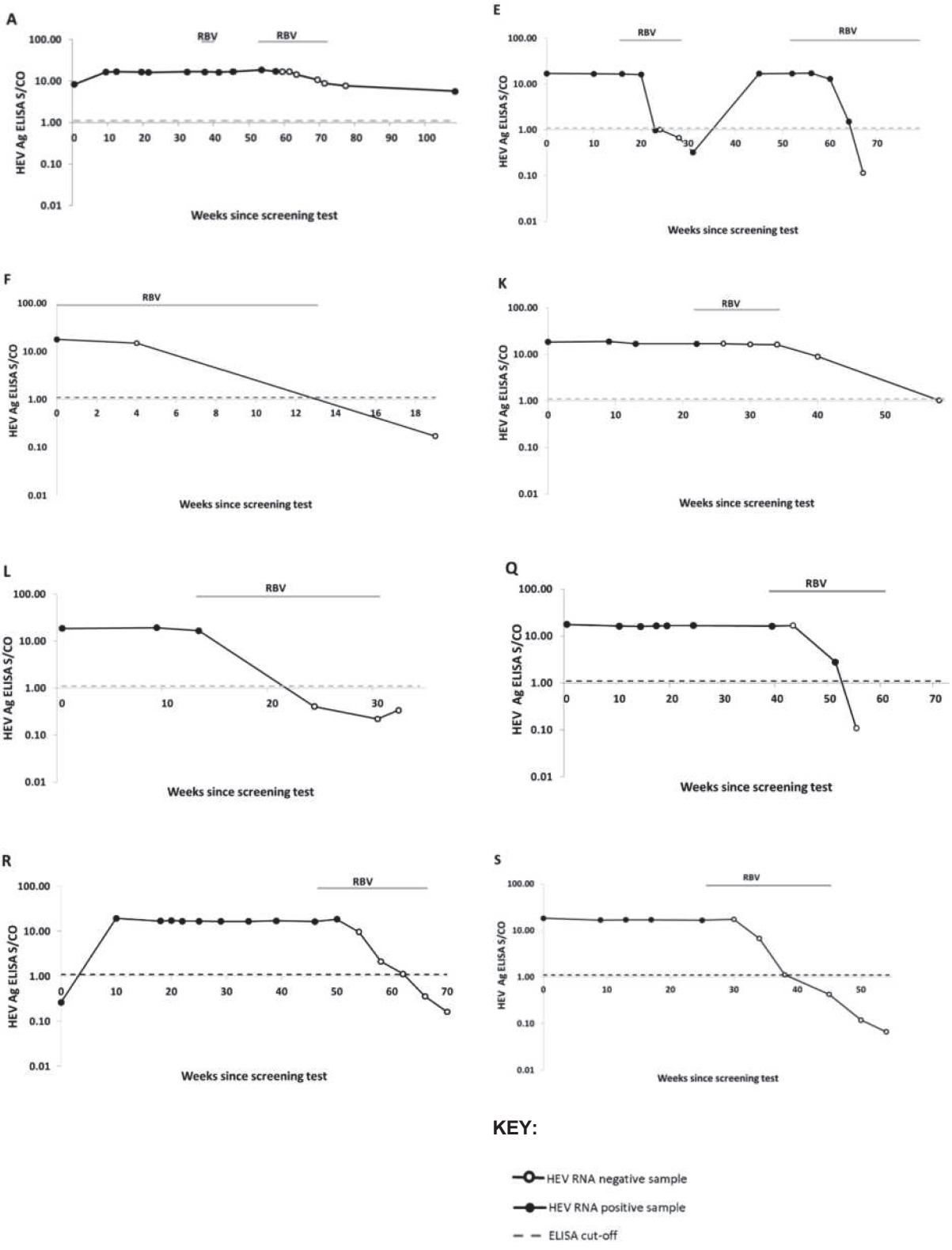
Figure 3.8 Plasma HEV-Ag in untreated patients



Correlation of plasma HEV RNA detection and HEV-Ag in untreated patients. Five patients (Patients D, G, I, Y and Z) with active HEV infection who received no treatment during follow-up were tested for HEV-Ag in baseline plasma samples and subsequent follow-up samples. Data shown are the HEV-Ag ELISA results during follow-up. Follow-up varied between 11.0 weeks (patient Z) and 22.3 weeks (patient G). Open circles represent samples without detectable HEV RNA and filled-in circles represent samples harbouring detectable HEV RNA. The dashed line represents the manufacturer's cut off threshold for defining a reactive sample. See supplementary figure A2.1 for the HEV-Ag reactivity plotted alongside the HEV RNA viral load quantitation.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV Ag, Hepatitis E virus antigen; S/CO, sample over cut-off ratio.

Figure 3.9 Plasma HEV-Ag in antiviral treated patients

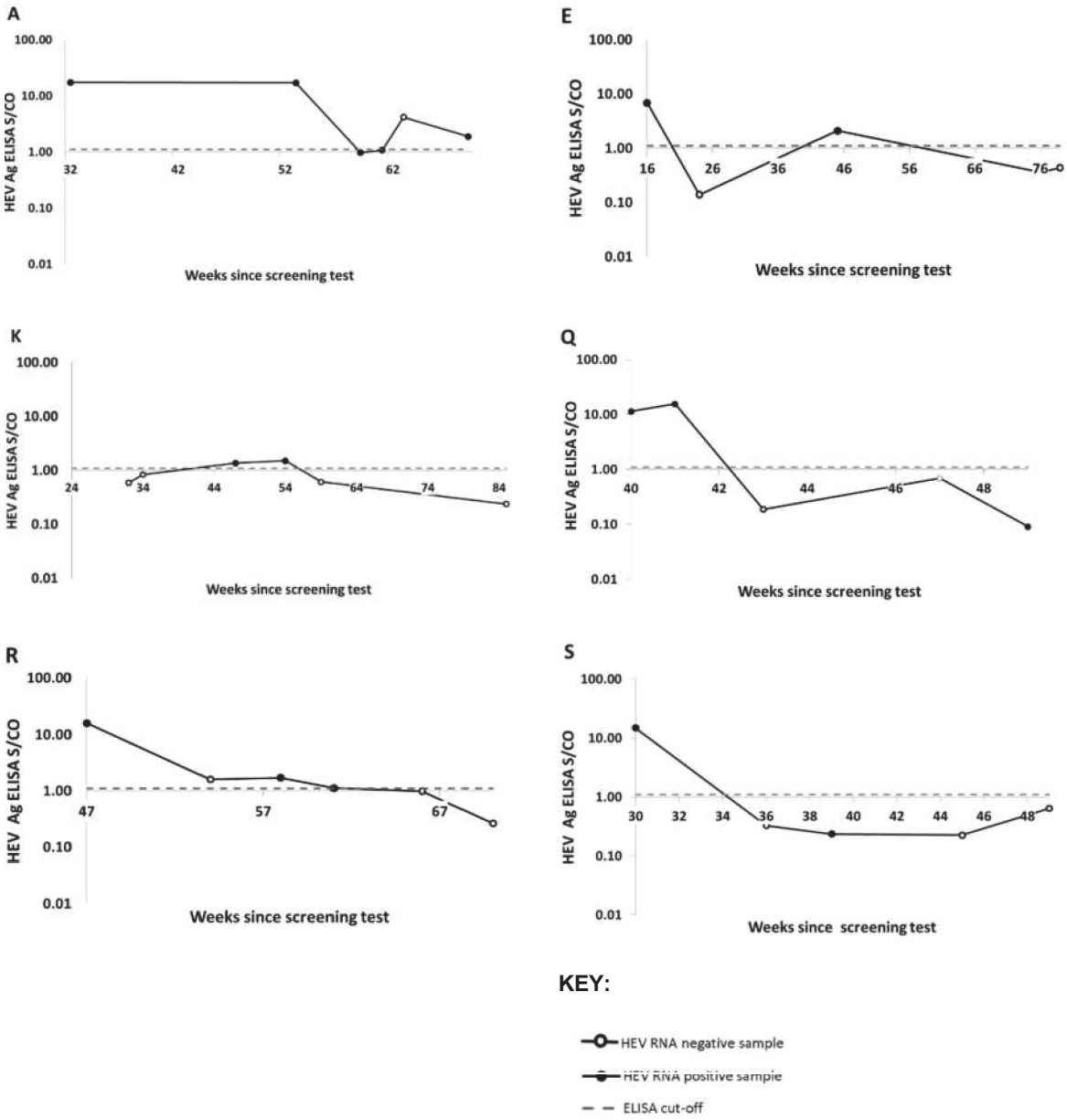


Legend for Figure 3.9:

Correlation of plasma HEV RNA detection and HEV-Ag in antiviral-treated patients. Eight patients who had persistent HEV infection (Patients A, E, F, K, L, Q, R and S) and were treated with Ribavirin were tested for HEV-Ag at baseline and throughout follow-up. Data shown are the HEV-Ag ELISA results during follow-up which varied between 19.0 weeks (patient F) and 108.1 weeks (patient A). Open circles represent samples without detectable HEV RNA and filled circles represent samples harbouring detectable HEV RNA. The dashed line represents the manufacturer's cut-off threshold for defining a reactive sample. See supplementary figure A2.2 for the HEV-Ag reactivity plotted alongside the HEV RNA viral load quantitation.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV Ag, Hepatitis E virus antigen; S/CO, sample over cut-off ratio.

Figure 3.10 Stool HEV-Ag in antiviral-treated patients



Correlation of HEV RNA and HEV-Ag detection in stool samples of antiviral-treated patients. Six patients treated with ribavirin for persistent HEV infection (Patients A, E, K, Q, R and S) were tested for HEV-Ag in stool samples and compared with HEV RNA quantified results in stool. Open circles represent stool samples without detectable HEV RNA and filled circles represent samples harbouring detectable HEV RNA. The dashed line represents the manufacturer's cut-off threshold for defining a reactive sample.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV Ag, Hepatitis E virus antigen; S/CO, sample over cut-off ratio.

3.3 Discussion

Diagnosis of HEV infection in immunocompetent patients is typically made by the demonstration of reactive anti-HEV IgM and IgG antibodies in sera, however in immunocompromised patients HEV RNA testing is recommended because of delayed or non-existent serological responses in these cohorts [216]. The availability of a commercial assay to detect the HEV ORF2 protein (HEV-Ag ELISA, Wantai diagnostics) opens up a number of possibilities, including screening vulnerable populations for persistent infections, as a diagnostic assay for active HEV infections in resource-limited settings and in the study of the dynamics of HEV-Ag detection throughout the course of a persistent infection. The HEV-Ag is translated from ORF2 to form the capsid protein which contains important immunogenic epitopes. It has been detected in the plasma and urine of HEV infected patients and in multiple extrahepatic sites in animals including the kidney, gut and central nervous system [134, 286, 287].

The first aim of this chapter was to develop a protocol for confirming the specificity of plasma, stool and urine samples reactive for HEV-Ag. We initially demonstrated that plasma from ten blood donors in convalescence from HEV infection was able to neutralise antigen incubated at either for an hour at room temperature or at 4°C. The four plasma samples with the highest antigen neutralising activity were then pooled to generate a putative neutralising reagent. This pooled plasma was able to neutralise HEV antigens derived from a variety of sources, including clinical specimens, HEV tissue culture supernatant, VLPs and the HEV-Ag ELISA positive kit control. Interestingly, the plasma was equally potent at neutralising G1 VLPs when compared with G3 VLPs, despite all the

donors being in convalescent from G3 HEV infections, therefore this reagent could be used for suspected G1 infections also. For the purposes of antigen neutralisation, either tissue culture supernatant or VLPs could be used as control material, however the use of VLPs would be optimal because they are relatively easy to generate in abundance, well characterised and do not pose the laboratory infection risk that clinical specimens or tissue culture supernatant appear to have. The stability testing data also show that G3 VLPs remain consistently reactive in the HEV-Ag assay in three diverse storage conditions over at least a period of 21 days.

A notable practical caveat is that any samples with high S/CO ratios should be diluted to ensure the neutralising reagent is not saturated by excess antigen. It was notable that attempts to neutralise HEV-Ag reactivity on undiluted samples which were on the plateau were unsuccessful. For example, HEV-Ag pool at a 1 $\log_{10}$ dilution (S/CO 17.10) could only be neutralised by 5%. This has been demonstrated in other settings where high titre HBsAg samples could only be neutralised following dilution [288]. In the current study the addition of 5µl of undiluted neutralising reagent was consistently able to neutralise HEV-Ag when the absorbance value was off the plateau of the standard curve (S/CO <15.0), but unable to fully neutralise pooled HEV-Ag with absorbance values on the plateau (S/CO >17.0). Therefore it is necessary to dilute any sample with a very high S/CO result prior to any attempt at neutralisation so that there is no risk of ascribing a genuinely reactive sample as non-neutralisable [288].

This novel antigen neutralisation step is also effective on both urine and stool samples. Such non-plasma analytes are inherently susceptible to causing non-

specific reactivity in ELISA assays. In using confirmation by antigen neutralisation it is possible to confidently differentiate a true signal from a non-specific signal even in the absence of detectable HEV RNA.

The second aim of this chapter was to determine the performance of the commercial HEV-Ag assay as a screening assay for active HEV infection in immunocompromised patients. In studies of immunocompetent patients the sensitivity of HEV-Ag detection varied between 60% in acute G1 infections and 88% in acute G3 infections in separate studies [280, 289]. In acute G3 infections in immunocompromised patients the detection of HEV-Ag was found to be higher at 94% [280]. We found the assay had a sensitivity of 90.5% for viraemia at the single random time-point of screening which included patients with persistent infection and acute HEV infection. Similar to Behrendt *et al*, we found that patients with acute HEV infection were less likely to have detectable HEV-Ag than those with established infection. All the patients in our study were infected with G3 HEV, but a similar sensitivity has been reported for G4 HEV infections [290].

The reason for the reduced sensitivity of HEV-Ag detection compared with HEV RNA detection in acute infections is not clear. The kinetics of HEV-Ag in early human HEV infections is not fully understood, nor is the relationship between ORF2 antigen and HEV RNA. We did find a correlation between the HEV RNA plasma viral load and the HEV-Ag S/CO result (Pearson's correlation, 0.8184 ($p < 0.001$), however the recent description of multiple forms of ORF2 antigen which are released from infected cells via different secretory pathways suggest a more complex association [141, 291]. Secreted forms of ORF2 antigen which are not associated with infectious virions are suspected to be the major circulating

antigens in patient sera and the predominant antigen detected by the Wantai commercial assay [291, 292]. Therefore, the most likely explanation is that samples taken from patients with acute infection are within a window period prior to the presence of high levels of secreted ORF2, however it is notable that in humanized mice inoculated intrasplenically with HEV (G1 or G3 HEV), HEV-Ag was detectable in plasma of some mice prior to the detection of plasma HEV RNA which is in contrast to our findings [293]. Nevertheless, antigen kinetics may differ in this humanised murine model because only the human hepatic tissue is susceptible to HEV infection and the route of inoculation is different [294].

We found the HEV-Ag assay to be highly specific amongst immunocompromised patients (99.15%), a finding confirmed in another UK-based study in liver transplant recipients [295]. In using the antigen neutralisation assay we were also able to identify one patient who had ongoing antigenaemia in the absence of detectable HEV RNA at the point of screening, indicating a clearing HEV infection. The clinical significance of antigenaemia in the absence of RNA is not known, but has been described in ribavirin-treated patients previously, however to our knowledge this patient was not known to be HEV-infected and had not received ribavirin [217].

Finally, we explored HEV-Ag kinetics in a subset of patients identified with HEV infection. One previously published study found that HEV-Ag may be detectable for more than 100 days in plasma after HEV RNA clearance in ribavirin-treated immunocompromised patients [217]. We found that plasma antigenaemia persisted despite undetectable HEV RNA in five of eight ribavirin-treated patients and two of three untreated patients. We found that HEV-Ag was detectable

despite cleared HEV RNA in plasma for a longer duration in ribavirin-treated patients than patients who spontaneously clear HEV infection and was detectable for over 200 days after HEV RNA clearance in one patient. The half-life of HEV-Ag in plasma is unknown, but disappears within seven days after injection into nonhumanized mice, suggesting that the HEV-Ag we are detecting may be due to ongoing secretion of ORF2 antigen in the absence of HEV RNA replication [292, 293]. The differences between ribavirin-treated patients and untreated patients suggest it may be due to the differential effects of ribavirin on the replication of HEV RNA compared with the translation and secretion of ORF2 antigen [292].

The detection of HEV RNA in stool samples has become critical in guiding antiviral treatment duration in patients with persistent HEV infection, however little is known about the rate of HEV-Ag decline in the stool of treated patients or its clinical significance [225]. We detected HEV-Ag in only two of sixteen samples which harboured no detectable HEV RNA. In particular, we found no evidence of prolonged HEV-Ag detection in stool samples in contrast to plasma. We also found a relatively poor correlation between HEV RNA quantitation and HEV-Ag OD values (Pearson's correlation, 0.4467), which could be due to the stochastic nature of stool testing when very low levels of virions are present. However, there appears to be a fundamental difference between HEV-Ag kinetics in plasma when compared with stool. Sayed *et al* observed that in stool samples from experimentally infected human-liver chimeric mice viral RNA was higher than ORF2 antigen, whereas in plasma ORF2 antigen levels were markedly higher than viral RNA levels [293]. If ORF2 antigen in stool is mostly virion associated

and in plasma mostly not associated with virions then this would explain our findings. Our findings suggest that testing for HEV-Ag in stool is unlikely to provide any enhanced diagnostic information, however prolonged antigenaemia in plasma may indicate ongoing secretion of ORF2 from infected cells and could help guide duration of antiviral therapy and should be studied prospectively.

There are a number of limitations of this study. The relative rarity of HEV infection amongst all immunocompromised patients limited the ability to determine accurately the sensitivity of HEV-Ag detection for the diagnosis of HEV infection in this group. The small numbers of patients followed up to assess HEV-Ag kinetics, the sporadic sampling determined by clinical follow-up and the availability of samples for testing allowed us to comment on some interesting observations, but was insufficient to draw firm conclusions. Furthermore, within the scope of this chapter we did not characterise the nature of the HEV-Ag we were detecting in plasma and stool to determine whether it was virion-associated.

3.4 Conclusions

- HEV-Ag is a both highly sensitive and specific for the detection of persistent HEV infections in immunocompromised cohorts, however in early infection sensitivity is reduced.
- HEV-Ag neutralisation can be a useful confirmatory assay in confirming the specificity of HEV-Ag ELISA reactivity and can be used on a number of analytes including plasma, urine and stool.
- HEV-Ag remains detectable in a proportion of ribavirin-treated patients beyond HEV clearance in plasma, but this is not observed in stool. This may indicate ongoing viral replication and warrants further investigation.

4 HEV infection in immunocompromised cohorts

4.1 Introduction

Persistent G3 HEV infections are typically asymptomatic or pauci-symptomatic with only a mild elevation in transaminases and are considered to be under-recognised in many patient cohorts [168]. Determinants and factors leading to persistent HEV infection are not well characterised or understood in immunocompromised patients, exemplified by cases in patients with undefined immune defects [172, 296]. Hence, it is difficult to identify cohorts highly susceptible to persistent HEV infection.

Certain immunocompromised patient cohorts outside of solid organ and stem cell transplantation may be particularly susceptible. Persistent and fatal courses of HEV infection have occurred in patients with haematological malignancies outside the context of allogeneic-HSCT [170, 297-299]. In one study five of 14 (36%) patients with haematological malignancies acquiring acute HEV infection developed a persistent infection [199]. However, risk of persistent HEV in patients with haematological malignancies is unknown, with no systematic studies in patients who have not been treated with allogeneic-HSCT [185, 191]. Haematological malignancies are heterogeneous and are treated with increasingly diverse immunosuppressive medications, some of which have profound and long-lasting immunosuppressive effects. In addition, the excess blood transfusion requirements in this patient group leads to increased exposure to HEV from donors [22].

A second group theoretically susceptible to persistent HEV infection are patients with antibody deficiency, characterised by low immunoglobulins or the functional failure of immunoglobulins. This may be caused by a primary immunodeficiency syndrome or secondary causes including haematological malignancy and medications [300]. Typically, this manifests as recurrent infections with encapsulated bacteria but also with a range of viral, fungal or protozoan pathogens. For example, prolonged rhinovirus infections, severe hepatitis C virus and enterovirus infections are described in patients with primary antibody deficiency, whilst norovirus can lead to severe prolonged enteropathy in common variable immune deficiency (CVID) [301-304]. Only two studies have attempted to address the risk of persistent HEV infection in this cohort. A study of 73 patients with CVID in Germany in 2012 and more recently, a study of 27 patients with primary antibody deficiency and abnormal liver enzymes in the UK, found no evidence of persistent HEV infection [300, 305].

Prevalence studies of HEV infection in immunocompromised patients are biased towards solid organ transplant recipients, with other immunocompromised cohorts being underrepresented. Current studies show marked differences geographically and by patient group, likely as result of different exposure risks through diet or substances of human origin and varying host susceptibility. The prevalence of persistent HEV infection in cohorts of SOT recipients varies between 0.7% and 1.5% in Western Europe, but as high as 3.2% in one small study of lung transplant recipients [185, 191-193, 306]. A Dutch study in HSCT recipients found HEV viraemia in 2.4% of patients [194].

The prevalence of persistent HEV infections in immunocompromised cohorts in the UK is unknown. However, the risk of HEV acquisition in Western Europe has risen in recent years [15]. Given the aforementioned uncertainty of the immunological dysfunction required to tolerate persisting HEV replication and the increase in HEV exposure within the UK, prevalence studies are required to inform testing strategies. Within this context, the aims of the following chapter are:

1. to determine the point prevalence of HEV viraemia in three distinct immunocompromised cohorts in the UK:
    a. SOT and HSCT patients
    b. Haemato-oncology patients
    c. Antibody-deficient patients
2. to identify predictive factors of HEV viraemia.

4.2 Results

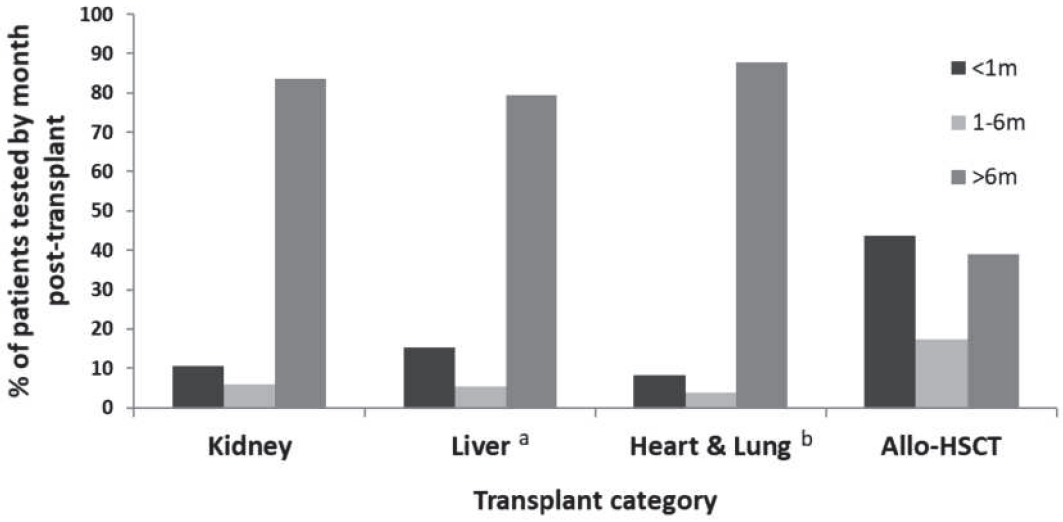
4.2.1 HEV infection in transplant recipients

4.2.1.1 Characteristics of transplant patients

All patients lived in England or Wales, 96% lived within 100 miles of central Birmingham. A minimum dataset of 2822 patients were included in the statistical analysis and consisted of 2419 SOT patients (1181 kidney, 869 liver, 229 heart, 110 lung, 21 kidney/liver, 6 heart/lung, 2 heart/kidney and 1 lung/liver), 144 allograft HSCT patients and 259 patients with no available transplant history. The majority of SOT patients were greater than six months from the transplant date at the time of screening for HEV RNA, whilst the majority of the HSCT patients were within six months of the transplant date (Figure 4.1). Seven hundred and thirteen patients were prescribed ciclosporin, 2066 tacrolimus, 42 sirolimus and one everolimus.

At the time of HEV screening the mean serum alanine aminotransferase (ALT) value was 61 IU/L amongst all screened patients (n=2765). Higher ALT values were observed in patients who had undergone transplantation within one month prior (Figure 4.2).

Figure 4.1 Timing of HEV screen in relation to transplant

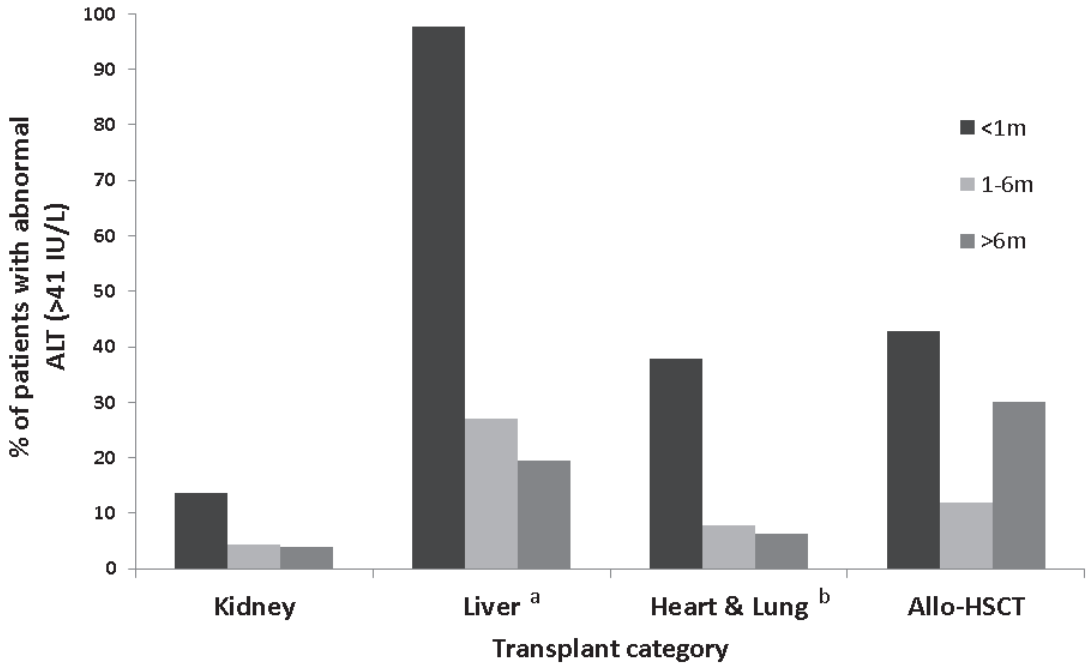


[a] Includes liver/kidney dual transplant (n=21) and lung/liver dual transplant (n=1).

[b] Includes heart/kidney (n=2)

Abbreviations: allo-HSCT, allogeneic haematopoietic stem cell transplant; m, months.

Figure 4.2 Proportion of patients with abnormal ALT in relation to transplant



[a] Includes liver/kidney (n=21) and lung/liver (n=1).

[b] Includes heart/kidney (n=2).

Abbreviations: Allo-HSCT, allogeneic haematopoietic stem cell transplant; m, months.

4.2.1.2 Viraemic transplant patients

Nineteen minipools containing HEV RNA were resolved to identify 19 viraemic patients, giving an overall RNA prevalence of one in 149 (0.67%, 95% CI of 0.43-1.05%). Individual viraemia levels ranged from 352 IU/ml to 9.09 x 10^6 IU/ml. Phylogenetic analysis demonstrated all samples to harbour HEV G3 viruses of which six (31.6%) were clade 1 (subtypes e,f,g) and 13 (68.4%) were clade 2 (subtypes a,b,c,h,i,j). Three of the viraemic patients (15.8%) were allogeneic HSCT recipients with a median duration of time since transplant of 11.3 months [interquartile range 2.3-23.0]. Sixteen (84.2%) were SOT recipients (6 kidney, 9 liver, 1 heart) with a median duration of time since transplant of 88.4 months [interquartile range 19.3-122.6] (Table 4.1).

Full clinical details were available on 16 of the viraemic patients. The diagnosis of hepatitis E infection was only considered clinically in one. Four had rises in ALT which were thought not to be clinically significant. The working diagnoses in the remainder were graft versus host disease (n=2), graft rejection (n=2), autoimmune hepatitis (n=2), statin induced liver injury (n=1), EBV associated hepatitis (n=1), alcohol excess (n=1), recurrent primary biliary cirrhosis (n=1) and recurrent primary sclerosing cholangitis (n=1).

A follow up sample taken at a median of nine weeks after the initial diagnosis of HEV infection (range 1.6-22.1 weeks) as part of the routine clinical care of the viraemic patient was available for 15 individuals, all but one of whom remained viraemic. The follow up sample for the patient who cleared HEV viraemia was taken 22.1 weeks after the initial sample (patient 6, Table 4.1). Two further

patients who were viraemic at initial follow up cleared their virus within three months of the initial screening test (patients 7 and 9, Table 4.1).

At the time of writing during the follow up period 12 patients had evidence of established persistent infection; viraemia >12 weeks (n=11) or an unchanged viral load over a period of at least eight weeks (n=1). Four patients had insufficient follow up to comment.

Table 4.1 Characteristics of HEV viraemic transplant patients

Pt No.	Transplant	Time since transplant (yrs)	Age (yrs)	Sex	Immune suppression	Bilirubin umol/L	ALT IU/L	Serology		HEV viral load IU/ml
								IgM S/CO	IgG S/CO	
1	Liver	0.17	27	F	Azathioprine, Prednisolone, Tacrolimus	16	15	0.01; NEG	-0.01; NEG	8.57E+02
2	Liver	5.49	65	F	Tacrolimus	6	47	13.44; POS	23.38; POS	9.97E+05
3	Liver	9.13	69	M	Tacrolimus	8	57	9.37; POS	6.61; POS	1.86E+05
4	Liver	16.89	55	F	Tacrolimus	11	78	13.52; POS	19.25; POS	1.30E+05
5	Liver	1.22	52	M	Prednisolone, Sirolimus	8	127	11.11; POS	23.45; POS	8.38E+05
6	Liver	2.06	21	M	MMF, Tacrolimus	11	229	6.60; POS	24.60;POS	3.58E+04
7	Liver	0.01	43	M	Basiliximab, Prednisolone, Tacrolimus	38	298	2.01; POS	-0.17; NEG	8.58E+03
8	Liver	14.15	57	F	Prednisolone, Tacrolimus	361	602	10.95; POS	15.32; POS	2.27E+04
9	Liver	1.61	61	M	Tacrolimus	10	794	14.24; POS	19.46; POS	4.32E+04
10	Kidney	10.22	58	M	Tacrolimus	129	93	9.64; POS	24.74; POS	7.56E+04
11	Kidney	8.69	66	F	MMF, Prednisolone, Tacrolimus	5	156	7.38; POS	23.80; POS	3.48E+05
12	Kidney	7.70	24	M	Prednisolone, Tacrolimus	9	272	13.51; POS	23.35; POS	3.08E+05
13	Kidney	7.37	52	M	Tacrolimus	-	-	11.91; POS	24.84; POS	3.56E+05
14	Kidney	12.61	56	F	Tacrolimus	-	-	15.20; POS	26.27; POS	1.16E+06
15	Kidney	-	61	M	Ciclosporin	25	415	12.30; POS	0.16;NEG	9.09E+06
16	Heart	2.58	67	M	MMF, Prednisolone, Tacrolimus	9	53	4.44; POS	2.59; POS	7.52E+05
17	Allo-HSCT	1.92	36	F	Ciclosporin	10	161	13.22; POS	18.37; POS	7.50E+05
18	Allo-HSCT	0.19	44	F	Ciclosporin	14	17	3.26; POS	17.45; POS	3.52E+02
19	Allo-HSCT	0.94	34	M	Ciclosporin	24	392	0.00; NEG	0.00; NEG	1.10E+06

Abbreviations: Allo-HSCT, allogeneic haematopoeitic stem cell transplant; M, male; MMF, mycophenolate mofetil; F, female; ND, not detected; NEG, negative; POS, positive; S/CO, sample over cut-off ratio of optical density values; yrs, years.

4.2.1.3 Predictive factors for HEV viraemia in transplant patients

The characteristics of HEV viraemic patients were compared with uninfected aviraemic patients. At the single time point of random screening the HEV viraemic patients had statistically significantly higher ALT ($p<0.0001$), bilirubin ($p=0.01$), tacrolimus levels ($p=0.002$) and ciclosporin levels ($p=0.02$), with the caveat of relatively small numbers (Table 4.2).

The median ALT was 156 IU/L [IQR 57-298] in HEV viraemic patients compared to a median ALT of 19 IU/L [IQR 13-30] in HEV RNA aviraemic patients (Figure 4.3). A sub analysis of only liver transplant patients found a similar difference with a median ALT of 127 IU/L [IQR 57-298] in HEV viraemic patients compared to a median ALT of 24 IU/L [IQR 15-62] in HEV RNA-negative patients ($p=0.005$) (Table 4.3). Comparison of transplant types did not identify any particular SOT category as being a risk factor for being HEV viraemic, however when SOT patients were compared with HSCT patients there was borderline evidence ($p=0.09$) that HSCT patients were more likely to be viraemic. Gender, drug administered (ciclosporin or tacrolimus), age and log(time since transplant) were not statistically significant univariate risk factors for HEV viraemia in this study.

Table 4.2 HEV viraemic and HEV RNA-negative transplant patients

	HEV viraemic patients (n=19)	HEV RNA-negative patients (n=2803)	p-value
Gender			
Male	11 (58%)	1662 (59%)	>0.99
Female	8 (42%)	1141(41%)	
Age [years]			
Median [IQR]	55 (36-61)	54 (42-63)	0.6
Number included	19	2803	
Transplant type			
SOT	16 (84%)	2403 (94%)	
Kidney	6	1175	
Liver[a]	9	882	0.09
Heart/Lung[b]	1	346	
HSCT	3 (16%)	141 (6%)	
Allo-HSCT	3	141	
Not reported	0	259	
Log (Time since transplant [yrs])			
Median [IQR]	3.8 (2.7-4.7)	3.8 (2.4-4.8)	0.9
Number included	18	2586	
Drug administered			
Tacrolimus	14 (74%)	2052 (73%)	
Ciclosporin	4 (21%)	709 (25%)	0.3
Other	1 (5%)	42 (2%)	
Tacrolimus level [µg/L]			
Median [IQR]	8.4 (7-8.9)	5.8 (4.4-7.7)	0.002
Number included	14	2052	
Ciclosporin level [µg/L]			
Median [IQR]	166.5 (118.5-434.5)	73 (43-119)	0.02
Number included	4	709	
ALT [IU/L]			
Median [IQR]	156 (57-298)	19 (13-30)	<0.0001
Log(ALT) Median [IQR]	5.0 (4.0-5.7)	2.9 (2.6-3.4)	<0.0001
Number included	17	2746	
Bilirubin [µmol/L]			
Median [IQR]	11 (9-24)	8 (6-13)	0.01
Log(Bilirubin) Median [IQR]	2.4 (2.2-3.2)	2.1 (1.8-2.6)	0.01
Number included	17	2803	

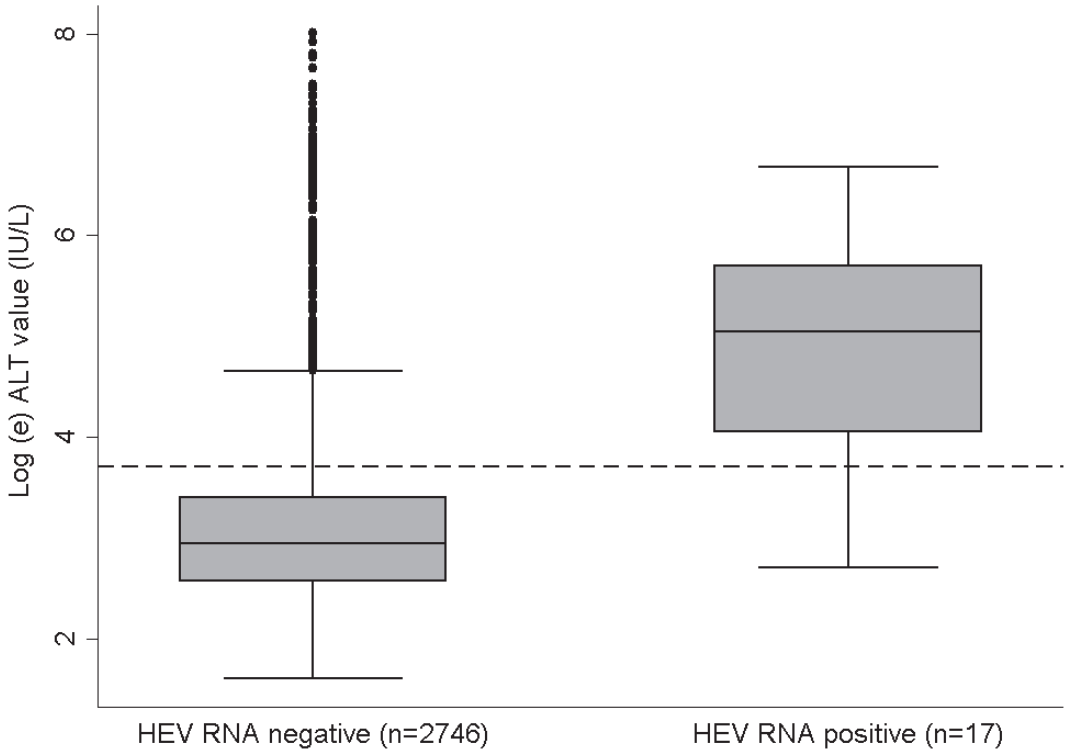
Selected demographic, clinical and biochemical parameters of HEV viraemic patients and HEV RNA-negative patients. Categorical values were compared using Fisher's exact test and continuous variables are compared using Wilcoxon two sample test.

[a] Includes kidney/liver (n=21) and lung/liver dual transplants (n=1).

[b] Includes heart/kidney dual transplants (n=2).

Abbreviations: IQR, interquartile range; HSCT, haematopoietic stem cell transplant.

Figure 4.3 Comparison of ALT values of HEV viraemic and aviraemic transplant patients



Distribution of ALT values at the time of screening for HEV amongst viraemic and aviraemic transplant patients. Median ALT values were significantly higher in the HEV viraemic patients (ALT 156 IU/L) compared to the aviraemic patients (ALT 19 IU/L) ($p < 0.0001$). Represented here are $\log_e$ transformed values. The hatched line represents the laboratory upper limit of normal for ALT (41 IU/L). The boxes are defined by the first and third quartiles and the band represents the median value. Outlier values are represented by black circles. Abbreviations: ALT, alanine aminotransferase; IU; international units; L, litre; log, logarithmic.

Table 4.3 Comparison of ALT values of HEV viraemic and aviraemic patients by transplant group

	HEV viraemic patients Median ALT, IU/L (IQR)	HEV RNA-negative patients Median ALT, IU/L (IQR)
Renal	214 (124.5-343.5) n=4[c]	16 (12-22) n=1120[d]
Liver[a]	127 (57-298) n=9	24 (15-62) n=882
Heart/Lung[b]	53 (-) n=1	18 (14-26) n=346
Allo-HSCT	161 (17-392) n=3	29 (18-50) n=141

Comparison of ALT values at the random time-point of screening for HEV viraemic patients and aviraemic patients by transplant group.

[a] Includes kidney/liver (n=21) and lung/liver dual transplants (n=1)

[b] Includes heart/kidney dual transplants (n=2).

[c] Data missing for 2 patients.

[d] Data missing for 55 patients.

Abbreviations: Allo, allogeneic; ALT, alanine aminotransferase; HSCT, haematopoietic stem cell transplant; IU; international units; L, litre; log, logarithmic.

4.2.1.4 Predictive value of a raised serum ALT value for HEV viraemia

Of the HEV viraemic patients with an available ALT result (n=17), fifteen (88.2%) had an abnormal ALT value at the time of screening (>41 IU/L) compared with only 452 (16%) of the HEV RNA-negative patients. The positive predictive value (PPV) of an abnormal ALT result (>41 IU/L) as a surrogate for HEV infection in this cohort was 3.2% and did not rise significantly by raising the ALT threshold (PPV 3.7%/sensitivity 70.6% for ALT >57 IU/L, PPV 5.2%/sensitivity 47.1% for ALT >156 IU/L, PPV 3.8%/sensitivity 23.5% for ALT >298 IU/L). No correlation was observed between ALT value and plasma HEV viral load (correlation co-efficient 0.11, p=0.7).

Both of the two patients with a normal ALT (patients 1 and 18, Table 4.1) at the time point of screening subsequently developed an abnormal ALT result during follow-up with a rising viral load (352 rising to 3.0×10^5 IU/ml, 8.57×10^2 rising to 7.6×10^4 IU/ml, respectively,) suggesting that the screening test was during early infection.

4.2.1.5 HEV markers in viraemic patients

Most patients, 15/19 (78.9%), were seropositive for IgM and IgG anti-HEV, two (10.5%) were seropositive for IgM anti-HEV only and two (10.5%) were seronegative (patients 1 and 19, Table 4.1). Of the two patients who were seronegative at the time of screening, one was an allogeneic HSCT recipient (HEV RNA 1.10×10^6 IU/ml) and one was a liver transplant recipient (HEV RNA 8.57×10^2 IU/ml). Both patients subsequently seroconverted for IgM and IgG

antibody but had remained seronegative for at least four months and two months respectively from the time of first testing.

4.2.2 HEV infection in patients with haematological malignancies

4.2.2.1 Characteristics of haemato-oncology patients

The characteristics of the 1591 patients tested for HEV RNA are detailed in Table 4.4. Most patients had underlying lymphoma (34.9%) or a plasma cell dyscrasia (32.7%), but the cohort also included 260 patients (16.3%) with chronic leukaemia and 130 patients (8.2%) with acute leukaemia. The majority were within five years of diagnosis of the haematological disorder (67.1%) and either in complete or partial remission (65.2%). Four hundred and eighty patients (30.2%) were lymphopenic (<1.2 x 10^9/L); seventy five (4.7%) were neutropenic (<1.0 x 10^9/L). One fifth (330/1591, 20.7%) of the cohort had received a prior autograft; 286 patients with a plasma cell disorder, 40 with underlying lymphoma and 4 had been historically treated with an autograft for acute leukaemia.

Most patients had been treated with at least one course of treatment (78.2%); over half of the patients (55.9%) tested had received a small molecule immunomodulator or monoclonal antibody for their haematological disorder.

Over a third had received immunosuppressive chemotherapy in the preceding six months (39.6%) (Table 4.5 and further details in Appendix A3.1); 129 of the total cohort had received rituximab therapy in this period.

Of the patients tested, haematology and biochemistry blood results were available for 99.9% of them, 93.2% of which were within 14 days of the HEV RNA

test. Overall, 205 patients (12.9%) had an abnormal ALT value (>35 IU/L for women, >50 IU/L for men) and 58 (3.6%) had an abnormal bilirubin (>20 µmol/L) at the time of screening for HEV infection.

Table 4.4 Haemato-oncology patients screened for HEV

Characteristic		No. (%)
Sex	Male	886 (55.7)
	Female	705 (44.3)
	Age, yrs, Median [IQR]	65.8 [54.9–73.8]
Underlying Haematological Disorder	Lymphoma	556 (34.9)
	Plasma Cell Disorder[a]	521 (32.7)
	Chronic leukaemia	260 (16.3)
	Acute leukaemia	128 (8.0)
	Myelodysplastic syndrome	77 (4.8)
	MPN	36 (2.3)
	Aplastic Anaemia	13 (0.8)
Time since diagnosis, yrs	<1	363 (22.8)
	1-5	704 (44.2)
	>5	524 (32.9)
Disease status	Complete or partial remission	1037 (65.2)
	No remission	446 (28.0)
	Progressive disease	108 (6.8)
Treatment of underlying disease	No treatment	347 (21.8)
	1-2 lines	908 (57.1)
	>2 lines	336 (21.1)
	Prior Autograft	330 (20.7)
	Prior small molecule inhibitors or monoclonal antibodies	889 (55.9)
	Immunosuppressive therapy in prior 6m[b]	628 (39.5)
	Rituximab in prior 6m	129 (8.1)
Transfusions in 5 yrs prior[c]	Nil	1150 (72.3)
	1-10	292 (18.4)
	11-20	46 (2.9)
	21-50	70 (4.4)
	>50+	33 (2.10)
Blood results, Median [IQR]	ALT (IU/L)	22 [16 – 31]
	Bilirubin (µmol/L)	7 [5 -10]
	Total WCC (x 10^9/L)	6.1 [4.4 – 8.3]
	Neutrophils (x 10^9/L)	3.4 [2.3 – 4.6]
	Lymphocytes (x 10^9/L)	1.6 [1.1 – 2.4]
	Platelets (x 10^9/L)	198 [149 – 245]

Clinical characteristics of patients witn a haematological malignancy tested for HEV RNA.

[a] Includes Multiple Myeloma and monoclonal gammopathy of uncertain significance.

[b] Transfusions only recorded if within the centre in the five-year period prior to enrolment excluding time between 10/4/17 and enrolment (HEV screened products given after that 10/4/17 at the centre).

[c] For treatments given in the preceeding six months see Table 4.5.

Abbreviations: IQR, interquartile range; m, months; MPN, Myeloproliferative neoplasm; yrs, years.

Table 4.5 Immunosuppression of haemato-oncology patients prior to HEV testing

Treatment	No.
Plasma Cell Disorders, n=271	
High intensity chemotherapy[a]	45
Standard intensity chemotherapy[b]	138
Other combination chemotherapy	1
Single agents +/- corticosteroid	84
CAR-T therapy	1
Radiotherapy	2
Acute Leukaemia, n=75	
High intensity chemotherapy[c]	53
Low intensity chemotherapy[d]	22
Chronic Leukaemia, n=67	
Low intensity chemotherapy[e]	25
Single agents - targeted small molecule inhibitors	41
Single agents - monoclonal antibodies	1
Lymphoma, n=171	
High intensity chemotherapy[f]	34
Moderate intensity chemotherapy[g]	99
Low intensity chemotherapy	26
Single agents - targeted small molecule inhibitors	4
Single agents - monoclonal antibodies	7
Radiotherapy	1
MDS, n=15	
Low intensity chemotherapy	13
Single agents – monoclonal antibodies[h]	2
MPN, n=27	
Low intensity chemotherapy PLUS targeted small molecule inhibitor	3
Low intensity chemotherapy	4
Very low intensity chemotherapy	6
Single agents - targeted small molecule inhibitors	14
Aplastic Anaemia, n=2	
Single agent immunosuppression	2
Total	**628**

Legend for Table 4.5:

Iatrogenic immunosuppression of haemato-oncology patients in the six months prior to HEV RNA testing. A total of 628 patients received iatrogenic immunosuppression in the six months prior to HEV screening. Where patients were in clinical trials, if the trial drug administered was known this was recorded, in blinded randomised trials only the known backbone drugs were recorded. The commonest regimes in each category are given below; a full version of this table with all chemotherapy regimes in each category is available in Appendix A3.1.

[a] e.g. autograft and DTPACE.

[b] e.g. combination chemo PLUS –imid drug/proteasome inhibitor PLUS corticosteroid.

[c] e.g DA-based regimes, FLA(G)-IDA and MidAC.

[d] e.g. low dose ARA-C and azacitidine..

[e] e.g. Rituximab+idelalisib, FLAIR clinical trial.

[f] e.g. R-CHOP+HD MTX, R-CODOX-M+R-IVAC, ABVD+BEACOPP

[g] e.g. ABVD, R-CHOP, R-Bendamustine.

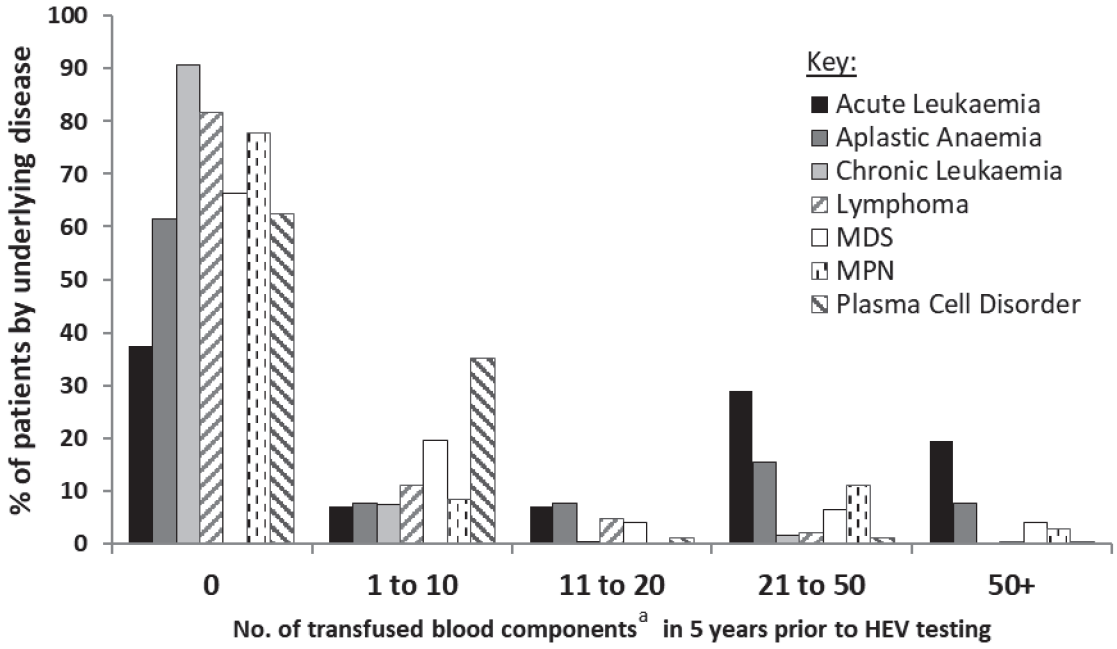
[h] Alemtuzumab in both cases.

Abbreviations: ABVD, Doxorubicin, Bleomycin, Vinblastine, Dacarbazine; ARA-C, Cytarabine; BEACOPP, Bleomycin Etoposide Doxorubicin Cyclophosphamide Vincristine Procarbazine Prednisolone; DA, daunorubicin, cytarabine; DTPACE, dexamethasone, thalidomide, cisplatin, doxorubicin; FLA(G)-IDA, Fludarabine cytarabine, Idarubicin; HD MTX, High Dose Methotrexate; MiDAC, mitoxantrone, cytarabine; R-CODOXM/R-IVAC, Vincristine, Doxorubicin, Cyclophosphamide, Cytarabine, methotrexate, Ifosfamide, Etoposide; R-CHOP, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone

4.2.2.2 Transfusion exposure to HEV infection in haemato-oncology patients

A total of 441 patients (27.7%) had been transfused with at least one blood component within the hospital trust in the five years prior to enrolment (Figure 4.4). Of those with a history of transfusions the mean number of transfusions was 15.5 (range 1-346); the majority of transfusions were either packed red cells (53.5%) or platelets (43.4%). As previously stated all transfusions included in the analysis were transfused before April 2017 and were therefore unscreened for HEV.

Figure 4.4 Patients receiving HEV unscreened transfusions prior to HEV testing



Proportion of patients receiving HEV unscreened transfusions in the five years prior to HEV RNA testing by diagnosis.

[a] Transfusions only recorded if within five years preceeding HEV testing and before the introduction of the universal screening of blood donations for HEV (10th April 2017). Patients with acute leukaemia with no transfusions were either transfused after the introduction of universal screening or were in long-term remission and transfusions may have occurred prior to the 5-year cut-off.

Abbreviations: MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; no., number.

4.2.2.3 Viraemic haemato-oncology patients

Of the 1591 patients tested for HEV RNA, two viraemic patients were identified giving a prevalence of 0.13% (95% CI, 0.02-0.45%). Both patients harboured a genotype 3c virus.

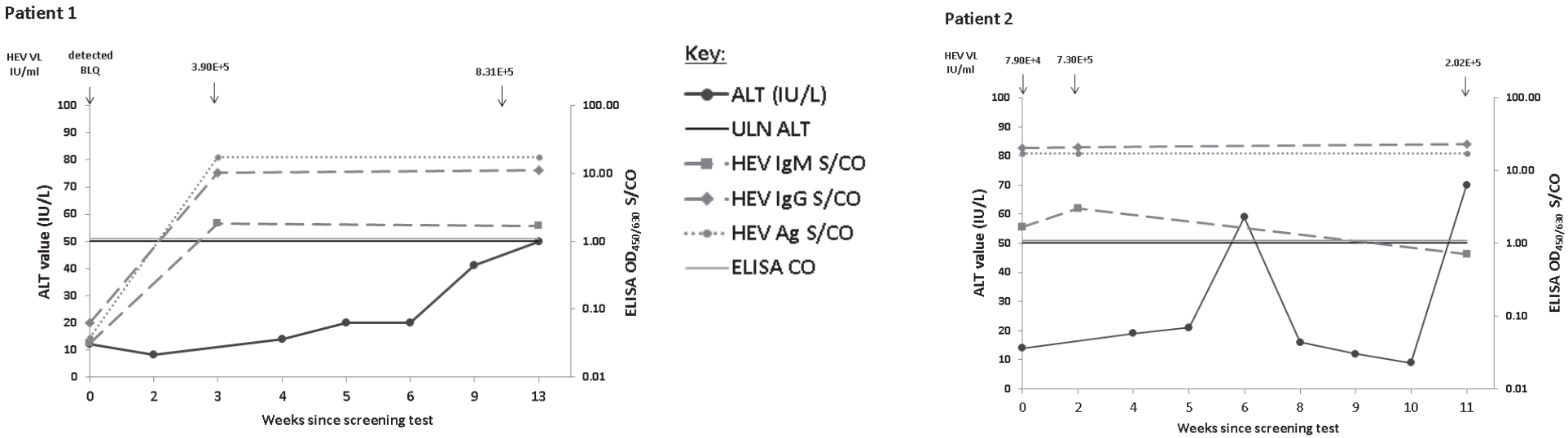
Both viraemic patients had underlying progressive Multiple Myeloma, diagnosed over 8 years and 16 years previously, and had received immunosuppressive treatment (Bortezomib + Dexamethasone + Panabinostat or Lenalidomide + Cyclophosphamide + Dexamethasone) within five weeks of HEV testing. Neither patient had extensive transfusion histories in the five previous years; patient 1 had received three packed red cells between 23-128 weeks prior to HEV screening and patient 2 had received no transfusions prior to HEV screening. Both patients died during follow-up of their underlying haematological disorder and did not receive any treatment for HEV infection.

At the time of screening both patients had unremarkable results for ALT, white cell count, neutrophils and platelets; however patient 2 was lymphopenic (0.39 x 10^9/L). The two patients had different virological profiles; the HEV infection in patient 1 was detected during established infection when the plasma viral load was quantified at 7.9 x 10^4 IU/ml with detectable plasma anti-HEV IgM (S/CO 1.69) and IgG (S/CO 20.32). In contrast, patient 2 was in the early phase of HEV infection when the plasma viral load was below the limit of quantitation (<1.0E+2 IU/ml) and there was no detectable plasma anti-HEV IgM (S/CO 0.03) or IgG (S/CO 0.06). It is notable that during follow-up over 13 weeks, patient 2 seroconverted for anti-HEV IgM and IgG. However, the ALT value remained within the normal range and patient 1 only had two of eight ALT readings outside

the normal range during follow-up, despite high level viraemia. The full diagnostic markers are displayed in Figure 4.5.

Figure 4.5 Diagnostic markers in HEV viraemic haemato-oncology patients

	ALT IU/L (range 10-35)	Bilirubin µmol/L (range 0-20)	WCC x 10^9/L (range 3-10)	Neutrophils x 10^9/L (range 2-7.5)	Lymphocytes x 10^9/L (range 1.2-3.65)	Platelets x 10^9/L (range 150-400)
Patient 1	14	9	6.85	4.84	1.53	145
Patient 2	12	28	3.31	2.40	0.39	102



Diagnostic markers in the two HEV viraemic patients identified by screening in the haemato-oncology cohort. The left Y-axis shows ALT levels, while the secondary y axis represents values for the anti-HEV IgM, anti-HEV IgG and HEV-Ag ELISA assays. Abbreviations: ALT, alanine aminotransferase; ELISA, enzyme-linked immunosorbent assay; S/CO, sample over cut-off of optical density values; HEV Ag, hepatitis E virus antigen; ULN, upper limit of normal; CO, cut-off; BLQ, below the limit of quantitation; IU, international units; VL, viral load.

4.2.2.4 Anti-HEV IgG seroprevalence in haemato-oncology patients

The odds of a patient being seroreactive for anti-HEV increased with age and the numbers of red cells/platelet transfusions in the univariable analysis. The odds reduced with increasing numbers of lines of treatment and was influenced by underlying disease and disease status. Treatment with rituximab in the prior six months did not influence anti-HEV IgG status (OR 1.09, 95% CI 0.68-1.74, p=0.727) (Table 4.6). A recent transfusion in the preceding 28 days was statistically significant in the univariable analysis, but had no effect on the odds of a patient being seroreactive in the multivariable model. In the final adjusted multivariable analysis increasing age and underlying haematological disease were the strongest factors associated with a patient being seropositive, such that patients with plasma cell disorders were least likely to be seroreactive, whilst patients with acute leukaemia had the highest odds. For every ten years increase in age the odds of a patient being seroreactive increased by 37% (OR 1.37, 95% CI 1.24-1.52) and for every ten transfusion episodes the likelihood of a patient being seroreactive increased by 11% (OR 1.11, 95% CI 1.02-1.20).

Table 4.6 Factors affecting likelihood of anti-HEV IgG seroreactivity

Factor		HEV IgG positive[a] /total (%) n=1591	Univariable analysis OR (95% CI)	Univariable analysis P value[c]	Multivariable analysis[b] OR (95% CI)	Multivariable analysis[b] P value[c]
Sex	F	110/705 (15.6)	-		-	
	M	156/886 (17.6)	1.16 (0.89-1.51)	0.287	1.08 (0.82-1.43)	0.572
Age, yrs	<40	17/165 (10.3)				
	40-59	46/413 (11.1)				
	60-79	149/836 (17.8)		<0.001		
	>80	54/177 (30.5)				
	Effect per 10 years	-	1.37 (1.24-1.51)		1.37 (1.24-1.52)	<0.001
Diagnosis						
	Plasma Cell Disorder	53/521 (10.2)	-		-	
	Acute leukaemia	34/128 (26.6)	3.19 (1.97-5.18)		2.28 (1.25-4.16)	
	Chronic leukaemia	54/260 (20.8)	2.31 (1.53-3.50)	<0.001	1.75 (1.12-2.75)	<0.001
	Lymphoma	86/556 (15.5)	1.62 (1.12-2.33)		1.45 (0.97-2.18)	
	MDS	23/77(29.9)	3.76 (2.14-6.62)		2.41 (1.28-4.54)	
	MPN	15/36 (41.7)	6.31 (3.07-12.97)		4.13 (1.94-8.78)	
	Aplastic Anaemia	1/13 (7.7)	0.74 (0.09-5.77)		0.25 (0.01-4.36)	
Disease status						
	No remission	96/446 (21.5)	-			
	Complete Remission	115/678 (17.0)	0.74 (0.55-1.01)	0.001	-	-
	Partial remission	42/359 (11.7)	0.48 (0.33-0.72)			
	Progressive disease	13/108 (12.0)	0.50 (0.27-0.93)			
Lines of treatment						
	0	70/347 (20.2)	-		-	
	1	117/614 (19.1)	0.93 (0.67-1.30)	<0.001	1.17 (0.80-1.72)	0.017
	2	50/294 (17.0)	0.81 (0.54-1.21)		1.11 (0.71-1.74)	
	>2	29/336 (8.6)	0.37 (0.24-0.60)		0.56 (0.33-0.95)	
Transfusions[d]						
	0	187/1150 (16.3)				
	1-10	41/292 (14.0)				
	11-20	10/46 (21.7)	-	0.002		
	21-50	13/70 (18.6)				
	>50	15/33 (45.5)				
	Effect per 10 transfusions	-	1.11 (1.03-1.19)		1.11 (1.02-1.20)	0.015
RTX within last 6m						
	Y	23/129 (17.8)	1.09 (0.68-1.74)	0.727	-	-
	N	243/1462(16.6)	-			
Transfusion <28d[e]						
	Y	24/86 (27.9)	2.02 (1.24-3.30)	0.005	-	-
	N	242/1505 (16.1)	-			

Clinical factors were assessed for the correlation with anti-HEV IgG seroreactivity.

[a] Any sample with a S/CO >1.1.

[b] Univariable analysis p values were calculated using Fisher's exact test, multivariate analysis p values were calculated using logistic regression.

[c] The final multivariable model included age (linear), gender, underlying haematological disease, numbers of transfusions (linear) and numbers of lines of treatment.

[d] Transfusion data only considered when given in 5 years prior and prior to introduction of universal screening.

[e] All transfusions considered including those given after introduction of universal screening.

Abbreviations: CI, confidence interval; D, days; M, months; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; RTX, rituximab.

4.2.3 HEV infection in patients with antibody deficiency

4.2.3.1 Characteristics of antibody-deficient patients

The characteristics of the 245 patients tested for HEV RNA are presented in Table 4.7. The majority of patients (80.8%) had an underlying primary immunodeficiency syndrome and most (75.1%) were receiving intravenous immunoglobulin. Fifty-seven patients received additional immunosuppressive medication (Table 4.8). Ninety patients had CD4+ T-cell counts below the normal range (<0.5 x 10^9/L), whilst 108 patients had CD19+ B-cell counts below the normal range (<0.10 x 10^9/L). Twenty three of 43 patients with significant CD4+ T-cell deficiency (<0.35 x 10^9/L) had T-cell proliferation results available, of which 74% displayed significantly impaired proliferation to phytohaemagglutinin, 68% to CD3 and 44% to CD3/CD28 stimulation. Thirty-nine patients (16%) had an abnormal ALT value at the time of testing for HEV infection.

Table 4.7 Characteristics of antibody-deficient patients tested for HEV

Characteristic	No. (%)
Sex	
Male	91 (37.1)
Female	154 (62.9)
Age, yrs, Median [IQR]	56 [41-68]
Underlying Diagnosis	
CVID	138 (56.3)
XLA	15 (6.1)
Hyper IgM syndrome	3 (1.2)
Good's syndrome	1 (0.4)
Other primary antibody deficiency[a]	41 (16.7)
Secondary antibody deficiency[b]	47 (19.2)
Immunoglobulin Product/manufacturer	
IV Flebogamma DIF 5%/Grifols	39 (15.9)
Gammagard 10%/Shire	1 (0.4)
Gammaplex 10%/BPL	20 (8.2)
Gamunex 10%/Grifols	13 (5.3)
Intratect 10%/Biotest	11 (4.5)
Intratect 5%/Biotest	6 (2.5)
Kiovig 10%/Baxalta	26 (10.6)
Octagam 10%/Octapharma	15 (6.1)
Privigen 10%/CSL Behring	53 (21.6)
SC Gammanorm 16.5%/Octapharma	10 (4.1)
Hizentra 20%/CSL Behring	24 (9.8)
Subcuvia 16%/Baxalta	13 (5.3)
Subgam 16%/BPL	14 (5.7)

Characteristic	No. (%)
Iatrogenic immunosuppression	
Nil	188 (76.7)
Mono or combination therapy	57 (23.3)
Lymphocyte count, x 10^9/L, Median [IQR]	1.39 [1.01-1.89][c]
No. (%) < normal range (1.0)	58 (24.3)[c]
CLL patients only (n=6), Median [IQR]	3.20 [1.55-13.73]
Absolute CD3 count[d], x 10^9/L, Median [IQR]	1.08 [0.74-1.49]
No. (%) < normal range (0.7)	54 (22.0)
Absolute CD4 count[d], x 10^9/L, Median [IQR]	0.67 [0.41-0.83]
No. (%) <0.5	90 (36.7)
No. (%) <0.35	43 (17.6)
Absolute CD8 count[d], x 10^9/L, Median [IQR]	0.35 [0.21-0.62]
No. (%) < normal range (0.2)	56 (22.9)
Absolute CD19 count, x 10^9/L, Median [IQR]	0.11 [0.03-0.23][d]
No. (%) < normal range (0.10)	108 (45.2)[d]
CLL patients only (n=6), Median [IQR]	0.71 [0.42-11.95]
Absolute CD16+CD56[d], x 10^9/L, Median [IQR]	0.13 [0.07-0.21]
No. (%) < normal range (0.09)	78 (31.8)
ALT[d], IU/L, Median [IQR]	24 [19-33]
No. (%) > normal range[e]	39 (16.0)
Bilirubin[d], umol/L, Median [IQR]	6 [5-9]
No. (%) > normal range (21)	5 (2.0)

Legend for Table 4.7:

[a] Includes isolated IgG hypogammaglobulinaemia, IgG subclass deficiency and specific antibody deficiency.

[b] Lymphoma +/- rituximab (n=15), chronic lymphocytic leukaemia (n=6), multiple myeloma or monoclonal gammopathy of uncertain significance (n=7), allograft HSCT (n=3), rheumatoid or vasculitis+/- rituximab (n=10), medication induced (n=6).

[c] Excludes CLL patients (n=6) and missing data for 2 patients (2 further patients missing CD19+ counts).

[d] Missing data for 2 patients.

[e] ULN of ALT varied by year sampled and by gender (women: > 33IU/L in 2015, >35 in 2016/17, men: >41 in 2015, >50 in 2016/17.)

Abbreviations: ALT, alanine aminotransferase; CLL, chronic lymphocytic leukaemia; CVID, common variable immune deficiency; IQR, interquartile range; XLA, X-linked agammaglobulinaemia.

Table 4.8 Iatrogenic immunosuppression in antibody deficient patients

Drug	No.
nil	188
Single agent , n=39:	
Low dose CS only	23
High dose CS only[a]	2
Azathioprine	1
Chlorambucil	1
Dapsone	1
Hydroxychloroquine	3
Lenalidomide	1
Ibrutinib	2
Imatinib	1
MMF	1
Rituximab	1
Sulfasalazine	2
Combination therapy, n=18:	
Infliximab + mesalazine	1
Hydroxychloroquine + dapsone	1
Abatacept + CS	2
Abatacept + Hydroxychloroquine + CS	1
Hydroxychloroquine + CS	2
Lenalidomide + CS	1
MMF + CS	7
MMF+ Hydroxychloroquine + CS	1
MMF + Tacrolimus + CS	1
Rituximab + CS	1
History of rituximab use	10

Iatrogenic immunosuppression in antibody deficient patients at time of HEV RNA testing.

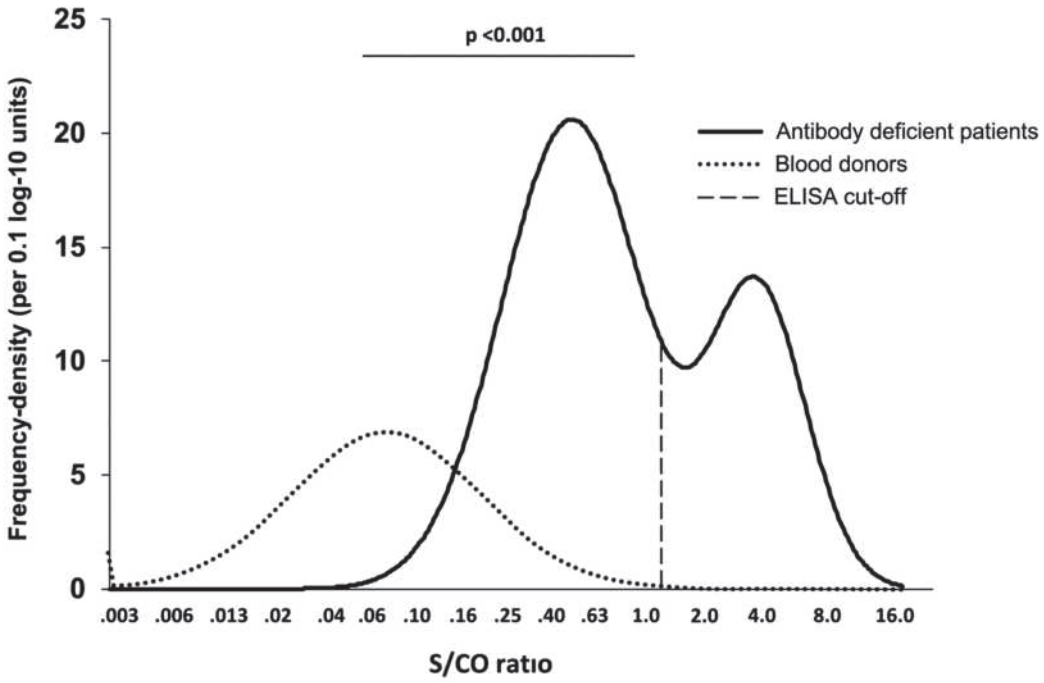
[a] Equivalent to >20mg prednisolone.

Abbreviations: CS, corticosteroids; MMF, Mycophenolate mofetil.

4.2.3.2 HEV markers in antibody-deficiency patients

HEV RNA was not detected in any plasma sample from the 245 patients, confirming that none were viraemic. Anti-HEV IgG was detected (S/CO >1.1) in the plasma from 38.8% of patients. However, the anti-HEV reactivity even in those samples considered negative by the ELISA manufacturers criteria had a significantly different distribution than the reactivity of samples from HEV known-susceptible blood donors (Figure 4.6).

Figure 4.6 Anti-HEV IgG reactivity in antibody-deficient patients compared with HEV-uninfected blood donors



Frequency density plot of anti-HEV IgG reactivity in antibody-deficient patients on immunoglobulin replacement therapy compared with HEV-uninfected blood donors. The lines represent fitted distributions which were a single normal distribution for blood donors and a mix of two normal distributions for the antibody deficient patients. Blood donor samples were archived plasma samples prior to the development of HEV infection to represent true anti-HEV IgG negative samples. There was a significant difference between the median S/CO values in HEV-uninfected blood donors (median, 0.06) and the antibody-deficient patients (median, 0.68) ($p<0.001$). Data were transformed to a log-scale for analysis; the axis labels were back-transformed for presentation. The hatched vertical line represents the manufacturer's cut-off (S/CO 1.1) for positive results.

Abbreviations: ELISA, enzyme linked immunosorbent assay; S/CO, sample over cut-off of optical density.

4.2.3.3 Factors associated with seropositivity for anti-HEV IgG

Comparison of serological reactivity of patients' plasma revealed the type of immunoglobulin product administered was the only statistically significant factor predicting anti-HEV IgG seropositivity ($p<0.001$) (Table 4.9). Specifically, the receipt of Kiovig 10%, Intratect 10% and Intratect 5% was significantly related to the detection of plasma anti-HEV IgG (S/CO >1.1). Patients given Kiovig 10% and those given Intratect 5% were 24 times (95% CI 4.8-122.7) and 136 times (95% CI 2.5-7501) more likely to have detectable plasma anti-HEV IgG when compared to patients who received Flebogamma DIF 5% (Table 4.10). No relationship was seen between the time since immunoglobulin infusion (in days) and the level of anti-HEV IgG detected in the patient's plasma (Pearson's correlation -0.1763, data not shown), even when products were analysed individually. However, the variability of timings was not wide; most patients (77.4%) had received their last dose of immunoglobulin within 20-30 days of testing. Age, sex, underlying diagnosis, receipt of iatrogenic immunosuppression, route of immunoglobulin administration and patients' lymphocyte subset results demonstrated no relationship with anti-HEV IgG seropositivity.

All ten immunoglobulin products tested (8 IVIG and 2 SCIG) contained detectable anti-HEV IgG (range 0.12-7.40 WHO IU/ml) (Table 4.11). There was some evidence of batch-to-batch variation in antibody titre, nevertheless all batches of Kiovig, Gamunex and Privigen were reactive for anti-HEV. The likelihood of a patient being seropositive for anti-HEV IgG strongly correlated with the level of anti-HEV IgG in the product ($p <0.001$, Figure 4.8). No demographic details of donors were available from the product manufacturers.

Table 4.9 Seropositive and seronegative antibody-deficient patients

		Seronegative (n=150)	Seropositive (n= 95)	p value
Age, y, median [IQR]		55.7 [41.2-66.7]	56.1 [41.4-68.8]	0.415
Sex, no (%)	F	96 (64)	58 (61.1)	0.685
	M	54 (36)	37 (38.9)	
Diagnosis, no (%)	CVID	88 (58.7)	50 (52.6)	0.520
	XLA	6 (4)	9 (9.5)	
	Hyper IgM syndrome	1 (0.7)	2 (2.1)	
	Good's syndrome	0 (0)	1 (1.1)	
	Other primary antibody deficiency[a]	29 (19.3)	12 (12.6)	
	Lymphoma +/- RTX	9 (6)	6 (6.3)	
	CLL	3 (2)	3 (3.2)	
	Myeloma or MGUS	4 (2.7)	3 (3.2)	
	Allograft HSCT	2 (1.3)	1 (1.1)	
	Rheumatoid or vasculitis +/- RTX	5 (3.3)	5 (5.3)	
	Medication induced	3 (2)	3 (3.2)	
Iatrogenic immunosuppression, no (%)	nil	117 (78)	71 (74.7)	0.642
	single agent or combination	33 (22)	24 (25.3)	
Product route, no (%)	IV	118 (78.7)	66 (69.5)	0.129
	SC	32 (21.3)	29 (30.5)	
Time since IVIG infusion[b], d, no (%)	<7	0 (0)	1 (2.2)	0.288[c]
	7-14	1 (11.3)	2 (4.4)	
	14-28	67 (76.1)	33 (73.3)	
	28+	20 (22.7)	9 (20)	
Product, no (%)	Flebogamma DIF 5%	33 (22)	6 (6.3)	<0.001
	Gammagard 10%	0 (0)	1 (1.1)	
	Gammanorm 16.5%	3 (2)	7 (7.4)	
	Gammaplex 10%	19 (12.7)	1 (1.1)	
	Gamunex 10%	10 (6.7)	3 (3.2)	
	Hizentra 20%	11 (7.3)	13 (13.7)	
	Intratect 10%	0 (0)	11 (11.6)	
	Intratect 5%	1 (0.7)	5 (5.3)	
	Kiovig 10%	7 (4.7)	19 (20)	
	Octagam 10%	9 (7.5)	6 (6.3)	
	Privigen 10%	39 (32.5)	14 (14.6)	
	Subcuvia 16%	4 (2.7)	9 (9.5)	
	Subgam 16%	14 (9.3)	0 (0)	
IgG in IVIG product[d], S/CO, no (%)	high (IgG >10.0)	51 (41.5)	58 (71.6)	<0.001
	medium (IgG 5-10.0)	22 (17.6)	16 (19.8)	
	low (IgG <5.0)	52 (41.6)	7 (8.6)	
Cell count[e], x10^9/L, median [IQR]	Lymphocytes[f]	1.43 [0.95-1.87]	1.37 [1.03-1.97]	0.832
	CD3+	1.09 [0.74-1.42]	1.075 [0.75-1.54]	0.522
	CD4+	0.60 [0.42-0.83]	0.59 [0.40-0.88]	0.888
	CD8+	0.33 [0.20-0.59]	0.37 [0.24-0.64]	0.307
	CD19+[f]	0.12 [0.04-0.25]	0.10 [0.03-0.21]	0.129
	NK cells	0.12 [0.07-0.19]	0.15 [0.07-0.02]	0.148
	ALT[c], IU/L	23 [19-32]	26.5 [19-36]	0.159
	Bilirubin[c], umol/L	6 [5-9]	6 [5-9]	0.461

Legend for Table 4.9:

[a] Includes isolated IgG hypogammaglobulinaemia, IgG subclass deficiency and specific antibody deficiency.

[b] Data included for patients where the infusion time was known (n=133)

[c] No statistical significance was seen with other time brackets (e.g. <28 /28+d or <21/21-28d/28d+)

[d] Not calculated for 39 patients on products Hizentra, Gammagard and Subgam as products not available for testing.

[e] Data missing for 2 patients.

[f] Excludes CLL patients (n=6) and missing data for 2 patients (2 further data points missing for CD19+ counts).

Continuous data were compared using the Wilcoxon two-sample signed-rank test whilst categorical data was compared using the Fisher's exact or chi squared test.

Abbreviations: RTX, rituximab therapy; CLL, chronic lymphocytic leukaemia; MGUS, monoclonal gammopathy of uncertain significance; HSCT, haematopoietic stem cell transplant.

Table 4.10 Correlation between immunoglobulin product type and patient seropositivity

IVIG Product	Odds Ratio[a]	P value[a]	95% CI[a]
Flebogamma DIF 5%	1.00		
Gammaplex 10%	0.52	0.588	0.05-5.58
Gamunex 10%	1.59	0.644	0.22-11.24
Intratect 10%	incalculable[b]	-	-
Intratect 5%	135.92	0.016	2.46-7500.68
Kiovig 10%	24.21	<0.001	4.78-122.69
Octagam 10%	4.49	0.076	0.85-23.59
Privigen 10%	1.71	0.504	0.36-8.13

Multivariable model examining the relationship between product type administered and a patient testing anti-HEV IgG reactive.

Data only considered if time since infusion was available (n=133). No data were available for Gammagard 10%, Gammanorm 16.5%, Hizentra 20%, Subcuvia 16% or Subgam 16%.

[a] Model adjusted for time since infusion (days).

[b] All patients on Intratect 10% tested anti-HEV IgG positive therefore an odds ratio could not be calculated.

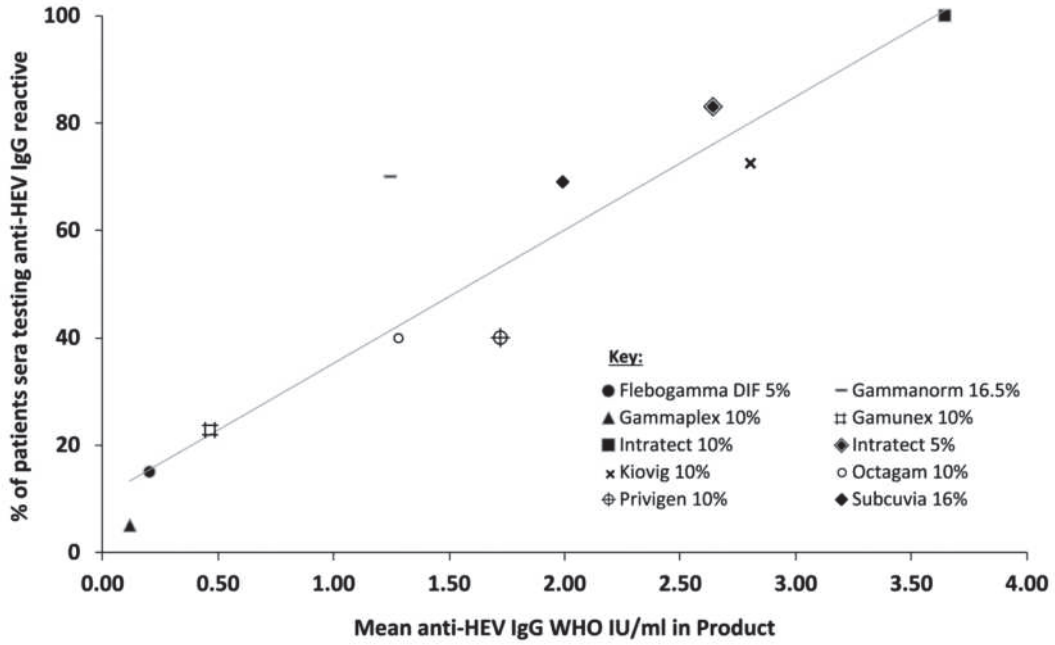
Abbreviations: CI, confidence interval; IVIG, intravenous immunoglobulin.

Table 4.11 Anti-HEV IgG reactivity in immunoglobulin products

	Product	Mean WHO IU/ml batch 1	Mean WHO IU/ml batch 2	Mean WHO IU/ml batch 3
IV	Flebogamma DIF 5%	0.20	-	-
	Gammaplex 10%	0.12	-	-
	Gamunex 10%	0.47	1.09	0.58
	Intratect 10%	3.65	-	-
	Intratect 5%	2.64	-	-
	Kiovig 10%	2.79	7.40	3.95
	Octagam 10%	1.28	-	-
	Privigen 10%	1.72	0.58	0.83
SC	Gammanorm 16.5%	1.25	-	-
	Subcuvia 16%	1.99	-	-

Ten immunoglobulin products, including three different batches for three products (Gamunex 10%, Kiovig 10% and Privigen 10%), were tested in duplicate for anti-HEV IgG. A mean was calculated and then each product ascribed a unitage of WHO IU/ml.
Abbreviations: IV, intravenous; SC, subcutaneous; WHO; world health organisation; IU, international units.

Figure 4.7 Correlation of patient seropositivity and anti-HEV IgG concentration in the immunoglobulin product



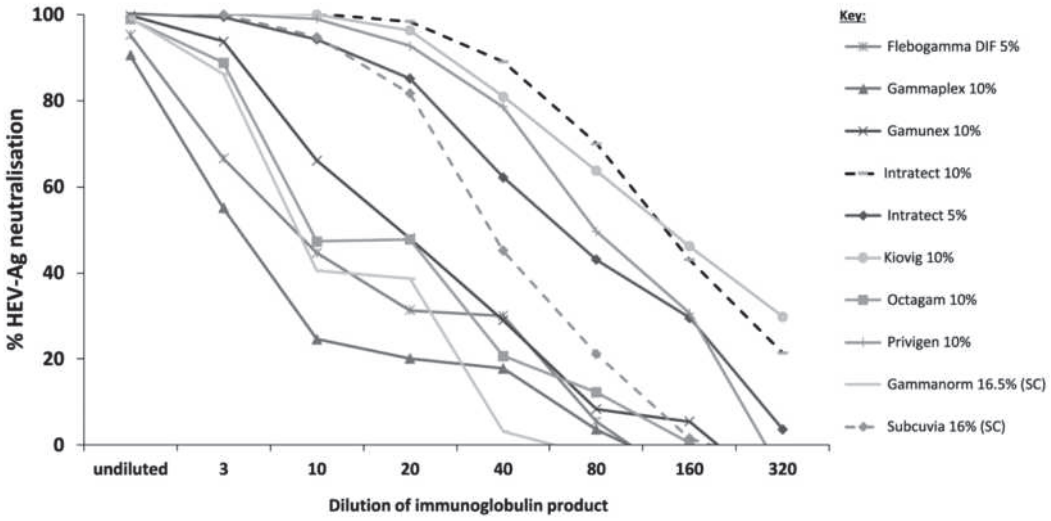
Correlation of the percentage of patients on each product testing anti-HEV IgG reactive and the IgG level (WHO IU/ml) detected in the product. Ten immunoglobulin products were tested in duplicate for anti-HEV IgG. The ascribed level of WHO IU/ml was plotted against the proportion of patients on each product testing anti-HEV IgG reactive (S/CO >1.1) and showed a good correlation (Pearson's correlation 0.900, p = 0.0004). The lowest result for any product was still reactive for anti-HEV IgG by manufacturer's criteria (mean S/CO of 2.44 for Gammaplex 10% equivalent to 0.12 WHO IU/ml).

Abbreviations: IV, intravenous. SC, subcutaneous. IU, international units.

4.2.3.4 HEV ORF2 antigen neutralising activity of patient plasma samples and immunoglobulin products

All ten immunoglobulin products were also able to neutralise HEV-Ag expressed in tissue culture and prevent reactivity in the HEV-Ag assay. Neutralising activity was detectable at a high level in half of the products tested even at a dilution of 1:20 (Figure 4.9). The extent of neutralisation correlated with the anti-HEV IgG S/CO value (Figure 4.10). In all nine patients, tested before and after infusion, the levels of anti-HEV IgG detected were higher in post-infusion samples (pre-infusion median S/CO 0.90; post-infusion median S/CO 1.96, p=0.008) and correlated with higher neutralising activity in eight of the nine patients (p=0.015) (Table 4.12).

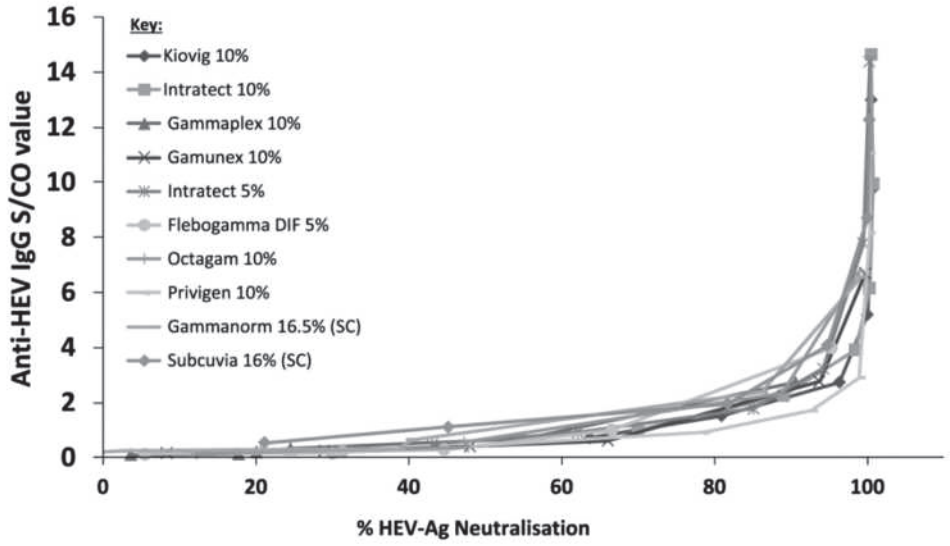
Figure 4.8 HEV-Ag neutralising activity of immunoglobulin products



HEV-Ag neutralising activity of immunoglobulin products at differing dilutions. Ten different immunoglobulin products were titrated in normal human plasma up to 1:320 and tested for the presence of antibodies capable of neutralising HEV-Ag derived from cell culture. The percentage of neutralisation was calculated as a percentage of the reduction in binding (Methods 2.2.2.7).

Abbreviations: HEV-Ag, hepatitis E virus antigen. SC, subcutaneous.

Figure 4.9 Correlation of Anti-HEV IgG level and HEV-Ag neutralisation



Abbreviations: HEV-Ag, hepatitis e virus antigen; SC, subcutaneous; S/CO, sample over cut-off value of optical densities.

Table 4.12 Pre and Post IVIG anti-HEV IgG levels and neutralisation

Pt no.	IVIG Product infused	Pre-IVIG		Post-IVIG	
		anti-HEV IgG S/CO	% Neutralisation	anti-HEV IgG S/CO	% Neutralisation
1	Privigen 10%	1.27	82.29	2.35	90.97
2	Intratect 10%	11.62	99.13	14.15	99.65
3	Privigen 10%	6.10	98.44	7.60	97.57
4	Kiovig 10%	0.36	36.98	3.02	97.92
5	Flebogamma DIF 5%	0.41	57.64	1.77	90.46
6	Privigen 10%	0.90	73.63	1.39	87.38
7	Gammaplex 10%	0.28	42.78	0.81	74.19
8	Gamunex 10%	0.76	47.27	1.96	95.23
9	Privigen 10%	1.05	74.76	1.75	81.49

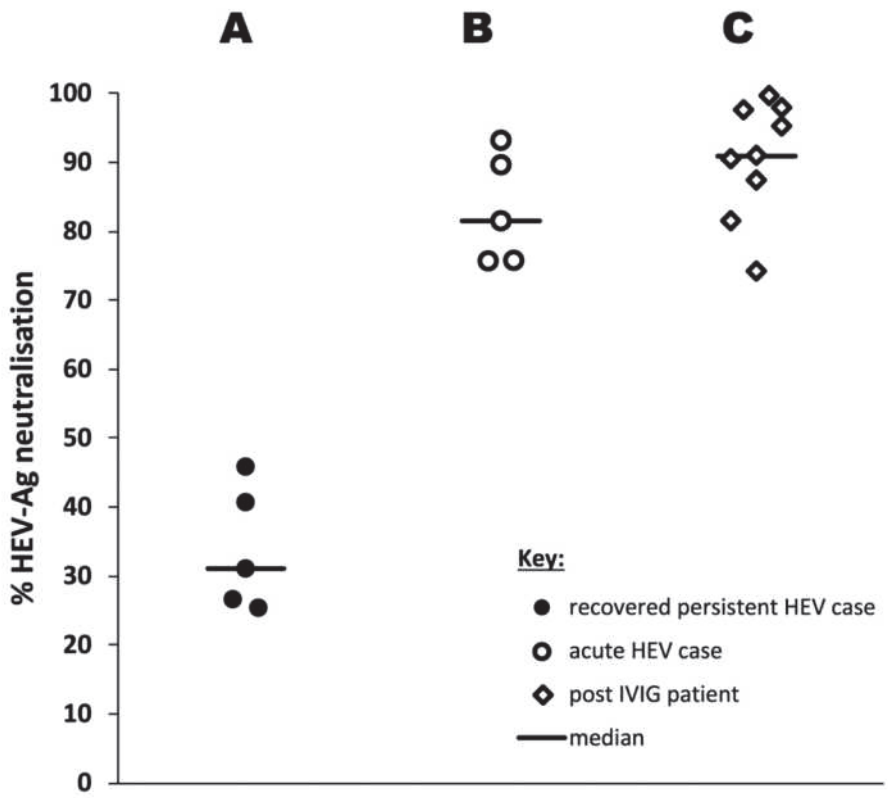
Nine patients were tested for anti-HEV IgG and the presence of HEV-Ag neutralising antibodies prior to IVIG infusion and immediately post infusion. Anti-HEV IgG rose in all nine patients (median OD change 0.2 IQR [0.1-0.3], p=0.008) and neutralising activity rose post-infusion in 8/9 patients (median change in % of neutralisation 13.7 IQR [6.7-32.8], p=0.015) (Wilcoxon signed-rank test).

Abbreviations: HEV-Ag, hepatitis E virus antigen; IVIG, intravenous immunoglobulin.

4.2.3.5 HEV antigen neutralising capacity in sera post-IVIG in comparison to sera from patients recovered from acute and persistent HEV infection

Five plasma samples from acute HEV cases and five from persistent HEV cases recently recovered after ribavirin therapy were tested for anti-HEV IgG in a half-log dilution series alongside the WHO antibody reference. A dilution of each sample containing equivalent levels of anti-HEV IgG (between 2 and 5 WHO IU/ml) was then tested for presence of HEV-Ag neutralising antibody. The neutralising capability was significantly higher in plasma samples from healthy patients recovered recently from acute HEV infection (median 81.5%, range 75.7-93.2%) compared with plasma from patients recovered from persistent HEV infection (median 31.1%, range 25.4-45.8%) (p=0.009) (Figure 4.10). A direct comparison of neutralising activity at the equivalent level of anti-HEV IgG in plasma from patients post-IVIG was not possible due to much lower anti-HEV in the latter (median S/CO 1.96, equivalent to ~0.2 WHO IU/ml). However, neutralising activity was broadly similar between post-IVIG patient samples (median 91.0%, range 74.2-99.7%) and plasma from acute cases (median 81.5%, range 75.7-93.2%) despite much lower anti-HEV IgG detected.

Figure 4.10 Comparison of HEV-Ag neutralisation by plasma from acute clinical cases of HEV, recovered persistent cases of HEV and patients post-IVIG



The neutralising activity of plasma samples diluted to have an equivalent level of anti-HEV IgG (between 2 and 5 WHO IU/ml) from (A) patients recently recovered from persistent HEV infection (median 31.1%, range 25.4-45.8%) was significantly lower than plasma samples from (B) patients in recovery from acute HEV infection (median 81.5%, range 75.7-93.2%) (p=0.009). Plasma samples from (C) post-IVIG patients were tested undiluted as they had lower anti-HEV IgG (median 0.22 WHO IU/ml, range 0.05-15.9) and therefore could not be diluted to comparable level of anti-HEV IgG, but still exhibited high neutralising activity similar to acute HEV cases (median 91.0%, range 74.2-99.7%).

4.3 Discussion

4.3.1 HEV infection in transplant recipients

The current study is the largest prevalence study of HEV viraemia in a transplant cohort and the first in the UK. There was a low but significant HEV RNA prevalence of one in 149 (0.67%), similar to studies in other European countries [185, 192, 193, 306, 307]. Studies from the UK published since the current study have found a prevalence of 0.20% amongst 490 liver transplant recipients tested in 2017 and 0.46% amongst 870 transplant recipients (kidney, liver and HSCT) sampled between 2013 and 2015 [295, 308].

During the time period of sampling the prevalence of HEV viraemia amongst blood donors in England was 1:1875 (0.05%) and in effect reflects the incidence rate for HEV infection in the English population (national screening of donors Feb-Sep 2016, England. NHSBT/PHE Epidemiology Unit, pers. comm. Dec 2016). The 12-fold higher prevalence rate in the transplant patients represents a cumulative prevalence of infection as a consequence of the failure to clear infection during iatrogenic immunosuppression.

The current study represents a single transplant centre experience in Birmingham where 96% of tested patients resided within a 100-mile radius. HEV exposure has been shown to vary geographically in some regions, such as southern France, considered to reflect regional dietary variation [40]. In England this heterogeneity is not evident; ongoing seroprevalence studies in English blood donors do not show marked variation by region (Steve Dicks, pers. comm.,

NHSBT, March 2017), therefore these data are likely to be representative of other transplant cohorts across the UK.

Higher tacrolimus and ciclosporin levels were found in the infected patients which would suggest that lower levels are associated with spontaneous clearance of viraemia. However, no association was found between different SOT categories even though they have different levels of immune suppression. The data suggested the possibility that HSCT patients, having a higher exposure to blood products, are more at risk of HEV infection (3/149, 2%). A larger population size may have reached statistical significance, but the patient selection criteria of those undergoing TDM will have biased the study to more heavily immunosuppressed HSCT patients by recruiting those closer to transplantation or with concomitant GvHD. However, any increased risk for this group is likely to diminish in the UK following the introduction of universal HEV screening of blood donations.

Crucially, 18 of the 19 HEV infections were previously undiagnosed, clearly showing that the burden of HEV infection in immunosuppressed patients is under-appreciated and relying on clinical suspicion to consider the diagnosis is insufficient. In four patients the derangement of liver enzymes was not considered significant for further evaluation and in at least 11 cases, alternative diagnoses were suggested, including many in which diagnoses where increasing immunosuppression would be a potential intervention. Such treatment would have the potential to amplify viral replication and exacerbate HEV disease.

The use of serology to diagnose chronic HEV infection in immunocompromised patients has been reported to be insufficient due to a delayed serological

response [216, 309]. In the current study 17/19 (89.5%) of viraemic patients had a detectable serological response (plasma IgM and IgG reactive or IgM reactive only) at the time of screening. This was higher than expected but due to the lack of stored samples for retrospective testing it is not possible to comment on whether these serological responses were significantly delayed in individual patients.

Plasma ALT levels were significantly higher in viraemic patients, however two HEV viraemic patients were identified who had normal ALT results. Both patients were likely to be in the early phase of infection as both returned elevated ALT levels when re-tested 29-30 weeks later with rising HEV RNA levels. If HEV screening was only performed on patients with an abnormal ALT it would have reduced the numbers of patients screened by over six-fold; from 2822 to 467 but at the expense of a reduced sensitivity (88.2%) through failing to identify two infections. Raising the ALT threshold any higher than the upper limit of normal only reduced sensitivity and did not increase the PPV of the patient being HEV viraemic.

The strengths of this study were the unbiased nature of testing, the size of the population and the range of different transplant populations screened. A major limitation was the single timepoint of testing at varying lengths of time post-transplant which rendered it difficult to ascribe specific infection risks, such as risk from blood components around transplant or accumulated dietary risk over time. The lack of stored samples prevented the determination of the duration of infection prior to screening which would have informed the data on ALT levels.

4.3.2 HEV infection in patients with haematological malignancies

An increasing number of reports describe persistent HEV infections in the context of haematological malignancy yet there are no systematic prevalence studies outside the HSCT setting. Haemato-oncology patients are of particular interest due to their immunosuppressed state with high transfusion requirements. In the current study, predominantly of patients with lymphoma and plasma cell dyscrasias, there was a very low prevalence of HEV viraemia (0.13%, 95% CI 0.015-0.45%), only slightly higher than found in healthy blood donors (0.04%) [22].

The rarity of active HEV infection in patients undergoing non-transplant chemo/immunotherapies is reassuring and does not suggest the need for routine screening of all patients. In comparison, prevalence rates of HEV viraemia as high as 2.4% are reported in allogeneic-HSCT recipients [194]. Patients with haematological malignancy outside allogeneic stem cell transplantation are a heterogeneous group of patients with immunosuppression that varies considerably throughout a therapeutic schedule. In the absence of T-cell subsets and immunoglobulin levels we characterised immunosuppression using surrogates of recent treatment history and absolute neutrophil and lymphocyte counts. A significant number (232 patients) had received moderate to highly intensive chemotherapy in the preceeding six months and nearly a third of patients were lymphopenic. However, many patients were on relatively novel agents and the degree of immunosuppression induced by these agents is not known. Of note is that bendamustine has recently been proposed as a risk for

persistent HEV; in our cohort only 23 patients had received bendamustine in the preceeding 6 months before testing [299].

Both HEV viraemic patients identified were under active treatment for multiple myeloma (2/521, 0.38%) and both developed persistent HEV infection which may suggest an increased risk of developing persistent HEV in multiple myeloma patients compared to other malignancies. This is in contrast to findings from a retrospective multicentre cohort study of 50 patients with HEV virus infection and haematological malignancy which found most patients had underlying non-hodgkin's lymphoma; only 6% in that cohort had underling multiple myeloma [310]. However, we also observed a low anti-HEV IgG seroprevalence in patients with plasma cell dyscrasias and patients with increasing numbers of lines of treatment, even after correcting for other factors in multivariable analysis. This could be due to lower prior HEV exposure, but plausibly the loss of anti-HEV IgG related to underlying disease and treatment; either would likely render this group more susceptible to primary HEV infections or even reinfection [311].

In the three month follow-up period both viraemic patients had normal ALT values at most time points. This makes it difficult to diagnose HEV infections clinically in such patients. The absence of raised liver enzymes is particularly intriguing; most cases of persistent infection described have modestly raised liver enzymes, which may be due to ascertainment bias. The significance of HEV viraemia in the absence of raised liver enzymes is unknown, particularly with regard to the risk of chronic liver disease and merits further study.

All patients were tested for anti-HEV IgG to characterise HEV exposure. The most influential factors in the multivariable analysis influencing IgG seroreactivity were

increasing age and the underlying haematological diagnosis. A linear relationship was observed between the numbers of transfusions received and the likelihood of being IgG seroreactive, which remained even after controlling for a recent transfusion, suggesting transfusional HEV acquisition. However, the lack of current active infections indicates that HEV clearance is the norm. Neither the source nor the timing of HEV infection in patients seroreactive for anti-HEV IgG could be ascertained.

There are several limitations to this current cross-sectional study. The study reflected a typical patient balance in a large tertiary haemato-oncology unit, thereby providing a clinically relevant insight into HEV risk in this cohort, however due to the relative rarity of certain conditions, such as MDS (n=77, it was not possible to test large numbers with specific diseases. Logistical challenges prevented the collection of patients' transfusion history at other hospitals outside the tertiary centre, therefore there could have been an underestimation of the transfusion burden in those who receive care (including transfusions) at their local hospital. Finally, dietary acquisition was not subject to systematic assessment and a formal look-back was not undertaken on the two HEV infected patients to ascertain the source of infection.

4.3.3 HEV infection in patients with antibody deficiency

In the current study no cases of persistent HEV infection were identified in a cohort of 245 patients with primary or secondary antibody deficiency, despite evidence of significant immunocompromise. Many patients had low CD19+ B-cell counts (45% <0.10 x 10^9/L), CD4+ T-cell counts (37% <0.5 x 10^9/L) and nearly a

quarter were also taking immunosuppressive medication. This is a significant finding in the current context of a high risk of HEV acquisition from dietary sources in the UK and the longstanding immune dysfunction in patients with antibody deficiency which renders them susceptible to a number of persistent and severe viral infections [15, 102, 301-304, 312, 313]. The results are consistent with studies in similar cohorts, neither of which displayed any evidence of persistent HEV infection amongst 73 CVID patients in Germany or 27 primary antibody deficient patients with deranged liver enzymes in the UK [300, 305]. The current study, which was larger and more heterogeneous, adds to the growing body of evidence that there is an extremely low risk of persistent HEV infection in these groups of patients. However, even larger studies may be required to detect a very small risk of HEV infection given that the prevalence of HEV viraemia in cohorts considered high risk for persistent HEV infection, such as solid organ transplant recipients in Western Europe is low (0.7-1.5%) [185, 192, 193, 306, 307]. Patients were tested for HEV RNA regardless of liver enzyme values because of the uncertainty of whether an abnormal ALT would be a sufficiently sensitive predictor of active HEV infection; at the time of HEV testing 16% of patients had abnormal ALT values.

The reason for the absence of persistent HEV infection in these patients could be due to a number of factors. Anti-HEV IgG was detectable in a high proportion of patient's plasma in this study (38.8%). Many plasma samples testing seronegative by the ELISA manufacturers' criteria nevertheless had higher anti-HEV IgG optical density values compared with plasma from blood donors known to be susceptible to HEV infection. This suggests the presence of low levels of

anti-HEV IgG in the patient samples but where the ELISA reactivity falls below the manufacturers' defined cut-off. Detectable anti-HEV IgG in plasma of antibody deficient patients could be the result of passive acquisition of antibodies from the immunoglobulin products, residual endogenous antibody production or a combination of the two. The most compelling explanation is passive acquisition of anti-HEV, supported by the correlation of patient plasma reactivity for anti-HEV IgG with both receipt of certain immunoglobulin products and the titre of anti-HEV IgG in those products. Rising titres of anti-HEV IgG observed post-IVIG in patients tested before and after infusion reinforce this assertion.

In this study it has been shown that patients' plasma and immunoglobulin products were able to bind to HEV ORF2 Ag and prevent or reduce reactivity in the HEV-Ag assay, referred to as antigen neutralisation. This antigen neutralising activity also rose in post-IVIG infusion samples in the subset of patients tested concordantly with rises in anti-HEV IgG levels. Recent studies exploring different forms of ORF2 antigen suggest that the antigen neutralisation we are detecting may be directed predominantly against the abundant secreted form of ORF2 which is not virion-associated [141, 291]. Nevertheless, this secreted form of ORF2 is still considered to harbour the major neutralizing epitopes found on the capsid form of ORF2 [141, 314]. The presence of pre-existing antigen neutralising antibodies circulating in their plasma may be sufficient to protect these patients from an enteric challenge of HEV and prevent early infection or the establishment of persistent infection.

It is notable that the source of anti-HEV IgG appears to influence the antigen neutralising capacity. It is well documented that patients with persistent infection

themselves produce anti-HEV antibodies without eliminating the virus, therefore plasma from five acute HEV cases and five recovered persistent HEV cases were tested for the ability of the detectable anti-HEV antibody to neutralise the tissue culture-derived antigen [168]. Antibodies from recovered persistent HEV cases had significantly lower antigen neutralising capacity when compared with acute HEV cases when tested at equivalent levels of anti-HEV IgG (between 2 and 5 WHO IU/ml). Due to lower anti-HEV IgG titres in the plasma from patients following IVIG infusions (7/9 samples had <1 WHO IU/ml) it was not possible to perform a direct comparison at an equivalent WHO unitage; despite this, the antigen neutralising activity was similar in post-IVIG samples compared to plasma from recovered acute cases. This higher antigen neutralising activity relative to the detected anti-HEV IgG titre may explain why these patients either do not develop initial HEV infection or do not develop persistence despite comparatively low concentrations of anti-HEV detected by ELISA.

The current study is unable to prove that passively transferred HEV antibodies are protective for these patients, since correlates of protection against HEV infection are undefined, even in vaccine studies [315]. However, as proof-of-concept, late stage convalescent plasma has been used successfully to prevent cynomolgus monkeys from developing hepatitis after an HEV challenge, therefore IVIG may prove useful for prevention of HEV infection [241]. Indeed IVIG has shown promise as a therapeutic agent in a small number of cases of HEV-associated neuralgic amyotrophy [316]. The recent description of a model for HEV infection using humanized homozygous uPA+/+-SCID mice may enable passive immunoprophylaxis to be studied further [317]. An alternative explanation

for the lack of persistent HEV infections in this study is that enough of the patients had sufficient preserved T-cell activity to clear HEV following infection. Suneetha *et al* have demonstrated the importance of T-cell responses for control of HEV infection [222]. The initial description of persistent HEV infections in solid organ transplant recipients found significantly lower total lymphocytes, CD2+, CD3+ and CD4+ cell counts in individuals developing persistent infection compared to those who resolved HEV infection [168]. In the current study 77% of patients had normal levels of CD8+ T-cells (>0.2 x 10^9/L) and higher median total lymphocyte count, CD3+ and CD4+ cell counts than found by Kamar *et al* in persistently infected transplant patients [168]. However, T-cell deficiency and iatrogenic immunosuppression were not uncommon in the current study and even in this subset no viraemic cases were detected.

4.3.4 Summary discussion

The aim of this chapter was to determine the point prevalence of HEV viraemia in three distinct immunocompromised cohorts and identify predictive factors of viraemia to inform testing strategies. There was a significant difference in the prevalence of HEV viraemia in the cohorts tested: 0.67% (95% CI 0.43-1.05%) in transplant patients undergoing therapeutic drug monitoring, 0.13% (95% CI 0.015-0.45%) in haemato-oncology patients under follow-up and 0% HEV RNA prevalence (97.5% one sided CI 0-1.49%) in antibody-deficient patients treated with replacement immunoglobulin therapy (fisher's exact test, p = 0.029). A number of factors including sample sizes, differing HEV risk through diet and transfusion and differing host susceptibility to supporting persistent HEV infection

may account for these differences. For example, the haemato-oncology cohort, due to the nature of patient selection were more heterogeneous in their immunosuppressive status compared with solid organ transplant patients on calcineurin inhibitors.

The low prevalence of active HEV infection made it challenging to identify predictive risk factors for viraemia. Viraemic patients in the transplant cohort had higher plasma levels of immunosuppressive drug (tacrolimus or ciclosporin), ALT and bilirubin compared with aviraemic patients. There were no demographic or clinical parameters that could be identified which increased the risk of being HEV viraemic. Amongst haemato-oncology patients ALT was not raised in either viraemic individual. Both patients had underlying Multiple Myeloma on high dose steroid treatment, however no other unique parameters were evident. It is difficult therefore to recognise HEV infected individuals amongst immunocompromised cohorts; in the transplant cohort in only one of 16 was HEV considered in the differential diagnosis of their abnormal ALT.

These three studies were designed in different ways reflecting practicalities in patient recruitment and data collection at the different centres. This introduces bias and hinders comparisons. For example, T-cell subsets are routinely measured at immunology outpatient visits, therefore, were available for patients with antibody deficiency but are not routinely measured regularly for transplant recipients or haemato-oncology with haematological malignancy. Nevertheless, the data from this study raise the question of whether structured systematic screening of transplant recipients for HEV infection by RNA testing or other virus-specific methods should be considered.

Notwithstanding the provision of HEV-screened blood for all patients in the UK, the predominant risk of HEV acquisition for the majority of patients is dietary so persistent infections will continue to occur [15, 102]. Therefore, further work on elucidating the relative importance of T-cell and B-cell responses for HEV clearance are needed to help stratify HEV risk in these and other immunocompromised cohorts. This will enable more targeted studies of patient groups on specific immunosuppressives or with specific immunological defects.

4.4 Conclusions

- Transplant recipients in the UK are at a low but significant risk of HEV infection. The majority of these infections go unrecognized despite increased awareness among the scientific and medical community.
- Patients with underlying haematological malignancy are also at risk of persistent HEV infection but the risk appears lower than amongst transplant recipients.
- We found no evidence of persistent HEV infection amongst a cohort of patients with primary and secondary antibody deficiency. The HEV-Ag–neutralizing antibodies detected in both immunoglobulin products and patients' plasma specimens may provide sufficient protection from developing HEV infection.

5 Demography, virology and outcomes of persistent HEV infection across England and Wales

5.1 Introduction

The understanding of the natural history and clinical outcomes of persistent HEV infections is derived from a number of case series, with most patients from hyperendemic areas of Southern France and provides a basis for the clinical monitoring and treatment of persistent HEV infection in SOT recipients [168, 207, 223]. The earliest descriptions of persistent courses of HEV infection were in SOT recipients, but have since been followed by case reports or small case series in other immunocompromised patient groups [168, 170, 194, 297, 299, 318, 319].

There is a significant knowledge gap with respect to the natural history of persistent HEV infection outside the transplant setting. Most studies to date are single-centre studies with a bias towards specific patient cohorts,without a broader perspective on the risk of HEV in all immuncompromised cohorts.

The blood borne virus unit (BBVU) within the virus reference department (VRD) at the National Infection Service, Colindale, Public Health England (PHE) has provided HEV RNA testing as a clinical service since 2003. In the same year PHE (formerly the Health Protection Agency) initiated a programme of enhanced surveillance for acute hepatitis E and within the scope of this research work, an enhanced surveillance system for persistent HEV infections across England and Wales has been established (see materials and methods). The first case of persistent HEV infection in the UK was recognised in an HIV infected patient in

2009 and since that time the numbers of recognised cases has steadily risen. The aims of this chapter are:

1. to establish an enhanced surveillance system for persistent HEV infections across England and Wales.
2. to identify patients identified by the national infection service who fulfil the criteria for persistent HEV infection.
3. to describe the demographics, virology, serological responses and clinical outcomes of persistent HEV infections across England and Wales

5.2 Results

5.2.1 Clinical case definitions and terms

For inclusion in this descriptive case series a persistent HEV infection was defined as a detectable plasma HEV RNA for more than 12 weeks. Virological outcomes were categorised as:

- viral clearance in stool: a minimum of one HEV RNA negative stool sample with no subsequent evidence of rebound;
- viral clearance in plasma: a minimum of one HEV RNA negative plasma sample when a stool sample was not available for testing and no subsequent evidence of rebound;
- ongoing viraemia: when the last sample demonstrated ongoing viraemia within the last 12 months;
- lost to follow up viraemic: when last sample demonstrated viraemia but no sample tested for over 12 months;
- death with viraemia: last sample prior to death was positive for HEV RNA with no evidence of recent change in viral load;
- rebound: viral recrudescence after treatment cessation (either no testing done at time of cessation or evidence of ongoing viraemia).

The term rebound is used as a broad term to describe any patient with an increase in viral load (plasma or stool) following cessation of therapy. However, relapse in this study is restricted to patients with HEV-RNA-negative samples (plasma sample and/or stool) at time of treatment cessation who subsequently developed a second period of detectable HEV viraemia. A sustained virological

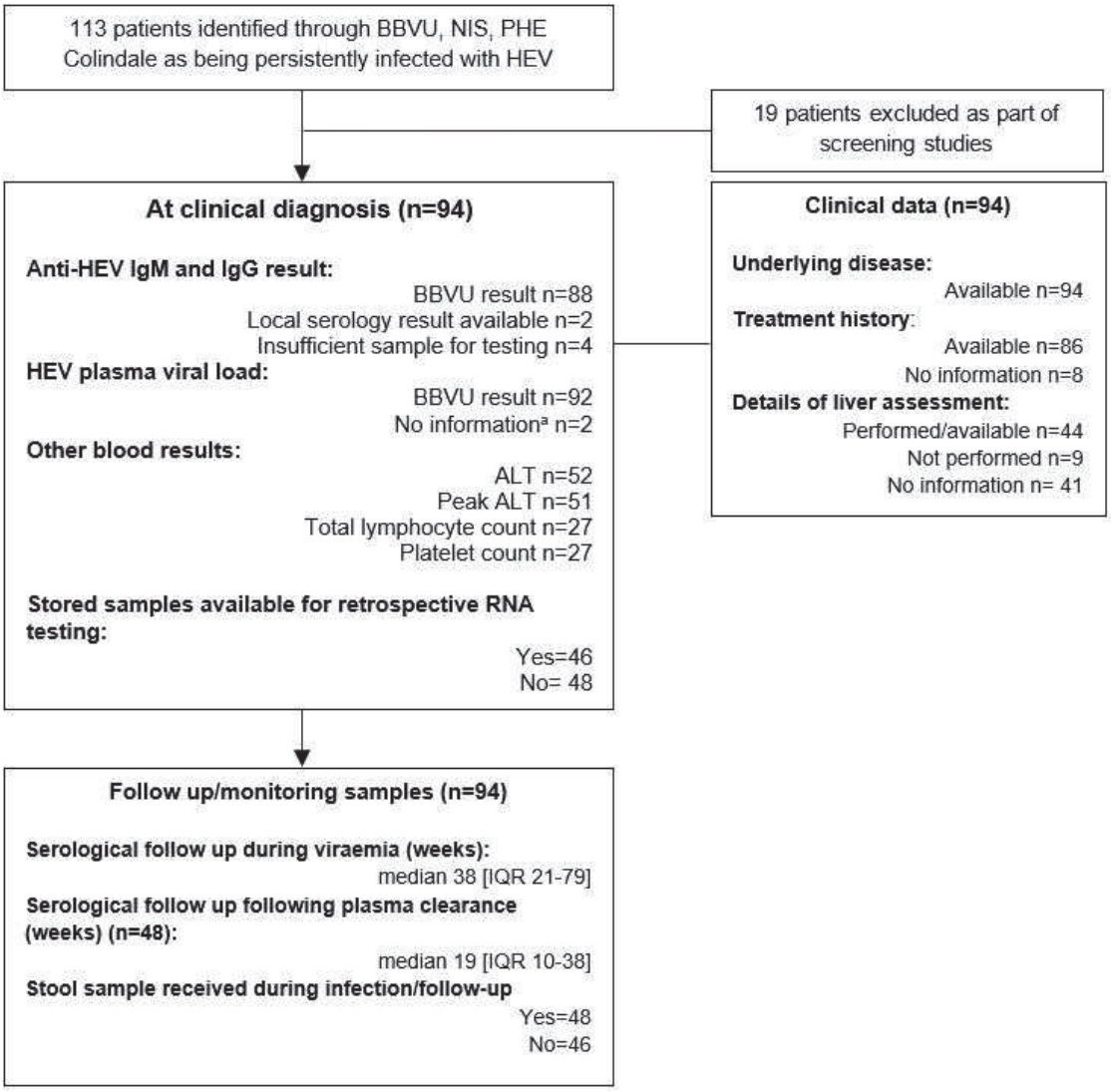
response (SVR) was defined as an undetectable HEV RNA in plasma and/or stool at 12 weeks (SVR 12) or 24 weeks (SVR 24) following the end of treatment.

5.2.2 Cases of persistent Hepatitis E diagnosed between 2009 and 2017

A total of 113 patients with persistent HEV infection were identified between January 2009 and December 2017. Of these, 19 cases were excluded as they were identified through separate screening studies. Demographic, Clinical, serological and molecular results were available for most patients (Figure 5.1). From 2009 there has been a steady increase in the numbers of diagnosed cases each year with a peak of 25 cases in 2016 (Figure 5.2).

The characteristics of the 94 patients with persistent HEV infection (median duration of demonstrable infection 45.5 weeks [IQR 22.3 – 89.8 weeks]) are summarised in Table 5.1. Male cases predominated (64%) with a median age of 52 years old. Whilst 66 cases were recipients of transplants (56 SOT and 10 haematopoietic stem cell transplant (HSCT) recipients), 16 patients had an underlying haematological malignancy without HSCT, six had underlying advanced HIV infection, four had auto-immune disease or other immunosuppression and one patient had no identified immunosuppression. The six patients with underlying HIV infection all had CD4 counts below <250 cells/mm3 (range 17-207 cells/mm3) at the point of HEV diagnosis with very low nadir CD4 counts (range 2-66 cells/mm3).

Figure 5.1 Available samples and clinical information from patients with persistent HEV infection

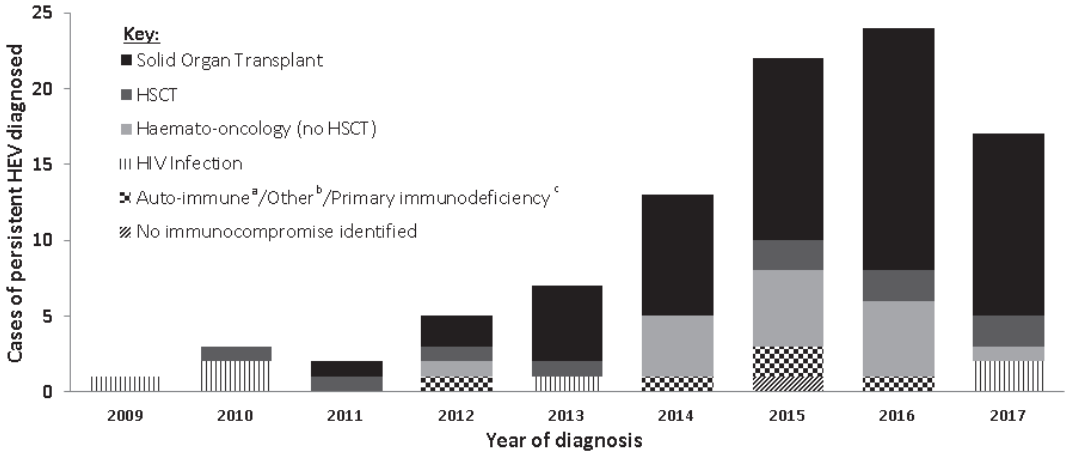


Flow chart of available diagnostic and follow-up samples and clinical information from patients identified through the NIS with persistent HEV infection, 2009-2017.

[a] No plasma viral load available due to the sample being tested prior to the introduction of a quantitative Taqman PCR (n=1) or to the first sample received being stool (n=1).

Abbreviations: ALT; alanine aminotransferase; BBVU, blood borne virus unit; IQR, interquartile range; NIS, national infection service; wks, weeks.

Figure 5.2 Cases of persistent HEV infection by year and diagnosis



Cases of persistent HEV infection across England & Wales by year and diagnosis.

Breakdown of the underlying diagnosis of patients with persistent HEV infection by year.

[a] Includes ANCA-positive vasculitis (n=2) and rheumatoid arthritis (n=1).

[b] Includes neurosarcoidosis (n=1).

[c] Includes severe combined immunodeficiency (SCID; JAK3 mutation, n=1).

Abbreviations: HSCT, haematopoietic stem cell transplant; HIV, human immunodeficiency virus.

Table 5.1 Characteristics of patients with persistent HEV infection

Characteristic		No. (%)
Sex		
	Male	60 (63.8)
	Female	34 (36.2)
Age, y median [IQR]		52.1 [36.7-63.5]
	0-20	4 (4.3)
	21-40	25 (26.6)
	41-60	34 (36.2)
	>60	31 (33.0)
Underlying immunocompromise		
	SOT:	56 (59.6)
	Kidney	35 (37.2)
	Liver	13 (13.8)
	Pancreas +/- kidney	4 (4.3)
	Heart +/- kidney	2 (2.1)
	Lung	2 (2.1)
haematological malignancy without HSCT:		16 (17.0)
	Lymphoma	12(11.7)
	CLL	2 (2.1)
	AML	1 (1.1)
	T-PLL	1 (1.1)
HSCT:		10 (10.6)
	Allograft	9 (9.6)
	Autograft	1 (1.1)
	HIV	6 (6.4)
	auto-immune/other immunosuppressives [a]	4 (4.3)
	primary immunodeficiency [b]	1 (1.1)
	no immunocompromise identified [c]	1 (1.1)
Duration of infection, wks median [IQR]		**45.5 [22.3-89.8]**

[a] Includes rheumatoid arthritis (n=1) and ANCA-vasculitits (n=2) and neurosarcoidosis (n=1).

[b] Patient with severe combined immunodeficiency (SCID; JAK3 mutation).

[c] Extensive (immunological) investigations have not identified an underlying immunological disorder/immunodeficiency.

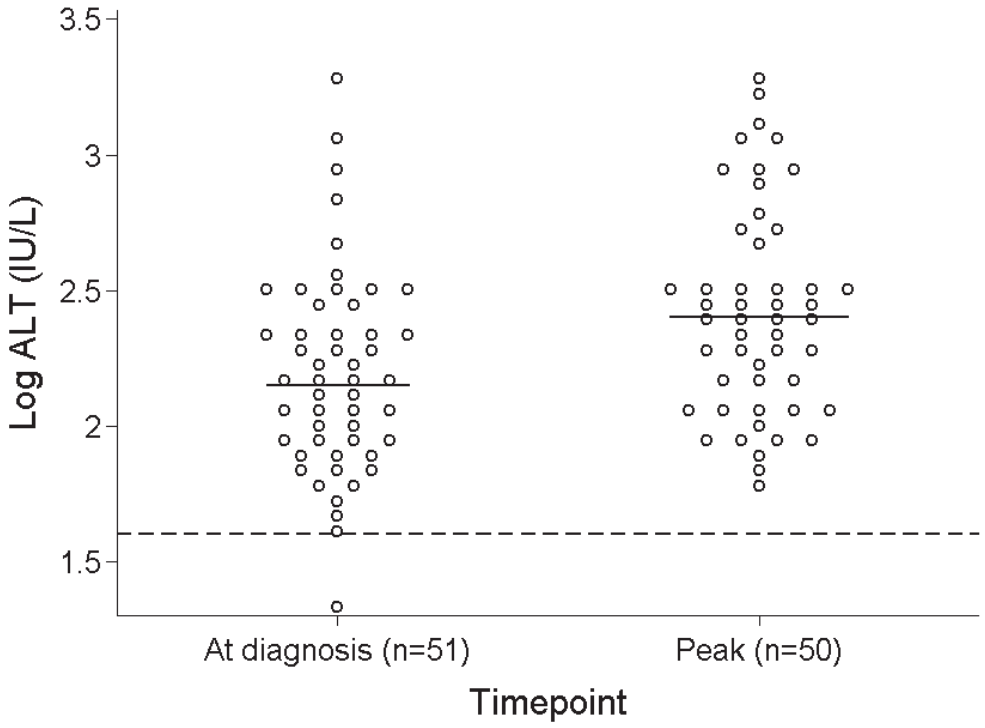
Abbreviations: SOT, solid organ transplant; CLL, chronic lymphocytic leukaemia; AML, acute myeloid leukaemia; T-PLL, T-cell prolymphocytic leukaemia.

5.2.3 Blood markers and liver disease at diagnosis

At the point of diagnosis, the median plasma total lymphocyte count was 1.0 x 10^9/L [IQR 0.6-1.5] (n=27) (lower limit of normal, 1.0 x 10^9/L) and the median platelet count was 208 x 10^9/L [IQR 158-246] (n=27) (lower limit of normal, 150 x 10^9/L). The median ALT at diagnosis was 145 IU/L [IQR 89-278] (n=52); and the peak recorded ALT was a median 260 IU/L [IQR 132-526] (n=51) (Figure 5.3).

Of 53 patients with details of baseline hepatic investigations, 44 underwent assessment by liver biopsy and/or imaging (ultrasound or hepatic transient elastography (Fibroscan®)). Eleven patients had advanced liver disease; either histological evidence of cirrhosis on biopsy or ultrasound (n=9) or a high hepatic stiffness measured by shear wave velocity elastography score (n=2) equivalent to METAVIR F4 (Table 5.2). These patients had been viraemic for a median duration of 64 weeks [IQR 26-80] prior to assessment (n=9). Eleven patients had evidence of mild to moderate hepatic inflammation and/or fibrosis and 22 patients had no evidence of significant liver disease.

Figure 5.3 Liver enzyme values in persistent HEV infection



ALT values at the point of diagnosis and the peak ALT value recorded for patients with persistent HEV infection.

The solid horizontal lines represent median values and the dashed horizontal line represents an ALT value of 40 IU/L illustrating a typical laboratory upper limit of normal (ULN).

Abbreviations: ALT, alanine aminotransferase.

Table 5.2 Liver imaging in persistent HEV infection

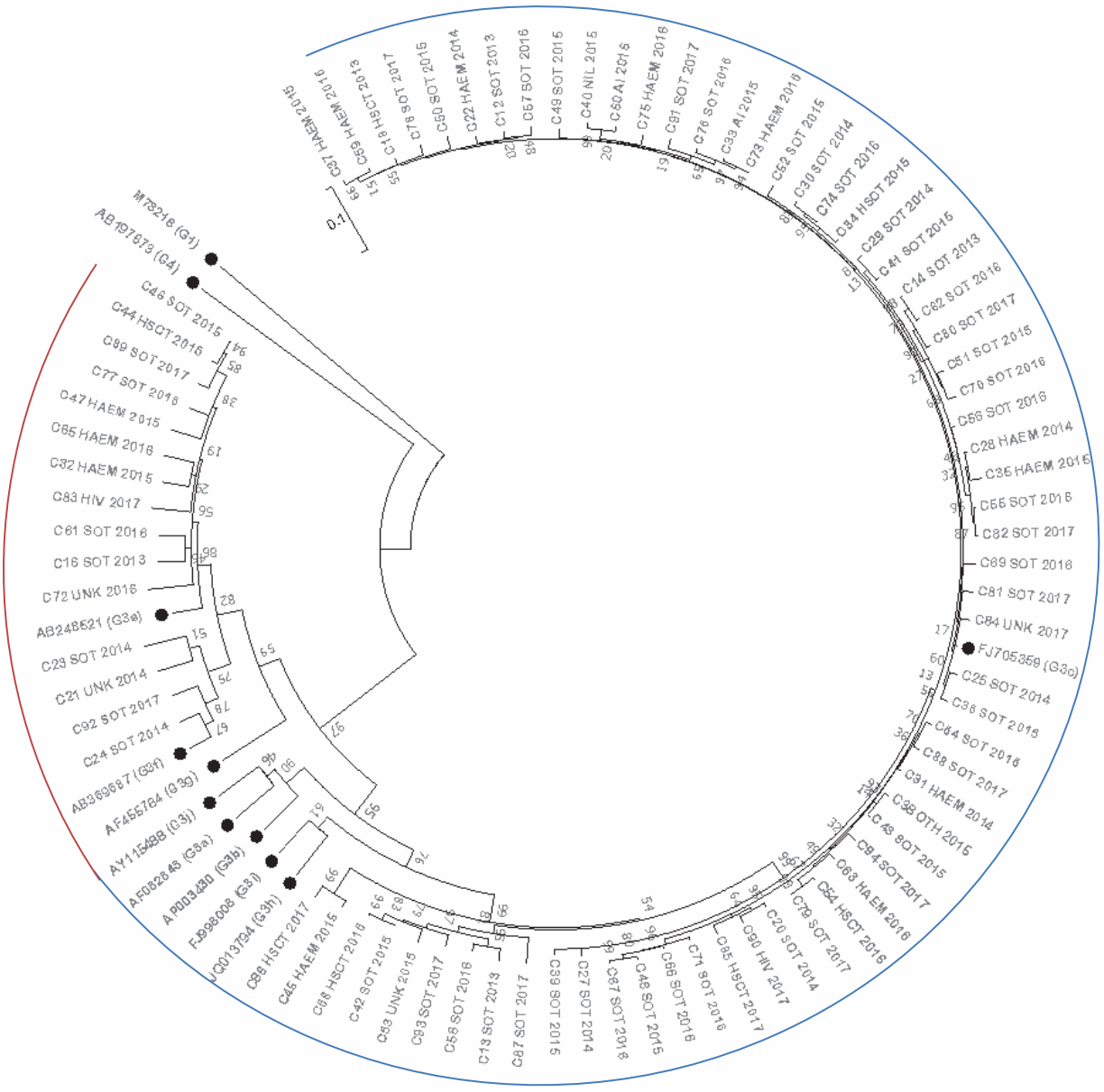
Investigation		No.
Liver Biopsy +/- USS/elastography	**n=14**	
cirrhosis		3
moderate inflammation +/- fibrosis		5
mild inflammation +/- fibrosis		3
no evidence of liver disease/inflammation		3
Elastography +/- USS	**n=13**	
F4/cirrhosis		3
F2-F3		3
F0-F1		7
USS only	**n=17**	
cirrhosis		4
normal scan		13
Post-mortem results	**n=1**	
cirrhosis		1
nil performed		9
no information		40
Total		**94**

Baseline liver imaging results for patients diagnosed with persistent HEV infection. Transient elastography performed by Fibroscan®. Fibrosis scores reported as F0-F1 if mean kPa score <7; F2-F3 if mean kPa score 7-14 and F4 if mean kPa score >14.
Abbreviations: USS, ultrasound scan; kPa, kilopascal units.

5.2.4 Virology and phylogenetic analysis of persistent HEV cases

Plasma HEV viral load at clinical diagnosis was a median of 1.80 x 10^6 IU/ml [IQR 3.80 x 10^5 – 5.43 x 10^6] (n=92) with the highest recorded viral load a median of 2.93 x 10^6 IU/ml [IQR 1.12 x 10^6 – 9.20 x 10^6] (n=93). All cases of persistent HEV infection were identified as G3 HEV; 79.8% were G3 clade 2 (subtypes a b c h i j) and 21.3% G3 clade 1 (subtypes e f g). One sample could not be accurately assigned a subtype within clade 2 (Figure 5.4). This proportion of clade 1 and clade 2 infections has not varied from 2013 onward.

Figure 5.4 Phylogeny of persistent HEV infections across England and Wales



Legend for Figure 5.4:

Phylogenetic tree based on open reading frame 2 nucleotide sequences of persistent HEV cases across England and Wales 2009-2017. Phylogeny based on 1.3kb region and was inferred by using the Maximum Likelihood method based on the General Time Reversible model conducted in MEGA (version 7.0.26). Bootstrapped values shown (1000 replicate). Fifteen samples (C1-C11, C15, C17, C19 and C26) are not included in the tree as sequencing was performed on the shorter Meng fragment (280bp) which gave poor bootstrapping values. For these samples, the percent identity to approved reference sequences was measured and were subtyped as follows: C1_HIV_2009 (3a, 91.4%), C2_HIV_2010 (unclassifiable), C3_HIV_2010 (3e, 92.5%), C4_HSCT_2010 (3c, 97.5%), C5_SOT_2011(3c, 96.1%), C6_HSCT_2011 (3c, 96.1%), C7_HAEM_2012 (3c, 96.4%), C8_SOT_2012 (3f, 88.9%), C9_SOT_2012 (3c, 96.1%), C10_HSCT_2012 (3f, 90.1%), C11_AI_2012 (3c, 95.4%), C15_SOT_2013 (3c, 96.8%), C17_HIV_2013 (3c, 96.8%), C19_HAEM_2014 (3e, 89.3%) and C26_SOT_2014 (3c, 95.0%) are reference sequences with accession numbers shown.

Abbreviations: AI, auto-immune disease; HAEM, haematological malignancy (no HSCT); HSCT, haematopoietic stem cell transplant; OTH, other immunocompromised; SOT, solid organ transplant.

5.2.5 Delays in the clinical diagnosis of persistent HEV infection

Retrospective analysis of stored samples in 46 patients demonstrated viraemia for at least a median of 38 weeks before HEV testing was initiated [IQR 17-68 weeks]. This delay in diagnosis has continued to occur in recent years; 2017 (median 41 weeks, n=5), 2016 (median 32 weeks, n=12), 2015 (median 43 weeks, n=11), 2014 (median 26 weeks, n=3), 2013 (median 34 weeks, n=6), 2012 (median 10 weeks, n=4), 2011 (median 33 weeks, n=1), 2010 (median 64 weeks, n=3), 2009 (80 weeks, n=1).

In 25 patients it was possible to identify precisely the timing of infection; this demonstrated viraemia a median of 33 weeks [IQR 17-73 weeks] prior to diagnosis.

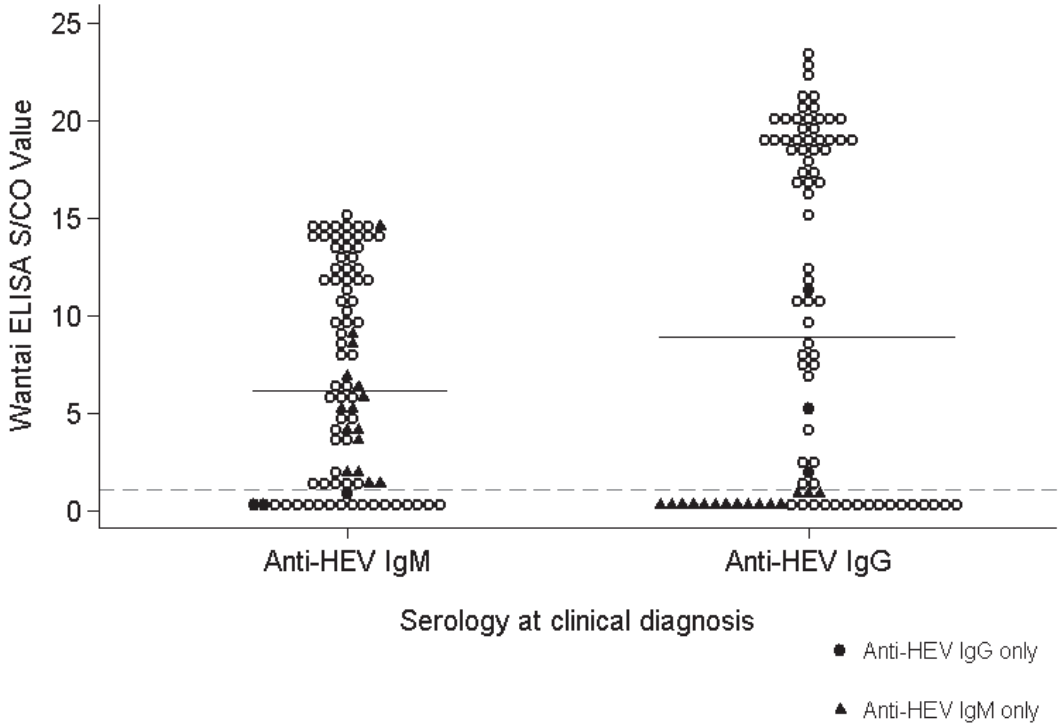
5.2.6 Serological responses at diagnosis

At the point of diagnosis, most patients (74/90, 82.2%) had a detectable serological response to HEV in the index diagnostic sample. Most commonly both anti-HEV IgM and IgG were detected (n=55), less frequently IgM (n=16) or anti-HEV IgG alone (n=3) were detected as single markers at clinical diagnosis. For 88 individuals, quantitative serological results for anti-HEV IgM and IgG were generated in the reference laboratory and display a wide range of reactivity (Figure 5.5).

Longitudinal sampling allowed definition of seroconversion for IgG antibody. Thirty-two patients were seronegative for anti-HEV IgG at diagnosis. Sixteen (50%) were seroreactive for IgM of whom 13 subsequently seroconverted to anti-HEV IgG, three only had transient IgM detected and did not seroconvert to anti-

HEV IgG during follow up (range 13-46 weeks). Sixteen patients (50%) were seronegative for both anti-HEV IgM and IgG, only three of these patients developed a durable anti-HEV IgG seroconversion. However, two patients developed an IgG transiently for 5 weeks and 16 weeks and one patient had intermittent low level anti-HEV IgG detectable in between intravenous immunoglobulin infusions.

Figure 5.5 Anti-HEV serological responses in persistent HEV infection



Anti-HEV serological responses at the point of diagnosis in 92 patients with persistent HEV infection. The solid horizontal lines represent median S/CO values (6.19 for anti-HEV IgM; 8.98 for anti-HEV IgG). The dashed horizontal line represents the manufacturer's threshold for a positive result (S/CO 1.1).

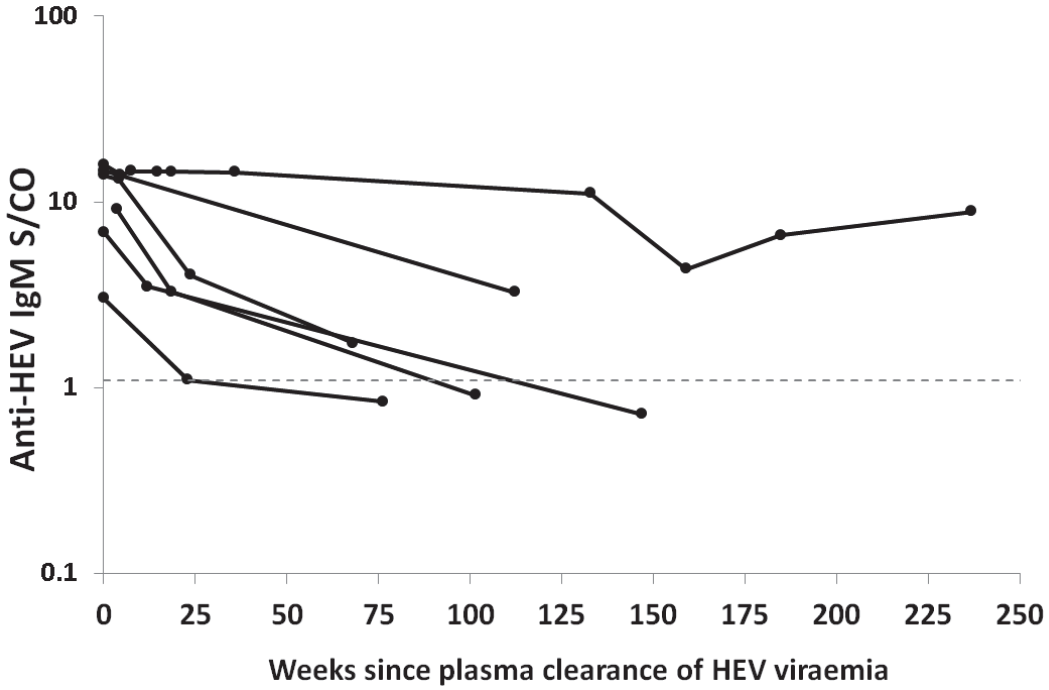
Abbreviations: ELISA, enzyme-linked immunosorbent assay; S/CO, sample over cut-off ratio of optical density values.

5.2.7 Sustained serological responses throughout and after HEV infection

Of the 81 patients who developed an anti-HEV IgM response at any timepoint, in 67 patients (82.7%) the IgM reactivity was detected throughout the viraemic period (median of 26.5 weeks [IQR 14-60.8]. Eleven patients lost anti-HEV IgM reactivity during viraemia (transient IgM only (n=3), transient IgM and IgG (n=1) and loss of IgM in the presence of IgG (n=7)). In six of these 11 cases HEV concentrations plateaued or rose when anti-HEV IgM was lost. Following plasma clearance of HEV RNA, detectable anti-HEV IgM persisted. In 19 patients followed up for one year or less following clearance of HEV viraemia all patients retained detectable anti-HEV IgM in their sera. Of six patients followed up for over one year following clearance of viraemia three still had detectable anti-HEV IgM for one year or more (Figure 5.6).

Anti-HEV IgG persisted throughout infection and also after clearance during follow-up in all but two patients who developed anti-HEV IgG. The first of these two a patient with underlying lymphoma, developed anti-HEV during viraemia (peak S/CO 10.5) but IgG seroreactivity subsequently fell over a period of three months which coincided with virological relapse (patient B, Table 5.4). The second patient developed a weak anti-HEV IgG response (S/CO 4.9) which was lost during viraemia; but subsequently seroconverted for anti-HEV IgG during viral clearance.

Figure 5.6 Persistence of anti-HEV IgM after clearance of HEV viraemia in persistent HEV infection



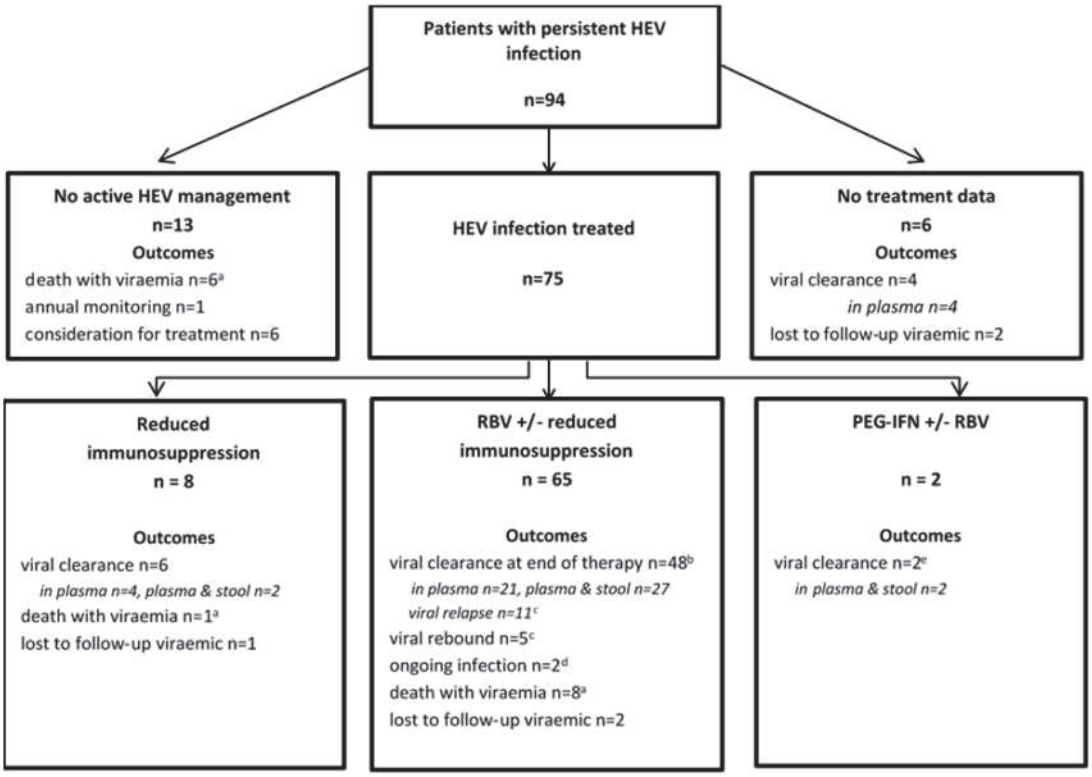
Persistence of anti-HEV IgM reactivity in patients following plasma clearance of HEV, Shown in the figure are six patients followed up for one year or more; in three anti-HEV IgM remained detectable for one year or more. Where samples were available for testing (5/6), HEV-Ag detection did not persist beyond three months in any patient (data not shown). The hatched line represents manufacturer's criteria for a positive result.
Abbreviations: S/CO, sample over cut-off ratio of optical density values.

5.2.8 Treatment and outcomes of patients with persistent HEV infection

Treatment and virological outcomes were available for 88 of the 94 patients (Figure 5.7). Fifteen patients died during the period of HEV infection; eight died of liver failure or a complication relating to liver decompensation in which HEV was considered contributory, three died of unrelated causes and in four the cause of death was unknown.

HEV was actively managed in 75 patients either via a reduction of immunosuppression alone (n=8), ribavirin with or without a reduction in immunosuppressive medication (n=65) or PEG-Interferon with or without ribavirin (n=2). Amongst eight patients who received a reduction of immunosuppression alone, viral clearance was achieved in six. Of the 65 ribavirin-treated patients, 48 (73.8%) had demonstrated viral clearance at the end of therapy either in plasma (n=21) or plasma and stool (n=27). The two patients treated with PEG-Interferon achieved viral clearance in plasma and stool. However, only 15 of the 67 patients treated with an antiviral had sufficient follow-up sampling to prove a sustained virological response at three months (n=3) or at six months (n=12) following cessation of treatment. Virological recurrence (rebound (n=5) or relapse (n=11)) was detected during follow-up in 16 patients treated with an antiviral (23.9%).

Figure 5.7 Treatment and outcomes in persistent HEV infection



[a] Of the 15 patients who died whilst viraemic; eight died of liver failure or complication related to decompensation to which HEV was considered contributory, three died of unrelated causes and in four the cause of death unknown.

[b] SVR was observed for 13 patients (12-week SVR n=3, 24-week SVR n=10).

[c] Viral rebound describes any patient with a quantitative increase in viral load (plasma or stool) post-cessation of therapy, however viral relapse is restricted to patients with HEV-RNA negative samples (plasma sample and/or stool) at time of treatment cessation who subsequently developed a second period of detectable HEV viraemia.

[d] Both patients undergoing prolonged antiviral treatment (>12m); includes one patient who has had PEG-IFN added to ribavirin.

[e] Both patients achieved a 24-week SVR.

Abbreviations: RBV, ribavirin; PEG-IFN, pegylated interferon alfa-2b; SVR, sustained virological response.

5.2.9 Outcomes in anti-HEV IgG seronegative patients

Sixteen patients, in whom the index diagnostic sample was either anti-HEV IgM reactive alone (n=3) or non-reactive for both anti-HEV IgM and IgG (n=13), did not seroconvert for anti-HEV IgG during a median duration of follow-up during active HEV infection of 45.0 weeks [IQR 34.3-83.8]). Twelve patients had an underlying haematological malignancy (lymphoma (n=10), acute myeloid leukaemia (n=1) or recent auto-HSCT (n=1)) and four were recipients of a SOT (renal (n=3) or liver (n=1)) (Table 5.3).

Twelve patients were treated with ribavirin alone (n=7) or ribavirin and concomitant reduction of immunosuppression (n=5) of whom only four cleared HEV infection in plasma and stool (n=3) or plasma alone (n=1). Five patients suffered a virological rebound (n=1) or relapse (n=4) on discontinuation of ribavirin, three have died (including one of the patients who relapsed) and in one patient antiviral treatment has not been completed. Of the four patients not treated with ribavirin, one had reduction of immunosuppression but was subsequently lost to follow up during persistent viraemia, two patients died during persistent viraemia and one patient had at the time of writing ongoing untreated persistent viraemia. In three of the five deaths HEV was considered contributory.

Table 5.3 Outcomes of patients who did not seroconvert to anti-HEV IgG

	SOT		Haematological malignancy		
	Kidney n=3	Liver n=1	Auto-HSCT n=1	Lymphoma n=10	AML n=1
Serological follow-up (mean, weeks)	79	82	17	61	46
Any detectable serological response during follow-up	2[a]	0	0	4[b]	1
Treatment					
Reduction IS alone (1)	1	-	-	-	-
Reduction IS and ribavirin (5)	2	1	-	2	-
Ribavirin alone (7)	-	-		6	1
Nil (3)	-	-	1	2	-
Virological Outcome					
Viral clearance	-	-	-	4	-
Rebound	1	-	-	-	-
Relapse	1	-	-	3	-
Ongoing treatment/infection	-	1	-	3	-
Lost to follow-up	1	-	-	-	-
Other Outcomes					
Death	-	-	1[c]	3[c]	1[c]

Follow-up and outcomes of 16 patients who did not develop an anti-HEV IgG response.

[a] One patient had a transient low level reactive anti-HEV IgM detected at diagnosis and one patient, who was lost to follow-up, had a transient IgG detectable for five weeks.

[b] One patient diagnosed with secondary hypogammaglobulinaemia had intermittent low level anti-HEV IgG detected (S/CO consistently <3.0) in between monthly IVIG infusions, another patient had a transient anti-HEV IgG detectable for 16 weeks which became undetectable during virological rebound, two patients had transient IgM detectable at diagnosis.

[c] Of these five patients who died with HEV viraemia, in three HEV was considered contributory to death.

Abbreviations: AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; HEV, hepatitis E virus; HSCT, haematopoetic stem cell transplant; IS, immunosuppression; RBV, ribavirin; SOT, solid organ transplant.

5.2.10 Virological recurrence after treatment

Viral recurrence was documented in 16 patients following ribavirin cessation. In five patients, low level detectable HEV RNA was present in stool and/or plasma on treatment, therefore viral rebound may have been predicted. The remaining eleven were considered true virological relapses, since there was no detectable HEV RNA in plasma (n=5) or plasma and stool (n=6) at the time of treatment cessation following ribavirin therapy (median 3.00 months [IQR 3.00-3.75 months]) (Table 5.4). Three of these eleven patients (27%) were anti-HEV IgG seronegative at treatment cessation. Starting daily doses of ribavirin in these 11 patients ranged from 480-1200mg. However, seven (64%) underwent a dose reduction of ribavirin due to side effects.

Seven of the relapse patients were confirmed by phylogenetic analysis as a relapse as opposed to re-infection. Of the other four patients, the viral load post-relapse was insufficient for phylogenetic analysis and in one patient the sample was sent to another diagnostic laboratory. Nevertheless, the timing of recurrent HEV viraemia was highly suggestive of relapse. Nine patients experienced relapse within six months of antiviral cessation; one patient (Patient G, Table 5.4) was only tested at 32 weeks following cessation and had a high HEV viral load at the time. In a further patient (Patient K, Table 5.4) the HEV relapse was identified approximately 24 months later; HEV re-infection cannot be excluded in this patient since post-relapse samples were unavailable for phylogenetic analysis.

Of the 11 patients who suffered virological relapse, eight were re-treated with ribavirin of whom two are still undergoing prolonged (>38 weeks) ribavirin treatment. Of the six patients who have completed re-treatment with ribavirin

therapy (median length 5.38 months [IQR 4.56-6.00]) only one has achieved a 24-week SVR, three suffered a second relapse and in two patients no significant change in viral load was observed after 14 and 24 weeks of ribavirin before treatment was discontinued.

Table 5.4 Patients with virological relapse

Pt	Age, years; Gender	Underlying condition	IS at diagnosis	ALT at diagnosis; peak ALT; at time of relapse (IU/L)	Reduction of IS?	RBV 1st course duration weeks (w) (daily dose; duration weeks)	eGFR	clearance in stool?	anti-HEV IgG at plasma clearance (S/CO)	time relapse identified
A	63; M	nil[a]	nil	173; 183; 33	-	11w (1000mg; 11w)	60-89	Y x 2	POS (19.18)	<18w
B	44; F	Lymphoma	Corticosteroids[b]	2192; 2447; 28	-	>8w[c] (600mg 8w)	60-89	NT	POS (3.10)	<8w
C	84; F	Lymphoma	Chemotherapy[d]	222; 260; 27	Y	16w (600mg 7w; 400mg 9w)	60-89, dropped to 45-60	NT	POS (18.84)	<8w
D	76; M	Lymphoma	nil in 4 years prior[e]	299; 304; 17	N	12w (800mg 4w; 400mg 8w)	60-89	Y x 2	POS (20.01)	<8w
E	59; M	Lymphoma	nil in 7month prior[f]	67; 113; 19	N	13w (1000mg 4w; 400mg 9w)	60-89	Y x 2	NEG (0.05)	<4w
F	21; F	Lymphoma[g]	nil in 2 years prior[h]	205; 574; 21	-	48w (800mg, 4w; 600mg 16w; 1200mg 28w)	>90	Y x 2	POS[i] (1.90)	<8w
G	50; M	Neurosarcoidosis	Methotrexate	109; 308; 70	N	14w (1200mg; 14w)	60-89	NT	POS (2.80)	<32w
H	66; M	Heart/kidney SOT	Tacrolimus, MMF, prednisolone	172; 250; 38	N	12w (600mg, 2w; 400mg 10w)	60-89	Y x 2	POS (9.86)	<16w
I	13; M	Liver SOT	Tacrolimus, prednisolone	112; 146; 62	Y	12w (480mg, 4w; 400mg 8w)	>90	Y x 1	NEG (0.07)	<12w
J	51; F	Kidney SOT	Sirolimus	299[j]; 557[j]; 137[j]	Y	17w (800mg 8w; 400mg 9w)	>90	NT	NEG (0.08)	<16w
K	32; F	SCID	nil	140; 197; 152	-	12w (1200mg 12w)	>90	NT	POS (9.14)	100w[k]

Legend for Table 5.4:

Patients who had evidence of virological relapse of HEV infection after apparently successful treatment.

[a] Extensive (immunological) investigations have not identified an underlying immunological disorder/immunodeficiency.

[b] Prior chlorambucil, rituximab, cyclophosphamide, vincristine, prednisolone (R-CVP) and splenectomy. Nil chemotherapy in year prior to diagnosis of HEV infection.

[c] Exact timings of treatment cessation not known.

[d] Patient undergoing chemotherapy containing rituximab, cyclophosphamide, vincristine, prednisolone (R-CVP).

[e] Patient received prior chlorambucil, rituximab, cyclophosphamide, vincristine, prednisolone (R-CVP) and rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone (R-CHOP).

[f] Prior rituximab and bendamustine.

[g] Patient diagnosed with secondary hypogammaglobulinaemia.

[h] Patient received prior rituximab and autologous stem cell transplant.

[I] Patient has intermittent low level anti-HEV IgG detected (S/CO consistently <3.0) in between monthly IVIG infusions.

[j] AST (aspartate aminotransferase) value recorded rather than ALT.

[k] Patient not followed-up and was re-referred with abnormal liver function tests two years after the last HEV RNA test. Sample was unavailable for sequencing.

Abbreviations: ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; F, female; IS, immunosuppression; M, male; MMF, mycophenolate mofetil; N, no; NEG, negative; NT, not tested as no sample sent; POS, positive; Pt, patient; RBV, ribavirin; S/CO, sample over cut-off ratio of optical density values; SCID, severe combined immunodeficiency; SOT, solid organ transplant; Y, yes.

5.3 Discussion

In this chapter, 94 patients are described who were diagnosed with persistent HEV infection through the Blood Borne Virus Unit at VRD, PHE Colindale between 2009 and 2017. An enhanced surveillance system was established to enable clinical data collection leading to the first report of a national series of cases of persistent HEV infections. Patients were excluded if they were diagnosed through separate defined screening studies outwith the routine diagnostic pathway within the NHS. These patients are expected to represent the majority of clinically recognised cases across England and Wales, whose samples were referred to VRD, due to the centralised testing for quantitative HEV RNA at PHE laboratories during this period. The lower incidence of cases diagnosed in 2017 at VRD may be the result of reduced referrals to the reference laboratory, but a true reduction in clinical cases in line with reducing numbers of acute HEV infections in England and Wales cannot be excluded [21]. The described patients however may only represent a small proportion of the true prevalence of persistent HEV infection owing to under-diagnosis as a result of the subclinical nature of this infection, as well as low awareness amongst clinicians.

Immunodeficiency is considered a prerequisite for the development of persistent HEV infection [172-174]. However, these results demonstrate that predisposition to persistent HEV infection is not limited to transplant recipients; just over one quarter (28%) of the patients were outside the context of stem cell or solid organ transplantation. Of specific interest in this patient series is the first recognition of

persistent HEV infection in a patient with a primary immunodeficiency syndrome and a patient with no apparent immunodeficiency; both will be reported in detail separately. Therefore, whilst transplant recipients appear to represent a uniquely high-risk cohort, it is important for specialists caring for immunosuppressed patients outwith transplantation, and even patients ostensibly not immunosuppressed, to be aware of the possibility of persistent HEV infection in patients with chronic hepatitis.

Hepatic disease was relatively common amongst the patients studied; 25% of those assessed at baseline had evidence of advanced liver disease (cirrhosis or F4 on hepatic elastography). These patients had been viraemic for many months prior to assessment (median 64 weeks). Whilst other aetiologies of liver disease in these patients could not be excluded and therefore causality for HEV cannot be inferred, earlier studies have demonstrated rapid progression to cirrhosis in SOT patients infected with HEV [207]. Therefore, prompt diagnosis is expected to prevent the development of advanced liver disease; amongst these patients significant delays in diagnosis were seen, with evidence of prolonged viraemia (median 38 weeks) prior to HEV testing. This is likely to be an underestimate because in 21 of 46 patients the earliest stored sample available for testing was viraemic and so the onset and duration of the infection could not be precisely timed.

Ribavirin remains the antiviral drug of choice to treat persistent HEV infection [223, 233, 234, 236]. Amongst these patients, 16 of the 67 antiviral-treated patients (23.9%) experienced virological rebound; this is lower than the 38% reported in a small controlled observational study of 24 SOT patients following

three months of ribavirin [225]. The current series of patients contained more diverse levels of underlying immunosuppression, with differing durations of ribavirin therapy (range 1.3-31.3 months) often tailored to stool testing. Importantly, many patients were not assessed for a SVR after attaining an end-of-treatment response and as a result late relapses may have been missed. Whilst virological rebound could have been predicted in five patients due to detectable HEV RNA in samples at the time of cessation, five patients relapsed despite yielding two sequential stool samples undetectable for HEV RNA. The recommendation to demonstrate virological clearance of HEV in stool has been incorporated into recently published guidelines in the UK and is increasingly being adopted by physicians caring for patients with persistent HEV infection [221, 225]. It is notable that in five patients viral relapse occurred despite apparent stool clearance, therefore whilst stool clearance should be a pre-requisite for cessation of therapy, it does not obviate the requirement for extended close follow-up monitoring both for a SVR and for late recrudescence of virus.

The use of serology is widely accepted to be inadequate for the diagnosis of HEV infection in immunocompromised patients [216]. These data confirm this, as sixteen patients did not seroconvert to anti-HEV IgG during prolonged HEV infection, this was seen mostly in patients with underlying haematological malignancy (n=12). The absence of anti-HEV IgG seroconversion was associated with a poor outcome. Only four of the twelve seronegative antiviral-treated patients (33.3%) achieved viral clearance and at least three of the five deaths amongst seronegative patients were considered to be HEV-associated. Whilst there are several confounders which are not accounted for in the seronegative

patients (in particular ribavirin dosing), a patient's serostatus could be a consideration when tailoring the duration and dosing of ribavirin therapy.

The serological data demonstrate that anti-HEV IgM can remain detectable in patient plasma for months and even up to several years following HEV RNA plasma clearance. This may lead to considerable diagnostic confusion if a patient were tested *de novo* at this time point. The mechanism leading to persistent antibody is unknown, but one may speculate could be the result of persistence in sanctuary sites including possibly cerebrospinal fluid or the urogenital tract [131, 320-324]. Animal models have suggested important extrahepatic sites of replication which may be reservoirs from which viral rebound and continuing antigen challenge may originate [243, 287]. Although whether a similar situation arises in the human host with HEV, particularly immunocompromised patients on ribavirin, is currently unknown.

The data presented in this chapter have limitations which are inherent in such an observational dataset. These include data missing from patients lost to follow-up, the differential timing of samples that are associated with routine clinical practice and some sample volumes being insufficient for further analysis. Patients were identified with persistent HEV infection only when samples were referred to the reference laboratory, which limits the case numbers. Clinical data collection was then reliant upon the completion of a questionnaire or paper form by the primary clinician or local infection specialist and supplemented with direct discussion for further details. Many cases were collected retrospectively which led to significant data gaps. In particular, it was not possible to demonstrate histological evidence of HEV-attributable liver disease; this is crucial to understand the pathogenesis

and natural history of hepatic fibrosis caused by HEV infection. A prospective study would overcome many of these limitations.

5.4 Conclusions

- A wide range of immunocompromised patients are susceptible to persistent HEV infection.
- Established liver disease was common, but causality by HEV could not be established. Eight patients (8.51%) died of liver disease to which HEV was considered to have had an important contribution.
- Serology is inadequate for the diagnosis of persistent HEV infection but it may be a helpful prognostic indicator for treatment response and this requires further study.
- The absence of detectable HEV RNA in a stool sample at the time of stopping therapy does not obviate the need for close follow-up to detect viral rebound as it may be an insufficient tool for indicating the safe discontinuation of ribavirin in some patients.

6 Cost-effectiveness analysis of screening for persistent HEV infection in SOT recipients

6.1 Introduction

A major/surprising finding from the prevalence study of HEV viraemia in transplant recipients in chapter 4 was the significant under-diagnosis of HEV infection amongst solid organ transplant patients in England. This could result in the development of progressive liver disease, cirrhosis and poor clinical outcomes [207]. A screening programme in the post-transplant period could identify patients with active HEV infection and enable early treatment prior to the development of end-stage liver disease. Treatment may include the modulation of immunosuppression, leading to clearance of HEV infection in 30% of patients, or antiviral therapy with ribavirin, which has shown high efficacy in observational studies [223]. Unselected HEV screening of organ transplant recipients is not undertaken in the UK, but has been introduced in regions in France and other countries [325]. British guidelines currently recommend testing patients with liver transaminases above the upper limit of normal for HEV infection, but in clinical practice testing for HEV infection is usually only performed when the clinician suspects HEV infection in the patient [221].

The optimal structure of a screening programme and the choice of diagnostic tests are not defined. Moreover, screening would utilise resources which may be better spent elsewhere. Therefore, this cost–effectiveness analysis will provide guidance on decisions to optimise desirable outcomes given resource limitations. In the UK an increasing number of regional clinical NHS laboratories are

incorporating HEV PCR to their laboratory repertoire of tests, so it is timely to consider the implications of a structured screening programme. The release of a commercial assay for the detection of HEV antigen (HEV-Ag ELISA, Fortress Diagnostics, Antrim, Northern Ireland, UK) in recent years also provides a less costly alternative to PCR testing.

The aim of this chapter is:

1. to determine the cost-effectiveness of four possible screening strategies for HEV infection in solid organ transplant recipients in the UK.

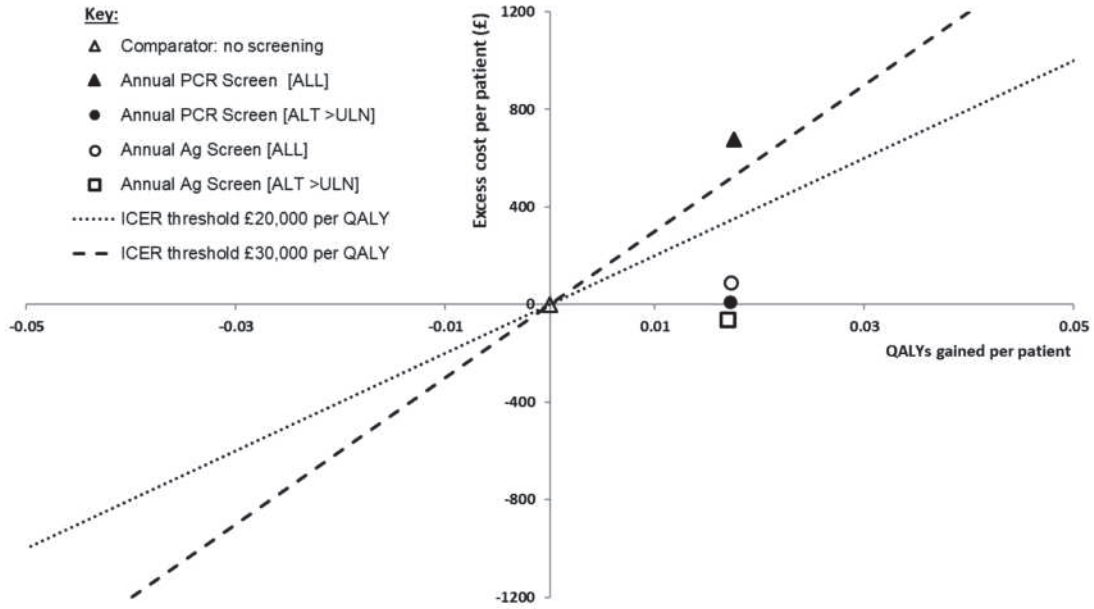
6.2 Results

6.2.1 Base-case results

All four screening options resulted in improved health outcomes compared to no screening (Figure 6.1). Over the lifetime of 1,000 SOT recipients HEV screening would be expected to prevent 7.0 cases of cirrhosis and 3.5 deaths related to HEV-induced cirrhosis (Table 6.1). Compared with the baseline strategy of no screening, the highest number of QALYs gained was seen in the screening arm testing all patients annually by PCR (strategy A), whereas the lowest numbers of QALYs gained amongst the screening options was testing those patients with an abnormal ALT by HEV-Ag (strategy D). The gain in QALYs was mainly driven by the reduction in the numbers of cases of cirrhosis and the lower utility values associated with this state.

In terms of costs, of the four screening strategies considered, restricted screening of those patients presenting an abnormal ALT value using HEV-Ag detection (strategy D) would be expected to be cost saving to the NHS compared to no screening, whereas testing all patients annually by PCR (strategy A) is likely to be the most costly option and was the only strategy that exceeded the threshold of £20,000 per QALY, as suggested by NICE [258].

Figure 6.1 Cost-effectiveness plane of base-case



Plotted values represent the average QALYs gained for each patient over their lifetime against the excess cost incurred for each patient for each of the screening options in comparison to not screening i.e. non-incrementally. Note that incrementally, only “Annual PCR testing of all patients” is below the conventional cost-effectiveness threshold used in England.

Abbreviations: ALT, alanine aminotransferase value; HEV-Ag, hepatitis E virus antigen; ICER, incremental cost-effectiveness ratio; PCR, polymerase chain reaction; QALY, quality-adjusted life year; ULN, upper limit of normal range.

Table 6.1 Projected preventable complications in base-case

Strategy	Annual screen assay	Size of cohort	Expected cases of cirrhosis (deaths)	Expected no. of liver transplants	Expected no. of neurological complications
	No screening	1000	8.95 (4.56)	1.48	1.73
	All patients:				
A	PCR	1000	1.91 (1.00)	0.33	0.37
B	HEV-Ag	1000	1.99 (1.04)	0.34	0.38
	Patients with ALT > ULN:				
C	PCR	1000	2.04 (1.07)	0.35	0.39
D	HEV-Ag	1000	2.12 (1.11)	0.36	0.41

Base-case results demonstrating the numbers of expected complications (cirrhosis, liver transplantation and neurological complication) in a cohort of 1,000 patients followed over the lifetime of the patient.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; PCR, polymerase chain reaction; ULN, upper limit of normal range.

6.2.2 Incremental cost-effectiveness analysis

Incrementally, testing those patients with an abnormal ALT for HEV-Ag (strategy D) was the most cost-effective option and strongly dominated the no screening strategy (i.e., it was both less costly and resulted in higher health gains). All other screening options had marginal health gains at increased costs with discounted ICERs above £300,000/QALY (Table 6.2).

Alternatively, when considering only strategies testing for HEV by PCR (i.e., strategy A, strategy C, and no screening), testing patients with a raised ALT (strategy A) was cost-effective (£660/QALY) while testing all patients (strategy C) was cost-ineffective with an ICER of £1 784 000/QALY (Table 6.3).

Table 6.2 Incremental cost-effectiveness for all HEV screening strategies

Strategy	Annual screen assay	Total lifetime cost per patient	Total LY per patient	Total QALYs per patient	Incremental lifetime costs per patient	Incremental LY gained per patient	Incremental QALYs gained per patient	ICER (£) per life year	ICER (£) per QALY
costs and benefits discounted (at 3.5%):									
-	No screening	£ 201	14.06355	10.72660	-	-	-	dominated	dominated
D	HEV-Ag [if ALT > ULN]	£ 140	14.08040	10.74354	- £ 61	0.016851	0.016939	cost-saving	cost-saving
C	PCR [if ALT > ULN]	£ 212	14.08064	10.74377	£ 72	0.000235	0.000233	£ 308 000	£ 311 000
B	HEV-Ag [ALL]	£ 289	14.08079	10.74393	£ 77	0.000154	0.000152	£ 499 000	£ 505 000
A	PCR [ALL]	£ 879	14.08102	10.74415	£ 590	0.000224	0.000222	£ 2 630 000	£ 2 660 000
costs and benefits undiscounted (0.0%):									
-	No screening	£ 417	21.31170	16.25228	-	-	-	dominated	dominated
D	HEV-Ag [if ALT > ULN]	£ 242	21.35427	16.29312	- £ 175	0.042578	0.040832	cost-saving	cost-saving
C	PCR [if ALT > ULN]	£ 348	21.35484	16.29365	£ 106	0.000562	0.000530	£ 189 000	£ 201 000
B	HEV-Ag [ALL]	£ 476	21.35520	16.29399	£ 128	0.000367	0.000345	£ 348 000	£ 370 000
A	PCR [ALL]	£ 1 400	21.35574	16.29449	£ 924	0.000534	0.000503	£ 1 729 000	£ 1 836 000

Incremental cost-effectiveness results for all HEV screening strategies (A-D) in the base-case analysis. Not fixing the no-screening option as baseline reference would not change results as 'no screening' is absolutely dominated by strategy D i.e. keeping it in the first row.
Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; ICER, incremental cost-effectiveness ratio; LY, life years; QALY, quality adjusted life year; ULN, upper limit of normal.

Table 6.3 Incremental cost-effectiveness for HEV screening using PCR only

Strategy	Annual screen assay	Total lifetime cost per patient	Total LY per patient	Total QALYs per patient	Incremental lifetime costs per patient	Incremental LY gained per patient	Incremental QALYs gained per patient	ICER (£) per life year	ICER (£) per QALY
costs and benefits discounted (at 3.5%):									
-	No screening	£ 201	14.06355	10.72660	-	-	-	-	-
C	PCR [if ALT > ULN]	£ 212	14.08064	10.74377	£ 11	0.017086	0.017172	£ 663	£ 660
A	PCR [ALL]	£ 879	14.08102	10.74415	£ 666	0.000378	0.000374	£ 1 765 000	£ 1 784 000
costs and benefits undiscounted (0.0%):									
-	No screening	£ 417	21.31170	16.25228	-	-	-	dominated	dominated
C	PCR [if ALT > ULN]	£ 348	21.35484	16.29365	- £ 69	0.043140	0.041361	cost-saving	cost-saving
A	PCR [ALL]	£ 1 400	21.35574	16.29449	£ 1 051	0.000901	0.000848	£ 1 167 000	£ 1 239 000

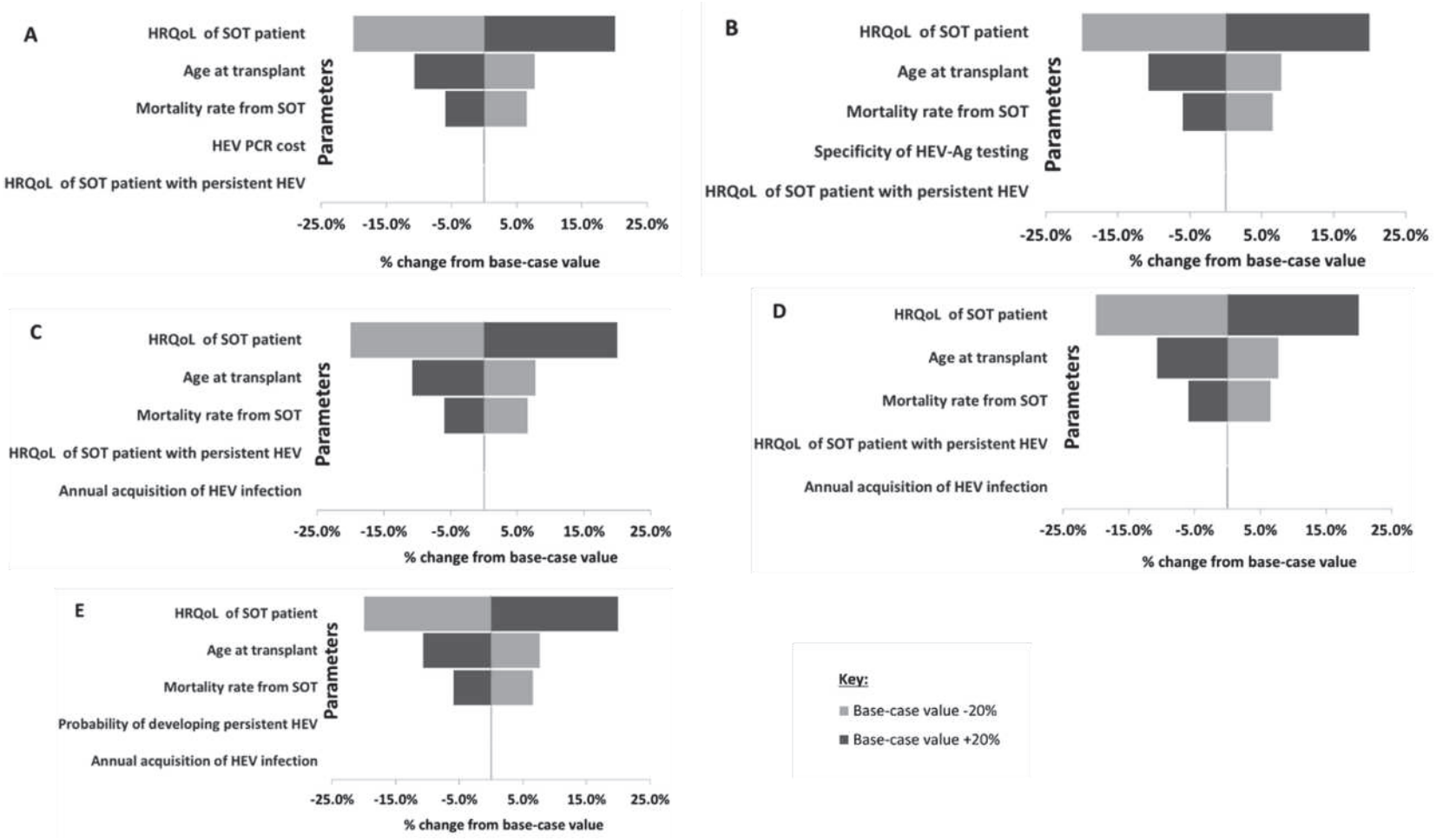
Incremental cost-effectiveness results for HEV screening strategies when using only PCR-based testing.

Abbreviations: ALT, alanine aminotransferase; ICER, incremental cost-effectiveness ratio; LY, life years; PCR, polymerase chain reaction; QALY, quality adjusted life year; ULN, upper limit of normal.

6.2.3 Deterministic sensitivity analysis (DSA)

In the DSA, of the 47 parameters tested by changing them individually to 80% and 120% of the base-case value, only three parameters changed the net benefit by more than 5%; in increasing order these were the mortality rate of a SOT patient, age at transplantation, and the HRQoL of SOT patients (Figure 6.2). The ranking of the optimal strategy was unchanged by changing the parameters in the DSA and also robust against setting all parameter values simultaneously to the worst level (data not shown).

Figure 6.2 Summary results of deterministic sensitivity analysis



Legend for figure 6.2:

Of 47 parameters tested, the top five parameters are shown which affected the net health benefit outcome the most when varied to 80% or 120% of the base-case value in the following cohorts: testing all by PCR (A), testing all by HEV-Ag (B), testing those with an abnormal ALT by PCR (C) or HEV-Ag (D), or no screening (E).

Abbreviations: HEV-Ag, hepatitis E virus antigen; HRQoL, health-related quality-of-life score; PCR, polymerase chain reaction; SOT, solid organ transplant.

6.2.4 Scenario analyses

Of the 15 performed scenario analyses, of specific interest are the annual attack rate of HEV, the probability of a SOT recipient developing persistent HEV infection, the annual rate of developing cirrhosis in persistent HEV infection, the probability of diagnosing HEV in the absence of screening, and the costs of testing (using PCR or HEV-Ag ELISA).

If the annual attack rate of HEV fell to as low as 0.06%, approximately a quarter of the base-case value, then only restricted testing by PCR (strategy C) or HEV-Ag (strategy D) generated a higher net benefit for each patient than not screening. However, HEV screening by any method no longer generated a higher net QALY gain when the annual attack rate falls to 0.01%. All screening options generated a higher net benefit than no screening if the annual attack rate rises above 0.4% (Figure 6.3).

If the probability of an HEV infected SOT recipient developing a persistent infection is actually 30% or lower then only restricted testing by PCR (strategy C) or HEV-Ag (strategy D) generated a higher net benefit for each patient than not screening. If it is actually lower still, at 10%, then only restricted testing by HEV-Ag generated a higher net benefit than not screening (Figure 6.4).

If the annual rate of developing cirrhosis amongst HEV-infected transplant recipients is lower than in the base-case, a restricted screening programme of those with an abnormal ALT (strategies C and D) will give the highest net benefit until it is as low as 2% (Figure 6.5). Higher rates than used in the base-case did not change the base-case results.

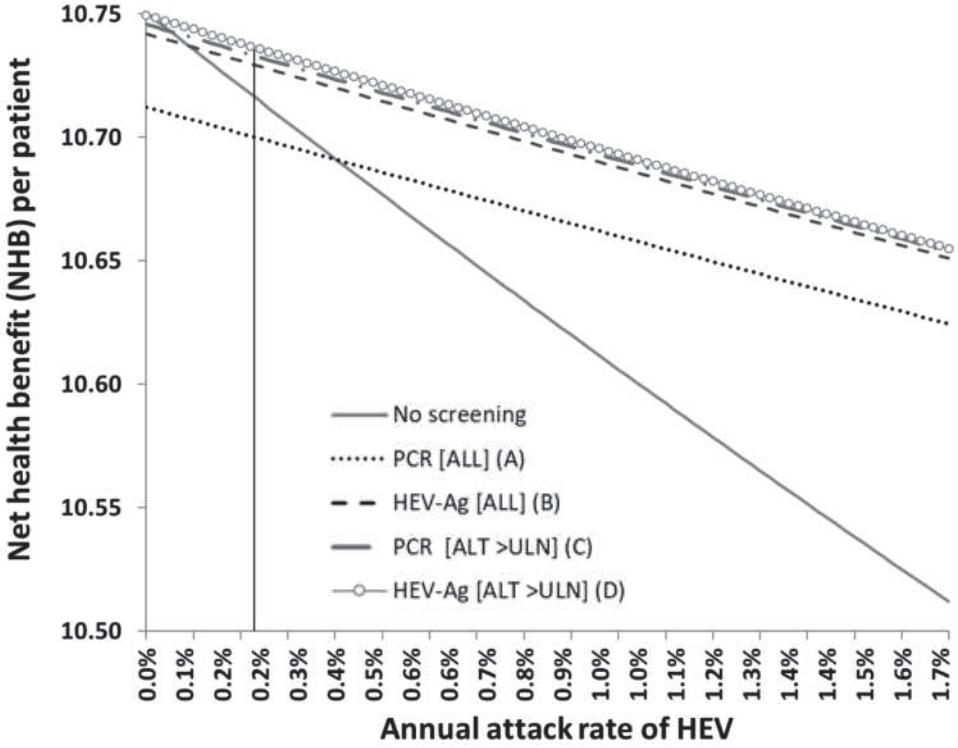
If the probability of diagnosing HEV infections in the absence of a screening programme was increased from the base-case value of 10% to 50% or 75%, restricted testing of patients with an abnormal ALT by either PCR (strategy C) or HEV-Ag (strategy D) still generated a higher net benefit than no screening, respectively (Figure 6.6).

Finally, if the cost of PCR testing fell to £15 or below, screening patients with an abnormal ALT (strategy C) would be expected to provide the highest net benefit per patient (Figure 6.7).

A further nine scenario analyses are presented in Appendix 3.2-3.10 exploring varying the age at transplantation, the mortality rate of a SOT patient, the HRQoL score for a SOT patient, the discounting rate of costs and utilities, the specificity of HEV-Ag testing, the healthcare costs of treating and monitoring a case of persistent HEV infection, the mortality rate of a patient with compensated cirrhosis, the mortality rate of a patient with decompensated cirrhosis and the probability an HEV-infected patient has an abnormal ALT results.

These findings were found to be robust when similar quantitative results were generated from a separate structural scenario analysis which changed the structure of the model to allow patients with compensated cirrhosis to be diagnosed, treated and returned to an uninfected state (Table 6.4).

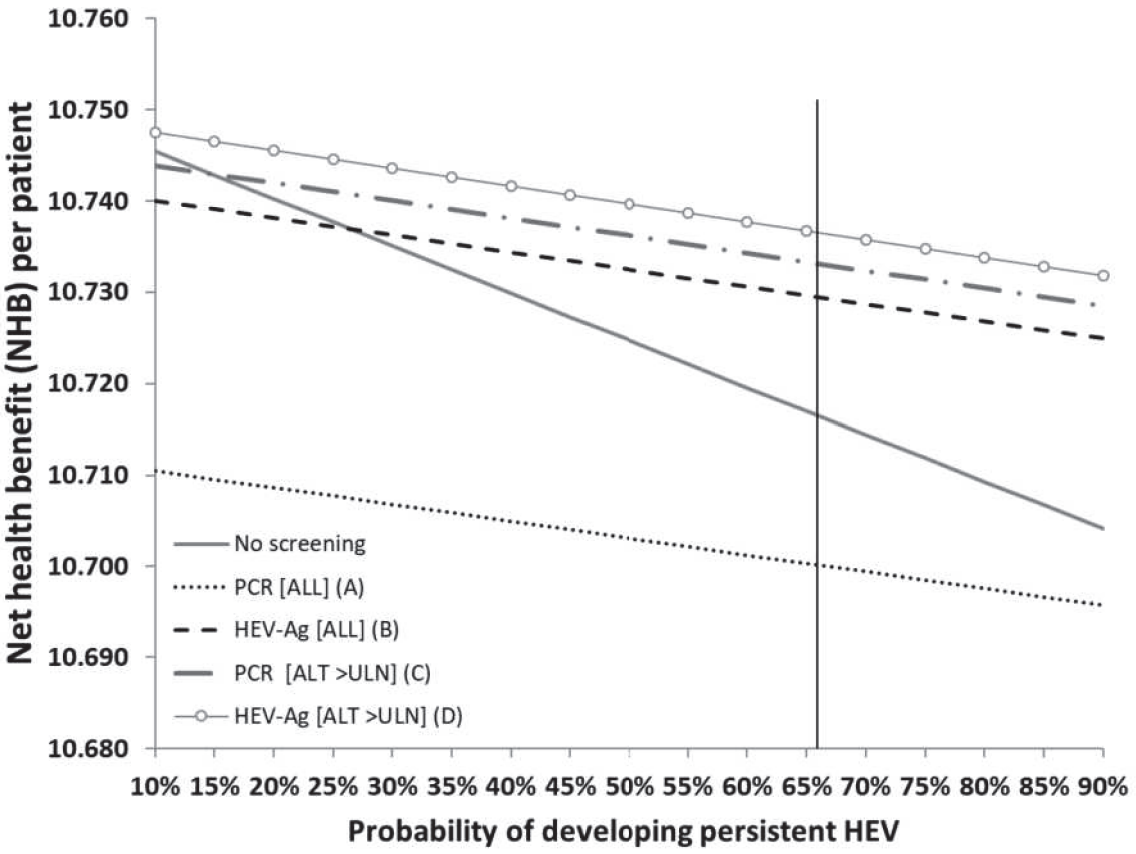
Figure 6.3 Altering the annual dietary risk of HEV



Comparison of the net health benefit per patient for each screening option at the threshold of £20,000/QALY when altering the annual attack rate of HEV infection. The vertical line represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; PCR, polymerase chain reaction; ULN, upper limit of normal.

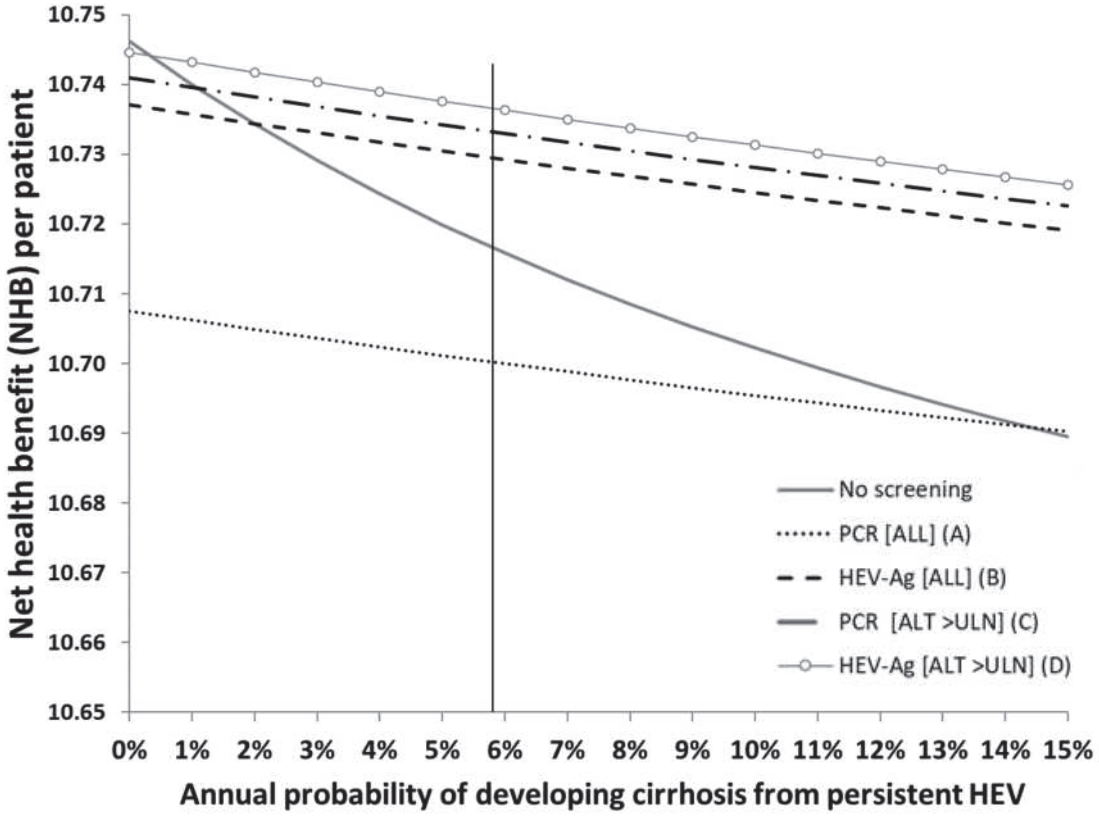
Figure 6.4 Altering the probability of developing persistent HEV infection



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the probability of developing persistent infection in a SOT recipient. The vertical line represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; SOT, solid organ transplant; ULN, upper limit of normal.

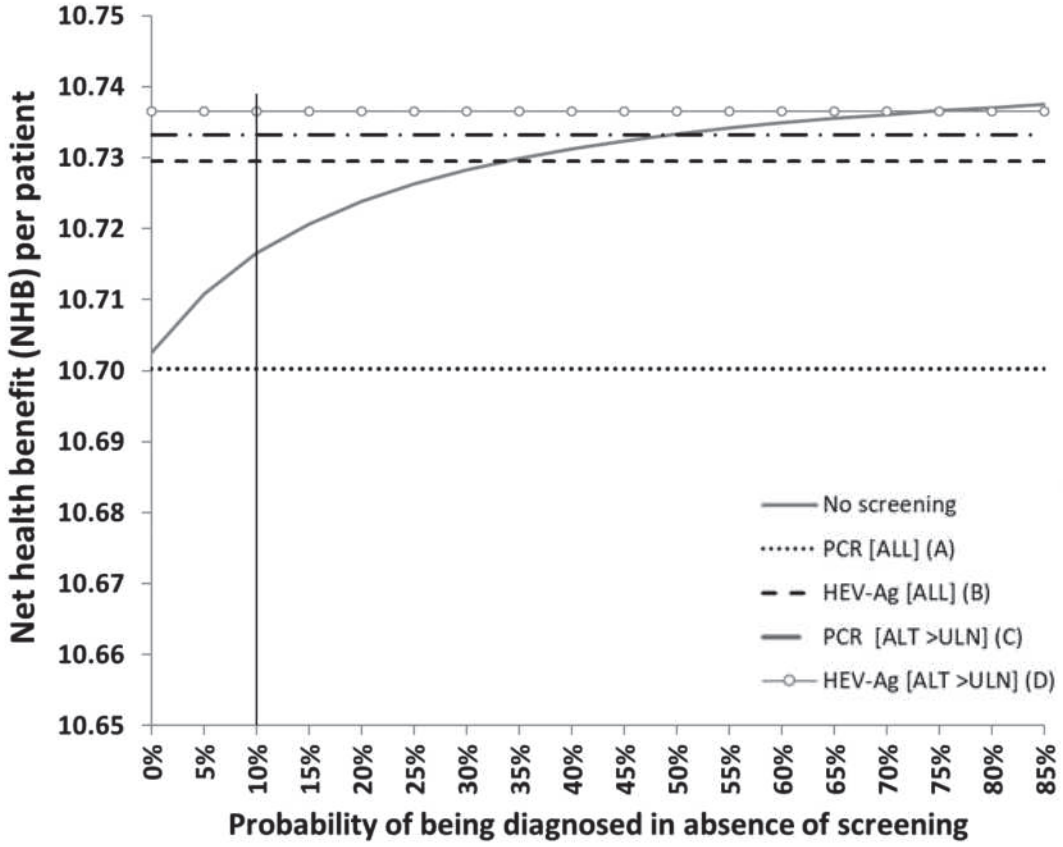
Figure 6.5 Altering the annual probability of developing cirrhosis



Comparison of the net health benefit per patient for each screening option at the threshold of £20,000/QALY when altering the annual probability of an HEV-infected SOT patient developing cirrhosis. The vertical line represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; PCR, polymerase chain reaction; SOT, solid organ transplant; ULN, upper limit of normal.

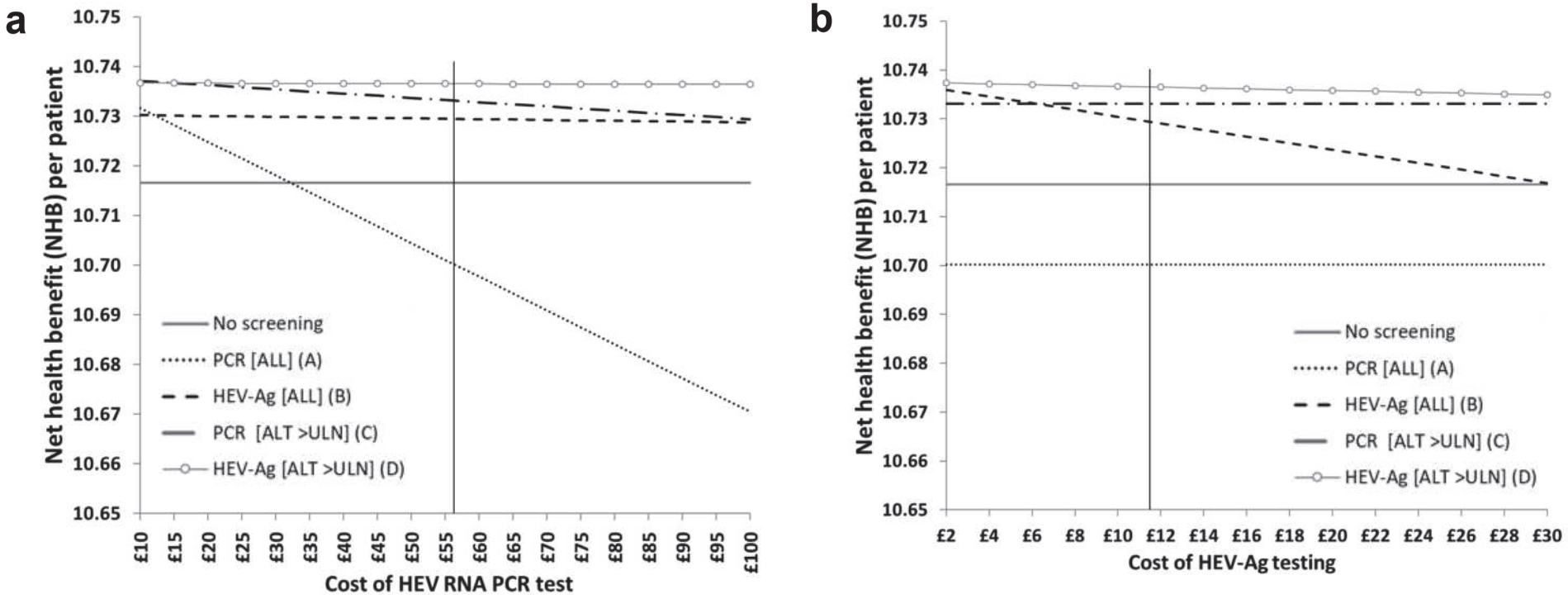
Figure 6.6 Altering the probability of being diagnosed in the absence of HEV screening



Comparison of the net health benefit per patient for each screening option at the threshold of £20,000/QALY when altering the probability a patient is diagnosed with HEV without systematic screening. The vertical line represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; PCR, polymerase chain reaction; ULN, upper limit of normal.

Figure 6.7 Altering the costs of HEV testing



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the assay costs of (a) HEV PCR testing or (b) HEV-Ag testing. The vertical lines represent the base-case values.

Abbreviations: Ag, hepatitis e virus antigen; ALT, alanine aminotransferase; HEV, hepatitis E virus; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

Table 6.4: Incremental cost-effectiveness of HEV screening using alternative model

Strategy	Annual screen assay	Total lifetime cost per patient	Total LY per patient	Total QALYs per patient	Incremental lifetime costs per patient	Incremental LY gained per patient	Incremental QALYs gained per patient	ICER (£) per life year	ICER (£) per QALY
costs and benefits discounted (at 3.5%):									
-	No screening	£ 170	14.06935	10.7326	-	-	-	dominated	dominated
D	HEV-Ag [if ALT > ULN]	£ 115	14.08534	10.7485	- £ 54	0.015988	0.015913	cost saving	cost saving
C	PCR [if ALT > ULN]	£ 188	14.08545	10.7486	£ 73	0.000111	0.000107	£ 657 459	£ 680 872
B	HEV-Ag [ALL]	£ 266	14.08552	10.7486	£ 77	0.000071	0.000068	£ 1 093 933	£ 1 133 841
A	PCR [ALL]	£ 856	14.08562	10.7487	£ 591	0.000100	0.000097	£ 5 900 570	£ 6 120 975
costs and benefits undiscounted (0.0%):									
-	No screening	£ 341	21.32687	16.2671	-	-	-	dominated	dominated
D	HEV-Ag [if ALT > ULN]	£ 189	21.36607	16.3043	- £ 152	0.039196	0.037177	cost saving	cost saving
C	PCR [if ALT > ULN]	£ 297	21.36632	16.3045	£ 108	0.000249	0.000230	£ 432 523	£ 469 350
B	HEV-Ag [ALL]	£ 426	21.36647	16.3046	£ 129	0.000158	0.000146	£ 814 823	£ 884 747
A	PCR [ALL]	£ 1 352	21.36670	16.3048	£ 926	0.000224	0.000206	£ 4 133 838	£ 4 491 313

An alternative model was built shown in which differed in that patients with persistent HEV infection in the compensated cirrhosis state could be screened, diagnosed and treated with identical diagnostic and treatment efficacy as compared with patients with persistent HEV infection without cirrhosis (Figure 2.1). The incremental cost-effectiveness analysis of the base-case results are presented above, with slightly lower costs and slightly higher QALY gains, leading to similar qualitative conclusions as the main analysis (Table 6.2).

6.2.5 Probabilistic sensitivity analysis

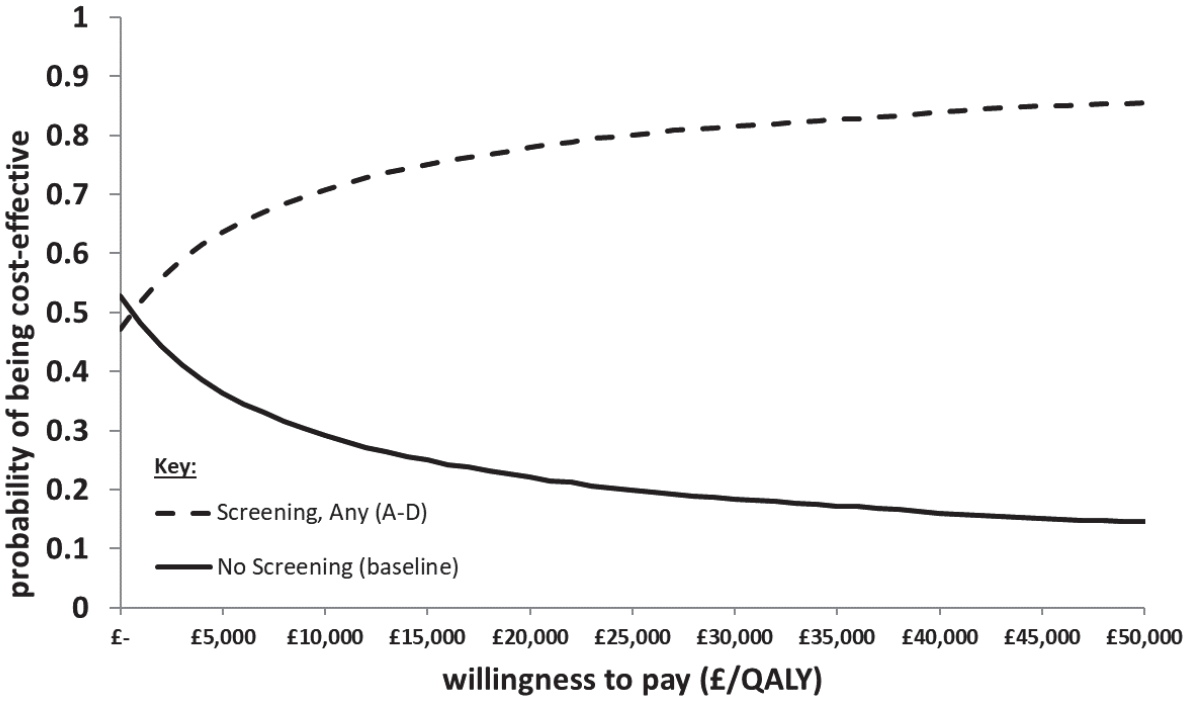
The probabilistic sensitivity analysis, incorporating the uncertainty around the parameters inputs, demonstrated similar results to the base-case results (see Appendix 3.1

for graphical representation of ICER values are for each of the 5000 simulations for each screening strategy).

At a willingness-to-pay of £0/QALY, i.e. if the NHS was unwilling to pay anything extra for any QALYs gained, adopting a systematic screening programme (using any strategy) had a probability of being cost-effective of 47.2% compared with no screening; which increased to 70.8% at £10,000/QALY, 77.9% at £20,000/QALY and up to 81.5% at £30,000/QALY (Figure 6.8 and 6.9).

The cumulative rank probability unambiguously preferred screening patients with an abnormal ALT for HEV-Ag at £20,000/QALY (Figure 6.10), and it offered the highest mean NHB up to £50,000/QALY among the five alternatives analysed (cost effectiveness acceptability frontier - Figure 6.11).

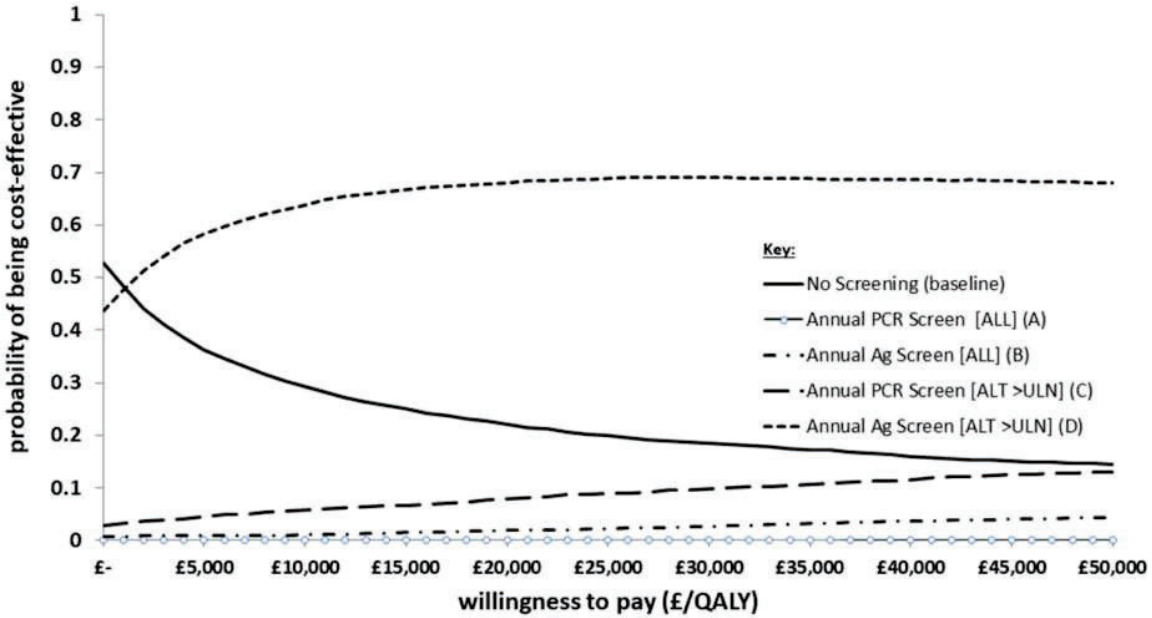
Figure 6.8 Cost-effectiveness acceptability curve for HEV screening by any strategy



Cost-effectiveness acceptability curve comparing screening by any strategy (A-D) with no screening at willingness-to-pay threshold per QALY ranging from £0 to £50,000.

Abbreviations: Ag, hepatitis E virus antigen; ALT, alanine aminotransferase; HEV, hepatitis E virus; PCR, polymerase chain reaction; QALY, quality-adjusted life year; ULN, upper limit of normal.

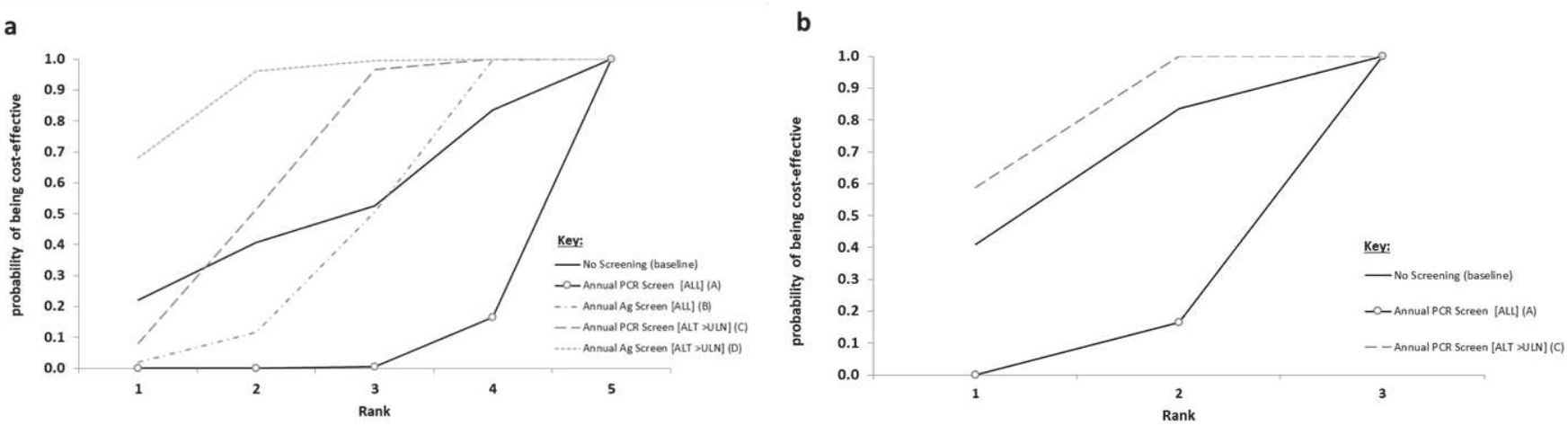
Figure 6.9 Cost-effectiveness acceptability curve for all HEV screening strategies considered (A-D)



Cost-effectiveness acceptability curve comparing all available screening options (A-D) and no screening at willingness-to-pay threshold per QALY ranging from £0 to £50,000.

Abbreviations: Ag, hepatitis E virus antigen; ALT, alanine aminotransferase; HEV, hepatitis E virus; PCR, polymerase chain reaction; QALY, quality-adjusted life year; ULN, upper limit of normal.

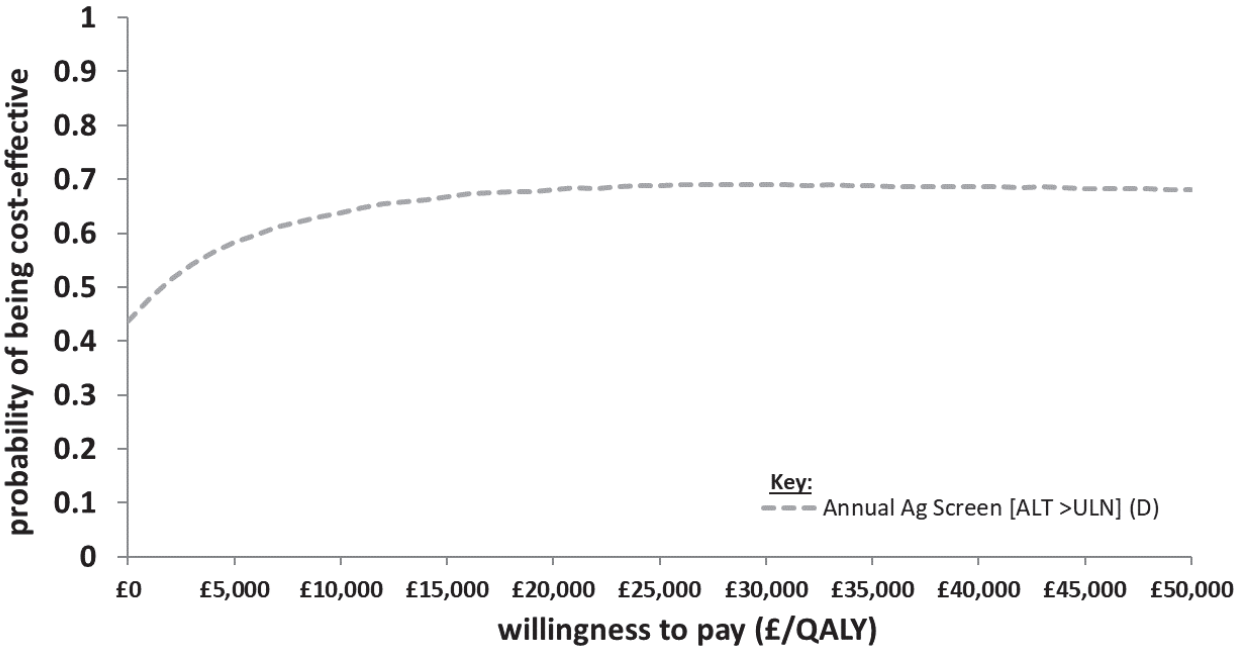
Figure 6.10 Cumulative rank probability of HEV screening being cost-effective



The rankogram depicts the probability that a given screening option (A-D) or no screening (baseline) would rank as being the cost-effective option at the threshold of £20,000/QALY. F(a) shows the rankogram when all options are considered whereas (b) shows the rankogram when only PCR testing is available. Both figures show that testing transplant patients with an abnormal ALT annually (either by HEV-Ag by ELISA or HEV RNA by PCR) have the highest cumulative rank probability of being cost-effective. However, testing all transplant patients annually by PCR irrespective of their ALT (strategy A) is always the least likely to be cost-effective.

Abbreviations: Ag, hepatitis E virus antigen; ALT, alanine aminotransferase; HEV, hepatitis E virus; NHB, net health benefit; PCR, polymerase chain reaction; QALY, quality-adjusted life year; ULN, upper limit of normal.

Figure 6.11 Cost-effectiveness acceptability frontier



Cost-effectiveness acceptability frontier showing the probability of cost-effectiveness of only the optimal option at each willingness-to-pay threshold, i.e. the strategy with the highest mean net benefit. Here, screening by HEV-Ag for patients with an abnormal ALT (strategy D) is optimal at all thresholds tested.

Abbreviations: Ag, hepatitis E virus antigen; ALT, alanine aminotransferase; HEV, hepatitis E virus; PCR, polymerase chain reaction; QALY, quality-adjusted life year; ULN, upper limit of normal.

6.3 Discussion

This chapter aimed to determine the cost-effectiveness of four possible screening strategies for HEV infection amongst solid organ transplant recipients in the UK. Analysis was restricted to SOT patients as they are a clearly defined group, with regular healthcare contact and known to be high risk for persistent HEV infection. To our knowledge, this is the first health-economic evaluation for screening of HEV in transplant recipients. Previous cost-effectiveness analyses for HEV focussed on screening of blood donations to protect vulnerable transfusion recipients in the Netherlands [103], and on a different intervention for different HEV genotypes, such as targeted immunisation of pregnant women or the elderly population in China [326, 327].

This model predicts that HEV screening would benefit the UK transplant population by preventing seven cases of cirrhosis and more than three deaths related to HEV-induced cirrhosis over the lifetime of every 1,000 SOT patients. However, the QALY gains per patient as compared to no screening were modest, and only testing those patients who have an abnormal ALT can be considered cost-effective (and, in fact, testing by HEV-Ag detection is cost-saving over the current practice of no screening). HEV screening of SOT patients by any of the methods considered had more than a 70% probability of being cost-effective above a willingness-to-pay threshold of £10,000/QALY. Annual testing by HEV-Ag detection in those patients who have an abnormal ALT is predicted to lead to the highest net benefit across the entire range of £0-£50,000/QALY.

Impact on policy

Despite universal screening of blood and tissue donors in the UK, the dominant residual dietary risk of HEV infection remains [102]. If systematic screening for HEV were implemented in SOT recipients, it would be predicted to identify early infections and provide the opportunity for treatment before substantial liver disease can evolve. In the case of HEV, the timing of screening is critical since cirrhosis can develop over a relatively short time in SOT recipients [207].

HEV-Ag detection is predicted to be the most cost-effective option; however, local decisions on the choice of screening assay may be influenced by practical considerations, including laboratory workflows which may be more suited to implementing an additional PCR to their testing repertoire rather than a manual plate-based ELISA. If PCR testing is available locally, then testing patients with an abnormal ALT is still expected to be cost-effective (Table 6.3).

A critical assumption of the model is that screening identifies more HEV infections than without screening. Results from Chapter 4 showed that only 10% of HEV infections were clinically diagnosed in transplant recipients. However, this was a single-centre study and in areas of higher awareness of HEV this assumption may be incorrect. However, the scenario analysis demonstrated that even if up to 50% of persistent HEV infections are clinically diagnosed in the absence of screening, selective testing of those with an abnormal ALT is still providing a higher net benefit than no screening at £20,000/QALY.

The results of this analysis can be regarded as conservative with regards to the assay costs, in particular for PCR, as reference laboratory costs were used as a proxy for the costs of PCR testing. However, with regional laboratories introducing commercial

HEV PCR assays lower costs may be negotiated. Furthermore, only testing of individual patient samples for HEV was considered, yet the high sensitivity of nucleic acid detection allows HEV PCR testing to be undertaken on pooled samples. This is commonly done in the blood transfusion service to reduce costs significantly, but is not conventional practice in NHS laboratories. The number of samples contributing to each pool can be optimised by consideration of the expected prevalence of infection to restrict the numbers of pools which will require individual resolution. In this model HEV-Ag reactive results were confirmed by PCR testing. However, in Chapter 3 the development of a neutralisation assay proved that the specificity of a reactive HEV-Ag ELISA could be confirmed by less costly methods than PCR, but this is not commercially available currently.

Lastly, the impact of a HEV vaccine becoming licensed for the UK market is unknown. So far, two vaccine candidates have reached advanced phases of clinical research, and one has been marketed in China [328]. However, it is unclear when a vaccine will become more widely available internationally, and whether it will demonstrate efficacy in preventing persistent HEV infection in transplant patients.

Strengths and limitations

This is the first cost-effectiveness analysis of screening for HEV in SOT patients and so this led to a level of uncertainty regarding the accuracy of parameter inputs, many of which were derived from small case series. As a result, extensive uncertainty analyses were performed which showed the qualitative conclusions to be robust, and many scenarios were explored which could be of future relevance in other contexts or countries.

The risk of HEV acquisition varies markedly over time and depends upon animal husbandry practices, importation patterns of pork meat, individual dietary habits and country-specific policies on screening blood, tissue and organ donors for HEV. The risk of dietary acquisition of HEV was based on blood donors in England in the base-case, which may not reflect the risk in SOT recipients adequately due to generic dietary advice given to this cohort [22, 102]. If annual attack rates dropped significantly to as low as 0.04%, as previously recorded in Scotland [27], the only strategy that generated a higher net health benefit than no screening at £20,000/QALY was screening patients with a raised ALT by HEV-Ag detection. Therefore, if screening were implemented it would be important to periodically monitor population HEV incidence to re-evaluate cost-effectiveness as the dietary risk of HEV changes.

An annual screen was considered as a pragmatic compromise given the potential ongoing dietary acquisition of HEV and clinical follow-up schedules of SOT recipients. More frequent screening may also be cost-effective for the NHS, but the limited granularity regarding the natural history of liver disease during HEV infection in transplant cohorts prevented assessment of more frequent testing schedules.

The model did not include intermediate stages of liver fibrosis prior to the development of cirrhosis due to limited data to inform this. Therefore, clinically relevant fibrosis, which may occur prior to diagnosis, was not explicitly accounted for. In contrast to modelling for HCV, the impact of HEV screening on secondary human cases was not considered since direct human-to-human transmission rarely occurs with G3 HEV. The model also assumed that SOT patients were at risk of HEV re-infection after clearance of a primary infection [256]. Whilst re-infections are known to occur, this assumption may not be true for patients who have partial immune reconstitution following the modulation of iatrogenic immunosuppression or if they alter their dietary habits.

The main problem faced in building the model was finding and deriving accurate figures for transition probabilities, healthcare costs and utilities to input into the model structure. There are few studies which cast light on the long-term consequences of HEV infection; available studies include small numbers of patients leading to imprecision in estimating complication rates. For example, the probability of developing persistent HEV infection, the annual probability of developing cirrhosis and the risk of decompensation and the requirement for liver transplantation were derived from relatively small cases series of less than 100 patients reported by Kamar *et al* [207, 329]. This is reflective of HEV having been underestimated in developed countries [330]. This meant certain parameters were derived from studies outside the context of organ transplantation and from other viral hepatitides, such as HCV, including health-related quality-of-life scores for patients undergoing treatment. However, findings were robust to changes of these parameters explored in sensitivity analysis. Similarly, all neurological complications were costed as for Guillain-Barré syndrome to simplify the analysis and, given that such neurological outcomes in transplant recipients are rare, yet in reality neurological complications of persistent HEV infection are more heterogeneous and may also include neuralgic amyotrophy or encephalitis [331]. Of note is that in contrast to established models for hepatitis C virus (HCV), a health state of hepatocellular carcinoma (HCC) was not included in the model given that an association between HCC and HEV infection has not been established, with only one published case of HCC complicating HEV-related cirrhosis [215, 332]. Lastly, in line with a few previous studies on HEV this model did not include a utility decrement for the health state of asymptomatic infection due to the absence of such information in HEV. These problems of data availability highlight the need for further research in HEV infections.

This analysis was conducted from the perspective of the UK NHS. Therefore, resources were valued using national NHS list prices and reference costs and expressed in pound sterling (GBP, £) [258-260]. Up-to-date UK-specific costs were used when available with 2017 as the base year; in other circumstances an inflation index up to 2017 was applied [333]. Due to this bias and because HEV attack rates vary markedly geographically the primary findings of the main analysis are not necessarily directly applicable in other geographic locales.

6.4 Conclusions

- Implementing a screening programme for HEV infection in SOT recipients has a very high probability of being cost-effective in the UK.
- Limiting testing to those SOT recipients who present an abnormal ALT would be optimal from the NHS perspective.

7 Evolution of HEV quasispecies during persistent infection

7.1 Introduction

HEV is a single-stranded positive-sense RNA virus which shows considerable diversity within genotypes, particularly within G3 and G4. The HEV G3 was initially classified into 10 subtypes a-j, now divided into three major clades known as clade 1 (subtypes efg), clade 2 (subtypes abchij) and a third clade (subtype ra) [287, 334-336]. The infecting subtype of G3 HEV may impact upon clinical outcome; in blood donors, clade 1 infections have been found to lead to more symptoms with higher viral loads than clade 2 infections [41]. HEV infections consist of a mixture of heterogeneous viruses known as quasispecies and the diversity found within a quasispecies may also be important in determining outcomes [337]. An increased heterogeneity in the M domain of ORF2 correlated with a higher risk of developing persistent infection in one study of solid organ transplant recipients [338].

In persistent HEV infection, ribavirin is commonly administered to patients to achieve viral clearance [223]. Ribavirin is a synthetic broad-spectrum antiviral, however the mechanism of action against HEV replication is not well characterised. In norovirus models, ribavirin increases quasispecies diversity *in vitro* and in a recent study in persistent HEV infection was found to increase viral heterogeneity in all open reading frames in a reversible manner [339]. Ribavirin-mediated increase in diversity seen in RNA viruses appears to be due to non-specific incorporation of the drug into the viral genome, leading to so-called error catastrophe and lethal mutagenesis [340]. Nevertheless, specific mutations are reported in the RNA-dependent RNA-polymerase

region associated with failure of ribavirin treatment, but which do not behave as drug resistance mutations in isolation. For instances, the G1634R mutation has been found in a number of non-responders and *in vitro* increases the replicative capacity of HEV, but is insufficient in isolation to lead to ribavirin resistance [245, 341]. Cases of non-response are rare and limited to case reports presently but lead to challenging treatment decisions [245, 247, 341, 342]. This chapter aims:

1. to investigate the evolution of mutations during persistent HEV infections under antiviral drug treatment.
2. to investigate the evolution of mutations during persistent HEV infections in untreated patients.
3. to characterise quasispecies diversity during persistent HEV infections in treated and untreated patients.

7.2 Results

7.2.1 Patient Characteristics

Eighteen patients with HEV G3 viraemia and defined virological outcomes were selected (summarised in Table 7.1). The median age of the cohort was 56 years (range 21-84 years) and 11 patients were male. One patient cleared HEV spontaneously (Patient A), two patients remain untreated (Patient B and C), two patients achieved a sustained virological response (SVR) following standard first-line treatment (Patient D and E) and 11 patients suffered virological rebound/relapse or breakthrough (Patient F-P). One patient developed apparent ribavirin resistance following relapse (Patient Q) and a second had a very poor response to a primary course of ribavirin (Patient R). Examples of two patients harbouring HEV phenotypically resistant to ribavirin therapy are shown in Figure 7.1; the virological profiles for all other patients, including the timing of all samples sent for HEV Illumina sequencing, can be viewed in Appendix A5.1.

7.2.2 Quality of sequencing data

Of 68 samples from 18 individuals, Illumina sequencing with HEV reference genome mapping successfully generated consensus sequence data covering over 70% of the genome for 47 samples. For 40 samples, coverage exceeded 90%. There was a strong correlation observed between the log HEV viral load in the primary sample and the percentage coverage achieved by consensus data (Pearson's correlation 0.8043 ($p<0.001$), Figure 7.2).

Of the 21 samples with consensus data covering less than 70% of the HEV genome, 16 of the prepared libraries were selected for repeat and underwent double enrichment. In only three cases was the HEV genome coverage higher following double enrichment compared to single enrichment, but none exceeded 70% coverage (Figure 7.3). Where viral loads were low in relapse samples, reference mapping was attempted using the consensus HEV sequence from an earlier sample derived from the same patient, however this did not yield higher genome coverage (data not shown).

Of the samples achieving more than 70% HEV genome coverage, six plasma samples were taken while on ribavirin therapy and eight samples were taken following an unsuccessful course of ribavirin therapy. The quality reports, genome coverage and full consensus sequence data for each sample can be found in Appendix A5.4.

Table 7.1 Characteristics of patients analysed by Illumina WGS

Pt	Underlying disease	Sex	Age, y	1st line treatment	Virological outcome	Samples sequenced[a] total/ pre-treatment/ on or post treatment	Time-span of sequenced samples, m	HEV viral load of failed samples (IU/ml)
A	Liver SOT	M	60	NA	spontaneous clearance	1/NA/NA	NA	1.5×10^3 (n=1)
B	Liver SOT	M	75	NA[b]	ongoing viraemia	4/NA/NA	64	1.4×10^4 (n=1)
C	Heart SOT	M	67	NA[b]	ongoing viraemia	3/NA/NA	14	-
D	Allogeneic-HSCT	F	36	RBV 5m	3/12 SVR	2/2/0	3	-
E	HIV	M	45	PEG-IFN 5.5m	6/12 SVR	3/3/0	120	-
F	Kidney SOT	F	42	RBV 6m	rebound	3/2/1	29	4.4×10^4 (n=1)
G	Kidney SOT	M	65	RBV 21m	rebound	2/1/1	3	1.2×10^3 -1.7×10^4 (n=4)
H	CLL	M	52	RBV 6.6m	rebound	2/1/1	14	6.8×10^3 (n=1)
I	Lymphoma	F	84	RBV 3.7m	relapse	3/1/2	13	4.9×10^4 (n=1)
J	Lymphoma	F	44	RBV 2m	relapse	2/2/0	4	4.3×10^3 (n=1)
K	Kidney SOT	F	51	RBV 3.9m	relapse	4/2/2	23	-
L	Liver SOT	M	52	RBV 2.8m	relapse	3/1/2	9	1.7×10^4 (n=1)
M	Liver SOT	F	27	RBV 4.2m	relapse	1/0/0	NA	4.4×10^4 - 7.6×10^4 (n=2)
N	Heart/Kidney SOT	M	66	RBV 2.8m	relapse	3/2/1	24	1.0×10^3 (n=1)
O	nil	M	63	RBV 2.5m	relapse	1/1/0	NA	1.4×10^3 – 3.0×10^4 (n=2)
P	Lymphoma	F	21	RBV 11m	rebound	2/2/0	31	8.8×10^2 – 1.0×10^3 (n=2)
Q	Lymphoma	M	76	RBV 3m	relapse[c]	5/1/4	22	5.5×10^4 (n=1)
R	Lymphoma	M	79	RBV 28.8m	poor primary response	3/3/0	15	1.5×10^4 (n=1)

Legend for Table 7.1:

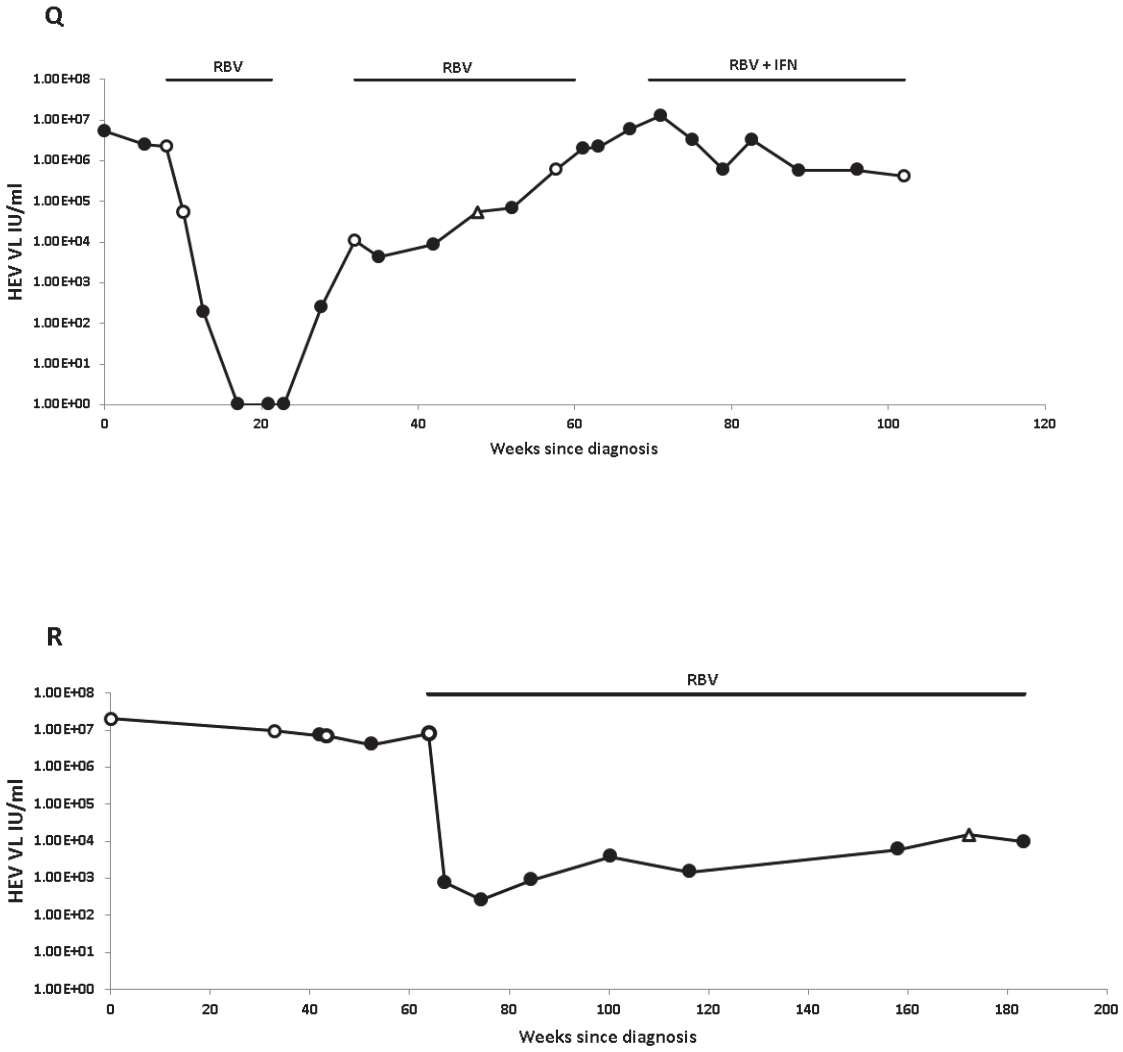
[a] With HEV genome coverage >70%.

[b] Patient undergoing regular monitoring without treatment.

[c] Patient had response to a second course of ribavirin.

Abbreviations: CLL, chronic lymphocytic leukaemia; HIV, human immunodeficiency virus; HSCT, haematopoietic stem cell transplant; m, months; NA, not applicable; PEG-IFN, pegylated interferon; Pt, patient; RBV, ribavirin; SOT, solid organ transplant; SVR, sustained virological response; WGS, whole genome sequencing; y, years.

Figure 7.1 Virological profiles of patients demonstrating ribavirin resistance

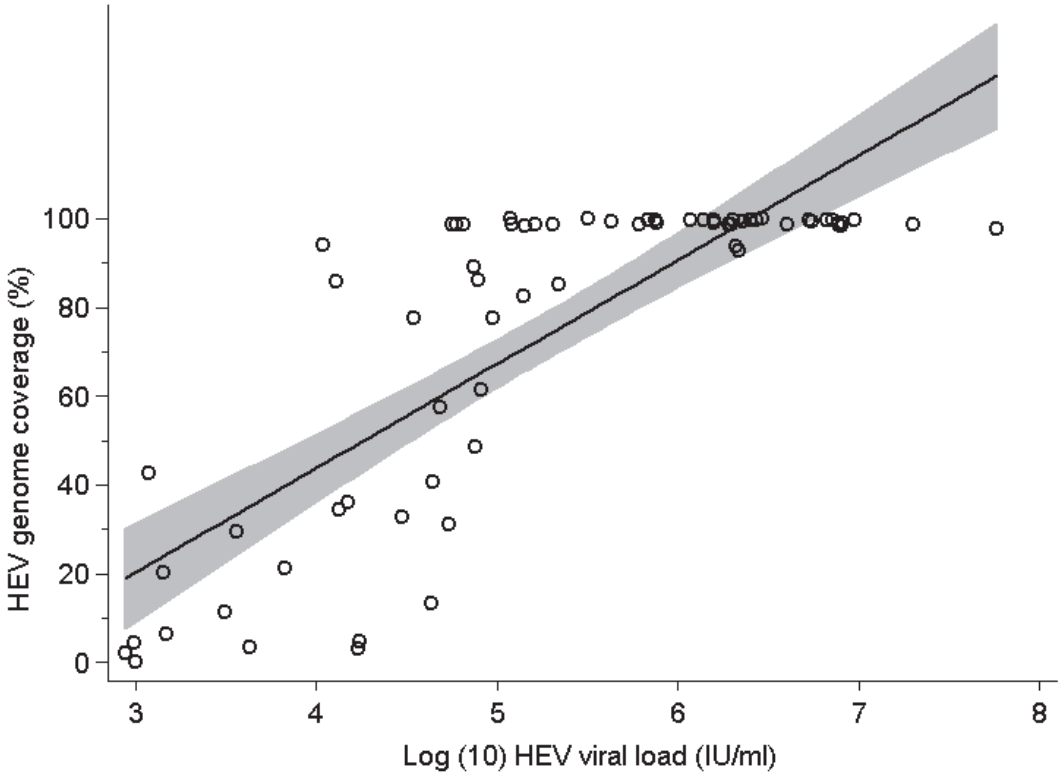


Virological profiles from serum samples of two patients infected with HEV with apparent phenotypic resistance to ribavirin are presented.

Open circles represent samples successfully sequenced and open triangles represent samples unsuccessfully sequenced.

Abbreviations: IFN, pegylated interferon; RBV, ribavirin; VL, viral load.

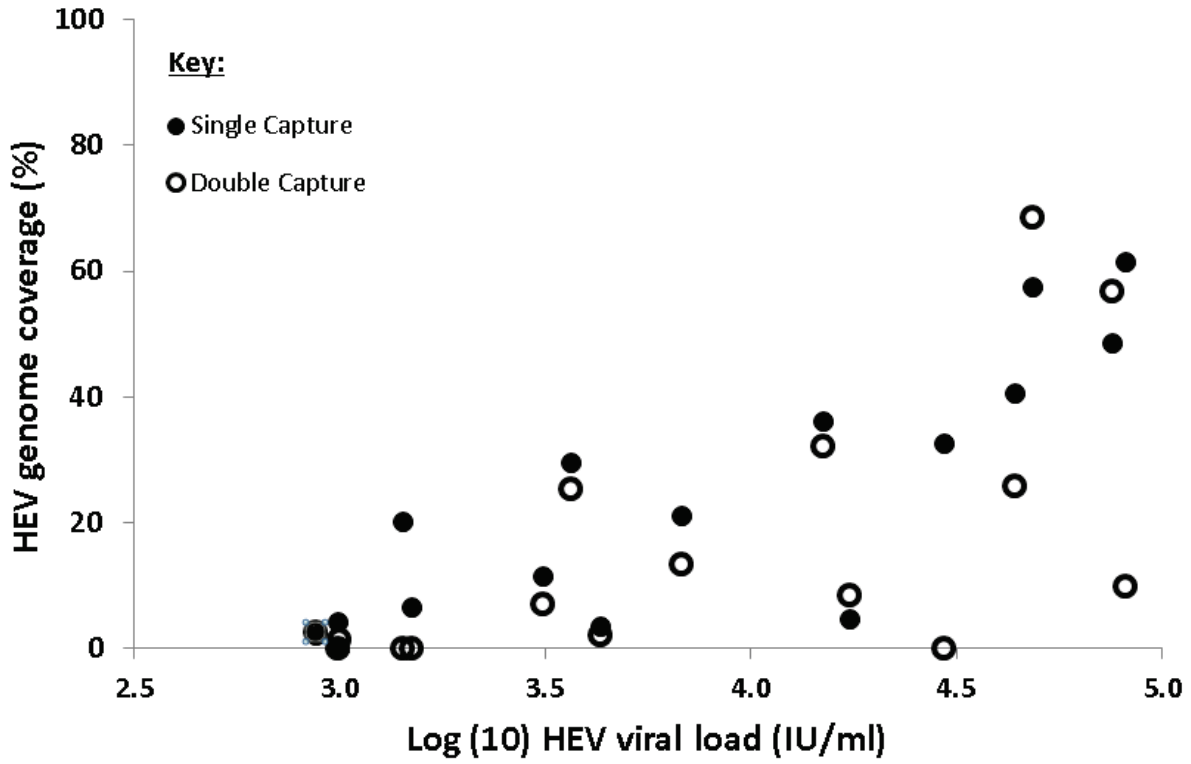
Figure 7.2 Correlation of viral load and genome coverage



Correlation of viral load in primary sample and percentage of genome covered by Illumina whole genome sequencing.earson's correlation 0.8043 ($p<0.001$).

Abbreviations: IU, international units; ml, millilitres.

Figure 7.3 Single and double probe target enrichment of HEV samples



Genome coverage by Illumina sequencing of samples following single and double probe target enrichment of HEV samples with viral loads lower than 5 log IU/ml.
Abbreviations: IU, international units.

7.1.1 HEV Polymerase region (RdRp) analysis

7.1.1.1 HEV Polymerase mutations detected in baseline samples

We looked for specific mutations in HEV RNA polymerase region of ORF1 that have been described in association with ribavirin treatment failure (Y1320H, K1383N, D1384G, K1398R, V1479I, Y1587F and G1634R/K) and found these mutations in the baseline consensus sequences of five patients (Table 7.2) [245-247]. These were detected in the baseline samples of patient E (V1479I), patient I (V1479I, G1634K), patient M (V1479I, G1634R), patient P (V1479I) and patient R (V1479I). Only one of these five patients achieved a sustained virological response; this patient was treated with pegylated interferon without ribavirin (Patient E, Table 7.1/Table 7.2).

7.1.1.2 Evolution of RdRp mutations after ribavirin treatment

In two patients several of the putative RdRp mutations associated with ribavirin treatment failure developed after initiating ribavirin treatment (Table 7.2). Patient Q had a strong primary response to ribavirin treatment, but relapsed and developed multiple mutations associated with treatment failure which appear to become fixed as the dominant amino acid in the viral quasispecies during re-treatment (V1305I, K1383N, D1384G, V1479I and G1634R) (Figure 7.4). In contrast other mutations developed in the RdRp region leading to amino acid changes (V1210M, L1227F, N1372S, V1479I, V1499I, F1543L, K1544R, W1614R, T1636M, A1666T) which were subsequently lost and did not appear to be fixed (Figure 7.5). Patient I developed the K1383N mutation in both samples following relapse but was not re-treated during the

follow-up of the study. Two other mutations developed in the initial relapse sample (R1318K and A1598V) but were subsequently lost (Figure 7.6).

7.2.2.1 Evolution of RdRp mutations in absence of ribavirin treatment

In three patients, mutations developed in the RdRp region of HEV in the absence of ribavirin therapy (Table 7.2). Patient C developed two mutations (V1285A and V1305I) which were subsequently lost. Patient E developed five mutations (V1305I, T1317A, V1365I, S1372N and S1454C) which appeared over a period of ten years of infection. Finally, patient F developed two mutations (P1453S and V1479I), one of which has been associated with ribavirin failure, prior to ribavirin initiation.

Table 7.2 Amino acid polymorphisms in RdRp of patient samples

Pt	Virological outcome	Virus genotype	Baseline RdRp mutations[a]	RdRp mutations after initiation of RBV[a]	Other AA switches after initiation of RBV[a]	AA switches in absence of RBV[a]
A	spontaneous clearance	3c	-	-	-	-
B	ongoing viraemia	3c	-	-	-	-
C	ongoing viraemia	3c	-	-	-	V1285A V1305I
D	3/12 SVR	3c	-	-	-	-
E	6/12 SVR	3 unassigned	V1479I	-	-	V1305I T1317A V1365I S1372N S1454C
F	rebound	3c	-	-	N1372S	P1453S V1479I
G	rebound	3c	-	-	-	-
H	rebound	3c	-	D1384N*	I1533V	-
I	relapse	3e	V1479I G1634K	K1383N D1384H*	R1318K A1598V	-
J	relapse	3c	-	-	-	-
K	relapse	3c	-	-	Q1690H	-
L	relapse	3c	-	-	E1329G I1533V	-
M	relapse	3 unassigned	V1479I G1634R	-	-	-
N	relapse	3c	-	-	-	-
O	relapse	3c	-	-	-	-
P	rebound	3c	V1479I Y1320F*	-	-	-
Q	relapse	3c	-	K1383N D1384G V1479I G1634K/R	V1210M L1227F V1305I N1372S V1499A F1543L K1544R W1614R T1636M A1666T	-
R	poor primary response	3c	V1479I		-	

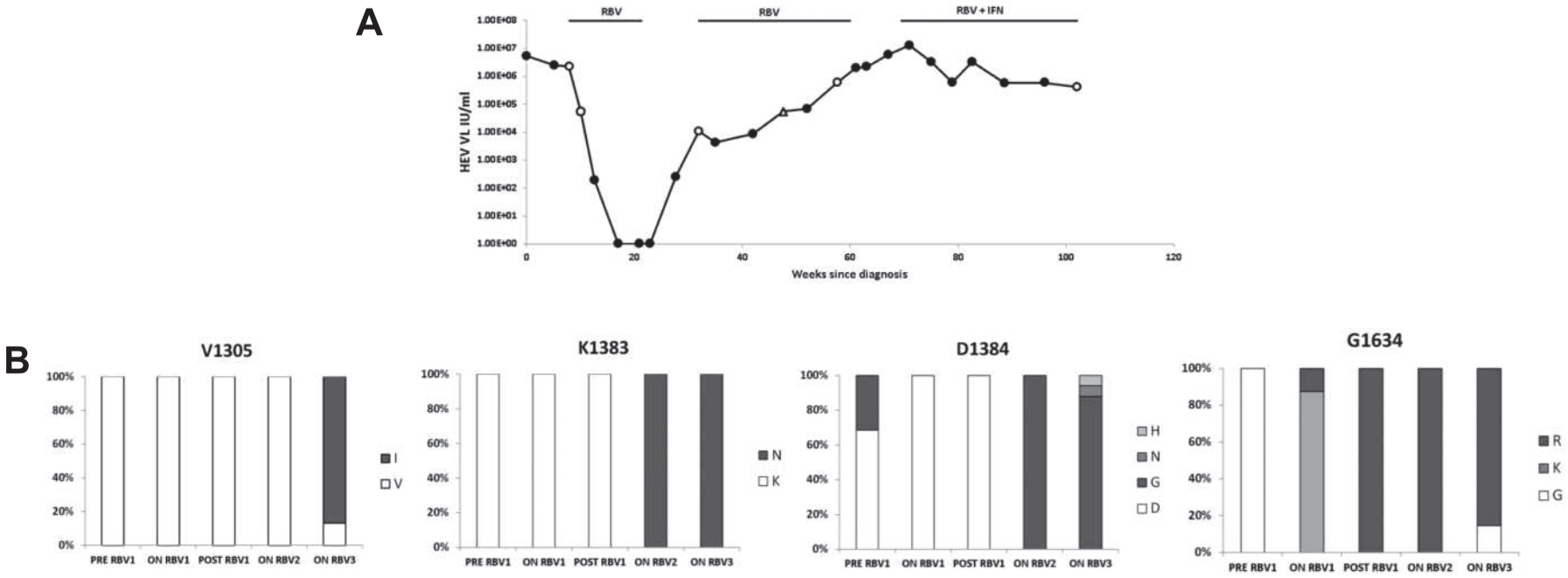
Amino acid polymorphisms in RdRp consensus sequences of patient samples.

[a] Reported only if polymorphism was dominant amino-acid and depth was >10 at site.

*Amino acid switch at loci where mutations associated with treatment failure are described but specific amino acid switch not reported previously.

Abbreviations: AA, amino acid; m, months; NA, not applicable; Pt, patient; RdRp, RNA-dependent RNA-polymerase; RBV, ribavirin; SVR, sustained virological response.

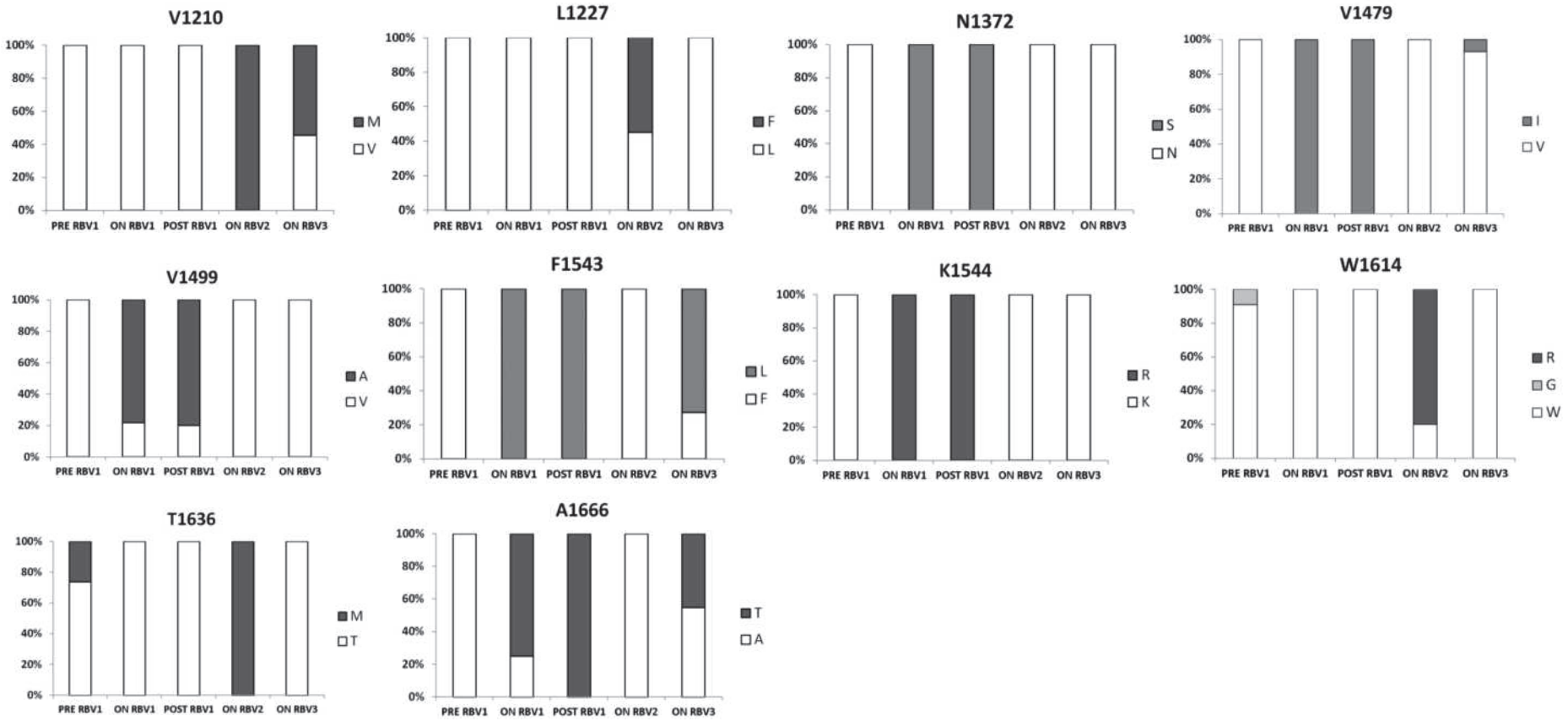
Figure 7.4 Evolution of fixed RdRp mutations associated with treatment failure in patient Q



Evolution of apparently fixed RdRp mutations associated with RBV treatment failure in patient Q

(A) Virological profile of patient Q where open circles represent samples successfully sequenced and open triangles represent samples not successfully sequenced. (B) Evolution of polymorphisms which are apparently becoming fixed associated with ribavirin treatment failure in patient Q. Courses of ribavirin are noted as 1, 2 and 3. Loci are labelled above each histogram with the dominant amino acid in the baseline sample Analysed using HEV GLUE offline (Version 0.1.152, Copyright (C) 2018 The University of Glasgow). Quality control acceptance criteria: minimum q score of 30 and frequency percentage of amino acid at 5%. Loci only analysed with a depth of 10 or above were recorded. Abbreviations: RBV, ribavirin; RdRp, RNA-dependent RNA-polymerase.

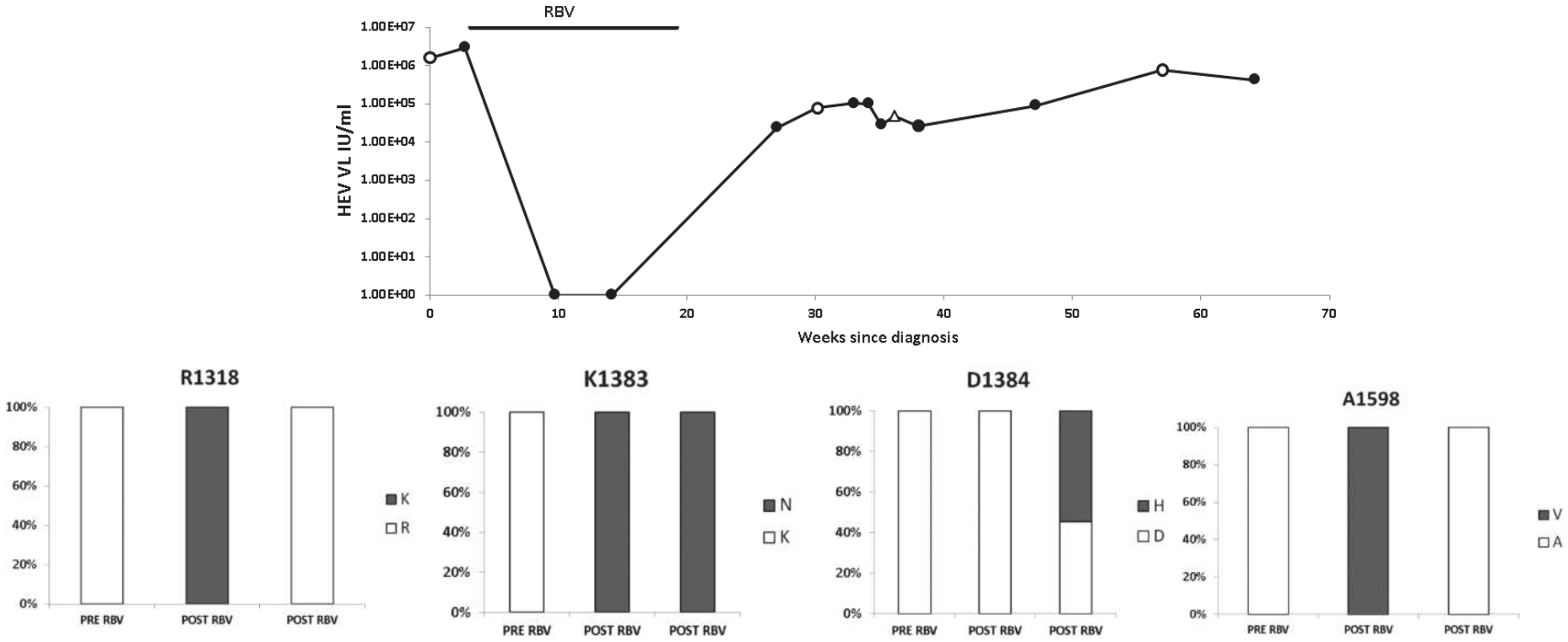
Figure 7.5 Evolution of transient RdRp polymorphisms in patient Q



Evolution of apparently transient RdRp polymorphisms in relation to ribavirin treatment in patient Q. Courses of ribavirin are noted as 1, 2 and 3. Loci are labelled above each histogram with the dominant amino acid in the baseline sample.

Abbreviations: RBV, ribavirin.

Figure 7.6 Evolution of all RdRp mutations in patient I



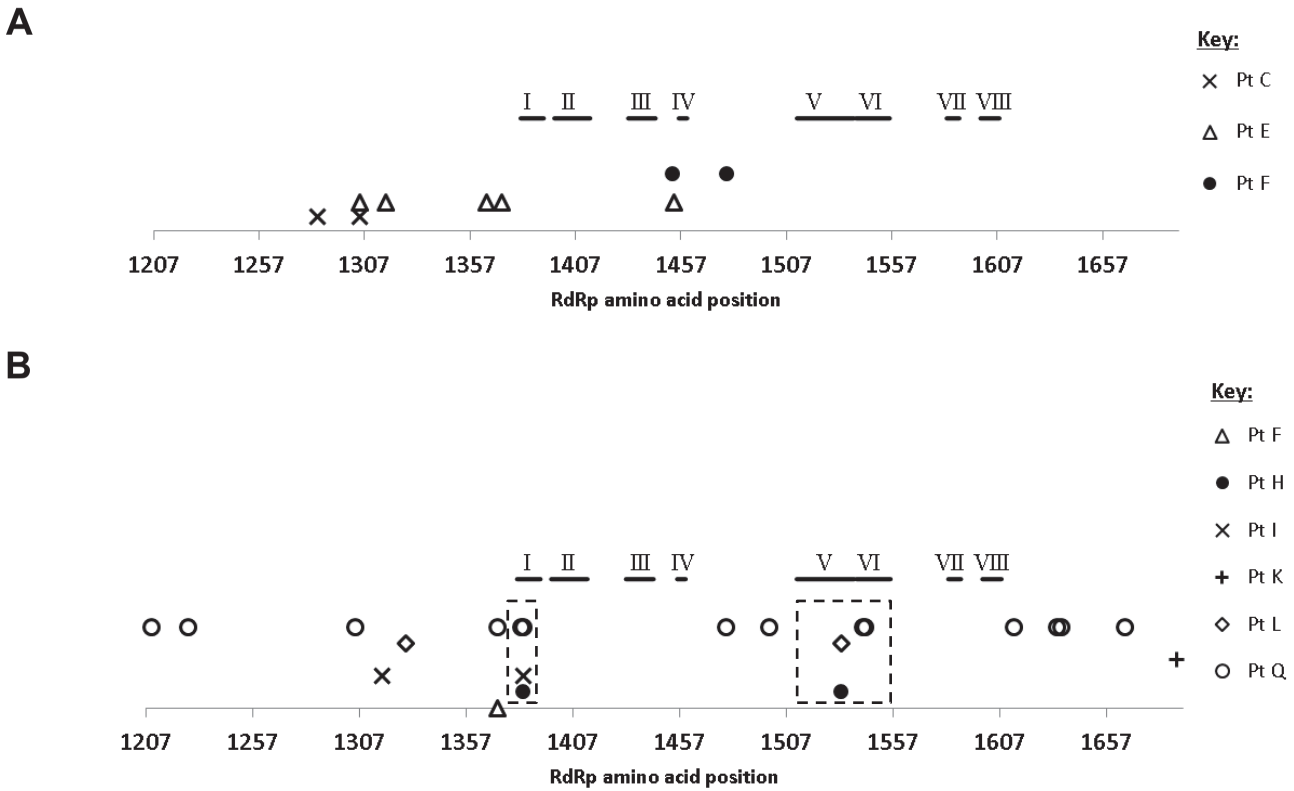
Evolution of all RdRp polymorphisms before and following ribavirin treatment in patient I. Open circles represent samples successfully sequenced and open triangles represent samples not sequenced successfully.

Abbreviations: RBV, ribavirin; RdRp, RNA-dependent RNA-polymerase.

7.2.2.2 Presence of RdRp mutations occurring in conserved regions

None of the mutations occurring in the absence of ribavirin therapy occurred in the recognised conserved motifs (I-VIII) of viral polymerases [343]. In contrast multiple mutations detected after the initiation of ribavirin therapy occurred in these conserved motifs; K1383N in motif I (patient I and Q), D1384N/H/G in motif I (patient H, I and Q), I1533V in motif V (patient H and L), F1543L in motif VI (patient Q) and K1544R (patient Q). No mutations were seen in the magnesium binding sequence (GDD) (motif VI) required for RdRp activity [344].

Figure 7.7 Location of RdRp mutations with or without drug pressure in conserved motifs



Location of amino acid switches occurring in the RdRp gene in 18 patients in the absence of ribavirin treatment (A) and with ribavirin treatment (B). Motifs I-VIII are shown which represent proposed conserved motifs found in the RdRp of positive-strand RNA viruses [343]. Mutations occurring within conserved motifs are highlighted in dashed rectangles.

Abbreviations: Pt, patient; RdRp, RNA-dependent RNA-polymerase.

7.2.2.3 Determination of frequency of RdRp mutations in published Genbank sequences

The proportion of published G3 HEV sequences in GenBank bearing the eight previously reported RdRp mutations, mutations occurring in conserved RdRp motifs and the V1305I mutation identified in the current study were investigated (Table 7.2). The V1479I was commonly found in certain subtypes; amongst 100% of subtypes 3e, 3f and 3g and 12.5% of 3c sequences. The G1634R was found in 94% of 3e sequences, 31% of 3f sequences and the G1634K in 100% of 3ra sequences. The other mutations were rarely found in previously published sequences.

Table 7.3 Frequency of HEV RdRp mutations in published sequences

Mutation Group	HEV Polymerase mutation	HEV subgenotype and ancestor-constraining reference sequence									
		3a	3b	3c	3e	3f	3g	3h	3i	3j	3ra
		AF082843	AP003430	FJ705359	AB248521	AB369687	AF455784	JQ013794	FJ998008	AY115488	FJ906895
A	Y1320H	0/35	0/73	0/6	0/16	1/26	0/1	0/1	0/2	0/1	0/16
	K1383N	0/35	0/73	0/6	0/16	1/26	0/1	0/1	0/2	0/1	0/16
	D1384G	0/34	0/73	0/6	0/16	0/26	0/1	0/1	0/2	0/1	0/16
	K1398R	0/35	0/73	0/7	0/16	0/26	0/1	0/1	0/2	0/1	0/16
	V1479I	1/110	1/76	11/88	63/63	97/97	4/4	0/3	2/5	0/1	16/16
	Y1587F	0/28	0/58	0/7	0/17	0/26	0/1	0/1	0/3	0/1	0/16
	G1634R	0/27	0/58	0/7	16/17	8/26	0/1	0/1	0/3	1/1	0/16
	G1634K	0/27	0/58	0/7	1/17	0/26	0/1	0/1	0/3	0/1	16/16
B	V1305I	0/26	0/57	0/6	0/16	0/26	0/1	0/1	0/2	0/1	0/16
C	D1384H	0/34	0/73	0/6	0/16	0/26	0/1	0/1	0/2	0/1	0/16
	D1384N	0/34	0/73	0/6	0/16	0/26	0/1	0/1	0/2	0/1	0/16
	I1533V	1/37	0/74	0/7	0/17	0/26	0/1	0/1	0/3	0/1	1/16
	F1543L	7/37	11/74	0/7	17/17	25/26	0/1	0/1	0/3	0/1	1/16
	K1544R	34/37	73/74	0/7	15/17	26/26	0/1	0/1	3/3	1/1	5/16

Frequency of HEV RdRp mutations in published sequences in GenBank. Mutations were selected on the basis that (A) they had been published in association with ribavirin failure or (B) there was apparent fixing during ribavirin failure in this study (V1305I) or (C) they occurred in conserved motifs. HEV GLUE off-line was used to interrogate GenBank published sequences as of August 2018. Any sequences containing the amino acid of interest was included.

Abbreviations: GT, genotype; RdRp, RNA-dependent RNA polymerase

7.2.3 HEV ORF2 analysis

Mutations also occurred in the ORF2 region both in the absence of ribavirin therapy and in the presence or after ribavirin therapy (Table 7.3). Most mutations occurred in the first 110 amino acids of ORF2 outside the shell, middle or protruding domain (Figure 7.8). There was a striking absence of mutations in the shell domain. Multiple mutations were detected occurring in the protruding domain where neutralisation epitopes are situated; patient B (N562D, T585A and T586A), patient E (F500L and I528T/V), patient F (C532Y), patient I (E448D and A467T), patient N (Y532H), patient Q (V600D) and patient R (F475L). These occurred irrespective of ribavirin treatment. Strikingly, a lot more mutations were observed in the ORF2 region of HEV viruses infecting patient Q, including the only mutations detected in the middle domain, illustrating a higher mutation frequency with this virus in a patient with extensive ribavirin treatment (Table 7.3).

Table 7.4 Amino acid polymorphisms in ORF2 of patient samples

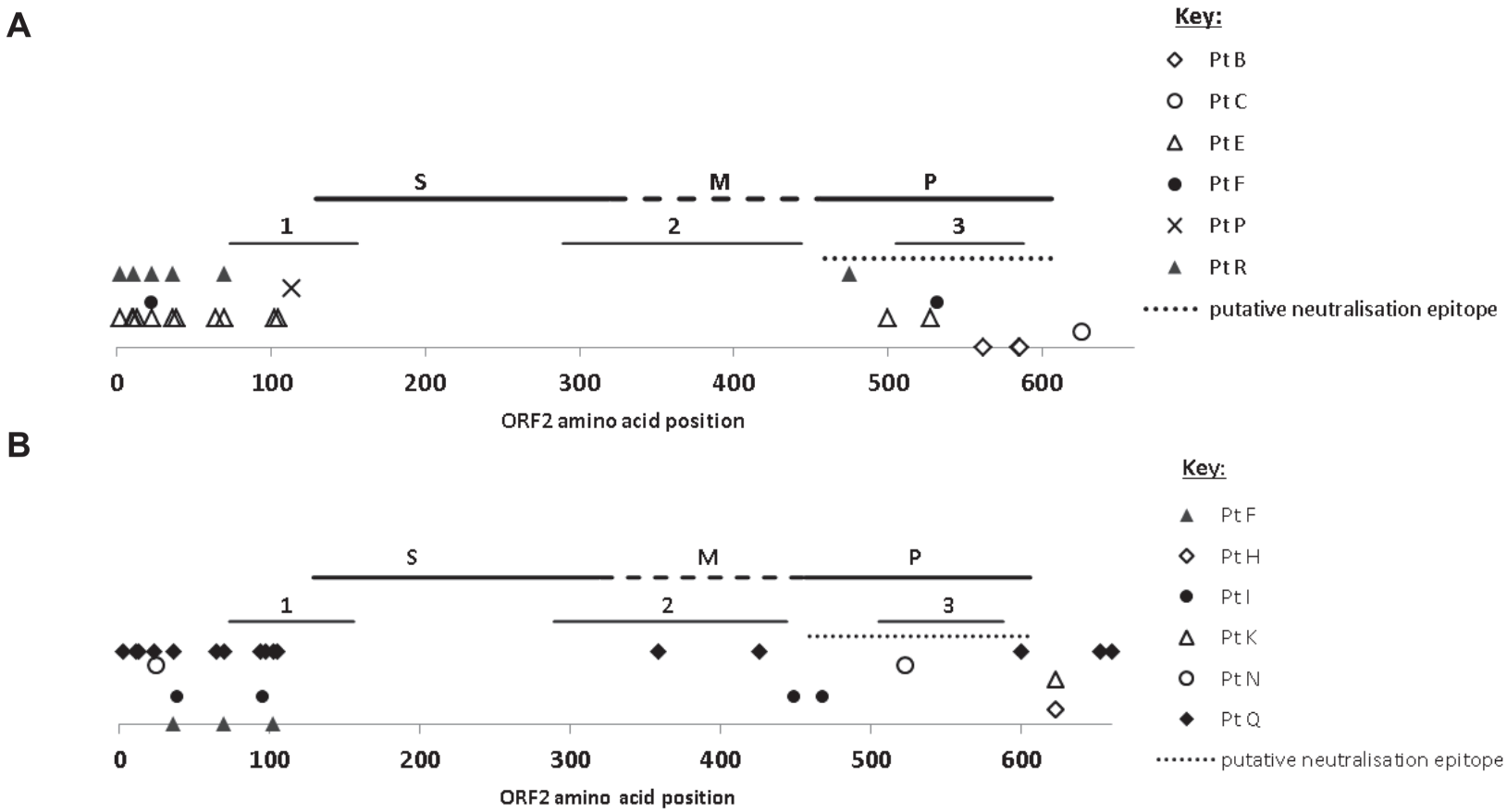
Pt	Virological outcome	Virus genotype	ORF2 mutations after initiation of RBV[a]		ORF2 mutations in absence of RBV	
A	spontaneous clearance	3c	-		-	
B	ongoing viraemia	3c			N562D T585A	T586A
C	ongoing viraemia	3c			F626S	
D	3/12 SVR	3c			-	
E	6/12 SVR	3 unassigned			C2R F10L L11F L13F S23G S36G S39G	T64A S70A S102L T105A F500L I528T/V
F	rebound	3c	G36S P70A	S102L	G23S C532Y	
G	rebound	3c	-		-	
H	rebound	3c	L623F		-	
I	relapse	3e	G38S P95H	E448D A467T	-	
J	relapse	3c	-		-	
K	relapse	3c	F623Y		-	
L	relapse	3c	N562D V595I	T614I F660V	-	
M	relapse	3 unassigned	-		-	
N	relapse	3c	P25S Y523H		-	
O	relapse	3c	-		-	
P	rebound	3c	-		T113I	
Q	relapse[c]	3c	R2C F11L L13F G23S G36S A64T S70A P94S	V97A S102L V105A T358M A426T V600D M652V F660S		
R	poor primary response	3c			C2R L11F S23G	S36G A70S F475L

Amino acid changes in the HEV ORF2 region during HEV infection.

[a] Reported only if polymorphism was dominant amino-acid and depth was >10 at site.

Abbreviations: ORF2, open reading frame 2; Pt, patient; RBV, ribavirin; SVR, sustained virological response.

Figure 7.8 Site of ORF2 mutations in relation to defined domains and T-cell epitopes



Location of amino acid switches occurring in the ORF2 of HEV (A) in the absence of ribavirin treatment and (B) with ribavirin treatment. Marked regions include the (S) shell domain, (M) middle domain and the (P) protruding domain [344]. Regions (1)(2) and (3) represent immunodominant T-cell epitopes [345]. The dotted line represents a putative neutralisation region [346].

7.1.2 Quasispecies diversity analysis

Seven patients (B, C, I, K, N, Q and R) were selected, who had three or more samples achieving over 70% HEV genome coverage, to assess diversity over time in three regions, namely RdRp, ORF2 and ORF3.

Initially samples were assessed for depth of coverage in these three regions and only loci with a depth of 100 or more were included in the analysis. This revealed uneven read depth in a number of samples within the regions of interest. For example, between 4-40% of the HEV RdRp region in the four samples from patient B were excluded due to depth of less than 100. The variation in depth led to artefacts in the diversity of the quasispecies, potentially leading to misleading results, therefore this analysis was not pursued further.

7.3 Discussion

The current study aimed to investigate the evolution of mutations during persistent HEV infections under antiviral drug pressure and in the absence of drug pressure, and to characterise quasispecies diversity during persistent HEV infections in treated and untreated patients. Ribavirin is the recommended antiviral for the treatment of persistent HEV infections, based on observational data of efficacy [223]. However, its mode of action is not fully understood and may have multiple antiviral effects, including depletion of intracellular GTP by inhibiting host inosine-5'-monophosphate dehydrogenase, the disruption of 7-methyl guanosine mRNA capping by inhibition of eukaryote initiation factor 4E or the modulation of the T-helper cell 1 response to HEV [229, 347, 348]. The metabolite ribavirin triphosphate may also have a direct antiviral effect by inhibiting the RNA polymerase, or as a mutagen leading to lethal mutagenesis [349].

Several case reports of ribavirin monotherapy treatment failures describe the emergence of a number of mutations in the HEV RdRp region [246, 247, 341]. Among the 18 patients in this study, multiple mutations in the RdRp were observed both with and without ribavirin treatment. No mutations were observed in any of the eight conserved motifs in the absence of ribavirin therapy [343]. However, several mutations occurred in these conserved motifs (K1383N, D1384N/H/G, I1533V, F1543L and K1544R) during or following ribavirin treatment, suggesting they may have functional importance under ribavirin pressure. Many were transiently detected; in the case of patient Q these transient mutations appeared to differ during discrete ribavirin treatment courses. This favours the randomness theory of ribavirin mutagenesis in which the mutation survives if doesn't affect replication fitness and has some

advantage over wild-type in presence of RBV, but then disappears after treatment is withdrawn. In contrast, we also observed that some mutations such as the K1383N (patient I and Q), D1384G (Patient Q) and I1533V (Patient L) appear to become fixed under ribavirin treatment. Both the K1383N and D1384G are recognised mutations associated with treatment failure; the I1533V is not. However, following relapse, patient L responded well to a second course of ribavirin [247, 341].

In the case of patient I the K1383N mutation was detected during relapse and did not revert to wild-type even without ribavirin selective pressure for six months. This was unexpected since ribavirin-induced mutagenesis is considered reversible when the treatment is stopped [246]. Even more surprising is this particular highly conserved lysine residue in motif I (F1 motif), important for GTP binding of the RdRp, results in abrogated viral fitness in a G3 replicon model *in vitro* [247]. In patient I, the K1383N occurred on a backbone of a potential compensatory residue (G1634K); partial restoration of viral fitness is described with G1634R in the same replicon system [247]. Nevertheless, the HEV viral load set-point post-relapse was established at 15-fold lower when compared with pre-treatment samples without the K1383N mutation, which may be due to the fitness cost of this residue change.

Two patients (patient Q and R) harboured HEV phenotypically resistant to ribavirin treatment, both of whom harboured G3c subtypes viruses. Patient R was infected with a virus containing the V1479I mutation which persisted in all subsequent samples. This mutation was described in one patient with treatment failure in conjunction with other mutations, however it is also found as a common variant (12.5%) in published subtype 3c sequences and 100% of clade 1 sequences (subtypes efg) [246]. Due to low viral loads, sufficient HEV reads could not be recovered from the sample during ribavirin therapy to assess for further RdRp mutations. The second patient (Patient Q)

demonstrated a number of mutations in the infecting dominant quasispecies (V1305I, K1383N, D1384G and G1634R) which apparently became fixed during relapse and the multiple ribavirin treatment courses (Figure 7.4). Only the D1384G could be detected as a minor variant in the baseline sample. Of these, only the V1305I has not been described in patients with treatment failure. The implications of this are unclear; it is not found in any published sequences to date and is found upstream of the conserved motifs in the RdRp (Table 7.2).

Indeed, the significance of many of the published putative resistance mutations remain unclear; both the D1384G and G1634R are described as evolving in patients with apparent phenotypic resistance, but how they contribute and in particular how the mutations interact is not known. No crystal structure is available for the HEV RdRp, so predicting the importance of specific residues changes is not easy. The G1634R leads to enhanced HEV replication *in vitro* but no specific resistance to ribavirin was observed [245]. The presence of the mutation was higher in solid organ transplant patients not achieving a SVR, but only marginally, and had no impact on the re-treatment success with a second course of ribavirin [245].

Due to time constraints analysis of the RdRp became the main focus of this study. However, analysis of the ORF2 mutations was also undertaken and revealed most non-synonymous mutations occurred in the first 110 amino acids of ORF2. This region upstream of the shell domain is the region of overlap with ORF3 and includes a newly identified signal sequence important in determining the fate of ORF2 as a secreted protein or in forming the nucleocapsid protein for intact virions [141, 350]. If time allowed analysis would have been extended to ORF3 and other regions of ORF1. Of note, analysis of the hypervariable region was excluded because of the challenges

with genome reconstruction with Illumina short read sequences and concerns of data bias with reference mapping.

It was not possible to achieve the second aim of this chapter of characterising quasipecies diversity during persistent HEV due to read depth variation. The inconsistent depth in coverage led to large areas of sequence being discarded, ultimately leading to a very truncated dataset, therefore this aim was not taken further. Previous studies highlight the potential importance of viral heterogeneity on the probability of developing persistent HEV infection in solid organ transplants and the progression of chronic liver disease to fibrosis, however HEV quasispecies evolution during persistent viraemia has only been studied by one research group [246, 338]. In that study, viral population heterogeneity did not vary over time significantly in untreated patients, but heterogeneity increased in all ORFs with ribavirin therapy [246]. We were interested in investigating this in our cohort, and a possible future analysis approach would be using newer bioinformatics software such as PhyloScanner, a powerful tool to quantify within-host viral diversity and assess longitudinal viral evolution [351]. It is also an efficient and accurate tool to identify and remove probable contaminating sequences, crucial in such studies.

Our study included a unique set of patients in which an ambitious aim was set. However, there were several challenges which limited the ability to achieve the aims effectively. This was an observational study constrained by small numbers, where sample availability and timing were restricted by clinical sampling and sample volumes. The Illumina whole genome sequencing methodology was unable to recover good quality sequences for many samples of interest; HEV genome coverage over 70% was achieved in 47/68 samples from 18 patients (69%). However, those with low genome coverage correlated with lower viral loads, particularly lower than 1 x 10^5

IU/ml. The same technique achieves near complete genome coverage in samples of 2.0 x 10^3 IU/ml for HCV, therefore the methodology requires further optimisation for HEV [352]. Lower viral loads are typically seen during drug pressure or in relapse samples, therefore an optimised methodology would facilitate similar studies investigating drug treatment failures in the future. Budget and time constraints prevented further experimentation investigating reasons leading to the low recovery of samples below 1 x 10^5 IU/ml. The correlation of genome coverage with viral load suggests a critical factor may be related to the ratio of viral RNA to host nucleic acids. In optimising probe enrichment techniques for Illumina sequencing of HCV samples the depletion of host DNA by DNase treatment of the RNA extract and modification of the library preparation method to accommodate low nucleic acid input has significantly increased genome coverage [353].

A second issue to consider is the integrity of the input samples. The patient samples were clinical specimens which were not taken exclusively for this study, so were handled extensively prior to extraction for Illumina sequencing. If sequences were over-fragmented either due to initial poor integrity or during the fragmentation process itself this would lead to poor hybridization to the capture probes and low reads of HEV sequences. The continual updating of capture probe design is likely to improve enrichment efficiency; HEV is highly divergent with relatively few published whole genome sequences therefore designing the probe set should be an iterative process whereby the probe design algorithm is updated with all available sequences. However, in the current study no specific areas of the HEV genome were consistently underrepresented in HEV sequence data, nor was there consistently poor sequencing from HEV strains derived from the same patient, suggesting this was not a major factor in the outcomes of sequencing in the current study. Finally, as bioinformatics tools

improve and more comparisons are performed it is clear that the choice of bioinformatics is crucial to reproducible and reliable outputs. Bioinformatic programmes were selected on the basis that they were designed specifically for viral analysis, but if time allowed analysis with other reference mapping tools may have yielded improved sequence data. Alternatively, *de novo* assembly tools for viral genomes may have yielded better results, particularly with the inherent high diversity of HEV which may even be higher after ribavirin therapy, due to the risk of reference mapping excluding the assembly of divergent reads.

The most clinically pertinent reason for this study was to investigate viral reasons for treatment failure. The main focus was on the RdRp region due to earlier studies identifying this as an important region for viral adaptation to ribavirin presence. This specific question may be answered by a simpler study using next generation sequencing on amplicons of the RdRp region which could supplement and corroborate the findings in this study. This would be particularly focused on patients Q and R who represent rare phenotypic resistance to ribavirin. However, this approach is liable be influenced by other confounding factors; the use of gene-specific primers may miss amplifying diverse variants and thus under-represent diversity and PCR amplification may introduce errors during RT-PCR.

In summary, in this study clinical phenotypic resistance to ribavirin was associated with the emergence of mutations seen in other published studies, some of which appear to be common variants, such as the V1479I and G1634R/K. Other mutations appear only to be detected in the context of antiviral failure (K1383N and D1384G). The K1383N is appears to be important because of the impact on GTP binding; it is also implicated in antiviral resistance of other viruses such as Chikungunya to favipiravir [354]. The V1305I mutation identified in this study may represent a novel

mutation of clinical importance and merits further study. The understanding of how these mutations interact and contribute to treatment failures requires larger studies and *in vitro* phenotypic experiments.

7.4 Conclusions

- Whole genome sequencing for HEV infection is a powerful tool with which to investigate intra-host evolution of viral quasispecies. However, further optimisation of the sequencing methodology is required to recover high genome coverage and adequate depth, particularly in samples with viral loads of less than 1 x 10^5 IU/ml, common under drug pressure or in relapse samples.
- In this chapter we observed that mutations in the conserved motifs of the RNA-dependent RNA polymerase region were only detected under ribavirin drug pressure. Clinical phenotypic resistance to ribavirin was associated with the emergence of common variants, such as the V1479I and G1634R/K in the RdRp region, while other mutations were detected which have only been described in the context of antiviral failure (K1383N and D1384G).

8 General discussion

HEV comprise a group of related viruses within the family Hepeviridae with a variable propensity to cause human infections. Until very recently, all strains of HEV isolated from human cases belonged to the species *orthohepevirus A*, in particular Genotypes 1-4 and 7 [4]. However, the capacity of cross-species infection is not fully understood [355, 356]. Highly divergent strains most closely related to rat HEV strains within *orthohepevirus C* (HEV-C) have recently been described to cause both acute and persistent human infections in Hong Kong [5, 7]. A further case was acquired whilst working in Gabon and the Democratic Republic of Congo [6]. HEV-C has been detected in wild rats in the UK and elsewhere in Europe but not in humans, however serological studies in Germany suggest human exposure occurs [357-361]. These cases would not have been diagnosed through routine laboratory diagnostics and emphasise our incomplete understanding of HEV infections in humans. Currently, it is believed that the majority of human HEV infections acquired in the UK arise from G3 infections via the consumption of insufficiently cooked pork and more rarely game meat, shellfish, through substances of human origin and occupational exposure [22, 73, 102, 362-366]. Ingestion of specific pork products including bacon, cured pork meats and pigs' liver have been associated with an increased risk of HEV infection in UK blood donors [68]. Other transmission routes including sexual transmission are plausible but appear to be very rare [124, 126, 129, 367]. However, other reservoirs of G3 HEV and even other types of HEV may exist in the UK [358, 368, 369]. Such infections can lead to persistent infections in immunocompromised patients who may develop significant liver disease. The experience of HEV diagnostic testing at the national reference laboratory (BBVU, VRD, PHE), serving England and Wales since

2003 and the testing of blood donors by NHSBT since 2015, has provided unique insights into the epidemiology of acute HEV infections in the UK [99, 102, 366, 370]. Following the first recognition of persistent HEV infection in an immunocompromised patient in the UK in 2009, further cases have arisen, but the prevalence and outcomes of persistent HEV infections in the UK has not been explored [371].

This thesis examined persistent HEV infection in immunocompromised cohorts in the UK. In doing so, the primary aims of this work were to define appropriate strategies for the diagnosis of persistent HEV infection, identify high-risk cohorts for persistent HEV infection, evaluate the cost-effectiveness of screening for HEV amongst SOT recipients, to describe clinical and virological outcomes of patients diagnosed with persistent HEV infection across England and Wales and, finally, to characterise viral quasi-species evolution of HEV in both treated and untreated patients.

Within the first objective the detection of HEV-Ag was assessed as an alternative diagnostic assay to PCR in immunocompromised patients. Both Gupta *et al* and Majumdar *et al* demonstrated HEV-Ag detection to be a useful adjunct to anti-HEV serology in early acute HEV infection in G1-endemic areas, and could be a practical diagnostic assay in outbreaks as it can be reformatted as a point-of-care test [289, 372, 373]. This has also been confirmed for acute G3 infections in Europe [280, 374]. However, a study in asymptomatic blood donors found HEV-Ag screening to be inferior to RNA for detecting current HEV infections [375]. In contrast to Vollmer *et al,* our data we showed that HEV-Ag performed well with both high specificity and sensitivity compared with PCR for HEV RNA as a screening assay for persistent HEV infections in immunosuppressed cohorts [375]. All the cases in our study were G3 HEV, however Zhang *et al* confirmed high specificity and sensitivity of the same assay for G4 HEV infections including in immunocompromised subjects [290]. The larger size of our

screened cohort meant we could confidently demonstrate the high specificity of the HEV-Ag assay. Further smaller studies have subsequently corroborated our findings [295].

The difference in sensitivity we found compared with data in blood donors is likely a reflection of the higher levels of HEV-Ag production in persistent infections and the wider diagnostic window presented by persistent HEV infections in immunosuppressed patients. In fact a number of studies have exploited this in trying to use HEV-Ag levels to predict clinical outcomes in both G1 infections and G3 infections [217, 376]. Behrendt *et al* initially described how the optical density reading of the HEV-Ag ELISA OD could infer the likelihood of the infection being acute or chronic [217]. In a separate cohort of immunocompromised patients, Marion *et al* also showed how the acute phase HEV-Ag titre could predict the development of a chronic infection [134].

Nevertheless, it is not only the HEV-Ag levels that are of interest, but also the HEV-Ag dynamics which may vary among immunocompetent and immunocompromised hosts. The study by Behrendt *et al* also reported on the persistence of detectable HEV-Ag beyond HEV RNA detection in the plasma of patients with persistent HEV infections treated with ribavirin [377]. The phenomenon of HEV-Ag persistence in the absence of RNA has been observed for a short duration in acute G1 infections, yet this was not replicated in Macaque challenge models using G1 and G4 HEV in work by Zhang *et al* [284, 372]. We found that amongst most cases of active infection HEV-Ag detection in plasma persisted beyond RNA detection, however this was very prolonged in a subset of patients treated with Ribavirin, thus confirming the findings of Behrendt *et al* [217]. A particular strength of our work was the development of an HEV-Ag neutralisation assay which allowed us to confirm the specificity of reactive HEV-Ag

ELISA results in both plasma and urine but most importantly stool, an analyte susceptible to generating non-specific reactivity. This allowed us to study HEV RNA and HEV-Ag kinetics in stool with more certainty. We observed for the first time that this dissociation between RNA detection and HEV-Ag detection seen in the plasma of treated patients was not seen frequently in the stool compartment. Specifically in our study HEV-Ag detection in stool never persisted beyond the plasma compartment. Our findings are supported by the more recent description of a secreted form of ORF2 antigen released in large quantities by infected hepatocytes into the plasma compartment but not the stool [141, 291]. This non-virion associated antigen is likely to be the predominant form detected by the HEV-Ag assay.

The second objective was to identify high-risk cohorts for persistent HEV infection. This was achieved by point prevalence studies of HEV viraemia in three distinct cohorts; transplant recipients, haemato-oncology patients and antibody-deficient patients. We confirmed SOT recipients to be a high-risk cohort with 0.7% of patients viraemic, broadly similar to other European studies, which range from 0.2% up to 4.4% of viraemic patients in a range of organ types [185-193, 202, 203, 378, 379]. The only other UK studies performed at a similar time found rates of viraemia of 0.2-0.5% among SOT patients [295, 308]. In contrast to Reekie *et al*, our study recruited all transplant recipients under follow-up undergoing therapeutic drug monitoring which therefore included patients with longstanding transplants [308]. This allowed us to capture the cumulative risk of persistent HEV infection following a patients' transplant rather than just the incidence in the peri-transplant period. The strength of our study was its large size; it remains the largest study performed to date of HEV prevalence in transplant patients in the UK and thus may reflect a more accurate prevalence rate.

Recipients of HSCT were also found to be a high-risk cohort for HEV viraemia with 2.1% of patients infected in our study. This is broadly similar to rates of viraemia found in the Netherlands (2.4-3.8%), but slightly higher than found in China (1.1%), Denmark (0.6%) and the only other UK study of HSCT recipients (0.4%) [194, 308, 380, 381]. A systematic review recently analysed these studies, concluding an overall prevalence of 1.5% of HEV viraemia among HSCT patients [382]. However, it must be noted that study size, differing study design and patient selection bias differed may account for some of the reported differences in risk. For example, studies by Abravanel *et al* in France and Koenecke *et al* in Germany were likely too small to identify any HEV risk [383, 384]. Whereas Reekie *et al* followed both recipients of allogeneic and autologous stem cell cells, our cohort only included allogeneic stem cell recipients undergoing TDM and hence were more highly immunosuppressed [308]. Furthermore, Tang *et al* studied only haploidentical HSCT recipients who also typically receive more immunosuppression and tested only those patients with unexplained abnormalities in liver enzyme blood tests [380].

When compared to transplant patients, we found haemato-oncology patients not undergoing HSCT to be lower risk of being HEV viraemic (0.13%). This is the first and only specific study assessing HEV infections in haemato-oncology patients not undergoing HSCT. Two HEV viraemic patients were identified, both of whom had underlying multiple myeloma and neither had abnormal liver enzymes at testing. The use of rituximab has previously been postulated to be a risk for persistent HEV infections

[297, 299, 319, 385]. In contrast to the retrospective European cohort study by von Felden *et al*, we found no association between a non-Hodgkin lymphoma diagnosis, treatment with both rituximab and bendamustine and the risk of persistent HEV

infection [310]. A particular strength of our study was the detailed analysis of diagnoses, treatment history including transfusion history, however with only two viraemic patients it limited our ability to define risk factors for HEV viraemia. The normal liver enzymes presented by both viraemic patients in our study is notable since these patients would have not been identified by studies relying on clinical diagnosis or by screening of patients with abnormal liver enzymes. In fact, lower peak ALT values have been associated with increased likelihood of persistent infection [207, 310].

We showed that anti-HEV IgG seropositivity increased with age and was influenced by the underlying haematological diagnosis. We confirmed that this cohort were being exposed to HEV infection from blood products with IgG seropositivity rising in a linear fashion associated with higher numbers of transfused blood products. Whilst substances of human origin are now widely accepted as routes of HEV transmission, this association was not replicated in a Danish study of 4023 immunocompromised patients by Harritshøj *et al* [378]. The correlation seen in our study was relatively small, however, compared with other risks such as increasing age which most likely reflects dietary risk. Therefore, studies in other countries with different rates of viraemia among blood donors or in patients with lower transfusional exposure may not identify such correlation.

The third cohort we investigated were antibody-deficient patients being treated with immunoglobulin therapy. We found no evidence of persistent HEV infection amongst a cohort of patients with primary and secondary antibody deficiency. Our study supports the findings of two similar studies, neither of which displayed any evidence of persistent HEV infection amongst 73 CVID patients in Germany or 27 primary antibody deficient patients with deranged liver enzymes in the UK [300, 305]. The particular strength of our study was its larger size and detailed characterisation of both

patients and immunoglobulin replacement therapy. We demonstrated that anti-HEV IgG was frequently detected in the plasma of antibody-deficient patients. Furthermore, their plasma following IVIG administration had higher antigen neutralising activity relative to the detected anti-HEV IgG titre when compared with the plasma of recovered persistent HEV cases and recovered acute recovered HEV cases. This suggests a plausible mechanism of protection from initial HEV infection, however this remains speculative since correlates of protection against HEV infection are not well defined, even amongst vaccinated individuals. Thus, the first hypothesis that persistent HEV infection is an emerging and under-recognised disease in the UK was proven in SOT recipients. The smaller sized cohorts of haemato-oncology patients and antibody deficient patients limited the ability to define accurately the true risk in such patients.

Factors associated with HEV infection among SOT recipients included higher plasma levels of immunosuppressive drug (tacrolimus or ciclosporin) and raised ALT or bilirubin. No other patient parameters were identified to help predict which patients may be infected with HEV.

The finding of many unrecognised HEV infections among SOT recipients and lack of predictive factors for infection prompted the exploration of four possible screening strategies in the post-transplant setting. We undertook a cost-effectiveness study by adapting a Markov model using data generated from the screening studies, including diagnostic assay performance. At a willingness-to-pay threshold of £20 000 per QALY, annual screening of SOT recipients by any of the methods considered had a high probability (>75%) of being cost-effective. Only two published studies have undertaken cost-effectiveness analyses in the context of HEV [103, 327]. Zhao et al considered the cost-effectiveness of HEV vaccination in pregnant women [327]. In contrast to de

Vos *et al*, who considered the cost-effectiveness of HEV RNA screening of blood donations, we also considered HEV-Ag screening as an alternative to RNA testing [103]. Screening patients with an abnormal ALT annually by detecting HEV-Ag is predicted to be cost-saving to the NHS. Such data are critical in the UK with a restricted health budget and a nationalised health service with the underlying objective of maximising health outcomes with finite resources. This part of the body of work was restricted in scope due to limited data from which to inform the model. Nevertheless, it was the first such study to explore screening of transplant recipients, which represents a more practical solution to capture both dietary-acquired and transfusion-acquired HEV. The strength of this study was our extensive uncertainty analyses to test the model with many different input parameters exploring situations of future relevance. Thus, proving the second hypothesis that screening for HEV infections in high-risk cohorts would be cost-effective, at least in SOT recipients in the context of current HEV infection rates.

The fourth objective; to describe the demographic, virological and clinical outcomes amongst patients with persistent HEV infection across England and Wales was achieved by establishing an enhanced surveillance system. This allowed the first national description of a series of 94 patients with persistent HEV infection and generated some interesting observations leading to important practical considerations for clinical practice. We confirmed that recipients of solid organ and stem cell transplants are a high-risk cohort, consistent with our previous HEV prevalence studies, representing 70.2% of all patients. Yet if our prevalence data is extrapolated to the estimated UK transplant population of some 48,000 patients with functioning transplants then we would expect over 300 to be viraemic at any point in time, indicating a significant discrepancy with expected infections and diagnosed clinical

cases [386]. A particular strength of our work was its national perspective; by identifying cases through a centralised diagnostic system it allowed us to describe the clinical outcomes of cases presenting to multiple hospital specialists, in contrast to previous cohort studies which have focussed on cases at either a single specialist centre or multiple centres via clinicians with a special interest in HEV [168, 201, 207, 310, 387]. We describe that nearly a third were non-transplant patients; the biggest contingent were patients with lymphoma, similar to the findings of a retrospective cohort study by Felden *et al* [310]. This is notable since we did not see any evidence of an increased risk of persistent HEV infection among the 556 lymphoma patients tested in our prevalence study, however this may have simply been too small a cohort to detect a risk.

By assessing serological responses at diagnosis and throughout the viraemic period, we observed that 17% of these patients did not develop a detectable serological response despite prolonged viraemia. Pischke *et al* previously described delayed or absent seroconversion in immunocompromised patients and anti-HEV IgG seroreversion has been noted in organ transplant recipients [188, 388]. However, we also saw a correlation between the absence of anti-HEV IgG seroconversion and poor outcomes. Our study is the first to report this; only 33.3% of the seronegative antiviral-treated patients achieved viral clearance and at least three of the five deaths amongst seronegative patients were considered to be HEV-associated. Whilst many confounders exist, this observation merits further study.

We observed a lower proportion of virological failure (24%) compared with Abravanels early controlled observational data among SOT recipients receiving three months of ribavirin (38%) [225]. Our cohort was more heterogenous both with respect to the underlying immunosuppression and the treatment schedules. A recent systematic

review of 395 ribavirin-treated patients including non-SOT patients and varying treatment schedules found more similar relapse rates (18%) to our cohort [219]. Crucially, relapse occurred in five patients despite demonstration of stool clearance of HEV in at least two stool samples after a median of 12 weeks of ribavirin (range 11-48 weeks). Abravanel *et al* initially described the high positive predictive value of detecting HEV RNA in stool after three months of ribavirin treatment with virological relapse [225]. Very recently Marion *et al* have proven that intestinal cells can not only support HEV replication *in vitro*, but furthermore ribavirin may have lower efficacy against the excretion of stool-derived HEV, suggesting a possible mechanism for prolonged stool detection in treated patients [244]. However, our study demonstrates that clearance of HEV RNA from stool is, in itself, an insufficient surrogate for absolute clearance of HEV infection. The data may simply indicate that HEV RNA detection is a poor surrogate for ongoing infection of intestinal cells, however there may be other important sites of extrahepatic replication which may lead to relapse. HEV infection and replication is supported by human neuronal cells, intestinal cells, endothelial cells, endometrial stromal cells and the human placenta as well as being detected in urine [134, 162, 244, 389-392]. In immunocompromised patients HEV has been detected in a number of other tissues including the reproductive tract and bone marrow [133, 393]. Specifically, HEV has been detected in the ejaculate in small numbers of patients more than nine months after viraemia ceased suggesting the testis could be a sanctuary site from which HEV could relapse following treatment [393]. Thus, the third hypothesis that an enhanced monitoring study of persistent HEV infections would help identify vital clinical monitoring parameters was proven.

The unique set of samples and follow-up data from both treated and untreated HEV infected patients facilitated the investigation of viral sequence changes during

persistent infection. Using Illumina whole genome sequencing, mutations were observed in the conserved motifs of the RNA-dependent RNA polymerase region under ribavirin drug pressure, but not in untreated patients. Clinical phenotypic resistance to ribavirin was associated with the emergence of common variants such as the V1479I and G1634R/K in the RdRp region, whilst other mutations were detected which have only been described in the context of antiviral failure (K1383N and D1384G). The role of such single nucleotide variants (SNV) and mutations remains unclear. We observed K1383N only in the context of G1634K/R, consistent with the hypothesis of Debing *et al* that the increased viral replication seen with G1634K/R and other SNVs may overcome the fitness loss and increased *in vitro* sensitivity to ribavirin associated with K1383N [247]. The K1383N is of particular interest as it is highly conserved among RNA viruses and may play a role of the fidelity of the RdRp [394]. However, we also report patients with a poor primary response to ribavirin and patients who relapsed despite none of these recognised mutations in the RdRp region so other mechanisms likely exist.

A strength of our study was the frequent longitudinal sampling from patients with different outcomes. However, this strength was not realised since a significant number of samples did not generate high quality sequence data, seen particularly with viral loads of less than 1 x 10^5 IU/ml. This is similar to Davis *et al* who used the same technique and were able to generate whole genome data for samples with as low as 1.25 x 10^4 IU/ml, but not any lower [395]. Low viral loads were observed in early relapse samples or under ribavirin drug pressure which were often samples of particular interest. This severely limited the analysis in this body of work and thus the investigations of intra-host evolution of viral quasi-species were not pursued further. Given more time and resources the probe capture technique for HEV would have been

optimised and *de novo* assembly bioinformatics software tested to achieve greater genome coverage and depth.

In conclusion, this thesis supports the notion that persistent HEV is under-recognised in SOT recipients in the UK. It is projected that annual screening for HEV infection would be cost-effective in the NHS, given current infection rates. Screening by HEV-Ag detection or RNA by PCR for persistent infection would both be acceptable, however the role of serology remains unclear in immunocompromised patients. Evidence of stool clearance during antiviral therapy does not obviate the need for close follow-up as relapses occur. Important future research questions include further characterising factors associated with persistent HEV infection, identifying more precisely predictive factors of virological relapse in order to guide antiviral therapy, understanding mechanisms of ribavirin failure and the ongoing surveillance of clinical outcomes of persistent HEV infection. The development of efficient cell culture models coupled with in silico modelling will enhance the development of new antivirals effective against HEV which are needed for the subset of patients failing ribavirin [232, 239, 240, 396-400].

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Appendices

Appendix 1. Chapter 2 supplementary materials and methods

Appendix 2. Chapter 4 supplementary results

Appendix 3. Chapter 6 supplementary results

Appendix 4. Chapter 7 supplementary results

Appendix 1. Chapter 2 supplementary materials and methods

A1.1 Clinical data collection tool for persistent HEV cases across England and Wales

Public Health England

National Chronic HEV Enhanced Surveillance PHE

Patient name_____ DOB_____ Weight (Kg)_____
Date of data collection_____
Referring centre_____ Clinician_____ | Email contact_____

Patient Co-morbidities & Immunosuppression

Primary underlying diagnosis_____
If patient has received a transplant, please state type_____ Date_____
Significant co-morbidities_____
Duration of preceding immunosuppression at HEV diagnosis_____
Immunosuppressive drugs at time of HEV diagnosis

1. Drug_____ Dose_____ recent trough level_____
2. Drug_____ Dose_____ recent trough level_____
3. Drug_____ Dose_____ recent trough level_____
4. Drug_____ Dose_____ recent trough level_____

Immunosuppressive drugs in preceding 3 months (if different from above)

1. Drug_____ Dose_____ dates_____
2. Drug_____ Dose_____ dates_____
3. Drug_____ Dose_____ dates_____
4. Drug_____ Dose_____ dates_____

Known pre-existing liver disease? Please state_____
Alcohol intake_____units/wk
Renal function

- ☐ No known renal disease
- ☐ CKD Stage 1 (eGFR 90+)
- ☐ CKD Stage 2 (eGFR 60-89)
- ☐ CKD Stage 3 (eGFR 30-59)
- ☐ CKD Stage 4 (eGFR 15-29)
- ☐ ESRF on dialysis

HEV Infection

Date of HEV diagnosis_____
Reason for investigation?

- ☐ Clinical hepatitis
- ☐ Asymptomatic transaminitis
- ☐ HEV Screening
- ☐ Unexplained neurology – please state_____
- ☐ Other – please state_____

Reported symptoms by patient:

- ☐ Fever
- ☐ Diarrhoea
- ☐ Abdominal Pain
- ☐ Headaches
- ☐ Nausea
- ☐ Vomiting
- ☐ Joint Pains
- ☐ Loss of appetite
- ☐ Weakness of limbs/tingling
- ☐ Jaundice
- ☐ Other, please specify_____

Lymphocyte count at HEV diagnosis_____ units_____date_____
Platelet count at diagnosis_____units_____date_____

Patient's Liver Function Assessment

- ☐ ALT at time of HEV diagnosis_____units_____date_____
- ☐ Peak ALT_____ ___units_____date_____
- ☐ Any Ultrasound results:
  - Report_____date_____
  - Report_____date_____

Any Fibroscan results:

- ☐ Result_____kPa date_____
- ☐ Result_____kPa date_____
- ☐ Result_____kPa date_____
- ☐ Result_____kPa date_____

- ☐ Any Liver Biopsy results
  - Report_____date_____
  - Report_____date_____
- ☐ Any synthetic derangement?_____

Treatment

Was there a trial of reduction of immunosuppression?

- ☐ Yes - If so, please state dose reduction/duration_____
- ☐ No – if not, please state reason_____

What was the indication of failure of this approach?_____

Treatment drug

- ☐ Ribavirin dose_____start date_____stop date_____
  If exact dates not available, estimated duration_____
- ☐ Pegylated Interferon a dose_____ start date_____stop date_____
  If exact dates not available, estimated duration_____
- ☐ Combination therapy

Toxicity & Other Adverse Outcomes

Was there any toxicity from treatment?

- ☐ Anaemia – please state severity_____
- ☐ Other – please state_____

Did this require additional adjustment of management?

- ☐ Monitoring only

- ☐ Ribavirin stopped
- ☐ Dose reduction of ribavirin - state dose/duration_____
- ☐ EPO/transfusion support - state duration of EPO_____ and no's of units transfused_____
- ☐ Other_____

Other adverse outcomes attributed to HEV (e.g. delayed treatment for primary diagnosis)

_____

Outcome

- ☐ Viral clearance
- ☐ Viral relapse – please state date of relapse and re-treatment strategy_____
  _____
- ☐ Viraemic at death
- ☐ Ongoing viraemia

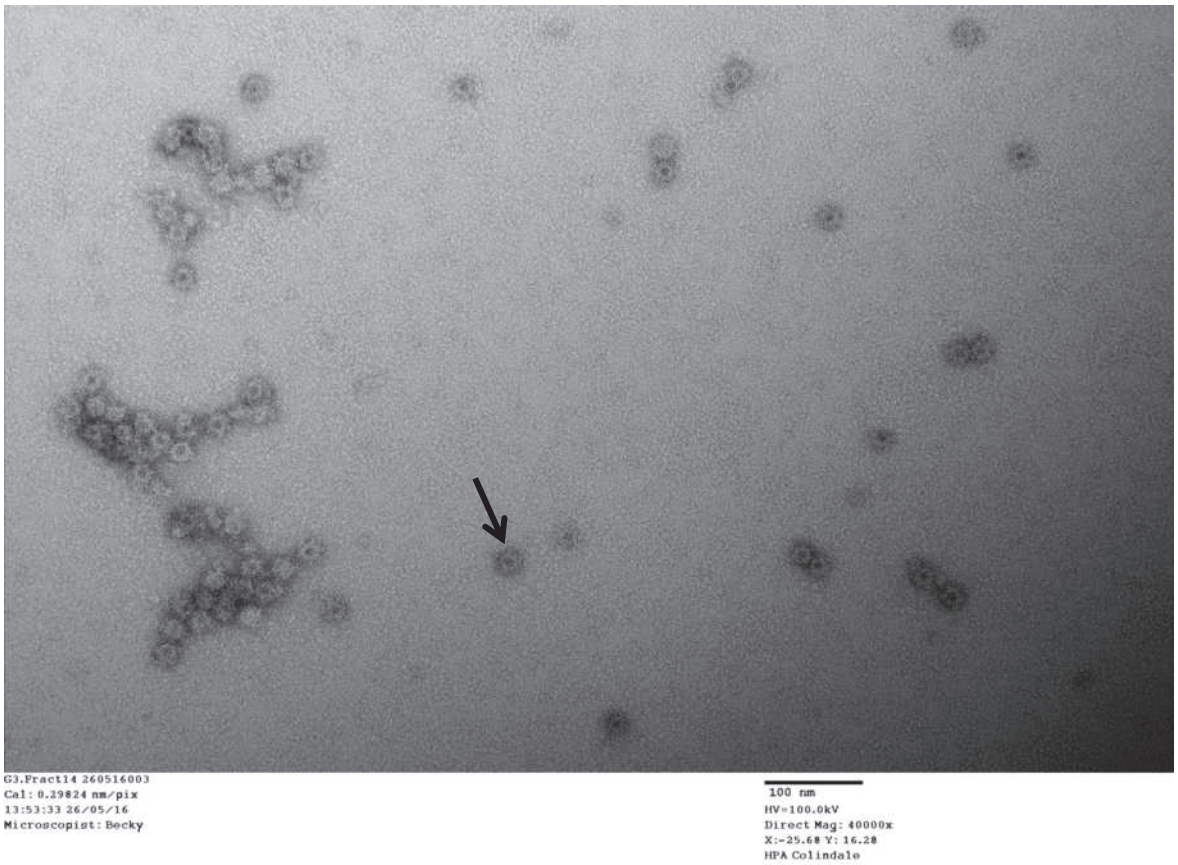
A1.2 Generation and purification of genotype 1 and 3 HEV virus-like particles

Virus-like particles (VLP) were generated using recombinant bacmids encoding amino acids 112-608 of either G1 or G3 HEV ORF2 created with the Bac-to-Bac® baculovirus expression system (Life Technologies), according to the manufacturer's protocol. Sf9 cells were cultured, harvested after five days and then lysed. The harvest, containing supernatant and lysed cells, was clarified, fractionated on Optiprep (Sigma) and fractions containing VLPs identified by SDS-PAGE and electron microscopy (A1.3 and A1.4).

A1.3 Electrophoresis image of denatured HEV VLPs.

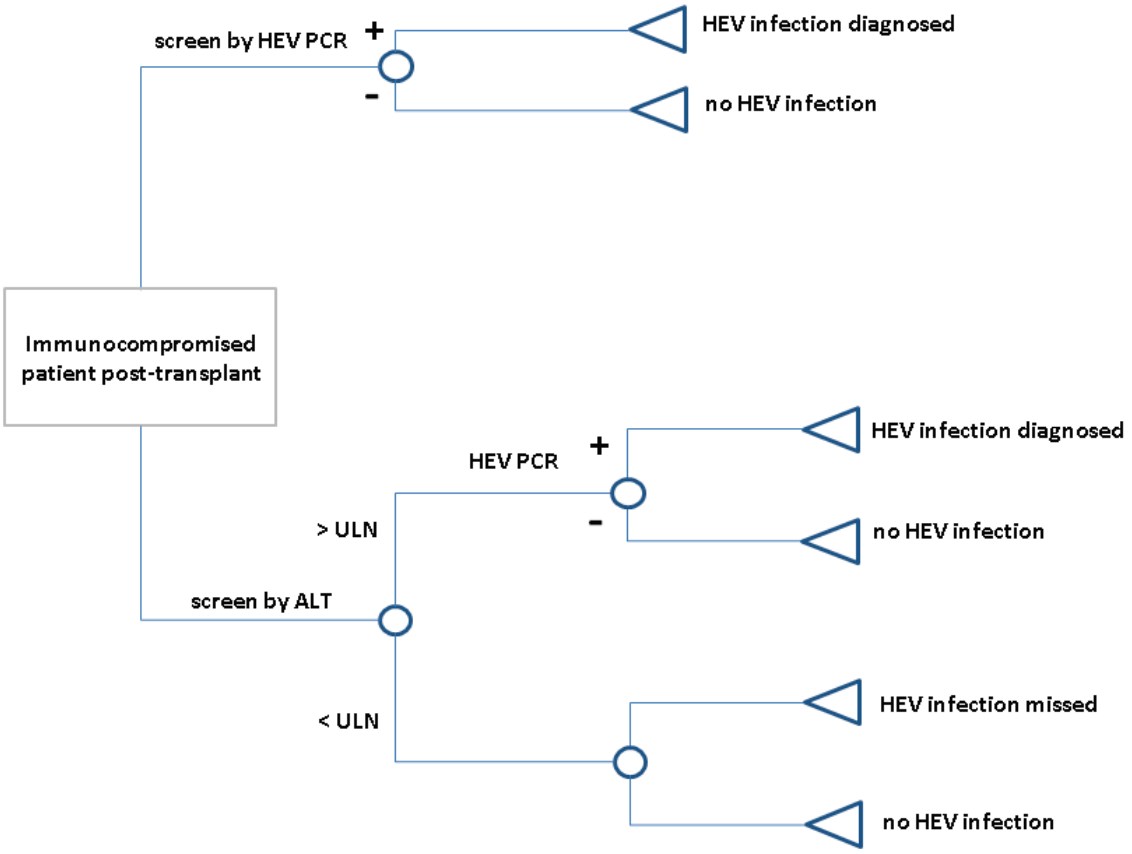
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) image of denatured HEV VLPs.Lanes 1-4 represent BSA of 200ng (1), 100ng (2), 20ng (3) and 4ng (4). Lanes 5-8 represent denatured G1 VLPs either neat (5) or at a dilution of 1:2 (6), 1:10 (7) or 1:50 (8). Lanes 9-12 represent denatured G3 VLPs either neat (9) or at a dilution of 1:2 (10), 1:10 (11) or 1:50 (12). The white rectangular box highlights the denatured HEV G3 VLPs at the expected size of 53kDa. MW= molecular weight ladder (Novex™ Sharp Pre-stained Protein Standard).

A1.4 Electron microscopy of HEV G3 VLPs.



Electron microscopy image of fractionated harvest of HEV G3 VLPs.
The arrow marks an example of an individual VLP. Negatively stained grids were viewed using the JEM-1400 transmission electron microscope (JEOL UK) and images captured using the AMT XR-600 digital camera (Deben UK) (40 000 x magnification, 2% uranyl acetate staining).

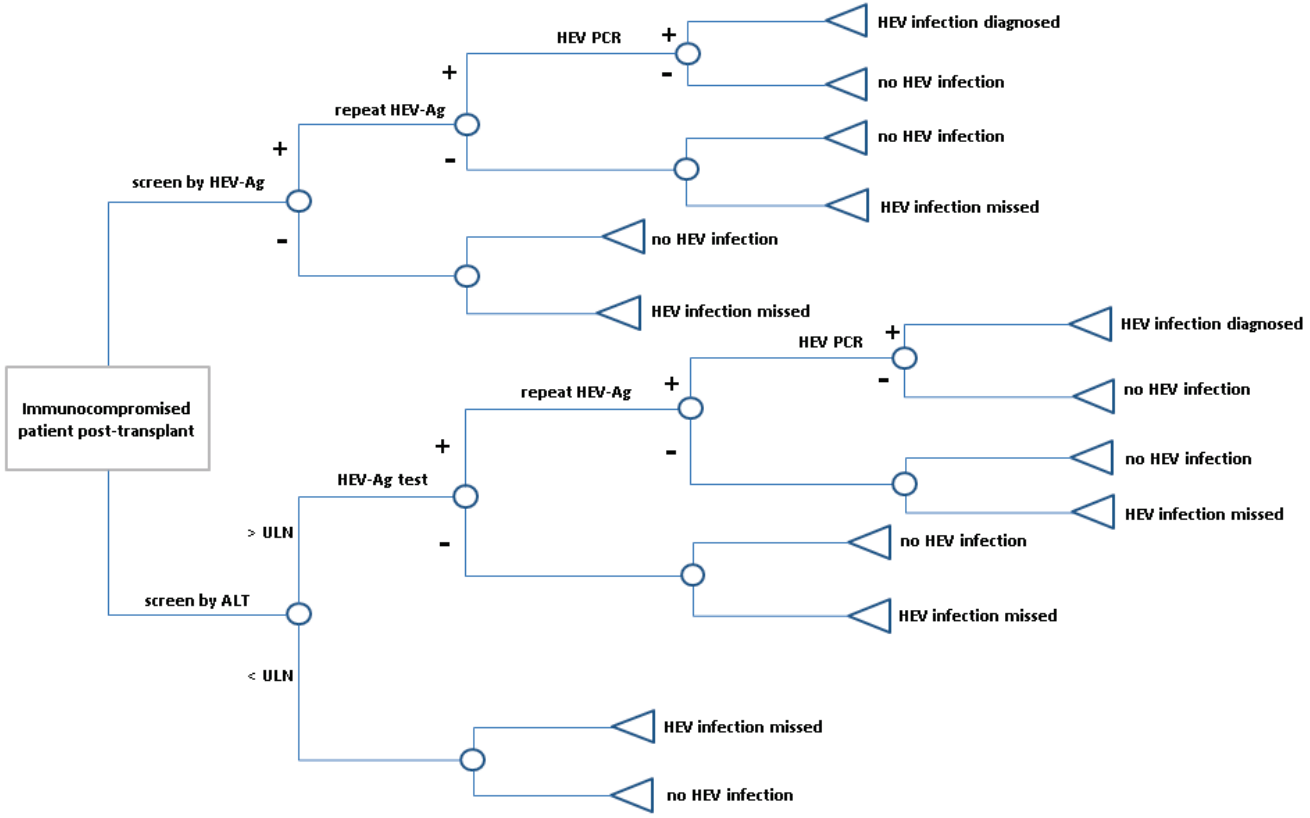
A1.5 Diagnostic testing algorithms for HEV considered in CEA



Proposed testing and confirmatory algorithm for solid organ transplant recipients with HEV RNA detection (PCR) or using an abnormal ALT value as an indicator for testing.

Abbreviations: ALT, alanine aminotransferase; CEA, cost-effectiveness analysis; ULN, upper limit of normal.

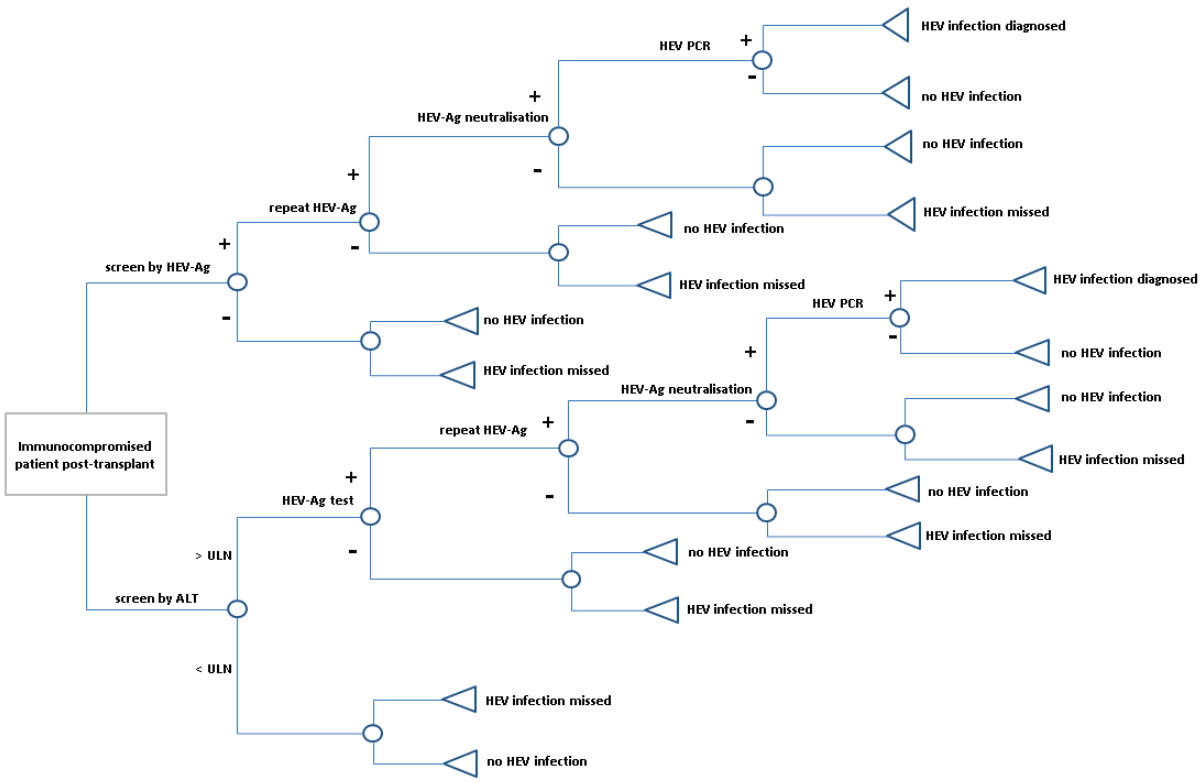
A1.6 Diagnostic testing algorithms for HEV considered in CEA



Proposed testing and confirmatory algorithm for solid organ transplant recipients with HEV antigen detection or using an abnormal ALT value as an indicator for testing.

Abbreviations: ALT, alanine aminotransferase; CEA, cost-effectiveness analysis; ULN, upper limit of normal.

A1.7 Diagnostic testing algorithms for HEV considered in CEA.



Proposed testing and confirmatory algorithm for solid organ transplant recipients with HEV antigen detection and confirmatory neutralisation or using an abnormal ALT value as an indicator for testing.

Abbreviations: ALT, alanine aminotransferase; ULN, upper limit of normal

A1.8 Linux commands used for whole genome sequence analysis

A1.8.1 HEV pipeline for mapping of short read sequences to reference genomes

```
#### Uses Sreenu Vattipally's CREATE KMERS and Tanoti programmes
#### Will produce sam alignment files, create consensus sequences and make a fast tree
#Addition to clean files with trim_galore and to use trimmed files only
ls *R1*fastq>r1
less r1|sed 's/R1/R2/g' >r2
paste r1 r2|sed 's/.fastq//g'>genolist-$$
echo "Trimming..."
while read file
do
fq1=`echo $file|awk '{print $1}' `;
fq2=`echo $file|awk '{print $2}' `;
trim_galore -q 30 $fq1.fastq $fq2.fastq >> $fq1.geno
done < genolist-$$
echo "HEV mapping pipeline has started..."
echo "Output files are as follows:
Input files (genolist)
Genotyping results (.geno)
Closest reference sequence (.newbest)
Alignment files (.sam)
Reference sequence are downloaded from e-utilities (named by accession number)
Consensus sequence file - allconfile
Consensus sequences file >90% - Consensus_90.fa)"
ls *R1*trimmed*fq>r1
less r1|sed 's/R1/R2/g' >r2
paste r1 r2|sed 's/.fq//g'>genolist_trim-$$
#Genotyping
#TROUBLESHOOTING Be careful that your reference file is in the correct format and that it is ok for
unix - you can fix this with tr -d '\r' <infile >outfile if needed
echo "Genotyping..."
while read file
do
fq1=`echo $file|awk '{print $1}' `;
fq2=`echo $file|awk '{print $2}' `;
```

```
CREATE_KMERS-FQ-T -i /home/HCV2/HEV_PHE/Refs/HEV_ref_seqs.fasta -1 $fq1.fastq -2
$fq2.fastq  -c 1 -p 1 -v >> $fq1.geno

done < genolist_trim-$$

echo "Removing dead wood..."

rm -rf  *.f5  *.f2  *.stats *.sorted *.newbest

echo "Sorting out the best reference..."

for gen in *.geno; do less $gen| cut -f5| sed 's/Genome coverage://g'| grep -v "All kmer matches"| grep
-v "Reading"| grep -v "Total"| grep -v "Reference"| grep -v "kmer"| grep -v "Time">$gen\.f5; done

for gen in *.geno; do less $gen| cut -f2| sed 's/Genome coverage://g'| grep -v "All kmer matches"| grep
-v "Reading"| grep -v "Total"| grep -v "Reference"| grep -v "kmer"| grep -v "Time">$gen\.f2; done

for gen in *.geno; do paste $gen\.f2 $gen\.f5>$gen\.stats; done

for stats in *.stats; do sort -k2 -n $stats>$stats\.sorted; done

for sorted in *.sorted; do tail -1 $sorted|cut -f1>$sorted\.newbest; done

rename \.geno\.stats\.sorted\.newbest \.newbest *newbest

# Pulls out the best genome for mapping

for br in *.newbest; do less $br|sed 's/All kmer matches//g'>>bestgenomes-$$; done

sort bestgenomes-$$| uniq | tr -d '\r' >uniquegenomes-$$

rm -rf a1 b1 uniquegenomes

# Downloads all genomes for mapping

echo "Downloading reference sequences from ncbi..."

exec < uniquegenomes-$$

while read id

do

wget -O $id
https://eutils.ncbi.nlm.nih.gov/entrez/eutils/efetch.fcgi?db=nuccore\&id=$id\&rettype=fasta\&retmode=t
ext

done

echo "Tanoti pipeline proceeding..."

#Tanoti prep

echo "Preparing tanoti files"

rm -Rf listpre tanlist foo1 foo2 *bad* constats Consensus_95.fasta allconfile samstats

ls *R1*fq>foo1

less foo1| sed 's/_R1_001_trimmed\.fq//g' > tanlist-$$

#Tanoti batch run

##while read file

##do

##fq1=`echo $file|awk '{print $1}' `;

##fq2=`echo $file|awk '{print $2}' `;

##tanoti -i $fq1.fastq $fq2.fastq -r $(less $fq1.newbest) -p 1 -u 0 -o $fq1-$(less
$fq1.newbest|##dos2unix).sam -m 50

##done < tanlist-$$
```

```
#Adjusted for use on rho
dos2unix *newbest
while read file
do
#ref=$(awk 'NR==1' $file\.newbest);
ref=$(echo $file"_R1_001_trimmed.newbest");
fq1=$(echo $file"_R1_001_trimmed.fq");
fq2=$(echo $file"_R2_001_trimmed.fq");
echo "tanoti -i $fq1 $fq2 -r $ref -p 1 -m 50 -o $fq1-$(cat $ref).sam";
tanoti -p 1 -i $fq1 $fq2 -r $ref -o $fq1-$(cat $ref).sam -u 0 -m 50
done < tanlist-$$
#Sam_stats
echo "Running SAM stats..."
for ST in *.sam; do echo $ST>>samstats-$$; SAM_STATS $ST>>samstats-$$; done
#Sam2consensus
for con in *.sam; do SAM2CONSENSUS -i $con>>allconfile-$$; done
#Consensus files from genomes of minimum 95%
echo "Selecting consensus sequences with >90% coverage..."
ConsensusSorter samstats-$$ allconfile-$$ 90 >> Consensus_90.fasta
echo "Aligning consensus sequences..."
cat Consensus_90.fasta /home/HCV2/HEV_PHE/Refs/HEV_ref_seqs.fasta
>>Consensus_plus_refs.fasta
mafft Consensus_90.fasta > Consensus_90_aligned.fasta
mafft Consensus_plus_refs.fasta > Consensus_plus_refs_aligned.fasta
echo "Building nj tree for quick look - to check for contamination..."
fasttree -nt Consensus_90_aligned.fasta > consensus_tree_$$
fasttree -nt Consensus_plus_refs_aligned.fasta > consensus_plus_refs_tree_$$
clear
echo "Analysis complete. Output is *.sam, samstats, allconfile (all consensus sequences) and
Consensus_90.fasta (all consensus sequences with coverage of >90%, an alignment file -
Consensus_90_aligned.fasta and a neighbour joining tree file called consensus_tree_no.)"
figtree consensus_plus_refs_tree_$$
#else
#echo "Error: Input R1 and R2 file number is not equal"; exit
```

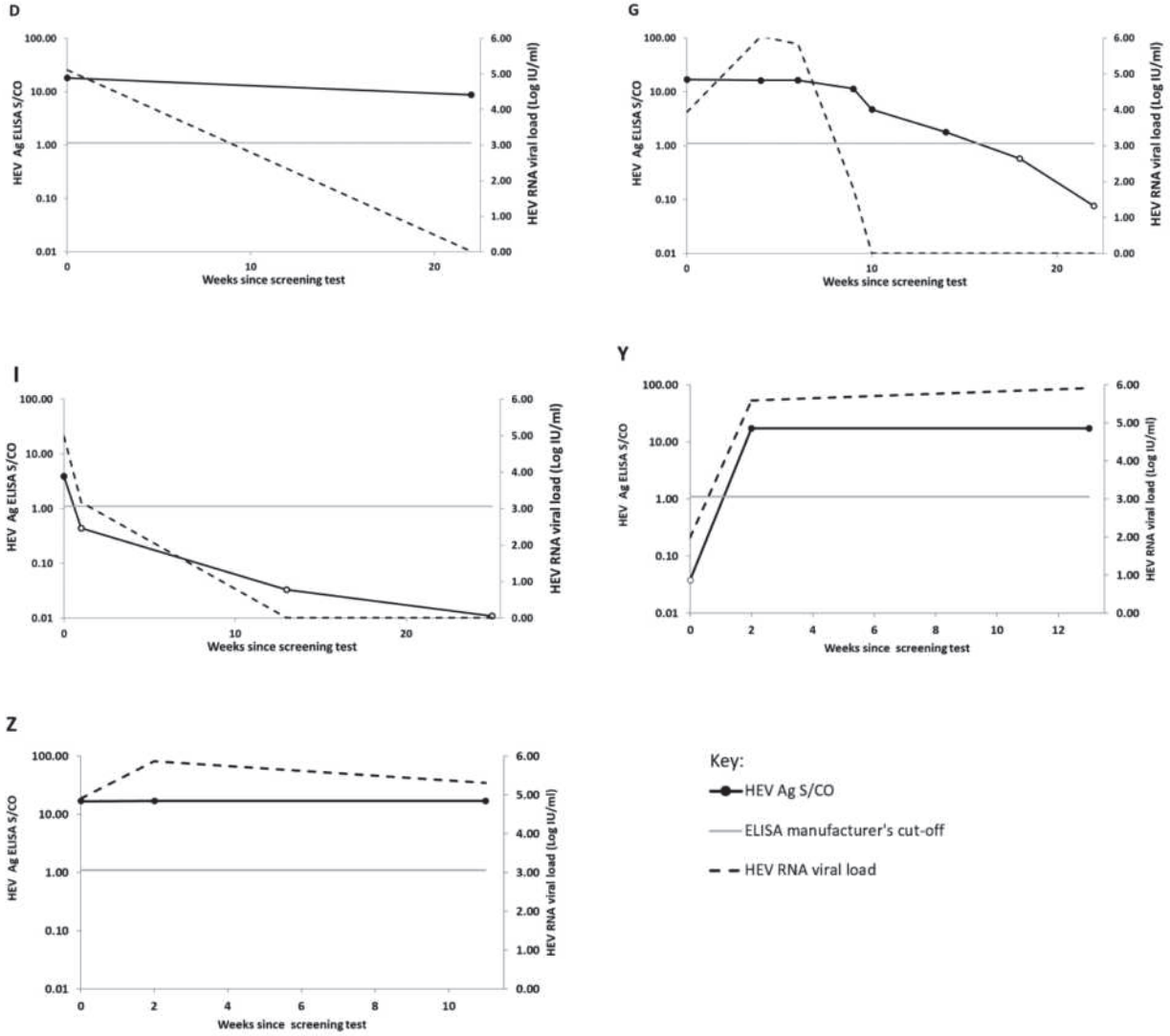
A1.8.2 HEV GLUE analysis command lines

module hevSamReporter amino-acid -i path/to/myNgsData.sam -r REF_MASTER_M73218 -f RdRp -p -P 5

alignment AL_3c amino-acid frequency -c -w "referenceMember=false" -r REF_MASTER_M73218 -f RdRp --labelledCodon 1630 1640

Appendix 2. Chapter 3 supplementary results

A2.1 Plasma HEV-Ag in untreated patients

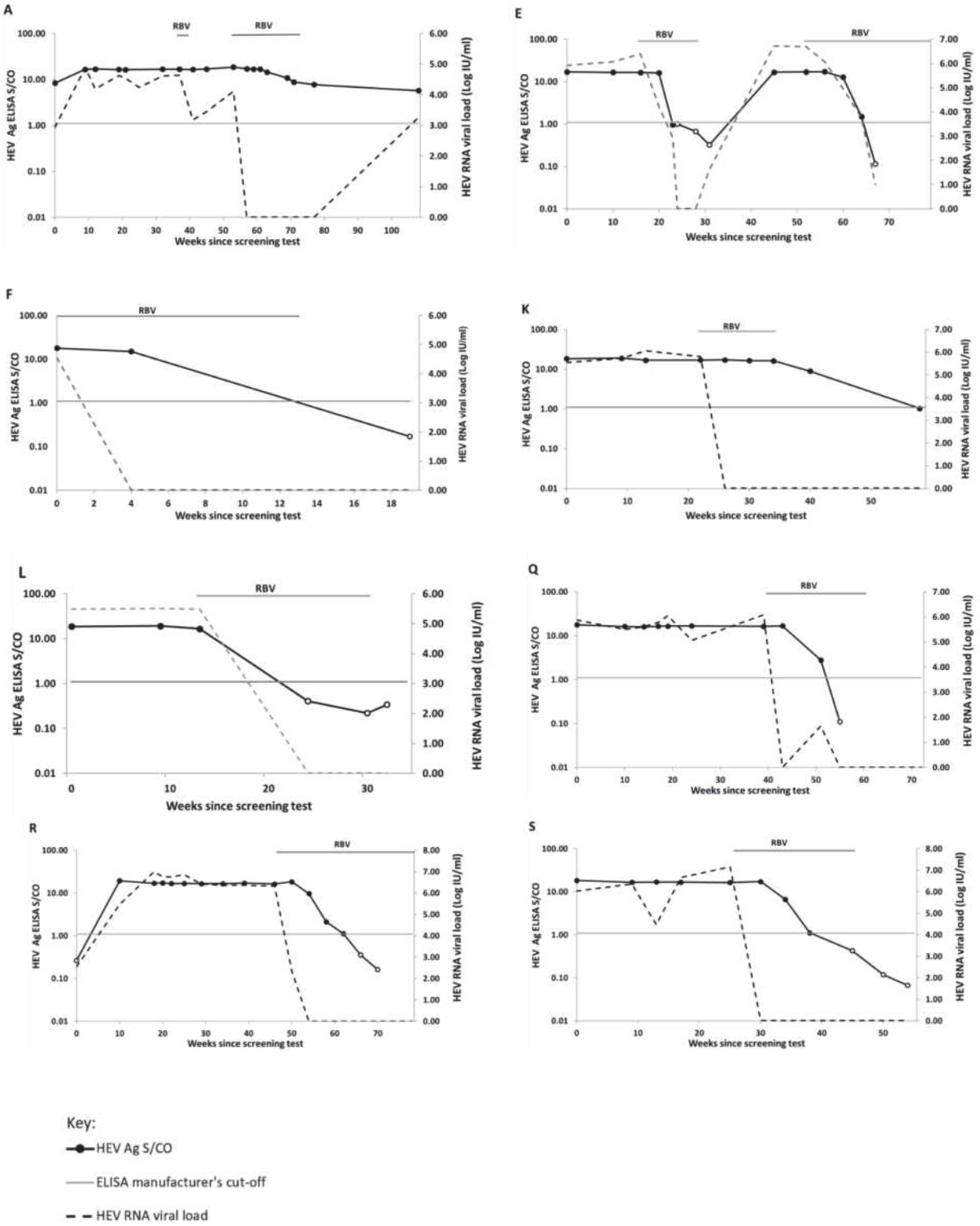


Correlation of plasma HEV RNA viral load and HEV-Ag reactivity in untreated patients. Five patients (Patients D, G, I, Y and Z) with active HEV infection who received no treatment during follow-up were tested for HEV-Ag in baseline plasma samples and subsequent follow-up samples. Data shown are the HEV-Ag ELISA results during follow-up. Follow-up varied between 11.0 weeks (patient Z) and 22.3 weeks (patient G).

Open circles represent samples negative for HEV-Ag by manufacturer's criteria and filled-in circles represent samples positive for HEV-Ag by manufacturer's criteria. The grey line represents the manufacturer's cut off threshold for defining a reactive sample.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV Ag, Hepatitis E virus antigen; IU, international units; S/CO, sample over cut-off ratio.

A2.2 Plasma HEV-Ag in antiviral treated patients



Correlation of plasma HEV RNA viral load and HEV-Ag reactivity in antiviral-treated patients. Eight patients who had persistent HEV infection (Patients A, E, F, K, L, Q, R and S) and were treated with Ribavirin were tested for HEV-Ag at baseline and throughout follow-up. Data shown are the HEV-Ag ELISA results during follow-up which varied between 19.0 weeks (patient F) and 108.1 weeks (patient A).

Open circles represent samples negative for HEV-Ag by manufacturer's criteria and filled-in circles represent samples positive for HEV-Ag by manufacturer's criteria. The grey line represents the manufacturer's cut off threshold for defining a reactive sample.

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HEV Ag, Hepatitis E virus antigen; S/CO, sample over cut-off ratio.

Appendix 3. Chapter 4 supplementary results

A3.1 Immunosuppressive medication given to patients with haematological malignancy prior to HEV RNA testing

Treatment	No.	Included regimes
Plasma Cell Disorders, n=271		
High intensity chemotherapy	45	Autograft, DTPACE
Standard intensity chemotherapy	138	Ixasomib+lenalidomide+dexamethasone, bortezomib+cyclophosphamide+dexamethasone,
Other combination chemotherapy	1	Carboplatin-based chemotherapy for non-haematological malignancy
Single agents +/- corticosteroid	84	Cyclophosphamide, lenalidomide, bortezomib, carfilzomib, ixazomib, CC-220
CAR-T therapy	1	-
Radiotherapy	2	-
Acute Leukaemia, n=75		
High intensity chemotherapy	53	DA60+GP/ARA-C, DA60+GO, DA50/ARA-C, FLAG-IDA, FLA, MidAC, Mini-FLAG-Ida+Gilterinib, clinical trials (UKALL14, UKALL 2011, UK11, UK60, AML19, AML17)
Low intensity chemotherapy	22	Low dose ARA-C, azacitidine, arsenic, imatinib, nilotinib, clinical trials (WT1 Trial Leukopheresis, IL3RA: KHK2823)
Chronic Leukaemia, n=67		
Low intensity chemotherapy	25	FC-R, ABVD, R-CVP, Rituximab+idelalisib+/-venetoclax, obinutuzumab+chlorambucil, ADCT 402+CHOP-R, venetoclax+idelalisib, ritxuximab+chlorambucil, clinical trials (FLAIR), single agents (cyclophosphamide, cladrabine, chlorambucil, methotrexate)
Single agents - targeted small molecule inhibitors	41	Idelalisib, ibrutinib, imatinib, nilotinib, dasatinib
Single agents - monoclonal antibodies	1	Rituximab
Lymphoma, n=171		
High intensity chemotherapy	34	IVE+/-GDP, ABVD+escalated BEACOPP, MAXI CHOP-R+/-ARA-C, CHOP-R+HD MTX , CHOP-R+bendamustine, ESHAP+brentuximab+bendamustine, R-CODOX-M+/-R-IVAC

Moderate intensity chemotherapy	99	ABVD, R-CHOP, R-CVP, FC-R, DHAP-R, ESHAP, R-BAC, R-bendamustine, R-GDP, R-GemOX, R-CVP+CHOP-R, ESHAP, MATRix, PMit-R, R-HDTMX, R-GDP
Low intensity chemotherapy	26	Chlorambucil, methotrexate, gemcitabine, hydroxycarbamide, ciclosporin
Single agents - targeted small molecule inhibitors	4	Ibrutinib and clinical trials (TAK-659 TRIAL)
Single agents - monoclonal antibodies	7	Rituximab, brentuximab, denusomab for non-haematological malignancy (n=1)
Radiotherapy	1	-
MDS, n=15		
Low intensity chemotherapy	13	Azacitidine, ciclosporin
Single agents - monoclonal antibodies	2	Alemtuzumab
MPN, n=27		
Low intensity chemotherapy PLUS targeted small molecule inhibitors	3	Ruxolitinib PLUS azacitidine or thalidomide
Low intensity chemotherapy	4	Azacitidine
Very low intensity chemotherapy	6	Hydroxycarbamide
Single agents - targeted small molecule inhibitors	14	Ruxolitinib
Aplastic Anaemia, n=2		
Single agent immunosuppression	2	Prednisolone, ciclosporin
Total	**628**	

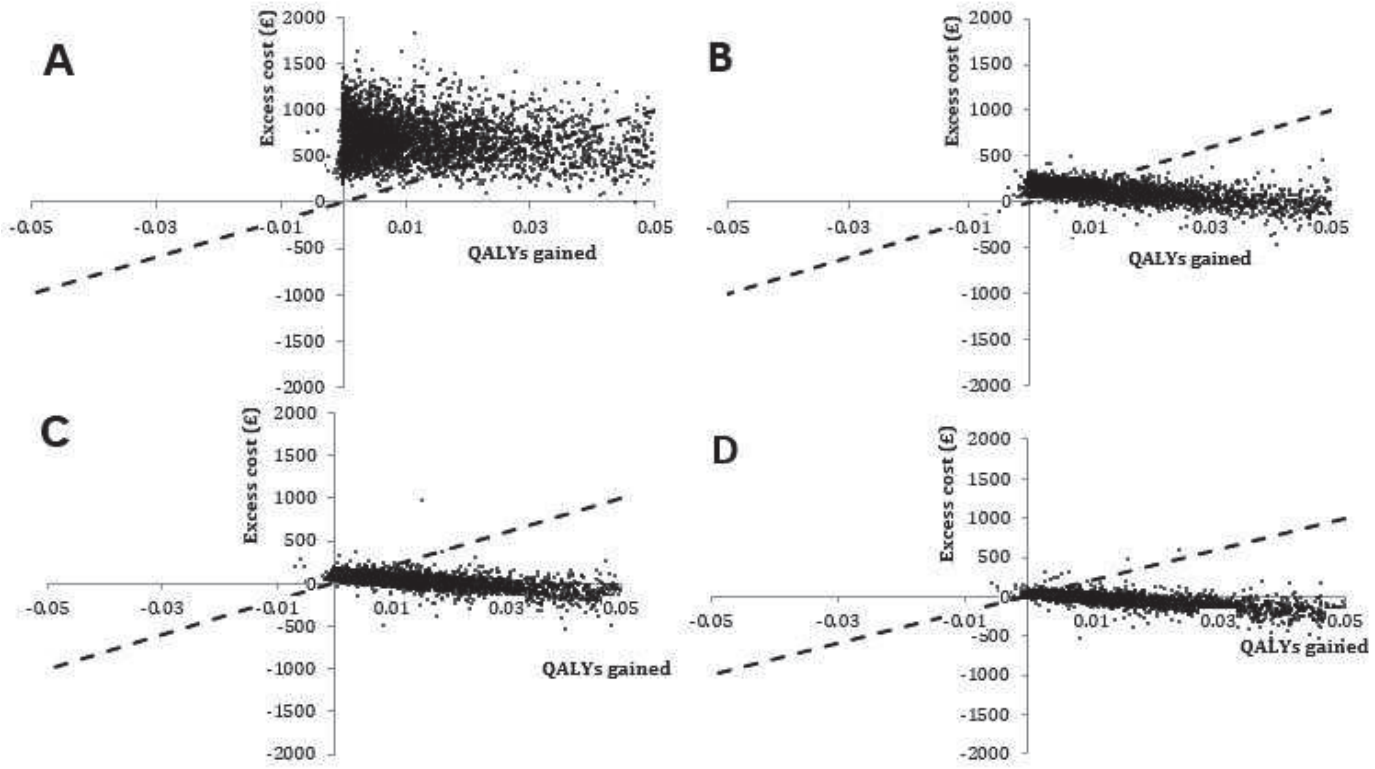
Legend for A3.1:

Modified version of Table 4.8 in main thesis detailing full immunosuppressive medication given to 628 patients with haematological malignancy in the six months prior to HEV RNA testing Where patients were in clinical trials, if the trial drug administered this was recorded, in blinded randomised trials only the known backbone drugs were recorded.

Abbreviations: ABVD, Doxorubicin, Bleomycin, Vinblastine, Dacarbazine; ARA-C, Cytarabine; BEACOPP, Bleomycin Etoposide Doxorubicin Cyclophosphamide Vincristine Procarbazine Prednisolone; CAR-T, chimeric antigen receptor T cell therapy; DA, daunorubicin, cytarabine; DHAP-R, Dexamethasone, high dose cytarabine, Cisplatin, Rituximab; DTPACE, dexamethasone, thalidomide, cisplatin, doxorubicin; ESHAP, Etoposide, Methylprednisolone, Cytarabine, Cisplatin; FC-R, Fludarabine, Chlorambucil, Rituximab; FLA(G)-IDA, Fludarabine cytarabine, Idarubicin; HDAC, high dose cytarabine; HD MTX, High Dose Methotrexate; IVE, Ifosfamide, Epirubicin, Etoposide; MATRIx, Methotrexate, Cytarabine, Thiotepa, Rituximab; MiDAC, mitoxantrone, cytarabine; PMitR, Prednisolone, Mitoxantrone, Rituximab; R-CHOP, Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, Prednisolone; R-CVP, Rituximab, Cyclophosphamide, Vincristine, Prednisolone; R-BAC, Rituximab, Bendamustine, Cytarabine; R-CODOXM/R-IVAC, Vincristine, Doxorubicin, Cyclophosphamide, Cytarabine, Methotrexate, Ifosfamide, Etoposide; R-GemOX, Gemcitabine, Oxaliplatin, Rituximab.

Appendix 4. Chapter 6 supplementary results

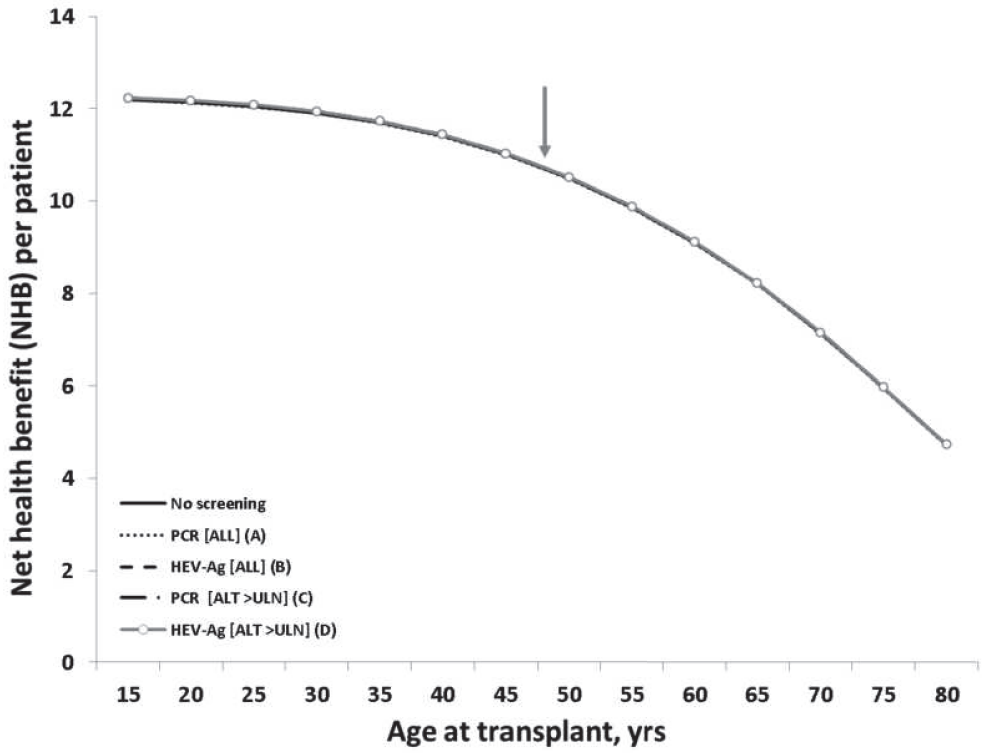
A4.1 Cost effectiveness planes for HEV screening strategies



CEA planes of 5000 Monte Carlo simulations for HEV screening of (A) all patients by PCR, (B) all patients by HEV-Ag detection (and PCR confirmation), (C) patient with an abnormal ALT by PCR and (D) patients with an abnormal ALT by HEV-Ag detection (and PCR confirmation),

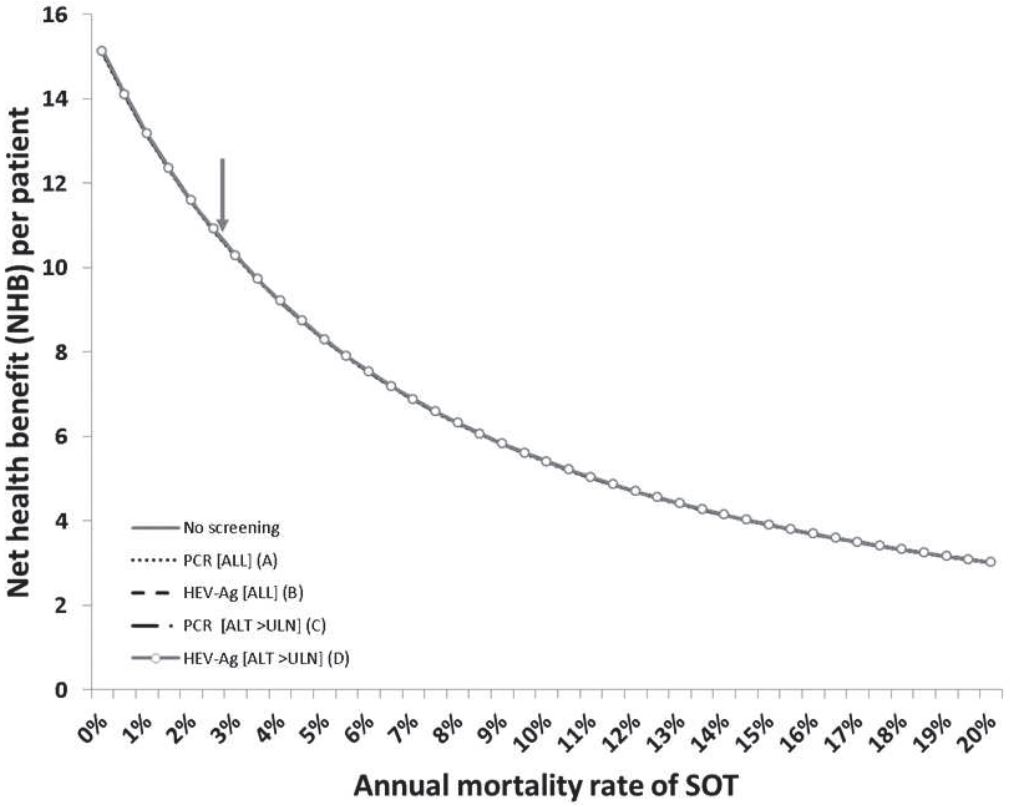
Abbreviations: ICER, incremental cost effectiveness ratio; QALY, quality-adjusted life year.

A4.2 Scenario analysis of the mean age at transplantation.



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the mean age of receiving a solid organ transplant. The arrow represents the base-case value. Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal; yrs, years.

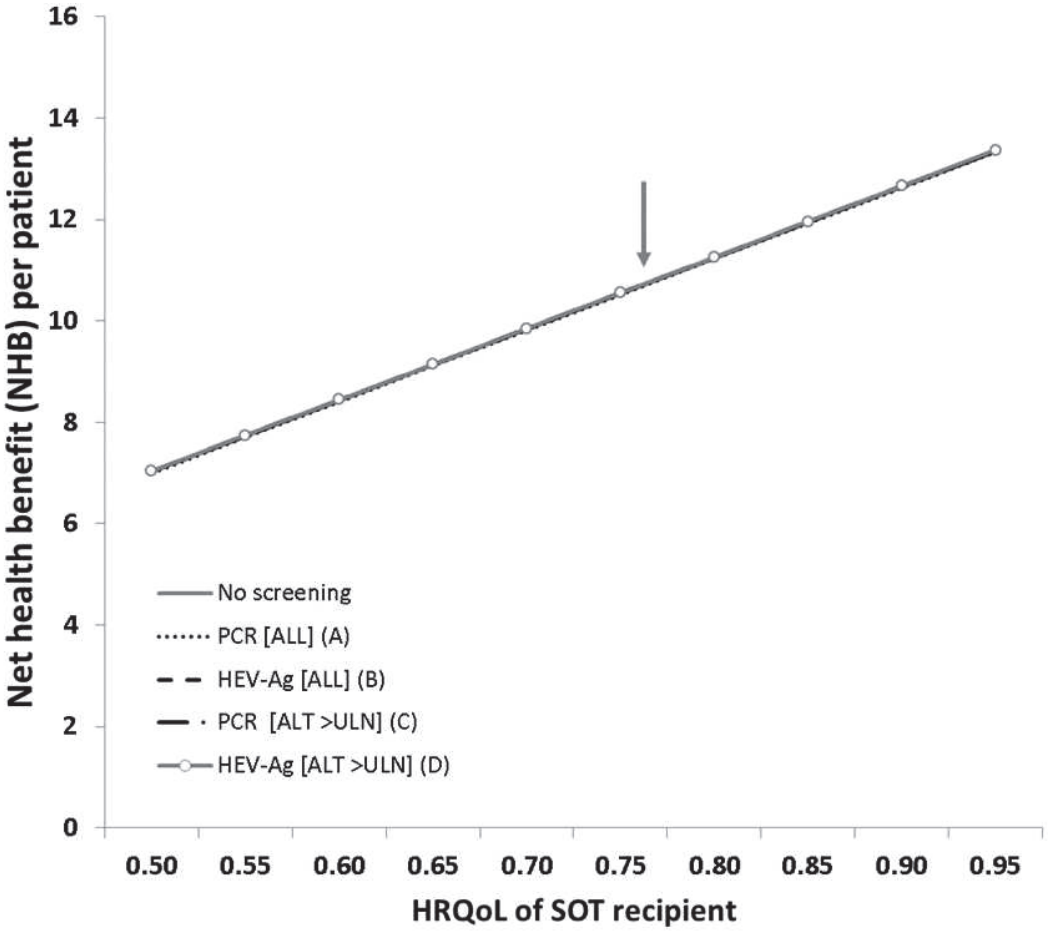
A4.3 Scenario analysis of the mortality rate of SOT recipient



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the annual mortality rate of SOT. The arrow represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; SOT, solid organ transplant; ULN, upper limit of normal.

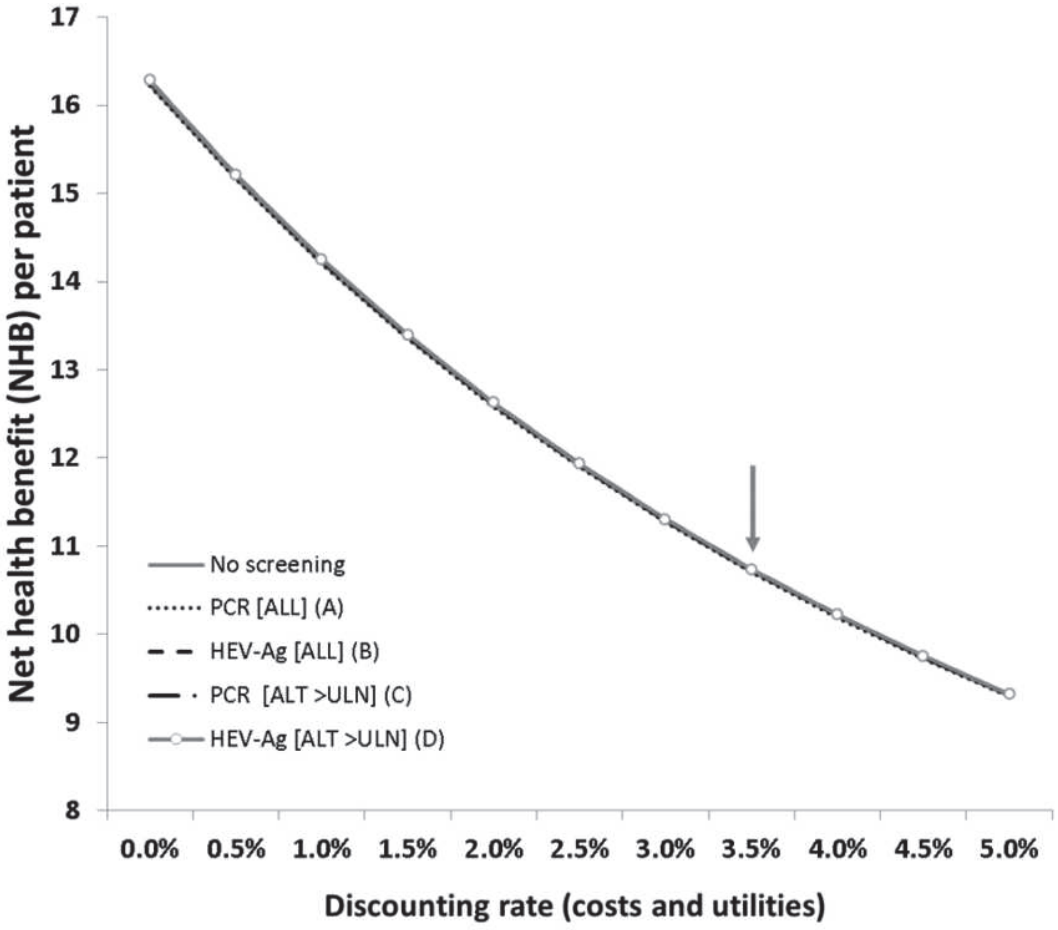
A4.4: Scenario analysis on the HRQoL of SOT recipient



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the HRQoL of a SOT recipient. The arrow represents the base-case value.

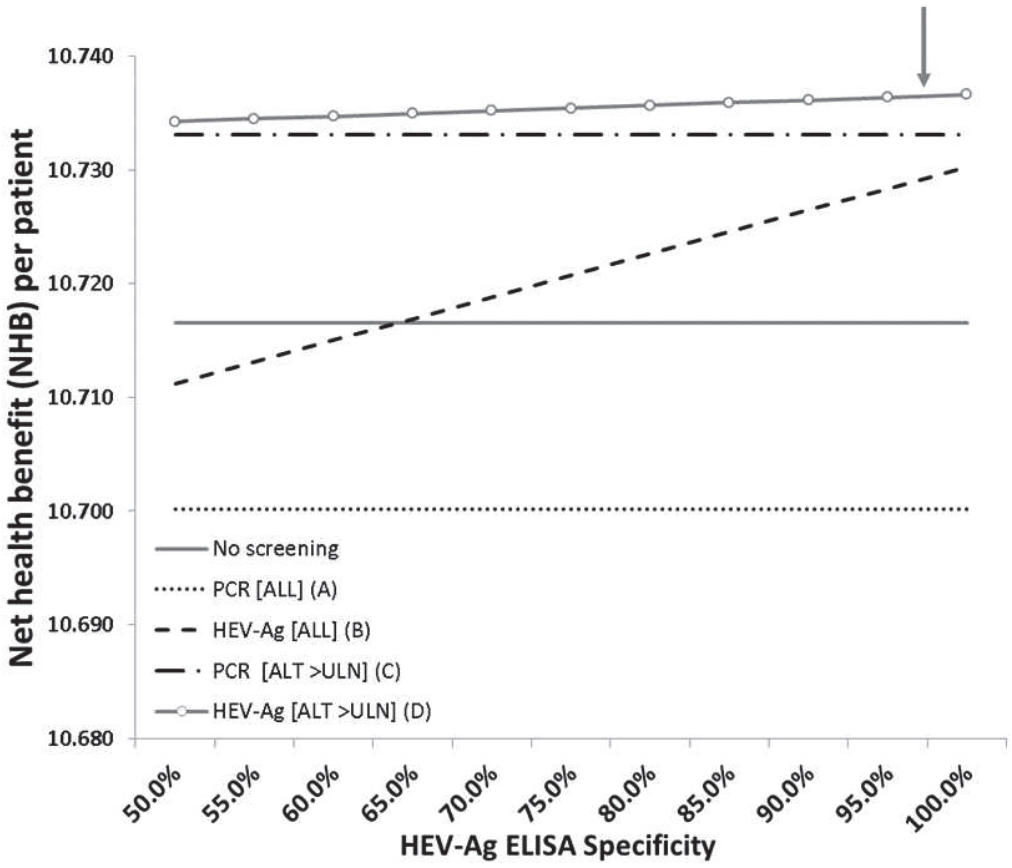
Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; HRQoL, health-related quality of life score; NHB, net health benefit; PCR, polymerase chain reaction; SOT, solid organ transplant; ULN, upper limit of normal.

A4.5: Scenario analysis on discounting of costs and utilities



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the discounting rates of costs and utilities. The arrow represents the base-case value.
Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

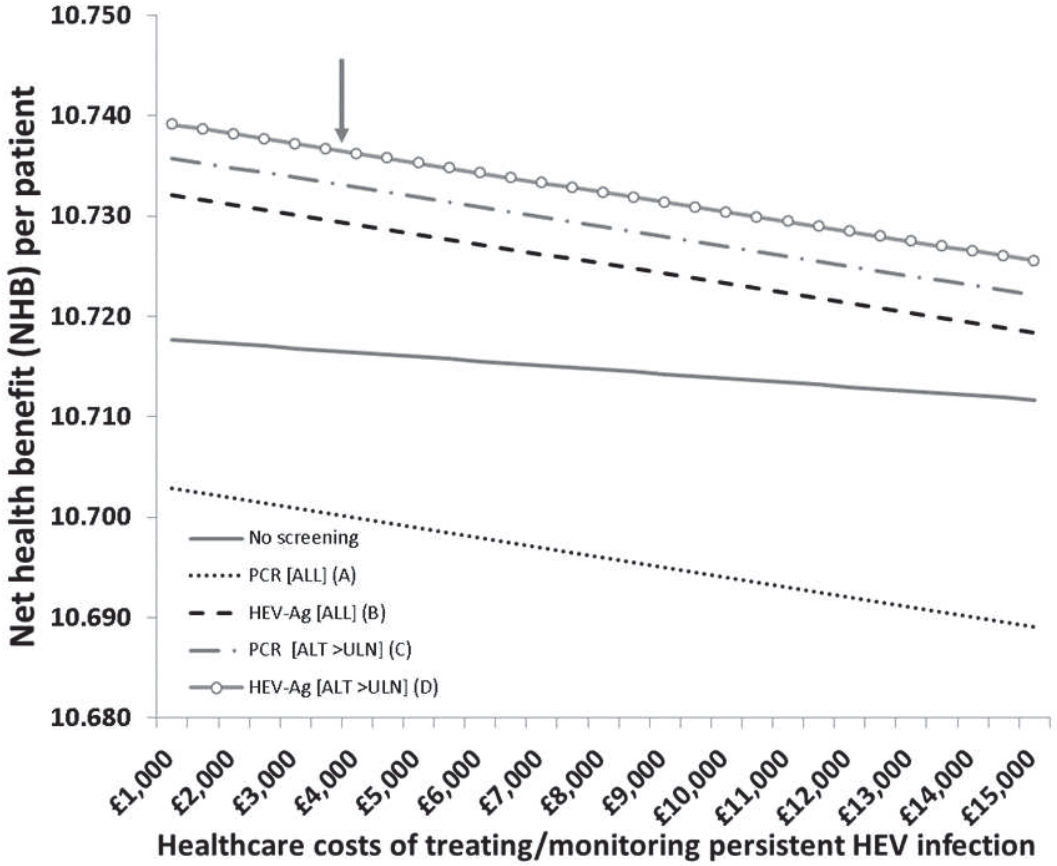
A4.6: Scenario analysis on the specificity of HEV-Ag testing



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the specificity of the HEV-Ag ELISA. The arrow represents the base-case value.

Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

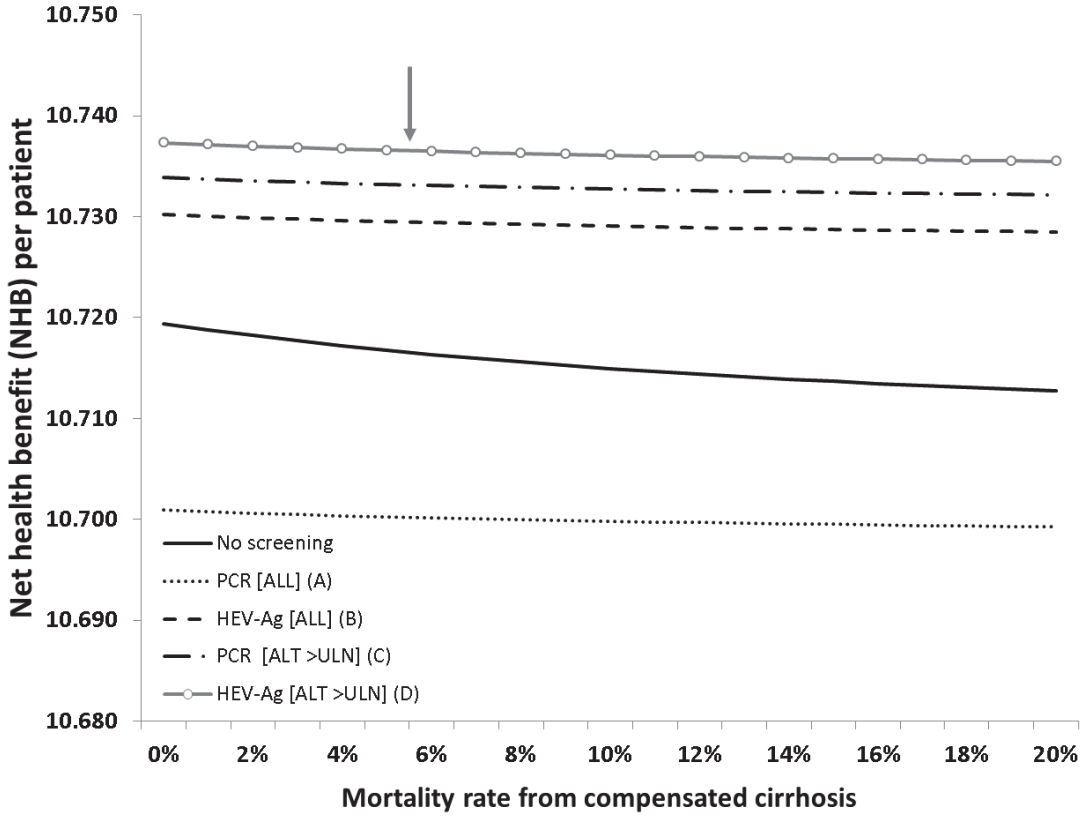
A4.7: Scenario analysis on healthcare costs of treating/monitoring persistent HEV infection



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the average annual healthcare costs of treating and monitoring persistent HEV infection. The arrow represents the base-case value.

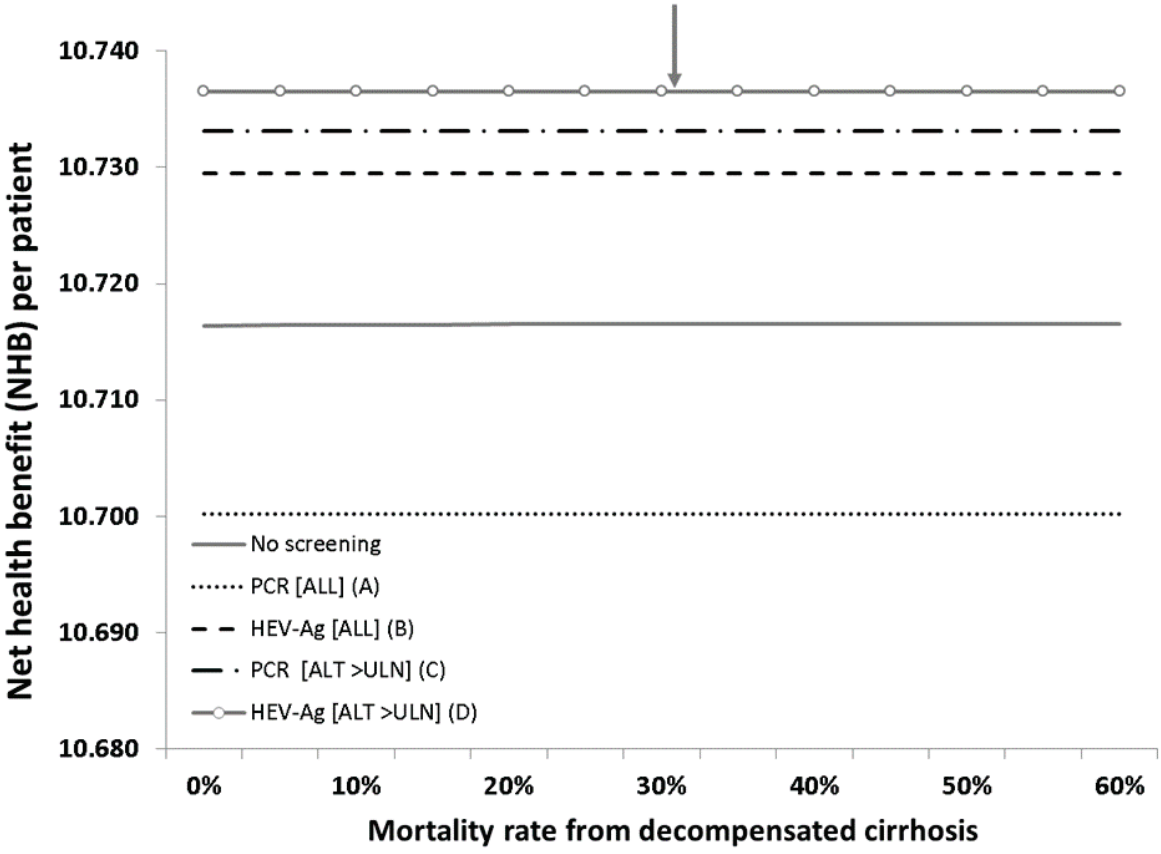
Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

A4.8: Scenario analysis on mortality rate from compensated cirrhosis



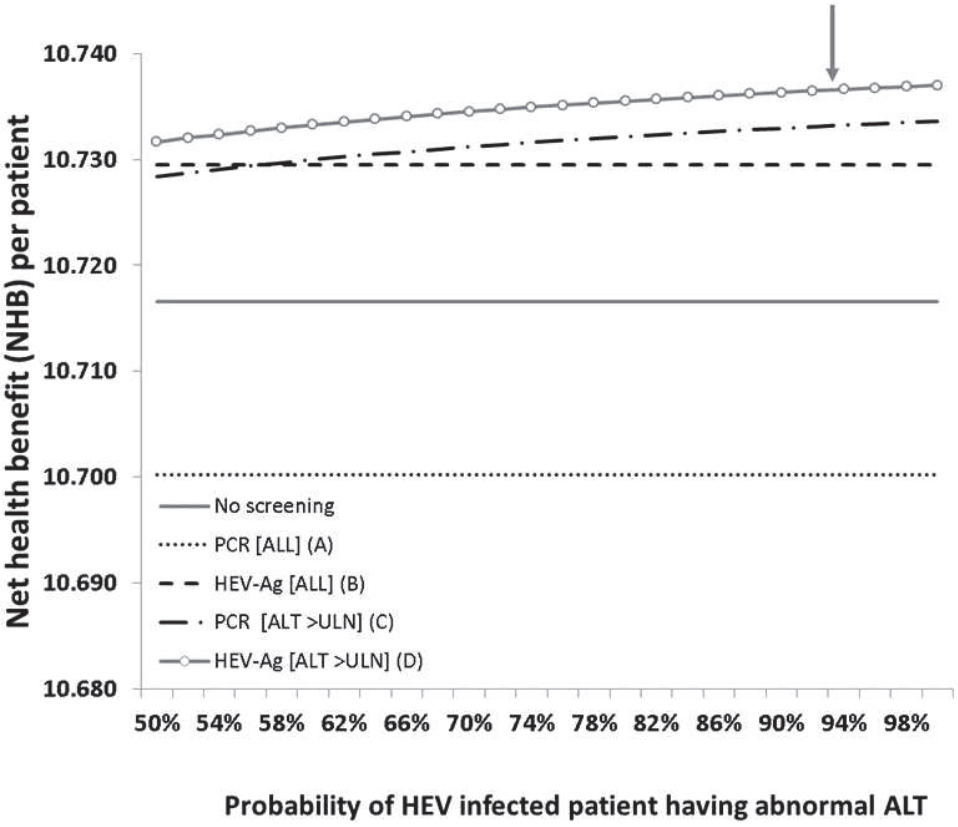
Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the annual mortality rate from compensated cirrhosis. The arrow represents the base-case value. Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

A4.9 Scenario analysis on mortality rate from decompensated cirrhosis.



Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the annual mortality rate from decompensated cirrhosis. The arrow represents the base-case value. Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

A4.10: Scenario analysis on the probability an HEV-infected patient has an abnormal ALT

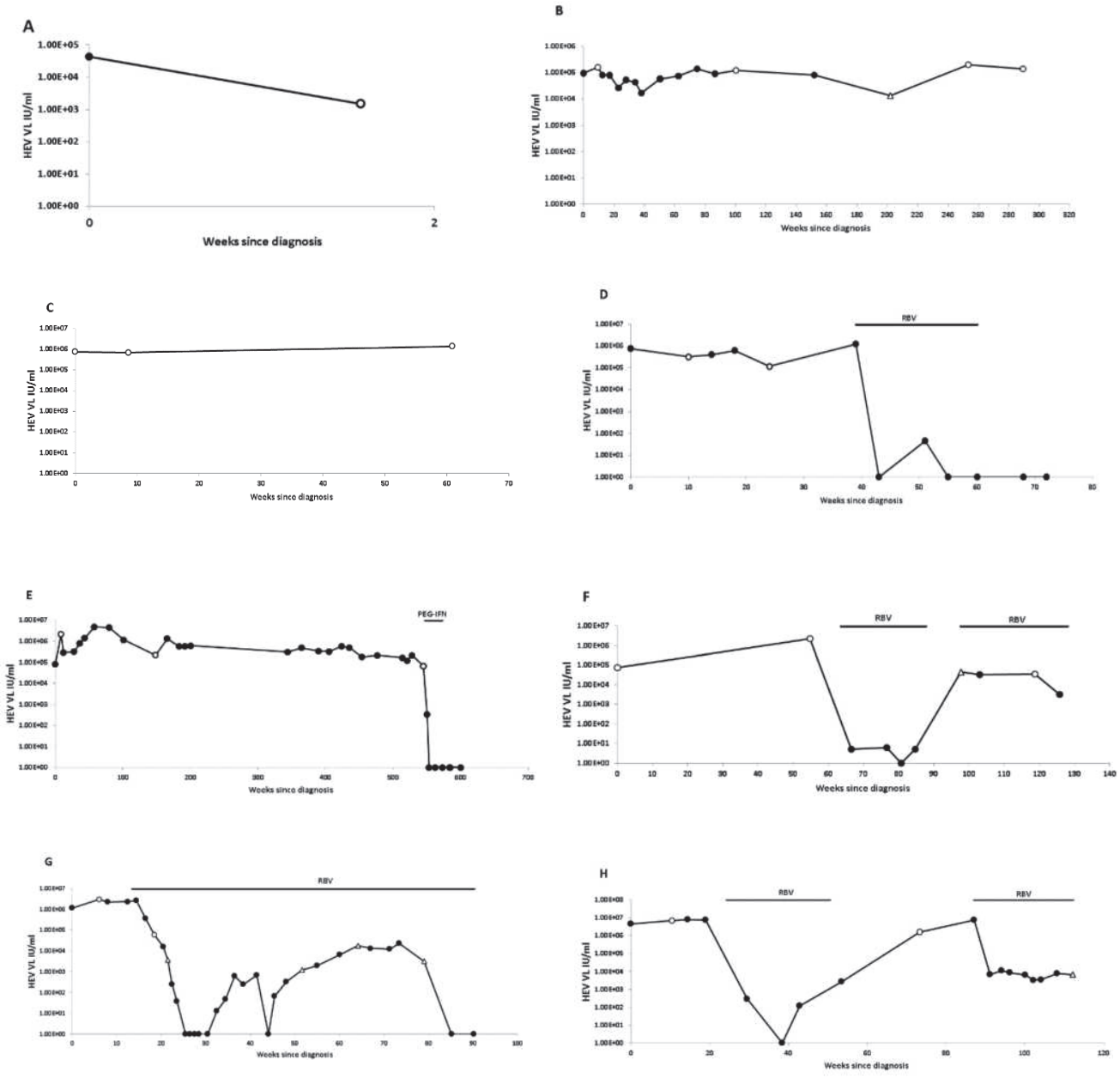


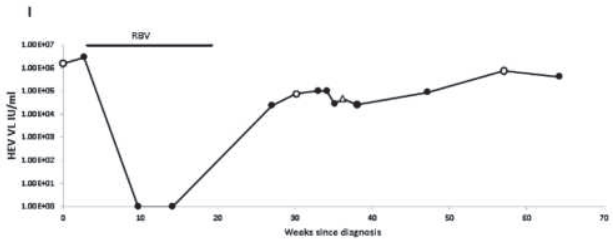
Comparison of the NHB per patient for each screening option at the threshold of £20,000/QALY when varying the annual probability an HEV-infected patient has an abnormal ALT. The arrow represents the base-case value.

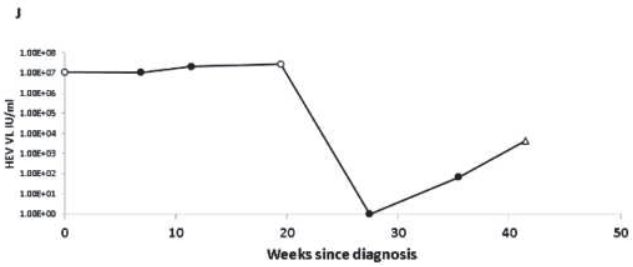
Abbreviations: ALT, alanine aminotransferase; HEV-Ag, hepatitis E virus antigen; NHB, net health benefit; PCR, polymerase chain reaction; ULN, upper limit of normal.

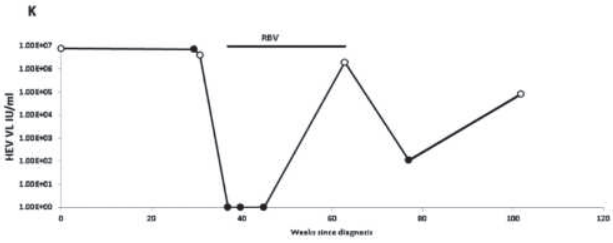
Appendix 5. Chapter 7 supplementary results

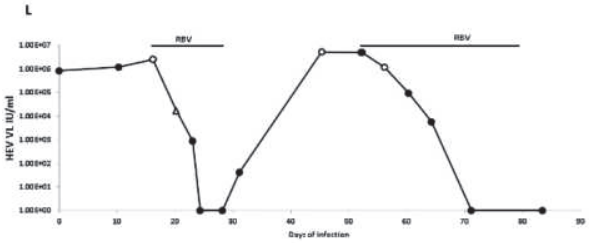
A5.1. Virological profiles of patients undergoing HEV Illumina whole genome sequencing

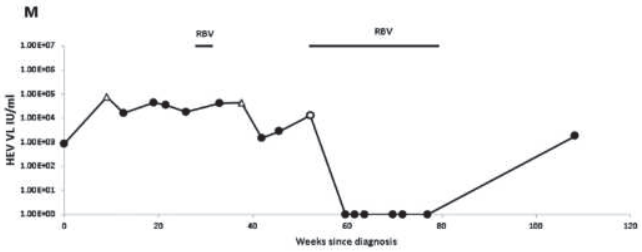


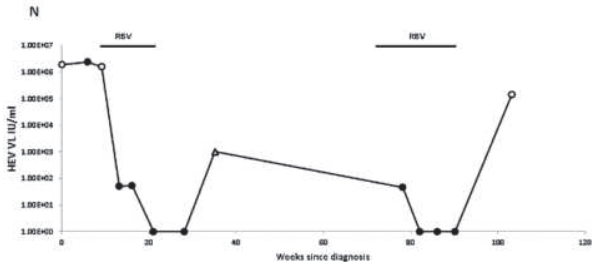

I
RBV
HEV VL IU/ml
Weeks since diagnosis

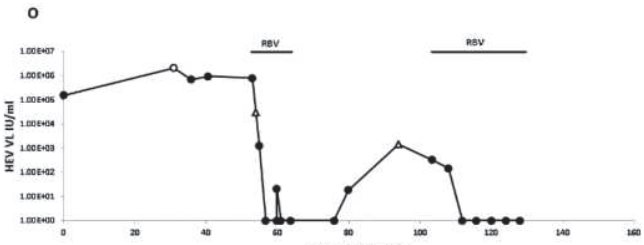

J
HEV VL IU/ml
Weeks since diagnosis

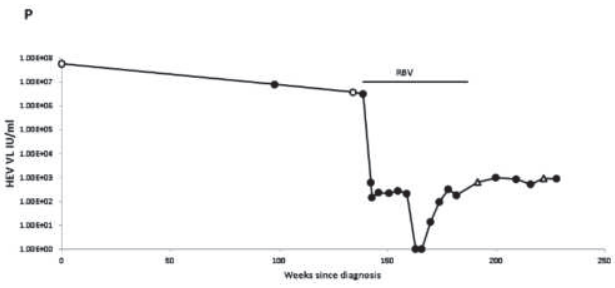

K
RBV
HEV VL IU/ml
Weeks since diagnosis

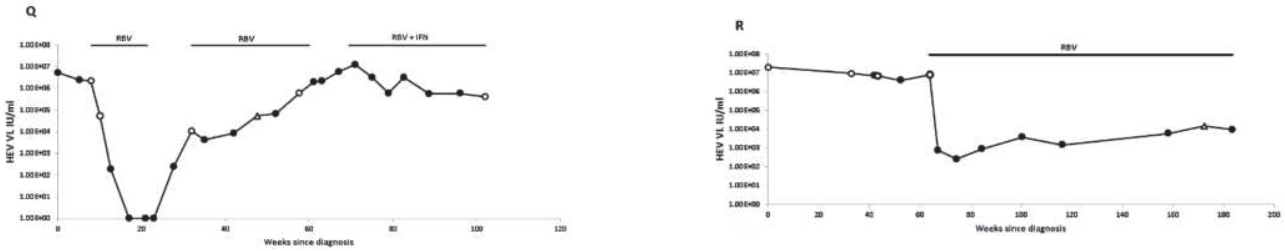

L
RBV
RBV
HEV VL IU/ml
Days of infection


M
RBV
RBV
HEV VL IU/ml
Weeks since diagnosis


N
RBV
RBV
HEV VL IU/ml
Weeks since diagnosis


O
RBV
RBV
HEV VL IU/ml
Weeks since diagnosis


P
RBV
HEV VL IU/ml
Weeks since diagnosis



Presented are the virological profiles in serum samples of eighteen patients with HEV G3 viraemia and defined virological outcomes which were selected for whole genome sequence analysis. Open circles represent samples successfully sequenced and open triangles represent samples not successfully sequenced.

Abbreviations: IFN, pegylated interferon; RBV, ribavirin; VL, viral load.

A5.2 Summary of HEV whole genome sequencing results

Sample	HEV VL (IU/ml)	Genome Size (nt)	Genome Coverage (%)	Reads mapped (%)	Average depth
A1 ACUTE	9.60E+04	7251	80.21	4.20	1852
B1 NO RBV	1.63E+05	7423	97.91	8.24	11499
B2 NO RBV	1.21E+05	7237	98.74	6.73	12306
B3 NO RBV	2.03E+05	7237	98.70	3.36	4722
B4 NO RBV	1.40E+05	7237	83.14	11.80	4469
C1 NO RBV	1.40E+06	7237	99.79	95.70	143724
C2 NO RBV	7.52E+05	7237	99.72	81.86	15149
C3 NO RBV	6.80E+05	7237	99.68	32.44	4441
D1 PRE RBV	3.20E+05	7110	100.00	50.44	78749
D2 PRE RBV	1.18E+05	7110	100.00	61.83	59842
E1 PRE IFN	2.10E+06	7251	94.44	100.00	1527
E2 PRE IFN	2.19E+05	7423	84.33	100.00	1183
E3 PRE IFN	6.56E+04	7251	98.14	100.00	4959
F1 PRE RBV	7.46E+04	7237	89.17	4.96	7046
F2 PRE RBV	2.20E+06	7237	95.38	100.00	1574
F3 ON RBV2	3.50E+04	7237	84.45	100.00	7
G1 PRE RBV	2.93E+06	7237	99.82	94.91	530748
G2 ON RBV	5.92E+04	7423	98.71	4.08	13123
H1 PRE RBV	6.72E+06	7237	99.72	70.59	19445
H2 POST RBV1	1.60E+06	7423	98.96	61.21	74280
I1 PRE RBV	1.60E+06	7237	99.34	100.00	93316
I2 POST RBV	7.87E+04	7251	88.88	5.66	5368
I3 POST RBV	7.70E+05	7251	99.56	100.00	26709
J1 PRE RBV	5.50E+06	7237	99.28	71.06	34456
J2 PRE RBV	2.73E+07	7237	99.82	92.94	123022
K1 PRE RBV	7.79E+06	7423	98.79	89.06	31755
K2 PRE RBV	4.04E+06	7237	99.82	79.10	21899
K3 POST RBV	1.98E+06	7423	98.73	24.75	3697

Sample	HEV VL (IU/ml)	Genome Size (nt)	Genome Coverage (%)	Reads mapped (%)	Average depth
L1 PRE RBV	2.53E+06	7216	99.53	81.58	17338
L2 POST RBV1	5.29E+06	7216	99.54	90.93	28257
L3 ON RBV2	1.18E+06	7237	99.75	17.16	8677
M1 PRE RBV	1.30E+04	7251	87.97	1.56	1197
N1 PRE RBV	1.90E+06	7423	98.73	88.08	42296
N2 PRE RBV	1.58E+06	7423	98.91	86.57	214254
N3 POST RBV2	1.43E+05	7237	99.47	37.43	42083
O1 PRE RBV	2.02E+06	7237	99.85	100.00	34231
P1 PRE RBV	7.99E+06	7423	98.21	100.00	48844
P2 PRE RBV	5.84E+07	7260	97.80	100.00	4440
Q1 PRE RBV1	2.30E+06	7185	99.28	100.00	30313
Q2 ON RBV1	5.60E+04	7251	98.59	100.00	2923
Q3 POST RBV1	1.10E+04	7251	96.43	100.00	369
Q4 ON RBV2	6.18E+05	7237	98.78	100.00	5130
Q5 ON RBV3	4.30E+05	7237	99.43	85.96	206778
R1 PRE RBV	2.02E+07	7237	98.63	100.00	5316
R2 PRE RBV	9.50E+06	7237	99.72	100.00	34488
R3 PRE RBV	7.20E+06	7237	99.75	69.82	22942
R4 PRE RBV	8.25E+06	7237	99.14	100.00	26268

Summary of samples processed for HEV whole genome sequencing. The name of the sample refers to the patient (A-R), the numbered sample for that patient (1-5) and the relationship of the sample with respect to therapy (Interferon or Ribavirin).

Abbreviations: IFN, pegylated interferon; IU, international units; nt, nucleotides; RBV, ribavirin; VL, viral load

A5.3. Reference sequences used by HEV GLUE

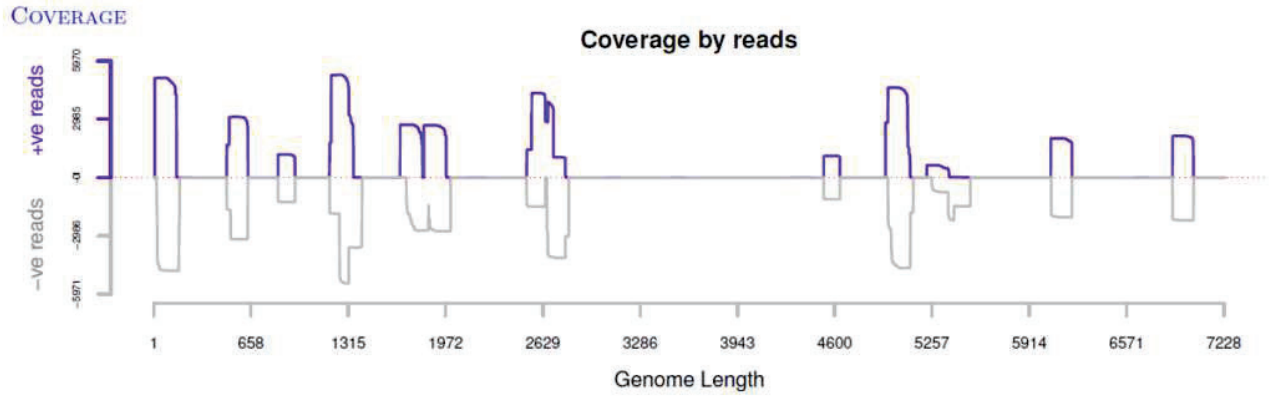
HEV Subgenotype	GenBank accession reference name
3a	AF082843
3b	AP003430
3c	FJ705359
3e	AB248521
3f	AB369687
3g	AF455784
3h	JQ013794
3i	FJ998008
3j	AY115488
3ra	FJ906895

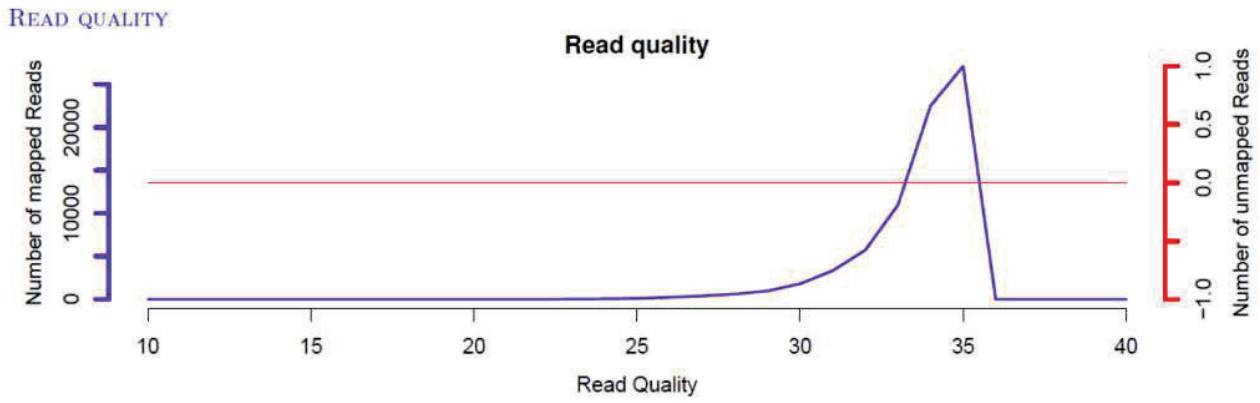
Reference subgenotypes used by bioinformatics software HEV GLUE in sequence analysis and the GenBank accession numbers.

A5.4 Summary reports for patient samples undergoing Illumina HEV whole genome sequencing.

A1-ACUTE

File name	**A1-ACUTE.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**73 832**
Mapped reads	**73 832 (100.00%)**
Read length	**140nt**
Coverage	**5 603nt (77.42%)**
Average depth	**1 418 reads/site**





A1-ACUTE Consensus sequence

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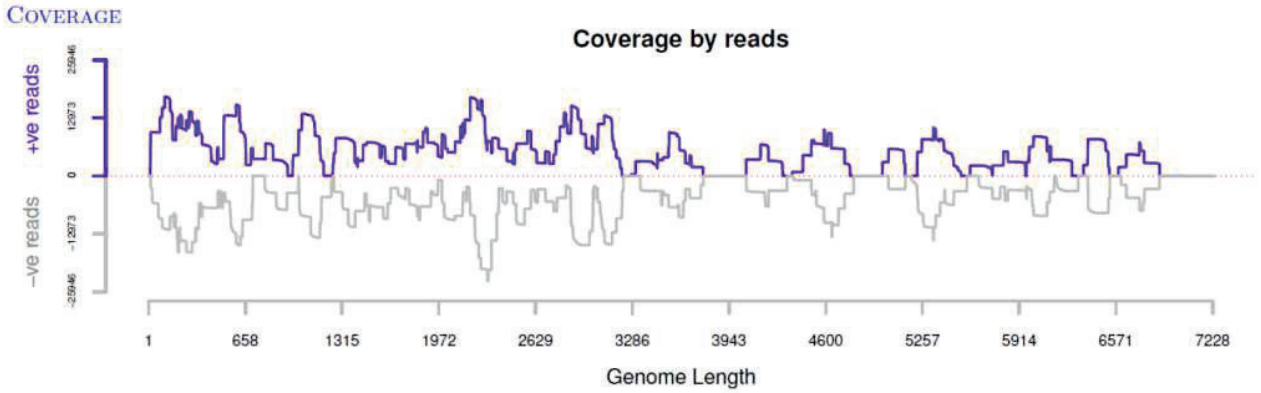
70	ATTGAGCAAG	CTGCTCTGGC	TGCGGCCAAT	TCTGCCCTGG	CGAATGCTGT	GGTGGTTCGG	CCGTTTTTAT
140	CTCGTGTACA	AACTGAGATT	CTTATTAATT	TGATGCAACC	CCGGCAGCTG	GTTTTCCGAC	CTGAAGTGCT
210	CTGGAATCAT	CCTATCCAAC	GGGTTATCCA	TAATGAACTT	GAACAGTACT	GTCGGGCCCG	GGCGGGTCGT
280	TGTTTTGAGA	TTGGGGCCCA	GCCAAGATCC	ATCAATGATA	ACCCGAATGT	TCTGCATCGC	TGCTTCCTAC
350	GACCAGTTGG	CAGAGATGTT	CAGCGCTGGT	ATTCAGCCCC	TACCCGTGGC	CCTGCGGCCA	ACTGTCGCCG
420	CTCTGCTTTG	CGTGGCCTTC	CCCCCGCTGA	CCGCACCTAN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
490	NNCGCAGAAA	CTGGGGTTGC	CCTTTACTCT	CTGCATGACC	TCTGGCCGGC	CGATGTTGCG	GAGGCTATGG
560	CTCGGCACGG	GATGACACGC	CTGTATGCTG	CACTACATCT	CCCTCCTGAA	GTACTACTAC	CACCCGGTAC
630	TTACCACACA	ACTTCATACC	TTCTCATCCA	CGACGGTGAT	CGTGCCGTTG	TGACTTACGA	GGGTGACACT
700	AGTGCAGGTT	ACAACCANNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
770	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNTGA
840	GCCGTCACCA	ATGCCTTATG	TTCCATACCC	CCGGTCGACA	GAGGTGTATG	TCCGCTCCAT	ATTCGGCCCT
910	GGCGGGTCCC	CATCCCTATT	TCCATCTGCT	TGCTCTACGA	AATCCNNNNN	NNNNNNNNNN	NNNNNNNNNN
980	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNC
1050	CTATCTCCGC	GGGATTAGTT	ACAAGGTCAC	TGTTGGCGCT	CTTGTCGCTA	ATGAGGGGTG	GAATGCTTCG
1120	GAGGACGCTC	TTACCGCTGT	TATTACTGCA	GCGTATTTAA	CCATTTGCCA	TCAGCGTTAT	CTCCGTACTC
1190	AAGCTATATC	CAAGGGTATG	CGCCGACTGG	AGGTTGAGCA	TGCCCAAAAA	TTTATTACAA	GACTTTATAG
1260	TTGGCTGTTC	GAAAAGTCCG	GCCGTGACTA	TATCCCTGGC	CGCCAGCTCC	AGTTCTATGC	ACAGTGCCGC
1330	CGCTGGTTAT	CGGCGGGTTT	CCATCTTGAC	CCAAGGGTGC	TTGTTTTCGA	TGAGGCTGTG	CCCTGCCGCT
1400	GCAGGACGTT	TCTTAAGAAG	GTTGCGGGTA	AGTTCTGCTG	TTTTATGAAG	TGGCTGGGGC	AGGAGTGCAC
1470	TTGCTTCCTG	GAACCAGCTG	AGGGCCTGGT	TGGTGACCAT	GGTCACGATA	ATGAAGCCTA	TGAGGGTTCT
1540	GAGGTCGACC	AGGCTGAGCC	CGCCCACCTC	GATGTCTCTG	GGACCTACGC	CGTCCATGGC	CGCCAACTCG
1610	AGGCCCTGTA	CAGGGCGCTC	AACATCCCAC	ATGNNNNNNN	NNNNCGAGCC	TCCCGTTTGA	CTGCCACCGT
1680	CGAACTCGTT	GCAAGTCCAG	ACCGTTTGGA	GTGCCGCACT	GTGCTTGGGA	ACAAGACTTT	CCGGACGACG
1750	GTGGTTGACG	GCGCTCATCT	TGAGGCGAAT	GGCCCCGAGC	AGTACGTTCT	TTCGTTTGAC	GCCTCTCGCC
1820	AGTCTATGGG	GGCCGGGCCG	CATAGTCTCT	CCTACGAGCT	CACCCCTGCT	GGTTTGCAGG	TCAAGATTTC
1890	ATCTAATGGC	CTGGATTGCA	CTGCAACATT	CCCTCCGGGC	GGGGCCCCTA	GCGCTGCTCC	GGGGGAGGTG
1960	GCAGCCTTCT	GCAGTGCCCT	CTACAGGTAC	AACAGGTTCA	CTCAGCGCCA	TTCGCTTACA	GGCGGTTTGT
2030	GGCTGCATCC	TGAGGGGCTA	TTGGGCATCT	TCCCTCCCTT	TTCCCCCGGG	CATCTTTGGG	AGTCCGCTAA
2100	CCCTTTTTGT	GGGGAGGGAA	CTTTGTATAC	CCGGACCTGG	TCAACATCTG	GTTTTTCTAG	TGATTTTTCC
2170	CCCCCTGAGG	CGGCTGCCGC	TGTACCGGCC	GCTACCACAG	GGTTACCCCA	CCCTACACCC	CCTGTTAGTG
2240	ANNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2310	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2380	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2450	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNA
2520	CCAACGCTAC	CCCGAATCTT	TCCATCCAAC	TGAGTTCATT	ATGCGCGACG	GCCTTGCCGC	GTACACTTTA
2590	ACTCCCCGGC	CTATTATTCA	TGCAGTGGCT	CCTGATTATA	GGGTTGAGCA	TAACCCAAAG	AGGCTTGAGG
2660	CAGCATACCG	AGAGACTTGC	TCCCGCCGCG	GTACCGCCGC	TTACCCACTC	CTCGGCTCGG	GTATATACCA
2730	AGTTCCCGTC	AGCCTCAGCT	TTGATGCTTG	GGAGCGTAAC	CACCGCCCCG	GAGATGAGCT	CTACCTAACC
2800	GACCTCGCCG	CTACCTGGTT	CGAGGCCAAT	AAGCCAGCAC	AACCGGCCCT	CACAATAACT	GAAGATACAG
2870	CNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNGGC	CGGGCATGTG	CCGGTTGTAC
2940	AGTTAGTCCT	GGGATTGTGC	ACTATCAATT	TACTGCCGGG	GTCCCAGGTT	CAGGGAAGTC	GCGATCTATA
3010	CAGCAAGGGG	ACGTCGATGT	GGTGGTCGTC	CCCACTCGGG	AGCTTCGGAA	TAGTTGGCGT	CGCCGGGGTT
3080	TTGCAGCTTT	TACGCCTCAC	ACGGCGGCCC	GTGTTACCAC	GGGCCGACGT	GTTGTGATTG	ATGAGGCCCC
3150	ATCCCTTCCG	CCGCACCTGC	TGCTTTTACA	TATGCAGCGG	GCCTCGTCGN	NNNNNNNNNN	NNNNNNNNNN
3220	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3290	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3360	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	GAAGTTGGTT
3430	TTTACGCAAG	CAGCTAAAGC	AGCCAACCCC	GGTGCAATTA	CTGTCCACGA	GGCCCAGGGT	GCCACTTTCA
3500	CGGAGACCAC	AATTATAGCC	ACGGCCGATG	CTAGGGGGCT	CATTCAATCC	TCCCGAGCCC	ATGCCATAGT
3570	CGCACTTACC	CGCCACACAG	AGAAGTGCGT	TATCCTTGAT	GCTCCCGGTT	TATTACGTGA	GGTNGGTATA
3640	TCTGACATAA	TTGTCAACAA	TTTCTTTCTC	GCCGGTGGGG	AAGTGGGCCA	CCACCGCCCC	TCTGTGATAC

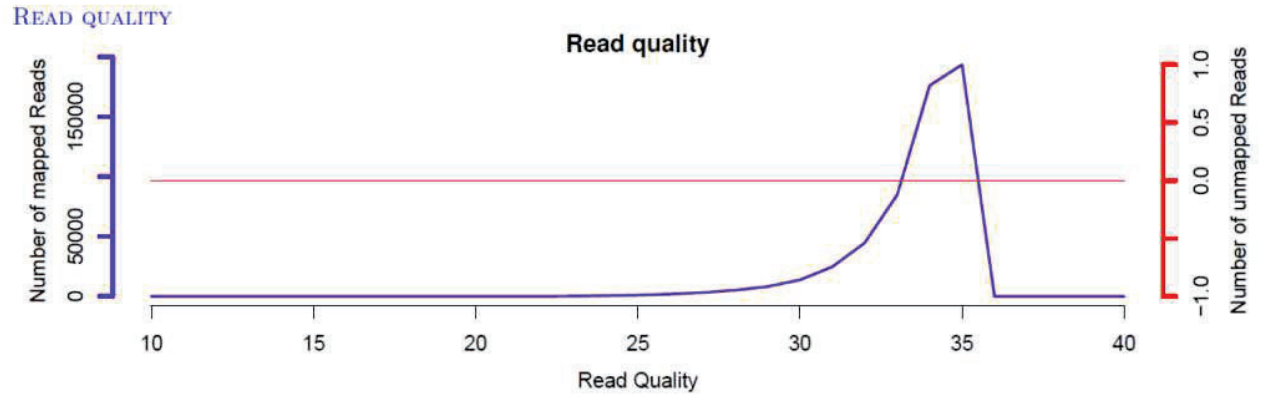
A1-ACUTE Consensus sequence

```
3710  CCCGCGGCAG TCCTGACCAG AACCTCGCGA CACTGCAGGC TTTTCCACCC TCCTGCCAGA TCAGTGCTTA
3780  CCATCAGTTA GCTGAGGAGC TGGGTCACCG CCCGGCCCCC GTCGCTGCCG TCCTGCCCCC CTGCCCNNNN
3850  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3920  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3990  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
4060  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
4130  GTCAGGACGG CTCTGCCGTA CTTGAGCTTG ATCTTTGCAA TCGCGATGTG TCGCGTATTA CATTCTTCCA
4200  GAAAGATTGT AACAAGTTCA CCACAGGGGA GACCATTGCT CATGGTAAGG TCGGCCAGGG CATTTCGGCT
4270  TGGAGCAAGA CCTTCTGTGC CCTCTTTGGC CCGTGGTTTC GTGCCATTGA AAAAGAAATA TTGGCCCTGC
4340  TCCCGCCCAA TATCTTCTAT GGCGATGCAT ATGAAGAGTC TGTGTTTGCT GCCGCTGTGT CTGGGGCAGG
4410  TTCATGCATG GTATTTGAGA ATGATTTTTC AGAGTTTGAC AGCACTCAAA ATAACTTCTC CCTTGGCCTC
4480  GAGTGTGTAG TCATGGAGGA GTGCGGCATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  CCTGGATCTT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AACCTGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTCCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGTGATGA TTCCGTGGTT CTCTGTAGCG ACTACCGTCA GGGCCGCAAC GCAGCGGCCC
4760  TGATTGCAGG CTGCGGACTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCCGGT TTGGGGACGC TACCCGATGT TGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCGGGGCCCG AGCGCGCCGA GCAGTTGCGC TTAGCTGTTT GTGACTTTCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTCGATGTT GTATCCCGTG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGCTGCAA ACCATAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT NNNNNGAATA ACATGTTTTG TGCATCGCCC ATGGGGTCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CCTCGTGCTT TTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG TACCACAATC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCCCCGCCG
5460  CCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCTATA TCACCNNNNN NNNNNNNNNN
5530  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5600  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5670  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNCATC TAATTATGCC CAGTATCGGG TTGTTCGAGC
5740  CACGATCCGT TATAGGCCAT TGGTGCCGAA TGCTGTCGGC GGCTACGCTA TTTCCATCTC TTTTTGGCCT
5810  CAGACTACTA CCACCCCTAC GTCTGTTGAC ATGAATTCTA TTACCTCCAC TGATGTTAGG ATCCTAGTTC
5880  AGCCCGGTAT CGCTTCCGAA CTAGTTATTC CTAGTGAACG CCTCCACTAC CGTAATCAAG GTTGGCGCTC
5950  TGTGGAGACC TCGGGTGTAG CTGAGGAGGA GGCTACTTCT GGTCTGGTGA TGCTTTGTAT CCATGGTTCC
6020  CCTGTTAATT CCTACACCAA TACCCCTTAT ACCGGGGCGC TTGGGCTCCT TGATTTCGCT TTGGAGCTTG
6090  AGTTTAGGAA CTTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GTTGCGCCGC GGTGCTGATG GCACCGCTGA ACTTACTACC ACAGCAGCTA CACGTTTCAT GAAGGACCTG
6230  CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGCCGTG GTATTGCTCT TACACTCTTT AATCTTGCTG
6300  ACACGCTCCT TGGCGGTTTG CCGACAGNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6370  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNG AGAATGCGCA GCAGGATAAA
6440  GGTATTGCTA TTCCGCATGA CATAGACTTA GGTGATTCCC GTGTGGTTAT TCAAGATTAT GACAATCAGC
6510  ATGAGCAGGA TCGGCCCACC CCTTCGCCCG CCCCATCTCG CCCTTTTTCG GTTCTTCGTG CTAATGATGT
6580  TTTATGGCTT TCCCTTACCG CTGCCGAGTA TGATCAGACT ACATATGGGT CGTCCACCAA CCCAATGTAT
6650  GTCTCAGATA CTGTTACATT TGTCAATGTG GCTACAGGAG CCCAGGCTGT TGCCCGCTCT CTTGACTGGT
6720  CTAAGGTCAC CCTAGACGGC CGCCCCCTCA CTACNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6790  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6860  NNNNNNNNNN NNNNNNNTTT GATTGAAAAT GCAGCTGGTC ATCGTGTTGC CATCTCCACG TACACTACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TTTAGCTCCA CATTCGGCTC TTGCAGTCCT
7000  TGAGGACACT ATTGACTACC CTGCCCGCGC CCACACTTTT GATGACCTCT GTCCAGAGTG TCGCACTCTT
7070  GGCTTGCAAG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7140  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7210  NNNNNNNNNN NNNNNNNNNN NNNNNNN
```

B1-NO-RBV

File name	**B1-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**557 681**
Mapped reads	**557 681 (100.00%)**
Average read length	**139nt**
Coverage	**7 180nt (99.21%)**
Average depth	**10 621 reads/site**





B1-NO-RBV Consensus sequence

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   1  NNNNNNNNNG TATGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATCAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC CACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAC TGCTTTGATG GATTTTCTCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC CCTTTATTCT TTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCTCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGTCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTTGGCCCT
 910  GGTGGGTCCC CATCTCTATT CCCATCAGCT TGCTCTACGA AATCTACATT TCATGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTTATGCTT TTTGGCGCTA CCCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TCACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA CCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATCACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGTTT CCACCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGCA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTTGATC AGGCCGAGCC TGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT CTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTTT CCTACGAGCT CACTCCTGCT GGTTTGCAGA TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TCACAGGTAC AATAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCATCC TGAGGGGTTA TTGGGTATTT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCT
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTATGCCA CCCTACACCT CCTGATAGTG
2240  CCATTTGGGT GTTACCACCA CCTTCTGAAG AATTTCTGGT TGATACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCC CCGCCGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAGCGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGCGGC GGCCTTTGCC ACGCCTTTTA
2520  CCAACGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GAGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GTATATACCA
2730  AGTCCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CACCGCCCCG GGGATGAGCT TTACCTAACC
2800  GACCTCGCCG CTACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCCGCTAC GGAGGTCGGC CGGGCCTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGT ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT GGTGGTTGTC CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GTGTCACCAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTACTGCA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
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3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGCGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGTTGCGGTC TTTATTCTGG AACGAGCCTG CCATCGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCGATCA CAGTCCACGA GGCCCAGGGC GCCACTTTTA
3500  CGGAAACTAC AATCATAGCC ACAGCTGATG CCAGGGGGCT CATCCAATCT TCCCGAGCAC ATGCCATAGT
3570  TGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTCGGTATA
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B1-NO-RBV Consensus sequence

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3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG TCCAGCTCCC GTCGCTGCTG TCCTGCCCCC TTGCCCTGAA
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3990  GTACGGCCGT CGGACGAAGT TGTATGAAGC AGCTCAGTCT GACGTCCGTG AGTCCCTGGC CAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ACGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AATAAGTTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCT
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCCAA TATCTTCTAT GGCGATGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  CTCAAGCATG GTATTTGAGA ATGATTTTTC AGAATTTGAT AGCACCCAAA ACAACTTCTC CCTTAGCCTC
4480  GAGTGTGCAG TTATGGAGGA ATGTGGCATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA CTCCGTGGTC CTTTGTAGTG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAACTGAAGG TTGATTACCG CCCCATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCCGGC CTGGGGACGC TACCCGATGT GGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCTGTTT GTGATTTCCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTCGATGTT GTATCCCGAG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTCA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT GGAATGAATA ACATGTTCTA TGCATTGCCC ATGGGATCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG CACCGCAATC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCTCCGCTG
5460  TCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCATA TCACCTGCTC CCGATACAGC
5530  CCCTGTCCCT GATGTTGACT CTCGCGGCNN NNNNNNNNNN NNNNNNNNNA ATTTATCCAC ATCCCCGCTT
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG GTTCTTTATG CTGCTCCGCT AAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATTCGT TACAGGCCAT TGGTGCCAAA TGCTGTTGGC GGTTATGCAA TATCCATCTC ATTTTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTCTAGTTC
5880  AGCCCGGCAT TGCTTCTGAG TTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT CCATGGCTCT
6020  CCTGTTAATT CCTACACCAA TACCCCCTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCC TTAGAGCTTG
6090  AGTTTAGGAA CCTTACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GCTGCGCCGT GGTGCTGATG GCACCGCGGA ACTTACCACC ACAGCGGCCA CGCGTTTCAT GAAGGACTTG
6230  CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCCCT CACACTCTTT AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGTTATTTT ACTCCCGCCC
6370  CGTTGTCTCA GCCAATGGCG AGCCGACCGT CAAGTTATAT ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA TATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAACCAGC
6510  ATGAGCAGGA TCGACCCACC CCCTCGCCTG CCCCTTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580  TTTATGGCTT TCTCTTACTG CCGCTGAGTA TGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTTGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGTCCTCTTA CTACTATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAATTATCTT TTTGGGAGGC CGGGACGACC AAGGCCGGCT ACCCCTATAA CTATAACACA
6860  ACTGCTAGTG ATCAGATTTT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TATACCACCA
6930  GTCTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTTGGTGT TTTAGCCCCA CATTCGGCCC TTGCAGTCCT
7000  TGAAGACACT ATTGACTACC CTGCCCGTGC CCACACATTT GATGATTTCT GCCCGGAGTG TCGCGCTCTT
7070  GGTTTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTCTC TTCTGCGGTT
7210  CGCGCTCCCT GGAAANNNNN NNNNNNN
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B2-NO-RBV

File name	**B2-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**600 859**
Mapped reads	**600 859 (100.00%)**
Average read length	**136nt**
Coverage	**184nt (99.27%)**
Average depth	**11 260 reads/site**

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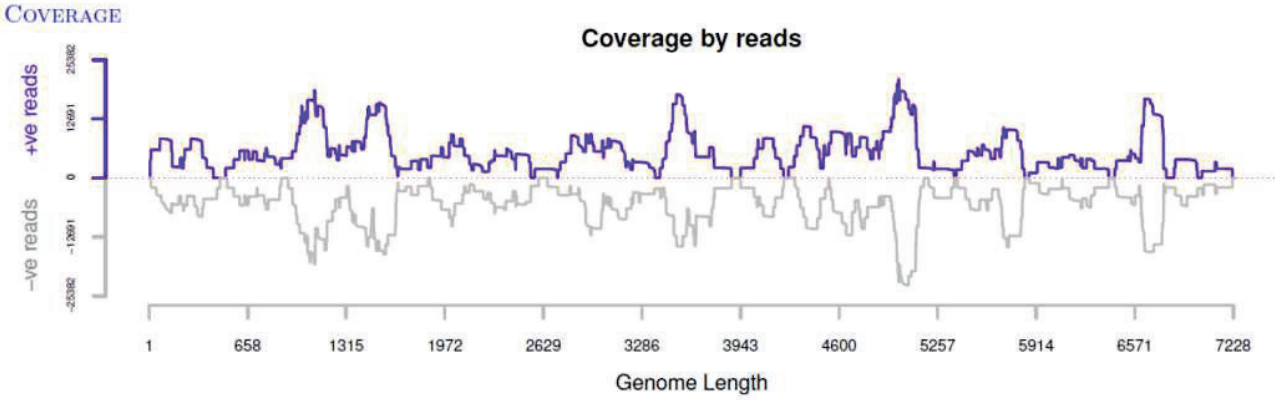

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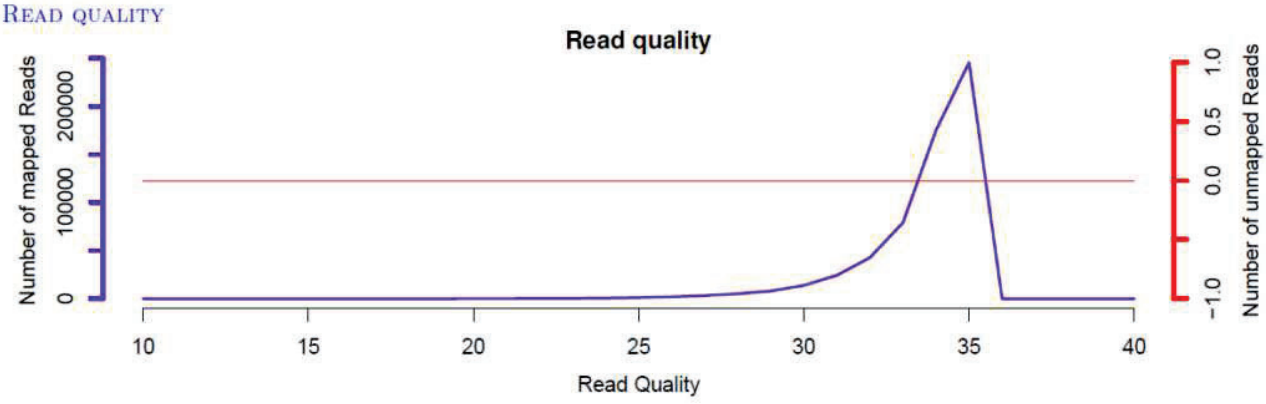

B2-NO-RBV Consensus sequence

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   1  NNNNNNNNCG TATGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATCAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC CACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAC TGCTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT TTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCTCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGTCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTTGGCCCT
 910  GGTGGGTCCC CATCTCTATT CCCATCAGCT TGCTCTACGA AATCTACATT TCATGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTTATGCTT TTTGGCGCTA CCCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TCACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA CCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATCACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGTTT CCACCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGCA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTTGATC AGGCCGAGCC TGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGCCTGGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT CTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTTT CCTACGAGCT CACTCCTGCT GGTTTGCAGA TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AATAGGTTCA CTCAGCACCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCATCC TGAGGGGTTA TTGGGTATTT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCT
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTATGCCA CCCTACACCT CCTGATAGTG
2240  CCATTTGGGT GTTACCACCA CCTTCTGAAG AATTTCTGGT TGATACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCC CCGCCGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAGCGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGCGGC GGCCTTTGCC ACGCCTTTTA
2520  CCAACGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GAGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GTATATACCA
2730  AGTCCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CACCGCCCCG GGGATGAGCT TTACCTAACC
2800  GACCTCGCCG CTACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCCGCTAC GGAGGTCGGC CGGGCCTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGT ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT GGTGGTTGTC CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GTGTCACCAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTACTGCA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCTATAGA CTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
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3570  TGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTCGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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B2-NO-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCACCT TCTTGCCAGA TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCAGCTCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAAG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGACAG TGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGT ATGGCTGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ACGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AATAAGTTCA CCACAGGGGA GANNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNCTCGGCT
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA TATCTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  CTCAAGCATG GTATTTGAGA ATGATTTTTC AGAATTTGAT AGCACCCAAA ACAACTTCTC CCTTAGCCTC
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5880  AGCCCGGCAT TGCTTCTGAG TTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
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6720  CTAAAGTTAC TCTGGACGGC CGTCCTCTTA CTACTATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAATTATCTT TTTGGGAGGC CGGGACGACC AAGGCCGGCT ACCCCTATAA CTATGACACA
6860  ACTGCTAGTG ATCAGATTTT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TATACCGCCA
6930  GTCTGGGCGC TGGCCCTGTG TCTGTCTCTG CAGTTGGTGT TTTAGCCCCA CATTCGGCCC TTGCAGTCCT
7000  TGAAGACACT ATTGACTACC CTGCCCGTGC CCACACTTTC GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGTTTACAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TCCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATTAATT TCCTTTGTGC CCCCTTCATA GCTTTGCTTT ATTTTTTTTC TTCTGCGGTT
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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B3-NO-RBV

File name	**B3-NO-RBV.sam**
Ref name	**FJ705359.**1
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**222 823**
Mapped reads	**222 823 (100.00%)**
Average read length	**139nt**
Coverage	**7 185nt (99.28%)**
Average depth	**4 268 reads/site**

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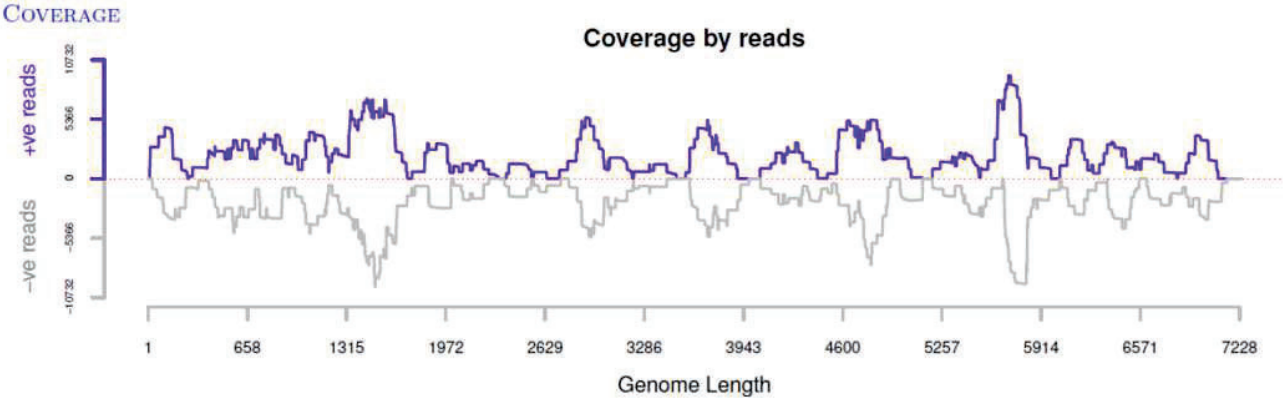

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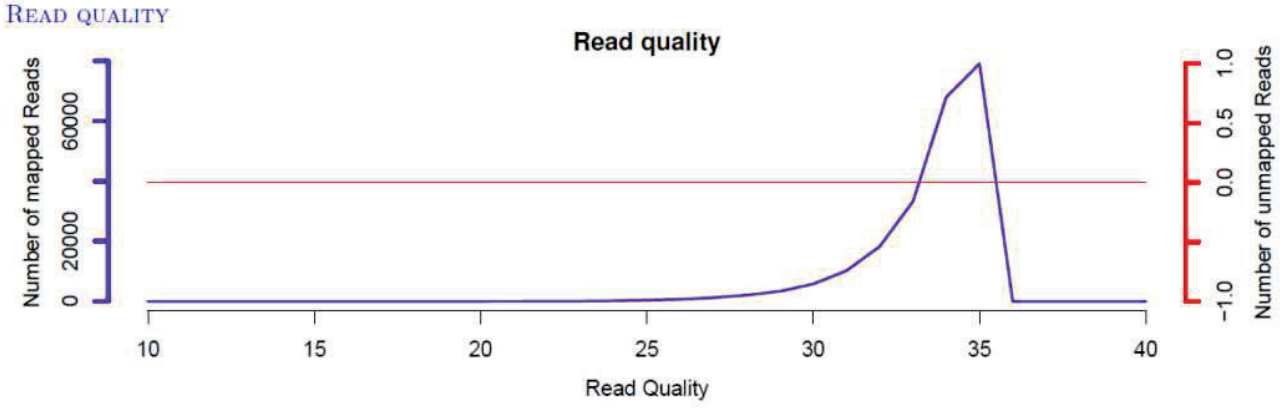

B3-NO-RBV Consensus sequence

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   1  NNNNNNNNCG TATGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATCAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC CACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAC TGCTTTGATG GATTTTCTCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC CCTTTATTCT TTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCTCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGTCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTTGGCCCT
 910  GGTGGGTCCC CATCTCTATT CCCATCAGCT TGCTCTACGA AATCTACATT TCATGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTTATGCTT TTTGGCGCTA CCCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TCACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA CCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATCACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGTTT CCACCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGCA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTTGATC AGGCCGAGCC TGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT CTCGTTTGAC GCCTCTCGCC
1820  AGTCCATGGG GGCCGGGCCG CATAGTCTTT CCTACGAGCT CACTCCTGCT GGTTTGCAGA TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AATAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCATCC TGAGGGGTTA TTGGGTATTT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCT
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTATGCCA CCCTACACCT CCAGATAGTG
2240  CCATTTGGGC GTTACCACCA CCTTCTGAAG AATTTCTGGT TGATACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCC CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAGCGCC ACCACCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  GCTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGCGGC GGCCTTTGCC ACGCCTTTTA
2520  CCAACGCTAC CCCGAGTCTT TCTACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GAGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GTATATACCA
2730  AGTCCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CACCGCCCCG GGGATGAGCT TTACCTAACT
2800  GACCTCGCTG CTACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCCGCTAC GGAGGTCGGC CGGGCCTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGT ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT GGTGGTTGTC CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GTGTCACCAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTACTGCA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCTATAGA CTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGTGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGTTGCGGTC TTTATTCTGG AACGAGCCTG CCATCGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCGATCA CAGTCCACGA GGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCAC ATGCCATAGT
3570  TGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTCGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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B3-NO-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCACCT  TCTTGCCAGA  TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCAGCTCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAAG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCTGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ACGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AATAAGTTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCT
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA TATCTTCTAC GGCGACGCAT ATGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  CTCAAGCATG GTATTTGAGA ATGATTTTTC AGAATTTGAT AGCACCCAAA ACAACTTCTC CCTTAGCCTC
4480  GAGTGTGCAG TTATGGAGGA ATGTGGCATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA CTCCGTGGTC CTTTGTAGTG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCTGGC CTAGGGACCC TACCCGATGT GGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCCGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCTGTTT GTGACTTCCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTTGATGTT GTATCCCGAG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTCA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NTGGGATCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG CACCGCAATC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCTCCGCTG
5460  TCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCATA TCACCTGCTC CCGATACAGC
5530  CCCTGTCCCT GATGTTGACT CTCGCGGCGC CATATTGCGG CGCCAGTATA ATTTATCCAC ATCCCCGCTT
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG GTTCTTTATG CTGCTCCGCT AAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAACACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATTCGT TACAGGCCAT TGGTGCCAAA TGCTGTTGGC GGTTATGCAA TATCCATCTC ATTTTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTCTAGTTC
5880  AGCCCGGCAT TGCTTCTGAG TTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT TCATGGCTCT
6020  CCCGTTAATT CCTACACCAA TACCCCCTAT ACCGGGGCGC TTGGACTCCT TGACTTCGCC TTAGAGCTTG
6090  AGTTTAGGAA CCTTACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GCTGCGCCGT GGTGCTGATG GCACCGCGGA ACTTACCACC ACAGCGGCCA CGCGTTTCAT GAAGGACTTG
6230  CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCCCT CACACTCTTT AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGTTATTTT ACTCCCGCCC
6370  CGTTGTCTCA GCCAATGGCG AGCCGACCGT CAAGTTATAT ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440  GGGATTGCTA TCCCACATGA TATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GATAACCAGC
6510  ATGAGCAGGA TCGACCCACC CCCTCGCCTG CCCCTTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580  TTTATGGCTT TCTCTTACTG CCGCTGAGTA TGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTTGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGTCCTCTTA CTACTATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAATTATCTT TTTGGGAGGC CGGGACGACC AAGGCCGGCT ACCCCTATAA CTATGACACA
6860  ACTGCTAGTG ATCAGATTTT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TATGCCGCCA
6930  GTCTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTTGGTGT TTTAGCCCCA CATTCGGCCC TTGCAGTCCT
7000  TGAAGACACT ATTGACTACC CTGCCCGTGC CCACACTTTC GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGTTTACAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TCCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATTAATT TCCTTTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTCTT TTCTGCGGTT
7210  CGCGCTCCCT GGAANNNNNN NNNNNNN
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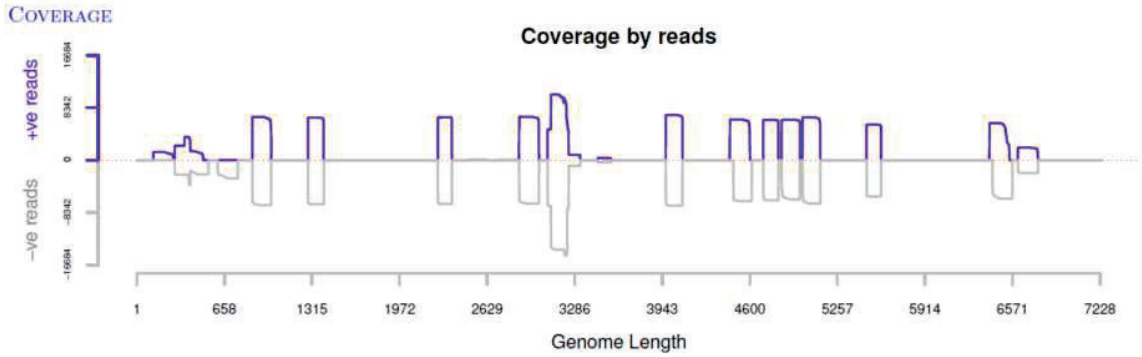
B4-NO-RBV

File name	**B4-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**189 446**
Mapped reads	**189 446 (100.00%)**
Average read length	**130nt**
Coverage	**5 979nt (82.62%)**
Average depth	**3 382 reads/site**



B4-NO-RBV Consensus sequence

1	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
70	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNGTTCGG	CCGTTTTTAT
140	CTCGTGTGCA	AACTGAGATT	CTTATTAATT	TGATGCAACC	CCGGCAGTTG	GTTTTTCGAC	CTGAAGTGCT
210	CTGGAATCAT	CCTATCCAAC	GGGTTATCCA	TAATGAACTT	GAACAGTACT	GTCGGGCCCG	GGCTGGTCGT
280	TGTCTAGAGG	TTGGGGCCCA	CCCAAGATCC	ATTAATGACA	ACCCAAATGT	TCTGCACCGG	TGCTTTCTAC
350	GACCAGTTGG	GAGAGATGTT	CAGCGCTGGT	ACTCCGCTCC	CACCCGCAGC	CCTGCGGCTA	ACTGCCGCCG
420	TTCTGCCTTG	CGTGGCCTCC	CCCCCGTTGA	TCGTACCTAC	TGCTTTGATG	GATTCTCTCG	CTGCTCATTT
490	GCCGCAGAAA	CTGGGGTTGC	TCTTTATTCT	TTGCATGACC	TCTGGCCGGC	CGATGTTGCG	GAGGCTATGG
560	CCCGGCACGG	GATGACACGC	TTGTATGCTG	CACTACATCT	CCCCCCTGAA	GTACTACTAC	CACCCGGTAC
630	TTACCATACA	ACTTCATACC	TTCTGATTCA	CGACGGTGAT	CGTGCTGTTG	TGACCTATGA	AGGTGATACT
700	AGTGCAGGCT	ACAACCATGA	TGTCTCCATA	CTTCGTGCAT	GGATCCGCAC	AACTAAGATA	ACCGGCAACC
770	ATCCGCTGGT	GATAGAGCGT	GTGCGGGCCA	TTGGTTGCCA	TTTTGTGCTG	CTGCTTACTG	CAGCCCCTGA
840	GCCGTCACCA	ATGCCTTATG	TCCCATACCC	CCGGTCGACG	GAGGTGTATG	TCCGCTCTAT	ATTTGGCCCT
910	GGCGGGTCCC	CATCTCTATT	CCCATCAGCT	TGCTCTACGA	AATCTACATT	TCATGCTGTC	CCGGTTCATA
980	TTTGGGACCG	GCTTATGCTT	TTTGGCGCTA	CCCTGGACGA	TCAGGCGTTT	TGCTGTTCAC	GGCTTATGAC
1050	TTACCNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNATGCTTCA
1120	GAGGACGCTC	TTACTGCTGT	TATTACTGCA	GCGTACTTAA	CTATTTGTCA	TCAGCGTTAC	CTCCGTACCC
1190	AGGCTATATC	CAAGGGTATG	CGCCGACTGG	AGGTTGAGCA	TGCTCAAAAA	TTCATTACAA	GACTTTATAG
1260	TTGGCTGTTT	GAGAAGTCCG	GCCGTGACTA	TATCCCCGGC	CGCCAGCTCC	AGTTCTATGC	ACAGTGCCGC
1330	CGTTGGTTAT	CGGCGGGTTT	CCACCTTGAC	CCAAGGGTGC	TTGTTTTTGA	TGAGTCTGTG	CCCTGCCGTT
1400	GTAGGACATT	TCTTAAGAAG	GCTGTGGGTA	AATTCTGNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1470	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1540	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1610	NNNNNNNNNN	NNNNNNNNNN	NNNNNCCCGC	ATGACATCGC	CGCCCGAGCC	TCCCGTCTGA	CTGCCACCGT
1680	CGAACTTGTT	GCAGGTCCAG	ACTACTTGGA	GTGCCGCACT	GTGCTCGGGA	ATAAGACTTT	CCGGACGACG
1750	GTGGTTGATG	GTGCCCATCT	TGAGGCGAAC	GGCCCCGAGC	AGTACGTTCT	TTCATTTGAC	GCCTCTCGCC
1820	AGTCTATGGG	GGCCGGACCG	CATAGTCTCA	CCTACGAGCN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNTC
1890	ATCTAATGGC	CTGGATTGCA	CTGCAACATT	CCCCCCGGGC	GGGGCCCCTA	GCGCTACTCC	GGGGGAGGTA
1960	ACAGCCTTTT	GCAGTGCTCT	TTATAGATAC	AACAGGTTCA	CTCAGCACCA	TTCACTTGTA	GGTGGCTTGT
2030	GGCTGCACCC	TGAGGGGTTG	TTGGGTATCT	TCCCCCCTTT	CTCTCCCGGG	NNNNNNNNNN	NNNNNNNNNN
2100	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNCATGG	TCAACATCTG	GTTTTTCTAG	TGACTTTTCT
2170	CCCCCTGAGG	CAGCCGTCGC	AGCGCCGGCT	GCTACTCCGG	GGTTATGCCA	CCCTACACCT	CCTGATAGTG
2240	CCATTTGGGT	GTTACCACCA	CCTTCTGAAG	AATTTCTGGT	TGATACAGCG	CCCGCTCCCC	CTGCCCCTGA
2310	GCCCGCTCAA	CCATCTAGCC	CCGCCGGGCC	GAAGGCTCCC	GTGCGTAAGC	CGCCAGCGCC	ACCATCCCCG
2380	CGCACCCGCC	GCCTTCTTTA	CACCTATCCG	GATGGGGCAA	AGGTGTATGC	GGGGTCACTG	TTTGAGTCTG
2450	ACTGTGATTG	GCTGGTTAAT	GCGTCGAATC	CCGGCCATCG	TCCTGGCGGC	GGCCTTTGCC	ACGCCTTTCA
2520	CCAACGCTAC	CCCGAGTCTT	TCTACTCAAC	TGAGTTCATT	ATGCGCGACG	GTCTTGCCGC	GTACACTTTA
2590	ACTCCCCGGC	CTATTATTCA	TGCAGTGGCC	CCTGATTATA	GAGTTGAGCA	TAACCCAAAG	AGGCTTGAGG
2660	CAGCATACCG	GGAGACTTGC	TCCCGCCGCG	GCACCGCCGC	TTACCCACTC	CTCGGCTCGG	GTATATACCA
2730	AGTCCCCGTC	AGCCTCAGCT	TTGACGCTTG	GGAGCGTAAC	CACCGCCCCG	GGGATGAGCT	TTACCTGACC
2800	GACCTCGCCG	CTACCTGGTT	CGAGGCTAAT	AAGCCAACAC	AGCCGGCCCT	TACAATAACT	GAGGNNNCAG
2870	CCCGCACAGC	CAACCTAGCA	CTGGAGATCG	ATGCCGCTAC	GGAGGTCGGC	CGGGCCTGTG	CCGGCTGTGC
2940	AGTTAGTCCT	GGGGTTGTGT	ACTATCAGTT	TACTGCTGGG	GTCCCAGGTT	CGGGGAAGTC	GCGTTCTATA
3010	CAGCAGGGNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3080	NCGCAGCTTT	TACACCTCAT	ACGGCGGCCC	GTGTCACCAC	GGGCCGTCGT	GTTGTGATTG	ATGAGGCCCC
3150	ATCCCTCCCA	CCGCATTTGT	TGCTACTGCA	CATGCAGCGG	GCCTCGTCGG	TCCACCTTCT	CGGCGACCCA
3220	AACCAGATCC	CTGCTATAGA	CTTCGAGCAC	GCCGGCCTGG	TCCCCGCAAT	ACGCCCTGAG	CTTGCGCCCA
3290	CCAGTTGGTG	GCATGTCACC	CACCGCTGCC	CTGCTGANNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNCCCAA
3360	AATCCAGACT	ACTAGCCGCG	TGCTGCGGTC	TTTATTCTGG	AATGAGCCCG	CCATTGGCCA	GAAGTTAGTC
3430	TTCACACAGG	CCGCTAAGGC	TGCCAACCCC	GGTGCGATCA	CAGTCCACGA	GGCCCAGGGC	GCCACTTTTA
3500	CGGAAACTAC	AATCATAGCC	ACAGCTGATG	CCAGGGGGCT	CATCCAATCT	TCCCGAGCCC	ATGCCATAGT
3570	CGCACTTACC	CGCCACACAG	AGAAGTGCAT	TATTCTTGAT	GCCCCCGGCT	TGTTACGTGA	AGTTGGTATA
3640	TCAGATGTGA	TTGTTAACAA	TTTTTTCCTT	GCCGGTGGGG	AGATGGGCCA	CCATCGCCCT	TCTGTGATAC

B4-NO-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AANNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNGCCAGA TCAGTGCTTA
3780  CCATCAGTTA GCTGAGGAGC TGGGTCACCG CCCGGCCCCC GTCGCTGCCG TCCTGCCCCC CTGCCCCGAA
3850  CTTGAGCAGG GCCTGCTGTA TATGCCACAA GAGCTTACGG TGTCTGACAG CGTGCTGGTC TTTGAACTCA
3920  CGGNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGGCGAAGC TGTACGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGTTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGCGATGTA TCGCGTATTA CATTTTTCCA
4200  GAAAGATTGT AATAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAAGG CATCTCGGCT
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCNNNNN
4340  NNNNNNNNNN NNNNNNNNNN NNNNNNGCAT ATGAAGAGTC TGTGTTTGCT GCCGCTGTGT CTGGGGCAGG
4410  TTCATGCATG GTATTTGAGA ATGACTTTTC AGAGTTTGAC AGCACCCAAA ACAACTTCTC CCTTAGCCTC
4480  GAGTGTGCAG TTATGGAGGA ATGTGGCATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGCG AGCCTNNNNN
4620  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
4690  NNNNNNNNNN NGGGAGATGA CTCCGTGGTC CTTTGTAGTG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCCGGC CTAGGGACCC TACCCGATGT GGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCCGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCTGTTT GTGACTTCCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG GGTTGATGTT GTATCCCGAG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTCA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5180  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNATGC GGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGGCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TGTCTGGGGT GACAGGGTTG ATTCTCACCC
5320  CTTCGCCCGC CCCTATATTC ATCCAACCAA CCCCTTTGAC GCCNNNNNNN NNNNNNNNNN NNNNNNNNNN
5390  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5460  NNNNNNNNNN NNNNTCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCATA TCACCTGCTC CCGATACAGC
5530  CCCTGTCCCT GATGTTGACT CTCGCGGCGC CATATTGCGG CGCCAGTATA ATTTATCCAN NNNNNNNNNN
5600  NNNNNNTCTG TTGCTTCAGG TACTAATCTG GTTCTTTATG CTGCCCCACT AAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATCCGT TACAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCAA TTTCCATCTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAATTCTA TTACTTCCAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT TGCTTCTGAG GTGGTGATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
5950  TGTAGAGACC TCTGGTGTGG CTGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT TCATGGCTCT
6020  CCTGTTAATT CCTACACCAA TACCCCCTAC ACCGGGGCGC TTGGACTCCT TGACTTTGCT TTAGAGCTTG
6090  AGTTTAGGAA CCTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGTACAG CCCGTCATCG
6160  ATTGCGCCGC GGTGCTGATG GCACCGCTGA ACTTACCACC ACAGCAGCCA CGCGCTTCAT GAAGGACCTG
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6300  ANNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN CAGTTGTTCT ACTCCCGCCC
6370  CGTCGTCTCG GCCAATGGCG AGCCGGCTGT CAAGTTATAT ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA TATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GATAACCAGC
6510  ATGAGCAGGA CCGACCCACC CCCTCGCCTG CCCCTTCTCG CCCTTTTTCG GTTCTTCGCN NNNNNNNNNN
6580  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTTGACTGGT
6720  CTAAAGTCAC TCTGGACGGC CGTCCTCTTA CTACTATCCA GCNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6790  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NGGGACGACT AAGGCCGGCT ACCCCTATAA TTACAACACA
6860  ACTGCGAGTG ATCAGATTCT GATTGAAAAT GCGGCTGGTC ATCGTGTTGT TATTTCCACG TATACCACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCCG CAGTTGGTGT CTTAGCCCCA CATTCGGCCC TCGCAGTCCT
7000  TGAAGACACG ATTGATTACC CTGCCCGTGC CCACACATTT GATGATTTCT GCCCGGAGTG TCGTGCTCTT
7070  GGTTTGCAGG GGTGTGCCTT CCAGNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7140  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7210  NNNNNNNNNN NNNNNNNNNN NNNNNNN
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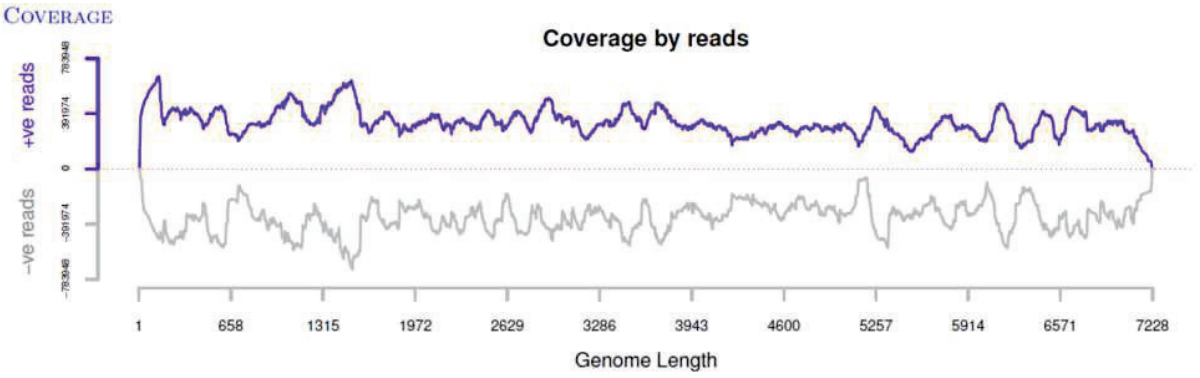
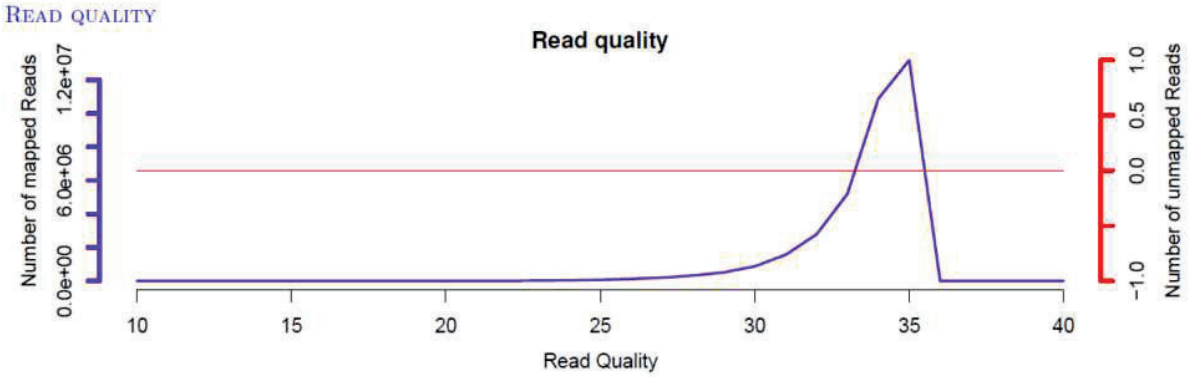
C1-NO-RBV

File name	**C1-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**35 778 732**
Mapped reads	**35 778 732 (100.00%)**
Average read length	**17nt**
Coverage	**7 222nt (99.79%)**
Average depth	**676 578 reads/site**





C1-NO-RBV Consensus sequence

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  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACCGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA TCCAAGATCC ATTAATGACA ACCCAAATGT TCTTCACCGG TGCTTTCTAC
 350  GACCAGTAGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ATTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAT TGCTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC CCTTTATTCC CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG CATGACACGC TTGTATGCCG CACTACATCT CCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGCGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGTTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCC TGCTCTACGA AATCCACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGCTCAC GACTTATGAC
1050  CTATCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGATTA CATCCCCGGT CGCCAGCTCC AGTTCTATGC ACAGTGCCGT
1330  CGTTGGTTAT CGGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTA GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCTGAGCC CGCCTGTCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGT TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGCC GCAGGTCCAG ACCGCCTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCACCT TGAGGCGAAC GGCCCCGAGC AGTATGTTCT TTCGTTCGAC GCCTCTCGCC
1820  AGTCAATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT TACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGATCC GGGGGAGGTA
1960  GCAGCCTTCT GCAGCGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT TTCTCCCGGA CACCTTTGGG AGTCCGCTAA
2100  TCCTTTTTGT GGGGAGGGAA CTTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCTGTTGC AGCGCCGGCT GCTACTCCGG GGTTACGCCA CCCCACACCC CCTGTTAGTG
2240  ACGTTCGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGATACAGCG CCCACTCCCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCCCCC GTGCGTAAGC CGCCAACGCT GCCATCCCCG
2380  CGCACTCGCC GCCTTCTTTA CACCTATCCA GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGTCATCG TCCTGGAGGC GGCCTTTGCC ATGCCTTTTA
2520  CCAGCGCTAT CCCGAGTCTT TCTACTCAAC TGAGTTCATC ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACCCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCGAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCTCGCCGCG GCACCGCCGC CTATCCACTC CTCGGTTCGG GCATATATCA
2730  AGTTCCCGTC AGCCTCAGCT TCGACGCTTG GGAGCGTAAC CACCGCCCCG GGGACGAGCT CTACCTAACC
2800  GACCTCGCCG CTACCTGGTT CGAGGCCAAT AAGCCAACAC AGCCGGCCCT CACAATAACT GAGGACGCAG
2870  CCCGCACAGC CAACCTAGCA TTGGAGATCG ATGCTGCTAC TGAGGTCGGC CGGGCTTGTG CCGGCTGCGC
2940  GGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA CAGTTGGCGT CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GTGTCACCAC GGGTCGTCGT GTTGTAATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATCTGT TGCTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCACCCA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATACGCGGGG CTTACCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC ATTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CCGCTAAGGC CGCCAACCCC GGTGCGATTA CAGTCCACGA GGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCC TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA CTTTTTCCTC GCCGGCGGCG AAGTGGGTCA TCATCGTCCC TCTGTGATAC
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C1-NO-RBV Consensus sequence

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3780  TCACCAGTTA GCTGAGGAAC  TTGGCCACCG  CCCAGCTCCT  GTCGCCGCCG  TCCTGCCCCC  TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA  TATGCCGCAG  GAGCTTACGG  TGTCTGATAG  CGTGCTGGTC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG  ATGGCAGCCC  CCAGCCAGCG  GAAGGCCGTC  CTATCGACGC  TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC  TGTACGAAGC  AGCTCACTCT  GACGTCCGTG  AGTCCCTGGC  TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA  GGCTACTACG  TGTGAGTTAT  ATGAGCTGGT  TGAGGCCATG  GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG  CTTGAGCTCG  ACCTTTGCAA  TCGTGATGTA  TCGCGTATCA  CATTTTTCCA
4200  GAAAGATTGT AACAAATTCA  CCACAGGGGA  GACCATTGCC  CACGGTAAGG  TCGGCCAGGG  CATCTCGGCT
4270  TGGAGCAAGA CCTTCTGTGC  CCTGTTTGGT  CCGTGGTTTC  GTGCTATCGA  AAAAGAAATA  CTAGCCCTGC
4340  TCCCGCCTAA TATTTTCTAC  GGCGACGCAT  ACGAGGAGTC  TGTGTTTGCC  GCCGCTGTGT  CGGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA  ATGATTTTTC  AGAGTTTGAT  AGCACCCAAA  ATAACTTTTC  CCTTGGTCTC
4480  GAGTGCGTAG TCATGGAGGA  ATGTGGCATG  CCCCAGTGGC  TAATCCGGTT  GTACCATCTG  GTTCGGTCGG
4550  CCTGGATCTT ACAGGCACCG  AAGGAGTCTC  TTAAGGGATT  TTGGAAGAAG  CATTCTGGTG  AGCCTGGCAC
4620  CCTCCTTTGG AACACTGTTT  GGAACATGGC  GATCATAGCA  CACTGCTATG  AATTTCGTGA  TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA  TTCCGTGGTC  CTCTGTAGCG  ACTACCGTCA  GAGCCGCAAT  GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC  AAACTGAAGG  TTGACTATCG  CCCTATTGGG  TTGTATGCTG  GTGTGGTGGT
4830  GGCCCCTGGC CTGGGGACGC  TACCCGATGT  GGTGCGCTTC  GCCGGCCGGC  TGTCTGAGAA  GAACTGGGGC
4900  CCTGGGCCGG AGCGAGCTGA  GCAGTTGCGC  CTAGCTGTTT  GTGACTTCCT  CCGAGGGTTA  ACGAATGTTG
4970  CGCAGGTATG TGTTGATGTT  GTATCCCGAG  TTTATGGAGT  TAGCCCTGGG  CTGGTACATA  ACCTTATTGG
5040  CATGTTGCAA ACCATAGCCG  ATGGCAAAGC  CCATTTCACA  GAGACTGTTA  AACCTGTGCT  TGACCTCACG
5110  AACTCTATCA TACAGCGGGT  GGAATGAATA  ACATGTTTTG  TGCATTGCCC  ATGGGATCAC  CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT  CTTCGTGCTT  TTGCCTATGC  TGCCCGCGCC  ACCGGCCGGC  CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC  AGCGGCGGTA  CCGGCAGTGG  TTTCTGGGGT  GACAGGGTTG  ATTCTCAGCC
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5740  TACGATTCGT TACAGGCCAT  TGGTGCCAAA  TGCCGTCGGC  GGTTATGCAA  TATCCATCTC  ATTCTGGCCT
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6090  AGTTTAGGAA CCTGACACCC  GGGAACACCA  ACACCCGTGT  GTCTCGGTAT  ACAAGCACAG  CCCGTCATCG
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6370  TGTCGTCTCA GCCAATGGCG  AGCCGACTGT  CAAGTTATAT  ACATCTGTAG  AGAATGCGCA  GCAGGATAAA
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7000  TGAAGACACT ATTGACTACC  CTGCCCGTGC  CCACACTTTT  GATGATTTCT  GTCCGGAGTG  TCGCGCTCTT
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7140  CCCGGGAGTT TTAATTAATT  TCCTTTGTGC  CCCCTTCATA  GCTTTGCTTT  ATTTCTTTTT  TTCTGCTTTT
7210  CGCGCTCCCT GGAAANNNNN  NNNNNNN
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C2-NO-RBV

File name	**C2-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7237**
Program used	**Tanoti Assembler 1.0**
Total reads	**714671**
Mapped reads	**714671 (100.00%)**
Average read length	**140nt**
Coverage	**7218nt (99.74%)**
Average depth	**13779 reads/site**

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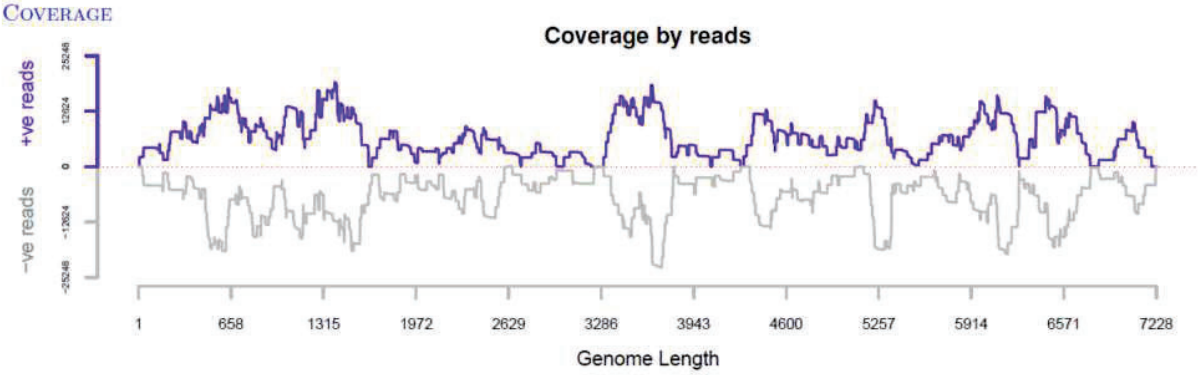

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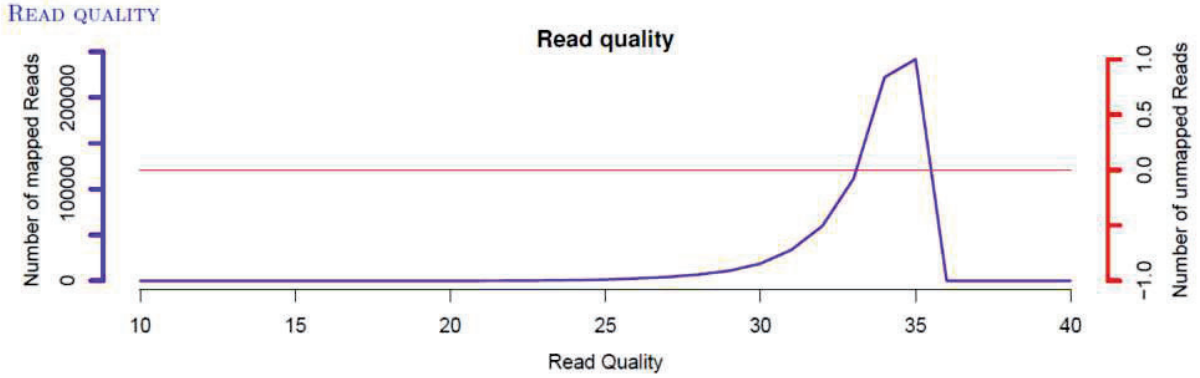

C2-NO-RBV Consensus sequence

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140	CCCGTGTGCA	AACCGAGATT	CTTATTAATT	TGATGCAACC	CCGGCAGTTG	GTTTTTCGGC	CTGAAGTGCT
210	CTGGAATCAT	CCTATCCAAC	GGGTTATCCA	TAATGAACTT	GAACAGTACT	GTCGGGCCCG	GGCTGGTCGT
280	TGTCTAGAGG	TTGGGGCCCA	TCCAAGATCC	ATTAATGACA	ACCCAAATGT	TCTTCACCGG	TGCTTTCTAC
350	GACCAGTAGG	GAGAGATGTT	CAGCGCTGGT	ACTCTGCTCC	TACCCGCGGC	CCTGCGGCTA	ATTGCCGCCG
420	TTCTGCCTTG	CGTGGCCTCC	CCCCCGTTGA	TCGTACCTAT	TGCTTTGATG	GATTCTCCCG	CTGCTCATTT
490	GCCGCAGAAA	CTGGGGTTGC	CCTTTATTCC	CTGCATGACC	TCTGGCCGGC	CGATGTTGCG	GAGGCTATGG
560	CCCGACACGG	CATGACACGC	TTGTATGCCG	CACTACATCT	CCCCCCTGAA	GTACTACTAC	CACCTGGTAC
630	TTACCATACA	ACTTCATACC	TTCTGATTCA	CGACGGTGAT	CGCGCTGTTG	TGACCTATGA	AGGTGATACT
700	AGTGCAGGCT	ACAACCATGA	TGTCTCCATA	CTTCGTGCAT	GGATCCGCAC	AACTAAGATA	GTCGGCGATC
770	ATCCGCTGGT	GATAGAGCGT	GTGCGGGCTA	TTGGCTGCCA	TTTTGTGCTG	CTGCTTACTG	CAGCCCCTGA
840	GCCGTCACCA	ATGCCTTATG	TTCCATACCC	CCGGTCGACA	GAGGTGTATG	TCCGTTCTAT	ATTCGGCCCT
910	GGCGGGTCCC	CATCCCTATT	CCCATCAGCC	TGCTCTACGA	AATCCACATT	TCACGCTGTC	CCGGTTCATA
980	TTTGGGACCG	GCTCATGCTT	TTTGGCGCTA	CTCTGGATGA	TCAGGCGTTT	TGCTGCTCAC	GACTTATGAC
1050	CTATCTCCGC	GGGATTAGTT	ACAAGGTCAC	TGTTGGCGCC	CTTGTCGCTA	ACGAGGGGTG	GAATGCTTCG
1120	GAGGACGCTC	TTACCGCTGT	TATTACTGCA	GCGTATTTGA	CCATTTGCCA	TCAGCGTTAC	CTCCGTACTC
1190	AAGCTATATC	CAAGGGTATG	CGCCGACTGG	AGGTTGAGCA	TGCCCAAAAA	TTCATTACAA	GACTTTATAG
1260	TTGGCTGTTC	GAGAAGTCCG	GCCGTGATTA	CATCCCCGGT	CGCCAGCTCC	AGTTCTATGC	ACAGTGCCGT
1330	CGTTGGTTAT	CGGCGGGTTT	CCATCTTGAC	CCAAGGGTGC	TTGTTTTTGA	TGAGTCTGTG	CCCTGCCGTT
1400	GTAGGACATT	TCTTAAGAAG	GCTGTGGGTA	AGTTCTGCTG	TTTTATGAAG	TGGTTGGGAC	AGGAGTGCAC
1470	CTGCTTTTTA	GAACCAGCAG	AGGGTCTAGT	TGGCGACCAT	GGCCACGATA	ATGAAGCCTA	TGAGGGCTCT
1540	GAGGTCGATC	AGGCTGAGCC	CGCCTGTCTC	GATGTTTCTG	GGACTTATGC	CGTCCATGGC	CGCCAACTTG
1610	AGGCCCTGTA	TAGGGCGCTT	AACATCCCGC	ATGACATCGT	TGCCCGAGCC	TCCCGTTTGA	CTGCCACCGT
1680	CGAACTCGCC	GCAGGTCCAG	ACCGCCTGGA	GTGCCGCACT	GTGCTCGGGA	ATAAGACTTT	CCGGACGACG
1750	GTGGTTGATG	GCGCCCACCT	TGAGGCGAAC	GGCCCCGAGC	AGTATGTTCT	TTCGTTCGAC	GCCTCTCGCC
1820	AGTCAATGGG	GGCCGGGCCG	CATAGTCTCT	CCTACGAGCT	TACTCCTGCT	GGTTTGCAGG	TCAAGATTTC
1890	ATCTAATGGC	CTGGATTGCA	CTGCAACATT	CCCCCCGGGC	GGGGCCCCTA	GCGCTGATCC	GGGGGAGGTA
1960	GCAGCCTTCT	GCAGCGCCCT	TTACAGGTAC	AACAGGTTCA	CTCAGCGCCA	TTCGCTTATA	GGTGGCTTGT
2030	GGCTGCATCC	TGAGGGGTTG	TTGGGTATCT	TCCCCCCTTT	TTCTCCCGGA	CACCTTTGGG	AGTCCGCTAA
2100	TCCTTTTTGT	GGGGAGGGAA	CTTTGTACAC	CCGGACATGG	TCAACATCTG	GTTTTTCTAG	TGACTTTTCC
2170	CCCCCTGAGG	CAGCTGTTGC	AGCGCCGGCT	GCTACTCCGG	GGTTACGCCA	CCCCACACCC	CCTGTTAGTG
2240	ACGTTCGGGT	GTTACCGCCA	CCTTCTGAAG	AATTTCAGGT	TGATACAGCG	CCCACTCCCC	CTGCCCCTGA
2310	GCCCGCTCAA	CCATCTAGCT	CCGCTGGGCC	AAAGGCCCCC	GTGCGTAAGC	CGCCAACGCT	GCCATCCCCG
2380	CGCACTCGCC	GCCTTCTTTA	CACCTATCCA	GATGGGGCAA	AGGTGTATGC	GGGGTCACTG	TTTGAGTCTG
2450	ACTGTGATTG	GCTGGTTAAT	GCGTCGAATC	CCGGTCATCG	TCCTGGAGGC	GGCCTTTGCC	ATGCCTTTTA
2520	CCAGCGCTAT	CCCGAGTCTT	TCTACTCAAC	TGAGTTCATC	ATGCGCGACG	GTCTTGCCGC	GTACACTTTA
2590	ACCCCCCGGC	CTATTATTCA	TGCAGTGGCT	CCTGATTATA	GGGTTGAGCA	TAACCCGAAG	AGGCTTGAGG
2660	CAGCATACCG	AGAGACTTGC	TCTCGCCGTG	GCACCGCCGC	CTATCCACTC	CTCGGTTCGG	GCATATATCA
2730	AGTTCCCGTC	AGCCTCAGCT	TCGACGCTTG	GGAGCGTAAC	CACCGCCCCG	GGGACGAGCT	CTACCTAACC
2800	GACCTCGCCG	CTACCTGGTT	CGAGGCCAAT	AAGCCAACAC	AGCCGGCCCT	CACAATAACT	GAGGACGCAG
2870	CCCGCACAGC	CAACCTAGCA	TTGGAGATCG	ATGCTGCTAC	TGAGGTCGGC	CGGGCTTGTG	CCGGCTGCGC
2940	GGTTAGTCCT	GGGGTTGTGC	ACTATCAGTT	TACTGCTGGG	GTCCCAGGTT	CGGGGAAGTC	GCGTTCTATA
3010	CAGCAGGGGG	ATGTTGACGT	GGTGGTTGTT	CCCACTCGGG	AGCTCCGGAA	CAGTTGGCGT	CGCCGGGGTT
3080	TCGCAGCTTT	TACACCTCAT	ACGGCGGCCC	GTGTCACCAC	GGGTCGTCGT	GTTGTAATTG	ATGAGGCCCC
3150	ATCCCTCCCA	CCGCATCTGT	TGCTACTACA	CATGCAGCGG	GCCTCGTCGG	TCCACCTTCT	CGGCGACCCA
3220	AACCAGATCC	CTGCCATAGA	CTTCGAGCAT	GCCGGCCTGG	TCCCCGCAAT	ACGCCCTGAG	CTTGCACCCA
3290	CCAGTTGGTG	GCATGTCACC	CACCGCTGCC	CCGCTGATGT	GTGCGAGCTT	ATACGCGGGG	CCTACCCCAA
3360	AATCCAAACC	ACTAGCCGCG	TGCTGCGGTC	ATTATTCTGG	AATGAGCCTG	CCATTGGCCA	GAAGTTAGTT
3430	TTCACCCAGG	CCGCTAAGGC	CGCCAACCCC	GGTGCGATTA	CAGTCCACGA	GGCCCAGGGC	GCCACTTTCA
3500	CGGAAACTAC	AATCATAGCC	ACGGCTGATG	CTAGGGGGCT	CATCCAATCC	TCCCGAGCTC	ATGCCATAGT
3570	CGCACTTACC	CGCCACACAG	AGAAGTGCGT	TATTCTTGAC	GCCCCCGGCT	TGTTACGTGA	GGTTGGTATA
3640	TCGGATGTGA	TTGTCAACAA	CTTTTTCCTC	GCCGGCGGCG	AAGTGGGTCA	TCATCGTCCC	TCTGTGATAC

C2-NO-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCACCT  TCCTGCCAGA  TCAGCGCCTA
3780  TCACCAGTTA GCTGAGGAGC  TTGGCCACCG  CCCAGCTCCT  GTCGCCGCCG  TCCTGCCCCC  TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA  TATGCCGCAG  GAGCTTACGG  TGTCTGATAG  CGTGCTGGCC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG  ATGGCAGCCC  CCAGCCAGCG  GAAGGCCATC  CTATCGACGC  TCGTGGGCAG
3990  GTACGGCCGT CGGACGAAGC  TGTACGAAGC  AGCTCACTCT  GACGTCCGTG  AGTCCCTGGC  TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA  GGCTACTACG  TGTGAGTTAT  ATGAGCTGGT  TGAGGCCATG  GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG  CTTGAGCTCG  ACCTTTGCAA  TCGTGATGTA  TCGCGTATCA  CATTTTTCCA
4200  GAAAGATTGT AACAAATTCA  CCACAGGGGA  GACCATTGCC  CACGGTAAGG  TCGGCCAGGG  CATCTCGGCT
4270  TGGAGCAAGA CCTTCTGTGC  CCTGTTTGGT  CCGTGGTTTC  GTGCTATCGA  AAAAGAAATA  CTAGCCCTGC
4340  TCCCGCCTAA TATTTTCTAC  GGCGACGCAT  ACGAGGAGTC  TGTGTTTGCC  GCCGCTGTGT  CGGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA  ATGATTTTTC  AGAGTTTGAT  AGCACCCAAA  ATAACTTTTC  CCTTGGTCTC
4480  GAGTGCGTAG TCATGGAGGA  ATGTGGCATG  CCCCAGTGGC  TAATCCGGTT  GTACCATCTG  GTTCGGTCGG
4550  CCTGGATCTT ACAGGCACCG  AAGGAGTCTC  TTAAGGGATT  TTGGAAGAAG  CATTCTGGTG  AGCCTGGCAC
4620  CCTCCTTTGG AACACTGTTT  GGAACATGGC  GATCATAGCA  CACTGCTATG  AATTTCGTGA  TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA  TTCCGTGGTC  CTCTGTAGCG  ACTACCGTCA  GAGCCGCAAT  GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC  AAACTGAAGG  TTGACTATCG  CCCTATTGGG  TTGTATGCTG  GTGTGGTGGT
4830  GGCCCCTGGC CTGGGGACGC  TACCCGATGT  GGTGCGCTTC  GCCGGCCGGC  TGTCTGAGAA  GAACTGGGGC
4900  CCTGGGCCGG AGCGAGCTGA  GCAGTTGCGC  CTAGCTGTTT  GTGACTTCCT  CCGAGGGTTA  ACGAATGTTG
4970  CGCAGGTATG TGTTGATGTT  GTATCCCGAG  TTTATGGAGT  TAGCCCTGGG  CTGGTACATA  ACCTTATTGG
5040  CATGTTGCAA ACCATAGCCG  ATGGCAAAGC  CCATTTCACA  GAGACTGTTA  AACCTGTGCT  TGACCTCACG
5110  AACTCTATCA TACAGCGGGT  GGAATGAATA  ACATGTTTTG  TGCATTGCCC  ATGGGATCAC  CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT  CTTCGTGCTT  TTGCCTATGC  TGCCCGCGCC  ACCGGCCGGC  CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC  AGCGGCGGTA  CCGGCAGTGG  TTTCTGGGGT  GACAGGGTTG  ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC  ATCCAACCAA  CCCCTTTGCC  GCCGATGTCG  TACCGCAATC  CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCGCC  CCGCCCCCTC  GGCTCCTCTT  GGCGCGATCA  GTCCCAGCGC  CCCTCCGCTG
5460  CCCCACGCCG TCGATCTGCC  CCAGCCGGGG  CTGCGCCGCT  GACTGCCATA  TCACCTGCCC  CCGATACAGC
5530  CCCTGTACCT GATGTTGACT  CACGTGGTGC  CATATTGCGA  CGCCAGTACA  ATTTATCCAC  ATCCCCGCTT
5600  ACATCATCTG TTGCTTCGGG  TACTAATCTG  GTTCTTTACG  CTGCCCCGCT  AAACCCTTTG  CTGCCCCTTC
5670  AGGATGGCAC TAATACTCAC  ATCATGGCCA  CTGAGGCATC  TAATTATGCC  CAGTATCGGG  TTGTTCGAGC
5740  TACGATTCGT TACAGGCCAT  TGGTGCCAAA  TGCCGTCGGC  GGTTATGCAA  TATCCATCTC  ATTCTGGCCT
5810  CAGACAACTA CCACCCCCAC  GTCTGTTGAT  ATGAACTCTA  TTACTTCCAC  TGATGTTAGG  ATTCTAGTCC
5880  AGCCCGGCAT TGCTTCTGAG  TTGGTTATCC  CTAGTGAGCG  CCTCCACTAT  CGTAACCAGG  GCTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG  TTGAAGAGGA  GGCTACTTCT  GGTTTGGTAA  TGCTTTGTAT  CCATGGCTCC
6020  CCTGTTAACT CCTATACCAA  CACCCCCTAC  ACCGGGGCGC  TTGGGCTCCT  TGATTTCGCT  TTAGAGCTTG
6090  AGTTTAGGAA CCTGACACCC  GGGAACACCA  ACACCCGTGT  GTCTCGGTAT  ACAAGCACAG  CCCGTCATCG
6160  GCTGCGCCGC GGTGCTGATG  GCACCGCCGA  ACTTACCACC  ACAGCGGCCA  CGCGCTTCAT  GAAGGACCTG
6230  CACTTCACCG GTACGAATGG  GGTTGGTGAA  GTGGGTCGTG  GTATTGCTCT  CACACTCTTT  AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG  CCGACAGAAT  TAATTTCGTC  GGCTGGGGGA  CAGTTATTTT  ACTCCCGCCC
6370  TGTCGTCTCA GCCAATGGCG  AGCCGACTGT  CAAGTTATAT  ACATCTGTAG  AGAATGCGCA  GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA  TATAGATCTA  GGTGACTCCC  GTGTGGTCAT  CCAAGACTAT  GACAACCAGC
6510  ATGAGCAGGA TCGACCCACC  CCCTCGCCTG  CCCCTTCTCG  CCCTTTTTCG  GTCCTTCGCG  CTAATGATGT
6580  TTTATGGCTT TCTCTTACTG  CCGCCGAGTA  CGACCAGACC  ACATATGGGT  CGTCCACCAA  CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT  TGTCAACGTG  GCTACAGGAG  CCCAGGCTGT  CGCCCGTTCC  CTTGACTGGT
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6790  GCTTCGCGGG AAGTTATCCT  TTTGGGAGGC  CGGGACGACT  AAGGCCGGCT  ACCCCTATAA  TTACAACACA
6860  ACTGCTAGTG ATCAGATCTT  GATTGAAAAT  GCGGCTGGTC  ATCGTGTAGC  TATTTCCACG  TATACCACCA
6930  GCTTGGGCGC AGGTCCTGTG  TCTGTTTCTG  CAGTCGGTGT  TTTAGCCCCA  CATTCGGCTC  TTGCAGTCCT
7000  TGAAGACACT ATTGACTACC  CTGCCCGTGC  CCACACTTTT  GATGATTCCT  GTCCGGAGTG  TCGCGCTCTT
7070  GGTTTGCAGG GGTGTGCTTT  CCAGTCTACT  GTTGCTGAGC  TTCAGCGTCT  TAAAATGAAG  GTAGGTAAAA
7140  CCCGGGAGTT TTAATTAATT  TCCTTTGTGC  CCCCTTCATA  GCTTTGCTTT  ATTTCTTTTT  TTCTGCTTTT
7210  CGCGCTCCCT GGAANNNNNN  NNNNNNN
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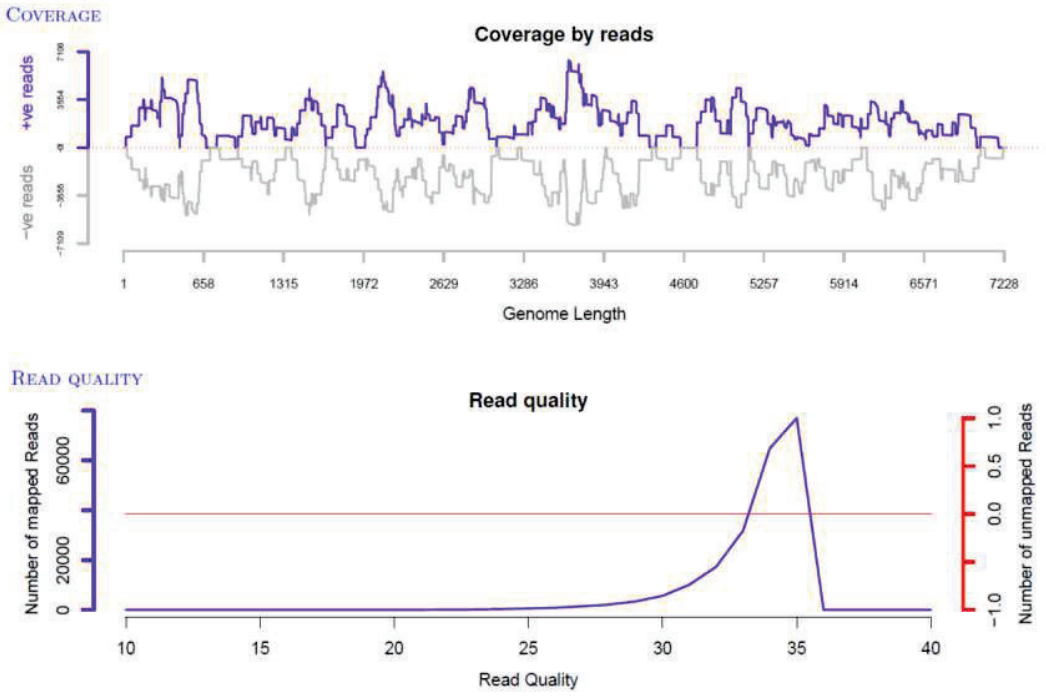
C3-NO-RBV

File name	**C3-NO-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**215 515**
Mapped reads	**215 515 (100.00%)**
Average read length	**136nt**
Coverage	**7 214nt (99.68%)**
Average depth	**4 042 reads/site**



C3-NO-RBV Consensus sequence

1	NNNNNNNNCG	TATGTGGTCG	ATGCCATGGA	GGCCCACCAG	TTTATTAAGG	CTCCTGGCAT	TACTACTGCC
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140	CTCGTGTGCA	AACCGAGATT	CTTATTAATT	TGATGCAACC	CCGGCAGTTG	GTTTTTCGGC	CTGAAGTGCT
210	CTGGAATCAT	CCTATCCAAC	GGGTTATCCA	TAATGAACTT	GAACAGTACT	GTCGGGCCCG	GGCTGGTCGT
280	TGTCTAGAGG	TTGGGGCCCA	TCCAAGATCC	ATTAATGACA	ACCCAAATGT	TCTTCACCGG	TGCTTTCTAC
350	GACCAGTAGG	GAGAGATGTT	CAGCGCTGGT	ACTCTGCTCC	TACCCGCGGC	CCTGCGGCTA	ATTGCCGCCG
420	TTCTGCCTTG	CGTGGCCTCC	CCCCCGTTGA	TCGTACCTAT	TGCTTTGATG	GATTCTCCCG	CTGCTCATTT
490	GCCGCAGAAA	CTGGGGTTGC	CCTTTATTCC	CTGCATGACC	TCTGGCCGGC	CGATGTTGCG	GAGGCTATGG
560	CCCGACACGG	CATGACACGC	TTGTATGCCG	CACTACATCT	CCCCCCTGAA	GTACTACTAC	CACCTGGTAC
630	TTACCATACA	ACTTCATACC	TTCTGATTCA	CGACGGTGAT	CGCGCTGTTG	TGACCTATGA	AGGTGATACT
700	AGTGCGGGCT	ACAACCATGA	TGTCTCCATA	CTTCGTGCAT	GGATCCGCAC	AACTAAGATA	GTCGGCGATC
770	ATCCGCTGGT	GATAGAGCGT	GTGCGGGCTA	TTGGCTGCCA	TTTTGTGCTG	CTGCTTACTG	CAGCCCCTGA
840	GCCGTCACCA	ATGCCTTATG	TTCCATACCC	CCGGTCGACA	GAGGTGTATG	TCCGTTCTAT	ATTCGGCCCT
910	GGCGGGTCCC	CATCCCTATT	CCCATCAGCC	TGCTCTACGA	AATCCACATT	TCACGCTGTC	CCGGTTCATA
980	TTTGGGACCG	GCTCATGCTT	TTTGGCGCTA	CTCTGGATGA	TCAGGCGTTT	TGCTGCTCAC	GACTTATGAC
1050	CTATCTCCGC	GGGATTAGTT	ACAAGGTCAC	TGTTGGCGCC	CTTGTCGCTA	ACGAGGGGTG	GAATGCTTCG
1120	GAGGACGCTC	TTACCGCTGT	TATTACTGCA	GCGTATTTGA	CCATTTGCCA	TCAGCGTTAC	CTCCGTACTC
1190	AAGCTATATC	CAAGGGTATG	CGCCGACTGG	AGGTTGAGCA	TGCCCAAAAA	TTCATTACAA	GACTTTATAG
1260	TTGGCTGTTC	GAGAAGTCCG	GCCGTGATTA	CATCCCCGGT	CGCCAGCTCC	AGTTCTATGC	ACAGTGCCGT
1330	CGTTGGTTAT	CGGCGGGTTT	CCATCTTGAC	CCAAGGGTGC	TTGTTTTTGA	TGAGTCTGTG	CCCTGCCGTT
1400	GTAGGACATT	TCTTAAGAAG	GCTGTGGGTA	AGTTCTGCTG	TTTTATGAAG	TGGTTGGGAC	AGGAGTGCAC
1470	CTGCTTTTTA	GAACCAGCAG	AGGGTCTAGT	TGGCGACCAT	GGCCACGATA	ATGAAGCCTA	TGAGGGCTCT
1540	GAGGTCGATC	AGGCTGAGCC	CGCCTGTCTC	GATGTTTCTG	GGACTTATGC	CGTCCATGGC	CGCCAACTTG
1610	AGGCCCTGTA	TAGGGCGCTT	AACATCCCGC	ATGACATCGT	TGCCCGAGCC	TCCCGTTTGA	CTGCCACCGT
1680	CGAACTCGCC	GCAGGTCCAG	ACCGCCTGGA	GTGCCGCACT	GTGCTCGGGA	ATAAGACTTT	CCGGACGACG
1750	GTGGTTGATG	GCGCCCACCT	TGAGGCGAAC	GGCCCCGAGC	AGTATGTTCT	TTCGTTCGAC	GCCTCTCGCC
1820	AGTCAATGGG	GGCCGGGCCG	CATAGTCTCT	CCTACGAGCT	TACTCCTGCT	GGTTTGCAGG	TCAAGATTTC
1890	ATCTAATGGC	CTGGATTGCA	CTGCAACATT	CCCCCCGGGC	GGGGCCCCTA	GCGCTAATCC	GGGGGAGGTA
1960	GCAGCCTTCT	GCAGCGCCCT	TTACAGGTAC	AACAGGTTCA	CTCAGCGCCA	TTCGCTTATA	GGTGGCTTGT
2030	GGCTGCATCC	TGAGGGGTTG	TTGGGTATCT	TCCCCCCTTT	TTCTCCCGGA	CACCTTTGGG	AGTCCGCTAA
2100	TCCTTTTTGT	GGGGAGGGAA	CTTTGTACAC	CCGGACATGG	TCAACATCTG	GTTTTTCTAG	TGACTTTTCC
2170	CCCCCTGAGG	CAGCTGTTGC	AGCGCCGGCT	GCTACTCCGG	GGTTACGCCA	CCCCACACCC	CCTGTTAGTG
2240	ACGTTCGGGT	GTTACCGCCA	CCTTCTGAAG	AATTTCAGGT	TGATACAGCG	CCCACTCCCC	CTGCCCCTGA
2310	GCCCGCTCAA	CCATCTAGCT	CCGCTGGGCC	AAAGGCCCCC	GTGCGTAAGC	CGCCAACGCT	GCCATCCCCG
2380	CGCACTCGCC	GCCTTCTTTA	CACCTATCCA	GATGGGGCAA	AGGTGTATGC	GGGGTCACTG	TTTGAGTCTG
2450	ACTGTGATTG	GCTGGTTAAT	GCGTCGAATC	CCGGTCATCG	TCCTGGAGGC	GGCCTTTGCC	ATGCCTTTTA
2520	CCAGCGCTAT	CCCGAGTCTT	TCTACTCAAC	TGAGTTCATC	ATGCGCGACG	GTCTTGCCGC	GTACACTTTA
2590	ACCCCCCGGC	CTATTATTCA	TGCAGTGGCT	CCTGATTATA	GGGTTGAGCA	TAACCCGAAG	AGGCTTGAGG
2660	CAGCATACCG	AGAGACTTGC	TCTCGCCGCG	GCACCGCCGC	CTATCCACTC	CTCGGTTCGG	GCATATATCA
2730	AGTTCCCGTC	AGCCTCAGCT	TCGACGCTTG	GGAGCGTAAC	CACCGCCCCG	GGGACGAGCT	CTACCTAACC
2800	GACCTCGCCG	CTACCTGGTT	CGAGGCCAAT	AAGCCAACAC	AGCCGGCCCT	CACAATAACT	GAGGACGCAG
2870	CCCGCACAGC	CAACCTAGCA	TTGGAGATCG	ATGCTGCTAC	TGAGGTCGGC	CGGGCTTGTG	CCGGCTGCGC
2940	GGTTAGTCCT	GGGGTTGTGC	ACTATCAGTT	TACTGCTGGG	GTCCCAGGTT	CGGGGAAGTC	GCGTTCTATA
3010	CAGCAGGGGG	ATGTTGACGT	AGTGGTTGTC	CCCACTCGGG	AGCTCCGGAA	CAGTTGGCGT	CGCCGGGGTT
3080	TCGCAGCTTT	TACACCTCAT	ACGGCGGCCC	GTGTCACCAC	GGGTCGTCGT	GTTGTAATTG	ATGAGGCCCC
3150	ATCCCTTCCA	CCGCATCTGT	TGCTACTACA	CATGCAGCGG	GCCTCGTCGG	TCCACCTTCT	CGGCGACCCA
3220	AACCAGATCC	CTGCCATAGA	CTTCGAGCAT	GCCGGCCTGG	TCCCCGCAAT	ACGCCCTGAG	CTTGCACCCA
3290	CCAGTTGGTG	GCATGTCACC	CACCGCTGCC	CCGCTGATGT	GTGCGAGCTT	ATACGCGGGG	CTTACCCCAA
3360	AATCCAAACC	ACTAGCCGCG	TGCTGCGGTC	ATTATTCTGG	AATGAGCCTG	CCATTGGCCA	GAAGTTAGTT
3430	TTCACCCAGG	CCGCTAAGGC	CGCCAACCCC	GGTGCGATTA	CAGTCCACGA	GGCCCAGGGC	GCCACTTTCA
3500	CGGAAACTAC	AATCATAGCC	ACGGCTGATG	CTAGGGGGCT	CATCCAATCC	TCCCGAGCTC	ATGCCATAGT
3570	CGCACTTACC	CGCCACACAG	AGAAGTGCGT	TATTCTTGAC	GCCCCCGGCT	TGTTACGTGA	GGTTGGTATA
3640	TCGGATGTGA	TTGTCAACAA	CTTTTTCCTC	GCCGGCGGCG	AAGTGGGTCA	TCATCGTCCC	TCTGTGATAC

C3-NO-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCACCT  TCCTGCCAGA  TCAGCGCCTA
3780  TCACCAGTTA GCTGAGGAAC  TTGGCCACCG  CCCAGCTCCT  GTCGCCGCCG  TCCTGCCCCC  TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA  TATGCCGCAG  GAGCTTACGG  TGTCTGATAG  CGTGCTGGTC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG  ATGGCAGCCC  CCAGCCAGCG  GAAGGCCGTC  CTATCGACGC  TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC  TGTACGAAGC  AGCTCACTCC  GACGTCCGTG  AGTCCCTGGC  TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA  GGCTACTACG  TGTGAGTTAT  ATGAGCTGGT  TGAGGCCATG  GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG  CTTGAGCTCG  ACCTTTGCAA  TCGTGATGTA  TCGCGTATCA  CATTTTTCCA
4200  GAAAGATTGT AACAAATTCA  CCACAGGGGA  GACCATTGCC  CACGGTAAGG  TCGGCCAGGG  CATCTCGGCT
4270  TGGAGCAAGA CCTTCTGTGC  CCTGTTTGGT  CCGTGGTTTC  GTGCTATCGA  AAAAGAGATA  CTAGCCCTGC
4340  TCCCGCCTAA CATTTTCTAC  GGCGACGCAT  ACGAGGAGTC  TGTGTTTGCC  GCCGCTGTGT  CGGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA  ATGATTTTTC  AGAGTTTGAT  AGCACCCAAA  ATAACTTTTC  CCTTGGTCTC
4480  GAGTGCGTAG TCATGGAGGA  ATGTGGCATG  CCCCAGTGGC  TAATCCGGTT  GTACCATCTG  GTCCGGTCGG
4550  CCTGGATCTT ACAGGCACCG  AAGGAGTCTC  TTAAGGGATT  TTGGAAGAAG  CATTCTGGTG  AGCCTGGCAC
4620  CCTCCTCTGG AACACTGTTT  GGAACATGGC  GATCATAGCA  CACTGCTATG  AATTTCGTGA  TTTTAGGGTT
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4760  TGATTGCAGG TTGCGGGCTC  AAACTGAAGG  TTGACTATCG  CCCTATTGGG  TTGTATGCTG  GTGTGGTGGT
4830  GGCCCCTGGC CTGGGGACGC  TACCCGATGT  GGTGCGCTTC  GCCGGCCGGC  TGTCTGAGAA  GAACTGGGGC
4900  CCTGGGCCGG AGCGAGCTGA  GCAGTTGCGC  CTAGCTGTTT  GTGACTTCCT  CCGAGGGTTA  ACGAATGTTG
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5110  AACTCTATCA TACAGCGGGT  GGAATGAATA  ACATGTTTTG  TGCATTGCCC  ATGGGGTCAC  CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT  CTTCGTGCTT  TTGCCTATGC  TGCCCGCGCC  ACCGGCCGGC  CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC  AGCGGCGGTA  CCGGCAGTGG  TTTCTGGGGT  GACAGGGTTG  ATTCTCAGCC
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6020  CCTGTTAACT CCTATACCAA  CACCCCCTAC  ACCGGGGCGC  TTGGGCTCCT  TGATTTCGCT  TTAGAGCTTG
6090  AGTTCAGGAA CCTGACACCC  GGGAACACCA  ACACCCGTGT  GTCTCGGTAT  ACAAGCACAG  CCCGTCATCG
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6230  CACTTCACCG GTACGAATGG  GGTTGGTGAA  GTGGGTCGTG  GTATTGCTCT  CACACTCTTT  AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG  CCGACAGAAT  TAATTTCGTC  GGCTGGGGGA  CAGTTATTTT  ACTCCCGCCC
6370  TGTCGTCTCA GCCAATGGCG  AGCCGACTGT  CAAGTTATAT  ACATCTGTAG  AGAATGCGCA  GCAGGATAAA
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7140  CCCGGGAGTT TTAATTAATT  TCCTTTGTGC  CCCCTTCATA  GCTTTGCTTT  ATTTCTTTTT  TTCTGCTTTT
7210  CGCGCTCCCT GGNNNNNNNN  NNNNNNN
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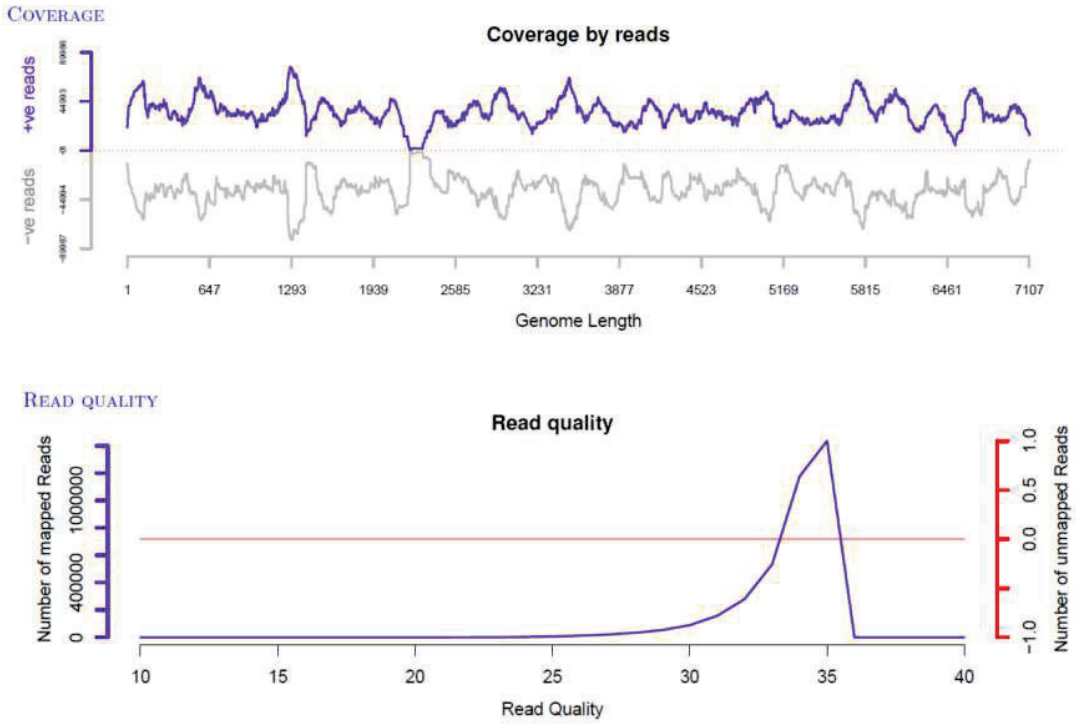
D1-PRE-RBV

File name	**D1-PRE-RBV.sam**
Ref name	**KJ701409.1**
Ref length	**7 110nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**3 794 466**
Mapped reads	**3 794 466 (100.00%)**
Average read length	**138nt**
Coverage	**7 110nt (100.00%)**
Average depth	**72 711 reads/site**
Average insert length	**3 433nt**



D1-PRE-RBV Consensus sequence

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 140  GAGATTCTTA TTAACTTGAT GCAACCCCGG CAGCTGGTTT TCCGACCTGA AGTGCTCTGG AATCATCCTA
 210  TCCAACGGGT TATTCATAAT GAATTAGAAC AGTACTGCCG GGCCCGGGCT GGTCGCTGTT TAGAGGTTGG
 280  GGCCCACCCA AGATCCATCA ATGATAACCC GAATGTTCTG CATCGCTGCT TCCTACGACC AGTTGGCAGA
 350  GATGTTCAGC GCTGGTATTC AGCCCCTACC CGTGGCCCTG CGGCTAACTG TCGCCGCTCT GCTTTGCGTG
 420  GCCTTCCCCC CGCTGACCGC ACCTACTGTT TTGATGGATT CTCTCGTTGT TCGTTTGCTG CGGAGACTGG
 490  TGTTGCCCTT TATTCATTGC ATGACCTCTG GCCGGCCGAT GTTGCAGAGG CTATGGCCCG GCACGGGATG
 560  ACACGCTTAT ATGCTGCACT ACATCTCCCC CCTGAAGTAT TACTACCACC TGGTACATAC CACACAACTT
 630  CATACCTTCT CATCCACGAC GGTGATCGTG CCGTTGTGAC TTACGAGGGT GACACTAGTG CAGGTTACAA
 700  CCACGATGTC TCCATACTCC GCGCATGGAT CCGCACAACC AAGATAGTTG GCGACCATCC GCTGGTGATA
 770  GAGCGTGTGC GGGCTATCGG CTGCCACTTT GTGCTGCTGC TCACTGCGGC CCCTGAGCCG TCACCGATGC
 840  CTTATGTCCC ATACCCCCGG TCGACAGAGG TGTACGTTCG TTCCATCTTC GGCCCCGGCG GGTCTCCGTC
 910  CTTGTTTCCA TCAGCTTGCT CTACAAAATC TACATTTCAT GCCGTCCCAG TTCACATTTG GGACCGGCTC
 980  ATGCTCTTTG GTGCTACCCT GGACGATCAG GCGTTTTGCT GTTCACGGCT TATGACTTAC CTTCGTGGGA
1050  TTAGTTACAA GGTTACTGTA GGCGCCCTTG TTGCTAATGA AGGGTGGAAT GCCTCGGAAG ACGCTCTTAC
1120  TGCTGTTATT ACCGCAGCAT ACTTGACCAT TTGCCATCAA CGTTACCTCC GCACTCAAGC TATATCCAAA
1190  GGCATGCGCC GGCTGGAGGT TGAGCATGCT CAAAAATTTA TCACAAGACT CTATAGTTGG CTGTTTGAGA
1260  AGTCTGGCCG TGACTACATC CCCGGCCGCC AGCTCCAGTT CTATGCACAG TGCCGCCGCT GGTTATCGGC
1330  GGGTTTCCAC CTCGATCCAA GGGTGCTTGT TTTTGATGAG TCTGCGCCCT GCCGCTGTAG GACGTTTCTT
1400  AAGAAGGTTG CGGGTAAGTT CTGCTGTTTT ATGAAGTGGC TGGGGCAGGA GTGCACTTGC TTCCTGGAAC
1470  CAGCTGAGGG TCTGGTTGGT GACCATGGTC ACGATAATGA AGCCTATGAG GGTTCTGAGG TCGACCAGGC
1540  TGAGCCTGCC CATCTTGATG TCTCTGGGAC CTATGCCGTC CATGGGAGCC AACTCGAGGC CCTGTACAGG
1610  GCGCTTAACA TCCCACATGA CATTGCCGCT CGAGCCTCCC GTTTAACCGC CACTGTTGAA CTTACTGCAG
1680  GTCCAGACCG TTTGGAGTGT CGCACTGTGC TCGGGAATAA GACCTTCCGG ACAACGGTGG TTGATGGCGC
1750  CCACCTTGAG GTGAATGGCC CCGAGCAGTA TGTCCTTTCG TTTGACGCCT CCTGTCAGTC TATGGGGGCC
1820  GGGCCGCATA ACCTCACCTA TGAGCTTACT CCTGCTGGTT TGCAGGTTAA GATTTCATCT AATGGTCTGG
1890  ATTGCACTGC AACATTTCCC CCGGGTGGAG CCCCTAGTGC AGCTCCGGGG GAGGTAGCAG CCTTTTGCAG
1960  TGCCCTCTAT AGGTACAACA GGTTCACCCA ACGCCATTCG CTTACAGGCG GTTTGTGGCT GCATCCTGAG
2030  GGGCTATTGG GCATCTTCCC TCCCTTTTCC CCCGGGCATC TTTGGGAGTC CGCTAACCCT TTTTGTGGGG
2100  AGGGAACTTT GTATACCCGG ACTTGGTCAA CATCTGGTTT TTCTAGTGAT TTTTCCCCCC CTGAGGCGGC
2170  TGCCGCTGTA CCGGCCGCTA CCACAGGGTT ACCCCACCCT ACACCCCCTG TTAGTGACAT TTGGGTGTTG
2240  CCGCCACCCT CTGAGGAGTC TCAGGTTGAT GCAGCGCCTG CTCCTCCAGC CCCTGAGCCT GCTCAACCGC
2310  CCGACCCTGT CGGGCTGAGG GCCTCCGTGC GTAAGCCACC AACACCATCG CCTCCGCGTA CCCGCCGCCT
2380  CCTCTATACT TATCCGGATG GGGCAAAGGT GTATGCGGGG TCATTGTTTG AGTCAGACTG TGATTGGCTG
2450  GTCAATGCGT CGAATCCCGG CCATCGCCCC GGAGGTGGCC TCTGCCATGC TTTTTACCAA CGCTATCCCG
2520  AGTCTTTCCA CCCAACTGAG TTCATTATGC GTGATGGTCT CGCCGCATAT ACCTTAACTC CTCGGCCTAT
2590  TATCCACGCG GTGGCTCCCG ATTATAGGGT TGAGCAGAAC CCAAAGAGGC TTGAGGCAGC ATACCGGGAG
2660  ACTTGCTCCC GCCGCGGCAC CGCCGCTTAC CCACTCCTCG GCTCGGGCAT ATACCGAGTC CCCGTCAGCC
2730  TCAGTTTTGA CGCTTGGGAG CGCAACCACC GTCCCGGGGA TGAGCTTTAC TTGACCGACC TCGCCGCTAC
2800  CTGGTTCGAG GCCAACAAGC CAGCACAACC GGCCCTCACA ATAACTGAAG ATACAGCTCG CACAGCCAAC
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3010  CGATGTGGTG GTTGTCCCCA CTCGGGAGCT TCGGAATAGT TGGCGTCGCC GGGGTTTTGC AGCTTTTACG
3080  CCTCACACGG CGGCCCGTGT TACCACGGGC CGACGTGTTG TGATTGATGA GGCCCCATCC CTTCCGCCGC
3150  ACCTGCTGCT TTTACATATG CAGCGGGCCT CGTCGGTCCA CCTTCTCGGC GACCCAAATC AGATCCCTGC
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3570  ACACAGAGAA GTGCGTTATC CTTGATGCTC CCGGTTTGTT ACGTGAGGTC GGTATATCTG ACATAATTGT
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D1-PRE-RBV Consensus sequence

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3780  AGGAGCTGGG TCACCGCCCG GCCCCCGTCG CTGCCGTCCT GCCCCCCTGC CCCGAACTTG AGCAGGGCCT
3850  GCTGTATATG CCACAAGAGC TCACGGTGTC TGATAGCGTG CTGGTTTTTG AACTCACGGA CATAGTTCAT
3920  TGTCGGATGG CCGCCCCTAG CCAGCGGAAG GCCGTCCTAT CGACGCTTGT GGGTAGGTAT GGCCGCCGGA
3990  CAAAGCTGTA TGAGGCGGCT CACTCCGATG TCCGTGAGTC TCTGACAAGA TTTATCCCTA CCATCGGGCC
4060  CGTTCAGGCA ACTACGTGTG AGTTATACGA ACTGGTTGAG GCCATGGTGG ACAAGGGTCA GGACGGCTCT
4130  GCCGTACTTG AGCTTGATCT TTGCAATCGC GATGTGTCGC GTATTACATT CTTCCAGAAA GATTGTAACA
4200  AGTTCACCAC AGGGGAGACC ATTGCTCATG GTAAGGTCGG CCAGGGCATT TCGGCTTGGA GCAAGACCTT
4270  CTGTGCCCTC TTTGGCCCGT GGTTTCGTGC CATTGAAAAA GAAATATTGG CCCTGCTCCC GCCCAATATC
4340  TTCTATGGCG ATGCATATGA AGAGTCTGTG TTTGCTGCCG CTGTGTCTGG GGCAGGTTCA TGCATGGTAT
4410  TTGAGAATGA TTTTTCAGAG TTTGACAGTA CTCAAAATAA CTTCTCCCTT GGCCTCGAGT GTGTAGTCAT
4480  GGAGGAGTGC GGCATGCCCC AGTGGCTAAT CCGGTTGTAC CACTTGGTTC GGTCGGCCTG GATTCTACAG
4550  GCGCCGAAGG AGTCACTTAA AGGGTTTTGG AAGAAGCATT CTGGTGAGCC CGGCACCCTC CTTTGGAACA
4620  CCATTTGGAA TATGGCGATC ATAGCACATT GCTATGAGTT TCGTGATCTT AGGGTTGCCG CCTTCAAGGG
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4760  GGGCTCAAAC TGAAGGTTGA TTACCGCCCT ATTGGGTTGT ATGCTGGTGT GGTGGTGGCC CCCGGCCTGG
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5390  GCCACCCCGC CCCCTCGGCT CCTCTTGGCG TGACCAGTCC CAGCGCCCCT CCGCTGCCTC ACGCCGTCGA
5460  CCTGCCCCAA CTGGGGCTGC GCCGCTGACT GCTATATCAC CAGCTCCTGA TACAGCCCCC GTGCCTGATG
5530  TTGACTCTCG TGGCGCTATA TTGCGGCGCC AGTATAATTT ATCTACATCC CCACTCACAT CATCTGTTGC
5600  TTCCGGCACT AATCTGGTTC TTTATGCTGC CCCGCTAAAT CCCTTGTTAC CTCTTCAGGA TGGCACTAAC
5670  ACTCATATCA TGGCTACTGA GGCATCTAAC TATGCCCAGT ACCGGGTTGT CCGAGCTACG ATCCGTTACA
5740  GGCCGTTGGT GCCGAATGCT GTCGGCGGCT ATGCAATCTC CATTTCATTC TGGCCTCAGA CCACTACCAC
5810  CCCTACGTCT GTTGACATGA ATTCTATTAC CTCCACTGAT GTTAGGATCC TAGTTCAGCC CGGTATTGCT
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6020  CACCAATACC CCTTATACCG GGGCGCTTGG GCTCCTTGAC TTTGCTCTAG AACTTGAGTT TAGGAACTTG
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6720  GATGGTCGCC CCCTCACTAC CATTCAACAG TACTCTAAAA CATTTTATGT TCTCCCACTG CGCGGGAAGT
6790  TGTCCTTTTG GGAGGCTGGG ACAACTAAAG CTGGTTATCC TTATAATTAT GACACGACTG CTAGTGACCA
6860  GATTTTAATT GAAAATGCAG CTGGCCACCG TGTTGCTATC TCTACTTATA CTACCAGCCT TGGCGCTGGC
6930  CCTGTGTCAG TTTCTGCAGT CGGTGTGTTA GCCCCACATT CGGCTCTTGC AGTTCTTGAG GATACTACTG
7000  ACTACCCTGC CCGCGCCCAC ACTTTTGATG ACTTCTGTCC AGAGTGTCGC ACTCTTGGCT TGCAAGGGTG
7070  TGCCTTCCAG TCTACTATTG CTGAGCTTCA GCGTCTTAAA
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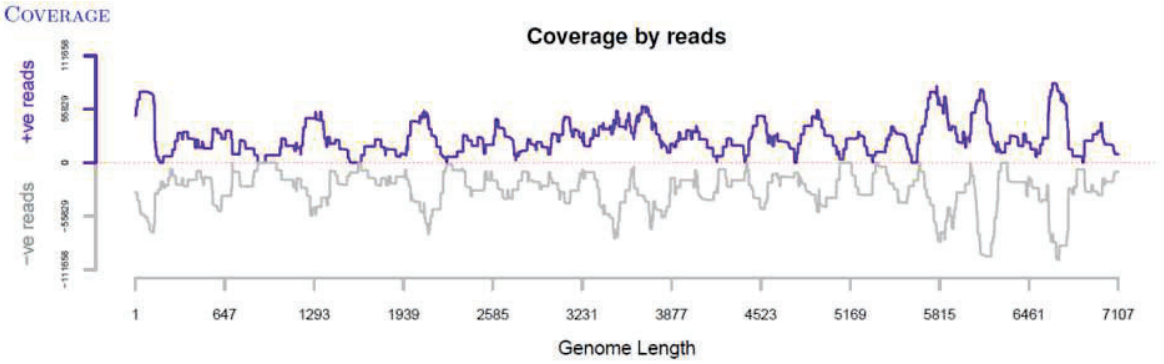
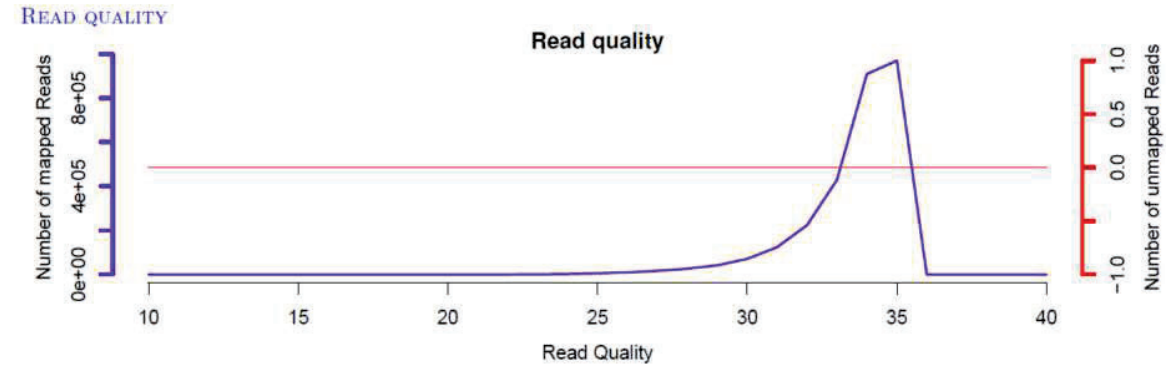
D2-PRE-RBV

File name	**D2-PRE-RBV.sam**
Ref name	**KJ701409.1**
Ref length	**7 110nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**2 830 383**
Mapped reads	**2 830 383 (100.00%)**
Average read length	**141nt**
Coverage	**7 110nt (100.00%)**
Average depth	**54 888 reads/site**





D2-PRE-RBV Consensus sequence

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  70  TCTGGCTGCG GCCAATTCTG CCCTGGCGAA TGCTGTGGTG GTTCGGCCGT TTTTATCGCG TGTGCAAACT
 140  GAGATTCTTA TTAACTTGAT GCAACCCCGG CAGCTGGTTT TCCGACCTGA AGTGCTCTGG AATCATCCTA
 210  TCCAACGGGT TATTCATAAT GAATTAGAAC AGTACTGCCG GGCCCGGGCT GGTCGCTGTT TAGAGGTTGG
 280  GGCCCACCCA AGATCCATCA ATGATAACCC GAATGTTCTG CATCGCTGCT TCCTACGACC AGTTGGCAGA
 350  GATGTTCAGC GCTGGTATTC AGCCCCTACC CGTGGCCCTG CGGCTAACTG TCGCCGCTCT GCTTTGCGTG
 420  GCCTTCCCCC CGCTGACCGC ACCTACTGTT TTGATGGATT CTCTCGTTGT TCGTTTGCTG CGGAGACTGG
 490  TGTTGCCCTT TATTCATTGC ATGACCTCTG GCCGGCCGAT GTTGCAGAGG CTATGGCCCG GCACGGGATG
 560  ACACGCTTAT ATGCTGCACT ACATCTCCCC CCTGAAGTAT TACTACCACC TGGTACATAC CACACAACTT
 630  CATACCTTCT CATCCACGAC GGTGATCGTG CCGTTGTGAC TTACGAGGGT GACACTAGTG CAGGTTACAA
 700  CCACGATGTC TCCATACTCC GCGCATGGAT CCGCACAACC AAGATAGTTG GCGACCATCC GCTGGTGATA
 770  GAGCGTGTGC GGGCTATCGG CTGCCACTTT GTGCTGCTGC TCACTGCGGC CCCTGAGCCG TCACCGATGC
 840  CTTATGTCCC ATACCCCCGG TCGACAGAGG TGTACGTTCG TTCCATATTC GGTCCTGGCG GATCCCCATC
 910  CCTATTCCCA TCAGCTTGCT CTACGAAATC CACATTTCAT GCCGTCCCAG TTCACATTTG GGACCGGCTC
 980  ATGCTCTTTG GTGCTACCCT GGACGATCAG GCGTTTTGCT GTTCACGGCT TATGACTTAC CTTCGTGGGA
1050  TTAGTTACAA GGTTACTGTA GGCGCCCTTG TTGCTAATGA AGGGTGGAAT GCCTCGGAGG ACGCTCTTAC
1120  TGCTGTTATT ACCGCAGCAT ACTTGACCAT TTGCCATCAA CGTTACCTCC GCACTCAAGC TATATCCAAA
1190  GGCATGCGCC GGCTGGAGGT TGAGCATGCT CAAAAATTTA TCACAAGACT CTATAGTTGG CTGTTTGAGA
1260  AGTCTGGCCG TGACTACATC CCCGGCCGCC AGCTCCAGTT CTATGCACAG TGCCGCCGCT GGTTATCGGC
1330  GGGTTTCCAC CTCGATCCAA GGGTGCTTGT TTTTGATGAG TCTGCGCCCT GCCGCTGTAG GACGTTTCTT
1400  AAGAAGGTTG CGGGTAAGTT CTGCTGTTTT ATGAAGTGGC TGGGGCAGGA GTGCACTTGC TTCCTGGAAC
1470  CAGCTGAGGG TCTGGTTGGT GACCATGGTC ACGATAATGA AGCCTATGAG GGTTCTGAGG TCGACCAGGC
1540  TGAGCCTGCC CATCTTGATG TCTCTGGGAC CTATGCCGTC CATGGGAGCC AACTCGAGGC CCTGTACAGG
1610  GCGCTTAACA TCCCACATGA CATTGCCGCT CGAGCCTCCC GTTTAACCGC CACTGTTGAA CTTACTGCAG
1680  GTCCAGACCG TTTGGAGTGT CGCACTGTGC TCGGGAATAA GACCTTCCGG ACAACGGTGG TTGATGGCGC
1750  CCACCTTGAG GTGAATGGCC CCGAGCAGTA TGTCCTTTCG TTTGACGCCT CCTGTCAGTC TATGGGGGCC
1820  GGGCCGCATA ACCTCACCTA TGAGCTTACT CCTGCTGGTT TGCAGGTTAA GATTTCATCT AATGGTCTGG
1890  ATTGCACTGC AACATTTCCC CCGGGTGGAG CCCCTAGTGC AGATCCGGGG GAGGTAGCAG CCTTTTGCAG
1960  TGCCCTCTAT AGGTACAACA GGTTCACCCA ACGCCATTCG CTTACAGGCG GTTTGTGGCT GCATCCTGAG
2030  GGGCTATTGG GCATCTTCCC TCCCTTTTCC CCCGGGCATC TTTGGGAGTC CGCTAACCCT TTTTGTGGGG
2100  AGGGAACTTT GTATACCCGG ACTTGGTCAA CATCTGGTTT TTCTAGTGAT TTTTCCCCCC CTGAGGCGGC
2170  TGCCGCTGTA CCGGCCGCTA CCACAGGGTT ACCCCACCCT ACACCCCCTG TTAGTGACAT TTGGGTGTTG
2240  CCGCCACCCT CTGAGGAGTC TCAGGTTGAT GCAGCGCCTG CTCCTCCAGC CCTTGAGCCT GCTCAACCGC
2310  CCGACCCTGT CGGGCCGAGG GCCTCCGTGC GTAAGCCACC AACACCATCG CCTCCGCGTA CCCGCCGCCT
2380  CCTCTATACT TATCCGGATG GGGCAAAGGT GTATGCGGGG TCATTGTTTG AGTCAGACTG TGATTGGCTG
2450  GTCAATGCGT CGAATCCCGG CCATCGCCCC GGAGGTGGCC TCTGCCATGC TTTTTACCAA CGCTATCCCG
2520  AGTCTTTCCA CCCAACTGAG TTCATTATGC GTGATGGTCT CGCCGCATAT ACCTTAACTC CTCGGCCTAT
2590  TATCCACGCG GTGGCTCCCG ATTATAGGGT TGAGCAGAAC CCAAAGAGGC TTGAGGCAGC ATACCGGGAG
2660  ACTTGCTCCC GCCGCGGCAC CGCCGCTTAC CCACTCCTCG GCTCGGGCAT ATACCGAGTC CCCGTCAGCC
2730  TCAGTTTTGA CGCTTGGGAG CGCAACCACC GTCCCGGGGA TGAGCTTTAC TTGACCGACC TCGCCGCTAC
2800  CTGGTTCGAG GCCAACAAGC CAGCACAACC GGCCCTCACA ATAACTGAAG ATACAGCTCG CACAGCCAAC
2870  CTGGCACTAG AGATCGACGC TGCTACGGAG GTTGGCCGGG CATGTGCCGG TTGTACAGTT AGTCCTGGGA
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3080  CCTCACACGG CGGCCCGTGT TACCACGGGC CGACGTGTTG TGATTGATGA GGCCCCATCC CTTCCGCCGC
3150  ACCTGCTGCT TTTACATATG CAGCGGGCCT CGTCGGTCCA CCTTCTCGGC GACCCAAATC AGATCCCTGC
3220  TATAGACTTT GAGCATGCCG GCCTGGTCCC CGCAATACGT CCTGAGCTCG CTCCCACCAG TTGGTGGCAT
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3430  TAAGGCTGCC AACCCCGGTG CGATTACAGT CCATGAGGCC CAGGGTGCCA CTTTCACGGA GACCACAATT
3500  ATAGCCACGG CCGATGCTAG GGGGCTCATT CAATCCTCCC GAGCCCATGC CATAGTCGCA CTTACCCGCC
3570  ACACAGAGAA GTGCGTTATC CTTGATGCTC CCGGTTTGTT ACGTGAGGTC GGTATATCTG ACATAATTGT
3640  CAACAATTTC TTCCTCGCCG GTGGGGAAGT GGGCCACCAC CGCCCCTCTG TGATACCCCG CGGCAGTCCT
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D2-PRE-RBV Consensus sequence

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3710  GACCAGAACC TCGCGACACT GCAGGCTTTT CCACCCTCCT GCCAGATCAG TGCTTACCAT CAGTTAGCTG
3780  AGGAGCTGGG TCACCGCCCG GCCCCCGTCG CTGCCGTCCT GCCCCCCTGC CCCGAACTTG AGCAGGGCCT
3850  GCTGTATATG CCACAAGAGC TCACGGTGTC TGATAGCGTG CTGGTTTTTG AACTCACGGA CATAGTTCAT
3920  TGTCGGATGG CCGCCCCTAG CCAGCGGAAG GCCGTCCTAT CGACGCTTGT GGGTAGGTAT GGCCGCCGGA
3990  CAAAGCTGTA TGAGGCGGCT CACTCCGATG TCCGTGAGTC TCTGACAAGA TTTATCCCTA CCATCGGGCC
4060  CGTTCAGGCA ACTACGTGTG AGTTATACGA ACTGGTTGAG GCCATGGTGG ACAAGGGTCA GGACGGCTCT
4130  GCCGTACTTG AGCTTGATCT TTGCAATCGC GATGTGTCGC GTATTACATT CTTCCAGAAA GATTGTAACA
4200  AGTTCACCAC AGGGGAGACC ATTGCTCATG GTAAGGTCGG CCAGGGCATT TCGGCTTGGA GCAAGACCTT
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7070  TGCCTTCCAG TCCACTATTG CTGAGCTTCA GCGTCTTAAA
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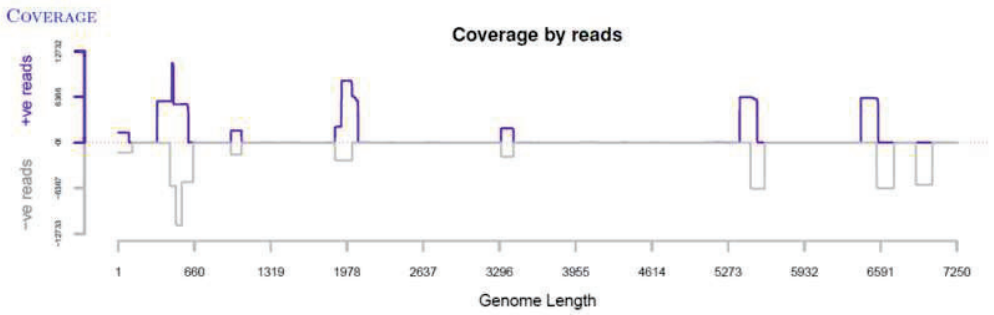
E1-PRE-IFN

File name	**E1-PRE-IFN.sam**
Ref name	**KT159771.1**
Ref length	**7 251nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**75 905**
Mapped reads	**75 905 (100.00%)**
Average read length	**145nt**
Coverage	**6 861nt (94.62%)**
Average depth	**1 405 reads/site**



E1-PRE-IFN Consensus sequence

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 140  CCTGAAGTTT TGTGGAATCA CCCGATCCAG CGAGTTATAC ACAATGAGCT TGAGCAGTAC TGCCGTGCCC
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 280  GTGTTTCCTT CGCCCGGTCG GGAGAGATGT ACAGCGTTGG TATTCCGCCC CGACTCGCGG CCCTGCGGCC
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 420  GTTGCGCTTT TGCTGCGGAG ACCGGTGTGG CCCTCTATTC TCTACATGAC CTTTGGCCAG CTGATGTTGC
 490  TGAGGCTATG GCCCGCCACG GGATGACACG CCTGTATGCC GCACTGCACC TTCCCCCTGA GGTGTTGTTA
 560  CCACCTGGCA CCTACCACAC AACCTCATAC CTCCTGATTC ATGACGGTAA CCGTGCCGTC GTGACTTATG
 630  AGGGTGATAC CAGTGCAGGT TATAACCATG ATGTGTCCAT TCTTCGCGCA TGGATCCGCA CAACTAAGAT
 700  AGTTGGCGAT CATCCGCTGG TGATAGAGCG TGTGCGGGCT ATTGGCTGCC ATTTTGTGTT GCTGCTTACT
 770  GCAGCCCCTG AGCCGTCACC AATGCCTTAT GTCCCATATC CCCGGTCGAC AGAGGTGTAT GTCCGCTCTA
 840  TATTCGGCCC TGGCGGGTCC CCATCTCTAT TCCCATCAGC TTGCTCTACG AAATCCACAT TTCACGCCGT
 910  CCCGGTTCAT ATTTGGGATC GGCTCATGCT TTTTGGCGCC ACTCTGGATG ATCAGGCGTT TTGCTGTTCA
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1120  CCTCCGTACC CAAGCTATAT CTAAGGGCAT GCGCCGACTG GAGGTTGAAC ATGCTCAGAA ATTCATCACA
1190  AGACTCTATA GTTGGTTGTT TGAGAAGTCC GGTCGAGATT ATATCCCCGG CCGTCAGCTC CAGTTTTATG
1260  CACAGTGCCG CCGTTGGCTG TCGGCAGGTT TTCATCTTGA CCCAAGAGTG CTTGTCTTCG ATGAGTCTGT
1330  ACCCTGCCGC TGCAGGACGT TTCTTAAGAA GGCTGCCGGC AAGTTCTGCT GCTTTATGAA GTGGCTGGGG
1400  CAGGAGTGCA CCTGCTCTCT GGAGCCGCCC GAGGGCCTGG TTGGCGACCA TGGTCATGAT AATGAGGCCT
1470  ATGAGGGCTC TGAGGTCGAC CAGGCTGAGC CCGCCCACCT AGATGTTTCT GGGACTTATG CTGTCCATGG
1540  GCGCCAGCTT GAGGCCTTGT ACAGGGCGCT CAACATCCCA CATGACATTG CGGCCCGNNN NNNNNNNNNN
1610  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNCAC TGTGCTTGGG AATAAGACTT
1680  TCCGGACGAC GGTGGTTGAT GGCGCCCATC TTGAGGCGAA CAGCCCCGAG CAGTACGTCC TTTCGTTTGA
1750  CGCCTCTCGC CAGTCTATGG GGGCCGGGCC GCATAGTCTC TCCTACGAGC TCACTCCTGC TGGTTTGCAG
1820  GTCAAGATTT CATCTAATGG CCTGGATTGC ACTGCAACAT TCCCTCCGGG TGGGGCCCCC AGTGCTGCGC
1890  CGGGGGAGGT AGCGGCTTTT TGTACTGCCC TTTACAGATA TAACAGATTC ACTCAGCGAC ACTCGCTGAC
1960  TGGTGGGTTG TGGTTACACC CTGAGGGGCT GGTCGGCATT TTCCCCCCCT TTTCCCCCGG GCATATTTGG
2030  GAGTCTGCCA ATCCTTTCTG CGGAGAGGGG ACTCTGTACA CCCGCACCTG GTCAACCTCC GGCTTCTCTA
2100  GTGATTTCTC CCCCCCTGAA GCGGCCGCCC TTGCACCGGC TGTTGCCCCA GGGCCGCCAC ATCCTACCCC
2170  ACCTGTCAGT GATATCTGGG TGTTACCGCC GCCTTCTGAA GAACTTCAGG TTGACACAGC GCCCGCTCCC
2240  CCTNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNCC CGTGCGTAAG CCATCTGTAC
2310  CTCCATCTTC TCGCACCCGC CGTCTCCTTT ACACCTATCC TGACGGCGCT AAGGTGTATG CGGGGTCATT
2380  GTTTGAGTCT GACTGTGATT GGCTGGTTAA TGCGTCAAAT CCTGGCCATC GTCCTGGCGG CGGCCTTTGC
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2800  TGAGGACACA GCCCGTACGG CTAACCTAGC ACTAGAGATC GATGCTGCCA CGGAGGTTGG CAGGGCTTGT
2870  GCCGGCTGTA CAGTTAGACC TGGGGTTGTG CATTATCAAT TCACTGCCGG GGTGCCAGGT TCGGGGAAGT
2940  CGCGGTCTAT ACAGCAGGGG GATGTCGATG TTGTGGTTGT TCCTACCCGG GAGCTCCGGA ATAGTTGGCG
3010  CCGCCGGGGC TTTGCTGCTT TTACACCTCA TACGGCGGCC CGCGTCACTG CCGGCCGACG TGTTGTGATT
3080  GATGAGGCGC CTTCGCTTCC ACCACATTTG CTGCTGCTTC ATATGCAGCG AGCTTCGTCA GTCCACCTTC
3150  TCGGCGACCC CAACCAGATT CCCGCCATTG ATTTCGAGCA TGCAGGCCTA GTGCCAGCGA TCCGCCCTGA
3220  GCTTGCCCCA ACCAGTTGGT GGCACGTTAC ACACCGTTGC CCTGCCGACG TGTGTGAGCT CATACGCGGG
3290  GCTTATCCCA AAATCCAAAC CACGAGCCGT GTGCTACGGT CTCTGTTTTG GAATGAACCG GCTACCGGCC
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3500  CATGCCATAG TTGCACTCAC CCGCCACACA GAAAAATGCG TCATTCTTGA CGCCCCCGGC CTGTTACGTG
3570  AGGTTGGTAT CTCGGATATA ATTGTTAACA ATTTTTTCCT CGCCGGCGGG GAGGTTGGTC ACCACCGCCC
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E1-PRE-IFN Consensus sequence

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3850  CTTCGAGCTC ACGGACATAG TCCACTGCCG GATGGCCGCC CCTAGCCAGC GGAAGGCCGT CCTATCGACG
3920  CTTGTGGGCA GGTACGGCCG CCGGACGAAG CTGTACGAAG CAGCCCACTC TGACGTCCGC GAGTCCCTGG
3990  CGAGGTTTAT CCCTACCATC GGACCAGTTC AGGCCACCAC ATGTGAGTTG TACGAGTTGG TAGAGGCTAT
4060  GGTTGAGAAG GGTCAGGACG GTTCCGCTGT GTTAGAGCTT GATCTCTGTA GCCGTGATGT CTCGCGTATC
4130  ACATTTTTCC AGAAGGACTG TAATAAGTTC ACAACAGGTG AGACCATTGC CCACGGCAAG GTGGGCCAGG
4200  GCATTTCGGC CTGGAGTAAG ACCTTTTGTG CCCTGTTTGG TCCGTGGTTT CGTGCTATTG AAAAGGAAAT
4270  ATTAGCCCTG CTCCCGCCTA ATATCTTCTA CGGCGACGCC TATGAGGAGT CAGTGTTTGC TGCCGCCGTG
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4620  ATCTTAGGGT TGCCGCCTTC AAGGGAGATG ACTCCGTAGT CCTCTGTAGC GACTACCGCC AAAGCCGCAA
4690  TGCGGCTGCC CTAATTGCGG GCTGCGGACT CAAACTGAAN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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4970  AACCTTATTG GCATGCTGCA AACCATAGCC GATGGCAAGG CCCATTTTAC TGAGACTGTT AAACCTGTAC
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5110  CCATGTGCCC TCGGGCTGTT CTGTTGTTGT TCCTCGTGCT TTTGCCTATG CTGCCCGCGC CACCGGCCAG
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5460  CCTGACACAG CCCCTGTACC TGATGTCGAC TCCCGCGGCG CCATTCTGCG CCGCCAGTAC AATTTATCCA
5530  CATCCCCGCT TACGTCATCT GTCGCCTCGG GTACTAATTT GGTTCTTTAT GCCGCCCCGC TCAATCCCCT
5600  CTTACCTCTC CAGGATGGCA CCAATACTCA TATCATGGCG ACTGAGGCGT CTAACTATGC TCAATATCGG
5670  GTTGTCCGAG CCACAATCCG TTATCGCCCT TTGGTGCCGA ATGCTGTTGG AGGCTATGCA ATTTCTATTT
5740  CCTTTTGGCC CCAGACTACA ACTACTCCCA CCTCTGTTGA TATGAATTCT ATTACCTCTA CTGATGTTAG
5810  GATTTTAGTT CAGCCTTGCA TAGCCTCCGA GTTGGTTATN NNNAGTGAGC GCCTCCATTA TCGCAACCAG
5880  GGCTGGCGTT CCGTTGAGAC CTCGGGTGTG GCTGAGGAGG AGGCGACATC TGGCCTGGTT ATGCTCTGTA
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6020  TCTCGAGCTC GAGTTTAGGA ACTTGACCCC TGGGAACACT AACACCCGTG TGTCCCGGTA CACAAGCACA
6090  GCCCGTCATC GGCTGCGCCG CGGTGCTGAT GGCACTGCCG AGCTCACCAC CACGGCGGCC ACGCGTTTCA
6160  TGAAGGACCT GCATTTCACC GGTACGAACG GGGTCGGTGA GGTGGGTCGT GGTATTGCTC TCACACTCTT
6230  TAATCTTGCT GATACGCTTC TTGGTGGTTT GCCGACAGAA TTAATTTCGT CGGCTGGGGG ACAGCTATTC
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6580  ACCCCATGTA TGTCTCTGAT ACAGTTACAT TTGTTAATGT GGCTACTGGT GCTCAGGCTG TGGCCCGCTC
6650  CCTTGATTGG TCTAAAGTTA CTCTGGATGG CCGCCCTCTC ACTATCACCC AGCAGTACTC TAAAATATTT
6720  TATGTTCTCC CGCTTCGAGG TAAGCTGTCC TTCTGGGAGG CCGGGACGAC TAAGGCCGGA TACCCCTATA
6790  ATTATAATAC AACTGCTAGT GACCAGATTC TGATAGAAAA TGCAGCCGGC CACCGTGTTG CTATATCCAC
6860  CTACACTACT AGCCTTGGTG CTGGTCCTGT GTCTGTTTCA GCGGTCGGTG TGCTAGCCCC GCACTCGGCC
6930  CTTGCTGTAC TTGAGGATAC TGTTGACTAC CCTGCCCGCG CTCATACTTT TGATGATTTT TGCCCGGAGT
7000  GCCGCACCCT TGGTCTACAG GGTTGTGCCT TTCAGTCCAC TATCGCTGAG CTTCAGCGTC TTAAAGTGAA
7070  GGTAGGCAAA ACCCGGGAGT CTTAATTAAT TCCATCTGTG CCCCCTTCAN AGGTCTTGGT TTATTTCTTT
7140  CTTCTGCGTT TCGCGCTCCC TGGAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
7210  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A
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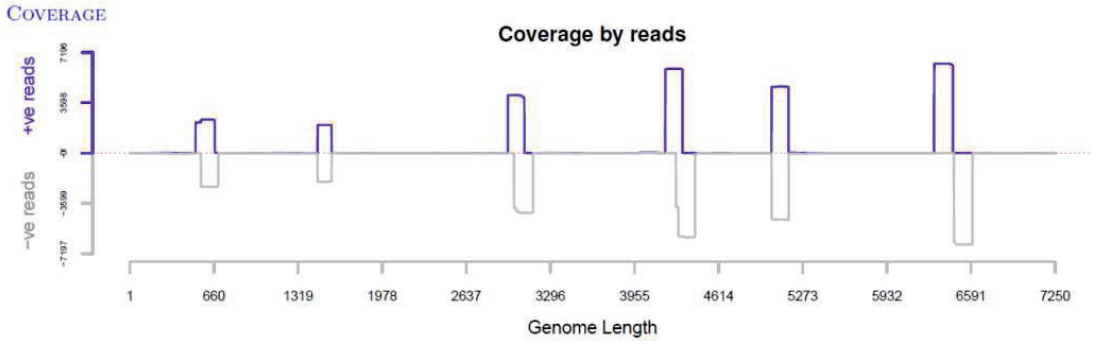
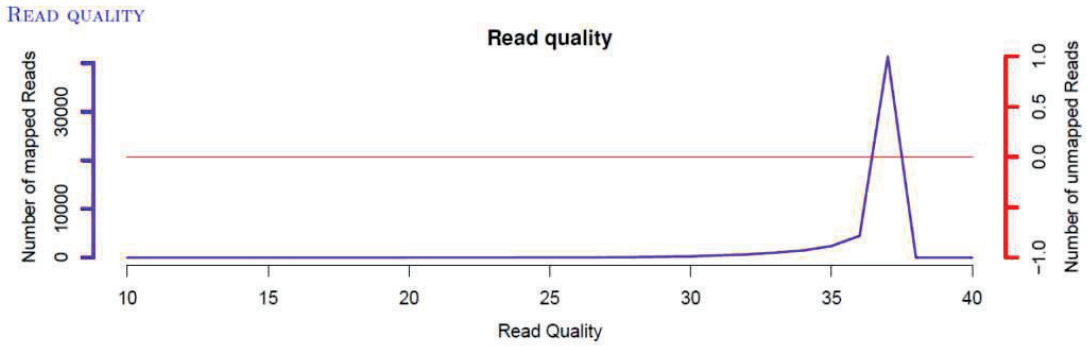
E2-PRE-IFN

File name	**E2-PRE-IFN.sam**
Ref name	**KT159771.1**
Ref length	**7 251nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**52 390**
Mapped reads	**52 390 (100.00%)**
Average read length	**146nt**
Coverage	**6 305nt (86.95%)**
Average depth	**991 reads/site**





E2-PRE-IFN Consensus sequence

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  70  GCCGTTTTTA TCTCGTGTGC AAACTGAGAT TCTCATTAAT TTGATGCAAC CCCGGCAGCT CGTTTTCCGA
 140  CCTGAAGTGC TCTGGAATCA CCCGATCCAG CGAGTTATAC ACAATGAGCT TGAGCAGTAC TGCCGTGCCC
 210  GCGCCGGTCG CTGCCTGGAG GTCGGGGCTC ACCCAAGATC CATTAATGAC AACCCTAACG TCCTGCACCG
 280  GTGTTTCCTT CGCCCGGTCG GGAGAGATGT ACAGCGTTGG TATTCCGCCC CGACTCGCGG CCCAGCTGCC
 350  AACTGCCGGC GTTCCGCATT ACGTGGCCTG CCCCCTGTCG ACCGTACTTA CTGTTTCGAC GGGTTCTCCC
 420  GCTGTGCTTT TGCTGCTGAG ACTGGAATTG CTTTGTATTC ACTACATGAC CTCTGGCCTG CCGATGTTGC
 490  GGAGGCCATG GCCCGACACG GGATGACACG CCTGTATGCC GCACTGCACC TTCCCCCTGA GGTGTTGTTA
 560  CCACCTGGCA CCTACCACAC AACCTCATAC CTCCTGATCC ATGATGGTAA CCGTGCCGTC GTGACTTATG
 630  AGGGTGATAC CAGTGCAGGC TATAACCATG ATGTTTCCAT ACTCCGTGCG TGGATCCGCA CAACTAAGAT
 700  AGTTGGCGAT CATCCGCTGG TGATAGAGCG TGTGCGGGCT ATTGGCTGCC ATTTTGTGTT GCTGCTTACT
 770  GCGGCCCCTG AGCCGTCCCC TATGCCCTAT GTCCCGTACC CCCGGTCGAC GGAGGTCTAT GTCCGGTCCA
 840  TATTCGGCCC CGGCGGCTCG CCATCTCTGT TCCCATCAGC NNNNNNNNNN NNNNNNNNAT TTCACGCCGT
 910  CCCGGTTCAT ATTTGGGATC GGCTCATGCT TTTTGGCGCC ACTCTGGATG ATCAGGCGTT TTGCTGTTCA
 980  CGGCTTATGA CCTACCTCCG CGGGATTAGT TACAAGGTCA CTGTTGGCGC CCTTGTCGCT AATGAGGGTT
1050  GGAACGCCTC AGAGGATGCG CTCACTGCTG TGATCACTGC AGCCTACTTG ACCATCTGTC ACCAGCGCTA
1120  CCTCCGTACC CAAGCTATAT CTAAGGGCAT GCGCCGACTG GAGGTTGAAC ATGCTCAGAA ATTCATCACA
1190  AGACTCTATA GTTGGTTGTT TGAGAAGTCT GGCCGTGACT ACATCCCCGG CCGTCAGCTC CAGTTTTATG
1260  CACAGTGCCG CCGTTGGCTG TCGGCAGGTT TTCATCTTGA CCCAAGAGTG CTTGTCTTCG ATGAGTCTGT
1330  ACCCTGCCGC TGCAGGACGT TTCTTAAGAA GGNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
1400  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN GAGGGCCTGG TTGGCGACCA TGGTCATGAT AATGAGGCCT
1470  ATGAGGGCTC TGAGGTCGAT CCAGCTGAAC CTGCACACCT CGATGTTTCG GGGACCTACG CCGTCAGTGG
1540  GCACCAGCTT GAGGCCCTCT ATAGGGCACT CAACATCCCG CATGATATTG CCGCCCGAGC NNNNNNNNNN
1610  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNTGCCGTAC AGTGCTCGGA AATAAGACCT
1680  TCCGCACATC GATAATGGAT GGCGCTCACC TCGAGGCTAA CGGCCCTGAG CAGTATGTTT TAACATTTGA
1750  CGACTCCCGC CAAGCGATGG GGGCTGGGTC GCATAGTCTN NNNNNNNAGC TCACTCCTGC TGGTTTGCAG
1820  GTCAAGATTT CATCTAATGG CCTGGATTGC ACTGCAACAT TCCCTCCGGG TGGGGCCCCT AGCGCTGCTC
1890  CGGGGGAGGT GGCAGCCTTT TGCAGTGCCC TCTACAGGTA CAACAGGTTC ACTCAGCGGC ACTCGCTTAC
1960  AGGTGGCTTG TGGCTACATC CTGAGGGGTT ATTGGGCATT TTCCCCCCTT TTNCTCCCGG GCATCTTTGG
2030  GAGTCCGCGA ATCCTTTTTG CGGGGAGGGA ACCTTGTACN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2100  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2170  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2240  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2310  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNACCTATCC GGATGGGGCA AAGGTGTATG CGGGGTCACT
2380  GTTTGAGTCT GACTGTGATT GGCTGGTTAA TGCGTCGAAT CCTGGCCATC GTCCTGGCGG CGGCCTTTGC
2450  CATGCCTTCT ACCAACGCTA CCCCGAGTCT TTNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2520  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2590  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNC GGTACCGCCG CCTATCCACT CCTCGGCTCG
2660  GGTATATACC AAGTTCCCGT CAGCCTCAGC TTTGACGCTT GGGAGCGTAA CCATCGCCCC GGAGACGAGC
2730  TTTACCTAAC CGACCTCGCA GCTACCTGGT TCGAGGCTAA CAAACCAACA CAGCCGGCCC TCACAATAAC
2800  TGAGGACACA GCCCGTACGG CTAACCTAGC ACTAGAGATC GATGCTGCCA CGGAGGTTGG CAGGGCTTGT
2870  GCCGGCTGTA CAGTTAGACC TGGGGTTGTG CATTATCAAT TCACTGCCGG GGTGCCAGGT TCGGGGAAGT
2940  CGCGGTCTAT ACAGCAGGGG GATGTTGATG TGGTGGTTGT CCCCACCCGG GAGCTTCGCA ACAGCTGGCG
3010  CCGCCGGGGG TTTGCGGCGT TTACACCTCA CACAGCCGCC CGTGTCACTA TCGGCCGCCG CGTTGTGATC
3080  GACGAGGCCC CGGCACTCCC GCCGCACTTG CTGCTGCTGC ATATGCAGCG GGCCTCTTCG GTCCATCTTC
3150  TTGGTGACCC AAATCAGATT CCTGCCCTAG ACTTCGAGCA CGCCGGCCTC GTTCCCGCAA TACGCCCCGA
3220  GCTTGCGCCA ACCAGCTGGT GGCATGTCAC CCACCGCTGC CCCGCTGATG TGTGCGAGCT TATTCGCGGG
3290  GCTTATCCCA AGATCCAAAC CACCAGCCGT GTGCTGCGGT CTTTATTCTG GAATGAGCCT GCCATTGGCC
3360  AGAAGTTAGT TTTCACTCAG GCCGCTAAGG CCGCTAACCC CGGTGCGATT ACAGTCCATG AGGCTCAGGG
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3500  CACGCCATAG TTGCACTTAC CCGCCACACA GAAAAATGCG TCATTCTTGA CGCCCCTGGC CTGTTACGTG
3570  AGGTTGGTAT CTCGGATGTG ATTGTTAACA ATTTTTTCCT TGCCGGCGGG GAGGTGGGCC ACCATCGCCC
3640  CTCCGTGATA CCCCGCGGTA ATCCTGACCA GAACCTCGCG ACACTTCAGG CCTTCCCGCC CTCTTGCCAG
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E2-PRE-IFN Consensus sequence

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3710  ATTAGTGCCT ATCACCAGTT AGCTGAGNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3780  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3850  NNNNNNNNNC ACGGACATAG TCCACTGCCG GATGGCCGCC CCTAGCCAGC GGAAGGCCGT CCTATCGACG
3920  CTTGTGGGCA GGTACGGCCG CCGGACTAAG CTGTACGAAG CAGCCCACTC TGACGTCCGC GAGTCCCTGG
3990  CTAGATTCAT CCCTACCATC GGACCAGTTC AGGCCACCAC ATGTGAGTTG TACGAGTTGG TAGAGGCTAT
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6930  CTTGCAGTTC TTGAGGATAC TATTGATTAC CCCGCCCGCG CCCACNNNNN NNNNNNNNNN NNNNNNNNNN
7000  NNNNNNNNNN NNNNNNNCAG GGCTGTGCTT TTCAATCCAC TATCGCTGAG CTTCAGCGTC TTAAAGTGAA
7070  GGTAGGCAAA ACCCGGGAGT CTTAATTAAT TCCATCTGTG CCCCCTTCAN AGGTCTTGGT TTATTTCTTT
7140  CTTCTGCGTT TCGCGCTCCC TGGAAACAAA AAAAAAAAAA AAGCAAAAAA AAAAAAAAAA AAAAACATAT
7210  CAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A
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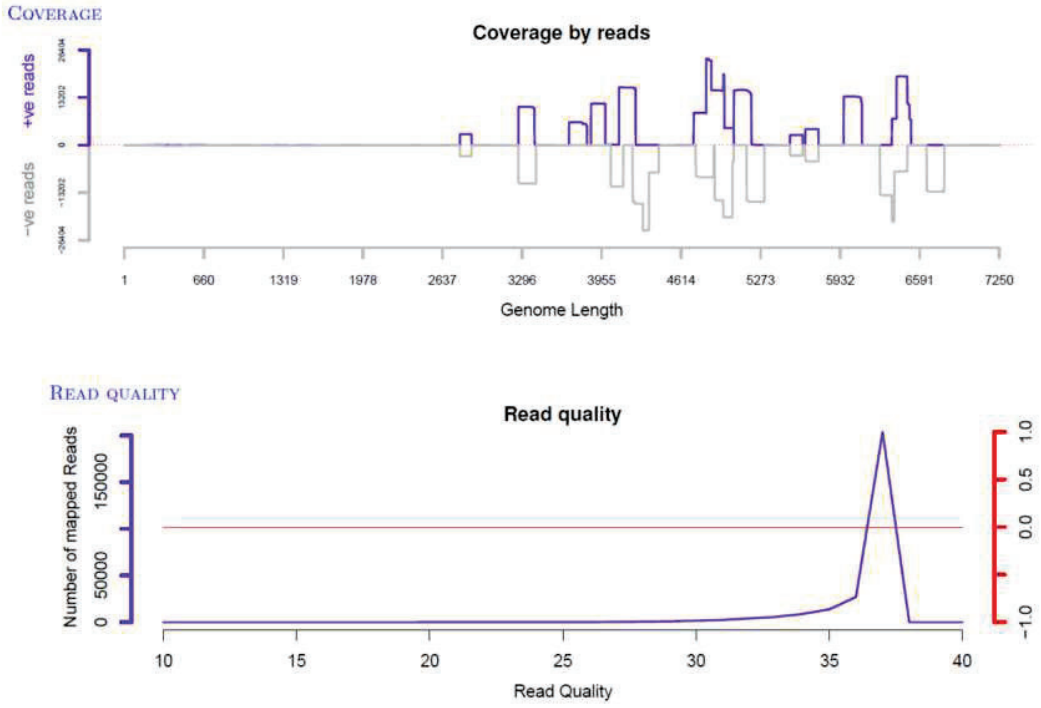
E3-PRE-IFN

File name	**E3-PRE-IFN.sam**
Ref name	**KT159771.1**
Ref length	**7 251nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**268 985**
Mapped reads	**268 985 (100.00%)**
Average read length	**145nt**
Coverage	**7 186nt (99.10%)**
Average depth	**4 901 reads/site**



E3-PRE-IFN Consensus sequence

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  70  GCCGTTTTTG TCCCGTGTTC AGACTGACAT TCTCATCAAT TTGATGCAAC CCCGGCAGCT GGTTTTCCGA
 140  CCTGAAGTGC TCTGGAATCA TCCTATCCAA CGGGTTATAC ACAATGAGCT TGAGCAGTAC TGCCGTGCCC
 210  GCGCCGGTCG CTGCCTGGAG GTCGGGGCTC ATCCGAGATC CATTAATGAC AACCCTAACG TCCTGCACCG
 280  GTGTTTCCTT CGCCCGGTCG GGAGAGATGT ACAGCGTTGG TATTCCGCCC CGACTCGCGG CCCAGCTGCC
 350  AACTGCCGGC GTTCCGCATT ACGTGGCCTG CCCCCTGTCG ACCGTACTTA CTGTTTCGAC GGGTTCTCCC
 420  GCTGTGCTTT TGCTGCTGAG ACTGGAATTG CTTTGTATTC ACTACATGAC CTCTGGCCTG CCGATGTTGC
 490  GGAGGCCATG GCCCGACACG GGATGACACG CCTATATGCT GTACTACATC TTCCCCCTGA AGTACTACTA
 560  CCACCTGGTA CCTACCACAC AACCTCATAC CTTCTGATTC ACGACGGTGA TCGTGCTGTT GTGACCTATG
 630  AAGGTGATAC TAGTGCAGGT TACAACCATG ATGTTTCCAT ACTTCGCGCA TGGATCCGCA CAACTAAGAT
 700  AGTTGGCGAT CATCCGCTGG TGATAGAGCG TGTGCGGGCT ATTGGCTGCC ATTTTGTGTT GCTGCTTACT
 770  GCAGCCCCTG AGCCGTCACC AATGCCTTAT GTCCCATACC CCCGGTCGAC AGAGGTGTAT GTCCGGTCTA
 840  TATTTGGCCC CGGCGGCTCG CCATCTCTGT TCCCGTCAGC CTGCTCTACT AAATCCACAT TTCACGCCGT
 910  CCCGGTTCAT ATTTGGGATC GGCTCATGCT TTTTGGCGCC ACTCTGGATG ATCAGGCGTT TTGCTGTTCA
 980  CGGCTTATGA CCTACCTCCG CGGGATTAGT TACAAGGTCA CTGTTGGCGC CCTTGTCGCT AATGAGGGTT
1050  GGAACGCCTC AGAGGATGCG CTCACTGCTG TGATCACTGC AGCCTACTTG ACCATCTGTC ACCAGCGCTA
1120  CCTCCGTACC CAAGCTATAT CTAAGGGCAT GCGCCGACTG GAGGTTGAAC ATGCTCAGAA ATTCATCACA
1190  AGACTCTATA GTTGGTTGTT TGAGAAGTCC GGTCGAGATT ATATCCCCGG CCGTCAGCTC CAGTTTTATG
1260  CACAGTGCCG CCGTTGGCTG TCGGCAGGTT TTCATCTTGA CCCAAGAGTG CTTGTCTTCG ATGAGTCTGT
1330  ACCCTGCCGC TGCAGGACGT TTCTTAAGAA GGCTGCCGGC AAGTTCTGCT GCTTTATGAA GTGGCTGGGA
1400  CAGGAGTGCA CTTGCTTTCT GGAGCCGGCC GAGGGCCTGG TTGGCGACCA TGGTCATGAT AATGAGGCCT
1470  ATGAGGGCTC TGAGGTCGAC CAGGCTGAGC CCGCCCACCT AGATGTTTCT GGGACTTATG CTGTCCATGG
1540  GCGCCAGCTT GAGGCCTTGT ACAGGGCGCT CAACATCCCA CATGACATTG CGGCCCGNNN NNNNCGTTTG
1610  ACTGCCACCG TCGAGCTCGT TGCAGGTCCA GACCGCTTAG AGTGCCGCAC TGTGCTTGGG AATAAGACTT
1680  TCCGGACGAC GGTGGTTGAT GGCGCCCATC TTGAGGCGAA CGGCCCCGAG CAGTACGTCC TTTCGTTTGA
1750  CGCCTCCCGC CAGTCTATGG GGGCTGGGCC GCATAGTCTT ACCTATGAGC TCACTCCTGC TGGTTTGCAG
1820  GTCAAGATTT CATCTAATGG CCTGGATTGC ACTGCAACAT TCCCTCCGGG TGGGGCCCCT AGCGCTGCTC
1890  CGGGGGAGGT GGCAGCCTTT TGCAGTGCCC TCTACAGGTA CAACAGGTTC ACTCAGCGAC ACTCGCTTAC
1960  AGGCGGCTTG TGGCTGCACC CTGAGGGGCT GCTGGGCATC TTCCCCCCCT TTTCCCCCGG GCATCTCTGG
2030  GAGTCCGCAA ATCCTTTTTG CGGTGAGGGC ACACTTTACA CCCGTACTTG GTCTACGTCT GGCTTTTCTA
2100  GTGATTTCTC TCCCCCTGNN NCAGCCGTTG TAGCGCCGGC TGCTACTCCG GGGTTACGCT ACCCTACACC
2170  TCCTGTTAGT GACATTTGGG TGTTACCGCC ACCTTCTGAA GAATTTCAGG TTGACACAGC GCCCGCTCCC
2240  CCTGCCCCTG GGCCCGCTCA ACCATCTAGC CCTGTTGGGC CGAAGGCTCC CGTGCGTAAG CCGCCAACGC
2310  CACCATCCCC GCGCACCCGC CGCCTTCTTT ACACCTATCC GGATGGGGCA AAGGTGTATG CGGGGTCACT
2380  GTTTGAGTCT GACTGTGATT GGCTGGTTAA TGCGTCAAAT CCTGGCCATC GTCCTGGCGG CGGCCTTTGC
2450  CATGCCTTCT ATCAACGCTA CCCCGAGTCT TTCTATCCGA CTGATTTCAT TATGCGCGAG GGCCTTGCCG
2520  CCTACACNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNAACCCAAA
2590  GAGGCTTGAG GCAGCATATC GAGAGACTTG TTCTCGCCGC GGTACCGCCG CCTATCCACT CCTCGGCTCG
2660  GGTATATACC AAGTTCCCGT CAGCCTCAGC TTTGACGCTT GGGAGCGTAA CCATCGCCCC GGAGACGAGC
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2800  GGAAGATACG GCCCGTACGG CCAACCTAGC GTTAGAGATC GATGCCGCTA CAGAGGTTGG CCGTGCCTGT
2870  GCCGGCTGTA CAGTTAGACC TGGGGTTGTG CATTATCAAT TCACTGCTGG GGTCCCAGGT TCGGGGAAGT
2940  CGCGGTCTAT ACAGCAGGGG GATGTCGATG TTGTGGTTGT TCCTACCCGG GAGCTCCGGA ATAGTTGGCG
3010  CCGCCGGGGT TTTGCAGCTT TTACACCTCA TACGGCGGCC CGCGTCACTG CCGGCCGACG TGTTGTGATT
3080  GATGAGGCCC CTTCGCTTCC ACCACATTTG CTGCTGCTTC ACATGCAGCG AGCTTCGTCA GTCCACCTTC
3150  TCGGCGACCC CAACCAGATT CCCGCCATAG ACTTCGAGCA TGCCGGCCTC GTTCCCGCAA TACGCCCTGA
3220  GCTTGCCCCA ACCAGTTGGT GGCATGTCAC CCACCGCTGC CCCGCTGATG TATGCGAGCT CATACGCGGA
3290  GCCTATCCTA AAATCCAGAC CACGAGCCGT GTGCTACGAT CTCTGTTTTG GAATGAACCG GCTACCGGCC
3360  AGAAGTTGGT TTTTACGCAG GCTGCTAAGG CTGCTAACCC TGGTGCAATC ACAGTCCATG AGGCTCAGGG
3430  CGCCACTTTC ACGGAAACTA CAATTATAGC CACAGCTGAT GCTAGGGGAC TTATCCAATC TTCTCGGGCT
3500  CATGCCATAG TTGCACTCAC CCGCCACACA GAAAAATGCG TCATTCTTGA CGCCCCTGGC CTGTTACGTG
3570  AGGTTGGTAT CTCGGATACA ATTGTTAACA ATTTTTTCCT CGCTGGTGGG GAGGTGGGCC ACCATCGCCC
3640  CTCCGTGATA CCCCGAGGTA ATCCTGACCA GAACCTCGCG ACACTACAGG CCTTCCCGCC GTCTTGCCAG
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E3-PRE-IFN Consensus sequence

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3850  CTTCGAGCTC ACGGACATAG TCCATTGCCG CATGGCCGCT CCAAGCCAGC GGAAGGCTAT CCTCTCAACA
3920  CTCGTTGGGA GGTACGGCCG CAGGGCGAAA TTATATGAGG CAGCACATTC TGATGTCCGT GAGTCCCTGG
3990  CCAGGTTTAT CCCTACCATC GGACCAGTTC AGGCCACCAC ATGTGAGTTA TATGAGCTGG TAGAGGCCAT
4060  GGTGGAGAAG GGCCAGGACG GCTCCGCCAT CCTGGAGCTT GACCTCTGCA ACCGCGACGT CTCGCGTATC
4130  ACATTCTTTC AAAAGGATTG TAATAAGTTT ACAACTGGTG AAACTATCGC CCATGGCAAG GTTGGTCAGG
4200  GTATATCGGC CTGGAGTAAG ACTTTTTGCG CCCTGTTCGG CCCATGGTTC CGAGCCATCG AAAAAGAAAT
4270  ATTGGCCTTG CTCCCACCTA ACATCTTTTA CGGCGATGCC TATGAGGAGT CAGTGTTTGC TGCCGCCGTG
4340  TCCGGGGCGG GGTCTTGCAT GGTGTTTGAG AATGATTTTT CAGAGTTTGA CAGTACTCAG AATAATTTCT
4410  CTCTTGGCCT TGAGTGTGTT ATTATGGAGG AGTGTGGCAT GCCCCAGTGG CTAATACGGT TGTACCACTT
4480  AGTTCGGTCG GCCTGGATAC TACAGGCGCC GAAGGAGTCT CTTAAAGGAT TCTGGAAGAA GCACTCTGGT
4550  GAGCCCGGCA CCCTCCTCTG GAACACCGTC TGGAACATGG CGATCATAGC GCACTGCTAT GAATTCCGTG
4620  ATCTTAGGGT TGCCGCCTTC AAGGGAGATG ACTCCGTAGT CCTCTGTAGC GACTACCGCC AAAGCCGCAA
4690  TGCGGCTGCC CTAATTGCGG GCTGCGGGCT CAAACTGAAG GTGGACTATC GCCCTATTGG GCTGTATGCT
4760  GGTGTGGTGG TTGCCCCTGG TCTGGGGACG TTGCCCGATG TGGTACGTTT TGCTGGCCGG CTGTCTGAAA
4830  AGAATTGGGG CCCCGGCCCT GAGCGAGTTG AGCAGCTGCG TCTTGCTGTT TGTGATTTTC TTCGAGGGTT
4900  AACGAATGTT GCGCAGGTCT GTGTTGACGT TGTATCTCGT GTCTATGGAG TTAGCCCTGG GCTGGTACAT
4970  AACCTTATAG GCATGCTGCA GACTATCGCC GATGGCAAGG CCCATTTCAC AGAGACTATT AAACCTGTGC
5040  TTGACCTCAC AAATTCAATC ATACAACGGA CGGAATGAAT AACATGTTTT GTGCATCGCC CATGGGTTCA
5110  CCATGCGCCC TAGGGCTGTT CTGCTGCTGT TCTTCGTGTT TCTGCCTATG CTGCCCGCGC CACCGGCCGG
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5740  CTTTTTGGCC TCAGACTACA ACTACTCCCA CCTCTGTTGA TATGAATTCT ATTACCTCTA CTGATGTTAG
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5880  GGCTGGCGTT CTGTTGAGAC CTCGGGTGTG GCTGAGGAGG AGGCTACATC GGGCTTGGTT ATGCTCTGTA
5950  TCCATGGTTC CCCTGTTAAC TCTTACACTA ATACGCCCTA CACTGGTGCA CTGGGACTCC TTGATTTTGC
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6090  GCCCGCCACC GCCTGCGCCG CGGTGCCGAT GGGACTGCCG AGCTCACGAC CACTGCGGCC ACGCGCTTCA
6160  TGAAGGACCT GCATTTCACC GGGACGAATG GGGTCGGCGA GGTGGGCCGT GGCATTGCCC TCACATTCTT
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6790  ATTATAATAC AACTGCTAGT GATCAGATTC TGATTGAAAA TGCAGCCGGT CATCGTGTTG CTATTTCCAC
6860  CTATACTACC AGCCTGGGCG CTGGTCCTGT GTCAGTCTCC GCAGTCGGTG TGCTTGCCCC ACATTCGGCT
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7070  GGTAGGCAAA ACCCGGGAGT CTTAATTAAT TCCATCTGTG CCCCCTTCAT AGGTCTTGGT TTATTTCTTT
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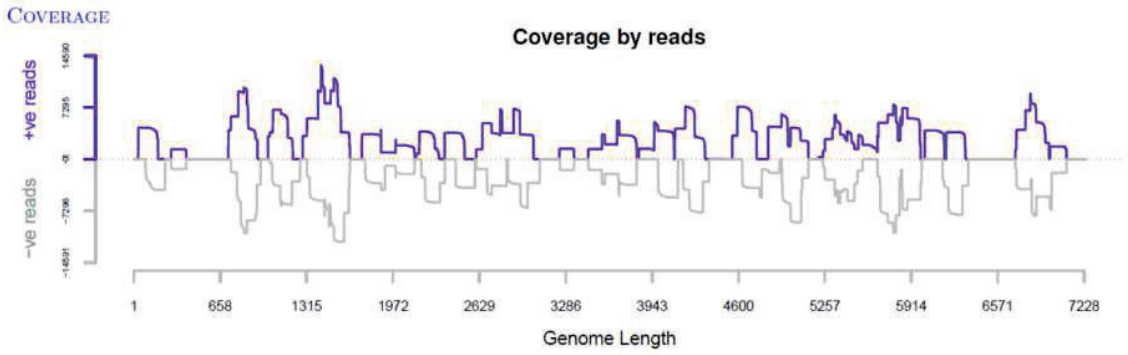
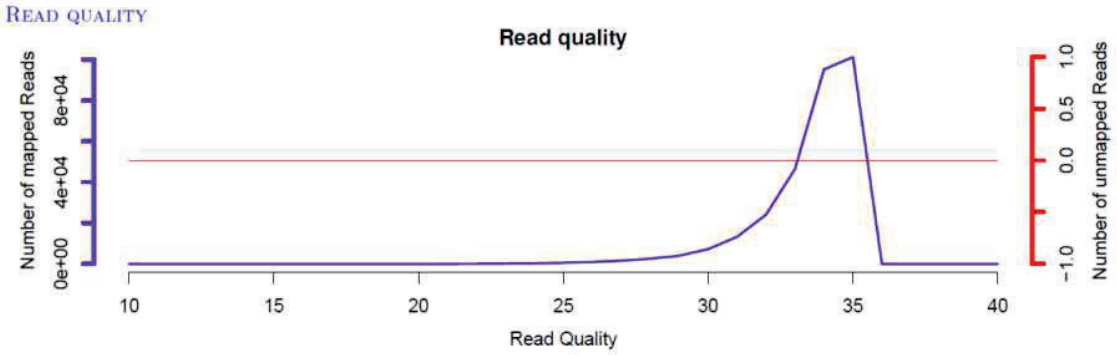
F1-PRE-RBV

File name	**F1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**297 130**
Mapped reads	**297 130 (100.00%)**
Average read length	**140nt**
Coverage	**6 452nt (89.15%)**
Average depth	**5 710 reads/site**





F1-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAACT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATCAATGACA ACCCGAATGT TCTGCACCGG TGCTTTTTAC
 350  GGCCAGTTGG GAGAGATGTT CAGCGCTGGT ATTCTGCTCC TACCCACGGC CCCGCGGCTA ACTGCCGCCG
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 560  CCCGGCACGG GATGACACGC TTATATGCTG CACTACATCT CCCCCCTGAA GTAATACTAC CACCTGGTAC
 630  ATACCACACA ACTTCATACC TTCTCATCCA CGACGGTGAT CGTGCCGTTG TGACTTACGA GGGTGACACT
 700  AGTGCAGGTT ACAACNNNNN TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACCAAGATA GTCGGCGACC
 770  ATCCGCTGGT CATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTTACCG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGTTCTACGA AATCTACATT TCACGNNNNN NNNNNNNNNN
 980  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NCAGGCGTTT TGCTGCTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATCTGA CCATCTGCCA TCAGCGTTAC CTCCGCACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCNNNNN NNNNNNNNNN NNNNNGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGCTGGTTAT CGGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGTTG TTTTATGAAG TGGTTGGGAC AGGAGTGTAC
1470  CTGTTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACTTATGC TGTCCATGGC CGCCAGCTTG
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1680  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGTGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGACTGCA CCGCAACATT TCCCCCGGGC GGGGCCCCTA GCGCCGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TCACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCATCC TGAGGGGTTA TTGGGTATTT TCCCCCCTTT CTCCCCCGGG CACCTTTGGG AGTCTGCCAA
2100  CCCTTTTTGT GGAGAGGGAA CCTTGTACAC CCGGACATNN NNNNNNNCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAAG CAGCCGTTGC AGCGCCGGCT GCTACTCCGG GGTTACGCCA CCCCACACCT CCTGTTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT CGATACAGCA CCCGTTCCCC CCGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCC CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ATCACCCCCG
2380  CGCACCCGCC GCCTCCTTTA TACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGCGGT GGCCTCTGCC ATGCCTTTTA
2520  CCAGCGTTAT CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTACA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GCATATACCA
2730  AGTTCCCATC AGCCTCAGCT TTGACGCTTG GGGGCGTAAC CACCGCCCCG GGGACGAGCT CTACTTAACC
2800  GATCTCGCCG CTACCTGGTT TGAGGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAAGTTGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGAG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TCGCAGCNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3150  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3220  NNNNNNNNNN NNNNNNTAGA TTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CATCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGCGGGG CTTACCCCAA
3360  AATTCAAACC ACTAGCCGCG TGCTGCGGTC TTTATTCTGG AATGAGCCCG CCATTGGCCA GAAGTTAGTC
3430  TTCACACAGG CCGCTAAGGC TGCCAACCCC GGTGCGATCA CAGTCCACGA GGCTCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCT ACAGCTGATG CCAGGGGGCT CATCCAATCC TCCCGAGCTC ATGCCATAGT
3570  TGCACTTACC CGCCATACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCTTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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F1-PRE-RBV Consensus sequence

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3710  CCCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCACCT TCCTGCCAGA TTAGCGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGTCACCG CCCAGCTCCC GTCGCTGCTG TTTTGCCCCC CTGCCCTGAA
3850  CTTGAGCAAG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGTCGG ATGGCTGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
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4200  GAAAGATTGC AACAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCA
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5110  AACTCTATCA TACAGCGGGT GNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
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5390  GCTCGCCCTC GACAGCCACC CCGTCCCCTC GGCTCCTCTT GGCGTGACCA GTCCCAGCGC CCCTCCGCTG
5460  CCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCATA TCACCTGCTC CCGATACAGC
5530  CCCTGTGCCT GATGTTGATT CGCGTGGCGC CATATTGCGG CGCCAGTACA ATTTATCCAC ATCCCCGCTC
5600  ACATCATCTG TTGCTTCGGG CACTAATCTG GTTCTTTATG CTGCCCCGCT AAACCCCTTG CTGCCCCTTC
5670  AGGATGGTAC TAACACTCAC ATCATGGCTA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTTCGAGC
5740  TACGATCCGT TATAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCGA TATCCATCTC ATTTTGGCCT
5810  CAGACTACTA CCACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT TGCTTCTGAG TTGGTTATCC CCAGTGAGCG CCTCCATTAT CGTAACCAAG GTTGGCGCTC
5950  TGTAGAGACC TCGGGCGTGG CTGAGGAGGA GGCTACTTCT GGNNNNNNNN NNNNNNNNNN NNNNNNCTCC
6020  CCTGTTAATT CCTACACCAA TACCCCCTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCT TTAGAGCTTG
6090  AGTTTAGGAA TCTGACACCC GGGAATACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GTTGCGCCGC GGTGCTGACG GCACCGCCGA ACTTACCACC ACAGCGGCCA CGCGCTTCAT GAAGGACTTG
6230  CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCTCT CACACTTTTT AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGNNN NNNNNNNNNN NNNNNNNNNN
6370  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6440  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6510  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNCG CCCTTTTTCG GTTCTTCGTG CTAATGATGT
6580  TTTATGGCTT TCCCTTACCG CTGCCGAGTA TGATCAGACT ACATATGGGT CGTCCACCAA CCCAATGTAT
6650  GTCTCAGATA CTGTTACATT TGTCAATGTG GCTACAGGAG CCCAGGCTGT TGCCCGTTCC CTCGACTGGT
6720  CTAAGGTTAC TCTGGATGGC CGCCCCCTTA CTACCATCCA GCAGTGCTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAATTATCTT TCTGGGAGGC CGGGACGACT AAGGCCGGCT ATCCCTATAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TACACCACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CGGTTGGTGT TTTGGCCCCA CATTCGGCCC TTGCAGTCCT
7000  TGAAGACACT ATTGATTACC CTGCCCGTGC CCACACTTTC GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGCTTGCAGG GGTGCGCCTT CCAGTNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7140  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
7210  NNNNNNNNNN NNNNNNNNNN NNNNNNN
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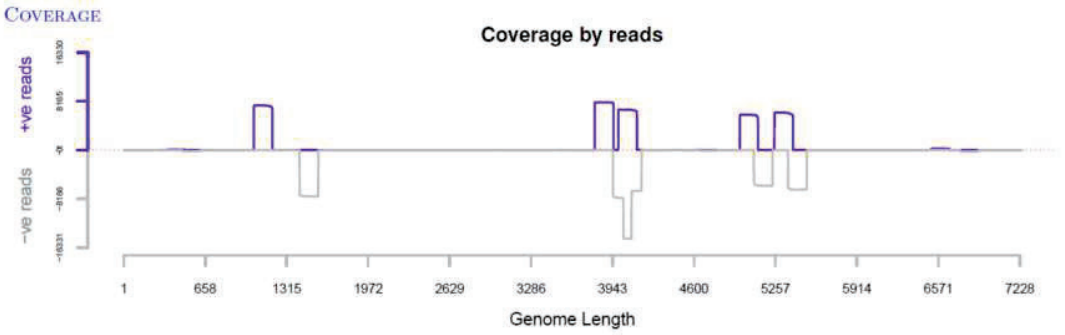
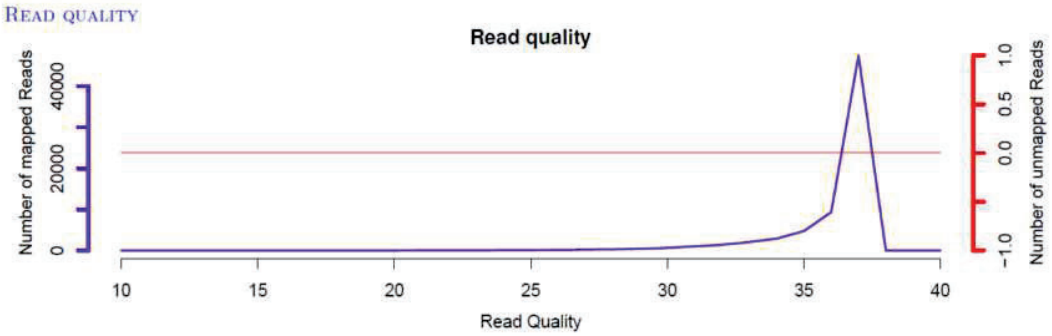
F2-PRE-RBV

File name	**F2-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref lenth	**7237**
Program used	**Tanoti Assembler 1.0**
Total reads	**71013**
Mapped reads	**71013 (100.00%)**
Average read length	**149nt**
Coverage	**6776nt (93.63%)**
Average depth	**1461 reads/site**





F2-PRE-RBV Consensus sequence

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   1  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
  70  NNNNNNNNNG CTGCTCTGGC TGCGGCTAAC TCCGCCTTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTGT
 140  CCCGTGTTCA GACTGACATT CTCATCAATT TGATGCAACC CCGGCAGCTC GTTTTCCGAC CTGAAGTTTT
 210  GTGGAATCAC CCGATCCAGC GAGTTATACA CAATGAGCTT GAGCAGTACT GCCGTGCCCG CGCCGGTCGC
 280  TGCCTGGAGG TCGGGGCTCA TCCGAGATCC ATTAATGACA ACCCTAACGT CCTGCACCGG TGTTTCCTTC
 350  GCCCGGTCGG GAGAGATGTT CAGCGCTGGT ATTCTGCTCC TACTCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCTTTG CGTGGTCTCC CCCCCGCTGA TCGTACCTAT TGTTTTGATG GGTTCTCCCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC CCTCTATTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCCGAA GTACTACTAC CACCTGGTAC
 630  CTACCACACA ACCTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGTT ACAACCATGA TGTTTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATATCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCTGCC TGCTCTACGA AATCTACATT TCACGCTGTC CCGGTTCATA
 980  TCTGGGACCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGCTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ATAAGGTCAC TGTTGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATCTGA CCATCTGCCA TCAGCGTTAC CTCCGCACCC
1190  AAGCTATATC CAAGGGCATG CGCCGACTGG AGGTTGAACA TGCTCAGAAA TTCATCACAA GACTCTATAG
1260  TTGGTTGTTT GAGAAGTCCG GCCGCGACTA CATCCCCGGC CGTCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGCTGT CGGCAGGTTT TCATCTTGAC CCAAGAGTGC TTGTCTTCGA TGAGTCTGTA CCCTGCCGCT
1400  GCAGGACGTT TCTTAAGAAG GCTGTGGGTA AGTTCTGTTG TTTTATGAAG TGGTTGGGAC AGGAGTGTAC
1470  CTGTTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACTTATGC TGTCCATGGG CGCCAGCTTG
1610  AGGCCTTGTA CAGGGCGCTC AACATCCCAC ATGACNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
1680  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
1750  NNNNNNGATG GCGCTCACCT CGAGTCTAAC GGCCCTGAGC AGTATGTTTT AACATTTGAC GACTCCCGCC
1820  AAGCGATGGG GGCTGGGCCG CATAGTCTTA CATATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT CTACAGGTAC AACAGGTTCA CTCAGCGCCA CTCGCTGACC GGCGGGCTGT
2030  GGCTGCACCC TGAGGGGCTG GTCGGCATTT TCCCCCCCTT TTCCCCCGGG CATGTCTGGG AGTCCGCAAA
2100  TCCTTTTTGC GGTGAGGGCA CCCTGTACAC CCGGACTTGG TCTACGTCTG GTTTTTCTAG TGACTTCTCC
2170  CCCCCTGAGG CAGCTNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2240  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCAAATC CTGGCCATCG TCCTGGCGGC GGCCTTTGCC ATGCCTTCTA
2520  CCAACGCTAC CCCGAGTCTT TCCATTCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTACA GGGTTGAGCA GAATCCAAAG AGGCNNNNNN
2660  NNNNNNNNCG AGATACTTGT TCTCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT CACAATAACT GAGGACACAG
2870  CCCGTACGGC TAACCTAGCA CTAGAGATCG ATGCTGCTAC GGAGGTGGGC CGTGCTTGTA CCGGCTGTAC
2940  TGTTAGCCCT GGCGTTGTCC ATTATCAGTT TACTGCCGGG GTGCCAGGTT CGGGGAAGTC GCGGTCTATA
3010  CAGCAGGGGG ATGTCGATGT TGTGGTTGTT CCTACCCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TTGCAGCTTT TACACCTCAT ACGGCGGCCC GCGTCACTGC CGGCCGACGT GTTGTGATTG ATGAGGCCCC
3150  TTCGCTTCCA CCACATTTGC TGCTGCTTCA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CATCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
3360  GATCCAAACC ACCAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTC
3430  TTTACGCAGG CTGCTAAGGC TGCTAACCCC GGTGCGATTA CAGTTCATGA GGCCCAGGGT GCCACTTTCA
3500  CTGAAACTAC AATCATAGCC ACAGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  TGCACTCACC CGCCACACAG AAAAATGCGT CATTCTTGAC GCCCCTGGCC TGTTACGTGA GGTTGGTATT
3640  TCTGATATAA TTGTTAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGCCA CCATCGCCCT TCCGTGATAC
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F2-PRE-RBV Consensus sequence

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3710  CCCGCGGTAA TCCTGACCAG AATCTCGCGA CACTTCAGGC CTTTCCACCT TCCTGCCAGA TTAGCGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGTCACCG CCCAGCTCCC GTCGCTGCTG TTTTGCCCCC CTGCCCTGAA
3850  CTTGAGCAAG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGTCGG ATGGCTGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT CGAGGCCATG GTGGAGAAGG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAAGGACTGT AATAAGTTTA CAACAGGTGA GACCATTGCC CACGGTAAGG TCGGTCAGGG CATTTCAGCC
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGC CCGTGGTTTC GTGCTATTGA AAAGGAAATA CTGGCCCTGC
4340  TCCCGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNGCAGG
4410  TTCTAGTATG GTTTTTGAGA ATGATTTCTC TGAGTTTGAC AGTACCCAAA ACAATTTCTC CCTCGGCCTT
4480  GAGTGTGTTA TTATGGAGGA GTGTGGCATG CCCCAGTGGC TAATACGGTT GTACCACTTA GTTCGGTCGG
4550  CCTGGATACT ACAGGCGCCG AAGGAGTCTC TTAAAGGATT CTGGAAGAAG CACTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTCT GGAATATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TCTTAGGGTC
4690  GCCGCTTTCA AGGGTGATGA TTCCGTGGTC CTTTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCT
4760  TGATTGCAGG TTGCGGGCTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCTCCTGGT TTGGGGACGC TACCCGATGT TGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACCGGGGC
4900  CCGAGTCCCG AGCGCGCCGA GCAGTTACGC TTGGCTGTTT GTGACTTCCT TCGAAAGTTA ACGAATGTTG
4970  CGCAGGTATG TGTCGATGTT GTATCCCGAG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCGTAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT GGAATGAATA ACATGTTTTG TGCATCGCCC ATGGGATCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCAGC CAGCCGTCTG
5250  GCCGTCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGACGTCG TACCGCAACC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGTCCCCTC GGCTCCTCTT GGCGTGACCA GTCCCAGCGC CCCTCCGCTG
5460  CCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCATA TCACCTGCTC CTGATACAGC
5530  CCCTGTGCCT GATGTTGATT CGCGCGGCGC TATACTGCGG CGACAGTATA ATCTATCTAC ATCCCCACTT
5600  ACATCATCTG TCGCTTCGGG CACTAATCTG GTTCTTTATG CTGCCCCGCT AAACCCCTTG CTGCCCCTTC
5670  AGGATGGTAC TAACACTCAC ATCATGGCTA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTTCGAGC
5740  TACGATCCGT TATAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCGA TATCCATCTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC CTCTGTTGAT ATGAATTCTA TTACCTCTAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT AGCCTCCGAG TTGGTTATTC CAAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTTGAGACC TCGGGTGTGG CCGAGGAGGA GGCGACCTCT GGTCTGGTGA TGCTCTGTAT CCATGGCTCC
6020  CCTGTAAACT CTTATACCAA CACGCCCTAN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
6090  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNA ACACCCGTGT GTCCCGGTAC ACCAGCACAG CCCGCCACCG
6160  CCTACGCCGT GGCGCCGATG GGACTGCCGA GCTCACGACC ACTGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CACTTCACCG GCACGAATGG CGTCGGCGAG GTGGGCCGTG GTATTGCTCT CACACTCTTT AATCTTGCTG
6300  ATACGCTTCT TGGCGGTTTG CCGACAGAAT TGATTTCGTC GGCCGGAGGT CAACTCTTTT ACTCCCGTCC
6370  AGTTGTCTCG GCCAATGGCG AGCCGACTGT TAAATTATAC ACCTCCGTCG AGAATGCACA GCAGGATAAA
6440  GGGATCGCTA TTCCACATGA CATAGATCTG GGAGATTCCC GTGTGGTTAT TCAGGATTAT GACAATCAAC
6510  ATGAGCAGGA CCGACCTACC CCCTCGCCTG CCCCCTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580  TCTATGGCTT TCTCTTACTG CCGCCGAGTA CGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTTACCCT CGTTAACGTG GCAACGGGAG CCCAGGCTGT CGCCCGCTCC CTTGATTGGT
6720  CTAAGGTCAC TCTGGATGGT CGCCCTCTTA CTACCATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAATTATCTT TCTGGGAGGC CGGGACGACT AAGGCCGGCT ATCCCTATAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTGCCACG TATACCACCA
6930  GCTTGGGCGC TGGCCCTGTG GCTGTTTCCG CAGTCGGCGT CCTTGCCCCC CATTCTGCTC TAGCTGTGCT
7000  TGAGGATACT ATTGATTACC CCGCCCGCGC CCACACTTTT GATGATTTCT GCCCTGAGTG CCGTAATCTC
7070  GGCCTACAGG GCTGTGCTTT TCAATCCACT ATCGCTGAGC TTCAGCGTCT TAAAGTGAAG GTAGGCAAAA
7140  CCCGGGAGTC TTAATTAATT CCATCTGTGC CCCCTTCAAG GTCTTGGTTT ATTTCTTNTC TTCTGCGTTT
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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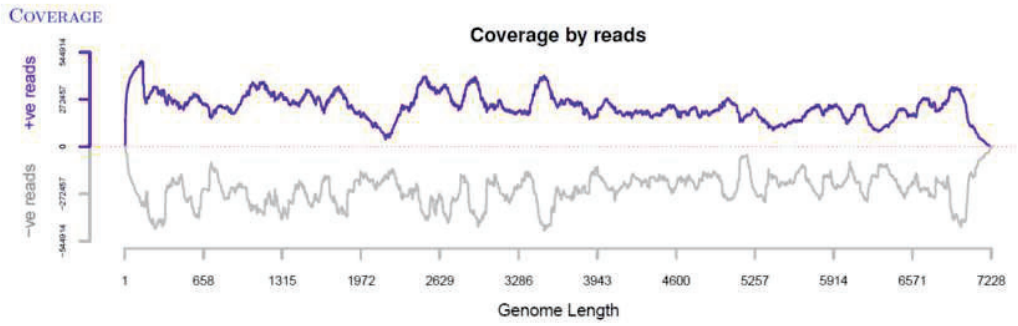
G1-PRE-RBV

File name	**G1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**25 373 167**
Mapped reads	**25 373 167 (100.00%)**
Average read length	**138nt**
Coverage	**7 225nt (99.83%)**
Average depth	**480 380 reads/site**



G1-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGC
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCT ATTAATGACA ATCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGTGGC CCCGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGTCTTC CCCCTGTTGA TCGCACCTAC TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCCCCTGAG GTACTATTAC CACCCGGTAC
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 700  AGTGCAGGCT ACAACCATGA TGTTTCCATA CTCCGTGCAT GGATCCGCAC AACTAAGATA ACCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTACG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCCACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTATCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCT CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTAA CCATTTGCCA TCAGCGTTAT CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTAG AGGTTGAGCA CGCCCAAAAA TTTATTACAA GACTCTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGTTT TCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGGC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAGGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC TGTCCACGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCTCGTCTGA CCGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGTCTGGA GTGCCGCACT GTGCTCGGGA ACAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCCATGGG GGCTGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCC GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCTCCTA GCGCAGCTCC GGGGGAGGTA
1960  GCAGCCTTCT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA CTCGCTTATA GGCGGCCTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCCCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCGTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCCG GTTTTTCTAG TGACTTTTCC
2170  CCCCCCGAGG CAGCCGTCGC AACGCCGGCT GCTGCCCCGG AGCTACGCCA CCCTACACCC CCTGTTAGTG
2240  ACATTTGGGT GCTACCGCCA CCTTCTGAAG AATTTCAGGT TGATACAGCG CTCGCTACCC CTGCTCCTGA
2310  GCCCGCTCAG CCATCCAGCC CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAATGCC ACCACCCCCG
2380  CGCACCCGCC GACTTCTTTA CACCTACCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGAGGC GGCCTTTGCC ATGCCTTCTA
2520  TCAACGCTAC CCTGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTG
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGACTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GCATATACCA
2730  AGTTCCTGTT AGCCTCAGCT TTGACGCATG GGAGCGTAAC CACCGCCCCG GGGATGAGCT CTACCTAACC
2800  GACCTCGCCG CTACCTGGTT CGAGGCTAAT AAGCCAGCAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGTACAGC CAACCTAGCA CTAGAGATCG ATGCTGCTAC GGAGGTTGGC CGGGCTTGTG CCGGCTGCGT
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC ACGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCGGCTTT TACACCTCAT ACGGCGGCCC GCGTTACCAC GGGCCGCCGT GTTGTGATTG ACGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCCATAGA TTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAA CTTGCGCCCA
3290  CCAGTTGGTG GCATGTTACC CATCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGCGGGG CTTATCCTAA
3360  AATTCAAACT ACTAGCCGCG TACTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC TGCCAATCCC GGTGCGATTA CAGTCCACGA AGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCCC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAT GCCCCCGGCT TGTTACGTGA GGTCGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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G1-PRE-RBV Consensus sequence

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3710  CTCGCGGCAA TCCTGACCAG AACCTCGCAA CACTACAGGC CTTTCCACCT TCCTGCCAGA TTAGTGCCTA
3780  TCACCAGCTG GCTGAGGAAC TTGGCCACCG CCCAGCACCC GTCGCTGCTG TCTTACCTCC CTGCCCTGAA
3850  CTTGAGCAGG GCTTGCTATA TATGCCCCAG GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCCC CCAGCCAGCG GAAGGCCGTC CTGTCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTCATC
4060  CCCACCATTG GGCCCGTTCG GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGCATCA CATTTTTCCA
4200  GAAAGATTGT AACAAATTCA CCACAGGGGA GACCATTGCC CATGGTAAGG TCGGCCAGGG CATCTCGGCT
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTCGGT CCGTGGTTCC GTGCTATTGA GAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA CATCTTCTAC GGTGACGCAT ATGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA ATGATTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGTCTC
4480  GAGTGTGTAG TTATGGAAGA GTGTGGCATG CCCCAGTGGC TAATCCGGCT GTACCATTTG GTCCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGCG AGCCCGGTAC
4620  CCTCCTCTGG AACACCGTTT GGAACATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGGGATGA TTCCGTGGTC CTCTGTAGTG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAGCTGAAGG TTGATTATCG CCCCATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCTGGT CTGGGGACGC TACCCGATGT GGTGCGCTTC GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCTGGGCCGG AGCGAGCTGA GCAGTTGCGC TTAGCTGTTT GTGACTTTCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTTGATGTT GTATCTCGAG TTTACGGAGT TAGCCCTGGG CTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC CCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT GGAATGAATA ACATGTCTTG TGCATCGCCC ATGGGATCAC CATGCGCCCT
5180  AGGGCTGTTT TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTA CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCA CACCGCAATC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTT GGCTCCTCTT GGCGCGATCA GTCCCAGCGC CCCCCCGCTG
5460  TCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCCGTA TCACCCGCTC CCGATACAGC
5530  TCCTGTACCT GATGTCGACT CGCGTGGCGC TATATTGCGA CGCCAGTACA ATTTATCCAC ATCCCCGCTC
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG GTTCTTTATG CTGCCCCGCT AAACCCTTTG CTGCCCCTTC
5670  AGGATGGTAC TAACACTCAT ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATCCGT TATAGGCCAT TGGTACCAAA TGCTGTCGGC GGCTATGCAA TATCCATCTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTCTAGTTC
5880  AGCCCGGTAT TGCTTCTGAG TTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAAGAGGA GGCCACTTCT GGTCTGGTAA TGCTTTGTAT CCATGGTTCT
6020  CCTGTCAATT CCTACACCAA TACCCCCTAT ACCGGGGCGC TTGGACTCCT TGACTTCGCT TTAGAGCTCG
6090  AGTTTAGGAA CCTGACACCC GGGAACACTA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GCTGCGCCGC GGTGCCGACG GCACCGCCGA ACTTACCACC ACAGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CACTTCACCG GTACGAATGG TGTCGGTGAG GTGGGTCGTG GTATTGCTCT CACACTCTTT AATCTTGCTG
6300  ATACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGTTATTCT ACTCCCGCCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT CAAGTTATAT ACATCTGTAG AGAATGCGCA GCAGGACAAA
6440  GGGATCGCTA TCCCACATGA TATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAACCAGC
6510  ATGAGCAGGA TCGACCCACC CCTTCGCCTG CCCCTTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580  TCTATGGCTT TCTCTTACCG CCGCTGAGTA CGACCAGACC ACATATGGGT CGTCCACTAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAATGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTCGACTGGT
6720  CTAAAGTTAC TTTGGACGGC CGCCCTCTTA CTACTATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAGTTATCCT TTTGGGAGGC CGGGACGGCT AAGGCCGGCT ACCCCTACAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATCGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCTACG TACACCACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTTGGTGT TTTAGCCCCA CATTCGGCTC TTGCAGTCCT
7000  TGAAGACACT ACTGACTACC CTGCCCGTGC CCACACTTTT GATGATTTTT GCCCGGAGTG CCGCACTCTT
7070  GGTTTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTTTGTGC CCCCTTCATA GCTTTGCTTT ATTTCTTTTT TTCTGCGGTT
7210  CGCGCTCCCT GGAAANNNNN NNNNNNN
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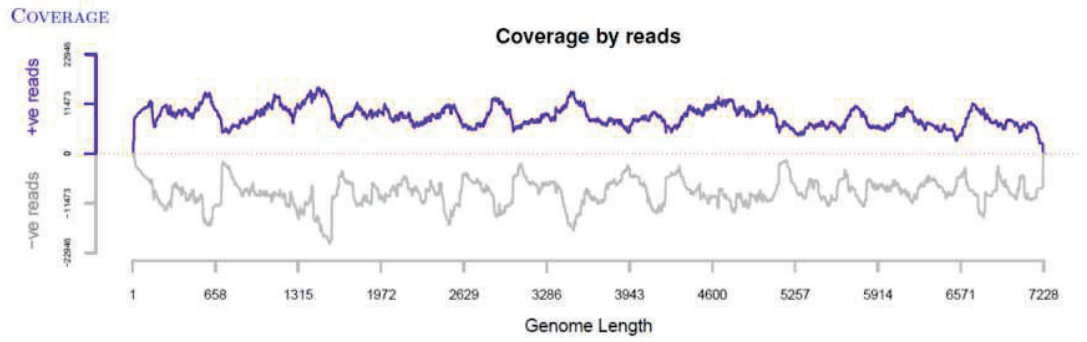
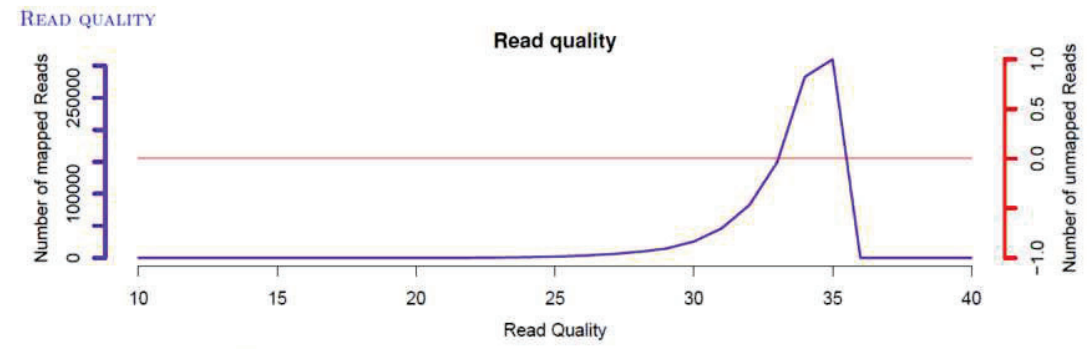
H1-PRE-RBV

File name	**H1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**933 333**
Mapped reads	**933 333 (100.00%)**
Average read length	**137nt**
Coverage	**7 218nt (99.74%)**
Average depth	**17 548 reads/site**





H1-PRE-RBV Consensus sequence

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   1  NNNNNNCACG TACGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCCATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGCCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ATCCAAATGT TCTGCACCGG TGCTTCCTAC
 350  GACCAGTTGG GAGAGACGTT CAGCGCTGGT ACTCTGCTCC TACTCGTGGC CCCGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCTGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG TTGTTCATTT
 490  GCCGCAGAAA CTGGAGTTGC CCTTTATTCT CTGCATGACC TCTGGCCGGC CGATGTTGCA GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTATTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGCGATACT
 700  AGTGCAGGCT ACAACCATGA TGTTTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGTGATC
 770  ATCCGTTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGTCA CTTTGTGCTG CTGCTCACTG CAGCTCCTGA
 840  GCCGTCACCA ATGCCTTACG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCTACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CCCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTCATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGCACTC
1190  AAGCTATATC CAAGGGTATG CGTCGACTGG AGGTTGAGCA TGCCCAAAAG TTCATTACAA GGCTTTACAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCCGCGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT CGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACCTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGAGCGCTT AACATCCCGC ATGATATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCCTGGA GTGCCGCACT GTGCTCGGGA ACAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCCATGGG GGCCGGACCG CATAGTCTCT CCTACGAACT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTAGATTGCA CTGCAATATT CCCCCCGGGC GGGGCCCCTA GCGCCGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCACCA TTCGCTCATA GGTGGCCTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTCTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTCTCC
2170  CCCCCTGAGG CAGCCGCCGC AGCGCCGGCT GCTGCCCCGG GGCTGCGCCA CCCTACACCT CCTGTCAGTG
2240  ACATTCGGGT GCTACCGCCA CCCTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCTCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAACC CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CACTAATGCC ACCATCCCCG
2380  CGCACCCGTC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGATCACTG TTTGAGTCTG
2450  ATTGTGACTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG CCCTGGAGGC GGCCTTTGCC ACGCCTTCTA
2520  CCAACGTTAT CCTGAGTCTT TCCACTCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTCTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GCATATACCA
2730  AGTTCCTGTT AGCCTCAGCT TTGACGCATG GGAGCGTAAC CATCGCCCCG GGGATGAGCT CTACTTACCC
2800  GACCCCGCCG CCACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTCGGC CGGGCTTGTG CCGGCTGCGC
2940  AATTAGCCCT GGGGTTGTGC ACTATCAGTT TACCGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGAG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TTGCGGCTTT TACACCTCAT ACGGCGGCCC GTGTCACCAC AGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTTCCA CCGCATTTGT TGTTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCA
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTTACC CATCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGCGGGG CATATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATCGGCCA AAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC TGCCAACCCC GGTGCGATTA CAGTCCACGA GGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTAACC CGCCACACAG AGAAGTGCGT TATTCTTGAT GCCCCCGGCT TGCTACGTGA GGTCGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTAGGTCA CCACCGCCCC TCTGTGATAC
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H1-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCACCT TCTTGTCAGA TTAGTGCCTA
3780  TCACCAGTTG GCTGAGGAAC TTGGCCACCG CCCAGCACCC GTTGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAGG GCCTGTTATA TATGCCTCAG GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCCC CCAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACTAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA CCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AACAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCT
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATCGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA TATTTTCTAC GGTGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  CTCAAGTATG GTATTTGAGA ATGATTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGTCTT
4480  GAGTGTGTAG TCATGGAGGA GTGTGGCATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTCCGGTCGG
4550  CCTGGATCCT GCAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCTGGCAC
4620  CCTTCTCTGG AATACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTCCGCGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA TTCCGTGGTC CTTTGTAGCG ACTACCGCCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAATTGAAGG TTGATTATCG CCCCATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCCCCTGGC CTGGGGACGC TACCCGATGT GGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAATTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCAGTTT GTGACTTTCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTGGATGTT GTATCCCGAG TTTACGGAGT TAGCCCTGGG CTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC TCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT AGAATGAATA ACATGTTTTG TGCATTGCCC ATGGGATCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGTCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTA CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG TACCGCAATC CGGGGCTGGA
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5600  ACATCATCTG TTGCCTCAGG TACTAACCTG GTTCTTTATG CTGCCCCGCT GAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAACACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATCCGT TACAGGCCAT TGGTGCCAAA TGCTGTCGGC GGCTATGCAA TATCCATCTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TCACTTCCAC TGATGTTAGG ATTCTAGTTC
5880  AGCCCGGTAT TGCTTCTGAG CTGGTCATTC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT TCATGGCTCC
6020  CCTGTTAATT CCTACACCAA TACCCCCTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCT TTGGAGCTTG
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6160  GCTGCGCCGC GGTGCTGATG GCACTGCCGA ACTTACCACC ACGGCGGCCA CGCGCTTCAT GAAGGACCTG
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6300  ATACGCTTCT CGGTGGCTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGTTATTCT ACTCCCGCCC
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6860  ACTGCTAGTG ATCAGATTTT GATCGAAAAT GCGGCTGGTC ATCGCGTCGC CATTTCCACC TATACCACTA
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7140  CCCGGGAGTT TTAATTAATT TCCTTTGTGC CCCCTTCACA GCTTTGCTTT ATTTCTTCTC TTCTGCGTTT
7210  CGCGCTCCCT GGAANNNNNN NNNNNNN
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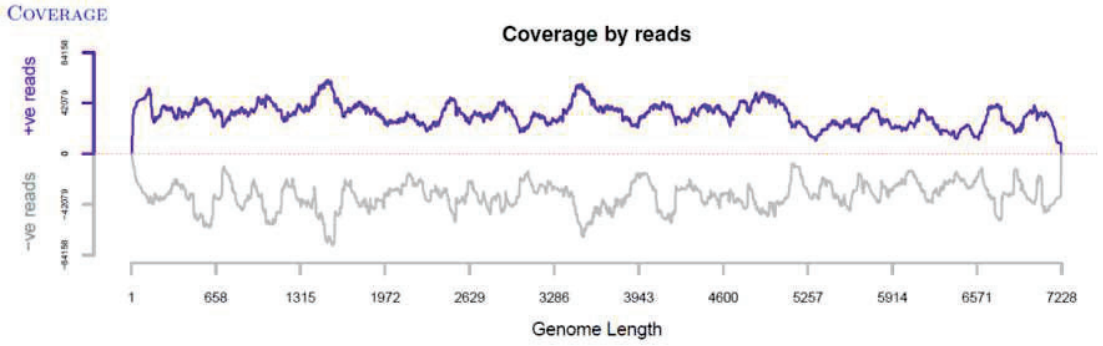
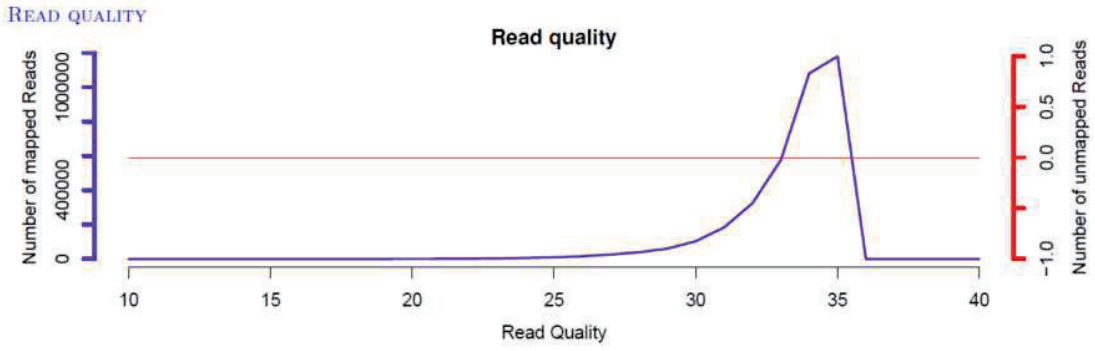
H2-POST-RBV1

File name	**H2-POST-RBV1.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**3 602 560**
Mapped reads	**3 602 560 (100.00%)**
Average read length	**137nt**
Coverage	**7 224nt (99.82%)**
Average depth	**67 813 reads/site**





H2-POST-RBV1 Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCCATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGCCGT
 280  TGTTTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ATCCAAATGT TCTGCACCGG TGCTTCCTAC
 350  GACCAGTTGG GAGAGACGTT CAGCGCTGGT ACTCTGCTCC TACTCGTGGC CCCGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCTGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG TTGTTCATTT
 490  GCCGCAGAAA CTGGAGTTGC CCTTTATTCT CTGCATGACC TCTGGCCGGC CGATGTTGCA GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTATTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGCGATACT
 700  AGTGCAGGCT ACAACCATGA TGTTTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGTGATC
 770  ATCCGTTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGTCA CTTTGTGCTG CTGCTCACTG CAGCTCCTGA
 840  GCCGTCACCA ATGCCTTACG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCTACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CCCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTCATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGCACTC
1190  AAGCTATATC CAAGGGTATG CGTCGACTGG AGGTTGAGCA TGCCCAAAAG TTCATTACAA GGCTTTACAG
1260  TTGGCTGTTC GAGAAGTCTG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCCGCGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT CGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACCTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGAGCGCTT AACATCCCGC ATGATATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCCTGGA GTGCCGCACT GTGCTCGGGA ACAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCCATGGG GGCCGGACCG CATAGTCTCT CCTACGAACT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTAGATTGCA CTGCAATATT CCCCCCGGGC GGGGCCCCTA GCGCCGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCACCA TTCGCTCATA GGTGGCCTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTCTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTCTCC
2170  CCCCCTGAGG CAGCCGCCGC AGCGCCGGCT GCTGCCCCGG GGCTGCGCCA CCCTACACCT CCTGTCAGTG
2240  ACATTCGGGT GCTACCGCCA CCCTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCTCC CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAACC CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CACTAATGCC ACCATCCCCG
2380  CGCACCCGTC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGATCACTG TTTGAGTCTG
2450  ATTGTGACTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG CCCTGGAGGC GGCCTTTGCC ACGCCTTCTA
2520  CCAACGTTAT CCTGAGTCTT TCCACTCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTCTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGC TCTCGCCGCG GTACCGCCGC CTACCCACTC CTCGGCTCGG GCATATACCA
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3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGAG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
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3290  CCAGTTGGTG GCATGTTACC CATCGCTGCC CCGCTGACGT GTGCGAGCTT ATACGCGGGG CATATCCCAA
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3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTAGGTCA CCACCGCCCC TCTGTGATAC
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H2-POST-RBV1 Consensus sequence

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4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA CCGTGATGTA TCGCGTATCA CATTTTTCCA
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4550  CCTGGATCCT GCAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCTGGCAC
4620  CCTTCTCTGG AATACTGTTT GGAATATGGC GGTCATAGCA CACTGCTATG AATTCCGCGA TTTTAGGGTT
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5530  TCCTGTACCT GATGTTGACT CGCGCGGCGC CATACTGCGA CGCCAGTACA ATTTATCCAC ATCCCCGCTC
5600  ACATCATCTG TTGCCTCAGG TACTAACCTG GTTCTTTATG CTGCCCCGCT GAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAACACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
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6090  AGTTTAGGAA CTTGACACCC GGGAACACTA ACACCCGTGT GTCCCGGTAC ACAAGCACAG CCCGTCATCG
6160  GCTGCGCCGC GGTGCTGATG GCACTGCCGA ACTTACCACC ACGGCGGCCA CGCGCTTCAT GAAGGACCTG
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6440  GGGATCGCTA TCCCACATGA CATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAACCAGC
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6580  CCTATGGCTT TCTCTTACTG CCGCTGAGTA CGACCAGACT ACATATGGGT CGTCCACTAA CCCGATGTAT
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6860  ACTGCTAGTG ATCAGATTTT GATCGAAAAT GCGGCTGGTC ATCGCGTCGC CATTTCCACC TATACCACTA
6930  GCTTGGGCGC CGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TTTAGCCCCA CATTCGGCTC TTGCAGCTCT
7000  TGAAGACACC ATTGACTACC CTGCCCGTGC CCACACCTTT GATGATTCTT GCCCGGAGTG CCGCGCTCTT
7070  GGTTTGCAGG GGTGCGCCTT CCAGTCTACC ATTGCTGAGC TTCAGCGTCT CAAAATGAAG GCAGGTAAAA
7140  CCCGGGAGTT TTAATTAATT TCCTTTGTGC CCCCTTCACA GCTTTGCTTT ATTTCTTCTC TTCTGCGTTT
7210  CGCGCTCCCT GGAAANNNNN NNNNNNN
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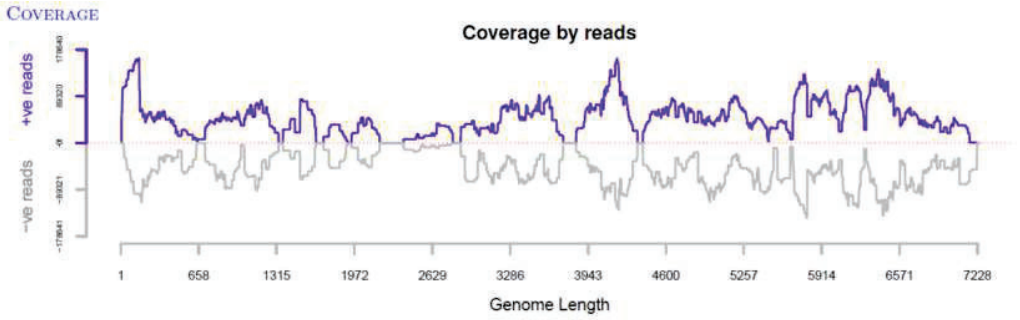
I1-PRE-RBV

File name	**I1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**5 306 159**
Mapped reads	**5 306 159 (100.00%)**
Average read length	**147nt**
Coverage	**7 216nt (99.71%)**
Average depth	**96 580 reads/site**



I1-PRE-RBV Consensus sequence

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 140  CCCGTGTTCA GACTGACATT CTCATCAATT TGATGCAACC CCGGCAGCTC GTATTCCGAC CTGAAGTTTT
 210  GTGGAATCAC CCGATCCAGC GAGTTATACA CAATGAGCTT GAGCAGTACT GCCGTGCCCG CGCCGGTCGC
 280  TGCCTGGAGG TCGGGGCTCA TCCGAGATCC ATTAATGACA ACCCTAACGT CCTGCACCGG TGTTTCCTTC
 350  GCCCGGTCGG GAGAGATGTA CAGCGTTGGT ATTCCGCCCC GACTCGCGGC CCAGCTGCCA ACTGCCGGCG
 420  TTCCGCATTA CGTGGCCTGC CCCCTGTCGA CCGTACTTAC TGTTTCGACG GGTTCTCCCG CTGTGCTTTT
 490  GCTGCTGAGA CTGGAATTGC TTTGTATTCA CTACATGACC TCTGGCCTGC CGATGTCGCG GAGGCCATGG
 560  CCCGTCATGG GATGACACGC CTGTACGCAG CCCTCCATTT ACCCCCTGAG GTTCTGTTAC CACCTGGTAC
 630  TTACCATACC ACCTCTTATT TGTTGATTCA TGACGGCAAC CGCGCCGTCG TAACTTATGA GGGGGATACC
 700  AGCGCGGGTT ATAACCATGA TGTGTCCATT CTTCGCGCAT GGATTCGCAC AACTAAAATA GTTGGCGACC
 770  ATCCGTTGGT TATAGAAAGG GTCCGTGCTA TCGGCTGCCA TTTTGTACTA CTCCTTACTG CTGCCCCTGA
 840  GCCATCCCCT ATGCCTTATG TCCCATACCC TCGTTCAACG GAGGTGTATG TCAGGTCCAT ATTTGGCCCC
 910  GGCGGCTCGC CATCTCTGTT CCCGTCAGCC TGCTCTACTA AATCCACATT TCATGCTGTT CCGGTCCATA
 980  TCTGGGACCG GCTCATGCTC TTCGGCGCCA CTCTAGACGA CCAAGCCTTT TGTTGCTCGC GGCTTATGAC
1050  TTATCTCCGC GGGATTAGTT ATAAGGTGAC CGTTGGTGCA CTCGTCGCCA ACGAAGGTTG GAACGCCTCA
1120  GAGGATGCGC TCACTGCTGT GATCACTGCA GCCTACTTGA CCATCTGTCA CCAGCGCTAC CTCCGAACTC
1190  AGGCTATATC TAAAGGCATG CGTCGGCTAG AAGTCGAGCA TGCCCAGAAG TTTATTACTA GACTTTACAG
1260  CTGGCTGTTT GAAAAGTCCG GTCGAGATTA TATCCCCGGC CGTCAACTTC AGTTTTACGC CCAGTGTCGC
1330  CGTTGGCTGT CGGCAGGTTT TCATCTTGAC CCAAGAGTGC TTGTCTTCGA TGAGGCGGTT CCCTGTCGTT
1400  GTAGGAGTTT TCTGAAGAAG GCTGCCGGCA AGTTCTGCTG CTTTATGAAG TGGCTGGGGC AGGAGTGCAC
1470  TTGTTTTCTG GAGCCGGCTG AGGGCTTGAT CGGCGATTGT GGTCACGATA ATGAGGCCTA CGAGGGTTCC
1540  GAAGTAGATC CTGCTGAGCC CGCCCACCTC GATGTCTCTG GAACCTACAC AGTTCATGGC CGTCAACTTG
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1680  GGAGCTCACC GCCGGCCCAG GTCGCCTCGA ATGCCGTACA GTGCTCGGAA ATAAGACCTT CCGCACATCG
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1820  AAGCGATGGG GGCTGGGCCG CATAGTCTTA CATATGAGCT CACCCCTGCT GGTTTGCAGG TTAAGATATC
1890  CTCTAATGGC CTAGATTGCA CTGCTGTTTT CCCTCCCGGT GGGGCGCCAA GTGCCGAACC GGGTGAGGTT
1960  GCAGCCTTCT GCAGTGCTTT ATATAGATAC AATAGATTTA CCCAGCGCCA TTCGCTAACC GGCGGACTGT
2030  GGCTGCACCC TGAGGGGCTT CTGGGCTTGT TTCCCCCCTT TTCCCCCGGG CATGTCTGGG AGTCCGCAAA
2100  TCCTTTTTGC GGTGAGGGCA CACTTTACAC CCGTACTTGG TCTACGTCTG GTTTTTCTAG TGATTTCTCT
2170  CCCCCTGAGG CAGCTGTCGC AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTTGGGT GCTACCGCCG CCTTCTGAAG AACTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GGCTTCTCTA CACCTACCCA GATGGAGCTA AGGTCTATGC AGGCTCCTTG TTCGAGTCAG
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2590  ACCCCACGCC CAATTATTCA TGCTGTGGCT CCTGATTATA GGGTTGAGCA GAACCCCAAG AGGTTGGAGG
2660  CGGCGTACCG CGAGACTTGC TCTCGCCTTG GCACAGCCGC TTACCCACTA CTGGGGTCGG GGATTTATCA
2730  AGTCCCCGTG GGTCTAAGTT TTGATGCCTG GGAGCGTAAC CATCGCCCGG GCGATGAGCT CTATCTCACC
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2940  TGTTAGCCCT GGCATTGTCC ATTATCAGTT TACTGCCGGG GTGCCTGGCT CCGGGAAGTC TAGGTCTATA
3010  CAACAAGGGG ACGTCGATGT TGTGGTTGTG CCAACCCGCG AGCTTCGCAA CAGCTGGCGG CGCCGTGGCT
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3570  TGCACTTACT CGCCACACAG AGAAATGTGT TATTTTGGAC GCCCCCGGCC TACTGCGTGA GGTCGGTATT
3640  TCTGATATAA TTGTTAATAA TTTTTTCTTA GCCGGCGGTG AAGTCGGCCA CCACCGCCCT TCCATTATAC
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I1-PRE-RBV Consensus sequence

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3780  CCACCAGTTG GCTGAGGAAC TAGGCCACCG CCCAGCCCCT GTCGCCGCTG TCCTGCCGCC CTGCCCTGAG
3850  CTTGAGCAGG GTTTACTTTA CATGCCGCAA GAGCTCACAG TGTCCGACAG CGTTCTAGTC TTCGAGCTCA
3920  CGGACATAGT TCACTGCCGA ATGGCGGCCC CTAGTCAACG GAAGGCTGTC CTTTCAACGC TTGTTGGCAG
3990  GTACGGCCGC CGAACGAGGC TGTATGAGGC AGCTCACTCC GATGTCCGCG AGTCTCTGGG GAGGTTTATC
4060  CCTACCATCG GACCAGTTCA GGCCACCACA TGTGAGTTGT ACGAGTTGGT AGAGGCTATG GTTGAGAAGG
4130  GTCAGGACGG TTCCGCTGTG TTAGAGCTTG ATCTCTGTAG CCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAAGGACTGT AATAAGTTTA CAACAGGTGA GACTATTGCT CATGGCAAGG TGGGCCAGGG CATTTCGGCC
4270  TGGAGCAAGA CCTTTTGTGC CCTATTCGGA CCGTGGTTCC GTGCCATTGA GAAGGAGATC TTAGCCCTAC
4340  TCCCACCTAA TATCTTCTAT GGTGATGCCT TTGAAGAGTC AGTGTTCTCT GCGGCCATTT CTGGAGCAGG
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7210  CGCGCTCCCT GGAANNNNNN NNNNNNN
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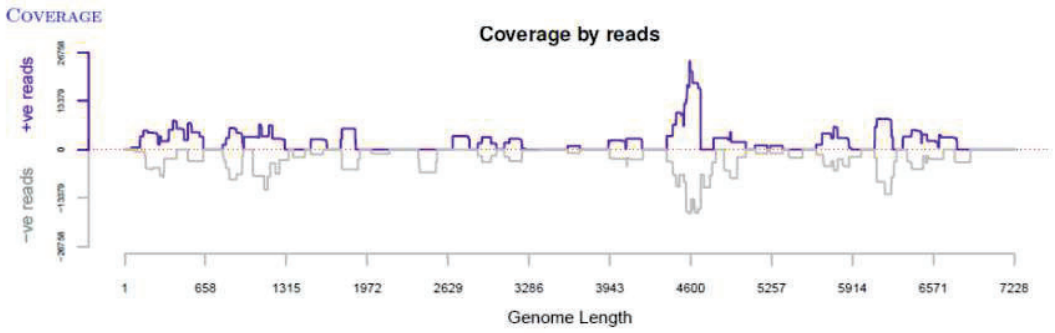
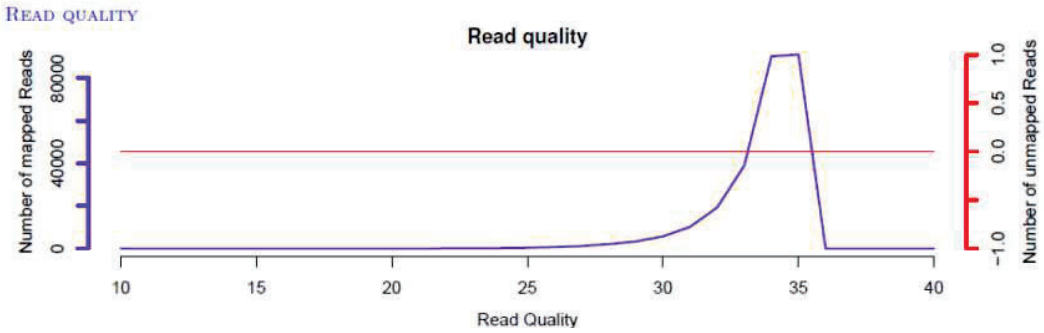
I2-POST-RBV

File name	**I2-POST-RBV.sam**
Ref name	**FJ705359**.1
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**263 021**
Mapped reads	**263 021 (100.00%)**
Average read length	**143nt**
Coverage	**6 074nt (83.93%)**
Average depth	**4 557 reads/site**





I2-POST-RBV Consensus sequence

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 140  CCCGTGTTCA GACTGACATT CTCATCAATT TGATGCAACC CCGGCAGCTC GTATTCCGAC CTGAAGTTTT
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 280  TGCCTGGAGG TCGGGGCTCA TCCGAGATCC ATTAATGACA ACCCTAACGT CCTGCACCGG TGTTTCCTTC
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 490  GCTGCTGAGA CTGGAATTGC TTTGTATTCA CTACATGACC TCTGGCCTGC CGATGTCGCG GAGGCCATGG
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 840  GCCATCCCCT ATGCCTTATG TCCCATACCC TCGTTCAACG GAGGTGTATG TCAGGTCCAT ATTTGGCCCC
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 980  TCTGGGACCG GCTCATGCTC TTCGGCGCCA CTCTAGACGA CCAAGCCTTT TGTTGCTCGC GGCTTATGAC
1050  TTATCTCCGC GGGATTAGTT ATAAGGTGAC CGTTGGTGCA CTCGTCGCCA ACGAAGGTTG GAACGCCTCA
1120  GAGGATGCGC TCACTGCTGT GATCACTGCA GCCTACTTGA CCATCTGTCA CCAGCGCTAC CTCCGAACTC
1190  AGGCTATATC TAAAGGCATG CGTCGGCTAG AAGTCGAGCA TGCTCAGAAG TTTATCACTA GACTTTACAG
1260  CTGGCTGTTT GAAAAGTCCG GTCGAGATTA TATCCCCGGC CGTCAACTTC AGTTTTACGC CCAGTGTCGC
1330  CGTTGGTTAT CGGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTCTTTGA TGAGACGGTT CCCTGTCGTT
1400  GTAGGAGTTT TCTGAAGAAG GCTGCCGGCA AGTTCTGCTG CTTTATGAAG TGGTTGGGGC AGGAGTGCAC
1470  TTGTTTTTTG GAGCCAGCTG AGGGTCTGGT TGGCGACCAT GGTCACGATA ATGAGGCCTA CGAGGGTTCC
1540  GAAGTAGATC CTGCTGAGCC CGCCCACCTC GATGTCTCTG GAACCTACAC AGTTCATGGC CGTCAACTTG
1610  AGGCTCTATA CAGGGCGCTC AACATCCCAC ATGACATTGT GGCCCGNNNN NNNNNNNNNN NNNNNNNNNN
1680  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
1750  NNNNTTGATG GCGCTCACCT CGAGGCTAAC GGCCCTGAGC AGTATGTTTT AACATTTGAT GACTCCCGCC
1820  AAGCGATGGG GGCTGGGCCG CATAGTCTTA CATATGAGCT CACCCCTGCT GGTTTGCAGG TTAAGATATC
1890  CTCTAATGGC CTAGATTGCA CNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
1960  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCAGCGCCA TTCGCTAATT GGCGGATTGT
2030  GGCTGCACCC TGAGGGGCTT CTGGGCTTGT TTCCCCCCTT TTCCCCCGGG CATGTCTGGG AGTCCGCAAA
2100  TCCTTTTTGC GGTGAGGGCA CACTTTACAC CCGTACTTGG TCTACGTCTG GTNNNNNNNN NNNNNNNNNN
2170  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2240  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2310  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2380  NNNACCCGCC GGCTTCTCTA CACCTACCCA GATGGAGCTA AGGTCTATGC AGGCTCCTTG TTCGAGTCAG
2450  ATTGCGACTG GCTGGTTAAC GCGTCCAATC CTGGCCACCG CCCTGGTGGC GGCCTTTGCC ATGCTTTTTA
2520  CCAGCGCTTC CCAGAGTCTT TCCACCCNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2590  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNAGG
2660  CGGCATACCG CGAGACTTGT TCTCGCCTTG GCACAGCCGC TTACCCACTA CTGGGGTCGG GGATTTATCA
2730  AGTCCCCGTG GGTCTAAGTT TTGATGCCTG GGAGCGTAAC CATCGCCCAG GCGATGAGCT CTATCTCACC
2800  GACCNNNNNN NNNNNNNNNN CGAGGCTAAT AAGCCAGCAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGCACGGC TAACCTCGCG CTTGAGATTG ACTCTGCTAC CGAGGTGGGC CGTGCTTGTA CCGGCTATAC
2940  TGTTAGCCCT GGCATTGTCC ATTATCAGTT TACTGCTGGG GTGCCTGGCT CCGGGAAGTC TAGGTCTATA
3010  CAACAAGGGG ACGTCGATGT TGTGGTTGTG CCAACCCGCG AGCTTCGCAA CAGCTGGCGG CGCCGTGGCT
3080  TTGCTGCTTT TACACCTCAT ACGGCGGCCC GCGTCACTCC CGGCCGACGT GTTGTGATTG ATGAGGCGCC
3150  TTCGCTTCCA CCACATTTGC TGCTGCTTCA TATGCAGCGA GCTTCGTCAG TCCACCTTCT CGGCGACCCC
3220  AACCAGATTC CCGCCATTGA TTTCGAGCAT GCAGGCCTAG TGCCAGCGAT CCGCCCTGAG CTTGCCCCAA
3290  CCAGTTGGTG GCACGTTACA CACCGTTGCC CTGCCGACGT GTGTGAGCTC ATACGCGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC ATTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CCGCTAAGGC CGCCAACCCC GGTGCGATTA CAGTCCATGA GGCCCAGGGT GCCACTTTCA
3500  CGGAGACCAC AATTATAGCC ACGGCCGATG CTAGGGGGCT CATTCAATCC TCCCGAGCCC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTTTGGAC GCCCCCGGCC TACTGCGTGA GGTCGGTATT
3640  TCTGATATAA TTGTTAATAA TTTTTTCTTA GCCGGCGGTG AAGTCGGCCA CCACCGCCCC TCTGTGATAC
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I2-POST-RBV Consensus sequence

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3710  CCCGCGGCAG TCCCGACCAG AACCTCGCGA CACTGCAGGC TTTTCCACCN NNNNNNNNNN NNNNNNNNNN
3780  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3850  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
3920  NNNNNNNNNN NCACTGCCGA ATGGCGGCCC CTAGTCAACG GAAGGCAGTC CTTTCAACGC TTGTAGGCAG
3990  GTACGGCCGC CGAACAAAGC TGTATGAAGC AGCTCACTCC GATGTCCGCG AGTCTCTGGG GAGGTTTATC
4060  CCTACCATCG GACCAGTTCA GGCCACCACA TGTGAGTTGT ACGAGTTGGT AGAGGCTATG GTTGAGAAGG
4130  GTCAGGACGG TTCCGCTGTG TTAGAGCTTG ATCTCTGTAG CCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAATGACTGT AATAAGTTCA CCACAGGGGA GACCATTGCT CATGGTAAGG TCGGCCAGGG CATTTCGGCT
4270  TGGAGCAAGA CCTTCTGTGC CCTCTTCGGC CCGTGGTTCC GTGCCATTGA GAAGGAGATC TTAGCCCTAC
4340  TCCCACCTAA TATCTTCTAT GGTGATGCCT TTGAAGAGTC AGTGTTCTCT GCGGCCATTT CTGGAGCAGG
4410  TTCTAGTATG GTTTTTGAGA ACGATTTCTC TGAGTTTGAC AGTACCCAAA ACAATTTCTC CCTCGGCCTT
4480  GAGTGTGTTA TTATGGAGGA GTGTGGCATG CCCCAGTGGC TCATTAGGTT ATACCATTTA GTCAGGTCGG
4550  CCTGGACTTT GCAGGCCCCG AAGGAGTCCC TGAAAGGCTT TTGGAAGAAG CACTCTGGTG AGCCCGGTAC
4620  ACTCCTCTGG AATACTGTCT GGAACATGGC AATCATAGCG CATTGCTACG AATTTCGTGA CCTGAGAGTG
4690  GCAGCGTTTA AAGGGGATGA TTCAGTAGTC CTTTGCAGTG ATTACCGCCA GAGCCGTAAT GCAGCTGCCT
4760  TGATCGCTGG TTGTGGGTTA AAATTGAAGG TTGATTACCG CCCCATAGGG TTGTATGCCG GTGTTGTGGT
4830  GGCCCCGGGT CTCGGGGTAC TCCCTGACGT CGTTCGGTTC GCCGGTCGGT TGTCTGAGAA GAATTGGGGC
4900  CCGAGTCCCG AGCGCGCCGA GCAGTTACGC TTGGCTGTTT GTGACTTCCT TCGAAAGTTA ACGAATGTTG
4970  CGCAGGTTTG TGTTGATGTT GTGTCCCGTG TTTATGGGGT TAGTCCTGGG CTAGTACATA ACCTTATTGG
5040  CATGTTACAG ACTATAGCTG ATGGGAAGGC CCATTTTACT GAGACTGTTA AACCTGTNNN NNNNNNNNNN
5110  NNNNNNNNNN TACAGAGGTT GGAATGAATA ACATGTTGTG TGCATCGCCC ATGGGTTCAC CATGCGCCCT
5180  CGGGCTGTTC TGTTGTTGTT CCTCGTGCTT TTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCAGTG CCAGCGGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTTGCCCTC CCCTATATTC ATCCAACCAA CCCCNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5390  NNNCGCCTTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGACCA GTCCCAGCGC CCCCACGCCG
5460  CCCCCCGCCG TCGATCTACC CCAGCTGGGG CTGCGCCGTT AACTGCCATN NNNNNNNNNN NNNNNNNNNN
5530  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
5600  NNNNNNNNNN NNNNNNNNGG TACTAATCTC GTTCTGTATG CTGCCCCGCT TAATCCTCTT TTACCTCTTC
5670  AGGATGGCAC CAATACTCAT ATCATGGCGA CTGAGGCATC TAACTATGCT CAATATCGGG TTGTCCGAGC
5740  CACAATCCGT TATCGCCCTT TGGTGCCGAA TGCTGTTGGA GGCTATGCAA TTTCTATTTC CTTTTGGCCC
5810  CAGACTACAA CTACTCCCAC CTCTGTTGAT ATGAATTCTA TTACCTCTAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT AGCCTCCGAG TTGGTTATTC CAAGCGAGCG CCTCCATTAT CGTAATCAGG GTTGGCGTTC
5950  TGTTGAGACC TCGGGTGTGG CTGAGGAGGA GGCGACATCG GGCCTAGTTA TGCTCTGTAT CCATGGTTCC
6020  CCTGNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNGAGCTCG
6090  AATTTAGGAA CTTAACCCCT GGGAACACTA ACACCCGTGT GTCCCGGTAC ACCAGCACAG CCCGCCACCG
6160  CCTACGCCGT GGCGCCGATG GGACTGCCGA GCTCACGACC ACTGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CATTTTACCG GGATGAATGG CGTCGGCGAG GTGGGCCGTG GTATAGCTCT NNNNNNNNNN NNNNNNNNNN
6300  NNNNNNNNNN NNNNNNNNNN NNNACAGAAT TGATTTCGTC GGCCGGAGGT CAACTCTTTT ACTCCCGTCC
6370  AGTTGTCTCA GCCAATGGCG AGCCGACTGT TAAATTATAC ACCTCCGTCG AGAATGCACA GCAGGACAAG
6440  GGCATTGCCA TACCACATGA CATAGATCTG GGAGATTCCC GTGTGGTTAT TCAGGATTAT GACAATCAAC
6510  ATGATCAGGA TAGGCCTACC CCCTCGCCAG CCCCGTCTCG CCCCTTCTCG GTTCTTCGCA CTAACGATGT
6580  TTTGTGGCTC TCCCTCACTG CTGCCGAATA TGATCAAACT ACTTATGGTT CGTCGACCAA CCCTATGTAT
6650  GTTTCAGATA CTGTTACCCT TGTTAACGTG GCAACGGGAG CCCAGGCTGT CGCCCGCTCT CTTGATTGGT
6720  CTAAGGTCAC TCTAGATGGT CGACCTCTCA CTACTATTCA GCAGTACTCT AAGACATTTT ATGTTCTCCC
6790  ACTCCGCGGG AAGCTTTCTT TCTGGGAGGC TGGCACCACT AAGGCCGGGT ATCCTTACAA TTATAATACC
6860  ACTGCTAGTG ACCAGATTTT AATTGAAAAT GCAGCTGGCC ACCGTGTTGC TATNNNNNNN NNNNNNNNNN
6930  NNNNNGGCGC TGGCCCTGTG TCAGTTTCTG CAGTCGGTGT GTTAGCCCCA CATTCGGCTC TTGCAGTTCT
7000  TGAGGATACT ACTGACTACC CTGCCCGCGC CCACACTTTT GATGACTTCT GTCCAGAGTG TCGCACTCTT
7070  GGCTTGCAGG GGTGTGCTTT CCAGTCTACT GTTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATTAATT TCCTTTGTGC CCCCTTCATA GCTTTGCTTT ATTTCTTNTT TTCTGCTTTT
7210  CGCGCTCCCN NNNNNNNNNN NNNNNNN
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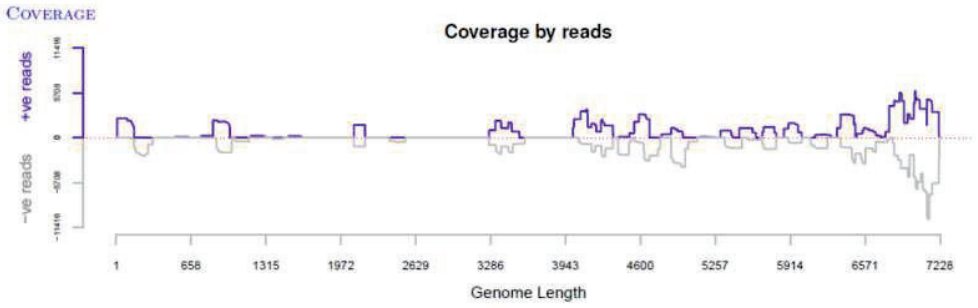
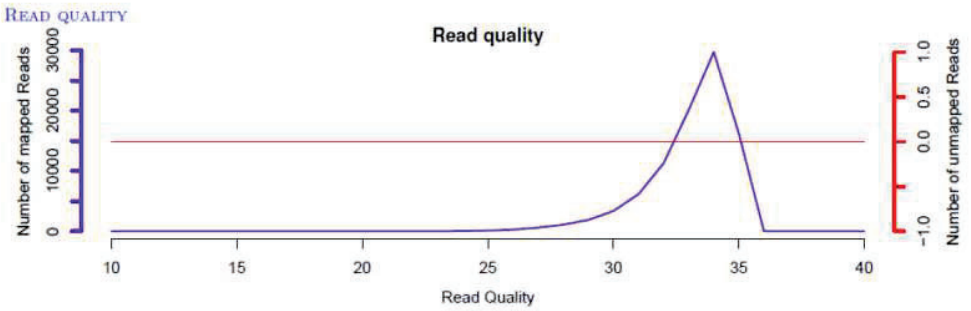
I3-POST-RBV

File name	**I3-POST-RBV.sam**
Ref name	**FJ705359**.1
Ref length	**7237**
Program used	**Tanoti Assembler 1.0**
Total reads	**90925**
Mapped reads	**90925 (100.00%)**
Average read length	**140nt**
Coverage	**5245nt (72.47%)**
Average depth	**1553 reads/site**





I3-POST-RBV Consensus sequence

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70	ATTGAGCAGG	CTGCTCTGGC	TGCGGCTAAC	TCCGCCTTGG	CGAATGCTGT	GGTGGTTCGG	CCGTTTTTGT
140	CCCGTGTTCA	GACTGACATT	CTCATCAATT	TGATGCAACC	CCGGCAGCTC	GTATTCCGAC	CTGAAGTTTT
210	GTGGAATCAC	CCGATCCAGC	GAGTTATACA	CAATGAACTT	GAGCAGTACT	GCCGTGCCCG	CGCCGGTCGC
280	TGCCTGGAGG	TCGGGGCTCA	TCCGAGATCC	ATTAATGACA	ACNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
350	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
420	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
490	NNNNNNNNNN	NNNNNNNNNN	NNNNTATTCA	CTACATGACC	TCTGGCCTGC	CGATGTTGCG	GAGGCCATGG
560	CCCGTCATGG	GATGACACGC	CTGTACGTAG	CCCTCCATTT	ACCCCCTGAG	GTTCTGTTAC	CACTTGGTAC
630	TTACCATACA	ACNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNTATGA	GGGTGATACC
700	AGCGCGGGTT	ATAACCATGA	TGTGTCCATT	CTTCGCGCAT	GGATTCGCAC	AACTAAAATA	GTTGGCGACC
770	ACCCGTTGGT	TATAGAAAGG	GTCCGTGCTA	TCGGCTGCCA	TTTTGTACTA	CTCCTTACTG	CTGCCCCTGA
840	GCCATCCCCT	ATGCCTTATG	TCCCATACCC	TCGTTCAACG	GAGGTGTATG	TCAGGTCCAT	ATTTGGCCCC
910	GGCGGCTCGC	CATCTCTGTT	CCCGTCAGCC	TGCTCTACTA	AATCCACATT	TCATGCTGTT	CCGGTCCATA
980	TCTGGGACCG	GCTCATGCTC	TTCGGCGCCA	CTCTAGATGA	CCAAGCCTTT	TGTTGCTCGC	GGCTTATGAC
1050	TTATCTCCGC	GGGATTAGTT	ATAAGGTGAC	CGTTGGTGCA	CTCGTCGCCA	ACGAAGGTTG	GAACGCCTCA
1120	GAGGATGCGC	TCACTGCTGT	GATCACTGCA	GCCTACTTGA	CCATNNNNNN	NNNNNNNNNN	CTCCGAACTC
1190	AGGCTATATC	TAAAGGCATG	CGTCGGCTAG	AAGTCGAGCA	TGCCCAGAAG	TTTATTACTA	GACTTTACAG
1260	CTGGCTGTTT	GAAAAGTCCG	GTCGAGATTA	TATCCCCGGC	CGTCAACTTC	AGTTTTACGC	CCAGTGTCGC
1330	CNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNGTGC	TTGTCTTCGA	TGAGGCGGTT	CCCTGTCGTT
1400	GTAGGAGTTT	TCTGAAGAAG	GCTGCCGGCA	AGTTCTGCTG	CTTTATGAAG	TGGCTGGGGC	AGGAGTGCAC
1470	TTGTTTTCTG	GAGCCGGCAG	AGGGNNNNNN	NNNNNNNNNN	NNNCACGATA	ATGAGGCCTA	TGAGGGTTCC
1540	GAAGTAGATC	CTGCTGAGCC	CGCCCACCTC	GATGTATCTG	GAACCTACAC	AGTTCATGGC	CGTCAACTTG
1610	AGGCTCTATA	CAGGGCGCTN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1680	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1750	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1820	NNNNNATGGG	GGCTGGGCCG	CATAGTCTTA	CATATGAGCT	CACCCCTGCT	GGTTTGCAGG	TTAAGATATC
1890	ATCTAATGGC	CTAGATTGCA	CTGCNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1960	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNCAGCGCCA	TTCGCTAACC	GGCGGACTGT
2030	GGCTGCACCC	TGAGGGGCTT	CTGGGCTTGT	TTCCCCCCTT	TTCCCCCGGG	CATATCTGGG	AGTCCGCAAA
2100	TCCTTTTTGC	GGTGAGGGCA	CACTTTACAC	CCGTACTTGG	TCTACATCTG	GTTTTTCTAG	TGATTTCTCT
2170	CCCCCTGAGG	CAGNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2240	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2310	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2380	NNNNNNNNNN	NNNNNNNNTA	CACCTACCCA	GATGGAGCTA	AGGTCTATGC	AGGCTCCTTG	TTTGAGTCAG
2450	ATTGCGACTG	GCTGGTTAAC	GCGGCCAATC	CTCGCCACCA	CCCTGGTGGC	GGCCTTTGCC	ATGCTTTTTA
2520	CCAGCGCTTC	CCAGAGTCTT	TNNNNNNNNN	NNNNNNNNTC	ATGCGTGAGG	GCCTCGCTGC	ATACACCTTG
2590	ACCCCCCGCC	CAATTATTCA	TGCTGTGGCT	CCCGATTATA	GGGTTGAGCA	GAACCCCAAG	AGGNNNNNNN
2660	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2730	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2800	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2870	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
2940	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3010	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3080	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3150	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3220	NNNNNNNNNN	NNNNNNNNNN	NNNCGAGCAT	GCAGGCCTAG	TACCAGCGAT	CCGCCCTGAG	CTTGCCCCAA
3290	CCAGTTGGTG	GCACGTTACA	CACCGTTGCT	CTGCCGACGT	GTGTGAGCTC	ATACGCGGGG	CTTATCCCAA
3360	AATCCAAACT	ACTAGCCGCG	TGGTGCGGTC	TCTGTTTTGG	AATGAGCCTG	CGGTGGGCCA	GAAATTGGTT
3430	TTTACGCAAG	CAGCTAAGGC	AGCCAACCCC	GGTGCAATTA	CTGTTCACGA	GGCCCAGGGT	GCTACATTCA
3500	CAGAAACTAC	CATTATTGCT	ACGGCCGATG	CTAGGGGCCT	TATTCAGTCC	TCTCGTGCAC	ATGCAATTGT
3570	TGCACTTACT	CGCCANNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3640	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN

I3-POST-RBV Consensus sequence

3710	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3780	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NTGCCCTGAG
3850	CTTGAGCAGG	GTTTACTTTA	CATGCCGCAA	GAGCTCACAG	TGTCCGACAG	CGTTCTAGTC	TTCGAGCTCA
3920	CGGACATAGT	TCACTGCCGA	ATGGCGGCCC	CNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
3990	NNNNNNNNNN	NNNNNNNNGC	TGTATGAGGC	AGCTCACTCT	GATGTCCGCG	AGTCTCTGGG	GAGGTTTATC
4060	CCTACCATCG	GACCAGTTCA	GGCCACCACA	TGTGAGTTGT	ACGAGTTGGT	AGAGGCTATG	GTTGAGAAGG
4130	GTCAGGACGG	TTCCGCTGTG	TTAGAGCTTG	ATCTCTGTAG	CCGTGATGTC	TCGCGTATCA	CATTTTTCCA
4200	GAATGACTGT	AATAAGTTTA	CAACAGGTGA	GACTATTGCT	CATGGCAAGG	TGGGCCAGGG	CATTTCGGCC
4270	TGGAGCAAGA	CCTTTTGTGC	CCTATTCGGA	CCGTGGTTCC	GTGCCATTGA	GAAGGAAATC	TTAGCCCTAC
4340	TCCCACCTAA	TATCTTCTAT	GGTGATGCCT	TTGAAGAGTC	AGTGTTCTCT	GCGGCCATTT	CTGGAGCAGG
4410	TTCTAGTATG	GTTTTTGAGA	ATGATTTCTC	TGAGTTTGAC	AGTACCCAAA	ACAATTTCTC	CCTCGGCCTT
4480	GAGTGTGTTA	TTATGGAGGA	GTGTGGCATG	CCCCAGTGGC	TCATTAGGCT	ATACCATTTA	GTTAGGTCGG
4550	CCTGGACTTT	GCAGGCCCCG	AAGGAGTCCC	TGAAAGGCTT	TTGGAAGAAG	CACTCTGGTG	AGCCCGGTAC
4620	ACTCCTCTGG	AATACTGTCT	GGAACATGGC	AATCATGGCG	CATTGCTATG	AATTTCGTGA	CCTGAGAGTG
4690	GCAGCGTTTA	AAGGGGATGA	TTCAGTAGTC	CTTTGCAGTG	ATTACCGCCA	GAGCCGTAAT	GCAGCTGCCT
4760	TGATCGCTGG	TTGNNNNNNN	NNNNTGAAGG	TTGATTACCG	CCCCATAGGG	TTGTACGCCG	GTGTTGTGGT
4830	GGCCCCGGGT	CTCGGGGCAC	TCCCTGACGT	CGTTCGGTTC	GCCGGCCGGT	TGTCTGAGAA	GAATTGGGGC
4900	CCGAGTCCCG	AGCGCGCTGA	GCAGTTACGC	TTGGCTGTTT	GTGACTTCCT	TCGAAAGTTA	ATGAATGTTG
4970	CGCAGGTTTG	TGTTGATGTT	GTGTCCCGTG	TTTATGGGGT	TAGTCCTGGG	CTGGTACATA	ACCTTATTGG
5040	CATGTTACAG	ACTATAGCCG	ATGGGAAGGC	CCATTTTACT	GAGACTGTTA	AACCTGTNNN	NNNNNNNNNN
5110	NNNTCTATCA	TACAGAGGTT	GGAATGAATA	ACATGTTGTG	TGCATCGCCC	ATGGGTTCAC	CATGCGCCCT
5180	CGGGCTGTTC	TGTTGTTGTT	CGTCGTGCTT	TTGCCTATGC	TGCCCGCGCC	ACCGGCCGGC	CAGCCGTCTG
5250	GCCGCCGTNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNCAGGGTTG	ATTCTCAGCC
5320	CTTTGCCCTC	CCCTATATTC	ATCCAACCAA	CCCCTTTGCC	GCCGACGTCG	TATCACAACC	CGGGGCTGGA
5390	GCTCGCCTTC	GACAACCACC	CCGCCCCCTC	GGCTCCTCTT	GGCGTGACCA	GTCCCAGCGC	CCCCCCGCCG
5460	CCCCCCGCCG	TCGATCTACC	CCAGCTGGGG	CTGCGCCGTT	AACTGCCGTC	TCCCCTGCGC	CTGATACTGC
5530	CCCAGTCCCC	GATGTTGATT	CTCGCGGTGC	TATTTTGCGC	CGCCAGTATA	ATTTGTCTAC	TTCCCCTCTG
5600	ACTTCTTCTG	TCGCCTCTGG	CACTAATCTC	GTTCTGTATG	CTGCCCCGNN	NNNNNNNNNN	NNNNNNNNNC
5670	AGGATGGCAC	CAATACTCAT	ATCATGGCGA	CTGAGGCATC	TAACTATGCT	CAATATCGGG	TTGTCCGAGC
5740	CACAATCCGT	TATCGCCCTT	TGGTGCCAAA	TGCTGTTGGA	GGCTATGCAA	TTTCTATTTC	CTTTTGGCCC
5810	CAGACTACAA	CTACCCCCNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNAC	TGATGTTAGG	ATTTTAGTTC
5880	AGCCTGGCAT	AGCCTCCGAG	TTGGTTATTC	CGAGCGAGCG	CCTCCATTAT	CGTAATCAGG	GTTGGCGTTC
5950	TGTTGAGACC	TCGGGTGTGG	CTGAGGAGGA	GGCGACATCG	GGTCTGGTTA	TGCTCTGTAT	CCATGNNNNN
6020	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
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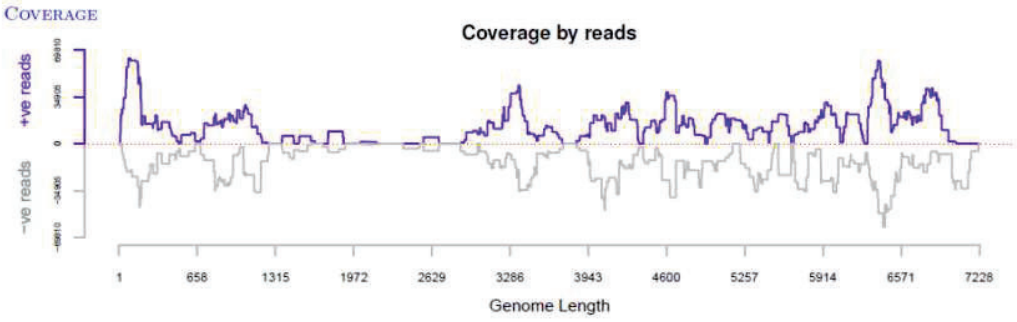
6160	CCTACGCCGT	GGCGCCGATG	GGACTGCTGA	GCTCACGACC	ACTGCGGCCA	CGCGCTTCAT	GAAGGACCTG
6230	CATTTTACCG	GGATGAATGG	CGTCGGCGAG	GTGGGCCGTG	GCATTGCNNN	NNNNNNNNNN	NNNNNNNNNN
6300	NNNNNNNNNN	NNNNNNNNNN	NNNNCAGAAT	TGATTTCGTC	GGCTGGAGGT	CAACTCTTTT	ACTCCCGTCC
6370	AGTTGTCTCG	GCCAATGGCG	AGCCAACTGT	TAAATTATAC	ACCTCCGTCG	AGAATGCACA	GCAGGACAAG
6440	GGCATTGCCA	TACCACATGA	CATAGATCTG	GGAGATTCCC	GTGTGGTTAT	TCAGGATTAT	GACAATCAAC
6510	ATGAGCAGGA	TAGGCCTACC	CCTTCGCCGG	CCCCGTCTCG	CCCCTTCTCG	GTTCTTCGCG	CTAACGACGT
6580	TCTGTGGCTC	TCCCTCACTG	CTGCCGAATA	TGATCAAACT	ACTTATGGTT	CGTCGACCAA	CCCTATGTAT
6650	GTTTCAGATA	CTGTTACCCT	CGTTAACGTG	GCAACGGGAG	CCCAGGCTGT	CGCCCGCTCT	CTTGATTGGT
6720	CTAAGGTCAC	TCTAGATGGT	CGACCTCTCA	CTACTATTCA	GCAGTACTCT	AAGACATTTT	ATGTTCTCCC
6790	ACTCCGTGGG	AAGCTTTCTT	TCTGGGAGGC	TGGCACCACT	AAGGCCGGGT	ATCCTTACAA	TTATAATACC
6860	ACTGCTAGTG	ACCAAATTCT	GATAGAAAAT	GCAGCCGGCC	ACCGTGTTGC	TATATCTACC	TACACTACTA
6930	GCCTTGGTGC	TGGTCCTGTG	TCTGTCTCTG	CGGTAGGTGT	GCTTGCCCCC	CATTCTGCTT	TAGCTGTGCT
7000	TGAGGATACT	ATTGATTACC	CCGCTCGTGC	CCATACTTTT	GATGATTTCT	GCCCTGAGTG	CCGTGATCTC
7070	GGCCTACAGG	GCTGTGCTTT	TCAATCCACT	ATCGCTGAGC	TTCAGCGTCT	TAAAGTGAAG	GTAGGCAAAA
7140	CCCGGGAGTC	TTAATTAATT	CCATCTGTGC	CCCCTTCAAG	GTATTGGTTT	ATTTCTTCTC	TTCTGCGTTT
7210	CGCGCTCCCT	GGANNNNNNN	NNNNNNN				

I4-POST-RBV

File name	**I4-POST-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 455 884**
Mapped reads	**1 455 884 (100.00%)**
Average read length	**148nt**
Coverage	**7 216nt (99.71%)**
Average depth	**26 632 reads/site**



I4-POST-RBV Consensus sequence

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  70  ATTGAGCAGG CTGCTCTGGC TGCGGCTAAC TCCGCCTTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTGT
 140  CCCGTGTTCA GACTGACATT CTCATCAATT TGATGCAACC CCGGCAGCTC GTATTCCGAC CTGAAGTTTT
 210  GTGGAATCAC CCGATCCAGC GAGTTATACA CAATGAGCTT GAGCAGTACT GCCGTGCCCG CGCCGGTCGC
 280  TGCCTGGAGG TCGGGGCTCA TCCGAGATCC ATTAATGACA ACCCTAACGT CCTGCACCGG TGTTTCCTTC
 350  GCCCGGTCGG GAGAGATGTA CAGCGTTGGT ATTCCGCCCC GACTCGCGGC CCAGCTGCCA ACTGCCGGCG
 420  TTCCGCATTA CGTGGCCTAC CCCCTGTCGA CCGTACTTAC TGTTTCGACG GGTTCTCCCG CTGTGCTTTT
 490  GCTGCTGAAA CTGGAATTGC TTTGTATTCA CTACATGACC TCTGGCCTGC CGATGTCGCG GAGGCCATGG
 560  CCCGTCATGG GATGACACGC CTGTACGCAG CCCTCCATTT ACCCCCTGAG GTTCTGTTAC CACCTGGTAC
 630  TTACCATACC ACCTCTTATT TGTTGATTCA TGACGGCAAT CGCGCCGTCG TAACTTATGA GGGGGATACC
 700  AGCGCAGGTT ATAACCATGA TGTGTCCATT CTTCGCGCAT GGATTCGCAC AACTAAAATA GTTGGCAACC
 770  ATCCGTTGGT TATAGAAAGG GTCCGTGCTA TCGGCTGCCA TTTTGTACTA CTCCTTACTG CTGCCCCTGA
 840  GCCATCCCCT ATGCCTTATG TCCCATACCC TCGTTCAACG GAGGTGTATG TCAGGTCCAT ATTTGGCCCC
 910  GGCGGCTCGC CATCTCTGTT CCCGTCAGCC TGCTCTACTA AATCCACATT TCATGCTGTT CCGGTCCATA
 980  TTTGGGACCG GCTCATGCTC TTCGGCGCCA CTCTAGACGA CCAAGCCTTT TGTTGCTCGC GGCTTATGAC
1050  TTATCTCCGC GGGATTAGTT ATAAGGTGAC CGTTGGTGCA CTCGTCGCCA ACGAAGGTTG GAACGCCTCA
1120  GAGGATGCGC TCACTGCTGT GATCACTGCA GCCTACTTGA CCATCTGTCA CCAGCGCTAC CTCCGAACTC
1190  AGGCTATATC TAAAGGCATG CGTCGGCTAG AAGTCGAGCA TGCCCAGAAG TTTATTACTA GACTTTATAG
1260  CTGGCTGTTT GAAAAGTCCG GTCGAGATTA TATCCCCGGT CGTCAACTTC AGTTTTACGC CCAGTGTCGC
1330  CGTTGGCTGT CGGCAGGTTT TCATCTTGAC CCAAGAGTGC TTGTCTTTGA TGAGGCGGTT CCCTGTCGTT
1400  GTAGGAGTTT TCTGAAGAAG GCTGCCGGCA AGTTCTGCTG CTTTATGAAG TGGCTGGGGC AGGAGTGCAC
1470  TTGTTTTCTG GAGCCGGCTG AGGGTTTGAT CGGCGATTGT GGTCACGATA ATGAGGCCTA CGAGGGTTCT
1540  GAAGTAGATC CTGCTGAGCC CGCCCACCTC GATGTCTCTG GAACCTACAC AGTTCATGGC CGTCAACTTG
1610  AGGCTCTATA TAGGGCGCTC AACATCCCAC ATGACATTGT GGCCCGTGCG TCCCGCCTGA CAGCCACTGT
1680  GGAACTCACT GCCGGCCCAG ATTGCCTCGA ATGCCGTACA GTGCTCGGAA ATAAGACCTT CCGCACATCG
1750  ATAATGGATG GCGCTCACCT CGAGGCTAAC GGCCCTGAGC AGTATGTTTT AACATTTGAC GACTCCCGCC
1820  AAGCGATGGG GGCTGGGCCG CATAGTCTTA CATATGAGCT CACCCCTGCT GGTTTGCAGG TTAAGATATC
1890  CTCTAATGGC CTAGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGTGAGGTT
1960  GCAGCCTTCT GCAGTGCTTT ATATAGATAC AATAGATTTA CCCAGCACCA TTCGCTAACC GGCGGACTGT
2030  GGCTGCACCC TGAGGGGCTT CTGGGCTTGT TTCCCCCCTT TTCCCCCGGG CATGTCTGGG AGTCCACAAA
2100  TCCTTTTTGC AGTGAGGACA CACTTTACAC CCGTACTTGG TCTACGTCTG GTTTTTCTAG TGATTTCTCT
2170  CCCCCTGAGG CAGCTGCCGC AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GGCTTCTCTA TACCTACCCA GATGGAGCTA AGGTCTATGC AGGCTCCTTG TTCGAGTCAG
2450  ATTGCGACTG GCTGGTTAAC GCGGCCAATC CTCGCCACCA CCCTGGTGGC GGTCTTTGCC ATGCTTTTTA
2520  CCAGCGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATC ATGCGTGAGG GCCTCGCCGC GTACACCTTG
2590  ACCCCACGCC CAATTATTCA TGCTGTGGCT CCTGATTATA GGGTTGAGCA GAACCCCAAG AGATTGGAGG
2660  CGGCGTACCG CGAGACTTGC TCTCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT CACAATAACT GAGGACACAG
2870  CCCGCACGGC TAACCTCGCA CTTGAGATTG ACTCTGCTAC TGAGGTGGGC CGTGCTTGTA CCGGCTGTAC
2940  TGTTAGCCCT GGCATTGTCC ATTATCAGTT TACTGCCGGG GTGCCTGGCT CCGGGAAGTC TAGGTCTATA
3010  CAACAAGGGG ACGTCGATGT TGTGGTTGTA CCAACCCGCG AGCTTCGCAA TAGCTGGCGG CGTCGTGGCT
3080  TTGCTGCTTT TACACCTCAT ACGGCGGCCC GCGTCACTGC CGGCCGACGT GTTGTGATTG ATGAGGCGCC
3150  TTCGCTTTCA CCACATTTGC TGCTGCTTCA TATGCAGCGA GCTTCGTCAG TCCACCTTCT CGGCGACCCC
3220  AACCAGATTC CCGCCATTGA TTTCGAGCAT GCAGGCCTAG TGCCAGCGAT CCGCCCTGAG CTTGCCCCAA
3290  CCAGTTGGTG GCACGTTACA CACCGTTGCC CTGCCGACGT GTGTGAGCTC ATACGCGGGG CTTATCCCAA
3360  AATCCAAACT ACTAGCCGCG TGGTGCGGTC TCTGTTTTGG AATGAGCCTG CGGTGGGCCA GAAGTTGGTT
3430  TTTACGCAAG CAGCTAAGGC AGCCAACCCC GGTGCAATTA CTGTTCACGA GGCCCAGGGT GCTACATTCA
3500  CAGAAACTAC CATTATTGCT ACGGCCGATG CTAGGGGCCT TATTCAGTCC TCTCGTGCAC ATGCAATCGT
3570  TGCACTTACT CGCCACACAG AGAAATGTGT TATTTTGGAC GCCCCCGGCC TACTGCGTGA GGTCGGTATT
3640  TCTGATATAA TTGTTAATAA TTTTTTCTTA GCCGGCGGTG AAGTCGGCCA CCACCGCCCT TCCATTATAC
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I4-POST-RBV Consensus sequence

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3780  CCACCAGTTG GCTGAGGAGC TGGGCCACCG CCCAGTCCCA GTCGCCGCTG TACTGCCGCC CTGCCCTGAG
3850  CTTGAGCAGG GTTTACTTTA CATGCCGCAA GAGCTCACAG TGTCCGACAG CGTTTTAGTC TTCGAGCTCA
3920  CGGACATAGT TCACTGCCGA ATGGCGGCCC CTAGTCAACG GAAGGCTGTC CTTTCAACGC TTGTTGGCAG
3990  GTACGGCCGC CGAACGAGGC TGTATGAGGC AGCTCACTCC GATGTCCGCG AGTCTCTGGG GAGGTTTATC
4060  CCTACCATCG GACCAGTTCA GGCCACCACA TGTGAGTTGT ACGAGTTGGT AGAGGCTATG GTTGAGAAGG
4130  GTCAGGACGG TTCCGCTGTG TTAGAGCTTG ATCTCTGTAG CCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAATCACTGT AATAAGTTTA CAACAGGTGA GACTATTGCT CATGGCAAGG TGGGCCAGGG CATTTCGGCC
4270  TGGAGCAAGA CCTTTTGTGC CCTATTCGGA CCGTGGTTCC GTGCCATTGA GAAGGAGATC TTAGCCCTAC
4340  TCCCACCTAA TATCTTCTAT GGTGACGCCT TTGAAGAGTC AGTGTTCTCT GCGGCCATTT CTGGAGCAGG
4410  TTCTAGTATG GTTTTTGAGA ATGATTTCTC TGAGTTTGAC AGTACCCAAA ACAATTTCTC CCTCGGCCTT
4480  GAGTGTGTTA TTATGGAGGA GTGTGGCATG CCCCAGTGGC TCATTAGGTT ATACCATTTA GTCAGGTCGG
4550  CCTGGACTTT GCAGGCCCCG AAGGAGTCCC TGAAAGGCTT TTGGAAGAAG CACTCTGGTG AGCCCGGTAC
4620  ACTCCTCTGG AATACTGTCT GGAACATGGC AATCATAGCG CATTGCTACG AATTTCGTGA CCTGAGAGTG
4690  GCAGCGTTTA AAGGGGATGA TTCAGTAGTC CTTTGCAGTG ATTACCGCCA GAGCCGTAAT GCAGCTGCCT
4760  TGATCGCTGG TTGTGGGTTA AAATTGAAGG TTGATTACCG CCCCATAGGG TTGTACGCCG GTGTTGTGGT
4830  GGCCCCGGGT CTCGGGGCAC TCCCTGACGT CGTTCGGTTC GCCGGCCGGT TGTCTGAGAA GAATTGGGGC
4900  CCGAGTCCCG AGCGCGCCGA GCAGTTACGC TTGGCTGTTT GTGACTTCCT TCGAAAGTTA ACGAATGTTG
4970  CGCAGGTTTG TGTTGATGTT GTGTCCCGTG TTTATGGGGT TAGTCCTGGG CTGGTACATA ACCTTATTGG
5040  CATGTTACAG ACTATAGCCG ATGGGAAGGC CCATTTTACT GAGACTGTTA AACCTGTACT GGATCTTACA
5110  AATTCTATCA TACAGAGGTT GGAATGAATA ACATGTTGTG TGCATTGCCC ATGGGTTCAC CATGCGCCCT
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5320  CTTTGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGACGTCG TATCACAACC CGGGGCTGGA
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5530  CCCAGTCCCC GATGTTGATT CTCGCGGTGC TATTTTGCGC CGCCAGTATA ATTTGTCTAC TTCCCCTCTG
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5670  AGGATGGCAC CAATACTCAT ATCATGGCGA CTGAGGCATC TAACTATGCT CAATATCGGG TTGTCCGAGC
5740  CACAATCCGT TATCGCCCTT TGGTGCCGAA TGCTGTTGGA GGCTATGCAA TTTCTATTTC CTTTTGGCCC
5810  CAGACTACAA CTACTCCCAC CTCTGTTGAT ATGAATTCTA TTACCTCTAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT AGCCTCCGAG TTGGTTATTC CAAGCGAGCG CCTCCATTAT CGTAATCAGG GCTGGCGTTC
5950  TGTTGAGACC TCGGGTGTGG CTGAGGAGGA GGCGACATCG GGCCTGGTTA TGCTCTGTAT CCATGGTTCC
6020  CCTGTTAATT CCTATACTAA TACGCCCTAC ACTGGGGCTT TGGGACTTCT TGACTTTGCA CTCGAGCTCG
6090  AATTTAGGAA CTTGACCCCT GGGAACACTA ACACCCGTGT GTCCCGGTAC ACCAGCACAG CCCGCCACCG
6160  CCTACGCCGT GGCGCCGATG GGACTGCCGA GCTCACGACC ACTGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CATTTTACCG GGATGAATGG CGTCGGCGAG GTGGGCCGTG GCATAGCCCT AACTTTATTT AATCTCGCTG
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6580  TCTGTGGCTC TCCCTCACTG CTGCCGAATA TGATCAAACT ACTTATGGTT CGTCGACCAA CCCTATGTAT
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6860  ACTGCTAGTG ACCAGATTCT GATAGAAAAT GCAGCCGGCC ACCGTGTTGC TATATCTACC TACACTACTA
6930  GCCTTGGTGC TGGTCCTGTG TCTGTCTCTG CGGTAGGTGT ACTTGCCCCC CATTCTGCTT TAGCTGTACT
7000  TGAGGATACT ATTGATTACC CCGCTCGTGC CCATACTTTT GATGATTTCT GCCCTGAGTG CCGTAATCTC
7070  GGCCTACAGG GCTGTGCTTT TCAATCCACT ATCGCTGAGC TTCAGCGTCT TAAAGTGAAG GTAGGCAAAA
7140  CCCGGGAGTC TTAATTAATT CCATCTGTGC CCCCTTCAAG GTCTTGGTTT ATTTCTTCTC TTCTGCGTTT
7210  CGCGCTCCCT GGAANNNNNN NNNNNNN
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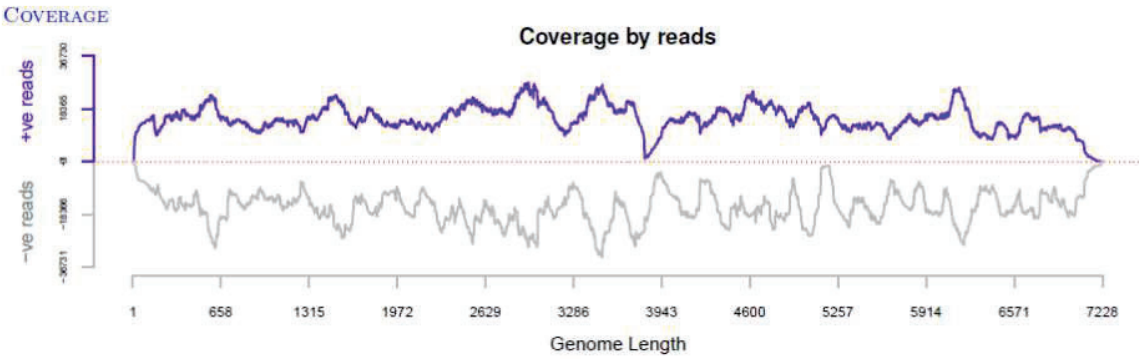
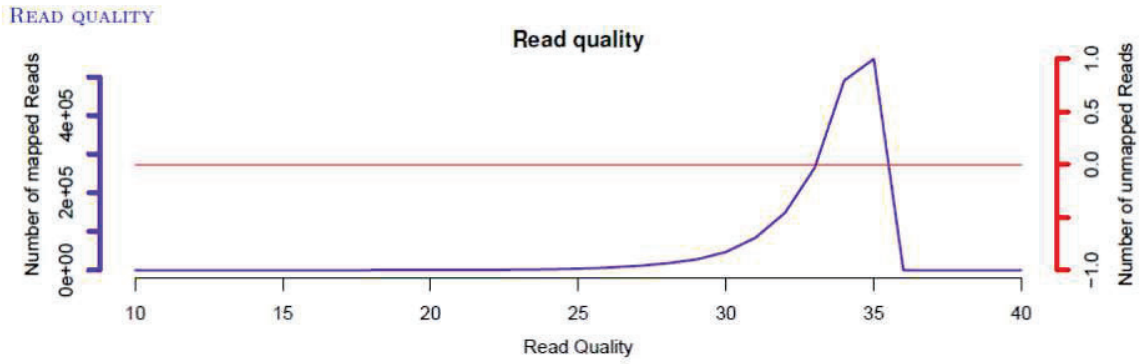
J1-PRE-RBV

File name	**J1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 651 056**
Mapped reads	**1 651 056 (100.00%)**
Average read length	**136nt**
Coverage	**7 224nt (99.82%)**
Average depth	**30 831 reads/site**





J1-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGA TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGTGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGTCTCC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCTCG CTGCTCATTT
 490  GCTGCGGAAA CTGGGGTTGC TCTTTACTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCTCCTGAA GTACTACTAC CACCTGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG TTGCTTACTG CAGCCCCTGA
 840  GCCGTCGCCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCTCTATT CCCATCTGCT TGCTCTACGA AGTCCACATT TCACGCTGTC CCGGTTCATA
 980  TCTGGGACCG GCTCATGCTT TTTGGCGCAA CTCTGGATGA TCAGGCATTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGTGCC CTTGTCGCTA ACGAAGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTACCTGA CCATTTGTCA TCAGCGTTAC CTCCGTACTC
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1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCTGAGCC CGCCTGTCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGAGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCTCGCTTGA CTGCCACCGT
1680  CGAACTCGTT GCAAGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT TCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCT CCTATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGACTGCA CTGCAACATT CCCTCCGGGC GGGGCCCCTA GTGCTGCCCC GGGGGAAGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCCTGTACAC CCGAACATGG TCAACATCTG GTTTTTCTAG TGATTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCC CCTGTTAGTG
2240  ACATCTGGGT GTTACCGCCG CCTTCTGAAG AATTTCAGGT TGACACAGTG CCCGCTCCCT CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACTCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGACTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGGGGC GGCCTTTGCC ATGCCTTCTA
2520  CCAACGCTTC CCCGAGTCTT TCCACCCAAC TGAGTTTATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATCATCCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACCTGC TCCCGCCGCG GTACTGCCGC CTACCCACTC CTTGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CACCGCCCCG GGGACGAGCT CTACCTAACC
2800  GACCCCGCAG CTACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAGGTCGGC CGGGCTTGTG CCGGTTGTGC
2940  AGTTAGTCCT GGGGTTGTCC ATTATCAGTT TACTGCTGGG GTCCCGGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCAGCTTT TACACCACAT ACGGCGGCCC GTGTCACCAC GGGCCGTCGT GTTGTAATTG ATGAGGCCCC
3150  ATCTCTCCCA CCGCATTTGT TGCTACTACA TATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCG
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3290  CCAGTTGGTG GCATGTTACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATACGCGGGG CTTATCCTAA
3360  AATTCAAACC ACTAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
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3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
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J1-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCACCT  TCCTGCCAGA  TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG  CCCAGCCCCC  GTCGCCGCTG  TCTTGCCCCC  CTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAG  GAGCTTACGG  TGTCAGATAG  CGTGCTAGTC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCTGCCC  CCAGCCAGCG  GAAGGCCGTC  CTATCGACGC  TTGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC  AGCTCACTCT  GACGTCCGTG  AGTCCCTGGC  TAGGTTCATC
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4200  GAAAGATTGT AATAAATTCA CCACAGGGGA  GACCATTGCC  CACGGCAAGG  TCGGCCAGGG  CATCTCGGCT
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4830  GGCCCCTGGC TTGGGGACGC TACCCGATGT  GGTGCGCTTT  GCCGGCCGGC  TGTCTGAGAA  GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAACTGCGC  CTAGCTGTTT  GTGACTTCCT  TCGAGGGTTA  ACGAATGTTG
4970  CGCAGGTGTG TGTTGATGTC GTATCCCGAG  TTTATGGAGT  TAGCCCTGGG  TTGGTACATA  ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC  CCATTTTACA  GAGACTGTTA  AACCTGTGCT  TGACCTCACG
5110  AACTCTATTA TACAGCGGGT GGAATGAATA  ACATGTTTTG  TGCATTGCCC  ATGGGATCAC  CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CCTCGTGCTT  CTGCCTATGC  TGCCCGCGCC  ACCGGCCGGC  CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTA  CCGGCAGTGG  TTTCTGGGGT  GACAGGGTTG  ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA  CCCCTTTGCC  GCCGATGTCG  TACCGCAATC  CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTC  GGCTCCTCTT  GGCGTGATCA  GTCCCAGCGC  CCCTCCGCTG
5460  CCCCACGCCG TCGATCTGCC CCAGCTGGGG  CTGCGCCGCT  GACCGCCATA  TCACCTGCTC  CTGATACAGC
5530  TCCTGTACCT GATGTTGACT CGCGCGGCGC  CATATTGCGA  CGCCAGTACA  ATTTATCTAC  ATCTCCGCTC
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG  GTTCTCTATG  CTGCCCCGCT  AAACCCTTTG  CTGCCCCTTC
5670  AGGATGGCAC TAACACTCAC ATTATGGCCA  CTGAGGCATC  TAATTATGCC  CAGTATCGGG  TCGTCCGAGC
5740  TACGATCCGT TATAGGCCAT TGGTGCCAAA  TGCTGTCGGT  GGTTATGCAA  TATCCATCTC  ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT  ATGAACTCTA  TTACTTCCAC  TGATGTTAGG  ATTTTAGTCC
5880  AGCCTGGCAT TGCTTCTGAG TTGGTTATCC  CTAGTGAGCG  CCTCCATTAT  CGTAACCAGG  GCTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAGGAGGA  GGCTACTTCT  GGTTTAGTAA  TGCTTTGTAT  CCATGGCTCC
6020  CCTGTTAATT CCTATACCAA TACCCCCTAT  ACCGGGGCGC  TTGGACTCCT  TGACTTCGCT  TTAGAGCTTG
6090  AGTTTAGGAA CCTGACACCC GGGAACACCA  ACACCCGTGT  GTCCCGGTAC  ACAAGCACAG  CCCGTCATCG
6160  GCTACGCCGC GGTGCTGATG GCACCGCCGA  ACTTACCACC  ACAGCAGCCA  CGCGCTTCAT  GAAGGACCTG
6230  CACTTCACCG GTACGAATGG GGTTGGTGAG  GTGGGTCGTG  GTATTGCTCT  CACACTCTTT  AATCTTGCTG
6300  ACACGCTTCT CGGTGGCTTG CCGACAGAAT  TAATTTCGTC  GGCTGGGGGA  CAGTTATTCT  ACTCCCGCCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT  CAAGTTATAT  ACATCTGTAG  AGAATGCGCA  GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA CATAGATCTG  GGTGACTCCC  GTGTGGTCAT  CCAGGACTAT  GACAACCAGC
6510  ATGAACAGGA TCGGCCCACC CCCTCACCTG  CCCCTTCTCG  CCCTTTTTCG  GTTCTTCGCG  CTAATGATGT
6580  TCTATGGCTT TCTCTCACTG CCGCCGAGTA  TGACCAGACT  ACATATGGGT  CGTCCACCAA  CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG  GCTACAGGAG  CTCAGGCTGT  CGCCCGTTCT  CTCGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGCCCTCTTA  CTACTATCCA  GCAGTATTCC  AAAACATTTT  ATGTTCTCCC
6790  GCTTCGCGGG AAGCTATCTT TCTGGGAGGC  CGGGACGACT  AAGGCCGGCT  ACCCCTATAA  TTACAACACA
6860  ACTGCTAGTG ATCAGATTTT GATTGAAAAT  GCAGCTGGCC  ATCGTGTTGC  TATTTCCACG  TATACCACCA
6930  GCTTGGGCGC CGGCCCTGTG TCTGTCTCTG  CAGTCGGTGT  TTTAGCCCCA  CATTCAGCTC  TTGCAGTCCT
7000  TGAAGACACT ATTGACTACC CTGCCCGTGC  CCATACTTTT  GATGATTTTT  GCCCGGAGTG  TCGTGCCCTT
7070  GGTCTGCAGG GGTGTGCCTT CCAGTCTACT  ATTGCTGAGC  TTCAGCGTCT  TAAAATGAAG  GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTTTGTGC  CCCCTTCATA  TCTTTGCTTT  ATTTCTTTTT  TTCTGCGGTT
7210  CGCGCTCCCT GGAANNNNNN NNNNNNN
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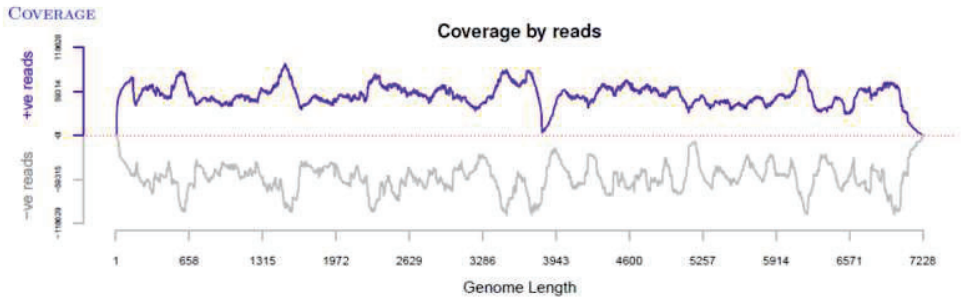
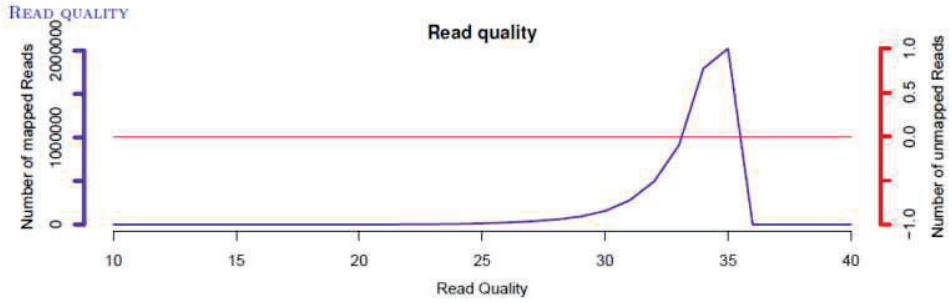
J2-PRE-RBV

File name	**J2-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**5 883 395**
Mapped reads	**5 883 395 (100.00%)**
Average read length	**138nt**
Coverage	**7 225nt (99.83%)**
Average depth	**111 533 reads/site**





J2-PRE-RBV Consensus sequence

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   1  GCAGACCACG TATGTGGTCG ATGCCATGGA GGCCCACCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGATTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTTTAGAGA TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGTGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGTCTCC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCTCG CTGCTCATTT
 490  GCTGCGGAAA CTGGGGTTGC TCTTTACTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC CTGTATGCTG CACTACATCT CCCTCCTGAA GTACTACTAC CACCTGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGATGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG TTGCTTACTG CAGCCCCTGA
 840  GCCGTCGCCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCTCTATT CCCATCTGCT TGCTCTACGA AGTCCACATT TCACGCTGTC CCGGTTCATA
 980  TCTGGGACCG GCTCATGCTT TTTGGCGCAA CTCTGGATGA TCAGGCATTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGTGCC CTTGTCGCTA ACGAAGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTACCTGA CCATTTGTCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC TAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTT GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTACGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGTTT TCATCTTGAC CCTAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCTGAGCC CGCCTGTCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGAGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCTCGCTTGA CTGCCACCGT
1680  CGAACTCGTT GCAAGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT TCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCT CCTATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGACTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GTGCTGCCCC GGGGGAAGTA
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCCTGTACAC CCGAACATGG TCAACATCTG GTTTTTCTAG TGATTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCC CCTGTTAGTG
2240  ACATCTGGGT GTTACCGCCG CCTTCTGAAG AATTTCAGGT TGACACAGTG CCCGCTCCCT CTGCCCCTGA
2310  GCCCGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACTCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGACTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGGGGC GGCCTTTGCC ATGCCTTCTA
2520  CCAACGCTTC CCCGAGTCTT TCCACCCAAC TGAGTTTATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATCATCCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACCTGC TCCCGCCGCG GTACTGCCGC CTACCCACTC CTTGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CACCGCCCCG GGGACGAGCT CTACCTAACC
2800  GACCCCGCAG CTACCTGGTT CGAGGCTAAT AAGCCAACAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAGGTCGGC CGGGCTTGTG CCGGTTGTGC
2940  AGTTAGTCCT GGGGTTGTCC ATTATCAGTT TACTGCTGGG GTCCCGGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCAGCTTT TACACCACAT ACGGCGGCCC GTGTCACCAC GGGCCGTCGT GTTGTAATTG ATGAGGCCCC
3150  ATCTCTCCCA CCGCATTTGT TGCTACTACA TATGCAGCGG GCCTCGTCGG TCCACCTTCT CGGCGACCCG
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTTACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATACGCGGGG CTTATCCTAA
3360  AATTCAAACC ACTAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCGATTA CAGTCCACGA GGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACAGCTGATG CTAGGGGGCT TATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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J2-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCACCT  TCCTGCCAGA  TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG  CCCAGCCCCC  GTCGCCGCTG  TCTTGCCCCC  CTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAG  GAGCTTACGG  TGTCAGATAG  CGTGCTAGTC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCTGCCC  CCAGCCAGCG  GAAGGCCGTC  CTATCGACGC  TTGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC  AGCTCACTCT  GACGTCCGTG  AGTCCCTGGC  TAGGTTCATC
4060  CCTACCATTG GGCCCGTTCA GGCGACTACG  TGTGAGTTAT  ATGAGCTGGT  TGAGGCCATG  GTGGAGAAAG
4130  GTCAGGACGG CTCTGCCGTG CTTGAGCTCG  ACCTCTGCAA  TCGTGATGTA  TCGCGTATCA  CATTTTTCCA
4200  GAAAGATTGT AATAAATTCA CCACAGGGGA  GACCATTGCC  CACGGCAAGG  TCGGCCAGGG  CATCTCGGCT
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGT  CCGTGGTTTC  GTGCCATTGA  AAAAGAAATA  TTAGCCCTGC
4340  TCCCGCCTAA TATTTTCTAC GGCGACGCAT  ACGAGGAGTC  TGTGTTTGCC  GCCGCTGTGT  CAGGGGCAGG
4410  CTCAAGCATG GTATTTGAGA ATGATTTTTC  AGAGTTTGAT  AGCACCCAAA  ATAACTTCTC  CCTTGGTCTC
4480  GAGTGTGTGG TCATGGAGGA ATGTGGCATG  CCCCAGTGGT  TAATCCGGTT  GTACCATTTG  GTTCGGTCAG
4550  CCTGGATCCT ACAGGCGCCG AAGGAGTCTC  TTAAGGGATT  TTGGAAGAAG  CATTCTGGTG  AGCCCGGCAC
4620  CCTCCTCTGG AATACTGTTT GGAACATGGC  GATCATAGCA  CACTGCTATG  AATTTCGTGA  TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA TTCCGTGGTC  CTCTGTAGCG  ACTACCGTCA  GAGCCGCAAC  GCAGCGGCCC
4760  TGATTGCAGG TTGCGGACTC AAACTGAAGG  TTGATTATCG  CCCTATTGGG  TTGTATGCTG  GTGTGGTGGT
4830  GGCCCCTGGC TTGGGGACGC TACCCGATGT  GGTGCGCTTT  GCCGGCCGGC  TGTCTGAGAA  GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAACTGCGC  CTAGCTGTTT  GTGACTTCCT  TCGAGGGTTA  ACGAATGTTG
4970  CGCAGGTGTG TGTTGATGTC GTATCCCGAG  TTTATGGAGT  TAGCCCTGGG  TTGGTACATA  ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGCAAAGC  CCATTTTACA  GAGACTGTTA  AACCTGTGCT  TGACCTCACG
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5180  AGGGCTGTTC TGTTGCTGTT CCTCGTGCTT  CTGCCTATGC  TGCCCGCGCC  ACCGGCCGGC  CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTA  CCGGCAGTGG  TTTCTGGGGT  GACAGGGTTG  ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA  CCCCTTTGCC  GCCGATGTCG  TACCGCAATC  CGGGGCTGGA
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5460  CCCCACGCCG TCGATCTGCC CCAGCTGGGG  CTGCGCCGCT  GACCGCCATA  TCACCTGCTC  CTGATACAGC
5530  TCCTGTACCT GATGTTGACT CGCGCGGCGC  CATATTGCGA  CGCCAGTACA  ATTTATCTAC  ATCTCCGCTC
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG  GTTCTCTATG  CTGCCCCGCT  AAACCCTTTG  CTGCCCCTTC
5670  AGGATGGCAC TAACACTCAC ATTATGGCCA  CTGAGGCATC  TAATTATGCC  CAGTATCGGG  TCGTCCGAGC
5740  TACGATCCGT TATAGGCCAT TGGTGCCAAA  TGCTGTCGGT  GGTTATGCAA  TATCCATCTC  ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT  ATGAACTCTA  TTACTTCCAC  TGATGTTAGG  ATTTTAGTCC
5880  AGCCTGGCAT TGCTTCTGAG TTGGTTATCC  CTAGTGAGCG  CCTCCATTAT  CGTAACCAGG  GCTGGCGCTC
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6090  AGTTTAGGAA CCTGACACCC GGGAACACCA  ACACCCGTGT  GTCCCGGTAC  ACAAGCACAG  CCCGTCATCG
6160  GCTACGCCGC GGTGCTGATG GCACCGCCGA  ACTTACCACC  ACAGCAGCCA  CGCGCTTCAT  GAAGGACCTG
6230  CACTTCACCG GTACGAATGG GGTTGGTGAG  GTGGGTCGTG  GTATTGCTCT  CACACTCTTT  AATCTTGCTG
6300  ACACGCTTCT CGGTGGCTTG CCGACAGAAT  TAATTTCGTC  GGCTGGGGGA  CAGTTATTCT  ACTCCCGCCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT  CAAGTTATAT  ACATCTGTAG  AGAATGCGCA  GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA CATAGATCTG  GGTGACTCCC  GTGTGGTCAT  CCAGGACTAT  GACAACCAGC
6510  ATGAACAGGA TCGGCCCACC CCCTCACCTG  CCCCTTCTCG  CCCTTTTTCG  GTTCTTCGCG  CTAATGATGT
6580  TCTATGGCTT TCTCTCACTG CCGCCGAGTA  TGACCAGACT  ACATATGGGT  CGTCCACCAA  CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG  GCTACAGGAG  CTCAGGCTGT  CGCCCGTTCT  CTCGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGCCCTCTTA  CTACTATCCA  GCAGTATTCC  AAAACATTTT  ATGTTCTCCC
6790  GCTTCGCGGG AAGCTATCTT TCTGGGAGGC  CGGGACGACT  AAGGCCGGCT  ACCCCTACAA  TTACAACACA
6860  ACTGCTAGTG ATCAGATTTT GATTGAAAAT  GCAGCTGGCC  ATCGTGTTGC  TATTTCCACG  TATACCACCA
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7000  TGAAGACACT ATTGACTACC CTGCCCGTGC  CCATACTTTT  GATGATTTTT  GCCCGGAGTG  TCGTGCCCTT
7070  GGTCTGCAGG GGTGTGCCTT CCAGTCTACT  ATTGCTGAGC  TTCAGCGTCT  TAAAATGAAG  GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTTTGTGC  CCCCTTCATA  TCTTTGCTTT  ATTTCTTTTT  TTCTGCGGTT
7210  CGCGCTCCCT GGAAANNNNN NNNNNNN
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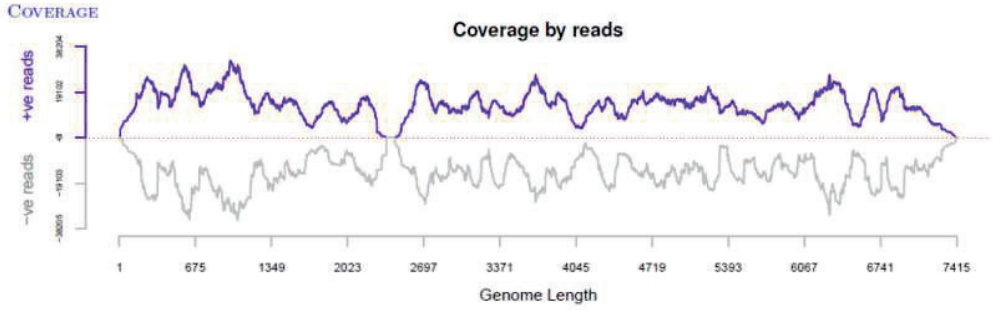
K1-PRE-RBV

File name	**K1-PRE-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 591 019**
Mapped reads	**1 591 019 (100.00%)**
Average read length	**134nt**
Coverage	**7 338nt (98.85%)**
Average depth	**28 503 reads/site**



K1-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTCCGAC CTGAAGTACT
 210  CTGGAATCAC CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTATT GTCGGGCCCG GGCTGGTCGC
 280  TGTCTGGAGG TTGGGGCCCA CCCAAGATCC ATTAATGATA ACCCAAATGT TCTGCACCGG TGCTTTTTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC TCTTTACTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAG GTACTACTAC CACCTGGTAC
 630  TTACCATACA ACATCATACC TTCTGATTCA CGACGGAGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGTAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTCACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTCTATG TCCGCTCTAT ATTTGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCCACATT TCACGCTGTT CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTC TTTGGTGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGATG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCCTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  CTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGCTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTCTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAC GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAAGTCGACC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AATATCCCGC ATGACATCGC AGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGCCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCAAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGTCCG CATAGTCTCT CCTATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATCTC
1890  ATCTAATGGG CTGGATTGCA CTGCAACATT CCCCCCGGGC GGCGCCCCAA GCGCTGCCCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAT AACAGGTTTA CTCAGCGCCA TTCGCTTATA GGCGGCTTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCCCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGC AGTGCCGGCC GCTACTCCGG GGTCACGCCG CCCTACACCT CCTGCTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TAACACAACT CCCGCCCCCC TTGCCCCCGG
2310  GCCCGCTCAA CCATCTAGCC CCGCTGAGCC AAAAGCTCCC GTGCGTAAGC CGCCAANNNN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNGA CCGCCACCTT
2450  CTGAAGAATT TCAGGTTAAC ACAACTCCCG CCCCCCTTGC CCCCGGGCCC GCTCAACCAT CTAGCCCCGC
2520  TGAGCCAAAA GCTCCCGTGC GTAAGCCGCC AACACCACCA TCCCCGCGCA CCCGCCGCCT TCTTTACACC
2590  TATCCTGATG GGGCAAAGGT GTACGCGGGG TCATTGTTTG AGTCTGACTG TGATTGGCTG GTCAATGCGT
2660  CGAATCCCGG CCATCGTCCT GGAGGCGGCC TTTGCCACGC CTTCTATCAA CGCTACCCTG AGTCCTTCCA
2730  CCCAACTGAG TTCATCATGC GCGACGGTCT TGCCGCTTAC ACTTTAACTC CCAGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCATAAC CCAAAGAGGC TTGAGGCAGC ATACCGAGAA ACTTGCTCTC
2870  GCCGCGGTAC CGCCGCCTAT CCACTCCTCG GCTCAGGCAT ATACCAAGTC CCCGTCAGCC TCAGCTTTGA
2940  CGCCTGGGAG CGTAACCACC GCCCCGGGGA TGAGCTCTAC CTAACTGACC TCGCTGCTAC CTGGTTTGAG
3010  GCTAATAAGC CAACACAACC GGCCCTCACA ATAACTGAGG ATGCGGCCCG TACAGCCAAC TTGGCACTGG
3080  AGATCGATGC TGCTACGGAG GTCGGCCGGG CTTGTGCCGG CTGTGCAGTT AGTCCTGGGG TTGTGCACTA
3150  TCAGTTTACT GCTGGGGTCC CAGGCTCGGG GAAGTCGCGT TCTATACAGC AGGGGGATGT TGACGTAGTG
3220  GTTGTTCCTA CTCGGGAGCT CCGGAATAGT TGGCGTCGTC GGGGTTTCGC AGCTTTTACA CCTCATACGG
3290  CAGCCCGTGT CACTACGGGC CGTCGTGTTG TGATTGATGA GGCCCCATCC CTCCCACCGC ATTTGTTGCT
3360  ACTACACATG CAGCGGGCCT CGTCGGTCCA CCTTCTCGGC GACCCAAACC AGATCCCTGC CATAGACTTT
3430  GAGCATGCCG GCCTGGTCCC CGCTATACGC CCTGAGCTCG CGCCCACCAG TTGGTGGCAT GTCACCCATC
3500  GCTGCCCCGC TGACGTGTGC GAGCTTATAC GCGGGGCTTA TCCCAAAATC CAAACCACTA GCCGCGTGCT
3570  ACGGTCTCTA TTCTGGAATG AGCCTGCCAT TGGCCAGAAG TTAGTTTTCA CCCAGGCTGC TAAGGCCGCC
3640  AACCCTGGTG CGATTACAGT CCACGAGGCC CAGGGCGCCA CCTTCACGGA AACTACAATC ATAGCCACGG
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K1-PRE-RBV Consensus sequence

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3710  CTGATGCTAG GGGGCTTATC CAATCTTCCC GAGCTCACGC CATAGTCGCA CTCACCCGCC ACACAGAGAA
3780  GTGCGTTATT CTTGACGCCC CCGGCTTGTT ACGTGAGGTT GGTATATCGG ATGTGATTGT TAACAATTTT
3850  TTCCTCGCCG GTGGAGAGGT GGGTCACCAT CGCCCCTCTG TGATACCTCG CGGTAATCCT GACCAGAACC
3920  TCGCGACACT ACAGGCCTTT CCACCCTCCT GCCAAATTAG TGCCTATCAC CAGTTAGCTG AGGAACTTGG
3990  CCACCGCCCA GCCCCCGTCG CCGCTGTCTT GCCCCCTTGC CCTGAACTTG AGCAAGGCTT GTTATATATG
4060  CCGCAGGAGC TTACGGTGTC TGATAGCGTG CTGGTCTTCG AACTCACGGA CATAGTTCAC TGCCGGATGG
4130  CTGCCCCCAG CCAGCGGAAG GCCGTCCTAT CGACGCTCGT GGGTAGGTAC GGCCGTCGGA CGAAGCTGTA
4200  TGAAGCAGCT CACTCTGATG TCCGTGAGTC CCTGGCTAGA TTCATCCCCA CCATTGGGCC CGTTCAGGCT
4270  ACTACATGTG AGTTATATGA GCTGGTTGAG GCCATGGTGG AGAAAGGTCA GGATGGCTCT GCCGTGCTCG
4340  AGCTCGACCT CTGCAATCGT GATGTATCGC GTATCACATT TTTCCAGAAA GATTGTAACA AATTCACCAC
4410  AGGGGAGACC ATTGCCCACG GTAAGGTCGG CCAGGGCATC TCGGCTTGGA GTAAGACCTT CTGTGCCCTG
4480  TTTGGTCCGT GGTTTCGTGC TATTGAAAAA GAAATACTAG CCCTGCTCCC GCCTAATATT TTCTACGGCG
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4620  TTTTTCAGAG TTTGATAGCA CCCAAAATAA CTTCTCCCTT GGTCTCGAGT GCGTAGTCAT GGAGGAATGT
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4760  AGTCTCTTAA GGGATTTTGG AAGAAGCATT CTGGTGAGCC AGGCACCCTC CTCTGGAACA CTGTTTGGAA
4830  CATGGCGATC ATAGCACACT GCTATGAATT TCGTGATTTT AGGGTTGCCG CTTTCAAGGG AGATGATTCT
4900  GTGGTCCTCT GTAGCGACTA CCGTCAGAGC CGCAATGCAG CGGCCCTGAT TGCAGGTTGC GGGCTCAAAC
4970  TGAAGGTTGA TTATCGCCCT ATTGGGTTGT ATGCTGGTGT GGTGGTGGCC CCTGGCCTGG GGACACTACC
5040  CGATGTGGTG CGCTTTGCCG GCCGGCTGTC TGAGAAGAAC TGGGGCCCTG GGCCGGAGCG GGCCGAGCAG
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5250  CAAAGCCCAT TTTACAGAGA CTGTTAAACC TGTGCTTGAC CTCACGAATT CTATTATACA GCGGGTGGAA
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5670  CCGGGGCTGC GCCGCTGACT GCCATATCAC CTGCTCCTGA TACAGCTCCT GTACCTGATG TTGACTCGCG
5740  CGGCGCCATA TTGCGACGCC AGTACAATTT ATCCACATCC CCGCTCACAT CATCTGTTGC TTCGGGTACT
5810  AATTTGGTTC TTTATGCCGC CCCGCTAAAC CCTTTGCTGC CCCTTCAGGA TGGCACTAAC ACTCACATCA
5880  TGGCCACTGA GGCATCTAAC TATGCCCAGT ATCGGGTTGT CCGAGCTACG ATCCGTTACA GGCCATTGGT
5950  GCCGAATGCC GTCGGCGGCT ATGCAATATC CATCTCATTC TGGCCTCAGA CTACTACTAC CCCCACATCT
6020  GTTGATATGA ACTCTATTAC TTCCACTGAT GTTAGGATTC TAGTTCAGCC CGGTATTGCT TCTGAGTTGG
6090  TTATCCCCAG TGAGCGCCTC CATTATCGTA ACCAGGGCTG GCGCTCTGTG GAGACCTCGG GTGTGGCTGA
6160  AGAGGAGGCT ACTTCTGGTT TGGTAATGCT TTGCATCCAT GGTTCTCCTG TTAATTCCTA CACCAATACC
6230  CCTTATACCG GGGCGCTTGG ACTCCTTGAT TTTGCTTTAG AGCTTGAGTT TAGGAACTTG ACACCTGGAA
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6370  CGCCGAACTT ACCACTACAG CGGCTACGCG CTTCATGAAG GACCTGCACT TCACCGGTAC GAATGGGGTC
6440  GGTGAGGTGG GTCGTGGTAT TGCTCTCACA CTCTTTAATC TTGCTGACAC GCTTCTTGGT GGTTTGCCGA
6510  CAGAATTAAT TTCGTCGGCT GGGGGACAGT TATTTTACTC CCGCCCCGTC GTCTCGGCCA ATGGCGAGCC
6580  GACTGTCAAG TTATACACAT CTGTTGAGAA TGCGCAGCAG GATAAGGGGA TCGCTATTCC ACATGACATA
6650  GATCTGGGTG ACTCCCGTGT GGTCATCCAA GACTATGACA ACCAGCATGA GCAGGATCGA CCCACCCCCT
6720  CGCCTGCCCC TTCTCGCCCT TTTTCGGTCC TTCGCGCTAA TGATGTTCTT TGGCTCTCTC TTACTGCCGC
6790  TGAGTACGAC CAGACTACAT ATGGGTCGTC CACCAACCCG ATGTATGTCT CGGATACTGT CACATTTGTC
6860  AACGTGGCTA CAGGAGCCCA GGCTGTCGCC CGTTCCCTCG ACTGGTCTAA AGTCACTCTG GACGGCCGCC
6930  CTCTCACTAC TATCCAGCAG TACTCCAAAA CATTTTATGT TCTCCCGCTT CGCGGGAAGT TATCTTTCTG
7000  GGAGGCCGGG ACGACTAAGG CCGGTTATCC CTATAATTAC AACACAACTG CTAGTGATCA GATTTTGATT
7070  GAAAATGCGG CTGGTCATCG TGTTGCTATT TCCACGTATA CCACCAGCTT GGGTGCCGGT CCTGTGTCTG
7140  TTTCTGCAGT TGGTGTTTTA GCCCCACATT CGGCCCTTGC AGTCCTTGAA GATACTATTG ATTACCCTGC
7210  CCGTGCCCAC ACTTTTGATG ATTTTTGCCC GGAGTGTCGC GCTCTTGGTT TGCAGGGGTG TGCCTTCCAG
7280  TCTACTATTG CTGAGCTTCA GCGCCTTAAA ATGAAGGTAG GTAAAACCCG GGAGTTTTAA TCAATTTCCT
7350  TTGTGCCCCC TTCATAGCTT TTGCTTTATT TCTTTTTTCT GCGGTTCGCG CTCCCTGGAA NNNNNNNNNN
7420  NNN
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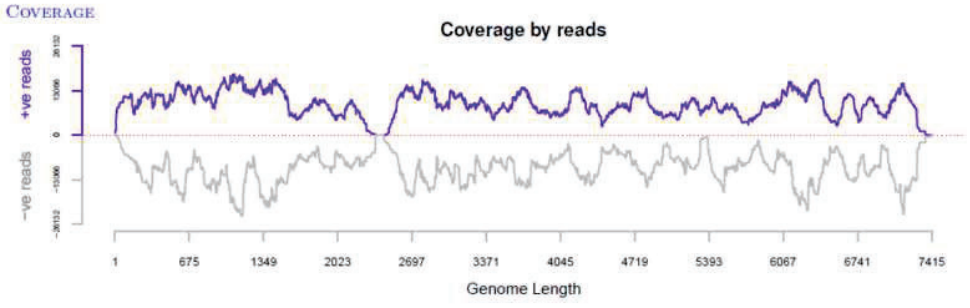
K2-PRE-RBV

File name	**K2-PRE-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 046 889**
Mapped reads	**1 046 889 (100.00%)**
Average read length	**136nt**
Coverage	**7 338nt (98.85%)**
Average depth	**19 004 reads/site**



K2-PRE-RBV Consensus sequence

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  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTCCGAC CTGAAGTACT
 210  CTGGAATCAC CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTATT GTCGGGCCCG GGCTGGTCGC
 280  TGTCTGGAGG TTGGGGCCCA CCCAAGATCC ATTAATGATA ACCCAAATGT TCTGCACCGG TGCTTTTTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC TCTTTACTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAG GTACTACTAC CACCTGGTAC
 630  TTACCATACA ACATCATACC TTCTGATTCA CGACGGAGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGTAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTCACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTCTATG TCCGCTCTAT ATTTGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCCACATT TCACGCTGTT CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTC TTTGGTGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGATG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCCTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  CTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCGGGCTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTCTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAC GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAAGTCGACC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
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1680  CGAACTTGTT GCAGGCCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCAAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGTCCG CATAGTCTCT CCTATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATCTC
1890  ATCTAATGGG CTGGATTGCA CTGCAACATT CCCCCCGGGC GGCGCCCCAA GCGCTGCCCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAT AACAGGTTTA CTCAGCGCCA TTCGCTTATA GGCGGCTTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCCCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGC AGTGCCGGCC GCTACTCCGG GGTCACGCCG CCCTACACCT CCTGCTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TAACACAACT CCCGCCCCCC TTGCCCCCGG
2310  GCCCGCTCAA CCATCTAGCC CCGCTGAGCC AAAAGCTCCC GTGCGTAAGC CGCCAATNNN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNA CCGCCACCTT
2450  CTGAAGAATT TCAGGTTAAC ACAACTCCCG CCCCCCTTGC CCCCGGGCCC GCTCAACCAT CTAGCCCCGC
2520  TGAGCCAAAA GCTCCCGTGC GTAAGCCGCC AACACCACCA TCCCCGCGCA CCCGCCGCCT TCTTTACACC
2590  TATCCTGATG GGGCAAAGGT GTACGCGGGG TCATTGTTTG AGTCTGACTG TGATTGGCTG GTCAATGCGT
2660  CGAATCCCGG CCATCGTCCT GGAGGCGGCC TTTGCCACGC CTTCTATCAA CGCTACCCTG AGTCCTTCTA
2730  CCCAACTGAG TTCATCATGC GCGACGGTCT TGCCGCTTAC ACTTTAACTC CCAGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCATAAC CCAAAGAGGC TTGAGGCAGC ATACCGAGAA ACTTGCTCTC
2870  GCCGCGGTAC CGCCGCCTAT CCACTCCTCG GCTCAGGCAT ATACCAAGTC CCCGTCAGCC TCAGCTTTGA
2940  CGCCTGGGAG CGTAACCACC GCCCCGGGGA TGAGCTCTAC CTAACTGACC TCGCTGCTAC CTGGTTTGAG
3010  GCTAATAAGC CAACACAACC GGCCCTCACA ATAACTGAGG ATGCGGCCCG TACAGCCAAC TTGGCACTGG
3080  AGATCGATGC TGCTACGGAG GTCGGCCGGG CTTGTGCCGG CTGTGCAGTT AGTCCTGGGG TTGTGCACTA
3150  TCAGTTTACT GCTGGGGTCC CAGGCTCGGG GAAGTCGCGT TCTATACAGC AGGGGGATGT TGACGTAGTG
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3290  CAGCCCGTGT CACTACGGGC CGTCGTGTTG TGATTGATGA GGCCCCATCC CTCCCACCGC ATTTGTTGCT
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K2-PRE-RBV Consensus sequence

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3710  CTGATGCTAG GGGGCTTATC CAATCTTCCC GAGCTCACGC CATAGTCGCA CTCACCCGCC ACACAGAGAA
3780  GTGCGTTATT CTTGACGCCC CCGGCTTGTT ACGTGAGGTT GGTATATCGG ATGTGATTGT TAACAATTTT
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3920  TCGCGACACT ACAGGCCTTT CCACCCTCCT GCCAAATTAG TGCCTATCAC CAGTTAGCTG AGGAACTTGG
3990  CCACCGCCCA GCCCCCGTCG CCGCTGTCTT GCCCCCTTGC CCTGAACTTG AGCAAGGCTT GTTATATATG
4060  CCGCAGGAGC TTACGGTGTC TGATAGCGTG CTGGTCTTCG AACTCACGGA CATAGTTCAC TGCCGGATGG
4130  CTGCCCCCAG CCAGCGGAAG GCCGTCCTAT CGACGCTCGT GGGTAGGTAC GGCCGTCGGA CGAAGCTGTA
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4620  TTTTTCAGAG TTTGATAGCA CCCAAAATAA CTTCTCCCTT GGTCTCGAGT GCGTAGTCAT GGAGGAATGT
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7350  TTGTGCCCCC TTCATAGCTT TTGCTTTATT TCTTTTTTCT GCGGTTCGCG CTCCCTGGAA NNNNNNNNNN
7420  NNN
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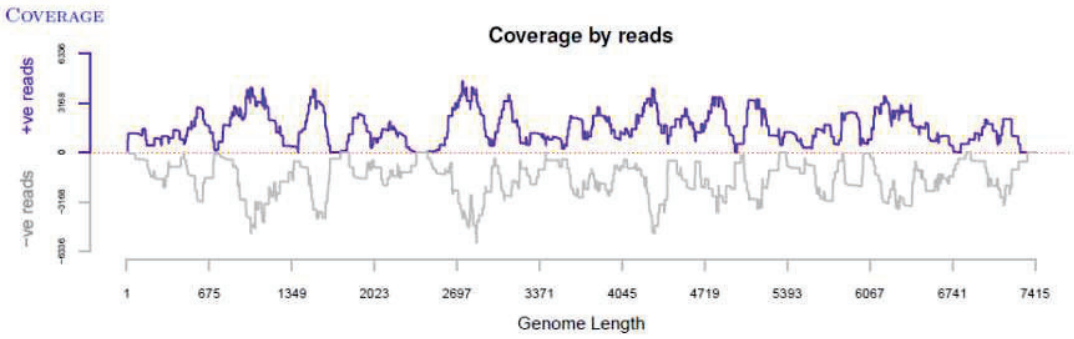
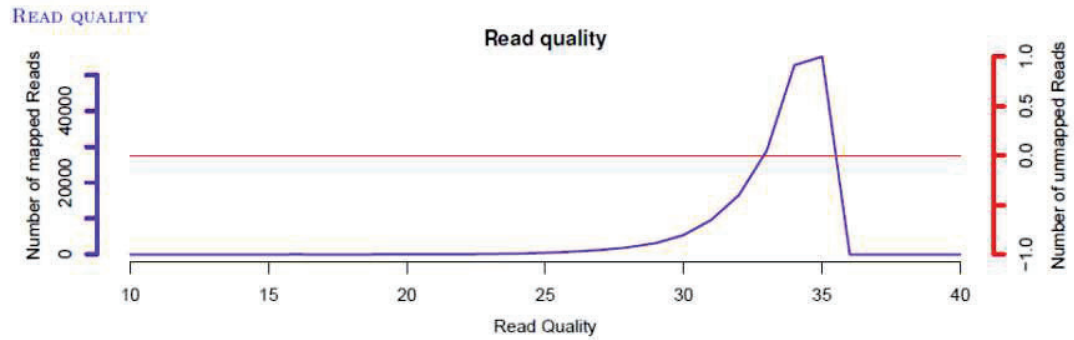
K3-POST-RBV

File name	**K3-POST-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**176 656**
Mapped reads	**176 656 (100.00%)**
Average read length	**138nt**
Coverage	**7 321nt (98.63%)**
Average depth	**3 256 reads/site**





K3-POST-RBV Consensus sequence

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 280  TGTCTGGAGG TTGGGGCCCA CCCAAGATCC ATTAATGATA ACCCAAATGT TCTGCACCGG TGCTTTTTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
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 490  GCCGCAGAAA CTGGGGTTGC TCTTTACTCT CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
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 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGTAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTCACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTCTATG TCCGCTCTAT ATTTGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCCACATT TCACGCTGTT CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTC TTTGGTGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTTAC TGTTGGCGCC CTTGTCGCTA ATGAGGGATG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCCTATTTGA CCATTTGCCA TCAGCGTTAC CTCCGTACTC
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1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTCTTG AAACCAGCAG AGGGTCTAGT TGGCGACCAC GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAAGTCGACC AGGCCGAGCC CGCCCATCTC GATGTTTCTG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AATATCCCGC ATGACATCGC AGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGCCCAG ACCGCTTGGA ATGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCAAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGTCCG CATAGTCTCT CCTATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATCTC
1890  ATCTAATGGG CTGGATTGCA CTGCAACATT CCCCCCGGGC GGCGCCCCAA GCGCTGCCCC GGAGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAT AACAGGTTTA CTCAGCGCCA TTCGCTTATA GGCGGCTTGT
2030  GGCTGCATCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCCCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGC AGTGCCGGCC GCTACTCCGG GGTCACGCCG CCCTACACCT CCTGCTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAA AATTTCAGGT TAACACAACT CCCGCCCCCC TTGCCCCCGG
2310  GCCCGCTCAA CCATCTAGCC CCGCTGAGCC AAAAGCTCCC GTGCGTAAGC CGCCAANNNN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNCTT
2450  CTGAAGAATT TCAGGTTAAC ACAACTCCCG CCCCCCTTGC CCCCGGGCCC GCTCAACCAT CTAGCCCCGC
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2660  CGAATCCCGG CCATCGTCCT GGAGGCGGCC TTTGCCACGC CTTCTATCAA CGCTACCCTG AGTCCTTCCA
2730  CCCAACTGAG TTCATCATGC GCGACGGTCT TGCCGCTTAC ACTTTAACTC CCAGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCATAAC CCAAAGAGGC TTGAGGCAGC ATACCGAGAA ACTTGCTCTC
2870  GCCGCGGTAC CGCCGCCTAT CCACTCCTCG GCTCAGGCAT ATACCAAGTC CCCGTCAGCC TCAGCTTTGA
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3010  GCTAATAAGC CAACACAACC GGCCCTCACA ATAACTGAGG ATGCGGCCCG TACAGCCAAC TTGGCACTGG
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K3-POST-RBV Consensus sequence

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4900 GTGGTCCTCT GTAGCGACTA CCGTCAGAGC CGCAATGCAG CGGCCCTGAT TGCAGGTTGC GGGCTCAAAC
4970 TGAAGGTTGA TTATCGCCCT ATTGGGTTGT ATGCTGGTGT GGTGGTGGCC CCTGGCCTGG GGACACTACC
5040 CGATGTGGTG CGCTTTGCCG GCCGGCTGTC TGAGAAGAAC TGGGGCCCTG GGCCGGAGCG GGCCGAGCAG
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5670 CCGGGGCTGC GCCGCTGACT GCCATATCAC CTGCTCCTGA TACAGCTCCT GTACCTGATG TTGACTCGCG
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5810 AATTTGGTTC TTTATGCCGC CCCGCTAAAC CCTTTGCTGC CCCTTCAGGA TGGCACTAAC ACTCACATCA
5880 TGGCCACTGA GGCATCTAAC TATGCCCAGT ATCGGGTTGT CCGAGCTACG ATCCGTTACA GGCCATTGGT
5950 GCCGAATGCC GTCGGCGGCT ATGCAATATC CATCTCATTC TGGCCTCAGA CTACTACTAC CCCCACATCT
6020 GTTGATATGA ACTCTATTAC TTCCACTGAT GTTAGGATTC TAGTTCAGCC CGGTATTGCT TCTGAGTTGG
6090 TTATCCCCAG TGAGCGCCTC CATTATCGTA ACCAGGGCTG GCGCTCTGTG GAGACCTCGG GTGTGGCTGA
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6230 CCTTATACCG GGGCGCTTGG ACTCCTTGAT TTTGCTTTAG AGCTTGAGTT TAGGAACTTG ACACCTGGAA
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6580 GACTGTCAAG TTATACACAT CTGTTGAGAA TGCGCAGCAG GATAAGGGGA TCGCTATTCC ACATGACATA
6650 GATCTGGGTG ACTCCCGTGT GGTCATCCAA GACTATGACA ACCAGCATGA GCAGGATCGA CCCACCCCCT
6720 CGCCTGCCCC TTCTCGCCCT TTTTCGGTCC TTCGCGCTAA TGATGTTCTT TGGCTCTCTC TTACTGCCGC
6790 TGAGTACGAC CAGACTACAT ATGGGTCGTC CACCAACCCG ATGTATGTCT CGGATACTGT CACATTTGTC
6860 AACGTGGCTA CAGGAGCCCA GGCTGTCGCC CGTTCCCTCG ACTGGTCTAA AGTCACTCTA GACGGCCGCC
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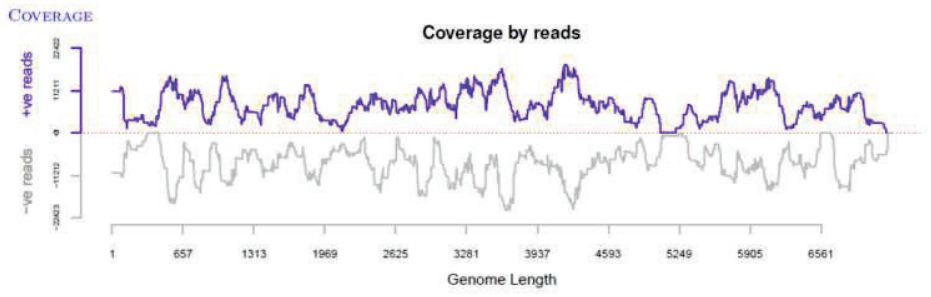
7000 GGAGGCCGGG ACGACTAAGG CCGGTTATCC CTATAATTAC AACACAACTG CTAGTGATCA GATTTTGATT
7070 GAAAATGCGG CTGGTCATCG TGTTGCTATT TCCACGTATA CCACCAGCTT GGGTGCCGGT CCTGTGTCTG
7140 TTTCTGCAGT TGGTGTTTTA GCCCCACATT CGGCCCTTGC AGTCCTTGAA GATACTATTG ATTACCCTGC
7210 CCGTGCTCAC ACTTATGATG ATTTTTGCCC GGAGTGTCGC GCTCTTGGTT TGCAGGGGTG TGCCTTCCAG
7280 TCTACTATTG CTGAGCTTCA GCGCCTTAAA ATGAAGGTAG GTAAAACCCG GGAGTTTTAA TCAATTTCCT
7350 TTGTGCCCCC TTCATAGCTT TTGTTTTATT TCTTTTTTCT GCGTTCCGCG CTCCCTGGAA NNNNNNNNNN
7420 NNN

L1-PRE-RBV

File name	**L1-PRE-RBV.sam**
Ref name	**KX462160.1**
Ref length	**7 216nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**815 209**
Mapped reads	**815 209 (100.00%)**
Average read length	**139nt**
Coverage	**7 182nt (99.53%)**
Average depth	**15 397 reads/site**



L1-PRE-RBV Consensus sequence

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   1  AGGCTCCTGG CATCACTACT GCCATTGAGC AAGCTGCTCT GGCTGCGGCC AATTCTGCCC TGGCGAATGC
  70  TGTGGTGGTT CGGCCGTTTT TGTCCCGTGT ACAAACTGAG ATTCTTATTA ATTTAATGCA ACCCCGACAG
 140  CTGGTTTTCC GACCTGAAGT GCTTTGGAAT CATCCAATCC AACGGGTTAT TCATAATGAA TTGGAACAGT
 210  ACTGCCGGGC CCGGGCTGGT CGTTGTCTTG AAGTTGGGGC CCACCCAAGA TCCATCAATG ACAACCCGAA
 280  TGTTTTGCAT CGGTGTTTTT TACGACCAGT CGGGAGGGAC GTTCAGCGCT GGTACTCTGC CCCTACCCGC
 350  GGCCCTGCGG CTAATTGCCG CCGCTCCGCT TTGCGTGGCC TCCCCCCTGC TGATCGTACC TATTGTTTTG
 420  ACGGGTTCTC CCGCTGCTCG TTTGCTGCAG AGACTGGGGT GGCTCTCTAC TCTTTGCATG ACCTCTGGCC
 490  GGCCGATGTT GCGGAGGCCA TGGCCCGACA CGGGATGACA CGCTTATATG CTGCACTACA TCTCCCTCCT
 560  GAAGTACTAC TACCGCCAGG TACATACCAC ACAACATCAT ACCTTTTGAT CCACGACGGC GATCGTGCTG
 630  TTGTGACCTA TGAAGGTGAC ACTAGTGCAG GCTACAACCA TGATGTTTCC ATACTCCGCG CATGGATCCG
 700  CACAACTAAA ATAGTTGGCG ACCATCCGCT CGTGATAGAG CGTGTGAGGG CTATTGGCTG CCACTTTGTG
 770  TTGCTGCTTA CTGCAGCCCC TGAGCCGTCA CCAATGCCTT ATGTCCCATA CCCCCGGTCG ACAGAGGTGT
 840  ATGTCCGCTC TATATTCGGC CCTGGCGGGT CCCCGTCCCT TTTCCCGTCA GCTTGTTCTA CAAAGTCCAC
 910  ATTTCATGCT GTCCCAGTCC ATATTTGGGA CCGACTTATG CTCTTTGGCG CTACCCTGGA TGATCAGGCG
 980  TTTTGTTGCT CACGGCTTAT GACCTATCTC CGTGGGATTA GTTACAAGGT CACTGTCGGC GCCCTCGTTG
1050  CTAACGAGGG ATGGAATGCT TCGGAGGACG CCCTTACCGC TGTTATTACT GCGGCGTATC TGACCATTTG
1120  CCACCAACGC TACCTCCGTA CTCAGGCTAT ATCTAAGGGT ATGCGCCGAC TTGAGGTTGA GCATGCTCAA
1190  AAATTTATCA CAAGACTTTA CAGTTGGCTG TTTGAGAAGT CTGGCCGTGA CTACATCCCC GGTCGTCAGC
1260  TCCAGTTCTA TGCACAGTGC CGCCGCTGGT TATCGGCGGG TTTCCACCTT GATCCAAGGG TGCTTGTTTT
1330  TGATGAGTCT GTGCCCTGCC GTTGCAGGAC GTTCCTTAAG AAGGTTGCAG GTAAATTCTG TTGTTTTATG
1400  AAGTGGCTTG GACAGGAGTG CACCTGCTTT CTGGAACCAG CGGAGGGCCT GGTTGGCGAC CATGGCCATG
1470  ATAATGAAGC CTATGAGGGC TCTGAGGTCG ACCAGGCTGA ACCCGCCCAT CTAGATGTTT CTGGGACCTA
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1820  GCTGGCTTGC AGGTTAGGAT TTCATCTAAT GGCCTGGATT GTACTGCAAC ATTCCCCCCG GGCGGGGCCC
1890  CTAGCGCCGC TCCGGGAGAG GTGGCAGCCT TCTGCGGTGC CCTCTACAGG TATAATAGGT TCACCCAGCG
1960  GCATTCGCTT ACGGGCGGCT TGTGGCTGCA TCCTGAGGGG TTATTGGGTA TCTTCCCCCC CTTTTCCCCC
2030  GGGCATATCT GGGAGTCCGC TAATCCCTTT TGCGGGGAGG GAACTTTGTA CACTCGGACT TGGTCAACAT
2100  CTGGTTTTTC TAGTGATTTT TCCCCCCCCG AAGCGGCTGC CGCTGCGCCG GCTGATGTTC CGGGGTTACC
2170  CCACCCTACA CCCCCTGTTA GTGATATCCG GGTGTTGCCG CCACCCTCCG AAGAACTACA GGTTGATGCA
2240  GTACCTGCCC CTCCTGCCCC TGAGCCTGTT CTACTGCCCA GCCCCGTTGA GCCAAGGGTC CCCGTGCGTA
2310  AGCCGGCGGC ACTACCGCCT CCGCGCACCC GCCGGCTTCT TTATACTTAC CCGGACGGGG CAAAAGGTGT
2380  ATGCAGGGTT CATTGTTTGA GTCTGACTGT GACTGGTTGG GGTTAATGCG TCGAACCCTG GTCATCGCCC
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2520  GCGACGGTCT TGCCGCGTAC ACTTTAACTC CCCGGCCCAT CATTCATGCG GTAGCCCCTG ATTATCGGGT
2590  TGAGCAGAAC CCAAAGAGGC TTGAGGCAGC ATATCGAGAG ACTTGTTCCC GCCGTGGCAC TGCTGCTTAC
2660  CCGCTCCTTG GTTCGGGCAT ATACCAAGTT CCGGTTAGCC TCAGCTTTGA CGCCTGGGAG CGTAACCATC
2730  GCCCCGGGGA CGAGCTTTAT CTGACTGACC TCGCCGCTAC CTGGTTCGAG GCTAATAAGC CAACACAGCC
2800  GGCCCTCACA ATAACTGAGG ATACAGCCCG TACGGCCAAC CTAGCACTGG AGATCGATGC TGCCACTGAG
2870  GTTGGTCGGG CTTGCGCCGG CTGTACAGTT AGTCCCGGGA TTATACACTA TCAATTTACT GCCGGGGTAC
2940  CAGGTTCGGG GAAGTCGCGG TCTATACAGC AGGGGGATGT CGATGTGGTG GTTGTTCCCA CTCGAGAGCT
3010  CCGGAACAGC TGGCGCCGCC GGGGTTTTGC AGCTTTCACA CCTCACACGG CGGCCCGTGT CACCACGGGC
3080  CGTCGCGTTG TGATTGATGA AGCCCCTTCT CTCCCACCGC ACTTGCTGCT ACTACACATG CAGCGGGCCT
3150  CGTCGGTCCA TCTTCTTGGC GACCCAAACC AGATCCCTGC TATAGACTTT GAGCATGCCG GCCTGGTCCC
3220  TGCAATACGC CCTGAGCTTG CGCCTACCAG CTGGTGGCAT GTTACCCATC GTTGCCCCGC CGATGTGTGT
3290  GAGCTTATAC GCGGGGCCTA CCCTAAAATC CAGACTACCA GCCGCGTGTT GCGATCATTA TTCTGGAATG
3360  AGCCCGCCAT TGGCCAGAAG TTAGTCTTTA CACAGGCTGC TAAGGCTGCC AACCCTGGTG CGATTACAGT
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3500  CAGTCTTCCC GGGCTCACGC TATAGTCGCA CTCACCCGCC ATACAGAGAA GTGCGTTATT CTTGATGCCC
3570  CCGGTTTGTT ACGAGAAGTT GGTATCTCGG ATATAATTGT TAACAATTTT TTCCTCGCTG GTGGAGAAGT
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L1-PRE-RBV Consensus sequence

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3780  CTGCCGTTCT GCCCCCTTGC CCTGAGCTTG AGCAGGGCTT GCTGTATATG CCGCAGGAAC TCACGGTGTC
3850  TGATAGCGTG CTGGTCTTTG AGCTCACGGA CATAGTCCAC TGCCGCATGG CCGCCCCTAG CCAGCGGAAG
3920  GCCGTCCTAT CGACGCTTGT GGGCAGGTAC GGCCGTAGGA CAAAGCTGTA TGAAGCAGCC CACTCTGACG
3990  TCCGTGAGTC CCTCGCTAGA TTCATCCCCA CCATTGGGCC CGTTCAGGCT ACTACGTGCG AGCTATATGA
4060  GCTGGTTGAG GCCATGGTGG AGAAGGGTCA GGATGGTTCA GCCGTGCTTG AGCTTGACCT TTGCAATCGT
4130  GATGTATCGC GTATCACATT TTTCCAGAAA GATTGTAACA AGTTCACCAC AGGGGAGACC ATTGCCCACG
4200  GCAAAGTCGG TCAGGGCATC TCTGCTTGGA GTAAGACCTT TTGTGCCCTG TTTGGTCCGT GGTTCCGTGC
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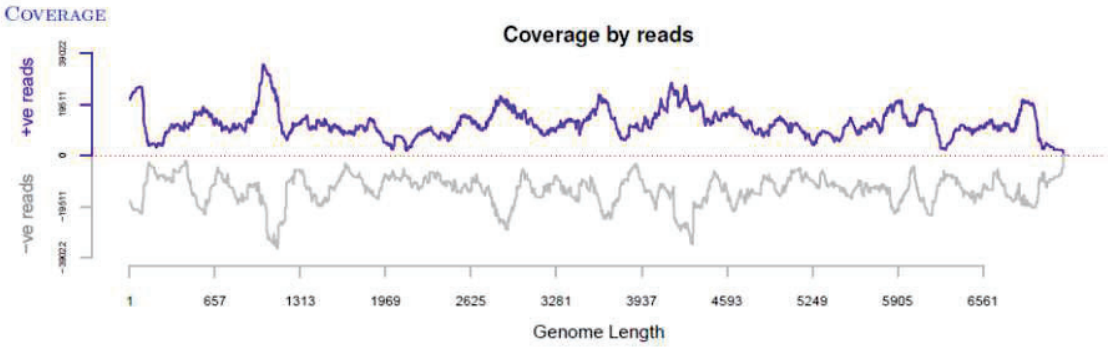
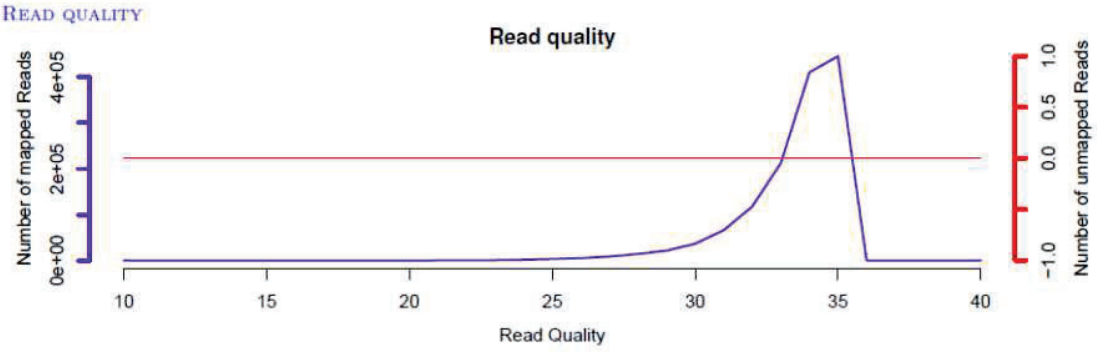
L2-POST-RBV1

File name	**L2-POST-RBV1.sam**
Ref name	**KX462160.1**
Ref length	**7 216nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 343 901**
Mapped reads	**1 343 901 (100.00%)**
Average read length	**140nt**
Coverage	**7 182nt (99.53%)**
Average depth	**25 534 reads/site**





L2-POST-RBV1 Consensus sequence

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 210  ACTGCCGGGC CCGGGCTGGT CGTTGTCTTG AAGTTGGGGC CCACCCAAGA TCCATCAATG ACAACCCGAA
 280  TGTTTTGCAT CGGTGTTTTT TACGACCAGT CGGGAGGGAC GTTCAGCGCT GGTACTCTGC CCCTACCCGC
 350  GGCCCTGCGG CTAATTGCCG CCGCTCCGCT TTGCGTGGCC TCCCCCCTGT TGATCGTACC TATTGTTTTG
 420  ACGGGTTCTC CCGCTGCTCG TTTGCTGCAG AGACTGGGGT GGCTCTCTAC TCTTTGCATG ACCTCTGGCC
 490  GGCCGATGTT GCGGAGGCCA TGGCCCGACA CGGGATGACA CGCTTATATG CTGCACTACA TCTCCCTCCT
 560  GAAGTACTAC TACCGCCAGG TACATACCAC ACAACATCAT ACCTTTTGAT CCACGACGGC GATCGTGCTG
 630  TTGTGACCTA TGAAGGTGAC ACTAGTGCAG GCTACAACCA TGATGTTTCT ATACTCCGCG CATGGATCCG
 700  CACAACTAAA ATAGTTGGCG ACCATCCGCT CGTGATAGAG CGTGTGAGGG CTATTGGTTG CCACTTTGTG
 770  TTGCTGCTTA CTGCAGCCCC TGAGCCGTCA CCAATGCCTT ATGTCCCATA CCCCCGGTCG ACAGAGGTGT
 840  ATGTCCGCTC TATATTCGGC CCTGGCGGGT CCCCGTCCCT TTTCCCGTCA GCTTGTTCTA CAAAGTCCAC
 910  ATTTCATGCT GTCCCAGTCC ATATTTGGGA CCGACTCATG CTCTTTGGCG CTACCCTGGA TGATCAGGCG
 980  TTTTGTTGCT CACGGCTTAT GACCTATCTC CGTGGGATTA GTTACAAGGT CACTGTCGGC GCCCTCGTTG
1050  CTAACGAGGG ATGGAATGCT TCGGAGGACG CCCTTACCGC TGTTATTACT GCGGCGTATC TGACCATTTG
1120  CCACCAACGC TACCTCCGTA CTCAGGCTAT ATCTAAGGGT ATGCGCCGAC TTGAGGTTGA GCATGCTCAA
1190  AAATTTATCA CAAGACTTTA CAGTTGGCTG TTCGAGAAGT CTGGCCGTGA CTACATCCCC GGTCGTCAGC
1260  TCCAGTTCTA TGCACAGTGC CGCCGCTGGT TATCGGCGGG TTTCCACCTT GATCCAAGGG TGCTTGTTTT
1330  TGATGAGTCT GTGCCCTGCC GTTGCAGGAC GTTCCTTAAG AAGGTTGCAG GTAAATTCTG TTGTTTTATG
1400  AAGTGGCTTG GACAGGAGTG CACCTGCTTT CTGGAACCAG CGGAGGGCCT GGTTGGCGAC CATGGCCATG
1470  ATAATGAAGC CTATGAGGGC TCTGAGGTCG ACCAAGCTGA ACCCGCCCAT CTAGATGTTT CTGGGACCTA
1540  TGCCGTCCAC GGACGCCAAC TTGAGGCCTT GTATAGGGCG CTTAACATCC CGCATGACAT CGCAGCTCGA
1610  GCCTCCCGTC TGACCGCCAC TGTTGAGCTC GTTGCAAGCC TAGACCGCTT GGAGTGTCGC ACCGTGCTCG
1680  GGAATAAGAC TTTCCGAACA ACGGTGGTTG ATGGCGCCCA TCTTGAGGCG AACGGCCCCG AGCAGTACGT
1750  CCTTTCGTTC GACGCCTCCC GTCAGTCTAT GGGTGCCGGG TCGCATAGCC TCACTTATGA GCTCACCCCT
1820  GCTGGCTTGC AGGTTAGGAT TTCATCTAAT GGCCTGGATT GTACTGCAAC ATTCCCCCCG GGCGGGGCCC
1890  CTAGCGCCGC TCCGGGAGAG GTGGCAGCCT TCTGCGGTGC CCTCTACAGG TATAATAGGT TCACCCAGCG
1960  GCATTCGCTT ACGGGCGGCT TGTGGCTGCA TCCTGAGGGG TTATTGGGTA TCTTCCCCCC CTTTTCCCCC
2030  GGGCATATCT GGGAGTCCGC TAATCCCTTT TGCGGGGAGG GAACTTTGTA CACTCGGACT TGGTCAACAT
2100  CTGGTTTTTC TAGTGATTTT TCCCCCCCCG AAGCGGCTGC CGCTGCGCCG GCTGATGTTC CGGGGTTACC
2170  CCACCCTACA CCCCCTGTTA GTGATATCCG GGTGTTGCCG CCACCCTCCG AAGAACTACA GGTTGATGCA
2240  GTACCTGCCC CTCCTGCCCC TGAGCCTGTT CTACTGCCCA GCCCCGTTGA GCCAAGGGTC CCCGTGCGTA
2310  AGCCGACGGC ACTACCGCCT CCGCGCACCC GCCGGCTTCT TTATACTTAC CCGGACGGGG CAAAAGGTGT
2380  ATGTAGGGTT CATTGTTTGA GTCTGACTGT GACTGGTTTG GGTTAATGCG TCGAACCCTG GTCATCGCCC
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2520  GCGACGGTCT TGCCGCGTAC ACTTTAACTC CCCGGCCCAT CATTCATGCG GTAGCCCCTG ATTATCGGGT
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2800  GGCCTTCACA ATAACTGAGG ATACAGCCCG TACGGCCAAC CTAGCACTGG AGATCGATGC TGCCACTGAG
2870  GTTGGTCGGG CTTGCGCCGG CTGTACAGTT AGTCCCGGGA TTATACACTA TCAATTTACT GCCGGGGTAC
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3010  CCGGAACAGC TGGCGCCGCC GGGGTTTTGC AGCTTTCACA CCTCACACGG CGGCCCGTGT CACCACGGGC
3080  CGTCGCGTTG TGATTGATGA AGCCCCTTCT CTCCCACCGC ACTTGCTGCT ACTACACATG CAGCGGGCCT
3150  CGTCGGTCCA TCTTCTTGGC GACCCAAACC AGATCCCTGC TATAGACTTC GAGCATGCCG GCCTGGTCCC
3220  TGCAATACGC CCTGAGCTTG CGCCTACCAG CTGGTGGCAT GTTACCCATC GTTGCCCCGC CGATGTGTGT
3290  GAGCTTATAC GCGGGGCCTA CCCTAAAATC CAGACTACCA GCCGCGTGTT GCGATCATTA TTCTGGAATG
3360  AGCCCGCCAT TGGCCAGAAG TTAGTCTTTA CACAGGCTGC TAAGGCTGCC AACCCTGGTG CGATTACAGT
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3500  CAGTCTTCCC GGGCTCACGC TATAGTCGCA CTCACCCGCC ATACAGAGAA GTGCGTTATT CTTGATGCCC
3570  CCGGTTTGTT ACGAGAAGTT GGTATCTCGG ATATAATTGT TAACAATTTT TTCCTCGCTG GTGGAGAAGT
3640  GGGCCACCAC CGCCCCTCCG TGATACCCCG CGGTAGCCCT GACCAGAACC TCGCTACACT ACAGGCCTTT
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L2-POST-RBV1 Consensus sequence

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3780 CTGCCGTTCT GCCCCCTTGC CCTGAGCTTG AGCAGGGCTT GCTGTATATG CCGCAGGAAC TCACGGTGTC
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3990 TCCGTGGGTC CCTCGCTAGA TTCATCCCCA CCATCGGGCC CGTTCAGGCT ACTACGTGTG AGCTATATGA
4060 GCTGGTTGAG GCCATGGTGG AGAAGGGTCA GGATGGTTCA GCCGTGCTTG AGCTTGACCT TTGCAATCGT
4130 GATGTGTCGC GTATCACATT TTTCCAGAAA GATTGTAACA AGTTCACCAC AGGGGAGACC ATTGCCCACG
4200 GCAAAGTCGG TCAGGGCATC TCTGCTTGGA GTAAGACCTT TTGTGCCCTG TTTGGTCCGT GGTTCCGTGC
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6370 CATCTGTAGA GAATGCGCAG CAAGATAAAG GGATTGCCAT CCCACATGAT ATAGACTTGG GTGACTCCCG
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7070 TCAGCGTCTT AAAATGAAGG TAGGTAAAAC CCGGGAGGTT TAATTAATTT CCTTTGTGCC CCCTTCATAG
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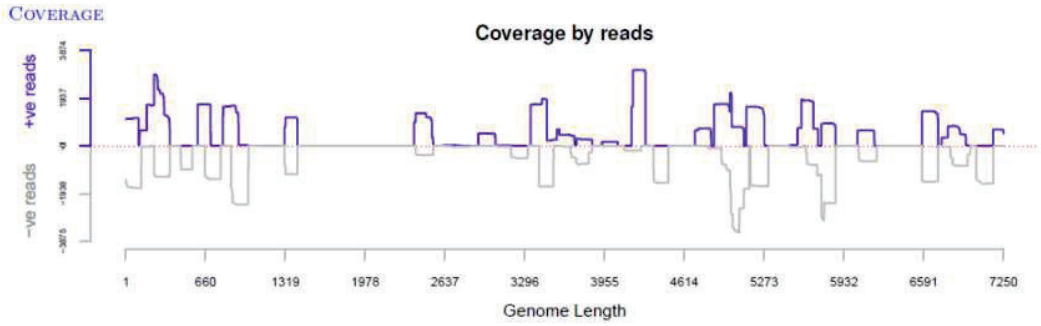
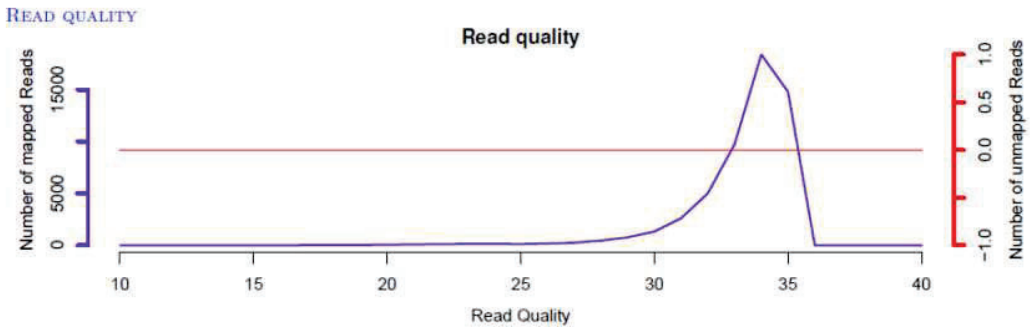
7210 NNNNNN

M1-PRE-RBV

File name	**M1-PRE-RBV.sam**
Ref name	**KT159771.1**
Ref length	**7 251nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**54 122**
Mapped reads	**54 122 (100.00%)**
Average read length	**145nt**
Coverage	**6 273nt (86.51%)**
Average depth	**954 reads/site**





M1-PRE-RBV Consensus sequence

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210	GTGCTGGCCG	CTGTCTGGAG	GTCGGGGCCC	ATCCAAGATC	CATCAATGAT	AACCCTAATG	TATTGCACCG	
280	GTGTTTCCTT	CGCCCAGTCG	GGAGAGATGT	GCAGCGTTGG	TACTCTGCTC	CGACTCGCGG	CCCAGCGGCT	
350	AACTGCCGCC	GTTCNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNGTACCTA	CTGTTTCGAC	GGGTTTTCCC	
420	GTTGTGCCTT	TGCCGCTGAG	ACTGGGATTG	CTTTATATTC	ACTGCATGAC	CTCTGGCCTG	CGGATGTCGC	
490	GGAGGCCATG	GCTCGCCACG	GGATGACGCG	CCTGTACGCG	GCCCTCCATC	TACCCCCTGA	GGTAATATTA	
560	ACAACCGGTA	CTTACAATAC	AACTTCGTAC	CTTCTGATTC	ACGACGGCGA	TCGCGCCGTT	GTAACTTATG	
630	AAGGAGATAC	CAGCGCGGGT	TATAACCACG	ATGTATCCAT	TCTCCGGGCA	TGGATCCGTA	CAACTAAGAT	
700	AACTGGTGAC	CACCCGCTGG	TCATAGAGAG	GGTTCGTGCC	ATCGGCTGCC	ATTTTGTACT	ACTTCTCACT	
770	GCCGCCCCTG	AGCCGTCTCC	TATGCCCTAC	GTTCCATACC	CTCGTTCAAC	AGAGGTGTAT	GTTAGATCTA	
840	TATTTGGCCC	CGGTGGATCG	CCCTCTCTCT	TCCCGTCAGC	CTGCTCTACT	AAATCCACAT	TCCACGCTGT	
910	TCCAGTCCAT	ATCTGGGACC	GGCTCATGCT	CTTCGGTGCC	ACTCTGGACG	ACCAAGCCTT	TTGTTGCTCG	
980	CGGCTTATGA	CCTATCTCCG	CGGGATCAGT	TACAAGGTCA	CTGTTGGCGC	ACTTGTCGCT	AACGAGGGGT	
1050	GGAATGCTTC	GGAGGACGCT	CTTACAGCTG	TTATTACTGC	ATCGTATTTG	ACCATTTGCN	NNNNNNNNTA	
1120	CCTCCGAACC	CAAGCTATAT	CTAAAGGTAT	GCGTCGCCTG	GAGGTTGAAC	ACGCCCAGAA	GTTCATTACT	
1190	AGACTTTACA	GTTGGTTGTT	CGAGAAGTCC	GGTCGTGACT	ACATCCCCGG	TCGCCAATTA	CAGTTCTATG	
1260	CGCAGTGNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNGTG	CTTGTTTTCG	ATGAGGCTGT	
1330	CCCTTGTCGC	TGTAGGAGCT	TTTTGAAGAA	AGTTGCTGGC	AAGTTTTGCT	GTTTTATGAA	ATGGCTGGGC	
1400	CAGGAGTGCA	CCTGCTTTTT	GGAACCAGCA	GAGGGTCTAG	TTGGCGACCA	TGGCCACGAT	AATGAAGCCT	
1470	ATGAGGGCGC	TGAGGTCGAT	CAGGCCGAGC	CCGCCCGTCT	CGATGTTTCT	GGGACTTATG	CTGTCCACGG	
1540	CCGCCAACTT	GAGGCCCTGT	ATAGGGCGCT	TAACATCCCG	CATGACATCG	NNNNNNNNNN	NNNNNNNNNN	
1610	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	
1680	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNGCCCCGAG	CAGTACGTTC	TTTCGTTTGA	
1750	CGCCTCTCGC	CAGTCCATGG	GGGCTGGGCC	GCATAGTCTC	TCCTACGAGC	TCACTCCTTC	CGGTTTGCAG	
1820	GTCAAGATTT	CATCTAATGG	CCTGGATTGC	ACTGCANNNN	NNCCCCCTGG	TGGGGCGCCA	AGCGCCGAAC	
1890	CGGGTGAGGT	TGCAGCCTTC	TGCAGTGCCT	TATATAGATA	CAACCGGTTT	ACCCAGCGTC	ACTCGCTAAC	
1960	CGGCGGGTTA	TGGCTGCACC	CTGAGGGNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	
2030	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	
2100	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	
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2240	TCTGCCCCTG	AGCCCGCTCA	ACCATCTAGC	TCCGCTGGGC	CAAAGGCTCC	CGTGCGTAAG	CCGCCAACNN	
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2380	GTTTGAGTCA	GACTGTGACT	GGCTGGTTAA	TGCGTCCAAC	CCTGGTCACC	GCCCCGGTGG	CGGCCTCTGT	
2450	CATGCCTTTT	ACCAGCGCTT	CCCAGAGTCG	TTCTACCCGA	CTGATTTTAT	TATGCGCGAG	GGTCTCGCCG	
2520	CGTACACTTT	GACTCCCCGN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNGTTGAGC	TTCACCCATA	
2590	GAGGCTTGAT	GCAGCATAGC	GAGAGACTTG	CTCCCGCCGC	GGTACTGCCG	CCTACCCACT	CCTTGGCTCG	
2660	GGTATATACC	AAGTTCCCGT	CAGCCTCAGC	TTTGACGCTT	GGGAGCGTAA	CCACCGCCCC	GGGGACGAGC	
2730	TCTACCTAAC	CGACCCCGCA	GCTACCTGGT	TCGAGGCTAA	TAAGCCAACA	CAGCCGGCCC	TCACAATAAC	
2800	TGAGGATGCA	GCCCGTACAG	CCAACCTAGC	ACTGGAGATC	GATGCTGCCA	CCGAGGTGGG	CCGTGCTTGT	
2870	ACCGGCTGTA	CTGTTAGCCC	CGGGGTTGTC	CATTATCAAT	TCACTGCTGG	GGTACCTGGT	TCCGGGAAGT	
2940	CCAGGTCTAT	ACAACAAGGG	GACGTCGATG	TCGTGGTTGT	GCCAACCCGC	GAGCTCCGTA	ATAGCTGGCG	
3010	GCGCCGCGGC	TTTGCTGCTT	TTACACCCCA	CACGGCAGCC	CGTGTCACCA	CGGGCCGTCG	TGTTGTAATT	
3080	GATGAGGCCC	CATCTCTCCC	ACCGCATTTG	TTGCTACTAC	ATATGCAGCG	GGCCTCGTCG	GTCCACCTTC	
3150	TCGGCGACCC	GAACCAGATC	CCTGCCATAG	ACTTTGAGCA	TGCGGGTTTG	GTGCCGGCGA	TCCGTCCTGG	
3220	GCTTGCCCCA	ACCAGTTGGT	GGCACGTCAC	ACACCGCTGC	CCTGCCGATG	TGTGTGAGCT	CATACGCGGG	
3290	GCTTACCCCA	AGATCCAAAC	TACTAGCCGN	NNNNNNNNNN	NNNNNNNNNN	NNNTGAGCCT	GCAGTGGGCC	
3360	AGAAATTGGT	CTTCACGCAG	GCAGCTAAGG	CTGCCAACCC	TGGTGCAATT	ACCGTCCATG	AGGCTCAGGG	
3430	TGCTACATTC	ACAGAGACCA	CCATTATAGC	CACGGCCGAT	GCTAGGGGCC	TTATTCAGTC	CTCTCGTGCA	
3500	CATGCGATTG	TTGCACTCAC	CCGCCATACA	GAAAAATGTA	TCATTTTGGA	TGCCCCCGGT	TTGTTACGTG	
3570	AGGTCGGTAT	ATCGGATGTG	ATTGTCAACA	ATTTTTTCCT	CGCCGGCGGG	GAGGTGGGTC	ACCATCGCCC	
3640	CTCTGTGATA	CCTCGCGGCA	ATCCTGACCA	GAACCTCGCA	ACACTACAGG	CCTTTCCACC	TTCCTGCCAG	

M1-PRE-RBV Consensus sequence

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3780  CCTGCCCTGA ACTTGAGCAG GGCTTGCTAT ATATGCCCCA GGAGCTTACG GTGTCTGATA GCGTGCTGGT
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4060  GGTTGAGAAA GGTCAGGATG GCTCTGCCGT GCTTGAGCTC GACCTCTGCA ATCGTGATGT CTCGCGTATT
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5180  CCAGCCGTCT GGCCGCCGTC GTGGGCGGCG CAGCGGCGGT ACCGGCGGTG GTTTCTGGGG TGACAGGGTT
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5460  CCCGATACAG CTCCTGTACC TGATGTTGAC TCGCGCGGCG CCATATTGCG ACGCCAGTAC AATTTATCTA
5530  CATCTCCGCT CACATCATCT GTTGCTTCTG GCACCAATCT TGTTTTATAC GCTGCCCCGC TCAACCCCCT
5600  TCTACCCCTC CAGGATGGCA CCAATACTCA CATCATGGCG ACTGAGGCGT CTAATTACGC CCAGTATCGG
5670  GTTGTCCGGG CCACAATCCG TTATCGCCCT TTGGTGCCCA ATGCTGTTGG CGGCTACGCA GTTTCTATTT
5740  CCTTTTGGCC CCAGACTACA ACTACTCCCA CCTCTGTTGA TATGAATTCC ATTACTTCTA CCGATGTTAG
5810  GATTTTAGTC CAGCCCGGTA TAGCCTCTGA GTTGGTCATC CCAAGTGAGC GCCTCCATTA TCGTAACCAG
5880  GGCTGGCGCT CTGTGGAGAC CTCGGGTGTG GCTGAAGAGG AGGCCACTTC TGGTCTGGTA ATGCTTTGTA
5950  TCCATGGTTC TCCTGTCAAT TCCTACACCA ATACCCCNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN
6020  NNNNNNNNNN NNNNNNNNN NNCTGACCCC TGGGAACACC AACACACGTG TGTCTCGGTA CACCAGCACC
6090  GCTCGTCACC GCCTACGTCG TGGCGCTGAT GGGACTGCCG AGCTCACGAC TATTGCAGCC ACGCGATTTA
6160  TGAAGGACCT GCACTTCACC GGGATGAACG GCGTGGGTGA GGNNNNNNNN NNNNNNNNN NNNNNNNNN
6230  NNNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNNNNN NNNNNGGGGG ACAGTTATTC
6300  TACTCCCGCC CCGTCGTCTC AGCCAATGGC GAGCCGACTG TCAAGTTATA TACATCTGTA GAGAATGCGC
6370  AGCAGGACAA AGGGAGCGCT ATCCCACTTG ATATAGNNNN NNNNNNNNNC CGTGTGGTCA TCCAAGACTA
6440  TGACTACCAG CATGAGCAGG ATCGACCCAC CCCCTCGACT GCCCCTTCTC GCGCTTTCTC AGTTCTTCGC
6510  GCTAATGATG TTTTATGGCT TTCTCTTACA GCCGCCGAGT ATGACCAGAN NACATATGGT TCGTCGACCA
6580  ACCCTATGTA TGTCTCAGAC ACTGTTACCT TTGTCAATGT GGCTACGGGA GCCCAGGCTG TTGCCCGCTC
6650  CCTTGATTGG TCTAAGGTCA CTCTGGATGG TCGACCTCTT ACCACAATTC AGCAGTATNN NNNNNNNNN
6720  NNNNNNNNNN NNNNNNNCGG GAAGTTATCC TTTTGGGAGG CCGGGACGGC TAAGGCCGGC TACCCCTACA
6790  ATTACAACAC AACTGCTAGT GATCAGATTC TGATCGAAAA TGCGGCTGGT CATCGTGTTG CTATTTCTAC
6860  GTACACCACC AGCTTGGGCG CTGGCCCTGT GTCTGTTTCT GCAGTTGGTG TTTTAGCCCC ACATTCGGCT
6930  CTTGCAGTCC TTGAAGACAC TACTGACTAC CCTGCCCGTA CCCACACTTT TGATGATTCT TGCCCGGAGT
7000  GTCGTGCCCT TGGTCTGCAG GGGTGTGCTT TCCAATCTAC TATTGCTGAA CTTCAGCGTC TTAAAATGAA
7070  GGTAGGTAAG ACCCGGGAGT CTTAATTAAT TTCATTTGTA CCCCCTTCTA AGCCTTTGTT TTATTTCTTT
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7210  AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA A
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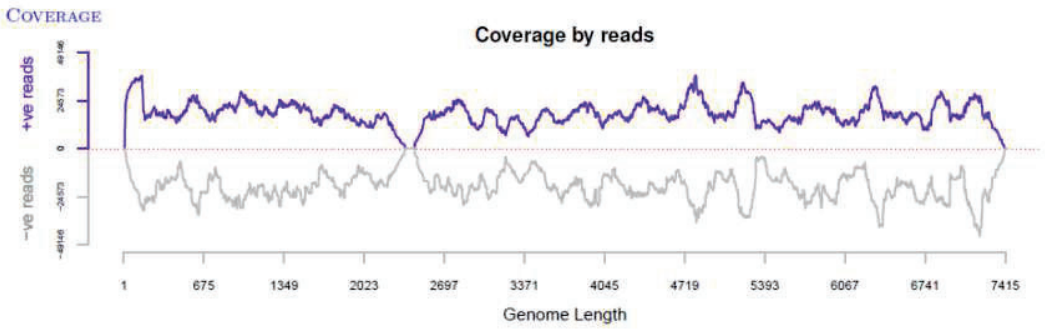
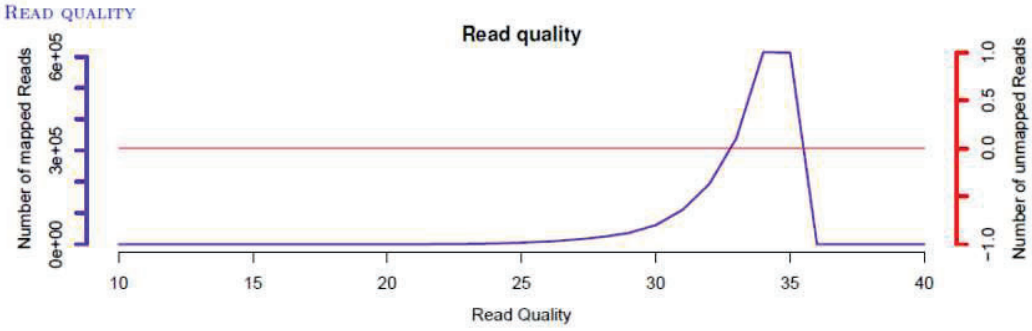
N1-PRE-RBV

File name	**N1-PRE-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**2 025 094**
Mapped reads	**2 025 094 (100.00%)**
Average read length	**138nt**
Coverage	**7 334nt (98.80%)**
Average depth	**37 269 reads/site**





N1-PRE-RBV Consensus sequence

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  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTCCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCAAAATGT TCTACACCGG TGCTTTCTAC
 350  GGCCGGTCGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGTGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTTC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTACTCT CTGCATGATC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGGCACGG GATGACACGT TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA CTTTGTGCTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT TCCATCTGCT TGCTCTACGA AATCCACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGT GGGATTAGTT ACAAGGTCAC TGTCGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACTGCTGT TATTACTGCA GCGTACTTAA CTATTTGTCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCTCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTT GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CAGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGGC AGGAGTGCAC
1470  CTGCTTTTTA GAACCAGCAG AGGGTCTAGT TGGTGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC CGTCCATGGC CGTCAACTTG
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1680  CGAACTCGTT GCAGGCCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCACCC TGAGGGGTTG TTAGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTCTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGATTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ACATCTGGGT GTTACCGCCG CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GTCCGCTCAG CCATCCAGCC CCGCAGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAATGCN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNGA CCGCCACCTT
2450  CTGAAGAATT TCAGGTTGAC ACAGCGCCCG CTCCCCCTGC CCCTGAGTCC GCTCAGCCAT CCAGCCCCGC
2520  AGGGCCAAAG GCTCCCGTGC GTAAGCCGCC AACGCCACCG CCCCCGCGCA CCCGCCGCCT TCTCTACACC
2590  TACCCGGATG GGGCAAAGGT GTATGCGGGG TCACTGTTTG AGTCTGACTG TGATTGGCTG GTTAATGCGT
2660  CGAATCCCGG CCATCGTCCT GGAGGCGGCC TTTGCCATGC CTTCTACCAA CGCTACCCCG AGTCTTTCCA
2730  CTCAACTGAG TTCATTATGC GCGACGGTCT TGCCGCGTAT ACTTTAACTC CCCGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCACAAC CCAAAGCGGC TTGAGGCAGC ATACCGAGAG ACCTGCTCCC
2870  GCCGCGGTAC TGCCGCCTAC CCACTCCTCG GCTCGGGCAT ATATCAAGTT CCTGTCAGCC TCAGCTTTGA
2940  CGCCTGGGAG CGTAACCACC GCCCTGGGGA CGAGCTCTAC CTGACCGACC TCGCAGCTAC CTGGTTTGAG
3010  GCTAATAAGC CAACACAGCC GGCCCTCACA ATAACTGAGG ATGCAGCCCG CACAGCCAAC CTAGCACTGG
3080  AGATCGATGC TGCTACAGAG GTCGGCCGGG CTTGTGCCGG CTGTGCAGTT AGTCCTGGGG TTGTGCACTA
3150  TCAGTTTACC GCTGGGGTCC CAGGTTCGGG CAAGTCGCGC TCTATACAGC AGGGGGATGT CGACGTAGTG
3220  GTTGTTCCCA CTCGGGAGCT CCGGAATAGT TGGCGTCGCC GGGGTTTCGC GGCTTTTACA CCTCATACGG
3290  CGGCCCGTGT CACCACGGGC CGTCGTGTTG TGATCGATGA GGCCCCATCA CTCCCACCGC ATTTGTTGCT
3360  ATTACACATG CAGCGGGCCT CGTCGGTCCA CCTCCTCGGC GACCCGAACC AGATCCCTGC CATAGACTTC
3430  GAGCATGCCG GCCTGGTCCC TGCAATACGC CCCGAGCTTG CGCCCACCAG TTGGTGGCAT GTCACCCATC
3500  GCTGCCCCGC TGATGTGTGC GAGCTTATAC GCGGGGCTTA TCCCAAAATT CAAACCACCA GCCGCGTGCT
3570  GCGGTCTTTA TTCTGGAATG AGCCTGCCAT TGGCCAGAAG TTAGTTTTCA CCCAGGCTGC TAAGGCCGCC
3640  AACCCTGGTG CGATTACAGT CCACGAGGCC CAGGGCGCCA CTTTCACGGA AACCACAATC ATAGCCACGG
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N1-PRE-RBV Consensus sequence

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3780 GTGTGTTATT CTTGACGCCC CCGGCTTGTT ACGTGAGGTT GGTATATCGG ATGTGATTGT CAACAATTTT
3850 TTCCTCGCCG GCGGGGAAGT GGGCCACCAT CGCCCCTCTG TGATACCTCG TGGTAATCCT GACCAGAACC
3920 TCGCGACACT ACAGGCCTTC CCACCTTCCT GCCAGATTAG TGCTTATCAT CAGTTAGCTG AGGAACTTGG
3990 CCACCGCCCA GCCCCCGTCG CCGCTGTCTT GCCCCCTTGC CCTGAACTTG AACAAGGCTT GTTATACATG
4060 CCACAGGAGC TCACGGTGTC TGACAGCGTG CTTGTCTTTG AACTCACGGA CATAGTCCAC TGCCGGATGG
4130 CTGCCCCCAG CCAGCGGAAG GCCGTCCTAT CGACGCTCGT GGGTAGGTAC GGCCGTCGGA CGAAGCTGTA
4200 TGAAGCAGCT CACTCTGACG TCCGTGAGTC CCTGGCTAGA TTTATTCCCA CCATTGGGCC CGTTCAGGCT
4270 ACTACGTGTG AGTTATATGA GCTGGTTGAG GCCATGGTGG AGAAAGGTCA GGATGGCTCT GCCGTGCTTG
4340 AGCTCGACCT CTGCAATCGT GATGTATCGC GTATTACATT TTTCCAGAAA GATTGTAATA AATTCACCAC
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4480 TTTGGTCCGT GGTTTCGTGC TATTGAAAAA GAAATACTAG CCCTGCTCCC GCCTAATATT TTCTACGGCG
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4620 TTTTTCAGAG TTTGATAGCA CCCAAAATAA CTTTTCCCTC GGTCTCGAGT GTGTAGTCAT GGAGGAATGT
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4760 AGTCTCTTAA GGGATTTTGG AAGAAGCATT CCGGTGAGCC CGGCACCCTC CTCTGGAACA CTGTTTGGAA
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6020 GTTGATATGA ATTCTATTAC TTCCACTGAT GTTAGGATTT TAGTTCAGCC TGGCATTGCT TCTGAGTTGG
6090 TTATCCCTAG TGAGCGCCTC CATTATCGTA ACCAGGGTTG GCGCTCTGTG GAGACCTCGG GTGTGGCCGA
6160 AGAGGAGGCT ACTTCTGGTT TAGTAATGCT TTGTATCCAT GGCTCTCCTG TTAATTCCTA CACCAATACC
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7070 GAAAATGCAG CTGGTCATCG TGTTGCCATT TCCACGTATA CCACCAGTTT GGGCGCTGGC CCTGTGTCTG
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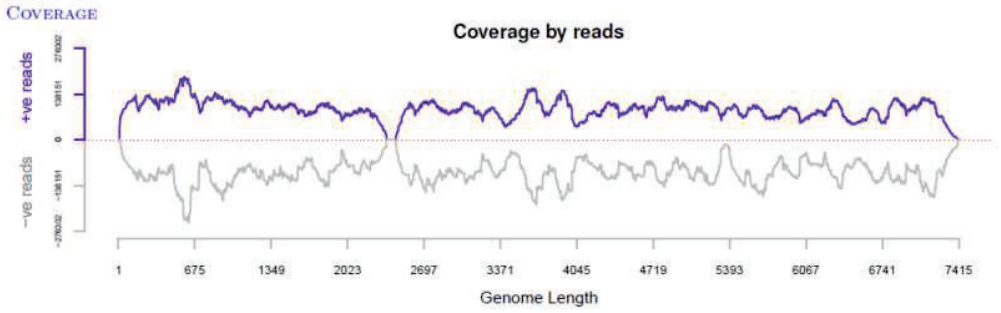
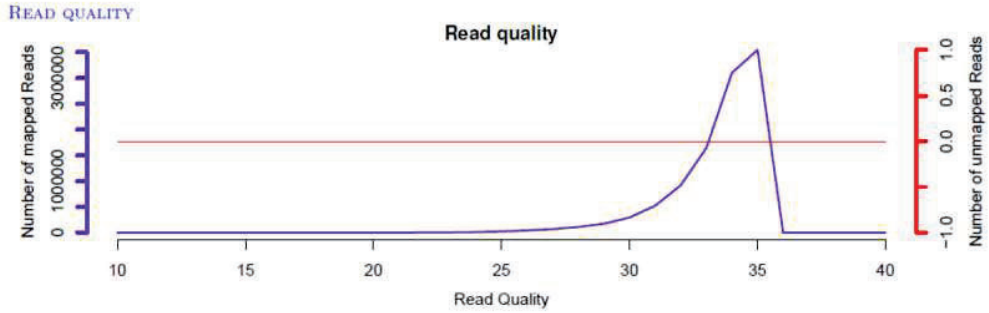
7420 NNN

N2-PRE-RBV

File name	**N2-PRE-RBV.sam**
Ref name	**pKC618403.1**
Ref len	**7423**
Program used	**Tanoti Assembler 1.0**
Total reads	**10470491**
Mapped reads	**10470491 (100.00%)**
Average read length	**137nt**
Coverage	**7343nt (98.92%)**
Average depth	**191047 reads/site**





N2-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTCCGAC CTGAAGTGCT
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 350  GGCCGGTCGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGTGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTTC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTACTCT CTGCATGATC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGGCACGG GATGACACGT TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA CTTTGTGCTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT TCCATCTGCT TGCTCTACGA AATCCACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGT GGGATTAGTT ACAAGGTCAC TGTCGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACTGCTGT TATTACTGCA GCGTACTTAA CTATTTGTCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCTCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTT GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CAGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGGC AGGAGTGCAC
1470  CTGCTTTTTA GAACCAGCAG AGGGTCTAGT TGGTGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC CGTCCATGGC CGTCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGATATCGC TGCCCGAGCC TCCCGTCTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGCCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCACCC TGAGGGGTTG TTAGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTCTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGATTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ACATCTGGGT GTTACCGCCG CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GTCCGCTCAG CCATCCAGCC CCGCAGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAATGCN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNGA CCGCCACCTT
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2730  CTCAACTGAG TTCATTATGC GCGACGGTCT TGCCGCGTAT ACTTTAACTC CCCGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCACAAC CCAAAGCGGC TTGAGGCAGC ATACCGAGAG ACCTGCTCCC
2870  GCCGCGGTAC TGCCGCCTAC CCACTCCTCG GCTCGGGTAT ATATCAAGTT CCTGTCAGCC TCAGCTTTGA
2940  CGCCTGGGAG CGTAACCACC GCCCTGGGGA CGAGCTCTAC CTGACCGACC TCGCAGCTAC CTGGTTTGAG
3010  GCTAATAAGC CAACACAGCC GGCCCTCACA ATAACTGAGG ATGCAGCCCG CACAGCCAAC CTAGCACTGG
3080  AGATCGATGC TGCTACAGAG GTCGGCCGGG CTTGTGCCGG CTGTGCAGTT AGTCCTGGGG TTGTGCACTA
3150  TCAGTTTACC GCTGGGGTCC CAGGTTCGGG CAAGTCGCGC TCTATACAGC AGGGGGATGT CGACGTAGTG
3220  GTTGTTCCCA CTCGGGAGCT CCGGAATAGT TGGCGTCGCC GGGGTTTCGC GGCTTTTACA CCTCATACGG
3290  CGGCCCGTGT CACCACGGGC CGTCGTGTTG TGATCGATGA GGCCCCATCA CTCCCACCGC ATTTGTTGCT
3360  ATTACACATG CAGCGGGCCT CGTCGGTCCA CCTCCTCGGC GACCCGAACC AGATCCCTGC CATAGACTTC
3430  GAGCATGCCG GCCTGGTCCC TGCAATACGC CCCGAGCTTG CGCCCACCAG TTGGTGGCAT GTCACCCATC
3500  GCTGCCCCGC TGATGTGTGC GAGCTTATAC GCGGGGCTTA TCCCAAAATT CAAACCACCA GCCGCGTGCT
3570  GCGGTCTTTA TTCTGGAATG AGCCTGCCAT TGGCCAGAAG TTAGTTTTCA CCCAGGCTGC TAAGGCCGCC
3640  AACCCTGGTG CGATTACAGT CCACGAGGCC CAGGGCGCCA CTTTCACGGA AACCACAATC ATAGCCACGG
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N2-PRE-RBV Consensus sequence

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3780  GTGTGTTATT CTTGACGCCC CCGGCTTGTT ACGTGAGGTT GGTATATCGG ATGTGATTGT CAACAATTTT
3850  TTCCTCGCCG GCGGGGAAGT GGGCCACCAT CGCCCCTCTG TGATACCTCG TGGTAATCCT GACCAGAACC
3920  TCGCGACACT ACAGGCCTTC CCACCTTCCT GCCAGATTAG TGCTTATCAT CAGTTAGCTG AGGAACTTGG
3990  CCACCGCCCA GCCCCCGTCG CCGCTGTCTT GCCCCCTTGC CCTGAACTTG AACAAGGCTT GTTATACATG
4060  CCACAGGAGC TCACGGTGTC TGACAGCGTG CTTGTCTTTG AACTCACGGA CATAGTCCAC TGCCGGATGG
4130  CTGCCCCCAG CCAGCGGAAG GCCGTCCTAT CGACGCTCGT GGGTAGGTAC GGCCGTCGGA CGAAGCTGTA
4200  TGAAGCAGCT CACTCTGACG TCCGTGAGTC CCTGGCTAGA TTTATTCCCA CCATTGGGCC CGTTCAGGCT
4270  ACTACGTGTG AGTTATATGA GCTGGTTGAG GCCATGGTGG AGAAAGGTCA GGATGGCTCT GCCGTGCTTG
4340  AGCTCGACCT CTGCAATCGT GATGTATCGC GTATTACATT TTTCCAGAAA GATTGTAATA AATTCACCAC
4410  AGGGGAGACC ATTGCCCACG GTAAGGTCGG CCAGGGCATC TCGGCTTGGA GTAAGACCTT CTGTGCCCTG
4480  TTTGGTCCGT GGTTTCGTGC TATTGAAAAA GAAATACTAG CCCTGCTCCC GCCTAATATT TTCTACGGCG
4550  ACGCATATGA GGAGTCTGTG TTTGCCGCCG CTGTGTCAGG GGCAGGTTCA AGCATGGTAT TTGAAAATGA
4620  TTTTTCAGAG TTTGATAGCA CCCAAAATAA CTTTTCCCTC GGTCTCGAGT GTGTAGTCAT GGAGGAATGT
4690  GGCATGCCCC AGTGGCTAAT TCGGTTGTAC CATTTGGTTC GGTCGGCCTG GATCCTACAG GCGCCGAAGG
4760  AGTCTCTTAA GGGATTTTGG AAGAAGCATT CCGGTGAGCC CGGCACCCTC CTCTGGAACA CTGTTTGGAA
4830  TATGGCGATC ATAGCACACT GCTATGAATT TCGTGATTTT AGGGTTGCCG CTTTCAAGGG AGATGATTCC
4900  GTGGTCCTCT GTAGCGACTA CCGTCAGAGC CGCAACGCAG CGGCCCTGAT TGCAGGTTGC GGGCTCAAGC
4970  TGAAGGTTGA TTACCGCCCC ATTGGGTTGT ATGCCGGTGT GGTGGTGGCC CCCGGTTTGG GGACGCTACC
5040  CGATGTGGTG CGCTTTGCCG GCCGGCTGTC TGAGAAGAAC TGGGGCCCTG GGCCGGAGCG GGCTGAGCAG
5110  TTGCGCCTAG CTGTTTGTGA CTTCCTTCGA GGGTTAACGA ATGTTGCGCA GGTATGTGTC GATGTTGTAT
5180  CCCGAGTTTA TGGAGTTAGC CCTGGGTTGG TACATAACCT TATTGGCATG TTGCAAACCA TAGCTGATGG
5250  CAAAGCCCAT TTTACAGAGA CTGTTAAACC TGTGCTTGAC CTCACGAACT CTATCATACA GCGGGTGGAA
5320  TGAATAACAT GTTCTGTGCA TTGCCCATGG GATCATCATG TGCCCTAGGG CTGTTCTGTT GTTGTTCCTC
5390  GTGCTTCTGC CTATGCTGCC CGCGCCACCG GCCGGCCAGC CGTCTGGCCG CCGTCGTGGG CGGCGCAGCG
5460  GCGGTACCGG CAGTGGTTTC TGGGGTGACA GGGTTGATTC TCAGCCCTTC GCCCTCCCCT ATATTCATCC
5530  AACCAACCCC TTTGCCGCCG ATGTCGTACC GCAATCCGGG GCTGGAGCTC GCCCTCGACA GCCACCCCGC
5600  CCCCTCGGCT CCTCTTGGCG TGACCAGTCC CAGCGCCCCT CCGCTGTCCC ACGTCGTCGA TCTGCCCCAG
5670  TTGGGGCTGC GCCGCTGACT GCCATATCAC CTGCTCCCGA CACGGCTCCT GTACCTGATG TTGACTCGCG
5740  TGGCGCTATA TTGCGACGCC AGTACAATTT ATCCACATCC CCGCTCACAT CATCTGTTGC TTCGGGTACT
5810  AATCTGGTTC TTTATGCTGC CCCGCTAAAC CCTTTGCTGC CCCTTCAGGA TGGCACTAAC ACTCACATCA
5880  TGGCCACTGA GGCATCTAAT TATGCCCAGT ATCGGGTTGT TCGAGCTACG ATCCGTTACA GGCCATTGGT
5950  GCCAAATGCT GTCGGTGGTT ATGCAATATC CATCTCATTC TGGCCTCAGA CTACTACTAC CCCCACGTCT
6020  GTTGATATGA ATTCTATTAC TTCCACTGAT GTTAGGATTT TAGTTCAGCC TGGCATTGCT TCTGAGTTGG
6090  TTATCCCTAG TGAGCGCCTC CATTATCGTA ACCAGGGTTG GCGCTCTGTG GAGACCTCGG GTGTGGCCGA
6160  AGAGGAGGCT ACTTCTGGTT TAGTAATGCT TTGTATCCAT GGCTCTCCTG TTAATTCCTA CACCAATACC
6230  CCCTATACTG GGGCGCTTGG ACTCCTTGAC TTTGCTTTAG AGCTTGAGTT TAGGAACCTG ACACCCGGGA
6300  ACACCAACAC CCGTGTGTCC CGGTATACAA GCACAGCCCG TCATCGGCTG CGCCGCGGTG CTGATGGCAC
6370  CGCTGAACTT ACCACCACAG CAGCCACGCG CTTCATGAAG GACCTGCACT TCACCGGTAC GAATGGGGTC
6440  GGTGAGGTGG GTCGTGGTAT TGCTCTTACA CTCTTTAATC TTGCTGACAC GCTTCTCGGT GGTTTGCCGA
6510  CAGAATTAAT TTCGTCGGCT GGGGGACAGT TATTCTACTC CCGCCCCGTC GTCTCAGCCA ATGGCGAGCC
6580  GACTGTCAAG TTATACACAT CTGTAGAGAA TGCGCAGCAG GATAAAGGGA TCGCCATCCC ACATGACATA
6650  GATCTGGGTG ACTCCCGTGT GGTCATTCAA GACTATGACA ACCAGCATGA GCAGGATCGA CCCACCCCCT
6720  CGCCTGCCCC TTCTCGCCCT TTTTCGGTTC TTCGCGCTAA TGATGTTTTA TGGCTTTCTC TTACCGCCGC
6790  CGAGTACGAC CAGACTACAT ATGGGTCGTC CACCAACCCG ATGTATGTCT CGGATACTGT CACATTTGTT
6860  AATGTGGCCA CAGGAGCCCA GGCTGTCGCC CGTTCCCTTG ACTGGTCCAA AGTTACTCTG GACGGCCGCC
6930  CTCTTACTAC TATCCAGCAG TATTCCAAAA CATTTTATGT TCTCCCGCTT CGCGGGAAGC TATCTTTTTG
7000  GGAGGCCGGG ACGACCAAGG CCGGCTACCC CTATAATTAC AACACAACTG CTAGTGATCA GATTCTGATT
7070  GAAAATGCAG CTGGTCATCG TGTTGCCATT TCCACGTATA CCACCAGTTT GGGCGCTGGC CCTGTGTCTG
7140  TTTCTGCAGT TGGTGTTTTA GCTCCACATT CGGCTCTTGC AGTCCTTGAA GACACTATTG ACTACCCTGC
7210  CCGTGCCCAC ACTTTTGATG ATTTTTGCCC GGAGTGTCGC GCTCTTGGTT TGCAGGGGTG TGCCTTCCAG
7280  TCTACTATTG CTGAGCTTCA GCGTCTTAAA ATGAAGGTAG GTAAAACCCG GGAGTTTTAA TCAATTCCTT
7350  TTGTGCCCCC TTCATAGCTT TTGTTTTATT TCTTTTTTCT GCGGTTCGCG CTCCCTGGAA AANNNNNNNN
7420  NNN
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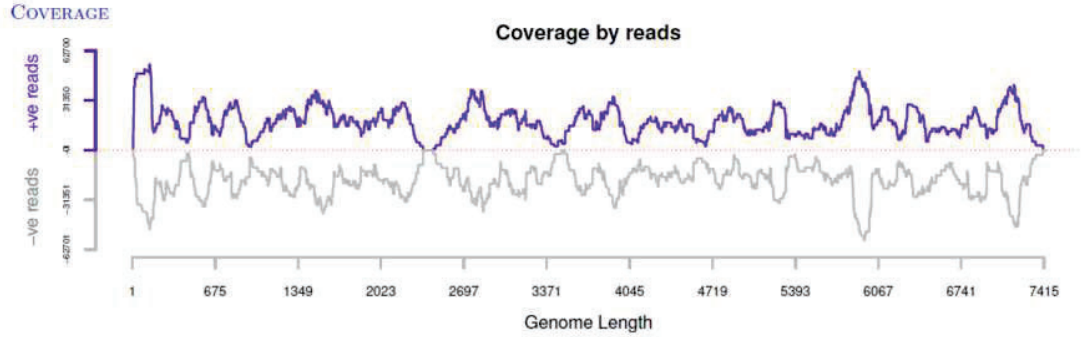
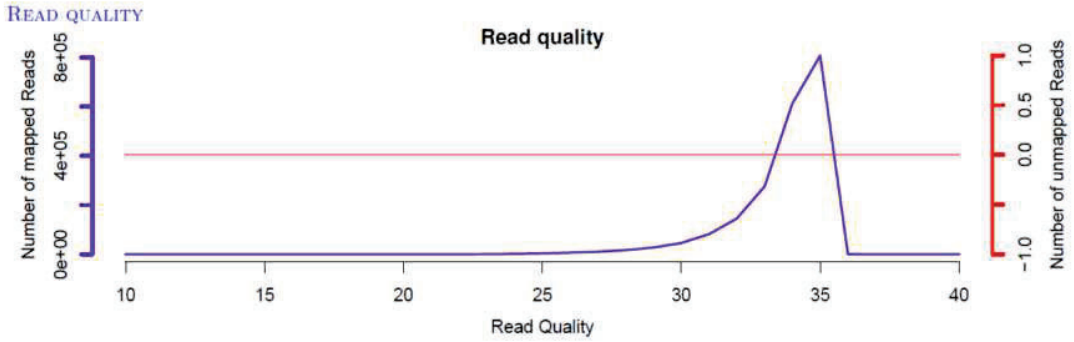
N3-POST-RBV2

File name	**N3-POST-RBV2.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**2 036 030**
Mapped reads	**2 036 030 (100.00%)**
Average read length	**138nt**
Coverage	**7 333nt (98.79%)**
Average depth	**37 569 reads/site**





N3-POST-RBV2 Consensus sequence

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  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTCCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GCCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCAAAATGT TCTACACCGG TGCTTTCTAC
 350  GGCCGGTCGG GAGAGATGTT CAGCGCTGGT ACTCTGCACC TACCCGTGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTTC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTACTCT CTGCATGATC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGGCACGG GATGACACGT TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTCGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT TCCATCTGCT TGCTCTACGA AATCCACATT TCACGCTGTC CCAGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGT GGGATTAGTT ACAAGGTCAC TGTCGGCGCC CTTGTCGCTA ACGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACTGCTGT TATTACTGCA GCGTACTTAA CTATTTGTCA TCAGCGTTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCTCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTT GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CAGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTTTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT CCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGGC AGGAGTGCAC
1470  CTGCTTTTTA GAACCAGCAG AGGGTCTAGT TGGTGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC TGTCCATGGC CGTCAACTTG
1610  AGGCCTTGTA TAGGGCGCTT AACATCCCGC ATGATATCGC TGCCCGAGCC TCCCGTCTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGCCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GTGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGC GGGGCCCCTA GCGCTGCTCC GGAGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCCTGT
2030  GGCTGCACCC TGAGGGGTTG TTAGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTCTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGATTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGCGCCGGCT GCTACTCCGG GGTTACGCCA GCCTACACCT CCTGTTAGTG
2240  ACATCCGGGC GTTACCGCCG CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGA
2310  GTCCGCTCAG CCATCCAGCC CCGCAGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAATGCN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNGA CCGCCACCTT
2450  CTGAAGAATT TCAGGTTGAC ACAGCGCCCG CTCCCCCTGC CCCTGAGTCC GCTCAGCCAT CCAGCCCCGC
2520  AGGGCCAAAG GCTCCCGTGC GTAAGCCGCC AACGCCACCG CCCCCGCGCA CCCGCCGCCT TCTTTACACC
2590  TACCCGGATG GGGCAAAGGT GTATGCGGGG TCACTGTTTG AGTCTGACTG TGATTGGCTG GTTAATGCGT
2660  CGAATCCCGG CCATCGTCCT GGAGGCGGCC TTTGCCATGC CTTCTACCAA CGCTACCCCG AGTCTTTCTA
2730  CTCAACTGAG TTCATTATGC GCGACGGTCT TGCCGCGTAT ACTTTAACTC CCCGGCCTAT TATTCATGCA
2800  GTGGCTCCTG ATTATAGGGT TGAGCACAAC CCAAAGCGGC TTGAGGCAGC ATACCGAGAG ACCTGCTCCC
2870  GCCGCGGTAC TGCCGCCTAC CCACTCCTCG GCTCGGGTAT ATATCAAGTT CCTGTCAGCC TCAGCTTTGA
2940  CGCCTGGGAG CGTAACCACC GCCCTGGGGA CGAGCTCTAC CTGACCGACC TCGCAGCTAC CTGGTTTGAG
3010  GCTAATAAGC CAACACAGCC GGCCCTCACA ATAACTGAGG ATGCAGCCCG CACAGCCAAC CTAGCACTGG
3080  AGATCGATGC TGCTACAGAG GTCGGCCGGG CTTGTGCCGG CTGTGCAGTT AGCCCTGGGG TTGTGCACTA
3150  TCAGTTTACC GCTGGGGTCC CAGGTTCGGG CAAGTCGCGC TCTATACAGC AGGGGGATGT CGACGTAGTG
3220  GTTGTTCCCA CTCGGGAGCT CCGGAATAGT TGGCGTCGCC GGGGTTTCGC GGCTTTTACA CCTCATACGG
3290  CGGCCCGTGT CACCACGGGC CGTCGTGTTG TGATCGATGA GGCCCCATCA CTCCCACCGC ATTTGTTGCT
3360  ATTACACATG CAGCGGGCCT CGTCGGTCCA CCTCCTCGGC GACCCGAACC AGATCCCTGC CATAGACTTC
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3500  GCTGCCCCGC TGATGTGTGC GAGCTTATAC GCGGGGCTTA TCCCAAAATT CAAACCACCA GCCGCGTGCT
3570  GCGGTCTTTA TTCTGGAATG AGCCTGCCAT TGGCCAGAAG TTGGTTTTCA CCCAGGCTGC TAAGGCCGCC
3640  AACCCTGGTG CGATTACAGT CCACGAGGCC CAGGGCGCCA CTTTCACGGA AACCACAATC ATAGCCACGG
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N3-POST-RBV2 Consensus sequence

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3710    CTGATGCTAG AGGGCTCATC CAATCTTCCC GAGCTCATGC CATAGTCGCA CTTACTCGCC ACACAGAGAA
3780    GTGTGTTATT CTTGACGCCC CCGGCTTGTT ACGTGAGGTT GGTATATCGG ATGTGATTGT CAACAATTTT
3850    TTCCTCGCCG GCGGGGAAGT GGGCCACCAT CGCCCCTCTG TGATACCTCG TGGTAATCCT GACCAGAACC
3920    TCGCGACACT ACAGGCCTTC CCACCTTCCT GCCAGATTAG TGCTTATCAT CAGTTAGCTG AGGAACTTGG
3990    CCACCGCCCA GCCCCCGTCG CCGCTGTCTT GCCCCCTTGC CCTGAACTTG AACAAGGCTT GTTATACATG
4060    CCACAGGAGC TCACGGTGTC TGACAGTGTG CTTGTCTTTG AACTCACGGA CATAGTCCAC TGCCGGATGG
4130    CTGCCCCCAG CCAGCGGAAG GCCGTCCTAT CGACGCTCGT GGGTAGGTAC GGCCGTCGGA CGAAGCTGTA
4200    TGAAGCAGCT CACTCTGACG TCCGCGAGTC CCTGGCTAGA TTTATTCCCA CCATTGGGCC CGTTCAGGCT
4270    ACTACGTGTG AGTTATATGA GCTGGTTGAG GCCATGGTGG AGAAAGGTCA GGATGGCTCT GCCGTGCTTG
4340    AGCTCGACCT CTGCAATCGC GATGTATCGC GTATTACATT TTTCCAGAAA GATTGTAATA AATTCACCAC
4410    AGGGGAGACC ATTGCCCACG GTAAGGTCGG CCAAGGCATC TCGGCTTGGA GTAAGACCTT CTGTGCCCTG
4480    TTTGGTCCGT GGTTTCGTGC TATTGAAAAA GAAATACTAG CCCTGCTCCC GCCTAATATT TTCTACGGCG
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4620    TTTTTCAGAG TTTGATAGCA CCCAAAATAA CTTTTCCCTC GGCCTCGAGT GTGTAGTCAT GGAGGAATGT
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5040    CGATGTGGTG CGCTTTGCCG GCCGGCTGTC TGAGAAGAAC TGGGGCCCTG GGCCGGAGCG GGCTGAGCAG
5110    TTGCGCCTAG CTGTTTGTGA TTTCCTTCGA GGGTTAACGA ATGTTGCGCA GGTATGTGTC GATGTTGTAT
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5600    CCCCTCGGCT CCTCTTGGCG TGACCAGTCC CAGCGCCCCT CCGCTGTCCC ACGTCGTCGA TCTGCCCCAG
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5810    AATCTGGTTC TTTATGCTGC CCCGCTAAAC CCTTTGCTGC CCCTTCAGGA TGGCACTAAC ACTCACATCA
5880    TGGCCACTGA GGCATCTAAT TATGCCCAGT ATCGGGTTGT TCGAGCTACG ATCCGTTACA GGCCATTGGT
5950    GCCAAATGCT GTCGGTGGTT ATGCAATATC CATCTCATTC TGGCCTCAGA CTACTACTAC CCCCACGTCT
6020    GTTGATATGA ATTCTATTAC TTCCACTGAT GTTAGGATTT TAGTTCAGCC TGGCATTGCT TCTGAGTTGG
6090    TTATCCCTAG TGAGCGCCTC CATTATCGTA ACCAGGGTTG GCGCTCTGTG GAGACCTCGG GTGTGGCCGA
6160    AGAGGAGGCT ACTTCTGGTT TAGTAATGCT TTGTATCCAT GGCTCTCCTG TTAATTCCTA CACCAATACC
6230    CCCTATACTG GGGCGCTTGG ACTCCTTGAC TTTGCTTTAG AGCTTGAGTT TAGGAACCTG ACACCCGGGA
6300    ACACCAACAC CCGTGTGTCC CGGTATACAA GCACAGCCCG TCATCGGCTG CGCCGCGGTG CTGATGGCAC
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6650    GACCTGGGTG ACTCCCGTGT GGTCATTCAA GACTATGACA ACCAGCATGA GCAGGATCGA CCCACCCCCT
6720    CGCCTGCCCC TTCTCGCCCT TTTTCGGTTC TTCGCGCTAA TGATGTTTTA TGGCTTTCTC TTACCGCCGC
6790    CGAGTACGAC CAGACTACAT ATGGGTCGTC CACCAACCCG ATGTATGTCT CGGATACTGT CACATTTGTT
6860    AATGTGGCCA CAGGAGCCCA GGCTGTCGCC CGTTCCCTTG ACTGGTCCAA AGTTACTCTG GACGGCCGCC
6930    CTCTTACTAC TATCCAGCAG CATTCCAAAA CATTTTATGT TCTCCCGCTT CGCGGGAAGC TATCTTTTTG
7000    GGAGGCCGGG ACGACCAAGG CCGGCTACCC CTATAATTAC AACACAACTG CTAGTGATCA GATTCTGATT
7070    GAAAATGCAG CTGGTCATCG TGTTGCCATT TCCACGTATA CCACCAGTTT GGGCGCTGGC CCTGTGTCTG
7140    TTTCTGCAGT TGGTGTTTTA GCTCCACATT CGGCTCTTGC AGTCCTTGAA GACACTATTG ACTACCCTGC
7210    CCGTGCCCAC ACTTTTGATG ATTTTTGCCC GGAGTGTCGC GCTCTTGGTT TGCAGGGGTG TGCCTTCCAG
7280    TCTACTATTG CTGAGCTTCA GCGTCTTAAA ATGAAGGTAG GTAAAACCCG GGAGTTTTAA TCAATTCCTT
7350    CTGTGCCCCC TTCATAACTT TTGTTTTATT TCTTTCTTCT GCGGTTCGCG CTCCCAGGAA NNNNNNNNNN
7420    NNN
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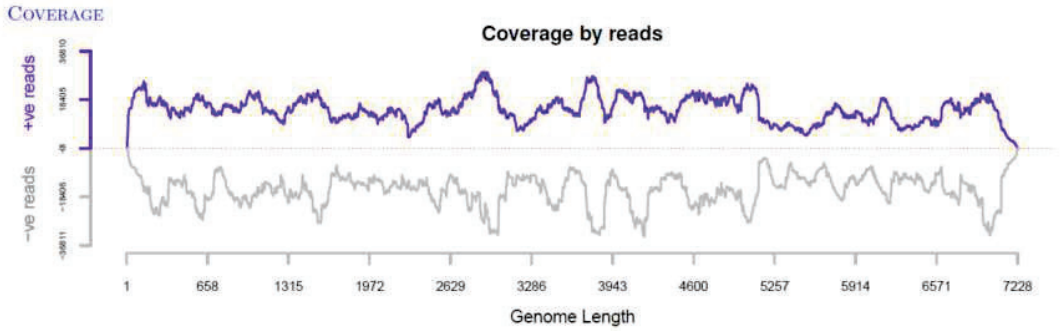
O1-PRE-RBV

File name	**O1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 634 741**
Mapped reads	**1 634 741 (100.00%)**
Average read length	**136nt**
Coverage	**7 226nt (99.85%)**
Average depth	**30 561 reads/site**



O1-PRE-RBV Consensus sequence

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 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCTTTG CGTGGTCTCC CCCCCGTTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
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 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACACCT CCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  TTACCGTACA ACTTCATATC TTTTGATTCA CGACGGTGAT CGTGCTGTTG TGACTTATGA GGGTGACACT
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 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCCACATT TCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGTTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT CATTACTGCA GCGTATTTGA CCATCTGTCA TCAGCGCTAC CTCCGTACTC
1190  AAGCTATATC CAAGGGTATG CGTCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  CTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTTTATGC ACAGTGCCGT
1330  CGTTGGTTAT CGGCGGGTTT CCATCTTGAC CCAAGGGTGC TTGTCTTTGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGTTG TTTCATGAAG TGGTTGGGGC AGGAGTGCAC
1470  CTGTTTCTTG GAACCAGCAG AGGGTCTAGT TGGAGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC CGTCCATGGT CGCCAACTTG
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1680  CGAACTCGTT GCAGGTCCTG ACCGTTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
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1890  ATCTAATGGC CTGGATTGCA CTGCAACATT TCCCCCGGGC GGCGCCCCGA GCGCTGCCCC GGGGGAGGTA
1960  GCAGCCTTCT GCAGTGCCCT TTACAGGTAT AACAGGTTTA CTCAGCGCCA TTCGCTTATA GGCGGCTTGT
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2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGC AGTGCCGGCT GCTACTCCGG GGCTACGCCA CCCTACACCT CCTGCCAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACTCAGCT CTTGCTCCCC CCGCCCCTGA
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2380  CGCACCCGCC GTCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTACGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTCAAT GCGTCGAATC CTGGCCATCG TCCTGGAGGT GGCCTTTGTC ATGCCTTTTA
2520  CCAACGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTACACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTACA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGT TCTCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GCATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCCTG GGAGCGTAAC CATCGCCCCG GGGATGAGCT TTACTTAACC
2800  GACCTCGCCG CTACCTGGTT CGAGGCTAAC AAGCCAACAC AGCCGGCCCT CACAATAACT GAGGATGCAG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTTGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGACCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGCTGGCGT CGCCGGGGTT
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3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAATCCC GGTGCGATTA CAGTCCACGA GGCCCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATTATAGCT ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ACGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
3640  TCGGATGCGA TTGTCAACAA CTTTTTCCTC GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCTGTGATAC
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O1-PRE-RBV Consensus sequence

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3710  CTCGTGGTAA TCCAGACCAG AACCTCGCGA CACTACAGGC CTTTCCACCT TCCTGCCAGA TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCAGCTCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAGG GCTTGTTATA TATGCCACAG GAACTTACGG TGTCTGACAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT TCACTGCCGG ATGGCTGCCC CCAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
3990  GTACGGCCGC CGGACGAAGC TGTATGAAGC GGCTCACTCT GACGTCCGTG AGTCCCTGGC TAGATTCATC
4060  CCCACCATCG GGCCCGTTCG GGCTACTACG TGTGAGTTAT ATGAGTTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATTA CATTTTTCCA
4200  GAAGGACTGT AACAAATTTA CCACAGGGGA GACCATTGCT CACGGTAAGG TTGGCCAGGG CATCTCGGCT
4270  TGGAGTAAGA CTTTCTGTGC CCTGTTTGGC CCGTGGTTCC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
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4620  CCTCCTTTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AGTTTCGTGA TTTTCGGGCT
4690  GCCGCCTTCA AGGGAGATGA TTCCGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
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5460  TCCCACGTCG TCGATCTGCC CCAGCTGGGG CTGCGCCGCT GACTGCTATA TCGCCTGCTC CCGATACAGC
5530  TCCCGTGCCT GATGTTGACT CGCGTGGCGC CATATTGAGA CGCCAGTACA ATTTATCCAC ATCCCCGCTC
5600  ACATCATCCG TCGCTTCGGG TACCAATTTG GTTCTTTATG CTGCCCCGCT AAACCCCTTG CTGCCCCTTC
5670  AGGACGGCAC TAACACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TCGTCCGAGC
5740  CACGATTCGT TACAGGCCAT TGGTGCCAAA TGCTGTTGGC GGTTATGCAA TATCCATTTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACCTCCAC TGATGTTAGG ATTCTAGTCC
5880  AGCCCGGTAT TGCTTCTGAG TTGGTTATCC CCAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
5950  TGTGGAGACC TCGGGTGTTG CAGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT CCATGGTTCT
6020  CCTGTTAACT CCTACACCAA TACCCCCTAC ACCGGGGCAC TTGGACTCCT TGATTTCGCT TTAGAGCTAG
6090  AGTTTAGGAA CCTGACACCC GGGAATACCA ACACCCGTGT GTCTCGGTAT ACAAGCACAG CCCGTCATCG
6160  GTTGCGCCGC GGTGCTGATG GCACCGCCGA ACTCACCACC ACAGCCGCCA CGCGCTTCAT GAAGGACCTG
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6300  ATACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGAGGA CAGTTATTCT ACTCCCGCCC
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6580  TCTTTGGCTT TCTCTTACTG CCGCCGAGTA CGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTTACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTCGATTGGT
6720  CTAAAGTCAC TCTGGACGGC CGTCCTCTTA CTACTACCCA GCAGCACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAGTTATCTT TCTGGGAGGC CGGGACAACT AAGGCTGGTT ATCCCTATAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TACACCACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TTTAGCCCCA CATTCTGCCC TCGCAGTCCT
7000  TGAAGACACT ATTGATTACC CTGCCCGTGC CCACACTTTT GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGTCTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT CTAATCAATT TCCTTTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTTTT TTCTGCGGTT
7210  CGCGCTCCCT GGAAAANNNN NNNNNNN
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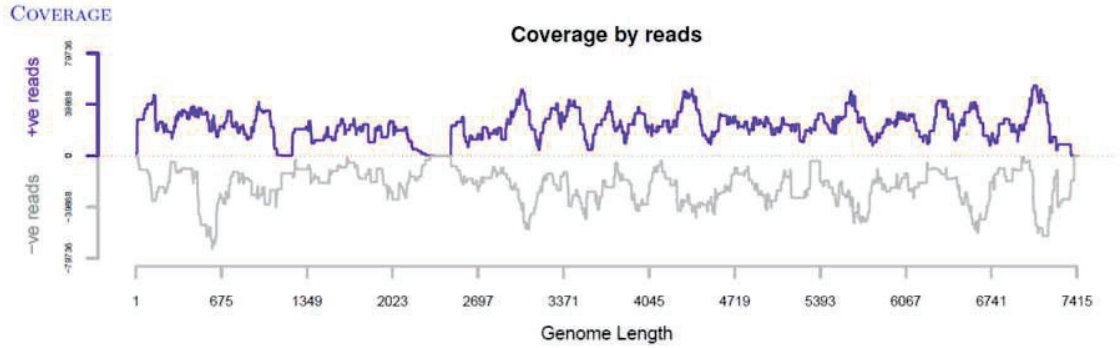
P1-PRE-RBV

File name	**P1-PRE-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**2 456 094**
Mapped reads	**2 456 094 (100.00%)**
Average read length	**147nt**
Coverage	**7 323nt (98.65%)**
Average depth	**46 965 reads/site**



P1-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATC CTTATCAATC TAATGCAACC CCGGCAACTG GTTTTCCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA CAATGAACTA GAACAGTACT GCCGGGCTCG GGCTGGTCGC
 280  TGTCTGGAAG TTGGGGCCCA CCCAAGATCT ATCAATGACA ACCCGAATGT TTTGCATCGG TGTTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGTTGGT ATTCTGCTCC CACCCGTGGC CCTGCAGCTA ATTGTCGCCG
 420  CTCCGCCTTA CGCGGCCTCC CCCCCGTTGA TCGTACCTAT TGCTTTGATG GATTCTCCCG CTGCTCGTTT
 490  GCTGCGGAAA CTGGTATTGC CCTTTATTCT TTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCCATGG
 560  CCCGACACGG GATGACACGC CTATATGCTG TACTACATCT TCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  CTACCACACA ACCTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTACGA AGGTGACACT
 700  AGTGCAGGCT ACAATCATGA TGTTTCCATA CTCCGAGCAT GGATCCGCAC AACTAAAATA GTAGGCGATC
 770  ATCCGCTGGT GATAGAACGT GTGCGGGCCA TTGGTTGCCA CTTTGTGTTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCCTATG TCCCGTATCC CCGGTCGACA GAGGTCTATG TCCGTTCTAT ATTCGGCCCA
 910  GGTGGGTCCC CATCTCTCTT TCCATCAGCT TGCTCTACAA AATCCACATT TCATGCTGTC CCGGTTCACA
 980  TCTGGGACCG GCTCATGCTC TTCGGCGCCA CCCTCGATGA TCAGGCGTTT TGCTGTTCAC GCCTTATGAC
1050  TTATCTCCGT GGGATTAGCT ACAAGGTCAC CGTTGGTGCC CTCGTTGCCA ATGAGGGGTG GAACGCTTCA
1120  GAGGACGCTC TTACCGCTGT TATTACTGCG GCATACCTGA CTATCTGCCA TCAGCGTTAC CTCCGCACTC
1190  AAGCTATATC CAAGGGCATG CGTCGACTGG AGGTTGAACA CGCTCAAAAA TTTATCACAA GACTTTACAG
1260  CTGGCTGTTT GAGAAGTCTG GCCGTGACTA CATTCCCGGC CGTCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGCTGGTTAT CTGCAGGTTT TCACCTTGAT CCTAGGGTGC TTGTCTTTGA TGAGTCCGTG CCCTGCCGTT
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1470  CTGTTTTTTG GAACCAGCGG AGGGCCTGGT TGGTGATCAT GGCCACGATA ACGAAGCCTA TGAGGGTTCT
1540  GAGGTCGACC AGGCTGAGCC TGCCTATCTT GATGTTTCTG GGACTTATGC CGTCCATGGG CGTCAGCTTG
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1680  CGAGCTCGTT GCAGGTCCAG ATCGCTTGGA GTGCCGTACT GTGCTCGGGA ATAAGACCTT TCGGACAACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCTAAC GGCCCTGAGC AATACGTCCT CTCGTTTGAT GCCTCCCGCC
1820  AGTCTATGGG GGCTGGATCG CATAATCTTA CCTATGAGCT TACCCCTGCT GGCCTACAGG TTAAGATCTC
1890  ATCTAATGGC TTAGATTGCA CTGCAACATT CCCCCCGGGC GGCGCCCCTA GCGCTGCTCC GGAGGAGGTG
1960  GCAGCTTTCT GCAGTGCCCT TTACAGGTAT AACAGGTTCA CCCAGCGACA CTCGCTTACA GGCGGCCTGT
2030  GGTTGTATCC CGAGGGGTTG CTGGGTATCT TCCCCCCCTT CTCCCCCGGT CATATTTGGG AGTCCGCCAA
2100  TCCTTTCTGT GGGGAAGGAA CGTTATACAC CCGGACCTGG TCAACATCTG GTTTTTCTAG CGACTTTTCC
2170  CCCCCAGAGG CGGCTGTCGC TGCGCCGGCT GCTGCCCCGG GGCTGCCCCA CCCCACACCC CCTGTTAGTG
2240  ATATTTGGGT GCTACCGTCA CTTTCTGAAG AATCTCAGGT TGATGCAACG CCAGCCATCC CTGCCCCTGA
2310  GCCCGCTCAA CCGCCCAGCC CTGCTGGGTC AAAGACCCCC GTGCGTAAGC CGCCAANNNN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNCTT
2450  CTGAAGAATC TCAGGTTGAT GCAACGCCAG CCATCCCTGC CCCTGAGCCC GCTCAACCGC CCAGCCCTGC
2520  TGGGTCAAAG ACCCCCGTGC GTAAGCCATC AACACCACCA CCCCCGCGCA CCCGCCGCCT CCTTTATACT
2590  TATCCGGACG GGGCAAAGGT GTACGCGGGG TCGTTGTTTG AGTCTGACTG TGACTGGCTG GTCAATGCAT
2660  CGAACCCTGG CCATCGTCCT GGGGGTGGCC TTTGCCATGC CTTTTACCAA CGCTATCCTG AGTCTTTCTA
2730  CTCAACTGAG TTCATCATGC GCGACGGTCT CGCCGCATAT ACTTTAACTC CCCGGCCCAT TATCCATGCA
2800  GTGGCCCCTG ACTACCGAGT CGAGCAAAAC CCAAAGAGGC TTGAGGCAGC ATATCGAGAG ACCTGCTCTC
2870  GCCGCGGTAC CGCCGCTTGT CCGCTTCTCG GCGCAGGTAT ATATCAAGTC CCCGTCAGCC TCAGCTTTGA
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3080  AGATCGATGC TGCCACGGAG GTTGGCAGGG CTTGTGCCGG CTGTACAGTT AGACCTGGGG TTGTGCATTA
3150  TCAATTCACT GCCGGGGTTC CAGGTTCAGG AAAGTCTCGG TCTATACAGC AGGGGGATGT TGATGTGGTG
3220  GTTGTCCCCA CCCGGGAGCT TCGGAATGGT TGGCGTCGCC GAGGTTTTGC AGCTTTTACA CCTCATACGG
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P1-PRE-RBV Consensus sequence

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3780  ATGCGTCATT CTTGACGCCC CTGGCCTGTT ACGTGAGGTC GGTATATCGG ATATTATTGT TAACAATTTC
3850  TTTCTTGCTG GTGGGGAAGT GGGCCATCAC CGCCCCTCTG TGATACCTCG TGGTAGTCCT GACCAAAATC
3920  TCGCGACGTT ACAGGCCTTC CCACCTTCCT GTCAGATTAG TGCCTACCAT CAGCTGGCCG AGGAACTAGG
3990  TCACCGCCCA GCCCCTGTCG CCGCTGTCCT GCCCCCTTGC CCCGAACTTG AGCAGGGCTT GTTATATATG
4060  CCCCAGGAGC TCACGGTGTC TGACAGTGTG CTGGTCTTTG AACTTACGGA CATAGTCCAC TGCCGGATGG
4130  CTGCCCCTAG TCAGCGGAAG GCCGTCCTGT CGACGCTTGT AGGTAGGTAC GGCCGTAGAA CGAAGCTGTT
4200  TGAGGCGGCC CATTCTGACG TCCGTGAGTC CCTGGCTAGA TTCATCCCCA CCATTGGGCC TGTTCAGGCC
4270  ACCACGTGTG AGCTATACGA GCTGGTTGAG GCCATGGTGG AGAAGGGTCA GGATGGCTCG GCCGTACTTG
4340  AGCTCGACCT TTGCAATCGT GATGTGTCGC GCATCACATT CTTCCAGAAA GATTGCAATA AGTTCACTAC
4410  AGGGGAGACC ATTGCTCACG GTAAGGTCGG TCAGGGCATT TCAGCTTGGA GTAAGACCTT TTGTGCCCTG
4480  TTCGGCCCGT GGTTTCGCGC TATTGAAAAG GAAATATTGT CCCTGCTCCC GCCTAATATT TTCTACGGCG
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4620  TTTTTCCGAG TTTGATAGCA CTCAGAACAA CTTCTCCCTA GGTCTCGAGT GTGTAATTAT GGAGGAATGT
4690  GGCATGCCCC AGTGGCTAAT ACGGTTGTAC CACTTAGTTC GGTCGGCCTG GATACTACAG GCGCCGAAGG
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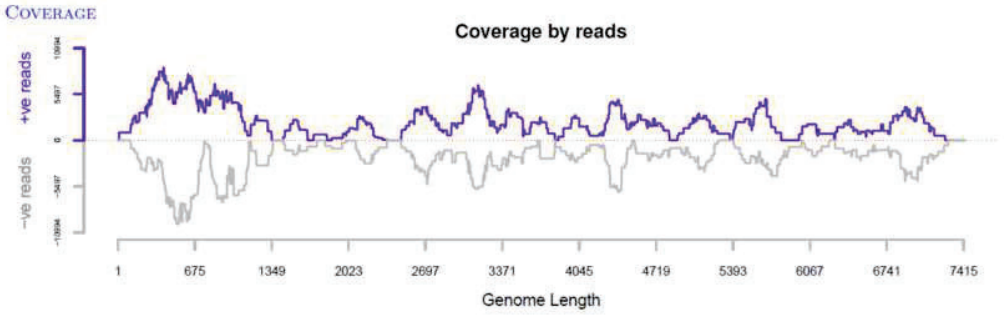
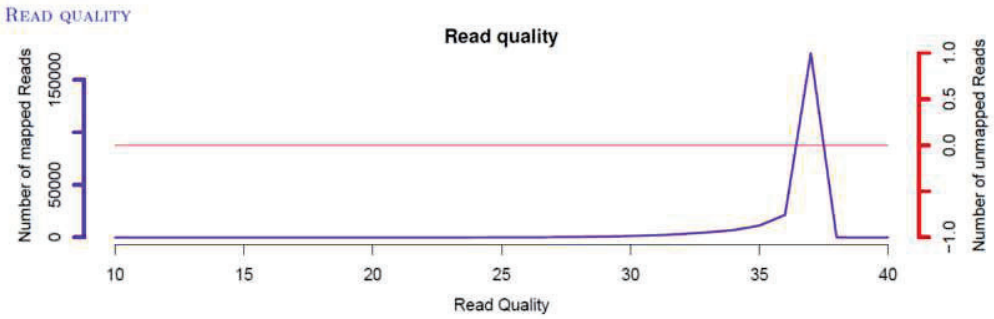
P2-PRE-RBV

File name	**P2-PRE-RBV.sam**
Ref name	**KC618403.1**
Ref length	**7 423nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**228 241**
Mapped reads	**228 241 (100.00%)**
Average read length	**143nt**
Coverage	**7 315nt (98.55%)**
Average depth	**4 248 reads/site**





P2-PRE-RBV Consensus sequence

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 280  TGTCTGGAAG TTGGGGCCCA CCCAAGATCT ATCAATGACA ACCCGAATGT TTTGCATCGG TGTTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGTTGGT ATTCTGCTCC CACCCGTGGC CCTGCAGCTA ATTGTCGCCG
 420  CTCCGCCTTA CGCGGCCTCC CCCCCGTTGA TCGTACCTAT TGCTTTGATG GATTCTCCCG CTGCTCGTTT
 490  GCTGCGGAAA CTGGTATTGC CCTTTATTCT TTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCCATGG
 560  CCCGACACGG GATGACACGC CTATATGCTG TACTACATCT CCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  CTACCACACA ACCTCATACC TTCTGATTCA CGACGGCGAT CGTGCTGTTG TGACCTACGA AGGTGACACT
 700  AGTGCAGGTT ACAATCATGA TGTTTCCATA CTCCGAGCAT GGATCCGCAC AACTAAAATA GTAGGCGATC
 770  ATCCGCTGGT GATAGAACGT GTGCGGGCCA TTGGTTGCCA CTTTGTGTTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCCTATG TCCCGTATCC CCGGTCGACA GAGGTCTATG TCCGTTCTAT ATTCGGCCCA
 910  GGTGGGTCCC CATCTCTCTT TCCATCAGCT TGCTCTACAA AATCCACATT TCATGCTGTC CCGGTCCACA
 980  TCTGGGACCG GCTCATGCTC TTCGGCGCCA CCCTCGATGA TCAGGCGTTT TGCTGTTCAC GCCTTATGAC
1050  TTATCTCCGT GGGATTAGCT ACAAGGTCAC CGTTGGTGCC CTCGTTGCCA ATGAGGGGTG GAACGCTTCA
1120  GAGGACGCTC TTACCGCTGT TATTACTGCG GCATACCTGA CTATCTGCCA TCAGCGTTAC CTCCGCACTC
1190  AAGCTATATC CAAGGGCATG CGTCGACTGG AGGTTGAACA CGCTCAAAAA TTTATCACAA GACTTTACAG
1260  CTGGCTGTTT GAGAAGTCTG GCCGTGACTA CATTCCCGGC CGTCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGCTGGTTAT CTGCAGGTTT TCACCTTGAC CCAAGAGTGC TTGTCTTCGA TGAGTCTGTA CCCTGCCGCT
1400  GCAGGACGTT TCTTAAGAAG GCTGCCGGCA AGTTCTGCTG CTTTATGAAA TGGTTGGGGC AGGAATGCAC
1470  CTGTTTTTTG GAACCAGCGG AGGGCCTGGT TGGTGATCAT GGCCACGATA ACGAAGCCTA TGAGGGTTCT
1540  GAGGTCGACC AGGCTGAGCC TGCCTATCTT GATGTTTCTG GGACTTATGC CGTCCATGGG CGTCAGCTTG
1610  AGGCCCTGTA TAGGGCGCTT AATATCCCGC AAGACATTGT TGCTCGAGCC TCTCGTCTAA CCGCCACTGT
1680  CGAGCTCGTT GCAGGTCCAG ATCGCTTGGA GTGCCGTACT GTGCTCGGGA ATAAGACCTT TCGGACAACG
1750  GTGGCTGATG GCGCCCATCT TGAGGCTAAC GGCCCTGAGC AATACGTCCT CTCGTTTGAT GCCTCCCGCC
1820  AGTCTATGGG GGCTGGATCG CATAATCTTA CCTATGAGCT TACCCCTGCT GGCCTACAGG TTAAGATCTC
1890  ATCTAATGGC TTAGATTGCA CTGCAACATT CCCCCCGGGC GGCGCTCCTA GCGCTGCTCC GGAGGAGGTG
1960  GCAGCTTTCT GCAGTGCCCT TTACAGGTAT AACAGGTTCA CCCAGCGACA CTCGCTTACA GGCGGCCTGT
2030  GGTTGCATCC CGAGGGGTTG CTGGGTATCT TCCCCCCCTT CTCCCCCGGT CATATTTGGG AGTCCGCCAA
2100  TCCTTTCTGT GGGGAAGGAA CGTTATACAC CCGGACCTGG TCAACATCTG GTTTTTCTAG CGACTTTTCC
2170  CCCCCAGAGG CGGCTGTCGC TGCGCCGGCT GCTGCCCCGG GGCTGCCCCA CCCCACACCC CCTGTTAGTG
2240  ATATTCGGGT GCTACCGTCA CTCTCTGAAG AATCTCAGGT TGATGCAACG CCAGCCATCC CTGCCCCTGA
2310  GCCCGCTCAA CCGCCCAGCC TTGCTGGGCC AAAGACCCCC GTGCGTAAGC NNNNNNNNNN NNNNNNNNNN
2380  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNT
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2730  CTCAACTGAG TTCATCATGC GCGACGGTCT CGCCGCATAT ACTTTAACTC CCCGGCCCAT TATCCATGCA
2800  GTGGCCCCTG ACTACCGAGT CGAGCAAAAC CCAAAGAGGC TTGAGGCAGC ATATCGAGAG ACCTGCTCTC
2870  GCCGCGGTAC CGCCGCTTGT CCGCTTCTCG GCTCAGGTAT ATATCAAGTC CCCGTCAGCC TCAGCTTTGA
2940  TGCTTGGGAG CGTAATCACC GCCCTGGGGA CGAGCTGTAC CTTACCGACC TTGCCGCTGC CTGGTTTGAG
3010  GCCAATAAGC CGACACAGCC GGCCCTCACA ATAACTGAGG ACACAGCCCG TACGGCTAAC CTAGCACTAG
3080  AGATCGATGC TGCCACGGAG GTTGGCAGGG CTTGTGCCGG CTGTACAGTT AGACCTGGGG TTGTGCATTA
3150  TCAATTCACT GCCGGGGTTC CAGGTTCAGG AAAGTCCCGG TCTATACAGC AGGGGGATGT TGATGTGGTG
3220  GTTGTCCCCA CCCGGGAGCT TCGGAATAGT TGGCGTCGCC GAGGTTTTGC AGCTTTTACA CCTCATACGG
3290  CGGCCCGTGT CGCTGCGGGC CGCCGTGTTG TGATTGATGA GGCCCCGTCC CTCCCACCGC ACTTGTTGTT
3360  ACTGCACATG CAGCGGGCTT CGTCGGTCCA TCTCCTTGGC GACCCAAATC AGATCCCTGC CATAGACTTC
3430  GAGCATGCCG GCCTCGTTCC CGCAATACGC CCCGAGCTTG CGCCAACCAG CTGGTGGCAT GTAACCCATC
3500  GCTGCCCCGC TGATGTGTGC GAGCTTATAC GTGGGGCTTA CCCCAAAATC CAGACCACTA GTCGCGTGTT
3570  GCGGTCTCTA TTCTGGAATG AGCCTGCCAT TGGCCAGAAG TTGGTTTTTA CACAGGCCGC TAAGGCCGCT
3640  AACCCTGGTG CGATTACTGT TCACGAGGCC CAGGGCGCCA CCTTTACGGA AACCACAGTT ATAGCCACAG
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P2-PRE-RBV Consensus sequence

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3780 ATGCGTCATT CTTGACGCCC CTGGCCTGTT ACGTGAGGTC GGTATATCGG ATGTGATTGT TAACAATTTC
3850 TTCCTTGCTG GTGGGGAAGT GGGCCATCAC CGCCCCTCTG TGATACCTCG TGGTAGTCCT GACCAAAATC
3920 TCGCGACGTT ACAGGCCTTC CCACCTTCCT GTCAGATTAG TGCCTACCAT CAGCTGGCCG AGGAACTAGG
3990 TCACCGCCCA GCCCCTGTCG CCGCTGTCCT GCCCCCTTGC CCCGAACTTG AGCAGGGCTT GTTATATATG
4060 CCCCAGGAGC TCACGGTGTC TGACAGTGTG CTGGTCTTTG AACTTACGGA CATAGTCCAC TGCCGGATGG
4130 CTGCCCCTAG TCAGCGGAAG GCCGTCCTGT CGACGCTTGT AGGTAGGTAC GGCCGTAGAA CGAAGCTGTT
4200 TGAGGCGGCC CATTCTGACG TCCGTGAGTC CCTGGCTAGA TTCATCCCCA CCATTGGGCC TGTTCAGGCC
4270 ACCACGTGTG AGCTATACGA GCTGGTTGAG GCCATGGTGG AGAAGGGTCA GGATGGCTCG GCCGTACTTG
4340 AGCTCGACCT TTGCAATCGT GATGTGTCGC GCATCACATT CTTCCAGAAA GATTGCAATA AGTTCACTAC
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4480 TTCGGCCCGT GGTTTCGCGC TATTGAAAAG GAAATATTGT CCCTGCTCCC GCCTAATATT TTCTACGGCG
4550 ATGCATATGA GGAGTCTGTG TTTGCCGCCG CTGTTTCTGG TGCAGGTTCA TGCATGGTGT TTGAGAATGA
4620 TTTTTCCGAG TTTGATAGCA CTCAGAACAA CTTCTCCCTA GGTCTCGAGT GTGTAATTAT GGAGGAATGT
4690 GGCATGCCCC AGTGGCTAAT ACGGTTGTAC CACTTAGTTC GGTCGGCCTG GATACTACAG GCGCCGAAGG
4760 AGTCTCTTAA AGGATTCTGG AAGAAGCACT CTGGTGAGCC CGGCACCCTT CTCTGGAACA CCGTCTGGAA
4830 CATGGCGATC ATAGCGCACT GCTATGAATT TCGTGATCTT AGGGTTGCCG CCTTCAAGGG AGATGACTCC
4900 GTAGTCCTCT GTAGCGACTA CCGCCAAAGC CGCAATGCGG CTGCCCTAAT TGCGGGCTGT GGGCTCAAAT
4970 TGAAGGTTGA TTATCGCCCT ATTGGATTGT ATGCTGGTGT CGTGGTGGCC CCCGGCCTGG GGACGCTACC
5040 CGATGTAGTG CGCTTTGCCG GCCGGCTGTC CGAGAAGAAC TGGGGCCCTG GGCCGGAGCG GGCTGAGCAG
5110 CTGCGCCTTG CTGTCTGTGA TTTCCTTCGA GGGTTGACGA ATGTTGCGCA GGTATGTGTT GATGTTGTGT
5180 CCCGAGTTTA TGGAGTTAGC CCTGGGCTGG TACATAACCT TATTGGCATG CTTCAGACCA TTGCTGATGG
5250 CAAAGCCCAC TTTACAGAGA CTGTTAAACC TGTGCTTGAC CTCACAAATT CTATTATACA GCGGGTGGAA
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5460 GCGGTTCCGG CGGTGGTTTC TGGGGTGACA GGGTTGATTC TCAGCCCTTC GCCCTCCCCT ATATTCATCC
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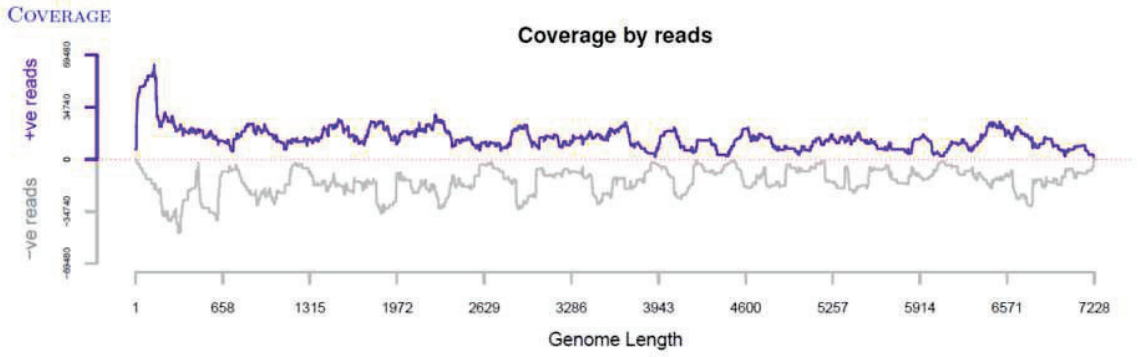
5880 TGGCCACTGA GGCATCTAAT TATGCCCAGT ATCGGGTTGT CCGAGCCACG ATCCGTTATA GGCCACTGGT
5950 GCCAAATGCT GTTGGTGGTT ATGCAATATC TATTTCATTC TGGCCCCAAA CTACTACTAC CCCTACGTCT
6020 GTCGATATGA ACTCTATTAC CTCTACTGAT GTTAGGATTC TGGTTCAGCC TGGTATTGCT TCCGAGCTGG
6090 TCATTCCCAG TGAGCGTCTC CATTATCGCA ACCAGGGCTG GCGTTCCGTA GAGACCTCAG GTGTGGCTGA
6160 AGAGGAGGCT ACTTCTGGTT TGGTGATGCT TTGTATTCAT GGTTCCCCTG TTAATTCTTA CACTAATACC
6230 CCCTATACTG GGGCGCTCGG GCTTCTTGAT TTTGCTTTAG AGCTTGAGTT TAGGAACTTG ACACCCGGGA
6300 ACACTAACAC TCGTGTCTCC CGGTACACAA GTACAGCCCG CCATCGGCTG CGTCGCGGTG CTGACGGCAC
6370 CGCCGAGCTC ACCACTACAG CGGCCACGCG TTTCATGAAA GACTTGCACT TCACCGGAAC GAATGGGGTC
6440 GGTGAGGTGG GCCGTGGTAT AGCTCTCACT CTCTTTAATC TTGCTGATAC GCTTCTTGGT GGTCTGCCGA
6510 CAGAATTAAT TTCGTCGGCT GGGGGACAGT TGTTCTATTC CCGCCCCGTC GTCTCAGCCA ATGGCGAGCC
6580 GGCTGTCAAG CTATATACAT CTGTAGAGAA TGCGCAGCAG GATAAAGGGA TCGCCATCCC ACATGATATA
6650 GACCTGGGTG ACTCTCGTGT CGTCATTCAG GATTATGATA ATCAGCATGA GCAGGATCGG CCCACCCCTT
6720 CGCCTGCCCC ATCTCGCCCT TTTTCGGTTC TTCGTGCTAA TGATGTTTTA TGGCTCTCTC TCACCGCTGC
6790 TGAGTATGAC CAGACTACAT ATGGGTCGTC CACCAACCCG ATGTATGTCT CGGATACTGT CACGTTTGTC
6860 AATGTGGCTA CAGGGGCTCA GGCTGTCGCC CGCTCCCTTG ACTGGTCCAA AGTTACCCTG GATGGTCGCC
6930 CCCTTACTAC TATTCAGCAG TACTCTAAAA TATTTTATGT TCTCCCGCTT CGAGGTAAGC TGTCCTTCTG
7000 GGAGGCCGGG ACGACTAAGG CCGGATACCC CTATAATTAC AATACAACTG CTAGTGATCA GATTTTGATT
7070 GAAAATGCGG CCGGTCACCG TGTTGCTATT TCTACCTATA CCACTAGTTT GAGTGCCGGC CCTGTATCAA
7140 TCTCTGCAGT CGGTGTATTG GCCCCGCATT CGGCTCTTGC AGTTCTTGAG GATACTATTG ATTACCCCGC
7210 CCGCGCCCAT ACTTTTGATG ATTTCTGCCC GGAGTGTCGC ACCCTTGGCT TACAGGGGTG TGCCTTCCAA
7280 TCCACTATCG CTGAGCTTCA GCGTCTTAAA GTGAAGGTAG GCAAAACCCG GGAGTCTTAA TTAATTCCAT
7350 CTGTGCCCCC TTCANAGGTC TTGGTTTATT TCTTTCTTCT GCGTTTCGCG CTCCCNNNNN NNNNNNNNNN
7420 NNN

Q1-PRE-RBV1

File name	**Q1-PRE-RBV1.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 486 849**
Mapped reads	**1 486 849 (100.00%)**
Average read length	**144nt**
Coverage	**7 224nt (99.82%)**
Average depth	**29 223 reads/site**



Q1-PRE-RBV1 Consensus sequence

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   1  GCAGACCACG TATGTGGTCG ATGCCATGGA GGCCCACCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTCATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GAGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCAGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGCTGA TCGTACCTAT TGTTTTGATG GATTCTCCCG CTGCTCATTT
 490  GCCGCAGAAA CTGGGGTTGC CCTCTATTCC CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA CGTCTCCATA CTTCGTGCAT GGATCCGCAC AACCAAGATA ACCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATATCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCTACATT CCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAAGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGCTGGTTAT CGGCGGGTTT CCATCTCGAC CCAAGGGTGC TTGTTTTCGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGTTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACCTATGC CGTCCACGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC CGCCCGAGCC TCCCGTCTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCA CCTACGAGCT CACTCCTGCC GGTCTGCAGA TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCTCT TTATAGATAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCCTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGT AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTTGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCTC CTGCCCCTGA
2310  GCCTGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA TACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCGAATC CCGGCCATCG TCCTGGCGGC GGCCTTTGCC ATGCCTTCTA
2520  TCAACGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC ATATACTTTA
2590  ACCCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGT TCTCGCCGCG GTACCGCCGC CTACCCACTC CTTGGCTCGG GCATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAT CATCGCCCCG GGGACGAGCT CTACTTAACC
2800  GACCTCGCCG CCACCTGGTT CGAAGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCGG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT AGTGGTTGTT CCTACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GCGTCACCAC AGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTATTACA TATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGCGACCCA
3220  AATCAGATCC CTGCTATAGA CTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTCGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
3360  GATCCAAACC ACCAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACTCAGG CTGCTAAGGC CGCTAACCCC GGTGCGATTA CAGTCCATGA GGCTCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATTATAGCC ACAGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCAC ACGCCATAGT
3570  TGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
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Q1-PRE-RBV1 Consensus sequence

3710 CTCGCGGTAA TCCTGACCAG AATCTCGCGA CACTTCAGGC CTTCCCACCC TCTTGCCAGA TTAGTGCTTA
3780 CCACCAGTTA GCTGAGGAAC TCGGCCACCG TCCAGCTCCC GTCGCTGCTG TCCTGCCCCC TTGCCCTGAA
3850 CTTGAGCAGG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGACAG TGTGCTGGTC TTTGAACTCA
3920 CGGACATAGT CCACTGCCGT ATGGCTGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TCGTGGGTAG
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4060 CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130 GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200 GAAAGACTGT AATAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCC
4270 TGGAGTAAGA CCTTCTGTGC CCTGTTTGGC CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
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4900 CCTGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCTGTTT GTGATTTCCT TCGAGGGTTA ACGAATGTTG
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6230 CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCCCT TACACTCTTT AATCTTGCTG
6300 ACACACTTCT TGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGCTATTCT ACTCCCGCCC
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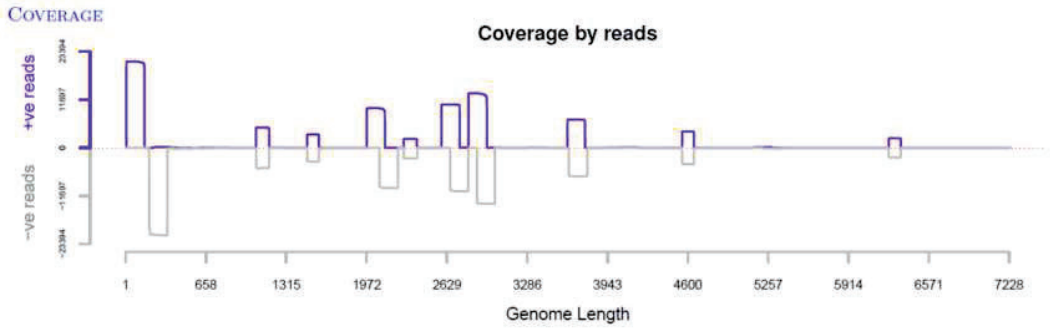
7000 TGAAGACACG ATTGATTACC CTGCCCGTGC CCACACATTT GATGATTTCT GCCCGGAGTG TCGCGCTCTT
7070 GGTTTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140 CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTCTT TTCTGCGGTT
7210 CGCGCTCCCT GGAANNNNNN NNNNNNN

Q2-ON-RBV1

File name	**Q2-ON-RBV1.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**159 325**
Mapped reads	**159 325 (100.00%)**
Average read length	**139nt**
Coverage	**7 186nt (99.30%)**
Average depth	**3 031 reads/site**



Q2-ON-RBV1 Consensus sequence

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  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTCATCAATT TGATGCAACC CCGGCAGCTG GTTTTTCGAC CTGAAGTGCT
 210  CTGGAATCAT CCTATTCAAC GAGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTCCTTC
 350  GCCCGGTCGG GAGAGATGTA CAGCGTTGGT ATTCCGCCCC GACTCGCGGC CCAGCTGCCA ACTGCCGGCG
 420  TTCCGCATTA CGTGGCCTGC CCCCTGTCGA CCGTACTTAC TGTTTCGACG GGTTCTCCCG CTGTGCTTTT
 490  GCTGCTGAGA CTGGAATTGC TTTGTATTCA CTACATGACC TCTGGCCTGC CGATGTTGCG GAGGCCATGG
 560  CCCGACACGG GATGACACGC CTATATGCTG TACTACATCT TCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  CTACCACACA ACCTCATACC TTCTGATTCA CGACGGTGAT CGTGCTGTTG TGACCTACGA AGGTGACACT
 700  AGTGCAGGTT ACAATCATGA TGTTTCCATA CTTCGCGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGTTGCCA CTTTGTGTTG CTGCTTACTG CGGCCCCTGA
 840  GCCGTCACCA ATGCCCTATG TCCCGTATCC CCGGTCGACA GAGGTCTATG TCCGTTCTAT ATTCGGCCCA
 910  GGTGGGTCCC CATCTCTTTT TCCATCAGCC TGCTCTACTA AATCCACATT TCACGCCGTC CCGGTTCATA
 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAAGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA GCAGCGCTAC CTCCGTACCC
1190  AAGCTATATC TAAGGGCATG CGCCGACTGG AGGTTGAACA TGCTCAGAAA TTCATCACAA GACTCTATAG
1260  TTGGTTGTTT GAGAAGTCCG GCCGCGACTA CATCCCCGGC CGTCAGCTCC AGTTTTATGC ACAGTGCCGC
1330  CGTTGGCTGT CGGCAGGTTT TCATCTTGAC CCAAGAGTGC TTGTCTTCGA TGAGTCTGTA CCCTGCCGCT
1400  GCAGGACGTT TCTTAAGAAG GCTGCCGGCA AGTTCTGCTG CTTTATGAAG TGGCTGGGGC AGGAGTGCAC
1470  TTGCTTTCTG GAGCCGGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACTTATGC TGTCCATGGG CGCCAGCTTG
1610  AGGCCTTGTA CAGGGCGCTC AACATCCCAC ATGACATNNN NNNNNNNNNN NNNNNNNNNN NNGCCACTGT
1680  CGAGCTCGTT GCAGGTCCAG ATCGCTTGGA GTGCCGTACA GTGCTCGGAA ATAAGACCTT CCGCACATCG
1750  ATAATGGATG GCGCTCACCT CGAGGCTAAC GGCCCTGAGC AGTATGTTTT AACATTTGAC GACTCCCGCC
1820  AAGCGATGGG GGCTGGGCCG CATAGTCTTA CATATGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCTCT TTATAGATAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCCTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGT AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTCGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCTTC CTGCCCCTGA
2310  GCCTGCTCAA CCATCTAGCT CCGCTGGTCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCAAATC CTGGCCATCG TCCTGGCGGC GGCCTTTGCC ATGCCTTCTA
2520  TCAACGCTAC CCCGAGTCTT TCCATCATAC TGGTTTTGTC ATGCGTGAGG GCCTCGCTGC GTACACTTTA
2590  ACCCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GGGTTGAACA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGGCTTGT TCTCGCCGCG GTACCGCCGC CTACCCACTC CTTGGCTCGG GCATATACCA
2730  AGTCCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAT CATCGCCCCG GGGACGAGCT CTACTTAACC
2800  GACCTCGCCG CCACCTGGTT CGAAGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCGG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTCGATGT TGTGGTTGTT CCTACCCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TTGCAGCTTT TACACCTCAT ACGGCGGCCC GCGTCGCTGC CGGCCGCCGT GTTGTGATTG ATGAGGCCCC
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3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
3360  GATCCAAACC ACCAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
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3500  CGGAAACTAC AATTATAGCC ACAGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCAC ACGCCATAGT
3570  TGCACTTACC CGCCACACAG AAAAATGCGT CATTCTTGAC GCCCCTGGCT TGTTACGTGA GGTTGGTATA
3640  TCAGATGTGA TTGTTAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGCCA CCATCGCCCT TCTGTGATAC
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Q2-ON-RBV1 Consensus sequence

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3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCAGCCCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAA GAGCTCACAC TGTCCGACAG CGTTTTAGTC TTCGAGCTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TTGTGGGCAG
3990  GTACGGCCGC CGGACTAAGC TGTACGAAGC AGCCCACTCT GACGTCCGCG AGTCCCTGGC GAGGTTTATC
4060  CCTACCATCG GACCAGTTCA GGCCACCACA TGTGAGTTGT ACGAGTTGGT AGAGGCTATG GTTGAGAAGG
4130  GTCAGGACGG TTCCGCTGTG TTAGAGCTTG ATCTCTGTAG CCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAAGGACTGT AATAAGTTTA CAACAGGTGA GACTATTGCT CATGGCAAGG TGGGCCAGGG CATTTCGGCC
4270  TGGAGCAAGA CCTTTTGTGC CCTGTTCGGC CCATGGTTCC GAGCCATCGA AAAAGAAATA TTGGCCTTGC
4340  TCCCACCTAA CATCTTTTAC GGCGACGCCT ATGAGGAGTC AGTGTTTGCT GCCGCCGTGT CCGGGGCAGG
4410  TTCTAGTATG GTTTTTGAGA ATGATTTCTC TGAGTTTGAC AGTACCCAAA ACAATTTCTC CCTCGGCCTT
4480  GAGTGTGTTA TTATGGAGGA GTGTGGCATG CCCCAGTGGC TAATACGGTT GTACCACTTA GTTCGGTCGG
4550  CCTGGATCCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCG CACTGCTATG AATTCCGTGA TCTTAGGGTT
4690  GCCGCCTTCA AGGGAGATGA CTCCGTAGTC CTCTGTAGCG ACTACCGCCA AAGCCGCAAT GCGGCTGCCC
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4830  GGCCCCTGGT CTGGGGACGC TACCTGATGT GGTGCGCTTT GCCGGCCGGT TGTCTGAGAA GAATTGGGGC
4900  CCGAGTCCCG AGCGCGCCGA GCAGTTACGC TTGGCTGTTT GTGACTTCCT TCGAAAGTTA ACGAATGTTG
4970  CGCAGGTTTG TGTTGATGTT GTGTCCCGTG TTTATGGGGT TAGTCCTGGG CTGGTACATA ACCTTATTGG
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5110  AACTCTATTA TACAGCGGTT GGAATGAATA ACATGTTGTG TGCATCGCCC ATGGGTTCAC CATGTGCCCT
5180  CGGGCTGTTC TGTTGTTGTT CCTCGTGCTT TTGCCTATGC TGCCCGCGCC ACCGGCCAGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCAGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
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5670  AGGATGGCAC CAATACTCAT ATCATGGCGA CTGAGGCGTC TAACTATGCT CAATATCGGG TTGTCCGAGC
5740  CACAATCCGT TATCGCCCTT TGGTGCCGAA TGCTGTTGGA GGCTATGCAA TTTCTATTTC CTTTTGGCCC
5810  CAGACTACAA CTACTCCCAC CTCTGTTGAT ATGAATTCTA TTACCTCTAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT AGCCTCCGAG TTGGTTATTC CAAGCGAGCG CCTCCATTAT CGCAACCAGG GCTGGCGTTC
5950  TGTTGAGACC TCGGGTGTGG CCGAGGAGGA GGCGACTTCT GGTTTGGTGA TGCTCTGCAT TCATGGCTCC
6020  CCTGTTAACT CTTACACCAA CACCCCCTAN NNNGGGGCGC TCGGGCTTCT TGATTTTGCT TTAGAGCTTG
6090  AGTTTAGGAA CCTGACACCC GGGAACACTA ACACTCGTGT CTCCCGGTAC ACCAGCACAG CCCGCCACCG
6160  CCTGCGCCGC GGTGCTGATG GCACTGCCGA GCTCACCACC ACGGCGGCCA CGCGTTTCAT GAAGGACCTG
6230  CATTTCACCG GGACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCCCT TACACTCTTT AATCTTGCTG
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6720  CTAAAGTTAC TCTGGATGGT CGCCCTCTTA CTACTATTCA GCAGTACTCT AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAGCTGTCCT TCTGGGAGGC CGGGACGACT AAGGCCGGAT ACCCCTATAA TTATAATACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCAGCCGGCC ACCGTGTTGC TATATCTACC TACACTACTA
6930  GCCTTGGTGC TGGTCCTGTG TCTGTCTCTG CGGTAGGTGT ACTTGCCCCC CATTCTGCTT TAGCTGTACT
7000  TGAGGATACT ATTGATTACC CCGCTCGTGC CCATACTTTT GATGATTTCT GCCCTGAGTG CCGTAATCTC
7070  GGCCTACAGG GCTGTGCTTT TCAATCCACT ATCGCTGAGC TTCAGCGTCT TAAAGTGAAG GTAGGCAAAA
7140  CCCGGGAGTC TTAATTAATT CCATCTGTGC CCCCTTCAAG GTCTTGGTTT ATTTCTTNTC TTCTGCGTTT
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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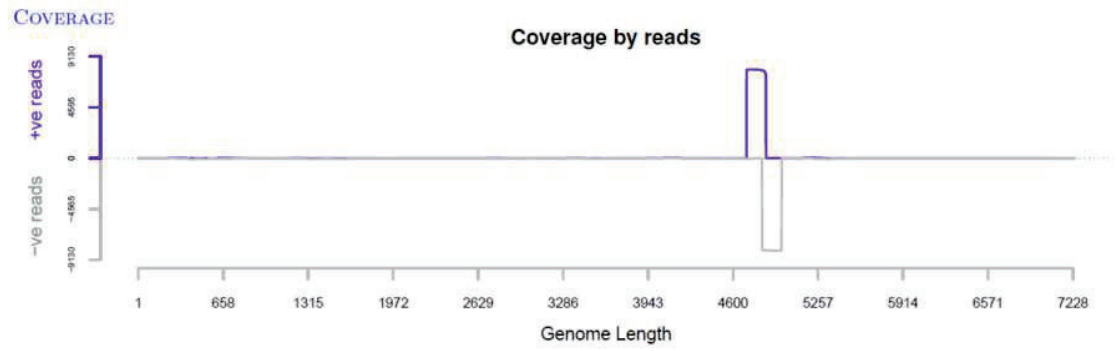
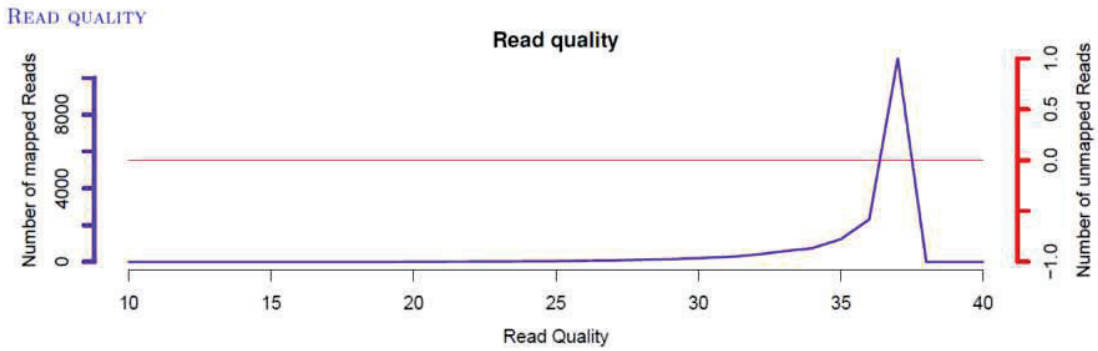
Q3-POST-RBV1

File name	**Q3-POST-RBV1.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**17 337**
Mapped reads	**17 337 (100.00%)**
Average read length	**150nt**
Coverage	**6 861nt (94.80%)**
Average depth	**358 reads/site**





Q3-POST-RBV1 Consensus sequence

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 140  CCCGTGTTCA GACTGACATT CTCATCAATT TGATGCAACC CCGGCAGCTC GTTTTCCGAC CTGAAGTGCT
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 350  GCCCGGTCGG GAGAGATGTA CAGCGTTGGT ATTCCGCCCC GACTCGCGGC CCAGCTGCCA ACTGCCGGCG
 420  TTCCGCATTA CGTGGCCTGC CCCCTGTCGA CCGTACTTAC TGTTTCGACG GGTTCTCCCG CTGTGCTTTT
 490  GCTGCTGAGA CTGGAATTGC TTTGTATTCA CTACATGACC TCTGGCCTGC CGATGTTGCG GAGGCCATGG
 560  CCCGACACGG GATGACACGC CTATATGCTG TACTACATCT TCCCCCTGAA GTACTACTAC CACCTGGTAC
 630  CTACCACACA ACCTCATACT TGTTGATTCA TGACGGCAAC CGCGCCGTCG TAACTTATGA GGGGGATACT
 700  AGTGCGGGTT ACAACCATGA TGTGTCCATT CTTCGCGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG CTGCTTACTG CTGCCCCTGA
 840  GCCGTCCCCT ATGCCTTATG TCCCATACCC CCGGTCGACG GAGGTGTATG TCAGGTCCAT ATTTGGCCCC
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 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGCTG GAACGCCTCA
1120  GAGGATGCGC TTACTGCTGT GATCACTGCA GCCTACTTGA CCATCTGTCA CCAGCGCTAC CTCCGTACCC
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1330  CGTTGGCTGT CGGCAGGTTT TCATCTTGAC CCAAGAGTGC TTGTCTTCGA TGAGTCTGTA CCCTGCCGCT
1400  GCAGGACGTT TCTTAAGAAG GCTGCCGGCA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
1470  CTGTTTTTTG GAACCAGCAG AGGGCCTGGT TGGCGACCAT GGTCATGATA ATGAGGCCTA TGAGGGCTCT
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1750  GTGGTGGATG GCGCTCACCT CGAGGCTAAC GGCCCTGAGC AGTATGTTTT AACATTTGAC GACTCCCGCC
1820  AAGCGATGGG GGCTGGGCCG CATAGTCTCA CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT CTACAGGTAC AACAGGTTCA CTCAGCNNNN NNNNNNNNNN NNNNNNNNNN
2030  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN
2100  NNNNNNNNNN NNNNNGGGAA CCCTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGT AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTCGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCTC CTGCCCCTGA
2310  GCCTGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCAAATC CTGGCCATCG TCCTGGCGGC GGCCTTTGCC ATGCCTTCTA
2520  TCAACGCTAC CCCGAGTCTT TCCATCCGAC TGACTTCATC ATGCGCGACG GCCTCGCCGC ATACACTCTG
2590  ACTCCCCGGC CCATCATCCA CGCTGTCGCC CCTGACTATA GGGTCGAGCA GAACCCCAAG AGGCTGGAGG
2660  CGGCGTACCG CGAGACTTGC TCTCGCCTCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT CACAATAACT GAGGACACAG
2870  CCCGTACGGC TAACCTAGCA CTAGAGATCG ATGCTGCCAC GGAGGTTGGC AGGGCTTGTG CCGGCTGTAC
2940  AGTTAGACCT GGGGTTGTGC ATTATCAATT CACTGCCGGG GTCCCAGGTT CGGGGAAGTC GCGGTCTATA
3010  CAGCAGGGGG ATGTCGATGT TGTGGTTGTT CCTACCCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TTGCAGCNNN NNNNNNNNNN NNNGCGGCCC GCGTCACTGC CGGCCGACGT GTTGTGATTG ATGAGGCGCC
3150  TTCGCTTCCA CCACATTTGC TGCTGCTTCA TATGCAGCGA GCTTCGTCAG TCCACCTTCT CGGCGACCCC
3220  AACCAGATTC CCGCCATTGA TTTCGAGCAT GCAGGCCTAG TGCCAGCGAT CCGCCCTGAG CTTGCCCCAA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
3360  GATCCAAACC ACCAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACGCAGG CAGCTAAGGC CGCTAACCCC GGTGCGATTA CAGTCCATGA GGCTCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATTATAGCC ACAGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  TGCACTTACC CGCCACACAG AAAAATGCGT CATTCTTGAC GCCCCTGGCC TGTTACGTGA GGTTGGTATC
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Q3-POST-RBV1 Consensus sequence

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3710  CCCGAGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCGCCT TCTTGCCAGA TTAGTGNNNN
3780  NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NTGCCCTGAG
3850  CTTGAGCAGG GTTTACTTTA CATGCCGCAA GAGCTCACAG TGTCCGACAG CGTTTTAGTC TTCGAGCTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCCC CTAGCCAGCG GAAGGCCGTC CTTTCGACGC TTGTGGGCAG
3990  GTACGGCCGC CGGACTAAGC TGTACGAAGC AGCCCACTCT GACGTCCGCG AGTCCCTGGC GAGGTTTATC
4060  CCTACCATCG GACCAGTTCA GGCCACCACA TGTGAGTTGT ACGAGTTGGT AGAGGCTATG GTTGAGAAGG
4130  GTCAGGACGG TTCCGCTGTG TTAGAGCTTG ATCTCTGTAG CCGTGATGTC TCGCGTATCA CATTTTTCCA
4200  GAAGGACTGT AATAAGTTTA CAACAGGTGA GACTATTGCT CATGGCAAGG TGGGCCAGGG CATTTCGGCC
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5810  CAGACTACTA CTACCCCCAC CTCTGTTGCT ATGACCTCTA TTACCTCCAC TGATGTTAGG CTTTTAGTTC
5880  AGCCTGGCAT TGCTTCCGAG TTGGTTATTC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTTGAGACC TCGGGTGTGG CCGAGGAGGA GGCTACCTCG GGTTTGGTTA TGCTCTGTAT CCATGGCTCC
6020  CCTGTTAATT CCTATACTAA TACGCCCTAC ACCGGGGCGC TCGGACTTCT TGACTTTGCT TTAGAGCTTG
6090  AGTTTAGGAA CTTGACACCC GGGAACACTA ACACCCGTGT GTCCCGGTAC ACCAGCACAG CCCGCCACCG
6160  CCTGCGCCGC GGCGCCGATG GCACCGCCGA GCTCACCACC ACTGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CATTTTACCG GGATGAATGG CGTCGGCGAG GTGGGCCGTG GNNNNNNNNN NNNNNNNNNN AATCTCGCTG
6300  ATACACTTCT TGGTGGTTTG CCGACAGAAT TGATTTCGTC GGCCGGAGGT CAACTCTTTT ACTCCCGTCC
6370  AGTTGTCTCG GCCAATGGCG AGCCGACTGT TAAATTATAC ACCTCCGTCG AGAATGCACA GCAGGACAAG
6440  GGTATTGCCA TACCACATGA CATAGATCTG GGAGATTCCC GTGTGGTCAT CCAGGACTAT GACAATCAGC
6510  ATGAGCAGGA TAGACCNNNN NNNNNNNNNN CCCCGTCTCG CCCCTTCTCG GTTCTTCGCG CTAACGACGT
6580  TCTGTGGCTC TCCCTCACTG CTGCCGAATA TGATCAAACT ACTTATGGTT CGTCGACCAA CCCTATGTAT
6650  GTTTCAGATA CTGTCACATT TGTCAACGTG GCAACGGGAG CCCAGGCTGT CGCCCGCTCT CTTGATTGGT
6720  CTAAGGTCAC TCTAGATGGT CGCCCTCTCA CTACTATTCA GCAGTACTCT AAGACATTTT ATGTTCTCCC
6790  ACTCCGCGGG AAGCTGTCCT TCTGGGAGGC CGGGACGACT AAGGCCGGAT ACCCCTATAA TTATAATACA
6860  ACTGNNNNNN NNCAGATATT GATTGAAAAT GCAGCCGGAC ATCGTGTTGC TATTTCCACC TATACCACCA
6930  GCCTGGGCGC TGGCCCTGTG TCTGTTTCCG CAGTTGGTGT CTTAGCCCCA CATTCGGCCC TCGCAGTCCT
7000  TGAAGACACG ATTGATTACC CTGCCCGTGC CCACACTTTT GATGATTTCT GCCCGGAGTG CCGCGCTCGC
7070  GGCCTGCAGG GCTGTGCTTT TCAATCCACT ATCGCTGAGC TTCAGCGTCT TAAAGTGAAG GTAGGCAAAA
7140  CCCGGGAGTC TTAATTAATT CCATCTGTGC CCCCTTCAAG GTCTTGGTTT ATTTCTTNTC TTCTGCGTTT
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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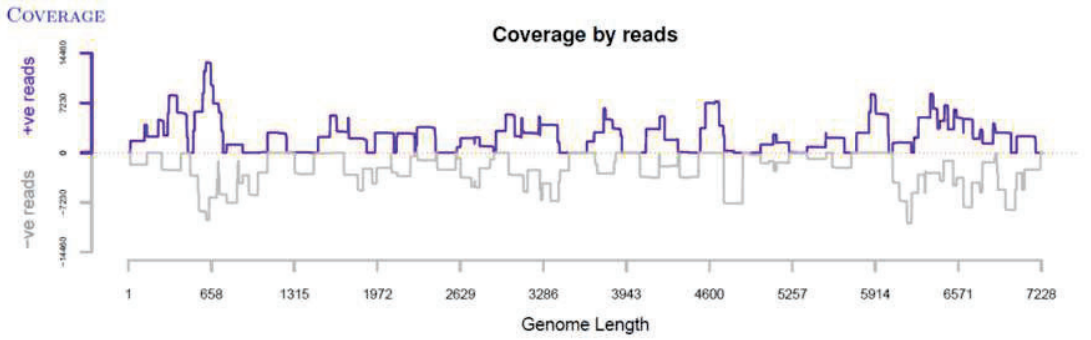
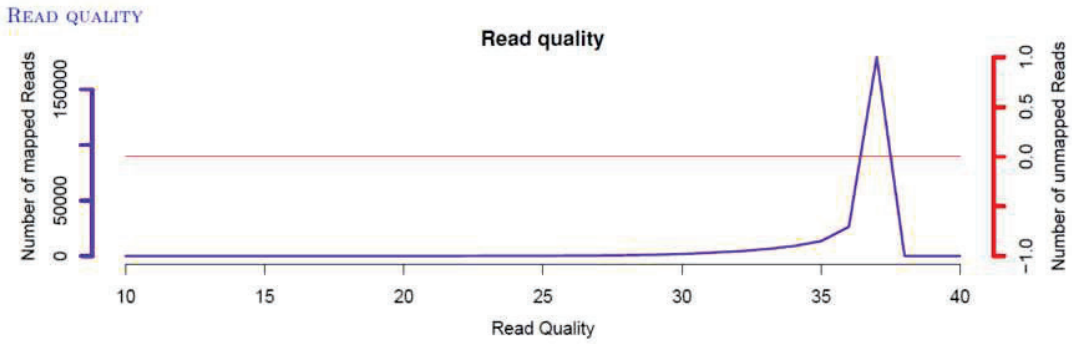
Q4-ON-RBV2

File name	**Q4-ON-RBV2.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**245 654**
Mapped reads	**245 654 (100.00%)**
Average read length	**147nt**
Coverage	**7 185nt (99.28%)**
Average depth	**4 934 reads/site**





Q4-ON-RBV2 Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTCATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGAC CTGAAGTACT
 210  CTGGAATCAT CCTATCCAAC GAGTTATCCA TAATGAACTT GAACAATACT GTCGGGCCCG GGCTGGTCGT
 280  TGTCTAGAGG TTGGGGCCCA CCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCAGCTA ACTGCCGCCG
 420  TTCTGCTTTG CGTGGCCTTC CCCCCGCTGA TCGTACTTAT TGTTTTGATG GGTTCTCCCG CTGTGCTTTT
 490  GCCGCAGAAA CTGGGGTTGC TCTCTATTCC CTGCATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CCCGACACGG GATGACACGC TTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA CGTCTCCATA CTTCGTGCAT GGATCCGCAC AACCAAGATA ACCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATATCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCTACATT CCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAAGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAAGGCATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTCTATAG
1260  TTGGTTGTTT GAGAAGTCTG GCCGCGACTA TATCCCCGGC CGCCAGCTTC AGTTCTATGC ACAGTGTCGC
1330  CGCTGGTTAT CGGCGGGTTT CCATCTCGAC CCAAGAGTGC TTGTTTTCGA TGAGTCTGTG CCCTGCCGTT
1400  GTAGGACATT TCTTAAGAAG ACTGTGGGTA AGTTCTGTTG TTTTATGAAG TGGTTGGGAC AGGAGTGCAC
1470  CTGCTTTTTG GAACCAGCAG AGGGCCTGGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTTGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACCTATGC CGTCCACGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCAC ATGACATCGC CGCCCGAGCC TCCCGTCTGA CTGCCACCGT
1680  CGAACTTGTT GCAGGTCCAG ACCGCTTGGA GTGCCGCACT GTGCTCGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTATGTTCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGACCG CATAGTCTCA CCTACGAGCT CACTCCTGCC GGTCTGCAGA TTAAGATTTC
1890  ATCTAATGGC CTGGATTGTA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  ACAGCCTTTT GCAGTGCTCT TTATAGATAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTAT
2030  GGTTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCCTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCATTGT AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTCGAGT GTTACCGCCA CCTTCTGAAN NNNNNNNGGT TGACACAGCG CTCGCTCCTC CTGCCCCTGA
2310  GTCTGCTCAA CCATCTCGCT CCGTTGGGCC AAAGGCTCCC GTGCGTAAGT CGCCAACGCC ACCATCCCCG
2380  CGCACCCGTC ACCTTCTTTA TACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAGTCTG
2450  ACTGTGATTG GCTGGTTAAT GCATCGAATC CCGGCCATCG TCCTGGTGGC GGCCTTTGTC ATGCCTTCTA
2520  TCAACGCTAC CCCGAGTCTT TCCACCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC ATATACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCC CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CGGCATACCG AGAGACTTGT TCTCGCCGCG GTACCGCCGC CTACCCACTC CTTGGCTCGG GCATATACCA
2730  AGTCCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAT CATCGCCCCG GGGACGAGCT CTACTTAACC
2800  GACCTCGCCG CCACCTGGTT CGAAGCTAAT AAGCCAACAC AGCCGGTCCT TACAATAACT GAGGATGCGG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CAGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT AGTGGTTGTT CCTACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCAGCCC GCGTCACCAC AGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTATTACA TATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGTGACCCA
3220  AATCAGATCC CTGCTATAGA CTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTCGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
3360  GATCCAAACC ACCAGCCGTG TGCTGCGGTC TTTATTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACTCAGG CTGCTAAGGC CGCTAACCCC GGTGCGATTA CAGTCCATGA GGCTCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATTATAGCC ACAGCTGATG CTAGGGGACT TATCCAATCT TCTCGGGCTC ATGCCATAGT
3570  TGCACTCACC CGCCACACAG AAAAATGCGT CATTCTTGAC GCCCCTGGCC TGTTACGTGA GGTTGGTATA
3640  TCAGATGTGA TTGTTAACAA TTTTTTCCTT GCCGGTGGGG AGATGGGCCA CCATCGCCCT TCTGTGATAC
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Q4-ON-RBV2 Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AATTTCGCGA CACTTCAGGC CTTTCCACCC TCTTGCCAGA TTAGTGCTTA
3780  CCACCAGTTA GCTGAGGAAC TTGGCCACCG TCCAGCTCCC GTCGCTGCTG TCCTGCCCCC TTGCCCTGAA
3850  CTTGAGCAGG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGACAG TGTGCTGGTC TTCGAGCTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACGC TTGTGGGCAG
3990  GTACGGCCGC CGGACTAAGC TGTACGAAGC AGCCCACTCT GACGTCCGCG AGTCCCTGGC TAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAAGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAATGGCTGT AATAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCC
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGC CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA TATCTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CGGGGGCAGG
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4480  GAGTGTGTAG TTATGGAGGA ATGTGGAATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  TTTGGATCCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TTTTAAGGTC
4690  GCCGCTTTCA AGGGAGATGA CTCTGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
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4900  CCGAGTCCCG AGCGCGCCGA GCAGTTGCGC CTAGCTGTTT GTGATTTCCT TCGAAGGTTA ATGAATGTTG
4970  CGCAGGTATG TGTCGATGTT GTATCCCGAG TTTACGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGCTGCAA GCCATAGCTG ATGGCAAAGC CCACTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
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5250  GCCGCCGTCG TGGGCGGCGC AGCGGCAGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTTGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC ACCGATGTCG TACCGCAATC CGGGGCTGGA
5390  GCACGCCCTC GACAGCCACC CCGCCCCCTT GGCTCCTCTT GGCGTGATCA GTCCCAGCGC TCCTCCGCTG
5460  TCCCACGTCG TCGATCTGCC CCAGTTGGGG CTGCACCGTT GACTGCCATA TCACCTGCCC CCGATACAGC
5530  TCCTGTGCCT GATGTTGACT CTCGCGGTGC CATATTGCGG CGCCAGTACA ATTTGTCCAC ATCCCCACTC
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG GTTCTTTATG CTGCCCCGCT AAACCCTTTG CTGCCCCTTC
5670  AAGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCGTC TAATTATGCC CAGTATCGAG TTGTCCGAGC
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5880  AGCCCGGCAT TGCTTCTGAG CTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
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6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT TGCCCGTTCC CTTGACTGGT
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7000  TGAAGACACA ATTGATTACC CTGCCCGTGC CCACACATTT GATGATTTCT GCCCGGAGTG TCGTGCTCTT
7070  GGTTTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTCTT TTCTGCGGTT
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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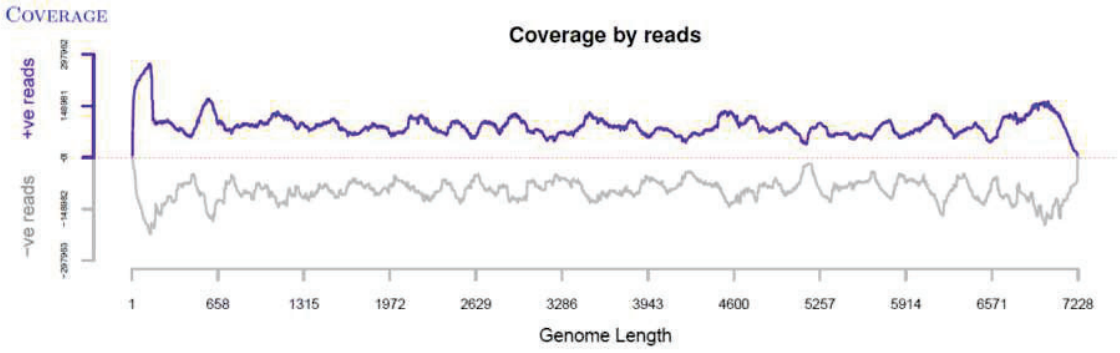
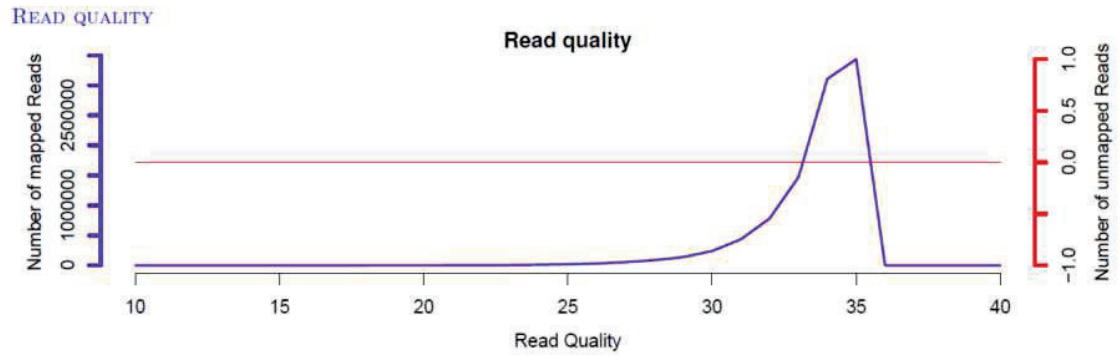
Q5-ON-RBV3

File name	**Q5-ON-RBV3.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**9 811 385**
Mapped reads	**9 811 385 (100.00%)**
Average read length	**141nt**
Coverage	**7 224nt (99.82%)**
Average depth	**188 636 reads/site**





Q5-ON-RBV3 Consensus sequence

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 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTGAT CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGCT ACAACCATGA CGTCTCCATA CTTCGTGCAT GGATCCGCAC AACCAAGATA ACCGGCGACC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCCA TTGGCTGCCA TTTTGTGCTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TCCCATATCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGTCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCAGCT TGCTCTACGA AATCTACATT CCACGCTGTC CCGGTTCATA
 980  TTTGGGACCG GCTCATGCTT TTTGGCGCTA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAAGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TTACCGCTGT TATTACTGCA GCGTATTTGA CCATCTGCCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTCATTACAA GACTTTATAG
1260  TTGGCTGTTC GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
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1470  CTGCTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAAGCCTA TGAGGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTT GATGTTTCTG GGACCTATGC CGTCCACGGC CGCCAACTTG
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1820  AGTCTATGGG GGCCGGACCG CATAGTCTCA CCTACGAGCT CACTCCTGCC GGTCTGCAGA TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCTCT TTATAGATAC AACAGGTTCA CTCAGCACCA TTCGCTTGTA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGTATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCTGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CCTTGTACAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTTGT AGCGCCGGCT GCTACTCCGG GGTTACGCTA CCCTACACCT CCTGTTAGTG
2240  ACATTCGGGT GTTACCGCCA CCTTCTGAAG AATTTCAGGT TGACACAGCG CCCGCTCCTC CTGCCCCTGA
2310  GCCTGCTCAA CCATCTAGCT CCGCTGGGCC AAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
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2800  GACCTCGCCG CCACCTGGTT CGAAGCTAAT AAGCCAACAC AGCCGGCCCT TACAATAACT GAGGATGCGG
2870  CCCGTACAGC CAACCTAGCA CTGGAGATCG ATGCTGCCAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC GCGTTCTATA
3010  CAGCAGGGGG ATGTTGATGT AGTGGTTGTT CCTACTCGGG AGCTCCGGAA TAGTTGGCGC CGCCGGGGTT
3080  TCGCAGCTTT TACACCTCAT ACGGCGGCCC GCGTCACCAC AGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCCCTCCCA CCGCATTTGT TGCTATTACA TATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGCGACCCA
3220  AATCAGATCC CTGCTATAGA CTTCGAGCAC GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTCGCGCCCA
3290  CCAGTTGGTG GCATGTCACC CACCGCTGCC CCGCTGATGT GTGCGAGCTT ATTCGCGGGG CTTATCCCAA
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3430  TTCACTCAGG CTGCTAAGGC CGCTAACCCC GGTGCGATTA CAGTCCATGA GGCTCAGGGC GCCACTTTCA
3500  CGGAAACTAC AATTATAGCC ACAGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCAC ACGCCATAGT
3570  TGCACTTACC CGCCACACAG AGAAGTGCGT TATTCTTGAC GCCCCCGGCT TGTTACGTGA GGTTGGTATA
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Q5-ON-RBV3 Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AATCTCGCGA CACTTCAGGC CTTCCCACCC TCTTGCCAGA TTAGTGCTTA
3780  CCACCAGTTA GCTGAGGAAC TTGGCCACCG TCCAGCTCCC GTCGCTGCTG TCCTGCCCCC TTGCCCTGAA
3850  CTTGAGCAGG GCTTATTATA TATGCCGCAG GAGCTTACGG TGTCTGACAG TGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGT ATGGCTGCCC CTAGTCAGCG GAAGGCCATC CTATCAACGC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCACTCT GACGTCCGTG AGTCCCTGGC CAGATTTATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTGGAGAAAG
4130  GTCAAGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAATGGCTGT AATAAATTCA CCACAGGGGA GACCATTGCC CACGGTAAGG TCGGCCAGGG CATCTCGGCC
4270  TGGAGTAAGA CCTTCTGTGC CCTGTTTGGC CCGTGGTTTC GTGCTATTGA AAAAGAAATA CTAGCCCTGC
4340  TCCCGCCTAA TATCTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CGGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA ATGACTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGCCTC
4480  GAGTGTGTAG TTATGGAGGA ATGTGGAATG CCCCAGTGGC TAATCCGGTT GTACCATTTG GTTCGGTCGG
4550  TTTGGATCCT ACAGGCGCCG AAGGAGTCTC TTAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620  CCTCCTCTGG AACACTGTTT GGAATATGGC GATCATAGCA CACTGCTATG AATTTCGTGA TTTTAAGGTC
4690  GCCGCTTTCA AGGGAGATGA CTCCGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG TTGCGGGCTC AAACTGAAGG TTGATTATCG CCCTATTGGG CTGTATGCTG GTGTGGTGGT
4830  GGCCCCTGGT CTGGGGACGC TACCCGATGT GGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAGTTGCGC CTAGCTGTTT GTGATTTCCT TCGAAGGTTA ACGAATGTTG
4970  CGCAGGTATG TGTCGATGTT GTATCCCGAG TTTACGGAGT TAGCCCTGGA TTGGTACATA ACCTTATTGG
5040  CATGCTGCAA GCCATAGCTG ATGGCAAAGC CCACTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCCATCA TACAGCGGGT GGAATGAATA ACATGTTCTG TGCATTGCCC ATGGGATCAC CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
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5390  GCACGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCTCCGCTG
5460  TCCCACGTCG TCGATCTGCC CCAGTTGGGG CTGCGCCGTT GACTGCCATA TCACCTGCCC CCGATACAGC
5530  TCCTGTGCCT GATGTTGACT CTCGCGGTGC CATATTGCGG CGCCAGTACA ATTTGTCCAC ATCCCCGCTC
5600  ACATCATCTG TTGCTTCGGG TACTAATCTG GTTCTTTATG CTGCCCCGCT AAACCCTTTG CTGCCCCTTC
5670  AGGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCGTC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  TACGATCCGT TACAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCAA TTTCCATCTC ATTTTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTTTAGTTC
5880  AGCCCGGCAT TGCTTCTGAG CTGGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GCTGGCGCTC
5950  TGTAGAGACC TCTGGTGTGG CTGAAGAGGA GGCTACTTCT GGTTTGGTAA TGCTTTGTAT CCATGGCTCT
6020  CCTGTTAATT CCTACACCAA TACCCCCTAC ACCGGGGCGC TTGGACTCCT TGACTTTGCT TTAGAGCTTG
6090  AGTTTAGGAA CCTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGTACAG CCCGTCATCG
6160  GCTGCGCCGC GGTGCTGATG GCACCGCGGA ACTTACCACC ACAGCAGCCA CGCGCTTCAT GAAGGACTTG
6230  CACTTCACCG GTACGAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCCCT TACACTCTTT AATCTTGCTG
6300  ACACACTTCT TGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGCTATTCT ACTCCCGCCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT CAAGTTATAC ACATCTGTAG AGAATGCACA GCAGGATAAA
6440  GGGATCGCTA TCCCACATGA CATAGATCTG GGTGATTCCC GTGTGGTCAT CCAAGACTAT GACAACCAGC
6510  ATGAGCAGGA TCGACCCACC CCCTCGCCTG CCCCTTCTCG CCCTTTCTCG GTTCTTCGCG CTAATGATGT
6580  TTTATGGCTT TCTCTTACAG CCGCCGAGTA TGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT TGCCCGTTCC CTTGACTGGT
6720  CTAAAGTTAC TCTCGACGGC CGCCCTCTTA CTACTATCCA GCAGTACTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGCGGG AAGTTATCTT TCTGGGAGGC TGGGACGACT AAGGCCGGCT ACCCCTATAA TTACAACACA
6860  ACTGCGAGTG ATCAGATTCT GATTGAAAAT GCGGCTGGTC ATCGTGTTGC TATTTCCACG TATACCACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCCG CAGTTGGTGT CTTAGCCCCA CATTCGGCCC TCGCAGTCCT
7000  TGAAGACACG ATTGATTACC CTGCCCGTGC CCACACATTT GATGATTTCT GCCCGGAGTG TCGTGCTCTT
7070  GGTTTGCAGG GGTGTGCCTT CCAGTCTACT ATTGCTGAGC TTCAGCGTCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGCTTT ATTTTCTCTT TTCTGCGGTT
7210  CGCGCTCCCT GGAAANNNNN NNNNNNN
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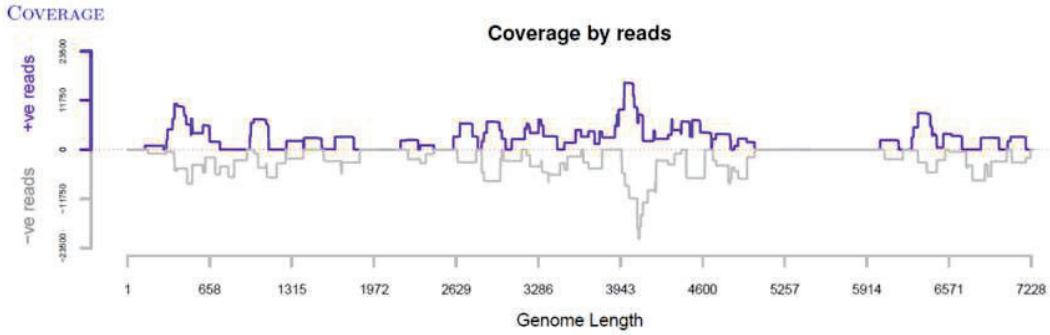
R1-PRE-RBV

File name	**R1-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**258 035**
Mapped reads	**258 035 (100.00%)**
Average read length	**145nt**
Coverage	**7 175nt (99.14%)**
Average depth	**5 098 reads/site**



R1-PRE-RBV Consensus sequence

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   1  NNNNNNNNCG TATGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAC TCTGCCTTGG CGAATGCTGT GGTGGTTCGG CCGTTTTTGT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGCCGT
 280  TGTCTAGAGG TTGGGGCCCA TCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACTTAC TGTTTTGATG GATTCTCTCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT CTACATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CTCGGCACGG GATGACACGC CTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTAAC CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGTT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCT ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCTTATT CCCATCTGCT TGCTCTACGA AATCCACATT TCACGCCGTC CCGGTTCATA
 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGATG GAATGCTTCG
1120  GAGGACGCTC TGACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGTCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTTATTACAA GACTCTATAG
1260  TTGGCTGTTT GAGAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTATTTGA TGAGTCCGTG CCCTGCCGCT
1400  GTAGGACGTT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
1470  CTGTTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAGGCCTA TGAAGGCTCT
1540  GAGGTCGATC AGGCTGAGCC CGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCTTAGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCCCCGGGC GGGGCCCCTA GCGCTGCTCC GGGGGAGGTA
1960  GCAGCCTTTT GCAGTGCTCT TTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACTTGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CGGCCGTCGC AGTGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ATATCTGGGT GCTACCGCCG CCTTCTGAAG AACTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAATCTG
2450  ACTGTGATTG GCTGGTTAAT GCGTCAAATC CTGGCCATCG TCCTGGCGGC GGCCTTTGCC ATGCCTTCTA
2520  TCAACGCTAC CCCGAGTCTT TCCANNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNNNACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCCCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC ACGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TTGCAGCTTT TACACCCCAT ACGGCGGCCC GTGTCACTAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCTCTCCCA CCGCATTTGC TGCTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGCGACCCG
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACT CATCGCTGCC CCGCTGACGT GTGTGAGCTT ATACGCGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC CTTGTTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCAATTA CAGTCCACGA GGCCCAGGGT GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CCAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT CATATTTGAC GCTCCCGGCC TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCCGTGATAC
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R1-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCGCCT TCTTGCCAGA TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCAGCCCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAA GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCTC CTAGCCAGCG GAAGGCCGTC CTATCGACAC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCATTCT GACGTCCGTG AGTCCCTGGC TAGGTTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTAGAGAAGG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AATAAATTCA CCACAGGGGA GACCATCGCC CACGGCAAGG TCGGCCAGGG CATCTCGGCC
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA TTAGCCCTGC
4340  TCTCGCCTAA TATTTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA ATGATTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGTCTC
4480  GAGTGTGTGA TCATGGAGGA ATGCGGCATG CCCCAGTGGC TAATTCGGCT GTACCATTTG GTTCGGTCGG
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4620  CCTTCTCTGG AACACTGTCT GGAATATGGC GATCATAGCA CACTGCTATG AATTCCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA TTCCGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG CTGCGGACTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCTCCTGGT TTGGGGACGC TACCCGATGT TGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAATTGCGC CTGGCTGTTT GTGACTTCCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTTTG TGTCGATGTT GTATCCCGTG TTTATGGAGT TAGCCCTGGG ATGGTACATA ACCTTATTGG
5040  CATGTTACAG ACTATAGCCG ATGGGAAGGC CCATTTTACT GAGACTGTTA AACCTGTACT GGATCTTACA
5110  ACTTCGGTCA TACACAGGTT GGAATGAATA ACATGTTGTG TGCATCGCCC ATGGGTTCAC CATGTGCCCT
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5670  AGGATGGCAC TAATACTCAT ATCATGGCGA CTGAGGCGTC TAACTATGCC CAGTATCGGG TTGTCCGAGC
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5880  AGCCTGGCAT TGCTTCTGAG TTAGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAGGAGGA GGCTACCTCT GGTTTAGTAA TGCTTTGCAT CCATGGCTCT
6020  CCTGTTAATT CCTACACTAA TACCCCTTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCT TTAGAGCTTG
6090  AGTTTAGGAA CTTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GTTGCGCCGC GGTGCTGATG GCACCGCTGA GCTTACTACC ACGGCGGCCA CGCGCTTCAT GAAGGACCTG
6230  CACTTCACCG GCACAAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCTCT CACACTCTTT AATCTTGCTG
6300  ACACGCTTCT CGGTGGTCTG CCGACAGAAT TAATTTCGTC GGCCGGGGGG CAGTTATTCT ACTCCCGTCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT CAAGTTATAC ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440  GGGATCGCTA TTCCACACGA CATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAATCAGC
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6580  TTTATGGCTT TCTTTTACTG CCGCCGAGTA CGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTCGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGTCCACTTA CTACCATCCA GCAGTATTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGTGGG AAGCTATCTT TCTGGGAGGC CGGGACGACT AAGGCCGGCT ACCCCTACAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCTGCTGGTC ATCGTGTTGC TATCTCCACC TATACTACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TCTAGCTCCA CATTCGGCTC TTGCGGTCCT
7000  CGAAGACACT ATTGACTATC CTGCCCGTGC CCACACTTTT GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGTTTGCAGG GGTGTGCTTT CCAGTCTACT ATTGCTGAGC TTCAGCGCCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGTTTT ATTTCTTTTT TTCTGCGTTC
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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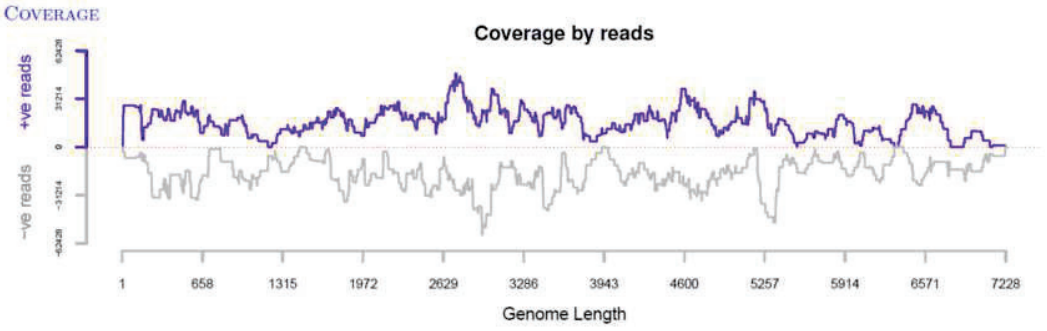
R2-PRE-RBV

File name	**R2-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 675 576**
Mapped reads	**1 675 576 (100.00%)**
Average read length	**145nt**
Coverage	**7 215nt (99.70%)**
Average depth	**33 269 reads/site**



R2-PRE-RBV Consensus sequence

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 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
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 280  TGTCTAGAGG TTGGGGCCCA TCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACTTAC TGTTTTGATG GATTCTCTCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT CTACATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CTCGGCACGG GATGACACGC CTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTAAC CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGTT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
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 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
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1120  GAGGACGCTC TGACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGTCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTTATTACAA GACTCTATAG
1260  TTGGCTGTTT GAAAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTATTTGA TGAGTCCGTG CCCTGCCGCT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
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1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCTTAGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT CTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGTGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ATATCCGGGT GCTACCGCCG CCTTCTGAAG AACTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAATCTG
2450  ACTGTGATTG GCTGGTTAAT GCATCGAACC CCGGCCATCG TCCTGGAGGC GGCCTTTGCC ATGCCTTCTA
2520  CCAACGTTAC CCCGAGTCTT TCCATCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCCCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
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2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC ACGTTCTATA
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3080  TTGCAGCTTT TACACCCCAT ACGGCGGCCC GTGTCACTAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
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3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACT CATCGCTGCC CCGCTGACGT GTGTGAGCTT ATACGCGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC CTTGTTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCAATTA CAGTCCACGA GGCCCAGGGT GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT CATATTTGAC GCTCCCGGCC TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCCGTGATAC
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R2-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCGCCT TCTTGCCAGA TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCGGCCCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAA GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCTC CTAGCCAGCG GAAGGCCGTC CTATCGACAC TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCATTCT GACGTCCGTG AGTCCCTGGC TAGGTTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTAGAGAAGG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200  GAAAGATTGT AATAAATTCA CCACAGGGGA GACCATCGCC CACGGCAAGG TCGGCCAGGG CATCTCGGCC
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA TTAGCCCTGC
4340  TCTCGCCTAA TATTTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA ATGATTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGTCTC
4480  GAGTGTGTGA TCATGGAGGA ATGCGGCATG CCCCAGTGGC TAATTCGGCT GTACCATTTG GTTCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TCAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCTGGCAC
4620  CCTTCTCTGG AACACTGTCT GGAATATGGC GATCATAGCA CACTGCTATG AATTCCGTGA TTTTAGGGTT
4690  GCCGCTTTCA AGGGAGATGA TTCCGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760  TGATTGCAGG CTGCGGACTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830  GGCTCCTGGT TTGGGGACGC TACCCGATGT TGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900  CCTGGGCCGG AGCGGGCTGA GCAATTGCGC CTGGCTGTTT GTGACTTCCT TCGAGGGTTA ACGAATGTTG
4970  CGCAGGTTTG TGTCGATGTT GTATCCCGTG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040  CATGTTGCAA ACCATAGCTG ATGGTAAAGC CCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110  AACTCTATCA TACAGCGGGT GGAATGAATA ACATGTTTTG TGCATTGCCC ATGGGATCAT CATGCGCCCT
5180  AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250  GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320  CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG TACCACAATC CGGGGCTGGA
5390  GCTCGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCTCCGCTG
5460  CCCCACGTCG TCGACTTGCC CCAGCTGGGG CTGCGCCGCT GACCGCTATA TCACCTGCTC CTGATACAGC
5530  TCCTGTACCT GATGTTGACT CGCGCGGTGC CATATTGCGA CGTCAGTACA ATTTATCCAC ATCTCCGCTC
5600  ACATCATCTG TTGCTTCGGG CACTAATCTG GTTCTTTATG CTGCCCCGTT AAACCCTCTG CTGCCCCTTC
5670  AGGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740  CACGATCCGT TATAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCGA TATCCATCTC ATTCTGGCCT
5810  CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTTTAGTTC
5880  AGCCTGGCAT TGCTTCTGAG TTAGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950  TGTGGAGACC TCGGGTGTGG CTGAGGAGGA GGCTACCTCT GGTTTAGTAA TGCTTTGCAT CCATGGCTCT
6020  CCTGTTAATT CCTACACTAA TACCCCTTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCT TTAGAGCTTG
6090  AGTTTAGGAA CTTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160  GTTGCGCCGC GGTGCTGATG GCACCGCTGA GCTTACTACC ACGGCAGCCA CGCGCTTCAT GAAGGACCTG
6230  CACTTCACCG GCACAAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCTCT CACACTCTTT AATCTTGCTG
6300  ACACGCTTCT CGGTGGTTTG CCGACAGAAT TAATTTCGTC GGCTGGGGGA CAGTTATTCT ACTCCCGTCC
6370  CGTCGTCTCA GCCAATGGCG AGCCGACTGT CAAGTTATAC ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440  GGGATCGCTA TTCCACACGA CATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAATCAGC
6510  ATGAGCAGGA TCGACCCACC CCCTCGCCTG CCCCCTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580  TTTATGGCTT TCTCTTACTG CCGCCGAGTA CGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650  GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTCGACTGGT
6720  CTAAAGTTAC TCTGGACGGC CGTCCACTTA CTACCATCCA GCAGTATTCC AAAACATTTT ATGTTCTCCC
6790  GCTTCGTGGG AAGCTATCTT TCTGGGAGGC CGGGACGACT AAGGCCGGCT ACCCCTACAA TTACAACACA
6860  ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCTGCTGGTC ATCGTGTTGC TATCTCCACC TATACTACCA
6930  GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TCTAGCTCCA CATTCGGCTC TTGCGGTCCT
7000  CGAAGACACT ATTGACTATC CTGCCCGTGC CCACACTTTT GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070  GGTTTGCAGG GGTGTGCTTT CCAGTCTACT ATTGCTGAGC TTCAGCGCCT TAAAATGAAG GTAGGTAAAA
7140  CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGTTTT ATTTCTTCTT TTCTGCGTTC
7210  CGCGCTCCCT GGANNNNNNN NNNNNNN
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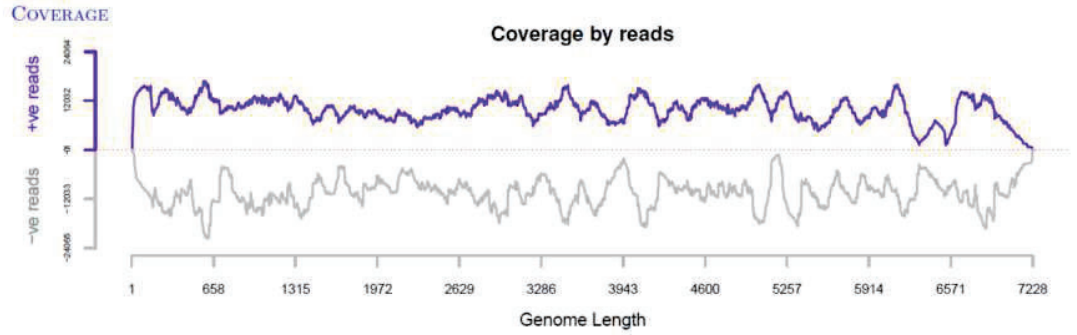
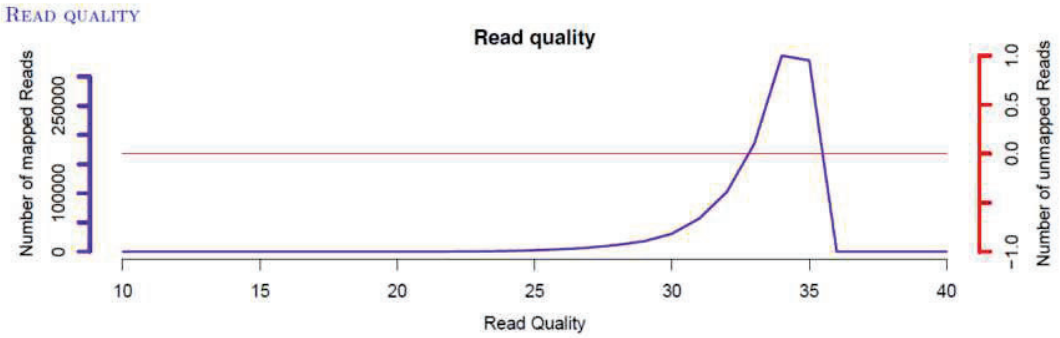
R3-PRE-RBV

File name	**R3-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 084 297**
Mapped reads	**1 084 297 (100.00%)**
Average read length	**138nt**
Coverage	**7 232nt (99.93%)**
Average depth	**20 499 reads/site**





R3-PRE-RBV Consensus sequence

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   1  NNNNNCCACG TATGTGGTCG ATGCCATGGA GGCCCATCAG TTCATTAAGG CTCCTGGCAT TACTACTGCC
  70  ATTGAGCAAG CTGCTCTGGC TGCGGCCAAT TCTGCCCTGG CGAATGCTGT GGTGGTCCGG CCGTTTTTAT
 140  CTCGTGTGCA AACTGAGATT CTTATTAATT TGATGCAACC CCGGCAGTTG GTTTTTCGGC CTGAAGTGCT
 210  CTGGAATCAT CCTATCCAAC GGGTTATCCA TAATGAACTT GAACAGTACT GTCGGGCCCG GGCTGGCCGT
 280  TGTCTAGAGG TTGGGGCCCA TCCAAGATCC ATTAATGACA ACCCAAATGT TCTGCACCGG TGCTTTCTAC
 350  GACCAGTTGG GAGAGATGTT CAGCGCTGGT ACTCTGCTCC TACCCGCGGC CCTGCGGCTA ACTGCCGCCG
 420  TTCTGCCTTG CGTGGCCTCC CCCCCGTTGA TCGTACTTAC TGTTTTGATG GATTCTCTCG CTGCTCATTT
 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT CTACATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CTCGGCACGG GATGACACGC CTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTAAC CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGTT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCTGCT TGCTCTACGA AATCCACATT TCACGCCGTC CCGGTTCATA
 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
1050  CTACCTCCGC GGGATTAGTT ACAAGGTCAC TGTTGGCGCC CTTGTCGCTA ATGAGGGGTG GAATGCTTCG
1120  GAGGACGCTC TGACCGCTGT TATTACTGCA GCGTATTTGA CCATTTGTCA TCAGCGTTAC CTCCGTACCC
1190  AAGCTATATC CAAGGGTATG CGCCGACTGG AGGTTGAGCA TGCCCAAAAA TTTATTACAA GACTCTATAG
1260  TTGGCTGTTC GAAAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTATTTGA TGAGTCCGTG CCCTGCCGCT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
1470  CTGTTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAGGCCTA TGAAGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCTTAGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT CTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGTGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ATATCCGGGT GCTACCGCCG CCTTCTGAAG AACTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAATCTG
2450  ACTGTGATTG GCTGGTTAAT GCATCGAACC CCGGCCATCG TCCTGGAGGC GGCCTTTGCC ATGCCTTCTA
2520  CCAACGTTAC CCCGAGTCTT TCCATCCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCCCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC ACGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TTGCAGCTTT TACACCCCAT ACGGCGGCCC GTGTCACTAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCTCTCCCA CCGCATTTGC TGCTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGCGACCCG
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACT CATCGCTGCC CCGCTGACGT GTGTGAGCTT ATACGCGGGG CTTATCCCAA
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3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCAATTA CAGTCCACGA GGCCCAGGGT GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT CATATTTGAC GCTCCCGGCC TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCCGTGATAC
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R3-PRE-RBV Consensus sequence

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3710  CTCGCGGTAA TCCTGACCAG  AACCTCGCGA  CACTACAGGC  CTTTCCGCCT  TCTTGCCAGA  TTAGTGCCTA
3780  TCACCAGTTA GCTGAGGAAC TTGGCCACCG  CCCGGCCCCC  GTCGCCGCTG  TCTTGCCCCC  TTGCCCTGAA
3850  CTTGAGCAAG GCTTGTTATA TATGCCGCAA  GAGCTTACGG  TGTCTGATAG  CGTGCTGGTC  TTTGAACTCA
3920  CGGACATAGT CCACTGCCGG ATGGCCGCTC  CTAGCCAGCG  GAAGGCCGTC  CTATCGACAC  TCGTGGGTAG
3990  GTACGGCCGT CGGACGAAGC TGTATGAAGC  AGCTCATTCT  GACGTCCGTG  AGTCCCTGGC  TAGGTTCATC
4060  CCCACCATTG GGCCCGTTCA GGCTACTACG  TGTGAGTTAT  ATGAGCTGGT  TGAGGCCATG  GTAGAGAAGG
4130  GTCAGGATGG CTCTGCCGTG CTTGAGCTCG  ACCTCTGCAA  TCGTGATGTA  TCGCGTATCA  CATTTTTCCA
4200  GAAAGATTGT AATAAATTCA CCACAGGGGA  GACCATCGCC  CACGGCAAGG  TCGGCCAGGG  CATCTCGGCC
4270  TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT  CCGTGGTTTC  GTGCTATTGA  AAAAGAAATA  TTAGCCCTGC
4340  TCTCGCCTAA TATTTTCTAC GGCGACGCAT  ACGAGGAGTC  TGTGTTTGCC  GCCGCTGTGT  CAGGGGCAGG
4410  TTCAAGCATG GTATTTGAGA ATGATTTTTC  AGAGTTTGAT  AGCACCCAAA  ATAACTTCTC  CCTTGGTCTC
4480  GAGTGTGTGA TCATGGAGGA ATGCGGCATG  CCCCAGTGGC  TAATTCGGCT  GTACCATTTG  GTTCGGTCGG
4550  CCTGGATTCT ACAGGCGCCG AAGGAGTCTC  TCAAGGGATT  TTGGAAGAAG  CATTCTGGTG  AGCCTGGCAC
4620  CCTTCTCTGG AACACTGTCT GGAATATGGC  GATCATAGCA  CACTGCTATG  AATTCCGTGA  TTTTAGGGTT
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7210  CGCGCTCCCT GGAAAAAAAA AAAAAAA
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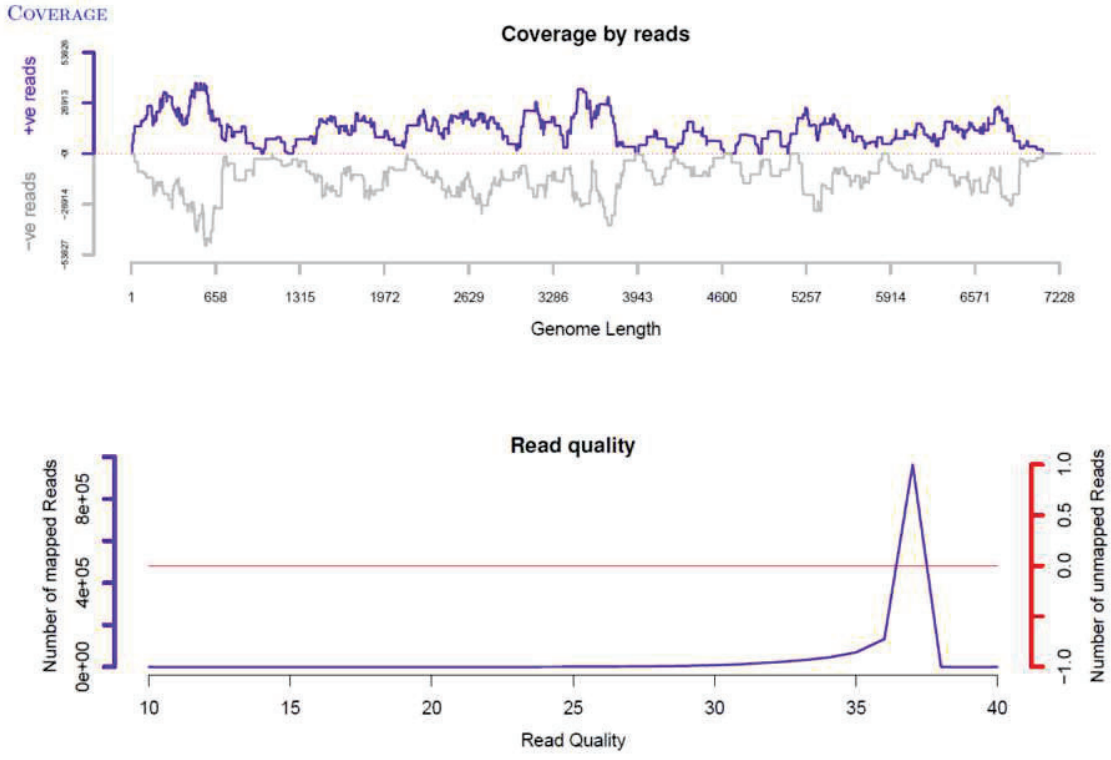
R4-PRE-RBV

File name	**R4-PRE-RBV.sam**
Ref name	**FJ705359.1**
Ref length	**7 237nt**
Program used	**Tanoti Assembler 1.0**
Total reads	**1 293 526**
Mapped reads	**1 293 526 (100.00%)**
Average read length	**143nt**
Coverage	**7 216nt (99.71%)**
Average depth	**25 411 reads/site**



R4-PRE-RBV Consensus sequence

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 490  GCTGCAGAAA CTGGGGTTGC CCTTTATTCT CTACATGACC TCTGGCCGGC CGATGTTGCG GAGGCTATGG
 560  CTCGGCACGG GATGACACGC CTGTATGCTG CACTACATCT CCCCCCTGAA GTACTACTAC CACCCGGTAC
 630  TTACCATACA ACTTCATACC TTCTGATCCA CGACGGTAAC CGTGCTGTTG TGACCTATGA AGGTGATACT
 700  AGTGCAGGTT ACAACCATGA TGTCTCCATA CTTCGTGCAT GGATCCGCAC AACTAAGATA GTTGGCGATC
 770  ATCCGCTGGT GATAGAGCGT GTGCGGGCTA TTGGCTGCCA TTTTGTGTTG CTGCTTACTG CAGCCCCTGA
 840  GCCGTCACCA ATGCCTTATG TTCCATACCC CCGGTCGACA GAGGTGTATG TCCGCTCTAT ATTCGGCCCT
 910  GGCGGGTCCC CATCCCTATT CCCATCTGCT TGCTCTACGA AATCCACATT TCACGCCGTC CCGGTTCATA
 980  TTTGGGATCG GCTCATGCTT TTTGGCGCCA CTCTGGATGA TCAGGCGTTT TGCTGTTCAC GGCTTATGAC
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1260  TTGGCTGTTC GAAAAGTCCG GCCGTGACTA TATCCCCGGC CGCCAGCTCC AGTTCTATGC ACAGTGCCGC
1330  CGTTGGTTAT CGGCAGGTTT CCATCTTGAT CCAAGGGTGC TTGTATTTGA TGAGTCCGTG CCCTGCCGCT
1400  GTAGGACATT TCTTAAGAAG GCTGTGGGTA AGTTCTGCTG TTTTATGAAG TGGTTAGGAC AGGAGTGCAC
1470  CTGTTTTTTG GAACCAGCAG AGGGTCTAGT TGGCGACCAT GGCCACGATA ATGAGGCCTA TGAAGGCTCT
1540  GAGGTCGATC AGGCCGAGCC CGCCCATCTC GATGTTTCCG GGACTTATGC CGTCCATGGC CGCCAACTTG
1610  AGGCCCTGTA TAGGGCGCTT AACATCCCGC ATGACATCGC TGCCCGAGCC TCCCGTTTGA CTGCCACCGT
1680  CGAACTCGTT GCAGGTCCAG ACCGCTTAGA GTGCCGCACT GTGCTTGGGA ATAAGACTTT CCGGACGACG
1750  GTGGTTGATG GCGCCCATCT TGAGGCGAAC GGCCCCGAGC AGTACGTCCT TTCGTTTGAC GCCTCTCGCC
1820  AGTCTATGGG GGCCGGGCCG CATAGTCTCT CCTACGAGCT CACTCCTGCT GGTTTGCAGG TCAAGATTTC
1890  ATCTAATGGC CTGGATTGCA CTGCAACATT CCCTCCGGGT GGGGCCCCTA GCGCTGCTCC GGGGGAGGTG
1960  GCAGCCTTTT GCAGTGCCCT CTACAGGTAC AACAGGTTCA CTCAGCGCCA TTCGCTTATA GGTGGCTTGT
2030  GGCTGCACCC TGAGGGGTTG TTGGGCATCT TCCCCCCTTT CTCTCCCGGG CACCTTTGGG AGTCCGCTAA
2100  CCCTTTTTGT GGGGAGGGAA CTTTGTATAC CCGGACATGG TCAACATCTG GTTTTTCTAG TGACTTTTCC
2170  CCCCCTGAGG CAGCCGTCGC AGTGCCGGCT GCTACCCCGG GGTTACGCCA CCCTACACCT CCTGTTAGTG
2240  ATATCCGGGT GCTACCGCCG CCTTCTGGAG AACTTCAGGT TGACACAGCG CCCGCTCCCC CTGCCCCTGG
2310  GCCCGCTCAA CCATCCAGCC CTGTTGGGCC GAAGGCTCCC GTGCGTAAGC CGCCAACGCC ACCATCCCCG
2380  CGCACCCGCC GCCTTCTTTA CACCTATCCG GATGGGGCAA AGGTGTATGC GGGGTCACTG TTTGAATCTG
2450  ACTGTGATTG GCTGGTTAAT GCATCGAACC CCGGCCATCG TCCTGGAGGC GGCATTTGCC ATGCCTTCTA
2520  CCAACGTTAC CCCGAGTCTT TCTATTCAAC TGAGTTCATT ATGCGCGACG GTCTTGCCGC GTATACTTTA
2590  ACTCCCCGGC CTATTATTCA TGCAGTGGCT CCTGATTATA GGGTTGAGCA TAACCCAAAG AGGCTTGAGG
2660  CAGCATACCG AGAGACTTGC TCCCGCCGCG GTACCGCCGC CTATCCACTC CTCGGCTCGG GTATATACCA
2730  AGTTCCCGTC AGCCTCAGCT TTGACGCTTG GGAGCGTAAC CATCGCCCCG GAGACGAGCT TTACCTAACC
2800  GACCTCGCAG CTACCTGGTT CGAGGCTAAC AAACCAACAC AGCCGGCCCT TACAATAACT GAGGATGCAG
2870  CCCGCACAGC CAACCTAGCA CTGGAGATCG ATGCTGCTAC GGAGGTCGGC CGGGCTTGTG CCGGCTGTGC
2940  AGTTAGTCCT GGGGTTGTGC ACTATCAGTT TACTGCTGGG GTCCCAGGTT CGGGGAAGTC ACGTTCTATA
3010  CAGCAGGGGG ATGTTGACGT AGTGGTTGTT CCCACTCGGG AGCTCCGGAA TAGTTGGCGT CGCCGGGGTT
3080  TTGCAGCTTT TACACCCCAT ACGGCGGCCC GTGTCACTAC GGGCCGTCGT GTTGTGATTG ATGAGGCCCC
3150  ATCTCTCCCA CCGCATTTGC TGCTACTACA CATGCAGCGG GCCTCGTCGG TCCACCTTCT TGGCGACCCG
3220  AACCAGATCC CTGCCATAGA CTTCGAGCAT GCCGGCCTGG TCCCCGCAAT ACGCCCTGAG CTTGCGCCCA
3290  CCAGTTGGTG GCATGTCACT CATCGCTGCC CCGCTGACGT GTGTGAGCTT ATACGCGGGG CTTATCCCAA
3360  AATCCAAACC ACTAGCCGCG TGCTGCGGTC CTTGTTCTGG AATGAGCCTG CCATTGGCCA GAAGTTAGTT
3430  TTCACCCAGG CTGCTAAGGC CGCCAACCCC GGTGCAATTA CAGTCCACGA GGCCCAGGGT GCCACTTTCA
3500  CGGAAACTAC AATCATAGCC ACGGCTGATG CTAGGGGGCT CATCCAATCT TCCCGAGCTC ATGCCATAGT
3570  CGCACTTACC CGCCACACAG AGAAGTGCGT CATATTTGAC GCTCCCGGCC TGTTACGTGA GGTTGGTATA
3640  TCGGATGTGA TTGTCAACAA TTTTTTCCTT GCCGGCGGGG AGGTGGGTCA CCATCGCCCC TCCGTGATAC
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R4-PRE-RBV Consensus sequence

3710 CTCGCGGTAA TCCTGACCAG AACCTCGCGA CACTACAGGC CTTTCCGCCT TCTTGCCAGA TTAGTGCCTA
3780 TCACCAGTTA GCTGAGGAAC TTGGCCACCG CCCGGCCCCC GTCGCCGCTG TCTTGCCCCC TTGCCCTGAA
3850 CTTGAGCAAG GCTTGTTATA TATGCCGCAA GAGCTTACGG TGTCTGATAG CGTGCTGGTC TTTGAACTCA
3920 CGGACATAGT CCACTGCCGG ATGGCCGCCC CTAGCCAGCG GAAGGCCGTC CTATCGACAC TCGTGGGTAG
3990 GTACGGCCGT CGGACGAAGC TGTATGAAGC AGCTCATTCT GACGTCCGTG AGTCCCTGGC TAGGTTCATC
4060 CCCACCATTG GGCCCGTTCA GGCTACTACG TGTGAGTTAT ATGAGCTGGT TGAGGCCATG GTAGAGAAGG
4130 GTCAGGATGG CTCTGCCGTG CTTGAGCTCG ACCTCTGCAA TCGTGATGTA TCGCGTATCA CATTTTTCCA
4200 GAAAGATTGT AATAAATTCA CCACAGGGGA GACCATCGCC CACGGCAAGG TCGGCCAGGG CATCTCGGCC
4270 TGGAGTAAGA CCTTTTGTGC CCTGTTTGGT CCGTGGTTTC GTGCTATTGA AAAAGAAATA TTAGCCCTGC
4340 TCTCGCCTAA TATTTTCTAC GGCGACGCAT ACGAGGAGTC TGTGTTTGCC GCCGCTGTGT CAGGGGCAGG
4410 TTCAAGCATG GTATTTGAGA ATGATTTTTC AGAGTTTGAT AGCACCCAAA ATAACTTCTC CCTTGGTCTC
4480 GAGTGTGTGA TCATGGAGGA ATGCGGCATG CCCCAGTGGC TAATTCGGCT GTACCATCTG GTTCGGTCGG
4550 CCTGGATTCT ACAGGCGCCG AAGGAGTCTC TCAAGGGATT TTGGAAGAAG CATTCTGGTG AGCCCGGCAC
4620 CCTTCTCTGG AACACCGTCT GGAACATGGC GATCATAGCG CACTGCTATG AATTCCGTGA TTTTAGGGTT
4690 GCCGCTTTCA AGGGAGATGA TTCCGTGGTC CTCTGTAGCG ACTACCGTCA GAGCCGCAAT GCAGCGGCCC
4760 TGATTGCAGG CTGCGGACTC AAACTGAAGG TTGATTATCG CCCTATTGGG TTGTATGCTG GTGTGGTGGT
4830 GGCTCCTGGT TTGGGGACGC TACCCGATGT TGTGCGCTTT GCCGGCCGGC TGTCTGAGAA GAACTGGGGC
4900 CCTGGGCCGG AGCGGGCTGA GCAATTGCGC CTGGCTGTTT GTGACTTCCT TCGAGGGTTA ACGAATGTTG
4970 CGCAGGTTTG TGTCGATGTT GTATCCCGTG TTTATGGAGT TAGCCCTGGG TTGGTACATA ACCTTATTGG
5040 CATGTTGCAA ACCATAGCTG ATGGTAAAGC CCATTTTACA GAGACTGTTA AACCTGTGCT TGACCTCACG
5110 AACTCTATCA TACAGCGGGT GGAATGAATA ACATGTTTTG TGCATTGCCC ATGGGATCAT CATGCGCCCT
5180 AGGGCTGTTC TGTTGCTGTT CTTCGTGCTT CTGCCTATGC TGCCCGCGCC ACCGGCCGGC CAGCCGTCTG
5250 GCCGCCGTCG TGGGCGGCGC AGCGGCGGTG CCGGCAGTGG TTTCTGGGGT GACAGGGTTG ATTCTCAGCC
5320 CTTCGCCCTC CCCTATATTC ATCCAACCAA CCCCTTTGCC GCCGATGTCG TACCACAATC CGGGGCTGGA
5390 GCTCGCCCTC GACAGCCACC CCGCCCCCTC GGCTCCTCTT GGCGTGATCA GTCCCAGCGC CCCTCCGCTG
5460 CCCCACGTCG TCGACTTGCC CCAGCTGGGG CTGCGCCGCT GACCGCTATA TCACCTGCTC CTGATACAGC
5530 TCCTGTACCT GATGTTGACT CGCGCGGTGC CATATTGCGA CGTCAGTACA ATTTATCCAC ATCTCCGCTC
5600 ACATCATCTG TTGCTTCGGG CACTAATCTG GTTCTTTATG CTGCCCCGTT AAACCCTCTG CTGCCCCTTC
5670 AGGATGGCAC TAATACTCAC ATCATGGCCA CTGAGGCATC TAATTATGCC CAGTATCGGG TTGTCCGAGC
5740 CACGATCCGT TATAGGCCAT TGGTGCCAAA TGCTGTCGGC GGTTATGCGA TATCCATCTC ATTCTGGCCT
5810 CAGACTACTA CTACCCCCAC GTCTGTTGAT ATGAACTCTA TTACTTCCAC TGATGTTAGG ATTTTAGTTC
5880 AGCCTGGCAT TGCTTCTGAG TTAGTTATCC CTAGTGAGCG CCTCCATTAT CGTAACCAGG GTTGGCGCTC
5950 TGTGGAGACC TCGGGTGTGG CTGAGGAGGA GGCTACCTCT GGTTTAGTAA TGCTTTGCAT CCATGGCTCT
6020 CCTGTTAATT CCTACACTAA TACCCCTTAT ACCGGGGCGC TTGGACTCCT TGATTTCGCT TTAGAGCTTG
6090 AGTTTAGGAA CTTGACACCC GGGAACACCA ACACCCGTGT GTCCCGGTAT ACAAGCACAG CCCGTCATCG
6160 GTTGCGCCGC GGTGCTGATG GCACCGCTGA GCTTACTACC ACGGCAGCCA CGCGCTTCAT GAAGGACCTG
6230 CACTTCACCG GCACAAATGG GGTCGGTGAG GTGGGTCGTG GTATTGCTCT CACACTCTTT AATCTTGCTG
6300 ACACGCTTCT CGGTGGTCTG CCGACAGAAT TAATTTCGTC GGCCGGGGGG CAGTTATTCT ACTCCCGTCC
6370 CGTCGTCTCA GCCAATGGCG AGCCGACTGT CAAGTTATAC ACATCTGTAG AGAATGCGCA GCAGGATAAA
6440 GGGATCGCTA TTCCACACGA CATAGATCTG GGTGACTCCC GTGTGGTCAT CCAAGACTAT GACAATCAGC
6510 ATGAGCAGGA TCGACCCACC CCCTCGCCTG CCCCCTCTCG CCCTTTTTCG GTTCTTCGCG CTAATGATGT
6580 TTTATGGCTT TCTCTTACTG CCGCCGAGTA CGACCAGACT ACATATGGGT CGTCCACCAA CCCGATGTAT
6650 GTCTCGGATA CTGTCACATT TGTCAACGTG GCTACAGGAG CCCAGGCTGT CGCCCGTTCC CTCGACTGGT
6720 CTAAAGTTAC TCTGGACGGC CGTCCACTTA CTACCATCCA GCAGTATTCC AAAACATTTT ATGTTCTCCC
6790 GCTTCGTGGG AAGCTATCTT TCTGGGAGGC CGGGACGACT AAGGCCGGCT ACCCCTACAA TTACAACACA
6860 ACTGCTAGTG ATCAGATTCT GATTGAAAAT GCTGCTGGTC ATCGTGTTGC TATCTCCACC TATACTACCA
6930 GCTTGGGCGC TGGCCCTGTG TCTGTTTCTG CAGTCGGTGT TCTAGCTCCA CATTCGGCTC TTGCGGTCCT
7000 CGAAGACACT ATTGACTATC CTGCCCGTGC CCACACTTTT GATGATTTTT GCCCGGAGTG TCGCGCTCTT
7070 GGTTTGCAGG GGTGTGCTTT CCAGTCCACT ATCGCTGAGC TTCAGCGCCT TAAAATGAAG GTAGGTAAAA
7140 CCCGGGAGTT TTAATCAATT TCCTCTGTGC CCCCTTCATA GCTTTGTTTT ATTTCTTNTT TTCTGCGTTC
7210 CGCGCTCCCT GGAANNNNNN NNNNNNN