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CHARACTERIZATION OF INTRACELLULAR INTERACTIONS BETWEEN DENGUE VIRUS AND HOST PROTEINS

by

DUMRONG MAIRIANG

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2012

MAJOR: MOLECULAR BIOLOGY AND GENETICS

Approved by:

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Advisor Date

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DEDICATION

To my grandmothers

Payoong Tongchua and Eua-aree Mairiang

แด่

คุณยายพยุง ทองเชื้อ และคุณย่าเอื้ออารี ไม้เรียง

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I would like to thank Dr. Prida Malasit and Dr. Thawornchai 'Tom' Limjindaporn for allowing me to work in their laboratory in 2005-2006. My project is derived from Dr. Prida's idea to combine dengue protein interactions with Drosophila protein interaction map. Tom helped me construct many dengue yeast two-hybrid baits, which were used in this project. During the course of this project, many reagents were obtained from Thailand thanks to Dr. Prida's and Tom's coordinations. I would like to thank Dr. Gerardus C. Tromp who taught me how to write a program. Programming was an essential and powerful tool to analyze a huge amount of data generated by this study.

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CHAPTER 1

INTRODUCTION

1.1 Dengue virus: a global health issue

1.1.1 The disease

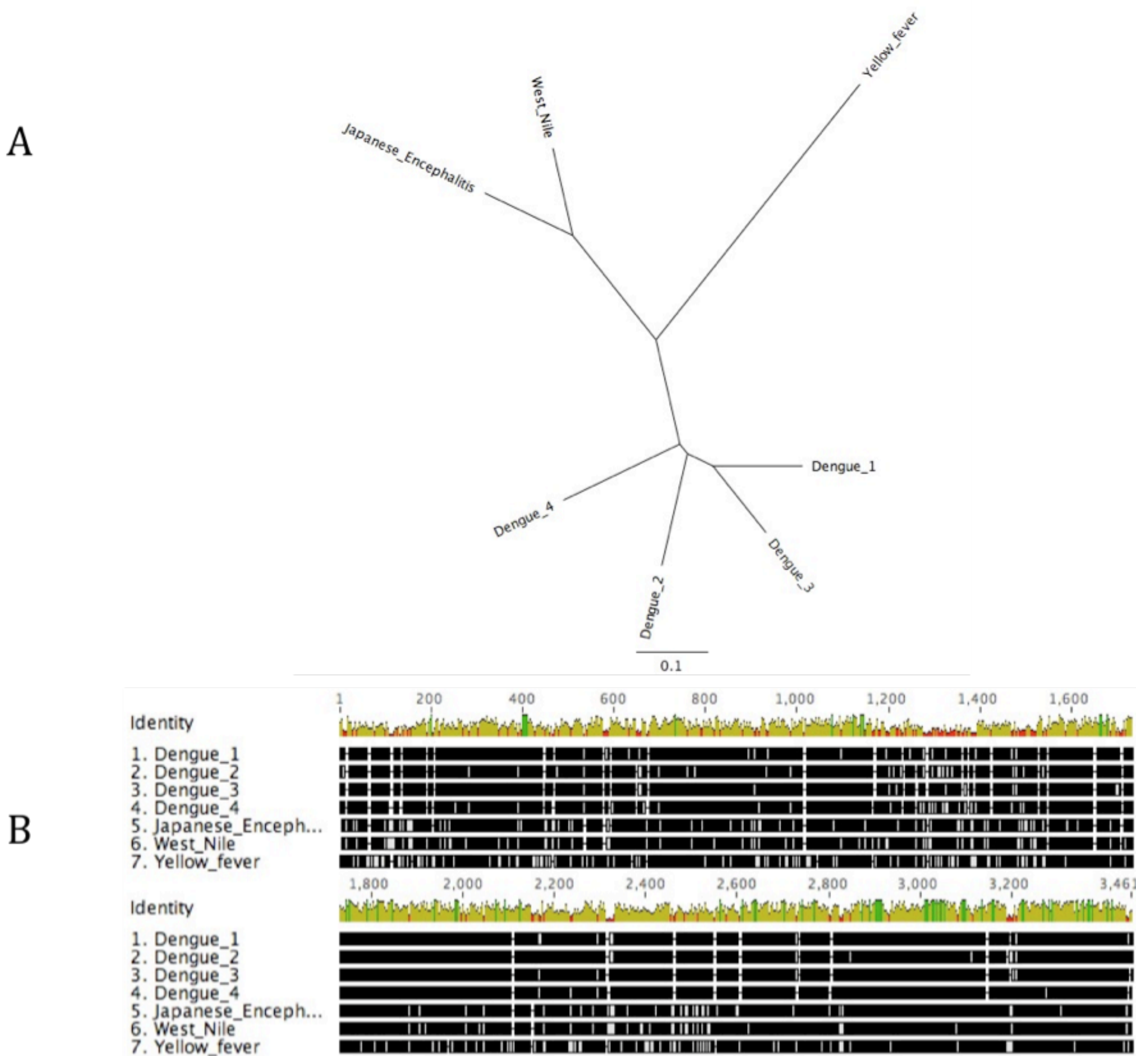
Dengue fever is a potentially deadly disease caused by the dengue fever virus. It is one of the major emerging/reemerging mosquito-borne diseases along with malaria (Snowden, 2008). The World Health Organization has estimated that two-fifths of the world population live in dengue endemic areas, which are tropical and subtropical regions (WHO, 1997). The usual transmission of the virus from one person to another is by mosquito bite (Gubler, 1988), though vertical transmission in both human and mosquito have also been reported (Rosen et al., 1983; Chye et al., 1997; Joshi et al., 2002; Guo et al., 2007; Angel and Joshi, 2008; Chuang et al., 2008; Tambyah et al., 2008). The prevalence of the disease is closely associated with the presence of the mosquito *Aedes aegypti* (*A. aegypti*), the major mosquito vector, and to a lesser extent, *Aedes albopictus (A. albopictus)* (Gubler, 1988; Gubler, 1998). There have been concerns about the spread of dengue fever due to an introduction or re-introduction of virus into mosquito-infested regions. One such case, for example, has been reported in Key West, USA between 2009 and 2010 (CDC, 2010; Radke et al., 2012). Another risk that might help spread the disease is climate change, which can expand the habitable environment for the mosquito vector. One study predicted that 44% and 52% of the world population might be at risk of dengue infection by 2055 and 2085, respectively (Hales et al., 2002).

The rate of dengue infection may be as high as 100 million cases annually and about 500,000 cases per year require hospitalization (Gubler, 1998; Halstead, 2007). The majority of hospitalized cases are children and teenagers, and these patients frequently develop serious symptoms and complications from the disease (WHO, 1997). Symptomatic infections usually result in dengue fever (DF) symptoms, which include high fever, body aches and rashes. DF patients usually fully recover within a week (Gubler, 1998). In some cases, a patient may develop hemorrhagic manifestations, which indicate a severe form of the disease called dengue hemorrhagic fever (DHF) (Gubler, 1998). The most severe complication of dengue infection is dengue shock syndrome (DSS), a hypovolemic shock caused by excessive plasma leakage from blood vessels as an over-response to the infection (Gubler, 1998). The prognosis of DSS is usually poor, and the outcome may be fatal (WHO, 1997). The mortality of the dengue diseases is around 25,000 cases per year (Gubler, 1998).

1.1.2 Prevention and treatment

Currently, no vaccine or antiviral drug is commercially available, even though vaccine development efforts have been ongoing for several decades (Barban et al., 2012). The first generation of vaccines was developed with live attenuated virus (LAV) (Innis and Eckels, 2003) or inactivated virus (Robert Putnak et al., 2005). However, the LAV vaccines have not been effective due to insufficient immunogenicity, excess reactogenicity, and an imbalanced response to each dengue serotype (Edelman, 2007). The vaccine derived from inactivated virus has not been tested in a human trial (Webster et al., 2009). A second-generation vaccine is being developed based on a

recombinant chimeric virus in which the viral structural proteins of one serotype were replaced by those from other serotypes. There are four antigenically distinct serotypes of the dengue virus (Westaway, 1997). The amino acid homologies among serotypes vary between 65-70% (see Figure 1-1). The hope is that the chimeric virus will safely and effectively induce a balanced immune response against all four of the dengue serotypes (Durbin et al., 2006). A third-generation vaccine is under development based on a replication-defective virus, which is capable of infecting a cell but not disseminating to other cells; this may be a promising alternative to vaccine candidates from previous generations (Suzuki et al., 2009). One major concern of vaccine development is the antibody-dependent enhancement (ADE) of infection (Dejnirattisai et al., 2010). ADE was proposed by Halstead et al., in 1977 to explain the finding that a secondary infection by a serotype different from that of the primary infection resulted in worse outcomes, such as a higher rates of developing DHF (Halstead and O'Rourke, 1977). In ADE, antibodies against one serotype of dengue virus may bind to another serotype without neutralizing the virus. This results in active antibody-virus complexes, which are then engulfed by phagocytes via opsonization (Diamond et al., 2008). Since the virus is still active, the opsonization does not eliminate the virus, but instead helps the virus infect the phagocytes (Dejnirattisai et al., 2010). Dejnirattisai et al., confirmed that antibodies against the viral membrane and envelope proteins, which are more likely to cross-react between serotypes, caused the ADE (Dejnirattisai et al., 2010). To avoid ADE, an effective vaccine must be capable of inducing production of antibodies that specifically recognize each of the four serotypes, but do not cross-react with other serotypes (Webster et al., 2009).



<u>Figure 1-1.</u> The similarity among flaviviruses. (A) A dendrogram shows amino acid sequence similarities among flaviviruses: yellow fever virus, West Nile virus, Japanese encephalitis virus, dengue virus serotype 1, 2, 3 and 4. The scale bar indicates amino acid substitution per site. (B) An alignment of amino acid sequences of seven flaviviruses. The identity track indicates consensus agreements. Green means an amino acid at the position is the same in all viruses. Olive means an amino acid at the position is the same in four or more viruses. Red means an amino acid at the position is the same in less than four viruses.

Antiviral drugs may also be vital tools to combat dengue infection. Ribavirin works against hepatitis C virus (HCV) (Torriani et al., 2004), which belongs to the same family, *flaviviridae*, as dengue virus (Lindenbach et al., 2006). The drug was shown to be effective against dengue virus *in vitro* (Takhampunya et al., 2006); however, it was not as effective in an animal model (Schul et al., 2007). Several adjustments to ribavirin administration are under development to improve its effectiveness and safety. For example, ribavirin treatment in combination with an α-glucosidase inhibitor has been shown to reduce dengue viremia in mice (Chang et al., 2011).

Another potential strategy to combat dengue fever is control of the mosquito vector (Gubler, 1998). Bed nets, which are useful for combating malaria transmission by *Anopheles* mosquitoes (Phillips-Howard et al., 2003), are not as effective against *Aedes* mosquitoes since they are daytime biters (Gibbons and Vaughn, 2002). Mosquito repellents applied to skin or clothing are recommended as a method to prevent mosquito bite (Fradin and Day, 2002; Wilder-Smith and Schwartz, 2005). Larvicides and pesticides have been used to reduce the mosquito population (Gubler, 1998). DDT was very effective for mosquito eradication in the early to mid twentieth century (Najera et al., 2011), but the program was not very well sustained. As the mosquito population was seemingly controlled, the resources were then reallocated to other competing health programs resulting in the rebound of mosquitoes and diseases (Gubler, 1998). Larvicide, such as Temephos, is used to kill larvae in water reservoirs and containers, which is the most widely used technique against *Aedes* mosquitoes (WHO, 1997). But a population with resistance has already emerged (Lima et al., 2003). Additionally, the larvae can grow in small water containers in urban areas (Gubler, 1998), which may be

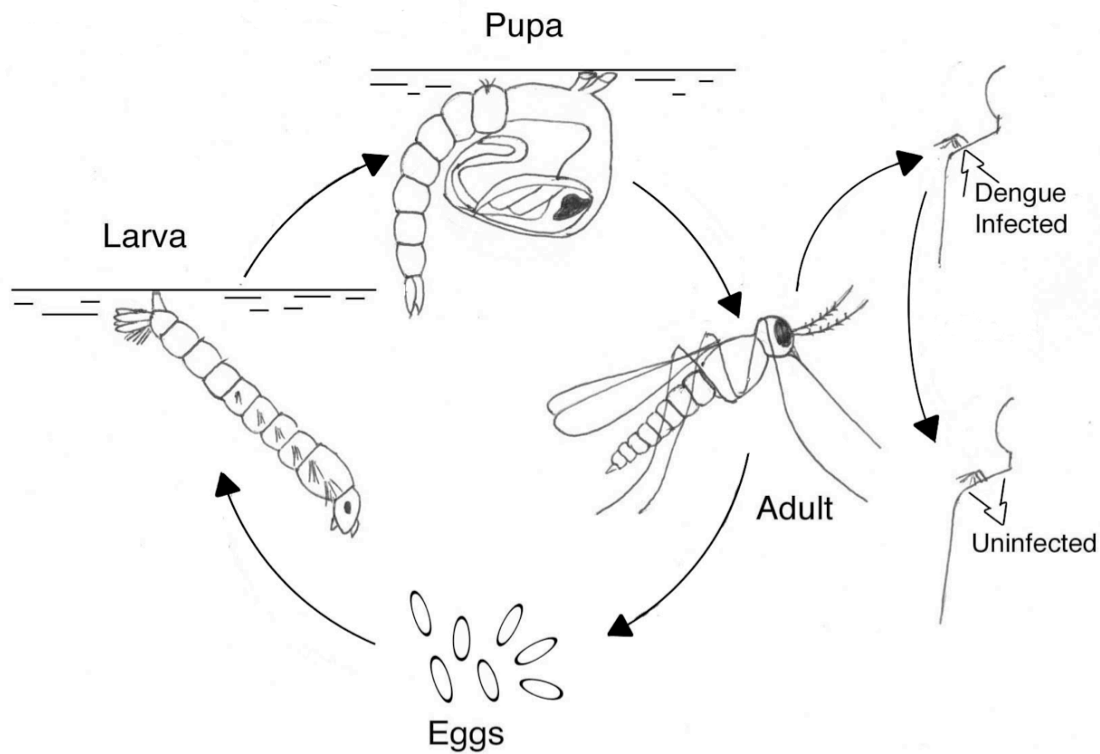
hidden or neglected from larvicide treatments. Other strategies under development include release of irradiated sterile or genetically modified (GM) mosquitoes into the wild to reduce mosquito population (Dame et al., 2009; Hoffmann et al., 2011). The sterile insect technique (SIT) was successful for controlling agricultural pests such as Medflies (Hendrichs et al., 2002) so the technique was adopted and tested for mosquito control (Dame et al., 2009). The rationale for the technique is that sterile males released into the wild will compete against wild-type males (Knipling, 1955). SIT may not be suitable for mosquito vector control because of its several shortcomings including, 1) a very large number of sterile males must be generated and released periodically in order to significantly and sustainably reduce the population of the insect (Alphey, 2002),and 2) sterilization techniques, such as irradiation, reduce the fitness of the insects, which could reduce their ability to effectively compete with the wild population (Lance et al., 2000). One method under development is to genetically engineer a late-acting lethal gene into mosquitoes so that they can mate with the wild population resulting in normal embryos and larvae, but the offspring containing the lethal gene will die once they begin pupation (Phuc et al., 2007). The transgenic larvae may compete with the wild type larvae for resources resulting in the decrease of adult mosquitoes (Phuc et al., 2007). A similar method was developed by Wise de Valdez et al., to generate a repressible female-specific flightless phenotype that causes all female offspring to be unable to survive and mate (Wise de Valdez et al., 2011). Another promising method involves infecting mosquitoes with the insect parasitic microbe, *Wolbachia*. Mosquitoes infected with *Wolbachia* will have cytoplasmic incompatibility, which kills the embryos produced by uninfected females after mating with infected males, but not vice versa (McMeniman

et al., 2009). In addition, *Wolbachia* infection shortens the lifespan of mosquitoes, which could reduce the number of dengue transmissions per infective mosquito within its life-time (McMeniman et al., 2009). There is also evidence showing that *Wolbachia* infection confers some protection against infection of the mosquito by other pathogens, by possibly priming immune response pathways, such as the toll pathway (Pan et al., 2012). Recently, studies have been initiated in Australia in which *Wolbachia*-infected mosquitoes were released into the wild, and the infected mosquitoes successfully invaded two natural populations in the two experimental sites (Hoffmann et al., 2011).

1.2 Dengue virus – genome and life cycle

1.2.1 Dengue is a flavivirus

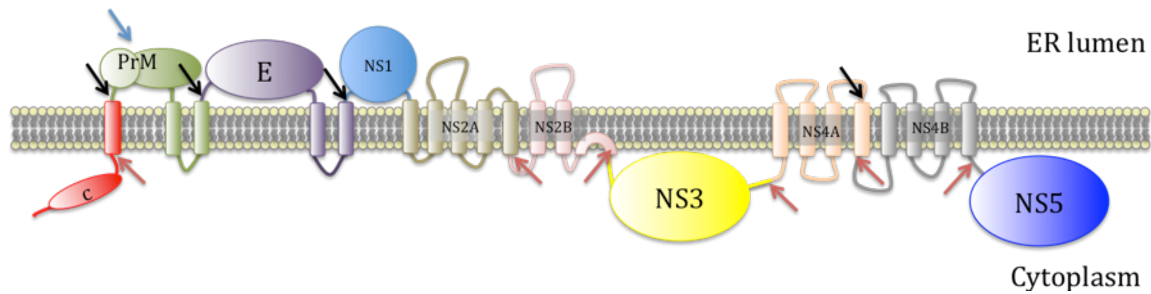
Dengue virus, the causative agent of dengue fever, belongs to the genus Flavivirus in the Flaviviridae family (Lindenbach et al., 2006). Other well-known viruses in this genus are West Nile virus (WNV), yellow fever virus (YFV) and Japanese encephalitis virus (JEV) (Westaway, 1997) (See Figure 1-1). Flaviviruses are arthropod-borne or Arboviruses, which means they require an insect as a host to complete their life cycle (Mackenzie et al., 2004) (See Figure 1-2 for dengue life cycle). Flaviviruses cause neurotropic and/or viscerotropic diseases (Lindenbach et al., 2006). Dengue virus rarely causes neurotropic disease differentiating it from other flaviviruses such as JEV and WNV (Gubler et al., 2006). Dengue virus and YFV are different from other flaviviruses because human is a natural host required to complete the YFV and dengue virus life cycles (Gubler et al., 2006). In contrast, human is an accidental host of other



<u>Figure 1-2.</u> The life cycles of *Aedes* mosquitoes and dengue virus. The mosquitoes have four developmental stages: eggs/embryos, larvae, pupae and adults. All stages, except the adults, are aquatic. Female adult mosquitoes require blood meals to produce eggs. Consequently, they may transmit dengue virus when the blood meal is taken from an infected human, and the next blood meal is from an uninfected one.

flaviviruses, such as WNV and JEV; infection of humans with these viruses may cause a disease, but does not result in a sufficient level of viremia for transmission to an insect (Gubler et al., 2006).

The genome of flavivirus consists of one molecule of positive single-stranded RNA. The genome is encapsulated in a viral capsid protein shell, which is enveloped by a membrane derived from a host cell (Lindenbach et al., 2006). Like other flaviviruses, the dengue genome encodes ten proteins. Three are structural proteins, which are capsid, the precursor of membrane protein (PrM) and envelope protein (E), while the rest are non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) (Lindenbach et al., 2006). The virus uses E protein to bind receptors on the host cell surface inducing endocytosis of the virion (Modis et al., 2004). A decrease of pH in the endosomal compartment induces a structural change of the virion resulting in release of viral RNA (van der Schaar et al., 2008). Next, the RNA is translated into a polyprotein inserted in the host endoplasmic reticulum (ER) membrane. The polyprotein is cleaved by the host proteases, furin and siganalase, and by a viral protease, NS2B/NS3 complex, to form the ten individual viral proteins (Sampath and Padmanabhan, 2009) (see Figure 1-3 for polyprotein). Viral RNA in the cytoplasm is transferred to replication complexes containing NS5, a viral RNA-dependent RNA polymerase, which generates more genomic RNA (Mackenzie, 2005). This takes place in vesicle packets, unique ER membrane-derived structures induced by the virus infection (Mackenzie, 2005). Viral RNA is coated with capsid forming a nucleocapsid, which buds into the ER lumen and acquires a virus envelope from the ER membrane (Sampath and Padmanabhan, 2009).



<u>Figure 1-3. The</u> polyprotein of dengue virus. A virus genome is translated as the polyprotein inserted into the endoplasmic reticulum membrane. The polyprotein contains ten viral proteins: capsid (C), precursor of membrane protein (PrM), envelope protein (E), non-structural protein 1 (NS1), NS2A, NS2B, NS3, NS4A, NS4B and NS5. Red arrows indicate the sites cleaved by NS2B/NS3. Black arrows indicate the sites cleaved by host signalase. A blue arrow indicates the site cleaved by host furin.

The virus envelope also contains PrM and E proteins embedded during dengue polyprotein translation at the ER membrane (Sampath and Padmanabhan, 2009). During maturation, the PrM protein of virions in the lumen of the ER and Golgi apparatus are further cleaved by host protease, and then the mature virions are transported out of the host cell via the secretory pathway (Yu et al., 2008b).

The primary target of infection in a mosquito is midgut epithelium, which is the very first barrier to come in contact with a dengue-infected blood meal (Black et al., 2002; Mercado-Curiel et al., 2006). The virus must then escape the midgut into the hemocele, and disseminate to other organs such as brain, ovaries, and most importantly, salivary glands to make the mosquito infective and start a new round of transmission (Black et al., 2002; Mercado-Curiel et al., 2006). The virus life cycle in the mosquito usually takes eight to ten days before the host becomes infective (Gubler and Rosen, 1976). When the virus is transmitted to human by a mosquito bite, the primary sites of infection are cells the mononuclear phagocyte lineage (Kyle et al., 2007). The virus can also infect secondary sites such as liver cells and may cause injuries in those tissues (Kuo et al., 1992; Souza et al., 2004).

1.2.2 Dengue virus proteins

Below I discuss dengue proteins and their known functions. I also address the dengue proteins whose functions are still unclear.

1.2.2.1 Capsid (C)

Capsid is a building block for a shell of nucleocapsid (Kuhn et al., 2002). The 11 kDa, 100 amino acid protein contains several basic amino acids giving it

a total positive charge (isoelectric point = 12.5) (Ma et al., 2004; Lazo et al., 2007). Newly translated capsid has a transmembrane domain at its C-terminus, which anchors it to the ER membrane as a part of the dengue polyprotein, while the N-terminus is exposed to the cytoplasm (Lindenbach et al., 2006). During virus maturation, the transmembrane domain is cleaved by the dengue serine protease, NS2B/NS3 (Ma et al., 2004). Capsid naturally forms homodimers that become part of the icosahedral nucleocapsid shell (Kuhn et al., 2002; Ma et al., 2004). Dimerized capsids have two surfaces. One surface has positive charges that interact with the viral RNA, while the other surface remains in contact with the ER membrane (Ma et al., 2004). Interestingly, capsid contains several potential nuclear localization signals (NLS) and has been reported to be nuclear and nucleolar localized in a few cell lines (such as HepG2 cells) through an interaction with host importin (Wang et al., 2002; Sangiambut et al., 2008; Bhuvanakantham et al., 2009; Netsawang et al., 2010). Nuclear localized capsid interacts with death-domain associated protein (DAXX), a multi-functional protein having a role in mediation of apoptosis (Netsawang et al., 2010). Nuclear capsid can sensitize cells to undergo apoptosis probably through CD137 signaling (Nagila et al., 2011). Other roles of nuclear and nucleolar localized capsid remain unclear.

1.2.2.2 Membrane protein (PrM and M)

The precursor of the membrane protein (PrM) is a 26 kDa glycoprotein located in the ER lumen and anchored to the ER membrane during synthesis

(Lindenbach et al., 2006). PrM binds to the envelope protein to prevent premature fusion to the Golgi membrane (Zhang et al., 2003). PrM contains a N-terminal domain, which is cleaved by the host protease, furin, in the Golgi apparatus during maturation (Yu et al., 2008b). PrM was proposed to contribute to ADE because antibodies against PrM were shown to be highly cross-reactive but non-neutralizing, at least *in vitro* (Dejnirattisai et al., 2010). A mixture of mature and immature disseminating virions is found in human cell cultures and infected mosquitoes (van der Schaar et al., 2007; Junjhon et al., 2008; Zybert et al., 2008). It has been hypothesized that the mixture may help dengue virus evade immunity since the uncleaved PrM of an immature virion elicits non-neutralizing antibodies. These antibodies bind the virion and induce opsonization, whereas antibodies against a mature virion usually neutralize the virus (Rodenhuis-Zybert et al., 2010). The ectodomain of M interacts with Tctex-1, a dynein light chain that functions in cargo binding (Brault et al., 2011). Silencing of Tctex-1 significantly reduces dengue replication in cell culture (Brault et al., 2011). The mechanism behind the Tctex-1 requirement remains unclear, but it does not involve microtubule-dependent retrograde transport of the dynein motor complex.

1.2.2.3 Envelope protein (E)

Envelope protein is a 53 kDa glycoprotein located in the ER lumen and anchored to the ER membrane during synthesis (Lindenbach et al., 2006). E functions as a virus receptor to target host cells. E protein binds proteins on the

surface of host cells, initiating receptor-mediated endocytosis (Modis et al., 2004). E protein has three domains, Domain I, Domain II and Domain III (Mukhopadhyay et al., 2005). Domain I and II form a hinge involved in a pH-induced structural change of the E protein, which takes place in the endosome and leads to envelope-membrane fusion and RNA release to cytoplasm (Mukhopadhyay et al., 2005). Domain III is involved in receptor binding (Crill and Roehrig, 2001) so it is a potential target for neutralizing antibodies (Li et al., 2012). Studies in *A. albopictus* cells and *A. aegypti* tissues has shown that the dengue virion or E protein can interact with laminin-binding protein (Sakoonwatanyoo et al., 2006), prohibitin (Kuadkitkan et al., 2010), tubulin-like protein (Chee and AbuBakar, 2004) and several unidentified proteins with molecular weight from 35 to 80 kDa (Salas-Benito and del Angel, 1997; Munoz et al., 1998; Yazi Mendoza et al., 2002; Reyes-del Valle and del Angel, 2004; Mercado-Curiel et al., 2006; Salas-Benito et al., 2007; Mercado-Curiel et al., 2008; Cao-Lormeau, 2009). One or more of these may be parts of a host cell receptor complex. Several additional human proteins and glycolipids were proposed to be members of a host receptor complex including heat-shock proteins 90 and 70 (Reyes-Del Valle et al., 2005), neolactotetraosylceramide (Aoki et al., 2006), CD14 (Chen et al., 1999), GRP78/BiP (Jindadamrongwech et al., 2004), 37-kDa/67-kDa laminin receptor (Thepparit and Smith, 2004), DC-SIGN (Tassaneetrithep et al., 2003; Lozach et al., 2005), the mannose receptor (Miller et al., 2008) and CLEC5A (Chen et al., 2008). Nevertheless, the full details and mechanisms of binding and endocytosis are still under investigation.

E protein also interacts with UBE2I, a SUMO conjugation enzyme (Chiu et al., 2007). Over-expression of UBE2I significantly reduces dengue virus production, but the role of sumoylation in dengue virus is not yet clear. Antibodies against E protein were shown to be cross-reactive and non-neutralizing, and thus may have a role in ADE (da Silva Voorham et al., 2012).

1.2.2.4 Non structural protein 1 (NS1)

NS1 is a 46 kDa glycoprotein (Lindenbach et al., 2006). The function of NS1 remains mostly elusive. Immature NS1 as a part of the dengue polyprotein resides on the lumenal side of the ER membrane (Lindenbach et al., 2006). It does not have a transmembrane domain. NS1 is cleaved by host signalase during virion maturation (Lindenbach et al., 2006). It can form homodimers that retain an association to the membrane by an unknown mechanism (Winkler et al., 1989). Hexameric NS1 is a secreted form of the protein (Flamand et al., 1999). This form is used as a marker to detect dengue infection in a suspected patient (Hang et al., 2009; Fry et al., 2011). Intracellular NS1 co-localizes with the viral dsRNA (Mackenzie et al., 1996; Welsch et al., 2009). Mutagenesis studies have shown that NS1 is required for efficient RNA replication in several flaviviruses, but the mechanism is unknown (Muylaert et al., 1996; Lindenbach and Rice, 1997; Muylaert et al., 1997; Lindenbach and Rice, 1999; Blaney et al., 2003). Secreted NS1 and cell surface-associated NS1 seem to interact with host immune factors contributing to immune evasion or pathogenesis. NS1 binds to components of the complement reaction, such as C1, C4 and C4BP, suppressing

their activity (Avirutnan et al., 2010; Avirutnan et al., 2011). Recently, secreted NS1 was found to form a lipoprotein mimicking HDLs, which may contribute to the acute vascular dysfunction and the associated life-threatening hypovolemic shock of DSS (Gutsche et al., 2011).

1.2.2.5 Non structural protein 2A (NS2A)

NS2A is a 22 kDa transmembrane protein whose function is not very well studied (Lindenbach et al., 2006). In Kunjin virus, NS2A is essential for virion assembly since a mutation of NS2A disrupts virion assembly (Liu et al., 2003; Leung et al., 2008). Another potential function of NS2A is to inhibit interferon signaling since it was shown that expression of dengue NS2A in human cells could suppress interferon-beta-stimulated gene expression (Munoz-Jordan et al., 2003).

1.2.2.6 Non structural protein 2B (NS2B)

NS2B is a 14 kDa transmembrane protein (Lindenbach et al., 2006). It functions as a cofactor of NS3, a viral serine protease (Falgout et al., 1991). Other functions of NS2B have not been described.

1.2.2.7 Non structural protein 3 (NS3)

NS3 is a 70 kDa cytoplasmic protein without a transmembrane (Lindenbach et al., 2006). NS3 has two functional domains, an N-terminal serine protease domain and a C-terminal RNA helicase domain (Lindenbach et al., 2006). The protease activity of NS3 is essential since it is required to cleave dengue

polyprotein into individual proteins on the cytoplasmic side of the ER membrane (Gorbalenya et al., 1989; Cahour et al., 1992; Lindenbach et al., 2006). Consequently, protease inhibitors of NS3 are attractive candidates for an effective anti-dengue therapy, and several of them are under development (Sampath and Padmanabhan, 2009). The helicase domain of NS3 is required for viral RNA replication (Li et al., 1999; Matusan et al., 2001; Lindenbach et al., 2006). NS3 also has an RNA triphosphatase activity, which plays a role in RNA 5'-capping (Bartelma and Padmanabhan, 2002). NS3 may also play a role in the apoptosis that can result from dengue infection (Gagnon et al., 1999; Morchang et al., 2011; Silveira et al., 2011) and NS3 has been shown to induce apoptosis in Vero cells (Shafee and AbuBakar, 2003). This has led to the hypothesis that NS3 might cleave or interact with host proteins that initiate apoptosis (Doolittle and Gomez, 2011). Consistent with this idea, West Nile virus NS3 was shown to directly interact with and cleave caspase-8 and initiate apoptosis (Ramanathan et al., 2006). It remains unclear whether NS3-induced apoptosis contributes to the tissue injuries observed in dengue patients. NS3 also physically interacts with fatty acid synthase (FASN) recruiting it to the replication site (Heaton et al., 2010). Consequently, FASN activity is increased resulting in an enhancement of lipid biosynthesis in infected cells (Heaton et al., 2010). An interaction of NS3 with autoantigen La (SSB) was also reported (Garcia-Montalvo et al., 2004), though no functional studies have confirmed the interaction or shown its significance. NS3 is truncated by cleavage during the infection of some

flaviviruses, but the importance of the truncation is unknown (Arias et al., 1993; Teo and Wright, 1997).

1.2.2.8 Non structural protein 4A (NS4A)

NS4A is a 16 kDa transmembrane protein whose function is not very well studied (Lindenbach et al., 2006). NS4A co-localizes with the replication complex and, therefore, may have a role in virus replication (Anwar et al., 2009). NS4A was reported to have a role in ER membrane rearrangement since the expression of NS4A in Huh-7/T7 cells alone induces membrane rearrangements similar to those observed in dengue-infected cells (Miller et al., 2007). The expression of NS4A in human cells can also suppress interferon-beta-stimulated gene expression (Munoz-Jordan et al., 2003). A recent study showed that expression of NS4A in epithelial cells up-regulates PI3K-dependent autophagy and prevents the cell death observed in infected cells (McLean et al., 2011). The mechanisms of these effects of NS4A are not known. NS4A interacts and is co-localized with polypyrimidine tract binding protein 1 (PTB) (Jiang et al., 2009). Silencing of PTB in dengue-infected cells showed that PTB is required for effective viral negative strand RNA replication. However, the mechanism for how NS4A-PTB interaction effects virus RNA replication is not clear.

1.2.2.9 Non structural protein 4B (NS4B)

NS4B is a 27 kDa transmembrane protein whose function is not very well studied (Lindenbach et al., 2006). It was shown along with NS2A and NS4A to inhibit the interferon pathway, with NS4B being the strongest inhibitor (Munoz-

Jordan et al., 2003). It was recently shown that expression of NS4B or 2K_NS4B, but not an immature form, NS4A_2K_NS4B, in human microvascular endothelial cells and THP-1 monocytes could elevate the secretion of DHF-associated immunomediator like interferon-gamma, IL-6 and IL-8(Kelley et al., 2011; Kelley et al., 2012).

1.2.2.10 Non structural protein 5 (NS5)

NS5 is a 103 kDa multi-functional protein (Lindenbach et al., 2006). It has an N-terminal methyltransferase domain (MTase) and a C-terminal RNA-dependent RNA polymerase (RdRp) (Lindenbach et al., 2006). The N-terminal domain is required for RNA capping (Egloff et al., 2002) while the C-terminal domain is required for RNA replication (Ackermann and Padmanabhan, 2001). Ribavirin 5'-triphosphate, a derivative of a widely used antiviral drug, interferes with the MTase activity of NS5 *in vitro (Benarroch et al., 2004)*; therefore, the drug has potential as an anti-dengue therapy, and studies of Ribavirin and its derivatives are ongoing (Chang et al., 2011). NS5 physically interacts with NS3 to form a complex (Kapoor et al., 1995; Johansson et al., 2001; Brooks et al., 2002; Yon et al., 2005), which may be essential for virus replication. The RdRp activity from NS5 and the helicase activity from NS3 are both required for RNA replication (Li et al., 1999; Ackermann and Padmanabhan, 2001; Matusan et al., 2001; Lindenbach et al., 2006). Interestingly, although viral replication occurs in the cytoplasm, NS5 contains a nuclear localization signal, physically interacts with importin, and has been observed in the nucleus (Johansson et al., 2001;

Brooks et al., 2002). The nuclear localization of NS5 was shown to reduce interleukin-8 (IL-8) production and secretion (Medin et al., 2005; Pryor et al., 2007; Rawlinson et al., 2009). NS5 also contains a nuclear export signal, which interacts with exportin 1 (CRM-1), implying that the nuclear import and export of NS5 is dynamically regulated by CRM-1 and importin (Rawlinson et al., 2009). NS5 was also shown to interfere with the interferon pathway by binding to STAT2 and promoting its degradation (Jones et al., 2005; Ashour et al., 2009; Mazzon et al., 2009). NS5 can be phosphorylated by protein kinase G (PKG), which increases virus production by an unknown mechanism (Bhattacharya et al., 2009). NS5 also interacts with SSB and zona occludens 1 (ZO-1) (Garcia-Montalvo et al., 2004; Ellencrona et al., 2009). However, the significance of these interactions has not been demonstrated.

1.3 Functional screens

Because the dengue genome encodes only ten viral proteins, the virus needs to hijack host proteins to help its replication. To determine what cellular processes may be required for the dengue virus, functional screens have been conducted. Additionally, functional screens have been used to investigate how host cells try to combat infection, or to determine which immune pathways the virus needs to perturb to evade the host's immune defense.

1.3.1 Host factors required for viral replication

Sessions et al., performed a genome-wide RNA interference screen in dengue-infected Drosophila cells to identify Dengue Virus Host Factors (DVHFs), defined as genes required for effective replication of dengue virus (Sessions et al., 2009). They found 116 *Drosophila* DVHFs. Out of 116 DVHFs, 82 genes had human homologs of which 42 were confirmed as DVHFs by siRNA assays with dengue-infected human cells. A limited set of RNA interference assays was also conducted in mosquitoes with three homologs of *Drosophila* DVHFs. Only one DVHF, a mosquito homolog of lola, was confirmed as a mosquito DVHFs. A limitation of this study was that the dengue virus used was deliberately mutated by multiple passages in *Drosophila* cells to overcome the fact that *Drosophila* is not naturally susceptible to dengue infection.

1.3.2 Host genes induced by dengue infection

Xi et al., used microarrays to identify transcriptional responses to dengue infection in *A. aegypti* mosquitoes (Xi et al., 2008). They implicated the Toll immune pathway and the Jak-STAT immune pathway as a major and a minor immune response to dengue infection, respectively. In another study, Fink et al., conducted microarray assays with dengue-infected patients and cell lines to identify transcriptional responses to infection in human (Fink et al., 2007). They were able to implicate three major pathways; NF-κB initiated immune responses, type I interferon, and the ubiquitin proteasome pathway.

1.4 Protein-protein interactions (PPI) of dengue virus

Data from functional screens may identify cellular pathways that a virus hijacks or perturbs, but they do not reveal the mechanisms for how the virus directly interacts with these pathways. Physical interactions, such as PPI, are crucial data that can complement the data from functional screens. Physical PPI data, for example, may implicate an interface between the virus and host that could be exploited for development of antiviral drugs targeting the interaction. In other words, PPI can be used to generate hypotheses of how the virus interacts with its hosts and how to develop tools to combat it. When I began this study in 2007, very few PPI data were available for host-virus interactions. Since then, useful PPI data have begun to emerge from several studies with a variety of viruses. Large-scale virus-host PPI studies, for example, have been conducted with Epstein-Barr virus (EBV) (Calderwood et al., 2007) and Hepatitis C virus (HCV) (de Chassey et al., 2008). Recently, several other collections of virus-host protein-protein PPI have become available. The HIV-1, Human Protein Interaction Database available at NCBI (http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/) is an archive for published PPI of HIV (Ptak et al., 2008; Fu et al., 2009; Pinney et al., 2009). VirusMINT (http://mint.bio.uniroma2.it/virusmint/) is another archive for published virus-host PPI including data from more than a hundred virus strains (Chatr-aryamontri et al., 2009). Below I document PPI studies that have been applied to the dengue virus.

1.4.1 Literature-curated dengue-host PPI

Several studies attempting to identify specific dengue-host PPI began since a few decades ago. These studies relied on low-throughput screens focusing on an

individual gene or a pathway related to dengue biology. For example, NS5 and C of dengue were known to locate in the cytoplasm (Lindenbach et al., 2006), but their interactions with importin hinted at the possibility of nuclear localization (Johansson et al., 2001; Sangiambut et al., 2008; Bhuvanakantham et al., 2009), which was further investigated in follow-up studies (Pryor et al., 2007; Rawlinson et al., 2009; Netsawang et al., 2010; Nagila et al., 2011). I searched the literature for additional PPI identified with low-throughput methods and found those summarized in Table 1-1.

1.4.2 PPI from large-scale yeast two-hybrid (Y2H) screens

Y2H is an economic and versatile tool for detecting PPI (Fields and Song, 1989). The technique is also compatible with high-throughput screens. It was used, for example, in large-scale PPI screens to detect tens of thousands of PPI for *Saccharomyces cerevisiae, Drosophila melanogaster, Caenorhabditis elegans,* and human (Uetz et al., 2000; Giot et al., 2003; Li et al., 2004; Stanyon et al., 2004; Rual et al., 2005). Y2H has been used to construct PPI maps for virus-host interactions including HCV (de Chassey et al., 2008), EBV (Calderwood et al., 2007), influenza virus (Shapira et al., 2009) and vaccinia virus (Zhang et al., 2009). Y2H has also been used in small-scale studies to detect dengue-host interactions (see Table 1-1). Recently, two groups carried out large-scale Y2H screens for PPI between dengue and human. In one study, Khadka et al., screened all ten dengue proteins against a human liver Y2H library (Khadka et al., 2011). They identified 139 interactions involving 105 human proteins. Most of the interactions had not been detected before. The screen implicated human proteins involved in the complement and coagulation cascade, the centrosome, and the

Table 1-1 Dengue-host PPI previously identified in low-throughput studies.

Dengue protein	Interactor	Technique/Method	Publication
NS3	NRBP	Y2H; Co-AP	(Chua et al., 2004)
NS5	KPNB1	Y2H; Co-AP	(Johansson et al., 2001)
NS5	NS3	Y2H; Co-AP	(Johansson et al., 2001)
E	HSPA5	Y2H; Co-AP	(Limjindaporn et al., 2009)
E	CANX	Y2H; Co-AP	(Limjindaporn et al., 2009)
E	CALR	Y2H; Co-AP	(Limjindaporn et al., 2009)
NS1	C4B	Co-AP	(Avirutnan et al., 2011)
NS5	PRKG1	Phosphorylation assay	(Bhattacharya et al., 2009)
NS5	XPO1	Co-Complex; Co-localization	(Rawlinson et al., 2009)
NS5	SSB	Co-Complex	(Garcia-Montalvo et al., 2004)
NS3	SSB	Co-Complex	(Garcia-Montalvo et al., 2004)
C	H2	Co-AP	(Colpitts et al., 2011a)
C	H4	Co-AP	(Colpitts et al., 2011a)
NS1	HNRNPC	TAP-MS; Co-AP	(Noisakran et al., 2008)
E	HSP90AA1	Affinity Chromatography	(Reyes-Del Valle et al., 2005)
E	HSPA4	Affinity Chromatography	(Reyes-Del Valle et al., 2005)
C	DAXX	Y2H; Co-AP	(Limjindaporn et al., 2007)
C	HNRNPK	Co-AP	(Chang et al., 2001)
NS5	TJP1	Co-AP	(Ellencrona et al., 2009)
NS4A	PTBP1	Y2H; Co-AP	(Jiang et al., 2009)
E	NCR2	ELISA	(Hershkovitz et al., 2009)
M	DYNLT1	Y2H; Co-AP	(Brault et al., 2011)
NS5	STAT2	Co-Complex	(Mazzon et al., 2009)
NS5	STAT2	Co-AP	(Ashour et al., 2009)

NS5	KPNB1	Co-AP; Domain Mapping	(Brooks et al., 2002)
NS1	CLU	Co-Complex	(Kurosu et al., 2007)
NS1	STAT3	Y2H; Co-AP	(Chua et al., 2005)
E	UBE2I	Y2H; Co-AP	(Chiu et al., 2007)
E	CD209	Co-AP	(Lozach et al., 2005)
NS3	FASN	Y2H	(Heaton et al., 2010)

cytoskeleton. They further investigated the functions of 12 dengue interactors by siRNA assays in cells containing a synthetic dengue replicon, and showed that six of them (CALR, DDX3X, ERC1, GOLGA2, TRIP11 and UBE2I) are essential for replication. In the other study, Le Breton et al., screened NS3 and NS5 from several flaviviruses including dengue against human cDNA libraries from liver, brain, spleen and bronchial epithelia (Le Breton et al., 2011). They detected 108 human proteins interacting with NS3, NS5, or both. Out of these proteins 29 proteins interacted with NS3 from dengue virus serotype 2, while 11 proteins interacted with NS5 from dengue virus serotype 1. Functional enrichment of all proteins detected in the screen implicated RNA binding, transcription regulation, vesicular transport, and innate immune response regulation. Interestingly, only one interaction (NS5 and MATR3) was detected in both Y2H studies suggesting that dengue-human PPI screenings are still far from saturation.

1.4.3 Co-affinity purification and co-complex purification

While Y2H could detect a large number of binary physical PPI, it has limitations if a library of proteins from the appropriate organism or tissue does not exist or if the genome of a species used for a study is not well annotated. Y2H assays also require proteins to be expressed in yeast cells, which may not imitate their natural locations (Fields and Song, 1989). Protein affinity purification is an alternative method to avoid such limitations (Rigaut et al., 1999). In this method, one protein is expressed as a bait in a target cell, and then purified with antibodies or an affinity reagent that recognizes a tag on the bait. Any proteins co-purified with the bait are potential interactors and may be identified by subsequent analyses such as mass-spectrometry (Rigaut et al., 1999).

This method reveals the proteins belonging to stable complexes, but the binary interactions among these proteins are not shown. The data derived from this method may, therefore, compliment the binary interaction data derived from Y2H assays, and vice versa. Colpitts et al used tandem affinity purification along with mass-spectrometry to identify dengue-mosquito PPI (Colpitts et al., 2011b). They expressed N-TAP tagged C, E, NS2A and NS2B from dengue virus and West Nile virus as baits in *A. albopictus* C6/36 cells and purified complexes. They identified 18 dengue-mosquito protein interactions involving 14 mosquito proteins. Despite the limited number of interactors detected, most virus proteins seemed to interact with one or more host proteins involved in cytoskeleton and cellular trafficking.

1.4.4 Computational predictions and data integration

Significant resources, time, and labor are required for conducting high throughput screening for PPI. An alternative approach is to use computational methods to predict PPI. These methods use presently available data to narrow down a number of potential PPI, which may be further experimentally investigated. Doolittle et al., computationally predicted dengue-human and dengue-mosquito PPI using structural similarity between human proteins and dengue proteins, and available human PPI databases (Doolittle and Gomez, 2011). They predicted that a human protein that interacts with another human protein containing a domain structurally similar to a dengue protein would also potentially interact with that dengue protein. Since there is no mosquito PPI database, they relied on *Drosophila* PPI data to predict dengue-mosquito interactions (Yu et al., 2008c). Similar to the dengue-human PPI predictions, a *Drosophila* protein that interacts

with another *Drosophila* protein containing a domain structurally similar to a dengue protein would also potentially interact with that dengue protein. Next, they assumed that mosquito orthologs of the *Drosophila* protein would also interact with the same dengue protein. The predictions gave more than 4000 potential dengue-human PPI and 176 potential dengue-mosquito PPI. They further used Gene Ontology (Ashburner et al., 2000) of a cellular component to select a dengue protein and the similar host protein sharing at least one GO term. The filter reduced the number of potential interactions to around 2000 for dengue-human PPI and 18 dengue-mosquito PPI. Interferon signaling, transcriptional regulation, stress, and the unfolded protein response are pathways to which a significant number of the predicted interactors belong. Another effort to predict dengue-host PPI was done by Guo et al., (Guo et al., 2010). Since there is no comprehensive PPI database for *Aedes* mosquito, Guo et al., used PPI data from yeast (Uetz et al., 2000), *D. melanogaster* (Giot et al., 2003) and *C. elegans* (Li et al., 2004) to construct a predicted mosquito PPI network. Next, they used data from functional studies, physical interaction assays, genome-wide RNA interference (RNAi) screens (Krishnan et al., 2008) and microarray assays (Xi et al., 2008) to predict mosquito proteins that may interact physically or functionally with dengue virus. From the predicted interactors, several cellular pathways, such as the toll pathway and the JAK/STAT pathway, were implicated as involved in dengue replication.

1.5 Unanswered questions

1.5.1 Cellular pathways connected to dengue virus

The unfolded protein response (UPR) in the ER is up-regulated under stress conditions, including virus infection. All three branches of the UPR, including ATF-6, PERK and IRE-1, are activated during dengue infection (Yu et al., 2006; Umareddy et al., 2007; Pena and Harris, 2011). However, disruption of the ATF-6 pathway does not seem to have any effect on dengue replication (Pena and Harris, 2011). Interestingly, it seems that dengue virus can either up- or down-regulate UPR over time to suit its replication (Pena and Harris, 2011). Doolittle et al., computationally predicted dengue-host PPI that may play roles in regulation of the UPR: NS4B and PPP1R15A, NS2A and NFYA, NS4B and NFYA, C and NFYA, E and BCL2, NS4B and BCL2L11, E and BCL2L1, NS3 and BCL2L1, and NS3 and BCL2L10 (Doolittle and Gomez, 2011). However, these PPI have not been tested or validated with any conventional experiment. Interestingly, envelope protein E2 of HCV, a distant relative to dengue virus, physically binds to PERK and inhibits PERK-mediated eIF2α phosphorylation (Pavio et al., 2003). It remains to be seen whether any dengue protein functions the same way as HCV E2 does. The mechanism for how dengue regulates the UPR and the reason why dengue targets the pathway is still a mystery.

Several viruses hijack the ubiquitination-proteasome pathway (Viswanathan et al., 2010). Dengue up-regulates the expression of ubiquitination-proteasome components (Fink et al., 2007). Moreover, disruption of the pathway has a negative effect on dengue replication (Kanlaya et al., 2010). One apparent example of the association of dengue virus and the pathway is an NS5-STAT2 interaction leading to

ubiquitination and degradation of STAT2. This interrupts interferon signaling (Jones et al., 2005; Ashour et al., 2009; Mazzon et al., 2009). Recently, Khadka et al., detected protein interactions between ubiquitin-conjugating enzyme E2I (UBE2I) and NS2B, NS4B, and NS5 (Khadka et al., 2011). They also showed that the silencing of UBE2I disrupted viral replication. Nevertheless, the overall significance and function of the ubiquitin-proteasome pathway during dengue replication is not clear.

Lipid and cholesterol biosynthesis seem to have some roles during dengue replication. Disruption of cholesterol biosynthesis inhibits dengue replication in cell culture, probably, by reducing virion assembly (Rothwell et al., 2009; Martinez-Gutierrez et al., 2011; Poh et al., 2012). However, the mechanism for how dengue hijacks or regulates cholesterol biosynthesis is unclear. In mosquito cells, dengue virus was found to alter lipid homeostasis, which may be contributed to the membrane rearrangement observed in dengue-infected cells (Perera et al., 2012). The NS3-FASN interaction seems to be the way that the virus hijacks lipid biosynthesis (Heaton et al., 2010). Again, how the virus precisely modulates lipid biosynthesis and homeostasis is unsolved.

Autophagy is required for effective dengue replication, and the virus seems to induce autophagosome formation (Lee et al., 2008; Khakpoor et al., 2009; Panyasrivanit et al., 2009; Heaton et al., 2010). Autophagy in dengue-infected cells seems to stimulate lipid and energy biosynthesis enhancing virus replication (Heaton and Randall, 2010). NS4A can up-regulate autophagy (McLean et al., 2011) so it might be an interface between dengue virus and the autophagic machinery. Nevertheless, the mechanism that dengue uses to hijack autophagy is not known.

Dengue virus needs to evade the innate immune response to effectively replicate. Dengue virus induces interferon response in human (Nasirudeen et al., 2011). Dengue employs several strategies to evade the response, including the NS5-STAT2 interaction, which induces the degradation of STAT2 and interrupts interferon signaling (Jones et al., 2005; Ashour et al., 2009; Mazzon et al., 2009). NS4B interferes with phosphorylation of STAT1 also interrupting the interferon response (Munoz-Jordan et al., 2005). Among the three branches of innate immune response in the mosquito, the Toll pathway and the JAK-STAT pathway seem to play a major role and a minor role in responding against dengue infection, respectively, based on microarray and gene-silencing assays, while the Imd pathway is irrelevant (Xi et al., 2008). After an infection has taken hold, all three branches of the mosquito innate immune response are suppressed by an unknown mechanism (Sim and Dimopoulos, 2010).

1.5.2 Serotype-specific characteristics

There are four antigenically distinct serotypes of dengue virus (DENV-1, DENV-2, DENV-3 and DENV-4) (Westaway, 1997). It has been reported that a given serotype of dengue virus is associated with certain symptoms in humans. For example, DENV-1 is associated with increased vascular permeability, while DENV-2 is associated more with shock and internal hemorrhage (Balmaseda et al., 2006). A study in dengue-infected cells has shown that DENV-1 and DENV-2 modulate the UPR at different points (Umareddy et al., 2007). DENV-1 is more potent than DENV-2 at inducing the production of PPP1R15A, which plays a role in the negative feedback loop to dephosphorylate eIF2α and restart transcription activities turned off by the UPR-

mediated phosphorylation of eIF2α (Lee et al., 2009). On the other hand, DENV-2 induces more production XBP1, a transcription factor that regulates genes functioning in the stress response (Lee et al., 2003). The mechanisms underlying these serotype specific properties of dengue viruses are not well understood. Serotype-specific dengue-host PPI may play a role in the observed serotype-specific chrematistics.

1.5.3 Dengue-host interaction data are incomplete

Despite several large-scale dengue-host PPI screens (Colpitts et al., 2011b; Khadka et al., 2011; Le Breton et al., 2011) and literature-curated interaction data (see Table 1-1), the dengue-host interactome is still far from complete. For example, there is only one interaction, NS5 and human MATR3, found in both dengue-human Y2H screens (Khadka et al., 2011; Le Breton et al., 2011) showing that these screens were not saturated. Many PPI were detected in only one study and may be false positives. One method to resolve this is to use orthogonal experiments to validate PPI (Uetz et al., 2000; Ito et al., 2001; Deane et al., 2002; von Mering et al., 2002; Giot et al., 2003; Stanyon et al., 2004; Schwartz et al., 2009). Thus far, however, dengue-host PPI confirmed by two or more independent experiments are rare. Thus, additional PPI screens are required to identify missing PPI and to validate existing PPI.

1.5.4 Summary of questions that identification of new PPI could address

In Section 1.5.1, I discussed possible cellular pathways that connect to dengue virus. The virus has to hijack or disrupt the pathways to replicate in host cells. However, direct connections between these pathways and dengue virus have not been identified.

For example, dengue virus can control the UPR, but the proteins in the UPR pathways that interact with the virus are unknown. A PPI study may help reveal such proteins and identify targets for antiviral intervention.

In Section 1.2.2, I reviewed the functions of dengue proteins. Interestingly, C and NS5 can localize in the nucleus and nucleolus, which are not virus replication sites. Some roles of nuclear localized dengue proteins have started to be revealed. To better understand the roles of these dengue proteins in the nucleus, a PPI study may identify other nuclear and nucleolar host proteins and, therefore, hint at new functions of the dengue proteins.

In Section 1.5.2, I discussed serotype-specific characteristics of dengue virus. However, little is known of how each serotype differentially interacts with host cellular mechanisms. A PPI study may identify serotype-specific PPI that are responsible for serotype-specific characteristics.

1.6 Project outline

I hypothesize that viral-host interactions will provide clues about the functions of viral proteins, and potential targets for drug intervention. In this project, I used Y2H assays to generate dengue-host PPI data as described in Chapter 2. At the beginning of the project there were no large-scale physical PPI data for dengue and its hosts. I set out to construct PPI maps for dengue-mosquito and dengue-human interactions using yeast two-hybrid assays. I also constructed the first mosquito Y2H cDNA library. I recognized the potential inaccuracy of Y2H results, which may include PPI that do not occur during virus infection. I used co-affinity purifications and cross-serotype Y2H

screens to obtain additional evidence for each interaction. I also used computational analyses to identify conserved interactions, gene ontology annotation enrichment, domain enrichment and interactions found for other viruses.

In Chapter 3, I set up a functional study by focusing on the capsid-nucleosome assembly protein 1 (NAP1) interaction, which was identified in both the human and mosquito screens. I generated a human cell line, HepG2, expressing capsid with a myc-tag fusion at the N-terminus. Next, I silenced and over-expressed nucleosome assembly protein 1-like 1 (NAP1L1) in the cell line and found a change in capsid localization. I also mapped the NAP1-interacting domain of capsid using Y2H and co-affinity purification assays. I found that the C-terminus of capsid is necessary for efficient interaction with human NAP1L1 and mosquito AAEL005567.

In Chapter 4, I describe a tool for studying the significance of dengue-host PPI. I designed and constructed a non-infectious dengue replicon to enable monitoring of replication levels by observing a reporter gene in live cells. I also tested mosquito cells for RNA interference (RNAi), which was successful. Combined with the replicon, RNAi could be used to test the importance of individual PPI to virus replication in the mosquito cells. However, the replicon failed to work in either human or mosquito cells.

The data presented in this dissertation may be used to generate hypotheses for future studies. My preliminary study with the capsid-NAP1L1 serves as one example. I summarize all findings and discuss some interesting points arising from my project in Chapter 5. I also propose further studies to expand and utilize the results from my project.

CHAPTER 2

DENGUE-HOST PROTEIN INTERACTOMES

Part of the work described in this chapter has been submitted for publication (Mairiang et al., 2012).

2.1 Introduction

Currently, there are more than 3,000 complete genome sequences available for several organisms and strains (Pagani et al., 2012). The genomic data are a useful tool to identify novel genes from gene and protein sequence structure and to predict gene function from sequence homology. However, sequence-based methods have failed to predict the functions of as many as 50% of the open-reading frames of any given genome (Skolnick and Brylinski, 2009). One method that may help identify or predict the function of a protein-coding gene is to identify interactions between its product and other proteins. Finding an interaction partner that has a known function or that participates in a known pathway can transitively link a poorly studied protein with that function or pathway (Pandey and Mann, 2000). One example of using protein-protein interaction data as a hypothesis generator was a study by Welzel et al., showing that ataxin-1, which plays a role in causing spinocerebellar ataxia type 1, interacts with and regulates the activity of FOX-2, which in turn regulates the splicing of ataxin-2 (Welzel et al., 2012). The authors used a published protein interaction network based on proteins involved in human inherited ataxias and disorders of Purkinje cell degeneration to help generate their hypothesis (Lim et al., 2006). Another example comes from two studies

showing that Drosophila Cyclin Y and a putative cyclin-dependent kinase, Eip63E, are a Cyclin/CDK pair that regulates the Wnt signaling pathway (Davidson et al., 2009; Liu and Finley, 2010). These studies were based on the interaction between Cyclin Y and Eip63E that was previously identified by large-scale PPI screens (Stanyon et al., 2004). Thus, PPI data are valuable tools that complement genomic data (Ito et al., 2001). Construction of large PPI networks, however, requires extensive resources and labor. To date, there are only a few large PPI networks available and they are still far from complete (Schwartz et al., 2009; Venkatesan et al., 2009).

Viruses have limited genomes and are obligated to infect host cells and hijack cellular mechanisms in order to replicate. To achieve this, viruses also need to evade or suppress host antiviral responses. An understanding of how viruses interact with host cellular machineries to survive and replicate is important for the development of methods to better combat pathogenic viruses. One way that viruses interact with their hosts is by PPI. Therefore, indentifying PPI between virus and host proteins may hint at the function of virus proteins. The importance of PPI has been recognized in recent studies aimed at identifying the host-virus PPI for several viruses, such as human immunodeficiency virus-1 (HIV-1) (Ptak et al., 2008; Fu et al., 2009; Pinney et al., 2009), hepatitis C virus (HCV) (de Chassey et al., 2008), Epstein-Barr virus (EBV) (Calderwood et al., 2007), influenza virus (Shapira et al., 2009) and vaccinia virus (Zhang et al., 2009). These studies are beginning to be useful since they hint at certain interactions worth further investigation. For example, Hagemeier et al., chose to study the interaction between EBV Na protein and tumor necrosis factor receptor-associated factor 2 (TRAF2) based on a EBV-human PPI network (Hagemeier et al., 2011). They

found that the Na-TRAF2 interaction was required to induce Jun N-terminal protein kinase (JNK) activation of lytic gene expression. In another example, Engeland et al., further investigated the interaction between HIV-1 Gag and the human protein Lyric, identified in a large-scale HIV-1 PPI screen (Engeland et al., 2011). Their result hinted at a role for the interaction in regulating infectivity since disrupting the interaction by mutating the GAG-binding domain of Lyric resulted in a reduction of infectivity.

The genome of dengue virus encodes only ten proteins: capsid, membrane protein, envelope protein, and non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5 (Lindenbach et al., 2006). The functions of some dengue proteins are not fully understood as described in Chapter 1. I reasoned that a dengue-host interactome may provide clues about dengue protein functions. At the beginning of this dissertation project, no large-scale PPI screens had been done for dengue virus. I proposed to construct dengue-host PPI using Y2H screens. While this study was in progress, Khadka et al. and Le Breton et al. published results from dengue-human PPI screens (Khadka et al., 2011; Le Breton et al., 2011). However, the PPI identified by these groups seem to be incomplete as there is very little overlap between the two datasets. It is known that large-scale PPI screens can generate false positives and false negatives (Schwartz et al., 2009). My dengue-human PPI data may help expand and verify the current PPI data.

Dengue virus requires both human and *Aedes* mosquitoes to complete its life cycle (Gubler, 1998). Therefore, it is also important to understand how the virus interacts with cellular machineries in mosquito cells. A better understanding of dengue-mosquito interactions, for example, may help improve vector control strategies to

combat the virus. The only attempt to construct a dengue-mosquito interactome with physical protein interactions was done by Colpitts et al., using tandem affinity purification with mass spectrometry (Colpitts et al., 2011b). They used a limited set of dengue baits and identified small number of PPIs. To date, no large-scale screen for binary dengue-mosquito PPI have been reported.

In this chapter, I started by subcloning proteins from dengue virus serotype 2 into Y2H plasmid vectors, and then set up a matrix Y2H screen to test intraviral PPI. Next, I reared mosquitoes, collected RNA, and synthesized the first mosquito cDNA library for yeast two-hybrid screening. I screened the mosquito cDNA library and a human peripheral blood leukocyte (PBL) cDNA library with dengue baits. The potential interactors were validated by reproducibility and specificity tests. Since Y2H may confer false positive results having no biological relevance during an actual dengue infection, I rescreened the dengue interactors against dengue proteins from serotype 1, 3 and 4. My rationale for these cross-serotype screens is based on the assumption that each dengue protein has the same major function in all four serotypes and, therefore, should interact with a similar set of host proteins. I also performed a co-affinity purification as an orthogonal assay because an interaction detected by two or more independent methods is more likely to be biologically relevance (Uetz et al., 2000; Ito et al., 2001; Deane et al., 2002; von Mering et al., 2002; Giot et al., 2003; Stanyon et al., 2004; Schwartz et al., 2009). In conclusion, I generated a list of potential dengue interactors, which may be used to select candidate genes for further functional studies.

2.2 Materials and methods

2.2.1 Dengue cDNA and subcloning of dengue Genes

cDNA of dengue virus serotype 1 (Hawaii), 2 (16681), 3(H87) and 4 (H241) were obtained from Dr. Prapat Suriyaphol (Siriraj hospital, Mahidol University, Bangkok, Thailand). Each dengue gene was PCR amplified using primers described in Appendix C, and attB sequences were added to the 5' and 3' ends of the dengue gene with a second PCR amplification using primers DM1 (5'- GGG GAC AAG TTT GTA CAA AAA AGC AGG CT -3') and DM2 (5'- GGG GAC CAC TTT GTA CAA GAA AGC TGG GT -3'). Each PCR reaction was performed using Herculase polymerase (Agilent Technologies: 600310) as per vendor instructions. PCR products were analyzed by 1% agarose gel electrophoreses in 1X TBE (90 mM Tris , 90 mM Boric acid , 2 mM EDTA) at 100V for 30 minutes. A DNA extraction was performed for any PCR product containing non-specific DNA bands using QIAquick Gel Extraction Kit (Qiagen: 28704). For any PCR reaction failing to generate a sufficient product, a new pair of long primers containing attB sequences and gene-specific sequences was used to repeat the reaction (see Appendix C). PCR products were subcloned into a plasmid vector, pDONR221 (Life Technologies: 12536017), by site-specific recombination reactions using BP clonase II (Life Technologies: 11789020), according to the manufacturer's protocol. The plasmids containing dengue genes were used to transform *E. coli* strain OmniMax2 (Life Technologies: C854003) and transformants were selected on LB-kanamycin (100 µg/ml) media. The plasmids were then sequenced (see Appendix D). The dengue gene in pDONR221 was transferred to Gateway destination vectors by LR clonase reaction (Life Technologies: 11791019).

2.2.2 *E.coli* strains, yeast strains and plasmid vectors

OmniMax2 (Life Technologies: C854003) was the main *E. coli* strain used for general transformation and plasmid storing. The genotype of OmniMax 2 is *F′ proAB+ lacIq lacZΔM15 Tn10(TetR) Δ(ccdAB) mcrA Δ(mrr-hsdRMS-mcrBC) φ80(lacZ)ΔM15 Δ(lacZYA-argF) U169 endA1 recA1 supE44 thi-1 gyrA96 relA1 tonA panD*. DH10B (Life Technologies: 18290015) was the *E. coli* strain used for electroporation. The genotype of DH10B is *F⁻ endA1 recA1 galE15 galK16 nupG rpsL ΔlacX74 Φ80lacZΔM15 araD139 Δ(ara,leu)7697 mcrA Δ(mrr-hsdRMS-mcrBC) λ⁻*. KC8 (Struhl et al., 1987-1997) was the *E. coli* strain used for homologous recombination cloning, gap repair, and plasmid rescue from a yeast lysate by auxotrophic selection. The genotype of KC8 is *pyrF::Tn5 hsdR leuB600 trpC9830 lacΔ74 strA galK hisB436.*

RFY231 (Kolonin et al., 2000) is a yeast strain used for the AD Y2H plasmid. The genotype is *MATα trp1::hisG his3 ura3–1 leu2::3Lexop-LEU2*. RFY309 was a yeast strain used for the BD Y2H plasmid. RFY309 is derived from RFY206 (*MATa trp1Δ::hisG his3Δ200 leu2-3 lys2Δ201 ura3-52 mal-*) that contains the *lacZ* reporter plasmid, pSH18-34(URA3+) (Finley and Brent, 1994).

pDORN221 contains a kanamycin resistance gene and a Gateway cassette as described in the manufacturer's manual (Life Technologies: 12536017). pDONR221 was used for BP clonase reactions, which generated an "entry clone" for storing a DNA inserts that may be transferred to other plasmid vectors by the LR clonase reaction. pDONR223, which is similar to pDORN221 but contains a spectinomycin resistance gene, was used for the human ORF library (Lamesch et al., 2007) was also used in this study. pNLex_attR (Stanyon et al., 2003) is a BD Y2H plasmid, which expresses N-

terminal LexA binding domain fusions. pJZ4_attR (Stanyon et al., 2003) is an AD Y2H plasmid, which expresses N-terminal activation domain fusions. pNLex_attR and pJZ4_attR contain a Gateway cassette compatible with the LR clonase reaction. pRF4-5o (Finley and Brent, 1994) is the AD Y2H plasmid used for mosquito cDNA library construction. pJG4-5 (Gyuris et al., 1993) is the AD Y2H plasmid used for the human PBL cDNA library synthesized by Origene Technologies. pJM-1 is an AD Y2H vector previously used for an aptamer library construction (Colas et al., 1996). pHZ12 and pHZ13 are plasmid vectors for expressing an N-terminal myc tag and a TAP tag, respectively. The protein expression of these vectors is driven by the Gal4-responsive upstream activating sequence (UAS). pHZ12attR and pHZ13attR are the Gateway versions of pHZ12 and pHZ13, respectively. pHZ12_attR and pHZ13_attR were constructed by inserting a Gateway destination vector cassette (Invitrogen) into the cloning sites of pHZ12 and pHZ13. Briefly, the Gateway cassette was PCR amplified from pJZ4_attR with primers, DM138 and DM139 (Appendix C) and then digested with *Xba*I and inserted into pHZ12 and pHZ13 digested with *Pme*I and *Xba*I. The ligations were used to transform *E. coli,* OmniMAXII (Invitrogen). Transformants with plasmids containing a Gateway cassette were selected on LB-Chloramphenicol/Ampicillin media. pMT-Gal4 (Klueg et al., 2002) is a plasmid containing a Gal4 gene driven by a Cu^{+2}-inducible metallothionine promoter, which then drives the protein expression of pHZ12 and pHZ13. All DNA plasmid extractions were performed with Qiagen Miniprep, Midiprep and Maxiprep (Qiagen: 27106, 12143 and 12163) depending on the desired amount. A PCR purification kit (Qiagen: 28104) was used to clean PCR products or restriction digestion reactions.

2.2.3 Mosquito rearing and RNA collections

A. aegypti embryos were a gift from Dr. Mark Brown (University of Georgia). Embryos were synchronously hatched into 200 ml de-ionized water by applying a vacuum (13 to 15 inHg) for 20-40 minutes. For the first 24 hours, larvae were fed with 1-2 ml of ground rat food. After that, about 200-250 larvae were transferred to a tray containing 700-800 ml de-ionized water and three pellets of dry cat food (Friskies Senior). About 120-144 hours after hatching, larvae started to turn into pupae so they were transferred into a mosquito cage. Adults started to emerge about a week after hatching. They were fed with 10% sucrose or blood from a mouse (a gift from Dr. Eduardo Palomino, Department of Biological Sciences, Wayne State University). All stages of mosquito development were maintained at 27°C, 70-90% relative humidity in a 8-hour dark/16-hour light cycle. The mosquito handling protocol is described in more detail elsewhere (Munstermann, 1997).

The mosquito tissues were collected from ten stages: 1) less than three-month-old embryos, 2) one-day-old larvae, 3) two-day-old larvae, 4) three-day-old larvae, 5) four-day-old larvae, 6) five-day-old larvae, 7) six-day-old larvae, 8) pupae, 9) adults and 10) adults collected three hours after a blood meal. In order to collect enough RNA, eggs from several layings were independently collected and pooled; the oldest eggs in the pool were aged less than three months. Larvae were collected every 24 hours for six days. Pupae were collected at 120 hours after egg hatching. Adults were collected 3 days after emerging from pupae. Tissues were quick frozen by ethanol dry ice before homogenizing in RNA isolation buffer (Qiagen, RNeasy Midi Kit: 75142) by a dounce homogenizer for 20 strokes. Embryos were treated with 1% bleach for 10-15 minutes to

soften their shells and immediately homogenized without quick freezing. The adults fed with a blood meal were collected three hours later so that the genes responding to blood ingestion were sufficiently expressed (Sodja et al., 2007). Total RNA from each tissue sample was isolated with the RNeasy Midi Kit (Qiagen: 75142). The RNA was treated with RNase-free DNase (Qiagen) at room temperature for 15 minutes. Next, poly-adenylated RNA (poly(A) RNA) was enriched from the total RNA with the Poly(A)Purist Kit (Ambion: AM1916). Each RNA sample was analyzed by gel electrophoresis with a 1.2% agarose formaldehyde gel in formaldehyde buffer (20 mM 3-[N-morpholino]propanesulfonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA, 250 mM formaldehyde, pH 7.0) at 100V for 60 minutes (Figure 2-1 and Table 2-1).

2.2.4 Mosquito cDNA library construction

Aliquots of 0.5 µg poly(A) RNA from each tissue sample were pooled and diluted in RNase-free water to a total volume of 20 µl, heated at 65°C for five minutes and treated at room temperature with 10 mM methylmercury hydroxide for one minute and then 100 mM β-mercaptoethanol for five minutes. The pooled RNA then was used for first-strand cDNA synthesis with AccuScript Reverse Transcriptase and an oligo(dT) linker-primer according to the manufacturer's protocol (Stratagene: 200401). A product from the first-strand synthesis was transferred to the second-strand synthesis reaction containing DNA polymerase I, RNase H and [α-^{32}P]dATP according to the manufacturer's protocol (Stratagene: 200401). The product was blunted with *Pfu* DNA polymerase, purified by phenol-chloroform extraction and precipitated in ethanol. *EcoR*I adapters (Figure 2-2) were added to the product by ligation. Next, the product was

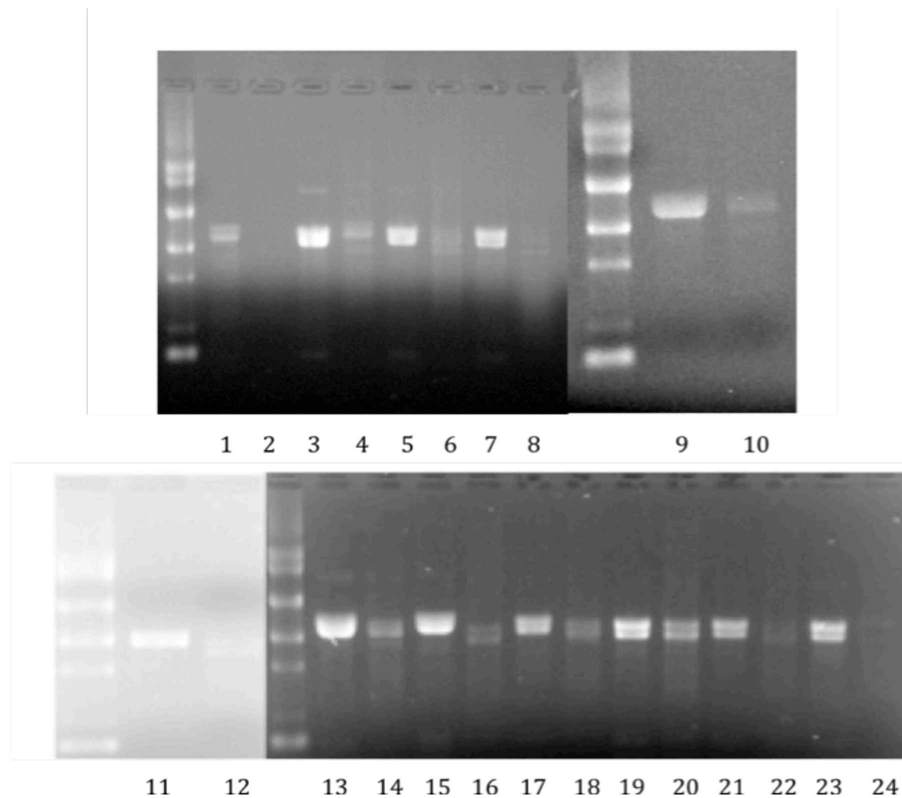


__Figure 2-1.__ **Gel electrophoreses showing the qualities of total RNA and poly-A RNA from mosquito tissues.** 1 μg of RNA sample was loaded each lane of 1.2% agarose formaldehyde gels. The electrophoreses were conducted at 100V for 60 minutes. Samples are: (1) 24 hr larvae total RNA set I, (2) 24 hr larvae poly(A) RNA set I, (3) 48 hr larvae total RNA, (4) 48 hr larvae poly(A) RNA, (5) embryos total RNA, (6) embryos poly(A) RNA, (7) adults total RNA, (8) adults poly(A) RNA, (9) 24 hr larvae total RNA set II, (10) 24 hr larvae poly(A) RNA set II, (11) 96 hr larvae total RNA, (12) 96 hr larvae poly(A) RNA, (13) 72 hr larvae total RNA, (14) 72 hr larvae poly(A) RNA, (15) pupae total RNA, (16) pupae poly(A) RNA, (17) 120 hr larvae total RNA, (18) 120 hr larvae poly(A) RNA, (19) 144 hr larvae total RNA, (20) 144 hr larvae poly(A) RNA , (21) adults total RNA, (22) adults poly(A) RNA, (23) adults 3 hr post-blood meal total RNA, (24) adults adults 3 hr post-blood meal poly(A) RNA.

Table 2-1 Amount of poly(A) enriched RNA from ten stages of *A. aegypti* mosquitoes.

Sample	Total amount (µg)	Concentration (ng/µl)
Embryos	7.2	48
24 hr Larvae	23.8	79.3
48 hr Larvae	10.2	68
72 hr Larvae	16.8	112
96 hr Larvae	13.23	44.1
120 hr Larvae	11.7	78
144 hr Larvae	15.3	102
Pupae	7.8	52
Adults	31.8	106
Adults 3 hr post-blood meal	11.7	78

Figure 2-2. The *EcoRI* adaptor used for adding *EcoRI* site to the 5' blunted end of double stranded cDNA. The hanging sequence lacked a phosphate group to prevent self-ligation. After the adaptors were ligated to cDNAs, the cDNAs were phosphorylated to convert the hanging sequence to a sticky end required for a ligation into a plasmid.

treated with polynucleotide kinase to phosporylate *EcoR*I ends. Finally, the product was digested with *Xho*I to create sticky ends derived from oligo(dT)linker primers.

The product was passed through Sepharose CL-2B gel filtration medium to size fractionate cDNA. Sample fractions were collected for every 100 µl eluted. Fractions were then analyzed by alkaline agarose gel elctrophoresis (Figure 2-3). Fractions 1 to 6 containing medium to large cDNA were combined into one sample, purified by phenol-chloroform extraction, precipitated by ethanol and resuspended in 50µl of RNase-free water. The cDNA protocol synthesis is described in more detail in the manufacturer's protocol (Stratagene: 200401). cDNA synthesis from adult RNA failed to generate sufficient cDNA (Figure 2-3A).

cDNA was ligated into the AD Y2H plasmid, pRF4-5o. The plasmid was first digested with *EcoR*I and *Xho*I, fractionated by a Centricon 100 (Millipore) and purified by phenol-chloroform extraction. The ligation was performed with 0.5 µl of cDNA, 6 ng of linearized pRF4-5o and 0.4 U of T4 ligase per 20 µl reaction according to the manufacturer's protocol (Roche: 10481220001). The ligation product was purified by phenol-chloroform extraction and precipitated by ethanol. The product was washed with 70% ethanol twice or more to thoroughly eliminate salt, which may interfere with electroporation. The DNA was then resuspended in 10 µl of sterile distilled water.

2.2.5 *E. coli* transformation, electroporation and yeast transformation.

For general *E. coli* transformations, a chemo-competent method was used as previously described (Walhout et al., 2000). Briefly, 500 ml *E. coli* culture in mid-log phase was washed and resuspended in 25 ml LB media (pH 6.1) containing 10 mM

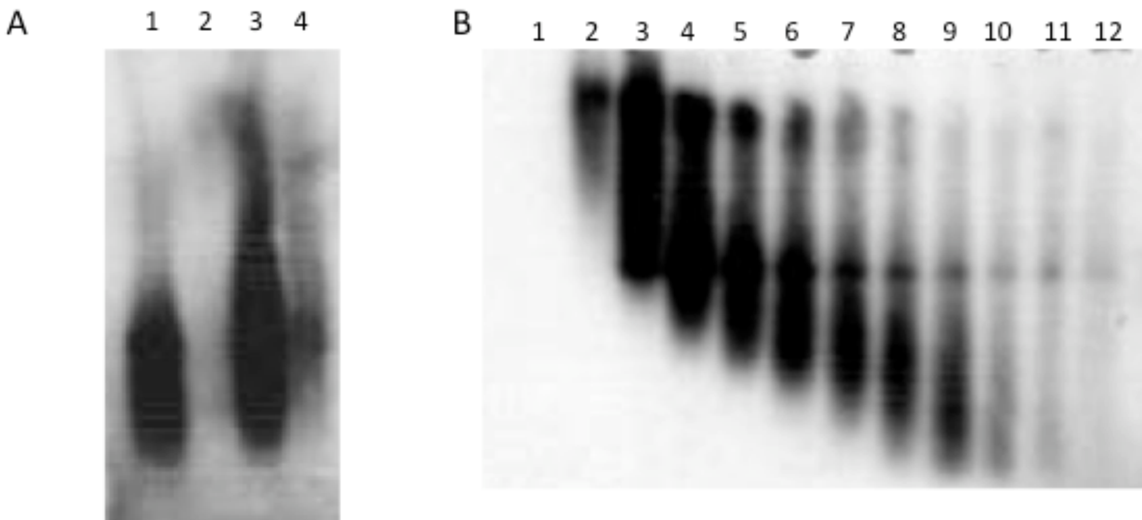


Figure 2-3. Autoradiographs showing cDNA from cDNA synthesis reactions. (A) The alkaline gel electrophoresis was conducted at 100 mA for 2 hours in 1% agarose gel. Next, the gel containing radioactive cDNA was used to expose an X-ray film for 90 minutes. (1) The first strand synthesis from adult mosquito RNA lacked large products as seen with (3) the first strand synthesis of pooled RNA. (2) The second strand synthesis from adult mosquito RNA produced a small quantity of products compared to (4) the second strand synthesis of pooled RNA. (B) The pooled RNA was fractionated through a Sepharose CL-2B column. 12 fractions of the pooled RNA were analyzed by 1% agarose gel electrophoresis at 100V for 60 minutes. Next, the gel containing radioactive cDNA was used to expose an X-ray film for 3 hours. Fractions 1 to 6 were combined to be used to construct a Y2H AD vector.

$MgCl_2$, 10 mM $MgSO_4$, 10% (w/v) PEG, 5% (v/v) DMSO and 10% (w/v) glycerol. The *E. coli* was aliquoted and frozen at -80°C for storage. To prepare DNA for transformation, <10 ng of a plasmid vector, 2 µl of ligation reaction, 2 µl of BP/LR clonase reaction or 100 ng of a linearized plasmid plus DNA insert with 1:3 molar ratio of plasmid to insert for gap-repair was resuspended in 100 µl of transformation buffer containing 100 nM KCl, 30 mM $CaCl_2$ and 50 mM $MgCl_2$. Next, 100 µl of chemo-competent *E. coli* was added to the DNA solution and incubated on ice for 20 minutes. The reaction was then incubated at room temperature for 10 minutes. Next, 1 ml of the SOC media (20 g/L Bacto Tryptone, 5 g/L Bacto Yeast Extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$ and 20 mM glucose) was added to the transformation reaction and incubated in a shaker-incubator at 37°C, 250 rpm for 1-1.5 hours. Finally, an appropriate amount of *E. coli* that would give isolated colonies was plated on selective medium and incubated at 37°C overnight.

For cDNA library construction or large DNA plasmid (>10 kb), eletro-competent *E. coli* was used for transformation by electroporation. Commercially prepared elctro-competent *E. coli*, *MegaX DH10B*™ T1R Electrocomp™ Cells (Life Technologies: C640003), were used for electroporation. The method is described in the manufacturer's protocol (Life Technologies: C640003). Briefly, 10 µl of cells were mixed with 2 µl of ligation reaction and loaded into an ice-cold 0.1-cm cuvette (Bio-Rad: 165-2083). The cuvette was then loaded into the Gene Pulser (Bio-Rad) and electroporated at 2.0 kV, 25 µF and 200 Ω. The cells were then resuspended in 1 ml SOC media and incubated in a shaker-incubator at 37°C, 250 rpm for 1-1.5 hours. Finally, an appropriate amount of *E. coli* was plated on selective medium and incubated at 37°C

overnight. For the mosquito cDNA library construction, *E. coli* transformants were counted from a 1:500 dilution for each electroporation (pooled from ~5 cuvettes before plating). The ligation and electroporation were repeated until >1 x 10^7 transformants were reached. All colonies were scraped into 2 L LB media. The plasmid containing cDNA library was isolated with Qiagen Gigaprep (Qiagen: 12191).

A yeast transformation protocol based on a previously described method was used (Gietz et al., 1992). Briefly, 50 ml culture of yeast in mid-log phase was washed and resuspended in 250 µl 100 mM lithium acetate in pH 7.5 Tris-EDTA (10 mM Tris, 1 mM EDTA) solution (LiOAc/TE). 30 µg of salmon sperm DNA and 10% (v/v) DMSO were added to a 50 µl aliquot of resuspended yeast. At this step, the yeast cells may be stored at -80°C for future use. Next, 1 µg or less of plasmid DNA and 300 µl of 40% PEG in LiOAc/TE solution was added to the transformation reaction, which was then mixed by gently inverting two to three times. The reaction was incubated at 30°C for 30 minutes and then 42°C for 15 minutes. Finally, the whole reaction was plated onto selective medium and grown at 30°C for more than three days until isolated colonies appeared. This transformation method was also applied for cloning by yeast homologous recombination (Orr-Weaver and Szostak, 1983), or yeast ‘gap-repair,’ by adding a 1:3 molar ratio of a linearlized vector to a DNA insert containing homologous sequences for recombination, with the maximal amount of total DNA being less than 1 µg. The size of the transformation reaction may be adjusted to comply with an experimental setup, such as 10 µl of competent yeast cells for a 96-well format batch transformation. For the mosquito cDNA and PBL cDNA libraries, 1.1 X 10^8 and 2.2 X 10^8 colonies of transformant yeasts were scraped into a freezing solution containing 50

mM $MgSO_4$, 5 mM Tris (pH 7.5) and 30% glycerol and stored in 1 ml aliquots at -80°C until use. The plating efficiency of frozen stocks of mosquito cDNA and PBL cDNA libraries was 0.35 x 10^8 CFU/100 μl and 2.41 x 10^8 CFU/100 μl, respectively.

2.2.6 Yeast two-hybrid screens

The Y2H assay was based on a previously described method (Kolonin et al., 2000; Golemis et al., 2001). To set up a mating, RFY309 containing a BD bait vector was cultured in 30 ml minimal media containing 2% glucose without uracil and histidine (Glu/CM-Ura, -His) until OD_{600} reached 1.0-2.0, which corresponds to 2-4 x 10^7 cells/ml. The yeast cells were washed and resuspended in 1 ml sterile water to a concentration of about 1 x 10^9 cells/ml. A frozen aliquot of RFY231 transformed with the AD library was thawed, and >1 x 10^8 CFU of RFY231 was mixed with at least two-fold more CFU of RFY309 cells (e.g. 2 x 10^8 RFY309 cells per 1 x 10^8 RFY231 CFU). The mixture was then plated onto YPD medium and incubated at 30°C overnight. The resulting diploids were harvested by scraping and resuspended in 25 ml of Gal/Mal/Raff/CM-Ura, -His, -Trp media. The diploids were incubated in a shaker-incubator at 30°C, 200 rpm shaking for 4 hours. After incubation, the diploids were washed and resuspended in 5 ml of freezing solution (described in Section 2.2.5). A small aliquot of diploids were serially diluted and plated on Gal/Mal/Raff/CM-Ura, -His, -Trp media or Gal/Mal/Raff/CM-Ura, -His, -Trp, -Leu media to calculate total diploids/ml or leucine prototrophic diploids/ml, respectively. All AD plasmids containing dengue genes in RFY309 were also tested for auto-activation by mock Y2H library screen against an empty vector, pJZ4, in RFY231. The details of each Y2H screen are described in Table 2-2.

Table 2-2. The setup for Y2H screens with a number of yeast diploids that passed through each stage of the screen.

baited	cDNA Library	Total diploid (DFU)	Leucine+ Diploid	Colonies picked	Galactose dependent leucine+ colonies	Passed reproducibility and specificity tests	AluI unique
CA	mosquito	118200000	100	96	47	39	21
CV	mosquito	56300000	560	192	85	53	27
PrM	mosquito	49200000	120	96	6	3	3
M	mosquito	153000000	10	96	0	0	0
E	mosquito	58100000	150	96	0	0	0
Eiii	mosquito	75000000	400	96	0	0	0
NS1	mosquito	187000000	100	96	0	0	0
NS2A	mosquito	57900000	20	96	4	0	0
NS2B	mosquito	65000000	20	96	3	0	0
NS3	mosquito	63000000	2340	384	269	105	84
NS3D1-160	mosquito	41000000	3000	288	11	6	5
NS4A	mosquito	65200000	80	96	3	2	1
NS4B	mosquito	51200000	70	96	5	3	1
NS5	mosquito	196000000	24000	1248	611	480	436
CA	human PBL	119800000	350	96	38	27	18
CV	human PBL	230000000	800	192	46	19	18
PrM	human PBL	31400000	350	96	0	0	0
M	human PBL	99500000	70	96	0	0	0
E	human PBL	63500000	40	96	0	0	0
Eiii	human PBL	101000000	500	96	0	0	0
NS1	human PBL	78000000	0	0	0	0	0
NS2A	human PBL	79100000	60	96	0	0	0
NS2B	human PBL	49000000	20	96	1	0	0
NS3	human PBL	179000000	1700	288	85	53	43
NS3D1-160	human PBL	56000000	7700	480	35	11	6
NS4A	human PBL	36800000	170	96	2	0	0
NS4B	human PBL	34700000	90	96	0	0	0
NS5	human PBL	190000000	7500	480	243	146	112
NS2B	Aptamer	147000000	50	96	0	n/a	n/a
NS3	Aptamer	169000000	33000	480	339	n/a	n/a
NS3D1-160	Aptamer	96000000	15000	288	51	n/a	n/a

The diploids from each screen were plated onto Gal/Mal/Raff/CM-Ura, -His, -Trp, -Leu media and incubated at 30°C for more than three days. The number of colonies needed to cover the entire library was calculated (Table 2-2) and that number was picked and rearrayed onto 96-well “PIM plates” containing 150 µl Glu/CM-Ura, -His, -Trp media per well. The plates were incubated in the shaker-incubator at 30°C, 200 rpm shaking for more than two days. Yeast culture (3-5 µl) from each well of the PIM plate was spotted onto four indicator plates: Glu/CM-Ura, -His, -Trp, -Leu; Gal/Mal/Raff/CM-Ura, -His, -Trp, -Leu; Glu/CM-Ura, -His, -Trp X-Gal and Gal/Mal/Raff/CM-Ura, -His X-Gal. After incubating indicator plates for three days at 30°C, the phenotype of each diploid was scored based on the scoring standard (Figure 2-4). A reporter score (C_SUM) was calculated by this formula: [(score of growth on Gal/Mal/Raff/CM-Ura, -His, -Trp, -Leu) – (score of growth on Glu/CM-Ura, -His, -Trp, -Leu)] + [(score of LacZ activity on Gal/Mal/Raff/CM-Ura, -His X-Gal) – (score of LacZ activity on Glu/CM-Ura, -His X-Gal)]. Only galactose-dependent leucine prototrophic diploids were selected and rearrayed onto PIM plates containing 150 µl Glu/CM-Ura, -His, -Trp media. The plates were again incubated in the shaker-incubator at 30°C, 200 rpm shaking for more than two days until yeast started to precipitate at the bottom of each well..

To perform reproducibility and specificity tests, 3-5 µl of yeast culture from each well of the PIM plate was spotted on a Gal/Mal/Raff/CM-Ura, -His, -Trp, -Leu plate and incubated at 30°C for more than three days. The PIM plates were saved as stock at -80°C. Colony PCR was performed using yeast diploids on the plates as templates. The total volume of PCR reaction for each diploid was 30 µl, which contained 0.3 µl of 10 µM of BCO1 primer (5’ CCA GCC TCT TGC TGA GTG GAG ATG 3’), 0.3 µl of 10 µM

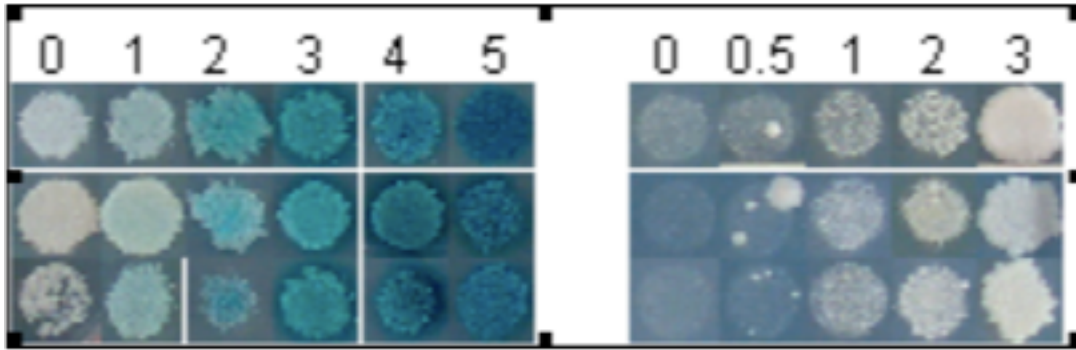
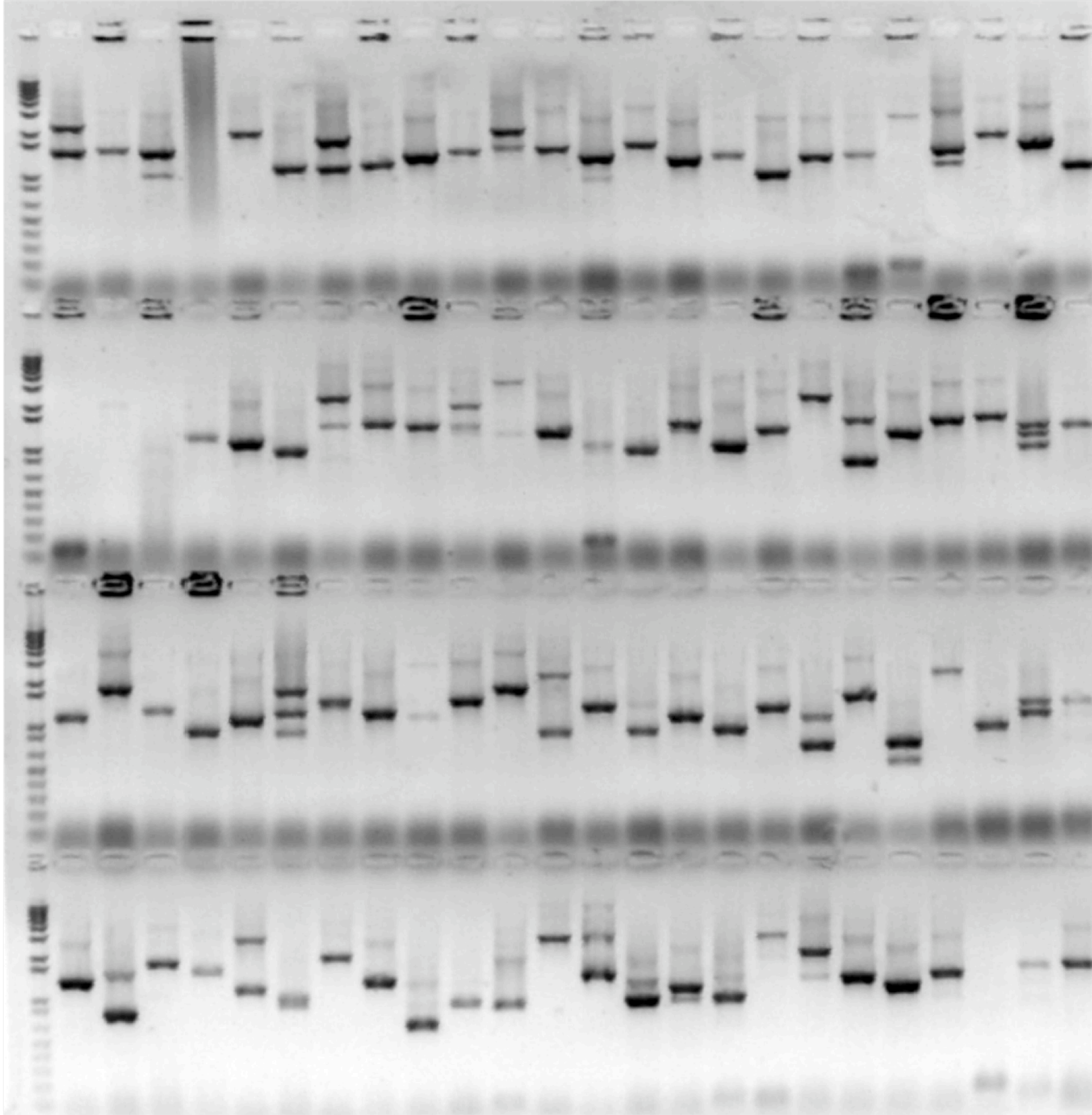


Figure 2-4. The standard for reporter activity scoring. The score for LacZ activity is between 0 for the weakest to 5 for the strongest. The score for leucine-protrophic growth is between 0 for no growth to 3 for the strongest growth.

of BCO2 primer (5’ GAC AAG CCG ACA ACC TTG ATT GGA 3’), 3 µl 10X PCR buffer, 3 µl of 2.5 mM dNTP Mix, 1.5 µl of 50 mM $MgCl_2$ and 0.3 µl of *Taq* polymerase (Invitrogen). Alternatively, 15 µl of 2X GoTAQ polymerase mix (Promega), 0.3 µl of 10µ M of BCO1 primer and 0.3 µl of 10 µM of BCO2 primer were also used for colony PCR yielding similar results. 10 µl of each colony PCR product was analyzed by 1% agarose gel electrophoresis in 1XTBE at 100V for 30 minutes (see Figure 2-5 for an example). 5 µl of each PCR product was added to 10 µl of competent yeast cells containing linearized pRF4-5o prepared as described in Section 2.2.4 and 2.2.5. The transformation was performed based on the description in Section 2.2.5. The yeast homologous recombination machinery automatically inserted cDNA into the linearized pRF4-5o, thereby generating a fresh AD yeast strain that was used for the reproducibility and specificity tests. Yeast transformants were plated onto Glu/CM-Trp media and incubated at 30°C for three days for selection. Transformant yeast colonies were picked and rearrayed onto PIM plates containing 150 µl Glu/CM-Trp, which were incubated at 30°C, 200 rpm shaking for three days. BD yeast strains containing dengue genes or specificity controls, *D. melanogaster* Cyclin J or Eip63E, were incubated in 10 ml of Glu/CM-Ura, -His at 30°C, 200 rpm shaking for three days. After incubations, 3-5 µl of each AD yeast culture was spotted onto YPD plates and allowed to dry. Next, 3-5 µl of BD yeast culture was spotted onto the same spot of AD yeast culture to set up a matrix mating. The YPD plates were incubated at 30°C overnight. Yeast diploids were then transferred onto four indicator plates by velvet cloth. A phenotype of each matrix-mated diploid was scored as described above (Figure 2-4). Any AD strain that generated a galactose-dependent leucine-prototroph diploid when mated with the same

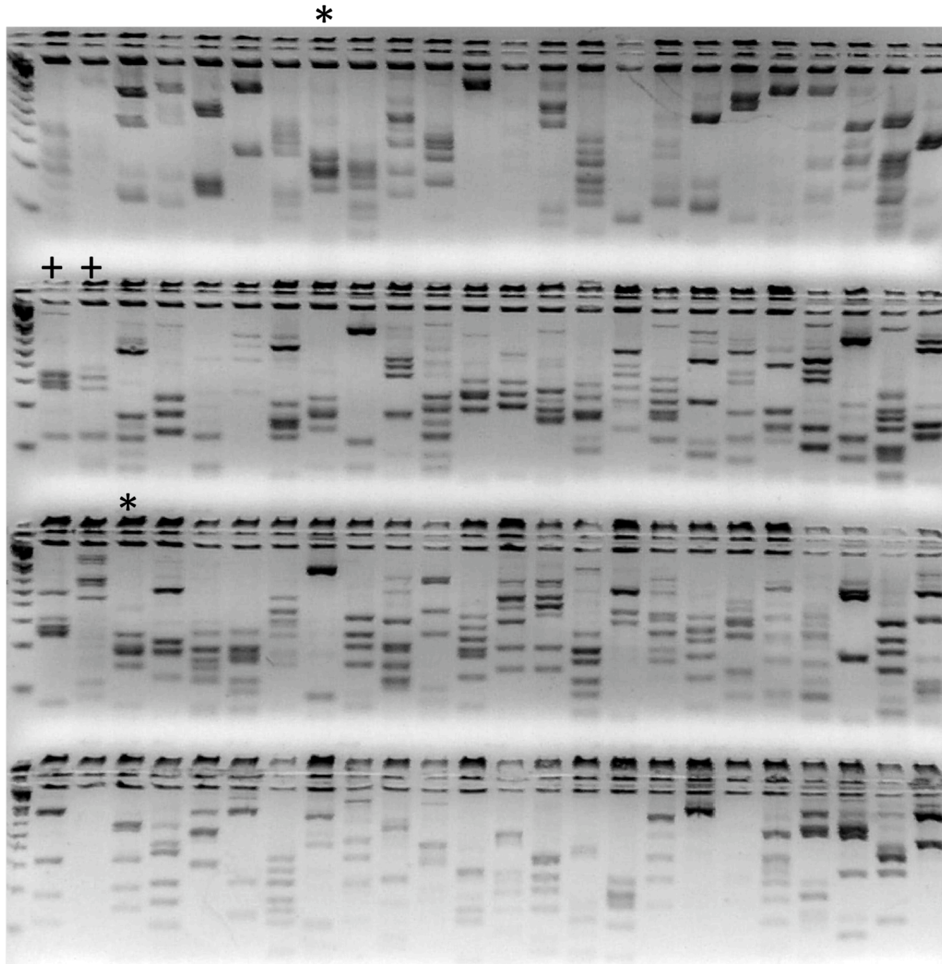


<u>Figure 2-5.</u> An example of a 96-well plate yeast colony PCR. 96 yeast colonies containing mosquito cDNA that encode putative dengue NS5 interactors were picked for colony PCR. 10 µl of the total 30 µl PCR products were loaded onto 1% agarose gel in 1XTBE. The gel electrophoresis was conducted at 100V for 30 minutes. The DNA markers were 300 ng of 1 kB Plus DNA ladder (Invitrogen).

dengue BD strain originally used to isolate it, was classified as a reproducible interactor. Any AD strain, which did not simultaneously generate galactose-dependent, leucine-prototroph diploids when mated with the Cyclin J and Eip63E BD strains, was classified as a specific interactor. Any AD strain that was a reproducible and specific interactor was rearrayed onto PIM plates containing 150 μl Glu/CM-Trp per well. 10 μl of colony PCR product of each reproducible and specific interactor was digested with *Alu*I in 20 μl reactions (2 μl 10X buffer 4 and 0.2 μl of 10,000 U/ml *Alu*I) at 37°C for 3 hours and analyzed by 2% agarose gel electrophoresis in 1X TBE at 100V for 40 minutes (see Figure 2-6 for an example). Clones with identical digestion patterns were grouped, and 5 μl of PCR product of a representative from each group was sequenced with BCO1 primer. The number of times that each cDNA was isolated with the same BD strain was calculated based on the number of clones with identical *Alu*I patterns plus identical DNA sequences (Table 2-2). Serotype specificity tests were performed using a matrix mating protocol as described above. All of yeast strains generated from the Y2H screens were aliquoted and kept as frozen yeast stocks. To make the frozen stock of the diploids 15% (v/v) glycerol was added to liquid media.

2.2.7 Computational analyses

DNA sequencing results were first filtered to eliminate failed reads. Standalone BLAST (Camacho et al., 2009) was used to analyze the sequencing results against the human Refseq database, *A. aegypti* transcript database, and plasmid sequences. The BLAST analysis and data parsing were performed using a python/biopython script,



<u>Figure 2-6.</u> An example of a 96-well plate *Alu*I digestion mapping. 96 PCR products of mosquito's dengue NS5 interactors were cut with AluI at 37°C for 3 hours. The whole reaction (20μl) was loaded onto a 2% agarose gel in 1XTBE. The gel electrophoresis was conducted at 100V for 40 minutes. The DNA markers were 300 ng of 1 kB Plus DNA ladder (Invitrogen). Clones with unambiguously identical *Alu*I patterns (e.g. * and +) were considered identical cDNAs.

blast_all.py (See Appendix E). The cutoff for BLAST results was <0.05 e-score.

For enrichment analysis of *Aedes* mosquito dengue interactors, a gene ontology annotation (GOA) file was downloaded from UniProt-GOA (ftp://ftp.ebi.ac.uk/pub/databases/GO/goa/proteomes/31436.A_aegypti.goa) (Barrell et al., 2009), and an OBO file version 1.2 was downloaded from The Gene Ontology project (http://www.geneontology.org/ontology/obo_format_1_2/gene_ontology_ext.obo) (Ashburner et al., 2000). A tree for InterPro domains was downloaded from EMBL-EBI (ftp://ftp.ebi.ac.uk/pub/databases/interpro/interpro.xml.gz) (Hunter et al., 2012). Cytoscape (Smoot et al., 2011) with the BINGO plug-in (Maere et al., 2005) was used to analyze GO annotation and IntePro domain enrichments of dengue interactors. Since the GOA file for *Aedes* mosquito and the tree for InterPro domains were not compatible with the BINGO plug-in, the customized files (AedesGO_for_bingo.txt and IPRtree_isa.txt) were generated with the python scripts, AedesGO_for_bingo.py and IPRtree.py, respectively (see Appendices F and G)

For homology analyses between mosquito proteins and human proteins, the following files were downloaded from Inparanoid database (http://inparanoid.sbc.su.se/) (Ostlund et al., 2009): InParanoid.D.melanogaster-H.sapiens.orthoXML, InParanoid.A.aegypti-D.melanogaster.orthoXML and InParanoid.A.aegypti-H.sapiens.orthoXML. These files were parsed into tables, Dro_to_hum_ID.txt , Aedes_to_Dro_ID.txt and Ae_to_hum_ID.txt, using the python scripts, inparanoid_dro_hum.py, inparanoid_Dro_Ae.py and inparanoid_ae_hum.py, respectively (see Appendices H, I and J). I found some genes that were not correctly clustered in the same homology group. 1008 human genes were predicted to be

orthologs of *D. melanogaster* genes, and 612 *A. aegypti* genes were predicted to be orthologs of the identical set of *D. melanogaster* genes. However, Inparanoid database failed to cluster these human genes and *A. aegypti* genes in the same homology group. Consequently, I used the python scripts, cross_fbgn.py, clusterInpara_dro.py, clusterInpara_droaedes.py and clusterInpara_droaedeshum.py, to re-cluster human, *D. melanogaster* and *A. aegypti* genes into an improved database (see Appendices K, L, M and N and the supplementary file 'New_Inparanoid_cluster_For_HumanAedes.xls'). The total number of human-*A. aegypti* homology groups were 8,007 clusters. They also included 499 clusters that were newly generated by my python scripts.

2.2.8 Drosophila cells and co-affinity purification

S2R+ cells are derived from *Drosophila melanogaster* embryos (Yanagawa et al., 1998). The cells were cultured in Schneider's media supplemented with 10% FBS and 100 μg/ml gentamicin at 25°C. The cells were passaged weekly by a 1:10 to 1:4 dilution. To dislodge the surface-attached cells, they were treated with 0.25% Trypsin-EDTA for about 5 minutes at room temperature.

The co-affinity purification protocol was based on a previously described method (Liu and Finley, 2010). Briefly, 1 x 10^6 S2R+ cells in 1 ml media were seeded into each well of a 12-well plate one day prior to DNA transfection. The DNA transfection was performed using Qiagen Effectene (Qiagen: 301425). 250 ng of pHZ12 with an insert, 250 ng of pHZ13 with an insert and 250 ng of pMT-Gal4 were diluted with Effectene EC buffer (Qiagen) to a total volume of 75 μl. Next, 6μl of Effectene enhancer (Qiagen) was added to the DNA mixture. The mixture was vortexed for 2 seconds and incubated at

room temperature for 5 minutes. Next, 15µl of Effectene was added to the mixture, which was vortexed for 10 seconds and incubated at room temperature for 10 minutes. The mixture was diluted in 400 µl of FBS-supplemented Schnieder's media, and then added drop-wise to the seeded cells. The next day, the media was replaced with complete Schneider's media supplemented with 1 mM $CuSO_4$. Three days after $CuSO_4$ induction, the cells were harvested by vigorous pipetting. The cells were washed twice with ice-cold 1X PBS and resuspended in 120 µl of NET lysis buffer (50 mM Tris-HCl pH 7.4, 180 mM NaCl, 5 mM EDTA, 1% NP-40 (v/v) and 10% Glycerol) supplemented with 1X protease inhibitor cocktail, 50 mM NaF, 1 mM Na_3VO_4 and 1 mM PMSF. The cells were passed through a 21½ G needle with 20 syringe strokes. The cell lysis reaction was incubated on ice for 45 minutes with 10 seconds of vortexing every 5 minutes. The lysate was centrifuged at 13,000 rpm for 30 minutes at 4°C. The supernatant was collected, and the protein content was quantified by the Bradford assay. Expression of fusion proteins was determined by Western blot analysis of cell lysates using anti-NTAP (Rockland Immunochemicals**)** and anti-Myc (Santa Cruz Biotechnology) antibodies for proteins expressed from pHZ13 and pHZ12, respectively.

If the expression of both proteins was successful, the cell lysate was used for co-affinity purification by incubating with 20 µl of pre-washed IgG agarose beads diluted in NET lysis buffer to a total volume of 500 µl. The incubation was done with a nutator at 4°C for 2 hours. The beads were spun down at 2,500 rpm for 5 minutes at 4°C. The supernatant was discarded, and the beads were washed with 400 µl of NET lysis buffer on the nutator for 5 minutes at room temperature. Next, the beads were spun down and washed repeatedly with NET lysis buffer at least five times. Finally, the beads were

resuspended in 60μl 1X LDS buffer (Invitrogen) containing 1X NuPAGE reducing agent (Invitrogen). The sample was heated to 70°C for 10 minutes before Western analysis. The co-purified proteins were detected by anti-Myc.

2.3 Results and discussion

2.3.1 A yeast two-hybrid cDNA library for *A. aegypti*

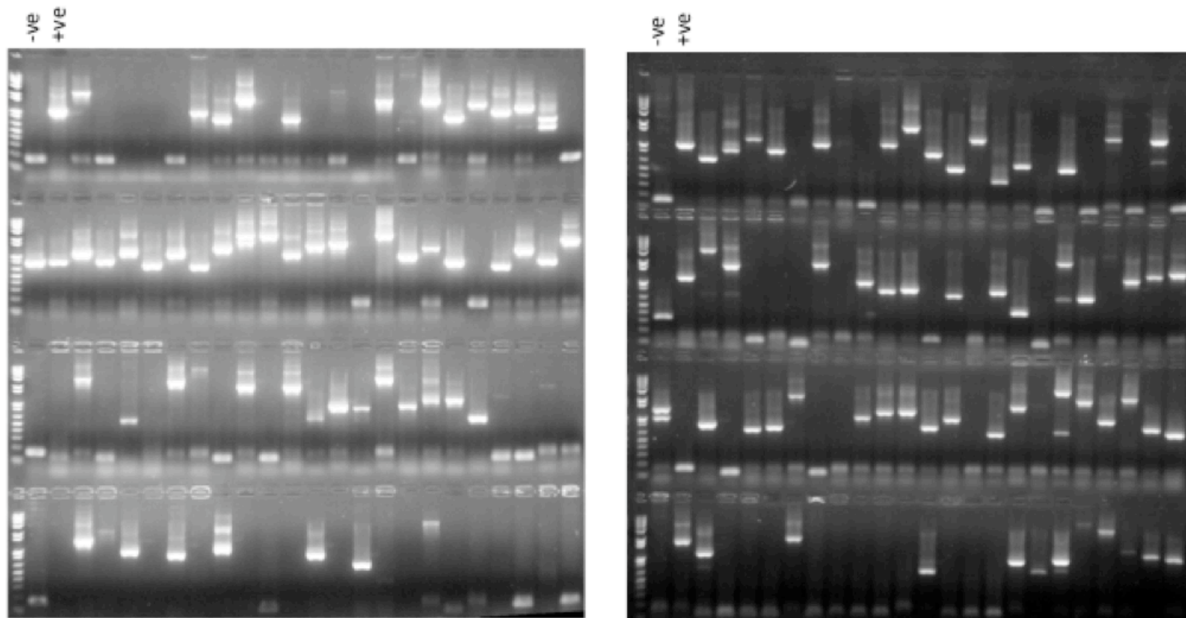
To date, most dengue-host PPI studies have focused on dengue-human PPI since various resources, such as Y2H cDNA libraries for many human tissues, are more available in comparison to the resources required for studying dengue-mosquito PPI. The dengue-mosquito PPI data are important due to the fact that the virus requires a mosquito host to complete its life cycle (Mackenzie et al., 2004). The only attempt to identify dengue-mosquito PPI on a large scale was done by tandem affinity purification-mass spectrometry (TAP-MS) assay, and provided limited results; 18 interactions involving four dengue proteins and 14 mosquito proteins (Colpitts et al., 2011b). In addition, the complexes detected by TAP-MS do not reveal binary PPI. A new tool like a mosquito Y2H cDNA library is required to identify binary dengue-mosquito PPI.

In this study, I have constructed the first Y2H cDNA library for *A. aegypti.* I collected and pooled RNA from ten stages: 1) less than three-month-old embryos, 2) one-day-old larvae, 3) two-day-old larvae, 4) three-day-old larvae, 5) four-day-old larvae, 6) five-day-old larvae, 7) six-day-old larvae, 8) pupae, 9) adults and 10) adults collected three hours after a blood meal. The pooled RNA was used to synthesize the Y2H cDNA, which was subcloned into the Y2H AD vector, pRF4-5o*.* To assess the quality of the library, I transformed *E. coli* with the library and randomly picked 188 colonies for colony PCR

(Figure 2-7). About 64% of the colonies had inserts. The sizes of the cDNA inserts were between 300 to 4,000 bp, with an average of about 1,400 bp. More than 1 x 10^7 *E. coli* colonies containing the cDNA library were harvested and plasmid DNA was extracted; 25 mg of library DNA was obtained. About 200 µg of the library DNA was then used to transform yeast resulting in 1.1 x 10^8 yeast colonies, which were scraped and resuspened in freezing solution (described in Section 2.2.5). The yeast was then aliquoted into 1 ml stocks and frozen. The plating efficiency of frozen stocks was 0.35 x 10^8 CFU/100 µl. This library in yeast is sufficient for more than 550 screens. The library DNA that I prepared would be sufficient for more than 65,000 screens. This library should be a valuable resource for studies on mosquito PPI and virus-mosquito PPI.

2.3.2 Intraviral protein-protein interaction

To identify interactions with dengue proteins, I subcloned open reading frames (ORFs) from dengue virus serotype 2 (strain 16681) into the Y2H bait vector for expression of the proteins with an N-terminal LexA DNA binding domain (DBD). I constructed a total of 14 baits (Figure 2-8B). These included baits for all ten full-length dengue proteins: nascent capsid protein (C), precursor of membrane protein (PrM), E, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. These ten proteins are individually cleaved from the viral polypeptide during maturation (Rice et al., 1985; Smith and Wright, 1985; Lindenbach et al., 2006). I also constructed baits for mature capsid protein (CV) (Lobigs, 1993), mature membrane protein (M) (Dejnirattisai et al., 2010), domain III of envelope protein (Eiii), and a fragment of NS3 lacking the N-terminal 160



<u>Figure 2-7.</u> Colony PCR of 188 randomly picked colonies from transformants derived from mosquito cDNA cloning by ligation. 20µl of the total 30µl PCR products were loaded onto 1% agarose gel in 1XTBE. . The gel electrophoresis was conducted at 100V for 30 minutes. The DNA markers were 300 ng of 1 kB Plus DNA ladder (Invitrogen). The negative control was pRF4-5o without an insert (-ve). The positive control was pRF4-5o with *D. melanogaster* cdi2 as an insert (+ve).

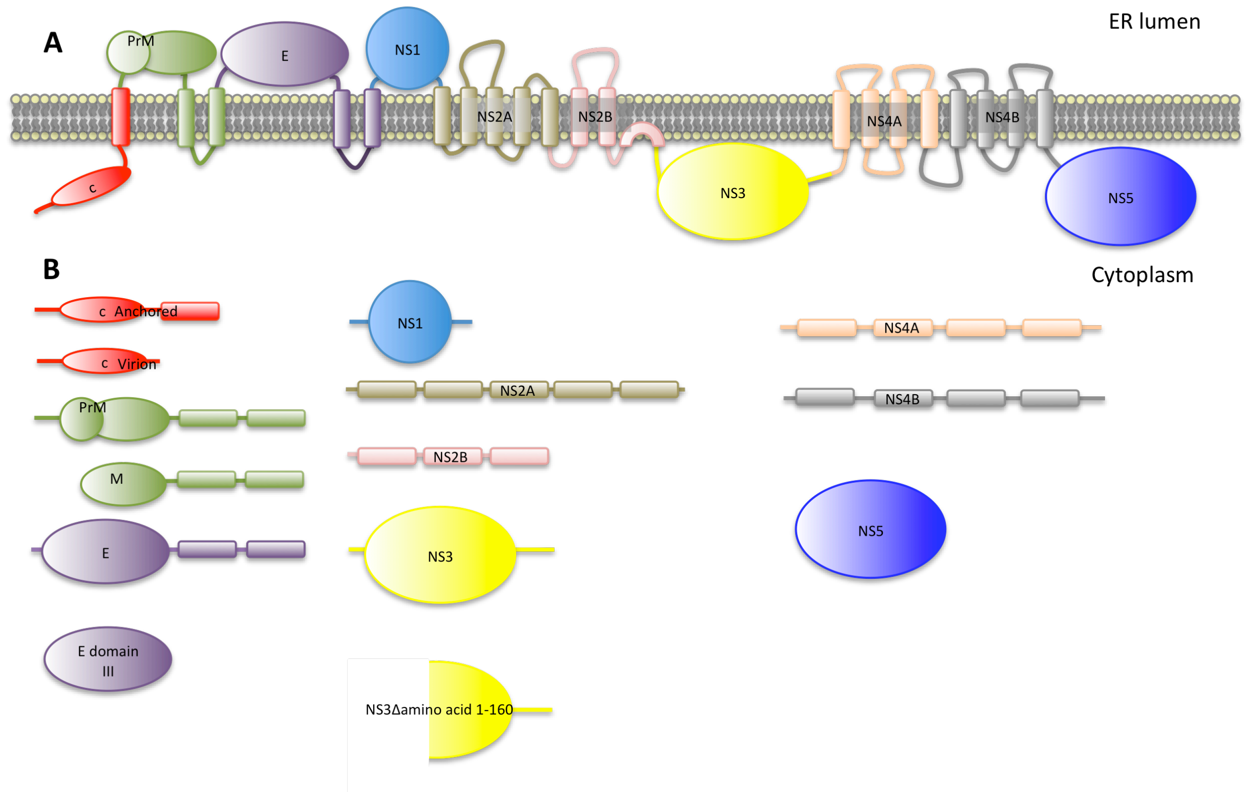
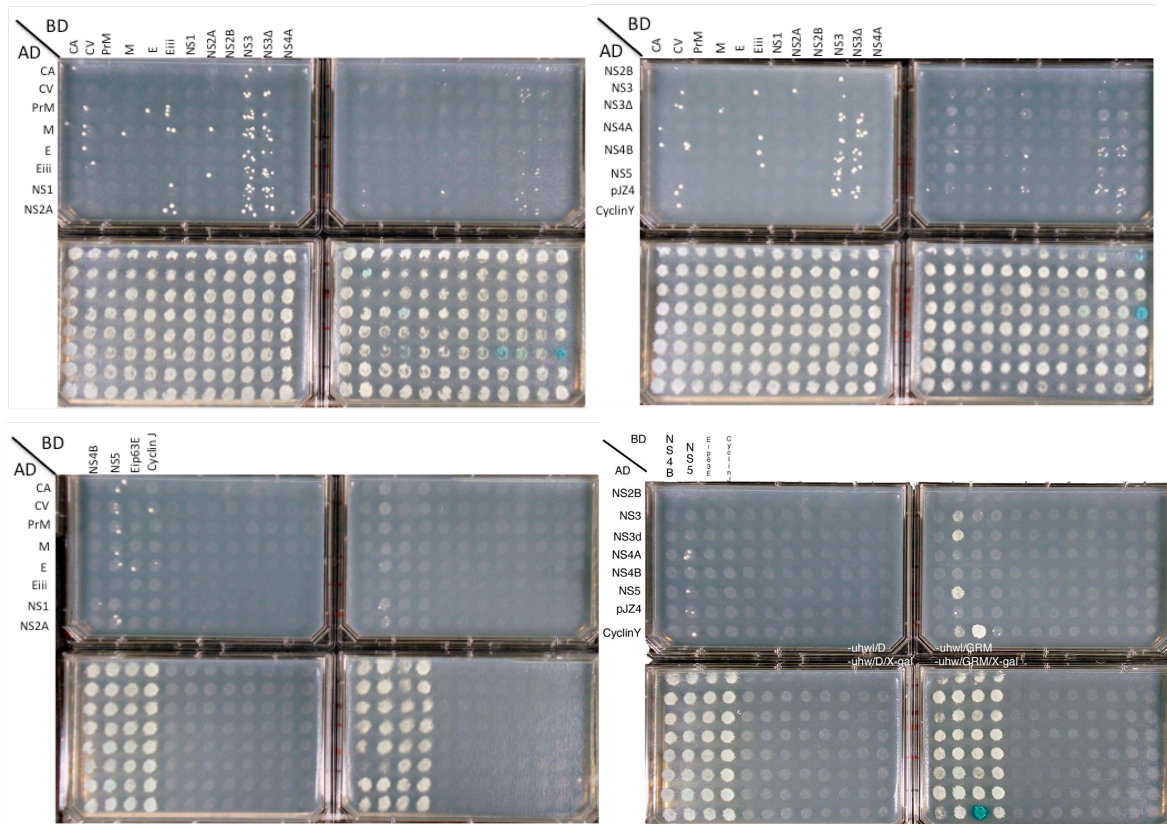


Figure 2-8. Dengue virus proteins. (A) The dengue polyprotein prior to processing is depicted in the ER membrane. (B) Fourteen dengue virus proteins and partial peptides as shown were cloned into Y2H plasmids.

amino acids (NS3Δ1-160) (Figure 2-8B). I analyzed the sequences of all dengue ORFs by BLAST analysis against the GenBank database and found that all ORFs had at least 98% amino acid identity to their archived sequences (see Appendix D). Since the dengue genome is known to contain some variations due to a high rate of mutation (Dunham and Holmes, 2007), the variations in the dengue ORFs were in an acceptable range. I subcloned the same 14 dengue ORFs into the Y2H activation domain (AD) vector. This enabled us to test for interactions among the 14 dengue proteins. I used a Y2H matrix mating assay to test all 14 DBD fusion proteins against all 14 AD fusion proteins (Materials and Methods). I detected interactions between NS5 and both NS3 and NS3Δ1-160 (Figure 2-9). The interaction between NS5 and the C-terminal region of NS3, which contains the helicase domain, was previously demonstrated by Y2H and co-immunoprecipitation assays (Johansson et al., 2001; Brooks et al., 2002) and NS3 was shown to be associated with cytoplasmic NS5 in dengue-infected cells (Kapoor et al., 1995). The complex of NS3 and NS5 may be essential for viral replication since NS3 contributes the helicase to unwind a viral dsRNA intermediate allowing NS5 to synthesize a new RNA molecule I also detected an interaction between the NS5 DBD and NS5 AD clones. The NS5 homodimer was also observed in another Y2H study (Vasudevan et al., 2001). No other novel interactions were detected. I failed to detect previously reported interactions between NS2B and NS3 (Arias et al., 1993) or between PrM and E, which were originally detected by co-purification assays and not by Y2H (Arias et al., 1993; Wang et al., 1999; Johansson et al., 2001). It was not possible to detect a reported interaction between NS2B/NS3 and NS4B/NS5 (Clum et al., 1997) because I did not co-express NS2B and NS3, or NS4B and NS5 in this screen.



<u>Figure 2-9. </u>Intraviral protein-protein interactions. Intraviral protein-protein interactions. Interactions were identified by the galactose-dependent growth of diploid yeasts expressing two dengue proteins. Each panel is a group of four indicator plates: Glucose CM –leucine –uracil –histidine -tryptophan (top-left), Galactose CM –leucine –uracil –histidine –tryptophan (top-right), Glucose CM –uracil –histidine –tryptophan +X-gal (bottom-left) and Galactose CM –uracil –histidine –tryptophan +X-gal (bottom-left). An interaction is indicated by galactose-dependent growth on the plates lacking leucine (top two plates in each panel) or galactose-dependent blue colony color on the X-Gal plates (bottom two plates in each panel). *D. melanogaster* Cyclin Y and Eip63E were used as a positive control while *D. melanogaster* Cyclin Y and Cyclin J were used as a negative control.

2.3.3 Dengue-mosquito protein-protein interactions

To identify mosquito proteins that interact with the dengue proteins, I constructed a Y2H AD library for *A. aegypti* using mRNA pooled from ten stages of development ranging from egg to adult (see Section 2.3.1 and Materials and Methods). I used a library mating assay to screen the mosquito library with each of 14 individual dengue bait proteins (Materials and Methods). To verify Y2H interactions, I subcloned the mosquito cDNAs from initial positives into new AD vectors and retested them for interaction with the original dengue bait proteins. At the same time, I tested for interactions with baits unrelated to the dengue baits to identify proteins that may nonspecifically interact with random proteins. In all, I identified 102 interactions that were reproducible and specific by this definition (Table 2-3). These interactions involved eight viral bait proteins representing C, NS3, NS5, or variants of these proteins, and PrM (Table 2-4). I did not find mosquito proteins interacting with the membrane proteins M, E, NS2A and NS2B, or with the luminal proteins Eiii and NS1. None of the mosquito–dengue interactions that I identified had previously been identified.

The 102 interactions involved 93 unique mosquito proteins, 58 of which have clear human orthologs. Two of the mosquito-dengue interactions that I detected had been previously detected for the human orthologs (Table 2-5). These included NS5 interactions with the mosquito E3 ubiquitin ligase Seven In Absentia (AAEL009614) and the human Seven In Absentia Homolog, SIAH2 (ENSG00000181788), which was previously detected by Le Breton et al. (Le Breton et al., 2011); and the interactions between NS5 and mosquito Paramyosin (AAEL010975) and human cingulin like-1

<u>Table 2-3.</u> Dengue – mosquito protein interactions. "-" in Expression Result for Co-AP means the host protein failed to express in the cell lysate, while "+" means both the host and the dengue protein were detected in the cell lysate. "-" in Co-AP result means the interaction was not detected by Co-AP while "+" means the interaction was detected. "NS" means a Myc-tagged protein was co-precipitated with an NTAP tag alone, which means an interaction was not assayable. "N/A" means no Co-AP was performed. See a supplementary file 'Table_2-3.txt' for a higher resolution.

VectorBaseID of transcript	Name of transcript	baitID	c_LEU	C_LACZ	Reporter Total (C_SUM)	Matrix Detections	Times isolated (ISTs_RFCs)	Reporter Total nonspecific bait 2 (DmEip63E)	Reporter Total nonspecific bait 1 (DmCycJ)	Expression result for Co-AP	Co-AP result	number_serotype	Dengue_1	Dengue_3	Dengue_4	Human ortholog(s)	Human interaction tested	Human interaction detected
AAEL005037	seryl-tRn/a synthetase	NS5	1.5	0	1.5	2	2	.5	0	+	-	2	Yes	No	No	ENSG00000031698	ENSG00000031698	
AAEL002565	titin	NS3	2.5	2	4.5	2	1	0	.5	+	-	4	Yes	Yes	Yes	ENSG00000042781		
AAEL011960	conserved hypothetical protein	CV	1.5	1	2.5	2	1	0	0	+	-	4	Yes	Yes	Yes	ENSG00000052749		
AAEL014012	membrane-associated guanylate kinase (maguk)	NS5	1.5	3	4.5	2	1	0	0	+	-	3	Yes	No	Yes	ENSG00000072415		
AAEL011708	heat shock protein	NS3	2.5	1	3.5	2	1	.5	0	+	+	4	Yes	Yes	Yes	ENSG00000080824;ENSG00000096384	ENSG00000096384; ENSG00000080824	ENSG00000096384
AAEL014843	heat shock protein	NS3	2.5	0	2.5	2	2	0	.3	+	+	4	Yes	Yes	Yes	ENSG00000080824;ENSG00000096384		
AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	NS3	2.5	0	2.5	2	1	.5	0	+	+	4	Yes	Yes	Yes	ENSG00000083720;ENSG00000198754	ENSG00000083720; ENSG00000198754	
AAEL005165	chaperone protein dnaj	NS3	0.5	0	0.5	2	6	.1	.5	+	-	3	Yes	Yes	No	ENSG00000086061;ENSG00000140403	ENSG00000086061; ENSG00000140403	
AAEL005165	chaperone protein dnaj	NS5	0.5	0	0.5	2	2	.1	.5	+	-	3	Yes	Yes	No	ENSG00000086061;ENSG00000140403	ENSG00000086061; ENSG00000140403	
AAEL010821	60S acidic ribosomal protein P0	NS3d	1.5	0	1.5	2	1	.7	0	+	NS	2	Yes	No	No	ENSG00000089157	ENSG00000089157	
AAEL010821	60S acidic ribosomal protein P0	NS5	1.5	0	1.5	2	6	.7	0	+	+	3	Yes	Yes	No	ENSG00000089157	ENSG00000089157	
AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	NS5	1.5	0	1.5	2	18	.3	1.1	+	-	3	Yes	Yes	No	ENSG00000092054;ENSG00000197616;ENSG00000109061;ENSG00000125414;ENSG00000133020;ENSG00000141048;ENSG00000109063;ENSG00000006788;ENSG00000078814;ENSG00000144821		
AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	NS5	2.5	0	2.5	2	1	0	0	+	-	3	Yes	Yes	No	ENSG00000092054;ENSG00000197616;ENSG00000109061;ENSG00000125414;ENSG00000133020;ENSG00000141048;ENSG00000109063;ENSG00000006788;ENSG00000078814;ENSG00000144821	ENSG00000078814	
AAEL012095	26S protease regulatory subunit	NS5	2.5	3	5.5	2	36	.3	.4	+	+	4	Yes	Yes	Yes	ENSG00000100764;LOC646791		
AAEL010360	nucleotide binding protein 2 (nbp 2)	NS5	1.5	1	2.5	2	1	.5	0	+	-	3	Yes	Yes	No	ENSG00000103274		
AAEL003676	myosin I homologue, putative	CV	0.5	2	2.5	2	2	0	0	-	N/A	4	Yes	Yes	Yes	ENSG00000104637		
AAEL004783	ornithine decarboxylase antizyme,	NS5	2.5	0	2.5	2	3	0	2	+	+	3	Yes	Yes	No	ENSG00000104904;ENSG00000180304		
AAEL003750	conserved hypothetical protein	CA	2	0	2	2	2	0	2.5	+	-	4	Yes	Yes	Yes	ENSG00000107833;ENSG00000158806	ENSG00000107833; ENSG00000158806	
AAEL014281	conserved hypothetical protein	NS5	1.5	0	1.5	2	4	0	0	+	+	3	Yes	No	Yes	ENSG00000109445		
AAEL003104	tripartite motif protein trim2,3	NS5	1.5	0	1.5	2	2	.5	0	+	+	3	Yes	Yes	No	ENSG00000110171;ENSG00000109654	ENSG00000109654	ENSG00000109654
AAEL003415	lamin	NS5	1.5	5	6.5	2	2	2	0	+	-	3	Yes	Yes	No	ENSG00000113368;ENSG00000176619;ENSG00000160789	ENSG00000113368; ENSG00000160789	
AAEL000951	elongation factor 1-beta2	NS5	2.5	0	2.5	2	1	.5	0	+	-	2	Yes	No	No	ENSG00000114942	ENSG00000114942	ENSG00000114942
AAEL011742	eukaryotic peptide chain release factor subunit	NS3	3	1	4	2	1	0	0	+	-	4	Yes	Yes	Yes	ENSG00000120705	ENSG00000120705	
AAEL009285	dead box atp-dependent rna helicase	CV	1.5	0	1.5	2	1	0	0	-	N/A	4	Yes	Yes	Yes	ENSG00000123064	ENSG00000123064	
AAEL013583	60S ribosomal protein L23	CV	1.5	2	3.5	2	2	0	0	+	-	4	Yes	Yes	Yes	ENSG00000125691	ENSG00000125691	
AAEL012237	bhlhzip transcription factor max/bigmax	NS5	2.5	0	2.5	2	1	0	1	+	-	4	Yes	Yes	Yes	ENSG00000125952	ENSG00000125952	

AAEL010975	paramyosin, long form	NS5	1.5	4	5.5	2	3	0	1.2	+	-	4	Yes	Yes	Yes	ENSG00000128849;ENSG00000143375		
AAEL006577	aspartyl-tRn/a synthetase	NS5	1.5	0	1.5	2	2	.5	0	+	-	3	Yes	Yes	No	ENSG00000134440	ENSG00000134440	
AAEL003064	conserved hypothetical protein	NS4B	1	3	4	2	3	0	0	-	N/A	2	Yes	No	No	ENSG00000134955;ENSG00000160190	ENSG00000134955	
AAEL000950	conserved hypothetical protein	NS3d	0.5	0	0.5	2	1	.5	0	+	NS	2	Yes	No	No	ENSG00000135521	ENSG00000135521	
AAEL000436	conserved hypothetical protein	NS5	2.5	2	4.5	2	12	0	.4	+	+	3	Yes	Yes	No	ENSG00000135632		
AAEL003739	M-type 9 protein, putative	NS5	1.5	0	1.5	2	9	0	2.9	+	-	4	Yes	Yes	Yes	ENSG00000135736		
AAEL009766	lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase	NS3	2.5	4	6.5	2	1	0	4.5	+	-	4	Yes	Yes	Yes	ENSG00000137992		
AAEL007201	glutamyl aminopeptidase	NS3	2.5	2	4.5	2	4	.3	0	+	-	4	Yes	Yes	Yes	ENSG00000138792		
AAEL010066	microfibril-associated protein	NS5	2.5	0	2.5	2	1	0	3	+	+	4	Yes	Yes	Yes	ENSG00000140259	ENSG00000140259	
AAEL003973	conserved hypothetical protein	NS5	1.5	1	2.5	2	2	.5	0	+	+/-	4	Yes	Yes	Yes	ENSG00000145088;ENSG00000144597	ENSG00000145088; ENSG00000144597	ENSG00000145088; ENSG00000144597
AAEL005790	malic enzyme	NS5	1.5	1	2.5	2	1	2	0	+	+	4	Yes	Yes	Yes	ENSG00000151376;ENSG00000065833		
AAEL010012	gtp-binding protein sar1	NS5	2.5	1	3.5	2	2	0	0	+	-	2	Yes	No	No	ENSG00000152700;ENSG00000079332	ENSG00000079332; ENSG00000152700	
AAEL005524	adenosylhomocysteinase	NS5	0.5	0	0.5	2	2	0	0	+	-	2	Yes	No	No	ENSG00000158467;ENSG00000168710	ENSG00000168710	
AAEL010782	carboxypeptidase	CV	0.5	0	0.5	2	1	0	0	+	-	4	Yes	Yes	Yes	ENSG00000158516;ENSG00000144410;ENSG00000128510;ENSG00000091704;ENSG00000158525;ENSG00000153002;ENSG00000080618;ENSG00000165078;ENSG00000163751	ENSG00000080618; ENSG00000153002; ENSG00000158516; ENSG00000091704; ENSG00000165078; ENSG00000158525; ENSG00000128510; ENSG00000163751	
AAEL000752	conserved hypothetical protein	NS5	1.5	0	1.5	2	2	.5	0	+	-	3	Yes	No	Yes	ENSG00000160058		
AAEL011985	conserved hypothetical protein	CV	0.5	3	3.5	2	3	0	0	+	-	4	Yes	Yes	Yes	ENSG00000160818	ENSG00000160818	
AAEL010784	conserved hypothetical protein	NS5	1.5	1	2.5	2	1	.5	0	+	-	3	Yes	Yes	No	ENSG00000163956		
AAEL010585	spermatogenesis associated factor	NS3	2.5	2	4.5	2	1	0	.5	+	+	4	Yes	Yes	Yes	ENSG00000165280		
AAEL002508	26S protease regulatory subunit 6a	NS3	2.5	0	2.5	2	2	1.5	.5	+	-	4	Yes	Yes	Yes	ENSG00000165916		
AAEL012827	endoplasmin	NS3	2.5	2	4.5	2	11	.1	1.3	+	+	4	Yes	Yes	Yes	ENSG00000166598	ENSG00000166598	
AAEL012827	endoplasmin	NS5	2.5	2	4.5	2	1	.1	1.3	+	-	3	Yes	Yes	No	ENSG00000166598	ENSG00000166598	
AAEL004500	eukaryotic translation elongation factor	NS5	0.5	0	0.5	2	2	0	0	+	+	3	Yes	Yes	No	ENSG00000167658		
AAEL014396	protein farnesyltransferase alpha subunit	NS3	2.5	1	3.5	2	1	.1	0	+	-	4	Yes	Yes	Yes	ENSG00000168522	ENSG00000168522	
AAEL014396	protein farnesyltransferase alpha subunit	NS5	2.5	1	3.5	2	7	.1	0	+	+	4	Yes	Yes	Yes	ENSG00000168522	ENSG00000168522	
AAEL003345	argininosuccinate lyase	NS3	2.5	2	4.5	2	1	.5	2	+	+	4	Yes	Yes	Yes	ENSG00000169910		
AAEL014104	conserved hypothetical protein	NS5	2.5	0	2.5	2	1	.5	0	+	-	3	Yes	No	Yes	ENSG00000172775		
AAEL009101	eukaryotic translation initiation factor 3f, eif3f	NS3	1.5	3	4.5	2	1	.5	0	+	+	4	Yes	Yes	Yes	ENSG00000175390	ENSG00000175390	
AAEL011988	tRNA selenocysteine associated protein (secp43)	NS5	1.5	0	1.5	2	4	.5	0	+	-	2	Yes	No	No	ENSG00000180098	ENSG00000180098	
AAEL009614	seven in absentia, putative	NS5	1.5	0	1.5	2	2	1	0	+	-	3	Yes	Yes	No	ENSG00000181788		
AAEL012348	splicing factor 3a	NS5	1.5	2	3.5	2	1	.5	0	+	-	4	Yes	Yes	Yes	ENSG00000183431	ENSG00000183431	
AAEL012686	ribosomal protein S12, putative	NS4A	0.5	0	0.5	2	1	0	.5	-	N/A	2	Yes	No	No	ENSG00000186468		
AAEL005567	nucleosome assembly protein	CA	2.5	0	2.5	2	3	0	.8	-	N/A	3	Yes	Yes	No	ENSG00000187109;ENSG00000205531		
AAEL005567	nucleosome assembly protein	NS3	2.5	0	2.5	2	6	0	.8	+	+	4	Yes	Yes	Yes	ENSG00000187109;ENSG00000205531		
AAEL013933	serine protease inhibitor, serpin	NS3	2.5	1	3.5	2	1	0	2	+	-	3	Yes	Yes	No	ENSG00000197249;ENSG00000165951;ENSG00000196136;ENSG00000100665;ENSG00000170099;ENSG00000188488;ENSG00000186910;ENSG00000165953;ENSG00000123561;ENSG00000170054;ENSG00000140093	ENSG00000188488; ENSG00000197249; ENSG00000100665; ENSG00000196136; ENSG00000165953; ENSG00000140093; ENSG00000123561; ENSG00000170099	
AAEL009357	myosin v	NS5	1.5	1	2.5	2	1	0	1	+	-	3	Yes	Yes	No	ENSG00000197535;ENSG00000167306;ENSG00000128833	ENSG00000128833	
AAEL008852	conserved hypothetical protein	CV	0.5	3	3.5	2	24	0	.9	+	-	4	Yes	Yes	Yes	ENSG00000198301		
AAEL008700	conserved hypothetical protein	NS5	1.5	2	3.5	2	5	0	.6	+	+	2	Yes	No	No	ENSG00000205571;ENSG00000172062	ENSG00000205571; ENSG00000172062	
AAEL000005	hypothetical protein	CV	1.5	0	1.5	2	1	0	1	+	-	4	Yes	Yes	Yes			
AAEL000136	conserved hypothetical protein	NS3	2.5	5	7.5	2	3	0	6.5	+	-	4	Yes	Yes	Yes			
AAEL000292	conserved hypothetical protein	CV	1.5	0	1.5	2	2	0	0	+	-	4	Yes	Yes	Yes			
AAEL001553	conserved hypothetical protein	NS3	2.5	4	6.5	2	4	0	8	+	+	4	Yes	Yes	Yes			
AAEL001892	conserved hypothetical protein	NS3	2.5	4	6.5	2	1	0	7	+	+	4	Yes	Yes	Yes			
AAEL001984	hypothetical protein	CV	1.5	3	4.5	2	7	0	6	-	N/A	4	Yes	Yes	Yes			

AAEL002057	conserved hypothetical protein	CV	0.5	0	0.5	2	1	0	1	+	-	4	Yes	Yes	Yes			
AAEL002145	gonadotropin inducible transcription factor	NS3	0.5	0	0.5	2	1	0	0	+	-	1	No	No	No			
AAEL002572	myosin regulatory light chain 2 (mlc-2)	NS3	2.5	0	2.5	2	18	.2	.2	+	-	2	Yes	No	No			
AAEL002828	hypothetical protein	NS5	0.5	2	2.5	2	2	0	0	+	-	3	Yes	Yes	No			
AAEL003815	zinc finger protein	NS3	2.5	2	4.5	2	1	0	.5	+	-	4	Yes	Yes	Yes			
AAEL003929	conserved hypothetical protein	NS5	1.5	2	3.5	2	2	0	.5	+	-	3	Yes	Yes	No			
AAEL004100	hypothetical protein	NS3	1.5	1	2.5	2	1	.3	0	+	-	4	Yes	Yes	Yes			
AAEL004100	hypothetical protein	NS5	1.5	1	2.5	2	1	.3	0	+	-	4	Yes	Yes	Yes			
AAEL004316	hypothetical protein	CV	0.5	1	1.5	2	1	0	.8	+	-	4	Yes	Yes	Yes			
AAEL004316	hypothetical protein	NS5	0.5	1	1.5	2	1	0	.8	+	-	4	Yes	Yes	Yes			
AAEL004484	predicted protein	NS3	2.5	0	2.5	2	4	.3	.3	+	+	3	Yes	No	Yes			
AAEL004869	hypothetical protein	CV	1.5	3	4.5	2	3	0	5	+	-	4	Yes	Yes	Yes			
AAEL006572	troponin C	NS5	1.5	1	2.5	2	1	.5	0	+	-	4	Yes	Yes	Yes			
AAEL007406	Conserved hypothetical protein	PrM	0.5	0	0.5		1	0	.5	-	N/A	2	Yes	No	No			
AAEL007850	hypothetical protein	NS3	2.5	0	2.5	2	2	.3	0	+	+	3	Yes	No	Yes			
AAEL007850	hypothetical protein	NS3d	2.5	0	2.5	2	2	.3	0	+	NS	1	No	No	No			
AAEL007980	hypothetical protein	NS5	2.5	1	3.5	2	2	0	0	+	-	3	Yes	Yes	No			
AAEL008052	hypothetical protein	NS3	2.5	5	7.5	2	2	0	2	+	-	4	Yes	Yes	Yes			
AAEL008746	hypothetical protein	NS3	2.5	5	7.5	2	3	.2	.2	+	-	4	Yes	Yes	Yes			
AAEL009182	zinc finger protein, putative	NS5	2.5	0	2.5	2	5	.3	1	+	-	3	Yes	Yes	No			
AAEL009460	conserved hypothetical protein	NS5	1.5	5	6.5	2	1	1	0	+	-	4	Yes	Yes	Yes			
AAEL009484	conserved hypothetical protein	NS5	0.5	0	0.5	2	3	0	4.5	+	-	4	Yes	Yes	Yes			
AAEL009948	aldehyde dehydrogenase	NS3	0.5	2	2.5	2	1	.3	0	+	-	4	Yes	Yes	Yes			
AAEL010005	conserved hypothetical protein	NS5	1.5	4	5.5	2	1	0	0	+	-	2	Yes	No	No			
AAEL010266	hypothetical protein	PrM	3	0	3	2	1	0	3	+	-	4	Yes	Yes	Yes			
AAEL010507	hypothetical protein	NS5	1.5	0	1.5	2	2	4	0	+	+	4	Yes	Yes	Yes			
AAEL011129	alcohol dehydrogenase	NS3	2.5	3	5.5	2	1	0	0	+	-	4	Yes	Yes	Yes			
AAEL012527	conserved hypothetical protein	NS5	1.5	1	2.5	2	3	0	3	+	-	3	Yes	Yes	No			
AAEL012556	Ofd1 protein, putative	NS3	2.5	0	2.5	2	3	0	5.5	+	-	4	Yes	Yes	Yes			
AAEL012680	Juvenile hormone-inducible protein, putative	NS5	1.5	1	2.5	2	1	0	0	+	-	4	Yes	Yes	Yes			
AAEL013075	conserved hypothetical protein	CA	1	0	1	2	2	0	0	+	+	4	Yes	Yes	Yes			
AAEL013086	hypothetical protein	NS5	2.5	0	2.5	2	4	0	0	+	-	3	Yes	Yes	No			
AAEL014845	heat shock protein	NS3	2.5	0	2.5	2	1	0	.5	+	+	4	Yes	Yes	Yes			

Table 2-4. **Number of host interactors for each dengue protein identified by Y2H screens.**

Dengue Protein	Mosquito	Human
C	16	20
PrM/M	1	0
NS3	34	15
NS4A	1	0
NS4B	1	0
NS5	49	11
E, NS1, NS2A, NS2B	0	0

Table 2-5. Mosquito proteins with human orthlogs that interact with proteins from other viruses.

Mosquito gene	Dengue Gene (this study)	Human Ortholog	Dengue interactions (from other sources)	HAdV interactions	HCV interactions	Herpes viruses interactions	HIV interactions	HPV interactions
AAEL002508	NS3	ENSG00000165916		HAdV-5_E1A			integrase; Vif; Tat	
AAEL002565	NS3	ENSG00000154358, ENSG00000042781			NS3			
AAEL003415	NS5	ENSG00000113368, ENSG00000176619, ENSG00000160789	NS3	HAdV-2_E1B	p7	HHV-1_UL31; HHV-1_ICP8; HHV-1_UL34	Vpr; Tat	
AAEL004500	NS5	ENSG00000167658					Vpr	
AAEL005567	C;NS3	ENSG00000187109, ENSG00000205531			NS3; NS5A	HHV-1_ICP8; EBV_EBNA1	Envelope surface glycoprotein gp120	HPV-8_E2; HPV-18_E2
AAEL005656	NS5	ENSG00000197616, ENSG00000092054, ENSG00000109061, ENSG00000125414, ENSG00000141048, ENSG00000133020, ENSG00000109063, ENSG00000006788, ENSG00000078814, ENSG00000144821					retropepsin	
AAEL005733	NS5	ENSG00000197616, ENSG00000092054, ENSG00000109061, ENSG00000125414, ENSG00000141048, ENSG00000133020, ENSG00000109063, ENSG00000006788, ENSG00000078814, ENSG00000144821					retropepsin	
AAEL008700	NS5	ENSG00000205571, ENSG00000172062						HPV-11_E2; HPV-16_E2; HPV-18_E2
AAEL011708	NS3	ENSG00000080824, ENSG00000096384	E		NS5A; Whole virus		Tat	
AAEL012095	NS5	ENSG00000100764					integrase; Vif; Tat	HPV-16_E7
AAEL012237	NS5	ENSG00000125952					Envelope surface glycoprotein gp120	
AAEL012686	NS4A	ENSG00000186468				EBV_EBNA-LP		
AAEL013583	C	ENSG00000125691				EBV_EBNA-LP		
AAEL013933	NS3	ENSG00000197249, ENSG00000165951, ENSG00000196136, ENSG00000100665, ENSG00000170099, ENSG00000188488, ENSG00000186910, ENSG00000165953, ENSG00000123561, ENSG00000170054, ENSG00000140093					Envelope surface glycoprotein gp160, precursor	
AAEL014843	NS3	ENSG00000080824, ENSG00000096384	E		NS5A; Whole virus		Tat	
AAEL010975	NS5	ENSG00000128849	NS5					
AAEL009614	NS5	ENSG00000181788	NS5					

(ENSG00000128849), previously detected by Khadka et al., (Khadka et al., 2011). None of the other mosquito-dengue interactions that I detected have potential human-dengue counterparts found in other studies. While some of these may be genuine species-specific dengue interactions, it is also likely that the lack of overlap with previous studies is largely due to differences in the techniques and libraries used. I used library screening and directed assays (described further below), to detect 9 additional human-dengue interactions that correspond to 8 of the mosquito-dengue interactions, indicating that at least some of the mosquito-dengue interactions may be conserved interologs (Table 2-3).

It has been reported that some human proteins interact with proteins from a range of different viruses, perhaps because these human proteins are common viral targets or part of common cellular responses to viral infections (Dyer et al., 2008; Khadka et al., 2011). I found that 15 of the mosquito proteins that I identified have human orthologs that interact with other viral proteins (Table 2-5). These include several interactions with Hepatitis C virus (HCV) proteins that could be considered conserved interactions or interologs. For example, I detected an interaction between dengue NS3 and mosquito titin (AAEL002565), an ortholog of human obscurin (OBSCN), which was shown to interact with HCV NS3 in a large-scale Y2H screen (de Chassey et al., 2008). Similarly, I detected an interaction between NS3 and mosquito nucleosome assembly protein (AAEL005567), an ortholog of human nucleosome assembly protein 1-like 1 (NAP1L1), which was shown to interact with HCV NS3 (de Chassey et al., 2008). The NS3 protein from both dengue and HCV contain serine protease and RNA helicase

domains and function similarly during the maturation of the viruses (Lindenbach et al., 2006).

In all, I identified 34 NS3-interacting mosquito proteins. To explore the NS3 domains that may interact with the host proteins, I tested all of them against both full-length NS3 and NS3Δ1-160 (Table 2-6). As expected, all of the host proteins interacted with full-length NS3, including the three proteins that were originally isolated with NS3Δ1-160. Interestingly, most host proteins also interacted with NS3Δ1-160, indicating that they interact with the C-terminal half of NS3, which contains the helicase domain. Five host proteins were incapable of interacting with NS3Δ1-160, suggesting that they require the N-terminal protease domain of NS3 for interaction (Table 2-6). The NS3-interacting proteins were enriched for proteins with the gene ontology annotation “response to stress” and for proteins with the domain “heat shock protein” (Table 2-7), primarily because they include several heat shock proteins. Human Hsp90 and Hsp70 were previously reported to be parts of the dengue virus receptor complex (Reyes-Del Valle et al., 2005), but no intracellular role for heat shock proteins during virus replication has been reported.

We identified 49 NS5-interacting mosquito proteins. The top enriched domains among these interactors were associated with myosin, found in myosin heavy chain, nonmuscle or smooth muscle (AAEL005656 and AAEL005733), myosin v (AAEL009357), long form paramyosin (AAEL010975) and a hypothetical protein (AAEL014104) (Table 2-7). Although there is no evidence linking myosin and NS5, myosin Vc was reported to be involved in the release of dengue virus from HepG2 cells (Xu et al., 2009). Colpitts et al., detected several myosin proteins by co-affinity

Table 2-6. NS3 domain analysis

Host	Host Gene	Dengue Gene	NS3 bait originally used	Interacts with NS3 or NS3Δ1-160	likely interface of interaction
Mosquito	AAEL000136	NS3	Full length	NS3	N-terminus
Mosquito	AAEL000950	NS3	NS3Δ1-160	both	C-terminus
Mosquito	AAEL001553	NS3	Full length	NS3	N-terminus
Mosquito	AAEL001892	NS3	Full length	both	C-terminus
Mosquito	AAEL002145	NS3	Full length	NS3	N-terminus
Mosquito	AAEL002508	NS3	Full length	both	C-terminus
Mosquito	AAEL002565	NS3	Full length	both	C-terminus
Mosquito	AAEL002572	NS3	Full length	both	C-terminus
Mosquito	AAEL003345	NS3	Full length	both	C-terminus
Mosquito	AAEL003815	NS3	Full length	both	C-terminus
Mosquito	AAEL004100	NS3	Full length	both	C-terminus
Mosquito	AAEL004484	NS3	Full length	both	C-terminus
Mosquito	AAEL005165	NS3	Full length	both	C-terminus
Mosquito	AAEL005567	NS3	Full length	both	C-terminus
Mosquito	AAEL007201	NS3	Full length	both	C-terminus
Mosquito	AAEL007850	NS3	NS3Δ1-160	both	C-terminus
Mosquito	AAEL008052	NS3	Full length	both	C-terminus
Mosquito	AAEL008746	NS3	Full length	both	C-terminus
Mosquito	AAEL009101	NS3	Full length	both	C-terminus
Mosquito	AAEL009766	NS3	Full length	NS3	N-terminus
Mosquito	AAEL009948	NS3	Full length	both	C-terminus
Mosquito	AAEL010585	NS3	Full length	both	C-terminus
Mosquito	AAEL010821	NS3	NS3Δ1-160	both	C-terminus
Mosquito	AAEL011129	NS3	Full length	both	C-terminus
Mosquito	AAEL011137	NS3	Full length	both	C-terminus
Mosquito	AAEL011708	NS3	Full length	both	C-terminus
Mosquito	AAEL011742	NS3	Full length	both	C-terminus
Mosquito	AAEL012556	NS3	Full length	NS3	N-terminus
Mosquito	AAEL012827	NS3	Full length	both	C-terminus
Mosquito	AAEL013933	NS3	Full length	both	C-terminus
Mosquito	AAEL014396	NS3	Full length	both	C-terminus
Mosquito	AAEL014843	NS3	Full length	both	C-terminus
Mosquito	AAEL014845	NS3	Full length	both	C-terminus
Human	ANP32B	NS3	Full length	both	C-terminus
Human	CALCOCO2	NS3	Full length	both	C-terminus
Human	CORO1A	NS3	Full length	both	C-terminus
Human	DNTTIP2	NS3	Full length	both	C-terminus
Human	GOLGB1	NS3	Full length	both	C-terminus

Human	HBB	NS3	Full length	both	C-terminus
Human	LRRFIP1	NS3	NS3Δ1-160	both	C-terminus
Human	MTF1	NS3	Full length	both	C-terminus
Human	NFKBIA	NS3	NS3Δ1-160	both	C-terminus
Human	NRBP1	NS3	Full length	NS3	N-terminus
Human	OS9	NS3	Full length	both	C-terminus
Human	RILPL2	NS3	Full length	NS3	N-terminus
Human	RPL24	NS3	Full length	NS3	N-terminus
Human	ZNF410	NS3	Full length	both	C-terminus

Table 2-7 Enrichment of GO annotations and protein domains in mosquito proteins that interact with dengue proteins.

Dengue protein	GO-ID	p-value	corr p-value	Description	Genes in test set
Capsid	GO:003676	1.86E-04	4.64E-03	nucleic acid binding	AAEL011985; AAEL003676; AAEL002057; AAEL001984; AAEL009285; AAEL004869; AAEL000292; AAEL003750; AAEL000005
	GO:005622	8.95E-04	1.12E-02	intracellular	AAEL005567; AAEL013583; AAEL011985; AAEL003676; AAEL002057; AAEL001984; AAEL009285; AAEL004869; AAEL000292; AAEL000005
NS3	GO:006950	1.12E-05	4.83E-04	response to stress	AAEL012827; AAEL005165; AAEL014843; AAEL002145; AAEL011708; AAEL014845
	GO:005737	2.93E-05	6.30E-04	cytoplasm	AAEL009101; AAEL002508; AAEL012827; AAEL010821; AAEL005165; AAEL011742; AAEL011137; AAEL014843; AAEL003345; AAEL011708
	GO:008152	1.97E-04	2.40E-03	metabolic process	AAEL009101; AAEL012827; AAEL014396; AAEL002565; AAEL014843; AAEL011708; AAEL014845; AAEL009766; AAEL002508; AAEL011129; AAEL010821; AAEL005165; AAEL011742; AAEL011137; AAEL003345; AAEL002145; AAEL007201; AAEL001553; AAEL009948
	GO:019538	2.24E-04	2.40E-03	protein metabolic process	AAEL009101; AAEL002508; AAEL012827; AAEL010821; AAEL014396; AAEL005165; AAEL002565; AAEL011742; AAEL014843; AAEL011708; AAEL007201; AAEL014845
	GO:005622	6.32E-04	5.44E-03	intracellular	AAEL009101; AAEL005567; AAEL012827; AAEL000136; AAEL003815; AAEL014843; AAEL011708; AAEL004484; AAEL002508; AAEL010821; AAEL005165; AAEL011742; AAEL011137; AAEL003345; AAEL002145; AAEL007850
	GO:044238	1.16E-03	8.35E-03	primary metabolic process	AAEL009101; AAEL012827; AAEL014396; AAEL002565; AAEL014843; AAEL011708; AAEL014845; AAEL002508; AAEL010821; AAEL005165; AAEL011742; AAEL003345; AAEL002145; AAEL007201; AAEL001553
NS5	GO:005622	3.06E-06	1.62E-04	intracellular	AAEL010975; AAEL013086; AAEL012827; AAEL007980; AAEL010784; AAEL003415; AAEL010012; AAEL009182; AAEL005037; AAEL000951; AAEL005656; AAEL008700; AAEL009614; AAEL012237; AAEL010821; AAEL009357; AAEL005165; AAEL003739; AAEL014281; AAEL012348; AAEL006577; AAEL005733; AAEL012095; AAEL010360
	GO:043226	1.92E-05	5.09E-04	organelle	AAEL010975; AAEL013086; AAEL007980; AAEL010784; AAEL003415; AAEL010012; AAEL009182; AAEL005656; AAEL008700; AAEL009614; AAEL012237; AAEL010821; AAEL009357; AAEL014281; AAEL003739; AAEL012348; AAEL005733
	GO:000166	4.55E-05	7.23E-04	nucleotide binding	AAEL012827; AAEL004500; AAEL010012; AAEL005037; AAEL005656; AAEL009357; AAEL005165; AAEL009460; AAEL006577; AAEL005790; AAEL011988; AAEL005733; AAEL012095; AAEL010360
	GO:003774	5.46E-05	7.23E-04	motor activity	AAEL010975; AAEL009357; AAEL005733; AAEL005656
	GO:005737	1.20E-04	1.25E-03	cytoplasm	AAEL008700; AAEL012827; AAEL010821; AAEL005165; AAEL010784; AAEL006577; AAEL010012; AAEL000951; AAEL005037; AAEL012095; AAEL010360

	GO:005856	1.41E-04	1.25E-03	cytoskeleton	AAEL010975; AAEL009357; AAEL003415; AAEL005733; AAEL005656
	GO:043234	5.09E-04	3.85E-03	protein complex	AAEL010975; AAEL009357; AAEL003415; AAEL000951; AAEL005733; AAEL005656; AAEL012095
	GO:005623	3.73E-03	2.47E-02	cell	AAEL010975; AAEL013086; AAEL012827; AAEL007980; AAEL010784; AAEL003415; AAEL010012; AAEL009182; AAEL005037; AAEL000951; AAEL005656; AAEL008700; AAEL009614; AAEL012237; AAEL010821; AAEL009357; AAEL005165; AAEL003739; AAEL014281; AAEL012348; AAEL006577; AAEL005733; AAEL012095; AAEL010360
	GO:005575	4.53E-03	2.67E-02	cellular_component	AAEL010975; AAEL012827; AAEL007980; AAEL010066; AAEL009182; AAEL000951; AAEL005037; AAEL005656; AAEL008700; AAEL012237; AAEL009614; AAEL009357; AAEL010821; AAEL005165; AAEL014281; AAEL003739; AAEL006577; AAEL005733; AAEL012095; AAEL010360; AAEL013086; AAEL010784; AAEL003415; AAEL010012; AAEL012348
	GO:005634	9.01E-03	4.78E-02	nucleus	AAEL008700; AAEL013086; AAEL012237; AAEL009614; AAEL007980; AAEL003739; AAEL014281; AAEL012348; AAEL009182

Dengue protein	Interpro ID	p-value	corr p-value	Description	Genes in test set
Capsid	IPR000467	1.67E-04	3.47E-03	D111/G-patch	AAEL011985; AAEL003676
	IPR007087	4.90E-04	3.47E-03	Zinc finger, C2H2	AAEL002057; AAEL001984; AAEL004869; AAEL000292; AAEL000005
	IPR015880	6.03E-04	3.47E-03	Zinc finger, C2H2-like	AAEL002057; AAEL001984; AAEL004869; AAEL000292; AAEL000005
	IPR004301	1.16E-03	3.47E-03	Nucleoplasmin	AAEL003750
	IPR007949	1.16E-03	3.47E-03	SDA1	AAEL008852
	IPR012541	1.16E-03	3.47E-03	DBP10CT	AAEL009285
	IPR012977	1.16E-03	3.47E-03	Uncharacterised domain NUC130/133, N-terminal	AAEL008852
	IPR012978	1.16E-03	3.47E-03	Uncharacterised domain NUC173	AAEL011960
	IPR016024	1.81E-03	4.83E-03	Armadillo-type fold	AAEL011960; AAEL005567; AAEL008852
	IPR012934	2.77E-03	6.65E-03	Zinc finger, AD-type	AAEL002057; AAEL001984; AAEL000292
	IPR000218	4.62E-03	1.01E-02	Ribosomal protein L14b/L23e	AAEL013583
	IPR002164	5.77E-03	1.15E-02	Nucleosome assembly protein (NAP)	AAEL005567
	IPR003146	1.38E-02	2.55E-02	Proteinase inhibitor, carboxypeptidase propeptide	AAEL010782
	IPR009020	2.63E-02	4.51E-02	Proteinase inhibitor, propeptide	AAEL010782
	IPR000834	3.30E-02	4.96E-02	Peptidase M14, carboxypeptidase A	AAEL010782

	IPR014014	3.30E-02	4.96E-02	RNA helicase, DEAD-box type, Q motif	AAEL009285
NS3	IPR020575	4.58E-10	3.67E-08	Heat shock protein Hsp90, N-terminal	AAEL012827; AAEL014843; AAEL011708; AAEL014845
	IPR001404	1.07E-09	4.27E-08	Heat shock protein Hsp90	AAEL012827; AAEL014843; AAEL011708; AAEL014845
	IPR003594	1.50E-08	3.99E-07	ATPase-like, ATP-binding domain	AAEL012827; AAEL014843; AAEL011708; AAEL014845
	IPR020568	1.05E-06	2.10E-05	Ribosomal protein S5 domain 2-type fold	AAEL012827; AAEL014843; AAEL011708; AAEL014845
	IPR005938	2.48E-03	1.98E-02	ATPase, AAA-type, CDC48	AAEL010585
	IPR007307	2.48E-03	1.98E-02	Low temperature viability protein	AAEL000950
	IPR009049	2.48E-03	1.98E-02	Argininosuccinate lyase	AAEL003345
	IPR012791	2.48E-03	1.98E-02	3-oxoacid CoA-transferase, subunit B	AAEL011137
	IPR012792	2.48E-03	1.98E-02	3-oxoacid CoA-transferase, subunit A	AAEL011137
	IPR014388	2.48E-03	1.98E-02	3-oxoacid CoA-transferase	AAEL011137
	IPR003959	4.81E-03	2.33E-02	ATPase, AAA-type, core	AAEL002508; AAEL010585
	IPR001790	4.95E-03	2.33E-02	Ribosomal protein L10/acidic P0	AAEL010821
	IPR004165	4.95E-03	2.33E-02	Coenzyme A transferase	AAEL011137
	IPR004167	4.95E-03	2.33E-02	E3 binding	AAEL009766
	IPR005140	4.95E-03	2.33E-02	eRF1 domain 1/Pelota-like	AAEL011742
	IPR005141	4.95E-03	2.33E-02	eRF1 domain 2	AAEL011742
	IPR005142	4.95E-03	2.33E-02	eRF1 domain 3	AAEL011742
	IPR001078	7.42E-03	2.93E-02	2-oxoacid dehydrogenase acyltransferase, catalytic domain	AAEL009766
	IPR001305	7.42E-03	2.93E-02	Heat shock protein DnaJ, cysteine-rich domain	AAEL005165
	IPR003338	7.42E-03	2.93E-02	CDC48, N-terminal subdomain	AAEL010585
	IPR000362	9.88E-03	2.93E-02	Fumarate lyase	AAEL003345
	IPR002088	9.88E-03	2.93E-02	Protein prenyltransferase, alpha subunit	AAEL014396
	IPR003031	9.88E-03	2.93E-02	Delta crystallin	AAEL003345
	IPR008251	9.88E-03	2.93E-02	Chromo shadow	AAEL004484
	IPR009010	9.88E-03	2.93E-02	Aspartate decarboxylase-like fold	AAEL010585
	IPR018125	9.88E-03	2.93E-02	Chromo shadow, subgroup	AAEL004484
	IPR022761	9.88E-03	2.93E-02	Lyase 1, N-terminal	AAEL003345

	IPR002164	1.23E-02	3.40E-02	Nucleosome assembly protein (NAP)	AAEL005567
	IPR008948	1.23E-02	3.40E-02	L-Aspartase-like	AAEL003345
	IPR000641	1.48E-02	3.70E-02	CbxX/CfqX	AAEL010585
	IPR017984	1.48E-02	3.70E-02	Chromo domain subgroup	AAEL004484
	IPR023780	1.48E-02	3.70E-02	Chromo domain	AAEL004484
	IPR005937	1.72E-02	4.18E-02	26S proteasome subunit P45	AAEL002508
	IPR000089	1.97E-02	4.63E-02	Biotin/lipoyl attachment	AAEL009766
NS5	IPR002928	1.44E-07	1.60E-05	Myosin tail	AAEL010975; AAEL005733; AAEL005656
	IPR001609	2.37E-05	1.32E-03	Myosin head, motor domain	AAEL009357; AAEL005733; AAEL005656
	IPR004009	6.69E-05	2.47E-03	Myosin, N-terminal, SH3-like	AAEL005733; AAEL005656
	IPR000048	1.83E-04	4.78E-03	IQ motif, EF-hand binding site	AAEL009357; AAEL005733; AAEL005656
	IPR000533	2.15E-04	4.78E-03	Tropomyosin	AAEL010975; AAEL014104; AAEL005733; AAEL005656
	IPR006195	1.14E-03	2.11E-02	Aminoacyl-tRNA synthetase, class II	AAEL006577; AAEL005037
	IPR002317	3.39E-03	2.68E-02	Seryl-tRNA synthetase, class IIa	AAEL005037
	IPR002993	3.39E-03	2.68E-02	Ornithine decarboxylase antizyme	AAEL004783
	IPR009066	3.39E-03	2.68E-02	Alpha-2-macroglobulin receptor-associated protein, domain 1	AAEL010784
	IPR009730	3.39E-03	2.68E-02	Micro-fibrillar-associated 1, C-terminal	AAEL010066
	IPR010483	3.39E-03	2.68E-02	Alpha-2-macroglobulin RAP, C-terminal	AAEL010784
	IPR010531	3.39E-03	2.68E-02	NOA36	AAEL014281
	IPR015866	3.39E-03	2.68E-02	Seryl-tRNA synthetase, class IIa, N-terminal	AAEL005037
	IPR004827	5.21E-03	2.68E-02	Basic-leucine zipper domain	AAEL005733; AAEL005656
	IPR020568	5.21E-03	2.68E-02	Ribosomal protein S5 domain 2-type fold	AAEL012827; AAEL004500
	IPR000043	6.76E-03	2.68E-02	Adenosylhomocysteinase	AAEL005524
	IPR001322	6.76E-03	2.68E-02	Intermediate filament, C-terminal	AAEL003415
	IPR001790	6.76E-03	2.68E-02	Ribosomal protein L10/acidic P0	AAEL010821
	IPR002539	6.76E-03	2.68E-02	MaoC-like dehydratase	AAEL003929
	IPR002710	6.76E-03	2.68E-02	Dilute	AAEL009357

	IPR002957	6.76E-03	2.68E-02	Keratin, type I	AAEL005656
	IPR004522	6.76E-03	2.68E-02	Asparaginyl-tRNA synthetase, class IIb	AAEL006577
	IPR010304	6.76E-03	2.68E-02	Survival motor neuron	AAEL008700
	IPR014038	6.76E-03	2.68E-02	Translation elongation factor EF1B, beta/delta subunit, guanine nucleotide exchange	AAEL000951
	IPR014717	6.76E-03	2.68E-02	Translation elongation factor EF1B/ribosomal protein S6	AAEL000951
	IPR015878	6.76E-03	2.68E-02	S-adenosyl-L-homocysteine hydrolase, NAD binding domain	AAEL005524
	IPR016044	6.76E-03	2.68E-02	Filament	AAEL003415
	IPR018444	6.76E-03	2.68E-02	Dil domain	AAEL009357
	IPR001305	1.01E-02	3.12E-02	Heat shock protein DnaJ, cysteine-rich domain	AAEL005165
	IPR001664	1.01E-02	3.12E-02	Intermediate filament protein	AAEL005656
	IPR001891	1.01E-02	3.12E-02	Malic oxidoreductase	AAEL005790
	IPR005607	1.01E-02	3.12E-02	BSD	AAEL000752
	IPR008374	1.01E-02	3.12E-02	SF-assemblin	AAEL010975
	IPR012301	1.01E-02	3.12E-02	Malic enzyme, N-terminal	AAEL005790
	IPR012302	1.01E-02	3.12E-02	Malic enzyme, NAD-binding	AAEL005790
	IPR020591	1.01E-02	3.12E-02	Chromosomal replication control, initiator DnaA-like	AAEL012095
	IPR002088	1.35E-02	3.84E-02	Protein prenyltransferase, alpha subunit	AAEL014396
	IPR002418	1.35E-02	3.84E-02	Transcription regulator Myc	AAEL012237
	IPR005517	1.35E-02	3.84E-02	Translation elongation factor EFG/EF2, domain IV	AAEL004500
	IPR009053	1.59E-02	4.41E-02	Prefoldin	AAEL005733; AAEL005656
	IPR013010	1.68E-02	4.56E-02	Zinc finger, SIAH-type	AAEL009614
	IPR011598	1.75E-02	4.63E-02	Helix-loop-helix DNA-binding	AAEL012237; AAEL003739
	IPR002312	2.02E-02	4.66E-02	Aspartyl/Asparaginyl-tRNA synthetase, class IIb	AAEL006577
	IPR004145	2.02E-02	4.66E-02	Domain of unknown function DUF243	AAEL009484

	IPR004364	2.02E-02	4.66E-02	Aminoacyl-tRNA synthetase, class II (D/K/N)	AAEL006577
	IPR018121	2.02E-02	4.66E-02	Seven-in-absentia protein, TRAF-like domain	AAEL009614
	IPR018150	2.02E-02	4.66E-02	Aminoacyl-tRNA synthetase, class II (D/K/N)-like	AAEL006577
	IPR020575	2.02E-02	4.66E-02	Heat shock protein Hsp90, N-terminal	AAEL012827
	IPR000640	2.35E-02	4.92E-02	Translation elongation factor EFG/EF2, C-terminal	AAEL004500
	IPR000690	2.35E-02	4.92E-02	Zinc finger, C2H2-type matrin	AAEL012348
	IPR001404	2.35E-02	4.92E-02	Heat shock protein Hsp90	AAEL012827
	IPR005937	2.35E-02	4.92E-02	26S proteasome subunit P45	AAEL012095
	IPR014775	2.35E-02	4.92E-02	L27, C-terminal	AAEL014012

purification from mosquito cells, but NS5 was not used in their study (Colpitts et al., 2011b).

I identified 16 capsid-interacting mosquito proteins. Three of these were identified using the anchored capsid bait that contained the C-terminal membrane-spanning domain, while the others were identified using the mature capsid bait. I tested all of the capsid-interacting proteins to see if they were capable of interacting with each capsid bait protein and found that all but two proteins were capable of interacting with both baits (Table 2-8). The Y2H reporter activity was generally less with the anchored capsid compared to the mature capsid, which could explain my failure to isolate these proteins with the anchored capsid bait even though they were capable of interacting with it. This could be due to a lower expression level of the anchored capsid or to an impaired ability of the membrane domain to enter the yeast nucleus and fold properly. The capsid-interacting mosquito proteins are enriched for “nucleic acid binding” proteins and proteins with “Zn finger” domains (Table 2-7). Among the nucleic acid binding capsid-interacting proteins, hypothetical protein (AAEL011985), putative myosin I (AAEL003676) and DEAD box ATP-dependent RNA helicase (AAEL009285) are potentially RNA binding proteins, according to the functions of their human orthologs. Moreover, the top protein domain enriched among the capsid interactors was the “G-patch” domain, which functions as an RNA-binding domain found in mRNA processing proteins and some retroviruses (Aravind and Koonin, 1999; Gifford et al., 2005). Since dengue capsid also directly binds to viral genomic RNA (Ma et al., 2004), it may be interesting to investigate whether interaction between capsid and G-patch proteins has any role in packaging the genome into the viral particle.

Table 2-8. Capsid domain analysis

Host	Host Gene	Dengue Gene	Capsid bait originally used	Interacts with Virion or Anchored Capsid	likely interface of interaction
Mosquito	AAEL000005	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL000292	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL001984	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL002057	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL003676	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL003750	C	Anchored	both	cytoplasmic domain of Capsid
Mosquito	AAEL004316	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL004869	C	Virion	Virion	cytoplasmic domain of Capsid
Mosquito	AAEL005567	C	Anchored	both	cytoplasmic domain of Capsid
Mosquito	AAEL008852	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL009285	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL010782	C	Virion	Virion	cytoplasmic domain of Capsid
Mosquito	AAEL011960	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL011985	C	Virion	both	cytoplasmic domain of Capsid
Mosquito	AAEL013075	C	Anchored	both	cytoplasmic domain of Capsid
Mosquito	AAEL013583	C	Virion	both	cytoplasmic domain of Capsid
Human	ANKRD12	C	Virion	both	cytoplasmic domain of Capsid
Human	AP3B1	C	Anchored	both	cytoplasmic domain of Capsid
Human	BIRC2	C	Anchored	both	cytoplasmic domain of Capsid
Human	BOD1L	C	Anchored	both	cytoplasmic domain of Capsid
Human	CD3E	C	Virion	Virion	cytoplasmic domain of Capsid
Human	CD3G	C	Anchored	both	cytoplasmic domain of Capsid
Human	DENND1C	C	Virion	Virion	cytoplasmic domain of Capsid
Human	GTPBP4	C	Virion	both	cytoplasmic domain of Capsid
Human	HBA1	C	Virion	both	cytoplasmic domain of Capsid
Human	HBB	C	Anchored	both	cytoplasmic domain of Capsid
Human	HBB	C	Virion	both	cytoplasmic domain of Capsid
Human	NAP1L1	C	Anchored	both	cytoplasmic domain of Capsid
Human	OS9	C	Anchored	both	cytoplasmic domain of Capsid
Human	RPL5	C	Virion	both	cytoplasmic domain of Capsid
Human	RPL6	C	Virion	both	cytoplasmic domain of Capsid
Human	RPS27	C	Anchored	Virion	cytoplasmic domain of Capsid
Human	RPS7	C	Virion	both	cytoplasmic domain of Capsid
Human	RRP12	C	Virion	both	cytoplasmic domain of Capsid
Human	S100A9	C	Anchored	Anchored	transmembrane domain of Capsid
Human	TMF1	C	Virion	both	cytoplasmic domain of Capsid
Human	ZNF394	C	Virion	Virion	cytoplasmic domain of Capsid

2.3.4 Dengue-human protein-protein interactions

While dengue-human protein interactions have been explored more extensively than dengue-mosquito protein interactions, the lack of overlap among validated interactions from different screens suggests that the dengue-human interactome is still incomplete. For example, if any one screen were complete, one would expect it to identify all validated PPI from all other screens. To complement the dengue-mosquito interactome and other dengue-human studies, I conducted Y2H screens using the 14 dengue protein baits (Figure 2-8B) and a cDNA library from human peripheral blood leukocytes (PBL). PBL contains a population of cells of the mononuclear phagocyte lineage, which are the primary target of dengue virus infection in human (Kyle et al., 2007). The library screens and the reproducibility and specificity tests were conducted as in the mosquito library screens (Materials and Methods). Similar to the mosquito library screen, I did not find human proteins interacting with M, E, NS1, NS2A, NS4A and NS4B; nor did I find interactors for PrM. In total I identified 46 reproducible specific interactions between 35 human proteins and five bait proteins representing dengue C, NS3, NS5 (Table 2-4 and 2-10). Only six of the interactions had previously been detected or predicted (Table 2-9). These included two interactions (capsid-beta hemoglobin (HBB) and capsid- ribosomal protein L5 (RPL5)) that had been predicted based on structural similarity between dengue virus and host proteins (Doolittle and Gomez, 2011) and four interactions (NS3- nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), NS3- nuclear receptor binding protein 1 (NRBP1), NS3-golgin B1 (GOLGB1), and NS5-Rab interacting lysosomal protein-like 2

(RILPL2)) that were identified in separate Y2H screens (Chua et al., 2004; Khadka et al., 2011; Le Breton et al., 2011).

My library screens identified four putative conserved interactions, where both human and mosquito orthologs were identified as interacting with the same dengue proteins (Table 2-3). 54 out of the remaining 93 mosquito genes that I identified have human orthlogs that I failed to detect in screens of the human PBL library. My failure to isolate human orthologs of these 54 mosquito genes could be because they are missing from the PBL cDNA library, or because the human orthologs actually do not interact with dengue proteins. To distinguish between these possibilities and to identify additional human-dengue interactions, I set out to test whether the human orthologs of the mosquito proteins interact with the same dengue protein. Sequence analysis identified 96 potential human orthologs for the 54 mosquito genes (Table 2-3). I was able to retrieve and subclone 55 of these from a human ORF library (Lamesch et al., 2007). These 55 human genes are potential orthologs of 31 mosquito genes. I made Y2H AD clones for these 55 and screened them against the corresponding dengue virus proteins. This resulted in identification of an additional five human-dengue interactions corresponding to four of the mosquito-dengue protein interactions (Table 2-3).

Combined, my human cDNA library screens and directed tests of mosquito orthologs identified 52 interactions involving 47 human proteins and three dengue proteins, capsid, NS3 and NS5 (Table 2-3 and Table 2-9). These include 46 novel interactions that were not previously detected or predicted; nine of these were detected with both human and mosquito orthologs. A global analysis of the human dengue-interacting proteins reveals no enriched GO annotations or protein domains. Similar to

Table 2-9 Dengue-human protein interactions. “-” in Expression Result for Co-AP means the host protein failed to express in the cell lysate, while “+” means both the host and the dengue protein were detected in the cell lysate. “-” in Co-AP result means the interaction was not detected by Co-AP while “+” means the interaction was detected. “NS” means a Myc-tagged protein was co-precipitated with an NTAP tag alone, which means an interaction was not assayable. “N/A” means no Co-AP was performed. See a supplementary file ‘Table_2-9.xls’ for a higher resolution.

Human Symbol	Ensembl ID for gene	Name of transcript	baitID	c_LEU	C_LACZ	Reporter Total _C_SUM _	Matrix Detections	Times isolated (ISTs_RFCs)	Reporter Total nonspecific bait 1 (DmCycJ)	Reporter Total nonspecific bait 2 (DmEip63E)	Expression result for Co-AP	Co-AP result	number_ serotype	Dengue _1	Dengue _3	Dengue _4	Human ORF tested	Human ORF interaction Y2H c_SUM	Human ORF interaction Co-AP result	Interaction previously reported in publication (PMID)
BIRC2	ENSG00000110330	Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	CA	2	0	2	2	4	0	0	+	+	4	Yes	Yes	Yes	No			
BOD1L	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	CA	3	1	4	2	7	0	2	+	+	4	Yes	Yes	Yes	No			
NAP1L1	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	CA	1	0	1	2	3	0	3.3	+	+	4	Yes	Yes	Yes	Yes	3	+	
AP3B1	ENSG00000132842	Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	CA	2	2	4	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
CD3G	ENSG00000160654	Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G), mRNA	CA	.5	0	.5	2	6	0	-.3	+	-	4	Yes	Yes	Yes	No			
HBB	ENSG00000244734	Homo sapiens hemoglobin, beta (HBB), mRNA	CA	.5	0	.5	2	1	.1	2.1	+	-	1	No	No	No	No			
OS9	ENSG00000135506	Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	CA	3	3	6	2	3	0	0	+	-	4	Yes	Yes	Yes	No			
S100A9	ENSG00000163220	Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	CA	3	1	4	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
RPS27	ENSG00000177954	Homo sapiens ribosomal protein S27 (RPS27), mRNA	CA	1	0	1	2	2	0	0	-	N/A	4	Yes	Yes	Yes	No			
ZNF394	ENSG00000160908	Homo sapiens zinc finger protein 394 (ZNF394), mRNA	CV	2.5	3	5.5	2	1	0	2.5	+	+	3	Yes	No	Yes	No			
ANKRD12	ENSG00000101745	Homo sapiens ankyrin repeat domain 12 (ANKRD12), transcript variant 2, mRNA	CV	2.5	3	5.5	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
DENND1C	ENSG00000205744	Homo sapiens DENN/MADD domain containing 1C (DENND1C), mRNA	CV	.5	0	.5	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
GTPBP4	ENSG00000107937	Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	CV	1.5	2	3.5	2	1	0	0	+	-	3	Yes	No	Yes	No			
HBA1	ENSG00000206172	Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	CV	1.5	0	1.5	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
HBB	ENSG00000244734	Homo sapiens hemoglobin, beta (HBB), mRNA	CV	1.5	0	1.5	2	1	.1	2.1	+	-	1	No	No	No	No			21358811
RPL5	ENSG00000122406	Homo sapiens ribosomal protein L5 (RPL5), mRNA	CV	2.5	2	4.5	2	1	0	0	+	-	4	Yes	Yes	Yes	No			21358811
RPS7	ENSG00000171863	Homo sapiens ribosomal protein S7 (RPS7), mRNA	CV	2.5	2	4.5	2	3	0	0	+	-	4	Yes	Yes	Yes	No			
RRP12	ENSG00000052749	Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	CV	2.5	3	5.5	2	2	0	0	+	-	4	Yes	Yes	Yes	No			
TMF1	ENSG00000144747	Homo sapiens TATA element modulatory factor 1 (TMF1), mRNA	CV	1.5	3	4.5	2	1	0	0	+	-	4	Yes	Yes	Yes	No			
RPL6	ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	CV	2.5	3	5.5	2	1	0	0	-	N/A	4	Yes	Yes	Yes	No			
CALCOCO2	ENSG00000136436	Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	NS3	.5	4	4.5	2	2	0	7	+	+	4	Yes	Yes	Yes	No			

NRBP1	ENSG00000115216	Homo sapiens nuclear receptor binding protein 1 (NRBP1), mRNA	NS3	2.5	3	5.5	2	22	0	.5	+	+	4	Yes	Yes	Yes	Yes	7	+	15084397
RPL24	ENSG00000114391	Homo sapiens ribosomal protein L24 (RPL24), mRNA	NS3	.5	0	.5	2	1	0	0	+	+	1	No	No	No	No			
DNTTIP2	ENSG00000067334	Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	NS3	.5	4	4.5	2	1	0	8	+	-	4	Yes	Yes	Yes	No			
GOLGB1	ENSG00000173230	Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	NS3	.5	1	1.5	2	1	.5	3	+	-	4	Yes	Yes	Yes	No			21911577
HBB	ENSG00000244734	Homo sapiens hemoglobin, beta (HBB), mRNA	NS3	.5	0	.5	2	1	.1	2.1	+	-	2	No	No	Yes	No			
ANP32B	ENSG00000136938	Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32 family, member B (ANP32B), mRNA	NS3	.5	1	1.5	2	1	0	3	+	-	4	Yes	Yes	Yes	No			
RILPL2	ENSG00000150977	Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	NS3	.5	2	2.5	2	2	0	3.3	+	-	3	Yes	No	Yes	Yes	5	+	
ZNF410	ENSG00000119725	Homo sapiens zinc finger protein 410 (ZNF410), mRNA	NS3	.5	2	2.5	2	2	0	3.5	+	-	4	Yes	Yes	Yes	Yes	4	-	
CORO1A	ENSG00000102879	Homo sapiens coronin, actin binding protein, 1A (CORO1A), mRNA	NS3	.5	1	1.5	2	1	0	0	-	N/A	4	Yes	Yes	Yes	No			
LOC100288418		PREDICTED: Homo sapiens similar to OK/SW-CL.16 (LOC100288418), mRNA	NS3	.5	1	1.5	2	1	0	0	-	N/A	4	Yes	Yes	Yes	No			
MTF1	ENSG00000188786	Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	NS3	1.5	0	1.5	2	1	.5	0	-	N/A	4	Yes	Yes	Yes	No			
OS9	ENSG00000135506	Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 4, mRNA	NS3	.5	1	1.5	2	1	0	0	-	N/A	4	Yes	Yes	Yes	No			
LRRFIP1	ENSG00000124831	Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	NS3d	.5	0	.5	2	1	1	0	+	NS	2	Yes	No	No	No			
NFKBIA	ENSG00000100906	Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA	NS3d	2.5	2	4.5	2	8	.3	0	+	-	2	Yes	No	No	No			22014111
CYTIP	ENSG00000115165	Homo sapiens cytohesin 1 interacting protein (CYTIP), mRNA	NS5	1.5	2	3.5	2	1	0	2	+	+	4	Yes	Yes	Yes	Yes	4	+	
FAM192A	ENSG00000172775	Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	NS5	2.5	0	2.5	2	2	.8	0	+	+	4	Yes	Yes	Yes	Yes	7	+	
FMR1	ENSG00000102081	Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	NS5	2.5	1	3.5	2	1	0	0	+	+	4	Yes	Yes	Yes	No			
DERL2	ENSG00000072849	Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	NS5	2.5	1	3.5	2	1	0	5	+	-	4	Yes	Yes	Yes	No			
HBB	ENSG00000244734	Homo sapiens hemoglobin, beta (HBB), mRNA	NS5	2.5	1	3.5	2	16	.1	2.1	+	-	4	Yes	Yes	Yes	No			
IMPDH2	ENSG00000178035	Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	NS5	2.5	3	5.5	2	1	.5	0	+	-	2	Yes	No	No	No			
PSMC1	ENSG00000100764	Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1 (PSMC1), mRNA	NS5	1.5	1	2.5	2	11	0	3.4	+	-	4	Yes	Yes	Yes	No			
RILPL2	ENSG00000150977	Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	NS5	2.5	2	4.5	2	72	0	3.3	+	-	4	Yes	Yes	Yes	Yes	0	Did not test	21911577
RPL12	ENSG00000197958	Homo sapiens ribosomal protein L12 (RPL12), mRNA	NS5	1.5	1	2.5	2	1	0	2	+	-	4	Yes	Yes	Yes	No			
WWP1	ENSG00000123124	Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	NS5	2.5	3	5.5	2	1	0	6	+	-	4	Yes	Yes	Yes	Yes	1	Failed to express	
HSPA5	ENSG00000044574	Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	NS5	1.5	0	1.5	2	2	.5	0	-	N/A	3	Yes	Yes	No	No			
EAF1	ENSG00000144597	Homo sapiens ELL associated factor 1	NS5	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	1	-	
EAF2	ENSG00000145088	Homo sapiens ELL associated factor 2	NS5	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	1	Failed to express	
EEF1B2	ENSG00000114942	Homo sapiens eukaryotic translation elongation factor 1 beta 2	NS5	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	2	-	
TRIM2	ENSG00000109654	Homo sapiens tripartite motif containing 2	NS5	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	1	Failed to express	
XPA	ENSG00000136936	Homo sapiens xeroderma pigmentosum, complementation group A	NS5	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	1	-	
HSP90AB1	ENSG00000096384	Homo sapiens heat shock protein 90kDa alpha (cytosolic), class B member 1	NS3	N/A	N/A	N/A	1	N/A	0	0	N/A	N/A	N/A	N/A	N/A	N/A	Yes	1	+	

my finding with the mosquito proteins, a significant proportion of the dengue-interacting human proteins (19 out of 47) have been shown to interact with proteins from other viruses (Table 2-10) (Calderwood et al., 2007; Ptak et al., 2008; Chatr-aryamontri et al., 2009; Fu et al., 2009; Pinney et al., 2009; Shapira et al., 2009; Kwofie et al., 2011). These include at least two interactions that could be thought of as orthologous, or interologs: Dengue NS3 interacted with zinc finger protein 410 (ZNF410) and calcium binding and coiled-coil domain 2 (CALCOCO2) both of which have been shown to interact with HCV NS3 by a Y2H screen (de Chassey et al., 2008).

It has been shown that proteins from other viruses frequently interact with hub proteins, which are host proteins that have a large number of interactions in the host interactome (Dyer et al., 2008). To evaluate the numbers of interactions for the host proteins that I identified, my co-author for the submitted paper (Mairiang et al., submitted) assembled a human protein interactome from several public databases. The human interactome contains 44 (94%) of the 47 dengue-interacting proteins that I identified. It also contains 143 (72%) of the 198 dengue-interacting human proteins identified exclusively in other screens (Chang et al., 2001; Johansson et al., 2001; Brooks et al., 2002; Garcia-Montalvo et al., 2004; Chua et al., 2005; Lozach et al., 2005; Reyes-Del Valle et al., 2005; Chiu et al., 2007; Kurosu et al., 2007; Limjindaporn et al., 2007; Noisakran et al., 2008; Ashour et al., 2009; Bhattacharya et al., 2009; Ellencrona et al., 2009; Hershkovitz et al., 2009; Jiang et al., 2009; Limjindaporn et al., 2009; Mazzon et al., 2009; Rawlinson et al., 2009; Heaton et al., 2010; Avirutnan et al., 2011; Brault et al., 2011; Colpitts et al., 2011a; Folly et al., 2011; Khadka et al., 2011; Le Breton et al., 2011), and 52 (83%) of the 63 human orthologs of mosquito proteins that I

<u>Table 2-10.</u> Human proteins that also interact with other viruses.

Dengue Gene (this study)	Host Gene	Dengue interactions (from other sources)	HCV_interactions	Herpes_family_interactions	HIV_interactions	HPV_interactions	Other_interactions
C	ANKRD12		NS3				
C	BIRC2				Vpr		
C	OS9		NS5B				
C	TMF1		NS5A				
C	CD3G				capsid; Tat; Envelope surface glycoprotein gp120; Envelope transmembrane glycoprotein gp41; Nef		
C	NAP1L1		NS3; NS5A	HHV-1_ICP8; EBV_EBNA1		HPV-8_E2; HPV-18_E2	
C	CD3E				capsid; Tat; Envelope surface glycoprotein gp120; Envelope transmembrane glycoprotein gp41; Nef		
C	RPL5				Rev		
C; NS3	HBB			EBV_BPLF1			
NS3	NFKBIA			HHV-1_ICP27	Vpr; Tat; Rev; Vpu; Envelope surface glycoprotein gp160, precursor; Envelope surface glycoprotein gp120; Nef		
NS3	ZNF410		NS3				
NS3	OS9		NS5B				
NS3	CALCOCO2	NS4A	NS3				
NS5	HSPA5	E	E1; E2	EBV_EBNA-LP	Pr55(Gag); matrix; Vpr; Tat; Envelope surface glycoprotein gp160, precursor; Envelope surface glycoprotein gp120		
NS5	WWP1						HTLV-1::gag
NS5	IMPDH2			HHV-1_ICP8			
NS5	HBB			EBV_BPLF1			
NS5	PSMC1				integrase; Vif; Tat	HPV-16_E7	
NS5	RPL12			EBV_EBNA-LP			
NS3	HSP90AB1		NS5A; Whole virus		Tat		

identified. For each of these gene sets, my co-author found that the average number of interactions (or degree) per protein was significantly higher than for random samples of similar numbers of proteins. For example, the average degree of dengue-interacting proteins in my dataset was 44.0, whereas the average degree of similarly sized random samples of proteins was 22.4 (p-value = 9.3×10^{-4}) (Bulich and Aaskov, 1992). The dengue interactors from mosquito were also enriched for proteins with many interactions (p-value = 2.7×10^{-3}), as were the dengue-interacting proteins identified by other studies (p-value= 6.4×10^{-7}). It has been suggested that the tendency of viral proteins to interact with hub proteins may represent a feature of viral pathogenesis since the disruption of a hub is more likely to impair the cell's protein network than the disruption a non-hub (Yook et al., 2004; Dyer et al., 2008). While these results are consistent with this hypothesis, they could also be explained by the possibility that some proteins are particularly interactive in the protein interaction assays that have been used to detect the human interactome, including the Y2H assay. Thus a more thorough test of the hypothesis that dengue viral proteins tend to target hubs will require a larger set of functionally validated dengue-host interactions.

We identified a number of potentially relevant NS3 interactors. CALCOCO2 (also known as NDP2) is a component of Nuclear Domain 10 (ND10) bodies, which play a role in the intrinsic cellular defense mechanisms against some viruses (Everett and Chelbi-Alix, 2007). Interestingly, another major component of ND10 is DAXX, which has been shown to interact with dengue capsid (Limjindaporn et al., 2007). These interactions may be involved in the interplay between host defense mechanisms and viral strategies to circumvent them. NS3 also interacted with two additional proteins that

play roles in the innate immune response, NFKBIA and leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1). NFKBIA negatively regulates the innate immune response by inhibiting the NF-kappaB transcription factor (Jacobs and Harrison, 1998). LRRFIP1 is a regulator of the toll-like receptor signaling pathway and was shown to associate with dsRNA-containing endosomes/lysosomes (Arakawa et al., 2010), which are generated in response to virus dsRNA intermediates during replication (Johnsen et al., 2006). Another NS3 interactor, amplified in osteosarcoma 9 (OS9), plays an important role in the unfolded protein response (UPR) (Alcock and Swanton, 2009), which is often observed in dengue-infected cells (Umareddy et al., 2007). As I did for the mosquito proteins, I tested the human NS3 interacting proteins against both variants of NS3 baits (Figure 2-1B) and found that the N-terminal 160 amino acids of NS3 was required for only three interactions, including the previously identified interaction, NS3-NRBP1 (Table 2-6). The remaining human proteins interacted with both the full-length and the C-terminal half of NS3.

The capsid interactors were isolated using the anchored capsid bait or the cytoplasmic capsid bait (Figure 2-1B). I tested all of the capsid interactors against both baits and found that most were able to interact with both the anchored and the cytoplasmic capsid proteins (Table 2-8), indicating that the C-terminal membrane spanning domain is not required for and does not dramatically interfere with most interactions. GO and protein domain enrichment analysis of the 20 capsid interactors failed to implicate any specific biological function or process (Table 2-11). However, the

Table 2-11. Enrichment of GO annotations and protein domains in human proteins that interact with dengue proteins

Dengue protein	GO-ID	p-value	corr p-value	Description	Genes in test set
Capsid	GO:005198	1.99E-05	1.26E-03	structural molecule activity	RPS27; CD3G; RPL6; CD3E; RPL5; RPS7
	GO:005840	3.87E-05	1.26E-03	ribosome	RPS27; RPL6; RPL5; RPS7
	GO:006412	1.79E-04	3.88E-03	translation	RPS27; RPL6; RPL5; RPS7
	GO:005730	9.96E-04	1.62E-02	nucleolus	GTPBP4; RRP12; RPL5; RPS7
	GO:005829	1.38E-03	1.80E-02	cytosol	RPS27; RPL6; RPL5; HBB; BIRC2; RPS7
NS3	none				
NS5	GO:030968	1.58E-04	2.25E-02	endoplasmic reticulum unfolded protein response	DERL2; HSPA5
	GO:034620	1.58E-04	2.25E-02	cellular response to unfolded protein	DERL2; HSPA5
	GO:010498	2.43E-04	2.25E-02	proteasomal protein catabolic process	DERL2; PSMC1; HSPA5
	GO:043161	2.43E-04	2.25E-02	proteasomal ubiquitin-dependent protein catabolic process	DERL2; PSMC1; HSPA5
	GO:030433	2.63E-04	2.25E-02	ER-associated protein catabolic process	DERL2; HSPA5
	GO:071445	2.63E-04	2.25E-02	cellular response to protein stimulus	DERL2; HSPA5
	GO:034976	4.18E-04	2.68E-02	response to endoplasmic reticulum stress	DERL2; HSPA5
	GO:044424	4.25E-04	2.68E-02	intracellular part	DERL2; CYTIP; EEF1B2; FMR1; FAM192A; XPA; TRIM2; RILPL2; EAF1; WWP1; PSMC1; RPL12; EAF2; HSPA5; HBB; IMPDH2
	GO:006984	4.69E-04	2.68E-02	ER-nucleus signaling pathway	DERL2; HSPA5
	GO:044265	5.35E-04	2.75E-02	cellular macromolecule catabolic process	XPA; DERL2; PSMC1; HSPA5
	GO:005515	6.73E-04	2.90E-02	protein binding	DERL2; CYTIP; EEF1B2; FMR1; XPA; TRIM2; RILPL2; EAF1; WWP1; PSMC1; EAF2; HSPA5; HBB; IMPDH2
	GO:005622	7.07E-04	2.90E-02	intracellular	DERL2; CYTIP; EEF1B2; FMR1; FAM192A; XPA; TRIM2; RILPL2; EAF1; WWP1; PSMC1; RPL12; EAF2; HSPA5; HBB; IMPDH2
	GO:071216	8.70E-04	2.90E-02	cellular response to biotic stimulus	DERL2; HSPA5
	GO:009057	8.77E-04	2.90E-02	macromolecule catabolic process	XPA; DERL2; PSMC1; HSPA5
	GO:060904	8.99E-04	2.90E-02	regulation of protein folding in endoplasmic reticulum	HSPA5
	GO:030176	9.06E-04	2.90E-02	integral to endoplasmic reticulum membrane	DERL2; HSPA5
	GO:006986	1.57E-03	3.47E-02	response to unfolded protein	DERL2; HSPA5
	GO:031227	1.57E-03	3.47E-02	intrinsic to endoplasmic reticulum membrane	DERL2; HSPA5
	GO:044267	1.59E-03	3.47E-02	cellular protein metabolic process	DERL2; EEF1B2; WWP1; PSMC1; RPL12; HSPA5; EAF2
	GO:003938	1.80E-03	3.47E-02	IMP dehydrogenase activity	IMPDH2

	GO:006177	1.80E-03	3.47E-02	GMP biosynthetic process	IMPDH2
	GO:006987	1.80E-03	3.47E-02	activation of signaling protein activity involved in unfolded protein response	HSPA5
	GO:021577	1.80E-03	3.47E-02	hindbrain structural organization	HSPA5
	GO:021589	1.80E-03	3.47E-02	cerebellum structural organization	HSPA5
	GO:046037	1.80E-03	3.47E-02	GMP metabolic process	IMPDH2
	GO:006511	1.80E-03	3.47E-02	ubiquitin-dependent protein catabolic process	DERL2; PSMC1; HSPA5
	GO:019941	1.89E-03	3.47E-02	modification-dependent protein catabolic process	DERL2; PSMC1; HSPA5
	GO:043632	1.89E-03	3.47E-02	modification-dependent macromolecule catabolic process	DERL2; PSMC1; HSPA5
	GO:051603	2.50E-03	4.32E-02	proteolysis involved in cellular protein catabolic process	DERL2; PSMC1; HSPA5
	GO:044257	2.57E-03	4.32E-02	cellular protein catabolic process	DERL2; PSMC1; HSPA5
	GO:030185	2.70E-03	4.32E-02	nitric oxide transport	HBB
	GO:032075	2.70E-03	4.32E-02	positive regulation of nuclease activity	HSPA5
	GO:030163	3.19E-03	4.61E-02	protein catabolic process	DERL2; PSMC1; HSPA5
	GO:031398	3.23E-03	4.61E-02	positive regulation of protein ubiquitination	PSMC1; HSPA5
	GO:032991	3.26E-03	4.61E-02	macromolecular complex	EEF1B2; EAF1; WWP1; PSMC1; FMR1; RPL12; EAF2; HBB
	GO:006414	3.36E-03	4.61E-02	translational elongation	EEF1B2; RPL12
	GO:005853	3.59E-03	4.61E-02	eukaryotic translation elongation factor 1 complex	EEF1B2
	GO:030492	3.59E-03	4.61E-02	hemoglobin binding	HBB
	GO:032069	3.59E-03	4.61E-02	regulation of nuclease activity	HSPA5
	GO:042149	3.59E-03	4.61E-02	cellular response to glucose starvation	HSPA5
	GO:044248	4.30E-03	4.95E-02	cellular catabolic process	XPA; DERL2; PSMC1; HSPA5
	GO:030970	4.49E-03	4.95E-02	retrograde protein transport, ER to cytosol	DERL2
	GO:043008	4.49E-03	4.95E-02	ATP-dependent protein binding	HSPA5
	GO:051787	4.49E-03	4.95E-02	misfolded protein binding	HSPA5
	GO:031324	4.50E-03	4.95E-02	negative regulation of cellular metabolic process	WWP1; PSMC1; FMR1; EAF2
	GO:016607	4.61E-03	4.95E-02	nuclear speck	EAF1; EAF2
	GO:005829	4.82E-03	4.95E-02	cytosol	RILPL2; EEF1B2; RPL12; HBB; IMPDH2
	GO:043234	4.82E-03	4.95E-02	protein complex	EEF1B2; EAF1; WWP1; PSMC1; FMR1; EAF2; HBB
	GO:010605	4.83E-03	4.95E-02	negative regulation of macromolecule metabolic process	WWP1; PSMC1; FMR1; EAF2
	GO:051789	4.85E-03	4.95E-02	response to protein stimulus	DERL2; HSPA5
	GO:019538	4.92E-03	4.95E-02	protein metabolic process	DERL2; EEF1B2; WWP1; PSMC1; RPL12; HSPA5; EAF2

Dengue protein	Interpro_ID	p-value	corr p-value	Description	Genes in test set

Capsid	IPR006414	2.00E-06	1.98E-04	ATPase, P-type, potassium/sodium efflux, fungal	RPS27; RPL6; RPL5; RPS7
	IPR001757	1.32E-05	5.65E-04	ATPase, P-type, K/Mg/Cd/Cu/Zn/Na/Ca/Na/H-transporter	RPS27; RPL6; RPL5; RPS7
	IPR003735	1.71E-05	5.65E-04	Protein of unknown function DUF156	RPS27; RPL6; RPL5; RPS7
	IPR005840	3.25E-05	6.46E-04	Ribosomal protein S12 methylthiotransferase RimO	RPS27; RPL6; RPL5; RPS7
	IPR005839	4.94E-05	6.46E-04	Methylthiotransferase	RPS27; RPL6; RPL5; RPS7
	IPR023970	4.94E-05	6.46E-04	Methylthiotransferase/B12-binding/radical SAM-type	RPS27; RPL6; RPL5; RPS7
	IPR000771	5.22E-05	6.46E-04	Ketose-bisphosphate aldolase, class-II	RPS27; RPL6; RPL5; RPS7
	IPR006412	5.22E-05	6.46E-04	Fructose-bisphosphate aldolase, class II, Calvin cycle subtype	RPS27; RPL6; RPL5; RPS7
	IPR005515	5.02E-04	5.00E-03	Vitelline membrane outer layer protein I (VOMI)	GTPBP4; RRP12; CD3G; CD3E; S100A9; NAP1L1; BIRC2; OS9; TMF1; RPS7; RPS27; RPL5; HBB; AP3B1
	IPR007166	5.69E-04	5.00E-03	Class III signal peptide motif	CD3G; CD3E; BIRC2
	IPR022625	6.06E-04	5.00E-03	Type I restriction and modification enzyme, subunit R, C-terminal	RPL6; RPL5
	IPR022627	6.06E-04	5.00E-03	Domain of unknown function DUF3502	RPS27; RPS7
	IPR005730	9.83E-04	6.73E-03	Carboxynorspermidine decarboxylase	GTPBP4; RRP12; RPL5; RPS7
	IPR000836	1.02E-03	6.73E-03	Phosphoribosyltransferase	OS9
	IPR008097	1.02E-03	6.73E-03	CX3X chemokine fractalkine	RPL5
	IPR000878	2.49E-03	1.54E-02	Tetrapyrrole methylase	RPL5; RPS7; TMF1
	IPR002669	3.06E-03	1.78E-02	Urease accessory protein UreD	CD3E
	IPR006364	3.89E-03	2.14E-02	Cobalamin (vitamin B12) biosynthesis CobI/CbiL, precorrin-2 C20-methyltransferase, core	RPL5; RPS7
	IPR006461	4.76E-03	2.48E-02	Uncharacterised protein family Cys-rich	CD3G; CD3E
	IPR005678	6.10E-03	2.82E-02	Mitochondrial inner membrane translocase complex, subunit Tim17	NAP1L1
	IPR022408	6.10E-03	2.82E-02	Acyl-CoA-binding protein, ACBP, conserved site	GTPBP4
	IPR004888	6.42E-03	2.82E-02	Glycoside hydrolase, family 63	CD3G; CD3E
	IPR006621	7.12E-03	2.82E-02	Nose resistant-to-fluoxetine protein, N-terminal	OS9
	IPR006622	7.12E-03	2.82E-02	Iron sulphur-containing domain, CDGSH-type, subfamily	AP3B1
	IPR018967	7.12E-03	2.82E-02	Iron sulphur-containing domain, CDGSH-type	AP3B1
	IPR005829	7.55E-03	2.87E-02	Sugar transporter, conserved site	RPS27; RPL6; RPL5; BIRC2; RPS7
	IPR007172	8.13E-03	2.98E-02	Domain of unknown function DUF374	CD3E
	IPR001772	1.12E-02	3.95E-02	Kinase-associated KA1	CD3E
	IPR005344	1.32E-02	4.50E-02	Uncharacterised protein family UPF0121	HBB
	IPR005634	1.41E-02	4.66E-02	Male specific sperm protein	RPS27; GTPBP4; RRP12; ANKRD12; S100A9; NAP1L1; RPL5; ZNF394; BIRC2; TMF1
NS3	IPR000836	8.50E-04	4.46E-02	Phosphoribosyltransferase	OS9
	IPR021554	8.50E-04	4.46E-02	Protein of unknown function DUF3202	RPL24
	IPR011991	1.57E-03	4.46E-02	Winged helix-turn-helix transcription repressor DNA-binding	CALCOCO2; LRRFIP1

	IPR005938	1.95E-03	4.46E-02	ATPase, AAA-type, CDC48	CORO1A; NRBP1
	IPR001845	2.55E-03	4.46E-02	HTH ArsR-type DNA-binding domain	CORO1A
	IPR001891	2.55E-03	4.46E-02	Malic oxidoreductase	CORO1A
	IPR010458	3.39E-03	4.46E-02	Trichodiene synthase, ascomycetes	RPL24
	IPR024652	3.39E-03	4.46E-02	Trichodiene synthase	RPL24
	IPR005515	4.63E-03	4.79E-02	Vitelline membrane outer layer protein I (VOMI)	HSP90AB1; CORO1A; NRBP1; MTF1; CALCOCO2; NFKBIA; RPL24; LRRFIP1; HBB; GOLGB1; OS9
	IPR007253	5.09E-03	4.79E-02	Putative cell wall binding repeat 2	NFKBIA
	IPR010888	5.09E-03	4.79E-02	CblD-like pilus biogenesis initiator	NFKBIA
	IPR006621	5.93E-03	4.79E-02	Nose resistant-to-fluoxetine protein, N-terminal	OS9
	IPR010038	5.93E-03	4.79E-02	MoaD, archaeal	MTF1
NS5	IPR005515	3.81E-04	3.77E-02	Vitelline membrane outer layer protein I (VOMI)	DERL2; CYTIP; EEF1B2; FMR1; XPA; TRIM2; EAF1; WWP1; PSMC1; EAF2; HSPA5; HBB; IMPDH2

capsid interactors include a preponderance of ribosomal proteins, including RPL5, ribosomal protein L6 (RPL6), ribosomal protein L7 (RPL7), and ribosomal protein L27 (RPL27), all of which are subunits of the 60S ribosome. Capsid also interacted with mosquito RPL23, and with a ribosomal RNA processing protein (RRP12) from both mosquito and human. Based in part on these ribosomal proteins and on a GTP-binding protein, GTPBP4, the capsid-interacting proteins are enriched for proteins annotated as being associated with the nucleolus (Table 2-11). Interestingly, dengue capsid has previously been found to accumulate in nucleoli in several cell lines (Tadano et al., 1989; Wang et al., 2002; Sangiambut et al., 2008), though the functional significance of this localization has not been determined. Many other viruses interact with nucleoli, and in some cases nucleoli have been shown to be essential for virus replication (Hiscox, 2007; Hiscox et al., 2010). The capsid proteins from two other flaviviruses, West Nile virus and Japanese encephalitis virus, each interact with specific nucleolar proteins, and in each case, these nucleolar proteins have been shown to be important for efficient viral replication (Tsuda et al., 2006; Yang et al., 2008; Xu et al., 2011). Further studies with the capsid-interacting proteins that I identified may provide insights into the role and mechanisms for accumulation of dengue capsid at the nucleolus.

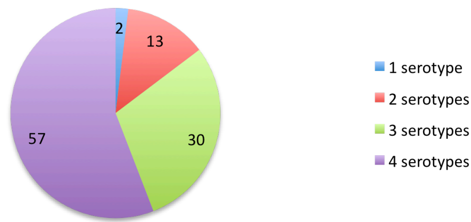
Among the NS5 interactors, “unfolded protein response (UPR)” is the top enriched GO annotation (Table 2-11). This enrichment is based on interactions with DERL2, an ER membrane protein involved in targeting misfolded glycoproteins for degradation (Lilley et al., 2006; Oda et al., 2006), and HSPA5/Grp78/BiP, an ER protein involved in protein folding (Malhotra and Kaufman, 2007; Wang et al., 2010). The UPR is known to be activated during dengue infection; however, its importance for virus

replication is still undetermined (Yu et al., 2006; Umareddy et al., 2007; Fischl and Bartenschlager, 2011; Pena and Harris, 2011).

2.3.5 Confirmation of protein interactions using additional assays

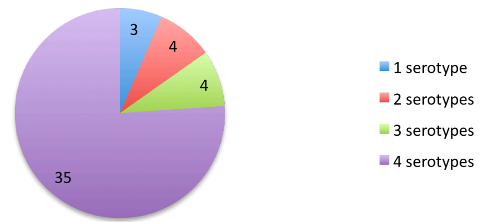
Y2H studies frequently detect false positive interactions that have no biological relevance. One way to gain confidence in a Y2H interaction is to detect it using additional assays. I used two approaches to test the confidence of interactions that I detected in the library screens. First, I reasoned that biologically relevant virus-host protein interactions are likely to be conserved across the four dengue serotypes. There is 63-68% amino acid sequence homology among the four serotypes (Lindenbach et al., 2006). An interaction between a host protein and the same dengue protein from multiple serotypes may imply that the interaction is more likely to have functional relevance because significant variation in the dengue protein does not interrupt the interaction. To test for conservation of interactions I repeated Y2H assays for all dengue-host interactions using dengue proteins from serotypes 1, 3 and 4. I found that 57 out of 102 (56.9%) dengue-mosquito protein interactions and 34 out of 46 (73.9%) dengue-human protein interactions were serotype independent; i.e., the host proteins interacted with corresponding dengue proteins from all four serotypes (Figure 2-10, Tables 2-4 and 2-10). This provides additional evidence that these host proteins genuinely interact with the dengue proteins, and further points to conserved sequences or structural elements in the dengue proteins as potential interaction interfaces. A minority of the host proteins interacted with only one or a subset of the dengue serotypes (Figure 2-10, Tables 2-4 and 2-10). While these interactions may be false positives, some may be biologically

A **Number of dengue-mosquito interactions (102 total)**



55.9% serotype independent

B **Number of dengue-human interactions (46 total)**



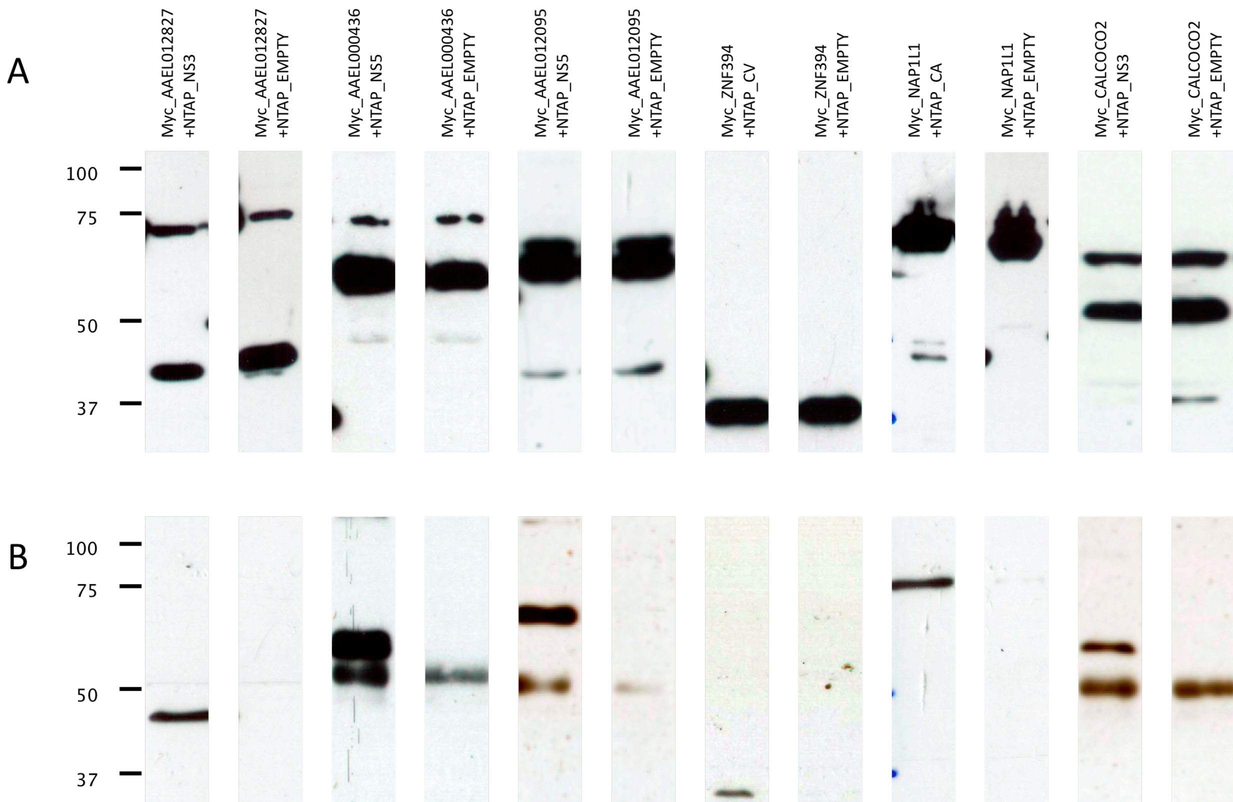
76% serotype independent

Figure 2-10. Serotype specificity of dengue – host protein interactions. Interactions identified by Y2H assays against dengue serotype 2 proteins were screened against the same protein from dengue serotypes 1, 3 and 4. (A) Serotype specificity of dengue-mosquito PPIs. (B) Serotype specificity of dengue-human PPIs.

some may be biologically relevant serotype-specific dengue-host interactions. If so, such interactions may mediate some of the serotype-specific dengue characteristics that are clinically observed (Balmaseda et al., 2006). Further investigation will be required to validate serotype specific interactions.

I also set up Y2H matrix matings for each dengue interactor against all 56 dengue BD proteins representing the 14 bait proteins from each of the four serotypes. I intended to use these screens to detect host proteins that interact with multiple dengue proteins. The screens resulted in about 8,000 mating pairs with 1,713 potential interactions including those already identified from the Y2H library screens with dengue serotype 2 (Appendix A). Because of limited time and resources, I did not further analyze PPIs found only in the matrix screens. They will require reproducibility tests or orthologonal assays for validation.

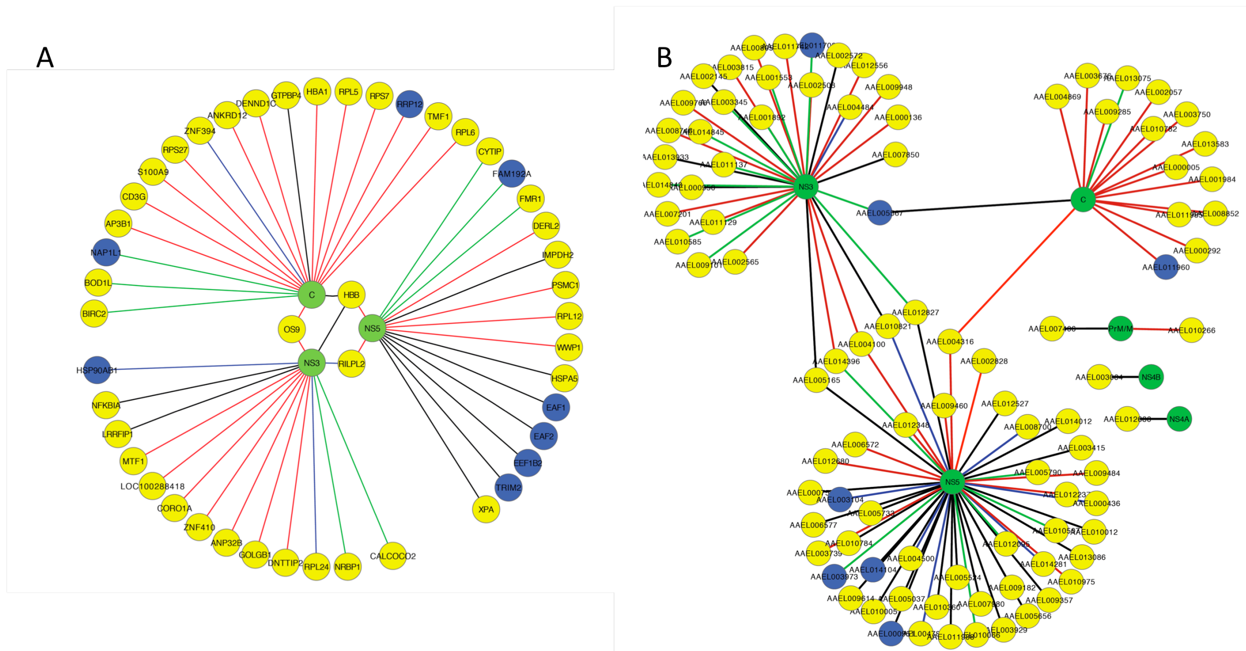
Next I employed an orthogonal assay, co-affinity purification (co-AP), to test most of the dengue-host interactions that I identified by Y2H assays. Myc-tagged versions of the mosquito and human proteins were expressed in cultured *Drosophila* cells along with NTAP-tagged dengue proteins (Materials and Methods). The tagged dengue proteins were purified and tested for co-purification of the host proteins by immunoblotting with myc antibodies (Figure 2-11 and Appendix O). If one of the two proteins failed to express in the cell lysate, I tried the experiment in the opposite orientation, giving the dengue protein a myc tag and the host protein an NTAP tag. I was able to express and test by co-AP 135 pairs of proteins, and I detected 38 interactions (27.9%) (Table 2-3 and 2-10). This confirmation rate is similar to that reported for other large-scale tests of protein interactions by orthogonal assays



<u>Figure 2-11.</u> Examples of co-affinity purification results. Host proteins were fused to a myc-tag while dengue proteins were fused to a NTAP-tag. The fusion proteins were expressed in S2R+ cells. NTAP-dengue proteins were purified from cell lysates, and then host proteins were detected with α-myc. (A) An α-myc immunoblot of cell lysates shows expression of mosquito and human proteins. (B) An α-myc immunoblot of NTAP-tag affinity purified samples.

(Yu et al., 2008a; Yu et al., 2011), but lower than the rate reported in some specific Y2H studies (Rual et al., 2005; Lim et al., 2006). One possible explanation for the discrepancy is that I define a Y2H positive based on reproducible activity of a highly sensitive *LEU2* reporter, and thus I may detect weaker protein-protein interactions than studies that require activation of multiple less sensitive Y2H reporters. However, the combined Y2H reporter activity (*LEU2* and *lacZ*) was not significantly higher for interactions that were positive by co-AP assays (average 3.4) than for interactions that were negative in co-AP assays (average 2.9).

Figure 2-12 shows a summary of the dengue-host interactions that I identified. The dengue-human interaction map includes 13 proteins, which had orthologs in the dengue-mosquito map and were involved in PPI that were detected in both species. Three human proteins and seven mosquito proteins interacted with more than one dengue protein. The maps also show whether or not each interaction was detected with all four dengue serotypes and whether or not it was also detected by co-AP

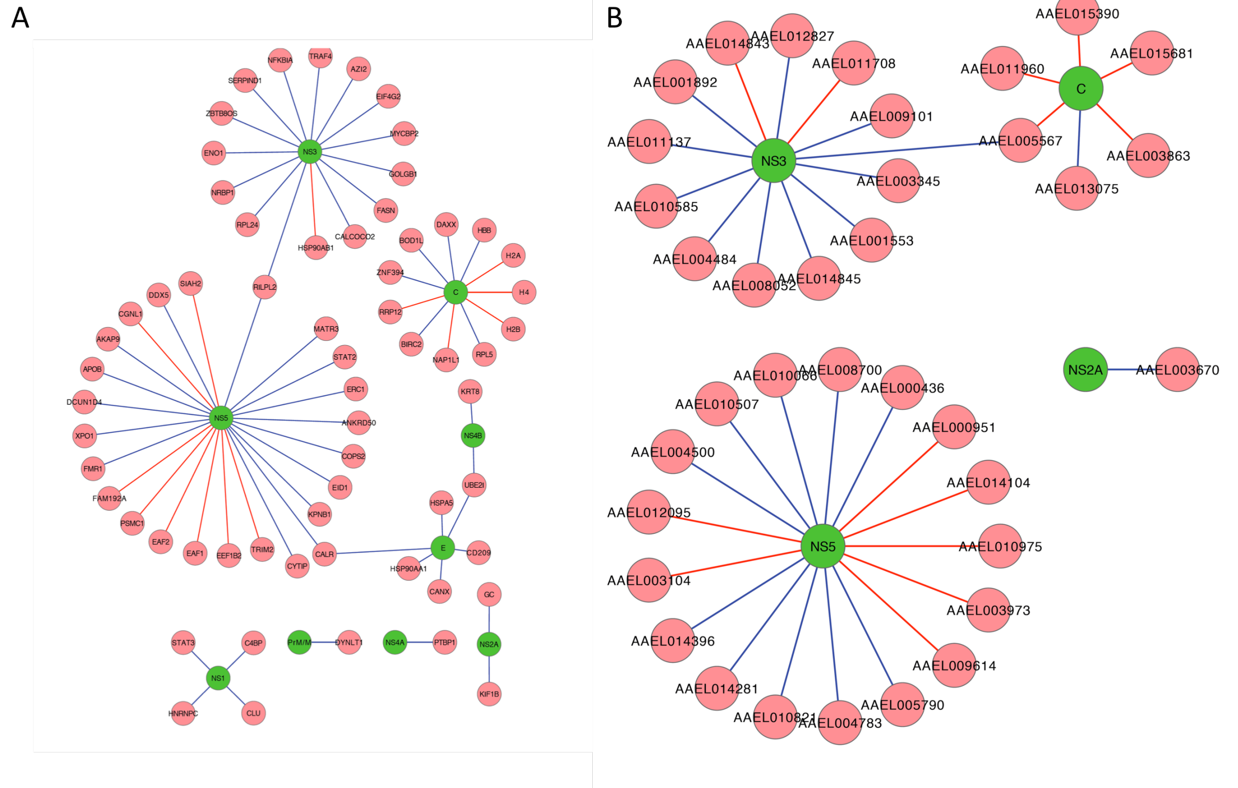


<u>Figure 2-12.</u> Dengue – host protein networks derived from Y2H screens and co-AP assays in this study. (A) Human-dengue interaction map. (B) Mosquito-dengue interaction map. Black edges represent protein-protein interactions. Red edges represent protein-protein interactions universally detected for all four serotypes. Blue edges represent protein-protein interactions confirmed by co-AP assays. Green edges represent the universal interactions that were confirmed by co-AP assays. Green nodes represent dengue proteins. Yellow nodes represent host proteins. Blue nodes represent host proteins of which potential orthologs were detected in both human and mosquito.

2.3.6 A snapshot of the dengue-host interactome

It is often noted that a virus such as dengue with only 10 proteins of its own should need to interact with a number of host proteins to carry out its replication cycle. My study combined with other large-scale and small-scale studies has identified 403 interactions between proteins from dengue and its hosts, not counting the more than 4,000 interactions that have been computationally predicted (Chang et al., 2001; Johansson et al., 2001; Brooks et al., 2002; Chua et al., 2004; Garcia-Montalvo et al., 2004; Chua et al., 2005; Lozach et al., 2005; Reyes-Del Valle et al., 2005; Chiu et al., 2007; Kurosu et al., 2007; Limjindaporn et al., 2007; Noisakran et al., 2008; Ashour et al., 2009; Bhattacharya et al., 2009; Ellencrona et al., 2009; Hershkovitz et al., 2009; Jiang et al., 2009; Limjindaporn et al., 2009; Mazzon et al., 2009; Rawlinson et al., 2009; Heaton et al., 2010; Avirutnan et al., 2011; Brault et al., 2011; Colpitts et al., 2011a; Colpitts et al., 2011b; Doolittle and Gomez, 2011; Folly et al., 2011; Khadka et al., 2011; Le Breton et al., 2011). Since I know that most protein interaction screens and assays produce false positives, it seems likely that a number of the dengue-host PPI detected thus far are not relevant to the virus or the host's defenses against it. Among the 403 experimentally detected PPI, only seventeen PPI have been studied further and shown to potentially have functional significance (Lozach et al., 2005; Chiu et al., 2007; Bhattacharya et al., 2009; Limjindaporn et al., 2009; Rawlinson et al., 2009; Avirutnan et al., 2010; Heaton et al., 2010; Brault et al., 2011; Khadka et al., 2011). How then can researchers decide which of the remainder of interactions merit further investigation? The number of validated PPI is too small to use as a gold standard for developing a statistical scoring system to rank all PPI, as has been done for other interactomes (Braun et al., 2009; Yu

and Finley, 2009; Yu et al., 2012). Thus, I propose the use of two criteria for prioritizing the dengue-host PPI for further study. The first criterion is based on the observation that PPI detected by multiple independent assays or studies are more likely to be biologically relevant (Uetz et al., 2000; Ito et al., 2001; Deane et al., 2002; von Mering et al., 2002; Giot et al., 2003; Stanyon et al., 2004; Schwartz et al., 2009). Assuming that this is also true for the dengue-host interactions, I counted the number of assays and the number of studies that detected each of the physical interactions. Any orthogonal assay was counted as an individual piece of evidence. Two similar assays that detected the same PPI, but that was conducted by two independent groups, were also counted as two pieces of evidence. By this criterion, 67 of the 403 dengue PPI were detected thus far by more than one assay or study. The second criterion proposed here is based on the fact that many biologically relevant PPI are conserved (Yu et al., 2004), and thus detection of the same interaction in two different species is tantamount to detecting the interaction more than once. Applying this criterion to the dengue-host interactions, I counted a PPI as a potentially conserved interolog if it was found in both mosquito and human. 28 PPI (14 PPI of each species) were detected in both species. I also counted an interaction as having multiple forms of supporting evidence if it was experimentally detected and also computationally predicted (Doolittle and Gomez, 2011). Taking these criteria together, I derive a list of 35 dengue-mosquito PPI and 65 dengue-human PPI with multiple forms of supporting evidence (Figure 2-13; Appendix B). These interaction maps provide a snapshot of the dengue-host PPI that are currently supported by multiple forms of evidence and therefore high priority candidates for further



<u>Figure 2-13.</u> Dengue-host interactions supported by multiple forms of evidence. Pink nodes represent host proteins. Green nodes represent dengue proteins. Red edges represent PPI with conserved interologs. (A) Dengue-human interactome. (B) Dengue-mosquito interactome. Red edges represent PPI conserved in both networks.

investigation. Finally, these data should be useful for developing antiviral drugs and vector control strategies.

2.4 Summary

I identified 102 dengue-mosquito interactions involving 93 unique mosquito proteins and 46 dengue-human interactions involving 35 unique human proteins by Y2H assays using dengue proteins from dengue virus serotype 2. I then re-tested each dengue-host PPI using corresponding dengue proteins from serotypes 1, 3 and 4 to identify 57 out of 102 (56.9%) dengue-mosquito protein interactions and 34 out of 46 (73.9%) dengue-human protein interactions that were serotype independent. I also employed co-affinity purification as an orthogonal assay, which detected 38 out of the 136 interactions (27.9%) previously identified by Y2H screens. Finally, I proposed a list of dengue-host protein interaction candidates for further studies using multiple pieces of supporting evidence as criteria. I hope that the dengue-host interaction data from this project will be useful to generate hypotheses that may be used to develop antiviral drugs, vector control strategies or dengue vaccines to help combat this re-emerging dengue virus.

CHAPTER 3

ANALYSIS OF THE INTERACTION BETWEEN DENGUE CAPSID AND THE HOST NUCLEOSOME ASSEMBLY PROTEIN, NAP1L1

3.1 Introduction

In Chapter 2, I identified several dengue-host PPI for human and mosquito. Out of these, seven PPI were found to be conserved in both human and mosquito based on Y2H assays. Since many biologically relevant PPI are conserved (Yu et al., 2004), these conserved PPI are worthy of further investigation. Among these conserved PPI, I identified human nucleosome assembly protein 1-like 1 (NAP1L1) and mosquito nucleosome assembly protein (AAEL005567) as interactors of dengue capsid using Y2H assays. I also found that capsid from all dengue serotypes interacted with NAP1L1 by Y2H assays, while capsid from serotypes 1, 2 and 3 interacted with AAEL005567. The capsid-NAP1L1 was also detected by co-AP. The capsid-NAP1L1 interaction is, therefore, supported by several forms of evidence, so I set out to study this interaction further.

Although dengue virus replicates in the cytoplasm, capsid has been found in the nucleus, and specifically in the nucleolus (Tadano et al., 1989; Wang et al., 2002; Sangiambut et al., 2008). The function of capsid in the nucleus is not yet clear, but it seems to involve the apoptosis pathway. Capsid can interact with death domain-associated protein (DAXX) in the nucleus resulting in the induction of apoptosis by an unknown mechanism (Limjindaporn et al., 2007; Netsawang et al., 2010). Capsid can

also bind histones resulting in the disruption of nucleosome formation in cells, and potentially altering host gene expression to suit viral replication (Colpitts et al., 2011a). Similarly, capsid proteins from other flaviviruses have been shown to interact with nuclear and, specifically, nucleolar proteins. In West Nile virus (WNV), capsid binds and sequesters the HDM2 ubiquitin ligase into the nucleolus, and thereby prevents the formation of the HDM2 and p53 complex (Yang et al., 2008). Consequently, p53 is stabilized resulting in p53-mediated apoptosis. Capsid of WNV also interacts with a nucleolar helicase, DDX56, in the nucleolus and translocates DDX56 to the cytoplasm (Xu et al., 2011). DDX56 is not required for viral replication, but it does enhance replication 100-fold compared to replication in DDX56-depleted cells (Xu et al., 2011). In Japanese encephalitis virus (JEV), capsid interacts with a nucleolar protein, B23, which seems to be important for virus replication since a dominant negative B23 reduces replication (Tsuda et al., 2006). These studies suggested that nuclear localization of flaviviral capsid is potentially significant for viral replication and pathogenesis.

Nucleosome assembly protein 1 (NAP-1) is a highly conserved protein involved in chromatin assembly (Ishimi et al., 1983; Ishimi and Kikuchi, 1991; Ito et al., 1996; Steer et al., 2003). It functions as a histone chaperone, which loads histones onto naked DNA to form a nucleosome and unloads histones from the nucleosome to disassemble it (Bowman et al., 2011). Yeast (*Saccharomyces cerevisiae*) has one NAP-1, while there are six NAP1 paralogs in human, including nucleosome assembly protein 1-like 1 (NAP1L1), NAP1L2, NAP1L3, NAP1L4, NAP1L5, and NAP1L6. NAP1L1 and NAP1L4 are ubiquitously expressed, while NAP1L2, NAP1L3 and NAP1L5 are neuron-specific (Attia et al., 2011). NAP1L6 is potentially a pseudogene (The UniProt

Consortium(2012)). In mosquito, there are five paralogs of yeast NAP-1, including AAEL005567, AAEL000432, AAEL012289, AAEL013951 and AAEL010809 (Lawson et al., 2009). It is not clear which of these various human and mosquito NAP1 family members are functional orthologs. However, AAEL005567 is more evolutionarily related to Drosophila nap1 and to the six human NAP1Ls than to any of the other mosquito NAP1 orthologs (Figure 3-1). The genes of the NAP1 family seem to have other poorly studied functions in addition to chromosome assembly. Yeast NAP-1, for example, has been shown to play a role in the regulation of mitotic events as it interacts with Cyclin B, kinase Gin4 and NAP1 binding protein 1 (NBP1) (Kellogg and Murray, 1995; Altman and Kellogg, 1997; Shimizu et al., 2000). The interactions of yeast NAP1 with Cyclin B and Gin4 are required for switching from polar to isotropic bud growth (Kellogg and Murray, 1995; Altman and Kellogg, 1997). The interaction between yeast NAP1 and NBP1 is required for the G2/M transition (Shimizu et al., 2000). Human NAP1L1 and the SET nuclear oncogene (SET) share structural similarity, and both proteins can function as host factors required for Adenovirus genome transcription and replication *in vitro* (Kawase et al., 1996). Human NAP1L1 also interacts with a transcriptional coactivator, p300, augmenting p300-dependent transcription, including the transcriptional activities of p53 and E2F (Shikama et al., 2000).

Although the name of the protein suggests that human NAP1L1 locates to the nucleus, the protein is predominantly found in the cytoplasm in various cells (Marheineke and Krude, 1998), a finding that I confirmed in HepG2 cells (Figure 3-2). During M, G1 and S phases, a small amount of human NAP1L1 is observed in the

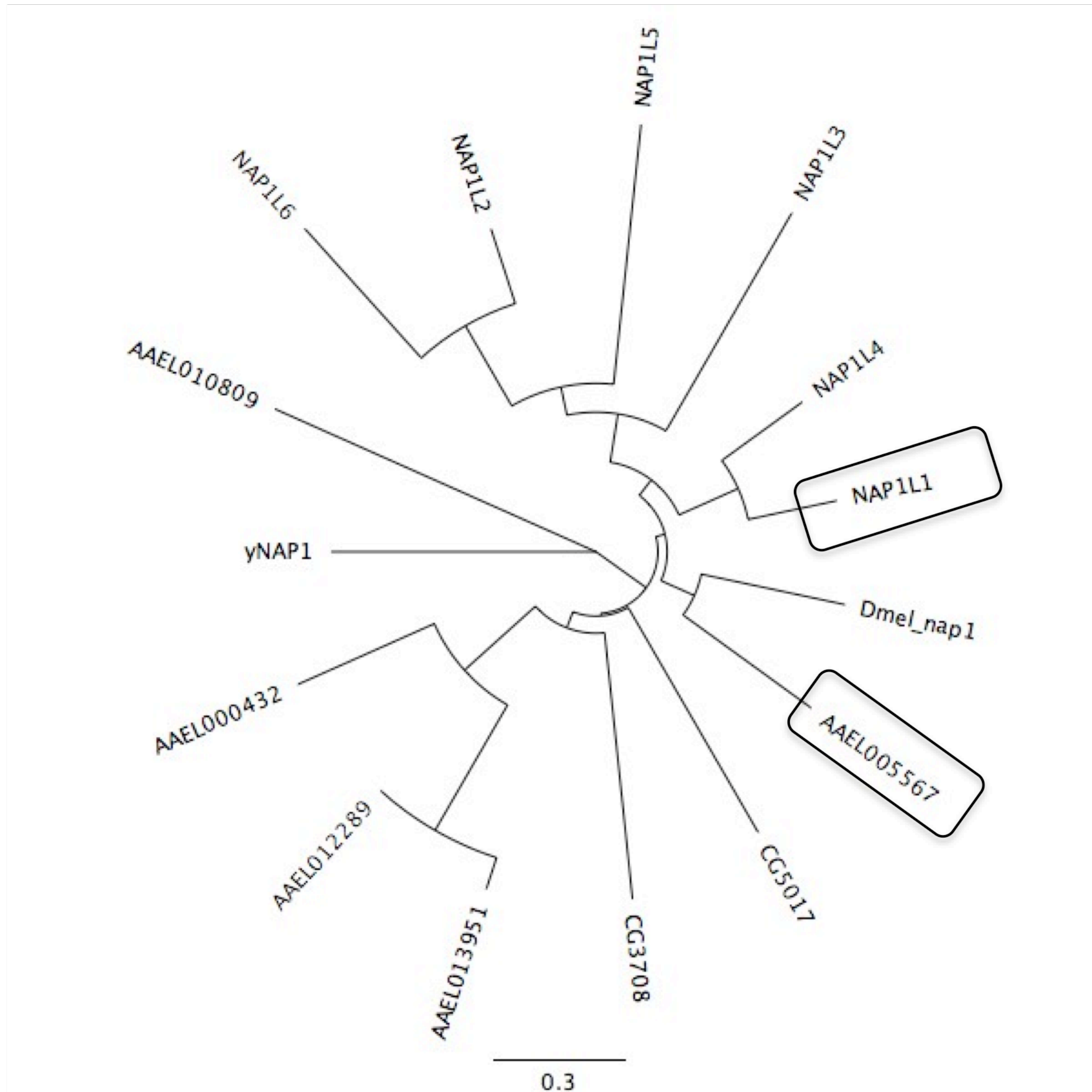


Figure 3-1. The similarity among genes in the Nucleosome Assembly Protein 1 family. A dendrogram shows amino acid sequence similarities among NAP1 genes: *Saccharomyces cerevisiae* NAP1 (yNAP1), *Drosophila melanogaster* NAP1 (Dmel_nap1, CG5017 and CG3708), *A. aegypti* proteins (AAEL005567, AAEL013951, AAEL012289, AAEL000432 and AAEL010809) and human NAP1(NAP1L1, NAP1L2, NAP1L3, NAP1L4, NAP1L5 and NAP1L6). The scale bar indicates amino acid substitution per site. yNAP1 was used as an outgroup. Proteins identified to interact with dengue capsid are circled.

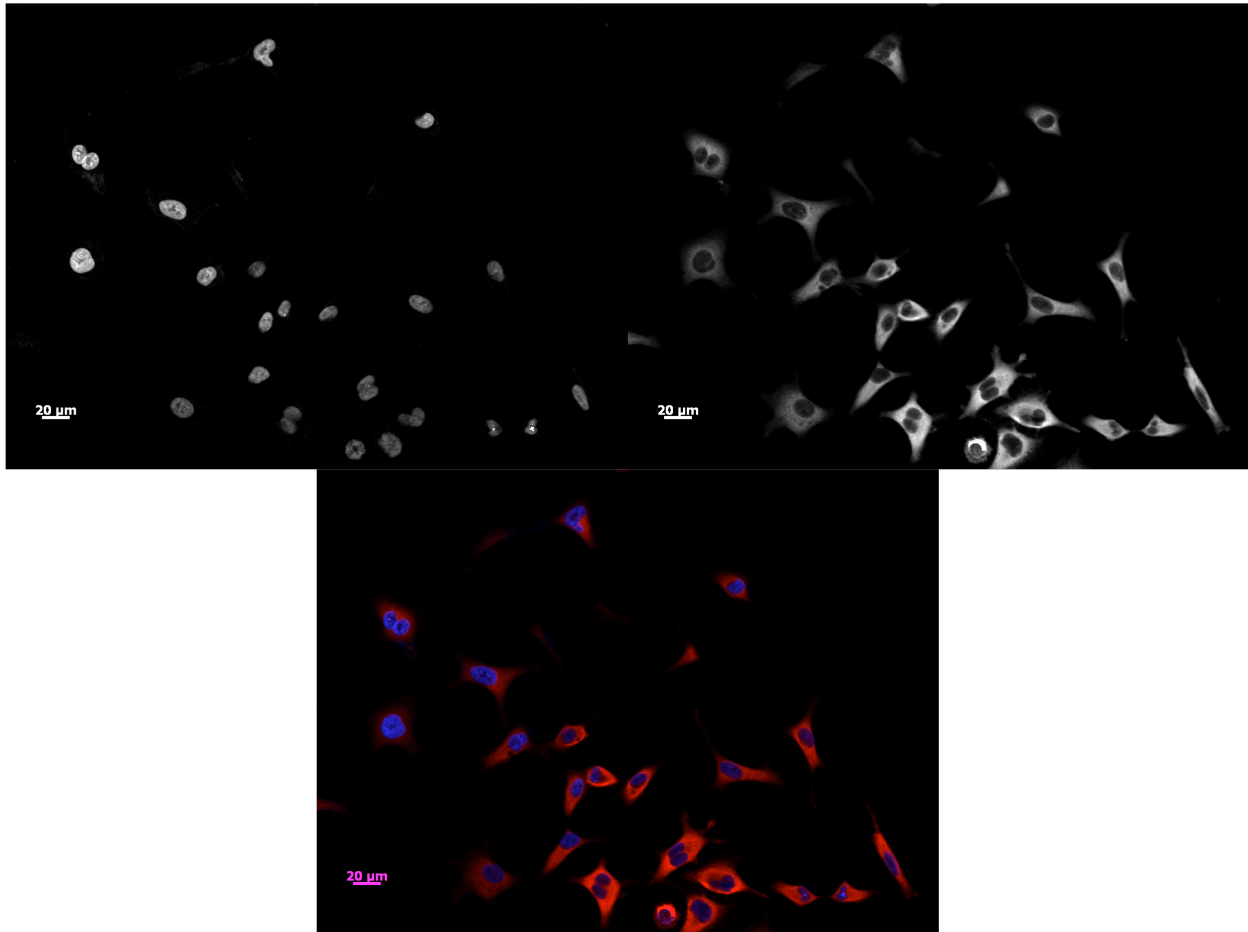


Figure 3-2. The localization of NAP1L1 in A549 cells. A549 cells were stained with anti-NAP1L1 antibody (red in merge) and DAPI. NAP1L1 was mostly localized in the cytoplasm.

nucleus (Marheineke and Krude, 1998). Yeast NAP1 contains a nuclear export sequence (NES), which plays a role in its nucleocytoplasmic shuttling during mitosis (Miyaji-Yamaguchi et al., 2003). Yeast NAP1 also mediates the nucleocytoplasmic transport of other proteins. Yeast NAP1 interacts with yeast Importin (Kap114p) to increase the affinity of Kap114p for the NLS of histone 2A and histone 2B, thereby enchancing nuclear transport of the histones (Mosammaparast et al., 2002). Human NAP1L1 has been shown to interact with the NLS of diacylglycerol kinase zeta (DGKζ) blocking the transport of DGKζ to the nucleus (Okada et al., 2011). It is unknown whether human NAP1L1 and mosquito AAEL005567 also control nucleocytoplasmic transport of other NLS-containing proteins.

A previous study has shown that expression of capsid in human HepG2 cells results in Importin-dependent nuclear localization of capsid (Bhuvanakantham et al., 2009). Because NAP1 proteins are known to play roles in localizing other proteins, I hypothesized that the capsid-NAP1L1 interaction that I discovered may have a role in the nuclear localization of capsid. To test this hypothesis, I first mapped the domains of capsid that are required for interaction with NAP1L1 and AAEL005567 using Y2H assays and co-affinity purification. I also mapped the capsid domains required for interaction with other capsid interactors using Y2H assays. Next, I created stable human cell lines expressing capsid and determined the effect of either silencing or over-expressing NAP1L1 on capsid nuclear localization. The results from this study have suggested an involvement of NAP1L1 in suppressing capsid nuclear localization.

3.2 Materials and methods

3.2.1 Cell lines

HepG2 cells, a human liver carcinoma cell line (Aden et al., 1979), were a gift from Dr. Kezhong Zhang (Wayne State University, Detroit, Michigan). The cells were maintained in DMEM/high glucose + sodium pyruvate (Thermo scientific: SH30243.01) supplemented with 10% FBS and 1X Antibiotic/Antimycotic Solution (Thermo scientific: SV30079.01) at 37°C and 5% CO_2. The cells were passaged weekly at 1:4 to 1:8 dilution. The media was changed every 3-4 days. To dislodge the surface-attached cells, they were treated with 0.05% Trypsin-EDTA for about 5-7 minutes at 37°C. The materials and methods involving Drosophila cells were described in Section 2.2.8.

3.2.2 Plasmids

pcDNA4_Myc_Dest was modified from pcDNA4/TO (Invitrogen) to include an N-terminal Myc tag and a Gateway cassette. First, pcDNA4/TO was cut with *EcoR*V and *Xba*I. Next, a Myc-Gateway cassette was PCR amplified from pHZ12attR with primers MYC3FWD (5'- GCG CAA TTG CAA AAT GCA CCA TCA CCA CCA TCA CGG ATT CGA GCT ATG CGG C-3') and DM140 (See Appendix C for primer sequences). The PCR product was digested with *Xba*I and ligated into the previously digested pcDNA4/TO. The plasmid then was transformed into *E. coli* and selected on LB-Ampicilin/Chloramphenicol. pcDNA4_GFP_Dest was modified from pcDNA4/TO (Invitrogen) to include an N-terminal GFP tag and a Gateway cassette. First, pcDNA4/TO was cut with *EcoR*V and *Xho*I. Next, a GFP-Gateway cassette was PCR amplified from pAGW (The Drosophila Gateway™ Vector Collection, Carnegie

Institution of Washington, Baltimore, Maryland) with primers DM195 and DM196 (See Appendix C for primer sequences). The PCR product was digested with *EcoR*V *and Xho*I and ligated into previously digested pcDNA4/TO. The plasmid then was transformed into *E. coli* and selected on LB-Ampicilin/Chloramphenicol.

3.2.3 Immunostaining

Cover slips were immersed in nitric acid for at least 2 minutes to clean the surfaces. Next, the cover slips were washed with a large volume of de-ionized water. Then, the cover slips were immersed in water, and the pH of the water was measured. If the pH was not near 7.0, the cover slips were rinsed again. After that the cover slips immersed in water were autoclaved for 30 minutes with a liquid cycle. After sterilized cover slips were cool, they were transferred into each well of a 6-well plate within a biosafety cabinet. Then 100-200 μl of Concanavalin A (ConA) was evenly spread on the surface of each cover slip. The cover slips were then incubated at 37°C for 2 hours. After incubation, 1 ml of sterile 1X PBS (8 g/l of NaCl, 0.2 g/l of KCl, 1.44 g/l of Na_2HPO_4, 0.24 g/l of KH_2PO_4, pH to 7.4) was repeatedly added and discarded to each well to wash the cover slips for three times. At this point, the ConA-coated sterilized cover slips were ready to be used or immersed in sterile 2 ml of 1X PBS and kept at 4°C until use. To seed cells onto the prepared cover slip, 1×10^5 cells resuspended in 100-200 μl culture media were spread on each cover slip and allowed to settle for 15-30 minutes. Insect cells were ready for cell fixing or further applications at this point. For human cells, 1 ml of complete media was added to each cover slip, and the cells were

incubated overnight at 37°C in 5% CO_2. The cells could then be fixed or used for further applications like transfection or RNAi assays.

To fix the cells, cover slips with seeded cells were washed twice with 1X PBS pre-warmed at 37°C (at this point cells did not need to be under sterile conditions). 1 ml of 4% (w/v) para-formaldehyde in 1X PBS pre-warmed at 37°C was added to each cover slip. The cover slips were incubated at room temperature for 10 minutes. Next, the cover slips were washed with 1 ml of 1X PBS three times with a 10-minute agitation at room temperature between washing. 1 ml of blocking solution containing 0.2% Triton-x, 5% BSA and 1X PBS was added to each cover slip and incubated for 1 hour at room temperature or 4°C overnight to three days. To stain the cells, the primary antibodies were diluted to the appropriate concentrations in 200 µl of staining buffer containing 5% BSA in 1X PBS supplement with 0.05% (v/v) Tween-20 (1X PBS-T) (1:100 for anti-myc from Santa Cruz Biotechnology and 1:500 for anti-NAP1L1 from Abcam). The 200 µl of diluted antibody solution was dropped onto a piece of parafilm, and the coverslip was then put on top of it with the cells facing down. The cover slip was incubated for 1-3 hours at room temperature. Next, the cover slip was placed back into a 6-well plate with the cells facing up and washed three times with 1X PBS for 10 minutes of agitation. The secondary antibodies were diluted to the appropriate concentrations in 200 µl of staining buffer (1:1,000 for Dylight goat anti-mouse from Invitrogen and 1:200 TexasRed or FITC conjugated goat anti-rabbit from Invitrogen). The 200 µl of diluted antibody solution was dropped onto a piece of parafilm, and the coverslip was then put on the top of it with the cells facing down. The cover slip was incubated for 1 hour at room temperature. Next, the cover slip was placed back into a 6-well plate with the cells facing up and washed

with 1X PBS with 10 minutes of agitation for three times. Finally, the cover slip was mounted in 20 µl of anti-fade solution (0.466 g of DABCO, 15 ml of glycerol, 5 ml of 1X PBS) containing DAPI on top of a slide. The edges of the cover slip were sealed with nail polish. The slide could be kept at 4°C in the dark for up to one year.

3.2.4 Human cell transfections

For DNA transfection, $2x10^5$ cells were seeded onto a 6-well plate one day before transfection. Cells seeded onto the cover slip may also be used for transfection. For stable transfection, a plasmid must be linearlized with a restriction enzyme. For example, pcDNA4/TO must be cut with *Pci*I. On the day of transfection, 1 µg of plasmid DNA was diluted in 100 µl of EC buffer (Qiagen Effectene Transfection Kit), and then 8 µl of enhancer (Qiagen Effectene Transfection Kit) was added to the dilution followed by vortexing for 2 seconds. The transfection reaction was incubated at room temperature for 5 minutes. Next, 10 µl of Effectene (Qiagen Effectene Transfection Kit) was added to the reaction followed by vortexing for 10 seconds. The reaction was incubated for 10 minutes at room temperature. At the same time, the media of the seeded cells was discarded and replaced with 1.5 ml of fresh media. After incubation, 600 µl of media was used to dilute the transfection reaction. The reaction was then added to the cells dropwise. The plate was gently swirled to evenly distribute transfection complexes and then put back into the cell culture incubator. The next day, the media was changed to remove transfection reagents, which may be cytotoxic under prolonged exposure. After further incubation for 48 hours, the cells may be used for immunostaining or Western analysis, or 200 µg/ml of Zeocin (Invitrogen) may be added to cells to select for a stable

cell line. Selection for the stable cell line may take 3-5 weeks. During selection, the media supplemented with Zeocin was changed twice a week. Once cell foci were visible, they were individually picked by using a 6x8 mm cloning cylinder (Fisher Scientific). Briefly, the cylinder and vacuum gel were sterilized by autoclaving before use. The cylinder was gently touched onto the vacuum gel so that a thin film of gel covered one side of the cylinder. Next, the cylinder was placed to encircle the focus of cells. 30 µl of Trypsin was added into the cylinder followed by incubating at 37°C for 5-7 minutes. The cells were dislodged by repeatedly pipetting, and then transferred into a 24-well plate with the fresh media supplemented with Zeocin for propagation.

siRNA transfection was performed with HiPerFect Transfection Reagent (Qiagen). The siRNAs were purchased from Qiagen (Hs_NAP1L1_5, Hs_NAP1L1_6, Hs_NAP1L1_10, Hs_NAP1L1_4, AllStars Negative Control siRNA and AllStars Hs Cell Death Positive Control siRNA). One day before transfection, the cells were seeded onto the cover slip as described above. 300 ng of siRNA was diluted in 100 µl of serum-free medium, and then 12 µl of HiPerFect Transfection Reagent was added to the dilution followed by vortexing for 2 seconds. The mixture was incubates for 10 minutes at room temperature. Next, the mixture was gently added dropwise onto the cells. The plate was then gently swirled to evenly distribute transfection complexes. The cells were incubated overnight, and then the medium was changed. The cells were incubated until the cells that were transfected with the positive control siRNA started dying. Then, the cells were fixed and immunostained.

3.2.5 Cell lysis and Western analysis for human cells

Human cell lysis was performed as described in Section 2.2.8, but the transfection step was perform as in Section 3.2.4, and NET lysis buffer was replaced with RIPA lysis buffer (50 mM Tris pH 7.2, 150 mM NaCl, 0.1% (w/v) NaCl, 0.5% (w/v) sodium deoxycholate, 1% (v/v) NP-40, 1X protease inhibitors cocktails, 1 mM PMSF) or nuclear lysis buffer (0.5 M NaCl, 50 mM Tris pH 7.5, 1% (v/v) NP-40, 1% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 2 mM EDTA and 1X protease inhibitor cocktails, 1 mM PMSF). In addition, the lysis reaction was adjusted to a 6-well plate format. Western analysis was performed as described in Section 2.2.8. In addition to anti-Myc, Rabbit anti-NAP1L1 (Abcam) and Mouse anti-capsid (a gift from Dr. Chunya Puttikhunt, Siriraj Hospital, Mahidol University, Bangkok, Thailand) were used as a primary antibody with a 1:1000 dilution and 1:3 in 5% (w/v) milk in 1X PBS, respectively.

3.2.6 Capsid domain mapping

Three deletion mutants of dengue capsid were generated by PCR amplification of different fragments from pDONR221_D2CA using Herculase polymerase (Agilent Technologies) according to the manufacturer's instructions. CΔ1-9 was PCR amplified with primers, DM193 and DM5. CΔNLS (or CΔ85-100) was PCR amplified with primers, DM3 and DM178. CΔ73-100 was PCR amplified with primers, DM3 and DM194. Cmut containing mutations, R85A and K86A, was a gift from Dr. Thawornchai Limjindaporn (Siriraj hospital, Mahidol University, Bangkok, Thailand) (Netsawang et al., 2010). Cmut was PCR amplified by primers, DM3 and DM5. All PCR products of capsid mutants were PCR amplified by primers, DM1 and DM2, to add attB tags. Finally, PCR products

were transferred into pDONR221 by BP clonase (Invitrogen). Entry clones were verified by DNA sequencing. Capsid mutants were then transferred to the destination vectors, pNLex_attR (Stanyon et al., 2003) and pHZ13attR, by LR clonase (Invitrogen). Capsid mutants in pNLex_attR were transformed into Yeast strain RFY309 and used in Y2H assays against all capsid interactors. Capsid mutants in pHZ13attR were used in Co-AP experiments using methods described in Section 2.2.8.

Human NAP1L1 in pDONR223 was retrieved from a human ORF library (Lamesch et al., 2007). Full-length mosquito AAEL005567 was PCR amplified from mosquito Y2H cDNA library by primers, DM190 and DM191. The PCR product was cloned into pDONR221 by BP clonase (Invitrogen). Human NAP1L1 and mosquito AAEL005567 in pDONR were transferred to pJZ4_attR (Stanyon et al., 2003) and pHZ12attR by LR clonase (Invitrogen). NAP1L1 and AAEL005567 in pJZ4_attR were transformed into Yeast strain RFY231 and used for Y2H assays against capsid and its mutants. NAP1L1 and AAEL005567 in pHZ12attR were used in Co-AP experiments using methods described in Section 2.2.8.

3.3 Results and discussion

3.3.1 The C-terminus of capsid potentially mediates the interaction between capsid and NAP1L1, and between capsid and AAEL005567

In Chapter 2, I found that dengue capsid interacts with human NAP1L1 and mosquito AAEL005567 suggesting a conserved protein interaction (interolog). Interestingly, previous studies have shown that capsid shuttles between the nucleus and the cytoplasm (Tadano et al., 1989; Wang et al., 2002; Sangiambut et al., 2008).

Capsid contains three potential nuclear localization signals (NLS) at amino acid residues 6-9, 73-76 and 85-100 (Figure 3-3) (Bulich and Aaskov, 1992). The NLS at residues 85-100 of capsid has been found to mediate the interaction with Importin alpha in capsid from both dengue virus and WNV (Bhuvanakantham et al., 2009). This NLS is classified as a bipartite signal (Wang et al., 2002), which is the same type as the NLS found in another NAP1L1-binding protein, DGKζ (Okada et al., 2011). Thus, I hypothesized that capsid interacts with NAP1L1 and AAEL005567 using its bipartite NLS.

To test this hypothesis, I constructed four mutants of capsid in which each NLS was either deleted or mutated (Figure 3-3). Next, I screened all mutants against NAP1L1 and AAEL005567 using Y2H assays (Figure 3-4). Interestingly, deletion of the bipartite sequence (residues 85-100) either alone or along with the middle NLS (residues 73-100) dramatically reduced LacZ reporter activity indicating impaired interaction with both NAP1L1 and AAEL005567. Activation of the more sensitive Leu2 reporter, however, was affected only for the interaction between CΔ73-100 and NAP1L1. Deletion of the N-terminal NLS (residues 1-9) did not affect the interactions. These results indicate that residues 85-100 of capsid are important but not essential for the interaction between capsid and NAP1L1 or AAEL005567. I also tested a capsid mutant (Cmut) with a defective bipartite NLS. Cmut, which has two single amino acid changes (R85A, K86A), has been shown to be defective for nuclear localization and for interaction with DAXX (Netsawang et al., 2010). The defective bipartite sequence did not interfere with the interaction between capsid and NAP1L1 or AAEL005567. These data suggest that while the bipartite NLS contributes to the interaction with NAP1L1 or

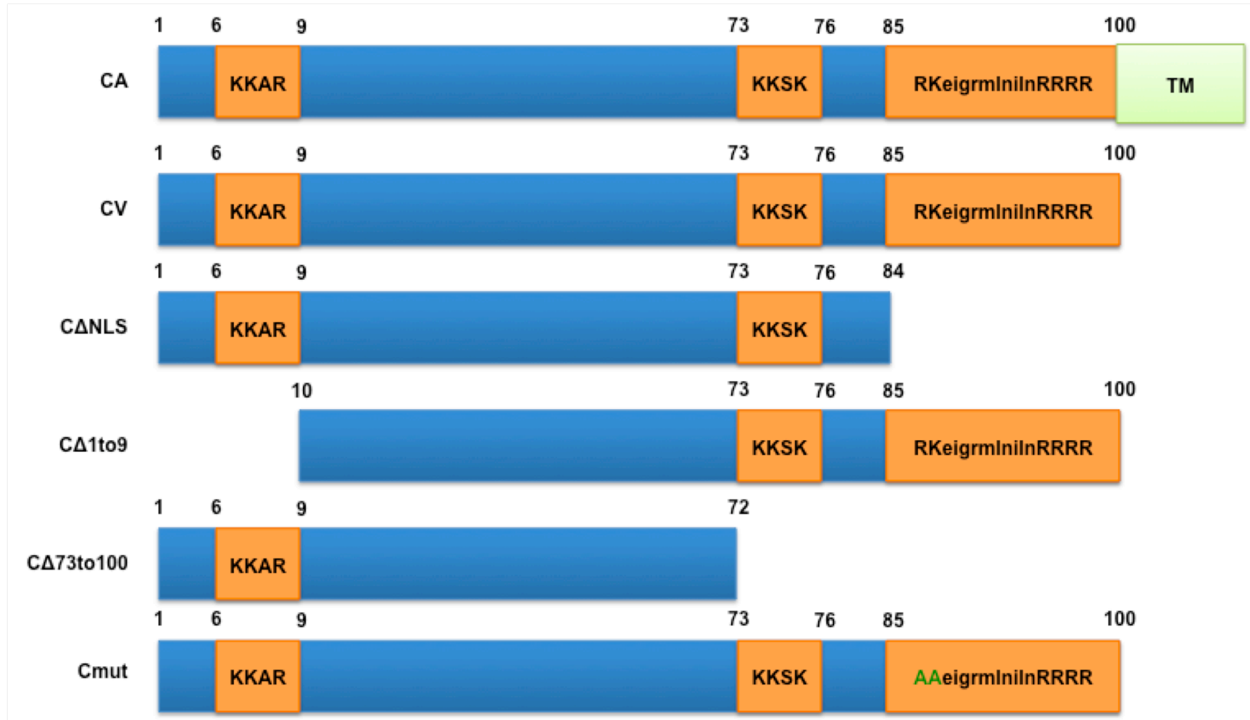


Figure 3-3. The constructs of dengue capsid used in this project. CA is the immature capsid still containing the transmembrane domain (TM). CV is the mature capsid used as the wild-type. CΔNLS is the capsid with the bipartite NLS (residues 85-100) deleted. CΔ1-9 is the capsid with the N-terminal NLS (residues 1-9) deleted. CΔ73to100 is the capsid with the middle NLS (residues 73-76) and the bipartite NLS deleted. Cmut is the capsid with two amino acid substitutions (green) in the bipartite NLS (R85A and K86A).

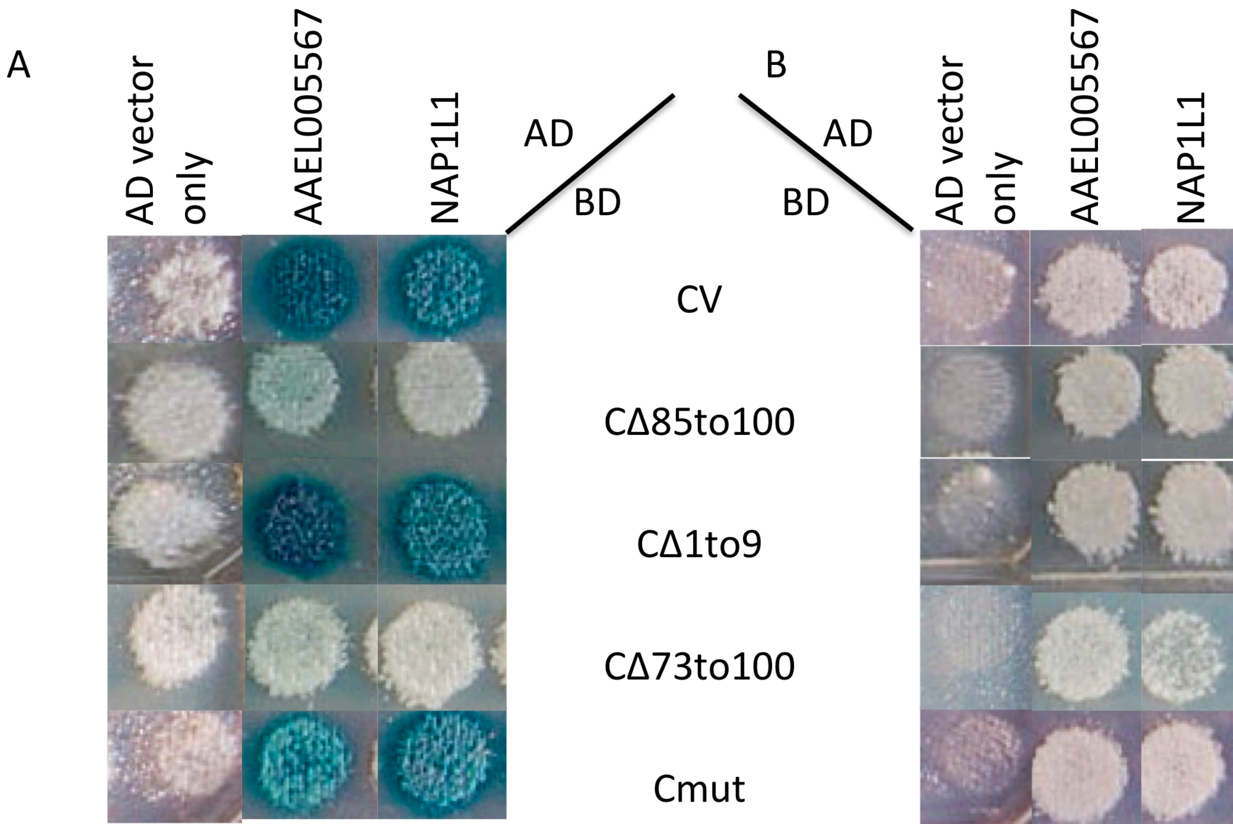


Figure 3-4. Y2H results from capsid domain mapping. Capsid and its mutants were expressed in Y2H BD strains (rows), while human NAP1L1 (NAP1L1) and mosquito NAP1 (AAEL005567) were expressed in Y2H AD strains (columns). The diploids from the screen were plated on media containing X-gal (A) or lacking leucine (B). Growth on the plates lacking leucine indicates an interaction. Blue on X-gal plates indicates a strong interaction.

AAEL005567, it does not need to be functional for nuclear localization. I also tested the interactions between the capsid mutants and AAEL005567 by Co-AP in S2R+ cells (Figure 3-5). The results were similar to those for Y2H. Deletion of the bipartite sequence (residues 85-100) dramatically reduced but did not eliminate the amount of AAEL005567 protein that co-purified with capsid. Deletion of the N-terminal NLS (residues 1 -9) did not reduce the amount of co-purified AAEL005567. Again, these results suggest that the C-terminal bipartite NLS of capsid is important for the interaction with AAEL005567 protein, but it is not essential.

Surprisingly, mutations of capsid did not reduce the amount of co-purified human NAP1L1 (Figure 3-6). This result contradicted the Y2H data and the Co-AP data with AAEL005567 protein. The Co-AP control sample showed a high background of co-purified NAP1L1 with the NTAP tag alone, so the contradicting Co-AP results might be due to non-specific binding of NAP1L1 to the agarose beads. This could be tested in a repeat experiment using less protein and more washing steps to try to reduce the background. Alternatively, the different result with mosquito AAEL005567 and human NAP1L1 may be due to expression of the human NAP1L1 in insect cells, which is not its natural cellular environment. Thus, it will be important to test the capsid-NAP1L1 interaction in human cells.

I also screened the capsid mutants against the other host capsid interactors in order to identify the regions of capsid that are required for their interactions (Figure 3-7 and Table 3-1). Out of 33 host proteins tested, three required residues 10-72, eight required residues 73-84, and six required residues 73-100. Twelve interactors required

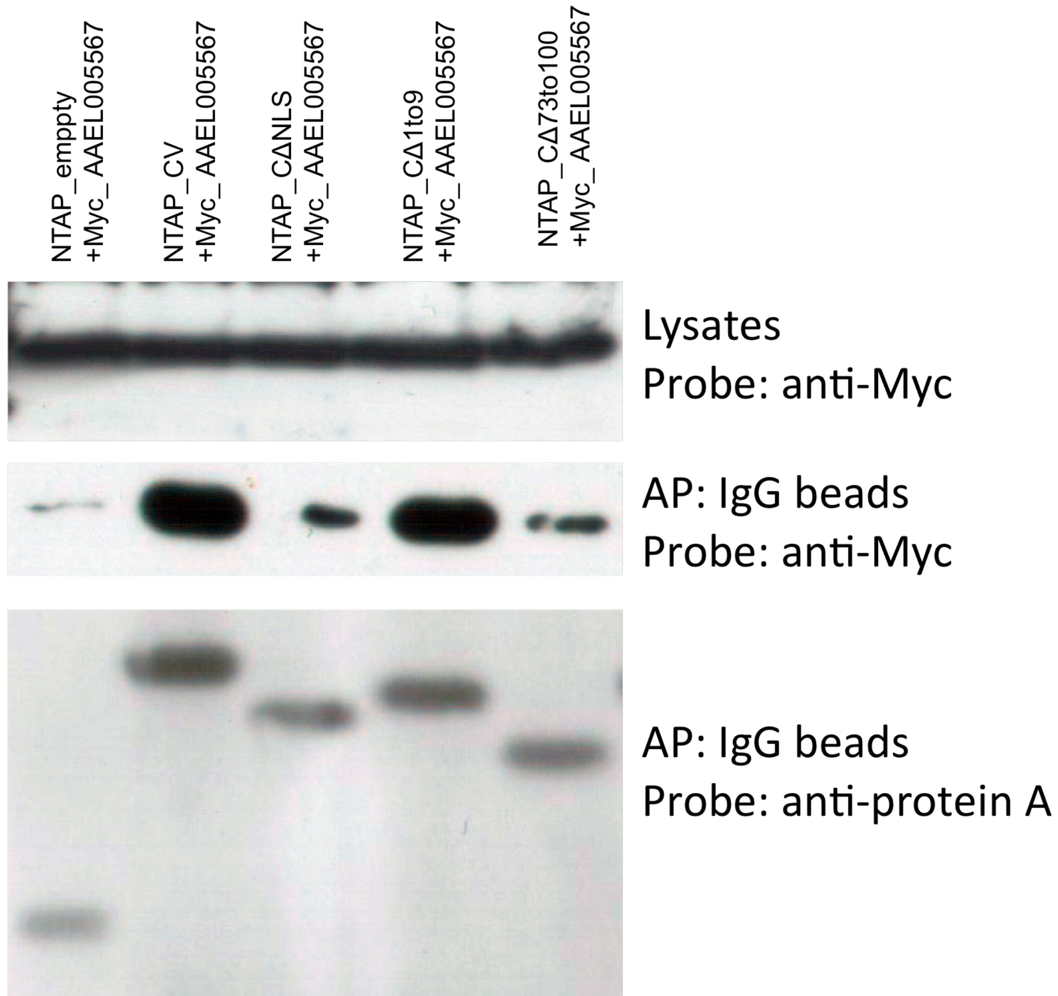


Figure 3-5. Co-affinity purification of mosquito AAEL005567 with capsid and its mutants. The capsid and its mutants were affinity purified with IgG agarose beads. Purified samples were probed with anti-Myc and anti-Protein A.

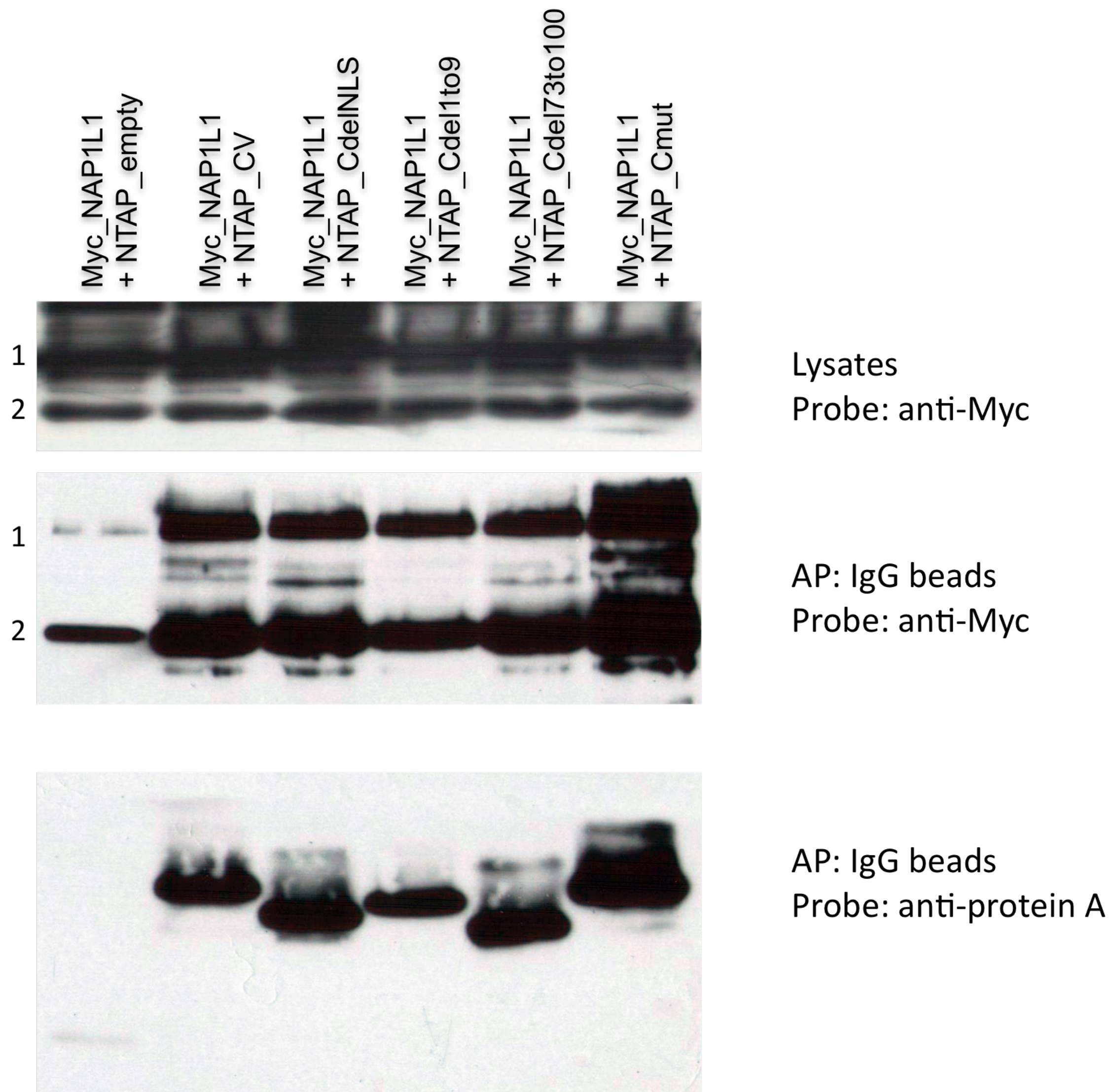
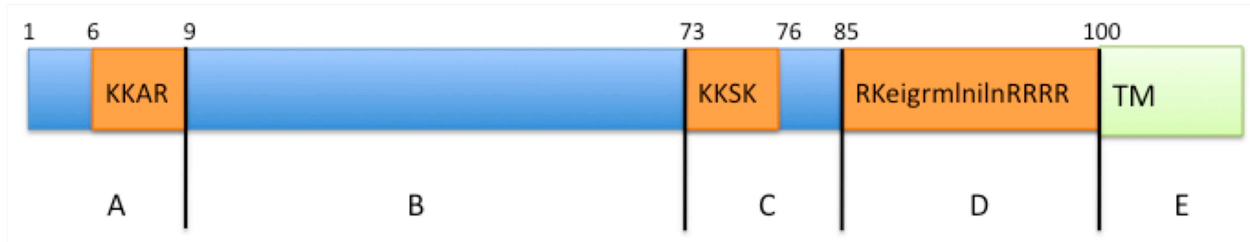


Figure 3-6. Co-affinity purification of human NAP1L1 with capsid and its mutants. Capsid and its mutants were affinity purified with IgG agarose beads. Purified samples were probed with anti-Myc and anti-Protein A. The antibody against human NAP1L1 recognized two bands (noted by numbers 1 and 2).



<u>Figure 3-7.</u> Y2H domain mapping. A is the region between amino acid residues 1 to 9. B is the region between residues 10 to 72. C is the region between residues 73 to 84. D is the region between residues 85 to 100. E is the transmembrane domain. The description here is used for Table 3-1

<u>Table 3-1.</u> Domain mapping of capsid for the region that is responsible for the interactions with host proteins. Capsid mutants are shown in Figure 3-7. Interface categories are denoted by letters (B, C, D and U), which are explained in Figure 3-7.

gene_ID	Name_of_gene	CV _growth	CV _score	CA _growth _diffCV	CA _score _diffCV	Cdel1to9 _growth _diffCV	Cdel1to9 _score _diffCV	Cdel73to100 _growth _diffCV	Cdel73to100 _score _diffCV	CdelNLS _growth _diffCV	CdelNLS _score _diffCV	Cmut _growth _diffCV	Cmut _score _diffCV	Interface _catagorize
AAEL003750	conserved hypothetical protein	1	2	0	1	0	3	0	-1	0	0	0	1	B
AAEL013075	conserved hypothetical protein	1	3	0	-1	0	1	0	1	0	-2	0	-2	B
ENSG00000132842	Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	1	0	4	0	1	0	1	0	2	0	0	B
AAEL004855	adp,atp carrier protein	1	4	0	-3	0	0	-1	-4	0	-3	0	0	C
AAEL008852	conserved hypothetical protein	1	5	0	-3	0	2	-1	-5	0	-3	0	2	C
AAEL009285	dead box atp-dependent rna helicase	1	5	-1	-5	0	-1	-1	-5	0	-4	0	-2	C
AAEL011960	conserved hypothetical protein	1	4	0	0	0	1	-1	-3	0	-1	0	0	C
AAEL011985	conserved hypothetical protein	1	6	0	-3	0	0	-1	-6	0	-4	0	0	C
AAEL013583	60S ribosomal protein L23	1	4	0	-2	0	1	-1	-4	0	-2	0	-1	C
ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	1	5	0	-3	0	0	-1	-5	0	-4	0	-1	C
ENSG00000107937	Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	1	3	-1	-3	0	1	-1	-3	0	-2	0	-1	C
AAEL000005	hypothetical protein	1	5	0	-3	0	1	-1	-5	0	-3	0	-1	C+D
AAEL001984	hypothetical protein	1	6	0	-4	0	1	-1	-6	0	-4	0	-1	C+D
ENSG00000110330	Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	5	0	-1	0	3	0	-2	0	-1	0	0	C+D
ENSG00000122406	Homo sapiens ribosomal protein L5 (RPL5), mRNA	1	4	-1	-4	0	1	0	-3	0	-2	0	-2	C+D
ENSG00000171863	Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	5	0	-2	0	-1	0	-4	0	-4	0	-2	C+D
ENSG00000206172	Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	1	3	0	-2	0	0	-1	-3	0	-2	-1	-3	C+D
AAEL000292	conserved hypothetical protein	1	5	0	-4	0	1	-1	-5	-1	-4	0	0	D
AAEL002057	conserved hypothetical protein	1	4	-1	-4	0	1	-1	-4	-1	-4	0	0	D
AAEL003676	myosin I homologue, putative	1	6	-1	-6	0	1	-1	-6	-1	-6	0	0	D
AAEL004316	hypothetical protein	1	5	-1	-5	0	-2	-1	-5	-1	-5	0	-2	D
AAEL004869	hypothetical protein	1	6	-1	-6	0	-1	-1	-6	-1	-6	0	-1	D
AAEL010782	carboxypeptidase	1	3	-1	-3	0	1	-1	-3	-1	-3	0	0	D
ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	7	0	-2	0	1	0	-3	0	-4	0	0	D
ENSG00000101745	Homo sapiens ankyrin repeat domain 12 (ANKRD12), transcript variant 2, mRNA	1	5	0	-2	0	1	-1	-5	-1	-5	0	-2	D
ENSG00000144747	Homo sapiens TATA element modulatory factor 1 (TMF1), mRNA	1	3	-1	-2	0	4	-1	-3	-1	-3	0	1	D
ENSG00000160654	Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G), mRNA	1	1	0	0	0	1	-1	-1	-1	-1	0	1	D
ENSG00000160908	Homo sapiens zinc finger protein 394 (ZNF394), mRNA	1	5	-1	-5	0	0	-1	-5	-1	-5	0	-3	D
ENSG00000205744	Homo sapiens DENN/MADD domain containing 1C (DENND1C), mRNA	1	2	-1	-2	0	2	-1	-2	-1	-2	0	0	D

the bipartite sequence (residues 85-100), and four interactors could not be classified. In addition, 15 host proteins seemed to require the functional bipartite NLS of capsid for efficient binding. It is worth noting that three out of the four unclassified interactors were detected with immature capsid containing a transmembrane domain, but not with the virion capsid. These mapping results require further validation with Co-AP assays. These results also demonstrated that all capsid mutants were stable enough to interact with some proteins in Y2H assays. Thus, the domain mapping of dengue capsid with NAP1L1 and AAEL005567 are unlikely to be false results caused by the instability of the mutant proteins.

3.3.2 NAP1L1 may regulate the nuclear localization of capsid in human cells

From Section 3.3.1, Y2H and Co-AP assays have suggested that NAP1L1 and AAEL005567 may modulate the nuclear localization of capsid since NAP1L1 and AAEL005567 require the bipartite NLS of capsid for efficient binding. To examine this, I set out to test whether a change in NAP1L1 or AAEL005567 expression can affect the nuclear localization of capsid. First, I established stable human cell lines expressing capsid. I generated two HepG2 cell lines expressing either N-terminal Myc-tagged capsid or N-terminal GFP-tagged capsid. I found that both myc-capsid and GFP-capsid localized to the nucleus and to concentrated regions within the nucleus that may correspond to the nucleolus (Figure 3-8). These results are similar to the previously reported localization of capsid (Wang et al., 2002). I also detected Myc-tagged capsid in the stably transfected cells by immunoblotting (Figure 3-9).

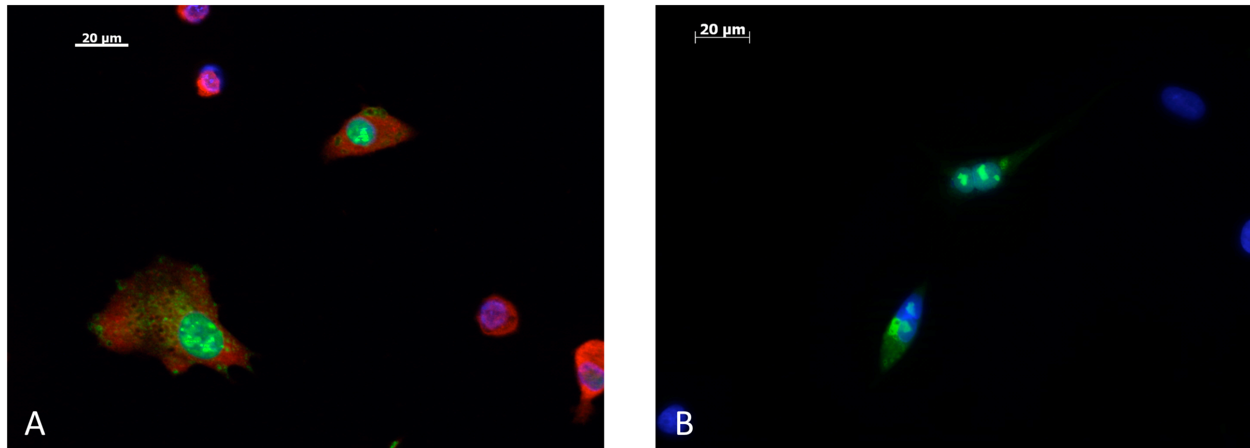
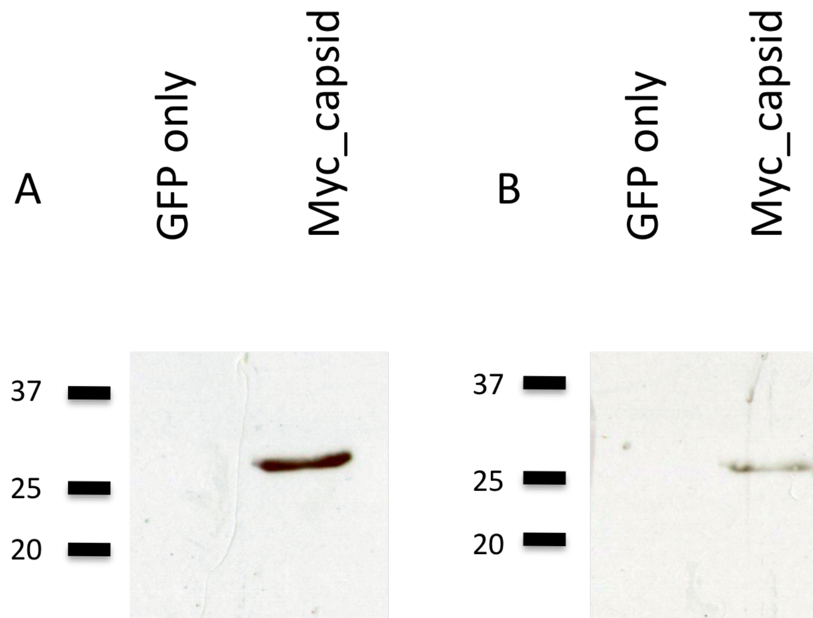


Figure 3-8. Stable HepG2 cells expressing capsid. (A) HepG2 cells expressing Myc-capsid. The cells were stained with anti-myc (green), anti-NAP1L1 (red) and DAPI (blue). (B) HepG2 cells expressing GFP-capsid (green). The cells were stained with DAPI.



<u>**Figure 3-9.**</u> **Expression of capsid in the stable cell line.** The lysate of GFP-transfected HepG2 cells (GFP only) and the lysate of HepG2 cells stably expressing Myc-capsid (Myc_capsid) were analyzed by Western blot. (A) The membrane was probed with anti-Myc. (B) The membrane was probed with anti-capsid.

Next, I set out to test the role of NAP1L1 in the nuclear localization of dengue capsid. First, I tested whether depletion of NAP1L1 would affect the localization of capsid. I treated HepG2 cells stably expressing Myc-capsid with four NAP1L1 siRNAs. Among these, Hs_NAP1L1_5 depleted most of NAP1L1 while Hs_NAP1L1_4 and Hs_NAP1L1_6 resulted in no and modest depletion of NAP1L1, respectively (Figure 3-10). I failed to obtain lysate from cells treated with Hs_NAP1L1_10. Immunostaining of Hs_NAP1L1_5-treated cells also showed that NAP1L1 expression was reduced compared to negative siRNA-treated cells (Figure 3-11). I did not observe any change in the nuclear localization of Myc-capsid as a result of NAP1L1 knock down (Figure 3-11). Next, I tested whether over-expression of NAP1L1 would affect the localization of capsid. I transfected either GFP or GFP-NAP1L1 into HepG2 cells stably expressing Myc-capsid. I found that capsid was localized more in the cytoplasm in some GFP-NAP1L1-transfected cells compared to GFP-transfected cells (Figure 3-12). This suggests that over-expressed NAP1L1 may inhibit the nuclear localization of dengue capsid.

The results from over-expressing NAP1L1 are still preliminary. Since not all GFP-NAP1L1-transfected cells showed the same change in capsid nuclear localization, they have to be statistically analyzed to determine the proportion of cells affected by NAP1L1 over-expression. Furthermore, a Co-AP assay is required to determine whether endogenous NAP1L1 would co-purify with capsid. Finally and importantly, the role of NAP1L1 and AAEL005567 during live virus replication should be investigated.

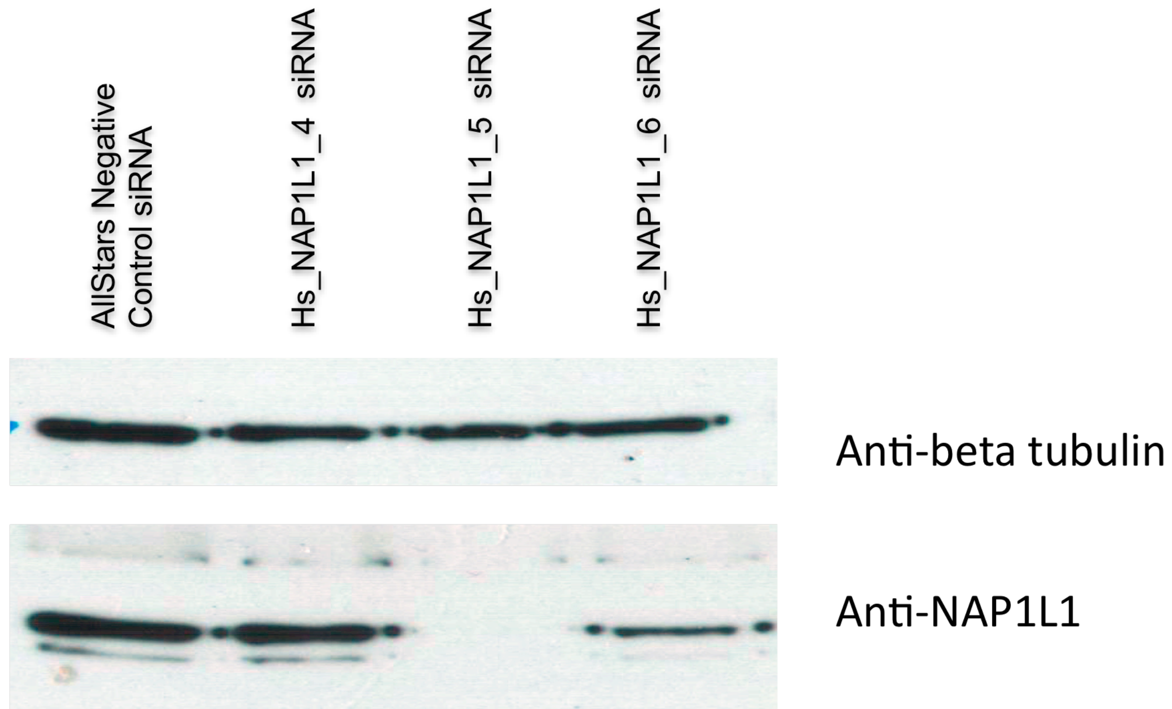
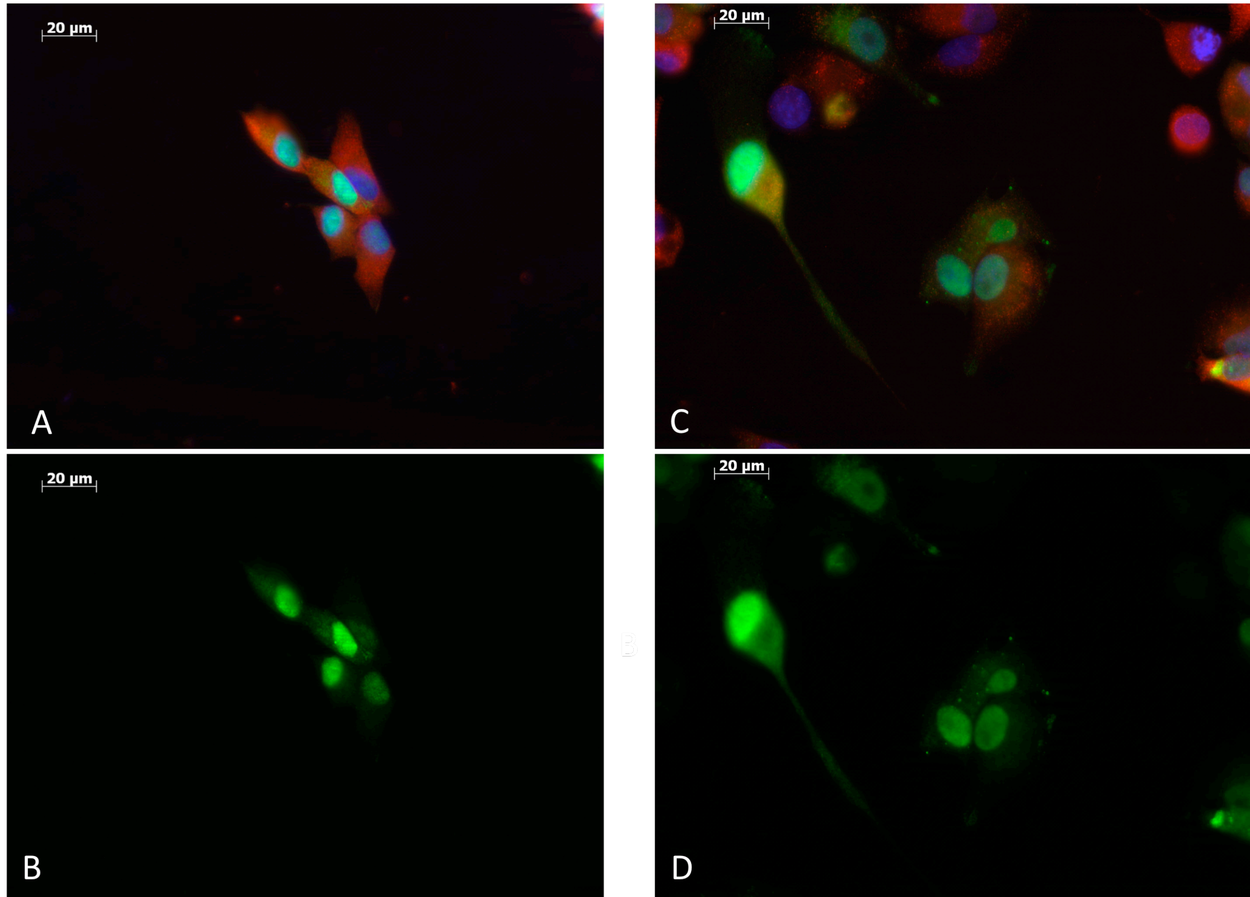
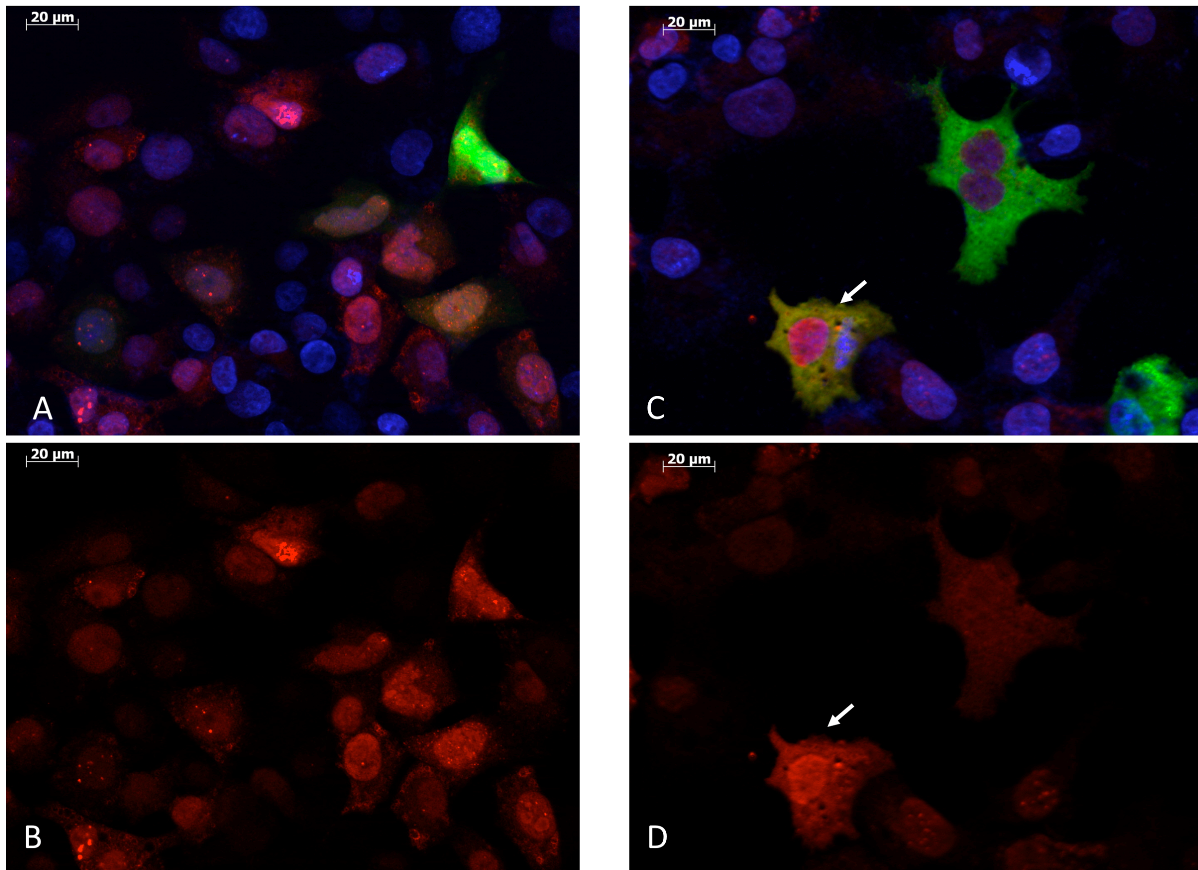


Figure 3-10. Expression of NAP1L1 when the cells were treated with siRNA. The HepG2 cells stably expressing Myc-capsid were treated with siRNA. The lysates were analyzed by Western analysis. Anti-beta tubulin was used to quantify total proteins.



<u>Figure 3-11.</u> Nuclear localization of capsid is not altered by silencing of NAP1L1. (A) HepG2 cells stably expressing Myc-Capsid treated with AllStars Negative Control siRNA were stained with anti-Myc (green), anti-NAP1L1 (red) and DAPI. (B) Only the green channel (anti-myc) is shown. (C) HepG2 cells stably expressing Myc-Capsid treated with Hs_NAP1L1_5 siRNA were stained with anti-Myc (green), anti-NAP1L1 (red) and DAPI. (D) Only the green channel (anti-myc) is shown. The red channel was equally exposed for 5 seconds for each sample.



<u>Figure 3-12.</u> Over-expression of NAP1L1 affects localization of capsid. (A) HepG2 cells stably expressing Myc-capsid transfected with GFP (green) were stained with anti-myc(red) and DAPI (blue). (B) Only the red channel (anti-myc) is shown. (C) HepG2 cells stably expressing Myc-capsid transfected with GFP-NAP1L1 were stained with anti-Myc (red) and DAPI (blue). (D) Only the red channel (anti-myc) is shown. The red channel was equally exposed for 5 seconds for each sample. Cells expressing NAP1L1 have increased Myc-capsid in the cytoplasm (arrows)

3.4 Summary

I hypothesized that the NLS of dengue capsid might be required for the interaction with human NAP1L1 and mosquito AAEL005567. I constructed mutant capsid proteins with one or more NLS deleted. I also included the mutant of capsid containing amino-acid substitution at the bipartite NLS that has been shown to disrupt an interaction with Importin (Netsawang et al., 2010). Using Y2H assays and Co-AP assays with these mutants, I found that the bipartite NLS (amino acid residues 85-100) of dengue capsid is required for efficient interaction with NAP1L1 and AAEL005567. However, the amino acid substitution did not disrupt the interaction suggesting that the interface of interaction of capsid with NAP1L1 and AAEL005567 may be different from that of Importin. I also mapped the regions of capsid that might be required for its interactions with human and mosquito proteins. Finally, I have shown that over-expression of NAP1L1 may inhibit the nuclear localization of capsid. These results have to be further investigated in models of virus infection and replication. In Chapter 5, I further discuss the implications of these results and I suggest future experiments to explore the functional significance of the capsid-NAP1L1 interaction.

CHAPTER 4

TOWARDS A FUNCTIONAL ASSAY FOR DENGUE-HOST INTERACTIONS

4.1 Introduction

Identification of a protein-protein interaction (PPI) may hint at the functions of a protein by associating it with the known functions of its interacting partner. However, the functional consequences of a PPI, such as inhibition or activation of one protein by another, are seldom revealed by the identification of that PPI alone. Further assays are required to understand the function of a PPI. In some cases, functional screens have been implemented regardless of the knowledge of PPI. Sessions et al., for example, used RNA interference assays in Drosophila cells to identify host factors that are either essential for or repressive against dengue replication (Sessions et al., 2009). They identified 116 Drosophila genes that when silenced led to the suppression of dengue replication. They called these genes dengue virus host factors (DVHF). The human orthologs of 42 of the Drosophila DVHFs were shown to be required for dengue replication in human cells. These DVHFs may be targets for the development of an antiviral drug. One of DVHFs, TRIP11, was subsequently found to physically interact with NS5 by Y2H (Khadka et al., 2011). However, we have no clue how the virus interacts with the other DVHFs. The DVHFs could be tested for PPI with dengue proteins to determine how they interact with the virus. However, these DVHFs may indirectly interact with dengue virus by interacting with other host proteins that physically interact with dengue proteins.

An alternative way to identify functional PPI is to first identify PPI and then assay for their functions. Khadka et al., for example, screened dengue proteins against a human liver library using a yeast two-hybrid assay (Khadka et al., 2011). They identified 139 dengue-human PPI involving 109 human genes. They then selected twelve dengue interactors for RNA interference assays and found that six of them were essential for efficient viral replication. These results provided a connection between the PPI data and the functions of the PPI. Such information could be used, for example, to design antiviral therapies by targeting the PPI found to be essential for the virus.

PPI identified by my study have expanded our knowledge of how dengue virus may interact with its hosts, but I still do not know the significance and function of most of these PPI. It would be ideal to study the effects of disrupting dengue interactors on live virus replication. However, a live virus can be dangerous to handle and difficult to assay for replication. An alternative method is to use a non-infectious replicon of a dengue virus. Such a replicon can replicate but it cannot form infectious virions, usually because it lacks proteins required for packaging or dissemination. If the replicon has a reporter gene, it can be used to study and calculate replication levels. Ng et al., constructed such a dengue replicon, which stably replicated in human cells under puromycin selection (Ng et al., 2007). The replicon lacked the capsid, membrane protein and envelope protein genes, but contained *Renilla* luciferase, which can be quantified and correlated to the level of RNA replication. This replicon has been used to study several aspects of dengue-host interactions, such as the roles of cholesterol biosynthesis (Rothwell et al., 2009) and of pyrimidine biosynthesis (Wang et al., 2011) in dengue

replication. However, the replicon was designed specifically for human cells, but not for mosquito cells that are also an essential host of dengue virus.

The overall goal of the work described in this chapter was to develop a system for testing the importance of specific mosquito genes for dengue replication. My approach was to design a non-infectious replicon that would work in mosquito cells. If successful, I could then knock down specific mosquito genes by RNAi and test whether the replicon is affected. First, I demonstrated that the mosquito cell line AAG2 is susceptible to a dsRNA-bathing assay similar to the one used to identify DVHFs in dengue-infected Drosophila cells (Sessions et al., 2009). My results have indicated that large-scale RNA interference screens similar to the one used by Sessions et al., would be feasible to identify mosquito factors required by dengue virus. Next, I designed and constructed a dengue RNA replicon and tested its replication and its reporter genes. Unfortunately, the replicon did not work and would require further development. I discuss my troubleshooting efforts and suggest specific strategies to improve the replicon.

4.2 Materials and methods

4.2.1 Cell lines

AAG2 cells, *A. aegypti* cells derived from embryonic tissue (Lan and Fallon, 1990), were a gift from Dr. Ann Fallon (University of Minnesota, St. Paul, Minnesota). The cells were maintained in Schneider's media supplemented with 10% FBS and 100 µg/ml gentamicin at 28°C. The cells were passaged once per week at 1:10 to 1:20

dilutions. To dislodge the surface-attached cells, they were treated with 0.25% Trypsin-EDTA for about 5 minutes at room temperature.

A549 cells, adenocarcinomic human alveolar basal epithelial cells (Giard et al., 1973), were a gift from Dr. Lawrence Grossman (Wayne State University, Detroit, Michigan). HepG2 cells, a human liver carcinoma cell line (Aden et al., 1979), were a gift from Dr. Kezhong Zhang (Wayne State University, Detroit, Michigan). Both human cell lines were maintained in DMEM/high glucose + sodium pyruvate (Thermo scientific: SH30243.01) supplemented with 10% FBS and 1X Antibiotic/Antimycotic Solution (Thermo scientific: SV30079.01) at 37°C in 5% CO_2. The cells were passaged once per week at 1:4 to 1:8 dilutions. The media was changed every 3-4 days. To dislodge the surface-attached cells, they were treated with 0.05% Trypsin-EDTA for about 5-7 minutes at 37°C.

4.2.2 RNA interference (RNAi) assays for insect cells and fluorescence-activated cell sorting (FACS) analysis

dsRNAs targeting *A. aegypti* IAP1 (AAEL009074) were designed using SnapDragon (http://www.flyrnai.org/cgi-bin/RNAi_find_primers.pl) . The primers used were DMRNA1 (5'-GGGCGGGT ATC AGT GCC GAT TTC GTA CC -3') and DMRNA2 ('5-GGGCGGGT CGG TGC TGA TAG TTG CTG AA-3') for an N-terminal part of *A. aegypti* IAP1(ae_IAP(1/2)), and DMRNA3 ('5-GGGCGGGT TTC AGC AAC TAT CAG CAC CG-3') and DMRNA4 ('5- GGGCGGGT TCA TCA CTA CTG CAG CCG AC-3') for a C-terminal part of *A. aegypti* IAP1(ae_IAP(3/4)). These primers were used to PCR amplify the template for dsRNA syntheses from the mosquito cDNA Y2H library using

methods previously described (Guest et al., 2011). dsRNA synthesis was as described in the same study (Guest et al., 2011) and evaluated by gel electrophoresis (Figure 4-1). dsRNA targeting the green fluorescent protein (GFP) was used as a negative control. dsRNAs targeting *D. melanogaster* genes involved in the wingless signaling pathway were synthesized as described (Guest et al., 2011) and used in this study.

The RNAi and FACS protocol used in this study was based on the published study (Guest et al., 2011). The experiment with S2R+ cells exactly followed the described protocol. Some modifications were required for experiments with AAG2 cells. Briefly, AAG2 cells were treated with trypsin to dislodge them from the culture flask. The number of viable cells was counted using trypan blue dye and a hemacytometer. The cells were spun down and resuspended with serum-free Schneider's media to 4×10^5 cells/ml. 75 µl of cells were added to each well of the 96-well plate already containing 5µl of 200 ng/µl appropriate dsRNA. Each dsRNA was loaded into two wells to assay as a duplicate. The content in each well was thoroughly mixed by pipetting. The plate was incubated at room temperature for 90 minutes. Next, 150µl of Schneider's media supplemented with 10% FBS was added to each well. The plate was incubated under culture conditions for 5 days. After the incubation, the cells were treated for 4-5 minutes at room temperature with 0.25% Trypsin-EDTA supplemented with extra 5 mM EDTA to better prevent AAG2 cells from clumping together. The cells in each well were then resuspended in 200µl of Schneider's media supplemented with 10% FBS and 3µl of Vybrant DyeCycle Orange/ 1 ml of media. The cells were transferred to a U-bottom 96-well plate. The plate was loaded to the FACS machine as previously described (Guest et al., 2011). The percentage of cells with DNA content similar to G1, sub-G1, G2/M

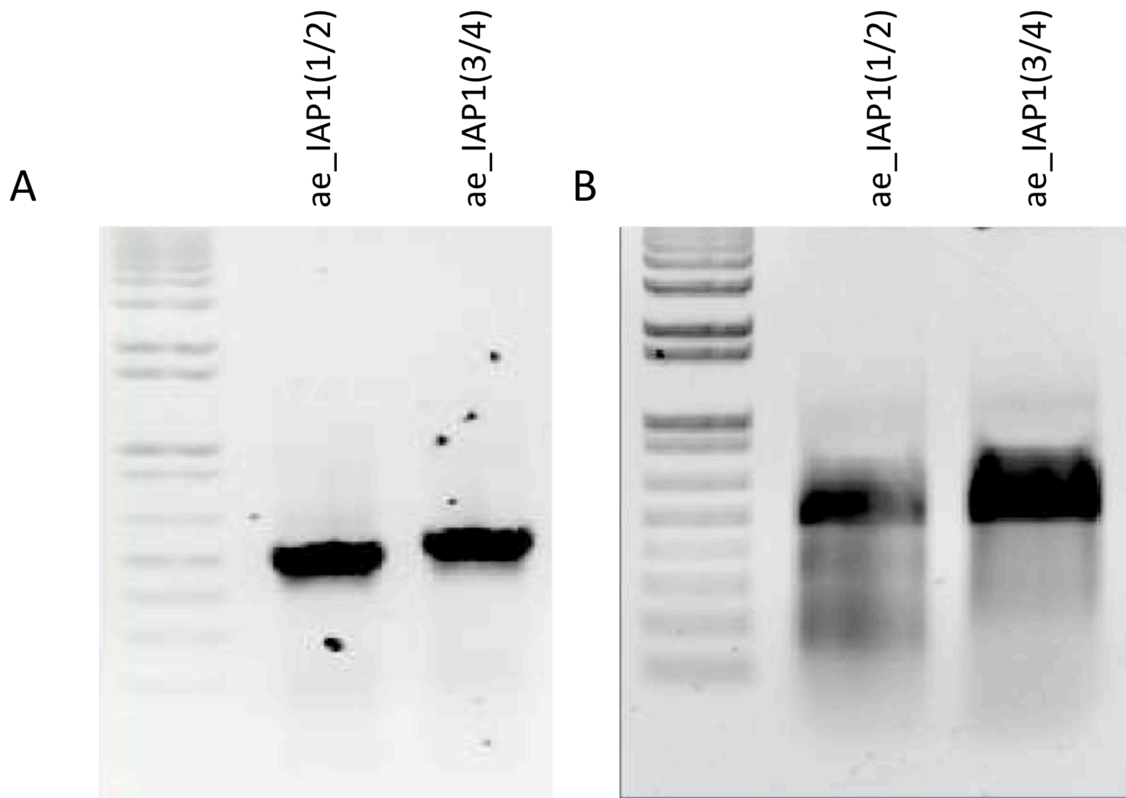


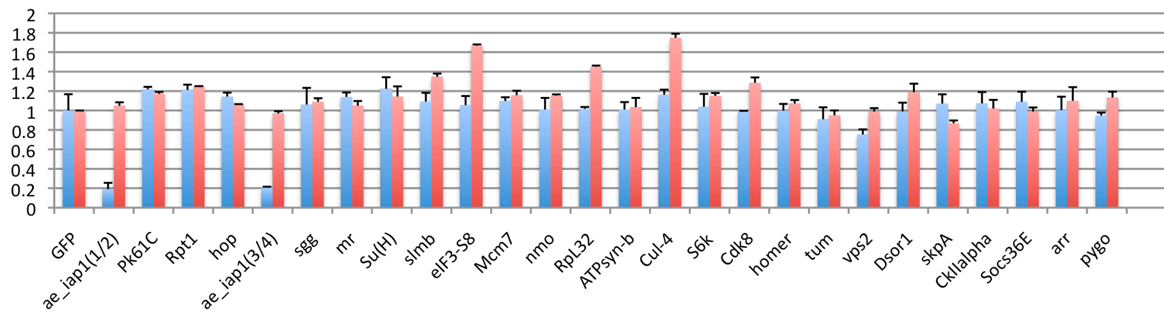
Figure 4-1. dsRNA targeting mosquito Inhibitor of Apoptosis Protein 1 (IAP1). (A) DNA templates for dsRNA synthesis. (B) dsRNAs. ae_IAP1(1/2) targets the 5' end of the IAP1 transcript, while ae_IAP1(3/4) targets the 3' end. 3µl of the total 30µl PCR products and 1µl of 20µl *in vitro* transcription products were loaded onto 1% agarose gel in 1XTBE. Gel electrophoresis was conducted at 100V for 30 minutes. The DNA markers were 300 ng of 1 kB Plus DNA ladder (Invitrogen).

and more than that of G2/M populations were retrieved. The data were normalized by dividing the percentage of cells observed in each population set with the percentage observed in GFP dsRNA control. For example, 10% of cells treated with dsRNA for gene A are in the G1 population, while 25% of cells treated with dsRNA for GFP are in the G1 population. Thus, the normalized G1 data for dsRNA for gene A is 10/25 = 0.4 comparing to GFP dsRNA treatment. Since each experimental set was duplicated, the average and standard deviation were calculated and used to plot the graphs (Figure 4-2).

4.2.3 Dengue replicon construction

The cloning strategy for constructing a dengue subgenomic replicon is shown in Figure 4-3. The cDNA of dengue virus serotype 2 (strain 16681) was used as the template for PCR amplification using Phusion High-Fidelity DNA Polymerase (NEB) according to the manufacturer's instruction (see Appendix C for sequences of the primers). Fragment 1 was constructed by sewing three fragments together. Fragment 1.1 was constructed by PCR amplification of the 5' UTR and the first 22 amino acids of capsid from dengue virus cDNA with primers, DM118 and DM119. Fragment 1.2 was constructed by PCR amplifying the puromycin resistant gene from pPur (Clonetech) using primers, DM120 and DM121. Fragment 1.3 was constructed by PCR amplifying overlapping primers, DM122 and DM123, without a template. Fragment 1.1, 1.2 and 1.3 were sewn by overlap extension PCR using primers, DM134 and DM123. The PCR product of Fragment 1 was column purified (Qiagen) and digested with *Pst*I (NEB).

A



B

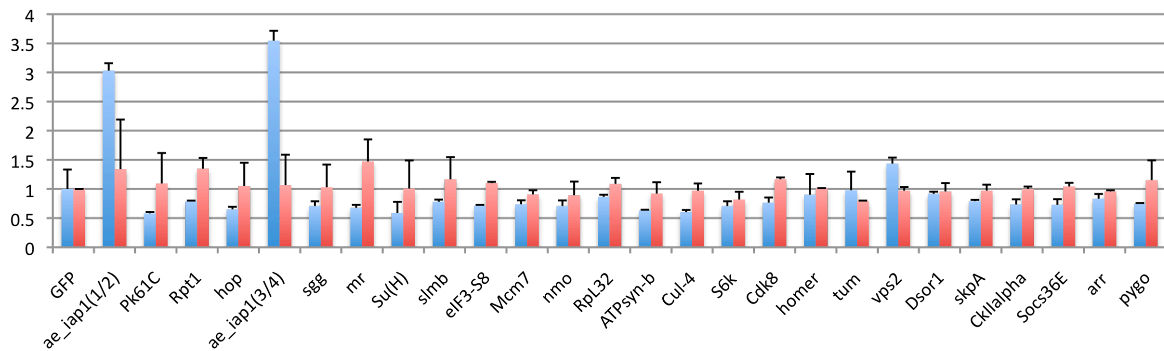
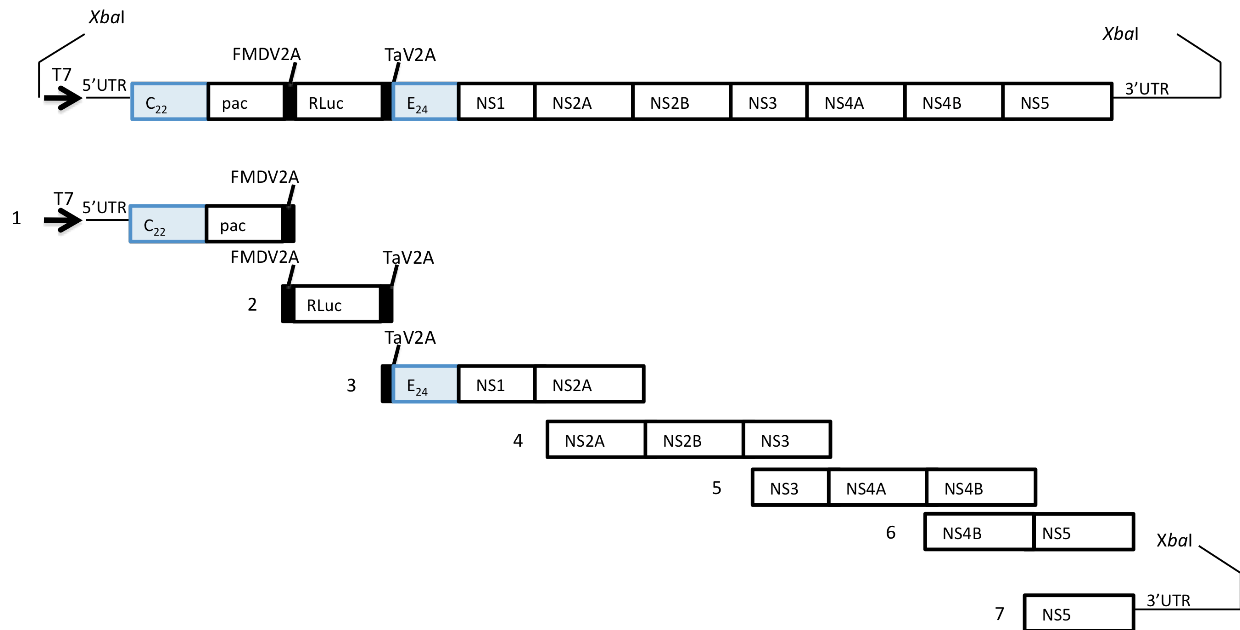


Figure 4-2. The results of FACS analysis. Values on the Y-axis represent the proportion of cells in each cell cycle stage according to their DNA content/cell compared to the GFP dsRNA control. The error bars are standard deviation. (A) G1 population. (B) Sub-G1 population. Blue columns represent the data from mosquito AAG2 cells. Red columns represent the data from Drosophila S2R+ cells. All dsRNAs target the indicated Drosophila genes, except GFP, ae_IAP1(1/2) and ae_IAP1(3/4). Knock down of eIF3-S8, RpL32 and Cul-4 are known to cause G1 arrest in Drosophila cells.

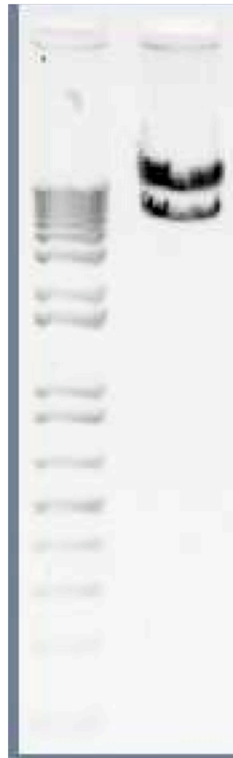


<u>Figure 4-3.</u> The cloning strategy for constructing a dengue subgenomic replicon. The replicon (TOP) was subcloned into the yeast shuttle vector YRp7. The replicon was constructed by assembling seven fragments (1 to 7). The details of the construction are described in Section 4.2.3. T7 is the T7 promoter. C_{22} is the sequence encoding the first 22 amino acids of the capsid. pac is the puromycin-resistance gene. FMDV2A is the 2A sequence from the foot-and-mouth disease virus. RLuc is the *Renilla* luciferase gene. TaV2A is the 2A sequence from *Thosea asigna* virus. E_{24} is the sequence encoding the last 24 amino acids of the envelope protein gene. UTR is the untranslated region.

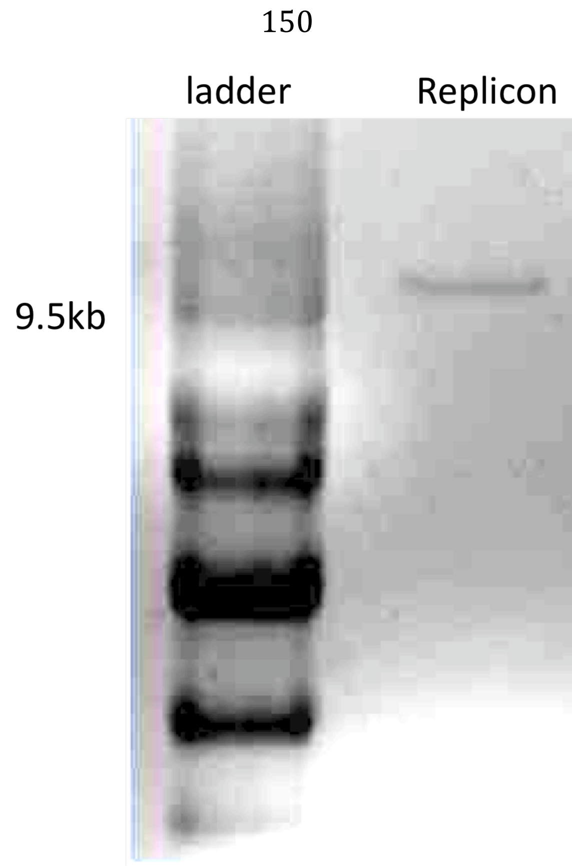
Fragment 1 then was ligated into pUC19, which was digested with *Pst*I and *Sma*I (NEB), using T4 ligase (Invitrogen). Fragment 3 was constructed by sewing two fragments together. Fragment 3.1 was PCR amplified from overlapping primers, DM124 and DM125, without a template. Fragment 3.2 was PCR amplified from dengue cDNA using primers, DM16 and DM126. Fragment 3.1 and 3.2 were sewn by overlap extension PCR using primers, DM16 and DM135. Fragment 3 was column purified and then digested with *Pst*I and *Sal*I (NEB). Fragment 3 then was ligated into pUC19, which was digested with *Pst*I and *Sal*I. Fragment 3 was digested from pUC19_Fragment 3 with *Pst*I and *Sal*I. Fragment 3 then was ligated into pUC18_Fragment 4, which was digested with *Pst*I and *Sal*I. Fragment 1 was PCR amplified from pUC19_Fragment 1 with primer, DM123 and DM134. Fragment 1 then was digested with *Pst*I and ligated into pUC19_Fragment 3+4, which was digested with *Hpa*I and *Pst*I. Fragment 4 was PCR amplified from dengue cDNA using primers, DM15 and DM21. Fragment 4 was digested with *Sal*I and *EcoR*I, and then was ligated into pUC18, which was digested with *Sal*I and *EcoR*I. Fragment 5 was PCR amplified from dengue cDNA using primers, DM20 and DM25. An *Xba*I site was added to the 3' end of Fragment 5 by PCR amplification with primer, DM20 and DM136. Fragment 5 was digested with *Xho*I and *Xba*I and ligated into pUC19, which was digested with *Sal*I and *Xba*I. (*Sal*I and *Xho*I have compatible sticky ends.) Fragment 6 was PCR amplified from dengue cDNA with primers, DM24 and DM29. An *Xba*I site was added to the 3' end of Fragment 6 by PCR amplification with primer, DM24 and DM136. Fragment 6 was digested with *Apa*I and *Xba*I and ligated into pUC19_Fragment 5, which was digested by *Apa*I and *Xba*I Fragment 7 was PCR amplified from dengue cDNA using primers, DM31 and DM127.

Fragment 7 was digested with *BsrG*I and *Xba*I and ligated into pUC19_Fragment5+6, which was digested by *BsrG*I and *Xba*I. Fragment 1+3+4 was PCR amplified from pUC18_Fragment 1+3+4 using primers DM153 and DM154. Fragment 5+6+7 was PCR amplified from pUC19_Fragment 5+6+7 using primers M13F(-21) and M13R, and then digested with *Xho*I. Fragment 1+3+4 and Fragment 5+6+7 were sewn together by overlap extension PCR using primers, DM154 and DM157. Fragment 1+3+4+5+6+7 was cloned into YRp7, which was digested with *Cla*I and *Bam*HI*,* using yeast recombination. Fragment 2 was PCR amplified from pGL4.75[hRLuc/CMV] (Promega) using primers DM158 and DM159. Fragment 2 was cloned into YRp7_Fragment 1+3+4+5+6+7, which was digested with *Not*I, using yeast recombination.

For *in vitro* RNA transcription, the YRp7_dengue_Replicon was digested with *Xba*I. The digestion products were then separated by gel electrophoresis and the ~11 kb replicon was purified by phenol and phenol-chloroform extraction, and precipitated by ethanol (Figure 4-4). The replicon DNA was treated with 200 µg/ml of proteinase K and 0.5% SDS for 30 min at 50°C to eliminate RNase. The DNA was then purified by phenol-chloroform extraction and precipitated by ethanol. The DNA was used as a template for RNA transcription with MEGAscript Kit (Ambion) with 1:4 GTP to cap analog according to the manufacturer’s protocol. After 2 hours of transcription at 37°C, the reaction was treated with 1 µl of RNase-free DNase at 37°C for 15 minutes and analyzed by formaldehyde gel electrophoresis (Figure 4-5). The RNA was purified by phenol-chloroform extraction, precipitated by ethanol and resuspended in RNase-free water to 1 µg/µl. The RNA replicon was kept at -80°C until use.



<u>Figure 4-4.</u> The YRp7_dengue_Replicon digested with *Xba*I. The top band is the DNA of replicon (~11 kb). The bottom band is the YRp7 plasmid backbone (~6 kb). 3 μl of the total 30 μl digestion products were loaded onto 1% agarose gel in 1X TBE. The gel electrophoresis was conducted at 100V for 30 minutes. The DNA markers were 300 ng of 1 kB Plus DNA ladder (Invitrogen).



<u>Figure 4-5.</u> Replicon RNA. 2 µl of 20 µl in vitro transcription reaction was loaded to 1.2% agarose formaldehyde gels. Electrophoresis was conducted at 100V for 60 minutes. The RNA marker was 3 µg of 0.24-9.5 Kb RNA ladder (Invitrogen).

4.2.4 Cell viability and *Renilla* luciferase assay

Before measuring *Renilla* luciferase activity, cell viability was measured with CellTiter-Glo Assay (Promega) according the manufacturer's instructions. Briefly, each sample of the cells was treated with an appropriate amount of trypsin and resuspended in an appropriate amount of media so that every sample contained a similar number of cells (~4 x 10^5 cells/ml). Next, 100 µl of each sample was added into each well of an opaque 96-well plate. One well contained media without cells so that background luminescence could be measured. 100 µl of pre-mixed CellTiter-Glo reagent was added to each sample. The plate was shaken on an orbital shaker for 2 minutes and incubated at room temperature for 10 minutes. The plate was then read by GLOMAX 96 microplate luminometer (Promega) according to the manufacturer's instructions. EnduRen Live Cell Substrate (Promega) was used to measure *Renilla* luciferase activity according to the manufacturer's instructions. Briefly, each sample of cells was treated with an appropriate amount of trypsin and resuspended in an appropriate amount of media so that every sample contained a similar number of cells (~4 x 10^5 cells/ml). Next, 100 µl of each sample was added into each well of an opaque 96-well plate. EnduRen substrate was added to each cell sample so that the final concentration of the substrate was 60 µM. The plate was incubated under cell culture conditions for at least 90 minutes to 24 hours. Finally, the plate was read by GLOMAX 96 microplate luminometer (Promega).

4.2.5 DNA and RNA transfection

mRNA transfection was done with the TransIT-mRNA Transfection Kit (Mirus). Briefly, $1x10^5$ cells were seeded into a well of a 12-well plate one day before transfection. On the day of transfection, the transfection reagents were warmed to room temperature. 1 µg of mRNA was diluted in 100 µl of serum-free media. 1 µl of mRNA boost reagent was then added to the RNA mixture followed by gently pipetting to mix the reaction well. Next, 1 µl of TransIT-mRNA reagent was added to the RNA mixture followed by gently pipetting to mix the reaction well. The reaction was then incubated at room temperature for 2-5 minutes. The reaction was gently added dropwise to the cells, and the plate was gently swirled to evenly distribute complexes. Two days after transfection, the cells were used for further analyses or for selection for a stable cell line with 5 µg/ml of puromycin. DNA or RNA transfection was also done with electroporation with the Neon Transfection System (Invitrogen). The electroporation was performed according to the manufacturer's protocol with some modifications. Briefly, 500 ng of DNA or RNA and $5x10^6$ cells/ml of A549 cells were loaded into a 10 µl tip. The tip was electroporated by two pulses of 1,200 volts with 30 ms of pulse width. After two days, the cells were used for further analysis or for selection for a stable cell line with 5 µg/ml of puromycin. The DNA transfection methods for Drosophila cells and human cells are described in Section 2.2.8 and Section 3.2.4, respectively.

4.3 Results and discussion

4.3.1 AAG2 cells are susceptible to a dsRNA bathing technique

The dsRNA bathing technique, in which cells are directly incubated with dsRNA, is widely used as a means to silence genes in Drosophila cells since the cells are capable of directly taking up dsRNA (Clemens et al., 2000). Because it is simple and economic, the technique has been used in several hundred large-scale RNA interference studies with *Drosophila* cell lines (Mohr et al., 2010), including studies of the pathways responsible for innate immunity (Foley and O'Farrell, 2004; Kleino et al., 2005). It was also used for a genome-wide study to identify DVHFs in dengue-infected *Drosophila* cells as mentioned earlier (Sessions et al., 2009). However, Drosophila is not a natural host for dengue virus and the Drosophila cells could only be infected with an extensively mutated virus (Sessions et al., 2009). The natural dengue host, mosquito, has been shown to be susceptible to dsRNA injection into its thorax for gene silencing, hinting at the potential for direct up-take of dsRNA (Zhu et al., 2003). It is also worth noting that C6/36 cells from *A. albopictus*, which have been widely used to propagate dengue virus, have been shown to have defective RNA interference machinery (Brackney et al., 2010) so they may not be used with this technique. However, no studies had tested whether the dsRNA bathing technique would work in cultured *A. aegypti* cells. Thus, I set out to test this possibility.

To test RNAi in mosquito AAG2 cells, I selected a mosquito inhibitor of apoptosis 1, AAEL009074 (ae_IAP1), because the phenotype observed after silencing its Drosophila ortholog, DIAP-1, is extensive cell death. I designed two dsRNAs targeting

two non-overlapping regions of ae_IAP1. ae_IAP1(1/2) targeted base positions 96-552 of the cDNA, while ae_IAP1(3/4) targeted base positions 533-953 of the cDNA (1,137 bp) (Figure 4-1). I applied the protocol for dsRNA bathing with minor modifications to AAG2 cells (see Section 4.2.2.) By observing the cells under a microscope, I found that both ae_IAP1(1/2) and ae_IAP1(3/4) induced dramatic cell death 5 days after dsRNA bathing. The fact that two dsRNAs from the same gene gave the same expected phenotype suggests that they are not due to off-target effects (Kulkarni et al., 2006; Ma et al., 2006). I confirmed these results by FACS analysis, which detected an elevated level of cells in the sub-G1 population, characteristic of dying cells, after the treatment of each one of dsRNAs (Figure 4-2). A control dsRNA targeting a GFP gene did not induce cell death. Taken together, I found that AAG2 cells are compatible with the dsRNA bathing technique. During the course of this project, a similar study showing identical results to my findings was published (Wang and Clem, 2011).

Although *A. aegypti* and *D. melanogaster* shared the most recent common ancestor 225-280 million years ago (Simmons and Weller, 2001), I decided to see whether dsRNA targeting a *Drosophila* gene is also capable of silencing a mosquito gene to some degree, and vice versa. Thus, I selected dsRNAs targeting a number of Drosophila genes, including eIF3-S8, RpL32 and Cul-4, which have been reported to significantly induce G1 arrest when knocked down (Guest et al., 2011). The results showed that dsRNA could not silence the ortholog of its original target since no G1 arrest was observed (Figure 4-2). Thus, unfortunately, the extensive RNAi reagents available for Drosophila cannot be used for the mosquito cell lines.

4.3.2 The dengue replicon failed to replicate in insect and human cells

The dengue RNA replicon constructed by Ng et al., has been shown to work well in human cell lines (Ng et al., 2007). The replicon contains most of the genome of dengue virus serotype 2 (strain NGC), but lacks a part of the capsid gene, a part of the envelope protein gene and the entire membrane protein gene to eliminate its ability to assemble a mature virion. These genes were replaced with a puromycin-resistance gene and a *Renilla* luciferase gene. The replicon has one "2A" sequence derived from the foot-and-mouth disease virus inserted between the puromycin-resistance gene and the *Renilla* luciferase gene, while a stop codon and an internal ribosome entry site (IRES) are at the 3' end of the luciferase gene. The 2A sequence, a short peptide sequence found in the peptides encoded by picornaviruses, can disrupt a ribosome from generating the peptide bond between the last two amino acids of the sequence without terminating the translation process resulting in two separate peptides from a single mRNA (Doronina et al., 2008). Thus, the puromycin-resistance gene is translated and separated from luciferase and the rest of the dengue proteins. The translation continues and then is terminated at the stop codon at the 3' end of the luciferase gene. The IRES initiates a new round of translation of the rest of the dengue genome. However, this IRES from encephalomyocarditis virus can be recognized by the human protein translation machinery, but not as efficiently by that of an arthropod (Finkelstein et al., 1999; Woolaway et al., 2001).

I set out to create a modified version of this replicon that could replicate in AAG2 and S2R+ cells. The most important modification of the original replicon was to replace the IRES with a second 2A sequence modified from the 2A sequence from *Thosea*

asigna virus (TaV). I chose this 2A sequences so that the two 2A sequences were not so similar that they would result in homologous recombination. The use of multiple 2A sequences has been reported to successfully generate separate peptides from a single mRNA encoding four CD3 proteins (Szymczak et al., 2004). I failed to retrieve the original replicon from the authors so I constructed my own dengue replicon from dengue cDNA as described in Section 4.2.3 and Figure 4-3. The DNA template of the replicon was successfully generated and sequenced. The RNA replicon was also successfully transcribed (Figure 4-5).

I attempted to transfect the replicon RNA into AAG2 cells, S2R+ cells, and A549 cells. However, none of these cell lines became puromycin-resistant. I tried to use several methods for transfection including various transfection reagents and electroporation, but none of the methods resulted in puromycin-resistant cells. Thus, I set out to test several potential problems that may cause the replicon to fail. First, I tested the puromycin-resistance gene by subcloning it into pHZ12_attR (Figure 4-6). The resulting vector, pHZ12_pac, was then transfected into S2R+ cells. Interestingly, the transfected S2R+ cells were puromycin-resistant for at least one week in comparison to the non-transfected cells, which were >90% killed after the second day of puromycin treatment. Thus, the puromycin-resistance gene was functional.

Second, I hypothesized that the two 2A sequences may not be functional, and thus may fail to generate three complete separate peptides. To test the 2A sequences, I subcloned two parts of the replicon into pHZ12_attR (Figure 4-6). The first part contained the puromycin-resistance gene, the 2A sequence from FMDV and the luciferase gene with a stop codon (pHZ12_ pac_FDMV_RLuc). The second part

pHZ12_NS1

pHZ12_pac

pHZ12_RLuc

pHZ12_pac_FDMV_RLuc

pHZ12_CtoNS1

Figure 4-6. Parts of the dengue replicon cloned into pHZ12_attR to test expression in Drosophila S2R+ cells. pHZ12_NS1 contained NS1 from dengue virus serotype 2. pHZ12_pac contained a puromycin-resistance gene. pHZ12_RLuc contained a *Renilla* luciferase gene. pHZ12_pac_FDMV_RLuc contained a puromycin-resistance gene and a *Renilla* luciferase gene, which were separated by a 2A sequence from foot-and-mouth disease virus. pHZ12_CtoNS1 contained a part of the replicon from capsid to NS1. All constructs were N-terminally tagged with Myc.

contained the puromycin-resistance gene, the 2A sequence from FMDV, the luciferase gene, the 2A sequence from TaV, and a part of envelope protein and NS1 with a stop codon (pHZ12_CtoNS1). Interestingly, these two plasmids successfully conferred puromycin-resistance to S2R+ cells for at least one week. Thus, the puromycin-resistance gene and the FDMV 2A sequence were functional. Next, I set out to test the luciferase gene. I subcloned the luciferase gene into pHZ12 (Figure 4-6) to be used as a positive control for luciferase activity (pHZ12_RLuc). S2R+ cells transfected with pHZ12_RLuc, pHZ12_ pac_FDMV_RLuc or pHZ12_CtoNS1 were tested for luciferase activity. Interestingly, the cells produced luciferase from all three constructs (Figure 4-7). Finally, I set out to test the TaV 2A sequence and NS1. I subcloned NS1 into pHZ12 (Figure 4-6) to be used as a positive control for NS1 detection (pHZ12_NS1). Both pHZ12_NS1 and pHZ12_CtoNS1 properly expressed NS1 in S2R+ cells. However, pHZ12_NS1 showed multimeric NS1 as previously reported (Gutsche et al., 2011), while pHZ12_CtoNS1 showed faint bands that might be multimeric NS1 or long peptides produced by the 2A sequences partially failing to separate the peptide bonds (Figure 4-8). Nevertheless, the above results showed that the puromycin-resistance gene and the luciferase gene functioned properly, while the 2A sequences mostly resulted in three separate peptides with a small amount of fused products.

Since the modified part of the replicon functioned properly, I considered two other potential causes for its failure. First, the methods that I used for RNA transfection might not work. I tested this by using RNA encoding β-galatosidase (LacZ) + EYFP as a control since its size was similar to that of the replicon. I first transferred the LacZ gene from pIB/V5-His/GW-LacZ (Invitrogen) into pDONR221 by BP clonase and then to

pcDNA 6.2/C-YFP-DEST (Invitrogen) by LR clonase. This plasmid was used as a template to transcribe RNA. The RNA and the plasmid were separately electroporated into A549 cells. I found a small number of EFYP positive cells from the DNA electroporation, but none for the RNA (Figure 4-9). This result hinted that RNA transfection might be inefficient, which would prevent the replicon from entering the cells. Repeat experiments with other RNA are required to further test this possibility. A second potential problem is that the part of replicon containing the dengue genome may contain mutations that prevent replication. However, sequencing results did not show deletions or insertions in the dengue genome. However, there were a few point mutations (see Appendix P). To determine whether the viral genome part of the replicon functions properly, I may need to generate live dengue virus from the original cDNA to determine its infection and replication efficiency. However, our current facility does not permit such experiments. Thus, the second potential problem is still unanswered.

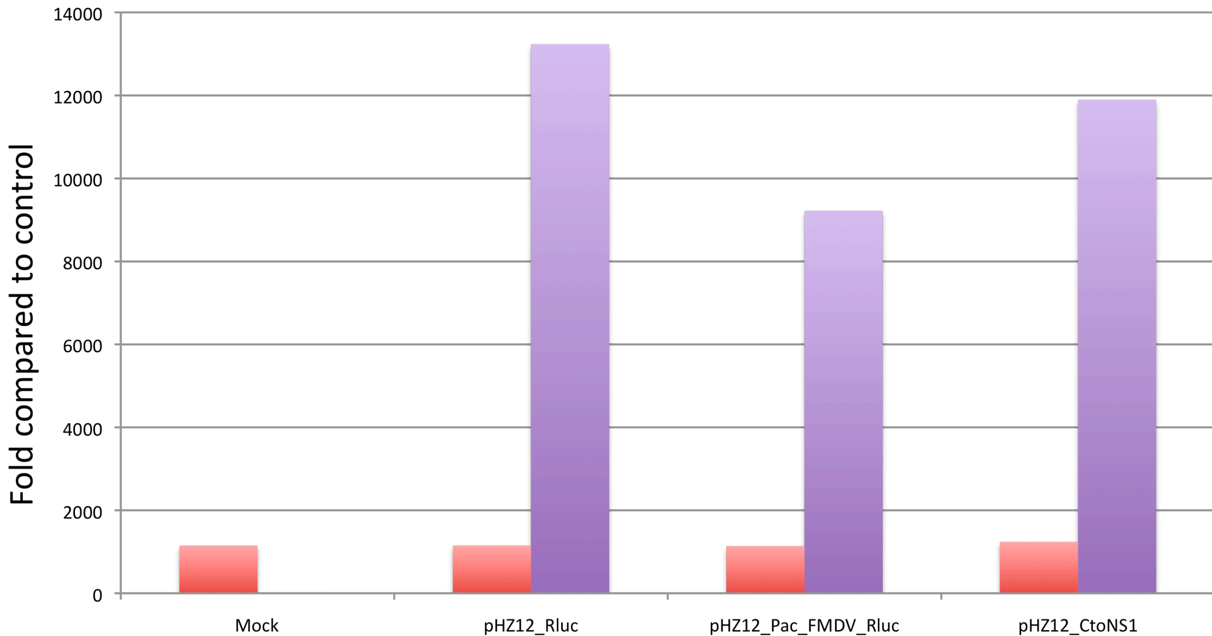


Figure 4-7. S2R+ cell viability and *Renilla* luciferase assays. The red bars represent cell viabilities measured with CellTiterGlo (Promega). The cell viabilities were divided by the background luminescence from the Schneider's media without cells for normalization. The cell viability assay showed that every sample contained a similar number of cells. The purple bars represent luciferase activities measured with EnduRen substrate (Promega). The activities were divided by the background luminescence from mock-transfected S2R+ cells for normalization.

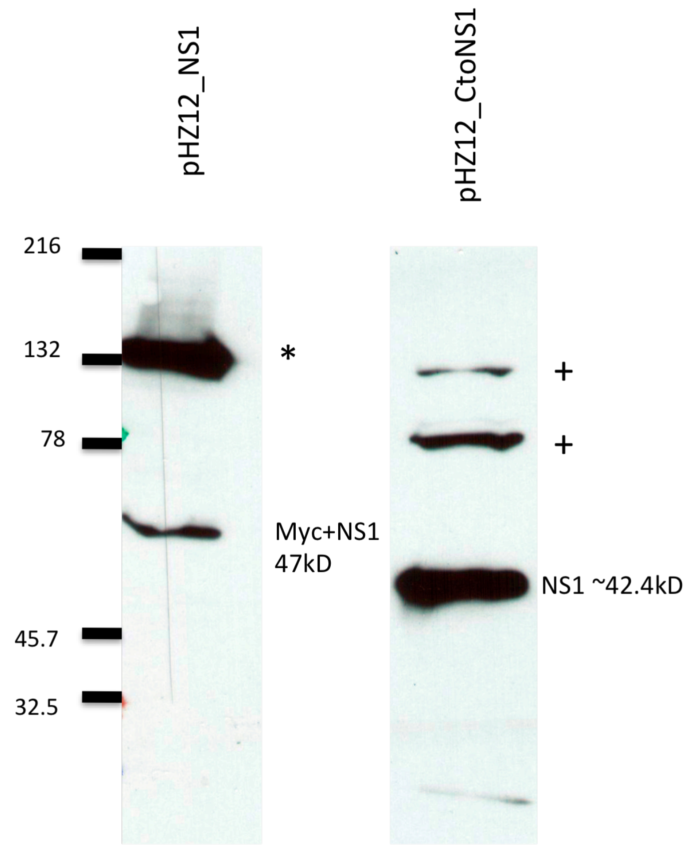
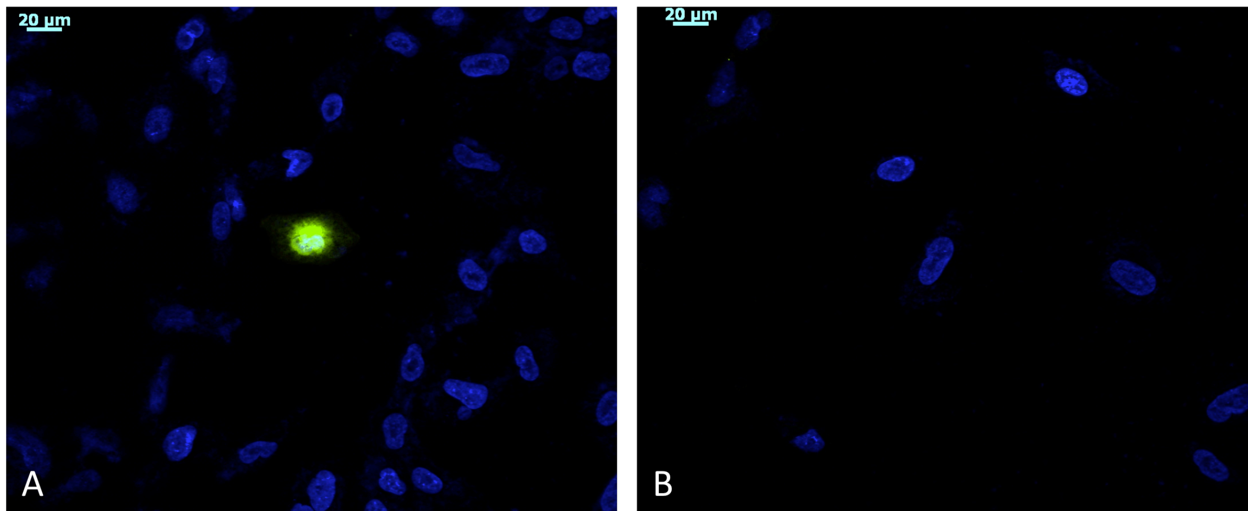


Figure 4-8. Western analysis for NS1 expression. S2R+ cells were transfected with either pHZ12_NS1 or pHZ12_CtoNS1. The cell lysate was run on a SDS-PAGE gel, and proteins were transferred to a membrane for immunoblotting. NS1 was probed with anti-NS1 (a gift from Dr. Chunya Puttikhunt, Mahidol University, Thailand). * indicates possible multimeric NS1 as previously reported (Gutsche et al., 2011) . + indicates larger bands that may be due to either multimeric NS1 or transcriptional read through of the 2A sequences.



<u>Figure 4-9.</u> The efficiency of electroporation of A549 cells. (A) pcDNA 6.2/LacZ_C-YFP was electroporated into the cells. The cells were stained with DAPI. Transfection efficiency is less than 1%. (B) RNA transcribed from pcDNA 6.2/LacZ_C-YFP was electroporated into the cells. The cells were stained with DAPI. No YFP was detected among 1 x 10^5 cells observed three days after electroporation.

4.4 Summary

I have shown that AAG2 cells are compatible with the dsRNA bathing technique. Thus, large-scale RNA interference studies in mosquito cells can now be contemplated, similar to the study that identified dengue *Drosophila* host factors (Sessions et al., 2009). However, the dsRNAs used with *Drosophila* have been shown to be incompatible with AAG2 cells so new dsRNAs for silencing mosquito genes must be generated. Finally, I tried but failed to generate a dengue replicon. However, I have shown that the idea of using two 2A sequences to generate three separate peptides is feasible. Thus, this idea may be applied to the construction of a new replicon in the future.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

5.1 Summary

In this study, I set out to identify dengue-human and dengue-mosquito PPI by employing Y2H screens. I then used co-affinity purifications and Y2H tests with different dengue serotypes to confirm PPI and to identify those that are most likely to have biological relevance. I also assembled all dengue-host PPI identified to date and proposed a prioritized subset to be further investigated based on multiple pieces of evidence and potential conservation. I then focused on the interaction between capsid and nucleosome assembly protein and showed initial results suggesting a role for human nucleosome assembly protein 1-like 1 (NAP1L1) in the nucleocytoplasmic shuttling of capsid. I also tested a mosquito cell line, AAG2, for its susceptibility to the dsRNA bathing technique. The results demonstrated that large-scale RNA interference studies could be performed with AAG2. Finally, I tried but failed to construct a dengue sub genomic replicon, but I showed that replicon designs in which two 2A sequences generate three separate functional peptides are feasible. Below I summarize and discuss further some of the important findings from my project.

5.2 An *Aedes aegypti* cDNA library for yeast two-hybrid screening

I constructed the first *A. aegypti* cDNA library for the Y2H system using pooled RNA collected from ten developmental stages. I showed that the library contains

various sizes of cDNAs from 300 to 4,000 bp, and that more than 60% of the independent *E.coli* clones contain a cDNA insert. The number of independent *E.coli* clones was greater than $1x10^7$ clones. Our group has subcloned full-length cDNAs from the library for this and other studies. We successfully retrieved mosquito nucleosome assembly protein (AAEL005567), cyclin J (AAEL008256), cyclin B (AAEL010094), gus (AAEL011069), spindle A (AAEL006080), Cks30A (AAEL004492), Cks85A (AAEL005232), cdk1 (AAEL008621) and cdk2 (AAEL012339). This library may be used for mapping PPI to better understand the biology of the mosquito since it is a vector for many diseases, including dengue fever, yellow fever, and Chikungunya. In addition, identification of mosquito PPI may help identify potentially conserved PPI. The conserved PPI may have significant biological functions. This library may also be used for studying host-parasite PPI. Other mosquito-borne pathogens, such as yellow fever and Chikungunya, may be studied using this library to identify pathogen-mosquito PPI, which may help the development of methods to better combat these pathogens. Recently, an intracellular insect parasite *Wolbachia* has been tested as a means to control the mosquito population (Hoffmann et al., 2011). The parasite confers resistance against dengue virus to the mosquito by priming its innate immunity (Pan et al., 2012). However, the priming mechanism is not fully understood. Thus, this library may be used to study *Wolbachia*-mosquito PPI to better understand the parasite-host interaction.

5.3 Dengue-host interactomes

5.3.1 Novel dengue-host PPI

I set up three Y2H screens to identify intraviral PPI, dengue-mosquito PPI, and dengue-human PPI. The intraviral screen yielded only three PPI, all of which were already known. The dengue-mosquito screen yielded 102 PPI involving 98 mosquito proteins. None of these PPI were previously identified. The dengue-human screen produced 46 PPI involving 35 human proteins. Six PPI were previously identified or predicted.

Protein interaction screens frequently generate false positives, which are PPI that do not occur in a natural biological context. In addition, there is no “gold standard” or large set of known interactions that can be used to develop a scoring or ranking system for real dengue-host PPI. To address this problem, I employed Co-AP as an orthogonal assay, since many studies have shown that PPI identified by two or more independent assays are more likely to be biologically relevant (Uetz et al., 2000; Ito et al., 2001; Deane et al., 2002; von Mering et al., 2002; Giot et al., 2003; Stanyon et al., 2004; Schwartz et al., 2009). The Co-AP confirmed 36 out of the 138 testable PPI. I also repeated Y2H assays to test each host protein with the dengue proteins from different serotypes. I proposed that biologically relevant virus-host PPI are likely to be conserved across the four dengue serotypes. The screen showed that 57 out of 102 (56.9%) dengue-mosquito PPI and 34 out of 46 (73.9%) dengue-human PPI interacted with the corresponding dengue proteins from all four serotypes.

Large-scale PPI screens also generate false negatives, which are true PPI not detected by the screens. False negatives may result from limitations of the assay used to identify PPI. For example, Y2H assays are poor at detecting PPI with membrane proteins because the aqueous environment in the yeast nucleus may induce misfolding of the hydrophobic membrane proteins (Stagljar and Fields, 2002). Another reason for false negatives is that the screens may be sub-saturating, which means a PPI may be theoretically detectable using Y2H, but the PPI is not tested or it is missed during the screens. For example, some cDNAs may be underrepresented or missing altogether from the Y2H cDNA library. In addition, different cDNA libraries represent different sets of proteins so some proteins may not be in a certain library and, therefore, would not be detected in the screens. I compared my results with other studies (Colpitts et al., 2011b; Folly et al., 2011; Khadka et al., 2011; Le Breton et al., 2011) and found very small levels of overlap. I identified only two of the PPI detected by Khadka et al., and only one of the PPI detected by Le Breton et al. I found no PPI in common with the studies by Copitts et al., and Folly et al. The overlap among PPI found in other studies is also very small. For example, only one PPI was detected by both Le Breton et al., and Khadka et al. None of the PPI detected by Colpitts et al., or Folly et al., were detected in other large-scale studies. It is formally possible that one of these studies is comprehensive while the others detected mostly false positives, resulting in such small overlap. If this assumption is true, that screen should have identified at least all of the functionally verified PPI from the other screens. However, when I looked at PPI detected by small-scale studies and extensively verified for their functional significance, none of the screens could detect such PPI better than any other. Thus, PPI data from any one of

these large-scale studies, including mine, appear to be incomplete. Therefore, further studies are still required to complete the dengue-host interactome.

To help address the problem of false negatives and false positives, I collected all 403 experimentally detected dengue-host PPI from my study and other studies. I then proposed a prioritization of these PPI for further investigation based on multiple pieces of evidence and potentially conserved interologs. The prioritized list contains 38 dengue-mosquito PPI and 65 dengue-human PPI. Seven PPI in this list are potentially conserved interlogs identified in my study. This list should help in the selection of candidates for further functional studies.

5.3.2 Expanding the dengue-host interactomes

Dengue-host PPI data appear to be incomplete. Additional Y2H screens may detect more dengue-host PPI. It may be useful, for example, to screen the dengue Y2H baits designed and used by other studies against the mosquito cDNA Y2H library. For example, Khadka et al., used the cytoplasmic and ER luminal parts of dengue membrane proteins for the Y2H screenings to avoid improper folding of these proteins (Khadka et al., 2011). New techniques such 454 sequencing and interaction sequence tag (IST) concatenating may improve Y2H screens resulting in detection and confirmation of more PPI (Hastie and Pruitt, 2007; Yu et al., 2011). Furthermore, if an ORF library for *A. aegypti* becomes available and affordable, the Y2H two-phase mating technique used for Drosophila interaction screens could be applied for the dengue-mosquito PPI screen (Zhong et al., 2003).

In Y2H assays, proteins tested must be expressed in the yeast nucleus, where extracellular or membrane proteins might not be properly folded resulting in false negatives. To overcome this limitation of Y2H assays, other methods may be employed. For example, a split-ubiquitin assay is a modified Y2H assay designed to test membrane proteins (Johnsson and Varshavsky, 1994; Stagljar et al., 1998). It works by fusing one protein with one half of ubiquitin and the other protein with the other half of ubiquitin and a transcription factor. If the proteins interact, the two halves of ubiquitin will be brought into close proximity and cleaved by a ubiquitin-specific protease releasing the transcription factor, which in turn activates a reporter gene. The split-ubiquitin assay has been used for a large-scale PPI screen for yeast membrane proteins (Miller et al., 2005). This assay may be useful to identify dengue-host PPI of membrane proteins such as NS2A, NS2B, NS4A and NS4B. Extracellular dengue proteins including membrane protein, envelope protein, and NS1 may not fold properly within cells so they may require an alternative method. For example, an avidity-based extracellular interaction screen (AVEXIS) has been designed to test extracellular PPI (Bushell et al., 2008). In AVEXIS, a bait protein is used to coat a microtiter plate, while a prey protein is fused with a reporter protein like β-lactamase. The prey is added into the plate and washed. A substrate for a reporter protein like nitrocefin is added to assess the reporter activity.

Another limitation of Y2H assays is that proteins of other species may not be folded properly in yeast cells due to different cellular environments. For example, a protein may lack a post-translational modification necessary for a PPI (Guo et al., 2004). To avoid this problem, protein-fragment complementation assays (PCA) could be used.

In PCA, a reporter protein, such as a fluorescent protein, is split into halves, and each half is fused with one of the tested proteins (Hu et al., 2002). The PCA may be conducted within native cells to test a PPI in its natural cellular environment. Similarly, co-localization supplemented with Förster resonance energy transfer (FRET) (Sekar and Periasamy, 2003) or bioluminescence resonance energy transfer (BRET) (Xu et al., 2007) may also be used to visualize PPI in native cells. Information about protein complexes is also important so further Co-AP studies, like the one conducted by Colpitts et al., (Colpitts et al., 2011b) would be useful to expand the dengue-host protein complex data. Because they could be performed in dengue-infected cells, each of these assays (PCA, Co-AP/MS and co-localization) could be conducted at several time points during the course of dengue virus infection to assess the dynamics of dengue-host PPI.

5.4 Functional studies of dengue-host PPI

In this study, I identified several dengue-host PPI that may have significant roles in the virus life cycle. I also employed orthogonal assays to identify the PPI that are most likely to be biologically relevant. However, all of the data were generated from artificial systems (e.g., yeast or cultured Drosophila cells). It will be important, therefore, to study some of these PPI in the context of live virus infection and replication. This may be done by over-expressing or silencing the dengue interactors in the host cells before infecting them with dengue virus. The replication level may be measured with the expression level of viral proteins in the cells by immunostaining and image analyses, as demonstrated by Sessions et al (Sessions et al., 2009). In addition, plaque assays as described by Fink et al., may assess the infectivity of the virus disseminating from the

experimental cells (Fink et al., 2007). However, for many reasons study of live dengue virus is difficult or inconvenient. Thus, I prepared two new tools. First, I tested and modified the dsRNA bathing technique for mosquito cells. Second, I attempted to construct a dengue replicon to allow an analysis of the importance of host-virus interactions to viral replication without using a live virus.

5.4.1 RNAi screens in mosquito cells

I showed that the dsDNA bathing technique induced AAG2 cells to undergo apoptosis using dsRNA against an inhibitor of apoptosis protein 1. Thus, it should be feasible to conduct large-scale RNA interference studies as has been done in Drosophila cells (Sessions et al., 2009). C6/36, the other widely used mosquito cell line, has been shown to have defective RNA interference machinery (Brackney et al., 2010) so it is not compatible with this technique. This technique will be valuable for identifying the functional significance of dengue-mosquito PPI detected by this study and others.

5.4.2 Dengue replicons for functional studies

An alternative to using live virus is to use a non-infectious replicon to follow virus replication. I attempted to construct a replicon as described in Chapter 4, but the replicon failed to replicate in either human cells or mosquito cells. Since the cDNA I used for constructing the replicon was fragmented, I had to employ several cloning and ligating steps, which may have introduced mutations that rendered the RNA replicon defective. In addition, the cDNA used for replicon construction was derived from a virus strain that was never propagated through AAG2 cells. Thus, replication of the original

virus was never assessed in these cells. A study by Vasilakis et al., has shown that propagating the virus in human cells increases the fitness of the virus in these cells, but decreases the fitness in mosquito cells (Vasilakis et al., 2009). To generate a replicon that can replicate in AAG2 cells, therefore, it may be necessary to propagate the live virus in mosquito cells for several replication cycles and then use cDNA from the AAG2-competent virus to construct the replicon. Note that selection of AAG2-competent virus could take as long as 4 months, which is how long it took to create a virus that could propagate efficiently in Drosophila cells (Sessions et al., 2009).

Other designs for a dengue replicon have also been published. Hsu et al., replaced capsid and PrM with firefly luciferase and inserted a neomycin-resistance gene between NS5 and the 3’ UTR (Hsu et al., 2012). However, this replicon contains an IRES for the neomycin-resistance gene, which may be incompatible with AAG2 cells. Leardkamolkarn et al., created several subgenomic replicons by replacing one of the structural proteins, C, PrM, or E, with GFP (Leardkamolkarn et al., 2012). However, these replicons do not contain a selectable marker so they may not be stably maintained in the cells. The same group also constructed a replicon containing GFP and an IRES-neomycin resistance gene replacing structural proteins (Leardkamolkarn and Sirigulpanit, 2012). Again, this replicon required a human IRES, which may not be compatible with AAG2 cells. Other published replicons also require human or human virus IRES, or lack a selectable marker (Alcaraz-Estrada et al., 2010; Lee et al., 2010). These designs might be modified, however, to construct an AAG2-compatible replicon. Interestingly, Massé et al., replaced dengue structural proteins with an EGFP-puromycin resistance gene fusion in their replicon suggesting that a reporter gene and a selectable

marker may be functional as a fusion protein, so that a 2A sequence is not required for their separation (Masse et al., 2010). This is another design that might be useful for constructing an AAG2-compatible replicon.

5.5 Nucleosome assembly protein and its role in the nuclear localization of dengue capsid

From the prioritized list in Chapter 2, I selected for further study the interaction between capsid and nucleosome assembly protein 1 (human NAP1L1 and mosquito AAEL005567) for two reasons. First, the PPI appears to be conserved in mosquito and human. Second, a recent publication has hinted at a role for nucleosome assembly protein 1 in the nucleocytoplasmic shuttling of diacylglycerol kinase zeta (DGKζ) (Okada et al., 2011). Human NAP1L1 binds the bipartite nuclear localization signal (NLS) of DGKζ, which blocks importin from binding to the same site. Thus, in the presence of NAP1L1, DGKζ accumulates in the cytoplasm. I hypothesized that similar binding and blocking of capsid's bipartite NLS by NAP1L1 may also occur as illustrated in Figure 5-1A.

First, I set out to map the NAP1L1 and AAEL00567 binding domain of capsid. I used Y2H assays to screen capsid and its mutants against NAP1L1. The results indicated that amino acid residues 85-100, which includes the bipartite NLS, were required for efficient interaction. I also used Co-AP to confirm that mosquito AAEL005567 requires residues 85-100 of capsid for efficient binding. However, the function of the bipartite NLS itself seemed to have no role in the interaction, since a point mutant that disrupts NLS, still interacted with capsid.

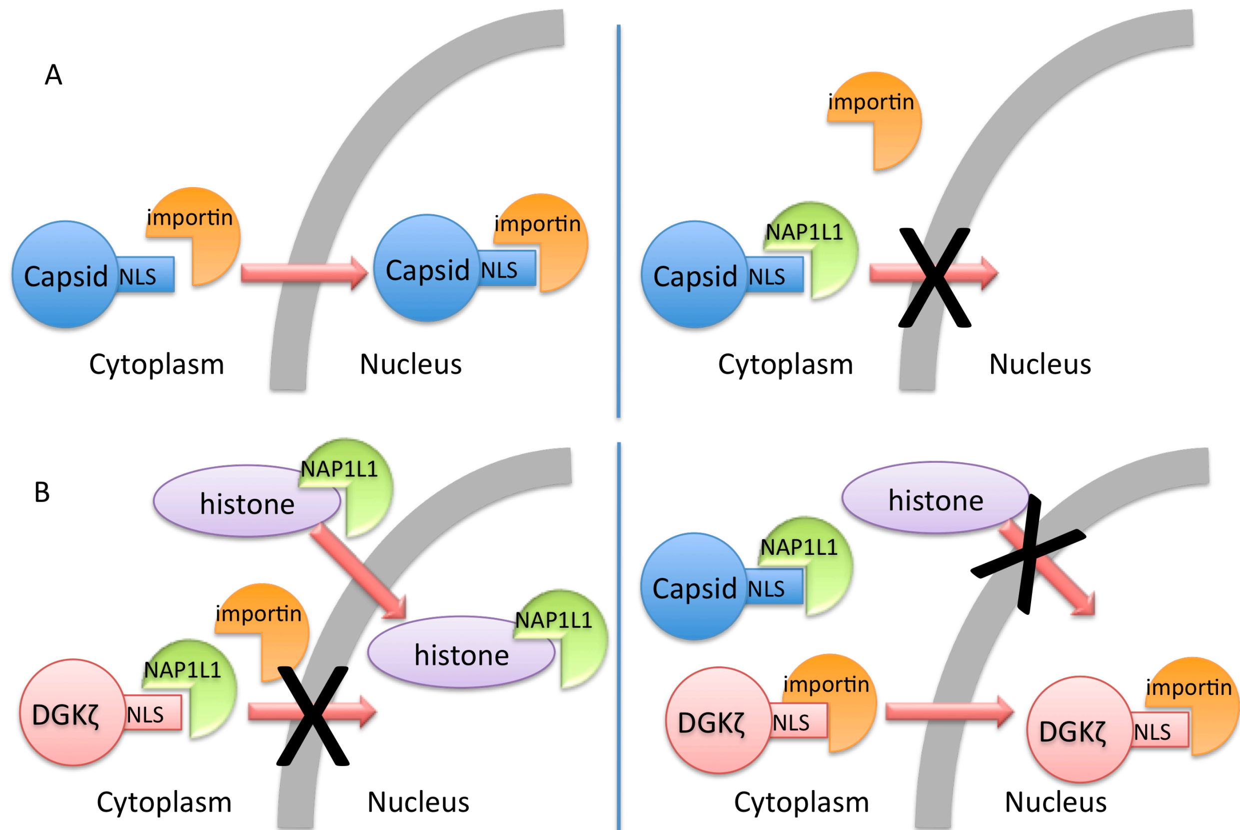


Figure 5-1. Hypothetical models for the role of the capsid-NAP1L1 interaction. (A, left) The bipartite NLS of capsid has been shown to interact with importin resulting in capsid nuclear localization. (A, right) Under unknown cellular conditions, NAP1 might bind to the bipartite NLS of capsid blocking importin binding so the nuclear localization of capsid is reduced. (B, left) Under normal conditions, diacylglycerol kinase zeta (DGKζ) and histone are bound by NAP1L1. The binding sequesters DGKζ in the cytoplasm by blocking the bipartite NLS of DGKζ from Importin (Okada et al., 2011). On the other hand NAP1L1 has been shown to transport histone into the nucleus (Okuwaki et al., 2010). (B) During dengue virus infection, capsid may sequester NAP1L1 so it cannot bind DGKζ or histone. Thus, nuclear localization of DGKζ would be increased, while histone transport into the nucleus would be diminished.

I attempted to test the biological significance of the capsid-NAP1L1 interaction. I generated two HepG2 cell lines that stably express dengue capsid. One expresses Myc-tagged capsid while the other expresses GFP-tagged capsid. Both cell lines showed the accumulation of capsid in the nucleus as previously observed (Tadano et al., 1989; Wang et al., 2002; Sangiambut et al., 2008). I then over-expressed or silenced human NAP1L1. I found that NAP1L1 over-expression resulted in more capsid localizing in the cytoplasm. The results preliminarily support my hypothesis that NAP1L1 inhibits the nuclear localization of capsid.

The functional significance of this interaction is still unknown. Since the capsid protein can bind histones and disrupt nucleosome formation (Colpitts et al., 2011a), hosts might use nucleosome assembly protein 1 as a tool to sequester capsid in the cytoplasm. An interesting observation supporting this hypothesis is that capsid and histones share structural similarities (Colpitts et al., 2011a) so nucleosome assembly protein 1, which is a histone chaperone, might bind capsid protein similar to its binding to histones. On the other hand, capsid-nucleosome assembly protein interaction might be used by the virus to alter or hijack cellular processes to suit its replication. Since nucleosome assembly protein 1 also plays a role in nucleocytoplasmic shuttling of some proteins, such as DGKζ (Park and Luger, 2006; Okada et al., 2011), the capsid protein might bind to nucleosome assembly protein 1 and block the shuttling of those proteins. I have tried to capture these possibilities in the model illustrated in Figure 5-1B.

For further study, capsid-nucleosome assembly protein 1 interaction could be tested *in vitro* by determining whether the interaction between capsid and importin can be disrupted in a dose-dependent manner by increasing levels of nucleosome assembly

protein 1. Co-affinity purification of capsid and Importin along with inducible expression of nucleosome assembly protein 1 could also be used. Finally and importantly, the significance of capsid- nucleosome assembly protein 1 interaction must be shown during the live virus infection. For example, a cell line stably over-expressing nucleosome assembly protein 1 could be infected with dengue virus. Next, the localization of capsid could be determined by immunostaining and microscopy during the course of virus replication. At the same time, the titer of virus generated from the cell line could be measured and compared to those generated by a mock-transfected cell line. On the other hand, the localization of host proteins regulated by nucleosome assembly protein 1, such as histones and DGKζ, may be followed to detect any changes in their localizations during the course of virus replication, compared to that in uninfected cells.

Humans have six paralogs of nucleosome assembly protein 1 (NAP1L1, NAP1L2, NAP1L3, NAP1L4, NAP1L5 and NAP1L6), but only NAP1L1 was detected in this study. It would be interesting to see whether some or all of the other paralogs interact with capsid. Y2H assays or co-affinity purification may be used to test each of the NAP1Ls. I expect that capsid will not interact with NAP1L2, NAP1L3 and NAP1L5 since they are only expressed in neurons (Attia et al., 2011), which are not targets for dengue infection so they never encounter dengue capsid and may lose the interaction interface for capsid found in the ancestral gene during evolution. NAP1L6 is potentially a pseudogene (UniProt Consortium (2012)) so its interaction with capsid would not be biologically relevant. On the other hand, NAP1L4 is closely related to NAP1L1 (Figure

3-1), so it might interact with capsid. Interestingly, both NAP1L1 and NAP1L4 were found to regulate nucleocytoplasmic shuttling of DGKζ (Okada et al., 2011).

5.6 Roles of conserved interologs during the virus life cycle

Many biologically important PPI are conserved among species (Yu et al., 2004). I identified seven potentially conserved interologs by Y2H screens as described in Chapter 2. I also retrieved PPI data from other publications and found six additional conserved interologs. I focused on the capsid-nucleosome assembly protein 1 interaction in Chapter 3. However, the other potentially conserved interologs may also be worth further investigation. A good place to start would be to map the interaction domain of dengue and host proteins to see whether the same domain is required for the interaction in human and mosquito, which would further support the idea that these interactions are functionally conserved.

The thirteen potentially conserved interlogs represent a small fraction of the PPI identified by this study and other publications. Since the dengue-host screens appear to miss many interactions, there may be additional interologs that were not detected. It would be interesting to directly test human or mosquito othologs of the available dengue-host PPI to see whether they are potentially conserved interologs. For example, a mosquito ortholog of a human dengue interactor may be individually cloned from the mosquito cDNA libraries and tested for an interaction by Y2H assays, and vice versa. This might expand the dengue-host interactome or hint at disparity between the dengue-human interactome and the dengue-mosquito interactome. If disparity between the two interactomes is detected, it might be used to explain some observed

phenomena, such as the reduction of fitness in mosquito cells of dengue virus propagated in human cells, and vice versa (Vasilakis et al., 2009).

5.7 Roles of nucleolar proteins during the virus life cycle

In Chapter 2, I showed that nucleolar proteins were enriched among dengue interactors. This is interesting because many viruses have been reported to interact with the nucleolus or disrupt its formation (Hiscox, 2007; Hiscox et al., 2010). The viruses need to interact with the nucleolus to either hijack the host nucleolar proteins or to alter the activity of the nucleolus to suit their replication (Hiscox, 2007; Hiscox et al., 2010). Dengue capsid has been shown to accumulate in the nucleolus during infection (Wang et al., 2002), but the role of nucleolar capsid during dengue replication is not yet clear. The role of nucleolar proteins has been studied in other flaviviruses. WNV and JEV interact with nucleolar proteins, DDX56 and B23, respectively (Tsuda et al., 2006; Xu et al., 2011). However, these nucleolar proteins have no role in dengue replication.

The role of nucleolar proteins identified in this study may be determined by over-expressing or silencing the proteins in the cells before dengue infection as described in Section 5.2.1. In addition, targeted disruption of the nucleolus structure or function may hint at the role of the nucleolus in dengue replication. For example, microinjection of an antibody against upstream binding factor (UBF), which is required for the transcription of ribosomal DNA genes, disrupts nucleolus formation without damaging host DNA (Rubbi and Milner, 2003). This method could be used to determine whether nucleolar integrity is important for viral replication. Nucleolin or transcription initiation factor TIF-IA silencing has also been reported to disrupt nucleolus formation, but it also induces cell

cycle arrest and apoptosis so the method must be assessed to see whether it is appropriate for a dengue infection assay (Yuan et al., 2005; Ugrinova et al., 2007).

5.8 Final remarks

The recent dengue-host interactome studies, including mine, have rapidly expanded our knowledge of potential dengue-host PPI compared to the low-throughput methods previously used. The studies have generated a large amount of data that may be useful for developing antiviral drugs and mosquito control strategies. However, these PPI data are mostly from experiments performed under artificial conditions. More effort is required to expand and validate these data so that we may eliminate the virus and its threat and rid the world of a major health problem.

APPENDIX A . THE RESULTS FOR Y2H MATRIX SCREEN FOR SEROTYPE SPECIFIC/INDEPENDENT INTERACTIONS.

Bait	Gene_ID	Description	Dengue 1	Dengue 2	Dengue 3	Dengue 4	Number_of_Positive_serotype
CA	AAEL000005	hypothetical protein	1	1	1	1	4
CA	AAEL000136	conserved hypothetical protein	0	0	0	1	1
CA	AAEL000292	conserved hypothetical protein	1	1	1	0	3
CA	AAEL001892	conserved hypothetical protein	1	0	0	0	1
CA	AAEL001984	hypothetical protein	1	1	1	1	4
CA	AAEL002057	conserved hypothetical protein	1	1	1	1	4
CA	AAEL002508	26S protease regulatory subunit 6a	0	0	1	0	1
CA	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	1	1	4
CA	AAEL003415	lamin	1	0	0	0	1
CA	AAEL003676	myosin I homologue, putative	1	1	1	0	3
CA	AAEL003739	M-type 9 protein, putative	1	0	0	0	1
CA	AAEL003750	conserved hypothetical protein	1	1	1	1	4
CA	AAEL004100	hypothetical protein	0	0	1	0	1
CA	AAEL004316	hypothetical protein	1	1	1	0	3
CA	AAEL004484	predicted protein	1	0	0	0	1
CA	AAEL004855	adp,atp carrier protein	1	1	1	1	4
CA	AAEL004869	hypothetical protein	0	0	1	0	1
CA	AAEL005165	chaperone protein dnaj	1	1	0	0	2
CA	AAEL005567	nucleosome assembly protein	1	0	1	0	2
CA	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	1	0	0	0	1
CA	AAEL006572	troponin C	0	1	0	0	1
CA	AAEL007980	hypothetical protein	1	1	1	1	4
CA	AAEL008052	hypothetical protein	1	0	0	0	1
CA	AAEL008852	conserved hypothetical protein	1	1	1	1	4
CA	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	1	0	0	0	1
CA	AAEL009182	zinc finger protein, putative	1	1	1	1	4
CA	AAEL009285	dead box atp-dependent rna helicase	1	1	1	1	4
CA	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	1	1	4
CA	AAEL009948	aldehyde dehydrogenase	1	0	0	0	1
CA	AAEL010266	hypothetical protein	1	0	0	0	1
CA	AAEL011129	alcohol dehydrogenase	1	0	0	0	1
CA	AAEL011960	conserved hypothetical protein	1	1	1	1	4
CA	AAEL011985	conserved hypothetical protein	1	1	1	1	4

CA	AAEL012527	conserved hypothetical protein	1	0	0	1	2
CA	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
CA	AAEL013075	conserved hypothetical protein	1	1	1	1	4
CA	AAEL013086	hypothetical protein	1	0	0	0	1
CA	AAEL013583	60S ribosomal protein L23	1	1	1	1	4
CA	AAEL014012	membrane-associated guanylate kinase (maguk)	1	0	0	0	1
CA	AAEL014104	conserved hypothetical protein	1	0	0	0	1
CA	AAEL000752	conserved hypothetical protein	1	0	0	0	1
CA	AAEL000951	elongation factor 1-beta2	1	0	0	0	1
CA	AAEL002828	hypothetical protein	1	0	0	0	1
CV	AAEL000005	hypothetical protein	1	1	1	1	4
CV	AAEL000292	conserved hypothetical protein	1	1	1	1	4
CV	AAEL000950	conserved hypothetical protein	0	1	0	0	1
CV	AAEL001892	conserved hypothetical protein	0	1	0	0	1
CV	AAEL001984	hypothetical protein	1	1	1	1	4
CV	AAEL002057	conserved hypothetical protein	1	1	1	1	4
CV	AAEL002508	26S protease regulatory subunit 6a	0	1	0	0	1
CV	AAEL002565	titin	1	1	0	0	2
CV	AAEL002572	myosin regulatory light chain 2 (mlc-2)	0	1	0	0	1
CV	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	1	1	4
CV	AAEL003415	lamin	1	0	0	0	1
CV	AAEL003676	myosin I homologue, putative	1	1	1	1	4
CV	AAEL003739	M-type 9 protein, putative	1	1	0	1	3
CV	AAEL003750	conserved hypothetical protein	1	1	1	1	4
CV	AAEL003815	zinc finger protein	1	1	0	1	3
CV	AAEL003929	conserved hypothetical protein	0	1	0	0	1
CV	AAEL004100	hypothetical protein	1	1	1	1	4
CV	AAEL004316	hypothetical protein	1	1	1	1	4
CV	AAEL004484	predicted protein	1	1	0	0	2
CV	AAEL004855	adp,atp carrier protein	1	1	1	1	4
CV	AAEL004869	hypothetical protein	1	1	1	1	4
CV	AAEL005567	nucleosome assembly protein	1	1	0	1	3
CV	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	1	3
CV	AAEL005790	malic enzyme	1	1	0	1	3
CV	AAEL006572	troponin C	1	1	0	1	3
CV	AAEL007201	glutamyl aminopeptidase	0	1	0	1	2
CV	AAEL007850	hypothetical protein	1	1	0	0	2
CV	AAEL007980	hypothetical protein	1	1	1	1	4
CV	AAEL008052	hypothetical protein	1	0	0	0	1
CV	AAEL008700	conserved hypothetical	1	1	0	0	2

		protein					
CV	AAEL008852	conserved hypothetical protein	1	1	1	1	4
CV	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	0	1	0	0	1
CV	AAEL009182	zinc finger protein, putative	1	1	1	1	4
CV	AAEL009285	dead box atp-dependent rna helicase	1	1	1	1	4
CV	AAEL009357	myosin v	0	1	0	0	1
CV	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	1	1	4
CV	AAEL009948	aldehyde dehydrogenase	1	1	0	0	2
CV	AAEL010005	conserved hypothetical protein	0	1	0	0	1
CV	AAEL010066	microfibril-associated protein	1	1	0	0	2
CV	AAEL010266	hypothetical protein	1	1	0	0	2
CV	AAEL010360	nucleotide binding protein 2 (nbp 2)	0	1	0	0	1
CV	AAEL010585	spermatogenesis associated factor	1	1	0	1	3
CV	AAEL010782	carboxypeptidase	1	1	1	1	4
CV	AAEL010784	conserved hypothetical protein	1	1	0	1	3
CV	AAEL010821	60S acidic ribosomal protein P0	1	1	0	1	3
CV	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	0	1	0	0	1
CV	AAEL011708	heat shock protein	0	1	0	0	1
CV	AAEL011742	eukaryotic peptide chain release factor subunit	0	1	0	0	1
CV	AAEL011960	conserved hypothetical protein	1	1	1	1	4
CV	AAEL011985	conserved hypothetical protein	1	1	1	1	4
CV	AAEL011988	tRNA selenocysteine associated protein (secp43)	0	1	0	0	1
CV	AAEL012095	26S protease regulatory subunit	1	1	0	0	2
CV	AAEL012527	conserved hypothetical protein	1	1	0	0	2
CV	AAEL012556	Ofd1 protein, putative	0	1	0	0	1
CV	AAEL012680	Juvenile hormone-inducible protein, putative	1	1	0	1	3
CV	AAEL012686	ribosomal protein S12, putative	1	1	0	1	3
CV	AAEL012827	endoplasmin	0	1	0	0	1
CV	AAEL013075	conserved hypothetical protein	1	1	1	1	4
CV	AAEL013086	hypothetical protein	1	1	0	1	3
CV	AAEL013583	60S ribosomal protein L23	1	1	1	1	4
CV	AAEL013933	serine protease inhibitor, serpin	0	1	0	0	1
CV	AAEL014012	membrane-associated guanylate kinase (maguk)	1	1	0	0	2
CV	AAEL014104	conserved hypothetical protein	1	1	0	1	3
CV	AAEL014281	conserved hypothetical protein	1	1	0	0	2
CV	AAEL014396	protein farnesyltransferase alpha subunit	0	1	0	0	1
CV	AAEL014843	heat shock protein	1	1	0	0	2

CV	AAEL014845	heat shock protein	1	1	0	1	3
CV	AAEL000752	conserved hypothetical protein	1	1	0	0	2
CV	AAEL000951	elongation factor 1-beta2	1	1	1	1	4
CV	AAEL002828	hypothetical protein	1	1	1	0	3
CV	AAEL003104	tripartite motif protein trim2,3	0	1	0	0	1
CV	AAEL003973	conserved hypothetical protein	1	1	0	0	2
CV	AAEL003973	conserved hypothetical protein	1	1	0	0	2
CV	AAEL004500	eukaryotic translation elongation factor	1	1	0	0	2
CV	AAEL005037	seryl-tRn/a synthetase	1	1	0	0	2
CV	ENSG00000044574	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	1	1	0	0	2
CV	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
CV	AAEL006577	aspartyl-tRn/a synthetase	0	1	0	0	1
E	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
Eiii	AAEL000005	hypothetical protein	1	1	0	1	3
Eiii	AAEL000950	conserved hypothetical protein	1	0	0	0	1
Eiii	AAEL001553	conserved hypothetical protein	0	0	0	1	1
Eiii	AAEL001892	conserved hypothetical protein	1	0	0	1	2
Eiii	AAEL001984	hypothetical protein	1	1	0	1	3
Eiii	AAEL002057	conserved hypothetical protein	1	1	0	1	3
Eiii	AAEL002508	26S protease regulatory subunit 6a	1	1	0	1	3
Eiii	AAEL002565	titin	1	0	0	1	2
Eiii	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	0	0	0	1
Eiii	AAEL003345	argininosuccinate lyase	1	0	0	1	2
Eiii	AAEL003415	lamin	1	0	0	0	1
Eiii	AAEL003676	myosin I homologue, putative	0	1	0	0	1
Eiii	AAEL003815	zinc finger protein	1	1	0	1	3
Eiii	AAEL003929	conserved hypothetical protein	1	0	0	0	1
Eiii	AAEL004100	hypothetical protein	1	1	0	1	3
Eiii	AAEL004316	hypothetical protein	1	1	0	0	2
Eiii	AAEL004484	predicted protein	1	1	0	1	3
Eiii	AAEL004855	adp,atp carrier protein	0	1	0	0	1
Eiii	AAEL004869	hypothetical protein	1	1	0	1	3
Eiii	AAEL005165	chaperone protein dnaj	1	0	0	1	2
Eiii	AAEL005524	adenosylhomocysteinase	1	0	0	0	1
Eiii	AAEL005567	nucleosome assembly protein	1	1	0	1	3
Eiii	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	0	1	0	0	1
Eiii	AAEL005790	malic enzyme	0	1	0	0	1
Eiii	AAEL006572	troponin C	1	1	0	0	2

Eiii	AAEL007201	glutamyl aminopeptidase	1	0	0	1	2
Eiii	AAEL007850	hypothetical protein	1	0	0	0	1
Eiii	AAEL007980	hypothetical protein	1	0	0	0	1
Eiii	AAEL008052	hypothetical protein	1	0	0	1	2
Eiii	AAEL008700	conserved hypothetical protein	1	0	0	0	1
Eiii	AAEL008746	hypothetical protein	1	0	0	1	2
Eiii	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	1	0	0	1	2
Eiii	AAEL009182	zinc finger protein, putative	1	1	0	1	3
Eiii	AAEL009285	dead box atp-dependent rna helicase	1	1	0	1	3
Eiii	AAEL009357	myosin v	1	1	0	0	2
Eiii	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	0	1	0	0	1
Eiii	AAEL009484	conserved hypothetical protein	1	0	0	1	2
Eiii	AAEL009766	lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase	1	0	0	1	2
Eiii	AAEL009948	aldehyde dehydrogenase	1	1	0	1	3
Eiii	AAEL010066	microfibril-associated protein	1	0	0	1	2
Eiii	AAEL010266	hypothetical protein	1	1	0	0	2
Eiii	AAEL010360	nucleotide binding protein 2 (nbp 2)	0	1	0	0	1
Eiii	AAEL010585	spermatogenesis associated factor	1	1	0	1	3
Eiii	AAEL010782	carboxypeptidase	1	1	0	1	3
Eiii	AAEL010784	conserved hypothetical protein	1	1	0	1	3
Eiii	AAEL010821	60S acidic ribosomal protein P0	1	1	0	1	3
Eiii	AAEL011129	alcohol dehydrogenase	1	0	0	1	2
Eiii	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	1	1	0	1	3
Eiii	AAEL011708	heat shock protein	1	1	0	1	3
Eiii	AAEL011742	eukaryotic peptide chain release factor subunit	1	0	0	1	2
Eiii	AAEL011960	conserved hypothetical protein	0	1	0	0	1
Eiii	AAEL011985	conserved hypothetical protein	1	0	0	0	1
Eiii	AAEL012095	26S protease regulatory subunit	1	1	0	1	3
Eiii	AAEL012527	conserved hypothetical protein	1	1	0	0	2
Eiii	AAEL012556	Ofd1 protein, putative	1	0	0	1	2
Eiii	AAEL012680	Juvenile hormone-inducible protein, putative	1	1	0	1	3
Eiii	AAEL012686	ribosomal protein S12, putative	1	1	0	1	3
Eiii	AAEL012827	endoplasmin	1	1	0	0	2
Eiii	AAEL013075	conserved hypothetical protein	1	1	0	1	3
Eiii	AAEL013583	60S ribosomal protein L23	1	1	0	0	2
Eiii	AAEL013933	serine protease inhibitor, serpin	1	0	0	1	2
Eiii	AAEL014012	membrane-associated guanylate kinase (maguk)	1	0	0	0	1

Eiii	AAEL014104	conserved hypothetical protein	1	1	0	0	2
Eiii	AAEL014281	conserved hypothetical protein	1	1	0	0	2
Eiii	AAEL014396	protein farnesyltransferase alpha subunit	1	1	0	1	3
Eiii	AAEL014843	heat shock protein	1	1	0	1	3
Eiii	AAEL014845	heat shock protein	1	1	0	1	3
Eiii	AAEL000752	conserved hypothetical protein	0	1	0	0	1
Eiii	AAEL000951	elongation factor 1-beta2	1	1	0	0	2
Eiii	AAEL002828	hypothetical protein	1	1	0	1	3
Eiii	AAEL003104	tripartite motif protein trim2,3	0	1	0	0	1
Eiii	AAEL003973	conserved hypothetical protein	1	1	0	0	2
Eiii	AAEL003973	conserved hypothetical protein	1	1	0	1	3
Eiii	AAEL004500	eukaryotic translation elongation factor	1	0	0	1	2
Eiii	AAEL005037	seryl-tRn/a synthetase	1	1	0	0	2
Eiii	ENSG00000044574	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	1	1	0	1	3
Eiii	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
Eiii	AAEL006577	aspartyl-tRn/a synthetase	1	1	0	1	3
M	AAEL007406	Conserved hypothetical protein	1	0	0	0	1
M	AAEL010266	hypothetical protein	1	1	0	0	2
M	AAEL010782	carboxypeptidase	0	1	0	0	1
M	AAEL012686	ribosomal protein S12, putative	0	1	0	0	1
M	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	0	1	0	0	1
NS1	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	0	0	0	1
NS1	AAEL003750	conserved hypothetical protein	1	0	0	0	1
NS1	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	0	0	0	1
NS1	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
NS1	AAEL013075	conserved hypothetical protein	1	0	0	0	1
NS2B	AAEL010266	hypothetical protein	1	0	0	0	1
NS2B	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
NS3	AAEL000005	hypothetical protein	1	1	1	1	4
NS3	AAEL000136	conserved hypothetical protein	1	1	1	1	4
NS3	AAEL000292	conserved hypothetical protein	0	1	0	1	2
NS3	AAEL000950	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL001553	conserved hypothetical protein	1	1	1	1	4

NS3	AAEL001892	conserved hypothetical protein	1	1	1	1	4
NS3	AAEL001984	hypothetical protein	1	1	1	1	4
NS3	AAEL002057	conserved hypothetical protein	1	1	0	1	3
NS3	AAEL002508	26S protease regulatory subunit 6a	1	1	1	1	4
NS3	AAEL002565	titin	1	1	1	1	4
NS3	AAEL002572	myosin regulatory light chain 2 (mlc-2)	1	1	0	0	2
NS3	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	0	1	0	0	1
NS3	AAEL003345	argininosuccinate lyase	1	1	1	1	4
NS3	AAEL003415	lamin	0	1	0	0	1
NS3	AAEL003739	M-type 9 protein, putative	1	1	0	0	2
NS3	AAEL003750	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL003815	zinc finger protein	1	1	1	1	4
NS3	AAEL003929	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL004100	hypothetical protein	1	1	1	1	4
NS3	AAEL004316	hypothetical protein	1	1	0	1	3
NS3	AAEL004484	predicted protein	1	1	0	1	3
NS3	AAEL004869	hypothetical protein	1	1	1	1	4
NS3	AAEL005165	chaperone protein dnaj	1	1	1	0	3
NS3	AAEL005524	adenosylhomocysteinase	0	1	0	0	1
NS3	AAEL005567	nucleosome assembly protein	1	1	1	1	4
NS3	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
NS3	AAEL005790	malic enzyme	1	1	0	1	3
NS3	AAEL006572	troponin C	1	1	0	0	2
NS3	AAEL007201	glutamyl aminopeptidase	1	1	1	1	4
NS3	AAEL007850	hypothetical protein	1	1	0	1	3
NS3	AAEL007980	hypothetical protein	1	1	0	1	3
NS3	AAEL008052	hypothetical protein	1	1	1	1	4
NS3	AAEL008700	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL008746	hypothetical protein	1	1	1	1	4
NS3	AAEL008852	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	1	1	1	1	4
NS3	AAEL009182	zinc finger protein, putative	1	1	0	1	3
NS3	AAEL009285	dead box atp-dependent rna helicase	1	1	0	0	2
NS3	AAEL009357	myosin v	1	1	0	1	3
NS3	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	0	1	0	0	1
NS3	AAEL009484	conserved hypothetical protein	1	1	1	1	4
NS3	AAEL009766	lipoamide acyltransferase component of branched-chain alpha-keto acid dehydrogenase	1	1	1	1	4
NS3	AAEL009948	aldehyde dehydrogenase	1	1	1	1	4

NS3	AAEL010005	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL010012	gtp-binding protein sar1	1	1	0	0	2
NS3	AAEL010066	microfibril-associated protein	1	1	0	0	2
NS3	AAEL007406	Conserved hypothetical protein	0	1	0	0	1
NS3	AAEL010266	hypothetical protein	0	1	0	0	1
NS3	AAEL010360	nucleotide binding protein 2 (nbp 2)	1	1	0	0	2
NS3	AAEL010585	spermatogenesis associated factor	1	1	1	1	4
NS3	AAEL010782	carboxypeptidase	1	1	0	0	2
NS3	AAEL010784	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL010821	60S acidic ribosomal protein P0	1	1	1	0	3
NS3	AAEL010975	paramyosin, long form	1	1	1	0	3
NS3	AAEL011129	alcohol dehydrogenase	1	1	1	1	4
NS3	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	1	1	1	1	4
NS3	AAEL011708	heat shock protein	1	1	1	1	4
NS3	AAEL011742	eukaryotic peptide chain release factor subunit	1	1	1	1	4
NS3	AAEL011960	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL011985	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL011988	tRNA selenocysteine associated protein (secp43)	0	1	0	0	1
NS3	AAEL012095	26S protease regulatory subunit	1	1	1	1	4
NS3	AAEL012527	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL012556	Ofd1 protein, putative	1	1	1	1	4
NS3	AAEL012680	Juvenile hormone-inducible protein, putative	1	1	1	1	4
NS3	AAEL012686	ribosomal protein S12, putative	1	1	1	0	3
NS3	AAEL012827	endoplasmin	1	1	1	1	4
NS3	AAEL013075	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL013933	serine protease inhibitor, serpin	1	1	1	0	3
NS3	AAEL014012	membrane-associated guanylate kinase (maguk)	1	1	0	0	2
NS3	AAEL014104	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL014281	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL014396	protein farnesyltransferase alpha subunit	1	1	1	1	4
NS3	AAEL014843	heat shock protein	1	1	1	1	4
NS3	AAEL014845	heat shock protein	1	1	1	1	4
NS3	AAEL000752	conserved hypothetical protein	1	1	0	0	2
NS3	AAEL000951	elongation factor 1-beta2	1	1	0	0	2
NS3	AAEL002828	hypothetical protein	1	1	0	0	2
NS3	AAEL003104	tripartite motif protein trim2,3	0	1	0	0	1
NS3	AAEL003973	conserved hypothetical protein	0	1	0	0	1
NS3	AAEL003973	conserved hypothetical protein	1	1	1	1	4

NS3	AAEL004500	eukaryotic translation elongation factor	1	1	1	1	4
NS3	AAEL005037	seryl-tRn/a synthetase	1	1	0	0	2
NS3	ENSG00000044574	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	1	1	0	0	2
NS3	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
NS3	AAEL006577	aspartyl-tRn/a synthetase	1	1	1	1	4
NS3d	AAEL000005	hypothetical protein	1	1	0	0	2
NS3d	AAEL000292	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL000950	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL001892	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL001984	hypothetical protein	1	1	0	0	2
NS3d	AAEL002057	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL002508	26S protease regulatory subunit 6a	1	1	0	0	2
NS3d	AAEL002565	titin	1	1	0	0	2
NS3d	AAEL002572	myosin regulatory light chain 2 (mlc-2)	1	1	0	0	2
NS3d	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	0	1	0	0	1
NS3d	AAEL003345	argininosuccinate lyase	0	1	0	0	1
NS3d	AAEL003415	lamin	0	1	0	0	1
NS3d	AAEL003676	myosin I homologue, putative	0	1	0	0	1
NS3d	AAEL003739	M-type 9 protein, putative	1	1	0	0	2
NS3d	AAEL003815	zinc finger protein	1	1	0	1	3
NS3d	AAEL003929	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL004100	hypothetical protein	1	1	0	1	3
NS3d	AAEL004316	hypothetical protein	1	1	0	0	2
NS3d	AAEL004484	predicted protein	1	1	0	0	2
NS3d	AAEL004869	hypothetical protein	1	1	0	0	2
NS3d	AAEL005165	chaperone protein dnaj	0	1	0	0	1
NS3d	AAEL005567	nucleosome assembly protein	1	1	0	0	2
NS3d	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
NS3d	AAEL005790	malic enzyme	1	1	0	0	2
NS3d	AAEL006572	troponin C	1	1	0	0	2
NS3d	AAEL007201	glutamyl aminopeptidase	1	1	0	0	2
NS3d	AAEL007850	hypothetical protein	0	1	0	0	1
NS3d	AAEL007980	hypothetical protein	0	1	0	0	1
NS3d	AAEL008052	hypothetical protein	0	1	0	0	1
NS3d	AAEL008746	hypothetical protein	0	1	0	0	1
NS3d	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	0	1	0	0	1
NS3d	AAEL009182	zinc finger protein, putative	1	1	0	0	2
NS3d	AAEL009285	dead box atp-dependent rna helicase	0	1	0	0	1
NS3d	AAEL009357	myosin v	0	1	0	0	1

NS3d	ENSG00000038219	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	0	1	0	0	1
NS3d	AAEL009948	aldehyde dehydrogenase	1	1	0	0	2
NS3d	AAEL010005	conserved hypothetical protein	1	0	0	0	1
NS3d	AAEL010066	microfibril-associated protein	1	1	0	0	2
NS3d	AAEL010266	hypothetical protein	0	1	0	0	1
NS3d	AAEL010360	nucleotide binding protein 2 (nbp 2)	1	1	0	0	2
NS3d	AAEL010585	spermatogenesis associated factor	1	1	0	0	2
NS3d	AAEL010782	carboxypeptidase	1	1	0	0	2
NS3d	AAEL010784	conserved hypothetical protein	1	1	0	1	3
NS3d	AAEL010821	60S acidic ribosomal protein P0	1	1	0	0	2
NS3d	AAEL011129	alcohol dehydrogenase	0	1	0	0	1
NS3d	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	1	1	0	0	2
NS3d	AAEL011708	heat shock protein	1	1	0	0	2
NS3d	AAEL011742	eukaryotic peptide chain release factor subunit	1	1	0	0	2
NS3d	AAEL011960	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL011985	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL012095	26S protease regulatory subunit	1	1	0	0	2
NS3d	AAEL012527	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL012556	Ofd1 protein, putative	1	0	0	1	2
NS3d	AAEL012680	Juvenile hormone-inducible protein, putative	1	1	0	0	2
NS3d	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
NS3d	AAEL012827	endoplasmin	1	1	0	0	2
NS3d	AAEL013075	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL013933	serine protease inhibitor, serpin	1	1	0	0	2
NS3d	AAEL014012	membrane-associated guanylate kinase (maguk)	0	1	0	0	1
NS3d	AAEL014104	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL014281	conserved hypothetical protein	0	1	0	0	1
NS3d	AAEL014396	protein farnesyltransferase alpha subunit	1	1	0	0	2
NS3d	AAEL014843	heat shock protein	1	1	0	0	2
NS3d	AAEL014845	heat shock protein	0	1	0	0	1
NS3d	AAEL000752	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL000951	elongation factor 1-beta2	0	1	0	0	1
NS3d	AAEL002828	hypothetical protein	1	1	0	0	2
NS3d	AAEL003104	tripartite motif protein trim2,3	1	1	0	0	2
NS3d	AAEL003973	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL003973	conserved hypothetical protein	1	1	0	0	2
NS3d	AAEL004500	eukaryotic translation	0	1	0	0	1

		elongation factor					
NS3d	AAEL005037	seryl-tRn/a synthetase	1	1	0	0	2
NS3d	ENSG00000044574	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	1	1	0	0	2
NS3d	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	1	1	0	0	2
NS3d	AAEL006577	aspartyl-tRn/a synthetase	1	1	0	0	2
NS4A	AAEL003064	conserved hypothetical protein	0	1	0	0	1
NS4A	AAEL010266	hypothetical protein	1	1	0	0	2
NS4A	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
NS4B	AAEL003064	conserved hypothetical protein	0	1	0	0	1
NS4B	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
NS5	AAEL000005	hypothetical protein	1	1	1	1	4
NS5	AAEL000136	conserved hypothetical protein	0	0	1	0	1
NS5	AAEL000292	conserved hypothetical protein	1	1	1	0	3
NS5	AAEL000436	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL000950	conserved hypothetical protein	1	0	0	0	1
NS5	AAEL001553	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL001892	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL001984	hypothetical protein	1	1	1	1	4
NS5	AAEL002057	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL002508	26S protease regulatory subunit 6a	1	1	1	0	3
NS5	AAEL002565	titin	1	1	1	0	3
NS5	AAEL002572	myosin regulatory light chain 2 (mlc-2)	1	1	0	0	2
NS5	ENSG00000187109	Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	0	0	2
NS5	AAEL003064	conserved hypothetical protein	0	1	0	0	1
NS5	AAEL003345	argininosuccinate lyase	1	1	0	0	2
NS5	AAEL003415	lamin	1	1	1	0	3
NS5	AAEL003676	myosin I homologue, putative	1	1	0	0	2
NS5	AAEL003739	M-type 9 protein, putative	1	1	1	1	4
NS5	AAEL003750	conserved hypothetical protein	1	0	0	0	1
NS5	AAEL003815	zinc finger protein	1	1	1	0	3
NS5	AAEL003929	conserved hypothetical protein	1	1	1	0	3
NS5	AAEL004100	hypothetical protein	1	1	1	1	4
NS5	AAEL004316	hypothetical protein	1	1	1	1	4
NS5	AAEL004484	predicted protein	1	1	1	0	3
NS5	AAEL004855	adp,atp carrier protein	0	1	1	0	2
NS5	AAEL004869	hypothetical protein	1	1	1	0	3
NS5	AAEL005165	chaperone protein dnaj	1	1	1	0	3

NS5	AAEL005524	adenosylhomocysteinase	1	1	0	0	2
NS5	AAEL005567	nucleosome assembly protein	1	1	1	0	3
NS5	AAEL005656	myosin heavy chain, nonmuscle or smooth muscle	1	1	1	0	3
NS5	AAEL005790	malic enzyme	1	1	1	1	4
NS5	AAEL006572	troponin C	1	1	1	1	4
NS5	AAEL007201	glutamyl aminopeptidase	1	1	1	0	3
NS5	AAEL007850	hypothetical protein	1	1	0	0	2
NS5	AAEL007980	hypothetical protein	1	1	1	0	3
NS5	AAEL008052	hypothetical protein	1	0	0	0	1
NS5	AAEL008700	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL008746	hypothetical protein	1	1	0	0	2
NS5	AAEL008852	conserved hypothetical protein	0	1	0	0	1
NS5	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	1	1	0	0	2
NS5	AAEL009182	zinc finger protein, putative	1	1	1	0	3
NS5	AAEL009285	dead box atp-dependent rna helicase	1	1	0	0	2
NS5	AAEL009357	myosin v	1	1	1	0	3
NS5	ENSG0000003821 9	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	0	0	2
NS5	AAEL009484	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL009948	aldehyde dehydrogenase	1	1	1	0	3
NS5	AAEL010005	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL010012	gtp-binding protein sar1	1	1	0	0	2
NS5	AAEL010066	microfibril-associated protein	1	1	1	1	4
NS5	AAEL010266	hypothetical protein	1	1	1	0	3
NS5	AAEL010360	nucleotide binding protein 2 (nbp 2)	1	1	1	0	3
NS5	AAEL010585	spermatogenesis associated factor	1	1	1	0	3
NS5	AAEL010782	carboxypeptidase	1	1	1	0	3
NS5	AAEL010784	conserved hypothetical protein	1	1	1	0	3
NS5	AAEL010821	60S acidic ribosomal protein P0	1	1	1	0	3
NS5	AAEL010975	paramyosin, long form	1	1	1	1	4
NS5	AAEL011129	alcohol dehydrogenase	1	0	0	0	1
NS5	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	1	1	1	0	3
NS5	AAEL011708	heat shock protein	1	1	0	0	2
NS5	AAEL011742	eukaryotic peptide chain release factor subunit	1	1	0	1	3
NS5	AAEL011960	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL011985	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL011988	tRNA selenocysteine associated protein (secp43)	1	1	0	0	2
NS5	AAEL012095	26S protease regulatory subunit	1	1	1	1	4
NS5	AAEL012527	conserved hypothetical protein	1	1	1	0	3

NS5	AAEL012556	Ofd1 protein, putative	1	1	1	0	3
NS5	AAEL012680	Juvenile hormone-inducible protein, putative	1	1	1	1	4
NS5	AAEL012686	ribosomal protein S12, putative	1	1	1	0	3
NS5	AAEL012827	endoplasmin	1	1	1	0	3
NS5	AAEL013075	conserved hypothetical protein	1	1	0	0	2
NS5	AAEL013086	hypothetical protein	1	1	1	0	3
NS5	AAEL013583	60S ribosomal protein L23	1	0	0	0	1
NS5	AAEL013933	serine protease inhibitor, serpin	1	1	0	0	2
NS5	AAEL014012	membrane-associated guanylate kinase (maguk)	1	1	0	1	3
NS5	AAEL014104	conserved hypothetical protein	1	1	0	1	3
NS5	AAEL014281	conserved hypothetical protein	1	0	0	1	2
NS5	AAEL014396	protein farnesyltransferase alpha subunit	1	1	1	1	4
NS5	AAEL014843	heat shock protein	1	1	1	0	3
NS5	AAEL014845	heat shock protein	1	1	0	0	2
NS5	AAEL000752	conserved hypothetical protein	1	1	0	1	3
NS5	AAEL000951	elongation factor 1-beta2	1	1	0	0	2
NS5	AAEL002828	hypothetical protein	1	1	1	0	3
NS5	AAEL003104	tripartite motif protein trim2,3	1	1	1	0	3
NS5	AAEL003973	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL003973	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL004500	eukaryotic translation elongation factor	1	1	1	0	3
NS5	AAEL005037	seryl-tRn/a synthetase	1	1	0	0	2
NS5	ENSG0000004457 4	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	1	1	1	0	3
NS5	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	1	1	1	0	3
NS5	AAEL006577	aspartyl-tRn/a synthetase	1	1	1	0	3
PrM	AAEL000005	hypothetical protein	0	1	0	0	1
PrM	AAEL001984	hypothetical protein	0	1	0	0	1
PrM	AAEL002057	conserved hypothetical protein	0	1	0	0	1
PrM	AAEL004100	hypothetical protein	0	1	0	0	1
PrM	AAEL004869	hypothetical protein	0	1	0	0	1
PrM	AAEL005165	chaperone protein dnaj	0	1	0	0	1
PrM	AAEL005567	nucleosome assembly protein	0	1	0	0	1
PrM	AAEL006572	troponin C	0	1	0	0	1
PrM	AAEL009182	zinc finger protein, putative	0	1	0	0	1
PrM	ENSG0000003821 9	Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	0	1	0	0	1
PrM	AAEL007406	Conserved hypothetical protein	1	1	0	0	2
PrM	AAEL010266	hypothetical protein	1	1	1	1	4
PrM	AAEL010360	nucleotide binding protein	0	1	0	0	1

		2 (nbp 2)					
PrM	AAEL010585	spermatogenesis associated factor	0	1	0	0	1
PrM	AAEL010782	carboxypeptidase	1	1	0	0	2
PrM	AAEL010784	conserved hypothetical protein	0	1	0	0	1
PrM	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	0	1	0	0	1
PrM	AAEL011708	heat shock protein	0	1	0	0	1
PrM	AAEL012556	Ofd1 protein, putative	0	1	0	0	1
PrM	AAEL012686	ribosomal protein S12, putative	1	1	0	0	2
PrM	AAEL014104	conserved hypothetical protein	0	1	0	0	1
PrM	AAEL014396	protein farnesyltransferase alpha subunit	0	1	0	0	1
PrM	AAEL014843	heat shock protein	0	1	0	0	1
PrM	AAEL000752	conserved hypothetical protein	0	1	0	0	1
PrM	AAEL002828	hypothetical protein	0	1	0	0	1
PrM	AAEL004500	eukaryotic translation elongation factor	0	1	0	0	1
PrM	AAEL005037	seryl-tRn/a synthetase	0	1	0	0	1
PrM	ENSG00000044574	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	0	1	0	0	1
PrM	AAEL005733	myosin heavy chain, nonmuscle or smooth muscle	0	1	0	0	1
CA	AAEL010507	hypothetical protein	0	0	1	0	1
CA	AAEL012237	bhlhzip transcription factor max/bigmax	1	1	0	1	3
CA	AAEL012348	splicing factor 3a	0	0	1	0	1
CA	AAEL004783	ornithine decarboxylase antizyme,	0	1	0	0	1
CA	ENSG00000197958	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	1	1	4
CA	ENSG00000132842	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	1	1	1	4
CA	ENSG00000123124	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	1	1	4
CA	ENSG00000110330	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	1	1	1	4
CA	ENSG00000067334	DNTTIP2; Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	0	0	1	0	1
CA	ENSG00000107937	GTPBP4; Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	1	1	1	1	4
CA	ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	1	1	1	1	4
CA	ENSG00000102879	Coro1a; Homo sapiens coronin, actin binding protein, 1A (CORO1A),	1	1	1	1	4

		mRNA					
CA	ENSG0000012240 6	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	1	1	4
CA	ENSG0000003821 9	BOD1L; Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	1	1	4
CA	ENSG0000014474 7	TMF1; Homo sapiens TATA element modulatory factor 1 (TMF1), mRNA	1	1	1	0	3
CA	ENSG0000018878 6	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	0	0	0	1
CA	ENSG0000020574 4	DENND1C; Homo sapiens DENN/MADD domain containing 1C (DENND1C), mRNA	0	0	1	0	1
CA	ENSG0000016322 0	S100A9; Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	1	1	1	1	4
CA	ENSG0000013693 8	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	1	1	1	4
CA	ENSG0000010174 5	ANKRD12; Homo sapiens ankyrin repeat domain 12 (ANKRD12), transcript variant 2, mRNA	1	1	1	1	4
CA	ENSG0000016065 4	Cd3g; Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G), mRNA	1	1	1	1	4
CA	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	1	1	1	1	4
CA	ENSG0000018710 9	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	1	1	4
CA		Homo sapiens haplogroup K1c1 mitochondrion, complete genome	0	1	0	0	1
CA	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	0	0	0	1
CA	ENSG0000015097 7	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	1	0	0	1	2
CA	ENSG0000017186 3	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	1	1	1	4
CA	ENSG0000017795 4	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	1	1	1	1	4
CA	ENSG0000005274 9	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant	1	1	1	1	4

		2, mRNA					
CA	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	1	1	4
CV	AAEL009460	conserved hypothetical protein	1	1	0	0	2
CV	AAEL009614	seven in absentia, putative	0	1	0	0	1
CV	AAEL010507	hypothetical protein	1	1	1	1	4
CV	AAEL012237	bhlhzip transcription factor max/bigmax	1	1	0	1	3
CV	AAEL012348	splicing factor 3a	1	1	0	0	2
CV	AAEL004783	ornithine decarboxylase antizyme,	0	1	0	0	1
CV	ENSG00000197958	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	1	1	4
CV	ENSG00000160908	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	1	1	0	1	3
CV	ENSG00000132842	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	1	0	1	3
CV	ENSG00000123124	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	1	1	4
CV	ENSG00000110330	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	1	1	1	4
CV	ENSG00000067334	DNTTIP2; Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	1	1	1	1	4
CV	ENSG00000107937	GTPBP4; Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	1	1	0	1	3
CV	ENSG00000178035	Impdh2; Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	0	1	0	0	1
CV	ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	1	1	1	1	4
CV	ENSG00000122406	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	1	1	4
CV	ENSG00000038219	BOD1L; Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	1	1	4
CV	ENSG00000144747	TMF1; Homo sapiens TATA element modulatory factor 1 (TMF1), mRNA	1	1	1	1	4
CV	ENSG00000188786	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	1	0	0	2
CV	ENSG00000205744	DENND1C; Homo sapiens DENN/MADD domain	1	1	0	1	3

		containing 1C (DENND1C), mRNA					
CV	ENSG0000016322 0	S100A9; Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	1	0	0	0	1
CV	ENSG0000013693 8	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	1	1	1	4
CV	ENSG0000010174 5	ANKRD12; Homo sapiens ankyrin repeat domain 12 (ANKRD12), transcript variant 2, mRNA	1	1	1	1	4
CV	ENSG0000007284 9	Derl2; Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	0	1	0	0	1
CV	ENSG0000017323 0	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	1	0	0	0	1
CV	ENSG0000016065 4	Cd3g; Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G), mRNA	1	1	1	1	4
CV	ENSG0000010208 1	FMR1; Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	0	1	0	0	1
CV	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	1	1	1	1	4
CV	ENSG0000012483 1	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	1	1	0	1	3
CV	ENSG0000018710 9	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	0	1	3
CV	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	0	1	3
CV	ENSG0000011972 5	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	1	1	0	0	2
CV	ENSG0000015097 7	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	0	1	0	0	1
CV	ENSG0000010076 4	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	0	1	0	0	1
CV	ENSG0000024473 4	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	0	1	0	0	1
CV	ENSG0000017186 3	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	1	1	1	4
CV	ENSG0000017277 5	fam192a; Homo sapiens family with sequence	1	1	1	1	4

		similarity 192, member A (FAM192A), mRNA					
CV	ENSG00000177954	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	1	1	0	1	3
CV	ENSG00000052749	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	1	1	1	1	4
CV	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	0	1	0	0	1
Eiii	AAEL009460	conserved hypothetical protein	1	0	0	0	1
Eiii	AAEL009614	seven in absentia, putative	1	0	0	0	1
Eiii	AAEL010507	hypothetical protein	1	0	0	1	2
Eiii	AAEL012237	bhlhzip transcription factor max/bigmax	1	1	0	0	2
Eiii	AAEL012348	splicing factor 3a	1	1	0	0	2
Eiii	AAEL004783	ornithine decarboxylase antizyme,	1	0	0	1	2
Eiii	ENSG00000197958	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	0	1	3
Eiii	ENSG00000160908	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	1	0	0	0	1
Eiii	ENSG00000132842	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	0	0	0	1
Eiii	ENSG00000123124	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	0	1	3
Eiii	ENSG00000067334	DNTTIP2; Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	1	0	0	1	2
Eiii	ENSG00000107937	GTPBP4; Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	0	1	0	0	1
Eiii	ENSG00000178035	Impdh2; Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	1	0	0	0	1
Eiii	ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	1	0	0	0	1
Eiii	ENSG00000102879	Coro1a; Homo sapiens coronin, actin binding protein, 1A (CORO1A), mRNA	1	0	0	0	1
Eiii	ENSG00000122406	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	0	1	3
Eiii	ENSG00000114391	Homo sapiens ribosomal protein L24, mRNA (cDNA clone MGC:2240 IMAGE:3349215), complete cds	1	0	0	0	1

Eiii	ENSG0000018878 6	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	0	0	1	2
Eiii	ENSG0000013693 8	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	0	0	1	2
Eiii	ENSG0000007284 9	Derl2; Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	1	0	0	0	1
Eiii	ENSG0000017323 0	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	1	0	0	1	2
Eiii	ENSG0000019885 1	CD3E; Homo sapiens CD3e molecule, epsilon (CD3-TCR complex) (CD3E), mRNA	1	0	0	0	1
Eiii	ENSG0000010208 1	FMR1; Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	1	0	0	1	2
Eiii	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	1	0	0	1	2
Eiii	ENSG0000012483 1	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	1	1	0	0	2
Eiii	ENSG0000018710 9	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	0	0	0	1
Eiii		Homo sapiens haplogroup K1c1 mitochondrion, complete genome	1	0	0	0	1
Eiii	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	0	0	1	2
Eiii	ENSG0000011972 5	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	1	0	0	1	2
Eiii	ENSG0000010076 4	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	1	0	0	1	2
Eiii	ENSG0000024473 4	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	1	1	0	0	2
Eiii	ENSG0000013643 6	CALCOCO2; Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	1	0	0	1	2
Eiii	ENSG0000011521 6	nrbp1; Homo sapiens nuclear receptor binding protein 1 (NRBP1), mRNA	1	0	0	1	2
Eiii	ENSG0000017186 3	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	1	0	0	2
Eiii	ENSG0000017277 5	fam192a; Homo sapiens family with sequence	1	1	0	1	3

		similarity 192, member A (FAM192A), mRNA					
Eiii	ENSG00000177954	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	1	0	0	0	1
Eiii	ENSG00000052749	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	1	1	0	1	3
Eiii	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	0	0	1	2
NS1	ENSG00000132842	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	0	0	0	1
NS1	ENSG00000110330	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	0	0	0	1
NS1	ENSG00000163220	S100A9; Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	1	0	0	0	1
NS1	ENSG00000136938	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	0	0	0	1
NS1	ENSG00000187109	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	0	0	0	1
NS1	ENSG00000177954	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	1	0	0	0	1
NS1	ENSG00000052749	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	1	0	0	0	1
NS1	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	0	0	0	1
NS2B	ENSG00000206172	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	0	1	0	0	1
NS3	AAEL009460	conserved hypothetical protein	1	1	1	1	4
NS3	AAEL009614	seven in absentia, putative	1	0	1	0	2
NS3	AAEL010507	hypothetical protein	1	1	0	1	3
NS3	AAEL012237	bhlhzip transcription factor max/bigmax	1	1	0	1	3
NS3	AAEL012348	splicing factor 3a	1	1	0	1	3
NS3	AAEL004783	ornithine decarboxylase antizyme,	1	1	1	1	4
NS3	ENSG00000197958	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	1	1	4
NS3	ENSG00000160908	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	1	1	0	1	3

NS3	ENSG0000013284 2	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	1	1	0	0	2
NS3	ENSG0000012312 4	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	1	1	4
NS3	ENSG0000011033 0	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	1	0	0	2
NS3	ENSG0000006733 4	DNTTIP2; Homo sapiens deoxynucleotidyltransferas e, terminal, interacting protein 2 (DNTTIP2), mRNA	1	1	1	1	4
NS3	ENSG0000010793 7	GTPBP4; Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	1	1	0	0	2
NS3	ENSG0000017803 5	Impdh2; Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	1	1	1	1	4
NS3	ENSG0000008900 9	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	0	1	0	0	1
NS3	ENSG0000010287 9	Coro1a; Homo sapiens coronin, actin binding protein, 1A (CORO1A), mRNA	1	1	1	1	4
NS3	ENSG0000012240 6	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	1	1	4
NS3	ENSG0000014474 7	TMF1; Homo sapiens TATA element modulatory factor 1 (TMF1), mRNA	0	1	0	0	1
NS3	ENSG0000018878 6	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	1	1	1	4
NS3	ENSG0000020574 4	DENND1C; Homo sapiens DENN/MADD domain containing 1C (DENND1C), mRNA	1	1	0	0	2
NS3	ENSG0000016322 0	S100A9; Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	1	1	0	1	3
NS3	ENSG0000013693 8	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	1	1	1	4
NS3	ENSG0000007284 9	Derl2; Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	1	1	1	1	4
NS3	ENSG0000017323 0	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	1	1	1	1	4
NS3	ENSG0000010208 1	FMR1; Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	1	1	1	1	4

NS3	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	0	1	0	0	1
NS3	ENSG0000012483 1	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	1	1	0	1	3
NS3	ENSG0000018710 9	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	0	0	2
NS3		Homo sapiens haplogroup K1c1 mitochondrion, complete genome	1	1	1	1	4
NS3	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	1	1	4
NS3	ENSG0000011972 5	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	1	1	1	1	4
NS3	ENSG0000015097 7	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	1	1	0	1	3
NS3	ENSG0000010076 4	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	1	1	1	1	4
NS3	ENSG0000024473 4	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	0	1	0	1	2
NS3	ENSG0000013643 6	CALCOCO2; Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	1	1	1	1	4
NS3	ENSG0000011521 6	nrbp1; Homo sapiens nuclear receptor binding protein 1 (NRBP1), mRNA	1	1	1	1	4
NS3	ENSG0000017186 3	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	1	0	1	3
NS3	ENSG0000017277 5	fam192a; Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	1	1	0	1	3
NS3	ENSG0000010090 6	NFKBIA; Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA	1	1	0	1	3
NS3	ENSG0000017795 4	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	0	1	0	1	2
NS3	ENSG0000005274 9	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	0	1	0	1	2
NS3	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	1	1	4

NS3d	AAEL009460	conserved hypothetical protein	1	0	0	0	1
NS3d	AAEL010507	hypothetical protein	1	1	0	1	3
NS3d	AAEL012237	bhlhzip transcription factor max/bigmax	0	1	0	0	1
NS3d	AAEL012348	splicing factor 3a	1	1	0	0	2
NS3d	AAEL004783	ornithine decarboxylase antizyme,	0	1	0	0	1
NS3d	ENSG00000197958	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	0	0	2
NS3d	ENSG00000160908	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	0	1	0	0	1
NS3d	ENSG00000132842	AP3B1; Homo sapiens adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA	0	1	0	0	1
NS3d	ENSG00000123124	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	0	0	2
NS3d	ENSG00000067334	DNTTIP2; Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	1	1	0	1	3
NS3d	ENSG00000178035	Impdh2; Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	1	1	0	0	2
NS3d	ENSG00000089009	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	0	1	0	0	1
NS3d	ENSG00000102879	Coro1a; Homo sapiens coronin, actin binding protein, 1A (CORO1A), mRNA	0	1	0	0	1
NS3d	ENSG00000122406	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	0	0	2
NS3d	ENSG00000188786	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	1	0	0	2
NS3d	ENSG00000136938	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	0	1	0	0	1
NS3d	ENSG00000072849	Derl2; Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	1	0	0	0	1
NS3d	ENSG00000173230	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	0	1	0	0	1
NS3d	ENSG00000206172	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	0	1	0	0	1
NS3d	ENSG00000124831	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript	1	1	0	0	2

		variant 5, mRNA					
NS3d	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	0	0	2
NS3d	ENSG0000011972 5	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	1	1	0	0	2
NS3d	ENSG0000010076 4	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	1	1	0	0	2
NS3d	ENSG0000024473 4	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	1	1	0	0	2
NS3d	ENSG0000013643 6	CALCOCO2; Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	0	1	0	0	1
NS3d	ENSG0000017186 3	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	0	1	0	0	1
NS3d	ENSG0000017277 5	fam192a; Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	1	1	0	0	2
NS3d	ENSG0000010090 6	NFKBIA; Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA	0	1	0	0	1
NS3d	ENSG0000005274 9	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	1	0	0	0	1
NS3d	ENSG0000013550 6	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	0	0	2
NS4 A	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	0	1	0	0	1
NS4 B	ENSG0000020617 2	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	0	1	0	0	1
NS5	AAEL009460	conserved hypothetical protein	1	1	1	1	4
NS5	AAEL009614	seven in absentia, putative	1	0	1	0	2
NS5	AAEL010507	hypothetical protein	1	1	1	0	3
NS5	AAEL012237	bhlhzip transcription factor max/bigmax	1	1	1	1	4
NS5	AAEL012348	splicing factor 3a	1	1	1	1	4
NS5	AAEL004783	ornithine decarboxylase antizyme,	1	1	1	0	3
NS5	ENSG0000019795 8	RPL12; Homo sapiens ribosomal protein L12 (RPL12), mRNA	1	1	1	1	4
NS5	ENSG0000016090 8	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	1	1	0	0	2
NS5	ENSG0000013284	AP3B1; Homo sapiens	1	1	0	0	2

	2	adaptor-related protein complex 3, beta 1 subunit (AP3B1), mRNA					
NS5	ENSG0000012312 4	WWP1; Homo sapiens WW domain containing E3 ubiquitin protein ligase 1 (WWP1), mRNA	1	1	1	1	4
NS5	ENSG0000011033 0	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	1	1	0	0	2
NS5	ENSG0000006733 4	DNTTIP2; Homo sapiens deoxynucleotidyltransferase, terminal, interacting protein 2 (DNTTIP2), mRNA	1	1	1	0	3
NS5	ENSG0000010793 7	GTPBP4; Homo sapiens GTP binding protein 4 (GTPBP4), mRNA	1	1	0	0	2
NS5	ENSG0000017803 5	Impdh2; Homo sapiens IMP (inosine monophosphate) dehydrogenase 2 (IMPDH2), mRNA	1	1	0	0	2
NS5	ENSG0000008900 9	Homo sapiens ribosomal protein L6 (RPL6), transcript variant 2, mRNA	1	1	0	0	2
NS5	ENSG0000010287 9	Coro1a; Homo sapiens coronin, actin binding protein, 1A (CORO1A), mRNA	1	0	0	0	1
NS5	ENSG0000012240 6	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	1	1	1	1	4
NS5	ENSG0000003821 9	BOD1L; Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	1	1	0	0	2
NS5	ENSG0000018878 6	MTF1; Homo sapiens metal-regulatory transcription factor 1 (MTF1), mRNA	1	1	1	0	3
NS5	ENSG0000020574 4	DENND1C; Homo sapiens DENN/MADD domain containing 1C (DENND1C), mRNA	1	1	0	0	2
NS5	ENSG0000016322 0	S100A9; Homo sapiens S100 calcium binding protein A9 (S100A9), mRNA	1	1	0	0	2
NS5	ENSG0000013693 8	Homo sapiens cDNA, FLJ94333, Homo sapiens acidic (leucine-rich) nuclear phosphoprotein 32family, member B (ANP32B), mRNA	1	0	0	0	1
NS5	ENSG0000010174 5	ANKRD12; Homo sapiens ankyrin repeat domain 12 (ANKRD12), transcript variant 2, mRNA	1	1	0	0	2
NS5	ENSG0000007284 9	Derl2; Homo sapiens Der1-like domain family, member 2 (DERL2), mRNA	1	1	1	1	4
NS5	ENSG0000017323 0	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein	1	1	0	0	2

		(GOLGB1), mRNA					
NS5	ENSG00000198851	CD3E; Homo sapiens CD3e molecule, epsilon (CD3-TCR complex) (CD3E), mRNA	1	0	0	0	1
NS5	ENSG00000160654	Cd3g; Homo sapiens CD3g molecule, gamma (CD3-TCR complex) (CD3G), mRNA	1	1	0	0	2
NS5	ENSG00000102081	FMR1; Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	1	1	1	1	4
NS5	ENSG00000206172	HBA1; Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA	1	1	1	0	3
NS5	ENSG00000115165	CYTIP; Homo sapiens cytohesin 1 interacting protein (CYTIP), mRNA	1	1	1	1	4
NS5	ENSG00000124831	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	1	1	1	1	4
NS5	ENSG00000187109	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript variant 1, mRNA	1	1	0	0	2
NS5		Homo sapiens haplogroup K1c1 mitochondrion, complete genome	1	0	0	0	1
NS5	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	1	1	0	3
NS5	ENSG00000119725	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	1	1	1	0	3
NS5	ENSG00000150977	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	1	1	1	1	4
NS5	ENSG00000100764	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	1	1	1	1	4
NS5	ENSG00000244734	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	1	1	1	1	4
NS5	ENSG00000136436	CALCOCO2; Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	0	0	1	0	1
NS5	ENSG00000115216	nrbp1; Homo sapiens nuclear receptor binding protein 1 (NRBP1), mRNA	1	1	1	0	3
NS5	ENSG00000171863	rps7; Homo sapiens ribosomal protein S7 (RPS7), mRNA	1	0	0	0	1
NS5	ENSG00000172775	fam192a; Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	1	1	1	1	4
NS5	ENSG00000100906	NFKBIA; Homo sapiens nuclear factor of kappa light polypeptide gene	1	0	0	0	1

		enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA					
NS5	ENSG00000177954	RPS27 Homo sapiens ribosomal protein S27 (RPS27), mRNA	1	1	0	0	2
NS5	ENSG00000052749	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	1	1	1	1	4
NS5	ENSG00000135506	OS9; Homo sapiens osteosarcoma amplified 9, endoplasmic reticulum lectin (OS9), transcript variant 2, mRNA	1	0	1	0	2
PrM	AAEL010507	hypothetical protein	0	1	0	0	1
PrM	ENSG00000124831	Lrrfip1; Homo sapiens leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1), transcript variant 5, mRNA	0	1	0	0	1
PrM	ENSG00000119725	znf410; Homo sapiens zinc finger protein 410 (ZNF410), mRNA	0	1	0	0	1
PrM	ENSG00000172775	fam192a; Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	0	1	0	0	1
PrM	ENSG00000052749	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	0	1	0	0	1

APPENDIX B DENGUE-HOST PPIS HAVING MORE THAN TWO PIECES OF SUPPORTING EVIDENCE.

Dengue_gene	Host_Gene	Gene_description	Method	Publication	Interolog	Interolog_info
C	AAEL015681	Histone 2B	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306;PMID:21909430	C-Histone2B	MI:0004(affinity chromatography technology)+PMID:21909430
C	AAEL015390	Histone 2A	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306;PMID:21909430	C-Histone2A	MI:0004(affinity chromatography technology)+PMID:21909430
C	AAEL003863	Histone 4	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306;PMID:21909430	C-Histone4	MI:0004(affinity chromatography technology)+PMID:21909430
NS2A	AAEL003670	myelinprotein expression factor [Source:VB External Description;Acc:AAEL003670]	MI:0004(affinity chromatography technology);MI:0037(domain profile pairs)	PMID:21700306;PMID:21358811		
NS5	AAEL010975	paramyosin, long form	MI:0018(two hybrid));MI:0064(interologs mapping)	Mairiang et al. (this study);PMID:21911577	NS5-CGNL	MI:0018(two hybrid)+PMID:21911577
C	AAEL005567	nucleosome assembly protein	MI:0018(two hybrid));MI:0064(interologs mapping)	Mairiang et al. (this study)	C-NAP1L1	MI:0018(two hybrid))+Mairiang et al. (this study);MI:0004(affinity chromatography technology)+Mairiang et al. (this study)
NS5	AAEL014104	conserved hypothetical protein	MI:0018(two hybrid));MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-FAM192A	MI:0018(two hybrid))+Mairiang et al. (this study);MI:0004(affinity chromatography technology)+Mairiang et al. (this study)
C	AAEL011960	conserved hypothetical protein	MI:0018(two hybrid));MI:0064(interologs mapping)	Mairiang et al. (this study)	C-RRP12	MI:0018(two hybrid))+Mairiang et al. (this study)
NS5	AAEL003973	conserved hypothetical protein	MI:0018(two hybrid));MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-EAF1 and NS5-	MI:0018(two hybrid))+Mairiang et al. (this study)

					EAF2	
NS5	AAEL000951	elongation factor 1-beta2	MI:0018(two hybrid));MI:0064 (interologs mapping)	Mairiang et al. (this study)	NS5-EEF1B2	MI:0018(two hybrid))+Mairiang et al. (this study)
NS5	AAEL009614	seven in absentia, putative	MI:0018(two hybrid));MI:0064 (interologs mapping)	Mairiang et al. (this study);PMID:22014111	NS5-SIAH2	MI:0018(two hybrid)+PMID: 22014111
NS5	AAEL000436	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL005790	malic enzyme	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL008700	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL005567	nucleosome assembly protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL008052	hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL004484	predicted protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL014845	heat shock protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL010585	spermatogenesis associated factor	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL001553	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL011137	succinyl-coa:3-ketoacid-coenzyme a transferase	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		

NS3	AAEL003345	argininosuccinate lyase	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL001892	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL009101	eukaryotic translation initiation factor 3f, eif3f	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL004783	ornithine decarboxylase antizyme,	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL010066	microfibril-associated protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL010821	60S acidic ribosomal protein P0	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
C	AAEL013075	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL010507	hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL014281	conserved hypothetical protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL004500	eukaryotic translation elongation factor	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	AAEL014396	protein farnesyltransferase alpha subunit	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS3	AAEL014843	heat shock protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS3-HSP90AB1	MI:0018(two hybrid))+Mairiang et al. (this study);MI:0004 (affinity chromatography technology)+Mairiang et al. (this study)

NS3	AAEL011708	heat shock protein	MI:0018(two hybrid));MI:0004 (affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS3-HSP90AB1	MI:0018(two hybrid))+Mairiang et al. (this study);MI:0004 (affinity chromatography technology)+Mairiang et al. (this study)
NS5	AAEL012095	26S protease regulatory subunit	MI:0018(two hybrid));MI:0004 (affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-PSMC1	MI:0018(two hybrid))+Mairiang et al. (this study)
NS5	AAEL003104	tripartite motif protein trim2,3	MI:0018(two hybrid));MI:0004 (affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-TRIM2	MI:0018(two hybrid))+Mairiang et al. (this study)
NS3	AAEL012827	endoplasmin	MI:0018(two hybrid));MI:0004 (affinity chromatography technology)	Mairiang et al. (this study)		
NS5	SIAH2	E3 ubiquitin-protein ligase SIAH2	MI:0018(two hybrid);MI:0064(interologs mapping)	PMID:22014111;Mairiang et al. (this study)	NS5-AAEL009614	MI:0018(two hybrid)+Mairiang et al. (this study)
NS3	TRAF4	TNF receptor-associated factor 4	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:22014111		
NS3	AZI2	5-azacytidine induced 2	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:22014111		
NS3	NFKBIA	NFKBIA; Homo sapiens nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:22014111;Mairiang et al. (this study)		
NS5	MATR3	matrin 3	MI:0018(two hybrid)	PMID:21911577;PMID:22014111		
NS2A	GC	group-specific component (vitamin D binding protein)	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS2A	KIF1B	kinesin family member 1B	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		

NS3	EIF4G 2	eukaryotic translation initiation factor 4 gamma, 2	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS3	SERPIND1	serpin peptidase inhibitor, clade D (heparin cofactor), member 1	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS4B	KRT8	keratin 8	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS4B	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast)	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS5	DDX5	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	MI:0018(two hybrid);MI:0009(protein complementation assay);MI:0037(domain profile pairs)	PMID:21911577;PMID:21358811		
NS5	STAT2	signal transducer and activator of transcription 2, 113kDa	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:21911577;PMID:19754307;PMID:19279106		
NS3	MYCBP2	MYC binding protein 2	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	CGNL1	cingulin-like 1	MI:0018(two hybrid);MI:0064(interologs mapping)	PMID:21911577;Mairiang et al. (this study)	NS5-AAEL010975	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	ERC1	ELKS/RAB6-interacting/CAST family member 1	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS3	ZBTB8OS	zinc finger and BTB domain containing 8 opposite strand	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	AKAP9	A kinase (PRKA) anchor protein (yotiao) 9	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	ANKRD50	ankyrin repeat domain 50	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	APOB	apolipoprotein B (including Ag(x) antigen)	MI:0018(two hybrid);MI:0009(protein complementation	PMID:21911577		

			assay)			
NS5	COPS2	COP9 constitutive photomorphogenic homolog subunit 2 (Arabidopsis)	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	DCUN 1D4	DCN1, defective in cullin neddylation 1, domain containing 4 (S. cerevisiae)	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	EID1	EP300 interacting inhibitor of differentiation 1	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	CALR	calreticulin	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577		
NS5	RILPL 2	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:21911577;Mairiang et al. (this study)		
NS3	GOLG B1	GOLGB1; Homo sapiens golgin B1, golgi integral membrane protein (GOLGB1), mRNA	MI:0018(two hybrid)	PMID:21911577;Mairiang et al. (this study)		
PrM/M	DYNLT 1	DYNLT1 dynein, light chain, Tctex-type 1 [Homo sapiens]	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:21767858		
C	H4	Histone 4 [Homo sapiens]	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306	C-AAEL003863	MI:0004(affinity chromatography technology)+PMID:21700306
C	H2A	Histone 2A [Homo sapiens]	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306	C-AAEL015390	MI:0004(affinity chromatography technology)+PMID:21700306
C	H2B	Histone 2B [Homo sapiens]	MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	PMID:21700306	C-AAEL015390	MI:0004(affinity chromatography technology)+PMID:21700306

NS1	C4BP	complement component 4 binding protein, alpha	MI:0004(affinity chromatography technology);MI:1088(phenotype-based detection assay)	PMID:21642539		
NS3	ENO1	enolase 1, (alpha) [Source:HGNC Symbol;Acc:3350]	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21358811;PMID:22014111		
NS1	CLU	clusterin [Source:HGNC Symbol;Acc:2095]	MI:0037(domain profile pairs);MI:0004(affinity chromatography technology)	PMID:21358811;PMID:17825259		
E	UBE2I	ubiquitin-conjugating enzyme E2I (UBC9 homolog, yeast) [Source:HGNC Symbol;Acc:12485]	MI:0037(domain profile pairs);MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:21358811;PMID:17265167		
NS1	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor) [Source:HGNC Symbol;Acc:11364]	MI:0037(domain profile pairs);MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:21358811;PMID:15878791		
E	CD209	CD209 molecule [Source:HGNC Symbol;Acc:1641]	MI:0037(domain profile pairs);MI:0686(unspecified method)	PMID:21358811;PMID:15855154		
E	HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1 [Source:HGNC Symbol;Acc:5253]	MI:0004(affinity chromatography technology);MI:0037(domain profile pairs)	PMID:21358811;PMID:15795242		
C	HBB	HBB; Homo sapiens hemoglobin, beta (HBB), mRNA	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21358811;Mairiang et al. (this study)		
C	RPL5	Homo sapiens ribosomal protein L5, mRNA (cDNA clone IMAGE:3544216), complete cds	MI:0018(two hybrid);MI:0037(domain profile pairs)	PMID:21358811		

NS3	FASN	fatty acid synthase	MI:0018(two hybrid);MI:0009(protein complementation assay)	PMID:20855599;PMID:21911577		
NS4A	PTBP1	polypyrimidine tract binding protein 1	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:19450550		
NS5	XPO1	XPO1 exportin 1 (CRM1 homolog, yeast) [Homo sapiens]	MI:0018(two hybrid);MI:0004(affinity chromatography technology);MI:0586(inhibitor)	PMID:19297323		
E	CANX	calnexin [Source:HGNC Symbol;Acc:1473]	MI:0004(affinity chromatography technology);MI:0037(domain profile pairs)	PMID:19105951;PMID:21358811		
E	CALR	calreticulin [Source:HGNC Symbol;Acc:1455]	MI:0004(affinity chromatography technology);MI:0037(domain profile pairs)	PMID:19105951;PMID:21358811		
E	HSPA5	HSPA5; Homo sapiens heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa) (HSPA5), mRn/a	MI:0018(two hybrid);MI:0004(affinity chromatography technology);MI:0037(domain profile pairs)	PMID:19105951;PMID:21358811		
NS1	HNRNPC	HNRNPC heterogeneous nuclear ribonucleoprotein C (C1/C2) [Homo sapiens]	MI:0004(affinity chromatography technology)	PMID:18471994		
C	DAXX	DAXX death-domain associated protein [Homo sapiens]	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:17707345		
NS3	NRBP1	nrbp1; Homo sapiens nuclear receptor binding protein 1 (NRBP1), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:15084397;Mairiang et al. (this study)		
NS5	KPNB1	karyopherin (importin) beta 1	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	PMID:11257177;PMID:11347963;PMID:121052241		
C	NAP1L1	NAP1L1; Homo sapiens nucleosome assembly protein 1-like 1 (NAP1L1), transcript	MI:0018(two hybrid);MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	C-AAEL005567	MI:0018(two hybrid)+Mairiang et al. (this study)

		variant 1, mRNA				
C	BOD1L	BOD1L; Homo sapiens biorientation of chromosomes in cell division 1-like (BOD1L), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
NS5	FMR1	FMR1; Homo sapiens fragile X mental retardation 1 (FMR1), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
C	BIRC2	birc2; Homo sapiens baculoviral IAP repeat-containing 2 (BIRC2), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
NS5	CYTIP	CYTIP; Homo sapiens cytohesin 1 interacting protein (CYTIP), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
NS3	CALCOCO2	CALCOCO2; Homo sapiens calcium binding and coiled-coil domain 2 (CALCOCO2), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
C	ZNF394	ZNF394; Homo sapiens zinc finger protein 394 (ZNF394), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
NS3	RPL24	Homo sapiens ribosomal protein L24, mRNA (cDNA clone MGC:2240 IMAGE:3349215), complete cds	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		
NS3	HSP90AB1	HSP90AB;1 heat shock protein 90kDa alpha (cytosolic), class B member 1	MI:0018(two hybrid);MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS3-AAEL014843 and NS3-AAEL011708	MI:0018(two hybrid)+Mairiang et al. (this study);MI:0004(affinity chromatography technology)+Mairiang et al. (this study)

NS5	FAM192A	fam192a; Homo sapiens family with sequence similarity 192, member A (FAM192A), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL014104	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	TRIM2	TRIM2; tripartite motif containing 2	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL003104	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	PSMC1	Homo sapiens cDNA, FLJ93843, Homo sapiens proteasome (prosome, macropain) 26S subunit, ATPase, 1(PSMC1), mRNA	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL012095	MI:0018(two hybrid)+Mairiang et al. (this study);MI:0004 (affinity chromatography technology)+Mairiang et al. (this study)
C	RRP12	Rrp12; Homo sapiens ribosomal RNA processing 12 homolog (S. cerevisiae) (RRP12), transcript variant 2, mRNA	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	C-AAEL011960	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	EEF1B2	EEF1B2; eukaryotic translation elongation factor 1 beta 2	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL000951	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	EAF2	EAF2; ELL associated factor 2	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL003973	MI:0018(two hybrid)+Mairiang et al. (this study)
NS5	EAF1	EAF1; ELL associated factor 1	MI:0018(two hybrid);MI:0064(interologs mapping)	Mairiang et al. (this study)	NS5-AAEL003973	MI:0018(two hybrid)+Mairiang et al. (this study)
NS3	RILPL2	Rilpl2; Homo sapiens Rab interacting lysosomal protein-like 2 (RILPL2), mRNA	MI:0018(two hybrid);MI:0004(affinity chromatography technology)	Mairiang et al. (this study)		

APPENDIX C. OLIGONUCLEOTIDES USED IN THIS STUDY

Name	Sequence	Description
DM 1	GGGGACAAGTTTGTACAAAAAAGCAGGCT	universal attB1 adapter primer
DM 2	GGGGACCACTTTGTACAAGAAAGCTGGGT	universal attB2 adapter primer
DM 3	AAAAAGCAGGCTTGATGAATGACCAACGGAAA	(U) DEN2 Capsid Start: 97
DM 4	AGAAAGCTGGGTGCTACGCCATCACTGTTGGAA	(L) DEN2 Capsid-Anc End: 438
DM 5	AGAAAGCTGGGTCCTATCTGCGTCTCCTATTCAAGA	(L) DEN2 Capsid-Vir End: 396
DM 6	AAAAAGCAGGCTCCTTCCATTTAACCACACG	(U) DEN2 PrM/M Start: 439
DM 7	AAAAAGCAGGCTCCTCAGTGGCACTCGTTC	(U) DEN2 M Start: 712
DM 8	AGAAAGCTGGGTGCTATGTCATTGAAGGAGTGAC	(L) DEN2 M End: 936
DM 9	AAAAAGCAGGCTCAATGCGTTGCATAGGAATG	(U) DEN2 E Start: 937
DM 10	AGAAAGCTGGGTCCTAGGCCTGCACCATGACTCC	(L) DEN2 E End: 2421
DM 11	AAAAAGCAGGCTAACTCAAAGGAATGTCATAC	(U) DEN2 Eiii Start: 1816
DM 12	AGAAAGCTGGGTGCTATTTCTTAAACCAGTTG	(L) DEN2 Eiii End: 2118
DM 13	AAAAAGCAGGCTGGGATAGTGGTTGCGTTGTG	(U) DEN2 NS1 Start: 2422
DM 14	AGAAAGCTGGGTGTTAAGCTGTGACCAAGGAG	(L) DEN2 NS1 End: 3477
DM 15	AAAAAGCAGGCTGGGGACATGGGCAGGTCG	(U) DEN2 NS2A Start: 3478
DM 16	AGAAAGCTGGGTTCTACCTTTTCTTGCTGGTTC	(L) DEN2 NS2A End: 4131
DM 17	AAAAAGCAGGCTCCAGCTGGCCATTAAATGAG	(U) DEN2 NS2B Start: 4132
DM 18	AGAAAGCTGGGTCCTACCGTTGTTTCTTCACTTC	(L) DEN2 NS2B End: 4521
DM 19	AAAAAGCAGGCTTTGCCGGAGTGTTGTGGGATG	(U) DEN2 NS3 Start: 4522
DM 20	AAAAAGCAGGCTCCGTGAGTGCTATAGCCCAGAC	(U) DEN2 NS3d1-160 Start: 5005
DM 21	AGAAAGCTGGGTCCTACTTTCTTCCGGCTGCAAATTC	(L) DEN2 NS3 End: 6375
DM 22	AAAAAGCAGGCTCCTCTCTGACCCTGAACCTA	(U) DEN2 NS4A Start: 6376
DM 23	AGAAAGCTGGGTATTATGCCATGGTTGCGGCCAC	(L) DEN2 NS4A End: 6756/6825
DM 24	AAAAAGCAGGCTATAACGAGATGGGTTTCCTA	(U) DEN2 NS4B Start: 6826
DM 25	AGAAAGCTGGGTGCTACCTTCTTGCGTTGGTTG	(L) DEN2 NS4B End: 7569
DM 26	AAAAAGCAGGCTCCGGAACTGGCAACATA	(U) DEN2 NS5 Start: 7570
DM 27	AGAAAGCTGGGTCCTACCACAGAACTCCTGCTTC	(L) DEN2 NS5 End: 10269
DM 28	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCGGAACTGGCA ACATA	(U) DEN2 NS5 Start: 7570(7539)
DM 29	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCACAGAA CTCCTGCTTC	(L) DEN2 NS5 End: 10269(10252)
DM 30	AAGAAGCGTGCTCCAAGCCAC	(L) DEN2 NS5 End: 9000
DM 31	GCCATATTCACTGATGAGAAC	(U) DEN2 NS5 Start:8800
DM 32	CCTTCAAGATGGAGATTCCTTTCC	(L) DEN2 NS5 end: 8900
DM 33	TTGACTGTATCGCCG	5' RT
DM 34	CCGGAATTAGCTTGGCTGCAG	3' RT
DM 35	AAAAAGCAGGCTTGATGAACAACCAACGG	DEN3: Capsid(U)@95
DM 36	AGAAAGCTGGGTGCTAAGCAAGTGTTGCTGGTAA	DEN3: Capsid-A(L)@436
DM 37	AGAAAGCTGGGTACTACTTTTTCCGTTTGTTGATAATG	DEN3: Capsid-V(L)@394
DM 38	AAAAAGCAGGCTCTTTCCACTTAACTTCA	DEN3: PrM(U)@437
DM 39	AAAAAGCAGGCTCCTCAGTGGCGTTAGCTCCC	DEN3: M(U)@710

DM 40	AGAAAGCTGGGTTCTATGTCATGGATGGGGTAA	DEN3: M(L)@934
DM 41	AAAAAGCAGGCTTTATGAGATGTGTGGGA	DEN3: E(U)@935
DM 42	AGAAAGCTGGGTTCTAAGCTTGCACCACGACCCCCAGA	DEN3: E(L)@2413
DM 43	AAAAAGCAGGCTGTAGACTCAAGATGGAC	DEN3: Eiii(U)@aa284(1784)
DM 44	AGAAAGCTGGGTTCTAAACACCACCCACTGATCCAAAGTC	DEN3: Eiii(L)@aa426(2212)
DM 45	AAAAAGCAGGCTCTGACATGGGGTGTGTC	DEN3: NS1(U)@2414
DM 46	AGAAAGCTGGGTACTATGCTGAGGCTAGAGA	DEN3: NS1(L)@3469
DM 47	AAAAAGCAGGCTCAGGGAGTGGAAAGGTG	DEN3: NS2A(U)@3470
DM 48	AGAAAGCTGGGTCCTATCTCCTTTTGAGTGT	DEN3: NS2A(L)@4123
DM 49	AAAAAGCAGGCTGAAGCTGGCCACTGAAT	DEN3: NS2B(U)@4124
DM 50	AGAAAGCTGGGTTCTATCTTTGGGTTTGCTTTTG	DEN3: NS2B(L)@4513
DM 51	AAAAAGCAGGCTGGTCCGGCGTCCTATGGG	DEN3: NS3(U)@4514
DM 52	AAAAAGCAGGCTATGTTAGTGGAATAGCG	DEN3: NS3d1-161(U)@4997
DM 53	AGAAAGCTGGGTTCTACTTTCTGCCAGCTGCAAAATCCTT	DEN3: NS3(L)@6370
DM 54	AAAAAGCAGGCTTGTCAATCGCCCTTGAT	DEN3: NS4A(U)@6371
DM 55	AGAAAGCTGGGTTCTATCTCTGCTTTTCTGG	DEN3: NS4A(L)@6751
DM 56	AAAAAGCAGGCTCCAATGAAATGGGACTG	DEN3: NS4B(U)@6821
DM 57	AGAAAGCTGGGTTCTATCTCTTTCCTGTTCC	DEN3: NS4B(L)@7564
DM 58	AAAAAGCAGGCTCAGGAACAGGGTCACAA	DEN3: NS5(U)@7565
DM 59	AGAAAGCTGGGTCCTACCAAATGGCTCCCTC	DEN3: NS5(L)@10264
DM 60	AAAAAGCAGGCTTAATGAACCAACGAAAA	DEN4: Capsid(U)@(102)94
DM 61	AGAAAGCTGGGTCCTACGCCATTGCGGTGGG	DEN4: Capsid-A(L)@(440)432
DM 62	AGAAAGCTGGGTTCTACCTTTTTCTTCCATTCAAGATGT	DEN4: Capsid-V(L)@(398)390
DM 63	AAAAAGCAGGCTCGTTTCACTTGTCAACA	DEN4: PrM(U)@(441)433
DM 64	AAAAAGCAGGCTGCTCAGTAGCCCTAACACAAC	DEN4: M(U)@(714)706
DM 65	AGAAAGCTGGGTGCTATCCGTAGGATGGGGCGACC	DEN4: M(L)@(938)930
DM 66	AAAAAGCAGGCTTAATGCGATGCGTGGGA	DEN4: E(U)@(939)931
DM 67	AGAAAGCTGGGTCCTATGCGTGAACTGTGAA	DEN4: E(L)@(2423)2415
DM 68	AAAAAGCAGGCTCAAAGGGAATGTCATAC	DEN4: Eiii(U)@)1813
DM 69	AGAAAGCTGGGTACTATTTCCTGAACCAATG	DEN4: Eiii(L)@)2112
DM 70	AAAAAGCAGGCTCAGACACGGGTTGTGCG	DEN4: NS1(U)@(2424)2416
DM 71	AGAAAGCTGGGTCCTAGGCCGATACCTGTGA	DEN4: NS1(L)@(3479)3471
DM 72	AAAAAGCAGGCTCCGGACAGGGTACATCA	DEN4: NS2A(U)@(3480)3472
DM 73	AGAAAGCTGGGTTCTATCTCTTTGAAGCTCC	DEN4: NS2A(L)@(4133)4125
DM 74	AAAAAGCAGGCTCATCTTGGCCCCTTAAC	DEN4: NS2B(U)@(4134)4126
DM 75	AGAAAGCTGGGTACTATCTTTGTGTTTTCAC	DEN4: NS2B(L)@(4523)4515
DM 76	AAAAAGCAGGCTTATCAGGAGCCCTGTGGGACGTCCCCTCACCT	DEN4: NS3(U)@(4524)4516
DM 77	AAAAAGCAGGCTGTGATTACGTCAGTGCTA	DEN4: NS3d1-161(U)@)4993
DM 78	AGAAAGCTGGGTCCTACTTTCTTCCACTGGCAAACTC	DEN4: NS3(L)@(6377)6369
DM 79	AAAAAGCAGGCTTGAGCATAACCCTCGAC	DEN4: NS4A(U)@(6378)6370
DM 80	AGAAAGCTGGGTACTAGGCTGCTATGAGACC	DEN4: NS4A(L)@(6827)6819
DM 81	AAAAAGCAGGCTCCAACGAGATGGGGCTG	DEN4: NS4B(U)@(6828)6820
DM 82	AGAAAGCTGGGTCCTACCTCCTGGGGGTTTG	DEN4: NS4B(L)@(7562)7554
DM 83	AAAAAGCAGGCTGGGGAACTGGGACCACA	DEN4: NS5(U)@(7563)7555
DM 84	AGAAAGCTGGGTCCTACAGAACTCCTTCACT	DEN4: NS5(L)@(10262)10254
DM 85	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGTCCGGCGTCCTATGGG	DEN3: NS3(U)@4514
DM 86	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTACTTTCTGCC	DEN3: NS3(L)@6370

	AGCTGCAAAATCCTT	
DM 87	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAGGAACAGGGT CACAA	DEN3: NS5(U)@7565
DM 88	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACCAAATGG CTCCCTC	DEN3:NS5(L)@10264
DM 89	GGGGACAAGTTTGTACAAAAAAGCAGGCTGGGGAACTGGGA CCACA	DEN4: NS5(U)@(7563)7555
DM 90	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACAGAACTCC TTCACT	DEN4: NS5(L)@(10262)10254
DM 91	AAAAAGCAGGCTTGATGAACAACCAACGG	DEN1:Capsid(U)95
DM 92	AGAAAGCTGGGTTCTAACGCCAGGGCTGTGGGCA	DEN1:C-reverse(ANC) 436
DM 93	AGAAAGCTGGGTCCTATCTTTTTCTTCTATT	DEN1:C-reverse 394
DM 94	AAAAAGCAGGCTCGTTCCATCTGACCACA	DEN1:PrM 437
DM 95	AAAAAGCAGGCTGTTCCGTCGCACTGGCCCCACAT	DEN1:M 710
DM 96	AGAAAGCTGGGTGCTAGGCCATGGATGGTGT	DEN1:M-reverse 934
DM 97	AAAAAGCAGGCTCCATGCGATGCGTGGGA	DEN1:E 935
DM 98	AGAAAGCTGGGTACTACGCCTGAACCATGAC	DEN1:E-reverse 2419
DM 99	AAAAAGCAGGCTCCTTAAAAGGGATGTCA	DEN1:Eiii (1814)
DM 100	AGAAAGCTGGGTTCTATTTCTTGAACCAGCTTAGTTTCAAA	DEN1:Eiii-reverse(2116)
DM 101	AAAAAGCAGGCTGCGACTCGGGATGTGTA	DEN1:NS1 2420
DM 102	AGAAAGCTGGGTACTATGCAGAGACCAATGA	DEN1:NS1-reverse 3475
DM 103	AAAAAGCAGGCTCAGGGTCAGGAGAAGTG	DEN1:NS2A 3476
DM 104	AGAAAGCTGGGTACTATTTCCTTCCCCAGATTTG	DEN1:NS2A-reverse 4129
DM 105	AAAAAGCAGGCTTAAGTTGGCCCCTCAAT	DEN1:NS2B 4130
DM 106	AGAAAGCTGGGTCCTATCTCTGTTTCTTTTTCTG	DEN1:NS2B-reverse 4519
DM 107	GGGGACAAGTTTGTACAAAAAAGCAGGCTCATCAGGAGTGCT ATGG	DEN1:NS3 4520
DM 108	AAAAAGCAGGCTACGTCAGTGCCATAGCTC	DEN1:NS3d1:160 5003
DM 109	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATCTTCTTCC TGCTGCAAA	DEN1:NS3-reverse 6376
DM 110	AAAAAGCAGGCTCAAGCGTCTCAGGTGAT	DEN1:NS4A 6377
DM 111	AGAAAGCTGGGTGCTAGCGTTGTCTGTCTGG	DEN1:NS4A-reverse 6757
DM 112	AAAAAGCAGGCTCCAATGAGATGGGATTA	DEN1:NS4B 6827
DM 113	AGAAAGCTGGGTTCTATCTCCTACCTCCTCC	DEN1:NS4B-reverse 7573
DM 114	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAGGTACGGGAG CCCAAG	DEN1:NS5 7574
DM 115	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTACCAGAGTG CCCCTTC	DEN1:NS5-reverse 10270
DM 116	GGGGACAAGTTTGTACAAAAAAGCAGGCTTATCAGGAGCCCT GTGGGACGTCCCCTCACCT	DEN4: NS3(U)@(4524)4516
DM 117	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTACTTTCTTCC ACTGGCAAACTC	DEN4: NS3(L)@(6377)6369

DM119	CGTGGGCTTGTACTCGGTCATGCGGTTTCTCTCGCGTTT	Replicon C22 + pac (R)
DM120	CGCGAGAGAAACCGCATGACCGAGTACAAGCCCACGGTG	Replicon C22 + pac (F)
DM121	CAAAGTCTGTTTCACGGCACCGGGCTTGCGGGTCAT	Replicon pac + FDMV2A (R)
DM122	GTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGA GAC	Replicon FDMV2A(F)
DM125	TGGGCCAGGATTCTCCTCGACGTCACCGCATGTTAGCAGACT	Replicon Tav2A(R)
DM126	GAGGAGAATCCTGGCCCAAGCACCTCACTGTCTGTGACA	Replicon Tav2A + E24 (F)
DM127	GCCTCTAGAGAACCTGTTGATTCAACAGCACCATTCCATTTTC	Replicon Den2 3'UTR + XbaI (R)
DM128	GAAGGGGGGCCCCATATGACAAGTTTGTACAAAAAAGCTGA	pAS1_Myc+GatewayCassette (F)
DM129	GAGGTACCCTCGATTCGCCACCACTTTGTACAAGA	GWcassette + pAS1 (R)
DM130	GATGACGATAAGCTTATCACAAGTTTGTACAAAAAAGCT	pDL4_NTAP + GWCassette (F)
DM131	TTCACAAAGATCCTCTAGCACCACTTTGTACAAGA	GWcassette + pDL4
DM132	TTGACTGTATCGCCGGAATTCTACCCTTATGATGTGCCAGATT A	5'RT+HA tag (F) for cDNA cloning
DM133	CCGGAATTAGCTTGGCTGCAGCTAGCTAGCTAGAAGAAGTCC AAAGCTTCTCG	3'RT + STOPs+ XhoI sites (R) for cDNA cloning
DM134	ATATATCTGCAGTAATACGACTCACTATAGAGT	Fix DM118 by 2nd PCR
DM138	ATAATACTGCAGCGGCCGCGAGGGCAGAGGAAG	DM135 but add 6 more bases to make sure PstI cut optimally
DM139	TGACAAGTTTGTACAAAAAAGCTGA	DM137 fixed (F) add 2 bases for blunt end ligation with PmeI
DM140	GGGTCTAGAACCACTTTGTACAAGA	DM136 fixed (R) add XbaI sites
DM143	ATTACTATGCGGCCGCCATGGCTTCCAAGGTGTACG	hRluc + NotI (F)
DM144	ATTACTCTGCGGCCGCACTGCTCGTTCTTCAGCACG	hRluc + NotI (R)
DM145	ATATATGGATCCTCTAGACCTTCTTGCGTTGGTTG	DM25 + Xbai site + BamHI site
DM146	CTGCGACATCGTATAACGTTACTG	pETseqPrimer_close to sphI site(4657)
DM147	TACCTTGTCGTCGTCATCTGCACC	pETseqPrimer_close to xbai site(4916)
DM148	GACAGTCTGGAACAGGGTGTG	DEN2:NS5 10000
DM149	GCAGCAGCCTAGGTTAATTAGTG	pETseqPrimer_close to salI site(5029)
DM150	CGATGTGAGGCACGACGT	seqPrimer for RLuc <---150
DM151	GACGATCTGCCTAAGATGTTCAT	seqPrimer for RLuc 791--->
DM152	GCCAGTGAATTGTAATACGACTCACTATAGG	pRS315seq+T7promoter(F)
DM153	CTTTCTTCCGGCTGCAAATTC	DEN2: NS3(L) with no tags
DM154	GTTTGACAGCTTATCATCGATTAATACGACTCACTATAGG	YRp7seq+T7promoter(F)
DM155	GTTTGACAGCTTATCATCGAT GTGAGTGCTATAGCC	YRP7seq+NS3d(F)
DM156	GGCCACGATGCGTCCGGCGTAGAGTTTAAACCTTTCTTCCGG CTGCAAATTC	YRp7seq+PmeI+NS3(L)
DM157	GGCCACGATGCGTCCGGCGTAGAGTTTAAACTCTAGAGAAC CTGTTGATTCAACAGCA	YRp7seq+PmeI+DM127(L)
DM158	GGAGACGTGGAGTCCAACCCAGGGCCCATGGCTTCCAAGGT GTACGA	FMDV+hRLuc(F)
DM159	GCATGTTAGCAGACTTCCTCTGCCCTCCTGCTCGTTCTTCAG CACGC	hRLuc+TaV(R)

DM160	TTGACTGTATCGCCGATGACCGAGTACAAG	5'RT+PAC(F)
DM161	CCGGAATTAGCTTGGCTGCAGTCAGGCACCGGGCTT	PAC+3'RT(R)
DM162	TTGACTGTATCGCCGATGGCTTCCAAGGTGT	5'RT+hRLuc(F)
DM163	CCGGAATTAGCTTGGCTGCAGTTACTGCTCGTTCTTC	hRLuc+3'RT(R)
DM164	TAATACGACTCACTATAGGGAGAATCACTACCGTTTGAGTTCT TGTG	T7 promoter + part of Actin5c promoter (F)
DM165	CTCCCACACCTCCCCCTG	After SV40polyA of pAc5.1-HisB
DM166	GGAAACAGCTATGACCATGTCTAGATCATTTTTGACACCAGA CCAAC	M13R + XbaI+Stop+LacZ(R)
DM167	GGCCACGATGCGTCCGGCGTATCTAGATCATTTTTGACACCA GACCAAC	YRp7seq + XbaI + Stop +LacZ(R)
DM168	GTTTGACAGCTTATCATCGATCGCGTAAAACACAATCAAGTAT G	YRp7seq + IE1(promoter)(F)
DM169	TCGGTCCACGTAGACTAACAACTGTCACTTGGTTGTTCACGA TCTT	IE1(promoter)+5'UTR of Dengue
DM170	GTGCTGTTGAATCAACAGGTTCTAACAAAAAAAGTACGCTCA CGTAC	3'UTR of DenV + 3'UTR of pIE1(F)
DM171	CGTCCGGCGTAGAGTTTAAACAAGCTTAAAAGTAGGAGGAAC GG	3'UTR of pIE1 + YRp7seq(R)
DM172	AAAAAGCAGGCTTGATGAAGCTACTGTCTTCTATCGAACA	(U) Gal4
DM173	AGAAAGCTGGGTGCTCTTTTTTTGGGTTTGGTGG	(L) Gal4
DM174	GTTTGACAGCTTATCATCGATCATGATGATAAACAATGTATGG TGC	YRp7seq + OpIE2(promoter)(F)
DM175	GGTCCACGTAGACTAACAACTAACAGATGCTGTTCAACTGTG TTT	OpIE2(promoter)+5'UTR of Dengue(R)
DM176	AAAAAGCAGGCTCCATGAAGCTACTGTCTTCTATCGAACA	attB1 + NTAP tag (F)
DM177	AGAAAGCTGGGTCCCGGAATTAGCTTGGCTGCAG	attB2 + 3'RT (R)
DM178	AGAAAGCTGGGTTTTAGAACCCTCTCAAAACATTAATAGCTT	Den2:Capsid-NLS(85-100)(R)
DM179	AAAAAGCAGGCTCAATGACTGGTACAGAGAAGAACGC	AeAe NAP1L1 homolog (F)
DM180	AGAAAGCTGGGTACTATTGTTGTTGGCATTCGG	AeAe NAP1L1 homolog(R)
DM181	CGCAACGATCTGGTAAACAC	OpIE2_FWD(F)
DM182	GACAATACAAACTAAGATTTAGTCAG	OpIE2_REV(R)
DM183	CAACATGGTACCGGTCGCCACCATG	Kozak + MCS of RFP/ECFP/EYP plasmids (F)
DM184	TCAGCTTTTTTGTACAAACTTGTCAAAAGGAACAGATGGTGG CG	RFP + attR1 (R)
DM185	TCAGCTTTTTTGTACAAACTTGTCAACTTGTACAGCTCGTCCA TG	EYFP or ECFP + attR1 (R)
DM189	AGTCGACGTCACGACACCATGG	Universal 5' primer for pTaglox
DM190	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAATGACTGGTAC AGAGAAGAACGC	AeNAP+attP1(F)
DM191	GGGGACCACTTTGTACAAGAAAGCTGGGTACTATTGTTGTTG GCATTCGG	AeNAP+attP1(R)
DM192	CAACATGGACTACAAAGACGATGACGACAAGCATATGACAAG TTTGTACAAAAAAGCTGA	FLAG + attR1(F)
DM193	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAACACGC CTTTCAATATGCTG	D2CΔaa1 to 9 (F)
DM194	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAATTGTTCC CCATCTCTTC	CΔaa73 to 100 (R)
DM195	ATCGAGGCCTGTCTAGAGAAGC	5' of Drosophila GW collection cassette (F)
DM196	GGGCTCGAGACCACTTTGTACAAGA	DM140 replace XbaI with

		XhoI (R)

<u>APPENDIX D</u>. SEQUENCES OF DENGUE ORFS

Gene_ID	Serotype	Position_in_genome	Sequence	Amino Acid Identity	positive
D1CA	Dengue1	95 to 436	ATGAACAA CCAACGGAAA AAGACGGGTC GACCGTCTTT CAATATGCTG AAACGCGCGA GAAACCGCGT GTCAACTGTT TCACAGTTGG CGAAGAGATT CTCAAAAGGA TTGCTTTCAG GCCAAGGACC CATGAAATTG GTGATGGCTT TTATAGCATT CCTAAGATTT CTAGCCATAC CCCCAACAGC AGGAATTTTG GCTAGATGGG GCTCACTCAA GAAGAATGGA GCGATTAAAG TGCTACGGAG TTTCAAGAAA GAAATCTCAA ACATGCTGAG CATAATGAAT AGAAGAAAAA GATCCGTGAC CATGCTCCTT ATGCTGCTGC CCACAGCCCT GGCG	0.98	0.99
D1CV	Dengue1	95 to 394	ATGAACAACCAACGGAAAAAGACGGGTCGA CCGTCTTTCAATATGCTGAAACGCGCGAGA AACCGCGTGTCAACTGTTTCACAGTTGGCG AAGAGATTCTCAAAAGGATTGCTTTCAGGCC AAGGACCCATGAAATTGGTGATGGCTTTTAT AGCATTCCTAAGATTTCTAGCCATACCCCCA ACAGCAGGAATTTTGGCTAGATGGGGCTCA CTCAAGAAGAATGGAGCGATTAAAGTGCTA CGGGGTTTCAAGAAAGAAATCTCAAACATGC TGAGCATAATGAATAGAAGAAAAAGA	0.99	1
D1PrM	Dengue1	437 to 934	TTCCATCTGACCACACGAGGGGGAGAGCCG CACATGATAGTTAGCAAGCAGGAAAGAGGA AAGTCACTTTTGTTTAAGACCTCTGCAGGTG TCAACATGTGCACCCTTATTGCGATGGATTT GGGAGAGTTATGTGAGGACACAATGACCTA CAAATGCCCTCGGATCACTAAGGCGGAACC AGATGACGTTGACTGTTGGTGCAATGCCAC GGACACATGGGTGACCTATGGAACGTGTTC TCAAACTGGCGAACACCGACGAGACAAGCG TTCCGTCGCACTGGCCCCACATGTGGGGCT TGGTCTAGAAACAAGAGCCGAAACGTGGAT GTCCTCTGAAGGCGCTTGGAAACAAATACA AAAAGTGGAGACTTGGGCTCTGAGACACCC AGGATTCACGGTAATAGCCCTCTTTCTAGCA CATGCCATAGGAACATCCATCACCCAGAAA GGGATTATTTTCATTTTGTTGATGCTGGTAA CACCATCCATGGCC	1	1
D1M	Dengue1	710 to 934	TCCGTCGCAC TGGCCCCACA TGTGGGGCTT GGTCTAGAAA CAAGAGCCGA AACGTGGATG TCCTCTGAAG GCGCTTGGAA ACAAATACAA AAAGTGGAGA CTTGGGCTCT GAGACACCCA GGATTCACGG TAATAGCCCT CTTTCTAGCA CATGCCATAG GAACATCCAT CACCCAGAAA GGGATTATTT TCATTTTGTT GATGCTGGTA ACACCATCCA TGGCC	1	1

D1E	Deng ue1	935 to 2419	ATGCGATGCGTGGGAATAGGCAACAGAGAC TTCGTGGAAGGACTGTCAGGAGGAACGTGG GTGGATGTGGTACTGGAGCGTGGAAGTTGC GTCACCACCATGGCAAAAGATAAACCAACAT TGGACATTGAACTCTTGAAGACGGAGGTCA CAAACCCTGCCGTCCTGCGTAAACTGTGCA TTGAAGCTAAAATATCAAACACCACCACCGA TTCAAGATGTCCAACACAAGGGGAAGCCAC ACTGGTGGAAGAACAAGACGCGAACTTCGT GTGTCGACGAACGTTTGTGGACAGAGGCTG GGGCAATGGCTGTGGGCTTTTCGGAAAAGG TAGCCTAATAACGTGTGCTAAGTTCAAGTGT GTGACAAAACTGGAAGGAAAGATTGTTCAAT ATGAGAACTTGAAATATTCAGTGATAGTCAC CGTCCACACTGGTGACCAGCACCAGGTGGG AAATGAGACCACAGAACATGGAACAATTGCA ACCATAACACCTCAAGCTCCTACGTCGGAAA TACAGCTGACCGACTACGGAGCTCTTACATT GGATTGCTCACCCAGAACAGGGCTAGACTT TGATGAGATGGTGTTGTTGACAATGAAAGAA AAATCATGGCTTGTCCACAAACAATGGTTTC TAGACTTACCACTGCCCTGGACCTCGGGAG CTTCAACACCCCAAGAGACTTGGAACAGAG AAGATTTGCTGGTTACATTTAAGACAGCTCA TGCAAAGAAGCAGGAAGTAGTCGTACTAGG ATCACAAGAAGGAGCAATGCACACTGCGTT GACCGGAGCGACAGAAATCCAAACGTCTGG AACGACAAAAATTTTTGCAGGACACTTGAAA TGTAGACTAAAAATGGACAAACTGACCTTAA AAGGGATGTCATATGTGATGTGCACAGGCT CATTCAAGTTAGAGAAAGAAGTGGCTGAGA CCCAGCATGGAACTGTTCTAGTGCAGGTTA AATACGAAGGAACAGATGCACCATGCAAGA TCCCCTTTTCGACCCAAGATGAGAAAGGAG TAACCCAGAATGGGAGATTGATAACAGCCA ACCCCATAGTCACTGACAAAGAAAAACCAGT CAACATTGAGGCAGAACCACCTTTTGGTGA GAGTTACATCGTGGTAGGAGCAGGTGAAAA AGCTTTGAAACTAAGCTGGTTCAAGAAAGGA AGCAGCATAGGGAAAATGCTTGAAGCAACT GCCCGAGGAGCACGAAGGATGGCCATCCTA GGAGACACCGCATGGGACTTCGGTTCTATA GGAGGAGTGTTCACGTCTGTGGGAAAACTG GTACACCAGATCTTTGGAACTGCATATGGAG TTTTGTTCAGCGGTGTTTCCTGGACTATGAA AATAGGAATAGGGATTCTGCTGACATGGCTA GGATTAAATTCAAGGAGCACGTCCCTTTCGA TGACGTGCATTGCAGTTGGCATGGTTACACT GTACCTAGGAGTCATGGTTCAGGCG	0.99	0.99

D1Eiii	Dengue1	1814 to 2116	TTAAAAGGGA TGTCATATGT GATGTGCACA GGCTCATTCA AGTTAGAGAA AGAAGTGGCT GAGACCCAGC ATGGAACTGT TCTAGTGCAG GTTAAATACG AAGGAACAGA TGCACCATGC AAGATCCCCT TTTCGACCCA AGATGAGAAA GGAGTAACCC AGAATGGGAG ATTGATAACA GCCAACCCCA TAGTCACTGA CAAAGAAAAA CCAGTCAACA TTGAGGCAGA ACCACCTTTT GGTGAGAGTT ACATCGTGGT AGGAGCAGGT GAAAAAGCTT TGAAACTAAG CTGGTTCAAG AAA	1	1
D1NS1	Dengue1	2420 to 3475	GACTCGGGATGTGTAATTAACTGGAAAGGCAGAGAACTCAAATGTGGAAGTGGCATTTTTGTCACCAATGAAGTTCACACTTGGACAGAACAATACAAATTCCAGGCCGACTCCCCAAAGAGACTATCAGCAGCCATTGGGAAGGCATGGGAAGAGGGTGTGTGTGGAATTCGATCAGCCACTCGTCTCGAGAACATCATGTGGAAGCAGATATCAAATGAACTAAACCACATCTTACTTGAAAATGACATGAAATTCACAGTGGTCGTAGGAGATGTTAGTGGGATCTTGACCCAAGGAAGAAAAATGATTGGGCCACAACCCATGGAACACAAATACTCGTGGAAAAGCTGGGGAAAAGCCAAAATCATAGGAGCAGATGTACAGAACACCACCTTCATTATCGACGGCCCAAACACCCCAGAATGCCCTGATGACCAAAGAGCATGGAACATTTGGGAAGTTGAGGACTATGGATTTGGAATTTTCACGACAAATATATGGTTGAAATTGCGTGACTCCTACACCCAAGTGTGTGACCCCCGGCTAATGTCAGCTGCCATCAAGGACAGCAAGGCAGTTCATGCCGATATGGGATACTGGATAGAAAGTGAAAAGAACGAGACCTGGAAGCTGGCGAGAGCCTCCTTCATAGAAGTTAAGACATGCGTCTGGCCAAAATCCCACACTCTATGGAGCAACGGAGTTTTGGAAAGTGAAATGATAATCCCAAAGATATATGGAGGACCAATATCTCAGCACAACTACAGACCAGGATATTCCACACAAACAGCAGGACCGTGGCACCTAGGCAAGTTGGAACTAGATTTTGATTTGTGTGAGGGTACCACAGTTGTTGTGGATGAACATTGTGGAAATCGAGGACCATCTCTTAGAACCACAACAGTAACAGGAAAGATAATCCATGAATGGTGCTGTAGATCTTGTACGCTACCCCCCTTACGTTTCAAAGGAGAAGACGGGTGTTGGTACGGCATGGAAATCAGACCAGTCAAGGACAAGGAAGAGAACCTAGTAAAGTCATTGGTCTCTGCA	1	1

D1N S2A	Deng ue1	3476 to 4129	AGGGTCAGGA GAAGTGGATA GCTTTTCACT AGGACTGTTA TGCGTATCAA TAATGATCGA AGAGGTGATG AGATCCAGAT GGAGTAGAAA AATGCTGATG ACTGGAACAC TGGCTGTGTT CCTCCTTCTC ATAATGGGAC AATTGACATG GAATGATCTG ATCAGGTTAT GCATCATGGT TGGAGCCAAT GCTTCAGACA GGATGGGGAT GGGAACAACG TACCTAGCTC TGATGGCCAC TTTTAAAATG AGACCAATGT TCGCTGTCGG GTTATTATTT CGCAGACTAA CATCTAGAGA AGTTCTTCTT CTTACGATTG GATTGAGTCT GGTGGCATCT GTGGAGCTAC CAAATTCCTT GGAGGAGCTG GGGGATGGAC TTGCAATGGG CATCATGATT TTAAAATTAC TGACTGACTT TCAGTCACAT CAGCTGTGGG CTGCCCTGCT GTCCTTGACA TTTATCAAAA CAACTTTTTC ATTGCACTAT GCATGGAAGA CAATGGCTAT GGTACTGTCA ATTGTATCTC TCTTCCCTTT ATGCCTGTCC ACGACCTCTC AAAAAACAAC ATGGCTTCCG GTGCTGTTGG GATCTCTTGG ATGCAAACCA CTAACCATGT TTCTTATAGC AGAAAACAAA ATCTGGGGAA GGAAA	0.99	1
D1N S2B	Deng ue1	4130 to 4519	AGTTGGCCCCTCAATGAAGGAATCATGGCT GTTGGAATAGTTAGCATCCTACTAAGTTCAC TCCTCAAGAATGACGTGCCGCTAGCCGGCC CACTAATAGCTGGAGGTATGCTAATAGCATG TTATGTTATATCCGGAAGCTCAGCCGATTTA TCACTGGAGAAAGCGGCTGAGGTCTCCTGG GAAGAAGAAGCAGAACACTCTGGTGCCTCA CACAACATACTAGTGGAAGTCCAAGATGATG GAACCATGAAGATAAAAGATGAAGAGAGAG ATGACACACTCACCATTCTCCTTAAAGCAAC TCTGTTGGCAGTCTCAGGGGTGTACCCAAT ATCAATACCAGCGACCCTTTTTGTGTGGTAT TTTTGGCAGAAAAAGAAACAGAGA	1	1

D1NS3	Dengue1	4520 to 6376	GTCAGTGCCATAGCTCAAGCTAAAGCATCACAAGAAGGGCCTCTACCAGAGATTGAGGACGAGGTGTTTAGGAAAAGAAACTTAACAATAATGGACCTACATCCAGGATCGGGGAAAACAAGAAGATATCTTCCAGCCATAGTCCGTGAGGCTATAAAAAGGAAGCTGCGTACGCTAATCTTGGCTCCCACAAGAGTTGTCGCTTCTGAAATGGCAGAGGCGCTCAAGGGAATGCCAATAAGGTATCAGACAACAGCAGTGAAGAGTGAACACACAGGAAGGGAGATAGTTGACCTTATGTGCCATGCCACTTTCACCATGCGTCTCCTGTCTCCCGTGAGAGTTCCCAATTACAACATGATCATCATGGATGAAGCACATTTCACCGATCCAGCCAGTATAGCGGCCAGAGGGTACATCTCAACCCGGGTGGGCATGGGTGAAGCAGCTGCGATCTTCATGACAGCCACTCCCCCAGGATCGGTGGAGGCCTTTCCACAGAGCAATGCAGTTATCCAAGATGAGGAAAGAGACATTCCTGAGAGATCATGGAACTCAGGCTATGACTGGATCACTGATTTCCCAGGTAAAACAGTCTGGTTTGTTCCAAGCATTAAATCAGGAAATGACATTGCCAACTGTTTAAGAAAGAATGGGAAACGGGTGATCCAATTGAGCAGAAAAACCTTTGATACTGAGTACCAGAAAACAAAAAATAATGACTGGGACTATGTCGTCACAACAGACATTTCCGAAATGGGAGCAAACTTCCGAGCCGACAGGGTAATAGACCCAAGACGGTGTTTGAAACCGGTAATACTAAAAGATGGTCCAGAGCGTGTCATTCTAGCCGGACCGATGCCAGTGACTGTGGCCAGTGCCGCCCAGAGGAGAGGAAGAATTGGAAGGAACCAAAATAAGGAAGGTGATCAGTACATTTACATGGGACAGCCTTTAAACAACGATGAGGATCACGCTCATTGGACAGAAGCAAAAATGCTCCTTGACAACATAAACACACCAGAAGGGATTATCCCAGCCCTCTTTGAGCCGGAGAGAGGAAAAAGTGCAGCAATAGACGGGGAATACAGACTGCGGGGTGAAGCAAGGAAAACGTTCGTGGAGCTCATGAGAAGAGGAGATCTACCTGTCTGGCTATCCTACAAAGTTGCCTCAGAAGGCTTCCAGTACTCTGACAGAAAGTGGTGCTTTGATGGGGAAAGGAACAACCAGGTGTTGGAGGAGAACATGGACGTGGAGATCTGGACAAAAGAAGGAGAAAGAAAGAAACTACGACCCCGCTGGCTGGACGCCAGAACATACTCTGACCCACAGGCTCTGCGCGAGTTTAAAGAGTTTGCAGCAGGAAGAAGA	1	1

D1N S3d	Deng ue1	5003 to 6376	GGCGCTCAAG GGAATGCCAA TAAGGTATCA GACAACAGCA GTGAAGAGTG AACACACAGG AAGGGAGATA GTTGACCTTA TGTGCCATGC CACTTTCACC ATGCGTCTCC TGTCTCCCGT GAGAGTTCCC AATTACAACA TGATCATCAT GGATGAAGCA CATTTCACCG ATCCAGCCAG CATAGCGGCC AGAGGGTACA TCTCAACCCG GGTGGGCATG GGTGAAGCAG CTGCGATCTT CATGACAGCC ACTCCCCCAG GATCGGTGGA GGCCTTTCCA CAGAGCAATG CAGTTATCCA AGATGAGGAA AGAGACATTC CTGAGAGATC ATGGAACTCA GGCTATGACT GGATCACTGA TTTCCCAGGT AAAACAGTCT GGTTTGTTCC AAGCATTAAA TCAGGAAATG ACATTGCCAA CTGTTTAAGA AAGAATGGGA AACGGGTGAT CCAATTGAGC AGAAAAACCT T	1	1
D1N S4A	Deng ue1	6377 to 6757	AGCGTCTCAGGTGATCTAATATTAGAAATAG GGAAACTTCCACAACATTTGACGCAAAGGG CCCAGAATGCTCTGGACAACCTGGTCATGT TGCACAACTCCGAACAAGGAGGAAAAGCCT ATAGACATGCTATGGAAGAACTACCAGACAC CATAGAAACGTTGATGCTCCTAGCTTTGATA GCTGTGTTAACTGGTGGAGTGACGCTGTTC TTCCTATCAGGAAGAGGCCTAGGGAAAACA TCTATCGGCCTACTCTGCGTGATGGCTTCAA GCGTACTGTTATGGGTGGCCAGTGTGGAGC CCCATTGGATAGCGGCCTCCATCATACTGG AGTTCTTTCTGATGGTGCTGCTTATTCCAGA GCCAGACAGACAACGC	1	1
D1N S4B	Deng ue1	6827 to 7573	AATGAGATGG GATTATTGGA AACCACAAAG AAAGACCTAG GGATTGGCCA TGTGGCTGTT GAAAACCACC ACCATGCCAC AATGCTGGAC GTAGACTTAC GTCCAGCTTC AGCCTGGACC CTCTATGCAG TGGCCACAAC AATCATCACT CCCATGATGA GACACACAAT TGAAAACACA ACGGCAAATA TTTCCCTGAC AGCTATTGCA AACCAGGCAG CTATATTGAT GGGACTTGAC AAGGGATGGC CAATATCGAA GATGGACATA GGAGTTCCAC TCCTCGCCTT GGGGTGCTAT TCCCAGGTGA ACCCGCTGAC GCTGATAGCG GCGGTATTGA TGCTAGTGGC TCATTACGCC ATAATTGGAC CTGGACTGCA AGCAAAAGCC ACTAGAGAAG CTCAAAAAAG AACAGCGGCC GGAATAATGA AAAATCCAAC TGTCGACGGA ATTGTTGCAA TAGATCTGGA CCCTGTGGTT TATGATGCAA AATTTGAAAA ACAGCTAGGC CAAATAATGT TGTTGATACT TTGCACATCA CAGATTCTTT TAATGCGGAC TACATGGGCC TTGTGTGAAT CCATCACACT GGCTACTGGA CCTCTGACCA	1	1

			CGCTTTGGGA GGGATCTCCA GGAAAATTCT GGAACACCAC GATCGCGGTG TCCATGGCAA ACATTTTCAG GGGAAGTTAT CTAGCAGGAG CAGGTCTGGC CTTCTCATTA ATGAAATCTC TAGGAGGAGG TAGGAGA		
D1N S5	Deng ue1	7574 to 10270	GGTACGGGA GCCCAAGGGG AAACACTGGG AGAAAAATGG AAAAGACAGC TAAACCAACT GAGCAAGTCA GAATTCAACA CTTACAAAGG GAGTGGGATT ATGGAGGTGG ATAGATCTGA AGCTAAAGAG GGATTGAAAA GAGGAGAAAC AACCAAACAT GCAGTGTCGA GAGGAACAGC CAAACTGAGG TGGTTTGTGG AGAGGAACCT TGTGAAGCCG GAAGGGAAAG TCATAGACCT CGGTTGTGGA AGAGGTGGCT GGTCATATTA TTGTGCTGGG CTGAAGAAAG TCACAGAAGT GAAAGGATAT ACAAAAGGAG GACCTGGACA TGAGGAACCA ATCCCAATGG CGACCTATGG ATGGAACCTA GTAAAGCTAC ACTCCGGGAA AGATGTATTC TTTACACCAC CTGAGAAATG CGACACCCTT TTGTGTGATA TTGGTGAGTC CTCTCCGAAC CCAACTATAG AAGAAGGAAG AACGTTACGT GTTCTAAAGA TGGTGGAACC ATGGCTCAGA GGAAACCAAT TTTGCATAAA AATTCTAAAT CCCTATATGC CGAGTGTGGT GGAAACTCTG GAGCAAATGC AAAGAAAACA TGGAGGAATG CTAGTGCGAA ATCCACTCTC AAGAAATTCC ACCCATGAAA TGTACTGGGT TTCATGTGGA ACAGGAAACA TTGTGTCAGC AGTAAACATG ACATCTAGAA TGTTGCTAAA TCGGTTCACA ATGGCTCACA GGAAGCCAAC ATATGAAAGA GACGTGGACTTAG GTGCTGGAAC AAGACATGTG GCAGTGGAAC CAGAGGTAGC CAACCTAGAT ATCATTGGCC AGAGGATAGA GAATATAAAA AATGAGCATA AGTCAACATG GCATTATGAT GAGGACAATC CATACAAAAC ATGGGCCTAT CATGGATCAT	0.99	0.99

			ATGAGGTTAA GCCATCAGGA TCAGCCTCAT CCATGGTCAA TGGCGTGGTG AGATTGCTCA CCAAACCATG GGATGTTATC CCTATGGTCA CACAAATAGC CATGACTGAC ACTACACCCT TTGGACAACA GAGGGTGTTT AAAGAGAAAG TTGACACGCG CACACCAAAA GCAAAACGAG GCACAGCACA AATCATGGAG GTGACAGCCA GGTGGTTATG GGGTTTTCTT TCTAGAAACA AAAAACCAAG AATCTGCACA AGAGAGGAGT TCACAAGAAA AGTCAGGTCA AACGCAGCCA TTGGAGCAGT GTTCGTTGAT GAAAATCAAT GGAACTCAGC AAAAGAAGCG GTGGAAGATG AACGGTTCTG GGACCTTGTG CACAGAGAGA GGGAGCTCCA TAAACAGGGA AAATGTGCCA CGTGTGTTTA CAATATGATG GGGAAGAGAG AGAAAAAATT AGGAGAGTTC GGAAAGGCAA AAGGAAGTCG TGCAATATGG TACATGTGGT TGGGAGCACG CTTTCTAGAG TTCGAAGCCC TTGGTTTCAT GAACGAAGAT CACTGGTTCA GTAGAGAGAA TTCACTCAGT GGAGTGGAAG GAGAAGGACT CCACAAACTT GGATATATAC TCAGAGACAT ATCAAAGATT CCAGGGGGAA ATATGTATGC AGATGACACA GCCGGATGGG ACACAAGAAT AACAGAGGAT GATCTTCAGA ATGAGGCCAA AATCACTGAC ATCATGGAGC CCGAACATGC CCTATTGGCT ACGTCAATCT TTAAGCTGAC CTACCAAAAC AAGGTGGTAA GGGTGCAGAG ACCAGCAAAA AATGGAACCG TGATGGATGT CATATCCAGA CGTGACCAGA GAGGAAGTGG ACAGGTCGGA ACTTATGGCT TAAACACTTT CACTAACATG GAGGTCCAAC TAATAAGACA AATGGAGTCT GAGGGAATCT TTTCACCCAG CGAATTGGAG ACCCCAAATT TAGCCGAAAG AGTTCTCGAC TGGTTGGAAA AACATGGCGT CGAAAGGCTG AAAAGAATGG CAATCAGCGG AGATGACTGT GTGGTGAAAC CAACTGATGA CAGGTTCGCA ACAGCCTTAA CAGCTTT GAATGACATGGGAAAAGTAAGAAAAGACATA CCGCAATGGGAACCTTCAAAAGGATGGAAT GATTGGCAACAAGTGCCTTTTTGTTCACACC ATTTCCACCAGCTGATCATGAAGGATGGGA GGGAGATAGTGGCGCCATGCCGCAACCAA GATGAACTTGTGGGTAGGGCTAGAGTATCA CAAGGCGCCGGATGGAGCCTGAGAGAAACT GCATGCCTAGGCAAGTCATATGCACAGATG TGGCAGCTGATGTACTTCCACAGGAGAGAC CTGAGACTAGCGGCCAATGCCATCTGTTCA GCCGTTCCAATTGATTGGGTCCCAACCAGC CGCACCACCTGGTCGATCCATGCCCATCAT		

			CAATGGATGACAACAGAAGACATGTTGTCA GTGTGGAATAGGGTTTGGATAGAGGAAAAC CCATGGATGGAGGATAAAACCCATGTATCC AGTTGGGAAGATGTTCCATACTTAGGAAAAA GGGAAGATCAGTGGTGTGGATCCCTGATAG GCTTAACAGCAAGGGCCACCTGGGCCACTA ATATACAAGTGGCCATAAACCAAGTGAGAAG GCTTATTGGGAATGAGAATTATCTAGATTAC ATGACATCAATGAAGAGATTCAAGAATGAGA GTGATCTCGAAGGGGCACTCTGGTAA		
D2C A	Deng ue2	97 to 438	ATGAATGACC AACGGAAAAA GGCGAAAAAC ACGCCTTTCA ATATGCTGAA ACGCGAGAGA AACCGCGTGT CGACTGTGCA ACAGCTGACA AAGAGATTCT CACTTGGAAT GCTGCAGGGA CGAGGACCAT TAAAACTGTT CATGGCCCTG GTGGCGTTCC TTCGTTTCCT AACAATCCCA CCAACAGCAG GGATATTGAA GAGATGGGGA ACAATTAAAA AATCAAAAGC TATTAATGTT TTGAGAGGGT TCAGGAAAGA GATTGGAAGG ATGCTGAACA TCTTGAATAG GAGACGCAGA TCTGCCGGCA TGATCATTAT GCTGATTCCA ACAGTGATGG CG	0.99	1
D2C V	Deng ue2	97 to 396	ATGAATGACCAACGGAAAAAGGCGAAAAAC ACGCCTTTCAATATGCTGAAACGCGAGAGA AACCGCGTGTCGACTGTGCAACAGCTGACA AAGAGATTCTCACTTGGAATGCTGCAGGGA CGAGGACCATTAAAACTGTTCATGGCCCTG GTGGCGTTCCTTCGTTTCCTAACAATCCCAC CAACAGCAGGGATATTGAAGAGATGGGGAA	0.99	1

			CAATTAAAAAATCAAAAGCTATTAATGTTTTG AGAGGGTTCAGGAAAGAGATTGGAAGGATG CTGAACATCTTGAATAGGAGACGCAGA		
D2PrM	Dengue2	439 to 936	TTCCATTTAA CCACACGTAA CGGAGAACCA CACATGATCG TCAGCAGACA AGAGAAAGGA AAAAGTCTTC TGTTTAAAAC AGAGGATGGC GTGAACATGT GTACCCTCAT GGCCATGGAC CTTGGTGAAT TGTGTGAAGA CACAATCACG TACAAGTGTC CCCTTCTCAG GCAGAATGAG CCAGAAGACA TAGACTGTTG GTGCAACTCT ACGTCCACGT GGGTAACTTA TGGGACGTGT ACCACCATGG GAGAACATAG AAGAGAAAAA AGATCAGTGG CACTCGTTCC ACATGTGGGA ATGGGACTGG AGACACGAAC TGAAACATGG ATGTCATCAG AAGGGGCCTG GAAACATGTC CAGAGAATTG AAACTTGGAT CTTGAGACAT CCAGGCTTCA CCATGATGGC AGCAATCCTG GCATACACCA TAGGAACGAC ACATTTCCAA AGAGCCCTGA TTTTCATCTT ACTGACAGCT GTCACTCCTT CAATGACA	1	1
D2M	Dengue2	712 to 936	TCAGTGGCACTCGTTCCACATGTGGGAATG GGACTGGAGACACGAACTGAAACATGGATG TCATCAGAAGGGGCCTGGAAACATGTCCAG AGAATTGAAACTTGGATCTTGAGACATCCAG GCTTCACCATGATGGCAGCAATCCTGGCAT ACACCATAGGAACGACACATTTCCAAAGAG CCCTGATTTTCATCTTACTGACAGCTGTCAC TCCTTCAATGACA	1	1

D2E	Deng ue2	937 to 2421	ATGCGTTG CATAGGAATG TCAAATAGAG ACTTTGTGGA AGGGGTTTCA GGAGGAAGCT GGGTTGACAT AGTCTTAGAA CATGGAAGCT GTGTGACGAC GATGGCAAAA AACAAACCAA CATTGGATTT TGAACTGATA AAAACAGAAG CCAAACAGCC TGCCACCCTA AGGAAGTACT GTATAGAGGC AAAGCTAACC AACACAACAA CAGAATCTCG CTGCCCAACA CAAGGGGAAC CCAGCCTAAA TGAAGAGCAG GACAAAAGGT TCGTCTGCAA ACACTCCATG GTAGACAGAG GATGGGGAAA TGGATGTGGA CTATTTGGAA AGGGAGGCAT TGTGACCTGT GCTATGTTCA GATGCAAAAA GAACATGGAA GGAAAAGTTG TGCAACCAGA AAACTTGGAA TACACCATTG TGATAACACC TCACTCAGGG GAAGAGCATG CAGTCGGAAA TGACACAGGA AAACATGGCA AGGAAATCAA AATAACACCA CAGAGTTCCA TCACAGAAGC AGAATTGACA GGTTATGGCA CTGTCACAAT GGAGTGCTCT CCAAGAACGG GCCTCGACTT CAATGAGATG GTGTTGCTGC AGATGGAAAA TAAAGCTTGG CTGGTGCACA GGCAATGGTT CCTAGACCTG CCGTTACCAT GGTTGCCCGG AGCGGACACAC AAGGGTCAAA TTGGATACAG AAAGAGACAT TGGTCACTTT CAAAAATCCC CATGCGAAGA AACAGGATGT TGTTGTTTTA GGATCCCAAG AAGGGGCCAT GCACACAGCA CTTACAGGGG CCACAGAAAT CCAAATGTCA TCAGGAAACT TACTCTTCACAGGACATCTCAAGTGCAGGCT GAGAATGGACAAGCTACAGCTCAAAGGAAT GTCATACTCTATGTGCACAGGAAAGTTTAAA GTTGTGAAGGAAATAGCAGAAACACAACAT GGAACAATAGTTATCAGAGTGCAATATGAAG GGGACGGCTCTCCATGCAAGATCCCTTTTG AGATAATGGATTTGGAAAAAAGACATGTCTT AGGTCGCCTGATTACAGTCAACCCAATTGTG ACAGAAAAAGATAGCCCAGTCAACATAGAA GCAGAACCTCCATTCGGAGACAGCTACATC ATCATAGGAGTAGAGCCGGGACAACTGAAG CTCAACTGGTTTAAGAAAGGAAGTTCTATCG GCCAAATGTTTGAGACAACAATGAGGGGGG CGAAGAGAATGGCCATTTTAGGTGACACAG CCTGGGATTTTGGATCCTTGGGAGGAGTGT TTACATCTATAGGAAAGGCTCTCCACCAAGT CTTTGGAGCAATCTATGGAGCTGCCTTCAGT GGGGTTTCATGGACTATGAAAATCCTCATAG GAGTCATTATCACATGGATAGGAATGAATTC ACGCAGCACCTCACTGTCTGTGACACTAGT ATTGGTGGGAATTGTGACACTGTATTTGGGA GTCATGGTGCAGGCC	1	1

D2Eiii	Dengue2	1816 to 2118	CTCAAAGGAA TGTCATACTC TATGTGCACA GGAAAGTTTA AAGTTGTGAA GGAAATAGCA GAAACACAAC ATGGAACAAT AGTTATCAGA GTGCAATATG AAGGGGACGG CTCTCCATGC AAGATCCCTT TTGAGATAAT GGATTTGGAA AAAAGACATG TCTTAGGTCG CCTGATTACA GTCAACCCAA TTGTGACAGA AAAAGATAGC CCAGTCAACA TAGAAGCAGA ACCTCCATTC GGAGACAGCT ACATCATCAT AGGAGTAGAG CCGGGACAAC TGAAGCTCAA CTGGTTTAAG AAA	1	1
D2NS1	Dengue2	2422 to 3477	GATAGT GGTTGCGTTG TGAGCTGGAA AAACAAAGAA CTGAAATGTG GCAGTGGGAT TTTCATCACA GACAACGTGC ACACATGGAC AGAACAATAC AAGTTCCAAC CAGAATCCCC TTCAAAACTA GCTTCAGCTA TCCAGAAAGC CCATGAAGAG GGCATTTGTG GAATCCGCTC AGTAACAAGA CTGGAGAATC TGATGTGGAA ACAAATAACA CCAGAATTGA ATCACATTCT ATCAGAAAAT GAGGTGAAGT TAACTATTAT GACAGGAGAC ATCAAAGGAA TCATGCAGGC AGGAAAACGA TCTCTGCGGC CTCAGCCCAC TGAGCTGAAG TATTCATGGA AAACATGGGG CAAAGCAAAA ATGCTCTCTA CAGAGTCTCA TAACCAGACC TTTCTCATTG ATGGCCCCGA AACAGCAGAA TGCCCCAACA CAAATAGAGC TTGGAATTCG TTGGAAGTTG AAGACTATGG CTTTGGAGTA TTCACCACCA ATATATGGCT AAAATTGAAA GAAAACCAGG ATGTATTCTG CGACTCAAAA CTCATGTCAG CGGCCATAAA AGACAACAGA GCCGTCCATG CCGATATGGG TTATTGGATA GAAAGTGCAC TCAATGACAC ATGGAAGATA GAGAAAGCCT CTTTCATTGA AGTTAAAAAC TGCCACTGGC CAAAATCACA CACCCTCTGG AGCAATGGAG TGCTAGAAAG TGAGATGATA ATTCCAAAGA ATCTCGCTGG ACCAGTGTCT CAACACAACT ATAGACCAGG CTACCATACA CAAATAACAG GACCATGGCA TCTAGGTAAG CTTGAGATGG ACTTTGATTT CTGTGATGGA ACAACAGTGG TAGTGACTGA GGACTGCGGA AATAGAGGAC CCTCTTTGAG AACAACCACT GCCTCTGGAA AACTCATAAC AGAATGGTGC TGCCGATCTT GCACATTACC ACCGCTAAGA TACAGAGGTG AGGATGGGTG CTGGTACGGG ATGGAAATCA GACCATTGAA GGAGAAAGAA GAGAATTTGG TCAACTCCTT GGTCACAGCT	0.99	0.99

D2N S2A	Deng ue2	3478 to 4131	GGAC ATGGGCAGGT CGACAACTTT TCACTAGGAG TCTTGGGAAT GGCATTGTTC CTGGAGGAAA TGCTTAGGAC CCGAGTAGGA ACGAAACATG CAATACTACT AGTTGCAGTT TCTTTTGTGA CATTGATCAC AGGGAACATG TCCTTTAGAG ACCTGGGAAG AGTGATGGTT ATGGTAGGCG CCATTATGAC GGATGACATA GGTATGGGCG TGACTTATCT TGCCCTACTA GCAGCCTTCA AAGTCAGACC AACTTTTGCA GCTGGACTAC TCTTGAGAAA GCTGACCTCC AAGGAATTGA TGATGACTAC TATAGGAATT GTACTCCTCT CCCAGAGCAC CATACCAGAG ACCATTCTTG AGTTGACTGA TGCGTTAGCC TTAGGCATGA TGGTCCTCAA AATGGTGAGA AATATGGAAA AGTATCAATT GGCAGTGACT ATCATGGCTA TCTTGTGCGT CCCAAACGCA GTGATATTAC AAAACGCATG GAAAGTGAGT TGCACAATAT TGGCAGTGGT GTCCGTTTCC CCACTGTTCT TAACATCCTC ACAGCAAAAA ACAGATTGGA TACCATTAGC ATTGACGATC AAAGGTCTCA ATCCAACAGC TATTTTTCTA ACAACCCTCT CAAGAACCAG CAAGAAAGGT	0.99	0.99
D2N S2B	Deng ue2	4132 to 4521	AGCTGGCCAT TAAATGAGGC TATCATGGCA GTCGGGATGG TGAGCATTTT AGCCAGTTCT CTCCTAAAAA ATGATATTCC CATGACAGGA CCATTAGTGG CTGGAGGGCT CCTCACTGTG TGCTACGTGC TCACTGGACG ATCGGCCGAT TTGGAACTGG AGAGAGCAGC CGATGTTAAA TGGGAAGACC AGGCAGAGAT ATCAGGAAGC AGTCCAATCC TGTCAATAAC AATATCAGAA GACGGTAGCA TGTCGATAAA AAATGAAGAG GAAGAACAAA CACTGACCAT ACTCATTAGA ACAGGATTGC TGGTGATCTC AGGACTTTTT CCTGTATCAA TACCAATCAC GGCAGCAGCA TGGTACCTGT GGGAAGTGAA GAAACAACGG	1	1

D2N S3	Deng ue2	4522 to 6375	GCCGGAGTGTTGTGGGATGTTCCTTCACCC CCACCCATGGGAAAGGCTGAACTGGAAGAT GGAGCCTATAGAATTAAGCAAAAAGGGATTC TTGGATATTCCCAGATCGGAGCCGGAGTTT ACAAAGAAGGAACATTCCATACAATGTGGCA TGTCACACGTGGCGCTGTTCTAATGCATAAA GGAAAGAGGATTGAACCATCATGGGCGGAC GTCAAGAAAGACCTAATATCATATGGAGGAG GCTGGAAGTTAGAAGGAGAATGGAAGGAAG GAGAAGAAGTCCAGGTATTGGTACTGGAGC CTGGAAAAAATCCAAGAGCCGTCCAAACGA AACCTGGTCTTTTCAAAACCAACGCCGGAAC AATAGGTGCTGTATCTCTGGACTTTTCTCCT GGAACGTCAGGATCTCCAATTATCGACAAAA AAGGAAAAGTTGTGGGTCTTTATGGTAATGG TGTTGTTACAAGGAGTGGAGCATATGTGAGT GCTATAGCCCAGACTGAAAAAAGCATTGAA GACAACCCAGAGATCGAAGATGACATTTTCC GAAAGAGAAGACTGACCATCATGGACCTCC ACCCAGGAGCGGGAAAGACGAAGAGATACC TTCCGGCCATAGTCAGAGAAGCTATAAAAC GGGGTTTGAGAACATTAATCTTGGCCCCCA CTAGAGTTGTGGCAGCTGAAATGGAGGAAG CCCTTAGAGGACTTCCAATAAGATACCAGAC CCCAGCCATCAGAGCTGAGCACACCGGGC GGGAGATTGTGGACCTAATGTGTCATGCCA CATTTACCATGAGGCTGCTATCACCAGTTAG AGTGCCAAACTACAACCTGATTATCATGGAC GAAGCCCATTTCACAGACCCAGCAAGTATA GCAGCTAGAGGATACATCTCAACTCGAGTG GAGATGGGTGAGGCAGCTGGGATTTTTATG ACAGCCACTCCCCCGGGAAGCAGAGGCCC ATTTCCTCAGAGCAATGCACCAATCATAGAT GAAGAAAGAGAAATCCCTGAACGTTCGTGG AATTCCGGACATGAATGGGTCACGGATTTTA AAGGGAAGACTGTTTGGTTCGTTCCAAGTAT AAAAGCAGGAAATGATATAGCAGCTTGCCT GAGGAAAAATGGAAAGAAAGTGATACAACT CAGTAGGAAGACCTTTGATTCTGAGTATGTC AAGACTAGAACCAATGATTGGGACTTCGTG GTTACAACTGACATTTCAGAAATGGGTGCCA ATTTCAAGGCTGAGAGGGTTATAGACCCCA GACGCTGCATGAAACCAGTCATACTAACAG ATGGTGAAGAGCGGGTGATTCTGGCAGGAC CTATGCCAGTGACCCACTCTAGTGCAGCAC AAAGAAGAGGGAGAATAGGAAGAAATCCAA AAAATGAGAATGACCAGTACATATACATGGG GGAACCTCTGGAAAATGATGAAGACTGTGC ACACTGGAAAGAAGCTAAAATGCTCCTAGAT AACATCAACACGCCAGAAGGAATCATTCCTA GCATGTTCGAACCAGAGCGTGAAAAGGTGG ATGCCATTGATGGCGAATACCGCTTGAGAG GAGAAGCAAGGAAAACCTTTGTAGACTTAAT GAGAAGAGGAGACCTACCAGTCTGGTTGGC CTACAGAGTGGCAGCTGAAGGCATCAACTA CGCAGACAGAAGGTGGTGTTTTGATGGAGT	0.99	0.99

			CAAGAACAACCAAATCCTAGAAGAAAACGTGGAAGTTGAAATCTGGACAAAAGAAGGGGAAAGGAAGAAATTGAAACCCAGATGGTTGGATGCTAGGATCTATTCTGACCCACTGGCGCTAAAAGAATTTAAGGAATTTGCAGCCGGAAGAAAG		

D2N S3d	Deng ue2	5005 to 6375	GTGAGTGCTA TAGCCCAGAC TGAAAAAAGC ATTGAAGACA ACCCAGAGAT CGAAGATGAC ATTTTCCGAA AGAGAAGACT GACCATCATG GACCTCCACC CAGGAGCGGG AAAGACGAAG AGATACCTTC CGGCCATAGT CAGAGAAGCT ATAAAACGGG GTTTGAGAAC ATTAATCTTG GCCCCCACTA GAGTTGTGGC AGCTGAAATG GAGGAAGCCC TTAGAGGACT TCCAATAAGA TACCAGACCC CAGCCATCAG AGCTGAGCAC ACCGGGCGGG AGATTGTGGA CCTAATGTGT CATGCCACAT TTACCATGAG GCTGCTATCA CCAGTTAGAG TGCCAAACTA CAACCTGATT ATCATGGACG AAGCCCATTT CACAGACCCA GCAAGTATAG CAGCTAGAGG ATACATCTCA ACTCGAGTGG AGATGGGTGA GGCAGCTGGG ATTTTTATGA CAGCCACTCC CCCGGGAAGC AGAGGCCCAT TTCCTCAGAG CAATGCACCA ATCATAGATG AAGAAAGAGA AATCCCTGAA CGTTCGTGGA ATTCCGGACA TGAATGGGTC ACGGATTTTA AAGGGAAGAC TGTTTGGTTC GTTCCAAGTA TAAAAGCAGG AAATGATATA GCAGCTTGCC TGAGGAAAAATGG AAAGAAAGTG ATACAACTCA GTAGGAAGAC CTTTGATTCT GAGTATGTCA AGACTAGAAC CAATGATTGG GAC TTCGTGGTTACAACTGACATTTCAGAAATGG GTGCCAATTTCAAGGCTGAGAGGGTTATAG ACCCCAGACGCTGCATGAAACCAGTCATAC TAACAGATGGTGAAGAGCGGGTGATTCTGG CAGGACCTATGCCAGTGACCCACTCTAGTG CAGCACAAAGAAGAGGGAGAATAGGAAGAA ATCCAAAAAATGAGAATGACCAGTACATATA CATGGGGGAACCTCTGGAAAATGATGAAGA CTGTGCACACTGGAAAGAAGCTAAAATGCT CCTAGATAACATCAACACGCCAGAAGGAAT CATTCCTAGCATGTTCGAACCAGAGCGTGA AAAGGTGGATGCCATTGATGGCGAATACCG CTTGAGAGGAGAAGCAAGGAAAACCTTTGT AGACTTAATGAGAAGAGGAGACCTACCAGT CTGGTTGGCCTACAGAGTGGCAGCTGAAGG CATCAACTACGCAGACAGAAGGTGGTGTTTT GATGGAGTCAAGAACAACCAAATCCTAGAA GAAAACGTGGAAGTTGAAATCTGGACAAAA GAAGGGGAAAGGAAGAAATTGAAACCCAGA TGGTTGGATGCTAGGATCTATTCTGACCCAC TGGCGCTAAAAGAATTTAAGGAATTTGCAGC CGGAAGAAAG	0.99	0.99

D2N S4A	Deng ue2	6376 to 6825	TCTCTGACCC TGAACCTAAT CACAGAAATG GGTAGGCTCC CAACCTTCAT GACTCAGAAG GCAAGAGACG CACTGGACAA CTTAGCAGTG CTGCACACGG CTGAGGCAGG TGGAAGGGCG TACAACCATG CTCTCAGTGA ACTGCCGGAG ACCCTGGAGA CATTGCTTTT ACTGACACTT CTGGCTACAG TCACGGGAGG GATCTTTTTA TTCTTGATGA GCGGAAGGGG CATAGGGAAG ATGACCCTGG GAATGTGCTG CATAATCACG GCTAGCATCC TCCTATGGTA CGCACAAATA CAGCCACACT GGATAGCAGC TTCAATAATA CTGGAGTTTT TTCTCATAGT TTTGCTTATT CCAGAACCTG AAAAACAGAG AACACCCCAA GACAACCAAC TGACCTACGT TGTCATAGCC ATCCTCACAG TGGTGGCCGC AACCATGGCA	1	1
D2N S4B	Deng ue2	6826 to 7569	AACG AGATGGGTTT CCTAGAAAAA ACGAAGAAAG ATCTCGGATT GGGAAGCATT GCAACCCAGC AACCCGAGAG CAACATCCTG GACATAGATC TACGTCCTGC ATCAGCATGG ACGCTGTATG CCGTGGCCAC AACATTTGTT ACACCAATGT TGAGACATAG CATTGAAAAT TCCTCAGTGA ATGTGTCCCT AACAGCTATA GCCAACCAAG CCACAGTGTT AATGGGTCTC GGGAAAGGAT GGCCATTGTC AAAGATGGAC ATCGGAGTTC CCCTTCTCGC CATTGGATGC TACTCACAAG TCAACCCCAT AACTCTCACA GCAGCTCTTT TCTTATTGGT AGCACATTAT GCCATCATAG GGCCAGGACT CCAAGCAAAA GCAACCAGAG AAGCTCAGAA AAGAGCAGCG GCGGGCATCA TGAAAAACCC AACTGTCGAT GGAATAACAG TGATTGACCT AGATCCAATA CCTTATGATC CAAAGTTTGA AAAGCAGTTG GGACAAGTAA TGCTCCTAGT CCTCTGCGTG ACTCAAGTAT TGATGATGAG GACTACATGG GCTCTGTGTG AGGCTTTAAC CTTAGCTACC GGGCCCATCT CCACATTGTG GGAAGGAAAT CCAGGGAGGT TTTGGAACAC TACCATTGCG GTGTCAATGG CTAACATTTT TAGAGGGAGT TACTTGGCCG GAGCTGGACT TCTCTTTTCT ATTATGAAGA ACACAACCAA CGCAAGAAGG	0.99	0.99

| D2NS5 | Dengue2 | 7570 to 10269 | GGAACTGGCA ACATAGGAGA GACGCTTGGA GAGAAATGGA AAAGCCGATT GAACGCATTG GGAAAAAGTG AATTCCAGAT CTACAAGAAA AGTGGAATCC AGGAAGTGGA TAGAACCTTA GCAAAAGAAG GCATTAAAAG AGGAGAAACG GACCATCACG CTGTGTCGCG AGGCTCAGCA AAACTGAGAT GGTTCGTTGA GAGAAACATG GTCACACCAG AAGGGAAAGT AGTGGACCTC GGTTGTGGCA GAGGAGGCTG GTCATACTAT TGTGGAGGAC TAAAGAATGT AAGAGAAGTC AAAGGCCTAA CAAAAGGAGG ACCAGGACAC GAAGAACCCA TCCCCATGTC AACATATGGG TGGAATCTAG TGCGTCTTCA AAGTGGAGTT GACGTTTTCT TCATCCCGCC AGAAAAGTGT GACACATTAT TGTGTGACAT AGGGGAGTCA TCACCAAATC CCACAGTGGA AGCAGGACGA ACACTCAGAG TCCTTAACTT AGTAGAAAAT TGGTTGAACA ACAACACTCA ATTTTGCATA AAGGTTCTCA ACCCATATAT GCCCTCAGTC ATAGAAAAAA TGGAAACACT ACAAAGGAAA TATGGAGGAG CCTTAGTGAG GAATCCACTC TCACGAAACT CCACACATGA GATGTACTGG GTATCCAATGCTTCCG GGAACATAGT GTCATCAGTG AACATGATTT CAAGGATGTT GATCAACAGA TTTACAATGA GATACAAGAA AGCCACTTAC GAGCCGGATG TTGACCTCGG AAGCGGAACC CGTAACATCG GGATTGAAAG TGAGATACCA AACCTAGATA TAATTGGGAA AAGAATAGAA AAAATAAAGC AAGAGCATGA AACATCATGG CACTATGACC AAGACCACCC ATACAAAACG TGGGCATACC ATGGTAGCTA TGAAACAAAA CAGACTGGAT CAGCATCATC CATGGTCAAC GGAGTGGTCA GGCTGCTGAC AAAACCTTGG GACGTCGTCC CCATGGTGAC ACAGATGGCA ATGACAGACA CGACTCCATT TGGACAACAG CGCGTTTTTA AAGAGAAAGT GGACACGAGA ACCCAAGAAC CGAAAGAAGG CACGAAGAAA CTAATGAAAA TAACAGCAGA GTGGCTTTGG AAAGAATTAG GGAAGAAAAA GACACCCAGG ATGTGCACCA GAGAAGAATT CACAAGAAAG GTGAGAAGCA ATGCAGCCTT GGGGGCCATA TTCACTGATG AGAACAAGTG GAAGTCGGCA CGTGAGGCTG TTGAAGATAG TAGGTTTTGG GAGCTGGTTG ACAAGGAAAG GAATCTCCAT CTTGAAGGAA AGTGTGAAAC ATGTGTGTAC AACATGATGG GAAAAAGAGA GAAGAAGCTA GGGGAATTCG | 0.99 | 0.99 |

			GCAAGGCAAA AGGCAGCAGA GCCATATGGT ACATGTGGCT TGGAGCACGC TTCTTAGAGT TTGAAGCCCT AGGATTCTTA AATGAAGATC ACTGGTTCTC CAGAGAGAAC TCCCTGAGTG GAGTGGAAGG AGAAGGGCTG CACAAGCTAG GTTACATTCT AAGAGACGTG AGCAAGAAAG AGGGAGGAGC AATGTATGCC GATGACACCG CAGGATGGGA TACAAGAATC ACACTAGAAG ACCTAAAAAA TGAAGAAATG GTAACAAACC ACATGGAAGG AGAACACAAG AAACTAGCCG AGGCCATTTT CAAACTAACG TACCAAAACA AGGTGGTGCG TGTGCAAAGA CCAACACCAA GAGGCACAGT AATGGACATC ATATCGAGAA GAGACCAAAG AGGTAGTGGA CAAGTTGGCA CCTATGGACT CAATACTTTC ACCAATATGG AAGCCCAACT AATCAGACAG ATGGAGGGAG AAGGAGTCTT TAAAAGCATT CAGCACCTAA CAATCACAGA AGAAATCGCT GTGCAAAACT GGTTAGCAAG AGTGGGGCGC GAAAGGTTAT CAAGAATGGC CATCAGTGGA GATGATTGTG TTGTGAAACC TTTAGATGAC AGGTTCGCAA GCGCTTTAAC AGCTCTAAATGACATGGGAAAGATTAGGAAA GACATACAACAATGGGAACCTTCAAGAGGA TGGAATGATTGGACACAAGTGCCCTTCTGTT CACACCATTTCCATGAGTTAATCATGAAAGA CGGTCGCGTACTCGTTGTTCCATGTAGAAA CCAAGATGAACTGATTGGCAGAGCCCGAAT CTCCCAAGGAGCAGGGTGGTCTTTGCGGGA GACGGCCTGTTTGGGGAAGTCTTACGCCCA AATGTGGAGCTTGATGTACTTCCACAGACG CGACCTCAGGCTGGCGGCAAATGCTATTTG CTCGGCAGTACCATCACATTGGGTTCCAAC AAGTCGAACAACCTGGTCCATACATGCTAAA CATGAATGGATGACAACGGAAGACATGCTG ACAGTCTGGAACAGGGTGTGGATTCAAGAA AACCCATGGATGGAAGACAAAACTCCAGTG GAATCATGGGAGGAAATCCCATACTTGGGG AAAAGAGAAGACCAATGGTGCGGCTCATTG ATTGGGTTAACAAGCAGGGCCACCTGGGCA AAGAACATCCAAGCAGCAATAAATCAAGTTA GATCCCTTATAGGCAATGAAGAATACACAGA TTACATGCCATCCATGAAAAGATTCAGAAGA GAAGAGGAAGAAGCAGGAGTTCTGTGGTAG		

D3CA	Dengue3	95 to 436	ATGAACAACCAACGGAAAAAGACGGGAAAA CCGTCTATCAATATGCTGAAACGCGTGAGAA ACCGTGTGTCAACTGGATCACAGTTGGCGA AGAGATTCTCAAGAGGATTGCTGAACGGCC AAGGACCAATGAAATTGGTTATGGCGTTTAT AGCTTTCCTCAGATTTCTAGCCATTCCACCG ACAGCAGGAGTCTTGGCTAGATGGGGTACC TTTAAGAAGTCGGGGGCTATTAAGGTCTTAA AAGGCTTCAAGAAGGAGATCTCAAACATGCT GAGCATTATCAACAAACGGAAAAAGACATCG CTCTGTCTCATGATGATGTTACCAGCAACAC TTGCT	1	1
D3CV	Dengue3	95 to 394	ATGAACAACC AACGGAAAAA GACGGGAAAA CCGTCTATCA ATATGCTGAA ACGCGTGAGA AACCGTGTGT CAACTGGATC ACAGTTGGCG AAGAGATTCT CAAGAGGATT GCTGAACGGC CAAGGACCAA TGAAATTGGT TATGGCGTTT ATAGCTTTCC TCAGATTTCT AGCCATTCCA CCGACAGCAG GAGTCTTGGC TAGATGGGGT ACCTTTAAGA AGTCGGGGGC TATTAAGGTC TTAAAAGGCT TCAAGAAGGA GATCTCAAAC ATGCTGAGCA TTATCAACAA ACGGAAAAAG	1	1
D3PrM	Dengue3	437 to 934	TTCCACTTAA CTTCACGAGA TGGAGAGCCG CGCATGATTG TGGGGAAGAA TGAAAGAGGA AAATCCCTAC TTTTTAAGAC AGCCTCTGGA ATCAACATGT GCACACTCAT AGCCATGGAT TTGGGAGAGA TGTGTGATGA CACGGTCACT TACAAATGCC CCCACATTAC CGAAGTGGAG CCTGAAGACA TTGACTGCTG GTGCAACCTT ACATCGACAT GGGTGACTTA TGGAACATGC AATCAAGCTG GAGAGCATAG ACGCGATAAG AGATCAGTGG CGTTAGCTCC CCATGTCGGC ATGGGACTGG ACACACGCAC TCAAACCTGG ATGTCGGCTG AAGGAGCTTG GAGACAAGTC GAGAAGGTAG AGACATGGGC CCTTAGGCAC CCAGGGTTTA CCATACTAGC CCTATTTCTT GCCCATTACA TAGGCACTTC CTTGACCCAG AAAGTGGTTA TTTTTATACT ATTAATGCTG GTTACCCCAT CCATGACA	1	1
D3M	Dengue3	710 to 934	TCAGTGGCGTTAGCTCCCCATGTCGGCATG GGACTGGACACACGCACTCAAACCTGGATG TCGGCTGAAGGAGCTTGGAGACAAGTCGAG AAGGTAGAGACATGGGCCCTTAGGCACCCA GGGTTTACCATACTAGCCCTATTTCTTGCCC ATTACATAGGCACTTCCTTGACCCAGAAAGT GGTTATTTTTATACTATTAATGCTGGTTACCC CATCCATGACA	1	1

| D3E | Deng ue3 | 935 to 2413 | ATGAGATGTG TGGGAGTAGG AAACAGAGAT TTTGTGGAAG GCCTATCGGG AGCCACGTGG GTTGACGTGG TGCTCGAGCA CGGTGGGTGT GTGACTACCA TGGCTAAGAA CAAGCCCACG CTGGACATAG AGCTTCAGAA GACCGAGGCC ACCCAACTGG CGACCCTAAG GAAGCTATGC ATTGAGGGAA AAATTACCAA CATAACAACC GACTCAAGAT GTCCCACCCA AGGGGAAGCG ATTTTACCTG AGGAGCAGGA CCAGAACTAC GTGTGTAAGC ATACATACGT GGACAGAGGC TGGGGAAACG GTTGTGGTTT GTTTGGCAAG GGAAGCTTGG TGACATGCGC GAAATTTCAA TGTTTAGAAT CAATAGAGGG AAAAGTGGTG CAACATGAGA ACCTCAAATA CACCGTCATC ATCACAGTGC ACACAGGAGA CCAACACCAG GTGGGAAATG AAACGCAGGG AGTTACGGCT GAGATAACAT CCCAGGCATC AACCGCTGAA GCCATTTTAC CTGGATATGG AACCCTCGGG CTAGAATGCT CACCACGGAC AGGTTTGGAT TTCAATGAAA TGATTTTATT GACAATGAAG AACAAAGCAT GGATGGTACA TAGACAATGG TTCTTTGACT TACCCCTACC ATGGACATCA GGAGCTACAA CAGAAACACC AACTTGGAAC AGGAGAGAGC TTCTTGTGAC ATTTAAAAAT GCACATGCAA AAAAGCAAGA AGTAGTTGTC CTTGGATCACAGGAGGGAGC AATGCATACA GCACTGACAG GAGCTACAGA GATCCAAACC TCAGGAGGCACAAGTATTTTTGCGGGGCAC TTAAAATGTAGACTCAAGATGGACAAATTGG AACTCAAGGGGATGAGCTATGCAATGTGCT TGAATACCTTTGTGTTGAAGAAAGAAGTCTC CGAAACGCAGCATGGGACAATACTCATTAA GGTTGAGTACAAAGGGGAAGATGCACCCTG CAAGATTCCTTTCTCCACGGAGGATGGACA AGGGAAAGCTCACAATGGCAGACTGATCAC AGCCAATCCAGTGGTGACCAAGAAGGAGGA GCCTGTCAACATTGAGGCTGAACCTCCTTTT GGGGAAAGTAATATAGTAATTGGAATTGGAG ACAAAGCCCTGAAAATCAACTGGTACAGGA AAGGAAGCTCGATTGGGAAGATGTTCGAGG CCACTGCCAGAGGTGCAAGGCGCATGGCC ATCTTGGGAGACACAGCCTGGGACTTTGGA TCAGTGGGTGGTGTTTTGAATTCATTAGGGA AAATGGTCCACCAAATATTTGGGAGTGCTTA CACAGCCCTATTTAGTGGAGTCTCCTGGATA ATGAAAATTGGAATAGGTGTCCTCTTAACCT GGATAGGGTTGAATTCAAAAAACACTTCTAT GTCATTTTCATGCATTGCGATAGGAATCATC | 0.99 | 1 |

			ACACTCTATCTGGGGGTCGTGGTGCAAGCT		
D3Eiii	Dengue3	1784 to 2212	AGACTCAAGATGGACAAATTGGAACTCAAGGGGATGAGCTATGCAATGTGCTTGAATACCTTTGTGTTGAAGAAAGAAGTCTCCGAAACGCAGCATGGGACAATACTCATTAAGGTTGAGTACAAAGGGGAAGATGCACCCTGCAAGATTCCTTTCTCCACGGAGGATGGACAAGGGAAAGCTCACAATGGCAGACTGATCACAGCCAATCCAGTGGTGACCAAGAAGGAGGAGCCTGTCAACATTGAGGCTGAACCTCCTTTTGGGGAAAGTAATATAGTAATTGGAATTGGAGACAAAGCCCTGAAAATCAACTGGTACAGGAAGGGAAGCTCGATTGGGAAGATGTTCGAGGCCACTGCCAGAGGTGCAAGGCGCATGGCCATCTTGGGAGACACAGCCTGGGACTTTGGATCAGTGGGTGGTGTT	1	1

D3N S1	Deng ue3	2414 to 3469	GACATGGGGTGTGTCATAAACTGGAAAGGC AAAGAACTCAAATGTGGAAGTGGAATTTTCG TCACTAATGAGGTCCACACCTGGACAGAGC AATACAAATTTCAAGCAGACTCCCCCAAAAG ACTGGCAACAGCCATTGCAGGCGCTTGGGA GAATGGAGTGTGCGGAATTAGGTCAACAAC CAGAATGGAGAACCTCTTGTGGAAGCAAAT AGCCAATGAACTGAACTACATATTATGGGAA AACAACATCAAATTAACGGTAGTTGTAGGCG ACATAACTGGGGTCTTAGAGCAAGGGAAAA GAACACTAACACCACAACCCATGGAGCTAA AATATTCTTGGAAAACATGGGGAAAGGCAAA AATAGTGACAGCTGAAACACAAAATTCCTCT TTCATAATAGATGGGCCAAGCACACCGGAG TGTCCAAGTGCCTCAAGAGCATGGAATGTG TGGGAGGTGGAAGATCACGGGTTCGGAGTT TTCACAACCAACATATGGCTGAAACTCCGAG AGGTGTACACCCAACTATGTGACCATAGGC TAATGTCGGCAGCCGTCAAGGATGAGAGGG CCGTACACGCCGACATGGGCTATTGGATAG AAAGCCAAAAGAATGGAAGTTGGAAGCTAG AAAAAGCATCCCTCATAGAGGTGAAAACCTG CACATGGCCAAAATCACACACTCTTTGGAGC AATGGTGTGCTAGAGAGTGACATGATTATCC CAAAGAGTCTAGCTGGTCCCATTTCGCAACA CAACCACAGGCCCGGGTACCACACCCAAAC GGCAGGACCCTGGCACTTAGGAAAATTGGA GCTGGACTTCAACTATTGTGAAGGAACAACA GTTGTCATCTCAGAAAACTGTGGGACAAGA GGCCCATCATTGAGAACAACAACAGTGTCA GGGAAGTTGATACACGAATGGTGTTGCCGC TCGTGCACACTTCCTCCCCTGCGATACATG GGAGAAGACGGCTGCTGGTATGGCATGGAA ATCAGACCCATTAATGAGAAAGAAGAGAACA TGGTAAAGTCTCTAGCCTCAGCA	0.99	1
D3N S2A	Deng ue3	3470 to 4123	GGGAGTGGAA AGGTGGACAA CTTCACAATG GGTGTCTTGT GTTTGGCAAT CCTCTTTGAA GAGGTGATGA GAGGAAAATT TGGGAAAAAA CACATGATTG CAGGGGTTCT CTTCACGTTT GTGCTCCTCC TCTCAGGGCA AATAACATGG AGAGACATGG CGCACACACT CATAATGATT GGGTCCAGCG CCTCTGACAG AATGGGGATG GGCGTCACTT ACCTAGCTCT AATTGCAACA TTTAAAATTC AGCCATTCTT GGCTTTGGGA TTCTTCCTGA GGAAACTGAC ATCTAGAGAA AATTTATTGC TGGGAGTTGG GTTGGCCATG GCAGCAACGT TACGACTGCC AGAGGACATT GAACAAATGG CGAATGGAAT TGCTTTGGGG CTCATGGCTC TTAAACTGAT AACACAATTT GAAACATACC AACTATGGAC GTCATTAGTT TCCCTAACGT GTTCAAATAC AATTTTCACG TTGACTGTTG CCTGGAGAAC AGCCACTCTG ATTTTAGCCG GAATTTCGCT TTTGCCAGTG TGCCAGTCTT CGAGCATGAG	0.99	1

			GAAAACAGAT TGGCTCCCAA TGACTGTGGC AGCTATGGGA GTTCCACCCC TACCACTTTT TATTTTCAGT CTGAAAGATA CACTCAAAAG GAGA		
D3N S2B	Deng ue3	4124 to 4513	AGCTGGCCAC TGAATGAGGG GGTGATGGCA GTTGGACTTG TGAGCATTCT GGCTAGTTCT CTCCTTAGGA ATGATGTGCC CATGGCTGGA CCATTAGTGG CTGGGGGCTT GCTGATAGCG TGCTACGTCA TAACTGGCAC GTCAGCAGAC CTCACTGTAG AAAAAGCAGC AGATGTAACA TGGGAGGAAG AGGCCGAGCA AACAGGAGTG TCCCACAATT TAATGATCAC AGTTGATGAT GATGGAACAA TGAGAATAAA AGATGACGAG ACTGAGAACA TCTTAACAGT GCTTTTAAAA ACAGCACTAC TAATAGTATC AGGCATCTTT CCATACTCCA TACCCGCAAC ACTGTTGGTC TGGCATACTT GGCAAAAGCA AACCCAAAGA	1	1

D3NS3	Dengue3	4514 to 6370	TCCGGCGTCCTATGGGACGTACCCAGCCCC CCAGAGACACAGAAAGCGGAACTGGAAGAA GGGGTCTATAGGATCAAACAGCAAGGAATT TTTGGGAAAACCCAAGTGGGGGTTGGAGTA CAGAAAGAAGGAGTTTTCCACACCATGTGG CACGTCACAAGAGGGGCAGTGTTGACACAC AATGGGAAAAGACTGGAACCAAACTGGGCT AGCGTGAAAAAAGATCTGATCTCATACGGA GGAGGATGGAGATTGAGTGCACAATGGCAA AAGGGGGAGGAGGTGCAGGTTATTGCCGTA GAGCCTGGGAAGAACCCAAAGAACTTTCAA ACCATGCCAGGCATTTTTCAGACAACAACAG GGGAAATAGGAGCAATTGCACTGGATTTCA AGCCTGGAACTTCAGGATCTCCCATCATAAA CAGAGAGGGAAAGGTAGTGGGACTGTATGG CAATGGAGTGGTTACAAAGAATGGAGGCTA TGTYAGTGGAATAGCGCAAACAAATGCAGA ACCAGATGGACCGACACCAGAGTTGGAAGA AGAGATGTTCAAAAAGCGAAATCTAACCATA ATGGATCTTCATCCTGGGTCAGGAAAGACG CGGAAATATCTTCCAGCTATTGTTAGAGAGG CAATCAAGAGACGCTTAAGGACTCTAATTTT GGCACCAACAAGGGTAGTTGCAGCTGAGAT GGAAGAAGCATTGAAAGGGCTCCCAATAAG GTATCAAACAACTGCAACAAAATCTGAACAC ACAGGAAGAGAGATTGTTGATCTAATGTGTC ACGCAACGTTCACAATGCGCTTGCTGTCAC CAGTCAGGGTTCCAAACTACAACTTGATAAT AATGGATGAGGCTCATTTCACAGACCCAGC CAGTATAGCGGCTAGAGGGTACATATCAAC TCGTGTAGGAATGGGAGAGGCAGCCGCAAT TTTCATGACAGCAACACCCCCTGGAACAGC TGATGCCTTTCCTCAGAGCAACGCTCCAATT CAAGATGAAGAGAGAGACATACCGGAACGC TCATGGAATTCAGGCAATGAATGGATTACTG ACTTTGTTGGGAAGACAGTGTGGTTTGTCCC TAGCATCAAAGCCGGAAATGACATAGCAAA CTGCTTGCGGAAAAATGGAAAAAAGGTCATT CAACTCAGCAGGAAGACCTTTGACACAGAA TATCAAAAGACCAAACTGAATGATTGGGACT TTGTGGTGACAACAGACATTTCAGAAATGGG AGCCAATTTCAAAGCAGATAGAGTGATCGAC CCAAGAAGATGTCTCAAGCCGGTGATTTTGA CAAATGGACCCGAGCGGGTGATCCTGGCTG GACCAATGCCAGTCACCGTAGCGAGCGCTG CGCAAAGGAGAGGGAGAGTTGGCAGGAAC CCACAAAAAGAAAATGACCAGTACATATTCA TGGGCCAGCCTCTCAACAATGATGAAGACC ATGCTCACTGGACAGAAGCAAAAATGCTGC TGGACAACATCAACACACCAGAAGGGATTAT ACCAGCTCTCTTTGAACCAGAAAGGGAGAA GTCAGCCGCCATAGACGGCGAATACCGCCT GAAGGGTGAGTCCAGGAAGACTTTCGTGGA ACTCATGAGGAGGGGTGACCTCCCAGTTTG GCTAGCCCATAAAGTAGCATCAGAAGGGAT CAAATATACAGATAGAAAATGGTGCTTTGAT	0.99	1

			GGAGAACGTAATAATCAAATTTTAGAGGAGA ATATGGATGTGGAAATCTGGACAAAGGAAG GAGAAAAGAAAAAACTGAGACCTAGGTGGC TTGATGCCCGCACTTATTCAGATCCTTTAGC ACTCAAGGAATTCAAGGATTTTGCAGCTGGC AGAAAG		

D3NS3d	Dengue3	4997 to 6370	GT TAGTGGAATA GCGCAAACAA ATGCAGAACC AGATGGACCG ACACCAGAGT TGGAAGAAGA GATGTTCAAA AAGCGAAATC TAACCATAAT GGATCTTCAT CCTGGGTCAG GAAAGACGCG GAAATATCTT CCAGCTATTG TTAGAGAGGC AATCAAGAGA CGCTTAAGGA CTCTAATTTT GGCACCAACA AGGGTAGTTG CAGCTGAGAT GGAAGAAGCA TTGAAAGGGC TCCCAATAAG GTATCAAACA ACTGCAACAA AATCTGAACA CACAGGAAGA GAGATTGTTG ATCTAATGTG TCACGCAACG TTCACAATGC GCTTGCTGTC ACCAGTCAGG GTTCCAAACT ACAACTTGAT AATAATGGAT GAGGCTCATT TCACAGACCC AGCCAGTATA GCGGCTAGAG GGTACATATC AACTCGTGTA GGAATGGGAG AGGCAGCCGC AATTTTCATG ACAGCAACAC CCCCTGGAAC AGCTGATGCC TTTCCTCAGA GCAACGCTCC AATTCAAGAT GAAGAGAGAG ACATACCGGA ACGCTCATGG AATTCAGGCA ATGAATGGAT TACTGACTTT GTTGGGAAGA CAGTGTGGTT TGTCCCTAGC ATCAAAGCCG GAAATGACAT AGCAAACTGC TTGCGGAAAA ATGGAAAAAA GGTCATTCAACT CAGCAGGAAG ACCTTTGACA CAGAATATCA AAAGACCAAA CTGAATGATT GGGACTTTGT GGTGACAACA GACATTTCAGAAATGGGAGCCAATTTCAAAG CAGATAGAGTGATCGACCCAAGAAGATGTC TCAAGCCGGTGATTTTGACAAATGGACCCG AGCGGGTGATCCTGGCTGGACCAATGCCAG TCACCGTAGCGAGCGCTGCGCAAAGGAGA GGGAGAGTTGGCAGGAACCCACAAAAAGAA AATGACCAGTACATATTCATGGGCCAGCCTC TCAACAATGATGAAGACCATGCTCACTGGAC AGAAGCAAAAATGCTGCTGGACAACATCAA CACACCAGAAGGGATTATACCAGCTCTCTTT GAACCAGAAAGGGAGAAGTCAGCCGCCATA GACGGCGAATACCGCCTGAAGGGTGAGTCC AGGAAGACTTTCGTGGAACTCATGAGGAGG GGTGACCTCCCAGTTTGGCTAGCCCATAAA GTAGCATCAGAAGGGATCAAATATACAGATA GAAAATGGTGCTTTGATGGAGAACGTAATAA TCAAATTTTAGAGGAGAATATGGATGTGGAA ATCTGGACAAAGGAAGGAGAAAAGAAAAAA CTGAGACCTAGGTGGCTTGATGCCCGCACT TATTCAGATCCTTTAGCACTCAAGGAATTCA AGGATTTTGCAGCTGGCAGAAAG	0.99	1

D3NS4A	Dengue3	6371 to 6751	TCAATCGCCCTTGATCTTGTGACAGAAATAGGAAGAGTGCCTTCACACTTAGCCCACAGAACGAGAAACGCCCTGGACAATTTGGTGATGCTGCACACGTCAGAACATGGCGGTAGGGCCTACAGGCATGCAGTGGAGGAACTACCAGAAACGATGGAAACACTCTTACTCCTGGGACTGATGATCTTGTTAACAGGTGGAGCAATGCTCTTCTTGATATCAGGTAAAGGGATTGGAAAGACTTCAATAGGACTCATTTGTGTAATTGCTTCCAGCGGCATGTTATGGATGGCTGATGTCCCACTCCAATGGATCGCGTCGGCTATAGTCCTGGAGTTTTTTATGATGGTGTTGCTCATACCAGAACCAGAAAAGCAGAGA	1	1
D3NS4B	Dengue3	6821 to 7564	CAATGAAATGGGACTGTTGGAAACTACAAAGAGAGATTTAGGAATGTCTAAAGAACCAGGTGTTGTTTCTTCAACCAGCTATTTGGACGTGGACTTGCACCCAGCATCAGCCTGGACATTGTACGCCGTGGCCACAACAGTAATAACACCAATGTTGAGACACACCATAGAGAATTCCACAGCAAATGTGTCCCTGGCAGCCATAGCTAACCAGGCAGTGGTCCTGATGGGTTTAGACAAAGGATGGCCGATATCGAAAATGGACTTGGGCGTACCACTATTGGCACTGGGTTGCTATTCACAAGTGAACCCACTAACTCTTGCAGCGGCAGTACTTTTGCTAGTCACACATTATGCAATTATAGGTCCAGGATTGCAGGCAAAAGCCACTCGTGAAGCTCAGAAAAGGACAGCTGCTGGAATAATGAAGAATCCAACGGTGGATGGAATAATGACAATAGACCTAGATCCTGTAATATATGATTCAAAATTTGAAAAGCAACTAGGACAGGTTATGCTCCTGGTTCTGTGTGCAGTTCAACTTTTGTTAATGAGAACATCATGGGCCTTGTGTGAAGTTCTAACCCTAGCCACAGGACCAATAACAACACTCTGGGAAGGATCACCTGGGAAGTTCTGGAACACCACGATAGCTGTTTCCATGGCGAACATCTTTAGAGGGAGCTATTTAGCAGGAGCTGGGCTTGCTTTTTCTATCATGAAATCAGTTGGAACAGGAAAGAGA	0.99	0.99

D3NS5	Dengue3	7565 to 10264	GGAACAGGG TCACAAGGTG AAACCTTAGG AGAAAAGTGG AAAAAGAAAT TAAATCAGTT ATCCCGGAAA GAGTTTGACC TTTACAAGAA ATCCGGAATC ACCGAAGTGG ATAGAACAGA AGCCAAAGAA GGGTTAAAAA GAGGAGAAAT AACACACCAT GCCGTATCCA GAGGCAGCGC AAAACTTCAA TGGTTCGTGG AGAGAAACAT GGTCATTCCT GAAGGAAGAG TCATAGACTT AGGCTGTGGA AGAGGAGGCT GGTCATATTA CTGTGCAGGA CTGAAAAAAG TTACAGAAGT GCGAGGATAC ACAAAAGGCG GCCCAGGACA CGAAGAACCA GTACCTATGT CTACATACGG ATGGAACATA GTCAAGTTAA TGAGTGGAAA GGATGTTTTT TATCTGCCAC CTGAAAAGTG TGATACCCTA TTGTGTGACA TTGGAGAATC TTCACCAAGC CCAACAGTGG AAGAAAGCAG AACCATAAGA GTCTTGAAGA TGGTTGAACC ATGGCTAAAA AACAACCAGT TTTGCATTAA AGTATTGAAC CCATACATGC CAACTGTGAT TGAGCACTTA GAAAGACTAC AAAGGAAACA TGGAGGAATG CTTGTGAGAA ATCCACTCTC ACGAAACTCC ACGCACGAAA TGTATTGGAT ATCCAATGGTACAG GCAACATCGT CTCTTCAGTC AACATGGTAT CCAGATTGCT ACTGAACAGA TTCACAATGA CACACAGGAG ACCCACCATA GAGAAAGATG TGGATTTAGG AGCAGGAACC CGACATGTCA ATGCGGAACC AGAAACACCC AACATGGATG TCATTGGGGA AAGAATAAAA AGGATCAAAG AGGAGCATAG TTCAACATGG CACTATGATG ATGAAAATCC TTACAAAACG TGGGCTTACC ATGGATCCTA TGAAGTAAAA GCCACAGGCT CAGCCTCCTC CATGATAAAT GGAGTCGTGA AACTCCTCAC AAAACCATGG GATGTGGTGC CCATGGTGAC ACAGATGGCA ATGACAGATA CAACTCCATT TGGCCAGCAA AGAGTTTTTA AAGAGAAAGT GGACACCAGG ACACCTAGGC CCATGCCAGG AACAAGAAAG GTTATGGAGA TCACAGCGGA GTGGCTTTGG AGGACCCTGG GAAGGAACAA AAGACCCAGA TTATGCACAA GGGAGGAGTT CACAAAGAAG GTCAGAACCA ACGCAGCTAT GGGCGCTGTC TTCACAGAAG AGAACCAATG GGACAGTGCG AGAGCTGCTG TTGAGGACGA AGAATTTTGG AAACTTGTGG ACAGAGAACG TGAACTCCAC AAACTGGGCA AGTGTGGAAG CTGCGTTTAC AACATGATGG GCAAGAGAGA GAAAAAACTT GGAGAGTTTG GTAAAGCAAA	0.99	0.99

			AGGCAGTAGG GCTATATGGT ACATGTGGTT GGGAGCCAGG TACCTTGAGT TCGAGGCGCT CGGATTCCTC AATGAAGACC ACTGGTTCTC GCGTGAAAAC TCTTACAGTG GAGTAGAAGG AGAAGGACTG CACAAGCTGG GATACATCTT GAGAGATATT TCCAAGATAC CCGGAGGAGC CATGTATGCT GATGACACAG CCGGTTGGGA CACAAGAATA ACAGAAGATG ACCTGCACAA TGAGGAAAAA ATCACACAGC AGATGGACCC TGAACACAGG CAGCTAGCGA ACGCTATATT CAAGCTCACA TACCAAAACA AAGTAGTCAA AGTCCAACGA CCAACTCCAA AGGGCACGGT AATGGACATC ATATCTAGGA AAGACCAAAG AGGCAGTGGA CAGGTGGGAA CTTATGGTCT GAACACATTC ACCAACATGG AAGCCCAGCT AATCAGACAA ATGGAAGGAG AAGGCGTGTT GTCAAAGGCA GACCTCGAGA ACCCCCATCC GCTAGAGAAG AAAATTACAC AATGGTTGGA AACTAAAGGA GTGGAGAGGT TAAAAAGAAT GGCCATCAGC GGGGATGATT GCGTAGTGAA ACCAATCGAC GACAGATTCG CCAATGCCCT GCTTGCCC TGAACGATATGGGAAAGGTTAGGAAGGACA TACCTCAATGGCAGCCATCAAAGGGATGGC ATGATTGGCAACAGGTCCCTTTCTGCTCCCA CCACTTTCATGAATTGATCATGAAAGATGGA AGAAAGTTGGTAGTTCCCTGCAGACCCCAG GACGAACTAATAGGAAGAGCGAGAATCTCT CAAGGAGCAGGATGGAGCCTTAGAGAAACT GCATGTCTAGGGAAAGCCTACGCTCAAATG TGGACTCTCATGTATTTTCACAGAAGAGATC TTAGACTAGCATCCAACGCCATATGTTCAGC AGTACCAGTCCATTGGGTCCCCACGAGCAG AACGACATGGTCTATTCATGCTCACCATCAG TGGATGACTACAGAAGACATGCTTACTGTCT GGAACAGGGTGTGGATAGAGGACAATCCAT GGATGGAAGACAAAACTCCAGTCACAACAT GGGAAGATGTTCCATATCTAGGGAAGAGAG AAGACCAATGGTGCGGATCATTCATAGGTCT CACTTCCAGAGCAACCTGGGCCCAGAACAT ACTCACAGCAATCCAACAGGTGAGAAGCCT CATAGGCAATGAAGAGTTTCTGGACTACATG CCTTCGATGAAGAGATTCAGGAAGGAGGAG GAGTCAGAGGGAGCCATTTGG		

D4CA	Dengue4	94 to 432	ATGAACCAAC GAAAAAAGGT GGTTAGACCA CCTTTCAATA TGCTGAAACG CGAGAGAAAC CGCGTATCAA CCCCTCAAGG GTTGGTGAAG AGATTCTCAA CCGGACTTTT TTCCGGGAAA GGACCCTTAC GGATGGTGCT AGCATTCATC ACGTTTTTGC GAGTCCTTTC CATCCCACCA ACAGCAGGGA TTCTGAAAAG ATGGGGACAG TTGAAGAAAA ACAAGGCCAT CAAAATACTG ACTGGATTCA GGAAGGAGAT AGGCCGCATG CTGAACATCT TGAATGGAAG AAAAAGGTCA ACAATGACAT TGCTGTGCTT GATTCCCACC GCAATGGCG	1	1
D4CV	Dengue4	94 to 390	ATGAACCAAC GAAAAAAGGT GGTTAGACCA CCTTTCAATA TGCTGAAACG CGAGAGAAAC CGCGTATCAA CCCCTCAAGG GTTGGTGAAG AGATTCTCAA CCGGACTTTT TTCCGGGAAA GGACCCTTAC GGATGGTGCT AGCATTCATC ACGTTTTTGC GAGTCCTTTC CATCCCACCA ACAGCAGGGA TTCTGAAAAG ATGGGGACAG TTGAAGAAAA ACAAGGCCAT CAAAATACTG ACTGGATTCA GGAAGGAGAT AGGCCGCATG CTGAACATCT TGAATGGAAG AAAAAGG	1	1
D4PrM	Dengue4	433 to 930	TTTCACTTGT CAACAAGAGA TGGCGAACCC CTTATGATAG TGGCAAAACA CGAAAGGGGG AGACCTCTCT TGTTTAAGAC AACAGAGGGA ATCAACAAAT GCACTCTTAC TGCCATGGAC CTGGGTGAAA TGTGTGAGGA CACCGTCACG TATGAATGCC CTCTACTGGT CAATACCGAA CCTGAGGACA TTGATTGCTG GTGCAATCTC ACGTCTGCCT GGGTCATGTA TGGGACATGC ACTCAGAGTG GGGAACGGAG ACGGGAGAAG CGCTCAGTAG CCCTAACACC ACATTCAGGA ATGGGATTGG AGACAAGGGC TGAGACATGG ATGTCATCGG AAGGGGCTTG GAAACATGCT CAGAGGGTAG AGAGTTGGAT ACTCAGAAAC CCAGGATTCG CTCTCTTGGC AGGATTTATG GCCTATATGA TTGGGCAAAC AGGAATCCAG CGAACAGTCT TCTTTGTTCT AATGATGCTG GTCGCCCCAT CCTACGGA	1	1
D4M	Dengue4	706 to 930	TCAGTAGCCCTAACACCACATTCAGGAATG GGATTGGAGACAAGGGCTGAGACATGGATG TCATCGGAAGGGGCTTGGAAACATGCTCAG AGGGTAGAGAGTTGGATACTCAGAAACCCA GGATTCGCTCTCTTGGCAGGATTTATGGCCT ATATGATTGGGCAAACAGGAATCCAGCGAA CAGTCTTCTTTGTTCTAATGATGCTGGTCGC CCCATCCTACGGA	1	1

| D4E | Deng ue4 | 931 to 2415 | ATGCGATGC GTGGGAGTGG GGAACAGAGA CTTTGTGGAA GGAGTCTCAG GTGGAGCATG GGTCGATTTG GTGCTAGAAC ATGGAGGATG TGTCACAACC ATGGCCCAGG GAAAACCAAC CTTGGATTTT GAACTGATCA AGACAACAGC CAAGGAAGTG GCTCTGTTAA GAACCTATTG CATTGAAGCC TCGATATCAA ACATAACCAC GGCAACAAGA TGTCCAACGC AAGGAGAACC TTATCTCAAA GAGGAACAAG ATCAACAGTA CATTTGCCGG AGAGATGTGG TAGACAGAGG GTGGGGCAAT GGCTGTGGCT TGTTTGGGAA AGGAGGAGTT GTGACATGTG CGAAGTTTTC ATGCTCGGGG AAGATAACAG GCAATTTGGT CCAAATTGAG AACCTTGAAT ACACAGTAGT TGTAACAGTC CACAATGGAG ACACCCATGC AGTAGGAAAT GACATACCCA ACCATGGAGT GACAGCCACG ATAACCCCCA GGTCACCATC GGTAGAAGTT AAATTACCGG ATTATGGAGA ATTAACACTC GATTGTGAAC CCAGGTCCGG AATTGATTTT AATGAGATGA TTCTGATGAA AATGAAAAAG AAAACGTGGC TTGTGCACAA GCAATGGTTT TTGGATCTAC CTCTACCATG GGCAGCAGGAGCAGACA CATCAGAAGT TCATTGGAAT TACAAAGAGA GAATGGTGAC ATTCAAGGTT CCTCATGCCA AGAGACAGGA TGTGACAGTG CTAGGATCTC AGGAAGGAGC CATGCATTCT GCCCTCACCG GAGCTACAGAAGTGGATTCCGGTGATGGAA ACCACATGTTTGCAGGACATCTGAAATGCAA AGTTCGCATGGAGAAATTGAGAATTAAGGG AATGTCATACACGATGTGCTCAGGAAAGTTC TCAATTGACAAAGAGATGGCAGAAACACAG CATGGGACAACAGTGGTAAAAGTCAAGTAT GAGGGTGCTGGAGCTCCATGTAAAGTTCCC ATAGAGATAAGAGATGTGAACAAGGAAAAA GTGGTAGGGCGCATCATCTCATCTACCCCT TTTGCTGAGTATACCAACAGTGTAACCAACA TAGAATTAGAACCCCCCTTTGGGGACAGCT ACATAGTAATAGGTGTTGGAGACAGTGCATT AACACTCCATTGGTTCAGGAAAGGGAGTTC CATTGGCAAGATGCTTGAGTCCACATACAGA GGCGCAAAGCGAATGGCCATTCTAGGTGAA ACAGCCTGGGATTTTGGTTCTGTTGGTGGA CTGTTCACATCATTGGGAAAGGCTGTACACC AGGTTTTTGGTAGTGTGTATACAACTATGTT TGGAGGAGTCTCATGGATGGTTAGAATCCT AATTGGGTTCTTAGTGTTGTGGATTGGCACG AATTCGAGAAACACCTCAATGGCAATGACGT GCATAGCTGTTGGAGGAATCACTCTGTTTCT GGGTTTCACAGTTCACGCA | 0.99 | 0.99 |

D4Eiii	Dengue4	1813 to 2112	AAGGGAATGTCATACACGATGTGCTCAGGAAAGTTCTCAATTGACAAAGAGATGGCAGAAACACAGCATGGGACAACAGTGGTAAAAGTCAAGTATGAGGGTGCTGGAGCTCCATGTAAAGTTCCCATAGAGATAAGAGATGTGAACAAGGAAAAAGTGGTAGGGCGCATCATCTCATCTACCCCTTTTGCTGAGTATACCAACAGTGTAACCAACATAGAATTAGAACCCCCCTTTGGGGACAGCTACATAGTAATAGGTGTTGGAGACAGTGCATTAACACTCCATTGGTTCAGGAAA	1	1
D4NS1	Dengue4	2416 to 3471	GACACGGGTTGTGCGGTGTCATGGAGTGGGAAAGAATTGAAATGTGGAAGCGGAATCTTTGTAATTGACAACGTGCACACTTGGACAGAACAGTACAAATTTCAACCAGAGTCTCCAGCGAGACTAGCGTCCGCAATATTGAATGCCCACAAAGATGGGGTCTGTGGAATTAGATCAACCACGAGGCTGGAAAACATCATGTGGAAGCAAATAACCAACGAGTTGAACTATGTTCTCTGGGAAGGAGGACATGACCTCACTGTAGTGGCTGGGGATGTGAAAGGGGTGCTGTCCAAAGGCAAGAGAGCACTCGCACCCCCAGTGAATGATCTGAAATATTCATGGAAGACATGGGGAAAAGCAAAGATCTTTACTCCAGAAGCAAAAAATAGCACATTTCTAATAGACGGACCAGACACCTCCGAATGCCCCAATGAACGAAGAGCATGGAATTTTCTTGAGGTAGAAGACTATGGATTTGGCATGTTTACGACCAACATATGGATGAAATTTCGAGAAGGAAGTTCAGAAGTGTGTGACCACAGGTTGATGTCGGCGGCAATCAAAGACCAGAAAGCTGTGCATGCTGACATGGGCTATTGGATAGAGAGCTCAAAAAACCAGACCTGGCAGATAGAGAAAGCATCTCTCATTGAAGTGAAAACATGTCTGTGGCCCAAGACCCACACATTGTGGAGCAATGGAGTGCTAGAGAGCCAGATGCTCATCCCAAAAGCATATGCAGGCCCTTTTTCACAGCACAATTACCGCCAGGGCTATGCCACGCAGACCGTGGGCCCATGGCACTTGGGCAAATTGGAGATAGACTTTGGAGAATGCCCCGGAACAACAGTCACTATTCAAGAGGATTGTGACCATAGAGGCCCATCTTTGAGGACCACTACTGCATCTGGAAAATTGGTCACGCAGTGGTGCTGCCGCTCCTGCACGATGCCTCCCTTAAGGTTTTTGGGAGAGGATGGATGCTGGTATGGGATGGAAATTAGGCCCTTGAGTGAAAAAGAAGAGAACATGGTCAAATCACAGGTATCGGCC	1	1

D4N S2A	Deng ue4	3472 to 4125	GGACAGGGTACATCAGAAACTTTTTCTATGG GGCTGTTATGCCTGACTTTGTTTGTGGAAGA ATGCTTGAGGAGAAGAGTCACCAGGAAACA CATGATATTGGTTGTGGTGACCACCCTTTGT GCCATCATCCTAGGAGGTCTCACATGGATG GACTTACTACGAGCTCTTATCATGTTAGGGG ACACCATGTCTGGTAGAATGGGAGGACAGA TTCACTTAGCCATCATGGCAGTGTTCAAGAT GTCACCAGGATACGTGCTGGGTATATTTTTA AGGAAACTCACTTCAAGAGAGACAGCACTA ATGGTGATAGGAATGGCCATGACAACGGTG CTTTCAATTCCACATGACCTTATGGAATTCAT TGATGGAATATCACTGGGGTTAATCTTATTA AAAATGGTAACACATTTTGACAACACTCAAG TGGGAACCTTAGCTCTTTCCTTGACTTTCAT AAGATCAACAATGCCATTGGTCATGGCTTGG AGGACCATAATGGCTGTGTTGTTTGTGGTCA CACTCATTCCTTTATGCAGGACAAGCTGTCT TCAAAAGCAGTCACATTGGGTAGAAATAACA GCACTCATCCTGGGAGCCCAGGCTCTGCCA GTGTACCTAATGACTCTCATGAAAGGAGCTT CAAAGAGA	1	1
D4N S2B	Deng ue4	4126 to 4515	TCTTGGCCCC TTAACGAGGG TATAATGGCT GTGGGTTTGG TCAGTCTCTT GGGAAGCGCC CTCCTAAAGA ATGATGTCCC TTTAGCTGGC CCAATGGTGG CAGGAGGCTT ACTTCTGGCA GCCTATGTGA TGAGTGGTAG CTCAGCAGAC CTGTCACTAG AGAAGGCCGC CAATGTGCAG TGGGATGAGA TGGCAGACAT AACAGGCTCA AGCCCAATCA TAGAAGTGAA GCAGGATGAA GATGGCTCTT TCTCCATACG GGACATCGAG GAAACCAATA TGATAACCCT CTTAGTGAAA CTGGCACTGA TAACAGTGTC AGGTCTCTAC CCCTTGGCAA TTCCAGTCAC AATGACCCTA TGGTACATGT GGCAAGTGAA AACACAAAGA	1	1

D4N S3	Deng ue4	4516 to 6369	CCGGCGTCCTATGGGACGTACCCAGCCCCC CAGAGACACAGAAAGCGGAACTGGAAGAAG GGGTCTATAGGATCAAACAGCAAGGAATTTT TGGGAAAACCCAAGTGGGGGTTGGAGTACA GAAAGAAGGAGTTTTCCACACCATGTGGCA CGTCACAAGAGGGGCAGTGTTGACACACAA TGGGAAAAGACTGGAACCAAACTGGGCTAG CGTGAAAAAAGATCTGATCTCATACGGAGG AGGATGGAGATTGAGTGCACAATGGCAAAA GGGGGAGGAGGTGCAGGTTATTGCCGTAG AGCCTGGGAAGAACCCAAAGAACTTTCAAA CCATGCCAGGCATTTTTCAGACAACAACAG GGGAAATAGGAGCAATTGCACTGGATTTCA AGCCTGGAACTTCAGGATCTCCCATCATAAA CAGAGAGGGAAAGGTAGTGGGACTGTATGG CAATGGAGTGGTTACAAAGAATGGAGGCTA TGTYAGTGGAATAGCGCAAACAAATGCAGA ACCAGATGGACCGACACCAGAGTTGGAAGA AGAGATGTTCAAAAAGCGAAATCTAACCATA ATGGATCTTCATCCTGGGTCAGGAAAGACG CGGAAATATCTTCCAGCTATTGTTAGAGAGG CAATCAAGAGACGCTTAAGGACTCTAATTTT GGCACCAACAAGGGTAGTTGCAGCTGAGAT GGAAGAAGCATTGAAAGGGCTCCCAATAAG GTATCAAACAACTGCAACAAAATCTGAACAC ACAGGAAGAGAGATTGTTGATCTAATGTGTC ACGCAACGTTCACAATGCGCTTGCTGTCAC CAGTCAGGGTTCCAAACTACAACTTGATAAT AATGGATGAGGCTCATTTCACAGACCCAGC CAGTATAGCGGCTAGAGGGTACATATCAAC TCGTGTAGGAATGGGAGAGGCAGCCGCAAT TTTCATGACAGCAACACCCCCTGGAACAGC TGATGCCTTTCCTCAGAGCAACGCTCCAATT CAAGATGAAGAGAGAGACATACCGGAACGC TCATGGAATTCAGGCAATGAATGGATTACTG ACTTTGTTGGGAAGACAGTGTGGTTTGTCCC TAGCATCAAAGCCGGAAATGACATAGCAAA CTGCTTGCGGAAAAATGGAAAAAAGGTCATT CAACTCAGCAGGAAGACCTTTGACACAGAA TATCAAAAGACCAAACTGAATGATTGGGACT TTGTGGTGACAACAGACATTTCAGAAATGGG AGCCAATTTCAAAGCAGATAGAGTGATCGAC CCAAGAAGATGTCTCAAGCCGGTGATTTTGA CAAATGGACCCGAGCGGGTGATCCTGGCTG GACCAATGCCAGTCACCGTAGCGAGCGCTG CGCAAAGGAGAGGGAGAGTTGGCAGGAAC CCACAAAAAGAAAATGACCAGTACATATTCA TGGGCCAGCCTCTCAACAATGATGAAGACC ATGCTCACTGGACAGAAGCAAAAATGCTGC TGGACAACATCAACACACCAGAAGGGATTAT ACCAGCTCTCTTTGAACCAGAAAGGGAGAA GTCAGCCGCCATAGACGGCGAATACCGCCT GAAGGGTGAGTCCAGGAAGACTTTCGTGGA ACTCATGAGGAGGGGTGACCTCCCAGTTTG GCTAGCCCATAAAGTAGCATCAGAAGGGAT CAAATATACAGATAGAAAATGGTGCTTTGAT	0.99	1

			GGAGAACGTAATAATCAAATTTTAGAGGAGA ATATGGATGTGGAAATCTGGACAAAGGAAG GAGAAAAGAAAAAACTGAGACCTAGGTGGC TTGATGCCCGCACTTATTCAGATCCTTTAGC ACTCAAGGAATTCAAGGATTTTGCAGCTGGC AGAAAG		

D4N S3d	Deng ue4	4993 to 6369	GATTACGTCA GTGCTATAAC GCAAGCCGAA AGAACTGGTG AGCCAGATTA TGAAGTGGAT GATGACATTT TTCGAAAGAA AAGATTAACT ATAATGGACT TGCACCCCGG AGCCGGAAAG ACAAAAAGAA TTCTCCCATC AATAGTCAGA GAAGCCTTAA AAAGGAGGCT GCGAACCTTG ATTTTGGCTC CCACGAGAGT GGTGGCGGCC GAGATGGAAG AGGCCCTACG TGGACTGCCA ATCCGTTATC AGACCCCAGC TGTGAAATCA GAACACACAG GAAGAGAGAT CGTAGACCTC ATGTGTCATG CAACCTTCAC AACAAGACTT TTATCATCAA CCAGGGTTCC AAATTACAAC CTCATAGTGA TGGATGAAGC ACATTTCACA GACCCTTCTA GTGTCGCGGC TAGAGGATAC ATCTCAACCA GGGTGGAAAT GGGAGAGGCA GCAGCCATCT TTATGACTGC AACCCCTCCT GGAGCGACAG ATCCCTTCCC CCAGAGCAAC AGCCCAATAG AAGACATCGA GAGAGAAATT CCAGAAAGGT CATGGAACAC AGGGTTCGAC TGGATAACCG ACTACCAAGG GAAAACTGTG TGGTTTGTTC CCAGCATAAA AGCTGGAAAT GACATTGCAA ATTGCTTGAG AAAGTCGGGA AAGAAGGTGA TCCAATTGAG TAGAAAAACC TTTGACACAG AGTATCCAAA AACGAAACTT ACGGACTGGG ATTTTGTGGT TACCACAGAC ATATCAGAAA TGGGGGCCAA TTTTAGAGCT GGGAGAGTGA TAGACCCCAG GAGATGCCTC AAGCCAGTTA TCTCAACTGA CGGGCCAGAG AGAGTTATTT TGGCAGGTCC CATTCCAGTG ACTCCAGCAA GCGCTGCTCA GAGAAGAGGG CGAATAGGTA GGAACCCAGC ACAAGAAGAT GACCAATATG TCTTCTCCGG AGACCCACTA AAAAATGATG AAGATCATGC CCACTGGACA GAAGCAAAGA TGCTGCTTGA TAATATCTAC ACCCCGGAAG GGATCATTCC AACATTGTTT GGTCCGGAAA GAGAAAAAAA TCAAGCCATT GATGGAGAGT TCCGCCTCAG AGGGGAACAA AGGAAGACTT TTGTAGAATT AATGAGGAGA GGAGACCTTC CGGTGTGGCT GAGCTACAAG GTAGCTTCTG CTGGTATCTC TTACAAAGAC CGGGAATGGT GCTTCACAGG GGAAAGGAAT AACCAAATTC TAGAAGAAAA CATGGAGGTT GAAATTTGGA CTAGAGAGGG AGAGAAGAAA AAACTCAGGC CAAAATGGTT AGATGCACGT GTTTACGCTG ACCCCATGGC TTTGAAGGAT TTCAAGGAGT TTGCCAGTGG AAGAAAG	0.99	1

D4N S4A	Deng ue4	6370 to 6819	AGCATAACCCTCGACATCCTAACAGAGATTG CCAGTTTGCCAACTTACCTTTCCTCTAGGGC TAAGCTCGCCCTTGACAACATAGTCATGCTC CACACAACAGAAAGAGGAGGGAAGGCCTAC CAACATGCCCTGAACGAACTCCCGGAGTCA CTAGAAACACTCATGCTTGTAGCTTTACTGG GTGCTATGACAGCAGGCATCTTCTTGTTTTT CATGCAAGGAAAAGGAATAGGGAAACTGTC AATGGGTTTGATAGCCATTGCGGTAGCTAGT GGCTTGCTCTGGGTAGCAGAAATCCAGCCC CAGTGGATAGCGGCCTCAATCATACTAGAG TTCTTTCTCATGGTGTTGTTGATACCAGAAC CAGAAAAACAAAGGACCCCACAAGACAATC AATTGATCTACGTCATATTGACCATTCTCAC CATTATTGGTCTCATAGCAGCC	1	1
D4N S4B	Deng ue4	6820 to 7554	AACGAGATGG GGCTGATTGA AAAAACAAAA ACGGATTTTG GGTTTTACCA GGTAAAAACA GTAACCACCA TCCTCGATGT GGATTTGAGA CCAGCCTCAG CATGGACGCT CTATGCAGTA GCCACCACTA TTCTGACTCC CATGCTGAGA CACACCATAG AAAACACGTC TGCAAACCTA TCTCTAGCGG CCATTGCTAA CCAAGCAGCT GTCCTAATGG GGCTTGGAAA AGGATGGCCG CTCCACAGAA TGGACCTCGG TGTGCCGCTG TTGGCAATGG GATGCTATTC TCAAGTGAAC CCAACGACCT TGACAGCATC CTTAGTCATG CTTTTAGTCC ATTACGCAAT AATAGGTCCA GGACTGCAGG CAAAAGCCAC AAGAGAGGCT CAGAAAAGGA CAGCAGCTGG GATCATGAAG AACCCCACTG TGGACGGGAT AACAGTAATA GATCTAGAAC CAATATCCTA TGACCCAAAA TTTGAAAAGC AATTAGGGCA AGTCATGCTA CTAGTCTTGT GTGCTGGACA GCTACTCTTG ATGAGAACAA CATGGGCTTT CTGTGAAGTC TTGACTTTGG CCACAGGACC AGTCTTGACC CTGTGGGAGG GCAACCCGGG AAGGTTTTGG AACACGACTA TAGCCGTGTC CACTGCCAAT ATTTTCAGGG GAAGCTACTT GGCGGGAGCT GGACTGGCCT TTTCGCTCAT AAAGAATGCA CAAACCCCCA GGAGG	0.99	0.99

D4N S5	Deng ue4	7555 to 10254	GGAACTGG GACCACAGGA GAGACACTGG GAGAGAAGTG GAAGAGACAG CTAAACTCAC TAGATAGGAA GGAGTTTGAA GAGTACAAAA GAAGTGGAAT ACTAGAAGTG GACAGGACTG AAGCCAAGTC TGCCTTGAAA GATGGATCTA AAATCAAGCA TGCAGTGTCC AGAGGGTCCA GCAAGATTAG ATGGATTGTT GAAAGAGGGA TGGTAAAACC AAAAGGGAAA GTTGTGGATC TTGGTTGCGG GAGAGGAGGA TGGTCGTACT ACATGGCGAC ACTCAAGAAC GTGACCGAGG TGAAAGGGTA CACAAAAGGA GGTCCAGGAC ATGAAGAACC GATTCCCATG GCTACCTATG GCTGGAACTT GGTCAAACTC CATTCAGGGG TTGACGTGTT CTACAAACCC ACTGAGCAAG TGGATACCCT GCTCTGTGAT ATTGGGGAGT CATCTTCTAA TCCGACGATA GAGGAAGGAA GAACATTAAG AGTTTTGAAG ATGGTGGAAC CATGGCTCTC TTCAAAACCT GAATTCTGCA TCAAAGTCCT TAATCCCTAC ATGCCAACAG TCATAGAAGA GCTGGAGAAA CTGCAGAGAA AACATGGTGG AAGTCTTGTC AGATGCCCGC TATCTAGGAA TTCCACTCAC GAGATGTATT GGGTGTCAGG TGTGTCGGGA AACATCGTGA GCTCTGTAAA CACAACATCA AAGATGTTGT TGAACAGATT TACCAC AAGGCATAGA AAACCCACTT ATGAGAAGGA CGTAGACCTT GGAGCAGGAA CGAGAAGTGT CTCCACTGAA ACAGAAAAAC CGGACATGAC AATCATTGGG AGAAGGCTTC AGCGACTGCA AGAAGAGCAC AAAGAAACTT GGCACTATGA TCAGGAAAAC CCATACAGAA CCTGGGCGTA TCATGGAAGC TATGAAGCTC CTTCGACAGG CTCAGCATCC TCCATGGTGA ACGGGGTAGT AAAATTGCTG ACAAAACCCT GGGACGTGAT TCCAATGGTG ACCCAGTTGG CTATGACAGA CACAACCCCT TTTGGGCAAC AGAGAGTGTT TAAAGAGAAG GTGGACACCA GAACACCACA ACCAAAACCC GGCACACGAA TGGTTATGAC CACGACAGCC AACTGGCTGT GGGCTCTCCT CGGGAAGAAG AAAAATCCCA GACTGTGCAC AAGGGAAGAG TTCATCTCAA AAGTTAGGTC AAACGCAGCC ATAGGCGCAG TCTTTCAGGA AGAACAGGGA TGGACATCAG CCAGTGAAGC TGTGAATGAC AGCCGATTTT GGGAACTGGT TGACAAAGAA AGGGCTCTGC ACCAGGAAGG GAAATGTGAA TCGTGTGTCT ACAACATGAT GGGAAAACGT GAGAAAAAGT	1	1

			TAGGAGAGTT TGGTAGAGCC AAGGGAAGCC GAGCAATCTG GTACATGTGG CTGGGAGCGC GGTTTCTGGA ATTTGAAGCC CTGGGTTTTT TGAATGAAGA TCACTGGTTT GGCAGAGAAA ACTCATGGAG TGGAGTGGAA GGGGAAGGTC TGCATAGATT GGGATATATC CTGGAGGACA TAGACAAGAA GGATGGAGAC CTGATATATG CTGATGACAC AGCTGGTTGG GACACAAGAA TCACTGAAGA TGACCTTCTA AATGAAGAAC TGATCACGGA ACAGATGGCC CCTCACCATA AGATCCTAGC CAAAGCCATT TTCAAACTAA CTTATCAAAA CAAAGTGGTG AAAGTCCTCA GACCCACACC GAAAGGAGCG GTGATGGATA TCATATCCAG GAAAGACCAA AGAGGTAGTG GACAGGTTGG AACATATGGT TTGAACACAT TCACCAACAT GGAAGTACAA CTCATCCGCC AAATGGAAGC TGAAGGAGTC ATCACACAAG ATGACATGCA TAACCCAAAA GGGTTGAAAG AAAGAGTTGA GAAATGGCTG AAAGAGTGTG GTGTCGACAG GTTAAAGAGG ATGGCAATCA GTGGAGACGA TTGTGTGGTG AAGCCTCTGG ATGAGAGGTT CAGCACTTCC CTCCTCTTCTTGAACGACATGGGAAAGGTG AGGAAAGACATTCCGCAGTGGGAACCATCT AAGGGATGGAAAAACTGGCAAGAGGTTCCT TTTTGCTCCCACCACTTTCACAAGATCTTCA TGAAGGATGGCCGCTCACTAGTTGTTCCAT GTAGAAACCAGGATGAACTGATAGGGAGAG CCAGAATCTCGCAAGGGGCTGGATGGAGTT TAAGAGAAACAGCCTGCCTGGGCAAAGCTT ACGCCCAGATGTGGTCGCTCATGTACTTTCA TAGAAGGGACCTGCGTTTAGCCTCCATGGC GATATGCTCAGCAGTTCCAACAGAATGGTTT CCAACAAGCAGAACAACATGGTCAATCCAC GCCCATCATCAGTGGATGACCACTGAAGAT ATGCTCAAAGTGTGGAACAGAGTGTGGATA GAAGACAACCCTAATATGACTGACAAGACTC CAGTTCATTCGTGGGAAGACATACCTTACCT AGGAAAAAGAGAAGATTTGTGGTGTGGATC CTTGATTGGACTTTCTTCCAGGGCCACCTG GGCGAAGAACATTCACACAGCCATAACCCA GGTCAGGAACCTGATCGGGAAAGAGGAGTA TGTGGATTACATGCCAGTCATGAAAAGATAC AGCGCTCCTTTCGAGAGTGAAGGAGTTCTG		

APPENDIX E. PYTHON SCRIPT OF ‘blast_all.py’

```
#!/usr/bin/env python


# Biopython is required for running this program

# Import modules


import os

import re

from datetime import date

from Bio.Blast import NCBIStandalone

from Bio.Blast import NCBIXML


#-----------------------------------------------------------------------------
-----

# Define the locations of databases, files and the blast program.


# 'blastall' is a generic BLAST algorithm in NCBI standalone BLAST.

blastall = "/Applications/blast/blast-2.2.17/bin/blastall"


# The default folder for adding *.seq files.

ori_folder = "/Users/hzhang/raw_sequences/"


# ref_seq were downloaded from NCBI on 9/1/09.

refseq_rna= "/Applications/blast/blastdbs/refseq_rna"

refseq_pro= "/Applications/blast/blastdbs/refseq_protein"


# A. aegypti transcripts and peptides databases were downlod from
VectorBase.orgon 9/1/09.
```

```
ae_rna = "/Applications/blast/blastdbs/AeAe_transcript"
ae_pro = "/Applications/blast/blastdbs/AeAe_peptide"

# Vector sequences were taken from Jinkai's program.
vector_seq = "/Applications/blast/blastdbs/Yeast_Vector.seq"
BD_vector_seq="/Applications/blast/blastdbs/BD_vector.seq"

# *.seq files that do not passed the pattern test will not be BLASTed and
will be listed in this file.
fail_file = "/Users/hzhang/" + str(date.today()) + "_fail_sequences.txt"
out_fail_file = open(fail_file, 'w')
#--------------------------------------------------------------------------
------

# Ask for the location of *.seq files.
def menu_input():
      global ori_folder
      print 'Batch BLAST program is about to start'
      print 'Where are your *.seq files? If they are in the
\"raw_sequence\" folder,'
      choice = raw_input('press ENTER. Otherwise, enter a path to your
folder.\n:')
      if len(choice) == 0:
            return ori_folder
      else:
            return choice
#--------------------------------------------------------------------------
------
```

```
# Ask for the file in which the result will be listed.
def menu_output():
	print 'Where will your result file be?'
	print 'If you want to have a file at the default destination, press
ENTER.'
	choice = raw_input('Otherwise enter a path and a file name for your
result.\n:')
	if len(choice) == 0:
		return     "/Users/hzhang/"     +     str(date.today())     +
"_blast_result.txt"
	else:
		return choice
#--------------------------------------------------------------------------
------

# Created a list of files that will be BLASTed.
def list_check(folder):
	global fail_file
	global out_fail_file
	file_list = os.listdir(folder)
	seq_list = []
	for seq_file in file_list:
		#The file must end with .seq
		m = re.search('\.seq', seq_file)
		if m :
			seq = folder + seq_file
			in_file = open(seq, 'r')
```

```
                my_seq = in_file.read()
                #The file must contain a sequences that have at least 3
As/Ts/Cs/Gs or any combination of 3 bases listed consecutively.
                n = re.search('[ATCG]{3}', my_seq)
                if n:
                     seq_list.append(seq_file)
                else:
                # The file failing the pattern test won't be BLASTed, but
will be listed here.
                     out_fail_file.write(seq_file + "\n")
                in_file.close()
     return seq_list
#__________________________________________________________________________
_____

# Get the best hit from BLASTing a sequence against human database.
def best_hit_homo(blast_record, seq_file):
       global global_e_score
     global out_fail_file
     e_score = global_e_score
       try:
              sequence = "gi|na| Not matched with Homo sapiens\tna"
              for alignment in blast_record.alignments:
                     for hsp in alignment.hsps:
                            m = re.search("Homo", alignment.title)
                            if hsp.expect < e_score and m:
                                   e_score = hsp.expect
```

```
                              #This  condition  is  to  eliminate  bugs  in
Bio.Blast modules that concats name of entries into one line.
                              a = re.search('.+?(?=>gi)', alignment.title)
                              if a:
                                    alignment.title = a.group()
                                          sequence = alignment.title +"\t"+
str(e_score)
                  return sequence
      # To avoid an error from seq that passed the pattern test but failed
BLAST
      except:
            out_fail_file.write(seq_file + "\n")
            return "gi|na| BLAST failed\tna"
#---------------------------------------------------------------------------
-----

# Get the best hit from BLASTing a sequence against mosquito database.
def best_hit_Ae(blast_record, seq_file):
        global global_e_score
      e_score = global_e_score
            global out_fail_file
      try:
                  sequence = "AAEL0-na Not matched with Aedes aegypti\tn/a"
                  for alignment in blast_record.alignments:
                          for hsp in alignment.hsps:
                                  if hsp.expect < e_score:
                                          e_score = hsp.expect
```

```
                                        sequence = alignment.title +"\t"+
str(e_score)
          return sequence
     # To avoid an error from seq that passed the pattern test but failed
BLAST
     except:
          out_fail_file.write(seq_file + "\n")
          return "AAEL0-na BLAST failed\tn/a"
#-------------------------------------------------------------------------
-----
#BLAST nucleotide against nucleotide database.

def blast_n(seq_file, database):
     global blastall
       global global_e_score
     global out_fail_file
     result_handle,   error_handle   =   NCBIStandalone.blastall(blastall,
"blastn", database, seq_file, expectation = global_e_score)
     try:
          blast_record = NCBIXML.read(result_handle)
     # To avoid an error from seq that passed the pattern test but failed
BLAST
     except:
          out_fail_file.write(seq_file + "\n")
          blast_record = ''
     return blast_record
#-------------------------------------------------------------------------
----
```

```
#BLAST nucleotide against protein database.
def blast_p(seq_file, database):
      global blastall
        global global_e_score
      global out_fail_file
      result_handle,   error_handle   =   NCBIStandalone.blastall(blastall,
"blastx", database, seq_file, expectation = global_e_score)
      try:
            blast_record = NCBIXML.read(result_handle)
      # To avoid an error from seq that passed the pattern test but failed
BLAST
      except:
            out_fail_file.write(seq_file + "\n")
            blast_record = ''
      return blast_record
#---------------------------------------------------------------------------
-----
#Format the result from BLAST (human database)
def entry_homo(string):
      m = re.search('(?<=gi\|)\w+', string)
      gi_number = m.group()
      n = re.search('(?<=\|\s).+(?=\t)', string)
      gene_name = n.group()
      p = re.search('(?<=\t).+', string)
      e_score = p.group()
      entry = "gi" + gi_number + "\t" + gene_name + "\t" + e_score
      return entry
```

```
#-------------------------------------------------------------------------------
-----

#Format the result from BLAST (Ae database)
def entry_Ae(string):
      m = re.search('AAEL\d+-\w\w', string)
      Ae_number = m.group()
      n = re.search('(?<=-\w\w\s).+(?=\t)', string)
      gene_name = n.group()
      p = re.search('(?<=\t).+', string)
        e_score = p.group()
        entry = Ae_number + "\t" + gene_name + "\t" + e_score
      return entry
#-------------------------------------------------------------------------------
-----

#Translated from Jinkai's vectorcheck subroutine in
SeqValidationbyfolder2.pl

def vector_chk(seq_file, vector_seq, AD_or_BD):
      temp_out = "/tmp/alignment.tmp"
      os.system('/Applications/blast/blast-2.2.17/bin/bl2seq -p blastn -i
%s -j %s -o %s -F F -e 0.001' % (seq_file, vector_seq, temp_out))
      my_file = open(temp_out, 'r')
      my_text = my_file.read()
      m = re.search("No hits found", my_text)
      if m:
            check = "No " + AD_or_BD + " vector sequence detected"
```

```
    else:
        p = re.search('(?<=Identities).+\/\d+', my_text)
        f = re.search('\d+(?=\/)', p.group())
        b = re.search('(?<=\/)\d+', p.group())
        if int(f.group()) > 30 and int(b.group()) > 30:
            q = re.search('(?<=Query\:\s)\d+', my_text)
            pos_in_seq = q.group()
            check = AD_or_BD + " vector found! Begin at " +
pos_in_seq
        else:
            check = "No " + AD_or_BD +" vector sequence detected"
    my_file.close()
    return check
#---------------------------------------------------------------------------
------

#Main program

#Locate input files
in_folder = menu_input()

#Locate output files
out_file = open(menu_output(), 'w')

#Enter the header of the result file
header =
"seq_file\tgi_homo_blastn\tname_homo_blastn\te_homo_blastn\tgi_homo_blastp
```

```
\tname_homo_blastp\te_homo_blastp\tae_ae_blastn\tname_ae_blastn\te_ae_blas
tn\tae_ae_blastp\tname_ae_blastp\te_ae_blastp\tBD_chk\tAD_chk\n"
out_file.write(header)

#Ask for a cut off.
e_choice = raw_input('Please enter e-score cut off. 1 = No stringency
(defualt if no number is entered). 0 = Highest stringency.\n:')

if len(e_choice) == 0:
      global_e_score = 1
else:
      global_e_score = float(e_choice)

#Get the list of files for BLAST
seq_list = list_check(in_folder)

#Prepare a countdown.
number_all = len(seq_list)
number_down = number_all

print str(number_down) + " of " + str(number_all) + " left."

# Process one sequence at a time.
for file in seq_list:
      my_seq = ori_folder + file

#Run BLAST
      homo_blastn = blast_n(my_seq,refseq_rna)
```

```
        homo_blastp = blast_p(my_seq,refseq_pro)

        ae_blastn = blast_n(my_seq,ae_rna)

        ae_blastp = blast_p(my_seq,ae_pro)

        BD_chk = vector_chk(my_seq, BD_vector_seq,"BD")

        AD_chk = vector_chk(my_seq, vector_seq, "AD")

#Get the best hit
        homo_best_rna = best_hit_homo(homo_blastn, file)
        homo_best_pro = best_hit_homo(homo_blastp, file)
        ae_best_rna = best_hit_Ae(ae_blastn, file)
        ae_best_pro = best_hit_Ae(ae_blastp, file)

#Print entries.
        entry = file + "\t" + entry_homo(homo_best_rna) + "\t" +
entry_homo(homo_best_pro) + "\t" + entry_Ae(ae_best_rna) + "\t" +
entry_Ae(ae_best_pro) + "\t" + BD_chk + "\t" + AD_chk +"\n"
        out_file.write(entry)
        number_down = number_down - 1
        print str(number_down) + " of " + str(number_all) + " left."

out_fail_file.close()
out_file.close()
```

APPENDIX F. PYTHON SCRIPT OF 'AedesGO_for_bingo.py'

```
#!/usrs/bin/env python


import re

import string


# Open and read AedesGO.txt file.

#  AedesGO.txt  was  generated  by  using  excel's  'Data'  ->  'Text  to
Columns...' command to open 31436.A_aegypti.goa file. Then gene_ID column
and GO_ID column were selected and saved as AedesGo.txt

in_file = r"/Users/dmairiang/Desktop/Python_script/InterPro/AedesGO.txt"

input = open(in_file, 'r')


intxt = input.readlines()

input.close()


# Write data in AedesGO_for_bingo.txt

out_file                                                              =
r"/Users/dmairiang/Desktop/Python_script/InterPro/AedesGO_for_bingo.txt"

output = open(out_file, 'w')


# Add header required by BINGO to identify readable GOA file.


header = "(species=Aedes aegypti)(type=Biological Process)(curator=GO)\n"

output.write(header)


# Parsing a line readable by BINGO

# Each line is in a form of:
```

```
# Gene_ID = GO_ID


for member in intxt:

      m = re.search('(AAEL\d+)\tGO:(\d+)', member)

      if m:

            entry = m.group(1) + ' = ' + m.group(2) + '\n'

            output.write(entry)




output.close()
```

APPENDIX G. PYTHON SCRIPT OF 'IPRtree.py'

```
#!/usrs/bin/env python

import string
import re

# Open interpro.xml file.
in_file = r"/Users/dmairiang/Desktop/Python_script/InterPro/interpro.xml"
input = open(in_file, 'r')

intxt = input.read()
input.close()

# Write a tree with "is a" relationship into IPRtree_isa.txt file.
# Example: 'Mitosis cell cycle' is a 'Cell cycle.'
out_file1                                                                    =
r"/Users/dmairiang/Desktop/Python_script/InterPro/IPRtree_isa.txt"

#  Write  a  tree  with  "is  a"  and  "  part  of"  relationship  into
IPRtree_isa.txt file.
# Example: 'G2 phase' part of 'Cell cycle.'
#This file was not used because self-connection causing an infinite loop.
# A 'isa' B while B 'partof' A = an infinite loop.

out_file2                                                                    =
r"/Users/dmairiang/Desktop/Python_script/InterPro/IPRtree_isa_partof.txt"

# Write any error in IPRtree_fail.txt file.
```

```
out_fail_file                                                    =
r"/Users/dmairiang/Desktop/Python_script/InterPro/IPRtree_fail.txt"



output1 = open(out_file1, 'w')
output2 = open(out_file2, 'w')
outfail = open(out_fail_file, 'w')

# Separate each entry of interpro_ID.
list = intxt.split('</interpro>')

# Set ID 999999 as the highest hierachy, 'A mother of all nodes.'
default_isa = '[isa: 999999 ]'

# Write a header required by BINGO to specify a readable file.
header = '(curator=IPR) (type=domain)\n999999 = InterPro_domain\n'

output1.write(header)
output2.write(header)

# Look at each entry of interpro_ID.
# Find the description of that ID.
# Find the ID.
# Parse in a form of:
# interpro_ID = Description
def make_index(string):
      n = re.search('<name>(.*?)</name>', string)
      m = re.search('<interpro id=\"IPR(\d+)', string)
```

```
        index =  m.group(1) + ' = ' + n.group(1)
        return index

# Add an 'isa' relationship by looking at parents of the interpro_ID

def find_isa(string):
        try:
                tmp1 = string.split('parent_list')
                tmp_list1 = tmp1[1].split('\n')
                isa_list = []
                for member in tmp_list1:
                        m1 = re.search('IPR(\d+)', member)
                        if m1:
                                isa_list.append(m1.group(1))

                isa = '[isa: ' + ' '.join(isa_list) + ' ]'
                return isa
        except:
                isa = ''
                return isa


# Add a 'partof' relationship by looking at 'found_in' identifier of the
interpro_ID.
def find_partof(string):
        try:
                tmp2 = string.split('found_in')
                partof_list = []
```

```
            tmp_list2 = tmp2[1].split('\n')
            for member in tmp_list2:
                  m1 = re.search('IPR(\d+)', member)
                  if m1:
                        partof_list.append(m1.group(1))

            partof = '[partof: ' + ' '.join(partof_list) + ' ]'
            return partof
      except:
            partof = ''
            return partof



# Main script
for member in list:
      m = re.search('<interpro id=\"IPR(\d+)', member)
      if m:
            print m.group(1)
            if find_isa(member) != '':
                  entry  =  make_index(member)  +  '  '  +  find_isa(member)  +
'\n'
                  output1.write(entry)
            elif find_isa(member) == '':
                  entry = make_index(member) + ' ' + default_isa + '\n'
                  output1.write(entry)
for member in list:
      m = re.search('<interpro id=\"IPR(\d+)', member)
      if m:
```

```
            if find_isa(member) != '' or find_partof(member) != '':
                  entry = make_index(member) + ' ' + find_isa(member) + ' '
+ find_partof(member) + '\n'
                  output2.write(entry)

            elif find_isa(member) == '' and find_partof(member) == '':
                  entry = make_index(member) + ' ' + default_isa +'\n'
                  output2.write(entry)
            else:
                  outfail.write(member)
      else:
            outfail.write(member)




output1.close()
output2.close()
outfail.close()
```

APPENDIX H. PYTHON SCRIPT OF 'inparanoid_dro_hum.py'

```
#!/usrs/bin/evn/ python

# See a similar description in a file, inparanoid_ae_hum.py

import string
import re

in_file                                                                         =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/InParanoid.D.m
elanogaster-H.sapiens.orthoXML"

input = open(in_file, 'r')
txt_list = input.readlines()
input.close()

out_file = "Dro_to_hum_ID.txt"
output = open(out_file, 'w')

txt = ''.join(txt_list)

tmp1= txt.split('</scores>')

gene_id = tmp1[0]

orth_cluster = tmp1[1].split('</orthologGroup>')
for cluster in orth_cluster:
```

```
    m = re.search('<orthologGroup id="(\d+)">', cluster)
    if m:
        cluster_id = m.group(1)
        print cluster

        group = cluster.split('</geneRef>')

        print cluster_id
        member_list = []
        for member in group:
            m = re.search('<geneRef id="(\d+)">',member)
            if not m:
                print "somthing wrong!\n"
            if m:
                id = m.group(1)
                o      =      re.search('<score      id="inparalog"
value="(.*?)"/>', member)
                score = o.group(1)
                n     =     re.search('<gene     id="'+m.group(1)+'"
geneId="(.*?)" protId="(.*?)"/>', gene_id)
                if n != '':
                    gene = n.group(1)
                else:
                    gene = n.group(2)
                entry = id+';'+gene+';'+score
                print entry
                member_list.append(entry)
```

```
        if len(member_list) > 2:
            human = []
            fly = []
            for member in member_list:
                m = re.search('ENSG', member)
                if m:
                    human.append(member)
                else:
                    fly.append(member)
            for member1 in fly:
                for member2 in human:
                    final_entry = cluster_id + '\t' + member1 +
'\t' + member2
                    print final_entry
                    output.write(final_entry+'\n')
        elif len(member_list) == 2:
            final_entry = cluster_id + '\t' + '\t'.join(member_list)
            print final_entry
            output.write(final_entry + '\n')

output.close()
```

<u>APPENDIX I.</u> PYTHON SCRIPT OF 'inparanoid_Dro_Ae.py'

```
#!/usrs/bin/evn/ python

# See a similar description in a file, inparanoid_ae_hum.py
import string
import re



in_file                                                              =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/InParanoid.A.a
egypti-D.melanogaster.orthoXML"

input = open(in_file, 'r')
txt_list = input.readlines()
input.close()

out_file = "Aedes_to_Dro_ID.txt"
output = open(out_file, 'w')

txt = ''.join(txt_list)

tmp1= txt.split('</scores>')

gene_id = tmp1[0]

orth_cluster = tmp1[1].split('</orthologGroup>')
for cluster in orth_cluster:
```

```
    m = re.search('<orthologGroup id="(\d+)">', cluster)
    if m:
        cluster_id = m.group(1)
        print cluster

        group = cluster.split('</geneRef>')

        print cluster_id
        member_list = []
        for member in group:
            m = re.search('<geneRef id="(\d+)">',member)
            if not m:
                print "somthing wrong!\n"
            if m:
                id = m.group(1)
                o      =      re.search('<score      id="inparalog"
value="(.*?)"/>', member)
                score = o.group(1)
                n     =     re.search('<gene     id="'+m.group(1)+'"
geneId="(.*?)" protId="(.*?)"/>', gene_id)
                if n != '':
                    gene = n.group(1)
                else:
                    gene = n.group(2)
                entry = id+';'+gene+';'+score
                print entry
                member_list.append(entry)
```

```
        if len(member_list) > 2:
              aedes = []
              fly = []
              for member in member_list:
                    m = re.search('AAEL', member)
                    if m:
                          aedes.append(member)
                    else:
                          fly.append(member)
              for member1 in aedes:
                    for member2 in fly:
                          final_entry = cluster_id + '\t' + member1 +
'\t' + member2
                          print final_entry
                          output.write(final_entry+'\n')
        elif len(member_list) == 2:
              final_entry = cluster_id + '\t' + '\t'.join(member_list)
              print final_entry
              output.write(final_entry + '\n')

output.close()
```

APPENDIX J. PYTHON SCRIPT OF ‘inparanoid_ae_hum.py’

```
#!/usrs/bin/evn/ python

import string
import re

# Open a downloaded XML file.
in_file                                                                  =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/InParanoid.A.a
egypti-H.sapiens.orthoXML"

input = open(in_file, 'r')
txt_list = input.readlines()
input.close()

# Write data in 'Ae_to_hum_ID.txt' file.
out_file = "Ae_to_hum_ID.txt"
output = open(out_file, 'w')



# Concatanating the whole file into one string.
txt = ''.join(txt_list)

# Separate a gene_ID list part of the file from a cluster list part of the
file.
tmp1= txt.split('</scores>')
gene_id = tmp1[0]
```

```
# Look at the cluster list part of the file.
# Split the list into cluster groups.

orth_cluster = tmp1[1].split('</orthologGroup>')

# Look at each cluster group.
for cluster in orth_cluster:

# Collect cluster_ID.
      m = re.search('<orthologGroup id="(\d+)">', cluster)
      if m:
            cluster_id = m.group(1)
            print cluster
# Split genes in the cluster group.
            group = cluster.split('</geneRef>')

            print cluster_id
            member_list = []

# Collect a gene_ID from each gene.
            for member in group:
                  m = re.search('<geneRef id="(\d+)">',member)
                  if not m:
                        print "somthing wrong!\n"

# Collect a homologous score of each gene.
                  if m:
```

```
                        id = m.group(1)
                        o      =      re.search('<score      id="inparalog"
value="(.*?)"/>', member)
                        score = o.group(1)

# Search for a correct gene ID from the gene list part of the original XML
file.
                        n     =     re.search('<gene     id="'+m.group(1)+'"
geneId="(.*?)" protId="(.*?)"/>', gene_id)

# Collect a gene ID (ENSG....) if found.
                        if n != '':
                              gene = n.group(1)
# Collect a protein ID if gene ID is not found.

                        else:
                              gene = n.group(2)

# Put all cluster_ID, gene_ID/protein_ID and homologous score in a list.
                        entry = id+';'+gene+';'+score
                        print entry
                        member_list.append(entry)
# Parse the list into table
# If there are more than one human or mosquito gene in the list, genes
were sorted by species.
# Next, each mosquito gene is paired with each human gene.
# For example
```

```
# cluster 1 with mosquito genes (a, b and c) and human genes (A and B) are
listed as:
# 1   a     A
# 1   b     A
# 1   c     A
# 1   a     B
# 1   b     B
# 1   c     B

            if len(member_list) > 2:
                  aedes =[]
                  human = []
                  for member in member_list:
                        m = re.search('AAEL', member)
                        if m:
                              aedes.append(member)
                        else:
                              human.append(member)
                  for member1 in aedes:
                        for member2 in human:
                              final_entry = cluster_id + '\t' + member1 +
'\t' + member2
                              print final_entry
                              output.write(final_entry+'\n')

# If there are one human gene and one mosquito gene, one pairing is done.

            elif len(member_list) == 2:
```

```
                final_entry = cluster_id + '\t' + '\t'.join(member_list)
                print final_entry
                output.write(final_entry + '\n')

output.close()
```

APPENDIX K. PYTHON SCRIPT OF 'cross_fbgn.py'

```
#!/usrs/bin/env/ python

import string

import re

# Open a Dmel to Aedes ortholog list
in_file1                                                                        =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/Aedes_to_Dro_I
D.txt"

# Open a human to Dmal ortholog list
in_file2                                                                        =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/Dro_to_hum_ID.
txt"

input1 = open(in_file1, 'r')
a_to_d = input1.readlines()
input1.close()

input2 = open(in_file2, 'r')
d_to_h = input2.readlines()
input2.close()

# Write data in crossFBgn_Ae_to_hum.txt
out_file = r"crossFBgn_Ae_to_hum.txt"
```

```
output = open(out_file, 'w')


# Write a header

header = "Aedes_gene\tDro_gene\tHuman_gene\n"

output.write(header)



# Look for Aedes gene and Dmel gene in the Dmel to Aedes list

for member1 in a_to_d:

      human_list = []

      m = re.search('(AAEL\d+)', member1)

      n = re.search('(FBgn\d+)', member1)

      if m and n:

            AAEL = m.group(1)

            FBgn = n.group(1)


# Look for Dmel gene from the Dmel to Aedes list in the human to Dmel list

            for member2 in d_to_h:

                  o = re.search(FBgn, member2)

                  if o:

                        p = re.search('(ENSG\d+)', member2)

                        if p:

                              human_list.append(p.group(1))

# If found, cluster Aedes gene, Dmel gene and human gene together.

      if len(human_list) > 0:

            for member3 in human_list:

                  entry = AAEL + '\t' + FBgn +'\t' + member3 +'\n'

                  output.write(entry)
```

```
output.close()
```

APPENDIX L. PYTHON SCRIPT OF 'clusterInpara_dro.py'

```
#!/usrs/bin/env/ python



import string
import re

# Open the combined aedes, dmel and human ortholog cluster.
in_file1                                                                            =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/crossFBgn_Ae_t
o_hum_complete.txt"
input1 = open(in_file1, 'r')
temp1 = input1.readlines()
input1.close()

temp1 = ''.join(temp1)
cross_orth = temp1.split('\r')

# Open the list of all unique FBgn IDs.
in_file2                                                                            =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/uniFBgn.csv"
input2 = open(in_file2, 'r')
temp2 = input2.readlines()
input2.close()

temp2 = ''.join(temp2)
uniFBgn = temp2.split('\r')
```

```
# Assign an output file
out_file                                                                     =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/clusteredOrth_
step1.txt"
output = open(out_file, 'w')

# Write a header
header = "cluster_ID\tAedes_ID\tDro_ID\tHuman_ID\n"
output.write(header)

# Initiate new cluster_ID
count = 1

# Assign the same cluster ID to all groups that have the same Dmel gene by
looking for identical FBgn IDs.

for member1 in uniFBgn:
      for member2 in cross_orth:
            m = re.search(member1, member2)
            if m:
                  entry = str(count) +'\t' + member2 + '\n'
                  output.write(entry)
                  print entry
      count = count + 1

# Assing the new clusterID for any group lacking a Dmel gene as a member.
for member in cross_orth:
```

```
        m = re.search('FBgn', member)
        if not m:
                entry = str(count) +'\t'+member + '\n'
                output.write(entry)
                print entry
                count = count + 1
output.close()
```

APPENDIX M. PYTHON SCRIPT OF 'clusterInpara_droaedes.py'

```
#!/usrs/bin/env/ python

import string
import re

# Open a list of unique mosquito gene ID (AAEL######)
in_file1                                                                    =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/uniAAEL.csv"
input1 = open(in_file1, 'r')
temp1 = input1.readlines()
temp1 = ''.join(temp1)
uniAAEL = temp1.split('\r')
input1.close()

# Open the cluster the file previously processed by looking for identical
Dmel genes.
in_file2                                                                    =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/clusteredOrth_
step1.txt"
input2 = open(in_file2, 'r')
orth_list = input2.readlines()
input2.close()

# Assign an output file.
```

```
out_file                                                                    =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/clusteredOrth_
step2_aedes_dro.txt"
output = open(out_file, 'w')
header = "cluster_ID\tAedes\n"
output.write(header)

# Assign new cluster_ID for any group that has the same mosquito gene.
for member1 in uniAAEL:
      clust_ID = 100000000
      for member2 in orth_list:
            m = re.search(member1, member2)
            if m:
                  n = re.search('(\d+)\tAAEL', member2)
                  if int(n.group(1)) < clust_ID:
                        clust_ID = int(n.group(1))
      entry = str(clust_ID) + '\t' + member1 + '\n'
      print entry
      output.write(entry)

output.close()
```

APPENDIX N. PYTHON SCRIPT OF 'clusterInpara_droaedeshum.py'

```
#!/usrs/bin/env/ python

import string
import re

# Open a list of unique human gene_ID (ENSG)
in_file1                                                                 =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/uniENSG.csv"
input1 = open(in_file1, 'r')
temp1 = input1.readlines()
temp1 = ''.join(temp1)
uniENSG = temp1.split('\r')
input1.close()

# Open the cluster file previously processed for identical Dmal and Aedes
genes.
in_file2                                                                 =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/clusteredOrth_
step2_aedes_dro.tab"
input2 = open(in_file2, 'r')
orth_list = input2.readlines()
input2.close()
orth_list = ''.join(orth_list)
orth_list = orth_list.split('\r')
```

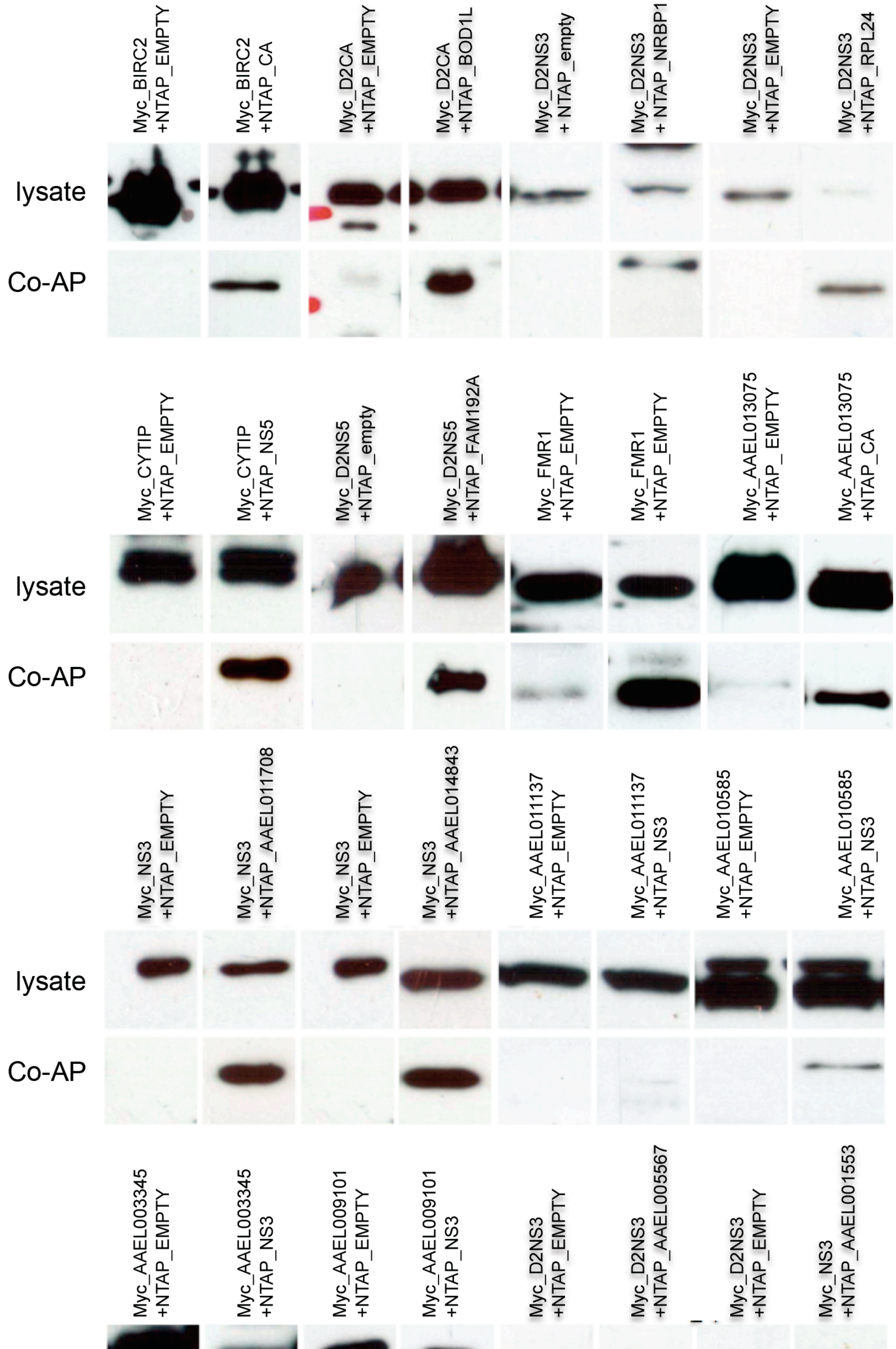
```
# Assign an output file
out_file                                                                    =
r"/Users/dmairiang/Desktop/Python_script/Inparanoid10012011/clusteredOrth_
step3_aedes_dro_hum.txt"
output = open(out_file, 'w')
header = "cluster_ID\thuman\n"
output.write(header)

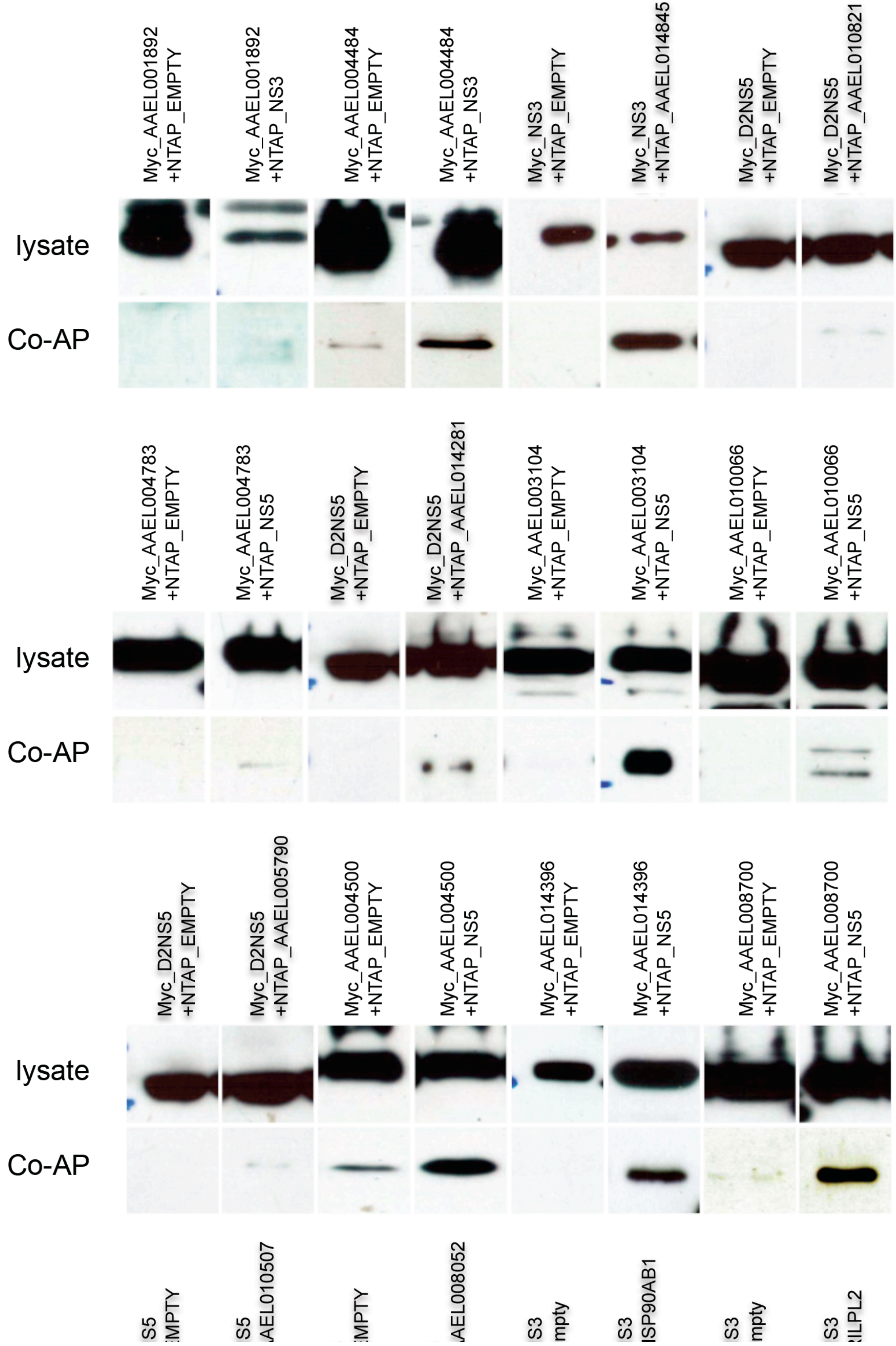
# Assign the same cluster_ID for any group having an identical human gene.
for member1 in uniENSG:
      clust_ID = 100000000
      for member2 in orth_list:
            m = re.search(member1, member2)
            if m:
                  n = re.search('^(\d+)\t', member2)
                  if n and int(n.group(1)) < clust_ID:
                        clust_ID = int(n.group(1))
      entry = str(clust_ID) + '\t' + member1 + '\n'
      print entry
      output.write(entry)

output.close()
```

APPENDIX O. CO-AFFINITY PURIFICATION ASSAYS FOR DENGUE-HOST PROTEIN INTERACTIONS.

Additional co-AP results not shown in Figure 2-11. The fusion proteins were expressed in S2R+ cells. NTAP-tagged proteins were purified from cell lysates, and then Myc-tagged proteins were detected with α-myc. (A) Lysate. (B) Co-AP.


Myc_BIRC2 +NTAP_EMPTY
Myc_BIRC2 +NTAP_CA
Myc_D2CA +NTAP_EMPTY
Myc_D2CA +NTAP_BOD1L
Myc_D2NS3 + NTAP_empty
Myc_D2NS3 + NTAP_NRBP1
Myc_D2NS3 +NTAP_EMPTY
Myc_D2NS3 +NTAP_RPL24
lysate
Co-AP
Myc_CYTIP +NTAP_EMPTY
Myc_CYTIP +NTAP_NS5
Myc_D2NS5 +NTAP_empty
Myc_D2NS5 +NTAP_FAM192A
Myc_FMR1 +NTAP_EMPTY
Myc_FMR1 +NTAP_EMPTY
Myc_AAEL013075 +NTAP_ EMPTY
Myc_AAEL013075 +NTAP_CA
lysate
Co-AP
Myc_NS3 +NTAP_EMPTY
Myc_NS3 +NTAP_AAEL011708
Myc_NS3 +NTAP_EMPTY
Myc_NS3 +NTAP_AAEL014843
Myc_AAEL011137 +NTAP_EMPTY
Myc_AAEL011137 +NTAP_NS3
Myc_AAEL010585 +NTAP_EMPTY
Myc_AAEL010585 +NTAP_NS3
lysate
Co-AP
Myc_AAEL003345 +NTAP_EMPTY
Myc_AAEL003345 +NTAP_NS3
Myc_AAEL009101 +NTAP_EMPTY
Myc_AAEL009101 +NTAP_NS3
Myc_D2NS3 +NTAP_EMPTY
Myc_D2NS3 +NTAP_AAEL005567
Myc_D2NS3 +NTAP_EMPTY
Myc_NS3 +NTAP_AAEL001553


lysate
Co-AP
Myc_AAEL001892 +NTAP_EMPTY
Myc_AAEL001892 +NTAP_NS3
Myc_AAEL004484 +NTAP_EMPTY
Myc_AAEL004484 +NTAP_NS3
Myc_NS3 +NTAP_EMPTY
Myc_NS3 +NTAP_AAEL014845
Myc_D2NS5 +NTAP_EMPTY
Myc_D2NS5 +NTAP_AAEL010821
lysate
Co-AP
Myc_AAEL004783 +NTAP_EMPTY
Myc_AAEL004783 +NTAP_NS5
Myc_D2NS5 +NTAP_EMPTY
Myc_D2NS5 +NTAP_AAEL014281
Myc_AAEL003104 +NTAP_EMPTY
Myc_AAEL003104 +NTAP_NS5
Myc_AAEL010066 +NTAP_EMPTY
Myc_AAEL010066 +NTAP_NS5
lysate
Co-AP
Myc_D2NS5 +NTAP_EMPTY
Myc_D2NS5 +NTAP_AAEL005790
Myc_AAEL004500 +NTAP_EMPTY
Myc_AAEL004500 +NTAP_NS5
Myc_AAEL014396 +NTAP_EMPTY
Myc_AAEL014396 +NTAP_NS5
Myc_AAEL008700 +NTAP_EMPTY
Myc_AAEL008700 +NTAP_NS5

APPENDIX P. DNA SEQUENCE ALIGNMENT BETWEEN THE DENGUE REPLICON AND DENGUE VIRUS SEROTYPE 2 (STRAIN 16681)

```
BLASTN 2.2.26+
Reference: Zheng Zhang, Scott Schwartz, Lukas Wagner, and
Webb Miller (2000), "A greedy algorithm for aligning DNA
sequences", J Comput Biol 2000; 7(1-2):203-14.


RID: 06MBHZFX111


Query= YRp7_Replicon

Length=15681


                                                                 Score        E
Sequences producing significant alignments:                     (Bits)     Value

ref|NC_001474.2|  Dengue virus 2, complete genome                1.533e+04  0.0

ALIGNMENTS
>ref|NC_001474.2| Dengue virus 2, complete genome
Length=10723

 Score = 1.533e+04 bits (8302),  Expect = 0.0
 Identities = 8350/8374 (99%), Gaps = 1/8374 (0%)
 Strand=Plus/Plus

Query  7154   AGCACCTCACTGTCTGTGACACTAGTATTGGTGGGAATTGTGACACTGTATTTGGGAGTC  7213
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  2350   AGCACCTCACTGTCTGTGACACTAGTATTGGTGGGAATTGTGACACTGTATTTGGGAGTC  2409

Query  7214   ATGGTGCAGGCCGATAGTGGCTGCGTTGTGAGCTGGGAAAACAAAGAACTGAAATGTGGC  7273
              |||||||||||||||||||| ||||||||||||||| ||||||||||||||||||||||||
Sbjct  2410   ATGGTGCAGGCCGATAGTGGTTGCGTTGTGAGCTGGAAAAACAAAGAACTGAAATGTGGC  2469

Query  7274   AGTGGGATTTTCATCACAGACAACGTGCACACATGGACAGAACAATACAAGTTCCAACCA  7333
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2470   AGTGGGATTTTCATCACAGACAACGTGCACACATGGACAGAACAATACAAGTTCCAACCA  2529

Query  7334   GAATCCCCTTCAAAACTAGCTTCAGCTATCCAGAAAGCCCATGAAGAGGGCATTTGTGGA  7393
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2530   GAATCCCCTTCAAAACTAGCTTCAGCTATCCAGAAAGCCCATGAAGAGGGCATTTGTGGA  2589

Query  7394   ATCCGCTCAGTAACAAGACTGGAGAATCTGATGTGGAAACAAATAACACCAGAATTGAAT  7453
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2590   ATCCGCTCAGTAACAAGACTGGAGAATCTGATGTGGAAACAAATAACACCAGAATTGAAT  2649

Query  7454   CACATTCTATCAGAAAATGAGGTGAAGTTAACTATCATGACAGGAGACATCAAAGGAATC  7513
              |||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  2650   CACATTCTATCAGAAAATGAGGTGAAGTTAACTATTATGACAGGAGACATCAAAGGAATC  2709

Query  7514   ATGCAGGCAGGAAAACGATCTCTGCGGCCTCAGCCCACTGAGCTGAAGTATTCATGGAAA  7573
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2710   ATGCAGGCAGGAAAACGATCTCTGCGGCCTCAGCCCACTGAGCTGAAGTATTCATGGAAA  2769

Query  7574   ACATGGGGCAAAGCAAAAATGCTCTCTACAGAGTCTCATAACCAGACCTTTCTCATTGAT  7633
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2770   ACATGGGGCAAAGCAAAAATGCTCTCTACAGAGTCTCATAACCAGACCTTTCTCATTGAT  2829

Query  7634   GGCCCCGAAACAGCAGAATGCCCCAACACAAATAGAGCTTGGAATTCGTTGGAAGTTGAA  7693
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2830   GGCCCCGAAACAGCAGAATGCCCCAACACAAATAGAGCTTGGAATTCGTTGGAAGTTGAA  2889
```

```
Query  7694  GACTATGGCTTTGGAGTATTCACCACCAATATATGGCTAAAATTGAAAGAAAAACAGGAT  7753
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2890  GACTATGGCTTTGGAGTATTCACCACCAATATATGGCTAAAATTGAAAGAAAAACAGGAT  2949

Query  7754  GTATTCTGCGACTCAAAACTCATGTCAGCGGCCATAAAAGACAACAGAGCCGTCCATGCC  7813
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2950  GTATTCTGCGACTCAAAACTCATGTCAGCGGCCATAAAAGACAACAGAGCCGTCCATGCC  3009

Query  7814  GATATGGGTTATTGGATAGAAAGTGCACTCAATGACACATGGAAGATAGAGAAAGCCTCT  7873
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3010  GATATGGGTTATTGGATAGAAAGTGCACTCAATGACACATGGAAGATAGAGAAAGCCTCT  3069

Query  7874  TTCATTGAAGTTAAAAACTGCCACTGGCCAAAATCACACACCCTCTGGAGCAATGGAGTG  7933
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3070  TTCATTGAAGTTAAAAACTGCCACTGGCCAAAATCACACACCCTCTGGAGCAATGGAGTG  3129

Query  7934  CTAGAAAGTGAGATGATAATTCCAAAGAATCTCGCTGGACCAGTGTCTCAACACAACTAT  7993
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3130  CTAGAAAGTGAGATGATAATTCCAAAGAATCTCGCTGGACCAGTGTCTCAACACAACTAT  3189

Query  7994  AGACCAGGCTACCATACACAAATAACAGGACCATGGCATCTAGGTAAGCTTGAGATGGAC  8053
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3190  AGACCAGGCTACCATACACAAATAACAGGACCATGGCATCTAGGTAAGCTTGAGATGGAC  3249

Query  8054  TTTGATTTCTGTGATGGAACAACAGTGGTAGTGACTGAGGACTGCGGAAATAGAGGACCC  8113
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3250  TTTGATTTCTGTGATGGAACAACAGTGGTAGTGACTGAGGACTGCGGAAATAGAGGACCC  3309

Query  8114  TCTTTGAGAACAACCACTGCCTCTGGAAAACTCATAACAGAATGGTGCTGCCGATCTTGC  8173
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3310  TCTTTGAGAACAACCACTGCCTCTGGAAAACTCATAACAGAATGGTGCTGCCGATCTTGC  3369
```

```
Query  8174   ACATTACCACCGCTAAGATACAGAGGTGAGGATGGGTGCTGGTACGGGATGGAAATCAGA  8233
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3370   ACATTACCACCGCTAAGATACAGAGGTGAGGATGGGTGCTGGTACGGGATGGAAATCAGA  3429

Query  8234   CCATTGAAGGAGAAAGAAGAGAATTTGGTCAACTCCTTGGTCACAGCTGGACATGGGCAG  8293
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3430   CCATTGAAGGAGAAAGAAGAGAATTTGGTCAACTCCTTGGTCACAGCTGGACATGGGCAG  3489

Query  8294   GTCGACAACTTTTCACTAGGAGTCTTGGGAATGGCATTGTTCCTGGAGGAAATGCTTAGG  8353
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3490   GTCGACAACTTTTCACTAGGAGTCTTGGGAATGGCATTGTTCCTGGAGGAAATGCTTAGG  3549

Query  8354   ACCCGAGTAGGAACGAAACATGCAATACTACTAGTTGCAGTTTCTTTTGTGACATTGATC  8413
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3550   ACCCGAGTAGGAACGAAACATGCAATACTACTAGTTGCAGTTTCTTTTGTGACATTGATC  3609

Query  8414   ACAGGGAACATGTCCTTTAGAGACCTGGGAAGAGTGATGGTTATGGTAGGCGCCATTATG  8473
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
Sbjct  3610   ACAGGGAACATGTCCTTTAGAGACCTGGGAAGAGTGATGGTTATGGTAGGCGCCACTATG  3669

Query  8474   ACGGATGACATAGGTATGGGCGTGACTTATCTTGCCCTACTAGCAGCCTTCAAAGTCAGA  8533
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3670   ACGGATGACATAGGTATGGGCGTGACTTATCTTGCCCTACTAGCAGCCTTCAAAGTCAGA  3729

Query  8534   CCAACTTTTGCAGCTGGACTACTCTTGAGAAAGCTGACCTCCAAGGAATTGATGATGACT  8593
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3730   CCAACTTTTGCAGCTGGACTACTCTTGAGAAAGCTGACCTCCAAGGAATTGATGATGACT  3789

Query  8594   ACTATAGGAATTGTACTCCTCTCCCAGAGCACCATACCAGAGACCATTCTTGAGTTGACT  8653
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3790   ACTATAGGAATTGTACTCCTCTCCCAGAGCACCATACCAGAGACCATTCTTGAGTTGACT  3849

Query  8654   GATGCGTTAGCCTTAGGCATGATGGTCCTCAAAATGGTGAGAAATATGGAAAAGTATCAA  8713
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Sbjct  3850   GATGCGTTAGCCTTAGGCATGATGGTCCTCAAAATGGTGAGAAATATGGAAAAGTATCAA  3909

Query  8714   TTGGCAGTGACTATCATGGCTATCTTGTGCGTCCCAAACGCAGTGATATTACAAAACGCA  8773
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3910   TTGGCAGTGACTATCATGGCTATCTTGTGCGTCCCAAACGCAGTGATATTACAAAACGCA  3969

Query  8774   TGGAAAGTGAGTTGCACAATATTGGCAGTGGTGTCCGTTTCCCCACTGTTCTTAACATCC  8833
              |||||||||||||||||||||||||||||||||||||||||||||||| |||||||||||
Sbjct  3970   TGGAAAGTGAGTTGCACAATATTGGCAGTGGTGTCCGTTTCCCCACTGCTCTTAACATCC  4029

Query  8834   TCACAGCAAAAAACAGATTGGATACCATTAGCATTGACGATCAAAGGTCTCAATCCAACA  8893
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Sbjct  4030   TCACAGCAAAAAACAGATTGGATACCATTAGCATTGACGATCAAAGGTCTCAATCCAACA  4089

Query  8894   GCTATTTTTCTAACAACCCTCTCAAGAACCAGCAAGAAAAGGAGCTGGCCATTAAATGAG  8953
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Sbjct  4090   GCTATTTTTCTAACAACCCTCTCAAGAACCAGCAAGAAAAGGAGCTGGCCATTAAATGAG  4149

Query  8954   GCTATCATGGCAGTCGGGATGGTGAGCATTTTAGCCAGTTCTCTCCTAAAAAAATGATATT  9013
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Sbjct  4150   GCTATCATGGCAGTCGGGATGGTGAGCATTTTAGCCAGTTCTCTCCTAAAAAAATGATATT  4209

Query  9014   CCCATGGCAGGACCATTAGTGGCTGGAGGGCTCCTCACTGTGTGCTACGTGCTCACTGGA  9073
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Sbjct  4210   CCCATGACAGGACCATTAGTGGCTGGAGGGCTCCTCACTGTGTGCTACGTGCTCACTGGA  4269

Query  9074   CGATCGGCCGATTTGGAACTGGAGAGAGCAGCCGATGTCAAATGGGAAGACCAGGCAGAG  9133
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Sbjct  4270   CGATCGGCCGATTTGGAACTGGAGAGAGCAGCCGATGTCAAATGGGAAGACCAGGCAGAG  4329

Query  9134   ATATCAGGAAGCAGCCCAATCCTGTCAATAACAATATCAGAAGATGGTAGCATGTCGATA  9193
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Sbjct  4330   ATATCAGGAAGCAGTCCAATCCTGTCAATAACAATATCAGAAGATGGTAGCATGTCGATA  4389

Query  9194   AAAAATGAAGAGGAAGAACAAACACTGACCATACTCATTAGAACAGGATTGCTGGTGATC  9253
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Sbjct  4390   AAAAATGAAGAGGAAGAACAAACACTGACCATACTCATTAGAACAGGATTGCTGGTGATC  4449

Query  9254   TCAGGACTTTTTCCTGTATCAATACCAATCACGGCAGCAGCATGKTACCTGTGGGAAGTG  9313
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Sbjct  4450   TCAGGACTTTTTCCTGTATCAATACCAATCACGGCAGCAGCATGGTACCTGTGGGAAGTG  4509

Query  9314   AAGAAACAACGGGCCGGAGTATTGTGGGATGTTCCTTCACCCCCACCCATGGGAAAGGCT  9373
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Sbjct  4510   AAGAAACAACGGGCCGGAGTATTGTGGGATGTTCCTTCACCCCCACCCATGGGAAAGGCT  4569

Query  9374   GAACTGGAAGATGGAGCCTATAGAATTAAGCAAAAAGGGATTCTTGGATATTCCCAGATC  9433
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Query  9434   GGAGCCGGAGTTTACAAAGAAGGAACATTCCATACAATGTGGCATGTCACACGTGGCGCT  9493
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Sbjct  4630   GGAGCCGGAGTTTACAAAGAAGGAACATTCCATACAATGTGGCATGTCACACGTGGCGCT  4689

Query  9494   GTTCTAATGCATAAAGGAAAGAGGATTGAACCATCATGGGCGGACGTCAAGAAAGACCTA  9553
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Sbjct  4690   GTTCTAATGCATAAAGGAAAGAGGATTGAACCATCATGGGCGGACGTCAAGAAAGACCTA  4749

Query  9554   ATATCATATGGAGGAGGCTGGAAGTTAGAAGGAGAATGGAAGGAAGGAGAAGAAGTCCAG  9613
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Sbjct  4750   ATATCATATGGAGGAGGCTGGAAGTTAGAAGGAGAATGGAAGGAAGGAGAAGAAGTCCAG  4809

Query  9614   GTATTGGCACTGGAGCCTGGAAAAAAATCCAAGAGCCGTCCAAACGAAACCTGGTCTTTTC  9673
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Sbjct  4810   GTATTGGCACTGGAGCCTGGAAAAAAATCCAAGAGCCGTCCAAACGAAACCTGGTCTTTTC  4869
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Query  9674   AAAACCAACGCCGGAACAATAGGTGCTGTATCTCTGGACTTTTCTCCTGGAACGTCAGGA  9733
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Sbjct  4870   AAAACCAACGCCGGAACAATAGGTGCTGTATCTCTGGACTTTTCTCCTGGAACGTCAGGA  4929

Query  9734   TCTCCAATTATCGACAAAAAAGGAAAAGTTGTGGGTCTTTATGGTAATGGTGTTGTTACA  9793
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Query  9794   AGGAGTGGAGCATATGTGAGTGCTATAGCCCAGACTaaaaaaaGCATTGAAGACAACCCA  9853
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Sbjct  4990   AGGAGTGGAGCATATGTGAGTGCTATAGCCCAGACTGAAAAAAGCATTGAAGACAACCCA  5049

Query  9854   GAGATCGAAGATGACATTTTCCGAAAGAGAAGACTGACCATCATGGACCTCCACCCAGGA  9913
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Sbjct  5050   GAGATCGAAGATGACATTTTCCGAAAGAGAAGACTGACCATCATGGACCTCCACCCAGGA  5109

Query  9914   GCGGGAAAGACGAAGAGATACCTTCCGGCCATAGTCAGAGAAGCTATAAAACGGGGTTTG  9973
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Sbjct  5110   GCGGGAAAGACGAAGAGATACCTTCCGGCCATAGTCAGAGAAGCTATAAAACGGGGTTTG  5169

Query  9974   AGAACATTAATCTTGGCCCCCACTAGAGTTGTGGCAGCTGAAATGGAGGAAGCCCTTAGA  10033
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Sbjct  5170   AGAACATTAATCTTGGCCCCCACTAGAGTTGTGGCAGCTGAAATGGAGGAAGCCCTTAGA  5229

Query  10034  GGACTTCCAATAAGATACCAGACCCCAGCCATCAGAGCTGAGCACACCGGGCGGGAGATT  10093
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Sbjct  5230   GGACTTCCAATAAGATACCAGACCCCAGCCATCAGAGCTGAGCACACCGGGCGGGAGATT  5289

Query  10094  GTGGACCTAATGTGTCATGCCACATTTACCATGAGGCTGCTATCACCAGTTAGAGTGCCA  10153
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5290   GTGGACCTAATGTGTCATGCCACATTTACCATGAGGCTGCTATCACCAGTTAGAGTGCCA  5349
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Query  10154  AACTACAACCTGATTATCATGGACGAAGCCCATTTCACAGACCCAGCAAGTATAGCAGCT  10213
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Sbjct  5350   AACTACAACCTGATTATCATGGACGAAGCCCATTTCACAGACCCAGCAAGTATAGCAGCT  5409

Query  10214  AGAGGATACATCTCAACTCGAGTGGAGATGGGTGAGGCAGCTGGGATTTTTATGACAGCC  10273
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5410   AGAGGATACATCTCAACTCGAGTGGAGATGGGTGAGGCAGCTGGGATTTTTATGACAGCC  5469

Query  10274  ACTCCCCCGGGAAGCAGAGACCCATTTCCTCAGAGCAATGCACCAATCATAGATGAAGAA  10333
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Sbjct  5470   ACTCCCCCGGGAAGCAGAGACCCATTTCCTCAGAGCAATGCACCAATCATAGATGAAGAA  5529

Query  10334  AGAGAAATCCCTGAACGTTCGTGGAATTCCGGACATGAATGGGTCACGGATTTTAAAGGG  10393
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Sbjct  5530   AGAGAAATCCCTGAACGTTCGTGGAATTCCGGACATGAATGGGTCACGGATTTTAAAGGG  5589

Query  10394  AAGACTGTTTGGTTCGTTCCAAGTATAAAAGCAGGAAATGATATAGCAGCTTGCCTGAGG  10453
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Query  10454  AAAAATGGAAAGAAAGTGATACAACTCAGTAGGAAGACCTTTGATTCTGAGTATGTCAAG  10513
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Query  10514  ACTAGAACCAATGATTGGGACTTCGTGGTTACAACTGACATTTCAGAAATGGGTGCCAAT  10573
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Sbjct  5710   ACTAGAACCAATGATTGGGACTTCGTGGTTACAACTGACATTTCAGAAATGGGTGCCAAT  5769

Query  10574  TTCAAGGCTGAGAGGGTTATAGACCCCAGACGCTGCATGAAACCAGTTATACTAACAGAT  10633
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Sbjct  5770   TTCAAGGCTGAGAGGGTTATAGACCCCAGACGCTGCATGAAACCAGTCATACTAACAGAT  5829

Query  10634  GGTGAAGAGCGGGCGATTCTGGCAGGACCTATGCCAGTGACCTACTCTAGTGCAGCACAA  10693
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Query  10694   AGAAGAGGGAGAATAGGAAGAAATCCAAAAAATGAGAATGACCAGTACATATACATGGGG  10753
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Query  10754   GAACCTCTGGAAAATGATGAAGACTGTGCACACTGGAAAGAAGCTAAAATGCTCCCAGAT  10813
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Sbjct  5950    GAACCTCTGGAAAATGATGAAGACTGTGCACACTGGAAAGAAGCTAAAATGCTCCTAGAT  6009

Query  10814   AACATCAACACGCCAGAAGGAATCATTCCTAGCATGTTCGAACCAGAGCGTGAAAAGGTG  10873
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Sbjct  6010    AACATCAACACGCCAGAAGGAATCATTCCTAGCATGTTCGAACCAGAGCGTGAAAAGGTG  6069

Query  10874   GATGCCATTGATGGCGAATACCGCTTGAGAGGAGAAGCAAGGAAAACCTTTGTAGACTTA  10933
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Sbjct  6070    GATGCCATTGATGGCGAATACCGCTTGAGAGGAGAAGCAAGGAAAACCTTTGTAGACTTA  6129

Query  10934   ATGAGAAGAGGAGACCTACCAGTCTGGTTGGCCTACAGAGTGGCAGCTGAAGGCATCAAC  10993
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Sbjct  6130    ATGAGAAGAGGAGACCTACCAGTCTGGTTGGCCTACAGAGTGGCAGCTGAAGGCATCAAC  6189

Query  10994   TACGCAGACAGAAGGTGGTGTTTTGATGGAGTCAAGAACAACCAAATCCTAGAAGAAAAC  11053
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Sbjct  6190    TACGCAGACAGAAGGTGGTGTTTTGATGGAGTCAAGAACAACCAAATCCTAGAAGAAAAC  6249

Query  11054   GTGGAAGTTGAAATCTGGACAAAAGAAGGGGAAAGGAAGAAATTGAAACCCAGATGGTTG  11113
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Sbjct  6250    GTGGAAGTTGAAATCTGGACAAAAGAAGGGGAAAGGAAGAAATTGAAACCCAGATGGTTG  6309

Query  11114   GATGCTAGGATCTATTCTGACCCACTGGCGCTAAAAGAATTTAAGGAATTTGCAGCCGGA  11173
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Sbjct  6310   GATGCTAGGATCTATTCTGACCCACTGGCGCTAAAAGAATTTAAGGAATTTGCAGCCGGA  6369

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Sbjct  6370   AGAAAGTCTCTGACCCTGAACCTAATCACAGAAATGGGTAGGCTCCCAACCTTCATGACT  6429

Query  11234  CAGAAGGTAAGAGACGCACTGGACAACTTAGCAGTGCTGCACACGGCTGAGGCAGGTGGA  11293
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Query  11294  AGGGCGTACAACCATGCTCTCAGTGAACTGCCGGAGACCCTGGAGACATTGCTTTTACTG  11353
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Query  11354  ACACTTCTGGCTACAGTCACGGGAGGGATCTTTTTATTCTTGATGAGCGGAAGGGGCATA  11413
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Sbjct  6550   ACACTTCTGGCTACAGTCACGGGAGGGATCTTTTTATTCTTGATGAGCGGAAGGGGCATA  6609

Query  11414  GGGAAGATGACCCTGGGAATGTGCTGCATAATCACGGCTAGCATCCTCCTATGGTACGCA  11473
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Sbjct  6610   GGGAAGATGACCCTGGGAATGTGCTGCATAATCACGGCTAGCATCCTCCTATGGTACGCA  6669

Query  11474  CAAATACAGCCACACTGGATAGCAGCTTCAATAATACTGGAGTTTTTTCTCATAGTTTTG  11533
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Sbjct  6670   CAAATACAGCCACACTGGATAGCAGCTTCAATAATACTGGAGTTTTTTCTCATAGTTTTG  6729

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Sbjct  6730   CTTATTCCAGAACCTGAAAAACAGAGAACACCCCAAGACAACCAACTGACCTACGTTGTC  6789

Query  11594  ATAGCCATCCTCACAGTGGTGGCCGCAACCATGGCAAACGAGATGGGTTTCCTAGAAAAA  11653
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Sbjct  6790   ATAGCCATCCTCACAGTGGTGGCCGCAACCATGGCAAACGAGATGGGTTTCCTAGAAAAA  6849
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Query  11654  ACGAAGAAAGATCTCGGATTGGGAAGCATTGCAACCCAGCAACCCGAGAGCAACATCCTG  11713
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Sbjct  6850   ACGAAGAAAGATCTCGGATTGGGAAGCATTGCAACCCAGCAACCCGAGAGCAACATCCTG  6909

Query  11714  GACATAGATCTACGTCCTGCATCAGCATGGACGCTGTATGCCGTGGCCACAACATTTGTT  11773
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Sbjct  6910   GACATAGATCTACGTCCTGCATCAGCATGGACGCTGTATGCCGTGGCCACAACATTTGTT  6969

Query  11774  ACACCAATGTTGAGACATAGCATTGAAAATTCCTCAGTGAATGTGTCCCTAACAGCTATA  11833
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Sbjct  6970   ACACCAATGTTGAGACATAGCATTGAAAATTCCTCAGTGAATGTGTCCCTAACAGCTATA  7029

Query  11834  GCCAACCAAGCCACAGTGTTAATGGGTCTCGGGAAAGGATGGCCATTGTCAAAGATGGAC  11893
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Sbjct  7030   GCCAACCAAGCCACAGTGTTAATGGGTCTCGGGAAAGGATGGCCATTGTCAAAGATGGAC  7089

Query  11894  ATCGGAGTTCCCCTTCTCGCCATTGGATGCTACTCACAAGTCAACCCCATAACTCTTACA  11953
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Query  11954  GCAGCTCTTTTCTTATTGGTAGCACATTATGCCATCATAGGGCCAGGACTCCAAGCAAAA  12013
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Sbjct  7150   GCAGCTCTTTTCTTATTGGTAGCACATTATGCCATCATAGGGCCAGGACTCCAAGCAAAA  7209

Query  12014  GCAACCAGAGAAGCTCAGAAAAGAGCAGCGGCGGGCATCATGAAAAACCCAACTGTCGAT  12073
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Query  12074  GGAATAACAGTGATTGACCTAGATCCAATACCTTATGATCCAAAGTTTGAAAAGCAGTTG  12133
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Sbjct  7270   GGAATAACAGTGATTGACCTAGATCCAATACCTTATGATCCAAAGTTTGAAAAGCAGTTG  7329
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Query  12134  GGACAAGTAATGCTCCTAGTCCTCTGCGTGACTCAAGTATTGATGATGAGGACTACATGG  12193
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Query  12194  GCTCTGTGTGAGGTTTTAACCTTAGCTACCGGGCCCATCTCCACATTGTGGGAAGGAAAT  12253
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Sbjct  7390   GCTCTGTGTGAGGCTTTAACCTTAGCTACCGGGCCCATCTCCACATTGTGGGAAGGAAAT  7449

Query  12254  CCAGGGAGGTTTTGGAACACTACCATTGCGGTGTCAATGGCTAACATTTTTAGAGGGAGT  12313
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Sbjct  7450   CCAGGGAGGTTTTGGAACACTACCATTGCGGTGTCAATGGCTAACATTTTTAGAGGGAGT  7509

Query  12314  TACTTGGCCGGAGCTGGACTTCTCTTTTCTATTATGAAGAACACAACCAACGCAAGAAGG  12373
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Sbjct  7510   TACTTGGCCGGAGCTGGACTTCTCTTTTCTATTATGAAGAACACAACCAACACAAGAAGG  7569

Query  12374  GGAACTGGCAACATAGGAGAGACGCTTGGAGAGAAATGGAAAAGCCGATTGAACGCATTG  12433
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  7570   GGAACTGGCAACATAGGAGAGACGCTTGGAGAGAAATGGAAAAGCCGATTGAACGCATTG  7629

Query  12434  GGAAAAAGTGAATTCCAGATCTACAAGAAAAGTGGAATCCAGGAAGTGGATAGAACCTTA  12493
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Query  12494  GCAAAAGAAGGCATTAAAAGAGGAGAAACGGACCATCACGCTGTGTCGCGAGGCTCAGCA  12553
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Query  12554  AAACTGAGATGGTTCGTTGAGAGAAACATGGTCACACCAGAAGGGAAAGTAGTGGACCTC  12613
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Sbjct  7750   AAACTGAGATGGTTCGTTGAGAGAAACATGGTCACACCAGAAGGGAAAGTAGTGGACCTC  7809

Query  12614  GGTTGTGGCAGAGGAGGCTGGTCATACTATTGTGGAGGACTAAAGAATGTAAGAGAAGTC  12673
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Query  12674   AAAGGCCTAACAAAAGGAGGACCAGGACACGAAGAACCCATCCCCATGTCAACATATGGG  12733
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Query  12794   GACACATTATTGTGTGACATAGGGGAGTCATCACCAAATCCCACAGTGGAAGCAGGACGA  12853
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Sbjct  7990    GACACATTATTGTGTGACATAGGGGAGTCATCACCAAATCCCACAGTGGAAGCAGGACGA  8049

Query  12854   ACACTCAGAGTCCTTAACTTAGTAGAAAATTGGTTGAACAACAACACTCAATTTTGCATA  12913
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Score = 300 bits (162), Expect = 7e-83

Identities = 162/162 (100%), Gaps = 0/162 (0%)

Strand=Plus/Plus

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APPENDIX Q - ABBREVIATIONS

Abbreviation	Full name
AD	Activation domain
ADE	Antibody-dependent enhancement
ATF-6	activating transcription factor 6
AVEXIS	avidity-based extracellular interaction screen
B23	nucleolar phosphoprotein B23, numatrin
BCL2	B-cell CLL/lymphoma 2
BCL2L1	BCL2-like 1
BCL2L10	BCL2-like 10 (apoptosis facilitator)
BCL2L11	BCL2-like 11 (apoptosis facilitator)
BD or DBD	DNA binding domain
bp	base pair(s)
BRET	bioluminescence resonance energy transfer
BSA	Bovine serum albumin
C	Capsid protein
C1	complement component 1
C4	complement component 4
C4BP	complement component 4 binding protein
CA	Nascent capsid
CALCOCO2	calcium binding and coiled-coil domain 2
CALR	calreticulin
CDK or Cdk	Cyclin-dependent kinase
CFU	Colony-forming unit
Cks30A	Cyclin-dependent kinase subunit 30A
Cks85A	Cyclin-dependent kinase subunit 85A
CLEC5A	C-type lectin domain family 5, member A
ConA	Concanavalin A
CRM-1	chromosome region maintenance 1 protein homolog (exportin)
Cul-4	Cullin-4
CV	Mature capsid
DABCO	1,4-diazabicyclo[2.2.2]octane
DAPI	4',6-diamidino-2-phenylindole
DAXX	Dead-domain associated protein
DC-SIGN	dendritic cell-specific intracellular adhesion molecules (ICAM)-3 grabbing non-integrin
DDX3X	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked
DDX56	DEAD (Asp-Glu-Ala-Asp) box helicase 56
DENV	dengue virus
DERL2	derlin 2
DF	Dengue fever
DGKζ	diacylglycerol kinase zeta (DGKζ)
DHF	Dengue hemorrhagic fever

DIAP-1	Drosophila Inhibitor of apoptosis protein 1
DMSO	Dimethyl sulfoxide
dsRNA	double-stranded RNA
DSS	Dengue shock syndrome
DVHF	Dengue Virus Host Factor
E protein	Envelope protein
EBV	Epstein-Barr virus
EDTA	ethylenediaminetetraacetic acid
EGFP	Enhanced Green fluorescent protein
eIF2α	eukaryotic translation Initiation Factor 2alpha
eIF3-S8	Eukaryotic translation initiation factor 3 subunit 8
Eiii	domain III of envelope protein
Eip63E	Ecdysone-induced protein 63E
ER	Endoplasmic reticulum
ERC1	ELKS/RAB6-interacting/CAST family member 1
EYFP	Enhanced yellow fluorescent protein
FACS	fluorescence-activated cell sorting
FASN	fatty acid synthase
FBS	Fetal bovine serum
FITC	Fluorescein isothiocyanate
FMDV	foot-and-mouth disease virus
FOX-2	RNA binding protein, fox-1 homolog (C. elegans) 2
FRET	Förster resonance energy transfer
GFP	Green fluorescent protein
GO	Gene ontology
GOLGA2	golgin A2
GOLGB1	golgin B1
GTPBP4	GTP-binding protein 4
gus	gustavus
HBB	hemoglobin, beta
HCV	Hepatitis C virus
HDLs	High-density lipoprotein
HDM2	p53 E3 ubiquitin protein ligase homolog (mouse)
HIV	Human immunodeficiency virus
Hsp70	heat shock protein 70
Hsp90	heat shock protein 90
HSPA5/Grp78/BiP	heat shock 70kDa protein 5
IAP	Inhibitor of apoptosis protein
IL-6	Interleukin 6
IL-8	Interleukin 8
IRE-1	endoplasmic reticulum to nucleus signaling 1
IRES	Internal ribosome entry site
Jak	Janus kinase
JEV	Japanese encephlitis virus

kDa	kilodalton
LAV	Live attenuated virus
LRRFIP1	leucine rich repeat (in FLII) interacting protein 1
M protein	Membrane protein
MATR3	matrin 3
Mtase	methyltransferase
N-TAP	N-terminal tandem affinity purification tag
NAP1	Nucleosome assembly protein 1
NAP1L1	Nucleosome assembly protein 1-like 1
NAP1L2	Nucleosome assembly protein 1-like 2
NAP1L3	Nucleosome assembly protein 1-like 3
NAP1L4	Nucleosome assembly protein 1-like 4
NAP1L5	Nucleosome assembly protein 1-like 5
NAP1L6	Nucleosome assembly protein 1-like 6
NBP1	NAP1 binding protein 1
ND10	Nuclear Domain 10
NES	nuclear export sequence
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NFYA	nuclear transcription factor Y, alpha
NLS	Nuclear localization signal
NRBP1	nuclear receptor binding protein 1
NS1	Non structural protein 1
NS2A	Non structural protein 2A
NS2B	Non structural protein 2B
NS3	Non structural protein 3
NS4A	Non structural protein 4A
NS4B	Non structural protein 4B
NS5	Non structural protein 5
OBSCN	obscurin
ORF	open reading frames
OS9	osteosarcoma 9
pac	Puromycin resistance gene
PBL	peripheral blood leukocyte
PCA	protein-fragment complementation assays
PEG	Polyethylene glycol
PERK	protein kinase RNA-like endoplasmic reticulum kinase
PKG	Protein kinase G
PMSF	phenylmethanesulfonylfluoride
PPI	Protein-protein interaction
PPP1R15A	protein phosphatase 1, regulatory subunit 15A
PrM	Precursor of membrane protein
PTB	polypyrimidine tract binding protein 1
RdRp	RNA-dependent RNA polymerase

RILPL2	Rab interacting lysosomal protein-like 2
Rluc	*Renilla* luciferase
RNAi	RNA interferene
RPL23	ribosomal protein L23
RPL27	ribosomal protein L27
RpL32	Ribosomal protein L32
RPL5	ribosomal protein L5
RPL6	ribosomal protein L6
RPL7	ribosomal protein L7
RRP12	ribosomal RNA processing protein
SET	SET nuclear oncogene
SIAH2	Seven In Absentia Homolog 2
siRNA	Small interfering RNA
SIT	sterile insect technique
SSB	Sjogren syndrome antigen B (autoantigen La)
STAT1	signal transducer and activator of transcription 1
STAT2	signal transducer and activator of transcription 2
SUMO	Small ubiquitin-like modifier
TAP-MS	by tandem affinity purification-mass spectrometry
TaV	*Thosea asigna* virus
TIF-IA	transcription initiation factor IA
TRAF2	tumor necrosis factor receptor-associated factor 2
TRIP11	thyroid hormone receptor interactor 11
UBE2I	ubiquitin-conjugating enzyme E2I
UBF	upstream binding factor
UPR	Unfolded protein response
UTR	Untranslated region
WNV	West Nile virus
XBP1	X-box binding protein 1
Y2H	Yeast two-hybrid
YFV	Yellow fever virus
ZNF410	zinc finger protein 410
ZO-1	zona occludens 1

REFERENCES

2012. Reorganizing the protein space at the Universal Protein Resource (UniProt). *Nucleic Acids Res*. 40:D71-75.

Ackermann, M., and R. Padmanabhan. 2001. De novo synthesis of RNA by the dengue virus RNA-dependent RNA polymerase exhibits temperature dependence at the initiation but not elongation phase. *J Biol Chem*. 276:39926-39937.

Aden, D.P., A. Fogel, S. Plotkin, I. Damjanov, and B.B. Knowles. 1979. Controlled synthesis of HBsAg in a differentiated human liver carcinoma-derived cell line. *Nature*. 282:615-616.

Alcaraz-Estrada, S.L., M.I. Manzano, R.M. Del Angel, R. Levis, and R. Padmanabhan. 2010. Construction of a dengue virus type 4 reporter replicon and analysis of temperature-sensitive mutations in non-structural proteins 3 and 5. *J Gen Virol*. 91:2713-2718.

Alcock, F., and E. Swanton. 2009. Mammalian OS-9 is upregulated in response to endoplasmic reticulum stress and facilitates ubiquitination of misfolded glycoproteins. *J Mol Biol*. 385:1032-1042.

Alphey, L. 2002. Re-engineering the sterile insect technique. *Insect Biochem Mol Biol*. 32:1243-1247.

Altman, R., and D. Kellogg. 1997. Control of mitotic events by Nap1 and the Gin4 kinase. *J Cell Biol*. 138:119-130.

Angel, B., and V. Joshi. 2008. Distribution and seasonality of vertically transmitted dengue viruses in Aedes mosquitoes in arid and semi-arid areas of Rajasthan, India. *J Vector Borne Dis*. 45:56-59.

Anwar, A., K.M. Leong, M.L. Ng, J.J. Chu, and M.A. Garcia-Blanco. 2009. The polypyrimidine tract-binding protein is required for efficient dengue virus propagation and associates with the viral replication machinery. *J Biol Chem*. 284:17021-17029.

Aoki, C., K.I. Hidari, S. Itonori, A. Yamada, N. Takahashi, T. Kasama, F. Hasebe, M.A. Islam, K. Hatano, K. Matsuoka, T. Taki, C.T. Guo, T. Takahashi, Y. Sakano, T. Suzuki, D. Miyamoto, M. Sugita, D. Terunuma, K. Morita, and Y. Suzuki. 2006. Identification and characterization of carbohydrate molecules in mammalian cells recognized by dengue virus type 2. *J Biochem*. 139:607-614.

Arakawa, R., A. Bagashev, L. Song, K. Maurer, and K.E. Sullivan. 2010. Characterization of LRRFIP1. *Biochem Cell Biol*. 88:899-906.

Aravind, L., and E.V. Koonin. 1999. G-patch: a new conserved domain in eukaryotic RNA-processing proteins and type D retroviral polyproteins. *Trends Biochem Sci*. 24:342-344.

Arias, C.F., F. Preugschat, and J.H. Strauss. 1993. Dengue 2 virus NS2B and NS3 form a stable complex that can cleave NS3 within the helicase domain. *Virology*. 193:888-899.

Ashburner, M., C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, and G. Sherlock. 2000. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet.* 25:25-29.

Ashour, J., M. Laurent-Rolle, P.Y. Shi, and A. Garcia-Sastre. 2009. NS5 of dengue virus mediates STAT2 binding and degradation. *J Virol*. 83:5408-5418.

Attia, M., A. Forster, C. Rachez, P. Freemont, P. Avner, and U.C. Rogner. 2011. Interaction between nucleosome assembly protein 1-like family members. *J Mol Biol*. 407:647-660.

Avirutnan, P., A. Fuchs, R.E. Hauhart, P. Somnuke, S. Youn, M.S. Diamond, and J.P. Atkinson. 2010. Antagonism of the complement component C4 by flavivirus nonstructural protein NS1. *J Exp Med*. 207:793-806.

Avirutnan, P., R.E. Hauhart, P. Somnuke, A.M. Blom, M.S. Diamond, and J.P. Atkinson. 2011. Binding of flavivirus nonstructural protein NS1 to C4b binding protein modulates complement activation. *J Immunol*. 187:424-433. .

Balmaseda, A., S.N. Hammond, L. Perez, Y. Tellez, S.I. Saborio, J.C. Mercado, R. Cuadra, J. Rocha, M.A. Perez, S. Silva, C. Rocha, and E. Harris. 2006. Serotype-specific differences in clinical manifestations of dengue. *Am J Trop Med Hyg.* 74:449-456.

Barban, V., J.L. Munoz-Jordan, G.A. Santiago, N. Mantel, Y. Girerd, S. Gulia, J.B. Claude, and J. Lang. 2012. Broad neutralization of wild-type dengue virus isolates following immunization in monkeys with a tetravalent dengue vaccine based on chimeric yellow fever 17D/dengue viruses. *Virology*. 429:91-98.

Barrell, D., E. Dimmer, R.P. Huntley, D. Binns, C. O'Donovan, and R. Apweiler. 2009. The GOA database in 2009--an integrated Gene Ontology Annotation resource. *Nucleic Acids Res.* 37:D396-403.

Bartelma, G., and R. Padmanabhan. 2002. Expression, purification, and characterization of the RNA 5'-triphosphatase activity of dengue virus type 2 nonstructural protein 3. *Virology*. 299:122-132.

Benarroch, D., M.P. Egloff, L. Mulard, C. Guerreiro, J.L. Romette, and B. Canard. 2004. A structural basis for the inhibition of the NS5 dengue virus mRNA 2'-O-methyltransferase domain by ribavirin 5'-triphosphate. *J Biol Chem*. 279:35638-35643.

Bhattacharya, D., Mayuri, S.M. Best, R. Perera, R.J. Kuhn, and R. Striker. 2009. Protein kinase G phosphorylates mosquito-borne flavivirus NS5. *J Virol*. 83:9195-9205.

Bhuvanakantham, R., M.K. Chong, and M.L. Ng. 2009. Specific interaction of capsid protein and importin-alpha/beta influences West Nile virus production. *Biochem Biophys Res Commun.* 389:63-69.

Black, W.C.t., K.E. Bennett, N. Gorrochotegui-Escalante, C.V. Barillas-Mury, I. Fernandez-Salas, M. de Lourdes Munoz, J.A. Farfan-Ale, K.E. Olson, and B.J. Beaty. 2002. Flavivirus susceptibility in Aedes aegypti. *Arch Med Res*. 33:379-388.

Blaney, J.E., Jr., G.G. Manipon, B.R. Murphy, and S.S. Whitehead. 2003. Temperature sensitive mutations in the genes encoding the NS1, NS2A, NS3, and NS5 nonstructural proteins of dengue virus type 4 restrict replication in the brains of mice. *Arch Virol*. 148:999-1006.

Bowman, A., R. Ward, N. Wiechens, V. Singh, H. El-Mkami, D.G. Norman, and T. Owen-Hughes. 2011. The histone chaperones Nap1 and Vps75 bind histones H3 and H4 in a tetrameric conformation. *Mol Cell*. 41:398-408.

Brackney, D.E., J.C. Scott, F. Sagawa, J.E. Woodward, N.A. Miller, F.D. Schilkey, J. Mudge, J. Wilusz, K.E. Olson, C.D. Blair, and G.D. Ebel. 2010. C6/36 Aedes albopictus cells have a dysfunctional antiviral RNA interference response. *PLoS Negl Trop Dis*. 4:e856.

Brault, J.B., M. Kudelko, P.O. Vidalain, F. Tangy, P. Despres, and N. Pardigon. 2011. The interaction of flavivirus M protein with light chain Tctex-1 of human dynein plays a role in late stages of virus replication. *Virology*. 417:369-378.

Braun, P., M. Tasan, M. Dreze, M. Barrios-Rodiles, I. Lemmens, H. Yu, J.M. Sahalie, R.R. Murray, L. Roncari, A.S. de Smet, K. Venkatesan, J.F. Rual, J. Vandenhaute, M.E. Cusick, T. Pawson, D.E. Hill, J. Tavernier, J.L. Wrana, F.P. Roth, and M. Vidal. 2009. An experimentally derived confidence score for binary protein-protein interactions. *Nat Methods*. 6:91-97.

Brooks, A.J., M. Johansson, A.V. John, Y. Xu, D.A. Jans, and S.G. Vasudevan. 2002. The interdomain region of dengue NS5 protein that binds to the viral helicase NS3 contains independently functional importin beta 1 and importin alpha/beta-recognized nuclear localization signals. *J Biol Chem*. 277:36399-36407.

Bulich, R., and J.G. Aaskov. 1992. Nuclear localization of dengue 2 virus core protein detected with monoclonal antibodies. *J Gen Virol*. 73 (Pt 11):2999-3003.

Bushell, K.M., C. Sollner, B. Schuster-Boeckler, A. Bateman, and G.J. Wright. 2008. Large-scale screening for novel low-affinity extracellular protein interactions. *Genome Res*. 18:622-630.

Cahour, A., B. Falgout, and C.J. Lai. 1992. Cleavage of the dengue virus polyprotein at the NS3/NS4A and NS4B/NS5 junctions is mediated by viral protease NS2B-

NS3, whereas NS4A/NS4B may be processed by a cellular protease. *J Virol*. 66:1535-1542.

Calderwood, M.A., K. Venkatesan, L. Xing, M.R. Chase, A. Vazquez, A.M. Holthaus, A.E. Ewence, N. Li, T. Hirozane-Kishikawa, D.E. Hill, M. Vidal, E. Kieff, and E. Johannsen. 2007. Epstein-Barr virus and virus human protein interaction maps. *Proc Natl Acad Sci U S A*. 104:7606-7611.

Camacho, C., G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, and T.L. Madden. 2009. BLAST+: architecture and applications. *BMC Bioinformatics*. 10:421.

Cao-Lormeau, V.M. 2009. Dengue viruses binding proteins from Aedes aegypti and Aedes polynesiensis salivary glands. *Virol J*. 6:35.

CDC. 2010. Locally acquired Dengue--Key West, Florida, 2009-2010. *MMWR*. 59:577-581.

Chang, C.J., H.W. Luh, S.H. Wang, H.J. Lin, S.C. Lee, and S.T. Hu. 2001. The heterogeneous nuclear ribonucleoprotein K (hnRNP K) interacts with dengue virus core protein. *DNA Cell Biol*. 20:569-577.

Chang, J., W. Schul, T.D. Butters, A. Yip, B. Liu, A. Goh, S.B. Lakshminarayana, D. Alonzi, G. Reinkensmeier, X. Pan, X. Qu, J.M. Weidner, L. Wang, W. Yu, N. Borune, M.A. Kinch, J.E. Rayahin, R. Moriarty, X. Xu, P.Y. Shi, J.T. Guo, and T.M. Block. 2011. Combination of alpha-glucosidase inhibitor and ribavirin for the treatment of dengue virus infection in vitro and in vivo. *Antiviral Res*. 89:26-34.

Chatr-aryamontri, A., A. Ceol, D. Peluso, A. Nardozza, S. Panni, F. Sacco, M. Tinti, A. Smolyar, L. Castagnoli, M. Vidal, M.E. Cusick, and G. Cesareni. 2009. VirusMINT: a viral protein interaction database. *Nucleic Acids Res*. 37:D669-673.

Chee, H.Y., and S. AbuBakar. 2004. Identification of a 48kDa tubulin or tubulin-like C6/36 mosquito cells protein that binds dengue virus 2 using mass spectrometry. *Biochem Biophys Res Commun*. 320:11-17.

Chen, S.T., Y.L. Lin, M.T. Huang, M.F. Wu, S.C. Cheng, H.Y. Lei, C.K. Lee, T.W. Chiou, C.H. Wong, and S.L. Hsieh. 2008. CLEC5A is critical for dengue-virus-induced lethal disease. *Nature*. 453:672-676.

Chen, Y.C., S.Y. Wang, and C.C. King. 1999. Bacterial lipopolysaccharide inhibits dengue virus infection of primary human monocytes/macrophages by blockade of virus entry via a CD14-dependent mechanism. *J Virol*. 73:2650-2657.

Chiu, M.W., H.M. Shih, T.H. Yang, and Y.L. Yang. 2007. The type 2 dengue virus envelope protein interacts with small ubiquitin-like modifier-1 (SUMO-1) conjugating enzyme 9 (Ubc9). *J Biomed Sci*. 14:429-444.

Chua, J.J., R. Bhuvanakantham, V.T. Chow, and M.L. Ng. 2005. Recombinant non-structural 1 (NS1) protein of dengue-2 virus interacts with human STAT3beta protein. *Virus Res*. 112:85-94.

Chua, J.J., M.M. Ng, and V.T. Chow. 2004. The non-structural 3 (NS3) protein of dengue virus type 2 interacts with human nuclear receptor binding protein and is associated with alterations in membrane structure. *Virus Res.* 102:151-163.

Chuang, V.W., T.Y. Wong, Y.H. Leung, E.S. Ma, Y.L. Law, O.T. Tsang, K.M. Chan, I.H. Tsang, T.L. Que, R.W. Yung, and S.H. Liu. 2008. Review of dengue fever cases in Hong Kong during 1998 to 2005. *Hong Kong Med J*. 14:170-177.

Chye, J.K., C.T. Lim, K.B. Ng, J.M. Lim, R. George, and S.K. Lam. 1997. Vertical transmission of dengue. *Clin Infect Dis*. 25:1374-1377.

Clemens, J.C., C.A. Worby, N. Simonson-Leff, M. Muda, T. Maehama, B.A. Hemmings, and J.E. Dixon. 2000. Use of double-stranded RNA interference in Drosophila cell lines to dissect signal transduction pathways. *Proc Natl Acad Sci U S A*. 97:6499-6503.

Clum, S., K.E. Ebner, and R. Padmanabhan. 1997. Cotranslational membrane insertion of the serine proteinase precursor NS2B-NS3(Pro) of dengue virus type 2 is required for efficient in vitro processing and is mediated through the hydrophobic regions of NS2B. *J Biol Chem*. 272:30715-30723.

Colas, P., B. Cohen, T. Jessen, I. Grishina, J. McCoy, and R. Brent. 1996. Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. *Nature*. 380:548-550.

Colpitts, T.M., S. Barthel, P. Wang, and E. Fikrig. 2011a. Dengue virus capsid protein binds core histones and inhibits nucleosome formation in human liver cells. *PLoS One*. 6:e24365.

Colpitts, T.M., J. Cox, A. Nguyen, F. Feitosa, M.N. Krishnan, and E. Fikrig. 2011b. Use of a tandem affinity purification assay to detect interactions between West Nile and dengue viral proteins and proteins of the mosquito vector. *Virology.* 417:179-187.

Crill, W.D., and J.T. Roehrig. 2001. Monoclonal antibodies that bind to domain III of dengue virus E glycoprotein are the most efficient blockers of virus adsorption to Vero cells. *J Virol*. 75:7769-7773.

da Silva Voorham, J.M., I.A. Rodenhuis-Zybert, N.V. Ayala Nunez, T.M. Colpitts, H. van der Ende-Metselaar, E. Fikrig, M.S. Diamond, J. Wilschut, and J.M. Smit. 2012. Antibodies against the envelope glycoprotein promote infectivity of immature dengue virus serotype 2. *PLoS One*. 7:e29957.

Dame, D.A., C.F. Curtis, M.Q. Benedict, A.S. Robinson, and B.G. Knols. 2009. Historical applications of induced sterilisation in field populations of mosquitoes. *Malar J*. 8 Suppl 2:S2.

Davidson, G., J. Shen, Y.L. Huang, Y. Su, E. Karaulanov, K. Bartscherer, C. Hassler, P. Stannek, M. Boutros, and C. Niehrs. 2009. Cell cycle control of wnt receptor activation. *Dev Cell*. 17:788-799.

de Chassey, B., V. Navratil, L. Tafforeau, M.S. Hiet, A. Aublin-Gex, S. Agaugue, G. Meiffren, F. Pradezynski, B.F. Faria, T. Chantier, M. Le Breton, J. Pellet, N. Davoust, P.E. Mangeot, A. Chaboud, F. Penin, Y. Jacob, P.O. Vidalain, M. Vidal, P. Andre, C. Rabourdin-Combe, and V. Lotteau. 2008. Hepatitis C virus infection protein network. *Mol Syst Biol*. 4:230.

Deane, C.M., L. Salwinski, I. Xenarios, and D. Eisenberg. 2002. Protein interactions: two methods for assessment of the reliability of high throughput observations. *Mol Cell Proteomics*. 1:349-356.

Dejnirattisai, W., A. Jumnainsong, N. Onsirisakul, P. Fitton, S. Vasanawathana, W. Limpitikul, C. Puttikhunt, C. Edwards, T. Duangchinda, S. Supasa, K.

Chawansuntati, P. Malasit, J. Mongkolsapaya, and G. Screaton. 2010. Cross-reacting antibodies enhance dengue virus infection in humans. *Science*. 328:745-748.

Diamond, M.S., T.C. Pierson, and D.H. Fremont. 2008. The structural immunology of antibody protection against West Nile virus. *Immunol Rev*. 225:212-225.

Doolittle, J.M., and S.M. Gomez. 2011. Mapping protein interactions between Dengue virus and its human and insect hosts. *PLoS*. 5:e954.

Doronina, V.A., C. Wu, P. de Felipe, M.S. Sachs, M.D. Ryan, and J.D. Brown. 2008. Site-specific release of nascent chains from ribosomes at a sense codon. *Mol Cell Biol*. 28:4227-4239.

Dunham, E.J., and E.C. Holmes. 2007. Inferring the timescale of dengue virus evolution under realistic models of DNA substitution. *J Mol Evol*. 64:656-661.

Durbin, A.P., J.H. McArthur, J.A. Marron, J.E. Blaney, B. Thumar, K. Wanionek, B.R. Murphy, and S.S. Whitehead. 2006. rDEN2/4Delta30(ME), a live attenuated chimeric dengue serotype 2 vaccine is safe and highly immunogenic in healthy dengue-naive adults. *Hum Vaccin*. 2:255-260.

Dyer, M.D., T.M. Murali, and B.W. Sobral. 2008. The landscape of human proteins interacting with viruses and other pathogens. *PLoS Pathog*. 4:e32.

Edelman, R. 2007. Dengue vaccines approach the finish line. *Clin Infect Dis*. 45 Suppl 1:S56-60.

Egloff, M.P., D. Benarroch, B. Selisko, J.L. Romette, and B. Canard. 2002. An RNA cap (nucleoside-2'-O-)-methyltransferase in the flavivirus RNA polymerase NS5: crystal structure and functional characterization. *EMBO J*. 21:2757-2768.

Ellencrona, K., A. Syed, and M. Johansson. 2009. Flavivirus NS5 associates with host-cell proteins zonula occludens-1 (ZO-1) and regulating synaptic membrane exocytosis-2 (RIMS2) via an internal PDZ binding mechanism. *Biol Chem*. 390:319-323.

Engeland, C.E., H. Oberwinkler, M. Schumann, E. Krause, G.A. Muller, and H.G. Krausslich. 2011. The cellular protein lyric interacts with HIV-1 Gag. *J Virol*. 85:13322-13332.

Everett, R.D., and M.K. Chelbi-Alix. 2007. PML and PML nuclear bodies: implications in antiviral defence. *Biochimie*. 89:819-830.

Falgout, B., M. Pethel, Y.M. Zhang, and C.J. Lai. 1991. Both nonstructural proteins NS2B and NS3 are required for the proteolytic processing of dengue virus nonstructural proteins. *J Virol*. 65:2467-2475.

Fields, S., and O. Song. 1989. A novel genetic system to detect protein-protein interactions. *Nature*. 340:245-246.

Fink, J., F. Gu, L. Ling, T. Tolfvenstam, F. Olfat, K.C. Chin, P. Aw, J. George, V.A. Kuznetsov, M. Schreiber, S.G. Vasudevan, and M.L. Hibberd. 2007. Host gene expression profiling of dengue virus infection in cell lines and patients. *PLoS Negl Trop Dis*. 1:e86.

Finkelstein, Y., O. Faktor, O. Elroy-Stein, and B.Z. Levi. 1999. The use of bi-cistronic transfer vectors for the baculovirus expression system. *J Biotechnol*. 75:33-44.

Finley, R.L., Jr., and R. Brent. 1994. Interaction mating reveals binary and ternary connections between Drosophila cell cycle regulators. *Proc Natl Acad Sci U S A.* 91:12980-12984.

Fischl, W., and R. Bartenschlager. 2011. Exploitation of cellular pathways by Dengue virus. *Curr Opin Microbiol*. 14:470-475.

Flamand, M., F. Megret, M. Mathieu, J. Lepault, F.A. Rey, and V. Deubel. 1999. Dengue virus type 1 nonstructural glycoprotein NS1 is secreted from mammalian cells as a soluble hexamer in a glycosylation-dependent fashion. *J Virol*. 73:6104-6110.

Foley, E., and P.H. O'Farrell. 2004. Functional dissection of an innate immune response by a genome-wide RNAi screen. *PLoS Biol.* 2:E203.

Folly, B.B., A.M. Weffort-Santos, C.G. Fathman, and L.R. Soares. 2011. Dengue-2 structural proteins associate with human proteins to produce a coagulation and innate immune response biased interactome. *BMC Infect Dis*. 11:34.

Fradin, M.S., and J.F. Day. 2002. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med*. 347:13-18.

Fry, S.R., M. Meyer, M.G. Semple, C.P. Simmons, S.D. Sekaran, J.X. Huang, C. McElnea, C.Y. Huang, A. Valks, P.R. Young, and M.A. Cooper. 2011. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis*. 5:e1199.

Fu, W., B.E. Sanders-Beer, K.S. Katz, D.R. Maglott, K.D. Pruitt, and R.G. Ptak. 2009. Human immunodeficiency virus type 1, human protein interaction database at NCBI. *Nucleic Acids Res*. 37:D417-422.

Gagnon, S.J., F.A. Ennis, and A.L. Rothman. 1999. Bystander target cell lysis and cytokine production by dengue virus-specific human CD4(+) cytotoxic T-lymphocyte clones. *J Virol*. 73:3623-3629.

Garcia-Montalvo, B.M., F. Medina, and R.M. del Angel. 2004. La protein binds to NS5 and NS3 and to the 5' and 3' ends of Dengue 4 virus RNA. *Virus Res*. 102:141-150.

Giard, D.J., S.A. Aaronson, G.J. Todaro, P. Arnstein, J.H. Kersey, H. Dosik, and W.P. Parks. 1973. In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst*. 51:1417-1423.

Gibbons, R.V., and D.W. Vaughn. 2002. Dengue: an escalating problem. *BMJ*. 324:1563-1566.

Gietz, D., A. St Jean, R.A. Woods, and R.H. Schiestl. 1992. Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* 20:1425.

Gifford, R., P. Kabat, J. Martin, C. Lynch, and M. Tristem. 2005. Evolution and distribution of class II-related endogenous retroviruses. *J Virol*. 79:6478-6486.

Giot, L., J.S. Bader, C. Brouwer, A. Chaudhuri, B. Kuang, Y. Li, Y.L. Hao, C.E. Ooi, B. Godwin, E. Vitols, G. Vijayadamodar, P. Pochart, H. Machineni, M. Welsh, Y. Kong, B. Zerhusen, R. Malcolm, Z. Varrone, A. Collis, M. Minto, S. Burgess, L. McDaniel, E. Stimpson, F. Spriggs, J. Williams, K. Neurath, N. Ioime, M. Agee, E. Voss, K. Furtak, R. Renzulli, N. Aanensen, S. Carrolla, E. Bickelhaupt, Y. Lazovatsky, A. DaSilva, J. Zhong, C.A. Stanyon, R.L. Finley, Jr., K.P. White, M. Braverman, T. Jarvie, S. Gold, M. Leach, J. Knight, R.A. Shimkets, M.P. McKenna, J. Chant, and J.M. Rothberg. 2003. A protein interaction map of Drosophila melanogaster. *Science.* 302:1727-1736.

Golemis, E.A., I. Serebriiskii, R.L. Finley, Jr., M.G. Kolonin, J. Gyuris, and R. Brent. 2001. Interaction trap/two-hybrid system to identify interacting proteins. *Curr Protoc Mol Biol.* Chapter:Unit 20.21.

Gorbalenya, A.E., A.P. Donchenko, E.V. Koonin, and V.M. Blinov. 1989. N-terminal domains of putative helicases of flavi- and pestiviruses may be serine proteases. *Nucleic Acids Res*. 17:3889-3897.

Gubler, D.J. 1988. Dengue. *In* Epidemiology of arthropod-borne viral diseases. Vol. II T.P. Monath, editor. CRC Press, Inc., Boca Raton, Fla. 223-260.

Gubler, D.J. 1998. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev.* 11:480-496.

Gubler, D.J., G. Kuno, and L. Markoff. 2006. Flaviviruses. *In* Fields Virology. D.M. Knipe and P.M. Howley, editors. Lippincott Williams & Wilkins, Philadelphia, USA. 1153-1252.

Gubler, D.J., and L. Rosen. 1976. A simple technique for demonstrating transmission of dengue virus by mosquitoes without the use of vertebrate hosts. *Am J Trop Med Hyg*. 25:146-150.

Guest, S.T., J. Yu, D. Liu, J.A. Hines, M.A. Kashat, and R.L. Finley, Jr. 2011. A protein network-guided screen for cell cycle regulators in Drosophila. *BMC Syst Biol*. 5:65.

Guo, D., T.R. Hazbun, X.J. Xu, S.L. Ng, S. Fields, and M.H. Kuo. 2004. A tethered catalysis, two-hybrid system to identify protein-protein interactions requiring post-translational modifications. *Nat Biotechnol*. 22:888-892.

Guo, X., Y. Xu, G. Bian, A.D. Pike, Y. Xie, and Z. Xi. 2010. Response of the mosquito protein interaction network to dengue infection. *BMC Genomics*. 11:380.

Guo, X., T. Zhao, Y. Dong, and B. Lu. 2007. Survival and replication of dengue-2 virus in diapausing eggs of Aedes albopictus (Diptera: Culicidae). *J Med Entomol*. 44:492-497.

Gutsche, I., F. Coulibaly, J.E. Voss, J. Salmon, J. d'Alayer, M. Ermonval, E. Larquet, P. Charneau, T. Krey, F. Megret, E. Guittet, F.A. Rey, and M. Flamand. 2011. Secreted dengue virus nonstructural protein NS1 is an atypical barrel-shaped high-density lipoprotein. *Proc Natl Acad Sci U S A*. 108:8003-8008.

Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell.* 75:791-803.

Hagemeier, S.R., E.A. Barlow, A.A. Kleman, and S.C. Kenney. 2011. The Epstein-Barr virus BRRF1 protein, Na, induces lytic infection in a TRAF2- and p53-dependent manner. *J Virol*. 85:4318-4329.

Hales, S., N. de Wet, J. Maindonald, and A. Woodward. 2002. Potential effect of population and climate changes on global distribution of dengue fever: an empirical model. *Lancet*. 360:830-834.

Halstead, S.B. 2007. Dengue. *Lancet*. 370:1644-1652.

Halstead, S.B., and E.J. O'Rourke. 1977. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med*. 146:201-217.

Hang, V.T., N.M. Nguyet, D.T. Trung, V. Tricou, S. Yoksan, N.M. Dung, T. Van Ngoc, T.T. Hien, J. Farrar, B. Wills, and C.P. Simmons. 2009. Diagnostic accuracy of

NS1 ELISA and lateral flow rapid tests for dengue sensitivity, specificity and relationship to viraemia and antibody responses. *PLoS Negl Trop Dis*. 3:e360.

Hastie, A.R., and S.C. Pruitt. 2007. Yeast two-hybrid interaction partner screening through in vivo Cre-mediated Binary Interaction Tag generation. *Nucleic Acids Res*. 35:e141.

Heaton, N.S., R. Perera, K.L. Berger, S. Khadka, D.J. Lacount, R.J. Kuhn, and G. Randall. 2010. Dengue virus nonstructural protein 3 redistributes fatty acid synthase to sites of viral replication and increases cellular fatty acid synthesis. *Proc Natl Acad Sci U S A*. 107:17345-17350.

Heaton, N.S., and G. Randall. 2010. Dengue virus-induced autophagy regulates lipid metabolism. *Cell Host Microbe*. 8:422-432.

Hendrichs, J., A.S. Robinson, J.P. Cayol, and W. Enkerlin. 2002. Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: The importance of mating behavior studies. *Florida Entomologist*. 85:1-13.

Hershkovitz, O., B. Rosental, L.A. Rosenberg, M.E. Navarro-Sanchez, S. Jivov, A. Zilka, O. Gershoni-Yahalom, E. Brient-Litzler, H. Bedouelle, J.W. Ho, K.S. Campbell, B. Rager-Zisman, P. Despres, and A. Porgador. 2009. NKp44 receptor mediates interaction of the envelope glycoproteins from the West Nile and dengue viruses with NK cells. *J Immunol*. 183:2610-2621.

Hiscox, J.A. 2007. RNA viruses: hijacking the dynamic nucleolus. *Nat Rev Microbiol*. 5:119-127.

Hiscox, J.A., A. Whitehouse, and D.A. Matthews. 2010. Nucleolar proteomics and viral infection. *Proteomics*. 10:4077-4086.

Hoffmann, A.A., B.L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P.H. Johnson, F. Muzzi, M. Greenfield, M. Durkan, Y.S. Leong, Y. Dong, H. Cook, J. Axford, A.G. Callahan, N. Kenny, C. Omodei, E.A. McGraw, P.A. Ryan, S.A. Ritchie, M. Turelli, and S.L. O'Neill. 2011. Successful establishment of Wolbachia in Aedes populations to suppress dengue transmission. *Nature*. 476:454-457.

Hsu, Y.C., N.C. Chen, P.C. Chen, C.C. Wang, W.C. Cheng, and H.N. Wu. 2012. Identification of a small-molecule inhibitor of dengue virus using a replicon system. *Arch Virol*. 157:681-688.

Hu, C.D., Y. Chinenov, and T.K. Kerppola. 2002. Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. *Mol Cell*. 9:789-798.

Hunter, S., P. Jones, A. Mitchell, R. Apweiler, T.K. Attwood, A. Bateman, T. Bernard, D. Binns, P. Bork, S. Burge, E. de Castro, P. Coggill, M. Corbett, U. Das, L. Daugherty, L. Duquenne, R.D. Finn, M. Fraser, J. Gough, D. Haft, N. Hulo, D. Kahn, E. Kelly, I. Letunic, D. Lonsdale, R. Lopez, M. Madera, J. Maslen, C. McAnulla, J. McDowall, C. McMenamin, H. Mi, P. Mutowo-Muellenet, N. Mulder, D. Natale, C. Orengo, S. Pesseat, M. Punta, A.F. Quinn, C. Rivoire, A. Sangrador-Vegas, J.D. Selengut, C.J. Sigrist, M. Scheremetjew, J. Tate, M. Thimmajanarthanan, P.D. Thomas, C.H. Wu, C. Yeats, and S.Y. Yong. 2012. InterPro in 2011: new developments in the family and domain prediction database. *Nucleic Acids Res*. 40:D306-312.

Innis, B.L., and K.H. Eckels. 2003. Progress in development of a live-attenuated, tetravalent dengue virus vaccine by the United States Army Medical Research and Materiel Command. *Am J Trop Med Hyg*. 69:1-4.

Ishimi, Y., and A. Kikuchi. 1991. Identification and molecular cloning of yeast homolog of nucleosome assembly protein I which facilitates nucleosome assembly in vitro. *J Biol Chem*. 266:7025-7029.

Ishimi, Y., H. Yasuda, J. Hirosumi, F. Hanaoka, and M. Yamada. 1983. A protein which facilitates assembly of nucleosome-like structures in vitro in mammalian cells. *J Biochem*. 94:735-744.

Ito, T., M. Bulger, R. Kobayashi, and J.T. Kadonaga. 1996. Drosophila NAP-1 is a core histone chaperone that functions in ATP-facilitated assembly of regularly spaced nucleosomal arrays. *Mol Cell Biol*. 16:3112-3124.

Ito, T., T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, and Y. Sakaki. 2001. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci U S A*. 98:4569-4574.

Jacobs, M.D., and S.C. Harrison. 1998. Structure of an IkappaBalpha/NF-kappaB complex. *Cell*. 95:749-758.

Jiang, L., H. Yao, X. Duan, X. Lu, and Y. Liu. 2009. Polypyrimidine tract-binding protein influences negative strand RNA synthesis of dengue virus. *Biochem Biophys Res Commun*. 385:187-192.

Jindadamrongwech, S., C. Thepparit, and D.R. Smith. 2004. Identification of GRP 78 (BiP) as a liver cell expressed receptor element for dengue virus serotype 2. *Arch Virol.* 149:915-927.

Johansson, M., A.J. Brooks, D.A. Jans, and S.G. Vasudevan. 2001. A small region of the dengue virus-encoded RNA-dependent RNA polymerase, NS5, confers interaction with both the nuclear transport receptor importin-beta and the viral helicase, NS3. *J Gen Virol.* 82:735-745.

Johnsen, I.B., T.T. Nguyen, M. Ringdal, A.M. Tryggestad, O. Bakke, E. Lien, T. Espevik, and M.W. Anthonsen. 2006. Toll-like receptor 3 associates with c-Src tyrosine kinase on endosomes to initiate antiviral signaling. *EMBO J*. 25:3335-3346.

Johnsson, N., and A. Varshavsky. 1994. Split ubiquitin as a sensor of protein interactions in vivo. *Proc Natl Acad Sci U S A*. 91:10340-10344.

Jones, M., A. Davidson, L. Hibbert, P. Gruenwald, J. Schlaak, S. Ball, G.R. Foster, and M. Jacobs. 2005. Dengue virus inhibits alpha interferon signaling by reducing STAT2 expression. *J Virol*. 79:5414-5420.

Joshi, V., D.T. Mourya, and R.C. Sharma. 2002. Persistence of dengue-3 virus through transovarial transmission passage in successive generations of Aedes aegypti mosquitoes. *Am J Trop Med Hyg*. 67:158-161.

Junjhon, J., M. Lausumpao, S. Supasa, S. Noisakran, A. Songjaeng, P. Saraithong, K. Chaichoun, U. Utaipat, P. Keelapang, A. Kanjanahaluethai, C. Puttikhunt, W. Kasinrerk, P. Malasit, and N. Sittisombut. 2008. Differential modulation of prM cleavage, extracellular particle distribution, and virus infectivity by conserved residues at nonfurin consensus positions of the dengue virus pr-M junction. *J Virol*. 82:10776-10791.

Kanlaya, R., S.N. Pattanakitsakul, S. Sinchaikul, S.T. Chen, and V. Thongboonkerd. 2010. The ubiquitin-proteasome pathway is important for dengue virus infection in primary human endothelial cells. *J Proteome Res*. 9:4960-4971.

Kapoor, M., L. Zhang, M. Ramachandra, J. Kusukawa, K.E. Ebner, and R. Padmanabhan. 1995. Association between NS3 and NS5 proteins of dengue virus type 2 in the putative RNA replicase is linked to differential phosphorylation of NS5. *J Biol Chem.* 270:19100-19106.

Kawase, H., M. Okuwaki, M. Miyaji, R. Ohba, H. Handa, Y. Ishimi, T. Fujii-Nakata, A. Kikuchi, and K. Nagata. 1996. NAP-I is a functional homologue of TAF-I that is required for replication and transcription of the adenovirus genome in a chromatin-like structure. *Genes Cells.* 1:1045-1056.

Kelley, J.F., P.H. Kaufusi, and V.R. Nerurkar. 2012. Dengue hemorrhagic fever-associated immunomediators induced via maturation of dengue virus nonstructural 4B protein in monocytes modulate endothelial cell adhesion molecules and human microvascular endothelial cells permeability. *Virology*. 422:326-337.

Kelley, J.F., P.H. Kaufusi, E.M. Volper, and V.R. Nerurkar. 2011. Maturation of dengue virus nonstructural protein 4B in monocytes enhances production of dengue hemorrhagic fever-associated chemokines and cytokines. *Virology*. 418:27-39.

Kellogg, D.R., and A.W. Murray. 1995. NAP1 acts with Clb1 to perform mitotic functions and to suppress polar bud growth in budding yeast. *J Cell Biol*. 130:675-685.

Khadka, S., A.D. Vangeloff, C. Zhang, P. Siddavatam, N.S. Heaton, L. Wang, R. Sengupta, S. Sahasrabudhe, G. Randall, M. Gribskov, R.J. Kuhn, R. Perera, and

D.J. LaCount. 2011. A physical interaction network of dengue virus and human proteins. *Mol Cell Proteomics*. 10:M111 012187.

Khakpoor, A., M. Panyasrivanit, N. Wikan, and D.R. Smith. 2009. A role for autophagolysosomes in dengue virus 3 production in HepG2 cells. *J Gen Virol*. 90:1093-1103.

Kleino, A., S. Valanne, J. Ulvila, J. Kallio, H. Myllymaki, H. Enwald, S. Stoven, M. Poidevin, R. Ueda, D. Hultmark, B. Lemaitre, and M. Ramet. 2005. Inhibitor of apoptosis 2 and TAK1-binding protein are components of the Drosophila Imd pathway. *EMBO J*. 24:3423-3434.

Klueg, K.M., D. Alvarado, M.A. Muskavitch, and J.B. Duffy. 2002. Creation of a GAL4/UAS-coupled inducible gene expression system for use in Drosophila cultured cell lines. *Genesis.* 34:119-122.

Knipling, E.F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* . 48:459-462.

Kolonin, M.G., J. Zhong, and R.L. Finley. 2000. Interaction mating methods in two-hybrid systems. *Methods Enzymol.* 328:26-46.

Krishnan, M.N., A. Ng, B. Sukumaran, F.D. Gilfoy, P.D. Uchil, H. Sultana, A.L. Brass, R. Adametz, M. Tsui, F. Qian, R.R. Montgomery, S. Lev, P.W. Mason, R.A. Koski, S.J. Elledge, R.J. Xavier, H. Agaisse, and E. Fikrig. 2008. RNA interference screen for human genes associated with West Nile virus infection. *Nature*. 455:242-245.

Kuadkitkan, A., N. Wikan, C. Fongsaran, and D.R. Smith. 2010. Identification and characterization of prohibitin as a receptor protein mediating DENV-2 entry into insect cells. *Virology*. 406:149-161.

Kuhn, R.J., W. Zhang, M.G. Rossmann, S.V. Pletnev, J. Corver, E. Lenches, C.T. Jones, S. Mukhopadhyay, P.R. Chipman, E.G. Strauss, T.S. Baker, and J.H. Strauss. 2002. Structure of dengue virus: implications for flavivirus organization, maturation, and fusion. *Cell*. 108:717-725.

Kulkarni, M.M., M. Booker, S.J. Silver, A. Friedman, P. Hong, N. Perrimon, and B. Mathey-Prevot. 2006. Evidence of off-target effects associated with long dsRNAs in Drosophila melanogaster cell-based assays. *Nat Methods*. 3:833-838.

Kuo, C.H., D.I. Tai, C.S. Chang-Chien, C.K. Lan, S.S. Chiou, and Y.F. Liaw. 1992. Liver biochemical tests and dengue fever. *Am J Trop Med Hyg*. 47:265-270.

Kurosu, T., P. Chaichana, M. Yamate, S. Anantapreecha, and K. Ikuta. 2007. Secreted complement regulatory protein clusterin interacts with dengue virus nonstructural protein 1. *Biochem Biophys Res Commun*. 362:1051-1056.

Kwofie, S.K., U. Schaefer, V.S. Sundararajan, V.B. Bajic, and A. Christoffels. 2011. HCVpro: hepatitis C virus protein interaction database. *Infect Genet Evol*. 11:1971-1977.

Kyle, J.L., P.R. Beatty, and E. Harris. 2007. Dengue virus infects macrophages and dendritic cells in a mouse model of infection. *J Infect Dis.* 195:1808-1817.

Lamesch, P., N. Li, S. Milstein, C. Fan, T. Hao, G. Szabo, Z. Hu, K. Venkatesan, G. Bethel, P. Martin, J. Rogers, S. Lawlor, S. McLaren, A. Dricot, H. Borick, M.E. Cusick, J. Vandenhaute, I. Dunham, D.E. Hill, and M. Vidal. 2007. hORFeome

v3.1: a resource of human open reading frames representing over 10,000 human genes. *Genomics.* 89:307-315.

Lan, Q., and A.M. Fallon. 1990. Small heat shock proteins distinguish between two mosquito species and confirm identity of their cell lines. *Am J Trop Med Hyg*. 43:669-676.

Lance, D.R., D.O. McInnis, P. Rendon, and C.G. Jackson. 2000. Courtship among sterile and wild Ceratitis capitata (Diptera: Tephritidae) in field cages in Hawaii and Guatemala. *Annals of the Entomological Society of America*. 93:1179-1185.

Lawson, D., P. Arensburger, P. Atkinson, N.J. Besansky, R.V. Bruggner, R. Butler, K.S. Campbell, G.K. Christophides, S. Christley, E. Dialynas, M. Hammond, C.A. Hill, N. Konopinski, N.F. Lobo, R.M. MacCallum, G. Madey, K. Megy, J. Meyer, S. Redmond, D.W. Severson, E.O. Stinson, P. Topalis, E. Birney, W.M. Gelbart, F.C. Kafatos, C. Louis, and F.H. Collins. 2009. VectorBase: a data resource for invertebrate vector genomics. *Nucleic Acids Res*. 37:D583-587.

Lazo, L., L. Hermida, A. Zulueta, J. Sanchez, C. Lopez, R. Silva, G. Guillen, and M.G. Guzman. 2007. A recombinant capsid protein from Dengue-2 induces protection in mice against homologous virus. *Vaccine*. 25:1064-1070.

Le Breton, M., L. Meyniel-Schicklin, A. Deloire, B. Coutard, B. Canard, X. de Lamballerie, P. Andre, C. Rabourdin-Combe, V. Lotteau, and N. Davoust. 2011. Flavivirus NS3 and NS5 proteins interaction network: a high-throughput yeast two-hybrid screen. *BMC Microbiol*. 11:234.

Leardkamolkarn, V., and W. Sirigulpanit. 2012. Establishment of a stable cell line coexpressing dengue virus-2 and green fluorescent protein for screening of antiviral compounds. *J Biomol Screen*. 17:283-292.

Leardkamolkarn, V., W. Sirigulpanit, N. Chotiwan, S. Kumkate, and C.Y. Huang. 2012. Development of Dengue type-2 virus replicons expressing GFP reporter gene in study of viral RNA replication. *Virus Res*. 163:552-562.

Lee, A.H., N.N. Iwakoshi, and L.H. Glimcher. 2003. XBP-1 regulates a subset of endoplasmic reticulum resident chaperone genes in the unfolded protein response. *Mol Cell Biol*. 23:7448-7459.

Lee, T.C., Y.L. Lin, J.T. Liao, C.M. Su, C.C. Lin, W.P. Lin, and C.L. Liao. 2010. Utilizing liver-specific microRNA-122 to modulate replication of dengue virus replicon. *Biochem Biophys Res Commun*. 396:596-601.

Lee, Y.R., H.Y. Lei, M.T. Liu, J.R. Wang, S.H. Chen, Y.F. Jiang-Shieh, Y.S. Lin, T.M. Yeh, C.C. Liu, and H.S. Liu. 2008. Autophagic machinery activated by dengue virus enhances virus replication. *Virology*. 374:240-248.

Lee, Y.Y., R.C. Cevallos, and E. Jan. 2009. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. *J Biol Chem*. 284:6661-6673.

Leung, J.Y., G.P. Pijlman, N. Kondratieva, J. Hyde, J.M. Mackenzie, and A.A. Khromykh. 2008. Role of nonstructural protein NS2A in flavivirus assembly. *J Virol*. 82:4731-4741.

Li, H., S. Clum, S. You, K.E. Ebner, and R. Padmanabhan. 1999. The serine protease and RNA-stimulated nucleoside triphosphatase and RNA helicase functional

domains of dengue virus type 2 NS3 converge within a region of 20 amino acids. *J Virol*. 73:3108-3116.

Li, P.C., M.Y. Liao, P.C. Cheng, J.J. Liang, I.J. Liu, C.Y. Chiu, Y.L. Lin, G.J. Chang, and H.C. Wu. 2012. Development of a Humanized Antibody with High Therapeutic Potential against Dengue Virus Type 2. *PLoS Negl Trop Dis*. 6:e1636.

Li, S., C.M. Armstrong, N. Bertin, H. Ge, S. Milstein, M. Boxem, P.O. Vidalain, J.D. Han, A. Chesneau, T. Hao, D.S. Goldberg, N. Li, M. Martinez, J.F. Rual, P. Lamesch, L. Xu, M. Tewari, S.L. Wong, L.V. Zhang, G.F. Berriz, L. Jacotot, P. Vaglio, J. Reboul, T. Hirozane-Kishikawa, Q. Li, H.W. Gabel, A. Elewa, B. Baumgartner, D.J. Rose, H. Yu, S. Bosak, R. Sequerra, A. Fraser, S.E. Mango, W.M. Saxton, S. Strome, S. Van Den Heuvel, F. Piano, J. Vandenhaute, C. Sardet, M. Gerstein, L. Doucette-Stamm, K.C. Gunsalus, J.W. Harper, M.E. Cusick, F.P. Roth, D.E. Hill, and M. Vidal. 2004. A map of the interactome network of the metazoan C. elegans. *Science*. 303:540-543.

Lilley, B.N., J.M. Gilbert, H.L. Ploegh, and T.L. Benjamin. 2006. Murine polyomavirus requires the endoplasmic reticulum protein Derlin-2 to initiate infection. *J Virol.* 80:8739-8744.

Lim, J., T. Hao, C. Shaw, A.J. Patel, G. Szabo, J.F. Rual, C.J. Fisk, N. Li, A. Smolyar, D.E. Hill, A.L. Barabasi, M. Vidal, and H.Y. Zoghbi. 2006. A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. *Cell*. 125:801-814.

Lima, J.B., M.P. Da-Cunha, R.C. Da Silva, A.K. Galardo, S. Soares Sda, I.A. Braga, R.P. Ramos, and D. Valle. 2003. Resistance of Aedes aegypti to

organophosphates in several municipalities in the State of Rio de Janeiro and Espirito Santo, Brazil. *Am J Trop Med Hyg*. 68:329-333.

Limjindaporn, T., J. Netsawang, S. Noisakran, S. Thiemmeca, W. Wongwiwat, S. Sudsaward, P. Avirutnan, C. Puttikhunt, W. Kasinrerk, R. Sriburi, N. Sittisombut, P.T. Yenchitsomanus, and P. Malasit. 2007. Sensitization to Fas-mediated apoptosis by dengue virus capsid protein. *Biochem Biophys Res Commun.* 362:334-339.

Limjindaporn, T., W. Wongwiwat, S. Noisakran, C. Srisawat, J. Netsawang, C. Puttikhunt, W. Kasinrerk, P. Avirutnan, S. Thiemmeca, R. Sriburi, N. Sittisombut, P. Malasit, and P.T. Yenchitsomanus. 2009. Interaction of dengue virus envelope protein with endoplasmic reticulum-resident chaperones facilitates dengue virus production. *Biochem Biophys Res Commun.* 379:196-200.

Lindenbach, B.D., and C.M. Rice. 1997. trans-Complementation of yellow fever virus NS1 reveals a role in early RNA replication. *J Virol*. 71:9608-9617.

Lindenbach, B.D., and C.M. Rice. 1999. Genetic interaction of flavivirus nonstructural proteins NS1 and NS4A as a determinant of replicase function. *J Virol*. 73:4611-4621.

Lindenbach, B.D., H.J. Thiel, and C.M. Rice. 2006. Flaviviridae: The Viruses and Their Replication. *In* Fields Virology. Vol. 2. D.M. Knipe and P.M. Howley, editors. Lippincott Williams & Wilkins, Philadelphia, USA. 1153-1252.

Liu, D., and R.L. Finley, Jr. 2010. Cyclin Y is a novel conserved cyclin essential for development in Drosophila. *Genetics*. 184:1025-1035.

Liu, W.J., H.B. Chen, and A.A. Khromykh. 2003. Molecular and functional analyses of Kunjin virus infectious cDNA clones demonstrate the essential roles for NS2A in virus assembly and for a nonconservative residue in NS3 in RNA replication. *J Virol*. 77:7804-7813.

Lobigs, M. 1993. Flavivirus premembrane protein cleavage and spike heterodimer secretion require the function of the viral proteinase NS3. *Proc Natl Acad Sci U S A.* 90:6218-6222.

Lozach, P.Y., L. Burleigh, I. Staropoli, E. Navarro-Sanchez, J. Harriague, J.L. Virelizier, F.A. Rey, P. Despres, F. Arenzana-Seisdedos, and A. Amara. 2005. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem*. 280:23698-23708.

Ma, L., C.T. Jones, T.D. Groesch, R.J. Kuhn, and C.B. Post. 2004. Solution structure of dengue virus capsid protein reveals another fold. *Proc Natl Acad Sci U S A.* 101:3414-3419.

Ma, Y., A. Creanga, L. Lum, and P.A. Beachy. 2006. Prevalence of off-target effects in Drosophila RNA interference screens. *Nature*. 443:359-363.

Mackenzie, J. 2005. Wrapping things up about virus RNA replication. *Traffic*. 6:967-977.

Mackenzie, J.M., M.K. Jones, and P.R. Young. 1996. Immunolocalization of the dengue virus nonstructural glycoprotein NS1 suggests a role in viral RNA replication. *Virology*. 220:232-240.

Mackenzie, J.S., D.J. Gubler, and L.R. Petersen. 2004. Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med*. 10:S98-109.

Maere, S., K. Heymans, and M. Kuiper. 2005. BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics.* 21:3448-3449.

Mairiang, D., H. Zhang, A. Sodja, T. Murali, P. Suriyaphol, P. Malasit, T. Limjindaporn, and R.L. Finley, Jr. . 2012. Identification of new protein interactions between dengue fever virus and its hosts, human and mosquito (submitted to publication).

Malhotra, J.D., and R.J. Kaufman. 2007. The endoplasmic reticulum and the unfolded protein response. *Semin Cell Dev Biol*. 18:716-731.

Marheineke, K., and T. Krude. 1998. Nucleosome assembly activity and intracellular localization of human CAF-1 changes during the cell division cycle. *J Biol Chem*. 273:15279-15286.

Martinez-Gutierrez, M., J.E. Castellanos, and J.C. Gallego-Gomez. 2011. Statins reduce dengue virus production via decreased virion assembly. *Intervirology*. 54:202-216.

Masse, N., A. Davidson, F. Ferron, K. Alvarez, M. Jacobs, J.L. Romette, B. Canard, and J.C. Guillemot. 2010. Dengue virus replicons: production of an interserotypic chimera and cell lines from different species, and establishment of a cell-based fluorescent assay to screen inhibitors, validated by the evaluation of ribavirin's activity. *Antiviral Res*. 86:296-305.

Matusan, A.E., M.J. Pryor, A.D. Davidson, and P.J. Wright. 2001. Mutagenesis of the Dengue virus type 2 NS3 protein within and outside helicase motifs: effects on enzyme activity and virus replication. *J Virol*. 75:9633-9643.

Mazzon, M., M. Jones, A. Davidson, B. Chain, and M. Jacobs. 2009. Dengue virus NS5 inhibits interferon-alpha signaling by blocking signal transducer and activator of transcription 2 phosphorylation. *J Infect Dis*. 200:1261-1270.

McLean, J.E., A. Wudzinska, E. Datan, D. Quaglino, and Z. Zakeri. 2011. Flavivirus NS4A-induced autophagy protects cells against death and enhances virus replication. *J Biol Chem*. 286:22147-22159.

McMeniman, C.J., R.V. Lane, B.N. Cass, A.W. Fong, M. Sidhu, Y.F. Wang, and S.L. O'Neill. 2009. Stable introduction of a life-shortening Wolbachia infection into the mosquito Aedes aegypti. *Science*. 323:141-144.

Medin, C.L., K.A. Fitzgerald, and A.L. Rothman. 2005. Dengue virus nonstructural protein NS5 induces interleukin-8 transcription and secretion. *J Virol*. 79:11053-11061.

Mercado-Curiel, R.F., W.C.t. Black, and L. Munoz Mde. 2008. A dengue receptor as possible genetic marker of vector competence in Aedes aegypti. *BMC Microbiol*. 8:118.

Mercado-Curiel, R.F., H.A. Esquinca-Aviles, R. Tovar, A. Diaz-Badillo, M. Camacho-Nuez, and L. Munoz Mde. 2006. The four serotypes of dengue recognize the same putative receptors in Aedes aegypti midgut and Ae. albopictus cells. *BMC Microbiol*. 6:85.

Miller, J.L., B.J. de Wet, L. Martinez-Pomares, C.M. Radcliffe, R.A. Dwek, P.M. Rudd, and S. Gordon. 2008. The mannose receptor mediates dengue virus infection of macrophages. *PLoS Pathog*. 4:e17.

Miller, J.P., R.S. Lo, A. Ben-Hur, C. Desmarais, I. Stagljar, W.S. Noble, and S. Fields. 2005. Large-scale identification of yeast integral membrane protein interactions. *Proc Natl Acad Sci U S A*. 102:12123-12128.

Miller, S., S. Kastner, J. Krijnse-Locker, S. Buhler, and R. Bartenschlager. 2007. The non-structural protein 4A of dengue virus is an integral membrane protein inducing membrane alterations in a 2K-regulated manner. *J Biol Chem*. 282:8873-8882.

Miyaji-Yamaguchi, M., K. Kato, R. Nakano, T. Akashi, A. Kikuchi, and K. Nagata. 2003. Involvement of nucleocytoplasmic shuttling of yeast Nap1 in mitotic progression. *Mol Cell Biol*. 23:6672-6684.

Modis, Y., S. Ogata, D. Clements, and S.C. Harrison. 2004. Structure of the dengue virus envelope protein after membrane fusion. *Nature.* 427:313-319.

Mohr, S., C. Bakal, and N. Perrimon. 2010. Genomic screening with RNAi: results and challenges. *Annu Rev Biochem*. 79:37-64.

Morchang, A., U. Yasamut, J. Netsawang, S. Noisakran, W. Wongwiwat, P. Songprakhon, C. Srisawat, C. Puttikhunt, W. Kasinrerk, P. Malasit, P.T. Yenchitsomanus, and T. Limjindaporn. 2011. Cell death gene expression profile: Role of RIPK2 in dengue virus-mediated apoptosis. *Virus Res*. 156:25-34.

Mosammaparast, N., C.S. Ewart, and L.F. Pemberton. 2002. A role for nucleosome assembly protein 1 in the nuclear transport of histones H2A and H2B. *EMBO J*. 21:6527-6538.

Mukhopadhyay, S., R.J. Kuhn, and M.G. Rossmann. 2005. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol*. 3:13-22.

Munoz, M.L., A. Cisneros, J. Cruz, P. Das, R. Tovar, and A. Ortega. 1998. Putative dengue virus receptors from mosquito cells. *FEMS Microbiol Lett*. 168:251-258.

Munoz-Jordan, J.L., M. Laurent-Rolle, J. Ashour, L. Martinez-Sobrido, M. Ashok, W.I. Lipkin, and A. Garcia-Sastre. 2005. Inhibition of alpha/beta interferon signaling by the NS4B protein of flaviviruses. *J Virol*. 79:8004-8013.

Munoz-Jordan, J.L., G.G. Sanchez-Burgos, M. Laurent-Rolle, and A. Garcia-Sastre. 2003. Inhibition of interferon signaling by dengue virus. *Proc Natl Acad Sci U S A.* 100:14333-14338.

Munstermann, L.E. 1997. Care and maintenance of Aedes mosquito colonies. *In* The molecular biology of insect disease vectors: a methods manual. . J.M. Crampton, C.B. Beard, and C. Louis, editors. Springer. 13-20

Muylaert, I.R., T.J. Chambers, R. Galler, and C.M. Rice. 1996. Mutagenesis of the N-linked glycosylation sites of the yellow fever virus NS1 protein: effects on virus replication and mouse neurovirulence. *Virology*. 222:159-168.

Muylaert, I.R., R. Galler, and C.M. Rice. 1997. Genetic analysis of the yellow fever virus NS1 protein: identification of a temperature-sensitive mutation which blocks RNA accumulation. *J Virol*. 71:291-298.

Nagila, A., J. Netsawang, C. Srisawat, S. Noisakran, A. Morchang, U. Yasamut, C. Puttikhunt, W. Kasinrerk, P. Malasit, P.T. Yenchitsomanus, and T. Limjindaporn. 2011. Role of CD137 signaling in dengue virus-mediated apoptosis. *Biochem Biophys Res Commun*. 410:428-433.

Najera, J.A., M. Gonzalez-Silva, and P.L. Alonso. 2011. Some lessons for the future from the Global Malaria Eradication Programme (1955-1969). *PLoS Med*. 8:e1000412.

Nasirudeen, A.M., H.H. Wong, P. Thien, S. Xu, K.P. Lam, and D.X. Liu. 2011. RIG-I, MDA5 and TLR3 synergistically play an important role in restriction of dengue virus infection. *PLoS Negl Trop Dis*. 5:e926.

Netsawang, J., S. Noisakran, C. Puttikhunt, W. Kasinrerk, W. Wongwiwat, P. Malasit, P.T. Yenchitsomanus, and T. Limjindaporn. 2010. Nuclear localization of dengue virus capsid protein is required for DAXX interaction and apoptosis. *Virus Res*. 147:275-283.

Ng, C.Y., F. Gu, W.Y. Phong, Y.L. Chen, S.P. Lim, A. Davidson, and S.G. Vasudevan. 2007. Construction and characterization of a stable subgenomic dengue virus type 2 replicon system for antiviral compound and siRNA testing. *Antiviral Res.* 76:222-231.

Noisakran, S., S. Sengsai, V. Thongboonkerd, R. Kanlaya, S. Sinchaikul, S.T. Chen, C. Puttikhunt, W. Kasinrerk, T. Limjindaporn, W. Wongwiwat, P. Malasit, and P.T. Yenchitsomanus. 2008. Identification of human hnRNP C1/C2 as a dengue virus NS1-interacting protein. *Biochem Biophys Res Commun*. 372:67-72.

Oda, Y., T. Okada, H. Yoshida, R.J. Kaufman, K. Nagata, and K. Mori. 2006. Derlin-2 and Derlin-3 are regulated by the mammalian unfolded protein response and are required for ER-associated degradation. *J Cell Biol.* 172:383-393.

Okada, M., Y. Hozumi, T. Ichimura, T. Tanaka, H. Hasegawa, M. Yamamoto, N. Takahashi, K. Iseki, H. Yagisawa, T. Shinkawa, T. Isobe, and K. Goto. 2011. Interaction of nucleosome assembly proteins abolishes nuclear localization of DGKzeta by attenuating its association with importins. *Exp Cell Res*. 317:2853-2863.

Okuwaki, M., K. Kato, and K. Nagata. 2010. Functional characterization of human nucleosome assembly protein 1-like proteins as histone chaperones. *Genes Cells*. 15:13-27.

Orr-Weaver, T.L., and J.W. Szostak. 1983. Yeast recombination: the association between double-strand gap repair and crossing-over. *Proc Natl Acad Sci U S A.* 80:4417-4421.

Ostlund, G., T. Schmitt, K. Forslund, T. Kostler, D.N. Messina, S. Roopra, O. Frings, and E.L. Sonnhammer. 2009. InParanoid 7: new algorithms and tools for eukaryotic orthology analysis. *Nucleic Acids Res*. 38:5.

Pagani, I., K. Liolios, J. Jansson, I.M. Chen, T. Smirnova, B. Nosrat, V.M. Markowitz, and N.C. Kyrpides. 2012. The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res*. 40:D571-579.

Pan, X., G. Zhou, J. Wu, G. Bian, P. Lu, A.S. Raikhel, and Z. Xi. 2012. Wolbachia induces reactive oxygen species (ROS)-dependent activation of the Toll pathway

to control dengue virus in the mosquito Aedes aegypti. *Proc Natl Acad Sci U S A*. 109:E23-31.

Pandey, A., and M. Mann. 2000. Proteomics to study genes and genomes. *Nature*. 405:837-846.

Panyasrivanit, M., A. Khakpoor, N. Wikan, and D.R. Smith. 2009. Co-localization of constituents of the dengue virus translation and replication machinery with amphisomes. *J Gen Virol*. 90:448-456.

Park, Y.J., and K. Luger. 2006. The structure of nucleosome assembly protein 1. *Proc Natl Acad Sci U S A*. 103:1248-1253.

Pavio, N., P.R. Romano, T.M. Graczyk, S.M. Feinstone, and D.R. Taylor. 2003. Protein synthesis and endoplasmic reticulum stress can be modulated by the hepatitis C virus envelope protein E2 through the eukaryotic initiation factor 2alpha kinase PERK. *J Virol*. 77:3578-3585.

Pena, J., and E. Harris. 2011. Dengue virus modulates the unfolded protein response in a time-dependent manner. *J Biol Chem*. 286:14226-14236.

Perera, R., C. Riley, G. Isaac, A.S. Hopf-Jannasch, R.J. Moore, K.W. Weitz, L. Pasa-Tolic, T.O. Metz, J. Adamec, and R.J. Kuhn. 2012. Dengue virus infection perturbs lipid homeostasis in infected mosquito cells. *PLoS Pathog*. 8:e1002584.

Phillips-Howard, P.A., B.L. Nahlen, M.S. Kolczak, A.W. Hightower, F.O. ter Kuile, J.A. Alaii, J.E. Gimnig, J. Arudo, J.M. Vulule, A. Odhacha, S.P. Kachur, E. Schoute, D.H. Rosen, J.D. Sexton, A.J. Oloo, and W.A. Hawley. 2003. Efficacy of permethrin-treated bed nets in the prevention of mortality in young children in an

area of high perennial malaria transmission in western Kenya. *Am J Trop Med Hyg*. 68:23-29.

Phuc, H.K., M.H. Andreasen, R.S. Burton, C. Vass, M.J. Epton, G. Pape, G. Fu, K.C. Condon, S. Scaife, C.A. Donnelly, P.G. Coleman, H. White-Cooper, and L. Alphey. 2007. Late-acting dominant lethal genetic systems and mosquito control. *BMC Biol*. 5:11.

Pinney, J.W., J.E. Dickerson, W. Fu, B.E. Sanders-Beer, R.G. Ptak, and D.L. Robertson. 2009. HIV-host interactions: a map of viral perturbation of the host system. *Aids*. 23:549-554.

Poh, M.K., G. Shui, X. Xie, P.Y. Shi, M.R. Wenk, and F. Gu. 2012. U18666A, an intra-cellular cholesterol transport inhibitor, inhibits dengue virus entry and replication. *Antiviral Res*. 93:191-198.

Pryor, M.J., S.M. Rawlinson, R.E. Butcher, C.L. Barton, T.A. Waterhouse, S.G. Vasudevan, P.G. Bardin, P.J. Wright, D.A. Jans, and A.D. Davidson. 2007. Nuclear localization of dengue virus nonstructural protein 5 through its importin alpha/beta-recognized nuclear localization sequences is integral to viral infection. *Traffic*. 8:795-807.

Ptak, R.G., W. Fu, B.E. Sanders-Beer, J.E. Dickerson, J.W. Pinney, D.L. Robertson, M.N. Rozanov, K.S. Katz, D.R. Maglott, K.D. Pruitt, and C.W. Dieffenbach. 2008. Cataloguing the HIV type 1 human protein interaction network. *AIDS Res Hum Retroviruses*. 24:1497-1502.

Radke, E.G., C.J. Gregory, K.W. Kintziger, E.K. Sauber-Schatz, E.A. Hunsperger, G.R. Gallagher, J.M. Barber, B.J. Biggerstaff, D.R. Stanek, K.M. Tomashek, and C.G.

Blackmore. 2012. Dengue outbreak in Key West, Florida, USA, 2009. *Emerg Infect Dis*. 18:135-137.

Ramanathan, M.P., J.A. Chambers, P. Pankhong, M. Chattergoon, W. Attatippaholkun, K. Dang, N. Shah, and D.B. Weiner. 2006. Host cell killing by the West Nile Virus NS2B-NS3 proteolytic complex: NS3 alone is sufficient to recruit caspase-8-based apoptotic pathway. *Virology*. 345:56-72.

Rawlinson, S.M., M.J. Pryor, P.J. Wright, and D.A. Jans. 2009. CRM1-mediated nuclear export of dengue virus RNA polymerase NS5 modulates interleukin-8 induction and virus production. *J Biol Chem*. 284:15589-15597.

Reyes-Del Valle, J., S. Chavez-Salinas, F. Medina, and R.M. Del Angel. 2005. Heat shock protein 90 and heat shock protein 70 are components of dengue virus receptor complex in human cells. *J Virol*. 79:4557-4567.

Reyes-del Valle, J., and R.M. del Angel. 2004. Isolation of putative dengue virus receptor molecules by affinity chromatography using a recombinant E protein ligand. *J Virol Methods*. 116:95-102.

Rice, C.M., E.M. Lenches, S.R. Eddy, S.J. Shin, R.L. Sheets, and J.H. Strauss. 1985. Nucleotide sequence of yellow fever virus: implications for flavivirus gene expression and evolution. *Science*. 229:726-733.

Rigaut, G., A. Shevchenko, B. Rutz, M. Wilm, M. Mann, and B. Seraphin. 1999. A generic protein purification method for protein complex characterization and proteome exploration. *Nat Biotechnol*. 17:1030-1032.

Robert Putnak, J., B.A. Coller, G. Voss, D.W. Vaughn, D. Clements, I. Peters, G. Bignami, H.S. Houng, R.C. Chen, D.A. Barvir, J. Seriwatana, S. Cayphas, N.

Garcon, D. Gheysen, N. Kanesa-Thasan, M. McDonell, T. Humphreys, K.H. Eckels, J.P. Prieels, and B.L. Innis. 2005. An evaluation of dengue type-2 inactivated, recombinant subunit, and live-attenuated vaccine candidates in the rhesus macaque model. *Vaccine*. 23:4442-4452.

Rodenhuis-Zybert, I.A., H.M. van der Schaar, J.M. da Silva Voorham, H. van der Ende-Metselaar, H.Y. Lei, J. Wilschut, and J.M. Smit. 2010. Immature dengue virus: a veiled pathogen? *PLoS Pathog*. 6:e1000718.

Rosen, L., D.A. Shroyer, R.B. Tesh, J.E. Freier, and J.C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: Aedes albopictus and Aedes aegypti. *Am J Trop Med Hyg*. 32:1108-1119.

Rothwell, C., A. Lebreton, C. Young Ng, J.Y. Lim, W. Liu, S. Vasudevan, M. Labow, F. Gu, and L.A. Gaither. 2009. Cholesterol biosynthesis modulation regulates dengue viral replication. *Virology*. 389:8-19.

Rual, J.F., K. Venkatesan, T. Hao, T. Hirozane-Kishikawa, A. Dricot, N. Li, G.F. Berriz, F.D. Gibbons, M. Dreze, N. Ayivi-Guedehoussou, N. Klitgord, C. Simon, M. Boxem, S. Milstein, J. Rosenberg, D.S. Goldberg, L.V. Zhang, S.L. Wong, G. Franklin, S. Li, J.S. Albala, J. Lim, C. Fraughton, E. Llamosas, S. Cevik, C. Bex, P. Lamesch, R.S. Sikorski, J. Vandenhaute, H.Y. Zoghbi, A. Smolyar, S. Bosak, R. Sequerra, L. Doucette-Stamm, M.E. Cusick, D.E. Hill, F.P. Roth, and M. Vidal. 2005. Towards a proteome-scale map of the human protein-protein interaction network. *Nature*. 437:1173-1178.

Rubbi, C.P., and J. Milner. 2003. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J*. 22:6068-6077.

Sakoonwatanyoo, P., V. Boonsanay, and D.R. Smith. 2006. Growth and production of the dengue virus in C6/36 cells and identification of a laminin-binding protein as a candidate serotype 3 and 4 receptor protein. *Intervirology*. 49:161-172.

Salas-Benito, J., J. Reyes-Del Valle, M. Salas-Benito, I. Ceballos-Olvera, C. Mosso, and R.M. del Angel. 2007. Evidence that the 45-kD glycoprotein, part of a putative dengue virus receptor complex in the mosquito cell line C6/36, is a heat-shock related protein. *Am J Trop Med Hyg*. 77:283-290.

Salas-Benito, J.S., and R.M. del Angel. 1997. Identification of two surface proteins from C6/36 cells that bind dengue type 4 virus. *J Virol*. 71:7246-7252.

Sampath, A., and R. Padmanabhan. 2009. Molecular targets for flavivirus drug discovery. *Antiviral Res*. 81:6-15.

Sangiambut, S., P. Keelapang, J. Aaskov, C. Puttikhunt, W. Kasinrerk, P. Malasit, and N. Sittisombut. 2008. Multiple regions in dengue virus capsid protein contribute to nuclear localization during virus infection. *J Gen Virol*. 89:1254-1264.

Schul, W., W. Liu, H.Y. Xu, M. Flamand, and S.G. Vasudevan. 2007. A dengue fever viremia model in mice shows reduction in viral replication and suppression of the inflammatory response after treatment with antiviral drugs. *J Infect Dis*. 195:665-674.

Schwartz, A.S., J. Yu, K.R. Gardenour, R.L. Finley, Jr., and T. Ideker. 2009. Cost-effective strategies for completing the interactome. *Nat Methods.* 6:55-61.

Sekar, R.B., and A. Periasamy. 2003. Fluorescence resonance energy transfer (FRET) microscopy imaging of live cell protein localizations. *J Cell Biol*. 160:629-633.

Sessions, O.M., N.J. Barrows, J.A. Souza-Neto, T.J. Robinson, C.L. Hershey, M.A. Rodgers, J.L. Ramirez, G. Dimopoulos, P.L. Yang, J.L. Pearson, and M.A. Garcia-Blanco. 2009. Discovery of insect and human dengue virus host factors. *Nature*. 458:1047-1050.

Shafee, N., and S. AbuBakar. 2003. Dengue virus type 2 NS3 protease and NS2B-NS3 protease precursor induce apoptosis. *J Gen Virol*. 84:2191-2195.

Shapira, S.D., I. Gat-Viks, B.O. Shum, A. Dricot, M.M. de Grace, L. Wu, P.B. Gupta, T. Hao, S.J. Silver, D.E. Root, D.E. Hill, A. Regev, and N. Hacohen. 2009. A physical and regulatory map of host-influenza interactions reveals pathways in H1N1 infection. *Cell*. 139:1255-1267.

Shikama, N., H.M. Chan, M. Krstic-Demonacos, L. Smith, C.W. Lee, W. Cairns, and N.B. La Thangue. 2000. Functional interaction between nucleosome assembly proteins and p300/CREB-binding protein family coactivators. *Mol Cell Biol*. 20:8933-8943.

Shimizu, Y., T. Akashi, A. Okuda, A. Kikuchi, and K. Fukui. 2000. NBP1 (Nap1 binding protein 1), an essential gene for G2/M transition of Saccharomyces cerevisiae, encodes a protein of distinct sub-nuclear localization. *Gene*. 246:395-404.

Silveira, G.F., F. Meyer, A. Delfraro, A.L. Mosimann, N. Coluchi, C. Vasquez, C.M. Probst, A. Bafica, J. Bordignon, and C.N. Dos Santos. 2011. Dengue virus type 3 isolated from a fatal case with visceral complications induces enhanced proinflammatory responses and apoptosis of human dendritic cells. *J Virol*. 85:5374-5383.

Sim, S., and G. Dimopoulos. 2010. Dengue virus inhibits immune responses in Aedes aegypti cells. *PLoS One*. 5:e10678.

Simmons, R.B., and S.J. Weller. 2001. Utility and evolution of cytochrome b in insects. *Mol Phylogenet Evol*. 20:196-210.

Skolnick, J., and M. Brylinski. 2009. FINDSITE: a combined evolution/structure-based approach to protein function prediction. *Brief Bioinform*. 10:378-391.

Smith, G.W., and P.J. Wright. 1985. Synthesis of proteins and glycoproteins in dengue type 2 virus-infected vero and Aedes albopictus cells. *J Gen Virol*. 66 (Pt 3):559-571.

Smoot, M.E., K. Ono, J. Ruscheinski, P.L. Wang, and T. Ideker. 2011. Cytoscape 2.8: new features for data integration and network visualization. *Bioinformatics.* 27:431-432.

Snowden, F.M. 2008. Emerging and reemerging diseases: a historical perspective. *Immunol Rev*. 225:9-26.

Sodja, A., H. Fujioka, F.J. Lemos, M. Donnelly-Doman, and M. Jacobs-Lorena. 2007. Induction of actin gene expression in the mosquito midgut by blood ingestion correlates with striking changes of cell shape. *J Insect Physiol*. 53:833-839.

Souza, L.J., J.G. Alves, R.M. Nogueira, C. Gicovate Neto, D.A. Bastos, E.W. Siqueira, J.T. Souto Filho, A. Cezario Tde, C.E. Soares, and C. Carneiro Rda. 2004. Aminotransferase changes and acute hepatitis in patients with dengue fever: analysis of 1,585 cases. *Braz J Infect Dis*. 8:156-163.

Stagljar, I., and S. Fields. 2002. Analysis of membrane protein interactions using yeast-based technologies. *Trends Biochem Sci*. 27:559-563.

Stagljar, I., C. Korostensky, N. Johnsson, and S. te Heesen. 1998. A genetic system based on split-ubiquitin for the analysis of interactions between membrane proteins in vivo. *Proc Natl Acad Sci U S A*. 95:5187-5192.

Stanyon, C.A., T. Limjindaporn, and R.L. Finley, Jr. 2003. Simultaneous cloning of open reading frames into several different expression vectors. *Biotechniques.* 35:520-522, 524-526.

Stanyon, C.A., G. Liu, B.A. Mangiola, N. Patel, L. Giot, B. Kuang, H. Zhang, J. Zhong, and R.L. Finley, Jr. 2004. A Drosophila protein-interaction map centered on cell-cycle regulators. *Genome Biol.* 5:R96.

Steer, W.M., A. Abu-Daya, S.J. Brickwood, K.L. Mumford, N. Jordanaires, J. Mitchell, C. Robinson, A.W. Thorne, and M.J. Guille. 2003. Xenopus nucleosome assembly protein becomes tissue-restricted during development and can alter the expression of specific genes. *Mech Dev*. 120:1045-1057.

Struhl, K., F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, and J.A. Smith. 1987-1997. Current Protocols in Molecular biology. Greene and Wiley-Interscience, New York.

Suzuki, R., E.R. Winkelmann, and P.W. Mason. 2009. Construction and characterization of a single-cycle chimeric flavivirus vaccine candidate that protects mice against lethal challenge with dengue virus type 2. *J Virol*. 83:1870-1880.

Szymczak, A.L., C.J. Workman, Y. Wang, K.M. Vignali, S. Dilioglou, E.F. Vanin, and D.A. Vignali. 2004. Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nat Biotechnol*. 22:589-594.

Tadano, M., Y. Makino, T. Fukunaga, Y. Okuno, and K. Fukai. 1989. Detection of dengue 4 virus core protein in the nucleus. I. A monoclonal antibody to dengue 4 virus reacts with the antigen in the nucleus and cytoplasm. *J Gen Virol*. 70 (Pt 6):1409-1415.

Takhampunya, R., S. Ubol, H.S. Houng, C.E. Cameron, and R. Padmanabhan. 2006. Inhibition of dengue virus replication by mycophenolic acid and ribavirin. *J Gen Virol*. 87:1947-1952.

Tambyah, P.A., E.S. Koay, M.L. Poon, R.V. Lin, and B.K. Ong. 2008. Dengue hemorrhagic fever transmitted by blood transfusion. *N Engl J Med*. 359:1526-1527.

Tassaneetrithep, B., T.H. Burgess, A. Granelli-Piperno, C. Trumpfheller, J. Finke, W. Sun, M.A. Eller, K. Pattanapanyasat, S. Sarasombath, D.L. Birx, R.M. Steinman, S. Schlesinger, and M.A. Marovich. 2003. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *J Exp Med*. 197:823-829.

Teo, K.F., and P.J. Wright. 1997. Internal proteolysis of the NS3 protein specified by dengue virus 2. *J Gen Virol*. 78 (Pt 2):337-341.

Thepparit, C., and D.R. Smith. 2004. Serotype-specific entry of dengue virus into liver cells: identification of the 37-kilodalton/67-kilodalton high-affinity laminin receptor as a dengue virus serotype 1 receptor. *J Virol*. 78:12647-12656.

Torriani, F.J., M. Rodriguez-Torres, J.K. Rockstroh, E. Lissen, J. Gonzalez-Garcia, A. Lazzarin, G. Carosi, J. Sasadeusz, C. Katlama, J. Montaner, H. Sette, Jr., S. Passe, J. De Pamphilis, F. Duff, U.M. Schrenk, and D.T. Dieterich. 2004.

Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. *N Engl J Med*. 351:438-450.

Tsuda, Y., Y. Mori, T. Abe, T. Yamashita, T. Okamoto, T. Ichimura, K. Moriishi, and Y. Matsuura. 2006. Nucleolar protein B23 interacts with Japanese encephalitis virus core protein and participates in viral replication. *Microbiol Immunol*. 50:225-234.

Uetz, P., L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover, T. Kalbfleisch, G. Vijayadamodar, M. Yang, M. Johnston, S. Fields, and J.M. Rothberg. 2000. A comprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. *Nature*. 403:623-627.

Ugrinova, I., K. Monier, C. Ivaldi, M. Thiry, S. Storck, F. Mongelard, and P. Bouvet. 2007. Inactivation of nucleolin leads to nucleolar disruption, cell cycle arrest and defects in centrosome duplication. *BMC Mol Biol*. 8:66.

Umareddy, I., O. Pluquet, Q.Y. Wang, S.G. Vasudevan, E. Chevet, and F. Gu. 2007. Dengue virus serotype infection specifies the activation of the unfolded protein response. *Virol J*. 4:91.

van der Schaar, H.M., M.J. Rust, C. Chen, H. van der Ende-Metselaar, J. Wilschut, X. Zhuang, and J.M. Smit. 2008. Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. *PLoS Pathog*. 4:e1000244.

van der Schaar, H.M., M.J. Rust, B.L. Waarts, H. van der Ende-Metselaar, R.J. Kuhn, J. Wilschut, X. Zhuang, and J.M. Smit. 2007. Characterization of the early events in dengue virus cell entry by biochemical assays and single-virus tracking. *J Virol*. 81:12019-12028.

Vasilakis, N., E.R. Deardorff, J.L. Kenney, S.L. Rossi, K.A. Hanley, and S.C. Weaver. 2009. Mosquitoes put the brake on arbovirus evolution: experimental evolution reveals slower mutation accumulation in mosquito than vertebrate cells. *PLoS Pathog*. 5:e1000467.

Vasudevan, S.G., M. Johansson, A.J. Brooks, L.E. Llewellyn, and D.A. Jans. 2001. Characterisation of inter- and intra-molecular interactions of the dengue virus RNA dependent RNA polymerase as potential drug targets. *Farmaco*. 56:33-36.

Venkatesan, K., J.F. Rual, A. Vazquez, U. Stelzl, I. Lemmens, T. Hirozane-Kishikawa, T. Hao, M. Zenkner, X. Xin, K.I. Goh, M.A. Yildirim, N. Simonis, K. Heinzmann, F. Gebreab, J.M. Sahalie, S. Cevik, C. Simon, A.S. de Smet, E. Dann, A. Smolyar, A. Vinayagam, H. Yu, D. Szeto, H. Borick, A. Dricot, N. Klitgord, R.R. Murray, C. Lin, M. Lalowski, J. Timm, K. Rau, C. Boone, P. Braun, M.E. Cusick, F.P. Roth, D.E. Hill, J. Tavernier, E.E. Wanker, A.L. Barabasi, and M. Vidal. 2009. An empirical framework for binary interactome mapping. *Nat Methods*. 6:83-90.

Viswanathan, K., K. Fruh, and V. DeFilippis. 2010. Viral hijacking of the host ubiquitin system to evade interferon responses. *Curr Opin Microbiol*. 13:517-523.

von Mering, C., R. Krause, B. Snel, M. Cornell, S.G. Oliver, S. Fields, and P. Bork. 2002. Comparative assessment of large-scale data sets of protein-protein interactions. *Nature*. 417:399-403.

Walhout, A.J., G.F. Temple, M.A. Brasch, J.L. Hartley, M.A. Lorson, S. van den Heuvel, and M. Vidal. 2000. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol*. 328:575-592.

Wang, H., and R.J. Clem. 2011. The role of IAP antagonist proteins in the core apoptosis pathway of the mosquito disease vector Aedes aegypti. *Apoptosis*. 16:235-248.

Wang, M., R. Ye, E. Barron, P. Baumeister, C. Mao, S. Luo, Y. Fu, B. Luo, L. Dubeau, D.R. Hinton, and A.S. Lee. 2010. Essential role of the unfolded protein response regulator GRP78/BiP in protection from neuronal apoptosis. *Cell Death Differ*. 17:488-498.

Wang, Q.Y., S. Bushell, M. Qing, H.Y. Xu, A. Bonavia, S. Nunes, J. Zhou, M.K. Poh, P. Florez de Sessions, P. Niyomrattanakit, H. Dong, K. Hoffmaster, A. Goh, S. Nilar, W. Schul, S. Jones, L. Kramer, T. Compton, and P.Y. Shi. 2011. Inhibition of dengue virus through suppression of host pyrimidine biosynthesis. *J Virol*. 85:6548-6556.

Wang, S., R. He, and R. Anderson. 1999. PrM- and cell-binding domains of the dengue virus E protein. *J Virol*. 73:2547-2551.

Wang, S.H., W.J. Syu, K.J. Huang, H.Y. Lei, C.W. Yao, C.C. King, and S.T. Hu. 2002. Intracellular localization and determination of a nuclear localization signal of the core protein of dengue virus. *J Gen Virol*. 83:3093-3102.

Webster, D.P., J. Farrar, and S. Rowland-Jones. 2009. Progress towards a dengue vaccine. *Lancet Infect Dis*. 9:678-687.

Welsch, S., S. Miller, I. Romero-Brey, A. Merz, C.K. Bleck, P. Walther, S.D. Fuller, C. Antony, J. Krijnse-Locker, and R. Bartenschlager. 2009. Composition and three-dimensional architecture of the dengue virus replication and assembly sites. *Cell Host Microbe*. 5:365-375.

Welzel, F., C. Kaehler, M. Isau, L. Hallen, H. Lehrach, and S. Krobitsch. 2012. FOX-2 Dependent Splicing of Ataxin-2 Transcript Is Affected by Ataxin-1 Overexpression. *PLoS One*. 7:e37985.

Westaway, E.G., and J. Blok. 1997. Taxonomy and evolutionary relationships of flaviviruses. *In* Dengue and dengue hemorrhagic fever. D.J. Gubler, editor. CAB International, London. 147-173.

WHO. 1997. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. World Health Organization, Geneva

Wilder-Smith, A., and E. Schwartz. 2005. Dengue in travelers. *N Engl J Med*. 353:924-932.

Winkler, G., S.E. Maxwell, C. Ruemmler, and V. Stollar. 1989. Newly synthesized dengue-2 virus nonstructural protein NS1 is a soluble protein but becomes partially hydrophobic and membrane-associated after dimerization. *Virology*. 171:302-305.

Wise de Valdez, M.R., D. Nimmo, J. Betz, H.F. Gong, A.A. James, L. Alphey, and W.C.t. Black. 2011. Genetic elimination of dengue vector mosquitoes. *Proc Natl Acad Sci U S A*. 108:4772-4775.

Woolaway, K.E., K. Lazaridis, G.J. Belsham, M.J. Carter, and L.O. Roberts. 2001. The 5' untranslated region of Rhopalosiphum padi virus contains an internal ribosome entry site which functions efficiently in mammalian, plant, and insect translation systems. *J Virol*. 75:10244-10249.

Xi, Z., J.L. Ramirez, and G. Dimopoulos. 2008. The Aedes aegypti toll pathway controls dengue virus infection. *PLoS Pathog*. 4:e1000098.

Xu, X., M. Soutto, Q. Xie, S. Servick, C. Subramanian, A.G. von Arnim, and C.H. Johnson. 2007. Imaging protein interactions with bioluminescence resonance energy transfer (BRET) in plant and mammalian cells and tissues. *Proc Natl Acad Sci U S A*. 104:10264-10269.

Xu, X.F., Z.T. Chen, N. Gao, J.L. Zhang, and J. An. 2009. Myosin Vc, a member of the actin motor family associated with Rab8, is involved in the release of DV2 from HepG2 cells. *Intervirology*. 52:258-265.

Xu, Z., R. Anderson, and T.C. Hobman. 2011. The capsid-binding nucleolar helicase DDX56 is important for infectivity of West Nile virus. *J Virol*. 85:5571-5580.

Yanagawa, S., J.S. Lee, and A. Ishimoto. 1998. Identification and characterization of a novel line of Drosophila Schneider S2 cells that respond to wingless signaling. *J Biol Chem*. 273:32353-32359.

Yang, M.R., S.R. Lee, W. Oh, E.W. Lee, J.Y. Yeh, J.J. Nah, Y.S. Joo, J. Shin, H.W. Lee, S. Pyo, and J. Song. 2008. West Nile virus capsid protein induces p53-mediated apoptosis via the sequestration of HDM2 to the nucleolus. *Cell Microbiol*. 10:165-176.

Yazi Mendoza, M., J.S. Salas-Benito, H. Lanz-Mendoza, S. Hernandez-Martinez, and R.M. del Angel. 2002. A putative receptor for dengue virus in mosquito tissues: localization of a 45-kDa glycoprotein. *Am J Trop Med Hyg*. 67:76-84.

Yon, C., T. Teramoto, N. Mueller, J. Phelan, V.K. Ganesh, K.H. Murthy, and R. Padmanabhan. 2005. Modulation of the nucleoside triphosphatase/RNA helicase and 5'-RNA triphosphatase activities of Dengue virus type 2 nonstructural protein

3 (NS3) by interaction with NS5, the RNA-dependent RNA polymerase. *J Biol Chem*. 280:27412-27419.

Yook, S.H., Z.N. Oltvai, and A.L. Barabasi. 2004. Functional and topological characterization of protein interaction networks. *Proteomics*. 4:928-942.

Yu, C.Y., Y.W. Hsu, C.L. Liao, and Y.L. Lin. 2006. Flavivirus infection activates the XBP1 pathway of the unfolded protein response to cope with endoplasmic reticulum stress. *J Virol*. 80:11868-11880.

Yu, H., P. Braun, M.A. Yildirim, I. Lemmens, K. Venkatesan, J. Sahalie, T. Hirozane-Kishikawa, F. Gebreab, N. Li, N. Simonis, T. Hao, J.F. Rual, A. Dricot, A. Vazquez, R.R. Murray, C. Simon, L. Tardivo, S. Tam, N. Svrzikapa, C. Fan, A.S. de Smet, A. Motyl, M.E. Hudson, J. Park, X. Xin, M.E. Cusick, T. Moore, C. Boone, M. Snyder, F.P. Roth, A.L. Barabasi, J. Tavernier, D.E. Hill, and M. Vidal. 2008a. High-quality binary protein interaction map of the yeast interactome network. *Science*. 322:104-110.

Yu, H., N.M. Luscombe, H.X. Lu, X. Zhu, Y. Xia, J.D. Han, N. Bertin, S. Chung, M. Vidal, and M. Gerstein. 2004. Annotation transfer between genomes: protein-protein interologs and protein-DNA regulogs. *Genome Res*. 14:1107-1118.

Yu, H., L. Tardivo, S. Tam, E. Weiner, F. Gebreab, C. Fan, N. Svrzikapa, T. Hirozane-Kishikawa, E. Rietman, X. Yang, J. Sahalie, K. Salehi-Ashtiani, T. Hao, M.E. Cusick, D.E. Hill, F.P. Roth, P. Braun, and M. Vidal. 2011. Next-generation sequencing to generate interactome datasets. *Nat Methods*. 8:478-480.

Yu, I.M., W. Zhang, H.A. Holdaway, L. Li, V.A. Kostyuchenko, P.R. Chipman, R.J. Kuhn, M.G. Rossmann, and J. Chen. 2008b. Structure of the immature dengue virus at low pH primes proteolytic maturation. *Science.* 319:1834-1837.

Yu, J., and R.L. Finley, Jr. 2009. Combining multiple positive training sets to generate confidence scores for protein-protein interactions. *Bioinformatics.* 25:105-111.

Yu, J., T. Murali, and R.L. Finley, Jr. 2012. Assigning confidence scores to protein-protein interactions. *Methods Mol Biol*. 812:161-174.

Yu, J., S. Pacifico, G. Liu, and R.L. Finley, Jr. 2008c. DroID: the Drosophila Interactions Database, a comprehensive resource for annotated gene and protein interactions. *BMC Genomics*. 9:461.

Yuan, X., Y. Zhou, E. Casanova, M. Chai, E. Kiss, H.J. Grone, G. Schutz, and I. Grummt. 2005. Genetic inactivation of the transcription factor TIF-IA leads to nucleolar disruption, cell cycle arrest, and p53-mediated apoptosis. *Mol Cell*. 19:77-87.

Zhang, L., N.Y. Villa, M.M. Rahman, S. Smallwood, D. Shattuck, C. Neff, M. Dufford, J.S. Lanchbury, J. Labaer, and G. McFadden. 2009. Analysis of vaccinia virus-host protein-protein interactions: validations of yeast two-hybrid screenings. *J Proteome Res*. 8:4311-4318.

Zhang, Y., J. Corver, P.R. Chipman, W. Zhang, S.V. Pletnev, D. Sedlak, T.S. Baker, J.H. Strauss, R.J. Kuhn, and M.G. Rossmann. 2003. Structures of immature flavivirus particles. *EMBO J*. 22:2604-2613.

Zhong, J., H. Zhang, C.A. Stanyon, G. Tromp, and R.L. Finley, Jr. 2003. A strategy for constructing large protein interaction maps using the yeast two-hybrid system: regulated expression arrays and two-phase mating. *Genome Res.* 13:2691-2699.

Zhu, J., L. Chen, and A.S. Raikhel. 2003. Posttranscriptional control of the competence factor betaFTZ-F1 by juvenile hormone in the mosquito Aedes aegypti. *Proc Natl Acad Sci U S A*. 100:13338-13343.

Zybert, I.A., H. van der Ende-Metselaar, J. Wilschut, and J.M. Smit. 2008. Functional importance of dengue virus maturation: infectious properties of immature virions. *J Gen Virol*. 89:3047-3051.

ABSTRACT

CHARACTERIZATION OF INTRACELLULAR INTERACTIONS BETWEEN DENGUE VIRUS AND HOST PROTEINS

by

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Dengue virus is the causative agent of dengue fever, dengue hemorrhagic fever and dengue shock syndrome. About two-fifths of world population live in areas where dengue is prevalent, leading to high levels of morbidity and mortality in many areas. Currently there are no vaccines or effective treatments. The virus is transmitted from one person to another by the yellow fever mosquito, *Aedes aegypti*. The genome of dengue virus encodes only ten proteins implying that the virus needs to interact with and utilize several host proteins for replication. In this project, I used high-throughput yeast two-hybrid screening to identify mosquito and human proteins that physically interact with dengue proteins. I detected 46 dengue-human and 102 dengue-mosquito protein interactions, including some that had been discovered previously and many novel interactions. I further confirmed 38 out of 136 testable interactions using co-affinity purification assays from cultured cells. I tested each host protein against the proteins from all four serotypes of dengue virus and found that 57 out of 102 (56.9%) dengue-mosquito PPI and 34 out of 46 (73.9%) dengue-human PPI interacted with corresponding dengue proteins from all four serotypes.

To further analyze biological significance of these protein interactions, I selected to study capsid-NAP1 interaction. I employed the domain mapping of capsid using yeast two-hybrid and co-affinity purification. I also over-expressed or silenced NAP1L1 in HepG2 cells stably expressing capsid. I found that NAP1L1 might bind the bipartite sequence of capsid blocking importin binding and sequestering capsid in the cytoplasm.

I also showed that the mosquito cells, AAG2, were capable of uptaking double stranded RNA without a transfection vehicle. Thus, a large-scale RNA interference study in AAG2 as previously published is feasible.

Finally, I showed that using two 2A sequences to generate three separate peptides form a single mRNA was possible in the insect cells. This construct may be applied to design a non-infectious dengue replicon, which may be a safer substitute of the live dengue virus.

The dengue-host interaction maps and the new tools that I generated should be useful for understanding how dengue interacts with its hosts and may provide candidates for drug targets and vector control strategies.

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