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Gonadal hypoplasia in Swedish Mountain cattle and other native Swedish cattle breeds

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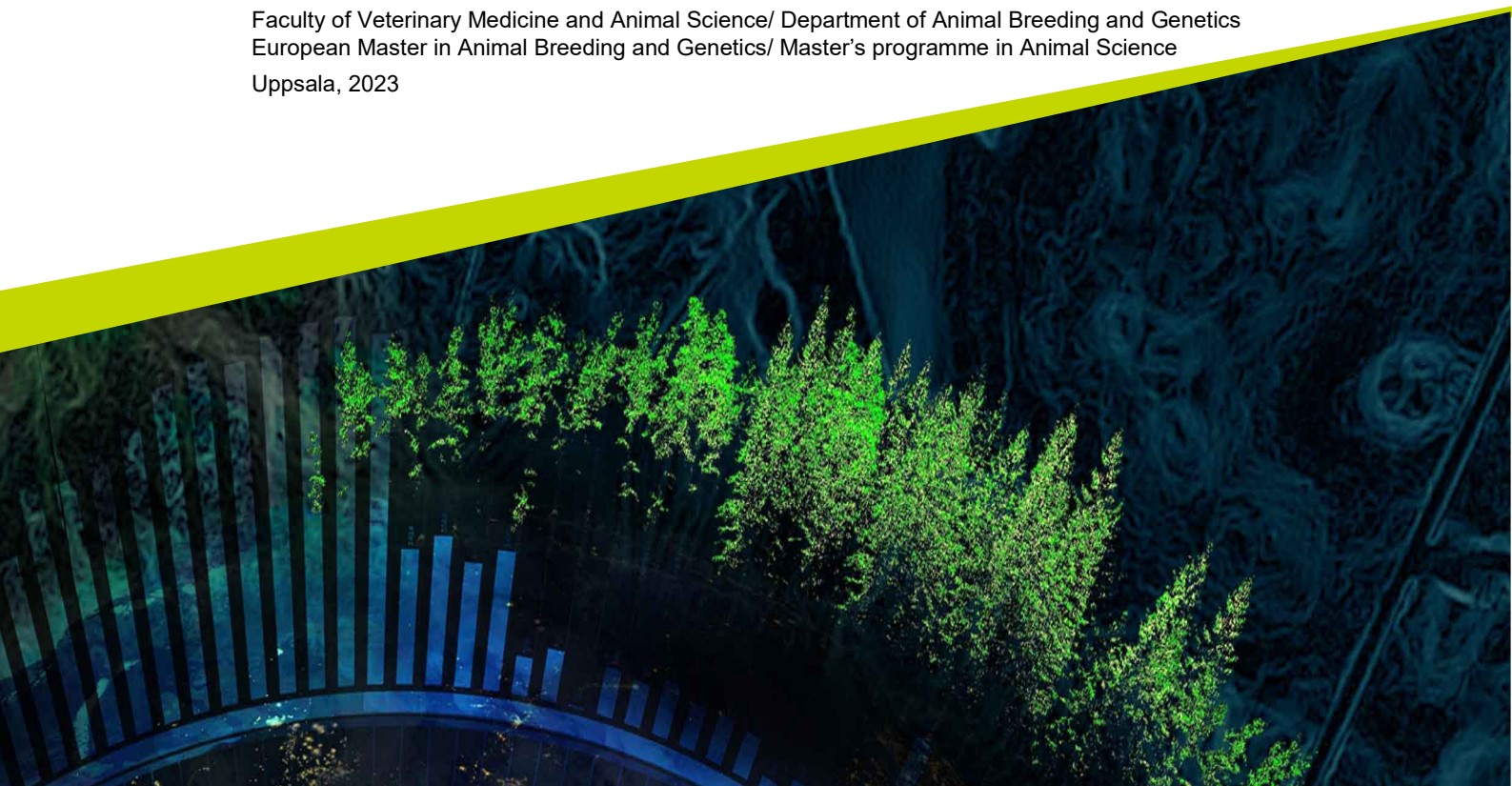

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Abstract

The Swedish Mountain cattle and several related breeds show the phenotype of colour-sidedness. The genetic reasons for this phenotype are a translocation and duplication from chr6 to chr29 (known as Cs_{29}) including the *KIT* gene and an additional translocation with part of the Cs_{29} translocation from chr29 back to chr6 (Cs_6). Besides the colour-sidedness the Cs_{29} translocation is known to be responsible for gonadal hypoplasia. Gonadal hypoplasia leads to an underdevelopment of the ovaries and testicles and can cause fertility problems. Breeders of the Swedish Mountain cattle have been aware of the appearance of gonadal hypoplasia since the early 20th century and have been trying to select against it ever since. However, there are still individuals found which show gonadal hypoplasia.

Therefore, the aim of this research was to investigate, how common the allele that is responsible for gonadal hypoplasia is in Swedish Mountain cattle and closely related breeds based on DNA samples from individuals born between 1976 and 2015. Therefore, 60 DNA samples were analysed using three primer pairs to identify the different genotypes of the Cs_{29} translocation and the additional Cs_6 translocation. The PCR was done using a multiplex of the primer pairs and the genotypes were identified by doing an electrophoresis in TapeStation.

The estimated allele frequencies for the Cs_{29} translocation were 0.22 for Swedish Polled, 0.27 for Fjällnära, 0.40 for Väneko, 0.56 for Bohus Polled and 0.57 for Swedish Mountain cattle. The results indicate that the Cs_{29} translocation, responsible for gonadal hypoplasia, is still common in Swedish Mountain cattle and can also be found in related breeds and other native Swedish cattle breeds that show the colour-sidedness phenotype. Thus, the integration of a DNA analysis to test for gonadal hypoplasia to detect the carriers of the Cs_{29} translocation would be favourable to increase the selection success against gonadal hypoplasia.

Keywords: gonadal hypoplasia, Swedish Mountain cattle, fjäll

Table of contents

List of tables

List of figures

Abbreviations

A	Adenine
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BWA	Burrows wheeler aligner
C	Cytosine
chr	Chromosome
Cs_6/Cs_{29}	Allele name of the translocation on specific chromosome
DNA	Deoxyribonucleic acid
G	Guanine
kbp	Kilobase pair
Mbp	Megabase pair
PCR	Polymerase chain reaction
SKB	Svensk Kullig Boskap (Swedish Polled)
SNP	Single nucleotide polymorphism
T	Thymine
VCF	Variant call format
VEP	Ensembl Variant Effect Predictor
WFFS	Warmblood fragile foal syndrome

1. Introduction

1.1 Swedish Mountain cattle

The Swedish Mountain cattle (Fjäll) is a native breed which is known to be healthy and robust and once played an important role for milk production in northern Sweden (Eriksson & Petitt, 2020; Johansson et al., 2020). In 2012 the breed consisted of 6,683 breeding individuals: 1,672 breeding males and 5,011 breeding females (Food and Agriculture Organisation of the United Nations, 2022c). Despite the small population size, the breed shows a relatively high genetic diversity (Upadhyay et al., 2019). Furthermore, Swedish Mountain cattle cows show a higher frequency of the β-casein variants A^1 and B as well as the κ-casein variant B compared to conventional milk production breeds (Poulsen et al., 2017). This and the low prevalence of non-coagulating milk makes the milk of Swedish Mountain cattle cows more suitable for cheese production compared to Swedish Red cows (Poulsen et al., 2017).

In 2016 a new breeding scheme was decided but due to the small population size breeding progress especially for yield traits is difficult (Svensk Fjällrasavel, 2016). Therefore, the Swedish Mountain cattle breeding association decided to mainly focus on improving casein production and udder quality (Svensk Fjällrasavel, 2016).

The breed was founded in the 1880s and in 1893 breeders came up with criteria for what Swedish Mountain cattle should look like (Svensk Fjällrasavel, 2022d). Based on these rules cows and bulls should have mainly white coat colour with red or black dots on their flanks and coloured ears (Food and Agriculture Organisation of the United Nations, 2022c; Johansson et al., 2020; Svensk Fjällrasavel, 2022d). With selection based on the white coat colour a problem was introduced in the breed that has still relevance nowadays: Gonadal hypoplasia (Svensk Fjällrasavel, 2022d). The breeding and selection based on the favoured white coat colour with the coloured sides spread the translocation that is responsible for gonadal hypoplasia through the population (Svensk Fjällrasavel, 2022d). Genetically the white coat colour is caused by a translocation on chromosome (chr) 6 and chr29.

Besides the white coat colour the translocation on chr29 can cause gonadal hypoplasia (Venhoranta et al., 2013). The major cause of gonadal hypoplasia is the underdevelopment of one or both gonads (Venhoranta et al., 2013).

1.1.1 Research aim

Therefore, the aim of this research was to investigate, how common the allele that is responsible for gonadal hypoplasia is in Swedish Mountain cattle and other native Swedish cattle breeds based on DNA samples from individuals born between 1976 and 2015. Part of the project was investigation and reanalysis of the translocation as well as allele frequency calculations. Furthermore, bioinformatic analysis was done to investigate if primers designed for previous research were suitable for this project.

1.2 Gonadal hypoplasia in Swedish Mountain cattle

Gonadal hypoplasia is described as the phenomenon that one or both gonads, ovaries in cows and testicles in bulls, are small and underdeveloped. The underdevelopment may lead to fertility problems (Venhoranta et al., 2013). In Swedish Mountain cattle the affected ovary or testicle is predominantly the left one, but cases with right sided or double-sided hypoplasia can be found, too (Lagerlöf & Settergren, 1953; Venhoranta et al., 2013). The side proportions of the affected gonads differ slightly between authors, but the left gonad is always the most common affected one (K. Eriksson, 1943; Lagerlöf & Settergren, 1953).

The origin of gonadal hypoplasia in Swedish Mountain cattle is suspected in Jämtland in 1900 (K. Eriksson, 1943). In the following years fertility problems as well as the number of affected animals increased, and researchers found out that hypoplasia was an inherited disorder (Lagerlöf & Settergren, 1953). Thus, in 1937 the Swedish Mountain cattle breeding association decided that hypoplasia affected bulls could no longer be included in the studbook (Lagerlöf & Settergren, 1953). Consequently, the occurrence of gonadal hypoplasia in the breeding population should be reduced (Lagerlöf & Settergren, 1953). From 1943 onwards only bulls from unaffected dams were considered as breeding bulls and since 1950 the dams dam of a breeding bull must be unaffected, too (Lagerlöf & Settergren, 1953).

Nowadays cows are still tested for hypoplasia by getting their ovaries checked by a veterinarian (Svensk Fjällrasavel, 2022b). Based on the recommended actions of the Swedish Mountain cattle breeding association, heifers should be tested at the

age of 12 months and cows during the time frame between their first calving and the first insemination after their first calving (Svensk Fjällrasavel, 2022b). A double check for hypoplasia, with the second check after the first calving was also mentioned by Lagerlöf and Settergren in 1953 (Lagerlöf & Settergren, 1953). Previous research showed that the incidence of gonadal hypoplasia in bulls and cows is equal (Lagerlöf & Boyd, 1953, cited after Lagerlöf, 1939).

The Swedish Mountain cattle is closely related to several other original Swedish cattle breeds. Three of them, Fjällnära, Bohus Polled (Bohuskulla) and Swedish Polled (Svensk Kullig Boskap) were included in the PCR research of this project. Besides the Väneko breed was included, which is not closely related to the Swedish Mountain cattle, but some individuals also show the colour-sidedness phenotype.

Besides, three additional original Swedish breeds, Väneko, Swedish Red Polled (Rödkulla) and Ringamålako were analysed for the gonadal hypoplasia translocation. The breeds developed in regions of Sweden that were more isolated and are nowadays at high risk for extinction (Food and Agriculture Organisation of the United Nations, 2022a, 2022b; Svensk Fjällrasavel, 2022a). Due to the shared origin and potential for gene flow between these breeds and the colour-sidedness it can be expected to find gonadal hypoplasia in them as well. However, the occurrence of gonadal hypoplasia in these related breeds has not been studied in the past.

1.2.1 Genetic reasons for gonadal hypoplasia in Swedish Mountain cattle

The known genetic mechanism responsible for gonadal hypoplasia is homozygosity of a ~500 kilobase pair (kbp) chromosomal segment that is translocated and duplicated from chr6 to chr29 (known as Cs_{29} allele) (Venhoranta et al., 2013). The duplicated segment includes the *KIT* gene which is known to encode a type III receptor protein of the tyrosine kinase family (Venhoranta et al., 2013). The *KIT* protein is needed for survival, proliferation and migration of melanocyte precursors and primordial germ cells during embryogenesis as well as regular development of hematopoietic stem cells (Bernex et al., 1996; Buehr et al., 1993; Nishikawa et al., 1991; Ogawa et al., 1993). Besides, the *KIT* gene is associated with white pigmentation and spotting in mammals as pigs (Andersson, 2020). Variants in the *KIT* gene were found to influence the fertility and to be one of the several reasons for infertility in human males (Galan et al., 2006). Furthermore, there might be other genetic variants that contribute to gonadal hypoplasia but are currently unknown (Venhoranta et al., 2013).

The translocated duplication is also one of the two known factors responsible for the colour-sidedness (Durkin et al., 2012). In humans' mutations in the *KIT* gene can lead to germ cell tumours (Cheng et al., 2011; Coffey et al., 2008). In Northern Finncattle and Swedish Mountain cattle the wild type *KIT* gene on chr6 only affects the coat colour, the translocated *KIT* gene as part of the Cs_{29} allele is associated with both, white coat colour and gonadal hypoplasia (Figure 1) (Venhoranta et al., 2013). The name Cs_{29} was introduced by Durkin et al., 2012 and described the translocated part from chr6 to chr29, while the Cs_{6} allele is the name for the translocated part from chr29 back to chr6. The different effects of the two *KIT* genes, the normal one on chr6 and the translocated version on chr29, showed that a predominantly white animal is not affected by gonadal hypoplasia in general (Venhoranta et al., 2013).

Venhoranta et al. (2013) also found homozygous animals for the Cs_{29} allele in the control group which should be unaffected from gonadal hypoplasia. This suggests that there must be other factors that influence the occurrence of gonadal hypoplasia, like one or more additional mutations. This makes it more difficult to detect animals that carry the mutation causing gonadal hypoplasia just by veterinary inspections. The assumption of additional genetic changes is supported by the fact, that colour-sidedness caused by the Cs_{6} and Cs_{29} allele translocation is widespread in several cattle breeds like Belgium blue, Gloucester cattle, Chillingham white cattle, Northern Finncattle, African Nguni cattle and Pinzgauer cattle (Artesi et al., 2020; Durkin et al., 2012; Hall et al., 2021; Szczerbal et al., 2017; Venhoranta et al., 2013). Gonadal hypoplasia was found in the Chillingham White cattle, African Nguni cattle and the Northern Finncattle (Hall et al., 2021; Kay et al., 1992; Venhoranta et al., 2013). The Northern Finncattle is closely related to the Swedish Mountain cattle and for the Chillingham white cattle a historic relationship with the Swedish Mountain cattle was assumed (Hall et al., 2021; Venhoranta et al., 2013). In the African Nguni cattle blood from European breeds was introduced in the 19th century (Szczerbal et al., 2017).

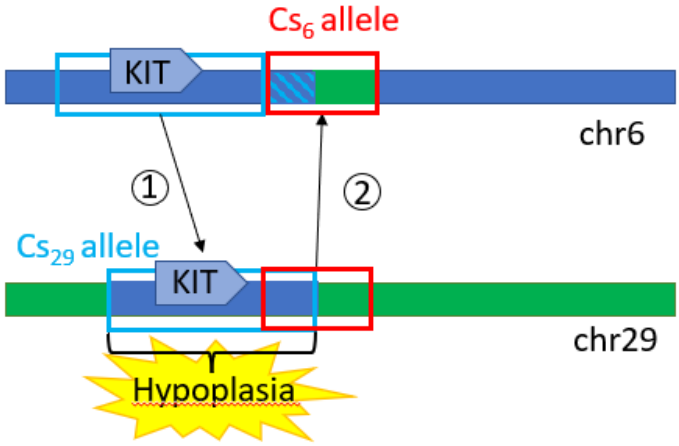


Figure 1 Sections responsible for the colour-sidedness and hypoplasia
Colour-sidedness occurs due to two factors: A translocation and duplication of a chromosomal segment that includes the KIT gene from chr6 to chr29 (Cs_{29} allele) (1) or a translocation of part of this fragment back to chr6 (Cs6) (2). The translocation from chr6 to chr29 is next to the colour-sidedness also responsible for hypoplasia. Own figure based on Durkin et al., 2012 and Venhoranta et al., 2013.

1.2.2 Genetic causes of gonadal hypoplasia in other species

Gonadal hypoplasia has been found in several mammalian species. Different types of gonadal hypoplasia have been reported in cats, dogs, sheep, alpacas, and humans as well as other cattle breeds (Arroyo et al., 2022; Bidarkar & Hutson, 2005; Neves et al., 2019; Palmieri et al., 2011; Romagnoli & Schlafer, 2006). Testicular hypoplasia was found in Nellore cattle (Neves et al., 2019). Here researchers found a polygenetic nature for the disease, but they detected no matching genomic regions as they were found by Venhoranta et al., 2013 (Neves et al., 2019). In cats' gonadal hypoplasia is triggered by abnormal chromosomal sex complements and in dogs it is caused by abnormal karyotypes (Romagnoli & Schlafer, 2006). In alpacas it is suspected that the gonadal hypoplasia is caused by a size difference of the autosome pair of chr36, one homologue was found to be significantly smaller than the other one in affected individuals (Fellows et al., 2014).

2. Material and Methods

2.1 Material

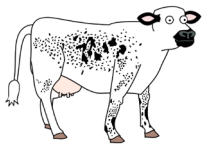
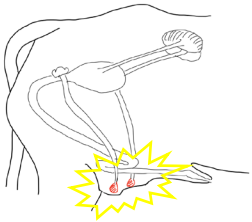
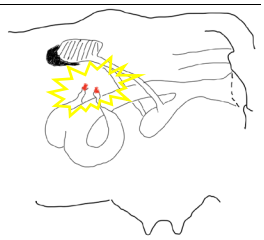
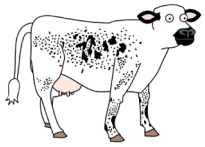
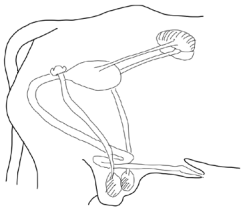
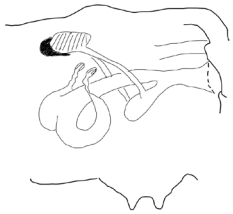
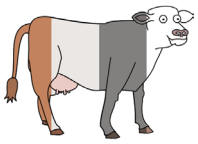
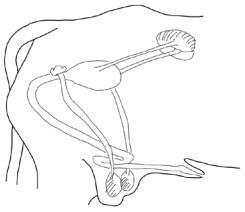
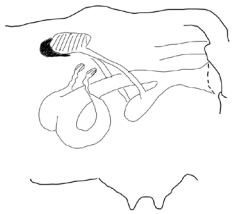
2.1.1 Primers

The six primer pairs used for this research were developed by Durkin et al., 2012 and Venhoranta et al., 2013 based on the UMD3.1 assembly of the bovine genome (Zimin et al., 2009). An overview of the primer pairs can be found in Appendix 1.

The primer pairs are used because they span breakpoints of the translocation and fusion points on the two chromosomes of interest, chr6 and chr29. This is important to distinguish between genetically affected and unaffected individuals as well as between the two translocations, because only the one from chr6 to chr29 (Cs_{29}) causes, next to the colour-sidedness, gonadal hypoplasia.

The primer pairs α-D, A-E and C-β mark the Cs_{29} allele which is responsible for the colour-sidedness and gonadal hypoplasia. The γ-B primer pair identifies the Cs_6 allele also known to cause colour-sidedness. The α-β and α-β_2 primer pair identifies the wild type allele on chr29. An overview, which primer pair identifies which phenotype is given in Table 1.

Table 1 Overview which primer pair marks which genotype with illustrations of the caused phenotypes.

Primer pair	Function	Coat colour	Gonads	
			Bull	Cow
α-D A-E C-β	Identify Cs_{29}			
		Colour-sidedness	Underdeveloped gonads	
γ-B	Identify Cs_6			
		Colour-sidedness	Normal gonads	
α-β α-β_2	Identify wild-type chr29			
		Various coat colours	Normal gonads	

2.1.2 Whole genome sequence data

For the bioinformatic analysis whole-genome sequence data from 30 individuals from different Swedish cattle breeds were available. The breed distribution can be seen in Table 2. This project used mapped reads (in binary alignment map files) and called variants (in variant call format files) that were generated previously and then made available for further analysis. Mapping of reads and variant calling was performed with the Sarek workflow (v. 2.7.1) (Garcia et al., 2020), where reads were aligned to the ARS-UCD1.2 reference genome (Rosen et al., 2018) with the burrows wheeler aligner (BWA) (Li & Durbin, 2009), and variants were called using the GATK HaplotypeCaller (v. 4.2.6.1) (Auwera, 2020) with default parameters. No phenotypes for gonadal hypoplasia were available.

Table 2 Number of whole-genome sequenced individuals from the different Swedish cattle breeds

Number	Breed
9	Swedish Red Polled (Rödkulla)
7	Swedish Mountain cattle (Fjäll)
5	Väneko
4	Fjällnära
3	Bohus Polled (Bohuskulla)
2	Ringamålako

2.1.3 Samples for laboratory assays

DNA from 60 individuals from different native Swedish cattle breeds was analysed. The DNA samples were already collected in the past for previous research. The DNA was extracted from blood and sperm samples and stored at -20°C. Individuals were from the Fjäll breed, from breeds which were related to the Fjäll breed or breeds which show the colour-sidedness phenotype. All breeds studied had their origin in Sweden. The distribution of the different breeds can be seen in Table 3. The analysed animals were cows as well as bulls and born between 1976 and 2015. A detailed overview of the samples is given in Appendix 2. For the 60 individuals no phenotypes for gonadal hypoplasia were available.

Table 3 Number of samples from the different Swedish cattle breeds for the DNA analysis.

Number	Breed
23	Swedish Mountain cattle (Fjäll)
15	Fjällnära
9	Swedish Polled (Svensk Kullig Boskap)
8	Bohus Polled (Bohuskulla)
5	Väneko

Samples for Sanger sequencing and PCR and TapeStation test run

To test the primer pairs, four individuals with known genotype from the earlier bioinformatic analysis were analysed with Sanger sequencing. To make sure the results could be visualised with the Agilent 4200 TapeStation the same four animals were taken for a test Polymerase chain reaction (PCR) and TapeStation run (Agilent Technologies, 2022). An overview of the four individuals and their genotypes can be found in Table 4

Table 4 Test individuals for Sanger sequencing, test PCR and TapeStation as well as their genotypes. Genotype was determined using "bedtools" and is shown in section 3.2.1.

Individual	Genotype
SE 432	Wildtype chr29
SE 012	Homozygous Cs_{29}
SE 425	Heterozygous Cs_{29}
SE 458	Heterozygous Cs_{29}| Cs_{6}

2.2 Methods

2.2.1 Bioinformatic analysis of the primer pairs

In Durkin et al., 2012 and Venhoranta et al., 2013 the primer pairs were developed based on the UMD3.1 assembly of the bovine genome (Zimin et al., 2009). However, there is a newer version of the bovine genome available called ARS-UCD1.2 (Rosen et al., 2018). Therefore, it was part of the project to analyse where the primer pairs map to the new cattle reference genome. This was done by using the Basic Local Alignment Search Tool (BLAST) (EMBL-EBI, 2022). With the BLAST web tool the primer pairs designed by Durkin et al., 2012 and Venhoranta et al., 2013 (Appendix 1) were searched against the new cattle reference genome ARS-UCD1.2 and their place of alignment was checked. An example of the settings can be seen in Appendix 3. To check for the best fitting result the length, E-value and percent identity were compared when multiple results were found. The best result has the same length as the primer and a percent identity of 100. Furthermore, the best fitting high-scoring segment pair on the karyotype is marked with a box. To check, if the primer pairs fall in the correct region of the new reference genome the primer pairs should be close to the *KIT* or *LUZP2* gene.

Afterwards the results were compared to the region where the primer pairs were expected to align based on the research of Durkin et al., 2012 and Venhoranta et al., 2013. To make sure that the primer pairs did not amplify any other unexpected region the UCSC in-silico PCR tool was used (Kent, 2022).

2.2.2 Bioinformatic analysis of sequence data

To locate the position of the hypoplasia mutation in the cattle genome the location from Figure 4 in the journal article from Venhoranta et al., 2013 was taken. Venhoranta et al., 2013 used the old cattle reference genome UMD3.1. To transfer the position from the UMD3.1 reference genome to the new ARS-UCD1.2 cattle reference genome the Lift Genome Annotations web tool was used (University of California Santa Cruz (UCSC), 2022). The change of the position of the responsible region for gonadal hypoplasia in the genome can be seen in Table 5.

For each of the 30 whole genome sequenced individuals (Table 2) the region of interest (Table 5) was divided into windows of 1 kbp of size using bedtools "makewindows" (Quinlan & Hall, 2010). Afterwards bedtools "coverage" was used to estimate the number of reads mapping on chr6 and chr29 for each of the 30 whole-genome sequenced individuals (Quinlan & Hall, 2010). The results were visualized using R (R Core Team, 2021) and the ggplot package (Hadley Wickham, 2016).

To investigate the potential function of the single nucleotide polymorphisms (SNPs) within the region of interest the Ensembl Variant Effect Predictor (VEP) software was used (McLaren et al., 2016). The SNPs found with the VEP software were filtered with R (R Core Team, 2021). As a filter the *KIT* gene as affected region, based on the name of the cattle *KIT* gene in the ARS-UCD1.2 reference genome on the Ensembl website was used (ENSBTAG00000002699) (Ensembl, 2022). Afterwards only entries with a high or moderate impact were kept for further analysis.

The location of the identified genes with high and moderate impact from the VEP analysis were used to define the area for the variant call format (VCF) tools analysis (Danecek et al., 2011). The area was defined from 70,200,000 base pairs (bp) to 70,220,000 bp. The matrix created with the VCF tools analysis was filtered for the genes found within the VEP analysis. Filtering was done with R (R Core Team, 2021).

Table 5 Change of the position of the region of interest for gonadal hypoplasia in the old reference genome (UMD3.1) to the new one (ARS-UCD1.2).

Chromosome	Old position (UMD3.1)	New position (ARS-UCD1.2)
6	71,000,000-72,500,000	69,351,066-70,814,795
29	19,500,000-21,000,000	19,334,261-20,791,745

2.2.3 Quantifying DNA samples

The DNA samples for the lab work were checked for their DNA quantity because the DNA samples were collected and used for different projects in the past. Quality control was done with NanoDrop (Thermo Fisher Scientific Inc., 2010). NanoDrop is a spectrophotometer (wavelength: 220-750 nm) to measure wavelength and does not require any preparation of the samples for the measurement. The analysis was done with the "Nucleic Acid" setting. All 60 samples were quantified.

Samples were additionally analysed with Qubit when the NanoDrop analysis gave a result below 10 ng DNA/µl or contaminations were detected (Invitrogen, 2010). NanoDrop as a spectrophotometric technique is sensitive to contamination that absorbs in the same wavelength region as DNA, the Qubit assay is less vulnerable to such contaminations. Twenty-one ADNA samples were run in a Qubit analysis. The DNA samples for which an additionally Qubit analysis was done can be found in Appendix 2. For the Qubit analysis a working solution based on the Qubit dsDNA BR assay kit was prepared following the manufactures guidelines (Invitrogen, 2010). Results can be found in Appendix 4.

2.2.4 Sequencing PCR with Sanger sequencing

Out of the 60 DNA samples four individuals, which were whole genome sequenced, were selected for breakpoint primer sequencing because their genotype is known from previous analysis (Table 4). The breakpoint primer sequencing was done using six different primer pairs using the Sanger sequencing technique (Crossley et al., 2020). Details about the primers are given in Appendix 1. Each of the four DNA samples was run twice for each primer, once forward and once reverse. This made in total 24 different reactions.

The preparation of the DNA samples for the sequencing was done in three steps. The first step contained the PCR for the specific primer regions. For the PCR a master mix was prepared. It consisted of 5.00 µl BigDye direct PCR mix, 2.50 µl nuclease free water and 1.50 µl of 0.80 µM primer mix. Each reaction for the regions PCR was built up out of 9.00 µl master mix and 1.00 µl DNA mix. The 1.00 µl DNA mix included 4.00 ng DNA. The DNA mix was calculated based on the results of the NanoDrop analysis to ensure the amount of 4.00 ng DNA in the DNA mix (Table 6). The 10.00 µl reaction mixture was placed in the ProFlex PCR system and ran 10 minutes at 95°C, followed by 35 cycles of 96°C for 3 seconds, 15 seconds at 58°C and 30 seconds at 68°C. Subsequently to the 35 cycles the reaction was heated up once to 72°C for 2 minutes and finally held at 12°C.

The second step contained the cycle sequencing reaction. Therefore, a mixture of 2.00 µl BigDye sequencing master mix was offset with 1.00 µl of a forward or reverse primer. The setting included the following steps, heating for 15 minutes up to 37°C, 80°C for 2 minutes, 96°C for 1 minute. Afterwards 25 cycles of 10 seconds at 96°C, 5 seconds at 50°C and 75 seconds at 60°C. Finally, the samples were cooled down to 4°C.

The third and final step included the cleaning of the samples. The cleaning was done by pipetting 45.00 µl SAM and 10.00 µl Xterminator into each sample, to remove unincorporated BigDye terminators and salts. For further process the company instructions were followed (Applied Biosystems, 2007). Sequencing was done by using the 3500xL Genetic Analyzer.

Afterwards the results of the Sanger sequencing were aligned by using Mega 11 (Tamura et al., 2021) and blasted by using the ensemble BLAST web tool (EMBL-EBI, 2022). In some samples the bases in the region of the primer pair deviated from the original primer pair bases as shown in Appendix 1. In this case the bases were corrected for the original primer pair base.

Table 6 Calculation of DNA mix for DNA sequencing.

		Calculation (µl)			
		Exact value		Rounded value	
Sample ID	Concentration (NanoDrop, ng/µl)	DNA	H_2O	DNA	H_2O
SE 012	952.20	0.42	99.58	0.50	99.50
SE 425	1426.00	0.28	99.72	0.50	99.50
SE 432	813.50	0.49	99.51	0.50	99.50
SE 458	1802.00	0.22	99.78	0.50	99.50

2.2.5 PCR

Test run

The test run of the standard PCR and TapeStation was done with the same four individuals which were used for the Sanger sequencing because their genotype and the functionality of the primer pairs were known. The selected animals are described in Table 4. For the PCR reaction a primer working solution of 10.00 µM was created. To meet the requirements of the HotStarTaq Plus Master Mix kit the DNA samples were diluted to create a DNA working solution with 10 ng DNA/µl.

The PCR working solution was based on the HotStarTaq Plus Master Mix of Quiagen (Qiagen, 2016). The PCR working solution was calculated once for single use of the primer pairs A-E, γ-B and α-β and once for a multiplex of the three primer pairs. In the multiplex all three primer pairs A-E, γ-B and α-β were combined in one reaction. This was possible, because the sizes of the used primer pairs were different from each other so it should be possible to distinguish between them in the TapeStation electrophoresis. Only three of the original six primer pairs designed by Durkin et al., 2012 and Venhoranta et al., 2013 were used for the test run because the Sanger sequencing showed that some primer pairs marked the same genotype. The primer pairs α-D, A-E and C-β all marked the Cs_{29} translocation and the α-β and α-β_2 both marked the wild type allele. The PCR reaction was built up from 9.00 µl PCR working solution and 1.00 µl DNA working solution which contained 10.00 ng DNA/µl. For each primer pair or multiplex a negative control was added to check the purity of the primers. Details for the composition of the PCR working solution are shown in Table 7. The multiplex is a combination of all three primer pairs in one tube.

The PCR reaction was performed in the ProFlex PCR system. The initial denaturation was done at 95°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 58°C for 1 minute and 72°C for 1 minute. The final extension was done at 72°C for 10 minutes. Afterwards the PCR products were cooled down to 15°C. The temperature cycle for the breakpoint PCR is different from the one used for the Sanger sequencing to meet the requirements of the HotStarTaq Plus Master Mix kit.

Table 7 Test run PCR working solution based on the HotStarTaq Plus Master Mix

	1x (single)	1x (multiplex)
HotStarTaq Plus Master Mix	5.00	5.00
Primer F working solution	0.50	3 x 0.25 = 0.75
Primer R working solution	0.50	3 x 0.25 = 0.75
H_2O	3.00	2.50
Sum	9.00	9.00

Final PCR

The final PCR was done in the same way as the test PCR. Only the amount of the γ-B primer pair in the PCR working solution was increased from 0.25 µl to 0.40 µl to make it more visible in the multiplex. Resulting from this the water volume was decreased from 2.50 µl to 2.20 µl. The final composition of the multiplex PCR working solution is described in Table 8.

Table 8 Final PCR working solution based on the HotStarTaq Plus Master Mix

	1x
HotStarTaq Plus Master Mix	5.00
Primer F working solution	
A-E	0.25
γ-B	0.40
α-β	0.25
Primer R working solution	
A-E	0.25
γ-B	0.40
α-β	0.25
H_2O	2.20
Sum	9.00

2.2.6 Visualizing PCR results with TapeStation

All PCR products were visualized using the Agilent 4200 TapeStation system using the High Sensitivity D1000 kit. The analysis was prepared following the steps in the High Sensitivity D1000 ScreenTape assay for TapeStation systems (Agilent Technologies Inc., 2021).

Rerunning samples in TapeStation with missing marker

The TapeStation results showed 20 samples with missing lower markers or different band sizes for the primer regions. For those samples a second TapeStation run was done. For the second run the PCR product was not added pure but diluted with 1.00 µl PCR product and 1.00 µl nuclease free water to get 2.00 µl PCR reaction. Otherwise, the TapeStation assay for the High Sensitivity D1000 ScreenTape was followed (Agilent Technologies Inc., 2021). The dilution of the PCR product should make it easier for the TapeStation to identify the markers.

2.2.7 Calculating frequencies

The results of the TapeStation analysis were used to calculate the genotype and allele frequencies of the Cs_{29} and Cs_6 translocation. To identify the different genotypes based on the TapeStation the results from the electrophoresis picture were compared with the band sizes in the sample intensity diagrams. The electrophoresis picture and the sample intensity diagram were compared within each TapeStation run not across them.

The smallest band showed the PCR product of the A-E primer pair and could therefore be seen as indicator of the Cs_{29} translocation. The band in the middle showed the α-β primer pair which marked the wildtype allele, and the largest band was the indicator for the Cs_6 translocation.

Genotype frequencies

To calculate the genotype frequencies the formula [1] was used, were $N_{subscript}$ is the number of individuals which have one of the four different genotypes (chr29+/+; Cs_{29}/+; Cs_{29}/Cs_{29}; Cs_6) and N the population size.

$$[1] \quad P = \frac{N_{subscript}}{N}$$

Allele frequencies

The allele frequencies were calculated using formula [2] where N is the number of alleles in the total population and $N_{Cs*/+}$ and $N_{Cs*/Cs*}$ represent the number of heterozygous and homozygous translocation alleles. The star (*) needs to be replaced with the Cs_6 or Cs_{29} translocation.

$$[2] \quad p_{Cs*} = \frac{\left(N_{Cs*/+} + 2 \cdot N_{Cs*/Cs*}\right)}{2N}$$

The 95% confidence intervals were calculated using a binomial test in R (R Core Team, 2021).

3. Results

3.1 Bioinformatic analysis of the primer pairs

The analysis of the primer pairs designed by Durkin et al., 2012 and Venhoranta et al., 2013 with the BLAST program showed that all primer pairs aligned on chr6 next to the *KIT* gene and on chr29 next to the *LUZP2* gene (Appendix 5). Therefore, the primer pairs are suited to be used with the new cattle reference genome ARS-UCD1.2 (Rosen et al., 2018). These two genes, *KIT* and *LUZP2* were mentioned by Durkin et al., 2012 to be close to the regions responsible for the colour-sidedness and can therefore be seen as landmarks for the correct position of the primer pairs in the genome.

The primer pairs can be divided into three groups. Three primer pairs (α-D, A-E, and C-β) marked the Cs_{29} translocation, one primer pair (γ-B) marked the Cs_{6} translocation, and two primer pairs (α-β and α-β_2) marked the wildtype allele. The chromosomes and regions where the primer pairs were expected to align in the genome are shown in Table 9 and the approximate position of them in the genome is illustrated in Figure 2. Table 10 shows which genotypes were marked by the different primer pairs.

The analysis of the primer pairs with the UCSC In-Silico PCR tool showed that the primer pairs did not amplify any other unexpected regions (Kent, 2022). For the primer pairs α-D, A-E, C-β and γ-B no matches were found when they were compared to the cattle reference genome ARS-UCD1.2 (Rosen et al., 2018). The primer pairs α-β and α-β_2 were found in the cattle reference genome ARS-UCD1.2 (Rosen et al., 2018). Detailed results of the analysis are shown in Appendix 6.

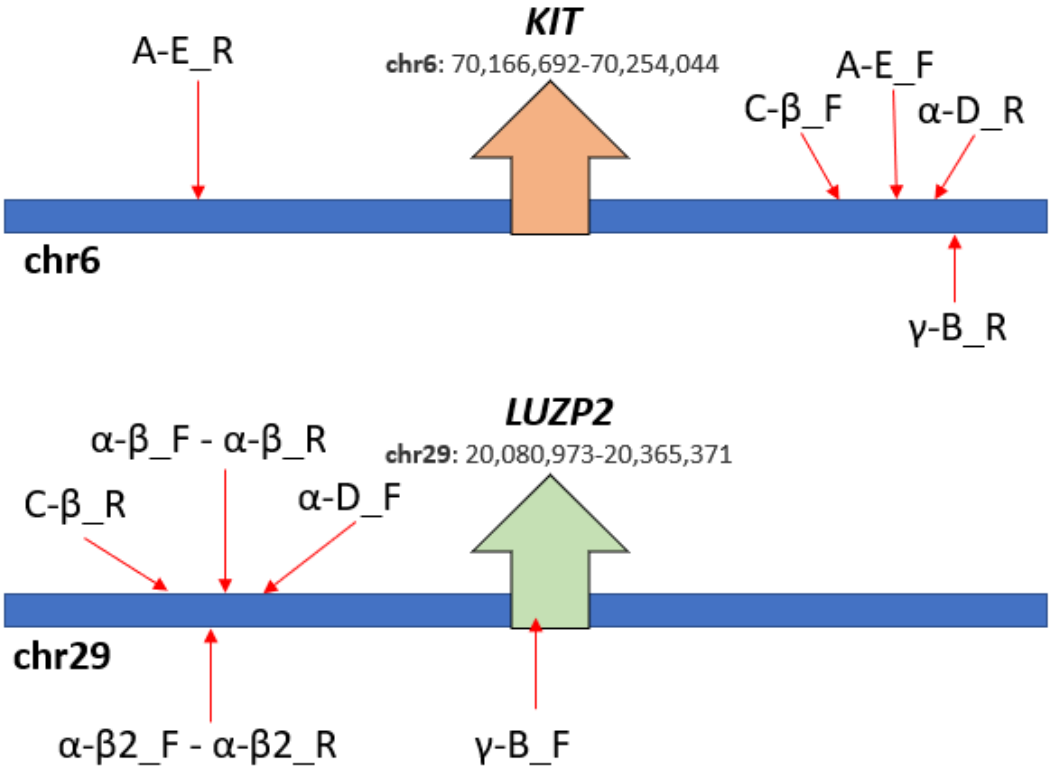


Figure 2 Illustration where the primers were located on chr6 and chr29.
_R = reverse primer; _F = forward primer

Table 9 Chromosome and region where the primer pairs align in the cattle reference genome ARS-UCD1.2.
Results were created by using the BLAST search (EMBL-EBI, 2022).

Primer pair	Forward		Reverse	
	chr	Region	chr	Region
α-D	29	19,703,504-19,703,524	6	70,444,887-70,444,907
A-E	6	70,466,799-70,466,819	6	69,975,266-69,975,286
C-β	6	70,444,222-70,444,242	29	19,703,813-19,703,833
γ-B	29	20,140,380-20,140,402	6	70,311,890-70,311,910
α-β	29	19,703,504-19,703,524	29	19,703,878-19,703,898
α-β_2	29	19,703,469-19,703,491	29	19,703,924-19,703,944

Table 10 Overview which genotypes are marked by the different primers.

	α-D	A-E	C-β	γ-B	α-β	α-β_2
Wildtype Cs29					X	X
Heterozygous Cs29	X	X	X		X	X
Homozygous Cs29	X	X	X			
Cs6				X		

3.2 Bioinformatic analysis of sequence data

3.2.1 Detection of the translocation

The results of the estimation of the depth of coverage of the 30 whole-genome sequenced individuals with bedtools are shown in Appendix 7. A collection of the illustration of all four genotypes is shown in Figure 3. The translocated region responsible for gonadal hypoplasia (Cs_{29}) was found in individuals of the Fjäll, Fjällnära, Väneko and Bohuskulla breed. Individuals which were homozygous for the hypoplasia translocation showed a duplication of ~500 kbp of size between 70.00 Mbp and 70.50 Mbp. The analysed individuals from the Rödkulla and Ringamålako breed did not show any genetic sign of gonadal hypoplasia.

The visualisation of the results in Appendix 7 made it possible to distinguish between homozygous and heterozygous individuals for the translocation from chr6 to chr29, named Cs_{29} allele (Description: Figure 1; name after Durkin et al., 2012). Individuals which were homozygous for the translocation had more reads for the translocated region on chr6 and therefore the difference between the baseline and the translocated region was bigger. Estimated homozygous individuals were Fjäll 1, Fjäll 12, Fjäll 29, Fjällnära 423, Väneko 475, Väneko 440 and Bohuskulla 463.

Heterozygous individuals also showed a higher number of reads for the translocated region, but less compared to homozygous individuals. Therefore, the difference between the baseline reads and the translocation reads was smaller. Inferred heterozygous individuals were Fjäll 14, Fjällnära 425, Väneko 434, Väneko 438, as well as Bohuskulla 453 and Bohuskulla 458.

Besides the translocation from chr6 to chr29 two individuals from the Bohuskulla breed (453 and 458) showed the additional translocation from chr29 to chr6, the Cs_6 allele (Description: Figure 1; name after Durkin et al., 2012). It was not possible to identify whether these two individuals were homo- or heterozygous for the Cs_6 translocation.

17 individuals did not show any sign of the translocation and can therefore be seen as genetically unaffected from gonadal hypoplasia. Unaffected individuals were found in the Fjäll, Fjällnära, Väneko, Bohuskulla, Rödkulla and Ringamålako breed. The Rödkulla and Ringamålako breed were found to be completely unaffected.

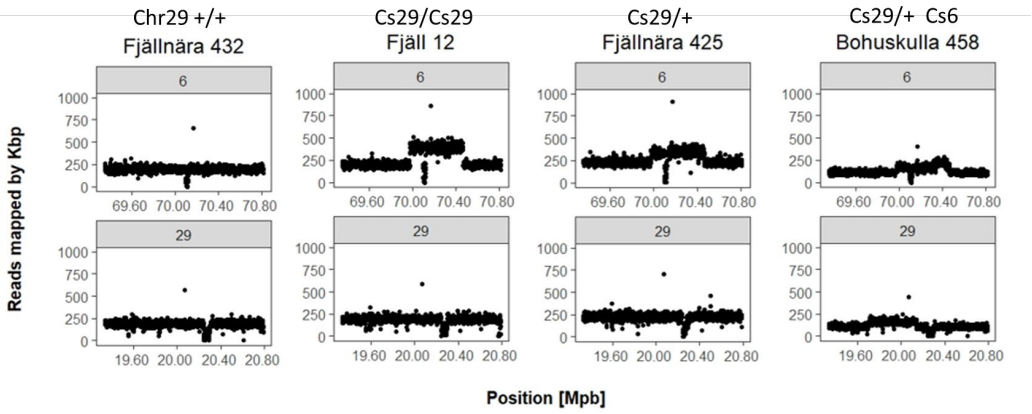


Figure 3 Read coverage for all four genotypes.
The numbers in the grey area represent the chromosome number. chr29+/+ = wildtype chr29; Cs29/Cs29 = homozygous Cs29; Cs29/+ = heterozygous Cs29; Cs6 = additional Cs6 translocation on chr6

3.2.2 Protentional functions of single nucleotide polymorphisms

The VEP analysis resulted in three SNPs of moderate impact which are part of the *KIT* gene. No SNPs with a high impact were found. All SNPs were transcripts and located on chr6. All of them caused a missense variant. The SNP at the position of 70,209,950 bp results in a change from the base adenine (A) to guanine (G). A change form G to A is the result of the second SNP at 70,209,970 bp. The third SNP is located at 70,214,244 bp and it effected a change of the base thymine (T) to cytosine (C). Results are shown in Table 11.

As illustrated in Appendix 8 a change in the SNP at 70,209,950 bp was found in one individual which was from the Fjäll breed and homozygous for the gonadal hypoplasia allele. The discovered SNP at 70,209,970 bp was also transformed in one individual from the Fjäll breed. This Fjäll individual was homozygous for the gonadal hypoplasia allele as well. A change from the reference allele to the non-reference allele in the third SNP was found for all analysed individuals. Most individuals (26/30), no matter which breed and genotype, had two non-reference alleles. In four Fjäll individuals one reference allele and one non-reference allele were found. These four Fjäll individuals were genetically unaffected from hypoplasia or heterozygous for the hypoplasia allele and carried therefore at least one copy of the wildtype allele.

Table 11 Consequences and impact of the SNPs located with the VEP analysis. All transcripts were part of the KIT gene (ENSBTAG00000002699)

Location	reference Allele	Non-reference Allele	Feature type	Consequence	Impact
chr6 - 70,209,950	A	G	Transcript	missense variant	moderate
chr6 - 70,209,970	G	A	Transcript	missense variant	moderate
chr6 - 70,214,244	T	C	Transcript	missense variant	moderate

3.3 Analysing breakpoints using Sanger sequencing

The Sanger sequencing gave results for all six primer pairs. As predicted during the bioinformatic analysis of the primer pairs (chapter 3.1) it was possible to distinguish between wildtype, homo- and heterozygous individuals for the Cs_{29} translocation. Besides it was feasible to identify carriers of the additional Cs_6 translocation. Due to a technical error the A-E reverse primer for SE 458 did not work. The primers α-D, A-E and C-β gave only results for individuals which were affected from gonadal hypoplasia and thus gave a result for hetero- and homozygous Cs_{29} carriers. The γ-B primer pair did only work in individuals which were carrier of the Cs_6 translocation. The primers α-β and α-β_2 gave a result for animals which carried a wildtype allele copy. Therefore, they tagged all individuals which were genetically unaffected form hypoplasia and animals which were heterozygous for the hypoplasia Cs_{29} allele. The results from the Sanger sequencing are shown in Table 12 and an overview of how to distinguish between the four different genotypes based on the functionality of the primer pairs is given in Table 10.

Table 12 Results of the Sanger sequencing.
grey: Primer pair did not work; white: Primer pair did work.

	1	2	3	4	5	6	
A	SE 432	SE 432	SE 432	SE 432	SE 432	SE 432	Forward
B	SE 012	SE 012	SE 012	SE 012	SE 012	SE 012	
C	SE 425	SE 425	SE 425	SE 425	SE 425	SE 425	
D	SE 458	SE 458	SE 458	SE 458	SE 458	SE 458	
E	SE 432	SE 432	SE 432	SE 432	SE 432	SE 432	Reverse
F	SE 012	SE 012	SE 012	SE 012	SE 012	SE 012	
G	SE 425	SE 425	SE 425	SE 425	SE 425	SE 425	
H	SE 458	SE 458	SE 458	SE 458	SE 458	SE 458	
	α-D	A-E	C-β	γ-B	α-β	α-β_2	

3.3.1 Align and BLAST Sanger sequencing results

The alignment of the Sanger sequencing results with Mega 11 (Tamura et al., 2021) showed that the sequences from the PCR products had slightly different sizes than the products expected from the primer pairs in the papers of Durkin et al., 2012 and Venhoranta et al., 2013. Blasting the results of the Sanger sequencing showed, that all primers align at different regions than assumed in the analysis of the primers in Table 9. The difference between the expected and the observed region where the primers aligned was for all primer pairs several hundred bases. For the primer pairs A-E reverse, α-β_2 forward, α-D forward, C-β reverse and γ-B forward the result with the second highest agreement given by the ensemble BLAST analysis fits the expected working region of the primer pair better than the first result. A detailed overview of the regions where the primers aligned based on previous analysis and where the products they produced in this study aligned is given in Appendix 9.

3.4 Test PCR and visualizing results

The analysis of the PCR products with TapeStation showed, that the four different genotypes can be distinguished by using the primer pairs A-E, γ-B and α-β and the multiplex made from all three different primer pairs. The primer pairs amplified as expected. The A-E primer pair marked the hypoplasia translocation Cs_{29} and was consequently not present in the wildtype SE 432 individual. The γ-B primer pair marked the Cs_6 translocation and was thus just present in the SE 458 individual. The α-β primer pair marked the wild type allele and was therefore not found in the homozygous Cs_{29} individual SE 012. The TapeStation results of the single primer pairs A-E, γ-B and α-β are shown in Appendix 10. The combined results of the primer pairs A-E, γ-B and α-β in the multiplex are illustrated in Figure 4.

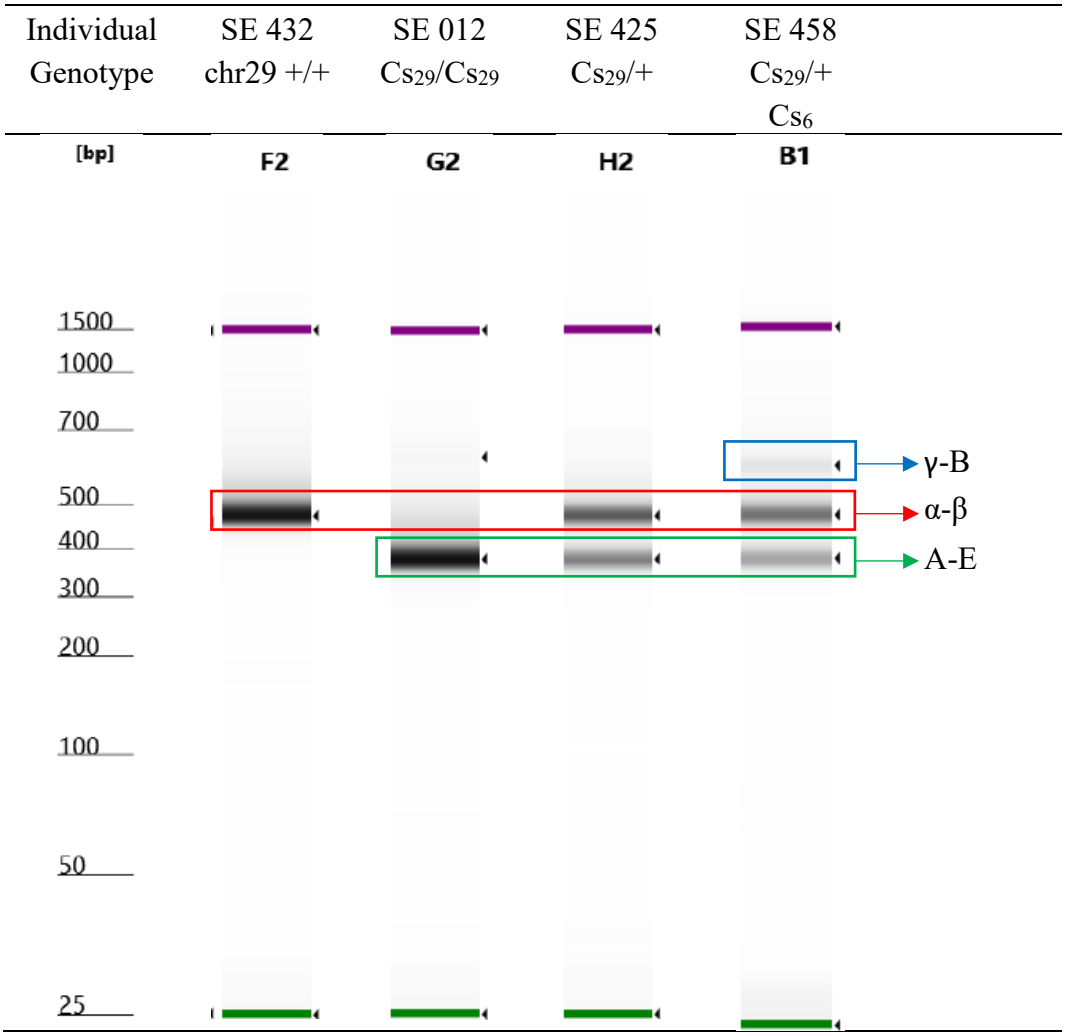


Figure 4 TapeStation results for the test run with the Sanger sequencing individuals.
Figure was edited by composing different runs from different parts of the original output.

3.5 PCR and visualizing results

36 out of the 56 samples were successfully analysed with the TapeStation in the first run. The 20 remaining samples were run again, due to different results in the sample intensity diagram or missing markers. Only sample SE 010 was left without result even after the repetition. The TapeStation results made it possible to distinguish between the three different hypoplasia genotypes (Cs_{29}) and to detect the additional Cs_6 translocation. In the analysis 20 individuals were found to have two copies of the wild type allele (chr29+/+). Furthermore, 24 individuals were heterozygous for the Cs_{29} translocation, and eleven individuals were homozygous for the Cs_{29} translocation. The additional Cs_6 translocation was found in eleven individuals independent from their Cs_{29} genotype. The genotype of the different individuals based on the TapeStation results is shown in Table 13. The

electrophoresis illustration from the TapeStation is shown in Appendix 11. The TapeStation electrophoresis illustrations for the revision run can be found in Appendix 12.

Table 13 Genotypes based on the TapeStation results.
Description: chr29+/+=wild type Cs_{29}; Cs_{29}/Cs_{29}=homozygous for gonadal hypoplasia; $Cs_{29}/+$=heterozygous for gonadal hypoplasia; chr6=wild type Cs_6; Cs_6=additional colour-sidedness translocation.
**=Crossbred individual. Percentual proportions are based on the pedigree.*

Sample ID	breed	genotype Cs_{29}	genotype Cs_6
SE 419	Bohuskulla	$Cs_{29}/+$	chr6 +/+
SE 449	Bohuskulla	$Cs_{29}/+$	chr6 +/+
SE 450	Bohuskulla	$Cs_{29}/+$	Cs_6
SE 451	Bohuskulla	Cs_{29}/Cs_{29}	chr6 +/+
SE 452	Bohuskulla	Cs_{29}/Cs_{29}	chr6 +/+
SE 454	Bohuskulla	$Cs_{29}/+$	chr6 +/+
SE 456	Bohuskulla	chr29 +/+	Cs_6
41773	Fjäll	$Cs_{29}/+$	chr6 +/+
51458	Fjäll	$Cs_{29}/+$	chr6 +/+
AJSE_101	Fjäll	chr29 +/+	chr6 +/+
AJSE_103	Fjäll	chr29 +/+	Cs_6
AJSE_110	Fjäll	$Cs_{29}/+$	Cs_6
AJSE_111	Fjäll	$Cs_{29}/+$	Cs_6
AJSE_114	Fjäll	Cs_{29}/Cs_{29}	chr6 +/+
AJSE_95	Fjäll	Cs_{29}/Cs_{29}	chr6 +/+
SE 004	Fjäll	$Cs_{29}/+$	chr6 +/+
SE 005	Fjäll	$Cs_{29}/+$	Cs_6
SE 006	Fjäll	chr29 +/+	chr6 +/+
SE 009	Fjäll	Cs_{29}/Cs_{29}	chr6 +/+
SE 010	Fjäll	lower marker not detected	
SE 013	Fjäll	$Cs_{29}/+$	Cs_6
SE 014	Fjäll	$Cs_{29}/+$	chr6 +/+
SE 016	Fjäll	Cs_{29}/Cs_{29}	Cs_6
SE 019	Fjäll	Cs_{29}/Cs_{29}	chr6 +/+
SE 022	Fjäll	chr29 +/+	Cs_6
SE 031	Fjäll	Cs_{29}/Cs_{29}	chr6 +/+
SE 032	Fjäll	Cs_{29}/Cs_{29}	Cs_6
SE 037	Fjäll	chr29 +/+	chr6 +/+
SE 038	Fjäll	$Cs_{29}/+$	Cs_6
43579	Fjällnära	$Cs_{29}/+$	Cs_6
SE 401	Fjällnära	chr29 +/+	chr6 +/+
SE 402	Fjällnära	chr29 +/+	chr6 +/+

SE 420	Fjällnära	Cs_{29}/+	chr6 +/+
SE 421	Fjällnära	chr29 +/+	chr6 +/+
SE 422	Fjällnära	Cs_{29}/Cs_{29}	Cs_6
SE 424	Fjällnära	Cs_{29}/+	chr6 +/+
SE 426	Fjällnära	chr29 +/+	chr6 +/+
SE 427	Fjällnära	Cs_{29}/Cs_{29}	chr6 +/+
SE 428	Fjällnära	chr29 +/+	chr6 +/+
SE 429	Fjällnära	chr29 +/+	Cs_6
SE 430	Fjällnära	chr29 +/+	Cs_6
SE 431	Fjällnära	chr29 +/+	Cs_6
AJSE_100	SKB	chr29 +/+	Cs_6
AJSE_102	SKB	Cs_{29}/+	Cs_6
AJSE_109	SKB	Cs_{29}/+	chr6 +/+
AJSE_112	SKB	chr29 +/+	Cs_6
AJSE_96	SKB	chr29 +/+	Cs_6
AJSE_98	SKB	Cs_{29}/+	chr6 +/+
42376	SKB; 45% Fjäll*	chr29 +/+	chr6 +/+
42377	SKB; 72% Fjäll*	chr29 +/+	Cs_6
49889	SKB; 72% Fjäll*	Cs_{29}/+	chr6 +/+
SE 433	Väneko	Cs_{29}/+	chr6 +/+
SE 435	Väneko	chr29 +/+	chr6 +/+
SE 436	Väneko	Cs_{29}/+	chr6 +/+
SE 441	Väneko	Cs_{29}/+	chr6 +/+
SE 473	Väneko	Cs_{29}/+	chr6 +/+

3.6 Genotype and allele frequencies

3.6.1 Genotype frequencies

Based on the three TapeStation results the genotype frequencies for the Cs_{29} and Cs_6 translocation were calculated. In total 36% of the individuals were found to be genetically unaffected from hypoplasia, 44% were heterozygous for the Cs_{29} translocation which is responsible for gonadal hypoplasia and 20% were homozygous for the Cs_{29} translocation. The additional Cs_6 translocation was found in 37% of the analysed individuals (Table 14).

Table 14 Distribution of genotypes for the Cs_{29} and Cs_6 translocation.

Translocation	chr29+/+	Cs_{29}/+	Cs_{29}/Cs_{29}	chr6+/+	Cs_6
No. of individuals	21	26	12	37	22
%	36	44	20	63	37

Genotype frequencies per breed

The three different genotypes connected to gonadal hypoplasia (Cs_{29}) were found in all five analysed breeds apart from the Swedish polled and Väneko breed. These two breeds were found to not have homozygous individuals for the Cs_{29} translocation. Besides the Väneko breed was the only one without individuals that had the additional Cs_6 translocation.

The wild type for chr29 was most common in the Fjällnära breed occurring in 60% of the analysed individuals and 56% in the Swedish Polled breed. Individuals showing the wild type for chr29 can be seen as genetically unaffected from gonadal hypoplasia. The Bohuskulla (63%) and Väneko (80%) breed were the ones that had the largest percentage for heterozygous individuals for the Cs_{29} translocation. Individuals which were homozygous for the Cs_{29} translocation were found to be most frequently in the Fjäll breed.

The distribution of the different genotypes per breed for the Cs_{29} translocation is illustrated in Figure 5 and the distributions between the different breeds for the Cs_6 translocation are shown in Figure 6.

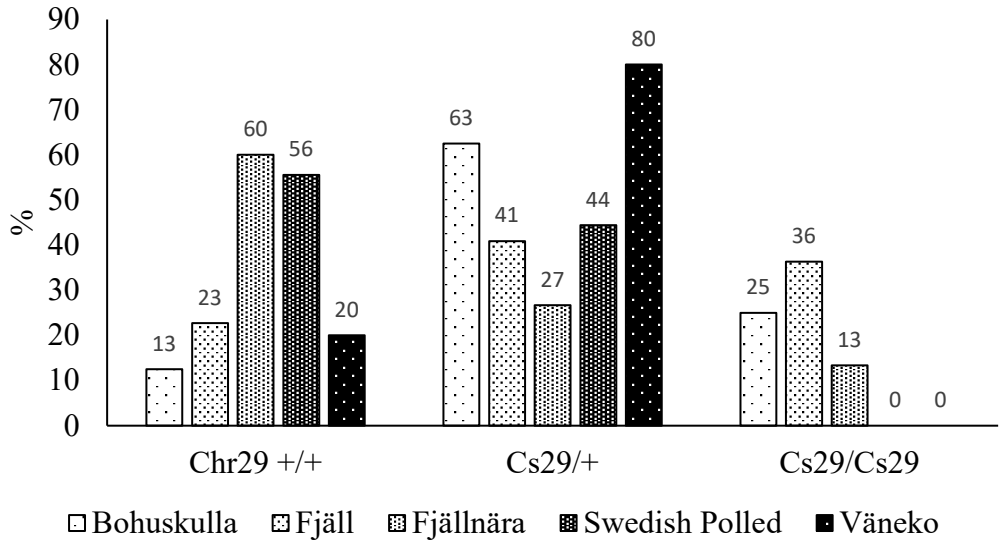


Figure 5 Percentual distribution of the Cs_{29} translocation in the different breeds. (chr29 +/+ =wild type; Cs_{29}/+ = heterogeneous; Cs_{29}/Cs_{29} = homozygous)

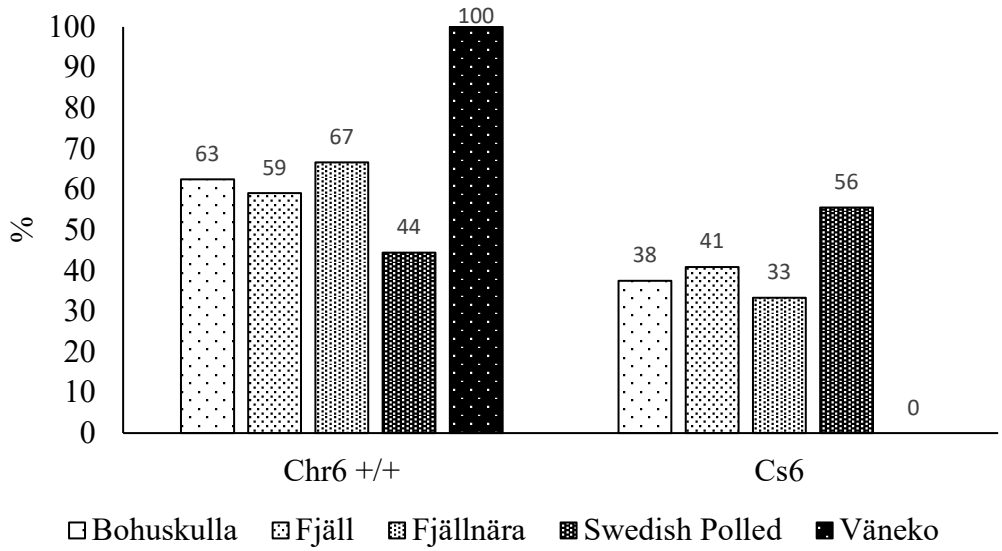


Figure 6 Percentual distribution of the Cs_6 translocation in the different breeds. (chr6 +/+ = wild type)

3.6.2 Allele frequencies

Cs_{29} allele

The overall allele frequency for the Cs_{29} translocation was found to be 0.42. The allele frequencies of the Cs_{29} translocation as well as the 95% confidence intervals for the different breeds are shown in Table 15.

Table 15 Allele frequencies of the Cs29 translocation in the different breeds.

Breed	Allele frequency Cs_{29}	95% Confidence interval
Bohuskulla	0.56	0.30 – 0.80
Fjäll	0.57	0.41 – 0.72
Fjällnära	0.27	0.12 – 0.46
SKB	0.22	0.06 – 0.48
Väneko	0.40	0.12 – 0.74

Cs_6 allele

For the Cs_6 allele no distinction between hetero- and homozygous individuals was possible. Therefore, the allele frequency for the Cs_6 allele is given as a range of possible values based on all individuals being heterozygous or all individuals being homozygous for the Cs_6 translocation. The overall allele frequency for all breeds was between 0.19 and 0.37. The ratios for the different breeds are given in Table 16.

Table 16 Possible allele frequencies for the Cs_6 translocation in the different breeds.

Breed	Allele frequency Cs6	
	All homozygous	All heterozygous
Bohuskulla	0.19	0.38
Fjäll	0.20	0.41
Fjällnära	0.17	0.33
SKB	0.28	0.56
Väneko	0	0

4. Discussion

The aim of this research was to investigate the occurrence of the allele responsible for gonadal hypoplasia in several native Swedish cattle breeds. The results showed that the Cs_{29} translocation that is known to be responsible for gonadal hypoplasia is not only common in the Swedish Mountain cattle breed but also in the Fjällnära, Bohus Polled, Väneko and Swedish Polled breed. This might be related to the fact that all breeds show the colour-sidedness phenotype and most of them are related to the Swedish Mountain cattle.

4.1 Comparing results to previous research

The found allele frequencies for the Cs_{29} translocation can be divided into two groups, breeds with high allele frequencies like the Swedish Mountain cattle (0.57) and Bohus Polled (0.56) and breeds with lower allele frequencies like Väneko (0.40), Fjällnära (0.27) and Swedish Polled (0.22). High allele frequencies for gonadal hypoplasia were also found by others (Venhoranta et al., 2013). They found an allele frequency of 0.60 in the Northern Finncattle and Swedish Mountain cattle. However, the sample sizes in this study were small, especially for Bohus Polled, Swedish Polled and Väneko, and mostly form individuals born before 2000. This makes the estimated allele frequencies error prone and uncertain. The small sample sizes might also be the reason, why no homozygous gene carriers were found in the Väneko, and Swedish Polled breed and it could explain the absence of the Cs_6 translocation in the Väneko breed.

The comparison of allele frequencies is due to the incomplete penetrance and the fact that it is not possible to detect heterozygous allele carriers by veterinarian inspection difficult. To adjust for the incomplete penetrance and not detected heterozygous allele carriers in phenotype investigations the corrected allele frequency is about 28.50%. It was calculated by dividing the number of heterozygous individuals (57%) by two. The result is high compared to the phenotypic frequencies of historic sources. K. Eriksson, 1943 examined about 6,000 individuals from the Swedish Mountain cattle starting in 1935. He reported frequencies for gonadal hypoplasia between 26% and 30% in 1935, 23% in 1937

and 8% in 1942. Lagerlöf & Settergren, 1953 quantified the incidence of gonadal hypoplasia in a total of 8,000 Swedish Mountain cattle cows. They found incidences of 18% for cows born until 1936, for cows born between 1937 and 1939 it was 15%, for cows born between 1940 and 1945 it was 11% and for cows born between 1946 and 1948 it was 9%. Lagerlöf & Boyd, 1953 estimated the incidence of gonadal hypoplasia in organs from cows slaughtered between 1950 and 1952. The incidence of total hypoplasia was 5.2% to 11.1% in different counties and the incidence of partial hypoplasia was 3.8% to 8.0% in different counties. Thus, all three studies reported a declining incidence for gonadal hypoplasia over time.

Therefore, the question arises, why the allele frequency of gonadal hypoplasia has increased that much even though there is a regulation program in the Swedish Mountain cattle since 1937. The control program to reduce gonadal hypoplasia seemed, based on the research of Lagerlöf & Boyd, 1953 and Lagerlöf & Settergren, 1953, to be successful in the beginning. Several scenarios might have had an impact on the increase of the occurrence of gonadal hypoplasia. One of them is the reduction of the Swedish Mountain cattle breeding population down to 440 individuals in 1997 and the successful increase of breeding females in the following years (Food and Agriculture Organisation of the United Nations, 2022c). This bottleneck scenario decreased the gene pool of the breed and to save and redevelop the breed breeders might not have been too strict with excluding affected individuals from breeding. Another contributing factor could have been the introduction of artificial insemination. Artificial insemination made the use of breeding bulls all over the country easier and some sires might have been used more often than others.

Besides, the sample size in this research was with 22 individuals from the Swedish Mountain cattle breed way smaller than the sample sizes in previous research. Thus, the actual frequencies might be different, because the taken sample size cannot be seen as representable for the whole population and the high number of affected individuals might be found by chance.

4.2 Possibilities for breeding organisations and breeders

The results of the DNA analysis showed that gonadal hypoplasia can still be found in the seven breeding bulls born in the 21st century. Interesting is, that not only heterozygous gene carriers were found, but also two homozygous ones. Based on the breeding goal of the Swedish Mountain cattle, homozygous bulls are not allowed in the herd book (Lagerlöf & Settergren, 1953). The occurrence of homozygous gene carriers can be seen as proof, that veterinarian inspections are not accurate enough to ensure the absence of gonadal hypoplasia. Therefore, the developed PCR multiplex technique with the combination of the three primer pairs could be an easy and quick solution to replace the veterinarian inspections. The breeding organisation already offers the possibility of DNA analysis for milk casein, so no extra DNA collection would be necessary, just an additional DNA test (Svensk Fjällrasavel, 2022c). The gene test for gonadal hypoplasia would give a clear genotype for the disease and make selection more accurate.

However, there is still the question, what to do with affected individuals. Excluding all allele carriers from breeding would narrow the genetic material and increase the risk of inbreeding. Thus, this strategy does not seem to be the one promising the most success. It seems more reasonable to exclude all homozygous bulls from breeding and avoid carrier – carrier mating. Therefore, both parents need to be tested for gonadal hypoplasia before the mating. The avoidance of carrier – carrier mating would reduce the risk of homozygous offspring for the gonadal hypoplasia translocation. A similar strategy is pursued in the breeding of horses. Here breeders try to avoid mating two individuals that carry the allele responsible for the warmblood fragile foal syndrome (WFFS) because homozygous individuals are unviable (Flanagan et al., 2021).

From a social point of view a more accurate detection of gonadal hypoplasia and corresponding selection could help to increase the profitability of Swedish Mountain cattle and other native Swedish cattle breeds which show the colour-sidedness phenotype. Less individuals suffering from gonadal hypoplasia would increase the fertility of both male and female individuals and thus decrease insemination costs. Furthermore, the tests could be already done on calves, so breeders do not have to raise the breeding individuals until they reach a certain age for the veterinarian investigation. This would avoid costs for raising heifers and potential breeding bulls which could suffer from infertility.

Furthermore, with the DNA testing method the animal welfare could be increased. The DNA samples are taken by the farmer itself when using hair samples like for the casein test (Svensk Fjällrasavel, 2022c). This would avoid the stress caused by a veterinarian inspection. Besides breeding for more healthy animals, in this case by avoiding the further spread of the mutation in the population, can be seen as an ethical consideration.

4.3 The Cs_6 translocation

The additional Cs_6 translocation which is also known to be responsible for the colour-sidedness but not influencing gonadal hypoplasia was found in all three genotypes, from hypoplasia genetically unaffected, hetero- and homozygous individuals. Based on the used primer pair a differentiation between hetero- and homozygous individuals was not possible. This is related to the complexity of the translocated region. Because the Cs_6 translocation includes part of the duplicated Cs_{29} duplication from chr29 the genome sequence of the Cs_6 translocation can theoretically be found three times in the genome of carrier individuals. Thus, it is difficult to develop a primer that produces a PCR product of the correct sequence when dividing between hetero- and homozygous individuals. The unavailability to distinguish between hetero- and homozygous individuals made it difficult to calculate allele frequencies. Therefore, the allele frequency was estimated by assuming all individuals were heterozygous for the Cs_6 translocation or all individuals were homozygous for the translocation. This gave a range of values in which the actual allele frequency can be expected. The estimated range of the allele frequencies was 0.19, when assuming all individuals were heterozygous and 0.37 when all individuals would be homozygous. Calculating the allele frequencies based on the same scheme for the study of Venhoranta et al., 2013 the value ranged from 0.31 supposing all individuals were heterozygous up to 0.61 if all individuals would be homozygous. Therefore, the Cs_6 translocation was more common in the Venhoranta et al., 2013 dataset.

4.4 Future research

Since the DNA samples were mostly from individuals born before 2000, the allele frequencies cannot be fully transferred to today's population. To get to know the current allele frequencies of gonadal hypoplasia in the Swedish Mountain cattle population and the other analysed Swedish cattle breeds, new DNA samples need to be collected. Furthermore, future research should be done with a larger number of individuals to avoid uncertain results and make the results more representable for the different breeds.

Besides further research of combined phenotype and genotype analysis should be done to test if incomplete penetrance as reported in the Northern Finncattle can also be found in other native Swedish Cattle breeds (Venhoranta et al., 2013). The incomplete penetrance indicates that other factors play a role in causing gonadal hypoplasia. This is supported by the fact, that several other breeds than the analysed ones (Belgium Blue, Gloucester Cattle, Chillingham White Cattle, Northern Finncattle, African Nguni cattle and Pinzgauer cattle) show the colour-sidedness phenotype but only in a few of them gonadal hypoplasia was found (Northern Finncattle, Chillingham White Cattel and African Nguni Cattle) (Artesi et al., 2020; Durkin et al., 2012; Hall et al., 2021; Szczerbal et al., 2017; Venhoranta et al., 2013). These breeds which are also known to be affected have at least a historic relationship with the Swedish Mountain cattle (Hall et al., 2021; Kay et al., 1992; Venhoranta et al., 2013). Therefore, more research is needed to identify these additional factors to get more precise information, what causes the disease and increase selection achievement.

4.5 Methodological concerns

The developed multiplex method is easy to realise, and the results are simple to interpret. However, the results of the TapeStation run showed that the method is error-prone for too high DNA quantities. Therefore, the PCR products of the multiplex should be diluted by using 1.00 µl of PCR product and 1.00 µl of nuclease free water instead of 2.00 µl PCR product when using the high sensitivity D1000 kit. Another possibility for improvement would be to use a different kit for the TapeStation, which can deal with higher DNA amounts. Furthermore, it might be possible to develop primer pairs that make it possible to distinguish between hetero- and homozygous individuals for the Cs_6 translocation. These primer pairs could, if their product has a different size than the existing primer pair products, easily added to the multiplex. This would make it possible to test for both translocation alleles in one step.

5. Conclusion

Gonadal hypoplasia still plays an important role in Swedish Mountain cattle and as now discovered also in several other native Swedish cattle breeds. The research showed that not only the Swedish Mountain cattle is affected, but also all four analysed native Swedish cattle breeds (Bohus Polled, Swedish Polled, Väneko and Fjällnära) have allele carriers. The fact that breeders try to select against gonadal hypoplasia since the beginning of the 20th century, but the disease is still present nowadays, the current way of investigation appears to be not accurate enough. Therefore, it is advisable to replace the veterinarian inspections with the developed PCR multiplex test. Taking into consideration that the frequencies for gonadal hypoplasia in the Swedish Mountain cattle have increased compared to previous research in the 1950s, the need for more detailed investigations is once again emphasised. Besides, breeding organisations need to decide new criteria how to deal with allele carriers in the breeding population. The new selection criteria should consider the prevention of genetic loss and the avoidance of inbreeding.

Since the DNA samples where not recent, further research is needed to study the allele frequency for gonadal hypoplasia of the current cattle populations. Furthermore, more research on the genetic effects which are responsible for gonadal hypoplasia is needed to investigate the reasons for the incomplete penetrance.

References

Agilent Technologies. (2022). *Agilent 4200 TapeStation System Manual*.

Agilent Technologies Inc. (2021). *High Sensitivity D1000 ScreenTape Assay for TapeStation Systems*.

Andersson, L. (2020). Mutations in Domestic Animals Disrupting or Creating Pigmentation Patterns. *Frontiers in Ecology and Evolution*, *8*, 116. https://doi.org/10.3389/FEVO.2020.00116/BIBTEX

Applied Biosystems. (2007). *Applied Biosystems BigDye ® XTerminator ™ Purification Kit Protocol*.

Arroyo, E., Patino, C., Ciccarelli, M., Raudsepp, T., Conley, A., & Tibary, A. (2022). Clinical and Histological Features of Ovarian Hypoplasia Dysgenesis in Alpacas. *Frontiers in Veterinary Science*, *9*. https://doi.org/10.3389/fvets.2022.837684

Artesi, M., Tamma, N., Deckers, M., Karim, L., Coppieters, W., van den Broeke, A., Georges, M., Charlier, C., & Durkin, K. (2020). Colour-sidedness in Gloucester cattle is associated with a complex structural variant impacting regulatory elements downstream of KIT. *Animal Genetics*, *51*(3), 461–465. https://doi.org/10.1111/AGE.12932

Auwera, G. A. V. der. (2020). *Genomics in the Cloud: Using Docker, GATK, and WDL in Terra* (B. D. O'Connor, Ed.) [Book]. O'Reilly.

Bernex, F., de Sepulveda, P., Kress, C., Elbaz, C., Delouis, C., & Panthier, J.-J. (1996). Spatial and temporal patterns of c-kit-expressing cells. *Development*, *122*, 3023–3033.

Bidarkar, S. S., & Hutson, J. M. (2005). Evaluation and management of the abnormal gonad. *Seminars in Pediatric Surgery*, *14*(2), 118–123. https://doi.org/10.1053/j.sempedsurg.2005.01.008

Buehr, M., Mclaren, A., Bartley, A., & Darling, S. (1993). Proliferation and Migration of Primordial Germ Cells in WIWe Mouse Embryos. In *DEVELOPMENTAL DYNAMICS*.

Cheng, L., Roth, L. M., Zhang, S., Wang, M., Morton, M. J., Zheng, W., Abdul Karim, F. W., Montironi, R., & Lopez-Beltran, A. (2011). KIT gene mutation and amplification in dysgerminoma of the ovary. *Cancer*, *117*(10), 2096–2103. https://doi.org/10.1002/CNCR.25794

Coffey, J., Linger, R., Pugh, J., Dudakia, D., Sokal, M., Easton, D. F., Bishop, D. T., Stratton, M., Huddart, R., & Rapley, E. A. (2008). Somatic KIT mutations occur predominantly in seminoma germ cell tumors and are not predictive of bilateral disease: report of 220 tumors and review of literature. *Genes, Chromosomes & Cancer*, *47*(1), 34–42. https://doi.org/10.1002/GCC.20503

Crossley, B. M., Bai, J., Glaser, A., Maes, R., Porter, E., Killian, M. L., Clement, T., & Toohey-Kurth, K. (2020). Guidelines for Sanger sequencing and molecular assay monitoring. *Journal of Veterinary Diagnostic Investigation*, *32*(6), 767–775. https://doi.org/10.1177/1040638720905833/ASSET/IMAGES/LARGE/10.1177_1040638720905833-FIG1.JPEG

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., & Durbin, R. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/BIOINFORMATICS/BTR330

Durkin, K., Coppieters, W., Drögüller, C., Ahariz, N., Cambisano, N., Druet, T., Fasquelle, C., Haile, A., Horin, P., Huang, L., Kamatani, Y., Karim, L., Lathrop, M., Moser, S., Oldenbroek, K., Rieder, S., Sartelet, A., Sölkner, J., Stålhammar, H., … Charlier, C. (2012). Serial translocation by means of circular intermediates underlies colour sidedness in cattle. *Nature*, *482*(7383), 81–84. https://doi.org/10.1038/nature10757

EMBL-EBI. (2022). *BLAST/BLAT search - Bos_taurus - Ensembl genome browser*. https://www.ensembl.org/Bos_taurus/Tools/Blast

Ensembl. (2022). *Gene: KIT (ENSBTAG00000002699)*. https://www.ensembl.org/Bos_taurus/Gene/Summary?db=core;g=ENSBTAG00000002699;r=6:70166692-70254044;t=ENSBTAT00000003498

Eriksson, C., & Petitt, A. (2020). Designing Cattle: The Social Practice of Constructing Breeds. *Anthrozoos*, *33*(2), 175–190. https://doi.org/10.1080/08927936.2020.1719758

Eriksson, K. (1943). Hereditary Forms of Sterility in Cattle. Biological and Genetical Investigations. I. In *Hereditary Forms of Sterility in Cattle. Biological and Genetical Investigations. I.* Lund: Hakan Ohlssons Boktryckeri.

Fellows, E., Kutzler, M., Avila, F., Das, P. J., & Raudsepp, T. (2014). Ovarian Dysgenesis in an Alpaca with a Minute Chromosome 36. *Journal of Heredity*, *105*(6), 964–968. https://doi.org/10.1093/JHERED/ESS069

Flanagan, S., Rowe, Á., Duggan, V., Markle, E., O'Brien, M., & Barry, G. (2021). Development of a real-time PCR assay to detect the single nucleotide polymorphism causing Warmblood Fragile Foal Syndrome. *PLOS ONE*, *16*(11), e0259316. https://doi.org/10.1371/JOURNAL.PONE.0259316

Food and Agriculture Organisation of the United Nations. (2022a). *Bohus Polled*. https://dadis-breed-datasheet-ext-ws.firebaseapp.com/?country=SWE&specie=Cattle&breed=Bohuskulla&lang=en

Food and Agriculture Organisation of the United Nations. (2022b). *Fjällnära*. https://dadis-breed-datasheet-ext-ws.firebaseapp.com/?country=SWE&specie=Cattle&breed=Fj%C3%A4lln%C3%A4ra%20boskap&lang=en

Food and Agriculture Organisation of the United Nations. (2022c). *Svensk Fjällras*. https://dadis-breed-datasheet-ext-ws.firebaseapp.com/?country=SWE&specie=Cattle&breed=Svensk%20Fj%C3%A4llras&lang=en

Galan, J. J., de Felici, M., Buch, B., Rivero, M. C., Segura, A., Royo, J. L., Cruz, N., Real, L. M., & Ruiz, A. (2006). Association of genetic markers within the KIT and KITLG genes with human male infertility. *Human Reproduction*, *21*(12), 3185–3192. https://doi.org/10.1093/HUMREP/DEL313

Garcia, M., Juhos, S., Larsson, M., Olason, P. I., Martin, M., Eisfeldt, J., DiLorenzo, S., Sandgren, J., Díaz De Ståhl, T., Ewels, P., Wirta, V., Nistér, M., Käller, M., & Nystedt, B. (2020). Sarek: A portable workflow for whole-genome sequencing analysis of germline and somatic variants. *F1000Research 2020 9:63*, *9*, 63. https://doi.org/10.12688/f1000research.16665.2

Hadley Wickham. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Hall, S. J. G., Brenig, B., Ashdown, R. A., & Curry, M. R. (2021). Conservation of rare wild-living cattle Bos taurus (L.): coat colour gene illuminates breed history, and associated reproductive anomalies have not reduced herd fertility. *Journal of Zoology*, *315*(4), 319–326. https://doi.org/10.1111/JZO.12929

Invitrogen. (2010). *Qubit*. https://www.ieg.uu.se/digitalAssets/176/c_176882-l_3-k_qubitquickrefcard.pdf

Johansson, A. M., Upadhyay, M., Strandberg, E., & Eriksson, S. (2020). Genetic differentiation between subpopulations of Swedish mountain (Fjäll and Fjällnära) cattle. *Acta Agriculturae Scandinavica A: Animal Sciences*, *69*(1–2), 39–46. https://doi.org/10.1080/09064702.2019.1704857

Kent, J. (2022). *UCSC In-Silico PCR*. https://genome.ucsc.edu/cgi-bin/hgPcr

Lagerlöf, N., & Boyd, H. (1953). Ovarian hypoplasia and other abnormal conditions in the sexual organs of cattle of the Swedish Highland breed; results of postmortem examination of over 6,000 cows. *Cornell Vet*, *43*, 64–79.

Lagerlöf, N., & Settergren, I. (1953). Results of seventeen years; control of hereditary ovarian hypoplasia in cattle of the Swedish Highland breed. *Cornell Vet*, *43*, 52–64.

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, *25*(14), 1754–1760. https://doi.org/10.1093/BIOINFORMATICS/BTP324

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., Flicek, P., & Cunningham, F. (2016). The Ensembl Variant Effect Predictor. *Genome Biology*, *17*(1), 1–14. https://doi.org/10.1186/S13059-016-0974-4/TABLES/8

Neves, H. H. R., Vargas, G., Brito, L. F., Schenkel, F. S., Albuquerque, L. G., & Carvalheiro, R. (2019). Genetic and genomic analyses of testicular hypoplasia in Nellore cattle. *Plos One*, *14*(1). https://doi.org/10.1371/journal.pone.0211159

Nishikawa, S., Kusakabe1, M., Yoshinaga2, K., Ogawa, M., Hayashi, S.-I., Kunisada, T., Era, T., Sakakura1, T., & Nishikawa, S.-I. (1991). In utero manipulation of coat color formation by a monoclonal anti-c-kit antibody: two distinct waves of c-kit-dependency during melanocyte development. In *The EMBO Journal* (Vol. 10, Issue 8).

Ogawa, M., Nishikawa, S., Yoshinaga, K., Hayashi, S.-I., Kunisada, T., Nakao, J., Kina, T., Sudo, T., Kodama, H., & Nishikawa, S.-I. (1993). Expression and function of c-Kit in fetal hemopoietic progenitor cells transition from the early c-Kit-independent to the late c-Kit-dependent wave of hemopoiesis in the murine embryo. *Development*, *117*, 1089–1098.

Palmieri, C., Schiavi, E., & Salda, L. della. (2011). Congenital and acquired pathology of ovary and tubular genital organs in ewes: A review. In *Theriogenology* (Vol. 75, Issue 3, pp. 393–410). https://doi.org/10.1016/j.theriogenology.2010.09.020

Poulsen, N. A., Glantz, M., Rosengaard, A. K., Paulsson, M., & Larsen, L. B. (2017). Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows. *Journal of Dairy Science*, *100*(11), 8722–8734. https://doi.org/10.3168/jds.2017-12920

Qiagen. (2016). *HotStarTaq ® Plus Master Mix Kit*. www.qiagen.com/HB-0450

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics*, *26*(6), 841–842. https://doi.org/10.1093/BIOINFORMATICS/BTQ033

R Core Team. (2021). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Romagnoli, S., & Schlafer, D. H. (2006). Disorders of sexual differentiation in puppies and kittens: A diagnostic and clinical approach. In *Veterinary Clinics of North America - Small Animal Practice* (Vol. 36, Issue 3, pp. 573–606). https://doi.org/10.1016/j.cvsm.2005.12.007

Rosen, B. D., Bickhart, D. M., Schnabel, R. D., Koren, S., Elsik, C. G., Zimin, A. V, Dreischer, C., Schultheiss, S. J., Hall, R. J., Schroeder, S. G., Tassell, C. P. Van, Smith, T. P. L., & Medrano, J. F. (2018). *Modernizing the Bovine Reference Genome Assembly*.

Svensk Fjällrasavel. (2016). *Breeding Scheme*. https://fjallko.se/avel/nytt-avelsupplagg

Svensk Fjällrasavel. (2022a). *Fjällnära*. https://fjallko.se/fjallnara/fjallnara-kor

Svensk Fjällrasavel. (2022b). *Hypoplasia*. https://fjallko.se/lasvart/viktigt-att-veta/hypoplasi

Svensk Fjällrasavel. (2022c). *Svensk Fjällrasavel - Hair samples for DNA analysis*. https://fjallko.se/mjolkkaseiner/kaseinlab

Svensk Fjällrasavel. (2022d). *Swedish Mountain Cattle*. https://fjallko.se/fjallrasen/fjallrasen

Szczerbal, I., Robinson, T. J., Duran, A., Davey, S. C., Andersson, M., & Switonski, M. (2017). Ectopic position of duplicated KIT gene in African Nguni cattle, associated with color sidedness, confirms its shared ancestry with the Bos taurus lineage. *Animal Genetics*, *48*(1), 122–123. https://doi.org/10.1111/AGE.12495

Tamura, K., Stecher, G., & Kumar, S. (2021). *MEGA11: Molecular Evolutionary Genetics Analysis Version 11*. https://doi.org/10.1093/molbev/msab120

Thermo Fisher Scientific Inc. (2010). *Nucleic Acid Thermo Scientific NanoDrop Spectrophotometers*.

University of California Santa Cruz (UCSC). (2022). *Lift Genome Annotations*. https://genome.ucsc.edu/cgi-bin/hgLiftOver

Upadhyay, M., Eriksson, S., Mikko, S., Strandberg, E., Stålhammar, H., Groenen, M. A. M., Crooijmans, R. P. M. A., Andersson, G., & Johansson, A. M. (2019). Genomic relatedness and diversity of Swedish native cattle breeds. *Genetics Selection Evolution*, *51*(1), 1–11. https://doi.org/10.1186/S12711-019-0496-0/FIGURES/6

Venhoranta, H., Pausch, H., Wysocki, M., Szczerbal, I., Hänninen, R., Taponen, J., Uimari, P., Flisikowski, K., Lohi, H., Fries, R., Switonski, M., & Andersson, M. (2013). Ectopic KIT Copy Number Variation Underlies Impaired Migration of Primordial Germ Cells Associated with Gonadal Hypoplasia in Cattle (Bos taurus). *PLoS ONE*, *8*(9). https://doi.org/10.1371/journal.pone.0075659

Zimin, A. v., Delcher, A. L., Florea, L., Kelley, D. R., Schatz, M. C., Puiu, D., Hanrahan, F., Pertea, G., van Tassell, C. P., Sonstegard, T. S., Marçais, G., Roberts, M., Subramanian, P., Yorke, J. A., & Salzberg, S. L. (2009). A whole-genome assembly of the domestic cow, Bos taurus. *Genome Biology*, *10*(4). https://doi.org/10.1186/gb-2009-10-4-r42

Popular science summary

The aim of this research project was to identify how common gonadal hypoplasia is nowadays in native Swedish cattle breeds which show the colour-sidedness coat colour. Gonadal hypoplasia is a disease were one or both testicles or ovaries are underdeveloped and smaller than normal. This disease can lead to fertility problems. In the Swedish Mountain cattle breeders were aware of the disease since the middle of the 20th century. To avoid the spread in the population it was decided in 1937 that affected breeding bulls could not be part of the herd book anymore and thus were excluded from the breeding population.

The DNA analysis of bulls and cows from five different native Swedish cattle breeds in this research showed that the genetic variant causing gonadal hypoplasia can still be found in the populations, also in breeding bulls. The results show that the veterinary investigations that have been standard practice so far to detect gonadal hypoplasia are not accurate enough. Therefore, it is recommended to replace the veterinarian investigations with the developed DNA test method used in this study.

The DNA test would help to identify all carriers of the disease, regardless of the phenotypic expression. This can help to increase the profitability of the breeds because fertility problems can be reduced. Furthermore, the DNA test allows for testing at calf age. Thus, breeders can already see the availability of an individual for breeding purposes at a young age. Costs for raising bulls and heifers to reach the necessary age for the veterinarian inspection would be avoided.

The DNA test was built by creating a multiplex genotyping method based on three different primer pairs that mark the different genotypes. It was possible to identify the translocation from chr6 to chr29 (Cs_{29}) and the second translocation from chr29 back to chr6 (Cs_{6}) as well as the wild type allele on chr29. Both translocations are known to be responsible for the colour-sidedness coat colour phenotype, but besides this the Cs_{29} translocation can cause gonadal hypoplasia. The primer pairs made it possible to distinguish between the two translocations and for the Cs_{29} translocation it was also possible to identify homozygous and heterozygous allele carriers.

The average allele frequency over all five breeds for the Cs_{29} allele was 0.42. The high allele frequency highlights the importance of the topic and indicates that more research is necessary to increase the selection success against this disease.

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Appendix 1

Primer name	Forward (5' - 3')	Reverse (3' - 5')	size bp	Region amplified bosTau4 (description based on Durkin et al., 2012; Venhoranta et al., 2013)
α-D	GGGGAGAATCTGTTTTCCTG	GGAAGGCCTTATTGCACACT	471	BTA29 to 6 breakpoint unique to BTA 29
A-E	GAAGCAACCCAGAGATGAGC	AAGGGAAGCCCATATGATGA	318	Fusion point on BTA6
C-β	TCAACGAGGGACAACATGAA	CAATTGACCCCTCATTTTGG	606	Common breakpoint, found on BTA6 & BTA29
γ-B	GCTGCAGAAAATGTTATTCCA	TCTTGAAGGGCCATAGCATC	525	BTA29 to 6 breakpoint unique to BTA 6
α-β	GGGGAGAATCTGTTTTCCTG	TAAAGTCGCCAGTGCAAGTG	394	Will not amplify in BTA 29 Cs/Cs (affected) animals
α-β_2	TGGGTAGACAGGTTTGTTTCC	TCTTGACCACTTGCATTGGA	475	Flanks insertion site of the wild type BTA29

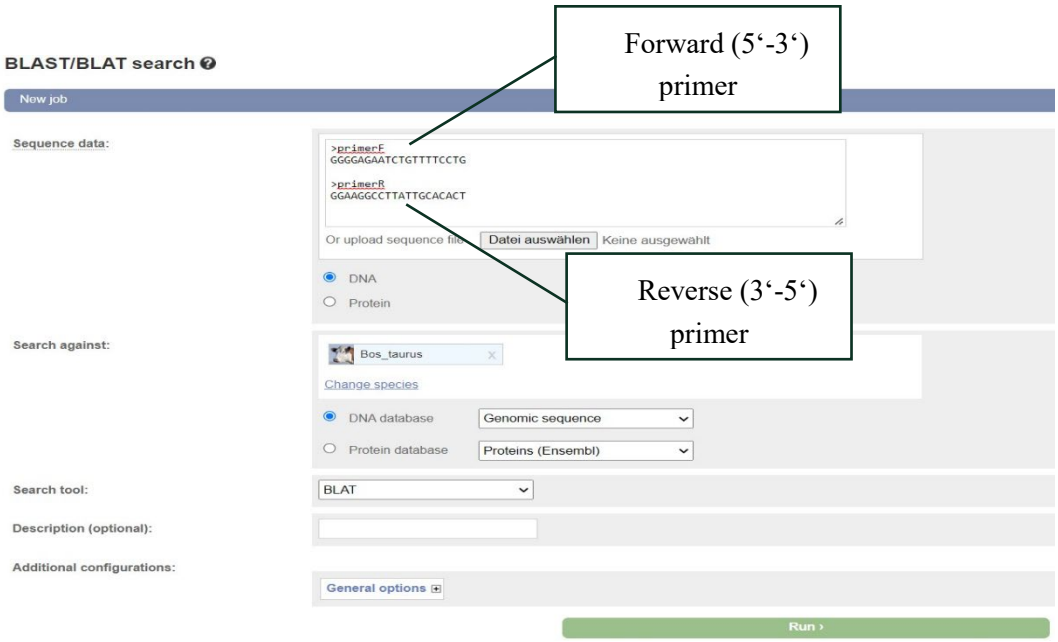
Appendix 2

Project DNA samples:

Sample ID	birth year	breed	Qubit analysis
43579	1991	Fjällnära	X
41773	1994	Fjäll	X
42376	1994	SKB	X
42377	1994	SKB	X
49889	1997	SKB	X
51458	1997	Fjäll	X
AJSE_100	1998	SKB	X
AJSE_101	2004	Fjäll	X
AJSE_102	2000	SKB	X
AJSE_103	2010	Fjäll	X
AJSE_109	2003	SKB	X
AJSE_110	2015	Fjäll	X
AJSE_111	2015	Fjäll	X
AJSE_112	2004	SKB	X
AJSE_114	2003	Fjäll	X
AJSE_95	2013	Fjäll	X
AJSE_96	2002	SKB	X
AJSE_98	1994	SKB	X
SE 004	1991	Fjäll	
SE 005	1991	Fjäll	
SE 006	1989	Fjäll	
SE 009	1993	Fjäll	
SE 010	1994	Fjäll	
SE 012	1993	Fjäll	
SE 013	1987	Fjäll	X
SE 014	1988	Fjäll	
SE 016	1993	Fjäll	
SE 019	1986	Fjäll	
SE 022	1993	Fjäll	
SE 031	1992	Fjäll	
SE 032	1987	Fjäll	
SE 037	1988	Fjäll	
SE 038	1989	Fjäll	

SE 401	1984	Fjällnära	
SE 402	1995	Fjällnära	
SE 419	1995	Bohuskulla	
SE 420	1982	Fjällnära	
SE 421	Unknown	Fjällnära	
SE 422	1992	Fjällnära	
SE 424	1991	Fjällnära	
SE 425	1992	Fjällnära	
SE 426	1985	Fjällnära	
SE 427	1980	Fjällnära	
SE 428	1985	Fjällnära	
SE 429	1990	Fjällnära	
SE 430	1989	Fjällnära	
SE 431	1991	Fjällnära	
SE 432	1989	Fjällnära	
SE 433	1995	Väneko	
SE 435	1976	Väneko	
SE 436	1984	Väneko	X
SE 441	1997	Väneko	
SE 449	Unknown	Bohuskulla	X
SE 450	1993	Bohuskulla	
SE 451	1994	Bohuskulla	
SE 452	1995	Bohuskulla	
SE 454	1996	Bohuskulla	
SE 456	Unknown	Bohuskulla	
SE 458	Unknown	Bohuskulla	
SE 473	1992	Väneko	

Appendix 3


Forward (5‘-3‘) primer
Reverse (3‘-5‘) primer
BLAST/BLAT search
New job
Sequence data:
>primerF
GGGGAGAATCTGTTTTCCTG
>primerR
GGAAGGCCTTATTGCACACT
Or upload sequence file
Datei auswählen
Keine ausgewählt
DNA
Protein
Search against:
Bos_taurus
Change species
DNA database
Genomic sequence
Protein database
Proteins (Ensembl)
Search tool:
BLAT
Description (optional):
Additional configurations:
General options
Run ›

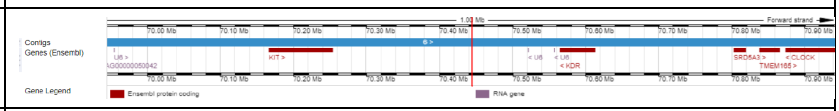
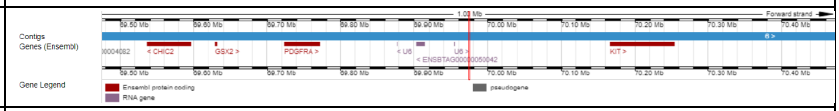
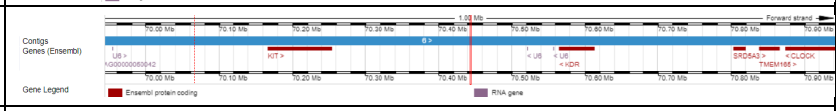
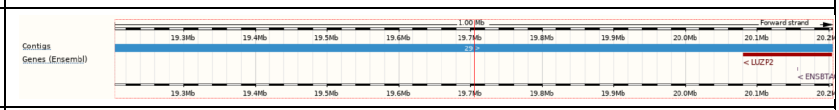
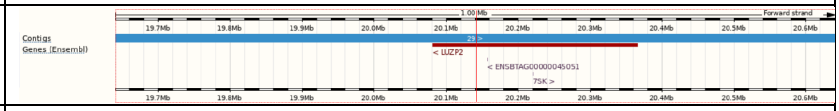
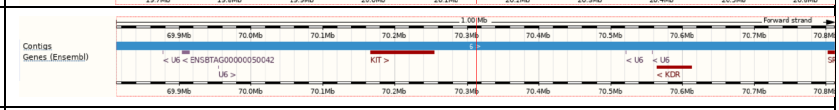
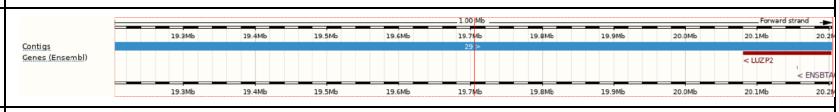
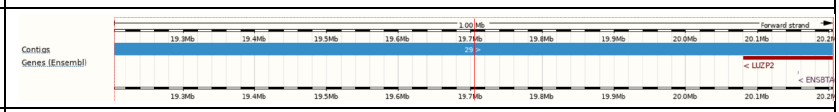
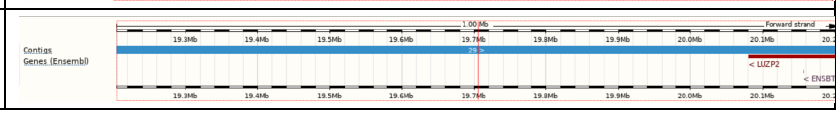
Appendix 4

DNA dilution for final PCR. Calculation made for 10 ng DNA per µl. When Qubit analysis was done, or DNA value was below 100 ng/µl the DNA was not diluted but pipetted pure in the PCR working solution. In this case columns four and five are blank.

	concentration		amount to pipette (based on 50 µl/100 µl total amount)	
Sample ID	NanoDrop	Qubit	DNA	H_2O
41773	80.90	110.00		
42376	113.70	94.60		
42377	134.80	78.20		
43579	28.80	30.00		
49889	53.90	21.40		
51458	26.30	36.00		
SE 004	2078.00		0.50	99.50
SE 005	1005.00		0.50	49.50
SE 006	761.70		0.66	49.34
SE 009	577.80		0.87	49.13
SE 010	594.20		0.84	49.16
SE 013	14.90	6.24		
SE 014	460.90		1.08	48.92
SE 016	909.00		0.55	49.45
SE 019	1414.00		0.70	99,30
SE 022	1411.00		0.70	99.30
SE 031	965.10		0.52	49.48
SE 032	2566.00		0.50	99,50
SE 037	14.50			
SE 038	1341.00		0.70	99.30
SE 401	1645.00		0.60	99.40
SE 402	2330.00		0.50	99.50
SE 419	2846.00		0.50	99.50
SE 420	641.40		0.78	49.22
SE 421	987.60		0.51	49.49

SE 422	530.20		0.94	49.06
SE 426	821.40		0.61	49.39
SE 427	1016.00		0.50	49.50
SE 428	525.90		0.95	49.05
SE 429	1281.00		0.78	99.22
SE 430	1595.00		0.62	99.38
SE 431	1349.00		0.74	99.26
SE 433	770.40		0.65	49.35
SE 435	287.00		1.74	48.26
SE 436	-10.30	<0.01		
SE 441	775.50		0.64	49.36
SE 449	-8.80	0.44		
SE 450	1887.00		0.50	99.50
SE 451	554.40		0.90	49.10
SE 452	1200.00		0.84	99.84
SE 454	434.60		1.15	48.85
SE456	362.40		1.38	48.62
SE 473	414.40		1.21	48.79
SE 424	478.60		1.04	48.96
AJSE 100	1254.00	7.20		
AJSE 101	8.70	3.26		
AJSE 102	-3.00	4.98		
AJSE 103	152.20	3.32		
AJSE 109	-125.40	8.28		
AJSE 110	-0.70	0.95		
AJSE 111	-25.80	4.40		
AJSE 112	350.00	3.46		
AJSE 114	-4.30	2.34		
AJSE 95	-0.80	3.48		
AJSE 96	-1.80	3.98		
AJSE 98	0.80	7.76		

Appendix 5

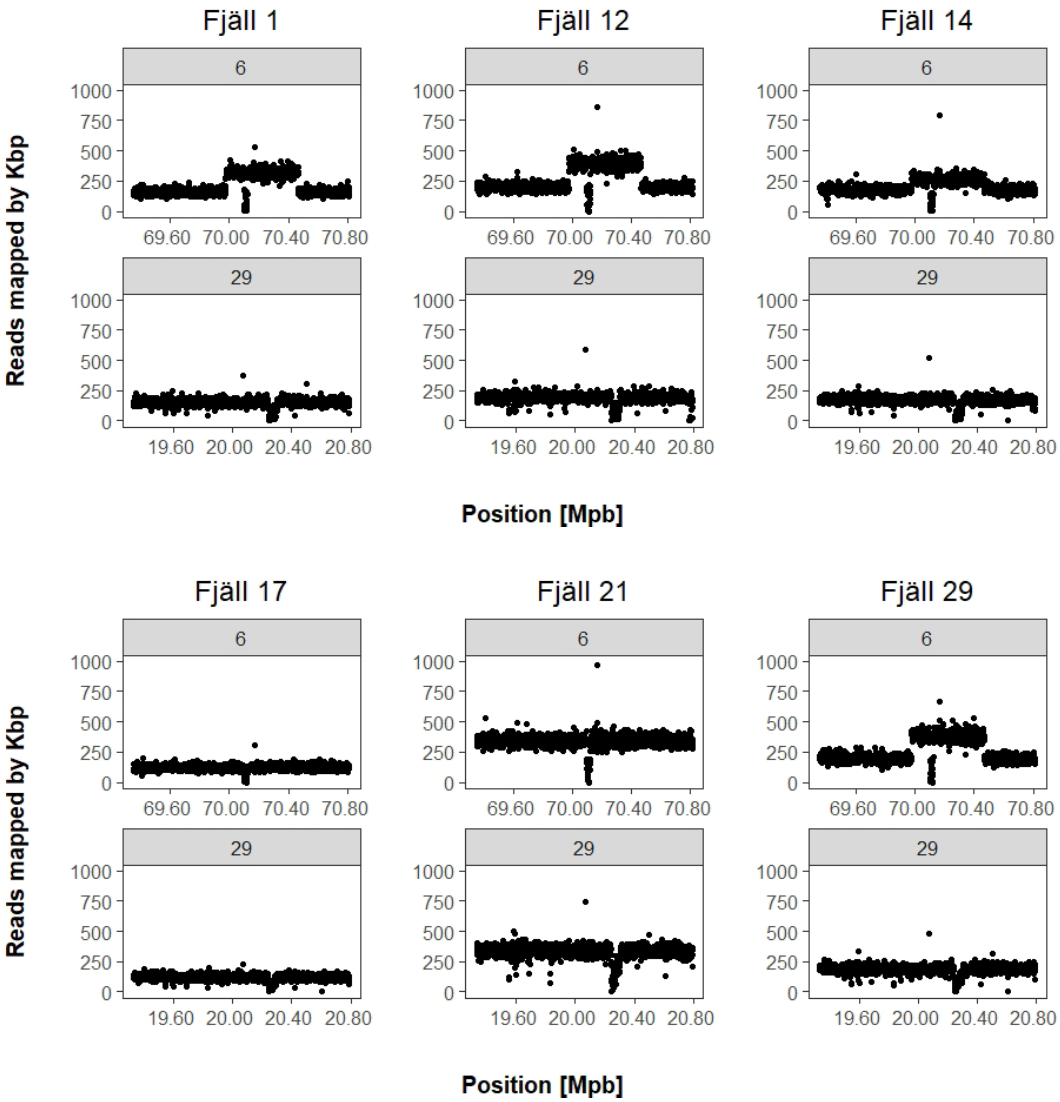
Primer name		Result from BLAST analysis (EMBL-EBI, 2022)
α-D	Forward	
	Reverse	
A-E	Forward	
	Reverse	
C-β	Forward	
	Reverse	
γ-B	Forward	
	Reverse	
α-β	Forward	
	Reverse	
α-β_2	Forward	
	Reverse	

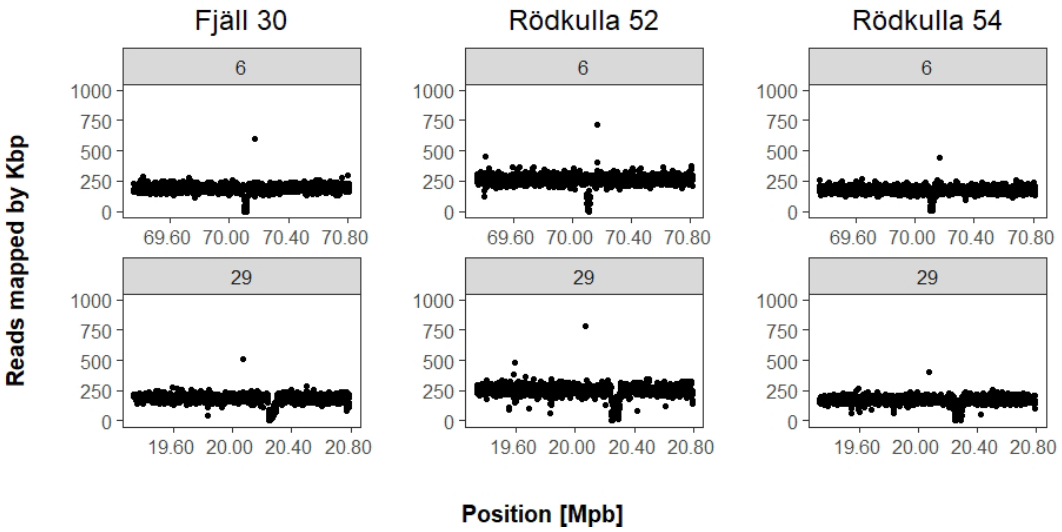
Appendix 6

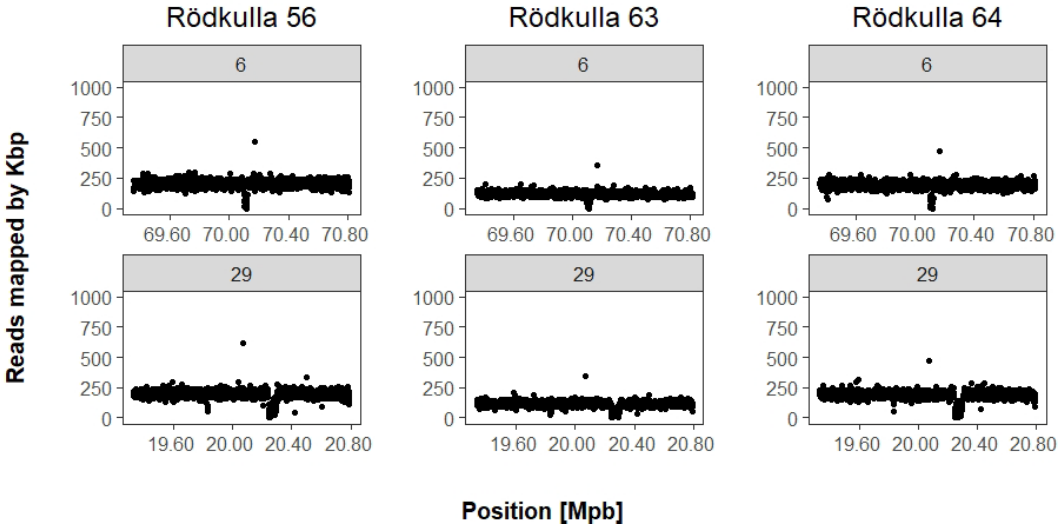
Primer name	Result from UCSC In-Silico PCR (Kent, 2022)
α-D	**UCSC In-Silico PCR** No matches to ggggagaatctgttttcctg ggaaggccttattgcacact in Cow Apr. 2018 (ARS-UCD1.2/bosTau9)
A-E	**UCSC In-Silico PCR** No matches to gaagcaacccagagatgagc aagggaagcccatatgatga in Cow Apr. 2018 (ARS-UCD1.2/bosTau9)
C-β	**UCSC In-Silico PCR** No matches to tcaacgagggacaacatgaa caattgacccctcattttgg in Cow Apr. 2018 (ARS-UCD1.2/bosTau9)
γ-B	**UCSC In-Silico PCR** No matches to gctgcagaaaatgttattcca tcttgaagggccatagcatc in Cow Apr. 2018 (ARS-UCD1.2/bosTau9)
α-β	**UCSC In-Silico PCR** >chr29:19703505+19703898 394bp GGGGAGAATCTGTTTTCCTG TAAAGTCGCCAGTGCAAGTG GGGGAGAATCTGTTTTCCTGttttttccagcttcaggagattgtcttcat tctttggtttatgaccatgcatcactccaacctcagcttctattgtcaca tctccttctctaacactgaccctattggttaaaagattcttgtgattaga ttgaatccttctgaataatattctatctgaataaatcctcctcttctcaa gatcattaacttatcacatctgcaaaaccctttggccatgtaaactaat atattcccaggttacaaggattagaaatggacattttgtggagagcatta ttttgcctaccaaaatgaggggtcaattgttccttctatcatagaggatt aatttgcacatataagattaaaatCACTTGCACTGGCGACTTTA
α-β_2	**UCSC In-Silico PCR** >chr29:19703470+19703944 475bp TGGGTAGACAGGTTTGTTTCC TCTTGACCACTTGCATTGGA TGGGTAGACAGGTTTGTTTCCttctggagggtctaggggagaatctgttt tcctgttttttccagcttcaggagattgtcttcattctttggtttatgac catgcatcactccaacctcagcttctattgtcacatctccttctctaaca ctgaccctattggttaaaagattcttgtgattagattgaatccttctgaa taatattctatctgaataaatcctcctcttctcaagatcattaacttatc acatctgcaaaaccctttttggccatgtaaactaatatattcccaggttac aaggattagaaatggacattttgtggagagcattattttgcctaccaaaa tgaggggtcaattgttccttctatcatagaggattaatttgcacatataa gattaaaatcacttgcactggcgactttagcggtccagtagttaagactt tgcctTCCAATGCAAGTGGTCAAGA

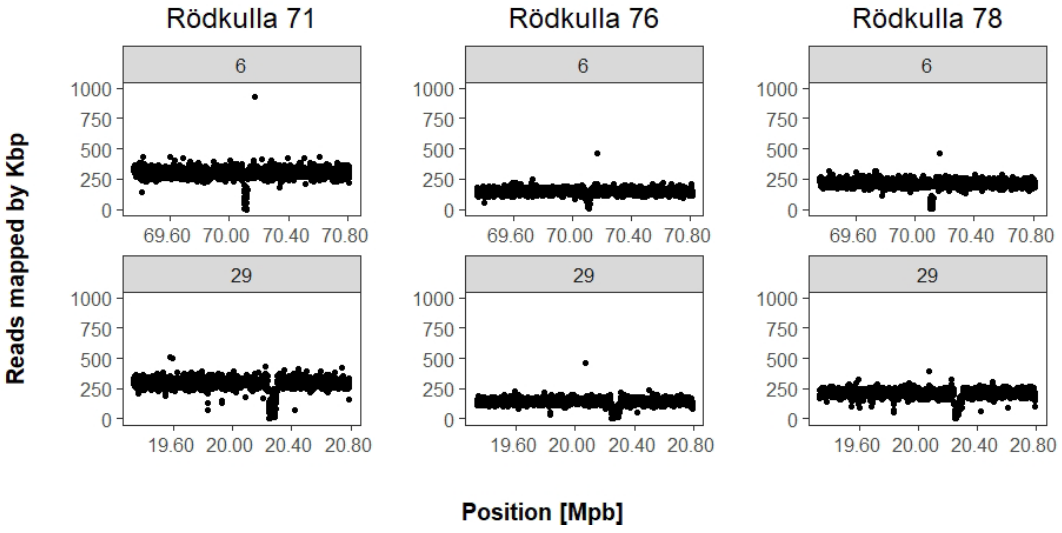
Appendix 7

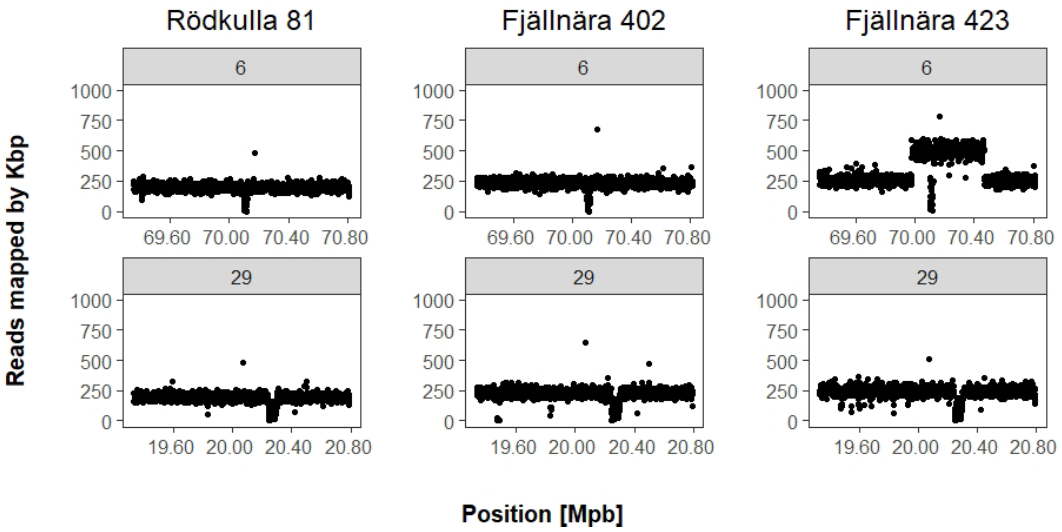
Read coverage in 1 Kbp windows on chr6 and chr29 in the genome of different native Swedish cattle breeds.

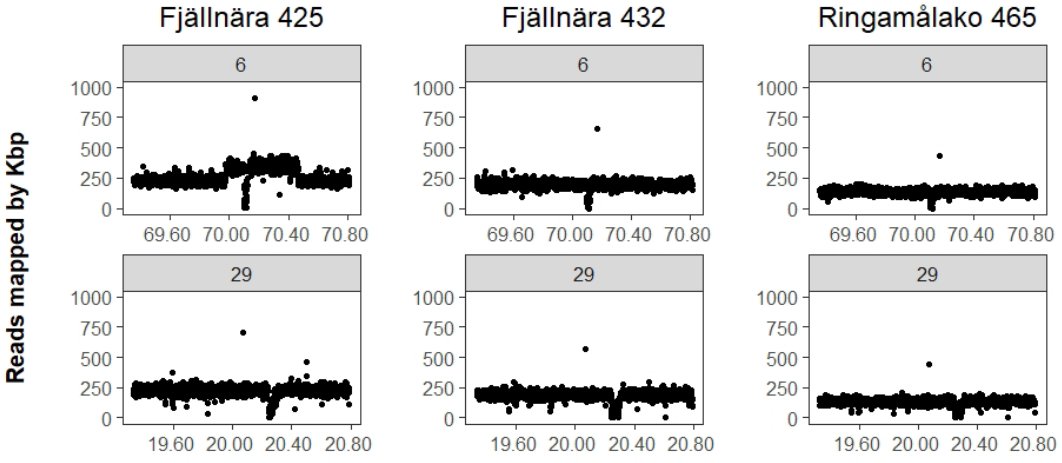


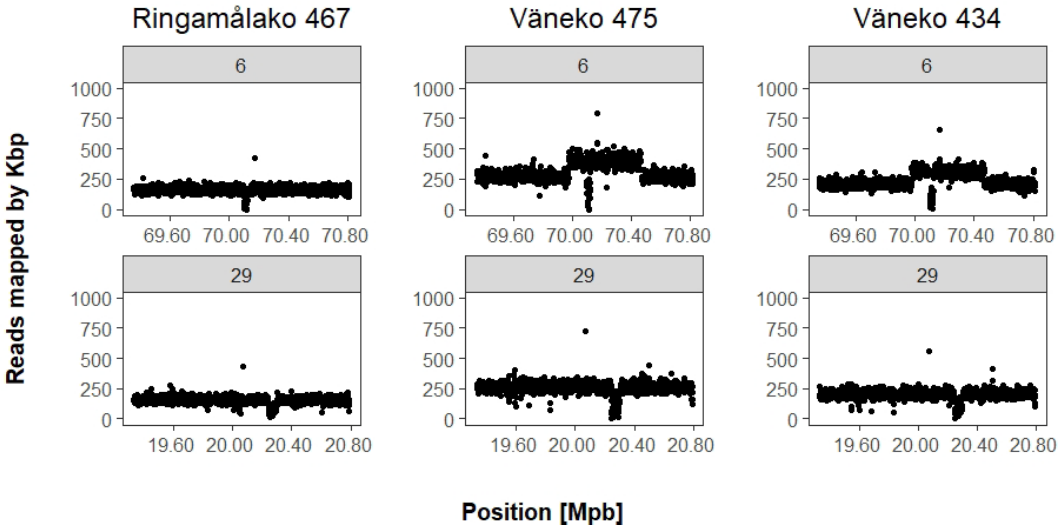

Fjäll 30
Rödkulla 52
Rödkulla 54
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]

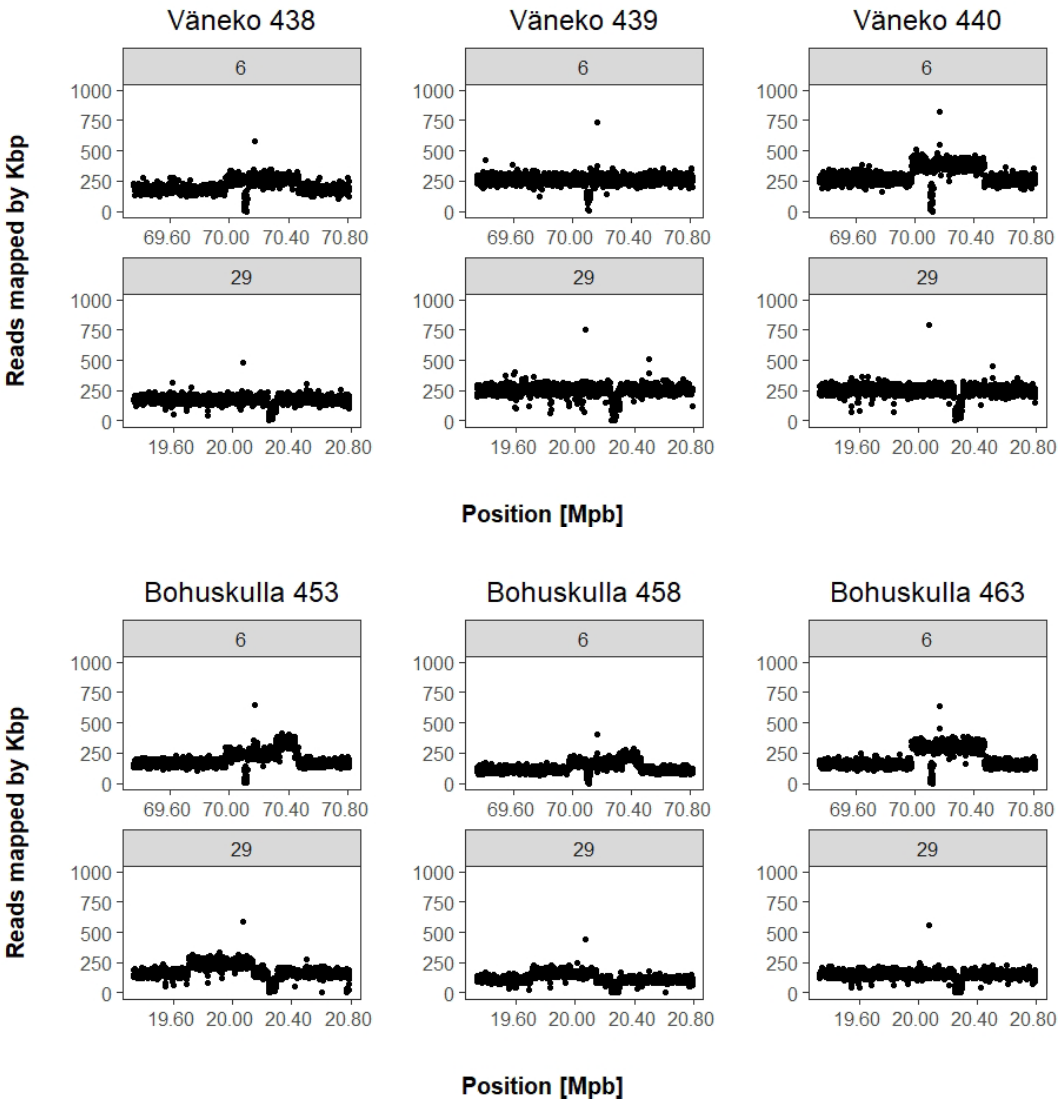

Rödkulla 56
Rödkulla 63
Rödkulla 64
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]


Rödkulla 71
Rödkulla 76
Rödkulla 78
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]


Rödkulla 81
Fjällnära 402
Fjällnära 423
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]


Fjällnära 425
Fjällnära 432
Ringamålako 465
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]


Ringamålako 467
Väneko 475
Väneko 434
6
29
Reads mapped by Kbp
0
250
500
750
1000
69.60
70.00
70.40
70.80
19.60
20.00
20.40
20.80
Position [Mpb]


Väneko 438
Väneko 439
Väneko 440
6
29
Reads mapped by Kbp
1000
750
500
250
0
69.60 70.00 70.40 70.80
19.60 20.00 20.40 20.80
Position [Mpb]
Bohuskulla 453
Bohuskulla 458
Bohuskulla 463

Appendix 8

Description: 0 = two reference alleles; 1 = one reference and one non-reference allele; 2 = two non-reference alleles
Status of hypoplasia was taken from the analysis of the results shown in Appendix 7.

Individual number	Breed	Hypo-plasia	70,209,950	70,209,970	70,214,244
BTA125	Swedish Red Cattle	-	0	0	2
BTA126	Swedish Red Cattle	-	0	0	2
BTA127	Swedish Red Cattle	-	0	0	2
BTA128	Swedish Red Cattle	-	0	0	2
BTA129	Swedish Red Cattle	-	0	0	2
BTA130	Swedish Red Cattle	-	0	0	2
BTA131	Swedish Red Cattle	-	0	0	2
BTA132	Swedish Red Cattle	-	0	0	2
BTA133	Swedish Red Cattle	-	0	0	2
RDCSWEM000000074941	Swedish Red Cattle	-	0	0	2
RDCSWEM000000007635	Swedish Red Cattle	-	0	0	2
RDCSWEM000000085409	Swedish Red Cattle	-	0	0	2
RDCSWEM000000086626	Swedish Red Cattle	-	0	0	2

RDCSWEM00000 0086688	Swedish Red Cattle	-	0	0	2
RDCSWEM00000 0091678	Swedish Red Cattle	-	0	0	2
RDCSWEM00000 0091804	Swedish Red Cattle	-	0	0	2
RDCSWEM00000 0093082	Swedish Red Cattle	-	0	0	2
SH-2270-SE-402	Fjällnära	-	0	0	2
SH-2270-SE-434	Väneko	Hetero-zygous	0	0	2
SH-2270-SE-438	Väneko	Hetero-zygous	0	0	2
SH-2270-SE-439	Väneko	-	0	0	2
SH-2270-SE-440	Väneko	Homo-zygous	0	0	2
SH-2270-SE-453	Bohuskull	Hetero-zygous	0	0	2
SH-2270-SE-458	Bohuskull	Hetero-zygous	0	0	2
SH-2270-SE-463	Bohuskull	Homo-zygous	0	0	2
SH-2270-SE-465	Ringamålako	-	0	0	2
SH-2270-SE-475	Väneko	Homo-zygous	0	0	2
SH-2271-SE-467	Ringamålako	-	0	0	2
SH-2271-SE-52	Rödkulla	-	0	0	2
SH-2271-SE-54	Rödkulla	-	0	0	2
SH-2271-SE-56	Rödkulla	-	0	0	2
SH-2271-SE-63	Rödkulla	-	0	0	2
SH-2271-SE-64	Rödkulla	-	0	0	2
SH-2271-SE-71	Rödkulla	-	0	0	2
SH-2271-SE-76	Rödkulla	-	0	0	2
SH-2271-SE-78	Rödkulla	-	0	0	2
SH-2271-SE-81	Rödkulla	-	0	0	2
SH-2272-SE-1	Fjäll	Homo-zygous	0	0	2
SH-2272-SE-12	Fjäll	Homo-zygous	0	1	2
SH-2272-SE-14	Fjäll	Hetero-zygous	0	0	1

SH-2272-SE-17	Fjäll	-	0	0	1
SH-2272-SE-21	Fjäll	-	0	0	1
SH-2272-SE-29	Fjäll	Homo-zygous	1	0	2
SH-2272-SE-30	Fjäll	-	0	0	1
SH-2272-SE-423	Fjällnära	Homo-zygous	0	0	2
SH-2272-SE-425	Fjällnära	Hetero-zygous	0	0	2
SH-2272-SE-432	Fjällnära	-	0	0	2

Appendix 9

Results of the BLAST analysis of the Sanger sequencing results. If there were multiple results the results were reduced to the two with the highest accordance.

Primer	Orientation	expected region of primer hit	Individual	ensembl BLAST
A-E	F	6: 70,466,799-70,466,819	SE 458	6:70466800-70467026 6:69975194-69975286
			SE 432	/
			SE 425	6:70466932-70467026 6:69975194-69975286
			SE 012	6:70466800-70467026 6:69975194-69975286
	R	6: 69,975,266-69,975,286	SE 458	/
			SE 432	/
			SE 425	6:70466800-70467026 6:69975194-69975286
			SE 012	6:70466800-70467026 6:69975194-69975286
α-β	F	29: 19,703,504-19,703,524	SE 458	29:19703596-19703898
			SE 432	29:19703628-19703898 29:19703505-19703584
			SE 425	29:19703596-19703898 29:19703505-19703584
			SE 012	no match found
	R	29: 19,703,878-19,703,898	SE 458	29:19703505-19703896
			SE 432	29:19703505-19703896
			SE 425	29:19703505-19703810
			SE 012	no match found
α-β_2	F	29: 19,703,469-19,703,491	SE 458	29:19703596-19703944 29:19703470-19703548
			SE 432	29:19703596-19703944

			SE 425	29:19703470-19703548 29:19703555-19703944
				29:19703470-19703547
			SE 012	no match found
	R	29: 19,703,924-19,703,944	SE 458	29:19703470-19703944
			SE 432	29:19703470-19703815 29:19703863-19703944
			SE 425	29:19703470-19703944
			SE 012	no match found
α-D	F	29: 19,703,504-19,703,524	SE 458	6:70444565-70444907 29:19703505-19703573
			SE 432	/
			SE 425	6:70444565-70444900 29:19703505-19703573
			SE 012	6:70444565-70444907 29:19703505-19703573
	R	6: 70,444,887-70,444,907	SE 458	6:70444565-70444907 29:19703505-19703573
			SE 432	/
			SE 425	6:70444565-70444907 29:19703505-19703573
			SE 012	6:70444565-70444907 29:19703505-19703573
C-β	F	6: 70,444,222-70,444,242	SE 458	6:70444223-70444560 29:19703569-19703833
			SE 432	/
			SE 425	6:70444223-70444560 29:19703569-19703833
			SE 012	6:70444223-70444560 29:19703569-19703833
	R	29: 19,703,813-19,703,833	SE 458	6:70444223-70444560 29:19703569-19703833
			SE 432	/
			SE 425	6:70444223-70444560 29:19703569-19703833
			SE 012	29:19703569-19703833 6:70444435-70444560
γ-B	F	29: 20,140,380-20,140,402	SE 458	6:70311557-70311910 29:20140381-20140478
			SE 432	/
			SE 425	/

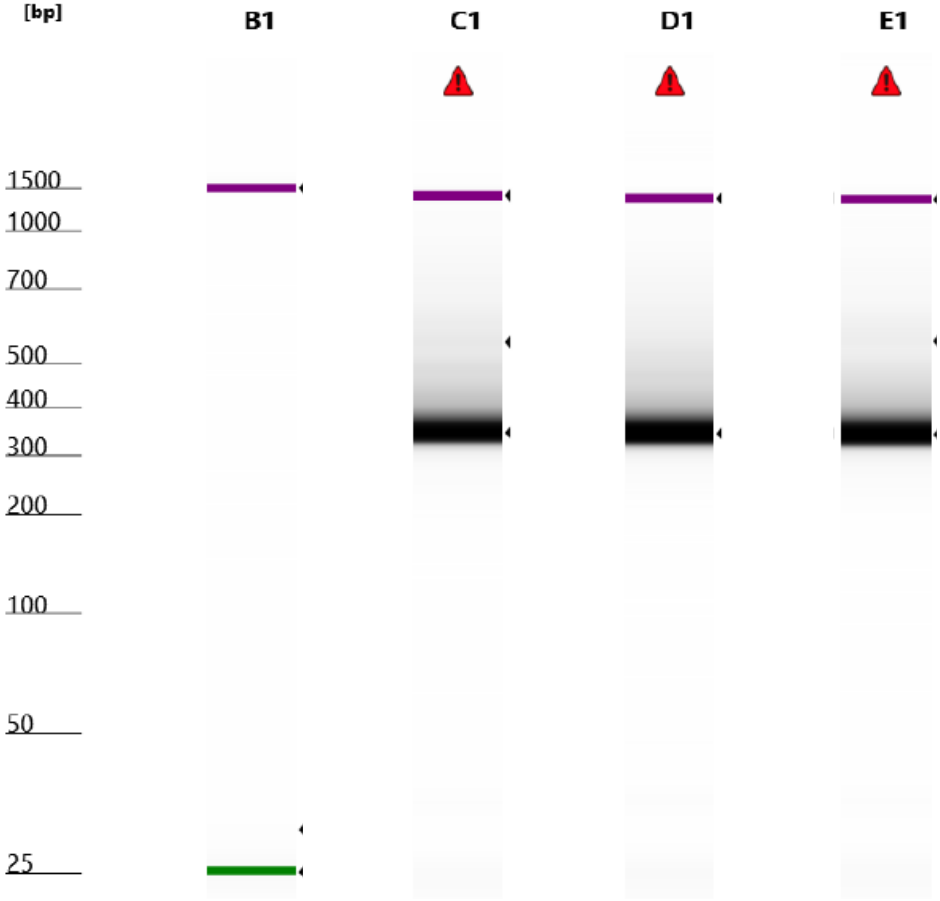
			SE 012	/
	R	6: 70,311,890-70,311,910	SE 458	6:70311557-70311910 29:20140381-20140478
			SE 432	/
			SE 425	/
			SE 012	no match found

Appendix 10

TapeStation results for the single primers A-E, γ-B and α-β.
Red triangles: Marker(s) not detected
Yellow triangles: Peak out of sizing range
B,C,D,E: Position of sample in TapeStation

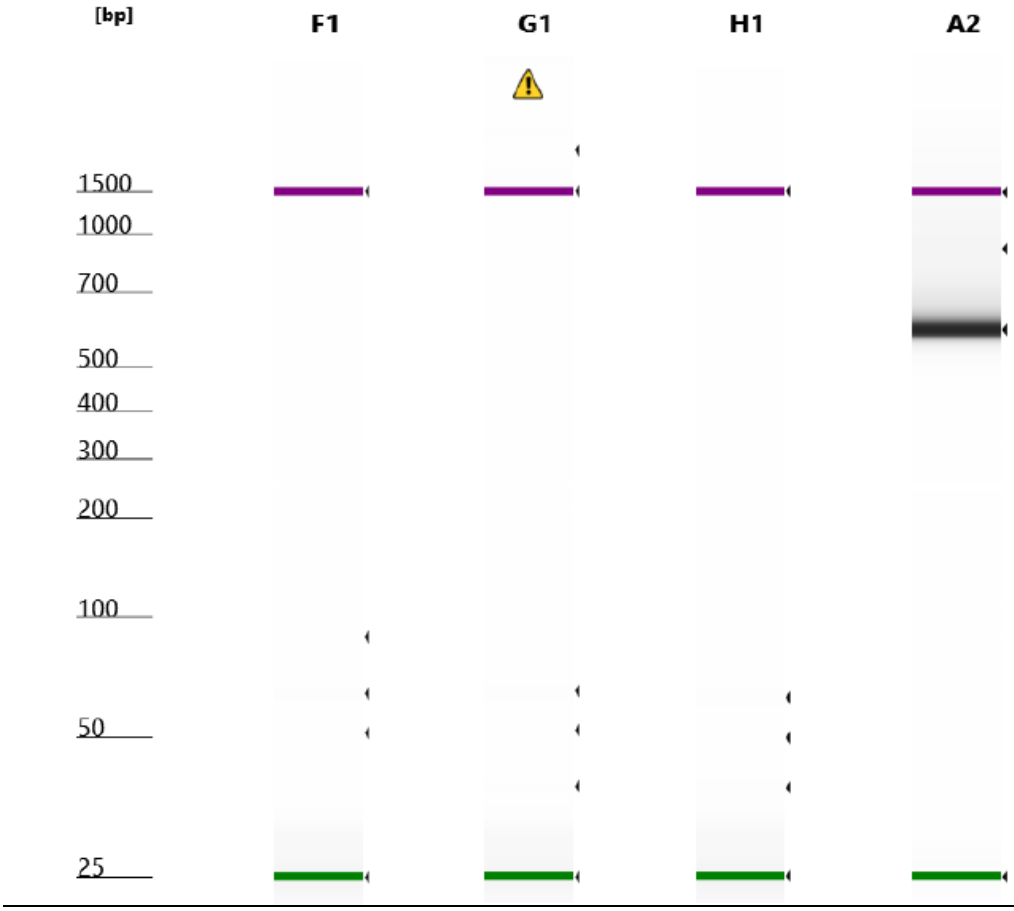
Primer pair A-E

Individual Genotype	SE 432 chr29+/+	SE 012 Cs_{29}/Cs_{29}	SE 425 $Cs_{29}/+$	SE 458 $Cs_{29}/+$ Cs_6



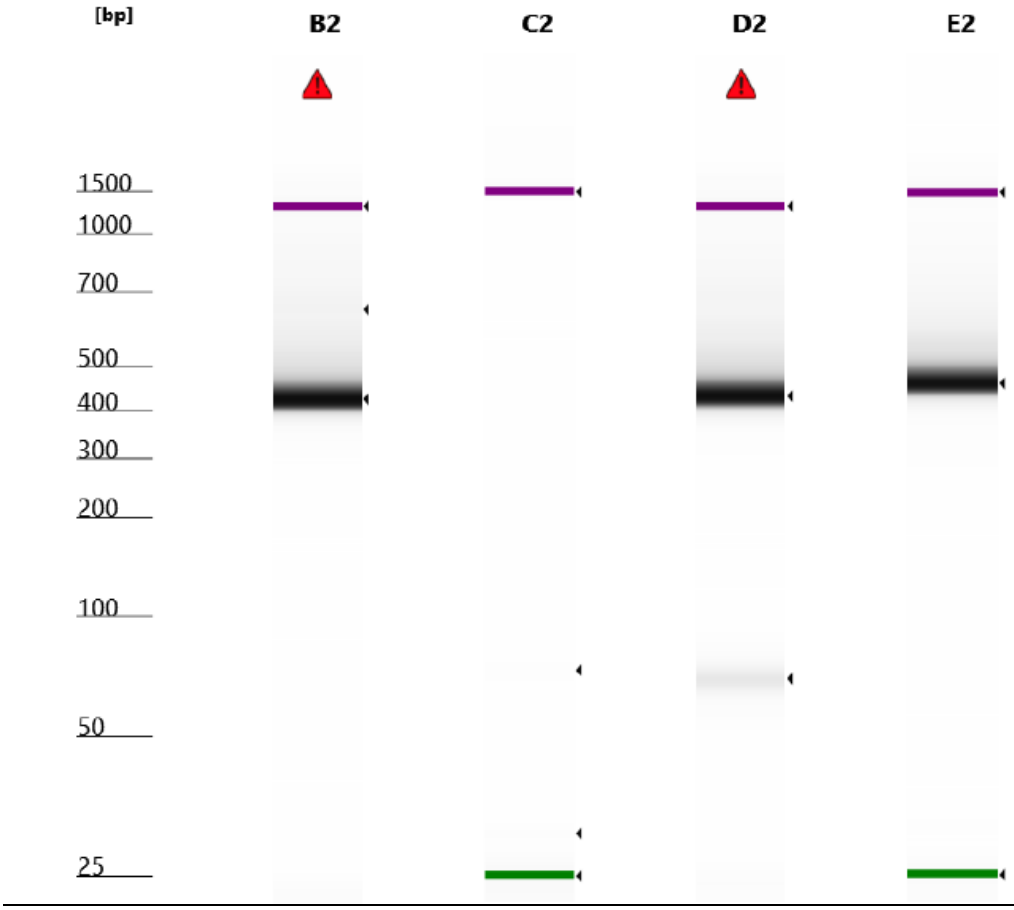
Primer pair γ-B

Individual Genotype	SE 432 chr29+/+	SE 012 Cs_{29}/Cs_{29}	SE 425 $Cs_{29}/+$	SE 458 $Cs_{29}/+$ Cs_{6}

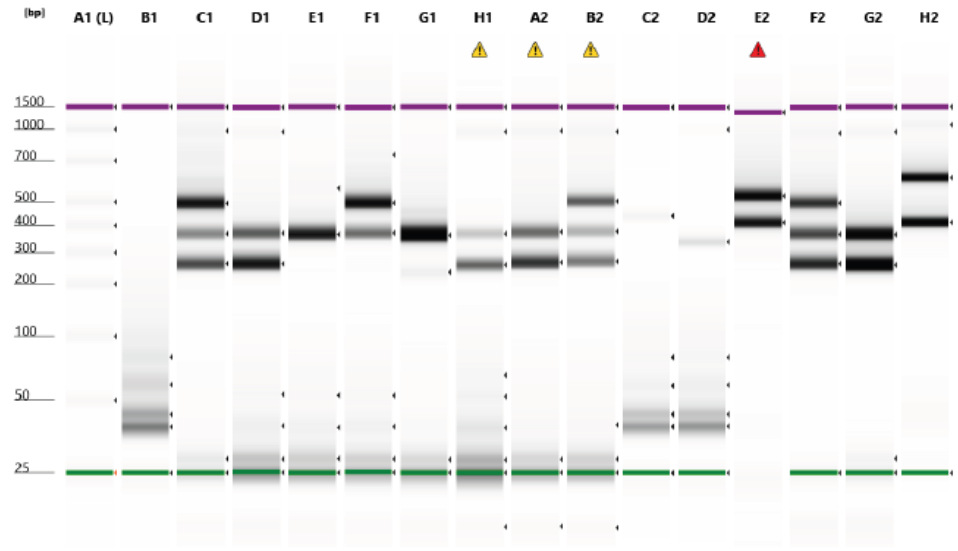


Primer pair α-β

Individual Genotype	SE 432 chr29+/+	SE 012 Cs_{29}/Cs_{29}	SE 425 $Cs_{29}/+$	SE 458 $Cs_{29}/+$ Cs_6



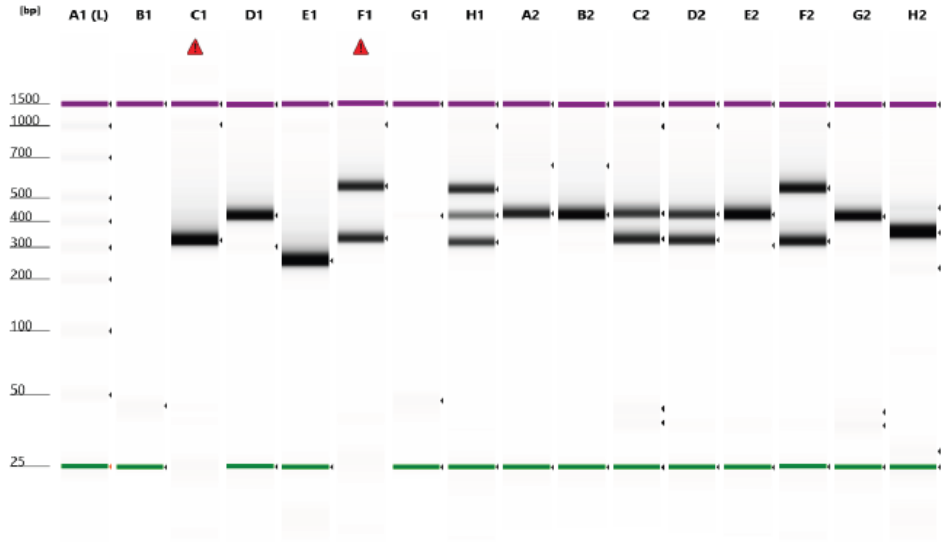
Appendix 11



Default image (Contrast 100%)

Sample Info

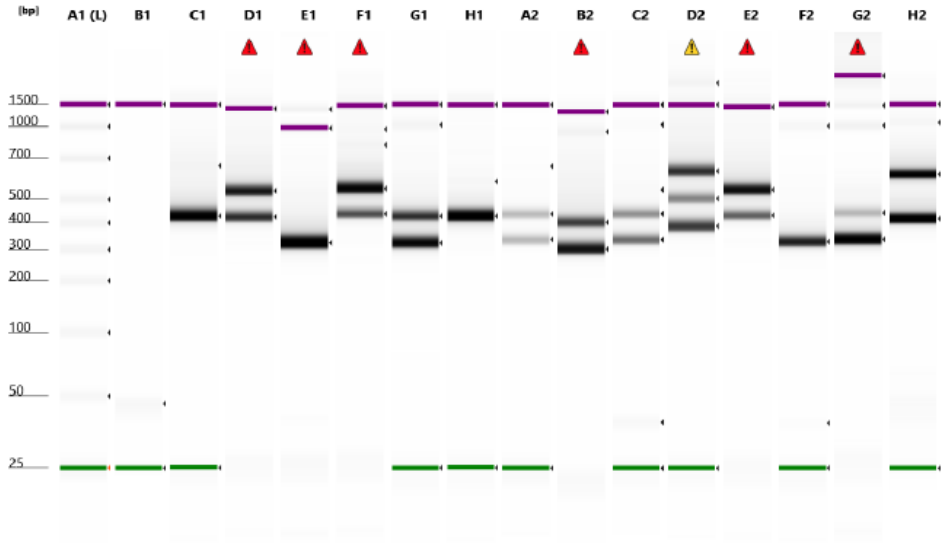
Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	2000	Ladder		Ladder
B1	13500	neg. conroll		
C1	16700	41773		
D1	20700	42376		
E1	12200	42377		
F1	15600	43579		
G1	14500	49889		
H1	11800	51458	⚠	Peak out of Sizing Range
A2	14900	SE 004	⚠	Peak out of Sizing Range
B2	12500	SE 005	⚠	Peak out of Sizing Range
C2	7730	SE 006		
D2	9310	SE 009		
E2	19900	SE 010	▲	Marker(s) not detected
F2	16600	SE 013		
G2	37000	SE 014		
H2	24500	SE 016		



Default image (Contrast 100%)

Sample Info

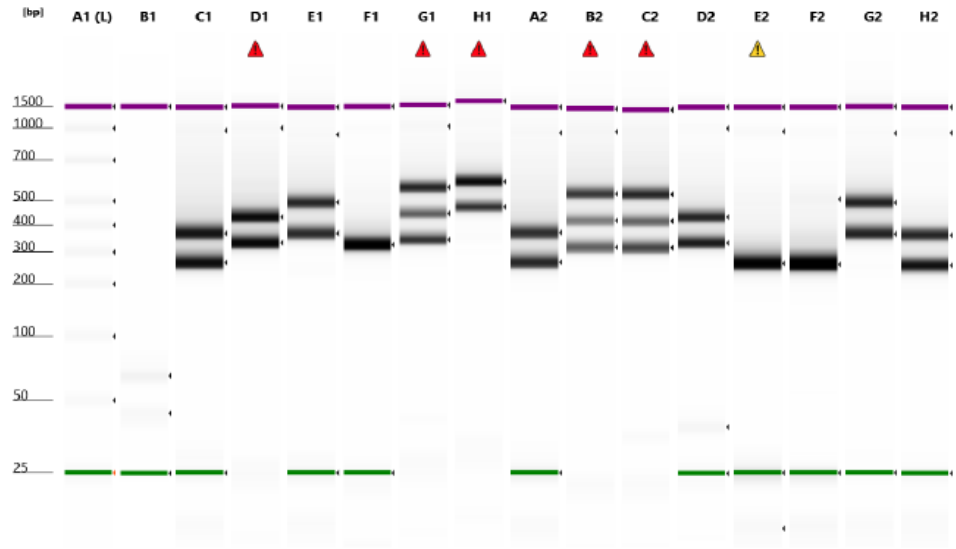
Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	2030	Ladder		Ladder
B1	444	neg. controll		
C1	29700	SE 019	⚠	Marker(s) not detected
D1	12600	SE 022		
E1	26000	SE 031		
F1	19600	SE 032	⚠	Marker(s) not detected
G1	494	SE 037		
H1	14900	SE 038		
A2	9670	SE 401		
B2	13200	SE 402		
C2	17600	SE 419		
D2	17300	SE 420		
E2	13700	SE 421		
F2	24600	SE 422		
G2	12100	SE 426		
H2	16700	SE 427		



Default image (Contrast 100%)

Sample Info

Well	Conc. [pg/ul]	Sample Description	Alert	Observations
A1	2140	Ladder		Ladder
B1	442	neg. controll		
C1	15000	SE 428		
D1	14300	SE 429	▲	Marker(s) not detected
E1	17100	SE 451	▲	Marker(s) not detected; Peak out of Sizing Range
F1	11000	SE 431	▲	Marker(s) not detected
G1	16500	SE 433		
H1	11700	SE 435		
A2	4060	SE 436		
B2	15000	SE 441	▲	Marker(s) not detected
C2	7720	SE 449		
D2	11300	SE 450	⚠	Peak out of Sizing Range
E2	10200	SE 430	▲	Marker(s) not detected
F2	10900	SE 452		
G2	22200	SE 454	▲	Marker(s) not detected
H2	32400	SE 456		

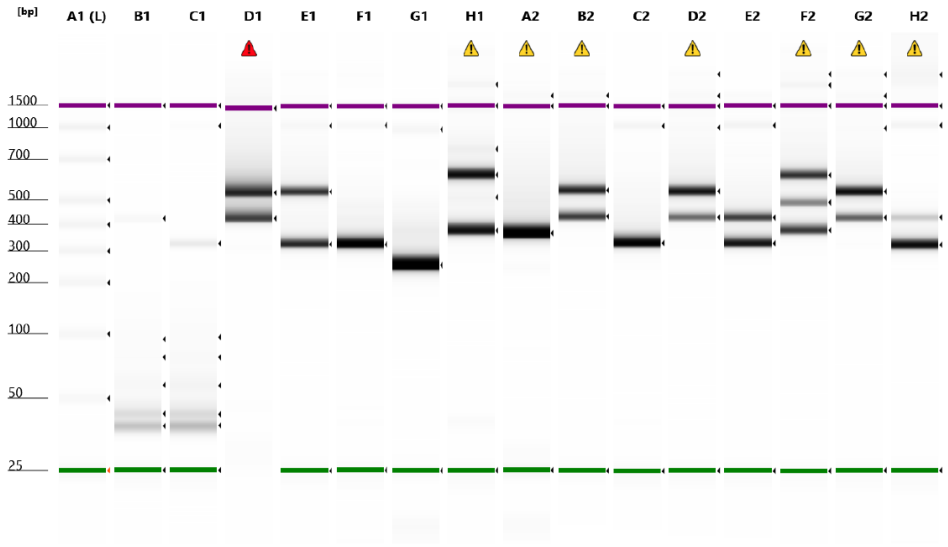


Default image (Contrast 100%)

Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	1990	Ladder		Ladder
B1	1020	neg. controll		
C1	26700	SE 473		
D1	30800	SE 424	⚠	Marker(s) not detected
E1	13300	AJSE 100		
F1	24100	AJSE 101		
G1	18200	AJSE 102	⚠	Marker(s) not detected
H1	14100	AJSE 103	⚠	Marker(s) not detected
A2	16600	AJSE 109		
B2	14700	AJSE 110	⚠	Marker(s) not detected
C2	20800	AJSE 111	⚠	Marker(s) not detected
D2	20100	AJSE 112		
E2	22500	AJSE 114	⚠	Peak out of Sizing Range
F2	29600	AJSE 95		
G2	13800	AJSE 96		
H2	18600	AJSE 98		

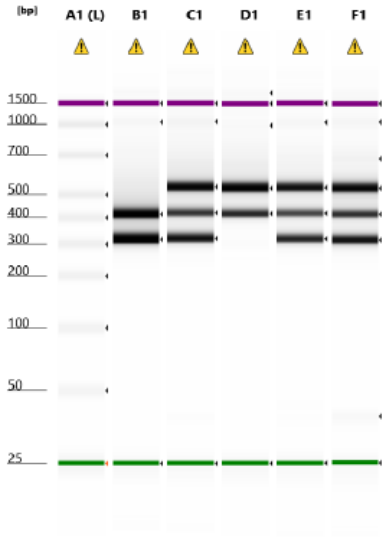
Appendix 12



Default image (Contrast 100%)

Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	2190	Ladder		Ladder
B1	4770	SE 006		
C1	6890	SE 009		
D1	16000	SE 010	▲	Marker(s) not detected
E1	16300	SE 016		
F1	23400	SE 019		
G1	28700	SE 031		
H1	16600	SE 032	⚠	Peak out of Sizing Range
A2	21000	SE 427	⚠	Peak out of Sizing Range
B2	13900	SE 429	⚠	Peak out of Sizing Range
C2	18200	SE 451		
D2	13600	SE 431	⚠	Peak out of Sizing Range
E2	17800	SE 441		
F2	13500	SE 450	⚠	Peak out of Sizing Range
G2	14900	SE 430	⚠	Peak out of Sizing Range
H2	15600	SE 454	⚠	Peak out of Sizing Range



Default image (Contrast 100%)

Sample Info

Well	Conc. [pg/µl]	Sample Description	Alert	Observations
A1	2240	Ladder	⚠	Caution! Expired ScreenTape device (used after two weeks of first use); Ladder
B1	26200	SE 424	⚠	Caution! Expired ScreenTape device (used after two weeks of first use)
C1	20900	AJSE 102	⚠	Caution! Expired ScreenTape device (used after two weeks of first use)
D1	14700	AJSE 103	⚠	Caution! Expired ScreenTape device (used after two weeks of first use); Peak out of Sizing Range
E1	17400	AJSE 110	⚠	Caution! Expired ScreenTape device (used after two weeks of first use)
F1	20700	AJSE 111	⚠	Caution! Expired ScreenTape device (used after two weeks of first use)

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