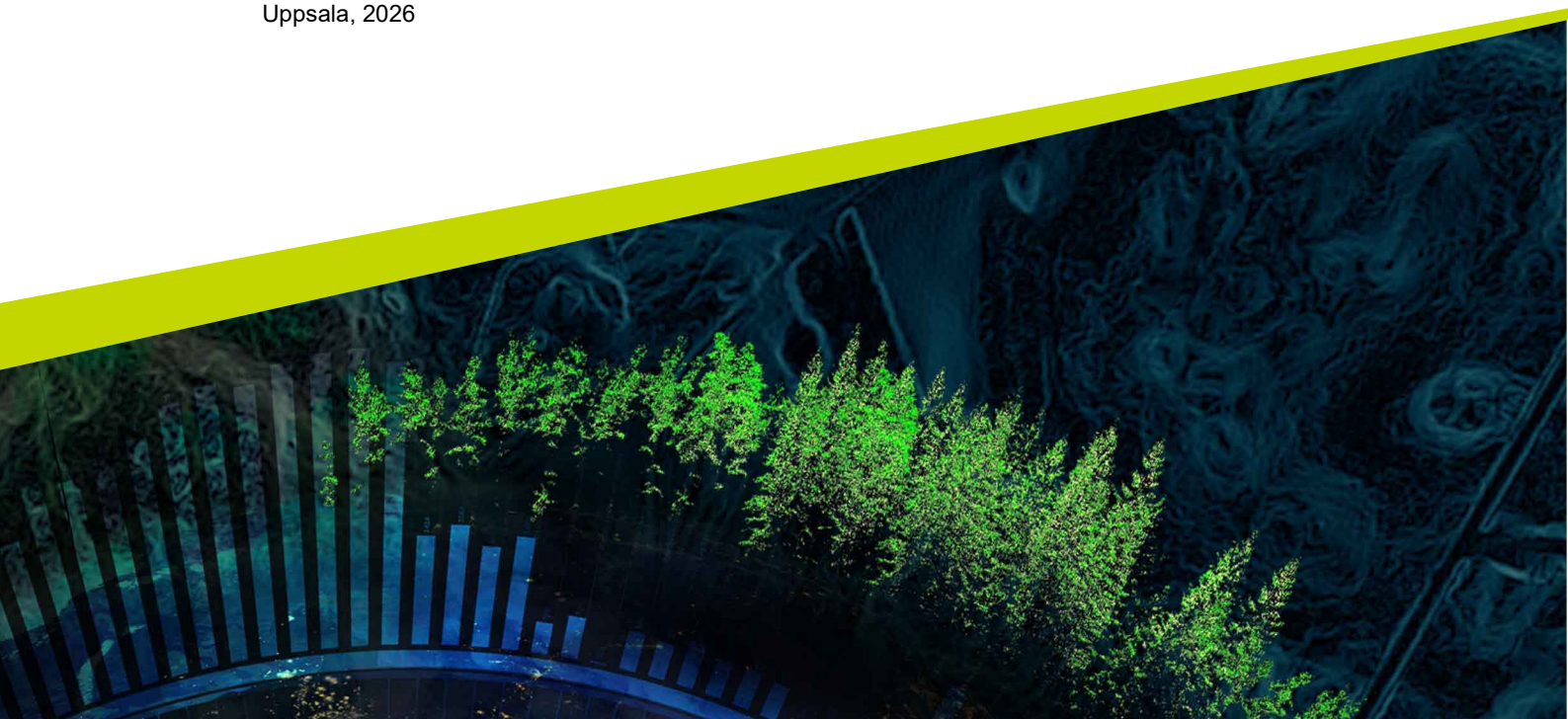
Genetic Characterization of the t(6;29) translocation and its association with gonadal hypoplasia in Swedish Mountain cattle

Michela Martina Sozzi

Independent project in Animal Science • 30 credits
Swedish University of Agricultural Sciences, SLU
Department of Animal Biosciences
Uppsala, 2026



Genetic Characterization of the t(6;29) translocation and its association with gonadal hypoplasia in Swedish Mountain cattle

Michela Martina Sozzi

Supervisor:	**Anna Maria Johansson, Sveriges lantbruksuniversitet, Department of Genetics, Breeding, Biochemistry and Biochemistry, Uppsala, Sweden**
Assistant supervisor:	Martin Johnsson, Sveriges lantbruksuniversitet, department of Quantitative Genetics and Breeding, Uppsala, Sweden
Examiner:	Gabriella Lindgren , Sveriges lantbruksuniversitet, Department of Molecular Genetics and Bioinformatics, Uppsala, Sweden

Credits:	30 credits
Level:	A2E
Course title:	Independent Project in Animal Science
Course code:	EX0870
Programme/education:	
Course coordinating dept:	Department of Animal Breeding and Genomics
Place of publication:	Uppsala, Sweden
Year of publication:	2026
Copyright:	All featured images are used with permission from the copyright owner.
Keywords:	Swedish mountain cattle, Fjällko, colour sidedness, fertility, chromosomal translocation, gonadal hypoplasia

Swedish University of Agricultural Sciences
Faculty Of Veterinary Medicine And Animal Science
Department Of Animal Breeding And Genetics

Abstract

The Swedish mountain cattle (Fjällko) has been selected for generations in order to maintain its characteristic phenotype of white colour coat with coloured pattern sides, unfortunately this led to the propagation of a recessive gene disorder, gonadal hypoplasia, characterized by underdeveloped gonads and reduced fertility. As established in previous studies both the colour-sided phenotype and the gonadal hypoplasia condition have been associated with the reciprocal translocation between chromosomes 6 and 29, denoted t(6;29). The Cs29 allele refers to the segment of chromosomes 29 that has been translocated to chromosome 6 as part of this rearrangement. However, what has been missing until now was a direct statistical association between homozygosity for the Cs29 allele (carrying two copies) and the hypoplasia phenotype in Swedish mountain cattle.

This is why this study aimed to validate and characterize the previously described molecular breakpoints of the t(6;29) translocation in the Swedish mountain cattle and across other Swedish cattle breeds genetically related to this breed and test for a statistical association between the Cs29 genotype and gonadal hypoplasia, this way providing additional evidence supporting the proposed genotype-phenotype relationship.

DNA from 29 individuals across four Swedish breeds (Fjällko, Väneko, Bohuskulla and SKB) was analysed using Sanger sequencing of three primer regions (A-E, alpha-D and C-beta) specific to the breakpoint junctions, and previously certified in other studies cited in this study. Samples from Väneko, Bohuskulla and SKB were originally collected for previous studies conducted in the same laboratory and were included in the present study for comparative sequence analysis only. Two Fisher's exact tests were performed exclusively on Fjällko samples as hypoplasia status and fertility records were not available for the other breeds. The first test focused on 20 Fjällko samples with confirmed hypoplasia status while the second expanded test included the same 20 samples with 8 additional Fjällko samples with documented fertility problems but unknown hypoplasia status.

The sequence analysis revealed a complete conservation of the three targeted breakpoint regions across all four breeds, supporting a common ancestral origin. Statistical analysis demonstrated a strong significant association between homozygosity for the Cs29 allele and gonadal hypoplasia. Among animals with confirmed hypoplasia 5 out of 6 were homozygotes for the Cs29 allele while only 1 out of 14 non hypoplastic animals carried this genotype. When other animals with certified fertility problems but no certain hypoplasia condition were

included, the association strengthened. In contrast the Cs6 allele, the segment of chromosome 6 that has been translocated to chromosome 29, showed no association with hypoplasia indicating its neutrality or its involvement in other traits.

This study provides an additional statistical link between Cs29 homozygosity to gonadal hypoplasia in Swedish mountain cattle. These findings demonstrate an association but do not establish a causal mechanism, which would require further functional studies. However, the molecular characterization of the breakpoints confirms their conservation across breeds and provides the base for developing targeted genetic screening tools to enable informed breeding decisions.

Keywords: Swedish mountain cattle, Fjällko, colour sidedness, fertility, chromosomal translocation, gonadal hypoplasia

Table of contents

List of tables

List of figures

Abbreviations

Abbreviation	Description
BTA6	Bos taurus autosome 6
BTA29	Bos taurus autosome 29
Cs6	Colour-sided allele on chromosome 6
Cs29	Colour-sided allele on chromosome 29
KIT	KIT proto-oncogene, receptor tyrosin kinase
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PGC	Primordial germ cell
ROH	Runs of Homozygosity
SKB	Svensk Kullig Boskap (Swedish Polled)
SNP	Single nucleotide polymorphism
t(6;29)	Translocation between chromosomes 6 and 29

1. INTRODUCTION

1.1 HISTORY OF THE BREED Fjällko

The Swedish Mountain Cattle (Fjällko) is a traditional landrace breed, shaped by the forest grazing and pasture-based husbandry practiced in northern Sweden for over two millennia. This history has produced a dairy cow adapted to harsh Nordic environments, a trait considered valuable for future sustainable agriculture (Svensk Fjällrasavel). Genetic and archaeological evidence indicates that Nordic cattle populations came from two distinct migration routes into Scandinavia. The Fjällko belongs to the eastern genetic lineage, together with the Finnish breeds, the Norwegian Sidet Trønderfe/Nordlandsfe (STN), and the Icelandic cattle (Harish et al., 2024). This lineage diverges from the southern lineage represented by older southern Swedish breeds. The precise arrival of this eastern type in Sweden remains unknown. The "Mid-Nordic" colonization theory, supported by archaeological findings of farm settlements along both the Swedish (Västernorrland) and Finnish coasts of the Gulf of Bothnia, provides the most plausible route. This theory suggests a trading network that allowed an easier northward spread of cattle via the present-day Finland into Norrland, Sweden, long before significant inland agricultural settlement. Consequently, the ancestors of the Swedish Mountain Cattle have been present in the region for at least 2,000 years (“Svensk Fjällrasavel,” n.d.). Historically, the northern Swedish cattle displayed considerable phenotypic variation in coat colour and pattern (black, red, grey, white, spotted, sided) and horn status. This variation was especially prominent in the fjällnära , a subgroup found on remote farms, whose isolation has preserved genetic characteristics. However, they were usually of a consistent light body frame and low musculature, a direct adaptation to centuries of scarce winter feeding intended to sustain the largest possible herd on limited resources. The Swedish Mountain cattle shows a distinct genetic identity without any documented crossbreeding with imported southern breeds throughout the 19th century (Johansson et al., 2020).

1.1.1 ESTABLISHMENT OF BREEDING PROGRAMS AND SELECTION STANDARDS

Formal systematic breeding of the Swedish Mountain Cattle began in the 1880s with the "Flach premium system". This initiative, led by Captain Sigge Flach, encouraged the use of phenotypically desirable bulls by offering monetary incentives to farmers who bred their cows to awarded sires. Regional breeding societies were granted autonomy to define eligible breeds within their counties.

Such systematic selection based on phenotypic uniformity, while effective for standardization, can inadvertently concentrate on specific genomic regions and reduce overall diversity (Mastrangelo et al., 2018; Senczuk et al., 2020). In 1893, things changed when the four northernmost county breeding societies decided to recognize only the Mountain breed. At a meeting in Östersund, they drafted the first official breed standards, a critical step in establishing the breed as a distinct breeding population. This standard initially described a white base coat with small red or black spots and a polled (hornless) condition. Early breeding heavily emphasized phenotypic uniformity, especially coat colour. This focus on achieving a "white suit," while aesthetically motivated, would later cause significant genetic consequences. To manage breed registrations, county-level studbooks were established, which were subsequently consolidated into a single national studbook in 1912. Performance data from this era indicate moderate initial production metrics: average milk yields of 1,200–1,400 kg/year and live weights of 180–250 kg. However, with improved nutrition and selection, yields rapidly increased, with some individuals producing over 4,000 liters a year in the early 1900s (“Svensk Fjällrasavel,” n.d.). The implementation of this phenotypic standards created a shortage of qualifying bulls. To address this, bull associations were formed in the early 1900s, supported by government funded loans schemes. This replaced the traditional practice of using seasonal bulls, showing a new commitment to improve the genetics of the breed. In 1903, the Breeding Centre (Avelscentrum or AC) Competitions were introduced as a more centralized and influential mechanism. Herds with at least ten phenotypically similar cows with documented lineage underwent a rigorous process of two-year performance tests. Selected "AC herds" gained considerable prestige and commercial advantage. Between 1905 and 1939, 34 herds achieved AC status. This program led to a profound genetic concentration around lineages from Jämtland county. By 1937, half of the 4,520 bulls in the national studbook were from sires linked to just 69 cow lines maintained by AC herds, with over 1,800 tracing back to Jämtland lines (“Svensk Fjällrasavel,” n.d.). This intense selection from a limited number of founder lineages created a genetic bottleneck, which has been shown to reduce genetic variation and increase the risk of propagating deleterious variants in subsequent generations (Johansson et al., 2020; Harish et al., 2024). Key founding herds, such as Nils Olof Olsson's at Rösta (source of the prolific Florastammen line) and Anders Mattson's at Överbyn (source of the influential Pukelinjen bull line), were established through this system, changing allele frequencies toward a narrow set of founder lineages. This pattern of genetic concentration around geographically restricted founder herds mirrors observations in other European native cattle breeds that underwent similar centralized breeding schemes (Mastrangelo et al., 2018). The long-term consequence of this breeding structure was a measurable reduction in genetic diversity within the Swedish Mountain

cattle, a finding consistent with genome-wide analyses showing that while the breed retains distinct ancestry, it has experienced significant founder effects and genetic drift (Upadhyay et al., 2019; Harish et al., 2024).

1.2 BREEDING CHALLENGE : THE LINK BETWEEN COLOR AND GONADAL HYPOPLASIA

The state-coordinated breeding effort, selecting for the white coat, unintentionally led to a hereditary issue. By the 1930s, breeders observed that as bulls got whiter, cases of gonadal hypoplasia (underdeveloped or missing tests) increased.

Comprehensive postmortem examinations of over 6,000 Swedish Mountain cattle cows confirmed that gonadal hypoplasia occurred almost exclusively in animals with white coat colour (Lagerlöf & Boyd, 1953). Studies later identified the cause as a recessive genetic disorder with incomplete penetrance, linked to the same chromosomal region controlling white coat pattern (Venhoranta et al., 2013; OMIA, 2025). According to clinical records from the 1930s–1950s, cases of gonadal hypoplasia in strong coat colour animals (e.g., fully red or black sides) were exceptionally rare or undocumented (Lagerlöf & Boyd, 1953; Venhoranta et al., 2013). To address this genetic defect while maintaining the breed, breeding authorities in the late 1930s and 1940s added strongly pigmented and side-coloured bulls from the related Norwegian STN breed to the breeding programs. At the same time, a strict screening protocol was implemented: all breeding animals needed to have a veterinary certification of normal gonad development. Since the 1940s, females needed proof of two normal ovaries, and males, normal testicles and a dam with normal ovaries to be registered in the herd book.

This shows the problems of strong artificial selection based on a single visual trait and the relationship between visible traits (like coat colour) and genes affecting health and fertility (Holečková et al., 2021).

1.3 GENETIC DIVERSITY IN THE SWEDISH MOUNTAIN CATTLE BREED

This history of intensive selection following a delimited breed standard, along with a genetic bottleneck and a past recessive disorder (Harish et al., 2024), helps understanding the study of structural genetic variants in the Swedish Mountain breeds that show low differentiation and have maintained a distinct gene pool with little influence from other European breeds(Upadhyay et al., 2019).

Two comprehensive genomic studies have since clarified the genetic status of the Swedish Mountain cattle relative to other native Swedish breeds. Upadhyay et al.

(2019) found that the Fjäll breed, in particular, has a large founder population and relatively high genetic diversity, evidenced by low runs of homozygosity (ROH) counts and rapid linkage disequilibrium decay, indicating a diverse ancestral population. In contrast, the southern breeds Väne and Ringamåla showed significant genetic isolation, low diversity, and high autozygosity due to small founder populations and limited gene flow. Interestingly, while the mountain breeds maintain a unique and authentic gene pool with little influence from other European cattle, the whole-genome sequencing study by Harish et al. (2024) revealed a more complex genealogy. Although the nuclear DNA confirms the distinct northern cluster, mitochondrial DNA analysis showed that the maternal ancestry of the mountain breed Bohuskulla is actually more similar to southern breeds like Väneko and Ringamåla, suggesting different maternal and paternal ancestries. Furthermore, Harish et al. (2024) confirmed that while the southern red breeds frequently carry the "recessive red" mutation (e) in the MC1R gene, the mountain breeds exhibit different coat colour alleles, including the dominant black allele (ED), highlighting a key genetic distinction linked to their unique phenotypes.

1.4 THE TRANSLOCATION

The specific translocation underlying colour sidedness and gonadal hypoplasia in several cattle breeds , including Swedish mountain cattle and Northern Finncattle is not a classical reciprocal translocation where two chromosomes exchange equal segments, instead , it's a serial translocation involving duplication and re-insertion (Durkin *et al.*, 2012). This process generates two main structural variants: the Cs29 allele and the Cs6 allele.

1.4.1 Formation of the Cs29 Allele

The Cs29 allele is the result of a duplication of 500 kilobase (kb) segment from BTA6. This segment contains the entire coding sequence of the KIT gene, which encodes a type III receptor tyrosine kinase critical for the development of melanocytes (pigment cells), primordial germ cells (precursor of gametes) and hematopoietic stem cells (Venhoranta *et al*., 2013). This segment , designated A-B-C-D-E, does not simply duplicate, it excises from BTA6 and circularizes generating a fusion between the ends of the segment (A-E fusion). The circular duplicate then reopens within the CD interval and integrates into BTA29 at the α–β interval creating two new fusion points, α-D and C-β (Durkin *et al.*, 2012). The result is the Cs29 allele, a BTA29 chromosome that carries an extra, ectopic copy of the KIT gene. The original intact KIT locus remains on BTA6 (Durkin *et al.*, 2012). This means that animals carrying the Cs29 allele have three KIT copies

if heterozygous (two on BTA6, one on BTA29) or four copies if homozygous (two on each chromosome) (Venhoranta *et al*., 2013).

1.4.2 Formation of the Cs6 Allele

Following the formation of the Cs29 allele, a second translocation events occurs. Part of the duplicated BTA6 segment that had been inserted into BTA29, designated B–C–β–γ excises from the Cs29 allele on BTA29, circularizes and is translocated back into the original KIT locus on BTA6. This creates the Cs6 allele that carries a fusion sequence containing DNA from both BTA6 and BTA29. The Cs6 allele is also associated with colour sidedness (Durkin *et al*., 2012; Venhoranta *et al.,* 2013; Hinken *et al.,* 2025).

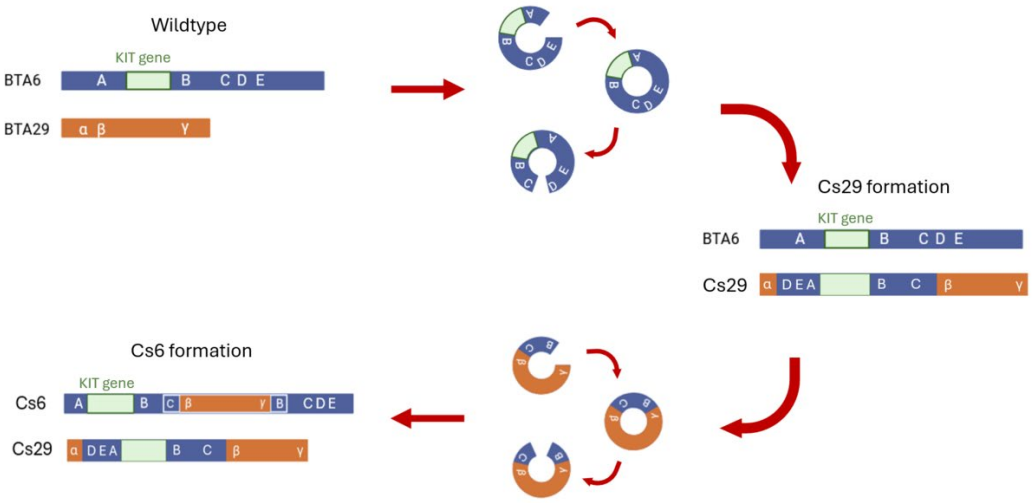


Figure 1. Schematic model of the serial translocation events generating the Cs29 and Cs6 alleles, adapted from Durkin et al. (2012).

Wildtype configuration. BTA6 carries the KIT gene (green box). BTA29 has no KIT.A 492 kb segment from BTA6 (A–E) excises and circularizes (E–A fusion), then reopens within the C–D interval, forming a circular intermediate. The opened circle integrates into BTA29 at the α–β interval via α–D and C–β fusions, generating the Cs29 allele. BTA29 now carries an ectopic KIT copy. Heterozygotes (Cs29/+) have three KIT copies and show colour-sidedness only. Homozygotes (Cs29/Cs29) have four KIT copies and are at risk for gonadal hypoplasia (Venhoranta et al., 2013).From the Cs29 allele, a fragment (B–C–β–γ) excises, circularizes, and reintegrates into the KIT locus on BTA6 via homologous recombination, generating the Cs6 allele. This allele carries a fused BTA6-BTA29 sequence and is associated only with colour-sidedness.

1.5 THE IMPORTANCE OF STUDYING THIS SPECIFIC TRANSLOCATION

Looking closely at the chromosomal translocation between chromosomes 6 and 29 in Swedish Mountain Cattle helps in understanding its role in the breed's genetic makeup and its real-world outcomes (e.g., effects on fertility, health or reproduction). Swedish cattle breeds, including the Mountain Cattle, have their own distinct genetic markers shaped by selection for adaptation and economic traits (Ghoreishifar et al., 2020), however they also show reduced genetic variation, increasing the risk of spreading deleterious variants (Johansson et al., 2020).

In particular, the translocation t(6;29) has been directly associated with the colour-sided coat pattern in Northern European cattle breeds, including Swedish Mountain cattle (Durkin et al., 2012). Furthermore, Venhoranta et al. (2013) demonstrated that a KIT copy number variation located within the rearranged region on chromosome 6 impairs the migration of primordial germ cells, leading to gonadal hypoplasia and reduced reproductive capacity in Northern Finncattle and Swedish Fjäll cattle. More recently, Hinken et al. (2025) confirmed that translocations associated with colour-sidedness are common specifically in northern Swedish cattle breeds, underscoring the clinical and breeding relevance of this structural variant. For this reason, early identification of carriers is important in breeding programs to avoid unwanted selection for lower fertility (Danielak-Czech et al., 2020; Ducos et al., 2008; Iannuzzi et al., 2021).

When an animal is homozygous for the Cs_{29} allele (carrying two copies of the ectopic KIT on BTA29), the resulting KIT gene dosage is altered – four copies instead of the usual two. This disruption impairs primordial germ cell migration, leading to gonadal hypoplasia (underdeveloped gonads) with a predominantly left-sided presentation (Venhoranta et al., 2013). Heterozygotes, carrying three KIT copies, typically show only the colour-sided coat pattern without fertility impairment, indicating a recessive disease model with incomplete penetrance (Venhoranta et al., 2013; Hinken et al., 2025). The Cs_6 allele, by contrast, is associated only with colour-sidedness and not with gonadal hypoplasia (Venhoranta et al., 2013). Understanding this molecular mechanism is critical for developing genetic screening tools to identify carriers and prevent the propagation of hypoplasia while preserving the breed's characteristic colour-sided appearance.

Defining a causal link between this variant and gonadal hypoplasia, independent or interacting with the coat colour phenotype, is a prime objective. This demands the use of cytogenomic approaches, which allow precise breakpoint mapping and the identification of disrupted genes or regulatory elements (Baptista et al., 2005).

1.6 STUDY OBJECTIVES

This study investigates the chromosomal translocation between chromosomes 6 and 29, previously tied to the breed's coat colour and gonadal hypoplasia (Durkin et al., 2012; Venhoranta et al., 2013), to validate its genetic structure and assess its distribution within the breed.

First, it is essential to recognize and identify carriers early in breeding programs to avoid spreading this structural variant, which is known to contribute to subfertility and reproductive loss in bovids (Iannuzzi et al., 2021). Establishing a connection between this specific translocation and reproductive impairment would provide valuable evidence for breeding decisions, particularly since reciprocal translocations in cattle are known to occur with notable frequency and are a documented cause of reduced fertility due to disruptions in chromosomal pairing and segregation during meiosis (De Lorenzi et al., 2012). Also, such translocations are often phenotypically linked to colour-sided patterns, suggesting an effect between coat colour and reproductive traits (Hinken et al., 2025). Therefore, clarifying whether the observed reproductive issues are associated with the translocation itself, or with linked loci, is vital for developing accurate genetic screening tools and breeding programmes that protect both the fertility and the genetic diversity of the Swedish Mountain Cattle.

Second, the study aims to validate and characterize the previously described breakpoint junctions on chromosomes 6 and 29 (Durkin et al., 2012). By confirming the molecular structure of the breakpoint, we can better understand whether the associated traits are a result of gene disruption at the breakpoint or a result of hitchhiking with linked colour-sided loci.

Thirdly it’s important to recognize the presence of this translocation in different Swedish breeds and determine whether sequence variations exist within the targeted regions.

Finally, this molecular characterization will support the development of targeted genetic tests. These tools are of great importance to create an informed conservation breeding, a sustainable management of genetic diversity, and to lower the reproductive and economic risks (Lewis et al., 2022) posed by this structural variant in the Swedish Mountain Cattle population.

2. MATERIALS & METHODS

2.1 STUDY POPULATION AND SAMPLES COLLECTION

DNA from a total of 29 individuals from different Swedish cattle breeds was sequenced. The distribution of the breeds is as follows: 17 Fjällko, four SKB, four Bohuskulla and four Väneko. However for the Fisher's exact tests, genotype and phenotype data from an additional 11 Fjällko samples from previous projects were included, resulting in a total of 28 Fjällko samples analyzed statistically. These 28 samples are listed in Table 1. The non Fjällko breeds (SKB, Bohuskulla, Väneko) were included only for comparative sequence analysis.

The primary objective was to characterize the t(6;29) translocation in these breeds. This translocation is of significant interest because the Cs29 allele, which causes the desirable colour-sided coat pattern, is also known to be the genetic mechanism responsible for gonadal hypoplasia when present in a homozygous state (Venhoranta *et al.*, 2013) . Despite decades of selective breeding, this variant persists in northern Swedish cattle populations and continues to be associated with fertility issues (Hinken *et al.*, 2025).

Among the samples of the Fjällko breed two were new and recently collected from affiliated farmers from a voluntary base, the samples were received as whole blood. The others Fjällko samples were previously extracted DNA stored at -20°C from earlier studies. For samples marked "unknown" in Table 1, the original DNA source could not be determined from laboratory records, however all samples had been stored under identical conditions. The sampled group comes from different herds (from 1 to 10) and were all sampled between 2023 and 2026.

Gonadal hypoplasia status was determined from breed association records but mostly from veterinary examinations. Animals were classified as "nohyp" if veterinary examination confirmed normal gonadal development, as "hyp" (hypoplasia present) if veterinary records documented underdeveloped gonads (testicular hypoplasia in males or ovarian hypoplasia in females) and as "fp" if they were recognized to have fertility problems (impossibility to get pregnant in females or low rate of offspring produced for males) but never associated through veterinary examination to gonadal hypoplasia, animals classified as "pregnant" had documented pregnancy but no veterinary examination for hypoplasia status and were included in the "no hypoplasia/no fertility problems" group for the expanded statistical analysis.

Table 1. List of Fjällko samples with informations regarding origin of the DNA samples, origin of the animals, genotype, hypoplasia status.

The marked samples "" are the ones sequenced during this study. Statistical analyses included all Fjällko and Fjällko related animals in this table (Fjällko, Fjällnära)*

DNA source	ID lab	breed	herd	A-E	Cs29 genotyp	Cs6 genotyp	Hypoplasia status
*nose swab/hair	FK740	Fjällko	1	yes	Cs29/Cs29		Hyp
*nose swab/hair	FK770	Fjällko	1	yes	Cs29/Cs29	Cs6	Hyp
*blood	FK053	Fjällko	1	yes	Cs29/+		NOHyp
*blood	FK054	Fjällko	1	yes	Cs29/Cs29		Hyp
*blood	FK056	Fjällko	1	yes	Cs29/+	Cs6	NOHyp
*blood	FK057	Fjällko	1	yes	Cs29/+		NOHyp
*blood	FK058	Fjällko	1	yes	Cs29/+	Cs6	NOHyp
*nose swab/hair	FK059	Fjällko	1	yes	Cs29/Cs29		Hyp
unknown	FK060	Fjällko	1	no	+/+		NOHyp
unknown	FK794	Fjällko	1	no	+/+		NOHyp
*nose swab/hair	FK002	fjällnära	2	yes	Cs29/+		NOHyp
unknown	FK001	fjällnära	2	no	+/+	Cs6	NOHyp
unknown	FK004	fjällnära	2	no	+/+	Cs6	NOHyp
*nose swab/hair	FK019	Fjällko	3	yes	Cs29/+	Cs6	fp
*nose swab/hair	FK022	Fjällko	3	yes	Cs29/+		Hyp
*nose swab/hair	FK029	Fjällko	4	yes	Cs29/Cs29		NOHyp
*nose swab/hair	FK049	Fjällko	5	yes	Cs29/+		fp
unknown	FK050	Fjällko	5	yes	Cs29/+		pregnant
unknown	FK062	fjällnära	6	no	+/+		pregnant
*nose swab/hair	FK063	fjällnära	6	yes	Cs29/+	Cs6	fp
unknown	FK064	fjällnära	6	no	+/+		pregnant
unknown	FK795	Fjällko	7	no	+/+		NOHyp
unknown	FK796	Fjällko	7	no	+/+	Cs6	NOHyp
unknown	FK835	fjällnära	8	no	+/+		NOHyp
*nose swab/hair	FK095	Fjällko	9	yes	Cs29/Cs29	Cs6	fp
*blood	FK140	Fjällko	10	yes	Cs29/+		NOHyp
*blood	FK141	Fjällko	10	yes	Cs29/Cs29	Cs6	Hyp
unknown	SE012	Fjällko	I	yes	Cs29/Cs29		fb

Additionally 12 samples were analyzed from different Swedish cattle breeds , two of which (Bohuskulla and SKB) historically related to the Swedish mountain cattle breed and one (Väneko) more distantly related to investigate the translocation and verify the genomic correlation (Table 2). The genetic relationships among the Swedish native cattle breeds have been characterized in previous genomic studies. Upadhyay *et al.* (2019) performed principal component

analysis (PCA) using genome-wide SNP data and demonstrated that the northern Swedish Mountain breeds (Fjäll, Fjällnära, Bohus Polled, and Swedish Polled) form a distinct genetic cluster, clearly separated from southern breeds including Väneko, Ringamåla, and Swedish Red. This genetic differentiation was subsequently confirmed by Harish *et al.* (2024) using whole-genome sequencing data. Consequently, Väneko was included in the present study as a genetically distant comparative breed and since we know from Hinken *at al*. (2025) that Cs29 is present in this breed as well. The breeds are Väneko, Bohuskulla and SKB (Svensk Kullig Boskap). These samples originated from different herds as well named A to I and are older samples used for earlier studies thus the hypoplasia status was not available for these breeds; they were included for comparative sequence analysis only.

Table 2. List of Väneko, Bohuskulla and SKB samples with informations regarding origin of the DNA samples, origin of the animals and genotype.

DNA source	**ID lab**	**birth year**	**breed**	**herd**	**AE**	**Cs29 genotype**	**Cs6 genotype**
sperm samples	AJSE_98	1994	SKB	A	yes	Cs29/+	
sperm samples	AJSE_102	2000	SKB	B	yes	Cs29/+	Cs6
sperm samples	AJSE_109	2003	SKB	C	yes	Cs29/+	
sperm samples	AJSE_112	2004	SKB	D	yes	Cs29/+	
- 70°C storage	SE 450	1993	Bohuskulla	E	yes	Cs29/+	Cs6
- 70°C storage	SE 451	1994	Bohuskulla	E	yes	Cs29/Cs29	
- 70°C storage	SE 452	1995	Bohuskulla	E	yes	Cs29/Cs29	
- 70°C storage	SE 454	1996	Bohuskulla	E	yes	Cs29/+	
- 70°C storage	SE 436	1984	Väneko	F	yes	Cs29/+	
- 70°C storage	SE 433	1995	Väneko	G	yes	Cs29/+	
- 70°C storage	SE 441	1997	Väneko	F	yes	Cs29/+	
- 70°C storage	SE 473	1992	Väneko	H	yes	Cs29/+	

"Confirmed primers" (table 3) were used to identify and characterize the sequence of the Cs29, and to verify them they were used for the same samples of Fjällko and the other breeds too, the first pair of primers and the confirmed one through literature is A-E, but also other two primer pairs were used and these are Alpha-D and C-beta (Durkin et al., 2012).

Table 3. Primers used with sequences, the region amplified and the size of the amplicon.

Primer	forward	reverse	description	Size bp
alpha-D	GGGGAGAATCTGTTTTCCTG	GGAAGGCCTTATTGCACACT	BTA29 to 6 breakpoint unique to BTA29	417
A-E	GAAGCAACCCAGAGATGAGC	AAGGGAAGCCCATATGATGA	Fusion point on BTA6	318
C-beta	TCAACGAGGGACAACATGAA	CAATTGACCCCTCATTTTGG	Common breakpoint, found on BTA6 & BTA29	606

2.2 DNA EXTRACTION AND QUANTIFICATION

For two new samples of Fjällko, FK140 and FK141, the DNA was extracted with a miniprep kit , quantified with Qubit dsDNA BR assay kit (Invitrogen), amplified using a HotStarTaq Plus Master Mix kit (Qiagen) following its protocol and firstly screened, to verify the presence of the translocation in these new samples, with a multiplex PCR (95°C x 5min, 94°c x 30sec, 58°C x 1min, 72°C x 1min, these last three were repeated for 32 cycles and lastly 12 °C until off). The results were visualized with two positive controls (FK053,genotype Cs29/+ and FK061, genotype Cs29/Cs29) and one negative control (Negk) the Agilent 4200 Tape Station system using the High Sensitivity D1000 kit (Agilent Technologies Inc.).

2.3 MOLECULAR CHARACTERIZATION OF THE TRANSLOCATION

To sequence the breakpoints of the t(6;29) translocation in 29 samples (17 Fjällko, four Väneko, four Bohuskulla and four SKB), the first step was the amplification PCR performed with the confirmed primers reported in Table 3 with an attached M13 tail. Primer pair A-E amplifies a 318bp product specific to the BTA6 fusion junction in carriers. Primer pair alpha-D amplifies a 417bp product specific to the BTA29->6 breakpoint. Primer pair C-beta amplifies a 606bp product spanning the common breakpoint region.

All primers were synthesized with M13 tails to facilitate sequencing as previously validated in Hinken *et al.* (2025).

These primers were chosen because they would allow to confirm whether the samples had any sign of the translocation of interest, since they are specific for unique breakpoints or common breakpoints and junctions between BTA6 and BTA29.

The amplifying PCR reactions were carried out following the BigDye Direct Cycle Sequencing kit (Applied Biosystems, Life Technologies)with the following settings: 1 cycle at 96° for 5 minutes, a first stage at 94°C for 30 sec then 60°C for 45 sec then 68°C for 45 secs repeated for 35 cycles, and 72°C for 2minutes for 1cycle and a final stage of 4°C hold until end.

The samples were then cycle sequenced with the following settings: first stage of 37°C for 15minutes, 80°C for 2minutes and 96°C for 10 secs, and second stage of 96°C for 10 secs, 50°C for 5secs, 60°C for 1min and 15secs for 25 cycles.

All PCR reactions were run on the ProFlex PCR system (Applied Biosystems, Life Technologies).

The next step was the purification of the products with the BigDye Xterminator Purification kit (Applied Biosystems, Life Technologies) according to the protocol to remove unincorporated terminators and salts that could obscure the data in the early part of the sequence and interfere with basecalling.

The purified samples were then run in the capillary electrophoresis (3500xL Genetic Analyzer) obtaining chromatograms.

2.4 DATA ANALYSIS & alignment

The sequences were firstly analyzed using Chromas.ink version 2.6.6 (Technelysium Pty Ltd) and then aligned through the software Bioedit version 7.7.1 (Ibis Biosciences). Each sequence was controlled singularly to verify the overall quality and then were grouped by type of sequenced amplicon (examples can be found as appendix), for the alignment the M13 tails were removed and each sequence was aligned to the best sequence obtained within the group. Almost all the 87 amplicons were successfully sequenced except some obtained from the earliest samples, probably due to the long storage.

To assess the association between translocation carrier status (Cs29/Cs29 or Cs29/+ vs. wild-type +/+) and hypoplasia phenotype, two Fisher's exact tests were performed using R version 4.5.2 (R Core Team, 2023). A P-value < 0.05 was considered statistically significant.

The analyses were based exclusively on the samples described in Table1. The first test was conducted on the 20 Fjällko animals with confirmed hypoplasia status, 13 newly sequenced in this study and seven additional samples from a previous project conducted in the same laboratory for which both genotype and confirmed hypoplasia status were available.

The second Fisher's exact test was then performed on the full set of 28 Fjällko animals, which includes the 20 animals with confirmed hypoplasia status with eight additional animals with documented fertility but lacked confirmed hypoplasia status.

3. RESULTS

The two new samples of Fjällko were successfully analysed and characterized. Fk140 (A1) known to not be affected by gonadal hypoplasia, was genotyped as Cs29/+, while FK141 (B1) with certified condition of gonadal hypoplasia was genotyped as Cs29/Cs29.

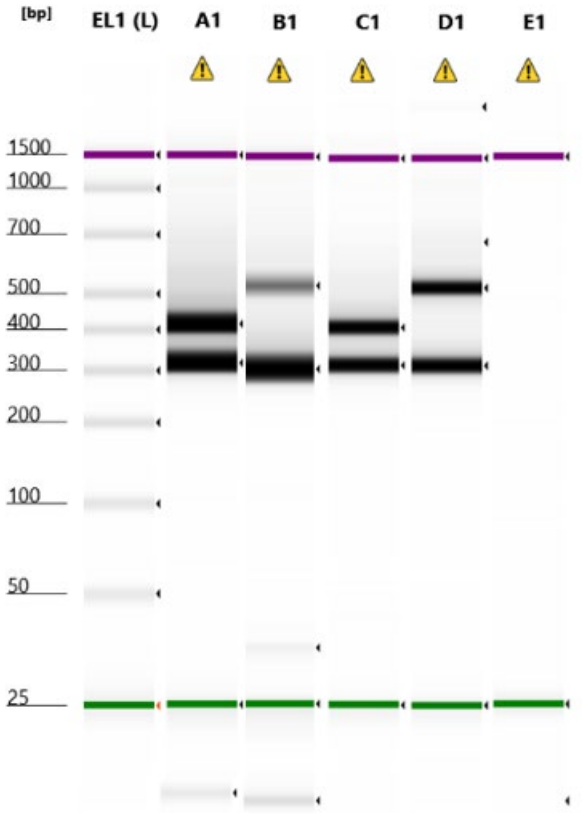


Figure 2. TapeStation results for FK140 and FK141.

EL1 electric ladder, A1 FK140, B1 FK141, C1 FK053(positive control), D1 FK061 (positive control), E1 Negk (negative control). The image was edited by composing different runs.

3.1 CHARACTERIZATION OF THE Cs6-29 BREAKPOINT IN FJALLRAS CATTLE

Analysis of the FK (Fjällko) samples across the A-E , Cbeta and alphaD regions reveals a highly conserved genetic profile with clear evidence of the Cs29 breakpoint signature. All FK samples (FK740, FK770, FK02, FK19, FK22, FK29, FK49, FK53, FK54, FK56, FK57, FK58) exhibit near-identical sequences, particularly within the conserved core domains of the Cbeta alignment, where the characteristic breakpoint region remains intact.

For the AE primer region, all Fjällko samples (FK740, FK770, FK02, FK19, FK22, FK29, FK49, FK53, FK54, FK56, FK57, FK58) displayed identical sequences across the entire ~330 bp alignment, with no ambiguous calls or evidence of polymorphic sites.

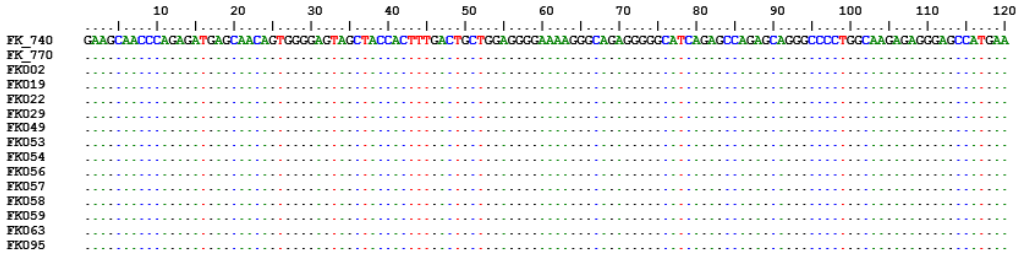


Figure 3. Alignment results for Fjällko samples with AE primer.

For the alphaD primer region, the same set of Fjällko samples showed perfect sequence conservation across the ~420 bp fragment.

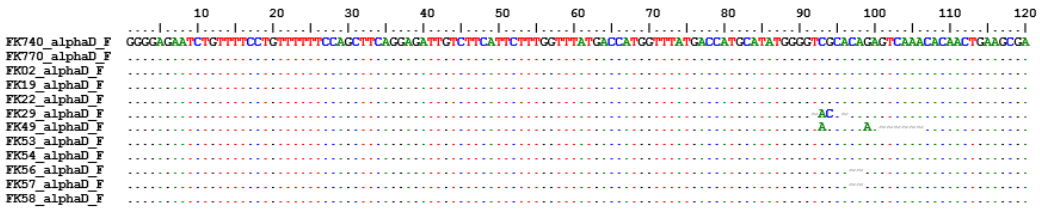


Figure 4. Alignment results for Fjällko samples with alphaD primer.

For the Cbeta primer region, all Fjällko samples exhibited near-identical sequences across the ~600 bp amplicon; the few ambiguous base calls observed (e.g., K, S, Y, M, W) are consistent with sequencing artifacts rather than genuine genetic variation. The absence of sequence heterogeneity across all three loci indicates that the three targeted breakpoint regions are fixed in the Fjällko breed, providing a robust reference haplotype for inter-breed comparisons.

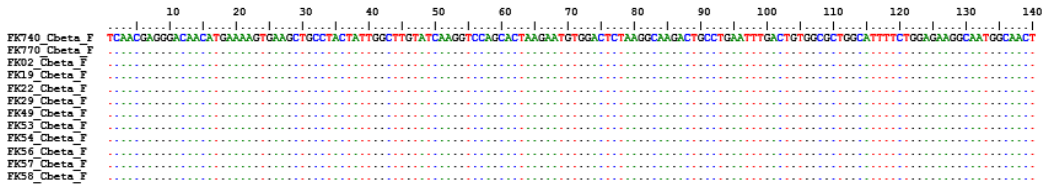


Figure 5. Alignment results for Fjällko samples with Cbeta primer.

Therefore, the Fjällko breed maintains a consistent and reliable Cs29 breakpoint signature across all tested individuals within the three amplified regions. The complete alignment for the Fjällko samples for each primers pair can be found in the appendix section, Appendix 1.

3.2 COMPARATIVE ANALYSIS ACROSS ALL SAMPLED POPULATIONS

Alignment of the AE, alphaD, and Cbeta sequences from Fjällko, Väneko, Bohuskulla, and SKB cattle demonstrated a high degree of inter-breed sequence identity across all three primer regions, with the notable limitation of several samples preservation.

For the AE primer alignment, sequences obtained from well-preserved Väneko, Bohuskulla, and SKB samples (AJSE_98, AJSE_102, AJSE_109, AJSE_112, SE_433, SE_441, SE_451, SE_473) were identical to the Fjällko reference sequence across the entire amplicon.

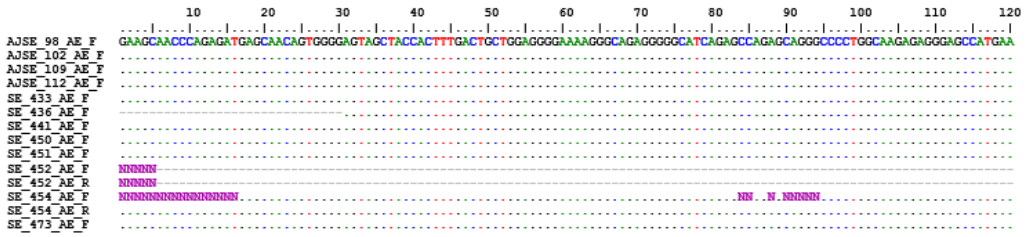


Figure 6. Alignment results for Väneko, Bohuskulla and SKB with AE primer.

For the alphaD primer alignment, the same set of samples showed complete sequence identity with the Fjällko consensus.

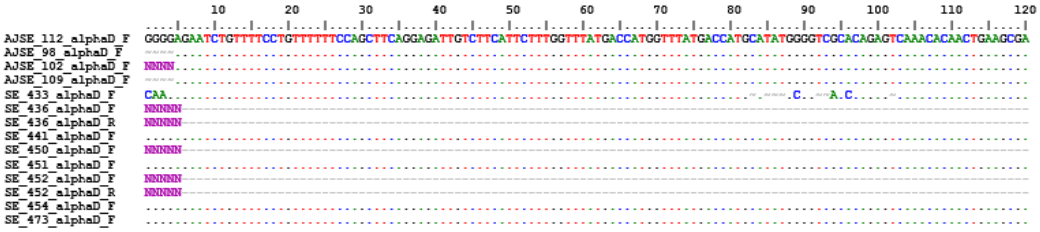


Figure 7. Alignment results for Väneko, Bohuskulla and SKB with alphaD primer.

For the Cbeta primer alignment, the high-quality sequences from these samples again aligned perfectly with the Fjällko reference, with only minimal, artifact-level discrepancies observed in a subset of samples.

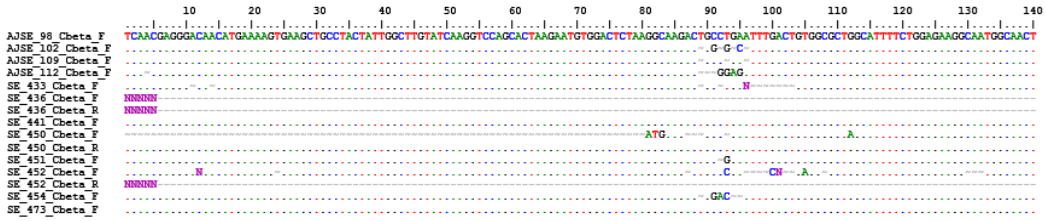


Figure 8. Alignment results for Väneko, Bohuskulla and SKB with Cbeta primer.

Several samples from the Väneko, Bohuskulla, and SKB breeds were later excluded from comparative analysis due to poor DNA quality resulting from extended storage, including SE436 (sampled 1984), SE452 (1995), SE450 (1993), and SE454 (1996). These samples exhibited extensive ambiguous base calls and shortened reads across all three primer regions. Despite these four samples representing three distinct genotypic classes (Cs29/+, Cs29/Cs29, and Cs29/+ with Cs6), their sequencing electropherograms were uniformly uninformative, producing predominantly N-calls and fragmented reads. This confirms that the absence of usable sequence data is attributable to DNA degradation rather than any underlying biological variation or genotype-specific amplification failure.

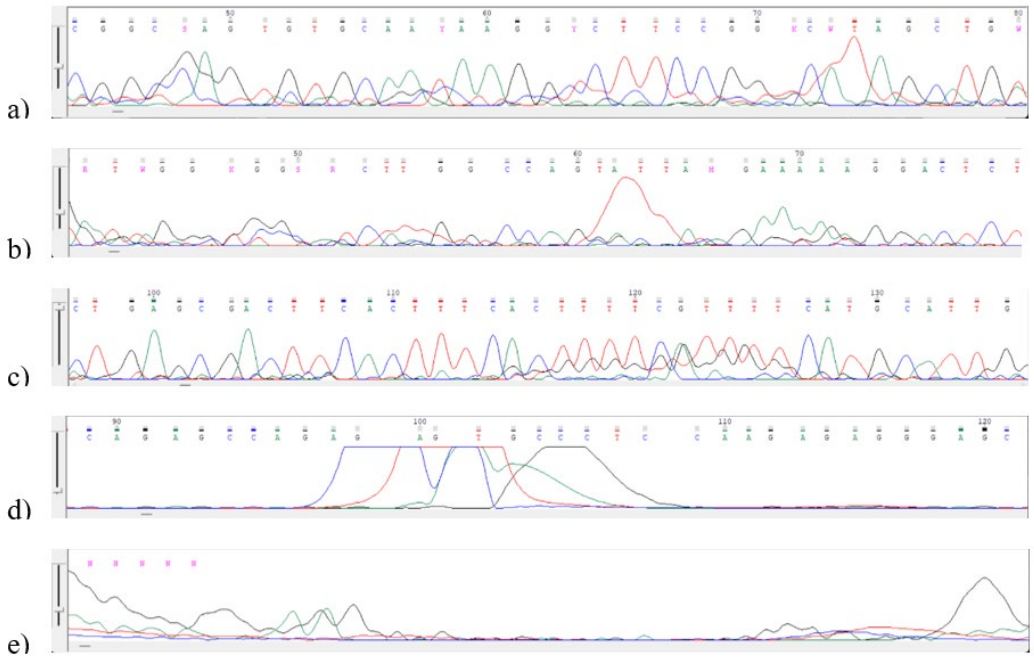


Figure 9. Examples of poor DNA quality, ambiguous base calls, gel artifact derived anomalies:
(a-c) shows respectively low quality sections of the sequence of SE452, SE450, SE436; d) gel artifact derived anomaly observed in SE454; e) complete sequencing failure in sample SE452.

Additionally, a number of other samples (e.g., AJSE_102, AJSE_109, SE_433, SE_450, FK_29, FK_49, FK_56, FK_57 and SE_452) displayed apparent indels or substitutions that, upon careful inspection, are attributable to gel artifacts introduced during the sample cleaning step prior to sequencing. These anomalies are not interpreted as genuine biological variation.

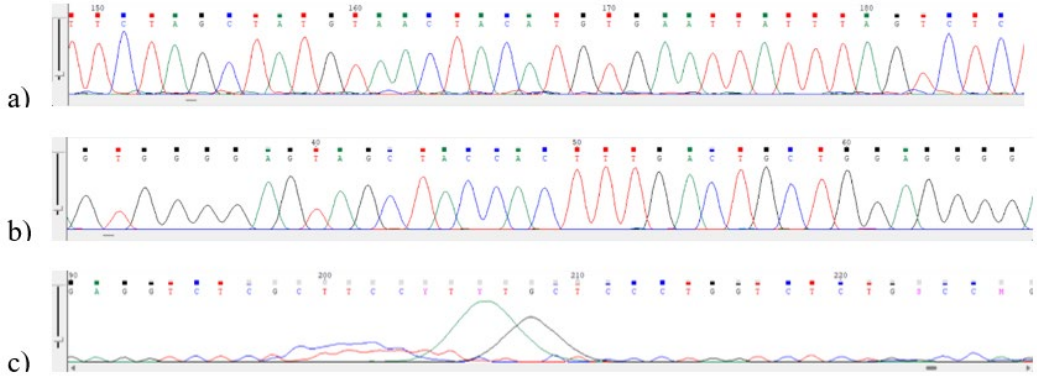


Figure 10. a) and b) Examples of good quality sequences taken from different breeds FK740 (Fjällko) and AJSE102 (SKB); c) example of gel artifact derived anomaly(FK049).

After accounting for sample degradation, gel artifact-derived anomalies, and the known genotypic diversity among excluded samples, the remaining high-quality sequence data from the AE, Cbeta, and alphaD primer alignments support a conclusion of complete sequence homogeneity across the Fjällko, Väneko, Bohuskulla, and SKB breeds at all three examined loci. No breed-specific polymorphisms were detected, and the poor sequence quality of degraded samples (SE436, SE452, SE450, SE454) does not correlate with their distinct Cs6-29 genotypes.

3.3 ASSOCIATION OF Cs6-29 GENOTYPES WITH HYPOPLASIA IN Fjällko CATTLE

To investigate whether the Cs6-29 breakpoint or the Cs6 allele is associated with the hypoplasia phenotype in Fjällko cattle, two Fisher's exact tests were performed. The first test analyzed only animals with confirmed hypoplasia status (20 animals) while for the second the analysis was expanded to include animals with fertility problems of unknown hypoplasia status (28 animals). The genotyping data from samples with confirmed hypoplasia status was collected before April 2026.

3.3.1 Analysis Of Confirmed Hypoplasia Status

When analyzing only animals with confirmed hypoplasia status (Table 1), the Fisher's exact test for the Cs29 genotype (comparing Cs29/Cs29 vs. Cs29/+ vs. +/+ across affected and unaffected animals) revealed a statistically significant association ($p = 0.002554$). Inspection of the contingency table reveals that 5 out of 6 hypoplastic animals were homozygous Cs29/Cs29, while only 1 out of 14 non-hypoplastic animals carried this genotype. In contrast, the Cs6 allele showed no significant association with hypoplasia ($p = 1.0$), with Cs6 carriers distributed evenly between hypoplastic (n=2) and non-hypoplastic (n=5) animals.

Table 4. number of animals with or without hypoplasia and with each genotype.

Genotype	Not Hypoplasia	Hypoplasia
Cs29/Cs29	1	5
Cs29/+	6	1
+/+	7	0
Cs6	5	2
not Cs6	9	4

3.3.2 Analysis Including Fertility Problems

When the analysis was expanded to include cows with fertility problems of unknown hypoplasia status (joined together with confirmed hypoplasia cases into a single "hypoplasia or fertility problems" category), the association for Cs29 became even stronger ($p = 0.0006136$) . The Cs29/Cs29 genotype was present in 6 out of 11 animals in the affected/fertility-problem group but in only 1 out of 17 animals in the unaffected group. The Cs6 allele remained non-significant ($p = 1.0$), indicating no significant association with either hypoplasia or fertility problems.

Table 5. number of animals with or without hypoplasia and recognized fertility problems divided based on each genotype.

Genotype	Not Hypoplasia, no fertility problems	Hypoplasia or fertility problems
Cs29/Cs29	1	7
Cs29/+	7	4
+/+	9	0
Cs6	7	5
not Cs6	10	6

3.3.3 Summary Of Statistical Findings

These results demonstrate a strong and statistically significant association between homozygosity for the Cs29 allele (Cs29/Cs29) and hypoplasia in Fjällko cattle. On the other hand, the Cs6 allele shows no association with the hypoplasia phenotype. This suggests that the Cs6-29 breakpoint (or a linked variant) may play a role in the pathogenesis of hypoplasia, whereas the Cs6 allele may be involved in a different biological pathway. Further functional studies are needed to investigate the mechanistic basis of this association.

4. DISCUSSION

4.1 ASSOCIATION BETWEEN CS29 GENOTYPE AND GONADAL HYPOPLASIA

The primary finding of this study is the strong and statistically significant association between homozygosity for the Cs29 allele (Cs29/Cs29) and gonadal hypoplasia in Fjällko cattle.

Fisher's exact test revealed a significant association ($p = 0.002554$) when analyzing animals with confirmed hypoplasia status. From Tables 4 and 5 we can see that 5 out of 6 hypoplastic animals were homozygous Cs29/Cs29, while only 1 out of 14 non-hypoplastic animals carried this genotype.

This pattern is consistent with recessive inheritance of the hypoplasia phenotype, where two copies of the Cs29 allele are required for the disease to manifest, while heterozygotes (Cs29/+) remain phenotypically normal. This finding aligns with the original observations by Lagerlöf and Boyd (1953) and the molecular mechanism further investigated by Venhoranta *et al.* (2013), who demonstrated that homozygosity for a ~500 kb chromosomal segment translocated from BTA6 to BTA29 damages the primordial germ cell migration via dysregulation of the *KIT* gene. However it is important to emphasize that this study demonstrates a statistical association and does not establish causality. The Venhoranta et al. (2013) study provided functional evidence for a mechanism, whereas the present study provides supporting statistical evidence for the association in a Swedish mountain cattle population.

In addition, the Cs6 allele showed no significant association with hypoplasia ($p = 1.0$), with Cs6 carriers distributed evenly between hypoplastic (n=2) and non-hypoplastic (n=5) animals. This suggests that the Cs6 allele may be involved in a different biological pathway, possibly related to the colour-sided phenotype without affecting fertility. However with the small sample size this null finding should be interpreted with caution.

4.1.1 Inclusion Of Fertility Problem Cases Strengthens The Association

When the analysis was extended to include animals with documented fertility problems but unknown hypoplasia status, the association for Cs29 became even stronger ($p = 0.0006136$). The Cs29/Cs29 genotype was present in 7 out of 11

animals in the affected/fertility-problem group but in only 1 out of 17 animals in the unaffected group.

This finding has two important implications. One suggests that many of the recorded fertility problems may represent undiagnosed cases of gonadal hypoplasia, as veterinary confirmation of hypoplasia requires specific examination that may not have been routinely performed. The other raises the possibility that the Cs29 allele is associated with broader fertility impairment beyond gonadal hypoplasia. But both interpretations are speculative and require further investigation.

4.2 MOLECULAR CONSERVATION OF THE BREAKPOINT REGION ACROSS BREEDS

Sequence analysis of the A-E, alpha-D, and C-beta primer regions revealed complete sequence homogeneity across all four sampled breeds (Fjällko, Väneko, Bohuskulla, and SKB) where high-quality DNA was available. No breed-specific polymorphisms were detected in the breakpoint regions. It is important to note that this conclusion of sequence conservation is limited to the regions amplified by these three primer pairs and should not be generalized to the entire breakpoint structure or to the full extent of the t(6;29) translocation. Other regions flanking these amplicons may harbor undocumented sequence variations that was not captured by the Sanger sequencing approach.

This finding is consistent with the known evolutionary origin of the Cs29 translocation. Durkin *et al.* (2012) demonstrated that the colour-sided translocation arose via a serial translocation mechanism involving circular intermediates, and that the same breakpoint junctions are present across multiple European cattle breeds showing the colour-sided phenotype. The sequence conservation observed in the three targeted regions suggests, but does not prove, that the translocation event in Swedish Mountain cattle shares a common ancestral origin with colour-sided breeds elsewhere, rather than representing an independent occurrence.

However, the poor sequence quality obtained from several older samples (SE436 from 1984, SE452 from 1995, SE450 from 1993, and SE454 from 1996) highlights an important methodological limitation: long-term storage of DNA can lead to degradation that compromises PCR amplification and Sanger sequencing. These samples produced predominantly vague base calls and shortened reads regardless of their underlying Cs29 genotype, confirming that the absence of usable sequence data is attributable to DNA degradation rather than biological variation.

4.3 COMPARISON WITH OTHER CATTLE BREEDS

The Cs29 translocation is not unique to Swedish Mountain cattle, colour-sided phenotypes associated with similar KIT-related translocations have been documented in Northern Finncattle (Venhoranta *et al.*, 2013) and several other European breeds (Durkin *et al.*, 2012). However, the sequence data used in the present study was limited to three amplicons and it cannot be concluded with certainty that the entire breakpoint structure is identical across breeds.

The presence of the same translocation breakpoints regions in geographically distant breeds is consistent with the hypothesis that this rearrangement predates the separation of these breeds and may have originated in ancestral European cattle populations. The exact age of the translocation remains unknown, but its distribution across Nordic and British breeds is consistent with cattle movement during the Viking Age (800–1050 CE) as suggested by Harish *et al.* (2024) for the eastern genetic lineage to which Swedish Mountain cattle belong.

4.4 POPULATION GENETICS IMPLICATIONS

The persistence of the Cs29 allele in Swedish Mountain cattle despite the decades of selective breeding against gonadal hypoplasia can be explained by its phenotypic effect to give the desired white coat pattern with coloured pattern sides (the "white suit" favoured by breeders since the 1890s). However, the same allele, when homozygous, produces the deleterious hypoplasia phenotype. This creates a scenario where in breeding populations the heterozygote individual (Cs29/+) have the desired colour pattern without the fertility impairment, maintaining the allele at intermediate frequency.

Hinken et al. (2025) recently confirmed that translocations associated with colour-sidedness remain common in northern Swedish cattle breeds, with the Cs29 allele frequency estimated at approximately 44% in Swedish Mountain cattle.

4.5 LIMITATIONS

Several limitations of this study should be acknowledged. The sample size for the statistical analysis, while sufficient to detect a strong association (20 animals with confirmed hypoplasia status), is relatively small. A larger cohort would allow for more precise estimation of the effect size and enable stratification by sex, age, and other covariates. Therefore, the significant association while encouraging should be interpreted cautiously.

The classification of hypoplasia status relied on veterinary records and breed association data rather than standardized, prospective examinations. This may introduce classification bias, particularly for the "fertility problems" group where hypoplasia status was unknown.

The poor DNA quality of several older samples (particularly from the 1980s and 1990s) limited our ability to confirm sequence conservation across all four breeds for all genotypic classes. Future studies should prioritize re-sampling of living animals from these breeds to obtain high-quality DNA.

Our study was limited to Sanger sequencing of three breakpoint regions. While this approach successfully characterized the conserved core sequences of these amplicons, it cannot detect structural variants (e.g., copy number variations, complex rearrangements) beyond the targeted regions. Whole-genome sequencing or long read sequencing of representative carriers and non-carriers would provide a more complete picture of the genomic architecture at the t(6;29) breakpoint.

Finally, the study did not include functional validation of the Cs29-hypoplasia association. While the statistical evidence is strong, direct demonstration that the Cs29 allele causes hypoplasia would require gene editing or functional studies in model systems.

4.6 FUTURE DIRECTIONS

Based on these findings, future research could focus on developing a routine genetic screening test for Cs29 carrier status as a priority, as the conserved breakpoint sequences identified here could serve as targets for a high-throughput genotyping assay (e.g., TaqMan or KASP) to enable low-cost, rapid screening of breeding stock.

On the other hand, a transcriptomic analysis of gonadal tissue from Cs29/Cs29 versus +/+ animals could identify disrupted gene expression pathways beyond KIT, potentially revealing therapeutic targets or biomarkers.

In addition, a comparative genomic analysis with colour-sided breeds from other regions (e.g., Northern Finncattle) using long-read sequencing would determine whether the breakpoint junctions are identical across breeds, supporting a single ancestral origin.

Finally, investigation of the Cs6 allele would be needed, although not associated with hypoplasia in this study, its biological role remains unknown. Cs6 carriers were present in the studied cohort, and further study is needed to determine whether Cs6 influences coat pattern intensity or other traits. Based on Durkin *et*

al. (2012) it is known that both Cs29 and Cs6 contribute to the colour sided phenotype so it may be possible to select for Cs6 while selecting against Cs29 thereby maintaining the distinctive phenotype of the breed while reducing the incidence of hypoplasia.

5. CONCLUSION

This study provides a detailed molecular characterization of three specific breakpoint regions (A-E, alpha-D and C-beta) of the t(6;29) translocation in Swedish Mountain cattle and demonstrates their homogeneity across the four Swedish breeds examined (Fjällko, Väneko, Bohuskulla and SKB), supporting a common ancestral origin, though this conservation is limited to the amplified regions and should not be generalized to the entire breakpoint structure.

The study demonstrates a strong, statistically significant association between homozygosity for the Cs29 allele and gonadal hypoplasia ($p = 0.0006136$) while the Cs6 allele resulted having no significant association with hypoplasia, indicating its likely neutrality or involvement in other traits. This findings provide additional statistical evidence supporting the proposed genotype-phenotype association but do not establish a causal mechanism, which would require further functional studies.

The persistence of the Cs29 allele that produces the historically valued white coat pattern, is associated with infertility when homozygous. Breeding programs should therefore avoid mating two Cs29 carriers together to prevent producing Cs29/Cs29 offspring. Given that both Cs29 and Cs6 contribute to the colour-sided phenotype selection for Cs6 may offer a path to maintain the breeds distinctive appearance while reducing the incidence of inherited hypoplasia. Routine genotyping of breeding bulls should be implemented to enable informed mating decisions.

The t(6;29) translocation or similar KIT-related colour-sided variants have been documented in Northern Finncattle and other European breeds. The presence of this translocation in geographically distant breeds suggests an ancient origin, possibly predating the Viking Age (800–1050 CE) when cattle were transported across the North Sea and Baltic regions.

In conclusion, this study confirms a strong statistical association between the Cs29 translocation and gonadal hypoplasia in Swedish mountain cattle. The molecular characterization of the breakpoint regions provides a foundation for developing targeted genetic screening tools that will enable breeders to maintain the breed's unique appearance while reducing the incidence of inherited infertility. These tools are essential for the sustainable conservation of this endangered Nordic genetic resource.

References

Baptista, J., Prigmore, E., Gribble, S.M., Jacobs, P.A., Carter, N.P., Crolla, J.A., 2005. Molecular cytogenetic analyses of breakpoints in apparently balanced reciprocal translocations carried by phenotypically normal individuals. Eur J Hum Genet 13, 1205–1212. https://doi.org/10.1038/sj.ejhg.5201488

Bouwman, A.C., Derks, M.F.L., Broekhuijse, M.L.W.J., Harlizius, B., Veerkamp, R.F., 2020. Using short read sequencing to characterise balanced reciprocal translocations in pigs. BMC Genomics 21, 576. https://doi.org/10.1186/s12864-020-06989-x

Buehr, M., McLaren, A., Bartley, A., Darling, S., 1993. Proliferation and migration of primordial germ cells in W^e/W^e mouse embryos. Developmental Dynamics. https://doi.org/10.1002/aja.1001980304

Danielak-Czech, B., Kozubska-Sobocińska, A., Smołucha, G., Babicz, M., 2020. Breeding and Economic Aspects of Cytogenetic Screening Studies of Pigs Qualified for Reproduction. Animals (Basel) 10, E1200. https://doi.org/10.3390/ani10071200

De Lorenzi, L., Morando, P., Planas, J., Zannotti, M., Molteni, L., Parma, P., 2012. Reciprocal translocations in cattle: frequency estimation. J Animal Breeding Genetics 129, 409–416. https://doi.org/10.1111/j.1439-0388.2011.00983.x

Ducos, A., Revay, T., Kovacs, A., Hidas, A., Pinton, A., Bonnet-Garnier, A., Molteni, L., Slota, E., Switonski, M., Arruga, M.V., van Haeringen, W.A., Nicolae, I., Chaves, R., Guedes-Pinto, H., Andersson, M., Iannuzzi, L., 2008. Cytogenetic screening of livestock populations in Europe: an overview. Cytogenet Genome Res 120, 26–41. https://doi.org/10.1159/000118738

Durkin, K., Coppieters, W., Drögemüller, 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., Zelenika, D., Zhang, Z., Leeb, T., Georges, M., Charlier, C., 2012. Serial translocation by means of circular intermediates underlies colour sidedness in cattle. Nature 482, 81–84. https://doi.org/10.1038/nature10757

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. F1000Res 9, 63. https://doi.org/10.12688/f1000research.16665.2

Ghoreishifar, S.M., Eriksson, S., Johansson, A.M., Khansefid, M., Moghaddaszadeh-Ahrabi, S., Parna, N., Davoudi, P., Javanmard, A., 2020. Signatures of selection reveal candidate genes involved in economic traits and cold acclimation in five Swedish cattle breeds. Genet Sel Evol 52, 52. https://doi.org/10.1186/s12711-020-00571-5

Harish, A., Lopes Pinto, F.A., Eriksson, S., Johansson, A.M., 2024. Genetic diversity and recent ancestry based on whole-genome sequencing of endangered Swedish cattle breeds. BMC Genomics 25, 89. https://doi.org/10.1186/s12864-024-09959-9

Heidaritabar, M., Vereijken, A., Muir, W.M., Meuwissen, T., Cheng, H., Megens, H.-J., Groenen, M. a. M., Bastiaansen, J.W.M., 2014. Systematic differences in the response of genetic variation to pedigree and genome-based selection methods. Heredity 113, 503–513. https://doi.org/10.1038/hdy.2014.55

Hinken, J., Vanhala, T., Gòdia, M., Johnsson, M., Johansson, A.M., 2025. Translocations associated with colour-sidedness are common in northern Swedish cattle breeds. Hereditas 162, 122. https://doi.org/10.1186/s41065-025-00481-w

Holečková, B., Schwarzbacherová, V., Galdíková, M., Koleničová, S., Halušková, J., Staničová, J., Verebová, V., Jutková, A., 2021. Chromosomal Aberrations in Cattle. Genes (Basel) 12, 1330. https://doi.org/10.3390/genes12091330

Iannuzzi, A., Demyda-Peyrás, S., Pistucci, R., Morales, R., Zannotti, M., Sbarra, F., Quaglia, A., Parma, P., 2024. A genomic biomarker for the rapid identification of the rob(1;29) translocation in beef cattle breeds. Sci Rep 14, 2951. https://doi.org/10.1038/s41598-024-53232-8

Iannuzzi, A., Parma, P., Iannuzzi, L., 2021. Chromosome Abnormalities and Fertility in Domestic Bovids: A Review. Animals (Basel) 11, 802. https://doi.org/10.3390/ani11030802

Iannuzzi, L., Ciotola, F., Albarella, S., Iannuzzi, A., Perucatti, A., Peretti, V., 2024. Abstracts of the 25th International Colloquium on Animal Cytogenetics and Genomics (25th ICACG), 26–29 June 2024, Naples, Italy. Biology and Life Sciences Forum 33, 1. https://doi.org/10.3390/blsf2024033001

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

Jourdain, J., Barasc, H., Faraut, T., Calgaro, A., Bonnet, N., Marcuzzo, C., Suin, A., Barbat, A., Hozé, C., Besnard, F., Taussat, S., Grohs, C., Kuchly, C., Iampietro, C., Donnadieu, C., Pinton, A., Boichard, D., Capitan, A., 2023. Large-scale detection and characterization of interchromosomal rearrangements in normozoospermic bulls using massive genotype and phenotype data sets. Genome Res. 33, 957–971. https://doi.org/10.1101/gr.277787.123

Lagerlöf, N., 1951. Hereditary Forms of Sterility in Swedish Cattle Breeds. Fertility and Sterility 2, 230–242. https://doi.org/10.1016/S0015-0282(16)30543-X

LAGERLOF, 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.

Lauvergne, J.J., 1970. Gonadal hypoplasia and white coat color in Swedish Highland cattle. J Hered 61, 43–44. https://doi.org/10.1093/oxfordjournals.jhered.a108031

Lewis, N.M., Canedo-Ribeiro, C., Rathje, C.C., Jennings, R.L., Danihel, M., Bosman, L.M., Silvestri, G., Griffin, D.K., 2022. The Economic Burden of Chromosome Translocations and the Benefits of Enhanced Screening for Cattle Breeding. Animals 12, 1982. https://doi.org/10.3390/ani12151982

Li, H., 2014. Aligning sequence reads, clone sequences and assembly con*gs with BWA-MEM. https://doi.org/10.6084/m9.figshare.963153.v1

Mastrangelo, S., Ciani, E., Ajmone Marsan, P., Bagnato, A., Battaglini, L., Bozzi, R., Carta, A., Catillo, G., Cassandro, M., Casu, S., Ciampolini, R., Crepaldi, P., D'Andrea, M., Di Gerlando, R., Fontanesi, L., Longeri, M., Macciotta, N.P., Mantovani, R., Marletta, D., Matassino, D., Mele, M., Pagnacco, G., Pieramati, C., Portolano, B., Sarti, F.M., Tolone, M., Pilla, F., 2018. Conservation status and historical relatedness of Italian cattle breeds. Genet Sel Evol 50, 35. https://doi.org/10.1186/s12711-018-0406-x

OMIA - Online Mendelian Inheritance in Animals [WWW Document], n.d. URL https://omia.org/home/

Raudsepp, T., Chowdhary, B.P., 2016. Chromosome Aberrations and Fertility Disorders in Domestic Animals. Annu. Rev. Anim. Biosci. 4, 15–43. https://doi.org/10.1146/annurev-animal-021815-111239

Senczuk, G., Mastrangelo, S., Ciani, E., Battaglini, L., Cendron, F., Ciampolini, R., Crepaldi, P., Mantovani, R., Bongioni, G., Pagnacco, G., Portolano, B., Rossoni, A., Pilla, F., Cassandro, M., 2020. The genetic heritage of Alpine local cattle breeds using genomic SNP data. Genet Sel Evol 52, 40. https://doi.org/10.1186/s12711-020-00559-1

Settergren, I., 1997. Ovarian hypoplasia in heifers due to germ cell weakness. Theriogenology 47, 531–539. https://doi.org/10.1016/S0093-691X(97)00011-3

Sugimoto, T., Inagaki, H., Mariya, T., Kawamura, R., Taniguchi-Ikeda, M., Mizuno, S., Muramatsu, Y., Tsuge, I., Ohashi, H., Saito, N., Hasegawa, Y., Ochi, N., Yamaguchi, M., Murotsuki, J., Kurahashi, H., 2023. Breakpoints in complex chromosomal rearrangements correspond to transposase-accessible regions of DNA from mature sperm. Hum Genet 142, 1451–1460. https://doi.org/10.1007/s00439-023-02591-9

Svensk Fjällrasavel [WWW Document], n.d. URL https://fjallko.se/fjallrasen/kortfattad-historik-kring-fjallrasen

Switonski, M., Szczerbal, I., Nowacka-Woszuk, J., 2025. From cytogenetics to cytogenomics: a new era in the diagnosis of chromosomal abnormalities in domestic animals. J Appl Genetics 66, 661–673. https://doi.org/10.1007/s13353-025-00943-x

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. Genet Sel Evol 51, 56. https://doi.org/10.1186/s12711-019-0496-0

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, e75659. https://doi.org/10.1371/journal.pone.0075659

Zhao, S., Zhang, B., 2015. A comprehensive evaluation of ensembl, RefSeq, and UCSC annotations in the context of RNA-seq read mapping and gene quantification. BMC Genomics 16, 97. https://doi.org/10.1186/s12864-015-1308-8

Popular science summary

The aim of this study was to determine whether and how the translocation t(6;29) is associated with gonadal hypoplasia in Swedish Mountain cattle (Fjällko). Gonadal hypoplasia is a condition where one or two gonads (testicles for males or ovaries for females) are underdeveloped and smaller than normal. This condition leads to fertility problems or complete infertility. Regarding Swedish Mountain cattle the condition have been known to breeders since the 1930s when it was firstly observed that the cases of gonadal hypoplasia occurred mainly in white coated animals and to control the spread breeding authorities implemented veterinary screening in the 1940s requiring all breeding animals to have certified normal gonads development before being registered in the herd book.

The DNA analysis of 28 Fjällko individuals in this research showed a strong and significant association between homozygosity for the Cs29 allele and gonadal hypoplasia. The results also demonstrated that the Cs6 allele shows no significant association with hypoplasia. This is an important finding because it suggests that breeders have a safer alternative to select for.

This shows how the veterinary investigations that have so far been standard practice may not be able to identify all cases therefore it would be recommended to complement veterinary inspections with a DNA-based test.

This study puts the basis for a future development of a specific DNA test by characterizing the molecular breakpoints of the t(6;29) translocation using three different primer pairs (A-E, alpha-D and C-beta) and demonstrating that these regions are conserved across Fjällko, Väneko, Bohuskulla and SKB breeds.

A future DNA test based on these findings would help identify carriers of the Cs29 allele reducing the incidence of fertility problems across the breed.

The findings of this study highlight the importance of genetic screening for the Cs29 allele and considering the continued presence of this variant in the breed indicates that more research and active informed selection are necessary to reduce the incidence of this inherited disease while preserving the breed’s distinctive colour sided appearance.

ACKNOWLEDGEMENTS

I would like to begin by thanking Anna Maria Johansson and Martin Johnsson for welcoming me into this project and trusting me with this research, despite the tight timeframe I had before needing to return to Italy. Their insightful guidance, constructive feedback, and constant encouragement have been instrumental throughout this journey, and I am truly grateful for the time and energy they have dedicated to supporting me over these past months.

A very special thank you goes to Tytti Vanhala, whose patient supervision in the laboratory was absolutely essential to the completion of this work. Her willingness to share her knowledge, guide me through every step, and always be available for questions made it possible to generate reliable results within such a short period. I could not have asked for a better mentor in the lab.

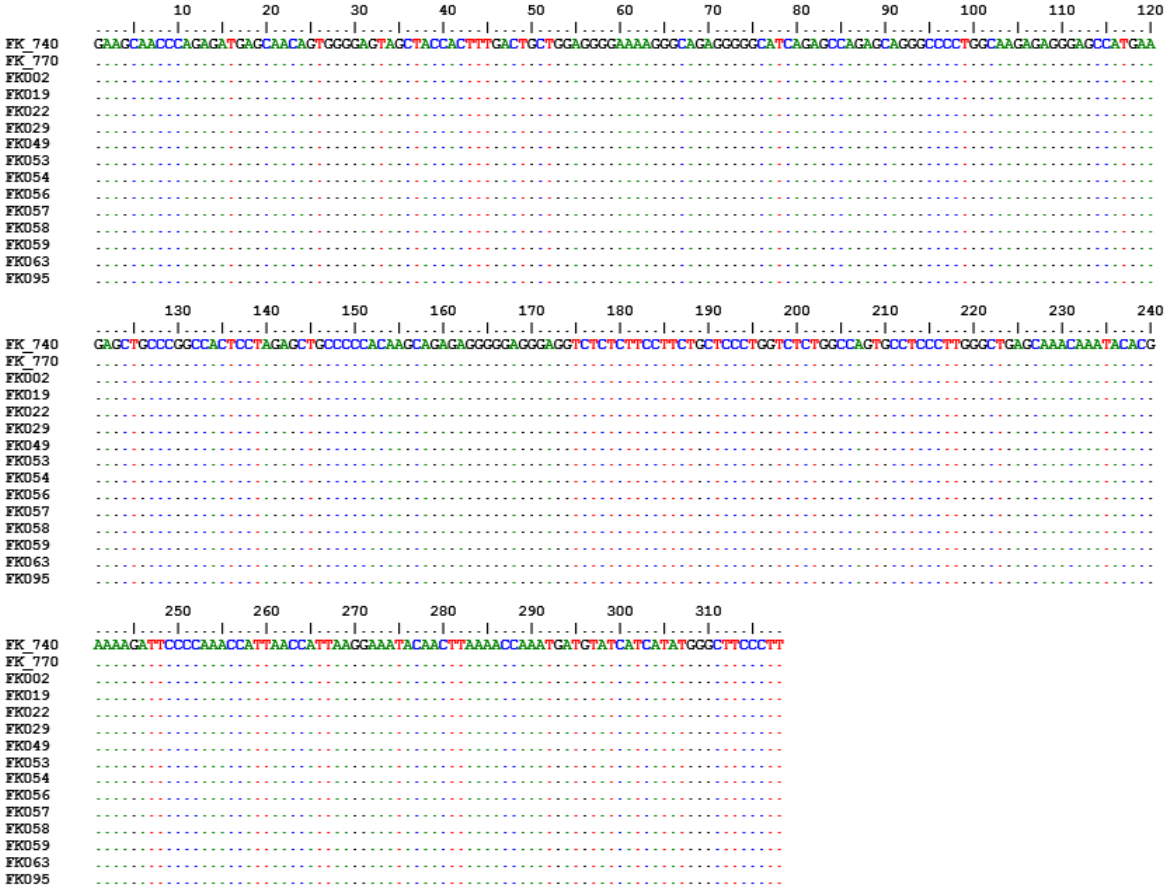
I would like to extend my sincere thanks to Gabriella Lindgren for agreeing to examine this thesis and for her valuable feedback.

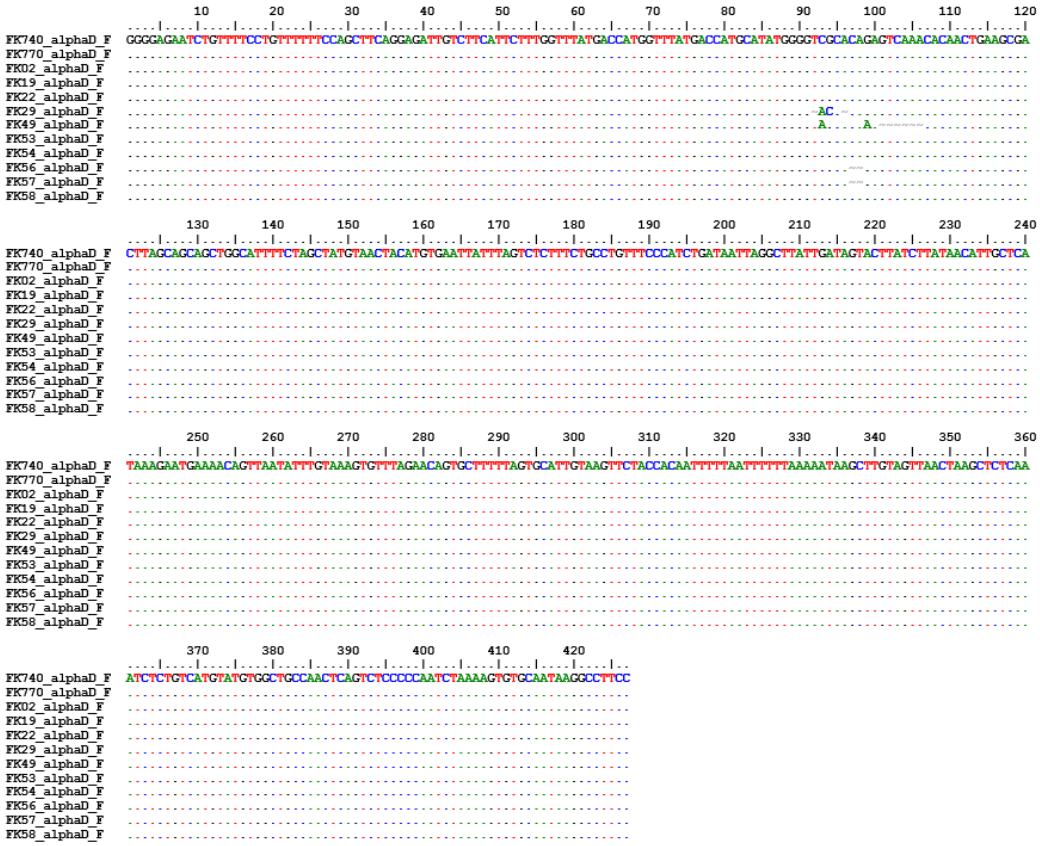
My deepest heartfelt gratitude goes to my family, whose love, unwavering support and continuous encouragement have been my foundation throughout this journey. Their belief in me, even from afar, has given me the strength to persevere. To my friends back home in Italy, thank you for your constant support and for always being there, even across the distance.

I am equally grateful to the new friends I have made far from home, here in Sweden, who made this experience not only possible but truly memorable. Your kindness, company, and shared moments made the long days of research and writing much lighter, and for that, I am deeply thankful.

This accomplishment would not have been possible without all of you. Thank you.

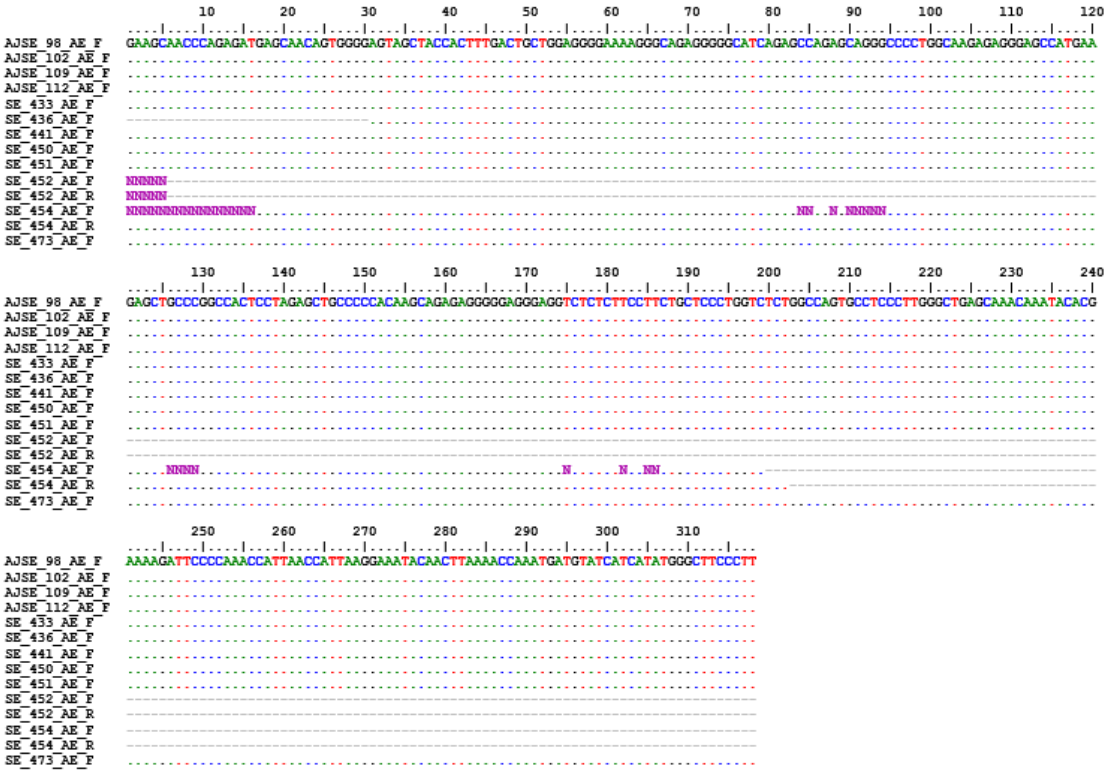
Appendix 1


10 20 30 40 50 60 70 80 90 100 110 120
FK_740 GAAGCAACCCAGAGATGAGCAACAGTGGGGAGTAGCTACCACTTTGACTGCTGGAGGGGAAAAGGGCAGAGGGGCATCAGAGCCAGAGCAGGGCCCCTGGCAAGAGAGGGAGCCATGAA
FK_770
FK002
FK019
FK022
FK029
FK049
FK053
FK054
FK056
FK057
FK058
FK059
FK063
FK095
130 140 150 160 170 180 190 200 210 220 230 240
FK_740 GAGCTGCCCGGCCACTCCTAGAGCTGCCCCCACAAGCAGAGAGGGGGAGGGAGGTCTCTCTTCCTTCTGCTCCCTGGTCTCTGGCCAGTGCCTCCCTTGGGCTGAGCAAACAAATACACG
FK_770
FK002
FK019
FK022
FK029
FK049
FK053
FK054
FK056
FK057
FK058
FK059
FK063
FK095
250 260 270 280 290 300 310
FK_740 AAAAGATTCCCCAAACCATTAACCATTAAGGAAATACAACTTAAAACCAAATGATGTATCATCATATGGGCTTCCCTT
FK_770
FK002
FK019
FK022
FK029
FK049
FK053
FK054
FK056
FK057
FK058
FK059
FK063
FK095


FK740_alphaD_F
FK770_alphaD_F
FK02_alphaD_F
FK19_alphaD_F
FK22_alphaD_F
FK29_alphaD_F
FK49_alphaD_F
FK53_alphaD_F
FK54_alphaD_F
FK56_alphaD_F
FK57_alphaD_F
FK58_alphaD_F


10 20 30 40 50 60 70 80 90 100 110 120 130 140
FK740_Cbeta_F TCAACGAGGGACAACATGAAAAGTGAAGCTGCCTACTATTGGCTTGTATCAAGGTCCAGCACTAAGAATGTGGACTCTAAGGCAAGACTGCCTGAATTTGACTGTGGCGCTGGCATTTTCTGGAGAAGGCAATGGCAACT
FK770_Cbeta_F
FK02_Cbeta_F
FK19_Cbeta_F
FK22_Cbeta_F
FK29_Cbeta_F
FK49_Cbeta_F
FK53_Cbeta_F
FK54_Cbeta_F
FK56_Cbeta_F
FK57_Cbeta_F
FK58_Cbeta_F
150 160 170 180 190 200 210 220 230 240 250 260 270 280
FK740_Cbeta_F CACTCCAGTGCTCTTGCCTGGTGAATCCCATGGACAGAGGAGCCTGATAGGCKGCAGTCCATGGCGTCGCTAGGAGTCAGACACGACKGAGSGACTTCACTTTCACTTTTCATTTYCATGCATTGGAGAAGGAAATGGCA
FK770_Cbeta_F
FK02_Cbeta_F
FK19_Cbeta_F
FK22_Cbeta_F
FK29_Cbeta_F
FK49_Cbeta_F
FK53_Cbeta_F
FK54_Cbeta_F
FK56_Cbeta_F
FK57_Cbeta_F
FK58_Cbeta_F
290 300 310 320 330 340 350 360 370 380 390 400 410 420
FK740_Cbeta_F ACCCACTCCAGKGTTCTTGCCTGGAGAATCCCAGGGACGGGGGAGCCTGGTGGGYTGCWTCACTCCATGCATCACTCCAACCTCAGCTTCCATTGTCACATCTCCTTTTCTAACACTGACCCTATTGGTTAAAAGATTCT
FK770_Cbeta_F
FK02_Cbeta_F
FK19_Cbeta_F
FK22_Cbeta_F
FK29_Cbeta_F
FK49_Cbeta_F
FK53_Cbeta_F
FK54_Cbeta_F
FK56_Cbeta_F
FK57_Cbeta_F
FK58_Cbeta_F
430 440 450 460 470 480 490 500 510 520 530 540 550 560
FK740_Cbeta_F TGTGATTAGATTGAATCCTTCTGAATAATATTCTATCTGAATAAATCCTCCTCTTCTCAAGATCATTAACTTATCACATCTGCAAAACCCTTTTGGCCATGTAAACTAAWATATTCCMAGGTTACAAGRATTAGAAATGG
FK770_Cbeta_F
FK02_Cbeta_F
FK19_Cbeta_F
FK22_Cbeta_F
FK29_Cbeta_F
FK49_Cbeta_F
FK53_Cbeta_F
FK54_Cbeta_F
FK56_Cbeta_F
FK57_Cbeta_F
FK58_Cbeta_F
570 580 590 600
FK740_Cbeta_F ACATTTTGTGGAGAGCATTATTTTGCCTACCAAAATGAGGGGTCAATTG
FK770_Cbeta_F
FK02_Cbeta_F
FK19_Cbeta_F
FK22_Cbeta_F
FK29_Cbeta_F
FK49_Cbeta_F
FK53_Cbeta_F
FK54_Cbeta_F
FK56_Cbeta_F
FK57_Cbeta_F
FK58_Cbeta_F

Appendix 2

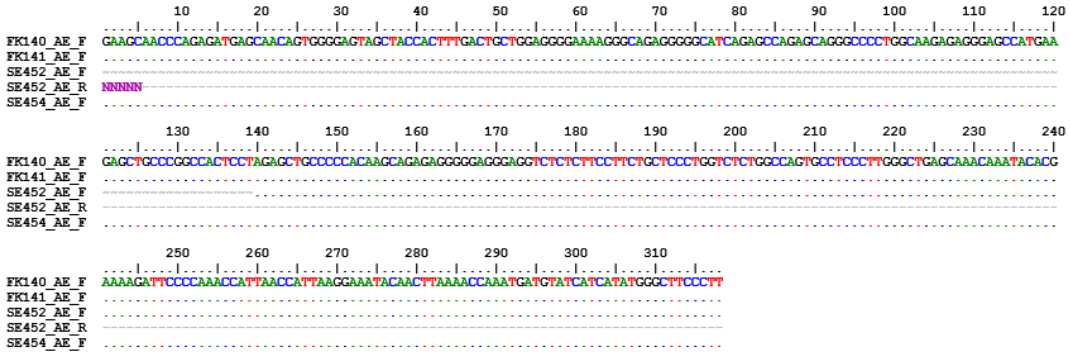

10 20 30 40 50 60 70 80 90 100 110 120
AJSE_98_AE_F GAAGCAACCCAGAGATGAGCAACAGTGGGGAGTAGCTACCACTTTGACTGCTGGAGGGGAAAAGGGCAGAGGGGGCATCAGAGCCAGAGCAGGGCCCCTGGCAAGAGAGGGAGCCATGAA
AJSE_102_AE_F
AJSE_109_AE_F
AJSE_112_AE_F
SE_433_AE_F
SE_436_AE_F
SE_441_AE_F
SE_450_AE_F
SE_451_AE_F
SE_452_AE_F NNNNN
SE_452_AE_R NNNNN
SE_454_AE_F NNNNNNNNNNNNNNNNN NN..N.NNNNN
SE_454_AE_R
SE_473_AE_F
130 140 150 160 170 180 190 200 210 220 230 240
AJSE_98_AE_F GAGCTGCCCGGCCACTCCTAGAGCTGCCCCCACAAGCAGAGAGGGGGAGGGAGGTCTCTCTTCCTTCTGCTCCCTGGTCTCTGGCCAGTGCCTCCCTTGGGCTGAGCAAACAAATACACG
AJSE_102_AE_F
AJSE_109_AE_F
AJSE_112_AE_F
SE_433_AE_F
SE_436_AE_F
SE_441_AE_F
SE_450_AE_F
SE_451_AE_F
SE_452_AE_F
SE_452_AE_R
SE_454_AE_F NNNN N N NN
SE_454_AE_R
SE_473_AE_F
250 260 270 280 290 300 310
AJSE_98_AE_F AAAAGATTCCCCAAACCATTAACCATTAAGGAAATACAACTTAAAACCAAATGATGTATCATCATATGGGCTTCCCTT
AJSE_102_AE_F
AJSE_109_AE_F
AJSE_112_AE_F
SE_433_AE_F
SE_436_AE_F
SE_441_AE_F
SE_450_AE_F
SE_451_AE_F
SE_452_AE_F
SE_452_AE_R
SE_454_AE_F
SE_454_AE_R
SE_473_AE_F

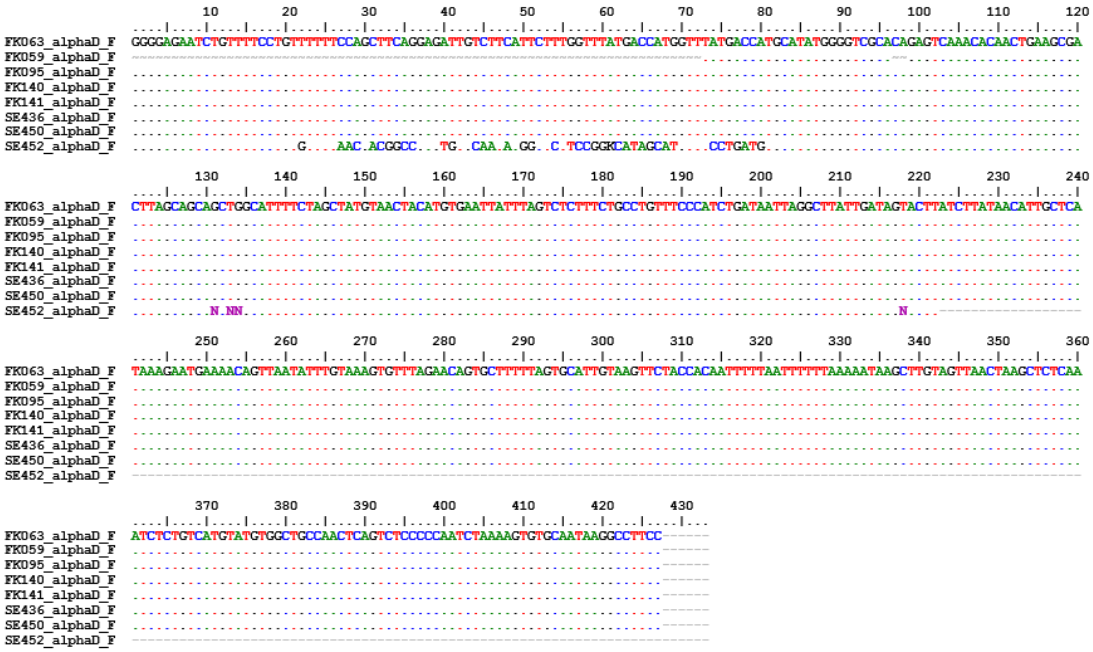

AJSE_112_alphaD_F
AJSE_98_alphaD_F
AJSE_102_alphaD_F
AJSE_109_alphaD_F
SE_433_alphaD_F
SE_436_alphaD_F
SE_436_alphaD_R
SE_441_alphaD_F
SE_450_alphaD_F
SE_451_alphaD_F
SE_452_alphaD_F
SE_452_alphaD_R
SE_454_alphaD_F
SE_473_alphaD_F


AJSE_98_Cbeta_F
AJSE_102_Cbeta_F
AJSE_109_Cbeta_F
AJSE_112_Cbeta_F
SE_433_Cbeta_F
SE_436_Cbeta_F
SE_436_Cbeta_R
SE_441_Cbeta_F
SE_450_Cbeta_F
SE_450_Cbeta_R
SE_451_Cbeta_F
SE_452_Cbeta_F
SE_452_Cbeta_R
SE_454_Cbeta_F
SE_473_Cbeta_F

Appendix 3

Ulterior alignments of samples FK140 AND FK 141 with the samples that resulted of poor quality to exclude the hypothesis of genotype-specific amplification failure.






FK059_Cbeta_F
FK063_Cbeta_F
FK140_Cbeta_F
FK141_Cbeta_F
FK095_Cbeta_F
FK095_Cbeta_R
SE436_Cbeta_F
SE436_Cbeta_R
SE450_Cbeta_F
SE452_Cbeta_F
SE452_Cbeta_R

Publishing and archiving

Approved students' theses at SLU can be published online. As a student you own the copyright to your work and in such cases, you need to approve the publication. In connection with your approval of publication, SLU will process your personal data (name) to make the work searchable on the internet. You can revoke your consent at any time by contacting the library.

Even if you choose not to publish the work or if you revoke your approval, the thesis will be archived digitally according to archive legislation.

You will find links to SLU's publication agreement and SLU's processing of personal data and your rights on this page:

- https://libanswers.slu.se/en/faq/228318

☒ YES, I, Michela Martina Sozzi, have read and agree to the agreement for publication and the personal data processing that takes place in connection with this

☐ NO, I/we do not give my/our permission to publish the full text of this work. However, the work will be uploaded for archiving and the metadata and summary will be visible and searchable.