P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.119

Efficiency of Diagnostic Methods for Hypotrichosis, Dilutor and Osteopetrosis in Local Cattle Breeds of the Republic of Kazakhstan

Nursaule Dyussekenova [1], Talgat Karymsakov [2], Pernebek Sailaubek [2], Makpal Junussova [3], Damir Khussainov [1,*], Zhuldyzay Kenzhebekova [1], Zhadyra Muslimova [1], Asset Turgumbekov [1], Raikhan Junussova [1] and Yessengali Ussenbekov

[1]Kazakh National Agrarian Research University, Almaty, Kazakhstan
[2]Kazakh Research Institute of Animal Husbandry and Forage Production, Almaty, Kazakhstan
[3]Asfendiyarov Kazakh National Medical University, Almaty, Kazakhstan
***Corresponding author:** dkhussainov@mymail.academy

Article History: 25-221 Received: 07-Aug-25 Revised: 19-Sep-25 Accepted: 28-Sep-25 Online First: 16-Oct-25

ABSTRACT

This study aimed to optimize tools for identifying carriers of inherited defects i.e., Hypotrichosis molecular-genetic diagnostic, molecular-genetic diagnostic tools Dilutor, and Osteopetrosis, in local cattle breeds of Kazakhstan. A special focus was given to the development and implementation of a Real-Time PCR method for detecting the Dilutor mutation. Genetic material from 346 cattle of various breeds, including Kazakh White headed, Kalmyk, Auliekol, Hereford, and Santa Gertrudis, was analyzed. TETRA-PRIMER ARMS-PCR and conventional PCR were used to detect Hypotrichosis, while both PCR-RFLP and Real-Time PCR assays were applied to screen for the Dilutor mutation in the SILV gene. The PMEL gene variant p.Ala612Glu, associated with coat color dilution, was assessed using PCR-RFLP. Screening for the Osteopetrosis-associated deletion in the SLC4A2 gene was also performed. A high diagnostic sensitivity was observed for Hypotrichosis detection, with carrier prevalence reaching 6.9% among Hereford cattle and 11.2% among breeding bulls in a major selection center. No heterozygous carriers of the Dilutor or Osteopetrosis mutations were identified. Real-time PCR was demonstrated to be a rapid and scalable method, enabling the genotyping of 96 DNA samples within 2.5 hours. Notably, heterozygosity at the PMEL locus was highest in the Kalmyk breed (25.6%), suggesting a possible link to coat color lightening. The applied molecular tools proved efficient for genetic monitoring in cattle populations. Periodic screening is recommended to prevent the spread of deleterious mutations, particularly in breeding herds.

Key words: KRT71 gene; SILV locus; PMEL mutation; SLC4A2 deletion; PCR-RFLP; TETRA-PRIMER ARMS-PCR

INTRODUCTION

According to the OMIA (Online Mendelian Inheritance in Animals) database, more than 1,800 hereditary disorders have been identified in livestock (OMIA 2025). These conditions often lead to reduced viability of offspring, congenital malformations, or embryonic mortality, causing notable economic losses to animal production. In cattle, over 690 genetic anomalies have been reported, and their number continues to grow, particularly among breeding animals. This trend is largely driven by intense selection and inbreeding, which increase the likelihood of recessive mutations being passed on (Khan et al. 2021; Makamu and Tyasi 2024).

While diagnostic methods for inherited conditions are well developed in dairy cattle breeds such as Holstein, Simmental, Jersey, and Ayrshire, beef breeds often remain under-monitored (Mahmood et al. 2022). In many cases, the lack of routine screening is linked to limited access to reliable molecular diagnostic tools, despite the successful application of PCR-based assays for detecting infectious and genetic conditions in cattle. (Avci et al. 2023). Nevertheless, a number of inherited disorders have been described in beef cattle populations globally (Ayuti et al. 2025). These include hypotrichosis (HY), dilutor (DL), idiopathic epilepsy (IE), maple syrup urine disease (MSUD), mandibulofacial dysostosis (MD), delayed blindness (DB), developmental duplication (DD), arthrogryposis multiplex (AM), osteopetrosis (OS), double muscling (M1) and arachnomelia syndrome (AS).

Cite This Article as: Dyussekenova N, Karymsakov T, Sailaubek P, Junussova M, Khussainov D, Kenzhebekova Z, Muslimova Z, Turgumbekov A, Junussova R and Ussenbekov Y, 2026. Efficiency of diagnostic methods for hypotrichosis, dilutor, and osteopetrosis in local cattle breeds of the Republic of Kazakhstan. International Journal of Veterinary Science 15(1): 143-152. https://doi.org/10.47278/journal.ijvs/2025.119

Most of these disorders are linked to specific point mutations, insertions, or deletions within coding regions of key genes (Konovalova et al. 2020).

One of the better-studied conditions is hypotrichosis in Hereford cattle, caused by an eight-base-pair deletion (c.334delTGTGCCCA) in the KRT71 gene. Affected calves are born with significantly reduced hair cover. Clinical reports describe a distinct phenotype: sparse, thin, curly or fuzzy hair, especially on the limbs. Histological analysis typically reveals alterations in the morphology of the inner root sheath of hair follicles, consistent with a loss-of-function mutation (Kuca et al. 2021).

Additional studies have shown that affected Hereford calves are often born partially or completely hairless, with a coat that later becomes curly or woolly. Pedigree analysis supports an autosomal recessive mode of inheritance (Markey et al. 2010; Jacinto et al. 2021). In one case reported in Switzerland, a calf presented with multiple alopecic patches on the legs, head and back. The animal was found to be homozygous for the deleterious variant in KRT71, linking the genotype to the observed phenotype (Romero-Benavente et al. 2023). To detect this mutation, PCR amplification was carried out using the following primers: HY_F: 5′-CGGAAGTCGGAGCCTTTACA-3′, HY_RN: 5′-ACGCACTTTCTGGATCTCGG-3′, HY_RM: 5′-CCAGGTCAGTTGGGCACAT-3′, with an annealing temperature of 65 °C (Markey et al. 2010).

A more comprehensive clinical study combined genotyping with histopathology and trichography. Skin, hair, and blood samples were collected from affected calves, their dams, and a Hereford sire. Results showed that the affected calves were homozygous for the KRT71 mutation, while the parents and one control animal were heterozygous carriers. These findings confirm that congenital hypotrichosis in Hereford cattle results from an eight-nucleotide deletion in KRT71, manifesting as follicular dysplasia (Romero-Benavente et al. 2023).

Russian researchers developed and applied DNA test systems based on allele-specific PCR (AS-PCR) and PCR-RFLP techniques to genotype four beef cattle breeds: Aberdeen Angus (n=701), Hereford (n=385), Simmental (n=286) and Belgian Blue (n=118). In Aberdeen Angus cattle, genotyping revealed that 2.38±0.31% of cows and 1.67±0.19% of bulls carried the AMC variant, while 0.65±0.07% of cows and 0.90±0.10% of bulls were identified as heterozygous for the DDC mutation. Among Hereford cattle, single individuals were found to carry mutations associated with MSUD and CWH (0.27±0.05%), as well as ICM and HY (0.16±0.03%). In Belgian Blue cattle, carriers of M1 and CMD1 were detected at a frequency of 0.84% (Konovalova et al. 2021). The prevalence of heterozygous carriers of the Hypotrichosis mutation in Hereford and Angus cattle was 4.16 and 1.66%, respectively (Dyussekenova et al. 2024).

Separate studies have described a case of epilepsy in a six-month-old Holstein calf, characterized by focal seizures progressing to generalized episodes. The seizures began with a short pre-ictal phase marked by depression and vocalization. During the ictal phase, the calf exhibited eyelid twitching, tongue contractions, nodding and excessive salivation, followed rapidly by convulsions involving bilateral tonic, clonic, or tonic-clonic activity with loss of consciousness. In the post-ictal phase, the calf was dull and disoriented, showed impaired obstacle perception, exaggerated movements, and a tendency to press its head against objects. Between seizures, the calf appeared clinically normal. Neuropathological examination revealed axonal degeneration in the brainstem and diffuse astrocytic hypertrophic gliosis. Whole-genome sequencing of the affected animal identified a heterozygous splice-site variant in the DYRK1B gene (NM_001081515.1: c.-101-1G>A), likely resulting in haploinsufficiency due to loss of function. These findings strongly suggest that the observed condition was associated with DYRK1B, positioning this gene as a likely candidate for inherited epilepsy in cattle (Jacinto et al. 2023).

Currently, the hereditary condition Idiopathic Epilepsy (IE) (Trait#232) in cattle is identified using molecular genetic diagnostic tools, with genotyping results typically classifying animals into three groups: IEF (free of mutation), IEC (carrier), and IEA (affected), as reported by Neogen's livestock genetic trait database (Neogen 2024a). However, available literature does not provide detailed insights into the genetic basis of this disorder, which presents challenges for developing robust diagnostic assays for IE.

In contrast, international researchers have successfully implemented molecular diagnostics to detect Dilutor mutation carriers in Hereford cattle, also documented by Neogen (Neogen 2024b). According to the Veterinary Genetics Laboratory at UC Davis, this condition is also referred to as Color Dilution (UC Davis VGL 2024). Genotyping results are labeled as DLF (free of the mutation), DLC (carrier) and DLA (infected).

Review of the literature reveals two known mechanisms responsible for the Color Dilution phenotype in cattle breeds such as Charolais, Simmental, Galloway, and Hereford. The first mechanism involves a point mutation in the PMEL17 gene, also known as SILV. In 2007, a single-nucleotide polymorphism (c.64G>A) was identified in Charolais cattle, resulting in a Gly22Arg amino acid substitution. This mutation, referred to as the Dc allele, is associated with a dilution phenotype that gives Charolais cattle their characteristic pale or white coat color (Gutiérrez-Gil et al. 2007).

A second mutation involves a three-base-pair deletion (delTTC) in exon 1 of PMEL17, which causes the loss of a leucine residue in the protein. This Dh variant has been associated with diluted coat color phenotypes in Highland, Simmental, Galloway, and Hereford breeds (Hecht 2006; Gutiérrez-Gil et al. 2007). It is believed to alter PMEL protein function and results in coat lightening, particularly of red and black pigmented hairs (Hartati et al. 2024). The degree of color dilution depends on both the number of Dh alleles (one or two copies) and the animal's MC1R gene status, which regulates red versus black pigment expression (Wang et al. 2023; Chen et al. 2024).

A recent study confirmed that coat color dilution in Kumamoto sub-breed Japanese Brown cattle is caused by a deletion in the PMEL gene (p.Leu18del). Interestingly, this causative allele has also been identified in genetically distant breeds, suggesting that PMEL could serve as a practical DNA marker for monitoring and managing coat color traits in cattle populations (Jolly et al. 2008; Kimura et al. 2022).

The influence of genetic variants in the TNFα gene on reproductive traits in cattle has also been documented. For

example, Holstein cows in Kazakhstan carrying the GG homozygous genotype demonstrated superior reproductive performance compared to animals with other genotypes (Bimenova et al. 2019).

In a large-scale screening of Aberdeen Angus cattle in Russia (n=4,480), the frequency of carriers for several hereditary disorders was reported as follows: 0.19±0.09% for M1, 0.53±0.03% for osteopetrosis (OS), 1.92±0.09% for arthrogryposis multiplex (AM), and 9.00±0.20% for developmental duplication (DD) (Konovalova et al. 2021). Detection of OS carriers was carried out using specific primers: OSF (5'-AGCCCCTACAGTCACAGTCA-3'), OSFn (5'-AGCAGCAGAGATCAGCTTGG-3') and OSFm (5'-CCGACCCCCTCACATTCAAA-3') with an annealing temperature of 60°C (Konovalova et al. 2020; Konovalova et al. 2021).

Further investigation of the SLC4A2 gene in both affected and healthy calves revealed a ~2.8 kb deletion in homozygous animals, encompassing exon 2 and nearly half of exon 3. This deletion disrupts the gene's function and impairs the formation of the SLC4A2 protein. RNA analysis from a confirmed heterozygous animal showed transcripts lacking exons 2 and 3. Genotyping of additional animals revealed complete concordance between the homozygous deletion and the osteopetrosis phenotype. For molecular diagnosis, a forward primer (5'-GGGAAGGGAAGCACTAAGACT-3') was paired with two reverse primers: one within the deleted segment (5'-TGGAGAGACAGCAGCAGAGAT-3'), producing a 475bp amplicon for the wild-type allele, and another spanning the deletion breakpoint (5'-GGTGGATGTGATGGGAAGACT-3'), yielding a 330bp product specific to the mutant allele (Meyers et al. 2010).

Recent genetic monitoring conducted in Kazakhstan showed that Arachnomelia syndrome was present in 4.1% of Angus and 2.5% of Hereford cattle. The prevalence of Developmental Duplication (DD) was 1.66% in Angus, while Arthrogryposis multiplex (AM) was found in 5% of Angus and 1.66% of Hereford cattle. Importantly, the Kazakh Whiteheaded breed showed no evidence of carrier status for these disorders. Real-Time PCR protocols have been developed and are actively used in Kazakhstan to identify DD carriers and bulls with subfertility syndrome (Ussenbekov et al. 2024a; Ussenbekov et al. 2025; Ussenbekov et al. 2025).

Kazakh researchers have also developed and optimized molecular diagnostic techniques primarily for Holstein and Jersey cattle. Genetic monitoring in these populations revealed the following carrier frequencies: HCD – 11.8%, HH1 – 3.2%, HH5 – 5.0%, BY – 6.2% and JH1 – 2.4% (Shormanova et al. 2023; Shormanova et al. 2024; Ussenbekov et al. 2024b; Ussenbekov et al. 2024c).

The main objective of the present study was to optimize existing PCR and PCR-RFLP assays for detecting carriers of three hereditary conditions—Hypotrichosis (HY), Dilutor (DL), and Osteopetrosis (OS)—and to develop a Real-Time PCR protocol for the identification of Dilutor mutation carriers in Santa Gertrudis, Kazakh Whiteheaded, Kalmyk, and Auliekol cattle.

MATERIALS AND METHODS

In our own work, a 279bp fragment of the KRT71 gene was successfully amplified using primers F: 5′-CAGTGGGAAGAGTGGAGGTT-3′ and R: 5′-CAATCCCTCTTGCTGCAACA-3′, as previously reported by Lirong et al. (2022).

The study material consisted of frozen blood samples and hair follicles collected from cows, breeding bulls, and young cattle of the Kazakh White headed, Kalmyk, Auliekol, Hereford, and Santa Gertrudis breeds. Samples were obtained from smallholder farms located in the Almaty, Zhetysu, and Kostanay regions of Kazakhstan.

Blood for DNA extraction was collected from the jugular vein (or the tail vein in some cases) in volumes of 2mL, using EDTA-coated vacuum tubes. Genomic DNA was extracted at the "Green Biotechnology and Cell Engineering" laboratory of the Kazakhstan-Japan Innovation Center at the Kazakh National Agrarian Research University. Two methods were applied: the classical phenol-chloroform extraction technique and the PureLink™ Genomic DNA Mini Kit, following the manufacturer's protocol.

Additionally, 37 cryopreserved semen samples from breeding bulls of Breeding Center No. 1 were used in the study. DNA extraction from semen was performed using a commercial DNA isolation kit, in accordance with the manufacturer's instructions.

The genetic defect Hypotrichosis (HY) was detected using the TETRA-PRIMER ARMS-PCR method, applying both outer and inner primers (Table 1).

Table 1: Primer sequences for genotyping beef cattle for genetic defects — Hypotrichosis (HY), Dilutor (DL) c.64G>A in exon I of the SILV gene, PMEL p.Leu18del, and Osteopetrosis (OS)

Gene / Diagnostic Method	Primer Sequences (5'→3')	Authors
KRT71, TETRA-PRIMER ARMS-PCR	FO- 5' ACCTGCAAGTCGGGAGCTGCTGCCAAGG-3', RO- 5' CAAGGGCTGTGTGCAGGTCCCCAGGTCC- 3', FI- 5' GTTTGGCAGCGTGGCCCTGGGGCCTAT -3', RI- 5' CTCCAGGTGGGCACACAGTTGGGCGCT -3'	Dyussekenova et al. 2024
KRT71, PCR	F - 5' – CGGAAGTCGGAGCCTTTACA-3' RN - 5' - ACGCACTTTCTGGATCTCGG-3' RM - 5' - CCAGGTCAGTTGGGCACAT-3'	Markey et al. 2010
SILV, c.64G>A, PCR-RFLP	F – 5' ACTGTCAATGAGTAGCAGGATGTC-3' R – 5' TGCACCCAAATCTTCATGTG -3'	Gutiérrez-Gil et al. 2007
PMEL, p.Ala612Glu, PCR-RFLP	F: AGCCAGGATCAAGACCAAG -3' R- GATAGCTGTTAAGTAAGTGG -3'	Kimura et al. 2022
SLC4A2, PCR	F – 5'-GGGAAGGGAAGCACTAAGACT-3' RM – 5'-TGGAGAGACAGCAGCAGAGAT-3' RN – GGTGGATGTGATGGGAAGACT-3'	Meyers et al. 2010

An alternative PCR approach was also used to identify carriers of the HY mutation. This method employed a common forward primer (HY_F: 5'-CGGAAGTCGGAGCCTTTACA-3') and two reverse primers specific to the wild-type (RN: 5'-ACGCACTTTCTGGATCTCGG-3') and mutant alleles (RM: 5'-CCAGGTCAGTTGGGCACAT-3'). The amplified fragment for homozygous wild-type genotypes measured 259bp (Table 1).

The amplified KRT71 gene fragment in healthy homozygous individuals was confirmed by sequencing and matched the following consensus sequence:

CGGAAGTCGGAGCCTTTACAACCTGGGCGGC
GTCCGGAGCATCTCCTTCAATGTGGCCAGCGGCA
GTGGGAAGAGTGGAGGTTATGGATTTGGCCGGG
GCCGGGCCAGTGGTTTCGCCGGCAGCATGTTTGG
CAGCGTGGCCCTGGGGCCCATGTGCCCAACTGTG
TGCCCACCTGGAGGCATCCACCAGGTCACTGTCA
ATGAGAGCCTCCTGGCCCCCCTCAACGTGGAGCT
GGACCCCGAGATCCAGAAAGTGCGT

For genotyping the c.64A>G mutation in exon 1 of the SILV gene (also known as PMEL), the following primers were used: forward primer: 5'-ACTGTCAATGAGTAGCAGGATGTC-3' and reverse primer: 5'-TGCACCCAAATCTTCATGTG-3', resulting in a 434bp PCR product (Table 1). Restriction analysis was performed using the SfcI endonuclease, which recognizes the sequence C/TRYAG (where R = A or G, and Y = C or T). Depending on the genotype, digestion of the amplified product with SfcI generated the following fragments: 434bp (undigested), 244bp, and 190bp. The recognized restriction sites included SfcI: CTACAG, CTGCAG, CTATAG, and CTGTAG.

A fragment of the SILV gene (also known as *PMEL*) located on chromosome 5 was analyzed. The amplified region contains a recognition site for the restriction enzyme SfcI. The target sequence is as follows:

ACTGTCAATGAGTAGCAGGATGTCCAGGGAC
CAGGATCCCTACTCAGTTCTTTCTCAGGTCCTTCT
GCTCCTCCCAGGATTTGCCTGGAGGGAGGGGAGG
AAGGGCTATGCAAATAAGCACCCCCTTCTTATAC
AGGGGTTTGTCCAATGCCCCAGAGTCTTTGGTTG
CTGGAAGGAAGAACAGGATGGATCTGGTGCTGA
GAAAATACCTTCTCCATGTGGCTCTGATGGGTGT
TCTTCTGGCTGTAGGGACCACAGAAGGTGAGTGT
GGGATGTTGGACATGAACAAGTGTGAATTTGGGG
TTGCACACCTGCTCTGGTTTTTCTCTCCCTAAAAT
GGAAGATATCAGTAGTGCTTCAGGTGTCTCCCAC
CCATTTGATTTAGTGAGGACATGGGCAACTGAGC
TCCCTCCCCACATGAAGATTTGGGTGCA

To detect the p.Ala612Glu mutation within the PMEL gene, primers with the sequences 5'-AGCCAGGATCAAGACCAAG-3' (forward) and 5'-GATAGCTGTTAAGTAAGTGG-3' (reverse) were used, amplifying a 463bp fragment as indicated in Table 1.

For allele discrimination, the BlpI restriction enzyme, which recognizes the sequence G/CTNAGC, where N can be any nucleotide (A, G, C, or T), was used. After digestion with BlpI, different fragment patterns were observed depending on the genotype: a 463bp fragment remained undigested in homozygous wild-type samples, while heterozygous or mutant samples showed digested fragments of 319bp and 144bp.

The PMEL gene fragment analyzed in this study is located on chromosome 5, and the precise position of the point mutation is within the BlpI restriction site (highlighted in red in the original sequence). The target region, including the recognition sequence and surrounding nucleotides, is presented below (ambiguous nucleotides denoted as N):

AGCCAGGATCAAGACCAAGTCAACCTGGGTT
ATGGTTTAGTCTTTTTTTTTTTTAGAGAAGCACA
AAGAGGTTGCCATTGACCACCACTAACCAGTATC
CCTGCTTTTCTCCCAATATCAGGCGAAGACTTAT
GAAGCAAGGCTCAGCAGTCCCCCTTCCCCAGCTG
CCACACGGTAGAACCCAGTGGCTACGTCTGCCCT
GGGTCTTCCGCTCTTGCCCCATTGGTGAGAGCAA
ACCCCTCCTCAGTGGACAGCAGGTCTGAGTGCTC
TTATGTGAAGTCATGATTTACCCAGGTGGAGAGC
AAGGCCTGTCTTTTCTCTGGTCTTCCCTCAGAGAC
TACCATTGCCTGAAATAAAGACTCAGAACTTGAN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNN

PCR diagnosis of carriers of the genetic defect Osteopetrosis was carried out using primers listed in Table 1. To detect carriers of the c.64 G>A mutation in exon 1 of the PMEL gene, an alternative approach was employed: Real-Time PCR diagnostics using the following primers — external forward FO: 5' GTGGCTCTGATGGGTGTTCTT 3', external reverse RO: 5' AACATCCCACACTCACCTTCTG 3', internal forward FI: 5' CTGGCTGTAGGGACCA 3', and internal reverse RI: 5' CTGGCTGTAAGGACCA 3'. The thermal cycling conditions included an initial denaturation at 95°C for 10min, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 1min, and elongation at 60°C for 30 seconds.

RESULTS

To develop molecular genetic methods for diagnosing hereditary defects, it is essential to have information on the genetic nature of these abnormalities and the localization of point mutations or deletions, as summarized in Table 3. Detection of heterozygous carriers of an 8-nucleotide deletion (g.27331221delTGTGCCCA) in the KRT71 gene was carried out using two approaches: the TETRA-PRIMER ARMS-PCR method and conventional polymerase chain reaction. In the TETRA-PRIMER ARMS-PCR assay, homozygous wild-type individuals produced two bands of 472bp and 234bp on the electropherogram, whereas heterozygous carriers showed three bands corresponding to 472bp, 292bp, and 234bp (Fig. 1). A key advantage of this method is that it eliminates the need for restriction enzyme digestion to distinguish between wild-type and mutant KRT71 alleles. A simpler, faster, and more accurate method for identifying carriers of Hypotrichosis involves PCR using a common forward primer (F: 5'-CGGAAGTCGGAGCCTTTACA-3') and two reverse primers — RN (5'-ACGCACTTTCTGGATCTCGG-3') for the wild-type allele and RM (5'-CCAGGTCAGTTGGGCACAT-3') for the mutant allele. In this assay, heterozygous animals displayed two fragments of 259bp and 159bp, while homozygous wild-type animals showed a single fragment of 259bp (Fig. 2).

Table 2: PCR conditions for genotyping DNA samples of cattle at KRT71, SILV, PMEL, and SLC4A2 gene loci

Amplification Conditions	KRT71, PRIMER ARMS-PCR	KRT71 (PCR)	SILV, c.64G>A	PMEL, p.Ala612Glu	SLC4A2
Initial denaturation	95℃ - 5min	95^0C - 5min	95^0C - 3min	95^0C - 5min	95^0C-5min
Denaturation Annealing	94℃ 30s	95℃ 30s	94℃ 45s	94℃ 30s	94℃ 45s
Elongation	64℃ 30s	60℃ 30s	60℃ 30s	60℃ 30s	63℃ 45s
	72℃ 30s	72℃ 30s	72℃ 40 s	72℃ 30s	72℃ 45s
Number of cycles	35	35	35	35	31
Final extension	72℃ 5min	72℃ 5min	72℃ 4min	72℃ 5min	72℃ 5min

Table 3: Genetic defects, their molecular basis, and mutation/deletion locations

Characteristics	Name of genetic defects			
	Hypotrichosis (HY)	Dilutor (DL) c.64A>G SILV	Dilutor (DL) PMEL p.Ala612Glu	Osteopetrosis (OS)
Gene	KRT71	SILV	PMEL	SLC4A2
Chromosome location	5	5	5	4
Gene length (bp)	8362	8813	8813	
Mutation type	8bp deletion (g.27331221delTGTGCCCA)	Point mutation (c.64G>A, exon I)	SNP (p.Ala612Glu)	2781bp deletion
Mutation localization (fragment)	*gtttggcagcgtggccctggggcc*catgt gcccaactgtgtgcccacctggag	atgggtgttcttctggct [g→a]tagggaccacaga	cttatgaagcaaggctcagcagtcccc cttcccc	
Diagnostic method	TETRA-PRIMER ARMS-PCR	PCR-RFLP	PCR-RFLP	PCR
Restriction enzyme used	None	SfcI (site: C/TRYAG, CTGTAG)	BlpI (site: G/CTNAGC)	None
Fragment patterns	472bp (outer), 234bp (T allele), 292bp (A allele)	434bp PCR product; after digestion: 244bp, 190bp	463bp; after digestion: 319bp, 144bp	475bp (wild-type), 330bp (mutant)

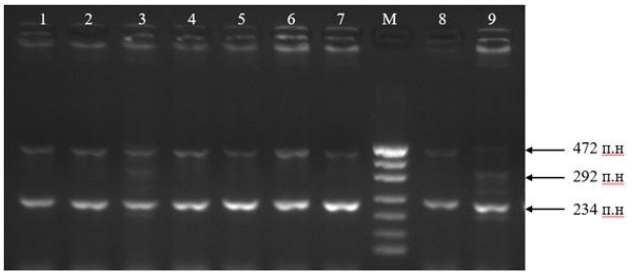

Fig. 1: Electropherogram of the KRT71 gene amplicon obtained using tetra-primers, 3% agarose gel. Lanes 3 and 9 represent the positive control (heterozygous carrier, fragments of 472bp, 292bp, and 234bp). Lanes 1–2, 4–7, and 8 correspond to homozygous wild-type individuals, showing fragments of 472bp and 234bp. M – DNA marker pUC19/MspI.

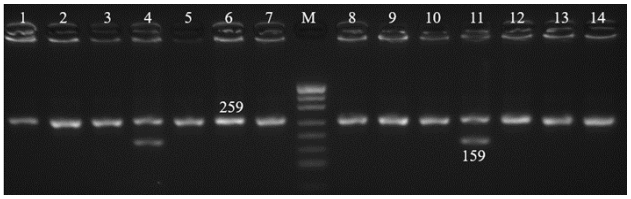

Fig. 2: Electropherogram of the KRT71 gene amplicon, 4% agarose gel. Lanes 1–3, 5–7, 8–10, and 12–14 show the homozygous genotype (259bp). Lanes 4 and 11 display the heterozygous genotype (259bp and 159bp). M – DNA marker pUC19/MspI.

Currently, two point mutations have been identified that are associated with the Dilutor (DL) genetic defect in beef cattle: one in the SILV gene (c.64A>G) and another in the PMEL gene (p.Ala612Glu). It is important to note that sequence analysis of the SILV and PMEL genes from the NCBI database suggests that these are the same gene, referred to under different names. The primers and PCR conditions used for detection are presented in Table 1 and 2. As shown in Fig. 3, PCR amplification of the SILV gene produced a 434bp fragment. In this case, allele identification was performed using the restriction enzyme SfcI, and all analyzed samples were found to be homozygous wild-type, yielding two bands of 244bp and 190bp on the electropherogram (Fig. 4). In cattle, coat color intensity and dilution are influenced by the expression of the PMEL gene (p.Ala612Glu, SNP). To investigate heterozygosity levels in the studied population, a PCR-RFLP assay was employed. Amplification of the PMEL gene resulted in a 463bp fragment (Fig. 5) and allele discrimination was performed through digestion of the PCR product with the restriction enzyme BlpI, which recognizes the site G/CTNAGC. The digested fragments revealed different genotypes among the tested animals: the presence of 319bp and 144bp fragments indicated a homozygous genotype, while heterozygous individuals showed all three fragments — 463bp, 319bp and 144bp (Fig. 6).

To identify carriers of the genetic defect Osteopetrosis, which results from a 2781bp deletion in the coding region of the gene, a polymerase chain reaction (PCR) method was employed using a common forward primer (F: 5'-GGGAAGGGAAGCACTAAGACT-3') and two reverse primers — RN (5'-GGTGGATGTGATGGGAAGACT-3') for the wild-type allele and RM (5'-TGGAGAGACAGCAGCAGAGAT-3') for the mutant allele. In this assay, heterozygous individuals displayed two fragments of 475bp and 330bp, while homozygous wild-type animals showed a single fragment of 475bp (Fig. 7).

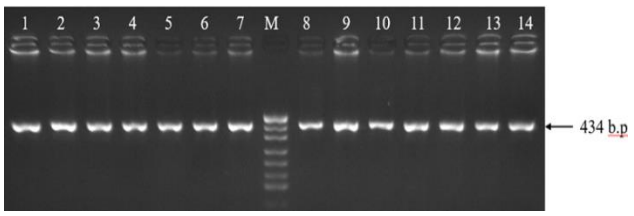

Fig. 3: Electropherogram of the SILV gene amplicon (c.64G>A), 3% agarose gel. Lanes 1–7 and 8–14 contain the PCR product with a length of 434bp. M – DNA marker pUC19/MspI.

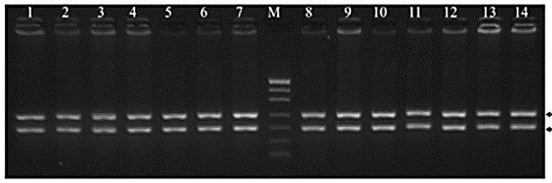

Fig. 4: Electropherogram of the PCR product after digestion with the SfcI endonuclease, 4% agarose gel. Lanes 1–7 and 8–14 show the homozygous genotype with fragments of 244bp and 190bp. M – DNA marker pUC19/MspI.

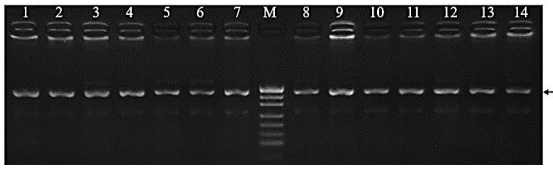

Fig. 5: Electropherogram of the PMEL gene amplicon (p.Ala612Glu), 3% agarose gel. Lanes 1–7 and 8–14 contain the PCR product with a length of 463bp. M – DNA marker pUC19/MspI.

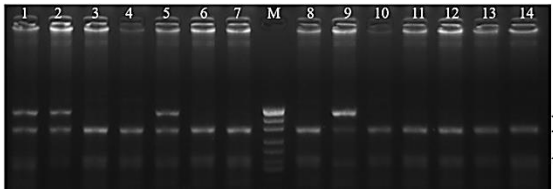

Fig. 6: Electropherogram of the PMEL gene amplicon (p.Ala612Glu) after digestion with the BlpI endonuclease, 4% agarose gel. Lanes 3–4, 6–7, 8 and 10–14 show the homozygous genotype with fragments of 319bp and 144bp. Lanes 1, 2, 5, and 9 show the heterozygous genotype with fragments of 463bp, 319bp, and 144bp. M – DNA marker pUC19/MspI.

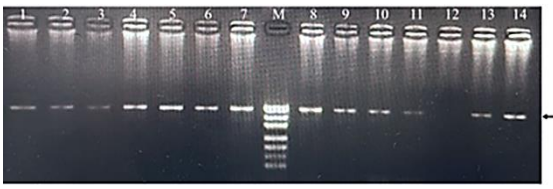

Fig. 7: Electropherogram of the SLC4A2 gene amplicon, 3% agarose gel. Lanes 1–7 and 8–14 show the homozygous genotype with a fragment of 475bp. M – DNA marker pUC19/MspI.

As an alternative diagnostic approach for detecting carriers of the c.64G>A point mutation in exon I of the PMEL gene, Real-Time PCR was performed using the Custom TaqMan™ SNP Genotyping Assay provided by Thermo Fisher Scientific. Analysis of the graphical output from the Real-Time PCR results demonstrated that all DNA samples exhibited a homozygous GG genotype at the PMEL locus, where the G allele is represented by the red curve and the A allele by the blue curve (Fig. 8). The allele discrimination plot further confirmed that all DNA samples had the homozygous GG genotype (Fig. 9).

DISCUSSION

To identify heterozygous carriers of the Hypotrichosis genetic defect in cattle of the Kazakh Whiteheaded, Kalmyk, Auliekol, Hereford, and Santa Gertrudis breeds, two methods were applied: the TETRA-PRIMER ARMS-PCR technique and a PCR assay using three primers (Table 4). The study population was relatively large (n=346) and included animals from three geographic regions of the Republic of Kazakhstan. No carriers of the Hypotrichosis mutation were detected among animals of the Kazakh Whiteheaded, Kalmyk, Auliekol, or Santa Gertrudis breeds. However, two heterozygous carriers of the 8-nucleotide deletion (g.27331221delTGTGCCCA) associated with Hypotrichosis were identified among the Hereford cattle, with a frequency of 6.9%.

The obtained carrier frequency of 6.9% in Herefords from our study is notably higher than the 0.16% reported in a large-scale Russian study (Konovalova et al. 2021) but aligns more closely with the 4.16% prevalence previously documented in Kazakh herds by Dyussekenova et al. (2024). This discrepancy may reflect regional variations in breeding practices and importation patterns of genetic material. We concur with the authors (Ussenbekov et al. 2024a) that such variability underscores the critical need for localized, breed-specific genetic monitoring programs to prevent the accumulation of deleterious alleles. The absence of carriers in local Kazakh breeds suggests possible genetic isolation or selective advantages, a phenomenon observed in other non-syndromic hypotrichosis cases (Jacinto et al. 2025).

The results of genetic monitoring also showed that no heterozygous carriers of the hidden mutations associated with Dilutor (DI), SILV (c.64A>G), or Osteopetrosis were detected in Kazakh Whiteheaded (n=15), Kalmyk (n=129), Auliekol (n=48), or Santa Gertrudis (n=125) animals. Among the analyzed genetic markers, the p.Ala612Glu SNP in the PMEL gene showed the greatest polymorphism. The Kalmyk breed exhibited the highest level of heterozygosity at 25.6%, based on a sample comprising adult cows, breeding bulls, and young animals of both sexes from the local population in the Almaty region. This SNP is associated with coat color dilution, and the observed loss of typical Kalmyk pigmentation may be the result of long-term uncontrolled crossbreeding with bulls of non-pure breeds.

The absence of Dilutor and Osteopetrosis carriers in local Kazakh breeds suggests these populations are currently free from these defects, consistent with recent reports on the genetic cleanliness of the Kazakh Whiteheaded breed (Ussenbekov et al. 2024a). The high heterozygosity (25.6%) at the PMEL p.Ala612Glu locus in the Kalmyk breed is significant and agrees with the documented role of PMEL mutations in coat colour dilution (Gutiérrez-Gil et al. 2007; Wang et al. 2023; Chen et al. 2024; Wei et al. 2024). Cases of coat-colour dilution with hair abnormalities in Hereford crossbreds support possible background interactions with other loci (Jolly et al. 2008). Reports on rare forms of hypotrichosis and associated mutations further emphasize the genetic diversity of such phenotypes and the need for comprehensive screening (Jacinto et al. 2021; Kuca et al. 2021).

The elevated frequency of the PMEL p.Ala612Glu allele in Kalmyk cattle likely results from uncontrolled crossbreeding, a concern also noted for dilution alleles in native populations (Hartati et al. 2024). This highlights the need for stricter pedigree control and genomic selection to preserve breed integrity. GWAS have

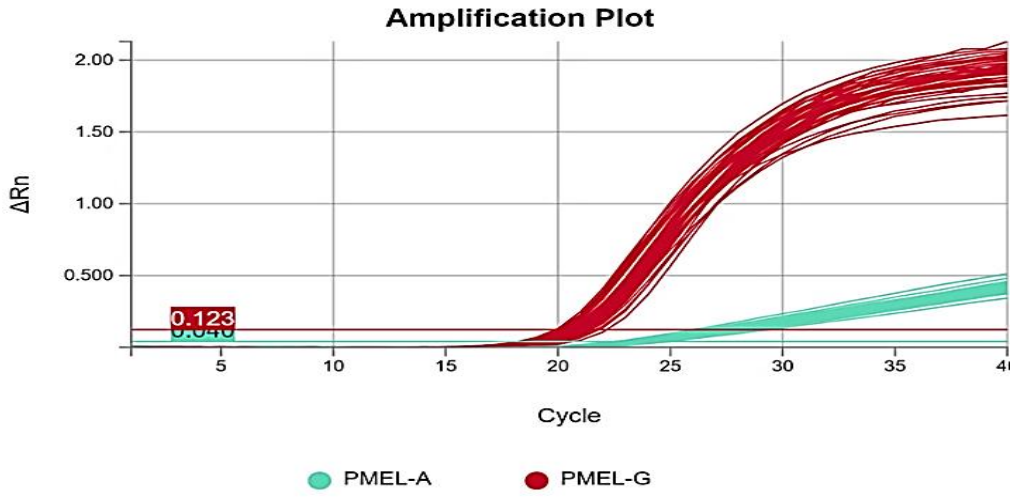

Fig. 8: Allelic discrimination plot from Real-Time PCR analysis showing a heterozygous carrier of the PMEL c.64G>A mutation (amplification with VIC probe for the wild-type G allele).

Table 4: Prevalence of heterozygous carriers of genetic defects in cows, bulls, and heifers from farms in Almaty, Zhetysu and Kostanay regions

Genetic Defect / Method	Breed and number of animals				
	Kazakh Whiteheaded (n=15)	Kalmyk (n=129)	Kazakh Whiteheaded (n=15)	Santa Gertrudis (n=125)	Kazakh Whiteheaded (n=15)
Hypotrichosis (HY), Tetra-Primer Arms-PCR		Hypotrichosis (HY), Tetra-Primer Arms-PCR		Hypotrichosis (HY), Tetra-Primer Arms-PCR	
wt/wt	15	129	48	125	27/93.1%
wt/mt	0	0	0	0	2/6.9%
mt/mt	0	0	0	0	0
Dilutor (DI), SILV, c.64A>G, PCR-RFLP, Real-Time PCR					
wt/wt	15	129	48	125	29
wt/mt	0	0	0	0	0
mt/mt	0	0	0	0	0
Dilutor (DI), PMEL p.Ala612Glu, PCR-RFLP					
wt/wt	14/93.3%	96/74.4%	45/93.7%	119/95.2%	27/93.1%
wt/mt	1/6.7%	33/25.6%	3/6.3%	6/4.8%	2/6.9%
mt/mt	0	0	0	0	0
Osteopetrosis (OS), PCR					
wt/wt	15	129	48	125	29
wt/mt	0	0	0	0	0
mt/mt	0	0	0	0	0

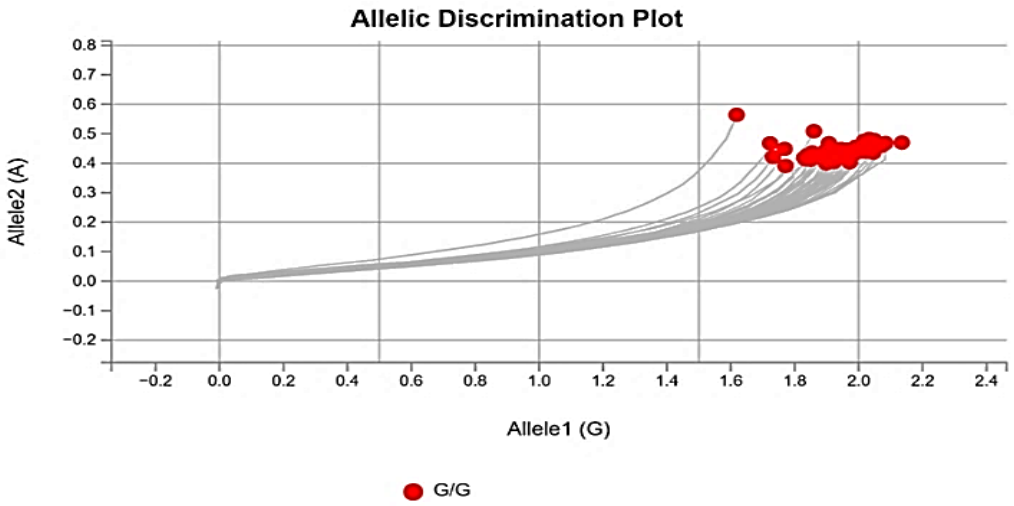

Fig. 9: Graphical representation of real-time PCR diagnostic results for homozygous samples with the PMEL c.64 G>A mutation (amplification using VIC probe, wild-type allele G).

Table 5: Prevalence of heterozygous carriers of genetic defects in breeding bulls from Breeding Center No. 1 (n=37)

Genetic Defect / Method	Breed and number of animals, diagnostic method				
	Kazakh Whiteheaded (n=13)	Angus (n=8)	Hereford (n=9)	Auliekol (n=5)	Kalmyk (n=2)
Hypotrichosis (HY), TETRA-PRIMER ARMS-PCR					
wt/wt	13	8	8/88.8%	5	2
wt/mt	0	0	1/11.2%	0	0
mt/mt	0	0	0	0	0
Dilutor (DI), SILV, c.64A>G, PCR-RFLP, Real-Time PCR					
wt/wt	13	8	9	5	0
wt/mt	0	0	0	0	0
mt/mt	0	0	0	0	0
Dilutor (DL) PMEL p.Ala612Glu, PCR-RFLP					
wt/wt	11/84.6%	7/87.5%	7/77.8%	5	2
wt/mt	2/15.4%	1/12.5%	2/22.2%	0	0
mt/mt	1	0	0	0	0
Osteopetrosis (OS), PCR					
wt/wt	13	8	8	5	2
wt/mt	0	0	0	0	0
mt/mt	0	0	0	0	0

consistently confirmed PMEL as a major candidate gene for coat color, with additional effects on birth weight in crossbred cattle (Wang et al. 2023; Hartati et al. 2024). Moreover, studies of rare white coat phenotypes in Simmental calves revealed unexpected genetic heterogeneity, suggesting similar complex interactions may exist in Kalmyk cattle (Jacinto et al. 2025).

In contrast, the heterozygosity levels for this SNP among Kazakh Whiteheaded, Auliekol, Hereford, and Santa Gertrudis cattle ranged from 4.8% to 6.9%.

A substantial number of animals (n=346) from various breeds and regions were tested for the Osteopetrosis (OS) genetic defect, but no carriers were found. Similarly, genetic monitoring studies conducted by Russian researchers revealed that the prevalence of Hypotrichosis in Hereford cattle (n=385) was very low, at just 0.16%. According to our earlier research, the frequency of heterozygous carriers of Hypotrichosis in Hereford and Angus cattle was 4.16% and 1.66%, respectively. In the first experimental group of Hereford cattle (n=29), two heterozygous carriers of the Hypotrichosis mutation were identified (6.9%). Among the nine breeding bulls tested from Breeding Center No. 1, one Hereford bull was found to carry the mutation, representing a prevalence of 11.2%.

The critical finding of an 11.2% carrier rate among breeding bulls at a major center highlights a significant vulnerability within the national breeding infrastructure. This is a cause for concern, as a single carrier bull can disseminate a harmful mutation widely through artificial insemination. Our data strongly support the recommendations of Shormanova et al. (2023) and Konovalova et al. (2021) for the mandatory and periodic genotyping of all sires used in artificial insemination programs. The development and implementation of the efficient Real-Time PCR assay in this study, which allows for high-throughput screening of 96 samples in 2.5 hours, provides a practical and scalable solution for such large-scale monitoring initiatives, enabling proactive management of genetic recessives.

These findings highlight the importance of conducting genetic screening to monitor the spread of harmful mutations in beef cattle, particularly among breeding bulls from artificial insemination centers. Data presented in Table 5 show that one Hereford bull from Breeding Center No. 1 was identified as a heterozygous carrier of the Hypotrichosis defect, with a carrier frequency of 11.2%. No carriers of hidden mutations associated with Dilutor (DL, SILV gene, c.64G>A) or Osteopetrosis (OS) were detected among the bulls from this center. The heterozygosity level for the p.Ala612Glu SNP was 15.4% in Kazakh Whiteheaded bulls, 12.5% in Angus bulls, and 22.2% in Hereford bulls. The number of tested bulls from the Auliekol (n=5) and Kalmyk (n=2) breeds was limited, and as a result, no genetic variation was observed at any of the studied loci in those groups.

In conclusion, while the local Kazakh breeds appear genetically robust against the specific defects targeted in this study, the presence of the Hypotrichosis allele in the Hereford population and the high dilution allele frequency in Kalmyk cattle demand attention. The results of this study, in agreement with global trends in livestock genetics (Avci et al. 2023; Mahmood et al. 2022), demonstrate that proactive genetic screening is not merely a diagnostic tool but a fundamental component of modern, sustainable animal breeding. Implementing the optimized protocols described here into national breeding programs will be crucial for safeguarding the genetic health and economic productivity of Kazakhstan's cattle populations in the long term. Furthermore, the discovered PMEL heterozygosity patterns warrant deeper investigation into potential pleiotropic effects on production traits, as suggested by recent GWAS findings (Kimura et al. 2022; Wang et al. 2023). The complex genetic architecture of coat color and its potential correlations with performance traits highlight the need for integrated genomic selection approaches rather than focusing on single genes.

Conclusion

Based on the results of genetic monitoring, only heterozygous carriers of the Hypotrichosis defect were identified, while no carriers of the genetic defects Dilutor (DL, SILV gene, c.64G>A) or Osteopetrosis were detected in the studied animals. These findings suggest that the populations of local cattle breeds—Kazakh Whiteheaded, Kalmyk, and Auliekol—are genetically clean and do not carry harmful mutations. Particularly noteworthy were the genotyping results for the PMEL gene at the p.Ala612Glu locus, which is associated with

coat color dilution. The Kalmyk breed showed a high level of heterozygosity at this locus, reaching 25.6%. Taken together, these results emphasize that integrating periodic genetic screening with optimized molecular diagnostic tools, such as Real-Time PCR, is critical for preventing the spread of recessive defects and for safeguarding both the genetic integrity and sustainable productivity of Kazakhstan's cattle breeding herds.

DECLARATIONS

Funding: This study was carried out within the framework of the project of the Ministry of Agriculture of the Republic of Kazakhstan titled «Development of the system of genetic perfection of meat breeds with application of innovative methods of molecular genetics, selection and digital technologies», project number BR22885686.

Acknowledgment: The authors thank the editorial team and reviewers for their constructive comments that improved the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The data are available by request from the corresponding author.

Ethics Statement: All procedures for field surveys and laboratory analyses complied with national sanitary and epidemiological safety standards of the Republic of Kazakhstan.

Author's Contribution: ND, TK, DK conceived the study and drafted the manuscript; PS and MJ designed the field survey and sampling strategy; ZhK and ZhM performed microbiological and molecular analyses; AT and RJ curated data and validated records; YU conducted statistical analysis; DK supervised the project and critically revised the manuscript. All authors read and approved the final version.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

Avci O, Bulut O, Dik I, Bulut Z, Dogan M, Kale M, Hasircioglu S, Yapici O, Simsek A, Yavru S and Orta YS, 2023. BoLA-DRB3.2 gene polymorphism in cattle naturally infected with bovine leukemia virus. Pakistan Veterinary Journal 43(1): 174-178. http://dx.doi.org/10.29261/pakvetj/2023.001

Ayuti SR, Shin S, Kim EJ, Lamid M, Warsito SH, Al Arif MA, Lokapirnasari WP, Rosyada ZNA, Khairullah AR, Akmal M, Ritonga MZ, Rimayanti R and Delima M, 2025. ACTA1 gene regulation in livestock: A multidimensional review on muscle development, meat quality, and genetic applications. Veterinary World 18(8): 2520–2541. https://doi.org/10.14202/vetworld.2025.2520-2541

Bimenova ZZ, Ussenbekov YeS, Terletskiy VP, Makashev YK, Tyshchenko VI and Kassymbekova SN, 2019. Reproductive function of cows with different genotypes for TNFα locus and estimation of sperm fertility by the DNA fragmentation method. Cytology and Genetics 53(1): 42–48. https://doi.org/10.3103/S0095452719010043

Chen C, Li J, Sun X, Jiang J, Lv S, Liu L, Wang G and Ren H, 2024. Understanding the skin blackening phenomenon in Youzhou Dark goats based on the histological characteristics of melanocytes. Journal of Advanced Veterinary and Animal Research 11(4): 1139. https://doi.org/10.5455/javar.2024.k865

Dyussekenova N, Nessipbayeva A, Buralkhiyev B, Khussainov D, Akimzhan N, Muslimova Z, Kalkayeva D, Turgumbekov A and Ussenbekov Y, 2024. Methods for diagnosing the DUMPS, BLAD, HY and OH1 hereditary abnormalities in cattle and determining their prevalence. International Journal of Veterinary Science 14(3): 595-604. https://doi.org/10.47278/journal.ijvs/2024.272

Gutiérrez-Gil B, Wiener P and Williams JL, 2007. Genetic effects on coat colour in cattle: dilution of eumelanin and phaeomelanin pigments in an F2-Backcross Charolais × Holstein population. BMC Genetics 8: 56. https://doi.org/10.1186/1471-2156-8-56

Gutiérrez-Gil B, Wiener P and Williams JL, 2007. Genetic effects on coat colour in cattle: dilution of eumelanin and phaeomelanin pigments in an F2-Backcross Charolais × Holstein population. BMC Genetics 8: 56. https://doi.org/10.1186/1471-2156-8-56

Hartati H, Putra WPB, Handiwirawan E, Ramon E, Firison J, Zubir Z, Suretno ND, Mariyono M, Yusriani Y, Robba DK, Destomo A, Anggraeni T, Anwar P, Irmawanti S, Aprisal A, Elieser S and Kurniawati D, 2024. Genome-wide association study of genetic markers of coat color patterns in Sumatran native cattle. Veterinary World 17(11): 2537–2543. https://doi.org/10.14202/vetworld.2024.2537-2543

Hecht BC, 2006. Sequence Analysis of PMEL17 as Candidate Gene for Causing Rat-Tail Syndrome in Cattle. MS Thesis, Brigham Young University.

Jacinto JG, Leuenberger T, Hauser M, Häfliger IM, Seefried FR, Letko A and Drögemüller C, 2025. Rare phenotypes of white coat color in Simmental calves: genetic causes of syndromic forms of albinism and depigmentation. Molecular Genetics and Genomics 300 (1): 1–16. https://doi.org/10.1007/s00438-025-02290-2

Jacinto JGP, Bolcato M, Häfliger IM, Oevermann A, Gentile A and Drögemüller C, 2023. DYRK1B haploinsufficiency in a Holstein cattle with epilepsy. Animal Genetics 54: 623–627. https://doi.org/10.1111/age.13348

Jacinto JGP, Markey AD, Veiga IMB, Paris JM, Welle M, Beever JE and Drögemüller C, 2021. A KRT71 Loss-of-Function Variant Results in Inner Root Sheath Dysplasia and Recessive Congenital Hypotrichosis of Hereford Cattle. Genes 12(7): 1038. https://doi.org/10.3390/genes12071038

Jolly RD, JL Wills, Kenny JE, Cahill JI and Howe L, 2008. Coat-Colour Dilution and Hypotrichosis in Hereford Crossbred Calves. New Zealand Veterinary Journal 56(2): 74-77. https://doi.org/10.1080/00480169.2008.36812

Khan MYA, Omar AI, He Y, Chen S, Zhang S, Xiao W and Zhang Y, 2021. Prevalence of nine genetic defects in Chinese Holstein cattle. Veterinary Medicine and Science 7(5): 1728-1735. https://doi.org/10.1002/vms3.525

Kimura S, Hatakeyama T, Koutaka T, Kubo K, Morita S, Eguchi K, Saitoh K, Yamauchi K, Imai S, Kashimura A, Inenaga T and Matsumoto H, 2022. PMEL p.Leu18del dilutes coat color of Kumamoto sub-breed of Japanese Brown cattle.

BMC Genomics 23: 694. https://doi.org/10.1186/s12864-022-08916-8

Kimura S, Hatakeyama T, Koutaka T, Kubo K, Morita S, Eguchi K, Saitoh K, Yamauchi K, Imai S, Kashimura A, Inenaga T and Matsumoto H, 2022. PMEL p.Leu18del dilutes coat color of Kumamoto sub-breed of Japanese Brown cattle. BMC Genomics 23: 694. https://doi.org/10.1186/s12864-022-08916-8

Konovalova E, Romanenkova O, Kostyunina O and Gladyr E, 2021. The molecular bases study of the inherited diseases for the health maintenance of the beef cattle. Genes 12(5): 678. https://doi.org/10.3390/genes12050678

Konovalova EN, Romanenkova OS, Volkova VV and Kostyunina OV, 2020. DNA analysis of the Russian populations of Aberdeen Angus, Hereford and Belgian Blue cattle. Archives Animal Breeding 63(2): 409–416. https://doi.org/10.5194/aab-63-409-2020

Kuca T, Marron BM, Jacinto JGP, Paris JM, Gerspach C, Beever JE and Drögemüller C, 2021. A nonsense variant in Hephaestin Like 1 (HEPHL1) is responsible for congenital hypotrichosis in Belted Galloway cattle. Genes 12(5): 643. https://doi.org/10.3390/genes12050643

Lirong H, Yuezhen T, Qin Ch, Abdul S, Shihui J, Xixia H, Zhenquan X, Shiyu H, Airong L, Hongjun W, Lin L, Wei W, Ying Y and Yachun W, 2022. Development and application of arachnomelia syndrome genetic detection in four Chinese dual-purpose cattle populations. Research in Veterinary Science 152: 323–332. https://doi.org/10.1016/j.rvsc.2022.08.009

Mahmood Q, Younus M, Sadiq S, Iqbal S, Idrees A, Khan S and Zia URR, 2022. Prevalence and associated risk factors of cystic echinococcosis in food animals – a neglected and prevailing zoonosis. Pakistan Veterinary Journal 42(1): 59-64. http://dx.doi.org/10.29261/pakvetj/2022.008

Makamu TC and Tyasi TL, 2024. Systematic Review of Single Nucleotide Polymorphisms of Cattle Beta-Lactoglobulin Gene and their Association with Milk Production Traits. American Journal of Animal and Veterinary Sciences 19(2): 121-132. https://doi.org/10.3844/ajavsp.2024.121.132

Markey AD, Taylor JF, Schnabel RD, McKay SD, McClure MC and Beever JE, 2010. A deletion mutation in Krt71 is associated with congenital hypotrichosis in Hereford cattle. Proceedings of Plant & Animal Genomes XVIII Conference, San Diego, United States; 9-13 January 2010, pp: 552.

Meyers SN, McDaneld TG, Swist SL, Marron BM, Steffen DJ, O'Toole D, O'Connell JR, Beever JE, Sonstegard TS and Smith TPL, 2010. A deletion mutation in bovine SLC4A2 is associated with osteopetrosis in Red Angus cattle. BMC Genomics 11: 337. https://doi.org/10.1186/1471-2164-11-337

Meyers SN, McDaneld TG, Swist SL, Marron BM, Steffen DJ, O'Toole D, O'Connell JR, Beever JE, Sonstegard TS and Smith TPL, 2010. A deletion mutation in bovine SLC4A2 is associated with osteopetrosis in Red Angus cattle. BMC Genomics 11: 337. https://doi.org/10.1186/1471-2164-11-337

Neogen, 2024a. Idiopathic Epilepsy (IE). Retrieved from: https://www.neogen.com/categories/livestock-genetic-traits-conditions/idiopathic-epilepsy-ie/

Neogen, 2024b. Coat Color Dilutor (DL). Retrieved from: https://www.neogen.com/categories/livestock-genetic-traits-conditions/coat-color-dilutor-dl/

Nicholas FW, Tammen I and Sydney I, 2025. Informatics Hub. Online Mendelian Inheritance in Animals (OMIA) [dataset]. https://doi.org/10.25910/2AMR-PV70

Romero-Benavente A, Briano-Rodriguez C and Dutra-Quintela F, 2023. Hypotrichosis congenita (KRT71 mutation) in Hereford cattle in Uruguay. Pesquisa Veterinária Brasileira 43: e07327. https://doi.org/10.1590/1678-5150-PVB-7327

Shormanova M, Makhmutov A, Shormanova A, Muslimova Z and Ussenbekov Y, 2024. Development of alternative diagnosis of HH1, HH3, HH5 and HCD fertility haplotypes and subfertility syndrome in cattle. Reproduction in domestic animals = Zuchthygiene 59(1): e14533. https://doi.org/10.1111/rda.14533

Shormanova MM, Nurpeisova RK, Makhmutov AK, Siyabekov ST and Ussenbekov YeS, 2023. Frequency of fertility haplotype carriers HH4, HH5, HCD in Holstein bulls and cows. Science and Education Part 1 1(70): 65–76.

UC Davis Veterinary Genetics Laboratory, Cattle Dilution Test. https://vgl.ucdavis.edu/test/cattle-dilution

Ussenbekov Y, Junussova R, Myrzaliyev A, Koibagarov K and Turgumbekov A, 2025. Prevalence of Arachnomelia, Developmental Duplication, Arthrogryposis Multiplex syndromes in Angus, Hereford, Kazakh White-headed breeds. International Journal of Veterinary Science 14(1): 219–226. https://doi.org/10.47278/journal.ijvs/2024.233

Ussenbekov YS, Bagdat AB, Junusova RZh, Muslimova ZhU, Serikov AY, Turganbaev RU and Khusainov DM, 2024. Method for diagnostics of carriers of the JH1 fertility haplotype in Jersey cattle by Real-Time PCR. Patent of the Republic of Kazakhstan, №37067.

Ussenbekov YS, Bagdat AB, Muslimova ZU, Junusova RZh, Serikov AY, Mambetnazarov AB and Khusainov DM, 2024. Method of diagnostics of JH1 fertility haplotype carriers in Jersey cattle by PCR-PDRF assay. Patent of the Republic of Kazakhstan, №37040.

Ussenbekov YS, Shormanova MM, Koibagarov KU, Khizat S, Serikov AY, Yesenbekova ZhS and Khusainov DM, 2024. Method of diagnostics of carriers of subfertility syndrome in bulls of producers by Real-Time PCR. Patent of the Republic of Kazakhstan, №37069.

Ussenbekov YS, Turgumbekov AA, Muslimova ZhU, Strochkov VM, Kassymbekova ShN, Turganbaev RU, Dyussekenova NT and Khusainov DM, 2025. Method for diagnosis of carriers of Developmental Duplication (DD) syndrome in Angus cattle by Real-Time PCR. Patent of the Republic of Kazakhstan, №37125.

Wang J, Fan T, Du Z, Xu L, Chen Y, Zhang L, Gao H, Li J, Ma Y and Gao X, 2023. Genome-Wide Association Analysis Identifies the PMEL Gene Affecting Coat Color and Birth Weight in Simmental × Holstein. Animals 13(24): 3821. https://doi.org/10.3390/ani13243821

Wei J, Brophy B, Cole S-A, Leath S, Oback B, Boch J, Wells DN and Laible G, 2024. Production of light-coloured, low heat-absorbing Holstein Friesian cattle by precise embryo-mediated genome editing. Reproduction, Fertility and Development 36(2): 112–123. https://doi.org/10.1071/RD23163