# Exploring Genotypes of Black and Brown Spotting in Dorper Sheep

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## Abstract

Dorper sheep exhibit two primary color phenotypes: white bodies with black or white heads. However, variations in these markings are observed. This quantitative study at Texas A&M University – Kingsville aims to identify mutations associated with coat color variation in Dorper sheep, providing livestock producers with insights into the effects of these mutations and the role of inbreeding on herds. The study sampled 106 Dorper sheep (thirty–eight males and sixty–nine females), capturing 360-degree photos to document black, brown, black and brown, tricolor spots, and white head patterns. DNA was extracted and analyzed using a candidate gene approach, focusing on the *melanocortin one receptor (MC1R)* and *tyrosinase-related protein 1 (TYRP1)* genes. Polymerase chain reaction (PCR) techniques were used to amplify regions of interest, followed by Sanger sequencing to identify mutations. Inbreeding was assessed through pedigree analysis using Pedigraph. The quantification of the black and brown spotting on all sheep revealed coat color variation across different patterns, and pedigree analysis indicated the patterns are not family specific. Analysis is ongoing to identify mutations within *MC1R* and *TYRP1* genes that are associated with the coat color patterns.

## Introduction

**Coat color:**
- Trait of aesthetic
- Economic significance
- Lens through which the genetic history and breeding practices of populations can be examined

**Screening candidate genes for additional mutations:**
- *MC1R* - Black pigmentation
  - Casual gene for black headed coat color in sheep (Zhou et al., 2023).
- *TYPR1* - Brown pigmentation
  - Casual gene for brown coat color in Jacob sheep (Posbergh, 2016).



Figure 1. Dorper sheep at TAMUK with different coat color variation.

**Inbreeding:** Mating of closely related individuals can lead to inbreeding depression, which negatively impacts growth, production, health, fertility, and survival traits (Binabaj et al., 2021).



Figure 2. Dorper sheep at TAMUK with a limb deformity.

## Objectives

1. Quantify the amount of spotting on the Dorper Sheep.
2. Investigate whether *MC1R* and *TYRP1* genetic mutations play a role in determining black versus brown spotting in Dorper sheep.
3. Analyze the level of inbreeding in Dorper sheep, to trace the origins of these mutations and determine how many generations have carried these genetic variations within the lineage.

## Methods

1. Sampled 106 sheep (38 males and 69 females) by taking 360-degree photos to quantify black and brown spots.
2. The sheep were categorized into five groups: fully white, brown spotting, black spotting, black and brown spotting, and tricolor/speckling.
3. DNA was extracted from blood samples to study the *MC1R* and *TYPR1* candidate genes.

## Methods

4. Gimp 2.0 software was used to quantify the amount of coat color. The calculation of the total percentage of spotting was calculated by taking the ratio of the spotting divided by the total ratio of the body.
5. Pedigraph was used to assessed pedigree analysis for inbreeding.
6. Statistical analysis was conducted using IBM SPSS. Shapiro Wilks Test was used to test for normality and Independent – Samples Kruskal – Wallis Test was used to test for statistically significant differences between two or more groups.
7. PCR techniques were used to amplify regions of interest, followed by Sanger sequencing.

## Results

We found that there was variation in the coat color distribution between control, brown spotting, black spotting, black and brown spotting, speckling, and tricolor (Figures 3-5).
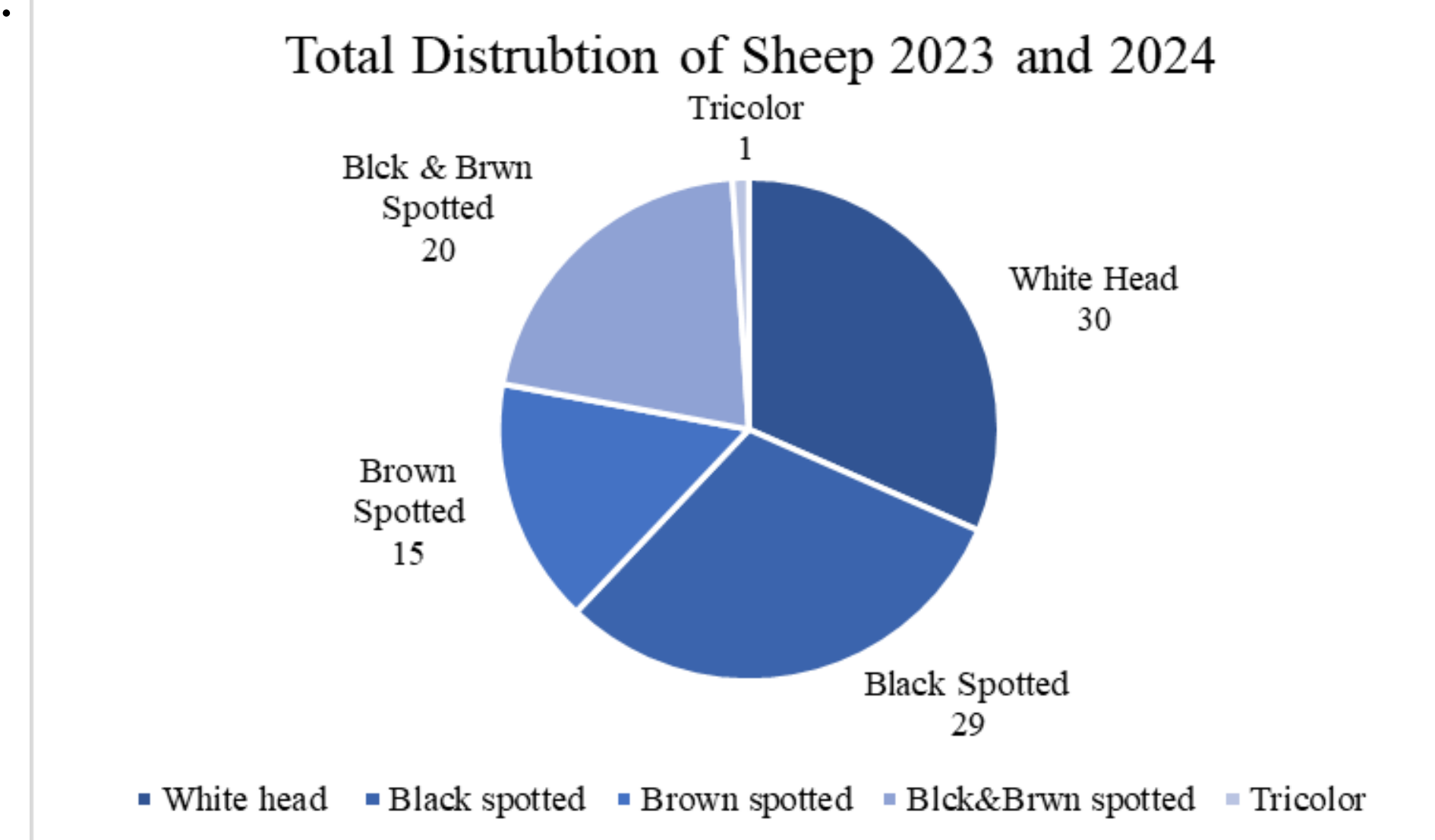


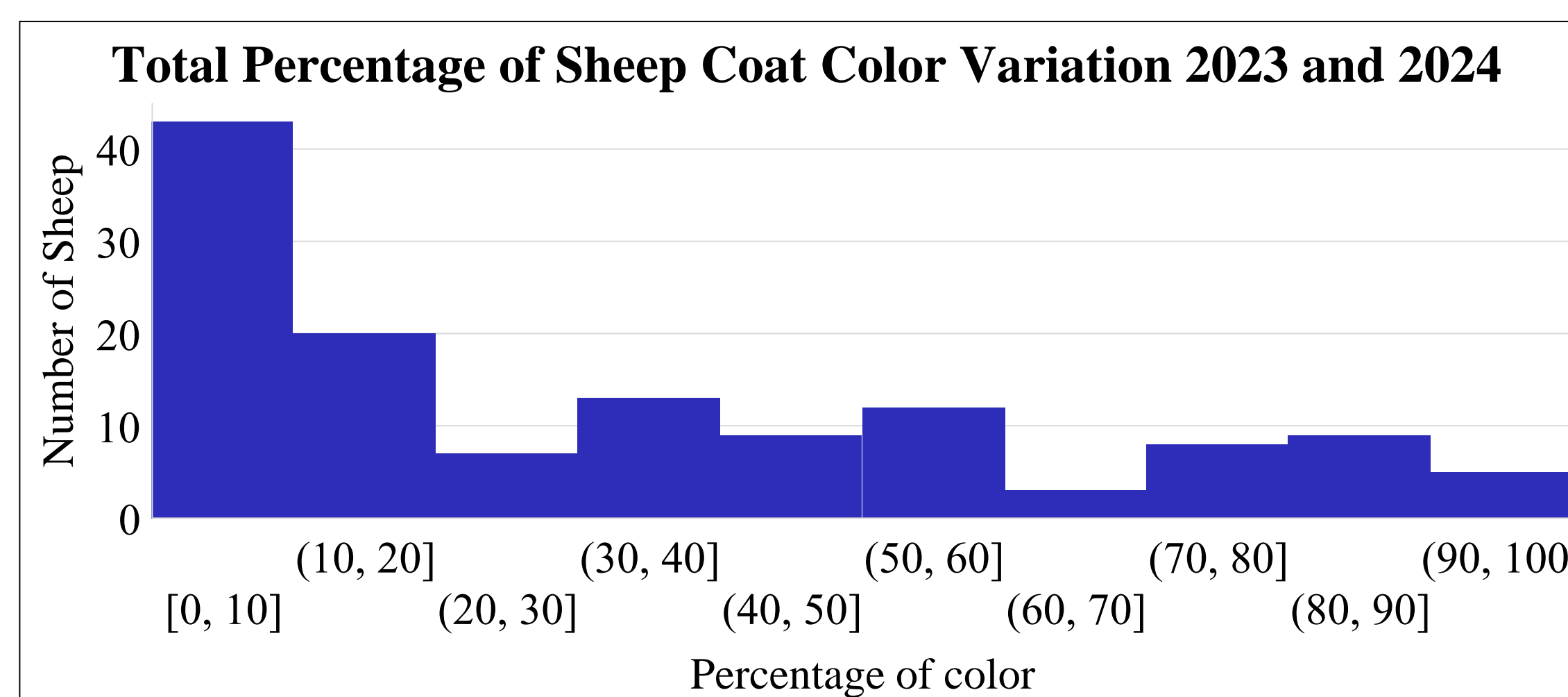Figure 3. Summary of all total sheep categorized.



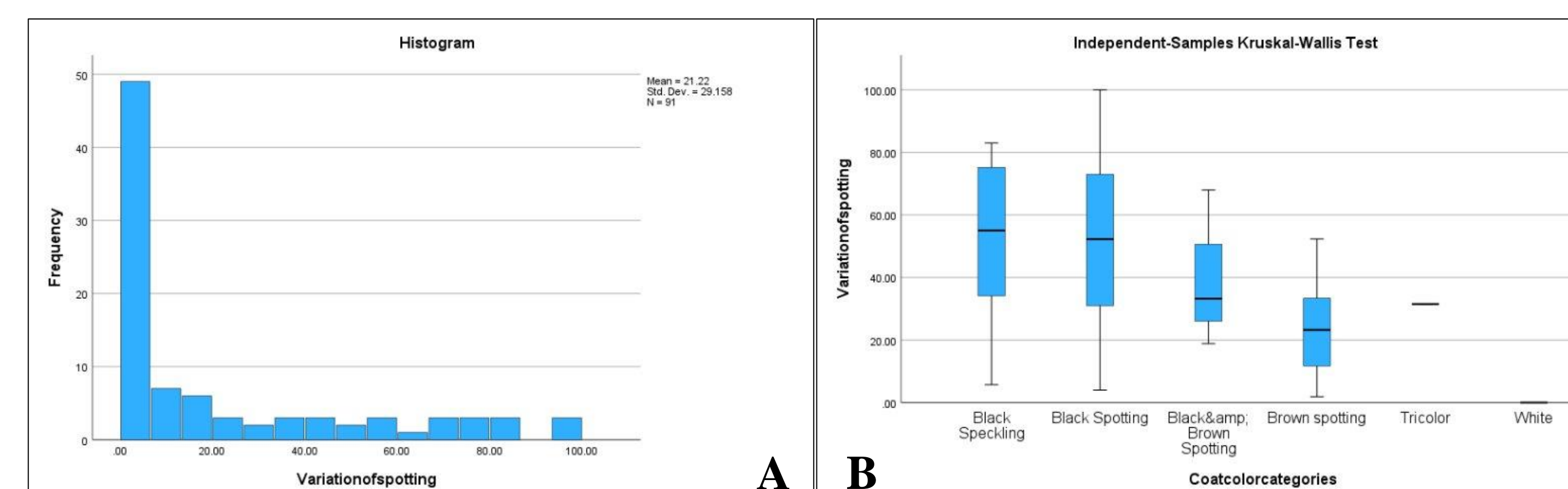Figure 4. Histogram of all sheep and the percentage of spotting on each of them.



Figure 5. A) Shapiro Wilks Test tested for normality. The analysis showed a significance level of *p< 0.001*, indicating the data is not normally distributed, which is driven by the higher number of white heads, but there is still variation in spotting across the different coat color categories.
B) Independent – Samples Kruskal – Wallis tested for the amount of spotting across all coat color categories is the same. The analysis showed *p< 0.001*, indicating that there are differences in the amount of spotting among all coat color categories.
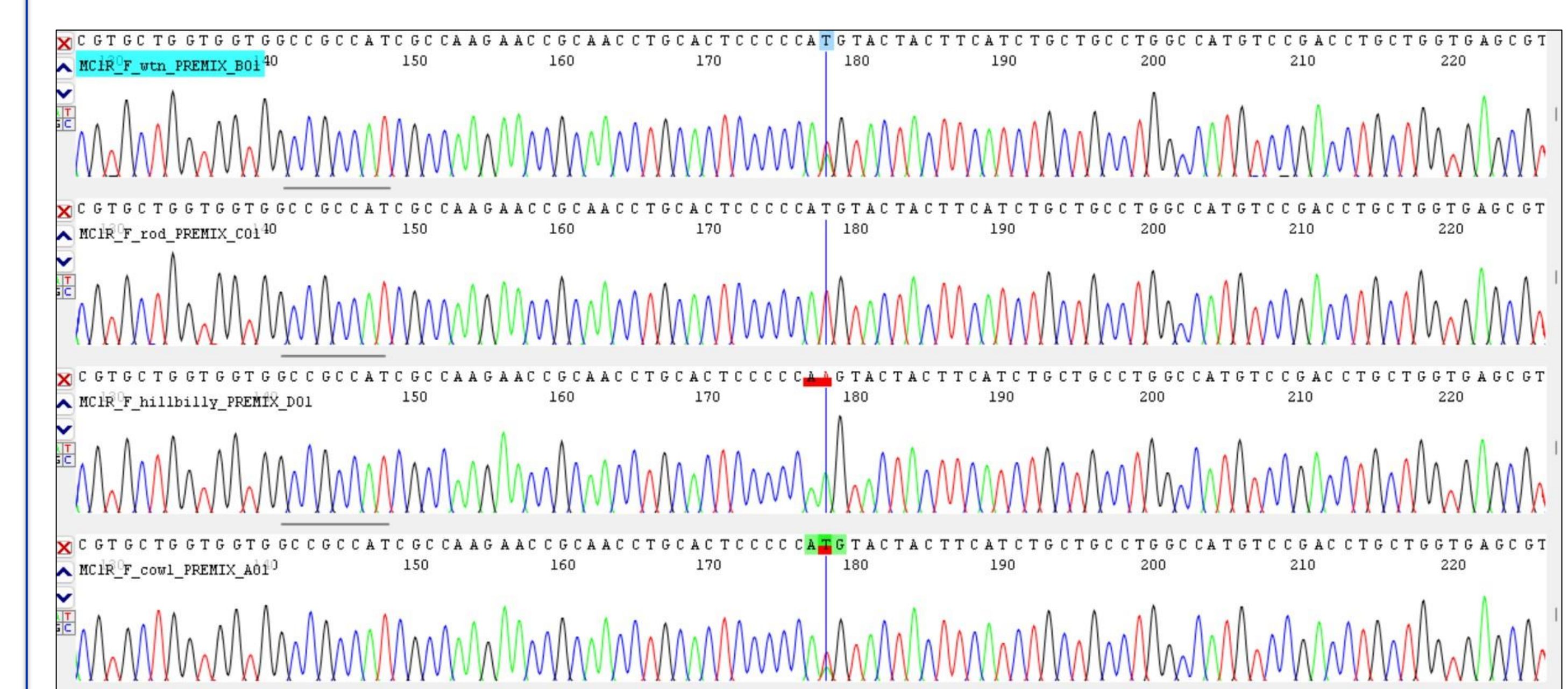


Figure 6. Sanger sequencing of four of the sheep genotyped for the *MC1R* gene. The blue vertical line highlights a mutation in the *MC1R* gene with all three genotypes detected.

## Discussion

- The quantification of the black and brown spotting on all sheep did show coat color variation between all different coat color categories.
  - From this we can see how prevalent mutations within the *MC1R* or *TYPR1* gene are within each color category.
- Through gel electrophoresis we confirmed our PCR worked and therefore can concluded we amplified *MC1R* and *TYPR1*.
- Using Sanger sequencing we have identified 2 mutations within two of the black spotted Dorper Sheep for the *MC1R* gene (Figure 6). Analysis is still ongoing.

## Future Studies

- The OvineSNP50 array will be utilized to provide genotypes for 50,000 + SNPs spread across the genome.
  - Identify regions in addition to the *MC1R* and *TYRP1* gene.
  - Assess inbreeding estimates from genotyping versus pedigree analysis.

## References

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## Acknowledgments