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# Phylogenetic analysis of KRT1.2 gene in Rambouillet sheep

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## Abstract
The objective of the present study was to investigate the phylogenetic analysis of KRT1.2 gene within the Rambouillet population, maintained at Sheep Breeding and Research Farm, Reasi, Jammu. DNA was isolated by HiPuraTM SPP Blood DNA Kit (HI-MEDIA). An amplicon of 480 bp of KRT1.2 gene was obtained. The coding sequence of the exon is of 347 bp that ranges from 65 bp to 412 bp with 116 amino acids in the coding sequence. A total of 15 nucleotide changes were found. Out of 15 changes 9 were transitions, 5 were transversions and one was multiple changes. Phylogenetic tree of different KRT1.2 gene sequences indicates that sequence KF718797 and KF718804 were the most distant sequences. The highest genetic identity value between different sequences was 99.58 %. The results will help to maintain variability within the population and hence, selection for wool will be more effective.

**Keywords:** Genetic identity, KRT1.2 gene, phylogenetic tree, Rambouillet sheep

## Introduction
The keratin intermediate-filament proteins (KRTs) and keratin-associated protein (KAPs) are the major proteins that make up the wool fibre. The KRTs form the skeletal structure of the wool fibre (microfibrils) and are embedded in a matrix of KAPs. More than 50 genes encode for these proteins (Pruett *et al.*, 2004) [4]. The efficiency of wool processing is dependent on the consistency of wool fibre and which in turn is dependent on these wool keratin proteins. Thus, the genes coding for these wool proteins have an impact on wool quality, and so are candidate genes for selective breeding. The use of such genetic markers greatly accelerates the efficiency of wool breeding programmes. Keeping in view the above discussed facts, study was undertaken with the objective to determine phylogenetic analysis of KRT gene in Rambouillet sheep.

## Materials & Methods
Five (5) ml blood sample was collected from external jugular vein aseptically in sterile EDTA coated vacutainer from 50 Rambouillet sheep including 25 individuals of each sex maintained at Sheep Breeding and Research Farm, Reasi, Jammu.
Genomic DNA was isolated from the venous blood samples by HiPurA$^{TM}$SPP Blood DNA Kit (HIMEDIA). DNA quality was checked through 0.8% horizontal submarine agarose electrophoresis. The genomic DNA samples having good quality intact bands (with no smearing) were used for further analysis.
Forward primer 5'-CACAACTGTGGCTTGGTGAACTTG-3' and reverse primer 5'-CTTAGCCATATCTCGGATTCCCTC-3', previously published by Rogers *et al.* (1993) [5], were used for amplification of KRT1.2 gene. PCR was carried out in a final volume of 25µl. For KRT1.2 gene, reactions were carried out in PCR tubes containing volume of 2µl of genomic DNA, 0.5µl of forward and reverse primers, 5µl of 5X PCR master-mix and 17µl of distilled water. The conditions were initial denaturation step at 94 ºC for 5 min. followed by 30 cycles of cyclic denaturation at 94 ºC for 30 sec annealing at 59 ºC for 1 min. extension at 72 ºC for 30 sec and final extension at 72 ºC for 10 min.
Direct sequencing of KRT1.2 gene was done using automated DNA sequencer by Sanger's di-deoxy method. A total of 10 amplicons of 480 bp of KRT1.2 gene were sequenced.
The sequenced products were subjected to BioEdit Software (Hall, 1999) [1] for identification of Single Nucleotide Polymorphisms (SNPs). Edited sequences were analyzed by multiple sequence alignment (CLUSTAL-W) to find out SNPs in the KRT1.2 gene and other bioinformatics analysis.

**Results and Discussion**

The PCR product of KRT1.2 gene was of 480 base pairs. Direct sequencing of KRT1.2 gene was done by using automated DNA sequencer by Sanger's di-deoxy method. A total of ten (10) amplicons of 480 base pair of the KRT1.2 was sequenced. All ten sequences submitted to NCBI with accession numbers KF718796, KF718797, KF718798, KF718799, KF718800, KF718801, KF718802, KF718803, KF718804 and KF469215. The coding region of KRT1.2 gene varies from 65-412 bp. After studying with NCBI sequence AY835598 [2], KF718796 sequence was considered as the reference sequence for the present Rambouillet population.

Multiple alignment results of these sequences revealed that in 480 bp KRT1.2 gene, there were a total of 15 nucleotide changes (Fig. 1). The changes were found at 84, 85, 86, 128, 160, 208, 223, 251, 294, 308, 314, 315, 340, 341 and 342 bp positions (Table 1). Out of 15 nucleotide changes, at 9 positions there were nucleotide substitutions for purine to purine and pyrimidine to pyrimidine, hence, in these positions the changes were transitions. At 5 different positions there were nucleotide substitutions for purine to pyrimidine and pyrimidine to purine, hence, in these positions the changes were transversions. As both transitions and transversions were found at 342 bp of KRT1.2 gene in Rambouillet population, it indicates the presence of multiple allele in KRT1.2 gene in the population. The sequencing results indicate that the KRT1.2 gene is highly polymorphic in the Rambouillet sheep population. Five alleles were reported for KRT1.2 genes in Merino sheep (Itenge-Mwezaa *et al.*, 2007) [3].

**Coding sequence of KF718796**

ATGTCTTTCAACTTCTGCCTGCCCAACCTGAGCTTCC
GCTCCAGCTGCTCCTCCAGGCCCTGCGTGCCCTCCAG
CTGCTGTGGCACCACCCTGCCCGGGGCCTGCAACAT
CCCCGCCAGCGTGGGCAGCTGCAACTGGTTCTGCGA
GGGCTCCTTCAACGGCAACGAGAAGGAGACCATGC
AGTTCCTGAACGACCGGCTGGCCAGCTACCTGGAGA
AGGTGCGGCAGCTGGAGCGGGAGAACACGGAGCTG
GAGAGACGCATCCTGGAGCGCAGTCAGCAGCAGGA
GCCCCTCGTGTGCCCCAACTACCAGTCCTACTTCCGG
ACCATCGAGGAGCTCCAGCAGAAG

**Amino acid sequence of reference sequence KF718796**

1 ATG TCT TTC AAC TTC TGC CTG CCC AAC CTG AGC TTC CGC TCC AGC 45
1 Met Ser Phe Asn Phe Cys Leu Pro Asn Leu Ser Phe Arg Ser Ser 15

46 TGC TCC TCC AGG CCC TGC GTG CCC TCC AGC TGC TGT GGC ACC ACC 90
16 Cys Ser Ser Arg Pro Cys Val Pro Ser Ser Cys Cys Gly Thr Thr 30

91 CTG CCC GGG GCC TGC AAC ATC CCC GCC AGC GTG GGC AGC TGC AAC 135
31 Leu Pro Gly Ala Cys Asn Ile Pro Ala Ser Val Gly Ser Cys Asn 45

136 TGG TTC TGC GAG GGC TCC TTC AAC GGC AAC GAG AAG GAG ACC ATG 180
46 Trp Phe Cys Glu Gly Ser Phe Asn Gly Asn Glu Lys Glu Thr Met 60

181 CAG TTC CTG AAC GAC CGG CTG GCC AGC TAC CTG GAG AAG GTG CGG 225
61 Gln Phe Leu Asn Asp Arg Leu Ala Ser Tyr Leu Glu Lys Val Arg 75

226 CAG CTG GAG CGG GAG AAC ACG GAG CTG GAG AGA CGC ATC CTG GAG 270
76 Gln Leu Glu Arg Glu Asn Thr Glu Leu Glu Arg Arg Ile Leu Glu 90

271 CGC AGT CAG CAG CAG GAG CCC CTC GTG TGC CCC AAC TAC CAG TCC 315
91 Arg Ser Gln Gln Gln Glu Pro Leu Val Cys Pro Asn Tyr Gln Ser 105

316 TAC TTC CGG ACC ATC GAG GAG CTC CAG CAG AAG 348
106 Tyr Phe Arg Thr Ile Glu Glu Leu Gln Gln Lys

In amino acid changes there were 6 synonymous changes and rest of the changes were non-synonymous (Table 1). Synonymous changes occurred at 128bp, 160bp, 208bp, 223bp, 251bp and 340bp positions. Non-synonymous changes were at 84, 85, 86, 294, 308, 314, 315, 341 and 342 bp positions.

The pictorial representation of phylogenetic tree of different KRT1.2 gene sequences indicates that sequence KF718797 and KF 718804 were the most distant sequences (Fig. 2).

Results depicted in Table 2 shows that the highest similarity was obtained between KF718801 with KF718796 & KF718799 and KF718803 and KF718799. The lowest value for identity matrix was 97.50% and it was between KF718804 and KF718797. The genetic similarities (homology) between all the sequences were very high. It was very close to unity as all the samples were from the same population of same breed i.e., Rambouillet sheep. These sample sequences may be genetically closely related.

Homology study with other NCBI sequence (AY835598) [2] suggested that similarity of different sequences varied from 97.12% (KF718797) to 99.58% (KF718801) (Table 3).

The protein distance matrix presented on Table 2 indicates that there was very much similarity between proteins of sequences of KF718800 and KF718803. The highest protein distance was obtained among KF718796 and KF718797. The overall protein distance were more for KF718804 sequence with all other sequences for the present study compared to other sequences' protein distance matrix.

The results of the present study indicates that there is very much similarity between the different sequences within the Rambouillet population. To improve the wool quality genetic variability within population should be maintained, so that, selection should be more effective. For this breeding of individuals with distance relationship or introduction of germplasm from outside will prove effective.

**Table 1:** Nucleotide changes at different positions

| Positions (bp) | Changes | 1st codon | 2nd codon | 3rd codon | Amino acid | Type of change |
|---|---|---|---|---|---|---|
| 83-85 | | C | T | G | Leucine (L) | NS |
| 84, 85 | T→C, G→T | C | C | T | Proline (P) | |
| 86-88 | | G | C | C | Alanine (A) | NS |
| 86 | C→G | C | C | C | Proline (P) | |
| 126-128 | | T | G | C | Cystine (C) | S |
| 128 | C→T | T | G | T | Cystine (C) | |
| 158-160 | | C | C | C | Proline (P) | S |
| 160 | T→C | C | C | T | Proline (P) | |
| 206-208 | | T | G | C | Cystine (C) | S |
| 208 | C→T | T | G | T | Cystine (C) | |
| 221-223 | | A | A | C | Asparagin (N) | S |
| 223 | C→T | A | A | T | Asparagin (N) | |
| 251-253 | | C | T | G | Leucine (L) | S |
| 251 | C→T | T | T | G | Leucine (L) | |
| 293-295 | | C | T | G | Leucine (L) | NS |
| 294 | T→G | C | G | G | Arginine (R) | |
| 308-310 | | A | C | G | Threonine (T) | NS |
| 308 | A→G | G | C | G | Alanine (A) | |
| 314-316 | | C | T | G | Leucine (L) | NS |
| 314, 315 | C→T, T→G | T | G | G | Tryptophan (W) | |
| 338-340 | | A | G | T | Serine (S) | S |
| 340 | T→C | A | G | C | Serine (S) | |
| 341-343 | | C | A | G | Glutamine (E) | NS |
| 341, 342 | C→A, A→G | A | G | G | Arginine (R) | |
| 341, 342 | C→A, A→T | A | T | G | Methionine (M) | NS |

S= Synonymous change; NS= Non-synonymous change

**Table 2:** Upper diagonal Similarity/Identity matrix (%) and below diagonal Protein distance matrix (%)

| | KF718796 | KF718797 | KF718798 | KF718799 | KF718800 | KF718801 | KF718802 | KF718803 | KF718804 | KF469215 |
|---|---|---|---|---|---|---|---|---|---|---|
| KF718796 | - | 97.92 | 98.13 | 99.17 | 98.96 | 99.58 | 97.92 | 98.75 | 98.75 | 98.96 |
| KF718797 | 4.01 | - | 98.34 | 98.75 | 98.54 | 98.33 | 98.75 | 98.75 | 97.50 | 98.54 |
| KF718798 | 2.80 | 2.40 | - | 98.13 | 97.93 | 98.55 | 98.13 | 97.72 | 97.72 | 98.34 |
| KF718799 | 1.60 | 2.39 | 2.81 | - | 99.38 | 99.58 | 98.75 | 99.58 | 98.75 | 99.38 |
| KF718800 | 2.00 | 2.80 | 3.21 | 1.19 | - | 99.38 | 98.96 | 99.79 | 98.54 | 99.17 |
| KF718801 | 0.80 | 3.21 | 2.00 | 0.80 | 1.20 | - | 98.34 | 99.17 | 99.17 | 99.38 |
| KF718802 | 3.62 | 1.99 | 3.20 | 2.00 | 1.60 | 2.81 | - | 99.17 | 97.92 | 98.54 |
| KF718803 | 2.40 | 2.39 | 3.62 | 0.79 | 0.40 | 1.60 | 1.19 | - | 98.33 | 99.38 |
| KF718804 | 2.40 | 4.84 | 3.63 | 2.41 | 2.81 | 1.60 | 3.62 | 3.22 | - | 98.54 |
| KF469215 | 2.00 | 2.80 | 2.40 | 1.19 | 1.59 | 1.20 | 2.40 | 1.19 | 2.81 | - |

**Table 3:** Homology of different sequences with NCBI reference sequence (AY835598)

| Sequence | Homology (%) |
|---|---|
| KF718796 | 99.17 |
| KF718797 | 97.12 |
| KF718798 | 98.55 |
| KF718799 | 99.17 |
| KF718800 | 98.96 |
| KF718801 | 99.58 |
| KF718802 | 97.92 |
| KF718803 | 98.75 |
| KF718804 | 98.75 |
| KF469215 | 99.38 |

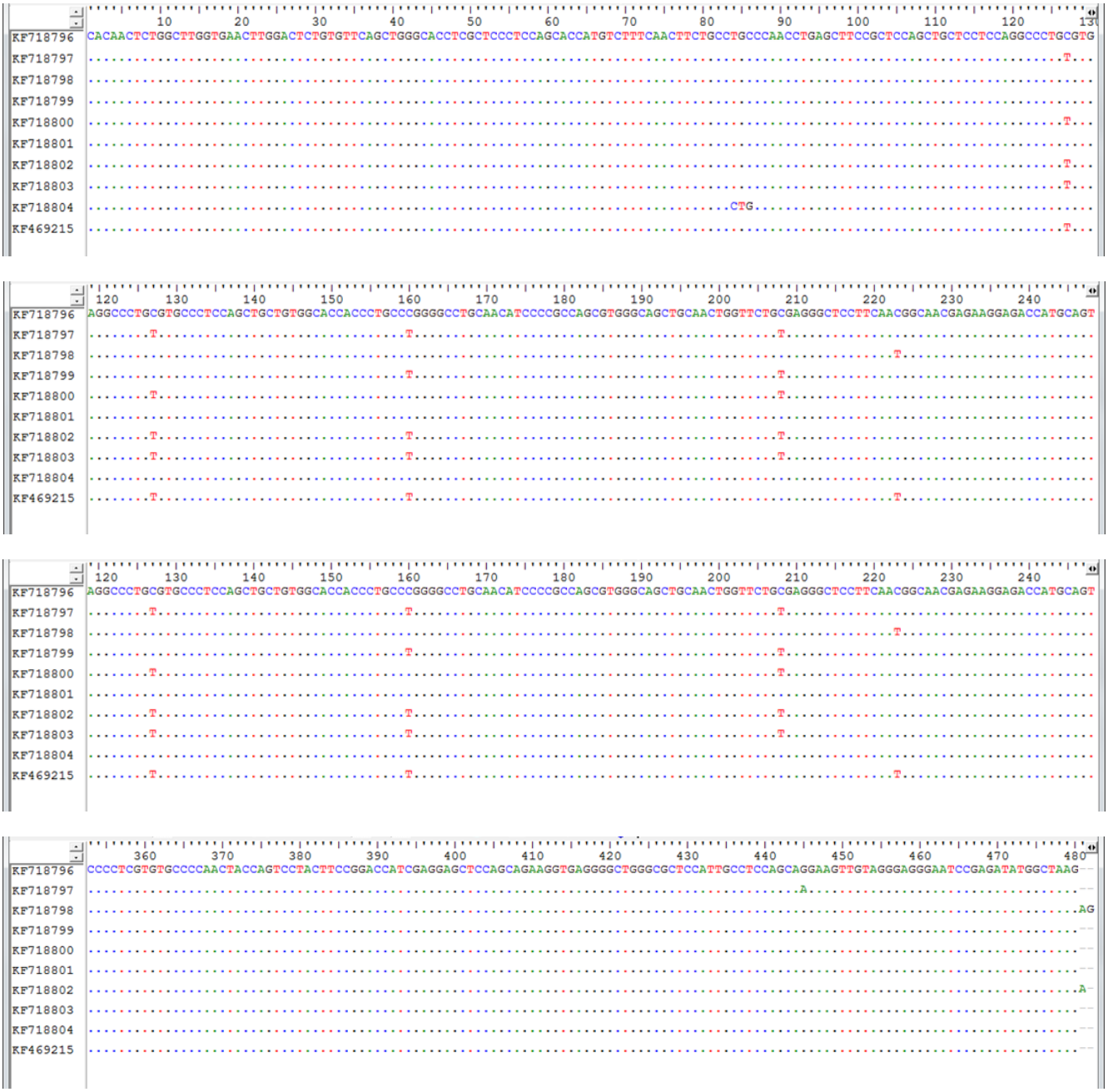


**Fig 1:** Multiple sequence alignment of 480 bp

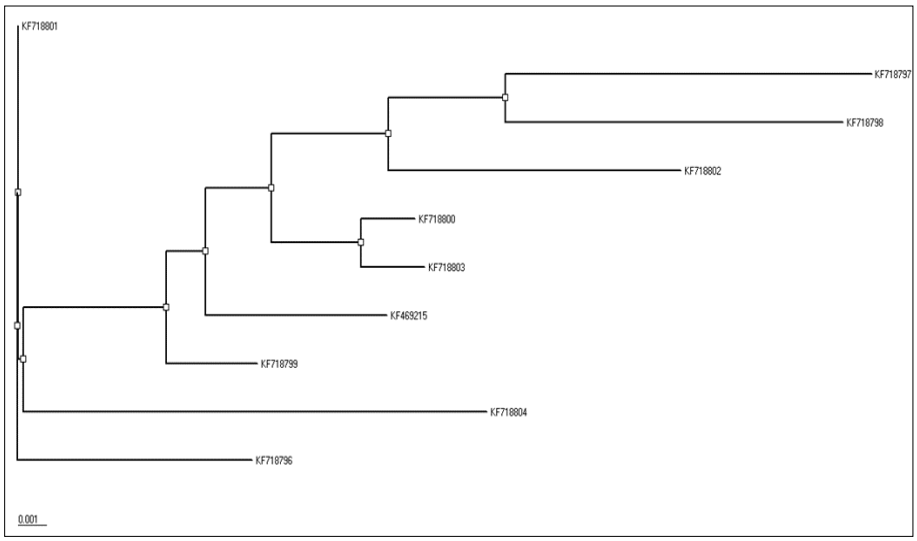


**Fig 2:** Phylogenetic tree for different sequences of KRT1.2

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