Supplemental Methods

Data analysis. Description of the procedure for sequence electropherogram correction for unequal genotyping efficiency and normalization:

The sequencing method needs a correction for unequal genotyping efficiency for the different alleles and this can be obtained comparing the peak height of the polymorphic base between known heterozygous individuals and the other DNA samples. However, in CNV it is not possible to be certain that heterozygous animals contain the 1:1 ratio for the two alleles, thus we used as reference samples i) two heterozygous samples for the g.180T>G mutation with similar electropherogram peak heights for the two polymorphic bases (T and G), pre-selected according to the RI value of the PCR-RFLP bands, and ii) two artificial heterozygous samples obtained mixing the same DNA amount, estimated using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA), of two different homozygous g.180TT and two different homozygous g.180GG animals. As reference samples for the g.187T>G SNP, two heterozygous animals that showed RI close to 0.30 were selected (a value close to the RI obtained for the reference samples constructed for the g.180T>G mutation). Sequencing peaks need also normalization before any ratio can be determined, as peak heights of different bases are not uniform in the electropherograms. The normalizing factor can be calculated for a polymorphic base, for example “T”, by taking the peak height of all “Ts” (or practically 10-20 “Ts”) before and after the polymorphic “T”. Thus, to normalize and estimate the copy ratio for the desired peak allele (*p*) in an unknown sample between the reference samples, the following formula was used:

$p = (B_{ref}/B_{unknown}) \times (b_{unknown}/b_{ref}) \times 0.5$,

where

p is the ratio for Allele 1, the allele with the base with the highest peak in the SNP position,

$q = 1 - p$ is the ratio of Allele 2,

B_{ref} is the average of all considered peak heights of the same base as Allele 1 in the considered reference sample(s) (except for the polymorphic base itself),

$B_{unknown}$ is the average of all considered peak heights of the same base as Allele 1 in the unknown sample (except for the polymorphic base itself),

$b_{unknown}$ is the peak height of Allele 1 of the polymorphic base in the unknown sample,

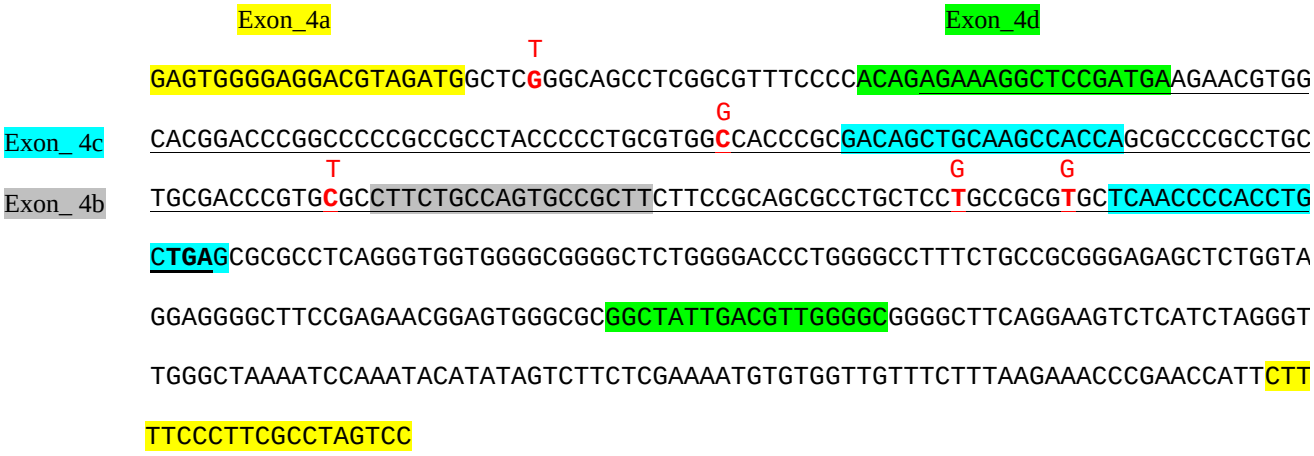
b_{ref} is the peak height of Allele 1 of the polymorphic base in the considered reference sample(s).

As no substantial difference in peak heights was evident between the two reference artificial heterozygous samples and the two reference selected heterozygous samples for the g.180T>G mutation, information of peak height from these four reference samples were averaged.

Differences of peak intensity were also detected for the intronic SNP, but as it was close to the forward primer of the Exon_4a amplified fragment it was not possible to obtain a reliable evaluation of the rate of this polymorphic site using the electropherogram patterns.

Supplemental Fig. 1. Sequence and primer pairs of the goat *ASIP* gene.

Sequence of the amplified region of the goat *ASIP* gene obtained with primer pair Exon_4a (included in EMBL ID: AM746057, without external primer sequences that have been designed on the bovine sequence) encompassing part of intron 3, the coding sequence of exon 4 (177 bp) and the complete 3'-untranslated region. The primer pair regions are indicated with different colours (Exon_4a, Exon_4b, Exon_4c, and Exon_4d). The forward primer of primer pair Exon_4b is the same of primer pair Exon_4a. The coding region is underlined. The stop codon is in bold. The five SNP positions are indicated in red.



Supplemental Table 1. PCR primers, PCR conditions, and use of the amplified products.

Genes/Primer pair	Forward and *reverse* primers (5'-3')	Amplified regions	Fragment length (bp)	PCR conditions [a]	Use
ASIP Exon_2	ggtcagagtaccagcccaaa *gtatcggcttggggagtgtt*	Part of intron 1, exon 2, and part of intron 2	352	58/1.5/TG	Sequencing
ASIP Exon_3	aaccctggggcttcctaga *caggacggttttggtagctt*	Part of intron 2, exon 3, and part of intron 3	299	56/2.0/TG	Sequencing; SQM-PCR
ASIP Exon_4a	gagtggggaggacgtagatg *ggactaggcgaagggaaaag*	Part of intron 3, the coding region of exon 4, the 3'-untranslated region and part of the 3'-flanking region	461	62/2.0/TG	Sequencing; PCR-RFLP analysis with *Hae*III (g.91C>G; and g.180T>G) or *Sac*II or *Cfr*42I (g.187T>G)
ASIP Exon_4b [b]	gagtggggaggacgtagatg *aagcggcactggcagaag*	Part of intron 3 and part of the coding region of exon 4	180	63/2.0/PT	PCR-RFLP analysis with *Hae*III (g.91C>G)
ASIP Exon_4c	gacagctgcaagccacca *ctcagcaggtggggttga*	Part of the coding region of exon 4	109	64/2.0/PT	PCR-RFLP analysis with *Hae*III (g.180T>G) or *Sac*II or *Cfr*42I (g.187T>G)
ASIP Exon_4d	acagagaaaggctccgatga *gccccaacgtcaatagcc*	Part of intron 3, the coding region of exon 4 and part of the 3'-untranslated region	298	64/2.0/PT	Sequencing; SQM-PCR
MC1R [c]	ctcgttggcctcttcatagc *gaagttcttgaagatgcagcc*	Part of exon 1 (GenBank: FM212940, *Capra hircus*)	267	59/2.0/PT	SQM-PCR
DGAT1	ccagtacctggtgagcatcc *atgagtgacagccacacagc*	Part of intron 15, exon 16, intron 16 and part of exon 17 (GenBank: DQ380250, *Capra hircus*)	215	59/2.0/PT	SQM-PCR
AHCY	gcctggatggtaaagtgcat *tctcctccccaagagcaaat*	Part of the 3'-untranslated region (GenBank: EU185100, *Ovis aries*)	294	59/2.0/PT	SQM-PCR

[a] PCR conditions: annealing temperature (°C)/[$MgCl_2$] mM/thermal cycler: PT = PT-100 (MJ Research, Watertown, MA, USA); TG = T-Gradient (Biometra, Göttingen, Germany).
[b] Primer forward of the *ASIP* Exon_4b primer pair is the same of *ASIP* Exon_4a primer pair.
[c] Primers already reported by Fontanesi et al. (2009).

Supplemental Table 2. Squared Mahalanobis distances between class means obtained clustering the RI values of the PCR-RFLP patterns in the Girgentana breed.
Squared Mahalanobis distances between class means (above diagonal) and probabilities for F statistics from squared distances (below diagonal) obtained clustering, as described in Material and Methods, the RI values of the PCR-RFLP bands of the g.180T>G and g.187T>G polymorphisms.

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	1	24.10	173.25	193.99
Cluster 2	<.0001	1	79.31	82.86
Cluster 3	<.0001	<.0001	1	13.89
Cluster 4	<.0001	<.0001	<.0001	1