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TBX3 and ASIP genotypes reveal discrepancies in officially recorded coat colors of Hucul horses

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Supplementary Table S2. Primer sequences and other amplification details.

Primer name and sequence	PCR product length (bp)	Annealing temp. °C	Source sequence or reference	Application
TBX3InDel_F1: ATAAAGTCAGGAGGCCTTTGC TBX3InDel_F2: TGGAAGGCAGAGGTTAGATCA TBX3InDel_R: CTTCTCCGGGGTCCTATTTT	223 (WT/WT)* 270 (-/-) 223 +270 (WT/-)*	61.0	NC009151.3** KT896509.1**	*TBX3* 1.6kb in/del genotyping (agarose electrophoresis)
NonDun1_F: CTGGAAGGACACTAACTTCTTGCT NonDun1_R: ATAGCTTCTCCACAAAGAGGGTTT	304	60.0	KT896509.1	*nd1* SNP genotyping (Sanger sequencing)
ASIP_F: CTTTTGTCTCTCTTTGAAGCATTG ASIP_R: GAGAAGTCCAAGGCCTACCTTG	105 (AA) 94 (aa) 105+94 (Aa)	58.5	*Rieder et al. 2001*	*ASIP* 11bp in/del genotyping (agarose electrophoresis)

TBX3InDel (F1, F2 and R) – PCR primers used for TBX3 1.6kb in/del polymorphism genotyping; NonDun1 (F and R) – PCR primers used for non-dun 1 SNP genotyping; ASIP (F and R) – PCR primers used for agouti signaling protein gene 11bp in/del polymorphism genotyping; TBX3 – the T-box 3 gene; WT – wild type; A – dominant *ASIP* allele, a – recessive *ASIP* allele, *nd1* SNP – non-dun 1 associated single nucleotide polymorphism.

PCR conditions: Initial denaturation (95°C, 5 min); 35 cycles of: denaturation (95°C, 30s), primers annealing (see above, 45s), and extension (72°C, 1 min); final extension (72°C, 10 min).

* for these genotypes an additional 1889bp fragment should be also amplified but due to using of short primer extension time it was invisible on agarose gel (its presence is not necessary for the precise genotyping); ** primers were designed on the basis of two sequences combination since dun 1.6kb insert is not present in the reference horse genome assembly