

Standard Operating Procedure for microsatellite based bovine breed identification

1. DNA analysis from blood, tissue or extracted hairs via one of the current standard protocols.
2. Microsatellite typing by an in-house routine protocol. The following 16 markers are tested:

Nr.	Name	Chr.	Primer sequence (5' -> 3')		Annealing temperature	Genbank (Accession Number)	Allele range
			Forward	Reverse			
1.	ETH225 (D9S1)	9	GATCACCTTGCCACTATTTCT	ACATGACAGCCAGCTGCTACT	55-65°C	Z14043	131-159
2.	INRA023 (D3S10)	3	GAGTAGAGCTACAAGATAAACTTC	TAACTACAGGGTGTAGATGAACTC	55°C	X67830	195-225
3.	ETH10 (D5S3)	5	GTTTCAGGACTGGCCCTGCTAACA	CCTCCAGCCCACTTTCTCTTCTC	55-65°C	Z22739	207-231
4.	CSSM66 (D14S31)	14	ACACAAATCCTTTCTGCCAGCTGA	AATTTAATGCACTGAGGAGCTTGG	55-65°C		171-209
5.	ETH3 (D19S2)	19	GAACCTGCCTCTCCTGCATTGG	ACTCTGCCTGTGGCCAAGTAGG	55-65°C	Z22744	103-133
6.	BM2113 (D2S26)	2	GCTGCCTTCTACCAAATACCC	CTTCTGAGAGAAGCAACACC	55-60°C	M97162	122-156
7.	BM1824 (D1S34)	1	GAGCAAGGTGTTTTTCCAATC	CATTCTCCAACCTGCTTCTTG	55-60°C	G18394	176-197
8.	BM1818 (D23S21)	23	AGCTGGGAATATAACCAAAGG	AGTGCTTTCAAGGTCCATGC	56-60°C	G18391	248-278
9.	ILSTS006 (D7S8)	7	TGTCTGTATTTCTGCTGTGG	ACACGGAAGCGATCTAAACG	55°C	L23482	277-309
10.	CSRM60 (D10S5)	10	AAGATGTGATCCAAGAGAGAGGCA	AGGACCAGATCGTGAAAGGCATAG	55-65°C		79-115
11.	HAUT27 (D26S21)	26	TTTTATGTTCAATTTTTGACTGG	AACTGCTGAAATCTCCATCTTA	57°C	X89252	120-158
12.	TGLA227 (D18S1)	18	CGAATTCCAAATCTGTTAATTTGCT	ACAGACAGAACTCAATGAAAGCA	55-56°C		75-105
13.	TGLA126 (D20S1)	20	CTAATTTAGAATGAGAGAGGCTTCT	TTGGTCTCTATTCTCTGAATATTCC	55-58°C		115-131
14.	TGLA122 (D21S6)	21	CCCTCCTCCAGGTAATCAGC	AATCACATGGCAAATAAGTACATAC	55-58°C		136-184
15.	TGLA53 (D16S3)	16	GCTTTCAGAAATAGTTTGCATTCA	ATCTTCACATGATATTACAGCAGA	55°C		143-191
16.	SPS115 (D15)	15	AAAGTGACACAACAGCTTCTCCAG	AACGAGTGTCTAGTTTGGCTGTG	55-60°C	X16451	234-258

Markers indicated in bold are also used in the Stockmarket kit from ABI for routine parentage testing

3. Standardization of allele lengths via comparison with a reference DNA samples (see the Table below) or via a comparison of allele frequencies with the data from the EU Resgen CT98-118 project on cattle genetic diversity (European Cattle Genetic Diversity Consortium, 2006; Negrini et al., 2007). The reference animals originate from Roslin Institute, UK (RH615, Dr. P. Wiener), Giessen (GI2000, Dr. G. Erhardt) and INRA (INRA2000, Dr. K. Moazami-Goudari).

Marker	Reference sample		
	RH 615	GI 2000	INRA 2000
ETH 225	145/149	143/149	157/157
INRA 023	199/201	215/217	199/215
ETH 10	215/215	217/219	207/219
CSSM 66	189/193	179/197	179/189
ETH 3	125/125	117/125	115/119
BM 2113	126/140	136/140	130/134
BM 1824	179/183	179/181	179/193
BM 1818	256/264	260/268	270/270
ILSTS 006	293/293	293/297	293/303
CSRM 60	97/99	97/105	93/103
HAUT 27	142/154	144/148	146/146
TGLA 227	83/87	91/99	79/89
TGLA 126	119/125	119/121	117/127
TGLA 122	140/142	150/160	142/142
TGLA 53	157/159	155/161	157/169
SPS 115	244/244	244/256	244/244

- Analysis of the results via the program GeneClass2 (Piry et al., 2004) with in the reference database genotypes from the Resgen CT98-118 project (available on request from J.A. Lenstra, Utrecht). For calculation of specificity and sensitivity, assignments with less than 90% likelihood are considered as negatives. Samples are first assigned to breeds and on the basis of this on the group of breeds allowed and not-allowed, respectively, in a specific PGI food product.

References

- European Genetic Cattle Diversity Consortium (2006) Marker-assisted conservation of European cattle breeds: an evaluation. *Anim. Genet.* **37**, 475-58
- Negrini, R., Nijman, I.J., Milanese, E., Moazami-Goudarzi, K., Williams, J.L. Erhardt, G. Dunner, S., Rodellar, C., Valentini, A., Bradley, D.G., Ajmone Marsan, P., Lenstra, J.A. & the European Cattle Genetic Diversity Consortium (2007). Differentiation of European cattle by AFLP fingerprinting. *Anim. Genet.* **38**, 60-66.
- Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., Estoup, A. (2004) GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. *Journal of Heredity* **9** Piry S, Alapetite A, Cornuet, J.-M., Paetkau D, Baudouin, L., Estoup, A. (2004) GeneClass2: A Software for Genetic Assignment and First-Generation Migrant Detection. *J. Hered.* **95**:536-5395, 536-539.