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Assessing SNPs in coat colour genes for cattle breed traceability

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ABSTRACT - Aim of this research was to identify a panel of SNPs in coat colour genes useful for breed traceability in Rendena, an autochthonous cattle breed raised in the province of Trento, and other 4 Italian cattle breeds. First, we sequenced some regions of several coat colour genes in 10 animals belonging to 5 breeds characterised by different coat colour phenotypes (Rendena, Italian Brown, Grey Alpine, Italian Friesian, and Italian Red Pied), and we detected 21 SNPs in 13 genes. These markers and 6 additional SNPs were used to genotype 180 animals of the same 5 breeds obtaining useful genotyping data for a total of 22 SNPs in 13 genes. Five out of the 22 SNP markers in the *MC1R*, *KIT*, *MLPH*, and *SILV* genes had the highest discriminating power. The panel of 22 SNPs is useful to trace Rendena particularly from Red Italian Pied and Italian Friesian.

Key words: Breed traceability, SNP, Pigmentation, Cattle.

Introduction - Tracing the breed of origin of animal products is helpful for the promotion of local food diversity with benefits for local economy, breed valorisation, and sustainable conservation of biodiversity. Traceability along the production chain plays an important role to protect the consumers from food risk and to support the marginal farmers and products from local breeds. DNA based methods could be useful to realise molecular traceability protocols to identify animals and animal derived products at breed level (Negrini *et al.*, 2008, 2009). Coat colour genes are good candidates for the traceability of farm animal breeds (Maudet and Taberlet, 2002). In fact, most breeds have been divergently selected by humans for coat colour and pattern, and still today pigmentation is one of the most important traits for breed identification. More than 100 genes are involved in mammalian pigmentation (Bennet and Lamoreux, 2000; Hoekstra, 2006), and molecular markers having fixed breed-specific allelic variants in these genes are actively sought in farm animals. Aim of this work was to identify SNPs in coat colour genes and to develop a panel of markers characterised by different frequencies useful for breed traceability purposes.

Material and methods - We sampled a total of 180 animals belonging to 5 cattle breeds. Sixty-two were young Rendena (REN) bulls raised in the genetic centre of the Rendena breed. The others belonged to the Italian Brown (BRU, n=27), Grey Alpine (GRI, n=30), Italian Red Pied (PRI, n=32), and Italian Friesian (FRI, n=29) breeds. Genomic DNA was extracted from frozen whole blood using a commercial kit (NucleoSpin Blood, Macherey-Nagel, Germany) and following the manufacturer's instructions. SNPs discovery was carried out by sequencing, aligning and comparing the PCR products of 2 animals of each of the 5 studied breeds. The panel of 180 animals was then genotyped for each of the identified SNPs and for additional SNPs from other projects by an out-sourcing service (http://kbioscience.co.uk). Using the PowerMarker v3.0 software (http://statgen.ncsu.edu/powermarker/), we calculated allele frequencies per breed and F_{st} index. The allocation tests were performed by the frequencies-based method of Paetkau *et al.* (1995) and the Bayesian-

based methods of Rannala and Mountain (1997), Badouin and Lebrun (2001) using software GeneClass2 (http://www1.montpellier.inra.fr/URLB/index.html). The probability of assignment was performed by a likelihood method without associated probabilities using as assignment threshold the value at 0.05 (Piry *et al.*, 2004). The efficiency of different algorithms was measured calculating: percentage of correct assignments (number of correct individuals allocated to breed "j"/number of animals sampled from breed "j"); overall average assignment probability (average of the probability of any correct assignment calculated per breed) and specificity (number of correct assignment to breed "j"/total (correct+incorrect) assignment to breed "j").

Results and conclusions - Sequence comparison among 10 animals from the 5 investigated breeds revealed 21 SNPs in 13 genes. We obtained a complete genotyping results for a total of 27 SNPs in 13 genes: the 21 SNPs revealed in this work, 4 SNPs from other project and 2 known *MC1R* polymorphisms (E^D and *e* alleles), for the entire panel of 180 animals. Five SNPs resulted monomorphic. The SNPs with the highest discriminating power in the 5 breeds considered (Table 1) were the 2 SNPs on *MC1R* gene with an F_{st} of 0.95-0.96 respectively, followed by 1 SNP in exon 2 of *KIT* gene (F_{st} 0.61), 1 SNP in exon 8 of *MLPH* gene (F_{st} 0.31), and 1 SNP in exon 6 of the *SILV* gene (F_{st} 0.28).

Table 1. Main genetic parameters on 22 SNP markers in 180 animals.

SNP	N° of obs gen	H expected	H observed	f[1]	F_{st}[2]	Reference
PAX3_b1_149_AC	2	0.0546	0.0562	-0.0261	0.0287	Negrini *et al.*, 2008, 2009
POMC_b1_63_CT	3	0.2303	0.2429	-0.0521	0.1343	Negrini *et al.*, 2008, 2009
MC1R_e1_E^D	3	0.2625	0.0169	0.9358	0.9513	Klungland *et al.*, 1995
MC1R_e1_e	3	0.3011	0.0170	0.9437	0.9573	Klungland *et al.*, 1995
MGRN1_exon 4	2	0.1383	0.0000	1.0000	0.2684	This work
TYRP1_exon 4	3	0.3315	0.3046	0.0841	0.0116	This work
TYRP2_exon 8	3	0.4955	0.4793	0.0357	0.0804	This work
MATP_exon 2	3	0.4547	0.4034	0.1155	0.1485	This work
MLPH_exon 8	3	0.4753	0.3450	0.2768	0.3082	This work
MLPH_exon 10	3	0.2790	0.2670	0.0458	0.1063	This work
PAX3_exon 5	3	0.4942	0.4859	0.0198	0.1016	This work
MITF_exon 10	3	0.1292	0.1167	0.1001	0.0830	This work
SILV_exon 2	3	0.4128	0.3941	0.0482	0.1521	This work
SILV_intron 2	3	0.4596	0.4545	0.0139	0.1578	This work
SILV_exon 6	3	0.4483	0.3626	0.1940	0.2840	This work
SILV_inton 6	3	0.1424	0.1314	0.0798	0.2277	This work
RAB38_intron 1	2	0.2437	0.0000	1.0000	0.0140	This work
KIT_exon 2	3	0.4914	0.2286	0.5369	0.6154	This work
KIT_exon 3	3	0.3418	0.3011	0.1218	0.2081	This work
MYO5a_intron 11_1	3	0.0882	0.0809	0.0855	0.0205	This work
MYO5a_intron 11_4	2	0.0609	0.0629	-0.0296	0.0429	This work
MYO5a_intron 11_5	3	0.4478	0.3913	0.1293	0.2028	This work
Overall		0.3083	0.2337	0.2446	0.2724	

H: heterozigosity; N° of obs gen: number of observed genotype; [1] and [2]: inbreeding-like effects within and among subpopulations.

The results of the three different allocation tests based on the panel of 22 SNPs genotyped on the 180 animals are reported in Table 2. It should be noted that the three methods gave consistent results. Considering as threshold a value of 85%, more than 80% of the animals correctly assigned with an average probability higher than 98% and a specificity higher than 95%. The breeds better assigned with the SNPs panel developed in this research are the Italian Red Pied and the Italian Friesian as expected due to the highest informative power of the SNP markers on *MC1R* and *KIT* genes that could be considered breed specific markers. Indeed, in the studied breed panel, the Italian Red Pied and the Italian Friesian are the only two breeds with eumelanic black pied and pheomelanic red pied co-

lours. The dark brown Rendena breed showed a percentage of animals correctly assigned higher than 70%. The animals belonging to the Italian Brown breed characterized by a pale brown solid coat colour were correctly assigned with percentages of 81.5% and with a specificity of 92%. The Grey Alpine breed was the worst assigned with 50% of corrected assignment. It is worth noting that specificity remained high, no animals belonging to other breeds were assigned to the Grey Alpine breed.

Table 2. Results of the allocation tests obtained with the 22 informative SNPs.

		Rannala and Mountain (1997)			Baudoin and Lebrun (2000)			Paetkau *et al.* (1995)		
Breed	N°	% CA	S	AP (%)	% CA	S	AP (%)	% CA	S	AP (%)
Italian Brown	27	81.48	0.92	98.25	81.48	0.92	98.07	85.19	0.92	97.48
Italian Friesian	29	100	1	99.87	100	1	99.54	100	1	98.59
Grey Alpine	30	50	0.94	98.50	50	0.94	98.10	50	0.94	98.16
Italian Red Pied	32	100	0.97	99.99	100	0.97	99.99	100	0.97	100
Rendena	62	72.58	0.98	97.26	74.19	1	97.13	74.19	0.98	97.72
overall	180	79.44	0.96	98.77	80.00	0.97	98.57	88.56	0.96	98.39

% CA: percentage of corrected assignment; S: specificity; AP: average assignment probability.

With the above reported SNPs is not possible to allocate efficiently all the breeds studied. At present, a new SNPs discovery step is in progress to improve Rendena breed traceability because a powerfully panel of SNPs may provide tool for adding value to animal food products from autochthonous breeds raised in a specific geographic area and for sustaining small farming and rural communities improving the economy of marginal areas.

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