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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 Fst index. The allocation tests were performed by the frequencies-based method of Paetkau et al. (1995) and the Bayesian-
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 180 animals: the 21 SNPs revealed in this work, 4 SNPs from other project and 2 known MC1R polymorphisms (E and e alleles), for the entire panel of 180 animals. Five SNPs resulted monomorphic.

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

| SNP              | N° of obs gen | H expected | H observed | f1 | f2 | 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_ED      | 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  | 0.3062 | This work |
| MLPH_exon 10    | 3             | 0.2790     | 0.2570     | 0.0458  | 0.1063 | This work |
| PA3X_exon 5     | 3             | 0.4942     | 0.4859     | 0.0198  | 0.1016 | This work |
| MTF_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_intron 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 |
| MYOSa_intron 11_1 | 2           | 0.0882     | 0.0809     | 0.0855  | 0.0205 | This work |
| MYOSa_intron 11_4 | 2           | 0.0609     | 0.0629     | -0.0296 | 0.0429 | This work |
| MYOSa_intron 11_5 | 3           | 0.4478     | 0.3913     | 0.1293  | 0.2028 | This work |
| Overall         |               | 0.3083     | 0.2337     | 0.2446  | 0.2724 | This work |

H: heterozigosity; N° of obs gen: number of observed genotype; f1 and f2: inbreeding-like effects within and among subpopulations.
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.

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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**REFERENCES -** Baudouin, L., Lebrun, P., 2001. An operational Bayesian approach for the identification of sexually reproduced cross-fertilized populations using molecular markers. Proc. Int. Symp. on Molecular Markers Eds. Doré, Dosba & Baril Acta Hort. 546:81-94. Bennet, D.C., Lamoreux, M.L., 2003. The colour loci of mice – A genetic century. Pigment Cell Res. 16:333-344. Hoekstra, H.E., 2000. Genetics, development and evolution of adaptive pigmentation in vertebrates. Heredity. 87:222-234. Klungland, H., Våge, D.I., Gomez-Raya, L., Adalsteinsson, S., Lien, S., 2008. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mamm. Genome. 19:636-663. Maudet, C., Taberlet, P., 2002. Holstein’s milk detection in cheeses inferred from melanocortin receptor 1 (MC1R) gene polymorphism. J. Dairy Sci. 85:707-715. Negrini, R., Nicoloso, L., Crepaldi, P., Milanesi, E., Marino, R., Perini, D., Pariset, L., Dunner, S., Leveziel, H., Williams, J.L., Ajmone-Marsan, P., 2008. Traceability of four European Protected Geographic Indication (PGI) beef products using Single Nucleotide Polymorphisms (SNP) and Bayesian statistics. Meat Sci. 80:1212-1217. Negrini, R., Nicoloso, L., Crepaldi, P., Milanesi, E., Colli, L., Chegdani, F., Pariset, L., Dunner, S., Leveziel, H., Williams, J.L., Ajmone-Marsan, P., 2009. Assessing SNP markers for assigning individuals to cattle populations. Anim. Genet. 40:18-26. Paetkau, D., Calvert, W., Stirling, I., Strobeck, C., 1995. Microsatellite analysis of population structure in Canadian polar bears. Mol. Ecol. 4:347-354. 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. 95:536-539. Rannala, B., Mountain, J.L., 1997. Detecting immigration by using multilocus genotypes. Proc. Natl. Acad. Sci. U.S.A. 94:9197-9221.

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

| Breed              | N° | % CA | S  | AP (%) | % CA | S  | AP (%) | % CA | S  | AP (%) |
|--------------------|----|------|----|--------|------|----|--------|------|----|--------|
| Italian Brown      | 27 | 81.48| 0.92| 98.25  | 50.5 | 9 | 98.07  | 85.19| 0.92| 97.48 |
| Italian Friesian   | 29 | 100  | 1   | 99.87  | 100  | 1 | 99.55  | 100  | 1   | 98.59 |
| Grey Alpine        | 30 | 50   | 0.94| 98.56  | 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  | 88.57| 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.**