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Short communication

Characterization of a SNPs panel for meat traceability in six cattle breeds

L. Orrù [a,*], G. Catillo [b], F. Napolitano [b], G. De Matteis [b], M.C. Scatà [b], F. Signorelli [b], B. Moioli [b]

[a] Genomic Research Centre (CRA-GPG), Via S Protaso 302, 29017 Fiorenzuola D'Arda, Italy
[b] Research Centre for Meat Production and Genetic Improvement (CRA-PCM), Via Salaria 31, 00016 Monterotondo, Italy

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ABSTRACT

Development of DNA technologies makes today possible implementation of conventional beef traceability systems with molecular methods. In the recent past, microsatellites have been the most used marker for individual assignment, however single nucleotide polymorphisms (SNPs) is now replacing them. With the aim to provide a set of SNPs useful for bovine meat traceability we have tested 63 SNPs for the ability to identify single individuals in six European cattle breeds. Eighteen highly informative SNPs located in different genes, have been selected. By using this panel of SNPs the probability that one individual is incorrectly assigned ranges from 1.39 to 0.07 out of 1 million, depending on the breed.

1. Introduction

On January 28th, 2002 the European Parliament adopted Regulation (EC) 178/2002 containing general provision for traceability (Schagele, 2005). Traceability is defined as the ability to trace and follow food, feed, and ingredients through all stages of production, processing and distribution.

The regulation requires that traceability must be verified at all stages of production, processing and distribution. The objective of the regulation is to ensure protection for the consumers. Applied to meat industry, traceability relies on a labelling system that ensuring connection between the individual animal and the beef at the retail. Several labelling system have been employed for the identification of cattle, as well as animal products; they include ear tags, tatoos and electronic transponders (Ammendrup & Fussel, 2001). However, after carcass disassembling, it is difficult to trace the identity of each single cut of meat through the distribution chain, and this opens risks of fraud. In the last years, the development of DNA technology allowed to combine the conventional labelling system with the analysis of DNA, therefore improving the traceability system. Because animals differ from each other's in their DNA sequences, the genotyping of polymorphic sites provides an unique DNA fingerprint, specific of each individual (Cunningam & Meghen, 2001; Dalvit, De Marchi, & Cassandro, 2007; Jobling & Gill, 2004). It is therefore possible to employ these methods to follow the meat samples along the retail chain, by generating a DNA profile that can be used to trace-back the identity of the individual animal from the carcasses or the meat cuts. To implement this method, biological samples of individual animals should be stored for a period of time.

In the last decade, microsatellites have been the most used DNA markers in animal identification and parentage determination (Orrù, Napolitano, Catillo, & Moioli, 2006; Sancristobal-Gaudy et al., 2000; Vignal, Milan, SanCristobal, & Eggen, 2002) however a new class of genetic markers named SNPs (Single Nucleotide Polymorphisms) is now replacing microsatellites as the most popular markers (Vignal et al., 2002). SNPs are single base change in DNA sequences and although biallelic, they show some advantages over microsatellites, mainly because they overtake the major problem of microsatellites, consisting in the lack of unicity in determining their size, when genotyping is performed on different analyzers and/or at different times. SNPs are highly abundant in the genome, i.e., averagely one SNP every 100–500 base pairs (Heaton et al., 2001) and several technologies are now well established for SNPs genotyping (MALDI TOF assay, primer extension, TaqMan, and several microchip technologies) (Bray, Boerwinkle, & Doris, 2001; Dearlove, 2002; Syvanen, 2005; Podder, Ruan, Tripp, Chu & Tebbutt, 2008). These technologies allow high throughput automated analysis and the SNPs databases so obtained are comparable even when changing genotyping platform. It is therefore possible to share databases between laboratories. In the present study a panel of 18 SNPs have been evaluated for the ability to trace the individual animal from biological samples.

2. Materials and methods

2.1. Sampled animals and genotyping

Samples from 528 animals, representing six cattle breeds that are widely used in Europe (103 Friesians, 40 Simmental, 107

* Corresponding author. Tel.: +39 0523 983758; fax: +39 0523 983750.
E-mail address: luigi.orru@entecra.it (L. Orrù).

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Piedmontese, 68 Chianina, 109 Charolaise and 101 Limousine) were used for polymorphism testing. Animals for genotyping were chosen in order to assure that they were a representative sample of each breed; in detail, for the Friesian breed two thirds were non-related cows of various Italian and Danish herds, and one third were AI sires, the semen of which was purchased from two Italian AI centres; for the Simmental and the Piedmontese, we used the young bulls from the performance testing stations of the corresponding Breed Societies; for the Chianina, sampled animals belonged to various herds of central Italy; animals of the Charolaise and Limousine breeds were provided from two slaughter houses, importing directly from France. DNA was extracted either from 5 ml blood, or from one semen dose, with the Genomix Kit (Talent), following manufacturer's protocols, or from 0.3 g muscle, with the Genomix Kit (Talent), modified as in Orrù et al. (2006). The tested SNPs were chosen from the NCBI dbSNP database (www.ncbi.nlm.nih.gov/projects/SNP/). In the dbSNP database, polymorphisms are reported with at least 100 bases of flanking sequence surrounding the variation. Using these sequences, for each SNP we perform a BLAST search against the Bos Taurus genome using the megaBLAST tool present in the NCBI database. In this way SNPs were assigned to their respective gene. In Table 1 the gene name, the symbol used, the chromosome position and the corresponding SNP are reported. SNPs were genotyped by KBioscience Ltd (http://www.kbioscience.co.uk/) using their own novel fluorescence-based competitive allele specific PCR (KASPar) assay. Details of the method used can be found at http://www.kbioscience.co.uk/.

2.2. Statistical analysis

Allele frequencies were estimated by direct counting. The probability of genotypic identity for two random individuals was

Table 1
List of gene names with the symbol used, chromosome numbers, and genotyped SNPs.

Gene name	Gene symbol	Chromosome	NCBI dbSNP ID
Acetyl-CoA carboxylase	ACACA	19	ss77832248
Alcohol dehydrogenase 7	ADH7	6	ss77831988
Aldehyde dehydrogenase 6	ALDH6	10	ss77831991
Bone morphogenetic protein 1	BMP1	8	ss77832007
Calmodulin 3	CALM3	18	ss77832019
Calpain	CAPN3	10	ss77832266
Calsequestrin1	CASQ1	3	ss77831823
Cytochrome oxidase	COX5B	11	ss77832287; ss77832285
Corticotropin releasing hormone receptor 1	CRHR1	19	ss77831799
Cathepsin F precursor	CTSF	29	ss77831850
Fatty acid binding protein	FABP4	14	ss77831725; ss77831853
Farnesyl diphosphate farnesyl transferase 1	FDFT1	8	ss77832045
Fibromodulin	FMOD	16	ss77831747
Growth differentiation factor 11	GDF11	5	ss77831747
Myostatin	GDF8	2	ss77831864
Growth hormone receptor	GHR	20	ss77832150
G protein-coupled receptor 24	GPR24	Unknown	ss77831802
Glucuronidase beta	GUSB	25	ss77832322
Glycogenin	GYG	1	ss77832333
Hydroxyacyl-Coenzyme A dehydrogenase	HADHSC	26	ss77832053
Histone deacetylase 1	HDAC1	2	ss77831869
Insulin-like growth factor 1	IGF1	5	ss77831727
Insulin-like growth factor 2 receptor	IGF2R	9	ss77831881
Insulin-like growth factor binding protein	IGFBP2	2	ss77832344; ss77832350
Insulin receptor	INSR	7	ss77831888
Leptina	LEP	4	ss77831750
Lysyl oxidase-like	LOXL1	21	ss77832172
Melanocortin-5 receptor	MC5R	24	ss77832364
Malic enzyme 3.	ME3	29	ss77831899; ss77831890
Matrix metallo proteinase 1	MMP1	15	ss77831924; ss77831917
Musculin	MSC	14	ss77831787
Myogenic factor 5	MYF5	5	ss77832180
Myogenic factor 6	MYF6	5	ss77831752
Myosin, heavy chain 1	MYH1	19	ss77832081; ss77832079
Myosin, heavy chain 2	MYH2	19	ss77832182
Myosin, light polypeptide 1	MYL1	2	ss77832194; ss77832193
Promyelocytic leukemia	MYL2	17	ss77832198
Nebulin	NEB	2	ss77832090
Paired box family of transcription factors	PAX3	12	ss77831861; ss77831953
Propionyl-CoA carboxylase beta chain	PCCB	1	ss77832098
Phosphoglycerate mutase 2	PGAM2	Unknown	ss77831774
Proopiomelanocortin	POMC	11	ss77832219
Pyruvate dehydrogenase phosphatase	PPM2C	14	ss77832222
Protein kinase, AMP-activated, alpha 2 catalytic subunit	PRKAA2	3	ss77831776
Protein kinase, AMP-activated, gamma 2 non-catalytic subunit	PRKAG2	7	ss77832377
Protein kinase, AMP-activated, gamma 3 non-catalytic subunit	PRKAG3	2	ss77832388
Secreted protein acidic cysteine rich	SPARC	7	ss77832111
Troponin T1	TNNT1	18	ss77831735
Troponin T2	TNNT2	2	ss77832396
Titin	TTN	2	ss77832244
Uncoupling protein 2	UCP2	15	ss77831780
Uncoupling protein 3	UCP3	15	ss77832410; ss77832403; ss77832405
Vitronectin	VTN	19	ss77832412