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# Molecular markers as guarantee of bovine meat traceability

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**SUMMARY** – The latest confidence crisis of consumers originated by the appearance of BSE have impelled the need to guarantee the origin of animals and their products. The objective of this work has been the establishment of a genetic traceability system using microsatellite DNA markers. Animals from two herds belonging to Parda de Montaña and Pirenaica cattle were monitored. A total of 674 samples have been analysed using the panel of microsatellites recommended by the International Society of Animal Genetics (ISAG) for identification studies. Different genetic parameters have been calculated and a program to verify the conventional traceability systems used has been designed. From a total of 111 meat samples obtained from slaughterhouse, it was possible to establish an objective link between an individual piece of meat, the animal and the farm in 102 cases, that is, 91.89%. From the remaining 8.11%, we found practically no links in 6 cases. Only in 2.7% of the total meat samples was it impossible to establish a correct link.

**Keywords:** Bovine, traceability, microsatellites.

**RESUMÉ** – "Les marqueurs moléculaires comme garantie de la traçabilité de la viande bovine". Les dernières crises de confiance des consommateurs par l'apparition de la maladie des vaches folles a entraîné la nécessité de garantir l'origine des animaux et de leurs produits. Un total de 674 échantillons appartenant à deux troupeaux (des races Parda de Montaña et Pirenaica) ont été analysés en utilisant des marqueurs des microsatellites recommandés par la Société Internationale de Génétique Animale (ISAG) pour des études d'identification. Différents paramètres génétiques ont été calculés et un programme pour vérifier les systèmes conventionnels de traçabilité déjà utilisés a été désigné. D'un total de 111 échantillons de viande obtenus à partir de l'abattoir, il était possible d'établir un lien objectif entre un morceau individuel de viande, l'animal et la ferme dans 102 cas, équivalant à 91,89%. Seulement chez 2,7% de tous les échantillons de viande des 8,11% restants il était impossible d'établir le correct lien.

**Mots-clés :** Bovin, traçabilité, microsatellites.

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## Introduction

Traceability of meat has become a very important aspect of food quality assurance for consumers and producers, mainly in the last years due to the precedent crises occurred in food industry as the major outbreak of BSE (Bovine Spongiform Encephalopathy).

Traceability in the context of food safety is defined in the European Regulation 178/2002 (EC, 2002) as the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporate into a food or feed, through all stages of production, processing and distribution. The application of traceability systems in the food chain has become obligatory since January 2005 (Neira, 2005).

Conventional traceability systems should ensure the animal origin of meat products, by means of ear tags, passports or labelling systems. Nevertheless this system could fail; the lost of document and the risk of cheating are real, that is why the need to develop techniques for verifying "a posteriori" the origin of meat (Loftus,2005).

The DNA identification technology offers a powerful means of authenticating and controlling these

conventional animal identification systems (Cunningham and Meghen, 2001) because the genetic information in each individual remains inalterable during its whole life, that is why this system displays an objectivity, repeatability and security not showed by other systems developed up to now.

The possibility to verify the animal products using scientist and objective methods increases the quality certification value (e.g. brands, PGI) allowing the development of some economic areas through the importance of typical products and given incentives for population conservation, keeping the biodiversity (Marsan *et al.*, 2004).

The aim of this work was to verify the traditional traceability system using microsatellites DNA markers in two herds from North Spain, and to develop an algorithm to compare DNA profiles that allows the establishment of a correct link between a meat sample, the animal and then the farm.

## Material and methods

### Samples

Animals from two herds belonging to Parda de Montaña and Pirenaica cattle were included in the study. Blood samples were obtained from every ancestor and descendant. In addition muscle pieces were sampled from offspring that went to slaughterhouse.

We got a total of 674 samples. Their accompanied data (identification number, sex, date of birth in case of offspring, data from parents in case of offspring, etc.) were entered in a database (Microsoft Access) to corroborate the origin of sample after genotyping.

### DNA extraction

DNA was extracted from 100 µL of whole blood using the GFX Genomic Blood DNA Purification Kit (Amersham Biosciences) and following the direct method described by the manufacturer. On the other hand, 200 mg of frozen meat sample were mixed with 1 ml of 1x RSB buffer pH 7.4 (10 mM Tris-HCl, 10 mM NaCl, 25 mM EDTA) and homogenized using a polytron homogenizer (OMNI GLH 220). Afterwards 40 µl SDS 20%, and 20 µl proteinase K 20 mg/ml were added and the mix was incubated at 50°C for 3 hours. Standard protocols were used for DNA purification.

The quality and quantity of DNA were testing using a GeneQuant pro RNA/DNA Calculator spectrophotometer.

### Microsatellite markers and PCR

Nine microsatellites recommended by the International Society of Animal Genetics (ISAG) for animal identification were tested. Data of primers sequences and fluorescent label at 5'position, chromosomal location and range are listed on Table 1.

A single multiplex PCR reaction was designed to amplify the nine microsatellites. All 5'modified primers were combined to get fragments with variable length and labelling. The PCR reaction was achieved using 50 ng of genomic DNA, the appropriate amount of each primer pairs and the QUIAGEN Multiplex PCR Kit 5x (QUIAGEN) in a 6 µl of final volume reaction. A thermocycler (2400 Applied Biosystems) was used under the following conditions: 1 cycle of 15' at 95°C; 30 cycles as follows: 30" at 95°C, 1' at 58°C, 1' at 72°C, and a final extension of 30' at 72°C.

Electrophoresis was performed in an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems) and the resulting profiles interpreted by GeneMapper v3.7 Software (Applied Biosystems).

### Statistical Analysis

Allele and genotype frequencies were calculated using the Genepop v3.4 Software (Raymond and

Rousset, 1995) and exclusion probabilities were also determined. The match probability (MP) (Weir, 1996), which is defined as the probability of two animals sharing an identical allelic profile by chance, was determined in order to evaluate the power of microsatellite loci to discriminate among different individuals.

Table1. Microsatellites, PCR primer sequences and 5' fluorescent label, amplicon size and chromosomal location

Loci	Primer's sequences, and label at 5' in reverse primer	Range (pb)	Chromosome
ETH225 (D9S1)	GATCACCTTGCCACTATTTCT <b>NED-</b> ACATGACAGCCAGCTGCTACT	140-158	9
INRA023 (D3S10)	GAGTAGAGCTACAAGATAAACTTC <b>NED-</b> TAACTACAGGGTGTAGATGAACTC	196-222	3
ETH10 (D5S3)	G TTCAGGACTGGCCCTGCTAACA <b>PET-</b> CCTCCAGCCCACTTTCTCTTCTC	209-223	5
BM2113 (D2S26)	GCTGCCTTCTACCAAATACCC <b>PET-</b> CTTCTGAGAGAAGCAACACC	125-143	2
BM1824 (D1S34)	GAGCAAGGTGTTTTTCCAATC <b>6-FAM-</b> CATTCTCCAAGTCTTCCTTG	178-190	1
TGLA227 (D18S1)	CGAATTCCAATCTGTTAATTTGCT <b>VIC-</b> ACAGACAGAACTCAATGAAAGCA	77-97	18
TGLA126 (D20S1)	CTAATTTAGAATGAGAGAGGCTTCT <b>6-FAM-</b> TTGGTCTCTATTCTCTGAATATTCC	113-125	20
TGLA122 (D21S6)	CCCTCTCCAGGTAAATCAGC <b>VIC-</b> AATCACATGGCAAATAAGTACATAC	137-183	21
SPS115 (D15)	AAAGTGACACAACAGCTTCTCCAG <b>VIC-</b> AACGAGTGTCTAGTTTGGCTGTG	246-260	15

### Application to traceability

An algorithm to compare DNA profiles was developed. It allows to establish the correct link between a meat sample, the animal and the farm. Moreover the algorithm allows to assign possible progenitors by searching in the DNA profiles database.

### Population assignment of individuals

The power of the WHICHRUN v4.1 software (Banks and Eichert, 1999), a computer program for population assignment of individuals based on multilocus genotype data to allocate individuals to their most likely source population, was evaluated for the assignment of a muscle sample to their breed using the data from the herds analysed and from data obtained in our laboratory in other cattle populations (Casta, Betizu, Serrana Negra, Mallorquina, Menorquina, Monchina, Pirenaica and Lidia)

### Results and discussion

The 674 samples were genotyped for the nine microsatellites. The number of alleles varied between 4 for locus TGLA 126 and 12 for TGLA 122, results that are in accordance with those reported by Vázquez *et al.* (2002) in "IGP carne de Asturias".

The probability of exclusion of microsatellite set was evaluated for all individuals in this study, showing that the panel of nine microsatellites is enough to get paternity exclusion upper than 99.9%. Excluding the TGLA 126 locus, which appears as the less polymorphic locus, we also obtain the

same results. So we could use an eight microsatellite panel for animal identification for routinely assays in traceability studies.

The probability of selecting two individuals by chance that share the same genotype in the 9 microsatellites was lower than  $10^{-8}$ , even when related animals were included in the study (Table 2). Considering commercialization conditions, the probability of an existing fraud not being detected will be the probability of two DNA profiles matching by chance (Vázquez *et al.*, 2004).

Table 2. Match probability in the different populations

Cattle population	Match probability (using nine microsatellites)
Parda	$2.29123 \times 10^{-9}$
Pirenaica I	$3.73857 \times 10^{-8}$
Pirenaica II	$9.15626 \times 10^{-9}$
Pirenaicas I+II	$8.24509 \times 10^{-9}$
Global	$1.4419 \times 10^{-9}$

### Application to traceability

The genotyping procedure allowed to reconstitute the two right blood-meat pairs. From a total of 111 meat samples obtained from slaughterhouse, it was possible to establish an objective link between an individual piece of meat, the animal and the farm in 102 cases, it means 91.89%. From the remaining 8.11%, we could found the causes of unlink in 6 cases. Then, only in a 2.7% of the total meat samples was impossible to establish a correct link. These results are summarized on Table 3.

Table 3. Possible causes of failures of matching

Meat sample (ID number)	Blood sample (ID number)	Result. Possible causes of failures of matching
5246 (I)	4842 (I)	No identical sample. No blood genotyped
5252 (II)	4847 (II)	No identical sample. Idem to blood sample 4857 (crossed samples), possible wrong labelled
5254 (III)	4857 (III)	No identical sample. Idem to blood sample 4847 (crossed samples), possible wrong labelled
5256 (IV)	4857 (IV)	No identical sample. Idem to 4872, maybe blood sample 4857 did not come and blood sample number 4872 came in the received tube, as they were received in the same date
5507 (V)	4932 (V)	No identical sample. Meat sample unknown origin, incompatible with father and mother, nevertheless the correspondent blood is compatible with the parents assigned in the database
5632 (VI)	5605 (VI)	No identical sample. Idem to 5574 received 0806 (the others blood and meat were received the same date, possible wrong labelled). Unknown origin
5767 (VII)	5195 (VII)	No identical sample. So many discordances. Unknown origin.
5774 (VIII)	5214 (VIII)	No identical sample. Idem to blood sample 5227 (crossed samples), possible wrong labelled
5781 (IX)	5227 (IX)	No identical sample. Idem to blood sample 5214 (crossed samples), possible wrong labelled

We could detect the possible practical causes of unlinked blood sample-meat in six from the nine cases. As we were working in a real live situation we could assume that this should be the percentage of error obtained routinely at slaughterhouse. So the percentage of error using traditional traceability

methods would be in the order of 8%. Using genetic methods we were able to minimize this value by detecting the causes of error/fraud as we have shown in Table 3.

## Population assignment of individuals

From the total of 111 meat samples we could assign correctly 105 samples to Pirenaica or Parda breeds, corresponding to 94.58%. In the remaining 5.41%, we found that three of these cases were the same than those that we could not link with blood, meaning that only 2.705% of cases could not be assigned correctly.

We also tested the assignment probability to herd using each Pirenaica farm as a different population. In this case, from a total of 66 meat samples, we could not assign correctly 8 cases, it means 87.88% from which 4 unassigned samples belonged to the first Pirenaica farm and the other 4 to the second. These results could be logical as we are working with the same breed, so the allelic frequencies are too similar.

Using the data obtained from others breeds (including a different Pirenaica population), we found that the 90.91% of meat samples were correctly assigned to Pirenaica breed and the remaining 9.09% was assign almost always to Serrana Negra breed, a population that could have been mixed with Pirenaica.

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