Available online at www.sciencedirect.com
ScienceDirect
Food Research International 41 (2008) 301–307
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

Genetic traceability of meat using microsatellite markers

C. Dalvit *, M. De Marchi, C. Targhetta, M. Gervaso, M. Cassandro

Dipartimento di Scienze Animali, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Received 23 August 2007; accepted 27 December 2007

Abstract

Traceability systems have become necessary, especially for beef products, to protect consumers' health. Aims of this study were to validate and to test a set of 12 microsatellite (STR) markers for the assessment of a genetic traceability system in six cattle breeds. The probability to find, by chance, two individuals sharing the same profile at the studied loci, was computed considering different number of STR, pooling the alleles in each breed, in the total population and in the dairy and beef population separately. Best results were then obtained considering match probabilities per breed. In this study, genotyping the five most polymorphic loci, the probability of finding two identical animals was approximately five in one million. Match probability values increased when the pooled marker sets were used, but were still satisfactory; moreover, use of the pooled marker sets will reduce the cost of analyses.

Keywords: Genetic traceability; Meat; Individual identification; Match probability; Cattle breeds; Microsatellite

1. Introduction

After the bovine spongiform encephalopathy outbreak, which caused a decrease in beef consumption in many European countries (Ciampolini, Leveziel, Mozzanti, Grohs, & Cianci, 2000), the development of a traceability system has become necessary to protect public health and ensure food safety. The European Union implemented, through regulations EC 1760/2000 and 1825/2000, a mandatory labeling system for beef and beef products. According to the mentioned legislation, every beef cut must show a label carrying the following information: an identification code referring to an animal or to a group of animals, and the country where the animal was born, fattened, slaughtered, and sectioned. However, as pointed out by several authors (Cunningham & Meghen, 2001; Orrù, Napolitano, Catillo, & Moioli, 2006; Sancristobal-Gaudy et al., 2000), frauds and mistakes along the production chain cannot be fully avoided by this system. Animal identification using DNA-based techniques could address this problem, as DNA is inalterable during all animal life and it is present in every tissue. Microsatellite (STR) markers, due to the high polymorphism, had already been widely investigated for many applications such as paternity analysis (Jobling & Gill, 2004) and breed assignment tests in many species (Bjørnstad & Røed 2001; Ciampolini et al., 2006; Koskinen, 2003); they were also investigated in studies on individual identification highlighting promising results (Orrù et al., 2006; Sancristobal-Gaudy et al., 2000; Vázquez et al., 2004).

The main objective of the present study was to set up a panel of STRs to implement a genetic traceability system in six cattle breeds. This goal consisted of two major tasks: to validate 12 STR markers on the basis of their genetic variation among and within cattle breeds, and to set up an efficient set of STR markers for individual identification of the studied breeds considering the genetic differentiation among them.

2. Materials and methods

2.1. Animal sampling and genotyping

The dataset consisted of 183 animals belonging to six cattle breeds, four of them are indigenous Italian beef

* Corresponding author. Tel.: +39 0498272616; fax: +39 0498272633.
E-mail address: chiara.dalvit@unipd.it (C. Dalvit).

0963-9969/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2007.12.010

breeds: Chianina (CH, $n = 27$), Marchigiana (MA, $n = 27$), Romagnola (RO, $n = 23$), and Piemontese (PI, $n = 33$), while two are cosmopolitan dairy breeds: Holstein Friesian (HF, $n = 29$) and Brown Swiss (BS, $n = 44$). Samples from beef breeds were collected in performance stations, samples from dairy breeds, in herds located in different geographical area. No pedigree data were recorded and samples were collected randomly to reproduce the market situation. Samples of the PI breed derived from animals of the ANABORAPI breeder association located in Cuneo (North-West Italy), the CH, MA and RO breed samples were collected from the ANABIC breeder association located in Perugia (Central Italy). Samples belonging to the HF and BS breeds were collected in six different farms of the Trentino Alto Adige region (North-East Italy). Blood samples were collected from each animal in 5 ml vacutainar tubes containing sodium citrate as anticoagulant, and stored at −20 °C until analyses were performed. DNA extraction was carried out with the use of the "Gentra System PUREGENE DNA purification kit" starting from 300 μl of whole blood. DNA samples were then amplified by PCR in correspondence of the following 12 STR loci: BM1818, ETH185, MM12, TGLA126, BM203, TGLA122, RM12, ILST008, SPS115, BL42, ETH3 and TGLA53 (Table 1). The investigated loci were chosen in accordance to ISAG/FAO Standing Committee Recommendations (2004) and consulting previous studies (Barendse, Vaiman, Kemp, et al. 1997; Bishop et al., 1994; Grosz et al., 1997; Kemp, Brezinsky, & Teale, 1993), to have highly polymorphic markers located all over the genome. For the amplification, 25 ng of DNA were added to a reaction mix containing: 1 pmol/μl of primer forward and reverse, 1× PCR buffer (16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl pH 8,8, 0.01% Tween 20), 0.26 mM of every dNTPs, 2.5 mM of $MgCl_2$ and 0.8 U of *Taq* DNA polymerase, in a final volume of 20 μl. The 12 STRs were individually analyzed by a PX2 Thermohybrad thermal cycle at the following conditions: initial denaturation step of 5 min at 95 °C, 40 cycles of 30 s at 94 °C, 1 min at the primer specific annealing temperature and 1 min at 72 °C, followed by a final extension of 10 min at 72 °C. Allele size was determined with a Perkin–Elmer ABI Prism 3730XL Genetic Analyzer, using GeneScan 2.0 and Genotyper 3.7 software (Perkin–Elmer).

2.2. Statistical analysis

Genetic variability of markers and breeds was analyzed aiming to validate the chosen STR set. Allelic frequencies and observed and expected heterozygosity, in the whole population and per breed, were calculated with Genetix 4.03 (Belkhir, Borsa, Goudet, Chikri, & Bonhomme, 1998). The Fstat 2.9.3 program (Goudet, 1995) was used in calculations of mean number of alleles, allelic richness,

Table 1
Investigated STRs, primer sequence, location, annealing temperature and size of the amplified fragments

Locus	Primer sequence	Chromosome	Annealing T (°)	Fragment size (bp)
BM1818	FW: AGCTGGGAATATAACCAAAGG RW: AGTGCTTTCAAGGTCCATGC	23	58	257–279
ETH185	FW: TGCATGGACAGAGCAGCCTGGC RW: GCACCCCAACGAAAGCTCCCAG	17	64	216–242
MM12	FW: CAAGACAGGTGTTTCAATCT RW: ATCGACTCTGGGGATGATGT	9	58	108–134
TGLA126	FW: CTAATTTAGAATGAGAGAGGCTTCT RW: TTGGTCTCTATTCTCTGAATATTCC	20	58	118–130
BM203	FW: GGGTGTGACATTTTGTTCCC RW: CTGCTCGCCACTAGTCCTTC	27	58	207–237
TGLA122	FW: CCCTCCTCCAGGTAAATCAGC RW: AATCACATGGCAAATAAGTACATAC	21	58	136–182
RM12	FW: CTGAGCTCAGGGGTTTTTGCT RW: ACTGGGAACCAAGGACTGTCA	7	58	103–107
ILST008	FW: GAATCATGGATTTTCTGGGG RW: TAGCAGTGAGTGAGGTTGGC	14	60	173–178
SPS115	FW: AAAGTGACACAACAGCTTCTCCAG RW: AACGAGTGTCCTAGTTTGGCTGTG	15	64	247–261
BL42	FW: CAAGGTCAAGTCCAAATGCC RW: GCATTTTTGTGTTAATTTCATGC	13	58	231–237
ETH3	FW: GAACCTGCCTCTCCTGCATTGG RW: ACTCTGCCTGTGGCCAAGTAGG	19	62	98–126
TGLA53	FW: GCTTTCAGAAATAGTTTGCATTCA RW: ATCTTCACATGATATTACAGCAGA	16	62	151–183