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Utilization of a 17 Microsatellites Set for Bovine Traceability in Czech Cattle Populations

Research Article

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ABSTRACT

For identification of individuals and parentage control performed by cattle breeders in the Czech Republic, a novel Finnish Bovine Genotypes™ Panel 3.1 was amplified by means of one multiplex polymerase chain reaction. Bovine Panel encompasses all the 12 STR loci recommended by the International Society for Animal Genetics (ISAG) for routine use in parentage testing and identification, including *TGLA227*, *BM2113*, *TGLA53*, *ETH10*, *SPS115*, *TGLA126*, *TGLA122*, *INRA23*, *ETH3*, *ETH225*, *BM1824* and *BM1818*. In addition, the kit included the following six microsatellites which were among the list of loci recommended by the Food and Agriculture Organization of the United Nations (FAO) for genetic studies of domestic animals: *SPS113*, *RM067*, *CSRM60*, *MGTG4B*, *CSSM66* and *ILSTS006*. Afterwards, the length of these microsatellite's polymorphisms was examined by fragment analysis of the amplified products. The investigated population consisted of 125 animals of three bovine breeds (Fleckvieh, n=50; Charolais, n=50 and Beef Simmental, n=25). A total of 337 alleles were identified and all microsatellite DNA markers showed high polymorphism. The probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion were 0.9942/0.9798/0.9999 (Fleckvieh), 0.9834/0.9744/0.9999 (Charolais), 0.9828/0.9682/0.9999 (Beef Simmental). Research data certified the possibility of using the Finnish panel of DNA microsatellites markers for the traceability purposes in the Czech cattle populations.

KEY WORDS cattle, microsatellites, multiplex PCR, parentage control.

INTRODUCTION

Short Tandem Repeat (STR) loci, microsatellites, are a class of nuclear DNA markers consisting of tandemly repeated sequence motifs of two to seven base pairs in length. Due to numerous technical and practical reasons, microsatellites have been widely used in the fields of human and animal identification and parentage testing, as well as in human forensics. The human identification and forensic testing communities have standardized and validated a set of STR loci encompassing mainly tetra nucleotide repeat. However, ISAG's cattle standing committee has chosen sets of dinucleotide STR loci for bovine testing markers (Koskinen, 2006). ISAG has currently recognized and validated numerous interlaboratory comparison tests of 12 microsatellite loci for routine use in bovine kinship analysis (http://www.isag.org.uk/ISAG/all/ISAG2008_CattleParentage.pdf).

New challenges are now emerging for genotyping laboratories because official organizations have initiated recommendations and minimum requirements for identity and kinship analysis. The International Committee for Animal Recording has recently instituted a working group on guidelines of accreditation of DNA paternity testing in cattle (www.icar.org). Recently, Budowle *et al.* (2005) have described the recommendations that need to be imple-

mented by different laboratories for animal DNA forensic and identity testing.

Multiplex polymerase chain reaction (MPCR) is a powerful technique typically used in genotyping applications, where, the simultaneous analysis of multiple markers is required. In parentage testing and individual identification easy and cost-effective MPCR is routinely used (Glowatzki-Mullis *et al*. 2006).

MPCRs with at least 10 microsatellite markers were developed for various animals by every laboratory or as commercial kits (Applied Biosystems, Finnzymes Diagnostics). In cattle there exist various commercially available kits; i.e. Finnzymes Diagnostics company offers several bovine STR typing kits: http://diagnostics.finnzymes.fi/bovine_genotypes.html. Bovine Genoty-pes™ Panel 3.1 encompasses all the 12 STR loci recommended by the International Society for Animal Genetics (ISAG, http://www.isag.org.uk/) for routine use in parentage testing and identification, including *TGLA227*, *BM2113*, *TGLA53*, *ETH10*, *SPS115*, *TGLA126*, *TGLA122*, *INRA23*, *ETH3*, *ETH225*, *BM1824* and *BM1818*. In addition, the kit includes the following six microsatellites which are among the list of loci recommended by the Food and Agriculture Organization of the United Nations (FAO, www.fao.org) for genetic studies of domestic animals: *SPS113*, *RM067*, *CSRM60*, *MGTG4B*, *CSSM66* and *ILSS006*.

The aim of this study was to characterize Fleckvieh, Charolais, and Beef Simmental using Bovine Genotypes™ Panel 3.1 for the analysis of the genetic variability of DNA microsatellite markers and to evaluate informativeness of these markers in parentage tests.

MATERIALS AND METHODS

Experimental animals

Samples of 125 animals from the following Czech cattle populations were analyzed: Fleckvieh (n=50), Charolais (n=50) and Beef Simmental (n=25).

Genotype determination

Bovine genomic DNA was extracted from blood using QIAamp® Blood kit (Qiagen, Valencia, CA, USA) and from hair roots using JETQUICK Tissue DNA Spin Kit (Genomed GmbH, Germany) following the protocol handbook.

Amplification of microsatellites sequences was performed by multiplex PCR reaction using commercial available Bovine Genotypes™ Panel 3.1 (Finnzymes Diagnostics, Espoo, Finland) and carried out in a GeneAmp™ PCR System 9700 cycler (Applied Biosystems, Foster City, CA). The commercial kit was used according to the manufacturer's instructions. The genotyping of microsatellite markers was performed on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) by fluorescent fragment analysis using 310 Data Collection software 3.1.0 and detected by Gene Mapper v4.0. The allele size was determinate in bp by comparing the length with a length standard GS LIZ 500 (Applied Biosystems). All loci were dinucleotide repeats. Reference samples distributed by ISAG Comparison test 2009/2010 were used to standardize allele sizes.

Statistical evaluation

The measures of genetic variability, including the number of alleles, were observed and theoretical heterozygosity were calculated for each locus (Equation 1). Exclusion probabilities (EP) and combined exclusion probabilities (CEPs) were calculated according to formulas published in Jamieson and Taylor (1997), Equation 2-4, where, k; is number of loci, x_i; is i allele frequency.

$$tH = 1 - \sum_{i=1}^{k} x_i^2$$

Equation 1 Theoretical heterozygosity (tH)

$$CEP(1) = 1 - 2\sum_{i=1}^{k} x_i^2 + \sum_{i=1}^{k} x_i^3 + 2\sum_{i=1}^{k} x_i^4 - 3\sum_{i=1}^{k} x_i^5 - 2\left(\sum_{i=1}^{k} x_i^2\right)^2 + 3\sum_{i=1}^{k} x_i^2 \sum_{i=1}^{k} x_i^2$$

Equation 2 Combined exclusion probability of CEP1: paternity exclusion

$$CEP(2) = 1 - 4\sum_{i=1}^{k} x_i^2 + 2\left(\sum_{i=1}^{k} x_i^2\right)^2 + 4\sum_{i=1}^{k} x_i^3 + 3\sum_{i=1}^{k} x_i^4$$

Equation 3 Combined exclusion probability of CEP2: one parental genotype unavailable

$$CEP(3) = 1 + 4\sum_{i=1}^{k} x_i^4 + 4\sum_{i=1}^{k} x_i^5 + 3\sum_{i=1}^{k} x_i^6 + 8\left(\sum_{i=1}^{k} x_i^2\right)^2 + 8\sum_{i=1}^{k} x_i^2 \sum_{i=1}^{k} x_i^2 + 2\left(\sum_{i=1}^{k} x_i^2\right)$$

Equation 4 Combined exclusion probability of CEP3: parentage exclusion

RESULTS AND DISCUSSION

The samples were genotyped for 17 microsatellites markers (*TGLA227*, *BM2113*, *ETH10*, *SPS115*, *TGLA126*, *TGLA122*, *INRA23*, *ETH3*, *ETH225*, *BM1824*, *BM1818*, *SPS113*, *RM067*, *CSRM60*, *MGTG4B*, *CSSM66* and *ILSTS006*) recommended by International Society of Animal Genetics (ISAG) and by the Food and Agriculture Organization of the United Nations (FAO). For locus description see Table 1 Electrophoretic separation by fragment analysis was done on ABI PRISM 310 laser sequencer (Ap-

Table 1 Locus description for the Bovine Genotypes™ Panel 3.1 of 18 microsatellites

Locus name	Dye color[1]	Repeat motif	Size range (bp)	Chromosome
TGLA227 (D18S1)	Blue	Di	63-115	18
BM2113 (D2S26)	Blue	Di	116-146	2
TGLA53 (D16S3)	Blue	Di	147-197	16
ETH10 (D5S3)	Blue	Di	198-234	5
SPS115 (D15)	Blue	Di	240-270	15
SPS113	Blue	Di	279 - 307	10
RM067	Green	Di	83-101	4
TGLA126 (D20S1)	Green	Di	104-132	20
TGLA122 (D21S6)	Green	Di	133-193	21
INRA23 (D3S10)	Green	Di	194-236	3
BM1818 (D23S21)	Green	Di	248-276	23
ETH3 (D19S2)	Black	Di	89-131	19
ETH225 (D9S1)	Black	Di	132-166	9
BM1824 (D1S34)	Black	Di	170-218	1
CSRM60 (D10S5)	Red	Di	79-15	10
MGTG4B	Red	Di	129-153	4
CSSM66 (D14S31)	Red	Di	171-209	14
ILST006 (D7S8)	Red	Di	277-309	7

[1] Dye colors are listed as they appear after electrophoresis with Filter Set G5 (Applied Biosystems).

Table 2 Alleles identified in Fleckvieh, Charolais and Beef Simmental

Locus	Fleckvieh	Charolais	Beef Simmental
BM1824	178, 180, 182, 188, 190	178, 180, 182, 188, 190	178, 180, 182, 188, 190
BM2113	125, 127, 131, 133, 135, 137, 139	125, 127, 131, 133, 135, 137, 139	127, 131, 133, 135, 137, 139
ETH3	117, 119, 125, 127	117, 119, **121**, **123**, 125	117, 119, 125, 127
ETH10	213, 217, 219, 221, 223	217, 219, 221, 223	213, 217, 219, 221
ETH225	140, **144**, 146, 148, 150, 152	**138**, 140, 148, 150, 152	140, 146, 148, 150, **158**
INRA23	198, 200, 202, 206, 208, 210, 214, 216, 218, **222**	198, 200, 202, **204**, 206, 208, **212**, 214, 216, 218	206, 208, 210, 214
SPS115	248, 252, 254, 256, 260	248, 252, 254, 256, 260	248, **250**, 252, 254, 256, 260
TGLA122	141, 143, 147, 151, 153, 161, **183**	141, 143, 147, 149, 151, 153, 155, **157**, **171**, **173**, **179**	141, 143, 147, 149, 151, 153, 155, 161, **181**
TGLA126	**111**, **113**, 115, 117, 121, 123, **125**	115, 117, **119**, 121, 123	115, 117, 121, 123
TGLA227	79, 81, 83, 89, 91, 93, 95, 97	**77**, 79, 81, 83, **85**, 89, 91, 93, 97	79, 81, 83, 89, 91, 93, 95, 97
SPS113	283, 285, 287, 291, 293, 295, 297	**279**, 283, 285, 287, 291, 293, 295, 297, **299**	283, 285, 287, **289**, 291, 295, 297
BM1818	258, 262, 264, 266, 268, 270	258, **260**, 262, 264, 266, 268, 270	258, 262, 264, 266, 268
RM067	90, 92, 94, 96, 98, 102	**88**, 90, 92, 94, 96, 98, 102, **104**, 106	90, 92, 94, 96, 98, 106
ILSTS006	284, 286, 288, 290, 292, 294, 296	282, 284, 286, 288, 290, 292, 294, 296, 298, **300**	282, 284, 286, 288, 290, 292, 294, 296, 298
MGTG4B	**125**, 135, 137, 139, 141, 145, 147, **149**, 151	**133**, 135, 137, 139, 141, 143, 145, 147, 151	135, 137, 139, 143, 145
CSSM66	**179**, 183, 185, 187, 189, 193, 197, 199	**181**, 183, 185, 187, 189, **191**, 193, 197	183, 185, 187, 189, 193, 197, 199
CSRM60	92, 96, 98, 100, 102, 104, **108**	92, 96, 98, 100, 102, 104, **112**	92, 96, 100, 102

bold=alleles found only in one breed; underlined=alleles with the highest frequency.

plied Biosystems) to identify five fluorescent dyes. The representative electrophoregram from one sample is on Figure 1.

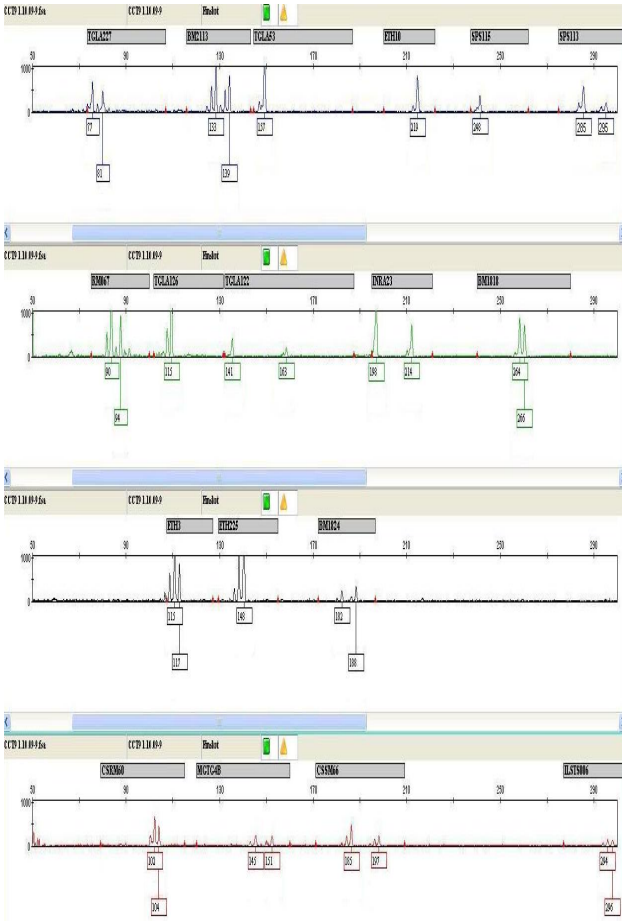

Figure 1 Electrophoregram of one representative sample (GeneMapper v4.0)

TGLA53 locus was excluded from the analysis. The efficiency of genotype determination was very low and problematic in some samples.

All 17 tested microsatellite markers were polymorphic and a total of 337 alleles were identified. The number of allele per each locus ranged from 4 (*ETH3, ETH10, INRA23, TGLA126* and *CSRM60*) to 11 (*TGLA122*) with a mean of 6.7 alleles for Fleckvieh (FC), 7.4 for Charolais (CHC) and 5.8 for Beef Simmental (BS). Table 2 shows the specific alleles identified in three bovine breed. Total number of 114, 125 and 98 alleles was found in cattle populations of Fleckvieh, Charolais and Beef Simmental, respectively. There were some typical alleles for a given breed. The greater number of typical alleles for breed was observed in Charolais; 22 alleles did not appear in Fleckvieh and Beef Simmental, while 10 alleles did not occur in animals of Charolais and Beef Simmental and were specific for Fleckvieh.

The alleles *ETH225-158* bp, *SPS115-250* bp, *TGLA122-181* bp and *SPS11-289* bp were present exclusively in Beef Simmental. Allele frequency and standard deviation were calculated for each locus, separately. There were alleles found with the highest allele frequency across all tested cattle breeds: *ETH3*-allele *117* bp, *ETH10*-allele *217* bp, *SPS115*-allele *248* bp, *TGLA122*-allele *151* bp, *RM067*-allele *90* bp, *ILSTS006*-allele *296* bp, *MGTG4B*-allele *135* bp and *CSSM66*-allele *185* bp.

Table 3 describes the number of founded alleles, their average theoretical heterozygosity (tH) and combined exclusion probabilities in Beef Simmental, Fleckvieh and Charolais cattle. The highest heterozygosity was observed for locus *TGLA227*-over 0.80 in all three breeds. A similarly high heterozygosity, above average, was ascertained for loci *ILSTS006*, *RM067*, *BM1818*, *BM1824*, *SPS113*, *TGLA122* and *INRA23*. The lowest value was determined for locus *ETH10* in CHC (0.15) and *SPS115* in CHC (0.51).

The probabilities of paternity exclusion/one parental genotype unavailable/and parentage exclusion were 0.9942/0.9798/0.9999 (Fleckvieh), 0.9834/0.9744/0.9-999 (Charolais), 0.9828/0.9682/0.9999 (Beef Simmental). Research data certified the possibility of using the Finnish Bovine panel of DNA microsatellites markers for the forensic purposes in the Czech cattle populations.

The results of this pilot experiment study indicated that the Bovine Genotypes™ Panel 3.1 of DNA microsatellites markers for the individual identification and parentage control, is suitable and comfortable for being used in genotyping of the Czech cattle populations. According to presented results (Table 2 and 3) and Koskien (2006), DNA microsatellites can be efficiently used to determine incorrect parentage attribution, due to high CEP indexes. Correct pedigree information is the basic condition for a successful breeding program (Řehout *et al*. 2006). The forensic usefulness of the cattle STR loci was further advanced by the recent publication (Civáňová and Putnová, 2003; Civáňová *et al*. 2003; Manga *et al*. 2007). All the analyzed loci showed high polymorphism and sufficient informativeness, though *ETH10* in Charolais cat- tle showed very low heterozygosity.

Most of the loci used in this study had been analyzed in previous studies with different breeds (Maudet *et al*. 2002; Radko *et al*. 2005; Cervini *et al*. 2006; Czerneková *et al*. 2006; Choroszy *et al*. 2006; Řehout *et al*. 2006; Stevanovic *et al*. 2009; Stevanovic *et al*. 2010; Radko, 2010; Montoya *et al*. 2010). The highest polymorphism for *TGLA227* and *INRA23* loci had also been found by Radko *et al*. (2005) in Polish Red cattle and Hereford. The *TGLA122* locus show-

Table 3 Number of founded alleles (NA), their average theoretical heterozygosity (tH) and combined exclusion probability of CEP1: paternity exclusion, CEP2: one parental genotype unavailable, CEP3: parentage exclusion in Fleckvieh cattle (FC), Charolais cattle (CHC) and Beef Simmental (BS)

Breed	FC					CHC					BS				
Locus	NA	tH	CEP1	CEP2	CEP3	NA	tH	CEP1	CEP2	CEP3	NA	tH	CEP1	CEP2	CEP3
BM1824	5	0.75				5	0.73				5	0.70			
BM2113	7	0.76				7	0.82				6	0.66			
ETH3	4	0.72				5	0.55				4	0.54			
ETH10	5	0.65				4	0.15				4	0.54			
ETH225	6	0.69				5	0.74				5	0.78			
INRA23	10	0.77				10	0.78				4	0.73			
SPS115	5	0.55				5	0.51				6	0.59			
TGLA122	7	0.74				11	0.74				9	0.78			
TGLA126	7	0.66	0.9942	0.9798	0.9999	5	0.58	0.9834	0.9744	0.9999	4	0.58	0.9828	0.9682	0.9999
TGLA227	8	0.83				9	0.84				8	0.81			
SPS113	7	0.88				9	0.77				7	0.84			
BM1818	6	0.73				7	0.78				5	0.72			
RM067	6	0.75				9	0.71				6	0.79			
ILSTS006	7	0.75				10	0.88				9	0.87			
MGTG4B	9	0.74				9	0.64				5	0.61			
CSSM66	8	0.65				8	0.76				7	0.70			
CSRM60	7	0.76				7	0.80				4	0.61			

ed also the highest allele polymorphism in Brazilian Nellore cattle (Cervini *et al*. 2006) and in Colombian cattle (Montoya *et al*. 2010). According to Table 2, microsatellite *TGLA*122 was the most polymorphic marker with 14 alleles. Furthermore, Maudet *et al*. (2002) informed about 19 alleles in native French cattle breeds. In accordance with other studies, our results showed as well that the *ETH10* locus had the lowest polymorphism (Table 2) (Radko *et al*. 2005).

Microsatellite DNA marker analysed in the Czech population of Simmental cattle in this study appeared less polymorphic than in other breeds of Simmental cattle (Serbia, Poland and Slovakia). The mean NA per locus observed in our population of Czech Simmental (5.8) was less than 8.3 found in Serbia Simmental cattle (Stevanovic *et al*. 2009; Stevanovic *et al*. 2010) or 7.5 found in Slovakian Pied (Czerneková *et al*. 2006) or 7.3 found in Simmental cattle from Poland (Choroszy *et al*. 2006). In Beef Simmental from Czech, tH ranged from 0.54 (*ETH3* and *ETH10*, 4 alleles) to 0.87 (*ILSTS006*, 9 alleles), with average value of 0.70, which is comparable to the values found in Serbian Simmental (0.75), Slovakian Pied (0.65) and Czech Pied (0.76) in the study of Stevanovic *et al*. (2009) and Czerneková *et al*. (2006).

The combined probability exclusion, estimated from different sets of microsatellite markers which were usually exceeded than 0.99 (Cervini *et al*. 2006; Radko, 2010). Based on the Bovine Genotypes™ Panel 3.1, CEP3 calculated for Fleckvieh, Charolais and Simmental population was 0.9999. The results of the present study confirmed the high polymorphism of the considered set of microsatellite DNA markers. Indeed, it proved the high heterozygosity of the analysed Fleckvieh, Charolais and Simmental population with reference to this Finnish group of markers.

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