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12-plex highly polymorphic microsatellite marker set for parentage analysis in Banija spotted pigs

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Abstract

Microsatellites (MS) have been, for the last two decades, widely used for parentage analysis in all types of livestock, including pigs. Conservation efforts on Banija spotted pigs included genetic characterization of the breed with MS markers. Since recent comparison of pedigree and MS data revealed some inconsistencies, the aim of this study was to develop a set of highly polymorphic and heterozygous MS markers, which could be used for parentage analysis and to prevent pedigree errors. 12 MS markers with the polymorphic information content (PIC) above 0.62 were chosen and combined into a single multiplex polymerase chain reaction (PCR). The combined non-exclusion probability for one candidate parent (NE-1P) was 0.00246149, the combined NE-1P given the genotype of a known parent of the opposite sex (NE-2P) was 0.00003739 and combined non-exclusion probability for a candidate parent pair (NE-PP) was 0.00000003. Due to a high information content of selected MS markers it was possible to obtain high accuracy in parentage assignment, which was confirmed by analyzing actual data with known genetic relationships.

Keywords: Banija spotted pig, microsatellite multiplex, parentage analysis

Introduction

Parentage analysis and parentage verification are very important for an efficient genetic management in livestock breeding (Yu et al., 2015). Traditionally, pedigree records are the foundation of the breeding programs, but if this records are incomplete or inaccurate, these data can lead to unexpected and undesired consequences and errors in further breeding. In order to avoid such errors, molecular markers have therefore been introduced into the livestock breeding programs.

Microsatellites (MS) have been, for the last two decades, widely used molecular markers to trace studbook information down to the individual level in all types of organisms, including pigs (Nechtelberger et al., 2001; Li et al., 2010). MS are highly polymorphic, informative and interspersed throughout the genome and have higher variability when compared to the same number of bi-allelic markers such as SNPs (Schlötterer, 2004). For that reason, they are still often used for parentage assignment, for the formulation of long-term breeding plans, designing of breeding programs and planning conservation strategies.

Banija spotted pig is an old Croatian pig breed, which was a few years ago on the verge of extinction. Conservation efforts included introduction and further management of a herd book, standardization of the breed based on phenotypic data and genetic characterization of the breed with MS markers (Salajpal et al., 2017). Recent comparison of pedigree and MS data revealed some inconsistencies, even though the number of animals in the population is low, and that currently only the fourth generation of Banija spotted pigs is recorded into a herd book.

Therefore, the aim of this study was to develop a set of highly polymorphic and heterozygous MS markers for potential use in parentage analysis to prevent pedigree errors, and also to provide a genetic basis for further breeding and conservation programs of Banija spotted pigs.

Materials and methods

24 MS markers from the ISAG/FAO recommendation list (ISAG/FAO, 2011) were selected and grouped into three multiplex reactions. First multiplex contained S0026, S0155, S0005, Sw2410, Sw830, S0355, Sw24, Sw632 and Swr1941, second multiplex contained Sw9366, S0218, S0228, Sw240, Sw2406, Sw122, Sw857 and S0097, and the third multiplex contained Sw72, S0226, SO090, Sw911, S0002, Sw1067 and S0101 MS markers.

Multiplex PCR reactions were performed with 2x Type-it Microsatellite PCR Kit (*Qiagen GmbH, Germany*) following the manufacturer instructions. MS cycling protocol began with initial activation step 6 min at 95 ºC, followed by 35 cycles of denaturation (30 s at 95 ºC), annealing (90 s at 58, 59 and 59.5 ºC), different temperature for each multiplex) and extension (60 s at 72 ºC). Cycling program ended with a final extension for 30 min at 60 ºC.

12 MS multiplex PCR for parentage analysis began with initial activation step 6 min at 95 ºC, followed by 35 cycles of denaturation (30 s at 95 ºC), annealing (90 s at 59 ºC), and extension (60 s at 72 ºC). Cycling program ended with a final extension for 30 min at 60 ºC. MS multiplex PCR products were sent to Macrogen (Macrogen Inc., Netherlands), where they were analyzed using GeneScan350 ROX internal standard size marker on ABI3730XL capillary gene analyzer.

Cervus 3.0.7. software (Marshall et al., 1998; Kalinowski et al., 2007) was used to test 12 MS loci for Hardy-Weinberg equilibrium (HWE), to calculate observed (H_O) and expected (H_E) heterozygosity at each locus and to calculate the polymorphism information content (PIC).

Parentage analysis was performed with Cervus 3.0.7. using 12 MS loci with high PIC values. Cervus generates likelihood ratios for parentage interference at a relaxed (80%) and strict (95%) level. Also average non-exclusion probability for one candidate parent (NE-1P), the average NE-1P given the genotype of a known parent of the opposite sex (NE-2P), and the average non-exclusion probability for a candidate parent pair (NE-PP) were determined with Cervus 3.0.7.

Results and discussion

HWE test on all loci showed that population was likely in HWE, and there was no evidence of null alleles. The heterozygosity of 23 typed MS loci was in range from 0.138 to 0.862, while the PIC ranged from 0.156 to 0.809. The number of alleles per locus ranged from 3 to 11. The selection of loci with high PIC values (≥0.62) revealed 12 MS loci, listed and described in Table 1.

Table 1. Genetic statistic of the 12 MS loci used in this study

Locus name	Number of alleles	Allele size (bp)		H_o	H_E	PIC
		Minimum	Maximum			
S0155	6	142	160	0.641	0.677	0.623
S0005	11	202	248	0.897	0.835	0.809
SW2410	7	102	116	0.795	0.799	0.759
SW830	8	154	182	0.864	0.803	0.762
S0355	7	210	230	0.615	0.745	0.691
SW936	7	90	114	0.816	0.765	0.72
SW240	8	92	112	0.789	0.803	0.766
SW122	8	112	126	0.895	0.793	0.752
SW857	6	146	162	0.711	0.800	0.758
S0097	6	210	248	0.868	0.798	0.755
S0002	8	182	216	0.437	0.704	0.66

Comparison of this 12 MS with MS sets, used for parentage analysis in other European commercial (Nechtelberger et al., 2001; Yu et al., 2015) and wild pigs (Costa et al., 2012) revealed that even though first set of 23 markers included

majority of MS, used for parentage analyses on other pig breeds, only about half of this markers are present in the 12 MS set, while the other half of the highly polymorphic markers in this set are specific for Banija spotted pigs.

The heterozygosity levels of selected 12 MS loci ranged from 0.437 to 0.897, while PIC values were between 0.623 and 0.809. The combined non-exclusion probability of identity was as low as $1.039E^{-13}$. The probability of identity with related individuals included in the samples (PISibs) was also low, $1.1E^{-5}$. The combined NE-1P was 0.00246149, the combined NE-2P was 0.00003739 and combined NE-PP was 0.00000003. These data are comparable or even better than data of other MS sets, used in parentage analysis in commercial pigs (Yu et al., 2015) and wild boars (Costa et al., 2012).

To assess the parentage assignment accuracy, 12-plex MS set was analyzed on 14 sampled Banija spotted pigs with known genetic relationships based on herd book data (9 offspring, 3 mothers and 2 fathers). Relationships of a part of the analyzed population are shown in Figure 1, where 6 offspring with two different mothers are sharing the same father.

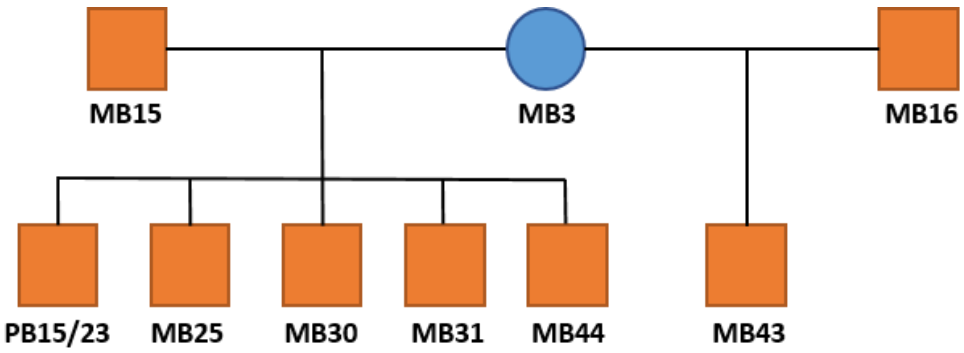

Figure 1. Genetic relationship of the 9 sampled Banija spotted pigs

Both, maternity and paternity analyses were able to correctly assign all offspring at relaxed (80%) and strict (95%) level of confidence when one parent was given and the other parent was known. When additional 5 female genotypes were added beside the three true mother genotypes in the analysis, at strict level of confidence assignments fell to 89%, but remain 100% at relaxed level.

Conclusions

Results of the presented work support the usefulness of selected highly informative 12 MS marker set, which could be performed in one single multiplex PCR, for parentage analysis in the Banija spotted pigs. Due to high information content of selected MS markers it was possible to obtain high accuracy in parentage assignment, which was confirmed by analyzing actual data with known genetic relationships.

Since Banija spotted pig is still at the beginning of its conservation attempts and the only genetic characterization of the breed is based on MS markers, the multiplex set of 12 highly polymorphic and heterozygous MS markers remains at the time the only reliable option for parentage analysis to prevent pedigree errors, and also to provide a genetic basis for further breeding and conservation programs of Banija spotted pigs.

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