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Research Note

Microsatellite panels suggested for parentage testing in cattle: informativeness revealed in Finnish Ayrshire and Holstein-Friesian populations

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Informativeness of eleven microsatellite markers suggested for parentage control in cattle by the International Society for Animal Genetics (ISAG) was studied in Finnish Ayrshire and Holstein-Friesian populations. Calculations were based on a sample of 100 non-sib artificial insemination bulls. Assuming one known parent the nine loci suggested for routine testing exhibited exclusion probabilities of 99.84% in the Ayrshires and 99.91% in the Holstein-Friesians. The addition of markers INRA23 and TGLA53, recommended for further investigations, increased the attained values to 99.94% in Ayrshires and to 99.98% in Holstein-Friesians. The recommended core set of six microsatellites provided a combined exclusion probability of 98.25% in Ayrshires and 99.32% in Holstein-Friesians. Although the combined values were high in general, a relatively low level of polymorphism was detected in some instances.

Key words: animal identification, bovine, exclusion probability, microsatellite

Introduction

Correct assignment of livestock pedigrees is particularly important when artificial insemination is widely used, as for example in cattle: errors have a direct effect to the genetic response (Arendonk et al. 1998). In recent years, nuclear DNA loci have become the markers of choice for parentage verification over the traditional polymorphisms, i.e. blood groups and serum proteins. Particularly, microsatellites (Weber and May 1989, Litt and Luty 1989) have proved suitable due to their unique polymorphism and Mendelian codominant inheritance (e.g. Litt and Luty 1989). In addition, these short tandem repeats (STR's) are readily typed and scored with automated procedures, which makes them amenable for routine laboratory practice (Goor et al. 1998).

In an ideal situation all laboratories would

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be using the same markers and recognising the same nomenclature for allele scoring. Therefore, in 1996 ISAG's (International Society for Animal Genetics) cattle standing committee suggested a set of nine microsatellites combined in three multiplex-PCR (Polymerase Chain Reaction) reactions as a standard protocol for parentage testing in bovines. Four additional markers were proposed for further investigations. In 1998 the committee recommended a core set of six loci as a minimum assay for all typed animals (http://www.immgen.com/html/primersequences.html).

We are routinely using eleven of these microsatellites to ensure correct pedigrees for cattle in Finland. Since 1996 over 2500 cattle, representing six different breeds (mainly Finnish Ayrshire and Holstein-Friesian), have been typed in FABALAB (laboratory of the Finnish Animal Breeding Association). The purpose of this work was to evaluate the efficiency of the three panels consisting of 11, 9 and 6 markers (suggested by ISAG), in detecting false parentage assignments in Finnish Ayrshire and Holstein-Friesian cattle.

Material and methods

Our sample consists of 100 bulls representing two of Finland's most common breeds, the Finnish Ayrshire (50) and the Holstein-Friesian (50). These individuals are all being used for artificial insemination purposes, thus having a high number of offspring, and representing the Finnish populations well.

DNA was extracted from hair bulbs using PCR buffer containing 8 µg of proteinase K / 40 µl reaction. Alternatively, extraction was performed from semen or blood using standard protocols. Microsatellites were amplified in three reactions: Multiplex 1, Multiplex 2 and Multiplex 3 (Table 1). Prior to electrophoresis, 0.5 µl of the PCR products were pooled, 12 µl formamide and 0.5 µl TAMRA 350 internal lane standard (Applied Biosystems) were added and the mixture was kept in 95°C for 3 minutes for denaturation and quickly cooled on ice. Fragment separation and allele size scoring were performed using the ABI 310 Genetic Analyser and the GeneScan v. 2.1 software (Applied Biosystems).

Table 1. Multiplexes, microsatellites and their PCR (Polymerase Chain Reaction) parameters.

Multiplex	Locus and its forward and reverse primer amounts		PCR parameters
1	BM1824	15pmol	94°C 3min
	BM2113	3pmol	27x 94°C 30s
	SPS115	7pmol	58°C 1min
			72°C 1min
			72°C 5min
2	ETH3	5pmol	94°C 3min
	ETH10	5pmol	27x 94°C 30s
	ETH225	5pmol	66°C 1min
			75°C 30s
			75°C 5min
3	TGLA227	5pmol	94°C 3min
	TGLA126	11pmol	29x 94°C 30s
	TGLA122	3pmol	55°C 1min
	TGLA53	5pmol *	75°C 30s
	INRA023	2pmol *	75°C 5min

* Markers recommended for further investigations included to Multiplex 3

Locus specific probabilities of excluding a falsely assigned sire (or dam) were calculated using formulae adapted from Jamieson (1994):

$$P_{En} = \Sigma p_i(1-p_i)^2 - \Sigma (p_i p_j)^2 [4-3(p_i + p_j)],$$

where P_{En} is the probability of exclusion in a given locus with n alleles and p_i and p_j refer to the frequencies of alleles.

Panel specific combined exclusion probabilities were calculated as:

$$P_{Ec} = 1-[(1-P_{E1})(1-P_{E2})\ldots(1-P_{En})],$$

where P_{Ec} is the combined exclusion probability over all loci in the panel and P_{E1}, $P_{E2}\ldots P_{En}$ are the exclusion probabilities in the individual loci.

This approach for calculating probabilities of exclusion is appropriate in situations where the

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the other parent's genotype is known. Thus, it can be used for farm animal populations (Jamieson and Taylor 1997).

In theory, the formula of Jamieson (1994) assumes that investigated marker alleles are inherited in Hardy-Weinberg (H-W) proportions. H-W exact probability tests of Guo and Thompson (1992), implemented by the computer program GENEPOP v.3.1b (Raymond and Rousset 1995), were therefore conducted for the eleven loci of Finnish Ayrshire and Holstein-Friesian populations. Corrections for multiple significance tests were performed by applying a sequential Bonferroni correction (Rice 1989).

Results

Twenty-two exact tests (2 populations, 11 loci) revealed no highly significant ($P<0.01$) deviations from H-W equilibrium. Genotypes of the locus BM1824 expressed marginal departures ($0.01<P<0.05$ in both populations) which, however, did not remain significant in either population after applying the Bonferroni correction for multiple tests. Hence, Jamieson's (1994) formulae are applicable for our population data.

Microsatellites BM2113, ETH3 and TGLA227 exhibited very high polymorphism with exclusion probabilities exceeding 52% in both breeds. Furthermore, loci BM1824, ETH10, ETH225, TGLA122, TGLA126 and TGLA53 were considerably informative, as in both breeds exclusion probabilities above 43% were attained. However, INRA23 was highly polymorphic only in the Holstein-Friesians, whereas SPS115 exhibited only moderate informativeness with its most common allele having a frequency of 0.69 in both breeds. Highest numbers of alleles were observed in Holstein-Friesians in TGLA122 (13 alleles), TGLA227 (10 alleles) and in TGLA53 (10 alleles) while in Ayrshires no more than 8 alleles were detected in any of the studied loci.

The nine microsatellites, suggested for routine parentage control, provided combined exclusion probabilities of 99.84% in the Finnish Ayrshire and 99.91% in the Holstein-Friesian populations. Adding INRA23 and TGLA53 increased the values to 99.94% in Ayrshires and to 99.98% in Holstein-Friesians. The core panel of six markers yielded combined values of 98.25% in Ayrshires and 99.32% in Holstein-Friesians, respectively (Table 2).

Table 2. Average exclusion probabilities exhibited by the eleven loci suggested for routine parentage testing in cattle by the International Society for Animal Genetics. Calculations according to Jamieson (1994) assuming one known parent.

Locus	Finnish Ayrshire	Holstein-Friesian
BM1824	0.418	0.531
BM2113	0.627	0.626
SPS115	0.266	0.313
TGLA122	0.430	0.677
TGLA126	0.530	0.457
TGLA227	0.589	0.678
Cumulative (6 loci)	*98.25%*	*99.32%*
ETH3	0.523	0.526
ETH10	0.570	0.479
ETH225	0.565	0.462
Cumulative (9 loci)	*99.84%*	*99.91%*
INRA23	0.334	0.562
TGLA53	0.437	0.577
Cumulative (11 loci)	*99.94%*	*99.98%*

Discussion

In a parentage verification test, DNA samples of both true parents are available under ideal conditions. The results obtained in this study imply very effective exclusion of false parents in such situations. This is true even for the core set of six markers. When exclusion based on only one marker is considered adequate, additional loci need rarely be used for solving parentages.

However, as calculated probabilities of exclusion are averages they do not always illustrate the problems one might confront using the minimum number of markers, i.e. the core set, particularly when the dam's genotype is missing. To demonstrate this, we randomly sampled bulls from our records and conducted calculations of true exclusion probabilities attained by the different multiplex sets for ten cases in both breeds: in a locus in a given population a parent's exclusion probability is the frequency of genotypes where neither of the parent's alleles exists (assuming the other parent's genotype unknown). We observed ranges of 99.0% to >99.9%, 93.7% to >99.9% and 79.1% to 99.2% in the Ayrshires and 97.3% to >99.9%, 95.4% to 99.7% and 88.1% to 99.3% in the Holstein-Friesians for the eleven, nine and six loci, respectively. These results emphasize the assay's efficiency but also point out the problems the minimum number of markers might give rise to.

Having an international agreement on microsatellites and fragment size calling serves when sperm or embryos are imported: the bull's readily available genotype greatly reduces problems with uncertain pedigrees. To avoid retyping it would be beneficial to have a high number of commonly investigated markers. With efficient multiplexing and stable assays the additional effort and cost is marginal. For instance, the set of eleven cattle microsatellites can be analysed in a single multiplex (Goor et al. 1998).

In setting up guidelines for testing cattle parentage it is important to survey as many breeds as possible as bovine microsatellite allele frequencies and exclusion probabilities tend to vary across breeds (e.g. Moazami-Goudarzi et al. 1997, Usha et al. 1995, Glowatzki-Mullis et al. 1995). Herein we report, to our knowledge, the first results of the informativeness of the combined sets of markers suggested by ISAG for cattle parentage testing. With this survey we conclude that the set of nine STR-loci provides high probabilities of exclusion in the Finnish Ayrshire and Holstein-Friesian populations. Addition of INRA23 and TGLA53 increases the attained values most likely enough for solving even the most troublesome cases. However, in the two studied breeds SPS115 exhibits considerably lower polymorphism than the other loci. If similar conclusions are made with other populations a possible exchange of the marker should perhaps be considered. Furthermore, at least in the Finnish Ayrshires, the minimum panel of six markers seems relatively inefficient for solving the most difficult disputes of parentage.

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SELOSTUS

Suomalaisten ayrshire- ja holstein-friisiläisrotuisten nautojen mikrosatelliitti-DNA:han perustuvan polveutumismäärityksen tehokkuus

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Kansainvälisen eläingenetiikan järjestö (ISAG) on ehdottanut mikrosatelliitti-DNA:han perustuvaa menetelmää yleisesti käytettäväksi nautojen polveutumisten varmistamisessa. Eri rotujen välillä esiintyvästä mikrosatelliittien polymorfian vaihtelusta johtuen ISAGin ehdottaman menetelmän tehokkuus vaihtelee rodun mukaan. Tämän tutkimuksen tarkoituksena oli selvittää ehdotettujen vaihtoehtojen (11, 9 tai 6 lokusta) tehokkuus suomalaisten ayrshire- ja holstein-friisiläisrotujen väärien isyyksien paljastajana. Tulokset osoittavat yhdeksän rutiinitestaukseen tarkoitetun mikrosatelliitin paljastavan väärät isyydet ayrshirellä 99,84 %:n ja holstein-friisiläisellä 99,91 %:n varmuudella. Kahden lisätutkimuksiin tarkoitetun mikrosatelliitin lisäys nostaa vastaavat arvot 99,94 %:iin ayrshirellä ja 99,98 %:iin holstein-friisiläisellä. Kuuden mikrosatelliitin muodostaman menetelmän avulla voidaan väärät isyydet paljastaa 98,25 %:n todennäköisyydellä ayrshirellä ja 99,32 %:n todennäköisyydellä holstein-friisiläisellä. Vaikka edellä mainitut todennäköisyydet osoittavat, että väärät isyydet voidaan sulkea pois tehokkaasti, havaitsimme tietyissä yksittäisissä tapauksissa (esim. lokus SPS115) suhteellisen vähäistä polymorfiaa. Jos vastaavia tuloksia saadaan jatkossa myös muilla roduilla, tulisi kyseisten lokusten mahdollista vaihtoa harkita. Ongelmat korostuvat esim. tilanteissa, joissa emästä ei ole saatavissa näytettä.