Short Communication

The Evaluation of Selected 13 Polymorphic Microsatellite Markers for the Parentage Test of Thai Holstein Cattle.

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Abstract

Nineteen families of 44 Thai Holstein cattle were evaluated for microsatellite characteristics and the parentage test. A total of 13 microsatellite markers were selected from the genome database and the genomic DNAs were isolated and used as templates for amplification. Multiplex PCR of these thirteen markers in a single reaction showed that only ten markers; b1, b2, b3, b4, b6, b7, b10, b11, b12 and b13 could amplify the fragments ranging from 5 to 12. Nine markers were highly informative with PIC of more than 0.5. An accuracy test revealed that seven markers (b2, b4, b6, b7, b10, b11 and b12) were completely matched with a known relationship. The combination of seven markers in pedigree testing showed 0.9915 and 0.9997 exclusion probabilities for one and both parents exclusion, respectively. We concluded that seven out of the thirteen selected microsatellite markers could be used as a tool in parentage testing in Thai Holstein cattle. This study is a good application for the evaluation of microsatellites DNA in parts of the breeding program and the improvement of livestock production.

Keywords : exclusion probability, microsatellite marker, multiplex PCR, parentage test, Thai Holstein cattle

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บทคัดย่อ

การประเมินการใช้ไมโครแซทเทลไลต์ จำนวน 13 ตัว เพื่อการตรวจพิสูจน์ พ่อ แม่ และลูกในโคนมไทยโฮสไตน์

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การศึกษานี้ได้เลือกเครื่องหมายพันธุกรรมไมโครแซทเทลไลต์ 1 ชุด จำนวน 13 ตัว เพื่อนำมาประเมินความเป็นไปได้ในการประยุกต์ใช้เครื่องหมายนี้เพื่อตรวจพิสูจน์ความเป็น พ่อ แม่และลูก จากตัวอย่างดีเอ็นเอที่นำมาจากโคนมไทยโฮสไตน์ จำนวน 44 ตัวอย่าง (19 ครอบครัว) ผลการเพิ่มจำนวนชิ้นดีเอ็นเอเพื่อวิเคราะห์ขนาดของไมโครแซทเทลไลต์ด้วยวิธี Multiplex PCR ในปฏิกริยาเดียว สามารถเพิ่มจำนวนได้ 10 ไมโครแซทเทลไลต์ (b1, b2, b3, b4, b6, b7, b10, b11, b12 และ b13) จากทั้งหมด 13 ไมโครแซทเทลไลต์ และสามารถหาขนาดของชิ้นดีเอ็นเอที่เพิ่มจำนวนขึ้นด้วยการใช้เครื่องวิเคราะห์อัตโนมัติ โดยขนาดของอัลลีลที่พบในแต่ละตำแหน่ง (locus) มีความแตกต่างระหว่าง 5 ถึง 12 แบบ นอกจากนี้ยังพบว่าไมโครแซทเทลไลต์จำนวน 9 ตัว (b1, b2, b3, b4, b6, b7, b10, b11 และ b13) ให้ค่า Polymorphism Information Content (PIC) มากกว่า 0.5 สำหรับการทดสอบความถูกต้องพบว่าไมโครแซทเทลไลต์ 7 ตัว (b2, b4, b6, b7, b10, b11 และ b12) ให้ผลสอดคล้องกับพันธุ์ประวัติจริงและให้ผล one and both parents exclusion probabilities เท่ากับ 0.9915 และ 0.997 ตามลำดับ ผลจากการศึกษานี้แสดงให้เห็นว่าเครื่องหมายพันธุกรรมที่คัดเลือกมาจำนวน 7 ตัวสามารถนำมาพิสูจน์ความเป็นพ่อ แม่ และลูกของโคนมไทยโฮสไตน์ และเป็นตัวอย่างของวิธีการประเมินเครื่องหมายพันธุกรรม

คำสำคัญ : Exclusion probability, เครื่องหมายพันธุกรรมไมโคแซทเทลไลต์, Multiplex PCR, การตรวจพิสูจน์ พ่อ แม่ และลูก, โคนมไทยโฮสไตน์

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Introduction

Microsattellite is a stretch of short tandem repeat DNA sequences cach of whose units is a 1-6 base pairs repeat. Typically, microsatellites span between twenty and a few hundred bases (Beckmann and Weber, 1992) and most of the microsatellites, about 30-67%, found in vertebrates' genome are dinucleotides. The first dinucleotide type, $(AC)_n$ repeat, is the most common dinucleotide motif which is 2.3 fold more frequent than $(AT)_n$, the second most general type of dinucleotids (Toth et al., 2000). However, in eukaryotic organisms, microsatellites have been shown to be have a non-random distribution pattern, being found more in noncoding regions (Metzgar et al., 2000).

Traditionally, pedigree verification in dairy cattle has been carried out using blood groups and protein polymorphism (Stormont, 1967) which have serious limitations. For instance, blood typing cannot be done retrospectively, after a sire is dead. Molecular techniques using DNA-based microsatellite markers offer several advantages over traditional blood typing. Any sample from an individual (for example, blood, hair, milk a saliva) can be used. In addition the technique can be noninvasive and retrospective from stored tissue or semen samples. The accuracy of the DNA test is much greater than for blood group markers, as DNA markers can have many alleles and a virtually unlimited supply of markers are available (Kappes et al., 1997). DNA-based markers have now been become the international standard system of identification verification in livestock. In most farm animals, there is now a wide choice of suitable co-dominantly inherited genetic markers of known chromosomal location (Barendse et al., 1994; Bishop et al., 1994; Rohrer et al., 1994; Burt et al., 1995;

Crawford et al., 1995). The present study aims to evaluate the utility of thirteen microsatellites for the parentage test in Thai Holstein cattle families. We found seven out of the thirteen combined to a marker set which had been evaluated under universal protocols. Finally, our study also contributes to the new knowledge, how to utilize candidate markers for parentage analysis in cattle.

Materials and Method

Genomic DNA samples used in this study were kindly provided by Dr Wiboon Tularaksa. The DNA samples were isolated from blood samples using a QIAGEN kit. A total of 44 dairy Thai-Holstein cattle (6 bulls, 19 cows and 19 daughters) from 19 families were included.

Selection of thirteen microsatellite markers: Thirteen microsatellite markers, named b1 to b13, were selected from cattle markers previously reported by Ihara et al. (2004). They were selected based on heterozygosity over 0.6 and the expected size had to be between 100 and 270 bp (table 1). The b1 to b13 markers corresponded to the markers previously reported as DIK1118, TGLA40, DIK023, MS2063, DIK1125, DIK4511, DIK2662, DIK5156, DIK5411, DIK116, DIK2733, DIK4356 and DIK4515, respectively (Ihara et al., 2004).

Amplification and fragment analysis of the thirteen microsatellite markers: Thirteen pairs of primers were used and each pair of primers was labeled with fluorescent dye. Details of the primers are shown in table 1. Multiplex PCR reaction was performed in a total volume of 50 µl. Each reaction was composed of 0.2 mM dNTPs, 4 mM $MgCl_2$, 5 pMoles of each 13 primers and 1 µ of *Taq* DNA polymerase in buffer (2 mM Tris-HCl pH 8.0, 10 mM KCl, 0.01 mM EDTA, 0.1 mM DTT, 5% glycerol, 0.05% Tween® 20 and 0.05% Nonidet® - P40) with 100 ng DNA template from DNA pellet suspensions above. The PCR reaction was started at 94°C for 7 min followed by 10 cycles of 94°C for 30 sec, the touch down annealing step was used at a temperature of 65 to 57°C for 30 sec and extension at 72°C for 15 sec. This reaction was followed by 25 cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 15 sec, and a final extension step of 72°C for 7 min. The amplified products were analyzed by gel electrophoresis using 1.5% agarose.

The sizes of amplified products were analyzed by MegaBACE 500 DNA Analysis System. The sample was heated at 95°C for 2 min prior to analyze by MegaBACE 500. Raw data obtained from MegaBACE Instrument Control Manager program version 2.5 was analyzed by the MegaBACE Genetic Profiler program version 2.0.

Characteristics of the thirteen microsatellite markers : The individuals were genotyped based on allele size data. Allele frequency, heterozygosity and polymorphism information content (PIC) was calculated using MS tools v3 software (Park, 2001). Each microsatellite marker was evaluated for its accuracy by comparing the genotypes obtained from a known relationship. All markers with more than 95% accuracy were further analyzed for exclusion probability/cumulative exclusion probability following the method of Jamieson et al. (1997).

Results and Discussion

A total of 44 genomic DNA samples from 19 families was used as templates to amplify thirteen microsatellite markers in a single reaction. Amplified fragments were analyzed and the result showed that fragment sizes of ten markers; b1, b2, b3, b4, b6, b7, b10, b11, b12 and b13 were obtained in all samples, whereas that of two markers, b5 and b9, were inconsistently amplified due to the efficiency of amplification. One marker, b8, was inapplicable. In addition, it was found that b5 contains di-nucleotide repeat instead of tri-nucleotide repeat as previously reported (table 1). The data of ten markers was then further analyzed.

The observed size of the 10 markers was between 103 and 266 bp, allele frequencies, expected/observed heterozygosities and PIC are as shown in tables 2 and 3.

An accuracy test by computing the percentage of matched families for each marker found that seven out of ten markers were 100% matched except for three markers; b1-94.74%, b3-89.47% and b13-94.74%. The seven markers (b2, b4, b6, b7, b10, b11 and b12) with completely matched pedigree were further computed for each exclusion probability and cumulative exclusion probabilities as shown in table 4.

All thirteen microsatellite markers, except b8, contained dinucleotide short tandem repeats. However, one set of primers, b8, could not give an application and two markers which were b5 and b9 could amplify only in some samples. This result indicated that the efficiency of all markers in a single reaction is different due to several factors such as type of template, length of amplified product and the number of repeats.

This study found that the ten markers have numbers of alleles ranging from 5 to 12 (table 2) which is polymorphic and useful for individual identification. In addition, these markers can be used for pedigree analysis. Based on the selective standard of microsatellite loci (Barker, 1994), microsatellite loci ought to have at least four alleles to be considered useful for the evaluation of genetic diversity.

Polymorphism information content or PIC is a parameter indicative of the degree of informativeness of a marker (Botstein et al., 1980). The PIC of the ten markers revealed that nine are highly informative (PIC > 0.5) and one (b12) is moderately informative (0.25 < PIC < 0.5) based on criteria of Botstein et al. (1980).

The accuracy test indicated that seven markers (b2, b4, b6, b7, b10, b11 and b12) were completely matched with a known relationship. These seven markers were calculated for exclusion probability (PE) which is the probability that two random individuals do not share any alleles, so that parentage can be excluded (Visscher et al., 2002). The seven markers had the highest PEs and the combination of these markers had 0.9915 and 0.9997 cumulative PEs for one parent exclusion and both parents exclusion, respectively (table 4). This study demonstrated that seven markers can be used for parentage testing based on the number of alleles, PIC and the exclusion test. However, further analysis of all parameters in a larger sample size is required to evaluate the reliability of the selected markers in parentage testing.

Conclusion

In this study it was revealed that the ten out of thirteen multiplexed PCR microsatellite markers which were assigned as b1, b2, b3, b4, b6, b7, b10, b11, b12 and b13, could amplify all 44 Thai Holstein genomic DNA samples. The numbers of alleles in all ten markers were between five and twelve and that is useful for parentage test. Nine out of ten markers were highly informative (more than 0.5). For the accuracy test at 95% confidence level, seven (b2, b4, b6, b7, b10, b11 and b12) out of the ten markers could be used for parentage testing with the highest cumulative PEs at 0.9915 and 0.9997 for the single and both parents exclusions, respectively.

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Table 1: Thirteen microsatellite markers with known characteristics were selected from the Genome database. Each pair of primer was labeled with fluorescent dye to be used in fragment analysis.

Marker name	Locus name	Chromosome	Relative position (cM)	Repeat type	Expected size (bp.)	Expected heterozygosity	Florescent dye
b1	DIK1118	10	7	di-nucleotide	102-118	0.821	Ned
b2	TGLA40	25	12.3	di-nucleotide	103-125	0.750	Fam
b3	DIK023	11	43	di-nucleotide	106-128	0.769	Hex
b4	MS2063	3	116.5	di-nucleotide	151-169	0.643	Ned
b5	DIK1125	9	59	tri-nucleotide	151-174	0.786	Fam
b6	DIK4511	6	44.2	di-nucleotide	152-170	0.679	Hex
b7	DIK2662	24	14.2	di-nucleotide	203-219	0.679	Ned
b8	DIK5156	20	64.3	tri-nucleotide	203-228	0.731	Hex
b9	DIK5411	18	2.8	di-nucleotide	204-218	0.808	Fam
b10	DIK116	16	40.6	di-nucleotide	244-270	0.654	Ned
b11	DIK2733	30	1.6	di-nucleotide	247-255	0.607	Fam
b12	DIK4356	5	77.6	di-nucleotide	248-268	0.786	Hex
b13	DIK4515	30	150.5	di-nucleotide	186-198	0.714	Fam

Table 2: Allele frequencies and observed fragment sizes obtained from ten markers.

b1		b2		b3		b4		b6		b7		b10		b11		b12		b13	
Allele	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*	*Allele*	*Freq.*
103	1.14	113	51.14	105	20.45	145	3.41	155	1.14	205	52.27	240	12.50	239	9.09	255	1.14	192	2.27
105	36.36	115	20.45	109	6.82	149	11.36	157	31.82	209	1.14	244	3.41	243	2.27	257	3.41	196	14.77
107	6.82	117	10.23	111	1.14	151	2.27	161	2.27	211	12.50	246	15.91	247	42.05	261	69.32	198	12.50
109	20.45	119	3.41	113	3.41	153	17.05	163	52.27	213	10.23	252	3.41	249	2.27	263	19.32	200	45.45
111	25.00	121	11.36	115	9.09	155	14.77	165	3.41	215	11.36	254	40.91	251	2.27	265	6.82	202	18.18
113	4.55	123	3.41	117	21.59	157	13.64	167	3.41	217	9.09	256	7.95	253	13.64			204	6.82
115	5.68			119	13.64	161	15.91	169	5.68	219	2.27	260	3.41	255	26.14				
				121	3.41	163	21.59			223	1.14	264	5.68	257	2.27				
				123	9.09							266	6.82						
				125	2.27														
				127	4.55														
				129	4.55														
Observed size		*Observed size*		*Observed size*		*Observed size*		*Observed size*		*Observed size*		*Observed size*		*Observed size*		*Observed size*		*Observed size*	
103-115 bp		113-123 bp		105-129 bp		145-163 bp		155-169 bp		205-223 bp		240-266 bp		239-257 bp		255-265 bp		192-204 bp	

Table 3: PIC analysis in ten markers.

	Marker name									
	b1	**b2**	**b3**	**b4**	**b6**	**b7**	**b10**	**b11**	**b12**	**b13**
Locus name	DIK1118	TGLA40	DIK023	MS2063	DIK4511	DIK2662	DIK116	DIK2733	DIK4356	DIK4515
No. alleles	7	6	12	8	7	8	9	8	5	6
Expected heterozygosity	0.762	0.679	0.875	0.854	0.626	0.687	0.783	0.734	0.482	0.726
Observed heterozygosity	0.705	0.500	0.909	0.864	0.682	0.795	0.841	0.659	0.500	0.636
Polymorphic information content (PIC)	0.716	0.633	0.851	0.824	0.559	0.651	0.752	0.687	0.434	0.683

Table 4: Exclusion probability and cumulative exclusion probability in seven markers.

	Marker name						
	b4	**b10**	**b11**	**b7**	**b2**	**b6**	**b12**
Locus name	MS2063	DIK116	DIK2733	DIK2662	TGLA40	DIK4511	DIK4356
Exclude a parent							
Exclusion probability (PE)	0.6847	0.5958	0.5052	0.4751	0.4481	0.3673	0.2633
Cumulative PE		0.8726	0.9369	0.9669	0.9817	0.9884	0.9915
Exclude both parents							
Exclusion probability (PE)	0.8543	0.7949	0.6970	0.6822	0.6436	0.5423	0.4197
Cumulative PE		0.9701	0.9909	0.9971	0.9990	0.9995	0.9997

References

Barendse, W, Armitage, S.M., Kossarek, L.M., Shalom A., Kirkpatrick, B.W., Ryan, A.M., Clayton, D., Li, N., Neibergs, H.L., Zhang, N., Grosse, W.M., Weiss, J., Creighton, P., McCarthy, F., Ron, M., Teale, A.J., Fries, R., McGraw, R.A., Moore, S.S., Georges, M., Soller, M., Womack, J.E. and Hetzel, D.J.S. 1994. A genetic linkage map of the bovine genome. Nat. Genet. 6: 227-235.

Barker, J.S.F. 1994. A global protocol for determining genetic distances among domestic livestock breeds. In: Proceedings of the 25th World Congress on Genetics Applied to Livestock Production, Guelph and Ontario, Canada 21, 501-508.

Beckmann, J.S. and Weber, J.L. 1992. Survey of human and rat microsatellites. Genomics. 12: 627-631.

Bishops, M.D., Kappes, S.M., Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D., Yoo, J.Y. and Beattie, C.W. 1994. A genetic linkage map for cattle. Genetics. 136: 619-639.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.W. 1980. Construction of a genetic linkage map in human using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Burt, D.W., Bumstead, N., Bitgood, J.J., Ponce De Leon, F.A. and Crittenden, L.B. 1995. Chicken genome mapping: a new era in avian genetics. Trends Genet. 11: 190-194.

Crawford, A.M., Dodds, K.G., Ede, A.J., Pierson, C.A., Montgomery, G.W., Garmonsway, H.G., Beattie, A.E., Davies, K., Maddox, J.F., Kappes, S.W., Stone, R.T., Nguyen, T.C., Penty, J.M., Lord, E.A., Broom, J.E., Buitkamp, J., Schewaiger, W., Epplen, J.T., Matthews, P., Matthews, M.E., Hulme, D.J., Beh, K.J., McGraw, R.A. and Beattie, C.W. 1995. An autosomal genetic linkage map of the sheep genome. Genetics. 140: 703-724.

Ihara, N., Tahasuga, A., Mizoshita, K., Takeda, H., Sugimoto, M., Mizoguchi, Y., Hirano, T., Itoh, T., Watanabe, T., Reed, K.M., Snelling, W.M., Kappes, S.M., Beattie, C.W., Bennett, G.L. and Sugimoto, Y. 2004. A comprehensive genomic map of the cattle genome based on 3802 microsatellites. Genome Res. 14: 1987-1998.

Jamieson, A. and Taylor, St.C.S. 1997. Comparisons of three probability formulae for parentage exclusion. Anim. Genetics. 28: 397-400.

Kappers, S.M., Keele, J.W., Stone, R.T., Sonstegard, T.S., Smith, T.P.L., McGraw, R.A., LopezCorrales, N.L. and Beattie, C.W. 1997. A second-generation linkage map of the bovine genome. Genome Res. 7: 235-249.

Metzgar, D., Bytof, J. and Wills, C. 2000. Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res. 10: 72-80.

Park, S.D.E. 2001. Trypanotolerance in west African cattle and the population genetic effects of selection [Ph.D. thesis] University of Dublin for "The Excel Microsatellite Tool Kit". [http://www.animalgenomics.ucd.ie/sdepark/ms-toolkit/]

Rohrer, G.A., Alexander, L.J., Keele, J.W., Smith, T.P. and Beattie, C.W. 1994. A microsatellite linkage map of the porcine genome. Genetics. 136: 231-245.

Stormont, C. 1967. Contribution of blood typing to dairy science progress. J. Dairy Sci. 50: 253-260.

Toth, G., Gaspari, Z. and Jurka, J. 2000. Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10: 967-981.

Visscher, P.M., Woolliams, J.A., Smith, D. and Williams, J.L. 2002. Estimation of pedigree errors in UK dairy population using microsatellite marker and the impact on selection. J. Dairy Sci. 85: 2368-2375.