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Paternity testing of crossbred beef cattle with 15 microsatellite markers

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Abstract Alleles from only 9 microsatellites were detected as BM1818, BM1824, BM2113, CSSM66, ETH10, ETH225, INRA023, INRA037, and INRA063. The highest number of alleles (23 alleles) was found in CSSM66, while the lowest number (13 alleles) was found in ETH 10. BM1818 had expressed an allele size between 239 to 316 bp, while the smallest sizes, 129 to 179 bp, were detected in BM2113. Twenty-nine pairs of sire-offspring obtained a positive LOD score, whereas B-066 with Carlos-sire obtained a negative LOD score. It meant the 29 pairs of sire-offspring were matched with the pedigree records. These 9 microstellites could be used for paternity testing.

Keywords: paternity testing, microsatellite markers, beef cattle, Thailand

Introduction

Beef cattle is an important economic livestock in Thailand. The number of cattle in 2018 was approximately 5.4 million (ICT, 2018). Most are *Bos indicus* cattle: Native and Brahman. High quality beef for premium market is produced from crossbreds between *Bos indicus* cow and *Bos taurus* sire. Pon Yang Kham Livestock Breeding Cooperative NSC. Ltd. is a famous beef producer in Thailand. The Cooperative was in Sakol Nakhon Province (17°04'26.7"N 104°11'53.1"E). Beef cattle from the members' farms were registered, intensively reared, and fattened in accordance with the cooperative management system. The officers or members recorded the cattle pedigree, consisting of identification of animal, sire, dam, breeds, etc. All cattle were crossbreds of Native x Brahman dam or Brahman dam with taurine sire (Charolais). Both domestic and imported frozen semen were used to inseminate the dams.

Parentage testing is popular for testing human genealogy. In the past, blood group was used to determine parentage. Currently, molecular approach, especially microsatellite markers, are used widely to analyse parentage. Microsatellite markers are popular because they are highly polymorphic, co-dominant, PCR-based, and repeatable (Pemberton, 2008). Research documents

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concerning parentage analysis, especially of livestock in Thailand, is still limited. The advantage of parentage analysis is accurate identification of animals and their parents that can help improve their economic traits, particularly the carcass and meat characteristics. For checking these characteristics, usually the progeny was tested. Heritability of the traits is high level, so the traits are heritable, commonly via the sire. Misidentification would negatively affect the estimation of the breeding value of the traits. Thus, as mentioned, the objectives of this study were determined the feasibility of using 15 microsatellite markers for paternity testing and compared the accuracy of the outcomes testing against the pedigree records.

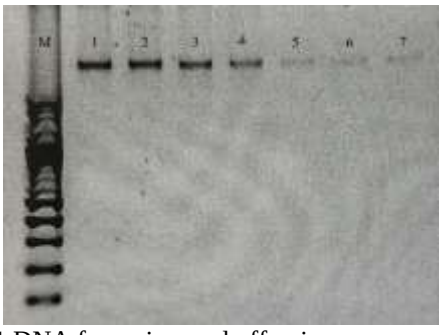
Materials and Methods

Animals, blood and semen collection

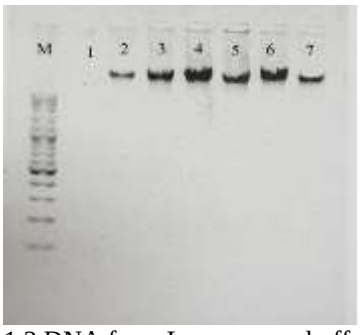
Thirty fattened crossbred steers (Native-Brahman cow or Brahman cow x Charolais sire) were raised by members of Pon Yang Kham Livestock Breeding Cooperative NSC. Ltd., located in Sakon Nakhon Province. About 10 ml of blood sample from jugular vein or ear vein (if blood from jugular vein was not accessible) from each animal was collected and mixed with 4%EDTA in a tube in order to prevent coagulation. All steers were offspring of 4 sires, namely Cowboy, Carlos, Lunaparc, and Canyon, which by their pedigree records, they sired 12, 6, 6, and 6 heads of offspring, respectively. The blood samples were kept at 4℃ in a plastic box and transported to Bangkok. Frozen semen of all sires was provided by the Cooperative. Both blood and frozen semen were stored at minus 20℃ for further DNA extraction.

DNA extraction

Blood and frozen semen samples were thawed to ambient temperature. DNA from the blood was extracted following the NucleoSpin Blood 50 preps kit (Macherey-Nagel) protocol, while DNA from the semen was extracted following the ISOLATE II Genomic DNA Kit 50 preps kit (Bioline) protocol. The absorbances of A260 and A260/280 that indicated the quantity and quality of both extracted DNA sources were measured with a spectrophotometer (SmartSpecTM Plus, BIO-RAD). The concentration of DNA was recorded. A further measurement of DNA quantity was performed using 1% agarose gel electrophoresis, see an example in Figure 1.



1.1 DNA from sires and offspring
M=100 bp marker, 1=Carlos, 2=Lunaparc, 3=Savant, 4=C198, 5-7=B-090, B-341, B-507



1.2 DNA from Lunaparc and offspring
M=100 bp marker, 1=Lunaparc, 2-7=B-403, B-501, B-502, B-503, B-504, B-505

Figure 1. DNA from sperm and blood sample

DNA amplification

Polymerase chain reaction (PCR) technique was used to amplify DNA with MyTaqTM HS Mix, 2x (Bioline). The PCR composition is shown in Table 1. The 15 microsatellite markers, recommended by FAO and used in this study, are shown in Table 2. All mixed samples were put in a PCR Machine (T100^{TM} Thermal Cycler, BIO-RAD). Table 3 showed the PCR cycling conditions.

Table 1. A PCR composition

Reagent	**Volumn (μL)**
5x My Taq Reaction Buffer	10
Template DNA	200 ng
Primer 10 μM _F	3 μL
Primer 10 μM _R	3 μL
MyTaq HS DNA Polymerase	0.5 μL
Water (ddH_2O)	up to 50 μL

The PCR products were analyzed by using 1% agarose (Vivantis) gel electrophoresis with 0.5X TBE buffer and were stained with 10000xGelRed Nucleic Acid Gel Stain in Water (Biotium) following a precast protocol. The standard marker was VC 100 bp DNA Ladder (Vivantis). The power supply (PowerNPac300, BIO-RAD) was set at 100 volts and 30 min. The DNA bands were visualized under UV (GelDocTM EZ Imager, BIO-RAD). In studying the feasibility of using the 15 markers for paternity testing, 3 DNA samples were tested at various melting temperatures (Tm) in accordance with FAO recommendation, as shown in Figure 2.

Table 2. The microsatellite primers used in the study (FAO, 2011)

Locus	Chromosome	Primer sequence (5′->3′) Forward Reverse	Tm°C	Size (bp)
BM1818	23	AGCTGGGAATATAACCAAAGG AGTGCTTTCAAGGTCCATGC	56-60	248-278
BM1824	1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	55-60	176-197
BM2113	2	GCTGCCTTCTACCAAATACCC CTTCCTGAGAGAAGCAACACC	55-60	122-156
CSRM60	10	AAGATGTGATCCAAGAGAGAGGCA AGGACCAGATCGTGAAAGGCATAG	55-65	79-115
CSSM66	14	ACACAAATCCTTTCTGCCAGCTGA AATTTAATGCACTGAGGAGCTTGG	55-65	171-209
ETH10	5	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55-65	207-231
ETH225	9	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	55-65	131-159
HEL1	15	CAACAGCTATTTAACAAGGA AGGCTACAGTCCATGGGATT	54-57	99-119
INRA023	3	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACTC	55	195-225
INRA037	10	GATCCTGCTTATATTTAACCAC AAAATTCCATGGAGAGAGAAAC	57-58	112-148
INRA063	18	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	55-58	167-189
TGLA53	16	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	55	143-191
TGLA122	21	CCCTCCTCCAGGTAAATCAGC AATCACATGGCAAATAAGTACATAC	55-58	136-184
TGLA126	20	CTAATTTAGAATGAGAGAGGCTTCT TTGGTCTCTATTCTCTGAATATTCC	55-58	115-131
TGLA227	18	CGAATTCCAAATCTGTTAATTTGCT ACAGACAGAAACTCAATGAAAGCA	55-56	75-105

Table 3. PCR cycling conditions

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	1 min	1
Denaturation	95 °C	15 s	34
Annealing	vary	15 s	
Extension	72 °C	10 s	
Final-extension	12 °C	∞	

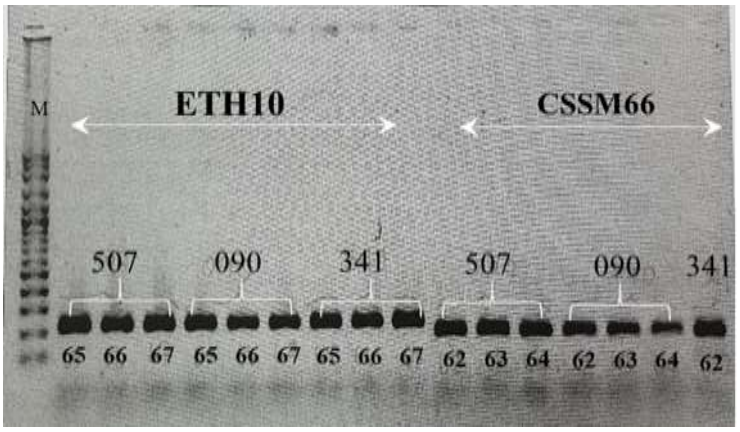


Figure 2. ETH10 and CSSM66 at Tm of 65, 66, and 67°C of B-507, B-090 and B-341 DNA sample (M=100 bp marker)

A test of nine primers that clearly showed DNA bands at a suitable Tm was repeated, beginning from amplifying the DNA from blood and semen samples to PCR product analysis. Then, the DNA bands were separated by a polyacrylamide gel (PAGE) technique and capillary electrophoresis (Bioanalyzer Model 2100, and DNA1000 kit chip, Agilent). See an example of a DNA allele in Figure 3.

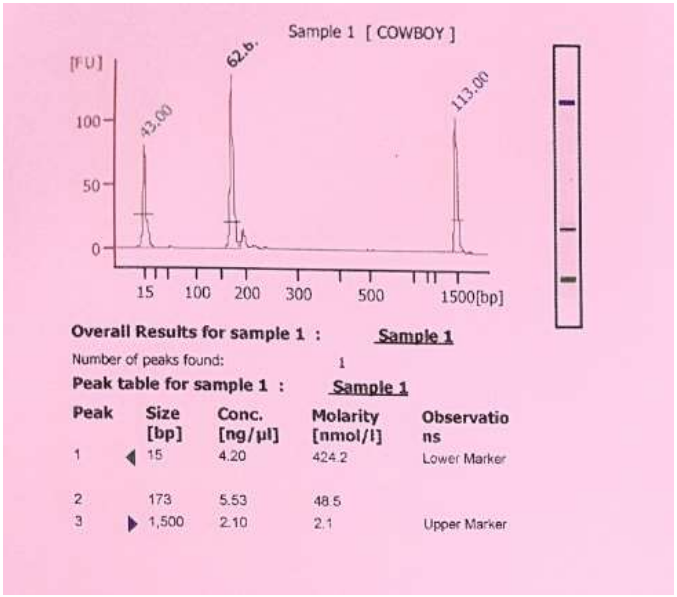


Figure 3. Allele of a Cowboy sire identified by capillary electrophoresis

Data analysis

Since there were 34 samples in total for each primer from 30 steers and 4 sires, but only 12 samples per chip could be run in one round of electrophoresis, the sizes of the alleles had to be overlaid both within and between chips with a standard of lower and upper markers by using the 2100 Bioanalyzer Expert Software (Agilent).

Cervus version 3.0.7 Software (Marshall *et al.*, 1998; Kalinowski *et al.*, 2007) was used to analyse observed heterozygosity (Ho), expected heterozygosity (He), polyamorphic information content (PIC), non-exclusion probability, and allele frequency. Then, an identity analysis, a simulation test, and a parentage assignment analysis were run. After that, the paternity was tested and the outcomes from the test were compared with the pedigree records.

Results

The feasibility of using the microsatellites for paternity testing

In determining the feasibility of using the 15 microsatellite markers for paternity testing, at the annealing step, the setting of melting temperature (Tm) of each primer was varied as recommended by FAO. Only 10 microsatellites had their DNA bands clearly detected (Table 4) at a specific Tm. The 10 primers were BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH10, ETH225, INRA023, INRA037, and INRA063. The highest suitable Tm (65℃) among all DNA samples was for ETH10, while the lowest at 55.5℃ was for BM1818, CSRM60, and INRA063.

Table 4. Suitable melting temperature (Tm) of the studied primers in the annealing step

No.	Locus	Tm (℃)	No.	Locus	Tm (℃)
1	BM1818	55.5	9	INRA023	56.0
2	BM1824	59.0	10	INRA037	56.0
3	BM2113	60.0	11	INRA063	55.5
4	CSRM60	55.5	12	TGLA53	NA
5	CSSM66	62.0	13	TGLA122	NA
6	ETH10	65.0	14	TGLA227	NA
7	ETH225	60.0	15	TGLA126	NA
8	HEL1	NA			

NA = Not available to determine the Tm of the primer

After the amplification step was repeated, the suitable Tm for each primer was set for the annealing step, and their DNA bands were separated by capillary electrophoresis. At this step, the allele from CSRM60 was not found, indicating that only 9 microsatellites had a feasibility to use for paternity testing. From the step of capillary electrophoresis after overlaying of the peak both within and between chips, the outputs were the allelic sizes of all primers. The genotypes of all animals indicated by these 9 microsatellites were recorded as a file.

Comparing the accuracy of the markers with the pedigree records

Allele frequency was analyzed with Cervus 3.0.7 software after the genotype file of all microsatellites was created. A list of statistical parameters of the studied loci is shown in Table 5. A total of 150 alleles were observed, with an average of 16.667 alleles per locus. The mean Ho and He were 0.997 and 0.901, respectively. The PIC ranged from 0.837 (INRA063) to 0.918 (CSSM66). The NE-1P was between 0.267 (CSSM66) and 0.449 (INRA063), while the NE-2P ranged from 0.154 (CSSM66) to 0.288 (INRA063). The highest null allele frequency (0.1071) was found in BM1818, and the lowest (0.0051) was found in INRA037. The sizes of the alleles and their corresponding frequencies for 9 microstellites were analyzed, shown in Table 6. The highest number of alleles (23 alleles) was observed in CSSM66, and the lowest (13 alleles) was noted in ETH10.

Table 5. Summary of the statistics of nine microsatellite loci

Locus	N	K	HObs	HExp	PIC	NE-1P	NE-2P	F(Null)
BM1818	34	15	0.706	0.884	0.858	0.411	0.258	0.1071
BM1824	34	14	0.971	0.903	0.880	0.365	0.222	0.0447
BM2113	34	17	1.000	0.878	0.855	0.409	0.256	0.0824
CSSM66	34	23	1.000	0.937	0.918	0.267	0.154	0.0408
ETH10	34	13	1.000	0.884	0.859	0.406	0.253	0.0738
ETH225	33	19	0.970	0.917	0.895	0.325	0.194	0.0362
INRA023	34	16	0.971	0.925	0.905	0.305	0.179	0.0315
INRA037	34	18	0.912	0.918	0.897	0.320	0.191	0.0051
INRA063	34	15	1.000	0.865	0.837	0.449	0.288	0.0862
Mean/Combined	16.667		0.997	0.901	0.878	9.443×10^{-5}	1.09×10^{-6}	

N = Number of samples, K= Number of alleles, HObs = Observed heterozygosity, HExp = Expected heterozygosity, PIC = Polymorphic information content, NE-1P = Non-exclusion probability of first parent, NE-2P = Non-exclusion probability of second parent, F(Null)= Estimation of null allele frequency.

Table 6. Alleles and their corresponding frequency for 9 microsatellites

BM1818		BM1824		BM2113		ETH10		CSSM66	
Allele	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**
239	0.0147	178	0.0588	129	0.0147	163	0.0147	183	0.0147
264	0.0294	180	0.1471	137	0.0147	218	0.0294	187	0.0147
269	0.0147	181	0.1471	143	0.0147	219	0.0147	189	0.0147
274	0.1618	182	0.0294	146	0.2941	220	0.1324	191	0.0147
275	0.0294	184	0.0147	147	0.0147	223	0.0294	192	0.0147
276	0.1765	185	0.0147	152	0.0294	227	0.2500	194	0.0882
281	0.1029	187	0.0588	153	0.0882	228	0.0294	195	0.0147
285	0.2059	189	0.0588	154	0.0294	232	0.0735	197	0.0882
297	0.0441	193	0.0735	158	0.0882	233	0.0882	199	0.0882
302	0.0147	195	0.1029	160	0.0147	234	0.0735	202	0.1029
305	0.0147	196	0.0147	161	0.0588	252	0.1324	205	0.1176
309	0.0588	201	0.0147	162	0.0294	253	0.0882	207	0.0147
311	0.1029	202	0.1765	164	0.0588	256	0.0441	209	0.0441
313	0.0147	204	0.0882	167	0.0147			210	0.0441
316	0.0147			171	0.1029			211	0.0147
				175	0.0294			212	0.0294
				179	0.1029			213	0.0147
								214	0.0147
ETH225		**INRA023**		**INRA037**		**INRA063**		219	0.0441
Allele	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	**Allele**	**Freq**	220	0.0147
155	0.0152	153	0.0294	139	0.0441	153	0.0294	221	0.0147
157	0.0152	160	0.0441	143	0.0147	160	0.0441	228	0.1324
158	0.0909	165	0.1029	144	0.0441	165	0.1029	232	0.0441
159	0.0303	179	0.0294	146	0.0588	179	0.0294		
162	0.0152	207	0.0294	148	0.0882	197	0.2647		
166	0.0303	209	0.0588	150	0.0294	201	0.0294		
167	0.0606	218	0.1029	153	0.0294	203	0.0588		
172	0.1061	224	0.1176	154	0.1176	206	0.0294		
173	0.1970	227	0.1471	155	0.0147	209	0.0147		
175	0.0455	235	0.0294	158	0.1176	210	0.0147		
178	0.0909	236	0.0882	159	0.1765	211	0.0147		
180	0.0758	237	0.1029	162	0.0147	223	0.1765		
185	0.0303	241	0.0588	163	0.0147	225	0.1618		
186	0.0152	245	0.0147	166	0.0147	234	0.0147		
187	0.0152	254	0.0294	168	0.0147	238	0.0147		
188	0.1212	260	0.0147	172	0.0294				
189	0.0152			173	0.1176				
192	0.0152			176	0.0588				
195	0.0152								

BM1818 had the biggest allelic size, ranging from 239 to 316 base pairs, whereas the smallest size was noticed in BM2113, ranging from 129-179 base pairs. INRA023 had a wider range of allele sizes, from 153 to 260 bp. The highest allele frequencies for the corresponding microsatellite (BM1818,

BM1824, BM2113, ETH10, ETH225, CSSM66, INRA023, INRA037, and INRA063) were 0.2059 (allele 285), 0.1765 (allele 202), 0.2941 (allele 146), 0.2500 (allele 227), 0.1970 (allele 173), 0.1324 (allele 228), 0.1471 (allele 227), 0.1765 (allele 159), and 0.2647 (allele 197).

The result of a simulation of parentage analysis in the case of father alone with all offspring is presented in Table 7. At a strict criterion, the assignment rate observed was 83%, meaning that 83% of offspring were assigned, and 5% were expected to be wrong. At a more relaxed criterion, 90% of offspring were assigned, and 20% were expected to be wrong. The delta score and LOD score were 5.22 and 3.50 for 95% and 80% of confidence levels, respectively.

Table 7. Delta criteria at different confidence levels for the case of father alone with all offspring

Level	Confidence (%)	Critical Delta	Assignments	Assignment Rate
			Observed (Expected)	Observed (Expected)
Strict	95.00	5.22	25 (5)	83% (16%)
Relaxed	80.00	3.50	27 (6)	90% (21%)
Unassigned			3 (24)	10% (79%)
Total			30 (30)	100% (100%)

A comparison of the parameters for the relationships between true parent with offspring is given in Table 8. Paired loci mismatchings were found in candidate sire Cowboy with B-072 (1 locus), Carlos with B-066 (3 loci), B-506 (1 locus), Lunaparc with B-505 (1 locus), and Canyon with B-792 (1 locus). It is interesting that most of pair LOD score was positive, whereas a negative pair LOD score (-5.33E+00) was found in the candidate sire Carlos with B-066. Their pair confidence was reported by the software as a blank, meaning that most of the offspring alleles mismatched with candidate sire alleles. Moreover, 25 pairs of sire-offspring were obtained at a strict (*) confidence level, while two pairs were obtained at a relaxed (+) confidence level, whereas another two pairs were obtained at a confidence level of less than 80 percent (-).

Table 8. Comparison of parameters for relationships between true parent with offspring

Offspring ID	Candidate father ID	Loci typed	Pair loci compared	Pair loci mismatching	Pair LOD score	Pair confidence
B-067	Cowboy	9	9	0	9.56E+00	*
B-072	Cowboy	9	9	1	1.68E+00	-
B-090	Cowboy	9	9	0	6.17E+00	*
B-223	Cowboy	9	9	0	5.60E+00	*

Table 8. Continue

Offspring ID	Candidate father ID	Loci typed	Pair loci compared	Pair loci mismatching	Pair LOD score	Pair confidence
B-315	Cowboy	9	9	0	7.28E+00	*
B-341	Cowboy	9	9	0	5.70E+00	*
B-344	Cowboy	9	9	0	5.57E+00	*
B-366	Cowboy	9	9	0	5.35E+00	*
B-507	Cowboy	9	9	0	7.55E+00	*
B-663	Cowboy	9	9	0	7.94E+00	*
B-855	Cowboy	9	9	0	4.84E+00	+
B-995	Cowboy	9	8	0	7.71E+00	*
B-041	Carlos	9	9	0	1.21E+01	*
B-066	Carlos	9	9	3	-5.33E+00	
B-410	Carlos	9	9	0	1.03E+01	*
B-506	Carlos	9	9	1	7.61E+00	*
B-508	Carlos	9	9	0	1.16E+01	*
B-616	Carlos	9	9	0	1.08E+01	*
B-043	Lunaparc	9	9	0	9.13E+00	*
B-501	Lunaparc	9	9	0	9.48E+00	*
B-502	Lunaparc	9	9	0	1.06E+01	*
B-503	Lunaparc	9	9	0	1.30E+01	*
B-504	Lunaparc	9	9	0	1.13E+01	*
B-505	Lunaparc	9	9	1	4.56E+00	+
B-002	Canyon	9	9	0	1.07E+01	*
B-006	Canyon	9	9	0	1.27E+01	*
B-437	Canyon	9	9	0	9.12E+00	*
B-664	Canyon	9	9	0	8.73E+00	*
B-792	Canyon	9	9	1	3.28E+00	-
B-889	Canyon	9	9	0	9.45E+00	*

Pair loci compared: comparison between the locus of the matched offspring alleles and the locus of the candidate sire alleles; **Pair loci mismatching**: comparison between the locus of the mismatched offspring alleles and the locus of the candidate sire allels; **Pair LOD score**: comparison between the Log-likelihood ratio score of the offspring and that of the candidate sire; A positive LOD score means that the candidate sire had a higher

probability of being the true sire than not being the true sire; A score of zero means that the candidate sire had equal probabilities of being and not being the true sire; A negative score means that the candidate sire had a lower probability of being the true sire than not being the true sire; Most negative scores were due to mismatching of alleles between the offspring and the candidate sire for at least 1 locus; **Pair confidence level**: confidence levels of matching offspring alleles with candidate sire alleles were strict (* = 95 percent confidence level), relaxed (+ = 80 percent confidance level), unassigned (- = less than 80 percent confidance level), and blank (most of the offspring alleles mismatched with the candidate sire alleles).

Discussion

With HEL1, TGLA53, TGLA122, TGLA227, and TGLA126 markers, no amplified alleles were found even when Tm was varied, and though some alleles were found with CSRM60, it showed no allele polymorphism. These results are in contrast with the results reported by Cervini *et al*. (2006), analyzing Brazilian Nellore cattle (*Bos indicus*), and those reported by Peelman *et al*. (1998), analyzing European cattle (*Bos taurus*). Both works found some polymorphism information content of the TGLA53, TGLA122, TGLA227, and TGLA126 markers.

Marshall *et al*. (1998) stated that the rule of thumb is that loci with an expected heterozygosity (He) of 0.5 or less are not useful for parentage analysis. Our mean He for the 9 loci was 0.901, much higher than 0.5. The PIC of the 9 loci in our study was at a high level, ranging from 0.837 to 0.918. The He was also greater than the PIC. These indicated that the nine loci were useful for identifying the sire of a steer.

The range of number of alleles and their size in our study were different from previous studies (Peelman *et al*., 1998; Curi and Lopes, 2002; Yoon, *et al*., 2005; Cervini *et al*., 2006; Van Eenennaam *et al*., 2007; Tian *et al*., 2008; Escobar *et al*., 2009; Stevanovic *et al*., 2010; Zhang *et al*., 2010; and Pei *et al*., 2018). It probably be because of the following reasons: 1) the genetics of the cattle, i.e., our animals were crossbreds between Zebu (Native or Brahman cow) and European cattle (Charolais sire), while the works above dealt with pure breeds of Indian cattle or European cattle or crossbreds of dairy cattle and beef cattle or with *Bos grunniens*; and 2) the procedures and instruments for DNA extraction, purification, and amplification, and alleles identification that we used were quite different from those that they used, i.e., we used a capillary electrophoresis Bioanalyzer 2100 with DNA1000 kit chip (Agilent), whereas they used an automated sequencer ABI PRISM 310 (Applied Biosystem). Some researchers used polyacrylamide gel electrophoresis.

Ten pairs of Cowboy sire-offspring from a total of 12 pairs were matched at a strict confidence level, meaning that Cowboy was likely to be the true sire of the 10 offspring, which matched perfectly with the recorded pedigree. On the other hand, B-995 was analyzed as Cowboy's offspring at a relaxed confidence level, i.e., Cowboy was likely to be the true parent of B-995 at 80% confidence level, but not as likely as the 10 offspring. From the pedigree records, B-072 was also Cowboy's offspring, but Cowboy was analyzed to match with B-072 only at a "-" confidence level (less than 80%), a lower confidence level than the eleven pairs mentioned above.

On the other hand, although the obtained pedigree records stated that B-066 was an offspring of Carlos, the negative pair LOD score of this candidate sire Carlos with B-066 indicated that probability was that Carlos was more likely not being the true parent of B-066. This might be that the pedigree records were plagued by human error, or it might be that the B-066 dam was inseminated twice with the frozen semen specimens of Carlos and another sire. A possibility is that the cooperative officer or the owner recorded only Carlos as the sire but not the other one. Misidentification would affect the estimation of the breeding value of sires. Thus, pedigree records of crossbred beef cattle should be confirmed by molecular approach, especially by the nine studied microsatellite markers.

We tested the feasibility of using 15 microsatellite markers for paternity testing and found that 9 markers: BM1818, BM1824, BM2113, CSSM66, ETH10, ETH225, INRA023, INRA037, and INRA063, can be used to test the paternity in crossbred beef cattle. Furthermore, our results will be even more useful when a progeny test of its carcass and meat qualities is undertaken and the breeding value of the sire is estimated more reliably. In order to improve the carcass and meat qualities of beef cattle in Thailand, the best sire with the best EBV should be used to inseminate cows.

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