Buffalo microsatellite markers and freemartin test

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Sampling

Forty blood samples of Taiwan Buffalos from Hualien Animal Propagation Station of LRI were collected and extracted for DNA.

Table 1. Recommended buffalo microsatellite markers list (FAO-MoDAD)

Nr.	Marker	chr.	Nr.	Marker	chr.	Nr.	Marker	chr.
1.	CSSM033	17 (17)	11.	BRN	11 (10)	21.	DRB3	2p (23)
2.	CSSM038	11 (10)	12.	CSSM032	1q (1)	22.	CSSM062	-
3.	CSSM043	1p (27)	13.	CSSM008	-	23.	CSSME070	3p (19)
4.	CSSM047	3q (8)	14.	CSSM045	2q (2)	24.	ETH121	2q (2)
5.	CSSM036	1p (27)	15.	CSSM022	4q (5)	25.	ILSTSO33	13 (12)
6.	CSSM019	1q (1)	16.	CSSM046	11 (10)	26.	ILSTS005	11 (10)
7.	CSRM060	11 (10)	17.	CSSM013	5p (29)	27.	ILSTS030	2q (2)
8.	CSSM029	9 (7)	18.	ETH003	3p (19)	28.	ILSTS008	15 (14)
9.	CSSM041	21 (22)	19.	CSSM061	-	29.	RM099	3p (19)
10.	CSSM057	9 (7)	20.	CSSM013	5p (29)	30.	HMH1R	21 (22)

Cattle chromosome assignments (in parentheses) - as given at

Table 2-1. Primer sequences and annealing temperature of microsatellite loci used in the study

Markers	Primer sequences	Annealing Temp. (℃)
CSSM019	* ttgtcagcaacttcttgtatcttt tgttttaagccacccaattatttg	55
CSSM033	* cactgtgaatgcatgtgtgtgagc cccatgataagagtgcagatgact	55
CSSM038	* ttcatataagcagtttataaacgc ataggatctggtaacttacagatg	55
CSSM041	* aatttcaaagaaccgttacacagc aagggacttgcagggactaaaaca	55
CSSM043	* aaaactctgggaacttgaaaacta gttacaaatttaagagacagagtt	55
CSSM045	* tagaggcacaagcaaacctaacac ttggaaagatgcagtagaactcat	55

* Lebal 6-Fam

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Table 2-2. Primer sequences and annealing temperature of microsatellite loci used in the study

Markers	Primer sequences	Annealing Temp. (℃)
CSSM047	* tctctgtctctatcactatatggc ctgggcacctgaaactatcatcat	55
CSRM060	* aagatgtgatccaagagagaggca aggaccagatcgtgaaaggcatag	55
CSSME032	* ttattttcagtgtttctagaaaac tataatattgctatctggaaatcc	47
CSSME070	* ttctaacagctgtcactcaggc atacagattaaatacccacctg	55
CSSM013	* ataagagattacccttcctgactg aggtaaatgttcctatttgctaac	55
BRN	* cctccacacaggcttctctgactt cctaacttgcttgagttattgccc	55

* Label 6-Fam

PCR condition

Ingredient	Concentration	Volume
Polymerase Taq	5 U / μ l	0.2 μ l
Primer (F)	10 μ M	0.5 μ l
Primer (R)	10 μ M	0.5 μ l
dNTP	2.5mM	2.0 μ l
10X PCR buffer		2.5 μ l
Distilled water		18.3 μ l
DNA template	100 ng / μ l	1.0 μ l
Total volume		25.0 μ l

- 96℃ 3mins
- 95 ℃ 30 secs
  55℃ 30 secs
  72℃ 45 secs
  35cycles
- 72℃ 10 mins
- 15℃ ∞ mins

- The PCR analyses were carried out using an Applied Biosystems GeneAmp® PCR System 9700 thermal cycler (ABI 9700)

Microsatellites analysis

- **For all microsatellites allele size was determined on all samples with a Perkin Elmer ABI Prism 3100 Genetic Analyzer using the GeneScan Software (Perkin Elmer).**
- **For the population and for each locus number of alleles (nA), observed heterozygosity (H0), unbiased expected heterozygosity (He) and polymorphic information content (PIC) were calculated using Excel Microsatellite Toolkit .**

Table 3. Number of alleles of twelve microsatellite markers detected in Taiwan Buffalo

Markers	Chr.	Fragments (bp)	Na
CSSM019	1	136, 138,142, 152, 154, 158	6
CSSM033	17	157, 167, 177	3
CSSM038	11	163, 171, 173	3
CSSM041	21	126, 136, 138, 140	4
CSSM043	1	220, 242, 246, 248	4
CSSM045	2	98	1
CSSM047	3	126, 128, 148, 150	4
CSRM060	11	88, 90, 92	3
CSSME032	1	211, 213, 215, 217, 221, 223	6
CSSME070	3	125, 127	2
CSSM013	5	165, 167, 169, 171	4
BRN	11	127, 129, 131, 139	4
Total			44

Chr.: Chromosome, Na: Number of allele

Table 4. Genetic diversity parameters in Taiwan Buffalo population

Markers	Observed heterozygosity	Expected heterozygosity	PIC
CSSM019	0.725	0.700	0.650
CSSM033	0.550	0.606	0.514
CSSM038	0.575	0.566	0.472
CSSM041	0.550	0.680	0.601
CSSM043	0.750	0.681	0.621
CSSM047	0.625	0.545	0.492
CSRM060	0.400	0.462	0.388
CSSME032	0.750	0.757	0.706
CSSME070	0.150	0.182	0.164
CSSM013	0.525	0.588	0.513
BRN	0.575	0.512	0.444
MEAN	0.561	0.570	0.506

PIC : polymorphic information content.

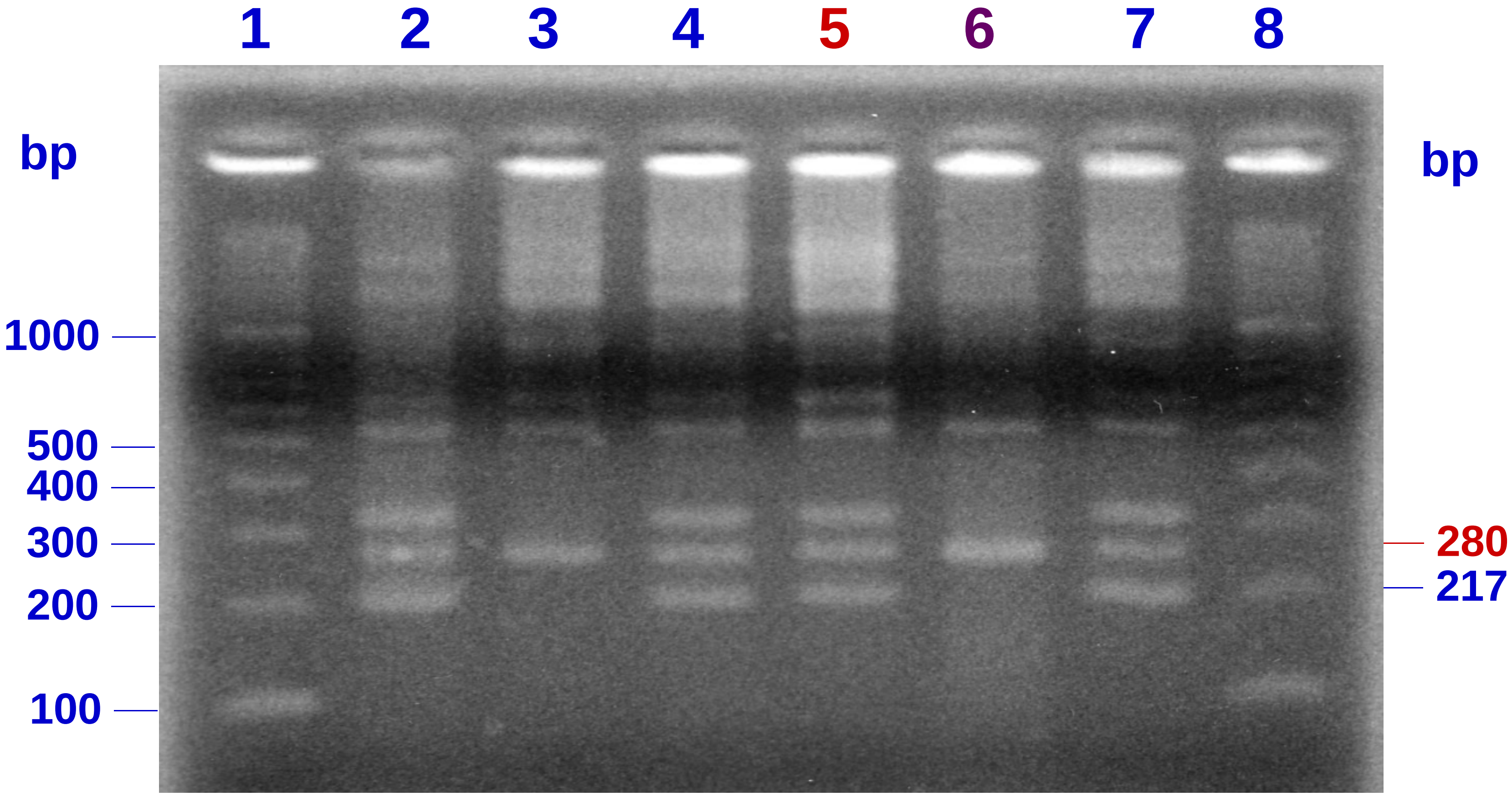

Fig. 1. Diagnosis of freemartinism by AMX/Y sexing marker. Lane 1, 8: 100bp ladder. Lane 2, 7: normal male genomic DNA. Lane 3: normal female DNA. Lane 4: heterosexual twin male DNA. Lane 5: heterosexual twin female DNA(freemartin). Lane 6: heterosexual twin female DNA(normal).

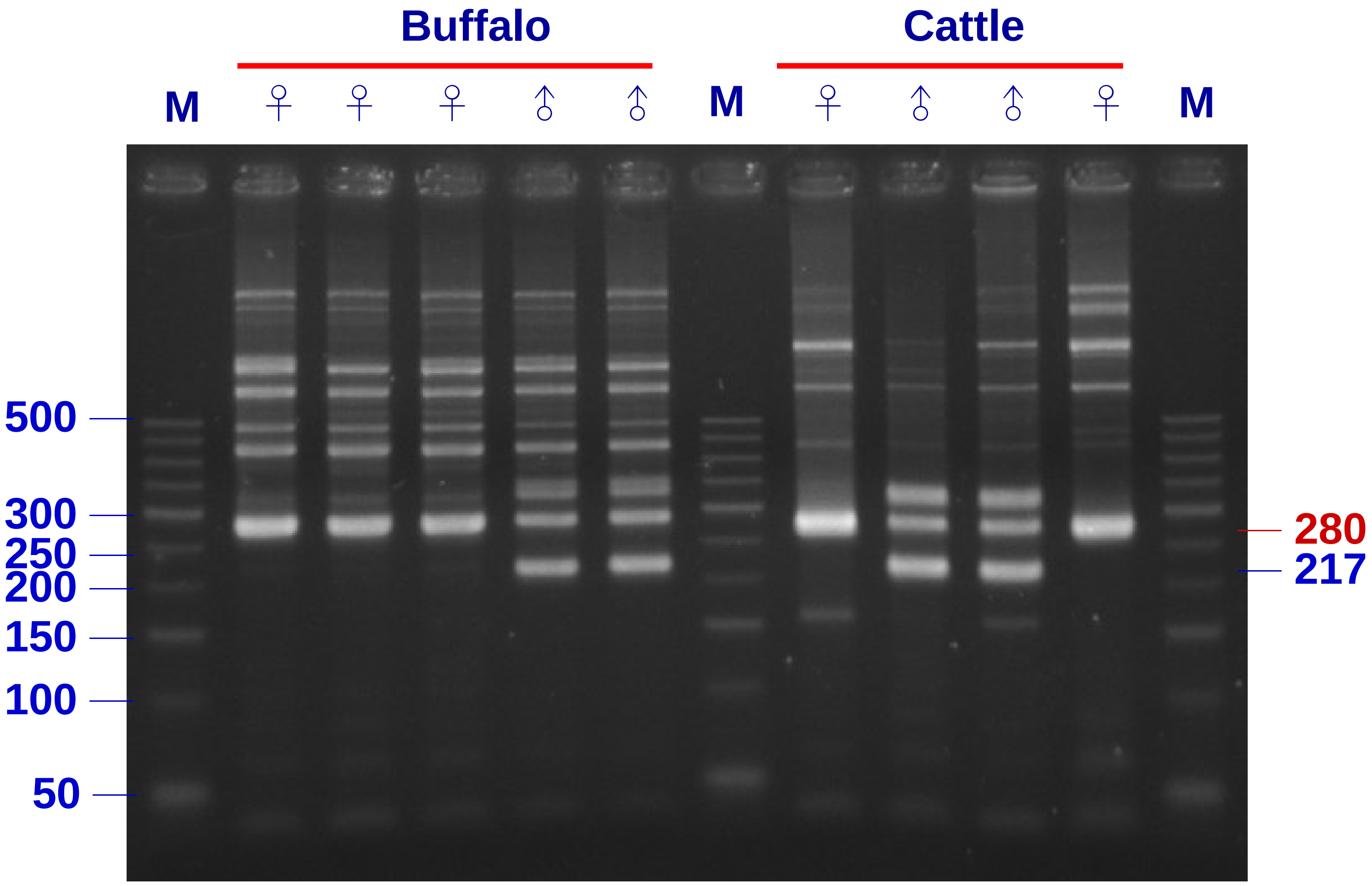

Fig. 2. DNA analysis of buffalo by bovine sexing marker, AMX/Y.

Summary

- **All Loci were polymorphic and generating 43 alleles with mean 3.91, except CSSM045.**
- **Number of allele in polymorphic loci ranged from 2 to 6.**
- **CSSME032 was the most polymorphic according to her polymorphic information content(PIC).**
- **CSSM019 and CSSME032 showed the highest and equal number of alleles but CSSME032 was more polymorphic than CSSM019 according to her PIC.**
- **In 12 markers, six markers were not highly informative with PIC less than 0.5 and one marker CSSM045 had only one allele detected. Therefore, geneticist should be aware of genetic diversity of Taiwan water buffalo.**
- **Bovine sexing marker could also be useed for buffalo freemartin test.**