OPTIMISATION OF PARENTAGE VERIFICATION METHODOLOGY USING MICROSATELLITE MARKERS THROUGH CONVENTIONAL PCR IN MALAYSIAN CATTLE

Ernie-Muneerah, M.A.[1*], Suriaty, R[2]., Mohd-Hafiz, A.R.[2], Salleh, S.I.[3] & Mohd-Hafizal, A.[2]

[1]Research & Innovation Division, Department of Veterinary Services, Putrajaya
[2]National Institute of Veterinary Biodiversity, Jerantut, Pahang.
[3]Livestock Resource Development Division, Department of Veterinary Services, Putrajaya
*Email: ernie@dvs.gov.my

Parentage analysis of livestock animals is becoming increasingly utilized in the agriculture industry for selection of breed to increase production traits. Microsatellite DNA marker are a powerful tool for parentage verification. Recent studies done by Mohd-Hafiz *et.al.*, 2012 (1) has evaluated thirteen microsatellite markers (13) that has been recognized by the International Society of Animal Genetics (ISAG)/Food and Agricultural Organization (FAO) and also complementary marker used by international labs. To further assist with parentage analysis, this study has been done to optimize the methodology using microsatellite markers through conventional PCR for various cattle breed in Malaysia.

Blood samples were collected from cattle breeds in vacutainer tube with 7.5 mg EDTA. Genomic DNA extraction was done using QIAamp DNA Blood Mini Kit (QIAGEN). Thirteen (13) microsatellite DNA regions were then amplified using conventional PCR technique and amplification reactions were done in Applied Biosystem, Veriti Model Thermocycler. PCR optimization was done using annealing temperature range from 46°C to 65°C. Amplified DNA fragments were separated using 3% MetaPhore agarose gel and were detected by Fluorosafe stain,1st Base and visualised under SYNGENE G:Box Gel Documentation and Analysis System.

For successful amplification of microsatellite DNA fragments, optimum annealing temperature is needed.This study managed to show the optimum annealing temperature and their microsatellite markers information.

No	Primer Name	Annealing Temperature	Notes	Marker References
1	MB009	54	• Complementary marker used by international lab	Ihara et al. 2004 (2)
2	CSRM60	58	• Recommended by ISAG/FAO	Moore et al. 1994 (3)
3	CSSM66	56	• Recommended by ISAG/FAO	Barendse et al. 1994(4)
4	INRA005	54	• Recommended by ISAG/FAO	Vaiman et al. 1992 (5)
5	HEL9	58	• Recommended by ISAG/FAO	Kaukinen &Varvio 1993(6)
6	BM2113	58	• Recommended by ISAG/FAO • Highest heterozigosiy for Brahman cattle(1)	Bishop et al. 1994 (7)
7	ETH152	56	• Recommended by ISAG/FAO	Steffen et al. 1993 (8)
8	TGLA227	56	• Recommended by ISAG/FAO	Georges & Massey 1992 (9)
9	TGLA53	56	• Recommended by ISAG/FAO	Georges &Massey 1992 (9)

10	TGLA122	58	• Recommended by ISAG/FAO	Georges & Massey 1992 (9)
11	ETH10	58	• Recommended by ISAG/FAO • Low exclusion probability for Brahman cattle(1)	Solinas Toldo et al. 1993 (10)
12	BM1824	52	• Recommended by ISAG/FAO • Highest heterozigosity for Mafriwal cattle(1) • Low exclusion probability y for Brahman cattle(1)	Bishop et al. 1994 (7)
13	INRA177	58	• Complementary marker used by international lab	Kappes et al. 1997 (11)

Table 1: List of microsatellite markers suitable for parentage verification in Malaysian cattle and their optimized annealing temperature.

Parentage verification is based on the concept that a calf possesses only two alleles of every locus, one of which inherited form the sire and the other from the dam. Besides parentage verification, this optimization result can also be used for other molecular study; such as population diversity and inbreeding study.

1. Mohd. Hafiz,A.R., Zawawi,I. Mohd-Hafizal,A., Salleh,S.I, & Ernie-Muneerah,M.A. 2012. Evaluation of Microsatellite Markers for Parentage Testing in Cattle, In Procceding of Konvensyen Penyelidikan Veterinar 2012, Serdang, Selangor. Pg 6-7.
2. Ihara,N., Takasuga,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,, Ben nett, G.L & Sugimoto, 2001. A comprehensive genetic map of the cattle genome based on 3802 microsatellites. Y. Genome Res. 1987-98.
3. Moore,S.S., Byrne, K. 1994.Characterization of 65 bovine microsatellites. Mammalian Genome 5:84-90.
4. Barendse,W., Armitage,S.M. 1994. A genetic linkage map of the bovine genome. Nature Genetics 6: 227-235.
5. Vaiman, D., Osta, D. 1992.Characterisation of five new bovine microsatellite repeats. Anim.Genet.23:537.
6. Kaukinen,J. & Varvio,S.L. 1993. Eight polymorphic bovine microsatellites. Anim. Genet. 24:148.
7. Bishop,M.D. & Kappes,S.M. 1994. A genetic linkage map for cattle. Genetics. 136:619-639
8. Steffen,P. & Eggen, A. 1993. Isolation and mapping of polymorphic microsatellites in cattle. Anim. Genet. 24: 121-124.
9. Georges,M. & Massey,J. 1992: Polymorphic DNA markers in Bovidae, WO. Publ. No. 92:13120.
10. Solinas-Toldo,S., Fries,R. 1993. Physically mapped, cosmid-derived microsatellite markers as anchor loci on bovine chromosomes. Mamm. Genome 4:720-727.
11. Kappes,S.M, Keele,J.W, Stone,R.T, McGraw,R.A., Sonstegard,T,S,, Smith, T,P,, Lopez-Corrales, N,L. & Beattie, C,W. 1997. A Second-generation linkage map of the bovine genome.Kappes Genome Res. 7(3):235-49.