ELUCIDATION OF RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS IN CROSSBRED CATTLE*

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Abstract

The PCR conditions for RAPD-PCR amplification were standardized for the crossbred cattle of Kerala. Nineteen random oligonucleotide primers were selected based on intensity, clarity and polymorphism of bands from 26 primers for amplification of unrelated DNA samples of crossbred cattle of Kerala. Number of bands obtained for different primers ranged from 2 to 16. The average number of bands produced by different primers ranged from 3.78 ± 0.15 in OPA 4 to 8.15 ± 0.25 in ILO 1127. The size of amplification products ranged from ~230 base pairs to ~3580 base pairs. The percentage of polymorphism represented by individual primers varied from 66.66 to 100 in different groups. Seven primers namely OPA 2, OPA 4, OPA 17, OPA 18, OPA 20, ILO 1127 and ILO 876 yielded 100% polymorphism in bands.

Key words : *RAPD, polymorphism, cross bred cattle*

Randomly Amplified Polymorphic DNA (RAPD) markers are arbitrary nucleotide sequences used to detect nucleotide sequence polymorphisms in DNA in a low stringency amplification reaction. They amplify pieces of DNA which are flanked by two inverted copies of the primer and are having the length between 200 to 3500 base pairs. Polymorphisms occur when there are sequence variations in the primer binding sites. Compared to the other molecular markers RAPD analysis requires only low quantities of template DNA (5-50ng per reaction). It is easy and quick and quite inexpensive. The technique generates multiple bands and is amenable for automation. Because of these reasons RAPD markers were one of the first choices for DNA studies in earlier days.

The major objective of the study was to delineate the RAPD marker polymorphisms in crossbred cattle of Kerala. The polymorphic patterns produced by different primers will be useful to study the molecular genetic characters of these animals.

Materials and Methods

The blood samples of 179 unrelated crossbred cattle were collected from the various organized farms and also from farmers' premises. Immediately after collection, the blood was transferred to sterile disposable polypropylene tubes with EDTA as anticoagulant @1mg / ml of blood. DNA was isolated from these samples using standard Phenol: Chloroform isolation procedure (Andersson *et al.*, 1986). Twenty six primers were used to find out the RAPD polymorphism of crossbred cattle. Commercially available primer kit (Operon Tecknologies Inc., USA) and custom synthesized primers (Life Teckno-

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logies India, Pvt., Ltd.) were used in this study. The random 10 base oligonucleotide primers selected in the study was OPA 1 to OPA 20 , ILO 1127, 1LO 876, OPAV 15, G_1 and G_2. The primers were suspended in sterile nuclease free distilled water to make the stock solution with a final concentration of 200 p*M* /µl.

For all the PCR reactions 10 µl volume was used. Each reaction tube had one microlitre each of template DNA (5p*M* /µl) and 10X pcr buffer (10 m*M* tris, pH 8.3; 50 m*M* KCl, 1.5 m*M* $MgCl_2$), 0.5 unit of taq DNA polymerase and one microlitre mixture of 1500 µ*M* each of the four dNTP solutions. The initial denaturation was done at 94°C for three minutes. Thirty five cycles of PCR were done. The denaturation was at 94°C for one minute, annealing temperature was 38°C for one minute and the DNA synthesis step was 72°C for one minute. A final extension of 72°C for 30 minutes was also done. The amplified products were stored at -20 °C till use.

The PCR products were run on 2% agarose gel. Electrophoresis was done at 100 volts and 300 mA current for two hours. TAE (Tris Acetic acid EDTA) was used as the electrophoresis buffer. The gel was then taken out and viewed through UV transilluminator. The good gels were saved in gel documentation system with quantity one programme. The bands were scored as present or absent. The frequency of different bands, their polymorphism and other characters were noted. The polymorphic pattern of each primer was assessed.

Results and Discussion

Out of twenty six random oligonucleotide primers used for the amplification of pooled DNA samples, twenty four primers yielded products. Based on intensity, clarity and

Table 1. Name, sequence and approximate size of bands amplified by different primers

Sl. No.	Primer	Sequence	Approximate size of bands	
			Range (kb)	Average
1	OPA 1	5'CAGGCCCTTC3'	0.29 - 2.24	1.14 ± 0.27
2	OPA 2	5'TGCCGAGCTG3'	0.37 – 2.34	1.78 ± 0.20
3	OPA 4	5'AATCGGGTTG3'	0.28 – 2.24	0.95 ± 0.17
4	OPA 7	5'GAACGGGCTG3'	0.29 – 2.35	1.13 ± 0.29
5	OPA 8	5'GTGACGTAGG3'	0.33 – 2.31	1.14 ± 0.20
6	OPA 9	5'GGGTAACGCC3'	0.40 – 2.10	1.05 ± 0.18
7	OPA 10	5'GTGATCGCAG3'	0.34 – 2.59	1.19 ± 0.27
8	OPA 12	5'TCGGCGATAG3'	0.39 – 1.50	0.87 ± 0.15
9	OPA 14	5'TCTGTGCTGG3'	0.30 – 2.40	1.14 ± 0.18
10	OPA 15	5'TTCCGAACCC3'	0.23 – 2.66	0.93 ± 0.20
11	OPA 16	5'AGCCAGCGAA3'	0.43 – 3.58	1.62 ± 0.31
12	OPA 17	5'GACCGCTTGT3'	0.28 – 2.74	1.19 ± 0.28
13	OPA 18	5'AGGTGACCGT3'	0.45 – 1.84	1.12 ± 0.21
14	OPA 19	5'CAAACGTCGG3'	0.31 – 2.09	0.99 ± 0.15
15	OPA 20	5'GTTGCGATCC3'	0.50 – 2.26	1.20 ± 0.20
16	PRG 1	5'GTGACGTAGG3'	0.46 – 2.27	1.20 ± 0.22
17	ILO 1127	5'CCGCGCCGGT3'	0.34 – 3.03	1.39 ± 0.29
18	ILO 876	5'GGGACGTCTC3'	0.23 – 2.02	0.96 ± 0.17
19	ILO 526	5'GCCGTCCGAG3'	0.30 – 2.25	1.01 ± 0.22

polymorphism of bands, nineteen primers were selected for finding out the RAPD polymorphic patterns in crossbred cattle of Kerala. The sequences, range and average size of bands produced by these primers are presented in Table 1. The smallest band identified was that of the primer ILO 896 (0.23kb) and the largest band observed was of 3.58 kb length from the primer OPA 16.

The average number of bands produced by different primers ranged from 3.78 ± 0.15 in OPA 4 to 8.15 ± 0.25 in ILO 1127 (Table 2). Number of different bands produced by various primers ranged from 7 in OPA 18 to 16 in ILO 1127. The percentage of polymorphism represented by selected primers varied from 66.66% to 100%.

The primers chosen for RAPD-PCR were of 10 base lengths in this study. Because of the short size of the primers, the specificity was also low. Very small alterations in the PCR conditions can affect the products of the reaction as they depend on the sequence and length of oligonucleotide primers and reaction conditions.

In the present study, 19 primers were chosen for depiction of RAPD polymorphisms of unrelated crossbred cattle. According to Bowditch *et al.* (1993), mutations that inhibit primer binding or otherwise interfere with amplification which can be detected by the absence of bands or presence of new bands in those individuals.

Kemp and Teale (1994) also used 26 random 10 base oligonucleotide primers in pooled samples. Aravindakshan and Nainar (1998) used three primers in individual analysis and Ganai *et al.* (2000) used four RAPD primers. Ramesha *et al.* (2002) evaluated seven primers in individual samples for detecting genetic variation in cattle breeds while Yeo *et al.* (2002) used 60

Table 2. Name of primers, average number of bands identified and percentage polymorphism of the different primers

Sl No	Primer	GC content of primers (%)	Number of bands		Percentage polymorphism (%)
			Range	Average	
1	OPA1	70	3 - 8	6.29 ± 0.11	70.00
2	OPA2	70	3 - 8	5.81 ± 0.14	100.00
3	OPA4	60	2 - 7	3.78 ± 0.15	100.00
4	OPA7	60	2 – 6	4.19 ± 0.12	87.50
5	OPA8	60	4 - 9	6.70 ± 0.16	91.66
6	OPA9	70	3 - 7	5.23 ± 0.15	90.00
7	OPA10	60	3 - 8	5.72 ± 0.17	85.00
8	OPA12	60	2 - 9	6.58 ± 0.16	77.77
9	OPA14	60	2 - 9	6.00 ± 0.20	91.66
10	OPA15	60	2 - 7	4.08 ± 0.21	92.31
11	OPA16	60	3 - 7	5.03 ± 0.21	90.91
12	OPA17	60	3 - 6	4.15 ± 0.12	100.00
13	OPA18	60	2 - 7	4.44 ± 0.16	100.00
14	OPA19	60	3 - 10	6.66 ± 0.41	92.31
15	OPA20	60	4 - 8	5.89 ± 0.12	100.00
16	PRG 1	60	3 - 8	6.46 ± 0.16	85.00
17	ILO 1127	90	3 – 10	8.15 ± 0.25	100.00
18	ILO 876	70	4 - 10	6.81 ± 0.16	100.00
19	ILO 526	80	4 – 7	5.81 ± 0.12	66.66

primers in their study on native Korean cattle. Ramesha *et al.* (2002) observed two breed specific bands, one for Malanad Gidda and the other in Amritmahal breed with the primer ILO 1127. Gwakisa *et al.* (1994) found that two RAPD primers ILO 1127 and ILO 1065 could differentiate three zebu cattle breeds of Tanzania. They could not identify any breed specific bands in Tanzanean zebu breeds of cattle with ILO 526 primer.

Antoniou and Skidmore (1995) were able to identify a 3100 bp specific fragment in bovines with primer OPA 6. All female samples failed to produce any product.

The number of bands produced in this study with the primer ILO 1127 was three to nine against those reported by Aravindakshan and Nainar in 1998 (3 to 10) and Ramesha *et al.* in 2002 (7 to 9). Ramesha *et al.* (2002) observed moderate to high levels of polymorphism with ILO 526 in South Indian cattle breeds. A specific product observed in Krishna Valley and Ongole breeds was found to be absent in Malanad Gidda, Amritmahal, Hallikar, Deoni and Khillari. Gwakisa *et al.* (1994) were unable to identify breed specific bands with ILO 526 in zebu cattle breeds of Tanzania.

In the present study, primer ILO 876 produced one to four bands in various genetic groups of dwarf cattle of Kerala. The polymorphism was high in all the four groups. Two bands ILO 876 d and ILO 876 e were common to all animals tested. Ramesha *et al.* (2002) was able to obtain 0.95 kb product similar to one reported in African zebu breeds. Similar findings were also reported by Nagaraja (1998). With ILO 1127 Ramesha *et al.* (2002) observed breed specific bands for Malanad Gidda and Amritmahal breed.

References

Andersson, L., Bohme, J., Rask, L. and Peterson, P.A. 1986. Genomic hybridization of bovine class II major histocompatibility genes: Extensive polymorphism of DQ_a and DQ_b genes. *Anim. Genet.*, **17**: 95 -112.

Antoniou, E. and Skidmore, C.J. 1995. A bovine Y specific marker amplified by RAPD. *Anim. Genet.*, **26**: 444 - 447.

Aravindakshan, T.V. and Nainar, A.M. 1998. Genetic variation in cattle and buffalo breeds analysed by RAPD markers. *Indian J. Dairy Sci.*, **51**: 368-374

Bowditch, B.M., Albright, D.G., Williams, J.G.K. and Braun, M.J. 1993. Use of randomly amplified polymorphic DNA markers in comparative genome analysis. *Methods in Enzymology,* **224**: pp 294-309

Gwakisa, P.S., Kemp, S.J. and Teale, A.J. 1994. Characterization of zebu cattle breeds in Tanzania using random amplified polymorphic DNA markers. *Anim. Genet.*, **25**: 89-94.

Kemp, S.J. and Teale, A.J. 1994. Randomly primed PCR amplification of pooled DNA reveals polymorphism in a ruminant repetitive DNA sequence which differentiate *Bos indicus* and *B. taurus*. *Anim. Genet.*, **25**: 83-88.

Nagaraja, C.S. 1998.Studies on breed specific molecular genetic markers in *B. taurus* and *B. indicus* cattle. *Ph.D. thesis.* University of Agricultural Sciences, Bangalore. 124 p

Ramesha, K.P., Saravanan, T., Rao, M.K., Appannavar, M.M. and Reddy, A.O. 2002. Genetic distance among South Indian breeds of zebu cattle using random amplified DNA markers. *Asian-Aust. J. Anim. Sci.*, **15**: 309-314

Yeo, J.S., Kim, J.W., Chang, T.K., Nam, D.H., Han, J.Y. and Choi, C.B. 2002. Detection of DNA fragment to differentiate Korean cattle. *Asian-Aust. J. Anim. Sci.*, **15**: 1071-75

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