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MOLECULAR CHARECTERIZATION OF HSP70 GENE IN KENGURI BREED OF SHEEP USING PCR-SSCP TECHNIQUE

DODAMANI, S.,[1] APPANNAVAR, M. M.,[2] RAMESHA, K. P.,[3] SURANAGI, M. D.,[2] TANDALE, M. K.[4] AND KARTIKESH, S. M.[5]

[1]Department of Animal Genetics and Breeding, Veterinary College, Hassan 577202 (Karnataka); [2]Department of AGB, Veterinary College, Bidar-585401; [3]Animal Production Department, SRS-NDRI, Adugodi, Bengaluru 560030; [4]Department of VGO, Veterinary College, Bidar-585401; [5]Department of Biochemistry, Veterinary College, Bidar 585401.
E. Mail: Shri537@gmail.com, Cell 0 9620784663

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Abstract: ***PCR-SSCP analysis of HSP70 gene was carried out in Kenguri breed of sheep to characterize HSP70 gene. The fragments consisting exon 1, 2, 3, 4 and 5 were of 490, 469, 525, 307 and 352 bp size. The exons 2, 3 and 4 of HSP70 gene showed monomorphism with similar pattern in all the 48 animals studied. Two unique SSCP patterns with a pattern frequency of 0.1875 and 0.8125, respectively were observed in fragment 1 comprising Exon1. Two SSCP patterns with a pattern frequency of 0.3541 and 0.6458 were observed in fragment 5 comprising Exon 5 of HSP70 gene. The analysis of fragment 1comprising Exon 1 revealed T170C (Methionineà Thrionine), A210G (Argininеà Glycine) and G504A (GlycineàArginine) amino acid substitution showing transition while for fragment 5 compromising Exon 5 the observed polymorphisms at G2033C (Glycineà Alanine) amino acid substitution showed transversion. The obtained polymorphism may be a characteristic of kenguri sheep.***

Key word: *HSP70 gene,* Kenguri sheep

INTRODUCTION

Kenguri breed of sheep, which is also known as Tenguri sheep, is found in Raichur, Koppal and parts of Gulbarga districts in Northern Karnataka. They tolerate high atmospheric temperature, fatigue and are resistant to many diseases. Kenguri sheep is also known to thrive well under scarcity condition and sparse vegetation. The initial survey conducted by NBAGR, Karnal has shown that the breed has high potential for meat production [1].

Body of animals synthesize group of proteins commonly known as heat stress or heat shock proteins (HSPs). The thresholds for expression of HSPs are correlated with levels of stress naturally undergone by the animals. HSP70 is one of the most abundant members of the HSP family and is present in all cells and increases when an individual is exposed to various stressors. Heat shock proteins 70 (HSP70) is an essential molecular chaperone of primary importance. It protects cells, tissue and organs from stress [2]. A single-nucleotide polymorphism (SNP, pronounced *snip*) is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual. SNP genotyping is the measurement of genetic variations of single nucleotide polymorphisms (SNPs) between members of a species. SNPs are one of the most common types of genetic variation and are conserved during evolution

and have been used as markers for use in quantitative trait loci (QTL) analysis and in association studies. SNPs can also provide a genetic fingerprint for use in identity analysis [3].

As HSP70 gene is associated with heat stress, therefore, study on genetic variation between HSP70 gene will aid in selective breeding for breed improvement. As less information on genetic variation in HSP70 gene in Kenguri sheep is available, the present study was proposed with the following objective to analyze genetic variation in Heat Shock Protein 70 (HSP70) gene and to evaluate allelic distribution of these haplotypes in Kenguri breed of sheep.

MATERIALS AND METHODS

The present study was conducted on 48 Kenguri sheep maintained at the Veterinary College, Bidar. About 10 ml of blood was collected aseptically from Jugular vein from each of the representative Kenguri sheep in a vacutainer tube containing 0.5 per cent EDTA. After collection, the samples were stored at 4°C and DNA was isolated within 24 hrs. DNA was isolated from the blood samples using modified High Salt Method [4].

The purity and concentration of DNA samples were estimated using 0.8% Agarose gel electrophoresis and Bio-photometer. Quantity of DNA was calculated using the formula: Quantity of DNA in (mg/ml) = OD = OD 260 ´ 50 ´ dilution factor samples showing an optical density ratio (260/280 nm) between 1.7 and 1.9 considered as high purity were used for further analysis (Fig 1).

Based on the bovine HSP 70 gene sequence (NCBI accession number U09861), 5 sets of overlapping primers were designed using primer 3 (V.0.4.0) online software for amplifying the entire coding region of HSP 70 gene. The primers used, product size and location are given in the Table 1. and the primers were procured from Europhins MWG Operon, Bangalore. Composition of reaction mixture for PCR amplifications of HSP70 gene are given in Table 2.

The thermal cycling conditions involved an initial denaturation at 94°C for 2 min, followed by 35 cycles with initial denaturation at 94°C for 30 seconds, primer specific annealing temperatures of 57°C for 1 min (to specifically amplify HSP70 Fragment-1), 60°C for 1 min (to specifically amplify HSP70 Fragment-2, Fragment-3 and Fragment-5 regions), 58.4°C for 1 min (to specifically amplify HSP70 Fragment-4), respectively, extension at 72°C for 1 min followed by a final extension at 72°C for 10 min.

The unique SSCP patterns obtained for the representative PCR products were segregated and further analyzed by direct sequencing (Amnion Bioscience Pvt. Ltd., Bangalore, India). The custom made forward and reverse allele specific PCR primer sets used for PCR-SSCP assay were utilized for direct sequencing. At least two individual animal samples representative of each unique PCR-SSCP patterns were given for direct sequencing to obtain representative sequences.

The complementary sequences representative of unique PCR-SSCP pattern were manually analyzed and sequenced. The retrieved sequences representing each of the unique PCR-SSCP patterns were further analyzed using Clustal-W multiple sequence alignment tool for detecting single nucleotide polymorphisms (SNP's) and their respective deduced amino acid variations. Sequence data were analyzed using, Bioedit software Clustal W multiple alignments for detecting single nucleotide polymorphisms.

RESULTS AND DISCUSSION

The present investigation was carried out to characterize heat shock protein 70 (HSP70) gene using PCR-SSCP technique in Kenguri breed of sheep. Molecular characterization of heat shock protein 70-1 gene of goat by Gade et al. [5] had revealed that the entire nucleotide sequence of goat HSP70-1 gene shows 97.8% homology with cattle, 96.3% with buffalo, 97.5% with yak, 99.4% with sheep, 95.3% with pig, 94.4% with horse, and 94.1% with human which indicates close evolutionary relationship. The yield of DNA ranged from 352.96 to 1408.32 µg/ml of blood with a mean yield of 686.95 ± 36.50 µg / ml. The purity of DNA (determined as O.D ratio at 260nm/280nm) ranged between 1.7 and 1.9 in all the samples, with a mean of 1.80 ± 0.01 indicating high purity of the extracted DNA. The quality of DNA is presented in Fig 1. This is supportive to the report of Montgomery and Sise [6] with mean yield of 640 µg/ml of blood from sheep.

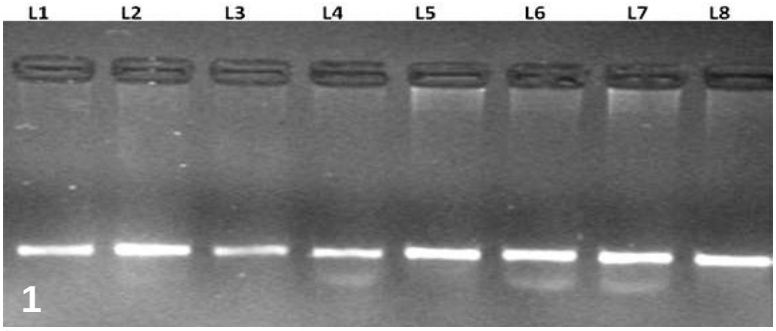

Fig. 1: Quality checking of DNA on 0.8% Agarose gel. Lane 1-8: Working DNA sample

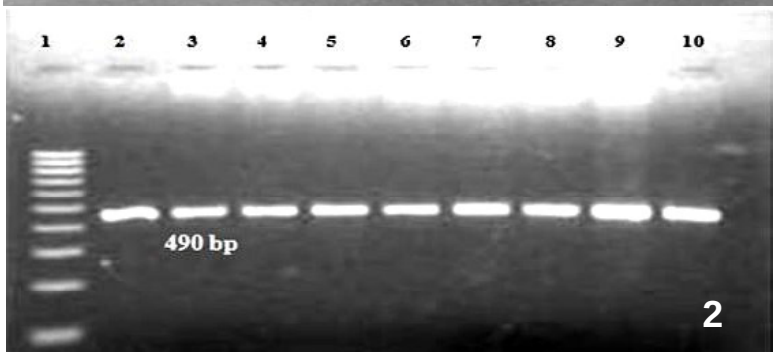

Fig. 2: Resolution of PCR amplified product of fragment-1 consisting exon 1 on 1.5% Agarose gel. Lane 1: 100 bp ladder molecular marker, Lane 2-10: PCR products

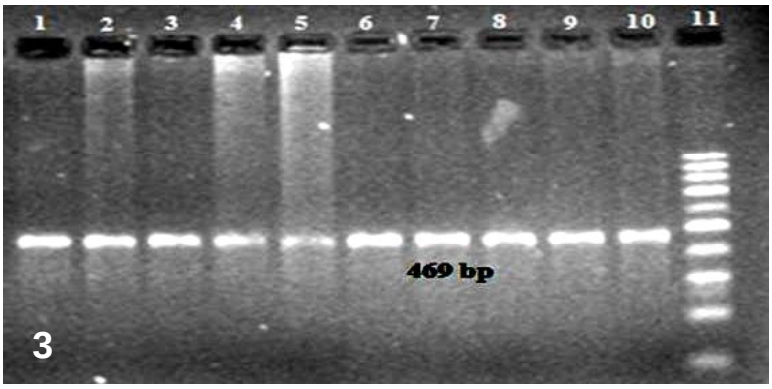

Fig. 3: Resolution of PCR amplified product of fragment-2 consisting exon 2 on 1.5% Agarose gel. Lane 1-10: PCR product (469bp),

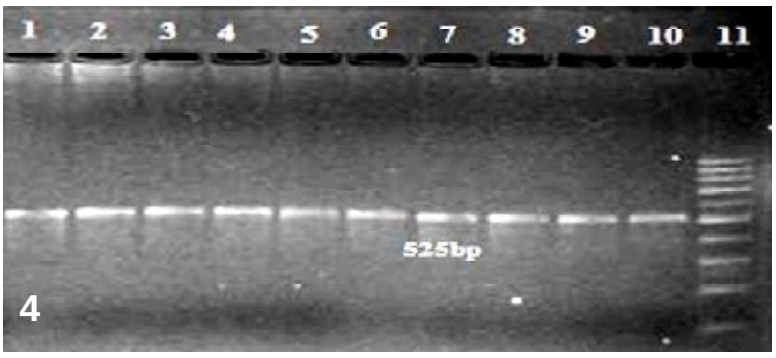

Fig. 4: Resolution of PCR amplified product of fragment-3 consisting exon 3

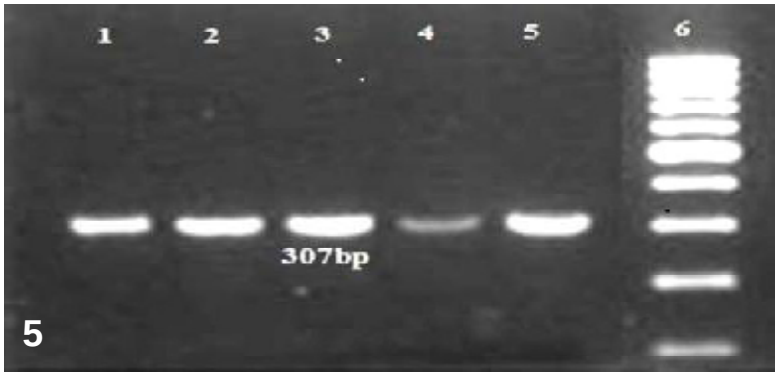

Fig. 5: Resolution of PCR amplified product of fragment-4 consisting exon 4 on 1.5% Agarose gel. Lane 1-5: PCR product (307bp), Lane 6: 100 bp ladder molecular marker

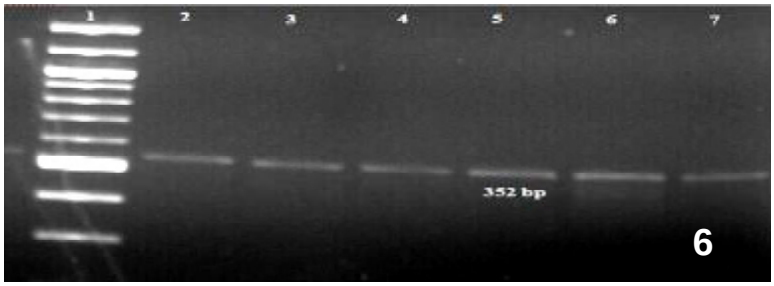

Fig. 6: Resolution of PCR amplified product of fragment-5 consisting exon 5 on 1.5% Agarose gel. Lane 2-7: PCR product (352bp), Lane 1: 100 bp ladder molecular marker

PCR conditions were standardized for each Primer. Five sets of primers were designed for coding region in such a way that each of the 5 exons of HSP70 gene was amplified and product size was below 1 kb (Fig. 2 to Fig. 6).

The PCR-SSCP analysis of HSP70 gene amplicons revealed varying degree of genetic polymorphisms with respect to each of the HSP70 gene fragments analyzed. PCR-SSCP analysis of amplicons of the fragment-2 comprising Exon 2, Fragment 3-comprising Exon 3 and Fragment-4 comprising Exon 4, showed monomorphism in Kenguri breed of sheep (Figs. 8-10). Thus the HSP70 gene Exon-2, Exon-3 and Exon-4 showed absence of polymorphisms indicating the probable absence/lack of mutation/s suggesting high degree of conservation of HSP70 gene in Kenguri breed of sheep. PCR-SSCP analysis of Fragment-1 comprising Exon 1, in HSP70 gene revealed two unique SSCP patterns with different mobility shifts. PCR-SSCP Pattern I of exon1 showed three distinct bands while pattern II showed two bands (Fig. 7).

Similarly the fragment-5 comprising Exon 5 of HSP70 gene in Kenguri breed of sheep also revealed two PCR-SSCP patterns with different mobility shifts. PCR-SSCP pattern I showed two distinct DNA bands and pattern II showed four distinct DNA bands (Fig. 11). The frequency of different patterns for Exon 1 & Exon 5 showed that the frequency of pattern I was less (0.1875) compared to pattern II (0.8125). Similarly the frequencies for pattern I was less (0.3541) than pattern II (0.6459) for Exon 5 (Table 3).

The frequencies observed in the present investigation suggest that the Kenguri breed of sheep have a diverse type of SSCP patterns for Exon 1 and 5 of HSP70 gene in the sampled population indicating the existence of variability.

The study on band patterns of HSP 70 gene in Deoni cattle revealed 14 bands while in Deoni X HF crossbred cattle it was eight [7]. Among 14 band patterns of Deoni cattle, eight bands were similar to those in Deoni X HF crossbred cattle while 6 bands were unique to Deoni cattle. Fragment 1 showed three band patterns (A, B and C) in Deoni cows and two bands (A and B) in Deoni X HF crossbred cattle. The frequency of "A" band was highest in both Deoni and Deoni X HF crossbred cows. Fragment 2 showed five bands in Deoni cattle and two bands in Deoni X HF crossbred cattle. The frequency of "A band" was higher in HF crossbred cows as compared to Deoni cows. Fragment 3 showed three bands in Deoni cattle and two bands in Deoni X HF crossbred cattle. The frequency of "A band" was 0.90 in Deoni X HF crossbred cattle while it was 0.50 in Deoni cattle. Similarly, fragment 5 also showed three bands in Deoni cattle and two bands in Deoni X HF crossbred cattle. But in this the frequency of "AB band" was 0.60 in Deoni X HF crossbred cattle whereas in Deoni cattle the pattern appeared with a low frequency of 0.05. The study in Deoni and Deoni X HF crossbred cattle showed the existence of variability in the sampled population of cattle similar to the variability observed in Kenguri sheep. This PCR-SSCP study for HSP70 gene may be the characteristic of Kenguri breed of sheep.

REFERENCES

[1] Jain, A., Kulkarni, V.S., Govindaiah, M.G., Aswathnarayana, T. and Sadana, D.K.: *Evaluation of Kenguri sheep breed of Karnataka*. National Symposium on livestock biodiversity vis-à-vis resource exploitation: an introspection at NBAGR, Karnal, 125: pp 11-12 (2004).

[2] Kiang, J.G. and Tsokos, G.C.: Pharmacol. Therap., 80(2): 183-201 (1998).

[3] Rapley, R. and Harbron, S.: *Molecular Analysis and Genome Discovery*. Chichester. John Wiley & Sons Ltd, New Jersey (2004).

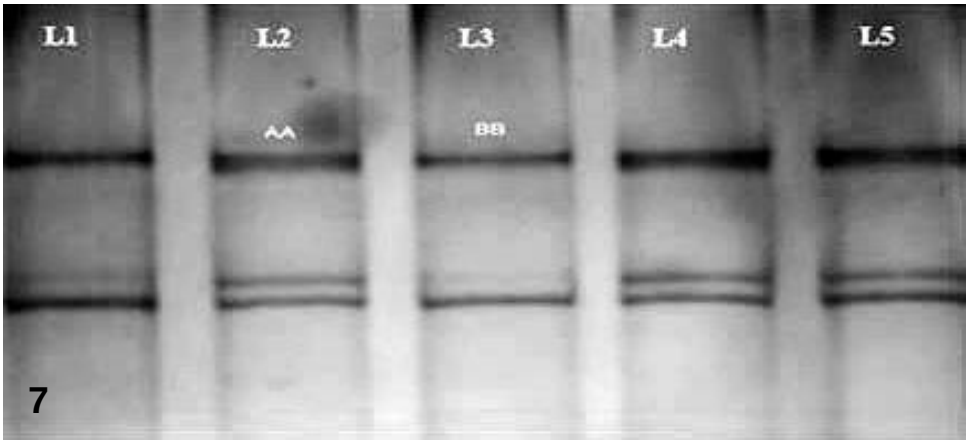

Fig. 7: PCR-SSCP patterns of HSP70 gene fragment-1 in Kenguri sheep. Silver stained PAGE representing PCR-SSCP band pattern for fragment-1. Lane 1, 3 shows allelic pattern BB. Lane 2,4,5 shows allelic pattern AA.

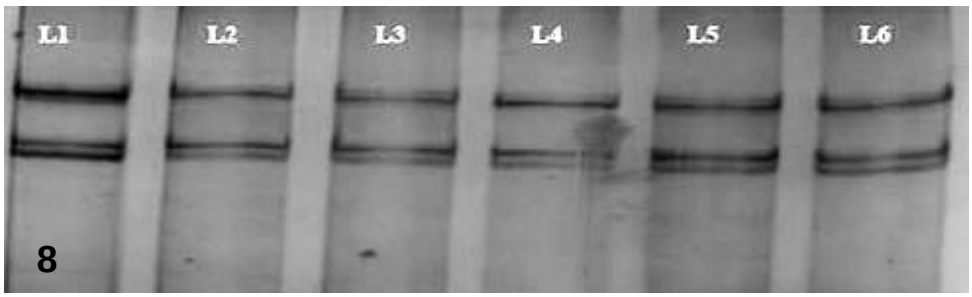

Fig. 8: PCR-SSCP patterns of HSP70 gene fragment-2 in Kenguri sheep. . Silver stained PAGE representing PCR-SSCP band pattern for fragment-2, All are monomorphic.

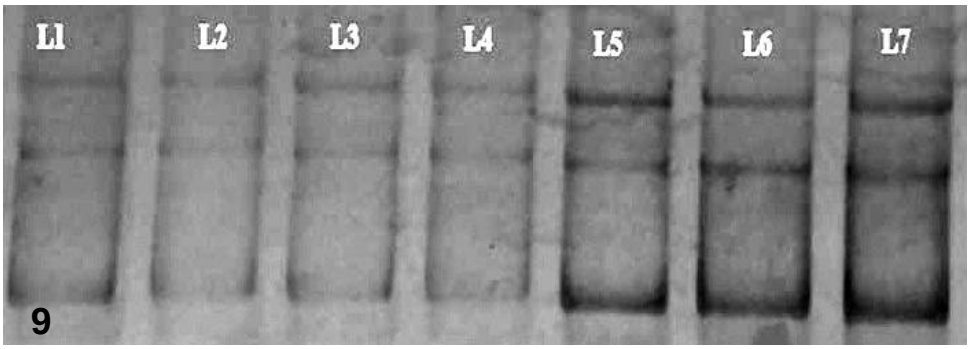

Fig. 9: PCR-SSCP patterns of HSP70 gene fragment-3 in Kenguri sheep. Silver stained PAGE representing PCR-SSCP band pattern for fragment-3, All are monomorphic.

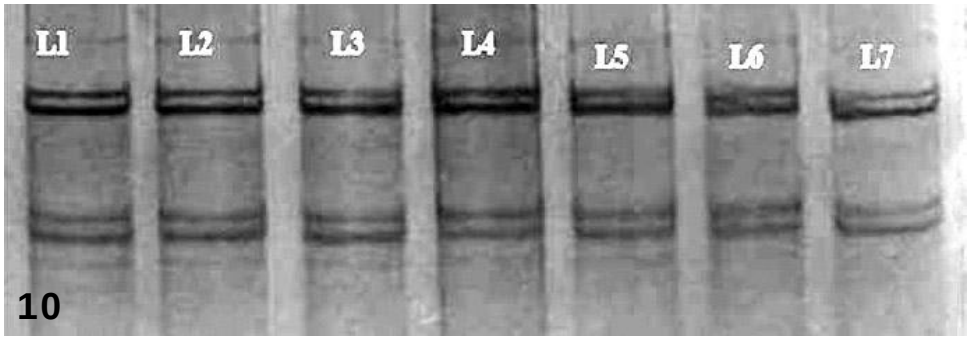

Fig. 10: PCR-SSCP patterns of HSP70 gene fragment-4 in Kenguri sheep Silver stained PAGE representing PCR-SSCP band pattern for fragment-4. All are monomorphic.

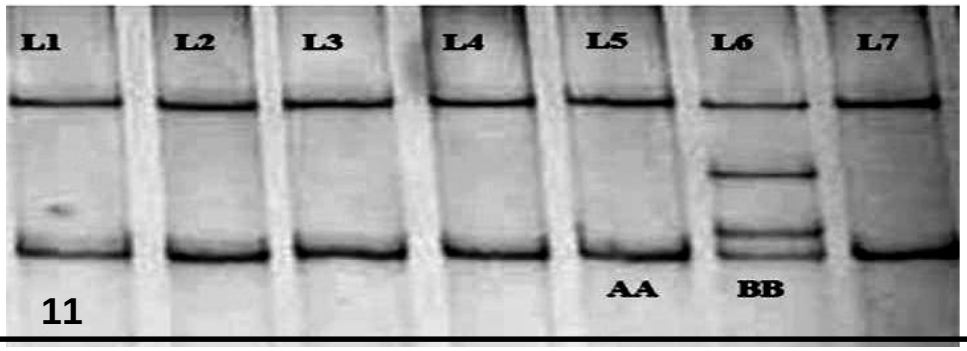

Fig. 11: PCR-SSCP patterns of HSP70 gene fragment-5 in Kenguri sheep: Silver stained PAGE representing PCR-SSCP band pattern for fragment-5. Lane-6 shows allelic pattern BB, Lane-1,2,4,5,7 show allelic pattern AA.

Table 1: Primers, Location and Product size of different fragments of HSP70 gene**.**

No.	Primers (5' ? 3')	Location	Product Size (bp)
1	Forward – TATCTCGGAGCCGAAAAGG Reverse – TCATCTTGGTCAGCACCATC	110-600	490
2	Forward – TGGTGCTGACCAAGATGAAG Reverse – GTGCTGGACGACAAGGTTCT	501-1100	469
3	Forward – CTCGTACACCTGGATCAGCA Reverse – GCCAAGAGAACCTTGTCGTC	951-1600	525
4	Forward – TTCTTGGCAGACACCCTCTC Reverse – TGCTGATCCAGGTGTACGAG	1451-1800	307
5	Forward – ACATGAGCAATCCAGGGAAG Reverse – GAGAGGGTGTCTGCCAAGAA	1731-2130	352

Table 2: Composition of reaction mixture for PCR amplifications of HSP70 gene.

Sl. No	Components	EXON1	EXON2	EXON3	EXON4	EXON5
1	Sigma water	16	16.2	16	16	16
2	10x Buffer (1X)	2.5	2.5	2.5	2.5	2.5
3	2.5 Mm dNTPs (100 µM each)	2.5	2.5	2	3	3
4	Forward Primer (20 pmol/µl)	0.5	0.4	0.5	0.5	0.5
5	Reverse Primer (20 pmol/µl)	0.5	0.4	0.5	0.5	0.5
6	DNA Template (50 ng/ µl)	2	2	2	2	2
7	Taq DNA Polymerase	1	1	1	1	1
8	Total Volume	25	25	25	25	25

Table 3: Frequencies of different patterns of Exon 1 and Exon 5 of HSP 70 gene. Note: The values in the parenthesis indicate the number of animals

Exons	Frequency	
	AA	BB
1	0.1875 (9)	0.8125 (39)
5	0.3541 (17)	0.6458 (31)

[4] MilleR, S.A., Dykes, D. D. and Polesky, H .F.: Nucleic Acids Res. 16: 1215 (1988).

[5] Gade, N., Mahapatra, R.K., Sonwane, A., Singh, V.K., Doreswamy, R.. and Saini, M.: Mol. Biol. Intern., 10(8): 420-429 (2010).

[6] Montgomery, G.W. and Sise, J.A.: J. Agri. Res., 33: 437-441(1990).

[7] Ramesha, K.P., Geetha, G.R., Rao, A. Hatkar, D.N., Krishnamurthy, L., Rao, T.V.L.N., Kataktalware, M.A. and Das, D.N.: *Sequence characterization and genetic variability analysis of HSP 70 gene in Bos Indicus (Deoni breed) cattle*. Indian Science Congress, Jan 3-7, Kolkatta (2012).