Original Research

Polymorphism of BoLA-DRB3.2 gene in Indian Holstein Crossbred Cattle by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism

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Abstract

Bovine lymphocyte antigen DRB3.2 (BoLA–DRB3.2) gene encodes for the beta chain of the major histocompatibility complex (MHC) class II molecule in cattle, which is a glycoprotein present on the surface of antigen-presenting cells. The objective of the present study was to genotype the BoLA-DRB3.2 locus in Holstein crossbred cattle (n = 40) by PCR-RFLP. The 304bp amplified region was separately digested with restriction endonucleases Hae III and Bst YI. Seven BoLA-DRB 3.2 genotypes were detected by digesting with Hae III. The frequencies of the genotypes were: 0.475, 0.225, 0.050, 0.100, 0.050, 0.050 and 0.050 for the genotype AA, AB, AE, BB, DD, EE and II, respectively. Digestion with Bst YI yielded three genotypes AA, AB and BB with frequency of 0.050, 0.625 and 0.325. On the basis of the above results it can be concluded that the DRB3.2 gene was found to be highly polymorphic in the crossbred cattle population.

Key words: Bovine Leukocyte Antigen, PCR-RFLP, Holstein-Crossbred Cattle

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Introduction

The major histocompatibility complex (MHC) is an organized cluster of firmly linked genes with immunological and non-immunological functions and is present in all vertebrates, except the jawless fish (Tizard, 2004). Major histocompatibility complex (MHC) genes, also called bovine leukocyte antigen (BoLA), have recently received attention because of their high degree of genetic polymorphism and association with immunity and milk production. The BoLA genes are located on the short arm of bovine chromosome number 23 Fries *et al.* (1986). In cattle the MHC can be divided into four regions: class I, class IIa, class IIb and class III. The class I region is the most telomeric part of the MHC, and the class III (or central) region is located between the class I and the class IIa region. The DRA, DRB, DQA, and DQB genes are located in the IIa region, while the DOB, DYA, DYB and DIB genes in the IIb region. There are

at least three DRB-like genes (DRB1, DRB2 and DBR3) in the BoLA region, among which only the DRB3 gene is expressed considerably and is highly polymorphic. Previously, several methods have been developed for BoLA typing. These include serology Emery *et al.* (1987), isoelectric focusing (IEF), direct sequencing Groenen *et al.* (1990) and Glass *et al.* (1992), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) Van Eijk *et al.* (1992), heteroduplex analysis Sitte *et al*. (1995), denaturing gradient gel electrophoresis Aldridge *et al*. (1998) and PCR sequence based typing Takeshima *et al*. (2001). Among these methods, PCR-RFLP developed by Van Eijk *et al.* (1992) has many advantages over the other methods for the genetic analysis of populations, as it requires small amount of genomic DNA and being adaptable to crude DNA preparations.

Materials and Methods

DNA Extraction

Blood samples were collected from 40 Holstein Friesian crossbred cows from Livestock Farm, College of Veterinary Science and A.H., Mhow, Shri Ahilyamata Jeev Dayamandal Trust Gaushala, Indore and Bull Mother Farm Bhadbhada, Bhopal. The extraction of genomic DNA from collected blood samples were done by John *et al*. (1991) with minor modifications. The working DNA concentration was adjusted to 50 to100 ng/µl.

Amplification of BoLA-DRB3 Exon 2

The DNA amplification was of BoLA-DRB3.2 gene was achieved by Polymerase Chain Reaction. The primers used for the amplification of BoLA DRB3.2 gene were selected on the basis of the previous reports Sigurdardottir *et al*. (1991).The primers were synthesized by Eurofins Genomics India Pvt. Ltd., Bangalore.

Forward: 5' GATGGATCCTCTCTCTGCAGCACATTTCCT- 3'

Reverse: 5' CTTGAATTCGCGCTCACCTCGCCGCTG – 3'

Reactions were carried out in a final volume of 30µl. Each 30 µl PCR reaction contained 50 to 100 ng of genomic DNA, 30µl of 10X PCR reaction buffer (10 mM Tris-pH-9.0, 50 mM KCl, 1.5 mM MgCl2 and 0.1% gelatin), 100 mM of each dNTP, 10 pmol of each primer and 1 unit of *Taq* DNA polymerase. A negative control, to which no genomic DNA was added, was also prepared to rule out any nonspecific amplification. The PCR was carried out in thermal cycler (Applied Biosystems). The thermal cycling profile was as follows: Initial denaturation for 2 min. at 94°C; followed by 30 cycles of 45 s at 92°C, 45 s at 66°C (annealing temperature) and 45 s at 72°C. The final extension step was for 10 min at 72°C.

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Restriction Endonuclease Digestion

The PCR-amplified products were digested separately with the restriction endonucleases *BstYI* and *HaeIII* (Thermoscientific, Lithuania). For restriction endonuclease digestion, 10 µl of the PCR products were digested for 3 h at 37°C with 5 units of *Hae III* and at 50°C with 5 units of *Bst YI* in a total volume of 15 µl. Digestions were performed in 200 µl PCR tubes, using a water bath. After heat denaturation of the enzymes, the restriction fragments were resolved in 3% agarose gel electrophoresis at 80 V for 1 to 2 h. Ethidium bromide (1%) was added at the rate of 5 µl/100 ml of gel solution. A 100-bp DNA ladder (Genedirex) was used as a DNA size marker. On completion of electrophoresis, the gel was visualized under UV transilluminator (UVITECH, Cambridge and U.K). The BoLA-DRB3.2 nomenclature, described by Van Eijk *et al.* (1992) was followed to identify the different allele types obtained in the present study from the different restriction enzyme patterns.

Results and Discussion

Genomic DNA of Holstein Crossbred cows was used for PCR and a PCR product of 304 bp was amplified after 2 % agarose gel electrophoresis detection (Fig. 1).

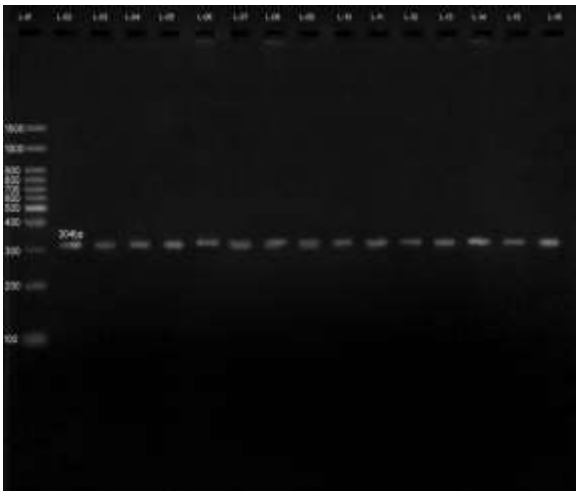

Fig.1: PCR product of 304 bp electrophoresed 2% agarose gel electrophoresis

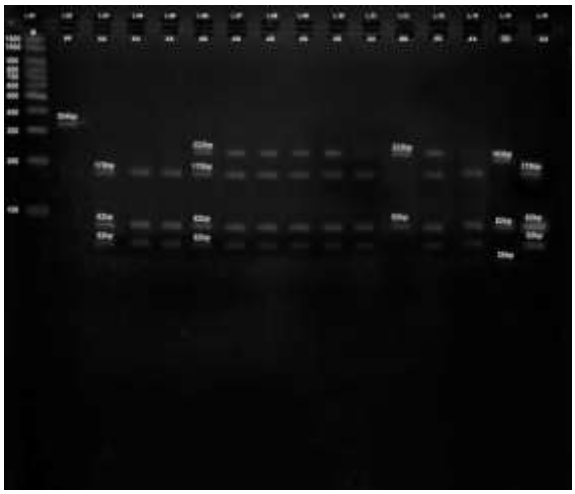

Fig. 2: PCR-RFLP digested with *Hae III* on electrophoresed on 3% agarose gel

Genotype Frequency and Allele Frequency of BoLA-DRB3.2 gene

Genotype frequency and allele frequency of BoLA-DRB3.2 gene in Holstein crossbred cows were shown in Table 1. Chi-square values obtained for testing correspondence between observed and expected genotypic frequencies for *Hae III* ($P < 0.01$) and *Bst YI* ($P < 0.05$) at this locus were found to be significant indicating that the population of cattle under study was not in Hardy Weinberg equilibrium at this locus .

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Table 1: Frequency of genotypes and alleles at BoLA-DRB3.2 locus in HF crossbreds

Hae III		*Bst YI*	
Genotype/Allele	**Frequency**	**Genotype/Allele**	**Frequency**
AA	0.475(19)	AA	0.050(02)
AB	0.225(09)	AB	0.625(25)
AE	0.050(02)	BB	0.325(13)
BB	0.100(04)	A	0.3625
DD	0.050(02)	B	0.6375
EE	0.050(02)	-	
II	0.050(02)	-	
A	0.6125	-	
B	0.2125	-	
D	0.05	-	
E	0.075	-	
I	0.05	-	
Chi-square	99.56**	Chi-square	4.96*

** Significant (P <0.05);** significant (P <0.01)*

In the present study, restriction endonucleases *Hae III* and *Bst YI* were used separately for digesting the PCR product in order to reveal the restriction fragment length polymorphism patterns. Analysis of band pattern of *Hae III* digested products revealed the existence of seven restriction patterns (genotypes) i.e., AA (170bp , 82bp, 52bp), AB (222 bp,170 bp,82 bp,52 bp), AE (170 bp,134 bp,82 bp,52 bp), BB (222 bp, 82 bp), DD (193 bp, 82 bp, 29 bp), EE (170 bp,134 bp) and II (304 bp) (Fig. 3) and five alleles viz., A, B, D, E, I indicating that BoLA DRB3.2 gene locus under study was polymorphic for restriction endonuclease *Hae III*.

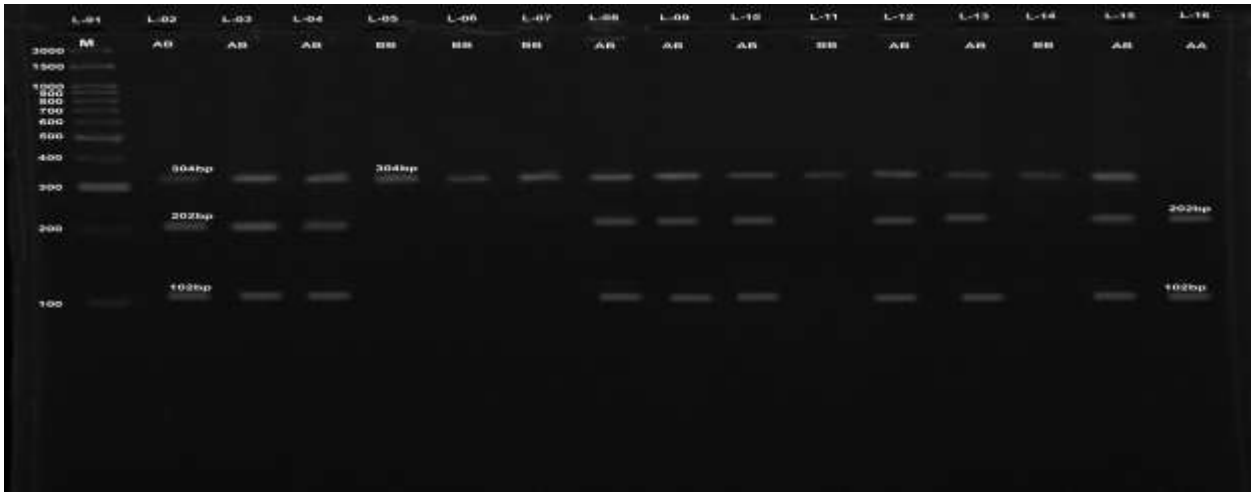

Fig. 3: PCR-RFLP digested with *Bst YI* electrophpresed on 3% agarose gel

These findings are in congruence with the findings of Gelhaus *et al*. (1995) in Holstein Friesian, N'Dama and Boran, Aravindakshan and Nainar (1999) in Jersey crossbred and Ongole and Behl *et al*. (2007) in

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Kankrej and Darshan Raj *et al*. (2012) in Amrithmahal, Amrithmahal crosses, Jersey crossbred and Red-Dane x Red-Sindhi crossbreds using same primer and restriction enzyme (*Hae III*). Since, the size of amplification product was same (304 bp) for all the animals of different breeds included in present study and earlier studies quoted above suggesting that this region is conserved in cattle. Analysis of band pattern of *Bst YI* digested products revealed three restriction patterns (genotypes) i.e., AA (202bp, 102bp), AB (304 bp, 202 bp, 102 bp) and BB (304 bp) and two alleles (A and B) indicating that BoLA DRB3.2 gene region under study was polymorphic for restriction endonuclease *Bst YI*. Earlier also polymorphism at this BoLA-DRB3.2 locus using same RE (*Bst YI*) and primer as used in present study has been reported by many workers. Gelhaus *et al.* (1995) reported five patterns in Holstein Friesian, N'Dama and Boran, Aravindakshan and Nainar (1999) reported two patterns in Jersey crossbreds and Ongole and Behl *et al.* (2007) reported four patterns in Kankrej cattle.

Frequencies of Genotypes and Alleles

The frequency of seven genotypes obtained by using *Hae III* was found to be: 0.475, 0.225, 0.050, 0.100, 0.050, 0.050 and 0.050 for the genotype AA, AB, AE, BB, DD, EE and II, respectively. The frequency of 5 alleles was found to be 0.6125, 0.2125, 0.050, 0.075 and 0.050, respectively for A, B, D, E and I. Genotype AA and allele A had the highest frequency. Aravindakshan and Nainar (1999) have reported seven patterns with highest frequency (0.63) of pattern 'a' (pattern same as 'AA' in present study) in Ongole and Jersey crossbred cattle. Darshan Raj *et al.* (2012) have reported five patterns in Amritmahal cattle and four in Jersey crossbred with highest frequency of pattern 'a' (same as 'AA' in present study); 0.52 and 0.66, respectively. Miretti *et al.* (2001) reported four patterns with gene frequency ranging from 0.036 to 0.631 in Argentinean Holstein; Paswan *et al.* (2005) reported five patterns with genotype frequency ranging from 0.01 to 0.53 in crossbred cattle; Wu *et al.* (2010) reported seven patterns with gene frequency ranging from 0.017 to 0.598 in Chinese Holstein; Chu *et al.* (2012) reported seven genotypes with their frequency ranging from 0.029 to 0.464 in Beijing Holstein and Chakraborty *et al.* (2015) have reported three patterns with frequency ranging from 0.014 to 0.714 in Sahiwal.

The frequency of three genotypes obtained by using *Bst YI* was found to be 0.050, 0.625 and 0.325 for genotypes AA, AB and BB, respectively. The frequencies of 2 alleles- A and B was 0.3625 and 0.6375, respectively. Comparatively, the frequency of genotype AB and allele B was higher. Contrary to present finding, Aravindakshan and Nainar (1999) could observe only two patterns ('a' and 'b') with higher frequency (0.73) of pattern 'a' (pattern same as 'AA' in present study) in 15 Ongole cattle. These differences in frequency of pattern / genotype may be attributed to breed difference and sampling fluctuations. Extensive genetic diversity at BoLA-DRB3.2 locus has been reported with different number of alleles and their varied frequencies in different breeds of cattle across the globe. Nascimento *et al.* (2006)

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reported 37 alleles with frequency ranging from 0.0005 to 0.19 in Brazilian Gyr cows; Behl *et al.* (2007) (frequencies 0.001 to 0.22) in Kankrej; Duangjinda *et al.* (2009) reported 40 alleles frequencies 0.005 to 0.139) in HF crossbreds; Pashmi *et al*. (2009) reported 28 alleles (frequency 0.19 to 17.94) in Iranian Holstein; Wu *et al.* (2010) reported 22 alleles (frequency 0.003 to 0.22) in Chinese Holstein and Suprovych *et al.* (2017) reported 28 alleles with frequency varying from 0.006 to 0.117 in Ukrainian cattle breeds.

These differences in genotypic / allelic frequencies might be due to the fact that the different breeds / populations maintained under the different sets of environmental conditions are subjected to different evolutionary forces to varying degree. In addition, sampling fluctuations may also contribute to these differences in genotypic and allelic frequencies observed in different breeds and populations.

Test for Genetic Equilibrium

The significant chi-square values observed in the present study for genotypic distributions obtained by both the restriction enzymes *Hae III* (99.56) and *Bst YI* (4.96) (Table 1) indicated that the observed genotypic frequencies in the population deviated significantly from expected frequencies and hence the population (HF crossbreds) under study was not in Hardy-Weinberg equilibrium at this locus. Genetic disequilibrium at BoLA- DRB3.2 locus has also been reported by Miretti *et al*. (2001) in South American cattle and Baltian *et al*. (2012) in Argentinean Holstein cows. However, Das *et al.* (2012) reported Malnad Gidda, Hallikar and Ongole breeds to be in Hardy-Weinberg equilibrium at this locus. The incongruence in observed and expected genotypic frequencies encountered in the present study indicates selective advantage of some genotype over other(s). Sampling fluctuations arising out of small population size under study might also contribute to this disequilibrium at this locus.

Conclusion

It may be concluded that PCR-RFLP may be used to study the polymorphism of the BoLA DRB3.2 gene because of its sensitivity for the determination of polymorphism in a functionally relevant domain. Crossbred cattle showed a moderate level of polymorphism with respect to the patterns reported by various previous workers. The results of the present investigation suggested that the DRB 3.2 gene is highly polymorphic in nature in our crossbred cattle population.

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References

1. Aldridge, B.M., McGuirk, S.M., Clark, Knapp, R.J., Watkins, L.A., and Lunn, D. P. (1998). Denaturing gradient gel electrophoresis: A rapid method for differentiating BoLA-DRB3 alleles. *Animal Genetics*, 9(5): 1361.

DOI 10.5455/ijlr.20180826072302

2. Aravindakshan, T.V. and Nainar, A.M. (1999). Genetic polymorphism of the BoLA DRB3 gene in Jersey crossbred and Ongole cattle revealed by PCR-RFLP. *Indian journal of Animal Science*, 69(6): 424-427.
3. Baltian, L. R., Ripoli, M. V., Sanfilippo, S., Takeshima, S. N., Aida, Y., and Giovambattista, G. (2012). Association between BoLA-DRB3 and somatic cell count in Holstein cattle from Argentina. *Molecular biology reports*, 39(7): 7215-7220.
4. Behl, J. D., Verma, N. K., Behl, R., Mukesh, M., and Ahlawat, S. P. S. (2007). Characterization of genetic polymorphism of the bovine lymphocyte antigen DRB3. 2 locus in Kankrej cattle (*Bos indicus*). *Journal of dairy science*, 90(6): 2997-3001.
5. Chakraborty, D., Singh, A., Tantia, M. S., Verma, A., and Chakravarty, A. K. (2015). Genetic Polymorphism of BoLA DRB3. 2 Locus in Sahiwa Cattle. *Animal Science Reporter*, 9(1): 33-40.
6. Chu, M. X., Ye, S. C., Qiao, L., Wang, J. X., Feng, T., Huang, D. W. and Chen, G. H. (2012).
7. Polymorphism of exon 2 of BoLA-DRB3 gene and its relationship with somatic cell score in Beijing Holstein cows. *Molecular biology reports*, 39(3): 2909-2914.
8. Darshan Raj, G., Nagaraja, C. S., Gowda, S. B., Govindaiah, M. G., and Jayashankar, M. R. (2012).
9. Comparative molecular genetic studies on Amrithmahal and crossbred cattle. *Thai Journal of Agricultural Science*, 45(1): 11-15.
10. Das, D. N., Sri Hari, V. G., Hatkar, D. N., Rengarajan, K., Saravanan, R., Suryanarayana, V. V. S. and Murthy, L. K. (2012). Genetic diversity and population genetic analysis of bovine MHC class II DRB3.2 locus in three *Bos indicus* cattle breeds of Southern India. *International journal of Immunogenetics*, 39(6): 508-519.
11. Duangjinda, M., Buayai, D., Pattarajinda, V., Phasuk, Y., Katawatin, S., Vongpralub, T. and Chaiyotvittayakul, A. (2009). Detection of bovine leukocyte antigen DRB3 alleles as candidate markers for clinical mastitis resistance in Holstein and Zebu. *Journal of Animal Science*, 87(2): 469–476.
12. Emery, D. L., Puri, N. K., Dufty, J. H., Gorrell, M.D. and Brandon, M. R. (1987). A functional and biochemical analysis of bovine class II MHC antigens using monoclonal antibodies. *Veterinary Immunology and Immunopathology*, 16: 215-234.
13. Fries, R., Hediger, R. and Stranzinger, G. (1986). Tentative chromosomal localization of the bovine MHC by *in situ* hybridization. *Animal Genetics*, 17: 287-294.
14. Gelhaus, A., Schnittger, L., Mehlitz, D., Horstmann, R. D. and Meyer. C. G. (1995). Sequence and PCR-RFLP analysis of 14 novel BoLA-DRB3 alleles. *Animal Genetics*, 26: 147–153.
15. Glass, E. J., Oliver, R. A., Williams, J. L. and Millar, P. (1992). Alloreactive T-cell recognition of bovine major histocompatibility complex class II products defined by one dimensional isoelectric focusing. *Animal Genetics*, 23:97-111.
16. Groenen, M., Van Der Poel, J., Dijkhof, R. and Giphart, M. (1990). The nucleotide sequence of bovine MHC class II DQB and DRB genes. *Immunogenetics*, 31:37-44.
17. John, S. W., Weitzner, G., Rozen, R. and Scriver, C. R. (1991). A rapid procedure for extracting genomic DNA from leukocytes. *Nucleic Acid Research*, 19(2): 408.
18. Miretti, M.M., Ferro, J.A., Lara, M.A. and Contel, E.P.B. (2001). Restriction Fragment Length Polymorphism (RFLP) in exon 2 of the BoLA-DRB3 gene in South American Cattle. *Biochemistry Genetics*, 39(9/10): 311-324.
19. Nascimento, C. S. D., Machado, M. A., Martinez, M. L., Silva, M. V. G., Guimarães, M. F. M., Campos, A. L., and Oliveira, D. A. A. (2006). Association of the bovine major histocompatibility complex (BoLA) BoLA-DRB3 gene with fat and protein production and somatic cell score in Brazilian Gyr dairy cattle (*Bos indicus*). *Genetics and Molecular Biology*, 29(4): 641-647.
20. Pashmi, M., Qanbari, S., Ghorashi, S. A., Sharifi, A. R. and Simianer, H. (2009). Analysis of relationship between bovine lymphocyte antigen DRB3. 2 alleles, somatic cell count and milk traits in Iranian Holstein population. *Journal of Animal Breeding and Genetics*, 126(4): 296-303.

 DOI 10.5455/ijlr.20180826072302

21. Paswan, C., Bhushan, B., Patra, B. N., Kumar, P., Sharma, A., Mukherjee, R. and Dutt, T. (2005). Relationship between the bovine major histocompatibility complex (BoLA) with somatic cell count in crossbred cattle. *Journal of Applied Animal Research*, 28(2): 129-132.
22. Sigurdardottir, S., Borsch, C., Gustafsson, K. and Andersson, L. (1991). Cloning and sequence analysis of 14 DRB alleles of the bovine major histocompatibility complex by using the polymerase chain reaction. *Animal Genetics*, 22: 199-209.
23. Sitte, K., East, I. J., Lavin, M. F. and Jazwinska, E. C. (1995). Identification and characterization of new BoLA-DRB3 alleles by heteroduplex analysis and direct sequencing. *Animal Genetics*, 26:413–417.
24. Suprovych, T., Suprovych, M., Berezhansky, A., Kolinchuk, R., Chornyi, I., Ostafin, M. M. and Bulski, K. (2017). Polymorphism of the gene bola-drb3 Ukrainian breeds of cattle. *Scientific Achievements in Agricultural Engineering, Agronomy and Veterinary Medicine* 2(1): 44-64.
25. Takeshima, S., Ikagami, M., Morita, M., Nakai, Y. and Aida, Y.(2001). Identification of new cattle BoLA-DRB3 alleles by sequence-based typing. *Immunogenetics*, 53: 74-81.
26. Tizard, I. R. (2004). In Veterinary Immunology an introduction, 8th Edn., Elsevier, Philadelphia, pp. 67-77.
27. Van Eijk, M. J., Stew vart-Haynes, J. A. and Lewin, H. A. (1992). Extensive polymorphism of the BoLA-DRB3 gene distinguished by PCR-RFLP. *Animal Genetics*, 23: 483-496.
28. Wu, X. X., Yang, P Z., Wang, L. X., Mao, Y. J., Li, C. S., Shi, K. X. and Chen, Y. (2010). Restriction fragment length polymorphism in the exon 2 of the BoLA-DRB3 gene in Chinese Holstein of the south China. *Journal of Biomedical Science and Engineering*, 3: 221-225.

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