RESEARCH ARTICLE

Isolation and characterization of the major histocompatibility complex *DQA1* and *DQA2* genes from gayal (*Bos frontalis*)

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Key words: Major histocompatibility complex (MHC), Gayal (*Bos frontalis*), *DQA1* and *DQA2* genes, Immunity, Polymorphisms.

Abstract

The species origin of Yunnan Gayal has been controversial since many years. However, few recent genetic studies have suggested that it has perhaps originated from the hybridization

between male *Bos frontalis* and female *Bos Taurus* or *Bos indicus*. Being an important semi wild bovid species, this has also been listed under the red list of International Union of Conservation of Nature and Natural Resources. However, there is limited information available about the immunogenicity of this precarious species of *Bos*. Major histocompatibility complex (MHC) plays a pivotal role in immune response to infectious diseases in vertebrates. In the present study, we have investigated the structural and functional characteristics and possible duplication of the MHC-DQA genes in gayal (*Bos frontalis)*. Two full length cDNA clones of the MHC-DQA genes were amplified and designated as Bofr-DQA1 (DQA*0101) and Bofr-DQA2 (DQA*2001) with GenBank accession numbers KT318732 and KT318733, respectively. A comparison between Bofr-DQA1, Bofr-DQA2 and to other MHC-DQA molecules from different animal species showed that nucleotide and encoded amino acid sequences of these two identified MHC-DQA genes have more similarity to alleles of specific DQA1 and DQA2 molecules from other *Ruminantia* species than to each other. The phylogenic investigation also demonstrated a large genetic distance between these two genes than to homologs from the other species. The large genetic distance between Bofr-DQA1 and Bofr-DQA2 and the presence of different bovine DQA putative motifs clarify that these sequences are non-allelic type. These results could suggest that duplication of the *DQA* genes has also occurred in gayal. The findings of the present study have strengthened our understanding to MHC diversity in rare ruminants and mutation of immunological functions, selective and evolutionary forces that affect MHC variation within and between species.

Key words: Major histocompatibility complex (MHC), Gayal (*Bos frontalis*), *DQA1* and *DQA2* genes, Immunity, Polymorphisms.

Introduction

The potential of an organism for evolutionary interactions with pathogens or other species as well as the fitness are related to immunological functions (Lazzaro et al., 2009). The extent of genetic diversity is known to be associated with the capacity for adaptation and evolution to environmental changes (Reed and Frankham, 2003). Diversity of genes was pivotal phenomenon for immune functions which could be associated with the resistance or susceptibility to pathogens (Trowsdale and Parham, 2004); Tibayrenc, 2004). A cluster of associated genes named the major histocompatibility complex (MHC) plays a key role in presenting antigenic peptides to T lymphocytes (Klein, 1986). In the vertebrate genome, MHC has been known to be the most variable genes which seem to be maintained by balancing selection, predating speciation events and reflecting the co-evolution of hosts with their pathogens (Bernatchez and Landry, 2003).

The class II genes of MHC encoded for α and β chains of DR and DQ dimer molecule which present antigenic peptides to the Helper T cells (McKinney et al. 2013). In rat, mouse, rabbit and pig, there was a single gene of the DQ genes whereas in dogs and human multiple DQ genes copies have been identified but only one of them appears to be expressed (Kappes and Strominger, 1988; Trowsdale, 2001). The number of DQ loci in ruminants varies in different species e.g. the haplotypes in cattle and buffalo contain two copies of DQ genes (Andersson and Rask, 1988; Sigurdardottir et al. 1992; Sena et al. 2011) and both genes are expressed (Russell et al. 1997). Hence, the polymorphisms as well as the duplication of DQ gene increase the differences at the cell surface by inter- and intra-haplotype pairing of α and β chains during dimerization. The formation of functional restriction elements is the result of inter-haplotype combination of DQA and DQB molecules with duplicated DQA haplotypes (Glass et al. 2009).

Gayal or mithun (*Bos frontalis*) is a natural inhabitant of hilly forests and kept in India by ethnic groups living in the hills of Tripura, Mizoram, Assam, Arunachal Pradesh, Nagaland and Chittagong Hill Tracts. They are also found in the Trung and Salween River basins in Northern Burma and Yunnan Province of China (Simoons, 1984). Gayal is an important source of meat in these areas than other cattle and is considered to possess high percentage resistance to diseases (Rajkhowa et al. 2004). Gayal normally intakes local bamboo and other plant leaves and grasses but possesses high rang (from cold to tropical regions) of adaptation to harsh environment (Zhao et al. 2003; Xi et al. 2007). However, its genetic composition has been controversial as many biologists regarded the gayal as the domestic gaur for morphological similarity between the gayal (*Bos frontalis*) and the gaur (*Bos gaurus*) (Walker et al. 1968; Lan et al. 1993; Nie et al. 1995). The findings of karyotyping, mt-DNA and Y- chromosome analysis have made the scenario little more complex. However, mostly studies have suggested that gaur has been one of the immediate species ancestor of gayal (Nie et al., 1995; Chi et al., 2005; Verkaar et al., 2004; Gou et al. 2010; Sun et al. 2014). A recent investigation of Yunnan gayal suggested that maternal lineages of both Yunnan gayal and cattle were the admixture of *B. indicus* and *B. Taurus*, while the Y chromosomal phylogeny indicated that their parental lineages are almost *B. frontalis* and *B. indicus*, respectively (Gou et al. 2010).

In the present study, we have isolated and characterized two cDNAs of DQA1 and DQA2 from gayal and compared with other homologues MHC sequences from other animal species in regards to determine the disease resistance and susceptibility genetic factors. This work will possibly strengthen our understanding to the disease control in pet animals as well as in knowing MHC diversity in common ruminants. The study will assist to explore new horizons to

investigate immunological functions, selective and evolutionary forces that affect MHC variation within and between species.

2. Materials and methods

Three healthy gayal (*Bos frontalis*) liver samples were collected from the National Jiumudang Stud Gayal Farm, Dulong Town, Gongshan County, Yunnan Province, China. The RNA extraction was performed using the commercial kit (Beijing Tiangen Biotech Co., Ltd, Beijing, China). The extracted RNA was incubated with DNase I to cleave the DNA contamination. The cDNA was constructed using the commercial RevertAid^{TM} First Strand cDNA synthesis kit (Fermentas Inc., Ontario, Canada), following the manufacturer's protocol.

The *Bofr-DQA1* (784bp) and *Bofr-DQA2* (801bp) fragments were amplified from the template cDNA of gayal using three primer pairs *i.e.* A1A2F and A1R, and A2R, published previously for swamp buffalo (Niranjan et al. 2009). The forward primer (A1A2F: 5'-ACCTTGAGAAGAGGATGGTCCTG-3') was shared on the consensus region. The other two reverse primers (A1R: 5'-ATTGCACCTTCCTTCTGGAGTGT-3' and A2R: 5'-TCATAGATCGGCAGAACCACCTT-3') were different for both the *DQA1* and *DQA2*. By using the combined primers A1A2F, A1R and A2R, the two primers (A1A2F and A1R) amplified the Bofr-DQA1 and the additional two primers (A1A2F and A2R) amplified the Bofr-DQA2 fragments, respectively. Using Bioer Life Express Thermocycler, the PCR was performed in a reaction volume of 25 µL, containing 2.0 µL template cDNA, 12.5 µl PCR Power Mix, 1.0 µL 10 pmoL µL-1 of each primer, and 8.5 µL double-distilled water. The PCR cycle was: denaturation at 94 °C for 3 min, followed by 35 cycles; at 94 °C for 1 min, 59 °C for 45 S and 72

°C for 45 S, with a final extension of 10 min at 72 °C. Finally, the PCR products were sequenced bi-directionally using an ABI 3730 DNA Analyzer (Applied Biosystems Inc).

The cDNA sequences were translated to amino acid sequences using GenScan software (http://genes.mit.edu/GENSCAN.html) and compared with the orthologous sequences. The theoretical isoelectric point (pI) and molecular weight (Mw) of the two putative proteins of the gayal genes were also computed using the online pI/Mw tool (http://www.expasy.org/tools/pi_tool.html). The sequence predictions were made using the ORF Finder software (http://www.ncbi.nlm.nih.gov/projects/gorf/ and the neighbor joining phylogenetic tree was constructed using MEGA software based on the coding regions of different orthologous DQA alleles from different species (Tamura et al. 2007). The Non synonymous (dn) and synonymous substitution (ds) ratios between Gayal and other livestock species in the genes *DQA1* and *DQA2* have been estimated using the software PAML (Yang et al. 2007) and the significant changes that has altered the amino acids among livestock with respect to gayal have been investigated by the web version of PAL2NAL (http://www.bork.embl.de/pal2nal/).

3. Results and discussion

We searched the most homologous sequences for Bofr-DQA1 and Bofr-DQA2 genes using the BLAST tool of NCBI server (http://www.ncbi.nlm.nih.gov/BlAST). The sequence similarity search has revealed that the two genes were not similar to any of the known gayal genes but possesses high similarity to other ruminant genes. The nucleotide sequences of DQA1 and DQA2 were deposited to the NCBI GenBank database with accession number KT318732 and KT318733, respectively. Then, the sequences were also deposited to Immunopolymorphism

database (www.ebi.ac.uk/ipd/mhc/bola/nomenclature) with the assigned official names as Bogr-DQA*0101 (for *Bofr-DQA1*) and DQA*2001 (for *Bofr-DQA2*). The sequence prediction showed that the 784bp and 801bp cDNA sequences only represent two single genes with an ORF of 768bp and both encoding a polypeptide of 255 amino acid residues. The computed pI of gayal *DQA1* and *DQA2* genes were 4.93 and 4.84, respectively. The computed molecular weight (Mw) of the two putative proteins were 28298.34 and 27953.88 Daltons for Bofr-DQA1 and Bofr-DQA2, respectively.

The nucleotide sequence comparison of *Bofr-DQA* with *BoLA-DQA* genes for homology showed that the *Bofr-DQA1* and -D*QA2* possess 91% and 100% sequence identities with that of *BoLA-DQA1* and *-DQA2*, respectively (Table 1). However, the nucleotide sequence identity between the Bofr-DQA1 and -DQA2 were 88% only. These findings corroborate to the study conducted by Niranjan et al. (2009) on water buffalo. However, these authors presented that the *Bubu-DQA* genes have different identity (93.9 and 97.7%) with that of cattle as compared to the sequence homology between the *DQA1* and *DQA2* genes (85.7%).

A considerable mutations of 49 amino acid polymorphisms were observed when Bofr-DQA1 and -DQA2 were compared to other alleles which resulted from 95 nucleotide polymorphisms within the coding regions (Fig. 1). A total of 29 amino acid replacements were found within the exon 2 motif (α1), deduced from the 51 of the nucleotide mutations. The remaining amino acid differences including 4 in SP domain, 11 in the α2 domain, 2 in the connecting peptide (CP), 2 in the transmembrane (TM) region and 1 in the cytoplasmic (CY) domain were observed. These results demonstrated that gayal has more amino acid substitutions than buffaloes with 45 amino acids variation (Niranjan et al. 2009).

Additionally, the peptide binding sites (PBS, marked by green arrow sign), one N-glycosylation (NFT) within the α1 domain and another (NIT) within the α2 domain, one intrapetide disulfide bond and the CD4+ binding site (marked by square) were identified, revealing the significance of maintaining their molecular conformation and function to against the invading pathogens (Rudd et al. 1999). There were 20 PBS (Fig. 1) which are specific functional motifs in contacting with the antigens (Brown et al. 1993; Kuduk et al. 2012). The highly conserved loci from different animal species were only 8 residues at positions 11, 25, 29, 35, 57, 60, 63 and 70 between DQA1 and DQA2 homologues. The other 12 PBS sites had the different amino acids in both the polypeptide chains, demonstrating that it could have associated with gayal adaptation to specific environment. Moreover, Indian buffaloes have extra 3 rare polymorphisms at the positions of 57 (hydrophilic > hydrophobic) and 36, 94 (hydrophobic > hydrophilic) resulting into the opposite water affinity (Niranjan et al. 2009). This may be from the animal germplasm because buffalo can well adapt to the tropical areas (Perera, 2011). However, the replacements within the α1 domain have impacted on the antigen binding groove and could reveal differential binding ability to wide profiles of pathogens in different environments during the evolution process for livestock (Germain, 1995; Williams et al. 2002). We conclude that the B*ofr-DQA1* and *-DQA2* genes were more identical with the corresponding sequences of their counterpart cattle. Similarly, the low nucleotide sequence homology between *Bofr-DQA1* and *-DQA2* as well as the high proportion of nucleotide and amino acid substitutions clearly reveal inconsistency as allelic form. Our results also support the findings of Ballingall (Ballingall et al. 1998), that the bovine DQA3*01 and DQA3*02 sequences as non-allelic types have 92% nucleotide homology and larger genetic distance within two genes cluster.

From the phylogenetic tree exploration based on the nucleotide sequences, it appears that

the split of the DQA1 and DQA2 sequences from the gayal and other ruminants into two major clades and further indicates their independently evolutionary relationship of the gayal DQA sequences (Fig. 2). We speculate from the results that gayal is genetically closer to cattle, which is in accordance to the previous studies (He et al. 2014; Sun et al. 2014). Furthermore, a large distance between the two clades indicated that the *Bofr-DQA1* and *-DQA2* belong to two separate loci. For cattle and buffalo, there are some results to confirm that the DQA molecules are in the form of duplicated types and can be expressed both (Russell et al. 1997; Niranjan et al. 2009) which also seems to be a similar case for gayal. These duplicated genes with more replacements could be useful to prompt the immunological defenses of gayal in adaptation to harsh environments.

There have been several non synonymous changes in the livestock species with respect to gayal that has altered the amino acid sequences in both *DQA1* and *DQA2* genes. A detailed results (dn/ds ratio) and synonymous/no synonymous substitution have been presented in the supplementary table (see table S1 & S2 for dn/ds ratio and S3 & S4 for synonymous/no synonymous substitutions).

Conclusion

The *Bofr-DQA1* and *-DQA2* genes have been characterized with extending our understanding to the MHC-DQA in rare ruminants. Like other animals DQA genes, the *Bofr-DQA* and *-DQA2* were also highly variable, especially in the α1 domain as in most ruminants. It would be more interesting to clarify the effect of mutations from *Bofr-DQA1* and *-DQA2* on the pathogen's resistance for gayal adaption in further studies.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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List of Figures

Figure 1. An alignment between the amino acid sequences of Bofr-DQA and orthologous DQA sequences [The arrows indicate the amino acids positions consulting part of peptide binding sites (PBS). The putative N-linked glycosylation sites are underlined (—). The square (■) indicates the position of residues associated with binding of CD4+ molecules. A point (.) indicates amino acid identity and hyphen (-) indicates gap inserted to maximize]. The reference GenBank accession numbers for DQA1 alignment are Y07898 (BoLA-DQA*0101), U80884 (BoLA-DQA*0102), U80872 (BoLA-DQA*0204), U80871 (BoLA-DQA*0401), AB257109 (BoLA-DQA*10011), Y07819 (BoLA-DQA*12011), D50454 (BoLA-DQA*12021), U80869 (BoLA-DQA*1401), DQ440647 (Bubu-DQA*0101) and M93430 (OLA-DQA1). The reference GenBank accession numbers for DQA2 alignment are Y07820 (BoLA-DQA*2201), D50045 (BoLA-DQA*22021), U80868 (BoLA-DQA*2401), Y14020 (BoLA-DQA*25012), Y14021 (BoLA-DQA*2602), Y14022 (BoLA-DQA*27012), AF037314 (BoLA-DQA*2801), DQ440648 (Bubu-DQA*2001), M93433 (OLA-DQA2) and AY464652 (CLA-DQA).

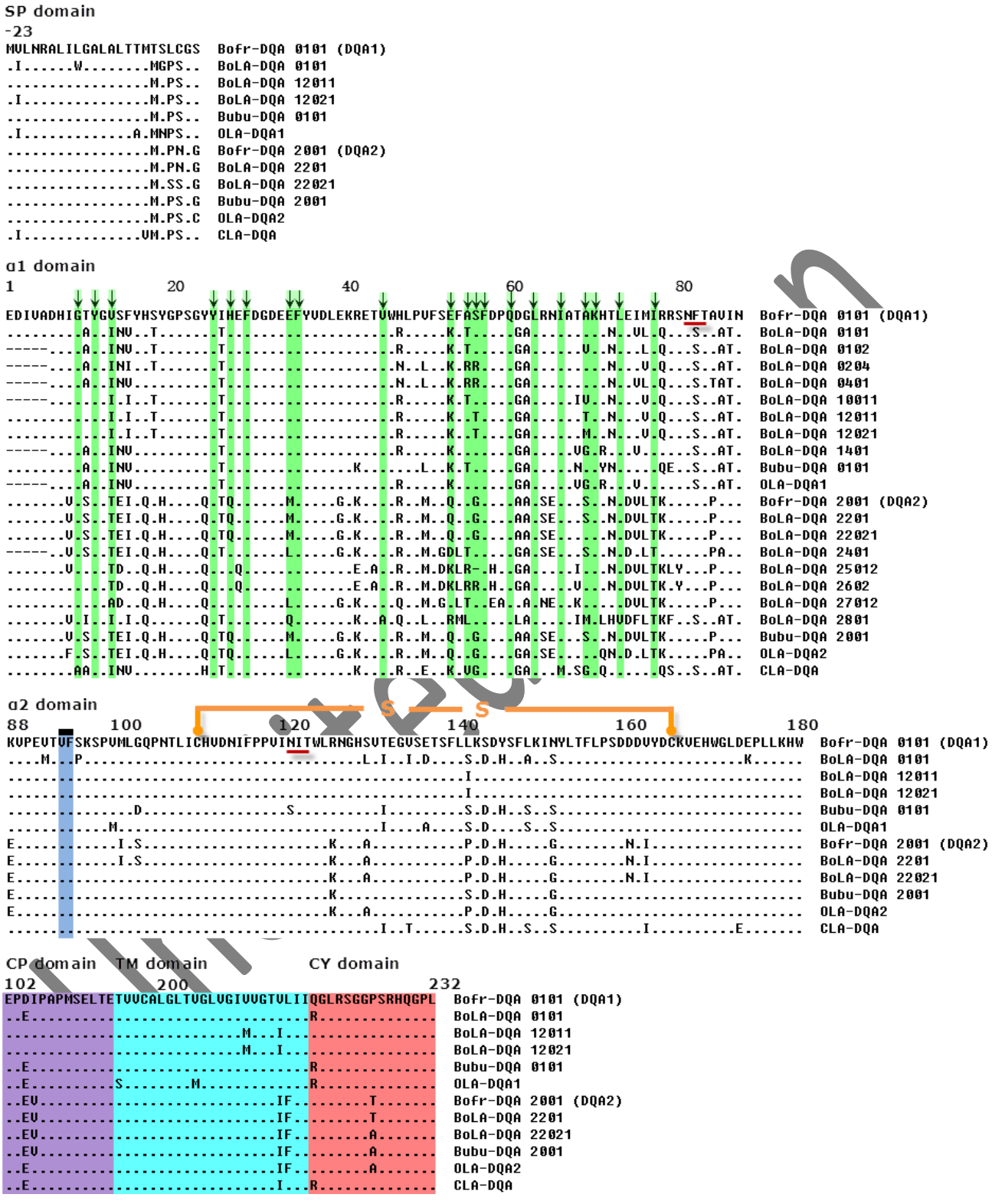

Figure 2. Phylogenetic tree based on DQA nucleotide sequences of gayal (Neighbor-joining method).

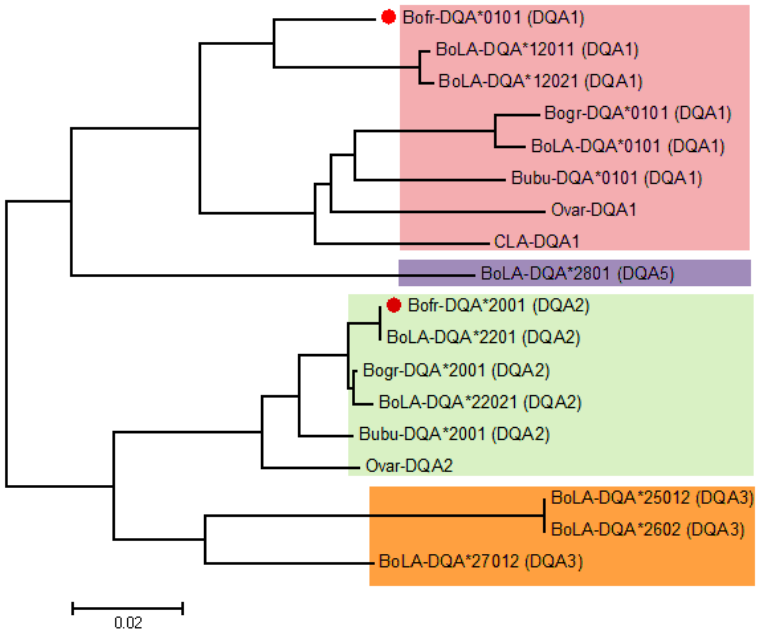

Table 1. Sequence comparison from the α1, α2, CP/TM/CY motifs between the *Bofr-DQA1/DQA2* and *BoLA-DQA1/DQA2* genes (The amino acid identity is shown in parentheses).

	*Bofr-DQA*2001 (DQA2)*	*BoLA-DQA*0101 (DQA1)*	*BoLA-DQA*2201 (DQA2)*
*Bofr-DQA*0101 (DQA1)*			
α1	81.0	91.0	81.0
α2	90.0	87.0	90.0
CP/TM/CY	92.0	94.0	92.0
Entire gene (protein)	88.0 (80.0)	91.0 (85.0)	88.0 (80.0)
*Bofr-DQA*2001 (DQA2)*			
α1		80.0	100.0
α2		87.0	100.0
CP/TM/CY		91.0	100.0
Entire gene (protein)		85.0 (78.0)	100.0 (100.0)
*BoLA-DQA*0101 (DQA1)*			
α1			80.0
α2			87.0
CP/TM/CY			91.0
Entire gene (protein)			85.0(78.0)

Table S1. dn/ds ratio of DQA1 gene sequences of different livestock species with respect to gayal (Bofr-DQA1)

Compare	>AB257109	>U80871	>D50454	>DQ440647	>KT318732	>M93430	>U80869	>U80872	>U80884	>Y07819	>Y07898
>AB257109	NA	3.875	0.824	0.804	0.849	0.919	1.228	1.241	1.379	0.616	1.938
>U80871	3.875	NA	4.429	0.888	1.286	1.086	2.364	1.390	NA	2.118	NA
>D50454	0.824	4.429	NA	1.264	0.849	1.072	2.740	1.516	1.930	0.280	2.491
>DQ440647	0.804	0.888	1.264	NA	0.684	1.399	4.429	0.688	0.807	0.942	0.811
>KT318732	0.849	1.286	0.849	0.684	NA	0.776	0.733	0.999	1.102	0.692	1.106
>M93430	0.919	1.086	1.072	1.399	0.776	NA	1.486	0.873	0.881	0.946	0.935
>U80869	1.228	2.364	2.740	2.547	0.733	1.486	NA	1.724	1.733	1.677	1.584
>U80872	1.241	1.390	1.516	0.688	0.999	0.873	1.724	NA	2.215	0.938	2.223
>U80884	1.379	NA	1.930	0.807	1.102	0.881	1.733	2.215	NA	1.125	NA
>Y07819	0.616	2.118	0.280	0.942	0.692	0.946	1.677	0.938	1.125	NA	1.129
>Y07898	1.938	NA	2.491	0.811	1.106	0.935	1.584	2.223	NA	1.129	NA

The reference GenBank accession numbers for DQA1 alignment are KT318732 (Bofr-DQA1), Y07898 (BoLA-DQA*0101), U80884 (BoLA-DQA*0102), U80872 (BoLA-DQA*0204), U80871 (BoLA-DQA*0401), AB257109 (BoLA-DQA*10011), Y07819 (BoLA-DQA*12011), D50454 (BoLA-DQA*12021), U80869 (BoLA-DQA*1401), DQ440647 (Bubu-DQA*0101) and M93430 (OLA-DQA1).

Compare	>AF037314	>AY464652	>D50045	>DQ440648	>KT318733	>Y14022	>Y14021	>Y14020	>Y07820	>U80868	>M93433
>AF037314	NA	0.670	0.840	0.840	0.840	0.735	0.587	0.494	0.840	0.679	0.804
>AY464652	0.651	NA	0.575	0.623	0.623	0.645	0.548	0.521	0.623	0.723	0.465
>D50045	0.789	0.575	NA	0.138	NA	1.019	1.024	0.775	NA	0.918	1.668
>DQ440648	0.789	0.623	0.138	NA	0.000	0.784	0.833	0.662	0.000	0.847	0.648
>KT318733	0.789	0.623	NA	0.000	NA	1.069	1.061	0.804	NA	0.847	1.945
>Y14022	0.716	0.662	1.019	0.784	1.069	NA	0.876	0.719	1.069	0.688	0.951
>Y14021	0.572	0.548	1.024	0.833	1.061	0.876	NA	0.399	1.061	0.830	0.970
>Y14020	0.483	0.521	0.775	0.662	0.804	0.719	0.399	NA	0.804	0.654	0.782
>Y07820	0.789	0.623	NA	0.000	NA	1.069	1.061	0.804	NA	0.847	1.945
>U80868	0.642	0.723	0.918	0.847	0.847	0.688	0.830	0.654	0.847	NA	0.624
>M93433	0.756	0.465	1.668	0.648	1.945	0.951	0.970	0.782	1.945	0.624	NA

Table S2. dn/ds ratio of DQA 2 gene sequences of different livestock species with respect of Gayal (Bofr-DQA2)

The reference GenBank accession numbers for DQA2 alignment are KT318733 (Bofr-DQA2), Y07820 (BoLA-DQA*2201), D50045 (BoLA-DQA*22021), U80868 (BoLA-DQA*2401), Y14020 (BoLA-DQA*25012), Y14021 (BoLA-DQA*2602), Y14022 (BoLA-DQA*27012), AF037314 (BoLA-DQA*2801), DQ440648 (Bubu-DQA*2001), M93433 (OLA-DQA2) and AY464652 (CLA-DQA).