

Molecular Characterization and Identification of SNP at Promoter Region of Bovine ITGB6 Receptor Gene



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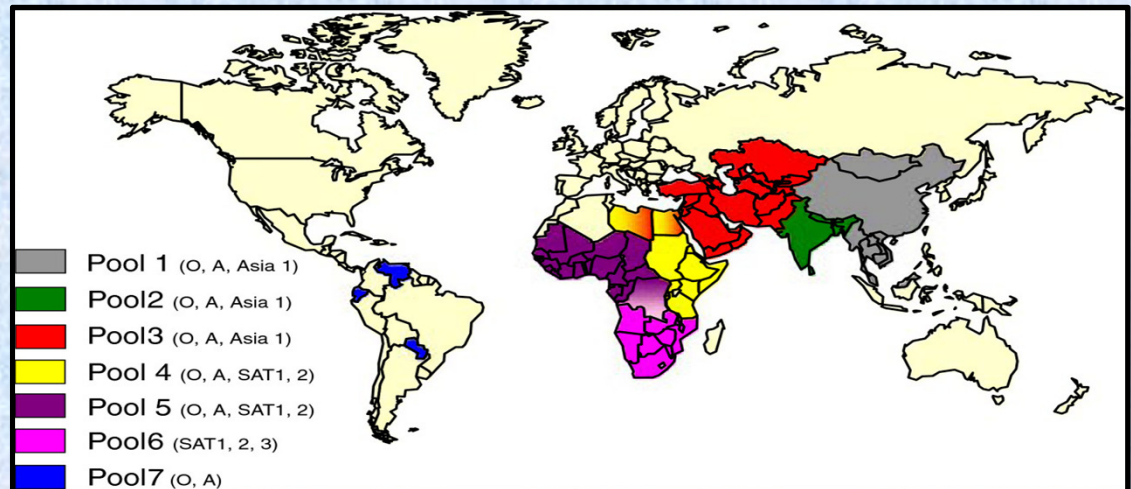
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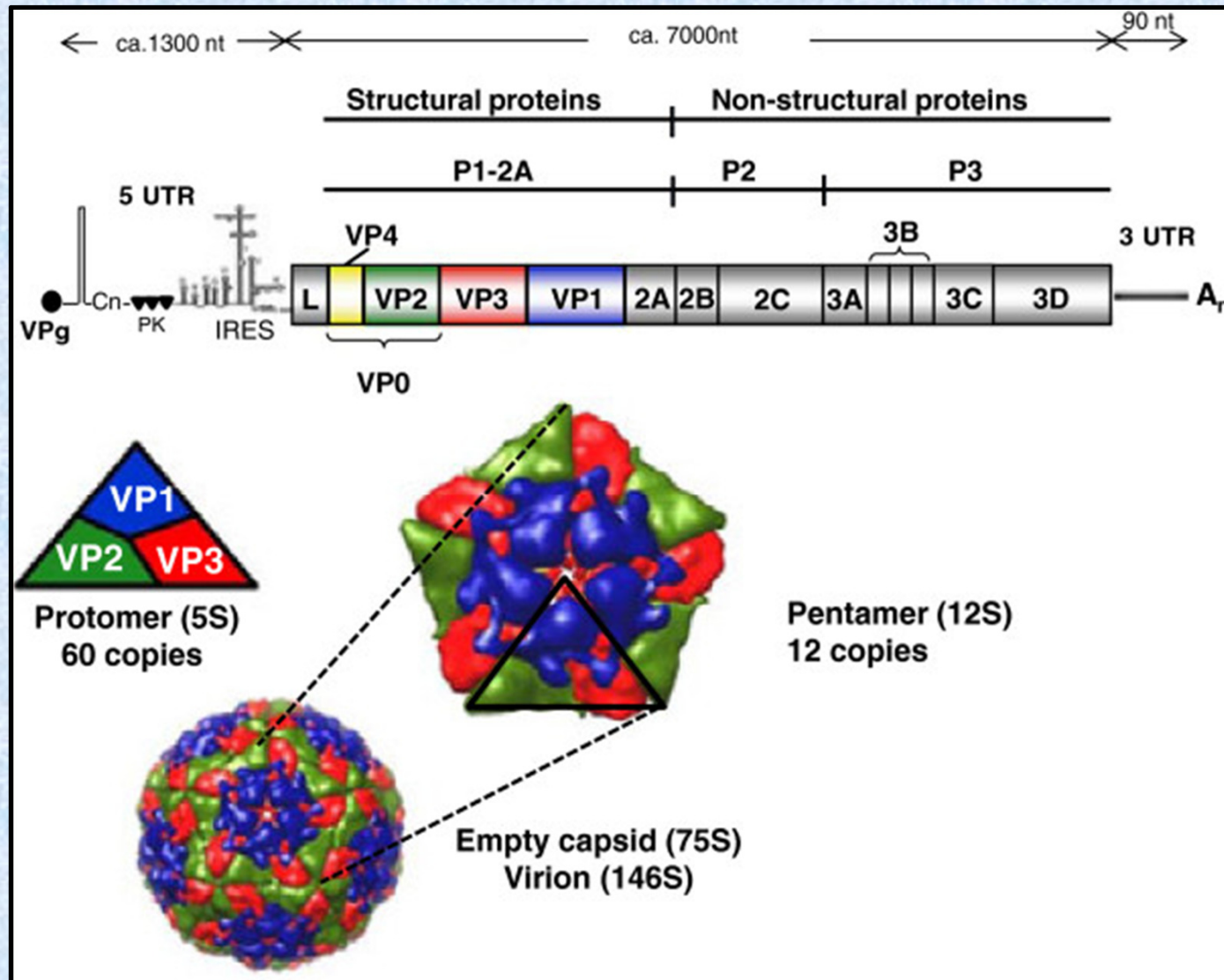
Foot and Mouth Disease

- At the national level in India, annual total economic loss due to FMD ranges from Rs 12,000 crore to Rs 14,000 crore (Singh et al., 2013).
- Zebu (*Bos indicus*) cattle are known to be resistant against Foot and Mouth disease virus (FMDV) compare to taurine (*Bos taurus*).
- Causes by positive stranded RNA viruses under the family of Picornaviridae characterized by vesicular lesions among the cloven-hoofed animals including cattle (Boothroyd et al., 1981).
- There are seven serotypes exist among the virus viz. O, A , C, Asia-1, South African Territories (SAT) types 1, 2 and 3.



- FMDV exhibits a strong tropism for cellular host epithelial cells and the uptake as well as viral replication machinery takes place in the pharynx and soft palate (Alexandersen et al., 2003).

Genomic organization of FMD virus





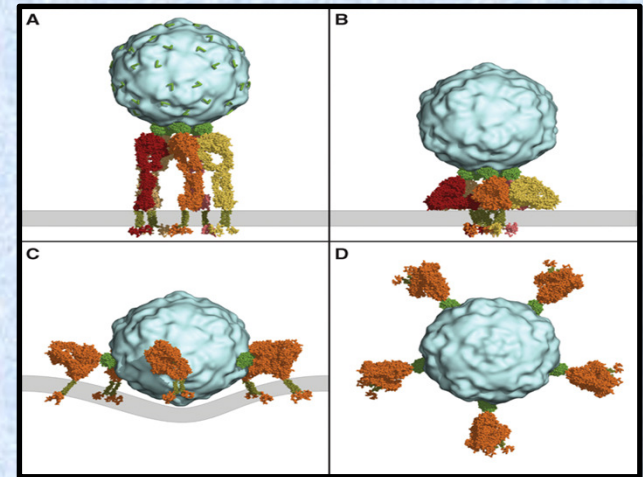
FMDV Host tropism

➤ FMD Virus get enter in host cell through receptor mediated endocytosis pathway (Jackson et al., 2003).

➤ Two kinds of cell-surface receptors have been identified for FMDV :

Heparan sulfate proteoglycans (HSPG)
(Jackson et al., 1996)

Integrins
(Jackson et al., 2002)



➤ Field isolates of FMDV attach to dependent integrin receptors through a long and conformationally flexible surface loop, the G-H loop of VP₁, which contains a highly conserved Arg-Gly-Asp (RGD) motif (Mason et al., 1994).

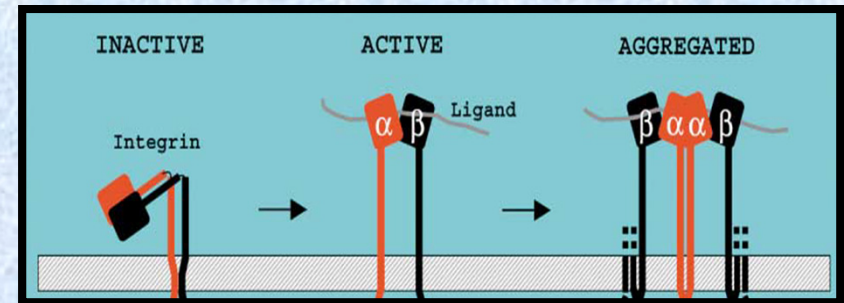
➤ Integrin has a more extensive role in mediating infection than heparan sulfate, and most FMDV strains infect susceptible cells via integrin (Jackson et al., 2003).

➤ Some strains can attach, internalize into cells and be multiply passaged through cultured cell lines only via heparan sulfate, for example O₁BFS strain (Baxt et al., 1995).



aVb6 receptor-major site for viral interaction

➤ Integrins are heterodimeric trans membrane glycoprotein that participate in a variety of cellular interactions.



➤ Out of known eight RGD binding integrins, FMDV utilizes four members of the av subgroup of integrins (avb1, avb3, avb6 and avb8) as receptors to initiate infection in vitro. (Berinstein et al., 1995; Jackson et al., 2000, 2002, 2004).

➤ Duque et al. (2004) reported that aVb6 integrin acts as a high-affinity receptor for type O FMDV, while aVb3 interacts with virus with a much lower affinity.

➤ Brown et al. (2006) have shown that integrin aVb6 appears to be constitutively expressed in normal airways of cattle which supports the premise that airway epithelium may serve as the primary site of infection with FMDV by aerosol.

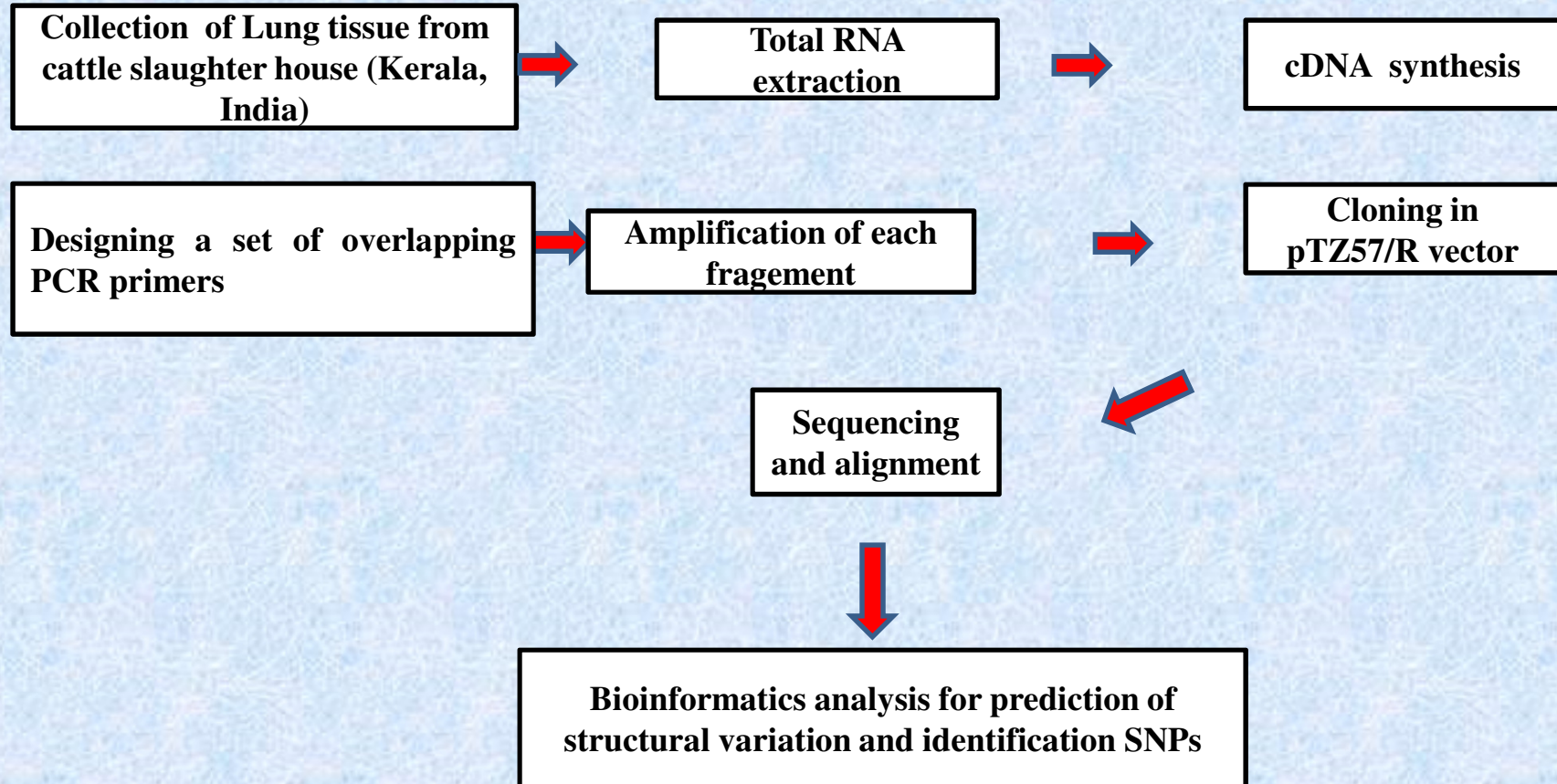
➤ However, whether sequence variation at aVb6 receptor gene involved in conferring resistant among native and taurine cattle are not yet known.



Objectives

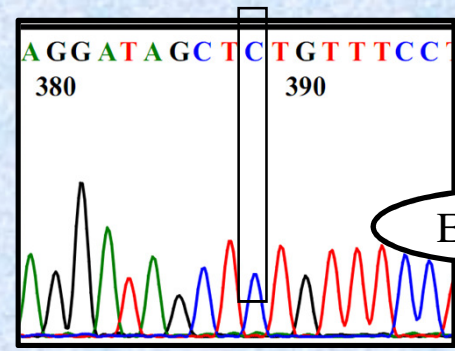
- Characterization of ITGB6 receptor gene of *Bos indicus* origin**
- Development of ARMS PCR based screening of genetic variation within 5'UTR region of ITGB6**
- Evaluation of the relationship between the genotype with FMD susceptibility among crossbred cattle**

Characterization of ITGB6 receptor gene of *Bos indicus* origin





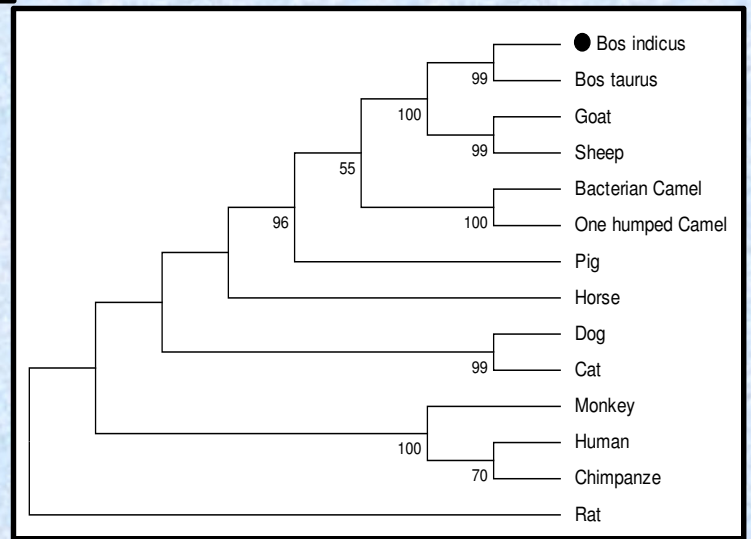
A	A	G	G	A	T	A	G	T	T	C	Majority
+	+	+	+	+	+	+	+	+	+	+	
1990										2000	
.	C	Bos_indicus- Indian Zebu_cattle KF886535
.	C	Bos_taurus_ (DQ867017)
.	Bactrian_Camel_ (EF613220)
.	One_humped_Camel_ (GQ443501)
.	G	Pig_ (EF432729)
.	C	Goat_ (JQ965817)
.	C	Sheep_ (EF367987)
.	C	Rat_ (NM001004263*)
.	G	Human_ (NM000888*)
.	Horse_ (XM005601534*)
.	Dog_ (XM852055*)
.	Cat_ (XM003990799*)
.	G	Monkey_ (XM001094740*)
.	G	Chimpanzee_ (XM001149234*)



T2145
C

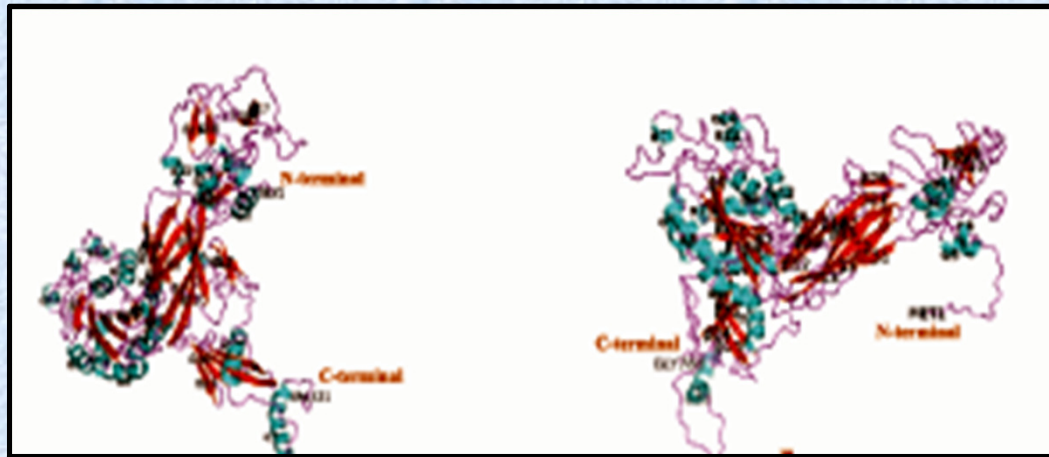
Exon- 14

TT: Phenylalanine
TC: Serine

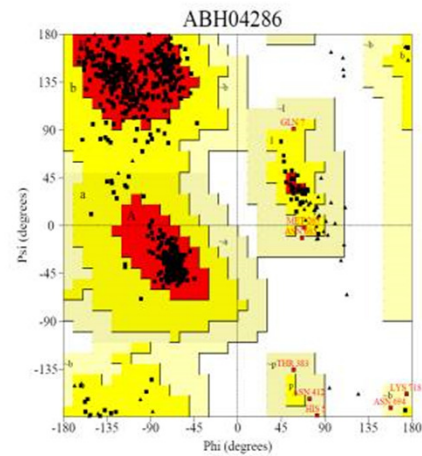


The predicted model through homology modelling using Discovery Studio 3.5. a) ABH04286 (Bos indicus) and b) AHI48847 (Bos taurus)

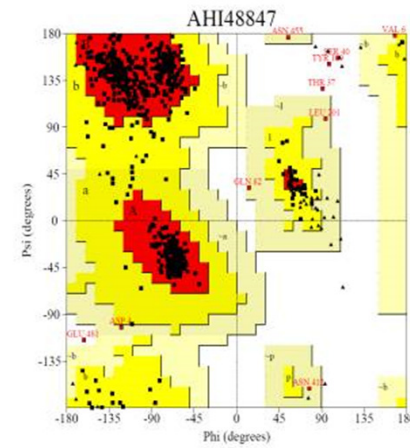
a



b



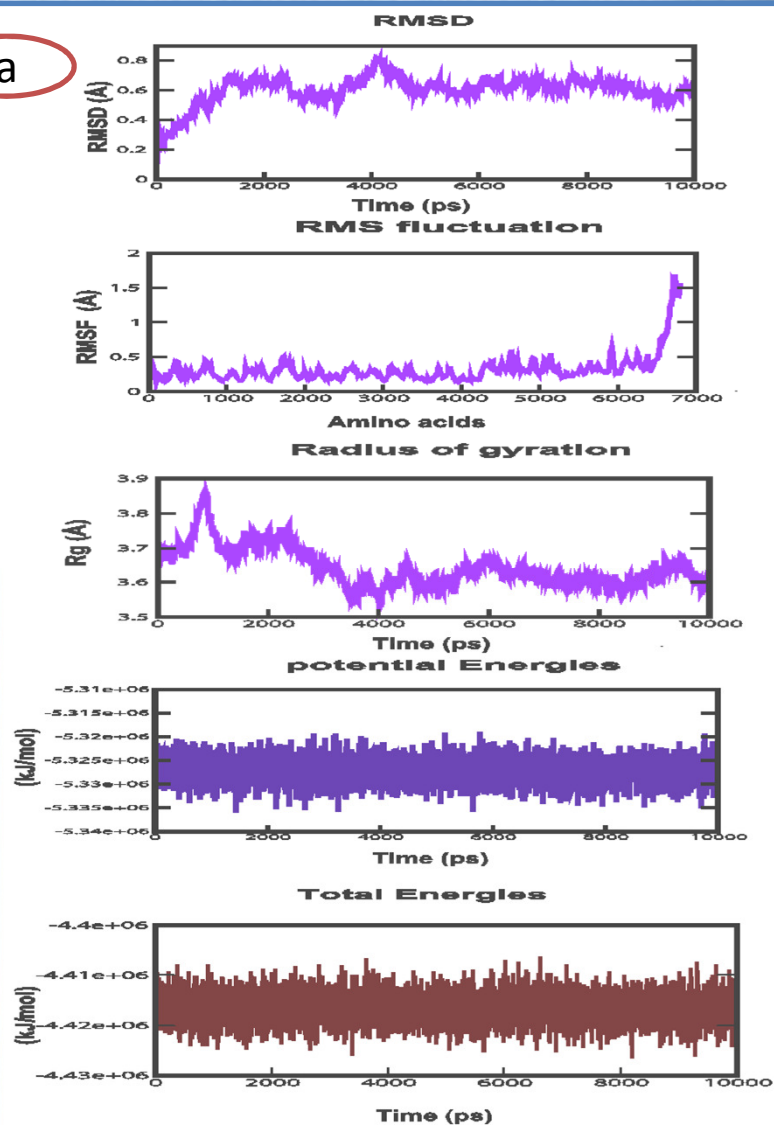
a



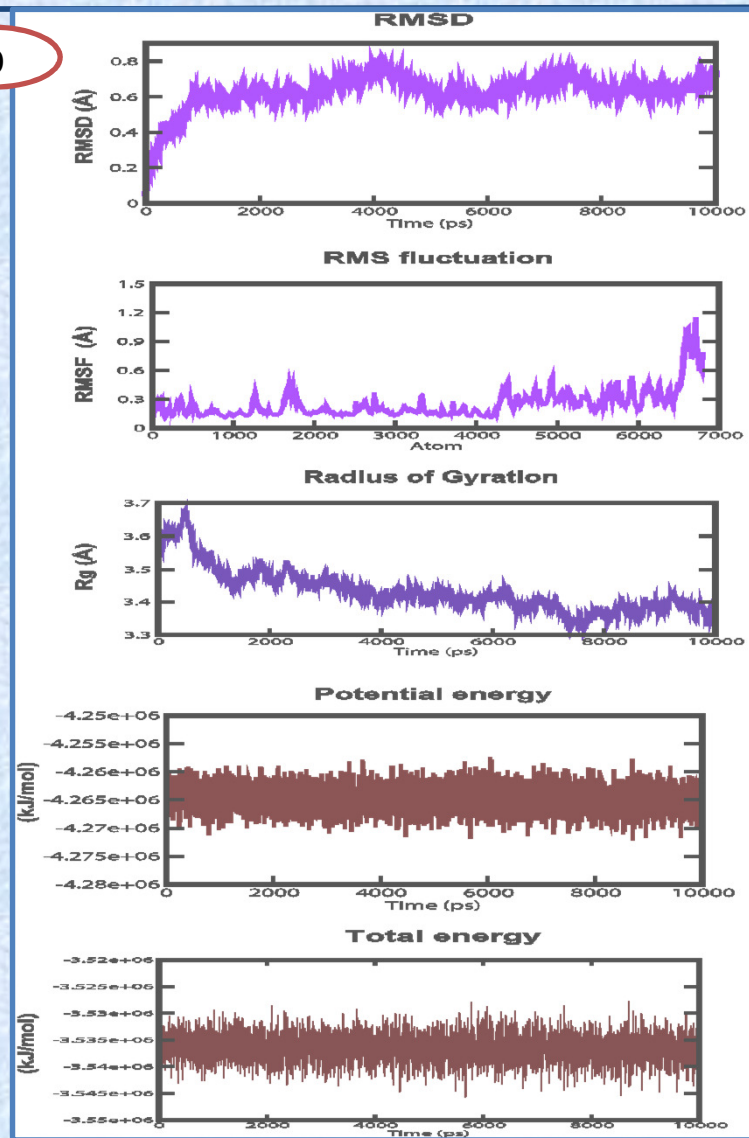
b

Ramachandran plot for the predicted model from the SAVEs server a) ABH04286 and b) AHI48847

a



b



Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of gyration, Potential energies and Total energies plots for the modelled protein throughout the MD simulation a) ABH04286 and b) AHI48847



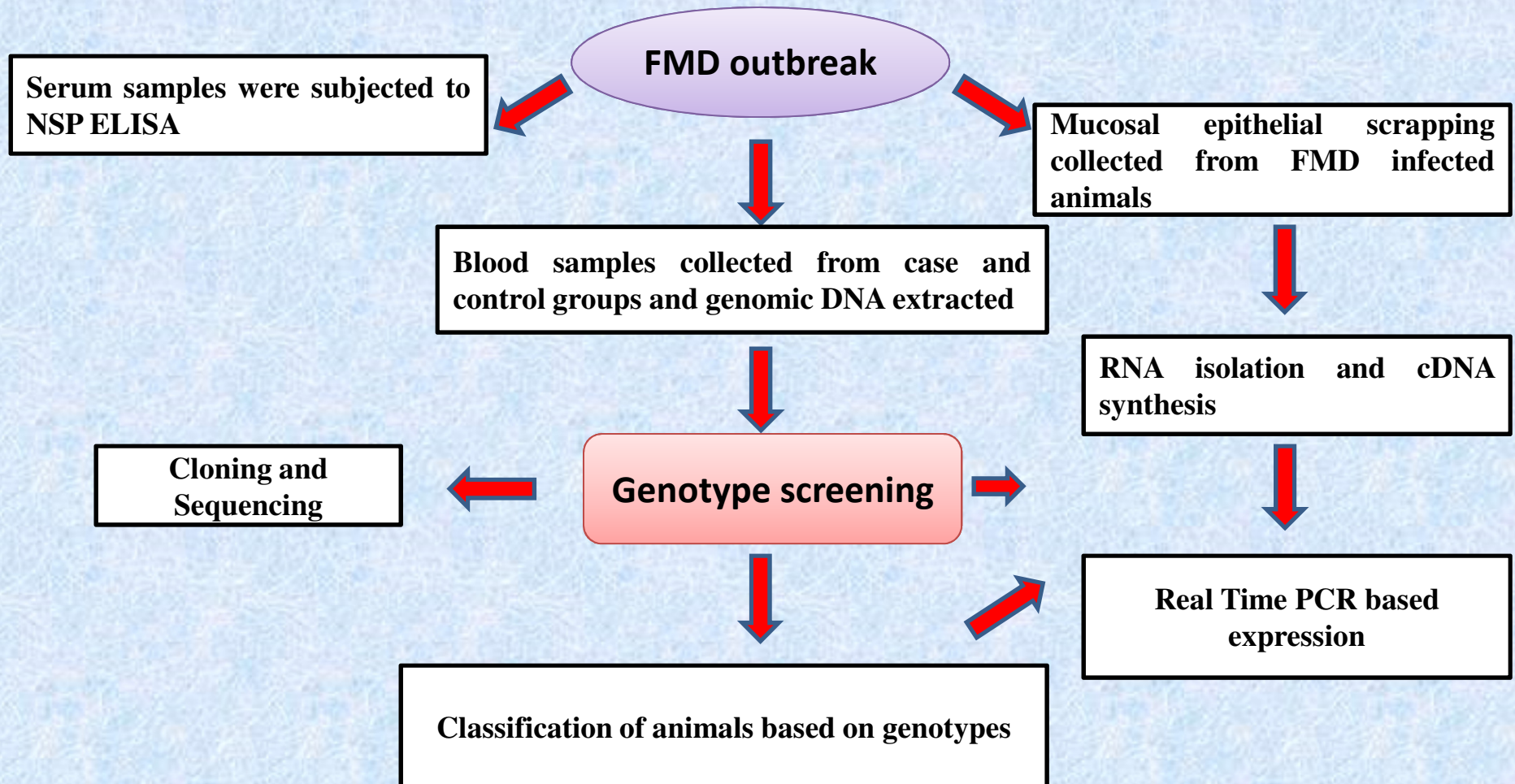
**The primary structure information i.e. the summary of ProtParam tool
(<http://expasy.org/cgi-bin/protparam>)**

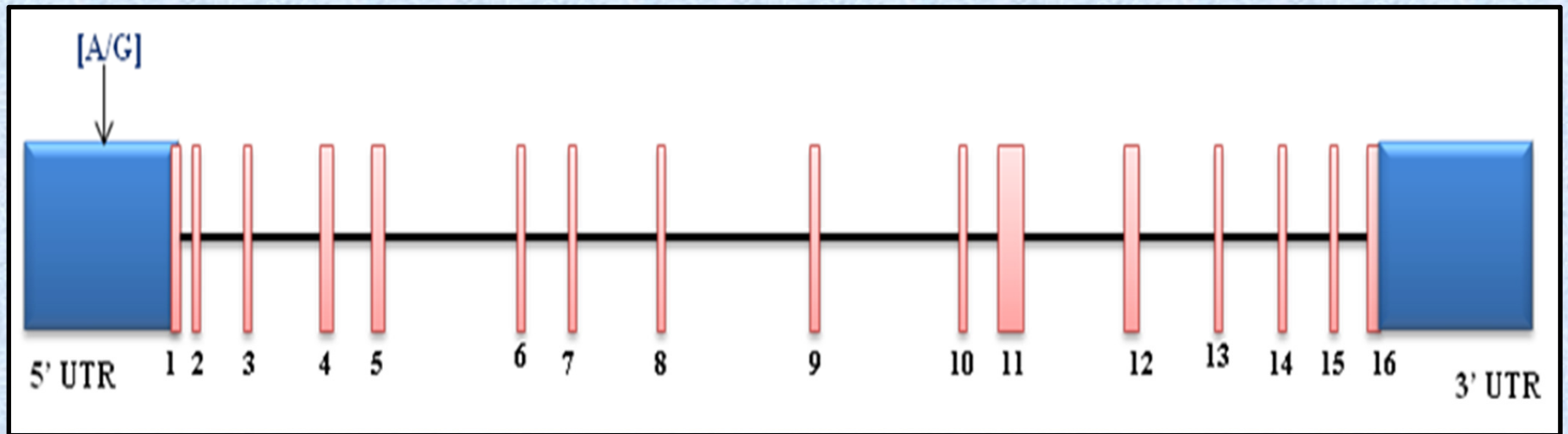
Sequence	Number of amino acids	Molecular weight	pI	Instability index	Grand average hydropathy	Aliphatic index
ABH04286	788	85954.9	5.08	43.50	-0.168	81.37
AHI48847	788	85832.8	5.05	43.72	-0.170	81.87

The secondary structure information using PSIPRED and CONCORD software

Sequence	No of Coil	Percentage of Coil	No of Helix	Percentage of Helix	No of Sheet	Percentage of Sheet	Total
PSIPRED							
ABH04286	514	65.2	135	17.2	139	17.6	788
AHI48847	521	66.1	124	15.7	143	18.2	788
CONCORD							
ABH04286	502	63.7	141	17.9	145	18.4	788
AHI48847	506	64.2	138	17.5	144	18.3	788

Evaluation of the relationship between the genotype with FMD susceptibility among crossbred cattle





Full length CDS of bovine ITGB6

Arch Virol
DOI 10.1007/s00705-014-2194-0

BRIEF REPORT

Development of a tetra-primer ARMS PCR-based assay for detection of a novel single-nucleotide polymorphism in the 5' untranslated region of the bovine ITGB6 receptor gene associated with foot-and-mouth disease susceptibility in cattle

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Sushil Kumar · Soumen Chakraborti · Sheetal Sharma ·
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Abstract ITGB6 is known to be one of the major receptor components involved in host tropism of foot-and-mouth disease (FMD) virus in cattle. A competitive PCR technique called ARMS PCR was adapted to identify a single-nucleotide polymorphism (SNP), G29A, at SNP Id: n109075046, in the 5' untranslated region (5'UTR) of the bovine ITGB6 gene. Genotype profiling identified three kinds of genetic variation within the targeted SNP among Friesian crossbred cattle. The occurrence of FMD in the three genotypes was further evaluated, revealing a clear role in the incidence of FMD in the studied population.

Keywords ARMS PCR · ITGB6 · Cattle · FMDV · 5'UTR

Foot-and-mouth disease (FMD) is a highly contagious viral disease of cloven-hoofed animals and is one of the most economically important diseases of livestock, producing an annual economic impact between US\$6.5 and 21 billion in

endemic regions in terms of visible production losses and vaccination [15]. At the national level in India, annual total economic loss due to FMD ranges from Rs 12,000 crore to Rs 14,000 crore [23]. In spite of all the control measures taken, FMD continues to be an economically important disease in India due to poor surveillance and inadequate control programs. Besides causing direct losses to the livestock economy, it also results in indirect losses due to severe trade restrictions [2, 3]. The disease is caused by foot-and-mouth disease virus (FMDV), a member of the family *Picornaviridae*, and is characterized by fever, profuse salivation, vesicles in the mouth and on the feet, and a drastic reduction in milk production. Sudden death in young stock may occur [21]. There are seven serotypes of FMD virus, viz., O, A, C, South African Territories (SAT) 1, SAT 2, SAT3 and Asia1, which produce immunologically distinct diseases. FMDV is a small, icosahedral, non-enveloped virus with a single-stranded (ss) positive-sense RNA genome. The capsid is composed of 60 copies of each of four structural proteins, VP1 to VP4, and has icosahedral symmetry [4]. Determination of the three-dimensional structure of FMDV has revealed a prominent surface feature formed by the loop between the G and H strands of VP1, which is partially disordered in nature [5]. The virus enters the host cell through a receptor-mediated endocytosis pathway [12]. FMDV exhibits strong tropism for epithelial cells, and following a natural infection, initial virus uptake and/or replication is thought to take place in the pharynx and soft palate [1, 19, 26]. Two kinds of cell-surface receptors have been identified for FMDV: heparan sulfate proteoglycans (HSPGs) [11] and, most commonly, integrins [12–14]. On the basis of synthetic peptide inhibition studies it has been reported that the G-H loop of VP1 gene contains a highly conserved arginine-glycine-aspartic acid (RGA) sequence that has been implicated in host

R. Singh and R. Deb contributed equally to this work.

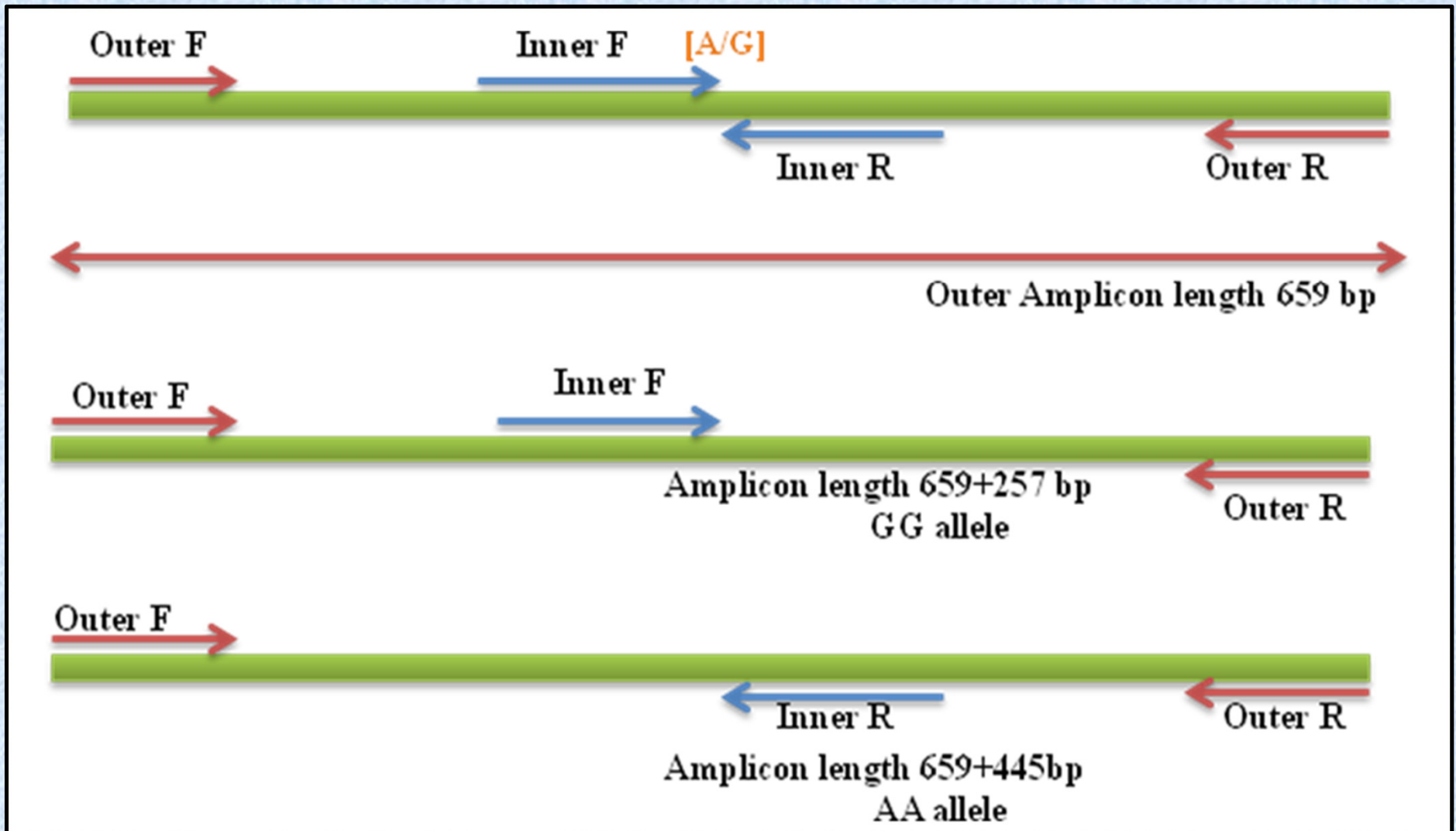
Electronic supplementary material The online version of this article (doi:10.1007/s00705-014-2194-0) contains supplementary material, which is available to authorized users.

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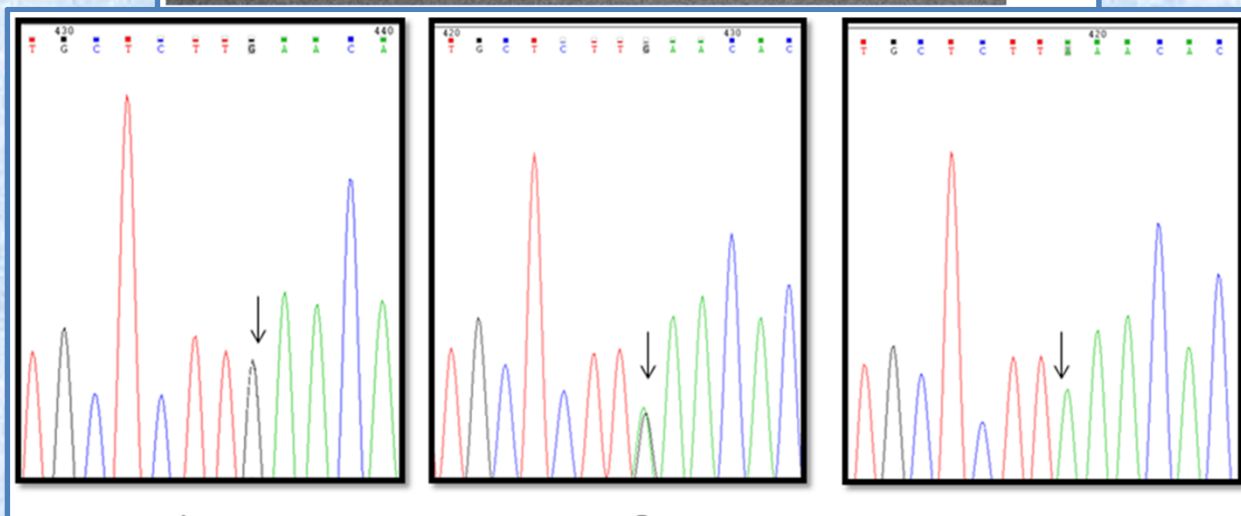
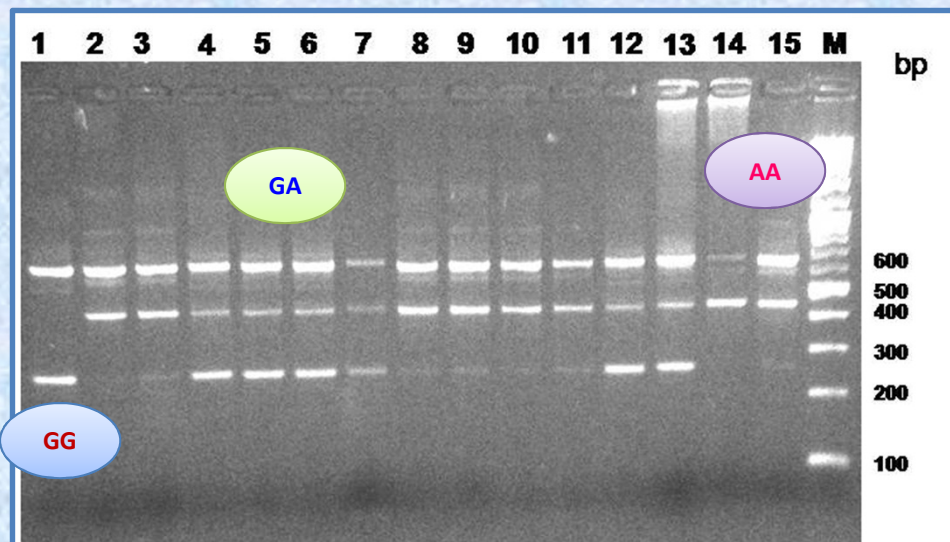
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Development of a strategy for genetic screening



Accession Number obtained: A. **GG** (KJ847280) B. **GA** (KJ847281) C. **AA** (KJ847282)



Genotype and Allele frequency of g>T533C mutation at 5'UTR region of ITGB6 gene among different breeds

Breeds	Genotype frequency			Allele frequency		Chi-Square
	AA	GA	GG	A	G	
Frieswal	65 (0.32)	125 (0.611)	14 (0.069)	0.626	0.374	19.25**
Sahiwal	34 (0.667)	17 (0.333)	-	0.833	0.167	2.04 ^{ns}
Kankrej	48	-	-	-	-	-
Ongole	38	-	-	-	-	-
Total	185	142	14			

** P < 0.001; ns-non-significant.

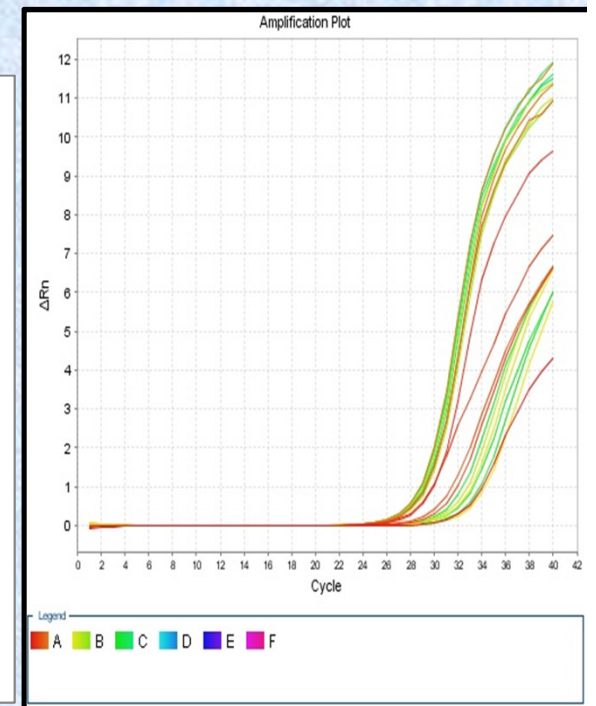
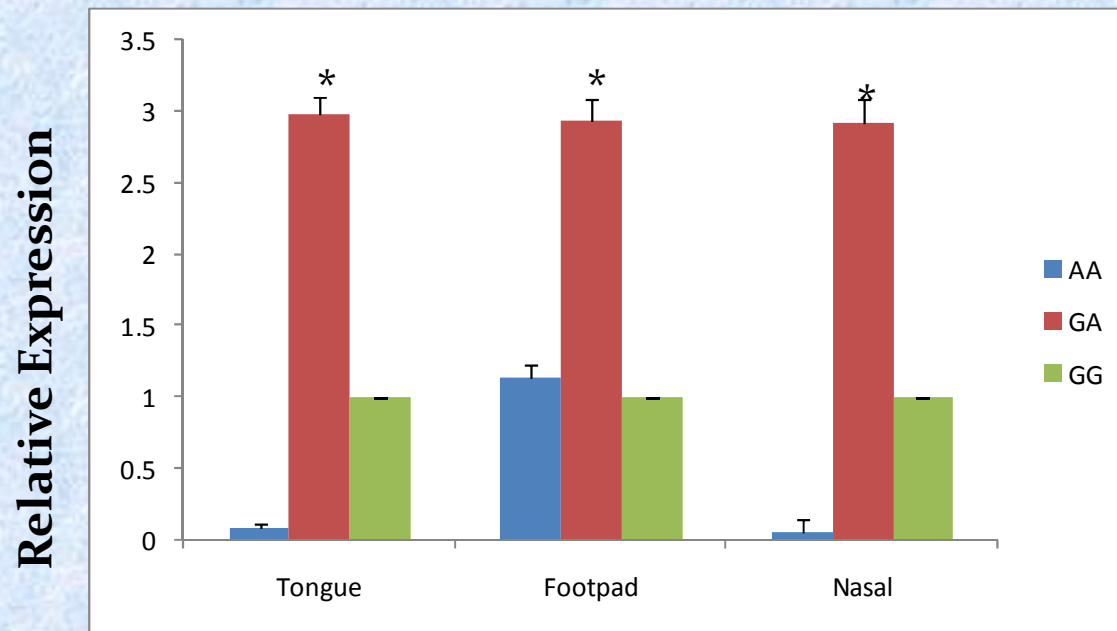


Genotype and Allele frequency of g>T533C mutation at 5'UTR region of ITGB6 gene among FMDV affected (case) and non affected (control) animals

Class	Genotype frequency			Total	Chi-Square
	AA	GA	GG		
Case	5	42	8	55	18.24**
Control	15	18	-	33	
Total	20	60	8	88	

** P<0.001

Relative Expression of ITGB6 receptor gene among different groups



*P<0.05



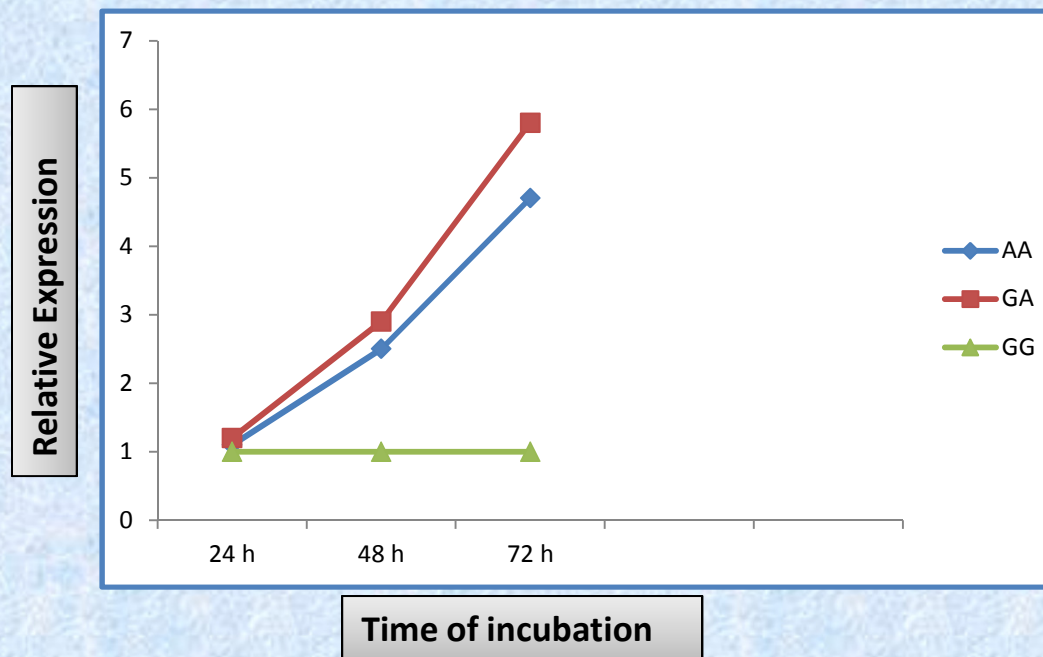
Epithelial cell of earpina from different genotypic groups were cultured Invitro



Inoculation with FMDV live viral vaccine strain (0.1 ml of a suspension of virus with $10 \log_{10}$ TCID₅₀/ml) was inoculated and incubation for different kinetic interval



Relative expression of FMDV specific 3-D gene was quantified





Conclusion

- ✓ The complete CDS of zebu ITGB6 was characterized.
- ✓ The zebu integrin share common structural and functional elements with taurine.
- ✓ A strategy was developed for genetic screening of FMD receptor gene in cattle.
- ✓ Genotype profiling identified three kinds of genetic variation exists within the targeted SNP at 5'UTR region of ITGB6.
- ✓ AA genotypes were most prevalent among zebu cattle compare to GA and GG.
- ✓ GA genotypes were associated with susceptibility to FMD in Frieswal bulls ($P < 0.01$), whereas AA were associated with resistance to FMD ($P < 0.001$).
- ✓ Expression profile of ITGB6 in different epithelial tissues revealed that, GA genotypes having significantly ($P < 0.05$) higher transcripts than AA and GG.



Further ongoing work

- Identification of the transcript variants of ITGB6 among different Zebu cattle
- Screening of SNPs within exon coding region of ITGB6 and their relation with FMD susceptibility
- Validation for the the intensity of *Invitro* FMDV infection in ITGB6 expressed primary epithelial cells among different genotypic groups are ongoing

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*Thank you very much for your
kind attention*