Original Research Article

# Bio-computational prediction of novel epitopes on VP2 protein of infectious bursal disease virus


## ABSTRACT

**Aim:** Recently, many viral Immunogenic peptides or epitopes have been used as potential vaccine and also immune-diagnostic candidates. In this study, we predicted different epitopic peptides on VP2 protein of infectious bursal disease virus (IBDV) by use of bioinformatics modelling which can be potential vaccine as well as diagnostic candidate for IBD, in future.

**Study design:** In the present study, B-cell epitopes (linear, continuous, and conformational) and T-cell epitopes were predicted on VP2 protein.

**Place and Duration of Study:** Bihar Animal Sciences University, Patna, and Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India between June and December 2023.

**Methodology:** For the prediction of linear B-cell epitopes, Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools were used, while Ellipro was used for conformational B-cell epitopes prediction. In the absence of immune-bioinformatics tool is available to predict poultry MHC-peptide binding, only those human MHC-I/II alleles having greater than 70% identities those of poultry MHC-I/II alleles were selected. NetMHCcons 1.1 and NetMHCIIpan - 4.0 tools were used to predict strength binding affinity of peptides with MHC-I and MHC-II, respectively.

**Results:** As per analysis by four different tools, the peptide 'SYDLGYVRLGDPIPAIGLDPKMVATCDSSDRPRVYTITAADDYQFSSQYQPGGV$^{164\text{-}217}$' ($Epitope_L$) was predicted as the most prominent linear B-cell epitope. Two peptides, i.e. ANLNSPLKIAG ($Epitope_C$ 1) and SSQYQPGGRTSVHGLGLTTGTDKSGGQAGDQMS ($Epitope_C$ 2) were predicted as potent conformational B-cell epitopes. During T-cell epitopes prediction, human HLA*B 40:06, HLA*B 41:03 and HLA*B 41:04 alleles chosen as homologues of poultry MHC class I alleles while DRB1:1310, DRB1:1366, DRB1:1445, and DRB1:1482 chosen as homologues of poultry MHC class II alleles. A 9-mer GELVFQTSV$^{236\text{-}244}$ peptide was predicted as MHC-I strong binder ability while, two 15-mer peptides, i.e. YTKLILSERDRLGIK$^{389\text{-}403}$ and QMLLTAQNLPASYNY$^{76\text{-}90}$ were predicted as MHC-II strong binder ability.

**Conclusion:** Using bio-computational analysis, one linear and two conformational B-cell epitopes were predicted on VP2 protein of IBDV. During T-cell epitopes prediction one 9-mer peptide and two 15-mer peptides were predicted as MHC-I and MHC-II strong binding peptides, respectively. After assessing protective immune responses through in vitro and in vivo studies, these predicted peptides could be potential candidates for developing subunit vaccines.



*Keywords:* Infectious Bursal Disease Virus, VP2 protein, B-cell epitope, T-cell epitopes

## 1. INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral infection affecting young chickens, especially between 3 and 6 weeks of age. The causative agent is the infectious bursal disease virus (IBDV), a member of *Birnaviridae* family. IBDV primarily targets the bursa of Fabricius and replicates within the B cells of the bursa, causing severe immunosuppression and compromising the chicken's ability to mount an effective immune response [1,2].

The IBDV has a bi-segmented double-stranded RNA genome. With the size of 3.2 kbp, segment A is the longer one, while segment B is smaller with size of 2.8 kbp [2]. Segment A has two partially overlapped open reading frames (ORFs). The first ORF encodes the non-structural VP5 protein of 17 kDa, and the second ORF encodes a 110 kDa polyprotein. After translation, the polyprotein is proteolytically cleaved into VPX, VP3 and VP4 proteins of 48 kDa, 32 kDa and 28 kDa, respectively. The VPX is further processed into 41 kDa VP2 protein [3]. Segment B has only one ORF which encodes 95 kDa VP1 protein [4]. The VP2 and VP3 are two structural proteins of IBDV [2]. The VP2 protein is the major structural protein of IBDV playing an important role in viral entry, assembly and immune recognition. It forms the bulk of the viral capsid and plays a crucial role in maintaining the structural integrity of the virus. The VP2 protein is involved in receptor binding and determines the tropism and host range of the virus. It also contains immunogenic epitopes that elicit an immune response in the host [5].

To prevent IBDV outbreaks, disease diagnosis, monitoring and vaccination of poultry are the common strategies [6]. For control of infectious disease, live attenuated and inactivated vaccines are two major types of vaccines used poultry [7]. However, both live-attenuated and inactivaced vaccine have certain limitation. The live-attenuated vaccine has potential risk of reverting back to virulent pathogen, while multiple doses, alongwith adjuvant, are needed in case inactivated vaccine to achieve protective immunity [8].

A lot of work has been done recently on peptide-based vaccines and diagnostics. Peptide vaccines are composed of antigenic epitopic regions (short peptide sequences) which are recognized by the immune systemleading to its activation. Peptides are also for development of diagnostic assays for infectious diseases [9]. Peptide-based diagnostic techniques include enzyme-linked immunosorbent assays (ELISA), peptide microarrays, and peptide-based biosensors. Peptide-based diagnostics are affordabile and having high sensitivity and specificity [10].

Considering the potential use of immunogenic peptides (epitopes) as vaccine and diagnostic assay candidates with high sensitivity and specificity, we predicted different epitopic peptides on VP2 protein of IBDV by use of bioinformatics modelling which can be potential vaccine as well as diagnostic candidate for IBD, in future.

## 2. MATERIALS & METHODS

### 2.1. Physiochemical properties prediction and 3D modelling

The amino acid (AA) sequence of VP2 protein of previously reported Indian isolate of IBDV (Acc. No.: AMQ81722; Region between 9 and 452 AA) was retrieved from the GenBank database of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/genbank). Physiochemical properties like molecular weight, isoelectric point (pI), amino acid composition, estimated half-life, and instability index were determined by the ProtParam programme (http://web.expasy.org/protparam/). The 3D model of VP2 protein was constructed using homology modeling on the Protein Data Bank (PDB) database (https://www.rcsb.org/). Based on the highest sequence identity along with a lower E-value, the crystal structure of the VP2 protein of IBDV, available on PDB, was selected for further study.

### 2.2. Linear/continuous B-cell epitopes prediction

Possible linear B-cell epitopes on VP2 protein were predicted by the immunomedicine group, Bepipred Linear Epitope Prediction 2.0 (BepiPred-2.0), SVMTriP, and BCPred tools. The immunomedicine group tool (http://imed.med.ucm.es/Tools/antigenic.pl), which has approximately 75% accuracy and is based upon Kolaskar and Tongaonkar [11] method was also used for epitopes prediction on VP2 protein. Linear B-cell epitopes were also predicted using BepiPred-2.0 tool (http://tools.iedb.org/bcell/). The BepiPred-2.0 tool is based upon a

random forest algorithm for epitopes annotated from antibody-antigen protein structures. It is a sequence-based epitope prediction tool based upon epitope data derived from solved 3D structures as well as epitopes database downloaded from IEDB [12]. The SVMTriP Tool is based on machine learning algorithms (http://sysbio.unl.edu/SVMTriP/). SVMTriP was developed to predict antigenic epitopes using the latest sequence input from IEDB database. In the SVMTriP Tool, Support Vector Machine (SVM) in combination with Tri-peptide similarity and Propensity scores has been utilized to achieve better prediction performance [13]. The linear B-cell epitopes were also predicted using BCPREDS (B-cell epitope prediction service; http://ailab.ist.psu.edu/bcpred/predict.html), which joined a subsequence kernel and a support vector machine (SVM) method with the output reliability of 74.57 percentage [14].

For the prediction of potential epitopic peptides with high specificity and sensitivity, consensus peptides from several epitopic peptide prediction methods should be selected [15]. In the current study, only those peptides or region of peptides common in three or more epitopic prediction methods were shorted as epitope.

### 2.3. Structure-based/conformational B-cell epitope prediction

Conformational B cell epitopes on VP2 protein was predicted using ElliPro (http://tools.iedb.org/ellipro/) tool based on the PDB 3D model of protein. The ElliPro is a web tool formulated by Thornton's method along with MODELLER program of a residue clustering algorithm and Jmol viewer [16]. The conformational B-cell epitopes predicted should have ElliPro score range between 0.5 and 0.8, and also have upper limit distance for residue clustering within 6.0 Å [17].

### 2.4. T-cell epitope prediction

MHC-I and MHC-II binding peptides have been considered as T cell immunogens [18]. Since, no immune-bioinformatics tool is available to predict poultry MHC-peptide binding, in the current study only those human MHC-I/II alleles having greater than 70% identities those of poultry MHC-I/II alleles were selected, as described by Thomsen et al. [19]. Human HLA*B 40:06, HLA*B 41:03 and HLA*B 41:04 alleles having more than 70% similarity with poultry MHC class I alleles while DRB1:1310, DRB1:1366, DRB1:1445, and DRB1:1482 alleles having more than 70% similarity with poultry MHC class II alleles [20].

NetMHCcons 1.1 (http://www.cbs.dtu.dk/services/NetMHCcons) and NetMHCIIpan - 4.0 (https://services.healthtech.dtu.dk/services/NetMHCIIpan-4.0/) tools were used to predict strength binding affinity of peptides with MHC-I and MHC-II, respectively. For *in silico* prediction of MHC-I binding affinity, the VP2 protein sequence was chopped into 9 amino acids long all possible polypeptides subsequently binding affinity of all these peptide with MHC-I were estimated as IC50 concentration (in nM) as described by Valdivia-Olarte et al. [21]. Peptides were categorised into two groups based on their estimeted affinities: "SB" or Strong Binder (for IC50 ≤ 2 nM) and "WB" or Weak Binder (for IC50 ≤ 2 nM ≤ 50 nM) with MHC-I. Peptides with 50 nM ≤ IC50 values were considered as non-binder of MHC-I. Similarly for prediction of MHC-II binding affinity, the VP2 protein sequence was chopped into 15 amino acids long all possible polypeptides subsequently binding affinity of all these peptide with MHC-II were estimated as % Rank as described by Andreatta et al. [18]. Peptide (s) having % Rank < 2 were considerd as strong binders (SB) with MHC-II.

## 3. RESULTS

### 3.1. Physiochemical properties of VP2 protein

Online available ProtParam programme-based physiochemical analysis indicated that VP2 is a negatively charged protein with an isoelectric point of5.12 and the molecular weight of 47.518 KDa. This protein contained 38 negatively charged residues (Asp + Glu) and 30 positively charged residues (Arg + Lys). The computationally calculated instability index of VP2 protein was 25.64. Glutamine (Gln; Q) was the N-terminal amino acid of this protein and its *in vitro* half-life was 0.8 hr in mammalian reticulocytes, while *in vivo* half-life in yeast, and in *E. coli* was 10 min, and 10 hr, respectively. The 3D structure of the VP2 protein of Indian isolate IBDV was predicted by homologous modeling using the PDB database. A total of eight reference templates were found on the PDB database having sequence identity by ranges between 99% and 44% and E-value range between 3.264e-288 and 1.608e-100. The

PDB code 3FBM (PDB code) (Fig. 1) having sequence identity (99%) and E-value (1.606e-287) [22,23] was selected as reference model for further study.

## 3.2. **Linear/continuous B-cell epitopes prediction**

The complete amino acid sequence of VP2 protein was analyzed for epitope prediction using various tools. Based upon Immunomedicine Group Tool calculation, VP2 protein had an average antigenic propensity score of 1.0329 (Fig. 2). A total of 18 segments (Supplementary Table 1) of VP2 protein had antigenic greater antigenic propensity than average value of 1.0329 and these could be potential epitopes. Similarly, based upon Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools analysis of VP2 protein, 16 (Fig. 3; Supplementary Table 2), 8 (Supplementary Table 3), and 10 (Supplementary Table 4) peptides, respectively were predicted as epitopes.

As the 'consensus method' described by Yang and Yu [15] for the prediction of epitopic peptides with high specificity and sensitivity, complete or partial common peptides predicted by any three or all four tools including Immunomedicine Group, Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools were shorted (Table 1). The peptide 'SYDLGYVRLGDPIPAIGLDPKMVATCDSSDRPRVYTITAADDYQFSSQYQPGGV$^{164-217}$' ($Epitope_L$) had complete or partial region of the predicted polypeptide by all four tools and was considered as the most prominent epitope on VP2 protein of IBDV.

## 3.3. Conformational B-cell epitopes prediction

With the help of Ellipro, conformational B-cell epitopes on VP2 protein (Table 2) were predicted by setting a minimum score of 0.8 and a maximum distance of 6.0 Å. The 3D structure of each of the Ellipro-predicted epitopes, as well as the relative orientation of the protein and peptide molecules, were all visualized in Jmol (Fig. 4). Jmol viewer also verified the placements of each projected epitope's amino acids. The top two highest-score conformational epitopes predicted by Ellipro were ANLNSPLKIAG ($Epitope_C$ 1) and SSQYQPGGRTSVHGLGLTTGTDKSGGQAGDQMS ($Epitope_C$ 2) with a score 0.922 and 0.901, respectively.

## 3.4. T-cell epitope prediction

Many 9-mer peptides on VP2 protein were predicted to bind (strongly as well as weekly) MHC-I alleles (Supplementary Table 5) out of which only four 9-mer peptides i.e. GEGVTVLSL$^{153-161}$, GELVFQTSV$^{236-244}$, YERVATGSV$^{346-354}$, and FELIPNPEL$^{363-371}$ were recognized to strongly binder to MHC-I alleles (Table 3) The peptide GELVFQTSV$^{236-244}$ was predicted to a strong binder to all the three human substitute alleles i.e. HLA-B40:06 HLA-B41:04, and HLA-B41:03, it was considered as strongest MHC-I binding T-cell epitope.

Many 15-mer peptide segments were predicted to bind (strongly as well as weekly) with four MHC-II alleles of the human being (Supplementary Table 6) out of which seven peptides i. e. QQIVPFIRSLLMPTT$^{1-15}$, QMLLTAQNLPASYNY$^{76-90}$, YNYCRLVSRSLTVRS$^{88-102}$, NYCRLVSRSLTVRSS$^{89-103}$, FSANIDAITSLSVGG$^{222-236}$, GGELVFQTSVHGLVL$^{235-249}$, and YTKLILSERDRLGIK$^{389-403}$ were recognized as strongly binder to MHC-II alleles (Table 4). Two peptides i.e. YTKLILSERDRLGIK$^{389-403}$ and QMLLTAQNLPASYNY$^{76-90}$ were predicted to be strong binders to three different out of four human substitute alleles for MHC-II and considered as stronger MHC-II binding T-cell epitope. Moreover, GGELVFQTSVHGLVL$^{235-249}$ (MHC-II binding peptide) also shares a common sequence (GELVFQTSV$^{236-244}$) with the MHC-I binding T-cell epitope (Table 3).

## 4. DISCUSSION

Identifying strong antigenic B cell and T cell epitopic peptides and the prior knowledge of the binding interactions of these peptides with MHC alleles and host immune cells are necessary for developing an effective vaccine against any infection [20]. Epitope identification could also contribute to the development of new diagnostics for infections [24]. In the current study, the identification of epitopic peptides of VP2 protein of IBDV was carried out for the peptide-based vaccine as well as diagnostic development in the future. *In silico* prediction of epitopes

for quick and affordable vaccine designing has been in practice for several diseases such as Hantavirus Cardiopulmonary Syndrome, acne vulgaris, dengue, hepatitis C, etc [20].

In the current study, we analyzed of the VP2 protein sequence of Indian isolate IBDV using the ProtParam program to estimate parameters determining antigenicity of protein molecule like molecular weight, pI, amino acid composition, half-life, and stability [25]. The VP2 protein was a negatively charged 47.518 KDa protein with a 5.12 pI value as it had higher (38 in number) content of negatively charged, hydrophilic, and polar amino acids (Asp + Glu). Hydrophilic amino acids are mainly present on the surface of antigens (Ingale, 2010) as they are accessible to adjoining aqueous environments. The stability of protein can be estimated by calculating the instability index and the protein having an instability index of less than 40 is considered as stable [24]. In present study, instability index valued of 25.64 indicated the stability of VP2 protein.

So far 3D structure of the VP2 protein of Indian isolate IBDV has not been determined. The 3D structure of proteins, for which no structure is available in PDB, can be determined by homology modeling [24]. A total of eight reference templates were found on the PDB database having sequence identity by ranges between 44% and 99%, while E-value range between 3.264e-288 and 1.608e-100. The reference model 3FBM had maximum homology with VP2 protein of Indian isolate IBDV. Therefore, reference model 3FBM was used for the prediction of epitopes.

It is commonly suggested that antigenic B-cell epitopes (linear and conformational) play the foremost role in the immunological response of the host [24]. We used Bepipred Linear Epitope Prediction 2.0, SVMTriP, and BCPred tools for prediction of linear B-cell epitopes. Several linear epitopes on VP2 protein were predicted using above tools. Subsequently, the consensus method [15] was used to short the most potent epitopes. Among several polypeptides, a partial or complete part of $Epitope_L$ polypeptide matched with at least one polypeptide predicted by each of four different linear epitope prediction tools. Hence, $Epitope_L$ was considered as the most potent linear epitope on VP2 protein. Ellipro tool-based prediction indicated $Epitope_C$ 1 and $Epitope_C$ 2 had scores above 0.9. Hence, $Epitope_C$ 1 and $Epitope_C$ 2 were considered the most potent conformational B-cell epitopes.

The binding of antigenic peptides to host MHC-I and MHC-II molecules is an essential prerequisite molecular event to trigger humoral and cell-mediated immune response [24]. For poultry MHC-peptide binding, currently, immune-bioinformatics tools are not available. Human HLA*B 40:06, HLA*B 41:03, and HLA*B 41:04 alleles have similarity greater than 70% with poultry MHC-I. Likewise, human DRB1:1310, DRB1:1366, DRB1:1445, and DRB1:1482 alleles have a similarity greater than 70% with poultry MHC-II [20]. Out of many strong MHC-I binding 9-mer peptides, the peptide GELVFQTSV$^{236-244}$ was predicted as the strongest MHC-I binding T-cell epitope due to its binding ability with all three human substitute MHC-I alleles. Similarly out of many strong MHC-II binding 15-mer peptides, two peptides i.e. YTKLILSERDRLGIK$^{389-403}$ and QMLLTAQNLPASYNY$^{76-90}$ were predicted as strongest MHC-II binding T-cell epitopes due to their binding capacity with three different (out of four) human MHC-II alleles. Moreover, the MHC-II binding GGELVFQTSVHGLVL$^{235-249}$ peptide was also predicted to bind MHC-I as it shared a common sequence (GELVFQTSV$^{236-244}$) with the MHC-I binding epitope.

## 5. CONCLUSION

We predicted $Epitope_L$ as the most prominent linear B-cell epitope on VP2 protein. Two peptide i.e. $Epitope_C$ 1 and $Epitope_C$ 2 were predicted as potent conformational B-cell epitopes. A 9-mer GELVFQTSV$^{236-244}$ peptide was predicted as strongest MHC-I binding peptide while, two 15-mer peptides i.e. YTKLILSERDRLGIK$^{389-403}$ and QMLLTAQNLPASYNY76-90 were as strongest MHC-II binding ability. Moreover, a stronger MHC-II binding GGELVFQTSVHGLVL$^{235-249}$ peptide share common sequence with MHC-I binding GELVFQTSV$^{236-244}$ peptide. These peptide should be assessed for protective immune response by *in vitro* and *in vivo* studies for development of subunit vaccine candidate. After proper validation, these peptides could be used for development of cost-effective and scalable vaccines for IBDV.

## RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

This article does not contain any studies with human participants or animals performed by any of the authors.

## Reference


1. Dey S, Pathak D, Ramamurthy N, Maity HK, Chellappa MM. Infectious bursal disease virus in chickens: prevalence, impact, and management strategies . *Vet Med Res Reports*. 2019;Volume 10:85-97. doi:10.2147/vmrr.s185159

2. Singh NK, Dey S, Madhan Mohan C, Mohan Kataria J, Vakharia VN. Evaluation of four enzyme linked immunosorbent assays for the detection of antibodies to infectious bursal disease in chickens. *J Virol Methods*. 2010;165(2):277-282. doi:10.1016/j.jviromet.2010.02.008

3. Lombardo E, Maraver A, Castón JR, et al. VP1, the Putative RNA-Dependent RNA Polymerase of Infectious Bursal Disease Virus, Forms Complexes with the Capsid Protein VP3, Leading to Efficient Encapsidation into Virus-Like Particles. *J Virol*. 1999;73(8):6973-6983. doi:10.1128/jvi.73.8.6973-6983.1999

4. Castón JR, Martínez-Torrecuadrada JL, Maraver A, et al. C Terminus of Infectious Bursal Disease Virus Major Capsid Protein VP2 Is Involved in Definition of the T Number for Capsid Assembly. *J Virol*. 2001;75(22):10815-10828. doi:10.1128/jvi.75.22.10815-10828.2001

5. Qin Y, Zheng SJ. Infectious bursal disease virus-host interactions: Multifunctional viral proteins that perform multiple and differing jobs. *Int J Mol Sci*. 2017;18(1). doi:10.3390/ijms18010161

6. Liebhart D, Bilic I, Grafl B, Hess C, Hess M. Diagnosing Infectious Diseases in Poultry Requires a Holistic Approach: A Review. *Poultry*. 2023;2(2):252-280. doi:10.3390/poultry2020020

7. Schijns VEJC, van de Zande S, Lupiani B, Reddy SM. Practical Aspects of Poultry Vaccination. In: *Avian Immunology: Second Edition*. ; 2013:345-362. doi:10.1016/B978-0-12-396965-1.00020-0

8. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. *Ann Med*. 2018;50(2):110-120. doi:10.1080/07853890.2017.1407035

9. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide vaccine: Progress and challenges. *Vaccines*. 2014;2(3):515-536. doi:10.3390/vaccines2030515

10. Aguilar-Montes de Oca S, Montes-de-Oca-Jiménez R, Carlos Vázquez-Chagoyán J, Barbabosa-Pliego A, Eliana Rivadeneira-Barreiro P, C. Zambrano-Rodríguez P. The Use of Peptides in Veterinary Serodiagnosis of Infectious Diseases: A Review. *Vet Sci*. 2022;9(10). doi:10.3390/vetsci9100561

11. Kolaskar AS, Tongaonkar PC. A semi-empirical method for prediction of antigenic determinants on protein antigens. *FEBS Lett*. 1990;276(1-2):172-174. doi:10.1016/0014-5793(90)80535-Q

12. Jespersen MC, Peters B, Nielsen M, Marcatili P. BepiPred-2.0: Improving sequence-based B-cell epitope prediction using conformational epitopes. *Nucleic Acids Res*. 2017;45(W1):W24-W29. doi:10.1093/nar/gkx346

13. Yao B, Zhang L, Liang S, Zhang C. SVMTriP: A Method to Predict Antigenic Epitopes Using Support Vector Machine to Integrate Tri-Peptide Similarity and Propensity. *PLoS One*. 2012;7(9). doi:10.1371/journal.pone.0045152

14. El-Manzalawy Y, Dobbs D, Honavar V. Predicting linear B-cell epitopes using string kernels. *J Mol Recognit*. 2008;21(4):243-255. doi:10.1002/jmr.893

15. Yang X, Yu X. An introduction to epitope prediction methods and software. *Rev Med Virol*. 2009;19(2):77-96. doi:10.1002/rmv.602

16. Ponomarenko J, Bui HH, Li W, et al. ElliPro: A new structure-based tool for the prediction of antibody epitopes. *BMC Bioinformatics*. 2008;9. doi:10.1186/1471-2105-9-514

17. Gurung RB, Purdie AC, Begg DJ, Whittington RJ. In silico identification of epitopes in

Mycobacterium avium subsp. paratuberculosis proteins that were upregulated under stress conditions. *Clin Vaccine Immunol*. 2012;19(6):855-864. doi:10.1128/CVI.00114-12

18. Andreatta M, Karosiene E, Rasmussen M, Stryhn A, Buus S, Nielsen M. Accurate pan-specific prediction of peptide-MHC class II binding affinity with improved binding core identification. *Immunogenetics*. 2015;67(11-12):641-650. doi:10.1007/s00251-015-0873-y

19. Thomsen M, Lundegaard C, Buus S, Lund O, Nielsen M. MHCcluster, a method for functional clustering of MHC molecules. *Immunogenetics*. 2013;65(9):655-665. doi:10.1007/s00251-013-0714-9

20. Aziz F, Tufail S, Shah MA, et al. In silico epitope prediction and immunogenic analysis for penton base epitope-focused vaccine against hydropericardium syndrome in chicken. *Virus Res*. 2019;273. doi:10.1016/j.virusres.2019.197750

21. Valdivia-Olarte H, Requena D, Ramirez M, et al. Design of a predicted MHC restricted short peptide immunodiagnostic and vaccine candidate for Fowl adenovirus C in chicken infection. *Bioinformation*. 2015;11(10):460-465. doi:10.6026/97320630011460

22. Irigoyen N, Garriga D, Navarro A, Verdaguer N, Rodríguez JF, Castón JR. *D431N Mutant VP2 Protein of Infectious Bursal Disease Virus; Derived T=1 Particles*.; 2009. doi:10.2210/pdb3fbm/pdb

23. Irigoyen N, Garriga D, Navarro A, Verdaguer N, Rodríguez JF, Castón JR. Autoproteolytic Activity Derived from the Infectious Bursal Disease Virus Capsid Protein. *J Biol Chem*. 2009;284(12):8064-8072. doi:10.1074/jbc.M808942200

24. Vedamurthy GV, Ahmad H, Onteru SK, Saxena VK. In silico homology modelling and prediction of novel epitopic peptides from P24 protein of Haemonchus contortus. *Gene*. 2019;703:102-111. doi:10.1016/j.gene.2019.03.056

25. Abbas A, Lichtman A, Pillai S. No Title. In: *Cellular and Molecular Immunology E-Book. Elsevier Health Sciences*. ; 2014:608.

26 Chowdhury A, Choudhury MD, Laskar MA. An In-silico Study Showing Potentials of Selected Anthocyanin Derivatives against Uropathogenic E. coli Adhesin Protein. S. Asian J. Res. Microbiol. [Internet]. 2022 Jun. 17 [cited 2024 May 16];12(4):15-26. Available from: https://journalsajrm.com/index.php/SAJRM/article/view/230

27. Hedayati M, Manafi M, Yari M, Avara A. The Influence of an Acidifier Feed Additive on Biochemical Parameters and Immune Response of Broilers. Ann. Res. Rev. Biol. [Internet]. 2014 Feb. 7 [cited 2024 May 16];4(10):1637-45. Available from: https://journalarrb.com/index.php/ARRB/article/view/507

28. Berg TP. Acute infectious bursal disease in poultry: a review. Avian pathology. 2000 Jun 1;29(3):175-94.

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**Fig. 1. 3D model of VP2 protein of IBDV.**

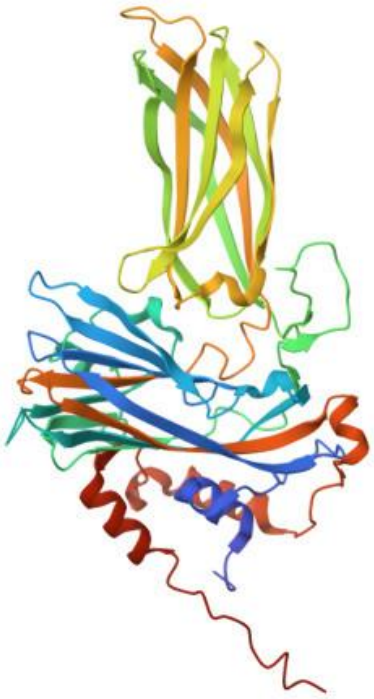

**Fig. 2. Antigenic peptides plot**

The x-axis of plot represents the residues number, and y-axis represents the average antigenic prosperity.

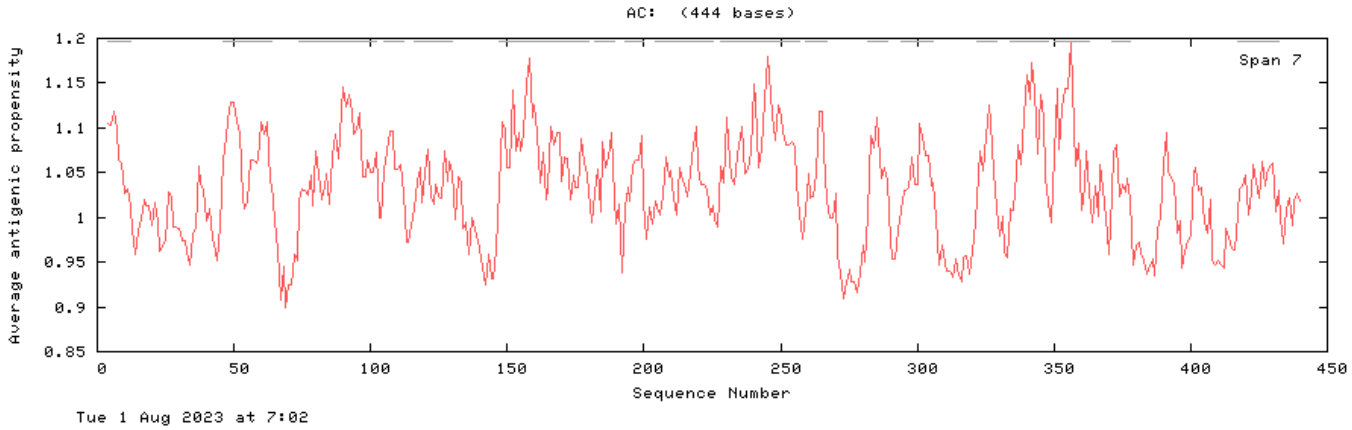

**Fig. 3. The graph of lineal B-cell epitopes of VP2 protein predicted by Bepipred software of IEDB.**

The x-axis of graph represents the position whereas y-axis represents score of predicted amino acids in the sequence. The threshold value for epitopes prediction is 0.5 and the yellow region is representing the linear B-cell epitopes in the VP2 protein.

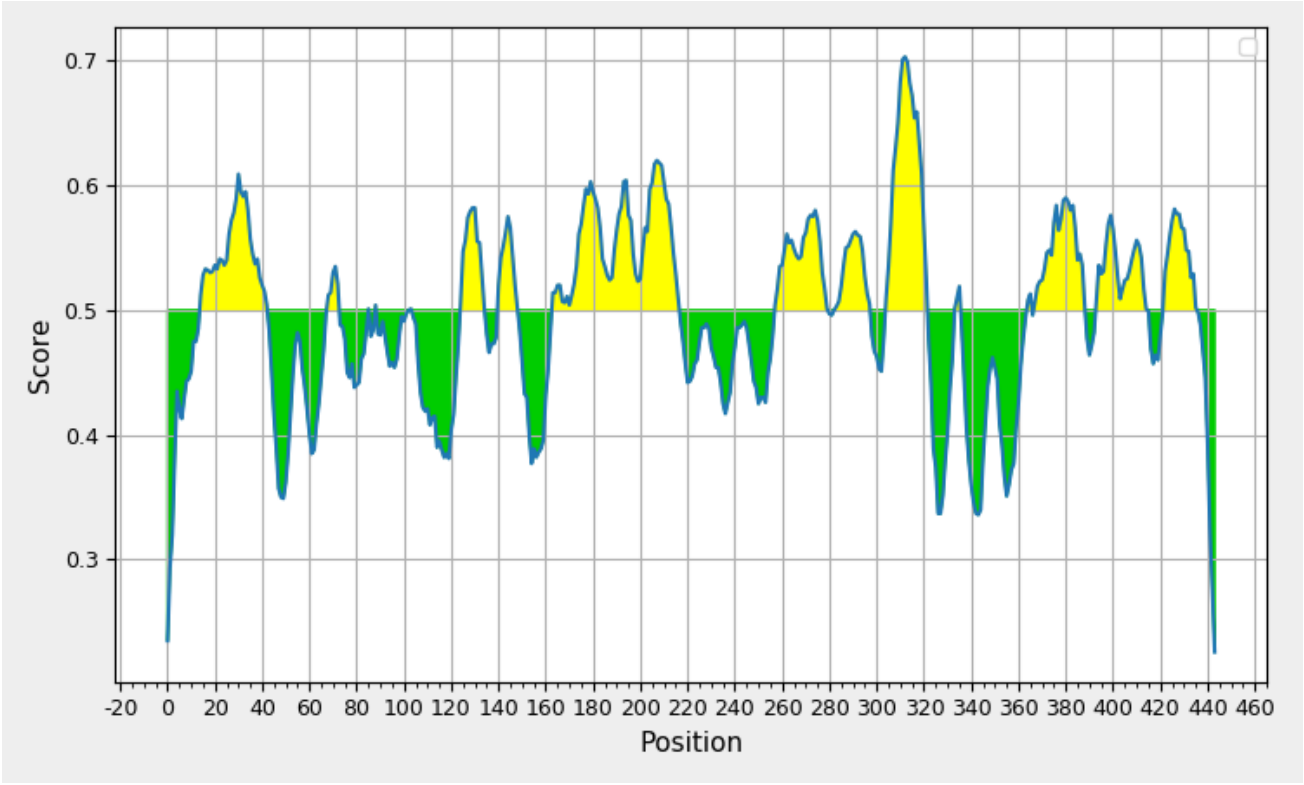

**Fig. 4. Conformational B-cell epitopes of VP2 protein predicted by Ellipro tools**

(A) 11 immunogenic residues with 92.2% of the residues exposed to the environment (B) 33 amino acid residues with a protrusion index of 90.1%. The immunogenic epitopes are depicted as globules on the ball and stick representation of the protein structure.

A B

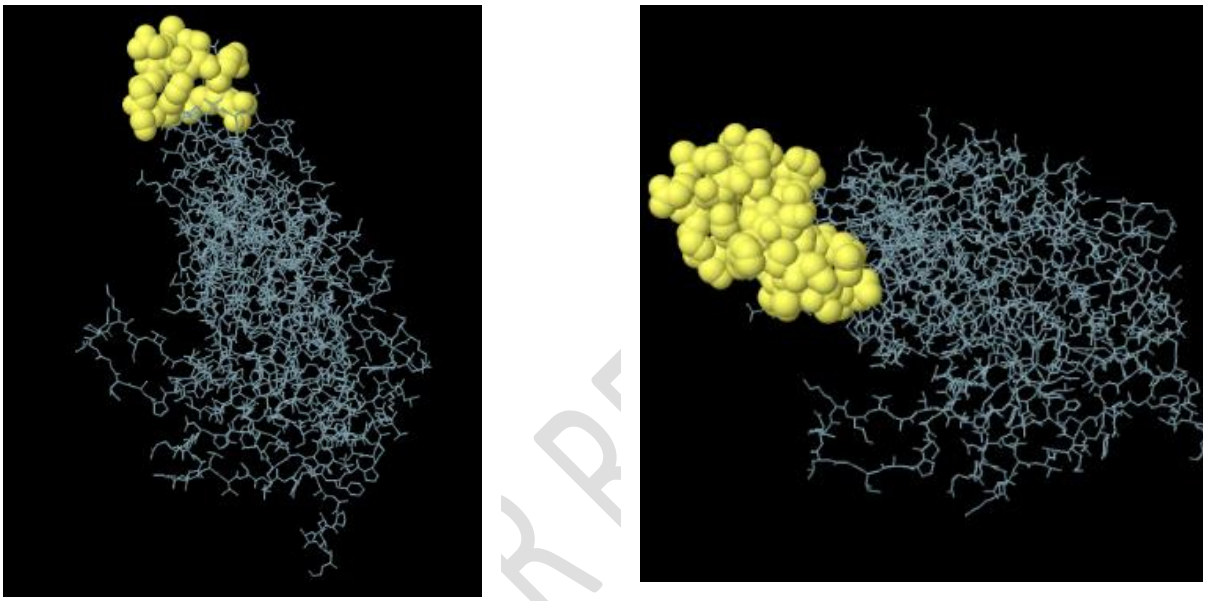

**Table 1: Shorted peptides predicted by any three or all four tools including Immunomedicine Group, Bepipred Linear Epitope Prediction 2.0. SVMTriP, and BCPred tools.**

| Immunomedicine Group Tool | BepiPred - 2.0 | SVMTriP Tool (Score) | BCPred Predictions (Score) |
|---|---|---|---|
| DDYQFSSQYQPGGVTITLFSAN | *SYDLGYVRLGDPIPAIG*LDPKMVATCDSSDRPRV YTITAADDYQFSSQYQPGGV | | AADDYQFSSQYQPGGV (0.958) |
| IGNVLVGEGVTVLSLPT*SYDLGYVRLG DPIPAIG* | | GEGVTVLSLPT*SYDLG* (0.511) | |
| DRPRVYTI | | | |
| DPKMVATC | | | |
| DGTAVITRA | FDGTAVITRAVAANNGLTTGTDN | | RAVAANNGLTTGTDNL (0.996) |
| | TGPASIPDDTLEKHTLRSETSTYNLTVGD | SIPDDTLEKHTLRSET (0.936) | PTTGPASIPDDTLEKH(1.000 ) |
| LAKNLVTE | NPELAKNLVTEYGRFDPGAMNY | | VTEYGRFDPGAMNYTK (0.929) |

**Note:** Same text color, text highlight color, underline color, and encircle color represent common peptides regions among polypeptide predicted by four different bioinformatics tools

**Table 2: Conformational B-cell epitopes on VP2 protein predicted by Ellipro server.**

| S. No | Epitopes | Number of residues | Score |
|---|---|---|---|
| 1 | A:A430, A:N431, A:L432, A:N433, A:S434, A:P435, A:L436, A:K437, A:I438, A:A439, A:G440 | 11 | 0.922 |
| 2 | A:S217, A:S218, A:Q219, A:Y220, A:Q221, A:P222, A:G223, A:G224, A:R249, A:T250, A:S251, A:V252, A:H253, A:G254, A:L255, A:G281, A:L282, A:T283, A:T284, A:G285, A:T286, A:D287, A:K316, A:S317, A:G318, A:G319, A:Q320, A:A321, A:G322, A:D323, A:Q324, A:M325, A:S326 | 33 | 0.901 |
| 3 | A:I184, A:P185, A:A186, A:I187, A:G188, A:L189, A:D190, A:P191, A:K192, A:M193 | 10 | 0.869 |
| 4 | A:V49, A:G50, A:D51, A:T52, A:T112, A:L113, A:P114, A:L120, A:N121, A:G122, A:V357, A:A358, A:T359, A:G360, A:S361 | 15 | 0.857 |
| 5 | A:L33, A:E34, A:K35, A:N376, A:P377, A:E378, A:A380, A:K381, A:N382, A:L383, A:V384, A:T385, A:E386, A:Y387, A:G388 | 15 | 0.824 |
| 6 | A:F390, A:D391, A:P392 | 3 | 0.809 |

**Table 3:** List of the strong MHC-I binders for VP2 protein of IBDV

| Pos | Allele | peptide | 1-log50k* | Affinity (nM) | %Rank# |
|---|---|---|---|---|---|
| 235 | HLA-B40:06 | GELVFQTSV | 0.564 | 111.72 | 0.08 |
| 362 | HLA-B40:06 | FELIPNPEL | 0.454 | 367.39 | 0.30 |
| 345 | HLA-B40:06 | YERVATGSV | 0.411 | 586.38 | 0.50 |
| 235 | HLA-B41:04 | GELVFQTSV | 0.615 | 64.10 | 0.12 |
| 345 | HLA-B41:04 | YERVATGSV | 0.548 | 133.13 | 0.30 |
| 362 | HLA-B41:04 | FELIPNPEL | 0.523 | 174.64 | 0.40 |
| 152 | HLA-B41:04 | GEGVTVLSL | 0.523 | 174.04 | 0.40 |
| 362 | HLA-B41:03 | FELIPNPEL | 0.639 | 49.97 | 0.25 |
| 152 | HLA-B41:03 | GEGVTVLSL | 0.637 | 51.06 | 0.25 |
| 235 | HLA-B41:03 | GELVFQTSV | 0.629 | 55.68 | 0.25 |

*** Predicted binding affinity (nano Molar IC50) in log scale from 0 to 1[20].**

**# Percentage Rank of predicted affinity compared to a set of 200,000 random natural peptides. This measure is not affected by inherent bias of certain molecules towards higher or lower mean predicted affinities[20].**

**Note:** The same text colour of peptide represent common peptide winding capacity with different MHC-I haplotype

**Table 4:** List of the strong MHC-II binders for VP2 protein of IBDV

| Seq | Allele | peptide | 1-log50k | Affinity |
|---|---|---|---|---|
| 388 | DRB1_1482 | YTKLILSERDRLGIK | 0.554 | 125.06 |
| 0 | DRB1_1482 | QQIVPFIRSLLMPTT | 0.550 | 130.07 |
| 88 | DRB1_1366 | NYCRLVSRSLTVRSS | 0.816 | 7.32 |
| 87 | DRB1_1366 | YNYCRLVSRSLTVRS | 0.779 | 10.87 |
| 75 | DRB1_1366 | QMLLTAQNLPASYNY | 0.774 | 11.58 |
| 234 | DRB1_1366 | GGELVFQTSVHGLVL | 0.752 | 14.67 |
| 0 | DRB1_1366 | QQIVPFIRSLLMPTT | 0.751 | 14.76 |
| 388 | DRB1_1366 | YTKLILSERDRLGIK | 0.742 | 16.33 |
| 88 | DRB1_1310 | NYCRLVSRSLTVRSS | 0.808 | 7.97 |
| 87 | DRB1_1310 | YNYCRLVSRSLTVRS | 0.772 | 11.80 |
| 388 | DRB1_1310 | YTKLILSERDRLGIK | 0.719 | 20.90 |
| 75 | DRB1_1310 | QMLLTAQNLPASYNY | 0.719 | 20.90 |
| 221 | DRB1_1310 | FSANIDAITSLSVGG | 0.694 | 25.86 |
| 234 | DRB1_1310 | GGELVFQTSVHGLVL | 0.694 | 27.29 |
| 75 | DRB1_1445 | QMLLTAQNLPASYNY | 0.520 | 180.56 |

**Note:** The same text colour of peptides represents common peptide winding capacity with different MHC-II haplotypes, and text highlight colour of peptides within the table and with the peptide of table 3 represent peptides having binding capacity with both MHC-I/II

**Supplementary Table 1**

| n | Start Position | Sequence | End Position |
|---|---|---|---|
| 1 | 4 | VPFIRSLLM | 12 |
| 2 | 46 | SGLIVFFPGFPGSIVGAHY | 64 |
| 3 | 74 | FDQMLLTAQNLPASYNYCRLVSRSLTVRS | 102 |
| 4 | 105 | LPGGVYAL | 112 |
| 5 | 116 | INAVTFQGSLSELTD | 130 |
| 6 | 147 | IGNVLVGEGVTVLSLPTSYDLGYVRLGDPIPAIG | 180 |
| 7 | 182 | DPKMVATC | 189 |
| 8 | 193 | DRPRVYTI | 200 |
| 9 | 204 | DDYQFSSQYQPGGVTITLFSAN | 225 |
| 10 | 228 | AITSLSVGGELVFQTSVHGLVLGATIYLIG | 257 |
| 11 | 259 | DGTAVITRA | 267 |
| 12 | 282 | MPFNLVIP | 289 |
| 13 | 294 | TQPITSIKLEIVT | 306 |
| 14 | 322 | RGSLAVTI | 329 |
| 15 | 334 | YPGALRPVTLVAYER | 348 |
| 16 | 350 | ATGSVVTVAGVSNF | 363 |
| 17 | 371 | LAKNLVTE | 378 |
| 18 | 417 | YFMEVADLNSPLKIAG | 432 |

**Supplementary Table 2**

| No. | Start | End | Peptide | Length |
|---|---|---|---|---|
| 1 | 15 | 43 | TGPASIPDDTLEKHTLRSETSTYNLTVGD | 29 |
| 6 | 125 | 135 | LSELTDVSYNG | 11 |
| 7 | 141 | 149 | ANINDKIGN | 9 |
| 8 | 164 | 217 | SYDLGYVRLGDPIPAIGLDPKMVATCDSSDRPRVYTITAADDYQFSSQYQPGGV | 54 |
| 9 | 258 | 280 | FDGTAVITRAVAANNGLTTGTDN | 23 |
| 10 | 283 | 298 | PFNLVIPTNEITQPIT | 16 |
| 11 | 305 | 322 | VTSKSGGQAGDQMSWSAR | 18 |
| 14 | 368 | 389 | NPELAKNLVTEYGRFDPGAMNY | 22 |
| 15 | 394 | 415 | LSERDRLGIKTVWPTREYTDFR | 22 |
| 16 | 423 | 436 | DLNSPLKIAGAFGF | 14 |

**Supplementary Table 3**

| Rank | Location | Epitope | Score | Recommend* |
|---|---|---|---|---|
| 1 | 251 - 266 | ATIYLIGFDGTAVITR | 1.000 | |
| 2 | 227 - 242 | DAITSLSVGGELVFQT | 0.942 | |
| 3 | 19 - 34 | SIPDDTLEKHTLRSET | 0.936 | |
| 4 | 90 - 105 | YCRLVSRSLTVRSSTL | 0.823 | |
| 5 | 336 - 351 | GALRPVTLVAYERVAT | 0.630 | |
| 6 | 124 - 139 | SLSELTDVSYNGLMSA | 0.618 | |
| 7 | 389 - 404 | YTKLILSERDRLGIKT | 0.580 | |
| 8 | 153 - 168 | GEGVTVLSLPTSYDLG | 0.511 | |

**Supplementary Table 4**

| Position | Epitope | Score |
|---|---|---|
| 13 | PTTGPASIPDDTLEKH | 1 |
| 266 | RAVAANNGLTTGTDNL | 0.996 |
| 403 | KTVWPTREYTDFREYF | 0.996 |
| 49 | IVFFPGFPGSIVGAHY | 0.995 |
| 327 | VTIHGGNYPGALRPVT | 0.994 |
| 99 | TVRSSTLPGGVYALNG | 0.986 |
| 32 | SETSTYNLTVGDTGSG | 0.984 |
| 202 | AADDYQFSSQYQPGGV | 0.958 |
| 307 | SKSGGQAGDQMSWSAR | 0.951 |
| 376 | VTEYGRFDPGAMNYTK | 0.929 |

**Supplementary Table 5**

| Allele | peptide | BindingLevel |
|---|---|---|
| HLA-B40:06 | GEGVTVLSL | WB |
| HLA-B40:06 | GELVFQTSV | SB |
| HLA-B40:06 | YERVATGSV | SB |
| HLA-B40:06 | FELIPNPEL | SB |
| HLA-B40:06 | REYFMEVAD | WB |
| HLA-B41:03 | GEGVTVLSL | SB |
| HLA-B41:03 | GELVFQTSV | SB |
| HLA-B41:03 | YERVATGSV | WB |
| HLA-B41:03 | FELIPNPEL | SB |
| HLA-B41:03 | REYTDFREY | WB |
| HLA-B41:03 | REYFMEVAD | WB |
| HLA-B41:04 | GEGVTVLSL | SB |
| HLA-B41:04 | SDRPRVYTI | WB |
| HLA-B41:04 | GELVFQTSV | SB |
| HLA-B41:04 | YERVATGSV | SB |
| HLA-B41:04 | FELIPNPEL | SB |
| HLA-B41:04 | REYTDFREY | WB |

**Supplementary Table 6**

| MHC-II | Peptide | BindLevel |
|---|---|---|
| DRB1_1482 | QQIVPFIRSLLMPTT | WB |
| DRB1_1482 | DPIPAIGLDPKMVAT | WB |
| DRB1_1482 | IPAIGLDPKMVATCD | WB |
| DRB1_1482 | DRPRVYTITAADDYQ | WB |
| DRB1_1482 | RPRVYTITAADDYQF | WB |
| DRB1_1482 | PRVYTITAADDYQFS | WB |
| DRB1_1482 | LFSANIDAITSLSVG | WB |
| DRB1_1482 | FSANIDAITSLSVGG | WB |
| DRB1_1482 | SANIDAITSLSVGGE | WB |
| DRB1_1482 | ANIDAITSLSVGGEL | WB |
| DRB1_1482 | ITQPITSIKLEIVTS | WB |
| DRB1_1482 | TQPITSIKLEIVTSK | WB |
| DRB1_1482 | IKLEIVTSKSGGQAG | WB |
| DRB1_1482 | KLEIVTSKSGGQAGD | WB |
| DRB1_1482 | YPGALRPVTLVAYER | WB |
| DRB1_1482 | PGALRPVTLVAYERV | WB |
| DRB1_1482 | RPVTLVAYERVATGS | WB |
| DRB1_1482 | PVTLVAYERVATGSV | WB |
| DRB1_1482 | GVSNFELIPNPELAK | WB |
| DRB1_1482 | VSNFELIPNPELAKN | WB |
| DRB1_1482 | SNFELIPNPELAKNL | WB |
| DRB1_1482 | AMNYTKLILSERDRL | WB |
| DRB1_1482 | MNYTKLILSERDRLG | SB |
| DRB1_1482 | NYTKLILSERDRLGI | SB |
| DRB1_1482 | YTKLILSERDRLGIK | SB |
| DRB1_1482 | TKLILSERDRLGIKT | SB |
| DRB1_1482 | LNSPLKIAGAFGFKD | WB |
| DRB1_1482 | NSPLKIAGAFGFKDI | WB |
| DRB1_1445 | QQIVPFIRSLLMPTT | WB |
| DRB1_1445 | DPIPAIGLDPKMVAT | WB |
| DRB1_1445 | PIPAIGLDPKMVATC | WB |
| DRB1_1445 | IPAIGLDPKMVATCD | WB |
| DRB1_1445 | PAIGLDPKMVATCDS | WB |
| DRB1_1445 | FSANIDAITSLSVGG | WB |
| DRB1_1445 | SANIDAITSLSVGGE | WB |
| DRB1_1445 | TQPITSIKLEIVTSK | WB |
| DRB1_1445 | SIKLEIVTSKSGGQA | WB |
| DRB1_1445 | IKLEIVTSKSGGQAG | WB |
| DRB1_1445 | KLEIVTSKSGGQAGD | WB |
| DRB1_1445 | LRPVTLVAYERVATG | WB |
| DRB1_1445 | RPVTLVAYERVATGS | WB |
| DRB1_1445 | PVTLVAYERVATGSV | WB |
| DRB1_1445 | GVSNFELIPNPELAK | WB |
| DRB1_1445 | VSNFELIPNPELAKN | WB |
| DRB1_1445 | MNYTKLILSERDRLG | WB |

| | | |
|---|---|---|
| DRB1_1445 | NYTKLILSERDRLGI | SB |
| DRB1_1445 | YTKLILSERDRLGIK | SB |
| DRB1_1445 | TKLILSERDRLGIKT | WB |
| DRB1_1366 | DPIPAIGLDPKMVAT | WB |
| DRB1_1366 | PIPAIGLDPKMVATC | WB |
| DRB1_1366 | IPAIGLDPKMVATCD | WB |
| DRB1_1366 | DRPRVYTITAADDYQ | WB |
| DRB1_1366 | RPRVYTITAADDYQF | WB |
| DRB1_1366 | GVSNFELIPNPELAK | WB |
| DRB1_1366 | VSNFELIPNPELAKN | WB |
| DRB1_1366 | SNFELIPNPELAKNL | WB |
| DRB1_1310 | DPIPAIGLDPKMVAT | WB |
| DRB1_1310 | IPAIGLDPKMVATCD | WB |
| DRB1_1310 | DRPRVYTITAADDYQ | WB |
| DRB1_1310 | RPRVYTITAADDYQF | WB |
| DRB1_1310 | GVSNFELIPNPELAK | WB |
| DRB1_1310 | VSNFELIPNPELAKN | WB |
| DRB1_1310 | SNFELIPNPELAKNL | WB |