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Bioinformatics analysis and expression of Erns protein of Bovine Viral Diarrhea Virus*

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Summary

To understand the immunogenicity and biological functions of the Erns protein of Bovine Viral Diarrhea Virus (BVDV) and express its protein, bioinformatics software was initially used for the analysis of BVDV Erns protein (physical and chemical properties, hydrophilicity, B-cell antigen epitopes, transmembrane regions, signal peptides, secondary and tertiary structures). Subsequently, using BVDV nucleic acid as a template, the BVDV-Erns gene was amplified by PCR and cloned into the pMD18-T vector, then transformed into DH5α competent cells to obtain the T-B-Erns recombinant plasmid. After that, both the T-B-Erns plasmid and the pFastBacHTB vector were subjected to double digestion with EcoR I and Hind III, and after ligation, they were again transformed into DH5α competent cells to obtain the H-B-Erns recombinant plasmid. After verification through double digestion, the H-B-Erns plasmid was transformed into DH10Bac competent cells to construct the recombinant shuttle plasmid B-B-Erns. After validation with M13 primer sequencing, it was transfected into Sf9 cells for expression and amplification of the target gene. The expressed recombinant protein was analyzed by Western blot. Results showed that the theoretical molecular weight of the BVDV Erns protein is 25,658.04 Da, with an isoelectric point of 7.77, classifying it as a stable protein. It exhibits strong hydrophilicity. Predictions on transmembrane domains and signal peptide analysis indicate that it is not a channel protein and lacks signal peptide characteristics. Prediction of antigenic epitopes revealed 11 dominant B-cell epitopes, suggesting potential application value in antibody production. Secondary structure analysis indicates that the Erns protein consists mainly of α-helices (41.85%) and random coils (39.21%), supplemented by β-sheets (6.61%) and extended strands (12.33%). The tertiary structure model of the Erns protein matches well with the secondary structure analysis. The Erns protein expressed through the baculovirus system shows a target band around 32 kDa, indicating successful expression of the BVDV-Erns protein, which possesses good immunogenicity, providing a theoretical foundation and material support for further research on this protein and preparation of antibodies.

Keywords: bovine viral diarrhea virus, bioinformatics analysis, baculovirus expression, Erns protein

Bovine viral diarrhea (BVD), caused by the bovine viral diarrhea virus (BVDV), is a significant infectious disease in cattle. BVDV, a single-stranded RNA virus of the *Pestivirus* genus within the Flaviviridae family, has been recognized as a critical pathogen in the global livestock industry since its initial identification in 1946. BVDV can induce a spectrum of complex clinical manifestations, including fever, mucosal lesions, diarrhea, reproductive issues, and immunosuppression (20). Of particular concern is the ability of BVDV to establish persistent infection, wherein infected animals are born with the virus and continuously shed it throughout their lives, serving as primary vectors for

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viral transmission (12). Consequently, persistently infected (PI) animals represent both a critical factor in the propagation of BVDV and a significant challenge in disease control efforts.

Based on their ability to induce cytopathic effects in cells cultured *in vitro*, BVDV strains are categorized into two primary biotypes: cytopathic (CP) and non-cytopathic (NCP) ones. Furthermore, based on sequence variations in the 5' untranslated region (5'-UTR), BVDV can be classified into three distinct genotypes: BVDV-1, BVDV-2, and BVDV-3 (1, 11). The high genetic diversity of BVDV complicates its control and management. Additionally, BVDV has the capability to cross species barriers, infecting other ruminants, such as sheep, goats, and deer, thereby expanding its potential host range and increasing transmission risks (4, 13).

BVDV represents a substantial threat to the global livestock sector, particularly in developing countries, where resource limitations pose significant challenges to the implementation of comprehensive prevention and control strategies. Effective management of BVDV hinges on precise diagnostic methods, well-designed vaccination programs, and stringent biosecurity protocols. While no advancements have been achieved in BVDV research, the ongoing evolution of this pathogen necessitates sustained efforts in research and innovation to enhance prevention and control measures, making it a critical focus in veterinary public health (11, 12).

The structural proteins of BVDV comprise the capsid protein C (P14) and glycoproteins Erns (gP48), E1 (gP25), and E2 (gp53). Notably, the Erns protein plays a pivotal role in several critical stages of viral infection, including virus particle assembly, receptor binding on host cells, and induction of the host immune response (13, 23). Cells infected with BVDV release substantial quantities of soluble Erns into the culture medium (14). Furthermore, the genetic and antigenic characteristics of the Erns protein exhibit high conservation across various isolates, rendering Erns an effective marker for identifying persistently infected (PI) animals (17, 22). Numerous studies have demonstrated that the Erns protein can be detected during the early stages of infection, rendering it an optimal candidate for early diagnosis (14, 15, 17, 22). Early detection is critical for the timely implementation of prevention and control measures, thereby reducing viral transmission. Furthermore, the Erns protein is not only useful for serological testing, but also holds potential for the development of rapid test kits and other antibody-based diagnostic tools. These applications are particularly valuable for large-scale screening and on-site detection (15, 23).

This study employed bioinformatics approaches to analyze the structural and functional characteristics of the BVDV Erns protein, aiming to elucidate its conserved sequences and antigenic properties. Additionally, the recombinant Erns protein was expressed to facilitate the preparation of monoclonal or polyclonal antibodies. The ultimate objective was to provide a robust theoretical foundation for advancing research on the BVDV Erns protein and the development of associated diagnostic kits.

Material and methods

Strain, plasmid, and expression vector. The BVDV strain, pFastBacHTB plasmid, Bacmid-GFP positive plasmid, and Sf9 cells were kindly provided by Kunming Customs Technical Center. The pMD18-T vector (TaKaRa, 6011, Dalian, China), DH5α competent cells (TaKaRa, 9057, Dalian, China), and DH10Bac competent cells (Veditek, DL1071S, Shanghai, China) were procured from Kunming Fengke Biotechnology Co., Ltd.

Reagents. TaKaRa MiniBEST Plasmid Purification Kit Ver.4.0 (TaKaRa, 9760, Dalian, China), TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 4.0 (TaKaRa, 9762, Dalian, China), Virus DNA/RNA Extraction Kit (4.0) (Tianlong, T038, Xi'an, China), PrimeScript™ One Step RT-PCR Kit Ver.2 (Dye Plus) (TaKaRa, RR057A, Dalian, China), TaKaRa LA Taq (TaKaRa, RR02MA, Dalian, China), Restriction endonuclease Hind III (TaKaRa, 1060, Dalian, China), EcoR I (TaKaRa, 1040, Dalian, China), DNA Ligation Kit Ver. 2.1 (TaKaRa, 6022Q, Dalian, China), His-tag antibody (TianGen, AB102, Beijing, China), Sheep anti-mouse HRP-IgG (Boster, BA1050, Wuhan, China), High-efficiency RIPA Lysis Buffer for Tissue/Cell (Sorlabio, R0010, Beijing, China), SDS-PAGE Gel Rapid Preparation Kit (Biosharp, BL522A, Hefei, China), Ultra-Sensitive ECL Chemiluminescence Kit (Beyotime, P0018S, Shanghai, China), Cellfectin II Transfection Kit (Invitrogen, 10362-100, California, USA), Grace's Insect Medium (Gibco, 11605102, California, USA). All the above reagents were purchased from Kunming Fengke Biotechnology Co., Ltd.

Bioinformatics analysis of the BVDV Erns gene. Based on the BVDV Erns gene sequence (GenBank accession number: MF278652.1), the corresponding amino acid sequence was retrieved. The alignment between the amino acid and nucleotide sequences was conducted using the SnapGene software. The physicochemical properties of the protein, including its molecular weight, theoretical isoelectric point, and amino acid composition, were analyzed using the ProtParam tool (https://web.expasy.org/protparam/). The hydrophilicity/hydrophobicity profile of the protein was predicted using the ProtScale tool (http://web.expasy.org/protscale/). Potential B-cell epitopes were identified using the Immune Epitope Database (IEDB) B-cell epitope prediction tool (http://tools.iedb.org/main/bcell/). Transmembrane domains were predicted using TMHMMServerv2.0 (https://services.healthtech.dtu.dk/service.php?DeepTMHMM-1.0). The presence of a signal peptide was evaluated using Novopro (https://www.novopro.cn/tools/signalp). The secondary structure of the protein was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsaautomat.pl?page=npsaso), while its tertiary structure was modeled using Phyre2 (https://www.sbg.bio.ic.ac.uk/phyre2).

Primer design and synthesis. Based on the BVDV Erns gene sequence, a pair of primers was designed using DNAMAN and SnapGene software: F: 5'-CCGGAATTC-CAGAAAACATAACACAGTGGAACC-3' (EcoR I); R: 5'-CCCAAGCTTAGCGTATGCTCCAAACCAC-3' (Hind III). The underlined sequences denote the respective restriction enzyme recognition sites. In accordance with the Bac-to-Bac baculovirus expression system user manual, the universal M13 primers for identifying the DH10Bac bacmid were synthesized as follows: F: 5'-GTTTTCCCAGTCAC-GAC-3'; R: 5'-CAGGAAACAGCTATGAC-3'. All primers were synthesized by Beijing Qingke Biotechnology Co., Ltd.

Amplification of the target gene. Nucleic acid extraction from BVDV samples was performed using an automated nucleic acid extraction instrument. Subsequently, RT-PCR amplification was conducted using Erns-specific primers, with the extracted BVDV nucleic acid serving as the template. The reaction mixture comprised 12.5 µL of 2 × One Step Buffer, 4.5 µL of dd H_2O, 5 µL of nucleic acid template, 1 µL of PrimeScript RT Enzyme Mix, and 1 µL each of forward and reverse primers (10 µmol/L). The amplification protocol was as follows: incubation at 50°C for 30 minutes; initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation (95°C for 30 seconds), annealing (52°C for 30 seconds), and extension (72°C for 45 seconds); and a final extension at 72°C for 10 minutes. Following amplification, the single PCR product band was subjected to sequencing analysis.

Construction of the cloning vector. The PCR products purified from the gel were ligated with the pMD18-T vector in a total reaction volume of 10 µL, comprising 4 µL of gel-purified PCR products, 1 µL of pMD18-T vector, and 5 µL of Solution I. After gentle mixing, the ligation reaction was incubated at 16°C for 2 hours. The resulting ligation products were subsequently transformed into competent *Escherichia coli* DH5α cells via heat shock. The transformed cells were then cultured in LB broth supplemented with 100 µg/mL ampicillin at 37°C with shaking at 180 rpm for 1.5 hours before being plated onto LB agar containing 100 µg/mL ampicillin for overnight incubation. On the following day, individual colonies were selected and cultured in LB broth supplemented with 100 µg/mL ampicillin at 37°C with shaking at 180 rpm for approximately 15 hours. Plasmids were extracted using a mini-prep plasmid extraction kit and verified by PCR. The confirmed recombinant plasmids were submitted for sequencing analysis. The sequencing results were compared against the known sequences in the NCBI database. Following verification, the recombinant plasmid was designated as T-B-Erns. The PCR product was subjected to double digestion using EcoR I and Hind III enzymes, followed by ligation with the pFastBacHTB vector that had undergone an identical enzymatic treatment. The resulting construct was then transformed into DH5α competent cells. Colonies were screened and analyzed using the same methodology as described above. Positive clones were identified and expanded for extraction of the recombinant donor plasmid H-B-Erns. Plasmid integrity was confirmed via double digestion (H-B-Erns 5 µL, EcoR I 1 µL, Hind III 1 µL, 10 × H Buffer 2 µL, dd H_2O 1 µL, total volume 10 µL). Plasmids with positive identification results were promptly stored at –80°C.

Baculovirus construction. The H-B-Erns plasmid was transformed into DH10Bac competent cells. The resulting bacterial suspension was spread onto LB agar plates supplemented with 50 µg/mL kanamycin, 7 µg/mL tetracycline, 10 µg/mL gentamicin, 100 µg/mL IPTG, and 40 µg/mL X-gal. The plates were incubated in an incubator at a constant temperature of 37°C for 48 hours. Well-formed, isolated white colonies were selected and inoculated into LB broth supplemented with 50 µg/mL kanamycin, 7 µg/mL tetracycline, and 10 µg/mL gentamicin. The cultures were incubated in a shaker at a constant temperature of 37°C for 13 hours. The recombinant shuttle plasmid B-B-Erns was extracted using an endotoxin-free plasmid extraction kit. The presence of the target gene was confirmed by PCR amplification using the recombinant plasmid as the template and M13 primers. The reaction system was composed as follows: 10 × LA Taq Buffer II (5 µL), TaKaRa LA Taq (0.5 µL), dNTP Mixture (8 µL), M13 forward primer (1 µL), M13 reverse primer (1 µL), bacterial lysate (5 µL), and dd H_2O (29.5 µL), resulting in a total volume of 50 µL. The PCR reaction protocol was as follows: an initial denaturation at 94°C for 4 minutes followed by 35 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 5 minutes; a final extension at 72°C for 10 minutes; and storage at 4°C.

Virus production and amplification. In accordance with the protocol outlined in the Invitrogen Bac-to-Bac Baculovirus Expression System manual, plasmid extraction kits were used to isolate the B-B-Erns and Bacmid-GFP. Subsequently, the bacmid was transfected into Sf9 cells using Cellfectin Reagent liposomes. Notably, the transfection of Bacmid-GFP served as a positive control, while untransfected Sf9 cells constituted the negative control group. Sf9 cells in the logarithmic growth phase were seeded into 6-well plates at a density of approximately 1×10^6 cells per well and cultured for 16 h at 27°C. The culture medium was aspirated from the wells, and the cells were washed 1 or 2 times with serum-free Grace's medium. Subsequently, 2 mL of serum-free Grace's medium was added to each well, and the cells were incubated at 27°C for 1 hour. For each well transfection sample, the complexes were prepared as follows: plasmid DNA was diluted in 100 µL of unsupplemented Grace Insect Medium (without antibiotics and serum), and 9 µL of Cellfectin II was mixed in 100 µL of unsupplemented Grace Insect Medium (without antibiotics and serum). To facilitate the self-assembly of the liposome-DNA complex, it was vortexed briefly to mix and incubated at room temperature for 45 minutes. Then, 800 µL of serum-free medium was added to dilute the solution to 1 mL to obtain the transfection complex. The original medium was removed from the cell culture plate, the transfection complex was added slowly, and incubation was performed at 27°C for 5 hours. After transfection, the medium was replaced with complete medium containing 10% fetal bovine serum, and culturing was continued until 80% to 90% of the cells showed virus-induced lesions. The

Sf9 cells displaying severe cytopathic effects were harvested along with the culture supernatant, and three cycles of freeze-thawing were performed, followed by centrifugation at 3000 rpm for 5 minutes to eliminate cellular debris and intact cells. The resulting supernatant was collected as the viral seed stock. This stock was used to infect fresh Sf9 cells, and incubation was performed at 27°C for 120 hours. Subsequently, the second (P2), third (P3), and fourth (P4) generations of recombinant viruses were obtained by the same procedure.

Protein analysis and verification. Using a highly efficient cell lysis buffer, 20 mL suspensions of both the P4 generation recombinant virus-infected cells and the normal Sf9 cells were lysed separately. Subsequently, the lysed cell suspensions were centrifuged at 500 to 1000 rpm for 5 minutes at 4°C. The supernatants were carefully aspirated and discarded. The cell pellets were resuspended in pre-chilled PBS, and the centrifugation step was repeated twice to ensure complete removal of residual culture medium components. A total of 100 µL of pre-cooled RIPA lysis buffer supplemented with 1% PMSF was added to the cell pellet and gently vortexed to ensure thorough mixing. The mixture was incubated on ice for 30 minutes and occasionally vortexed gently to promote complete lysis. The sample was centrifuged at 12,000 rpm for 15 minutes at 4°C, and the supernatant containing the target protein was carefully collected. The supernatant was subjected to SDS-PAGE using a 15% separating gel and a 4% stacking gel. Following electrophoresis, the protein bands were electrotransferred onto a PVDF membrane. The membrane was then blocked with 5% BSA for 1 hour at room temperature. Subsequently, it was washed three times with 1 × TBST for 5 minutes each and incubated overnight at 4°C with a mouse anti-His tag antibody diluted at a ratio of 1 : 5000. After washing three times with 1 × TBST for 5 minutes each to remove unbound primary antibody, the membrane was incubated with a goat anti-mouse IgG HRP-conjugated secondary antibody (diluted at 1 : 5000) in a shaker at room temperature for 1 hour. Finally, the membrane was washed three times with 1 × TBST for 5 minutes each to eliminate any unbound secondary antibody. The ECL chromogenic reagent was prepared according to the manufacturer's instructions and applied uniformly onto the membrane. The membrane was allowed to incubate for 1 to 2 minutes to facilitate the reaction between the chemiluminescent substrate and HRP. Subsequently, the membrane was placed in a light-tight container, and a chemiluminescence imaging system was used to expose and capture the image. Finally, the image obtained was documented.

Results and discussion

Physicochemical characteristics analysis. The fundamental physicochemical characteristics of the BVDV Erns protein were analyzed using the online software ProtParam. The results indicate that the Erns gene of the BVDV reference strain is 681 base pairs in length, encoding a polypeptide chain of 227 amino acids. The molecular formula of this protein is $C_{1123}H_{1747}N_{325}O_{338}S_{14}$, with a calculated molecular weight of 25,658.04 Da. It comprises a total of 3,547 atoms and has an isoelectric point (pI) of 7.77. The extinction coefficient is 1.957, and the instability index is 33.29, classifying this protein as a stable protein. Additionally, it has a grand average of hydropathicity (GRAVY) value of –0.548 and a fat index of 69.60.

Hydrophilic/hydrophobic prediction. The hydrophilic and hydrophobic properties of the BVDV Erns protein were analyzed using the ProtScale online software. The results, as illustrated in Figure 1, indicate that the majority of the scores for the Erns protein are negative, suggesting a pronounced hydrophilic nature.

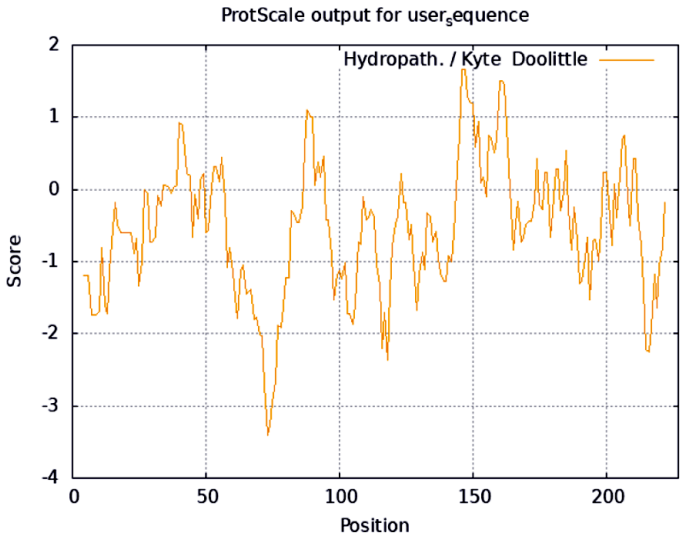

Fig. 1. Protein hydrophilicity and hydrophobicity

Protein domain prediction. The transmembrane domains of the protein were predicted using the TMHMMServer v. 2.0 online software. The analysis revealed that this particular protein fragment does not contain any transmembrane regions, as illustrated in Figure 2.

Protein signaling peptide prediction. Using the NovoPro online software for predicting protein signal

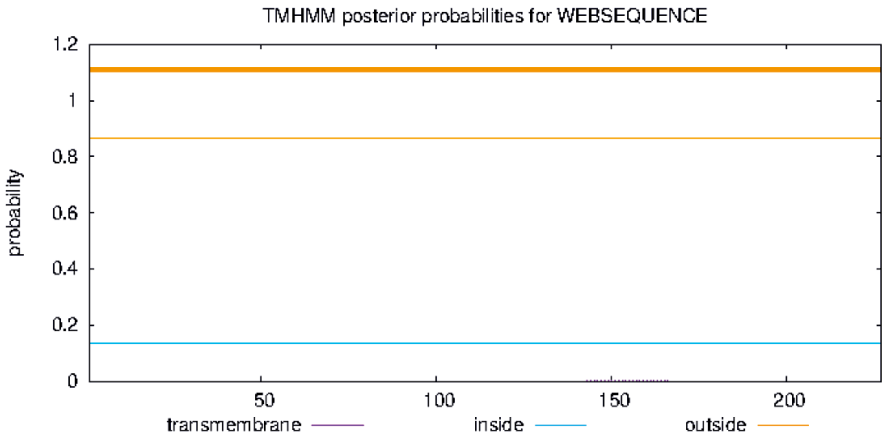

Fig. 2. Protein domain structure

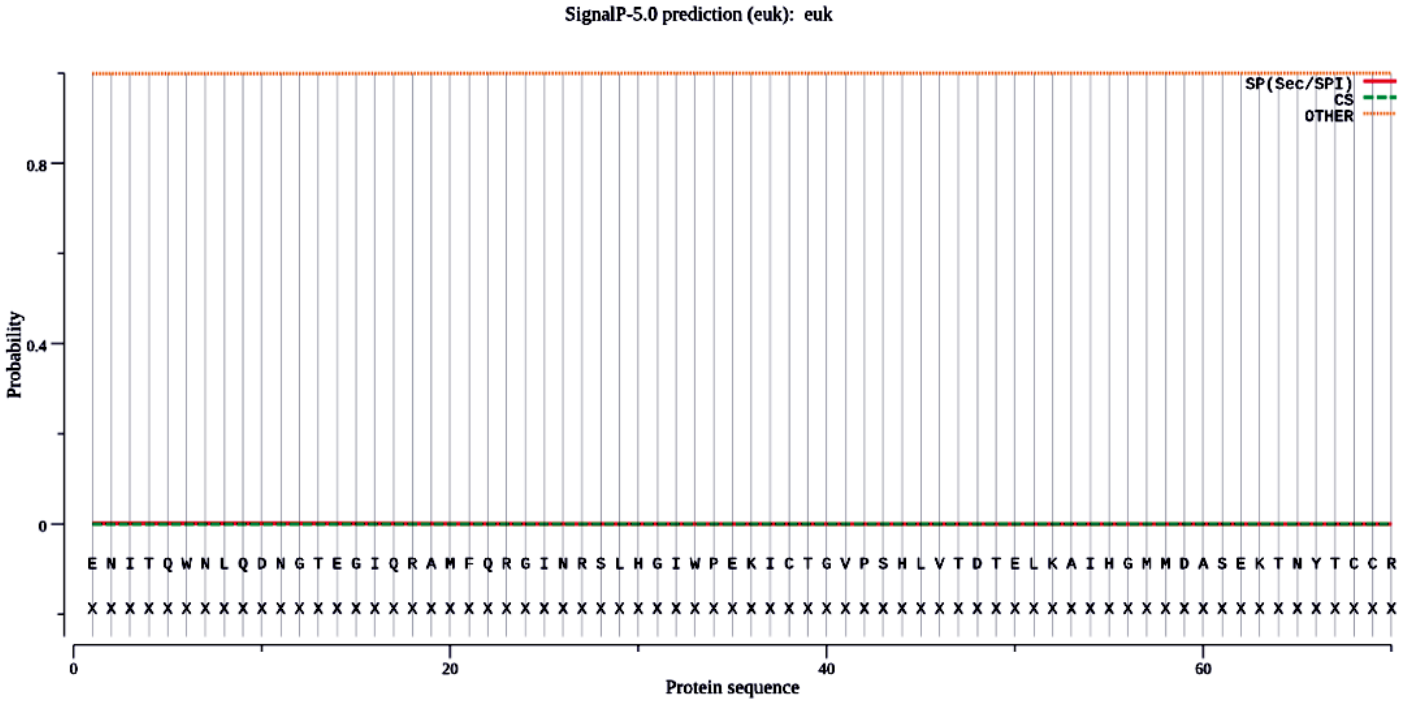

Fig. 3. Signal peptide prediction

Tab. 1. B cell epitope prediction

No.	The starting position of amino acids	The termination position of amino acids	Peptide fragment	bp
1	5	15	QWNLQDNGTEG	11
2	25	33	INRSLHGIW	9
3	39	52	TGVPSHLVTDTELK	14
4	58	67	MDASEKTNYT	10
5	70	82	RHQRHEWNKHGWC	13
6	98	107	QANLTEGQPP	10
7	117	144	DRDSDLNVVTQARDSPTPLTGCKKGKNF	28
8	155	158	CNFE	4
9	165	172	VLFKEHDCT	9
10	191	196	ESARQG	6
11	217	223	NKSKTWF	7

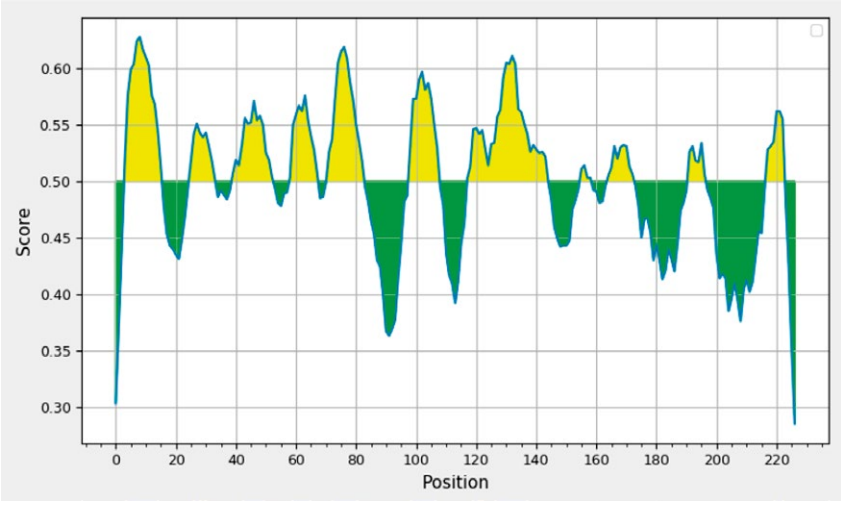

Fig. 4. Erns antigenicity prediction

peptides, it was determined that the probability of this full-length protein containing a signal peptide is 0.115% (Fig. 3).

Protein B cell epitope prediction. The epitope prediction analysis of the Erns amino acid sequence was performed using the IEDB online software. The results revealed the presence of 11 B-cell dominant antigenic epitopes (Tab. 1). The antigenicity profile of the protein is illustrated in Figure 4, where the X-axis denotes the position of the amino acids, and the Y-axis represents the corresponding antigenicity scores. A threshold of 0.5 was set; higher values indicate stronger antigenicity at specific positions. The yellow intervals highlight regions predicted to contain antigenic epitopes, covering 52.9% of the total amino acids within the entire protein peptide segment. Based on this analysis, it can be inferred that the Erns protein exhibits antigenic characteristics, suggesting its potential utility in the development of diagnostic reagents. However, further research is required to comprehensively understand its potential and limitations across different contexts.

Analysis of protein secondary and tertiary structures. The secondary structure of the Erns protein was analyzed using online bioinformatics tools, which revealed that it comprises 90 α-helices, 29 extended strands, 15 β-sheets, and 89 random coils. Specifically, α-helices constitute 41.85%, extended strands ac-

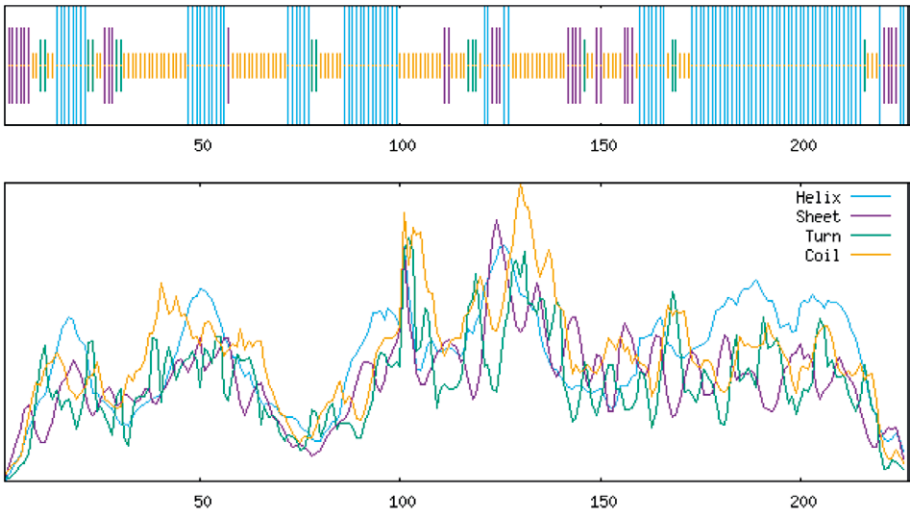

Fig. 5. Erns protein secondary structure
Explanations: Helix – α-helix; Sheet – β-sheet; Turn – β-turn, Coil – Random coil

count for 12.33%, β-sheets represent 6.61%, and random coils make up 39.21% of the total structure (Fig. 5). The tertiary structure model of the BVDV Erns protein was constructed using online software (Fig. 6). The left side is predominantly composed of yellow β-sheets, forming a relatively planar domain.

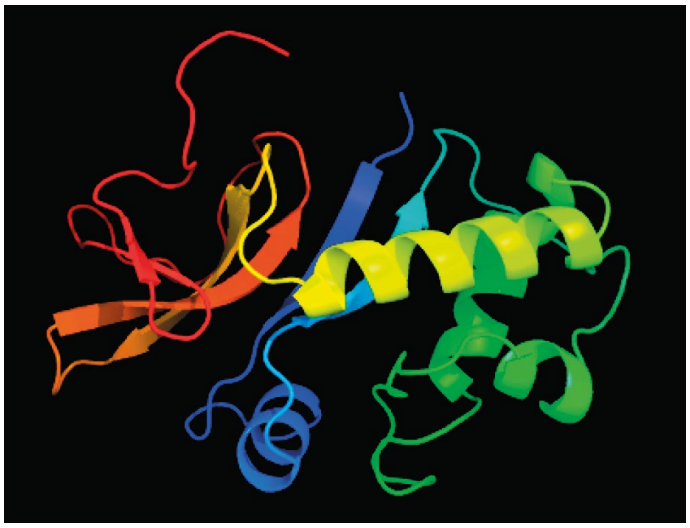

Fig. 6. Tertiary structure of the Erns protein
Explanation: Image coloured by rainbow N→C terminus

In contrast, the right side consists of blue and green α-helices, constituting a more compact helical domain. The central region contains random coils (orange) and turns (red), which serve to provide connectivity and flexibility. The structural analysis of the protein elucidates its intricate compositional characteristics. Integration of secondary structure predictions with tertiary structure models reveals that that this protein contains diverse secondary structure elements, including α-helices (depicted in blue), β-sheets (highlighted in purple), turns (marked in green), and random coils (shown in orange). These elements are extensively distributed within the amino acid sequence and exhibit distinct patterns. For instance, the region spanning residues 50~70 predominantly consists of random coils, whereas the segment from residues 80~100 encompasses both α-helices and β-sheets. The three-dimensional structural model reveals that these secondary structure elements interact to form a compact and organized three-dimensional conformation, where α-helices and β-sheets serve as the primary supporting structures. This endows the protein with its distinctive shape and functional characteristics. Collectively, this structural arrangement not only underscores the high complexity of the Erns protein, but also highlights its importance in executing specific functions within the organism.

Amplification results of the target gene. The BVDV Erns gene was successfully amplified using specific primers. The resulting PCR products were analyzed via agarose gel electrophoresis, revealing a distinct band at approximately 681 bp. This size is consistent with the expected length of the gene fragment, thereby confirming accurate amplification of the target gene (Fig. 7). The target fragments that are consistent can still be obtained after gel extraction (Fig. 8).

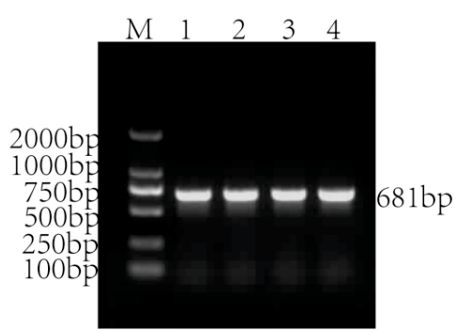

Fig. 7. Identification of gene fragments amplified by RT-PCR
Explanations: M – DL2000 Maker, 1-4 – RT-PCR product of BVDV-Erns

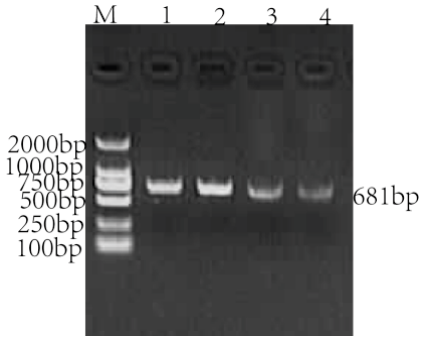

Fig. 8. Gel extraction of BVDV-Erns
Explanations: M – DL2000 Maker; 1-4 – Gel recovery product of BVDV-Erns

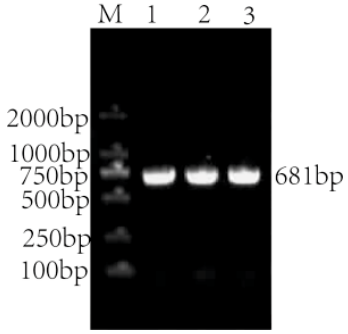

Fig. 9. PCR-amplified fragments
Explanations: M – DL2000 Maker; 1 – Colony 1; 2 – Colony 2; 3 – Colony 3

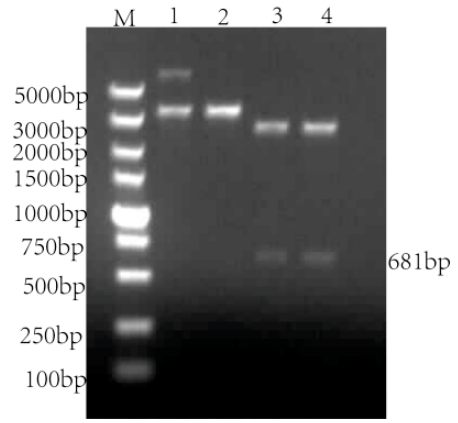

Fig. 10. Identification of recombinant expression plasmid by double enzyme digestion
Explanations: M – DL2000 Maker; 1 – Single enzyme digestion with EcoR I; 2 – Single enzyme digestion with Hind III; 3-4 – Double enzyme digestion with EcoR I and Hind III

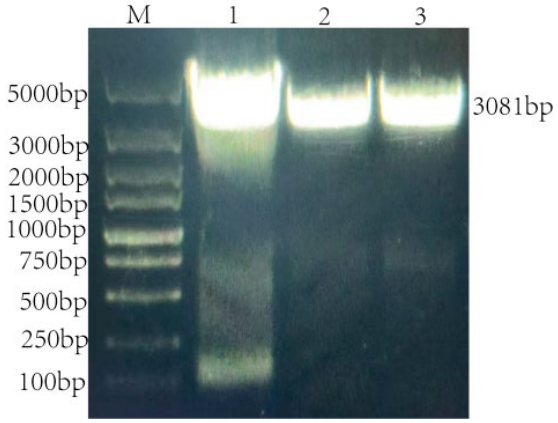

Fig. 11. Verification by the M13 primer
Explanations: M – DL5000 Maker; 1 – Positive control for Bacmid; 2 – Bacmid-BVDV-Erns 1; 3 – Bacmid-BVDV-Erns 2

Amplification of the target gene. The recombinant plasmid T-B-Erns served as the template for bacterial liquid PCR identification. The results revealed a clear band at 681 bp, which matched the expected size of the amplified fragment. This confirms the successful construction of the recombinant plasmid T-B-Erns (Fig. 9).

After double digestion of the PCR product and the pFastBacHTB vector with EcoR I and Hind III enzymes, ligation was performed, followed by identification with double digestion. Gel electrophoresis results showed a target gene band at 681 bp (Fig. 10), consistent with the expected size. The results of PCR, digestion, and sequencing confirmed that the size and sequence of the exogenous target fragment were correct, indicating successful construction of the recombinant donor plasmid. The positive plasmid was named H-B-Erns.

Identification of recombinant plasmids. Following the transfer of the H-B-Erns recombinant donor plasmid into DH10Bac competent cells, the cells were cultured on selective plates containing gentamicin, kanamycin, and tetracycline for 48 to 96 hours to facilitate screening. Subsequently, individual white colonies were isolated and expanded. PCR identification was performed using M13 primers. Successful transformation of DH10Bac strains with the plasmid was confirmed by the presence of a 3081 bp band, whereas untransformed DH10Bac strains exhibited a 300 bp band. As illustrated in Figure 11, a distinct band at 3081 bp was observed, confirming the successful transposition of the target fragment into Bacmid and the subsequent construction of the recombinant plasmid B-B-Erns.

Virus production and amplification. The recombinant shuttle plasmid was extracted and transfected into Sf9 cells in the logarithmic growth phase using liposome-mediated transfection. A blank control group was established, and all cells were cultured at 27°C. Microscopic observations revealed that after 120 hours of culture, the Sf9 cells transfected with B-B-Erns exhibited significant cytopathic effects. Compared to the normal cells (Fig. 12A), the plasmid-transfected cells appeared markedly enlarged and rounded, with signs of lysis (Fig. 12B). The positive control transfection demonstrated fluorescence under an inverted fluorescence microscope (Fig. 12C).

Expression of recombinant proteins. The lysate supernatant from P4 recombinant virus-infected Sf9 cells and the supernatant from uninfected control Sf9 cells were analyzed by SDS-PAGE using a 15% separating gel and a 4% stacking gel. Western blot analysis revealed that the recombinant protein was specifically

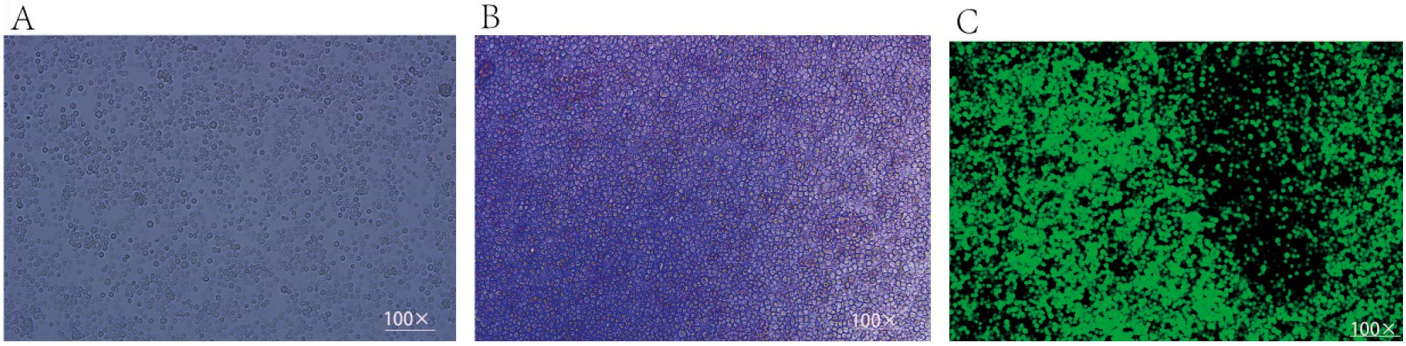

Fig. 12. Transfection cells
Explanations: A – Blank group; B – Sf9 cells transfected with B-B-Erns; C – Sf9 cells transfected with Bacmid-GFP

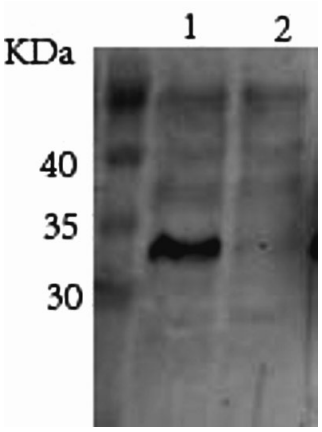

Fig. 13. Western blot analysis of Erns gene expression products
Explanations: 1 – Supernatant after cell lysis by P4 recombinant virus infection; 2 – Normal Sf9 cells

recognized by an anti-His tag antibody, with a distinct target band appearing at approximately 32 kDa. This molecular weight is consistent with the BVDV-Erns protein expressed in Chinese hamster ovary (CHO) cells, as reported by Li Yajun et al. (10), but exceeds the theoretical value, which may be attributed to post-translational modifications (16). No specific bands were detected in the supernatant of uninfected cells. These findings confirm the successful expression of the Erns recombinant plasmid in Sf9 cells.

BVD is a significant infectious disease affecting cattle, resulting in substantial economic losses for the global cattle industry. BVDV evades the host's innate antiviral immune responses by inducing extensive apoptosis of monocytes and inhibiting the interferon-mediated antiviral response, thereby facilitating viral persistence. While numerous potential mechanisms of BVDV-host interactions have been elucidated, the precise mechanisms governing viral replication, pathogenicity, and evasion of the host's innate immunity warrant further investigation. The Erns protein plays a pivotal role in both the replication of BVDV and the evasion of the host's immune responses, rendering its detection not only an indicator of viral infection, but also a valuable source of information regarding viral activity and the host's immune status (22). Given its immunogenicity, specificity, and essential function in the viral life cycle, the Erns protein is considered a promising antigen for BVDV detection. Bioinformatics analysis serves as an indispensable tool for gaining a comprehensive understanding of gene mechanisms and potential functions. It not only enhances our comprehension of gene structure and function, but also provides a robust scientific foundation for developing disease diagnosis and prevention strategies. By conducting bioinformatics analysis on the Erns gene, we can achieve a deeper insight into the structure, function, high conservation, and specificity of its protein. This approach enables us to accurately predict the three-dimensional structure of the Erns protein and identify its key functional domains, which is critical for elucidating its RNase activity and its role in the viral life cycle (8, 21). Furthermore, through the implementation of bioinformatics analysis to compare gene sequences across various strains, it is possible to achieve rapid and precise identification of viral strains (3, 5, 18, 19). Based on the variability of the Erns gene, researchers can design more effective diagnostic methodologies, develop specific antibody detection assays, or create subunit vaccines targeting the antigenic epitopes of the Erns protein. Studies have demonstrated that the Erns protein effectively induces the host to produce specific antibodies with high specificity, facilitating the differentiation of BVDV infection from other pathogen infections (17). During the early stages of infection, the Erns protein typically appears in the host earlier than other markers, making it a valuable antigen for detection. This is essential for early diagnosis of the disease and timely implementation of preventive and control measures. As a structural protein, Erns has been established as an essential component for the development of BVDV gene-engineered subunit vaccines and diagnostic antigens. For example, Kuhne et al. (9) successfully detected BVDV antigens in bovine ear tissue samples using antigen capture enzyme-linked immunosorbent assay (ACE), thereby validating that the Erns ELISA is as a sensitive and reliable detection method. Furthermore, a study conducted by Jaruwan Kampa et al. (7) demonstrated that the Erns capture ELISA exhibits superior applicability compared to the indirect immunoperoxidase assay (IPX) for detecting heat-inactivated samples and testing PI animals at an early age. Consequently, it can effectively serve as a viable alternative to IPX. Furthermore, research conducted by Todd E. Cornish (2) demonstrated that the antigen ELISA method targeting the Erns protein exhibited a relatively high degree of accuracy in testing skin biopsy samples (ear notches) for the presence of BVDV in PI cattle. E. Grego et al. (6) developed an ELISA for detecting antibodies against the BVDV Erns protein using a baculovirus expression system. They reported that the results obtained by this method exhibited high concordance with those from the NS2/3 competitive ELISA in three infected swine farms. Seyfi Abad Shapouri MR et al. (15) generated monoclonal antibodies against recombinant Erns by utilizing BVDV Erns as an immunogen, further validating its potential for laboratory diagnosis of BVDV. Therefore, antigen detection methods based on the Erns protein hold significant promise for monitoring the prevalence of BVDV PI animals in China.

This study conducted a comprehensive analysis of the BVDV Erns protein, revealing its significant physicochemical properties and structural characteristics. The Erns gene encodes a protein consisting of 227 amino acids, with a molecular weight of 25,658.04 Da and an isoelectric point of 7.77, classifying it as a stable

protein. This protein exhibits strong hydrophilicity, is not a channel-forming protein, and lacks signal peptide characteristics. Antigenic epitope prediction identified 11 B-cell dominant epitopes, with 52.9% of the amino acids demonstrating strong antigenicity. These findings suggest that the Erns protein holds potential for antibody production applications. Secondary structure analysis reveals that the Erns protein primarily consists of α-helices (41.85%) and random coils (39.21%), with minor contributions from β-sheets (6.61%) and extended strands (12.33%). The tertiary structure model of the Erns protein further elucidates its complexity, wherein α-helices and β-sheets serve as the principal scaffolding elements, collectively forming a three-dimensional conformation. The central region is characterized by random coils and turns, which function as linkers between distinct domains, imparting flexibility to the protein and playing a crucial role in its biological activity. Furthermore, the hydrophilic characteristics and distribution of antigenic epitopes on the protein highlight its potential utility in antigen-antibody interactions. Consequently, the distinctive structural attributes and biochemical properties of the Erns protein underscore its pivotal role in executing biological functions and reveal its promising application prospects in antigen-antibody reactions. This provides a critical theoretical foundation for elucidating the function of the Erns protein and its potential applications in areas such as antibody production and vaccine development. The Erns protein expressed using the Bac-to-Bac baculovirus expression system was specifically recognized by an anti-His tag antibody, confirming successful protein expression. This provides essential material for further investigation of the protein's properties and for the preparation of specific antibodies.

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