Review Article

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Advances in Classical Swine Fever Virus molecular detection and characterization using the E2 gene as a diagnostic target

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ABSTRACT

Classical Swine Fever (CSF) is a transboundary viral disease that continues to cause significant economic losses to the swine industry in various countries. Efforts to control this disease are crucially dependent on the availability of rapid, highly sensitive diagnostic methods capable of providing reliable epidemiological data. Classical Swine Fever Virus (CSFV) is a single-stranded, positive-polarity RNA virus with a single open reading frame, in which the E2 gene encodes the main structural glycoprotein that plays a crucial role in the infection process, antigenic properties, and induction of the host immune response. These characteristics make the E2 gene the most widely used molecular target for CSFV detection and characterization. This review article aims to comprehensively examine the latest developments in CSFV molecular detection and characterization methods, with an emphasis on the use of the E2 gene as a diagnostic target. Various E2 gene-based molecular approaches, ranging from conventional RT-PCR, real-time PCR, isothermal amplification methods, to sequencing, are discussed based on their working principles, sensitivity and specificity levels, and their application in clinical diagnosis and field surveillance activities. Furthermore, the function of the E2 gene in phylogenetic analysis, strain origin tracing, and monitoring of CSFV genetic diversity was also reviewed, particularly in the context of outbreak dynamics and evaluating the effectiveness of vaccination programs. The review results indicate that the E2 gene has a balanced combination of sequence conservation and genetic variation, making it effective for both virus detection and characterization. However, several challenges remain, including sequence mutations, limited diagnostic facilities, and the need for method standardization. Therefore, the integration of the latest molecular technologies, the application of multi-target approaches, and the harmonization of E2 gene-based diagnostic protocols are expected to improve control success and support sustainable CSF eradication efforts.

Introduction

Classical Swine Fever (CSF) is one of the most impactful viral diseases in the global swine sector, given its high transmissibility and significant economic consequences (Ganges *et al.*, 2020). The disease can infect both domestic and wild pigs, with a broad spectrum of clinical symptoms ranging from asymptomatic cases to acute infections resulting in high mortality (Khairullah *et al.*, 2024). Historically, CSF outbreaks have resulted in significant losses due to high livestock mortality, international trade restrictions, and the high costs of control and eradication efforts (Casal *et al.*, 2022). Although several countries have achieved CSF-free status, the disease remains endemic in parts of Asia, Eastern Europe, and Latin America, remaining a long-term threat to food security and the economic stability of the livestock industry (Brown and Bevins, 2018).

The etiologic agent of CSF is Classical Swine Fever Virus (CSFV), a member of the Pestivirus genus of the Flaviviridae family (Lamothe-Reyes *et al.*, 2023). This virus has a single-stranded, positive-stranded RNA genome approximately 12.3 kb in length, consisting of a large open reading frame (ORF) flanked by non-translated regions at the 5′ and 3′ ends (Liu *et al.*, 2022a). This ORF is expressed as a single polyprotein that is subsequently proteolytically processed into structural and non-structural proteins (Guo *et al.*, 2023). Among the structural proteins produced, the E2 glycoprotein is a major component of the virion surface and plays a crucial role in the process of virus attachment to host cells and in inducing humoral immune responses (Vuono *et al.*, 2021). These biological properties make the E2 gene not only crucial for viral function but also highly valuable as a molecular marker in the detection and genetic characterization of CSFV (Huong *et al.*, 2025).

Rapid and accurate detection is an essential component in CSF control efforts, given the similarity of its clinical manifestations to a number of other viral diseases in pigs, such as African swine fever and porcine reproductive and respiratory syndrome (Wang *et al.*, 2020a). Conventional diagnostic methods, including virus isolation and serological testing, despite their important diagnostic value, still face limitations related to long testing times, low sensitivity in the early stages of infection, and dependence on the host immune response (Kurniawan *et al.*, 2025). These limitations have the potential to hinder early detection, particularly in subclinical cases and in vaccinated livestock populations (Blome *et al.*, 2017). Therefore, the application of molecular-based diagnostic methods is gaining increasing attention as a means of rapid confirmation, with high sensitivity and good specificity (Muzykina *et al.*, 2024).

In the field of molecular diagnostics, the E2 gene is often chosen as an analysis target because it is able to accommodate a balance between the level of sequence conservation and significant genetic diversity (Chen *et al.*, 2010a). The relatively conservative portion of the E2 gene facilitates the design of consistent primers and probes for detecting various genotypes, while its sequence variation is useful for phylogenetic studies and tracing epidemiological relationships between isolates (Jiang *et al.*, 2013). The development of molecular methods, including real-time PCR, isothermal amplification techniques, and next-generation sequencing technology, has expanded the use of the E2 gene not only as a diagnostic tool but also to monitor viral evolution and analyze outbreak dynamics (Postel *et al.*, 2012).

Based on this description, this review article was prepared to com-

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prehensively examine the latest advances in the detection and molecular characterization of Classical Swine Fever Virus, with a primary focus on the use of the E2 gene as a diagnostic target. This review covers the biological aspects of the E2 gene, various molecular methods that have been developed, and their relevance to clinical diagnosis, epidemiological surveillance activities, and the formulation of sustainable CSF control strategies.

Method

This review was conducted through a narrative literature analysis focusing on molecular detection and genetic characterization of Classical Swine Fever Virus (CSFV) using the E2 gene as a diagnostic target. Relevant scientific publications were collected from electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar, using keywords such as Classical Swine Fever Virus, CSFV, E2 gene, molecular detection, RT-PCR, real-time PCR, isothermal amplification, sequencing, and phylogenetic analysis.

Articles were selected based on their relevance to E2 gene-based molecular diagnostics and characterization of CSFV. Priority was given to peer-reviewed studies describing conventional and advanced molecular techniques, their diagnostic performance, and applications in clinical diagnosis and epidemiological surveillance.

The selected literature was reviewed and summarized descriptively, with emphasis on methodological principles, advantages, limitations, and practical applicability of each approach. Information related to phylogenetic analysis, strain differentiation, and monitoring of CSFV genetic diversity was also included to provide a comprehensive overview of the role of the E2 gene in CSF control strategies.

Structure and function of the E2 gene

The E2 gene is one of the main elements in the CSFV genome that has a crucial role in the mechanism of infection, triggering the immune response in the host, and its use in the diagnosis and molecular characterization of the virus. Figure 1 illustrates the structural organization and multifunctional role of the E2 gene in CSFV.

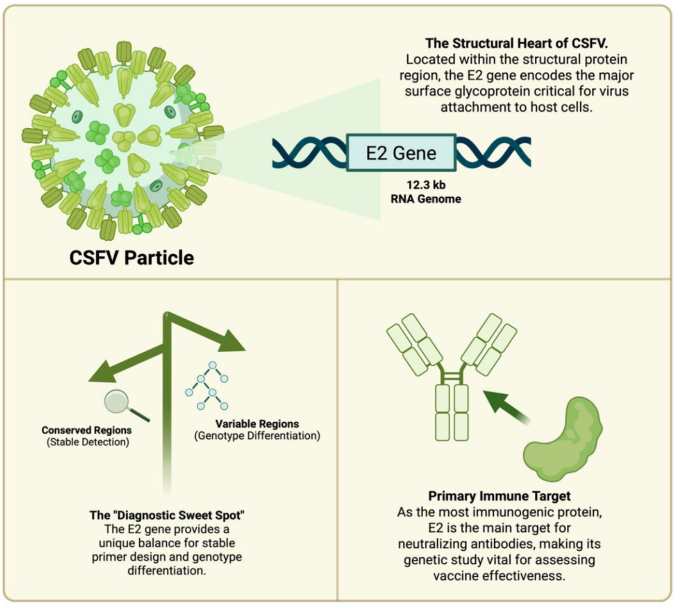


Figure 1. Structural and diagnostic significance of the E2 gene in CSFV

Location in the CSFV genome

The CSFV genome is composed of a single-stranded, positive-stranded RNA approximately 12.3 kb in size, containing a continuous ORF delimited by non-translated regions at the 5′ and 3′ ends (Li *et al.*, 2017). The ORF is translated into a polyprotein that subsequently undergoes proteolytic processing to produce structural and non-structural proteins with specific functions in the viral replication cycle (Lamp *et al.*, 2011). In this genome structure, the E2 gene is located in the structural protein section, located after the capsid (C) gene and before the Erns gene, thus having a strategic position in the sequence of viral protein expression (Hinojosa *et al.*, 2024).

The location of the E2 gene within the structural genome reflects its function as the primary glycoprotein expressed on the surface of the virion (Borca *et al.*, 2019). Genomically, the location of the E2 gene is relatively stable across CSFV isolates, both geographically and genotypically (Hao *et al.*, 2020). However, the nucleotide sequence of the E2 gene exhibits a higher degree of variation than that of several other genes in the CSFV genome, particularly in regions that act as antigenic determinants (Zhou, 2019). This combination of genomic stability and sequence diversity makes the E2 gene highly informative for molecular analysis (Chakraborty *et al.*, 2018).

From a diagnostic and molecular epidemiological perspective, the presence of the E2 gene in the CSFV genome supports its role as a stable target for analysis and offers high discrimination (Malik *et al.*, 2020). Its relatively constant location facilitates the design of specific primers and probes, while its genetic diversity allows for differentiation between strains and the exploration of phylogenetic relationships (Rios *et al.*, 2018). Thus, the position of the E2 gene in the CSFV genome not only reflects the structural organization of the virus but also provides an important foundation for its application in precise molecular detection and genetic characterization (Risatti *et al.*, 2005).

Protein E2

The E2 protein is the dominant structural glycoprotein in CSFV, expressed on the surface of the virion, and plays a crucial role in the viral infection cycle (Guo *et al.*, 2023). Structurally, E2 consists of several functional domains stabilized by disulfide bonds and glycosylation modifications, which contribute to its three-dimensional conformational stability (Wang *et al.*, 2025a). This configuration allows the E2 protein to interact directly with receptors on host cells, thus supporting the process of virus attachment and entry into target cells (Gladue *et al.*, 2014). Minor changes in the E2 structure, particularly those resulting from amino acid substitutions, can affect the efficiency of this step and subsequently impact the virus's replication ability and virulence (Huang *et al.*, 2021).

In terms of pathogenesis, the E2 protein not only mediates virus entry into cells but also plays a role in the interaction between the virus and the host immune system (Fan *et al.*, 2021). Expression levels and structural variations of the E2 protein can influence the process of virus recognition by immune cells and modulate the initial immune response, which in turn impacts the clinical course of infection (Sun *et al.*, 2023a). Several sequence variations in the E2 gene have been associated with differences in disease severity between CSFV strains, indicating that this protein is one of the molecular determinants contributing to viral virulence (Hu *et al.*, 2016).

In terms of immunogenicity, the E2 protein serves as the primary antigen of CSFV and is the dominant target of neutralizing antibodies produced both during natural infection and after vaccination (Yuan *et al.*, 2025). The majority of protective epitopes are located on the E2 protein, making it a central component in the induction of an effective humoral immune response (Liu *et al.*, 2006). Genetic diversity in the E2 antigenic region can influence antibody affinity and specificity, which in turn impacts the ability to neutralize the virus and the sensitivity of antigen- and antibody-based diagnostic methods (Chen *et al.*, 2010b). Therefore, the immunogenic properties of the E2 protein have direct implications for vaccine design, immune response assessment, and the development of reliable diagnostic techniques (Puente-Marin *et al.*, 2025).

The strong correlation between the structure, biological function, and immunogenic characteristics of the E2 protein explains why the E2 gene has been widely selected as a primary target in molecular detection and genetic characterization studies of CSFV (Yoo *et al*., 2018). A comprehensive understanding of these aspects is crucial for improving diagnostic accuracy, strengthening disease control strategies, and supporting the development of molecular approaches capable of adapting to the virus's genetic diversity.

Reasons for selecting E2 as a diagnostic target

The determination of the E2 gene as a diagnostic target in CSFV infection is based on its unique biological and molecular characteristics and its relevance to the need for precise and informative detection (Wang *et al*., 2020a). The E2 gene encodes a major structural glycoprotein that is consistently expressed on the virion surface and plays a crucial role in the interaction between the virus and host cells (Zhang *et al*., 2025a). This essential function causes the E2 gene to be maintained in all CSFV isolates, thus providing a stable target for various nucleic acid-based diagnostic methods (Zhang *et al*., 2025b). The presence of the E2 gene in all circulating strains makes it a reliable molecular marker for identifying the presence of the virus in various clinical and epidemiological contexts (Hu *et al*., 2016).

In addition to its functional conservation, the E2 gene also exhibits sufficient sequence diversity to distinguish CSFV genotypes and subgenotypes (Chen *et al*., 2008). This variation generally accumulates in specific regions that do not interfere with the protein's primary function, yet still provide valuable information for phylogenetic analysis and tracing the origins of the virus (Risatti *et al*., 2005). This characteristic provides an additional advantage in a diagnostic context, as E2 gene-based approaches not only allow confirmation of infection but also support concurrent genetic characterization of the virus (Chen *et al*., 2021a). Therefore, the E2 gene serves as a multifunctional target that can be utilized for both detection and molecular epidemiological analysis (Ceppi *et al*., 2005).

From a technical perspective, the E2 gene demonstrates high compatibility with various molecular diagnostic platforms, including conventional Reverse Transcription Polymerase Chain Reaction (RT-PCR), real-time PCR, isothermal amplification techniques, and sequencing approaches (Srivastava and Prasad, 2023). The length and structural characteristics of the E2 gene allow for the design of specific primers and probes with minimal risk of cross-reaction with other viruses in the Pestivirus genus (Chakraborty *et al*., 2018). This level of specificity plays a crucial role in reducing the possibility of false-positive results, particularly in endemic areas where multiple pestiviruses may co-circulate (Huang *et al*., 2021). Furthermore, the relatively stable expression of the E2 gene during viral replication contributes to detection sensitivity, even in samples with low viral loads (Chu *et al*., 2025).

Immunological aspects also strengthen the basis for selecting the E2 gene as a diagnostic target (Zhou, 2019). Given that the E2 protein acts as the primary antigen that induces the formation of neutralizing antibodies, this gene is often the focus of simultaneous serological and molecular assay development (Chen *et al*., 2025a). The relationship between E2 gene-based molecular detection results and the host immune response provides more comprehensive diagnostic and interpretive value, particularly in distinguishing natural infection, vaccination status, and the dynamics of the infection's course (Panyasing *et al*., 2018). Thus, the combination of genomic conservation, informative sequence variation, technical suitability, and immunological relevance makes the E2 gene the most logical and widely used diagnostic target for CSFV detection and molecular characterization (Fatima *et al*., 2021).

Molecular detection technique using the E2 target

Advances in molecular detection methods targeting the E2 gene have significantly improved the accuracy and speed of CSFV diagnosis. Figure 2 presents the spectrum of molecular diagnostic platforms that utilize the E2 gene as a primary target for the detection of CSFV. Table 1 summarizes the various molecular detection techniques for CSFV that utilize the E2 gene as the primary diagnostic target.

Conventional PCR

Conventional PCR is one of the initial approaches widely used in the molecular detection of CSFV, with the E2 gene as the primary target for amplification (Lu *et al*., 2017). The use of the E2 gene in this method is based on a balance between the level of sequence conservation and the

Table 1. CSFV molecular detection techniques based on the E2 gene targets.

Detection technique	Basic principle	Role of the E2 gene as a target	Main advantages	Limitations	References
Conventional PCR	Amplification of target sequences using specific primers, with end-product detection by gel electrophoresis	The E2 gene is used due to a balance between sequence conservation and variability, enabling cross-strain detection of CSFV	Simple procedure, relatively low cost, suitable for laboratories with limited facilities	Lower sensitivity compared to qPCR; dependent on RNA quality and visual interpretation	(Lu *et al*., 2017; Mukherjee *et al*., 2023; Sadchikova *et al*., 2024; Hoffmann *et al*., 2005)
Real-Time PCR (qPCR)	Simultaneous amplification and detection of the target using fluorescent probes (e.g., TaqMan)	The E2 gene provides high specificity for CSFV and enables detection at low viral loads	High sensitivity and specificity; allows viral RNA quantification; suitable for routine diagnosis and surveillance	Requires specialized equipment and higher operational costs	(Dias *et al*., 2014; Ciglenecki *et al*., 2008; Chakraborty *et al*., 2018; Huang *et al*., 2009)
Multiplex RT-PCR	Simultaneous amplification of multiple genetic targets in a single reaction	The E2 gene serves as a primary target combined with other genes to enhance diagnostic reliability	Time- and cost-efficient; improves accuracy and reduces false-negative results caused by genetic variation	Requires complex technical optimization to balance amplification of each target	(Liu *et al*., 2022a; Beer *et al*., 2015; Zhao *et al*., 2023a; de Arce *et al*., 2009)
Loop-Mediated Isothermal Amplification (LAMP)	Isothermal amplification using multiple primer sets without thermal cycling	The E2 gene is selected due to its specificity and sequence stability, enabling recognition of multiple target regions	Rapid, easy to perform, no need for sophisticated equipment; suitable for field diagnosis	Sensitivity depends on viral load; primer design is more complex	(Bohórquez *et al*., 2024; Mustafa *et al*., 2014; Li *et al*., 2022; Xu *et al*., 2022)
CRISPR-based molecular assays (SHERLOCK/ DETECTR)	Target recognition by Cas systems that trigger detectable signals through collateral cleavage	The E2 gene is used as a target due to its high specificity and genetic relevance to CSFV	Extremely high sensitivity, short detection time, adaptable for both laboratory and field formats	Still under development and validation; not yet widely standardized	(Rao *et al*., 2022; Mustafa and Makhawi, 2021; Huang *et al*., 2022; Chen *et al*., 2023)

presence of diagnostically valuable genetic variation (Mukherjee *et al.*, 2023). E2 primer design is generally directed at genomic regions that are relatively stable among CSFV isolates, allowing consistent target amplification without compromising the ability to detect differences between strains (Sadchikova *et al.*, 2024). During the design stage, primers are selected to avoid regions with high mutation rates that could potentially reduce amplification efficiency, while also considering the appropriate size of the PCR product for visualization by agarose gel electrophoresis (Bohórquez *et al.*, 2024).

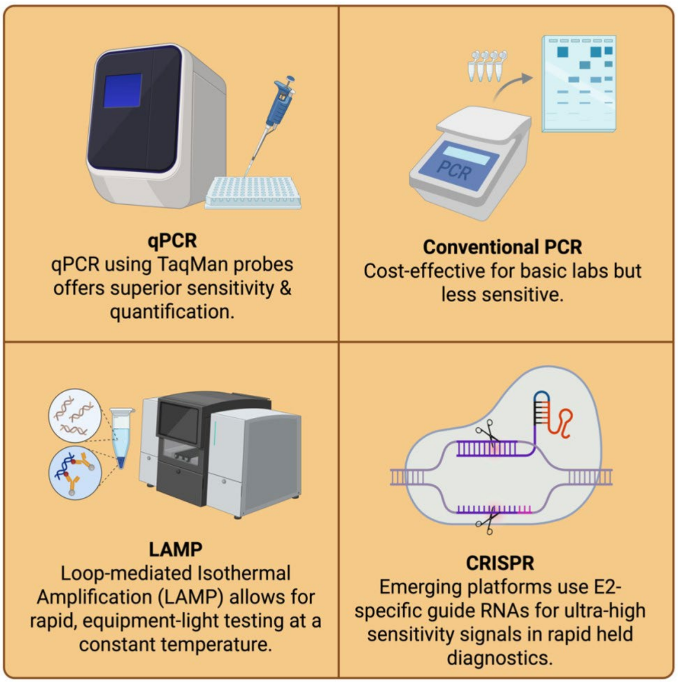


Figure 2. Molecular diagnostic platforms targeting the CSFV E2 gene (From qPCR to CRISPR-based detection)

The success of conventional PCR targeting the E2 gene is largely determined by the specificity of the primers to the CSFV sequence (Risatti *et al.*, 2003). This aspect is crucial given the presence of other viruses in the Pestivirus genus that share genomic similarities, such as Bovine Viral Diarrhea Virus and Border Disease Virus (Khalid *et al.*, 2023). Therefore, E2 primers are generally designed within sequence regions unique to CSFV to minimize the possibility of nonspecific amplification or cross-reactions (Hoffmann *et al.*, 2005). Furthermore, in silico evaluation and laboratory testing using a panel of related viruses are frequently performed to ensure that the resulting amplification products truly reflect the presence of CSFV (Coronado *et al.*, 2025a).

In terms of sensitivity, conventional PCR targeting the E2 gene is capable of detecting CSFV in samples with moderate to high viral loads, particularly during the acute phase of infection (Manessis *et al.*, 2024). However, the sensitivity of this method is generally lower than that of real-time PCR because it relies on visualization of the final amplification product (Hwang *et al.*, 2023). Various factors, including the quality of the starting RNA, the efficiency of the reverse transcription process, and the PCR reaction conditions, also influence the detection limit (Chen *et al.*, 2009). Nevertheless, conventional PCR remains important, especially in laboratories with limited facilities, given its relatively simple procedure, lower cost, and the lack of specialized equipment other than a thermal cycler (Wang *et al.*, 2020a).

Real-Time PCR (qPCR)

Real-time PCR or quantitative real-time PCR (qPCR) is one of the most widely used molecular techniques in CSFV diagnosis due to its ability to detect and quantify viral RNA with high sensitivity and specificity (Dias *et al.*, 2014). In its application, the E2 gene is often targeted by qPCR and combined with the use of fluorescently labeled probes, such as TaqMan probes, which are designed to bind specifically to the target sequence during the amplification process (Ciglenecki *et al.*, 2008). This approach increases the specificity of detection, as the fluorescence signal is only generated when the probe is degraded by the 5′–3′ exonuclease activity of DNA polymerase, thereby reducing the risk of nonspecific detection often encountered with methods using intercalating dyes (Tu *et al.*, 2021).

The qPCR probe targeting the E2 gene was designed in a genomic region that is relatively conserved among CSFV isolates, yet still offers good discrimination against other viruses in the Pestivirus genus (Chakraborty *et al.*, 2018). This level of specificity is one of the main advantages of using the E2 gene, particularly in endemic areas where co-circulation of Bovine Viral Diarrhea Virus or Border Disease Virus is possible (Panyasing *et al.*, 2018). Furthermore, the stability of E2 gene expression during viral replication supports high detection sensitivity, allowing CSFV identification in samples with low viral loads, including in early stages of infection and in animals with subclinical infections (Sánchez *et al.*, 2008). Therefore, E2 gene-based qPCR is highly suitable for routine diagnostic purposes, active surveillance activities, and rapid confirmation in outbreak situations (Huang *et al.*, 2009).

Compared with other molecular targets, such as the 5′UTR region and the NS5B gene, the E2 gene provides a more optimal balance between diagnostic sensitivity and genetic information value (Malik *et al.*, 2020). The 5′UTR region is highly conserved and commonly used for initial detection due to its sequence stability, but this high degree of conservation limits its ability to differentiate CSFV genotypes or strains (Desai *et al.*, 2010). Conversely, the NS5B gene tends to be more conservative than E2 and is often used for molecular confirmation purposes, but its limited sequence variation makes it less ideal for phylogenetic analysis (Sarma *et al.*, 2011). The E2 gene is positioned between these two targets, with sufficient diversity to support genetic characterization without compromising detection sensitivity and specificity (Huang *et al.*, 2014).

Multiplex RT-PCR

Multiplex RT-PCR is a molecular diagnostic method developed to detect two or more genetic targets simultaneously in a single amplification reaction, thereby increasing the efficiency and accuracy of CSFV diagnosis (Liu *et al.*, 2022a). In this approach, the E2 gene is generally used as the primary target and combined with other genomic regions, such as the 5′ UTR or non-structural genes, to enhance detection reliability (Beer *et al.*, 2015). This multi-target approach plays a crucial role in anticipating viral genetic variations that could potentially impact amplification success if only one gene is used as a target (Rios *et al.*, 2017). By incorporating the E2 gene, multiplex RT-PCR maintains a high level of specificity for CSFV while providing an additional layer of confirmation through co-targets (Zhao *et al.*, 2023a).

The development of multiplex RT-PCR requires careful technical optimization, particularly in the selection of compatible primers and adjustment of reaction conditions to ensure balanced amplification of each target (Chen *et al.*, 2021b). The E2 gene has sequence characteristics that support its application in multiplex systems, as it provides a stable, conservative region for primer binding while also possessing sufficient discriminatory power to distinguish CSFV from other pestiviruses (Gladue *et al.*, 2014). These advantages enable the E2 gene to serve as a reliable diagnostic marker, even when used in a single reaction involving multiple molecular targets simultaneously (Nazerke *et al.*, 2025).

In clinical practice, multiplex RT-PCR targeting the E2 gene offers significant advantages in supporting rapid and accurate CSFV diagnosis, particularly in field operations and disease surveillance (de Arce *et al.*, 2009). This approach allows for more time- and cost-efficient testing of large numbers of samples without compromising the quality of the results (Wang *et al.*, 2025b). Furthermore, the ability to detect multiple molecular targets in a single assay helps differentiate CSFV from other infectious agents with similar clinical symptoms, thus supporting informed decision-making in outbreak control and livestock health management (Coronado *et al*., 2025a). Therefore, multiplex RT-PCR involving the E2 gene is a relevant and strategic diagnostic strategy in current veterinary diagnostic practice (Zhao *et al*., 2008).

Loop-Mediated Isothermal Amplification (LAMP)

Loop-mediated isothermal amplification (LAMP) is a nucleic acid amplification technique developed as an alternative to conventional PCR, with the main advantage of a constant temperature amplification process without the need for heating and cooling cycles (Bohórquez *et al*., 2024). This approach is very suitable for the detection of CSFV in environments with limited laboratory facilities, because the LAMP reaction can be carried out using simple equipment such as a water bath or heat block (Akram, 2017). The LAMP method utilizes DNA polymerase with strand displacement activity and several sets of primers that recognize six to eight different regions in the target genome, resulting in rapid, efficient amplification with a high level of specificity (Mekata *et al*., 2009).

The use of the E2 gene as a target in the LAMP test is based on its relatively stable and specific sequence characteristics for CSFV (Mustafa *et al*., 2014). Designing LAMP primers targeting the E2 gene allows for the simultaneous recognition of multiple target regions, significantly reducing the risk of nonspecific amplification (Bi *et al*., 2025). Furthermore, the E2 gene offers a balance between conservation and genetic diversity, allowing the LAMP test to remain effective in detecting a wide range of CSFV isolates while maintaining discrimination against other closely related pestiviruses (Hinojosa *et al*., 2024). With these characteristics, the E2 gene is an appropriate target for the development of the LAMP method in the context of field diagnosis (Li *et al*., 2022).

In terms of diagnostic performance, the LAMP method targeting the E2 gene has a relatively fast reaction time, with results typically observed in less than an hour (Tran *et al*., 2024). Detection of amplification products can be achieved through various approaches, such as turbidity changes, the use of intercalating dyes, and fluorescence visualization, making it easier to read results without the need for complex detection equipment (Wong *et al*., 2018). The sensitivity of this method is reportedly equivalent to conventional PCR and, under certain conditions, can approach the sensitivity of real-time PCR, particularly when moderate to high amounts of viral RNA are available (Padzil *et al*., 2021).

In practical applications, the LAMP method targeting the E2 gene has great potential for active surveillance, rapid screening, and early response to CSF outbreaks, particularly in endemic areas or areas with limited laboratory access (Xu *et al*., 2022). The ease of implementation, rapid results, and high target specificity make this method a valuable complement to PCR-based molecular techniques (Chen *et al*., 2009). Thus, the E2 gene-based LAMP represents an adaptive and applicable diagnostic approach, supporting early detection and more effective control of CSF (Coronado *et al*., 2021).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based molecular assays

Advances in Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based technology have opened up new opportunities for developing molecular virus detection with very high levels of sensitivity and specificity (Rao *et al*., 2022). In the context of CSFV, CRISPR-based molecular assays, such as Specific High-sensitivity Enzymatic Reporter unlocking (SHERLOCK) and DNA Endonuclease Targeted CRISPR Trans Reporter (DETECTR), are beginning to be explored as innovative diagnostic approaches that have the potential to complement or replace conventional amplification methods (Mustafa and Makhawi, 2021). The working principle of both platforms utilizes the ability of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated (Cas) endonucleases to specifically recognize target sequences through guide RNA, which then triggers nonspecific cleavage activity (collateral cleavage) against labeled reporter molecules when the target is successfully detected (Huang *et al*., 2022).

The E2 gene is an excellent candidate target for CRISPR-based molecular testing of CSFV, given its sequence specificity for the virus and its role in genetic characterization (Chen *et al*., 2010b). In the SHERLOCK system, which typically utilizes Cas13, viral RNA generated through isothermal amplification or in vitro transcription is directly recognized by a Cas13–guide RNA complex designed specifically for the E2 gene (Kellner *et al*., 2019). In contrast, the Cas12-based DETECTR approach uses amplified target DNA, such as from RT-LAMP or RPA, to trigger a detection signal (Qian *et al*., 2023). In both methods, selecting a conservative yet discriminatory region of the E2 gene is key to ensuring accurate detection and preventing cross-reactions with other closely related pestiviruses (Chen *et al*., 2023).

The key advantage of CRISPR-based molecular tests targeting the E2 gene lies in their combination of high sensitivity, short detection time, and flexibility in readout formats (Wang *et al*., 2025c). Signal detection can be performed using either fluorescence or lateral flow approaches, enabling applications in both the laboratory and the field (Manessis *et al*., 2024). Furthermore, the guide RNA target recognition mechanism provides an additional level of selectivity beyond the amplification step, which is particularly useful for addressing the challenges of CSFV genetic diversity (Ju *et al*., 2025). This approach also has the potential to detect viral RNA at very low concentrations, making it relevant for early identification of infection and monitoring of subclinical cases (Ganges *et al*., 2020).

However, the application of CRISPR-based molecular assays for CSFV detection targeting the E2 gene is still in the development and validation stages (Du *et al*., 2022). Several challenges that need to be overcome include protocol standardization, optimization of guide RNA design to cov-

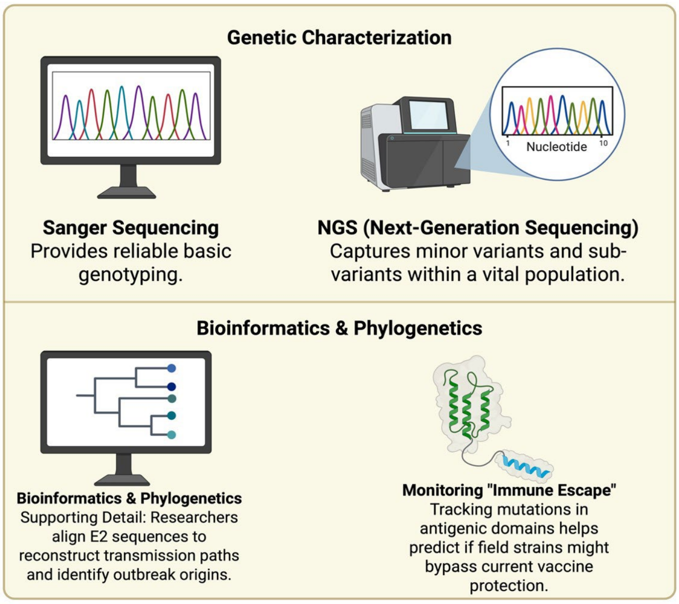
Table 2. E2 gene-based genetic characterization approach of CSFV

Approach	Analytical principle	Genetic information generated	Main advantages	Limitations	References
Sanger sequencing of the E2 gene	Determination of the nucleotide sequence of E2 gene fragments using the chain-termination method	CSFV genotyping and subgenotyping; basic phylogenetic relationship analysis; identification of major antigenic variations	High accuracy; relatively affordable cost; well-established and easy to implement	Limited sequence coverage; unable to detect minor variants within viral populations	(Fatima *et al*., 2021; Blome *et al*., 2010; Chakraborty *et al*., 2018; Rios *et al*., 2018)
Next-Generation Sequencing (NGS) of the E2 gene	High-throughput parallel sequencing of the E2 gene from single or multiple samples	Detection of minor genetic variants; viral population analysis; high-resolution phylogenetic inference	High sensitivity; capable of identifying sub-strains and minor variants; supports viral evolution analysis	Requires complex laboratory infrastructure and bioinformatics expertise; higher costs	(McDowell *et al*., 2025; Hilt and Ferrieri, 2022; Mukherjee *et al*,. 2023; Postel *et al*., 2012)
Bioinformatics and phylogenetic analysis	Sequence alignment and evolutionary inference using various computational algorithms	Genotype classification; reconstruction of evolutionary relationships; estimation of evolutionary dynamics and spread	Enables in-depth interpretation of sequence data; supports evidence-based molecular epidemiology	Dependent on sequence quality and appropriate selection of evolutionary models	(Liu *et al*., 2022b; Satam *et al*., 2023; Blacksell *et al*., 2004; Baele *et al*., 2025)

er various CSFV genotypes, and assessment of the method's performance using large clinical samples (Ciotti *et al.*, 2024). However, with the rapid development of CRISPR technology and its integration with isothermal amplification techniques, E2 gene-based assays have great potential to develop into next-generation diagnostic tools that are rapid, sensitive, and easy to implement in CSF surveillance and control systems (Masi *et al.*, 2023).

Genetic characterization based on E2

Genetic characterization of Classical Swine Fever Virus, focusing on the E2 gene, is the primary approach for studying genotypic diversity, phylogenetic relationships, and epidemiological dynamics of the virus. Figure 3 illustrates the framework for genetic characterization and evolutionary analysis of the E2 gene of CSFV using sequencing and bioinformatics approaches. Table 2 presents a summary of the main methods for E2-gene-based genetic characterization of CSFV, including sequencing approaches and bioinformatics analysis.



Sanger Sequencing
Provides reliable basic genotyping.

NGS (Next-Generation Sequencing)
Captures minor variants and sub-variants within a vital population.

Bioinformatics & Phylogenetics
Supporting Detail: Researchers align E2 sequences to reconstruct transmission paths and identify outbreak origins.

Monitoring "Immune Escape"
Tracking mutations in antigenic domains helps predict if field strains might bypass current vaccine protection.

Figure 3. Genetic characterization and evolutionary analysis of the CSFV E2 gene using sequencing and bioinformatics approaches

Sanger E2 sequencing

Sanger sequencing of the E2 gene has long been used as a standard method for molecular characterization of CSFV, particularly for genotyping and analyzing phylogenetic relationships between isolates (Fatima *et al.*, 2021). This approach allows for highly accurate nucleotide sequence determination of genetically informative fragments of the E2 gene, thus supporting the classification of CSFV into predefined genotypes and subgenotypes (Blome *et al.*, 2010). Given that the E2 gene exhibits a higher degree of sequence variation than other highly conserved regions of the genome, Sanger sequencing of this gene provides sufficient resolution to differentiate viral strains circulating in different geographic regions and over different time periods (Chakraborty *et al.*, 2018). This information plays a crucial role in molecular epidemiological studies, including tracing the source of infection and understanding the dynamics of viral spread (Moennig *et al.*, 2003).

Besides its use for genetic classification, Sanger sequencing of the E2 gene also plays a crucial role in the study of CSFV antigenic variation (Rios *et al.*, 2018). Nucleotide changes in the E2 gene can result in amino acid substitutions in the antigenic domain of the E2 protein, potentially affecting virus recognition by neutralizing antibodies (Nguyen *et al.*, 2022). E2 gene sequence analysis allows the identification of mutations associated with differences in antigenicity between strains, thus impacting vaccine effectiveness and the interpretation of serological test results (Chen *et al.*, 2010a). By comparing E2 sequences from field isolates and vaccine strains, researchers can assess the degree of antigenic match and identify potential immune escape mechanisms (Chen *et al.*, 2025a).

Although the data generated by Sanger sequencing is limited to specific genome fragments, this method remains highly relevant due to its ease of implementation, relatively low cost, and the reliability of the results obtained (Bold*et al.*, 2023). In various studies, Sanger sequencing of the E2 gene is often used as an initial step in molecular characterization before continuing with higher-resolution methods, such as next-generation sequencing (Leng *et al.*, 2017; Gao *et al.*, 2025). Thus, E2 gene-based Sanger sequencing remains important in mapping CSFV genotypes and understanding antigenic variations underlying the epidemiological dynamics and control strategies of CSF (Parchariyanon *et al.*, 2000).

Next-Generation Sequencing (NGS) E2

The use of next-generation sequencing (NGS) in E2 gene analysis has brought significant advances in the molecular characterization of CSFV, particularly through increased genetic resolution unattainable by conventional sequencing techniques (McDowell *et al.*, 2025). NGS technology enables massively parallel sequencing, allowing for the identification of minor nucleotide variations in the E2 gene with high sensitivity (Hilt and Ferrieri, 2022). This capability plays a crucial role in detecting CSFV substrains with subtle genetic differences, including variants that arise in response to immune selection pressure, vaccination, or host adaptation (Tang *et al.*, 2008). Therefore, the E2 gene-based NGS approach not only reveals the dominant sequences in a sample but also supports a comprehensive and in-depth analysis of the viral population (Jansz and Faulkner, 2024).

The high resolution offered by NGS technology allows for comprehensive mapping of genetic diversity in the E2 gene, including the identification of point mutations, insertions, deletions, and allele frequency variations within a single viral population (Mukherjee *et al.*, 2023). These data are crucial for studying the evolutionary dynamics of CSFV, particularly regarding antigenic changes in the E2 protein that could potentially influence the host immune response (Summerfield and Ruggli, 2015). Furthermore, the NGS approach allows for high-precision comparison of E2 gene sequences between isolates from different geographic regions and time periods, thus enhancing phylogenetic analysis and determining kinship relationships between strains (Blacksell *et al.*, 2004).

In molecular epidemiology studies, the use of E2 gene-based NGS plays a strategic role in tracing the source of infection and mapping the spread of CSFV (Postel *et al.*, 2012). High-resolution sequence information allows for the identification of epidemiological clusters, the reconstruction of transmission pathways, and the assessment of the interrelationships between outbreaks occurring in different geographic locations (Blome *et al.*, 2017). This approach is highly effective in distinguishing outbreaks resulting from the introduction of a new virus from the re-emergence of a previously circulating strain (Brown and Bevins, 2018). Furthermore, NGS allows for long-term monitoring of genetic changes in the E2 gene, which can be used as a basis for assessing the effectiveness of disease control strategies and vaccination programs (Chen *et al.*, 2025b).

Although the application of E2 gene-based NGS requires more complex laboratory facilities and bioinformatics analysis than conventional sequencing techniques, this method makes a significant contribution to CSFV research and surveillance activities (Parchariyanon *et al.*, 2000). Combining NGS data with phylogenetic analysis and field epidemiological information allows for a more comprehensive understanding of the virus's distribution patterns and evolutionary processes (Duault *et al.*, 2022). Thus, E2 gene-based NGS is increasingly recognized as a key component in contemporary molecular epidemiology and in the formulation of evidence-based control strategies for CSF (Postel *et al.*, 2012).

Bioinformatics and phylogenetic analysis

Bioinformatics approaches play a central role in the molecular analysis of the CSFV E2 gene, particularly in managing sequence data and drawing conclusions about the evolutionary relationships between isolates (Liu *et al*., 2022b). After E2 gene sequence data is obtained through Sanger or next-generation sequencing techniques, the initial step is often multiple sequence alignment to assess the level of nucleotide similarity and variation (Satam *et al*., 2023). Various software tools such as Clustal Omega, MUSCLE, and MAFFT are widely used because they can produce accurate and efficient alignment results, especially for datasets with moderate levels of diversity, such as the E2 gene (Eskioglu *et al*.,2024). The quality of this sequence alignment is crucial for the success of subsequent analyses, as errors in the initial stages can directly impact phylogenetic interpretation and the evaluation of genetic variation (Singh and Rajak, 2017).

Phylogenetic analysis of the CSFV E2 gene is generally conducted using various computational approaches, such as distance-based methods, maximum likelihood, and Bayesian inference, which are selected according to the research objectives and the level of complexity of the analysis (Blacksell *et al*., 2004). Software such as MEGA is widely used for phylogenetic tree construction using neighbor-joining or maximum likelihood methods, with easy-to-use interfaces, making it suitable for both routine analysis and surveillance activities (Sohpal *et al*., 2010). Meanwhile, for more in-depth and high-resolution phylogenetic studies, software such as PhyML, RAxML, and BEAST are more frequently applied because they are able to implement more complex evolutionary models and estimate important evolutionary parameters, including substitution rates and divergence times (Baele *et al*., 2025). Selecting the most appropriate nucleotide substitution model, which is generally determined through statistical approaches such as the Akaike Information Criterion, is a crucial step in improving the accuracy and reliability of the resulting phylogenetic tree (Abadi *et al*., 2019).

Interpretation of E2 gene sequence variations is not only used to group isolates into genotypes and subgenotypes but also includes analysis of the biological consequences of nucleotide and amino acid changes (Hao *et al*., 2020). Some variations in the E2 gene may reflect viral adaptation to immune selection pressures or environmental factors, and potentially influence the antigenic properties of the E2 protein (Hinojosa *et al*., 2024). Within the framework of molecular epidemiology, phylogenetic clustering patterns derived from E2 sequences are often utilized to assess the phylogenetic relationships among isolates from different outbreaks, trace the origin of the virus, and distinguish between newly introduced viruses and ongoing endemic circulation (Jemersić *et al*., 2003). Therefore, the combination of E2 gene-based bioinformatics and phylogenetic analyses provides a robust analytical framework for a more comprehensive understanding of the dynamics of CSFV evolution, spread, and genetic variation (Mahadevaswamy *et al*., 2025).

Evaluation of method performance

Performance assessment of molecular detection methods utilizing the E2 gene is a crucial aspect for determining diagnostic reliability, clinical validity, and readiness for implementation both in the laboratory and in the field.

Sensitivity and specificity

Sensitivity and specificity are key parameters in assessing the performance of CSFV molecular detection methods, particularly those targeting the E2 gene (Xu *et al*., 2022). Various E2 gene-based approaches, ranging from conventional PCR, real-time PCR, multiplex RT-PCR, to isothermal techniques and CRISPR-based assays, exhibit performance variations influenced by their working principles, assay design, and the context in which they are used (Liberty *et al*., 2025). E2 gene-based real-time PCR generally has the highest sensitivity, capable of detecting viral RNA at low concentrations, including in the early phase of infection or subclinical cases (Engstrom-Melnyk *et al*., 2015). This high sensitivity is supported by the use of specific probes that improve signal accuracy while reducing the possibility of non-specific amplification (Espy *et al*., 2006). In contrast, conventional E2 gene-based PCR, although showing good specificity, tends to have a higher detection limit because it relies on visualization of the final amplification product (Zhang *et al*., 2024).

Multiplex RT-PCR methods targeting the E2 gene offer a combination of sensitivity and diagnostic reliability through a multi-target strategy (Haines *et al*., 2013). By combining the E2 gene with other genomic targets, this approach maintains adequate sensitivity while increasing specificity, particularly for distinguishing CSFV from other genomically similar pestiviruses (Lee *et al*., 2025). Isothermal techniques such as E2 gene-based LAMP demonstrate competitive sensitivity under field conditions, although their performance can be affected by the quality of primer design and reaction control (Wang *et al*., 2020b). On the other hand, CRISPR-based assays targeting the E2 gene have the potential for very high sensitivity due to the additional layer of target recognition via guide RNA, although their implementation still requires further validation at the clinical scale (Wang *et al*., 2025c).

Variation in the E2 gene sequence is a crucial factor that can affect the sensitivity and specificity of this gene-based detection method (Reuscher *et al*., 2021). Nucleotide mutations, particularly in the regions where primers or probes bind, can potentially reduce amplification efficiency or even cause detection failure (Blome *et al*., 2017). This impact is more significant in methods that rely solely on a single target compared to multi-target systems (Malik *et al*., 2020). However, E2 sequence variation is generally focused on specific antigenic domains and rarely affects the conserved regions typically chosen for diagnostic primer design (Huang *et al*., 2021). Therefore, selecting the appropriate target site and periodically updating primer design are crucial strategies to maintain assay performance as CSFV evolves (Wang *et al*., 2025b).

Clinical and field trials

Clinical and field trials are crucial for assessing the feasibility of implementing E2 gene-based molecular detection methods for CSFV diagnosis outside of controlled laboratory conditions (Chu *et al*., 2025). Evaluation of clinical samples typically involves a variety of specimens, such as tonsil tissue, spleen, lymph nodes, blood, and swabs, obtained from animals with varying infection states, including acute, subclinical, and post-vaccination phases (Hochman *et al*., 2025). Targeting the E2 gene in clinical samples has been shown to provide consistent detection capabilities, as its expression remains relatively stable throughout viral replication (Huynh *et al*., 2024a). Field testing results provide a clear picture of the method's sensitivity to variations in sample quality, RNA degradation, and the presence of reaction inhibitors frequently encountered in field specimens (Wang *et al*., 2020a).

In the field, the performance of E2 gene-based detection methods is also influenced by operational factors, such as sampling time, storage methods, and transportation (Chakraborty *et al*., 2018). Field studies have shown that highly sensitive methods, such as real-time PCR and E2 gene-based LAMP, are more tolerant of these variations than conventional PCR (Chen *et al*., 2009). Furthermore, the application of E2 gene-based methods in active surveillance in endemic areas allows for early detection of infections before clinical symptoms appear, providing a strategic advantage in outbreak control (Moennig, 2015). Findings from field trials are also crucial for assessing the suitability of diagnostic methods for practical needs in the livestock and animal health sectors (Wang *et al*., 2020b).

Method validation is a crucial step in ensuring that E2 gene-based assays produce reliable, reproducible, and consistent data across laboratories (Robert *et al*., 2023). The validation process typically includes determining the limit of detection, evaluating both intra- and inter-assay precision, and testing specificity using a panel of related viruses (Coronado *et al*., 2025b). Furthermore, the method is often compared with internationally standardized reference methods to assess its diagnostic accuracy. The results of this validation process provide an important basis for the acceptance and implementation of E2 gene-based methods in national and regional surveillance programs (Manessis *et al*., 2024).

Standardization of E2 gene-based diagnostic procedures is crucial to ensure consistency of results between laboratories and regions (Nazerke *et al*., 2025). The standardization process includes harmonization of RNA extraction protocols, primer and probe design, reaction conditions, and data interpretation (Blome *et al*., 2017). International organizations and reference laboratories play a role in developing technical guidelines and providing standardized positive and negative control materials (Wang *et al*., 2020a). With adequate validation and standardization, E2 gene-based detection methods can be widely implemented as reliable diagnostic tools in CSF control programs (Cao *et al*., 2023).

Current technical limitations

Although the E2 gene is a primary target for the detection and molecular characterization of CSFV, this gene-based approach has several limitations that require critical consideration (Khairullah *et al*., 2024). One major challenge is the potential for false-negative results due to genetic variations in the E2 sequence (Rios *et al*., 2017). Nucleotide mutations, particularly in the primer or probe binding region, can reduce amplification efficiency or prevent optimal target recognition (Wang *et al*., 2024). This risk is higher with methods that rely solely on a single target, such as conventional PCR or singleplex qPCR, potentially leaving certain CSFV variants undetected (Dias *et al*., 2014). Although assays typically target a conserved region of the E2 gene, the dynamic evolution of the virus demands regular evaluation and updating of primer designs to maintain diagnostic sensitivity (Chakraborty *et al*., 2018).

In addition to genetic factors, limitations also arise in the field implementation of E2 gene-based methods (Wei *et al*., 2021). Highly sensitive methods, such as real-time PCR and CRISPR-based assays, require specialized equipment, high-quality reagents, and trained personnel, which are often not readily available in endemic or resource-limited areas (Zhang *et al*., 2023). Relatively high operational costs—including procurement of instruments, consumables, and maintenance of laboratory facilities—present a significant barrier to the widespread and sustainable implementation of molecular diagnostics (Singh-Moodley *et al*., 2020). This can limit the frequency of surveillance and slow the response to emerging outbreaks (Brown and Bevins, 2018).

Another challenge in the field relates to sample quality and handling (de Oliveira *et al*., 2020). RNA degradation due to suboptimal storage or transportation can reduce detection efficacy, especially with methods sensitive to template quality (Relova *et al*., 2017). Furthermore, the presence of reaction inhibitors in some clinical samples can interfere with the efficiency of E2 gene amplification, thereby reducing the accuracy of results (Coronado *et al*., 2025b). Therefore, despite the high diagnostic value of the E2 gene, these technical and operational limitations emphasize the importance of additional strategies, such as the use of multi-target systems, methods that are more tolerant to field conditions, and strengthening the diagnostic infrastructure to support effective control of CSF (Zhang *et al*., 2025c).

Comparison with other molecular targets

Various regions of the CSFV genome have been used as targets for molecular detection and characterization, each with distinct biological and diagnostic properties (Robert *et al*., 2023). The E2 gene is the most common target because it encodes the major structural glycoprotein, which plays a key role in the antigenicity and genetic diversity of the virus (Zhao *et al*., 2023b). Compared with other targets, the E2 gene offers a unique combination of functional conservation and sequence variation, making it not only effective for confirming the presence of the virus but also providing important information for phylogenetic analysis and genotyping (Luo *et al*., 2017). However, this genetic diversity requires careful primer and probe design to maintain method sensitivity across circulating genotypes (Hu *et al*., 2015).

The 5′ untranslated region (5′UTR) is a highly conserved target in the CSFV genome and is often used for early detection due to its high sequence stability (Deng *et al*., 2012). The primary advantage of the 5′UTR is its high sensitivity, particularly in detecting viral RNA at low concentrations (Nadar *et al*., 2011). However, this highly conserved level limits the 5′UTR's ability to differentiate CSFV genotypes or subgenotypes, making it less than ideal for molecular epidemiology studies or outbreak origin tracking (Hinojosa *et al*., 2024). Furthermore, the similarity of 5′UTR sequences across pestiviruses increases the risk of cross-reactivity if assay design is not specifically designed (Reuscher *et al*., 2021).

Non-structural genes such as NS5A and NS5B are also used as molecular targets due to their crucial role in viral replication and their relatively high degree of conservation (Lamp *et al*., 2013). These targets generally offer good specificity and diagnostic stability, and are therefore often used as confirmatory genes in multi-target systems (Sun *et al*., 2023b). However, sequence variation in NS5A/B is relatively low compared to the E2 gene, limiting its phylogenetic resolution (Nguyen *et al*., 2021). Therefore, these genes are more suitable for detecting the presence of the virus than for in-depth genetic characterization (Hao *et al*., 2020).

The Npro gene, which encodes a non-structural protein with a role as a protease and in modulating the host immune response, is also used as an alternative diagnostic target (Summerfield and Ruggli, 2015). This gene exhibits good specificity against CSFV and is relatively stable across isolates (Bauhofer *et al*., 2005). However, due to its limited sequence length and low variation, its informative value for genotyping and antigenic variation analysis is limited (Chen *et al*., 2010a). Therefore, Npro is more often used as an additional target in multiplex systems to improve detection reliability (Huang *et al*., 2025).

Based on the comparison of various genomic targets, the E2 gene is recommended as the primary target for applications requiring both detection and genetic characterization of CSFV, such as epidemiological surveillance, outbreak tracking, and viral evolution studies (Risatti *et al*., 2005). On the other hand, the 5′UTR and non-structural genes such as NS5A/B or Npro are more suitable as supporting or confirmatory targets, especially for early detection with high sensitivity (Qi *et al*., 2022). Diagnostic strategies that combine the E2 gene with one or more other molecular targets in a multi-target or multiplex system offer a balanced approach, increasing sensitivity, specificity, and informative value in CSFV detection (Chakraborty *et al*., 2018).

Clinical and epidemiological applications

The use of the Classical Swine Fever Virus detection and characterization method targeting the E2 gene plays a strategic role in supporting rapid diagnosis, epidemiological monitoring, and decision-making for disease control in the field.

Use in rapid diagnosis

The use of the E2 gene as a target for rapid CSFV diagnosis plays a crucial role in veterinary clinical practice and disease surveillance programs (Ma *et al*., 2023). Molecular detection methods targeting the E2 gene, such as real-time PCR, multiplex RT-PCR, and isothermal techniques, enable rapid and specific identification of viral RNA in clinical samples from suspected infected animals (Wang *et al*., 2025a). This speed and accuracy of detection are crucial in the clinical setting, as CSF symptoms are often nonspecific and can mimic other viral diseases in pigs

(Rauloand Lyytikäinen, 2007). By focusing on the E2 gene, which is consistently expressed during viral replication, diagnosis can be made even at an early stage of infection before clinical symptoms are apparent (Risatti *et al*., 2005).

In surveillance activities, the application of methods targeting the E2 gene supports the early identification of CSFV circulation in domestic and wild pig populations (Tsai *et al*., 2025). This rapid detection allows the identification of subclinical or latent infections that could potentially serve as sources of viral spread (Robert *et al*., 2024). Molecular data obtained from E2 gene-based surveillance can also be used to map the distribution of viral genotypes, providing critical information for monitoring the spatial and temporal dynamics of CSFV epidemiology (Jiang *et al*., 2013). This approach strengthens early warning systems and enhances preparedness for potential outbreaks (Beer *et al*., 2015).

The role of the E2 gene in rapid diagnosis is increasingly important to support the control of Classical Swine Fever outbreaks (Dewulf *et al*., 2000). Rapid and accurate molecular confirmation allows for the timely implementation of control measures, such as animal movement restrictions, quarantine, and selective culling in affected areas (Wang *et al*., 2020b). Furthermore, genetic information from the E2 gene can be used to assess familial relationships among outbreak cases, making it easier to distinguish between the introduction of a new virus and subsequent spread from a common source (Chakraborty *et al*., 2018). Therefore, the use of the E2 gene in rapid diagnosis serves not only as a clinical confirmation tool but also as a crucial component of a CSFV outbreak control strategy supported by scientific evidence and molecular epidemiological analysis (Moennig *et al*., 2003).

Monitoring of genetic variation

Monitoring CSFV genetic variation is crucial for understanding the virus's evolution and spread patterns, both locally and globally (Brown and Bevins, 2018). The E2 gene, which encodes the major surface glycoprotein, is a key target for genetic variation studies due to its vital role in antigenicity and the virus's interaction with the host immune system (Zhang *et al*., 2025b). Nucleotide and amino acid differences in the E2 gene allow for the identification of CSFV strains down to the genotype and subgenotype levels, providing high resolution for phylogenetic analysis (Jang and Lee, 2025). E2 gene-based approaches are widely used to trace the origins of outbreaks, map transmission pathways, and distinguish between endemic viral spread and the introduction of new strains into a region (Mukherjee *et al*., 2023).

Tracking CSFV strains through E2 gene sequence analysis provides important insights into changes in the viral population over time (Hu *et al*., 2016). Observed genetic mutations may reflect selection pressures caused by environmental conditions, pig population density, or control interventions such as vaccination and animal movement restrictions (Ji *et al*., 2014). E2-based phylogenetic data allow for the clustering of circulating virus isolates, facilitating the identification of epidemiological clusters and familial relationships among cases (Jiang *et al*., 2013). This information is invaluable in outbreak investigations, supporting evidence-based decision-making regarding the source of infection and the effectiveness of implemented control strategies (Blome *et al*., 2017).

In addition to strain tracking, E2 gene variation analysis also has a direct impact on the development and assessment of vaccination strategies (Huynh *et al*., 2024b). The E2 gene serves as the primary antigen that triggers neutralizing antibody responses, so changes in epitope sequences can affect the antigenic match between vaccine strains and viruses circulating in the field (Chen *et al*., 2010b). Continuous monitoring of E2 gene variation allows the identification of mutations that could potentially reduce vaccine effectiveness, particularly in areas where live attenuated vaccines are widely used (Xu *et al*., 2020). This data provides the scientific basis for adjusting vaccination policies, selecting more antigenically matched vaccine strains, and assessing the risk of vaccination failure in the field (Luo *et al*., 2017).

Future prospects

Advances in molecular technology offer opportunities to improve the accuracy, speed, and robustness of CSFV diagnostic systems targeting the E2 gene (Tong *et al*., 2020). One promising development is the use of new-generation detection technologies that overcome the limitations of conventional methods (Wei *et al*., 2021). For example, digital PCR allows absolute quantification of viral RNA without the need for a standard curve, thus increasing the precision in detecting low viral loads in the early stages of infection or subclinical cases (Bianconi *et al*., 2025). This technology is also more tolerant of variations in amplification efficiency, a key advantage in analyzing the E2 gene, which exhibits sequence variation between strains (Chakraborty *et al*., 2018). Furthermore, innovations in real-time sequencing based on portable devices enable E2 gene characterization directly in the field, accelerating genotype identification and supporting rapid response to outbreaks without relying entirely on reference laboratories (McDowell *et al*., 2025).

The integration of artificial intelligence (AI) and advanced bioinformatics methods is expected to further enhance the role of the E2 gene in CSFV diagnosis and surveillance (Nazerke *et al*., 2025). AI-based analysis can predict E2 gene mutation patterns by leveraging historical sequence data and viral evolutionary trends, helping to anticipate the emergence of variants that could potentially reduce the sensitivity of diagnostic assays (Hamelin *et al*., 2025). Furthermore, machine learning algorithms can be used to automatically design primers and probes that account for global sequence diversity, resulting in assays that are more robust to genetic variation (Xia *et al*., 2022). This approach also enables the integration of molecular data with epidemiological and geographic information, creating a more adaptive and evidence-based predictive surveillance system (Blome *et al*., 2017). In the future, the success of E2 gene-based CSFV detection will depend heavily on the implementation of international standardization (Gopinath *et al*., 2024). Harmonization of diagnostic protocols across countries and laboratories is crucial to ensure data comparability and result reliability, particularly in the context of global trade and cross-border disease control (Wang *et al*., 2020a). This standardization includes the selection of E2 gene target regions, recommended primer and probe designs, assay validation procedures, and the use of internationally calibrated control materials (Chakraborty *et al*., 2018). International organizations and reference laboratories play a crucial role in formulating technical guidelines that remain methodologically consistent yet flexible (Khairullah *et al*., 2024). With the support of cutting-edge technology, AI integration, and a global standardization framework, the use of the E2 gene as a CSFV diagnostic target is expected to become increasingly efficient in supporting a modern, rapid, and sustainable disease control system (Suryawanshi *et al*., 2025).

Conclusion

CSFV remains a serious threat to the swine industry worldwide, necessitating rapid, precise, and informative diagnostic methods. Based on the developments reviewed in this article, the E2 gene has proven to be the most relevant molecular target for CSFV detection and characterization because it acts as a major structural glycoprotein, influences the antigenic properties of the virus, and reflects genetic variation between genotypes and subgenotypes. The use of the E2 gene has increased the sensitivity and specificity of molecular diagnostic methods, while supporting phylogenetic analysis and epidemiological tracking of outbreaks with greater precision.

The development of diagnostic technologies utilizing the E2 gene, including real-time PCR, isothermal amplification techniques, and sequencing methods, has expanded the diagnostic role from mere clinical confirmation to a strategic tool for disease surveillance and control.

Furthermore, information on E2 genetic variation plays a crucial role in assessing vaccine effectiveness and monitoring the dynamics of viral evolution in the field. However, challenges such as sequence variation, limited facilities, and the need for standardization remain to be addressed through the development of multi-target methods, updated assay designs, and harmonization of diagnostic protocols.

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Conflict of interest

The authors declare that there are no commercial or financial relationships that could be construed as a potential conflict of interest in the conduct and publication of this work.

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