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Optimizing transient transfection conditions in mammalian cell production lines for expression of classical swine fever virus E2 antigen

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ABSTRACT

Introduction. Despite no cases of classical swine fever (CSF) have been recorded in the Russian Federation since 2021, gaining official recognition, as a disease-free zone, will require adoption of effective, safe vaccines compatible with the DIVA strategy. A range of expression systems is being evaluated as potential platforms for a recombinant subunit vaccine; synthesizing the E2 antigen in mammalian cells appears to be a particularly promising approach.

Objective. Optimizing transient transfection conditions in mammalian cell production lines for expression of classical swine fever virus (CSFV) E2 antigen.

Materials and methods. The nucleotide sequence encoding a 188-amino acid fragment of the E2 antigen was cloned into the pVAX1 vector. Transient transfection was performed using two common methods – calcium-phosphate and cationic (employing branched polyethylenimine, PEI) – on three established mammalian production cell lines: CHO-K1, PK-15, and BHK-21/13. Expression efficiency was controlled using immunofluorescence, quantitative reverse transcription polymerase chain reaction, and enzyme-linked immunosorbent assay.

Results. It was determined that all the cell lines evaluated underwent transfection with an efficiency ranging from 60 to 90%. Cellular viability 24 hours post-transfection was at least 87%, with the lowest rates observed following calcium-phosphate transfection using an initial 12-hour incubation period. In all cases, transfection was accompanied by expression of specific messenger RNAs. The highest yield of the 17.3 kDa recombinant E2 protein was achieved in the CHO-K1 cell line (up to 47.4 mg/L), while the lowest yield was observed in the BHK-21/13 line (up to 24.1 mg/L). The specificity ratio in the antigen variant of the indirect enzyme-linked immunosorbent assay using specific antisera ranged from 5.1 to 6.2 units for all the expressed protein variants.

Conclusion. All the cell lines presented in the study demonstrated satisfactory transfection efficiency. Combined with their properties – such as high proliferation rates and adaptation to serum-free media – this makes them suitable for stable expression. Both the calcium-phosphate and cationic methods provide high transfection efficiency, relatively low cytotoxicity, and good reproducibility. The combined use of these control methods is advisable during the design phase of expression systems. In a production setting, however, the primary metric of their functionality is the overall yield of the specific recombinant protein, as determined by antigen-specific enzyme-linked immunosorbent assay.

Keywords: classical swine fever, mammalian cells, transient gene expression, recombinant antigen, immunofluorescence, messenger RNAs, enzyme-linked immunosorbent assay

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Оптимизация условий транзientной трансфекции производственных линий клеток млекопитающих для экспрессии антигена E2 вируса классической чумы свиней

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РЕЗЮМЕ

Введение. Несмотря на отсутствие в Российской Федерации с 2021 г. зарегистрированных случаев классической чумы свиней, для получения статуса зоны, свободной от данного заболевания, необходимо внедрение эффективных и безопасных вакцин, соответствующих стратегии DIVA. В качестве потенциальных инструментов для создания рекомбинантной субъединичной вакцины рассматриваются различные системы экспрессии; перспективным представляется синтез антигена E2 в клетках млекопитающих.

Цель исследования. Оптимизация условий транзientной трансфекции производственных линий клеток млекопитающих для экспрессии антигена E2 вируса классической чумы свиней.

Материалы и методы. Нуклеотидная последовательность, кодирующая фрагмент антигена E2 протяженностью 188 а. о., была клонирована в вектор pVAX1. Транзientная трансфекция проводилась двумя общедоступными методами: кальций-фосфатным и катионным (при помощи разветвленного полиэтиленimina) – в отношении трех производственных клеточных линий млекопитающих: СНО-К1, РК-15, ВНК-21/13. Контроль эффективности экспрессии осуществлялся методами иммунофлуоресценции, количественной полимеразной цепной реакции с обратной транскрипцией, иммуноферментного анализа.

Результаты. Было установлено, что все рассматриваемые клеточные линии подвергались трансфекции с эффективностью от 60 до 90%. Выживаемость клеток через 24 ч после проведения трансфекции составляла не менее 87%, наименьшие показатели регистрировались при проведении кальций-фосфатной трансфекции с первичной инкубацией 12 ч. Проведение трансфекции во всех случаях сопровождалось экспрессией специфических матричных РНК. Наибольший выход рекомбинантного белка E2 молекулярной массой 17,3 кДа был характерен для линии СНО-К1 (до 47,4 мг/л), наименьший – для линии ВНК-21/13 (до 24,1 мг/л). Коэффициент специфичности в антигенном варианте непрямого иммуноферментного анализа со специфическими антисыворотками для всех вариантов экспрессируемого белка варьировал в диапазоне 5,1–6,2 ед.

Заключение. Все представленные в исследовании клеточные линии обладали удовлетворительной трансфицируемостью, что в совокупности с их свойствами (высокой скоростью пролиферации, адаптацией к бессывороточным средам) позволяет использовать их для стабильной экспрессии. И кальций-фосфатный метод, и катионный обеспечивают высокую эффективность трансфекции, относительно низкую цитотоксичность и воспроизводимость. Применение рассматриваемых методов контроля целесообразно в совокупности на этапе конструирования экспрессионных систем, однако в производственных условиях основным критерием их функциональности является тотальный выход специфического рекомбинантного белка, регистрируемый при помощи антигенного иммуноферментного анализа.

Ключевые слова: классическая чума свиней, клетки млекопитающих, транзientная экспрессия генов, рекомбинантный антиген, иммунофлуоресценция, матричные РНК, иммуноферментный анализ

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INTRODUCTION

Classical swine fever (CSF), caused by the RNA-containing *Pestivirus C*, is one of the most dangerous viral diseases of the *Suidae* family and is accompanied by hemorrhagic syndrome [1, 2]. Classical swine fever virus (CSFV) primarily targets endothelial cells and macrophages. Concurrently, it triggers T-cell apoptosis, leading to significant immunosuppression. In most cases, the infection follows an acute course, culminating in one of three outcomes: fatality, recovery with the development of virus-neutralizing antibodies, or progression to a chronic infection [3, 4]. In countries where CSF is endemic, effective live attenuated vaccines are commonly used. However, the ability of vaccine strains to replicate within the host complicates differentiating vaccinated animals from infected ones (the DIVA strategy).

This challenge underlies the restrictive vaccination policies adopted in non-endemic regions. Thus, within the Russian Federation, there is a clear need to develop effective and safe marker vaccines. Such vaccines are essential for controlling potential CSF outbreaks and are a prerequisite for eventually achieving CSF-free zone status, officially recognized by the World Organisation for Animal Health [5, 6].

In the context of developing experimental subunit vaccines, various expression systems have been successfully employed for the biosynthesis of the major glycoprotein E2. We previously generated a prokaryotic equivalent of glycoprotein E2 [7]. Its synthesis predominantly resulted in inclusion body formation, necessitating extensive chromatographic purification and refolding steps. Consequently, the yield of purified, bioactive product was relatively low.

As an alternative to *Escherichia coli* expression, gene expression in mammalian cells can be considered. Its primary advantages are post-translational modifications closely resembling the native ones, along with recombinant protein solubility and folding compatible with high-level overexpression [8]. To facilitate future scale-up, mammalian cell expression must be cost-effective and high yielding. This necessitated development of an optimized genetic construct, selection of a suitable production cell line as a potential producer of the recombinant vaccine antigen, and adaptation of established transfection protocols to meet specific requirements of this bioprocess.

The objective of this study was to optimize transient transfection conditions in mammalian production cell lines for CSFV E2 antigen expression. The following tasks were completed to achieve this objective:

- assess suitability of CHO-K1, PK-15, and BHK-21/13 cell lines for transient expression of a gene encoding a fragment of glycoprotein E2;
- compare efficiency of widely available non-viral DNA delivery methods (calcium-phosphate and cationic transfection);
- validate developed methods for monitoring expression efficiency: immunofluorescence assay (IFA) on cell culture, quantitative assessment of messenger RNA (mRNA), and antigen-capture enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Generation of recombinant DNA. A nucleotide sequence encoding a truncated E2 protein Shimen strain of CSFV (GenBank ID AF092448.2) was cloned into pVAX1 vector (Evrogen, Russia) using *Bam*HI and *Eco*RI restriction sites. Sanger sequencing confirmed the identity of the generated construct. The recombinant plasmid was transformed into *E. coli* strain DH5 α (Novagen, Germany), amplified, and isolated using a Midiprep system (Evrogen, Russia). Plasmid concentration was measured using a Nano-500 spectrophotometer (Allsheng, China). Sample purity was assessed by the absorbance ratio at 260 and 280 nm; a ratio between 1.8 and 2.0 indicates an absence of polypeptide or free-nucleotide contamination.

Cell culture. Monolayers of Chinese hamster ovary (CHO-K1, ATCC[®] CCL-61), Syrian hamster kidney (BHK-21/13, ATCC[®] CCL-10), and porcine kidney (PK-15, ATCC[®] CCL-33) cells were cultivated in 75 mL flasks and 100 mm diameter plates (Biologix Group Ltd., China). The growth medium consisted of DMEM-F12 (PanEco company, Russia) supplemented with 10% fetal bovine serum (HyClone, Australia), 100 IU/mL penicillin and streptomycin (PanEco company, Russia), and 20 mM L-glutamine (Sigma-Aldrich, USA). Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO₂, starting at an initial concentration of 10 × 10⁶ cells/mL. Routine contamination screening was performed. No contamination by bacteria, mycoplasma, or bovine viral diarrhea virus was detected.

Transfection process. The conditions for calcium-phosphate (CaP) and cationic (PEI) transfection of the production cell lines are detailed in the Table.

Cell viability was assessed 24 hours post-transfection in 96-well culture plates (Corning Incorporated, USA) using a colorimetric methyl thiazole tetrazolium (MTT) assay. To each well containing 100 μ L of fresh culture medium, 10 μ L of MTT reagent (Wuhan Servicebio Technology Co.,

Ltd., China) were added. The plates were then incubated for 3.5 hours under standard conditions. The formazan crystals that formed were dissolved by adding an equal volume of dimethyl sulfoxide. The absorbance in each well was then measured at a wavelength of 570 nm. The percentage of viable cells was calculated from the ratio of wells containing transfected cells to the wells with the intact cells.

Assessment of messenger RNA expression levels. The transcriptional activity of the E2 gene fragment was assessed by quantitatively determining its product – specific mRNAs. Nucleic acids were extracted from transfected cells using the RIBO-sorb reagent kit (Central Research Institute of Epidemiology of Rospotrebnadzor, Russia). The isolated samples were treated with DNase (Syntol, Russia) at a ratio of 1 unit per 10 ng of DNA and incubated for 1 hour at 37 °C, followed by enzyme inactivation at 80 °C for 5 minutes. For amplification, the following reaction mixture was prepared (per one 20 μ L sample): 4 μ L of prepared PCR 5 \times qPCRmix-HS SYBR premix, 5 pM each of forward (5'-CGTCAACCAATGAGATAGGGCTGT-3') and reverse (5'-GCACAGCCCGAATCGAAGT-3') primers, 100 units of MMLV reverse transcriptase (Evrogen, Russia), 15 ng of RNA template, and ddH₂O to a final volume of 20 μ L. A ten-fold serial dilution of the recombinant plasmid pVAX1-trE2 was used to generate a calibration curve. Real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) was performed using a C1000 thermocycler with a CFX96 optical module (Bio-Rad Laboratories, Inc., USA) according to the following program: 1 – reverse transcription at 37 °C for 5 minutes; 2 – DNA denaturation at 95 °C for 5 minutes; 3 – 35 cycles of: 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 25 seconds; 4 – final elongation at 72 °C for 10 minutes. Melting curve analysis of the amplicons was performed using the CFX Manager software (Bio-Rad Laboratories, Inc.,

Table
Transfection conditions on culture plates (\varnothing 100 mm)

Indicator	Calcium phosphate transfection (CaP method)	Cationic transfection (PEI method)
Seeding density	(6 – 10) × 10 ⁵ cells/mL	
Cell confluency at the time of transfection (%)	70	70–85
Mixture composition:		
Solution A	2.5 M CaCl ₂ – 30 μ L Plasmid DNA – 10–20 μ g ddH ₂ O – up to 300 μ L	Serum-free medium – 100 μ L Plasmid DNA – 2–4 μ g
Solution B	HBS buffer, pH 7.05 – 300 μ L Full medium – up to 5–6 mL	Serum-free medium – 100 μ L PEI – 6–16 μ L
Complex formation	Solution A was added drop by drop to solution B (3–5 min at room temperature)	Solution B was added drop by drop to solution A (15 min at room temperature)
Duration of incubation	6–12 hours	4–12 hours
Posttransfection cultivation	48–72 hours	48–72 hours

USA). The number of mRNA copies in each sample was normalized and is presented per 1 µg of the total RNA.

Immunofluorescence assay on cell culture. Transfected cells were stained with an FITC-conjugated polyclonal swine antiserum to CSFV (Federal Center for Toxicological, Radiation and Biological Safety) at a working dilution of 1:500, as previously described by the authors [9].

Isolation of the target protein. 48–72 hours after transfection, cells were mechanically removed from the surface of the culture vials using scrapers. Cells from the suspension were pelleted by centrifugation at 3,000 g for 15 minutes. The pellet was resuspended in 1 mL of chilled RIPA buffer (50 mM Tris-HCl, pH 7.4; 150 mM NaCl; 1 mM ethylenediaminetetraacetic acid; 1% Triton X-100; 0.1% sodium dodecyl sulfate; 0.1 mM phenylmethylsulfonyl fluoride) and incubated on ice for 30 minutes. Subsequently, the cells were pelleted again by centrifugation. Protein presence in the supernatant was confirmed by analytical electrophoresis on a 15% polyacrylamide gel under denaturing conditions. Reactivity with specific antisera was assessed in ELISA using a previously developed test system designed to monitor the specificity of the recombinant CSFV antigen [10].

Statistical analysis was performed using GraphPad Prism 10.4 software (GraphPad Software, USA).

RESULTS AND DISCUSSION

Bioinformatic analysis identified a 188-amino acid fragment (residues 690–878) characterized by the highest density of CSFV-specific B-cell epitopes. According to pBLAST analysis results, the sequence identity of this fragment among representatives of CSFV genotypes 1 and 2 ranges from 91.19 to 98.74%. Furthermore, it exhibits no significant homology with the corresponding region of the bovine viral diarrhea virus proteome, which is a critical factor in selecting a diagnostic antigen sequence. The final amino acid sequence, flanked by a hexahistidine tag, was reverse-translated. The synthesized nucleotide sequence, containing a Kozak consensus sequence upstream of the start codon, was then cloned into the target pVAX1 vector. The construct scheme is given in Figure 1.

Cell viability was assessed 24 hours after transfection in the CHO-K1, PK-15, and BHK-21/13 cell lines (Fig. 2).

The chart shows that the percentage of viable cells following both transfection methods, under the specified incubation periods with the transfection agents, was at least 87%. Furthermore, cell viability exceeded 93.6% when using cationic transfection with incubation period of 6–12 hours, as well as with CaP transfection when the primary incubation period was limited to no more than 6 hours. Lower viability rates (87.2–92.8%) were recorded for CaP transfection when the primary incubation was extended up to 12 hours. This is likely attributable to the cytotoxic effect exerted by calcium precipitates during extended incubation periods.

The next phase of the study involved a comparative assessment of various methods for monitoring transfection efficiency, specifically direct IFA on transfected cells, quantitative mRNA analysis in real-time RT-PCR, and antigen-capture ELISA.

According to IFA performed on cell cultures, transfection efficiency at 48 hours post-transfection varied from 60 to 90%. The highest efficiency was recorded in monolayers of the CHO-K1 line: 87% for CaP transfection and 90% for PEI transfection. A slightly lower proportion of fluorescent

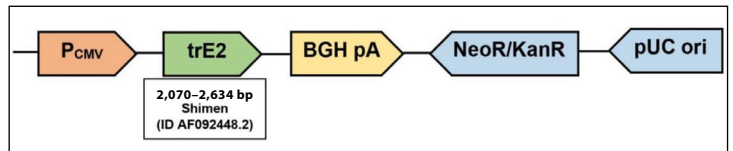


Fig. 1. Structure of the genetic construct based on the pVAX1 vector: P_{CMV} – cytomegalovirus promoter; trE2 – sequence encoding the truncated E2 protein of CSFV (nucleotides 2,070–2,634 of the Shimen strain); BGH pA – bovine growth hormone polyadenylation signal; NeoR/KanR – genes conferring resistance to selective antibiotics (neomycin and kanamycin); pUC ori – origin of replication

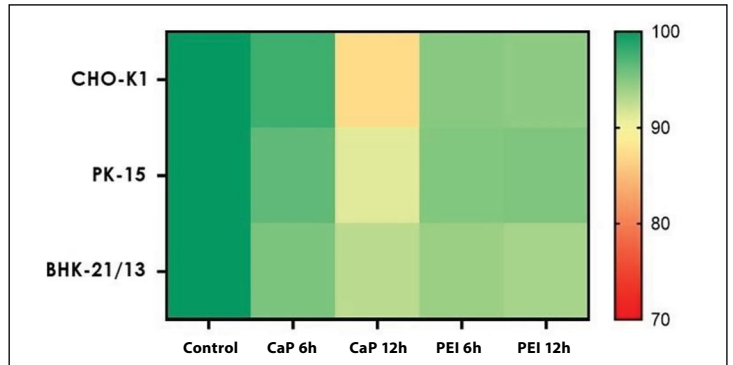


Fig. 2. Cell viability results for production cell lines 24 hours after calcium phosphate transfection and PEI using MTT assay

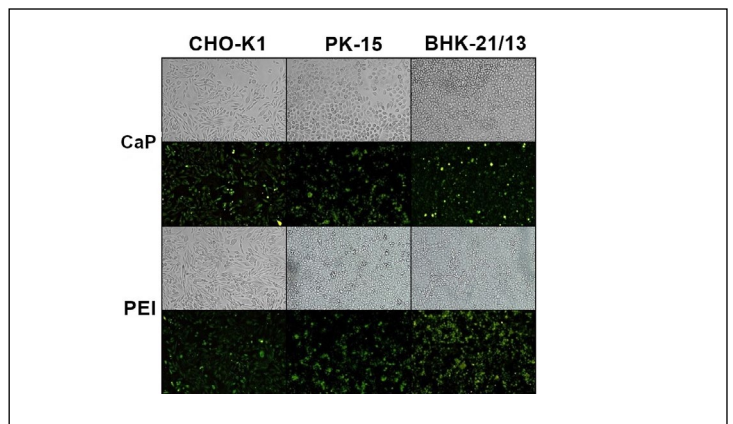


Fig. 3. Fluorescence-based monitoring of transfection (48 hours) in production cell lines

cells was observed for the PK-15 line: 82% for CaP and 85% for PEI transfection. The lowest efficiency was observed for the BHK-21/13 line: 80 and 65% for CaP and PEI transfection, respectively (Fig. 3).

In addition to IFA, which serves as a fundamental method for assessing transfection efficiency, an analysis of mRNA expression was conducted. It is established that measurements based on protein and mRNA levels are complementary and essential for a comprehensive assessment of gene expression. The real-time RT-PCR protocol was initially validated using a ten-fold serial dilution of the control plasmid pVAX1-trE2 (starting concentration 50 ng/µL). It was determined that the linearity of the reaction (R^2) was

0.994, the slope (Ct threshold between two DNA dilutions) was -3.748 , the theoretical limit of detection (y-intercept) was 29.288, and the reaction efficiency (E) was 94.8%. The results of specific mRNA level quantification, determined by real-time RT-PCR, are given in Figure 4.

It is known that within a cell, the ratio of protein to mRNA is governed by translation and protein degradation processes, which are regulated at the gene-specific level [11]. The overall correlation between mRNA and protein concentrations in multicellular eukaryotes is considered significant but moderate compared to that in bacteria and yeasts: the number of protein molecules per transcript can vary substantially [12, 13]. Therefore, a more objective assessment of the expression system's functionality, particularly in a production setting, is the quantitative protein yield coupled with the determination of its specificity using an accessible serological method (Fig. 5).

According to the calculated data, the rE2 protein, with a predicted molecular mass of 17.3 kDa, is stable and soluble. These parameters did not differ among the protein variants expressed by all three producer lines, which may indicate similar patterns of post-translational modifications. This is supported by the comparable activity of all protein variants in ELISA, with a positiveness degree ranging from 5.1 to 6.2. The highest recombinant protein yield was achieved using the CHO-K1 line (35.6–47.4 mg/L), while the lowest yield was observed with BHK-21/13 (19.3–24.1 mg/L). This finding aligns with the established influence of cell origin on transfection efficiency under comparable conditions [14]. Although cationic transfection demonstrated greater efficiency in terms of transcript levels, the CaP transfection method yielded an average of $(8.7 \pm 1.4)\%$ more functional product.

Literature describes production of the recombinant glycoprotein E2 of CSFV in mammalian cells. For instance, in the work of R.-H. Hua et al. [15], the generation of a stable BHK-21 cell line using the pCAG vector is reported, achieving a productivity of up to 45 mg/L. The recombinant protein provided 100% protection to pigs upon lethal challenge. Tian H. et al. [16] successfully created a truncated E2 protein containing CSFV-specific domains, which induced seroconversion in rabbits, i.e. in PK-15 cells transfected with

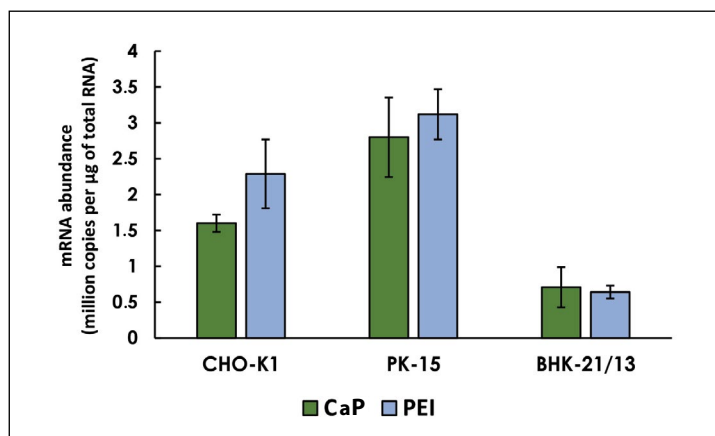


Fig. 4. Quantitative assessment of specific mRNA expression

the retroviral vector pBABE puro. The use of the CHO cell line also enabled significant yields of recombinant E2: L. Feng et al. [17] generated stable rCHO transgenic cells with a dynamic Txnip promoter, forming the basis for an inducible expression strategy. Thus, mammalian cell expression is an effective tool for the large-scale biosynthesis of complex glycoproteins and is applicable in the development of candidate subunit vaccines [18, 19, 20].

CONCLUSION

This study investigated the application of standard transfection methods on several mammalian production cell lines for the generation of the recombinant CSFV E2 antigen, along with methods for monitoring expression efficiency. It was established that the CHO-K1, PK-15, and BHK-21/13 cell lines are successfully transfected under standard conditions with an efficiency of up to 90%. Among these, the CHO-K1 line demonstrated the highest transfection efficiency as well as the highest overall yield of the target protein. Given that all cell lines considered are characterized by high proliferation rates, ease of cultivation (including in suspension), and adaptation to serum-free

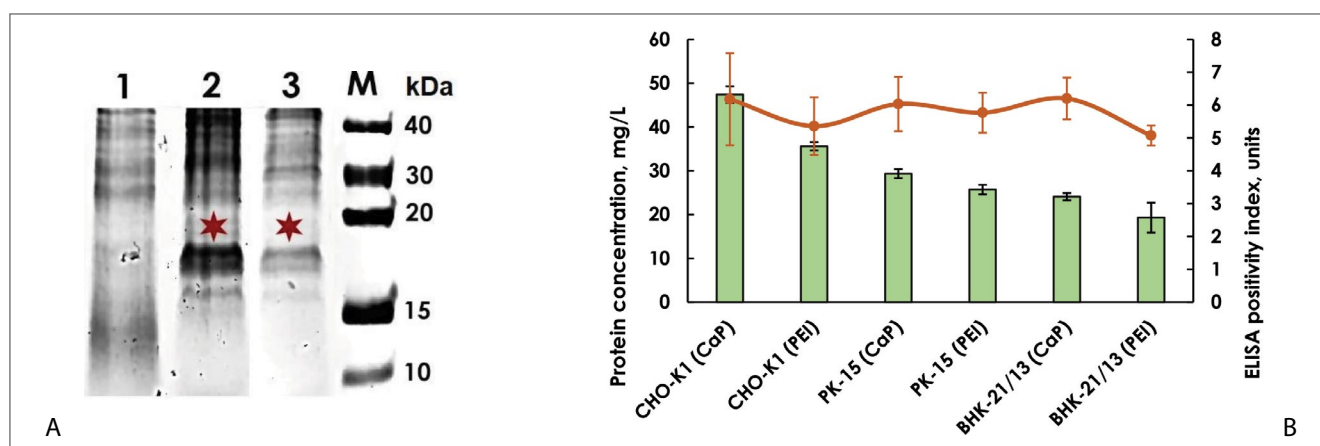


Fig. 5. Functional analysis of the expression systems based on the pVAX1 vector and cell lines CHO-K1, PK-15, and BHK-21/13.

A – electrophoretogram of cell lysates from the primary producer (CHO-K1): lane 1 – negative control (lysate of cells transfected with the original pVAX1 plasmid without insert); lanes 2 and 3 – lysates of cells transfected with the pVAX1-E2 plasmid (target polypeptide fraction marked with *); M – molecular weight marker Prestained Protein Marker IV (Wuhan Servicebio Technology Co., Ltd., China); B – assessment of the quantitative yield and specificity of the recombinant truncated E2 protein (ELISA data presented for antigen solutions at $1 \mu\text{g}/\text{mL}$ concentration)

media, they possess significant potential for generating clonal producer lines tailored to specific bioprocess requirements. It was also established that both transfection methods examined – CaP and PEI – provided sufficiently high transfection efficiency and comparable yields of the target protein. Their advantages include cost-effectiveness and reproducibility.

A comparative assessment of transfection efficiency monitoring methods – IFA, quantitative PCR, and indirect ELISA – demonstrated that their combined application is advisable during the expression system design phase. While conducting IFA with FITC-labeled polyclonal antibodies provides valuable information during the initial stages of protein synthesis, it necessitates continuous screening of cell lines and fetal bovine sera for contamination with bovine viral diarrhoea virus, which can lead to false-positive results. Quantitative mRNA detection does not always correlate with target product yield; therefore, the use of this method is justified primarily for an initial assessment of gene transcriptional activity. In a production setting, the most reliable indicator is the total yield of the target protein. This is conveniently monitored using quantitative or semi-quantitative ELISA, particularly when working with stable expression cell lines.

Although this study utilized a nucleotide sequence encoding a truncated antigen intended for diagnostic purposes – specifically, a fragment of the E2 glycoprotein with the highest density of CSFV-specific domains – the described approaches are applicable to the development of a recombinant subunit vaccine against CSFV. This application is viable provided there is a well-designed construct encoding the full-length glycoprotein.

REFERENCES

- Mahadevaswamy R., Muruganatham V., Ramesh V., Mambully S., Suresh K. P., Hiremath J., et al. Global population dynamics and evolutionary selection in classical swine fever virus complete genomes: insights from Bayesian coalescent analysis. *Virus Genes*. 2025; 61 (4): 464–473. <https://doi.org/10.1007/s11262-025-02154-2>
- Huang Y.-C., Deng M.-C., Huang Y.-L., Tsai K.-J., Liu H.-M., Liu I.-L., et al. Classical swine fever virus genotype 2.1 triggers stronger inflammatory and immune responses in porcine alveolar macrophages than genotype 3.4. *Developmental and Comparative Immunology*. 2025; 172:105496. <https://doi.org/10.1016/j.dci.2025.105496>
- Blome S., Staubach C., Henke J., Carlson J., Beer M. Classical swine fever – an updated review. *Viruses*. 2017; 9 (4):86. <https://doi.org/10.3390/v9040086>
- Yamashita M., Iwamoto S., Ochiai M., Yamamoto A., Sudo K., Narushima R., et al. Pathogenicity of genotype 2.1 classical swine fever virus isolated from Japan in 2019 in pigs. *Microbiology and Immunology*. 2024; 68 (8): 267–280. <https://doi.org/10.1111/1348-0421.13160>
- Ganges L., Núñez J. I., Sobrino F., Borrego B., Fernández-Borges N., Frías-Lepoureau M. T., Rodríguez F. Recent advances in the development of recombinant vaccines against classical swine fever virus: cellular responses also play a role in protection. *The Veterinary Journal*. 2008; 177 (2): 169–177. <https://doi.org/10.1016/j.tvjl.2007.01.030>
- Alekseev K. P., Zaberezhnyj A. D., Verkhovskij O. A., Musienko M. I., Shemelkov E. V., Yuzhakov A. G., Aliper T. I. Labeled subunit vaccine against classical swine fever, method of its preparation and use. Patent No. 2808703 Russian Federation, Int. Cl. A61K 39/187, C12N 7/00. OOO “Vetbiokhim”. No. 2023105741. Date of filing: 13.03.2023. Date of publication: 01.12.2023. Bull. No. 34. (in Russ.)
- Galeeva A. G., Akhunova A. R., Khammadov N. I., Yarullina G. M., Efimova M. A. Heterologous expression of recombinant classical swine fever virus and porcine circovirus type 2 antigens. *Veterinariya*. 2024; (10): 25–31. <https://doi.org/10.30896/0042-4846.2024.27.10.25-31> (in Russ.)
- Hopkins R. F., Wall V. E., Esposito D. Optimizing transient recombinant protein expression in mammalian cells. In: *Protein Expression in Mammalian Cells. Methods in Molecular Biology*. Ed. by J. L. Hartley. Vol. 801. Frederick: Humana Press; 2012; Chapter 16: 251–268. https://doi.org/10.1007/978-1-61779-352-3_16
- Ahunova A. A., Nasyrov Sh. M., Galeeva A. G., Arutyunyan G. S., Efimova M. A., Gulyukin M. I. Application of direct fluorescent antibodies test in process control of classical swine fever virus master seeds. *Veterinarian*. 2024; (3): 27–33. <https://elibrary.ru/mnswgm> (in Russ.)
- Nasyrov Sh. M., Akhunova A. R., Galeeva A. G., Yarullina G. M., Samerkhanov I. I., Andreeva A. V. Enzyme-linked immunosorbent assay for in-process control of the specificity of the CSF virus recombinant antigen. *Izvestia Orenburg State Agrarian University*. 2024; (3): 228–335. <https://doi.org/10.37670/2073-0853-2024-107-3-228-235> (in Russ.)
- Gebauer F., Hentze M. W. Molecular mechanisms of translational control. *Nature Reviews Molecular Cell Biology*. 2004; 5 (10): 827–835. <https://doi.org/10.1038/nrm1488>
- De Sousa Abreu R., Penalva L. O., Marcotte E. M., Vogel C. Global signatures of protein and mRNA expression levels. *Molecular BioSystems*. 2009; 5 (12): 1512–1526. <https://doi.org/10.1039/b908315d>
- Greenbaum D., Colangelo C., Williams K., Gerstein M. Comparing protein abundance and mRNA expression levels on a genomic scale. *Genome Biology*. 2003; 4 (9):117. <https://doi.org/10.1186/gb-2003-4-9-117>
- Kim T. K., Eberwine J. H. Mammalian cell transfection: the present and the future. *Analytical and Bioanalytical Chemistry*. 2010; 397 (8): 3173–3178. <https://doi.org/10.1007/s00216-010-3821-6>
- Hua R.-H., Huo H., Li Y.-N., Xue Y., Wang X.-L., Guo L.-P., et al. Generation and efficacy evaluation of recombinant classical swine fever virus E2 glycoprotein expressed in stable transgenic mammalian cell line. *PLoS ONE*. 2014; 9 (9):e106891. <https://doi.org/10.1371/journal.pone.0106891>
- Tian H., Liu X., Wu J., Shang Y., Jiang T., Zheng H., Xie Q. Expression of major antigen domains of E2 gene of CSFV and analysis of its immunological activity. *Virologica Sinica*. 2008; 23 (4): 247–254. <https://doi.org/10.1007/s12250-008-2956-5>
- Feng L., Chen L., Yun J., Cao X. Expression of recombinant classical swine fever virus E2 glycoprotein by endogenous Txnip promoter in stable transgenic CHO cells. *Engineering in Life Sciences*. 2020; 20 (8): 320–330. <https://doi.org/10.1002/elsc.201900147>
- Reinhart D., Damjanovic L., Kaisermayer C., Sommerer W., Gili A., Gasselhuber B., et al. Bioprocessing of recombinant CHO-K1, CHO-DG44, and CHO-S: CHO expression hosts favor either mAb production or biomass synthesis. *Biotechnology Journal*. 2019; 14 (3):e1700686. <https://doi.org/10.1002/biot.201700686>
- Opefi C. A., Tranter D., Smith S. O., Reeves P. J. Construction of stable mammalian cell lines for inducible expression of G protein-coupled receptors. *Methods in Enzymology*. 2015; 556: 283–305. <https://doi.org/10.1016/bs.mie.2014.12.020>
- Tossolini I., López-Díaz F. J., Kratje R., Prieto C. C. Characterization of cellular states of CHO-K1 suspension cell culture through cell cycle and RNA-sequencing profiling. *Journal of Biotechnology*. 2018; 286: 56–67. <https://doi.org/10.1016/j.jbiotec.2018.09.007>

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