Data Article

Data on production of mammalian stable cells expressing secretory BEFV transmembrane deleted G protein

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\section*{A B S T R A C T}

Generation of stable cell lines is a widely used technique for continuous recombinant protein production. Advantages of the constitutive stable over the transient protein expression are uniformity of the expression across cell populations as well as high quantity and consistency of the protein yields. This data describe step-by-step procedure for the production of glycoprotein without a transmembrane domain (G\textsuperscript{Δ}TM) of bovine ephemeral fever virus (BEFV) by mammalian stable cells. LentiX-293T cells were transfected with four plasmid constructs to generate a recombinant lentivirus. Subsequently, 293T cells were transduced by the recombinant virus and the polyclonal stable cell pools were then selected by puromycin. Next, limiting dilution was performed from each cell pool to isolate the monoclonal stable cells expressing G\textsuperscript{Δ}TM protein. Western blot analysis showed that all monoclonal cell clones could stably express G\textsuperscript{Δ}TM protein. The data confirms...
that the stable 293T cell line expressing the secretory GΔTM protein is an attractive platform for antigen production.

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1. Data

Generation of mammalian stable cells expressing BEFV GΔTM protein in secretory form resulted in constitutive expression of the target protein [1]. To produce the stable BEFV GΔTM secreting cells, recombinant lentiviral particles were generated and rescued by co-transfection of LentiX-293T cells with four plasmid constructs followed by determination of lentivirus titration. The lentivirus RNA copy number acquired from our experiment corresponded to a raw copy number of $1.2 \times 10^7$ copies by the qRT-PCR standard curve which equal to $4 \times 10^9$ copies/ml. This is typically 100-fold lower than normally observed in the infectious titer as measured by transduction units (TU/mL) [2]. Results of standard curve optimization and viral RNA copy number were shown in Tables 1 and 2, respectively. Subsequently, transduction of 293T cells with the recombinant lentivirus was performed and the transduced cells were selected by puromycin resistant characteristics. The optimal concentration of puromycin for the selection process was determined and it was 3 μg/ml (Fig. 1). Monoclonal stable cells secreting BEFV GΔTM were isolated from antibiotic resistant cell pools by the limiting dilution technique. All monoclonal stable cell clones stably expressing secretory BEFV GΔTM protein as examined by Western blot analysis (Fig. 2).
2. Experimental design, materials and methods

2.1. Production of recombinant lentivirus

pLVX_GΔTM construct used for transfection consisted of N-terminal BiP secretory signal sequence followed by GΔTM in which signal peptide, transmembrane domain, and cytoplasmic tail were deleted. To generate recombinant lentiviruses, LentiX-293T cells were seeded at 4.5 × 10⁶ cells in a 10 cm² plate and incubated overnight at 37 °C with 5% CO₂. The LentiX-293T cells were co-transfected with a
transfer vector containing G\(\Delta\)TM (pLVX_G\(\Delta\)TM) produced in our laboratory [1] and three helper plasmids including pMDLg/pRRE, pRSV-Rev, and pMD2.G [3] using polyethylenimine (PEI) transfecting reagent. The PEI stock solution was prepared by dissolving PEI (Sigma) in deionized water to reach 500 ng/\(\mu\)l. A total 4 \(\mu\)g of all four plasmid constructs including 1.2 \(\mu\)g of pMDLg/pRRE, 0.8 \(\mu\)g of pRSV_Rev, 0.4 \(\mu\)g of pMD2.G, and 1.6 \(\mu\)g of pLVX_G\(\Delta\)TM were diluted in 100 \(\mu\)l of 150 mM NaCl in a polypropylene tube. The PEI working solution (60 \(\mu\)g PEI per 1 \(\mu\)g DNA) was prepared by diluting 400 \(\mu\)l of the PEI stock in 350 \(\mu\)l of 150 mM NaCl. The PEI working solution was then transfer to the DNA tube. The DNA/PEI mixture was mixed gently and incubated at room temperature for 20–25 min. In the meantime, the culture medium was removed from the 10 cm\(^2\) plate containing LentiX-293T cells and gently replaced with 1 ml of OptiMEM (Gibco). Then, the DNA/PEI mixture was added into the culture plate in a drop wise manner and incubated at 37 °C with 5% CO\(_2\) for 5 h. After the incubation, the medium was removed and replaced with OptiMEM complete medium containing 10% FBS, 4% L-glutamine, and 1% streptomycin-ampicillin followed by further incubation for 48 h. The culture medium supernatant was then harvested and clarified by centrifugation at 2000 rpm for 10 min. The lentiviral titer was determined by quantitative RT-PCR (qRT-PCR).

### 2.2. Development of real time qRT-PCR for recombinant lentivirus detection

To generate GagP24 RNA transcript to be used as positive control for the recombinant lentivirus titration, GagP24 was amplified from pMDLg/pRRE plasmid [3] using the following primer pair:
BamHI_Gagp24_F (ATATGGATCCCATATAGTATGGGCAAGC) and XhoI_Gagp24_R (ATATCTCGAGTCTGGTACTGGTG). The PCR product was cloned into pGEMT-easy vector (Promega) to construct a recombinant plasmid containing GagP24 sequence (pGagP24). The sequence and direction of GagP24 were verified by DNA sequencing. Subsequently, the pGagP24 construct was cut with BamHI to linearize the plasmid which was the template to generate GagP24 RNA transcript by using T7 in vitro transcription kit (Promega) following the manufacturer instruction. The plasmid DNA was removed by incubation with 1 unit of DNasel (Promega) at 37 °C for 30 min followed by incubation with StopDNasel solution at 65 °C for 30 min. Then, ethanol precipitation was performed to concentrate the GagP24 RNA transcript [4]. GagP24 RNA copy number was calculated using an available online tool, NEBioCalculator™ (https://nebiocalculator.neb.com/#/ssrnaamt). For standard curve optimization, 500 ng of the GagP24 RNA transcript was 10-fold serially diluted from 50 ng to 5 fg and used as the template for qRT-PCR. The real time RT-PCR was performed in triplicate using iTaq universal SYBR green one-step kit (Bio-Rad) and GagP24 specific primers: Gagp24_F (CTGTTAGAAACATCAGAAGGCTG) and Gagp24_R (CACACAATAGAGGGTTGCTACTG). The thermal cycling protocol consisted of reverse transcription step at 50 °C for 10 min, polymerase activation and DNA denaturation step at 95 °C for 1 min, followed by 35 cycles of denaturation at 95 °C for 10 s and annealing/extension at 60 °C for 15 s. Melt-curve analysis was also performed at 65–95 °C with 0.05 °C increment.

2.3. Determination of optimal concentration of puromycin

Puromycin titration was performed to optimize the minimum concentration that completely killed all cells within 3–5 days. Initially, 293T cells were seed at 2 × 10⁵ cells/well in a 24-well plate and incubated overnight at 37 °C with 5% CO₂. On the next day, culture medium was removed from each well and then replaced with the culture medium containing puromycin (Sigma) at 0.5, 1, 3, 5, 8, 10, 25, 50, and 100 μg/ml, respectively. The medium was changed every 2–3 days and cell viability was examined every day under light microscope until all cells died.

2.4. Generation of a stable BEFV GΔTM secretory cell line

To generate stable cells expressing secretory GΔTM protein, 293T cells were transduced with the recombinant lentivirus. Initially, 293T cells were seeded at 1 × 10⁶ cells/well in a 6-well plate and incubated overnight. The recombinant lentivirus at multiplicity of infection of 5 was combined with OptiMEM medium containing 10 μg/ml polybrene (Sigma) before adding onto the 293T cells. At 48 h after the transduction, the culture supernatant was removed and replaced with a selecting culture medium containing 3 μg/ml puromycin (Sigma) to select stable protein expressing and antibiotic resistant cells. The selective culture medium was changed every 3 days until colonies of the stable protein expressing cells were observed. The puromycin survival cell clones were pooled for further clone isolation.

2.5. Isolation of monoclonal BEFV GΔTM secretory cell population

The pool of polyclonal stable cells that survived the puromycin selection was isolated by limiting dilution to obtain monoclonal stable cell lines. Briefly, the polyclonal stable cell pool was seeded into a T75 cm² cell culture flask to reach 60% confluent and incubated overnight. On the next day, the spent culture medium was collected and further used as a conditioned medium to enhance the single cell growth. The stable cell pool was separated into individual cells by trypsin treatment and pipetting up and down. The cells were enumerated by using a hemocytometer. The cell suspension was then diluted in the conditioned medium to reach the final concentration of 5 cells/ml. One hundred microliters of the cell suspension was seeded into each well of a 96-well plate and incubated at 37 °C with 5% CO₂. Eight days after incubation, wells containing a single colony were maintained while wells with more than a single colony were discarded. Each single colony was culture in the 96-well plate until confluent. Subsequently, the cells were culture in the selecting media while they were stepwise transferred to larger culture wells, e.g. from 48-, 24-, 12- and 6–well plates to culture flasks, respectively. During the
stable cells were cultured in 24-well plate, each of the monoclonal colonies was examined for the expression of GΔTM protein by Western blot analysis as described previously [5,6].

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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