

Fast and high yield recombinant proteins production by large-scale transfection of suspension-growing mammalian cells

SUMMARY

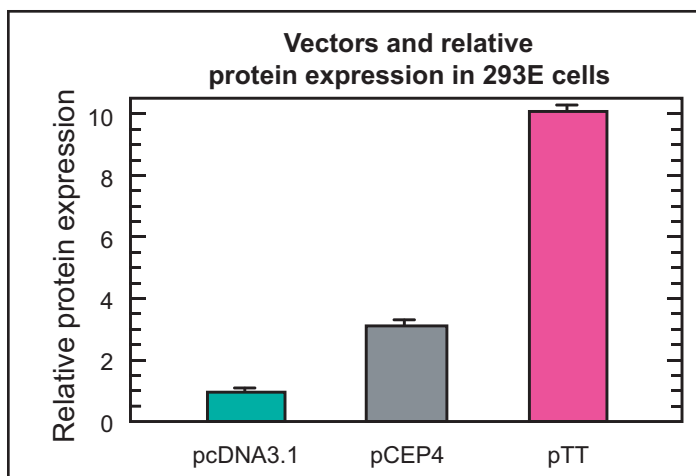
Mammalian expression systems allow for the production of active recombinant proteins (r-proteins) that possess relevant post-translational modifications. Current practices for the production of significant amounts of r-proteins with mammalian cells are often long, tedious, or of limited scalability. This technology provides an improved process for the production of r-proteins by the transient transfection of suspension-growing cells. The process carried out in a single step is easily scalable and achieves high expression levels in a very short period of time.

APPLICATIONS

- High yield and low cost protein production in a few days to a couple of weeks (mg or g of protein).
- Production of proteins, including antibodies, for High-throughput screening assays, antibody generation, structure-activity analyses, surface plasmon resonance, NMR or crystallography.

CONCEPT

Transient gene expression is often preferable to the establishment of stable transfectant as this latter approach is time consuming and requires that the expressed protein not adversely affect the growth of the cells. This technology provides a robust large-scale transient transfection process for fast production of milligram to gram amounts of r-proteins in suspension-growing mammalian cells. This easily scalable technology, amenable to high throughput production was obtained by combining optimized parameters in four key aspects namely the cell line, the expression vector, the transfection vehicle and the culture medium. The vectors, medium and cell line were optimized to allow highest expression levels in a short period of time.



In addition, recovery of secreted r-proteins is easily achieved as the process uses serum-free medium.

FEATURES AND BENEFITS

Optimized serum-free adapted cell line

A novel cell line, designated 293SFE, was established by stably transfecting serum-free adapted HEK293SF-3F6 cells with an EBNA1 (Epstein-Barr virus (EBV) Nuclear Antigen 1) expression plasmid. Although the commercially available HEK293E cell line also allows the episomal persistence of vectors containing the EBV origin of replication oriP, the 293SFE cell line offers the added advantage of being capable of growing in a serum-free medium.

Highly efficient expression vector

Transient protein expression is achieved using the small size pTT5 vector (4.4 kb), a family of expression vectors that contains the EBV oriP and an improved cytomegalovirus-based expression cassette. As illustrated, use of the pTT vector in HEK293E cells provided a 2-3 fold increase in transgene expression compared with the pCEP4 vector and a 10-fold increase compared to pcDNA3.1 vector (both from Invitrogen).

Fast and scalable transfection

Use of commercially-available transfection reagents led to the development of a robust transient transfection procedure that can be carried out at multi-liters scale. The single step procedure is fast and easy to perform as there is no need to change the culture medium.

High yield and cost-effective serum-free culture

Optimization of the culture conditions led to the formulation of a serum-free growth medium enriched with selected peptones. This medium reduces the costs associated with r-protein purification and allows higher transient gene expression in the absence of serum. Protein expression levels obtained with 293SFE cells grown in the optimized serum-free medium were nearly identical to those obtained with HEK293E cells cultured in a serum-supplemented medium.

Validated expression system

The resulting single step high performance system was validated by the successful production and purification (>95% purity was achieved following a single IMAC purification step) of over thirty r-proteins; scale ranged from 1 to 14-liters while expression yields ranged from 20 to 60 mg/L for non-toxic proteins.

PROTECTION STATUS

Enhanced production of recombinant proteins by transient transfection of suspension-growing mammalian cells (NRC no. 11266).

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