

A more efficient and productive method for large-scale transfection of CHO cells

SUMMARY

The biomedical research community and the biopharmaceutical industry make extensive use of Chinese Hamster Ovary (CHO) cells to produce recombinant proteins (r-proteins). Even though large scale transfection is gaining increasing recognition for rapid and productive expression of r-proteins, this approach has yet to be fully optimized when using these cells. Through crucial improvements in the processes, vectors, and cell line, this technology now allows for more efficient and cost-effective transient gene expression using CHO cells.

APPLICATIONS

- Production of r-proteins at high yield and low cost in CHO cells.
- Rapid production of r-proteins that require post-translation modification for research or pharmaceutical use.

CONCEPT

Increasing demand for fast and efficient production of proteins in expression systems capable of conducting elaborate post-translational modifications is spurring the need for alternatives to the development of stable mammalian cell lines. Large-scale transient transfection provides a mean for speeding up the production of milligram-to-gram quantities of r-proteins in mammalian cells. Although CHO cells still outweigh human embryonic kidney 293 cells (HEK293) for the stable expression of r-proteins, the latter is the most widely used cell line for their transient expression. This is attributable to the fact that the large-scale transfection of CHO cells suffers from low efficiency and low productivity when cost-effective transfection reagents are used, and that plasmid vectors used in CHO cells are not fully optimized for transient gene expression. Building on the knowledge and expertise gained through optimization of its HEK293 platform (PL-11565), NRC-BRI developed a technology that provides processes, expression

vectors, and engineered cell lines for more efficient and productive transfection of CHO cells at large scale. A fully deacetylated variety of the inexpensive cationic polymer polyethylenimine (PEI) was shown to provide a cost-effective alternative to currently used transfection reagents for the introduction of plasmid DNA into CHO cells. To enhance productivity, a CHO cell line stably expressing an optimized EBNA1 was developed. Such a system is well suited when using transient expression, as it increases r-protein expression levels by a factor of 3-4 when using plasmids containing the viral EBV origin of replication (oriP), such as the pTT family of vectors. Further increases in the transient expression and productivity of CHO cells were achieved through valproic acid potentiation combined with AKT and a temperature shift.

FEATURES AND BENEFITS

Validated expression system and host cell line

This is an alternative to the robust and well-established HEK293 transient gene expression platform of NRC-BRI (L-11266; PL-11565), a platform broadly validated by the successful production and purification of hundreds of r-proteins. The application of several features of this platform allows for the optimization of transient gene expression in CHO cells—a leading mammalian cell line for the production of r-proteins—particularly those that require post-translational modifications.

Efficient transfection process

Use of a fully deacetylated linear PEI as a transfection reagent improves transfection efficiency and productivity in CHO cells in comparison to the use of the usual linear 25kDa PEI. This potent reagent offers the advantages of being stable, non-toxic, easy to use, and commercially available. Further, it is more economical than established reagent formulations currently available on the market for the introduction of plasmid DNA into CHO cells.

Optimized expression toolbox

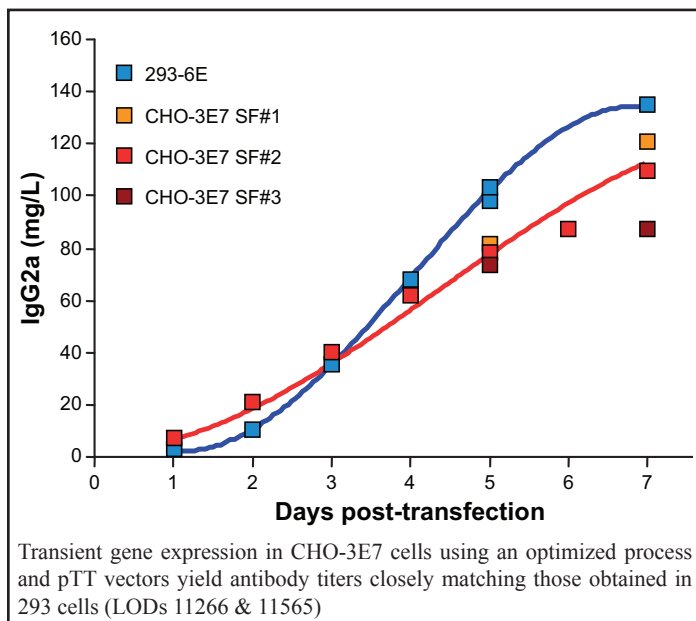
A stable CHO clone expressing a truncated but functional form of EBNA1 (CHO-3E7) was obtained and shown to enhance r-protein production when using pTT vectors. Placing the gene of interest under the control of the cytomegalovirus (CMV)-based expression cassette on an oriP-bearing pTT vector was also shown to increase productivity significantly compared to the use of the strong elongation factor 1 alpha (EF1 α) promoter. Further enhancement of transient gene expression and productivity was achieved following the co-expression of fibroblast growth factor 2 (FGF2) or the use of valproic acid in tandem with the co-expression of protein kinase B (AKT) followed by a temperature shift.

Convenient platform when using both CHO and HEK293 cells

Because of the higher r-protein yields achieved with the use of the pTT vectors family, the cumbersome practice of subcloning cDNAs into cell line-specific vectors can be avoided. Comparing product quality/activity in both cell lines can thus be easily performed in parallel while ensuring maximal productivity.

PROTECTION STATUS

Process, vectors and engineered cell lines for enhanced large-scale transfection (NRC no. 11992).



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