tebu-bio

# Transiently produced recombinant proteins to speed up the decision-making process and limit risks

tebu-bio offers recombinant protein production services using a robust, inexpensive and fast transient-expression system in Chinese hamster ovary (CHO) cells, for various research and development applications. This service is based on the valuable combination of (i) our proprietary expression vector (compliant with European Union and US regulations), (ii) a linear polyethyleneimine transfection reagent and (iii) CHO cells growing in suspension in serum-free conditions, allowing transient production of proteins containing proper post-translational modifications.



Demands for high-yield production and fast delivery of recombinant-protein batches using mammalian cells are increasing dramatically. The generation of stable recombinant protein–producing cell lines is laborious, expensive and time-consuming. This approach will not meet the demands in production of bulk amounts of properly folded, soluble and post-translationally modified proteins for a wide variety of early discovery studies and applications before entering any bioproduction process.

Among mammalian cell types commonly used for recombinant-protein production and adapted to grow in suspension under serum-free, chemically defined conditions, CHO cells are now the industry standard for protein production and manufacturing. To generate substantial amounts of recombinant protein in a short period of time, the scalability of the production process is of major interest. Thus, small- to large-scale transient transfection of mammalian cells growing in suspension is needed. However, because of the very high cost of transfection reagents, adoption of this modern expression system is still questionable.

tebu-bio has developed a protein expression platform in CHO cells that allows transient protein expression and selection of CHO cell lines stably expressing recombinant proteins in suspension cultures. Here we describe recent experiments using CHO cells for transient protein production with a low-cost transfection reagent. This system combines the use of a proprietary expression vector, pTBL_KN, with a CHO cell line adapted to grow in suspension under serum-free, animal origin–free medium and a low-cost transfection reagent, the 25-kDa linear polyethyleneimine (L-PEI). We tested this system using 30-ml cultures in shake flasks and were able to scale up the production process using the same transfection procedure irrespective of the final culture volume (5-liter maximum batch volume at tebu-bio facilities).

## Evaluation of transfection reagent

We evaluated L-PEI as a transfection reagent using a luciferase expression vector and compared transient transfection efficiency to that attained with different commercially available transfection reagents. Transfection efficiency of our chemically defined transfection agent was similar to that of other reagents (**Fig. 1**). Thus, L-PEI can be used for transfection at a substantially reduced cost.

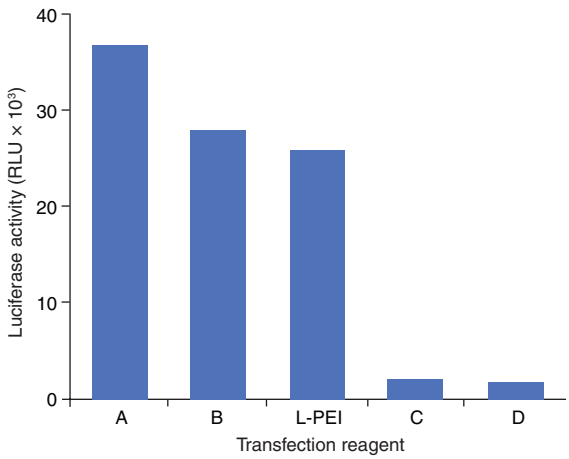


**Figure 1** | Transient transfection efficiency in CHO cells. We transfected $10^6$ cells/ml with a cell viability >95% growing in 30-ml shaker flasks with a luciferase expression vector. Luciferase expression was monitored 24 hours after transfection. Transfections with commercially available reagents (A, B, C, D and L-PEI) were done according to manufacturer's protocol.

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# APPLICATION NOTES

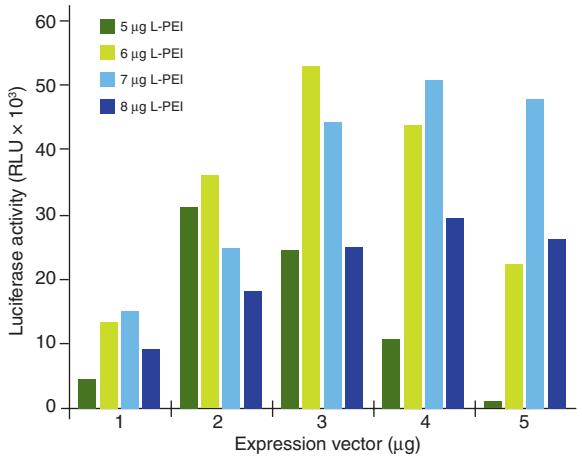


**Figure 2** | Transient transfection optimization. CHO cells at $10^6$ cells/ml with viability >95% growing in 30-ml shake flasks were transfected with various luciferase expression vector/L-PEI ratios (wt/wt). Luciferase activity was monitored 24 hours after polyplex transfection.

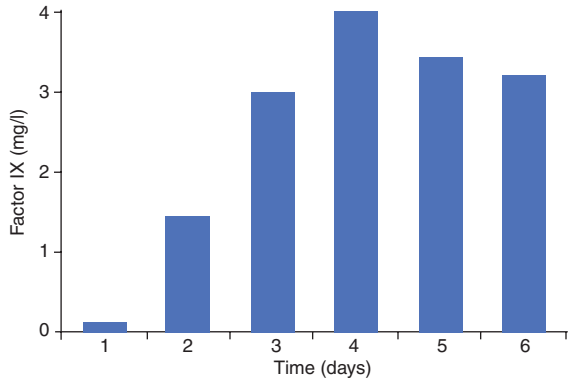


**Figure 3** | Time course of Factor IX transient production. A volume of 30 ml of CHO cells at $10^6$ cells/ml with viability >95% growing in shake flasks were transfected with an expression vector (pTBL_FIX) encoding Factor IX using the 3:6 polyplex ratio. An aliquot of the cells was collected daily and protein concentrations were determined by immunoassay.

## Optimization of expression conditions

What makes our system competitive, unique and reliable for transient and stable expression? It is the combination of our engineered expression vector with a low-cost transfection agent. This expression vector was constructed in accordance with US and European Union guidelines for bioproduction. It is a small vector that can be used in research and development applications, with limited licensing when shifting to large-scale production. This versatile vector can drive expression of several proteins from one construct and can be upgraded to a *Dhfr* derivative to allow gene amplification in adapted CHO cell lines.

A recent study[1] has demonstrated the efficiency and versatility of the L-PEI agent for gene delivery in several adherent cell lines. Researchers at tebu-bio performed experiments to optimize conditions of expression vector delivery in suspensions of CHO cells (**Fig. 2**).

The ratio of plasmid DNA to transfection agent within polyplexes had a major impact on transfection efficiency. Therefore, each expression vector needs a fine tuning of transfection conditions to enhance efficiency and reduce toxicity of the polyplex mixtures. An imbalance in one of the components led to a decrease of the transfection efficiency and also increased toxicity of polyplexes (data not shown).

## Protein production

We transiently produced a recombinant protein as proof of concept. Factor IX is a secreted glycoprotein used in hemophilia B treatment. We subcloned the cDNA encoding Factor IX into the same vector used to express luciferase protein. We transiently transfected CHO cells with 3:6 (plasmid DNA/L-PEI) polyplex ratio.

We obtained Factor IX yields of 4 mg/l over the 6 days of culture without any optimization of the culture process (**Fig. 3**).

## Conclusion and perspectives

tebu-bio has developed and subsequently improved a system to rapidly produce various recombinant proteins transiently and in high yields in CHO cells in suspension. This service is a powerful tool to increase the speed of the decision-making process and to limit risk assessment by performing early screenings and selection of proteins of interest[2]. The process we provide makes recombinant-protein production affordable for almost all applications requiring microgram- to milligram-scale batches of purified proteins with mammalian post-translational modifications. Direct transfer of this process to large-scale bioproduction will reduce the time for recombinant proteins to reach the market. Furthermore, tebu-bio can also generate stable recombinant protein–producing CHO lines.

1. Huh, S.-H. *et al.* Optimization of 25 kDa linear polyethylenimine for efficient gene delivery. *Biologicals* **35**,165–171 (2007).
2. Garnier, J.-P. Rebuilding the R&D engine in big pharma. *Harvard Bus. Rev.* 69–76 (May 2008).