Development of Recombinant Protein Overproducer Cell Lines with Lentiviral Expression Vectors

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Overview

We have developed a highly efficient and cost effective technology for overexpression of recombinant proteins in a wide range of cell lines using a lentiviral delivery system. A high level of production, up to several milligrams (mg) per ml, can be achieved directly from uncloned pools of HEK 293 cells transduced at high MOI with lentiviral expression constructs. Stable production (up to 50 passages) of 5-50 mg* of secreted cytokine protein per liter of media was achieved with a success rate of 80% for ten cytokines.

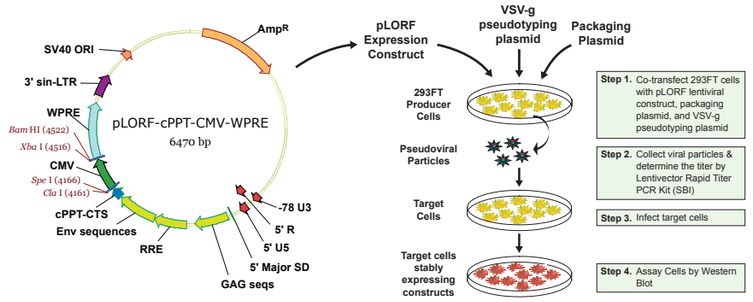
Custom overproducer cell line services are offered using the developed protocols, for the development of a wide range of cell lines overproducing recombinant proteins. Using Cellecta's lentiviral system, a construct containing the gene of interest can be packaged into VSV-g pseudotyped viral particles and delivered into a wide range of mammalian cells. With an average development time of 6 weeks, these cell lines dramatically improve the efficiency of process development and can be used for preparative and industrial-scale production of proteins.

* Submitted abstract incorrectly states that 0.1-5 mg of secreted cytokine protein per ml was achieved. We can achieve yields of 0.1-5 mg/ml for most other proteins.

Lentiviral vs. Transient Expression

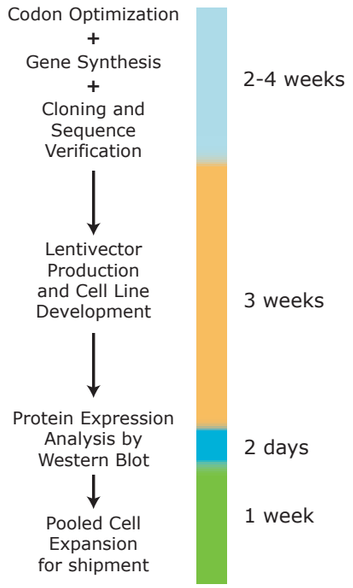
	Transient Plasmid-based Protein Production	Lentiviral-based Protein Production
Duration of expression:	1 week	50 cell passages
Delivery Efficiency:	Low - Medium	Medium - High
Construct Stability:	Unstable	Stable
Cell Types:	Commonly used, easy-to-transfect cell lines	Nearly all cell types, including non-dividing, primary, and stem cells

Fig. 1. Construction of Cytokine Expression Lentivectors



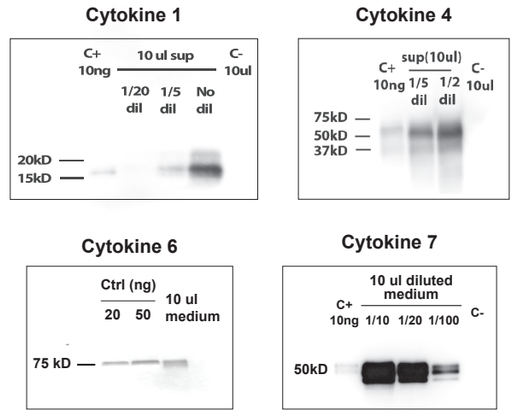
Full-length cytokine genes were codon-optimized for mammalian expression and cloned under a CMV promoter in an optimized lentiviral expression vector. Each construct was packaged into VSV-g pseudotyped viral particles using Lipofectamine transfection of 293FT cells (Invitrogen). Proteins expressed using this system will have mammalian post-translational modifications.

Fig. 2. Process and Timeline



The ability to produce substantial amounts of protein in a short period of time without clonal selection is one of the advantages of pooled cell line development.

Fig. 3. Western Blot Analysis



FreeStyle 293-F cells (Invitrogen) were transduced with cytokine-expressing lentiviral constructs. The resulting pooled cytokine producer 293-F cells were grown in 293SFM II serum-free medium (Invitrogen) under suspension-type culture conditions. Subcultured cells were collected once the cell density reaches 1 – 2 × 10^6 viable cells per ml and then frozen in 293SFM II Medium containing 7.5% DMSO at a density of 1 × 10^7 viable cells/ml.

The protein amount from each sample (Fig. 4) was detected by the Supersignal West Pico Chemiluminescent Detection system (Pierce Biotechnology).

Fig. 4. High Protein Expression

Results from the generation of 8 cytokine secretion overproducer cell lines

Protein Product	Protein size	Yield* (mg/L)	Cell Density** (cells/mL)
Cytokine 1	17.5 kD	50	2.0 × 10^6
Cytokine 2	35 kD	50	1.2 × 10^6
Cytokine 3	30 kD	50	1.5 × 10^6
Cytokine 4	55 kD	25	1.1 × 10^6
Cytokine 5	17.5 kD	10	1.3 × 10^6
Cytokine 6	75 kD	5	1.8 × 10^6
Cytokine 7	50 kD	50	1.2 × 10^6
Cytokine 8	50 kD	> 50	1.6 × 10^6

* From cell culture medium at 60 ml culture scale
** At which the culture medium was collected for testing

Advantages of the Cellecta System

- Substantial amounts of protein produced without clonal selection
- Efficient delivery and long-term protein expression in a wide range of cell lines
- Stable protein production (up to 50 passages) for preparative or industrial scale experiments, unlike transient transfections
- Mammalian, bacterial, or viral ORFs are codon-optimized for appropriate expression
- Cells are grown in suspension, in serum-free media
- Proteins can either be secreted or cytoplasmic

Contact Information

To order custom overproducer cell lines, please contact us by phone or email:

Toll-free: **(877) 938-3910**
Tel: **(650) 938-3910**
E-mail: **orders@cellecta.com**

Simply provide the following information and receive a custom quotation.

- name of cell line and any special growth media requirements
- full gene name, accession #, and sequence (up to 3kb)
- source of gene (donor vector, PCR product, etc.)
- marker required - copGFP, Puro, RFP, etc.
- required minimum level of protein production
- required assay (Western or biological activity)