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Recombinant Protein Expression in Mammalian system

Introduction

Multi-gram protein expression is a pre-requisite for many academic (for example functional analysis, crystal structure determination…) industrial and biopharmaceutical projects. For long, achieving high yields in recombinant protein productions using mammalian cells was considered as particularly challenging. Today, achieving yields over several grams per liters is regularly possible and thus overcomes the economic issues associated with the use of mammalian cell lines. These new advances in protein expression were encouraged by the fast increase of approved biotherapeutics expressed in mammalian cells.

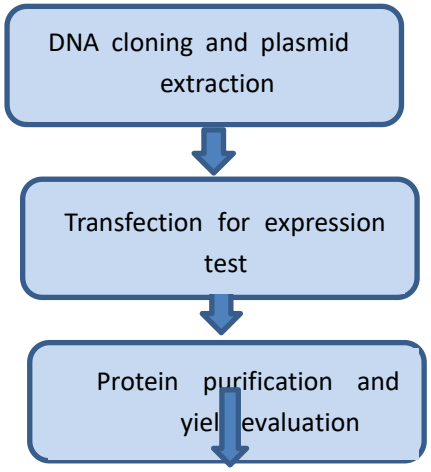
More and more investments are made to develop biopharmaceuticals such as therapeutic antibodies or proteins. In this context, the use of mammalian cells for protein production is of particular interest.

The main difference between bacterial and mammalian cell lines comes from the presence of post-translational metabolic machinery. Thus, obtaining protein expression with proper glycosylation profiles is only possible with protein production in mammalian cells. This is particularly relevant for recombinant therapeutic antibody production where proper glycosylation can induce increased efficacy, stability and safety. In contrast, antibody fragments can be produced in bacterial expression systems.

Mammalian cells protein expression also allows for proper protein folding, a determining criteria to prevent the loss of biological activity.

To conclude, one of the main ideas to keep in mind is that recombinant protein expression in mammalian cells increases their compatibility for further human use. That's why they are the reference expression systems when it comes to therapeutic antibody or protein production.

Experimental Outline



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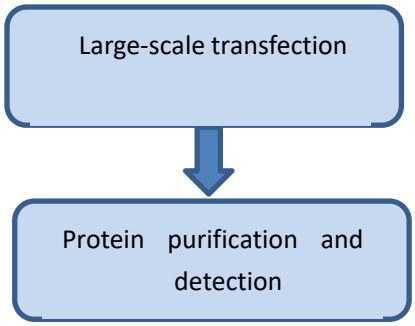

Protocol for Recombinant Protein Expression using the Mammalian Expression System

The following protocol can be a reference for both transient and stable gene expression using HEK293 and CHO cells.

1. Plasmid preparation

a) Prepare pure plasmids for cell transfection could maximize transfection efficiency and minimize cell death from endotoxin

b) Prepare 250 mL bacteria culture.

c) Purify with Endo-free Maxi Prep Kit or use cesium chloride purification instead.

2. Linearization of the expression plasmid

a) Recommended for stable cell lines.

b) Choose restriction site in non-essential location.

Please note that the selected location SHOULD NOT be within or close to genes that are to be expressed.

c) Chances are that integration doesn't occur in your inserted gene, which is the most common reason for growth of resistant cells that don't express your inserted gene.

3. Transfection

Optimal transfection is achieved when adherent cells reach about 90% confluency.

a) For a standard 15 cm tissue-culture dish (approximate surface area 176 cm2), 50 μg of plasmid DNA is required (quantities need to be scaled accordingly for the different surface areas of plates/flasks/bottles).

b) Mix DNA with PEI stock (1 mg/ml). Vortex briefly .

c) Incubate the solution at room temperature to allow DNA-PEI complex formation. Different DNA:PEI ratio should be tested.

d) During complex formation, change the media from the plates to be transfected, lower the serum concentration to 2%.

e) Finally, the DNA–PEI complex is added to the dish, which is then briefly rotated to allow mixing.

f) Place cells in the incubator.

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In this section, positive control will be included to monitor transfection efficiency.

4. Evaluation of the transfection efficiency

a) 2 days post transfection.
b) Counting the percentage of GFP-positive cells
c) Or, quantitate mRNA expression of the inserted gene by RT-PCR. This will require harvesting at least 10% of cells.
5. Harvest cells for experiment.

Besides, 10% of cells can be returned to flask for stable cell line generation (next).

6. Generate stable cell line

a) Add media with selection agent 48 hours post transfection.
b) Allow up to 2 weeks for selection and growth of resistant clones. Refresh media when old media starts to turn yellow.
c) Transfer visible clonal colonies to a 6-well plate.
i. Collect at least 6-12 colonies
ii. Change to media with antibiotics (Pen/Strep and half strength selection antibiotic, 400 ug/mL Geneticin) immediately before picking up colonies.
d) Allow to reach about 80% confluency and maintain concentration of antibiotics.
e) Harvest 90% of cells and evaluate for expression of the inserted gene.
i. RT-PCR (best for gene sequence variants)
ii. Activity for enzyme
iii. Western blot if appropriate antibody is available.
f) Transfer the best clones to T-75 for scale-up production.
7. Purification

a) Recombinant proteins are readily purified from conditioned media, with protocols that vary depending on the affinity tags used.
b) A final round of gel filtration is usually sufficient to yield proteins of over 90% purity.