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Transient Mammalian Cell Transfection with Polyethylenimine (PEI)

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Abstract

Standard protein expression systems, such as *E. coli*, often fail to produce folded, mono-disperse, or functional eukaryotic proteins (see Small-scale Expression of Proteins in *E. coli*). The expression of these proteins is greatly benefited by using a eukaryotic system, such as mammalian cells, that contains the appropriate folding and posttranslational machinery. Here, we describe methods for both small- and large-scale transient expression in mammalian cells using polyethylenimine (PEI). We find this procedure to be more cost-effective and quicker than the more traditional route of generating stable cell lines. First, optimal transfection conditions are determined on a small-scale, using adherent cells. These conditions are then translated for use in large-scale suspension cultures. For further details on generating stable cell lines please (see Rapid creation of stable mammalian cell lines for regulated expression of proteins using the Gateway® Recombination Cloning Technology and Flp-In T-REx® lines or Generating mammalian stable cell lines by electroporation).

1. THEORY

DNA can be introduced into a host cell by transfection with polyethylenimine (PEI), a stable cationic polymer (Boussif et al., 1995). PEI condenses DNA into positively charged particles that bind to anionic cell surfaces. Consequently, the DNA:PEI complex is endocytosed by the cells and the DNA released into the cytoplasm (Sonawane et al., 2003). Our laboratory uses PEI over other cell transfection reagents because of its low cost.

This protocol is appropriate for two suspension cell lines, CHO-S and HEK *293 GnTi-*. Many cell lines can be transfected successfully with PEI but in our experience these two cell lines express the highest level of protein compared to other cells.

2. EQUIPMENT

Laminar flow hood

CO_2 incubator

Platform shaker

Centrifuge

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Water bath (37 °C)

Inverted microscope

Hemacytometer

Sterile 0.22 μm filters

Sterile 250-ml polypropylene centrifuge tubes

Sterile 50-ml polypropylene conical tubes

Sterile 1.5-ml polypropylene tubes

Sterile 6-well tissue culture plates micropipettors

Sterile micropipettor tips

Sterile disposable serological pipettes

Sterile square polypropylene bottles

3. MATERIALS

Plasmid DNA directing your protein of interest

Fetal bovine serum (FBS, Invitrogen)

Polyethylenimine 'Max' (linear, MW 25 000) (Polysciences, Inc.)

L-Glutamine 100× (Invitrogen)

Sodium hydroxide (NaOH)

MEM α (containing Earl's Salts and l-glutamine, but no ribonucleosides, deoxyribonucleosides, $NaCO_3$; Invitrogen 12000)

DMEM/F12 (with L-glutamine, but no HEPES, $NaHCO_3$; Invitrogen 12500)

Freestyle™ 293 medium (Invitrogen 12338-026)

FreeStyle™ CHO-S (Invitrogen R800-07)

Hybridoma SFM (Invitrogen 12045)

Opti-MEM® (Invitrogen)

HEK293S GnTI- (ATCC# CRL-3022)

HEK293T/17 (ATCC# 11268)

Note	*Some of the stock solutions come with the pH indicator phenol red. This supplement does not affect the application and might be useful if the researcher wishes to visualize any pH changes that can occur in the solutions over time. In the case of non-CO_2 incubators (e.g., when scaling-up the production of adherent cells in roller bottles), HEPES-buffered media can be used to keep the pH stable.*
Note	*Catalog numbers are from the US website of Invitrogen and may differ on other local websites.*

3.1. Solutions & buffers

PEI ‘Max’		
Dissolve 1 g PEI ‘Max’ in 900 ml distilled water. Adjust the pH to 7.0 with 1 N NaOH. Add distilled water to 1 l		
Note: Stable at least 9 months at 4 °C		
Make smaller volumes depending on how much is needed. PEI ‘Max’ cannot be frozen!		
FreeStyle™ 293 ‘Completed’		
Component	**Stock**	**Amount**
FreeStyle™ 293 medium		1 l
FBS	100%	10 ml
L-Glutamine	200 mM	10 ml
DMEM:F12, 5% FBS		
Add 50 ml FBS to 1 l of DMEM:F12		
Alpha MEM, 5% FBS		
Add 50 ml FBS to 1 l of Alpha MEM		
Hybridoma SFM, 1% FBS		
Add 10 ml FBS to 1 l of Hybridoma SFM		

4. PROTOCOL

4.1. Preparation

Before transfection, sterile high-quality DNA must be prepared. The vector containing the appropriate expression promoter (see Molecular Cloning) and the gene of interest should be transformed into a recA- strain of *E. coli* (see Transformation of Chemically Competent *E. coli* or Transformation of *E. coli* via electroporation) and then the plasmid DNA isolated (see Isolation of plasmid DNA from bacteria). Commercially available, endotoxin-free kits for large-scale plasmid DNA isolation produce sufficiently high-quality DNA. High-quality DNA is characterized as having an OD260/280 ratio between 1.88 and 1.92, an OD260/230 ratio of 2.1–2.2, and a concentration above 0.5 mg ml^{-1} (see Explanatory Chapter: Nucleic Acid Concentration Determination).

Cells must be greater than five passages from liquid nitrogen, adapted to media, free of mycoplasma contamination, and single cells if in suspension culture. All steps are carried out using sterile technique in a laminar flow hood. Solutions should be sterile-filtered through 0.22-μm filters. All plastic and glassware, if not purchased as sterile, should be double autoclaved. Cell growth media should be warmed to 37 °C prior to contact with cells. Different growth media are needed for each cell line and growth condition. These media are listed in Table 18.1. Each time the protocol says to use ‘media,’ use the appropriate media as outlined in Table 1 for the specific cell line and growth conditions. Cell type used depends on specific needs of protein of interest. In our laboratory, optimized protein expression conditions determined for adherent cells translate well into large-scale suspension conditions for the same cell line.

4.2. Duration

Preparation	1 week
Protocol	1–2 weeks

See Fig. 18.1 for the flowchart of the complete protocol.

5. STEP 1 SMALL-SCALE TRANSIENT TRANSFECTION

5.1. Overview

This step will prescreen a variety of transfection conditions including media, cell type, ratio of PEI to DNA, and expression time to maximize protein expression before scaling up to a large-scale transfection.

5.2. Duration

5 days

Note	*Transfection must be done in the absence of antibiotics.*

6. STEP 1.1 SEED ADHERENT CELLS FOR TRANSFECTION

6.1. Overview

The appropriate amount of cells are transferred to a 6-well dish and allowed to become adherent.

6.2. Duration

1.5 days

1.1.1 Wash the cells with PBS, trypsinize, add 10ml DMEM:F12 + 5% FBS, and gently pipet the cells several times to ensure an even suspension before counting. Transfer the cells to a 15-ml sterile centrifuge tube. Count the cells using a hemacytometer.

1.1.2 For each transfection condition to be tested, aliquot 3×10^5 cells into a sterile centrifuge tube.

1.1.3 Spin the cells at 200 × g at room temperature for 5 min. Aspirate supernatant.

1.1.4 Add DMEM:F12 + 5% FBS to the cell pellet to a final concentration of 3×10^5 cells ml^{-1}.

1.1.5 Resuspend the cells with a serological pipette.

1.1.6 Add 2 ml of DMEM:F12+5% FBS to each well of a 6-well plate.

1.1.7 Transfer 1 ml of the cell suspension to each well.

1.1.8 Place dish in the 37 °C incubator, 5% CO_2.

1.1.9 After 24 h, remove the media from each well.

1.1.10 Add 2.7 ml of fresh DMEM:F12 + 5% FBS to each well.

1.1.11 Return the dish to incubator.

See Fig. 18.2 for the flowchart of Step 1.1.

7. STEP 1.2 TRANSIENTLY TRANSFECT CELLS

7.1. Overview

Cells are transfected by adding DNA and PEI 'Max' to the cells. The PEIDNA mixture is prepared and added to the cells on the same day as changing the media.

7.2. Duration

45 min active time; 4 days total

1.2.1 Dilute 9 μg of PEI 'Max' into a total volume of 150 μl of Opti-MEM. The amount of PEI can be varied.

1.2.2 Dilute 3 μg of DNA into a total volume of 150 μl of Opti-MEM.

1.2.3 Add the diluted PEI 'Max' to the diluted DNA.

1.2.4 Incubate the mixture at room temperature for 30 min.

1.2.5 Carefully add the PEI-DNA mixture to a well of adherent cells. Take care to gently pipette the solution down the side of the well and not on top of the cells, so as not to disrupt the adherent cells.

1.2.6 Return the dish to the 5% CO_2 incubator.

7.3. Tip

Opti-MEM can be replaced with Hybridoma Media without serum.

7.4. Tip

The protocol outlined here uses a 3:1 ratio of PEI to DNA (w/w). We have found this ratio to be optimal for most genes we have expressed. However, this ratio should be screened for each gene tested. We routinely screen ratios between 1:1 and 5:1.

7.5. Tip

In general, use 1 μg of DNA per 1 ml of culture to be transfected. PEI and DNA should each be diluted into 1/20 of the total culture volume before being combined.

7.6. Tip

Small-scale transfections can be performed with suspension-adapted cells. The protocol for small scale is essentially the same. For suspension culture, we use square plastic bottles designed to hold 125 ml; however, we add only 5–12 ml of cell medium for optimal aeration and agitation.

See Fig. 18.3 for the flowchart of Step 1.2.

8. STEP 1.3 HARVEST CELLS AND ANALYZE PROTEIN EXPRESSION

8.1. Overview

Harvest and lyse cells (see Lysis of mammalian and Sf9 cells). Analyze protein expression by Western blotting (see Western Blotting using Chemiluminescent Substrates).

8.2. Duration

1–2 days

1.3.1 96 h after transfection, collect samples to be analyzed. For secreted proteins collect and save the media. For membrane or intracellular proteins, remove media. Wash the cells with PBS and lyse the cells.

1.3.2 Analyze protein expression by Western blotting or ELISA as appropriate.

8.3. Tip

HEK293 GnTI-cells can be resuspended by pipetting gently up and down with a serological pipette. CHO-S cells adhere more tightly to the dish and need to be manually resuspended with a cell scraper.

8.4. Tip

Some protocols call for trypsin digestion to detach the *cells from the dish. This can be avoided by manual scraping of the cells. Trypsin could degrade the expressed protein if it is a membrane protein*.

8.5. Tip

Once a cell type, media, and optimal ratio of PEI to DNA are established, this protocol can be repeated and samples taken between 24 and 96 h posttransfection to optimize the length of expression.

See Fig. 18.4 for the flowchart of Step 1.3.

9. STEP 2 LARGE-SCALE TRANSIENT TRANSFECTION OF SUSPENSION CELLS

9.1. Overview

Preparative scale expression of protein in suspension culture. For this protocol, the parameters optimized in Step 1 are expanded to larger volume cultures. You will need 400 ml of cells at a density of $2–3 \times 10^6$ cells ml^{-1}.

9.2. Duration

4–8 days

10. STEP 2.1 PREPARE THE CELLS TO BE TRANSFECTED

10.1. Overview

Harvest and count suspension cells to ensure that they are at the proper density. Centrifuge the cells and resuspend them in a total of 360 ml of fresh suspension growth medium.

10.2. Duration

30 min

2.1.1 Grow 400 ml of cells in the appropriate suspension growth medium to a density between 2 and 3×10^6 cells ml^{-1}.

2.1.2 Transfer the cell suspension to sterile centrifuge bottles.

2.1.3 Spin the cells at 200 × g at room temperature for 5 min. Aspirate the supernatant.

2.1.4 Add 25 ml of the appropriate fresh suspension growth medium.

2.1.5 Gently resuspend the cells with a serological pipette.

2.1.6 Add the cells to 335 ml of fresh suspension growth medium in a sterile square bottle.

2.1.7 Do not tighten the bottle cap all the way.

2.1.8 Place the cells into a 37 °C incubator shaker set at 8% CO_2, with shaking at 130 rpm.

See Fig. 18.5 for the flowchart of Step 2.1.

11. STEP 2.2 TRANSFECT CELLS

11.1. Overview

Transfect the cells using the optimal ratio or PEI to DNA as determined above.

11.2. Duration

45 min active time, expression time as determined above

2.2.1 Dilute 400 μg of DNA in a total volume of 20 ml of Hybridoma SFM (without serum).

2.2.2 Dilute the appropriate amount of PEI ‘Max,’ as determined in Step 1, into a total volume of 20 ml of Hybridoma SFM (without serum).

2.2.3 Add the diluted PEI ‘Max’ to the diluted DNA and mix.

2.2.4 Incubate the mixture at room temperature for 30 min.

2.2.5 Add the PEI-DNA mixture to the suspension cells from Step 2.1.8.

2.2.6 Return the cells to the 37 °C incubator shaker, shaking at 130 rpm.

See Fig. 18.6 for the flowchart of Step 2.2.

12. STEP 2.3 HARVEST CELLS AND PROCESS PROTEIN AS NEEDED

12.1. Overview

Harvest the cells (or medium for a secreted protein). Purify the protein or process as needed for downstream applications (see Salting out of proteins using ammonium sulfate precipitation, Using ion exchange chromatography to purify a recombinantly expressed protein, Gel filtration chromatography (Size exclusion chromatography) of proteins, Use and Application of Hydrophobic Interaction Chromatography for Protein Purification or Hydroxyapatite Chromatography: Purification Strategies for Recombinant Proteins, or look up the chapters on affinity purification if tags have been added to the protein: Purification of His-tagged proteins, Affinity purification of a recombinant protein expressed as a fusion with the maltose-binding protein (MBP) tag, Purification of GST-tagged proteins, Protein Affinity Purification using Intein/Chitin Binding Protein Tags, Immunoaffinity purification of proteins or Strep-tagged protein purification).

12.2. Duration

About 1 h

2.3.1 After the appropriate amount of time, as determined in Step 1, centrifuge the cells at 200 ×g for 5 min at room temperature.

2.3.2 If the protein is secreted, collect the medium. Centrifuge the cells at 200 × g for 5 min at room temperature and sterile-filter the medium through a 0.22-μm filter. Add sodium azide to 0.02%. The medium can be stored at 4 °C for months until needed.

2.3.3 If the protein is to be purified, the cell pellet should be flash-frozen in liquid nitrogen and stored at −80 °C until needed.

12.3. Tip

In suspension cultures, single cells are transfected more efficiently than cells that have clumped together during growth. Growth conditions may need to be optimized for single cell growth.

12.4. Tip

Square bottles should be autoclaved in two consecutive dry cycles (45 min each, dry 15 min each) with the lids as loose as possible without falling off. They should be allowed to cool completely in the laminar flow hood before tightening the lids. If the bottles collapse inward, cells will not grow well.

12.5. Tip

To generate more protein, we have found that 24 h posttransfection the cells can be diluted between 1:2 and 1:5 in the appropriate media. The effect of dilution and optimal dilution ratios should be determined empirically.

See Fig. 18.7 for the flowchart of Step 2.3.

REFERENCES

Referenced Literature

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Small-scale Expression of Proteins in *E. coli*

Rapid creation of stable mammalian cell lines for regulated expression of proteins using the Gateway® Recombination Cloning Technology and Flp-In T-REx® lines

Generating mammalian stable cell lines by electroporation

Molecular Cloning

Transformation of Chemically Competent *E. coli*

Transformation of *E. coli* via electroporation

Isolation of plasmid DNA from bacteria

Explanatory Chapter: Nucleic Acid Concentration Determination

Lysis of mammalian and Sf9 cells

Western Blotting using Chemiluminescent Substrates

Salting out of proteins using ammonium sulfate precipitation

Using ion exchange chromatography to purify a recombinantly expressed protein

Gel filtration chromatography (Size exclusion chromatography) of proteins

Use and Application of Hydrophobic Interaction Chromatography for Protein Purification

Hydroxyapatite Chromatography: Purification Strategies for Recombinant Proteins

Purification of His-tagged proteins

Affinity purification of a recombinant protein expressed as a fusion with the maltose-binding protein (MBP) tag

Purification of GST-tagged proteins

Protein Affinity Purification using Intein/Chitin Binding Protein Tags

Immunoaffinity purification of proteins

Strep-tagged protein purification

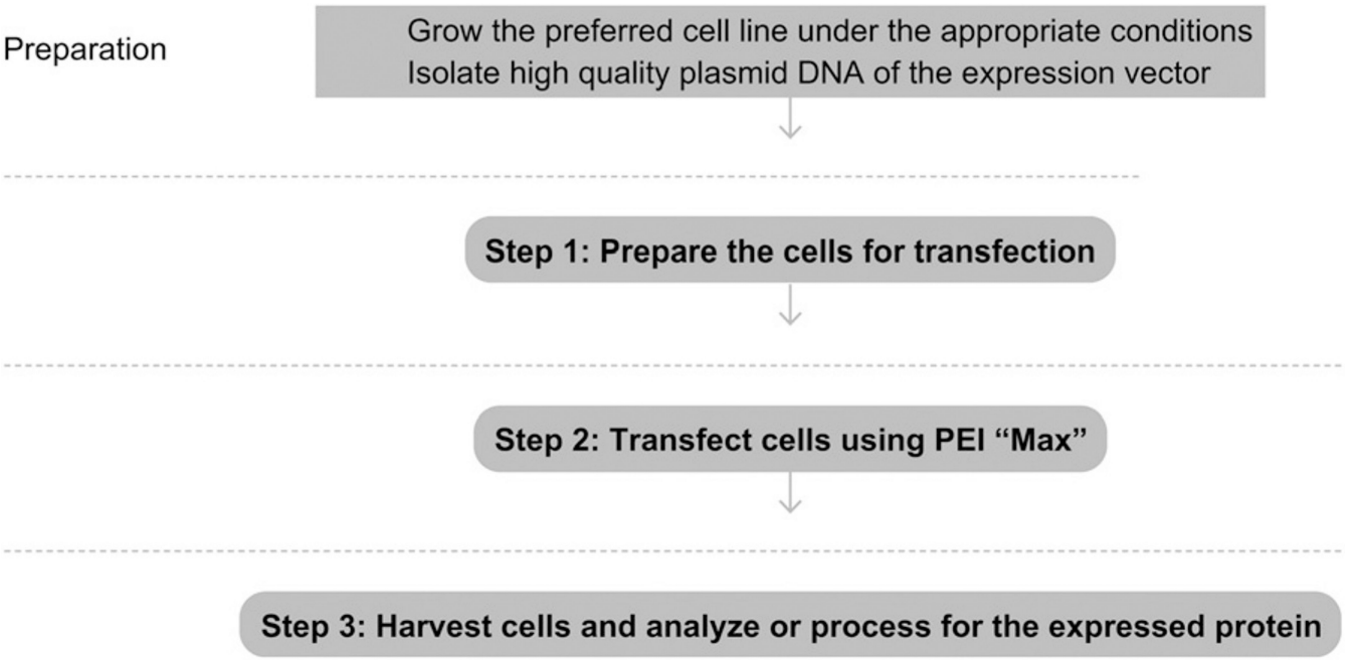

Figure 18.1.
Flowchart of the complete protocol, including preparation.

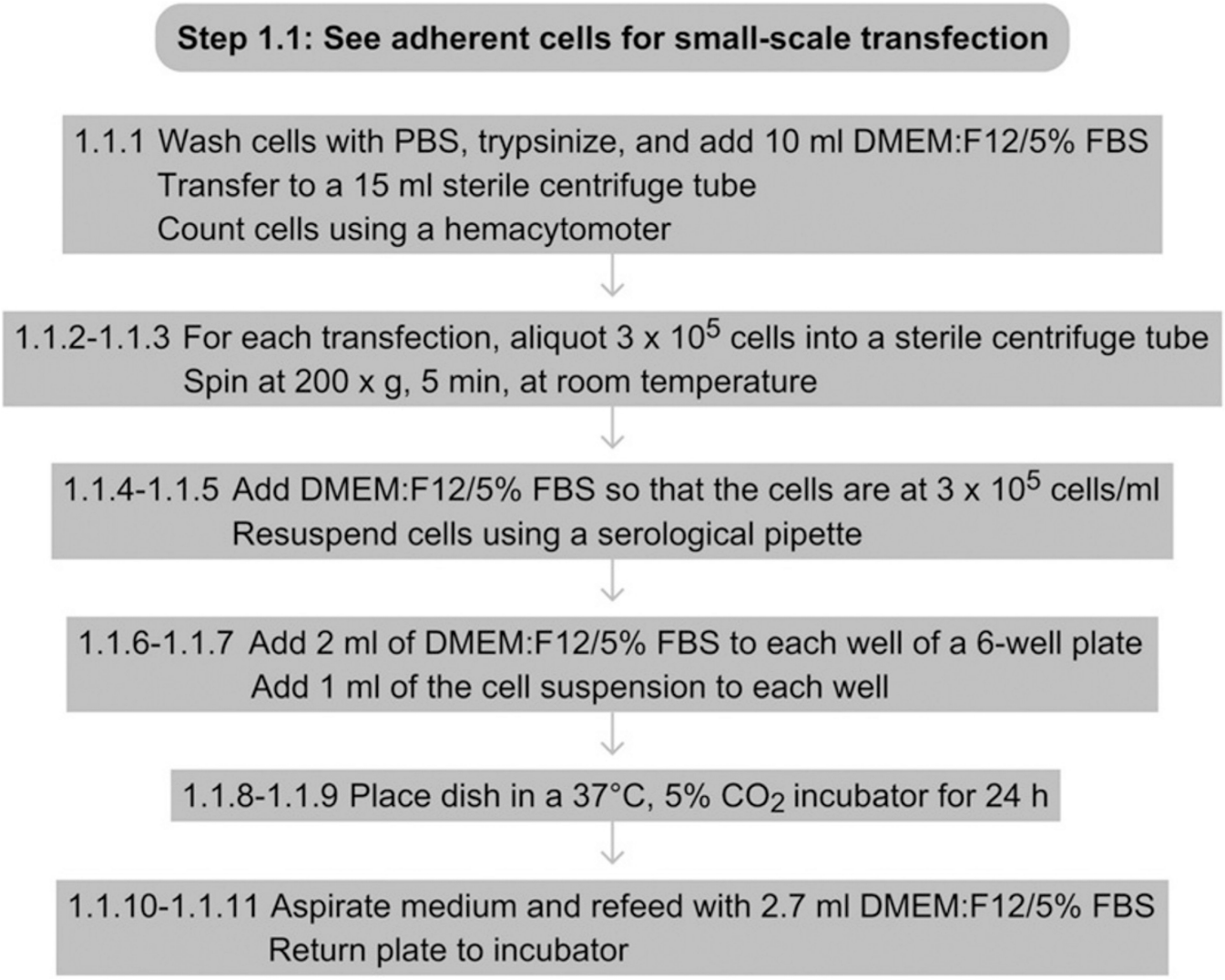

Figure 18.2.
Flowchart of Step 1.1.

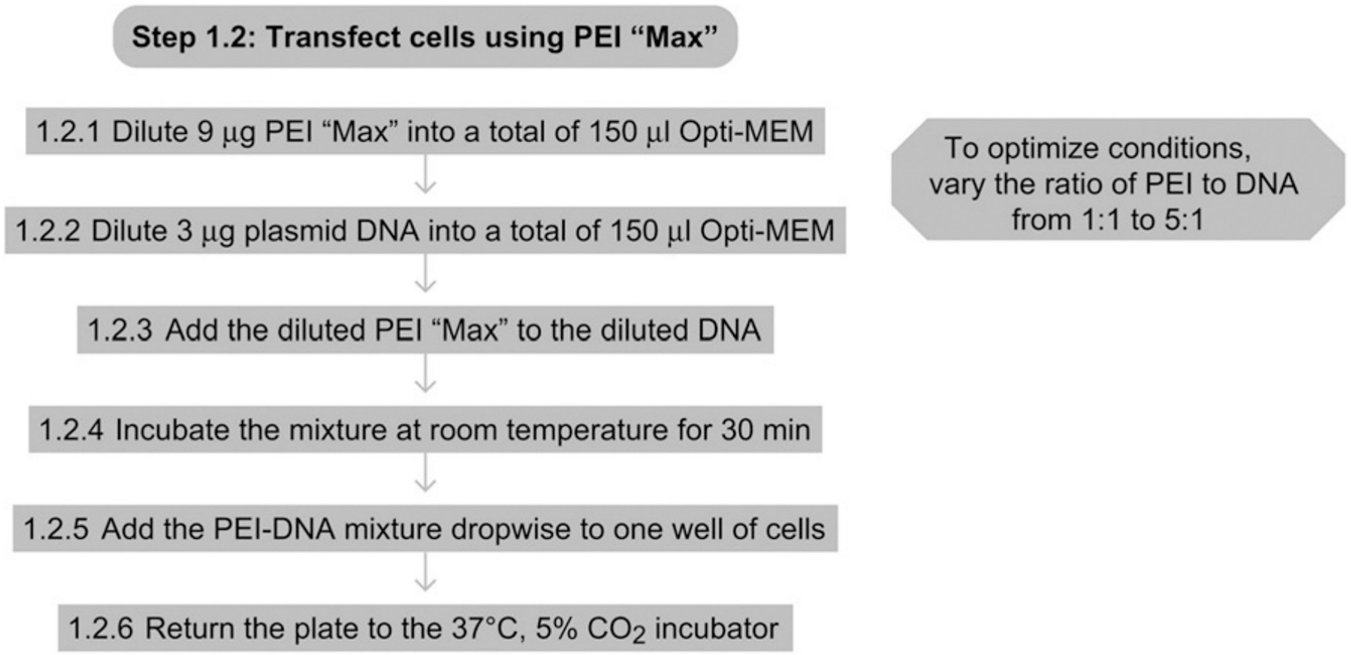

Figure 18.3.
Flowchart of Step 1.2.

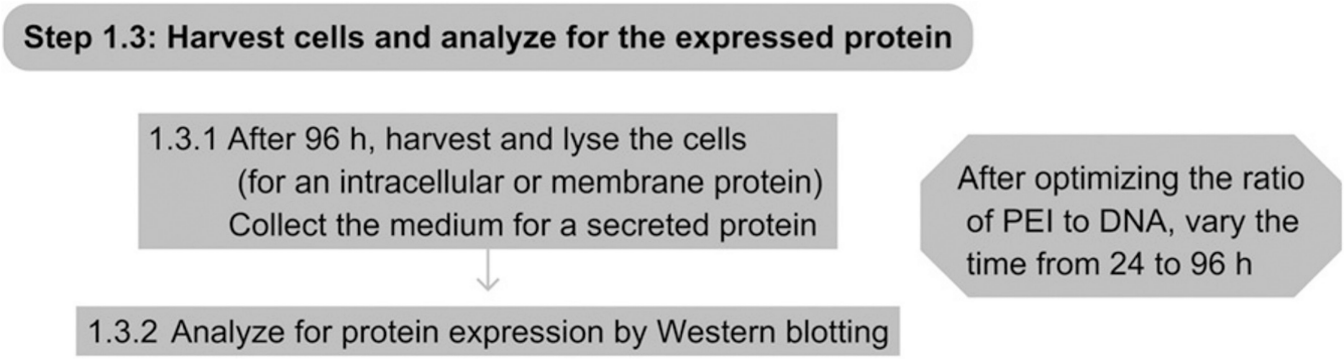

Figure 18.4.
Flowchart of Step 1.3.

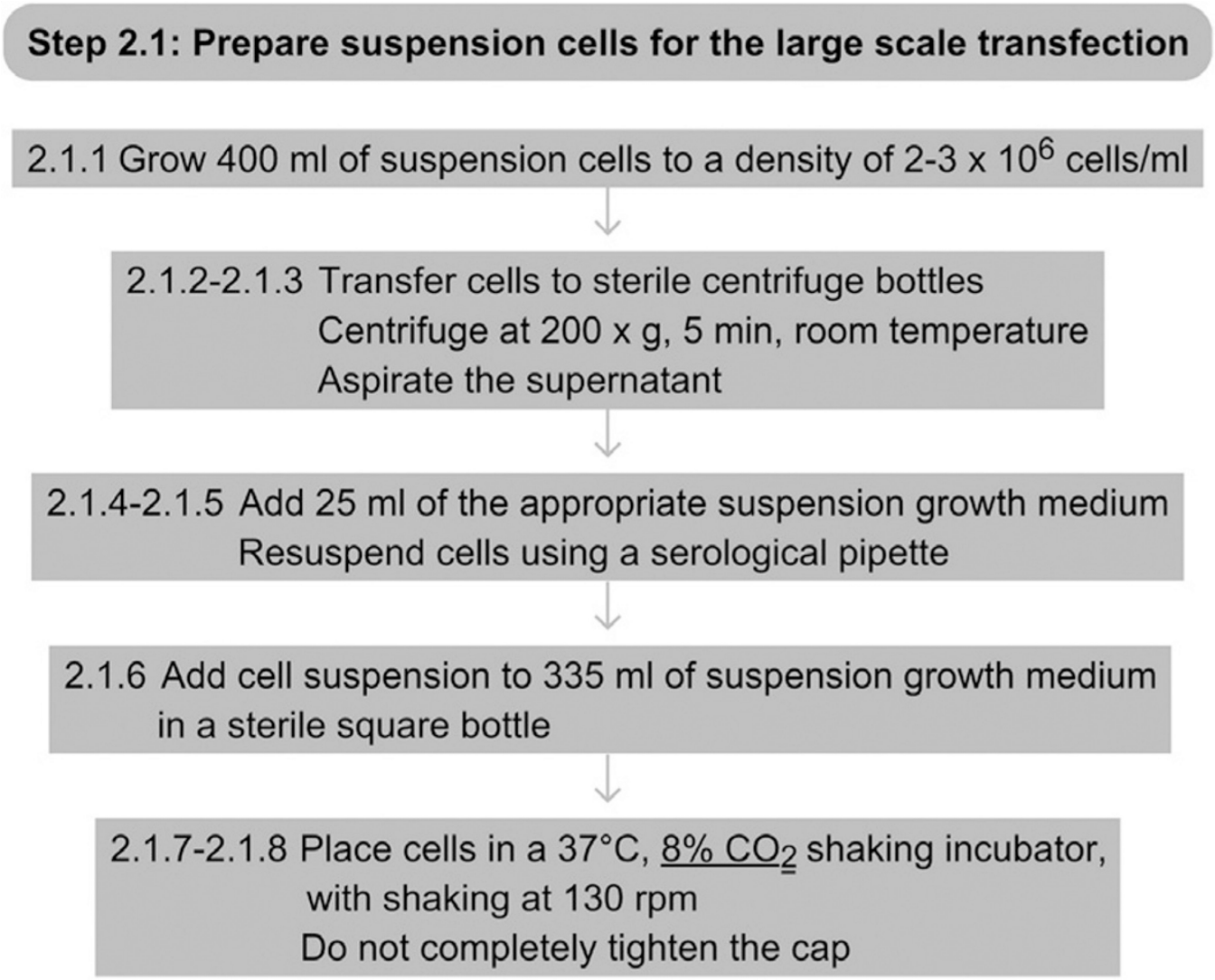

Figure 18.5.
Flowchart of Step 2.1.

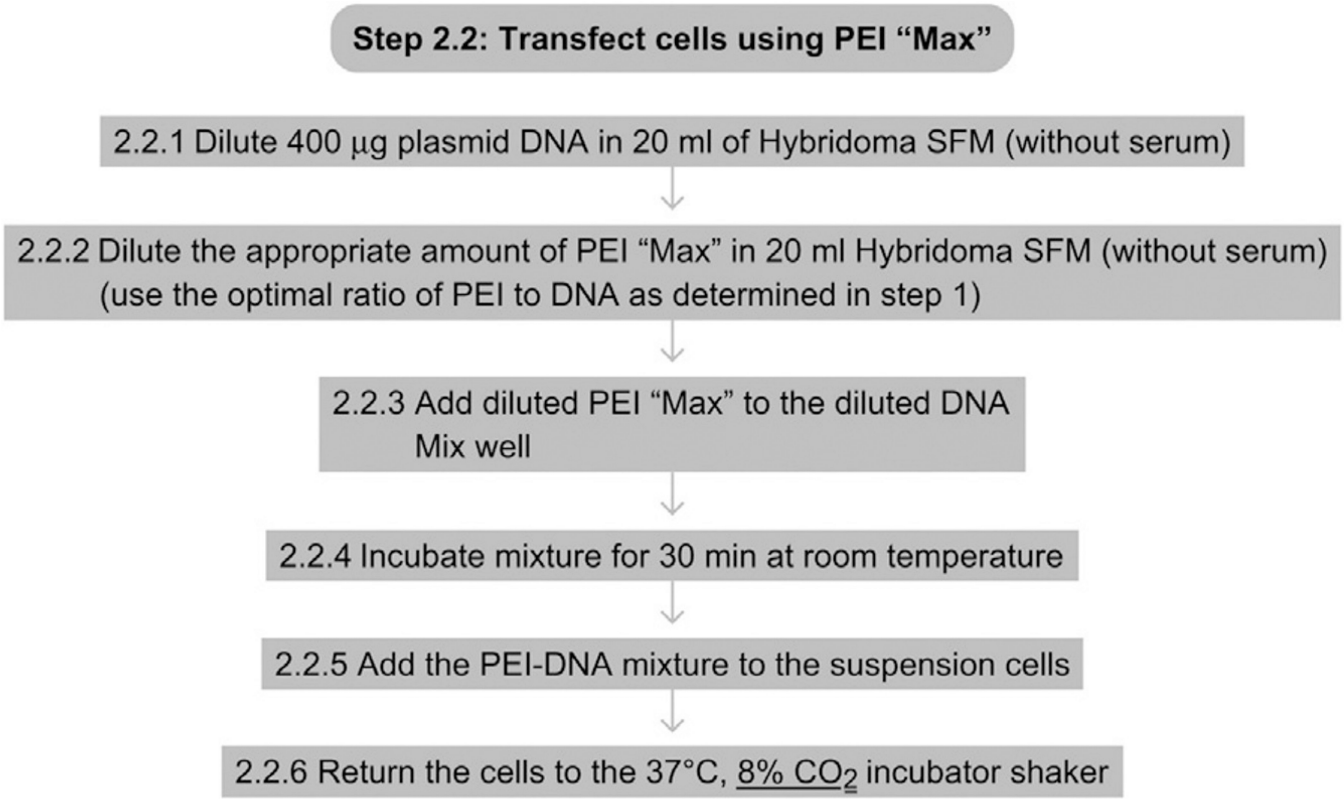

Figure 18.6.
Flowchart of Step 2.2.

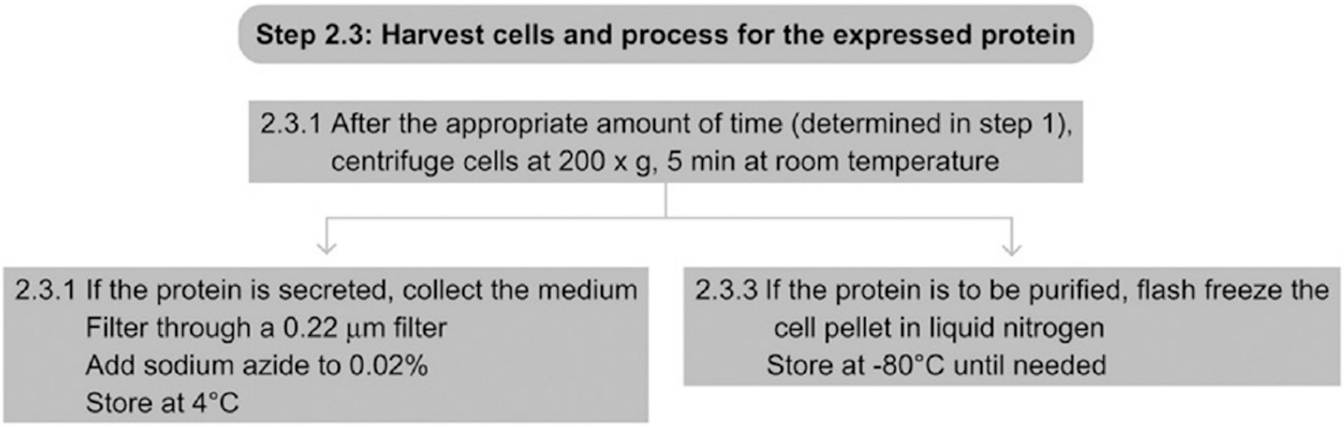

Figure 18.7.
Flowchart of Step 2.3.

Table 18.1

Cells: Growth characteristics and medium

Cell line	Growth type	Ideal medium	Cell source
HEK293S GnTI-	Suspension	Freestyle™ 293 ‘completed’	ATCC# CRL-3022
HEK293S GnTI-	Adherent	DMEM:F12, 5% FBS	ATCC# CRL-3022
HEK293T/17	Adherent	DMEM:F12, 5% FBS	ATCC# CRL-11268
CHO-S	Suspension	Hybridoma SFM, 1% FBS	Invitrogen R800-07
CHO-S	Adherent	DMEM:F12, 5% FBS	Invitrogen R800-07