MethodsX 3 (2016) 371–377

Contents lists available at ScienceDirect

MethodsX

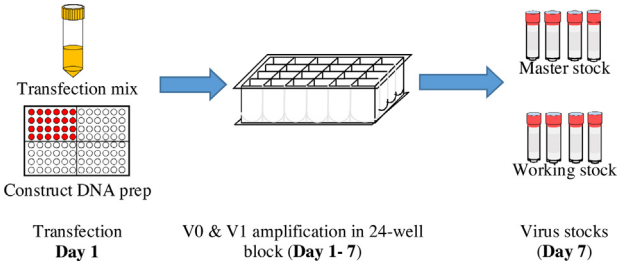
journal homepage: www.elsevier.com/locate/mex

Transfection of insect cell in suspension for efficient baculovirus generation

S. Roest, S. Kapps-Fouthier, J. Klopp, S. Rieffel, B. Gerhartz, B. Shrestha*

Protein Science Group (PSG), Novartis Institute of Biomedical Research (NIBR), Center for Proteomic Chemistry (CPC), Basel, Switzerland

GRAPHICAL ABSTRACT



ABSTRACT

Baculovirus (BV) mediated insect cell expression system utilizes transfection as a first step to introduce recombinant baculovirus DNA into insect cells. Many labs are still relying on the conventional liposome based transfection method in adherent culture. Here we describe a more efficient method that can replace the existing method. This method is economical and does not require any special adjustment in existing labs.

- An innovative method of transfecting insect cells in suspension using polyethyleneimine (PEI) is described here.
- The beauty of this method is minimal intermediate manipulation of culture during transfection and virus generation.
- The method significantly reduces the chances of cross contamination of viruses while handling multiple targets and constructs as well as the other microbial contamination.

ARTICLE INFO

Keywords: Transfection in suspension, Baculovirus, Insect cell, Sf9, Lipofectin, Polyethyleneimine (PEI), High-throughput

Article history: Received 26 January 2016; Accepted 26 April 2016; Available online 4 May 2016

* Corresponding author at: Protein Science Group (PSG), Novartis Institute of Biomedical Research (NIBR), Center for Proteomic Chemistry (CPC), Virchow 16-3.249.10, Novartis, CH-4056, Basel, Switzerland.

E-mail address: binesh.shrestha@novartis.com (B. Shrestha).

http://dx.doi.org/10.1016/j.mex.2016.04.011

Method details

We have described a superior method to transfect insect cells using polyethyleneimine (PEI) in suspension culture for the purpose of recombinant virus generation. The method is simple that allows transfection of multiple targets and constructs in parallel using 24-deep well block. Our lab uses FlashBac system to transfect Sf9 insect cells hence protocol is designed accordingly.

A. Preparation of polyethylenimine (PEI)

This protocol was adapted from "Transient Expression in HEK293-EBNA1 Cells," Chapter 12, in Expression Systems (eds. Dyson and Durocher). Scion Publishing Ltd., Oxfordshire, UK, 2007.

1. Take approximately 450 mL of Milli-Q water in a 500 mL glass beaker.
2. Add 500 mg of PEI (Cat no. 23966-2; Polysciences) in a beaker with gentle stirring.
3. Add 12 M HCl drop-wise to the solution until the pH drops <2.0.
4. Stir until the PEI is dissolved ($\sim$2–3 h). Monitor and maintain pH <2.0 throughout. Approximately 800 μL of 12 M HCl will be required for full PEI dissolution. There may still be some small fiber-like particles that will not dissolve.
5. Add concentrated NaOH dropwise to bring solution to neutral (pH 7.0).
6. Approximately 500 μL of 10 M NaOH will be required to neutralize the PEI solution
7. Pour the solution into a 500 mL glass cylinder. Adjust the final volume to 500 mL with Milli-Q water.
8. Filter sterilize the solution through a 0.22 μm membrane filtration using vacuum filtration device.
9. Store aliquots of the desired volume at $-20\,^{\circ}$C.

B. Routine growth and maintenance of cells

1. Sf9 cells adapted to suspension culture are maintained in Gibco® Sf-900™ III SFM medium (Cat. no. 12658-027, life technologies). This serves as a master stock. Cells were passaged once the viable count reaches approximately 10×10^6 cells per mL by adding fresh medium.
2. Take an aliquot of cells from the master stock and dilute to initiate 50 mL starter culture in 250 mL Erlenmeyer flask (Cat. no. 431144, Corning®). Recommended starting density is $(0.7–0.8) \times 10^6$ cells per mL.
3. Incubate diluted culture at 27 °C and 90 rpm in shaker incubator (Kuhner ISF-4-V: 50 mm rotating diameter).
4. Analyze cell count and viability using Vi-Cell™ XR cell counter (Beckman Coulter) or Neubauer's counting chamber. It is recommended to check cell parameters every day for first few days which will give an idea on frequency of passage required. We passage cells two times a week, on day 2 (eg. Tuesday) and day 5 (eg. Friday).

Note: If you are new to cell culture and starting your first cell culture in suspension, please follow supplier's instruction to revive cells from frozen stock.

C. Maintenance of cells for transfection

In order to manage the work flow and time, Wednesday is the best time to start transfection in our set up. Hence the procedure is described accordingly. Monday is taken as a Day 1 of a week. You can adjust it to fit best for your schedule.

1. Take a small volume of cell culture from master stock and dilute to $(0.7–0.8) \times 10^6$ cells per mL in Sf900III medium on Day 5 of a week i.e. on Friday.
2. The following week on Day 1 (e.g. Monday), dilute cells to $(0.7–0.8) \times 10^6$ cells per mL in Sf900III medium.
3. On Day 2 (i.e. Tuesday) dilute further to $(0.7–0.8) \times 10^6$ cells per mL in fresh medium.