



Production of High-Titer, Low-Passage Recombinant Baculovirus Stocks

Note: For all passages of baculovirus stock production, use healthy Sf9 cells that are in log phase of growth, growing in ESF 921. Alternatively, ESF AF can be used in place of ESF 921. ESF 921 or ESF AF can be used in place if the Transfection Medium although efficiency is increased with the Transfection Medium.

Cotransfection (Production of P0 stock)

Pre-warm reagents to room temperature before use.

Adherent method:

1. Plate out 0.9×10^6 cells per well of a 6-well plate. Use 2 ml of a 0.46×10^6 /ml solution. Let cells adhere 15-30 minutes.
2. Mix the following in polystyrene tubes:

Solution A: 2 ug recombinant transfer vector
.5 ug linearized viral DNA
100 ul Transfection Medium

Solution B: 6 ul Lipofection reagent of choice
100 ul Transfection Medium
Let the solutions sit for at least 5 minutes.
3. Combine solutions A and B, mix gently, and incubate at room temperature for 30 minutes
4. After incubation add 800 ul Transfection medium to tubes from step 3, mix gently.
5. Remove ESF 921 from cells adhered to plate.
6. Add transfection mix dropwise onto edge of well, being careful not to disrupt cell monolayer.
7. Incubate at 27° C for 4-5 hours.
8. At the end of incubation, remove the transfection mix and add 3 ml ESF 921. It is recommended to add an antibiotic at this step if there is a question of the sterility of the DNA. Use 10 ng/ml gentamicin.
9. Collect supernatant 4-5 days post transfection. Spin out cell debris.

Nonadherent method:

1. Plate out 1×10^6 cells in 0.5 ml ESF 921 per well of a 24 deep well block. (It is also acceptable to spin the cells down at 1000 rpm and resuspend in Transfection Medium).
2. Mix the cotransfection solution as above.



3. After incubation, add the 1 ml total cotransfection mix to the well of the deep well block.
4. Incubate at 27° C for 4-5 hours
5. Add 3 ml ESF 921 to the wells (final volume of 4 ml). It is possible to add an antibiotic at this step if there is a question of the cleanliness of the DNA. Leaving the lipofection reagent in the media does not appear to have a negative effect that outweighs the utility of performing large numbers of cotransfections. If following the adherent method, remove transfection mix.
6. Cover and incubate with shaking for 4-5 days.

Generation of P1 Stocks:

1. Seed a 125 ml Erlenmeyer culture flask with 30 ml ESF 921 media containing Sf9 cells at 10^6 cells per ml. These should be cells taken from a log phase culture. Let the cells grow overnight.
2. Inoculate flask with either 0.5 ml P0 supernatant (from adherent method) or 0.5 ml P0 culture (cells and sup from nonadherent method). Save remaining P0 sups. Note: These volumes are based upon moderate to good cotransfection efficiencies. If the cell count does not increase to between $3-4 \times 10^6$ /ml after inoculation, the volume of P0 was too high and resulting titers will be low. Repeat using less P0 if necessary. If the cell count increases more than 4×10^6 /ml, repeat with a greater volume of P0.
3. Incubate flasks at 27° C with shaking for 4-5 days.
4. Remove cells by centrifugation at 2500 rpm for 5 minutes. Titer. These P1 titers are very often $>10^8$ infectious units/ml.
5. Archive remaining P1 stocks at -80° C for long term storage. There will be a drop of 1 log in titer upon thaw so account for that loss when determining aliquot volumes. Use aliquots to generate working P2 stocks.

Generation of P2 Stocks:

1. Seed a shake flask at the desired volume with Sf9 cells at 10^6 /ml in ESF 921. Let the cells grow overnight.
2. Infect the flask at an MOI of 0.1 (It is possible to generate a high titer stock using an MOI of 0.01 but may not be as efficient).
3. Incubate flasks at 27° C with shaking for 3 days. Harvest. The viability should still be greater than 90%. There is no advantage to letting the culture become less viable. The initial titers will be similar but the cell debris associated with excessive cell death will negatively impact storage.
4. Remove cells by centrifugation. Speed and duration increase with an increase in volume.
5. Immediately filter virus stock through a 0.2 micron, low protein-binding filter. This step is important for virus titer stability. There is rarely a loss in titer due to the filtration but there is often a loss of titer due to binding of virus to cell debris.
6. Titer stock. This typically yields $>5 \times 10^8$ infectious units per ml.
7. Store stock at 4° C for up to 6 months.

**Generation of P3 or greater stocks.**

1. Follow the protocol as described for P2 generation.
2. It is not recommended to continually amplify successive passages as this will result in the amplification of undesirable variants of baculovirus resulting either from the cotranfection or random mutations.
3. Once a P1 or P2 stock has been generated of sufficient titer and volume, use this as a master stock to generate working stocks for expression.