Isolating P1 Viral Stock

Budded virus should be released into the medium 72 hours after transfection. However, if your transfection efficiency was not optimal, cells may not show all of the signs of viral infection until 4 or 5 days post-transfection. Beginning at 72 hours after transfection, you should visually inspect the cells for signs of infection (see below). Once the cells appear infected (i.e. demonstrate characteristics typical of late to very late infection), harvest the virus from the cell culture medium using the procedure below.
Virally-infected insect cells typically display the following characteristics as observed from visual inspection using an inverted phase microscope at 250-400X magnification. The time points provided below assume that the transfection was successful (i.e. transfection efficiency was high).

Signs of Infection

-Early (first 24 hours)
Increased cell diameter – A 25-50% increase in cell diameter may be seen.
Increased size of cell nuclei – Nuclei may appear to "fill" the cells.
-Late (24-72 hours)
Cessation of cell growth – Cells appear to stop growing when compared to a cell-only control. Granular appearance – Signs of viral budding; vesicular appearance to cells.
Detachment – Cells release from the plate or flask.
-Very Late (>72 hours)
Cell lysis – Cells appear lysed, and show signs of clearing in the monolayer
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Harvesting P1 virus

1. Once the transfected cells demonstrate signs of late stage infection (e.g. 72 hours post-transfection), collect the medium containing virus from each well (~2 ml) and transfer to sterile 15 mL tubes. Centrifuge the tubes at 500 x g for 5 minutes to remove cells and large debris.
2. Filter through 0.2 um sterile filter.
3. Transfer the clarified supernatant to fresh 15 mL tubes. This is the P1 viral stock. Store at 4°C, protected from light
4. For long-term storage, store an aliquot of the viral stock (1mL + 2% FBS) at -80°C for later re-amplification. Do not store routinely used viral stocks at temperatures below 4°C. **This method is NOT recommended. Repeated freeze/thaw cycles can result in a 10- to 100-fold decrease in virus titer.**

Amplifying the Baculoviral Stock

Note: We typically do not perform Plaque Assay to count the viral titer. Provided that the transfection to generate P1 was successful, a 10% (v/v) infection ratio is equivalent to an infection with stable MOI=1.

1. On the day of infection, prepare an Sf9 cell suspension in SF900II complete media and plate 50mL of cells at 2 x 10^6 cells/ml into two T225 flasks. Incubate cells at room temperature for 1/2 hour to allow attachment.
2. After 1/2 hour, inspect cells under an inverted microscope to verify attachment.
3. Aspirate the media and add 5mL or 10% (v/v) P1 viral stock to each flask. Incubate with the virus for 1 hour at 27°C, gently moving the flask every 20 minutes to ensure that the cells do not dry out.
4. Add 45ml of Sf900 II SFM complete media and incubate the cells for 48 hours in a 27°C humidified incubator.
5. 48 hours post-infection, collect the media containing virus and transfer to sterile 50 mL tubes. Centrifuge the tubes at 500 x g for 5 minutes to remove cells and large debris.

Note: It is possible to harvest virus at later times after infection, and we recommend harvesting at 72 hours after infection. Optimal harvest times can vary and should be determined for each baculoviral construct. Remember that culture viability will decrease over time as cells lyse.

6. Filter the supernatant through a sterile 0.2uM filter. This is the P2 viral stock. Store at 4°C, protected from light.

Once you have generated a high-titer P2 baculoviral stock, you may scale-up the amplification procedure to any volume of your choice. To produce this high-titer P3 stock, proportionally scale up the amount of cells and volume of virus used, and follow the guidelines and procedure outlined above. Infect SF9 cells in suspension for P3 and P4 virus at 10% v/v. We generally transfect the Ric15 cells with P3 or P4 virus and we do not amplify the virus beyond P4 as mutations will start to get introduced with every passage beyond that point.