Baculovirus (BV) Purification by Ultracentrifugation of Sucrose Solution

(Yi Zhu @ 06/02/2024; TCH commented)

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I. Material Preparation and Equipment

1). Preparation of **27% Sucrose Solution**

Dissolve 27 grams of Sucrose in 60ml of double-distilled water (ddH_2O) (using magnetic stirring bar if necessary);

Add ddH_2O to 100ml;

Autoclave the solution to sterilize it;

Store the sterilized 27% Sucrose solution at 4°C until use.

2). Beckman L7 Ultracentrifuge (Dept of Surgery, located in **Room AB-525**)

3). Rotor: **SW32Ti** and tubes (6-position; each tube holds ~**36ml**; either thin or thick wall)

II. Large Scale BV Amplification

1). Seed exponentially growing SF9 cells in **15~20 of 100mm petri dishes** (10ml medium/dish), or **2~3 of 250ml BV amplification bottles** on a shaking platform (~50ml medium/bottle) [Note: it is important not to over-infect the SF9 cells in order to obtain high BV titer preparation];

2). Maintain the infected SF9 cells in the insect medium (e.g., **ESF 921** Insect Cell Culture Medium; Thermo-Fisher Cat# NC9541308; or **SF-900™ III SFM**; without adding any FBS) at 27-29°C, without CO_2;

3). Collect the BV-containing culture medium at three days after infection, and keep in 4°C till use.

III. BV Purification by Ultracentrifugation in 27% Sucrose Solution

1). Centrifuge the BV supernatant at 1,500g for 20 minutes at 4°C to remove cell debris;

2). Transfer BV-containing supernatant to 50ml conical tubes;

3). To each SW32Ti tube, add **16 ml** of 27% Sucrose solution first, and then carefully overlay with **20ml** of the cleared BV supernatant on top of the sucrose solution (by adding the supernatant slowly against tube wall with 5ml serological pipets or sterile plastic Pasteur pipets);

4). [Optional: if the supernatant falls short to completely fill paired tubes, you can fill them with mineral oil since each tube needs to be filled >95% full];

5). Load the paired tubes to the SW32Ti rotor, and then carefully transfer/load the rotor to the Beckman L7 Ultracentrifuge (see the brief Operational Instructions below).
6). Centrifuge at 100,000g (approximately 28,000 RPM for the SW32Ti rotor) for 90 minutes at 4°C;
7). At the end of centrifugation, pour off all liquid, and the purified BV is in the pellet form at the bottom of the tubes;
8). Dissolve the BV-containing pellet in appropriate volume of sterile PBS (e.g., 500µl total volume for 150ml initial BV supernatant).
9). Aliquot the purified BV stocks (e.g., 50-100 µl/tube) and keep at -80°C for long-term storage.

IV. Titer Determination of the Purified BV Stocks (expressing GFP or RFP marker)

Plate four 35mm dishes of SF9 cells, two for supernatant after ultracentrifuge, and two for purified BV.

If BV is successfully purified, 1ul of purified BV is enough to get 100% fluorescence, while 10-50ul supernatant should show very little or no fluorescence.

SUPPLEMENTAL: Brief Instruction for Operating Beckman L7 Ultracentrifuge

1. **Equipment and Rotor:**
   The SW32Ti rotor with 36ml tubes (either thin wall or thick wall) is used in our lab.

2. **Power On:**
   Turn on the ultracentrifuge machine using the switch located on the right lateral side.

3. **Log Entry:**
   Record your usage in the log book (i.e., name/date/rotor/RPM/PI).

4. **Load the Tubes:**
   Place the prepared tubes into the metal tubes, ensuring they are securely closed with the correct number.
   Carefully insert the SW32Ti rotor into the ultracentrifuge machine, ensuring all tubes are correctly matched with their number.

5. **Operate the Ultracentrifuge:**
   Close the door of the ultracentrifuge.
   Press the “Vacuum” button to initiate the vacuum process.
   Set the required RPM, temperature, and time.
   Press “enter” to confirm the settings, then press “start” to begin the run.

6. **Post-Run Procedure:**
   After the run, record the final revolution count number in the log book.