GENE TRANSFER VECTOR CORE, UNIVERSITY OF IOWA

VIRAL PARTICLE PURIFICATION

Reagents

Light CsCl (1.2 gm/mL in 10 mM tris pH 8.1)

Heavy CsCl (1.45 gm/mL in 10 mM Tris pH 8.1)

10 mM Tris pH 8.1

3% Sucrose/PBS (3 L for Dialysis per virus)

50% Glycerol/BSA

Materials

Slide-A-Lyzer Cassettes from Pierce – Cat #:66425

Beckman Centrifuge Tubes, 1 x 3 inches – Cat #:344058

Beckman Centrifuge Tubes, $^{9}/_{16}$ x 3 inches – Cat #:344059

Procedure

Freeze/thaw the lysate 3 times, using a dry ice EtOH bath and 37°C waterbath.

Spin the lysate at 3500 rpm (g = 1177.8) for about 10 min.

First Cesium Chloride gradient.

a. 20 plate lysate -
   - Sw 28 rotor and buckets for Beckman Optima LE-80K ultracentrifuge.
   - Add 12 ml of light CsCl to Beckman centrifuge tube.
   - Add 12 ml of heavy CsCl very slowly under the light CsCl. Take 14 ml of heavy CsCl in the pipette so no air bubbles disrupt the gradient.
   - Add the clarified lysate very gently against the side of the tube on top of the CsCl gradient. Supplement with 10 mM Tris to about $^{1}/_{4}$" below the top of the tube. Make sure that the volume is balanced between the 2nd sample and/or the blank.

b. 10 plate and 5 plate lysate -
   - For Beckman Ultras, use the Sw 41 Ti set.
   - Add 3 ml of light CsCl to the centrifuge tube.
   - Add 3 ml of heavy CsCl very slowly under the light CsCl. Always take 4-6 ml of heavy CsCl in the pipette so no air bubbles disrupt the gradient.

- Add the clarified supernatant very gently against the side of the tube on top of the CsCl gradient. Supplement with 10 mM Tris to about $^{1}/_{4}$" below the top of the tube. Make sure the tubes are balanced.

Spin in the ultra centrifuge at 20K rpm (g = 33,735) at 4°C for a minimum of 3 h.

Pull the viral band. The viral band is the bottom white colored band that is present where the heavy and light CsCl meet:

a. Remove the cell debris at the top of the gradient, to about half an inch above the viral bands with a glass Pasteur pipette.

b. Place solution in waste bottle.

c. Use a 5 cc syringe with an 18 g 1.0" needle. Insert the needle through the side of the tube about four needle widths below the virus band.

d. Extract the virus in as small a volume as possible, leaving behind incomplete bands higher up the tube.

e. Dilute the band by raising the volume to 3 ml of 10 mM Tris in a snap cap tube. Place the syringe and needle inside the snap cap tube and pull up the 10 mM Tris. The band must be diluted by 50% with 10 mM Tris.

f. If you started with the 5 or 10 plate lysates, dilute your pulled band to only 2 mL.

Second gradient -

a. 20 plate lysate -
   - Use the Beckman Sw 41 Ti rotor and buckets.
   - Add 3 ml of light CsCl to centrifuge tube.
   - Add 3 ml of heavy CsCl very slowly under the light CsCl.
   - Add the virus/10 mMTris very gently against the side of the tube on top of the CsCl gradient. Supplement with 10 mM Tris to about $^{1}/_{4}$" below the top of the tube.

b. 10 or 5 plate lysate -
   - Use the Beckman Sw 60 or equivalent rotor, buckets and tubes.
   - For 1 mL gradients, following the above instructions to form the gradient and add the virus.

Spin in the ultra centrifuge at 20K rpm (g = 30,195) at 4°C overnight.

Pull the viral band as above using a 5 cc syringe. If not dialyzing, put the virus in a small, labeled snap cap tube, or eppendof microcentrfuge tube.

Store at –80°C

Dialysis -

a. In the syringe, add 1 ml of 3% Sucrose/PBS, about half the volume of the band. If the virus is too concentrated, it will precipitate out.

b. Inject the contents of the syringe into a "Slide-A-Lyzer" dialysis cassette from Pierce. Using the 5 cc syringe pull out the remaining air in the slide.

c. Place the dialyzer slide with a buoy in a bucket of 3% Sucrose/PBS. Use 1L Sucrose/PBS per slide.

d. Change the Sucrose/PBS once an hour, 2 times. You will need a total of 3 liters of 3% Sucrose/PBS for each virus prep.

e. To remove the virus from the dialyzer slide, use a 5 cc syringe and add 1 - 2 mL of air to the slide. Use a different port from step "b".

f. With syringe still in the Slide-A-Lyzer extract the virus.

g. Note total volume of virus in the syringe. Subtract 0.4 ml from the total volume.

h. Place virus in a snap cap tube.

i. For glycerol stock -
   - Transfer 250 μl of virus into a 2 ml screw cap tube.
   - Label the tube with virus name.
   - Add approximately 300 μl of 50% Glycerol/BSA to the virus. (For very thick bands, add 500 to 1000 μL.)

j. Turn the spectrophotometer on and allow it to warm up.

k. Prepare a blank of 13 μl of Sucrose/PBS in 987 μl of 10 mM Tris.

l. Dilute the virus to 1:75 dilution (13 μl of virus in 987 μl of 10 mM Tris).

m. Measure the absorbance at 260 nm wavelength.
   - 1.0 $O.D._{260} = 1.1 \times 10^{12}$ pt/ml
   - Calculate pt/ml concentration taking into consideration the 1:75 dilution factor.

n. Dilute in 3% Sucrose/PBS or Glycerol/PBS to the virus to 1.0×10^{12} particles/ml.

o. Target values -
   - 3% Sucrose/PBS preps: 1×10^{12} pt/ml for the working stock (between 1 - 1.5 is ok).
   - Glycerol/BSA stocks: 3×10^{12} pt/ml (between 3 - 4 is ok).

p. Aliquot and freeze at –80°C (sucrose/BSA) or –20°C (glycerol/BSA)
   - For 3% Sucrose/PBS preps:
     - 1 mL, 500 μL, 200 μl aliquots

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