Lentivirus Production and Infection

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This protocol was standardized using 20mL collected supernatant and 100uL of concentrated virus is enough to infect one well of a 12 well plate with 1:10 dilution of the virus (Vf=500uL). At this dilution, 30% of suspension cells (K562) were infected.

The drug selection marker on the lentiviral plasmids is blasticidin. 3.5ug/mL is the optimal amount to kill 100% of K562 cells that are not blasticidin resistant, 2ug/mL for HeLa S3 cells. The normal killing time is 10-14 days.

Procedure

Day 1

Plate 293FT cells in 10cm plate so that they will be ~90% confluent on the day of transfection (~5 x 10^6 cells in 10mL)

- Do not use 293FT cells that have been passed more than 20 times.
- Do not coat plates with poly lysine.

Day 2

Transfect cells:

1. Dilute 9ug of ViraPower Packaging mix and 3ug of pLenti6 expression vector in 1.5mL OMEM in a 15mL conical tube.
   - Use vectors that have been midi- or CsCl prepped only.
2. In a separate tube, dilute 36uL Lipo2000 in 1.5 mL OMEM.
3. Incubate 5 min RT.
4. Combine diluted DNA with diluted Lipo2000 and incubate 20 min RT.
5. Add DNA-Lipo mix DROPWISE to each plate of cells and incubate cells overnight.

Day 3

Remove media containing Lipo/DNA and replace with 10mL complete media

Day 4

- WARNING: From this point on, you are working with live virus, so be sure to double glove and wear a lab coat to protect yourself because the virus can infect you! Make sure to put everything that touches virus in a solution of 70% etoh/1% sds to inactivate the virus. Label everything that you store for others to know where the lentivirus is.

1. Collect supernatant from plates and store in foil wrapped conical tube at 4 deg.
2. Add 10mL fresh media.

Day 5

1. Collect supernatant from plates and combine with supernatant from Day 4 samples.
2. Centrifuge supernatants at 1000 rpm for 5 min at 4 deg to pellet cell debris.
3. Filter the supernatants through a 0.45um low protein-binding filter.
4. Add filtered supernatant, up to 30mL/ tube to 25x89 mm ultratubes (open top, thick walled, polycarbonate).
5. Spin for 90 min in SW 28 swinging bucket rotor at 25000 rpm, 4 deg.
6. Aspirate supernatant exhaustively until the tubes seem dry. Invert tubes over a Kimwipe to drain remaining supernatant for 5 min. Aspirate the media residue at the opening of the tubes.
7. Add 100uL PBS+Ca and Mg with NO bicarbonate to the tubes and seal with Parafilm.
8. Nutate the tubes at 4 deg overnight to resuspend viral pellet.
   Use virus immediately or store at –80 for up to 1 year.