Lentivirus production

Day 0 – Day before Transfection:

1. Split 4.5x10^6 cells 293T cells one day before transfection into 10 cm dish (9ml).

Day 1 – Day of Transfection

2. In a sterile tube, dilute total plasmid DNA (ug) in 500ul **diluent***. Use transfer vector: viral packaging (psPAX2):viral envelope (pMD2G) at 4:2:1 ratio (6:3:1.5 ug, respectively)
3. Add 42 ul of PEI (1ug/uL) to the diluted DNA. Mix immediately by pipeting up and down/ vortexing. The volume of PEI used is based on a 4:1 ratio of PEI (ug): total DNA (ug).
4. Incubate 10-15 minutes at RT (don't go over 20 min).
5. Add 500ul of DNA/PEI mixture to each plate of cells and incubate overnight.

Day 2 – 18-24hrs Post-Transfection

6. Aspirate the media and wash cell with pre-warmed 8 ml PBS once.
7. Overlay with pre-warmed 9-10ml transfection media (DMEM w/o Phenol Red supplemented with 3 to 4% FBS and glutamine).

Day 3

8. Collect viral supernatant, filter using 0.22µm, and store at 4°C. LV is stable for 1 week at 4°C without loosing titer.
   (Option – If making multiple plates of same virus, aliquot soup into 50ml conical tubes and spin O/N at 4ºC at 8500 g, aspirate media carefully (pellet can be loose), and resuspend at 100X in HBSS.)
9. Virus can be aliquoted and stored at -80 indefinitely. Freeze thaw cycles will decrease titer.

Reagents:

PEI (1ug/ul) – PEI is polyethylenimine 25kD linear from Polysciences (cat# 23966-2). To make a stock solution:

- Dissolve PEI in endotoxin-free dH_2O that has been heated to ~80°C.
- Let cool to room temperature
- Neutralize to pH 7.0, filter sterilize (0.22um), aliquot and store at -20°C, a working stock can be kept at 4°C (up to 1 month). Once thawed, you may see precipitate: then vortex until ppt goes into solution.

Diluent: 10 mM HEPES/ 150mM NaCl (pH 7.05) – No $NaCO_3$ buffering. Use 1L TC grade water (commercial), and add HEPES/NaCl, and wrap up the bottle with foil to prevent light. Store at room temp.