PEI Transfection

Transfection:

1. Split 293T cells one day before transfection in DMEM/10% FBS medium:
   a. **6 well dish:** $0.5x10^6$ cells
   b. **10cm dish:** $4.0x10^6$ cells
   c. **15cm dish:** $9.0x10^6$ cells

2. Prior to transfection bring all reagents to room temperature.

3. In a sterile tube dilutetotal plasmid DNA (ug) in **serum-free** DMEM w/o phenol red (volume of media is 10% of final volume in culture vessel). Use transgene: viral packaging(psPAX2):viral envelope(pMD2G) constructs at 4:2:1 DNA ratio

   a. **6 well dish:** 200ul+ 3ug of total DNA
   b. **10cmdish:** 1mL+ 7-8 ug of total DNA
   c. **15cmdish:** 2mL+ 11-12ug of total DNA

4. Add PEI (1ug/uL) to the diluted DNA. Mix immediately by vortexing or pipeting. The volume of PEI used is based on a 3:1 ratio of PEI(ug):total DNA(ug).

   a. **6 well dish:** 9ul of PEI(1ug/ul) = 9ug
   b. **10cmdish:** 21ul of PEI (1ug/ul) = 21ug
   c. **15cmdish:** 33ul of PEI(1ug/ul) = 33ug

5. Incubate 15 minutes at RT

6. Add DNA/PEI mixture to cells

7. Harvest transfected cells and/or viral supernatant at 48 hours post-transfection

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.