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 dilute total 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 + 3 ug of total DNA
    b. **10cm dish:** 1mL + 7-8 ug of total DNA
    c. **15cm dish:** 2mL + 11-12 ug 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
    a. **10cm dish:** 21ul of PEI (1ug/ul) = 21ug
    b. **15cm dish:** 33ul of PEI(1ug/ul) = 33ug

Helper	**6ug**
AAV9	**4ug**
crispr	**4ug**

PEI：42ul

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.