**General PEI Transfection**

**Materials: Bring these reagents to room temp prior to transfection**

* 10mM PEI (polyethylenimine, 25kDa) (Sigma, #40,872-7) (Stock made by Jun and Stored in the 4ºC refrigerator in the tissue culture room, take one tube and label it with your name.)
* Filtration causes loss of PEI. Twice filtrations totally deplete PEI!
* PEI solublize plastics, so use glass container!
* 5% glucose in PBS (sterile filtered)
* DNA

**Procedure:**

1. Culture the target cells to the appropriate confluency (50% is a good starting point for most cells; ask Yibin or labmates for suggestions about any particular cell line).
2. Prepare two autoclaved eppendorf tubes for each transfection:

Tube A: n ug DNA in m ul of PBS (5% glucose). Vortex briefly

Tube B: 4n ul of 10mM PEI and m ul of PBS (5% glucose). Vortex briefly

* + In this case, the PEI/DNA ratio is 4:1 (V/W). The PEI/DNA ratio is the most important factor and might varied between different cell lines and different PEI stock. For optimal results one should titer that ratio (from 1:1 to 4:1) with his/her own PEI and DNA prep.
  + For each well of 6-well plate, n=3, m=70; for each well of 12-well plate, n=1, m=40; for 10cm dish, n=10, m=300
  + If you are doing multiple wells of transfections for the same plasmid (e.g. luciferase reporter assays), multiple n (and m) in both Tube A and Tube B with the number of well you are transfecting and prepare a master PEI/DNA mix (see below) so that transfection efficiency is relatively consistent between different wells. For example, if you are transfecting a plasmid into 6 wells, prepare 18ug of DNA and 420ul 150mM NaCl in Tube A and 72ul PEI and 420 ul of 150mM NaCl in Tube B.

1. Slowly add the PEI (Tube B) into the DNA (Tube A) drop by drop, then vortex immediately
2. Incubate the DNA/PEI mixture for 15 minutes at room temp.
3. During the incubation, remove old medium, gently add x ml **serum-free medium** into each well/dish.

* For 6-well and 12-well plate, x=1 for each well ; for 10cm dish, x=5

1. Add PEI/DNA mixture, mix by shaking the plate forward and backward, then side to side (not swirling!) (add 600ul mixture into a 10cm plate, 140ul into each well of a 6-well plate, and 80ul into each well of the 12-well plate).

* Transfection efficiency may be increased by plate centrifugation immediately following the transfection (1000rpm, 2 min)

1. Put the plates/dishes back to 37°C incubate with 5% CO2, incubate for 4 hours

* Beyond a certain threshold the PEI has a strong toxic effect on the cells. It is possible to reduce the toxic effect by reducing incubation time (the time after transfection before returning the normal growing medium), or alternatively by including serum at a certain level during that incubation period. (In our experience, this is generally not necessary as the toxicity of PEI is low to most cells)

1. After the incubation period, replace transfection medium with the normal growing medium

* For 6-well plate, 2ml medium per well; for 10cm dish, 10ml medium; for 12-well plate, 1ml per well.