**PEI Transfection for Luciferase Reporter Assay in 12-well Plates**

**I. Materials: Bring all reagents to room temp prior to transfection**

**10mM PEI** (polyethylenimine, 25kDa) (Sigma, #40,872-7)

10 ml water

9 mg PEI liquid (50%w/v) (~5ul)

Mix well!

Neutralize the solution with HCl (pH6.5-7.5) (~80ul)

Filter (0.2um or 0.45 um Millipore filters)

Store in sterile glass vials as 2ml aliquots at 4°C

**150mM NaCl**

3 ml of sterile 5M NaCl stock solution

97 ml sterile water

**II. Procedures**

The following procedure is for 6-well transfection for each reporter plasmid so that there will be triplicates for -/+ conditions (for example, -/+ TGF). If -/+ condition is not needed (for example, testing the basal expression level of reporters in different cell lines), use half amount of the regents (e.g. 1ug of reporter instead of 2ug) listed below for each transfection (3 wells). All reagents and containers must be sterile.

***Day 0:***

1) Seed cells into 12-well plates (1ml media) at the optimal confluency for each particular cell line (e.g. 20% for HaCaT, 70% for MDA-MB-231).

**Note:**

* The total surface area table for each type of cell culture plate/dish was posted on fridge sitting right next to the tissue culture hood. Use that table to calculate how to split the cells so that the correct confluency on the wells can be achieved.
* Calculate how much volume do you need (12 ml for each plate) so that you can add 1ml of cell suspension into each well. Make a master cell suspension for each cell line so that the density of each cell line is consistent throughout all wells.
* Make sure cells are in single cell suspension. If the cells are in clumps, the transfection won’t work well.
* **DO NOT** shake or swirl the plates after you seed the cells onto the plates. Otherwise the cells will all concentrate in the middle of the well and ruin your transfection experiment.

***Day 1:***

1. Calculate and aliquot 2ug of each reporter plasmid into each 1.5ml eppendorf tube.
2. Prepare the following two master mix in two 15ml or 50ml falcon tubes:

Tube A: add (n+1) x 0.2 μg of rellina-luciferase plasmid in (n+1) x 150 μl 150mM NaCl. n is the number of transfections. For example, if you need to do 5 transfection (into 30 wells) of 5 different reporters, use 1.2ug rellina-luciferase and 900ul of 150mM NaCl. Vortex briefly .

Tube B: add (n+1) x 4 μl of 10mM PEI and (n+1) x150 μl of 150 mM NaCl. Vortex briefly.

3) Add 150ul of solutions in tube A into each eppendorf tube containing the reporter plasmid. Add another 150ul of solutions in tube B into each eppendorf tube.

4) Incubate 15-45 minutes at room temp.

5) In the interim, **COMPLETELY** remove (residual serum can lower transfection efficiency) old medium from each well on the plate. You can remove media from no more than 3 plates each time to avoid cell drying. You **DON’T** need to change Pasteur pipets between each well. Add 1 ml serum-free media per well.

6) Prepare n x 5ml faclon polystyrene tubes. Add 2ml serum-free media into each tube. Remove the 300ml PEI/DNA mixture from each eppendorf tube and add it into each 5ml falcon tube. Vortex briefly. Remove serum-free media from each well and Add 350 μl PEI/DNA mixture from each 5ml falcon tube into each well. **DO NOT** shake or swirl the plates. You can perform perform this procedure to no more than 3 plates at a time to avoid drying of cells.

7) Returned plates to incubator for 4 hours

8) Aspirate transfection medium and replace it with 1ml normal growing medium/well.

***Day 2:***

Add TGF (or any other drug) to the + wells if necessary. When adding TGF, remove media from all wells (both – and +), add fresh media into – wells and media with TGF into + wells.

***Day 3:***

Remove media from each well, add 200ul 1x lysis buffer into each well for reporter assays. If reporter assays can not be performed immediately, store the plates in -20ºC freezer.

**Important Considerations about PEI transfection reagents:**

* The PEI/DNA ratio is the most important factor. For transfection take a ratio of 1/1 to 3/1 of PEI/DNA (volume/weight)
* For optimal results one should titre that ratio with his/her own PEI and DNA prep.
* 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 incubataion period.
* Filtration causes loss of PEI. Twice filtrations totally deplete PEI!
* PEI solublize plastics, so use glass container!