Preparation of PEI stock solution(1mg/ml)

1. Polyethylenimine, linear from Polysciences, Inc. Cat# 23966

2. Dissolve the powder in H_2O. Adjust pH to 7.0 with HCl (the solution becomes clear as the pH is adjusted). Be patient. It may take hours to get completely dissolved. Suggest to make at least 100 ml each time since it's easier to work with larger volume.

3. Adjust the volume to the final concentration of 1 mg/ml.

4. Filtrate through 0.22 um membrane. You HAVE TO DO THIS, it's not only for sterility, you may not be able to see any transfection if you don't filter. The reason may be that the presence of undissolved PEI particles will precipitate DNA but cannot be transported into cells.

5. Make 0.2~1ml aliquots, and store at –80°C. Once thawed the tube should be kept at 4°C. It should be good for two months at least. But watch the transfection efficiency closely after two months. If you think there is a decline in transfection efficiency, discard and get a new tube.

6. For every batch of PEI, Test the transfection efficiency using different DNA/PEI ratio(mass:mass) (1:1, 1:2, 1:3 for example). Choose the best ratio for further experiments.

Transfect 293 cells in a 150 cm^2 flask

1. Remove half of the medium from the flask (usually 15 ml from 30 ml). for 6 wells. (1ml from 2 ml)
2. Add 40 μg of expression vector DNA to 1.5 ml Opti-MEM and mix. 4μg DNA to 100μl Opti-ME
3. Add 40 μl of 1mg/ml polyethyleneimine to the DNA/Opti-MEM and mix gently. 1:2 ratio → 80μl 1:3 ratio → 120μl of 1mg/ml
4. Leave it for 10 min at toom temp.
5. Add the PEI/DNA/Opti-MEM to 293 cells in the flask.
6. Optional: Add 15 ml of fresh medium on the next day. for 6 wells + 1 ml of fresh medium

Notes: 1. It was observed mixing DNA first gave better, reliable transfection.

2. You can proportionally reduce the amount of DNA/PEI for experiments carried in small dishes(using the surface area as a guide).

3. Hela cells transfect slightly less efficiently than 293, but comparable to other transfection reagents.