Transfection with Linear 25 kDa Reagent (for 6 well dish)

1. Seed cells 24 hrs before at 60% confluency (about 3×10^5 cells) since you do get about 20% - 30% cell death at a 3:1 ratio of the reagent.

2. Next day, wash cells 3 – 4 times with cell media shortly before transfecting. This is required since there is something that most cells secrete that will interfere with the reagent.

3. In an eppendorf tube, dilute 3 μg total in 400 μl of serum free media. Mix well by pipetting up and down.

4. Add PEI to the diluted DNA and vortex for 10 seconds immediately.
   Optimize for cells by trying 1:1, 2:1, and 3:1 ratios of PEI:DNA

5. Incubate for 15 minutes at room temperature.

6. Add DNA/PEI mix evenly over the 6 well dish. After adding all the samples, gently swirl plate for a few seconds to mix the components.

7. Next day (give 12-16 h), change media and harvest the following day.

Note: For **293 cells**, trypsinize cells as per normal (on the day of the transfection) and add to 6 well dish. Carry out the above protocol and then add to cells in the wells. Mix PEI/DNA with cells in each well.

Cell lines tried:

At 24 hours later:

Cell	Ratio	Death	Transf. efficiency
COS-7	3:1	< 5 %	~ 40 %
U2OS	1:1 and 2:1	20 %	~ 20%
H1299	2:1	20%	~ 30 %
293	3:1		

Reagent:

PEI is polyethyleimine, a 25 kDa linear from Polysciences. Make up solution at 1 μg/μl in sterile water, neutralize with HCl to pH 7.2 and filter sterilize using a 0.22 μm filter. Aliquot and store at – 80 ^{o}C. A 5 - 10 ml aliquot can be kept at 4 ^{o}C for up to 4 months.

Linear PEI 25 kDa	Polysciences Inc.	2g	23966-2	$45.95