PEI transfection protocol

24-well protocol

1. Preparation of the transfection solution:
   For one well of a 24 well plate: 100,000 cells, 2µg of DNA and 10µl of PEI (25KD, 10uM=250ug/ml).
   NB: These quantities and volumes should be scaled up according to the number of wells to be transfected.
   - Dilute 2µg of DNA in 50µl of NaCl 150mM. Vortex gently and spin down briefly.
   - Dilute 10µl of PEI (25KD) in 40µl of NaCl 150mM. Vortex gently and spin down briefly.
   - **Important:** add the 50µl of PEI (25KD) to 50µl of DNA (ref.5) (not the reverse order) and vortex-mix the solution immediately.
   - Incubate for 10 minutes at room temperature.

2. The 100µl of the PEI (25KD) /DNA mixture are added to each well.
   Generally, the volume of the PEI (25KD)/DNA mixture represents one tenth of the total volume of the culture medium.

3. Gently swirl the plate by a slow hand rotation.

4. Centrifuge, if possible, the culture plate for 5 min. at 280g and incubate at 37°C in a moist chamber.

5. Growth of the transfected cells:
   If the transfection has been made in a restricted volume, add culture medium and serum 2-3 hours after the transfection.
   Treat the cells as usual
   A contact of 30 min. can be sufficient to obtain a transfection efficiency compatible with a good cell recovery.

   The transfected gene expression can be monitored 24-48 hours (transient expression) or 10-15 days (stable expression) after the transfection.
100mm culture dish

1. **Preparation of the transfection solution:**
   60µg of DNA and 300µl of PEI (25KD, 10uM=250µg/ml).

2. Dilute 60µg of DNA in 1500µl of NaCl 150mM. Vortex gently and spin down briefly.
   - Dilute 300µl of PEI (25KD) in 1200µl of NaCl 150mM. Vortex gently and spin down briefly.
   - **Important:** add the 1.5ml of PEI (25KD) to 1.5ml of DNA (not the reverse order) and vortex-mix the solution immediately.
   - Incubate for 10 minutes at room temperature.

2. The 3ml of the PEI (25KD) /DNA mixture are added to each dish.
3. Gently swirl the plate by a slow hand rotation.
4. **Growth of the transfected cells :**
   5. change media 4 hours after the transfection.