**Lipofectamine or Turbofect transfection protocol**
Routinely used for cultured cells (HEK293T)

Transfection into 6-well plates:
1. Seed 0.4 to 0.5 x $10^6$ cells/well 1 day prior to transfection. Grow cells to ~80% confluency prior to transfection (may require seeding at higher densities, depending doubling time).

2. For each transfection (in each well), dilute 1µg pDNA (plasmid DNA) into 100µls serum-free prewarmed DMEM (also P/S – penicillin/streptomycin, Gln – glutamine, HEPES-free) in an Eppendorf (1.5ml) tube.

3. In a second tube, add 5µls of Lipofectamine (2000) or Turbofect to 100µls DMEM (serum/PS/Gln/HEPES-free) per 6-well transfection, and mix. Add the Lipofectamine or Turbofect/DMEM mixture to the diluted DNA; mix and incubate for 15 mins. at room temperature.

4. While DNA/lipid complexes are forming, wash cells twice with prewarmed 1x PBS, 1ml per wash per well. Then add 800µls DMEM (serum/PS/Glu/HEPES-free) and incubate the cells at 37°C.

5. Add the pDNA/Plus-reagent/lipid mixture to each well dropwise (200µls per well).

6. Incubate 3-5 hours at 37°C

7. Add 800µls of 2x DMEM (16% FBS, 2x P/S, Gln, HEPES)

Notes:
-Transfections can be scaled up or down to desired plate formats.
-Cells are generally transfected at lower densities on coverslips for immunofluorescence staining and analysis.