Lipofectamine2000 for PC12 transfection

1. The day before transfection, count the cells, plate them in 6-well plates at $2x10^5$ cells per well. Cells are plated in 1.5 ml of their normal growth medium containing serum and without antibiotics.

2. For each well of cells to be transfected, dilute 0.9 μg of the desired DNA plus 0.1 μg GFP into 1 ml DMEM(HG), No serum, No P/S and mix well.

3. For each well of cells, add 3 μl of the lipofectamine-2000 reagent into the DNA containing medium, vortex (at speed 5-6) within 5 min, then incubate for at least 20 min to allow the DNA-LF2000 reagent complexes to form.

4. During the 20 min, wash cells once with DMEM(HG), No serum, No P/S.

5. Add the DNA-LF2000 reagent complexes (1 ml) directly to each well and mix gently by rocking the plate back and forth.

6. Incubate the cells at 37°C in a CO_2 incubator for 1h, add 1 ml of DMEM containing 20% horse serum/10% FBS into each well. And then incubate the cells at 37°C in a CO_2 incubator for a total of 24h.

7. Remove the DNA-LF2000 medium, PBS wash once and add fresh culture medium containing the desired reagent (ex with 100 ng/ml NGF).

8. Incubate the cells for 3 more days and assess neurite outgrowth under a microscope.

Growth medium: DMEM(HG) + 10% HS + 5% FBS + P/S
For 24 well, use 1/5 vol of all the reagents, plating cells with more volume of growth medium (0.3-0.4 ml/well).