Transfection protocol for HPC7 using lipofectamine 2000- 96 well plate

Materials:

Lipofectamine 2000, plasmids after we diluted it, OptiMeM medium, growth medium without antibiotics.

1. **Just prior to preparing complexes, plate 8-16 x 10^6 cells in 100 µl of growth medium without antibiotics.**
2. **For each transfection sample, prepare complexes as follows:**
   ***the ratio between DNA (µg) to Lipofectamine (µl) which was chosen is 1:3.**

a. Dilute 200ng DNA in 25 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.

b. Mix Lipofectamine® 2000 gently before use, then dilute the appropriate amount (0.6 µl) in 25 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature. Note: Proceed to Step c within 25 minutes.

c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine® 2000 (total volume = 50 µl). Mix gently and incubate for 20 minutes at room temperature (solution may appear

cloudy). Note: Complexes are stable for 6 hours at room temperature.

3. **Add the 50 µl of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.**
4. **Incubate cells at 37°C in a CO2 incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.**
5. **For stable cell lines: Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) the following day.**