

Cell Transfection Protocol

For Lipofectamine 2000 transfection of a 35 mm dish of 80% confluent CHO cells.

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1. Prepare cells
 - a. Plate cells in a 35mm dish at least a day before transfection
 - b. Check and make sure to cells are 80% confluent
 - c. ensure cells are in 1 ml Ham's F12 + 10%FBS solution with NO antibiotics and NO selection agents. NOTE it is OK to plate cells in 1 ml of this media in step 1a
 - i. If there are antibiotics or selections media, rinse cells at least once with 2ml PBS and change media at least an hour before transfection
2. Prepare a diluted solution of Lipofectamine
 - a. In 1.5 ml tube, add **2ul of Lipofectamine 2000** and then add **100ul of Optimem**
 - b. Triturate mixture
3. Prepare DNA and GFP solution
 - a. In another 1.5 ml tube, add **0.5ug of ion channel DNA**, add **0.5 ug of EGFP DNA**, and then add **100ul of Optimem**
 - b. Triturate mixture
4. 5 min after diluting Lipofectamine, mix the diluted Lipofectamine solution with the DNA mixture
5. 20 minutes after mixing the Lipofectamine solution with the plasmid DNA solution, move cells to hood, apply the mixture to the cells, replace in incubator
6. After 4-6 hours, replace media with 2 ml Ham's F12 + 10% FBS + 1% pen/strep
7. On the following day, record percent efficiency of transfection
8. Patch cells in 2-3 days