

Two Methods for Transfection of Immortalized Mouse Embryonic Fibroblasts (MEFs): Lipofectamine and PEI

[This protocol is from the Kahn lab, optimized for MEFs being transfected in 6-well plates, with thanks to Laura Newman and Rachel Turn.]

Everything below should be done in a tissue culture hood, maintaining sterility. The same initial steps are used for transfecting MEFs for later use in (1) CRISPR gene editing, (2) immunofluorescence imaging of cells on cover slips, or (3) live cell imaging of cells on glass bottom dishes. See below for where these protocols diverge.

We described first the protocol for the more common means of transfection, lipid based, and later one (PEI-based) that we found to be less toxic to MEFs, particularly lines that may be stressed by cell cycle or other defects.

Lipofectamine 2000 based transfection

- 1) Seed or grow MEF cells to ~90% confluence (e.g., a confluent 10 cm plate taken up into 10 mL and 0.5 mL can be used to inoculate a well in a 6-well dish (to which you also add 1 mL of DMEM + 10% FBS) and they should be at about the right density (again ~90% confluence the next day).
- 2) In two separate Eppendorf microfuge tubes, add 250 μ L Optimem. Add 4 μ g DNA to one tube and 12 μ g Lipofectamine 2000 to the other tube and briefly vortex (Note: the ratio of 3 μ L Lipofectamine 2000 : 1 μ g DNA is important). Incubate 5 min at room temperature.
- 3) Mix the contents of the two tubes (now 0.5 mL), pipetting up and down a few times to mix; incubate 20 min at room temperature.
- 4) Change medium to 1 mL fresh Optimem per well of 6-well plate.
- 5) Add 0.5 mL DNA/Lipo2000 mixture to cells dropwise (1.5 mL final volume).
- 6) Put plate into incubator at 37°C for 4 hrs.

(A) For CRISPR gene editing:

7A) Replate cells into 10 cm plates. Remove medium from each well, add 1 mL 0.05% trypsin/EDTA, let sit at room temperature for ~ 5 min (time not critical), then add 2 mL DMEM with 10% FBS. Transfer all 3 mL to 10 cm dish containing 10 mL of same medium. Return to incubator and let cells grow and recover overnight.

8A) Next day: Aspirate medium and replace with 10 mL 3 μ g/mL puromycin in DMEM/10% FBS. Note: expect to see massive cell killing when growing in puromycin. Grow for 2 days, then replace with fresh 10 mL medium again containing 3 μ g/mL puromycin.

9A) After the second round of 2 day puromycin incubations, replace medium with 10 mL fresh DMEM/10% FBS (no puromycin) and let cells grow to confluence (about one week – no need to change medium).

10A) To each 10 cm plate of near confluent cells (80-90%), add 1 mL trypsin, incubate ~5 min, triturate to suspend cells and transfer all to tube containing 9 mL fresh DMEM/FBS. From this

10 mL I take 1 mL into Eppendorf tube for use in T7 endonuclease or immunoblot assay. Pellet those cells, aspirate medium and store frozen until needed. The rest of the cell suspension (9 mL) is pelleted, cells resuspended into 1 mL DMEM/FBS. Then put 0.45 mL into each of two cryotubes containing 50 μ L DMSO, mix gently, and freeze in -80°C freezer before moving to liquid nitrogen for storage. Because puromycin selection reduces the numbers of untransfected cells, surviving cells will initially be sparse. This may lead to cells growing slower and in clumps. You may wish to re-plate cells once before cells grow to final density before freezing down. You may also go directly into the cloning phase without freezing.

(B) Plating transfected cells for immunofluorescence analyses:

7B) To each well of the 6-well plate, add 1 mL trypsin (see above), incubate at room temperature for ~ 5 min. Add 2 mL DMEM/FBS. Take 0.5 mL and add to well of a 6-well plate containing 3-5 coverslips (pre-treated with Matrigel or your favorite cover slip coating). Incubate overnight and fix when optimal confluence achieved (typically ~ 24 hr).

(C) For live cell imaging

7C) Same as 7B, only difference is plate into glass bottom culture dish (e.g., Mat Tek P35 GC-1.5-14-C; 35 mm, 14 mm viewing area). Next day change to appropriate medium for imaging and off you go.

PEI Transfection Protocol for MEFs

Note: This mode of transfection is optimal for cells that are more sensitive to transfection conditions (e.g., ones with a cell cycle defect). In our experience, PEI transfection is gentler (*i.e.*, less toxic), though it may have a slightly reduced transfection efficiency compared to Lipofectamine 2000 (~ 10 - 20% versus 20%).

1. Day 0: Plate MEFs in wells of a 6-well dish so that they are 70-80% confluent the next day; e.g., from a 10 cm plate resuspended into 10 mL we use 0.5 mL per well of the 6-well plate (1/20 dilution) to which we also add 2 mL medium. Let cells grow overnight at 37°C in incubator with DMEM + 10% FBS.
2. Day 1: Aspirate medium from cells and replace with DMEM + 2% FBS.
3. Prepare the following master mix for each test condition (1 reaction per well of a 6-well dish). Aliquot 100 μ L serum-free medium into Eppendorf tube. Add DNA and PEI at 1:3 ratio (I typically use 4 μ g DNA : 12 μ g PEI). Triturate to mix PEI, DNA, and medium, and let incubate at room temperature in the hood for 20 minutes.
4. Add dropwise PEI-DNA complexes (product of Step 3) to respective well of 6-well dish, with gentle rotation to mix. Return to incubator.
5. Day 2: Next day, either replace the medium with fresh DMEM + 10% FBS if require later time points for expression, or, if 24 hours is sufficient for expression, can either harvest cells for Western or fix for immunofluorescence with appropriate fixative. Note that anecdotal evidence suggests that the percentage of transfected cells is higher after 48 hrs than after 24.