Transfection P19 cells with Lipofectamine 2000

(The same protocol can be used for 293T cells)

1. Maintain P19 cells undifferentiated in MEM with 10% serum (7.5% calf serum, 2.5% fetal bovine serum). Typically passage cells every 2-3 days. Maintain cell density at 80% confluence or lower. Higher densities can lead to spontaneous differentiation.
2. Passage cells one day before transfection.
3. Observe cell density before transfection. The density of the cell should be 80-90% confluent, and the cells should be evenly distributed and attached on the dish. If cell number is too little (<70%) or too dense (>90%), don't use the dish.
4. Remove culture medium and replace with serum free Opti-MEM medium in CO2 incubator until transfection mixture is ready to put on (at least 5 min).
5. *Prepare transfection mixture:*
    a. Add DNA to Opti-MEM in 1.5 ml microfuge tube (see table for amonts).
    b. Mix Lipofectamine 2000 gently before use. Add Lipofectamine 2000 to Opti-MEM in another 1.5 ml tube (see table for amounts) and gently mix.
    c. Incubate in hood for 5 min.
    d. Combine diluted DNA and diluted Lipofectamine 2000 together, and incubate in hood for 20 min.
5. Remove Optimum medium from cell dish
6. Add DNA/Lipofectamine 2000 diluted mixture on cell with gentle pipette by slightly lift up dish on one side and pipette tips against side of the dish avoiding dropping on cell surface.
7. Change medium after 6 h to culture medium.

Culture vessel	Relative area to 24 well	Volume of medium	Cells	DNA dilution	Lipofectamine dilution	Mixture (µl)
96 well	0.2	100 µl		10-100 ng in 25 µl	0.2-0.5 µl in 25 µl	50 µl
48 well	0.7	200 µl		50-100 ng in 25 µl	0.3-0.8 µl in 25 µl	50 µl
24 well	1	500 µl	2×10^5	100-200ng in 50 µl	0.5-1.5 µl in 50 µl	100 µl
12 well	2	1 ml	4×10^5	100-200ng in 100 µl	0.5-1.5 µl in 100 µl	200 µl
6 well	5	2 ml	1×10^6	0.5-1.0 µg in 250 µl	2.5-6 µl in 250 µl	500 µl
6 cm plate	10	4 ml	2×10^6	1.0-3.0 µg in 500 µl	5-12 µl in 500 µl	1000 µl

Notes:

1. It is not necessary to remove complex or change/add medium after transfection, but transfection complexes may be remove after 4-6 hours without affecting transefection efficiency.
2. Although DMEM or RPMI1940 can be used, Opti-MEM is preferable when making Lipofectamine 2000/DNA complexes.
3. Antibiotics can interfere with transfection.
4. It is best to use polypropylene instead of polystyrene tubes.
5. Transfection efficiency of P19 cells is between 30-50% (from 50-99% for many other stable cell lines).
6. To optimize the amount of Lipofectamine™ 2000 for transfection in a 24-well plate, start with cells at >90% confluency and use a fixed amount of DNA (0.8-1.2 μg). With cell number and DNA concentration held constant, vary the amount of Lipofectamine™ 2000 to determine the optimal concentration (usually 1.5-3 μl). In the same way, the cell number and amount of DNA can also be optimized.