

Liposome-Mediated Transfection

The following protocol was reprinted from *Current Protocols in Molecular Biology* eds. Ausubel F. M. *et al.*

Using liposomes to deliver DNA into different eukaryotic cell types results in higher efficiency and greater reproducibility than other transfection methods. This basic protocol describes a transient expression system while the alternate protocol involves stable transformation and expression of DNA integrated into the genome of the transfected cell. In both protocols, plasmid DNA derived from either crude (miniprep) or purified (through CsCl) preparations is mixed with liposome suspension comprised of cationic lipids and applied to monolayer cell cultures.

Transient Expression Using Liposomes

For transient expression of DNA in the nucleus, plasmid DNA is complexed with a liposome suspension in serum-free medium. This DNA/liposome complex is added directly to cells grown in tissue culture plates and after a 3 to 5 hour incubation period, fresh medium containing serum is added. The cells are incubated to allow expression of the transfected gene, harvested, and assayed.

Materials

- Exponentially growing mammalian cells (see table 1)
- Plasmid DNA (miniprep or CsCl purified)
- Complete Dulbeccos minimum essential medium, serum free (DMEM; or other appropriate growth medium)
- Complete DMEM containing 10% and 20% fetal calf serum (DMEM-10 and -20; or other appropriate complete growth medium)
- Liposome suspension

- 6 well, 35 mm tissue culture dishes
- Humidified, 5% CO₂ incubator at 37°C
- Polystyrene tubes (Falcon #2058 or Corning #25310)

1. Plate exponentially growing cells in 6 well tissue culture dishes at 5×10^5 cells/well and grow overnight in a CO₂ incubator at 37°C to 80% confluency.

If 100 mm dishes are used in place of 6 well dishes, grow cells to 80% confluency and scale up all amounts by a factor of 8.

Table 1 - Amount of DNA and Liposome Required for Liposome-Mediated Transfection^a

Cell Type	Plasmid DNA ^b (μ g)	Liposome Suspension (μ l)
BHk-21	0.5	5
COS-7	0.5	5
CV-1	1.0	10
HeLa	2.0	10

^a Amounts of DNA and liposome suspension are recommended for transfection of each cell type in a total volume of 1 to 1.5 ml DMEM-SF in a 6 well, 35 mm dish

^b Plasmid DNA can be prepared using a miniprep protocol or purified by CsCl/ethidium bromide equilibration centrifugation

2. Prepare DNA/liposome complex in a polystyrene tube as follows: dilute plasmid DNA into 1 ml complete DMEM-SF*, vortex 1 second, then add liposome suspension and vortex again. Incubate 5 to 10 minutes at room temperature to allow binding of DNA to cationic liposomes.

See table 1 for amounts of DNA and liposome suspension, according to cell type. Relatively small amounts of DNA are effectively delivered into the nuclei of these cell types. For other cell types, it is desirable to systematically examine the amounts of DNA and liposome suspension needed to obtain maximal transfection frequencies and optimal levels of expression.

It is very important to use a polystyrene rather than a polypropylene tube because the DNA/liposome complex apparently sticks to polypropylene. While the DNA/liposome complex may form immediately, a 5 minute incubation ensures that binding is complete.

3. Aspirate complete DMEM-10 from cells, wash cells once with 1 ml of complete DMEM-SF, and aspirate DMEM-SF. To each 35 mm well, add 1 ml DNA/liposome complex directly to the cells. Incubate 3 to 5 hours in a CO₂ incubator at 37°C.
4. To each well of cells, add 1 ml of complete DMEM-20 and incubate an additional 16 to 24 hours in a CO₂ incubator at 37°C.
5. Aspirate complete DMEM/DNA/liposome complex and add 2 ml of fresh, complete DMEM-10 to each well. Incubate an additional 24 to 48 hours in a CO₂ incubator at 37°C.
6. Harvest cells by scraping, trypsinization, or freeze-thaw lysis.
7. Perform appropriate expression assay.

Stable Transformation using Liposomes

Stable transformation of mammalian cells using liposome-mediated transfection is similar to the protocol for transient expression except that after recovery from transfection, the cells are grown in selective medium for expression of the desired marker.

1. Plate cells as described in step 1 of previous protocol and grow to 50% confluency.
2. Prepare DNA/liposome complex and transfect cells as in steps 2 and 3 above.
3. To each well of cells, add 1 ml of complete DMEM-20 and incubate for 48 hours in a CO₂ incubator at 37°C.
4. Aspirate DMEM and dilute cells into selective medium. Grow cells for the appropriate length of time to select true transfected colonies.

Reagents & Solutions

Complete DMEM

Dulbeccos minimum essential medium, high glucose formulation, containing:

5% FCS (DMEM-5), 10% FCS (DMEM-10) or 20% FCS (DMEM-20), heat-inactivated 1 hour at 56°C

1% nonessential amino acids

2 mM L-glutamine

50 µM βME

100 U/ml penicillin

100 µg/ml streptomycin sulfate

*DMEM-SF - Complete DMEM-Serum Free