Liposome-Mediated Transfection

Using liposomes to deliver DNA into different eukaryotic cell types results in higher efficiency and greater reproducibility than other transfection methods (*UNITS 10.13-10.15 & 10.17*). In this unit, the 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 a liposome suspension comprised of cationic lipids and applied to monolayer cell cultures.

NOTE: All incubations are performed in a humidified 37°C, 5% CO_2 incubator.

TRANSIENT EXPRESSION USING LIPOSOMES

BASIC PROTOCOL

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-hr 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 eukaryotic cells (Table 10.16.1)
Plasmid DNA (miniprep or CsCl purified; Table 10.16.1)
Complete DMEM, serum free (complete DMEM-SF), and complete DMEM-10 and -20 (or other appropriate growth medium; *APPENDIX 2)*
Liposome suspension (Table 10.16.1; Lipofectin or TransfectACE; GIBCO/BRL #8292SA and 8301SA, respectively)

6-well, 35-mm tissue culture dishes
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 incubate overnight 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 10.16.1 Amount of DNA and Liposome Required for Liposome-Mediated Transfection[a]

Cell type	Plasmid DNA[b] (μg)	Liposome suspension (μl)
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 (*UNIT 10.3*) or purified by CsCl/ethidium bromide equilibrium centrifugation (*UNIT 10.3*).

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

   See Table 10.16.1 for amounts of DNA and liposome suspension to be added, according to cell type. Relatively small amounts of DNA are effectively delivered into the nuclei of these cells. 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-min incubation ensures that binding is complete.

3. Aspirate medium from cells, wash cells once with 1 ml complete DMEM-SF, and aspirate medium. To each 35-mm well, add 1 ml DNA/liposome complex directly to the cells. Incubate 3 to 5 hr.

4. To each well of cells, add 1 ml complete DMEM-20 and incubate an additional 16 to 24 hr.

5. Aspirate complete DMEM/DNA/liposome complex and add 2 ml fresh, complete DMEM-10 to each well. Incubate an additional 24 to 48 hr.

6. Harvest cells by scraping, trypsinization (*UNIT 10.2*), or freeze-thaw lysis.

7. Perform appropriate expression assay.

   Protein expression can also be detected by fluorescence microscopy (Whitt et al., 1991), flow cytometry (Chapter 5), or by radiolabeling (UNIT 8.12) followed by immunoprecipitation (UNIT 8.3). RNA analysis and immunohistochemistry can also be carried out.

ALTERNATE PROTOCOL

STABLE TRANSFORMATION USING LIPOSOMES

Stable transformation of eukaryotic 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.

Additional Materials

Selective medium (*UNIT 10.17*)

1. Plate cells as described in step 1 of the basic protocol and grow to 50% confluency.

2. Prepare DNA/liposome complex and transfect cells as in steps 2 and 3 of the basic protocol.

3. To each well of cells, add 1 ml complete DMEM-20 and incubate 48 hr.

4. Aspirate medium and dilute cells into selective medium. Grow cells for the appropriate length of time to select true transfected colonies (*UNIT 10.17*).

COMMENTARY

Background Information

The mechanism by which liposomes containing cationic and neutral lipids mediate transfection of DNA into animal cells is not well understood. Negatively charged phosphate groups on DNA bind to the positively charged surface of the liposome, and the residual positive charge then presumably mediates binding to negatively charged sialic acid residues on the cell surfaces. The observed decrease in transfection frequencies at high DNA concentrations may be attributed to saturation of the positive charge on the liposome. Felgner et al. (1987) have presented evidence suggesting that liposomes fuse with the cell surface. An alternative possibility is that DNA bound to liposomes is taken up by endocytosis and some fraction of the DNA is then released into the cytoplasm by an unknown mechanism.

Various cationic lipids have been tested for transfection efficiencies. Among those tested, two preparations, Lipofectin (Felgner et al., 1987) and TransfectACE (Rose et al., 1991; Elroy-Stein and Moss, 1990; Whitt et al., 1991), have been highly effective at transfecting a wide range of cell types.

Liposomes have been shown to promote transfection frequencies as high as 95% when used in conjunction with the vaccinia/T7 cytoplasmic gene expression system. In this system, cells are infected with a recombinant vaccinia virus carrying the bacteriophage T7 RNA polymerase gene and are then transfected with plasmid DNA containing the gene of interest under control of the T7 promoter (Fuerst et al., 1986). The cloned target gene is transcribed efficiently by the T7 RNA polymerase, providing a reproducibly high level of expression. Alternatively, DNA is transfected into the cytoplasm of a cell line that expresses the T7 RNA polymerase constitutively (Elroy-Stein and Moss, 1990). For reasons that remain unclear, however, high-level expression requires infection by wild-type vaccinia virus.

Liposome-mediated transfection has facilitated studies of the association between the plasma membrane glycoprotein CD4 and a membrane-associated protein tyrosine kinase (Shaw et al., 1989), virus assembly (Whitt et al., 1989), and the interaction between the human immunodeficiency virus spike glycoprotein gp160 and CD4 (Buonocore and Rose, 1990; Crise et al., 1990).

Critical Parameters

The choice of a particular liposome mixture depends upon the cell line to be transfected. Two commercially available liposome reagents, Lipofectin and TransfectACE, are effective for transfecting a wide range of cell lines. TransfectACE is available at about half the cost of Lipofectin but may not be as effective in all cell types (Whitt et al., 1991). Alternatively, TransfectACE and other cationic lipid suspensions can be prepared in the laboratory, where their composition can be varied and tested for transfection efficiency (Rose et al., 1991). Lipofectin cannot be made easily in the laboratory, because the cationic lipid

> *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA)

is not commercially available; its chemical synthesis is outlined in Felgner et al. (1987).

The DNA/liposome complex is formed in serum-free DMEM rather than HEPES-buffered saline (HeBS) because incubating monolayer cells in HeBS for 3 hr at 37°C can result in the cells rounding-up and coming off the plate. For most cell lines, it is necessary to omit serum from the growth medium in the initial growth and recovery phase after transfection (step 3 of the basic protocol). For example, Felgner et al. (1987) observed that transfection of mouse L cells with Lipofectin is inhibited by serum-containing growth medium.

Certain parameters, including the appropriate lipid concentration, DNA concentration, and length of time for transfection should be optimized. Very small amounts of DNA are often sufficient for promoting high transfection frequencies. On the other hand, high concentrations of either DNA or liposomes can be toxic. It appears that cells do not have to be mitotic to be transfected with liposomes since 90% of the cells can be transfected in a nonsynchronized culture (M.W., L.B., and J.K.R., unpub. results). For each cell type, it is desirable to systematically examine the amounts of DNA and liposome mixture required to obtain maximal transfection frequencies and optimal levels of expression.

Anticipated Results

The use of liposomes to transfect mammalian cells has yielded transient expression frequencies 5- to 100-fold higher than those obtained with other transfection methods (Felg-

ner et al., 1987; Rose et al., 1991). In many cases, 60% to 80% of transfected cells are observed to express the desired protein. Liposome-mediated transfection is often more reproducible than other transfection methods because it appears to be less affected by contaminants, varying salt concentrations, and pH differences of various DNA preparations. When used in conjunction with the vaccinia/T7 cytoplasmic expression system, expression frequencies of >90% can be obtained (Whitt et al., 1991).

For many cell types, stable transformation using liposomes results in transfection frequencies that are 3- to 20-fold higher than those observed for other methods, in the range of 10^4 colonies/μg DNA.

Time Considerations

The actual transfection process requires 3 to 5 hr, with an additional 2 to 3 days of growth before harvesting cells for transient nuclear expression. Stable transformants require that colonies be grown in selective medium, which requires additional time. Once the transformants have been selected, expression can be assayed at any time.

Literature Cited

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Key References

Felgner et al., 1987. See above.

Rose et al., 1991. See above.

These two references present comparative studies on various liposome suspensions and their ability to promote transfection in a wide range of mammalian cell types.

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