Transfection of DNA into Eukaryotic Cells

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

Transfection Using Calcium Phosphate-DNA Precipitate Formed in HEPES

A precipitate containing calcium phosphate and DNA is formed by slowly mixing a HEPES-buffered saline solution with a solution containing calcium chloride and DNA. This precipitate adheres to the surface of cells and should be visible in the phase contrast microscope the day after transfection. Depending on the cell type, up to 10% of the cells on a dish will take up the DNA through an as yet undetermined mechanism. Glycerol or dimethyl sulfoxide shock increases the amount of DNA absorbed in some cell types.

Materials

- Exponentially growing eukaryotic cells (e.g. HeLa, BALB/c 3T3, NIH3T3, CHO or rat embryo fibroblasts)
- Complete medium (depending on cell line used)
- CsCl-purified plasmid DNA (10 to 50 μg per transfections)
- 2.5 M $CaCl_2$
- 2X HEPES-buffered saline (HeBS)
- Phosphate-buffered saline (PBS)
- 37°C, 5% CO_2 humidified incubator
- 10 cm tissue culture plates
- 15 ml conical tube

1. Split cells into 10 cm tissue culture plates the day before transfection. When transfecting adherent cells that double every 18 to 24 hours a 1:15 split from a confluent dish generally works well. On the day of the transfection, it is important that the cells are thoroughly separated on the dish, as the ability to take up DNA is related to the surface area of the cell exposed to the medium. Cells should be split in a manner that accomplishes this. Feed cells with 9.0 ml of complete medium 2 to 4 hours prior to precipitation.

   The desired density of cells on dishes to be transfected will vary with cell type and the reason for doing the transfection. The optimal density is that which produces a near confluent dish when the cells are harvested or split into selective media.

2. Ethanol precipitate the DNA to be transfected and air dry the pellet by inverting the microcentrifuge tube on a fresh Kimwipe inside a tissue culture hood. Resuspend the pellet in 450 μl of 2.5 M $CaCl_2$. The amount of DNA that is optimal for transfection varies from 10 to 50 μg per 10 cm plate, depending on the cell line to be transfected.

   DNA to be transfected should be purified twice by CsCl gradient centrifugation. Supercoiled DNA works well in transfections. Impurities in the DNA preparation can be deleterious to transfection efficiency.

   Ethanol precipitation sterilizes the DNA to be transfected. For transfections that will be harvested within 3 to 4 days (transient analysis), this is not necessary. For transient experiments, many researchers make a 450 μl aqueous solution containing the DNA directly, without ethanol precipitation. If this is done, care should be taken to keep the amount of Tris in the solution to a minimum, as Tris may alter the pH of the precipitate and therefore reduce transfection efficiency.

3. Place 500 μl of 2X HeBS in a sterile 15 ml conical tube. Use a mechanical pipettor attached to a plugged 1 or 2 ml pipet to bubble 2X HeBs and add the DNA/$CaCl_2$ solution dropwise with a Pasteur pipet. Immediately vortex the solution for 5 seconds.

   If no mechanical pipettor is available, the solution can be bubbled by blowing through rubber tubing that is attached to a pipet via a filter. The filter is necessary to maintain sterility. This does not give as reproducible results as the mechanical pipettor.

4. Allow precipitate to sit 20 minutes at room temperature.

5. Use a Pasteur pipet to distribute the precipitate evenly over a 10 cm plate of cells and gently agitate to mix precipitate and medium.

6. Incubate the cells for 4 to 16 hours under standard growth conditions. Remove the medium. Wash cells twice with 5 ml of 1X PBS and feed cells with 10 ml complete medium.

   The amount of time that the precipitate should be left on the cells will vary with cell type. For hardy cells such as HeLa, NIH 3T3 and BALB/c 3T3, the precipitate can be left on for 16 hours. Other cell types will not survive this amount of exposure to the precipitate.

7. For transient analysis, harvest the cells at the desired time point. For stable transformation, allow the cells to double twice before plating in selective media.

   [Invitrogen Note: Because it is difficult to determine when cells have doubled, we recommend changing the media to selective media after 48 hours.]

Reagents & Solutions

Phosphate-buffered saline (PBS) pH ~7.3
- 137 mM NaCl
- 2.7 mM KCl
- 4.3 mM $Na_2HPO_4 \cdot 7H_2O$
- 1.4 mM KH_2PO_4

Alternate Protocol for Transfection of Mammalian Cells

High-Efficiency Transfection Using Calcium Phosphate-DNA Precipitate Formed in BES

A solution of calcium chloride, plasmid DNA, and *N,N-bis*(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) buffer, pH 6.95, is added to a plate of cells containing culture medium. The plates are incubated overnight while a calcium phosphate-DNA complex forms gradually in the medium under an atmosphere of 3% CO_2. With this method, 10% to 50% of the cells on a plate stably integrate and express the DNA transfected. Transient expression under these conditions is comparable to that obtained with the basic protocol. Glycerol or DMSO shock does not increase the number of cells transformed.

Materials
- Exponentially growing mammalian cells
- Complete medium (Dulbecco modified Eagle medium containing 10% fetal calf serum)
- CsCl-purified plasmid DNA
- 2.5 M $CaCl_2$
- 2X BES-buffered solutions (BBS)
- Phosphate-buffered saline
- 10 cm tissue culture plates
- 35°C, 3% CO_2 humidified incubator
- 35°C to 37°C, 5% CO_2 humidified incubator
- Fyrite gas analyzer (optional; Fisher Scientific or Curtin Matheson)

1. Seed exponentially growing cells at 5×10^5 cells/10 cm tissue culture plate in 10ml complete medium the day prior to transfection. There should be $<10^6$ cells per plate just prior to infection. Enough surface area should remain on the plate for at least two more doublings.

2. Dilute plasmid DNA with TE buffer to 1μg/μl. Store the DNA solution at 4°C.

The optimum amount of plasmid to use can be determined by transfecting three plates of cells with 10, 20 and 30 μg of plasmid DNA and incubating overnight. The plates are then examined with a microscope at 100X. A coarse, clumpy precipitate will form at DNA concentrations that are too low, a fine (almost invisible) precipitate will form at concentrations that are higher than optimal, and an even, granular precipitate will form with optimal DNA concentrations.

3. Prepare 0.25M $CaCl_2$ from a 2.5M stock. Mix 20-30μg of plasmid DNA with 500μl of 0.25M $CaCl_2$. Add 500μl of 2X BBS, mix well, and incubate 10-20 minutes at room temperature.

4. Add the calcium phosphate-DNA solution dropwise onto the medium containing plate while swirling the plate. Incubate 15-24 hours in a 35°C, 3% CO_2 incubator.

   Level of carbon dioxide is critical. Use a Fyrite gas analyzer to measure percent of CO_2 prior to incubation.

5. Wash the cells twice with 5ml of PBS and add 10ml of complete medium. For stable transformation, incubate overnight in a 35°C to 37°C, 5% CO_2 incubator. For studies involving transient expression, incubate the cells for 48-72 hours after adding the DNA.

6. Split the cells before beginning to select stable transformants - 1:10 to 1:30 depending on the growth rate of the host cell. Incubate overnight in a 35°C to 37°C, 5% CO_2 incubator.

7. Start selection by changing the medium to selection medium or by incubating cells under appropriate selection conditions.

Reagents & Solutions

Phosphate-buffered saline (PBS) pH ~7.3

137mM NaCl
2.7mM KCl
4.3mM $Na_2HPO_4 \cdot 7H_2O$
1.4mM KH_2PO_4