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Transfection of mammalian cells by electroporation

Pulsed electrical fields can be used to introduce DNA into a wide variety of animal cells[1,2]. Electroporation works well with cell lines that are refractive to other techniques, such as calcium phosphate–DNA coprecipitation. But as with other transfection methods, the optimal conditions for electroporation of untested cell lines must be determined experimentally.

PROCEDURE

Preparation of the cells

1| Collect the cells to be transfected from cultures in the mid- to late-logarithmic phase of growth. Use either a rubber policeman or trypsin to release adherent cells. Centrifuge at 500*g* at 4 °C for 5 min.

2|Resuspend the cell pellet in 0.5× volume of the original growth medium and measure the cell number using a hemocytometer.

3| Collect the cells by centrifugation, as described in Step 1 and resuspend them in growth medium or phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4) at 15–25 °C at a concentration of 2.5×10^6 to 2.5×10^7 cells/ml.

4| Transfer 400-µl aliquots of the cell suspension (10^6–10^7 cells) into as many labeled electroporation cuvettes as needed. Place the loaded cuvettes on ice.

5| Set the parameters on the electroporation device. (A typical capacitance value is 1,050 µF.) Voltages range from 200 to 350 V, depending on the cell line, but generally average 260 V. Use an infinite internal resistance value. Discharge a blank cuvette containing PBS at least twice before electroporating cells.

Introduction of the DNA

6| Add 10–30 µg of plasmid DNA in a volume of up to 40 µl to each cuvette containing cells. (Some investigators add carrier DNA (for example, salmon sperm DNA) to bring the total amount of DNA to 120 µg.) Gently mix the cells and DNA by pipetting the solution up and down. Proceed to Step 7 without delay.
Do not introduce air bubbles into the suspension during the mixing step.

7| Immediately transfer the cuvette to the electroporator and discharge the device. After 1–2 min, remove the cuvette, place it on ice and proceed immediately to the next step.

8| Transfer the electroporated cells to a 35-mm culture dish using a micropipettor equipped with a sterile tip. Rinse out the cuvette with a fresh aliquot of growth medium and add the washings to the culture dish. Transfer the dish to a humidified incubator at 37 °C with an atmosphere of 5–7% CO_2.

9| Repeat Steps 6–8 until all of the DNA cell samples have been treated. Recording the actual pulse time for each cuvette will facilitate comparisons between experiments.

10| If the objective is stable transformation of the cells, proceed directly to Step 11. For transient expression, examine the cells 24–96 h after electroporation using an appropriate assay.

11| To isolate stable transfectants, incubate for 48–72 h in complete medium, trypsinize the cells and replate them in the appropriate selective medium. Change the selective medium every 2–4 d for 2–3 weeks to remove the debris of dead cells and to allow colonies of resistant cells to grow. Thereafter, clone individual colonies and propagate for the appropriate assay.

SOURCE

This protocol was adapted from "DNA transfection by electroporation" in *Molecular Cloning: A Laboratory Manual* (eds. Sambrook, J. & Russell, D.W.) 16.33–16.36 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; http://www.cshlpress.com/link/molclon3.htm).

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