JURKAT TRANSFECTION PROTOCOL USING THE ELECTROPORATION METHOD

Background:

Transfection, the introduction or delivery of nucleic acids into cultured mammalian cells, has provided a powerful way to analyze the function, regulation, and interaction of mammalian genes along with their gene products. Many methods have been developed to facilitate the chemical and physical transfection of nucleic acids into mammalian cells, including DEAE-dextran, calcium phosphate, liposome-mediated transfection, micro-injection, and electroporation. The technique that we describe here utilizes electroporation, a method which exposes the cells to a brief, defined electrical pulse to create transient pores to allow plasmid DNA to cross the cell membrane.

Cell line used for this procedure: TAg (SV40 large T antigen) Jurkat T lymphocytes (Leukemia cell line)

Materials:

TAg Jurkat cell media:

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<tr>
<th>Preparing the Media:</th>
<th>(Prepared under a sterile hood)</th>
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<td>Fresh RPMI 1640 (Bellco Biotech, pH 7.3) supplemented with:</td>
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<td>• 1% 1 M HEPES pH 7.4</td>
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<td>• 2 mM L-Glutamine</td>
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<td>• 100 units/ml Penicillin G</td>
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<td>• 100 ug/ml Streptomycin</td>
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<td>• 10% (v/v) Fetal Bovine Serum (GIBCO BRL)</td>
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<td>Mix well + filter</td>
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<td>Pre-warm to 37°C prior to Jurkat transfection</td>
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6-well flat-bottom multiwell Falcon plates
96-well flat-bottom microtiter plates
250 ml sterile polypropylene Corning centrifuge tubes with plug seal caps
UV Sterilized electroporation cuvettes (gap width 0.4 cm)

DNA Concentration for Transfection: Plasmid DNA used for transfection should be highly purified, sterile, and free from contaminants such as endotoxins. We suggest that the plasmid DNA should be prepared by 2X CsCl or Qiagen. Although the optimal DNA concentration for each transfection may vary, we recommend using 2 µg of purified DNA as a starting point.
Pre-aliquot DNA for transfection:
Pre-aliquot the appropriate volumes of target plasmid and reporter into sterile microfuge tubes. This facilitates the later step of adding the DNA directly to the cell culture, especially when many transfections are being performed simultaneously.

Cell Culture Density at time of transfection:
Approximately $10^7$ cells should be used per Jurkat transfection. A good density for transfection is usually $5.5 \times 10^5$ cells/ml. Therefore, for a cell density of $5.5 \times 10^5$ cells/ml:
Use: $(10^7 \div 5.5 \times 10^5) \times (# \text{ of transfections})$ mls of Jurkat cell culture.

Pre-transfection:
- Spin the proper volume of cell culture down in a 250 ml Corning centrifuge tube (plug-seal cap) at 1K for 5 minutes to pellet the cells.
- While waiting for cells to spin down, aliquot 10 ml of pre-warmed Jurkat cell culture media to each well in the 6-well flat-bottom multiwell Falcon plates.
- Aspirate the media from the pelleted cells.
- Use 300 µl of media per transfection. Depending on the number of transfections, use: $300 \mu l \times (# \text{ transfections})$ mls to resuspend the pellet.
- Add 300 ul of resuspended cells to pre-aliquoted target plasmid + reporter.
- Pipet to mix well.
- Transfer to electroporation cuvettes.

**Electroporation (using the Bio-Rad Gene Pulser)**
Electroporation volume: 300 µl
Set the appropriate voltage (for Jurkat cells, 250 Volts)
Check the capacitance (960 µF)
Gap Width: 0.4 cm
To zap, press both buttons down and wait
Flick the cuvettes after zapping to mix contents.

Post-transfection:
• Transfer the electroporated Jurkat cells to 6-well flat-bottom multiwell Falcon plates containing the pre-aliquoted media.

• Incubate transfected cells in the incubator (5% CO₂ at 37°C and 95 % humidified atmosphere)

• Harvest cells after 24 h
Perform the desired assay (Secreted Alkaline Phosphatase Assay used in the following protocol)

References:

- Aliquot harvested cells in triplicate into 96-well flat-bottom microtiter plates (2 x 10^5 cells/well in 100 µl of complete medium)
- Stimulate with ionomycin (1 µM) and phorbol myristate acetate (PMA, 20 ng/ml) in a final volume of 200 µl
- Reporter gene activity was measured 12-24 h after stimulation.
- Secreted alkaline phosphatase activity was measured 16-24 h after stimulation
- Microtiter plates were heated to 65˚C for 1.5-2.0 h
- 100 µl aliquots from each well were incubated with an equal volume of 2 M diethanolamine bicarbonate (pH 10.0), 1 mM methylumbelliferyl phosphate (Sigma) at 37˚C.
- Relative alkaline phosphatase activity was measured by quantitating the accumulation of fluorescent product using a Titertek Fluoroskan II (ICN) with an excitation wavelength of 355 nm and an emission wavelength of 460 nm

Preparation of cell extracts:

References:

Cells were washed once with cold phosphate buffered saline
Resuspended in buffer A (10 mM Hepes (pH 7.8)), 15 mM KCl, 2 mM MgCl2, 1 mM dithiothreitol, 0.1 mM EDTA
Pelleted by low speed centrifugation (Eppendorf microcentrifuge, setting 3 for 3 min)