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Electroporation of T-Cell and Macrophage Cell Lines

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Introduction

We have been investigating the regulation of gene expression of the simian immunodeficiency virus (SIV) in different lymphoid cell types. To facilitate these studies, we have employed recombinant plasmids containing the coding sequences of the bacterial chloramphenicol acetyl transferase gene (CAT) under the transcriptional control elements of the SIV long terminal repeat (LTR). These plasmids were introduced into a human T-cell line, HuT 78, and into two human monocyte/macrophage cell lines, U937 and GCT by electroporation. For monocyte/macrophage cells in particular, transfection of exogenous DNA by means such as coprecipitation with calcium phosphate or treatment with DEAE dextran, can be extremely inefficient. This holds for conditions that give excellent transfection results when done in parallel in the mouse fibroblast L-cell line (these authors, unpub. results). Over a wide range of capacitance settings and voltages, DNA can be successfully introduced into these cell lines using the Gene Pulser® apparatus. In general, higher capacitance settings (500 and 960 µF) produce greater transfection efficiencies when the assays are normalized on a per cell basis.

Materials and Methods

HuT 78 cells, a human T-cell lymphoma suspension cell line, U937 cells, a human monocyte-like lymphoma suspension cell line, and GCT cells, a human macrophage-like histiocytoma adherent cell line, are maintained in RPMI 1640 media + 10% fetal calf serum (FCS). All cell lines were obtained from the American Type Culture collection. Cells are used for electroporation only when they are in logarithmic growth. Thus cells were subdivided 24 hours prior to electroporation. This step is critical for optimal efficiency of electroporation.

The conditions for electroporation are modifications of the protocols of Chu *et al.* [*Nucl. Acids Res.*, **15**, 1311 (1987)] and Thomas and Capecchi [*Cell*, **51**, 503 (1987)]. Exponentially growing cells are harvested, and the number of viable cells are counted by dye exclusion. The cells are washed once with PBS and resuspended at a density of 1.3×10^7 viable cells/ml in RPMI media without FCS/10 mM dextrose/ 0.1 mM dithiothreitol. 0.3 ml of the cell suspension is used per electroporation in 0.4 cm cuvettes. Immediately prior to electroporation, the contents of the cuvette are gently mixed by tapping, being careful to avoid forming bubbles, and the cuvette is then placed into the electroporation chamber. The cells are maintained at room temperature prior to and after electroporation. At 5 to 10 minutes post electroporation, the cells are placed into growth media.

The DNAs used for electroporation are as follows. pSIV LTR/CAT contains the coding sequences of the bacterial chloramphenicol acetyltransferase (CAT) gene placed under the transcriptional control of the 3' long terminal repeat (LTR) of the simian immunodeficiency virus (SIV) (these authors, unpub. results). pSV/tat contains the coding sequences of the human immunodeficiency virus tat gene under the transcriptional control of the SV40 early region promoter [Peterlin, *et al.*, *Proc. Natl. Acad. Sci. USA*, **83**, 9734 (1986)]. For these experiments, 10 µg of supercoiled pSIV LTR/CAT and 15 µg of supercoiled pSV/tat were used per electroporation. A total of 10-20 µl of DNA in a volume of 20-40 µl were used per electroporation.

At 40 to 48 hours post electroporation, the cells are harvested and resuspended in a volume of 200 µl. 25 µl of cell-free extracts are used for the *in vitro* CAT reaction. The CAT assay is that of Nordeen *et al.* [DNA, **6**, 173 (1987)], and is based on the conversion of a [^{3}H] labeled substrate to a [^{3}H] labeled reaction product. The level of conversion is dependent on the level of CAT enzymatic activity present within the extracts of the electroporated cells. The reactions proceed overnight, and the results are within the linear range of the assay (data not shown).

Results

Table 1 represents electroporation experiments using HuT 78 cells, done according to the protocol above, varying the capacitance, voltage, and cuvette size. Due to the smaller size of the 0.2 cm cuvettes, the volume used per electroporation was 0.2 ml (versus 0.4 ml in the 0.4 cm cuvettes), the cell density was 2×10^7 cells per ml (versus 1×10^7 cells/ml with the 0.4 cm cuvettes), and the voltages used were 120 and 150 volts (versus 250 and 300 volts with the 0.4 cm cuvettes). Included in the table are the number and percent of viable cells determined at 48 hours post electroporation. The percent of viable cells is somewhat subjective, since in counting the number of non-viable cells it is difficult to discriminate between what represents dead cells and what represents portions of dead cells. The level of CAT enzymatic activity is given in counts per minute (CPM), representing the enzymatic conversion of he [^{3}H] labeled substrate to the [^{3}H] acetylated reaction product.

Table 1. Electroporation of HuT 78 Cells

10 mg pSIV LTR/CAT + 15 mg pSV/tat

Capacitance (μF)	Voltage (V)	Cuvette Size (cm)	# Viable Cells/ml	% Viable Cells	Time Constant (msec)	CAT Activity (CPM)
250	250	0.4	2.7×10^5	71	7.0	4.5×10^5
250	300	0.4	3×10^5	59	6.7	5.5×10^5
500	250	0.4	1.2×10^5	34	13.9	3.8×10^5
500	300	0.4	3.5×10^4	10	12.3	3.4×10^5
960	250	0.4	7.5×10^4	22	27.0	2.2×10^5
960	300	0.4	6.5×10^4	14	24.2	0.45×10^5
250	120	0.2	3×10^5	73	5.6	2×10^5
250	150	0.2	2.4×10^5	40	4.0	7.5×10^5
500	120	0.2	1.9×10^5	45	10.1	4.1×10^5
500	150	0.2	3.2×10^5	54	7.4	1×10^5
960	120	0.2	1×10^5	29	21.5	5.4×10^5
960	150	0.2	$<1 \times 10^4$	-	15.6	1×10^5
Control electroporation without DNA						
250	120	0.2	4.4×10^5	86	5.5	0.03×10^5

Tables 2 and 3 represent electroporation experiments using U937 and GCT cells, respectively, varying capacitance and voltage. Only the 0.4 cm cuvettes were used. The cells were prepared for electroporation as above. 10 μg of supercoiled pSIV LTR/CAT and 15 μg of supercoiled pSV/tat were used per electroporation.

Table 2. Electroporation of U937 Cells†

10 mg pSIV LTR/CAT + 15 mg pSV/tat

Cuvette Capacitance (μF)	Time Voltage (V)	CAT Size (cm)	# Viable Cells/ml	Constant (msec)	Activity (CPM)
250	300	0.4	4.7×10^6	7.0	27.5×10^3
500	200	0.4	4.8×10^6	14.2	11.6×10^3
500	300	0.4	4×10^5	12.5	4.6×10^3
960	200	0.4	2.7×10^6	28.0	45.8×10^3
Control electroporation without DNA					
250	300	0.4	6.5×10^6	6.6	0.5×10^3

† For these experiments, the U937 cells were harvested at 96 hours post electroporation.

Table 3. Electroporation of GCT Cells

10 mg pSIV LTR/CAT + 15 mg pSV/tat

Capacitance (μF)	Voltage (V)	Cuvette Size (cm)	# Viable Cells/ml	Time Constant (msec)	CAT Activity (CPM)
250	300	0.4	2×10^5	6.5	1.34×10^5
500	200	0.4	5×10^4	13.9	0.69×10^5
500	300	0.4	3.3×10^4	12.5	3.06×10^5
960	200	0.4	1.2×10^5	29.0	3.65×10^5
960	300	0.4	4×10^4	23.9	2.42×10^5
Control electroporation without DNA					
250	300	0.4	4.4×10^5	6.7	0.007×10^5

Discussion

We have been able to successfully introduce DNA into different lymphoid cell lines using the Gene Pulser transfection apparatus. DNA can be electroporated over a spectrum of capacitance and voltage settings. Repetitions of the electroporation experiments with smaller increments between voltages may identify a narrower range for optimal conditions. However, we now routinely use 960 μF and 200 volt settings for the cell types discussed here. Electroporation of the U937 cells has proven to be more efficient than DEAE-dextran mediated transfection of these cells. Under conditions that have allowed the successful transfection of the mouse fibroblast L-cell line, extremely poor results were obtained with the U937 cells (unpub. results). One series of experiments was performed using U937 cells that were overgrown at the time of electroporation. No electroporation of the cells was observed (data not shown), and therefore, we now use the cells only when they are in an active growth state. In addition, we have introduced proviral clones of SIV (constructed in both lambda phage and bacterial plasmid vectors) into HuT 78 cells, and have recovered infectious virus using the described electroporation parameters (data not shown). Thus, these conditions apply to both transient and stable transformation, and for both linear and supercoiled DNAs. Given the central role of HIV infection in macrophages, critical molecular experiments can now be performed by introducing macromolecules into this cell type using electroporation.

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