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DEAE-dextran enhances electroporation of mammalian cells

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Electroporation has proven to be a convenient method for transfection of many cell types. One drawback has been that it requires large amounts of DNA, typically 20 to 80 μg per transfection (1). Here we report that adding DEAE-dextran to the electroporation media dramatically increases electroporation efficiency in mammalian cells, lowering the amount of DNA required for efficient transfection. We routinely get efficient electroporation using 0.8 μg DNA per transfection using the method described below. With luciferase activity as an indicator, electroporation efficiency is increased 7- to 149-fold when DEAE-dextran is added to the electroporation media (Table 1). Using plasmid recovery as the indicator in transient-transfections, 8- to 14-fold more DNA is recovered from the cells when DEAE-dextran is added to the electroporation media (Table 2). The basis for the enhanced electroporation efficiency is likely to be that DEAE-dextran causes adherence of DNA to the surface of cells (2). The resulting higher local concentration of DNA at the cell surface may result in enhanced uptake of DNA upon electroporation.

Preparation of the cells and electroporation media is simple and rapid; as many as 12 DEAE-dextran electroporations can be completed within an hour. For this study, 3×10^6 cells in log phase growth were pelleted (440 *g* for 5 min), washed once with RPMI 1640, repelleted and resuspended in 0.8 ml of room temperature RPMI 1640 containing 1 μg/ml DNA (CsCl purified, supercoiled plasmid) and 5 or 10 μg/ml DEAE-dextran (optimal concentration varies with the cell line; the amounts used here are: Reh, CEM and PW, 5 μg/ml; Nalm-6 and 4364A, 10 μg/ml). The mixture was transferred to a 4 mm electroporation cuvette and pulsed once at room temperature using a BioRad Gene Pulser set at 960 μF. The voltages used were: Reh and 4364A, 250 V; Nalm-6, CEM and PW, 300 V. The cells were returned to the tissue culture incubator after addition of 4.2 ml of the normal growth media for the cell line (no additional wash steps).

For Table 1, we electroporated the cells with the luciferase expression vector pRSVL (3), and assayed 24 hours later using a previously described protocol (4). For Table 2, we electroporated the shuttle vector plasmid pGG50 into the cell line Reh and recovered the DNA 48 hours later using an alkaline lysis protocol (5). The recovered DNA was digested with *Dpn*I to fragment non-transfected plasmids (see below) and then electrotransformed into DH10B *E.coli* (Gibco BRL). Bacterial transformants were selected on LB/ 100 μg/ml ampicillin plates (pGG50 is a pBR322-based plasmid that confers ampicillin resistance in bacteria). The data in Table 2 are colony counts from the bacterial transformation step of each transfection, and thus represent an easy method for quantitating plasmid recovery from each transfection. The shuttle vector pGG50 also has the SV40 replication origin and large T coding region, and replicates when transfected into Reh. Eukayrotic replicated plasmids lose their prokayrotic *dam* methylation pattern, and are resistant the dam methylation-dependent endonuclease, *Dpn*I. Hence, only eukayrotic replicated (i.e. transfected) plasmids give rise to colonies upon bacterial transformation. In previous studies we

Table 1. DEAE-dextran enhancement of electrotransformation: luciferase activity in transfected cells

Cell line	Luciferase activity (RLU)[1] + DEAE-dextran	−DEAE-dextran	Increase
Reh[2]	5997	235	25×
Nalm-6[2]	63611	428	149×
PW[2]	102045	777	31×
CEM[2]	2937	407	7×
4364A[3]	8872	546	71×

The luciferase expression vector PRSVL (0.8 μg) was electroporated into each of the cell lines indicated (3×10^6 cells/transfection) with and without addition of DEAE−dextran to the electroporation media. Luciferase activity was assayed 24 hours after electroporation. [1]Luciferase activities are reported in relative light units (RLU) as assayed by an Analytical Luminescence Luminometer model 2010. RLU values are averages from duplicate transfections. [2]Human lymphoid cell lines. [3]Hamster fibroblast cell line.

Table 2. DEAE-dextran enhancement of electrotransformation: recovery of replicated DNA from transiently transfected cells

Transfection Pair	Bacterial Transformants from Recovered DNA + DEAE-dextran	−DEAE-dextran	Increase
1	9734	699	14×
2	11336	1073	11×
3	789	102	8×

The shuttle vector plasmid pGG50 (0.8 μg) was electroporated into the human lymphoid cell line, Reh (3×10^6 cells/transfection) with and without addition of DEAE-dextran to the electroporation media. Transfected plasmids were recovered 48 hours after electroporation by alkaline lysis, digested with *Dpn*I to destroy non-transfected plasmids (see text), and transformed into *E.coli*. Bacterial transformants were counted after selection on LB/ampicillin. Values are averages from duplicate transfections.

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have determined that eukayrotic replication of these shuttle vector plasmids is an accurate estimation of the plasmid entry into the eukayrotic cells (5).

The DEAE-dextran electroporation method described here should prove useful wherever high transfection efficiencies are necessary, for conserving DNA when many transfections are required, and as a possible alternative for cells recalcitrant to other transfection methods.

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