

Electroporation efficiency in mammalian cells is increased by dimethyl sulfoxide (DMSO)

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ABSTRACT

Electroporation is one of the most common methods used to transform mammalian cells with plasmids. This method is versatile and can be adapted to meet the requirements of many cell lines. However, sometimes the efficiency of this method is low. We demonstrate that dimethyl sulfoxide (DMSO) facilitated a better DNA uptake in four different cell lines (HL60, TR146, Cos-7 and L132). The cells were electroporated with a β -Gal expression plasmid in a medium containing DMSO (1.25%) during, and for 24 h after the pulse. In all these cells a dramatic (up to 8-fold) increase in transfection efficiency occurred after this treatment. This method opens up the possibility of using electroporation even in cells which are difficult to transfect.

For a mammalian expression system a high level of expression is, in most cases, a primary requirement. To improve transfection, several methods have been developed. In recent years electroporation has become the preferred method since it is very efficient and relatively easy to perform. However, some cell lines remain difficult to transfect, and reliable protocols to increase efficiency are rare.

Here we demonstrate an easy and inexpensive method to increase the efficiency of electroporation of mammalian cell lines up to 8-fold.

As an example for cells which can only be poorly transfected, we used HL60 (a promyelocyte cell line; ATCC CCL240) and TR146 cells [Squamous Cell Carcinoma Cell line (1)]. To determine whether electroporation efficiency can also be increased in cells which can generally be transfected efficiently, we used Cos-7 (SV40 transformed kidney cells; ATCC CRL 1650) and L132 cells (lung fibroblasts; ATCC CCL5).

All cells were adjusted to a concentration of 2.3×10^7 cells/ml. 7×10^6 cells (300 μ l) per cuvette were electroporated with a Gene Pulser (BioRad, München, Germany) with 30 μ l (30–40 μ g) plasmid DNA (pCMV β , Clontech, Palo Alto, CA; a β -Gal expression plasmid). Cells and DNA were incubated in RPMI medium containing 10% FCS for 1 min at room temperature under gentle agitation. Electroporation was performed in the same medium without (group n/n and n/D) (Fig. 1) or with 1.25% final concentration dimethyl sulfoxide (DMSO) (group D/n and D/D) using 960 μ F for all cells and 290 V for HL60, 250 V for Cos 7 and L132 and 260 V for TR146 cells. These parameters were used because they yield the best transfection results in these

cell lines. Immediately after the pulse, transfected cells from one cuvette were split in 2×5 ml medium either without (group D/n and n/n) or with 1.25% DMSO (group D/D and n/D). Previous experiments showed that during and after electroporation, DMSO concentrations of 1.25% were optimal.

The β -Gal activity was determined 24 h after electroporation using standard procedures (2). Data were normalized with respect to the n/n group (without DMSO during and after electroporation) of each cell line. All electroporations were performed in quadruplicates.

In all cell lines investigated, addition of DMSO resulted in a higher electroporation efficiency and in higher cell viability (Fig. 2). The efficiency was increased slightly when DMSO was only present during electroporation; an observation which has been made earlier for other transfection methods (3). Incubation of cells with DMSO for 24 h after the pulse resulted in an additional (up to 8-fold) increase of the activity of the reporter gene product. We conclude, therefore, that 8-fold more plasmid is taken up by the cells when DMSO is present after electroporation. The exact mechanisms of increasing transfection efficiency by DMSO remain unclear. It is possible (i) that membranes are more permeable for the plasmid under influence

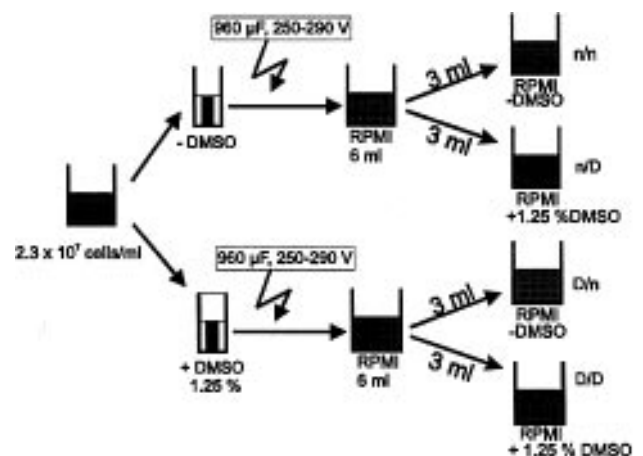


Figure 1. The diagram shows the experimental procedure to obtain the most comparable conditions. After the electrical pulse, the cells were resuspended in 6 ml RPMI/10% FCS, split into 2×3 ml and mixed with 2 ml RPMI/10% FCS with 3.125% DMSO (n/D and D/D) to give a final DMSO concentration of 1.25%, or without DMSO (n/n and D/n).

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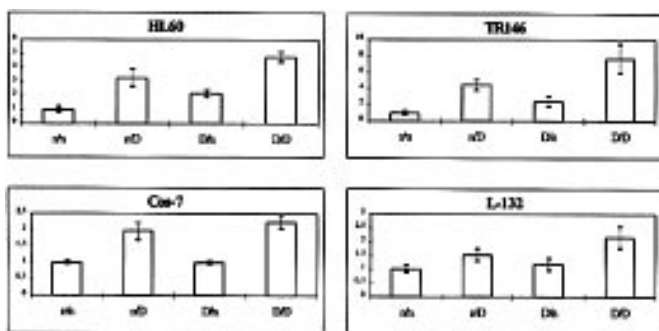


Figure 2. Comparison of the β -galactosidase activity of the pCMV β plasmid in cell lines with and without DMSO. Groups are as follows: n/n without DMSO during and after electroporation; n/D: no DMSO during, but 1.25% after, electroporation; D/n: 1.25% DMSO but not after electroporation; D/D: 1.25% DMSO during and after electroporation. The data are normalized with respect to the n/n group for better comparability. Data are shown as \pm SD of four completely individual experiments.

of DMSO and (ii) that DMSO may also stabilize cell membranes and thus ensure a better survival rate of the cells after electroporation

as observed when using DMSO as a protectant for frozen cells. Whatever the exact role of DMSO may be, the described method offers an alternative option for transcriptional studies in cell lines which usually cannot be transfected with high efficiency. It is obvious that the influence of DMSO on the cell line under investigation should be analyzed before using this compound as an aid for electroporation.

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REFERENCES

- 1 Rupniak, T.H., Rowlatt, C., Lane, E.B., Steele, J.G., Trejdosiewicz, L.K., Laskiewicz, B., Povey, S. and Hill, B.T. (1985) *J. Natl. Cancer Inst.* **75**, 621–635.
- 2 Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- 3 Aubin, R.A., Weinfeld, M., Mirzayans, R. and Paterson, M.C. (1994) *Mol. Biotechnol.* **1**, 29–48.