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# Efficient electrotransformation of yeast using bipolar electric pulses

**D. FOLOGEA, TATIANA VASSU, ILEANA STOICA, ORTANSA CSUTAK, ELENA SASARMAN, DIANA SMARANDACHE, ROBERTINA IONESCU**
*MICROGEN-Center for Research in Genetics, Microbiology and Biotechnology, University of Bucharest, Faculty of Biology, 1-3 Aleea Portocalilor, sector 5, 772011-Bucharest, Romania*

## Abstract

*Electroporation has become a routine technique for direct gene transfer in cells and tissue. Although electroporation is a universal phenomenon, transformation efficiency is different from one strain to another, due to physical, biochemical and biological features. There are certain strains where electroporation is leading to a very small number of transformed cells, as a consequence of the lethal effect of a very strong electric field.*

*In this paper we present an improved method for the electrotransformation of yeast strains, based on avoiding strong electric field using bipolar electric pulses.*

**Keywords:** Electrotransformation, electroporation, yeast

## Introduction

Electroporation is probably one of the most popular alternative methods used for the direct transfer of genes on a large variety of cells and tissues. When an electric potential is applied across a cell membrane, over a certain value, a dramatically increase in the permeability of the cell membrane appears. Keeping this transmembrane potential into a reasonable range, for a short time, membrane will remain fully functional [1].

This sudden change in permeability seems to be due to the small pores that come out as a result of a transmembrane potential in the range of 0.5-1V.

The main application of electroporation is direct gene transfer, although the exact mechanism of DNA penetrating cell membrane still remains unknown. As a direct gene transfer method, electroporation was applied on protoplasts [2], yeast [3], bacteria [4] or plant tissue [5, 6].

Electroporation is an asymmetrical process [7] and this is a limiting factor for transformation efficiency. The simplest way to avoid this asymmetry consists in periodically changing of electric field direction during the electroporation experiment.

In this paper, an improved method of direct gene transfer using bipolar electric pulses, in order to avoid electroporation asymmetry, is described.

## Materials and Methods

### Biological strains

*S. cerevisiae* SC589 (a ura3 his3 leu2 ade2) and TS5 (a ura3 leu2), grown on YEPD medium at $30^0$C for 24h, are washed twice with 20% glycerol and then resuspended in 2 ml of the same solution.

**Culture media [8]**

YEPD medium: 1% yeast extract, 1% peptone, 1% dextrose.

Minimal culture media: 0.17% Yeast Nitrogen Base, 0.5% ammonium sulphate, 2% glucose, 2% agar, supplemented with 1mM leu for TS5 and 1mM leu, 1mM his, 1mM ade for SC589, respectively.

**Vectors**

Two vectors, Yep352 (for SC589 strain) and Yep24 (for TS5 strain) are used for a fast identification and selection of ura $3^+$ transformants.

**Electrotransformation**

Electroporation has been performed using a home made electroporation device. It has an output as a succession of bipolar square electric pulses, with adjustable voltage and total pulses train length. Samples of 200 µl cell suspension are incubated on ice with 20 µl DNA solution (0.5 ng DNA) into electroporation cuvette (Aluminium electrodes, 2 mm aperture, BioRAD, USA). After 10 min, cells are subjected to the electric field, kept on ice for 10 min and transferred in YEPD medium (800 µl/sample) for 1 h. Afterwards, cells are plated on YNB media, transformation efficiency being estimated after 3 days. For the two strains, different electrical conditions are chosen. The total length of the pulses train was 4 msec for SC589 strain and 22 msec for TS5 strain. The electrical field varied for both cases in the range 0-3.5 kV/cm.

## Results and Discussions

**Electroporation asymmetry**

In the case of an electroporation experiment, the increased transmembrane potential is usually obtained using an external electric field. Under its action, due to polarization phenomena, on cell membrane, the value of induced potential will be given by: [1, 9]

$$V = -1.5 * R * E * \cos\theta \quad (1)$$

where R is the cell radius (a spherical cell), E – amplitude of the external electric field and θ the angle between the electric field direction and the cell radius to the current point of potential estimation on the cell membrane.

As a consequence of this relationship (1), is easy to observe that transmembrane potential is not uniform on the membrane surface, being related to θ angle value. Obviously, the induced potential have extreme values for $\theta=0^0$ and $\theta=180^0$.

The initial rate of formatting pores is depending on transmembrane potential according to the formula [7]:

$$K_f = D\int \exp[G(\phi y)^2 dy \quad (2)$$

where:

ϕ= maximal positive value of induced potential;

$$D = \frac{2\pi\nu R * \exp[-E_0 / kT]}{a_l}$$

$$G = \frac{0.5\pi C_m(\varepsilon_a / \varepsilon_m - 1)r_c^2}{kT}$$

y=cosθ
R = cell radius
ν = frecuency of lipid fluctuations
$E_0$ = energy needed for hydrophilic pores formation
$r_c$ = the critical radius of pores
$a_l$ = area of a lipidic molecule on membrane surface
$C_m$ = specific capacity of cell membrane

Living cells have a natural transmembrane potential of about -100 mV. Therefore, the exact value of transmembrane potential will be given by formula:

$$V_{total} = \phi * y - \phi_{natural}$$

Due to the algebraic sign of the induced potential (depending of the electric field direction), the resulted transmembrane potential will be different for cells hemispheres. Although the natural potential is only about 10% of critical potential for electroporation, due to the exponential formula (2), the formatting pores rates will be one order of magnitude different for cells hemispheres.

**Yeast electrotransformation**

The main results regarding yeast electrotransformation using bipolar electric pulses are presented in (**Figure 1**) and (**Figure 2**). Transformation efficiency is estimated as ratio of transformed cells number reported to initial cells number in the sample before electroporation. The shape of the curves representing transformation efficiency vs electric field is similar for both strains even the pulses parameters are different. The number of transformed cells and transformation efficiency are lower for weak electric fields, due to the fact that electroporation is a threshold phenomenon. On the other hand, at strong electric field, the lethal effect of electric field is present, so the transformation efficiency is dramatically decreased. Although the strains used in this experiment are geometrically similar, the maximal efficiency is obtained at different electric field, 1.125 kV/cm for TS5 and 2.25 kV/cm for SC589, respectively. The explanation for such a difference is the different length of the pulses trains.

The transformation efficiency is about $10^{-3}$ for both strains. This value is obviously higher than the reported ones for similar strains but electrotransformed using unipolar electric pulses or chemical transformation [10].

Also, according to Figure 1 and 2, two peaks of maximal efficiency are recorded, unlike the electroporation with unipolar electric pulses, where only one peak is present. On the other hand, the differences in electrotransformation efficiency with respect to unipolar pulses are at least one order. Therefore, we can assume an electrostimulation effect of bipolar electric pulses or/and another strong influence of these pulses on the electrotransformation process.

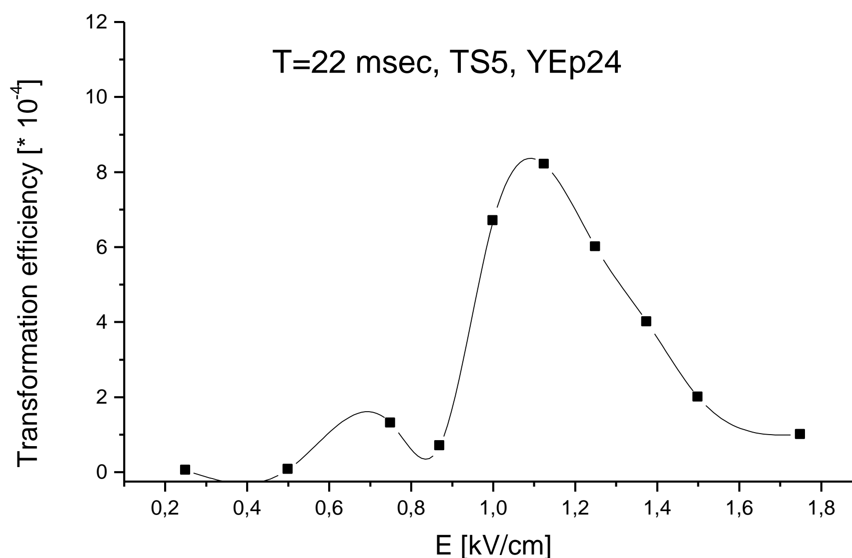


**Figure 1.** Transformation efficiency vs electric field for TS5 strain

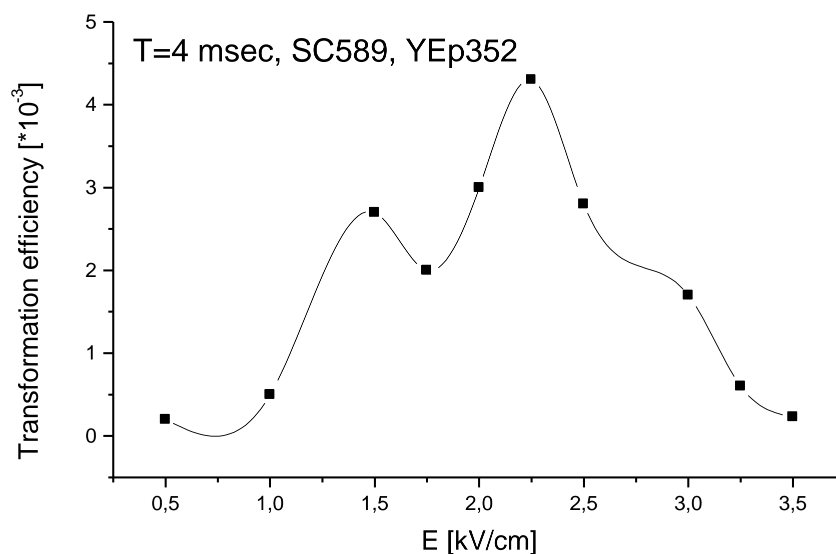


**Figure 2.** Transformation efficiency vs electric field for SC589 strain

## Conclusions

Electrotransformation efficiency is obviously increased using bipolar electric pulses. This could be very important in order to improve the gene transfer process for biological systems recalcitrant to other transformation methods. Also, lower electric field intensity could be use for electroporation, avoiding electrical arch into electroporation cuvette.

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