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Transformation of *Saccharomyces cerevisiae* by Electroporation

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A method for introducing heterologous DNA into *Saccharomyces cerevisiae* rapidly and efficiently by electroporation was developed. Transformant colonies appeared somewhat sooner than by the LiCl or spheroplast transformation method, and the time spent in manipulation was much less than for these two methods. The pores in the cell membrane formed by the high voltage of electroporation were resealed within 6 to 7 min after electroporation. At a capacitance of 25 μF, the optimum voltage was 2.0 to 2.25 kV/cm. Log-phase cells concentrated to 10 to 20 units at an optical density of 600 nm in 200 μl of fresh rich medium and electroporated at 2.25 kV/cm in the presence of 0.1 μg of supercoiled plasmid DNA will yield 1,000 to 4,500 colonies per μg of DNA.

Work by Rieman et al. (17), Zimmermann et al. (22), Vienken et al. (20), Neumann et al. (15), and Potter et al. (16), among others, led to the development of electroporation as a simple and rapid method for introducing macromolecules, including proteins (19), into mammalian cells. Since then, specialized equipment has become commercially available, which has resulted in the application of the technique to many different mammalian cell types from different species and to bacterial, plant, and yeast cells.

The transformation of yeast cells presents difficulties in that the cells are surrounded by a cell wall composed mostly of mannoproteins and glucans (23). Protocols for DNA-mediated transformation of yeast cells always involve either enzymatic removal of the cell wall with glusulase, lyticase, or zymolyase (1, 8, 12, 18) or chemical pretreatments such as polyethylene glycol, LiCl, or thiol compounds which render the cell wall leaky to macromolecules (3, 7, 9–11, 13, 21). While these methods are effective in introducing heterologous DNA into the cells, they are time-consuming. I adapted electroporation in a procedure that allows transformation of yeast cells with foreign DNA quickly and efficiently without removing or pretreating the cell wall.

MATERIALS AND METHODS

Materials. Yeast extract, yeast nitrogen base without amino acids, Casamino Acids (technical grade), Bacto-Peptone, and purified agar were purchased from Difco Laboratories (Detroit, Mich.). Glucose was obtained from Fisher Scientific Co. (Pittsburgh, Pa.). The electroporation method described in this work was optimized on a Gene Pulser (catalog no. 165-2075; Bio-Rad Laboratories, Richmond, Calif.) in Gene Pulser cuvettes with electrodes spaced at 0.4 cm (Bio-Rad catalog no. 165-2085).

Instrument setting. The capacitance of the Gene Pulser was set at 25 μF and the voltage at 900 V, except as noted.

Host. For the purpose of optimizing the electroporation conditions, I used SE7-6 (*matα trp pep4-3*), a strain of *Saccharomyces cerevisiae* obtained from S. G. Elliott (5) which has a deletion in the *TRP1* gene to prevent reversion to wild type during selection.

Growth conditions. Overnight cultures of *S. cerevisiae* grown in nonselective YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 30°C were inoculated at 1:100 in YPD medium and grown to an optical density at 600 nm (OD_{600}) of 0.35 to 1.0 or as noted in each experiment. The cells were centrifuged at approximately 2,000 × *g* for 10 min and resuspended in YPD medium at an OD_{600} of 10 or as indicated. Samples of 200 μl of the cells were electroporated, plated directly on selective plates (0.67% yeast nitrogen base without amino acids, 2% glucose, 0.5% acid-hydrolyzed Casamino Acids, 2% Bacto-Agar [Difco]), and grown at 30°C until colonies appeared (about 2 days).

Plasmid DNA. pYE, an *Escherichia coli*-yeast shuttle vector obtained from G. A. Bitter (2), was used in all the optimization experiments except as noted. The vector is stably maintained and is present at high copy number in yeast cells kept under selection. pYE is a large vector (11,532 base pairs) which contains the entire 2μm plasmid endogenous to yeasts, the *TRP1* gene for selection, and all of pBR322, which allows replication and selection in *E. coli*. In addition, I tested YRP7 (18), a pBR322 derivative (5,815 base pairs) which contains the *TRP1* gene for selection and *ARS1* for autonomous replication in yeast cells. YRP7 is a multicopy plasmid but is somewhat unstable in yeasts. pYE(CEN3)30, a plasmid made from YRP7 by insertion of a 635-base-pair fragment containing a yeast centromere (4, 6), was also tested. The presence of the centromere enhances the plasmid stability but reduces the plasmid copy number to one per cell. The plasmids were purified on CsCl gradients (14).

Viability assay. To measure cell viability during optimization of the electroporation procedure, I electroporated cells without DNA at 0, 700, 800, 900, 1,000, and 1,500 V. The cells were plated on nonselective YPD plates at three different dilutions, each in triplicate, so that at 0 V there were approximately 500, 200, or 100 cells per plate.

Cell recovery after electroporation. Viable cells are able to actively exclude dyes and other compounds from accumulating in the cytoplasm. Phloxin B (Sigma Chemical Co., St. Louis, Mo.), a red dye often used to measure cell viability, was used in this work to determine how quickly the cell membrane regained its integrity after electroporation. The dye was added before electroporation or 10 s, 5 min, or 20 min after electroporation. Samples of stained cells were examined microscopically 2 to 5 min after staining, and the red and white cells were counted.

RESULTS AND DISCUSSION

My goal was to devise a yeast transformation protocol that is rapid but has a reasonably high transformation efficiency. I tested the effects of voltage, cell development, cell density,

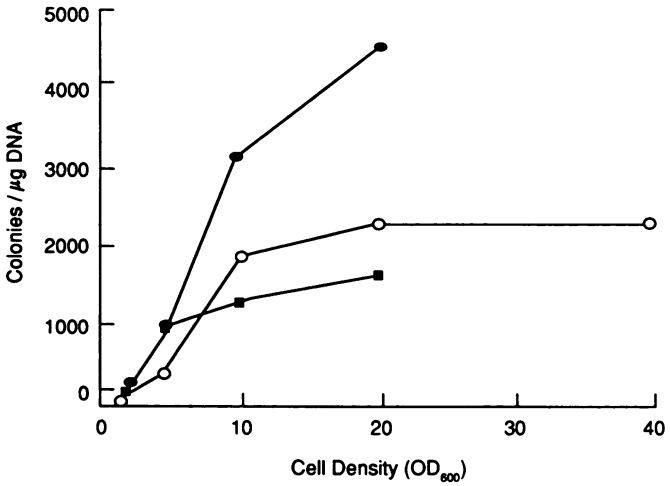

FIG. 1. Effect of voltage on transformation efficiency. Cells grown to an OD_{600} of 0.750 were concentrated by centrifugation to the concentrations given, and 200-μl samples were electroporated in the presence of 0.1 μg of pYE DNA. Symbols: ■, 1,000 V; ●, 900 V; ○, 800 V.

carrier DNA, and plasmid DNA concentration on the efficiency of transformation by electroporation.

The capacitance setting was chosen based on the length of the pulse delivery through 200 μl of YPD medium. At 1 μF, the pulse time was 0.5 ms, while at 25 μF, the pulse time was about 6 ms. The longer pulse allowed cell transformation at lower voltages and appeared be less deleterious to the cells. I optimized the voltage required to transform yeast cells with the capacitance set at 25 μF without further testing of the effect of capacitance or pulse length on the transformation efficiency of the cells.

Effect of voltage on transformation efficiency and cell survival. Optimization of the voltage is the most important step in the development of a transformation procedure by electroporation. The voltage must be high enough to create pores in the cell membrane, yet it must be low enough to avoid excessive cell death. As described below, the optimal voltage range is narrow. Furthermore, the optimal voltage varies from cell type to cell type.

The number of transformants per microgram of DNA increased with increasing voltage from 2,300 at 800 V to 4,500 at 900 V with cells at an OD_{600} of 20 (2.8×10^8 cells per ml), while electroporation at 1,000 V sharply reduced the number of transformants to 1,630/μg of DNA (Fig. 1). However, higher voltage also increased cell death. Cell death increased from about 52% at 900 V/0.4 cm to 84% at 1,000 V/0.4 cm (Table 1). Calculation of the transformation efficiency as a function of the number of viable cells after electroporation yielded different results. Using the data presented in Table 1 and Fig. 1, starting with 200 μl of cells at an OD_{600} of 20 (5.6×10^7 cells per 200 μl), at 900 V, the transformation efficiency would be 0.16% (or one transformant per 597 surviving cells), while at 1,000 V, the transformation efficiency would be 0.18% (or one transformant per 550 surviving cells). For most applications, I was interested in obtaining the greatest number of transformants per microgram of DNA, so I considered the optimum voltage for the transformation of yeast cells by electroporation to be 900 V, the voltage which produces the greatest number of colonies per microgram of DNA.

Effect of growth phase. Yeast cells in the log phase transformed more readily than resting cells. Similar transformation efficiencies were observed for cells grown to an OD_{600} of 0.3 to 1.0, while cells grown to an OD_{600} of 6 had a 4.5-fold lower transformation efficiency. At an OD_{600} of 11 (23 h of growth), the transformation efficiency was 22.5-fold lower than for cells at an OD_{600} of 0.35 (Fig. 2).

TABLE 1. Cell survival after electroporation[a]

Volts	10^6 Colonies/1.4 × 10^7 cells	% Survival
0	14.0	100
700	7.9	57
800	7.0	50
900	6.6	48
1,000	2.3	16
1,500	1.3	9

[a] Cells at an OD_{600} of 0.695 per ml were centrifuged and resuspended in YPD at an OD_{600} of 5. Samples of 200 μl (approximately 1.4×10^7 cells) were electroporated without DNA at 0, 700, 800, 900, 1,000, and 1,500 V and then diluted and plated onto SDCAA agar.

Effect of cell density. The best transformation efficiency for a given amount of DNA is achieved when the DNA becomes limiting for the cell density used. The effect of cell density was examined, keeping volume and DNA concentration constant. The number of transformants increased with cell density and was maximal when the cells were concentrated at an OD_{600} of 10 to 20 (1.4×10^8 to 2.8×10^8 cells per ml) (Fig. 1). Because of the high cell density (about 3×10^7 to 6×10^7 cells per 200 μl), it may be necessary to dilute the cells in selection medium and to plate on several selection plates to avoid cross feeding between the transformed and nontransformed cells. Another alternative would be to optimize the voltage with 20- to 50-μl aliquots of cells.

Effect of plasmid concentration. The transformation efficiency was examined as a function of plasmid concentration. Transformation efficiency can be expressed as transformants per microgram of DNA or transformants per electroporation. The maximum number of transformants per microgram of DNA was obtained with 0.1 μg of DNA, but the total number of transformants increased with DNA amounts up to 1.0 μg per electroporation (Table 2). Greater amounts of plasmid DNA did not reproducibly increase the number of colonies per plate, indicating that the plasmid DNA was present in saturating amounts for the cell density used. When screening a library, it would be preferable to electro-

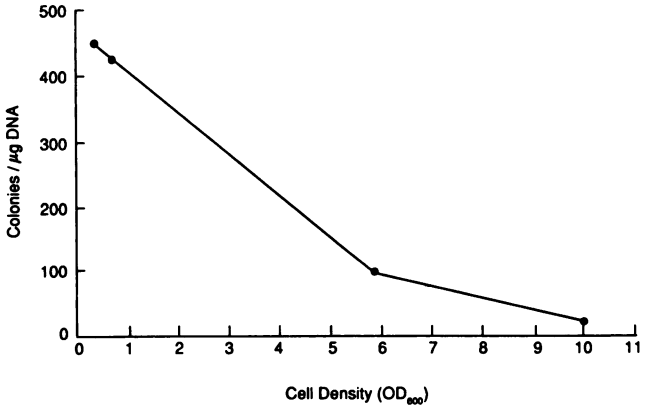

FIG. 2. Effect of cell development on transformation efficiency. Cells were grown to different growth phases, concentrated by centrifugation to an OD_{600} of 5, and then electroporated in the presence of 1 μg of pYE DNA.

TABLE 2. Effect of plasmid DNA concentration on transformation efficiency[a]

DNA (μg)	Expt 1[b]		Expt 2[c]	
	Colonies/ plate	Colonies/ μg of DNA	Colonies/ plate	Colonies/ μg of DNA
0.1	77	770	165	1,650
0.5	110	220		
1.0	110	110	302	302
2.0	149	75	290	145
5.0	112	22	266	53
10.0	122	12	358	35

[a] Cells were grown to an OD_{600} of 0.404, concentrated as indicated, and electroporated with pYE plasmid DNA.
[b] Experiment 1, 1.4×10^7 cells per 200 μl (OD_{600} of 5).
[c] Experiment 2, 2.8×10^7 cells per 200 μl (OD_{600} of 10).

porate 10 aliquots of cells with 0.1 μg of DNA each rather than to electroporate 1 aliquot of cells with 1 μg of DNA.

Effect of plasmid construction on transformation efficiency. The transformation efficiency for different yeast transformation methods varies with the method used, and within each procedure the efficiency also varies with the host and with the transforming plasmid. I found variations in the transformation efficiency of our TRP^- strains although they were derived from SE7-6, the strain I used in my optimization experiments. Some strains were transformed more efficiently and others less efficiently than SE7-6, but all efficiencies were within the range reported here. I noticed that strains unrelated to SE7-6 carrying the *ura3* marker were more difficult to transform than my *trp* mutant strains. Transformation by electroporation and with LiCl gave similar but low efficiencies when transforming *ura* mutants with *ura3*-containing plasmids.

I tested the effects of three different plasmid constructions on the transformation efficiency of yeast cells. All the plasmids tested carried the *TRP1* yeast gene for selection to eliminate the effect of different hosts on the transformation efficiency. pYE transformed by electroporation into SE7-6 yeasts had the best transformation efficiency of the three plasmids tested. pYE(CEN3)30 was 78% as efficient as pYE, and YRP7 was only 52% as efficient as pYE in transforming yeasts. The plasmid stabilities of pYE and pYE(CEN3)30, 95 to 99%, were the same with transformation by LiCl or by electroporation. The plasmid stability of YRP7 was reported to be 20 to 30% (2); I expect that such low stability would affect transformation efficiency. Whether the transformation efficiency is related to plasmid size (pYE, 11.5 kilobases; pYE(CEN3)30, 6.4 kilobases; YRP7, 5.8 kilobases) or, more likely, to the plasmid stability in yeasts was not determined, but the results suggest that construction of cDNA libraries in pYE- or pYE(CEN3)30-type plasmids, rather than in YRP7, might increase the chances of recovering the desired gene.

Effect of DNA quality. For routine transformation, when high transformation efficiency was not a consideration, 1 ml of log-phase cells was centrifuged and the cell pellet was resuspended in 200 μl of fresh YPD, electroporated with 0.1 to 4 μg of DNA obtained from a minipreparation by the boiling lysis method (14), and plated directly on selective plates. I estimated the amount of DNA in our preparations by agarose gel electrophoresis to separate the RNA from the plasmid DNA and compared the intensity of the DNA band with that of the standards. The DNA preparations were not digested with RNase before electroporation. I obtained efficiencies of 100 to 700 colonies per μg of DNA from these small-scale unpurified preparations. Higher concentrations

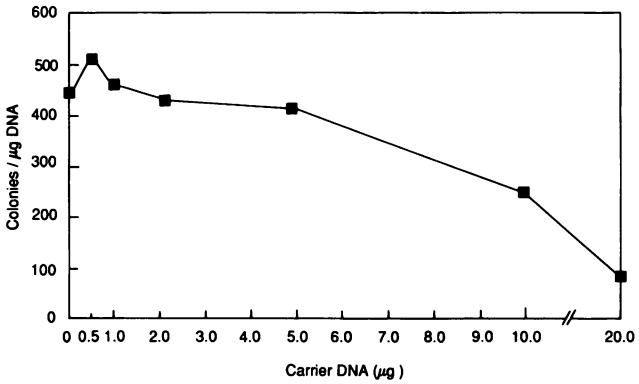

FIG. 3. Effect of carrier DNA on transformation efficiency. The cells were resuspended at an OD_{600} of 5 and electroporated at 800 V in the presence of 0.1 μg of pYE DNA.

of DNA from these preparations produced similar number of colonies per plate but much lower transformation efficiencies in terms of colonies per microgram of DNA.

Effect of carrier DNA. Carrier DNA is used in the transformation of yeast cells by the LiCl and spheroplast methods to increase the efficiency of plasmid DNA transformation. I wanted to know whether carrier DNA would have a similar effect when yeast cells are transformed by electroporation. The addition of 0.5 to 5.0 μg of sheared salmon sperm DNA to 200 μl of cells mixed with 0.1 μg of plasmid DNA had a small positive effect on the transformation efficiency (Fig. 3) that was reproducible in three experiments. This suggests that the transformation efficiency could be improved further by lowering the amount of plasmid DNA and electroporating in the presence of small amounts of carrier DNA. However, the addition of 10 and 20 μg of salmon sperm DNA lowered the transformation efficiency by about 45 and 77%, respectively.

Cell recovery after electroporation. To determine the effect of electroporation on cell membrane permeability, I examined the uptake of phloxin B. Phloxin B is normally excluded from living cells by the cell membrane, but leaky or dead cells take up the red dye. The dye was added before electroporation or at various times after electroporation. Uptake of the dye was determined by microscopic examination of the cells. Before electroporation, less than 1% of the cells took up the dye. When the cells were electroporated at 900 V in the presence of the dye, the dye entered 100% of the cells, indicating that all the cells had the capacity to take up DNA. When the dye was added within a few seconds after electroporation, 17 to 23% of the cells did not take up the dye, suggesting that some of the pores created by the electric field were already resealed. When the dye was added 6 to 7 min after electroporation, 50% of the cells excluded the dye, indicating that after electroporation the transient pores induced by electroporation were resealed in 100% of the viable cells (Table 3). The same was true of cells electroporated at 1,000 V, in which case about 10 to 20% of the cells were viable after electroporation (Tables 1 and 3). Cell survival was about 50% at the optimum voltage for transformation efficiency.

Colony formation after electroporation. The lag period before the appearance of transformed colonies depends on the type of plasmid and the host used for the transformation. In this work, only the plasmid construction was examined, since I used the same host for each plasmid. Cells transformed with pYE and pYE(CEN3)30 gave rise to colonies

TABLE 3. Cell recovery after electroporation[a]

Expt[b]	Exclusion of phloxin B (% white cells)				
	Before electro-poration	During electro-poration	10 s after electro-poration	6 to 7 min after electro-poration	20 min after electro-poration
1					
a	>99	0	23	50	49
b	>99	0	18	53	52
c	>99	0	20	42	48
d	>99	0	17	52	50
2		0	9	21	20

[a] The dye phloxin B was added at different times during the experiment. The cells were examined immediately for dye uptake by microscopy.

[b] Experiment 1, Four aliquots of cells were electroporated at 900 V, and one sample of each was examined. Experiment 2, Cells were electroporated at 1,000 V, and seven samples were examined and averaged.

about 1 mm in diameter, while the colonies from YRP7 transformation were 0.5 mm or smaller 40 h after plating on selective plates. In some experiments, pYE gave rise to tiny colonies which were visible 24 h after electroporation. Because the cells recover very quickly after electroporation, within 6 to 7 min, the appearance of transformed colonies occurs 12 to 30 h sooner than after LiCl, or about 2 days sooner than after spheroplast transformation.

Optimum conditions. Overnight cultures of *S. cerevisiae* grown in YPD medium were inoculated at 1:100 into fresh YPD medium, grown to an OD_{600} of 0.35 to 0.75, centrifuged, and resuspended in fresh YPD medium at an OD_{600} of 10 to 20 per ml. Samples of 200 μl of cells and 0.1 μg of cesium chloride-purified plasmid DNA were added to a Gene Pulser cuvette and electroporated at 900 V (2.25 kV/cm) with the Gene Pulser set at 25 μF. Samples of 100 or 200 μl of electroporated cells were spread directly on SDCAA plates (0.67% yeast nitrogen base without amino acids, 2% glucose, 0.5% acid-hydrolyzed Casamino Acids, 2% Bacto-Agar) and incubated at 30°C for 2 days.

Frequency of targeted DNA integration. I electroporated CM1, a *ura trp* strain derived from SE7-6, with pBR-u3, which consists of pBR322 with a *URA3* insert in the *Hin*dIII site. I obtained 11 colonies per μg of CsCl-purified plasmid DNA. This frequency of integration is comparable to that obtained with transformation by the spheroplast method (18).

Comparison of different yeast transformation procedures. The transformation efficiencies of YRP7 into yeast cells have been published, using spheroplast (18), LiCl (9), and a combination of spheroplast and electroporation (12) transformation methods, and can be compared with my electroporation method. My pYE plasmid is similar to Yep2 (18) in that it contains both the *E. coli* pBR322 plasmid and the yeast 2μm plasmid.

As with other methods, the transformation efficiency obtained by my method varied with the type of plasmid used. The transformation of YRP7 by my electroporation procedure was slightly more efficient than with the LiCl method (9) and within the range reported for the transformation method employing spheroplasts (18). My transformation efficiency by electroporation of intact cells was very similar to that reported for electroporation of spheroplasts with YRP7 (12). The transformation efficiency of pYE (1,000 to 4,500 colonies per μg of DNA) was less than that reported for transformation of Yep2 by the spheroplast method (5,000 to 20,000 colonies per μg of DNA) (18) but was as good as or better than the transformation efficiency by the LiCl method.

The major advantages in transforming yeast cells by the electroporation procedure presented here are the speed and simplicity of the procedure. The only manipulation prior to addition of the cells to plasmid DNA is a centrifugation and resuspension step to concentrate the cells. The electric pulse delivered lasts about 6 ms. After electroporation, the cells are plated directly from the cuvette onto selective plates, without any heat shock or additional incubations. There is no top agar required, no long regrowth period, and no risk of polyploid formation by cell fusion, in contrast to the spheroplast method. Another advantage of yeast transformation by electroporation is that the transformants do not need to be picked out of top agar as with the spheroplast method. The transformed colonies can be replica plated directly from the transformation plate for further characterization. Transformation by electroporation is particularly helpful when screening large numbers of either plasmid constructions or yeast mutants.

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