High efficiency transformation of *Schizosaccharomyces pombe* by electroporation

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The highest reported transformation efficiencies for *Schizosaccharomyces pombe* of 1×10^5 to 10^6 colonies per μg of DNA have been achieved by a highly efficient cation method (1) and by the addition of lipofectin to protoplasts (2). I have developed a method of *S. pombe* transformation by electroporation, based on a similar method for *Saccharomyces cerevisiae* (3), that achieves high efficiencies but requires less time, no special media, and no heat shock step which may be detrimental to temperature sensitive strains. Electroporation of *S. pombe* by this protocol involves very little manipulation and can be completed in one hour starting from log phase cells. Previously, it was shown that *S. pombe* could be transformed by electroporation with efficiencies of $1-8\times10^3$ transformants per μg of DNA (4). Typical efficiencies by the method reported here have been 1×10^5 to 10^6 colonies per μg DNA.

Electroporation

The apparatus used for electroporation was the Bio-Rad Gene Pulser™ with a pulse controller. This apparatus delivers exponential decay pulses with a 5 msec time constant at the settings used here (see below). Cells were electroporated in chilled cuvettes with a 0.2 cm electrode gap (Bio-Rad).

Cells to be electroporated were grown in either YE (2% glucose, 0.5% yeast extract) or dropout media (5) to a density of approximately 1×10^7/ml at 30°C and collected by centrifugation. Because the cell suspension must have an extremely low conductivity, cells were washed three times with ice cold filter sterilized 1.2 M sorbitol and resuspended in 1.2 M sorbitol to a cell concentration of 1×10^9/ml. 0.2 ml of the cell suspension was mixed with 1 ng to 1 μg of DNA then immediately transfered to an ice cold cuvette. Cells were pulsed at 2.25 kV (11.25 kV/cm), 200Ω and 25μF. Immediately after the pulse 0.5 ml of ice cold 1.2 M sorbitol was added to the cuvette. Electroporated cells were diluted in 1.2 M sorbitol such that 0.5 ml was spread on very dry selective minimal plates (0.67% yeast nitrogen base, 2% glucose, 2% agar and supplements as required). Higher transformation efficiencies were achieved when the cells were not diluted more than fivefold. Colonies appear in four to six days at 30°C.

The number of transformants obtained is roughly linear with the amount of DNA used from 1 ng to 1μg. The DNA should be added to the cells just prior to electroporation. Extended incubation leads to lower transformation efficiencies possibly due to nucleases in the cell suspension (6). The plasmids used were CsCl purified. Miniprep DNA did not give good results. A variety of autonomously replicating plasmids have been used with similar results. Linearized integrating vectors have also been electroporated successfully.

Because this method of transformation requires less time than the commonly used lithium acetate procedure (7), it is also useful for routine transformations. If cells are electroporated at 2.5 kV instead of 2.25 kV there is less of a background lawn and larger more distinct colonies. At this voltage the number of viable cells decreases reducing the background lawn of cells such that the number of transformed cells per number of viable cells increases (Table I). With these modifications transformation efficiencies of 1×10^4 to 1×10^5/μg have been obtained. For convenience electrocompetent cells may be prepared and frozen at −70°C with a loss in transformation efficiency.

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Table 1. The Effect of voltage on transformation efficiency

Voltage kV	Viability %	Efficiency*
0	100	0
0.8	109	0
1.25	101	4.2×10^3
1.5	84	2.7×10^4
1.75	80	7.9×10^4
2.0	73	1.8×10^5
2.25	43	1.9×10^5
2.47	13	2.0×10^4

*Efficiency is Ura+ transformants per μg DNA for 10 ng DNA.