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Transformation of *Schizosaccharomyces pombe* by electroporation

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Schizosaccharomyces pombe is considered an excellent alternative host to *Saccharomyces cerevisiae* for molecular cloning because of its closer similarities to higher eukaryotic cells (1). It has been shown that *S. pombe* chromosome III can replicate autonomously in mouse cells (2) and that *S. pombe* can correctly synthesize the gene products of human antithrombin III (3) and human CDC 2 (4). With the rapidly developing interest in molecular cloning in the fission yeast, there is an increased need for an efficient and easy method of genetic transformation. Beach *et al.* (5) developed a commonly used method involving protoplasting cells with Novo SP234 followed by the addition of plasmid DNA and treatment with $CaCl_2$ in 20% polyethylene glycol (PEG). To date this method produces the highest transformation efficiency in *S. pombe*, but obtaining protoplasts prior to transformation is time consuming. More recently, Bröker (6) developed a method circumventing the generation of protoplasts by pretreating intact yeast cells with LiCl in 50% PEG followed by a 25 minute heat pulse at 46°C in the presence of plasmid DNA. Although the transformation efficiencies by this method are lower than those of the standard protoplast method, it is less tedious and requires less time. We have developed a protocol for transformation of *S. pombe* by electroporation, a method which involves creating pores in the yeast, through which plasmid DNA can pass, by subjecting a cell suspension to a controlled electrical impulse. The transformation efficiencies using the conditions listed below are comparable to that of the LiCl/PEG treatment while requiring only approximately 10 minutes to perform from the cell harvesting to plating.

The electroporation procedure is as follows: a yeast culture grown to late log phase in 50 ml of YDP (1% yeast extract, 2% dextrose, 2% bactopeptone) was harvested by centrifugation and the subsequent pellet resuspended in 1 ml of TE buffer (pH 8.0). A 50 μl aliquot of the cell suspension was added to 50 μl of 60% PEG 4000 resulting in a cell concentration of approximately 4 $\times 10^8$ cells/100 μl. One μg of plasmid DNA is added to the suspension and vortexed to mix. The entire 100 μl is then loaded into a 0.2 cm gapped electroporation cuvette and pulsed once with a field strength of 8.5 kV/cm and decay time of 0.5 msec. The suspension is then diluted in the cuvette with 300 μl of TE and removed with a pasteur pipette. 10 μl and 100 μl are spread onto duplicate YNB (6.7% yeast nitrogen base without amino acids, 2% glucose, 1.5% agar) selection plates. Yeast transformant colonies appear in four to six days at 30°C. This protocol was developed using the strain *S. pombe* h− ura 4−294 with the cloning vector pFL20, which contains *S. pombe* autonomously replicating sequences and the *S. cerevisiae* ura3 gene which complements the *S. pombe* ura 4−294 mutation (7). Electroporation was performed with the BioRad Gene Pulser and Pulse Controller with 0.2 cm gapped electroporation cuvettes.

In addition to the electroporation technique, we have obtained transformation efficiencies similar to those reported for the LiCl/PEG/heat shock method (6) by omitting the LiCl step and increasing the PEG concentration to 60%.

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Table 1

Method	Efficiency*	Est. Time**	Reference
Electroporation	$1-8 \times 10^3$	10 minutes	—
Heat Shock	7×10^3	30 minutes	—
LiCl/PEG/heat	$4-9 \times 10^3$	2 hours	Bröker (6)
Protoplast	$2-3 \times 10^4$	3 hours	Beach *et al.* (5)

* Efficiency is Ura + transformants per μg of plasmid DNA.
**Estimated time to complete procedure (time from harvesting cells to plating on selective media).