A practical method for fission yeast transformation by electroporation

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ABSTRACT

High-voltage shock within a very short duration under the proper conditions causes cells to incorporate exogenous macromolecules. This technique, electroporation, has been widely used in recent years to transform many organisms. We determined optimum conditions for fission yeast transformation using this method. Of nineteen combinations of electric field strength and pulse time examined, 1.75 kV/0.2 cm, 4 msec pulse was found to provide approximately 4.0×10^5 transformants per μg of DNA. Other factors responsible for the transformation efficiency in fission yeast are also discussed.

1. INTRODUCTION

Transformation is required for manipulating recombinant DNAs, and a variety of techniques have been developed for working with many organisms. In yeast, transformation efficiency is much lower than that in bacteria, mainly because yeast cells are surrounded by a rigid cell wall composed of glucans and mannoproteins. Two methods have been used to resolve this problem; enzymatic digestion of the cell wall (Beggs, 1978; Hinnen et al., 1978; Struhl et al., 1979), and treatment of intact cells with alkali cations and polyethylene glycol (Ito et al., 1983; Yamakawa et al., 1985; Brzobohaty and Kovac, 1986; Okazaki et al., 1990). Electroporation is also used to transform budding yeast, *Saccharomyces cerevisiae*, taking advantage of the simple and rapid insertion of DNA into cells without chemical or heat shock treatment (Delorme 1989; Becker and Guarente, 1991). Physical permeabilization within a short duration makes it possible to minimize changes in physiological conditions of the cells during transformation. This is important for transforming certain mutants, including temperature sensitive ones. We adapted this method to the fission yeast, *Schizosaccharomyces pombe*, which is thought to differ from budding yeast in certain aspects, particularly in cell wall composition. In this paper, we determined optimum conditions for fission yeast transformation by electroporation, with respect to the growth phase, electric field strength and pulse time. Effect of the size of plasmid

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on transformation efficiency was also examined.

2. MATERIALS AND METHODS

Strains, plasmids and culture conditions

Schizosaccharomyces pombe strains, CP925 (h^- *cps3 ura4-D18*) and CP914 (h^- *cps3 leu1-32*) were used as the recipients for plasmids pAU-SK and pDB248', respectively. pAU-SK (5.6 kb in size) contains an *ars* element and *URA3* selective marker (Tanaka, K., personal communication), and pDB248' (12.6 kb in size) contains a portion of a 2 μ m DNA and *LEU2* marker (Beach et al., 1982). pD912-10 is a pDB248' bearing 10 kb insert DNA at the *HindIII* site, and was also used as a transforming plasmids. The strain, CP925 or CP914, was cultured aerobically in SD medium (0.7% yeast nitrogen base w/o amino acid, 2% glucose) supplemented with uracil (75 μ g/ml) or leucine (50 μ g/ml) at 28°C. Plate medium contained 2% agar.

Electroporation

The procedure of electroporation was based on the method of Becker and Guarente (1991). Cells were sedimented from 100 ml of culture that had reached a given growth phase, and washed twice with 100 ml of Milli Q water (18 M Ω /cm) and then washed again with 20 ml of 1.2 M sorbitol. The pellet was suspended in 1.2 M sorbitol to a final cell density of approximately 10^9 cells/ml. Routinely, 0.6 μ g of the plasmid DNA dissolved in TE buffer was added to 50 μ l of the cell suspension, mixed well and transferred to a electroporation cuvette with a 2 mm inter-electrode gap. Electroporation was performed using an EASYJECT PLUS (EquiBio). Setting of parameters (output voltage, capacitance and timing resistance) are indicated in the RESULTS AND DISCUSSION. One milliliter of 1.2 M sorbitol was added to the cuvette immediately following the pulse. An appropriate amount (10–50 μ l) of the electroporated cells were spread on a SD plate and incubated at 28°C for several days. Transformation efficiency was assessed based on the number of colonies grown on the plates.

3. RESULTS AND DISCUSSION

Growth phase

The strain CP925 was grown to a middle-log, late-log and stationary phases, respectively. The cells that had reached a given growth phase were tested for transformation efficiency using pAU-SK plasmids as described in the MATERIALS AND METHODS. Electroporation was performed at 1.5 kV/0.2 cm, 201 Ω and 25 μ F. The results are shown in Table 1. The highest transformation efficiency was obtained from the late-log phase culture (3.5×10^5 transformants/ μ g DNA).

Table 1. Effect of growth phase on transformation efficiency

Growth phase	Cell density ($\times 10^7$ cells/ml)	Transformants/ μ g DNA ^a
middle-log	0.6	1.4×10^4
late-log	1.5	3.5×10^5
stationary	2.0	2.6×10^4

^a pAU-SK plasmid was used. Electroporation was performed at 1.5 kV/0.2 cm, 201 Ω and 25 μ F.

Fig. 1. Effects of field strength (V/0.2 cm) and pulse time (msec) on transformation efficiency. The pulse times indicated are calculated by the equation, $T=R \cdot C$, in which T is expressed in seconds, R in Ohms and C in Farads. In this experiment, the capacitance is fixed at 25 μ F.

Electroporation conditions

The most critical parameters affecting transformation efficiency are electric field strength and pulse time. Nineteen different combinations of these two parameters were examined for selecting optimum electroporation conditions. The results are shown in Fig. 1. The range of pulse times that resulted in high transformation efficiencies appeared to become more narrow with increasing initial field strength from 1.25 kV to 1.85 kV/0.2 cm. The best combination of voltage and pulse time was 1.75 kV and 4 msec (156Ω , $25\mu\text{F}$) with an efficiency of approximately $4.0 \times 10^5/\mu\text{g}$ DNA.

Plasmids and other factors

Three *S. pombe*-*E. coli* shuttle vectors were used as transforming plasmids: a routinely used pAU-SK (5.6 kb in size), a pDB248' (12.6 kb) and a pDB248' bearing 10 kb insert DNA at the *Hind*III site (pD912-10). When the latter two plasmids were used, the efficiencies decreased to 1/2 and 1/8, respectively, in comparison with that obtained with pAU-SK (Table 2). The size of plasmid would thus appear to be a critical factor in determining transformation efficiency.

Prentice (1991) reported fission yeast transformation by electroporation. However, there are two discrepancies between his and our results. One is the amount of DNA added and the other, electric field strength required for the maximum transformation efficiency. In Prentice's study, the maximum efficiency was observed at 2.25 kV/0.2 cm, using 10 ng DNA. We noted, however, that the amount of DNA added to competent cells greatly affects transformation efficiency: up to 500 ng of DNA was required for the maximum efficiency for *S. pombe*. In *S. cerevisiae*, decreased efficiency was observed with increases in input DNA at more than 100 ng (Becker and Guarente, 1991). The amount of DNA required for the maximum number of transformants per μg DNA was thus much higher in *S. pombe* than in *S. cerevisiae*. This has also been reported in the case of *H. polymorpha* (Faber et al., 1994). In this study, a pulse of 2.25 kV/0.2 cm was noted too high to obtain the maximum efficiency. The reason for this is unclear, since the strain and plasmid used were not specified in Prentice's paper. In this study, several strains in addition to CP925 and CP914 were also transformed

Table 2. Effect of size of plasmid on transformation efficiency

Plasmid	Size (kb)	Transformants/ μg DNA ^a
pAU-SK	5.6	3.5×10^5
pDB248'	12.6	1.6×10^5
pD912-10 ^b	23.0	4.0×10^4

^a Late-log phase cells were used. Electroporation was performed at 1.5 kV/0.2 cm, 201Ω and $25\mu\text{F}$.

^b pD912-10 is a pDB248' bearing 10 kb insert DNA at the *Hind*III site.

under the standard condition (1.5 kV/0.2 cm, 201 Ω , 25 μ F). There was no significant difference in the transformation efficiency between CP925 (Ishiguro and Uhara, 1992) and other strains examined, CP903 (*h⁻ leu1-32*), CP938 (*h⁻ cps1 ura4-D18*) and CP941 (*h⁻ cdc23 ura4-D18*), with one exception, CP932 (*h⁻ cps8 ura4-D18*) that provided only 5×10^3 transformants/ μ g DNA.

As an effective method for fission yeast transformation, we recommend the following conditions: (1) the use of late-log phase cells in a concentrated suspension ($>10^9$ cells/ml) and more than 0.5 μ g purified DNA. (2) a pulse of 1.75 kV/0.2 cm for 4 msec (156 Ω , 25 μ F) or 1.25 kV/0.2 cm for 8.2 msec (329 Ω , 25 μ F). Certain strains are killed more easily by high-voltage shock, and for such strains, lower voltage should be used (1.25 kV). (3) plating of the electroporated cells on a selective medium in the absence of sorbitol. In contrast to *S. cerevisiae*, supplement of sorbitol in the plate medium rather reduced transformation efficiency for *S. pombe*. The present method for fission yeast transformation must be efficient for molecular cloning and gene disruption.

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NOTE In this study, SD medium was used for routine cell growth. We have noted, however, the cells cultivated in the medium enter stationary and subsequent death-phase much faster than in EMM medium, so EMM should be used instead of SD.

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