Electroporation of *Schizosaccharomyces pombe* by hyperosmotic post-pulse incubation

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BioTechniques 36:218-220 (February 2004)

The fission yeast *Schizosaccharomyces pombe* is attractive for molecular biological investigation because of its resemblance to higher eukaryotic cells. These days, *S. pombe* is transformed largely through electroporation; to obtain high-transformation efficiency, foreign DNA is introduced into intact cells (1–3). For still greater efficiency, several attempts have been made to pretreat intact *S. pombe* cells before the electric pulse is applied. These attempts have included pretreatment with dithiothreitol (DTT) (4) or hyperosmotic solution (5) and freezing in sorbitol with calcium (6). However, when *S. pombe* cells are pulsed in a polyethylene glycol (PEG) solution and continuously postincubated, the pores of the plasma membrane are maintained, but the efficiency is impractically low (7). We found that a switch to hyperosmotic solution buffer for the post-pulse incubation of *S. pombe* cells improved the transformation efficiency to the point of practicality.

The *S. pombe* strains ATCC38399 (*h⁻ leu1-32*), ATCC38436 (*h⁻ ura4-294*) (ATCC, Manassas, VA, USA), and TK107 (*h⁻ phh1::ura4⁺ ura4-D18 leu1-32*) were used as the recipients for plasmids pAL7 and pAU5 (8,9). The plasmids were isolated and purified using the Plasmid Mini Kit (Qiagen GmbH, Hilden, Germany).

The electroporation procedure was based on a cryopreservation method that has been previously described (10). *S. pombe* cells were grown in synthetic defined (SD) medium [0.67% Bacto™ yeast nitrogen base (Difco Laboratories, Detroit, MI, USA) without amino acid and 2% glucose] to a density of 1×10^7 cells/mL at 30°C. The culture medium was supplemented with 150 µg/mL leucine or uracil, depending on the particular strains. The culture was placed on ice for 15 min just before harvesting. The cells were collected by centrifugation at 1600× *g* for 5 min. The resulting pellet was washed three times with ice-cold sterilized water and then suspended in ice-cold 2.0 M sorbitol to give 5×10^8 cells/mL. Aliquots of 0.1 mL of the cell suspension were dispensed into 1.5-mL QSP® microcentrifuge tubes (Porex Bio Products, Petaluma, CA, USA), which were then slowly frozen for storage in a freezer at -80°C. For each electroporation, the frozen competent cells were quickly thawed in a water bath at 30°C, followed by washing once with 1 mL of ice-cold 1.0 M sorbitol by centrifugation at 1600× *g*. The final pellet was resuspended in 1.0 M sorbitol to give 1×10^9 cells/mL. The cell suspension was mixed with 0.1 ng of purified plasmid DNA and then transferred to a chilled cuvette with a 0.2-cm electrode gap. A high electric pulse was applied to the cell suspension at 2.1 kV, 25 µF, 200 Ω, using the Gene Pulser® II with Pulse Controller Plus (Bio-Rad Laboratories, Hercules, CA, USA). The electroporated cells were immediately transferred in hyperosmotic sorbitol solution buffer and postincubated at 30°C. A 0.2-mL aliquot was spread on minimal selection plates (0.67% yeast nitrogen base without amino acid, 2% glucose, and 2% agar). Transformant colonies appeared in 4–6 days at 30°C.

The transformation efficiency of *Saccharomyces cerevisiae* cells can be markedly increased by spreading them on a selection plate containing 1.0 M sorbitol as an osmotic stabilizer for a long-term period of 4–6 days until colonies form (11). Unfortunately, this sorbitol treatment reduces the efficiency of *S. pombe* cell transformation (3). Figure 1 shows the effects of each of the three sorbitol solution buffers on the transformation efficiency of *S. pombe* (*leu⁻*) during the postincubation period. The electroporated cells were postincubated in osmotic sorbitol solutions with 25 mM HEPES, pH 7.0, and then spread on selection plates without sorbitol. The efficiency at 1.0 M sorbitol increased by approximately 5-fold until 30 min of incubation but then gradually decreased. This result indicates that a long-term incubation on a selection plate with 1.0 M sorbitol is not suitable for *S. pombe*. By increasing the osmotic concentration from 1.0 to 2.0 M sorbitol, the efficiency further increased; the effective periods began sooner but did not last as long. Thus, the best efficiency was obtained at 2.0 M sorbitol for 5–15 min. Under these conditions, the efficiency was raised by approximately 1 order of magnitude. Efficiency decreased markedly after 30

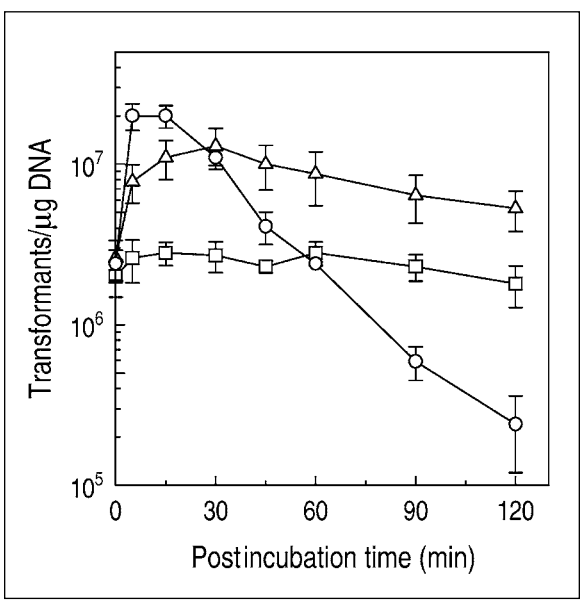

Figure 1. Effects of the postincubation period of the osmotic sorbitol solutions on transformation efficiency. *Schizosaccharomyces pombe* (*leu⁻*) cells were postincubated with 25 mM HEPES, pH 7.0, in 0.5 (□), 1.0 (Δ), and 2.0 M (○) sorbitol. Data represent the mean ($\bar{x}$ ± SD) of five experiments.

min or longer. Postincubation in 0.5 M sorbitol close to isosmotic solution had no effect on the efficiency.

Although the absence of pH buffer slightly increased the efficiencies at 1.0 and 1.5 M sorbitol compared to no postincubation treatment, the addition of HEPES, pH 7.0, significantly increased efficiency between 1.0 and 2.0 M sorbitol (Figure 2). This result shows that it was necessary to add pH buffer to the hyperosmotic sorbitol solution. The optimal pH was 7.0 even though the pH buffers ranged from pH 5.0 to 8.0.

Although electric pulse conditions certainly affect electroporation experiments, the transformation efficiency of postincubated *S. pombe* cells was constantly about one order of magnitude higher than in cells with no postincubation within 7.0–12.5 kV/cm. The optimum conditions for an electric field strength of 10–11 kV/cm were almost the same whether or not the cells underwent post-pulse incubation.

In electroporation procedures for other yeast species, postincubation in culture medium is rarely carried out for cell recovery after the application of an electric pulse (12,13). For *S. pombe*, however, the addition of SD medium to hyperosmotic sorbitol solution buffer did not sufficiently affect hyperosmotic postincubation. On the other hand, when *S. pombe* cells were pulsed in 30% PEG 4000 and then continuously postincubated, permeability increased as membrane pores became enlarged (7). In contrast, the efficiency decreased only gradually when the cells pulsed in 1.0 M sorbitol were immediately transferred and postincubated in 30% PEG 4000 with pH buffer.

A similar profile of sorbitol concentrations and incubation periods was observed in another strain of *S. pombe* (*ura*$^-$). The efficiency of the cells postincubated in 2.0 M sorbitol for 10 min increased approximately 15-fold. Furthermore, to determine whether or not the hyperosmotic postincubation would be applicable to osmo-sensitive strains, *S. pombe* (strain TK107) was tested (9,14). Efficiency at 2.0 M sorbitol for 10 min increased approximately 4-fold, but there was no effect at 1.0 M sorbitol for 30 min because of the longer exposure to high osmolarity.

We previously reported that pretreatment with DTT increased the transformation efficiency of *S. pombe* by electroporation (4). In the present study, a combination of DTT pretreatment with hyperosmotic postincubation further improved efficiency. A maximum transformation efficiency of 5.0 ± 1.1 × 10^7/µg DNA was obtained when *S. pombe* (*leu*$^-$) cells pretreated with DTT were postincubated in 2.0 M sorbitol with 25 mM HEPES, pH 7.0, at 30°C for 10 min. This simple and rapid procedure using hyperosmotic post-pulse incubation will help to fulfill the demand for high-transformation efficiency in gene expression and functional analyses.

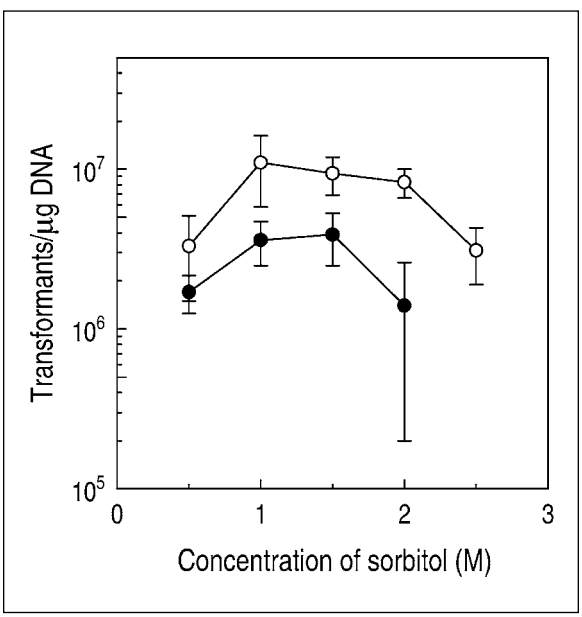

Figure 2. Effects of various concentrations of sorbitol on transformation efficiency. *Schizosaccharomyces pombe* (*leu*$^-$) cells were postincubated with 25 mM HEPES, pH 7.0 (o), and without pH buffer (●) for 60 min. The efficiency of the nonincubated cells was 2.2 ± 0.7 × 10^6/µg DNA. Data represent the mean ($\bar{x}$ ± SD) of five experiments.

ACKNOWLEDGMENTS

We would like to thank Dr. Koei Okazaki, Dr. Koichi Tanaka, and Dr. Hiroto Okayama for providing yeast strains and plasmids.

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Received 28 September 2003; accepted 12 November 2003.

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