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A simple method for rescuing autonomous plasmids from fission yeast

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Numerous features of the fission yeast *Schizosaccharomyces pombe* make it an attractive model system for molecular genetic studies of eukaryotic organisms (Ref. 1). Furthermore, because of the similarity of comparable cellular, genetic and molecular characteristics between *S. pombe* and mammalian cells, this yeast is a useful tool for cloning as well as analyzing genes from higher eukaryotes. It is often necessary to recover shuttle vector replicating in *S. pombe* back into *Escherichia coli* in order to obtain a large amount of plasmid DNA for molecular genetic studies with this yeast. In some cases, it has been difficult or impossible to recover plasmid from *S. pombe* into *E. coli*. This could be a plasmid-specific phenomenon which might be due either to loss of vector DNA essential for replication or selection in *E. coli* during yeast DNA preparations. Another problem more difficult to solve is the tendency of *S. pombe* to multimerize and rearrange plasmids (Ref. 2).

Plasmids are usually rescued from *S. pombe* using the method described by Moreno *et al.* (Ref. 3) We have used the method developed by Robzyk and Kassir (Ref. 4) for *Saccharomyces cerevisiae* with some modifications. This method is simple, rapid and inexpensive and routinely yields approximately 10^3 bacterial transformants using DNA prepared from 10 ml yeast culture.

Protocol

1. *Schizosaccharomyces pombe* transformants were grown in 10 ml yeast nitrogen base without amino acids [1.7 g/l yeast nitrogen base (Difco), 5 g/l $(NH_4)_2SO_4$, 0.5% (w/v) glucose] and appropriate supplements (for example, lacking uracil for the strain carrying a ura4-containing plasmid) at 30°C, 180 rev/min, overnight (10^8cells/ml).
2. Cells were harvested by centrifugation and resuspended in 100 μl of STET (8% sucrose, 50 mM Tris pH 8, 50 mM EDTA, 5% Triton X-100).
3. Acid-washed glass-beads (0.3 g of 0.45 mm diameter; treated with 70% nitric acid for 30 min, washed several times with distilled water and dried) were added and the tube was vortexed for 5 min at room temperature.
4. The supernatant was transferred to new tube, an equal volume of STET was added, the tube was vortexed briefly and placed in a boiling water bath for 3 min.
5. The tube was cooled on ice and centrifuged in a microfuge for 10 min at 4°C.
6. The supernatant (200 μl) was transferred to a fresh tube containing 100 μl of 7.5 M ammonium acetate, incubated at −20°C for 1 h and centrifuged at 10,000 rev/min for 5 min at 4°C.
7. Two volumes of ice-cold ethanol were added to the supernatant which was then left for 5 min at room temperature.
8. DNA was recovered by centrifugation (20,000 rev/min, 15 min, 4°C) and resuspended with 20 μl water.
9. Ten microlitres of the DNA was then used to transform super competent *E. coli* DH5α (Ref. 5).

We have found that one of the most important steps in this process was to make competent the *E. coli* strain DH5α. Using the super-competent cells (Ref. 5) ($1-3 \times 10^9$cfu/μg DNA) and DNA isolated from *S. pombe* ura4 D18 (a gift from C. Price, University of Sheffield, UK) carrying pREP42 which contains an ars1 fragment (Ref. 6), we have obtained approximately 10^3 ampicillin-resistant colonies.

Our method has some advantages over the method of Moreno *et al.* (Ref. 3) as we do not use any expensive compounds and spend extra time obtaining spheroblasts and cleaning the DNA.

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