Single-step purification of shuttle vectors from yeast for high frequency back-transformation into *E. coli*

Alister C.Ward
CSIRO Division of Biomolecular Engineering, 343 Royal Parade, Parkville and Russell Grimwade School of Biochemistry, University of Melbourne, Parkville, Victoria 3052, Australia

Submitted July 19, 1990

The yeast *Saccharomyces cerevisiae* is a convenient and efficient host for the expression and analysis of eukaryotic genes, having a wide array of shuttle vectors available. Current, widely-used methods for the isolation of plasmid from yeast (1, 2) are time-consuming, tedious and result in low transformation frequencies. New methods for rapid plasmid isolation (3) or for high transformation frequency (4) only overcome some of the difficulties in each instance. The method described here produces consistent high level transformation frequency without recourse to long and laborious protocols or expensive reagents and enzymes. The method requires only 20 minutes, and takes advantage of the finding that phenol/chloroform extraction in the presence of LiCl and Triton X-100 solubilises plasmid DNA, while precipitating cellular proteins and denatured chromosomal DNA. The plasmid isolation procedure is an adaption of a similar protocol used in the isolation of *E. coli* plasmids (5).

The modified protocol is as follows: a 1.5 ml culture of *S. cerevisiae* was grown in a selective medium for 16 h, and the cells harvested in an Eppendorf tube by centrifugation at 15000×g for 20 seconds. The cells were then suspended in 100 µl of 2.5 M LiCl, 50 mM Tris-HCl (pH 8.0), 4% Triton X-100, 62.5 mM Na_2EDTA, and to this was added an equal volume of phenol/chloroform (1:1 (w/v)) and 0.2 g Braun glass beads (0.45–0.50 mm). The mixture was vigorously vortexed for 2 minutes and centrifuged at 15000 ×g for 1 minute. The upper phase was collected and precipitated with ethanol. The entire procedure can be performed at 22°C without loss of transforming material. The nucleic acid was dried *in vacuo* and dissolved in 30 µl TE.

Table 1 shows typical transformation data for this method in comparison to results obtained with equivalent cultures using another rapid protocol (3). A >20-fold increase in transformants per transformation experiment is observed. Taking into account the difference in final volumes of the two methods, this represents a >3-fold increase in transformants per ml of starting culture. Ethanol precipitation of the transforming material from (3) failed to improve its relative transformation frequency. In addition, the procedure outlined above produced an increased number of transformants when a greater amount of plasmid preparation was used unlike the method in (3) which actually gave less transformants when more than 5 µl of plasmid preparation was used (Figure 1). This suggests the LiCl-Triton X-100 removes non-specific inhibitors of transformation. The higher number of transformants with this method, therefore, more readily facilitates the routine shuffling of plasmids from yeast to *E. coli* for genetic analysis or manipulation, relieving the frustration of repeated attempts of transformation to obtain a few transformants which is often encountered with some vectors and if cells are of moderate to low competency. Data presented is for pYELC5 (6) from strain DBY747 transformed into HB101. Similar results have been achieved using other vectors and a number of different *E. coli* strains.

REFERENCES

1. Davis,R.W., Thomas,M., Cameron,J., St. John,T.P., Scherer,S. and Padgett,R.A. (1980) *Meth. Enzymol.* **65**, 404–411.
2. *Methods in Yeast Genetics*—Laboratory Manual, eds F.Sherman, G.R.Fink, and J.B.Hicks (1983) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
3. Hoffman,C.S. and Winston,F. (1987) *Gene* **57**, 267–272.
4. Chow,T.Y-K. (1989) *Nucl. Acids Res.* **17**, 8391.
5. He,M., Wilde,A. and Kaderbhai,M.A. (1990) *Nucl. Acids Res.* **18**, 1660.
6. Macreadie,I.G. (1990) *Nucl. Acids Res.* **18**, 1078.

TABLE 1

	Volume used/ final volume of preparation	Number of *E. coli* transformants[a] (mean +/− S.E.)
This method	5 µl/30 µl	172+/−71
Other method (3)	5 µl/200 µl	8+/−2
EtOH prec of (3)	5 µl/30 µl	38+/−4

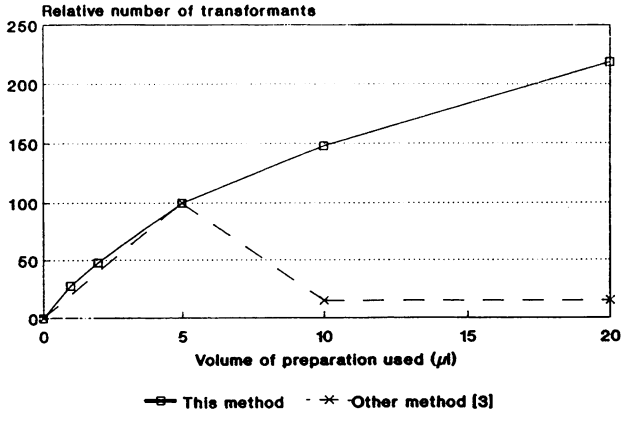

Figure 1.