

A rapid and inexpensive method for isolation of shuttle vector DNA from yeast for the transformation of *E.coli*

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Saccharomyces cerevisiae (bakers' yeast) is extensively used for the study of eucaryotic cellular processes. In particular, the wide variety of mutants available has resulted in its increasing use as a system for the isolation of complementing cDNAs from other eukaryotes using libraries constructed in yeast expression vectors. Most of these experiments require these vectors to be isolated from yeast and shuttled back into *E.coli* to facilitate further analysis.

Currently used methods for the isolation of DNA from yeasts use either zymolyase (1, 2), which dissolves the yeast cell wall and results in spheroplast formation, or glass beads (3, 4, 10), which shear the cells resulting in the release of DNA. Both these approaches involve the use of expensive reagents and are relatively time consuming.

The method described here for the isolation of plasmid DNA from yeast is quick and inexpensive, since neither zymolyase nor glass beads are required, and the plasmid can be successfully rescued back into *E.coli*. The modified protocol is as follows:

A 1.5 ml culture of *Saccharomyces cerevisiae* is grown in a medium selective for the plasmid to be recovered for 24 h to an $O.D._{600}$ of 1–1.5. Cells are pelleted in a microcentrifuge at 14000 rpm for 20 seconds. The cells are then suspended in 100 μl of 2.5 M LiCl, 50 mM Tris–HCl (pH 8.0), 20 mM EDTA (pH 8.0), 4% Triton X-100 and to this is added an equal volume of phenol/chloroform (1:1 v/v). The whole mixture is vortexed vigorously for 10 minutes. Alternatively, and more conveniently, samples are mixed vigorously on a Ika-Vibrax VXR shaker at motor speed 1800 for 10 minutes at room temperature. This facilitates the preparation of multiple samples. The suspension is then incubated at 65°C for 5 minutes without agitation. Samples are then centrifuged at 14000 rpm for 5 minutes. The aqueous phase is collected, taking care not to disturb the interphase, and precipitated with ethanol. The nucleic acid pellet is dried and resuspended in 30 μl of TE (10 mM Tris–Cl, 1 mM EDTA pH 8.0).

We find that this method gives sufficient transformants of *E.coli* when 2 μl of the DNA mixture is used, and that it is generally more efficient than the glass bead method (4) using equivalent samples (Table 1). Comparative data is presented here for the number of transformants obtained with the multicopy 2 μ-*ori*-*STB* based plasmid pFL61 (5) isolated from strains BF305-15d (ref. 6) and GY1 [cir$^+$] (ref 7) and transformed into *E.coli* by electroporation (efficiency 10^8 transformants per μg with control pFL61 DNA). Recovery has been demonstrated for the larger plasmid CV21*IR*$^-$ (12.9 kb; containing the complete 2 μ sequence) from the strain MY30-A (ref. 7), and also works well for the isolation of single copy centromere based plasmids, such as pZ61 (a derivative of pRS314; ref. 8), from the strain S150-2B (ref. 9).

Table 1. Comparison of numbers of *E.coli* colonies obtained by the glass bead method (4) and our method

Yeast strain	Plasmid	Experiment number	No. of colonies obtained This method	Glass bead method
BF305-15d	pFL61+	1	25	14
		2	26	8
		3	12	3
		4	5	4
		5–20	Mean = 15	Not done
S150-2B	pZ61		12	11
GY1 [cir+]	pFL61		19	10
MY30-A	CV21*IR*$^-$		15	18

+ refers to the cDNA inserts cloned in the *Not*I site of pFL61.
2 μl of DNA mixture was used in all the experiments and the efficiency of *E.coli* (HB101) cells was 10^8 transformants per microgram of DNA.

In short our method is rapid and inexpensive for the isolation of any shuttle plasmid from yeast cells for the subsequent transformation of *E.coli*.

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