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Purification of yeast–*E.coli* shuttle plasmid suitable for high transformation frequency in *E.coli*

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The wide variety of expression vectors available for the yeast, Saccharomyces cerevisiae, makes it an attractive host for analysis of eukaryotic genes. Current methods for plasmid isolation from yeast (1,2) and subsequent amplification in E. coli, however, result in low transformation frequencies. Often, only a few transformants were obtained after many attempts. The following method is a simple and efficient process of eliminating impurities present in yeast DNA preparations which contribute to the low transformability in E. coli. This method increases transformation frequency by at least 2 orders of magnitude.

Method: Plasmid DNA (in conjunction with cellular DNA) is isolated according to the method described above (1,2). The DNA is resuspended in 10 mM Tris-HCl pH7.5 containing 1 mM EDTA (TE), and passed through a NACS (BRL) column pre-equilibrated with TE plus 0.5 M NaCl. The column is then washed with 5 ml of the same buffer. Plasmid DNA is eluted with 300 ul of TE containing 2 M NaCl. The DNA is then precipitated with ethanol and resuspended in 50 ul TE.

Result: Plasmid DNA isolated from yeast were divided into two portions. One was used to transform E. coli directly while the other was passed through the NACS column (prepare by method above) prior to transformation. Only a few transformants were obtained using the crude DNA mixture whereas purification of the DNA with the NACS column resulted in two orders of magnitude increase in transformants (Table 1). This method, therefore, facilitates the shuttling of plasmids from yeast to E. coli.

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Table 1: Number of E. coli (DH5) transformants obtained with DNA isolated from yeast.

Amount of DNA (ng)	Number of E. coli transformants (mean ± S.E.) Crude DNA mixture no passage through a NACS column	DNA mixture after passage through a NACS column
50	-	200 ± 40 (3)[a]
100	4 ± 3 (4)	500 ± 50 (4)
200	6 ± 3 (4)	800 ± 50 (4)

[a]The number in parenthese is the number of independent measurement made to obtain the mean ± S.E.

Reference: 1) Methods in Yeast Genetics-Laboratory Manual. eds by F. Sherman, G.R. Fink, and J.B. Hicks (1983) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 2) Birnboim, H.C. and Doly, J. (1979) Nuc. Acid Res. 7:1513-1525.