Yeast Plasmids with the Least Trouble

Yeast Plasmid Isolation Using the Wizard® *Plus* SV Minipreps DNA Purification System

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Abstract

The isolation of plasmid DNA from yeast can be more difficult than from bacterial cells due to the yeast cell wall and lower plasmid copy number. In this article, we demonstrate the isolation of plasmid from yeast using a one-step modification of the silica-membrane technology Wizard® Plus *SV Minipreps DNA Purification System protocol.*

In our protocol, we performed an initial step to remove the exterior wall in order to obtain yeast protoplasts. Starting from protoplasts, we used the Wizard® *Plus* SV Minipreps DNA Purification System to isolate plasmid DNA from yeast.

Introduction

Plasmid DNA purification is one of the most common procedures performed in a molecular biology laboratory. The most prevalent method is to use a silica-membrane spin or vacuum column system such as the Wizard® *Plus* SV Minipreps DNA Purification System[(a,b)], as it offers high yield and consistent performance in an easy-to-use format.

Due to the presence of a more resistant cell wall than the one present in *E. coli*, the plasmid DNA isolation methods commonly used for the yeast *Saccharomyces cerevisiae* are more difficult and produce lower DNA yields than bacterial plasmid isolation methods.

In our protocol, we performed an initial step to remove the exterior wall from yeast in order to obtain yeast protoplasts. Starting from protoplasts, we used the Wizard® *Plus* SV Minipreps DNA Purification System to isolate plasmid DNA from yeast.

Preparation of Protoplasts from Transformed Yeast

The preparation of protoplasts was performed on MMY2-derived transformants using the protocol described for protoplast fusion preparation with minor modifications (1). The strain bears only a *URA3* marker. The library used was constructed in the pCT3 vector.

Table 1. Solutions for Generating and Testing Yeast Protoplasts.

Solution	Composition
Growth media	Rich (YPD medium) or selective (0.25% casamino acids [Difco], 0.67% yeast nitrogen base [Difco], and 2% glucose). When solid medium was needed, 2% agar was incorporated.
Tris buffer	100mM Trizma base buffer adjusted to pH 9.3 with HCl.
MP buffer	1M sorbitol, 1M NaCl, 0.01M acetic acid adjusted to pH 5.5 with 1M NaOH.
Protoplasting solution	50µl of 1M β-mercaptoethanol solution and 50µl of β-glucuronidase (127UI/ml; ICN) added to 3ml of MP buffer.
Regeneration medium	0.67% yeast nitrogen base, 2% glucose, 1M sorbitol and 2% agar.
Top agar	0.67% yeast nitrogen base, 2% glucose, 1M sorbitol and 1% agar.

A volume of 8ml of either rich or selective medium was inoculated with 80µl of a fresh yeast culture. The cells were grown at 30°C overnight until the middle exponential growth phase was reached. In our experience, this is equivalent to 5×10^6 to 2×10^7 cells per milliliter. The culture was centrifuged for 5 minutes at 3,500rpm in a clinical centrifuge. The pellet was washed twice with distilled sterile water and suspended in 3ml of Tris buffer with 50µl of 1M β-mercaptoethanol. The mixture was incubated at 30°C for 30 minutes.

Following the incubation, the mixture was centrifuged at 2,800rpm for 8 minutes and the pellet was washed twice with sterile distilled water. The obtained precipitate was resuspended in 3ml of protoplasting solution and incubated at 37°C for 50–60 minutes. To corroborate protoplast formation, aliquots of 50 or 100µl were taken every 15 minutes and diluted with 0.5ml of either 1M sorbitol, Tris buffer or distilled water. Comparison of turbidity among these three solutions indicated protoplast formation. Protoplasts will burst in Tris buffer and water resulting in transparent solutions, while intact protoplasts will cause the sorbitol solution to remain turbid. This test roughly indicated that most of the cells had been converted into protoplasts in 15–30 minutes.

As an additional test, protoplasts were plated with and without top agar. Protoplasts require top agar to regenerate the cell wall, so we would expect more colonies on plates using top agar. The protoplasts were collected at 1,400rpm in a clinical centrifuge. The pellet was washed with MP buffer and resuspended in 0.5ml of MP buffer. Two 30µl aliquots were diluted in 5ml of 1M sorbitol or Tris buffer. One hundred microliters of each dilution was inoculated in regeneration medium. One plate was covered with top agar, and the other remained uncovered. Plates were incubated at 30°C for 3–4 days. We observed that the plates without top agar had only 30% of the colonies found on plates with top agar, so we concluded that most of the cells were transformed into protoplasts by β-glucuronidase treatment.

Isolation of Plasmid DNA From Protoplasts with the Wizard® *Plus* SV Minipreps DNA Purification System

The remaining protoplast suspension (450µl) was centrifuged at 1,400rpm, and the obtained pellet was treated as described in Wizard® *Plus* SV Minipreps DNA Purification System Technical Bulletin #TB225. The pellet was treated the same as a pellet from 5ml of an overnight bacterial culture. To avoid an excessive dilution of the DNA, the column was eluted with 100µl of Nuclease-Free Water.

Transformation of *E. coli* with Purified Yeast Plasmid

To transform bacteria with the isolated DNA, 30µl of eluted DNA was mixed with 100µl of competent bacteria (4×10^7 cells/0.1ml) (2). For each transformation, 280 ± 30 colonies containing the required plasmid were obtained. This was established by analyzing isolated plasmid DNA by agarose gel electrophoresis. No difference was observed when the plasmid used was either the pCT3 vector alone or clones from the DNA library.

Similar results were obtained using plasmids based on Yep24 and other *S. cerevisiae* wildtype strains.

Conclusions

This protocol demonstrates the purification of plasmid DNA from *S. cerevisiae*. This simple procedure adds only one step to the Wizard® *Plus* SV Minipreps System protocol to prepare the yeast protoplasts. The remainder of the protocol is identical to the protocol presented in TB225. Purified plasmid DNA performs well in bacterial transformation.

Acknowledgments

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References

1. Curran, B.P.G. and Bugeja, V.C. (1996) In: *Yeast Protocols: Methods in Cell And Molecular Biology,* Evans, I.H., ed. Humana Press, Totowa., NJ, 45–9.
2. Davis, L.G., Dibner, M.D., Battey, J.F. (1986) In: *Basic Methods in Molecular Biology*, Elsevier, New York, 90–2.

Protocol

◆ *Wizard*® Plus *SV Minipreps DNA Purification System Technical Bulletin* #TB225, Promega Corporation.
(www.promega.com/tbs/tb225/tb225.html)

Ordering Information

Product	Size	Cat.#
Wizard® *Plus* SV Minipreps DNA Purification System	50 preps	A1330
	250 preps	A1460
Wizard® *Plus* SV Minipreps DNA Purification System + Vacuum Adapters	50 preps	A1340
	250 preps	A1470

For Laboratory Use.

[a]Australian Pat. No. 730718 and other patents and patents pending.

[b]U.S. Pat. No. 5,981,235, Australian Pat. No. 729932 and other patents pending.