YEASTMAKER™ Yeast Plasmid Isolation Kit Protocol-at-a-Glance

(PT3049-2)

Please read the *User Manual* before using this Protocol-at-a-Glance. This abbreviated protocol is provided for your convenience, but is not intended for first-time users. Note that the corresponding steps may be numbered differently here than in the User Manual.

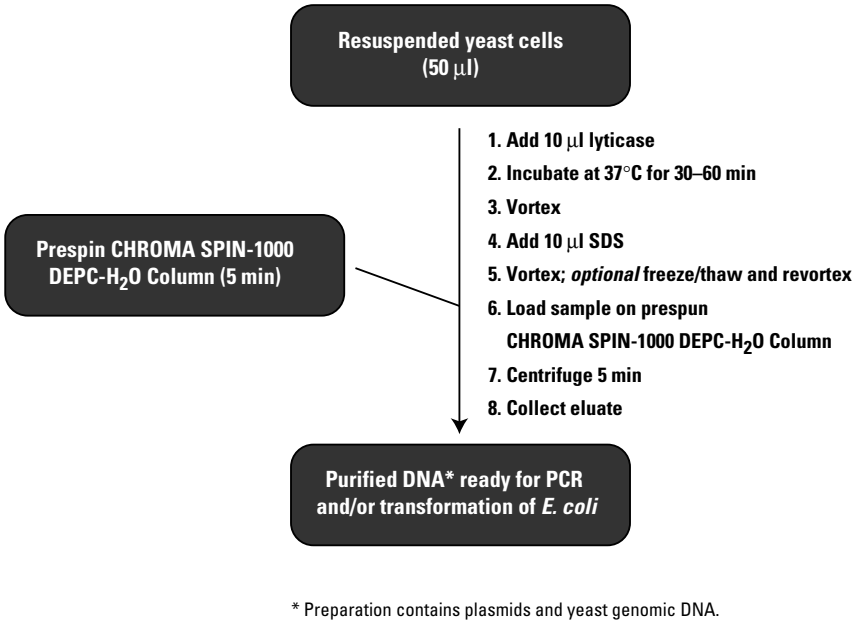

Figure 1. Flowchart for using the YEASTMAKER™ Yeast Plasmid Isolation Kit to prepare plasmid from yeast cells.

A. Prepare Cell Lysates

1. Prepare yeast cultures for lysis as described in Section IV.B.1. Each cell sample should be in a total volume of ~50 µl.
2. Add 10 µl of Lyticase solution to each tube. Pipette the solution up and down repeatedly to thoroughly resuspend the colony (or pellet).
3. Incubate at 37°C for 30–60 min.
4. Add 10 µl of 20% SDS to each tube and vortex vigorously for 1 min.
5. [Optional] Put samples through one freeze/thaw cycle (–20°C) and vortex again.
6. If necessary, samples can be stored frozen at –20°C. If samples have been frozen, vortex before using them.

B. Purify Plasmid DNA using CHROMA SPIN™-1000 DEPC-H_2O Columns

1. Invert and vortex the CHROMA SPIN Column several times to completely resuspend the gel matrix.
2. Remove the cap and break off the tip. Place the column into one of the 2-ml polypropylene tubes provided.
3. Centrifuge the column for 5 min at 700 x g in a swinging bucket rotor.
4. Remove the column from the tube and discard the buffer.

(PR14607; published 11 April 2001)

5. Place the tip of the column into a clean microcentrifuge tube.
6. Carefully apply the sample to the center of the gel bed's flat surface.
7. Centrifuge the column for 5 min at 700 x g (use a swinging bucket rotor).
8. The purified DNA solution is in the eluate. Store at –20°C.

C. Transforming chemically competent *E. coli* with Plasmid Isolated from Yeast

1. Prepare the chemically competent *E. coli* cells or thaw them on ice.
2. Add 10 µl of yeast plasmid solution to a prechilled 14-ml Falcon tube.
3. Add 100 µl of competent cells to the tube and mix well by gently tapping the tube.
4. Incubate on ice for 30 min.
5. Transfer the tube to a 42°C water bath and incubate for 45–50 sec.
6. Chill on ice for 2 min.
7. Add 1 ml of LB or (preferably) SOC medium with no antibiotic.
8. Incubate at 37°C for 1 hr with vigorous shaking (250 rpm).
9. Centrifuge at 2,500 rpm for 5 min in a table-top centrifuge.
10. Discard supernatant.
11. Resuspend pellet in 100 µl of the selection medium and spread on appropriate plates.
12. Incubate plates at 37°C for 24 hr (LB/amp selection only), or for 36–48 hr (for nutritional selection on M9 medium). Typically, 10–100 colonies will be seen on the plate for a successful transformation using plasmid isolated from yeast.
13. If you performed a parallel transformation using the control pUC19 DNA, calculate the transformation efficiency (i.e., cfu/µg DNA). The competent cells should have been transformed with an efficiency of $\geq 1 \times 10^7$ cfu/µg.