

Minipreparation (from “Nonconventional Yeasts in Biotechnology”, p.373)

This method is derived from Hoffman and Winston (1987).

1. Grow the strain in 10 ml YPD or YNB until stationary phase.
2. Spin and resuspend the pellet in 0.5 ml of water.
3. Transfer to an Eppendorf tube.
4. Spin 5s and pour off supernatant.
5. Spin 1s and resuspend the pellet in whatever supernatant is left.
6. Add 200 µl of the following mix:
 - 2 % Triton X-100
 - 1 % SDS
 - 100 mM NaCl
 - 10 mM Tris-HCl pH 8.0
 - 1 mM EDTA
7. Add 200 µl of phenol-chloroform and 0.3 g of glass beads (acid-washed, 45,um).
8. Vortex 3-4 min, then add 200 µl TE.
9. Spin 5 min, and save the aqueous phase in a new tube.
10. Add 1 ml 100% ethanol, mix by inverting the tube, spin 2 min, discard the supernatant. Do not dry at this step.
11. Resuspend the pellet in 400 µl of TE with 30 µg of RNase I (boiled).
12. Incubate for 5 min at 37°C.
13. Add 10 µl of 4 M ammonium acetate, then 1 ml of 100% ethanol. Mix by inverting the tube, spin 2 min, discard the supernatant.
14. Dry under vacuum, then resuspend in 50 µl of TE.
Use 5-10 µl for one digestion.

One should obtain about 2 µg of DNA; this is the method of choice for analyzing transformants by Southern hybridization. Bands up to 12 kb are detectable, higher ones might be sheared. Probably because of the presence of impurities, this method does not reproducibly yield transformants in *E. coli*.