Quick Yeast DNA Prep: Isolation of Total DNA (genomic and plasmid)

Linda Warfield, Hahn Lab 10/16/98

Grow a 5 ml YPD O/N culture inoculated with a single yeast colony at 30 deg.

Transfer culture to a small 13 x 100 glass tube. Spin down cells 2 min. in tabletop centrifuge. Pour off supernatant.

Wash cells with 5 ml H2O. Spin down cells 2 min. Pour off supernatant. Cell pellet can be stored at -20 deg.

Resuspend cells in 500 ul lysis buffer by vortexing.

Lysis buffer:	**20 ml:**
0.1M Tris pH 8.0	2 ml 1M Tris 8.0
50 mM EDTA	2 ml 0.5M EDTA
1% SDS	2 ml 10% SDS
14 ml H2O	

Add acid-washed glass beads (400-500 microns) to about 2 mm below meniscus. Vortex 30 sec. Add 25 ul 5M NaCl. Vortex 30 sec. Spin down 2 min. to decrease foam.

Remove lysed cells with P1000 at bottom of tube and transfer to a 1.5 ml microfuge tube.

Add 400 ul of TE-saturated phenol. Vortex. Spin 4 min. Transfer upper phase to a clean 1.5 ml microfuge tube (~400ul).

Add 400 ul phenol:chloroform (4:1). Vortex. Spin 4 min. Transfer upper phase to a clean 1.5 ml microfuge tube.

Add 1 ml 95% EtOH and mix. Spin 6 min. Pour off EtOH. Wash with 1 ml 70% EtOH. Vortex. Spin 6 min. Pour off EtOH. Dry pellet in hood or vacuum.

Resuspend in 50 ul TE (vortex, 37 deg. 10 min, vortex)