Retrieval of Shuttle Plasmids from Yeast


1. Inoculate 4 mls of minimal selective medium in 15 ml round-bottom plastic tube with a colony from a selective plate. Grow overnight on drum rotator at appropriate temperature.

2. Spin for 5 minutes at 2000 rpm in IEC centrifuge. Remove all supernatant. Unless otherwise indicated, all steps are done at room temperature.

3. Resuspend cell pellet in 1 ml ddH$_2$O. Transfer to microfuge tube. Spin 15 seconds at top speed.

4. Resuspend cell pellet in 200 µl of TENS buffer: 100 mM NaCl, 10 mM Tris HCl, pH 8, 1 mM EDTA, 0.1% SDS

5. Quickly transfer cells to 2.0 ml tube with 0.25 g glass beads. Vortex vigorously for 1 minute.

6. Add 200 µl of phenol. Vortex vigorously for 1 minute.

7. Spin in microfuge at top speed for 5 minutes. Transfer ~250 µl to fresh microfuge tube. Do not transfer any of the white interface; only the clear aqueous layer.

8. Extract aqueous layer with an equal volume of phenol:CHCl$_3$ as described above. Transfer ~250 µl to a fresh tube.

9. Add 1/10 volume of 3 M NaOAc, pH 5. Vortex. Add 2 volumes 100% EtOH. Hold at -20°C for 1 hour.

10. Spin in microfuge at 4°C for 20 minutes at top speed. Wash pellet with 70% EtOH. Spin again for 5 minutes. Dry pellet in Speed-Vac. Resuspend in 10 µl sterile ddH$_2$O.

11. Use 1-3 µl to transform electrocompetent E. coli (DH5α, HB101).

If transformation efficiency is low, clean up DNA with Silica method, which can be used to replace steps 9 and 10 above.

Silica Purification
A. Resuspend DNA in 250 µl. Add 250 µl of 6 M NaI. Mix at room temperature.
B. Add 1-2 µl of silica slurry. Place on rotator at 4°C for 15-30 minutes.
C. Continue with purification according to gel isolation method.
D. Repeat step 11 above.