KEN IZUMI : Extraction of DNA from YEAST



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Dear Erle,
Thank you the pleasant greetings. I hope you and Erin had a wonderful winter holiday. Below is the information about yeast DNA to E. coli transfers.

Yeast DNA transformation transfer to E. coli

1. Replicate yeast in selective media (10 cc)
2. Pellet 3000 rpm, 5 min. Aspirate off media.
3. Transfer with water to microcentrifuge tube (2.0 ml) which has ~300 microliters glass beads (425-600 micron) in it already.
4. Pellet briefly in micrcentrifuge. Remove water. Wash again with water. Pellet.
5. Add 0.5 ml 10mM Tris 8.0, 1.0 mM EDTA, 0.25% SDS. Vortex 3' (make sure yeast are suspended before starting time. Brief pelleting in the microcentrifuge avoids packing).
6. Add 0.5 ml buffer adjusted phenol. Vortex 1' and microcentrifuge 2'.
7. Remove bottom organic layer. Don't mess with the interface.
8. Phenol extract 1-2 times, choroform extract.
9. Add NaCl to adjust to 0.1M. Add 2 volumes ethanol (22 C)
10. Microcentrifuge 30 min. If you have the horizontal Eppendorf microcentrifuge rotor, use that one instead of the fixed angle.
11. Remove ethanol. Wash pellet with 70-75% ethanol. Pellet again 10'.
12. Remove ethanol. Warm room dry or gently speed vac.
13. Resuspend in 10 microliters TE pH8.0

Frozen competent XL-1 blue. (Pre-chill sterile water and sterile 10% glycerol in water).
1. Inoculate 15 ml SOC with XL-1 Blue. Replicate overnight at 37 C shaking
2. Inoculate large culture of SOC (pre-warm) (1 liter e.g.) with overnight. Start at about 0.1 OD(600 nm). Shake at 37 C to 0.6 OD.
3. Pellet cells 10 min, 5000 rpm at 4 C.
4. Wash with large volume of chilled water. Pellet again. Aspirate off water.
5. Wash again with chilled water. Pellet again.
6. Wash twice with 10% glycerol.
7. Final resuspension is 1/500 of culture volume.
8. Pipet into microtubes in 0.5 ml aliquots. Flash freeze in dry ice/ethanol bath.

DNA transformations.
1. In a cold eppendorf tube, mix 50 microliters of XL-1 blue (thaw on ice) with 1 microliter of DNA. More DNA is not better (may get electric arcs).
2. Mix by pipetting then transfer quickly to 0.1 mm gap cuvette (chilled on ice). Make sure bacteria go to the bottom. (Gently fling the cuvette to settle the bacteria at the bottom if they don't pipet in).
3. Electroporate 1500 volts, 50 microFarad, 500 ohms resistance. Dry off the cuvette before inserting into rig!
4. No arcing! Use 1 ml SOC to resuspend bacteria in cuvette. Transfer to 17X95 culture tube.
5. Recover at 37 C for 1-2 hr.
6. Spread on selective media plates (250, 100, 50 microliters per plate). Incubate at 37 C.

I hope this makes your experiments work well. I'll talk with you later.
Sincere regards,
Ken I