Smash and Grab

NOTE 1: A harsh treatment of yeast that yields plasmid DNA and sheared genomic DNA. This protocol is used to release plasmids from yeast, which are then transformed into bacteria for amplification. Usually done to rescue library plasmids and determine if the phenotype under study is plasmid linked (the purity and yields are low, so it's not much use for anything else).

SAFETY: When working with phenol always make sure lids are fully closed on the ependorffs you're vortexing – phenol burns are not pretty ….

1. Grow a 2mL culture of yeast overnight in media that selects for the plasmid of interest.
2. Transfer 1.5mL to a microcentrifuge tube and flash spin (up to 10K) to collect the cell pellet (should be ≈ 150µl pcv (packed cell volume))
3. Aspirate supernatant. Resuspend cells in 200µL breaking buffer.
4. Add an equal volume of small acid washed glass beads (Sigma G8772; gently pour in beads until they almost reach the meniscus of the yeast-buffer solution).
5. Add 200µL of 25:24:1 phenol / chloroform / isoamyl alcohol (PCI).
6. Vortex at highest speed for 2 minutes (one min vortex, one min ice, repeat).
7. Spin in microfuge (12K, 5m, RT°).
8. Remove 200µl supernatant to a new tube. Add 20µL 3M sodium acetate (pH 5.2) and 500µL ethanol (from –20°C). Spin 12K, 10m, RT° to precipitate DNA. Aspirate supernatant.
9. Resuspend pellet in 50µL TE. Use 2µL to transform *E.coli* (heat shock or electroporation).

NOTE 2: We use a relatively high competence E.coli (e.g Bioline α-select Silver (BIO-85026), $>10^8$ cfu/µg pUC19 DNA) for the transformation step – the yields are not great so don't expect a lawn of colonies.

Solutions

Breaking Buffer (store RT°)
2% triton X-100
1% SDS
100mM NaCl
10mM Tris-Cl, pH8.0
1mM EDTA

PCI (50mL) (**BE CAREFUL WITH THIS**)
25mL phenol
24mL chloroform
1mL isoamyl alcohol
(Store 4°C)