Copy code 6845

Bloom Lab Protocol
**Rapid DNA Isolation from Yeast**

This is a protocol to rapidly prepare total DNA and RNA from yeast cells. The DNA produced is suitable for PCR or plasmid rescue from yeast. The yield of genomic or plasmid DNA has not been established, and will likely vary depending on culture density and health. Use of the RNA generated in this protocol has not been investigated. The method uses glass beads to mechanically break open the cells during vortexing, and phenol:chloroform to denature proteins.

1. Grow 3-5ml overnight culture in appropriate media. Use selective media to maintain plasmids or YPD when able.
2. Transfer 1.5ml culture into eppendorf tubes.
3. Centrifuge ~10 seconds at max speed, RT (room temperature) to pellet cells
4. Decant supernatant and resuspend in 400ul **Yeast Lysis Buffer**
5. Add 300 mg (~400 ul) **Glass Beads**
6. Add 200ul Phenol:CHISMA (50:49:1 with Isoamyl alcohol) (In fridge) take from bottom piece (organic phase)
7. Shake 5 min in a multi-tube shaker
8. Centrifuge 5 min maximum speed, RT. When complete be cautious not to disturb white interphase.
9. Transfer 300 ul of aqueous (upper) layer to a fresh eppendorf tube without disturbing interphase.
10. ~~Add 300 ul Isopropanol (1 volume if 300 ul was not extracted above)~~ Add 2x volume ethanol (100%)
11. Invert to mix, let stand 2 min at room temperature to precipitate DNA
12. Centrifuge 10 min at maximum speed, RT
13. (optional) Wash pellet with 70% ethanol (-20c)
14. Resuspend in 20-~~100 ul~~ of water or preferred buffer. Typically 1-2ul of a 100ul resuspension will act as a template in a 100 ul PCR reaction.

→ add RNase
→ add equal volume chisma, shake 5m centrifuge 5min and remove top layer

decant, tip upside down on tissue rack for 10 min in 37°C oven

Yeast Lysis Buffer

| Final Concentration | For 100ml | | |
|---|---|---|---|
| 2% Triton X-100 | 2ml 100% Triton | 20 of 10% | 20 |
| 1% SDS | 5ml 20% SDS | 10 ml 10% | 10 |
| 100mM NaCl | 2ml 5M NaCl | | 2 |
| 10mM Tris, pH 8.0 | 1ml 1M Tris, pH 8.0 | | 1 |
| 1 mM EDTA | 0.2ml 0.5M EDTA | | 0.2 |
| | 90ml Water | 85ml $H_2O$ | 77ml $H_2O$ 33.2 |

77w

Glass Beads

Use 425-600 micron glass beads (Sigma G-8772). For this protocol, Acid Washed beads are NOT required. We have fashioned a glass bead scoop from a needle and a 1.7ml Eppendorf tube. Using a hot razor blade (please be careful here!!) cut the bottom off of the eppendorf tube at about the 500ul mark. This will be the "scoop". Heat the tip of large bore needle (18Ga 1.5 inch) and punch it through the scoop near the top to form a handle (Again please use caution if not common sense in stabbing something with a hot needle!!). The tip of the needle should just pass through one wall of the scoop, but remain embedded in the other so that the scoop is rigidly fastened to the needle and does not swing freely. Mark the scoop at the level where 300mg of beads (or 400ul of water) fills the scoop.

use Isopropanol @ step 10 for plasmid extraction →