

Yeast protein extraction with bead beating

Gabe Zentner

Modified from a protocol from the Biggins Lab (FHCRC)

-Make a scoop for beads by cutting the bottom off of a 0.5 mL PCR tube about halfway down the conical portion. Briefly place the point of a P1000 tip in the flame of a Bunsen burner and press the melted tip onto the tube portion

1. Inoculate 5 mL YPD or SC with a single colony and grow overnight
2. In the morning, subculture 50 μ L of the saturated overnight culture into 2 mL medium and grow for 3-4 hr
3. Harvest 1.5 mL cells in a microfuge tube. Flash spin to pellet cells and remove medium
4. Resuspend cells in 50 μ L 2X SDS sample buffer
5. Add one scoop of glass beads (425-600 μ m diameter, Sigma G8772)
6. Bead beat for 3 min using a bead beater or vortex at 4°C
7. Flash spin to collect lysate and beads at the bottom of tube
8. Heat lysate at 100°C for 5 min and load gel with 10-15 μ L