Glass bead lysis of whole yeast cells

For preparation of 5 OD_{600} units of whole-cell extract at a final concentration of 0.5 OD_{600} units per 20 µL of SDS-PAGE reducing sample buffer.

1. Dilute an overnight culture of yeast cells to 0.2 ODU/mL in 10 mL medium.
2. When the culture reaches mid-logarithmic stage (0.5 – 0.8 ODU/mL), centrifuge 5 ODU of cells at ~5,000 x g for 5 min at room temperature (RT).
3. Decant supernatant, resuspend cell pellet in 1 mL sterile water, and transfer entire volume to eppi tube.
4. Add 100 µL of 100% trichloroacetic acid (TCA) and vortex immediately.
5. Incubate on ice ≥ 20 min.
6. Centrifuge at full speed for 5 min at 4˚C.
7. Aspirate supernatant; keep pellets on ice.
8. Add 1 mL ice-cold acetone.
9. Resuspend pellet completely using a water bath sonicator.
10. Repeat steps 5 – 9.
11. Incubate on ice ≥ 20 min.
12. Centrifuge at full speed for 5 min at 4˚C.
13. Aspirate supernatant.
14. Dry pellets completely by speed-vac for 15 min (heat setting “high”).
15. Add 200 µL of 1X SDS-PAGE sample buffer + 0.2% β-mercaptoethanol
16. Add 100 µL of sterile glass beads.
17. Secure eppi’s with lid-locks.
18. Vortex 15 min.
19. Heat at 90 – 100˚C for 5 min.
20. Pulse-spin at RT to pellet beads and cellular debris.
21. Load 20 µL (equivalent to 0.5 OD_{600} units) onto an SDS-polyacrylamide gel.