Hoffman Winston DNA Prep

This protocol is the classic Hoffman Winston DNA preparation using bead bashing with amendments based on the Dunham Lab.

All spins are max speed in the microfuge.

Make a master mix of 400µL TE + 30µg RnaseA (from 100µg/µL stock in 4C room) based on the number of samples.

Be careful using phenol.

1. Grow an overnight culture in 5 ml YPD.
2. Spin 2 x 1.5mL to pellet in microfuge for 2 minutes. Decant supernatant. (Want to collect around 3 to 10 e8 cells)
3. Resuspend pellet in 500 µl water and transfer to a 2.0 mL tube. Or if desired you can store the cells in a Sorbitol solution and store at -80C.
4. Spin 2 minutes to pellet. Remove the supernatant, leaving just enough to resuspend the pellet completely.
5. Working in the fume hood, add to resuspended pellet:
   a. 200µl lysis buffer (recipe below)
   b. 200µl 25:24:1 phenol/chloroform/isoamyl alcohol (ensure that it is the buffered phenol).
6. Add mixture to 300mg acid-washed glass beads in a 1.5 ml Tube.
7. Vortex 8 minutes in the fume hood.
8. Touch spin in a low speed minifuge to get the phenol off the lid.
9. Add 200µl TE. Invert to mix.
10. Spin 5 min to separate aqueous and organic layers.
11. Transfer aqueous (top) layer to a new tube.
12. Add 1mL room temp 100% ethanol. Invert to mix.
13. Spin 2min. You should see a white pellet.
14. Remove supernatant and add 400µl TE+30 µg RnaseA.
15. Resuspend pellet using pipette tip and vortexing.
16. Incubate 30 minutes at 37C
17. Add 10µl 4M ammonium acetate and 1mL room temp 100% ethanol. Invert to mix.
18. Spin 2 min.
19. Remove supernatant completely and dry pellet by leaving the tube inverted on a kimwipe on the bench for 30 min.
20. Resuspend in 50µl Nuclease-Free H2O or TE.
21. Measure DNA concentration using a fluorometer.

Total yield should be 10-20µg.

**Lysis buffer**

- 2% Triton X-100
- 1% SDS
- 100 mM NaCl
- 10 mM Tris pH 8
- 1 mM EDTA

**Sorbitol Solution**

- 0.9 M Sorbitol
- 100 mM Tris pH 8
- 100 mM EDTA