Yeast Genomic DNA Prep

Reagents:

Sterile distilled water
β-mercaptoethanol
Sorbitol Buffer: 1 M sorbitol, 0.1 M sodium citrate (pH 5.8), 0.01 M EDTA (pH 8.0)
Saline-EDTA Buffer: 0.15 M NaCl, 0.1 M EDTA (pH 8.0), 0.1% SDS
Lyticase (or T100 Zymolase)
95% Ethanol
70% Ethanol
10 mg/ml Rnase
phenol
TE

Protocol:

1. Grow 25 ml of yeast cells in YPD to $5x10^7$ cells/ml.
2. Spin down cells in 50-ml conical tube at 3,000 rpms for 5 minutes.
3. Resuspend in 10 ml of sterile distilled (SD) water, then spin for 5 minutes at 3,000 rpms and decant off water.
4. Resuspend in 5 ml of SD water and add 100 μl of β-mercaptoethanol.
5. Incubate at room temperature for 15 minutes.
6. Spin at 3,000 rpms for 5 minutes then pour off supernatant.
7. Resuspend in 5 ml of sorbitol buffer
8. Add ~1.0 mg of Lyticase (or 0.25 mg Zymolase).
9. Incubate at 37°C for 1 hour (Check efficiency of spheroplasting after 30 minutes and if less than 80% incubate another 30 minutes).
10. Spin down spheroplasts for 3 minutes at 3,000 rpms and remove supernatant.

11. Resuspend in 1.2 ml Saline-EDTA Buffer (divide sample into two tubes).
12. phenol extract 3 times (make sure to avoid interface)
13. Ethanol precipitate (no salt)-allow to sit at -20°C for 15-60 minutes.
14. Spin for 30 minutes at maximum speed at 4°C.
15. Wash with 70% Ethanol and dry pellet.
16. Resuspend pellet in 0.5 ml TE.
17. Add 1μl of 10mg/ml RNase A and incubate at 37°C for 1 hour.
18. phenol:Chloroform sample.
19. Chloroform sample.
20. Ethanol precipitate – adding 1/10 the volume of 3 M sodium acetate and 2.5X the volume of ethanol. Place at -20°C for 1 hour.
21. Spin for 30 minutes at maximum speed at 4°C.
22. Wash with 70% ethanol and dry pellet.
23. Resuspend in 100 μl of water and determine DNA concentration.
24. Use 20 μg (12-20 μg) of Genomic DNA in Southern blot analysis.