Yeast DNA isolation (40ml)

1. Grow 40 ml of yeast cells in 250 ml flask
2. Harvest at 4000 RPM for 5 min
3. Wash once with sterile water
4. Resuspend in 3 ml of 0.9M sorbitol and 0.1 M EDTA pH 7.0)
5. Add 0.1 ml of 2.5 mg/ml zymolase 100T
6. Incubate at 30^0C for 1 hr
7. Harvest the spheroplast
8. Resuspend in 5 ml of 50 mM Tris and 20 mM EDTA (pH 7.0)
9. Add 0.5 ml of 10% SDS
10. Incubate at 60^0C for 30 min
11. Add 0.5 ml of 5M potassium acetate and store on ice for 1 hr
12. Centrifuge at 10,000 RPM for 10 min.
13. Transfer the supernatant to a fresh tube and add two volumes of 90% ethanol at RT
14. Centrifuge at 6000 RPM for 15 min and discard the supernatant
15. Dry the pellet and add re-suspend in 3 ml of TE. This may take few hrs.
16. Centrifuge to remove any remaining debris (10,000 RPM for 15 min) and transfer the supernatant to another tube.
17. Add 0.150 ml of 1 mg/ml DNAse free RNase and incubate for 30 min at 37^0C
18. Add one volume of 100 % isopropanol and shake gently to mix.
19. Remove the DNA, which look like a cocoon. (if you dot see a good cocoon, centrifuge 5000 RPM for 10 min, but was DNA with 70% ethanol twise)
20. Resuspend the DNA in TE and use it as you wish !!