Yeast Genomic DNA Preparation from Spheroplasts

1. Resuspend with 50 mM Tris, 25 mM EDTA (pH 8) in 10X the spheroplast pellet volume.

2. Immediately add 1/10 volume of 10% SDS. Mix.

3. Incubate 30 minutes at 65°C. Place tubes on ice 5 minutes.


5. Hold tubes on ice for 30-60 minutes. Transfer to two 30 ml Corex centrifuge tubes.

6. Centrifuge 30 minutes in SS-34 rotor at 10,000 rpm at 4°C.

7. Transfer supernatant to fresh 30 ml Corex tubes. Add equal volume of phenol:CHCl₃. Mix. Centrifuge 10 minutes in SS-34 rotor at 10,000 rpm at 4°C.

8. Transfer supernatant to fresh 30 ml Corex tubes. Measure volume. Add 2 volumes of 100% ethanol. Mix gently. DNA may precipitate immediately. Precipitate on ice for 1 hour.

9. Centrifuge 10 minutes in SS-34 rotor at 10,000 rpm at 4°C. Discard supernatant.

10. Wash with 70% ethanol. Gently break up pellets. Wash for at least 5 minutes at 25°C.

11. Centrifuge 10 minutes in SS-34 rotor at 10,000 rpm at 4°C. Discard supernatant.

12. Drain and dry pellets on bench. Dissolve each pellet completely in 2 mls TE (pH 8).

13. Add 20 µl 10 mg/ml RNase A (heat treated; DNase-free) to each tube. Incubate for 30 minutes at 37°C. The digestion may be stored overnight at 4°C at this point.

14. Centrifuge 5 minutes in SS-34 rotor at 10,000 rpm at 4°C. Transfer supernatant to a fresh 30 ml Corex tubes.

15. Add 1/4 volume 10M NH₄OAc. Mix completely. Add 2 volumes 100% EtOH. Precipitate on ice.

16. Centrifuge 10 minutes in SS-34 rotor at 10,000 rpm at 4°C. Discard supernatant.

17. Wash with 70% EtOH (several mls). Disperse pellets. Centrifuge 10 minutes in SS-34 rotor at 10,000 rpm at 4°C. Discard supernatant. Drain and dry pellets on bench.

18. Resuspend in total volume of 1 ml TE, pH 8. Store gDNA solution at 4°C. Do not freeze.

Run 1 µl of uncut gDNA on gel. Digest 5 µl for minigel and Southern blot. Use 1 µl for PCR.