Genomic DNA Extraction S. cerevisiae and S. pombe

Protocol:

1. Grow a 10ml overnight culture, spot onto YPD to check markers
2. Pellet cells, decant supernatant and resuspend in 0.5ml water
3. Transfer to a 1.7ml micro-centrifuge tube
4. Pellet 5 seconds and decant supernatant
5. Briefly vortex pellet to resuspend in remaining medium
6. Add: 0.2ml Smash and Grab Solution
   0.2ml Phenol-Chloroform
   0.3grams Acid-washed glass beads
7. Vortex for 2-5 minutes using the multi-tube holder or 10 minutes on multi-tube vortexer in the cold room
8. Add 0.2ml of TE to each tube
9. Spin 5 minutes in centrifuge
10. Transfer aqeous phase to new tube
11. Add 1.0ml EtOH and mix to ethanol precipitate
12. Spin down 5 minutes, decant supernatant
13. Resuspend pellet in 0.4ml TE
14. Add 30µg RNase and incubate for 5 minutes at 370
15. Add 10µl 4M ammonium acetate and 1.0ml EtOH
16. Ethanol precipitate on ice for 5 minutes
17. Spin down 10 minutes
18. Optional wash with 0.5ml 70% EtOH
19. Air dry pellet and resuspend in 100µl TE

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Smash and Grab Solution (100mL)

10 mL 10% SDS *(1%)*
2 mL 100x Triton-X100 *(2%)*
2 mL 5M NaCL *(100mM)*
1 mL 1M Tris pH 8.0 *(10mM)*
200 □L 0.5M EDTA pH 8.0 *(1mM)*

Mix in beaker, adding the detergents while the solution is mixing. Filter sterilize.

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