DNA Extraction for Southern Blot (Meiotic Recombination Intermediates)

1. Fix 10 ml cells in 70% EtOH at −20° C (can do 14 ml at OD ~1.2).
2. Wash once with spheroblast buffer (1 M sorbitol, 10mM $NaPO_4$ pH 7.0, 50 mM EDTA).
3. Resuspend in 0.5 ml spheroblast buffer + 3 µl β-mercaptoethanol + 0.5 µl zymolyase (100T 10 mg/ml).
4. 15 min at 37° C (or longer until spheroblasted — check with half-half 2% SDS on slide).
5. Spin 3 min at 4000 rpm.
6. Resuspend in 0.5 ml lyse solution (50 mM EDTA, 0.3% SDS) + 5 µl protK (20 mg/ml).
7. 30 min at 65° C.
8. Put on ice.
9. Add 0.2 ml 5 M KAc and invert several times.
10. 20 min on ice.
11. Centrifuge to remove cell debris.
12. 3 x phenol chloroform extraction (can rock ~30 min rather than vortex).
13. 1 x chloroform extraction (original protocol used ether — can use rocker here too).
14. 2 x EtOH precipitation (I add 1/10 x 3M NaOHAc (pH 5.2), mix then 2 x EtOH, 30 min at −20° C).
15. Resuspend (can use ~40 µl 10 mM TrisHCl) and store at 4° C.
16. Run 25% total on gel after digest (a lot, but looking for really rare events).