Quick yeast DNA preparation

1. Start with either a 10ml selective media or < 5ml YES saturated culture, or scrape cells off a plate.

2. Pellet. Resuspend in 1ml SCE, transfer to microfuge tube.

3. Pellet 20 sec. Resuspend in 150ul enzyme mix:-

   Mix = 1ml SCE
   8ul BMEtOH
   60ul 10mg/ml zymolyase 5000 (in 10% glycerol, 100mM NaCl)

4. Incubate with occasional shaking 37^{o}C 1 hour. Check spheroplasts if necessary.

5. Add 150ul of 10% SDS
   100mM Tris 9.0
   10mM EDTA
   Mix and vortex briefly.

6. Heat shock 65^{o}C 5 min.

7. Add 150ul 5M KAc (no need to pH). Vortex briefly. Leave on ice 1 hour.

8. Spin 10 min 4^{o}C. Remove 0.3ml supernatant and discard pellet.

9. Add 200ul 5M NH_4Ac
   1ml isopropanol
   and leave -20^{o}C 10 min.

10. Spin RT 3 min. Pour off supernatant (doing thorough job), and without extensive drying redissolve in 90ul H_2O. When dissolved, add 10ul 5M NH_4Ac, 200ul isopropanol. Mix...a fibrous DNA ptt will form. Let it sink to the bottom of the tube, where it will stick.

11. Pour off all the remaining liquid. Wash pellet with 1ml 80% EtOH, dry, dissolve in 50ul TE.

12. When dissolved, spin 2 min, transfer supernatant to a fresh tube (leaving any crud behind).

Yields about 10ug DNA, enriched for 2u and ribosomal DNA? This DNA contains > 70% plasmid DNA and is readily cleaved by enzymes. Use 5ul for genomic Southerns. 5ul of this is enough to rescue even cosmids into $CaCl_2$-competent E.coli directly.