DNA_6v1

Large scale genomic DNA preparation from S. pombe
w/ QIAGEN Genomic –tip100/G

(By Sigurd Braun. Original protocol from **Nurse's manual** with modifications)

1. Grow 100 ml cells in YE to OD595 of 2-3 (late stationary phase) with shaking at 25-35°C.
2. Spin down at 3000 rpm for 5 minutes and resuspend in 5ml of:
   50mM Citrate/ Phosphate pH 5.6 (0.71 g/100 mL Na2HPO4, 1.15 g/100 mL citric acid)
   40mM EDTA pH 8.0 (8ml of 0.5 M stock/100 mL)
   1.2M Sorbitol (21.9 g/100 mL)
3. Add 15 (5) mg Zymolyase-20T (100T) and incubate at 37°C for 30-60 (90) minutes.
4. Check digestion of cell walls using a phase contrast microscope on a 10 µl sample to which 1 µl of 10 % SDS has been added (the cells loose their characteristic refringence and become black).
5. Spin down at 3000 rpm for 5 minutes.
6. Resuspend in 15 ml of 5x TE (50 mM Tris-HCl pH7.5, 5mM EDTA). Add 1.5 ml 10% SDS, mix well. Recheck the lysis (if necessary cells can be incubated at 65°C for 5 minutes).
7. Add 5ml of 5M potassium acetate and keep on ice for 30 minutes. Centrifuge at 5000 rpm for 15 minutes. Pass the supernatant through a gauze and add 20 ml ice-cold isopropanol and leave for 5 minutes at -20°C.
   (Instead of filtering supernatant you may do a second spin at 5000 rpm for 15 min).
8. Centrifuge at 10000 rpm for 10 minutes, drain well and dry the pellet.
9. Resuspend in 3 ml 5 x TE and add RNAse to a final concentration of 20 µg/ml and incubate for 2 hours at 37°C.
10. Add 50 µl of ProteinaseK (20 mg/ml) and incubate for 30-60 min at 55ºC or o/n.
11. Do phenol/chloroform extraction by adding sequentially each 3 ml of phenol and chloroform, respetively. Mix well and transfer to phase-lock tubes. For each extraction step: Spin at 10000 rpm for 5-10 minutes.
12. Equilibrate QIAGEN genomic-tip 100/G with 4 ml of buffer QBT. Apply aqueous phase from previous step. Wash w/ 2x 7.5 ml buffer QC. Elute with 5 ml of buffer QF.
13. Transfer the upper aqueous phase to a 15 (30) ml Corex tube (transfer step can be omitted when DNA is recovered by spooling). Add 3.5 ml (0.7 volumes) of room-temperature isopropanol. Recover the precipitated DNA after inverting 10-20x either by
    13a) spooling the DNA using a glass rod or by
    13b) centrifuging at >5000x g for at least 15 min at 4ºC. Wash the pellet with 5 ml of cold 70% ethanol and dry under vacuum.
14. Finally, resuspend the DNA in 0.2 ml of TE and dissolve the DNA overnight on a shaker or at 55ºC for 1-2 hours. Read the OD260/280. A ratio of 1.6 indicates pure RNA, 1.8 pure DNA. The DNA can be quantified by running an aliquot on a gel alongside a previously determined sample.

1 haploid cell contain 14000 kb size genome = 1.53 x 10-14 g of DNA. Typical yields are about 20 µg per starting culture of 100 ml at stationary phase. This DNA can be used for restriction digestion and Southern blotting. For molecular cloning such as library construction the DNA should be further purified by CsCl centrifugation.