S.pombe - genomic DNA extraction

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NOTE 1: Also see Keogh lab protocol for the extraction of Genomic DNA from *S.cerevisiae*: similar, but also contains downstream analysis protocols.

SAFETY: Always take extreme care working with phenol. Ensure lids are fully closed on the tubes you are vortexing – **phenol burns are not pretty**.

1. Grow 10 ml culture to saturation (or even easier, collect cells from a freshly sectored plate). Harvest by centrifugation (pellet volume 30 – 50µl). Remove supernatant, resuspend cells in 0.5 ml ddH_2O and transfer to eppendorf. Flash spin (5 seconds at 12K RPM) to collect pellet and discard supernatant.

2. Add 200µl Breaking Buffer (2% triton X-100, 1% SDS, 100mM NaCl, 10mM Tris-HCl [pH 8.0], 1mM EDTA). Add 200µl phenol:chloroform:isoamyl alcohol, pH 8.0 (PCI; 25:24:1). Add acid-washed glass beads to the level of the meniscus of the solution (about 200-250µl).

3. Vortex 2 minutes (1 min vortex, 1 min on ice; ≈ 90% cell breakage).

5. Microfuge 6 minutes at 12K rpm (or Max speed). Transfer upper, aqueous layer (about 130µl) to fresh tube with 200µl Chloroform. Vortex 1 minute and transfer upper, aqueous layer (about 80µl) to fresh tube with 500µl 100% EtOH (-20°C).

6. Incubate ≥1hr (-20°C) and microfuge 10 min /12K / RT°C. Wash pellet with 70% EtOH and spin 10 min /12K / RT°C.

7. Air-dry 5 min RT°C. Resuspend in 30µl TE (+ 1µl 10µg/ml RNAseA). Incubate 30 min / 37°C. Use 3µl DNA / PCR reaction.

Preparing Chromosomal DNA from *S. pombe* (Method II)

(*for Southerns, PCR, etc. from Nurse Fission Yeast Handbook*)

1. Grow 10 ml culture to saturation. Harvest by centrifugation. Remove supernatant and resuspend cells in 0.5 ml dH2O. Transfer cells to screw-cap micro-centrifuge tube.
2. Spin 5 seconds in microfuge, decant sup, briefly vortex tube to resuspend pellet in residual liquid.
3. Add 0.2 ml of (2% triton X-100, 1% SDS, 100mM NaCl, 10mM Tris-HCl [pH 8.0], 1mM EDTA). Add 0.2 ml PCI (25:24:1). Add acid-washed glass beads to the level of the meniscus of the solution.
4. Vortex 5 minutes, or longer, until 90% cells are broken.
5. Microfuge 5 minutes. Transfer upper, aqueous layer to fresh tube. Add 1/10 vol 3M NaOAc, pH 5.2, and 2.5 vol 100% ethanol. Microfuge for 5 min.
6. Wash pellet once with 70% ethanol (-20°C), spin again.
7. Air dry. Resuspend in 20-30µl TE (+ RnaseA, or add during subsequent digest). Yield 10-20 µg DNA.