

Glass Bead DNA Preps of Time Course Samples

1. After the culture has reached between 5×10^6 and 1×10^7 cells/ml, spin down 50 mls of cells in a table top centrifuge. Use the 50 ml disposable conical polypropylene centrifuge tubes. Resuspend the cells in 400 μ l of extraction buffer.
2. Mix 500 μ l of glass beads with 400 μ l of phenol and the yeast cells suspended in extraction buffer. The glass/bead/phenol mixture may be prepared beforehand and stored at 4° or on ice in the dark (spin briefly in the microfuge to get the phenol to the bottom). See standard manuals for preparation of phenol.
3. Vortex tube for 5 min. on high with occasional manual shaking.
4. Let sit on ice for 1 min. or longer and spin in the microfuge for 10-15 minutes. All the samples from the various time points may be stored on ice and then spun all at once at the end. Allow the samples to sit on ice for 1 min. before spinning to improve phase separation.
5. Carefully remove the top aqueous layer to a new tube, avoiding the interface. Add 400 μ l of phenol to the new tube. Mix. Leave on ice 1-2 mins., centrifuge samples (10-15 mins.) and extract the aqueous layer.
6. Add 25 μ l of 5M NaCl and 600 μ l isopropanol. Mix.
7. Spin in microfuge for 10 min. Discard supernatant. Do not dry the pellet.
8. Add 300 μ l TE containing 10 μ g of RNase to each tube. Incubate at 37° for 30-60 minutes with occasional, minimal vortexing to resuspend pellet. RNA is mostly digested when the pellet dissolves.

9. Add 30 μ l of 5M NaCl and 300 μ l of isopropanol. Spin 10 mins. The DNA pellet may be small. Discard supernatant. Rinse with 95% (or 70%) ethanol. Vacuum dry. Resuspend in TE (200 μ l for each 50 mls of cells) and digest 20% of the sample for use on Southern blots (usually 40 μ l DNA in 200 μ l total will work).

Comments:

For time course samples, it is really only necessary to use one set of polypropylene tubes for the entire time course, while rinsing out the tubes with dH₂O in between time points. Sterility is not critical at this stage and cross contamination is negligible.

If preparing samples from cultures starting at different densities, proportionately adjust the volumes of the DNA samples to compensate for their starting cell densities. It is only an approximate way to equalize DNA quantities among strains, but it seems to work in most cases. An alternative is to run samples on a gel with calibration controls and quantitate the DNA using a densitometer/scanner.

The RNase step can be omitted and carried out at a later step.

Glass beads: Acid washed (425 to 600 microns from Sigma).

Extraction Buffer: 100 mM tris pH 8, 50 mM EDTA, 2% SDS

For the most recent version of this protocol see the Haber Lab web page:
<http://www.bio.brandeis.edu/haberlab/jehsite/protocol.html>.