Hoffman-Winston DNA prep for CGH

Annotated and modified by Maitreya Dunham and Cheryl Christianson (November 2005)

Before you start, make the lysis buffer and TE+RNase, weigh out the glass beads into individual servings, and label all your tubes. Process only the number of tubes you can fit in your vortexer at one time (for us that's batches of 8). Use phenol-resistant gloves.

Grow an overnight culture in 5 ml YPD (or ~10^8-10^9 cells however you like).
Spin to pellet. Decant super. Resuspend pellet in 500 μl water and transfer to a 1.5 ml screwtop or lidlock tube. This precaution is to limit the chance of the tube popping open and spraying phenol everywhere during the later vortex step.

If desired, the cells can be stored for later processing by resuspending the pellet in sorbitol solution (recipe below) instead of water and storing at –80C. Upon thawing, proceed as usual.

Spin to pellet again. Decant most of the super, leaving just enough to resuspend the pellet completely.
To resuspended pellet, add:

200 μl	lysis buffer (recipe below)
200 μl	25:24:1 phenol/chloroform/isoamyl alcohol (isoamyl alcohol optional)
300 mg	acid-washed glass beads (~500 micron size range)

Vortex 8 minutes. We've found this added vortexing increases the yield substantially without obviously shortening the DNA on a 1% gel. We use a small foam multi-tube attachment, ~4 inches in diameter. Do not use one of those funny rack vortexers or a large multitube attachment on a normal vortexer. You should make sure your setup actually vortexes the tubes adequately. If you get low yields, this is a key step to check.

Touch spin in a low speed minifuge to get the phenol off the lid.
Add 200 μl TE. Invert to mix.
Spin 5 min max speed in a microcentrifuge.
Carefully transfer aqueous (top) layer to a new tube without catching interphase junk.
Add 1 ml room temp 100% ethanol. Invert to mix.

Spin 2 min max speed.
Decant super and resuspend pellet in 400 μl TE+30 μg RNaseA. The pellet may not resuspend easily. As the incubation proceeds, you can usually get the whole thing to resuspend, though.
Incubate 30 minutes at 37C. We've lengthened this digestion from the original 5 min to reduce RNA contamination and to make sure the entire pellet gets into solution.
Add 10 μl 4 M ammonium acetate and 1 ml room temp 100% ethanol. Invert to mix.
Spin 2 min max speed.
Decant super completely and dry pellet. We leave the tube inverted on a kimwipe on the bench for about 10 min.
Resuspend in 50 μl TE.
Measure DNA concentration using a fluorometer or other DNA-specific method (i.e., NOT the spectrophotometer). Even with the RNase treatment and ammonium acetate precipitation, there's a lot of RNA contamination in these preps.
Total yield should be 10-20 μg. DNA should restriction digest easily.

Lysis buffer

We make this fresh each time for some unknown reason.

2% Triton X-100
1% SDS
100 mM NaCl
10 mM Tris pH 8
1 mM EDTA

Sorbitol Solution

0.9 M sorbitol
100 mM Tris pH 8
100 mM EDTA