Hoffman-Winston DNA prep for CGH

Annotated and modified by Maitreya Dunham and Cheryl Christianson in November 2005, and updated for the UW lab in 2010.

Make the TE+Rnase. You'll need 400ul TE plus 30ug Rnase per sample. Our stock solution of Rnase is 100mg/ml, and kept in a box on the chemical shelf in S410. Label all your tubes with an Ethanol proof marker. Process only the number of tubes you can fit in the Turbo mix vortexer attachment (that's batches of 12). Use the blue nitrile gloves, because they're more phenol-resistant, and plan to do everything up to the 37C incubation in the S403 hood.

Grow an overnight culture in 5 ml YPD (or ~10^8-10^9 cells however you like). Spin to pellet. Decant supernatant. 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. Remove the supernatant, 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. We have a tiny scoop that delivers this amount.

Vortex 8 minutes, with the hood closed. We've found this added vortexing increases the yield substantially without obviously shortening the DNA on a 1% gel. We use the TurboMix attachment for our Vortex genie 2's with good results. Be careful about what kind of attachment you use. 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. Dump the rest of the tubes contents into the Phenol Chloroform Waste bottle (there's a funnel too), and throw the tube in the tip disposal bucket.
Add 1 ml room temp 100% ethanol. Invert to mix.
Spin 2 min max speed. You should see a white pellet.

If this prep is only for pcr, you can stop here. Just resuspend the pellet in 50ul water. If you need a cleaner prep, proceed to the RNAse step.

Remove supernatant and add 400 μl TE+30 μg RnaseA. Vortex. The pellet may not resuspend easily, but go ahead and start the incubation.
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.
Remove supernatant completely and dry pellet. We leave the tube inverted on a kimwipe on the bench for 10-30 min.
Resuspend in 50 μl TE.
Measure DNA concentration using a fluorometer or other DNA-specific method (i.e., NOT the nanodrop). 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 keep a stock of this in the S403 hood, along with all the other supplies. If there isn't any, make more.

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

Sorbitol Solution
Plan ahead and make your own.

0.9 M sorbitol
100 mM Tris pH 8
100 mM EDTA