From O'Geen et al., BioTechniques 41(5), (November 2006)

Farnham Lab Whole Genome Amplification Protocol for ChIP-chip

(adapted from protocol provided with Sigma GenomePlex Kit)

**[Since the input material is sonicated chromatin, the initial series of fragmentation steps is skipped and one can go right to library preparation]

A. Library Preparation

1. Add 2 ul 1X Library Preparation Buffer to 10 ul of input material

[For the "input" sample, measure the concentration of reverse crosslinked, QIAquick purified DNA and add 10 ng to a total volume of 10 ul with H2O. For the ChIP sample, the concentration of nucleic acid is usually too low to get an accurate quantitation. Typically the entire 50ul of reverse crosslinked, QIAquick purified DNA is lyophilized and resuspended in 10 ul of H20]

Transfer samples to strip tubes or individual thin walled 0.2 ml PCR tubes

2. Add 1 ul Library Stabilization Solution, vortex or mix by pipetting. Quick spin and place at 95° for 2 minutes in thermal cycler

3. Immediately cool on ice, quick spin again

4. Add 1 ul Library Preparation Enzyme, vortex or mix by pipetting and quick spin if necessary

5. Incubate in thermal cycler as follows:
   16° for 20' (cycler should be precooled to this temperature)
   24° for 20'
   37° for 20'
   75° for 5'
   4° hold

9. Quick spin if necessary and either proceed to first amplification or freeze at –20° for up to three days

B. Amplification (round 1)

10. Prepare master mix for each sample containing:

7.5 ul of 10X Amplification Master Mix
47.5 ul Nuclease-free H20
5 ul WGA DNA polymerase

[For multiple samples, multiply above volumes by the number of samples then add 1/10 volume extra of each component]

11. Add 60 ul master mix to each sample, vortex or mix by pipetting and quick spin if necessary

12. Incubate in thermal cycler as follows:
    95° for 3' , then 14 cycles of
    94° for 15"
    65° for 5', then
    4° hold

At this point, amplified material is stable and can be stored at –20 ° indefinitely

13. Purify samples using QIAquick PCR cleanup columns or analogous product. It is important to elute the samples in water so that the subsequent labeling reactions are efficient.

[Since the amplified material contains both single- and double-stranded DNA that can be effectively labeled, the column purification method used should recover both.]

[At this stage, the purification column eluates for total and immunoprecipitated samples should be readily quantifiable by nanodrop, spectrometer, or dye intercalation, eg, picogreen (dye intercalation may underestimate amount due to single strand product). Optimally, total recovery for immunoprecipitated samples will be in the 1-4 ug range. This gives enough material for several labelings for downstream microarray analysis. If yields are less, or more product is desired, re-amplify material using Sigma GenomePlex WGA Reamplification Kit]

C. Reamplification (round 2)

1. Add 15 ng purified amplification product in 10 ul volume to strip tubes or individual thin walled 0.2 ml PCR tubes

[For input material start with the high concentration primary amplified stock]

2. Prepare master mix for each sample containing:

7.5 ul of 10X Amplification Master Mix
47.5 ul Nuclease-free H20
5 ul WGA DNA polymerase

For multiple samples, multiply above volumes by the number of samples then add 1/10 volume extra of each component

3. Add 60 ul master mix, vortex or mix by pipetting and quick spin if necessary

4. Incubate in thermal cycler as follows:
    95° for 3' , then 14 cycles of
    94° for 15"
    65° for 5', then
    4° hold

At this point, amplified material is stable and can be stored at –20 ° indefinitely

5. Purify samples using QIAquick PCR cleanup columns or analogous product.

[Since the amplified material contains both single- and double-stranded DNA that can be effectively labeled, the column purification method used should recover both.]