

# Integration of Sigma® GenomePlex® WGA with the NimbleGen™ Microarray ChIP-chip Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for genomic analysis on the NimbleGen™ platform, and can be readily integrated into existing NimbleGen workflows. NimbleGen recommends the use of GenomePlex WGA for amplification of immunoprecipitated genomic DNA for microarray applications ChIP on CHIP!<sup>1,2,3</sup>

- Prepare samples by following the **NimbleGen Sample Preparation Protocol for ChIP-chip, Step 7**. Amplify the experimental and control input DNA samples using the **Sigma GenomePlex Complete WGA 2 Kit (Cat. No. WGA2-50RXN)**, with additional amplification using **GenomePlex WGA 3** or **WGA 4** if necessary before proceeding to the NimbleGen labeling procedure.
- The GenomePlex WGA amplification product size ranges from ~200 to 1000 base pairs, averaging ~400 base pairs, making sonication unnecessary.
- Purify samples using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**).

## Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform a modified GenomePlex WGA amplification as described in the product bulletin for **GenomePlex WGA Kits**.

### Modification 1. Omit fragmentation steps 1-4.<sup>2,3</sup>

Omit

- Fragmentation

  1. Isolate DNA sample and quantify concentration by UV absorption (260 nm). Prepare DNA solution of 1 ng/μL
  2. Add 1 μL of 10× Fragmentation Buffer to 10 μL of DNA (1 ng/μL) sample in a PCR tube or multiwell strip/plate.
  3. Place the tube/plate in a thermal block or cycler at 95 °C for EXACTLY 4 minutes. Note, the incubation is very time sensitive. Any deviation may alter results.
  4. Immediately cool the sample on ice, then centrifuge briefly to consolidate the contents.

### **Modification 2.** If insufficient amplification product is generated, re-amplify with the **GenomePlex WGA Reamplification Kit**.<sup>2,3</sup>

2. Purify the amplification product using the GenElute PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

Note 1. Thirty microliters is the absolute minimum elution volume.

Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 μg, equivalent to the typical output of a **single** GenomePlex WGA amplification reaction.

3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
4. Fragmentation of GenomePlex WGA amplification product is unnecessary.

## Entry into NimbleGen™ Workflow

Enter NimbleGen Array User's Guide: ChIP-chip Analysis, v5.0, Chapter 2. "Sample Preparation & QC", pp 9-11.

We recommend using the GenElute™ PCR Cleanup kit (Cat.No NA1020).

### Sample Requirements

- High-quality experimental (IP) and control (input) DNA are required to obtain optimally labeled samples for array hybridization. The NimbleGen sample preparation protocol for ChIP-chip is available upon request from Roche Microarray Technical Support.
- Roche NimbleGen recommends starting with the following sample amounts for each hybridization. If your experimental (IP) sample quantity is less than the amount listed, amplify the experimental (IP) and control (input) samples using the Sigma GenomePlex Complete WGA 2 Kit (Catalog No. WGA2-50RXN) before labeling. Then purify samples using the Qiagen QIAquick PCR Purification Kit (Catalog No. 28106).

*Note: The success of the IP reaction depends on using antibodies validated with immunoprecipitation. Not all antibodies work well for this method.*

Starting Sample Amount Recommendations	385K Array	Each Sample for a 4x72K Array	2.1 M Array	Each Sample for a 3x720K Array
Experimental (IP) Sample	1.5µg	1.5µg	3.5µg	1.5µg
Control (Input) Sample	1.5µg	1.5µg	3.5µg	1.5µg

- For optimal results, samples should meet the following criteria:
  - A significant majority of the DNA  $\geq$  200 nucleotides in size.
  - A concentration of approximately 250ng/µl to 1,000ng/µl in nuclease-free water or 1X TE buffer (10mM Tris-HCl and 0.1mM EDTA, pH 7.5 - 8.0).
  - An  $A_{260}/A_{230} \geq 1.7$  and  $A_{260}/A_{280} \geq 1.6$ .

### Sample QC

1. Transfer 200ng of each sample to a sterile microcentrifuge tube. Store the remainder of your sample set at -20°C until required for labeling.
2. Analyze the samples using the Agilent Bioanalyzer and RNA 6000 Nano Assay Reagent Kit or by agarose gel electrophoresis.
3. Review Bioanalyzer traces (Figure 2 and Figure 3) or agarose gels (Figure 4) for sample degradation. Degraded samples detected using the Bioanalyzer appear as significantly lower intensity traces with the main peak area shifted to the left with typically more noise in the trace.

*Important: Samples exhibiting degradation should not be carried through labeling and hybridization due to the risk of poor results.*

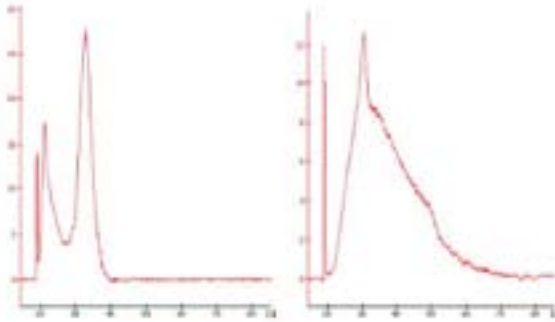


Figure 2: Examples of Bioanalyzer Traces of Nondegraded Samples

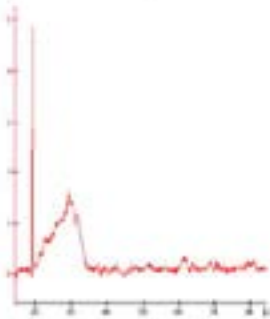


Figure 3: Example of Bioanalyzer Trace of a Degraded Sample

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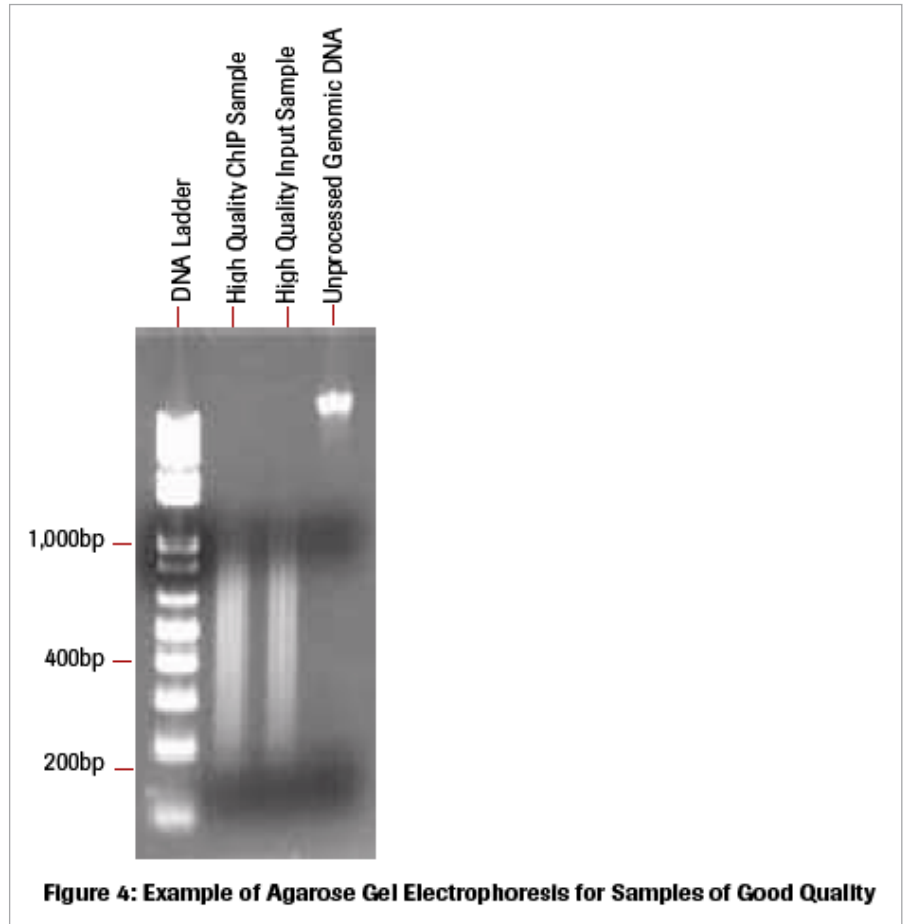
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## Safety-related Information

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## Global Locations

For a list of our global locations  
and contact details, visit  
sigma-aldrich.com/international



Proceed to Chapter 3, "Sample Labeling". Continue without deviation.

## References and Acknowledgements

1. NimbleGen™ ChIP-on-Chip Product Information,  
<http://www.nimblegen.com/products/chip/index.html>
2. NimbleGen Arrays User's Guide ChIP-chip Analysis,  
[http://www.nimblegen.com/products/lit/chip\\_userguide\\_v6p0.pdf](http://www.nimblegen.com/products/lit/chip_userguide_v6p0.pdf)
3. O'Geen, et al. *Biotechniques* (November, 2006) 41 (5): 577-580

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