

Genomics

Integration of Sigma® GenomePlex® WGA with the NimbleGen™ Comparative Genomic Hybridization Microarray 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 GenomPlex WGA for amplification of genomic DNA for CGH microarray applications.¹

- If your experimental genomic DNA sample quantity is less than the amount listed below, amplify the experimental and control (input) samples using the Sigma GenomePlex Complete WGA 2 Kit (**Cat. No. WGA2-50RXN**) before labeling.
- 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 GenomePlex WGA amplification as described in the product bulletin for GenomePlex WGA Kits.
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. Further fragmentation of GenomePlex WGA amplification product is unnecessary. Omit the sonification step found in the NimbleGen CGH Analysis procedure below.

Entry into NimbleGen Workflow

Enter **NimbleGen Arrays User's Guide: CGH Analysis**, v 6.0, Chapter 2.

Sample Requirements

- Purified, unamplified, and unfragmented genomic DNA (gDNA) is required for optimal sample labeling and hybridization.
- Roche NimbleGen recommends starting with the following gDNA amounts for each hybridization:

Sample Requirements	385K Array	Each Sample for a 4x72K Array	2.1M Array	Each Sample for a 3x720K Array	Each Sample for a 12x135K Array
Test gDNA	1.5µg	1.5µg	2.5µg	1.5µg	1.5µg
Reference gDNA	1.5µg	1.5µg	2.5µg	1.5µg	1.5µg

- Samples should be prepared at a concentration of 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).
- Samples should have an $A_{260}/A_{280} \geq 1.8$ and $A_{260}/A_{230} \geq 1.9$ for optimal labeling yields.

Note: Roche NimbleGen recommends running 250ng of gDNA on a NanoDrop Spectrophotometer to measure the A_{260}/A_{280} and A_{260}/A_{230} ratios.

Omit Sonication Step

Sample Preparation & QC

Note: Roche NimbleGen has tested several common genomes and found that the sonication step described below can be omitted without adversely affecting CGH data quality. Roche NimbleGen recommends determining the quality of unsonicated samples as described in step 5 below to ensure they show no signs of RNA contamination or degradation.

1. Dilute test and reference gDNA to 80µl with VWR water in a 1.5ml microcentrifuge tube.
2. Clean the sonicator tip with 70% ethanol and wipe dry with a tissue.
3. Select the following settings on a Branson model 450 Sonifier:

Note: If using another sonicator, adjust the settings as necessary to produce a smear from approximately 500bp to 2,000bp.

- Time = 10 seconds
- Amplitude = 10%
- Pulse On = 0.5 second
- Pulse Off = 0.5 second

4. Lower the sonicator tip to near the bottom of the tube and push the start button. Positioning of the probe near the bottom of the tube will prevent splashing and ensure complete sonication of your sample.

Important: Wear hearing protection when operating the sonicator.

5. To determine the quality of your samples, run 250ng of pre- and post-sonicated gDNA on a 1% agarose gel to ensure they show no signs of RNA contamination or degradation.

Important: Unsonicated genomic DNA should appear as a single prominent band greater than 12kb. If the sample appears as more than one band or as a smear, the DNA may be degraded or have a contaminant that could affect the labeling procedure. RNA contamination will result in a smear less than 200bp.

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Safety-related Information

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Global Locations

For a list of our global locations
and contact details, visit
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Omit Sonication Step

Sonicated sample should appear as a smear from approximately 500bp to 2,000bp with the majority of the fragments migrating between 500bp and 1,000bp. Genomic DNA exhibiting significant degradation (all bands < 500bp) is unsuitable for CGH analysis.

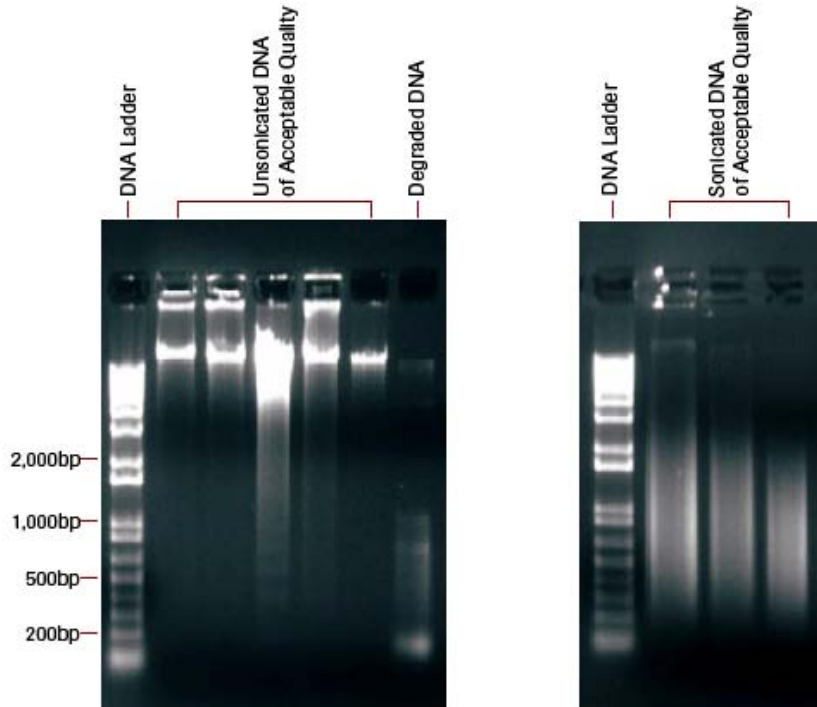


Figure 2: Examples of Agarose Gel Electrophoresis for Unsonicated DNA (left) and Sonicated DNA (right)

GenomePlex® WGA - amplified DNA will have an electrophoretic profile similar to that illustrated for sonicated DNA above.

Proceed to Chapter 3, "Sample Labeling", page 15. Continue without deviation.

References

1. NimbleGen specifically recommends GenomePlex WGA for its CHIP on CHIP and DNA Methylation array applications. GenomePlex WGA is efficacious where DNA samples are of small quantity or where amplification of a consumed stock is required.

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