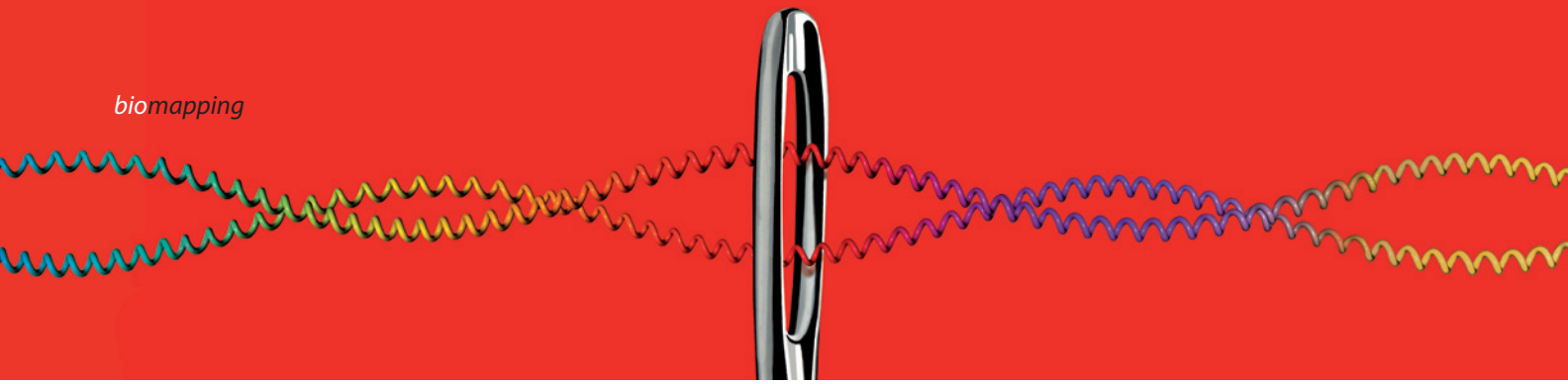

Integration of Sigma® GenomePlex® WGA with the Affymetrix CGH Microarray Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for expression analysis on the Affymetrix platform, and can be readily integrated into existing Affymetrix workflows for Comparative Genomic Hybridization (CGH) analysis.[1] The following modification is required:

- The GenomePlex WGA amplification product is **double-stranded cDNA**. Fragmentation and labeling is accomplished using the GeneChip® WT Double-Stranded DNA Labeling Kit, (**Affymetrix Cat. No. 900812**).

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***.

   Enter GenomePlex WGA2 Kit Procedure, page 2, *Fragmentation*.

   Modification 1. Fragmentation is not performed at this point in the procedure.[1]

Omit:

Fragmentation

1. Isolate DNA sample and quantity concentration by UV absorption (260 nm). Prepare DNA solution of 1ng/µL.
2. Add 1 µL of 10X Fragmentation Buffer to 10 µLof 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.

Replace with:

1. Place 10 µL of DNA (1 ng/ µL) sample (in nuclease-free H_2O) in a PCR tube or multiwell strip/plate.

Enter "Library Preparation" at Step 5

Library Preparation

5. Add 2 µL of 1X Library Preparation Buffer to each sample.
6. Add 1 µL of Library Stabilization Solution.
7. Vortex thoroughly, consolidate by centrifugation, and place in thermal cycler at 95°C for 2 minutes.
8. Cool the sample on ice, consolidate the sample by centrifugation, and return to ice.
9. Add 1 µL of Library Preparation Enzyme, vortex thoroughly, and centrifuge briefly.
10. Place sample in a thermal cycler and incubate as follows:
    16°C for 20 minutes
    24°C for 20 minutes
    37°C for 20 minutes
    75°C for 5 minutes
    4°C hold
11. Remove samples from thermal cycler and certrifuge briefly. Samples may be amplified immediately or stored at -20°C for three days.

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Modification 2. During the amplification step, dUTP is incorporated for subsequent Uracil-DNA glycosylase (UNG) fragmentation.[2]

Replace: Step 12 of Amplification Procedure

Amplification

12. A master mix may be prepared by adding the following reagents to the 15 µL reaction from step 11:
    7.5 µL of 10X Amplification Master Mix
    47.5 µL of Nuclease-Free Water
    5 µL of WGA DNA Polymerase

With:

12. Create amplificatoin mix. For each re-amplification reaction, add the following reagents to the 15µL reaction from step 11:
    46.9 µL of Nuclease-Free Water
    7.5 µL of 10X Amplification Master Mix (final volume = 60 µL)

    0.6 µL of dUTP (10 mM), final conc. = 80 µM
    5.0 µL of WGA DNA Polymerase

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. Elute with < 39 µL nuclease-free water.
   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.

References

1. Clifford Tepper, PhD., Assistant Research 2, Biochemist, UCD, Med, Biochemistry and Molecular Medicine, and Ryan Davis, Staff Research Assistant, University of Chalifornia-Davis Cancer Center, Sacramento, CA.
2. KDM8, a H3K36me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation
   Datsun A. Hsia, Clifford G. Tepper, Mamata R. Pochampalli, Elaine Y. C. Hsia, Chie Izumiya, Steve B. Huerta, Michael E. Wright, Hong-Wu Chen, Hsing-Jien Kung, and Yoshihiro Izumiya
   PNAS, May 2010; 107: 9671 - 9676.

Entry into Affymetrix Workflow

Enter the ***GeneChip® WT Double-Stranded DNA Target Assay***, Chapter Two "Target Preparation for Samples Requiring Amplification"; Procedure M, "Fragmentation of Double-Stranded DNA", page 34.

Proceed without deviation starting at step 1.

Procedures M and N require the use of the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (Affymatrix Cat. No. 900182).

1. Fragment the samples using the reactions described in Table 2.11.

Table 2.11
Fragmentation of Double-Stranded DNA

Component	Volume or Amount in 1 Reaction
Double-Stranded DNA	7.5 µg
10X Fragmentation Buffer	4.8 µL
UDG, 10 U/µL	1.5 µl
APE 1, 100 U/µL	2.25 µL
RNase-free Water	up to 48 µL
Total Volume	48.0 µL