SIGMA-ALDRICH®

sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

GenomePlex® Whole Genome Amplification (WGA) Kit

Product Number **WGA1**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

GenomePlex is a Whole Genome Amplification (WGA) method that allows the researcher to generate a representative, ~500-fold amplification of genomic DNA. The amplification yield is dependent on the purity and amount of starting material. The kit utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable OmniPlex Library molecules flanked by universal priming sites. The OmniPlex library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles.

WGA has been used in a variety of applications,[1,2] and is suitable for use with purified genomic DNA from a variety of sources including whole blood, plant, soil, buccal swabs, formalin fixed tissues, and cultured cells. GenomePlex WGA requires a minimum 10 ng of starting DNA, which after PCR can yield >10 μg of WGA product. After purification, the WGA product can be analyzed in a manner similar to any genomic or chromosomal DNA sample. A number of downstream applications may be performed including TaqMan® assays, microsatellite analysis, SNP analysis, sequencing, etc.

Components

Product Description	Catalog Number	Quantity
10× Fragmentation Buffer	F4304	55 μL
1× Library Preparation Buffer	L7167	110 μL
Library Stabilization Solution	L7292	55 μL
Library Preparation Enzyme	E0531	55 μL
10× Amplification Master Mix	A5604	410 μL
Water, Molecular Biology Reagent	W4502	2 × 1.5 mL
Control Human Genomic DNA	D7192	10 μL (5 ng/μL)

Materials and Reagents Required but Not Provided

- WGA DNA Polymerase (Component of GenomePlex WGA Reamplification Kit, WGA3)
- Thermal cycler
- DNA to be amplified
- Spectrophotometer
- 0.2 mL or 0.5 mL thin-walled PCR tubes or PCR multiwell plate
- Dedicated pipettes
- PCR pipette tips

Precautions and Disclaimer

The GenomePlex WGA Kit is for R&D use only, not for drug, household or other uses. Consult the MSDS for information regarding hazards and safe handling practices.

Storage/Stability

All components should be stored at –20 °C. When thawed for use, components should be kept on ice. Stability of the Library Preparation Enzyme will be affected if stored warmer than –20 °C or allowed to remain for long periods at temperatures over 4 °C.

Procedure

The WGA process is divided into fragmentation, OmniPlex library generation, and PCR amplification. The first two steps, fragmentation and library generation, should be carried out sequentially, as the ends of the library DNA can degrade thus affecting subsequent steps. OmniPlex library DNA, generated in the stepped isothermal reactions, can be stored up to three days at –20 °C without detectable differences in the process. The final WGA DNA should be stored at –20 °C, and is as stable as any comparably stored genomic DNA sample.

The starting amount of DNA is critical. When using a complex starting material, such as human genomic DNA, the gene bias in the resulting GenomePlex product is significantly altered if the quantity of input DNA is reduced. One ng of human genomic DNA affords product with gene representation that varies 2–10-fold from the original material, while product yield is only ~50% lower. Less complex genomes such as bacterial DNA can give good representation with as little as 1 ng of input DNA.

GenomePlex can be used on archival fixed tissue DNA or degraded samples provided that the extracted DNA is 200 bp or greater in size. However, greater quantities of damaged DNA are required to afford acceptable yields of final product. We recommend using up to 100 ng of fixed tissue DNA, and following the complete protocol, including fragmentation.

Experiments should be performed along with a positive control DNA sample, such as the Control Human Genomic DNA included in the kit.

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, and then centrifuge briefly to consolidate the contents.

Library Preparation

5. Add 2 µL of 1× 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 replace on 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 centrifuge briefly. Samples may be amplified immediately or stored at –20 °C for three days.

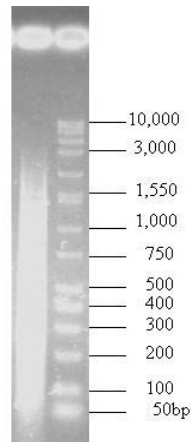
Amplification

12. A master mix may be prepared by adding the following reagents to the 15 µL reaction from Step 11:
    - 7.5 µL of 10× Amplification Master Mix
    - 47.5 µL of Water, Molecular Biology Reagent
    - 5 µL of WGA DNA Polymerase
13. Vortex thoroughly, centrifuge briefly and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermal cycler:

Initial Denaturation	95 °C for 3 minutes
Perform 14 cycles as follows:	
Denature	94 °C for 15 seconds
Anneal/Extend	65 °C for 5 minutes

After cycling is complete, maintain the reactions at 4 °C or store at –20 °C until ready for analysis or purification. The stability of WGA DNA is equivalent to genomic DNA stored under the same conditions.

The quality of the WGA DNA can be qualitatively determined by loading 5-10% (4-8 μL) of the final reaction onto a 1.5% agarose gel. The DNA size should range from 75–1500 bp, with the mean size ~400 bp.



Purification of the final product is recommended before being used in subsequent applications. GenomePlex WGA amplified DNA may be purified with PCR Cleanup Kit (Catalog Number NA1020) or standard purification methods that isolate single and double stranded DNAs. Once purified, the DNA can be quantified by measuring absorbance, assuming that one A_{260} unit is equivalent to 50 ng/μL DNA. Measurement techniques such as PicoGreen® dye will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.

Product Profile

All lots are functionally tested by the amplification of a 10 ng sample of standard human genomic DNA that must yield 3–7μg of product. The quality and representation of amplification is determined by real-time PCR using primer sets for eight separate loci.

References

1. Barker, D. L., *et al.,* Two methods of whole-genome amplification enable accurate genotyping across a 2320-SNP linkage panel. *Genome Res.*, **14**, 901–907 (2004).
2. Gribble, S., *et al.,* Chromosome paints from single copies of chromosomes. *Chromosome Res.*, **12**, 143–151 (2004).
3. Thorstenson, Y. R., *et al.,* An Automated Hydrodynamic Process for Controlled, Unbiased DNA Shearing. *Genome Res.*, **8**, 848–855 (1998).

Frequently Asked Questions

1. **How does GenomePlex work?** Genomic DNA is randomly fragmented and the resulting product is manipulated to attach a common sequence at each DNA end. This library of fragments is amplified using fourteen rounds of PCR.

2. **What if fragmentation is allowed to proceed for less or more than four minutes?** The 4 minute fragmentation time was found to give optimal results over a wide variety of DNA samples. Too little or no fragmentation will afford low yields and poor gene representation in the resulting WGA product. A ten minute fragmentation step will also give poor WGA yields in almost all cases because a significant fraction of the DNA has is now too small to allow efficient library production.

3. **What is the average size of fragmented DNA?** The mean size after the fragmentation step is ~0.4 kb.

4. **Can GenomePlex be used on archival fixed tissue DNA or degraded samples?** Yes, provided that the extracted DNA is 200 bp or greater in size. However, more damaged DNA is required to afford acceptable yields of final product. We recommend using up to 10 ng of fixed tissue DNA and following the complete protocol, including fragmentation. See the troubleshooting guide under low yield, input DNA is degraded.

5. **Can I use less input DNA in the GenomePlex protocol?** When using a complex starting material, such as human genomic DNA, the gene bias in the resulting GenomePlex output is significantly altered if the input DNA is reduced. An input of 1 ng of human genomic DNA affords product with gene representation that varies 2–10-fold over the original material, even though the yield is only ~50% lower. However, less complex genomes such as bacterial DNA can give good representation with only 1 ng of input DNA.

6. **How should WGA DNA be purified? Is there a preferred way to quantify GenomePlex DNA?** We recommend purifying GenomePlex DNA using Sigma's PCR cleanup kit (Catalog Number NA1020) before it is used in any downstream process. Once purified, the DNA can be quantified by measuring absorbance, assuming that one A_{260} unit is equivalent to 50 ng/μL DNA. Measurement techniques such as PicoGreen will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.

7. **How can I store GenomePlex DNA? Where can I stop during the GenomePlex process?** The WGA process can be divided into fragmentation, generation of the OmniPlex library, and PCR amplification. Fragmented DNA should be processed immediately, as the ends of this DNA can degrade and will affect subsequent steps. OmniPlex library DNA, generated in the stepped isothermal reactions, can be stored up to three days at –20 °C without any detectable differences to the process. The final WGA DNA should be stored at –20 °C, and is as stable as any comparably stored genomic DNA sample

8. **Will the GenomePlex process afford product with a negative control (no input DNA)?** No-template controls will occasionally afford product. This no-template product will not contain genes of interest if probed using PCR or hybridization techniques.

9. **What studies were conducted to determine sequence fidelity for this whole genome amplification method?** The GenomePlex method was tested for representation during development of the product by using 107 different human primer sets along with PCR and quantitative PCR. The sets are all from the National Center for Biotechnology UniSTS database.* While this subset of 107 DNA sequences represents a small fraction of the 20–30,000 human genes, it is a good statistical representation of the human genome.

   *http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists

Collaborators have also published work showing the representation of the amplification obtained using GenomePlex.[1,2]

As part of our QC protocol, every lot of our whole genome amplification (WGA) kit is run on human genomic DNA, which is subsequently tested using QPCR and eight different UniSTS primer sets. The results are compared to an equal amount of unamplifed DNA. This test demonstrates equal representation over several different regions.

View application data and recent protocols using GenomePlex WGA products by visiting the WGA home page at http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Molecular_Biology/PCR/Product_Lines/Whole_Genome_Amplification.html

Troubleshooting Guide

Observation	Cause	Recommended Solution
Low yield after cycling	Sample contains PCR inhibitors or high buffer salts	Dialysis in a suitable microdialysis unit may dilute the inhibiting components. Loss of DNA may occur in this process, and so quantitation of the dialyzed product is highly recommended.
	Input DNA is degraded or was less than 10 ng.	Amplification of insufficient DNA quantities often result in poor yield or poor representation in the final product. Some templates can be rendered amplifiable by using more input DNA. Successful WGA amplification has been performed with degraded samples by increasing starting template from 25–100 ng.
	More enzyme is required.	WGA yield suffers when limiting amounts of DNA polymerase are used. We recommend a minimum of 12 units hot start DNA polymerase per 75 μL reaction. This is preferable to adding cycles as the resulting DNA may suffer from amplification bias.
	The post reaction purification was inappropriate.	We recommend PCR Cleanup Kit (Catalog Number NA1020). The method must retain single and double stranded DNAs.
	The fragmentation reaction was too long or short.	The 4 minute fragmentation time was found to give optimal results over a wide variety of DNA samples. Too little or no fragmentation will afford low yields and poor gene representation in the resulting WGA product. A ten minute fragmentation step will give the same effect in almost all cases because a significant fraction of the DNA has is now too small to allow efficient library production.
qPCR shows significant bias in WGA representation for gene of interest.	Inappropriate controls	Genomic DNA can only be compared to GenomePlex WGA once the control DNA has been sheared. We recommend using several pooled samples that have been subjected to the fragmentation protocol (steps 1–4), or compare against DNA subjected to hydroshearing.[3]
	DNA sample is limited or degraded.	See low yield comments.

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PicoGreen is a registered trademark of Molecular Probes, Inc.

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