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Product Information

GenomePlex® WGA Reamplification Kit

Catalog Number **WGA3**
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 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.

The GenomePlex WGA Reamplification Kit allows subsequent reamplifications of the WGA product with little genetic bias. This kit contains an optimized enzyme that decreases the background in the reaction. The WGA DNA Polymerase successfully produces a negative control sample without product. This result provides more accuracy in downstream PCR or hybridization techniques.

The format of this kit, with the dNTPs separated, allows the user to create custom amplification mixes by supplementing with alternative nucleotide blends.

Reagents Provided

Product Description	Catalog Number	50 RXN
10× Amplification Master Mix	A5606	410 μL
10 mM dNTP Mix	D7295	0.2 ml
WGA DNA Polymerase	W3891	275 μL

Materials and Reagents Required but Not Provided

- Thermal cycler
- GenomePlex WGA DNA
- Spectrophotometer
- 0.2 ml or 0.5 ml Thin-Walled PCR Tubes or PCR multiwell plate
- Dedicated pipettes
- PCR pipette tips
- PCR grade water

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet 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.

Procedure

The WGA Reamplification process forgoes the initial fragmentation and OmniPlex library generation by using a previous WGA reaction as starting material. Simply add the defined amount of WGA product to a PCR reaction to reamplify. The reamplified WGA DNA should be stored at –20 °C and is as stable as any comparably stored genomic DNA sample. Be aware, this kit will not successfully amplify genomic DNA that has not previously been amplified by Genomeplex WGA technology.

It is critical to use at least 10 ng of WGA DNA. Insufficient starting material can result in poor genetic representation in the final reamplified WGA product.

Two reamplification procedures are provided for user convenience. The first procedure describes preparation of a single reamplification reaction. If performing multiple reactions, a master mix can easily be prepared using this procedure. Multiply the volume of each added reagent by the number of reactions needed, mix the calculated volumes of the reagents, and then transfer the appropriate volume of the resulting master mix into each individual reaction tube.

The second procedure describes addition of the appropriate volume of 10 mM dNTP mix to the 10× Amplification Master Mix upon receiving the kit. The resulting Working Amplification Master Mix is less than 10×, and therefore, the volume of reagents needed per reaction is modified accordingly. The first procedure requires 4 pipetting steps to set up a single reaction, while the second procedure requires only 3 pipetting steps after the preparation of Working Amplification Master Mix.

Reamplification Procedure A

1. Add 10 μL of 1 ng/μL WGA amplified DNA to a PCR tube or multiwell plate.
   Note: It is necessary to clean up the WGA reaction to decrease possible bias in the reamplification. We recommend using the Sigma's GenElute™ PCR Clean-Up Kit (Catalog Number NA1020) or standard purification methods that isolate single and double stranded DNA.

2. Create amplification mix. For each reamplification reaction, add the following to the WGA amplified DNA (step A1):

   49.5 μL of Nuclease-Free Water
   7.5 μL of 10× Amplification Master Mix
   3.0 μL of the 10 mM dNTP mix
   5.0 μL of WGA DNA Polymerase

3. Vortex thoroughly, centrifuge briefly, and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermocycler:

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

Reamplification Procedure B

The preparation of a Working Amplification Master Mix is only necessary if performing this procedure. Do not make a Working Amplification Master Mix if performing Procedure A.

1. Create Working Amplification Master Mix by adding 168 μL of 10 mM dNTP mix to the tube of 10× Amplification Master Mix. Mix until homogenous and label tube appropriately. Extra Working Amplification Master Mix can be stored at –20 °C for up to six months.

2. Add 10 μL of 1 ng/μL WGA amplified DNA to a PCR tube or multiwell plate.
   Note: It is necessary to clean up the WGA reaction to decrease possible bias in the reamplification. We recommend using the Sigma's GenElute PCR Clean-Up Kit (Catalog Number NA1020) or standard purification methods that isolate single and double stranded DNA.

3. Create amplification mix. For each reamplification reaction, add the following to the WGA amplified DNA (step B2):

   49.5 μL of Nuclease-Free Water
   10.5 μL of prepared Working Amplification Master Mix (step B1)
   5.0 μL of WGA DNA Polymerase

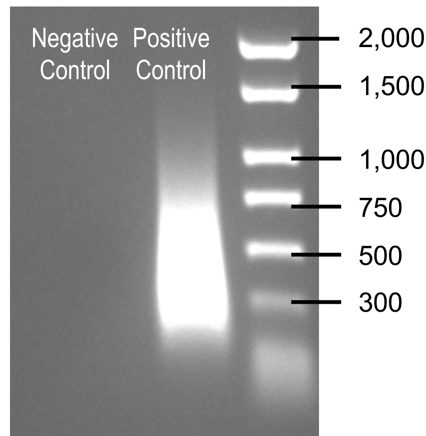
4. Vortex thoroughly, centrifuge briefly, and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermocycler:

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 completion of cycling in Procedure A or B, 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.

Purification of the final product is recommended before use in subsequent applications. Once again, we recommend Sigma's GenElute PCR Clean-Up Kit (Catalog Number NA1020) or standard purification methods that isolate single and double stranded DNA. 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.

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 100-1,000 bp, with the mean size ~400 bp.



Product Profile
All lots are functionally tested by the reamplification of a 10 ng sample of standard human genomic DNA that must yield 3–7 µg of product. A no template control must afford no or little yield by the gel. The quality and representation of amplification is determined by real-time PCR using primer sets for multiple 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).

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, so quantitation of the dialyzed product is highly recommended.
	Input DNA is severely degraded or was less than 10 ng.	Amplification of insufficient DNA quantities often results in poor yield or poor representation in the final product. Some templates can be amplified only by using more input DNA. Successful WGA amplification has been performed with degraded samples by increasing starting template to 25–100 ng.
	More enzyme is required.	WGA yield suffers when limiting amounts of DNA polymerase are used. We recommend a minimum of 5 µl of WGA DNA Polymerase per 70 µl reaction. This is preferable to adding cycles as the resulting DNA may suffer from amplification bias.
	Post reaction purification is necessary.	We recommend Sigma's PCR Cleanup Kit (NA1020). The method must retain single and double stranded DNA.
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.
	Too many reamplifications	We only recommend a maximum of five reamplifications. Excessive reamplifications can result in allele bias. Our R&D efforts have shown less than 10-fold allele bias after five successive reamplifications.
Negative (no template) control affords product.	Reagents have been contaminated by an outside source.	One or more reagents have been contaminated with DNA. While this may not affect your results, a clean no template control can be re-achieved only by replacing the affected component.

Frequently Asked Questions

1. **How does WGA Reamplification work?**
   Reamplification is simply PCR from a previous WGA reaction. The WGA product already contains universal sequences at its flanks. The WGA Reamplification Kit simplifies the Genomeplex technology by amplifying the WGA products with our 10× Amplification Master Mix and WGA DNA Polymerase.
2. **Why can't I use my own PCR Buffer and Polymerase to reamplify WGA product?**
   The supplied 10× Amplification Master Mix contains a unique solution that ensures recognition of the WGA product.
   The WGA DNA Polymerase was specifically chosen to eliminate potential product created in negative controls.
3. **Can I use less input DNA in the GenomePlex protocol?**
   We recommend using a minimum of 10 ng of WGA amplified DNA for any reamplification. The gene bias in the resulting reamplification can be significantly altered if the input DNA is reduced.
4. **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.
5. **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.

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

   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

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

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