

Product Information

GenomePlex® Tissue Whole Genome Amplification Kit

Product Number **WGA5**
Storage Temperature $-20\text{ }^{\circ}\text{C}$

TECHNICAL BULLETIN

Product Description

GenomePlex is a Whole Genome Amplification (WGA) method that allows the researcher to generate a representative 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.

This kit is formulated to amplify the genomic DNA from 1 mg of fresh, frozen, RNA/*later*®-preserved, or formalin-fixed paraffin-embedded (FFPE) tissue; however, as little as 0.1 mg of tissue has successfully been used with this product. The tissue is lysed and genomic DNA released in a 1 hour proteinase K/CelLytic™ Y incubation. An aliquot of lysate is then used directly in the WGA reaction. The kit doesn't require awkward organic solvent extractions to remove excess paraffin when working with FFPE tissues and produces yields that can exceed 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, STR analysis, etc.

It is well known that tissue fixation adversely affects DNA quality as a result of irreversible reactions that take place between monomethylol adducts and primary amine groups on nucleotides, proteins, and other chemical moieties. As a result, the quality of DNA present in FFPE tissues varies drastically depending on a number of factors including sample age and fixation conditions. An in-depth review of fixation parameters that affect DNA quality has been published.³ In some cases, DNA extracted from fixed tissue may not be of sufficient quality to allow molecular characterization, including WGA.

Multiplex PCR has been demonstrated to be an effective quality control tool for identifying such samples.⁵ These assays are based on amplifying PCR products of increasing size. The ability to amplify the larger PCR products is predictive of high quality DNA that is capable of successful DNA-based analysis.⁵

Components

Product Description	Catalog Number	10 Rxn	50 Rxn
CellLytic Y Lysis Solution	C8367	0.3 mL	1.5 mL
Proteinase K solution	P4850	100 µL	300 µL
1× Library Preparation Buffer	L7167	22 µL	110 µL
Library Stabilization Solution	L7292	12 µL	55 µL
Library Preparation Enzyme	E0531	12 µL	55 µL
10× Amplification Master Mix	A5604	80 µL	410 µL
WGA DNA Polymerase	W3891	55 µL	275 µL
Control Human Genomic DNA	D7192	10 µL (5 ng/µL)	10 µL (5 ng/µL)
Water, Molecular Biology Reagent	W4502	1 × 1.5 mL	2 × 1.5 mL

Materials and Reagents Required but Not Provided

- Thermal cycler
- fresh, frozen, RNA/*later*-preserved, or FFPE tissue
- Spectrophotometer
- 0.2 or 0.5 mL Thin-Walled PCR Tubes or PCR multiwell plate
- Dedicated pipettes
- PCR pipette tips
- GenElute™ PCR Clean-Up Kit, Catalog Number NA1020

Precautions and Disclaimer

The GenomePlex Tissue WGA Kit 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 $-20\text{ }^{\circ}\text{C}$. When thawed for use, components should be kept on ice. Stability of the Library Preparation Enzyme, Catalog Number E0531, will be affected if stored warmer than $-20\text{ }^{\circ}\text{C}$ or allowed to remain for long periods at temperatures over $4\text{ }^{\circ}\text{C}$.

Procedure

One procedure is provided for processing fresh, frozen, RNA/*later*-preserved, and/or FFPE tissues. The WGA process is divided into Lysis and Fragmentation, OmniPlex Library Generation, and PCR Amplification steps. Ideally these steps should be carried out sequentially without pause, as storage between steps may allow the ends of the library DNA to degrade, thus affecting subsequent steps. The final WGA DNA should be stored at $-20\text{ }^{\circ}\text{C}$ and is as stable as any comparably stored genomic DNA sample.

It is recommended all experiments should be performed along with a positive control DNA sample, such as the Control Human Genomic DNA included in the kit.

Lysis and Fragmentation

1. Weigh out a 1 mg sample of fresh, frozen, RNA/*later*-preserved, or FFPE tissue into a PCR-ready vessel. See step 4 for instructions on setting up a positive control reaction.
Note: As little as 0.1 mg of tissue has been successfully used with this kit. If use of less than 1 mg of FFPE tissue is desired, it is recommended to start with 1 mg of tissue and then empirically determine the minimum amount of tissue that can be processed.
2. To the sample, add 24 μL of CellLytic Y Lysis Solution, Catalog Number C8367, and 6 μL of Proteinase K solution, Catalog Number P4850.
3. Place the tube in a thermal cycler at $60\text{ }^{\circ}\text{C}$ for 60 minutes and then $99\text{ }^{\circ}\text{C}$ for 4 minutes.

OmniPlex Library Preparation

4. Combine 9 μL of Water, Molecular Biology Reagent, Catalog Number W4502, and 1 μL of the tissue lysate from step 3 into a fresh PCR-ready vessel. For a positive control reaction, combine 7 μL of Water, Molecular Biology Reagent, Catalog Number W4502.
2 μL of Control Human Genomic DNA, Catalog Number D7192, and 1 μL of CellLytic Y Lysis Solution, Catalog Number C8367.
5. Add 2 μL of 1 \times Library Preparation Buffer, Catalog Number L7167.
6. Add 1 μL of Library Stabilization Solution, Catalog Number L7292.
7. Mix thoroughly and place in thermal cycler at $95\text{ }^{\circ}\text{C}$ for 2 minutes.
8. Immediately cool the sample on ice, consolidate the sample by centrifugation, and replace on ice.
9. Add 1 μL of Library Preparation Enzyme, Catalog Number E0531, mix thoroughly, and centrifuge briefly.
10. Place sample in a thermal cycler and incubate as follows:
 - 16 $^{\circ}\text{C}$ for 20 minutes
 - 24 $^{\circ}\text{C}$ for 20 minutes
 - 37 $^{\circ}\text{C}$ for 20 minutes
 - 75 $^{\circ}\text{C}$ for 5 minutes
 - 4 $^{\circ}\text{C}$ hold
11. Remove samples from thermal cycler and centrifuge briefly. Samples may be amplified immediately or stored at $-20\text{ }^{\circ}\text{C}$ for up to three days.

PCR Amplification

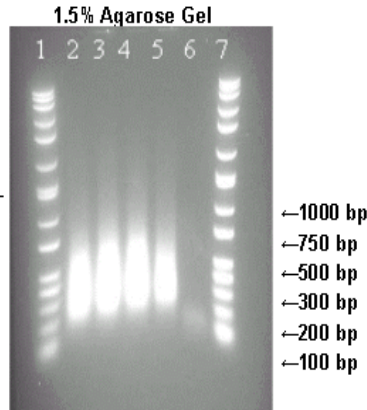
12. Add the following reagents to the entire 14 μL reaction:
 - 7.5 μL of 10 \times Amplification Master Mix
Catalog Number A5604
 - 48.5 μL of Water, Molecular Biology Reagent,
Catalog Number W4502
 - 5.0 μL of WGA DNA Polymerase
Catalog Number W3891
13. Mix thoroughly, centrifuge briefly, and begin thermal cycling. The following profile has been optimized for a PE 9700 or equivalent thermocycler:

Initial Denaturation	95 $^{\circ}\text{C}$ for 2 minutes
Perform 20 Cycles as follows:	
Denature	94 $^{\circ}\text{C}$ for 15 seconds
Anneal/Extend	65 $^{\circ}\text{C}$ for 4 minutes
Hold	4 $^{\circ}\text{C}$

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 WGA DNA can be determined by loading 5-10% (4-8µl) of the final product onto 1.5% agarose gel. The DNA size should range from 100-1000 bp with the mean size ~ 400 bp

Lane 1: Wide Range Marker
Lane 2: Human Uterus 1
Lane 3: Human Uterus 2
Lane 4: Human Gall Bladder
Lane 5: Human Colon
Lane 6: No Template Control
Lane 7: Wide Range Marker



Purification of the final product is recommended before use in subsequent applications. GenomePlex WGA amplified DNA may be purified with the GenElute PCR Cleanup 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® 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 5–10 µg of product. The quality and representation of amplification is determined by real-time PCR using primer sets for multiple loci. Negative (no template) controls must yield no detectable product.

References

1. Barker, D.L., *et al.*, Two methods of whole-genome amplification enable accurate genotyping across a 2320-SNP linkage panel. *Genomic Research*, **14**, 901-7 (2004).
2. Gribble, S., *et al.*, Chromosome paints from single copies of chromosomes. *Chromosome Research*, **12**, 143-51 (2004).
3. Srinivasan, M., *et al.*, Effect of fixatives and tissue processing on the content and integrity of nucleic acids. *American Journal of Pathology*, **161**, 1961-1971 (2002).
4. Thorstenson, Y.R., *et al.*, An Automated Hydrodynamic Process for Controlled, Unbiased DNA Shearing. *Genome Research*, **8**, 848-855 (1998).
5. Van Beers E.H., *et al.*, A multiplex PCR predictor for aCGH success of FFPE samples. *British Journal of Cancer*, **94**, 333-337 (2005).

Troubleshooting Guide

Observation	Cause	Recommended Solution
Low yield after cycling	WGA reaction may be inhibited due to contaminants in the tissue extract.	Dilute inhibitors in the tissue lysate created in step 3 with water by a dilution factor of 1:10 or more prior to continuing with the library preparation section. Alternatively, too much tissue was used, attempt using less tissue. To test for inhibition, include a DNA control and/or spike 10 ng of purified genomic DNA template into the proteinase K digestion in step 2.
	Poor quality template	Tissue stored improperly prior to WGA, leading to DNA degradation. If working with FFPE tissues, the formalin fixation process has irreversibly damaged DNA rendering the sample unsuitable for amplification. This can result if the fixation conditions are too harsh to afford high quality DNA, or if FFPE tissues have been stored for excessive amounts of time; the fixation process, and therefore DNA damage, often continue even after archiving FFPE tissues. Obtain properly fixed FFPE tissue samples per Srinivasan, M., <i>et al.</i> ³ Compare to the included control.
	Extraction is insufficient.	Digest samples at 60 °C longer than 1 hour in step 3. Samples can be incubated 2 hours to overnight followed by incubation at 99 °C for 4 minutes.
	Post reaction purification was inappropriate.	We recommend the GenElute PCR Cleanup Kit (Catalog Number NA1020). The method must retain single and double stranded DNA.
	Fragmentation reaction is too long or short.	The 99 °C four minute fragmentation time following proteinase K digestion (see step 3) 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 low yields in almost all cases, because a significant fraction of the DNA is now too small to allow efficient library production.
	Less than 0.1 mg of tissue weighed.	Insure that an adequate amount of tissue is used in the WGA reaction. When working with FFPE tissues, remove excess paraffin with a razor blade prior to weighing tissue. This ensures that an adequate amount of tissue is present in the subsequent proteinase K digestion.
Pieces of tissue are present after proteinase K digestion.	Incomplete proteinase K digestion of tissue	The GenomePlex Tissue Whole Genome Amplification Kit does not require the tissue to be completely digested. Sufficient DNA is released for WGA without completely digesting the tissue.
qPCR using WGA amplicons as template 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 fragmentation by heating at 99 °C for 4 minutes. Alternatively, compare against DNA subjected to hydroshearing. ⁴
	DNA sample is limited or degraded.	See low yield comments.
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 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 twenty rounds of PCR.
- 2. What is the average size of fragmented DNA?**
The mean size after the fragmentation step is ~0.4 kb.
- 3. Will the GenomePlex process afford product with a negative control (no input DNA)?**
No product will be generated without input of DNA if the procedure is performed correctly.
- 4. How should WGA DNA be purified? Is there a preferred way to quantify GenomePlex DNA?**
We recommend purifying GenomePlex DNA using the GenElute 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/ μ L DNA. Measurement techniques such as PicoGreen will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.
- 5. Where can I stop during the GenomePlex process? How can I store GenomePlex DNA?**
The WGA process can be divided into 3 steps: Lysis and Fragmentation, OmniPlex Library Generation, 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 $-20\text{ }^{\circ}\text{C}$ without any detectable differences to the process. The final WGA DNA should be stored at $-20\text{ }^{\circ}\text{C}$ and is as stable as any comparably stored genomic DNA sample.

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