

Product Information

GenomePlex® Single Cell Whole Genome Amplification Kit

Product Number **WGA4**
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 genome of a single cell. WGA from a single cell often results in a million-fold amplification yielding microgram quantities of gDNA. After purification, the Single Cell 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, comparative genomic hybridization, etc.^{1,2}

Materials and Reagents Required but not Provided

- Thermal cycler
- Isolated single cells
- 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 Single Cell GenomePlex 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. The stability of the Library Preparation Enzyme will be affected if stored warmer than $-20\text{ }^{\circ}\text{C}$ or allowed to remain for long periods at temperatures over $4\text{ }^{\circ}\text{C}$.

Reagents Provided

Product Description	Catalog Number	10 Rxn	50 Rxn	500 Rxn
10× Single Cell Lysis & Fragmentation Buffer	L1043	350 μL	1.8 mL	18 mL
Proteinase K Solution	P4850	25 μL	110 μL	1 mL
1× Single Cell Library Preparation Buffer	L0918	25 μL	110 μL	1.1 mL
Library Stabilization Solution	L7292	12 μL	55 μL	550 μL
Library Preparation Enzyme	E0531	12 μL	55 μL	550 μL
10× Amplification Master Mix	A5604	85 μL	410 μL	5 x 820 μL
WGA DNA polymerase	W3891	55 μL	275 μL	2 x 1.375 mL
Control DNA (5 ng/ μL)	D7192	10 μL	10 μL	10 μL
Water, Molecular Biology Reagent	W4502	1.5 mL	2 x 1.5 mL	2 x 20 mL

Procedure

The single cell WGA process is divided into lysis & fragmentation, OmniPlex library generation, and PCR amplification. These steps should be carried out sequentially without pause, as storage between steps may allow the ends of the library DNA to degrade. Such degradation will affect subsequent steps. The final WGA DNA should be stored at -20°C and is as stable as any comparably stored genomic DNA sample.

We recommend that all experiments should be performed along with a positive control DNA sample, such as the Control Human Genomic DNA, Catalog Number D7192, included in this kit.

Single Cell Lysis and Fragmentation

1. Isolate a single cell into a PCR-ready vessel using laser capture micro-dissection, cell sorting, or other method. If sorted, the buffer should be of low ionic strength, such as Tris EDTA (TE) buffer, and in the minimal sort volume.
2. Add a sufficient volume of water to the single cell sample for a final volume of $9\ \mu\text{L}$.
3. Prepare a working Lysis and Fragmentation Buffer Solution by adding $2\ \mu\text{L}$ of Proteinase K Solution into $32\ \mu\text{L}$ of the $10\times$ Single Cell Lysis & Fragmentation Buffer. Vortex thoroughly.
4. Add $1\ \mu\text{L}$ of the freshly prepared Proteinase K Solution to $10\times$ Single Cell Lysis & Fragmentation Buffer to the single cell sample. Mix thoroughly.
5. Incubate DNA mix at 50°C for 1 hour, then heat to 99°C for EXACTLY four minutes. Note that the incubation is very time sensitive. Any deviation may alter results. Cool on ice. Spin down sample prior to proceeding to Library Preparation.

Library Preparation

6. Add $2\ \mu\text{L}$ of $1\times$ Single Cell Library Preparation Buffer to each sample.
7. Add $1\ \mu\text{L}$ of Library Stabilization Solution.
8. Mix thoroughly and place in thermal cycler at 95°C for 2 minutes.
9. Cool the sample on ice, consolidate the sample by centrifugation, and replace on ice.
10. Add $1\ \mu\text{L}$ of Library Preparation Enzyme, mix thoroughly, and centrifuge briefly.
11. 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

12. Remove samples from thermal cycler and centrifuge briefly. Samples may be amplified immediately or stored at -20°C for three days.

Amplification

13. Add the following reagents to the entire $14\ \mu\text{L}$ reaction:
 - $7.5\ \mu\text{L}$ of $10\times$ Amplification Master Mix
 - $48.5\ \mu\text{L}$ of Water, Molecular Biology Reagent
 - $5.0\ \mu\text{L}$ of WGA DNA Polymerase
14. Mix thoroughly, centrifuge briefly, and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermal cycler:

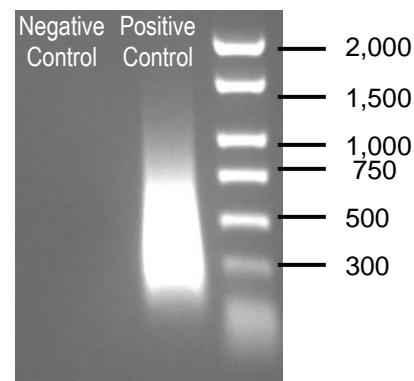
Initial Denaturation 95°C for 3 minutes

Perform 25 cycles as follows:

Denature 94°C for 30 seconds
 Anneal/Extend 65°C for 5 minutes
 Hold 4°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 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 100-1000 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 the GenElute™ 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 $1\ A_{260}$ unit is equivalent to $50\ \text{ng}/\mu\text{L}$ 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 100 pg sample of standard human genomic DNA that must yield 4 µg of product. The quality and representation of amplification is determined by real-time PCR using primer sets for eight separate 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. Thorstenson, Y. R., *et al.* An Automated Hydrodynamic Process for Controlled, Unbiased DNA Shearing. *Genome Research*, **8**, 848-855 (1998).

Troubleshooting Guide

Observation	Cause	Recommended Solution
Low yield after cycling	Sample contains salt or inhibitors.	The sorting process may carry over inhibitors or salts that disrupt the process.
	Poor quality template	The DNA in the single cell was degraded during the isolation process. The single cell was improperly stored. Performing WGA on more than one sample helps rule out quality issues that are random in nature, but will not help if the sorting process damages the sample.
	The post reaction purification was inappropriate.	We recommend GenElute PCR Cleanup Kit, Catalog Number NA1020. The method must retain single and double stranded DNA.
	A single cell was not captured.	Ensure you have a single cell present in your PCR-ready tube. In addition, make certain to thoroughly vortex after adding in the single cell lysis/fragmentation buffer.
qPCR shows significant bias in WGA representation for my gene of interest.	The DNA sample is limited or degraded.	See low yield comments.
	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 above (steps 1–5), or comparing against DNA subjected to hydroshearing. ³
Negative (no template) control affords product.	Reagents have been contaminated by 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-five rounds of PCR.

2. What if fragmentation (99 °C step) is allowed to proceed for less or more than four minutes?

The four-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 is now too small or degraded 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. 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.

5. 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 1 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.

6. How can I store GenomePlex DNA? Where can I stop during the GenomePlex process?

The WGA process can be divided into single cell lysis/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.

7. I have analyzed my sample for gene representation and have observed allelic dropout.

Studies have shown that allelic dropout is inherent in single cell amplification. Sigma's Single Cell WGA Kit minimizes allelic dropout. Our WGA methodology has been tested via quantitative PCR on multiple single cell WGA samples resulting in 30% allelic dropout.

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