molecular biology

Real-time Quantitative Representation Measurement of Eighty Loci Using GenomePlex® Whole Genome Amplification

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Application Notes

- The most time-efficient WGA method on the market
- A novel, random DNA fragmentation technique makes GenomePlex more advantageous than other WGA methods
- No detectable allele bias

Introduction

The methodologies of molecular biology have opened many fields of scientific research, allowing scientists to access the vast wealth of genetic information found in DNA. As these techniques have become more commonplace, researchers have worked diligently to push the limits using a variety of amplification schemes.[1] Whole genome amplification (WGA) has naturally become a goal for researchers who wish to perform multiple analyses on limited quantities of genomic DNA. Sigma has in the past year introduced the GenomePlex WGA method (WGA1), which is based on both isothermal and geometric amplification.

Unique, Quick, and Convenient Whole Genome Amplification

Successful application of the GenomePlex® Kit depends on several factors including the amount and quality of the input DNA; the correct application of the fragmentation, isothermal and amplification steps, and the appropriate choice of a purification method once the product is obtained. A schematic of the overall method is presented in Figure 1. An animated depiction of the whole process can be downloaded from our Web site: **sigma-aldrich.com/wga**.

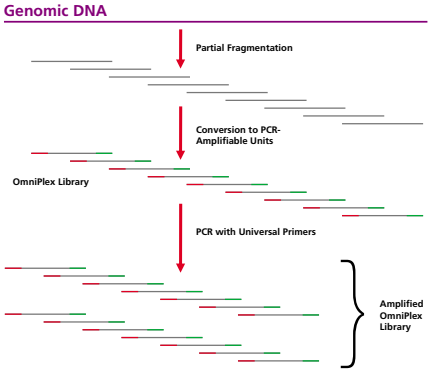

Figure 1. Depiction of the overall GenomePlex Whole Genome Amplification process.

The GenomePlex Whole Genome Amplification method uses input DNA to generate a library of amplified DNA fragments that faithfully represents the original input genome. The three step fragmentation, isothermal amplification, and limited geometric amplification method yields products ranging in size from ~100 bp to ~2 kb with an average size of ~450 bp. While other WGA methods require 6-16 hours to complete, the method is simple, taking approximately thirty minutes of hands-on time and a total of only three and one-half hours to complete.

GenomePlex begins with a unique, chemical fragmentation of DNA in an initial four-minute incubation step. The resulting fragments must be the correct size for optimal WGA. Too little fragmentation will afford sub-optimal representation and yield, while DNA that is less than 200 bp will not be efficiently primed and amplified in the following steps. The recommended fragmentation time works well for the vast majority of DNA samples, but samples that have been extensively digested will work better with shorter or no fragmentation.

The next two steps of amplification in the GenomePlex method generate denatured fragments that are subsequently primed by a proprietary, partially randomized primer set. Through a series of stepped isothermal incubations these primers generate DNA with fixed 5′ and 3′ sequences. The resulting library of 5′ and 3′ adaptor-appended fragments, termed the OmniPlex® library, can be stored at 4 °C overnight or –20 °C over the weekend.

GenomePlex finishes with a limited set of PCR amplification cycles, using a primer set targeting the newly appended 5′ and 3′ sequences. The resulting product yield ranges from 5-10 micrograms, depending on the quality of the initial DNA input.

The GenomePlex Whole Genome Amplification family of products includes:

- GenomePlex Whole Genome Amplification Kit (WGA1)
- GenomePlex Complete Whole Genome Amplification Kit (WGA2)
- GenomePlex WGA Reamplification Kit (WGA3)

The GenomePlex Whole Genome Amplification Kit (WGA1), contains all the necessary reagents to perform fragmentation and library preparation and allows the customer the flexibility of using their amplification enzyme of choice. The GenomePlex Complete WGA Kit (WGA2) contains the same reagents as well as an optimized enzyme, WGA DNA Polymerase. This enzyme provides for increased accuracy in amplification, as evidenced by producing no amplicon in the negative control reactions. The soon to be offered, GenomePlex WGA Reamplification Kit (WGA3), allows subsequent reamplifications of the initial WGA product. The successive reamplifications provide DNA with little genetic bias when compared to the original genome. The Reamplification Kit contains the WGA DNA Polymerase and the 10X Master Amplification Mix.

Representation of the final GenomePlex product was determined using quantitative PCR with over seventy-five different qPCR primer sets chosen from the National Center for Biotechnology UniSTS database **(http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unists**; data is shown in Figure 2). Real-time PCR was performed using 0.2 µM each primer (above) in a SYBR® Green containing reaction mix. Cycling was initiated with a 94 °C step for five minutes followed by forty cycles of 94 °C, 15 sec and 68 °C, 1 minute. Detection occurred at the end of the second PCR step. Cycle thresholds were determined after baseline correction was applied and the cycle thresholds set at 5x baseline signal. Each primer set was run using 10 ng samples from 3-5 different GenomePlex reactions. Relative gene quantities in the WGA product were determined by normalizing to a qPCR containing the same primer set starting with 10 ng total (unamplified) human genomic DNA.

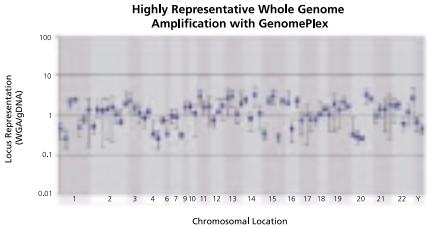

Figure 2. GenomePlex representation for eighty different human loci, normalized to an unamplified human DNA control. *Loci were selected from National Center for Biotechnology UniSTS database.*

As can be seen in Figure 2, GenomePlex affords a representative outcome, generating a product at ~500x the original input DNA without significant loci bias. Overall distribution of these single gene loci is less than four-fold across the entire panel. It is worth noting that all tested loci were found in the 80-member test panel. Similar findings have been published by independent testers.[2,3]

Summary

The GenomePlex Whole Genome Amplification Kit is a quick and convenient method to reproducibly amplify precious or rare DNA samples while maintaining genetic representation of the original sample information. GenomePlex WGA has been successfully applied to many different sample types including formalin fixed paraffin embedded tissues, blood samples from FTA cards, soil samples, plant leaf DNA, and a variety of other sources. GenomePlex WGA is offered exclusively by Sigma-Aldrich.

References

1. Hughes, S. *et al.,* The use of whole genome amplification in the study of human disease. *Prog. Biophys. Mol. Biol.* **88**(1), 173-189 (2005).
2. Barker, D.L. *et al.,* Two methods of whole-genome amplification enable accurate genotyping across a 2320-SNP linkage panel. *Genome Research,* **14,** 901-907 (2004).
3. Gribble, S. *et al.,* Chromosome paints from single copies of chromosomes. *Chromosome Research* **12,** 143-151 (2004).

Ordering Information

Product	Description	Unit
WGA1	GenomePlex Whole Genome Amplification (WGA) Kit	50 rxn
WGA2	GenomePlex Complete Whole Genome Amplification Kit	10 rxn 50 rxn
WGA3	GenomePlex WGA Reamplification Kit	50 rxn