GenomePlex® Whole Genome Amplification

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Introduction

Genomic testing has become a useful, practically ubiquitous tool in a wide variety of scientific disciplines. Often, DNA sample availability limits the analysis of environmental, clinical, and archived samples. Whole genome amplification (WGA) offers a means to immortalize DNA from these rare samples.

Over the years, a number of WGA technologies have emerged using strategies ranging from random priming to multiple strand displacement with phi29. Although all methods prove promising in their respective approach, each has specific drawbacks including inefficient primer binding and amplification bias. GenomePlex WGA technology (outlined in **Figure 1**) minimizes these problems through the random, non-enzymatic fragmentation of the genome followed by the conversion of these fragments into PCR-amplifiable units flanked by universal adaptor sequences. This library of fragments (the OmniPlex® library) is then subjected to a limited number of PCR cycles to generate sufficient material for subsequent use in a variety of downstream applications.

In this poster we demonstrate the capabilities of the GenomePlex WGA Kit offered by Sigma-Aldrich. We show the effect of DNA input quantity on GenomePlex yield, with as little as 100 pg of starting material needed. We show real-time quantitative PCR data for 79 loci throughout the human genome, which demonstrates both the low bias and reproducibility of this method. We also present pre- and post-WGA SNP data that demonstrate the utility of GenomePlex WGA in genotyping applications.

Overview of GenomePlex Whole Genome Amplification

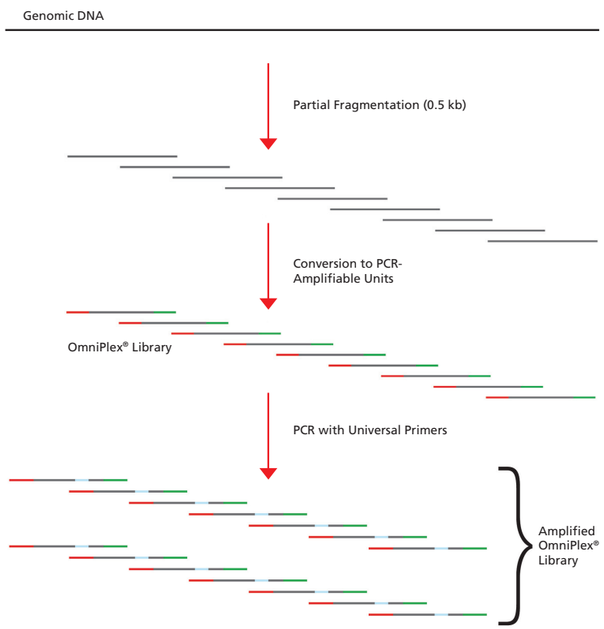

Figure 1. GenomePlex WGA begins with the random, non-enzymatic fragmentation of the genome followed by the conversion of these fragments into PCR-amplifiable units flanked by universal adaptor sequences. This library of fragments (the OmniPlex library) is then subjected to a limited number of PCR cycles to generate sufficient material for subsequent use in a variety of downstream applications.

Effect of Input DNA Quantity

Often, DNA sample availability limits the analysis of rare environmental, clinical, and archived samples. Naturally it is the goal of any WGA technology to generate as much representative DNA from as little starting material as possible. To investigate the effect of input DNA quantity, we performed GenomePlex WGA on 0, 0.01, 0.1, 1.0, 10, 100, and 1000 ng of human genomic DNA (Roche), analyzed the products on a 1.0% agarose gel, removed reaction components using the Genelute™ PCR Clean-Up Kit, and measured final DNA concentration by A_{260} using a Nanodrop® ND-1000 spectrophotometer (Nanodrop Technologies). We found that GenomePlex was able to amplify DNA from as little as 100 pg of starting material, with increasing input quantities resulting in larger OmniPlex fragment lengths (**Figure 2a**). Subsequent quantitation showed an inversely proportional relationship between yield and fold-amplification dependent on input DNA quantity (**Figure 2b**), with an optimum input quantity of ~10 ng.

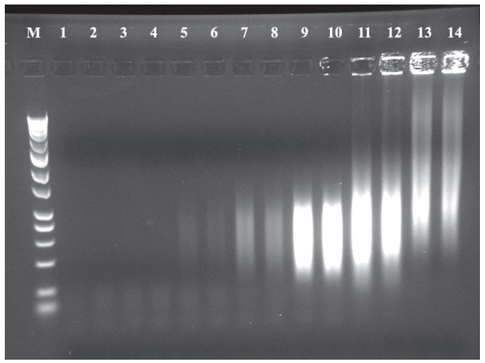

Figure 2a. Gel analysis of GenomePlex WGA products from reactions with increasing input human genomic DNA (Roche). A DNA marker (M) contains bands at 50, 100, 200, 300, 400, 500, 750, 1000, 1400, 1550, 2000, 3000, 4000, 6000, 8000, and 10000 bp. No-template controls (lanes 1–2) yielded no products; reactions with 0.01 ng of input DNA (lanes 3–4) yielded very little product (visible only with increased UV exposure) ranging in size from ~150 to ~750 bp; 0.1 ng of input DNA (lanes 5–6) yielded a smear ranging from ~150 to ~750 bp; 1 ng of input DNA (lanes 7–8) yielded a smear ranging from ~150 to ~750 bp; 10 ng of input DNA (lanes 9–10) yielded a smear ranging from ~200 to ~1000 bp; 100 ng of input DNA (lanes 11–12) yielded a smear ranging from ~200 to ~2000 bp; 1000 ng of input DNA (lanes 13–14) yielded a smear ranging from ~250 bp to >10 kb.

Effect of Input DNA Quantity on GenomePlex Yield

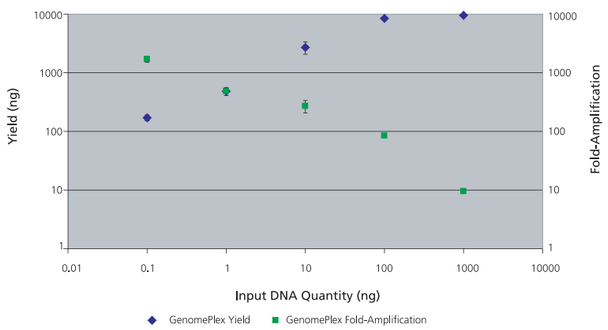

Figure 2b. Quantitation of GenomePlex WGA products post-purification to remove excess reaction components. Reactions with 0 and 0.01 ng of input DNA contained lower than detectable limits of DNA. Increasing input DNA from 0.1 to 1000 ng increased yield at the cost of fold-amplification.

Highly Representative Whole Genome Amplification

While many technologies are capable of producing large amounts of DNA from small input quantities, very few are able to amplify entire genomes efficiently with low bias. Above, we have demonstrated the ability of GenomePlex WGA to amplify input DNA approximately 2000-fold; to measure the bias of this amplification, we performed real-time quantitative PCR on human genomic DNA pre- and post WGA targeting 79 loci throughout the human genome. Ten nanograms of human genomic DNA (Roche) was amplified using the GenomePlex WGA Kit, purified, and quantified as described for figure 2. Ten nanograms of pre- and post-WGA DNA in 25 µL reactions were analyzed in triplicate for each locus. Thermal cycling was performed on an Opticon® II thermocycler (MJ Research) using SYBR® Green JumpStart™ Taq ReadyMix™ for Quantitative PCR with the following cycling conditions: 94 °C for 4 min 30 sec followed by 41 cycles of 94 °C for 30 sec, 60 °C for 30 sec, 72 °C for 30 sec. Fluorescence was read each cycle at 72 °C. Primer-dimers were excluded from data collection as measured by melt curve analysis. Data are normalized by Ct values and represented as the ratio of WGA/gDNA at each locus (**Figure 3**). We found GenomePlex WGA DNA to be highly representative of starting genomic material, with every locus represented , and only a four-fold maximum bias of representation between the 79 loci tested.

Highly Representative Whole Genome Amplification with GenomePlex

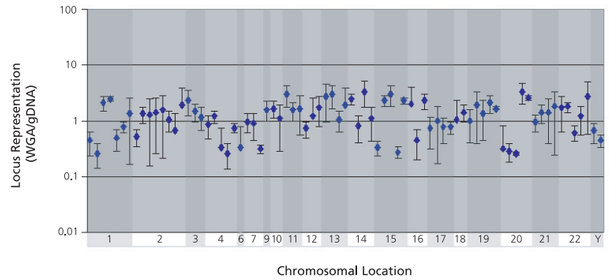

Figure 3. Real-time quantitative PCR was performed targeting 79 loci throughout the human genome on pre- and post-WGA DNA. When compared to the unamplified sample, 36 of the 79 loci were represented within two-fold in the GenomePlex WGA DNA. The maximum bias of representation throughout the remaining loci was found to be only four-fold.

SNP Genotyping

Hyperhomocysteinemia (characterized by elevated levels of homocysteine) is associated with an increased risk of arterial disease and veinous thrombosis. While environmental factors such as renal failure or deficiencies in B12, B6, or folate can contribute to this condition, genetic factors include a C677T transition in the methylene tetrahydrofolate reductase (MTHFR) gene. To demonstrate the utility of GenomePlex WGA in SNP genotyping, we analyzed DNA samples pre- and post-WGA from homozygous wt (CC), heterozygous (CT), and homozygous mutant (TT) individuals. Ten nanograms of human genomic DNA was amplified using the GenomePlex WGA Kit, purified, and quantified as described for figure 2. Ten nanograms of pre- and post-WGA DNA each in 20 µL reactions were used for SNP genotyping, which was performed in duplicate for each sample. Real-time quantitative PCR was performed on an ABI Prism® 7700 (Perkin Elmer) using the MGB Eclipse™ PCR Reagent Kit for SNPs. The probe sequence for the wt allele is MGB-Q-ATCGGCTCCCGC-FAM, and the probe sequence for the mutant allele is MGB-Q-AATCGACTCCCGC-YY (Yakima Yellow™). The melt curves and peak intensities of post-WGA samples closely matched those of pre-WGA samples, and correctly genotyped all three samples tested (**Figure 4**).

Methylene Tetrahydrofolate Reductase SNP Genotyping Pre- and Post-GenomePlex WGA

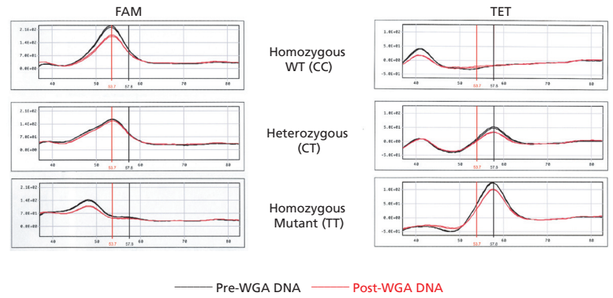

Figure 4. DNA samples from individuals homozygous wt (CC), heterozygous (CT), and homozygous mutant (TT) at nucleotide position 677 of the MTHFR gene were amplified using GenomePlex WGA and genotyped using real-time quantitative PCR. A FAM peak at 53.7 °C indicates the presence of the wild-type allele, while a TET peak at 57.8 °C indicates the presence of the mutant allele. The melt curves and peak intensities of post-WGA samples closely matched those of pre-WGA samples and correctly genotyped all three samples tested.

Conclusions

GenomePlex WGA presents a highly accurate and efficient method to amplify DNA samples of limited availability. As little as 100 pg of starting material can be amplified up to 2000-fold to create an unlimited supply of DNA suitable for a variety of downstream applications. We found fold-amplification inversely proportional to yield, which increased with increasing quantity of input DNA (tested up to 1 µg). In our assay of locus representation, GenomePlex WGA was found to accurately represent each of the 79 human loci tested, with a maximal bias of only four-fold. In addition to highly representative amplification across loci of the human genome, we also found GenomePlex WGA to maintain allelic representation in a standard SNP assay.

Related Sigma-Aldrich Products

GenomePlex® Whole Genome Amplification Kit	WGA1
GenElute™ PCR Clean-up Kit	NA1020
SYBR® Green JumpStart™ Taq ReadyMix™ for Quantitative PCR	S4438
MGB Eclipse™ PCR Reagent Kit for SNPs	M4693
SYBR®Green Quantitative RT-PCR Kit	QR0100

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