

# feature article

## Whole Genome Amplification: Moving Beyond the Limits of Traditional PCR

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### Introduction

The history of molecular biology is marked by technological advances that have opened up the investigation of both fundamental and applied biological problems. Consequently, specialized disciplines of molecular biology emerged along with the need for more powerful and dynamic analysis tools. A commonality among these diverse research areas has been the limitations on the availability of DNA. In response to the challenge of obtaining adequate quantities of specific DNAs, researchers began examining various methods for producing copies of DNA. In 1985, the discovery of the Polymerase Chain Reaction (PCR) was introduced to the research community by Dr. Kary Mullis.<sup>1</sup> PCR enabled researchers to exponentially amplify specific segments of a DNA template and ultimately, revolutionized many research areas ranging from viral identification to transcriptional regulation. Fueled by polymerase activity, PCR is based on the incorporation of nucleotides into a DNA sequence that is complementary to a template resulting in the exponential accumulation of a known DNA fragment. The specificity of the amplified DNA fragment is controlled by primer choice and can be evaluated by determining the size and sequence of the amplified fragment. Because analysis follows completion of the PCR cycle, this type of PCR is often referred to as "End-Point PCR."

As PCR became a fundamental research tool, the apparent limitations of sensitivity, specificity, and amplification of a limited target-specific fragment presented certain challenges. Incremental improvements of PCR technology such as hot-start polymerases and more advanced instrumentation have eased some of the constraints. However, given the rapid expansion of research in areas such as Drug Discovery and Functional Genomics, it has become a necessity to perform analysis on a genome-wide scale. For example, genotyping has increasingly become more routine in pathological studies where limited availability of genetic material limits testing and can cause valuable information to be lost. DNA (as a limiting resource) has impacted the extent of research and understanding in many areas of molecular analysis including pharmacogenomics, target discovery/validation, and population studies. Therefore, a technology that allows unbiased

replication of the entire genome is necessary to support continuous investigative endeavors and has become a critical focus. Traditional amplification methods are inadequate as they afford amplified fragments of a *targeted* sequence without adequate representation of the entire genome. The need for whole genome representation from minimal amounts of genomic DNA has led to the development of several innovative techniques capable of whole genome amplification.

### Discussion

Whole genome amplification (WGA) methods began with the introduction of Primer Extension Pre-amplification (PEP), Degenerate Oligonucleotide-Primed (DOP) PCR, and Tagged PCR (T-PCR) in the early 1990s. Each method utilized a different strategy in order to achieve amplification of low starting amounts of genomic DNA with the ultimate goal of generating a complete and unbiased representation of the entire genome. PEP-PCR is based on the frequent annealing of a totally degenerate 15-mer or 16-mer primer. DOP-PCR incorporates a partially degenerate primer, which binds at numerous sites throughout the genome. After a series of low-stringent annealing cycles, the temperature is increased to permit amplification of fragments that are tagged with a specific part of the primer sequence. T-PCR is a two-step procedure during which the initial low-stringent cycles use a primer with a constant 5' end and a random 3' end to allow priming at various sites. The unincorporated primers are then removed and amplification is carried out with a second primer containing only the constant 5' sequence of the above primer exposed to high-stringent conditions to allow exponential amplification.

Although all methods proved promising in their respective approach, each has had one specific drawback that affected its usefulness with many downstream genetic analyses. These early genome amplification methods all gave varying degrees of bias when compared to the representation present in the starting material. Additionally, each of these methods generates relatively short amplification of fragments — up to only about 500 bp in length. PEP-PCR with its short, completely degenerate primers resulted in the amplification of internal regions of previously amplified products leading to the accumulation of shorter products with each subsequent cycle. DOP-PCR suffered from similar bias, with whole sections of the genome under or unrepresented in the final product. Finally, the labor-intensive nature of T-PCR, which involved a purification step to remove unincorporated degenerate primers, suffered from the previously mentioned bias to a somewhat lesser extent, but the extra manipulation was cumbersome and resulted in the loss of precious genomic DNA.<sup>2</sup>

Integration of these methods into various analyses highlighted an inability to produce long products from very low (nanogram or picogram) quantities of genomic DNA. Efforts to improve existing technologies and develop innovative techniques became a necessity. This necessity was further compounded by periodic announcements for complete whole genome sequencing of various bacteria, plants, insects, rodents and humans. Genome sequencing has given researchers a tremendous opportunity to identify important biological information and determine its influence on gene expression. Access to information that may have seemed impossible just a decade ago has accelerated the need to develop a WGA technology that successfully amplifies low concentrations of genomic DNA, achieves high yield and fidelity, and provides equal genome representation.

The delineation of newly developed applications for WGA is founded on new means to reduce amplification bias. These methods are easily differentiated by their respective method of amplification. The crucial step required by all genome amplification technologies is the successful denaturation of the target DNA and competition of primer annealing over annealing of the opposite strand. Thermodynamics are unfavorable for the competition of intermolecular priming over intramolecular strand “rebound” for long strands of DNA, thus priming events occur too distant to allow effective PCR.

Of the various WGA methods available today, GenomePlex™ WGA best addresses the issues of amplification biases and primer binding. Developed by Rubicon Genomics, GenomePlex WGA is based on random fragmentation of the genome into a series of overlapping, short templates. The resulting, shorter DNA strands can be efficiently primed and amplified to generate a library of DNA fragments with defined 3' termini — the OmniPlex™ library. This library is replicated using a linear, isothermal amplification in the initial stages, followed by a limited round of geometric (PCR) amplifications. Competing technologies rely on a highly processive mesophilic polymerase to amplify the long regions between the sparse priming events. The method of amplification is crucial in determining the time required to generate microgram quantities of genomic DNA from sub-nanogram starting concentrations. Isothermal strand-displacement methods carry out amplification at a static temperature that requires a six-hour to overnight incubation. Amplification by traditional temperature cycling, as demonstrated with GenomePlex WGA technology, is completed in approximately three hours.

The practicality of GenomePlex WGA technology extends beyond incubation requirements as successful amplification may be achieved from any source of DNA, no matter what secondary structure or GC content, and has been demonstrated on many organisms including human, bacterial, and plant. This technology is further complimented for its ability to perform single-cell amplification without any significant background or allele bias. Additionally, the amplified DNA can be archived for further study allowing researchers to avoid the traditional collection and storage of whole samples.

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Traditional PCR is considered a primary method for amplifying a targeted sequence and also provides a precise manner for the introduction of a new sequence into a known template. Multiple time-consuming tasks such as purifying the genomic or cDNA template, designing sequence-specific primers, and optimizing reagents and cycling parameters have been recognized. Amplification of select genomic regions is desirable for many research areas. However, due to the previously mentioned thermodynamic bottlenecks, obtaining whole genome representation by standard PCR is not a reasonable expectation. GenomePlex WGA and traditional PCR differ not only in their respective output but also in relation to their input. Both methods utilize routine PCR cycling, however, GenomePlex technology utilizes an initial step of generating an OmniPlex library of PCR-amplifiable fragmented molecules prior to amplification whereas, traditional PCR relies on the amplification of a sequence-specific fragment template (Figure 1).

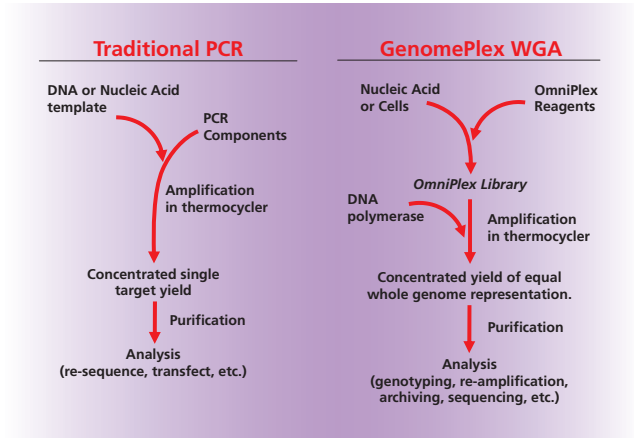


Figure 1.

### Genomic DNA

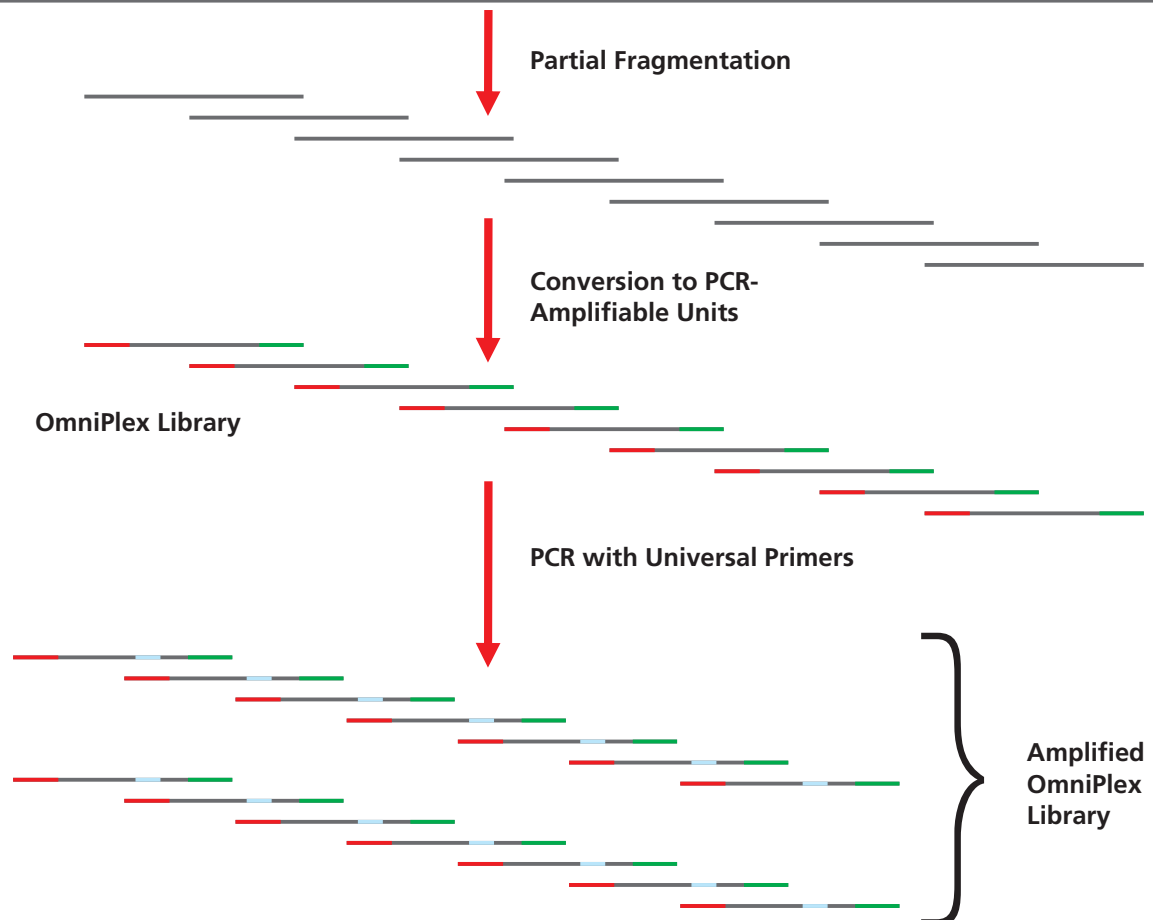


Figure 2. Overview of GenomePlex WGA

The concentration requirements of starting material also differ for the two amplification methods. Traditional PCR typically requires approximately 100 nanograms of starting material and produces on average microgram amounts of sequence-specific amplicon. Whole genome amplification by GenomePlex WGA technology provides much more versatility by requiring only sub-nanograms amounts of genomic DNA while still affording yields similar to traditional PCR.

GenomePlex WGA utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA. Following the fragmentation step, the samples are exposed to a one-hour incubation of cycling through various temperatures to facilitate the conversion of fragmented molecules into PCR-amplifiable molecules flanked by universal priming sites. Once the resulting OmniPlex library is combined with universal primers, WGA is carried out by PCR (Figure 2). Upon completion of PCR, amplified DNA may be purified by standard purification methods and used immediately for genetic or genomic analysis or it may be archived for future investigation. GenomePlex WGA technology is compatible with single-reaction PCR tubes and the high-throughput format of 96-well PCR plates.

Many research projects have been significantly impacted due to the limited availability of sufficient quantities of genomic DNA. With continuous efforts focusing on the development of more sensitive analytical research tools, this limitation has presented a continuous challenge for many disciplines of molecular research including tumor pathology and patient genotyping. Access to a constant source of genomic material is critical for proper identification of potential genetic markers. GenomePlex WGA technology bypasses this obstacle by ensuring equal representation of the whole genome with little or no bias (Figure 3). Unlike traditional clinical trial investigations which may require facilities to offer virtually unlimited storage capacity for samples, WGA provides a platform that minimizes collection and storage by generating concentrated high fidelity yields of genomic DNA from human blood or buccal swabs, thereby enabling researchers to easily store archived material or distribute it among their collaborators.

## Genotyping of Unamplified and GenomePlex WGA DNA

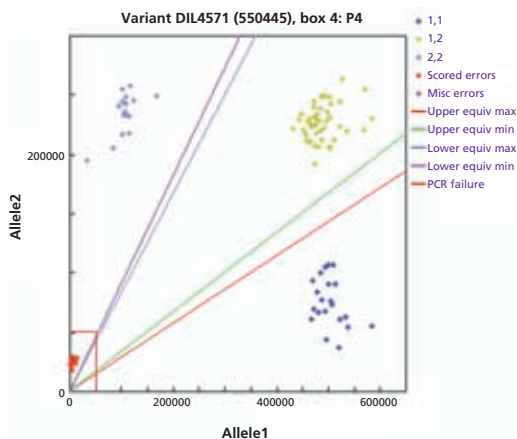


Figure 3. DNA samples isolated from buccal swabs were amplified using GenomePlex WGA and genotyped using a conventional TaqMan™ assay. The intensities and clustering of the data were comparable to those from unamplified DNA. Data provided by Rubicon Genomics, Inc.

Pathological examination of tissues or biopsies at the DNA level may occasionally be compromised due to the basic nature of tissue complexity. Often, primary tissue samples contain an inherent mixture of multiple cell populations that may alter the interpretation of results and lead to an incorrect conclusion or diagnosis. Advances in microdissection techniques have permitted the collection of purified cell populations. From the purified cell pool, multiple copies can be made by WGA leading to increased analytical possibilities. One caveat of most WGA methods is the exposure of the sample to a series of preparative manipulations prior to amplification. GenomePlex WGA technology eliminates the risk of losing precious DNA sample by utilizing a platform that does not require sample manipulations or purification methods prior to amplification.

Additional research areas such as genotyping have been significantly enhanced due to WGA. GenomePlex WGA provides a robust method for the generation of significant amounts of genomic DNA necessary for comprehensive genome scans.<sup>3</sup> In addition to sequence variation studies, single nucleotide polymorphisms (SNPs) analysis has proven useful in comparison studies with gene expression levels and consequently enabled scientists to dissect disease pathways and concentrate their efforts on the development of more applicable patient therapeutics (Figure 4).

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## Isolation and Analysis of Cancer Cell DNA by GenomePlex WGA

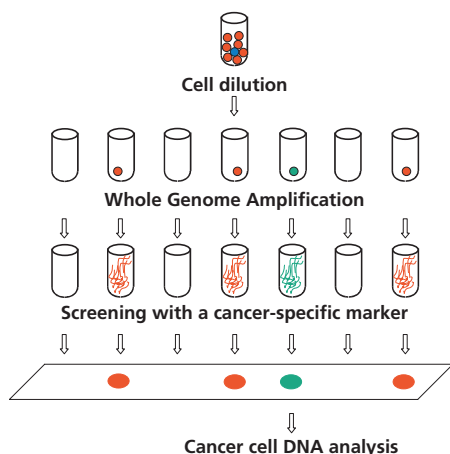


Figure 4.

**Summary**

Since the early 1990s, the evolution of whole genome amplification technologies has resulted in significant improvements that have ultimately eased the constraint of limited genomic DNA. The superior technology of GenomePlex WGA allows multiple disciplines of molecular research to extend beyond the limitations of traditional PCR. In less than three hours, nanogram amounts of genomic DNA from numerous sources, such as cell cultures, blood or even tissue, are amplified into microgram yields. This DNA can be archived for future use or analyzed by a variety of genetic tools. The unsurpassed yield and unlimited potential of the GenomePlex WGA technology makes it the obvious choice for genetic research.

As a leading supplier of biochemicals and kits for the Life Science and Biotechnology community, Sigma-Aldrich is committed to addressing the evolving needs of today's researcher. Through an exclusive world-wide licensing agreement with Rubicon Genomics, we have enhanced our Nucleic Acid Amplification product line by offering an accurate and robust method to preserve and expand very small amounts of precious DNA. GenomePlex WGA kits will be available through Sigma-Aldrich in late fall 2004. Learn more about this exciting technology at [www.sigmaaldrich.com/wga](http://www.sigmaaldrich.com/wga).

**References**

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3. Barker, D., et al., Two Methods of WGA Enable Accurate Genotyping Across a 2320-SNP Linkage Panel. *Genomic Research* **14**; 901-907 (2004).

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