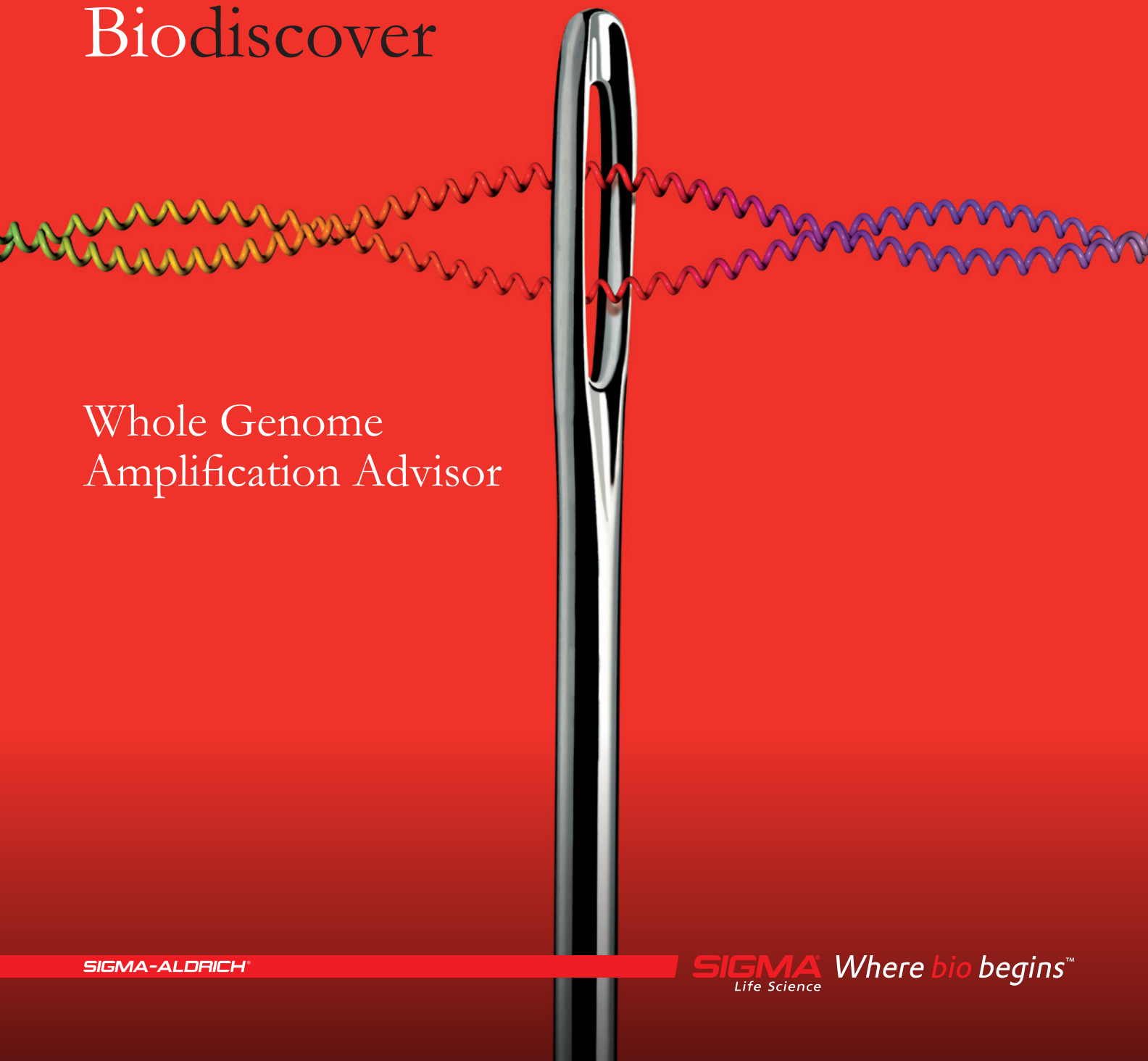
biomapping

Biodiscover



Whole Genome Amplification Advisor

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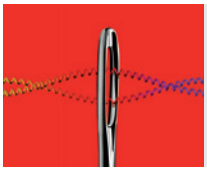


Biodiscover
Whole Genome Amplification Advisor

Table of Contents

Introduction to Whole Genome Amplification

The availability of sufficient quantities of genomic DNA is crucial for numerous analyses used in the study of human and animal disorders. In many cases, the DNA source is limited or the available DNA is damaged or degraded preventing the researcher from performing crucial analyses.

Genomic material is preserved in many ways including buccal swabs, fresh tissue samples, frozen tissue samples, blood cards, peripheral blood and formalin-fixed, paraffin-embedded (FFPE) tissues. Until recently, the genetic information from some of these sources could only be amplified by gene specific polymerase chain reaction (PCR). Unfortunately, there are drawbacks to gene specific PCR.

The technique leads to rapid depletion of precious genomic material and only allows for analysis of specifically amplified genes. An additional problem is that by only studying the amplified genes, important genetic information essential for making prognostic decisions can go unnoticed. As a result of the roadblocks presented by DNA starting material and the methods used to amplify DNA, the desire to preserve complete genomic DNA samples led to the development of whole genome amplification (WGA) technology. The technology emerged in the early 1990's and quickly evolved into a field of its own within molecular cell biology. WGA technology allows immortalization of a limited sample providing researchers with the capability to perform all desired analyses on one DNA sample.

WGA Methodologies

Whole Genome Amplification allows for representative amplification of the whole genome, preserving what was a limited sample and allowing for the analysis of any gene in the organism being studied. Currently, there are two different methodologies for performing WGA: variations of PCR amplification and isothermal DNA amplification. The PCR amplification category includes techniques to attach random primers, degenerate primers and adaptors to genomic DNA fragments to create universal priming regions. Whole genome amplification emerged initially with degenerate oligonucleotide primer (DOP) PCR and primer extension pre-amplification (PEP) PCR, which led to the development of the linker adaptor technique. The linker adaptor technique takes advantage of the fragmentation of DNA by enzymatic and chemical means prior to the attachment of adaptors and amplification via PCR. The second method for whole genome amplification, isothermal DNA amplification, utilizes multiple strand displacement (MSD) amplification using phi29 DNA polymerase.

Both PCR amplification and isothermal DNA amplification methodologies generate several micrograms of amplified DNA with as little as 1 nanogram of starting material. The choice of methodology for WGA is often dictated by the source from which the DNA was obtained. For instance, isothermal DNA amplification using multiple strand displacement requires high quality DNA samples in excess of 2 kb in size for successful amplification. Such DNA samples can be obtained from fresh tissues and blood samples, but not from FFPE tissues or damaged DNA. The PCR based methods are generally more tolerant of damaged DNA samples. As a result, PCR methods are applicable to a variety of sources.

When implementing WGA, it is critical to assess the entire research workflow and the desired results to assess which methodology may be more advantageous. It is also important to remember that handling of the sample, the DNA source, the quantity and the quality of the DNA, storage conditions, and the extraction technique are all factors that can affect WGA directly or indirectly. These factors should be taken into consideration when selecting the most appropriate WGA method. Regardless of the methodology used to amplify the genomic material, the ultimate result is that the amplified DNA is indistinguishable from the original genomic information.

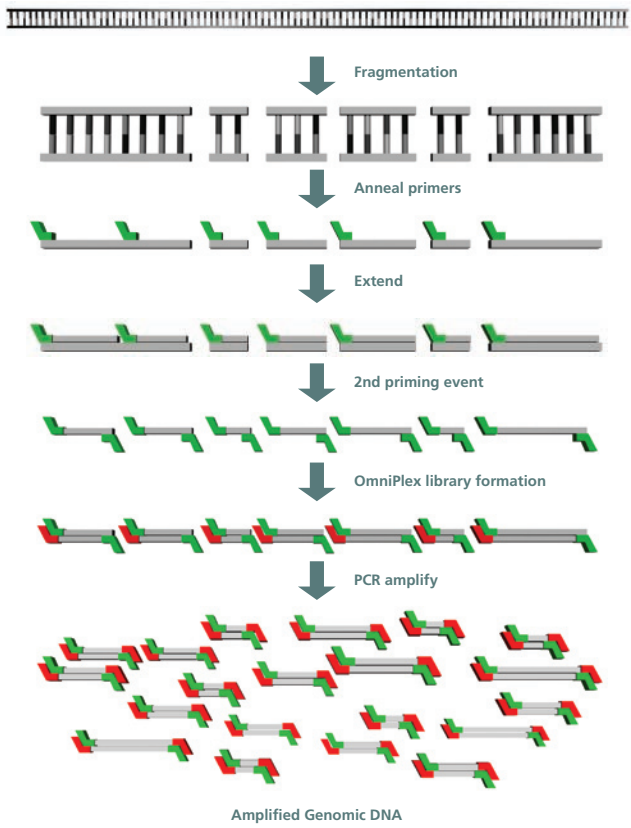
Unbiased amplification is desired and necessary for successful research employing whole genome amplification. A WGA method must result in high quality, representative samples every time. The WGA method used should also be applicable to a wide spectrum of sources. WGA methodology via PCR based on random fragmentation followed by the annealing of universal adapters not only results in high quality samples, but it is also applicable to a variety of source materials. This method provides a comparable yield to other WGA methods producing microgram quantities of DNA from as little as 1 nanogram of starting material; however, amplification is most successful with 10 nanograms of starting material yielding 5–10 micrograms of amplified product.

The random fragmentation WGA method has been used to successfully amplify genomic material from sources such as: saliva, whole blood, blood card, buccal swab, commercially available human genomic DNA, soil, bacterial artificial chromosome, formalin-fixed, paraffin-embedded tissues from rat and human, numerous plant sources and a variety of animal sources. The range of this methodology appears to have no limit. Random fragmentation is achieved by non-enzymatic digestion/cleavage, which leads to generation of a library of genomic fragments ranging in size from 100–1000 base pairs with an average size of approximately 400 base pairs. The fragments are subsequently amplified with a limited number of PCR cycles by taking advantage of a robust DNA polymerase. Annealing of universal adapters to the 5′ and 3′ ends of each of the fragments allows for specific priming with universal primers followed by amplification. The use of adapter-specific primers and optimized DNA polymerase enzyme results in specific amplification of the desired product. The GenomePlex® Whole Genome Amplification Kit from Sigma-Aldrich® utilizes the random fragmentation and annealing of adapters technique via PCR to generate high quality, amplified genomic DNA from a wide variety of sources.

GenomePlex® Whole Genome Amplification

Since its introduction to the market, the GenomePlex technology has evolved into five powerful kits for whole genome amplification. The GenomePlex Whole Genome Amplification Kit (**Cat. No. WGA1**) does not include a DNA polymerase providing researchers with the flexibility to use their polymerase of choice to proceed with amplification of the whole genome. The GenomePlex Complete Whole Genome Amplification Kit (**Cat. No. WGA2**) includes a DNA polymerase mix designed for optimal amplification. The GenomePlex Whole Genome Reamplification Kit (**Cat. No. WGA3**), allows for reamplification of products produced using any of the WGA kits resulting in an even higher yield of DNA. The GenomePlex Single Cell WGA Kit (**Cat. No. WGA4**) was developed for use with single cells and includes an optimized cell lysis protocol, which has been incorporated into the fragmentation step. The newest member of the GenomePlex product family is the GenomePlex Tissue Whole Genome Amplification Kit (**Cat. No. WGA5**). This product allows whole genome amplification directly from formalin-fixed, paraffin-embedded (FFPE), frozen, RNA*later*®-preserved or fresh tissue. The kit includes optimized reagents for tissue disruption and cell lysis eliminating the need for tedious organic extractions to remove excess paraffin or DNA purification prior to amplification. The GenomePlex technologies use the same amplification process whether using the WGA1, WGA2, WGA4, or the WGA5 Kit. The processes involved in amplification of DNA include: random fragmentation of input genomic DNA, generation of the OmniPlex® library and OmniPlex library amplification. When amplification is complete, it is strongly recommended to purify the amplified products. Once WGA has been performed using WGA1, WGA2, WGA4, or WGA5, reamplification can be performed using WGA3 if desired.

GenomePlex WGA Kit Process Overview



GenomePlex® Whole Genome Amplification

Overview of the GenomePlex WGA Workflow

Random Fragmentation

For successful whole genome amplification, the input genomic DNA must be appropriately diluted with PCR grade water to a concentration of 1 ng/µl. The incubation step (4 minutes at 95 °C) denatures double stranded DNA into single stranded DNA and facilitates non-enzymatic fragmentation. The incubation step is time and temperature sensitive and any deviation from the suggested incubation time and temperature may result in compromised amplification yields due to inappropriate fragmentation. The result is genomic DNA that is randomly fragmented into overlapping segments. The fragments range in size from 100–1000 base pairs with an average of about 400 base pairs. The randomly fragmented DNA must be immediately processed to the next step: generation of the OmniPlex library. Unprocessed samples may degrade as the DNA fragments are not stable.

Generation of the OmniPlex® Library

Upon addition of the preparation buffer that contains degenerate adapters and stabilization buffer, the randomly fragmented DNA is incubated at 95 °C for 2 minutes. The OmniPlex library is generated via a traditional PCR reaction. This process takes a little over an hour. The PCR reaction allows for annealing of universal adapters to 5′ and 3′ ends of each of the DNA fragments. The universal adapters provide sites to which adapter-specific primers can anneal in order to facilitate amplification. The OmniPlex library can immediately be amplified or it can be stored at –20 °C up to three days.

Library Amplification

The OmniPlex library is PCR amplified via a single proprietary primer. The universal adapters at the 5′ and 3′ ends allow for priming to occur. After 14 PCR cycles, the OmniPlex Library will have been amplified 500–1000 fold in a total processing time of three hours generating 5–10 µg of amplified genomic DNA.

Purification of Amplified Products

It is recommended to purify the amplified DNA with a PCR cleanup kit (**Cat No. NA1020**) to ensure removal of salts and free nucleotides before quantitation or downstream processing. GenomePlex amplified DNA can be stored at 2–8 °C for short periods of time, but should be kept at –20 °C for long-term storage. GenomePlex products can be used in any application using genomic DNA such as genotyping, sequencing, microarrays, microsatellite analysis, PCR, comparative genomic hybridization (CGH) analysis, TaqMan® assays, or single nucleotide polymorphism (SNP) analysis. GenomePlex Whole Genome Amplification technology can be applied in a wide range of fields including drug discovery, environmental biology, forensics and plant research.

Reamplification with GenomePlex

The GenomePlex Whole Genome Reamplification Kit (**Cat. No. WGA3**) allows the user to obtain even higher yields of desired DNA without disturbing the original, limited source of genomic DNA. The products from WGA1, WGA2, WGA4, and/or WGA5 can be reamplified using WGA3. WGA3 utilizes the same library amplification technology as WGA1 and WGA2. The procedure works optimally with at least 10 ng of input DNA. Using less than 10 ng of input DNA may result in lower yields. Additionally, the products from WGA1, WGA2, WGA4, or WGA5 can be reamplified up to five cycles with minimal allele dropout producing an even higher yield. It is important to note that a higher number of reamplification cycles may introduce slight bias representation.

Whole Genome Amplification for Single Cell Biology

By Ernie Mueller and Chad Brueck Sigma-Aldrich Corporation, St. Louis, MO, USA

Introduction

The ultimate biological unit lies within a single cell. Many biological disciplines have taken aim to elucidate the causes of cellular differentiation at this level. The secret triggers that signal human maturation, regeneration and genetic diseases all lie buried in a single cell that was originally part of the genetically clonal, multicellular organism. Despite careful work with sophisticated instrumentation available for the dissection of tissue samples, several studies suggest that pooled cell samples, thought to be homogenous, are often composed of cells with quite different phenotypes (**e.g. ref 1**).

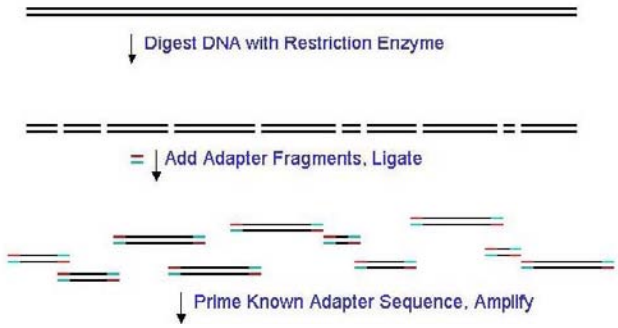
Development and commercialization of economical, easier-to-use single cell tools have enabled more researchers to explore this novel area. These include means to isolate single cells, such as fluorescence activated cell sorting, laser capture micro dissection, optical tweezers and atomic force microscopy. Once single cell samples were readily available, applications such as fluorescent *in situ* hybridization or FISH[2] and single cell PCR[3] could be used to identify the differences between populations of single cells. This has resulted in an explosion of work and speculation in the field of single-cell biology[4-6].

Adapting multicell/tissue techniques to single cell study often have limited utility because of technical shortcomings – mostly problems related to sensitivity. Despite the raw potential for single cell genomic analysis, the field has been restricted to comparative analysis of relatively few genomic loci for large numbers of single-cell isolates. Techniques such as FISH or single-cell PCR can be only used to probe a small number of DNA sequences before the cell is destroyed. Likewise, the small sample size of a single cell has so far allowed limited investigation of gene expression, proteomic make up and the characterization of cell metabolites.

Whole genome amplification (WGA) offers a means to overcome the above restrictions for single-cell genomic analyses. WGA has been described as a non-specific amplification technique that affords an amplified product completely representative of the initial starting material. Three different strategies for WGA have been described in the literature.

History

Linker adapter PCR was first described in 1989[9]. In this method, the target DNA is digested with an appropriate restriction enzyme and then each end is ligated to an adaptor. These known adaptor sequences are used to uniformly amplify each of the many DNA fragments representing the original sample. The method relies on absolutely efficient ligation and unbiased amplification between the identical primed regions.

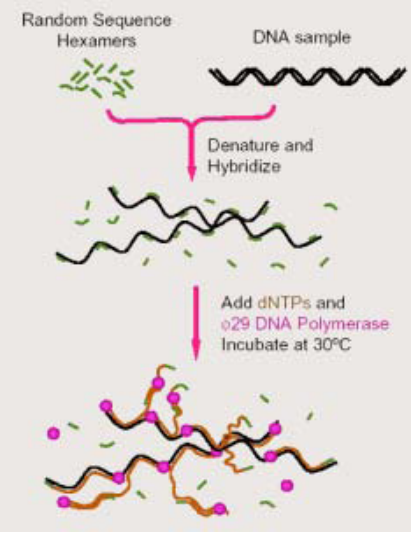


Primer extension pre-amplification (PEP) PCR, in contrast, uses a set of random hexamers to prime template DNA[10]. The subsequent thermal cycling conditions use very low (permissive) annealing temperatures and fifty or more cycles to create a series of fragments representing the original input DNA. Bias in the resulting PEP PCR product is due to non-uniformity of random hexamer annealing and extension -DNA sections with infrequent or distant priming events tend to be discriminated against in this method. These shortfalls were largely overcome with multiple strand displacement (MSD) amplification[11]. The MSD technique employs a unique and highly processive mesophillic DNA polymerase, phi29. The resulting product consists of long, ~10-50kb, fragments, but good amplification and representation.

Finally, degenerative oligonucleotide primer (DOP) PCR, also described as arbitrary PCR, relies on a set of oligos with a random 3'-end and partially fixed 5'-sequence[12]. These primers are designed

Whole Genome Amplification for Single Cell Biology

to anneal relatively evenly throughout the DNA sample. Once extended by a polymerase, these products are amplified using oligos targeting their fixed sequences. Primer design is critical for this technique – the oligo must bind relatively evenly throughout the DNA sequence but not bind to other oligonucleotides. This method has also been successfully applied to give representative samples.



Phi29 DNA Polymerase Extension Process

Random DNA hexamers act as primers, on Genomic DNA target. Isothermal amplification conditions enable the extension of new DNA templates, with the addition of dNTPs and phi29 DNA polymerase.

Single Cell Analysis

Each of these techniques has been applied to the problem of amplifying the genetic material in a single cell, and has met with some success. PEP PCR was the first to be applied to single cell WGA, and was successfully applied in several subsequent applications[13-14]. A variant of the DOP PCR, developed by Rubicon Genomics, was used to amplify single chromosomes[15], a feat very shortly followed by the use of a linker-adaptor PCR method to also completely amplify a single chromosome[16]. Finally, MSD with phi29 was used to amplify a series of single cells[8,17].

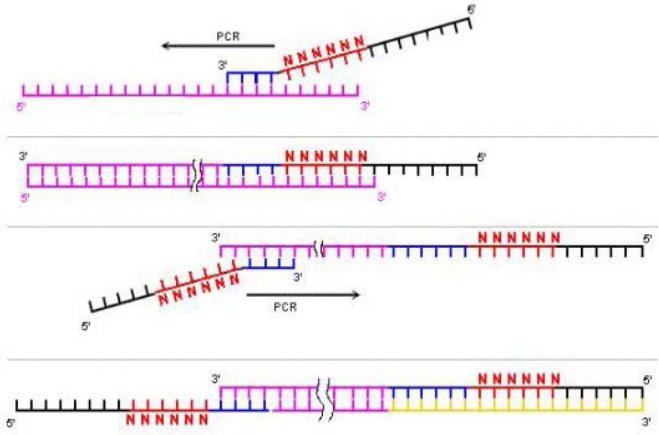
WGA methods differ in two respects: the amount of bias in the product when using limiting amounts of input template and the quality requirements for the input template. The former issue, which in single cell applications manifests itself as apparent loss of information or allelic drop out (ADO), is thought to be due to inequities of local distribution of the reagents near the target[28].

The latter phenomenon is dependent on the method, and the fact that damaged DNA can render certain loci unamplifiable.

WGA methods that generate long amplicons, like MSD, can be less robust because priming events are necessarily few and therefore any error in a long amplicon causes a relatively large loss of information. WGA that generates short amplicons, such as PEP PCR, linker-adaptor amplification and DOP PCR, lose less information in such circumstances.

Both DOP PCR and MSD amplification are now available in commercial molecular biology kits, but only the GenomePlex® kit offered by Sigma-Aldrich has been developed specifically for single cell applications. The technique builds on a product line that was introduced first by Rubicon, and acquired in the fall of 2004 by Sigma-Aldrich as the sole licensee and distributor. Since that time, Sigma-Aldrich has developed four WGA related products, including the original kit (WGA1), a kit with an optimized DNA polymerase (WGA2), a kit to reamplify WGA DNA (WGA3) and a kit for single cell applications (WGA4). At the heart of this product line is a PCR-based WGA method that employs degenerate oligonucleotides coupled with universal adaptors in a combination of PEP and DOP amplification methods. GenomePlex can faithfully amplify ten nanograms of genomic DNA in about three hours.

The single cell WGA kit, released in February 2006, is able to produce a million-fold amplification of a flow-sorted or laser micro-captured single cell resulting in approximately 5µg of final yield. The single



Single Primer PCR Amplifies GenomePlex Library

Traditional PCR annealing and extension are essentially single primer PCR amplification, once the GenomePlex library has been created. A pool of amplicons, all containing a universal priming site, are cycled using a single amplification primer.

cell method differs from the original kit in three ways. First of all, WGA4 includes a cell lysis protocol that combines an efficient lysis procedure with the original fragmentation. Secondly, the subsequent isothermal library preparation steps use a newly optimized primer that gives better coverage at low template but maintains low self-annealing and thus undetectable primer elongation. Finally, the amplification cycling protocol has been modified to use more cycles, allowing a greater total amplification. The single cell WGA product was tested using 96 separate SYBR® Green qPCR loci, and has a demonstrated 25-33% random Allelic Drop Out (ADO). The single cell WGA work was validated with multiple beta-testers using STR analysis, RPLF assays, quantitative PCR and microarray analysis.

Advances in single cell WGA will allow the researcher to uncover the contribution of genomics to single cell biology. Specifically, cancer and drug discovery research within genomics shows the greatest potential for opportunity. Chromosomal aberrations, as a result of cancer, could be better catalogued when comparing a single cancerous cell to its normal counterpart. In addition, comparing single cells from the 'treated' population to the 'untreated' to evaluate genomic effects can be used to screen drug candidates.

Understanding differences at the level of a single cell is the ultimate goal of biology. New, commercialized techniques, such as single cell WGA, are opening a new frontier for further study. Considerable work has already been accomplished toward the sensitive, unbiased amplification of single cell RNA to allow for single cell gene expression[7,22-27] and this area has already seen the development of commercialized kits to respond to this customer need. As researchers continue to find sensitive means to explore epigenetics, proteomics[18-19], metabolomics and cell signaling[20-21], the whole world of single cell biology will be revealed.

MicroArray Workflow

A DNA microarray is a proven technology used by molecular biology researchers to study a large number of genetic signatures at one time. It consists of an arrayed series of thousands of microscopic spots of DNA oligos, called features, each containing a specific DNA sequence, known as a probe. These can be a short section of a gene or other DNA element that are used to a DNA sample under high-stringency conditions. Probe-target hybridization is usually detected and quantified by detection of flourescent probe or chemiluminescence labeled targets to determine relative abundance of nucleic acid sequences in the target. Arrays can contain millions of probes, accelerating many types of investigation.

In standard microarrays, the probes are attached to a solid surface by a covalent bond to a chemical. The solid surface can be solid glass or a silicon chip. Other microarray platforms, use microscopic beads, instead of the large solid support.

DNA microarrays can be used to measure changes in gene expression levels, to detect single nucleotide polymorphisms (SNPs), or to genotype or resequence mutant genomes.

Protocols

Extraction Protocol—Blood Card

Blood cards provide the convenience of archiving small volumes of blood. However, many times genomic DNA from these samples is limited, which may hinder the researcher's ability to perform downstream analysis. This protocol provides a simple and convenient method to extract genomic DNA from a blood card. Once the DNA has been extracted, it can then be amplified using the amplification protocol on page 21.

Required Products

- GenElute™ Blood Genomic DNA Kit (**Cat. No. NA2000**)

Materials to be Supplied by the User

- Blood card
- 1.5 ml microcentrifuge tubes
- Ethanol (**Cat. No. E7023**)
- Microcentrifuge (with rotor for 2 ml tubes)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 55 °C water bath or heat block

Extraction of DNA from a Blood Card

The **GenElute Blood Genomic DNA Kit** (**Cat. No. NA2000**) is recommended for this procedure.

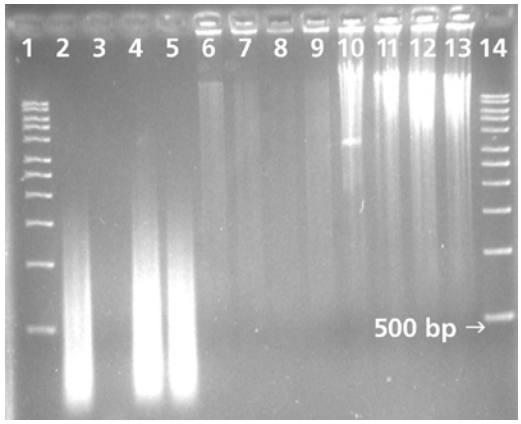
1. Cut a disc from a dried blood card (200 µl spotted) into several 2 mm by 2 mm pieces and place the pieces into a 1.5 ml microcentrifuge tube.
2. Add 40 µl Proteinase K and 1.0 ml Resuspension Solution.
3. Add 300 µl of Lysis Solution C and vortex thoroughly for 15 seconds.
4. Incubate at 55 °C for 10 minutes.
5. After the incubation, transfer the liquid (discard blood card remaining in the microcentrifuge tube) to a 15 ml conical tube.
6. Add 500 µl of Column Preparation Solution to the GenElute Miniprep Binding Column (red o-ring) and centrifuge at 12,000 × *g* for 1 minute.
7. Discard the flow-through liquid.

   Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
8. Add 900 µl of 95–100% ethanol to the lysate in the 15 ml conical tube and mix thoroughly by vortexing 5–10 seconds.
9. Transfer the contents of the tube into the treated column from step 6. Centrifuge at ≥6500 × *g* for 1 minute. Repeat until all of the lysate has been passed through the column.
10. Discard the collection tube and flow-through. Place the column in a new 2 ml collection tube.
11. Add 500 µl of Prewash Solution (be sure to dilute with ethanol prior to first use) and centrifuge for 1 minute at ≥6500 × *g*.
12. Discard the collection tube containing the flow-through and place the binding column into a new 2 ml collection tube.
13. Add 500 µl of Wash Solution (be sure to dilute with ethanol prior to first use) to the binding column and centrifuge at maximum speed (12,000–16,000 × *g*) for 3 minutes to dry the binding column.
14. Pipette 400 µl of Elution Solution onto the column and centrifuge for 1 minute at ≥6500 × *g* to elute the DNA.
15. Store the eluted DNA at –20 °C or proceed to the amplification step.

Amplification Protocol

See page 21.

Application Data



Amplified Human Genomic DNA from Blood Card

Products were amplified using the GenomePlex® Whole Genome Amplification Kit (**Cat. No. WGA1**) from Sigma, Supplier A's kit and Supplier Q's kit. Products were resolved on a 1.5% agarose gel. 5 µl of amplified product was added to each well. The products amplified using GenomePlex technology were of a smaller molecular weight as shown on the gel when compared to Supplier A and Q. This is due to the random fragmentation of genomic DNA prior to amplification. Sigma's amplified products are specific and there is no amplicon visible in the negative control (lane 2) indicating that only the desired genomic DNA is amplified. Both Suppliers A and Q yield a nonspecific signal in the negative control which is equal in size and intensity to the signal for the suppliers' positive control.

Lane 1—1 kb Marker
Lane 2—Sigma Positive Control
Lane 3—Sigma Negative Control
Lane 4—Sigma Blood Card
Lane 5—Sigma Blood Card
Lane 6—Supplier A Positive Control
Lane 7—Supplier A Negative Control
Lane 8—Supplier A Blood Card
Lane 9—Supplier A Blood Card
Lane 10—Supplier Q Positive Control
Lane 11—Supplier Q Negative Control
Lane 12—Supplier Q Blood Card
Lane 13—Supplier Q Blood Card
Lane 14—1 kb Marker

Extraction Protocol—Whole Blood

Whole blood is a common source of material used to perform genetic analysis. Many times genomic DNA isolated from whole blood samples is of low yield. This can hinder the researcher's ability to perform downstream analysis. The following protocol is a simple method to isolate DNA from fresh or aged whole blood products. Once the DNA is isolated, it can be amplified using the GenomePlex® Whole Genome Amplification protocol on page 21.

Required Products

- GenElute™ Blood Genomic DNA Kit (**Cat. No. NA2000**)

Materials to be Supplied by the User

- Whole blood
- 1.5 ml microcentrifuge tubes
- Ethanol (**Cat. No. E7023**)
- Microcentrifuge (with rotor for 2 ml tubes)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 55 °C water bath or heat block

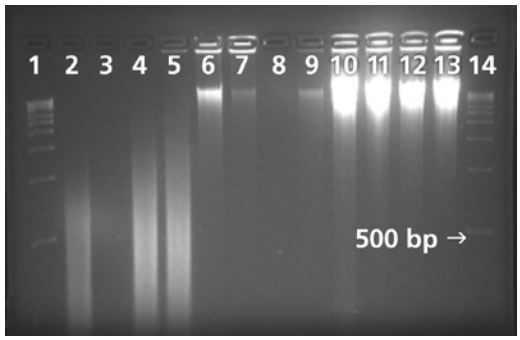
Extraction of DNA from Whole Blood

The GenElute Blood Genomic DNA Kit (**Cat. No. NA2000**) is recommended for this process.

1. Place 20 µl of Proteinase K into a 1.5 ml microcentrifuge tube and add 200 µl of whole blood to the tube.
2. Add 200 µl of Lysis Solution C and vortex thoroughly for 15 seconds.
3. Incubate at 55 °C for 10 minutes.
4. Add 500 µl of Column Preparation Solution to the GenElute Miniprep Binding Column (red o-ring) and centrifuge at 12,000 × *g* for 1 minute.

   Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
5. Discard the flow-through liquid.
6. Add 200 µl of 95–100% ethanol to the lysate from step 3 and mix thoroughly by vortexing 5–10 seconds.
7. Transfer the entire contents of the tube into the treated column (from step 4). Centrifuge at ≥6500 × *g* for 1 minute.
8. Discard the collection tube and flow-through. Place the column into a new 2 ml collection tube.
9. Add 500 µl of Prewash Solution (be sure to dilute with ethanol prior to first use) and centrifuge at ≥6500 × *g* for 1 minute.
10. Discard the collection tube containing the flow-through and place the column into a new 2 ml collection tube.
11. Add 500 µl of Wash Solution (be sure to dilute with ethanol prior to first use) to the binding column and centrifuge at maximum speed (12,000–16,000 × *g*) for 3 minutes to dry the binding column.
12. Pipette 200 µl of Elution Solution onto the column and centrifuge for 1 minute at ≥6500 × *g* to elute the DNA.
13. Store the eluted DNA at –20 °C or proceed with the amplification step.

Application Data

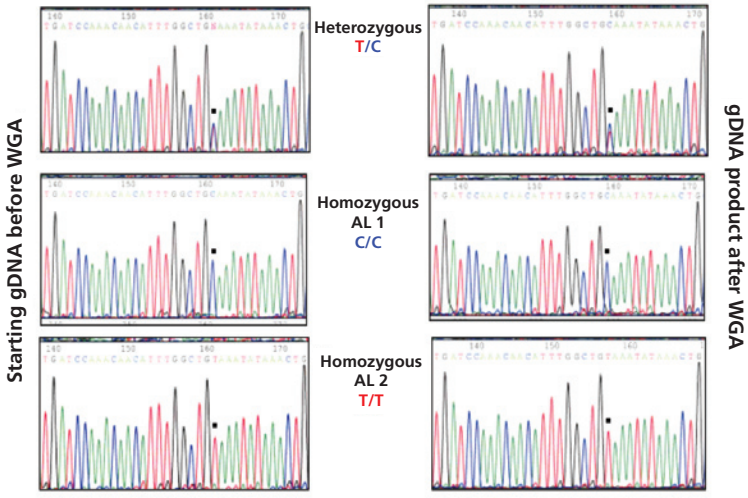


Human Whole Blood Amplified DNA-Comparison Data

Products were amplified using the GenomePlex Whole Genome Amplification Kit (**Cat. No. WGA1**) from Sigma, Supplier A's kit and Supplier Q's kit. Products were resolved on a 1.5% agarose gel. 5 µl of amplified product was added to each well. The products amplified using GenomePlex technology were of a smaller molecular weight as shown on the gel when compared to Supplier A and Q. This is due to the random fragmentation of genomic DNA prior to amplification. Sigma's amplified products are specific and there is no amplicon visible in the negative control (lane 2) indicating that only the desired genomic DNA is amplified. Both Suppliers A and Q yield a nonspecific signal in the negative control which is equal in size and intensity to the signal for the suppliers' positive control.

Lane 1—1 kb Marker
Lane 2—Sigma Positive Control
Lane 3—Sigma Negative Control
Lane 4—Sigma Blood Card
Lane 5—Sigma Blood Card
Lane 6—Supplier A Positive Control
Lane 7—Supplier A Negative Control
Lane 8—Supplier A Blood Card
Lane 9—Supplier A Blood Card
Lane 10—Supplier Q Positive Control
Lane 11—Supplier Q Negative Control
Lane 12—Supplier Q Blood Card
Lane 13—Supplier Q Blood Card
Lane 14—1 kb Marker

Extraction Protocol—Whole Blood



Performance of DNA Amplified with GenomePlex® WGA Identical to Non-Amplified DNA

Genomic DNA was extracted from a whole blood sample using the GenElute™ Blood Genomic DNA Kit (**Cat. No. NA2000**). 10 ng of genomic DNA was amplified using the GenomePlex WGA Kit (**Cat. No. WGA1**) followed by purification using the GenElute PCR Clean-up Kit (**Cat. No. NA1020**). SNP genotyping analysis was performed on non-amplified DNA and GenomePlex amplified DNA. GenomePlex WGA DNA genotyping provided the same accuracy and quality of scores to non-amplified DNA, indicating that the amplification process did not alter the original genomic sequence.

Whole Genome Amplification from Serum or Plasma

Whole genome amplification (WGA) of plasma and serum DNA presents a unique challenge due to the small amount of nucleic acid in such samples. One must use a robust DNA purification scheme capable of removing inhibitory substances, while at the same time, concentrating the DNA within the sample. The use of GenElute™ Blood Genomic DNA Kit in combination with one of two modified WGA protocols has been shown to work well with DNA isolated from serum or plasma. Both procedures typically produce approximately 5 µg of WGA product. Old serum and plasma samples may contain degraded DNA, and therefore may not be suitable for downstream applications such as WGA. The WGA procedures described below are modifications of the WGA2 and WGA4 protocols. The procedures work equally well, each typically producing approximately 5 µg of WGA product.

Required Products

- GenElute™ Blood Genomic DNA Kit (**Cat. No. NA2000**)

Materials to be Supplied by the User

- Serum or Plasma
- 1.5 ml microcentrifuge tubes
- Ethanol (**Cat. No. E7023**)
- Microcentrifuge (with rotor for 2 ml tubes)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 55 °C water bath or heat block

Extraction of DNA from Serum or Plasma

The GenElute Blood Genomic DNA Kit (**Cat. No. NA2000**) is recommended for this process. It is recommended to start with 0.1 ml to 1 ml serum or plasma. The protocol below is written for 0.1 ml to 0.2 ml serum or plasma. Scale GenElute reagents proportionally if using more than 0.2 ml serum or plasma.

1. Place 20 µl of Proteinase K into a 1.5 ml microcentrifuge tube and add 200 µl of plasma or serum to the tube.
2. Add 200 µl of Lysis Solution C and vortex thoroughly for 15 seconds.
3. Incubate at 55 °C for 10 minutes.
4. Add 500 µl of Column Preparation Solution to the GenElute Miniprep Binding Column (red o-ring) and centrifuge at 12,000 × *g* for 1 minute.

   Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
5. Discard the flow-through liquid.
6. Add 200 µl of 95–100% ethanol to the lysate from step 3 and mix thoroughly by vortexing 5–10 seconds.
7. Transfer the entire contents of the tube into the treated column from step 4. Centrifuge at 6500 × *g* for 1 minute.
8. Discard the collection tube and flow-through. Place the column into a new 2 ml collection tube.
9. Add 500 µl of Prewash Solution (be sure to dilute with ethanol prior to first use) and centrifuge at 6500 × *g* for 1 minute.
10. Discard the collection tube containing the flow-through and place the column into a new 2 ml collection tube.
11. Add 500 µl of Wash Solution (be sure to dilute with ethanol prior to first use) to the binding column and centrifuge at maximum speed (12,000–16,000 × *g*) for 3 minutes to dry the binding column.
12. Pipette 50 µl of Elution Solution onto the column and centrifuge for 1 minute at 6500 × *g* to elute the DNA.
13. Store the eluted DNA at –20 °C or proceed to the amplification step with WGA2 or WGA4.

Amplification with WGA2

1. Combine 10 µl of serum or plasma DNA, purified as outlined above, and 1 µl of 10× Fragmentation Buffer to a PCR tube or multiwell strip/plate.

   Note: Use 10 µl of DNA regardless of concentration, since spectrophotometric quantitation of very dilute DNA is not accurate. 10 µl is the maximum volume that can be used in WGA.
2. Continue with step 3 of the WGA2 protocol, however, perform 25 amplification cycles in step 13, as opposed to 14 cycles.

Amplification with WGA4

1. Combine 10 µl of serum or plasma DNA, purified as outlined above, and 1 µl of 10× Single Cell Lysis & Fragmentation Buffer to a PCR tube or multiwell strip/plate. Mix thoroughly.

   Note: Use 10 µl of DNA regardless of concentration, since spectrophotometric quantitation of very dilute DNA is not accurate. 10 µl is the maximum volume that can be used in WGA.
2. Place the tube/plate in a thermal block or cycler at 95 °C for EXACTLY 4 minutes. Immediately cool on ice. Spin down sample prior to proceeding to Library Preparation.

   Note: This incubation is very time sensitive. Any deviation may alter results.
3. Continue with the Library Preparation section (step 6) of the WGA4 technical bulletin.

Extraction Protocol—Buccal Swab

This protocol provides a simple and convenient method to isolate, amplify, and purify genomic DNA from buccal swabs. Buccal swabs are a convenient method of acquiring a DNA sample. Once the DNA is isolated using the following extraction protocol, it can be amplified using the GenomePlex® Whole Genome Amplification Protocol on page 21 to provide enough DNA for numerous downstream applications.

Required Products

- GenElute™ Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**)

Materials to be Supplied by the User

- Buccal swab
- 1.5 ml microcentrifuge tubes
- Ethanol (**Cat. No. E7023**)
- Microcentrifuge (with rotor for 2 ml tubes)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 55 °C water bath or heat block

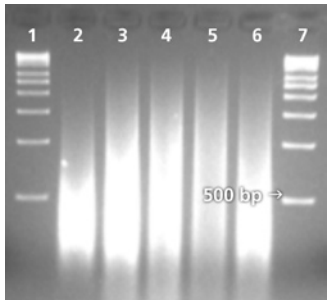
Extraction of DNA from Buccal Swab

It is recommended to use the GenElute Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**) for this process.

1. Dry the collected swabs at room temperature for 15 minutes.
2. Add 280 µl of Lysis Solution T and 20 µl of Proteinase K. Insert the swab and gently spin. Cap the tube and mix by vortexing.
3. Incubate the sample at 55 °C for 20 minutes with occasional vortexing.
4. Add 200 µl of Lysis Solution C and vortex thoroughly for 15 seconds.
5. Incubate at 70 °C for 10 minutes.
6. Add 500 µl of Column Preparation Solution to each GenElute Miniprep Binding Column (red o-ring) and centrifuge at 12,000 × *g* for 1 minute. Discard the flow-though liquid.

   Note: The Column Preparation Solution maximizes binding of DNA to the membrane resulting in more consistent yields.
7. Add 200 µl of 95–100% ethanol to the lysate from step 5.
8. Mix thoroughly by vortexing and add the entire contents of the tube into the binding column.
9. Centrifuge at ≥6500 × *g* for 1 minute.
10. Discard the collection tube containing the flow-through and place the binding column in a new 2 ml collection tube.
11. Add 500 µl of Wash Solution (be sure to dilute with ethanol prior to first use) and centrifuge at ≥6500 × *g* for 1 minute.
12. Discard the collection tube and flow-through and place the binding column in a new 2 ml collection tube.
13. Add another 500 µl of Wash Solution to the binding column and centrifuge at maximum speed (12,000–16,000 × *g*) for 3 minutes to dry the binding column.
14. Pipette 200 µl of Elution Solution onto the binding column and centrifuge for 1 minute at ≥6500 × *g*.
15. Store the eluted DNA at –20 °C or proceed to the amplification step.

Application Data



GenomePlex Whole Genome Amplification Performed on a Buccal Swab Sample

Amplified Sigma products are visualized on 1.5% agarose gel. 5 µl of amplified product was loaded per well. The GenomePlex amplified products result in an average size of 400 bp. The smear pattern varies by source as shown on the gel.

Lane 1—1 kb Ladder
Lane 2—Blood
Lane 3—Plant
Lane 4—Buccal Swab
Lane 5—Soil
Lane 6—Positive Control
Lane 7—1 kb Ladder

Extraction Protocol—Plant Tissues

Extracting DNA from plant tissue is a complicated process due to the tough cell wall that surrounds most plant cells. Genomic DNA from plant material can be damaged during the extraction process, resulting in low yields of high quality genomic material. As a result, the researcher's ability to perform downstream analysis is challenged. GenomePlex Whole Genome Amplification has been used to amplify genomic DNA from soybean, corn, tomato, purple coneflower, and ginseng.

Required Products

- GenElute™ Plant Genomic DNA Miniprep (**Cat. No. G2N10**)

Materials to be Supplied by the User

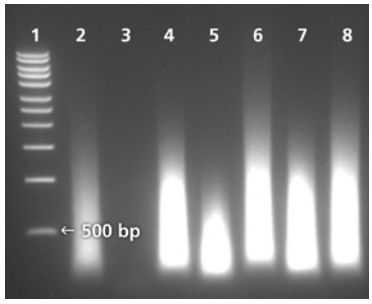
- Plant samples
- 1.5 ml microcentrifuge tubes (**Cat. No. T9661**)
- Ethanol (**Cat. No. E7023**)
- Microcentrifuge (with rotor for 2 ml tubes)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 55 °C water bath or heat block

Extraction of DNA from Plant Tissues

It is recommended to use the Sigma GenElute Plant Genomic DNA Miniprep Kit (**Cat. No. G2N10**) for this procedure.

1. Grind approximately 50 mg leaf punch into a fine powder with liquid nitrogen. Keep the sample on ice for immediate use or freeze at –70 °C.
2. Add 350 µl of Lysis Solution (Part A) and 50 µl of Lysis Solution (Part B) and thoroughly mix by vortexing. A white precipitate will form upon the addition of Lysis Solution Part B.
3. Incubate the mixture at 65 °C for 10 minutes with occasional inversion to dissolve the precipitate.
4. Add 130 µl of Precipitation Solution, mix by inversion, and place the sample on ice for 5 minutes.
5. Centrifuge at maximum speed (12,000–16,000 × *g*) for 5 minutes to pellet the cellular debris, proteins and polysaccharides.
6. Carefully pipette the supernatant onto a GenElute Filtration Column (blue insert with a 2 ml collection tube).
7. Centrifuge at maximum speed for 1 minute. Discard the Filtration Column and retain the collection tube.
8. Add 700 µl of Binding Solution directly to the flow-through (liquid from step 7). Mix thoroughly by inversion.
9. Insert the GenElute Miniprep Binding Column (red o-ring) into the provided microcentrifuge tube.
10. Add 500 µl of the Column Preparation Solution to each Miniprep Column and centrifuge at 12,000 × *g* for 1 minute. Discard the flow-through liquid.
11. Pipette 700 µl flow-through (from step 8) onto the Miniprep Column prepared in the previous step.
12. Centrifuge at maximum speed for 1 minute and discard the flow-through.
13. Apply the remaining lysate (from step 8) and repeat centrifugation for 1 minute at maximum speed and discard the flow-through.
14. Place the Binding Column in a fresh 2 ml collection tube and apply 500 µl diluted Wash Solution to the column (be sure to add ethanol to the Wash Solution Concentrate prior to first time use).
15. Centrifuge at maximum speed for 1 minute. Discard flow-through and retain the collection tube.
16. Add another 500 µl of diluted Wash Solution to the column and centrifuge at maximum speed for 3 minutes to dry the column.
17. Transfer the binding column to a fresh 2 ml collection tube.
18. Apply 100 µl of pre-warmed (65 °C) Elution Solution to the column and centrifuge at maximum speed for 1 minute. Repeat the elution.
19. Store the eluted DNA at –20 °C or proceed to the amplification step.

Application Data



GenomePlex® Whole Genome Amplification Performed on Plant Samples

Amplified Sigma products are resolved on a 1.5% agarose gel. 5 µl of amplified product was loaded per well. The GenomePlex amplified products result in an average size of 400 bp. The smear pattern varies by source as shown on the gel. Products amplified using GenomePlex technology are specific as evidenced by the lack of signal in the negative control (lane 3).

Lane 1—1 kb Marker
Lane 2—Positive Control
Lane 3—Negative Control
Lane 4—Corn
Lane 5—Ginseng
Lane 6—Purple Cornflower
Lane 7—Tomato
Lane 8—Soybean

Extraction Protocol—Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue and Diagnostic

Archived Formalin-fixed, Paraffin-embedded (FFPE) tissue samples are invaluable resources for profiling gene expression and studying a variety of diseases. Since the archived DNA is usually available in limited quantities, amplification of the samples is essential. Amplifying the FFPE tissue can be a difficult task due to the damaged template that results from the archiving process. This protocol provides a convenient method to purify and amplify genomic DNA from FFPE tissue. GenomePlex® Whole Genome Amplification has been used to amplify genomic DNA from rat and human FFPE tissue samples.

***Note:** The protocol below describes the process of DNA purification and subsequent whole genome amplification. We have dramatically streamlined this process with our GenomePlex Tissue Whole Genome Amplification Kit (Cat. No. **WGA5**). This kit includes optimized reagents for tissue disruption and cell lysis eliminating the need for tedious organic extractions to remove excess paraffin or DNA purification prior to amplification. For more information on WGA5 Amplification Protocol see p. 23.

Required Products

- GenElute™ Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**)

Materials to be Supplied by the User

- FFPE tissue
- 37 °C water bath or heat block
- Xylene
- 55 °C water bath or heat block
- Ethanol (**Cat. No. E7023**)
- 70 °C water bath or heat block
- Water, molecular biology reagent (**Cat. No. W4502**)
- Microcentrifuge (with rotor for 2 ml tubes)

Extraction of DNA from Formalin-Fixed, Paraffin-Embedded (FFPE) Tissue

This protocol was performed on rat liver tissue. The GenElute Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**) is recommended for this procedure.

1. Place a small section (20 mg) of paraffin-embedded tissue in a 2 ml microcentrifuge tube.
2. Add 1200 µl of xylene and vortex for 30 seconds.
3. Centrifuge at full speed for 5 minutes at room temperature.
4. Remove supernatant by pipetting. Do not remove any of the pellet.
5. Add 1200 µl of ethanol to the pellet to remove the residual xylene. Mix by vortexing.
6. Centrifuge at full speed for 5 minutes at room temperature.
7. Carefully remove the ethanol by pipetting. Do not remove any of the pellet.
8. Repeat steps 5–7 again.
9. Incubate the open microcentrifuge tube at 37 °C for 10–15 minutes to remove any residual ethanol by evaporation.
10. Digest Tissue: Resuspend the tissue pellet in 180 µl of Lysis Solution T.
11. Add 20 µl of Proteinase K. Mix by vortexing. Incubate at 55 °C overnight or until the tissue is completely lysed. Vortex occasionally during incubation.

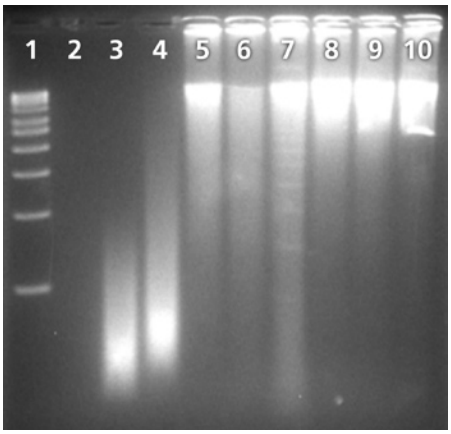
    Optional RNase treatment: If residual RNA is a concern, add 20 µl of RNase A solution and incubate at room temperature for 2 minutes.
12. Lyse cells: Vortex for 15 seconds. Add 200 µl of Lysis Solution C to the sample. Vortex thoroughly as a homogenous mixture is essential for efficient lysis. Incubate at 70 °C for 10 minutes.
13. Prepare column: Add 500 µl of the Column Preparation Solution to each pre-assembled GenElute Miniprep Binding Column and centrifuge at 12,000 × *g* for 1 minute.
14. Prepare for binding: Add 200 µl of ethanol to the lysed sample and mix by vortexing.
15. Load lysate: Transfer the entire contents of the sample tube into the treated binding column from step 14. Centrifuge at ≥6500 × *g* for 1 minute. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
16. First wash: Prior to first use, dilute the Wash Solution Concentrate with ethanol as described under preparation instructions. Add 500 µl of Wash Solution to the binding column and centrifuge for 1 minute at ≥6500 × *g*. Discard the collection tube containing flow-through liquid and place the binding column in a new 2 ml collection tube.
17. Second wash: Add another 500 µl of Wash Solution to the binding column and centrifuge for 3 minutes at maximum speed (12,000–18,000 × *g*) to dry the binding column. It is crucial that the binding column is free of ethanol before eluting DNA off the column. Centrifuge the column for an additional minute if residual ethanol is visible. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.

18. Elute DNA: Pipette 200 µl of the Elution Solution directly into the center of the binding column and incubate at room temperature for 5 minutes. Centrifuge for 1 minute at ≥6500 × *g* to elute the DNA.

19. Store DNA samples at –20 °C.

Note: This protocol can be performed without using xylene starting with step number 10. As a result of omitting the xylene treatment step, the amount of DNA will decrease by approximately 50% when compared to the protocol with a xylene step.

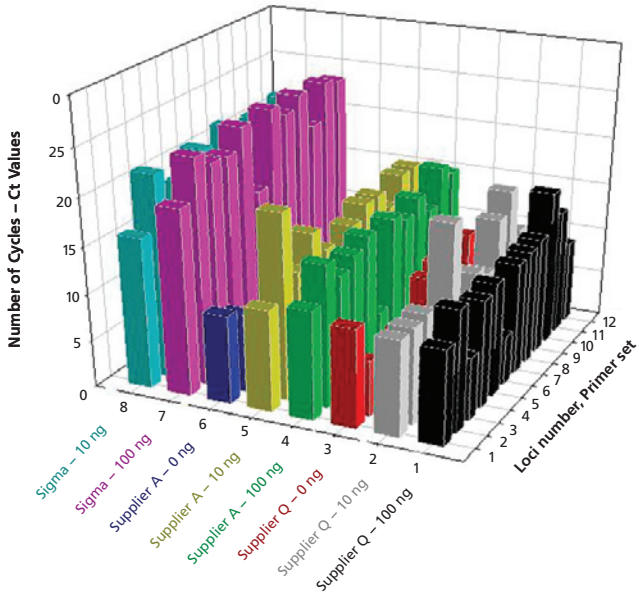
Application Data



GenomePlex® Whole Genome Amplification FFPE Tissue

Products were amplified using the GenomePlex Whole Genome Amplification Kit (Cat. No. **WGA1**) from Sigma, Supplier A, and Supplier Q. Products were resolved on a 1.5% agarose gel. 5 µl of amplified product was added to each well. The products amplified using GenomePlex technology were of a smaller molecular weight as shown on the gel when compared to Supplier A and Q. This is due to the random fragmentation of genomic DNA prior to amplification. Sigma's amplified products are specific and there is no amplicon visible in the negative control (lane 2) indicating that only the desired genomic DNA is amplified. Both Suppliers A and Q yield a nonspecific signal in the negative control which is equal in size and intensity to the signal for the suppliers' positive control.

Lane 1—1 kb Marker
Lane 2—Sigma Negative Control
Lane 3—Sigma 10 ng input DNA
Lane 4—Sigma 100 ng input DNA
Lane 5—Supplier A Negative Control
Lane 6—Supplier A 10 ng input DNA
Lane 7—Supplier A 100 ng input DNA
Lane 8—Supplier Q Negative Control
Lane 9—Supplier Q 10 ng input DNA
Lane 10—Supplier Q 100 ng input DNA



Performance of FFPE DNA Amplified with GenomePlex WGA

DNA was extracted from a sample of formalin-fixed, paraffin-embedded rat liver. 10 ng and 100 ng of genomic DNA was amplified using GenomePlex Complete WGA Kit (**Cat. No. WGA2**) followed by purification using the GenElute™ PCR Clean-up Kit (**Cat. No. NA1020**). Quantitative PCR (45 cycles) was performed on the WGA reaction using 12 different primer sets. GenomePlex demonstrates ~1000 fold (10 Ct) better representation compared to suppliers.

Qualitative Multiplex PCR Assay for Assessing DNA Quality from FFPE Tissues, and Other Sources of Damaged

The assessment of DNA quality is a crucial first step in acquiring meaningful data from formalin-fixed paraffin-embedded (FFPE) tissues, and other sources of damaged DNA.[1] Formalin reacts with nucleic acids to cause irreversible damage resulting in DNA samples of poor quality that may not work in downstream processes[2-3]. To address this issue, we've developed a simple, qualitative, gel-based multiplex PCR assay that can be used to determine DNA quality prior to performing tedious and expensive downstream processes such as array-based comparative genomic hybridization (aCGH). The assay consists of five primer sets, derived from the NCBI UniSTS database, that amplify products ranging from 132 bp to 295 bp (see table below). Some or all of these products will fail to amplify as DNA sample quality fades, allowing the classification of genomic DNA quality based on the number and size of fragments amplified, with high quality genomic DNA producing all five amplicons (**see figure 1**). This assay cannot predict the usefulness of damaged DNA without prior validation work. By performing correlative experiments to empirically determine a quality threshold, one can ensure the repeatability of their downstream experiments. For example, some applications may require that all five amplicons be produced in order to predict a successful outcome, where as others, such as qPCR, may only require the presence of a single band. In this way, one can compare samples from different sources and repeatability of experiments is ensured.

Materials to be Supplied by the User

- Water, molecular biology reagent (**Cat. No. W4502**)
- 25 mM $MgCl_2$ (**Cat. No. M8787**)
- 4% agarose gels (**Cat. No. P6097**)
- 10X TBE buffer (**Cat. No. T4323**)
- JumpStart™ REDTaq® ReadyMix™ PCR reaction mix (**Cat. No. P0982**)
- 0.2 µM fi nal concentration each primer (see table below)

Multiplex PCR Amplification of Damaged DNA (e.g. FFPE tissue DNA)

JumpStart REDTaq ReadyMix PCR reaction mix (**Cat. No. P0982**) is recommended for this process. Reagents may be scaled proportionally if performing PCR reactions of smaller volume.

Reagent Name	Sigma Cat. No.	Final Concentration	µl per Reaction
Water	**W4502 or equivalent**	NA	16
JumpStart RedTaq ReadyMix	**P0982**	1X	25
25 mM $MgCl_2$	**M8787**	3.5 mM*	3
10 µM MultiPlex Primer Mix	**NA**	0.2 µM each primer	1
TOTAL			**45**

Primer sequences

UniSTS number	Forward Primer Sequence	Reverse Primer Sequence	Amplicon Size (bp)	Chr
STB39J12.SP6	GCAAAATCCATACCCTTTCTGC	TCTTTCCCTCTACAACCCTCTAACC	132	4
STSG50529	GCTGTTAGAGCTTTTATTGCAGC	CTAGAAATTTCTGCATAAACCAACC	150	22
CSNPHARP	GCTGTTAGAGCTTTTATTGCAGC	TTGCCTCTTACAGAGGAGCAG	196	2
SHGC147491	TTTGATGTTAGGACACGCTGAAA	AAAAACGGAAGAAGTCTCTTGGC	235	12
SHGC105883	GTCAGAAGACTGAAAACGAAGCC	GCTTGCCACACTCTTCTTCAAGT	295	13

1. Prepare 10 µM MultiPlex Primer Mix by combining all ten primers in a suitable vessel at a final concentration of 10 µM each primer. Refer to the table above for primer sequences.
2. Combine the following reaction components in a suitable sized tube. Scale-up master mix appropriately for the number of reactions being performed. Make extra master mix to account for pipetting loss. **Note:** The final PCR reaction volume may be scaled up or down as long as reagent concentrations are unchanged.

   *JumpStart™ RedTaq® ReadyMix™ contains 2mM $MgCl_2$. The final $MgCl_2$ concentration is 3.5 mM after supplementing with 1.5 mM M8787.
3. Add 45 µl of the resulting master mix to an appropriate PCR tube or plate.
4. Add 5 µl of genomic DNA and mix until homogenous. For best results, use between 10 and 100 ng of template DNA per reaction. **Note:** Alternatively, 5µl of undiluted WGA5 tissue lysate, or 100 ng of WGA amplicons generated with any of Sigma's GenomePlex® products can be added instead of purified genomic DNA.
5. Place PCR tube(s) or plate in thermal cycler and amplify DNA using the following cycling parameters:
   - 94 °C for 2 minutes to denature
   - 35 cycles of:
     - 94 °C for 1 minute
     - 60 °C for 1 minute
     - 72 °C for 1 minute
   - 72 °C for 7 minutes
   - 4 °C hold
6. Resolve 5 µl of resulting amplicons on a 4% agarose gel. **Note:** JumpStart RedTaq ReadyMix contains gel-loading solution, which allows immediate sample loading onto an agarose gel after PCR. All five PCR amplicons (132 bp, 150 bp, 196 bp, 235 bp and 295 bp) will be generated with high quality genomic DNA. Low quality DNA may fail to produce any amplicons, or may present as faint bands for some amplicons **(See Fig 1**).

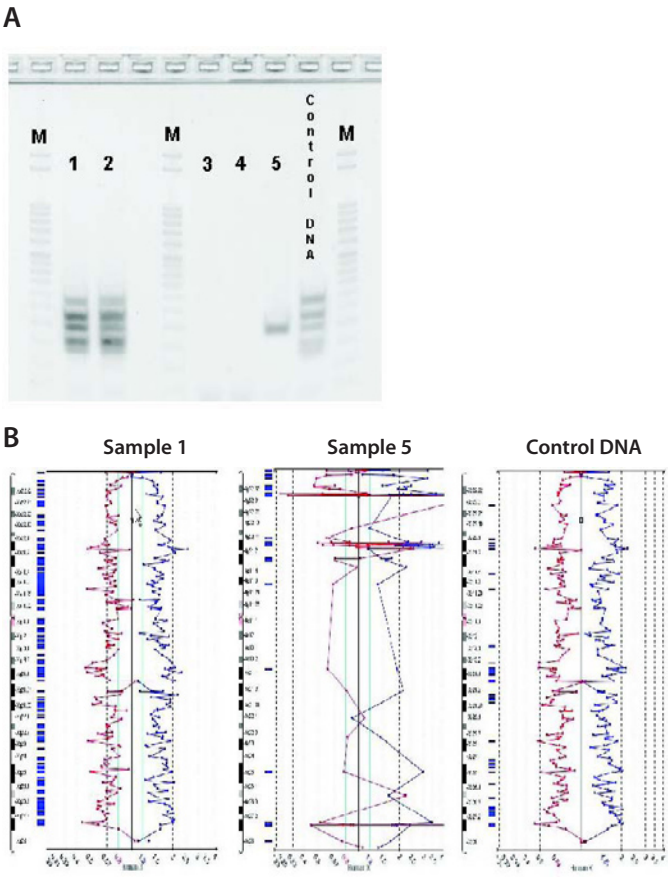


Figure 1: Approximately 1mg of tissue was collected from five FFPE tissue samples followed by processing with the GenomePlex Tissue Whole Genome Amplifi cation Kit (**Cat. No. WGA5**) as outlined in the technical bulletin. (A)5 µl of undiluted WGA5 tissue lysate was subjected to multiplex PCR amplifi cation as outlined above, and 5 µl of each reaction was resolved on a 4% agarose gel (gel info here). The 123 bp ladder (**Cat. No. D5042**) was used as a size standard. All five bands were amplified in lanes 1 and 2 indicating that these FFPE tissue lysates contain high quality genomic DNA, where as lanes 3, 4 and 5 contain low quality DNA since all, or most, of the multiplex PCR fragments were not amplifiable. Similar results were observed when purifi ed DNA or amplified WGA product derived from these FFPE tissues were used directly in the multiplex qPCR assay (data not shown). (B) aCGH was performed to demonstrate a correlation between the multiplex PCR results and aCGH performance. 1µg of WGA5 products were used for BAC aCGH analysis using PerkinElmer's Spectral Labeling Kit and Spectral Chip™ 2600 array platform per manufacturer's recommendations. The ideograms above are representative of the data obtained with this sample set. They were generated using PerkinElmer's SpectralWare™ BAC array analysis software. High quality array statistics and QC metrics were obtained with samples 1 & 2, where as samples 3, 4 and 5 produced irregular array statistics and poor QC metrics. Test and control hybridization samples are labeled in the figure above.

Extraction Protocol—Animal Tissue

Animal tissue is a common source of material when performing genetic analysis. The protocol below is a simple method of extracting DNA from the animal sample. Once the DNA has been isolated, it can then be amplified using the GenomePlex® Whole Genome Amplification Protocol on page 27. The GenomePlex products have been used to amplify genomic DNA from chicken, porcine, bovine, fish, and shrimp sources.

***Note:** The protocol below describes the process of DNA purification and subsequent whole genome amplification. We have dramatically streamlined this process with our GenomePlex Tissue Whole Genome Amplification Kit (**Cat. No. WGA5**). This kit includes optimized reagents for tissue disruption and cell lysis eliminating the need for DNA purification prior to amplification. For more information on WGA5 Amplification Protocol see p. 23.

Required Products

- GenElute™ Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**)

Materials to be Supplied by the User

- Animal tissue
- Microcentrifuge (with rotor for 2 ml tubes)
- Ethanol (**Cat. No. E7023**)
- 55 °C water bath or heat block
- Water, molecular biology reagent (**Cat. No. W4502**)
- 70 °C water bath or heat block

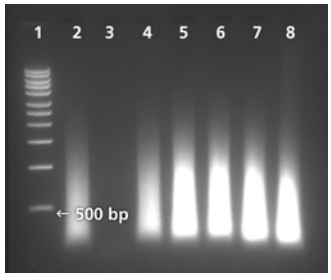
Extraction of DNA from Animal Tissue

It is suggested to use the GenElute Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**) for this procedure.

1. Place 20 mg of tissue into a microcentrifuge tube.
2. Digest Tissue: Resuspend the tissue pellet in 180 µl of Lysis Solution T.
3. Add 20 µl of proteinase K, mix by vortexing.
4. Incubate at 55 °C for 2–4 hours or overnight until the tissue is completely lysed. Vortex occasionally during incubation.

   Optional RNase treatment: If residual RNA is a concern, add 20 µl of RNase A solution and incubate at room temperature for 2 minutes.
5. Lyse cells: Vortex for 15 seconds. Add 200 µl of Lysis Solution C to the sample. Vortex thoroughly as a homogenous mixture is essential for efficient lysis. Incubate at 70 °C for 10 minutes.
6. Prepare column: Add 500 µl of the Column Preparation Solution to each pre-assembled GenElute MiniPrep Binding Column and centrifuge at 12,000 × *g* for 1 minute.
7. Prepare for binding: Add 200 µl of ethanol to the lysed sample and mix by vortexing.
8. Load lysate: Transfer the entire contents of the sample tube into the treated binding column from step 5. Centrifuge at ≥6500 × *g* for 1 minute. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
9. First wash: Prior to first use, dilute the Wash Solution Concentrate with ethanol as described under preparation instructions. Add 500 µl of Wash Solution to the binding column and centrifuge for 1 minute at ≥6500 × *g*. Discard the collection tube containing flow-through liquid and place the binding column in a new 2 ml collection tube.
10. Second wash: Add another 500 µl of Wash Solution to the binding column and centrifuge for 3 minutes at maximum speed (12,000–18,000 × *g*) to dry the binding column. It is crucial that the binding column is free of ethanol before eluting DNA off the column. Centrifuge the column for an additional minute if residual ethanol is visible. Finally, discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
11. Elute DNA: Pipette 200 µl of the Elution Solution directly into the center of the binding column and incubate at room temperature for 5 minutes. Centrifuge for 1 minute at ≥6500 × *g* to elute the DNA.
12. Store DNA samples at –20 °C.

Application Data



GenomePlex Whole Genome Amplification Performed on Various Animal Tissues

Amplified Sigma products are visualized on 1.5% agarose gel. 5 µl of amplified product was loaded per well. The GenomePlex amplified products result in an average size of 400 bp. The smear pattern varies by animal as shown on the gel. Products amplified using GenomePlex technology are specific as evidenced by the lack of signal in the negative control (lane 3).

Lane 1—1 kb Marker
Lane 2—Positive Control
Lane 3—Negative Control
Lane 4—Porcine
Lane 5—Bovine
Lane 6—Chicken
Lane 7—Catfish
Lane 8—Shrimp

Extraction of DNA from Saliva

Extraction Techniques

GenomePlex® Whole Genome Amplification can be performed on DNA extracted in many ways. As outlined in the Protocols section, Sigma-Aldrich offers many products for DNA extraction including the GenElute™ Blood Genomic DNA Kit (**Cat. No. NA2000**), GenElute Mammalian Genomic DNA Miniprep Kit (**Cat. No. G1N10**) and the GenElute Plant Genomic DNA M iniprep (**Cat. No. G2N10**). GenomePlex Whole Genome Amplification has also been used to amplify DNA from soil and saliva samples. DNA from saliva can be extracted using DNA Genotek's Oragene™ DNA Self-Collection Kit. DNA from soil can be extracted using the UltraClean™ Soil Kit by Mo Bio Laboratories, Inc. (#12800-50). The protocols are included below.

Extraction of DNA from Saliva

This protocol provides a simple and convenient method to isolate, amplify, and purify genomic DNA from saliva.

Required Products

- DNA Genotek's Oragene DNA Self-Collection Kit
- GenomePlex WGA Kit (**Cat. No. WGA1**)

Materials to be Supplied by the User

- Saliva
- 95% ethanol (**Cat. No. E7023**)
- Water, molecular biology reagent (**Cat. No. W4502**)
- JumpStart™ Taq DNA Polymerase (**Cat. No. D9307**)
  Note: If using WGA2 there is no need to supply DNA polymerase as the enzyme is provided with the kit.
- Microcentrifuge (with rotor for 2 ml tubes)
- 50 °C water bath or heat block

Protocol for Extraction of DNA from Saliva

It is recommended to use the Oragene™ DNA Self-Collection Kit from DNA Genotek, Inc. for this procedure.

Purification from 500 µl aliquot

1. Incubate the Oragene/saliva sample in the Oragene vial at 50 °C in a water bath or in an air incubator for a minimum of 1 hour. The sample may be incubated overnight if this is more convenient.
2. Transfer 500 µl of the Oragene/saliva sample to a 1.5 ml microcentrifuge tube. The remainder of the Oragene/saliva sample can be stored at room temperature until ready for further use.
3. Add 20 µl (or 1/25th of the total volume) of Oragene Purifier (supplied with kit) and mix gently by inversion. The sample will become turbid as impurities are precipitated.
4. Incubate on ice for 10 minutes.
5. Centrifuge for 3 minutes at 13,000 rpm at room temperature. Carefully pipette the clear supernatant into a fresh microcentrifuge tube without disturbing the pellet. Discard the pellet.
6. Add 500 µl (or an equal volume) of room temperature 95% ethanol to the supernatant and mix gently by inverting at least 5 times. A clot of DNA may be visible.
7. Let the solution stand for 10 minutes at room temperature so that the DNA is fully precipitated. Do not incubate at –20 °C because impurities will co-precipitate with the DNA.
8. Centrifuge for 1 minute at 13,000 rpm at room temperature. Discard the supernatant without disturbing the DNA pellet (may or may not be visible).
9. If necessary, centrifuge again for 10 seconds to remove excess ethanol.
10. Once all of the ethanol has been removed, dissolve the DNA pellet in 100 µl of TE buffer or other standard buffer. The expected concentration of the rehydrated DNA is 10 to 100 ng/µl.
11. To fully dissolve the DNA, vigorous vortexing followed by incubation for a minimum of 1 hour at room temperature or preferably overnight, is recommended Alternatively, incubation for 10 minutes at 50 °C is also effective.
12. Store the DNA at –20 °C or proceed to the amplification step.

Extraction of DNA from Soil

Genomic DNA from soil samples can be easily damaged by nucleases and contaminating debris resulting in low DNA yields. As a result, the researcher's ability to perform downstream analysis may be compromised. After isolating DNA from the soil sample, the GenomePlex® Whole Genome Amplification Protocol on page 27 can be used to amplify the genomic DNA resulting in a higher yield of DNA.

Required Products

- UltraClean™ Soil Kit (by Mo Bio Laboratories, Inc., #12800-50)

Materials to be Supplied by the User

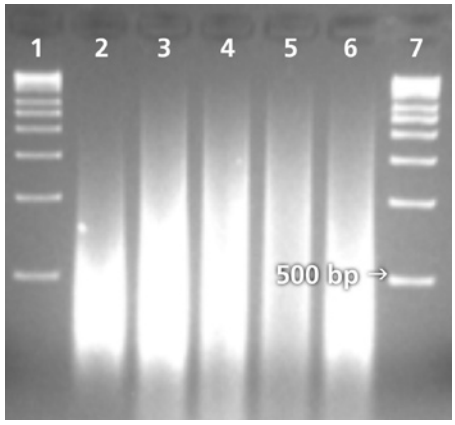
- Soil samples
- Ethanol (**Cat. No. E7023**)
- Water, molecular biology reagent (**Cat. No. W4502**)
- 1.5 ml microcentrifuge tubes (**Cat. No. T9661**)
- Microcentrifuge (with rotor for 2 ml tubes)

Protocol for Extraction of DNA from Soil

The UltraClean Soil Kit by MO Bio Laboratories Inc. is recommended for this procedure.

1. Add 0.5 gm of soil sample to the 2 ml Bead Solution tube provided.
2. Gently vortex to mix.
3. Add 60 µl of Solution 1 and invert several times.
4. Add 200 µl of Solution IRS (Inhibitor Removal Solution).
5. Vortex at maximum speed for 10 minutes.
6. Centrifuge at 10,000 × *g* for 30 seconds (be sure NOT to exceed 10,000 × *g* or the tubes may break).
7. Transfer the supernatant to a clean microcentrifuge tube (supernatant may contain some soil particles).
8. Add 250 µl of Solution S2 and vortex for 5 seconds.
9. Incubate at 4 °C for 5 minutes.
10. Centrifuge the tubes at 10,000 × *g* for 1 minute.
11. Transfer 450 µl of supernatant to a clean microcentrifuge tube.
12. Add 900 µl of Solution S3 to the supernatant and vortex for 5 seconds.
13. Load 700 µl of the solution onto a spin filter and centrifuge at 10,000 × *g* for 1 minute.
14. Discard the flow-through and add the remaining supernatant to the spin filter and centrifuge at 10,000 × *g* for 1 minute.
15. Add 300 µl of Solution S4 and centrifuge for 30 seconds at 10,000 × *g*. Discard the flow-through.
16. Centrifuge for 1 minute.
17. Carefully place the spin filter into a clean microcentrifuge tube.
18. Add 50 µl of Solution S5 to the filter membrane.
19. Centrifuge for 30 seconds and discard the spin filter.
20. Store the eluted DNA at –20 °C or proceed to the amplification step.

Application Data



GenomePlex Whole Genome Amplification Performed on a Soil Sample

Amplified Sigma products are resolved on a 1.5% agarose gel. 5 µl of amplified product was loaded per well. The GenomePlex amplified products result in an average size of 400 bp. The smear pattern is source specific as shown on the gel.

Lane 1—1 kb Ladder
Lane 2—Blood
Lane 3—Plant
Lane 4—Buccal Swab
Lane 5—Soil
Lane 6—Positive Control
Lane 7—1 kb Ladder

Quantitation Techniques

The amount of DNA amplified using GenomePlex Whole Genome Amplification can be detected with or without purification. For the highest quality samples of DNA, it is strongly recommended to purify the samples after amplification. The amplified products can be measured with the PicoGreen® dsDNA Quantitation Assay from Molecular Probes Inc. (#P-7589). Another method of detecting the amplified products is spectrophotometric absorption (OD_{260}) on a NanoDrop® spectrophotometer. This instrument can measure absorbance on 1 µl of sample over a large dynamic range, from 2–3700 ng/

Integration of Sigma® GenomePlex® WGA with the Affymetrix CGH Microarray Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for expression analysis on the Affymetrix platform, and can be readily integrated into existing Affymetrix workflows for Comparative Genomic Hybridization (CGH) analysis.[1] The following modification is required:

- The GenomePlex WGA amplification product is **double-stranded cDNA**. Fragmentation and labeling is accomplished using the GeneChip® WT Double-Stranded DNA Labeling Kit, (**Affymetrix Cat. No. 900812**).

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform a **modified** GenomePlex WGA amplification as described in the product bulletin for ***GenomePlex WGA Kits***.

   Enter GenomePlex WGA2 Kit Procedure, page 2, *Fragmentation*.

   Modification 1. Fragmentation is not performed at this point in the procedure.[1]

Omit:

Fragmentation

1. Isolate DNA sample and quantity concentration by UV absorption (260 nm). Prepare DNA solution of 1ng/µL.
2. Add 1 µL of 10X Fragmentation Buffer to 10 µL of DNA (1 ng/µL) sample in a PCR tube or multiwell strip/plate.
3. Place the tube/plate in a thermal block or cycler at 95°C for EXACTLY 4 minutes.
   NOTE: The incubation is very time sensitive. Any deviation may alter results.
4. Immediately cool the sample on ice, then centrifuge briefly to consolidate the contents.

Replace with:

1. Place 10 µL of DNA (1 ng/ µL) sample (in nuclease-free H_2O) in a PCR tube or multiwell strip/plate.

Enter "Library Preparation" at Step 5

Library Preparation

5. Add 2 µL of 1X Library Preparation Buffer to each sample.
6. Add 1 µL of Library Stabilization Solution.
7. Vortex thoroughly, consolidate by centrifugation, and place in thermal cycler at 95°C
   for 2 minutes.
8. Cool the sample on ice, consolidate the sample by centrifugation, and return to ice.
9. Add 1 µL of Library Preparation Enzyme, vortex thoroughly, and centrifuge briefly.
10. 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
11. Remove samples from thermal cycler and certrifuge briefly. Samples may be amplified immediately or stored at -20°C for three days.

1. **Modification 2.** During the amplification step, dUTP is incorporated for subsequent Uracil-DNA glycosylase (UNG) fragmentation.[2]

Replace: Step 12 of Amplification Procedure

Amplification

1. A master mix may be prepared by adding the following reagents to the 15 µL reaction from step 11:
   7.5 µL of 10X Amplification Master Mix
   47.5 µL of Nuclease-Free Water
   5 µL of WGA DNA Polymerase

With:

12. Create amplificatoin mix. For each re-amplification reaction, add the following reagents to the 15µL reaction from step 11:
    46.9 µL of Nuclease-Free Water
    7.5 µL of 10X Amplification Master Mix (final volume = 60 µL)

    0.6 µL of dUTP (10 mM), final conc. = 80 µM
    5.0 µL of WGA DNA Polymerase

2. Purify the amplification product using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Elute with < 39 µL nuclease-free water. Thirty microliters is the absolute minimum elution volume.

   Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** GenomePlex® WGA amplification reaction.

3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.

Entry into Affymetrix Workflow

Enter the ***GeneChip® WT Double-Stranded DNA Target Assay***, Chapter Two "Target Preparation for Samples Requiring Amplification"; Procedure M, "Fragmentation of Double-Stranded DNA", page 34.

Proceed without deviation starting at step 1.

Procedures M and N require the use of the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (**Affymatrix Cat. No. 900182**).

1. Fragment the samples using the reactions described in Table 2.11.

Component	Volume or Amount in 1 Reaction
Double-Stranded DNA	7.5 µg
10X Fragmentation Buffer	4.8 µL
UDG, 10 U/µL	1.5 µl
APE 1, 100 U/µL	2.25 µL
RNase-free Water	up to 48 µL
Total Volume	48.0 µL

Table 2.11
Fragmentation of Double-Stranded DNA

References

1. Clifford Tepper, PhD., Assistant Research 2, Biochemist, UCD, Med, Biochemistry and Molecular Medicine, and Ryan Davis, Staff Research Assistant, University of Chalifornia-Davis Cancer Center, Sacramento, CA.
2. KDM8, a H3K36me2 histone demethylase that acts in the cyclin A1 coding region to regulate cancer cell proliferation
   Datsun A. Hsia, Clifford G. Tepper, Mamata R. Pochampalli, Elaine Y. C. Hsia, Chie Izumiya, Steve B. Huerta, Michael E. Wright, Hong-Wu Chen, Hsing-Jien Kung, and Yoshihiro Izumiya, *PNAS*, May **2010**; 107: 9671 - 9676.

Integration of Sigma® GenomePlex® WGA with the Affymetrix Microarray ChIP-chip Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for expression analysis on the Affymetrix platform, and can be readily integrated into existing Affymetrix workflows for ChIP on CHIP analysis.[1,2] The following adaptation is required:

- **The GenomePlex WGA amplification product is double-stranded cDNA.** Fragmentation and labeling is accomplished using the **GeneChip® WT Double-Stranded DNA Labeling Kit,** (**Affymetrix Cat. No. 900812**).

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform a **modified** GenomePlex WGA amplification as described in the product bulletin for **GenomePlex WGA Kits**.

   Enter GenomePlex WGA2 Kit Procedure, page 2, "Fragmentation".

   Modification 1. Fragmentation is not performed at this point in the procedure.[1, 2]

Omit:

Fragmentation

1. Isolate DNA sample and quantity concentration by UV absorption (260 nm). Prepare DNA solution of 1ng/µL.
2. Add 1 µL of 10X Fragmentation Buffer to 10 µL of DNA (1 ng/µL) sample in a PCR tube or multiwell strip/plate.
3. Place the tube/plate in a thermal block or cycler at 95˚C for EXACTLY 4 minutes.
   NOTE: The incubation is very time sensitive. Any deviation may alter results.
4. Immediately cool the sample on ice, then centrifuge briefly to consolidate the contents.

Proceed with:

1. Place 10 µL of DNA (1 ng/ µL) sample (in nuclease-free H_2O) in a PCR tube or multiwell strip/plate.

Enter "Library Preparation" at Step 5

Library Preparation

5. Add 2 µL of 1X Library Preparation Buffer to each sample.
6. Add 1 µL of Library Stabilization Solution.
7. Vortex thoroughly, consolidate by centrifugation, and place in thermal cycler at 95°C
   for 2 minutes.
8. Cool the sample on ice, consolidate the sample by centrifugation, and return to ice.
9. Add 1 µL of Library Preparation Enzyme, vortex thoroughly, and centrifuge briefly.
10. 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
11. Remove samples from thermal cycler and certrifuge briefly. Samples may be amplified immediately or stored at -20°C for three days.

1. **Modification 2.** Amplification product is re-amplified with the **GenomePlex® WGA 3 Reamplification Kit**.[1] During amplification dUTP is incorporated for subsequent Uracil-DNA glycosylase (UNG) fragmentation.[2]

Replace: Step 2 of Re-Amplification Procedure A

Amplification

1. Create amplification mix. For each reamplification reaction, add the following to the WGA amplified DNA (step A1):
   49.5 µL of Nuclease-Free Water
   7.5 µL of 10X Amplification Master Mix (final volume = 60 µL)
   3.0 µL of the 10 mM dNTP mix
   5.0 µL of WGA DNA Polymerase

With:

1. Create amplificatoin mix. For each re-amplification reaction, add the following to the WGA amplified DNA (step A1)[3]:
   48.9 µL of Nuclease-Free Water
   7.5 µL of 10X Amplification Master Mix (final volume = 60 µL)
   0.6 µL of dUTP (10 mM), final conc. = 80 µM
   3.0 µL of the 10 mM dNTP mix
   5.0 µL of WGA DNA Polymerase

2. Purify the amplification product using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Elute with < 39 µL nuclease-free water. Thirty microliters is the absolute minimum elution volume.

   Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** GenomePlex WGA amplification reaction.

3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.

Entry into Affymetrix Workflow

Enter the ***GeneChip® WT Double-Stranded DNA Target Assay***, Chapter Two "Target Preparation for Samples Requiring Amplification"; Procedure M, "Fragmentation of Double-Stranded DNA", page 34.[2]

Proceed without deviation starting at step 1.

Procedures M and N require the use of the GeneChip® WT Double-Stranded DNA Terminal Labeling Kit (**P/N 900812**).

1. Fragment the samples using the reactions described in Table 2.11.

Component	Volume or Amount in 1 Reaction
Double-Stranded DNA	7.5 µg
10X Fragmentation Buffer	4.8 µL
UDG, 10 U/µL	1.5 µl
APE 1, 100 U/µL	2.25 µL
RNase-free Water	up to 48 µL
Total Volume	48.0 µL

Table 2.11
Fragmentation of Double-Stranded DNA

References

1. O'Geen, et al. *Biotechniques* (November, **2006**) 41 (5): 577-580
2. Clifford Tepper, PhD., Assistant Research Biochemist, UCD, Med, Biochemistry and Molecular Medicine, and Ryan Davis, Staff Research Assistant, University of California-Davis Cancer Center, Sacramento, CA.

Integration of Sigma® GenomePlex® WGA with the Agilent Genomic Microarray Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for genomic analysis on the Agilent platform, and can be readily integrated into existing Agilent workflows. The following modifications are required:

- **The GenomePlex WGA amplification product is double-stranded cDNA.** Labeling, hybridization, and wash procedures are performed using the workflow outlined in the **Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis ULS Labeling for Blood, Cells, Tissues or FFPE (with a High Throughput option)** (Version 3.1, August 2009).
- Fragmentation of the amplification product is *not* required.
- The **Agilent Genomic DNA ULS™ (Universal Linkage System) Labeling Kit** procedure (Agilent part # 5190-0419, allows for direct labeling of the microarray target, *preferable* to enzymatic incorporation of modified nucleotides during or following amplification.

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform GenomePlex WGA amplification as described in the product bulletin for **GenomePlex WGA Kits**.
2. Purify the amplification product using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Thirty microliters is the absolute minimum elution volume.

   Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a single Genomeplex WGA amplification reaction.

   Note 3. Divalent cation contamination (e.g. Mg^{2+}) will negatively affect ULS labeling efficiency. The GenElute PCR Cleanup kit adequately removes both chaotropic salt contaminants and divalent cation contaminants.
3. Determine concentration of purified amplification product using NanoDrop® spectrophotometry. Use the following table (adapted from the Agilent procedure) to assure you have the appropriate DNA concentration for target labeling for the respective Agilent array formats.

Microarray format *	DNA input amount [ng]†	Volume of DNA [µL]	Minimum DNA concentration [ng/µL]
1x microarray (non-FFPE samples)	1500	16.5	91
1x microarray (FFPE samples)	2000	16	125
2x microarray	1000	17	59
4x microarray	500	8	62.5
8x microarray	250	8	32

* Input DNA requirements and volumes are the same for both FFPE and non-FFPE samples for the 2x, 4x, and 8x arrays.

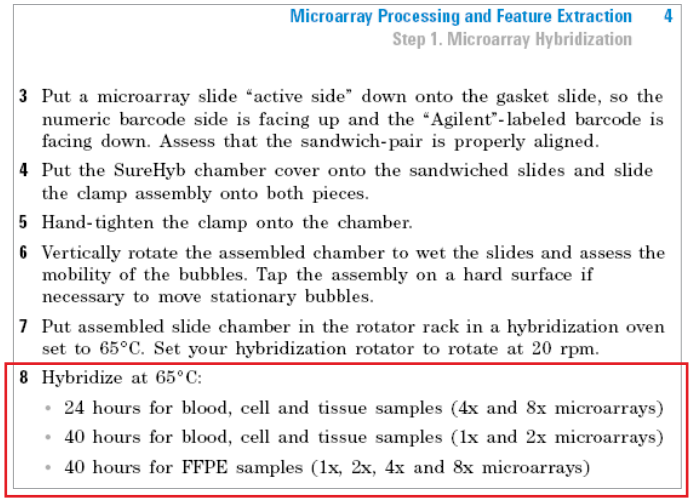
† You can use more DNA, but you will also need to use more ULS dye. Always use a ratio of 1 µL ULS dye per microgram DNA (see procedure below).

4. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
5. **Fragmentation** of GenomePlex WGA amplification product **is unnecessary**.

Entry into Agilent Workflow

1. Enter **Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis ULS™ Labeling for Blood, Cells, Tissues or FFPE** at Step 3. "ULS Labeling", page 38.
2. Proceed **without deviation** from the Agilent procedure.

   Note 4: Reference: Step 1. **Microarray Hybridization**, procedural step 8, page 53, describes the **hybridization incubation** times: Forty hours of 65° C hybridization incubation is **recommended** in all cases.



Microarray Processing and Feature Extraction 4
Step 1. Microarray Hybridization

3 Put a microarray slide "active side" down onto the gasket slide, so the numeric barcode side is facing up and the "Agilent"-labeled barcode is facing down. Assess that the sandwich-pair is properly aligned.

4 Put the SureHyb chamber cover onto the sandwiched slides and slide the clamp assembly onto both pieces.

5 Hand-tighten the clamp onto the chamber.

6 Vertically rotate the assembled chamber to wet the slides and assess the mobility of the bubbles. Tap the assembly on a hard surface if necessary to move stationary bubbles.

7 Put assembled slide chamber in the rotator rack in a hybridization oven set to 65°C. Set your hybridization rotator to rotate at 20 rpm.

8 Hybridize at 65°C:

- 24 hours for blood, cell and tissue samples (4x and 8x microarrays)
- 40 hours for blood, cell and tissue samples (1x and 2x microarrays)
- 40 hours for FFPE samples (1x, 2x, 4x and 8x microarrays)

References

Procedure developed by Ken Heuermann, Sr. Research Scientist and Jaimie Robert, Research Scientist, Life Science Division, Sigma-Aldrich Corporation, St. Louis, MO.

Integration of Sigma® GenomePlex® WGA with the Illumina Genomic Microarray Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for genomic analysis on the Illumina platform, and can be readily integrated into the existing Illumina Golden Gate workflow[1].

- **The GenomePlex WGA amplification product is double-stranded cDNA.** Labeling, hybridization, and wash procedures are preformed using the workflow outlined in the **Illumina GoldenGate Genotyping Assay Guide** (Cat. No. GT-901-1001 Part # 15004065 Rev. A, June 2009).
- Fragmentation of the amplification product is **not** required.

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform GenomePlex WGA amplification as described in the product bulletin for **GenomePlex WGA Kits**.
2. Purify the amplification product using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Thirty microliters is the absolute minimum elution volume.

   Note 2. The ablolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** GenomePlex WGA amplification reaction.
3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
4. Fragmentation of GenomePlex WGA amplification product **is unnecessary**.

Entry into Illumina Workflow

To include a DNA quantitation step (optional):

(Manual Protocol) Enter the Illumina GoldenGate Genotyping Assay at Chapter 2: Make DNA Quantitation Plate, "Prepare Sample QDNA Fluotrac Plate with PicoGreen™ and DNA", Step 2 , page 18;

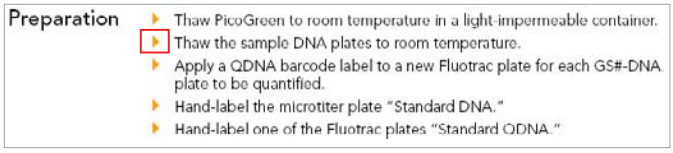
> **Prepare Sample QDNA Fluotrac plate with PicoGreen and DNA**
>
> In this process, you create a new Sample QDNA Fluotrac plate that contains DNA sample and PicoGreen.
>
> 1. Transfer 195 µl of the PicoGreen/1X TE dilution that you made earlier into each well of the new black flat-bottom plate labelled "Sample QDNA" (Figure 7).
> 2. Add 2 µl sample DNA to each well of the Sample QDNA plate.

(Manual)

OR

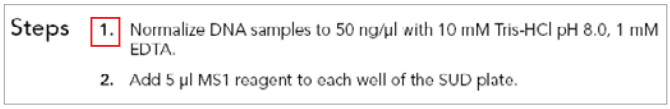
Continued on next page

(Automated Protocol) Enter at Chapter 3: Quantitate DNA, "Preparation", bullet 2, page 121


Preparation
- Thaw PicoGreen to room temperature in a light-impermeable container.
- Thaw the sample DNA plates to room temperature.
- Apply a QDNA barcode label to a new Fluotrac plate for each GS#-DNA plate to be quantified.
- Hand-label the microtiter plate "Standard DNA."
- Hand-label one of the Fluotrac plates "Standard QDNA."

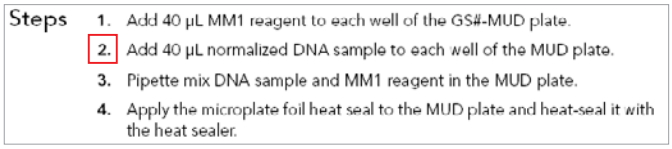
If DNA samples have been previously quantitated:

(Manual Protocol) Enter Make Single-Use DNA (SUD) Plate, Step 1, page 23.


Steps
1. Normalize DNA samples to 50 ng/µl with 10 mM Tris-HCl pH 8.0, 1 mM EDTA.
2. Add 5 µl MS1 reagent to each well of the SUD plate.

(Make Single-Use DNA (SUD) Plate)

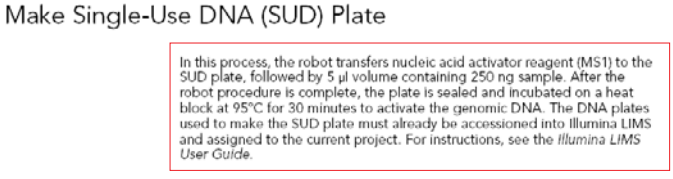
(Manual Protocol) Enter Chapter 2: Make Multi-Use DNA (MUD) Plate, Step 2, page 29.


Steps
1. Add 40 µL MM1 reagent to each well of the GS#-MUD plate.
2. Add 40 µL normalized DNA sample to each well of the MUD plate.
3. Pipette mix DNA sample and MM1 reagent in the MUD plate.
4. Apply the microplate foil heat seal to the MUD plate and heat-seal it with the heat sealer.

(Make Single-Use DNA (SUD) Plate)

OR

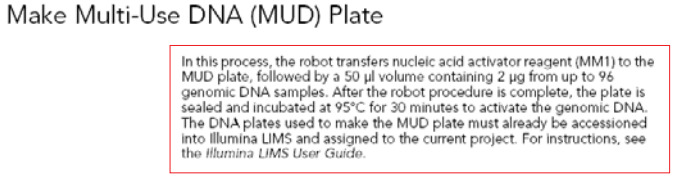
(Automated Protocol) Enter Chapter 3: Make Single-Use DNA (SUD) Plate, Step 1, page 131.


Make Single-Use DNA (SUD) Plate

In this process, the robot transfers nucleic acid activator reagent (MS1) to the SUD plate, followed by 5 µl volume containing 250 ng sample. After the robot procedure is complete, the plate is sealed and incubated on a heat block at 95°C for 30 minutes to activate the genomic DNA. The DNA plates used to make the SUD plate must already be accessioned into Illumina LIMS and assigned to the current project. For instructions, see the *Illumina LIMS User Guide*.

OR

(Automated Protocol) Enter Chapter 3: Make Single-Use DNA (MUD) Plate, Step 2, page 169.


Make Multi-Use DNA (MUD) Plate

In this process, the robot transfers nucleic acid activator reagent (MM1) to the MUD plate, followed by a 50 µl volume containing 2 µg from up to 96 genomic DNA samples. After the robot procedure is complete, the plate is sealed and incubated at 95°C for 30 minutes to activate the genomic DNA. The DNA plates used to make the MUD plate must already be accessioned into Illumina LIMS and assigned to the current project. For instructions, see the *Illumina LIMS User Guide*.

Proceed without deviation from the Illumina procedure.

References

1. I Agalliu, P.A. Schweitzer, SM Leanza, RD Burk, and TE Rohan. (Jan 2009) Illumina DNA test panel-based genotyping of whole genome amplified-DNA extracted from hair samples: performance and agreement with genotyping results from genomic DNA from buccal cells. Clin Chem Lab Med: 47(5): 516-22.

Special acknowledgement and thanks to Dr. Ilir Agalliu of the Albert Einstein College of Medicine, Bronx, NY, and Dr. Peter A. Schweitzer, Life Sciences Core Laboratories Center, Cornell University, Ithaca, NY for their assistance in the assessment and preparation of this procedure.

Integration of Sigma® GenomePlex® WGA with the NimbleGen™ Comparative Genomic Hybridization Microarray Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for genomic analysis on the NimbleGen™ platform, and can be readily integrated into existing NimbleGen workflows. NimbleGen recommends the use of GenomPlex WGA for amplification of genomic DNA for CGH microarray applications.[1]

- If your experimental genomic DNA sample quantity is less than the amount listed below, amplify the experimental and control (input) samples using the Sigma GenomePlex Complete WGA 2 Kit (**Cat. No. WGA2-50RXN**) before labeling.
- The GenomePlex WGA amplification product size ranges from ~200 to 1000 base pairs, averaging ~400 base pairs, making sonication unnecessary.
- Purify samples using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**).

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform GenomePlex WGA amplification as described in the product bulletin for GenomePlex WGA Kits.
2. Purify the amplification product using the GenElute PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Thirty microliters is the absolute minimum elution volume.

   Note 2. The ablosute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a single GenomePlex WGA amplification reaction.
3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
4. Further fragmentation of GenomePlex WGA amplification product is unnecessary. Omit the sonification step found in the NimbleGen CGH Analysis procedure below.

Entry into NimbleGen Workflow

Enter **NimbleGen Arrays User's Guide: CGH Analysis**, v 6.0, Chapter 2.

Sample Requirements

- Purified, unamplified, and unfragmented genomic DNA (gDNA) is required for optimal sample labeling and hybridization.
- Roche NimbleGen recommends starting with the following gDNA amounts for each hybridization:

Sample Requirements	385K Array	Each Sample for a 4x72K Array	2.1M Array	Each Sample for a 3x720K Array	Each Sample for a 12x135K Array
Test gDNA	1.5µg	1.5µg	2.5µg	1.5µg	1.5µg
Reference gDNA	1.5µg	1.5µg	2.5µg	1.5µg	1.5µg

- Samples should be prepared at a concentration of 250ng/µl to 1,000ng/µl in nuclease-free water or 1X TE buffer (10mM Tris-HCl and 0.1mM EDTA, pH 7.5 - 8.0).
- Samples should have an $A_{260}/A_{280} \geq 1.8$ and $A_{260}/A_{230} \geq 1.9$ for optimal labeling yields.

  Note: Roche NimbleGen recommends running 250ng of gDNA on a NanoDrop Spectrophotometer to measure the A_{260}/A_{280} and A_{260}/A_{230} ratios.

Omit Sonication Step

Sample Preparation & QC

Note: Roche NimbleGen has tested several common genomes and found that the sonication step described below can be omitted without adversely affecting CGH data quality. Roche NimbleGen recommends determining the quality of unsonicated samples as described in step 5 below to ensure they show no signs of RNA contamination or degradation.

1. Dilute test and reference gDNA to 80µl with VWR water in a 1.5ml micro-centrifuge tube.
2. Clean the sonicator tip with 70% ethanol and wipe dry with a tissue.
3. Select the following settings on a Branson model 450 Sonifier:

   Note: If using another sonicator, adjust the settings as necessary to produce a smear from approximately 500bp to 2,000bp.

   - Time = 10 seconds
   - Amplitude = 10%
   - Pulse On = 0.5 second
   - Pulse Off = 0.5 second
4. Lower the sonicator tip to near the bottom of the tube and push the start button. Positioning of the probe near the bottom of the tube will prevent splashing and ensure complete sonication of your sample.

   Important: Wear hearing protection when operating the sonicator.
5. To determine the quality of your samples, run 250ng of pre- and post-sonicated gDNA on a 1% agarose gel to ensure they show no signs of RNA contamination or degradation.

   Important: Unsonicated genomic DNA should appear as a single prominent band greater than 12kb. If the sample appears as more than one band or as a smear, the DNA may be degraded or have a contaminant that could affect the labeling procedure. RNA contamination will result in a smear less than 200bp.

Omit Sonication Step

Sonicated sample should appear as a smear from approximately 500bp to 2,000bp with the majority of the fragments migrating between 500bp and 1,000bp. Genomic DNA exhibiting significant degradation (all bands < 500bp) is unsuitable for CGH analysis.

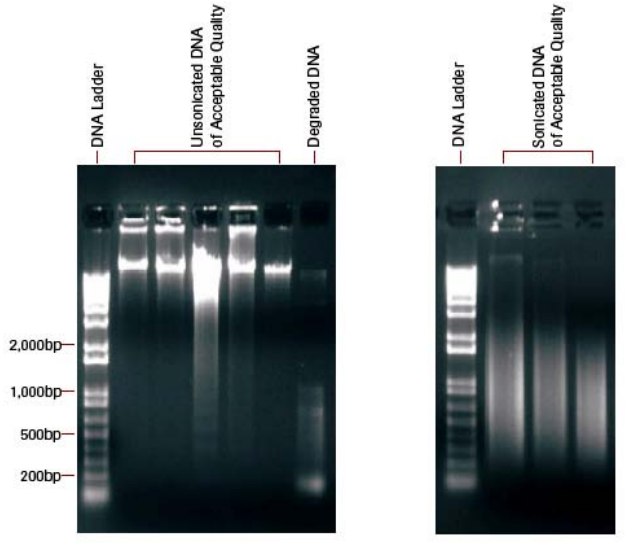


Figure 2: Examples of Agarose Gel Electrophoresis for Unsonicated DNA (left) and Sonicated DNA (right)

GenomePlex® WGA - amplified DNA will have an electrophoretic profile similar to that illustrated for sonicated DNA above.

Proceed to Chapter 3, "Sample Labeling", page 15. Continue without deviation.

References

1. NimbleGen specifically recommends GenomePlex WGA for its ChIP on CHIP and DNA Methylation array applications. GenomePlex WGA is efficacious where DNA samples are of small quantity or where amplification of a consumed stock is required.

Special acknowledgement and thanks to Simon Melov, PhD, Associate Professor and Director of the Genomics Core, and Krysta Felkey, BS, Research Scientist, the Buck Institute for Age Research, Novato, CA, for their assistance in the preparation of this procedure.

Integration of Sigma® GenomePlex® WGA with the NimbleGen™ Microarray ChIP-chip Workflow

The GenomePlex® WGA amplification product is suitable as a microarray target for genomic analysis on the NimbleGen™ platform, and can be readily integrated into existing NimbleGen workflows. NimbleGen recommends the use of GenomePlex WGA for amplification of immunoprecipitated genomic DNA for microarray applications ChIP on CHIP.[1,2,3]

- Prepare samples by following the **NimbleGen Sample Preparation Protocol for ChIP-chip, Step 7.** Amplify the experimental and control input DNA samples using the **Sigma GenomePlex Complete WGA 2 Kit** (**Cat. No. WGA2-50RXN**), with additional amplification using **GenomePlex WGA 3** or **WGA 4** if necessary before proceeding to the NimbleGen labeling procedure.
- The GenomePlex WGA amplification product size ranges from ~200 to 1000 base pairs, averaging ~400 base pairs, making sonication unnecessary.
- Purify samples using the GenElute™ PCR Cleanup kit (**Cat. No. NA1020**).

Preparation of GenomePlex WGA Amplification Product for Labeling

1. Perform a modified GenomePlex WGA amplification as described in the product bulletin for **GenomePlex WGA Kits**.

   Modification 1. Omit fragmentation steps 1-4.[2,3]

   Omit

   Fragmentation

   1. Isolate DNA sample and quantify concentration by UV absorption (260 nm). Prepare DNA solution of 1 ng/µL.
   2. Add 1 µL of 10× Fragmentation Buffer to 10 µl of DNA (1 ng/µl) sample in a PCR tube or multiwell strip/plate.
   3. Place the tube/plate in a thermal block or cycler at 95 °C for EXACTLY 4 minutes. Note, the incubation is very time sensitive. Any deviation may alter results.
   4. Immediately cool the sample on ice, then centrifuge briefly to consolidate the contents.

   Modification 2. If insufficient amplification product is generated, re-amplify with the **GenomePlex WGA Reamplification Kit**.[2,3]

2. Purify the amplification product using the GenElute PCR Cleanup kit (**Cat. No. NA1020**), eluting with sterile RNase-/DNase-free water (**Cat. No. W4502** or **W1754**).

   Note 1. Thirty microliters is the absolute minimum elution volume.

   Note 2. The absolute capacity of the GenElute PCR Cleanup filter cartridge is 10 µg, equivalent to the typical output of a **single** GenomePlex WGA amplification reaction.

3. If concentration of the amplification product is required, use vacuum-centrifugation to avoid loss of amplified product.
4. Fragmentation of GenomePlex WGA amplification product is unnecessary.

Entry into NimbleGen™ Workflow

Enter NimbleGen Array User's Guide: ChIP-chip Analysis, v5.0, Chapter 2. "Sample Preparation & QC", pp 9-11.

Sample Requirements

- High-quality experimental (IP) and control (input) DNA are required to obtain optimally labeled samples for array hybridization. The NimbleGen sample preparation protocol for ChIP-chip is available upon request from Roche Microarray Technical Support.
- Roche NimbleGen recommends starting with the following sample amounts for each hybridization. If your experimental (IP) sample quantity is less than the amount listed, amplify the experimental (IP) and control (input) samples using the Sigma GenomePlex Complete WGA 2 Kit (Catalog No. WGA2-50RXN) before labeling. Then purify samples using the Qiagen QIAquick PCR Purification Kit (Catalog No. 28106).*

  Note: The success of the IP reaction depends on using antibodies validated with immunoprecipitation. Not all antibodies work well for this method.

Starting Sample Amount Recommendations	385K Array	Each Sample for a 4x72K Array	2.1M Array	Each Sample for a 3x720K Array
Experimental (IP) Sample	1.5µg	1.5µg	3.5µg	1.5µg
Control (Input) Sample	1.5µg	1.5µg	3.5µg	1.5µg

- For optimal results, samples should meet the following criteria:
  - A significant majority of the DNA ≥ 200 nucleotides in size.
  - A concentration of approximately 250ng/µl to 1,000ng/µl in nuclease-free water or 1X TE buffer (10mM Tris-HCl and 0.1mM EDTA, pH 7.5 - 8.0).
  - An $A_{260}/A_{280} \geq 1.7$ and $A_{260}/A_{230} \geq 1.6$.

***We recommend using the GenElute™ PCR Cleanup kit (Cat.No NA1020).**

Sample QC

1. Transfer 200ng of each sample to a sterile microcentrifuge tube. Store the remainder of your sample set at -20°C until required for labeling.
2. Analyze the samples using the Agilent Bioanalyzer and RNA 6000 Nano Assay Reagent Kit or by agarose gel electrophoresis.
3. Review Bioanalyzer traces (Figure 2 and Figure 3) or agarose gels (Figure 4) for sample degradation. Degraded samples detected using the Bioanalyzer appear as significantly lower intensity traces with the main peak area shifted to the left with typically more noise in the trace.

   Important: Samples exhibiting degradation should not be carried through labeling and hybridization due to the risk of poor results.

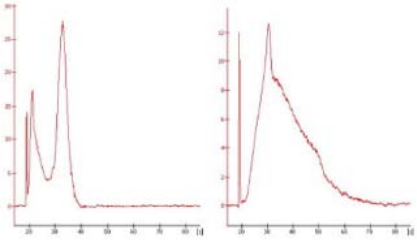


Figure 2: Examples of Bioanalyzer Traces of Nondegraded Samples

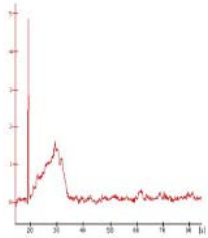


Figure 3: Example of Bioanalyzer Trace of a Degraded Sample

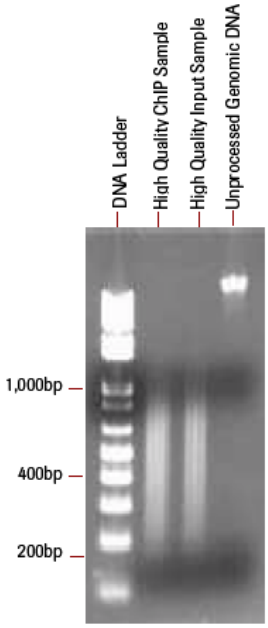


Figure 4: Example of Agarose Gel Electrophoresis for Samples of Good Quality

Proceed to Chapter 3, "Sample Labeling". Continue without deviation.

References and Acknowledgements

1. NimbleGen™ ChIP-on-Chip Product Information, http://www.nimblegen.com/products/chip/index.html
2. NimbleGen Arrays User's Guide ChIP-chip Analysis, http://www.nimblegen.com/products/lit/chip_userguide_v6p0.pdf
3. O'Geen, et al. Biotechniques (November, 2006) 41 (5): 577-580

Special acknowledgement and thanks to Simon Melov, PhD, Associate Professor and Director of the Genomics Core, and Krysta Felkey, BS, Research Scientist, the Buck Institute for Age Research, Novato, CA, for their assistance in the preparation of this procedure.

ChIP-chip Introduction

ChIP-chip is a powerful tool that combines chromatin immunoprecipitation (ChIP) with microarrays to study protein-DNA interactions genome-wide. The ChIP step utilizes an antibody directed against a target protein of interest, such as a transcription factor, polymerase, or modified histone present in nucleosomes. The ChIP step results in the enrichment of DNA fragments bound by the target protein. Normally ChIP DNA samples contain very small amounts of DNA, necessitating the need to amplify the samples before labeling and hybridization to a microarray. For researchers, the GenomePlex® Whole-genome Amplification Kits from Sigma-Aldrich® are the preferred method for the efficient, unbiased, amplification of these precious samples. Once amplified, the ChIP DNA is labeled and hybridized to a micro array, such as Roche NimbleGen's sensitive, high-density (with ≤2.1 million, long-oligonucleotides probes) ChIP-chip microarrays. GenomePlex has been used with Agilent and Affymetrix ChIP-chip arrays as well.

I have used WGA2 kit to amplify my Chipped DNA. I got brilliant results and amplification was very efficient. Recommend others to use the same kit in case they want to have reliable and fast results. WGA4 was very good on a small-scale, when I used micro amounts of Chipped DNA from Cell culture. I work with plants and its not easy sometimes to get a proper kit, but WGA kits were perfect!

Lena M.
Swiss Federal Institute of Technology Zurich (ETH)
Institute of Plant, Animal and Agroecosystem Sciences (IPAS)

Farnham Lab Whole Genome Amplification Protocol for ChIP-chip

From O'Geen et al., BioTechniques 41(5), (November 2006)
(adapted from protocol provided with Sigma GenomePlex Kit)

A. Library Preparation

1. 1. Add 2 ul 1X Library Preparation Buffer to 10 µl of input material

   [For the "input" sample, measure the concentration of reverse crosslinked, QIAquick purified DNA and add 10 ng to a total volume of 10 µl with H_2O. For the ChIP sample, the concentration of nucleic acid is usually too low to get an accurate quantitation. Typically the entire 50 µl of reverse crosslinked, QIAquick purified DNA is lyophilized and resuspended in 10 µl of H_20]

   Transfer samples to strip tubes or individual thin walled 0.2 ml PCR tubes

2. Add 1 ul Library Stabilization Solution, vortex or mix by pipetting. Quick spin and place at 95 °C for 2 minutes in thermal cycler
3. Immediately cool on ice, quick spin again
4. Add 1 µl Library Preparation Enzyme, vortex or mix by pipetting and quick spin if necessary
5. Incubate in thermal cycler as follows:
   - 16 °C for 20' (cycler should be precooled to this temperature)
   - 24 °C for 20'
   - 37 °C for 20'
   - 75 °C for 5'
   - 4 °C hold
6. Quick spin if necessary and either proceed to first amplification or freeze at –20 °C for up to three days

B. Amplification (round 1)

7. Prepare master mix for each sample containing:
   - 7.5 µl of 10× Amplification MasterMix
   - 47.5 µl Nuclease-free H_20
   - 5 µl WGA DNA polymerase

   [For multiple samples, multiply above volumes by the number of samples then add 1/10 volume extra of each component]

8. Add 60 µl master mix to each sample, vortex or mix by pipetting and quick spin if necessary

9. Incubate in thermal cycler as follows:
   - 95 °C for 3′, then 14 cycles of
   - 94 °C for 15″
   - 65 °C for 5′, then
   - 4 °C hold

   At this point, amplified material is stable and can be stored at –20 °C indefinitely

10. Purify samples using QIAquick PCR cleanup columns or analogous product. It is important to elute the samples in water so that the subsequent labeling reactions are efficient.

    [Since the amplified material contains both single- and double-stranded DNA that can be effectively labeled, the column purification method used should recover both.]

    [At this stage, the purification column eluates for total and immunoprecipitated samples should be readily quantifiable by NanoDrop®, spectrometer, or dye intercalation, eg, PicoGreen® (dye intercalation may underestimate amount due to single strand product). Optimally, total recovery for immunoprecipitated samples will be in the 1-4 µg range. This gives enough material for several labelings for downstream microarray analysis. If yields are less, or more product is desired, re-amplify material using Sigma GenomePlex® WGA Reamplification Kit]

C. Reamplification (round 2)

11. Add 15 ng purified amplification product in 10 µl volume to strip tubes or individual thin walled 0.2 ml PCR tubes

    [For input material start with the high concentration primary amplified stock]

12. Prepare master mix for each sample containing:
    - 7.5 µl of 10× Amplification MasterMix
    - 47.5 µl Nuclease-free H_20
    - 5 µl WGA DNA polymerase

    For multiple samples, multiply above volumes by the number of samples then add 1/10 volume extra of each component

13. Add 60 µl master mix, vortex or mix by pipetting and quick spin if necessary

14. Incubate in thermal cycler as follows:
    - 95 °C for 3′, then 14 cycles of
    - 94 °C for 15″
    - 65 °C for 5′, then
    - 4 °C hold

    At this point, amplified material is stable and can be stored at –20 °C indefinitely

15. Purify samples using QIAquick PCR cleanup columns or analogous product.

    [Since the amplified material contains both single- and double-stranded DNA that can be effectively labeled, the column purification method used should recover both.]

GenomePlex® Whole Genome Amplification (WGA1 or WGA2) Protocol

Whole genome amplification is performed with GenomePlex Whole Genome Amplification Kit (**Cat. No. WGA1**) and/or GenomePlex Complete Whole Genome Amplification Kit (**Cat. No. WGA2**).

Random Fragmentation

1. Prepare DNA solution of 1 ng/µl from extraction protocol described above.
2. Add 1 µl of 10× Fragmentation Buffer to 10 µl DNA (1 ng/µl) in a PCR tube.
3. Place the tube in a thermal cycler at 94 °C for exactly 4 minutes. **Note:** The incubation is time sensitive and any deviation may alter results.
4. Immediately cool the sample on ice and centrifuge briefly.

OmniPlex® Library Preparation

5. Add 2 µl of 1× Library Preparation Buffer.
6. Add 1 µl of Library Stabilization Solution.
7. Mix thoroughly and place in thermal cycler at 95 °C for 2 minutes.
8. Cool the sample on ice and centrifuge briefly.
9. Add 1 µl of Library Preparation Enzyme, mix thoroughly, and centrifuge briefly.
10. Place sample in 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
11. Remove samples from thermal cycler and centrifuge briefly.
12. Samples may be amplified immediately or stored at –20 °C for up to three days.

Whole Genome Amplification

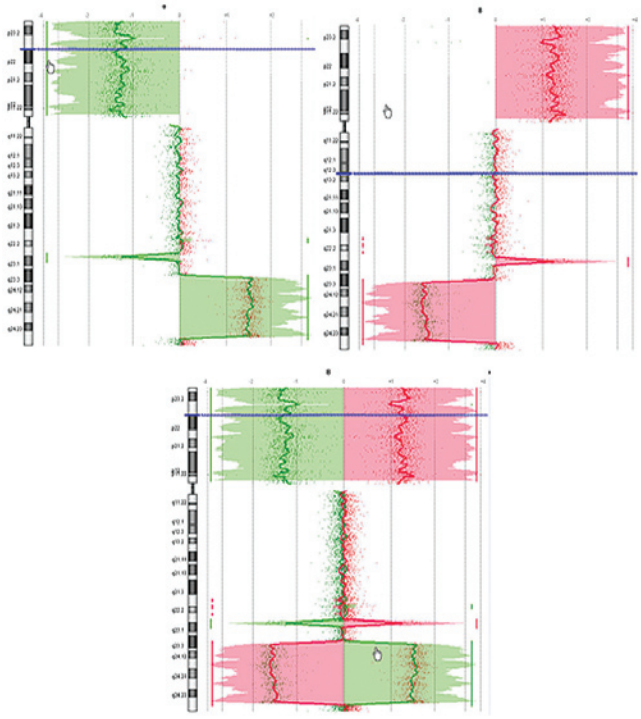
13. Add the following reagents to the entire 15 µl reaction:
    7.5 µl 10× Amplification Master Mix
    47.5 µl Nuclease Free Water
    5.0 µl JumpStart™ Taq DNA Polymerase (12.5 units) for WGA1

    -or-

    5.0 µl WGA DNA Polymerase for WGA2
14. Mix thoroughly, centrifuge briefly, and begin thermocycling:

    Initial Denaturation 95 °C for 3 minutes

    Perform 14 cycles as follows:
    Denature 94 °C for 15 seconds
    Anneal/Extend 65 °C for 5 minutes
15. After cycling is complete, maintain the reactions at 4 °C or store at –20 °C until ready for analysis or purification.



Achieve Robust Amplification Representative of the Original Input Genome

GenomePlex WGA was performed on genomic DNA isolated from HT29 colon carcinoma cells and from a healthy human male. 2.5 ug of WGA product was labeled with Cy3® or Cy5® dye using the Genomic DNA Labeling Kit PLUS (Agilent). The entire labeled sample was loaded onto an Agilent Human Genome CGH Microarray 105A. Specific activities were between 28 and 43 for all samples, and always within 50% of samples being compared. The dye swaps (A & B) demonstrate that there was no bias in the DNA labeling and the aberrations detected are consistent with the HT-29 karyotype.

Whole Genome Amplification from a Single Cell

Whole genome amplification from a single cell is now possible with our optimized GenomePlex® Single Cell Whole Genome Amplification Kit (**Cat. No. WGA4**). The single cell procedure differs very little from the previously described GenomePlex system, but for three procedural changes: (1) the kit includes a robust, optimized cell lysis protocol that is incorporated into the fragmentation steps; (2) the primers that have been optimized for increased sensitivity; and (3) the number of cycles have been increased to account for the minute amount of starting template. Single cells can be isolated by fluorescence-activated cell sorting (FACS), laser capture microdisection, dilution or any other applicable method. Single Cell WGA has been successfully applied to single cell Comparative Genome Hybridization, STR analysis of amniocentesis samples and genomic analysis of *in vitro* fertilized embryos.

WGA4 Protocol

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 µl.
3. Prepare a working Lysis and Fragmentation Buffer Solution by adding 2 µl of Proteinase K Solution into 32 µl of the 10× Single Cell Lysis and Fragmentation Buffer. Vortex thoroughly.
4. Add 1 µl of the freshly prepared Proteinase K Solution-10× Single Cell Lysis and 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 µl of 1× Single Cell Library Preparation Buffer to each sample.
7. Add 1 µl 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 µl 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 µl reaction:
    7.5 µl of 10× Amplification Master Mix
    48.5 µl of Nuclease-Free Water
    5.0 µl 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 20 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.

Whole Genome Amplification Directly from Archived Tissue

The flexibility of the GenomePlex® technology enables the use of compromised starting material, such as DNA isolated from FFPE tissue, for whole genome amplification. Whole genome amplification directly from formalin-fixed, paraffin-embedded (FFPE), frozen, RNA*later*®-preserved or fresh tissue is now possible with our GenomePlex Tissue Whole Genome Amplification Kit (**Cat. No. WGA5**). The kit includes optimized reagents for tissue disruption and cell lysis eliminating the need for tedious organic extractions to remove excess paraffin or DNA purification prior to amplification. This rapid and straightforward method yields microgram quantities of genomic DNA from one milligram of tissue.

WGA5 Protocol

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 CelLytic Y Lysis Solution (**Cat. No. C8367**) and 6 µL of Proteinase K Solution (**Cat. No. P4850**).

3. Place the tube in a thermal cycler at 60 °C for 60 minutes and then 99 °C for 4 minutes.

Library Preparation

4. Combine 9 µL of Nuclease-Free Water (**Cat. No. W4765**) 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 Nuclease-Free Water (**Cat. No. W4765**), 2 µL of Control Human Genomic DNA (**Cat. No. D7192**), and 1 µL of CelLytic Y Lysis Solution (**Cat. No. C8367**).

5. Add 2 µL of 1× Library Preparation Buffer (**Cat. No. L7167**).

6. Add 1 µL of Library Stabilization Solution (**Cat. No. L7292**).

7. Mix thoroughly and place in thermal cycler at 95 °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 (**Cat. No. E0531**), mix thoroughly, and centrifuge briefly.

10. 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

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

Amplification

12. Add the following reagents to the entire 14 µl reaction:
    7.5 µl of 10× Amplification Master Mix
    48.5 µl of Nuclease-Free Water
    5.0 µl of WGA DNA Polymerase

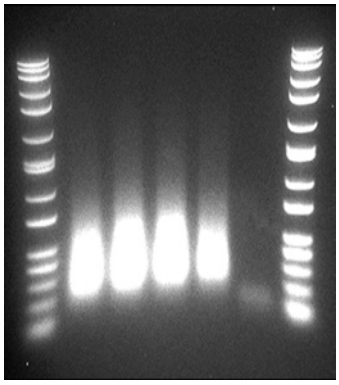
13. Mix thoroughly, centrifuge briefly and begin thermocycling. The following profile has been optimized for a PE 9700 or equivalent thermal cycler:

Initial Denaturation	95 °C for 2 minutes
Perform 20 cycles as follows:	
Denature	94 °C for 15 seconds
Anneal/Extend	65 °C for 4 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.

Whole Genome Amplification Directly from Archived Tissue

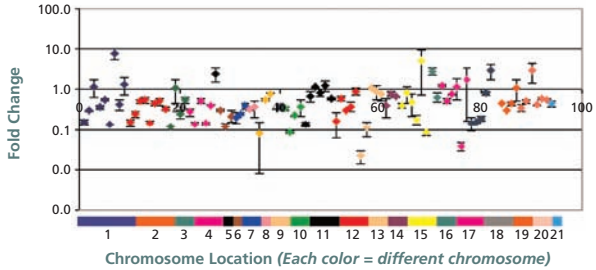
Application Data



Robust Amplification of FFPE Tissues

DNA was amplified directly from 1 mg of various formalin-fixed, paraffin-embedded human tissues with the GenomePlex Tissue WGA Kit (Cat. No. **WGA5**). DNA was purified with the GenElute™ PCR Clean-up Kit and analyzed on a 1% Agarose Gel. WGA yields were 5–6 micrograms for all samples.

1. Wide Range Marker
2. Uterus sample 1
3. Uterus sample 2
4. Gall Bladder
5. Colon
6. No Template control
7. Wide Range Marker



Comparison of WGA5 Amplified & Unamplified GenElute Purified FFPE Gallbladder DNA

Real-time qPCR was performed targeting 96 loci on unamplified human genomic DNA isolated directly from formalin-fixed, paraffin-embedded (FFPE) gallbladder tissue or DNA products generated with the GenomePlex® Tissue Whole Genome Amplification Kit starting with 1 mg of the same FFPE gallbladder tissue. Fold change was calculated as 2CT, where CT is the average CT value generated from GenomePlex amplified DNA minus the average CT value generated from unamplified DNA.

GenomePlex® Whole Genome Reamplification (WGA3) Protocol

1. Add 10 µl of 1 ng/µl WGA amplified DNA (amplified using WGA1, WGA2, or WGA4 Kit) to a PCR tube or multiwell plate.

Note: It is necessary to clean up the WGA reaction to decrease possible bias in the reamplification. We recommend using the Sigma GenElute™ PCR Clean-up Kit (**Cat. No. NA1020**) or a standard purification method that isolates single and double stranded DNA.

2. Create amplification mix. For each reamplification reaction, add the following to the WGA amplified DNA (step 1):

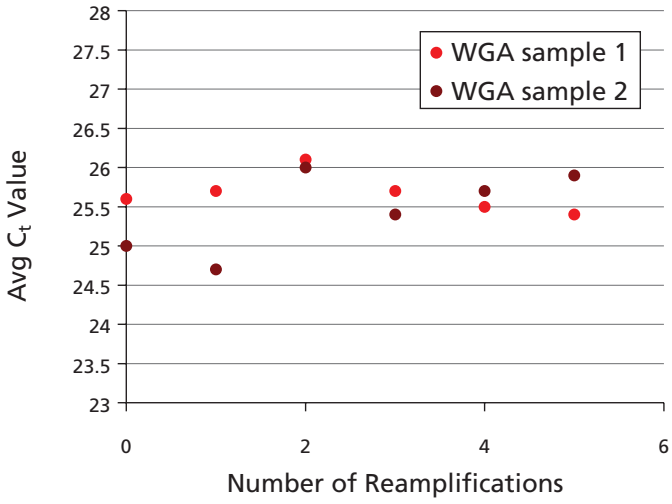
   47.5 µl of Nuclease-Free Water
   7.5 µl of 10× Amplification Master Mix
   5 µl of WGA DNA Polymerase

3. Vortex 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 14 cycles as follows:	
Denature	94 °C for 15 seconds
Anneal/Extend	65 °C for 5 minutes

4. 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.

Application Data



qPCR Effects of Reamplification

Two 10 ng samples of purified human genomic DNA were amplified with WGA2. Each WGA product was then reamplified five successive times with WGA3. QPCR was performed on each sample using primer pairs representative of eight different single copy loci and the average C_t was determined. Genomic representation was maintained after five successive reamplifications.

Clean-Up Procedure for WGA Amplicons

Purification of Amplified Products performed with **GenElute™ PCR Clean-up Kit** (**Cat. No. NA1020**)

1. Insert a GenElute Miniprep Binding Column (with a blue o-ring) into a provided collection tube.
2. Add 0.5 ml of the Column Preparation Solution to each Miniprep column and centrifuge at 12,000 × *g* for 30 seconds to 1 minute. Discard the eluate.

   Note: The Column Preparation Solution maximizes binding of the DNA to the membrane resulting in more consistent yields.
3. Add 5 volumes of Binding Solution to 1 volume of the PCR reaction and mix. **Example:** add 500 µl of Binding Solution to 100 µl of the PCR reaction.
4. Transfer the solution into the binding column. Centrifuge the column at maximum speed (12,000–16,000 × *g*) for 1 minute. Discard the eluate, but retain the collection tube.
5. Replace the binding column in the collection tube. Apply 0.5 ml of diluted Wash Solution to the column and centrifuge at maximum speed for 1 minute. Discard the eluate, but retain the collection tube.

   Note: Be sure to add ethanol to the Wash Solution Concentrate prior to first time use. See Preparation Instructions.
6. Replace the column in the collection tube. Centrifuge the column at maximum speed for 2 minutes, without any additional wash solution, to remove excess ethanol. Discard any residual eluate as well as the collection tube.
7. Transfer the column to a fresh 2 ml collection tube. Apply 50 µl of Elution Solution or water to the center of each column. Incubate at room temperature for 1 minute.

   Note: When eluting with water, make sure that the pH of the water is between 5.5 and 8.5. Elution may also be performed using the Elution Solution diluted 10-fold with water.
8. To elute the DNA, centrifuge the column at maximum speed for 1 minute. The PCR amplification product is now present in the eluate and is ready for immediate use or storage at –20 °C.

GenomePlex® Whole Genome Amplification

Products	Cat. No.
GenomePlex Whole Genome Amplification Kit	WGA1
GenomePlex Complete Whole Genome Amplification Kit	WGA2
GenomePlex Whole Genome Reamplification Kit	WGA3
GenomePlex Single Cell Whole Genome Amplification Kit	WGA4
GenomePlex Tissue Whole Genome Amplification Kit	WGA5
GenElute Blood Genomic DNA Miniprep Kit	NA0200
GenElute Mammalian Genomic DNA Miniprep Kit	G1N10
GenElute Plant Genomic DNA Miniprep Kit	G2N10
GenElute PCR Clean-up Kit	NA1020
Ethanol	E7023
Water, molecular biology reagent	W4502
SYBR® Green JumpStart™ Taq ReadyMix™ for High Throughput QPCR	S9194
SYBR Green JumpStart Taq ReadyMix for QPCR	S4438
SYBR Green JumpStart Taq ReadyMix without $MgCl_2$	S5193
JumpStart Taq ReadyMix for High Throughput Quantitative PCR	D6442
JumpStart Taq ReadyMix for Quantitative PCR	D7440

GenomePlex® Automation Resources

Genomic testing and characterization has become an important tool for understanding biological systems. Often, such analysis is hampered by the number of samples to be examined and the availability of sufficient quantities of genomic DNA. This is particularly a problem for rare and archived sources of DNA. The GenomePlex® Whole Genome Amplification (WGA) kits have been developed for use as a high-throughput system for the rapid and highly representative, amplification of genomic DNA from trace amounts of starting material. A semi-automated method has been developed and validated for the GenomePlex® WGA Kits. The following resources are available for download:

- Automated Method for use with the Sciclone ALH 3000 Workstation (Caliper Life Sciences)
- Automation Protocol (1.83 Mb pdf) for use with the Sciclone ALH 3000 Workstation (Caliper Life Sciences)
- Automated Method for use with the Biomek FX® Workstation (Beckman Coulter)
- Automation Protocol (1.86 Mb pdf) for use with the Biomek FX® Workstation (Beckman Coulter)

Features and Benefits

Rapid Procedure

96 samples can be prepared for WGA and amplified in under 4 hours using the Sciclone ALH 3000 Workstation.

Compatibility

The automated method for GenomePlex® WGA is suitable for use with a variety of source samples including: buccal swabs, whole blood, blood cards, plant tissue, and formalin-fixed, paraffin-embedded tissues.

Genomic DNA amplified using GenomePlex® WGA can be used in a variety of downstream applications including TaqMan® assays, SNP analysis, sequencing, CGH analysis, and microarray analysis.

Immortalizes Source Samples

Nanogram quantities of genomic DNA can be amplified up to 500-fold.

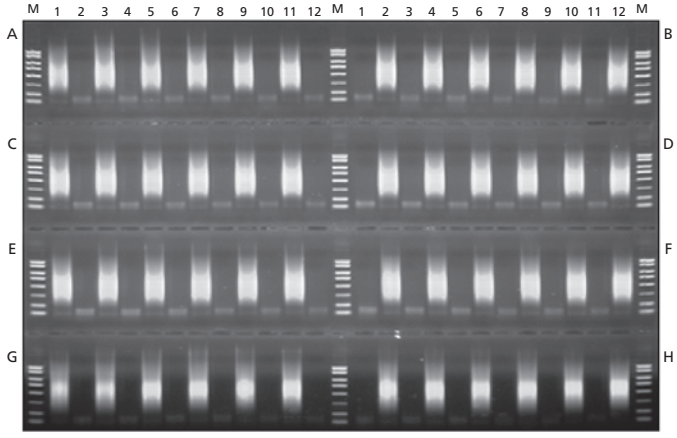
Representative Amplification

WGA is accomplished with minimal allelic drop out.

Reproducible

The automated GenomePlex® WGA method provides accurate reaction setup, while eliminating human error and cross contamination.

Application Data



Cross Contamination Analysis

10 ng of human genomic DNA samples or 8 µl of water were placed in alternating wells of an amplification plate. The plate was processed using the automated WGA procedure on the Sciclone ALH 3000 workstation. All samples were then subjected to amplification and 6 µl of the resultant products were electrophoresed on a 1% agarose gel. Amplified products were not observed in the wells containing water.

Troubleshooting Guide

Problem: No amplification or poor amplification.

Possible Solutions:

1. The DNA quantitation is incorrect.
   Try quantifying the amplified DNA in a different manner. If the absorbance readings and PicoGreen® assay fail to give accurate quantification, visualize the samples on a 1% agarose gel.
2. Low quality DNA.
   The damage done to the DNA is too excessive for the amplification reaction to occur. More than likely, the DNA has been sheared to fragments smaller than 200 bp preventing amplification from occurring or there are too many contaminants in the original sample.
3. Excessive contaminants are present.
   The best optimization step is to perform a dilution series of starting material. This will dilute contaminants and allow for the amplification to occur. Loss of DNA may occur in this process, so quantitation of dialyzed product is strongly recommended.
4. Excessive inhibitors are present.
   Dialysis in a suitable microdialysis unit may dilute the inhibitors. Loss of DNA may occur in this process, so quantitation of dialyzed product is strongly recommended.
5. Inactive enzyme.
   It is critical that the enzyme is stored properly at –20 °C. The freezer used to store the enzyme must be a frost-free unit.
6. Insufficient amount of starting material.
   Depending on the original source, the best results are obtained with 1–10 ng of input DNA for nondegraded DNA samples. Successful WGA amplification has been performed with degraded samples by increasing the starting template to 25–100 ng. Perform a broad titration of your starting material to establish the optimal conditions.
7. Inefficient denaturation.
   The fragmentation and library preparation steps are time and temperature sensitive and any deviation from the protocol may alter final results.
8. Post purification was inappropriate.
   We recommend using Sigma's PCR Clean-up Kit (**Cat. No. NA1020**).

Problem: No reamplification or poor reamplification.

Possible Solutions:

1. DNA that was amplified using a different WGA method (not GenomePlex®) was reamplified using Sigma-Aldrich's GenomePlex Whole Genome Reamplification Kit (**Cat. No. WGA3**). Reamplification can only be performed on products initially amplified with Sigma's GenomePlex WGA1 or WGA2 Kit. The reamplification kit is not compatible with any other commercially available WGA kit.
2. Amplified DNA was not purified prior to reamplification. Prior to reamplification, GenomePlex WGA1/WGA2 products must be cleaned up with a PCR Clean-up Kit (**Cat. No. NA1020**).
3. One or more reagents may have been contaminated. The best solution is to start with fresh components.
4. The DNA polymerase was potentially contaminated. When using WGA1, the user must provide the DNA polymerase. It is possible that the DNA polymerase is contaminated. Minute amounts of any contaminating DNA will result in amplification.

Problem: Negative (no template) control affords product.

Possible Solution:

1. One or more reagents have been contaminated by outside source. While this may not affect your results, a clean no-template control can be re-achieved only by replacing the affected component.

Contact Information

Technical Service: (800) 325-5832
www.sigmaaldrich.com

GenomePlex® Whole Genome Amplification FAQS

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 14 rounds of PCR.

2. **What if fragmentation is allowed to proceed for less or more than 4 minutes?**
   The 4 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 10 minute fragmentation step will also give poor WGA yields in almost all cases because a significant fraction of the DNA is now too small 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. **Can GenomePlex be used on archival fixed tissue DNA or degraded samples?**
   Yes, provided that the extracted DNA is 200 bp or greater in size. However, more damaged DNA is required to afford acceptable yields of final product. We recommend using up to 10 ng of fixed tissue DNA, and following the complete protocol, including fragmentation. See the troubleshooting guide under low yield, input DNA is degraded.

5. **Can I use less input DNA in the GenomePlex protocol?**
   When using a complex starting material, such as human genomic DNA, the gene bias in the resulting GenomePlex output is significantly altered if the input DNA is reduced. An input of 1 ng of human genomic DNA affords product with gene representation that varies 2–10-fold over the original material, even though the yield is only ~50% lower. However, less complex genomics such as bacterial DNA can give good representation with only 1 ng of input DNA.

6. **How should WGA DNA be purified? Is there a preferred way to quantify GenomePlex DNA?**
   We recommend purifying GenomePlex DNA using Sigma's PCR Clean-up Kit (**Cat. No. 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/µl DNA. Measurement techniques such as PicoGreen® will often underestimate the actual WGA DNA yield, since single stranded DNA might be generated during amplification.

7. **How can I store GenomePlex DNA? Where can I stop during the GenomePlex process?**
   The WGA process can be divided into 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.

8. **Will the GenomePlex process afford product with a negative control (no input DNA)?**
   No-template controls will occasionally afford product. This no-template product will not contain genes of interest if probed using PCR or hybridization techniques.

9. **What studies were conducted to determine sequence fidelity for this whole genome amplification method?**
   The GenomePlex method was tested for representation during development of the product by using 107 different human primer sets along with PCR and quantitative PCR. The sets are all from the National Center for Biotechnology UniSTS database.* While this subset of 107 DNA sequences represents a small fraction of the 20–30,000 human genes, it is a good statistical representation of the human genome.

   As part of our QC protocol, every lot of our whole genome amplification (WGA) kit is run on human genomic DNA, which is subsequently tested using QPCR and eight different UniSTS primer sets. The results are compared to an equal amount of unamplified DNA. This test demonstrates equal representation over several different regions.

   View application data and recent protocols using GenomePlex WGA products by visiting the WGA home page at **sigmaaldrich.com/wga**.

10. **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.

References

GenomePlex® WGA products have been utilized in a number of exciting publications since the product release in 2006. For an updated literature list, search popular data bases such as http://highwire.stanford.edu/ using the following keywords: GenomePlex, single cell, microarray, next generation sequencing

Selected open access PLoS-- Public Library of Science Publications:

Reconstruction of Cell Lineage Trees in Mice
Adam, Wasserstrom; Rivka, Adar; Gabi, Shefer; Dan, Frumkin; Shalev, Itzkovitz; Tomer, Stern; Irena, Shur; Lior, Zangi; Shai, Kaplan; Alon, Harmelin; Yair, Reisner; Dafna, Benayahu; Eldad, Tzahor; Eran, Segal; Ehud, Shapiro ... [+]
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Public Library of Science (PLoS) Journal – Apr 9, **2008**
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HECTD2 Is Associated with Susceptibility to Mouse and Human Prion Disease
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Detection of Mycobacterium leprae DNA from Archaeological Skeletal Remains in Japan Using Whole Genome Amplification and Polymerase Chain Reaction
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Klara, Stefflova; Matthew C., Dulik; Athma A., Pai; Amy H., Walker; Charnita M., Zeigler-Johnson; Serigne M., Gueye; Theodore G., Schurr; Timothy R., Rebbeck ... [+]
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