



Protocol for GenomePlex[®] Whole Genome Amplification from Blood Card

<i>Application Guide</i>	2
I. Description	2
II. Product Components	2
III. Materials to be Supplied by the User	2
IV. Protocol for Extraction of DNA from Blood Card	2
V. Protocol for GenomePlex Whole Genome Amplification from Blood Card	3
VI. Quantification of Amplified Products	4
VII. Purification of Amplified Products	4
<i>Appendix</i>	5
Application Data	5
References	6
Contact Information	6

Application Guide

I. Description

Blood cards provide the convenience of archiving small volumes of blood. Many times genomic DNA from samples can have low yield. This quantity can hinder the researcher's ability to perform downstream analysis. This method describes amplifying nanogram amounts of starting genomic DNA with little or no detectable bias resulting in microgram quantities. In addition, the protocol provides a simple and convenient method to extract the genomic DNA from the blood card, amplify the genomic DNA, and rapidly purify the PCR amplified products. The methods described are completed using the GenElute™ Blood Genomic DNA Kit, GenomePlex® WGA kit, and GenElute™ PCR Clean-up Kit.

GenomePlex® is a Whole Genome Amplification (WGA) method that allows the researcher to generate a representative, approximate 300 to 1000-fold amplification of genomic DNA. The amplification yield is dependent on the purity and amount of starting material. The kit utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable OmniPlex® Library molecules flanked by universal priming sites. The OmniPlex library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles. This technology maintains the genetic representation with concordance >99.8% in genotyping results from comparing genomic DNA and GenomePlex amplified DNA¹. In addition to SNP genotyping, downstream applications also include performing TaqMan® assays and BeadArray™ analysis.

II. Product Components

- GenElute™ Blood Genomic DNA Kit ([NA2000](#))
- GenomePlex® Whole Genome Amplification Kit ([WGA1](#))
- GenElute™ PCR Clean-Up Kit ([NA1020](#))

III. Materials to be Supplied by the User

- Blood Card
- 1.5 ml microcentrifuge tubes for lysis
- Microcentrifuge (with rotor for 2ml tubes)
- Ethanol (absolute) Product Code E7023
- Molecular Biology Reagent Water, Product Code W4502
- JumpStart™ Taq DNA Polymerase, Product Code D9307
- 55 C water bath or heating block

IV. Protocol for Extraction of DNA from Blood Card

- **Performed with GenElute Blood Genomic DNA (NA2000)**
 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 15ml conical tube.
6. Add 500 μ l of Column Preparation Solution to the GenElute Miniprep Binding Column (red o-ring) and centrifuge at 12,000 x g for 1 minute. Discard the flow-through liquid.
7. Add 900 μ l of 95-100% ethanol to the lysate in the 15 ml conical tube and mix thoroughly by vortexing 5 to 10 seconds.
8. Transfer the contents of the tube into the treated column from step 4. Centrifuge at \geq 6,500 x g for 1 minute. Repeat this until all of the lysate has been passed through the column.
9. Discard the collection tube and flow-through and place the column in a new 2 ml collection tube.
10. Add 500 μ l of Prewash Solution (be sure to dilute with ethanol prior to first use) and centrifuge for 1 minute at \geq 6,500 x g for 1 minute.
11. Discard the collection tube containing the flow through and place the binding column in a new 2 ml collection tube.
12. Add 500 μ l of Wash Solution (be sure to dilute with ethanol prior to first use) to the binding column and centrifuge at maximum speed (12,000 x g to 16,000 x g) for 3 minutes to dry the binding column.
13. Pipette 400 μ l of Elution Solution onto the column and centrifuge for 1 minute at \geq 6,500 x g to elute the DNA.
14. Store the eluted DNA at -20 °C.

V. Protocol for GenomePlex Whole Genome Amplification from Blood Card

- **Performed with GenomePlex Whole Genome Amplification Kit (WGA1)**

Fragmentation

1. Prepare DNA solution of 1 ng/ μ l from whole blood extraction protocol described above.
2. Add 1 μ l of 10X Fragmentation Buffer to 10 μ l DNA (1 ng/ μ l) in a PCR tube.
3. Place the tube in a thermal cycler at 95 °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.

Library Preparation

5. Add 2 μ l of 1x Library Preparation Buffer.
6. Add 1 μ l 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 μ l 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. Samples may be amplified immediately or stored at - 20 °C up to three days.

Amplification

12. Add the following reagents to the entire 15 µl reaction:
 - 7.5 µl 10x Amplification Master Mix
 - 47.5 µl Nuclease Free Water
 - 5.0 µl JumpStart Taq DNA Polymerase (12.5 units)
13. Mix thoroughly, centrifuge briefly, and begin thermocycling:

Initial Denaturation 95 °C for 3 minutes

Perform 14 cycles as follows:

Denature 95 °C for 15 seconds

Anneal/Extend 65 °C for 5 minutes

14. After cycling is complete, maintain the reactions at 4 °C or store at -20 °C until ready for analysis or purification.

VI. Quantification of Amplified Products

The amount of GenomePlex Whole Genome Amplification Kit products can be detected with or without purification. For the highest quality samples of DNA we strongly recommend cleaning up the samples after amplification. The amplified products can be measured with the PicoGreen™ dsDNA Quantitation Assay (Molecular Probes Inc. Product # P-7589). Another method of detecting the amplified products is spectrophotometric absorption (OD₂₆₀) on a NanoDrop® instrument.

VII. Purification of Amplified Products

- **Performed with GenElute PCR Clean-Up Kit (NA1020)**

1. Insert a GenElute Miniprep Binding Column (with a blue o-ring) into a provided collection tube, if not already assembled. Add 0.5 ml of the Column Preparation Solution to each miniprep column and centrifuge at 12,000 x 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.

2. Add 5 volumes of Binding Solution to 1 volume of the PCR reaction and mix. For example, add 500 µl of Binding Solution to 100 µl of the PCR reaction. Transfer the solution into the binding column. Centrifuge the column at maximum speed (12,000-16,000 Xg) for 1 minute. Discard the eluate, but retain the collection tube.
3. Replace the binding column into 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.

4. Replace the column into 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.
5. Transfer the column to a fresh 2 ml collection tube. Apply 50 μ l of Elution Solution or water to the center of each column. Incubate at room temperature for 1 minute.

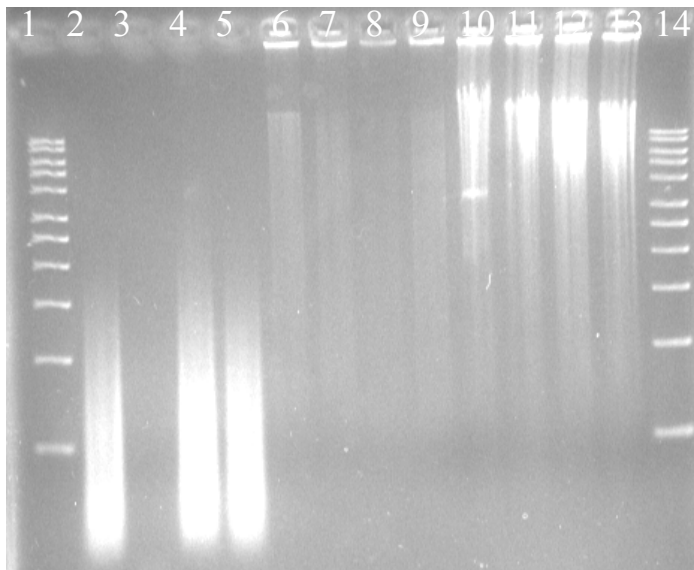
Note: When eluting with water, make sure that the pH of the water is between 5.5 and 8.5. Elution may also be performed using the Elution Solution diluted 10-fold with water.

6. 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 .

Appendix

Application Data

GenomePlex[®] WGA human genomic DNA from Blood Card



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

References

1. Barker, D. L., *et al.* Two methods of whole-genome amplification enable accurate genotyping across a 2320-SNP linkage panel. *Genomic Research*, **14**, 901-7 (2004).
2. Gribble, S., *et al.* Chromosome paints from single copies of chromosomes. *Chromosome Research*, **12**, 143-51 (2004).
3. Thorstenson, Y. R., *et al.* An Automated Hydrodynamic Process for Controlled, Unbiased DNA Shearing. *Genome Research*, **8**, 848-855 (1998).

Contact Information

For technical assistance please contact:

Technical Service

(800) 325-5832

www.techserv@sial.com

To learn more about GenomePlex WGA technology visit: www.sigmaaldrich.com

GenomePlex is a registered trademark of Rubicon Genomics, Inc.

GenElute and Jumpstart are trademarks of Sigma-Aldrich, Inc.

Nanodrop is a registered trademark of Nanodrop Technologies, Inc.

PicoGreen is a trademark of Molecular Probes, Inc.

BeadArray is a Trademark of Illumina, Inc.

TaqMan is a registered trademark of Roche Molecular Systems

Taq antibody licensed for *in vitro* research use under U.S. Patent No. 5,338,671 and 5,587,287, and corresponding patents in other countries.

This product is sold under license from Roche Molecular Systems, Inc. and Applied Biosystems.