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ProductInformation

Automated Protocol for GenomePlex® Whole Genome Amplification (WGA) Kit

Using the Sciclone ALH 3000 Workstation (Caliper Life Sciences)

WGA Product Codes **WGA1 and WGA2**

Automation Guide

I. Description

The GenomePlex Whole Genome Amplification (WGA) kit has been developed for use as a high-throughput system for the rapid and highly representative, 500-fold amplification of genomic DNA from trace amounts of sample. 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 amplified using universal oligonucleotide primers and a limited number of cycles.

The GenomePlex kit is suitable for use with purified genomic DNA from a variety of sources including buccal swabs, whole blood, blood card, and formalin-fixed, paraffin-embedded (FFPE) tissue. GenomePlex WGA kits require 10 ng of starting DNA, and yield 5–10 μg of WGA product after amplification.

A semi-automated method is available for use on the Sciclone ALH 3000 Liquid Handling Workstation from Caliper Life Sciences. Amplification of genomic DNA from various sources is accomplished in a few simple steps:

1. Fragmentation Buffer is added to genomic DNA in a 96-well plate and incubated off-line at 95 °C for 4 minutes.
2. A mixture of Library Preparation and Stabilization Solution is added to the samples followed by an off-line incubation at 95 °C for 2 minutes.
3. A mixture of Library Preparation Enzyme and Library Preparation Buffer is added to the samples. Samples are then subjected to a series of stepped isothermal reactions in a thermal cycler to generate the OmniPlex library.
4. An amplification master mix with a thermal stable DNA Polymerase is added to the samples followed by 14 cycles of thermal cycling to amplify the OmniPlex library.

All 96 DNA samples can be amplified in less than 4 hours.

II. Product Components

Reagents Provided	Product Code	GenomePlex Whole Genome Amplification Kit WGA1*	GenomePlex Complete Whole Genome Amplification Kit WGA2*
		50 reactions	50 reactions
10x Fragmentation Buffer	F4304	55 µl	55 µl
Library Preparation Buffer	L7167	110 µl	110 µl
Library Stabilization Solution	L7292	55 µl	55 µl
Library Preparation Enzyme	E0531	55 µl	55 µl
10x Amplification Master Mix	A5604	410 µl	410 µl
Nuclease-Free Water	W4765	2 x 1.5 ml	2 x 1.5 ml
Control Human Genomic DNA (5 ng/µl)	D7192	10 µl	10 µl
WGA DNA Polymerase	W3891	Not Included	275 µl

* To perform WGA on a 96-well plate of samples, 3 kits must be ordered. Alternatively, custom packaging is available. Contact your local Sigma sales representative for more information.

III. Storage

All components should be stored at –20 °C. Do not store in a frost-free freezer.

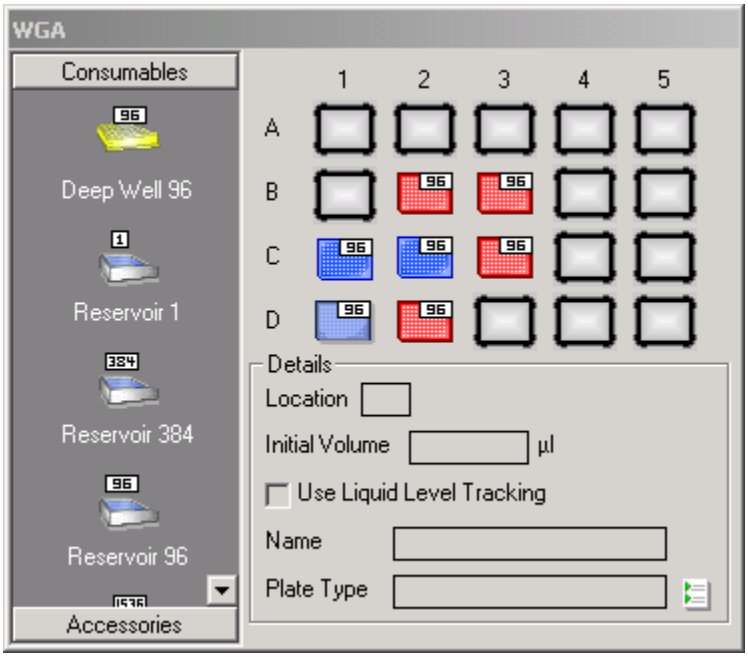
IV. Materials to Be Supplied by the User

1. Genomic DNA samples
2. Water, molecular biology reagent (Sigma, W4502)
3. (Optional) DNA Polymerase for use with WGA1 (Sigma, W3891 or WGA3)
4. 96-well PCR plates, with full skirt (Sigma, P4616)
5. 96-well PCR plates, with half skirt (ABgene, AB-1100)
6. Thermowell™ sealing mat (Fisher, 07200614)
7. Corning plate holder (Corning, 6525)
8. Sealing film, SealPlate™ (Sigma, Z369659)
9. 12 column reagent reservoir with low profile (Innovative Microplates, S30028)
10. (Optional) 12 column reagent reservoir with high profile (Innovative Microplates, S30019)
11. Thermal Cycler

V. Instrument Requirements for the Sciclone ALH 3000 Workstation

Part Description	Qty	Ordering Information
Z8™ Pipettor	1	Contact Caliper
Deck Locator	3	Contact Caliper
Tip Box Locator	4	# 76523 (Caliper)
80 µl Barrier Tip Box	4	# 68759 (Caliper)

VI. Deck Setup



Deck Position	Equipment
B2	80 µl Barrier Tips
B3	80 µl Barrier Tips
C1	96-well PCR plate with genomic DNA samples to be amplified (seated into a plate holder)
C2	96-well PCR plate with full skirt containing reagents
C3	80 µl Barrier Tips
D1	12 column reservoir for amplification master mix
D2	80 µl Barrier Tips

VII. Sample Requirements

1. Protocols for purifying genomic DNA from different biological sources may be downloaded from: http://www.sigmaaldrich.com/Area_of_Interest/Life_Science/Molecular_Biology/PCR/Product_Lines/Whole_Genome_Amplification/WGA_Protocols.html.

2. A minimum of 10 ng of genomic DNA is recommended for amplification with the GenomePlex WGA kit. DNA concentration is critical for successful whole genome amplification and needs to be determined by absorbance. Other methods tend to underestimate single stranded DNA present in a sample.

3. DNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue may contain impurities and should be quantified using PicoGreen® Quantitation Reagent. In this and other samples where DNA damage may have occurred, the starting concentration of genomic DNA may need to be increased. Successful WGA amplification has been performed with degraded samples using 10 ng of starting template, but may require increasing the starting template to 25–100 ng.

VIII. Reagent Preparation

1. *Genomic DNA Samples*
   The GenomePlex kit requires 10 ng of starting genomic DNA. Prepare DNA solutions at a concentration of 1.25 ng/μl and aliquot 8 μl of this solution into each well of a 96-well PCR plate located at position C1. Positive or negative controls can be added to the same plate.

2. *5x Fragmentation Buffer*
   To process a single plate of 96 samples, dilute the 10x Fragmentation Buffer 2-fold by adding 140 μl of water to 140 μl of Fragmentation Buffer. Aliquot 35 μl of the solution into each well the first column of the 96-well PCR plate located at position C2.

3. *Library Preparation and Stabilization Solution Mixture*
   To process a single plate of 96 samples, combine the Library Preparation and Stabilization Solution at a 1:1 ratio in a total volume of 280 μl. Aliquot 35 μl of the mixture into each well of the third column of 96-well PCR plate located at position C2.

4. *Library Preparation Buffer and Enzyme Mixture*
   To process a single plate of 96 samples, combine the Library Preparation Buffer and Enzyme at a 1:1 ratio in a total volume of 280 μl. Aliquot 35 μl of the mixture into each well of the fifth column of 96-well PCR plate located at position C2.

5. *Amplification Master Mix*
   To prepare the Master Mix, add water and DNA Polymerase to the 10x Amplification Master Mix as described in the table below.

	Water	Amplification Master Mix	DNA Polymerase
Stock		10x	50x
Working (6 ml)	5.175 ml	750 μl	75 μl

To process one 96-well plate, a total of 6 ml of Amplification Master Mix needs to be added to the first column of the 12-column low profile reservoir (S30028) located at position D1. If setting up more than one 96-well plate of the samples, it will be necessary to use a 12-column high profile reservoir (S30019).

IX. Automated Method Description

A. Methods

Three methods have been created for this application:

1. *MAIN_WGA*: Performs all of the steps necessary to amplify DNA from 96 samples.
2. *SUB_TipTouch(12ColumnTrough)*: This sub-routine is called up in the main method to perform the tip touching steps after aspirating the amplification master mix from the 12-column reagent trough. This method must be modified if using a different trough for the master mix.
3. *SUB_TipTouch(PCRPlate)*: This sub-routine is called up in the main method to perform the tip touching steps after dispensing the reagents into the amplification plate. This method must be modified if a different PCR plate is used.

B. Getting Started

1. Set up the deck layout by placing the tip boxes, plates and reservoirs at the appropriate positions on the deck as described in section VI.
2. Add reagents to the appropriate reservoirs as described in section VIII.
3. Run the method using Sciclone Software Version 3.2.
4. When prompted, place the sealing mat onto the amplification plate and move the plate to a thermal cycler for incubations or amplifications as indicated in the dialog box.
5. Store the amplified DNA samples at –20 °C until ready for analysis or purification.

C. Method Overview

Below is a summary of the steps for the *MAIN_WGA* method. For complete program details, the automation program can be downloaded from www.sigmaaldrich.com/automation

1. 5X Fragmentation Buffer (2 µl) is dispensed into each well of the amplification plate containing DNA samples and mixed 4 times.
2. The user is prompted to transfer the amplification plate to a thermal cycler for incubation at 95 °C for 4 minutes.
3. Return the amplification plate back to the deck of the Sciclone and click the “OK” button on the dialog box to continue the method.
4. Library Preparation and Stabilization Solution mixture (2 µl) is dispensed into each well of the amplification plate and mixed 4 times.
5. The user is prompted to transfer the amplification plate to a thermal cycler for incubation at 95 °C for 2 minutes.
6. Return the amplification plate back to the deck of the Sciclone and click the “OK” button on the dialog box to continue the method.
7. Library Preparation Enzyme and Buffer mixture (2 µl) is dispensed into each well of the amplification plate and mixed 4 times.
8. The user is prompted to transfer the amplification plate to a thermal cycler for amplifications.
9. Return the amplification plate back to the deck of the Sciclone and click the “OK” button on the dialog box to continue the method.
10. Amplification Master Mix (60 µl) is dispensed into each well of the amplification plate and mixed 4 times.
11. The user is prompted transfer the amplification plate to a thermal cycler for amplification.
12. Click the “OK” button on the dialog box to finish the program.

X. Recommended Parameters for PCR Amplification:

Step	Temperature	Time	Cycles
Initial Denaturation	95 °C	3 minutes	1
Denaturation	94 °C	15 seconds	14
Annealing/Extension	65 °C	5 minutes	
Hold	4 °C	Indefinitely	

XI. Method Customization

A. Use of a different PCR plate for Amplification

The automated method was created using half-skirt 96-well PCR amplification plates ABgene. Other PCR plates may be used in this method, but may require the creation of new labware in the Sciclone software. If different PCR plates are used, the *SUB_TipTouch(PCRPlate)* method may need to be adjusted. Tip touching is a critical step for the addition of the low volume of reagents to the reaction mixture.

B. Use of a different PCR plate or Reservoir for the Reagents

The automated method was created using full-skirt 96-well PCR plates from Sigma. Other PCR plates or reservoirs may be used in this method, but may require the creation of new labware in the Sciclone software. If different labware is used, the *SUB_TipTouch(PCRPlate)* method may need to be adjusted.

C. Use of a different Reservoir for the Amplification Master Mix

The automated method was created using a 12-column reservoir for holding the Amplification Master Mix. Other reservoirs may be used in this method, but may require the creation of new labware in the Sciclone software. If different labware is used, the *SUB_TipTouch(12ColumnTrough)* method may need to be adjusted.

XII. Performance Characteristics

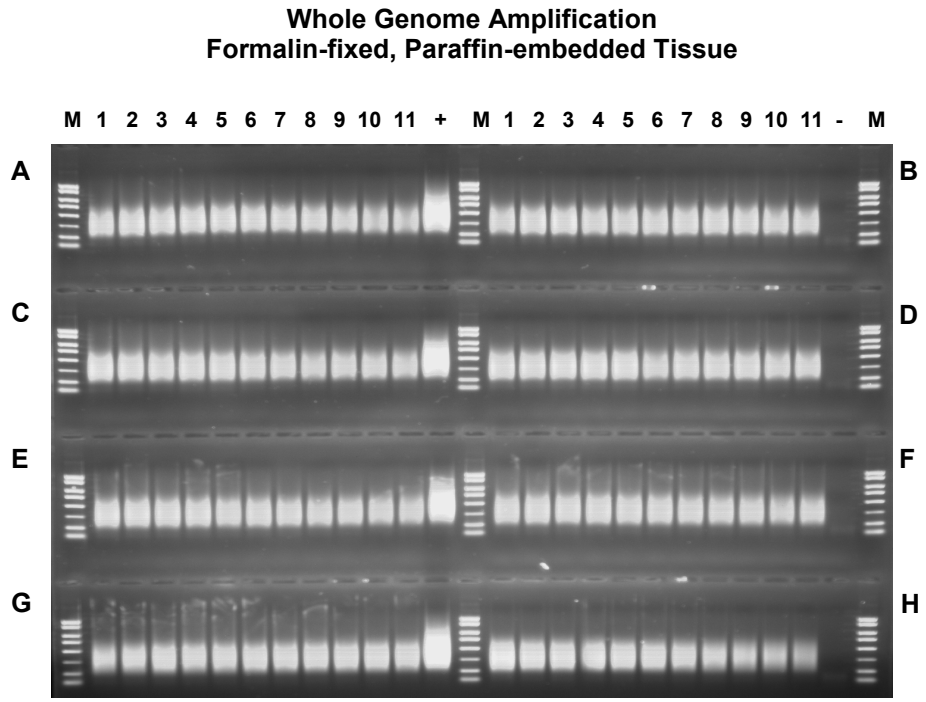


Figure 1. 10 ng of DNA isolated from 88 samples of formalin-fixed, paraffin-embedded (FFPE) rat liver tissue were amplified using the automated WGA procedure on the Sciclone ALH 3000 workstation. 6 µl of each amplified product were analyzed on a 1% agarose gel. M: PCR marker. (+): Human genomic DNA control. (-): No DNA template control.

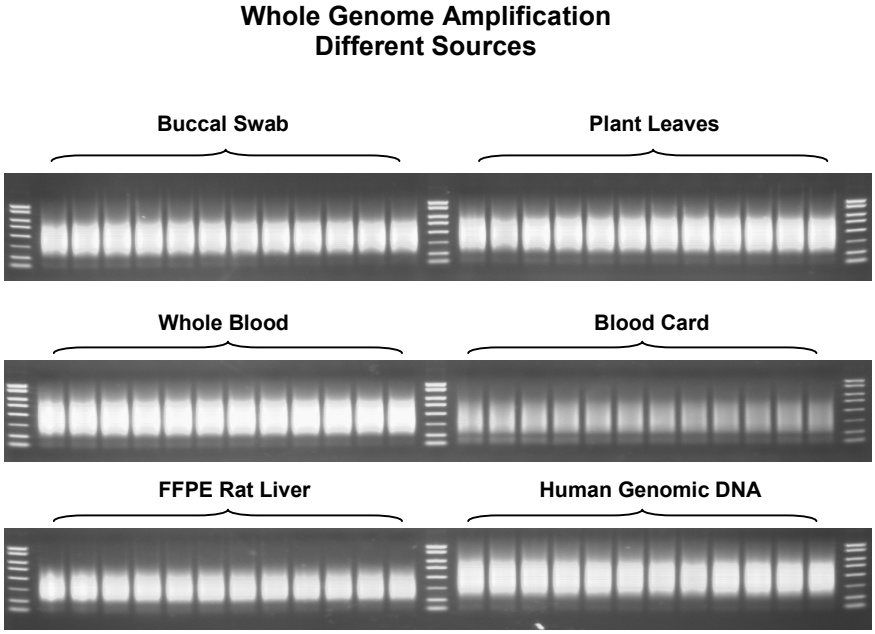


Figure 2. DNA isolated from buccal swab, tomato leaves, whole blood, blood card, and FFPE rat liver tissue was amplified using the automated WGA procedure on the Sciclone ALH 3000 workstation. 6 µl of each amplified product were analyzed on a 1% agarose gel.

Cross-contamination Analysis

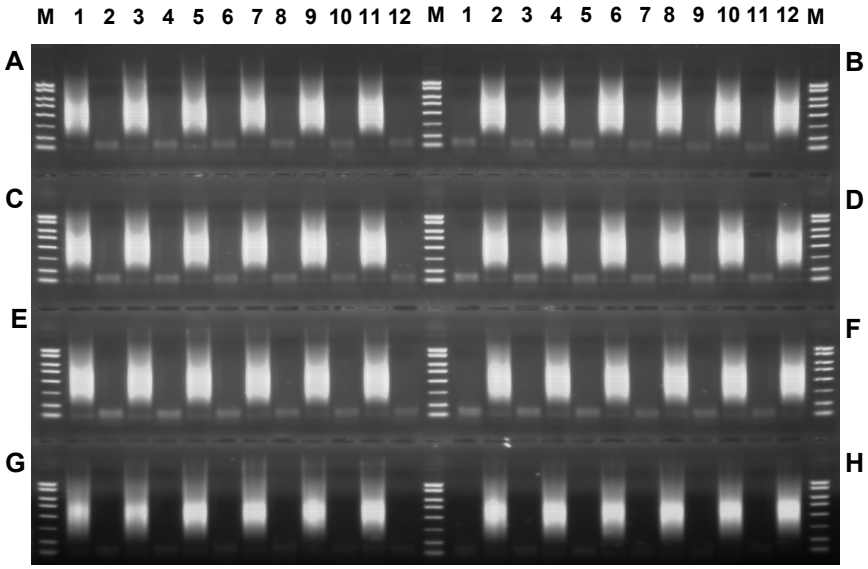


Figure 3. 10 ng of human genomic DNA samples or 8 μl of water were placed in alternating wells of an amplification plate. The plate was processed using the automated WGA procedure on the Sciclone ALH 3000 workstation. All samples were then subjected to amplification and 6 μl of the resultant products were electrophoresed on a 1% agarose gel. Amplified products were not observed in the wells containing water.

XIII. Troubleshooting

Problem	Cause	Solution
Low yield after cycling	Sample contains PCR inhibitors, or high buffer salts	Dialyze samples in a suitable microdialysis unit to remove the inhibiting components. Requantitate the dialyzed product as loss of DNA may occur in the dialysis process.
	Input DNA is degraded or less than 10 ng of DNA is present in the sample	Amplification of insufficient DNA quantities often result in poor yield or poor representation in the final product. Some templates can be rendered amplifiable by increasing input DNA. Successful WGA amplification has been performed with degraded samples by increasing starting template from 25–100 ng.
	More enzyme is required	WGA yield suffers when limiting amounts of DNA polymerase are used. We recommend a minimum of 12 units of DNA polymerase per 75 μl reaction. This is preferable to adding cycles as the resulting DNA may suffer from amplification bias.
	Liquid handler is not dispensing low volumes accurately.	Increase the tip travel distance inside well in the dispensing steps so that the reagents are added into the solution. Check the performance of liquid handler at low volume range.
	The mixing of reagents is not sufficient due to inefficient mixing by the liquid handler.	Increase the aspiration and dispensing speed and/or cycle times in the mixing steps. Increase the tip travel distance inside well in the mixing steps.
	Others	Refer to the Technical Bulletin of GenomePlex WGA Kit.
Negative control shows an amplified product.	Reagents are contaminated.	Use new labware and new batch of reagents. Test a reagent blank without DNA template to determine if the reagents are contaminated.

XIV. Contact Information

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