3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Protocol for GenomePlex® Whole Genome Amplification from Formalin-Fixed Parrafin-Embedded (FFPE) tissue

Application Guide

I. Description

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 precious material and usually available in limited quantities, amplification of the samples is essential. However, 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 amplify and purify genomic DNA from FFPE tissue. The methods described are completed using the GenElute™ Mammalian Genomic DNA Miniprep Kit, the 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 ~500-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 DNA[1]. In addition to SNP genotyping, downstream applications also include performing TaqMan® assays and BeadArray™ analysis.

II. Product Components

- GenElute™ Mammalian Genomic DNA Miniprep Kit (G1N10)
- GenomePlex® WGA kit (WGA1)
- GenElute™ PCR Clean-Up Kit (NA1020)

III. Materials to be Supplied by the User

- FFPE tissue
- Xylene
- JumpStart™ Taq DNA Polymerase, Product Code D9307
- Ethanol (absolute) Product Code E7023
- 37 °C water bath or heating block
- 55 °C water bath or heating block
- 70 °C water bath or heating block
- Microcentrifuge (with rotor for 2ml tubes)
- Molecular Biology Reagent Water, Product Code W4502

IV. Protocol for Extraction of DNA from FFPE tissue

- **GenElute Mammalian Genomic DNA Miniprep Kit**

1. Place a small section (20mg) 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 pipettting. 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 pallet.
8. Repeat steps 5-7 one more time.
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.
12. **Optional Rnase treatment.** If residual RNA is a concern add 20 μl of RNase A solution and incubate at room temperature for 2 minutes.
13. **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.
14. **Column preparation.** Add 500 μl of the Column Preparation Solution to each pre-assembled GenElut MiniPrep Binding Column and centrifuge at 12,000g for 1 minute.
15. **Prepare for binding.** Add 200 μl of ethanol to the lysed sample and mix by vortexing.
16. **Load lysate.** Transfer the entire contents of the sample tube into the treated binding column from step 14. Centrifuge at >6,500g for 1 minute. Discard the collection tube containing the flow-through liquid and place the binding column in a new 2 ml collection tube.
17. **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 >6,500g. Discard the collection tube containing flow-through liquid and place the binding column in a new 2 ml collection tube.
18. **Second wash.** Add another 500 μl of Wash Solution to the binding column and centrifuge for 3 minutes at maximum speed (12,000-18,000g) to dry the binding column. It is crucial that 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.
19. **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 >6,500g to elute the DNA.
20. Store DNA samples at -20°C.

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

V. Protocol for GenomePlex Whole Genome Amplification from FFPE tissue

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

Fragmentation

1. Prepare DNA solution of 1 ng/μl from the FFPE DNA
2. Add 1 μl of 10X Fragmentation Buffer to 10 μl DNA (1 ng/μl) 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 μl of 1x 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 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 (OD_{260}) 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 X*g*) 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 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.

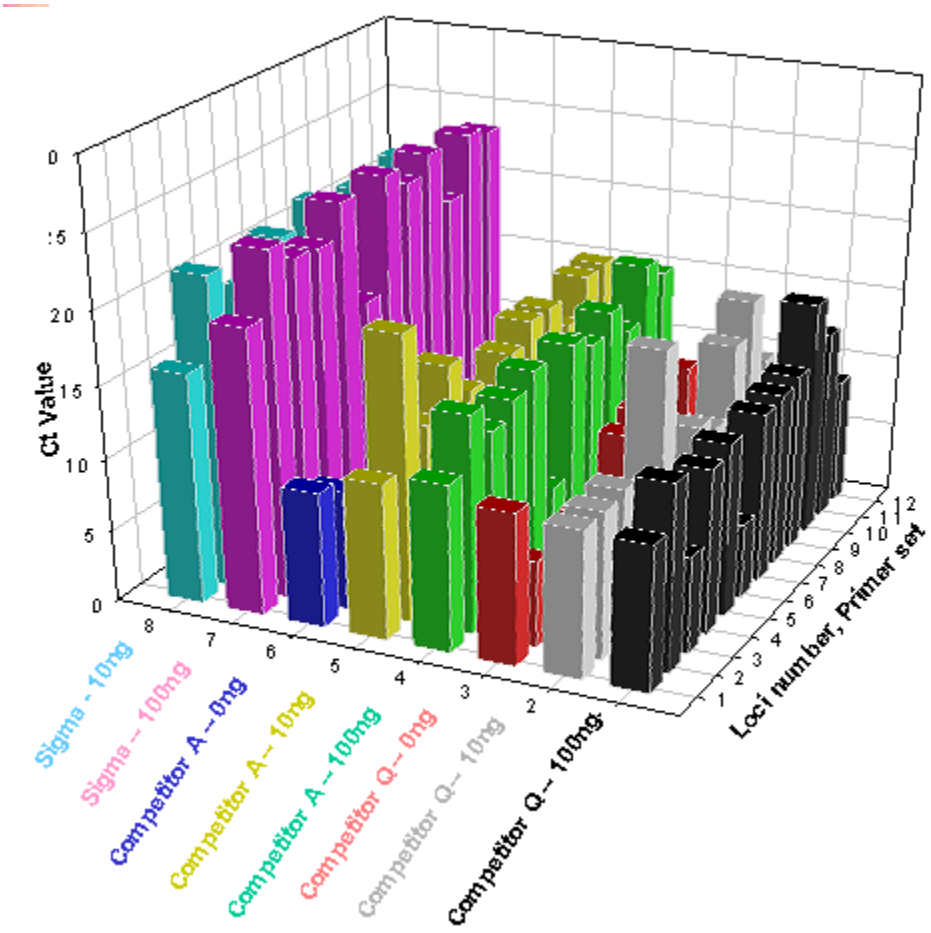
6. To elute the DNA, centrifuge the column at maximum speed for 1 minute. The PCR amplification product is now present in the eluate and is ready for immediate use or storage at –20 °C.

Appendix

Application Data

Performance of FFPE DNA Amplified with GenomePlex® WGA

DNA was extracted from a sample of formalin-fixed paraffin-embedded rat liver. 10ng and 100ng of genomic DNA was amplified using GenomePlex Complete WGA kit (WGA2) followed by purification using the GenElute™ PCR Clean Up Kit. Quantitative PCR (45 cycles) was performed on the WGA reaction using 12 different primer sets. GenomePlex amplifies more of the desired DNA, ~1000x more (10 Cts greater) over the competitors.



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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