Protocol for GenomePlex® Whole Genome Amplification from Plant

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

I. Description
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 and low yield. These qualities challenge the researchers ability to perform downstream analysis. GenomePlex® WGA provides a method of amplifying nanogram quantities of genomic DNA from plant with little or no detectable bias. The methods described use the GenElute™ Plant Genomic DNA Miniprep 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 1000-fold amplification of genomic DNA. 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. In addition to SNP genotyping, downstream applications also include performing TaqMan® assays and BeadArray™ analysis.

II. Product Components
- Sigma GenElute™ Plant Genomic DNA Miniprep (G2N10)
- GenomePlex® Whole Genome Amplification Kit (WGA1)
- GenElute™ PCR Clean-Up Kit (NA1020)

III. Materials to be Supplied by the User
- Plant samples
- 65 °C water bath or heating block
- 1.5 ml microcentrifuge tubes for lysis, Product Code T9661
- Microcentrifuge (with rotor for 2ml tubes)
- Ethanol (absolute), Product Code E7023
- Molecular Biology Reagent Water, Product Code W4502
- JumpStart™ Taq DNA Polymerase (D9307)

IV. Protocol for Extraction of DNA from Plant
- Performed with Sigma GenElute™ Plant Genomic DNA Miniprep (G2N10)
  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 to 16,000 x 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 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 x 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 min 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 Wash Solution Concentrate prior to first time use).
15. Centrifuge at maximum speed for 1 minute and 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.
19. Repeat the elution.
20. Store the eluted DNA at –20 °C.

V. Protocol for GenomePlex Whole Genome Amplification from Plant
- **Performed with GenomePlex Whole Genome Amplification Kit (WGA1)**

**Fragmentation**
1. Prepare DNA solution of 1 ng/µl from plant genomic DNA extraction described above.
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 amplified genomic DNA 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 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

References


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

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