Amplification of Plant DNA with GenomiPhi DNA Amplification Kit

**Keywords:** GenomiPhi | plant DNA | PCR | cloning | AFLP | RAPD analysis

Plant tissues often contain substances that interfere with DNA extraction. Polyphenols, for instance, are powerful oxidizing agents and can irreversibly bind to DNA, producing a brown gelatinous material (1). High levels of polysaccharides, as well, can make DNA pellets slimy and difficult to handle. In high concentrations, both of these compounds can also inhibit restriction enzymes and DNA polymerases, making extracted DNA unusable for many research applications including AFLP, RAPD analysis, PCR, and cloning. Other compounds that can complicate and prevent successful isolation of DNA in high concentrations include secondary compounds such as tannic acid, which is present in oak. Many protocols for improving DNA extraction from plants containing polyphenols and polysaccharides have been described (2, 3, 4, 5, 6, 7).

GenomiPhi™ DNA Amplification Kit offers a way to prepare high quality DNA from plant tissues (Fig 1). Genomic DNA is amplified by multiple-primed linear amplification using the highly processive enzyme Phi29 DNA polymerase (8, 9). The isothermal strand displacing enzyme has 3’–5’ exonuclease proofreading activity, ensuring representative amplification. The error rate is one in 10⁷—100-fold lower than Taq DNA polymerase (10).

The GenomiPhi DNA Amplification Kit can amplify DNA from leaves or seeds, generating microgram quantities of DNA overnight from just a small amount of input DNA. Often, only a simple lysis is required to produce sufficient input DNA for an amplification reaction. Because high levels of polysaccharides and polyphenols inhibit Phi29 DNA polymerase, plants containing high levels of these compounds may require additional sample purification.

### Products used

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Number</th>
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<tbody>
<tr>
<td>GenomiPhi DNA Amplification Kit</td>
<td>25-6600-01</td>
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<tr>
<td>Tween™ 20</td>
<td>US20605</td>
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<tr>
<td>MicroSpin™ G-50 columns</td>
<td>27-5330-01</td>
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</tbody>
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### Other materials required

- FTA™ card (Whatman BioScience)
- FTA purification reagent (Whatman BioScience)
- PicoGreen™ dsDNA Quantitation Kit (Molecular Probes Inc)

### Protocols

#### Leaf DNA preparation

For many plants, a simple alkaline lysis is sufficient to prepare DNA from leaf samples for amplification. For leaves containing high polysaccharide levels, such as strawberry, DNA extraction by CTAB (8) (Fig 2) or similar methods may be required prior to amplification. Failure to remove sufficient quantities of these compounds will result in inhibition of the GenomiPhi DNA amplification reaction.

**Alkaline lysis for leaves**

1. Grind 1 cm fresh leaf tissue using a mortar and pestle in liquid nitrogen.
2. Transfer into 1.5 or 0.7 ml microcentrifuge tube.
3. Add 150 µl 0.25 M NaOH. Incubate at 70 °C for 15 min. Note: Addition of 0.1 % Tween 20 v/v to NaOH may improve DNA extraction.
4. Add 150 µl of neutralization solution (80 mM Tris-HCl pH 8.0, 1 mM EDTA). Centrifuge at full speed for 5 min.
5. Remove supernatant and use appropriate amount in amplification reaction.
Seed DNA preparation

As for leaves, alkaline lysis is sufficient for most seeds, but CTAB extraction may be required for seeds with high polysaccharide or polyphenol levels (Fig 3). These methods [12] have been shown to work with wheat and soybean seeds. Methods may need to be modified for other plant species.

Alkaline lysis for seeds

1. Sterilize seeds by washing in 2% bleach solution on a shaker for 10 min followed by three water washes of 5 min each on the shaker.
2. Cut each seed in half using a sterile blade and place both halves in a 2 ml tube.
3. Add appropriate amount of lysis buffer (100 mM Tris-HCl, pH 9.5, 1 M KCl, 10 mM EDTA) depending on seed size (400 µl to soybean seeds and 100 µl to wheat seeds).
4. Incubate at 95 °C for 10 min.
5. Remove supernatant and use appropriate amount in amplification reaction.

CTAB extraction for seeds

1. Sterilize seeds by washing in 2% bleach solution on shaker for 10 min followed by three, 5-min washes in water on the shaker.
2. Grind seeds in liquid nitrogen using a sterile pestle and mortar. Transfer to a 40 ml conical tube with 500–800 µl of CTAB extraction buffer (2% CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA). Mix gently and incubate at 55 °C for 20 mins.
3. Centrifuge at 15 000 g for 5 min.
4. Transfer supernatant to a clean tube and add one volume of chloroform:isoamyl alcohol (24:1). Mix gently for 2 min.
5. Centrifuge at 15 000 g for 20 s.
6. Transfer upper aqueous phase to a new tube with 1/10 volume of 7.5 M ammonium acetate and two volumes of ice-cold ethanol.
7. Mix gently by inversion and incubate at -20 °C for 1 h.
8. Centrifuge at 15 000 g for 1 min and discard supernatant.
9. Wash pellet twice with 70% ethanol, mixing and centrifuging at 15 000 g for 30 s after each wash.
10. Dry pellet and resuspend in 50 µl of 1x TE buffer.
11. Use appropriate amount in amplification reaction.

Fig 3. DNA extraction from soybean.
CTAB extraction for leaves

This modified CTAB protocol (3, 11) has been shown to work with corn, wheat, and strawberry leaves. Methods may need to be modified for other plant species.

1. Weigh leaf tissue and grind to a fine powder in liquid nitrogen.

2. Add 1 ml of CTAB extraction buffer (2 % CTAB, 50 mM Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 1µM β-mercaptoethanol) per gram of leaf tissue. Mix well and transfer to fresh tubes, making sure that the total volume of the solution takes up no more than half of the total tube volume.

3. Incubate tubes for 30 min at 65 °C, mixing periodically (every 5–10 min).


5. Centrifuge at 13 000 g for 10 min at room temperature.

6. Transfer supernatant to a new tube; record the volume.

7. Add 10% CTAB solution (10% CTAB, 0.7 M NaCl in water); amount added should be equivalent to 1/10 tube volume. Mix gently.


9. Centrifuge at 13 000 g for 3 min at room temperature.

10. Transfer the supernatant to a new tube; record the volume (to transfer high viscosity supernatant, cut the end of the pipette tip).

11. Add an equal volume of the 1% CTAB solution (1 % CTAB, 50 mM Tris-HCl, pH 8.0). Mix gently.

12. Watch for pellet formation (solution will become slightly cloudy). If pellet is not seen immediately, let tube rest overnight at room temperature.

13. Centrifuge at 13 000 g for 3 min at room temperature.

14. Discard supernatant and dissolve pellet in 100 µl of 1 M NaCl.

15. Centrifuge at 13 000 g for 3 min at room temperature.

16. Transfer the supernatant to a new tube. Add an equal volume (100 µl) of ice-cold isopropanol.

17. Centrifuge at 13 000 g for 3 min at room temperature.

18. Wash three times with three volumes cold 70% ethanol.

19. Dissolve in an appropriate volume of ddH2O and use appropriate amount in amplification reaction.

Leaf samples immobilized to FTA cards

1. Punch out small disk of FTA matrix impregnated with plant material.

2. Wash disk for 5 min with 200 µl FTA purification reagent.

3. Discard wash solution. Repeat wash two additional times.

4. Wash disk for 5 min with 200 µl of TE (10mM Tris-HCL, 0.1 mM EDTA).

5. Discard wash solution and dry disk.

6. Use disk in amplification reaction.

Note: See www.whatman.com for more information on FTA paper and its compatibility with GenomiPhi DNA Amplification Kit.
Amplification

While the yield of extracted DNA varies by plant type and extraction method, alkaline lysis extraction generally gives lower and more variable yields than CTAB. In addition, the quality and quantity of input DNA in the amplification reaction directly affects the quality of the output amplification product. It is therefore important to quantitate the DNA sample prior to amplification. As shown in Figure 4, high levels of polyphenols or polysaccharides in the input sample will inhibit DNA amplification. Samples high in polyphenols will often appear orange or brown in color.

Genome size

Even though plant genomes vary considerably in size, from Arabadopsis at 126 Mb to wheat at 15996 Mb, yield from amplification reactions remains consistent (Fig 5). The variance in genome size, however, makes it important to calculate the appropriate amount of DNA to add to amplification reactions for any given plant species. We have found that a minimum of ~450–750 cells (3-5 ng of human DNA) is required for representative amplification.

Reaction scale

Amplification reactions can be scaled to produce different amounts of DNA, provided that all components, including the input DNA, are scaled proportionally (Fig 6). The recommended 20 µl amplification reaction will produce approximately 7 µg DNA, while a 5 µl reaction will produce 1–2 µg. We do not recommend reaction volumes lower than 5 µl.

Amplification product quantitation

The completed amplification reaction contains amplification product, unused hexamers, and dinucleotide mono- and di-phosphate. The amplification product cannot be directly quantitated by spectrophotometric absorption because of the presence of the nucleotides and hexamers. To determine the yield using absorption (OD260), the amplification product must first be purified by ethanol precipitation or a suitable column, such as MicroSpin G-50 Columns. We recommend PicoGreen dsDNA Quantitation reagent for accurate quantification of the nonpurified amplification product.

Using amplification product for genetic analysis

Amplification reaction products are high molecular weight (average size ~40 kb) concatamers of the input DNA. Amplification product can be used directly in downstream applications, though it may be necessary to re-optimize PCR conditions. If sufficient high quality DNA is used, the amplification product will be representative of the input DNA.

GenomiPhi amplified DNA has been successfully used in many applications including simple (Fig 7), multiplex, and real-time PCR, SNP genotyping (Third Wave Invader™ assay, MegaBACETM SNePe™ genotyping kit, GeneChip™ HuSNP™ Mapping Assay, Pyrosequencing™), STR and SSR genotyping (Fig 8), comparative genomic hybridization (CGH), cloning and library construction, heteroduplex analysis, slot and dot blots, and yeast-2-hybrid systems.

Fig 4. GenomiPhi DNA Amplification Kit products. DNA was extracted from wheat seeds using either alkaline lysis or CTAB methods. Extracted DNA and purified DNA were amplified with GenomiPhi DNA Amplification Kit. The sample used in lane 7 produced a lower yield, indicating inhibition of the amplification reaction.

Fig 5. Amplification kinetics and yield. Amplification of purified corn (2500 Mb), soybean (1115 Mb), wheat (15996 Mb), human (3000 Mb), and lambda DNA (40 Kb) with GenomiPhi DNA Amplification Kit. Yield and amplification kinetics are equivalent regardless of genome size. Yields determined by PicoGreen dsDNA quantitation reagent.
Polysaccharide and polyphenol compounds in plants can interfere with DNA extraction. GenomiPhi DNA Amplification Kit offers a way to prepare microgram quantities of DNA from leaves and seeds. Often a simple lysis is sufficient to prepare the DNA for amplification. In plants containing high polysaccharide or polyphenol levels, more extensive purification may be required prior to amplification. Alkaline-lysis-based extraction methods yield lower levels of DNA than CTAB procedures, but are much quicker and simpler to perform.

Amplification reactions produce high molecular weight concatamers of the input DNA. The amplification product will be representative of the input DNA provided sufficient amount of high quality DNA is used as input. No additional purification is necessary before using the amplification product for genetic analysis, but some reaction conditions, particularly PCR conditions, may require optimization.

**Conclusion**

**Fig 6.** Scaling GenomiPhi DNA amplification reactions. Amplification of purified corn, soybean, wheat, and human genomic DNA at different reaction volumes.

**Fig 7.** GenomiPhi and PCR amplification of plant DNA from FTA cards. Punches were taken from 35-month old leaf pressings immobilized to FTA cards and processed following recommended FTA protocols. DNA on disks was amplified with GenomiPhi DNA Amplification Kit. Rubisco activase gene (rca) was PCR amplified from the GenomiPhi amplification products and purified plant genomic DNA using PuReTaq™ Ready-To-Go™ PCR beads. (Data kindly provided by Whatman).

**Fig 8.** SSR analysis of white clover DNA. CTAB purified DNA (1 µl) was amplified with the GenomiPhi DNA Amplification Kit. In subsequent SSR analysis, equivalent results were obtained with the CTAB purified DNA (top two panels) and amplification products (bottom two panels). Data analysis performed with the MegaBACE Genetic Profiler v.2.0 software. (Data kindly provided by the Molecular Marker Technology Program, Plant Biotechnology Centre, Victoria, Australia).
References


