

# Genomic DNA Mini Kit (Plant)

*For research use only*

**Sample:** up to 100 mg of fresh plant tissue or up to 25 mg of dry plant tissue

**Yield:** 3-5 µg (100 mg *Arabidopsis thaliana* leaf), 20-25 µg (100 mg *Nicotiana tabacum* leaf)

**Format:** spin column

**Time:** within 30 minutes

**Elution volume:** 30-200 µl

**Storage:** dry at room temperature (15-25°C)



## Introduction

The Genomic DNA Mini Kit (Plant) provides a quick and easy method for purifying total DNA (including genomic DNA, mitochondrial DNA and chloroplast DNA) from various plant species. Homogenized samples are treated with RNase A then filtered to remove cell debris and salt precipitates. In the presence of the binding buffer, coupled with chaotropic salt, genomic DNA in the lysate binds to the glass fiber matrix of the spin column. Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer, TE or water. The procedure does not require DNA phenol extraction or alcohol precipitation, and can be completed in less than 30 minutes. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

## Quality Control

The quality of the Genomic DNA Mini Kit (Plant) is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Genomic DNA is isolated from 50 mg young leaf samples. More than 10 µg of genomic DNA is quantified with a spectrophotometer and analyzed by electrophoresis.

## Kit Contents

| Component                        | GP004            | GP100             |
|----------------------------------|------------------|-------------------|
| GP1 Buffer                       | 2 ml             | 50 ml             |
| GPX1 Buffer                      | 2 ml             | 50 ml             |
| GP2 Buffer                       | 1 ml             | 15 ml             |
| GP3 Buffer*<br>(Add Isopropanol) | 1.5 ml<br>(3 ml) | 30 ml<br>(60 ml)  |
| W1 Buffer                        | 2 ml             | 45 ml             |
| Wash Buffer**<br>(Add Ethanol)   | 1 ml<br>(4 ml)   | 25 ml<br>(100 ml) |
| Elution Buffer                   | 1 ml             | 30 ml             |
| RNase A (10 mg/ml)               | 25 µl            | 550 µl            |
| Filter Columns                   | 4                | 100               |
| GD Columns                       | 4                | 100               |
| 2 ml Collection Tubes            | 8                | 200               |

## Order Information

| Product                                    | Package Size     | Catalogue Number   |
|--|------------------|--------------------|
| Genomic DNA Mini Kit (Blood/Cultured Cell) | 100/300 preps    | GB100/300          |
| Genomic DNA Maxi Kit (Blood/Cultured Cell) | 10/25 preps      | GDM10/25           |
| Genomic DNA Mini Kit (Tissue)              | 50/100/300 preps | GT050/100/300      |
| gSYNC™ DNA Extraction Kit                  | 50/100/300 preps | GS050/100/300      |
| Genomic DNA Mini Kit (Plant)               | 100 preps        | GP100              |
| Genomic DNA Maxi Kit (Plant)               | 10/25 preps      | GPM010/25          |
| GENEzol™ DNA Reagent Plant                 | 100/200 rxns     | GR100/200          |
| Presto™ Mini gDNA Yeast Kit                | 100/300 preps    | GBY100/300         |
| Presto™ Mini gDNA Bacteria Kit             | 100/300 preps    | GBB100/101/300/301 |
| Geneius™ Micro DNA Extraction Kit          | 100/300 preps    | GMB100/300         |
| Presto™ Buccal Swab gDNA Extraction Kit    | 100/300 preps    | GSK100/300         |
| Presto™ 96 Well Blood gDNA Extraction Kit  | 4/10 x 96 preps  | 96GBP04/10         |
| Presto™ 96 Well Plant gDNA Extraction Kit  | 4/10 x 96 preps  | 96GPP04/10         |

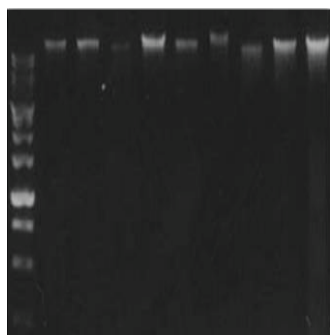
\*Add isopropanol (see the bottle label for volume) to the GP3 Buffer immediately prior to initial use

\*\*Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

## Caution

The components contain irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

## Genomic DNA Mini Kit (Plant) Functional Test Data



**Figure 1.** Genomic DNA was extracted from a variety of 100 mg plant species using the Genomic DNA Mini Kit (Plant). A 3 µl aliquot from each 200 µl eluate was analyzed by electrophoresis on a 1% agarose gel.

M = Geneaid 1 Kb DNA ladder

1. *Cinnamomun camphora* (Camphor tree)
2. *Pisum sativum* (Pea sprout)
3. *Arabidopsis thaliana* (Thale cress)
4. *Oryza sativa* (Rice)
5. *Ipomoea batatas* (Sweet potato vine)
6. *Rhizoma dioscoreae* (Chinese yam )
7. *Populus tremula* (Aspen)
8. *Flammulina velutipes* (Mushroom)
9. *Oxalis comiculats* (Clover)

M 1 2 3 4 5 6 7 8 9

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## Genomic DNA Mini Kit (Plant) Protocol

Various plant species contain different metabolites such as polysaccharides, polyphenols, and proteins. The standard protocol uses GP1 Buffer for lysis of most common plant species. Alternatively, GPX1 Buffer is provided with the kit to ensure efficient cell lysis of plant species with high polysaccharide content.

### IMPORTANT BEFORE USE

- Add isopropanol (see the bottle label for volume) to the GP3 Buffer immediately prior to initial use
- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional Requirements: microcentrifuge tubes, isopropanol, absolute ethanol

|                                  |  |
|----------------------------------|--|
| Step 1<br>Tissue<br>Dissociation | <ul style="list-style-type: none"><li>• Cut off <b>50 mg (up to 100 mg) of fresh or frozen plant tissue</b> or <b>10 mg (up to 25 mg) of dry sample</b>.</li><li>• Freeze the sample with liquid nitrogen.</li><li>• Grind the sample to a fine powder then transfer it to a 1.5 ml microcentrifuge tube.</li></ul> NOTE: Some plant samples can be ground sufficiently in the absence of liquid nitrogen.   |
| Step 2<br>Lysis                  | NOTE: Mix GP1 Buffer or GPX1 Buffer and RNase A immediately prior to use. <ul style="list-style-type: none"><li>• Add <b>400 µl of GP1 Buffer</b> or <b>GPX1 Buffer</b> and <b>5 µl of RNase A</b> into the sample tube and mix by vortex.</li><li>• Incubate at 60°C for 10 minutes. During incubation, invert the tube every 5 minutes.</li></ul> At this time, pre-heat the required <b>Elution Buffer</b> (200 µl per sample) to 60°C (for Step 5 DNA Elution). <ul style="list-style-type: none"><li>• Add <b>100 µl of GP2 Buffer</b> and mix by vortex then incubate on ice for 3 minutes.</li><li>• Place a <b>Filter Column</b> in a <b>2 ml Collection Tube</b> then transfer the mixture to the <b>Filter Column</b>.</li><li>• Centrifuge for 1 minute at 1,000 x g then discard the <b>Filter Column</b>.</li><li>• Carefully transfer the supernatant from the 2 ml collection tube to a new 1.5 ml microcentrifuge tube.</li></ul>  |
| Step 3<br>DNA<br>Binding         | <ul style="list-style-type: none"><li>• Add a 1.5 volume of <b>GP3 Buffer</b> (make sure isopropanol was added) then vortex immediately for 5 seconds.</li></ul> E.g. Add 750 µl of GP3 Buffer to 500 µl of lysate.<br>NOTE: If precipitate appears, break it up as much as possible with a pipette. <ul style="list-style-type: none"><li>• Place a <b>GD Column</b> in a <b>2 ml Collection Tube</b>.</li><li>• Transfer 700 µl of mixture (and any remaining precipitate) to the <b>GD Column</b>.</li><li>• Centrifuge at 14-16,000 x g for 2 minutes.</li><li>• Discard the flow-through then place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li><li>• Add the remaining mixture to the <b>GD Column</b> then centrifuge at 14-16,000 x g for 2 minutes.</li><li>• Discard the flow-through then place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li></ul>   |
| Step 4<br>Wash                   | <ul style="list-style-type: none"><li>• Add <b>400 µl of W1 Buffer</b> to the <b>GD Column</b> then centrifuge at 14-16,000 x g for 30 seconds.</li><li>• Discard the flow-through then place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li><li>• Add <b>600 µl of Wash Buffer</b> (make sure ethanol was added) to the <b>GD Column</b>.</li><li>• Centrifuge at 14-16,000 x g for 30 seconds.</li><li>• Discard the flow-through then place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li><li>• Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.</li></ul> <b>Optional Residual Pigment Removal Step</b><br>If pigments remain on the column, perform this optional step. <ul style="list-style-type: none"><li>• Following <b>Wash Buffer</b> addition, add <b>400 µl of absolute ethanol</b> to the <b>GD Column</b>.</li><li>• Centrifuge at 14-16,000 x g for 30 seconds.</li><li>• Discard the flow-through then place the <b>GD Column</b> back in the <b>2 ml Collection Tube</b>.</li><li>• Centrifuge for 3 minutes at 14-16,000 x g to dry the column matrix.</li></ul> |
| Step 5<br>DNA Elution            | Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to approximately 200 µl. <ul style="list-style-type: none"><li>• Transfer the dried <b>GD Column</b> to a clean 1.5 ml microcentrifuge tube.</li><li>• Add <b>100 µl of pre-heated Elution Buffer</b> or TE to the center of the column matrix.</li><li>• Let stand for 3-5 minutes to ensure the <b>Elution Buffer</b> or TE is completely absorbed.</li><li>• Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.</li></ul>  |

## Troubleshooting

| Problem   | Possible Reasons/Solution   |
|---|---|
| Column Clogged  | <b>Too much sample was used</b> <ul style="list-style-type: none"><li>• Reduce the sample volume or separate it into multiple tubes.</li></ul>  |
| Low Yield   | <b>Precipitate was formed at the DNA Binding step</b> <ul style="list-style-type: none"><li>• Reduce the sample material.</li><li>• Following GP3 Buffer addition, break up any precipitate as much as possible prior to loading GD Column.</li></ul> <b>Incorrect DNA Elution Step</b> <ul style="list-style-type: none"><li>• Ensure that the Elution Buffer or TE is added to the center of the GD Column matrix and is absorbed completely.</li></ul> |
| Eluted DNA does not perform well in downstream applications | <b>Incomplete DNA Elution</b> <ul style="list-style-type: none"><li>• Elute twice to increase yield.</li></ul> <b>Residual ethanol contamination</b> <ul style="list-style-type: none"><li>• Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.</li></ul>  |