# CYDN38-CHANGYU Plant Genomic DNA Maxi Kit

| Catalog number | Package |
|---|---|
| CYDN3801 | 10 preps |

Storage and shipping: 15-25 °C. Stable for 12 months at room temperature. RNase A should be stored at -20 °C.

## Description

This kit combines a DNA spin column with a unique solution system and is suitable for rapid and simple extraction of genomic DNA from plant samples containing phenolics, polysaccharides and enzyme inhibitors. Purification of DNA from one or more 1 g fresh or 200 mg dry plant samples can be completed in about 1 hour. The procedure does not require toxic organic solvents such as phenol or chloroform and does not require time-consuming isopropanol or ethanol precipitation; polysaccharides, phenolics, enzyme inhibitors and other impurities are removed rapidly and efficiently. The purified DNA can be used directly for PCR, restriction digestion, hybridization and similar experiments.

Fresh or dry plant tissue (cells) is ground and lysed with Lysis Buffer. Proteins, polysaccharides and cell debris are removed by precipitation. Genomic DNA then binds selectively to the silica membrane of the spin column under high-ionic-strength conditions. A series of rapid wash and centrifugation steps further removes polysaccharides, polyphenols, cell metabolites, proteins and other impurities, and purified genomic DNA is eluted from the membrane with low-salt Elution Buffer EB.

## Kit components

| Component | Storage | 10 preps (CYDN3801) |
|---|---|---|
| RNase A (10 mg/ml) | -20 °C | 500 µl |
| Buffer AP1 | 15-25 °C | 50 ml |
| Buffer AP2 | 15-25 °C | 20 ml |
| Buffer AP3/E | 15-25 °C | 40 ml |
| Wash Buffer WB | 15-25 °C | 25 ml × 2 |
| Elution Buffer EB | 15-25 °C | 20 ml |
| Spin Column AC | 15-25 °C | 10 |
| Collection Tube (50 ml) | 15-25 °C | 10 |

Before first use, add the indicated volume of absolute ethanol to Buffer AP3/E and Wash Buffer WB and tick the boxes on the labels. The kit is stable for 12 months at room temperature.

## Storage notes

Buffer AP1 and Buffer AP3/E may precipitate at low temperature. Warm in a 65 °C water bath for a few minutes to redissolve (AP3/E may be heated before ethanol is added but must not be heated after ethanol has been added). After the buffers become clear, cool to room temperature before use.

Avoid prolonged exposure of reagents to air, which causes evaporation, oxidation and pH changes. Keep all solutions tightly capped after use.

## Features

The silica membrane in the spin columns is a specially manufactured adsorption membrane; adsorption varies very little from column to column and reproducibility is good.

No toxic reagents such as phenol are required, and no ethanol precipitation step is needed.

Fast and simple: a single sample is usually processed within 1 hour.

Several polysaccharide- and polyphenol-removing components and repeated column washes ensure high purity. Typical OD260/OD280 is 1.7-1.9; the DNA is suitable for PCR, Southern-blot and restriction digestion.

### Notes before starting

**Note:** Preheat the required water bath to 65 °C before starting.

**Note:** Buffer AP3/E contains irritants. Wear latex gloves and avoid contact with skin, eyes and clothing. If skin, eyes or clothing are contaminated, rinse with plenty of water or saline.

**Note:** DNA yield differs between plant materials. Typical yield from 1 g fresh tissue is 30-260 µg.

**Note:** Elution Buffer EB contains no EDTA and does not interfere with downstream digestion or ligation. Water may also be used for elution if its pH is above 7.5; a low pH reduces elution efficiency, and DNA eluted with water should be stored at -20 °C. For long-term storage, DNA may be eluted with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), but EDTA may affect downstream restriction digestion, so the eluate may need to be diluted before use.

### ❖ Procedure

Read the notes before starting. Before first use, add the indicated volume of absolute ethanol to Wash Buffer WB and Buffer AP3/E, mix thoroughly and tick the boxes on the labels.

1. Preheat Buffer AP1 in a 65 °C water bath.

2. Take an appropriate amount of plant tissue (no more than 1 g fresh tissue or 200 mg dry tissue) and grind thoroughly to a fine powder in a mortar with liquid nitrogen.

3. Transfer the powder to a 15 ml centrifuge tube without thawing, add 5 ml Buffer AP1 (preheated to 65 °C) and 40 µl RNase A (10 mg/ml), and vortex thoroughly to help lysis.

If the tissue is difficult to lyse, a gentle homogenization step of 10 seconds may be added. In most cases there is no need to centrifuge out incompletely lysed tissue, because a centrifugation step later removes it.

4. Incubate in a 65 °C water bath for 10-15 minutes, inverting the tube 2-3 times during the incubation to mix the sample.

5. Add 1.8 ml Buffer AP2, mix thoroughly, place on ice for 10 minutes and centrifuge at 9,000 x g at room temperature for 10 minutes. Carefully transfer the supernatant to a new 50 ml centrifuge tube without disturbing the interface material.

**Note:** If a high-speed centrifuge is not available, centrifugation at 4,000-5,000 x g with a proportionally longer time is also acceptable.

6. Estimate the supernatant volume and add 1.5 volumes of Buffer AP3/E (check that ethanol has been added); vortex immediately to mix.

**Note:** A flocculent precipitate may appear when AP3/E is added; it does not affect DNA extraction. Add AP3/E directly to the supernatant and vortex immediately to mix.

7. Load the mixture from the previous step, including any precipitate, onto Spin Column AC placed in a collection tube. Centrifuge at 3,000-5,000 x g for 5 minutes and discard the flow-through.

8. Add 10 ml Wash Buffer WB (check that ethanol has been added), centrifuge at 3,000-5,000 x g for 4 minutes and discard the flow-through.

9. Add 10 ml Wash Buffer WB (check that ethanol has been added), centrifuge at 3,000-5,000 x g for 3 minutes and discard the flow-through.

10. Return Spin Column AC to the empty collection tube and centrifuge at maximum speed (preferably above 9,000 x g; if the centrifuge speed is lower, extend the time accordingly) for 10 minutes to dry residual ethanol from the membrane. Remove residual ethanol between the inner ring and the column wall with a pipette tip, and air-dry at room temperature or in an oven for a few minutes.

11. Place Spin Column AC in a clean centrifuge tube. Apply 1-2 ml Elution Buffer EB (prewarmed in a 65-70 °C water bath if desired) to the centre of the membrane, leave at room temperature for 5 minutes and centrifuge at 3,000-5,000 x g for 3 minutes to obtain the DNA. In addition, reload the eluate onto the column, leave at room temperature for 2 minutes and elute again to increase concentration and yield.

A larger elution volume gives higher elution efficiency; increase the elution volume if a high yield is expected and needed. For a higher DNA concentration the elution volume may be reduced, but it should not be less than 500 µl; too small a volume reduces elution efficiency and DNA yield.

Store the DNA at 2-8 °C; for long-term storage, keep at -20 °C.

## Troubleshooting

| Problem | Comments and suggestions |
|---|---|
| Low DNA yield | Excess starting material or incomplete lysis - use an appropriate amount of starting material and grind or homogenize thoroughly. Inappropriate binding conditions - estimate the supernatant volume accurately at step 5 and add exactly 1.5 volumes of AP3/E. |
| Residual RNA | Plant RNA content in the sample is too high - increase the RNase A concentration. |
| No DNA obtained | Absolute ethanol was forgotten in Wash Buffer WB - add the indicated volume of absolute ethanol to WB before the first experiment. |
| Spin column clogged | Insufficient grinding or lysis with many clumps; the lysate is too viscous; the centrifugal force is too low - see step 2 and add a centrifugation step to remove debris; reduce the amount of starting material and do not overload; increase the centrifugal force. |
| Coloured eluted DNA solution or visible pigment on the membrane | Insufficient washing - after step 8, wash once more with 10 ml ethanol. Excess starting material - reduce the amount of starting material and do not overload. |
| Low DNA yield in the eluate | Residual ethanol in the spin column or ethanol at the bottom of the tube - make sure step 9 was performed, otherwise residual ethanol reduces elution efficiency. Water or another non-optimal liquid was used instead of Elution Buffer - read step 10 and note 4 carefully and elute only with Elution Buffer EB. The elution volume is too low - elute with 2 ml Elution Buffer. |
| Abnormally high A260 | Silica membrane components co-eluted and interfered with absorbance - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant. |
| DNA cannot be cut or is only partially digested by restriction enzymes | Silica membrane components co-eluted and inhibited digestion - centrifuge the eluted genomic DNA again at 13,000 rpm for 1 minute and carefully use the supernatant. Residual ethanol in the spin column or at the bottom of the tube inhibited digestion - make sure step 9 was performed, then air-dry for a few minutes to let residual ethanol evaporate. |

**This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.**