PURIFYING GENOMIC DNA FROM PLANT

1 HOMOGENIZATION

Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material.
Proceed to cell lysis using Lysis Buffer PA1 (step 2.1) or alternatively Lysis Buffer PA2 (step 2.2).

2 LYSIS

2.1 CELL LYSIS WITH LYSIS BUFFER PA1

Transfer resulting powder to a new tube and add 400 µL Lysis Buffer PA1.
Vortex mixture thoroughly.
Add 10 µL RNase A solution and thoroughly mix sample.
Incubate at 65°C for 10 min.
Proceed to step 3.

2.2 CELL LYSIS WITH LYSIS BUFFER PA2

Transfer resulting powder to a new tube and add 300 µL Lysis Buffer PA2.
Vortex mixture thoroughly.
Add 10 µL RNase A solution and thoroughly mix sample.
Incubate at 65°C for 10 min.
Add 75 µL Precipitation Buffer PL3, mix thoroughly and incubate for 5 min on ice to precipitate SDS completely.
Proceed to step 3.

3 FILTER CRUDE LYSATE

Place ISOLATE II Filter (violet) into a new 2 mL Collection Tube and load lysate onto column.
Centrifuge 2 min at 11,000 x g. Collect clear flow-through and discard ISOLATE II Filter.
If a pellet is visible in flow-through, transfer clear supernatant without disturbing pellet to a new 1.5 mL microcentrifuge tube (not supplied).

4 ADJUST DNA BINDING CONDITIONS

Add 450 µL Binding Buffer PB.
Mix thoroughly by pipetting up and down 5 times or by vortexing.

5 BIND DNA

Place ISOLATE II Plant DNA Spin Column (green) into a new 2 mL Collection Tube and load sample (max. of 700 µL).
Centrifuge 1 min at 11,000 x g and discard the flow-through.

6 WASH AND DRY SILICA MEMBRANE

- Add 400 µL Wash Buffer PAW1.
  Centrifuge 1 min at 11,000 x g and discard flow-through.
- Add 700 µL Wash Buffer PAW2.
  Centrifuge 1 min at 11,000 x g and discard flow-through.
- Add another 200 µL Wash Buffer PAW2.
  Centrifuge 2 min at 11,000 x g to remove wash buffer and to dry silica membrane completely.

7 ELUTE DNA

Place ISOLATE II Plant DNA Spin Column into a new 1.5 mL microcentrifuge tube (not supplied).
Add 50 µL preheated Elution Buffer PG (65˚C) onto center of silica membrane.
Incubate 5 min at 65˚C.
Centrifuge 1 min at 11,000 x g.
Repeat this step with another 50 µL Elution Buffer PG (65˚C) and elute into same tube.