PURIFYING GENOMIC DNA FROM PLANT

1 **Homogenization**

Homogenize up to 100mg wet weight or up to 20mg 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 400µl Lysis Buffer PA1.
Vortex mixture thoroughly.
Add 10µl 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 300µl Lysis Buffer PA2.
Vortex mixture thoroughly.
Add 10µl RNase A solution and thoroughly mix sample.
Incubate at 65°C for 10 min.
Add 75µl 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 2ml 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 1.5ml microcentrifuge tube (not supplied).

4 **Adjust DNA binding conditions**

Add 450µl 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 2ml Collection Tube and load sample (max. of 700µl).
Centrifuge 1 min at 11,000 x g and discard the flow-through.

6 **Wash and dry silica membrane**

- Add 400µl Wash Buffer PAW1.
  Centrifuge 1 min at 11,000 x g and discard flow-through.
- Add 700µl Wash Buffer PAW2.
  Centrifuge 1 min at 11,000 x g and discard flow-through.
- Add another 200µl 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 1.5ml microcentrifuge tube (not supplied).
Add 50µl 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 50µl Elution Buffer PG (65°C) and elute into same tube.