Plant DNA extraction using the DNAeasy® Plant Mini Kit (Qiagen)

PS: ensure the water bath is set at 65°C before you commence extraction

A. Prepare and label extraction tubes

1.Eppendorf tube

2. QIAshredder columns (purple/pink)

3. DNA easy mini spin column (colourless)

NB: One tube for each represents one sample.

a) Label two sets of 2ml microcentrifuge (Eppendorf)tubes (1 for each sample). First set will be used for adding **homogenized sample** and the second set will be used for **binding DNA**.(Not provided with the kit).
b) Label the QIAshredder columns and the DNAeasy mini spin column
c) Label 1.5ml Eppendorf tube for final **DNA elution**- provided with the kit

B: Procedure

I. Tissue disruption/ homogenization

1. Disrupt Cells Grind plant tissue into a fine powder in liquid nitrogen using a mortar and pestle/genogrinder. Transfer up to 20 mg of the powder to a 2ml microcentrifuge tube. Keep the sample on ice for immediate use or freeze at –70 °C for future use.

II. Lyse Cells

2. Add **400 µL** of Buffer **AP1** and **4 µL** of **RNAse A**. Thoroughly mix by vortexing and inverting
   Incubate the mixture at 65 °C for 10 minutes with occasional inversion (2 to 3 times)

III. Precipitation of DNA and removal of Debris

3. Add **130** µL of **Buffer P3** to the mixture; mix completely by inversion and place the sample on ice for 5 minutes.
4. Centrifuge the sample at maximum speed (12,000–16,000 5 g) for 5 minutes to pellet the cellular debris, proteins, and polysaccharides.
5. Carefully pipette the supernatant from step 4 and transfer onto the QIAshredder spin column placed in a collection tube (purple/pink**). PS: Avoid disrupting the debris settled at the bottom while pipetting.** Centrifuge at a maximum speed for 2 minutes. This removes any cellular debris not removed in step 4 above

   NB: **Discard the filtration column but retain the collection tube since it will now have the flowthrough which contains the DNA.**

IV. Binding DNA

6. Transfer the **(~450 ml)** flowthrough (from 5 above) into a new 2ml eppendorf tube without disturbing the pellet if present. Then add 1.5 volumes **(~675ml)** of Buffer AW1 and mix by inverting 4 -5 times. **PS: hold onto the cap tightly to avoid spillage.**
7. Transfer **650 µL** of mixture from step 6 into a DNA Mini spin column(colourless/white) placed in a 2ml collection tube. Centrifuge for 1min at 8000rpm. **Discard the flow-through liquid; retain the collection tube.** Return the column to the collection tube. Apply the remaining mixture from step 6 onto the column. Repeat the centrifugation as above and discard the flow-through liquid and collection tube.

V. First and second column wash

8. Place the spin column into a new 2ml collection tube. Add **500 µL Buffer AW2**, and centrifuge for 1min at 8000rpm. Discard the flow through
9. Add another **500 µL Buffer AW2.** Centrifuge for 2minutes and maximum speed. **NB: Remove the spin column carefully from the collection tube so that it does not come into contact with the flow through.**

VI. Elution

10. Transfer the spin column to a new 1.5ml Eppendorf tube
11. Add 70 µL **Buffer AE** for elution. Incubate for 5 minutes at room temperature.Centrifuge for 1min at 8000rpm.

VII. Quantification of DNA

a. Nanodrop (spectrophotometer)

Materials Needed:

- DNA sample
- Buffer AE (for blanking)
- Lint-free tissue or Kimwipes
- Pipettes and tips

Protocol:

- Turn on the Nanodrop and select the Nucleic Acid mode.
- Blank the instrument:
- Open the pedestal and place 2 µL of buffer AE close the pedestal(gently).
- Lower the arm and press "Blank" to set the baseline.
- Wipe the pedestal clean with a lint-free tissue.

Measure the DNA sample:

- Pipette 2 µL of your DNA sample onto the lower pedestal.
- Lower the arm and press "Measure".

Record the results:

- Note the DNA concentration (ng/µL) and purity ratios (A260/A280, A260/A230).
- Clean the pedestal with a lint-free tissue before measuring the next sample.

Interpretation:
A260/A280 ratio (~1.8): Indicates pure DNA.
A260/A230 ratio (>2.0): Indicates minimal contamination.
Low ratios suggest protein, salt, or phenol contamination.

b. Qubit (fluorometer)

Materials Needed:

- Qubit dsDNA Broad Range (BR) Assay Kit (Includes buffer, dye, and standards)
- DNA sample
- Thin-walled 0.5 mL Qubit tubes
- Pipettes and tips

Protocol:

- Prepare the working solution: Mix 199 µL of Qubit buffer with 1 µL of Qubit BR dye per sample.
- Vortex briefly.
- Prepare standards and samples: Label Qubit tubes for two standards and your DNA samples.
- Pipette 190 µL of working solution into each standard tube.
- Add 10 µL of Standard #1 and Standard #2 to their respective tubes.
- For your samples, mix 198 µL of working solution with 2 µL of DNA sample in a new tube.
- Vortex all tubes briefly and incubate for 2 minutes at room temperature.

Measure on Qubit Fluorometer:

- Select dsDNA Broad Range assay mode.
- Insert Standard #1, press "Read", then insert Standard #2 and repeat.
- Insert your sample tube, press "Read", and record the concentration.

Interpret results:

- Qubit provides DNA concentration in ng/µL.
- If concentration is out of range, dilute the sample and remeasure.

VIII. **Electrophoresis Gel Preparation Protocol**

Prepare 1.8% Agarose Gel in 100 mL

1. To prepare a 1.8% agarose gel in 100 mL, you'll need 1.8 grams of agarose powder.
2. In a flask, add 1.8 grams of agarose powder to 100 mL of 1X TAE buffer.
3. Heat the mixture in a microwave or on a hot plate until the agarose is completely dissolved. Swirl the flask occasionally to ensure thorough mixing.
4. Let is cool to about 60°C
5. Add 3ul of Gel red (staining dye that enables visualization under UV). **NB: Ensure safety precautions are followed when handling ethidium bromide.**
6. Swirl the flask to ensure thorough mixing.
7. Pour the agarose solution into the gel tray, ensuring that no bubbles are trapped (Gel tray is assembled prior)
8. Let the gel solidify for about 20-30 minutes at room temperature.
9. Mix DNA samples with loading dye to make them denser and more visible during electrophoresis.
10. Gently mix the samples and centrifuge briefly to ensure proper mixing.
11. Carefully remove the combs from the gel tray.

    -Setup Electrophoresis Apparatus:
    - Fill the electrophoresis tank with 1X TAE buffer until the gel is completely submerged.
    - Carefully place the gel tray into the electrophoresis tank, ensuring that the wells are on the side with the negative (black) electrode.

12. Use a micropipette to load DNA samples into the wells of the gel.
13. Load a DNA ladder in one of the wells to serve as a size reference.
14. Run Electrophoresis: Connect the power supply to the electrophoresis apparatus. Set the voltage to about 100V and run the gel for approximately 45-60 minutes. Visualize the gel under a transilluminator and save photos

PCR protocol

Reagents from the Qiagen kit: AllTaq Master Mix

Reagent	concentration	n=1	Master mix n=10	Mastermix n=
AllTaq master mix 4X	1X	2.5µl	25	
Primer F (10 µM)	0.5µM	0.5	5	
Primer R (10 µM)	0.5µM	0.5	5	
RNAsefree water		5.5	55	
DNA	10ng	1 µl	1 ul @sample added separately	
aliquot		10ul	9 µl into each tube	

PCR cycle

Step	Temperature	Time	
Denaturation	95 °C	2 minutes	
Denaturation	95 °C	15 seconds	35cycles
Annealing	52 °C	30 seconds	
Extension	72 °C	1 minute	
Extension	72 °C	2 minutes	
Hold	12 °C	10 minutes	