PROTOCOL 1.

DNA isolation from herbarium leaf tissue using DNeasy Plant Mini Kit® and QIAcube® robotic workstation

Things to be done before the start:

- **All centrifugation steps are carried out at room temperature (15–25°C)**
- **Buffer AP1 and Buffer AW1 concentrate may form precipitates upon storage. If necessary, warm to 65°C to redissolve (before adding ethanol to Buffer AW1). Do not heat Buffer AW1 after ethanol has been added**
- **Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffers AW1 and AW2 as indicated on the bottles to obtain working solutions.**
- **The QIAcube performs fully automated processing of up to 12 samples.**
- **Pour AW1, AW2 and AE buffers into three 30 ml QIAcube reagent bottles.**
- **Place 30 ml QIAcube reagent bottles into the position 1 (AW1), 2 (AW2) and 3 (AE) of the QIAcube reagent bottles rack.**
- **Place DNeasy Mini spin column (white), QIAshredder spin column (violet column with cut off lid) from DNeasy® Plant Mini kit and 1,5 ml microcentrifuge tube into the position 1, 2 and 3 of the QIAcube® Rotor Adapter.**
- **Place the loaded QIAcube Rotor Adapter into the QIAcube centrifuge bucket.**
- **Place two prefilled tip racks onto the worktable of the QIAcube.**

1) Place ca. 20 mg of herbarium leaf tissues into a 2 ml safe lock (Eppendorf®) microcentrifuge tubes. Place the tubes into the TissueLyser Adapter Set 2 x 24 (Qiagen®). Add 5 mm autoclaved stainless steel bead into each microcentrifuge tube.

2) Fix TissueLyser Adapter Set 2 x 24 into the clamps of the TissueLyser (Qiagen®) and grind the samples for 1 min at 30 Hz.

3) Add 400 µl Buffer AP1, 4 µl 2-mercaptoethanol and 4 µl RNaseA stock solution into each microcentrifuge tube with disrupted leaf tissue and vortex vigorously using vortex mixer.

   - Carry out this step in a fume hood

4) Incubate the mixtures in microcentrifuge tubes for 10 min at 65°C. Mix 2-3 times during incubation using vortex mixer.

   - This step lyses the cells

5) Add 130 µl Buffer P3 to each lysate, mix, and incubate for 7 min on ice

   - This step precipitates detergent, proteins, and polysaccharides

6) Centrifuge the lysates for 5 min at 20,000 x *g*

7) Transfer the supernatants to a new 2 ml safe lock (Eppendorf®) microcentrifuge tubes.

   - Be careful not to pick up pellet at the bottom of the tube during pipetting process

8) Open the QIAcube door and place 2 ml tubes into the QIAcube shaker adapter. Place tube lids into the slot at the edge of the shaker adapter.

   - Remove caps from 30 ml QIAcube reagent bottles

9) Close the QIAcube door and launch QIAcube robotic workstation choosing protocol: Purification of total DNA from plant cells and tissues (see QIAcube Protocol Sheet).
   - In these steps DNA molecules are bounded, washed, dried and finally eluted from DNeasy Mini spin column as a pure DNA isolate.
10) When the protocol run has finished, a message is displayed in the touchscreen confirming that the samples have been processed.
11) Remove the 1,5 ml microcentrifuge tubes containing purified DNAs from the rotor adapters.
   - Discard sample tubes, used rotor adapters, and reagent according to safety regulations.
   - Replace the lids of the reagent bottles and close tightly
   - Empty the waste drawer
12) Isolated DNA solution should be kept at -20 °C for longer period or at 4-8 ° C for several days

References:

1) DNeasy® Plant Handbook
2) QIAcube® User Manual
3) QIAcube and DNeasy Plant Mini Kit - QIAcube® Protocol Sheet

Laboratory equipment and accessories used for DNA isolation

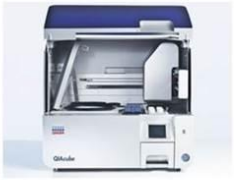

TissueLyser (Qiagen®)

Dneasy® Plant mini kit

GVLab vortex mixer (Gilson®).


Bio TDB-100 Dry Block Biosan®


Centrifuge Hettich® Mikro 200


QIAcube® robotic workstation


QIAcube® Filter-Tips 1000 µl


QIAcube® 30 ml Reagent Bottles


QIAcube® Rotor Adapter

Plant material