DNA Extraction (Qiagen Kit)

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3:02 PM

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Tissue Grinding

1. Label and weigh 2 ml microfuge tubes, be sure to use sterile tubes and used gloved hands to avoid contamination
2. Harvest leaf material, you want no more than 100 mg FW of tissue, this is approximately 1 fully expanded mature leaf, and immediately freeze in LN_2

To harvest leaf material, take 2 mL microfuge tube and place the leaf inside of the tube. Close the cap on top of the whole leaf. This will prevent contamination between different leaves.

**** Make sure there is not too much material, < 100 mg FW maximum, this is approximately 1 fully expanded mature leaf, or DNA yield will suffer****

3. After freezing, weigh again, to determine fresh weight
4. Store in -80º C until ready to continue with assay
6. Place Retch mill microfuge holder in Styrofoam box and cool with LN_2
7. Get plant samples from step 4 and place an autoclaved stainless ball in each sample tube
8. Place samples in cooled microfuge tube holder
9. Place and secure holder containing vials into Retsch Mill, you will need cryo gloves

** **Make sure each holder has a microfuge tube holder, however the second microfuge tube holder does not have to be filled with tubes****

10. Grind samples for 20 sec at a frequency of 30 (maximum speed)

First Time Kit is Used

Buffer AP1 and Buffer QP3/E concentrate may form precipitates upon storage. If necessary, warm to 65° C to redissolve (before adding ethanol to Buffer AP3/E). Do not heat Buffer AP3/E after ethanol has been added.

Buffer AW and Buffer AP3/E are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.

Procedure

1. **Preheat a water bath or heating block to 65°C.**
2. **Add 400 µL Buffer AP1 and 4 µL RNase A stock solution (100mg/mL) to a maximum of 100 mg (wet weight) or 20 mg (dried) disrupted plant or fungal tissue and vortex vigorously.**
   No tissue clumps should be visible. Vortex or pipet further to remove any clumps. Clumps of tissue will not lyse properly and will therefore result in a lower yield of DNA. In rare cases, where clumps cannot be removed by pipetting and vortexing, a disposable micropestle may be used.
   Note: Buffer AP1 may develop a yellow color upon storage. This does not affect the procedure.
   Note: Do not mix Buffer AP1 and RNase A before use.
3. **Incubate the mixture for 10 min at 65°C. Mix 2 or 3 times during incubation by inverting tube.**
   This step lyses the cells.
4. **Add 130 µL Buffer AP2 to the lysate, mix, and incubate for 5 min on ice.**
   This step precipitates detergent, proteins, and polysaccharides.
5. **Pipet the lysate into the QIAshredder Mini spin column (lilac) placed in a 2 mL collection tube, and centrifuge for 2 min at 20,000 x g (14,000 rpm).**
   It may be necessary to cut the end off the pipet tip to apply the lysate to the QIAshredder Mini spin column. The QIAshredder Mini spin column removes most precipitates and cell debris, but a small amount will pass through and form a pellet in the collection tube. Be sure not the disturb the pellet in step 6.
   Note: All centrifugation steps are carried out at room temperature (15-25°C) in a microcentrifuge.

6. **Transfer the flow-through fraction from step 5 into a new tube (not supplied) without disturbing the cell-debris pellet.**
   Typically 450 µL of lysate is recovered. For some plant species less lysate is recovered. In this case, determine the volume for the next step.
7. **Add 1.5 volumes of Buffer AP3/E to the cleared lysate, and mix by pipetting.**
   For example, to 450 µL lysate, add 675 µL Buffer AP3/E. Reduce the amount of Buffer AP3/E accordingly if the volume of lysate is smaller. A precipitate may form after the addition of Buffer AP3/E, but this will not affect the DNeasy procedure.
   Note: Ensure that ethanol has been added to Buffer AP3/E.
   Note: it is important to pipet Buffer AP3/E directly onto the cleared lysate and to mix immediately.
8. **Pipet 650 µL of the mixture from step 7 (keep excess for step 9), including any precipitate that may have formed, into the DNeasy Mini spin column placed in a 2 mL collection tube (supplied). Centrifuge for 1 min at ≥ 6000 x g (corresponds to ≥ 8000 rpm for most microcentrifuges), and discard the flow-through.* Reuse the collection tube in step 9.**
9. **Repeat step 8 with remaining sample. Discard flow-through* and collection tube.**
10. **Place the DNeasy Mini spin column into a new 2mL collection tube (supplied), add 400 µL Buffer AW, and centrifuge for 1 min at ≥6000 x g (≥8000 rpm). Discard the flow-through and reuse the collection tube in step 11.**
    Note: Ensure that ethanol is added to Buffer AW.
11. **Add 400 µL Buffer AW to the DNeasy Mini spin column, and centrifuge for 2 min at 20,000 x g (14,000 rpm) to dry the membrane.**
    It is important to dry the membrane of the DNeasy Mini spin column since residual ethanol may interfere with subsequent reactions. This centrifugation step ensures that no residual ethanol will be carried over during elution. Discard flow-through and collection tube.
    After washing with Buffer AW, the DNeasy Mini spin column membrane is usually only slightly colored. In the rare case that the membrane remains significantly colored after washing with Buffer AW, refer to "Darkly colored membrane or green/yellow eluate after washing with Buffer AW" on pg. 45 in DNeasy Plant Handbook.
    Note: Following the centrifugation, remove the DNeasy Mini spin column from the collection tube carefully so the column does not come into contact with the flow-through, as this will result in carryover of ethanol.
12. **Transfer the DNeasy Mini spin column to a 1.5 mL or 2 mL microcentrifuge tube (not supplied), and pipet 100 µL Buffer AE directly onto the DNeasy membrane. Incubate for 5 min at room temperature (15-25°C), and then centrifuge for 1 min at ≥6000 x g (≥8000 rpm) to elute.**
    Elution with 50 µL (instead of 100 µL) increases the final DNA concentration in the eluate significantly, but also reduces overall DNA yield. If larger amounts of DNA (>20 µg) are loaded, eluting with 200 µL (instead of 100 µL) increases yield. (for KO eluate once with 50 µL AE)
13. **Repeat step 12 once.**
    A new microcentrifuge tube can be used for the second elution step to prevent dilution of the first eluate. Alternatively, the mircocentrifuge tube can be used for the second elution step to combine the eluates.
    Note: More than 200 µL should not be eluted into a 1.5 mL microcentrifuge tube because the Dneasy Mini spin column will come into contact with the eluate.

*Flow-through fractions contain Buffer AP3/E, and are therefore not compatible with bleach.