**RNA Extraction Protocol for Basal Angiosperms**

RNA extraction Kits available commercially (e.g. RNA WIZ Kit from Promega, RNeasy Plant Mini Kit from Qiagen) are designed based on investigations of model plants (e.g., *Arabidopsis*). These kits have rarely been tested on other flowering plants, such as lineages of basal angiosperms. In our initial RNA extractions of basal angiosperm taxa (e. g., *Amborella* and *Nuphar*) we employed commercially available kits, and these efforts failed. We therefore developed our own protocol for isolating high yields of RNA from these difficult plant tissues. Our method combined parts of the CTAB DNA extraction protocol (Doyle and Doyle, 1987) with the subsequent use of the RNeasy Plant Mini Kit (Qiagen, Stanford, CA). This protocol has worked well in all of the basal angiosperm taxa that we have tested. One problem with this method, however, is DNA contamination. We recommend treating RNA extract with DNase before subsequent experiments.

**Combination of CTAB (Doyle and Doyle, 1987) and QIAGEN RNeasy Plant Mini Kit.**

CTAB Protocol

1. Start from 100mg of grinded tissue in the liquid nitrogen with 1.5ml microcentrifuge tube.
2. Add 500ml of 2X CTAB buffer + 1ul of B-mercarptoethanol.
3. incubate at 60C for 10 min.
4. Add 500ul of chloroform: isoamyl alcohol=24:1 then invert many times (vortexing is also possible)
5. Centrifuge at 15000rpm for 15 min.
6. Pipette aqueous solution and transfer to new 1.5ml microcentrifuge tuge
7. Add 2/3 volume of isopropanol (100% EtOH is also fineH).
8. Invert and Mix.
9. Place at –20C for more than 1hr.

(Go to number 6 step of the protocol in RNeasy mini Handbook (page 77) in the Kit.)

RNeasy Plant Mini Kit Protocol

10. Apply sample (usually 650ul) to an RNeasy mini column (pink color) placed in a 2 ml collection tube.
11. Centrifuge for 15 sec at 15000rpm. Discard the flow-through.
12. Add 700ul Buffer RW1 to the RNeasy column.
13. Centrifuge for 15 sec at 15000rpm. Discard the flow-through and collection tube.
14. Transfer the RNeasy column into a new 2ml collection tube
15. Add 500ul Buffer RPE on to the RNeasy column.
16. Centrifuge for 15 sec at 15000rpm. Discard the flow-through.
17. Repeat 15 and 16 and discard collection tube.
18. Transfer the RNeasy column to a new 1.5ml tube (RNase-free).
19. Add 40ul RNase-free water directly onto the RNeasy silica-gel membrane.
20. Centrifuge for 1min at 15000rpm.

    -- Load 5ul in the Formaldehyde agarose gel.

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