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RNA Extraction Protocol for Shorea V.2

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Manuscript citation:
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Protocol status: Working
We use this protocol and it's working

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Abstract

RNA extraction protocol using CTAB method optimized for leaf and bud samples from *Shorea curtisii.*
Adapted from extraction protocol for *Shorea beccariana* (see attached publication).

Protocol materials

Turbo DNA-free Kit **Invitrogen - Thermo Fisher Catalog #**AM1907

RNeasy Plant Mini Kit **Qiagen Catalog #**74904

Lysis

1h 10m

1 Weigh BUD 40 mg (25-40 mg) or LEAF 60 mg (50-70 mg) and transfer into a 1.5 mL tube. 15m

2 Ground in CTAB buffer (3% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl pH 8.0, 0.2% b-mercaptoethanol) using tissuelyzer. 10m

3 Incubate at 60 °C for 00:45:00 . 45m

Precipitation

25m

4 Add 1 volume of chloroform.
Mix by vortex but not too vigorous.
Ensure that the two phases of the mixture are homogenized well. 5m

5 Centrifuge at 5000 rpm, Room temperature, 00:01:00 .
Transfer the supernatant into a new 1.5 mL tube. 5m

6 Add 2/3 volume of isopropanol.
Mix gently (turning the tubes upside down 00:02:00). 5m

7 Centrifuge 5000 rpm, Room temperature, 00:05:00 10m
Discard solution carefully.

Expected result

Formation of white to translucent pellet.

Washing

25m

8 Add 700 µL ethanol. 5m
Mix gently (flicking the tube) until the pellet no longer stick to the tube.

9 Centrifuge 5000 rpm, Room temperature, 00:05:00 . 10m
Discard solution carefully.

10 Air dry the pellet 00:01:00 . 5m
Repeat Washing if necessary.

Resuspension 25m

11 Resuspend the pellet in 50 µL RNAse-free water.
Flick the tube until the pellet is no longer visible.

If the pellet is difficult to dissolve, leave it for ~30 min before flicking again.
If it still does not dissolve, add more RNAse-free water AND adjust the subsequent steps accordingly.

Genomic DNA Digestion 55m

12 Removed gDNA using
Turbo DNA-free Kit **Invitrogen - Thermo Fisher Catalog #**AM1907 .
Follow the manufacturer's protocol.

Purification 40m

13 Purification is done using RNeasy Plant Mini Kit **Qiagen Catalog #**74904 with 5m
minor adjustment to the protocol.
Add 350 µL RLT buffer.
Mix gently (flicking).

14 Add 250 µL absolute ethanol. 5m
Mix gently (flicking).

15 Transfer the solution into RNeasy Mini Spin column. 3m
Centrifuge 10000 rpm, Room temperature, 00:00:15 .
Discard the flow through.

16 Add 700 µL RW1 buffer to the column. 3m
Centrifuge 10000 rpm, Room temperature, 00:00:15 .
Discard the flow through.

17 Add 500 µL RPE buffer to the column. 3m
Centrifuge 10000 rpm, Room temperature, 00:00:15 .
Discard the flow through.

18 Add 500 µL RPE buffer to the column. 5m
Centrifuge 10000 rpm, Room temperature, 00:02:00 .
Discard the flow through.

19 Replace the collection tube. 3m
Centrifuge 14000 rpm, Room temperature, 00:01:00 to further dry the membrane.

20 Replace the collection tube with a new 1.5 mL tube. 8m
Add 50 µL RNAse-free water directly onto the membrane.
Incubate at Room temperature for 00:05:00 .

21 Centrifuge 10000 rpm, Room temperature, 00:01:00 . 5m
Store the extracted RNA in -20 °C or -80 °C for longer storage.

Quality Assessment

22 Evaluate the RNA quality using Agilent 2100 Bioanalyzer.