RNA Extraction Protocol for Shorea V.2

mpfsum [1]

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dx.doi.org/10.17504/protocols.io.b2ipqcdn

MP2Lab

mpfsum

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).

DOI

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protocol

Kobayashi MJ, Takeuchi Y, Kenta T, Kume T, Diway B, Shimizu KK. Mass flowering of the tropical tree *Shorea beccariana* was preceded by expression changes in flowering and drought-responsive genes. *Mol Ecol.* 2013 Sep;22(18):4767-82. doi: 10.1111/mec.12344. Epub 2013 May 8. PMID: 23651119; PMCID: PMC3817532.

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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 15m

tube.

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

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

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

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

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.

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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 RNeasy Plant Mini Kit **Qiagen Catalog #74904** 5m

Purification is done using with 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** .

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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.

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