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RNA Extraction Protocol for Shorea

mpfsum 1

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MP2Lab



ABSTRACT

RNA extraction protocol using CTAB method optimized for leaf and bud samples from Shorea sp.

PROTOCOL CITATION

mpfsum 2021. RNA Extraction Protocol for Shorea. **protocols.io** https://protocols.io/view/rna-extraction-protocol-for-shorea-btw9nph6

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- 15m Weigh BUD **□ 40 mg (25-40 mg)** or LEAF **□ 60 mg (50-70 mg)** and transfer into a 1.5 mL 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.
- 3 Incubate at & 60 °C for © 00:45:00.

45m

Precipitation

25m

5m

Add 1 volume of chloroform.

Mix by vortex but not too vigorous.

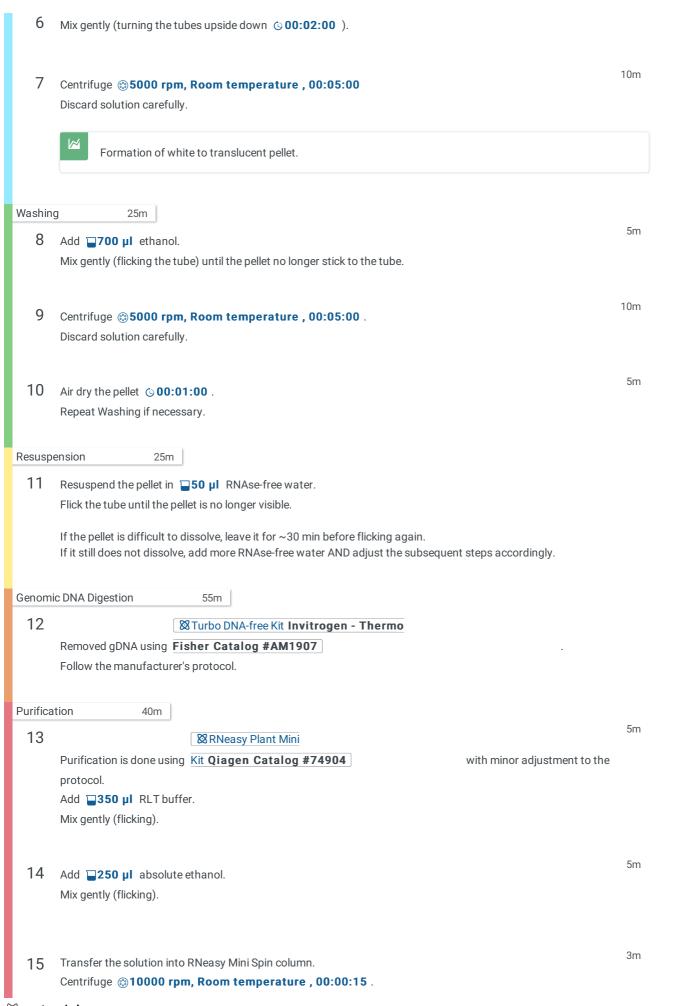
Ensure that the two phases of the mixture are homogenized well.

5 Centrifuge at **\$5000 rpm, Room temperature**, **00:01:00**.

Transfer the supernatant into a new 1.5 mL tube.

5m

Add 2/3 volume of isopropanol.



Discard the flow through. 3m 16 Add **□700 µI** RW1 buffer to the column. Centrifuge **10000 rpm, Room temperature**, **00:00:15**. Discard the flow through. 3m 17 Add $\mathbf{500} \mu$ RPE buffer to the column. Centrifuge **10000 rpm, Room temperature**, **00:00:15**. Discard the flow through. 5m 18 Add **□500** µI RPE buffer to the column. Centrifuge @10000 rpm, Room temperature, 00:02:00. Discard the flow through. 3m Replace the collection tube. 19 Centrifuge (3) 14000 rpm, Room temperature, 00:01:00 to further dry the membrane. 8m 20 Replace the collection tube with a new 1.5 mL tube. Add $\Box 50 \mu I$ RNAse-free water directly onto the membrane. 5m 21 Centrifuge @10000 rpm, Room temperature, 00:01:00.

Quality Assessment

22 Evaluate the RNA quality using Agilent 2100 Bioanalyzer.

Store the extracted RNA in § -20 °C or § -80 °C for longer storage.