RNA Extraction Full Protocol

Onkar Nath[1], Stephen Fletcher[1], Alice Hayward[1], Agnelo Furtado[1], Robert J Henry[1], Neena Mitter[1]

[1]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane 4072, Australia

Jun 24, 2021

1 *Works for me* Share This protocol is published without a DOI.

qaafi QAAFI, UQ

Onkar Nath

DISCLAIMER

ABSTRACT

RNA Extraction protocol suitable for all Avocado tissues. This protocol also suits extraction from many other plants like Capsicum, Finger lime and Cotton.

PROTOCOL CITATION

Onkar Nath, Stephen Fletcher, Alice Hayward, Agnelo Furtado, Robert J Henry, Neena Mitter 2021. RNA Extraction Full Protocol. **protocols.io** https://protocols.io/view/rna-extraction-full-protocol-bv2dn8a6

KEYWORDS

avocado, rna extraction, high quality RNA, CTAB

CREATED

Jun 23, 2021

LAST MODIFIED

Jun 24, 2021

PROTOCOL INTEGER ID

50981

GUIDELINES

for modifications required with different tissue types follow associate manuscript.

MATERIALS TEXT

Buffers and chemicals:

OB1:
CTAB Buffer (pH 7.6, filter sterilized with 33mm PES membrane filter unit): 2% CTAB, 1.4M NaCL, 10mM EDTA, 0.1M MES, 1% PEG
For DNA extraction 0.5% Na2SO3 (e.g. 0.05 g in 10 ml), 2% PVP (0.2 g in 10 ml), 2% β-mercaptoethanol (e.g. 20 µl in 10 ml) was added before use.
For RNA Extraction, 1/100th volume 10% SDS, 2% β-mercaptoethanol (e.g. 20 µl in 10 ml) was added before use.
For 100ml:
CTAB 2.0 g (hexadecyltrimethyl-ammonium bromide)
NaCl 8.18 g (sodium hydroxide)
EDTA 0.3723 g (ethylenediaminetetra acid disodium salt)
MES 1.96 g (2-(N-morpholino) ethanesulfonic acid hydrate)
PEG-8000 1.0 g (polyethylene glycol 8000)

OB2:
Buffer 2: 25mM sodium citrate, 0.5% lauroyl sarcosine, 1M sodium acetate, 2% PVP and 4.2M guanidine thiocyanate (optional)
For 100ml:
sodium citrate 0.535 g
lauroyl sarcosine 0.5 g
sodium acetate 13.605 g
PVP 2 g
guanidine thiocyanate 49.625 g

Optional Clean-up Buffer (OB3/SSTE) (Chang, Puryear, and Cairney 1993): 1M NaCl, 0.5% SDS, 10mM Tris pH 8.0 and 1mM EDTA pH 8.0.

chloroform:isoamyl alcohol (C:IAA) 24:1
Isopropanol 100%
Sodium dodecyl sulfate (SDS) 10% (To be used within two months as its efficiency reduces with time)

SAFETY WARNINGS

Use of various instruments and hazardous chemicals are required. Consult and follow GHS guidelines.

DISCLAIMER:

BEFORE STARTING

Prepare buffers and organise chemicals

Tissue Lysis 4h 45m

1 Finely grind frozen tissue. Grinding in the buffer is not recommended.

2 **Preparation on the Day of Use**
- Add **10 µl 10% SDS per 2ml OB1 buffer** and **2 % (v/v) β-mercaptoethanol** (Fume-hood should be used for working with 2ME)

- Heat the buffer to **65 °C** .

3 Add approximately **10 mg** (0.01 g) of ground frozen leaf tissue to **650 µl** hot OB1 buffer in 2 ml tube and incubate at **65 °C** for **00:05:00** . 5m

> Over-incubation leads to RNA degradation and delicate tissues for e.g. roots cannot tolerate this. Tissue-specific recommendations are reported in RNA extraction section

4 Centrifuge at **20817 x g** (14,000 rpm) for **00:10:00** at **Room temperature** , pour supernatant to another tube. This improves the cleaning process. 10m

5 Add an equal volume of **OB2 buffer**.

Removing Impurity 4h 30m

6 Add an equal volume of **chloroform isoamyl alcohol** (C:IAA); to be performed in fume-hood. Vortex tubes well.

7 Centrifuge at **20817 x g** (14,000 rpm) for **00:20:00** at **Room temperature** . 20m

8 Collect the supernatant and repeat chloroform wash.

Precipitation 4h 30m

9 Add an equal volume of 100% **Isopropanol** (Cold).

> Preferably precipitate with a half volume of **[M]10 Molarity (M)** LiCl instead of isopropanol, if the ratio of high molecular weight and low molecular weight RNA does not matter for the experiment.

10 10. Incubate at **-20 °C** for **01:00:00** or longer as needed. Incubating longer increases yield but adds more salt in precipitant. 1h

11 11. Centrifuge at **12857 x g, 4°C, 00:25:00** (10,000 rpm). 25m

12 12. Wash pellet with **1 mL** of **[M]75 % (v/v) ethanol**.

13 13. Centrifuge at **7674 x g, 4°C, 00:10:00** (8,500 rpm) 10m

Elution 4h 30m

14 14. Air Dry the pellet. Do not over-dry but get rid of maximum ethanol possible.

15 15. Dissolve in 20 µl of ultrapure DNase RNase free water.

15.1 Pool tubes if required at this stage for higher RNA content.

DNA Degradation 4h 30m

16 17. If DNA must be removed from the RNA sample, add 1/20th volume of DNase I Reaction Buffer - 6.0 ml **New England Biolabs Catalog #B0303S** and 1/20th volume of DNase I (RNase-free) **New England Biolabs Catalog #E6316** .

17 18. Incubate at **37 °C** for **00:30:00** . 30m

DNase Enzyme removal 4h 30m

18 19. Add water to make up **500 µl** of total volume.

RNAClean XP Bead protocol from Beckman Coulter can also be followed to remove DNase enzyme. TRISure protocol has been followed because of cost factor.

19 20. Add an equal volume of TRISure **Bioline Catalog #BIO-38033**

Use OB3 if RNA degrades with the use of TRISure.

20 21. Incubate for **00:05:00** . 5m

21 22. Add **100 µl** **chloroform**.

22 23. Centrifuge at **20817 x g, 4°C, 00:20:00** (14,000 rpm), collect supernatant. 20m

23 24. Add an equal volume of **isopropanol**

24 25. Incubate at **-20 °C** for **01:00:00** or longer. 1h

25 26. Centrifuge at **20817 x g, 4°C, 00:40:00** (14,000 rpm). 40m

26 27. Ethanol wash pellet.

27 28. Dissolve in 20 µl of ultrapure DNase RNase free water or 1x TE buffer as per requirement.

Allow the pellet to dissolve and store at -20 or -80 as required. Do not leave at room temperature or 4°C for long.

Optional clean up and assessment

27.1 Optionally if the pellet is still not clean, follow RNAClean XP Bead protocol from Beckman Coulter. Briefly, add beads to solution, separate beads attached to RNA using magnetic rack, ethanol wash and elute in DNase RNase free water (use 100µl buffer instead of the recommended, 90µl per 50µl sample) (Follow manuscript's RNA Extraction Section for specific recommendations and discussion).

28 30. Quality-assure using Nanodrop and Quantify using Quantifluor RNA, or Bioanalyzer.