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RNA Extraction CTAB Protocol

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We use this protocol and it's working

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Abstract

Protocol used and fine-tuned in the Joly laboratory

Reference

Gambino G., Perrone I., Gribaudo I. 2008. A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. Phytochemical Analysis. 19:520–525.

Troubleshooting

Preparation

1 **Working with RNA**

- Clean bench and pipettes with RNAzap
- Autoclave platics
- Be **ultra** careful!
- All reagents and chemicals used for RNA extractions should never be used for other purposes.
- Always manipulate everything with clean nitrile gloves.

2 **Tissue grinding**

- Use super clean mortar and pestles. Whash them well and ideally bake them overnight at 350 °C
- It is **very** important that your tissue samples stay frozen all the time. Keep all your tubes in liquid nitrogen all the time. Also, freeze the mortars before grinding by filling them with liquid nitrogen and letting them evaporate.
- Grind the tissues into a very fine powder and transfer them in 2 ml eppendorf tubes. Make some aliquots of 100 mg that will be used for the extractions.

3 **Before starting the extractions**

- Make sure all reagents are prepared, and that liquid nitrogen is available.
- Place water bath and vortex under a hood.
- Turn on water bath, add distilled water if necessary, and adjust temperature to 65 °C.
- Transfer the required volume of CTAB buffer to a Falcon tube (about 1 mL/sample), and add b-mercaptoethanol to a final concentration of 2% (v/v) (20 µL/mL CTAB buffer). Heat to 65 °C in water bath (approx. 30 minutes).
- Turn on centrifuge and adjust to 4 °C.
- It is convenient to extract RNA of 12 samples and run a chip on Bioanalyzer per day.
- Prepare 1.5 mL Eppendorf tubes. Two sets of tubes will be needed if samples will be ground and extracted in the original tubes. If ground tissue needs to be transferred to separate tubes, then three sets of tubes will be needed. The set used for the LiCl precipitations should be well labeled with the sample number, the extraction date, and “Total RNA”.

Extraction protocol

4 **Extraction protocol**

Note

Perform following steps 1-8 inside hood; collect liquid and solid waste into respective containers.

1. Add 750 µL CTAB + b-mercaptoethanol to ca. 100 mg ground tissue in 2 mL Eppendorf tubes. Immediately vortex or shake well to mix. (If tissue has already been ground, then you might choose to add tissue to buffer. Store tissue tubes in liquid N with a 2 inch cardboard box before adding extraction buffer).
2. Incubate tubes in water bath at 65 °C for 10 minutes, vortexing periodically.
3. Add an equal amount (750 µL) chloroform : isoamyl alcohol (24:1) to each tube, vortexing each tube vigorously.
4. Centrifuge at 21,000g (ca. 14000 rpm) for 10 min at 4 °C.
5. Remove the aqueous phase and transfer to a new tube. Take care to remove as much of this aqueous phase as possible in order to have better yields, but avoid the interphase.
6. Repeat steps 3-5 a second time. The aqueous phase should be transferred to the tubes with the full labels.
7. Determine the volume of the aqueous phase recovered after the second partitioning with chloroform : isoamyl alcohol (generally determined by drawing up this volume with a pipettor until the full volume is accurately drawn up). Add an equal volume of LiCl-EDTA (7.5 M LiCl, 50mM EDTA). Mix gently by inverting the tubes several times and place the tubes at -20 °C freezer for 30 minutes to one hour. Be consistent with your time of incubation!
8. Centrifuge for 15 minutes at 21,000g (ca. 14000 rpm) at 4 °C. Discard the supernatant.
9. Wash the pellet by adding 800 µL of 80% ethanol. Vortex briefly, then centrifuge at 21,000g (ca. 14000 rpm) at 4 °C for 5 minutes. Remove the supernatant. Do a quick spin to collect the supernatant at the bottom of the tubes. Remove remaining liquid using a pipettor with a 200 µl tip.
10. Let the pellets dry with the tube on a piece of clean paper towel until the edges begin to look a little transparent. Do not let the pellet dry too long or it will be very difficult to be resuspended.
11. Resuspend the pellet in autoclaved DEPC water – use between 10 and 40 µl, depending on the size of the pellet. Pippeting water to completely resuspend RNA and put tubes on ice.
12. Determine the relative quality and quantity of RNA using the spectrophotometer. Treat cuvette specific for RNA with Rnase away for 1 minute and rinse with Milli Q water twice. Use DEPC water as blank. Record readings of A220, A280, A320, ratio between A260 and A280, and concentration.
    For the spectrophotometer:
    i. A260: measures nucleic acids
    ii. A280: measures principally proteins and phenolics.
    iii. A230: measures carbohydrates
    iv. A320: measures any film or dirt on the cuvette and is often used to adjust the other calculations accordingly
    v. The 260 / 280 ratio for pure RNA should be 2 (1.8 for DNA).
13. Using the concentrations obtained from the spectrophotometer, prepare dilutions of the extracts in order to carry out analyses using the Bioanalyzer or gel

electrophoresis on agarose (1%) (WICH STAIN?). The ideal sample concentration should be 100-200 ng/µL, but the practical detection range of the instrument is between 25 and 500 ng/µL.

Solutions

5 **2X CTAB extraction Buffer**

	Reagent	Final concentration	For 500 ml
	CTAB	2%	10 g
	PVP	2%	10 g
	Sodium Chloride (NaCl)	2M	200 ml of 5M NaCl
	EDTA, disodium	25 mM	25 ml of 0.5M EDTA
	Tris-HCl	100 mM	50 ml of 1M Tris pH 8.0
	Spermidine	0.05%	0.25 g

Fill up to 500 ml with H2O, adjust to pH 8.0

6 **Chloroform**

- 24 parts chloroform
- 1 part Isoamyl

7 **Reference**

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