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RNA isolation protocol from unwilling plant tissue

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sa.parra[1]

[1]Universidad de Chile

Samuel Parra
Universidad de Chile

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

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Abstract

This protocol is intended for HIGH quality RNA extraction from plan tissues
We used this protocol after several attempts with Trizol, Qiagen RNeasy plant kit and other protocols with no ressults.
This procedure redered us between 300 to 1000 ng with good RNA

Materials

Extraction buffer:
2% CTAB (hexadecyltrimethylammonium bromide)
2% PVP (polyvinylpyrrolidinone K 30)
100 mM Tris-HCl (pH 8.0)
25 mM EDTA
2.0 M NaC1
0.5g/L spermidine
(mix and autoclave) Alt. microweave heat
2% [B-mercaptoethanol] (add just before)

Ethanol 100%
LiCl2 10M
Chloroform:IAA 24:1

1.5 mL tubes
2 mL tubes

SSTE buffer:
1M NaCl
0.5% SDS
10mM TrisHCl (pH 8)
1mM EDTA (pH 8)

RNA isolation protocol

1 This protocol is an adaptation of https://doi.org/10.1007/BF02670468 designed for extraction of RNA from pine needles with high polyphenolic compounds or high sugar content.

For all buffers use DEPC treated water or Nuclease free ddH2O

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SSTE buffer:
1M NaCl
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10mM TrisHCl (pH 8)
1mM EDTA (pH 8)

Procedure, Extraction

3 For 200 mg sample use 3 mL of extraction buffer. 10m
Grind the sample with liquid nitrogen until to a fine powder. Add the 3 ml of Extraction buffer, the frozen paste will become softener in time, use the cold paste to finish grinding any remanent plant tissue.
Separate the mixture in 3 separated 2mL tubes
Keep the tubes on ice

On ice

4 Add an equal volumen of ChCl:IAA and vortex x 30 sec 10m

Room temperature

Extract the WHOLE aquose phase, do not leave any of it from each tube.
ignore *any coloration of the aqueus phase*
**Optional : Gycogen can be added at this point*
Transfer the phase to 1.5 ml tubes and add 1/4 volume of 10M LiCl and mix by inverting

5 Precipitate overnight at 4°C 4 °C 12h

Phases can be formed due to cold SDS in the buffers, **ignore** every phase or precipitation and go on.

RNA cleaning 2h 30m

6 13000 rpm, Room temperature, 00:10:00 10m

Centrifuge 10m at 13000 at RT, discard the liquid, mind the pellet!

7 **If the SSTE buffer is below 25°C SDS on it will form flecks, warm untl sds is completely dissolved*

Dissolve the pellet with 500 µL of SSTE buffer.

Join the pellet from the 3 initial tubes using the same 500 ul of SSTE.

8 Extract with an equal volumen of CHCl:IAA by vortexing for 30 sec 10m

13000 rpm, Room temperature, 00:10:00 Centrifuge at 13000 rpm x 10m at RT

9 Recover the upper phase completely, do not leave ANY traces, a *white smudge between the phases must be retrieved. dont worry if SOME chcl2 is tranfered.* 2h

Add 2 volumes of cold ETOH100% and precipitate for at least 02:00:00 at

-20 °C

10 After precipitation centrifuge at 13000 rpm, 4°C, 00:10:00 10m

Discard the ethanol, DRY the rna pellet (might be invisible!)

Resuspend the pellet on 30-50 µL of ddH2O nuclease free or TE buffer