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Isolation of high quality RNA from tree leaves using Polyclar in the Spectrum Plant Total RNA Kit

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Genetic Technologies

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

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Disclaimer

No liability is assumed for any damage to persons or property.

Abstract

This protocol is based on the use of the Spectrum Plant Total RNA Kit from Sigma. The protocol has been adapted for the isolation of RNA from plant tissue of trees. It was tested with good results for pedunculate oak (*Quercus robur*), European beech (*Fagus sylvatica*), and poplar hybrids (*Populus tremula* × *P. alba*).

Guidelines

When working with RNA, you should bear in mind that RNA is rapidly degraded by ubiquitous RNases. Therefore, rapid processing without unnecessary lengths is highly recommended. Substances for the removal of active RNases, e.g. "RNase-Away", should be used to clean the workplace and required utensils.

Materials

- The isolation is based on the Spectrum Plant Total RNA Kit.
- Common laboratory material such as pipettes and pipette tips is needed.
- A swing mill or a bead ruptor are used here to homogenize the material, although similar instruments could also be used. If necessary, the material could be homogenized by hand in a mortar.
- A heating block and a centrifuge are used for the isolation protocol.

Safety warnings

Consider the safety regulations for handling chemicals, as many chemicals are hazardous substances, as well as the instructions for technical equipment. When handling liquid nitrogen, be aware of the dangers posed by the extremely low temperature and possible displacement of breathing air. Therefore, work with all the necessary technical and personal safety equipment such as a fume hood, safety goggles, gloves and cold protection gloves.

Ethics statement

As this is a protocol for processing plant material, no statement is to be made here.

Before start

Preheat a heatblock to 56 °C. Add ethanol to Wash Buffer 2 during the first extraction according the manufacturer's guidelines.

Sample preparation

30m

1 Harvest plant material and freeze it immediately in liquid nitrogen. Alternatively, you can use frozen plant material.

2 Grind approximately 100 mg using a swing mill (2-3 x 1:15 min at 17.5 Hz) with small metal balls or using a bead ruptor (3 × 15 s at 2.1 m/s).

Preparation of required solutions

20m

3 Take an aliquot of the Lysis Solution according to the number of your samples and transfer this volume into a separate vessel, such as an RNase-free centrifugation tube. For your calculations: 500 µL of Lysis Solution, including the additives, will be used per sample later.

4 Add β-Mercaptoethanol to the Lysis Solution (10 µL β-Mercaptoethanol per mL Lysis Solution).

5 Add 60 mg Polyclar per mL Lysis Solution.

6 Thaw DNase I Digestion Buffer and prepare the DNase digestion mix. Carefully mix 10 µL DNase I and 70 µL Digestion Buffer per sample. Store the digestion mix on ice until utilization.

RNA isolation procedure

1h 30m

7 Add 500 µL Lysis Solution supplemented with Polyclar and β-Mercaptoethanol to each sample and vortex vigorously for 30 s immediately.

8 Incubate for 3-5 min at 56 °C in the heatblock. During incubation you can start preparing the filtration columns (consider proper labelling to avoid mixing up samples).

9 Centrifuge for 3 min at 16,000 × g, while preparing filtration columns (blue).

10 Transfer the supernatant to the filtration column and close the lid of the tube. Do not pipette any of the pellet. Small suspended particles should not pose a problem.

11 Centrifuge for 1 min at max. speed (16,000 × g). Repeat this centrifugation if the lysate has not run through the column completely. **Do not discard the flow-through.**

12 Prepare the binding columns.

13 Continue with Protocol B according the manufacturer's manual: Add 250 µL Binding Solution to the filtered lysate, mix by pipetting (pipette up and down at least 5 times).

14 Pipette the mixture to the binding column and close the tube cap.

15 Centrifuge for 1 min at max. speed (16,000 × g).

16 Discard the flow-through and reuse the collection tube.

17 Add 300 µL of Wash Solution 1 to the column and close the cap.

18 Centrifuge for 1 min max. speed (16,000 × g).

19 Discard the flow-through and reuse the collection tube.

20 Pipette 80 µL of DNase I digestion mix onto the column and incubate at room temperature for at least 15 min (preferably 30 min).

21 Add 500 µL of Wash Solution 1 to the column and close the cap.

22 Centrifuge for 1 min max. speed (16,000 × g).

23 Discard the flow-through, and reuse the tube.

24 Add 500 µL of Wash Solution 2 to the column and close the cap.

25 Centrifuge for 30 s max. speed (16,000 × g).

26 Discard the flow-through and reuse the collection tube.

27 Repeat the wash cycle: Add 500 µL of Wash Solution 2 to the column and close the cap.

28 Centrifuge for 30 s max. speed (16,000 × g).

29 Discard the flow-through and reuse the collection tube.

30 Centrifuge for 1 min max. speed (16,000 × g) to dry the column.

31 Carefully place the column in a new collection tube.

32 Pipette 50 µL of Elution Solution into the center of the column matrix and incubate for 1-2 min at room temperature.

33 Centrifuge for 1 min max. speed (16,000 × g).

34 Place the samples on ice for immediate processing or freeze at -70°C for later use.

Protocol references

This protocol is largely based on the protocol intended for use with the Spectrum Plant Total RNA Kit from Sigma. The modifications were made in order to isolate high quality RNA from material from trees whose tissue is known to be difficult.