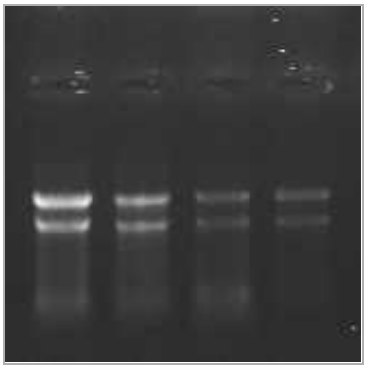

APR 16, 2023

RNA extraction from hairy roots of common bean (Phaseolus vulgaris L.) and cDNA synthesis

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Ronal's protocols

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ABSTRACT

Extracting RNA for subsequent quantification of transcript levels by RT-qPCR requires high purity and concentration. When the amount of tissue is not abundant, as is the case with hairy roots, the concentration of RNA is frequently low. Here we present an optimized protocol for TRIzol-mediated RNA extraction from hairy roots of common bean. This protocol is based on the manufacturer's instructions

TRIzol Reagent **Thermo Fisher Scientific Catalog #15596026**

MATERIALS

QIAzol Lysis Reagent **Contributed by users Catalog #79306**

DOI:
dx.doi.org/10.17504/protocols.io.8epv5jq24l1b/v1

Protocol Citation: Ronal Pacheco Sánchez, Noreide Nava 2023. RNA extraction from hairy roots of common bean (Phaseolus vulgaris L.) and cDNA synthesis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv5jq24l1b/v1

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

Created: Apr 10, 2023

Last Modified: Apr 16, 2023

PROTOCOL integer ID:
80277

Extraction of total RNA

1 Macerate root tissue using liquid nitrogen.

2 Load 100 mg of macerated tissue into a 1.5 mL Eppendorf tube and add 1 mL of TRIzol Reagent **Thermo Fisher Scientific Catalog #15596026** .

> Note
>
> If the amount of root tissue is less than 100 mg, add the equivalent amount of Trizol.

3 Mix by vortexing 00:00:15 and incubate for 00:05:00 at room temperature. 5m 15s

4 Add 200 µL chloroform:isoamyl alcohol 24:1 , mix by vortexing 00:00:15 and incubate fo 3m 15s 00:03:00 at room temperature.

5 Centrifuge 11800 rpm, 4°C, 00:15:00 15m

6 Transfer the aqueous phase to a new 1.5 mL Eppendorf tube.

7 Add 500 µL of isopropanol, mix by immersion, and incubate Overnight -20 °C . 10m

Note

The original protocol indicates incubating for 10 min at room temperature; however, we have had a low RNA concentration using these conditions. We strongly recommend incubating for at least 6 h at -20°C to get a higher concentration of RNA.

8 Centrifuge 11800 rpm, 4°C, 00:10:00 10m

9 Transfer the aqueous phase to a new 1.5 mL Eppendorf tube.

10 Add 500 µL of 4 M LiCl and rise the pellet, do not resuspend, vortex slowly.

Note

LiCl increased the RNA concentration; thereby, this is an important step to reaching a high concentration of RNA.

11 Centrifuge 5900 rpm, 4°C 00:20:00 20m

12 Discard the LiCl phase.

Note

LiCl is difficult to remove; so, try to remove all remanents using a micropipette or syringe.

13 Add 500 µL of tris-EDTA buffer pH 8 . Resuspend RNA by vortexing.

14 Add 500 µL chloroform:isoamyl alcohol 24:1 v/v and mix by vortexing.

15 Centrifuge 5900 rpm, 4°C, 00:10:00 10m

16 Transfer the aqueous phase to a new 1.5 mL Eppendorf tube.

17 Add 500 µL of isopropanol and 66 µL of 3 M sodium acetate pH 5.2 Mix by immersion and incubate Overnight -20 °C

> Note
>
> The original protocol does not include an overnight incubation step, but we strongly recommend incubating for at least 6 h at -20°C to get a higher concentration of RNA.

18 Centrifuge 11800 rpm, 4°C, 00:10:00 10m

19 Discard the aqueous phase and vacuum or air dry the RNA pellet.

20 Resuspend RNA pellets using nuclease-free water. Preferably, use DEPC-treated water.

Preparation of RNA samples for cDNA synthesis

35m

21 Check the integrity of RNA in a 1% agarose gel treated with bleach.

CITATION

Aranda PS, LaJoie DM, Jorcyk CL (2012). Bleach gel: a simple agarose gel for analyzing RNA quality..

LINK

https://doi.org/10.1002/elps.201100335

22 Prepare a dilution (1/10) of each RNA sample and quantify the concentration using a NanoDrop™ 2000c Spectrophotometer **Thermo Fisher Scientific Catalog #ND-2000C** or an equivalent instrument.

23 Prepare one aliquot 10 µL of each RNA sample at [M] 10 ng/µl

24 Add 1 µL of DNase I recombinant RNase-free **Merck MilliporeSigma (Sigma-Aldrich) Catalog #04716728001** and 1 µL of the corresponding buffer (10X) to each RNA sample.

Note

To reduce pipetting errors, prepare a mix of the DNase and the incubation buffer 1:1 v/v (total volume according to the number of samples) and add 2 µL of this mix to each RNA sample.

25 Incubate samples 00:30:00 37 °C

30m

Note

After this step, a qPCR assay should be performed to check if traces of genomic DNA are remaining. According to our experience,
DNase I recombinant RNase-free **Merck MilliporeSigma (Sigma-Aldrich) Catalog #04716728001**
efficiently remove all traces of genomic DNA by performing this step.

26 Add 1 µL of [M] 10 millimolar (mM) oligo (dT) to each sample and incubate 00:05:00 70 °C, then immediately incubate On ice 3 m . 5m

27

Synthesis of cDNA 1h 40m

28 Prepare a mix containing 1 µL of
RevertAid Reverse Transcriptase (200 U/µL) **Thermo Fisher Catalog #EP0442** ,
4 µL of the corresponding buffer (5X), and 2 µL dNTP mix . Add 7 µL of the mix to each RNA sample.

29 Incubate 01:30:00 at 42 °C and 00:10:00 70 °C . 1h 40m

30 Store cDNA samples at -20 °C .