RNA Isolation Protocol in Bixa orellana V.2

DOI
https://dx.doi.org/10.17504/protocols.io.v5ae82e

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Protocol Citation: Victor Carballo-Uicab, Yair Cárdenas-Conejo, Alba Vallejo-Cardona, Margarita Aguilar-Espinosa, Jacobo Rodríguez-Campos, Hugo Serrano Posada, José Iberto Narváez Zapata, Renata Rivera-Madrid . RNA Isolation Protocol in Bixa orellana. **protocols.io** https://dx.doi.org/10.17504/protocols.io.v5ae82e

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Created: December 04, 2018

Last Modified: December 04, 2018

Protocol Integer ID: 18306

Keywords: bixin, carotenoids, pigment, rna isolation protocol in bixa orellana, rna isolation protocol, commercial rna extraction protocol, length cdna from bixa orellana, bixa orellana, long distance reverse transcript pcr protocol, rna, large quantities of pigment, length cdna

Abstract

A reliable protocol is described for isolation of large full-length cDNA from Bixa orellana mature tissues containing large quantities of pigments, phenols, and polysaccharides. This protocol involves the optimization of a commercial RNA extraction protocol in combination with a long distance reverse transcript PCR protocol.

Guidelines

Rodrguez-vila et al. - 2009 - Full-le...

Materials

MATERIALS

Pure-LinkTM RNA Mini Kit **Ambion Catalog #**Cat. No. 12183018A

DNase I Amp Grade Deoxyribonuclease I Amplification Grade **Invitrogen - Thermo Fisher Catalog #**Cat. No 18068-015

SuperScript™ III Reverse Transcriptase **Invitrogen - Thermo Fisher Catalog #**Cat. No 18068-015

liquid nitrogen
Pure-Link RNA Mini Kit was used to extract RNA (Cat. No. 12183018A, Ambion®)
DNase (DNase I Amp Grade, Deoxyribonuclease I Amplification Grade, Invitrogen, Cat. No 18068-015)
SuperScript™ III Reverse Transcriptase (Invitrogen, Cat. No. 18080-093)
NanoDrop® ND-1000

1 Total RNA from *Bixa orellana* L. seeds was isolated from each of the five developmental stages in triplicate from three biological replication of N4P and P13W. Seeds (50 mg 50 mg) were ground in liquid nitrogen using a mortar and pestle.

2 A Pure-Link Micro-to-Midi kit was used to extract RNA (Cat. No. 18068-018 Invitrogen®), which was subsequently treated with DNase (DNase I Amp Grade, Deoxyribonuclease I Amplification Grade, Invitrogen, Cat. No 18068-015) and stored at -80 °C until analysis. The RNA concentration was measured by spectrophotometric analysis (NanoDrop® ND-1000), and its quality was verified by agarose gel electrophoresis at [M] 1.2 Mass Percent 1.2 % and ethidium bromide staining. Reverse transcription was performed with SuperScript™ III Reverse Transcriptase (Invitrogen, Cat. No. 18080-093) using 100 ng of total RNA from each tissue.