



RNA Isolation Protocol in Bixa orellana V.1

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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



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

Materials

liquid nitrogen

Pure-Link Micro-to-Midi kit was used to extract RNA (Cat. No. 18068-018 Invitrogen®)

DNase (DNase I Amp Grade, Deoxyribonuclease I Amplification Grade, Invitrogen, Cat. No 18068-015)

SuperScript™ III Reverse Transcriptase (Invitrogen, Cat. No. 18080-093)



- 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  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.