RNA extration for the R. crenulata genome

Yuanyuan Fu, Liangwei Li, Shijie Hao, Rui Guan, Guangyi Fan, Chengcheng Shi, Haibo Wan, Wenbin Chen, He Zhang, Guocheng Liu, Jihua Wang, Lulin Ma, Jianling You, Xuemei Ni, Zhen Yue, Xun Xu, Xiao Sun, Xin Liu, Simon Ming-Yuen Lee

Abstract

This protocol is used to clarify the process of RNA extration for our R. crenulata genome.

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https://www.protocols.io/view/rna-extration-for-the-r-crenulata-genome-hrjb54n

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Materials

- pBIOZOL by Contributed by users
- CTAB by Contributed by users
- Chloroform by Contributed by users
- Isoamylol by Contributed by users
- Phenol by Contributed by users
- Isopropanol by Contributed by users
- liquid nitrogen by Contributed by users
- 75% Ethanol by Contributed by users
- DEPC by Contributed by users

Protocol

Reagents preparation

Step 1.

Incubate a centrifuge tube with 1.5ml of pBIOZOL : CTAB (1:1) at 65°C in the Thermomixer.

AMOUNT
2 ml Additional info:

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 2ml

Reagents preparation

Step 2.

Transfer 400 µl of Chloroform : Isoamylol (24: 1) in a new centrifuge tube.

AMOUNT
40 µl Additional info:

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 2ml

Reagents preparation

Step 3.

Transfer two pieces of the mixture of 700 µl of water-saturated Phenol and 200 µl of Chloroform : Isoamylol (24: 1) in two new centrifuge tubes, respectively.

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 2ml

Reagents preparation

Step 4.

Transfer 850µl of Chloroform : Isoamylol (24: 1) in a new centrifuge tube.

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 2ml

Reagents preparation

Step 5.

Transfer 700 µl of Isopropanol in a new centrifuge tube.

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 1.5ml

Sample preparation

Step 6.

Add the sample tissue to a pre-cooling mortar and grind tissue into powder under the liquid nitrogen with a pre-cooling pestle.

Tissues lysis

Step 7.

Transfer the powder in the tube prepared in step1, after mixing, incubate the tube for 15min at 65°C.

DURATION
00:15:00

Phase separation

Step 8.

Cool the centrifuge tube to room temperature and centrifuge it at 12,000rpm for 5min at 4°C.

DURATION
00:05:00

Phase separation

Step 9.

Transfer the supernatant in the tube prepared in step2 and shake strongly for 10-20s to mix.

DURATION
00:00:20

Phase separation

Step 10.

Centrifuge the tube at 12,000rpm for 10min at 4°C.

DURATION
00:10:00

Phase separation

Step 11.

Transfer and divide the supernatant equally into two tubes prepared in step3, and then shake strongly for 10-20s to mix, respectively.

DURATION
00:00:20

Phase separation

Step 12.

Centrifuge the tube at 12,000rpm for 10min at 4°C.

Phase separation

Step 13.

Transfer and mix the supernatant from two tubes, and then add it to the tube prepared in step 4. Shake strongly for 10-20s to mix completely.

DURATION
00:00:20

RNA precipitation

Step 14.

Centrifuge the tube at 12,000rpm for 10min at 4°C.

DURATION
00:10:00

RNA precipitation

Step 15.

Transfer the supernatant in the tube prepared in step 5 and mix them by gently inverting.

RNA precipitation

Step 16.

Incubate the tube at -20°C for 2hours.

DURATION
02:00:00

RNA precipitation

Step 17.

Centrifuge at 13,600rpm for 20min at 4°C and remove the supernatant.

DURATION
00:20:00

RNA washing

Step 18.

Add 1ml of 75% of ethanol, flick the tube to resuspend the pellet, wash the RNA pellet for 3min. (wash 1/2)

DURATION
00:03:00

RNA washing

Step 19.

Centrifuge at 13,600rpm for 3min at 4°C, discard the liquid. (wash 1/2)

DURATION
00:03:00

RNA washing

Step 20.

Add 1ml of 75% of ethanol, flick the tube to resuspend the pellet, wash the RNA pellet for 3min. (wash 2/2)

DURATION
00:03:00

RNA washing

Step 21.

Centrifuge at 13,600rpm for 3min at 4°C, discard the liquid. (wash 2/2)

DURATION
00:03:00

RNA washing

Step 22.

Air dry the pellet for 3-5min at room temperature.

DURATION
00:05:00

Dissolve RNA

Step 23.

Add 50 µl of DEPC to dissolve the pellet.

AMOUNT
50 µl Additional info: