DNA 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 total DNA extration for our R. crenulata genome.

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https://www.protocols.io/view/dna-extration-for-the-r-crenulata-genome-hrmb546

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Materials

- RNase by Contributed by users
- TE buffer by Contributed by users
- 70% Ethanol by Contributed by users
- Fresh leaves by Contributed by users
- liquid nitrogen by Contributed by users
- CTAB lysis buffer by Contributed by users
- water by Contributed by users
- Phenol by Contributed by users
- Chloroform by Contributed by users
- Isoamylol by Contributed by users
- Isopropanol by Contributed by users
- ammonium acetate by Contributed by users

Protocol

Sample preparation

Step 1.

Collect and cut 200mg of fresh leaves.

AMOUNT

200 mg Additional info:

Sample preparation

Step 2.

Add tissues and liquid nitrogen in mortar, grind them into powder with a pestle.

Tissue lysis

Step 3.

Transfer the powder in a centrifuge tube with 1ml of pre-heated CTAB lysis buffer and then mix them.

AMOUNT
1 ml Additional info:

NOTES
GigaScience Database 25 Apr 2017

Volume of the tube: 2ml

Tissue lysis

Step 4.

Incubate the tube for 1 hour at 65°C in the recirculating water bath with mixing gently every 5-10 min.

DURATION
01:00:00

NOTES
GigaScience Database 25 Apr 2017

Prolong the lysis time to lyse completely if necessary.

Tissue lysis

Step 5.

Cool the centrifuge tube to room temperature and centrifuge it at 13,600rpm for 10min at 4°C.

DURATION
00:10:00

Tissue lysis

Step 6.

Transfer the supernatant in a new tube.

NOTES
GigaScience Database 25 Apr 2017

Tube -- 2.0ml, marked

GigaScience Database 25 Apr 2017

Tube -- 2.0ml, marked

Phase separation

Step 7.

Add the same volume of Phenol : Chloroform : Isoamylol (25: 24: 1) and mix by gentle inversion for 3-5 min at room temperature.

DURATION
00:05:00

Phase separation

Step 8.

Centrifuge at 13,600rpm for 10min at 4°C.

DURATION
00:10:00

Phase separation

Step 9.

Transfer the supernatant aqueous phase in a new tube.

NOTES
GigaScience Database 25 Apr 2017

Tube -- 2.0ml, marked

Phase separation

Step 10.

Add the same volume of Chloroform : Isoamylol (24 : 1) and mix by gently inverting for 3-5min at room temperature.

DURATION
00:05:00

Phase separation

Step 11.

Centrifuge at 13,600rpm for 10min at 4°C.

DURATION
00:10:00

Phase separation

Step 12.

Transfer the supernatant aqueous phase in a new tube.

NOTES
GigaScience Database 25 Apr 2017

Tube -- 2.0ml, marked

DNA precipitation

Step 13.

Add 2/3 volume of Isopropanol and 1/10 volume of 3M ammonium acetate, and then mix by slowy inverting.

DNA precipitation

Step 14.

Incubate the tube at -20°C for overnight.

DURATION

16:00:00

DNA precipitation

Step 15.

Centrifuge at 13,600rpm for 10min at 4°C and discard the supernatant.

DURATION

00:10:00

DNA washing

Step 16.

Add 1ml of 70% of ethanol, flick the centriguge tube to resuspend the pellet.

AMOUNT

1 ml Additional info:

DNA washing

Step 17.

Wash the DNA pellet for 3-5min.

DURATION

00:05:00

DNA washing

Step 18.

Centrifuge at 13,000rpm for 5min at 4°C, discard the ethanol.

DURATION

00:05:00

DNA washing

Step 19.

Add 1ml of 70% ethanol and mix gently.

AMOUNT

1 ml Additional info:

DNA washing

Step 20.

Centrifuge at 13,000rpm for 5min at 4°C, discard the ethanol.

DURATION
00:05:00

DNA washing

Step 21.

Centrifuge at 13,000rpm for 30s, discard the liquid.

DURATION
00:00:30

DNA washing

Step 22.

Air dry the pellet for 5-10min at room temperature.

DURATION
00:10:00

Dissolve DNA

Step 23.

Add RNase and 100µl of TE buffer (TE : RNase = 100:1), and then dissolve the pellet at 37°C for 30min in the Thermomixer.

DURATION
00:30:00