Genomic DNA extraction from sea cucumber body-wall tissue

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Abstract

This is a DNA extraction protocol from sea cucumber (Apostichopus japonicus) body-wall tissue. It accompanies the following *GigaScience* publication:

Jihoon Jo, et al. (2016): Draft genome of the sea cucumber Apostichopus japonicus and genetic polymorphism among color variants. *GigaScience*...

Citation: Jihoon Jo, Jooseong Oh, Hyun Gwan Lee, Hyun Hee Hong, Sung Gwon Lee, Seongmin Cheon, Elizabeth MA Kern, Soyeong Jin, Sung Jin Cho, Joong Ki Park, Chungoo Park Genomic DNA extraction from sea cucumber body-wall tissue. **protocols.io**

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Before start

Pre-heat the waterbath or hot-plate to lysis buffer incubation, and pre-heat the CTAB lysis buffer at 65℃.

Protocol

Sample preparation

Step 1.

Dissect the body-wall tissue (about 35 g) and cut them in small pieces with a razor blade.

NOTES

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Rinse the tissues with autoclaved sea-water for minimalize the bacterial contamination.

Sample preparation

Step 2.

Add tissues in mortar with liquid nitrogen and grinding tissue.

Tissue lysis

Step 3.

Transfer ground tissues in a 35ml centrifuge tube with 10ml of pre-heated CTAB lysis buffer, add Protease K in the mix and incubate it at 65℃ for 1hour with mixing by gentle inverting every 5-10 min.

DURATION
01:00:00

Tissue lysis

Step 4.

Add 10ml of Phenol solution and incubate at 65℃ for 10min.

DURATION
00:10:00

Phase separation 1/2

Step 5.

Add 10ml of Chloroform and mix by gently inverting at RT for 5min.

DURATION
00:05:00

Phase separation 1/2

Step 6.

Centrifuge at 12000xg for 10min at RT

DURATION
00:10:00

Phase separation 1/2

Step 7.

Transfer the supermatant aqueous phase in a new tube

Phase separation 2/2

Step 8.

Add 10ml of Chloroform and mix by gently inverting at RT for 5min.

DURATION
00:05:00

Phase separation 2/2

Step 9.

Centrifuge at 12000xg for 10min at RT

DURATION
00:10:00

DNA precipitation

Step 10.

Transfer the supermatant aqueous phase in a new tube, add the same volume of Isopropanol and 1/3 volume of 7.5M ammonium acetate.

NOTES

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7.5M ammonium acetate can be added to 0.5 volume of supernatant.

DNA precipitation

Step 11.

Gently mix the tube and incubate the tube at -80℃ for overnight.

DURATION

16:00:00

DNA precipitation

Step 12.

Centrifuge at 12000xg for 10min at 4℃.

DURATION

00:10:00

NOTES

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Do not touch the DNA pellet.

DNA precipitation

Step 13.

Add 70% of ethanol for washing the DNA pellet.

DNA precipitation

Step 14.

Centrifuge at 12000xg for 10min at 4℃.

DURATION

00:10:00

NOTES

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Do not touch the DNA pellet.

DNA precipitation

Step 15.

Discard the ethanol and air-dry the pellet for 10-20min at RT.

DURATION
00:20:00

DNA precipitation

Step 16.

Add 1-2ml of TE buffer (10mM Tris, 1mM EDTA) and dissolve the pellet.

NOTES
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Heat the tube at 60℃ for completely dissolve the pellet.

Warnings

Phenol, Chloroform and Isopropanol are toxic. Please read the MSDS before working with this chemical.