DNA extraction for vertebrate tissues using Phenol:Chloroform:Isoamylol

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Abstract

This protocol is used to clarity the process of total DNA extration for L. maculatus genome.

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Protocol

Sample preparation

Step 1.

1) Collect and cut muscle tissue of spotted sea bass.

2) The tissue was defrosted and washed with normal saline.Cut about 0.5g tissue.

REAGENTS
normal saline by Sinopharm Chemical Reagent Co.

Tissue lysis

Step 2.

Add STE, SDS (10%), protease K (20mg/ml) to mix. Heat 4-6h at 56℃, and shake in centrifuge at 500 rmp.

TEMPERATURE
56 °C :
REAGENTS
STE by Contributed by users
SDS by Ambion
protease K by New England Biolabs
DURATION
06:00:00 : heat

Phase separation

Step 3.

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

2) Centrifuge at 13600rpm for 10min at 4℃ and then transfer the supermatant aqueous phase in a new tube.

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

4) Centrifuge at 13600rpm for 10min at 4℃ and then transfer the supermatant aqueous phase in a new tube.

TEMPERATURE
4 °C : 2)
TEMPERATURE
4 °C : 4)
REAGENTS
Phenol : Chloroform : Isoamylol (25: 24: 1) by Sinopharm Chemical Reagent Co.
Chloroform : Isoamylol (24 : 1) by Sinopharm Chemical Reagent Co.
DURATION
00:05:00 : 1)
DURATION
00:10:00 : 2)
DURATION
00:05:00 : 3)
DURATION
00:10:00 : 4)

DNA precipitation

Step 4.

1) Add equal volume of Isopropanol, and then mix by slowy inverting.

2) Incubate the tube at -20℃ for overnight.

3) Centrifuge at 13600rpm for 10min at 4℃ and discard the supermatant.

TEMPERATURE
-20 °C : 2)
TEMPERATURE
4 °C : 3)
REAGENTS
Isopropanol by Sinopharm Chemical Reagent Co.
DURATION
00:10:00 : 3)

DNA washing

Step 5.

1) Add 1ml of 70% of ethanol, flick the centriguge tube to resuspend the pellet, wash the DNA pellet for 3-5min.

2) Centrifuge at 13000rpm for 5min at 4℃, discard the ethanol.

3) Add 1ml of 70% ethanol and mix gently. Centrifuge at 13000rpm for 5min at 4℃, discard the ethanol.

4) Centrifuge at 13000rpm for 30s, discard the liquid. Air dry the pellet for 5-10min at room temperature.

TEMPERATURE
4 °C : 2)
TEMPERATURE
4 °C : 3)
REAGENTS
70% of ethanol by Sinopharm Chemical Reagent Co.
DURATION
00:05:00 : 1)
DURATION
00:05:00 : 2)
DURATION
00:05:00 : 3)
DURATION
00:00:30 : 4)
DURATION
00:10:00 : 4)

Dissolve DNA

Step 6.

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

TEMPERATURE
37 °C :
REAGENTS
RNase by FERMENTAS Inc.
TE buffer by Invitrogen - Thermo Fisher
DURATION
00:30:00 :