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DNA extraction for vertebrate tissues using Phenol:Chloroform:Isoamylol

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Protocol status: Working

We use this protocol and it's working

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Abstract

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



Materials

STEP MATERIALS

☒ normal saline **Sinopharm Chemical Reagent Co.**

☒ STE

☒ SDS **Ambion**

☒ protease K **New England Biolabs**

☒ Phenol : Chloroform : Isoamylol (25: 24: 1) **Sinopharm Chemical Reagent Co.**

☒ Chloroform : Isoamylol (24 : 1) **Sinopharm Chemical Reagent Co.**

☒ Isopropanol **Sinopharm Chemical Reagent Co.**

☒ 70% of ethanol **Sinopharm Chemical Reagent Co.**

☒ RNase **FERMENTAS Inc.**

☒ TE buffer **Invitrogen - Thermo Fisher**

☒ normal saline **Sinopharm Chemical Reagent Co.**

☒ STE

☒ SDS **Ambion**

☒ protease K **New England Biolabs**

☒ Phenol : Chloroform : Isoamylol (25: 24: 1) **Sinopharm Chemical Reagent Co.**

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☒ Phenol : Chloroform : Isoamylol (25: 24: 1) **Sinopharm Chemical Reagent Co.**

☒ SDS **Ambion**

☒ STE

☒ RNase **FERMENTAS Inc.**



Sample preparation

- 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.

normal saline **Sinopharm Chemical Reagent Co.**

Tissue lysis

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

STE

SDS **Ambion**

protease K **New England Biolabs**

06:00:00 heat

56 °C

Phase separation

- 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°C and then transfer the supernatant 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°C and then transfer the supernatant aqueous phase in a new tube.

Phenol : Chloroform : Isoamylol (25: 24: 1) **Sinopharm Chemical Reagent Co.**

Chloroform : Isoamylol (24 : 1) **Sinopharm Chemical Reagent Co.**

00:05:00 1)

00:10:00 2)

4 °C 2)

00:05:00 3)

00:10:00 4)

4 °C 4)

DNA precipitation



- 4
- 1) Add equal volume of Isopropanol, and then mix by slowly inverting.
 - 2) Incubate the tube at -20°C for overnight.
 - 3) Centrifuge at 13600rpm for 10min at 4°C and discard the supernatant.

 Isopropanol **Sinopharm Chemical Reagent Co.**

 -20 °C 2)

 4 °C 3)

 00:10:00 3)

DNA washing

- 5
- 1) Add 1ml of 70% of ethanol, flick the centrifuge tube to resuspend the pellet, wash the DNA pellet for 3-5min.
 - 2) Centrifuge at 13000rpm for 5min at 4°C, discard the ethanol.
 - 3) Add 1ml of 70% ethanol and mix gently. Centrifuge at 13000rpm for 5min at 4°C, discard the ethanol.
 - 4) Centrifuge at 13000rpm for 30s, discard the liquid. Air dry the pellet for 5-10min at room temperature.

 70% of ethanol **Sinopharm Chemical Reagent Co.**

 00:05:00 1)

 00:05:00 2)

 4 °C 2)

 00:05:00 3)

 4 °C 3)

 00:00:30 4)

 00:10:00 4)

Dissolve DNA

- 6
- Add RNase and 100ul of TE buffer (TE : RNase = 100:1), and then dissolve the pellet at 37°C for 30min in the Thermomixer.

 RNase **FERMENTAS Inc.**

 TE buffer **Invitrogen - Thermo Fisher**

 00:30:00

 37 °C