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DNA extration for the Betta splendens genome

In 1 collection

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ABSTRACT

This protocol is used to clarify the process of total DNA extration for our Betta splendens genome.

MATERIALS

STEP MATERIALS

⊗ lysis buffer **Contributed by users**

⊗ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988**

⊗ isoamyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W20570**

⊗ isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907**

⊗ ethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1.07017**

⊗ TE Buffer **Roche Catalog #5618215001**

⊗ lysis buffer **Contributed by users**

⊗ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988**

⊗ isoamyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W20570**

⊗ isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907**

⊗ ethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1.07017**

⊗ TE Buffer **Roche Catalog #5618215001**

Sample preparation

1 1.Pour 1ml lysis buffer.

buffer:

100mmol Tris-Hcl(PH8.0);
25mmol EDTA(PH 8.0);
500mmol NaCl;
1% SDS

2. For tissue samples, grind about 200mg with liquid nitrogen into powder.

Safety information

Formaldehyde is toxic. Please read the MSDS before working with this chemical. Gloves and safety glasses should be worn and solutions made inside a fume hood.

Note

Thaw the formaldehyde for an hour at room temperature prior to beginning the assay

lysis buffer **Contributed by users**

Tissue lysis

- 2
 1. Transfer the powder samples into the 2 ml tube contain of 1ml lysis buffer.
Note: As seawater samples fix quickly, samples are ready for slide preparation after 10 min on ice.
 2. Incubate the sample at 56°C in a water incubator for 30~180min. Mix by inversion every 5-10min time period.
 3. Centrifuge at 16700×g for 10 minutes after cooling to room temperature.

00:10:00 1

00:30:00 2(1)

00:05:00 2(2)

00:10:00 3

Phase separation

- 3
 1. Transfer the supernatant to a new 2.0ml tube, add equal volume of supernatant of Chloroform/isoamyl alcohol(24:1). Gently invert each 3-5min to mixed liquid no obvious boundaries.
 2. Transfer the aqueous phase to a new 1.5mL tube; add equal volume of supernatant of isopropyl alcohol. Gently invert each 3-5min to mixed liquid no obvious boundaries. Centrifuge at 16700×g for 10 minutes.

00:03:00 1

00:03:00 2(1)

⌚ 00:10:00 2(2)

⊗ Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988**

⊗ isoamyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W205702**

⊗ isopropyl alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #W292907**

DNA precipitation

- 4
1. Transfer the aqueous phase to a new 1.5mL tube; add 2/3th volume of supernatant of isopropyl alcohol. Gently mix by inverting at least 3 times and place at -20°C for 2 hours for precipitation.
 2. Centrifuge at 16700×g for 10 minutes and remove the supernatant.

⌚ 02:00:00 1

⌚ 00:10:00 2

DNA washing

- 5
1. Wash the DNA pellet with 1 ml 75% cooling ethanol. Re-suspend the pellet and centrifuge at 16700×g for 5 minutes at 4°C and remove the supernatant
 2. Wash the DNA pellet with 500ul 75% cooling ethanol. Re-suspend the pellet and centrifuge at 16700×g for 5 minutes at 4°C and remove the supernatant.
 3. 16700×g for 30~60s, Completely remove the ethanol without disturbing the pellet.
 4. Air-dry the DNA pellet in the biosafety cabinet.

⌚ 00:05:00 1

⌚ 00:05:00 2

⌚ 00:00:30 3

⊗ ethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1.07017**

Dissolve DNA

- 6
1. Add 50μL of TE Buffer to dissolve the DNA pellet.

⊗ TE Buffer **Roche Catalog #5618215001**