JUN 11, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.qvedw3e

Protocol Citation: Kailong Ma 2018. DNA extraction for the Betta splendens genome. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.qvedw3e>

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Protocol status: Working

Created: Jun 11, 2018

Last Modified: Jun 12, 2018

PROTOCOL integer ID:
12934

DNA extraction for the Betta splendens genome

Forked from [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 extraction 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
#W205702

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
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Sample preparation

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
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isopropyl alcohol Merck MilliporeSigma (Sigma-Aldrich) Catalog
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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
- Add 50μL of TE Buffer to dissolve the DNA pellet.

⌚ TE Buffer Roche Catalog
#5618215001