DNA extration for the Betta splendens genome

Xin Liu

Abstract

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

Citation: Xin Liu DNA extration for the Betta splendens genome. **protocols.io** dx.doi.org/10.17504/protocols.io.qq6dvze
Published: 08 Jun 2018

Protocol

Sample preparation

Step 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.

REAGENTS
lysis buffer by Contributed by users

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.

NOTES
Hongling Zhou 06 Jun 2018

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

Tissue lysis

Step 2.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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 30180min. Mix by inversion every 5-10min time period.

3. Centrifuge at 16700×g for 10 minutes after cooling to room temperature.

Phase separation

Step 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.

REAGENTS

Chloroform 319988 by Sigma

isoamyl alcohol W205702 by Sigma

isopropyl alcohol W292907 by Sigma

DNA precipitation

Step 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.

DNA washing

Step 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 3060s, Completely remove the ethanol without disturbing the pellet.

4. Air-dry the DNA pellet in the biosafety cabinet.

REAGENTS

ethanol 1.07017 by Sigma

Dissolve DNA

Step 6.

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

REAGENTS

TE Buffer 5618215001 by Roche

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited