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RNA extration for the Betta splendens genome

In 1 collection

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ABSTRACT

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

MATERIALS

STEP MATERIALS

⊗ TRIZOL reagent Invitrogen - Thermo Fisher Catalog #15596-026

⊗ Chloroform / isoamyl alcohol(24:1) Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988/W205702

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

⊗ DEPC-treated water Ambion Catalog #AM9915G

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⊗ DEPC-treated water Ambion Catalog #AM9915G

Sample preparation

- 1
 1. Pour 1.5ml TRIZOL reagent.
 2. For tissue samples, grind about 60mg with liquid nitrogen into powder and transfer the powder samples into the 2 ml tube contain of 1.5ml Trizol reagent.

Tissues lysis

- 2 Homogenize 2 minutes and place the sample at rest horizontally for 5 minutes to permit the complete dissociation of nucleoprotein complexes.

⌚ 00:02:00 1

⌚ 00:05:00 2

Phase separation

- 3
1. Centrifuge at 12000×g for 5 minutes at 4°C. Transfer the supernatant to a new 2.0ml tube, add 0.3 ml of Chloroform / isoamyl alcohol(24:1) per 1.5 ml of Trizol reagent. Shake the tubes vigorously for 15 seconds.
 2. Centrifuge at 12000×g for 10 minutes at 4°C. After centrifugation, the mixture should separates into three layers: the lower phenol-chloroform phase, an interphase, and an upper aqueous phase. RNA remains in the aqueous phase.

⌚ 00:05:00 1(1)

⌚ 00:00:15 1(2)

⌚ 00:10:00 2

⌘ Chloroform / isoamyl alcohol(24:1) Merck MilliporeSigma (Sigma-Aldrich) Catalog #319988/W205702

RNA precipitation

- 4
1. Transfer the aqueous phase to a new 1.5mL tube; add equal volume of supernatant of isopropyl alcohol. Mixing well and place at -20°C for 2 hours for precipitation.
 2. Centrifuge at 13600rpm for 20 minutes at 4°C and remove the supernatant.

⌚ 02:00:00 1

⌚ 00:20:00 2

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

RNA washing

- 5
1. Wash the RNA pellet with 1 ml 75% ethanol. Re-suspend the pellet and centrifuge at 13600rpm for 3 minutes at 4°C. Repeat this step again, Completely remove the ethanol without disturbing the pellet.
 2. Air-dry the RNA pellet in the biosafety cabinet.

⌚ 00:03:00 1

Dissolve RNA

- 6 Add 50µL of DEPC-treated water to dissolve the RNA pellet.

DEPC-treated water Ambion Catalog #AM9915G