RNA extraction for the Betta splendens genome

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Abstract
This protocol is used to clarity the process of RNA extraction for our Betta splendens genome.

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Protocol

Sample preparation

Step 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.

REAGENTS

TRIZOL reagent 15596-026 by Invitrogen - Thermo Fisher

Tissues lysis

Step 2.

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

Phase separation

Step 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 remaims in the aqueous phase.

REAGENTS

Chloroform / isoamyl alcohol(24:1) 319988/W205702 by Sigma

RNA precipitation

Step 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.

REAGENTS

isopropyl alcohol W292907 by Sigma

RNA washing

Step 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.

Dissolve RNA

Step 6.

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

REAGENTS

DEPC-treated water AM9915G by Ambion