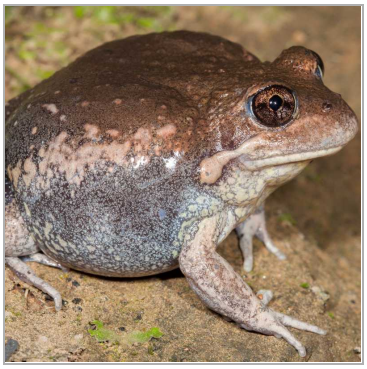

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Protocol status: Working

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DNA extraction protocol for the eastern banjo frog using the Gentra Puregene Tissue Kit

In 2 collections

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Qunfei Guo

ABSTRACT

Genomic DNA was extracted from the liver of an adult female *Limnodynastes dumerilii dumerilii* using the Gentra Puregene Tissue Kit (QIAGEN, Hilden, Germany) with modifications as outlined in this protocol.

MATERIALS

MATERIALS

2-propanol **Sigma Aldrich Catalog #I9516**

Ethanol (undenatured) **chem-supply Catalog #EA043**

Last Modified: Feb 27, 2023

PROTOCOL integer ID:
33534

Keywords: DNA extraction, DNA purification, Gentra Puregene Tissue Kit, the eastern banjo frog

1 The following protocol is a modification of the protocol for "DNA purification from 5-10mg fresh or frozen solid tissue" using the Gentra Puregene Genomic DNA Purification Kit.

The amounts listed in this protocol are for a single extraction. Four replicate extractions were made using this protocol.

Approximately 30mg of frozen liver tissue was minced with a scapel blade on ice.

2

Lysis

Dispense 900 µl Cell Lysis Solution into a 1.5 ml microfuge tube and add the minced tissue from the previous step. Add 6 µl of Proteinase K Solution (20mg/ml) and mix by inverting 25 times. Incubate at 55 °C overnight with agitation.

Heat RNase A Solution (12mg/ml) to 100 °C for 10 min.
Add 1.5 µl to the microfuge tube, mix by inverting 25 times and incubate at 37 °C for 60 min.

Cool to room temperature.

55 °C
Overnight
100 °C
00:10:00
37 °C

01:00:00

Room temperature

3

Protein precipitation

Add 300 µl Protein Precipitation Solution and vortex vigorously for 20s at high speed.

Centrifuge for 5min at 16000 x g.

.

00:00:20

00:05:00

4

DNA precipitation

Add 900 µl cold 100% Isopropanol to a clean 2 ml microfuge tube and add the supernatent from the previous step by pouring carefully. Gently invert the tube 50 times to mix.

Spool the precipitated DNA onto a sterile glass rod.

5

Wash with ethanol

Wash the spooled DNA by immersing in 300 µl 70% ethanol and then place in a clean microfuge tube with 500 µl 70% ethanol at -20 °C for 2h.

-20 °C

02:00:00

Note

This is the adapted step which is different from the manufacturer's instructions.

6

DNA hydration

Dry the DNA at room temperature for 1h by inverting the glass rod. Dissolve the DNA in 200 µl of the recommended elution buffer (add more elution buffer if the DNA does not fully dissolve).

Room temperature

01:00:00

Note

This is the adapted step which is different from the manufacturer's instructions.