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DNA Isolation from Reptile Blood using Gentra Puregene (Qiagen) DNA Isolation Kit

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Alexis Lindsey[1], Tonia S Schwartz[1]

[1]Auburn University

Schwartz Lab of Ecologi...

Alexis Lindsey
Auburn University

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We use this protocol and it's working

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Abstract

This protocol is used to isolate DNA from reptile blood (either whole or red blood cell pellets). Reptile blood has nucleated red blood cells and will produce considerably more DNA from the same volume of mammalian blood. This protocol is adapted from Gentra Puregene DNA Isolation A Kit.

The time estimates assumes you are processing 24 samples and you are well practiced.

Materials

Equipment

- Pipettes (p1000, p200, p20)
- Shaking tube incubator
- Benchtop Centrifuge

Consumables

- One 15 ml tube (DNA/RNA and DNAse free) to make 70% ETOH
- 1.5-mL microcentrifuge tubes (VWR Catalog Number 76332-068). Two tubes for each sample
- Filtered pipette tips (p1000, p200, p20)
- Serological pipette (10 or 20 ml) to make 75% ETOH
- latex or nitrile gloves

Reagents

- Qiagen Puregene Core Kit A (Catalog Number 158467)
  ·Cell Lysis Buffer
  ·Protein Precipitation Solution
  ·DNA Hydration Solution
- Proteinase K (20 mg/mL) (IBI Science, Product Number IB05406)
- Isopropanol (ThermoFisher Scientific, Catalog Number AC327272500)
- 70% ethanol (200 proof for dilutions: Deacon Labs Product Number 3916EA; Molecular Grade Water)
- Molecular Grade Water (QualityBiological, Catalog Number 351-029-131)
- 1.5 mL microcentriguge tubes (VWR Catalog Number 76332-068)

Troubleshooting

Notice for high quality (long undamaged DNA)

1 **Through out this protocol we **vortex** the samples for mixing. This works well and produces alot of good quality DNA that works for most everything. If you want to minimize damage to the DNA such as for long-read sequencing on the Nanopore or PacBIO, or assays to quantify DNA damage) **do not vortex.** Rather mix gently by flicking and inverting the tube. The quality will be higher but generally less yield.*

Setting Up

10m

2 Turn on shaking tube incubator and set to 55 °C

3 Make fresh 70% ETOH. For example, to make 15 ml in a 15 ml tube use a 10 ml sterile pipette to take 10.5 mL of [M] 100 % volume (200 proof) ETOH, and another 10 ml sterile pipette to add 4.5 mL of molecular grade water.

10m

Red Blood Cell Lysis

1h 45m

4 Set out the the blood samples to be used on ice to thaw, but stay cold.
Set out the correct number of 1.5 mL tubes into a clean tube rack.

5m

5 Add 300 µL of **Cell Lysis Solution** (from PureGene Kit) to each tube.

1m

Note

Do NOT use the Blood Lysis Solution

6 Add 10 µL **proteinase K** (20 mg/ml) to each tube.

1m

7 Close tubes and label each tube according to Sample IDs.

3m

8 Add 15 µL of **whole blood** to the correct tube and *vortex well** .

30m

Note

Note: This volume of blood can be reduced as low as 3 µl of whole blood with good results but considerably less DNA. If using packed red blood cells use 5-7 µl.

Note

***Importantly:** If you want to use the DNA for damage analysis, quantify telomere lengths, or long sequencing, it is recommended to NOT vortex throughout this protocol, only flick the tube with your finger and invert the tube to mix.

9 Place each tube in the shaking incubator and incubate at 300 rpm, 55°C, 01:00:00 . 1h

Vortex* the samples periodically.

Isolate DNA 1h 33m

10 Add 100 µL of **Protein Precipitation Solution** (from PureGene Kit) to each tube and vortex* vigorously. 5m

11 Place in ice for approximately 5 minutes. 5m

During this incubation do steps 12 and 13.

12 Obtain fresh 1.5 mL tubes and label top and side with DNA ID, Date, and "stock DNA" (these will be the tubes that the isolated DNA will be stored in). 10m

13 Use a pipette to add 300 µL of **isopropanol** into each of the newly labeled tubes. 2m

14 Centrifuge the samples for 16,000 x g, 00:01:00 . 1m

After removing the tubes from the centrifuge, there should be a yellow/brown pellet at the bottom of the tube. If there is no pellet, repeat this step for each tube missing the pellet.

15 Without dislodging the protein pellet, pour out the supernatant containing the DNA into the newly labeled tubes containing the isopropanol. Throw away the original tube with 1m

the protein pellet.

16 Mix the supernatant with the isopropanol by inverting 50 times. If there is a lot of DNA you can see the strands condensing at this step (looks like thin white threads). 2m

17 Place each tube into the centrifuge with the hinge facing out so the DNA pellet forms on that side of the tube. Centrifuge the samples for 16,000 x g, 00:01:00 to pellet the DNA. 1m

Expected result

There should be a small, white/clearish pellet of DNA present towards the bottom of the tube on the side of the hinge.

18 Without dislodging the DNA pellet, carefully pour the supernatant out into waste container with as little movement as possible. 2m

19 To wash the DNA pellet, add 300 µL **70% ethanol** to each tube and invert several times. 1m

20 Centrifuge for 16,000 x g, 00:01:00 .
Again, there should be a white/clear DNA pellet visible towards the bottom of each tube. 1m

21 Pour out the supernatant into waste container. 1m

22 OPTIONAL: Centrifuge the tubes again for 16,000 x g, 00:01:00 , and use a 10 µl tip to remove the residual ethanol. This will make the next step go faster. 1m *

23 Invert tubes on a paper town, with the tops open, until ethanol has completely evaporated. 1h

Resuspend the DNA 1h 1m

24 Once the ethanol has evaporated (but the DNA pellet is not over dry), add 50 µL **DNA Hydration Solution** (PureGene Kit) and vortex* to resuspend the DNA into solution. 1m

Expected result

This produces on average this produced 1 µg of DNA per µl. This volume of DNA hydration solution can be increased or lowered if you want a higher concentration or if you started with a smaller volume of blood.

25 Incubate the tubes for one hour at room temperature, or 4°C overnight to resuspend the DNA. 1h

Check Quality and Quantity

26 Run 5 µL of resuspended DNA on 1% agarose gel to visual the quality and estimate quality. DNA can be quantified with the Nanodrop. For sensitive procedures (DNA sequencing library preparation we recommend using the Agilent TapeStation or BioAnalyzer).