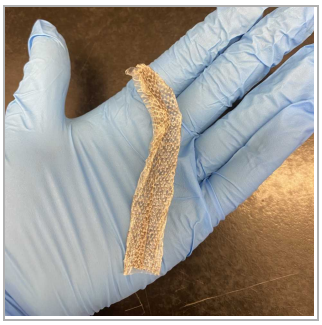

DEC 19, 2022

WORKS FOR ME 1

DNA Isolation from Snake Skin Shed

COMMENTS 0

DOI
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ABSTRACT

Purpose:
This protocol was developed for the Memphis Zoo's Louisiana Pine Snake Breeding Project.The protocol for skin shed DNA isolation was adapted from Fetzner (1999).

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

References: James W Fetzner (1999) Extracting High-Quality DNA from Shed Reptile Skins: A Simplified Method. BioTechniques 26:6

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KEYWORDS

snake, skin shed, DNA isolation, snake shed, shed

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MATERIALS TEXT

Equipment

- Sterile razor blades or scissors
- Dissection boards for cutting up the skin sheds

Consumables

- Filtered micropipette tips (p1000, p200)
- 1.5-mL microcentrifuge tubes (VWR Catalog Number 76332-068)
- 2-mL microcentrifuge tubes (VWR Catalog Number 20170-170)
- 15 ml conical tube for making 70% Ethanol.
- latex or nitrile gloves

Reagents

- RNase AWAY, which also degrades DNA (Molecular BioProducts Catalog Number 7002)
- Proteinase K (20 mg/mL) (IBI Science Product Number IB05406)
- Lysis Buffer (10mM Tris-base, 10mM EDTA, 2% sodium dodecyl sulfate (SDS), pH 8.0)*****
- TE Buffer (10mM Tris-base, 0.1 mM EDTA, pH 8.0; Growcells.com Catalog No: MRGF-4240)
- 70% ethanol (200 proof ETOH, Deacon Labs Product Number 3916EA in Molecular Grade Water (see below)
- 5M aqueous solution ammonium acetate (ThermoFisher Scientific (Alfa Aesar) Product Number J60688)
- Isopropanol (ThermoFisher Scientific (Acros Organics) Catalog Number AC327272500)
- Molecular Grade Water (QualityBiological Catalog Number 351-029-131)

*****Lysis Buffer Recipe: For a final volume of 100 mL:
95 mL molecular-grade water
1 mL 1M Tris-base (VWR Product Number E199)
2 grams SDS (VWR Product Number 0227)
2 mL 0.5M EDTA (VWR Code E177)

1h 10m

Set Up

10m

1 Turn on shaking incubator and set to 55 °C

Obtain Ice

Print list of samples

30m

2 Clean the razors, scissors, and dissection boards with RNAase away; rinse with molecular-grade water.

10m

3 Make fresh 70% ETOH. For example, to make 15 ml in a 15 ml tube use a 10 ml sterile pipette to take

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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

4 Set out and label 1.5 mL microcentrifuge tube for each sample to use in Step 6 (Digestion).
Add 900 µL **Cell Lysis Buffer**
Add 10 µL of **proteinase K(** 20 mg/mL).

10m

5 Set out and label a 2 mL tube for each sample to use in Step 13 (DNA precipitation).
Add containing 900 µL **isopropanol**.

4h 5m

Isolation: Lysis

1h

6 Cut up 1 in^2 piece of shed into smaller pieces with a sterile razor blade or scissors (change gloves and utensils between sheds).
Put shed pieces in the labeled 1.5 mL microcentrifuge tube containing the Cell Lysis Buffer and Proteinase K (prepared in Step 4).

3h

7 Place in shaking incubator 300 rpm, 55°C 3 hours to overnight Vortex occasionally for the first few hours.

5m

8 Cool samples to room temperature and vortex.

25m

Isolation: Precipitate Proteins

5m

9 Add 550 µL **5M ammonium acetate** to each tube and vortex for 10 seconds.

10m

10 Place samples on ice for Room temperature for 10 minutes.

5m

11 Centrifuge samples 17,000 x g, 00:05:00 to pellet protein and debris.

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12 Draw off as much supernatant as possible with a filtered tip into put in to a new 1.5 mL labeled tube. 2m

13 Centrifuge the supernantent at second time at 20,000 x g, 00:03:00 to pellet any residual protein and debris. 3m

Isolation: Precipitate DNA 1h 13m

14 With a filtered tip, transfer supernatant from the second spin into the prepared 2mL tubes containing the isopropanol (prepared in Step 4). 2m

15 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

16 Place each tube into the centrifuge with the hinge facing out so the DNA pellet forms on that side of the tube. Centrifuge samples at 16,000 x g, 00:02:00 to pellet the DNA. 2m

Expected result

17 Pour off isopropanol into waste container. 1m

18 Wash the DNA pellet by adding 500 µL of **70% ethanol**. 2m

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19 Centrifuge at 16,000 x g, 00:02:00. The DNA pellet should still be visable. 2m

20 Without dislodging the DNA pellet, carefully pour the supernatant out into waste container with as little movement as possible. 1m

21 Centrifuge the tubes again for 16,000 x g, 00:01:00, and use a 10 µl tip to remove the residual ethanol. 1m

* This will make the next step go faster.

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

Resuspension

1h 1m

23 Once the ethanol has evaporated (but the DNA pellet is not over dry), resuspend samples in 50 µL of **TE buffer**. 1m

Expected result

24 Sit at 300 rpm, 37°C, 01:00:00 or leave in 4°C overnight to fully resuspend the DNA. 1h

Check Quality

25 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.

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