VERSION 1

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**Protocol status:** Working
We use this protocol and it's working

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# DNA - Ball Python DNA Extraction from sheds V.1

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ABSTRACT

Protocol to extract DNA from Ball Python (Python regius) dry sheds using Phenol:Chloroform:Isoamyl Alcohol

## 1 EQUIPMENT

- Dry Bath / Heated Block

- Microcentrifuge
- DNA LoBind tubes 1.5mL
- Micropipettes
- Assorted pipette tips

## 2 REAGENTS

- Lysis Buffer ( [M] 10 millimolar (mM) Tris-base, [M] 100 millimolar (mM) EDTA, 2% SDS, [M] 5 Molarity (M) , NaCl, pH 8 )
- TE Buffer (EDTA [M] 1 millimolar (mM) , Tris-Cl [M] 10 millimolar (mM) )
- Proteinase K ( [M] 20 mg/mL )
- Phenol/Chloroform/Isoamyl Alcohol 25:24:1 (v/v)
- Ethanol 100 %
- Ethanol 70 % (Freshly prepared)
- Tris Acetate-EDTA (TAE) Buffer
- SYBR Safe DNA stain
- Agarose
- Loading Dye
- Wide Range Ladder (100-12,000 bp)

## 3 PROTEINASE K DIGESTION

30s

1. Cut a small piece of fresh tissue (3-5mm). Put in a 1,5mL microtube. Add 900 µL of lysis buffer. Add 9 µL Proteinase K ( [M] 20 mg/mL ) and vortex thoroughly for 00:00:30 seconds.

2. Incubate at 55 °C overnight .

## 4 PHENOL/CHLOROFORM/ISOAMYL EXTRACTION

5m 30s

1. Add 500 µL of Phenol/Chloroform/Isoamyl Alcohol 25:24:1 (v/v) and vortex thoroughly for 00:00:30 seconds.

2. Centrifuge at room temperature for 00:05:00 at 16000 x g .

3. Carefully remove the upper aqueous phase (~ 500 µL )and transfer the layer to a fresh tube. Be sure not to carry over any Phenol during pipetting.

## 5 ETHANOL PRECIPITATION

1h 50m

1. Add 1000 µL of [M] 100 % volume Ethanol, invert the tube and place the tube at -80 °C for 01:00:00 or at -20 °C Overnight .

2. Centrifuge the sample at 4 °C for 00:30:00 at 16000 x g to pellet the DNA.

3. Carefully remove the supernatant without disturbing the pellet.

4. Add 150 µL of [M] 70 % volume ethanol.

5. Centrifuge the sample at 4 °C for 00:05:00 at 16000 x g .

6. Carefully remove the supernatant without disturbing the pellet.

7. Repeat [M] 70 % volume ethanol wash once and remove as much of the remaining ethanol as possible.

8. Dry the DNA at Room temperature for 00:05:00 to 00:10:00 .

9. Re-suspend the DNA pellet in 200 µL of TE Buffer.

## 6 DNA QUALITY CHECK

40m

1. Prepare a 1% agarose gel with 1X TAE buffer and 1X SYBR Safe DNA stain.

2. Add 4 µL Wide Range Ladder (100-12,000 bp) in the first well.

3. Premix 9 µL of sample and 1 µL of 10X loading dye or 5 µL of sample and 1 µL of 6X loading dye. Load this mixture in each well.

3. Run the samples for 00:40:00 at a 100V.

4. Visualize in a trans illuminator and take a photo of the gel.