

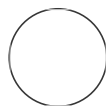


VERSION 1
MAR 27, 2023

DNA - Ball Python DNA Extraction from sheds V.1

Jose Avila Cervantes¹

¹Lolita Genomics



Jose Avila Cervantes

ABSTRACT

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

OPEN  ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.rm7vzb344vx1/v1

Protocol Citation: Jose Avila Cervantes 2023. DNA - Ball Python DNA Extraction from sheds. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.rm7vzb344vx1/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

Created: Oct 15, 2022

Last Modified: Mar 27, 2023

PROTOCOL integer ID:
71398

- 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 $\text{900 } \mu\text{L}$ of lysis buffer. Add $\text{9 } \mu\text{L}$ Proteinase K ([M] 20 mg/mL) and vortex thoroughly for 00:00:30 seconds.

2. Incubate at $\text{55 } ^\circ\text{C}$ overnight .

4 PHENOL/CHLOROFORM/ISOAMYL EXTRACTION

5m 30s



1. Add $\text{500 } \mu\text{L}$ 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 $\text{16000 } \times g$.

3. Carefully remove the upper aqueous phase ($\sim \text{500 } \mu\text{L}$)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  100 % volume Ethanol, invert the tube and place the tube at  $-80\text{ }^{\circ}\text{C}$ for  01:00:00 or at  $-20\text{ }^{\circ}\text{C}$  Overnight .
2. Centrifuge the sample at  $4\text{ }^{\circ}\text{C}$ 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  70 % volume ethanol.
5. Centrifuge the sample at  $4\text{ }^{\circ}\text{C}$ for  00:05:00 at  16000 x g .
6. Carefully remove the supernatant without disturbing the pellet.
7. Repeat  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.