VERSION 1

MAR 27, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.3byl4jb78lo5/v1

Protocol Citation: Jose Avila Cervantes 2023. DNA - Ball Python DNA Amplification with TFEC primers. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4jb78lo5/v1

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

Created: Oct 18, 2022

Last Modified: Mar 27, 2023

PROTOCOL integer ID:
71489

DNA - Ball Python DNA Amplification with TFEC primers V.1

Jose Avila Cervantes[1]

[1]Lolita Genomics

Jose Avila Cervantes

ABSTRACT

PCR amplification with Tfec set Exon 5 to genotype Piedball morph in Ball python (Python regius)

LEFT PRIMER	AACTCAGAGCACTCCATGACC
RIGHT PRIMER	CAGGTGTGCCCCTTTCATAA

1 REAGENTS

- 10X PCR Buffer

- MgSO4 [M] 20 millimolar (mM)
- Taq Polymerase 5U/ul
- Primer Tfec exon5 Left [M] 10 micromolar (µM)
- Primer Tfec exon5 Right [M] 10 micromolar (µM)
- DNTP's [M] 10 micromolar (µM)
- Ultra Pure Water
- 100bp ladder (100-2,000 bp)
- SYBR Safe DNA stain
- Loading dye
- Agarose

2 EQUIPMENT

- DNA LoBind tubes 1.5mL
- PCR 8-Strip tubes 0.2mL
- Micropipettes
- Thermal Cycler

3 MASTER MIX

30s

Below is the recommended volume for one sample. Adjust for the number of samples to process and add 10% to account for pipetting error. Thaw all reagents at room temperature before using them except the Taq DNA polymerase, which has to be kept on ice at all times. Premix all reagents and add the Taq DNA polymerase at the end, vortex thoroughly for 00:00:30 seconds. Aliquot the master mix in to each tube 28 µL and then add 2 µL of DNA sample. The final volume for each reaction is 30 µL .

	A	B
	Reagent	Volume (uL)
	10x PCR Buffer	3
	MgSO4	4.5
	Taq Polymerase	0.3
	Primer F	1.5
	Primer R	1.5
	DNTP's	0.6
	Template-DNA	2
	Water	16.6

Table 1. Master Mix for PCR.

4 PCR CONDITIONS

	A	B	C	D
	STEP	TIME	TEMPERATURE (°C)	
	Initial Denaturation	3 min	94	
	Denaturation	15 sec	94	25 Cycles
	Annealing	30 sec	53.2	
	Extension	30 sec	74	
	Final Extension	2 min	74	

Table 2. Thermocycler program

5 AMPLICON QUALITY CHECK

35m

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

2. Add 4 µL of 100bp ladder (100-2,000 bp) in the first well.

3. Premix 4 µL of sample and 1 µL of 10X loading dye or 3 µL of sample and 1 µL of 6X loading dye. Load this mixture in each well.

3. Run the samples for 00:35:00 at a 100V.

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

5. The product should be a clear, single band around 200 bp.