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

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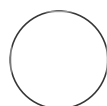
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## DNA - Ball Python DNA Amplification with TFEC primers V.1

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### 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

- MgSO<sub>4</sub> [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
MgSO <sub>4</sub>	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.