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Whitefly DNA extraction, partial mtCO1 gene amplification and gel electrophoresis

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1 Works for me dx.doi.org/10.17504/protocols.io.bdvri656

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MATERIALS

NAME	CATALOG #	VENDOR
Gel Loading Dye Orange (6X) - 4.0 ml	B7022S	New England Biolabs
Chelex 100	C7901-100G	Sigma Aldrich
70% ethanol		Fisher Scientific
TBE buffer (Tris-Borate-EDTA), 5x Solution	A00265.SIZE.4L	Bio Basic Inc.
PCR-Grade Water	W3500	Sigma Aldrich
Agarose	75510019	Thermo Fisher
GeneJET PCR Purification Kit	K0701	Thermo Fisher

1 Single whitefly adults were randomly chosen from each of the 1.5 ml Eppendorf tubes containing 70% ethanol, using a sterilized entomological pin.

2 Each whitefly was transferred to a clean 1.5 ml Eppendorf tube.

3 To extract insect DNA, 50 µl of 10% Chelex was added to the tube and the whitefly was crushed using a plastic pestle until a clear homogenised mixture was obtained.

4 The mixture was then incubated at 56°C for 20 minutes and further incubated at 100°C for five minutes.

5 The mixture was then centrifuged at 13,500 g for five minutes using the Eppendorf Centrifuge 5424 R and the supernatant was collected and stored at -20°C until use.

6 Two sets of primers, with different degrees of sensitivity, were used to amplify the mtCO1 fragments, which were the: (i) African specific 2195Bt (5'-TGRTTTTTTGGTCATCCRGAAGT-3') and C012/Bt-sh2 (5'-TTTACTGCACTTTCTGCC-3') primers [21] and (ii) generic CI-J-2195 (5'-TTGATTTTTTGGTCATCCAGAAGT -3') and TL2-N-3014 (TCCAATGCACTAATCTGCCATATTA -3') primers.

7 Polymerase chain reaction (PCR) was run on an Applied Biosystems 2720 thermal cycler for 35 cycles.

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8 The PCR program conditions were set at (i) initial denaturation at 94°C for 2 minutes, (ii) denaturation at 94°C for 20 seconds, (iii) annealing at 52°C for 30 seconds, (iv) extension at 72°C for one minute and (v) final extension at 72°C for five minutes.

9 Agarose gel electrophoresis (1% w/v in 0.5 X Tris/borate/EDTA buffer) was used to confirm PCR amplification.

10 The GeneJETPCR purification kit (Thermo Scientific)was used to purify amplicons of interest as described by the kit manufacturer.

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