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Supplemental Lab-Protocol for Barcoding Primers: dEURYT-BRBM2, LCO1490-JJ, LCO1490-JJ2 & LCO1490-JJ3

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

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Keywords: dEURYT-BRBM2, LCO1490-JJ, LCO1490-JJ2, LCO1490-JJ3, DNA Barcoding, COI, PCR, Touchdown PCR conditions, protocol for barcoding primer, barcoding primer, extended supplement to astrin, primer, astrin, lco1490

Abstract

As a detailed and extended supplement to Astrin & Stüben, 2008; Astrin et al., 2016; Rulik et al., 2017; Jafari et al., 2023; Jaume-Schinkel et al., 2024.

Guidelines

Follow the general laboratory etiquette. Wear gloves to avoid contamination of the samples. Clean the work bench with 96% EtOH before starting.

Materials

Present protocol is based on Qiagen products:

DNeasy Blood & Tissue Kit: https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/dneasy-blood-and-tissue-kit?catno=69504

BioSprint 96 DNA Blood Kit (384): https://www.qiagen.com/us/products/discovery-and-translational-research/dna-rna-purification/dna-purification/genomic-dna/biosprint-dna-blood-kits?catno=940057

QIAGEN Multiplex PCR Kit (100): https://www.qiagen.com/us/products/discovery-and-translational-research/pcr-qpcr-dpcr/pcr-enzymes-and-kits/end-point-pcr/qiagen-multiplex-pcr-kit?catno=206143

QIAGEN Multiplex PCR *Plus* Kit (100): https://www.qiagen.com/de-de/products/discovery-and-translational-research/pcr-qpcr-dpcr/pcr-enzymes-and-kits/end-point-pcr/qiagen-multiplex-pcr-plus-kit?catno=206152

Safety warnings

None.

Before start

Make sure all chemicals & buffers are prepared and ready to use before starting.

DNA extraction

1 Prepare 96 block format or single tube with 20 µL Proteinase K and 180 µL ATL Buffer per well or tube → centrifuge briefly.

2 Put a small subsample (leg, piece of tissue ca. 1-2mm) of each sample or whole body of the specimen into the well/tube.

3 Sterilize the instruments (forceps, scissors etc.) by flame-scarfing and wiping with ethanol-soaked tissue paper after each sample or use a glass microsphere steriliser like Steri 250.

4 Incubate at 56 °C in an thermomixer with gentle shaking overnight (we use constant shaking with 300 rpm) → for more detailed information see:

Citation

Jaume-Schinkel S, Müller B, Avila-Calero S, Kukowka S, Rduch V, Mengual X (2024) . Preserving morphology while extracting DNA: a non-destructive field-to-museum protocol for slide-mounted specimens.

https://doi.org/10.3897/BDJ.12.e119448 LINK

5 DNA isolation is performed using the magnetic bead-based BioSprint 96 DNA Blood Kit or DNeasy Blood and Tissue Kits (both QIAGEN GmbH - Germany).

6 Quantity and quality check of the extracted DNA by e.g. NanoDrop Spectrophotometer, Quantus™ Fluorometer or capillary electrophoresis by Agilent Fragment Analyzer Systems.

7 Using DNeasy® Blood & Tissue kit, follow the manuals guidelines: https://www.qiagen.com/us/resources/resourcedetail?id=68f29296-5a9f-40fa-8b3d-1c148d0b3030&lang=en

8 Using BioSprint 96 DNA Blood Kit, follow the manuals guidelines: https://www.qiagen.com/us/resources/resourcedetail?id=64902c5d-9c3c-4fe3-a3f7-668c4704d9eb&lang=en

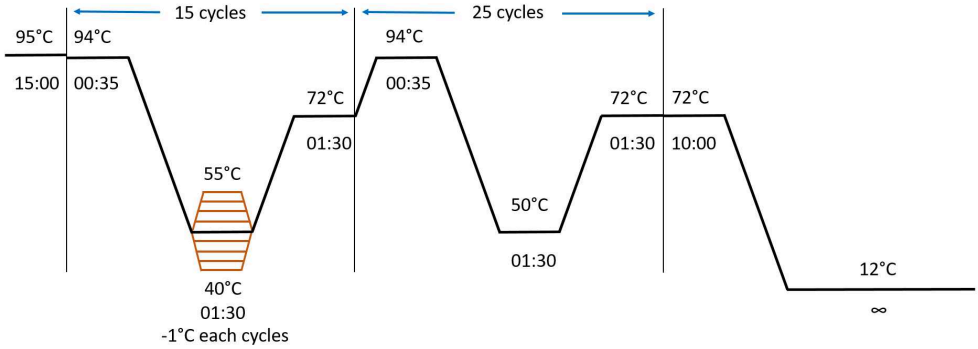
PCR chemicals & conditions

9 PCR was done with the QIAGEN Multiplex PCR Kit [Cat. No. 206143] or QIAGEN Multiplex PCR *Plus* Kit [Cat. No. 206152]. Other than the given volume of 50 µL per sample according to the instructions of the manufacturer, here we used a reduced reaction volume of 20 µL per sample:

The master mix for a 96 PCR well plates is composed as follows: 1000 µL Multiplex, 200 µL Q-Solution, 440 µL RNase-free water, 80 µL forward primer (10 pmol/µl) and 80 µL reverse primer (10 pmol/µl).
Each well/tube is filled with 18 µL PCR Mastermix and 2 µL DNA.

10 PCR: Initial 00:15:00 at 95 °C, followed by 94 °C denaturation for 00:00:35, 55 °C annealing for 00:01:30 and 72 °C elongation for 00:01:30. Denaturation, annealing and elongation are repeated 15 times, but the annealing temperature is decreased by 1 °C in each cycle. After reaching an annealing temperature of 40 °C, there is no further reduction in temperature. Denaturation, annealing and elongation are repeated 25 times as described above, with a constant annealing temperature of 50 °C. A final elongation at 72 °C for 00:10:00 terminates the PCR and it is then cooled to 12 °C permanently. 28m 35s

Touchdown PCR conditions according to GBOL and GBOL III: Dark Taxa



Conditions for touchdown PCR with forward primers dEURYT-BRBM2, LCO1490-JJ, LCO1490-JJ2 and LCO1490-JJ3 according to GBOL and GBOL III: Dark Taxa

11 Verification of the PCR products by gel electrophoresis.

Protocol references

Citation

Jonas J. Astrin and Peter E. Stüben (2008)
. Phylogeny in cryptic weevils: molecules, morphology and new genera of western Palaearctic Cryptorhynchinae (Coleoptera : Curculionidae).
Invertebrate Systematics.

https://doi.org/10.1071/IS07057 LINK

Citation

Astrin JJ, Höfer H, Spelda J, Holstein J, Bayer S, Hendrich L, Huber BA, Kielhorn KH, Krammer HJ, Lemke M, Monje JC, Morinière J, Rulik B, Petersen M, Janssen H, Muster C
(2016). Towards a DNA Barcode Reference Database for Spiders and Harvestmen of Germany.

https://doi.org/10.1371/journal.pone.0162624 LINK

Citation

Björn Rulik, Jonas Eberle, Laura von der Mark, Jana Thormann, Manfred Jung, Frank Köhler, Wolfgang Apfel, Andreas Weigel, Andreas Kopetz, Jonas Köhler, Frank Fritzlar, Matthias Hartmann, Karl Hadulla, Joachim Schmidt, Thomas Hörren, Detlef Krebs, Florian Theves, Ute Eulitz, André Skale, Dirk Rohwedder, Andreas Kleeberg, Jonas J. Astrin, Matthias F. Geiger, J. Wolfgang Wägele, Peter Grobe, Dirk Ahrens
(2017). Using taxonomic consistency with semi-automated data pre-processing for high quality DNA barcodes.
Methods in Ecology and Evolution.

https://doi.org/10.1111/2041-210X.12824 LINK

Citation

Jafari S, Müller B, Rulik B, Rduch V, S Peters R (2023)
. Another crack in the Dark Taxa wall: a custom DNA barcoding protocol for the species-rich and common Eurytomidae (Hymenoptera, Chalcidoidea).

https://doi.org/10.3897/BDJ.11.e101998 LINK

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Jaume-Schinkel S, Müller B, Avila-Calero S, Kukowka S, Rduch V, Mengual X (2024)
. Preserving morphology while extracting DNA: a non-destructive field-to-museum protocol for slide-mounted specimens.

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Citations

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Step 4

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