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Amplification of cytb gene of Plasmodium spp. in blood spots from wild animals

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We use this collection and it's working

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Abstract

Amplification of the Plasmodium mitochondrial cytochrome oxidase b gene (cytb) for detection of the Plasmodium genus in wildlife samples.

Guidelines

A) To detect *Plasmodium* spp. in humans and non human primates use the primers:

Procedure:
ROUND 1 PCR

1. Hydrate the primers with water without DNAases and dilute the primers for Plasmodium spp. (DW2 and DW4) to a concentration of 5uM.

Note

Final effective concentration in PCR is 0.5 µM for 20uL volume.

2. Volumes for the pcr mix for 1 reaction (1 rx) were:

Primer DW2 [5uM]: 2 µL
Primer DW4 [5uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 1 µL
Sample DNA: 5 µL

3. PCR conditions:

94°C --- 00:03:00
94°C --- 00:00:20
62°C --- 00:00:30
72°C --- 00:01:00
The last 3 steps are repeated for 40 cycles.
72°C --- 00:10:00
4°C --- store at this temperature until electrophoresis

Electrophoresis
4. Add 15 ul of PCR product to each well of the agarose gel. Then run on a 1.5% agarose gel prepared with TAE buffer at 95 volts for 00:40:00

Note

Before nested PCRs are performed, dilute the PCR products for Plasmodium spp. (DW2 and DW4) by 20-fold, i.e. 2ul of PCR product in 38ul of DNAase-free water. Then use this dilution as template DNA for the following nested PCR.

B) nested PCR cytb in humans and non human primates use the primers:

ROUND 2 PCR

1.Hydrate the primers with water without DNAases and dilute the primers for *Plasmodium* spp. (FP3 and RP3) to a concentration of 5uM.

Note

Final effective concentration in PCR is 0.5 µM for 20uL volume.

2.Volumes for the pcr mix for 1 reaction (1 rx) were:

FP3 primer [5uM]: 2 µL
RP3 Primer [5uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 4 µL
Diluted PCR product (1/20): 2 µL

3.PCR conditions:

94°C --- 00:03:00
94°C --- 00:00:20
52°C --- 00:00:30
72°C --- 00:01:00
The last 3 steps are repeated for 40 cycles

72°C --- 00:10:00

4°C --- store at this temperature until electrophoresis

Electrophoresis

4. Add 15 ul of PCR product to each well of the agarose gel. Then run on a 2% agarose gel prepared with TAE buffer at 95 volts for 00:45:00 . Visualize bands under UV or blue light. Expected size: ~776 bp.

Materials

- DW2 and DW4 primers
- Platinum II Hot Start PCR Master Mix (2X)
- Nuclease-free water
- Template DNA (from blood spot samples)
- PCR tubes
- Thermal cycler
- Agarose, TAE buffer
- Gel electrophoresis system
- DNA stain
- DNA ladder (100 bp)

Before start

Materials

- DW2 and DW4 primers
- Platinum II Hot Start PCR Master Mix (2X)
- Nuclease-free water
- Template DNA (from blood spot samples)
- PCR tubes
- Thermal cycler
- Agarose, TAE buffer
- Gel electrophoresis system
- DNA stain
- DNA ladder (100 bp)

To detect *Plasmodium* spp. in humans and non human primates use the primers:
ROUND 1

DW2: 5'-TAA TGC CTA GAC GTA TTC CTG ATT ATC CAG-3'
DW4: 5'-TGT TTG CTT GGG AGC TGT AAT CAT AAT GTG-3'

Nested PCR cytb in humans and non human primates use the primers:
ROUND 2

FP3: 5'-TAT ATA ACT TAT TTT TTG ATA TG-3'
RP3: 5'-GTR ATW GCA TTA TCT GGA TGT GA-3'

For the PCR Mix (conventional and nested) we used the **Platinum™ II Hot-Start PCR Master Mix (2X)** from Invitrogen.

Files

SEARCH

Protocol references

MARTINSEN, E. S., PAPERNA, I. & SCHALL, J. J. Morphological versus molecular identification of avian Haemosporidia: an exploration of three species concepts. *Parasitology* **133**, 279–288 (2006).

Acknowledgements

This protocol has been cited in our peer-reviewed publication:
https://doi.org/10.1016/j.meegid.2024.105554

It has now been updated to include step-by-step instructions, in compliance with protocols.io formatting requirements.