Jun 10, 2025

Amplification of *P. vivax/P. malariae/P. falciparum cox3* gene in humans and non human primates

DOI
dx.doi.org/10.17504/protocols.io.4r3l2ooxpv1y/v1

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Collection Citation: Gabriela Ulloa Urizar 2025. Amplification of P. vivax/P. malariae/P. falciparum cox3 gene in humans and non human primates. **protocols.io https://dx.doi.org/10.17504/protocols.io.4r3l2ooxpv1y/v1**

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

Created: February 14, 2022

Last Modified: June 10, 2025

Collection Integer ID: 58167

Keywords: Plasmodium spp., zoonosis, malaria, wild animals, non human primates

Funders Acknowledgements:
ERANet-LAC Research projects
Grant ID: N°ERANet17/HLH-0271-WildEmerg
CONCYTEC-FONDECYT
Grant ID: Nº136-2018-FONDECYT
Instituto de Salud Carlos II
Grant ID: NºAC18/00054
Fundació Autònoma Solidària
Grant ID: NºFS-XXXVI-FS1
Global GreenGrants Funds
Grant ID: Nº2020-4226
CNPq
Grant ID: SWE2019(201546/2020-5)

Disclaimer

It is not an original protocol, it is a protocol adapted from the methodology used by Isozumi R, et al, Improved detection of malaria cases in island settings of Vanuatu and Kenya by PCR that targets the Plasmodium mitochondrial cytochrome c oxidase III (cox3) gene, Parasitology International (2014),http://dx.doi.org/10.1016/j.parint.2014.09.006

Abstract

Amplification of *Plasmodium* mitochondrial cytochrome c oxidase III (cox3) gene to detection *Plasmodium* spp. by conventional PCR. Also, detection of *Plasmodium falciparum*, *Plasmodium vivax/simium* and *Plasmdium malariae/brasilianum* in humans population and non human primates (NHP) by Nested-PCR.

Guidelines

ROUND 1 PCR

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

1. Hydrate the primers with water without DNAases and dilute the primers for *Plasmodium* spp. (spp1F and spp1R) to a concentration of 2uM.

Note

Final effective concentration in PCR is 0.2 µM for 20uL volume.

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

Primer spp1F [2uM]: 2 µL
Primer spp1R [2uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 1 µL
Sample DNA: 5 µL

3. PCR conditions:

96°C --- 00:01:00
96°C --- 00:00:10
63°C --- 00:01:00
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% agarose gel prepared with TAE buffer and DNA stain at 95 volts for 00:30:00

Note

Before nested PCRs are performed, dilute the PCR products for Plasmodium spp. (spp1F and spp1R) by 50-fold, i.e. 2ul of PCR product in 98ul of DNAase-free water. Then use this dilution as template DNA for the following nested PCRs.

ROUND 2 PCR

B) To detect *P. falciparum* in humans and non human primates use the primers:

1.Hydrate the primers with water without DNAases and dilute the primers for ***P. falciparum*** **(FALF and FALR)** to a concentration of 4uM.

Note

Final effective concentration in PCR is 0.4 µM for 20uL volume.

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

FALF primer [4uM]: 2 µL
FALR Primer [4uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 4 µL
Diluted PCR product: 2 µL

3.PCR conditions:

96°C --- 00:01:00
96°C --- 00:00:10
54°C --- 00:01:30
72°C --- 00:01:00
The last 3 steps are repeated for 30 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 and DNA stain at 95 volts for 00:35:00

C) To detect *P. vivax/P. simium* in humans and non human primates use the primers:

1.Hydrate the primers with water without DNAases and dilute the primers for ***P. vivax*** **(VIVF and VIVR)** to a concentration of 4uM.

Note

Final effective concentration in PCR is 0.4 µM for 20uL volume.

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

VIVF primer [4uM]: 2 µL
VIVR Primer [4uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 4 µL
Diluted PCR product: 2 µL

3.PCR conditions:

96°C --- 00:01:00
96°C --- 00:00:10
54°C --- 00:01:30
72°C --- 00:01:00
The last 3 steps are repeated for 30 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 and DNA stain at 95 volts for 00:35:00

D) To detect *P. malariae/P. brasilianum* in humans and non human primates use the primers:

1.Hydrate the primers with water without DNAases and dilute the primers for *P. malariae* (MALF and MALR) to a concentration of 4uM.

Note

Final effective concentration in PCR is 0.4 µM for 20uL volume.

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

MALF primer [4uM]: 2 µL
MALR Primer [4uM]: 2 µL
Master Mix (2X): 10 µL
Free water: 4 µL
Diluted PCR product: 2 µL

3.PCR conditions:

96°C --- 00:01:00
96°C --- 00:00:10
58°C --- 00:01:30
72°C --- 00:01:00
The last 3 steps are repeated for 30 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 and DNA stain at 95 volts for 00:35:00

Materials

- 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

- 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**:

SPP1F: 5'-CTC GCC ATT TGA TAG CGG TTA ACC-3'
SPP1R: 5'-CCT GTT ATC CCC GGC GAA CCT TC-3'

if the band you get is very faint you can do a nested PCR with the following primers:
(in my case it wasn't necessary)

SPP2F: 5'-GTA AAC ATG CWG TCA TAC ATG ATG CAC-3'
SPP2R: 5'-CCC CGG CGA ACC TTC TTA CCG T-3'

Primers for *P. falciparum* detection:

FALF: 5'-GAA CAC AAT TGT CTA TTC GTA CAA TTA TTC-3'
FALR: 5'-CTT CTA CCG AAT GGT TTA TAA ATT CTT TC-3'

Primers for *P. vivax/P. simium* detection:

VIVF: 5'-CTA GCT TTT AAC ACA ATA TTA TTG TCT ATA C-3'
VIVR: 5'-GTT CTT TTT CTA TTC AGA ATA ATG AAT ATA T-3'

Primers for *P. malariae/P. brasilianum* detection:

MALF: 5'-CTA GCT TTG TAC ACA AAT TAA TTC GTC TAC-3'
MALR: 5'-CTT TAT AAG AAT GAT AGA TAT TTA TGA CAT A-3'

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

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Files

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Protocol references

Isozumi, R. *et al.* Improved detection of malaria cases in island settings of Vanuatu and Kenya by PCR that targets the Plasmodium mitochondrial cytochrome c oxidase III (cox3) gene. *Parasitol Int* **64**, 304–308 (2015).