S5. Amplification of *Plasmodium falciparum* dihydrofolate reductase (*dhfr*) and cytochrome b (*cytb*) genes and *Plasmodium vivax* putative multidrug resistance-associated protein 1 (mrp1) gene by polymerase chain reaction (PCR)

To increase analytical sensitivity, nested PCR (n-PCR) or semi-nested PCR (hn-PCR) methodologies were used. These approaches consisted in two sequentially performed PCR reactions, in which an aliquot of the product from the first reaction is used as analyte in the second amplification reaction.

PCR amplifications were performed in a final volume of 25 µL using 2 µL of purified DNA. Reaction mixtures contained: 0.04 U/µL DNA polymerase (Platinum™ SuperFi™ DNA Polymerase), 0.2 µM of each primer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs. The reaction volume was adjusted using nuclease-free, water. One microliter (1 µL) of the first PCR was used as template in the second amplification reaction.

P. falciparum mitochondrial cytochrome-b (*cytb*) gene amplification

The amplification of *cytb* gene was performed by hn-PCR, using the outer primers PfCytoB1_F and PfCytoB2_R and the inner primer PfCytoB3_F. The thermal profile consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of: 95 °C for 30 s, 56 °C for 30 s and, 72 °C for 60 s. Finally, the reaction proceeded for 5 min at 72 °C followed by a hold at 4 °C. The first amplification reaction used the primers: PfCytoB1_F and PfCytoB2_R (expected size 937 bp) and the second PCR used the primers PfCytoB2_R and PfCytoB3_F (expected size 371 bp).

P. falciparum nuclear dihydrofolate reductase (*dhfr*) gene amplification

Amplification *dhfr* gene, located on chromosome 4, was accomplished by hn-PCR using the outer primers PfDHFR_F3 and PfDHFR_M5 and the inner primer PfDHFR_AL3504. The cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of: 95 °C for 30 s, 52 °C for 60 s and, 72 °C for 30 s. The reaction continued for 5 min at 72 °C and ended with a hold at 4 °C. The first PCR used the primers PfDHFR_F3 and PfDHFR_M5 (expected size 653 bp) and the second reaction used the primers PfDHFR_AL3504 and PfDHFR_M5 (expected size 645 bp).

P. vivax putative multidrug resistance-associated protein 1 (*mrp1*) gene amplification

The *mrp1* gene was amplified n-PCR using outer primers Pvmrp1R1 and Pvmrp1F1 and the inner primers Pvmrp1R2 and Pvmrp1F2. The cycling profile consisted of an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of: 95 °C for 30 s, annealing temperature for 30 s and, 72 °C for 75 s. The reaction ended with an extension for 5 min at 72 °C followed by a hold at 4 °C. The annealing temperature was set at 60 °C (expected size 1,257 bp) and 58°C (expected size 1,163 bp for the first and second reaction respectively).

Analysis of amplification reactions

PCR products were analyzed by electrophoresis in 2% (w/v) agarose gels dissolved in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA) and copolymerized with GelRed Nucleic Acid Gel Stain (Biotium). Fragments were resolved at 120 V for 60 minutes in TAE buffer followed by photodocumentation on a UV transilluminator equipped with a digital camera.

Amplicons, at the expected size, were purified from agarose slices using QIAquick Gel Extraction Kit (QIAGEN) following the manufacturer's recommendations. Purified amplicons were submitted to Sanger dideoxy sequencing.

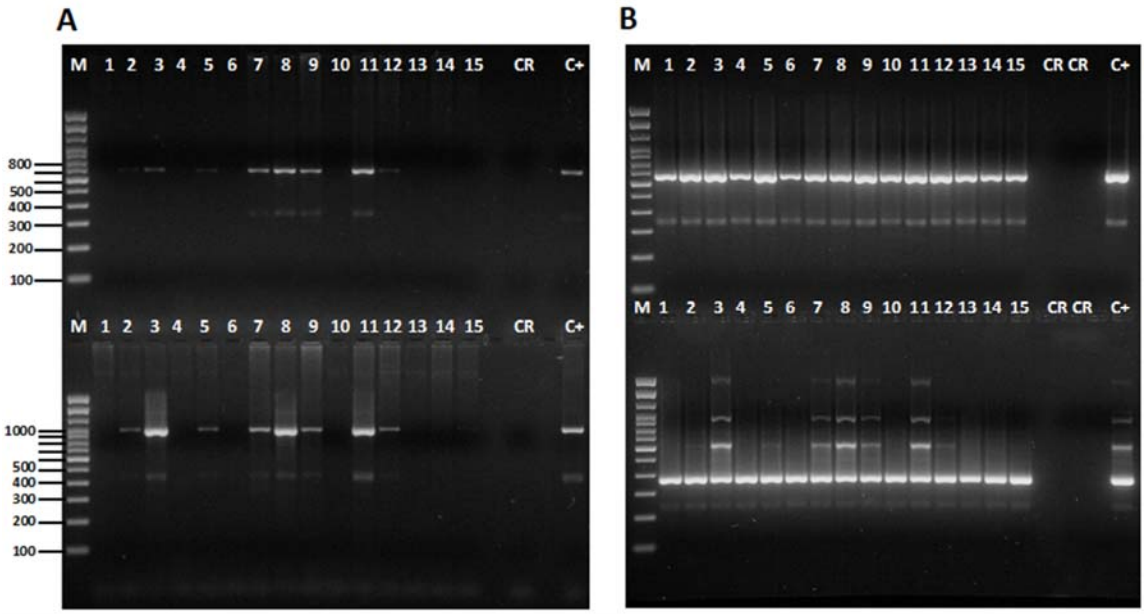

Figure 1. Analysis of amplification products of *P. falciparum* dihydrofolate reductase (*dhfr*) (upper panel) and *cytb* (lower panel) genes. Ten microliters of the PCR reaction were examined by electrophoresis in 2% agarose gels at 120 V for 60 min. **A**) Analysis of the products from the first amplification reaction. **B**) Analysis of the products of the second amplification reaction. M: molecular size marker, sizes in bp are shown on the left; CR: non template control, C+: positive control. Samples:1, 19-MALP-7; 2, 19-MALP-20; 3, 19-MALP-24; 4, 19-MALP-25; 5, 19-MALP-31; 6, 19-MALP-55; 7, 20-MALP-6; 8, 20-MALP-14; 9, 21-MALP-5; 10, 21-MALP-14; 11, 21-MALP-15; 12, 21-MALP-20; 13, 21-MALP-24; 14, 21-MALP-29 and 15, 21-MALP-30.

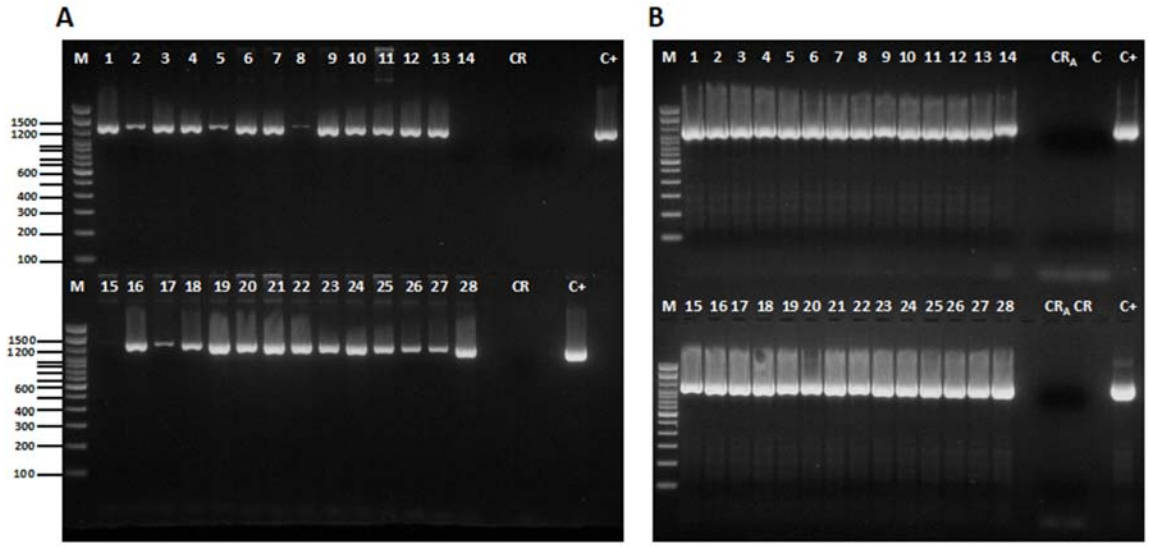

Figure 2. Analysis of amplification products of the *P. vivax* putative multidrug resistance-associated protein 1 (*mrp1*) gene. Ten microliters of the PCR reaction were examined by electrophoresis in 2% agarose gels at 120 V for 60 min. **A**) Analysis of the products from the first amplification reaction. **B**) Analysis of the products of the second amplification reaction. M: molecular size marker, sizes in bp are shown on the left; CR: non template control, CR_A: non template control from the first reaction; C+: positive control. Samples: **Upper wells**: 1, 19-MALP-9; 2, 19-MALP-11; 3, 19-MALP-15; 4, 19-MALP-16; 5, 19-MALP-19; 6, 19-MALP-28; 7, 19-MALP-30; 8, 19-MALP-33; 9, 19-MALP-34; 10, 19-MALP-35; 11, 19-MALP-44; 12, 19-MALP-45; 13, 19-MALP-49; 14, 19-MALP-50; **Lower wells**: 15, 19-MALP-52; 16, 19-MALP-56; 17, 19-MALP-57; 18, 19-MALP-63 19, 19-MALP-66 20, 20-MALP-9; 21, 20-MALP-10; 22, 21-MALP-6; 23, 21-MALP-9; 24, 21-MALP-12; 25, 21-MALP-28 26, 21-MALP-33 27, 21-MALP-34 and 28, 21-MALP-38.