

Supplementary table 1: RT-PCR and nested PCR primer sequences

Gene	Primer	Sequence 5'-3'	Bases	Reference
18s RNA	18s Pf F	GTAATTGGAATGATAGGAATTACAAGGT	29	Hermsen et al.2001
	18s Pf R	TCAACTACGAACGTTTAACTGCAAC	26	
	18s Pf probe	AACAATTGGAGGGCAAG	17	
dhps	dhps_extF	CTAACACGTGCTGTTCAAAGAATG	23	Designed in this study
	dhps_extR	GTGGATACTCATCATATACATG	22	
	dhps_intF	[MID tag]-GATAATGAAGGTGCTAGTGT	21	
	dhps_intR	GAGTTTAATAGATTGATCATG	21	
k13	k13_extF	CTTAACCTCTTAAGAAATCCG	21	Designed in this study
	k13_extR	AGGCATATGGAAATTGTTCCC	21	
	k13_intF	[MID tag]-GAAGCCTGTTGAAAGAACGAG	22	
	k13_intR	CACCATTAGTTCCACCAATGAC	22	
dhfr	dhfr_extF	CTCCTTTTATGATGGAACAACT	23	Designed in this study
	dhfr_extR	CTTTCTAAAAATTCTTGATAAAC	24	
	dhfr_intF	[MID tag]-GAACAAGTCTGCGACGTTTC	21	
	dhfr_intR	CTTGATAAAACAACCGAACCTCC	22	
mdr1	mdr1_extF	GAGTTGAACAAAAAGAGTACCG	22	Designed in this study
	mdr1_extR	CCATTAAAGCCTTCTATAATGG	24	
	mdr1_intF	[MID tag]-GTATGTGCTGTATTATCAGG	20	
	mdr1_intR	GTATTGTTATTATATAACAAAG	22	
crt	crt76_F	GGTGGAGGTTCTGTCTTGG	20	Okombo et al. 2014
	Crt76_R	ATAAAAGTTGAGTTTCGGATG	22	
	crt145_extF	CTTATGACCTTTTAGGAACG	21	Designed in this study
	crt145_extR	CTTAATATTAAAAAGCAGAAGAAC	24	
	crt145_intF	AGGAACGACACCGAAGCT	18	
	crt145_intR	GCAGAAGAACATATTAATAGG	21	
ama1	ama1_ext_F	GGAGAAGATGCTGAAGTAGCTGG	23	Designed in this study
	ama1_ext_R	GGTATATCTTCACAATTCCATCG	24	
	ama1_int_F	[MID tag]-GAAATGCCAGTATTGGTAAAGG	24	
	ama1_int_R	CCCATAATCCGAATTTCGCATTC	23	

Supplementary Table 2: Multiplex identifier (MID) 10bp sequence sets

MID tag	sequence	MID tag	sequence
MID-1	ACGAGTGC GT	MID-9	TCTCTATGCC G
MID-2	ACGCTCGACA	MID-10	TGATACGTCT
MID-3	AGACGCAC TC	MID-11	CATA GTAGTG
MID-4	AGCACTGTAG	MID-12	CGAGAGATAC
MID-5	ATCAGACACG	MID-13	ATACGACGTA
MID-6	ATATCGCGAG	MID-14	TCACGTACTA
MID-7	CGTGTCTCTA	MID-15	CGTCTAGTAC
MID-8	CTCGCGTGTC	MID-16	TCTACGTAGC

Amplicons were generated using forward primers tagged on the 5' end with the above listed MIDs. This enabled the unique identification of sequences from each sample during sequence data demultiplexing.

References

Okombo J, Kamau AW, Marsh K, Sutherland CJ, Ochola-Oyier LI. Temporal trends in prevalence of Plasmodium falciparum drug resistance alleles over two decades of changing antimalarial policy in coastal Kenya. *Int J Parasitol Drugs Drug Resist.* 2014. 4(3):152-63.

Hermsen CC, Telgt DSC, Linders EHP, Van De Locht LATF, Eling WMC, Mensink EJBM, et al. Detection of Plasmodium falciparum malaria parasites in vivo by real-time quantitative PCR. *Mol Biochem Parasitol.* 2001. 118(2):247–51.