Supplemental Table 1

Gene	**Chromosome**	**Genomic Coordinates**	**Target Codons**	**Primer**	**Final Concentrations**	**Thermocycler**	**Reference**
crt	7	Start: 403484 End: 403690	72S 74I 75E 76T	F: AACAGATGGCTCACGTTTAGG R: TGTGAGTTTCGGATGTTACAAAA	1. 10X AccuPrime™ PCR Buffer II: 1x 2. AccuPrime™ HiFi Taq Polymerase: 0.2 U 3. Forward Primer: 800 nM 4. Reverse Primer: 800 nM 5. BSA: 0.05 mg/mL	1. 95°C for 2 min 2. 35 cycles of 95°C for 30 secs, 50°C for 30 secs, and 68°C for 1 min 3. 68°C for 10 min	1
kelch *	13	Start: 1724994 End: 1725490	539T 580Y	F: AGGCTTTATTTGAAACTGAGGTGT R: TCTGGTACACCATTTAGAAATTGCC	1. 10X AccuPrime™ PCR Buffer II: 1x 2. AccuPrime™ HiFi Taq Polymerase: 0.2 U 3. Forward Primer: 200 nM 4. Reverse Primer: 200 nM 5. BSA: 0.05 mg/mL	1. 95°C for 2 min 2. 35 cycles of 95°C for 30 secs, 58°C for 30 secs, and 68°C for 1 min 3. 68°C for 10 min	*
mdr1	5	Start: 957959 End: 958493	86Y 184F	Outer F: AGGTTGAAAAAGAGTTGAAC Outer R: ATGACACCACAAACATAAAT Inner F: ACAAAAAGAGTACCGCTGAAT Inner R: AAACGCAAGTAATACATAAAGTC	*Outer & Nested Step* 1. 10X AccuPrime™ PCR Buffer II: 1x 2. AccuPrime™ HiFi Taq Polymerase: 0.2 U 3. Forward Primer: 200 nM 4. Reverse Primer: 200 nM 5. BSA: 0.05 mg/mL	*Outer*/***Nested*** 1. 94°C for 3 min 2. 30 cycles of 94°C for 30 secs, 55°C/**60°C** for 30 secs, and 68°C for 1 min 3. 68°C for 5 min	2**
mdr1	5	Start: 961527 End: 961847	1246Y	F: TTCAAACCAATCTGGATCTGC R: TTCTCTGTTTTTGTCCACCTGA	1. 10X AccuPrime™ PCR Buffer II: 1x 2. AccuPrime™ HiFi Taq Polymerase: 0.2 U 3. Forward Primer: 400 nM 4. Reverse Primer: 400 nM 5. BSA: 0.05 mg/mL	1. 95°C for 15 min 2. 40 cycles of 94°C for 30 secs, 55.5°C for 30 secs, and 68°C for 1 min 3. 68°C for 10	3***

Supplemental Table 1. Summary of the genomic targets, primers, recipes, and thermocycler conditions for the amplicons generated in this study. The genomic coordinates are 1-based and are base off of the Pf3D7 version 3 reference genome. The forward (F) and reverse primer (R) are listed as 5' to 3'. Primer sequences for *crt* were adapted from Juliano *et al.* 2007 and the thermocycler conditions were adapted for the AccuPrime™ HiFi Taq Polymerase. Similarly, the primer sequences for the mdr1 amplicon targeting loci 86 and 184 were taken from Humphreys *et al.* 2007 and the thermocycler conditions were adapted for the AccuPrime™ HiFi Taq Polymerase. Finally, the primers for the *mdr1* amplicon targeting loci 1246 were adapted from Kwansa-Bentum *et al.* 2011. Each PCR reaction used 5 µL of template DNA and had a total volume of 20 µL.

*The primers for the *kelch* amplicon were designed using the *kelch* reference sequence from PlasmoDB (accessed May 2, 2017) and the Primer3/BLAST engine from NCBI. The forward and reverse primers were requested to fall between the local coordinates of 1415-1515 and 1838-1938, respectively. Default settings were used with the exception of excluding *Homo sapiens* from Refseq mRNA under the heading Primer Pair Specificity Checking Parameters.

** Thermocycler conditions were adapted from original protocol for the AccuPrime™ HiFi Taq Polymerase.

*** Primers were adapted for this study and thermocycler conditions were adapted for AccuPrime™ HiFi Taq Polymerase.

Supplemental Table 2

Pool	Gene Target	Frequency	Haplotype	Haplotype Count	SNP Amplicon Position	SNP	SNP Gene Position	Codon	Referent AA	Alternate AA	Mutant Type
Pool 1	crt	0.52	PfCRT.0	82							
Pool 1	crt	0.48	PfCRT.1	75	115	T	222	74	M	I	N
Pool 1	crt	0.48	PfCRT.1	75	116	G	223	75	N	E	N
Pool 1	crt	0.48	PfCRT.1	75	118	A	225	75	N	E	N
Pool 1	crt	0.48	PfCRT.1	75	120	C	227	76	K	T	N
Pool 1	k13	1.00	K13.0	91							
Pool 1	mdrE	1.00	MDR1E.0	445							
Pool 1	mdrL	1.00	MDR1L.0	208228							
Pool 2	crt	0.64	PfCRT.1	2502	115	T	222	74	M	I	N
Pool 2	crt	0.64	PfCRT.1	2502	116	G	223	75	N	E	N
Pool 2	crt	0.64	PfCRT.1	2502	118	A	225	75	N	E	N
Pool 2	crt	0.64	PfCRT.1	2502	120	C	227	76	K	T	N
Pool 2	crt	0.36	PfCRT.0	1416							
Pool 2	k13	1.00	K13.0	19666							
Pool 2	mdrE	0.54	MDR1E.0	349							
Pool 2	mdrE	0.46	MDR1E.1	295	460	T	551	184	Y	F	N
Pool 2	mdrL	1.00	MDR1L.0	14881							
Pool 3	crt	0.56	PfCRT.0	93760							
Pool 3	crt	0.44	PfCRT.1	74736	115	T	222	74	M	I	N
Pool 3	crt	0.44	PfCRT.1	74736	116	G	223	75	N	E	N
Pool 3	crt	0.44	PfCRT.1	74736	118	A	225	75	N	E	N

Pool 3	crt	0.44	PfCRT.1	74736	120	C	227	76	K	T	N
Pool 3	k13	1.00	K13.0	302947							
Pool 3	mdrE	0.83	MDR1E.1	25516	460	T	551	184	Y	F	N
Pool 3	mdrE	0.11	MDR1E.0	3331							
Pool 3	mdrE	0.06	MDR1E.3	1974	140	A	231	77	V	V	S
Pool 3	mdrL	1.00	MDR1L.0	187805							
Pool 4	crt	0.83	PfCRT.0	145205							
Pool 4	crt	0.17	PfCRT.1	29439	115	T	222	74	M	I	N
Pool 4	crt	0.17	PfCRT.1	29439	116	G	223	75	N	E	N
Pool 4	crt	0.17	PfCRT.1	29439	118	A	225	75	N	E	N
Pool 4	crt	0.17	PfCRT.1	29439	120	C	227	76	K	T	N
Pool 4	k13	1.00	K13.0	213592							
Pool 4	mdrE	0.62	MDR1E.0	15278							
Pool 4	mdrE	0.38	MDR1E.1	9384	460	T	551	184	Y	F	N
Pool 4	mdrL	1.00	MDR1L.0	88103							
Pool 5	crt	0.54	PfCRT.0	159398							
Pool 5	crt	0.46	PfCRT.1	133898	115	T	222	74	M	I	N
Pool 5	crt	0.46	PfCRT.1	133898	116	G	223	75	N	E	N
Pool 5	crt	0.46	PfCRT.1	133898	118	A	225	75	N	E	N
Pool 5	crt	0.46	PfCRT.1	133898	120	C	227	76	K	T	N
Pool 5	k13	1.00	K13.0	236833							
Pool 5	mdrE	0.82	MDR1E.0	55800							
Pool 5	mdrE	0.18	MDR1E.1	12317	460	T	551	184	Y	F	N
Pool 5	mdrL	1.00	MDR1L.0	284040							
Pool 6	crt	0.91	PfCRT.1	264462	115	T	222	74	M	I	N
Pool 6	crt	0.91	PfCRT.1	264462	116	G	223	75	N	E	N
Pool 6	crt	0.91	PfCRT.1	264462	118	A	225	75	N	E	N
Pool 6	crt	0.91	PfCRT.1	264462	120	C	227	76	K	T	N
Pool 6	crt	0.09	PfCRT.0	25200							

Pool 6	k13	1.00	K13.0	191824							
Pool 6	mdrE	0.58	MDR1E.1	20539	460	T	551	184	Y	F	N
Pool 6	mdrE	0.42	MDR1E.0	14708							
Pool 6	mdrL	1.00	MDR1L.0	167846							
Pool 7	crt	0.60	PfCRT.0	312661							
Pool 7	crt	0.40	PfCRT.1	207787	115	T	222	74	M	I	N
Pool 7	crt	0.40	PfCRT.1	207787	116	G	223	75	N	E	N
Pool 7	crt	0.40	PfCRT.1	207787	118	A	225	75	N	E	N
Pool 7	crt	0.40	PfCRT.1	207787	120	C	227	76	K	T	N
Pool 7	k13	1.00	K13.0	167241							
Pool 7	mdrE	0.52	MDR1E.0	4123							
Pool 7	mdrE	0.48	MDR1E.1	3823	460	T	551	184	Y	F	N
Pool 7	mdrL	1.00	MDR1L.0	65259							
Pool 8	crt	0.53	PfCRT.0	164053							
Pool 8	crt	0.47	PfCRT.1	147664	115	T	222	74	M	I	N
Pool 8	crt	0.47	PfCRT.1	147664	116	G	223	75	N	E	N
Pool 8	crt	0.47	PfCRT.1	147664	118	A	225	75	N	E	N
Pool 8	crt	0.47	PfCRT.1	147664	120	C	227	76	K	T	N
Pool 8	k13	1.00	K13.0	316202							
Pool 8	mdrE	0.56	MDR1E.1	25951	460	T	551	184	Y	F	N
Pool 8	mdrE	0.33	MDR1E.0	15305							
Pool 8	mdrE	0.10	MDR1E.2	4787	215	C	306	102	G	G	S
Pool 8	mdrL	1.00	MDR1L.0	216942							
Pool 9	crt	0.83	PfCRT.0	196471							
Pool 9	crt	0.17	PfCRT.1	40066	115	T	222	74	M	I	N
Pool 9	crt	0.17	PfCRT.1	40066	116	G	223	75	N	E	N
Pool 9	crt	0.17	PfCRT.1	40066	118	A	225	75	N	E	N
Pool 9	crt	0.17	PfCRT.1	40066	120	C	227	76	K	T	N
Pool 9	k13	1.00	K13.0	271346							

Pool 9	mdrE	0.79	MDR1E.1	49191	460	T	551	184	Y	F	N
Pool 9	mdrE	0.21	MDR1E.0	13154							
Pool 9	mdrL	1.00	MDR1L.0	247796							

Supplemental Table 2. Summary of the haplotypes for each pool and targeted as determined by the SeekDeep (V2.6.0) program. Of note, the targets mdrE and mdrL correspond to the amplicons targeting amino acid positions 86, 184 and 1246, respectively (Supplementary Table 1). The haplotype names and the number of consensus reads for each haplotype from the SeekDeep (V2.6.0) output are given under the haplotype and haplotype count columns, respectively. The relative location of the SNP within the amplicon is provided under the column SNP Amplicon position, while the relative location of the SNP within the gene is provided under the SNP gene position column. Finally, the resulting amino acid changes are described in the last three columns with N and S corresponding to a nonsynonymous and synonymous mutation, respectively.

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3. Kwansa-Bentum B, Ayi I, Suzuki T, Otchere J, Kumagai T, Anyan WK, Osei JHN, Asahi H, Ofori MF, Akao N, Wilson MD, Boakye DA, Ohta N. Plasmodium falciparum isolates from southern Ghana exhibit polymorphisms in the SERCA-type PfATPase6 though sensitive to artesunate in vitro. *Malar J*. 2011;10:187. doi:10.1186/1475-2875-10-187.