

Supplementary Information for

**Mutations in Plasmodium falciparum Actin-binding Protein Coronin Confer Reduced Artemisinin Susceptibility**

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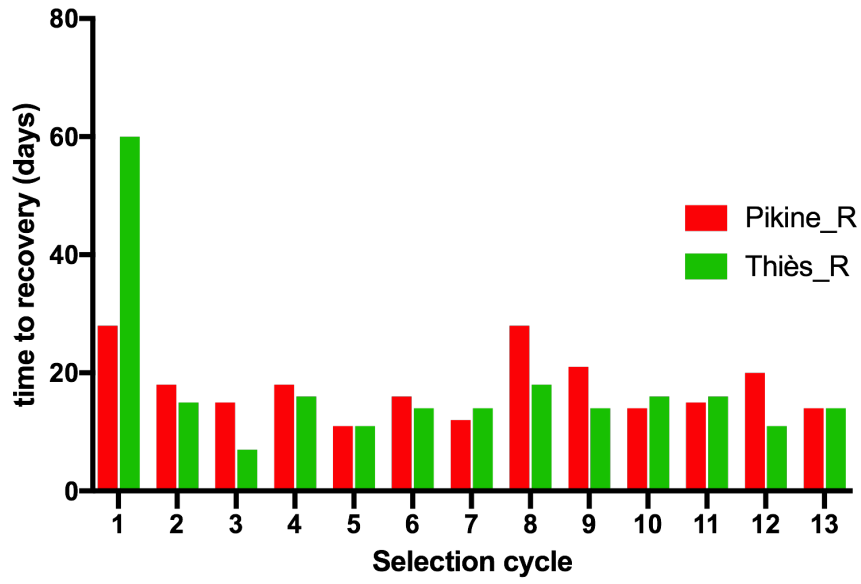
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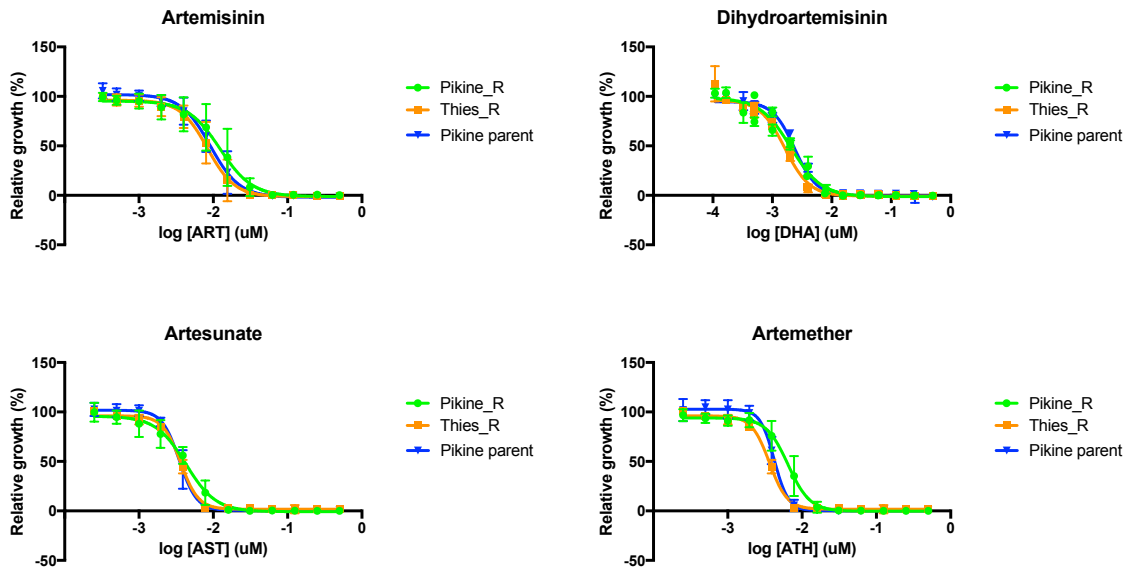
**This PDF file includes:**

Figs. S1 to S8

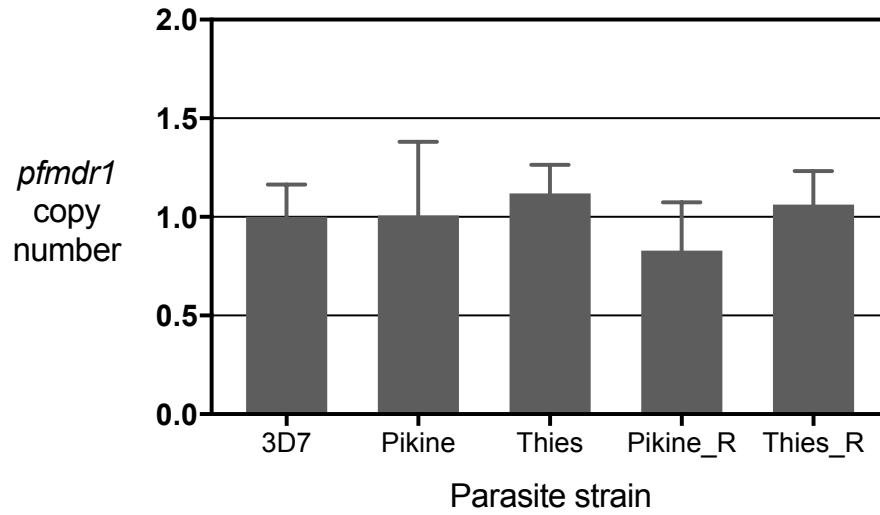
Tables S1 to S2



**Fig. S1.** Recovery period for selected lines Pikine\_R and Thiès\_R following each Dihydroartemisinin (DHA) pulse over 13 selection cycles with increasing drug concentration.

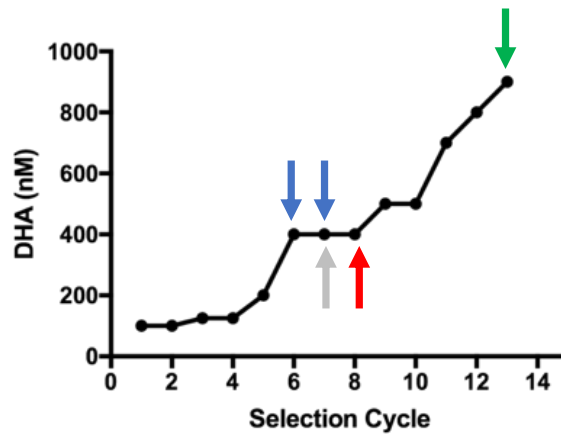


**Fig. S2.** Selected lines Pikine\_R and Thies\_R after 13 selection cycles under increasing drug pressure show no change in EC<sub>50</sub> in response to artemisinin or its derivatives. Parasite drug sensitivity was measured by 72-hour *in vitro* assays with SYBR Green. The Thies parent response is not shown as it is virtually indistinguishable from that of the Pikine parent.



**Fig. S3.** Copy number of *pfmdr1* as estimated from quantitative real-time polymerase chain reaction assays in 3D7, a laboratory line, both parental lines, and selected lines Pikine\_R and Thies\_R after 13 selection cycles under increasing drug pressure. The *pfmdr1* copy number was normalized to that of the gene for seryl tRNA.

Gene Name	Gene ID	Pikine_R	Thiès_R
conserved Plasmodium protein unknown function	PF3D7_1433800	S1054F	I575M
transporter putative	PF3D7_0209600	D1035N	
coronin	PF3D7_1251200	R100K, E107V	G50E
autophagy-related protein 7, putative (ATG7)	PF3D7_1126100	N1041S	



**Fig. S4.** Timeline of the appearance for some of the mutations after intermittent Dihydroartemisinin (DHA) pulse. The mutations were identified via Sanger sequencing after PCR amplification of the bulk gDNA. *PF3D7\_1433800* I575M in Thiès\_A was the first mutation to appear after six selection cycles followed by S1054F (*PF3D7\_1433800*) and D1035N (*PF3D7\_0209600*) in Pikine\_R. All mutations in *pfcoronin* appeared after eight selection cycles.

**A. Homology region for G50E replacement: 501bp**

GTATAGCTTGTAGTGCTGGATATATTGCTgtaaggaaaaaaaaaaaaataataataataaaaaattaaaa  
 tgtacaaatgatgcagtatatatgtatatgtatattatgtatatatattatgtatatatgtatgtacatattt  
 atgtatgtatatatatttgggtgcgctttaagattaattcattattcatatatatcgttttatgatgtttggt  
 catattatagGTACCATGGCAAGTTGAGGGTGAAGGAATGATCGGAGTTATCAGATTAGAAAAATCAAGTGA  
 GAAATCCCCCTGTAATAAAATTGAAGAGTCATACATCTCCCATCCTTGATTTGTCATTTAACCCGTGTTAT  
 AGTGAGATATTAGCTTCATGTTTCAGAAGATATGTCTATAAGAATATGGGAGATACGTCATGAGGATGAGAA  
 TGTGAATGAGGTAAAGGATCCTTTATGTATATTAATGGTCATAAGAAAAAAGTAAATATATTATCATGGA  
ATCC

Guide RNA for Cas9 plasmid

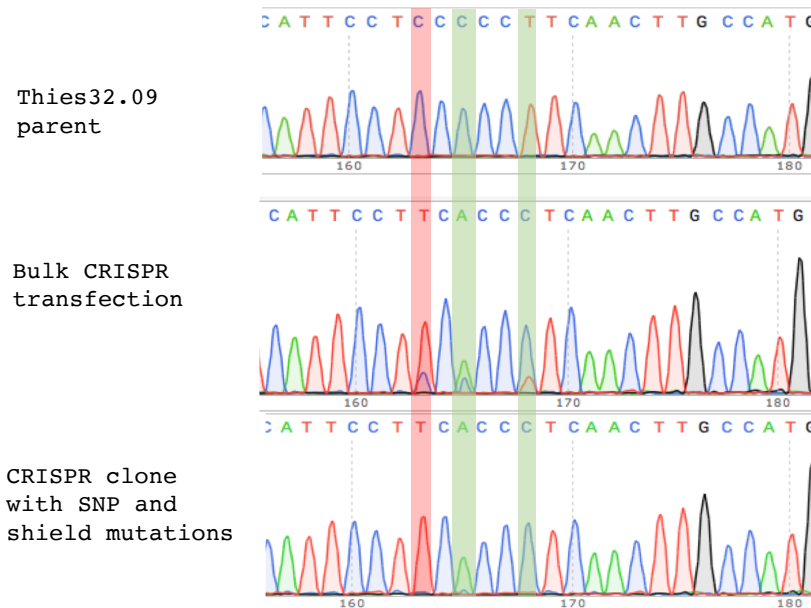
Guide 1 5' CCATGGCAAGTTGAAGGGGG

Guide 2 5' ATCCTTGATTTGTCATTTAA

WT AA Sequence: VPWQVEGGGMIGVIRLEN

Modified AA Sequence: VPWQVEGEMIGVIRLEN

**B.**



**Fig. S5. A.** CRISPR gene editing strategy for generating *pfcoronin* mutants in SenTh032.09

(Thiès) parental background. Homology region with primer sequences underlined, *pfcoronin* mutated site indicated in red, shield mutations in green, protospacer adjacent motif (PAM) sequences highlighted in yellow. Shield mutations were added for guide 1 but not guide 2. **B.** Sanger sequencing confirmation of CRISPR edited parasite gDNA from bulk transfection and an individual clone highlighting the target SNP in red and shield mutations in green.

**A.** Homology region for R100K E107V replacement: 500bp

ggtgcgctttaagattaattcattattcatatatatcgttttatatgatttgttcatattatagGTACCA  
TGGCAAGTTGAAGGGGGAGGAATGATCGGAGTTATCAGATTAGAAAATCAAGTGAGAAATCCCCCTGTAAT  
AAAATTGAAGAGTCATACATCTCCCATCCTTGATTTGTCATTTAACCCGTGTTATAGTGAGATATTAGCTT  
CATGTTTCAAGATATGTCTATAAAAATATGGGAGATTCGACATGTGGATGAGAATGTGAATGAGGTAAAG  
GATCCTTTTATGTATATTAATGGTCATAAGAAAAAAGTAAATATATTATCATGGAATCCTATGAATTATTT  
TATATTATCATCTACCTCTTTTGATTCTTCTGTTAATATATGGGATATAGAAAATGAGAAGAAAGCCTTTG  
AAATAAATATGCCAAAGAAATTAAGTTCTTTACAATGGGATATCGGTGGTAATTTATTAAGTGGAACTTGT  
CAG

Guide RNA for Cas9 plasmid

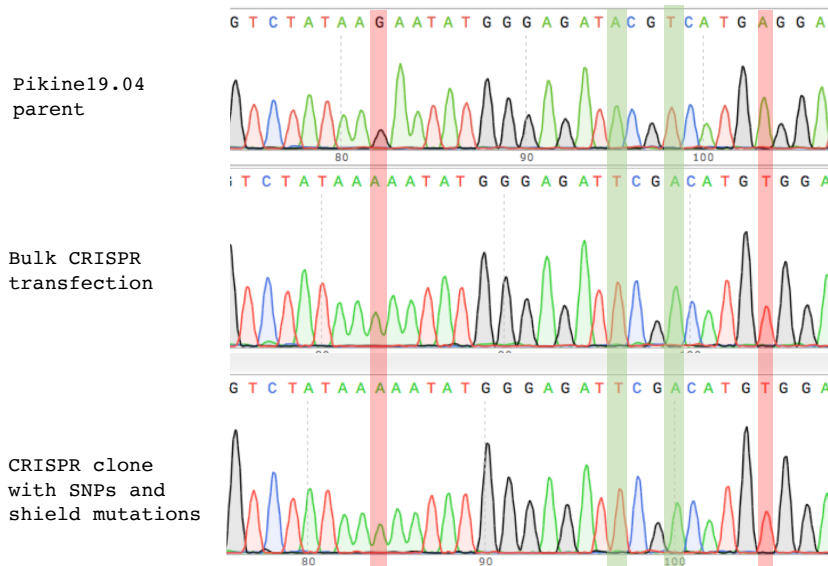
Guide 1 5' GAATATGGGAGATACGTCATG

Guide 2 5' TGAGGATGAGAATGTGAATG

WT AA Sequence: SEDMSIRIWEIRHEDENVNEV

Modified AA Sequence: SEDMSIKIWEIRHVDENVNEV

**B.**

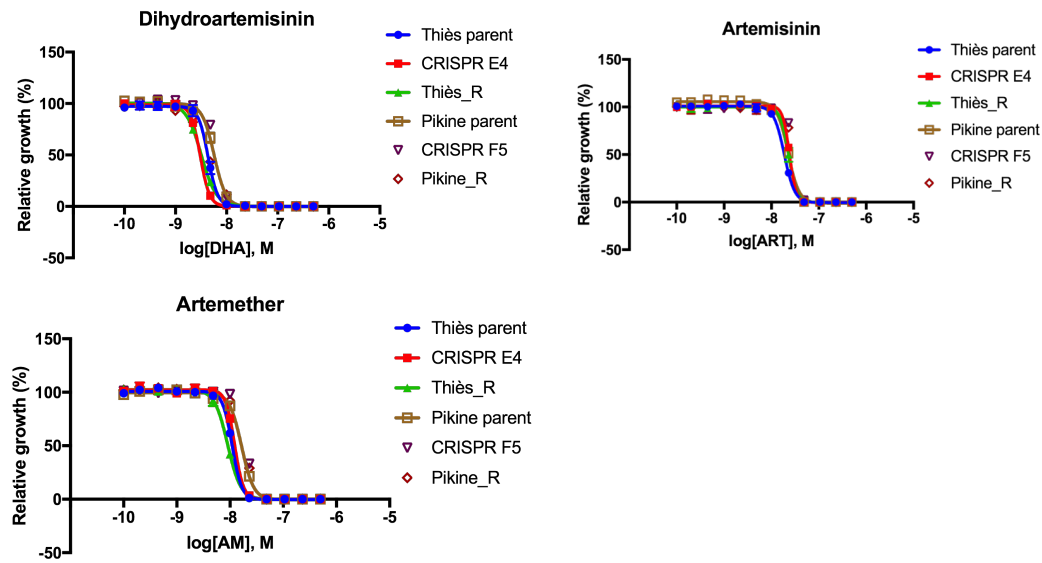


**Fig. S6. A.** CRISPR gene editing strategy for generating *pfcoronin* mutants in SenP019.04

(Pikine) parental background. Homology region with primer sequences underlined, *pfcoronin* mutated sites indicated in red, shield mutations in green, protospacer adjacent motif (PAM) sequences highlighted in yellow. Shield mutations were added for guide 1 but not guide 2.

**B.** Sanger sequencing confirmation of CRISPR edited parasite gDNA

highlighting the target SNPs in red and shield mutations in green. The bulk transfection and individual clone sequencing chromatograms were virtually indistinguishable.



**Fig. S7.** Pikine and Thiès CRISPR *pfcoronin* mutants show no change in  $EC_{50}$  in response to artemisinin (ART) and its derivatives. *Pfcoronin* mutations identified in Pikine\_R (R100K, E107V) and Thiès\_R (G50E) were knocked into their respective parents to obtain independent clones in each background Thiès (CRISPR E4) and Pikine (CRISPR F5). Parasite drug sensitivity was measured by 96-hour *in vitro* assays with SYBR Green. Only results for one CRISPR clone per background are shown as the results were virtually indistinguishable from other clones.





TgondiiTGME49_216970	ADSQLAQE-LQSEVASLKAQLTELDRLRKENEELKANGG---DTAALLQEN---QELKAN	473
PfalciparumPF3D7_1251200	SKSILIQDNNNPKKGSVMRQFTKKFTFRKKKGTVEIQGEIIGETKSSIEADFEVLESKEN	467
PgaboniPGSY75_1251200	SKSIIIQDNNNPKKDSIMRQFTKKFTFRKKKGTVEIQGEIIGETKSSIEADFEVLESKEN	435
PvivaxPVP01_1468300	FNSIIIGEEYTSKRTSIIROFTKKFTFFKKGLHNDGFSSVDSFKESV-----	454
PcoatneyiPCOAH_00051520	FNSIIIGEEYTAKRTSIIROLTKKFTFFKKGPHNDGFSSVGSFKESV-----	454
PknowlesiPKNH_1471200	FNSIIIGEDYTSKRTSIIROLTKKFTFFKKGIHNDGFSSVNSFKESV-----	454
PyoeliiPY01337	MDSILIGDPSLDKKSFIIRQFTKRFTFFKKNNNIEFNNDNDDDD-----	440
PbergheiPBANKA_1464100	MDSILIGDPCLDKKSFIIRQFTKRFTFFKKNNNIEFNNN-----	447
PchabaudiPCHAS_1466400	MDSILIGDPCLDKRSFIIRQFTKRFTFFKKNNNIEFNNNLN-----	449
	. * : : : : * : * : . .	
TgondiiTGME49_216970	AQE--LE----TLR---KENAELKAKIKELSAQSAMAVPSTSEDPQLKMRVSELAEALS	523
PfalciparumPF3D7_1251200	KKGNKLNEAPKFLFACEDVEICHLDNVDDDDYLIIVNGTNEPYEETVIKTNENENYKE--	525
PgaboniPGSY75_1251200	KNANKLNEDPKFLFASDVEVCHLDNVDDDDYLIINGANQPYEETVIKTNENEHYKE--	493
PvivaxPVP01_1468300	-----FIYPKSFK-----EKGLLTEQGGAQFSSSNSLARGAEAAARE--	491
PcoatneyiPCOAH_00051520	-----FIYPKSFK-----EKWLLTEKGGQFSGKNSLEKGPTEKEEQ--	491
PknowlesiPKNH_1471200	-----FIYPKSFK-----EKWLLTEKGGAQFSSNSLARGAEAAARE--	491
PyoeliiPY01337	-----NNHNSSESSFNINDSYQNDE--	460
PbergheiPBANKA_1464100	-----SSESFNINDSKNEG--	462
PchabaudiPCHAS_1466400	-----ISSESFNINDSEQTEE--	466
	. : :	
TgondiiTGME49_216970	NEKSTTAQLEARLRDLEGRFISAASKQAAEQEAETLKERVQELEAKNRELKTQMEA--	581
PfalciparumPF3D7_1251200	NNDS-----SIQSIRS-----NSKSIEKND-----DDNNNN---N---NDNTLQ	558
PgaboniPGSY75_1251200	NNDT-----SIQSIRS-----NSKSIDNND-----DNNNNN---NKDNDTTCQ	528
PvivaxPVP01_1468300	AAEE-----HP-----DEQ-----PDEHPDEQFPLEGEPFPCD	518
PcoatneyiPCOAH_00051520	GQQE-----EHSPLENEQPCD	507
PknowlesiPKNH_1471200	GQQT-----GQ-----QTGQET-----GQQDELEEFPLESEQPCD	521
PyoeliiPY01337	NKKT-----RF-----FVKDKD-----DDNDENQ-----NCM	482
PbergheiPBANKA_1464100	NKKT-----KI-----SIEDK-----DNDENK-----NSV	482
PchabaudiPCHAS_1466400	KKKI-----KI-----SIEDI-----DNDKKN-----SGE	486
	.	
TgondiiTGME49_216970	---HGTLH---RAATLSGLSDMKNELNEMRDFFRDILHQAQDEAA-----	621
PfalciparumPF3D7_1251200	SEEN-EEHL-----KHISII-HEENNPKNFFKNVLDNILDMMCKSTATVL	602
PgaboniPGSY75_1251200	SQEN-QQHL-----KQISSI-HEENNPKNFFKNVLDNILDMMCKSTATVL	572
PvivaxPVP01_1468300	GTSRGTSELVPRSGDPVRLARGR-TRRGSG---ANCFDALRCARLCRRREF--	565
PcoatneyiPCOAH_00051520	GYSRGTSELV-----RLSRGE-TQRKTC---VTCFDALRCARLCRRKEPQE	550
PknowlesiPKNH_1471200	GESRGTSELV-----GLSRGK-TQRAGG---SNCFNLRCARLCRGRK---	560
PyoeliiPY01337	TSGRMKEEC-VQ-TETSEFN-KI-KNDNNS---NKCLDTITCKKLFQKTN---	525
PbergheiPBANKA_1464100	ISDKVKEEY-IQ-TETSEFN-KI-EYNTNS---NKCLGTITCKKLFQKTN---	525
PchabaudiPCHAS_1466400	FSDRLKEEY-VQ-TETIEFN-KI-EENTNS---NKFLDTITCKKWFQKPN---	529
	.	

**Fig. S8.** Multiple sequence alignment of Coronin protein sequences from several *Plasmodium* species and *Toxoplasma gondii*. Highlighted in grey is the *Plasmodium falciparum* Coronin full amino acid sequence and highlighted in yellow are G50, R100 and E107 residues, which were found to be mutated in the DHA selected Senegalese parasites Thiès\_R and Pikine\_R. Analyses were done using the Clustal Omega multiple sequence alignment tool through EMBL.

**Table S1.** Summary of RSA values for all parasite lines

<b>Parasite line</b>	<b>RSA survival (%)</b>	<b>Standard error of measurement (SEM)</b>
Pikine19.04 (parent)	0.99	0.26
Pikine_R (8 cycles)	7.18	0.80
Pikine_R (13 cycles)	7.83	0.98
Pikine19.04 (parent)	0.55	0.08
Pikine CRISPR F4 (R100K, E107V)	6.75	0.91
Pikine CRISPR F5 (R100K, E107V)	9.35	1.09
Thiès32.09 (parent)	0.78	0.16
Thiès_R (8 cycles)	8.16	0.76
Thiès_R (13 cycles)	7.62	1.46
Thiès32.09 (parent)	0.48	0.09
Thiès CRISPR D4 (G50E)	5.22	1.38
Thiès CRISPR E4 (G50E)	5.30	0.72

**Table S2.** PCR resequencing primers to amplify candidate genes and *Pfkelch13*

(PF3D7\_1343700). For mutagenesis primers, shield mutations are highlighted in green and targeted mutation(s) are highlighted in red.

Primer target	Primer name	Sequence 5'-3'
PF3D7_1343700	kelch13 Fwd1	ATGGAAGGAGAAAAAGTAAAAACAAAAGC
PF3D7_1343700	kelch13 Rev1	ACGGTTTTCTAATTCTTTGTACAATCGTAC
PF3D7_1343700	kelch13 Fwd2	GAAACGGAATTAAGTGATGCTAGTGA
PF3D7_1343700	kelch13 Rev2	CCAGCATTGTTGACTAATATCTAATAATTCCA
PF3D7_1343700	kelch13 Fwd3	CATTCCCATTAGTATTTTGTATAGGTGGAT
PF3D7_1343700	kelch13 Rev3	TTATATATTTGCTATTAACGGAGTGACCAA
PF3D7_1251200	coronin fwd1	ATGTATAATGTTCCCTTAATCAAGA
PF3D7_1251200	coronin rev1	CTTGGCATATTTATTCAAAGG
PF3D7_1251200	coronin fwd2	GTGGTATAGCTTGTAGTGCT
PF3D7_1251200	coronin rev2	CTTTAAACTCCATAATTTCAATTCTC
PF3D7_1251200	coronin fwd3	AAGTTCTTTACAATGGGATATCG
PF3D7_1251200	coronin rev3	CTGTCTCATGACAGAACCCT
PF3D7_1251200	coronin fwd4	GATTTATATCCTCCTATTATTATGAG
PF3D7_1251200	coronin rev4	TACCGTTGCTGTACTTTTACAC
PF3D7_1251200	coronin fwd5	<u>GAGCTCAAGCTT</u> GTATAGCTTGTAGTGCTGGA TATATTG
PF3D7_1251200	coronin rev5	<u>GAGCTCAAGCTT</u> GGATTCCATGATAATATATTT ACTTTTTTCTTATG
PF3D7_1251200	coronin mutfw1	gGTACCATGGCAAGTTGAGGGTGAAGGAATGA TCGGAGTTATC
PF3D7_1251200	coronin mutrv1	CTGATAACTCCGATCATTCTTCAACCCTCAACT TGCCATGGTAC
PF3D7_1251200	coronin fwd6	GGTGCGCTTTAAAGATTAATTC
PF3D7_1251200	coronin rev6	CAGAAGAATCAAAAGAGGTAGATG
PF3D7_1251200	coronin seq1	CCTCATTACATTCTCATCCTCATG
PF3D7_1251200	coronin fwd7	<u>GAGCTGCTAGCGG</u> TGCGCTTTAAAGATTAATT C
PF3D7_1251200	coronin rev7	<u>GAGCTCAAGCTT</u> CTGACAAGTTCCACTTAATA AATTAC
PF3D7_1251200	coronin mutfw2	GTCTATAAAATATGGGAGATTCGCATGTGG ATGAGAATGTG

PF3D7_1251200	coronin mutrv2	CTCATTACATTCTCATCCACATGTCGAATCTC CCATATTTTATAG
PF3D7_1251200	coronin fwd8	GAAATCCCCCTGTAATAAAATTG
PF3D7_1251200	coronin rev8	CTTGTTTTCTAGGATCTATTATATGTATCTG
PF3D7_1251200	coronin seq2	CCACCGATATCCCATTGTAAAGAAC
PF3D7_0209600	PF3D7_0209600 fwd	GTACTATATATTGGAAATATAGAAACC
PF3D7_0209600	PF3D7_0209600 rev	ATGGGAAAAGAAACAATAGGAGC
PF3D7_1126100	ATG7 fwd	CAAAGGTAATGGATATACCTATGC
PF3D7_1126100	ATG7 rev	TGCTACCGTAATACCTAAAGGAG
PF3D7_1433800	PF3D7_1433800 SNP1 fwd	CCATACAAATGTCAAAATATAACAAACC
PF3D7_1433800	PF3D7_1433800 SNP1 rev	CTAGTTGCACGATTTTGATAATTTCC
PF3D7_1433800	PF3D7_1433800 SNP2 fwd	GATGAATAATAATATGTTTTATGATAATGG
PF3D7_1433800	PF3D7_1433800 SNP2 rev	AACTGATAATAATTTAAATGATGTTGG
PF3D7_1121900	PF3D7_1121900 fwd	GATCAAAATTCAAACAATGCGTTCTAG
PF3D7_1121900	PF3D7_1121900 rev	CTGTTGAACCATCAAATGGAACGG
PF3D7_1324300	PF3D7_1324300 fwd	AGTTCAGTTGTTATCATGTTC
PF3D7_1324300	PF3D7_1324300 rev	TGGCACATTCTTCGTTTTCC
PF3D7_1422400	PF3D7_1422400 fwd	CGTTTACCATGAGTTATGTACATCAAG
PF3D7_1422400	PF3D7_1422400 rev	ACACGGAACAATAAATATTTCTGTACTC