

Overlap Extension Barcoding for the Next Generation Sequencing and Genotyping of Plasmodium Falciparum in Individual Patients in Western Kenya

Brandt Levitt B.S. ¹, Andrew Obala Ph.D. ², Scott Langdon Ph.D. ³, David Corcoran Ph.D. ⁴, Wendy Prudhomme O'Meara Ph.D. ⁵, Steve M Taylor M.D. M.P.H* ⁵

1 Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA

2 Moi University School of Medicine, College of Health Sciences, Nandi Rd, Eldoret, Kenya

3 Duke University DNA Analysis Facility, Department of Immunology, Duke University Medical Center, Durham, NC, USA

4 Duke Center for Genomic and Computational Biology, Duke University Medical Center, Durham NC, USA

5 Division of Infectious Diseases and Duke Global Health Institute, Duke University Medical Center, Durham, NC, USA

*Corresponding Author. Correspondence to Steve.Taylor@duke.edu

Primer Name			Sequence (5'-3')	Barcode
IonTorrent Adaptor A forward			CCATCTCATCCCTGCGTGTCTCCGACTCAG	
Reverse Complement	Linker Sequence	TCACCGCTCGGATC		
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IonTorrent	Adaptor	A	reverse	Barcode_158	TCACCGCTCGGATC/GTGTTGGCTCCA/CTGAGTCGGAGACACGCA	TGGAGCCACAC
IonTorrent	Adaptor	A	reverse	Barcode_159	TCACCGCTCGGATC/GAATCGATTAGA/CTGAGTCGGAGACACGCA	TCTAATCGATTC
IonTorrent	Adaptor	A	reverse	Barcode_160	TCACCGCTCGGATC/GATTGGTGG/CTGAGTCGGAGACACGCA	CCACCAATAC
IonTorrent	Adaptor	A	reverse	Barcode_161	TCACCGCTCGGATC/GTCGAATCCAAG/CTGAGTCGGAGACACGCA	CTTGGAATTCGAC
IonTorrent	Adaptor	A	reverse	Barcode_162	TCACCGCTCGGATC/GATAATCCAGAA/CTGAGTCGGAGACACGCA	TTCTGGATTATC
IonTorrent	Adaptor	A	reverse	Barcode_163	TCACCGCTCGGATC/GCCAGAAGAA/CTGAGTCGGAGACACGCA	TTCTTCTGGC
IonTorrent	Adaptor	A	reverse	Barcode_164	TCACCGCTCGGATC/GAGTCTCAGGA/CTGAGTCGGAGACACGCA	TCCTGAGACTC
IonTorrent	Adaptor	A	reverse	Barcode_165	TCACCGCTCGGATC/GTCTTGTTCAG/CTGAGTCGGAGACACGCA	CTGGAACAGAC
IonTorrent	Adaptor	A	reverse	Barcode_166	TCACCGCTCGGATC/GATTAAGCAAGA/CTGAGTCGGAGACACGCA	TCTTGCTTAATC
IonTorrent	Adaptor	A	reverse	Barcode_167	TCACCGCTCGGATC/GTCCAATTGGAG/CTGAGTCGGAGACACGCA	CTCCAATTGGAC
IonTorrent	Adaptor	A	reverse	Barcode_168	TCACCGCTCGGATC/GACCTTCCTTAG/CTGAGTCGGAGACACGCA	CTAAGGAAGGTC
IonTorrent	Adaptor	A	reverse	Barcode_169	TCACCGCTCGGATC/GAGGTGCCTTCA/CTGAGTCGGAGACACGCA	TGAGGCACCTC
IonTorrent	Adaptor	A	reverse	Barcode_170	TCACCGCTCGGATC/GAACCGGATTGT/CTGAGTCGGAGACACGCA	ACAATCCGGTTC
IonTorrent	Adaptor	A	reverse	Barcode_171	TCACCGCTCGGATC/GTTCTGTAAGGA/CTGAGTCGGAGACACGCA	TCCTTACAGAAC
IonTorrent	Adaptor	A	reverse	Barcode_172	TCACCGCTCGGATC/GTTCGATTCA/CTGAGTCGGAGACACGCA	TGAATCGAAC
IonTorrent	Adaptor	A	reverse	Barcode_173	TCACCGCTCGGATC/GACGGCTTCAAG/CTGAGTCGGAGACACGCA	CTTGAAGCCGTC
IonTorrent	Adaptor	A	reverse	Barcode_174	TCACCGCTCGGATC/GATTGATCTCAA/CTGAGTCGGAGACACGCA	TTGAGATCAATC
IonTorrent	Adaptor	A	reverse	Barcode_175	TCACCGCTCGGATC/GTCGAATTGCTG/CTGAGTCGGAGACACGCA	CAGCAATTTCGAC
IonTorrent	Adaptor	A	reverse	Barcode_176	TCACCGCTCGGATC/GATTAGCTTCG/CTGAGTCGGAGACACGCA	CGAAGCTAATC
IonTorrent	Adaptor	A	reverse	Barcode_177	TCACCGCTCGGATC/GTCAGCCTTAAG/CTGAGTCGGAGACACGCA	CTTAAGGCTGAC
IonTorrent	Adaptor	A	reverse	Barcode_178	TCACCGCTCGGATC/GTGGTTCTCCAG/CTGAGTCGGAGACACGCA	CTGGAGAACCAC
IonTorrent	Adaptor	A	reverse	Barcode_179	TCACCGCTCGGATC/GATTCCAAGTA/CTGAGTCGGAGACACGCA	TACTTGGAATC
IonTorrent	Adaptor	A	reverse	Barcode_180	TCACCGCTCGGATC/GAGGAGGCCTAG/CTGAGTCGGAGACACGCA	CTAGGCCTCCTC
IonTorrent	Adaptor	A	reverse	Barcode_181	TCACCGCTCGGATC/GTTGTCTCGG/CTGAGTCGGAGACACGCA	CCGAGAACAAC
IonTorrent	Adaptor	A	reverse	Barcode_182	TCACCGCTCGGATC/GACGTCTTAA/CTGAGTCGGAGACACGCA	TTAAGACGTC
IonTorrent	Adaptor	A	reverse	Barcode_183	TCACCGCTCGGATC/GCGGATCTTAG/CTGAGTCGGAGACACGCA	CTAAGATCCGC
IonTorrent	Adaptor	A	reverse	Barcode_184	TCACCGCTCGGATC/GATGAAGCCA/CTGAGTCGGAGACACGCA	TGGCTTCATC
IonTorrent	Adaptor	A	reverse	Barcode_185	TCACCGCTCGGATC/GACAAATTGTTG/CTGAGTCGGAGACACGCA	CGAACAATTGTC
IonTorrent	Adaptor	A	reverse	Barcode_186	TCACCGCTCGGATC/GAACACCTTGAA/CTGAGTCGGAGACACGCA	TTCAAGGTGTTT
IonTorrent	Adaptor	A	reverse	Barcode_187	TCACCGCTCGGATC/GTGGTGGTTAAG/CTGAGTCGGAGACACGCA	CTTAACCAACCAC
IonTorrent	Adaptor	A	reverse	Barcode_188	TCACCGCTCGGATC/GAACGGTCCGGA/CTGAGTCGGAGACACGCA	TCCGGACCGTTC
IonTorrent	Adaptor	A	reverse	Barcode_189	TCACCGCTCGGATC/GCATGCTCAAGG/CTGAGTCGGAGACACGCA	CCTTGAGCATGC
IonTorrent	Adaptor	A	reverse	Barcode_190	TCACCGCTCGGATC/GAATATCTAAGA/CTGAGTCGGAGACACGCA	TCTTAGATATTC
IonTorrent	Adaptor	A	reverse	Barcode_191	TCACCGCTCGGATC/GTAATTCAAG/CTGAGTCGGAGACACGCA	CCTGAATTAC
IonTorrent	Adaptor	A	reverse	Barcode_192	TCACCGCTCGGATC/GTTGGTTGGCTT/CTGAGTCGGAGACACGCA	AAGCCAACCAAC
Linker sequence					CGATCCGAGCGGTGA	
Linker- <i>pfam1</i> -forward					CGATCCGAGCGGTGA/CCATCAGGGAAATGTCCAGT	
Linker- <i>pfmsp</i> -forward					CGATCCGAGCGGTGA/ACAATCAAGGTAATGGACAAGG	
Linker- <i>pfk13</i> -forward					CGATCCGAGCGGTGA/TGCTGTATTGAATAATTTCT	
TrP1 Sequence					CCTCTCTATGGGCAGTCGGTGAT	
<i>pfmsp</i> -TrP1-reverse					CCTCTCTATGGGCAGTCGGTGAT/TTACGACATTAAACACACTGGAAC	
<i>pfam1</i> -TrP1-reverse					CCTCTCTATGGGCAGTCGGTGAT/TTTTTATCTTTGTTCATATAACAGC	
<i>pfk13</i> -TrP1-reverse					CCTCTCTATGGGCAGTCGGTGAT/TTTATTATCAAAAGCAAC	
Final Forward					CCATCTCATCCCTGCGTGTCTCCGACTCAG	
Final Reverse					CCTCTCTATGGGCAGTCGGTGAT	

Table S1. Primer Sequences.

<i>pf-csp</i>			<i>pf-ama1</i>		
Position	Nucleotide	SNP	Position	Nucleotide	SNP
39	862	G862A	54	486	T486A
79	902	G902A	59	491	C491T
82	905	C905A	68	500	C500A
126	949	A949G	83	515	G515A
129	952	G952C	91	523	T523G
140	963	C963G	104	536	G536
142	965	A965G	106	538	G538A
147	970	C970A	108	540	T540G
150	973	A973T	113	545	C545
156	979	C979A	127	559	G559A
231	1054	A1054G	129	561	A561T
232	1055	A1055G	134	566	T566A
237	1060	C1060T	138	570	G570A
246	1069	G1069C	154	586	G586A
258	1081	G1081	157	589	G589C
259	1082	C1082	158	590	A590G
268	1091	T1091	159	591	A591T
270	1093	G1093	166	598	C598G
271	1094	A1094	167	599	A599T
279	1102	A1102	169	601	T601C
282	1105	T1105	171	603	T603G
283	1106	G1106	178	610	G610A
284	1107	T1107	184	616	A616G
285	1108	A1108	187	619	T619G
301	1124	C1124	197	629	A629
			204	636	T636
			205	637	G637A
			218	650	G650
			242	674	T674A
			266	698	A698T

Table S2. Alleles used to construct haplotypes.

ID	Date	Sex	Age	Location	RDT	Symptomatic
BC13	4/2014	male	<10	maraka	positive	
BC14	4/2014	male	<10	Ndivisi	positive	
BC15	4/2014	female	<10	Misikhu	positive	
BC16	4/2014	male	<10	Misikhu	positive	
BC17	4/2014	female	<10	Misikhu	positive	
BC18	4/2014	male	>10	Misikhu	positive	Yes
BC19	4/2014	male	<10	Misikhu	positive	
BC20	4/2014		<10	Sitikho	positive	
BC21	4/2014	male	<10	Misikhu	positive	
BC22	4/2014	female	>10	Webuye	positive	
BC23	4/2014	female	<10	Webuye	positive	Yes
BC24	4/2014	female	<10	Webuye	positive	
BC25	4/2014	female	>10	Sitikho	positive	
BC26	4/2014	male	>10	Sitikho	positive	Yes
BC27	5/2014	female	<10	Miendo	positive	
BC28	5/2014	male	<10	Miendo	positive	Yes
BC29	5/2014	male	<10	Miendo	positive	
BC30	5/2014	female	<10	Misikhu	positive	
BC31	5/2014	male	<10	Misikhu	positive	
BC32	5/2014	male	>10	Misikhu	positive	Yes
BC33	5/2014		<10	Sitikho	positive	
BC34	5/2014	female	<10	Sitikho	positive	Yes
BC35	9/2014	male	<10	Sitikho	positive	
BC36	9/2014	female	<10	Sitikho	positive	
BC37	9/2014	female	>10	Sitikho	positive	Yes
BC38	9/2014	male	<10	Sitikho	positive	Yes
BC39	9/2014	male	<10	Sitikho	positive	
BC40	5/2014	male	<10	Maraka	positive	
BC41	5/2014	male	<10	Sitikho	positive	
BC42	4/2014	male	<10	Yalusi	positive	
BC43	9/2014		<10	Muji	positive	
BC44	9/2014	male	<10	Sitikho	positive	
BC45	5/2014	male	<10	Matulo	positive	
BC46	5/2014	male	<10	Matulo	positive	Yes
BC47	5/2014	male	<10	Matulo	positive	Yes
BC48	9/2014	female	<10	Sitikho	positive	
BC49	9/2014	female	>10	Sitikho	negative	
BC50	5/2014	male	<10	Muchi	positive	
BC51	5/2014	male	<10	Muchi	positive	Yes
BC52	5/2014	male	<10	Webuye	positive	
BC53		female	<10	Makemo	positive	
BC54	4/2014	female	<10	matulo	positive	
BC55	9/2014		<10	Maraka	positive	
BC56	9/2014	female	>10	Maraka	positive	
BC57	9/2014	female	<10	matulo	positive	
BC58		female	<10	Maraka	positive	
BC59	5/2014		<10	Webuye	positive	
BC60			<10	Muji	positive	
BC61	5/2014	female	<10	Webuye	positive	
BC62	5/2014	male	<10	Miendo	positive	
BC63	5/2014	male	<10	Misikhu	positive	
BC64	5/2014	male	<10	Maraka	positive	
BC65		female	<10	Maraka	positive	
BC66	5/2014	female	>10	Muchi	positive	Yes
BC67	5/2014	female	<10	Muchi	positive	Yes
BC68	5/2014	female	<10	Muchi	positive	Yes
BC69	5/2014	female	<10	Muchi	positive	Yes
BC70	5/2014	male	<10	Ndivisi	positive	
BC71	5/2014	female	<10	Ndivisi	positive	Yes
BC72	5/2014	female	<10	Webuye	positive	
BC73	5/2014	male	<10	Sitikho	positive	
BC74	5/2014	male	>10	Misikhu	positive	Yes
BC75		male	>10	Sitikho	positive	Yes
BC76		female	<10	Sitikho	positive	
BC77		female	>10	Sitikho	positive	
BC78		female	>10	Sitikho	positive	
BC79		female	<10	Matisi	positive	
BC80		female	>10	Matisi	positive	
BC81	7/2014	male	<10	Webuye	positive	
BC82	7/2014		<10	Webuye	positive	
BC83	7/2014	female	>10	Webuye	positive	

BC84	7/2014	male	<10	Maraka	positive	
BC85	7/2014	female	<10	Sitikho	positive	
BC86	7/2014	female	<10	Sitikho	positive	Yes
BC87	7/2014	female	<10	Misikhu	positive	
BC88	7/2014	male	<10	Matulo	positive	
BC89	7/2014	female	<10	Sitikho	positive	
BC90	7/2014	female	<10	Sitikho	positive	Yes
BC91	7/2014	female	>10	Sitikho	positive	Yes
BC92	7/2014	female	>10	Sitikho	positive	Yes
BC93		male	<10	Misikhu	positive	Yes
BC94	7/2014	male	<10	Muchi	Positive	
BC95	7/2014		<10	Muchi	Positive	
BC96	7/2014	female	<10	Sitikho	positive	
BC97	7/2014	male	<10	Sitikho	positive	yes
BC98	7/2014	female	>10	Sitikho	positive	yes
BC99	7/2014	female	<10	Chetambe	positive	
BC100	9/2014		<10	Sitikho	positive	
BC101			<10	Webuye	positive	
BC102		female	>10	Webuye	positive	
BC103		female	>10	Webuye	positive	Yes
BC104		female	<10	Webuye	positive	
BC105	9/2014	male	<10	Webuye	positive	
BC106		male	<10	Maraka	positive	Yes
BC107		female	<10	Maraka	positive	Yes
BC108		male	>10	Maraka	positive	Yes
BC109		female	>10	Maraka	positive	Yes
BC110	9/2014	male	<10	Matulo	positive	
BC111		male	>10	Miendo	positive	Yes
BC112	9/2014	male	>10	Sitikho	positive	
BC113	7/2014	female	>10	Webuye	positive	Yes
BC114	7/2014	male	<10	Webuye	positive	Yes
BC115		female	<10	Chetambe	positive	Yes
BC116	7/2014	male	>10	Sitikho	positive	
BC117		female	>10	Mihuu	positive	Yes
BC118		male	<10	Mihuu	positive	Yes
BC119	9/2014	male	>10	Sitikho	positive	
BC120	9/2014	female	<10	Matulo	positive	
BC121	9/2014	female	>10	Matulo	positive	Yes
BC122	9/2014	male	<10	Matulo	positive	Yes
BC123		female	>10	Maraka	positive	Yes
BC124		female	<10	Maraka	positive	Yes
BC125		male	<10	Maraka	positive	Yes
BC126	9/2014	female	<10	Sitikho	positive	Yes
BC127	8/2014	male	>10	Misikhu	positive	
BC128	8/2014	male	>10	Sitikho	positive	
BC129	8/2014	male	<10	Sitikho	positive	
BC130	8/2014	female	<10	Sitikho	positive	Yes
BC131	8/2014	male	<10	Sitikho	positive	Yes
BC132	8/2014	male	>10	Sitikho	positive	
BC133	8/2014		<10	Sitikho	positive	Yes
BC134	8/2014	female	>10	Sitikho	positive	
BC135	7/2014	male	<10	Misikhu	positive	Yes
BC136		female	>10	Mihuu	positive	Yes
BC137		female	>10	Mihuu	positive	Yes
BC138		female	>10	Mihuu	positive	
BC139		female	<10	Misikhu	positive	
BC140		female	<10	Misikhu	positive	
BC141	7/2014	female	>10	Sitikho	positive	
BC142	7/2014	female	>10	Sitikho	positive	
BC143	7/2014	male	>10	Sitikho	positive	
BC144	8/2014	male	<10	Misikhu	positive	Yes
BC145	8/2014	female	>10	Misikhu	positive	Yes
BC146	7/2014	female	<10	Sitikho	positive	Yes
BC147	7/2014	male	<10	Sitikho	positive	Yes
BC148	7/2014	male	>10	Sitikho	positive	Yes
BC149		female	>10	Misikhu	positive	Yes
BC150		female	<10	Misikhu	positive	Yes
BC151		female	<10	Misikhu	positive	Yes
BC152	7/2014	male	>10	Misikhu	positive	
BC153	7/2014	female	<10	Misikhu	positive	
BC154	7/2014	female	<10	Misikhu	positive	
BC155	7/2014	female	>10	Matulo	positive	Yes
BC156	7/2014	female	>10	Matulo	positive	
BC157	7/2014	male	>10	Matulo	positive	

BC158	8/2014	female	>10	Sitikho	positive	Yes
BC159	8/2014	female	<10	Maraka	positive	Yes
BC160	8/2014	male	>10	Maraka	positive	Yes
BC161	8/2014	female	>10	Misikhu	positive	Yes
BC162	8/2014	male	>10	Misikhu	positive	Yes
BC163	8/2014	male	<10	Webuye	positive	Yes
BC164	8/2014	female	>10	Sitikho	negative	
BC165	8/2014	female	>10	Sitikho	positive	Yes
BC166	8/2014	female	>10	Sitikho	positive	Yes
BC167	7/2014	female	>10	Sitikho	positive	Yes
BC168	7/2014	male	<10	Sitikho	Positive	
BC169	7/2014	male	<10	Sitikho	positive	Yes
BC170		male	>10	Sitikho	positive	
BC171		male	>10	Sitikho	positive	
BC172		female	>10	Maraka	positive	Yes
BC173		female	<10	Maraka	positive	Yes
BC174		female	<10	Webuye	positive	Yes
BC175		male	>10	Sitikho	positive	Yes
BC176		female	>10	Sitikho	positive	Yes
BC177		male	>10	Sitikho	positive	
BC178	7/2014	male	>10	Sitikho	positive	Yes
BC179	7/2014	female	>10	Sitikho	positive	Yes
BC180	8/2014	female	>10	Matulo	positive	Yes
BC181	8/2014	male	>10	Matulo	positive	
BC182	8/2014	female	>10	Matulo	positive	Yes
BC183	8/2014		<10	Matulo	positive	
BC184	8/2014	female	<10	Matulo	positive	Yes
BC185	7/2014		<10	Muchi	positive	
BC186	7/2014	female	>10	Muchi	negative	
BC187	7/2014	female	>10	Muchi	positive	Yes
BC188	7/2014	female	<10	Muchi	positive	

Table S3. Clinical and demographic information all patients. ID indicates patient identifier by barcode number. Date refers to the date of collection of blood sample. RDT status indicates whether they tested positively by the *P. falciparum* antigen HRP2 (Standard Diagnostics SD Bioline Malaria Ag Pf (HRPII))

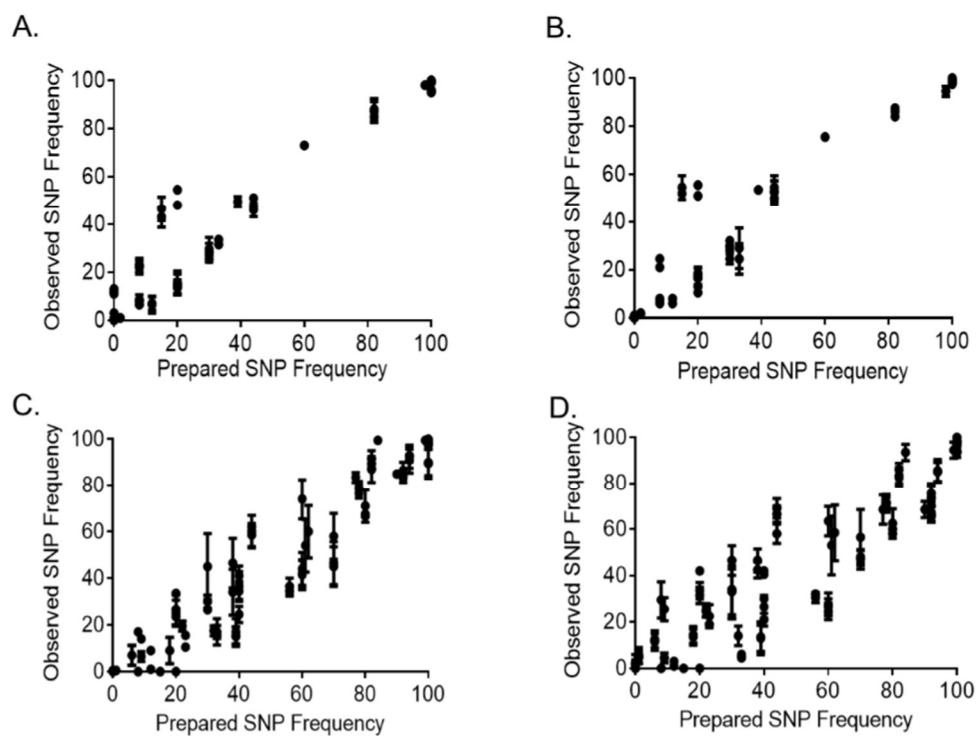


Figure S1. Experimental replicates of IonTorrent analysis. A. IonTorrent replicate 1 for *pf-csp*. B. IonTorrent replicate 2 for *pf-csp*. C. IonTorrent replicate 1 for *pf-ama1*. D. IonTorrent replicate 2 for *pf-ama1*.

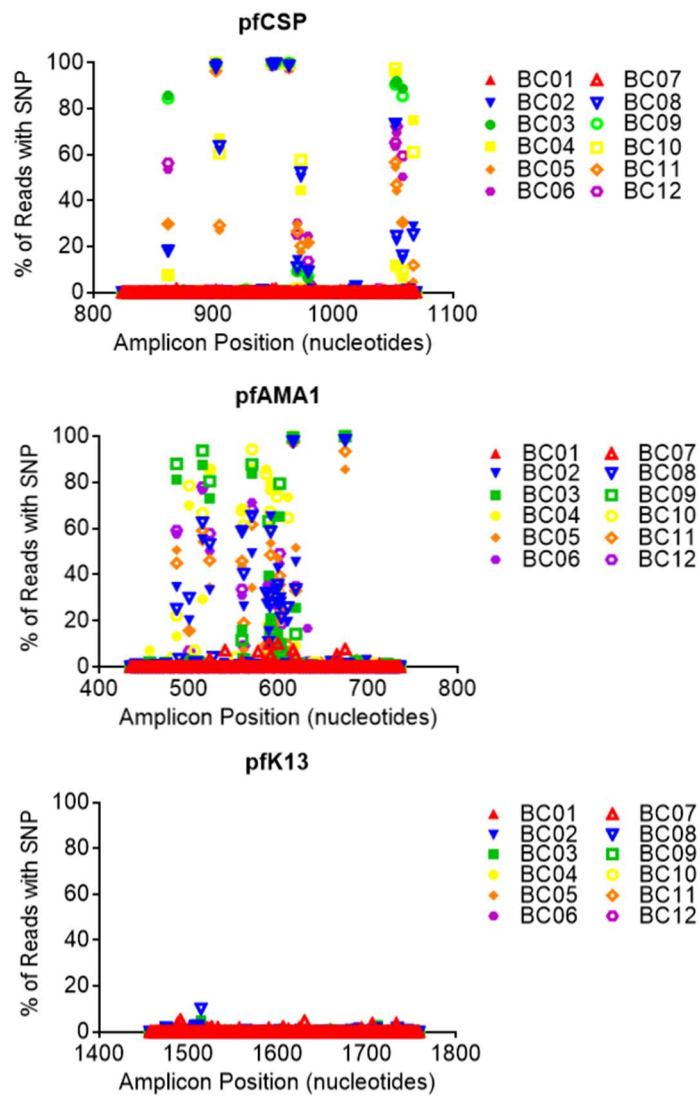


Figure S2. Positions and frequency of SNPs in mock-infected controls for each gene target.

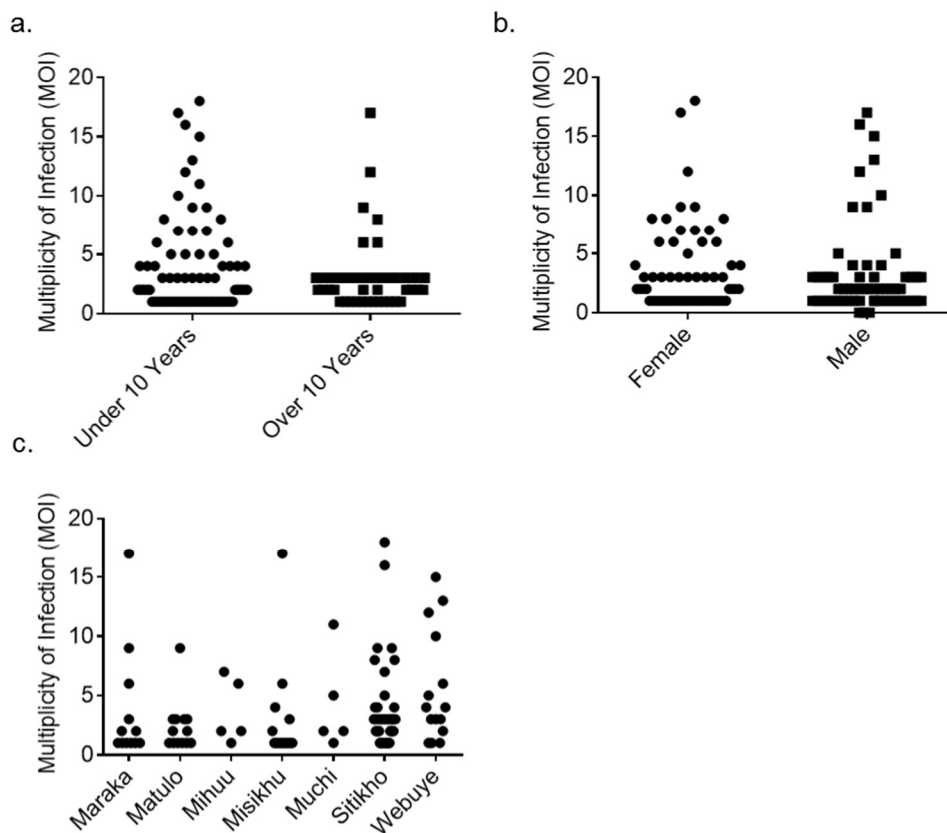
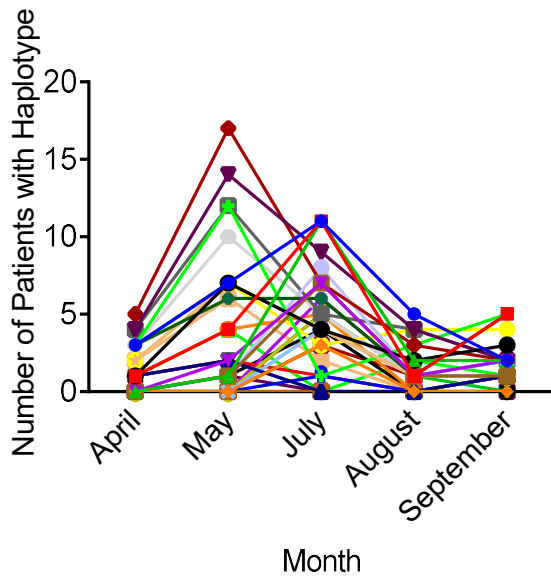


Figure S3. Multiplicity of infection (MOI) stratified by clinical and demographic features. a. Sorted by age. b. Sorted by gender. c. Sorted by location

a



b.

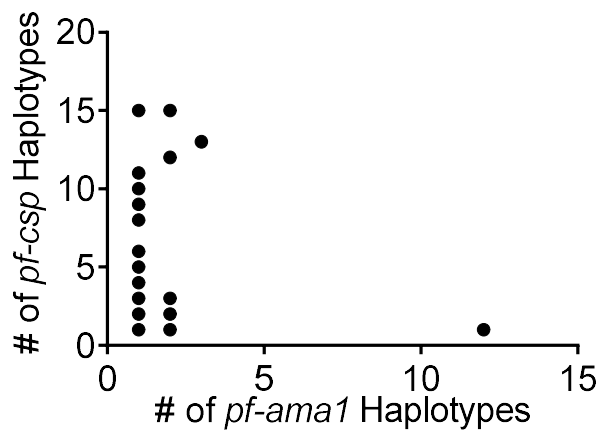


Figure S4. Analysis of Haplotypes in Field Collected Samples. a. Seasonal trends of each of the *pf-csp* haplotypes over several months. b. Concordance of number of *pf-ama1* and *pf-csp* haplotypes.