Genetic Report Card User Guide

Updated July 2023

Contents

1 Introduction

The Genetic Report Card (GRC) contains malaria parasite genetic data, derived from the analysis of patient blood samples. For each sample, we have determined genotypes at 101 single nucleotide polymorphisms (SNPs) that have been chosen to analyses the diversity between parasites. Also, 36 amino acid changes known to be associated with resistance to antimalarial drugs and details of associated haplotypes are reported. The Report additionally contains data on Plasmodium species found in the samples and on the complexity of sample infection.

The following is a guide to interpreting the Genetic Report Card data, linking the genotyped mutations (molecular markers) to resistance to different antimalarial drugs.
The Genetic Report Card is detailed over multiple tabs:

- **GRC** - gives sample metadata details, species call, complexity of infection, sample SNP barcode and drug resistant gene haplotypes.
- **GRC2** - shows the individual amino acid changes for the genotypes highlighted in the drug resistant haplotypes and additional mutations linked to amino acid changes that have been identified as significant drug resistant markers.
- **Barcode** - details the genotype result for each SNP, which is shown concatenated in GRC tab.

2 Sample Characteristics

2.1 Species Co-infections

We detect the presence of different *Plasmodium* species by sequencing two conserved regions of the parasite mitochondria. SNPs within these regions are able to differentiate the five human-infecting species of *Plasmodium* (*P. falciparum, P. vivax, P. malariae, P. ovale,* and *P. knowlesi*). We report a list of all species detected e.g. Pf/Pv. The order of the list has no biological significance.

In rare cases it is possible that due to missing sequence data certain species are indistinguishable, these will be indicated on the GRC (P-).

2.2 Complexity of Infection (COI)

We use the sample barcodes to infer individual sample estimates of Complexity of Infection (COI). Previously we reported COIL[1] and The real McCOIL[2] but from 2023 we only report the latter, and the COIL column does not contain data. The COI is expressed as the estimated number of genetically distinct parasites within the infection from which the sample was taken.

This is an approximation that greatly simplifies the complexities of mixed infections. However, the prevalence of highly mixed infections is a useful indicator of transmission intensity, which

can be used when comparing sites or seasons. Additionally, highly polyclonal infections are sometimes favorable to exclude from certain population analyses.

2.3 Sample Barcodes

The sample barcodes are formed by concatenated genotypes at 101 SNPs across the *P. falciparum* nuclear genome (see Release Change Log). These SNPs are all biallelic, i.e. only two alleles are observed; they were chosen for their usefulness in the analyses of relationships between parasites, due to their high heterozygosity, and are not associated with drug resistance. The full list of SNPs used is given in **Appendix Table 1**. SNPs within the barcode are represented by the observed homozygous nucleotide (A, T, C and G). If the genotype is missing (could not be detected), the symbol "X" is used. The symbol "N" indicates that both alleles were observed (heterozygous call). These loci were seen to be biallelic in a global set of whole-genome sequenced parasites, however it is possible that sporadic alternate alleles do exist at very low levels in populations.

Barcodes can be used for a variety of purposes, such as:

- The proportion of positions with identical alleles gives a measure of the genetic distance between two samples and can be used for generating population clusters.
- The proportion of heterozygous calls can be used to estimate the complexity of infection (COI).
- Identifying several samples with (almost) identical barcodes indicates a fast- expanding strain.
- The proportion of missingness in the barcode can give you a quick indication of sample quality.

3 Drug Resistance Genes and Haplotypes

Several columns in the Genetic Report Card show the genotypes of drug resistance mutations. For consistency with published literature, these genotypes are reported as their amino acid translations and positions eg. V127M. For genes with multiple SNPs of interest we translate each SNP to the amino acid and concatenate these to report an amino acid haplotype eg. NCSI, which is also common in the literature. Genotypes are called using a read based approach where an allele must have 5 high quality supporting reads at a minimum proportion of 10% of all high quality reads. (See Release Change Log).

For example, in the *pfdhfr* gene, we genotype amino acid positions 51, 59, 108 and 164, which are implicated in resistance to antifolates (see section 3.1.6 "Pyrimethamine Drug Resistance Mutations" below). The wild-type (WT) amino acids at these positions are N, C, S and I respectively - we represent this haplotype as "NCSI". If a parasite is reported to carry the haplotype "IRNI", it means it is a triple mutant at positions 51 (I →N), 59 (C →R) and 108 (S →N).

The following table shows the antimalarial drug and gene associated in resistance to that drug, also the amino acid positions associated with the haplotype and the wild type, non-mutated, form.

Column	Antimalarial	Gene	Amino Acid Positions	Wild Type
Kelch (see Release notes)	artemisinin	pfkelch13	any mutation seen in BTB/POZ and propeller domains	WT
P23:BP (Plasmepsin 2/3 Breakpoint)	piperaquine	Plasmepsin 2/3	N/A	WT
PfCRT	chloroquine	pfcrt	72, 73, 74, 75, 76	CVMNK
PfDHFR	pyrimethamine	pfdhfr	51, 59, 108, 164	NCSI
PfDHPS	sulfadoxine	pfdhps	436, 437, 540, 581, 613	SAKAA
PfEXO	piperaquine	exonuclease	415	E
PfMDR1	chloroquine, amodiaquine, lumefantrine, mefloquine	pfmdr1	86, 184, 1246	NYD
PGB (ART-R genetic background)	artemisinin	pfarps10	127, 128	VDDNIT
		ferredoxin	193	
		pfcrt	326, 356	
		pfmdr2	484	

In the haplotypes, we also use the following special characters:

- A dash (“-”) indicates a missing genotype, either because the sample could not be genotyped at that position, or because the assay is still under development/refinement
- If two alleles were detected (a heterozygous call), then both appear in brackets (e.g. [L/I]).
- Further loci are typed outside of “canonical” drug resistance haplotypes. These accessory mutations have less associated evidence and require further independent validation, these additional amino acid changes are found in GRC2.

Details of how genetic mutations relate to resistance to specific drugs can be found in the following sections.

3.1 Resistance to Specific Antimalarials

3.1.1 Artemisinin Drug resistance

Non-synonymous Kelch13 mutations
Several non-synonymous mutations in two domains (BTB/POZ and propeller) of the kelch13 (PF3D7_1343700) gene have been associated with delayed clearance of artemisinin.[3] Many kelch13 mutations have been reported around the world[4], but not all have been validated. We provide a list of associated mutations in **Appendix Table 2.** We carried out targeted discovery sequencing of this region, and detected mutations conferring non-synonymous amino acid changes are reported for each sample (see Release Change Log).

We report a list of the non-synonymous amino acid mutation changes and positions. If all mutations in the list are heterozygous we indicate this by appending a WT at the beginning of the list eg. WT V517I

WT independently is only reported if 95% of the kelch BTB/POZ and propeller domain is successfully genotyped.

Parasite genetic background (PGB) mutations
A study showed evidence of a genetic background of mutations that allowed for the emergence of *kelch13* mutations.[4] These mutations are:

- V127M and D128Y/H in the *pfarps10* (PF3D7_1460900) protein,
- D193Y in *ferredoxin* (*pffd*, PF3D7_1318100),
- N326S and I356T in *pfcrt* (PF3D7_0709000), and
- T484I in *pfmdr2* (PF3D7_1447900).

These are displayed in concatenated haplotype form, the reference allele (WT) being VDDNIT.

3.1.2 Chloroquine Drug resistance Mutations

Chloroquine drug resistance is primarily mediated by mutations in the chloroquine resistance transporter (*pfcrt*, PF3D7_0709000).[5] An accessory mutation at position 86 in the multidrug resistance protein (*pfmdr1*, PF3D7_0523000) has been shown to accentuate this resistance phenotype in parasites.[6] The loci in *pfcrt* are represented as a 5-amino acid haplotype at positions 72-76, the wild-type (WT) haplotype being CVMNK.[7] The CVIET haplotype is the most widespread resistant haplotype in Asia and Africa, while SVMNT is common in resistant parasites in South America and Oceania.

The *pfmdr1* mutation at position 86 is the first in the 3 amino-acid haplotype reported for this gene. The WT variant is N, while the *pfmdr1* 86Y variant enhances resistance to chloroquine.

3.1.3 Amodiaquine Drug resistance Mutations

Mutations in the multidrug resistance protein (*pfmdr1*, PF3D7_0523000) have been associated with parasite response to amodiaquine. There is limited evidence that mutations at positions 86 and 1246 can mediate response to this drug.[8] We report the haplotype positions at 86, 184 and 1246. In vitro experiments have shown that haplotypes containing the mutant 86Y have increased IC50's to chloroquine and amodiaquine.[9]

3.1.4 Mefloquine and Lumefantrine Drug Resistance Mutations

Mutations in multidrug resistance protein (*pfmdr1*, PF3D7_0523000) have been associated with parasite response to the drugs mefloquine and lumefantrine.[10] There is limited evidence that variations at positions 86, 184, and 1246 increase susceptibility to these drugs.[10,11] Additional mutations in the multidrug resistance protein (*pfmdr1*, PF3D7_0523000) have been shown to affect the parasite response to mefloquine, chloroquine, quinine, and halofantrine *in vitro.* The mutations at positions 1034, 1042, & 1226 were typed to be able to monitor these mutations in field isolates.[10,12] These additional mutations are not part of the haplotype and can be found in GRC2.

3.1.5 Piperaquine Drug Resistance

In a recent genome-wide association study (GWAS), a SNP in a putative *exonuclease* gene (*pfexo*, PF3D7_1362500) was associated with ex vivo piperaquine IC50 of parasite isolates from Cambodia.[13] This molecular marker is at position 415, and the 415G allele was shown to be significantly associated with increased tolerance of piperaquine with respect to the wild-type allele (415E). Gene amplification of a section of chromosome 14 involving the genes plasmepsin 2 and plasmepsin 3 has been associated with increased resistance to piperaquine. An assay designed to detect the breakpoint of a hybrid gene product was used to detect gene amplifications.[14] The presence or absence of this specific breakpoint is reported in the B23:BP column.

Various mutations in pfcrt, including at amino acid positions 93, 97, 218 and 353, have been associated with resistance to piperaquine.[15]

3.1.6 Pyrimethamine, Sulfadoxine, and Cycloguanil Drug Resistance Mutations

Pyrimethamine drug resistance is mediated by mutations in the bifunctional dihydrofolate reductase-thymidylate synthase (*pfdhfr*, PF3D7_0417200).[16] Four non- synonymous mutations at amino acid positions 51, 59, 108 and 164 have been established as important in drug resistance.[17] Resistant parasites are often characterized by the number of mutations they carry (single, double, triple, quadruple mutants), which is taken to be an indicator of the level of resistance to the drug.

Sulfadoxine drug resistance is mediated by mutations in the dihydropteroate synthetase

(*pfdhps*, PF3D7_0810800). A variety of mutation combinations at positions 436, 437, 540, 581 and 613 are thought to confer resistance, and parasites with higher numbers of mutations often have higher levels of resistance.[17] Some mutations are geographically isolated, while others are seen globally.

Cycloguanil is the active metabolite of the drug proguanil, which is often used in conjunction with atovaquone in malaria prophylaxis. Mutation in the bifunctional dihydrofolate reductase-thymidylate synthase (*pfdhfr*, PF3D7_0417200) have been associated with resistance to cycloguanil. The four mutations associated with resistance to pyrimethamine may cause differing levels of cross-resistance to cycloguanil, where the mutation of the residue at amino acid position 16 is only associated with resistance to proguanil.[18] *Pfdhfr* amino acid position 16 is detailed in GRC2.

Pfdhps mutations are often seen in combination with *pfdhfr* mutations, since sulfadoxine and pyrimethamine are mostly used in combination (SP). One combination of particular importance is the quintuple mutant carrying mutations at positions 51, 59 and 108 in *pfdhfr*, and at positions 437 and 540 in *pfdhps* (IRNx + xGExx in respective haplotypes). Such a combination of alleles is strongly predictive of SP treatment failure.[19]

4 Release Change Log

- The 16 June 2017 release fixed a barcode ordering problem that occurred on some (but not all) samples in previous releases. As a result of this problem, several barcodes were concatenated in sequence different from that in the documentation, and direct barcode comparison gave incorrect results**. Please note that drug-resistance genotypes were not affected by this issue.**
- The GRC is a data output of the Amplicon toolkit bioinformatics pipeline at the Wellcome Sanger Institute, and assays are undergoing continued development and optimization.
- Barcode SNP Pf3D7_08_v3:701557 is undergoing development and will appear missing for all samples.
- References provided are not the only evidence for association of molecular markers with clinical and *in vitro* phenotypes. Further research should be done before drawing conclusions on results and reporting.
- The June 2022 release introduced a stricter genotyping read cut off and corrected for genotyping errors in kelch13 by manually curating out all heterozygous mutations that do not appear a) in the list as defined by the WHO (**Appendix Table 2**), b) in the MalariaGEN Pf7 whole-genome data release, or c) as homozygous mutations in previous GRCs which have been manually validated.

5 References

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6 Appendix

Table 1 – List of the 101 barcode SNPs.

The SNPs are presented in the order in which they are concatenated in the barcode. For each SNP, we show: the chromosome and position within the chromosome; the reference (3D7 strain) and non-reference (alternative) alleles; the ID and description of the gene containing the SNP; whether the SNP is synonymous or non-synonymous; the amino acid mutations caused by the SNP in that gene; and the coding strand for this gene (+ = sense, - = antisense).

Num	Chr	Pos	Ref	Nonref	GeneId	GeneDescription	MutType	MutName	Strand
1	Pf3D7_02_v3	376222	A	G	PF3D7_0209000	6-cysteine protein (P230)	N	K1929E	+
2	Pf3D7_02_v3	470013	G	A	PF3D7_0211700	protein kinase, putative (TKL1)	N	G75E	+
3	Pf3D7_03_v3	656861	T	G	PF3D7_0316200	conserved Plasmodium protein, unknown function	S	129V	-
4	Pf3D7_04_v3	110442	C	T	PF3D7_0401900	acyl-CoA synthetase (ACS6)	N	G285E	-
5	Pf3D7_04_v3	881571	A	G	PF3D7_0419900	phosphatidylinositol 4-kinase, putative	S	1081R	+
6	Pf3D7_05_v3	350933	G	A	PF3D7_0508500	guanidine nucleotide exchange factor (RCC1)	S	1369N	-
7	Pf3D7_05_v3	369740	T	C	PF3D7_0508900	conserved Plasmodium protein, unknown function	S	907L	+
8	Pf3D7_06_v3	900278	G	A	PF3D7_0622100	conserved Plasmodium protein, unknown function	N	P696S	-
9	Pf3D7_07_v3	1044052	T	C	PF3D7_0724700	conserved Plasmodium protein, unknown function	S	686K	-
10	Pf3D7_08_v3	1314831	G	A	PF3D7_0830800	surface-associated interspersed gene 8.2 (SURFIN8.2) (SURF8.2)	S	1342K	+
11	Pf3D7_08_v3	413067	A	G	PF3D7_0808100	conserved Plasmodium protein, unknown function	S	1044V	+
12	Pf3D7_09_v3	900277	A	G	PF3D7_0922100	ubiquitin-like protein, putative	S	1534E	+
13	Pf3D7_11_v3	1018899	T	C	PF3D7_1126100	ThiF family protein, putative	S	1199L	-
14	Pf3D7_11_v3	1815412	C	G	PF3D7_1145800	conserved Plasmodium protein, unknown function	N	E765Q	-
15	Pf3D7_13_v3	1056452	T	C	PF3D7_1325400	conserved Plasmodium protein, unknown function	S	1234D	+
16	Pf3D7_13_v3	1466422	G	C	PF3D7_1335900	sporozoite surface protein 2+(TRAP)	N	N66K	-
17	Pf3D7_14_v3	137622	T	C	PF3D7_1403800	nuclear formin-like protein (MISFIT)	S	1179V	-
18	Pf3D7_14_v3	2164225	A	G	PF3D7_1452600	conserved Plasmodium protein, unknown function	S	2830S	+
19	Pf3D7_01_v3	145515	T	A	PF3D7_0103300	conserved Plasmodium protein, unknown function	S	294I	-

20	Pf3D7_03_v3	548178	C	A	PF3D7_0313400	conserved Plasmodium protein, unknown function	N	R2L	-
21	Pf3D7_04_v3	1102392	A	T	PF3D7_0424400	surface-associated interspersed gene 4.2, (SURFIN4.2) (SURF4.2)	N	E808D	+
22	Pf3D7_04_v3	139051	G	T	PF3D7_0402300	normocyte binding protein 1, reticulocyte binding protein homologue 1+(RH1)	N	K438N	+
23	Pf3D7_04_v3	286542	G	T	PF3D7_0405300	sequestrin	N	H586N	-
24	Pf3D7_04_v3	529500	G	A	PF3D7_0411900	DNA polymerase alpha	S	1477Y	-
25	Pf3D7_05_v3	796714	A	G	PF3D7_0519300	cytochrome c+oxidase assembly protein (heme A:+farnesyltransferase), putative	S	396K	+
26	Pf3D7_07_v3	1256331	C	T	PF3D7_0729500	mRNA (N6-adenosine)-methyltransferase, putative	N	L321F	+
27	Pf3D7_07_v3	461139	G	A	PF3D7_0710100	conserved Plasmodium protein, unknown function	N	M361I	+
28	Pf3D7_07_v3	619957	G	C	PF3D7_0713600	mitochondrial ribosomal protein S5 precursor, putative	S	675R	+
29	Pf3D7_08_v3	417335	C	T	PF3D7_0808200	plasmepsin X	N	R244K	-
30	Pf3D7_09_v3	163977	C	T	PF3D7_0903500	conserved Plasmodium protein, unknown function	S	403D	+
31	Pf3D7_10_v3	317581	A	T	PF3D7_1007900	eukaryotic translation initiation factor 3+subunit 7, putative	S	311I	+
32	Pf3D7_10_v3	336274	A	G	PF3D7_1008100	conserved Plasmodium protein, unknown function	N	I1677V	+
33	Pf3D7_11_v3	1020397	C	T	PF3D7_1126100	ThiF family protein, putative	N	G700E	-
34	Pf3D7_11_v3	1294107	C	T	PF3D7_1133400	apical membrane antigen 1+(AMA1)	S	84A	+
35	Pf3D7_11_v3	1935227	T	A	PF3D7_1148700	Plasmodium exported protein (PHISTc), unknown function (GEXP12)	N	R73S	-
36	Pf3D7_11_v3	477922	C	T	PF3D7_1112500	conserved Plasmodium protein, unknown function	N	H147Y	+
37	Pf3D7_12_v3	1663492	A	G	PF3D7_1239800	conserved Plasmodium protein, unknown function	S	1014E	+
38	Pf3D7_12_v3	2171901	T	A	PF3D7_1253100	Plasmodium exported protein (PHISTa), unknown function	N	V140D	+
39	Pf3D7_13_v3	1233218	T	C	PF3D7_1329100	myosin C+(MyoC)	N	N277S	-
40	Pf3D7_13_v3	1867630	G	C	PF3D7_1346400	conserved Plasmodium protein, unknown function	N	M4911I	+
41	Pf3D7_13_v3	2377887	C	A	PF3D7_1359600	conserved Plasmodium protein, unknown function	S	2002S	+
42	Pf3D7_14_v3	2355751	T	A	PF3D7_1457400	conserved Plasmodium protein, unknown function	N	H1589Q	+
43	Pf3D7_14_v3	3046108	C	T	PF3D7_1474400	conserved Plasmodium protein, unknown function	S	417V	+
44	Pf3D7_02_v3	529709	T	A	PF3D7_0212800	multidrug efflux pump, putative	N	F487L	+
45	Pf3D7_02_v3	714480	T	C	PF3D7_0217200	conserved Plasmodium protein, unknown function	N	D258G	-
46	Pf3D7_03_v3	155697	A	G	PF3D7_0302900	exportin 1, putative	S	150P	-
47	Pf3D7_04_v3	1037656	A	T	PF3D7_0422500	pre-mRNA-splicing helicase BRR2, putative (BRR2)	S	2776I	+

48	Pf3D7_04_v3	648101	G	A	PF3D7_0414200.1	calmodulin-like protein	S	51V	-
49	Pf3D7_05_v3	1204155	A	C	PF3D7_0529400.1	conserved Plasmodium protein, unknown function	S	1338I	+
50	Pf3D7_06_v3	1282691	G	A	PF3D7_0630600	conserved Plasmodium protein, unknown function	S	803K	+
51	Pf3D7_06_v3	1289212	A	G	PF3D7_0630800	conserved Plasmodium protein, unknown function	S	125T	+
52	Pf3D7_07_v3	1066698	G	A	PF3D7_0725100	conserved Plasmodium membrane protein, unknown function	N	G483S	+
53	Pf3D7_07_v3	1213486	G	A	PF3D7_0728200	actin-like protein, putative	N	S543N	+
54	Pf3D7_07_v3	704373	A	G	PF3D7_0716000	RNA binding protein, putative	S	389E	+
55	Pf3D7_08_v3	1313202	T	C	PF3D7_0830800	surface-associated interspersed gene 8.2 (SURFIN8.2) (SURF8.2)	S	799F	+
56	Pf3D7_08_v3	339406	A	G	PF3D7_0806300	ferlin like protein, putative	S	1283C	-
57	Pf3D7_08_v3	701557	T	G	PF3D7_0814500	conserved Plasmodium protein, unknown function	S	394G	+
58	Pf3D7_09_v3	452690	A	G	PF3D7_0910000	SET domain protein, putative (SET4)	S	1018I	-
59	Pf3D7_09_v3	599655	G	C	PF3D7_0914000	pseudouridylate synthase, putative	N	E654D	+
60	Pf3D7_10_v3	1383789	A	C	PF3D7_1034900	methionine-tRNA ligase, putative	N	N114H	+
61	Pf3D7_10_v3	1385894	C	T	PF3D7_1034900	methionine-tRNA ligase, putative	S	815P	+
62	Pf3D7_11_v3	1006911	A	T	PF3D7_1125700	kelch protein, putative	N	D124E	-
63	Pf3D7_11_v3	1295068	G	A	PF3D7_1133400	apical membrane antigen 1+(AMA1)	N	E405K	+
64	Pf3D7_11_v3	1802201	G	A	PF3D7_1145400	dynamin-like protein (DYN1)	S	450S	-
65	Pf3D7_12_v3	1667593	T	C	PF3D7_1239800	conserved Plasmodium protein, unknown function	S	2381N	+
66	Pf3D7_12_v3	1934745	G	A	PF3D7_1246500	conserved Plasmodium protein, unknown function	S	241L	-
67	Pf3D7_12_v3	858501	C	A	PF3D7_1221400	membrane skeletal protein, putative (ALV3)	N	Q469K	+
68	Pf3D7_13_v3	1419519	T	C	PF3D7_1335100	merozoite surface protein 7+(MSP7)	N	Q208R	-
69	Pf3D7_13_v3	159086	A	G	PF3D7_1303100	methyltransferase-like protein, putative	S	21R	+
70	Pf3D7_13_v3	2161975	T	A	PF3D7_1354200	inositol-polyphosphate 5-phosphatase, putative	N	D252V	-
71	Pf3D7_13_v3	2573828	A	C	PF3D7_1364200	conserved Plasmodium protein, unknown function	N	I1153M	-
72	Pf3D7_13_v3	388365	A	C	PF3D7_1308400	conserved Plasmodium protein, unknown function	N	S1236R	-
73	Pf3D7_14_v3	2625887	C	G	PF3D7_1464700	ATP synthase (C/AC39) subunit, putative	N	M238I	-
74	Pf3D7_14_v3	3126219	C	T	PF3D7_1475900	conserved Plasmodium protein, unknown function	N	S628F	+
75	Pf3D7_14_v3	438592	A	C	PF3D7_1410900	conserved Plasmodium protein, unknown function	N	N348T	+
76	Pf3D7_01_v3	179347	A	G	PF3D7_0104100	conserved Plasmodium membrane protein, unknown function	S	311G	+
77	Pf3D7_01_v3	180554	G	A	PF3D7_0104100	conserved Plasmodium membrane protein, unknown function	N	D714N	+
78	Pf3D7_01_v3	283144	C	G	PF3D7_0106700	small ribosomal subunit assembling AARP2 protein (AARP2)	N	H664D	+

79	Pf3D7_01_v3	535211	C	T	PF3D7_0113800	DBL containing protein, unknown function	S	2521F	+
80	Pf3D7_02_v3	839620	T	C	PF3D7_0220800	cytoadherence linked asexual protein 2+(CLAG2)	S	260L	+
81	Pf3D7_04_v3	426436	A	C	PF3D7_0408900.1	peptidase, M22 family, putative	N	D560A	+
82	Pf3D7_04_v3	531138	G	T	PF3D7_0411900	DNA polymerase alpha	N	A992E	-
83	Pf3D7_04_v3	891732	A	C	PF3D7_0419900	phosphatidylinositol 4-kinase, putative	N	R4468S	+
84	Pf3D7_05_v3	172801	G	A	PF3D7_0504400	ATP-dependent helicase, putative	N	E218K	+
85	Pf3D7_06_v3	574938	A	C	PF3D7_0613800	transcription factor with AP2 domain(s) (ApiAP2)	N	I2934L	+
86	Pf3D7_07_v3	1308383	C	T	PF3D7_0730500	conserved Plasmodium protein, unknown function	N	G1945R	-
87	Pf3D7_07_v3	1358910	A	G	PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	N	K286E	+
88	Pf3D7_07_v3	1359218	A	T	PF3D7_0731500	erythrocyte binding antigen-175 (EBA175)	N	K388N	+
89	Pf3D7_07_v3	635985	T	C	PF3D7_0713900	conserved Plasmodium protein, unknown function	N	T598A	-
90	Pf3D7_08_v3	1056829	C	A	PF3D7_0824200	conserved Plasmodium protein, unknown function	N	L474I	+
91	Pf3D7_08_v3	150033	T	C	PF3D7_0802000	glutamate dehydrogenase, putative (GDHc)	S	1315I	+
92	Pf3D7_08_v3	399774	C	T	PF3D7_0807800	proteasome subunit alpha type 5, putative	S	421K	-
93	Pf3D7_09_v3	1379145	G	A	PF3D7_0935400	cytoadherence linked protein	N	R398Q	+
94	Pf3D7_10_v3	1386850	C	T	PF3D7_1035000	U2 snRNA/tRNA pseudouridine synthase, putative	S	927K	-
95	Pf3D7_11_v3	1935031	T	A	PF3D7_1148700	Plasmodium exported protein (PHISTc), unknown function (GEXP12)	N	I139L	-
96	Pf3D7_11_v3	408668	T	A	PF3D7_1110200	pre-mRNA-processing factor 6, putative (PRPF6)	S	1058I	+
97	Pf3D7_11_v3	828596	T	C	PF3D7_1121800	petidase, M16 family	N	K240E	-
98	Pf3D7_12_v3	857245	A	G	PF3D7_1221400	membrane skeletal protein, putative (ALV3)	N	E50G	+
99	Pf3D7_14_v3	107014	G	A	PF3D7_1402900	conserved Plasmodium protein, unknown function	S	215K	+
100	Pf3D7_14_v3	1757603	A	G	PF3D7_1442900	guanine nucleotide exchange factor, putative	N	D1365G	+
101	Pf3D7_14_v3	2733656	C	T	PF3D7_1466800	conserved Plasmodium protein, unknown function	S	557C	+

Table 2 - Associated and validated *kelch13* resistance mutations.
This classification, based on published studies, is provided by WHO in a Report on antimalarial drug efficacy, resistance and response: 10 years of surveillance (2010-2019), published November 2020. (https://www.who.int/publications/i/item/9789240012813)

Validated	Candidate or associated
F446I	P441L
N458Y	G449A
M476I	C469F/Y
Y493H	A481V
R539T	R515K
I543T	P527H
P553L	N537I/D
R561H	G538V
P574L	V568G
C580Y	R622I
	A675V