

Molecular characterization of *Plasmodium falciparum* in Tanzania

Supplementary material

Table S1 Primer sequences for detection *Plasmodium* species, SP and artemisinin resistance markers in *P. falciparum*.

Detections	Primers/Probes	Sequence (5'-3')	Product size (bp)
Nest1- <i>pf</i> <i>dhfr</i>	<i>pf</i> <i>dhfr</i> _PrF	ATGGARSAMSTYTSMGABGTWTTY [ATGGA(G/A)(G/C)A(A/C)(G/C)T(T/C)T(G/C)(A/C)GA(G/T/C)GT(A/T)TTY]	714
	<i>pf</i> <i>dhfr</i> _M5	AGTATATACATCGCTAACAGA	
Nest2- <i>pf</i> <i>dhfr</i>	<i>pf</i> <i>dhfr</i> _PrNF	TSMGABGTWTTYGAYATTTAYGC [T(G/C)(A/C)GA(G/T/C)GT(A/T)TT(T/C)GA(T/C)ATTTA(T/C)GC]	710
	<i>pf</i> <i>dhfr</i> _M5	AGTATATACATCGCTAACAGA	
Nest1- <i>pf</i> <i>dhps</i>	<i>pf</i> <i>dhps</i> _F	GTTGAACCTAAACGTGCTGT	880
	<i>pf</i> <i>dhps</i> _R/	AATTGTGTGATTGTCCACAA	
Nest2- <i>pf</i> <i>dhps</i>	<i>pf</i> <i>dhps</i> _R2	AACCTAAACGTGCTGTTCAA	648
	<i>pf</i> <i>dhps</i> _R/	AATTGTGTGATTGTCCACAA	
Nest1- <i>pf</i> <i>kelch13</i>	K13_c.1F	TGGAAGGAGAAAAAGTAAAAACAAAA	2283
	K13_c.2283R	TGTGCATGAAAAATAAATATTAAGAAG	
Nest2- <i>pf</i> <i>kelch13</i>	K13_K189T_F	ACCAAGTAATTATCAAGAACATT	1245
(F1&F2)	K13_c.1538R	CGATCATACACCTCAGTTTCAA	
Nest2- <i>pf</i> <i>kelch13</i>	K13_c.1344F	AGGTGGATTGATGGTGTAGAA	940
(F3)	K13_c.2283R	TGTGCATGAAAAATAAATATTAAGAAG	
Multiplex <i>hrp2</i> exon 2 / Pf	Pfhrp2exon2N2F	ATTATTACACGAAACTCAAGCAC	791
	Pfhrp2exon2N1R	AATAAATTTAATGGCGTAGGCA	
	EdwinFor	GCTCTTTCTTGATTCTTGGATG	100
	EdwinRe	AGCAGGTAAAGATCTCGTTCG	

Table S2 The prevalence reports of *pfdhfr* mutations in study site, Tanzania between 2021 and 2024.

Years	<i>Pfdhfr</i> gene					Count (N)	Proportions (%)
	A16V	N51I	C59R	S108N	I164L		
2021	A	N	C	A	I	1	1.1
(n = 99)	A	N	<u>R</u>	<u>N</u>	I	5	5.1
	A	<u>I</u>	C	<u>N</u>	I	5	5.1
	A	<u>I</u>	<u>R</u>	<u>N</u>	I	61	61.6
	A	<u>I</u>	<u>C/R</u>	<u>N</u>	I	18	18.2
	A	<u>N/I</u>	<u>R</u>	<u>N</u>	I	6	6.1
	A	<u>N/I</u>	<u>C/I</u>	<u>N</u>	I	3	3
	A	N	<u>R</u>	<u>N</u>	I	11	10.2
2022	A	N	<u>R</u>	<u>N</u>	I	11	10.2
(n = 108)	A	<u>I</u>	C	<u>N</u>	I	10	9.3
	A	<u>I</u>	<u>R</u>	<u>N</u>	I	56	51.9
	A	N	<u>C/R</u>	<u>N</u>	I	4	3.7
	A	<u>I</u>	<u>C/R</u>	<u>N</u>	I	23	21.3
	A	<u>N/I</u>	<u>C/R</u>	<u>N</u>	I	2	1.9
	A	<u>I</u>	<u>C/R</u>	<u>S/N</u>	I	2	1.9
	A	N	<u>R</u>	<u>N</u>	I	4	4
2023	A	N	<u>R</u>	<u>N</u>	I	4	4
(n = 99)	A	<u>I</u>	C	<u>N</u>	I	6	6.1
	A	<u>I</u>	<u>R</u>	<u>N</u>	I	37	37.4
	A	N	<u>C/R</u>	<u>N</u>	I	2	2
	A	<u>I</u>	<u>C/R</u>	<u>N</u>	I	42	42.4
	A	<u>N/I</u>	<u>R</u>	<u>N</u>	I	5	5.1
	A	<u>N/I</u>	<u>C/R</u>	<u>N</u>	I	3	3
	A	N	C	S	I	2	1.9
2024	A	N	C	S	I	2	1.9
(n = 108)	A	N	<u>R</u>	<u>N</u>	I	3	2.8
	A	<u>I</u>	C	<u>N</u>	I	3	2.8
	A	<u>I</u>	<u>R</u>	<u>N</u>	I	33	30.6
	A	N	<u>C/R</u>	<u>N</u>	I	4	3.7
	A	<u>I</u>	<u>C/R</u>	<u>N</u>	I	59	54.6
	A	<u>N/I</u>	<u>R</u>	<u>N</u>	I	2	1.9
	A	<u>N/I</u>	<u>C/R</u>	<u>N</u>	I	1	0.9
	A	<u>I</u>	<u>C/R</u>	<u>S/N</u>	I	1	0.9
	A	<u>I</u>	<u>C/R</u>	<u>S/N</u>	I	1	0.9

Table S3 The prevalence reports of *pf dhps* mutations in study site, Tanzania between 2021 and 2024.

Years	<i>Pfdhps</i> gene						Count (N)	Proportions (%)
	I431 <u>V</u>	S436 <u>F/A</u>	A437 <u>G</u>	K540 <u>E</u>	A581 <u>G</u>	A613 <u>S/T</u>		
2021	I	S	A	K	A	A	8	8.3
(n = 96)	I	S	A	<u>E</u>	A	A	1	1
	I	<u>A</u>	A	K	A	A	2	2.1
	I	<u>H</u>	A	K	A	A	2	2.1
	I	<u>F</u>	A	K	A	<u>S</u>	1	1
	I	<u>F</u>	A	<u>E</u>	A	<u>S</u>	1	1
	I	S	<u>G</u>	<u>E</u>	A	A	57	59.4
	I	S	A	<u>K/E</u>	A	A	1	1
	I	<u>C</u>	A	<u>K/E</u>	A	A	1	1
	I	S	<u>G</u>	<u>K/E</u>	A	A	2	2.1
	I	S	<u>A/G</u>	<u>E</u>	A	A	5	5.2
	I	S	<u>A/G</u>	<u>K/E</u>	A	A	11	11.5
	I	<u>S/A</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1
	I	<u>S/G</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1
	I	<u>S/F</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1
	I	<u>S/F</u>	<u>A/G</u>	<u>K/E</u>	A	<u>A/S</u>	1	1
2022	I	S	A	K	A	A	13	11.8
(n = 110)	I	<u>A</u>	A	K	A	A	2	1.8
	I	<u>H</u>	A	K	A	A	2	1.8
	I	<u>F</u>	A	K	A	A	2	1.8
	I	<u>A</u>	<u>G</u>	K	A	A	1	0.9
	I	S	<u>G</u>	<u>E</u>	A	A	74	67.3
	I	S	<u>G</u>	<u>E</u>	<u>G</u>	A	1	0.9
	I	S	A	<u>K/E</u>	A	A	1	0.9
	I	S	<u>A/G</u>	K	A	A	1	0.9
	I	S	<u>G</u>	<u>K/E</u>	A	A	1	0.9
	I	S	<u>A/G</u>	<u>E</u>	A	A	4	3.6
	I	S	<u>A/G</u>	<u>K/E</u>	A	A	5	4.6
	I	<u>S/A</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	0.9
	I	<u>S/Y</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	0.9
	I	<u>S/F</u>	<u>A/G</u>	<u>K/E</u>	A	<u>A/S</u>	1	0.9

Table S3 The prevalence reports of *pfldhps* mutations in study site, Tanazia between 2021 and 2024
(cont.).

Years	<i>Pfdhps</i> gene						Count (n)	Proportions (%)
	I431 <u>V</u>	S436 <u>F/A</u>	A437 <u>G</u>	K540 <u>E</u>	A581 <u>G</u>	A613 <u>S/T</u>		
2023	I	S	A	K	A	A	4	4.3
(n = 93)	I	<u>A</u>	A	K	A	A	2	2.2
	I	<u>H</u>	A	K	A	A	1	1.1
	I	<u>P</u>	A	K	A	A	1	1.1
	I	S	<u>G</u>	<u>E</u>	A	A	63	67.7
	I	<u>F</u>	A	<u>E</u>	A	<u>S</u>	1	1.1
	I	S	A	<u>K/E</u>	A	A	2	2.2
	I	S	<u>A/G</u>	<u>E</u>	A	A	2	2.2
	I	S	<u>A/G</u>	<u>K/E</u>	A	A	14	15.1
	I	<u>A</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1.1
	I	<u>S/C</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1.1
	I	<u>S/A</u>	<u>A/G</u>	<u>K/E</u>	A	A	1	1.1
2024	I	S	A	K	A	A	9	8.5
(n = 106)	I	<u>A</u>	A	K	A	A	3	2.8
	I	<u>C</u>	A	K	A	A	1	0.9
	I	<u>H</u>	A	K	A	A	7	6.6
	I	S	A	<u>E</u>	A	A	1	0.9
	I	<u>F</u>	A	K	A	<u>S</u>	1	0.9
	I	S	<u>G</u>	<u>E</u>	A	A	75	70.8
	I	S	<u>A/G</u>	K	A	A	1	0.9
	I	S	<u>A/G</u>	<u>E</u>	A	A	1	0.9
	I	S	<u>G</u>	<u>K/E</u>	A	A	2	1.9
	I	S	<u>A/G</u>	<u>K/E</u>	A	A	5	4.7

Table S4 The prevalence of *pfdhfr/dhps* combination haplotypes in Tanzania between 2021 and 2024.

Category	Haplotype	% Frequency			
		2021	2022	2023	2024
		(n = 94)	(n = 104)	(n = 92)	(n = 106)
Wild type	ANCSI + ISAKAA	1.1			
Single mutant	ANCSI + <u>I</u> HAKAA				0.9
Double mutant	ANCSI + IS <u>G</u> EAA				0.9
Triple mutant	AN <u>R</u> NI + ISAKAA		1		
	A <u>I</u> C <u>N</u> I + ISAKAA		1	1.1	
	AN <u>R</u> NI + I <u>F</u> AKAA		1		
	AN <u>R</u> NI + <u>I</u> HAKAA		1		0.9
	AN <u>R</u> NI + ISAK <u>E</u> /AA		1	1.1	
	AN <u>C</u> / <u>R</u> NI + I <u>C</u> AKAA				0.9
	AN <u>I</u> / <u>R</u> NI + ISAKAA				0.9
	A <u>I</u> C <u>N</u> I + I <u>A</u> AKAA		1		
	A <u>I</u> C <u>N</u> I + <u>I</u> HAKAA	3.2			
	A <u>I</u> C <u>N</u> I + ISAK <u>E</u> /AA	1.1			
	A <u>I</u> R <u>N</u> I + ISAKAA	3.2	7.7	2.2	5.7
	A <u>I</u> C/ <u>R</u> NI + ISAKAA	1.1	2.9	1.1	1.9
	AN <u>R</u> NI + IS <u>G</u> EAA	4.3	4.8	3.3	1.9
	AN <u>R</u> NI + IS <u>A</u> / <u>G</u> K/ <u>E</u> AA		1.9		
	AN <u>C</u> / <u>R</u> NI + I <u>A</u> GKAA		1		
Quadruple mutant	AN <u>C</u> / <u>R</u> NI + IS <u>G</u> EAA		1.9	2.2	1.9
	AN <u>C</u> / <u>R</u> NI + IS <u>A</u> / <u>G</u> K/ <u>E</u> AA		1		
	AN <u>C</u> / <u>R</u> NI + IS <u>G</u> K/ <u>E</u> AA				0.9
	AN <u>I</u> / <u>R</u> NI + I <u>A</u> AKAA				0.9
	AN <u>I</u> / <u>R</u> NI + I <u>P</u> AKAA			1.1	
	A <u>I</u> C <u>N</u> I + IS <u>G</u> EAA	4.3	6.7	4.4	2.8
	A <u>I</u> R <u>N</u> I + I <u>A</u> AKAA	2.1	1	2.2	
	A <u>I</u> R <u>N</u> I + I <u>F</u> AKAA		1		
	A <u>I</u> R <u>N</u> I + <u>I</u> HAKAA	2.1	1	1.1	0.9
	A <u>I</u> R <u>N</u> I + IS <u>A</u> <u>E</u> AA	1.1			0.9

Table S4 The prevalence of *pfdhfr/dhps* combination haplotypes in Tanzania between 2021 and 2024 (cont).

Category	Haplotype	% Frequency			
		2021	2022	2023	2021
		(n = 94)	(n = 104)	(n = 92)	(n = 94)
Quintuple mutant	A IR NI + IS A /GEAA		1.9		
	A IR NI + IS A /GK/EAA		1		
	A IR NI + IS A /GKAA		1		
	A IC /RNI + I A AKAA				1.9
	A IC /RNI + I H AKAA				3.8
	A IC /RNI + IS A /GKAA				0.9
	A IC /RNI + IS A /K/EAA			1.1	
	A IC NI + I F A E AS	1.1		1.1	
	A IC NI + IS/AA/GK/EAA		1		
	A IR NI + I F AKAS	1.1			0.9
	A IR NI + ISGEAA	1.1	37.5	23.9	20.8
	A IR NI + I C A K /EAA	35.1			
	A IR NI + IS A /GEAA	1.1		1.1	0.9
	A IR NI + ISGK/EAA	3.2	1		0.9
	A IR NI + IS A /GK/EAA	1.1		5.4	0.9
	A N /IRNI + IS A /GEAA	9.6		1.1	
	A N /IRNI + ISGEAA	2.1		3.3	
	A N /IC/RNI + IS A /GK/EAA	4.3			
	A N /IC/RNI + ISGEAA	1.1		2.2	0.9
	A N /IC/RNI + IS A /GEAA		0.96		
	A IC /RNI + ISGEAA	1.1	12.5	28.3	40.6
	A IC /RNI + ISGK/EAA	11.7			
	A IC /RNI + IS A /GEAA		1		
	A IC /RNI + IS A /GK/EAA	1.1	1	9.8	3.8
	A IC /RS/N I + ISGEAA		1.9		0.9
Sextuple mutant	A IR NI + IS/FA/GK/EAA	1.1			
	A N /IC/RNI + IS/GA/GK/EAA	1.1			
	A IC /RNI + IS/AA/GK/EAA	1.1			

Table S4 The prevalence of *pfdhfr/dhps* combination haplotypes in Tanzania between 2021 and 2024 (cont).

Category	Haplotype	% Frequency			
		2021	2022	2023	2024
		(n = 94)	(n = 104)	(n = 92)	(n = 106)
Septuple mutant	A IC/RNI + I AA/GK/EAA			1.1	
	A IC/RNI + I S/CA/GK/EAA			1.1	
	A IC/RNI + IS GEGA		1	1.1	
	A IC/RNI + I S/YA/GK/EAA		1		
	A IRNI + I S/FA/GK/EAA/S	1.1			
	A IC/RNI + I S/FA/GK/EAA/S		1		

Table S5 The prevalence reports of *pfkelch13* mutations in study site, Tanzania between 2021 and 2024.

Gene	Year	Haplotype	Count (N)	Percentage (%)
<i>Pfkelch13</i>	2021	Wild type	22	75.9
		n = 29		
		<u>NN</u> at codon 142	1	3.4
	2022	K189 <u>T</u>	6	20.7
		Wild type	38	63.3
		n = 60		
	2022	<u>N</u> at codon 142	1	1.7
		<u>NN</u> at codon 142	3	5
		<u>NNN</u> at codon 142	1	1.7
		I132 <u>S</u>	1	1.7
		K189 <u>N</u>	1	1.7
		K189 <u>T</u>	9	15
		K189 <u>T/K</u>	1	1.7
		T192 <u>P/T</u>	1	1.7
		R255 <u>K</u>	1	1.7
		R398 <u>R</u>	1	1.7
		P417 <u>P</u>	1	1.7
		C469 <u>C</u>	1	1.7
	2023	Wild type	69	65.1
		n = 106		
		<u>N</u> at codon 142	5	4.7
		<u>NN</u> at codon 142	2	1.9
		H136 <u>N</u>	1	0.9
		K189 <u>T</u>	8	7.6
		G220 <u>G</u>	1	0.9
		K189 <u>T/K</u>	13	12.3
		R223 <u>K/R</u>	1	0.9
		N138 <u>N/N</u> , K189 <u>T</u>	1	0.9
		K189 <u>T/K</u> , L251 <u>L</u>	1	0.9
		G220 <u>S/G</u> , G220 <u>F/G</u>	1	0.9
		C469 <u>C</u>	1	0.9
		V666 <u>L/V</u>	1	0.9
		K189 <u>N/K</u> , C469 <u>C/C</u>	1	0.9

Table S5 The prevalence reports of *pfkelch13* mutations in study site, Tanzania between 2021 and 2024 (cont.).

Gene	Year	Haplotype	Count (N)	Percentage (%)
	2024	Wild type	135	61.6
	n = 219	<u>N</u> at codon 142	4	1.8
		<u>NN</u> at codon 142	4	1.8
		<u>NNN</u> at codon 142	1	0.5
		K189 <u>N</u>	1	0.5
		K189 <u>N/K</u>	2	0.9
		K189 <u>T</u>	29	13.2
		K189 <u>T</u> , L222 <u>L</u>	1	0.5
		K189 <u>T/K</u>	16	7.3
		L258 <u>L/M</u>	1	0.5
		L152 <u>L/L</u>	1	0.5
		M157 <u>V</u>	1	0.5
		L258 <u>M</u>	2	0.9
		Q271 <u>Q</u>	1	0.5
		R255 <u>K</u>	3	1.4
		R398 <u>R</u>	1	0.5
		P417 <u>P</u>	1	0.5
		C469 <u>C</u>	1	0.5
		A504 <u>A</u>	1	0.5
		G538 <u>G</u>	1	0.5
		P715 <u>P</u>	1	0.5
		C469 <u>C/C</u>	2	0.9
		C473 <u>P/C</u>	1	0.5
		P574 <u>S/P</u>	1	0.5
		A578 <u>S/A</u>	1	0.5
		A676 <u>A/A</u>	1	0.5
		K189 <u>N</u> , C469 <u>C</u>	2	0.9
		K189 <u>T</u> , C469 <u>C</u>	1	0.5
		K189 <u>T</u> , P441 <u>P/P</u> , P443 <u>P/P</u>	1	0.5
		K189 <u>T</u> , N594 <u>S/N</u> , V647 <u>I</u>	1	0.5

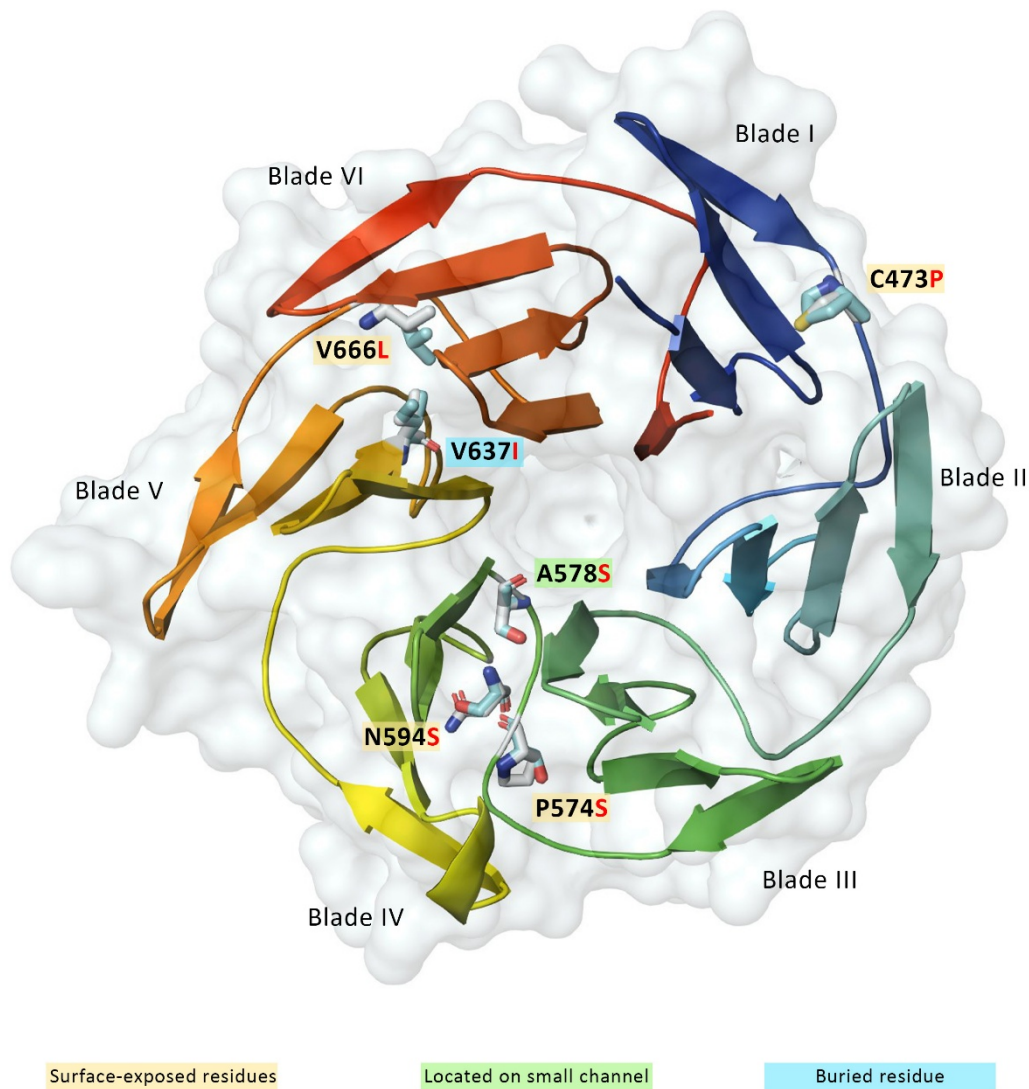


Figure S1. The built K13 propeller domain (amino acid 442nd – 726th) structure of *P. falciparum* from Tanzania. The six detected mutations were shown as a cyan stick, while the wild type was represented as a grey stick. The location of mutated residues on the structure was highlighted in yellow, green, and blue boxes for surface-exposed, close to the small channel, and buried residues, respectively.

