

Supplementary data

Supplementary Table 1. Primers used in this work.

Supplementary Table 2. Codon polymorphisms of the *pvdhfr* sequence in non-human primate adapted lines.

Supplementary Figure 1. Aligned *pvdhfr* sequences from the Pyr-sensitive wild-type Chesson line, edited Chesson #4909 parasites, the pvDC2- ZFN^{*pvdhfr*} plasmid and the ZFN binding site.

Chesson: ACCACATAACGAAGTAGGAGTAACCGCTTAACCCATGGAAGACCTTTCAGATGTATTTGACATTTACGCCATCTGCGCAT
 edited Chesson #4909: ACCACATAACGAAGTAGGAGTAACCGCTTAACCCATGGAAGACCTTTCAGATGTATTTGACATTTACGCCATCTGCGCAT
 pvDC2- ZFN^{pvdnfr}: -----GGAAGACCTTTCAGATGTATTTGACATTTACGCCATCTGCGCAT
 ZFN binding site: -----

Chesson: GCTGCAAGGTCGCCCCACCAGTGAAGGGACAAAGAATGAACCGTTCAGCCCGCGGACCTTTAGGGGTCTGGGCAATAAG
 edited Chesson #4909: GCTGCAAGGTCGCCCCACCAGTGAAGGGACAAAGAATGAACCGTTCAGCCCGCGGACCTTTAGGGGTCTGGGCAATAAG
 pvDC2- ZFN^{pvdnfr}: GCTGCAAGGTCGCCCCACCAGTGAAGGGACAAAGAATGAACCGTTCAGCCCGCGGACCTTTAGGGGTCTGGGCAATAAG
 ZFN binding site: -----

Chesson: GGGACTCTCCCATGGAAATGCAACTCCGTCGATATGAAGTACTTCAGCTTCGGTGAACACCTACGTGGATGAGTCAAAGTA
 edited Chesson #4909: GGGACTCTCCCATGGAAATGCAACTCCGTCGATATGAAGTACTTCAGCTTCGGTGAACACCTACGTGGATGAGTCAAAGTA
 pvDC2- ZFN^{pvdnfr}: GGGACTCTCCCATGGAAATGCAACTCCGTCGATATGAAGTACTTCAGCTTCGGTGAACACCTACGTGGATGAGTCAAAGTA
 ZFN binding site: -----

171 174 182

Chesson: TGAGAAGCTAAAGTGGAAGAGGGAGAGGTACCTACGAATGGAAGCCTCACAGGGGGGGGGTGACAACACACACGGTGGTG
 edited Chesson #4909: TGAGAAGCTAAAGTGGAAGAGGGAGAGGTACCTACGAATGGAAGCCTCACAGGGGGGGGGTGACAACACACACGGTGGTG
 pvDC2- ZFN^{pvdnfr}: TGAGAAGCTAAAGTGGAAGAGGGAGAGGTACCTACGAATGGAAGCCTCACAGGGGGGGGGTGACAACACACACGGTGGTG
 ZFN binding site: -----

Chesson: ACAACGCGGACAAGCTGCAAAAACCTCGTGGTCAATGGGGAGATCCACTGGGAGATCCATCCCCAAGCAGTACAAGCCGCTC
 edited Chesson #4909: ACAACGCGGACAAGCTGCAAAAACCTCGTGGTCAATGGGGAGATCCACTGGGAGATCCATCCCCAAGCAGTACAAGCCGCTC
 pvDC2- ZFN^{pvdnfr}: ACAACGCGGACAAGCTGCAAAAACCTCGTGGTCAATGGGGAGATCCACTGGGAGATCCATCCCCAAGCAGTACAAGCCGCTC
 ZFN binding site: -----TCGTGGTCAATGGGGAGATCCACTGGGAGATCCATCCCCAAGCAGTACAAGCCGCTC-----

350

- Mutations linked to pyrimethamine resistance
- Silent mutations engineered to identify transgenic parasites

Supplementary Table 1.
Primers used in this work

Name	Sequence 5'-3'	Description
rPLU5	CCTGTTGTTGCCTTAAACTCC	Primer for <i>Plasmodium sp</i> detection [1]
rPLU6	TAAAATTGTTGCAGTTAAACG	Primer for <i>Plasmodium sp</i> detection [1]
rVIV1	CGCTTCTAGCTTAATCCACATAACTGATAC	Primer for <i>P. vivax</i> detection by nested PCR [1]
rVIV2	ACTTCCAAGCCGAAGCAAAGAAAGTCCTTA	Primer for <i>P. vivax</i> detection by nested PCR [1]
<i>pvdhfr</i> -5'UTR-F	TCACCGCACCAGTTGATTC	<i>pvdhfr-ts</i> 5'UTR forward
<i>pvdhfr</i> -R1	CCACCTTGCTGTAAACCAAAAAGTCCAGAG	<i>pvdhfr-ts</i> internal reverse [2]
MS50-F	CAAAGGAACATGCTCGAT	microsatellite MS50 forward [3] ^a
MS50-R	GAATTCTGAAGGAATTAGG	microsatellite MS50 reverse [3]
MS315-F	TTAACGGTTAATCCTCTATT	microsatellite MS315 forward [3] ^b
MS315-R	TTGTGTCTATTTGGCCATT	microsatellite MS315 reverse [3]
MS92-F	TCACTGATCTTTTCGCATG	microsatellite MS92 forward [3] ^c
MS92-R	TAGTAGCATAGTGGTAGTA	microsatellite MS92 reverse [3]
MS38-F	AACAACGCAATGTGGATAA	microsatellite MS38 forward [3] ^d
MS38-R	TTACGCTTAACTCATTCGT	microsatellite MS38 reverse [3]
MS40-F	ATTTGCGTACGGTTAAGAT	microsatellite MS40 forward [3] ^e
MS40-R	CAGGGTTATTCAATTTGCT	microsatellite MS40 reverse [3]
MS206-F	TCTTTATGTTGTACTGCTC	microsatellite MS206 forward [3] ^f
MS206-R	ACCACTTACAAAAGTGTGA	microsatellite MS206 reverse [3]
MS116-F	AAATGCAAGATCCAAGAAAT	microsatellite MS116 forward [3] ^g
MS116-R	GTCGCTCTTCATGTGGCA	microsatellite MS116 reverse [3]
MS196-F	ATCGTATGTGCGAAGTTAT	microsatellite MS196 forward [3] ^h
MS196-R	AATTATGCTATACGACTTAC	microsatellite MS196 reverse [3]
p1	gggccCATCGTTGCCTTTGAAAGAGC	Apal <i>vivax Calmodulin</i> 5'UTR forward
p2	TcctaggTGTGCTATTCGGCTATGC	AvrII <i>vivax Calmodulin</i> 5'UTR reverse
p3	ccctcgagCCGGAAGGGTGGAGATGA	XhoI <i>vivax hsp86</i> 3' UTR forward
p4	cgggatcCCATGCGCACGCGCAAGTGC	BamHI <i>vivax hsp86</i> 3' UTR reverse
p5	gggcccGGAGGACCTTTCAGATGTATTTG	Apal <i>pvdhfr-ts</i> forward
p6	CGAGCTCCATGCATATTTGTGTATTACAGACC	SacI <i>pvdhfr-ts</i> reverse
p7	GGTgATGGGGAGAtcCAcCTGGGAGtcCATCCCCAAGCAGTACAAGCCG	SDM ZFN silent binding site mutations reverse
p8	GgaCTCCCAGtGgaTCTCCCCATcACCACCACGTTTTGCAGCTTGTCG	SDM ZFN silent binding site mutations forward
p9	CTTgAGgTCGGTgAtGACCTACGTGGATGAGTCAAAGTATGAGAAGC	SDM F57L, S58R and T61M forward
p10	GGTCaTCACCGAcTcAAGTACTTCATATCGACGGAGTTGCATTTC	SDM F57L, S58R and T61M reverse
p11	GGAGAAGCAcCTGGGAGAGCATCCCCAAGCAGTACAAGCCGC	SDM S117T forward
p12	GCTCTCCCAGtGCTTCTCCCCATGACCACGACGTTTTGC	SDM S117T reverse

^a MS50 (chromosome 6) microsatellite product size was 124 bp from AMRU-1 and 128 bp from both Chesson and transgenic Chesson parasites

^b MS315 (Chromosome 3) microsatellite product size was 202 bp from AMRU-1 and 202 bp from both Chesson and transgenic Chesson parasites

^c MS92 (Chromosome 5) microsatellite product size was 114 bp from AMRU-1 and 120 bp from both Chesson and transgenic Chesson parasites

^d MS38 (Chromosome 6) microsatellite product size was 390 bp from AMRU-1 and 202 bp from both Chesson and transgenic Chesson parasites

^e MS40 (Chromosome 6) microsatellite product size was 157 bp from AMRU-1 and 140 bp from both Chesson and transgenic Chesson parasites

^f MS206 (Chromosome 12) microsatellite product size was 180 bp from AMRU-1 and 168 bp from both Chesson and transgenic Chesson parasites

^g MS116 (Chromosome 10) microsatellite product size was 178 bp from AMRU-1 and 158 bp from both Chesson and transgenic Chesson parasites

^h MS196 (Chromosome 8) microsatellite product size was 230 bp from AMRU-1 and 210 bp from both Chesson and transgenic Chesson parasites

[1] Snounou G, Viriyakosol S, Zhu XP, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. *Mol Biochem Parasitol* 1993; 61:315-20.

[2] Lu F, Lim CS, Nam DH, et al. Mutations in the antifolate-resistance-associated genes dihydrofolate reductase and dihydropteroate synthase in *Plasmodium vivax* isolates from malaria-endemic countries. *Am J Trop Med Hyg* 2010; 83:474-9.

[3] Mu J, Duan J, Makova KD, et al. Chromosome-wide SNPs reveal an ancient origin for *Plasmodium falciparum*. *Nature* 2002; 418:323-6.

Supplementary Table 2.**Codon polymorphisms of the *pvdhfr* sequence in non-human primate adapted lines**

Line	codon 57 (169-171)	codon 58 (172-174)	codon 61 (181-183)	codon 117 (349-351)
Drug-sensitive pattern (ARI/Pakistan Accession #: X98123)	F (TTC)	S (AGC)	T (ACG)	S (AGC)
Indonesia-I/CDC	F (TTC)	S (AGC)	T (ACG)	S (AGC)
Vietnam-IV	L (CTC)	S (AGC)	T (ACG)	S (AGC)
AMRU-I	L (TTG)	R (AGG)	M (ATG)	T (ACC) at 367-369
Indonesia-XIX	F (TTC)	R (AGG)	T (ACG)	N (AAC)
Brazil-I/CDC	F (TTC)	R (AGA)	T (ACG)	N (AAC)
Chesson	F (TTC)	S (AGC)	T (ACG)	S (AGC)