# An open dataset of *Plasmodium vivax* genome variation in 1,895 worldwide samples

MalariaGEN *Plasmodium vivax* Genome Variation Project

## Mapping genetic markers to resistance status classification

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In the accompanying data release we have classified all samples into different types of drug resistance based on published genetic markers including SNPs and copy number variations (CNVs). The methods of classification are heuristic and represent a best attempt based on the available data. Our aim is to improve the accuracy of classification in future data releases, as new sources of evidence become available on the relationship between genotype and drug resistance phenotype.

Each type of resistance was considered to be either present, absent or unknown for a given sample. We have not attempted to make a quantitative assessment of the level of drug resistance, which may depend on complex genetic interactions that remain poorly understood.

This is more problematic for some types of resistance than others. For example, it is thought that increased copy number of the *mdr1* gene is a reliable marker of mefloquine resistance, whereas there are various degrees of resistance to sulfadoxine-pyrimethamine (SP) that are determined by complex interactions between multiple mutations in the *dhfr* and *dhps* genes. Where appropriate, we used the simplest approach, e.g. the *dhfr* 117T allele appears sufficient for clinically significant treatment failure, and so parasites were classified as pyrimethamine resistant if this mutation was present irrespective of other *dhfr* and *dhps* alleles.

This document describes the heuristic utilised for each of the following drugs or combination of drugs:

- Pyrimethamine
- Sulfadoxine
- Mefloquine
- Sulfadoxine-Pyrimethamine (SP) when used for the treatment of uncomplicated cases

For each drug, the rules have priorities and are applied sequentially according to the “Step” column with the first successful criterion determining the classification and stopping the procedure.

The inferred resistance status classifications for all samples can be found on the resource page at https://www.malariagen.net/resource/30.

# Pyrimethamine

**Info on the drug:** https://en.wikipedia.org/wiki/Pyrimethamine

**Locus utilized:** PVP01_0526600 (*dhfr*)

**Codon:** 117

**Workflow:**

| Step | Genetic change | Interpretation | Classification |
|---|---|---|---|
| 1 | 117 S/T heterozygote | Heterozygous mutant | Undetermined |
| 2 | 117 missing | Missing | Undetermined |
| 3 | S117 | Wild type | Sensitive |
| 4 | 117N | Mutant | Sensitive |
| 5 | 117 S/N heterozygote | Heterozygous mutant | Sensitive |
| 6 | 117T | Mutant | Resistant |
| 7 | otherwise | Unknown mutant | Undetermined |

**References:**


- Hastings, M. D. *et al.* Novel Plasmodium vivax dhfr Alleles from the Indonesian Archipelago and Papua New Guinea: Association with Pyrimethamine Resistance Determined by a Saccharomyces cerevisiae Expression System. *Antimicrob. Agents Chemother.* **49**, 733–740 (2005)
- Hastings, M. D. *et al.* Dihydrofolate Reductase Mutations in *Plasmodium vivax* from Indonesia and Therapeutic Response to Sulfadoxine plus Pyrimethamine. *J. Infect. Dis.* **189**, 744–750 (2004)


**Notes:**

- Although the PvP01 sequence translates as a N at 117, this is thought to be the mutant allele, i.e. PvP01 does not have the wild type S allele.
- The allele 117T appears to be necessary and sufficient for clinical resistance to pyrimethamine. However different set of mutations in the gene have been associated with various levels of

resistance. To date the link between those mutations and the clinical outcome is not completely understood hence the decision to not consider those mutations in the classification.

- Hastings, *et al*. 2004 report that common double mutant 58R/117N does not confer resistance (based on genotyping after determination of therapeutic response), hence our classification of 117N as sensitive.

# Sulfadoxine

**Info on the drug:** https://en.wikipedia.org/wiki/Sulfadoxine

**Locus utilized:** PVP01_1429500 (*dhps*)

**Codon:** 383

**Workflow:**

| Step | Genetic change | Interpretation | Classification |
|---|---|---|---|
| 1 | 383 A/G heterozygote | Heterozygous mutant | Undetermined |
| 2 | 383 missing | Missing | Undetermined |
| 3 | A383 | Wild type | Sensitive |
| 4 | 383G | Mutant | Resistant |
| 5 | otherwise | Unknown mutant | Undetermined |

**References:**


- Pornthanakasem, W. *et al.* Role of Plasmodium vivax Dihydropteroate Synthase Polymorphisms in Sulfa Drug Resistance. *Antimicrob. Agents Chemother.* **60**, 4453–4463 (2016)


**Notes:**

# Mefloquine

**Info on the drug:** https://en.wikipedia.org/wiki/Mefloquine

**Locus utilized:** PVP01_1010900 (*mdr1*)

**Codons:** Amplification status of whole gene

**Workflow:**

| Step | Genetic change | Interpretation | Classification |
|---|---|---|---|
| 1 | Missing | Missing | Undetermined |
| 2 | Heterozygous duplication | Heterozygous mutant | Undetermined |
| 3 | Single copy | Wild type | Sensitive |
| 4 | Multiple copies | Mutant | Resistant |

**References:**


- Suwanarusk, R. *et al.* Amplification of pvmdr1 associated with multidrug-resistant Plasmodium vivax. *J. Infect. Dis.* **198**, 1558–64

# Sulfadoxine-Pyrimethamine (treatment)

**Info on the drugs combination:** https://en.wikipedia.org/wiki/Sulfadoxine/pyrimethamine

**Locus utilized:** PVP01_0526600 (*dhfr*)

**Codons:** 57, 58, 61, 111, 117 and 173

**Workflow:**

| Step | Genetic change | Interpretation | Classification |
|---|---|---|---|
| 1 | F57 or S117 | Not quadruple mutant (at least one allele is WT) | Sensitive |
| 2 | (57I or 57L) and 117T and ( (58R and 61M) or (111L and 173F) ), all homozygous | Mutant | Resistant |
| 3 | otherwise | Missing or unknown combination | Undetermined |

**References:**


- Hastings, M. D. *et al.* Dihydrofolate Reductase Mutations in *Plasmodium vivax* from Indonesia and Therapeutic Response to Sulfadoxine plus Pyrimethamine. *J. Infect. Dis.* **189**, 744–750 (2004)
- Imwong, M. *et al.* Novel point mutations in the dihydrofolate reductase gene of Plasmodium vivax: evidence for sequential selection by drug pressure. *Antimicrob. Agents Chemother.* **47**, 1514–21 (2003)
- Hastings, M. D. *et al.* Novel Plasmodium vivax dhfr Alleles from the Indonesian Archipelago and Papua New Guinea: Association with Pyrimethamine Resistance Determined by a Saccharomyces cerevisiae Expression System. *Antimicrob. Agents Chemother.* **49**, 733–740 (2005)


**Notes:**

- Also known as the “quadruple mutant”
- Only need one wild-type allele to determine sample is “Sensitive”, even if other alleles are mutant or missing