

**Supplementary Table 1. Within-sample diversity in Malaysia**

| FWS            | Malaysia 2019 | Malaysia 2010-2017 |
|----------------|---------------|--------------------|
| N              | 80            | 253                |
| Mean           | 0.949         | 0.945              |
| Median         | 0.956         | 0.949              |
| Q1             | 0.952         | 0.944              |
| Q3             | 0.958         | 0.953              |
| N (FWS > 0.95) | 63            | 114                |
| N (FWS > 0.90) | 76            | 249                |

Data was generated on 333 independent Malaysian *P. falciparum* samples using 23,575 biallelic SNPs.

**Supplementary Table 2. Summary of genotype calls at drug resistance-associated variants in samples collected from the Philippines in 2013-2018.**

| Gene                 | Variant     | Sample Proportion With Alt Allele <sup>1</sup> | Total Samples <sup>2</sup> | Samples with Hets <sup>3</sup> | Drug Assoc. <sup>4</sup> | WHO Status |
|----------------------|-------------|------------------------------------------------|----------------------------|--------------------------------|--------------------------|------------|
| <b><i>Pfcr</i></b>   | 47V>47M     | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 72C>72S     | 7.69%                                          | 39                         | 3                              | CQ                       | Validated  |
|                      | 76K>76T     | 7.69%                                          | 39                         | 3                              | CQ                       | Validated  |
|                      | 78F>78L     | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 220A>220S   | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 273H>273N   | 41.03%                                         | 39                         | 10                             |                          |            |
|                      | 280W>280R   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 319F>319L   | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 326N>326D   | 7.50%                                          | 40                         | 3                              |                          |            |
|                      | 333T>333A   | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 356I>356L   | 2.56%                                          | 39                         | 1                              | PPQ                      | Candidate  |
|                      | 371R>371G   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 372E>372K   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 378F>378S   | 2.56%                                          | 39                         | 1                              |                          |            |
| <b><i>Pfmdr1</i></b> | 86N>86Y     | 2.50%                                          | 40                         | 0                              |                          |            |
|                      | 90D>90G     | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 115C>115Y   | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 117D>117N   | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 130E>130K   | 7.50%                                          | 40                         | 3                              |                          |            |
|                      | 977E>977K   | 5.00%                                          | 40                         | 2                              |                          |            |
|                      | 1022R>1022G | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 1025I>1025N | 2.50%                                          | 40                         | 1                              |                          |            |
|                      | 1245R>1245K | 5.41%                                          | 37                         | 2                              |                          |            |
|                      | 1261F>1261S | 2.70%                                          | 37                         | 1                              |                          |            |
|                      | 1269I>1269V | 2.78%                                          | 36                         | 1                              |                          |            |
|                      | 1277T>1277I | 2.78%                                          | 36                         | 1                              |                          |            |
| <b><i>Pfk13</i></b>  | 683I>683V   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 678L>678M   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 676A>676T   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 671M>671I   | 2.56%                                          | 39                         | 1                              |                          |            |
|                      | 663L>663Q   | 2.56%                                          | 39                         | 1                              |                          |            |

|                      |           |        |    |   |     |           |
|----------------------|-----------|--------|----|---|-----|-----------|
|                      | 638G>638A | 2.56%  | 39 | 1 |     |           |
|                      | 567E>567G | 2.50%  | 40 | 1 |     |           |
|                      | 566V>566L | 2.50%  | 40 | 1 |     |           |
|                      | 515R>515G | 7.50%  | 40 | 3 |     |           |
|                      | 454V>454L | 2.50%  | 40 | 1 |     |           |
| <b><i>Pfdhfr</i></b> | 15C>15R   | 2.56%  | 39 | 1 |     |           |
|                      | 20V>20I   | 2.56%  | 39 | 1 |     |           |
|                      | 21E>21K   | 2.56%  | 39 | 1 |     |           |
|                      | 25E>25K   | 2.56%  | 39 | 1 |     |           |
|                      | 29N>29S   | 2.56%  | 39 | 1 |     |           |
|                      | 54D>54N   | 2.50%  | 40 | 1 |     |           |
|                      | 59C>59R   | 53.85% | 39 | 8 | PYR | Candidate |
|                      | 59C>59Y   | 2.56%  | 39 | 1 |     |           |
|                      | 68S>68L   | 2.56%  | 39 | 1 |     |           |
|                      | 108S>108N | 28.21% | 39 | 4 | PYR | Candidate |
|                      | 109W>109R | 5.13%  | 39 | 2 |     |           |
| <b><i>Pfdhps</i></b> | 410E>410D | 2.56%  | 39 | 1 |     |           |
|                      | 421M>421V | 2.56%  | 39 | 1 |     |           |
|                      | 423N>423S | 2.56%  | 39 | 1 |     |           |
|                      | 424E>424K | 2.56%  | 39 | 1 |     |           |
|                      | 426A>426V | 2.56%  | 39 | 1 |     |           |
|                      | 437G>437A | 76.92% | 39 | 3 | SP  | Candidate |
|                      | 461K>461E | 2.56%  | 39 | 1 |     |           |
|                      | 511P>511L | 2.63%  | 38 | 1 |     |           |

<sup>1</sup>Sample Proportion with Alt Allele indicates the proportion of samples carrying alternate allele (either homozygous or heterozygous) with a read depth  $\geq 5$

<sup>2</sup>Total Samples indicates Number of samples (N Samples) with a minimum read depth  $\geq 5$  at variant position

<sup>3</sup>Number of samples with a heterozygous call at the variant position (N Het.) No. Het. = Number of samples with heterozygous alleles at that position; Genotyping source = variant detected using whole genome sequencing (WGS) data and/or SpotMalaria derived amplicon sequencing data.

<sup>4</sup>Antimalarials include chloroquine (CQ), amodiaquine (AQ), artemisinin (ART), pyrimethamine (PYR), sulfadoxine-pyrimethamine (SP).

**Supplementary Table 3. Result of *pfcr* ONT long sequencing for Malaysian and Philippines samples with their available real-time assay results.**

| Country     | Sample     | Realtime qPCR assay |                        |    | PfCRT ONT Haplotype Sequencing |                                  |           | CN | Remark                                          |
|-------------|------------|---------------------|------------------------|----|--------------------------------|----------------------------------|-----------|----|-------------------------------------------------|
|             |            | Avg FC              | SD FC (range)          | CN | Total depth                    | Estimated ratio over total depth | Haplotype |    |                                                 |
| Malaysia    | KF1        | 0.9844              | 0.0075 (0.9776-0.9912) | 1  | 7281                           | 0.827                            | H2        | 1  |                                                 |
|             | NKD4-101   | 0.9085              | 0.0181 (0.8920-0.9250) | 1  | 973                            | 0.793                            | H2        | 1  |                                                 |
|             | NKD4-111   | -                   | -                      | -  | 35                             | 0.343                            | H2        | 1  | Low quality sample                              |
|             | NKD4-115   | 0.9083              | 0.0147 (0.8949-0.9217) | 1  | 4330                           | 0.78                             | H2        | 1  |                                                 |
|             | NKD4-167   | 0.9863              | 0.0106 (0.9766-0.9960) | 1  | 5058                           | 0.835                            | H2        | 1  |                                                 |
|             | NKD4-64    | 1.0459              | 0.0021 (1.0440-1.0479) | 1  | 6213                           | 0.761                            | H2        | 1  |                                                 |
|             | QEM969     | 0.995               | 0.0022 (0.9930-0.9970) | 1  | 8430                           | 0.798                            | H1a       | 1  |                                                 |
|             | KF3        | 2.096               | 0.0205 (2.0772-2.1147) | 2  | 7394                           | 0.417                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.295                            | H2        |    |                                                 |
|             | NKD4-103   | 1.7494              | 0.0261 (1.7256-1.7732) | 2  | 8371                           | 0.379                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.373                            | H2        |    |                                                 |
|             | NKD4-114   | -                   | -                      | -  | 37                             | 0.333                            | H2        | 1  | Low quality sample                              |
|             | NKD4-118   | -                   | -                      | -  | 41                             | 0.354                            | H1b       | 2  | Low quality sample                              |
|             |            |                     |                        |    |                                | 0.463                            | H2        |    |                                                 |
|             | NKD4-154   | 1.7908              | 0.0546 (1.7410-1.8407) | 2  | 528                            | 0.4                              | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.438                            | H2        |    |                                                 |
|             | NKD4-179   | 1.8611              | 0.0150 (1.8475-1.8748) | 2  | 5995                           | 0.415                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.341                            | H2        |    |                                                 |
|             | NKD4-22    | 2.0152              | 0.2415 (1.7948-2.2356) | 2  | 75                             | 0.331                            | H1b       | 2  | Low quality sample                              |
|             |            |                     |                        |    |                                | 0.513                            | H2        |    |                                                 |
|             | NKD4-25    | 2.1313              | 0.0233 (2.1100-2.1526) | 2  | 262                            | 0.431                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.379                            | H2        |    |                                                 |
|             | NKD4-37    | 1.7587              | 0.0336 (1.7280-1.7894) | 2  | 1759                           | 0.374                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.38                             | H2        |    |                                                 |
|             | NKD4-43    | 1.9717              | 0.0310 (1.9434-2.0000) | 2  | 55                             | 0.291                            | H1b       | 2  | Low quality sample                              |
|             |            |                     |                        |    |                                | 0.455                            | H2        |    |                                                 |
|             | NKD4-70    | 1.8722              | 0.0151 (1.8584-1.8860) | 2  | 4033                           | 0.374                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.365                            | H2        |    |                                                 |
|             | NKD4-79    | 2.0411              | 0.1072 (1.9433-2.1390) | 2  | 785                            | 0.499                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.363                            | H2        |    |                                                 |
|             | NKD4-83    | 1.8655              | 0.1167 (1.7590-1.9721) | 2  | 11385                          | 0.378                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.292                            | H2        |    |                                                 |
|             | NQE4-28    | 1.9628              | 0.0315 (1.9340-1.9916) | 2  | 5441                           | 0.42                             | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.333                            | H2        |    |                                                 |
|             | QEM615     | -                   | -                      | -  | 138                            | 0.349                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.43                             | H2        |    |                                                 |
|             | QEM668     | 1.8389              | 0.0656 (1.7790-1.8988) | 2  | 2594                           | 0.377                            | H1b       | 2  |                                                 |
|             |            |                     |                        |    |                                | 0.407                            | H2        |    |                                                 |
| Philippines | PF-2013-03 | 0.955               | 0.0055 (0.9500-0.9600) | 1  | 1292                           | 0.854                            | H1b       | 1  |                                                 |
|             | PF-2013-17 | 0.905               | 0.0383 (0.8700-0.9400) | 1  | 813                            | 0.924                            | H1a       | 1  |                                                 |
|             | PF-2013-40 | 1.01                | 0.0438 (0.9700-1.0500) | 1  | 170                            | 0.191                            | H1a       | 1  | Probably polyclonal; asymmetric haplotype ratio |
|             |            |                     |                        |    |                                | 0.709                            | H1b       |    |                                                 |
|             | PF-2013-73 | 1.01                | 0.0329 (0.9800-1.0400) | 1  | 17                             | 0.882                            | H1a       | 1  | Low quality sample                              |
|             | PF-2013-75 | 1.025               | 0.1150 (0.9200-1.1300) | 1  | 125                            | 0.904                            | H1a       | 1  |                                                 |
|             | PF-2013-78 | 0.925               | 0.0164 (0.9100-0.9400) | 1  | 17697                          | 0.869                            | H1a       | 1  |                                                 |
|             | PF-2013-83 | 1.05                | 0.0548 (1.0000-1.1000) | 1  | 974                            | 0.923                            | H1a       | 1  |                                                 |
|             | PF-2015-11 | 0.86                | 0.0000 (0.8600-0.8600) | 1  | 281                            | 0.826                            | H1b       | 1  |                                                 |
|             | PF-2015-12 | 0.92                | 0.0548 (0.8700-0.9700) | 1  | 742                            | 0.906                            | H1a       | 1  |                                                 |
|             | PF-2015-15 | 1.195               | 0.1917 (1.0200-1.3700) | 1  | 56                             | 0.875                            | H1a       | 1  | Low quality sample                              |
|             | PF-2015-16 | 0.995               | 0.0493 (0.9500-1.0400) | 1  | 1413                           | 0.89                             | H1b       | 1  |                                                 |
|             | PF-2015-18 | 0.86                | 0.0000 (0.8600-0.8600) | 1  | 697                            | 0.898                            | H1a       | 1  |                                                 |

|               |        |                        |   |      |       |     |   |                                                |
|---------------|--------|------------------------|---|------|-------|-----|---|------------------------------------------------|
| PF-2015-19    | 0.935  | 0.0822 (0.8600-1.0100) | 1 | 178  | 0.362 | H1a | 1 | single-copy pfct with minor sequence variation |
|               |        |                        |   |      | 0.537 | H1b |   |                                                |
| PF-2015-20    | 0.985  | 0.0383 (0.9500-1.0200) | 1 | 1652 | 0.883 | H1a | 1 |                                                |
| PF-2015-21    | 1.035  | 0.0164 (1.0200-1.0500) | 1 | 40   | 0.9   | H1a | 1 |                                                |
| PF-2015-22    | 0.935  | 0.0712 (0.8700-1.0000) | 1 | 462  | 0.92  | H1a | 1 |                                                |
| PF-2015-39    | 0.72   | 0.0767 (0.6500-0.7900) | 1 | 15   | 0.933 | H1a | 1 | Low quality sample                             |
| PF-2015-42    | 1.08   | 0.1315 (0.9600-1.2000) | 1 | 17   | 0.882 | H1a | 1 | Low quality sample                             |
| PF-2015-43    | 1.105  | 0.2136 (0.9100-1.3000) | 1 | 5931 | 0.897 | H1b | 1 |                                                |
| PF-2015-44    | 1.015  | 0.0931 (0.9300-1.1000) | 1 | 227  | 0.899 | H1a | 1 |                                                |
| PF-2015-46    | 0.965  | 0.0602 (0.9100-1.0200) | 1 | 2542 | 0.852 | H1a | 1 |                                                |
| PF-2015-48    | 0.995  | 0.0164 (0.9800-1.0100) | 1 | 40   | 0.9   | H1a | 1 | Low quality sample                             |
| PF-2015-49    | 1.085  | 0.1260 (0.9700-1.2000) | 1 | 113  | 0.823 | H1b | 1 |                                                |
| PF-2015-50    | -      | -                      | - | 24   | 0.917 | H1a | 1 | Low quality sample                             |
| PF-2015-59    | 1.05   | 0.1095 (0.9500-1.1500) | 1 | 229  | 0.572 | H1b | 1 |                                                |
| PF-2015-76    | 0.98   | 0.1643 (0.8300-1.1300) | 1 | 50   | 0.94  | H1a | 1 |                                                |
| PF-2015-80    | 1.105  | 0.1917 (0.9300-1.2800) | 1 | 112  | 0.821 | H1b | 1 |                                                |
| PF-2015-82    | 1.13   | 0.1862 (0.9600-1.3000) | 1 | 1644 | 0.918 | H1a | 1 |                                                |
| PF-2017-18-36 | -      | -                      | - | 942  | 0.914 | H1a | 1 |                                                |
| PF-2018-15    | 1.04   | 0.0657 (0.9800-1.1000) | 1 | 2697 | 0.916 | H1a | 1 |                                                |
| PF-2018-37    |        |                        |   | 2382 | 0.903 | H1a | 1 |                                                |
| PF-2018-52    |        |                        |   | 9623 | 0.901 | H1a | 1 |                                                |
| PF-2018-60    | 0.965  | 0.0164 (0.9500-0.9800) | 1 | 62   | 0.903 | H1a | 1 | Low quality sample                             |
| PF-2018-64    | 0.93   | 0.0329 (0.9000-0.9600) | 1 | 3729 | 0.894 | H1a | 1 |                                                |
| PF-2013-72    | 2.11   | 0.0329 (2.0800-2.1400) | 2 | 674  | 0.591 | H1b | 2 |                                                |
|               |        |                        |   |      | 0.305 | H3  |   |                                                |
| PF-2015-47    | 1.95   | 0.1643 (1.8000-2.1000) | 2 | 165  | 0.255 | H1a | 2 |                                                |
|               |        |                        |   |      | 0.639 | H2  |   |                                                |
| QEM192*       | 2.0389 | 0.1084 (1.9400-2.1379) | 2 | 5112 | 0.32  | H1b | 2 |                                                |
|               |        |                        |   |      | 0.287 | H4  |   |                                                |

\*Imported case to Malaysia (2011), with travel history to the Philippines.

**Supplementary Table 4. Summary of the demography of Malaysian 2019 outbreak cases.**

| Site  | Country  | Collection period    | Age category (years) |                    |                    | % Male patients    |
|-------|----------|----------------------|----------------------|--------------------|--------------------|--------------------|
|       |          |                      | <5                   | 5-15               | >15                |                    |
| Sabah | Malaysia | Jan 2019 - June 2023 | 11/109<br>(10.09%)   | 61/109<br>(55.96%) | 37/109<br>(33.94%) | 62/109<br>(56.88%) |

**Supplementary Table 5. TaqMan *pfcr*t copy number quantitation assay primer and probe sequences and *pfcr*t ONT sequencing primers**

| Primer label           | Sequences (5'-3')              |
|------------------------|--------------------------------|
| Pfcrt_TaqMan_F         | ACGACACCGAAGCTTTAATTTAC        |
| Pfcrt_TaqMan_R         | TTTCCAGTAGTTCTTGTAAGACCT       |
| Pfcrt_TaqMan_Probe     | TGCTATATCCATGTTAGATGCCTGTTCACT |
| B-tubulin_Taqman_F     | CCCATTCCCACGTTTACATTTT         |
| B-tubulin_Taqman_R     | GGCACAGTTAAGGCTCTGTAT          |
| B-tubulin_Taqman_Probe | CGGGTTTGCTCCTTTAACTAGTAGAGGC   |
| Pfcrt_ONT_F            | CCGTTAATAATAAATACACGCAG        |
| Pfcrt_ONT_R            | TCCTTATAAAGTGTAATGCGATAGC      |