

**A multiplex qPCR approach for detection of *pfhrp2* and *pfhrp3* gene deletions in multiple strain infections of *Plasmodium falciparum***

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**Supplementary file 1.** Sequence alignment (first 350 bp) of pfhrp2 and pfhrp3 genes including intron sequence (grey). qHRP2/3-del primer (black) and probe (green) binding regions are highlighted. Nucleotide sequence differences in oligo binding regions between *pfhrp2* and *pfhrp3* are indicated in red.

|        |     |                                                     |                                                |    |     |
|--------|-----|-----------------------------------------------------|------------------------------------------------|----|-----|
| pfhrp2 | 1   | ATGGTTTCCTTCTCAAAAAATAAA                            | <u>GTATTATCCGCTGCCGTTTTTGCC</u>                | TC | 50  |
| pfhrp3 | 1   | ATGGTTTCCTTCTCAAAAAATAAA                            | <u>ATATTATCCGCTGCCGTTTTTGCT</u>                | TC | 50  |
| pfhrp2 | 51  | CGTACTTTTGTTAGATAAC                                 | CGTAAGCATTTTAATTGCAAATAGAAATAAAAT              |    | 100 |
| pfhrp3 | 51  | CGTACTTTTGTTAGATAAC                                 | CGTAAGTATTTTAATTGCAAATATAAATAAAAT              |    | 100 |
| pfhrp2 | 101 | ATATCATTC                                           | AATTATAAAAAATACATATAATAATAATATATATATATATAT     |    | 150 |
| pfhrp3 | 101 | ATAACACTTACTT                                       | TATAAAAAATACATATAATAATATTATATATATATATATAT      |    | 150 |
| pfhrp2 | 151 | ATATATTTATATAT                                      | -----TGTATATATATAAAATTTTTTCATTTTTAAAT          |    | 195 |
| pfhrp3 | 151 | ATATATATATATATATATATATATATATATGTACATTTTACATTTTTAAAT |                                                |    | 200 |
| pfhrp2 | 196 | GCTTTTTTATTTTTATATAGAATAA                           | <u>TCCGCATTTAATAATAACTTGTGT</u>                |    | 245 |
| pfhrp3 | 201 | GATTTTTCATTTT--TATA                                 | GAATAA <u>CTCCGAATTTAACAATAACTTGT</u>          |    | 248 |
| pfhrp2 | 246 | <u>AGC</u> AAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTAC | <u>ACGAAAC</u>                                 |    | 295 |
| pfhrp3 | 249 | <u>AGC</u> AAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTAC | <u>ACGAAAG</u>                                 |    | 298 |
| pfhrp2 | 296 | <u>TCAAGCACATG</u>                                  | <u>TAGA</u> TGATGCCCATCATGCTCATCATGTAGCCGATGCC |    | 345 |
| pfhrp3 | 299 | <u>TCAAGCACATG</u>                                  | <u>CAGG</u> TGATGCCCATCATGCACATCATGTAGCTGATGCC |    | 348 |

**Supplementary file 2.** Sequence alignment (first 360 bp) of *pfhrp2* for five reference strains from Africa (3D7, NF54, NF166.C8), Brazil (7G8) and Cambodia (NF135.C10). The oligo binding sites are highlighted in grey.

|       |                                                               |     |
|-------|---------------------------------------------------------------|-----|
| NF135 | ATGGTTTCCTTCTCAAAAAATAAAGTATTATCCGCTGCCGTTTTTGCC              | 60  |
| 3D7   | ATGGTTTCCTTCTCAAAAAATAAAGTATTATCCGCTGCCGTTTTTGCC              | 60  |
| 7G8   | ATGGTTTCCTTCTCAAAAAATAAAGTATTATCCGCTGCCGTTTTTGCC              | 60  |
| NF54  | ATGGTTTCCTTCTCAAAAAATAAAGTATTATCCGCTGCCGTTTTTGCC              | 60  |
| NF166 | ATGGTTTCCTTCTCAAAAAATAAAGTATTATCCGCTGCCGTTTTTGCC              | 60  |
| ***** |                                                               |     |
| NF135 | TTAGATAACGTAAGCATTTTAAATTGCAAATAGAAATAAATATATCATTCAATTATAAAAA | 120 |
| 3D7   | TTAGATAACGTAAGCATTTTAAATTGCAAATAGAAATAAATATATCATTCAATTATAAAAA | 120 |
| 7G8   | TTAGATAACGTAAGCATTTTAAATTGCAAATAGAAATAAATATATCATTCAATTATAAAAA | 120 |
| NF54  | TTAGATAACGTAAGCATTTTAAATTGCAAATAGAAATAAATATATCATTCAATTATAAAAA | 120 |
| NF166 | TTAGATAACGTAAGCATTTTAAATTGCAAATAGAAATAAATATATCATTCAATTATAAAAA | 120 |
| ***** |                                                               |     |
| NF135 | TACATATAATAATAATATATATATATATATATATATTTATATATTGTATATATATAAATT  | 180 |
| 3D7   | TACATATAATAATAATATATATATATATATATATATTTATATATTGTATATATATAAATT  | 180 |
| 7G8   | TACATATAATAATAATATATATATATATATATATATTTATATATTGTATATATATAAATT  | 180 |
| NF54  | TACATATAATAATAATATATATATATATATATATATTTATATATTGTATATATATAAATT  | 180 |
| NF166 | TACATATAATAATAATATATATATATATATATATATTTATATATTGTATATATATAAATT  | 180 |
| ***** |                                                               |     |
| NF135 | TTTTTCATTTTTAAATGCTTTTTTATTTTTATATAGAATAATTCCGCATTTAATAATAACT | 240 |
| 3D7   | TTTTTCATTTTTAAATGCTTTTTTATTTTTATATAGAATAATTCCGCATTTAATAATAACT | 240 |
| 7G8   | TTTTTCATTTTTAAATGCTTTTTTATTTTTATATAGAATAATTCCGCATTTAATAATAACT | 240 |
| NF54  | TTTTTCATTTTTAAATGCTTTTTTATTTTTATATAGAATAATTCCGCATTTAATAATAACT | 240 |
| NF166 | TTTTTCATTTTTAAATGCTTTTTTATTTTTATATAGAATAATTCCGCATTTAATAATAACT | 240 |
| ***** |                                                               |     |
| NF135 | TGTGTAGCAAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTACACGAAACTCAAG  | 300 |
| 3D7   | TGTGTAGCAAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTACACGAAACTCAAG  | 300 |
| 7G8   | TGTGTAGCAAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTACACGAAACTCAAG  | 300 |
| NF54  | TGTGTAGCAAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTACACGAAACTCAAG  | 300 |
| NF166 | TGTGTAGCAAAAATGCAAAAGGACTTAATTTAAATAAGAGATTATTACACGAAACTCAAG  | 300 |
| ***** |                                                               |     |
| NF135 | CACATGTAGATGATGCCCATCATGCTCATCATGTAGCCGATGCCCATCATGCTCATCATG  | 360 |
| 3D7   | CACATGTAGATGATGCCCATCATGCTCATCATGTAGCCGATGCCCATCATGCTCATCATG  | 360 |
| 7G8   | CACATGTAGATGATGCCCATCATGCTCATCATGTAGCCGATGCCCATCATGCTCATCATG  | 360 |
| NF54  | CACATGTAGATGATGCCCATCATGCTCATCATGTAGCCGATGCCCATCATGCTCATCATG  | 360 |
| NF166 | CACATGTAGATGATGCCCATCATGCTCATCATGTAGCCGATGCCCATCATGCTCATCATG  | 360 |
| ***** |                                                               |     |

**Supplementary file 3.** Sequence alignment (first 360 bp) of *pfhrp3* for five reference strains from Africa (3D7, NF54, NF166.C8), Brazil (7G8) and Cambodia (NF135.C10). The oligo binding sites are highlighted in grey.

|       |                                                              |     |
|-------|--------------------------------------------------------------|-----|
| NF166 | ATGGTTTCCTTCTCAAAAAATAAAATATTATCCGCTGCCGTTTTTGCTTCCGTACTTTTG | 60  |
| NF135 | ATGGTTTCCTTCTCAAAAAATAAAATATTATCCGCTGCCGTTTTTGCTTCCGTACTTTTG | 60  |
| 3D7   | ATGGTTTCCTTCTCAAAAAATAAAATATTATCCGCTGCCGTTTTTGCTTCCGTACTTTTG | 60  |
| 7G8   | ATGGTTTCCTTCTCAAAAAATAAAATATTATCCGCTGCCGTTTTTGCTTCCGTACTTTTG | 60  |
| NF54  | ATGGTTTCCTTCTCAAAAAATAAAATATTATCCGCTGCCGTTTTTGCTTCCGTACTTTTG | 60  |
|       | *****                                                        |     |
| NF166 | TTAGATAACGTAAGTATTTTAATTGCAAATATAAATAAATAAACACTTACTTATAAAAA  | 120 |
| NF135 | TTAGATAACGTAAGTATTTTAATTGCAAATATAAATAAATAAACACTTACTTATAAAAA  | 120 |
| 3D7   | TTAGATAACGTAAGTATTTTAATTGCAAATATAAATAAATAAACACTTACTTATAAAAA  | 120 |
| 7G8   | TTAGATAACGTAAGTATTTTAATTGCAAATATAAATAAATAAACACTTACTTATAAAAA  | 120 |
| NF54  | TTAGATAACGTAAGTATTTTAATTGCAAATATAAATAAATAAACACTTACTTATAAAAA  | 120 |
|       | *****                                                        |     |
| NF166 | TACATATAATAATATTATATATATATATATATATATATATATATATATATATATATATGT | 180 |
| NF135 | TACATATAATAATATTATATATATATATATATATATATATATATATATATATATATATGT | 180 |
| 3D7   | TACATATAATAATATTATATATATATATATATATATATATATATATATATATATATATGT | 180 |
| 7G8   | TACATATAATAATATTATATATATATATATATATATATATATATATATATATATATATGT | 180 |
| NF54  | TACATATAATAATATTATATATATATATATATATATATATATATATATATATATATATGT | 180 |
|       | *****                                                        |     |
| NF166 | ACATTTTACATTTTAAATGATTTTTCATTTTATAGAATAACTCCGAATTTAACAATA    | 240 |
| NF135 | ACATTTTACATTTTAAATGATTTTTCATTTTATAGAATAACTCCGAATTTAACAATA    | 240 |
| 3D7   | ACATTTTACATTTTAAATGATTTTTCATTTTATAGAATAACTCCGAATTTAACAATA    | 240 |
| 7G8   | ACATTTTACATTTTAAATGATTTTTCATTTTATAGAATAACTCCGAATTTAACAATA    | 240 |
| NF54  | ACATTTTACATTTTAAATGATTTTTCATTTTATAGAATAACTCCGAATTTAACAATA    | 240 |
|       | *****                                                        |     |
| NF166 | ACTTGTTTAGCAAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTACACGAAAGTC | 300 |
| NF135 | ACTTGTTTAGCAAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTACACGAAAGTC | 300 |
| 3D7   | ACTTGTTTAGCAAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTACACGAAAGTC | 300 |
| 7G8   | ACTTGTTTAGCAAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTACACGAAAGTC | 300 |
| NF54  | ACTTGTTTAGCAAAAATGCAAAAGGACTTAATTCAAATAAGAGATTATTACACGAAAGTC | 300 |
|       | *****                                                        |     |
| NF166 | AAGCACATGCAGGTGATGCCCATCATGCACATCATGTAGCTGATGCCCATCATGCACATC | 360 |
| NF135 | AAGCACATGCAGGTGATGCCCATCATGCACATCATGTAGCTGATGCCCATCATGCACATC | 360 |
| 3D7   | AAGCACATGCAGGTGATGCCCATCATGCACATCATGTAGCTGATGCCCATCATGCACATC | 360 |
| 7G8   | AAGCACATGCAGGTGATGCCCATCATGCACATCATGTAGCTGATGCCCATCATGCACATC | 360 |
| NF54  | AAGCACATGCAGGTGATGCCCATCATGCACATCATGTAGCTGATGCCCATCATGCACATC | 360 |
|       | *****                                                        |     |

**Supplementary file 4.** Evaluation of *pfrnr2e2* as a biomarker for detection and quantification of *P. falciparum* parasites during controlled human malaria infection (CHMI). Bland-Altman plot of average parasitaemia density (x-axis) and ratio of parasitaemia levels calculated between internal control of qHRP2/3-del assay, *pfrnr2e2*, and qPCR assays based on 18S rDNA detection (y-axis). Average ratio (black line) and 95 % limits of agreement (dashed line) are depicted.

