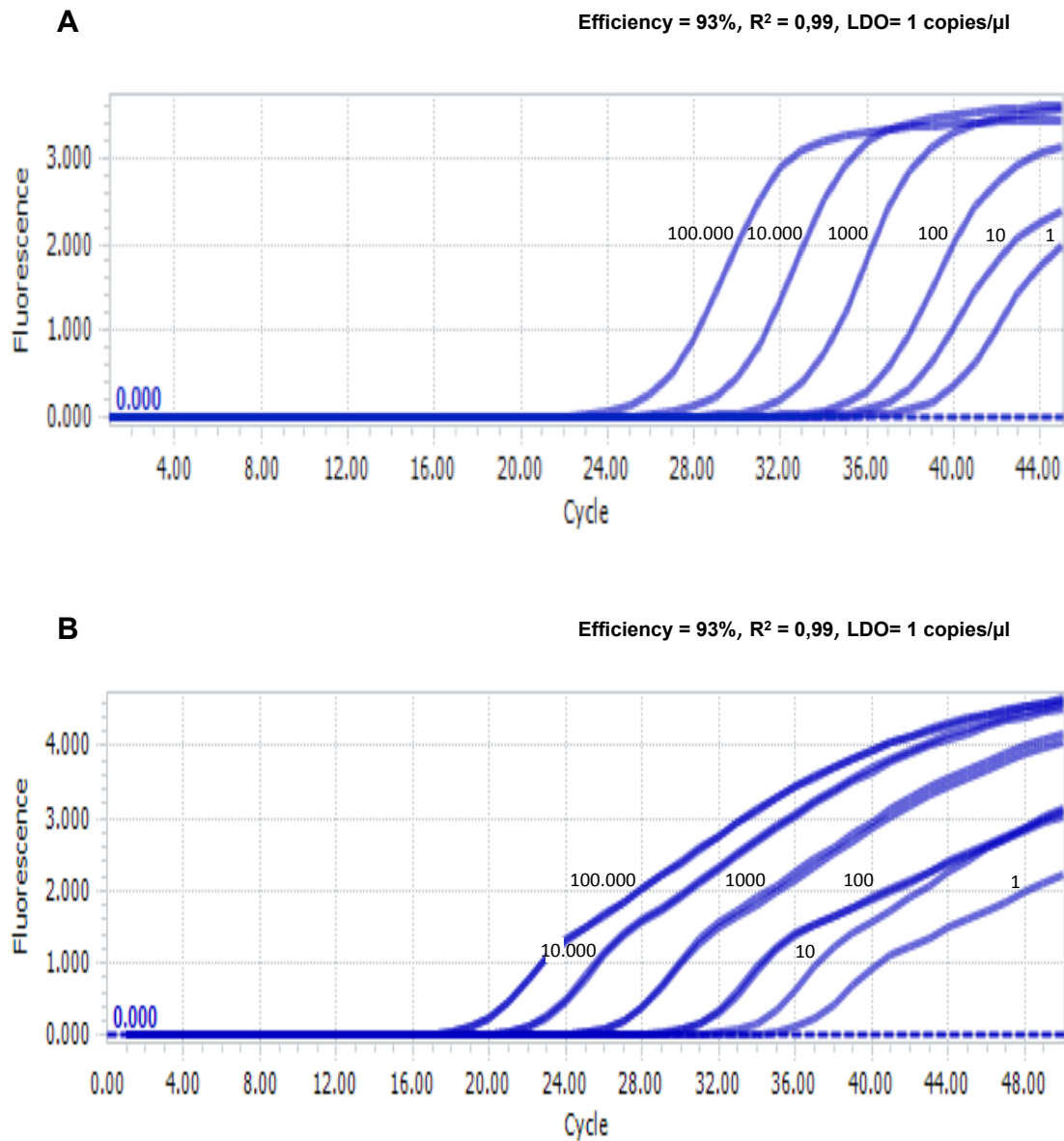


# A new high-resolution melting analysis for the detection and identification of *Plasmodium* in human and *Anopheles* vectors of malaria

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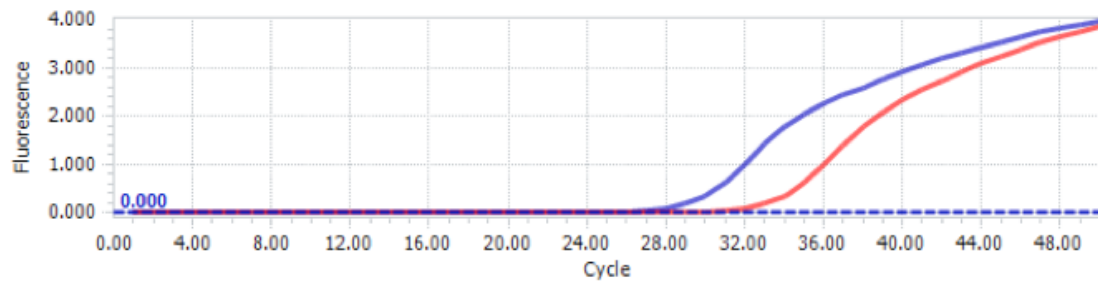
**Supplementary Table S1. Theoretical melting temperatures of the three species of *Plasmodium* studied.** The theoretical melting temperatures ( $T_m$ ) were calculated using OligoCalc (<http://biotools.nubic.northwestern.edu/OligoCalc.html>) and uMelt v2.0.2 (<http://www.dna.utah.edu/umelt/um.php>), for the three species studied: *P. vivax*, *P. falciparum* and *P. malariae*. The GenBank accession numbers listed were used to simulation of theoretical temperature. The sequences were aligned in Mega 6. Only the sequences with the full amplicons were analysed.

| <i>Plasmodium</i><br>species | GenBank<br>accession | Endmal18SF-R<br>theoretical $T_m$ |           | GenBank<br>accession | Amzmal18SF-R<br>theoretical $T_m$ |           |
|------------------------------|----------------------|-----------------------------------|-----------|----------------------|-----------------------------------|-----------|
|                              |                      | uMelt                             | OligoCalc |                      | uMelt                             | OligoCalc |
| <i>P. vivax</i>              | X13926               | 79                                | 79.05     | X13926               | 77                                | 76.15     |
| <i>P. vivax</i>              | LT635613             | 79                                | 79.05     | LT615241             | 77                                | 76.15     |
| <i>P. vivax</i>              | LT615265             | 79                                | 79.05     | JQ627158             | 77                                | 76.15     |
| <i>P. vivax</i>              | LT615257             | 79                                | 79.05     | U83877               | 77                                | 76.15     |
| <i>P. vivax</i>              | LT615240             | 79                                | 79.05     | JQ627155             | 77                                | 76.15     |
| <i>P. vivax</i>              | U07367               | 79                                | 79.05     | JQ627156             | 77                                | 76.15     |
| <i>P. vivax</i>              | U03079               | 79                                | 79.05     | JQ627157             | 77                                | 76.15     |
| <i>P. vivax</i>              | LT635621             | 79                                | 79.05     | JQ627153             | 77                                | 76.15     |
| <i>P. falciparum</i>         | M19172               | 77.5                              | 77.64     | XR_002273101         | 74                                | 73.91     |
| <i>P. falciparum</i>         | XR_002273101         | 77.5                              | 77.64     | XR_002273080         | 74                                | 73.91     |
| <i>P. falciparum</i>         | XR_002273080         | 77.5                              | 77.64     | CP016997             | 74                                | 73.91     |
| <i>P. falciparum</i>         | CP016997             | 77.5                              | 77.64     | CP016995             | 74                                | 73.91     |
| <i>P. falciparum</i>         | CP016995             | 77.5                              | 77.64     | AL844506             | 74                                | 73.91     |
| <i>P. falciparum</i>         | AL844506             | 77.5                              | 77.64     | AL844504             | 74                                | 73.91     |
| <i>P. falciparum</i>         | AL844504             | 77.5                              | 77.64     | pdb 3J7A A           | 74                                | 73.91     |
| <i>P. falciparum</i>         | pdb 3J7A A           | 77.5                              | 77.64     | JQ627151             | 74                                | 73.91     |
| <i>P. malariae</i>           | M54897               | 78.2                              | 78.22     | AB489195             | 74.5                              | 73.68     |
| <i>P. malariae</i>           | LT594631             | 78.2                              | 78.22     | AB489196             | 74.5                              | 73.68     |
| <i>P. malariae</i>           | LT594624             | 78.2                              | 78.22     | LT594491             | 74.5                              | 73.68     |
| <i>P. malariae</i>           | LT594624             | 78.2                              | 78.22     | -----                | -----                             | -----     |
| <i>P. malariae</i>           | AF488000             | 78.2                              | 78.22     | -----                | -----                             | -----     |
| <i>P. malariae</i>           | AF487999             | 78.2                              | 78.22     | -----                | -----                             | -----     |

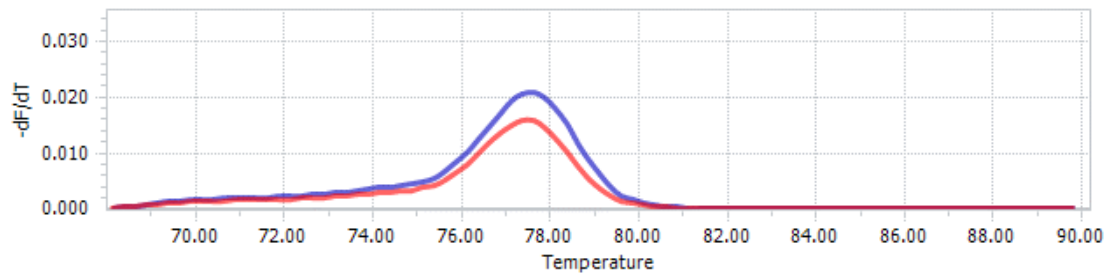


**Supplementary Figure S1. PCR-HRM standard curves.** Standard curve with six point of dilution on base 10, of a plasmid of *P. falciparum* 3D7, using LightCycler® 96 v1.1.0.1320. Primers: **A.** Endmal18SF-R. **B.** Amzmal18SF-R.

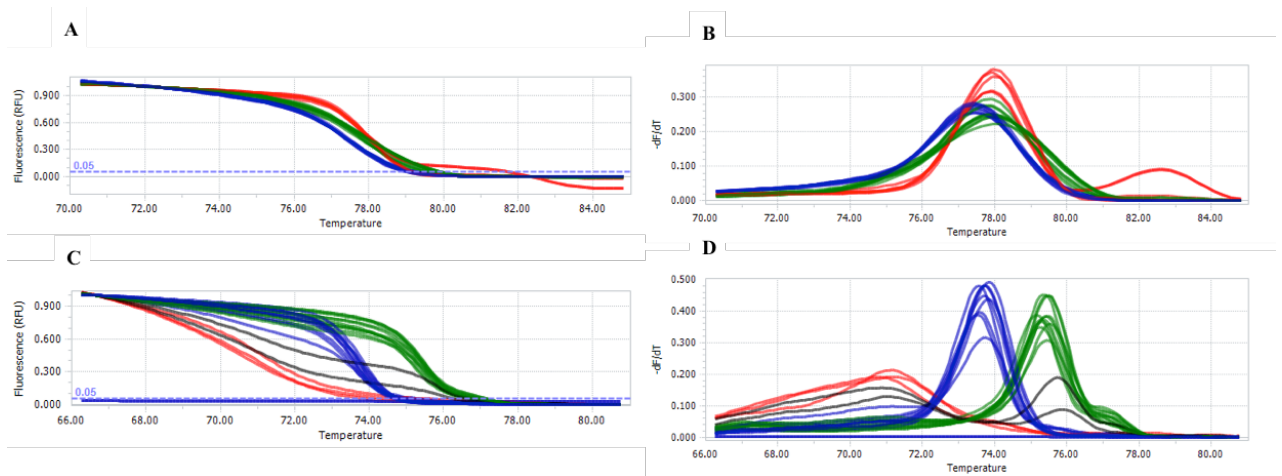
**A**



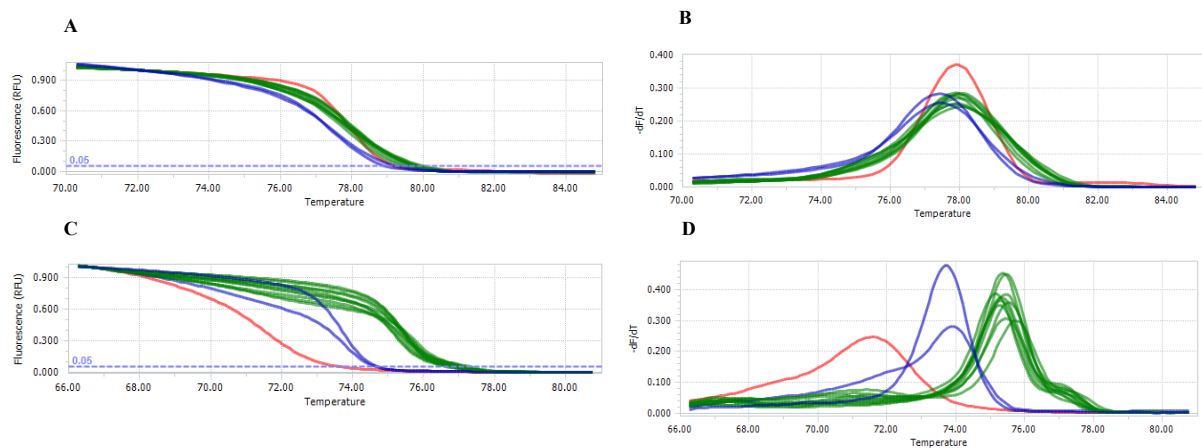
**B**



**Supplementary Figure S2. DNA interfering Assay.** For Endmal18sF-R primers: **A.** Amplification curve. **B.** Melting curve of *P. falciparum* 3D7. 10ng of *P. falciparum* 3D7 was used in a 1:1 (blue) and 1:100 ratio (red).



**Supplementary Figure S3. PCR-HRM analysis of clinical samples.** A and B. Normalised melting curves and peaks using the Endmal18sF-R primers. C and D. Normalised melting curves and peaks using the Amzmal18sF-R primers. Blue curve: *P. falciparum*. Green curve: *P. vivax*. Red curve: *P. malariae*. Black curves with the Amzmal18sF-R are mixed infection of clinical samples.



**Supplementary Figure S4. PCR-HRM analysis of mosquitoes samples.** A and B. Normalised melting curves and peaks using the Endmal18sF-R primers. C and D. Normalised melting curves and peaks using the Amzmal18sF-R primers. Blue curve: *P. falciparum*. Green curve: *P. vivax*. Red curve: *P. malariae*.

**Supplementary Tables S2.** Melting temperatures of the clinical and mosquito samples.

| Melting temperatures of the clinical samples with the primers Endmal18sF-R |              |                      |          |                 |                    |                      |
|----------------------------------------------------------------------------|--------------|----------------------|----------|-----------------|--------------------|----------------------|
| Sample ID                                                                  | Blood smears | Snounou's nested PCR | PCR-HRM  | Tm (°C)         |                    |                      |
|                                                                            |              |                      |          | <i>P. vivax</i> | <i>P. malariae</i> | <i>P. falciparum</i> |
| HUMAN 1                                                                    | +            | +                    | POSITIVE |                 |                    | 77.37                |
| HUMAN 2                                                                    | +            | +                    | POSITIVE | 77.66           |                    |                      |
| HUMAN 3                                                                    | +            | +                    | POSITIVE | 77.94           |                    |                      |
| HUMAN 4                                                                    | +            | +                    | POSITIVE |                 |                    | 77.44                |
| HUMAN 5                                                                    | +            | +                    | POSITIVE | 77.67           |                    |                      |
| HUMAN 6                                                                    | -            | -                    | POSITIVE |                 | 78.76              |                      |
| HUMAN 7                                                                    | -            | -                    | POSITIVE |                 | 78.28              |                      |
| HUMAN 8                                                                    | -            | -                    | POSITIVE | 77.91           |                    |                      |
| HUMAN 9                                                                    | -            | -                    | POSITIVE | 77.95           |                    |                      |
| HUMAN 10                                                                   | +            | +                    | POSITIVE |                 |                    | 77.20                |
| HUMAN 11                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 12                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 13                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 14                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 15                                                                   | +            | +                    | POSITIVE |                 |                    | 77.50                |
| HUMAN 16                                                                   | +            | +                    | POSITIVE |                 |                    | 77.39                |
| HUMAN 17                                                                   | +            | +                    | POSITIVE |                 |                    | 77.44                |
| HUMAN 18                                                                   | +            | +                    | POSITIVE |                 |                    | 77.43                |
| HUMAN 19                                                                   | +            | +                    | POSITIVE |                 |                    | 77.38                |
| HUMAN 20                                                                   | +            | +                    | POSITIVE |                 |                    | 77.42                |
| HUMAN 21                                                                   | +            | +                    | POSITIVE |                 |                    | 77.52                |
| HUMAN 22                                                                   | +            | +                    | POSITIVE |                 |                    | 77.25                |
| HUMAN 23                                                                   | +            | +                    | NEGATIVE |                 |                    |                      |
| HUMAN 24                                                                   | +            | +                    | POSITIVE |                 |                    | 77.15                |
| HUMAN 25                                                                   | -            | -                    | POSITIVE |                 |                    | 77.46                |
| HUMAN 26                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 27                                                                   | +            | +                    | POSITIVE |                 |                    | 77.47                |
| HUMAN 28                                                                   | +            | +                    | POSITIVE | 77.7            |                    |                      |
| HUMAN 29                                                                   | +            | +                    | POSITIVE | 77.77           |                    |                      |
| HUMAN 30                                                                   | +            | +                    | POSITIVE | 77.76           |                    |                      |
| HUMAN 31                                                                   | +            | +                    | POSITIVE | 77.74           |                    |                      |
| HUMAN 32                                                                   | -            | +                    | NEGATIVE |                 |                    |                      |
| HUMAN 33                                                                   | -            | +                    | POSITIVE | 77.72           |                    |                      |
| HUMAN 34                                                                   | -            | +                    | POSITIVE |                 | 78.23              |                      |
| HUMAN 35                                                                   | -            | +                    | POSITIVE | 77.89           |                    |                      |
| HUMAN 36                                                                   | +            | +                    | POSITIVE | 77.68           |                    |                      |
| HUMAN 37                                                                   | +            | +                    | POSITIVE | 77.8            |                    |                      |
| HUMAN 38                                                                   | -            | -                    | POSITIVE |                 |                    | 77.27                |
| HUMAN 39                                                                   | -            | -                    | POSITIVE |                 |                    | 77.27                |
| HUMAN 40                                                                   | +            | +                    | POSITIVE |                 |                    | 77.16                |
| HUMAN 41                                                                   | +            | +                    | POSITIVE | 77.72           |                    |                      |
| AVERAGE                                                                    |              |                      |          | 77.78           | 78.42              | 77.36                |
| Sd                                                                         |              |                      |          | 0.15            | 0.34               | 0.11                 |

| Melting temperatures of the clinical samples with the primers Amzmal18sF-R |              |                      |          |                 |                    |                      |
|----------------------------------------------------------------------------|--------------|----------------------|----------|-----------------|--------------------|----------------------|
| Sample ID                                                                  | Blood smears | Snounou's nested PCR | PCR-HRM  | Tm (°C)         |                    |                      |
|                                                                            |              |                      |          | <i>P. vivax</i> | <i>P. malariae</i> | <i>P. falciparum</i> |
| HUMAN 1                                                                    | +            | +                    | POSITIVE |                 |                    | 73.67                |
| HUMAN 2                                                                    | +            | +                    | POSITIVE | 75.28           |                    |                      |
| HUMAN 3                                                                    | +            | +                    | POSITIVE | 75.7            | 71                 |                      |
| HUMAN 4                                                                    | +            | +                    | POSITIVE |                 |                    | 73.77                |
| HUMAN 5                                                                    | +            | +                    | POSITIVE | 75.4            |                    |                      |
| HUMAN 6                                                                    | -            | -                    | POSITIVE |                 | 71.08              |                      |
| HUMAN 7                                                                    | -            | -                    | POSITIVE |                 | 71.05              |                      |
| HUMAN 8                                                                    | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 9                                                                    | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 10                                                                   | +            | +                    | POSITIVE |                 |                    | 73.77                |
| HUMAN 11                                                                   | -            | -                    | POSITIVE |                 |                    | 73.58                |
| HUMAN 12                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 13                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 14                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 15                                                                   | +            | +                    | POSITIVE |                 |                    | 73.7                 |
| HUMAN 16                                                                   | +            | +                    | POSITIVE |                 |                    | 73.68                |
| HUMAN 17                                                                   | +            | +                    | POSITIVE |                 |                    | 73.82                |
| HUMAN 18                                                                   | +            | +                    | POSITIVE |                 |                    | 73.79                |
| HUMAN 19                                                                   | +            | +                    | POSITIVE |                 |                    | 73.66                |
| HUMAN 20                                                                   | +            | +                    | POSITIVE |                 |                    | 73.5                 |
| HUMAN 21                                                                   | +            | +                    | POSITIVE |                 |                    | 73.49                |
| HUMAN 22                                                                   | +            | +                    | POSITIVE |                 |                    | 73.49                |
| HUMAN 23                                                                   | +            | +                    | POSITIVE |                 |                    | 73.39                |
| HUMAN 24                                                                   | +            | +                    | POSITIVE |                 |                    | 73.49                |
| HUMAN 25                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 26                                                                   | -            | -                    | POSITIVE | 75.3            |                    |                      |
| HUMAN 27                                                                   | +            | +                    | POSITIVE |                 |                    | 73.87                |
| HUMAN 28                                                                   | +            | +                    | POSITIVE | 75.1            |                    |                      |
| HUMAN 29                                                                   | +            | +                    | POSITIVE | 75.17           |                    |                      |
| HUMAN 30                                                                   | +            | +                    | POSITIVE | 75.48           |                    |                      |
| HUMAN 31                                                                   | +            | +                    | POSITIVE | 75.26           |                    |                      |
| HUMAN 32                                                                   | -            | +                    | POSITIVE | 75.42           |                    |                      |
| HUMAN 33                                                                   | -            | +                    | POSITIVE | 75.43           |                    |                      |
| HUMAN 34                                                                   | -            | +                    | POSITIVE | 75.72           | 70.7               |                      |
| HUMAN 35                                                                   | -            | +                    | POSITIVE | 75.25           |                    |                      |
| HUMAN 36                                                                   | +            | +                    | POSITIVE | 75.49           |                    |                      |
| HUMAN 37                                                                   | +            | +                    | POSITIVE | 75.39           |                    |                      |
| HUMAN 38                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 39                                                                   | -            | -                    | NEGATIVE |                 |                    |                      |
| HUMAN 40                                                                   | +            | +                    | POSITIVE |                 |                    | 73.39                |
| HUMAN 41                                                                   | +            | +                    | POSITIVE | 75.67           | 71.1               |                      |
| AVERAGE                                                                    |              |                      |          | 75.40           | 70.99              | 73.64                |
| Sd                                                                         |              |                      |          | 0.19            | 0.16               | 0.16                 |

| Melting temperatures of mosquito samples with the primers<br>Endmal18sF-R |                 |                    |                      |
|---------------------------------------------------------------------------|-----------------|--------------------|----------------------|
| Sample ID                                                                 | Tm (°C)         |                    |                      |
|                                                                           | <i>P. vivax</i> | <i>P. malariae</i> | <i>P. falciparum</i> |
| MOSQUITO 1                                                                | 77.68           |                    |                      |
| MOSQUITO 2                                                                | 77.86           |                    |                      |
| MOSQUITO 3                                                                | 77.94           |                    |                      |
| MOSQUITO 4                                                                | 77.91           |                    |                      |
| MOSQUITO 5                                                                | -               |                    | 77.52                |
| MOSQUITO 6                                                                | 77.76           |                    |                      |
| MOSQUITO 7                                                                | 77.97           |                    |                      |
| MOSQUITO 8                                                                | 77.8            |                    |                      |
| MOSQUITO 9                                                                | 77.88           |                    |                      |
| MOSQUITO 10                                                               | 77.76           |                    |                      |
| MOSQUITO 11-342                                                           | NEGATIVE        |                    |                      |
| <b>AVERAGE</b>                                                            | <b>77.840</b>   |                    | <b>77.52</b>         |
| <b>Sd</b>                                                                 | <b>0.096</b>    |                    |                      |

| Melting temperatures of mosquito samples with the primers<br>Amzmal18sF-R |                 |                    |                      |
|---------------------------------------------------------------------------|-----------------|--------------------|----------------------|
| Sample ID                                                                 | Tm (°C)         |                    |                      |
|                                                                           | <i>P. vivax</i> | <i>P. malariae</i> | <i>P. falciparum</i> |
| MOSQUITO 1                                                                | 75.14           |                    |                      |
| MOSQUITO 2                                                                | 75.6            |                    |                      |
| MOSQUITO 3                                                                | 75.74           |                    |                      |
| MOSQUITO 4                                                                | 75.42           |                    |                      |
| MOSQUITO 5                                                                | NEGATIVE        |                    | 73.82                |
| MOSQUITO 6                                                                | 75.5            |                    |                      |
| MOSQUITO 7                                                                | 75.49           |                    |                      |
| MOSQUITO 8                                                                | 75.28           |                    |                      |
| MOSQUITO 9                                                                | 75.63           |                    |                      |
| MOSQUITO 10                                                               | 75.43           |                    |                      |
| MOSQUITO 11-342                                                           | NEGATIVE        |                    |                      |
| <b>AVERAGE</b>                                                            | <b>75.47</b>    |                    | <b>73.82</b>         |
| <b>Sd</b>                                                                 | <b>0.182</b>    |                    |                      |

**Supplementary Table S3. Theoretical melting temperatures of *P. brasilianum* and *P. simium*.** The theoretical melting temperatures ( $T_m$ ) were calculated using OligoCalc (<http://biotools.nubic.northwestern.edu/OligoCalc.html>) and uMelt v2.0.2 (<http://www.dna.utah.edu/umelt/um.php>), for *P. brasilianum* and *P. simium*. The GenBank accession numbers listed were used to simulation of theoretical temperature. The sequences were aligned in Mega 6. Only the sequences with the full amplicons were analysed.

| <i>Plasmodium</i> species | GenBank accession | Amzmal18SF-R theoretical $T_m$ |           |
|---------------------------|-------------------|--------------------------------|-----------|
|                           |                   | uMelt                          | OligoCalc |
| <i>P. brasilianum</i>     | KC906728          | 71.4                           | 74.25     |
| <i>P. simium</i>          | U69605            | 76.7                           | 76.8      |