**Additional File 2. Detail of PCR primers used for detection of malaria parasite species in Anopheles mosquito specimens**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Target | Genus/species | Primer name | Sequence (5’ - 3’) | Annealing temp. (oC) | Size of PCR product (bp) | Reference |
| SSU-rRNA | *Plasmodium* genus | rPLU1 | TCAAAGATTAAGCCATGCAAGTGA | 55 | 1640 | [1] |
| rPLU5 | CCTGTTGTTGCCTTAAACTCC | [1] |
| rPLU3 | TTTTTATAAGGATAACTACGGAAAAGCTGT | 62 | 240 | [1] |
| rPLU4 | TACCCGTCATAGCCATGTTAGGCCAATACC | [1] |
| SSU-rRNA | *P. coatneyi* | PctF1 | CGCTTTTAGCTTAAATCCACATAACAGAC | 62 | 504 | [2] |
| PctR1 | GAGTCCTAACCCCGAAGGGAAAGG | [2] |
| *P. inui* | PinF2 | CGTATCGACTTTGTGGCATTTTTCTAC | 60 | 479 | [2] |
| INAR3 | GCAATCTAAGAGTTTTAACTCCTC | [2] |
| *P. fieldi* | PfldF1 | GGTCTTTTTTTTGCTTCGGTAATTA | 66 | 421 | [2] |
| PfldR2 | AGGCACTGAAGGAAGCAATCTAAGAGTTTC | [2] |
| *P. cynomolgi* | CY2F | GATTTGCTAAATTGCGGTCG | 60 | 137 | [2] |
| CY4R | CGGTATGATAAGCCAGGGAAGT | [2] |
| *P. knowlesi* | PkF1140 | GATTCATCTATTAAAAATTTGCTTC | 50 | 424 | [3] |
| PkR1550 | GAGTTCTAATCTCCGGAGAGAAAAGA | [3] |
| *P. falciparum* | NewPLFshort | CTATCAGCTTTTGATGTTAG | 53 | 370 | [4] |
| FARshort | GTTCCCCTAGAATAGTTACA | [4] |
| *P. vivax* | NewPLFshort | CTATCAGCTTTTGATGTTAG | 53 | 476 | [4] |
| VIRshort | AAGGACTTCCAAGCC | [4] |
| *P. malariae* | NewPLFshort | CTATCAGCTTTTGATGTTAG | 53 | 241 | [4] |
| MARshort | TCCAATTGCCTTCTG | [4] |
| *P. ovale* | NewPLFshort | CTATCAGCTTTTGATGTTAG | 53 | 407 | [4] |
| OVRshort | AGGAATGCAAAGARCAG | [4] |

[1]Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg. 1999;60(4):687-692.

[2]Lee KS, Divis PCS, Zakaria SK, Matusop A, Julin RA, Conway DJ et al. *Plasmodium knowlesi*: Reservoir hosts and tracking the emergence in humans and macaques. Plos Pathog. 2011;7:4.

[3]Imwong M, Tanomsing N, Pukrittayakamee S, Day, NPJ, White NJ, Snounou G. Spurious amplification of a *Plasmodium vivax* small-subunit RNA gene by use of primers currently used to detect *P. knowlesi.* J Clin Microbiol. 2009;47(12):4173-4175.

[4]Ta TH, Hisam S, Lanza M, Jiram AI, Ismail NP, Rubio JM. First case of a naturally acquired human infection with *Plasmodium cynomolgi*. Malaria J. 2014;13:68.