**A mismatch-tolerant reverse transcription loop-mediated isothermal amplification method and its application on simultaneous detection of all four serotype of dengue viruses**

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**Supplementary Information**

**Supplementary Table S1. Primers and probes used in the study.**

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| **Aim** | **Primers** | **Sequence** (5’-3’) | **Ref.** |
| Mutant construction | F3-Mu-A(F) | CAAACCGTGCTGCCTGAAGCTCCGCCAAT | This study |
| F3-Mu-A(R) | TCAGGCAGCACGGTTTGAATCGTCG |
| F3-Mu-G(F) | CAAACCGTGCTGCCTGGAGCTCCGCCAAT |
| F3-Mu-G(R) | CCAGGCAGCACGGTTTGAATCGTCG |
| F3-Mu-C(F) | CAAACCGTGCTGCCTGCAGCTCCGCCAAT |
| F3-Mu-C(R) | GCAGGCAGCACGGTTTGAATCGTCG |
| F2-Mu-A(F) | CACGGAAGCTGTACGAGTGGCATATTGGA |
| F2-Mu-A(R) | TCGTACAGCTTCCGTGGCGCATGGCCTC |
| F2-Mu-T(F) | CACGGAAGCTGTACGTGTGGCATATTGGA |
| F2-Mu-T(R) | ACGTACAGCTTCCGTGGCGCATGGCCTC |
| F2-Mu-G(F) | CACGGAAGCTGTACGGGTGGCATATTGGA |
| F2-Mu-G(R) | CCGTACAGCTTCCGTGGCGCATGGCCTC |
| F1-Mu-A(F) | GGTTAGAGGAGACCCCATCCCATCACTGAC |
| F1-Mu-A(R) | TGGGGTCTCCTCTAACCGCTAGTCCA |
| F1-Mu-C(F) | GGTTAGAGGAGACCCCCTCCCATCACTGAC |
| F1-Mu-C(R) | GGGGGTCTCCTCTAACCGCTAGTCCA |
| F1-Mu-G(F) | GGTTAGAGGAGACCCCGTCCCATCACTGAC |
| F1-Mu-G(R) | CGGGGTCTCCTCTAACCGCTAGTCCA |
| BLP-Mu-G(F) | CAGAGATCCTGCTGTCTGTGCAACATCA |
| BLP-Mu-G(R) | CAGACAGCAGGATCTCTGGTCTTTCCCAG |
| BLP-Mu-A(F) | CAGAGATCCTGCTGTCTATGCAACATCA |
| BLP-Mu-A(R) | TAGACAGCAGGATCTCTGGTCTTTCCCAG |
| BLP-Mu-T(F) | CAGAGATCCTGCTGTCTTTGCAACATCA |
| BLP-Mu-T(R) | AAGACAGCAGGATCTCTGGTCTTTCCCAG |
| RT-LAMP assay of DENV | F3/134 | CAAACCGTGCTGCCTGT | Teoh et al., 2013 |
| F3/2 | TGAGTAAACTATGCAGCCTGT |
| B3/123 | ACCTGTTGATTCAACAGCACC |
| B3/4 | ACCTGTTGGATCAACAACACC |
| FIP/123 | AGGGGTCTCCTCTAACCRCTAGTCTTTCAAACCRTGGAAGCTGTACGC |
| FIP/4 | AGGGGTCTCCTCTAACCRCTAGTCTTTTTTGCCACGGAAGCTGTACGC |
| BIP/123 | ACAGCATATTGACGCTGGGARAGACGTTCTGTGCCTGGAATGATGCTG |
| BIP/4 | ACAGCATATTGACGCTGGGARAGACGCTCTGTGCCTGGATTGATGTTG |
| BLP/1234 | CAGAGATCCTGCTGTCTC |
| RT-qPCR assay of DENV | Pan-DENV-F | AAGGACTAGAGGTTAGAGGAGAC | [Go et al., 2016](#_ENREF_1) |
| Pan-DENV-R | GGCGTTCTGTGCCTGGAATGAT |
| Pan-DENV-P | FAM-CCAGAGATCCTGCTGTCTC-MGB-NFQ |

The artificially introduced mutations are highlighted in red.



**Supplementary Figure S1. Sequence comparison among four DENV standard strains.** Only the primer regions having mutations are shown.

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| **30 minutes** | F:\学生进展报告\周毅文章\MS\Revision\抗突变LAMP回复意见材料\30min.tiff |
| **40 minutes** | 40 min |
| **60 minutes** | F:\学生进展报告\周毅文章\MS\Revision\抗突变LAMP回复意见材料\60min.tiff |

**Supplementary Figure S2. Visual detection of four DENV serotypes using the novel mismatch-tolerant and the conventional RT-LAMP assays at 30-, 40- and 60-minute time points.** The color changes from violet to azure for HNB and from burgundy to orange or yellow for cresol red were considered as positive (+).

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**Supplementary Figure S3. Numbers of DENV-positive samples detected by four different assays.** Because 3 samples were negative by all the four assays, the total number of samples was 150 in this Venn diagram.



**Supplementary Figure S4. Sequence analysis of the samples with a Tt difference of more than 15 minutes between the novel and the conventional RT-LAMP assays.** Only the F2 region is shown because it is where mutations were detected in some samples.