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Dengue genotyping by E gene amplification and sequencing

PLOS Neglected Tropical Diseases

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ABSTRACT

1)The partial E gene of dengue virus is amplified before sequencing by using four sets of serotype-specific oligonucleotides as referenced in the manuscript text.
2) The reaction mixture is prepared as the following:
12.5ul of MyTaq RT-PCR (Bioline, Korea)
4.5ul of Nuclease-Free Water
1.0 ul of Foward Primer for the respective serotype (10uM)
1.0 ul of Reverse Primer for the respective serotype (10uM)
0.5ul of RNase inhibitor
0.5ul of Reverse Transcriptase (RT)
3) Aliquot 20ul in each PCR tubes.
4) 5ul of Dengue RNA (with known serotype) is added to the corresponding PCR tube.
5) PCR amplification is performed on CFX-96 (BioRad) conventional PCR thermal cycler.
6) The PCR is performed with the following cycling profile: 35 cycles (94 °C for 1 min, 55 °C for 1 min and 72 °C for 1.5 min) followed by an extension reaction at 72 °C for 5 min.
7) A 25 µl aliquot of each PCR reaction is analyzed on 1.5 % pre-stained agarose by gel electrophoresis, run for 30 min at 90 V.
8) The gel is then viewed under UV illumination.
9) The expected amplicon size for each serotype is 578bp for DENV1, 617bp for DENV 2, 582bp for DENV3 and 572bp for DENV 4 The corresponding amplicons are extracted from the agarose gel and purified by Gel Extraction Kit (Qiagen, USA) according to the manufacturer 's instruction.
10) The final elution volume is 30 µl of purified PCR amplicons. Then, 5 µl of these are reanalyzed on 1.5 % agarose gel to substantiate the accuracy of purification step. The purified PCR amplicons were outsourced for sequencing.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

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