DENV E gene Amplification for Genotyping

(Version.1.2 20240111)

MATERIALS AND CONSUMABLES

- TransStart®FastPfu Fly DNA Polymerase Kit — TransGen — AP231
- RNase-free H_20 — - — -
- DL 2,000 DNA marker — Takara — 3427
- Primer:

D1F1: CACATGCCATAGGAACATCCA	D1R1: ATGAGCCTGTGCACATCACA
D1F2: GGAACAGACAAGATTTGCTGGT	D1R2: TGCCACTTCCACATTTGAGT
D2F1: TGGCATACACCATAGGAACGA	D2R1: CCTTTGAGCTGTAGTTTGTCCA
D2F2: CCTCGACTTCAATGAGATGGT	D2R2: TTGAAGGGGATTCTGGTTGGA
D3F1: AGGGTTCACAATACTAGCCCTA	D3R1: GGGCTACAACAGAAACACCA
D3F2: GTTCTCCATTCTGGTTGTCGA	D3R2: GTTCTCCATTCTGGTTGTCGA
D4F1: AGCTGGATACTCAGAAACCCAGGATT	D4R1: ACATCCTGTCTCTTGGCATGAGG
D4F2: CCTCATGCCAAGAGACAGGATGT	D4R2: AATTTGTACTGTTCTGTCCAAGTGTG

PROCEDURES

I. Total Viral RNA extraction & Reverse transcription

II. PCR

☐ 1. Prepare PCR reaction as follow,

COMPONENT	AMOUNT (μl)	SAMPLE NUM	OPERATE
Template	2.00	-	-
☐ Nuclease-free water	31.00		
☐ Forward primers (10 μmol/l)	1.00		
☐ Reverse primers (10 μmol/l)	1.00		
☐ 5 × TransStart FastPfu Fly Buffer	10.00	×_____	
☐ tNTPs (2.5 mmol/l)	4.00		
☐ TransStart FastPfu Fly DNA Polymerase	1.00		
TOTAL		50.0	

☐ 2. Place in a thermocycler and run the following program,

STEP	TEMPERATURE	TIME	CYCLE
Pre-denature	98	2 min	1
Denature	98	10 s	
Anneal	59	20 s	40
Extend	72	15 s	
Re-Extend	72	5 min	1
Stop	16	-	-

☐ 3. Analysis the product by Agarose gel electrophoresis.

III. The PCR products Sanger sequencing

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