Dengue virus subtyping of serotypes 1 through 4 using end-point PCR.

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This protocol describes the end-point polymerase chain reaction with reverse transcription (RT-PCR) approach developed and optimized for the detection and typing of Denguevirus (DENV) 1 through 4 viruses. Dengue virus are group IV ((+)ssRNA) species of an unassigned order, Flaviviridae family, Flavivirus genus related to the Zika, yellow fever, Japanese encephalitis, and West Nile viruses. DENV have genomes of 11 kb encoding three structural proteins: capsid protein (C), membrane protein (M) and envelope protein (E) as well as seven nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5).

Oligonucleotides

Name	Sequence	bp	%GC	Tm	Hair	HomD	HetD	Amplicon
DENV1-F	5′-CAT-ggg-CCT-ATC-ATg-gAT-CAT-3′	21	47.6	54	-5.75	-9.28	-8.09	401 bp
DENV1-R	5′-AAC-ggT-TCT-ggg-ACC-TTg-Tg-3′	20	55	54	-4.22	-4.41	-8.09	
DENV2-F	5′-AgA-CAg-ATT-CTT-TgA-ggg-AgC--3′	21	47.6	52	-3.28	-3.17	-4.74	300 bp
DENV2-R	5′-ggC-TTT-TgA-TTT-TTT-AAT-TgT-TC-3′	23	26.1	46	-2.99	-5.36	-4.74	
DENV3-F	5′-CTC-TgA-TgA-ACA-ACC-AAC-gg-3′	20	50	43	-2.13	-3.61	-5.24	353 bp
DENV3-R	5′-gTg-gAA-AgC-AAg-TgY-TgC-Tg-3′	20	50	49	-6.19	-8.64	-5.24	
DENV4-F	5′-TCT-ggA-AAA-ATg-AAC-CAA-CgA-3′	21	38.1	37	-2.61	-5.02	-7.32	451 bp
DENV4-R	5′-TgC-ATT-TgT-TgA-TCC-CCT-CT-3′	20	45	57	-3.76	-7.05	-7.32	

Note 1: Reverse oligonucleotide primer sequences given in this table are the reverse-complement of sequence present in alignments and as they should be ordered for synthesis; Hairpin (Hair), homodimer (homD) and heterodimer (HetD) ΔQ's given in kcal/mol.

Dual (separate RT and PCR) components and conditions

Dual (separate RT and PCR) relies on the use of Vivantis M-MULV (Cat. No.: ME2305) RT Enzyme and Biorad iTaq (Cat. No.: 1725150) qPCR Master Mix.

First strand synthesis (RT)		1x
dH_2O	cf	10 µL
10 µM Reverse oligo	500 nM	1 µL
10 mM dNTPs	250 µM	0.5 µL
RNA	-	5 µL
		vf: 16.5 µl

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Run RT-1 program in Axygen →

Total time: 5 min		
95 ºC	2 min	1 cycle
4 ºC	2 min	

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RT Buffer	1x cf	2 µL
RT Enzyme 250 IU/µl	100 IU	0.5 µL
DTT	0.1 M	1 µL
		vf: 20 µl

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Run RT-2 program in Axygen →

Total time: 1:12 hrs		
42 ºC	60 min	1 cycle
95 ºC	5 min	
4 ºC	5 min	

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Polymerase Chain Reaction (PCR)		1x
dH_2O	cf	6.2 µL
2x Master mix	1 x	7.5 µL
10 µM Forward oligo	100 nM	0.15 µL
10 µM Reverse oligo	150 nM	0.15 µL
cDNA Template	10 ng	1 µL
		vf: 10 µl

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Run DENV program in Axygen →

Total time: 2:26 hrs		
95 ºC	3 min	
94 ºC	15 sec	38 cycles
60 ºC	60 sec	
95 ºC	15 sec	
60 ºC	20 sec	
95 ºC	15 sec	

References

1. Optimized diagnosis of acute dengue fever in Swedish travelers by a combination of reverse transcription-PCR and immunoglobulin M detection. Lindegren G, Vene S, Lundkvist A, Falk KI. J Clin Microbiol. 2005 Jun;43(6):2850-5. PMID:15956408

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Changes to document format only.

4.0 Optimize for use in MiniAmp Plus cyclers.