Dengue virus type 4 (DENV-4) TaqMan (DENV4-TM2017) assay

Ian Mackay,Judy Northill

Abstract

This protocol was designed and developed at this laboratory.

The assay specifically targets the 3' UTR region of DENV-4 strains and is designed as a qualitative screening test for human cases of DENV-4 infection, but not for infection due to other known DENVs.

Citation: Ian Mackay,Judy Northill Dengue virus type 4 (DENV-4) TaqMan (DENV4-TM2017) assay. **protocols.io** dx.doi.org/10.17504/protocols.io.n7pdhmn
Published: 03 Apr 2018

Before start

- If using a different brand or model of real-time thermocycler, check the concentration of ROX is adequate.
- Method assumes the user is familiar with the thermocycler and software used to run the protocol and with PCR in general.

Materials

SuperScript® III First-Strand Synthesis System 18080-051 by Thermo Scientific

Protocol

Oligonucleotide sequences

Step 1.

Name	Sequences (5'-3')
D4-3UTR-for	CTGTAGCTCCGCCAA**H**AA**Y**GG
D4-3UTR-rev	CTCCTCTAACC**R**CTAGTCCAATA
D4-3UTR-Prb	6FAM - TGCGCCACGGAAGCTGTACGCGTGG - BHQ

Reagents

Step 2.

REAGENTS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit 11732088 by Life Technologies

Reaction set-up

Step 3.

Reagent	Vol (µl) x1	Final reaction concentration
Nuclease-free water	4.37	N/A
D4-3UTR-for (200µM)	0.07	700nM
D4-3UTR-rev (200µM)	0.09	900nM
D4-3UTR-Prb (100µM)	0.03	150nM
2X Reaction Mix[1]	10	1X
ROX reference dye (25µM)	0.04	50nM
SuperScript™ III/Platinum™ Taq Mix[1]	0.4	1X
TOTAL	15	

[1]Superscript™III Platinum™ One-step qRT-PCR kit

- Dispense 15µL to each reaction well.
- Add 5µL of template (extracted RNA, controls or NTC [nuclease-free water]).
- Total reaction volume is 20µL

Amplification

Step 4.

50°C	5min	1X
95°C	2min	1X
95°C	3sec	| 40X
60°C	30sec[1]	|

[1]Fluorescence acquisition step

Result analysis

Step 5.

The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:

1. A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase

2. A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)

3. A **defined threshold (C_T) value** which the fluorescent curve has clearly exceeded (Fig.1 arrow), which sits early in the log-linear phase and is <40 cycles

A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T >40 cycles is considered a negative result. NTCs should not produce a curve.

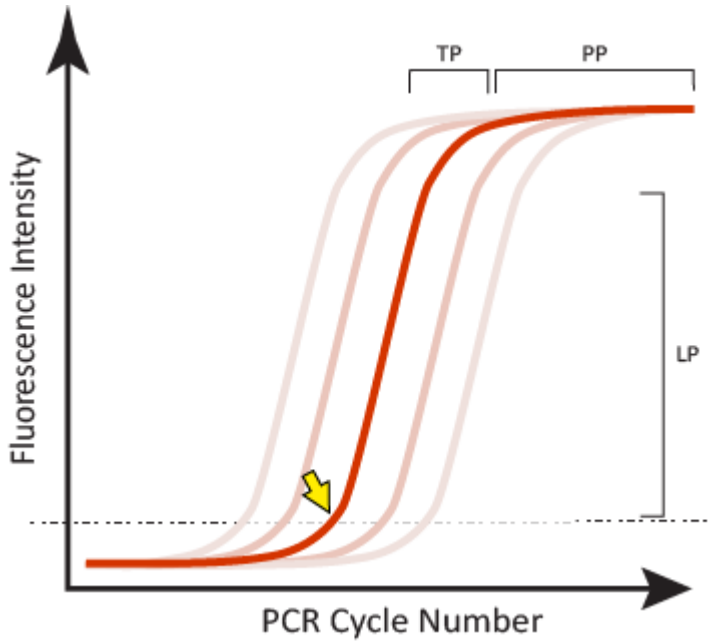

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.