Dengue virus type 2 (DENV-2) prMG-Multiplex TaqMan assay (no longer in use; see Guidelines)

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Abstract

This protocol was designed and developed at this laboratory by Greg A. Smith and colleagues for Public Helath Virology, Queensland's Department of Health, prior to his departure. It has not been previously published.

The protocol aims explicitly to amplify DENV-2 viruses and not other dengue viruses. The assay targets the membrane glycoprotein precursor region and is designed as a qualitative test for investigating suspected human cases of DENV-2 infection.

This assay has been superseded by the Dengue virus type 2 (DENV-2) MGB TaqMan (DENV2-2016MGB) assay.

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Guidelines

This was a past assay that we no longer in use.

For the best DENV-2 TaqMan assay, please refer to our recommended protocol:

Dengue virus type 2 (DENV-2) MGB TaqMan (DENV2-2016MGB) assay

https://www.protocols.io/view/dengue-virus-type-2-denv-2-mgb-taqman-denv2-2016mg-n7kdhkw

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

Protocol

Oligonucleotide sequences

Step 1.

Name	Sequence 5'-3'

D2 prMG FWD	GACCACACG**Y**AACGGAGAA
D2 prMG REV	TTTGTCTTAAACAGAAG
D2prM(b)-f	ACCACACGCAACGGAGAAC
D2prM(b)-r	CTCTGTTTT**R**AACAG**R**AGACTTTT
D2 prMG[C]	*FAM* - CTTGTCTACTGACGATCAT – *TAMRA*
D2 prMG[A]	*FAM* - CTTGTATACTGACGATCAT – *TAMRA*

Reagents

Step 2.

READAGENTS

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

Reaction set-up

Step 3.

The assay has been used on both a Rotor-Gene 6000 and a Rotor-Gene Q real-time thermocycler

Prepare sufficient mix for the number of reactions.
Include a suitable 'dead volume' as necessary if using a robotic dispenser.

MIX PREPARATION

Reagent	Volume (µl) x1	Final reaction concentration
Nuclease-free water	4.374	N/A
D2 prMG FWD 200pmol/µl	0.031	310nM
D2 prMG REV 200pmol/µl	0.031	310nM
D2prM(b)-f 200pmol/µl	0.031	310nM
D2prM(b)-r 200pmol/µl	0.031	310nM
D2 prMG[C] 100pmol/µl	0.031	155nM
D2 prMG[A] 100pmol/µl	0.031	155nM
2X Reaction Mix[1]	10	1X
SuperScript® III/Platinum® *Taq* Mix[1]	0.4	1X
ROX Reference Dye (25µM)	0.04	0.05µM
Template	5	N/A
TOTAL	20	

[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.

CYCLING CONDITIONS

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

[1]Fluorescence acquisition step

Result Analylsis

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

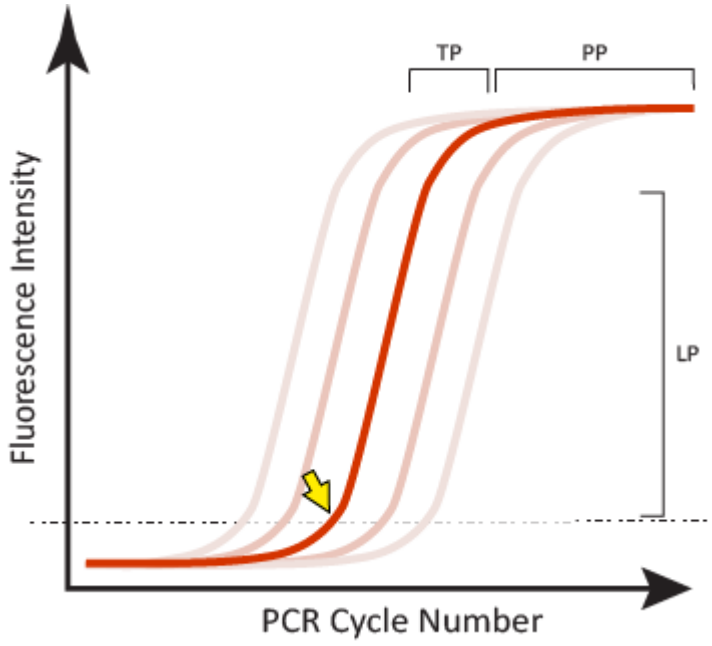

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) 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.