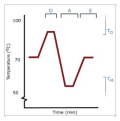

May 08, 2019

Working

Measles virus TaqMan RT-PCR (no longer in regular use; see Guidelines)

Version 2

Mitchell Finger[1], Michael Lyon[1], Judy Northill[1], Ian Mackay[1]

[1]Public Health Virology, Forensic and Scientific Services

dx.doi.org/10.17504/protocols.io.2qvgdw6

Ian M. Mackay
Public Health Virology, Forensic and Scientific Services

ABSTRACT

- This real-time TaqMan-MGB RT-PCR protocol aimed to amplify measles virus (MeV) strains and not other viruses.
- Michael Lyon and Mitchell Finger designed the assay in 2009 using Primer Express software.
- The method was later published by Greg Smith in 2010 (see below).
- The assay targets the fusion (F) gene region and is designed as a qualitative test for investigating MeV infection of humans.
- This was a past assay that we no longer in use. For our favoured Measles virus TaqMan test, please refer to the *MeV N TaqMan* protocol.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Smith G. (2010) Measles Virus. In: Schuller M., Sloots T., James G., Halliday C., Carter I. (eds) PCR for Clinical Microbiology. Springer, Dordrecht

STEPS MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
SuperScript™ III Platinum™ One-Step qRT-PCR Kit	11732088	Life Technologies		

BEFORE STARTING

- 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.

Oligonucleotide sequences

1

Name	Sequence 5'-3'
Primer Measles MGB FP	GCTCAAATTGCTCAGATACTATACAGAAA
Primer Measles MGB RP	GCAGATATGGGGTCCCGTAA
Probe Measles MGB Probe	FAM - CCTGTCATTATTTGGCC - MGBNFQ

Reagents

2

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

Reaction set-up

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.

Reagent	Volume (µl) x1	Final reaction concentration
Nuclease-free water	4.45	N/A
Measles MGB FP 150pmol/µl	0.04	300nM
Measles MGB RP 150pmol/µl	0.04	300nM
Measles MGB Probe 100pmol/µl	0.03	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

4

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

1-Fluorescence acquisition step

Result Analysis

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

- A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase
- A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)
- 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

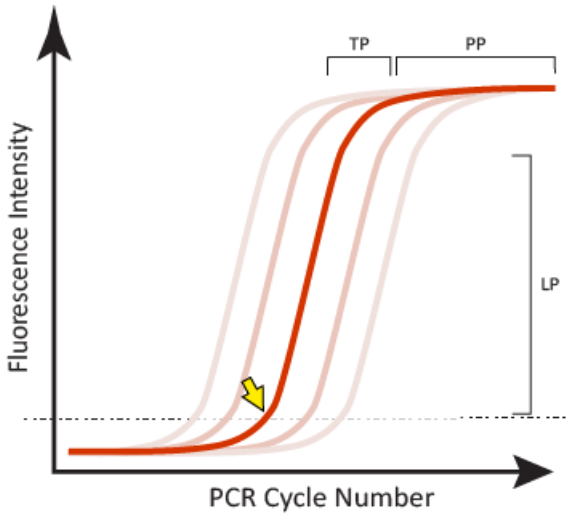

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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