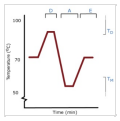

May 08, 2019

Working

Measles Vaccine Virus Taqman-MGB

Version 3

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.2qugdww

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

ABSTRACT

This previously unpublished protocol aims to amplify genotype A measles virus (MeV) strains but not non-mealses viruses.
Mitchell Finger and Michael Lyon developed this in-house test in 2010.
The assay targets the intergenic region between the M (matrix) and F (fusion) genes, designed as a qualitative test for investigating measles vaccine virus (MVV) strains.
Numbering indicates the oligonucleotide location on the sequence with MeV strain Edmonston (Moraten vaccine), complete genome, GenBank accession number AF266287.

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'
Measles F 4729 Vac	AAACCCCCAGCAATTGGAA
Measles R 4795 Vac	GGTCACCTCGGTCGCTTGT
Measles Probe 4757	FAM - CCCTCTTCCTCAACACA - MGBNFQ

Reagents

2

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

Reaction set-up

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	4.42	N/A
Measles F 4729 Vac 150pmol/µl	0.04	300nM
Measles R 4795 Vac 150pmol/µl	0.04	300nM
Measles Probe FAM 100pmol/µl	0.06	300nM
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 - SuperscriptTMIII PlatinumTM 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

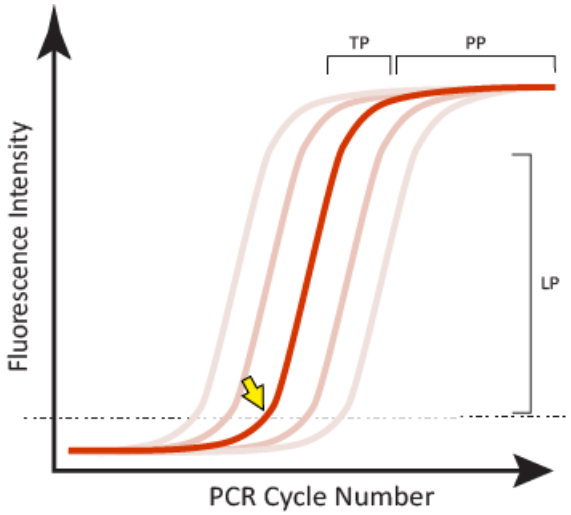

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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