Barmah Forest virus TaqMan 2017 (BF-VTM2017) Version 2

Ian Mackay,Ina Smith,Judy Northill

Abstract

The protocol aims explicitly to amplify BFV viruses and not other viruses.

The assay targets the E2 gene region and is designed as a qualitative test for investigating BFV infection of humans and arthropods.

Citation: Ian Mackay,Ina Smith,Judy Northill Barmah Forest virus TaqMan 2017 (BF-VTM2017). **protocols.io** dx.doi.org/10.17504/protocols.io.rsvd6e6

Published: 19 Jul 2018

Guidelines

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'
BFV-F	AGTGTGGCAGTACAACTCCCAAT
BFV-R	AAGGCACATGGATCTTTCCTTTC
BFV-FAM	FAM - CGTGCCCAGGTCCGAAGTTACGG- BHQ

Reagents

Step 2.

REAGENTS

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	4.47	N/A
BFV-F 200pmol/µl	0.03	300nM
BFV-R 200pmol/µl	0.03	300nM
BFV-FAM 100pmol/µl	0.03	150nM
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

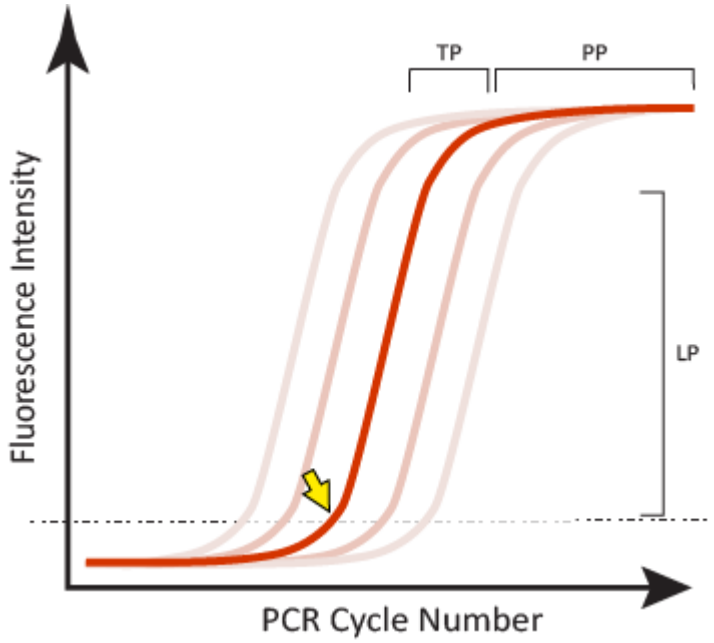

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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