Yellow fever virus real-time RT-PCR

Judy Northill,Alyssa Pyke,Ian Mackay

Abstract

A real-time RT-PCR targeting the 5' untranslated region of Yellow fever virus.
This protocol was designed and developed at this laboratory.

Citation: Judy Northill,Alyssa Pyke,Ian Mackay Yellow fever virus real-time RT-PCR. **protocols.io** dx.doi.org/10.17504/protocols.io.rszd6f6
Published: 19 Jul 2018

Guidelines

- 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.

Materials

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

Protocol

Oligonucleotide sequences

Step 1.

Name	5'-3'
YF-TaqFor	TGTGCTAATTGAGGTGCATTGG
YFV wildrev	TCTCTGCTAATCGCTCAACGAA
YFV-Prob	6FAM-AATCGAGTTGCTAGGCAATAAACACATTTGGA-BHQ1

Reaction set-up

Step 2.

- Assay has been used on both a Rotor-Gene 6000 / Rotor-Gene Q 5-plex using 100-place rotor discs.
- Total reaction volume is 20µL.
- Prepare sufficient for number of reaction plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

Reagent	Vol (µL) x1	Final reaction concentration
Nuclease free water	4.41	
YF-TaqFor (200pmol/µl)	0.03	300nM
YFV wildrev (200pmol/µl)	0.09	900nM
YFV-Prob (100pmol/µl)	0.03	150nM
2 X Reaction mix[1]	10	1X
SuperScript® III/Platinum® *Taq* Mix [1]	0.4	
ROX Reference Dye (25µM)[1]	0.04	
TOTAL	15	

[1] Superscript III Platinum One-step qRT-PCR kit, Cat no. 11732088

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 3.

The assay has been optimised and validated for the Rotor-Gene 6000 and Rotor-Gene Q thermocyclers.

RT-PCR

50°C	5min	
95°C	2min	
95°C	3s	40X
60°C	30s*	

*Florescence acquisition step

Result Analysis

Step 4.

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) and 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 value >40 cycles is considered a negative result
5. NTCs should not produce a curve

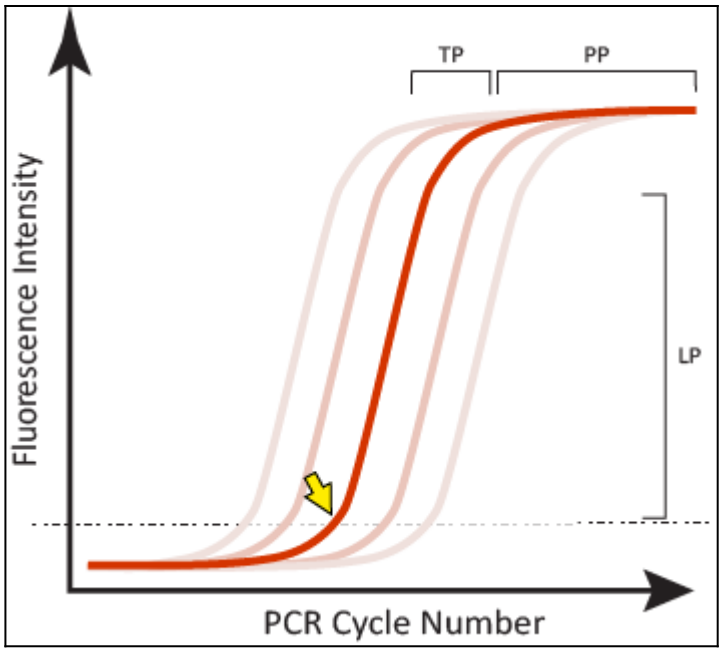

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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