Orthopoxvirus real-time PCR

Judy Northill,David Warrilow,Ian Mackay

Abstract

A real-time PCR targeting the DNA-dependent RNA polymerase of Orthopoxviruses.

Citation: Judy Northill,David Warrilow,Ian Mackay Orthopoxvirus real-time PCR. **protocols.io** dx.doi.org/10.17504/protocols.io.nxzdfp6
Published: 23 Mar 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

SensiFAST™ Probe Lo-ROX Kit BIO-84002 by Bioline

Protocol

Oligonucleotide sequences

Step 1.

Name	5'-3'
OPV2018-F	CGTACMGGAACACTRGCTAGA
OPV2018-R	AGCGTATTACCTATAACTACTTGTCCGTA
OPV2018-FAM	6FAM- ATCATTAAAAAGATGGAGGATATGGTGGTHGA -BHQ1

Step 2.

REAGENTS

SensiFAST™ Probe Lo-ROX Kit BIO-84002 by Bioline

REACTION SET-UP

Step 3.

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	Volume (uL)	Final concentration
Nuclease-free water	4.87	
SensiFast Probe Lo-Rox mix	10	1X
OPV2018-F	0.05	500nM
OPV2018-R	0.05	500nM
OPV2018-FAM	0.03	150nM
TOTAL VOLUME	**15**	

AMPLIFICATION

Step 4.

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

PCR

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

*Data aquisition step

RESULT ANALYSIS

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) 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

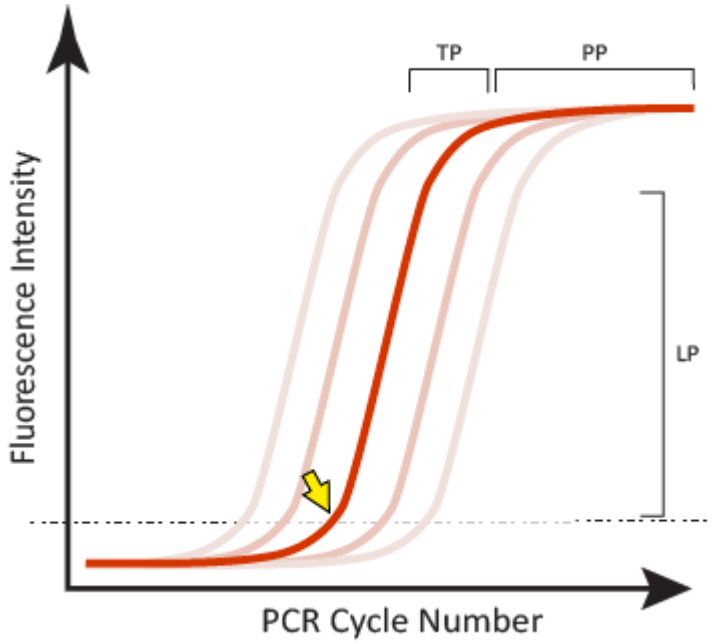

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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