Parapoxvirus real-time PCR

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Abstract

A real-time PCR for Parapoxvirus targeting the DNA polymerase. It is used to screen human samples where Parapoxvirus is suspected.

This protocol is based on the published RVSS assay by Das et al 2017. Oligonucleotides have been modified and a different PCR kit is used.

Citation: Judy Northill,Ian Mackay Parapoxvirus real-time PCR. **protocols.io**
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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.

Protocol

Oligonucleotide sequences

Step 1.

Name	5'-3'
PPV2018TM-F2	GATGGCYGTGCAGCTCTT
PPV2018TM-R	CGTACAAGATCACKGCCAACT
PPV2018TM-FAM	6FAM-CGGAARCCCATGAGCCCGTACA-BHQ1

Oligonucleotides have been modified from the RVSS assay linked below.

LINK: http://journals.sagepub.com/doi/abs/10.1177/1040638716680676

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.

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	4.89	
PPV2018TM-F2 200pmol/µl	0.03	300nM
PPV2018TM-R 200pmol/µl	0.05	500nM
PPV2018TM-FAM 100pmol/µl	0.03	150nM
SensiFast Probe Lo-ROX mix[1]	10.0	1X
TOTAL VOLUME	15	

[1]Bioline SensiFast™ Probe Lo-ROX kit

Dispense 15µL to each reaction well.

Add 5µL of template, extracted DNA, controls or NTC (nuclease-free water).

Total reaction volume is 20µL

REAGENTS

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

AMPLIFICATION

Step 3.

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*	

*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.

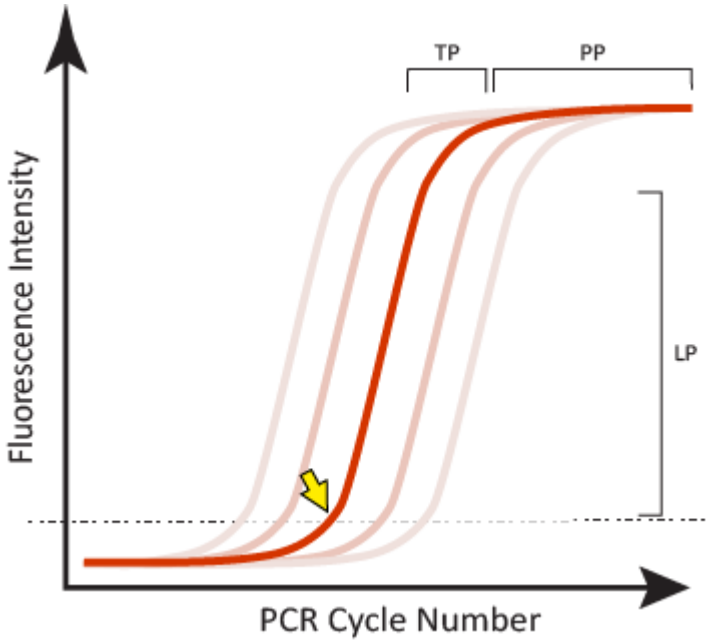

Figure 1. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C_T) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary)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