# Orthopoxvirus real-time PCR Version 3

**Judy Northill,David Warrilow,Ian Mackay**

## Abstract

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

This protocol was designed and developed at this laboratory.

**Citation:** Judy Northill,David Warrilow,Ian Mackay Orthopoxvirus real-time PCR. **protocols.io** dx.doi.org/10.17504/protocols.io.n43dgyn
**Published:** 29 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 |

### 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



This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

using a robotic dispenser.

| Reagent | Volume X1 (µL) | Final concentration |
|---|---|---|
| Nuclease-free water | 4.87 | |
| SensiFast Probe Lo-Rox mix | 10 | 1X |
| OPV2018-F (200pmol/µL) | 0.05 | 500nM |
| OPV2018-R (200pmol/µL) | 0.05 | 500nM |
| OPV2018-FAM (100pmol/µL) | 0.03 | 150nM |
| **TOTAL VOLUME** | **15** | |

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* | |

*Data aquisition 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



This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

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

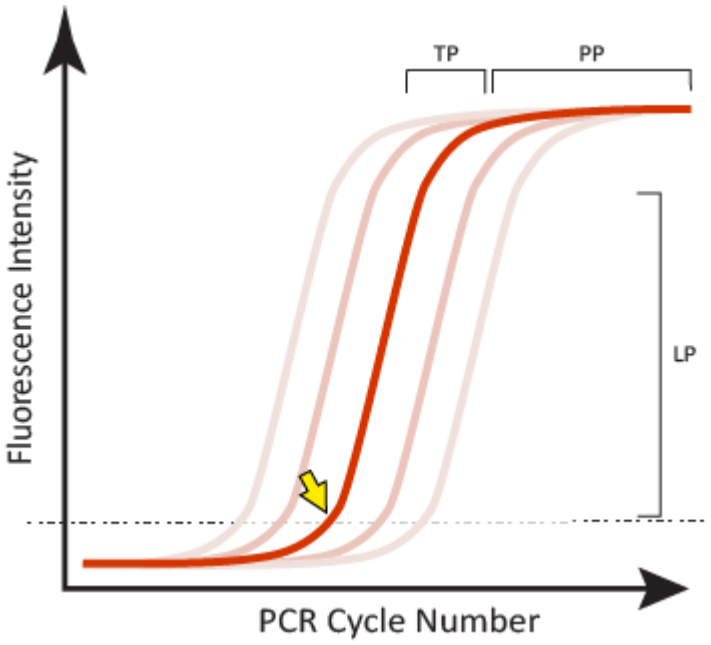


**Figure 1**. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle ($C_T$) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation,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



This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited