VERSION 2

MAY 14, 2018

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.p4kdquw

External link:
<http://journals.sagepub.com/doi/abs/10.1177/1040638716680676>

Protocol Citation: Judy A Northill, Ian M Mackay 2018. Parapoxvirus real-time PCR. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.p4kdquw>

License: 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

Protocol status: Working
 We use this protocol and it's working

Created: May 14, 2018

Last Modified: May 14, 2018

Parapoxvirus real-time PCR V.2

Judy A Northill¹, Ian M Mackay¹

¹Public Health Virology, Forensic and Scientific Services

Public Health Virology, Forensic and Scientific Services

Judy A Northill

Public Health Virology, Forensic and Scientific Services

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.

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

STEP MATERIALS

⊗ SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

⊗ SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

PROTOCOL integer ID:
 12140

Keywords: parapoxvirus,
 parapox, poxvirus, Orf,
 pseudocowpox, real-time,
 PCR, BPSV, PCPV

Oligonucleotide sequences

1

Name	5' - 3'
PPV2018TM-F2	GATGGCYGTGCAAGCTCTT
PPV2018TM-R	CGTACCAAGATCACCKGCCAAT

6	F	A	M
-	C	G	A
A	R	C	C
C	A	T	G
A	G	C	C
C	G	T	A
C	A	-	B
H	Q	1	

Oligonucleotides have been modified from the RVSS assay linked below.

<http://journals.sagepub.com/doi/abs/10.1177/1040638716680676>

Reaction set-up

- 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(μ L)x1	Final reaction concentration
Nuclease free water	4.89	
PPV2018TM-F22000 pmol/ μ L	0.03	300 nM

PPV2018TMR2000ppm/μl	0.05	500nM
PPV2018TFAM1000ppm/μl	0.03	150nM
SensiFastProbeLcRQXmix 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

SensiFAST™ Probe Lo-ROX Kit **Bioline Catalog #BIO-84002**

AMPLIFICATION

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

5
0
°
C
5
m
in
9
5
°
C
2
m
in
9
5
°
C
3
s
4
0
X
6
0
°
C
3
0
s
*

*Florescence acquisition step

RESULT ANALYSIS

- 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

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.