

# Molluscum contagiosum virus real-time PCR version 2

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## Abstract

A real-time PCR method for the detection of Molluscum contagiosum virus from human samples. This assay targets the MC021L gene and detects both subtype 1 and 2 of the virus.

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## Before start

- 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'
MCVp43kF (forward primer)	GCTCACGTACGACTGCTTYGAC
MCVp43kR (reverse primer)	CGTGGAGCGCAGATTGC
MCVp43kP (probe)	6FAM-CGCTCATCTCGCAGAC-MGB

### 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 and an ABI 7500 using 96-well plates.

Total reaction volume is 20µL and is suitable for both formats.

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	4.91	
MCVp43kF 200pmol/µL	0.03	300nM
MCVp43kR 200pmol/µL	0.03	300nM
MCVp43kP 100pmol/µL	0.03	150nM
<sup>1</sup> SensiFast Probe Lo-ROX mix (2X)	10	1X
<b>TOTAL VOLUME</b>	<b>15</b>	

<sup>1</sup>Bioline product [BIO-84005](#)

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*	

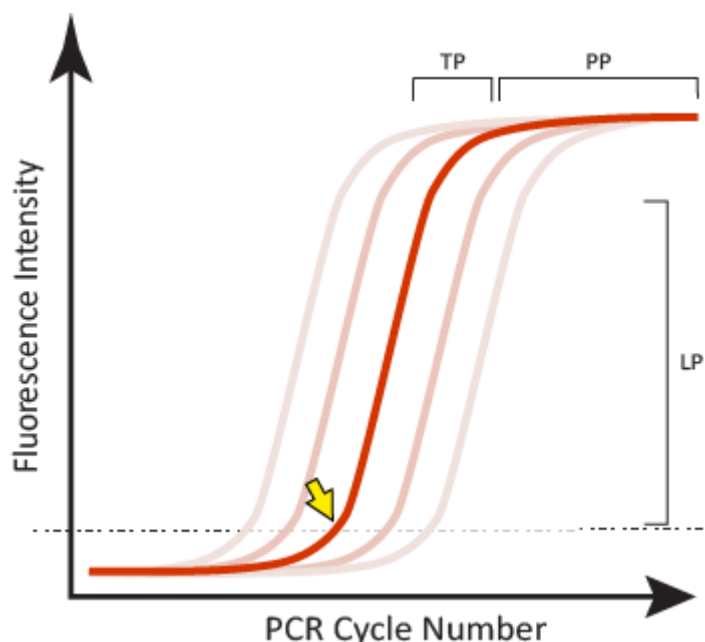
\*Florescence acquisition step

## 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.