Molluscum contagiosum real-time PCR

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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 familar with the thermocycler and software used to run the protocol.

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
[1]TaqMan™ Fast Universal PCR Master Mix (2X)	10	1X
Template	**5**	

[1]Thermofisher product 4352042

Dispense 15µL to each reaction well.

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

Total reaction volume is 20µL

REAGENTS

TaqManTM Fast Universal PCR Master Mix (2X) 4352042 by Applied Biosystems

Amplification

Step 3.

The assay has been optimised and validated for the ABI 7500. It also is used on 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 threshold should be placed in the exponential range above any background noise within the assay.

A positive result is one where the C_T is <40 and produces a sigmoidal curve.

NTC should not produce a curve and should be greater than $40C_T$.