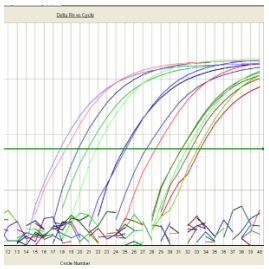
Nov 06, 2017 Version 1

Molluscum contagiosum real-time PCR V.1



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Manuscript citation:

1. Molluscum contagiosum virus detection by 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.

Attachments

AVG2009_Poster_JNort.
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818KB

Materials

STEP MATERIALS

TaqManTM Fast Universal PCR Master Mix (2X) **Applied Biosystems (ThermoFisher Scientific) Catalog #**4352042

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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.

Oligonucleotide sequences

1

	Name	5'-3'
	MCVp43kF (forward primer)	GCTCACGTACGACTGCTTYGAC
	MCVp43kR (reverse primer)	CGTGGAGCGCAGATTGC
	MCVp43kP (probe)	6FAM-CGCTCATCTCGCAGAC-MGB

Reaction set-up

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

TaqManTM Fast Universal PCR Master Mix (2X) **Applied Biosystems (ThermoFisher Scientific) Catalog #**4352042

Amplification

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

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.