Oct 21, 2025

Dual-target detection of Mpox virus

DOI
dx.doi.org/10.17504/protocols.io.n2bvj3b8plk5/v1

Alan O'Dwyer[1], Tracy Lee[1], Michael Chan[1], Branco Cheung[1], Frankie Tsang[1], John Tyson[1,2], Kathleen Kolehmainen[1], Natalie Prystajecky[1,2], Agatha Jassem[1,2]

[1]British Columbia Centre for Disease Control Public Health Laboratory, Vancouver, British Columbia, Canada; [2]Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, British Columbia, Canada

BCCDC Public Health La...

BCCDC Public Health Laboratory
BCCDC

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n2bvj3b8plk5/v1

Protocol Citation: Alan O'Dwyer, Tracy Lee, Michael Chan, Branco Cheung, Frankie Tsang, John Tyson, Kathleen Kolehmainen, Natalie Prystajecky, Agatha Jassem 2025. Dual-target detection of Mpox virus. **protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvj3b8plk5/v1**

Protocol status: Working
This protocol is currently in use at British Columbia Centre for Disease Control Public Health Laboratory.

Created: December 01, 2023

Last Modified: October 21, 2025

Protocol Integer ID: 91715

Keywords: target detection of mpox virus, detecting mpox virus, mpox virus, mpxv gp113 gene, tnf receptor deletion, targeting tnf receptor gene, tnf receptor gene, enabled pcr assay, undetectable by the g2rg tnf, hbg multiplex qpcr for the detection, human betaglobin, g2rg tnf, human betaglobin as endogenous control, targeted qpcr, secondary mpxv target, existing g2rg target, mpxv, cases of mpox, hbg multiplex qpcr, mpox, g2rg target in order

Abstract

This procedure provides instructions for detecting Mpox virus and human beta-globin using a laboratory developed fast-enabled PCR assay on a Quantstudio 7 real-time PCR instrument.

To address the 2022 Mpox virus (MPXV) outbreak a real-time PCR targeting TNF receptor gene (G2RG) as described in the Journal of Virological Methods 2010 publication by Li et al. (1) was assessed and multiplexed with human betaglobin as endogenous control.

In September 2022, the CDC released an alert on its Laboratory Outreach Communication System stating that three cases of Mpox were detected in California with a significant deletion in the TNF receptor gene. These cases were undetectable by the G2RG TNF-targeted qPCR (2). In response, a secondary MPXV target was required to multiplex with the existing G2RG target in order to detect any future cases with a TNF receptor deletion. Several targets were trialed and the MPXV GP113 gene was selected.

This validation report presents the data supporting the use of a G2RG/GP113/HBG multiplex qPCR for the detection of Mpox virus.

Guidelines

This procedure describes testing of extracted DNA. Sample extraction is not included.
Prepare PCR master mixes in a Clean Room.
Perform all manipulations of samples and DNA in a genomic level PCR laboratory

Materials

Samples: DNA extracts from suspected Monkeypox cases.

Materials

	Reagents	Equipment	Supplies
	TaqMan Fast Advanced Master Mix 2x (Cat No. 4444557)	ABI QuantStudio 7 Pro real time PCR instrument	Applied Biosystems Fast Optical 96-well plates
	Primers and probes (see below)	Appropriate pipettes	Pipette tips
	IDTE 1x TE Buffer pH 8.0		MicroAmp Optical Adhesive Film and Applicator
	UltraPURE DNAse/RNAse Free water		Gloves & gowns

Materials

PRIMERS

	Name	Sequence (5' → 3')	Target	Amplicon length	Author
	G2RG-Fmod	ATGGAAARTGTAAAGACAACGAATAC	TNF receptor gene	92 bp	Li et al. 2010
	G2RG-Rmod2	GCTATCACATAATCTGRAAGCGTA			
	GP113-F	TATTGGTACCGTCTTCCGACAA	CDS of S-S bond formation pathway protein	83 bp	Tracy Lee 2022
	GP113-R	CATTGTACTGAATCCGCCTAAGC			
	HBG-F	ACCCAGAGGTTCTTTGAGTCCTTT	Human beta-globin	82 bp	HSV/VZV assay

	Name	Sequence (5' → 3')	Target	Amplicon length	Author
	HBG-R	TGCCATGAGCCTTCACCTTAG	gene		

Primers - Supplied by Life Technologies and/or IDT

PROBES

	Name	Sequence (5' → 3')	Dye/Quencher	Target	Author
	G2RG-P	AAGCCGTAATCTATGTTGTCTATCGTGTCC	FAM/ZEN	G2RG	Li et al. 2010
	GP113-P	ATCCGAGCATGTAGAAGA	VIC-NFQ-MGB	GP113	Tracy Lee 2022
	HBG-P	CACTCCTGATGCTGTTATG	NED-MGB	HBG	HSV/VZV assay

Probes - Supplied by Life Technologies and/or IDT

20X Mix oligo recipe

	Reagents	Stock Conc (µM)	Final Conc (nM)	Volumes for 100µL 20X mix
	G2RG-Fmod	100 µM	900	18
	G2RG-Rmod2			18
	G2RG-P		250	5
	GP113-F		450	9
	GP113-R			9
	GP113-P		150	3
	HBG-F		100	2

	Reagents	Stock Conc (µM)	Final Conc (nM)	Volumes for 100µL 20X mix
	HBG-R			2
	HBG-P		100	2
	IDTE Buffer	-	-	32
	TOTAL	-	-	100

20x mix - Oligo recipe for 100µL of 20x mix

Quality Control

1. Positive Extraction Control
2. Negative Extraction Control
3. PCR Control (AmpliRun Monkeypox Virus DNA Control Cat# MBC146-R)
4. No Template Control

Troubleshooting

Before start

Prepare a 20x oligo mix per the recipe in the materials section

Prepare master mix

1 Prepare the master mix cocktail according to the following table. Invert mixture gently several times to mix and quickly spin down.

	Reagents	1 x Rxn (µL)	100x Rxn (µL)
	TaqMan Fast Advanced MasterMix 2x	10	1000
	PCR grade water	4	400
	20x Oligo Mix	1	100

Table 1: Mastermix recipe

1.1 Aliquot 15µL of master mix to each well of a MicroAmp Fast Optical 96 well reaction plate (0.1mL). Transfer plate to the PCR Genomic Room

Mpox PCR

2 In a Biological Safety Cabinet, transfer 5µL of sample extract and controls to the optical plate in order:

1. Patient Sample Extracts
2. Positive and Negative Extraction Controls
3. MPXV Positive PCR control (Amplirun Monkeypox Virus DNA Control)
4. Matrix (BG) control
5. No Template Control (NTC)

Seal the plate with an optical plate film

2.1 Load the plate onto an ABI QuantStudio 7 Real-Time PCR instrument. Refer to Table 2 for fluorophore information. Refer to Table 3 for thermocycling conditions. Refer to Table 4 for recommended manual target threshold settings.

	Target	Reporter	Quencher
	G2RG	FAM	ZEN
	GP113	VIC	NFQ
	HBG	NED	None

Table 2 Probe dyes

		Stage	Temp (°C)	Time (Min)
	40 cycles	Initial Denaturation	95	0:20
		Denaturation	95	0:03
		Annealing/Extension	60	0:30

Table 3 Thermocycling conditions

	Target	Threshold
	G2RG	0.2
	GP113	0.1
	HBG	0.03

Table 4 Manual target thresholds

2.2 Run the PCR and analyze results.

Protocol references

1. Li Y, Zhao H, Wilkins K, Hughes C, Damon IK. Real-time PCR assays for the specific detection of monkeypox virus
West African and Congo Basin strain DNA. *J Virol Methods*. 2010;169(1):223-227. doi:10.1016/j.jviromet.2010.07.012

**2. Lab Alert: MPXV TNF Receptor Gene Deletion May Lead to False Negative Results with Some MPXV
Specific LDTs. CDC's Laboratory Outreach Communication Systems (LOCS) – 09/02/2022**