Elsevier has created a Monkeypox Information Center in response to the declared public health emergency of international concern, with free information in English on the monkeypox virus. The Monkeypox Information Center is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

In-silico primer designing and PCR for detection of monkeypox virus (MPXV)

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Dear Editor,

Monkeypox has been declared as a global health emergency as infections soar on 23 July 2022. MPXV was first identified in Democratic Republic of the Congo in 1970 [1]. Monkeypox was not reported outside Africa until 2003 [1]. In 2003, a cargo of rats from Ghana introduced MPXV to Illinois pet prairie dogs, infecting more than 70 people in the US [1]. Adult travellers from Nigeria were diagnosed with monkeypox in Israel in 2018; in the UK in 2018, 2019, and 2021; in Singapore in 2019; in the US in 2019 [1]. Monkeypox is a viral zoonosis (a virus transmitted to humans from animals) with symptoms include fever, swollen lymph nodes, and a rash that forms blisters and then crusts over [2]. The severity leads to severe illness (a temperature of ⩾38.3 °C), pneumonia, encephalitis, and eye infections, which can be fatal [3,4]. MPXV is transmitted from infected person through fluids secretion from the respiratory tract, skin lesions, body and contaminated materials [1]. An ongoing outbreak of MPXV was confirmed in May 2022, MPXV started spreading quickly in 75 countries of the world. Regions of the Americas and European Region became hotspots of MPXV outbreak [5]. Till date (21 July 2022, 16:00 EST), 15,734 laboratory confirmed cases, including five deaths have been reported [5,6]. The case fatality rate has been around 3–6% recently [1]. Owing to rise in number of potential cases in this global pandemic situation, the clinician is under pressure to develop a quick, easier-to-use diagnostic test with high sensitivity that can be used in the early stages of both symptomatic and asymptomatic disease. Polymerase Chain (PCR) or real time PCR has been considered as a specific and sensitive molecular method to detect the viral titre in the humans [7]. Our aim of the study is to design monkeypox specific primers. In this study, Bioinformatics tools (Primer quest and MFE primer 3.1) were used to design and verify the monkeypox specific primers. qPCR primer and probe were designed within conserved region from DNA dependent RNA polymerase and envelope of MPXV isolated from Rivers State, Nigeria (Gene bank No: NC_063383). The details of primer, specificity, sensitivity and conditions were provided in Table 1. PCR primers were validated with tools such as SerialCloner 2.6.1, Primer-BLAST, and BLAST which allow to investigate the amplification targets of primers and probes to ensure adequate specificity and sensitivity. No cross reactivity of primer set A28-F1/R1 or A28-Probe-F1/A28-R1, H3-F2/R2 or H3-R2/H3-Probe-R1, and DDRP-F5/R5 or DDRP-Probe-F5/DDRP-R5 with other human-infecting Orthopoxviruses viz. Camelpox, Cowpox, Vaccinia and Variola (Variola major and Variola minor) were observed. Our study showed that all the primers (Table 1) either based on SYBR Green chemistry had binding capacity (ΔG) ranged from − 19.64 to − 23.26 (kcal/mole) while based on TaqMan probes had binding capacity (ΔG) ranged from − 26.48 to − 29.59 (kcal/mole). Higher binding capacity of primers in MFE primer 3.1 showed higher specificity against virus [8]. All these sets of primers of monkeypox were highly specific and any of the primer set either SYBR Green based or TaqMan probe based may be useful for commercial PCR based kit development for the molecular diagnosis of MPXV. The assay based on qPCR detection will be sensitive, rapid and specific to detect MPXV in swab of roof or fluid from vesicles and pustules, and dry crusts, and from cell culture supernatant, however in vitro validation and standardization of these primers are needed on patient samples. In conclusion, our study has designed primer set A28-F1/R1 or A28-Probe-F1/A28-R1, H3-F2/R2 or H3-R2/H3-Probe-R1, and DDRP-F5/R5 or DDRP-Probe-F5/DDRP-R5 as the potential monkeypox specific primers.

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Table 1
List of PCR primers synthesised in this study.

No	Primer ID	Sequence (5′ → 3′)	Size (bp)	GC (%)	Tm (°C)	ΔG (kcal/mol)	Sensitivity (%)[a]	Specificity (%)[b]	Specificity (%)[c]	Specificity (%)[d]	Specificity (%)[e]
1	A28-F1	CCTGGATAAACCACACATCTCC	22	50.00	59.04	-22.40	100	100	1.4	32.6	100
	A28-R1	ACTCATGCAGCATTCGAGTATT	22	40.91	58.78	-22.31	100	100	100	100	100
	A28-Probe-F1	ACTCTCCTATCTAATGCCGGTGTTCCA	27	48.15	65.66	-28.74	100	100	1.4	19.6	100
2	A28-F2	ACATTGTCGCATCGTGTTAAAT	22	36.36	57.90	-22.00	100	20.6	100	62.0	100
	A28-R2	AGTGGAGATGTGTGGTTTATCC	22	45.45	58.37	-21.97	100	100	1.4	29.3	100
	A28-Probe-R2	TGGATTTCAGGCAGAAGTTGGACCC	25	52.00	65.62	-27.54	100	100	2.8	19.6	100
3	H3-F1	CAGCGCCGTAGTAACTCTAATAA	23	43.48	58.73	-22.95	100	15.9	1.4	18.4	100
	H3-R1	ACCGAGCTTGTAATAGACAAAGA	23	39.13	58.19	-22.41	99.6	100	100	100	0
	H3-Probe-R1	CAGGAGGGTATGATGTTAGCTTATCCGC	28	50.00	65.40	-29.59	99.9	7.9	1.4	3.3	0
4	H3-F2	TTAGCAGCTACCGTTCCTATTC	22	45.45	58.27	-22.07	99.6	100	100	100	100
	H3-R2	ACACGATCCTCGTCTTGTTG	20	50.00	58.50	-21.41	99.7	7.9	100	80.4	0
	H3-Probe-R2	ACACCGCTTCGAAACCATGAAACC	24	50.00	64.92	-26.97	100	100	100	91.3	100
5	H3-F3	ACGTGTACATAACTCCTGGATAAC	24	41.67	58.69	-23.24	96.2	11.1	100	0	0
	H3-R3	CCGCTTCGAAACCATGAAAC	20	50.00	58.60	-21.61	100	20.6	50.7	78.3	100
	H3-Probe-F3	AGCAGCTACCGTTCCTATTCTAGACCA	27	48.15	65.42	-28.66	99.9	100	100	100	100
6	DDRP-F1	GGCAGACACGGACGATATTA	20	50.00	57.71	-20.95	100	100	100	83.7	100
	DDRP-R1	AGTGACTCTCCATCTTCTTCATC	23	43.48	57.92	-22.21	100	11.1	43.7	3.3	0
	DDRP-Probe-F1	TCCGATGATCTCACCGAATACGAGGA	26	50.00	65.35	-28.18	99.6	93.7	38	32.6	0
7	DDRP-F2	CTTCATGGTGGGAATATGCTCTA	23	43.48	58.29	-22.33	100	7.9	22.5	0	0
	DDRP-R2	AACCCGCATTGGCTACAT	18	50.00	57.40	-19.64	99.9	20.6	100	95.7	100
	DDRP-Probe-R2	AGCTGTCAATGAGGAATGGCTAACTGC	27	48.15	66.02	-29.21	100	7.9	2.8	0	0
8	DDRP-F3	CTCTACAGCAGTTAGCCATTCC	22	50.00	59.05	-22.49	99.9	20.6	100	56.5	100
	DDRP-R3	ATCGTCGTTGAACTCGAACC	20	50.00	58.79	-21.61	100	7.9	2.8	0	0
	DDRP-Probe-F3	ACTACTCCAATGTTTAACAAGGGCCA	26	42.31	63.20	-26.48	100	100	100	63.0	100
9	DDRP-F4	AGCCATTCCTCATTGACAGC	20	50.00	58.74	-21.25	99.8	12.7	0	0	0
	DDRP-R4	TCCACGGGAAGAGAATTCAATC	22	45.45	58.64	-22.12	100	7.9	0	0	0
	DDRP-Probe-F4	TAGCCAATGCGGGTTCGAGTTCAA	24	50.00	65.49	-26.99	100	100	100	97.8	100
10	DDRP-F5	CAACGTGTATCCTGGAGTATGG	22	50.00	58.80	-22.40	99.7	9.5	0	0	0
	DDRP-R5	GATCACAAGGCTGGTACAGATAA	23	43.48	58.50	-22.53	99.8	100	100	100	100
	DDRP-Probe-F5	TCGTGGATACTTGTGCGAAGCCAT	24	50.00	65.28	-26.96	99.9	61	100	0	100
11	DDRP-F6	TACCGGAACACTGGCTAGAA	20	50.00	58.58	-21.07	100	7.9	0	51	0
	DDRP-R6	CTGCGTACTTGATGAGCGTATTA	23	43.48	59.22	-23.26	100	46	0	7	100
	DDRP-Probe-F6	TATGGTGGTCGACGGATACGGACA	24	54.17	65.59	-27.11	99.5	7.9	1.4	42	0

[a] Sensitivity = (ts/Tts)* 100 (ts: number of target strains detected, Tts: total number of target strains tested), blasted over 1006 strains of monkeypox from NCBI database
[b] Specificity = (nts/Tnts)* 100 (nts: number of non-target species undetected, Tnts: total number of non-target species tested), blasted over all verified 63 Variola from NCBI database
[c] Specificity = (nts/Tnts)* 100 (nts: number of non-target species undetected, Tnts: total number of non-target species tested), blasted over all verified 71 Vaccinia from NCBI database
[d] Specificity = (nts/Tnts)* 100 (nts: number of non-target species undetected, Tnts: total number of non-target species tested), blasted over all verified 92 Cowpox from NCBI database
[e] Specificity = (nts/Tnts)* 100 (nts: number of non-target species undetected, Tnts: total number of non-target species tested), blasted over all verified 10 Camelpox from NCBI database

Ethical approval

Not required.

Competing interests

The authors have no conflicts of interest to disclose.

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Ying Wei Khoo [a,c], Shifang Li [a,b], Khim Phin Chong [c,*]
[a] *State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China*
[b] *Key Laboratory of Integrated Pest Management on Tropical Crops, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China*
[c] *Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia*
E-mail address: chongkp@ums.edu.my (K.P. Chong).

* Corresponding author.