MGI's ATOPlex MPXV Targeted Sequencing Package Empowers the Investigation into the Epidemiology, Genetic Characteristics and Clinical Manifestations of the First Monkeypox Outbreak in Shenzhen

The Shenzhen Center for Disease Control and Prevention used MGI's ATOPlex MPXV (monkeypox virus) targeted sequencing package to perform whole genome sequencing on all identified monkeypox positive cases to investigate the virus tracing of the first monkeypox case in Shenzhen. A comprehensive detection and epidemiological study of the first monkeypox outbreak in Shenzhen were conducted to establish a strong reference for effective prevention and response strategies for future monkeypox outbreaks.

The relevant results were published in *Biosafety and Health* in 2023, titled *Investigation into the Epidemiology, Genetic Characteristics, and Clinical Manifestations of the First Monkeypox Outbreak in Shenzhen, China*[1].

Recommended application: Pathogenic microorganisms – identification and traceability of monkeypox virus

Recommended models: DNBSEQ-G99RS, DNBSEQ-E25RS, DNBSEQ-G50RS, DNBSEQ-G400RS

- **ATOPlex platform enables the precise targeted detection and traceability of monkeypox virus**

  The MPXV targeted sequencing package,developed based on ATOPlex multiplex PCR technology, can identify and trace the monkeypox virus in a rapid, accurate and targeted manner, achieving the real-time monitor of monkeypox epidemics.

- **A complete monkeypox detection and traceability combination product**

  The package includes nucleic acid extraction and library preparation kit, automation system, DNBSEQ sequencing platform, and bioinformatics analysis software, enabling the detection and traceability of monkeypox.

- **The automation system highly matches the entire process of sample treatment and library preparation**

  Efficient and fast experimental processes of nucleic acid extraction and library preparation of monkeypox can be achieved with MGI's self-developed automation platform.

- **Sequencing data is efficient and high-quality**

  The DNBSEQ sequencing technology has outstanding features such as high accuracy, low duplicate rate and low index hopping rate; DNBSEQ-G99 has fast sequencing speed, a built-in computing module, and integrated sequencing and bioinformatics, which can complete SE100+10+10 sequencing in 4.5 hours, with efficient and high-quality data output.

Background

Monkeypox is a zoonotic infectious disease caused by the monkeypox virus (MPXV), with sporadic infections and outbreaks occurring mainly in forested areas of West and Central Africa. In 1958, the monkeypox virus was first discovered in Denmark, in the monkeys used for research. In 1970, the first case of human infection was found in a 9-month-old boy in the Democratic Republic of the Congo. The epidemiological paradigm of the disease changed in 2022, with the virus spreading beyond its endemic areas, resulting in cases in multiple countries. As of June 2023, monkeypox has spread across 111 countries or regions around the world. This significant geographic spread and subsequent international impact prompted the World Health Organization (WHO) to list monkeypox as a public health emergency of international concern in July 2022. China's National Health Commission has decided to include monkeypox in the management of Class B infectious diseases as stipulated in the *Law of the People's Republic of China on the Prevention and Control of Infectious Diseases* from September 20, 2023, and to adopt prevention and control measures for Class B infectious diseases.

The main feature of the disease is an extensive rash, and some people may firstly experience fever, myalgia or sore throat. The rash typically starts on the face and spreads throughout the body. The rash begins as flat sores and then develops into fluid-filled blisters that may itch or be painful. After the rash heals, the lesions dry up, then scab over and fall off[2, 3].

MPXV is a 197kb double-stranded DNA virus consisting of approximately 200 genes. Nucleic acid amplification and serological tests can quickly diagnose monkeypox cases at a low cost, but these tests cannot be used to monitor the source, lineage, transmission and genomic variation of MPXV. Newly emerged mutations may lead to an underestimate of the viral load in the sample, or even misdiagnosis[4]. However, genomics can achieve rapid and accurate identification and traceability of monkeypox virus, with the characteristics of high sensitivity, high specificity, and low false positive rate.

Study Description

At 22:00 on June 8, 2023, the Shenzhen Center for Disease Control and Prevention received a suspected case of monkeypox reported by the Futian District Center for Disease Control and Prevention, and immediately conduct an epidemiological investigation. A large number of samples were collected for molecular detection and virus sequencing.

The MPXV targeted sequencing package developed based on MGI's ATOPlex multiplex PCR technology was used to complete virus tracing, including the whole genome sequencing of MPXV, the detection of mutation sites, consensus sequences and monkeypox virus lineages, and finally a highly reliable full-length genome sequence was obtained in a short time.

Materials and Methods

Sample Preparation

The index case (Case 1) was found to be infected with monkeypox virus during COVID-19 treatment. Subsequent cases (Cases 2 and 3) were found during the screening of the close contacts. All three cases were unmarried males aged 25, 27 and 26 years, living in Shenzhen since May 2023, with no history of international travel or contact with overseas individuals or animals.

During the fever of these three cases, they were tested for SARS-CoV-2 antigen, and a large number of samples were collected, including nasopharyngeal swabs, oropharyngeal swabs, blister fluid, blood (anticoagulant and non-anticoagulant), anal swabs, and urine samples for molecular detection and genome sequencing of monkeypox virus.

Monkeypox virus nucleic acid extraction and purification were performed with a commercial kit (qEx-DNA/RNA virus, Tianlong Technology) and a nucleic acid extractor (Tianlong GeneRotex96, Tianlong Technology). About 200μL of fully mixed sample solution (including serum, rash material, scabs, blister fluid, and oropharyngeal or nasopharyngeal secretions of suspected patients) was used for nucleic acid extraction. The extracted nucleic acid were subjected to qPCR detection and monkeypox whole genome sequencing.

Library Preparation and Sequencing

The MGI's ATOPlex MPXV targeted sequencing package used the MGI's ATOPlex platform (https://atoplex.mgi-tech.com/) to perform primer design, optimization and development based on the monkeypox reference genome (NC_063383.1), and to target the monkeypox virus genome for enrichment and sequencing library construction. This study used this package and 10μL of extracted viral DNA for sequencing library construction. For detailed procedures, please refer to the relevant manual. The prepared library was sequenced on DNBSEQ-G99 using the SE100+10+10 strategy. It is worth noting that MGI's automation system is integrated in this package for automated extraction and library construction, which can greatly save manpower and improve efficiency in large-scale sample situations.

Analysis Process

Sequencing data were analyzed using MGI's MPXV software to identify mutation sites, consensus sequences, and monkeypox virus lineages. High-quality sequencing reads were aligned to the monkeypox reference genome (NC_063383.1) using the bwa mem algorithm with default options[5]. Freebayes software was then used for mutation detection[6], and only mutation sites with a frequency greater than 40% were retained. The assembled consensus sequence was obtained to determine the lineage of the monkeypox virus.

Nucleic acid extraction (~1h)

- MGIEasy Microbiome DNA Extraction Kit (MD01T-99)

MGISP-960RS

Library preparation (~9h)

ATOPlex MPXV Library Preparation Set

- MGISP-100RS

or

- MGISP-960RS

or

- DNBelab-D4

Sequencing (~5-50h)

DNBSEQ-G99RS
- SE100, ~5 h
- PE150, ~12h

or

DNBSEQ-E25RS
- SE100, ~5 h
- PE150,~20h

or

MGISEQ-200RS
- SE100 (FCS), ~10 h
- SE100 (FCL),~13 h
- PE150 (FCS), ~28 h
- PE150 (FCL),~40 h

or

MGISEQ-2000RS
- SE100 (FCS), ~13 h
- SE100 (FCL),~25 h
- PE150 (FCS), ~31 h
- PE150 (FCL),~50 h

Analysis (~1-3h)

MGI MPXV Assembly And Phylogenetic Software

- SE100/5M:~1h /16 Samples
- SE100/20M: ~2.5h/16 Samples
- PE150/1.5 M:~50 min /16 Samples
- PE150/6 M: ~3 h /16 Samples

Platform of microocrganisms Fast Identifcation

The above analysis time is based on platform of microorganisms Fast Identification.

Results

Real-time qPCR and SARS-CoV-2 Antigen Detection

First, real-time qPCR identification of monkeypox virus and SARS-CoV-2 antigen detection were carried out. As shown in Table 1, the nasopharyngeal swabs and herpes swabs of three cases were positive for monkeypox virus, and the oropharyngeal swabs and anal swabs of two cases were also positive. Each patient tested positive for SARS-CoV-2 antigen during the fever period, indicating a secondary infection. Therefore, it is impossible to rule out the possibility that the related symptoms were caused by SARS-CoV-2 infection.

Sample	Case 1	Case 2	Case 3
Nasopharyngeal swab	35	35	37
Oropharyngeal swab	Negative	29	31
Blister fluid	31	27	34
Anal swab	28	34	Negative
Urine	Negative	Negative	Negative
Nasopharyngeal swab (SARS-CoV-2 antigen detection)	Positive	Positive	Positive

Table 1. Results of real-time qPCR and SARS-CoV-2 antigen detection of three samples.

Whole Genome Sequencing and Traceability Analysis of Monkeypox Virus

As shown in Figure 1, different MPXV lineages collected from GISAID were compared with the 3 MPXV sequences analyzed in this study, and phylogenetic trees of different MPXV lineages were constructed based on the maximum likelihood estimation approach. Subsequently, the 3 MPXV sequences in this study were further compared with 45 MPXV sequences downloaded from GISAID (samples were collected from January 1, 2023 to July 3, 2023) to construct a phylogenetic tree as shown in Figure 2, and then analyzed by Nextclade of genome sequencing (https://clades.nextstrain.org/). The results showed that all 3 cases in Shenzhen were infected with human monkeypox virus (hMpxV, B.1.3 evolutionary branch).

The next step is to further explore the genomic correlation between the three cases in this study and other worldwide monkeypox cases. Based on SNPs in the core genome, using the genome sequences of 71 monkeypox cases since 2023 downloaded from GISAID, a phylogenetic tree containing 6 domestic cases (3 from Shenzhen, 2 from Chongqing, and 1 from Hangzhou) was constructed using the maximum likelihood estimation approach and refined the tree by using iTOL. The results showed that the cases in Shenzhen and Hangzhou belonged to the same minor evolutionary branch and had a high homology. The China-SZ-2023-06-case3 in Shenzhen is a new variant genome produced by the continuous evolution and mutation from the genome of the China-SZ-2023-06-case2. The possibility that those 3 cases belong to the same transmission chain cannot be ruled out. Furthermore, phylogenetic tree analysis of all B.1.3 gene sequences worldwide revealed that the four local cases in Shenzhen and Hangzhou were on the same evolutionary branch as the recent Japanese monkeypox cases. From an evolutionary perspective, these four Chinese cases are highly homologous to strains from Japan collected in January, February, and March 2023 (GISAID ID: EPI_ISL_17445514, EPI_ISL_17445515, EPI_ISL_17692269).

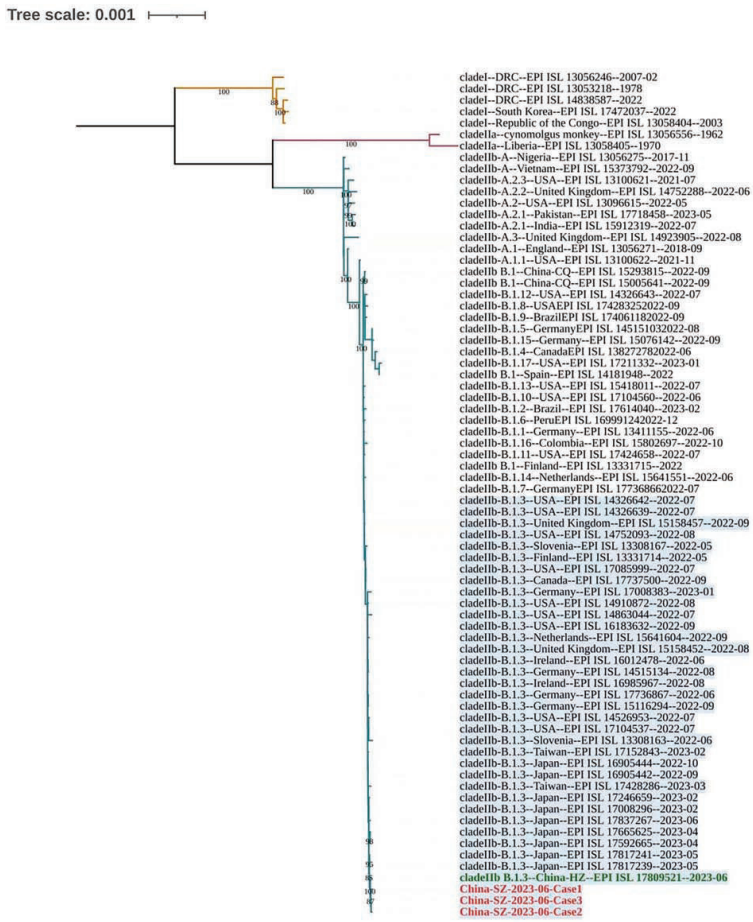

Figure 1. Phylogenetic tree of different lineages of MPXV constructed based on the maximum likelihood estimation approach.

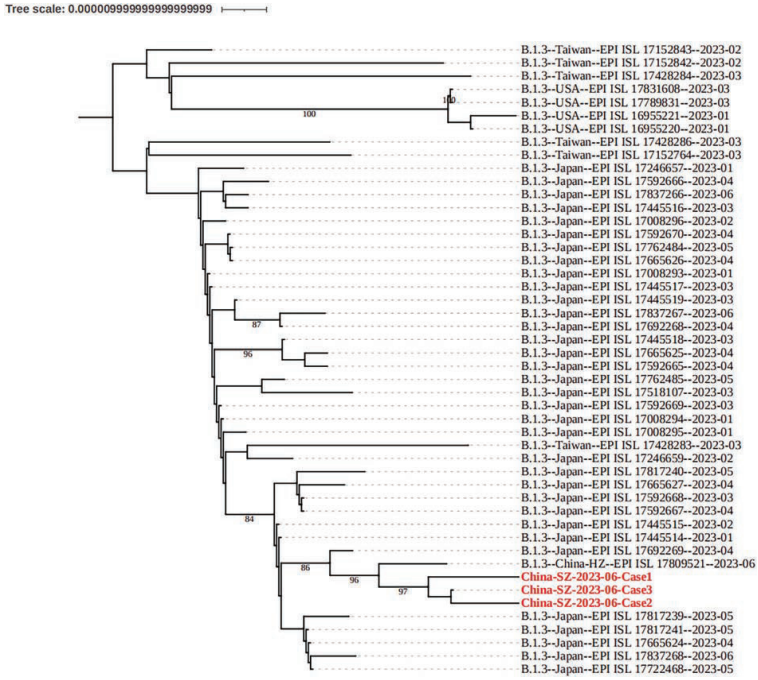

Figure 2. Phylogenetic tree constructed by comparing 3 MPXV cases found in Shenzhen, China with 45 MPXV whole genome sequences downloaded from GISAID (samples were collected from January 1, 2023 to July 3, 2023).

Summary

This study used three suspected cases of monkeypox found in Shenzhen as the subjects, and used the MPXV targeted sequencing package developed by MGI based on ATOPlex multiplex PCR technology. In addition to monkeypox virus extraction and purification as well as targeted library construction reagents, the package also includes MGI's DNBSEQ sequencing platform and MPXV analysis software, as well as DNBelab-D4, MGISP-100 or MGISP-960 automated system, etc. The package could achieve full-process support from sample to report, full-length genome detection of monkeypox virus, and relative quantitation of the virus. This full-process solution played an important role in the prevention and control of monkeypox in Shenzhen, providing a powerful tool for monitoring and tracing the source of the monkeypox virus.

The study and observation of this monkeypox outbreak laid the foundation for surveillance and epidemiological information reporting to play a key role in managing and controlling future outbreaks. A comprehensive epidemiological information reporting system can help to provide real-time dynamic updates and help authorities make prompt decisions. Given the global mobility of people, the surveillance system in Shenzhen and other parts of the world shall attract more attention and be strengthened.

Genetic Sequencer DNBSEQ-G99RS

MGISP-100 DNA Sequencing Library Preparation System

References

1. J. Wan, *et al.* Investigation into the epidemiology, genetic characteristics, and clinical manifestations of the first monkeypox outbreak in Shenzhen, China. *Biosafety and Health*. 2023.
2. WHO, 2022–23 Mpox (monkeypox) outbreak: Global trends, World Health Organization produced on June 13 2023. https://worldhealthorg.shinyapps.io/mpx_global/, 2023 (accessed 6 June 2023).
3. N. Girometti, *et al.* Demographic and clinical characteristics of confirmed human monkeypox virus cases in individuals attending a sexual health centre in London, UK: an observational analysis. *Lancet Infectious Diseases*. 2022.
4. CC. Wu, *et al.* Rapid identification of full-length genome and tracing variations of monkeypox virus in clinical specimens based on mNGS and amplicon sequencing. *Virologica Sinica*. 2023.
5. H. Li, *et al.* Fast and accurate short read alignment with Burrows–Wheeler Transform. *Bioinformatics*. 2009.
6. E. Garrison, *et al.* Haplotype-based variant detection from short-read sequencing [Preprint]. *arXiv*. 2012.

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G99ARS	900-000560-00
	MGISP-100 DNA Sequencing Library Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System (Config 2)	900-000147-00
	MGISP-960RS Automated Sample Preparation System(Config 7)	900-000152-00
Bioinformatics	MGI MPXV Assembly and Phylogenetic Software Analysis Package (16 reports)	970-000311-00
Sample Extraction	MGIEasy Microbiome DNA Extraction Kit MD01T-96 (96 Preps)	1000027955
Library Prep reagent	ATOPlex MPXV Library Preparation Set (96 RXN)	940-002482-00
	DNBSEQ OneStep DNB Make Reagent Kit (4 RXN)	1000026466
Sequencing reagent	DNBSEQ-G99RS High-throughput Sequencing Set（G99 FCL PE150）	940-001269-00

MGI Tech Co.,Ltd

Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083

Version: December 2024

+86-4000-688-114

en.mgi-tech.com

MGI-service@mgi-tech.com

Authors: Jing Zhao

Editor-in-Charge: Qiwei Wang

Reviewer: Yao Jiang