The BBS Mode of DNBSEQ-G99 Facilitates the Rapid Analysis of the First Imported Monkeypox Case in China

MGI's Metagenomic Sequencing Strategy Enables Unknown Pathogen Discovery, Genome Sequencing and Tracing

The first suspected case of monkeypox in mainland China was found to be infected with B.1 clade of the West African strain of monkeypox with whole-genome sequencing based on the MGI's metagenomic sequencing strategy by staff from Chongqing Municipal Center for Disease Control and Prevention, Chongqing Municipal Key Laboratory for High Pathogenic Microbes, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, and Nan'an Center for Disease Control and Prevention, Nan'an District, Chongqing Municipality[1].

The related work was published in *China CDC Weekly* in 2022, entitled "The First Imported Case of Monkeypox in the Mainland of China — Chongqing Municipality, China, September 16, 2022".

Recommended applications: Pathogenic microorganisms -Monkeypox virus

Recommended model: DNBSEQ-G99ARS, DNBSEQ-G50RS

- **Fast detection**

  The DNBSEQ-G99 genetic sequencer with multi-point data output mode (Bioanalysis by Sequencing, BBS) can complete virus identification in 9 hours and genome assembly in 16 hours.

- **High automation**

  The automated sample preparation system MGISP-100 and analysis software can achieve automated library preparation and data analysis, which minimizes the manual manipulation.

- **Excellent data quality**

  DNBSEQ sequencing technology provides high quality sequencing data for downstream analysis.

- **Full analysis function**

  MGI's self-developed software can satisfy the needs of microbial identification, genome assembly, variant detection and tracing.

Background

Monkeypox is a zoonotic viral disease caused by infection with monkeypox virus (MPXV) of the Poxviridae family and Orthopoxvirus[2]. MPXV was first identified in 1958 in monkeys originating from Singapore, which may be the reason why the disease is named monkeypox[2]. However, the natural hosts of MPXV are more likely to be rodents and other small mammals. Disseminated infection with monkeypox virus in the population first emerged in several countries in Africa in the 1970s and has spread widely across the continent in the last two decades[3].

Fortunately, the symptoms in humans following monkeypox are similar to those of smallpox but with a lower mortality rate[3]. MPXV is a double-stranded DNA virus with a genomic DNA size of approximately 197 kb encoding a total of approximately 190 genes[4]. The MPXV genome contains three structural regions: the core region, the left arm region and the right arm region. The core region is relatively conserved and encodes genes related to viral replication and assembly; the left and right arms are relatively more diverse and encode genes related to host range and pathogenicity[4]. MPXV has two branching clades, the Central African clade and the West African clade, which differ in genome length by approximately 900 bp and show a similarity of not less than 95%[2, 4]. Among them, the Central African clade was more toxic, with an average mortality rate of 10.6%, while the West African clade had a lower lethality rate at 3.6%[5].

Since May 2022, there has been a dramatic increase in monkeypox cases worldwide, leading the World Health Organization (WHO) to declare monkeypox as a global health emergency[2]. Approximately 60,000 cases have been reported to date in more than 100 countries and regions worldwide, posing a significant health challenge to the world. MPXV is transmitted mainly by rodents[6] , mainly through blood and body fluids between humans. Smallpox vaccination can provide effective protection against monkeypox, and one of the possible causes of the current outbreak is the general decline in smallpox immunity in the global population and the policy of ending smallpox vaccination[7].

Research description

One Chinese patient had travelled to Germany and France in September 2022, and returned to Chongqing, China, on September 14, 2022, where he was quarantined for exhibiting symptoms of fever. The medical history revealed that the man had developed a dry and itchy throat with fever and a red rash and pustules on his right thigh on September 91. All indications are that the man presented with symptoms associated with monkeypox and may have been infected with the monkeypox virus[1].

In response to the relevant situation, the Chongqing Municipal Center for Disease Control and Prevention collected the clinical sample of the patient and confirmed that he was positive for monkeypox virus by qPCR. Afterwards, whole-genome sequencing of the relevant clinical sample was performed utilizing the MGI metagenomic sequencing strategy based on the DNBSEQ-G99 sequencing platform[1]. The results showed that the MPXV (China-CQ202209) that the man was infected with belonged to clade B.1 of the West African strain and was highly homologous to the strain found in Germany on June 21, 2022 (GISAID ID: EPI_ISL_13889435). This was the first confirmed imported case of monkeypox in mainland of China[1].

Materials and Methods

Specimen collection

A total of four samples (herpes fluid, mouth swab, nasal swab and blood samples) were collected from this case and subjected to qPCR test, and the herpes fluid and nasal swab samples were used for genetic sequencing.

Library preparation and sequencing

The nucleic acid was first extracted from herpes fluid and nasal swab samples and then quantitated.

The library preparation was then performed using MGIEasy Fast FS DNA Library Prep Set (940-000029-00) and DNB preparation was performed using the DNBSEQ One Step DNB Make Reagent Kit (OS-DB) (1000026466). Detailed operation procedure can be referred to in the instructional manual.

Finally, the DNBSEQ-G99ARS genetic sequencer (900-000609-00) equipped with DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE150) (940-000410-00) was used for sequencing. Based on the multi-temporal data output mode (Bioanalysis by Sequencing, BBS) integrated in DNBSEQ-G99ARS, the sequencing data can be analyzed simultaneously in SE40, SE100 and PE100 sequencing recipe, as shown in Figure 1.

Bioinformatics analysis

Sequencing data are analyzed with MGI MPXV software, which was developed specifically for microbial pathogen sequencing data analysis and is suitable for metagenome and multiplex PCR sequencing data analysis, which enables identification, genome assembly, variant detection and tracing of individual pathogenic microorganisms. The analysis process is shown in Figure 2.

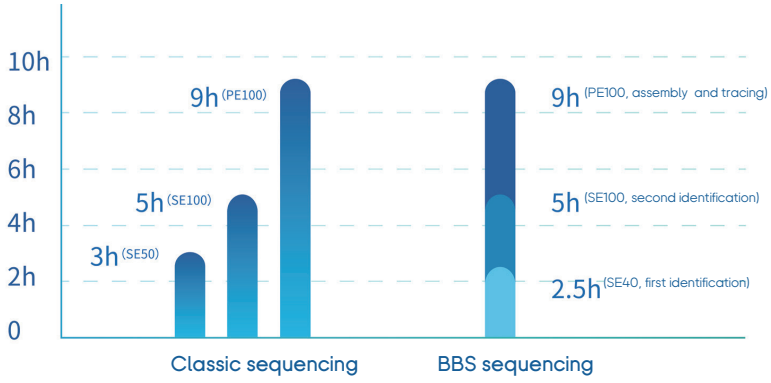

Figure 1. The schematic diagram of BBS sequencing mode compared with the classic sequencing mode.

FASTQ QC	Aligned to the reference genome	Variation detection	Sequence assembly

Figure 2. The schematic diagram of the data analysis process.

Sample collection	Library preparation and sequencing	Bioinformatics Analysis	Result analysis
Herpes fluid, mouth swabs, nasal swabs and blood samples	 MGIEasy Fast FS DNA Library Prep Set  DNBSEQ-G99ARS Genetic Sequencer	MGI MPXV Software	Genome assembly; Evolutionary tracing Analysis

Results

qPCR test results

All four samples tested positive for MPXV by qPCR, where mouth swabs, nasal swabs and blood samples were tested positive for the West African strain. The Ct values in Table 1 show that the herpes fluid samples had the highest viral load.

Quality control of sequencing data

The Q30 of sequencing data was 95.64% with excellent quality, and the data yield was 98.21 M reads, which reached the theoretical expected data yield of DNBSEQ-G99ARS (80 M reads), and the sequencing quality and data yield fully satisfied the demand of subsequent analysis.

Primary analysis results

The sequencing data were compared to the reference genome of monkeypox virus by MGI MPXV software, and the data of average sequencing depth and genome coverage were obtained, as shown in Table 2. The data from herpes fluid samples performed better, with an average sequencing depth of up to 200× and genomic coverage of over 99%, as shown in Figures 3 and 4, and the excellent data quality was sufficient for advanced analysis.

Evolutionary tracing

The phenotypic features of this patient were highly compatible with the symptoms of monkeypox disease, as shown in Figure 5(A). The structural features of this virus strain are consistent with monkeypox virus, as shown in Figure 5(B). Evolutionary tracing analysis of this strain by MGI MPXV software revealed that this MPXV (China-CQ202209) belongs to clade B.1 of the West African strain and is highly homologous to the strain found in Germany on June 21, 2022 (GISAID ID: EPI_ISL_13889435)[1], as shown in Figure 5(C). The tracing results were consistent with the typing results of qPCR, which further confirmed the case is the first imported case of monkeypox in mainland China.

Specimen	Ct-F3L	Ct-J2R	Ct-D14L	MPXV	West Africa strain of MPXV
Blister fluid swab	20.76	ND	ND	positive	ND
Oropharyngeal swab	27.81	31.29	Neg	positive	positive
Nasopharyngeal swab	31.00	33.25	Neg	positive	positive
Blood	33.65	35.54	Neg	positive	positive

Abbreviation: MPXV=monkeypox virus; qPCR=quantitative real-time polymerase chain reaction; ND=not determined; Neg=negative.

Table 1. PCR test results.

Reads	Average sequencing depth (×)		Coverage % (10×)	
	Herpes fluid	Nasal swabs	Herpes fluid	Nasal swabs
SE40	33.55	1.82	99.12	0.13
SE100	110.28	5.62	99.87	9.58
PE100	200.28	9.9	99.91	49.96

Table 2. Sequencing depth and coverage.

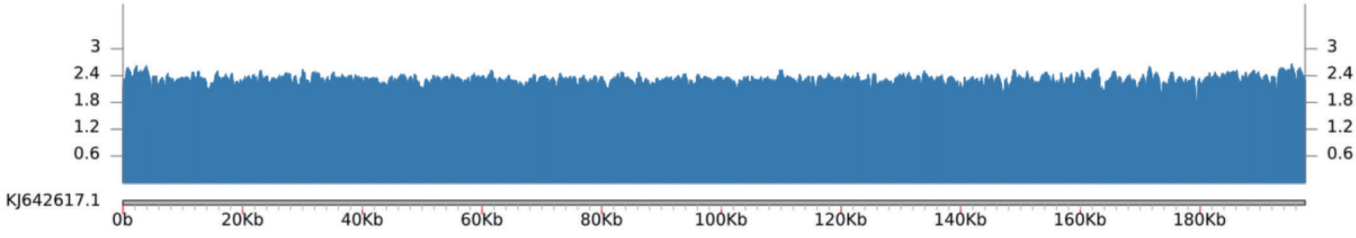

Figure 3. Whole-genome coverage of herpes fluid samples.

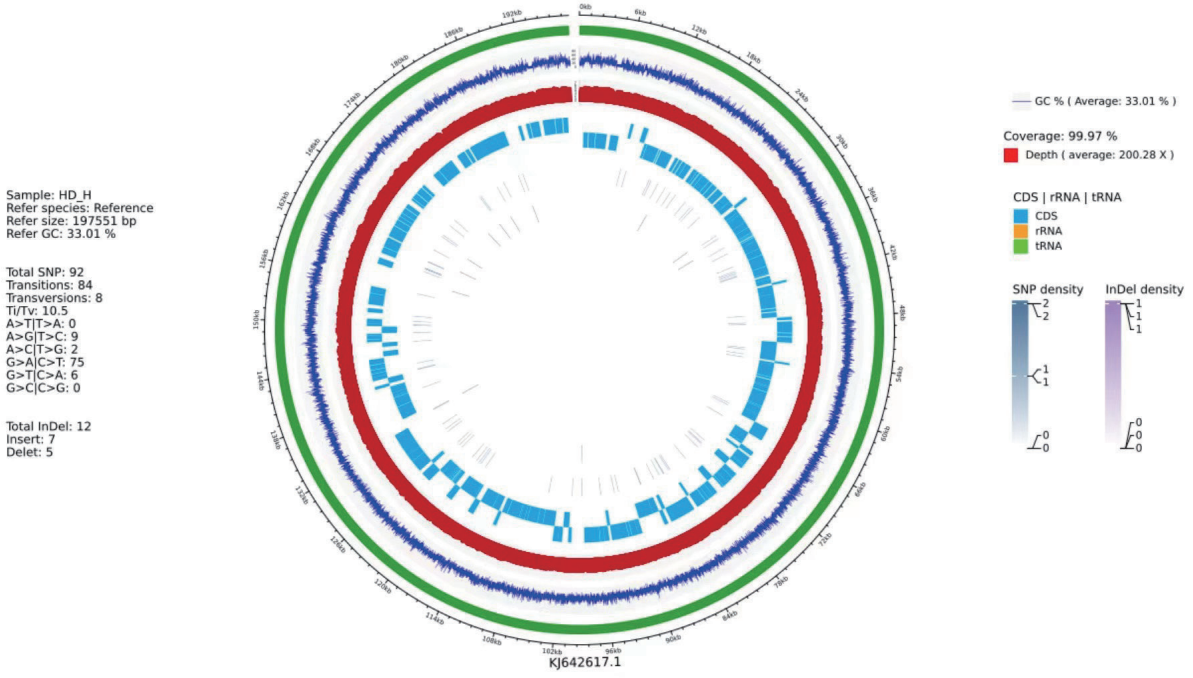

Figure 4. Density distribution of coverage and depth of herpes fluid sample.

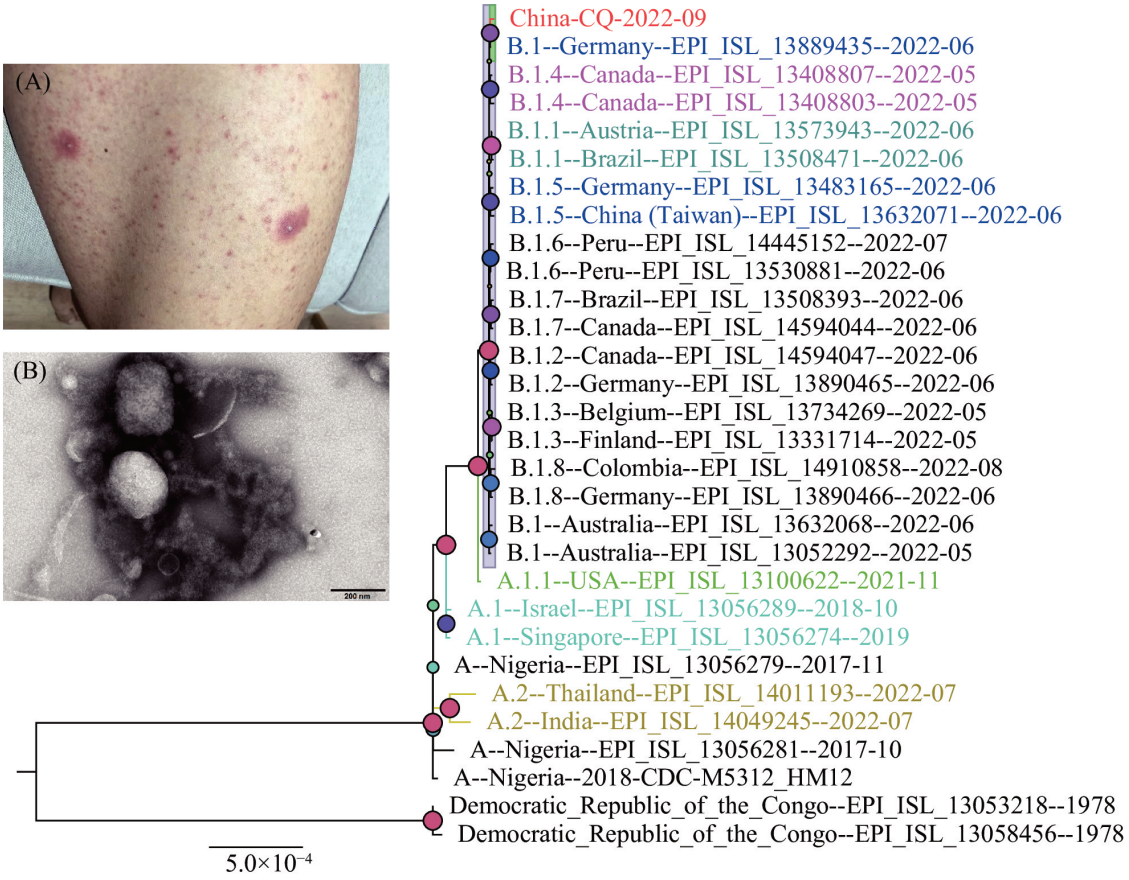

Figure 5. (A) Characterization photos of the patient (B) Electron microscopy test image (C) Whole-genome sequencing evolutionary traceability analysis[1].

Conclusion

The neglected zoonotic monkeypox has once again become a global concern, and the first imported case of monkeypox in mainland China is indeed considered a wake-up call to prevent the importation and spread of MPXV. Of these, MGI's metagenomic sequencing strategy and DNBSEQ-G99ARS sequencing platform provided strong support for the confirmation of the case and the sequencing and tracing of the monkeypox virus genome[1].

With fast detection, excellent data quality, complete analysis functions and high automation, the MGI's metagenomic sequencing strategy played a key role in the whole-genome sequencing of the first imported human monkeypox virus in mainland China, which provided a powerful tool for the identification, genome assembly and tracing of monkeypox virus.

Genetic Sequencer DNBSEQ-G99ARS

References

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7. Reynolds, M. G. & Damon, I. K. Outbreaks of human monkeypox after cessation of smallpox vaccination. *Trends Microbiol* 20, 80-87,doi:10. 1016/j.tim.2011.12.001 (2012).

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	Genetic Sequencer DNBSEQ-G99ARS	900-000609-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
Software	MGI MPXV Software	970-000311-00
Library Prep	MGIEasy Fast FS DNA Library Prep Set (16 RXN)	940-000029-00
	DNBSEQ One Step DNB Make Reagent Kit (OS-DB) (4 RXN)	1000026466
Sequencing Reagents	DNBSEQ-G99RS High-throughput Sequencing Set (G99 SM FCL PE150)	940-000410-00

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2. For HotMPS sequencers: This sequencer is only available in selected countries, and its software has been specially configured to be used in conjunction with MGI's HotMPS sequencing reagents exclusively.
3. For HotMPS reagents: This sequencing reagent is only available in selected countries.