The Application of High-throughput Sequencing in the Diagnosis of Severe Psittacosis

MGI's DNBSEQ platform based metagenomic sequencing shows better performance in etiological diagnosis

A team of researchers investigated psittacosis using metagenomic next-generation sequencing (mNGS) on DNBSEQ platform and compared it to another sequencing platform. Compared to MinION and Illumina platforms, the DNBSEQ sequencing platform generated more data and offered the most comprehensive genomic data for *Chlamydia psittaci* L99.

The research was published on *BMC Genomics* in 2021, under the title "Metagenomic diagnosis of severe psittacosis using multiple sequencing platforms"[1].

Recommended application: Pathogenic microorganism - *Chlamydia psittaci*

Recommended model: DNBSEQ-G99ARS

- ### Outstanding performance in metagenomic sequencing

  Metagenomic sequencing based on the DNBSEQ sequencing platform generates more total reads, and has higher genome coverage, sequencing depth and detection sensitivity.

- ### Flexible and diverse product solutions

  MGI techniques, such as sample pre-processing, automatic library construction, and automatic sequencing data interpretation, can cover processes from sample input to report output, thereby enhancing efficiency and reducing labor cost.

Background

For emerging infectious diseases, it is critical to identify novel pathogens for disease prevention and control[1]. As depicted in Fig. 1, red represents emerging infectious diseases, blue represents that have reappeared following a prior epidemic, and black represents "man-made" diseases. Multiple new infectious diseases have threatened people' s lives throughout history. Our public health system is under constant threat from these infectious diseases[2-4]. Fever and infections caused by pathogenic microorganisms (e.g., psittacosis) are the most problematic diseases during clinicians' diagnosis. These pathogens tend to be diverse and complex and pose a threat to human health. Traditional detective methods of pathogenic microorganisms are limited by the long cycle, complex process, and low sensitivity. However, timely and precise diagnosis of diseases is an important prerequisite for effective treatment, disease monitoring, and control of disease spread.

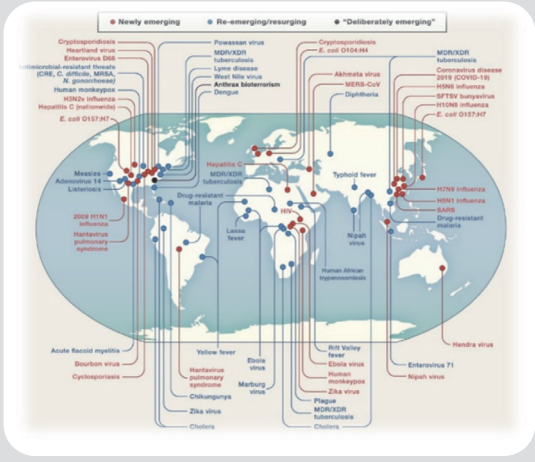

Fig. 1. Emerging infectious diseases from 1981 to 2020[1].

Microbiome analyzing methods and standards have developed rapidly in the past few years[5]. As an emerging detecting method, metagenomic sequencing can be utilized to comprehensively analyze genetic materials (DNA and RNA) from patient samples to achieve rapid identification and in-depth analysis of unknown pathogens (Fig. 2) [6]. Currently, this method is widely used to analyze the blood, cerebrospinal fluid, alveolar lavage fluid, sputum, pleural effusion, and other samples from patients with clinically unexplained fever and highly suspected infection. Metagenomic sequencing provides strong clinical evidence for identifying the pathogens of a variety of challenging uncommon diseases and it' s changing the manner in which doctors diagnose and treat infectious diseases[7].

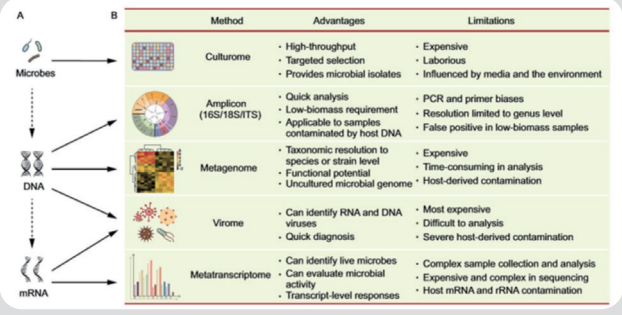

Fig. 2. Advantages and limitations of high-throughput sequencing technique in micro-organism studies[6].

Study description

Psittacosis is a zoonotic intracellular pathogen that can cause a range of serious illnesses, including asymptomatic transient carriage, mild pneumonia, and severe pneumonia that can lead to respiratory and multi-organ system failure, and even death in rare cases. However, early diagnosis is confounded with nonspecific clinical manifestations and low clinical suspicion of rare infections. Furthermore, limited diagnostic analysis in most clinical laboratories constrain the diagnosis of psittacosis.

In this study, a research team reported a case of lethal psittacosis diagnosed using mNGS. The results indicated that both sample type and sequencing platform selection are critical in the diagnosis of the etiology of severe pneumonia7. The greatest advantage of metagenomic sequencing is the identification of unknown pathogens. Although the type of pathogen is unknown, the pathogen genome or transcriptome can be comprehensively analyzed by comparing with the database, to search for clues and identify the disease-causing microorganisms. For pathogenic microorganism detection, MGI provides a one-stop product portfolio for metagenomic sequencing (Fig. 3).

Fig. 3. The process of metagenomic detection of pathogenic microorganisms.

Materials and Methods

Sample collection

Lower respiratory tract samples (bronchoalveolar lavage fluid and sputum) and blood samples from a patient (60 years old, female) with severe psittacosis were collected to extract DNA and RNA, and the RNA was reversely transcribed into double-stranded DNA.

Library preparation and sequencing

For the metagenomic DNA of the above three samples, the team used MGIEasy DNA Rapid Library Prep Kit (ceased production; it is suggested using MGIEasy Universal DNA Library Prep Set) to construct the DNA library with the following process: The extracted metagenomic DNA was fragmented to 300bp in length, then underwent purification and adaptor ligation; then amplified with PCR. The obtained PCR product was denatured and circularized to get DNA nanoball (DNB). Finally, the DNB libraries were loaded onto the DNBSEQ sequencer for sequencing with paired-end 100bp (PE100) sequencing recipe.

At the same time, Illumina and MinION were used for metagenomic sequencing of the three samples. Dealing with a large number of samples, MGI can provide automatic machine for sample extraction and library preparation, thereby reducing labor cost and increasing work efficiency.

Data analysis

The team filtered low-quality sequencing data with PycoQC and SOAPnuke and sorted high-quality sequencing data with Centrifuge; and used Bowtie2 and MEGAHIT for alignment and assembly, respectively. Then, the team phylogenetically analyzed the genomes obtained from the samples with other *C. psittaci* genomes in the database and identified drug-resistant genes and virulence factors in sample genomes.

Sample collection	Library preparation and sequencing	Bioinformatics analysis	Result analysis
Lower respiratory tract sample and blood sample from a patient with severe psittacosis	 MGIEasy FS DNA Library Prep Set  DNBSEQ-G99ARS Genetic Sequencer	PycoQC SOAPnuke Centrifuge Bowtie2 MEGAHIT	Pathogen identification, Phylogenetic analysis, Identification of drug resistance genes and virulence factors

Results

Accurate identification of major pathogens in pathological samples

The MGI DNBSEQ sequencing platform generated sequencing data of 10.2Gb, 11.3Gb, and 8.7Gb based on bronchoalveolar lavage fluid, sputum, and blood samples, respectively with the read length of 100bp. The analysis results showed that *C. psittaci* is the main pathogen, with 39,385, 30,320, and 14,478 reads in bronchoalveolar lavage fluid, sputum, and blood samples, respectively (Table 1). The results of genomic analysis showed that *C. psittaci* in this case belongs to the E genotype of the ompA locus, which is named as *C. psittaci* L99 isolate.

Sequencing data can be used for accurate genome assembly

The generated sequencing reads from the DNBSEQ sequencing platform were mapped to *C. psittaci* L99 isolate genome and it showed that the coverage of bronchoalveolar lavage fluid, sputum, and blood samples could reach 99.5%, 99.0%, and 88.0%, with coverage depths of 6.8×, 5.2×, and 2.5×, respectively. Moreover, the results also indicated that bronchoalveolar lavage fluid is the most appropriate sample type for the detection of *C. psittaci* (Fig. 5).

Top ten species	Number of unique reads(n)		
	BALF	Sputum	Blood
Chlamydia psittaci[a]	39,385	30,320	14,478
Propionibacterium acnes[a]	64	59	171
Stenotrophomonas maltophilia[a]	54	102	150
Chlamydia abortus	46	23	/
Klebsiella pneumoniae[a]	29	22	24
Salmonella enterica[a]	27	56	43
Pseudomonas aeruginosa[a]	20	16	29
Moraxella osloensis[a]	17	9	26
Micrococcus luteus	10	8	/
Escherichia coli[a]	8	10	50
Enterococcus faecium	/	/	11
Staphylococcus epidermidis	/	/	11

[a]indicates species that were detected in all three sample types. "/" indicates species that were not among the top ten species of the corresponding sample

Table 1. Main species detected by the MGI platform from the three samples.

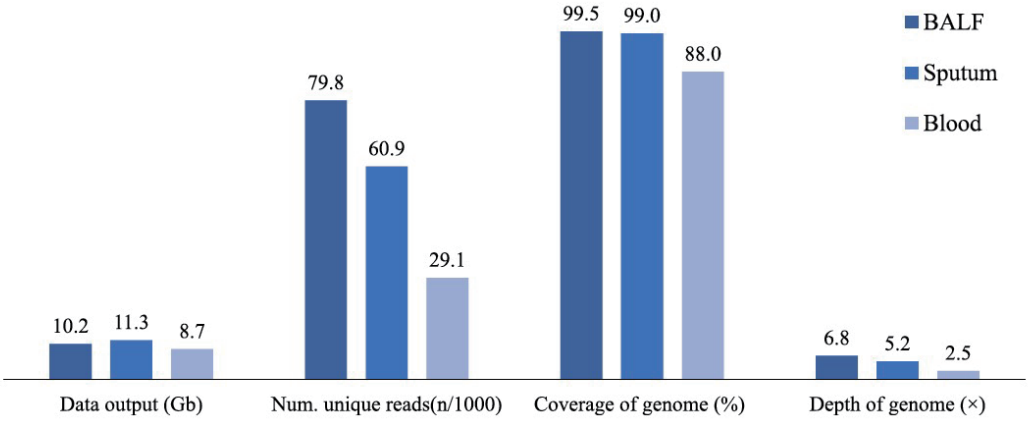

Fig. 5. The MGI DNBSEQ platform based sequencing results of *C. psittaci* from different types of samples.

MGI sequencing platform exhibited superior performance

By comparing the sequencing data of the DNBSEQ sequencing platform with those of Illumina and MinION, it was found that the DNBSEQ sequencing platform performed better, and had more sequencing reads, higher genome coverage, and higher sequencing depth (Fig. 6).

The comparative analysis of all the sequencing data showed no mutations in single bases in the sequencing results of the three platforms. Based on conjoint analysis of sequencing data obtained from the MGI platform and other platforms, the *C. psittaci* genome containing 34 Contigs was assembled. The phylogenetic analysis showed that the L99 isolate is highly homologous to the MN isolate (NC_018627.1) and 01DC12 isolate (NC_019391.1) of the reference genome *C. psittaci* (Fig. 7). At the same time, high-quality genomic analysis based on these sequencing data showed that this isolate had no resistance and virulence factors.

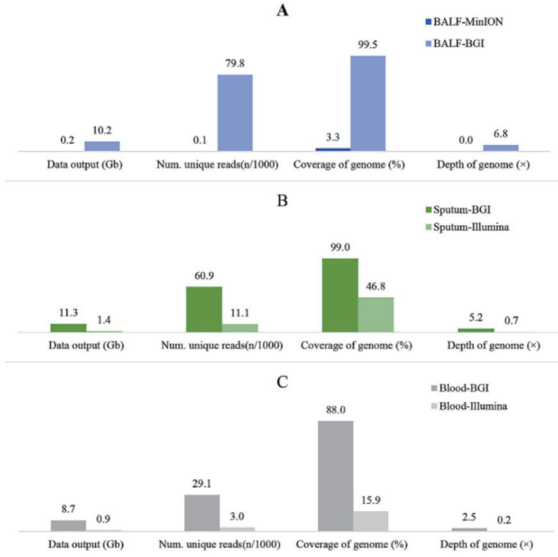

Fig. 6. Comparison of sequencing results between the MGI DNBSEQ sequencing platform and other platforms.

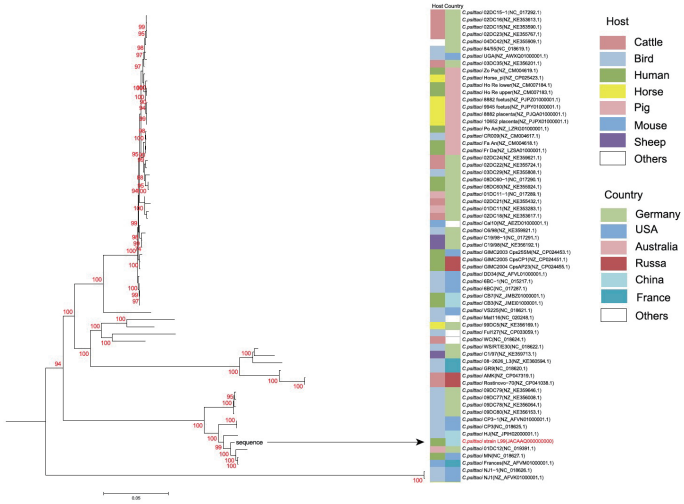

Fig. 7. Whole-genome phylogeny of *C. psittaci* strain L99.

Summary

Based on metagenomic sequencing analysis of three body fluid samples from a patient with severe psittacosis, three sequencing platforms—including the MGI DNBSEQ sequencing platform—were compared in this study, to identify the pathogenic species causing the disease, and evaluate the performance of the three sequencing platforms. The MGI sequencing platform produced the largest amount of effective data, with the widest coverage of the whole genome, the deepest sequencing depth, and the highest detection sensitivity. The MGI sequencing platform is suitable for pathogen detection of multiple samples and can generate accurate detection results.

For pathogenic microorganism detection based on a large number of samples, MGI can provide a whole process of automated solution from sample preparation, DNA extraction, library construction, sequencing, and data analysis, to result reports. In addition, MGI can also provide a platform of microorganism fast identification (PFI) for rapid and accurate identification of diverse and important pathogens, thus providing strong support for the prevention and control of related epidemics.

DNBSEQ-G99ARS Genetic Sequencer

Reference

1. Wang, K. *et al.* Metagenomic diagnosis of severe psittacosis using multiple sequencing platforms. *BMC Genomics* 22, 1-6 (2021).
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4. Morens, D. M. & Fauci, A. S. Emerging infectious diseases in 2012: 20 years after the institute of medicine report. *mBio* 3, e00494-12 (2012).
5. Knight, R. *et al.* Best practices for analysing microbiomes. *Nat Rev Microbiol* 16, 410-422, (2018).
6. Qin, J. *et al.* A human gut microbial gen ecatalogue established by metagenomic sequencing. *Nature* 464, 59-65 (2010).
7. Liu, Y.-X. *et al.* A practical guide to amplicon and metagenomic analysis of microbiome data. *Protein Cell* 12, 315-330, (2021).

Recommended Ordering Information

Category	Product	Cat. NO.
Instruments	DNBSEQ-G99ARS Genetic Sequencer	900-000609-00
	MGISP-100RS Automated Sample Preparation System	900-000206-00
	MGISP-960RS Automated Sample Preparation System	900-000146-00
Software	Platform of microorganisms Fast Identification and assembly evolution	900-000399-00
Library Prep reagents	MGIEasy Universal DNA Library Prep Set（16 RXN）	1000006985
Sequencing Reagents	DNBSEQ-G99RS High-throughput Sequencing Set （G99 SM FCL PE150）	940-000410-00

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1. For StandardMPS and CoolMPS: Unless otherwise informed, StandardMPS and CoolMPS sequencing reagents, and sequencers for use with such reagents are not available in Germany, Spain, UK, Sweden, Belgium, Italy, Finland, Czech Republic, Switzerland, Portugal, Austria and Romania. Unless otherwise informed, StandardMPS sequencing reagents, and sequencers for use with such reagents are not available in Hong Kong. No purchase orders for StandardMPS products will be accepted in the USA until after January 1, 2023.
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.