PFI
One click Pathogen Fast Identification Server

Product Introduction

PFI is a solution that combines the functions of software and hardware. It involves a large microorganism database with analyzing function, and is able to manage the whole process including inputting samples and generating reports via ZLIMS. The entire system is loaded on the server of Bioinformatics analysis accelerator.

PFI can rapidly, accurately and comprehensively identify microorganisms from the original samples at a metagenome/meta-transcriptome level, and automatically generate identification reports and analytical results. It can also provide assistance and references for the diagnosis and treatment of infectious diseases.

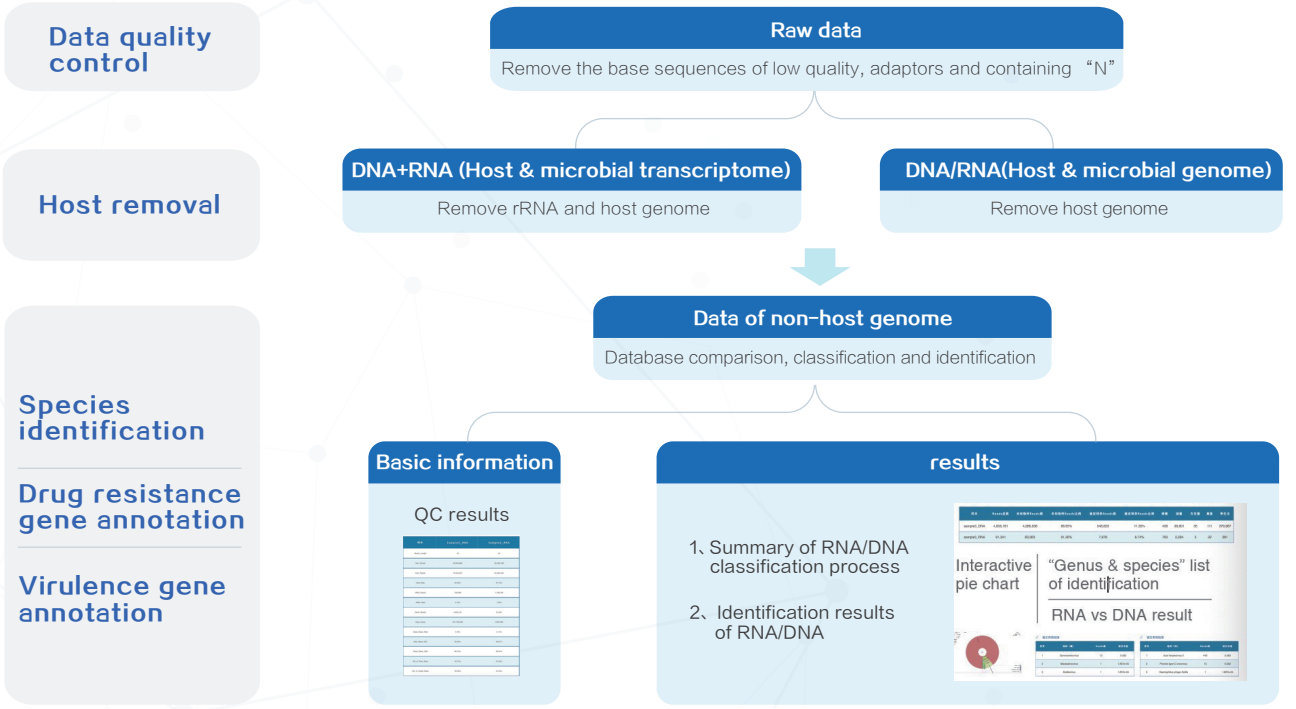
Database

The PFI system collects more than 27,000 microbial genomic sequences (the information of species that have multiple reference genomes is also included) for the database to provide rapid and precise detection.

Microbial classification	Species	Genus
Bacteria	8335+	1925+
Archaea	361+	130+
Viruses	9461+	2606+
Fungi	9031+	1050+
Parasites	642+	361+

Workflow

- Format of the input data： FASTQ



Total analysis time

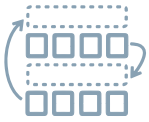
No. Reads/sample	No. Samples/time	Read lengths	Total analysis time (hour)
100M	1	SE50	0.5
	1	SE100	0.85
	1	PE50	0.92
	1	PE100	1.53
100M	5	SE50	1.18
	5	SE100	1.77
	5	PE50	3.23
	5	PE100	2

The server

Highly-stable and reliable HP Z8 G4 workstation

- CPU: Intel Xeon Gold 6240
- Memory: 128GB DDR4
- SSD: 2TB+256GB
- HDD: 30TB 7200 SATA 3.5 inch

Our advantages



Simplified operation process

Web system, one-click operation, easy to use, no need of Linux command and programming.

With Zlims to enable the one-stop automatic operation (sequencing and analyzing); totally automatic, no manual calculating process is needed.

Large database

Host database: reference sequences of human beings, pigs, goats, sheep, mice, rats, carp, domestic geese, chicken, ducks, cattle, cats, dogs, rabbits and other common animals; automatically filter those sequences or customized according to customer needs.

Microbial database: entire NCBI RefSeq genome sequences, including bacteria (including archaea), viruses, fungi, and parasites.

Fully functional analysis

Able to undertake analysis on RNA meta-transcriptome sequencing and DNA metagenome sequencing independently, RNA meta-transcriptome sequencing and DNA metagenome sequencing at the same time.

Solve the problem of background microorganisms' interference, identify the samples more precisely, find the active pathogenic microorganisms.

A wide range of samples

- Including environmental and clinical samples
- Compatible with samples from various sources such as blood of human or animals, alveolar lavage fluid, intestines, etc.

Case sharing

Pathogen Fast Identification helps understand the diversity of samples by sequencing all nucleotides from both host and microbes. As this method does not require preliminary knowledge of pathogenic microbial genomes, it can identify unknown pathogens in infectious disease. Importantly, the unique identification technique supports to develop strategies to control and prevent human and animal infectious diseases.

Case 1
Identification of a novel or variant pathogen strain

Overview

A 4-year-old boy was hospitalized with clinical presentations of hand-foot-and-mouth disease including fever and vesicular exanthema on his hands, feet, oral mucosa, and anus for 1 week. The qRT-PCR results revealed that the causative agent was HEV instead of EV71 or CVA16. To further verify the pathogen, a stool sample was collected from the patient for metagenomic sequencing.

Solution

The stool specimen was collected for Pathogen Fast Identification with automated RNA isolation, library preparation and high-throughput sequencing.

Result

Ten non-overlapping contigs were assembled after high-throughput sequencing and verified by mapping to the genomes of three pathogens: human coxsackievirusA24, enterovirus96, and human poliovirus 1. Primers were used to amplify the sequences and analsyis suggested that all the contigs belonged to a consensus sequence of new strain EV-96. It is the first time that metagenomic sequencing has been used to identify an EV-96 strain as the cause of HFMD.

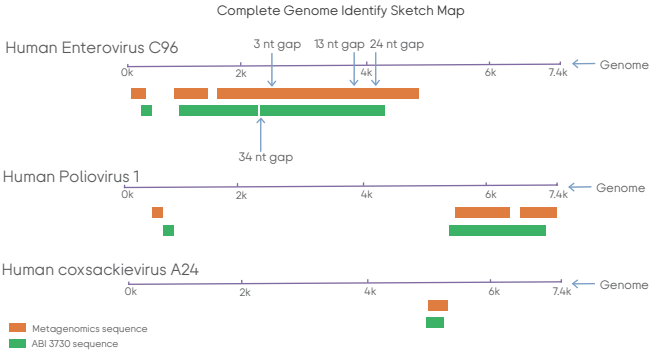


Fig.1 Complete genome identify sketch map. A total of 10 contigs were assembled after metagenomic sequencing (yellow-highlighted bars). Sequencing was performed with an ABI 3730 genetic analyzer. the sequence was verified,and the gaps were filled with overlap for the whole genome (green-highlighted bars) (Color figure online)

Paper

A novel Enterovirus 96 circulating in China causes hand, foot, and mouth disease published on Virus Genes on February 7th, 2017

A novel Enterovirus 96 circulating in China causes hand, foot, and mouth disease

Case 2
Identification of co-infections

> Overview

A 26-year-old woman developed a mild respiratory illness on Jan. 28, 2017, but symptoms progressed to recurrent fever, cough, chills, expectoration, slight hemoptysis, muscle and joint pain in the following days. On February 3, the patient was hospitalized due to worsening symptoms of cyanotic lips, fever of 39.3 ° C, heart rate of 144 beats/ min and diagnosed with severe pneumonia with ARDS. She was treated with antibiotics and antiviral therapies and then discharged on February 17. Blood and respiratory secretions were collected during her hospitalization for pathogen testing. The screening results from bacteria and fungi culture-based test, G-test and GM-test were all negative.
In addition, HIV, HBV, influenza viruses, SARS-CoV, MERS- CoV and other coronaviruses were negative by ELISA and/or (RT-)PCR assays.

> Solution

Pulmonary secretions from the patient were collected on the first day of hospitalization and analyzed using metagenomic sequencing to determine the cause of infection.

> Result

Two respiratory viruses, HRV and HBoV were identified in high abundance using sequencing and confirmed by specific (RT-)qPCR assays and a report generated to diagnose acute co-infection of HBoV1 and HRV-C.

In this case, metagenomic sequencing showed a significant advantage in detection of the causative agents of severe illness over traditional methods such as culture, ELISA, PCR, etc. because prior knowledge was not assumed or required.

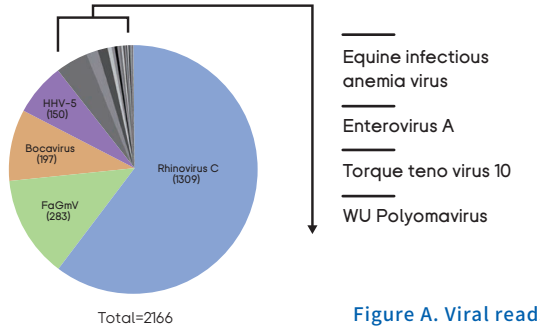


Figure A. Viral reads distribution.

> Paper

In a case published on Journal of Infection in March 2018 of an adult suffering from severe pneumonia.

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Journal of Infection (2017) ■■, ■■–■■

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LETTER TO THE EDITOR

Metagenomic analysis identified co-infection with human rhinovirus C and bocavirus 1 in an adult suffering from severe pneumonia

Dear Editor,

Human rhinovirus (HRV) and bocavirus (HBoV) are two of the most common respiratory viruses associated with acute respiratory tract infections among children.[1,2] Recent articles in this journal have highlighted the role of bocavirus and rhinovirus in potentially severe pneumonia in adults and younger children.[3,4] However co-infection with both viruses was very rare among adults. A previous study reported a 16-month child who was infected by HBoV1 and also showed presence of HRV, developed severe pneumonia.[5] Here we reported co-infection with HBoV1 and HRV-C in an adult woman with acute respiratory distress syndrome (ARDS).

A 26-year-old woman caught a cold on Jan. 28, 2017, and developed symptoms of recurrent fever, cough, chills, expectoration, slight hemoptysis, muscle and joint pain in the following days (Fig. 1A). She felt chest tightness and shortness of breath on February 1, and visited Guangzhou Eighth People's Hospital for further examination and treatment on February 3 since her condition continuously worsened with cyanotic lips and paraesthesia.

The patient had fever of 39.3 °C, heart rate of 144 beats/min, respiratory rate of 38 breaths/min, blood pressure of 112/66 mmHg, and pulse oxygen saturation (SpO2) of 85%. Laboratory evaluation showed white blood cell count $2.2\text{–}3.77 \times 10^9$/L(normal, $3.5\text{–}9.5 \times 10^9$/L), lymphocyte 0.61×10^9/L (normal, $1.1\text{–}3.2 \times 10^9$/L), platelet count (PLT) 72×10^9/L (normal, $100\text{–}350 \times 10^9$/L), C-reactive protein 89.5 mg/L (normal, <8.0 mg/L), hemoglobin (HB) 108 g/L (normal, 110–150 g/L), oxygenation index (OI) 105 mmHg (normal, 400–500 mmHg), CD3 T cell 197/mm³ (normal, 500–1300/mm³), CD4 T cell 197/mm³ (normal, 300–1200/mm³), and CD8 T cell 121/mm³ (normal, 238–874/mm³) (Supplementary Table S1). Auscultation of the lungs revealed extensive moist rales and computed tomographic (CT) scan showed bilateral diffuse infiltration (Fig. 1A). She was diagnosed with severe pneumonia with ARDS, and admitted immediately to the ICU with high flow oxygen to maintain the SPO2 at approximately 90% on February 3. During this period, she showed polypnea, dysphoria, frequent cough and slight hemoptysis. Abundant hemorrhagic secretions were observed through bronchofiberscope. She was treated with antiviral and antibiotic therapies in combination with ulinastatin (Fig. 1A).

Antiviral drug peramivir was used in the first three days, followed by oseltamivir in another three days. Antibiotics (imipenem, cilastatin and moxifloxacin) were given for 10 days. Her body temperature returned normal on the fourth day and CT showed significant improvement in the patient's condition. She was switched to sequential non-invasive ventilation from February 10 to 12, and then transferred to general ward and treated with low-flow oxygen and prednisone. The patient was discharged from the hospital on February 17 and showed a sustained recovery in two subsequent visits on February 28 and March 15.

Throat swabs and sera were collected during her hospitalization. Blood and respiratory secretions were negative for bacteria, and fungi in cultures, and G-test and GM-test, respectively. HIV, HBV, influenza viruses, SARS-CoV, MERS-CoV and other coronaviruses were negative by ELISA and/or (RT-)PCR assays. Pulmonary secretions from the first day of hospitalization were further analyzed using metagenomic sequencing as described previously.[6] Of 64,024,857 total reads, 2166 (0.0034%) were of viral origin. Two respiratory viruses, HRV (1309 reads, 60.4%) and HBoV (197 reads, 9.1%) were found with high percentages (Fig. 1B). The presence of HRV and HBoV were confirmed by specific (RT-)qPCR assays, and HBoV1 RNA was also detected at all sample points. HRV-C and HBoV1 were determined by phylogenetic analyses of HRV VP4/VP2 and HBoV VP1/VP2 fragments, respectively (Fig. 1C). Specific IgM and IgG responses against HBoV1 and HRV-C were further investigated by ELISA (Feiya BioTech, China). The serum IgG and IgM levels of the patient showed 2.2 to 4.7 fold increases for HRV-C and 5.1 to 2.6 fold increases for HBoV1 from February 4 to February 15, respectively (Fig. 1D). Above results indicated an acute co-infection with HBoV1 and HRV-C in the patient. We further characterized the dynamics of both viruses using (RT-)qPCR assays (Fig. 1E). After 2 days of treatment, the viral load of HRV-C and HBoV1 among swabs showed a rapid decrease from 1.3×10^6 copy/ml to below the detection limit, and from 1.2×10^8 copy/ml to 2.7×10^5 copy/ml, respectively. Then, HRV-C viral load remained undetectable among swabs and sera, while HBoV1 viral load started a slowdown from 2.7×10^5 copy/ml to 1.2×10^5 copy/ml during whole treatment, indicating a persistent infection of HBoV1.[7] Accompanied by the antiviral treatment, the patient progressively recovered and bilateral lung opacity improved rapidly, indicating that the treatment was effective. It was difficult to determine which virus was mainly responsible for the case. But the recovery of the patient from the rapid elimination of HRV-C and a persistent presence of HBoV1 among swabs and sera suggested that HRV-C may be the principal causative agent for the severe

Case 3
Diagnosis of rare pathogen

Overview

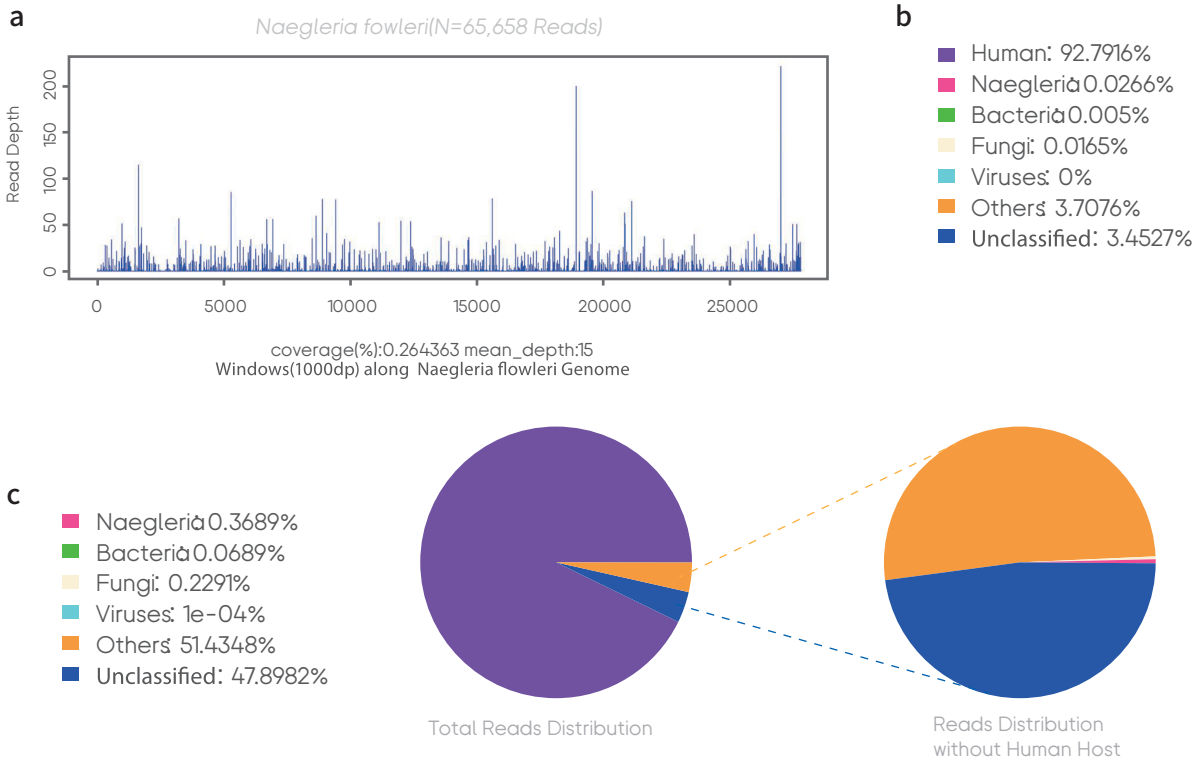
On August 20, 2018, A 42-year-old man was hospitalized after presenting with symptoms of severe headache, fever of 38.4 ° C, and elevated CSF leukocyte and protein levels. 24 hours post-presentation, the patient spoke incoherently, had breathing difficulties, became comatose and was subsequently transferred to ICU. Further examination by CT scan showed hydrocephalus and brain edema. Four days later, a culture from cerebrospinal fluid samples showed negative results for bacteria and fungi, therefore, to identify the pathogen, the sample was further analyzed using high-throughput sequencing on August 31. Results were reported to clinicians 2 days later.

Solution

The cerebrospinal fluid sample collected from the patient was analyzed on the MGI sequencing platform using the Pathogen Fast Identification workflow.

Result

Interestingly the results revealed a low level (0.0266%) of sequencing reads that identified as Naegleria fowleri, a rare amebic pathogen that can cause primary amebic menigioencephalitis (PAM).



PAM caused by Naegleria fowleri infection is extremely rare in China but almost always fatal. The patient went to the Songkran Festival prior to the onset of illness and may have come into contact with sewage. In this case, traditional methods failed to detect the pathogen, however, the MGI high-throughput sequencing platform successfully identified the rare pathogen.

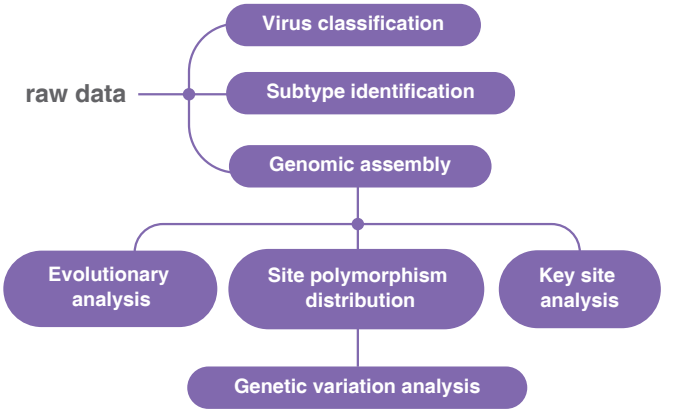
Case 4
Public health issue

Overview

A 45-year-old male returning to China from Angola showed symptoms of Rift Valley fever including fever (38.8 ° C), chills, headache, arthralgia, anorexia and enervation on July 13 and was hospitalized for treatment. BGI assisted the Entry-exit Inspection and Quarantine of China to obtain a whole genome sequence of Rift Valley fever virus from the individual using NGS technology. As a result, BGI helped identify, quarantine and treat the individual and prevent a local outbreak of RVF in China.

Solution

RVFV isolation and culture identification were done in biosafety lab of Guangdong Inspection and Quarantine Technology Center. BGI laboratory performed high-throughput sequencing of the sample to gather genomic information about RVFV.

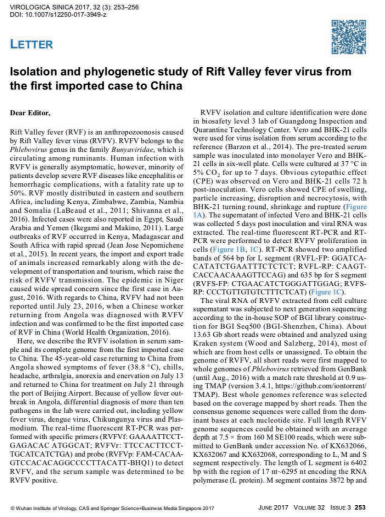


Result

Alignment of the full genome sequence of the RVFV isolate (named RVFV-Beijing strain) revealed 100% identity of three gene segments and 98% homologous to RVFV Kakamas isolate in South Africa.

Paper

Isolation and phylogenetic study of Rift Valley fever virus from the first imported case to China
Published on VIROLOGICA SINICA in June 2017



VIROLOGICA SINICA 2017,32 (3): 253–256
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LETTER

Isolation and phylogenetic study of Rift Valley fever virus from the first imported case to China

Dear Editor,

Rift Valley fever (RVF) is an anthropozoonosis caused by Rift Valley fever virus (RVFV). RVFV belongs to the *Phlebovirus* genus in the family *Bunyaviridae*, which is circulating among ruminants. Human infection with RVFV is generally asymptomatic, however, minority of patients develop severe RVF diseases like encephalitis or hemorrhagic complications, with a fatality rate up to 50%. RVF mostly distributed in eastern and southern Africa, including Kenya, Zimbabwe, Zambia, Namibia and Somalia (LaBeaud et al., 2011; Shivanna et al., 2016). Infected cases were also reported in Egypt, Saudi Arabia and Yemen (Ikegami and Makino, 2011). Large outbreaks of RVF occurred in Kenya, Madagascar and South Africa with rapid spread (Jean Jose Nepomichene et al., 2015). In recent years, the import and export trade of animals increased remarkably along with the development of transportation and tourism, which raise the risk of RVFV transmission. The epidemic in Niger caused wide spread concern since the first case in August, 2016. With regards to China, RVFV had not been reported until July 23, 2016, when a Chinese worker returning from Angola was diagnosed with RVFV infection and was confirmed to be the first imported case of RVF in China (World Health Organization, 2016).

Here, we describe the RVFV isolation in serum sample and its complete genome from the first imported case to China. The 45-year-old case returning to China from Angola showed symptoms of fever (38.8 °C), chills, headache, arthralgia, anorexia and enervation on July 13 and returned to China for treatment on July 21 through the port of Beijing Airport. Because of yellow fever outbreak in Angola, differential diagnosis of more than ten pathogens in the lab were carried out, including yellow fever virus, dengue virus, Chikungunya virus and Plasmodium. The real-time fluorescent RT-PCR was performed with specific primers (RVFV-F: GAAAATTCCT-GAGACAC ATGGCAT; RVFV-: TTCCACTTCCT-TGCATCATCTGA) and probe (RVFV-P: FAM-CACAA-GTCCACACAGGCCCCTTACATT-BHQ1) to detect RVFV, and the serum sample was determined to be RVFV positive.

RVFV isolation and culture identification were done in biosafety level 3 lab of Guangdong Inspection and Quarantine Technology Center. Vero and BHK-21 cells were used for virus isolation from serum according to the reference (Barzon et al., 2014). The pre-treated serum sample was inoculated into monolayer Vero and BHK-21 cells in six-well plate. Cells were cultured at 37 °C in 5% CO_2 for up to 7 days. Obvious cytopathic effect (CPE) was observed on Vero and BHK-21 cells 72 h post-inoculation. Vero cells showed CPE of swelling, particle increasing, disruption and necrocytosis, with BHK-21 turning round, shrinkage and rupture (Figure 1A). The supernatant of infected Vero and BHK-21 cells was collected 5 days post inoculation and viral RNA was extracted. The real-time fluorescent RT-PCR and RT-PCR were performed to detect RVFV proliferation in cells (Figure 1B, 1C). RT-PCR showed two amplified bands of 564 bp for L segment (RVFL-FP: GGATCA-CATATCTGAATTTCTCTCT; RVFL-RP: CAAGT-CACCAACAAAGTTCCAG) and 635 bp for S segment (RVFS-FP: CTGAACATCTGGGATTGGAG; RVFS-RP: CCCTGTTGTGTCTTTCTCAT) (Figure 1C).

The viral RNA of RVFV extracted from cell culture supernatant was subjected to next generation sequencing according to the in-house SOP of BGI library construction for BGI Seq500 (BGI-Shenzhen, China). About 13.63 Gb short reads were obtained and analyzed using Kraken system (Wood and Salzberg, 2014), most of which are from host cells or unassigned. To obtain the genome of RVFV, all short reads were first mapped to whole genomes of *Phlebovirus* retrieval from GenBank (until Aug., 2016) with a match rate threshold at 0.9 using TMAP (version 3.4.1, https://github.com/iontorrent/TMAP). Best whole genomes reference was selected based on the coverage mapped by short reads. Then the consensus genome sequences were called from the dominant bases at each nucleotide site. Full length RVFV genome sequences could be obtained with an average depth at 7.5 × from 160 M SE100 reads, which were submitted to GenBank under accession No. of KX632066, KX632067 and KX632068, corresponding to L, M and S segment respectively. The length of L segment is 6402 bp with the region of 17 nt–6295 nt encoding the RNA polymerase (L protein). M segment contains 3872 bp and

Case 5
Diagnosis of animal disease

Overview

A group of goats, infected with unknown pathogens developed scabby lesions around their lips, muzzle,and in their mouth. To efficiently control the unknown infection, throat swab samples from the affected goats were tested using MGI Pathogen Fast Identification system with a report generated within two days.

Solution

The throat swab samples from goats were processed using the MGI Pathogen Fast Identification system with automatic DNA and RNA sample extraction.

Result

A large proportion of sequencing reads in both DNA (64.2%) and RNA (44%) samples mapped directly to the Orf virus which associates closely with the clinical symptoms presented.

Table 1 The microorganism identification of DNA and RNA sample from goat swab

Rank	name of pathogen	DNA		RNA	
		reads number	relative abundance	reads number	relative abundance
1	Orf_virus	358593	64.20%	11311	44%
2	Pseudocowpox_ virus	26658	4.80%	650	2.50%
3	Bacillus_subtilis	3130	0.60%	518	2.00%
4	Pseudomonas_aeruginosa	2011	0.40%	217	0.80%
5	Staphylococcus_aureus	1158	0.20%	220	0.90%

Direct comparison of the obtained sequencing reads to reference genome of Orf virus genome showed 86.7% identity, 87.6% average coverage and 200X depth. (see Figure below)

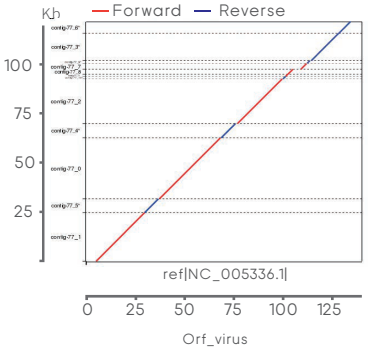

Fig.1 Alignment linear graph of assembled sequence and viral genome

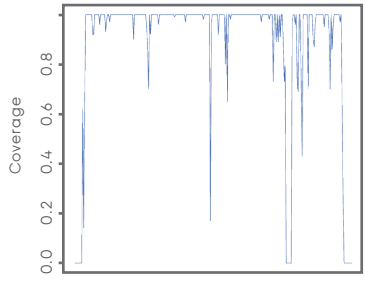

Fig.2 Average coverage of viral genome

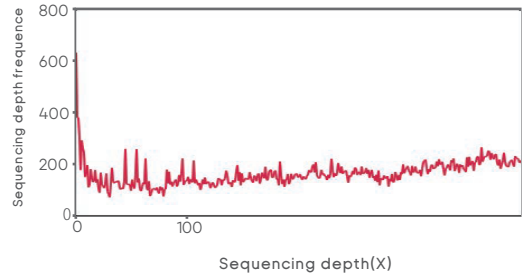

Fig.3 Average sequencing depth of viral genome

To verify the result, a traditional PCR assay was then performed and it confirmed the accuracy of the result. The MGI sequencing technology is highly accurate for pathogen identification and capable of rapid diagnosis and treatment of animal diseases.

MGI Global Presence

China

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R&D centre

Shenzhen
R & D Center
Production Center
Training Center
Logistics Centre
After-sale Center

Wuhan
R & D Center
Production Center
Training Center
Logistics Centre
After-sale Center
Quality Center
Qualification Center

Hong Kong
Logistics Centre
After-sale Center

Latvia

Riga
Qualification Center
After-sale Center
Training Center
Logistics Centre

UAE

Dubai
After-sale Center

Japan

Kobe
After-sale Center

US

San Jose
R & D Center
Production center
After-sale Center

Ordering Information

Product	Part No.
Fast identification of pathogenic infections (with server)	510-000164-00

MGI Tech Co., Ltd.

Service & Support
MGI has accumulated rich experience in gene sequencing with an excellent team of scientists and engineers, who are committed to providing comprehensive technical support in each section: from the installation, testing and operation, training, maintenance to subsequent upgrades, as well as the laboratory system construction, experiment scheme design and sequencing data analysis. You will experience an unprece-dented journey of sequencing.

Contact Us
Address.: Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, China 518083
Email: MGI-service@mgi-tech.com
Website: en.mgi-tech.com
Tel: 4000-966-988
https://www.linkedin.com/company/mgi-bgi
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