Rapid identification of unknown pathogen in breeding base

MGI Microbiological Detection Total Package

Highlight

Comprehensive and Constantly Updated Database

- A database of the complete genomes of 27,830 microorganisms, including viruses, fungi, archaea, bacteria, and parasites

Fast, Short Identification

- No need for virus isolation and cell culture, no need for empirical

High Compatibility

- Open system, supports for third party kit and analysis software

Introduction

ORFV is prevalent worldwide, especially in sheep-raising countries. In China, it occurs in Xinjiang, Qinghai, Inner Mongolia and other regions where sheep breeding are concentrated, bringing huge losses to sheep breeding industry[1,2]. The traditional ORFV detection method is PCR technology. However, due to the limitation of the type and quantity of primers, the PCR detection may cause many problems such as: fewer pathogens detected,missed diagnosis, misdiagnosis, cannot detect unknown and new pathogens.

A number of green goats in a breeding base were infected with unknown pathogen. The main symptoms were pustule and scab on the lip. In order to control the epidemic situation, swab samples of several sheep with severe disease were tested with MGI Microbiological Detection Total Package, and the pathogen identification results were obtained within two days. This paper briefly introduces the workflow and data results for the rapid identification of unknown pathogens in the breeding base.

Experimental Method

Library preparation

MGIEasy FS DNA library prep set V2.0 (16 RXN, Cat. No. 1000005252) and MGIEasy RNA library prep set V3.0 (16 RXN, Cat. No. 1000006383) were used respectively to construct DNA and RNA library. And MGIEasy cyclization kit V2.0 (16 RXN, Cat. No. 1000005259) was used to cyclize the libraries. After the reaction, DNB was made according to the DNBSEQ-G50RS high-throughput sequencing set* (SE100, Cat. No. 1000019856) instructions (FIG. 1). These kits are all suitable for automation system MGISP-100 (MGI, Cat. No. 900-000051-00).

Figure 1. MGI Library Preparation Kits

Sequencing

Based on DNBSEQ-G50* (FIG. 2, Cat. NO. 900-000354-00) high-throughput sequencer, SE100 sequencing is carried out. The single-end sequencing can effectively detect the virus and shorten the time. We also provide DNBSEQ-G400* (Cat. No. 900-000170-00) and DNBSEQ-T7* (Cat. No. 900-000128-00) for instrument selection, and SE50 and PE100 for sequencing reads.

Figure 2. DNBSEQ-G50 high-throughput sequencer

Data analysis

Pathogen Fast identification software (PFI, Cat. No. 510-000164-00) provides host filtering, pathogeny identification, identification of virulence factor and identification of resistance genes. By comparing with the MGI-constructed database (Table 1), PFI presents the top 10 identification result of bacteria, fungi, virus, archaea, protozoa, respectively, including the reads number and relative abundance on genus level and species level.

If the sample can be identified, researcher can use third party open-source assembly software (e.g. SPAdes, IDBA and ABySS) to complete genome based on the identified pathogeny sequencing reads. Also, we provide Microbial Genome Analysis Pipeline(MGAP, Cat. No. 970-000109-00) software for data analysis selection.

Table 1. Microbial database

Classification of Microbes	Number of Species	Number of Family
Bacteria	8335	1925
Archaea	361	130
Fungi	9461	2606
Virus	9031	1050
Parasite	642	361

Results

Identification of *Orf_virus*

After sequencing, DNBSEQ-G40 sequencer generated 8.1GB data with a total of 81.1 M metagenome sequencing reads. Combined with the Pathogen Fast Identification software, a total of 138, 492 reads were identified (Table 2).

Table 2 Microbial database

NO.	Species	Reads Number	Relative Abundance(%)
1	*Orf_virus*	138,492	15.487
2	*Pseudocowpox virus*	10694	1.196
3	*Bovine papular stomatitis virus*	497	0.056
4	*Parapoxvirus of red deer in New Zealand*	461	0.052
5	*Seal parapoxvirus*	76	0.009
6	*Staphylococcus virus EW*	56	0.007
7	*Jaagsiekte sheep retrovirus*	41	0.005
8	*Enzootic nasal tumour virus of goats*	35	0.004
9	*Klebsiella virus Matisse*	14	0.002
10	*Klebsiella virus KP27*	11	0.001

Assembly and verification of *Orf_virus*

The viral genome sequence with a full length of 122.9 kb and a GC content of 64.57% was obtained by assembling the 138, 492 reads from all the sequences. The alignment rate with *Orf_virus* (135 kb) was as high as 86.7% (FIG. 3) and the average coverage was as high as 87.6% (FIG. 4).

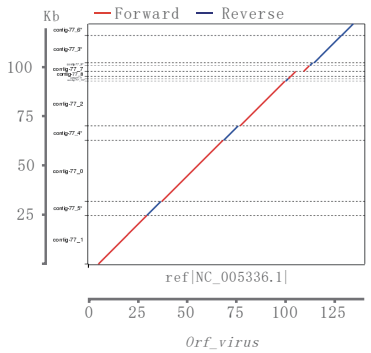

Figure 3. The assembled sequence is aligned linearly with the viral genome

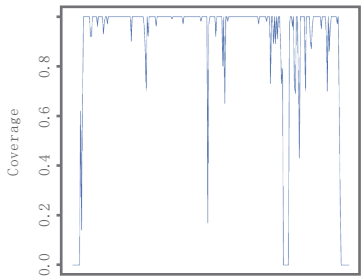

Figure 4. Average coverage

Finally, the infected goats were verified by PCR, and *Orf_virus* was identified in all of them. The results further explained the accuracy of the MGI Microbiological Detection Total Package, and provided important information for the control of the epidemic situation.

Reference

1. Hosamani M, Scagliarini A, Bhanuprakash V, et al. Orf: an update on current research and future perspectives. Expert review of anti-infective therapy, 2009,7(7):879-893.
2. Zhao K, Song D, He W, et al. Identification and phylogenetic analysis of an Orf virus isolated from an outbreak in sheep in the Jilin province of China. Veterinary microbiology, 2010,142(3-4):408-415.

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Microbiological Detection Total Package

Ordering information

Equipment

Product Name	Catalog No.	Configuration
DNA Sequencing Library Preparation System MGISP-100	900-000051-00	RUO
Genetic Sequencer DNBSEQ-G50RS*	900-000354-00	RUO
Genetic Sequencer DNBSEQ-G400RS*	900-000170-00	RUO
Genetic Sequencer DNBSEQ-T7RS*	900-000128-00	RUO

Reagents

Product Name	Catalog No.	Configuration
MGIEasy FS DNA Library Prep Set	1000006987	16 RXN, RUO
MGIEasy RNA Library Prep Set	1000006383	16 RXN, RUO
MGIEasy Circularization Kit	1000005259	16 RXN, RUO
DNBSEQ-G50RS High-throughput Sequencing Set*	1000019856	SE100, RUO

Analysis

Product Name	Catalog No.	Configuration
Fast identification of pathogenic infections (PFI, with server)	510-000164-00	Server + software, RUO
Fast identification of pathogenic infections (PFI)	057-000060-00	software, RUO
Microbial Genome Analysis Pipeline (MGAP)	970-000109-00	software, RUO

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*Unless otherwise informed, StandardMPS and CoolMPS sequencing reagents, and sequencers for use with such reagents are not available in Germany, USA, Spain, UK, Hong Kong, Sweden, Belgium, Italy, Finland, Czech Republic, Switzerland and Portugal.

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