MGI Respiratory Microorganisms Genome Sequencing Package

Highlight

High-quality technologies integration

For library preparation, the ATOPlex multiplex PCR technology can enrich targeted regions and the MGISP-100RS automated sample preparation can complete Fast PCR-FREE library preparation.For sequencing and data processing, DNBSEQ-G50RS* genetic sequencer is used to perform PE100 rapid sequencing. After the sequencing is completed, FluTrack can seamlessly perform automated data processing.

High sequencing detection sensitivity

It is suitable for high-throughput sequencing of samples with Ct value of influenza A/B virus ≤ 32. The ATOPlex multiplex PCR technology makes it possible to obtain high-coverage sequencing data of the influenza A/B virus whole genome.

Automated experiments and data processing

With the MGISP-100RS automated sample preparation system, multiple steps in the entire experiment can be realized through automated operations. The FluTrack software can seamlessly connect the sequencing data and the data processing, realizing the automatic result generation, which vastly reduces the dependence on manual labor in the whole process.

Excellent data quality

DNB and cPAS technologies ensure the sequencing accuracy and efficiency, providing high-quality sequencing data.

Product Description

The MGI Respiratory Microorganisms Genome sequencing package is based on self-developed compatible reagents, automated sample preparation systems, high-throughput sequencing platforms, and data processing systems for influenza A/B virus whole-genome sequencing, covering the processes from RNA to result generation. Parts of experimental steps and all data processing can realize automatic operation. This package can quickly amplify the 8 viral genome segments of influenza positive sample, including influenza A HA (1-16), NA (1-9) subtypes, and influenza B Yamagata and Victoria subtypes. It performs fast, accurate, and comprehensive high-throughput sequencing, thereby providing important references for applications such as whole genome amplification, typing , assembly and tracing of influenza A and B viruses.

Method

Use influenza A and B viruses RNA standard. Dilute viral RNA standard to reactions containing 500, 1000 and 10000 copies (samples of influenza A are named as S500-A, S1000-A and S10000-A, respectively; samples of influenza B are named as S500-B, S1000-B and S10000-B, respectively) . Use MGIEasy Respiratory Microorganisms Genome Amplification Kit (Cat. No. 940-000059-00) to perform reverse transcription and multiplex PCR amplification. Use MGIEasy Fast PCR-FREE FS Library Prep Set (16 RXN, Cat. No. 940-000019-00) in the MGISP-100RS automated sample preparation system (Cat. No. 900-000206-00) for automated library preparation.

Use DNBSEQ-G50RS High-throughput Rapid Sequencing Set (FCS PE100)*(Cat. No. 1000019861) for PE100+10+10 sequencing.

Use MGI FluTrack software (Cat. No. 970-000225-00) to perform data filtering and quality control of sequencing data, identification of influenza A and B viruses, viral genome assembly, and phylogenetic analysis.

Results

Data Overview

Table1 Data overview

Sample ID	Raw Reads	Raw Q30	GC	Clean rate	Influenza reads Pct
S10000-B	7,260,100	92%	41%	99.90%	99.88%
S10000-A	6,729,326	93%	44%	99.72%	99.84%
S1000-B	9,669,614	93%	41%	99.61%	99.26%
S1000-A	9,080,198	93%	43%	98.81%	96.41%
S500-B	9,618,600	93%	40%	99.88%	99.01%
S500-A	9,062,940	94%	43%	99.81%	96.53%

Identification Result

FluTrack completes primer excision, filtering, comparison and identification of sequencing data fq through bioinformatic analysis of sequencing downstream data; assembly and traceability analysis of Influenza A and Influenza B samples, thus obtaining the assembly sequences of Influenza A and Influenza B, specifically the identification results and traceability results of Influenza A and Influenza B. The identification result is as follows.

Table2 FluTrack identification result

Sample ID	InfluenzaA Identification Result	InfluenzaA Reads Pct	InfluenzaB Identification Result	InfluenzaB Reads Pct	InfluenzaA Type	InfluenzaB Type
S10000-B	Negative	NA	Positive	99.88%	-	Yamagata
S10000-A	Positive	99.84%	Negative	NA	H1N1	-
S1000-B	Negative	NA	Positive	99.26%	-	Yamagata
S1000-A	Positive	96.41%	Negative	NA	H1N1	-
S500-B	Negative	NA	Positive	99.01%	-	Yamagata
S500-A	Positive	96.53%	Negative	NA	H1N1	-

Fragment coverage

MGIEasy Respiratory Microorganisms Genome Amplification Kit can amplify the 8 viral genome segments. After sequencing, they were compared and analyzed by FluTrack software. The coverage of each fragment exceeded 95%, and the fragment coverage was as follows.

Table3 Viral genome segments coverage

Sample ID	PB1	PB2	PA	HA	NP	NA	M	NS
S10000-B	99.92%	99.46%	99.05%	98.25%	99.19%	99.87%	98.74%	96.26%
S10000-A	99.40%	99.87%	98.75%	99.55%	97.89%	98.84%	99.12%	99.55%
S1000-B	98.90%	99.33%	99.26%	96.01%	99.67%	99.87%	99.83%	96.72%
S1000-A	99.32%	99.23%	98.66%	99.55%	99.23%	97.88%	98.34%	99.55%
S500-B	98.69%	99.33%	99.39%	95.27%	99.84%	99.87%	98.74%	97.63%
S500-A	99.23%	99.19%	98.75%	98.76%	97.96%	98.84%	99.42%	99.55%

Phylogenetic Analysis

The analysis results include traceability results, and the software uses the assembled sequences and database sequences for multiple sequence comparison, and visualizes the 100 species with the most similar origin through the evolutionary tree. Influenza A uses three traceability methods, whole genome, HA (Hemagglutinin), and NA (Neuraminidase) traceability (if only Influenza A can be identified, but its subtype is unknown, only NA and HA traceability results will be included in the report); Influenza B uses whole genome traceability. The whole-genome traceability means that the database contains all 8 viral fragment genomes and the assembled genomes for evolutionary tree construction; HA traceability means that the database contains only HA genomes and the assembled HA fragments for evolutionary tree construction; NA traceability means that the database refers to only NA genomes and the assembled NA fragments for evolutionary tree construction. The phylogenetic analysis results of influenza A and B are as follows.

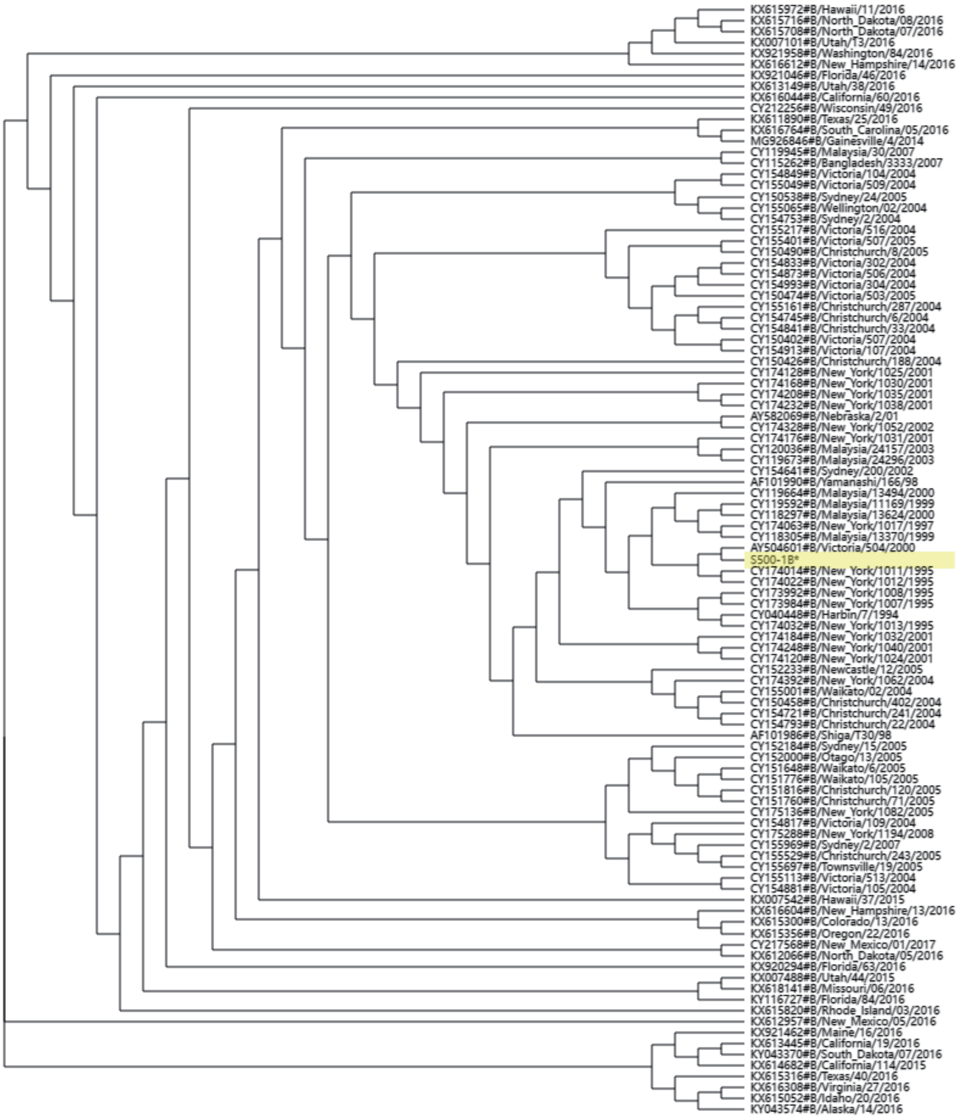

Figure 1 phylogenetic analysis of influenza B virus

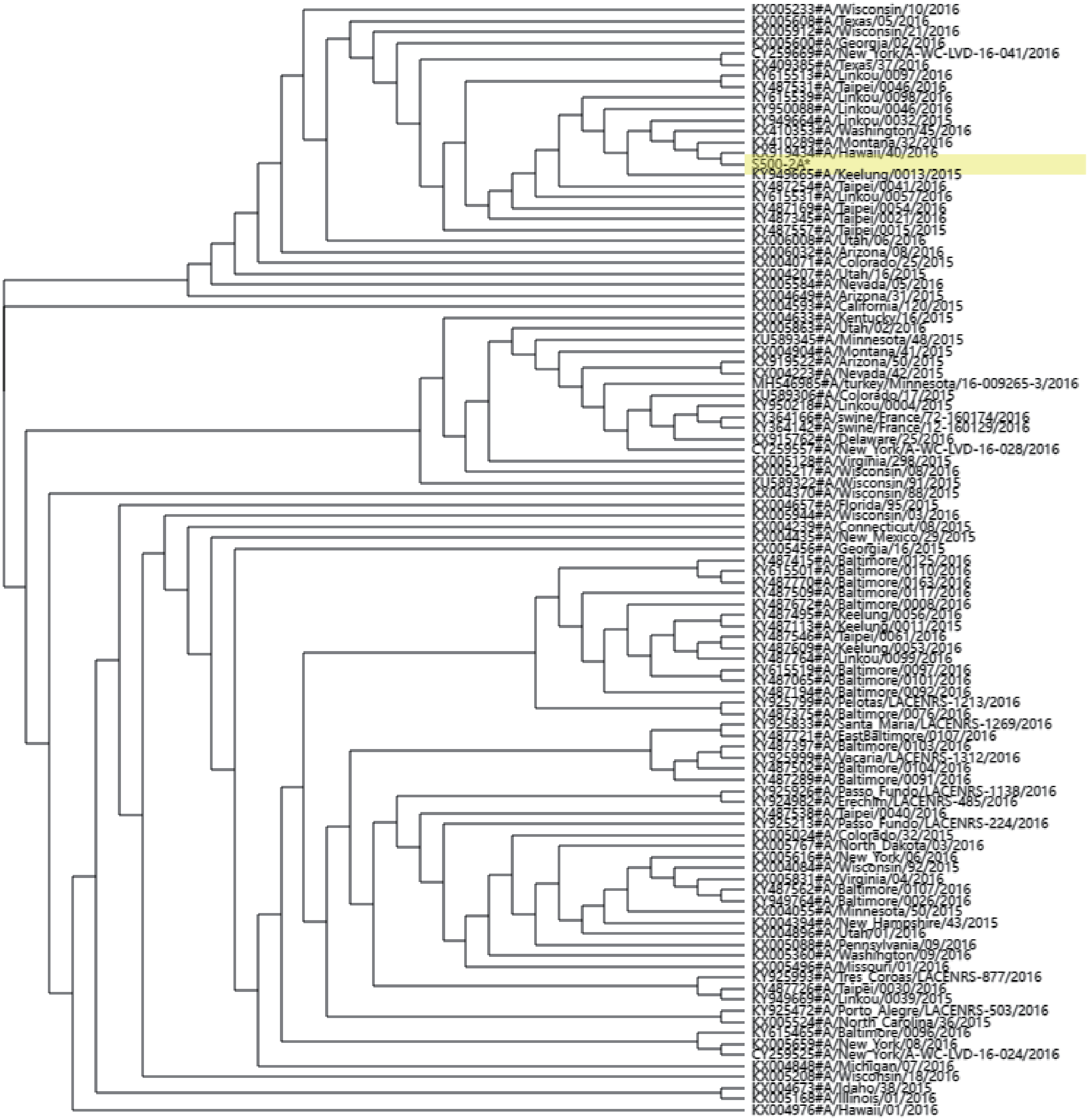

Figure 2 Phylogenetic analysis of influenza A virus

Summary

Equipped with MGI's self-developed reagents, automated sample preparation system, high-throughput sequencing platform, and FluTrack software, the MGI Respiratory Microorganisms Genome sequencing package enables to perform influenza A/B virus whole-genome sequencing, alignment, identification and phylogenetic analysis., etc. Therefore, This MGI package is ideal for influenza virus whole genome sequencing and analysis, which can help customers achieve their research goals faster, more conveniently, and more accurately.

Ordering Information

Product	Specification	Cat. No.
Automated Sample Preparation System and Sequencer		
MGISP-100RS Automated Sample Prep System	Standard Configuration	900-000206-00
Genetic Sequencer DNBSEQ-G50RS*	Configuration 2	900-000354-00
Library and DNB Prep Reagent		
MGIEasy Respiratory Microorganisms Genome Amplification Kit	16 RXN	940-000059-00
MGIEasy Fast PCR-FREE FS DNA Library Prep Set	16 RXN	940-000019-00
MGIEasy Dual Barcode Circularization Kit	16 RXN	1000020570
Sequencing Reagent		
DNBSEQ-G50RS High-throughput Rapid Sequencing Set*	FCS PE100	1000019861
CPAS Barcode Primer 3 Reagent Kit	3.5 ml	1000020834
Data processing system		
MGI FluTrack	V1.0	970-000225-00

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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.