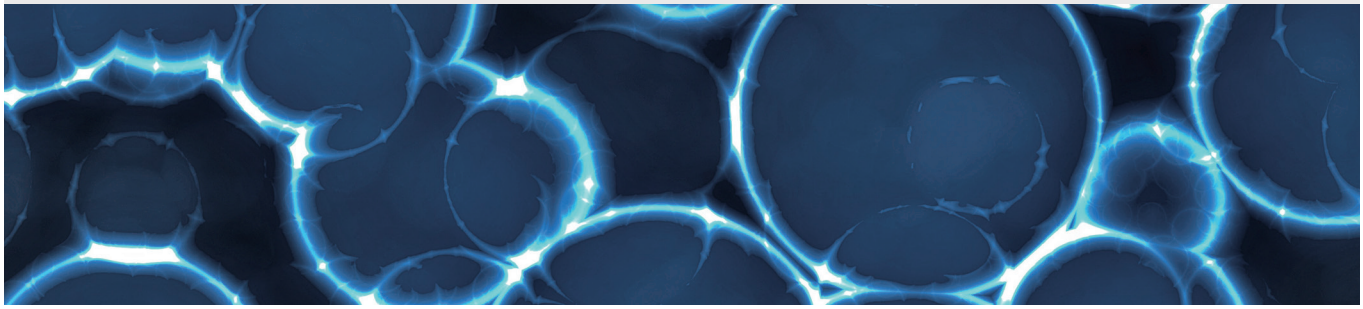
MGI

Respiratory Microorganisms Genome Sequencing Package

FAQ

Package characteristics



What are the product forms and characteristics of this package?

MGI Respiratory Microorganisms Genome Sequencing Package consists of the reagents independently researched and developed by MGI, an automated sample preparation system, a high-throughput sequencing platform, and a data processing system. It covers the entire process from RNA to result output. Part of the experiment operations and all data processing processes can be realized through automatic operation.

The package can be used to perform rapid, accurate, and comprehensive high-throughput sequencing of positive RNA samples of Influenza A and B, providing important references for assembling, identifying and tracing the genome sequence of Influenza A and B samples.

Specifically, after RNA reverse transcription and multiplex PCR amplification are completed in the library preparation process, you can perform library preparation (8 or 16 throughput) by using the MGISP-100RS automated sample preparation system, which adopts the Fast PCR-FREE library preparation technology.

In the sequencing and data processing process, you can use the DNBSEQ-G50RS genetic sequencer to carry out rapid sequencing of PE100, and realize automatic data analysis through MGI FluTrack.

What subtypes of influenza A and B can be identified by MGI FluTrack?

The influenza A database of MGI FluTrack contains 16 HA subtypes (H1-H16) and 9 NA subtypes (N1-N9). The software can identify the combination of influenza A subtypes existed in the database, such as the representative subtypes circulating in the population: A(H1N1), A(H3N2), etc.; for influenza B virus, the software is able to identify the lineage of samples based on their whole genome: B/Yamagata or B/Victoria.

What are the features of the amplification and library preparation kits in this package?

- **MGIEasy Respiratory Microorganisms Genome Amplification Kit:**

  (1) The primers for influenza A and B viruses are mixed into one tube, and there is no need to distinguish the types of influenza viruses in the sample before amplification;

  (2) During amplification, one-step RT-PCR can realize the amplification of the whole genome of eight fragments in one tube.

  (3) Compared with other multiplex PCR reactions, this kit adopts the technology of relative quantification based on the external reference. By adding a known amount of external reference, the virus concentration in the sample can be relatively quantified.

- **MGIEasy Fast PCR-Free FS DNA Library Prep Kit:**

  (1) Using high-quality one-step enzymes for fragmentation and end repair, which can simplify the experimental processes;

  (2) Combined with dual unique barcode technology, impressively reducing the risk of cross-contamination of samples.

Can the package be used for microbial identification and tracing besides Influenza A and B?

No. This package only applies to whole-genome amplification, library preparation, sequencing and data processing of Influenza A and B, and to obtain results of Influenza A and B identification, assembling and tracing, etc.

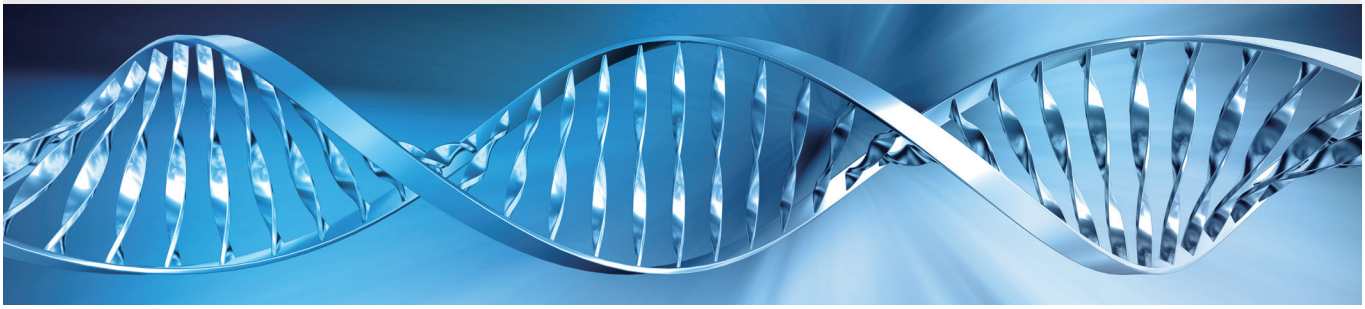
How long does it take for this package to complete the whole process of experiment and data processing?

This package covers the entire process from RNA to sequencing result output, which takes 31 to 44 hours in total.

Table 1 Processing time of the package

Step	Run times
Library preparation (RNA→DNB)	8 RXN：about 11.5 h；16 RXN：about 12.5 h；
Sequencing and data processing	FCS, DNBSEQ-G50RS Configuration 1, 31 to 34 h； DNBSEQ-G50RS Configuration 2, about 22 h
Total time	FCS, DNBSEQ-G50RS Configuration 1, 43.5 to 46.5 h； DNBSEQ-G50RS Configuration 2, 32.5 to 34.5 h

Library preparation



Which types of sample are applicable to library preparation for MGI Respiratory Microorganisms Genome Sequencing Package?

The package is suitable for the total RNA extracted from a variety of samples, including cultured strains and buccal swabs, etc. It applies to the whole genome enrichment, typing and assembly of influenza A and B viruses.

Is it necessary to specifically remove the host DNA or rRNA before library preparation?

No. This product uses an MGIEasy Respiratory Microorganisms Genome Amplification Kit，and its multiplex primers can specifically identify and amplify the genome sequence of influenza A and B, so there is no need to remove the host DNA or rRNA.

What special consumables are needed for the experiment?

It is recommended to use filter tips throughout the experiment. If tips without filters are used during the experiment, it is highly possible to cause aerosol contamination via pipettes, and even cross-contamination between samples.

How do you avoid aerosol contamination?

In addition to using filter tips during experiment, strictly dividing the experiment operation area into a pre-amplification area and a post-amplification area is necessary. Pipettes, pipette tips, magnetic stands and lab coats of each area cannot be used mixedly.

Table 2 Experimental operation partition

Experimental area	Pre-PCR Area	Post-PCR Area
Experimental operation	RT-PCR product purification and Homogenization	RT-PCR amplification reaction
	RT-PCR product purification reagent preparation	RT-PCR product cleanup, quantification and homogenization,
	Fast PCR-FREE library preparation reagent preparation	Fast PCR-FREE library preparation, quantification and homogenization
	/	Circularization, DNB reagent preparation and DNB preparation

Can the automatic PCR instrument integrated in MGISP-100RS effectively prevent cross-contamination?

The MGISP-100RS internally-integrated PCR module has a temperature-controllable hot lid, and a PCR seal is installed on the hot lid. Under the action of pressure, a complete sealing effect is formed between the rubber pad and the PCR plate. After verification, its sealing effect is equivalent to that of the heat-sealing film, which can effectively avoid cross-contamination between samples during the experiment. After each use, you should follow the MGISP-100 and MGISP-960 Equipment Cleaning Instructions to complete the [post-clean] process.

What steps can you use MGISP-100RS during the experiments of MGI Respiratory Microorganisms Genome Sequencing Package?

Library preparation of influenza RNA sample includes RT-PCR amplification, RT-PCR amplification product purification, FAST PCR-FREE library preparation, library circularization and DNB preparation. MGISP-100RS can be used for RT-PCR amplification product purification (8 or 16 sample throughput) and FAST PCR -FREE library preparation (8 or 16 sample throughput).

Table 3 Package experiment

Steps	Operation	Run times
RT-PCR amplification	Manual	About 5 h
RT-PCR amplification product purification	Automated	8RXN throughput：about 30 min；16RXN throughput：40 min
Fast PCR-FREE library preparation	Automated	8RXN throughput：1 h 55 min；16RXN throughput：2 h 25 min
Library circularization and DNB preparation	Manual	About 3 h

When using MGISP-100RS, how to dispense reagents for library preparation?

When using the MGIEasy Fast PCR-FREE FS Library Prep Set (16 RXN, Cat.No.: 940-000019-00) for library preparation on MGISP-100RS, it can perform twice with 8 RXN throughput, but you need to manually dispense reagents before each library preparation and then use MGISP-100RS for automated reaction, or you can directly use the original tube of reagents for library preparation with a 16RXN throughput without manual dispensing.

If you use the MGIEasy Fast PCR-FREE FS Library Prep Set (96 RXN, Cat. No.: 940-000021-00) for library preparation on MGISP-100RS, you need to manually dispense the reagents according to relevant instructions.

What quality control indicator is involved in library preparation?

The quality control indicator of library preparation refers to the concentration of library.

Table 4 The quality control indexes of library preparation

Quality control indexes	Quality control ranges	Measurement method
The concentration of RT-PCR amplification products purified	≥5 ng/μL	Qubit dsDNA quantitative detection
Fast PCR-FREE library concentration	≥0.8 ng/μL	Qubit dsDNA quantitative detection
Circularization purification product	≥10 ng	Qubit ssDNA quantitative detection
DNB Concentration	≥8 ng/μL	Qubit ssDNA quantitative detection

How to deal with abnormal DNB concentration?

- **When DNB concentration is lower than 8 ng/μL, perform the following steps:**

  1)Check whether the reagent kits used is out of date.

  2)Confirm whether the operations are carried out according to the instructions;

  3)Check whether the concentration and fragment distribution of the FAST PCR-FREE library are normal. Fig. 1 shows the normal fragment distribution of the FAST PCR-FREE library. The size in the figure is larger than the actual one, because the PCR-FREE adapter is a non-complete double-stranded structure, so the electrophoretic migration rate is slower.

  4)When re-preparing DNB under normal conditions of the library, increase the amount of purified circularization product input appropriately.

- **When DNB concentration is higher than 40 ng/μL, do the following:**

  It needs to be diluted to 20 ng/μL with DNB Loading Buffer I before use.

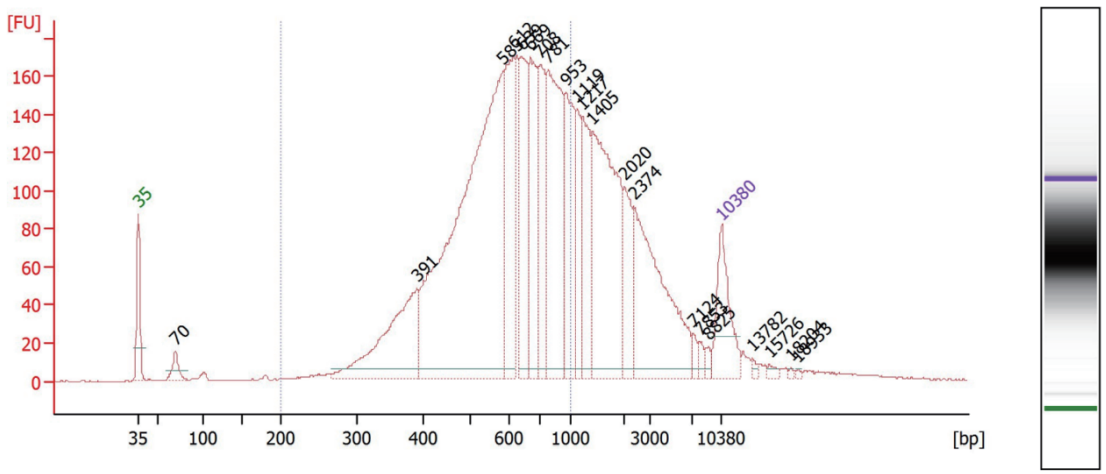


Fig.1 The fragment distribution of the library of the packages under normal conditions

High-throughput sequencing

What should you do if sequencing reagents are thawed but cannot be used on time?

- If a kit has been thawed (including dNTPs) and cannot be used on time, it can go through freeze-thaw cycle once again.
- If a kit has been thawed (including dNTPs) and cannot be used on time, it can be temporarily stored at 4 ℃ and used within 24 h. The reagent cartridge needs to be re-mixed before use.
- If dNTPs and enzymes have been added to a reagent cartridge, the reagent cartridge has been well prepared. If it cannot be used in time, it can be temporarily stored at 4 ℃ and used within 24 h. The reagent cartridge needs to be re-mixed before use.
- If a reagent cartridge has been well prepared when dNTPs and enzymes have been added. Then sequencer's needle has been inserted into it. If it cannot be used in time, make sure to seal it with tinfoil, store it at 4 ℃, and use it within 24 h. Gently mix the reagent cartridge before use. Be careful not to spill the reagent from needle holes when mixing to avoid cross-contamination.

Data processing

What is reference genome sequence information used for data processing?

The reference genomes are derived from the NCBI public database.

Can MGI FluTrack software be used to analyze samples from other sources besides the analysis of Influenza A and B viruses?

No. The MGI FluTrack software is a bioinformatics tool dedicated to the analysis of the Influenza A and B viral genome. Its functions include Influenza identification and phylogenetic analysis.

How long does it normally take to analyze the sequencing data when using the FluTrack software?

If the sequencing data produced by the DNBSEQ-G50RS High-throughput Sequencing Reagent Set (FCS PE100) contains 16 samples, it usually takes about 1.6 h.

What should you do if automated sequencing data analysis by MGI FluTrack fails?

- If the sequencing is successful, a manual analysis process can be initiated on the MGI FluTrack. Please open the rawdata folder on the server desktop and search for the Fastq file with the sequencing flow cell ID to check whether the Fastq file of the sequencing data has been completely transferred to the corresponding server of FluTrack.
- If the sequencing data has been completely transmitted to the server. For details, refer to step 2 to step 5 of Scenario 2: Only analysis server (Manual analysis by ZLIMS Lite) in the MGI FluTrack User Manual, then manually start data analysis.
- If the sequencing data has not been transmitted to the server or the transmission is incomplete, manually import the sequencing Fastq file from the sequencer to the server. For details about data import, refer to step1 of Scenario 2: Only analysis server (Manual analysis by ZLIMS Lite) in the MGI FluTrack User Manual, then manually start data analysis

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