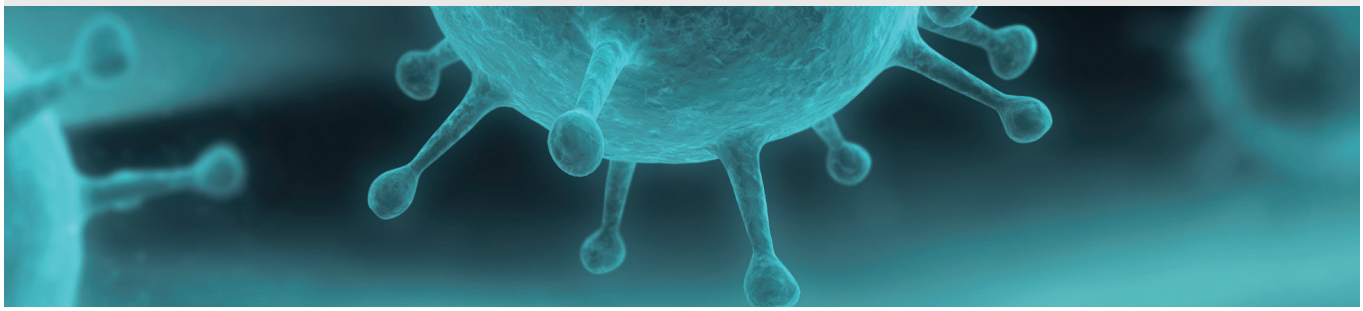
MGI

SARS-CoV-2 Sequencing Package (V3.1)

FAQ

Package characteristics



What are the product forms and characteristics of this package?

The MGI SARS-CoV-2 Sequencing Package (V3.1) 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 SARS-CoV-2, providing important references for detecting mutations in SARS-CoV-2 samples, annotating mutation sites, clade and lineage assignment and tracing, etc.

Specifically, after RNA reverse transcription and multiple PCR amplification, you can perform library preparation (8 or 16 throughput) and make DNB (1 to 4 throughput) by using the MGISP-100RS automated sample preparation system, which adopts the Fast PCR-FREE library preparation technology and One Step DNB preparation technology.

In the sequencing and data processing process, you can use the DNBSEQ-G50RS* genetic sequencer to carry out the rapid sequencing of SE100, and realize automatic data processing through MGI metargetCOVID.

Can the package be used for microbial identification and tracing besides SARS-CoV-2?

No. This package only applies to whole-genome amplification, library preparation, sequencing and data processing of SARS-CoV-2, and to obtain results of SARS-CoV-2 variants detection, variants annotation and mutation branch identification, etc.

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

This package facilitates the entire process from RNA to sequencing result output, and it takes 19 to 22 hours in total.

Table1 The process time of the package

Steps	Run times
library preparation (RNA ⟶ DNB)	8 RXN：~8 h；16 RXN：~9 h；
sequencing and data processing	FCS，11~13 h
In total	FCS, 19~22 h

Library preparation

Which are the suitable sample types for library preparation for ATOPlex RNA Multiplex PCR sequencing packages?

It is suitable for RNA extracted from a variety of samples, including total RNA of blood, tissue, and buccal swabs, etc.

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

No. This product uses a multiplex PCR amplification kit designed by the ATOPlex platform. Multiple primers can specifically identify and amplify the genome sequence of SARS-CoV-2, so there is no need to remove 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, aerosol contamination can be easily caused through the use of pipette, which may cause cross-contamination between samples.

How do you avoid aerosol contamination?

It is recommended to use filter tips in the experiment, and physically divide the experiment operation area into at least the pre-PCR area and the post-PCR area. Pipettes, pipette tips, magnetic stands, lab coats, etc. of each area cannot be cross-mixed.

Table 2 Experimental operation partition

Experimental area	Pre amplification region	Post amplification region
Experimental operation	RNA reverse transcription	Multiplex PCR amplification reaction
	Multiplex amplification reagent preparation and sample adding	Multiplex PCR product purification and normalization
	Reagent preparation of multiplex PCR product purification	Fast PCR-FREE library preparation
	Reagent preparation of Fast PCR-FREE library preparation	DNB reagent preparation and DNB preparation

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

PCR module integrated in the MGISP-100RS 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, the user should follow the **MGISP-100 and MGISP-960 Equipment Cleaning Instructions** to complete the post-clean process.

What steps can I use MGISP-100RS in the experiments of the SARS-CoV-2 package?

Library preparation of SARS-CoV-2 RNA sample includes reverse transcription, multiplex PCR amplification, multiplex PCR product purification, fast PCR-free library preparation, DNB preparation. MGISP-100RS can be used for reverse transcription amplification product purification (8 or 16 sample throughput), fast PCR-free library preparation (8 or 16 sample throughput), and DNB preparation (1 to 4 sample throughput).

Table3 Package experiment

Steps	Operation	Run times
Reverse transcription	Manual	~30 min
Multiplex PCR amplification	Manual	~2 h 20 min
Multiplex PCR product purification	Automated	8RXN throughput：~30 min；16RXN throughput：~40 min
Fast PCR-FREE library preparation	Automated	8RXN throughput：1 h 55 min；16RXN throughput：2 h 25 min
DNB preparation	Automated	1~4RXN throughput：~50 min

When using MGISP-100RS, should I dispense the 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 16 RXN 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 indexes are involved in library preparation?

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

Table4 The quality control indexes of library preparation

Quality control indexes	Quality control ranges	Measurement method
Concentration of Multiplex PCR purification Products	≥5 ng/μL	Qubit dsDNA quantitative detection
Concentration of Fast PCR-FREE Library	≥0.8 ng/μL	Qubit dsDNA quantitative detection
DNB Concentration	≥8 ng/μL	Qubit ssDNA quantitative detection

If the DNB concentration does not meet the requirement, what should I do?

- When DNB concentration is lower than 8 ng/μL, do the following:

  1) Check whether the reagent kits used are expired.

  2) Check 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 picture 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 Fast PCR-FREE library input appropriately.

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

  The DNB needs to be diluted to 20 ng/μL with DNB Load Buffer I before loading.

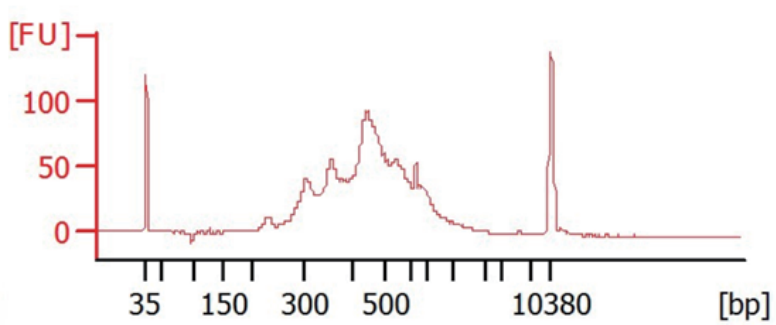


Fig.1 The fragment distribution of the library of the packages under normal conditions

High-throughput sequencing

What should I do if sequencing reagents have been thawed but cannot be used on time?

- If a kit has been thawed (including dNTPs) and cannot be used on time, it can be frozen and thawed 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

Can the metargetCOVID software be used to analyze samples from other sources besides the analysis of SARS-CoV-2?

No. The MGI metargetCOVID software is a bioinformatics tool dedicated to the analysis of the SARS-CoV-2 genome. Its functions include variants detection, variants annotation, clade and lineage assignment, and tracing.

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

If the sequencing data produced by the DNBSEQ-G50RS High-throughput Sequencing Reagent Set* (FCS SE100) contains 16 samples, it usually takes about 40 minutes.

What should I do if automated sequencing data analysis by metargetCOVID fails?

- If the sequencing is successful, 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 metargetCOVID.
- If the sequencing data has been completely transmitted to the server, manually start data analysis. For details, refer to the **Scenario 2: External Sample Sequencing Data Analysis (Manual Analysis)** in **MGI metargetCOVID User Manual.**
- 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 and manually start data analysis. For details about the data import, refer to **Upload Sequencing Data** in **MGI metargetCOVID User Manual.**

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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.