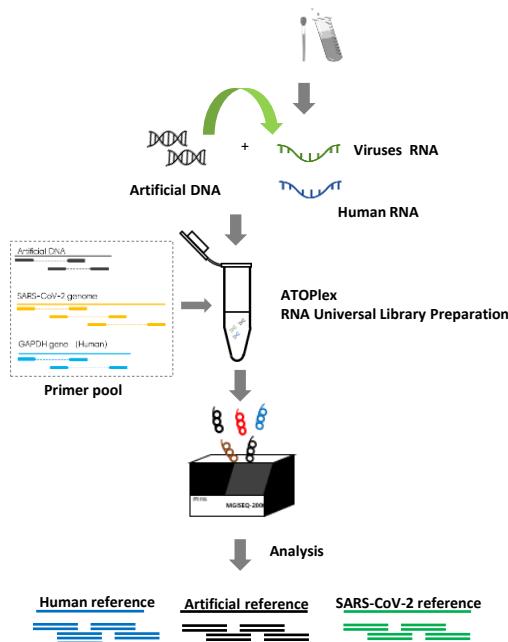
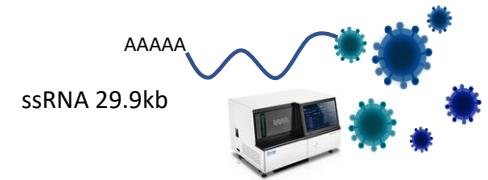


ATOplex RNA Library Prep Set for Virus Research



Introduction

The MGI ATOplex mPCR-based Massively Parallel Sequencing (MPS) platform provides a streamlined workflow for complete genome sequencing and epidemiological study of SARS-CoV-2. By integrating three sets of highly specific universal primers into one tube and utilizing dual barcode anti-crosstalk technology, all relevant genomic segments are amplified. Subsequent sequencing on a DNBSEQ platform combined with these features achieves highly accurate coronavirus genotyping.

In comparison to other multiplex PCR-based methods, the ATOplex RNA Universal Library Preparation Kit combined with Spike-in Control Primer Pool and Dual Barcode Primers can not only analyze the whole viral genome, but more importantly, also quantify viral load. All amplicons are amplified together in one tube which minimizes cross-contamination risk.

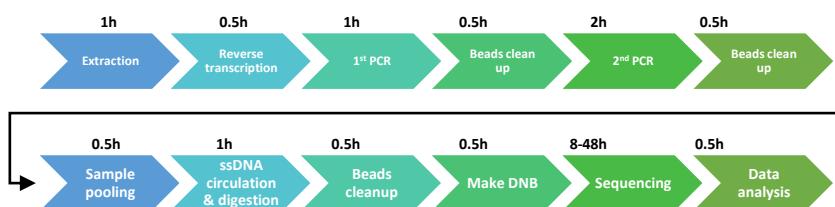


Figure1. Workflow of virus detection and full-length genome analysis

The following **FAQ** highlights some common questions about the operation of the ATOplex RNA Universal Library Preparation Kit.

FAQ

1. Is it necessary to remove host DNA or rRNA before the ATOplex library preparation ?

No. We use multiplex PCR-based technology which can specifically amplify the viral sequences, so there is no need to remove host DNA or rRNA before library preparation.

2. Does the presence of carrier RNA in the nucleic acid extraction process affect subsequent library construction ?

At present, we have not experienced the failure of library construction—due to carrier RNA. However, excess carrier RNA may cause the decrease of reverse transcription efficiency with random hexamers. Therefore, it is strongly recommended to use the nucleic acid extraction kit without adding carrier RNA.

3. What special equipment is needed for the experiment?

This experiment requires pipette tips with filters. Tips without filters can easily allow liquid to enter the pipette which can cause cross-contamination of different samples. Therefore, filter tips must be used.

4. How do you avoid aerosol contamination?

PCR can enrich DNA molecules millions of times, so strict partitioning operation is required. As shown below, if there are space constraints, the operations in R1 and R2 can be merged and R4 and R5 can be merged. Pipettes, magnetic racks, lab coats, etc. cannot be cross-used amongst operations.

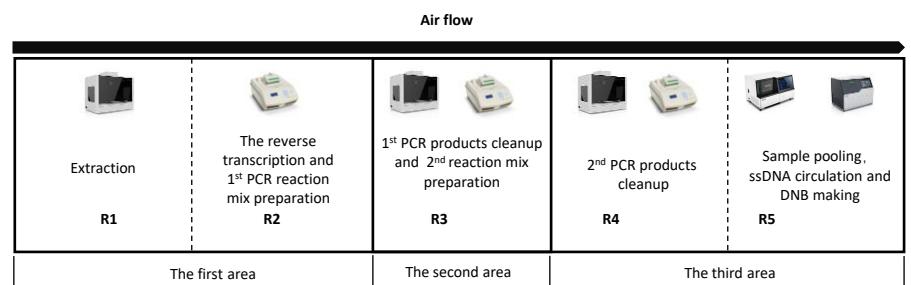


Figure2. Laboratory requirements

5. What is the role of artificial DNA in this kit?

The artificial DNA serves as a spike-in control which is used to relatively quantify viral loads and monitor for successful completion of the 2-step PCR. Artificial DNA has been pre-incorporated into the amplification primer pool.

6. How does the clean system work?

In the pre-PCR stage, the clean enzyme can specifically recognize and digest the 2nd PCR products, which are incorporated modified dNTPs (PCR additives), to reduce aerosol contamination.

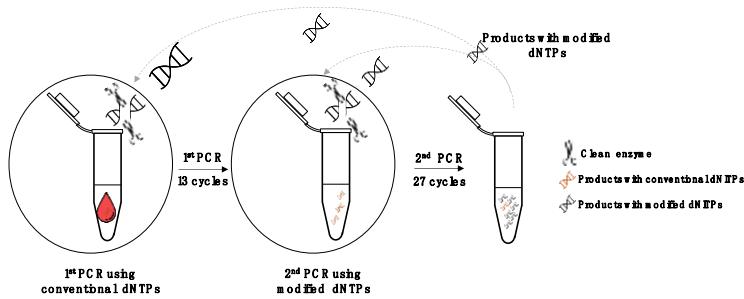


Figure3. Laboratory requirements

7. What is the role of the GAPDH primers in this kit?

The GAPDH primers serve as internal control and have two functions: 1. They are used to relatively quantify the concentration of the virus; 2. They are used for monitoring whether the nucleic acid extraction process is successful. If the GAPDH primers fail to amplify, it is likely that the nucleic acid extraction has failed.

8. What are the criteria for successful library construction?

The concentration of the library should be > 4 ng/μL, amounting to a total of 25 μL and the fragment distribution should be between 240 bp to 350 bp. If the concentration of the library is >80 ng/μL, you can reduce 1-2 cycles at the 2nd PCR stage.

9. Low circularization efficiency during circularization ?

The PCR product cannot be left on the PCR instrument for too long. This can cause the addition of a non-specific base at the 3' end, and this will result in failure of circularization. It is recommended that the purification be completed within 30 minutes of PCR completion. The low complexity of the amplicon library allows for easy renaturation during the denaturation and quenching process, resulting in low circularization efficiency.

Generally, the recommended input amount of amplicon library in the circularization process is >400 ng and it needs to be digested and purified. The efficiency of the circularization is >3%.

10. DNB concentration is low?

It is recommended to input 10 ng of cyclization product to make DNB. Concentration of DNB products >8 ng/μL can meet the sequencing requirements. If the concentration of DNB products is too low, it is recommended to input all cyclization products to make DNB.

11. How many samples can be measured on one lane, on DNBSEQ platform?

Assuming you need to assemble the full genome of the virus, you need to adjust based on viral load and the sequencing platform used. Assuming you're sequencing on the DNBSEQ-G400* and one lane produces 400 M reads, after accounting for the balanced library (100 M reads), 300 M reads are enough for the assembly of 60-300** samples.

Table1 Sequencing capacity for SARS-CoV-2 full-length genome

| CT value of RT-PCR | Data recommended | Max samples on DNBSEQ-G400* | Max samples on DNBSEQ-G50* |
|--------------------|------------------|-----------------------------|----------------------------|
| <30 | 1 M reads | 300/lane | 300/lane |
| 31~34 | 2.5 M reads | 120/lane | 120/lane |
| 35~36 | 5 M reads | 60/lane | 60/lane |

**MGI only provides 96 dual-barcode primers at present, More dual barcode primers need to be synthesized by the customer from the third-party agent (check it out at https://en.mgi-tech.com/Download/download_file/id/191)

12. Does the ATOPlex library need to be mixed with a balanced library for sequencing? What type of balanced library is needed?

The complexity of ATOPlex libraries is low, which can cause the separation of certain bases and affect the accuracy of base calling. Therefore, the addition of a mixed balanced library for sequencing is required. The mixed balanced library must be dual barcoded (ATOPlex Dual Barcode Balance Library Reagent), added according to the DNB amount and the mixing ratio between the ATOPlex library and balanced library should be 3:1.

13. How do you perform dual barcode sequencing? Can dual barcodes also be used for single-end sequencing?

Under the condition that the existing sequencing reagent set is unchanged, dual barcode sequencing can be performed by purchasing an additional dual barcode sequencing primer module (CPAS Barcode Primer 3 Reagent Kit for pair-end sequencing, CPAS Barcode Primer 4 Reagent Kit for single-end sequencing). Dual barcode can also be used for single-end sequencing.

Table2 Reagent list for virus research

| ATOPlex RNA Library Prep Set (940-000183-00) | Cat. No: |
|---|---------------|
| ATOPlex RNA Universal Library Preparation Kit | 940-000181-00 |
| Internal Control Primer Pool | 940-000241-00 |
| ATOPlex PCR Dual Barcode Primer Module (01-96) | 1000021626 |
| ATOPlex Dual Barcoded Balance Library Reagent | 1000022270 |
| Sequencing reagent recommended list | Cat. No: |
| MGIEasy Dual Barcode Circularization kit | 1000020570 |
| CPAS Barcode Primer 3 Reagent Kit (PE Sequencing) | 1000020834 |
| CPAS Barcode Primer 4 Reagent Kit (SE Sequencing) | 1000014048 |

14. How do you conduct data analysis?

There are two ways to analyze: 1) process of data analysis has been disclosed on git hub and can be found at https://github.com/MGI-tech-bioinformatics/SARS-CoV-2_Multi-PCR_v1.0 ; 2) could purchase iGVD software to start automatic analysis.

15. How do you obtain artificial DNA sequences, primer sequences and targeted bed file information?

The required information can be obtained at <https://pan.genomics.cn/ucdisk/s/ERnMVz>

16. How to obtain full-length viral sequence data?

For amplicon sequencing, primers need to be excised and then assembled to obtain accurate full-length sequence. The primer sequence information can be found at <https://pan.genomics.cn/ucdisk/s/ERnMVz>

17. Can this kit detect other pathogenic microorganisms?

This kit only detects the novel coronavirus and cannot detect other pathogenic microorganisms. If you need to detect other microorganisms, you can submit a customized requirement on the ATOPlex page on MGI's official website. We can provide a customized detection panel for pathogenic microorganisms to meet various application needs. <https://www.mgitech.cn/products/atoplex/>

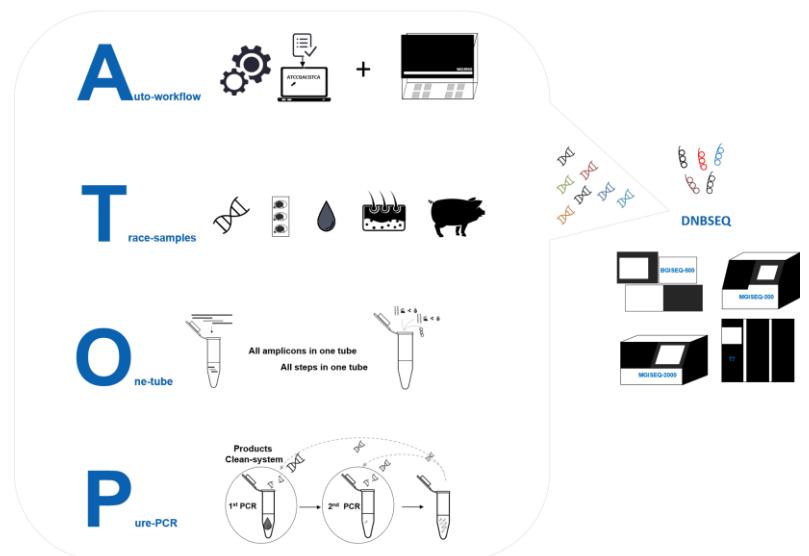
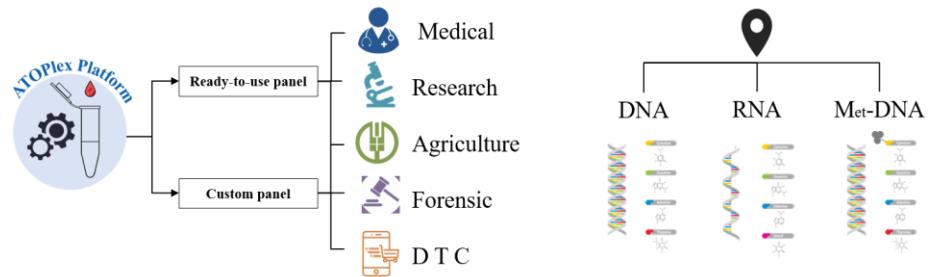
Table 3 The Parameters of the ATOPlex SARS-CoV-2 Full Length Genome Panel

| Specification | Performance |
|-------------------------|---|
| Targeted region | Artificial DNA (10 plex) Human GAPDH gene (4 plex) SARS-CoV-2 Full length genome (259 plex) |
| Amplicons length | 240-333 bp (Insert size 106-199 bp) |
| Amplicons number | 273 amplicons in one tube |
| Sample Input | 10ul total RNA |
| Mutations | SNV, Indel |
| Sample type | Throat swab, plasma, anal swab and BALF etc. |
| Total time | 5.0 hours (sample to library) |
| Uniformity | ≥95%# |
| Specificity | ≥95%# |
| Coverage | ≥99%# |
| Sequencing type | PE100+10+10 |
| Data recommended | 0.5-3 M reads for virus full length genome |

#Input more than 10 copies

About the ATOPlex platform

ATOPlex is a targeted MPS solution based on MGI's proprietary ultra-high multiplex PCR-based enrichment technique. It can be applied to DNA, RNA and DNA methylation sequencing in multiple fields such as medicine, forensics, agriculture, DTC etc. MGI provides a total targeted sequencing solution which includes product design, library construction kits, automated workflow, sequencers etc.



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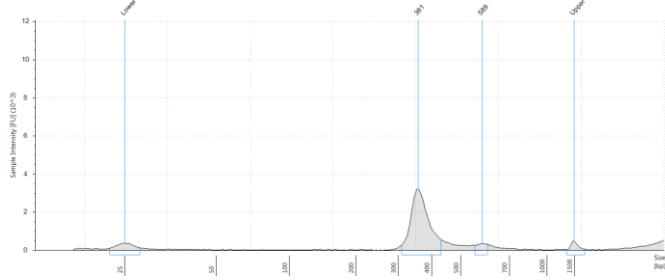
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*Unless otherwise informed, All sequencers and sequencing reagents are not available in Germany, the US, Spain, the UK, HKSAR and Sweden.

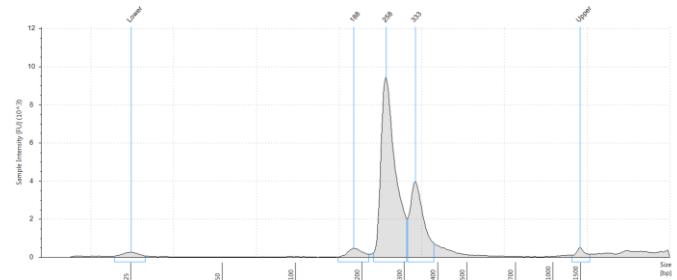
Appendix

The size distribution of ATOPlex SARS-CoV-2 full-length genome panel library varies from sample to sample. The figure below shows that we used the Agilent 4200 TapeStation system to obtain the size distribution of the library from COVID-19 positive swab samples, COVID-19 negative swab samples and NF- water.

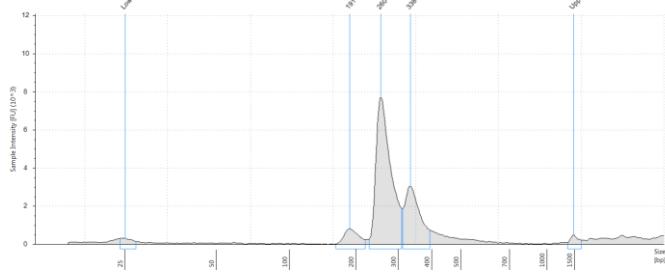
The size distribution of COVID-19 positive sample , ct value=20



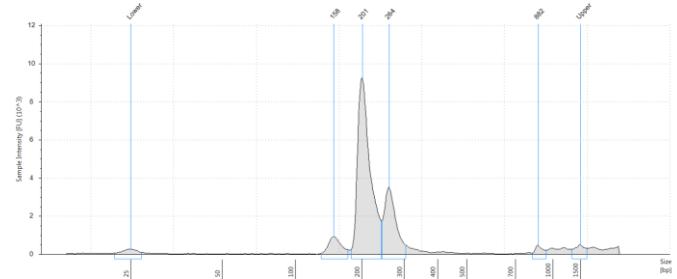
The size distribution of COVID-19 positive sample , ct value =37



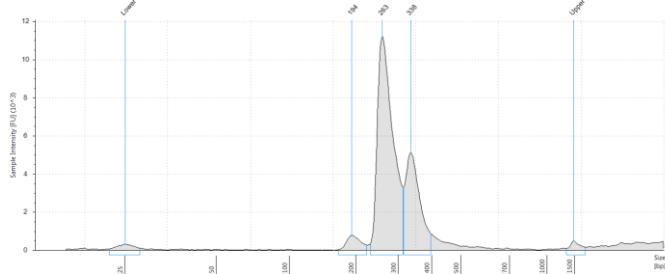
The size distribution of COVID-19 positive sample , ct value = 30



The size distribution of COVID-19 negative sample



The size distribution of COVID-19 positive sample , ct value = 35



NF-Water

