ATOPlex RNA Library Prep Set for Virus Research

——Accurate, fast, cost-efficient, sensitive and simple for virus detection and full-length genome analysis

Highlight

Ultra-sensitive Detection	Analyze samples with as low as 10 copies/mL viral load
Accurate Quantification	The ability to accurately quantify viral load by spike-in control
High Coverage	It covers >99% of the viral genome and variants in challenging sample

Introduction

ATOPlex RNA Library Prep Set is a 2-step multiplex PCR-based library preparation set， which provides a streamlined workflow for SASR-CoV-2 whole genome enrichment and amplification. Combined with DNBSEQ-based high-throughput sequencing platform, it can obtain the full-length genome sequences of SARS-CoV-2 and achieve relative quantification of SARS-CoV-2 for population-scale virus detection, surveillance and tracing.

Product Parameters

Product Name	ATOPlex RNA Library Prep Set
Configuration	96 Preps/kit
Sample Types	Total Nucleic Acid from Throat Swabs, BALF， Saliva, plasma etc
Application	Surveillance, Variation and Evolution Analysis of SARS-CoV-2
Detection Region	SARS-CoV-2 full-length genome
Amplicons Size	106-199 bp
Amplicons Numbles	273 Amplicons in one tube
cDNA input	>10 copies genome for full-length，>10 copies/mL for detection
Variant Types	SNP，InDel
Total Time (sample to library)	5.0 Hours
Uniformity (0.1X)	95%
On Target Aligned Reads	⩾95%
Recommend Sequence Type	PE100 for Full Length Genome，SE50 for Detection
Recommend Total Reads	5-20 M Reads/sample

Workflow

ATOPlex Prep Set utilizes a 2-step multiplex PCR method to enrich and amplify the entire genome of SARS-CoV-2 in one tube. It converts the extracted RNA into a DNA library and ready for subsequent DNB making and sequencing (Figure 1).

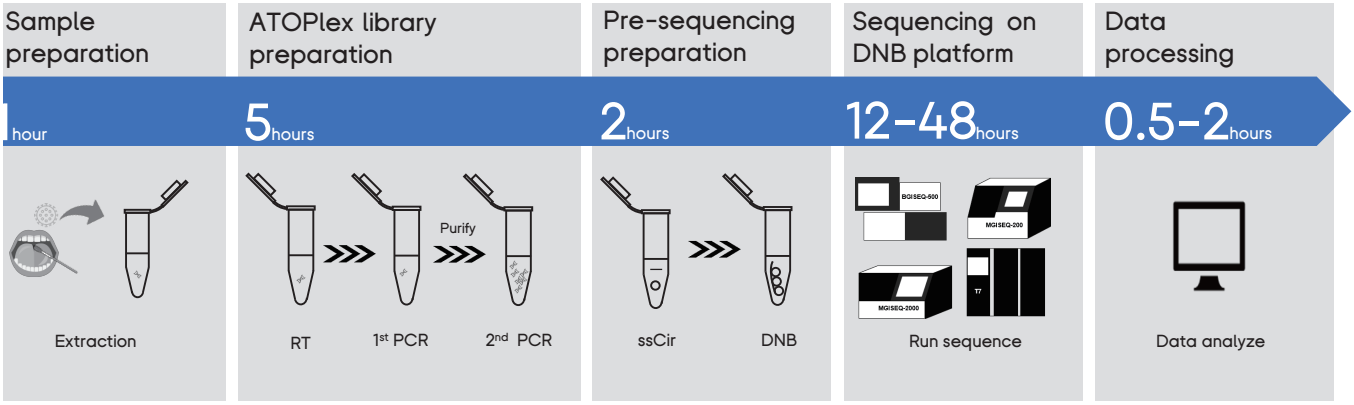

Figure1 Workflow of ATOPlex Massively Parallel Sequencing (ATOPlex MPS)

Performance

Ultra-Sensitive

6 serial dilutions of cultured isolate subjected to direct ATOPlex MPS and RT-qPCR (Figure 2). According to the results (Table 1), ATOPlex MPS can detect and assemble nearly full-length genome with 10-6gradient dilutions (about 10 copies/mL vial load).

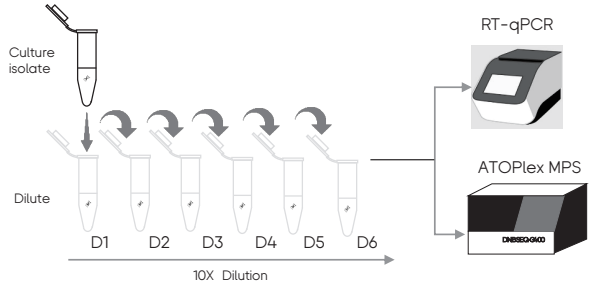

Figure 2 Workflow of subjection to direct ATOPlex MPS and RT-qPCR

Table1 Comparison of mPCR-based MPS and RT-PCR results

ID	Raw reads	ATOPlex MPS			RT-PCR
		SARS-CoV-2 Reads	SARS-Cov-2 Mean Depth	100X Coverage%	Ct Value
Dilution 10^{-1}	**9,455,876**	**9,135,710**	**61102.3**	**99.8**	**24.3**
Dilution 10^{-2}	**10,232,235**	**8,823,284**	**59012.7**	**99.8**	**27.1**
Dilution 10^{-3}	**9,122,357**	**4,655,942**	**31140.3**	**99.8**	**30.6**
Dilution 10^{-4}	**5,965,846**	**441,279**	**2951.4**	**99.8**	**33.5**
Dilution 10^{-5}	**4,536,254**	**154,987**	**1036.6**	**95.3**	**36.9**
Dilution 10^{-6}	**17,563,253**	**30,935**	**206.9**	**75.4**	**NO CT**

Accurate Quantitative

Equimolar artificial DNA has been pre-incorporated into the amplification primer pool. The artificial DNA serves as a spike-in control which is used to relatively quantify viral loads. To evaluate the detection performance, 6 serial dilutions of cultured isolate were detected by ATOPlex MPS and RT-qPCR, and the results of ATOPlex MPS were highly correlated with dilution gradient and RT-qPCR (Figure. 2).

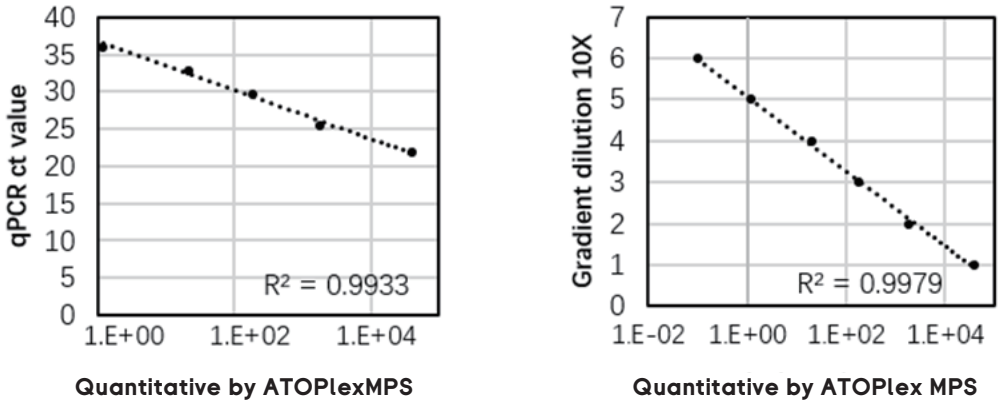

Figure 2 Quantitative Results of ATOPlex MPS. Quantify the copy number of SARS-CoV-2 using definite artificial DNA, the X-axis represents the copy number of SARS-CoV-2 relative to artificial DNA, the y-axis from the left to the right figure is the number of RT-qPCR Ct value and gradient dilution, respectively.

Genome Assembly with Nearly Full-Length Virus Genome

ATOPlex Prep Set was used to prepare the library of the clinical sample with ct value of 35, and then sequencing on DBNSEQ-G400 with PE100+10+10. We assembled a nearly full-length SRAS-CoV-2 genome of 99.54% with >100× coverage and identified a total of 13 variants（Table 2，9 SNP loci, 1 INS loci, and 3 DEL loci）. At the same time, a maximum likelihood phylogenetic tree was constructed according to the SARS-CoV-2 consensus, which provided a powerful basis for virus traceability (Figure. 3).

Table2 SARS-CoV-2 Genome Assembly Information

Assembly length	Non-N ratio(%)	Number of SNPs	Number of INSs	Number of DELs
29903 bp	**99.54**	**9**	**1**	**3**

Figure3 Maximum likelihood phylogenetic tree of SARS-CoV-2 (genomes obtained from GISAID as of early March,2020. Total 26 countries included.)

Summary

ATOPlex RNA Library Prep Set utilizes spike-in control can not only obtian the nearly full-length genome of virus at RT-qPCR ct value of 36.9, but more importantly, also can accurately quantify viral load of clinical sample. Extracted virus RNA was reverse transcribed and amplified in one tube with a simple flow that converts virus RNA to sequence library in 5 hours. ATOPlex MPS workflow based on DNBSEQ high-throughput sequencing platform, enables users to identify SARS-CoV-2, examine biological functions and track genetic changes, thereby allowing for rapid responses to outbreaks.

Ordering information

Cat. No.	Product Name
1000027431	ATOPlex RNA Library Prep Set

About ATOPlex Platform

ATOPlex platform is a targeted MPS customized package 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 package which includes customized panel, automated system and DNB sequencers etc.

Contact Us

MGI Tech Co.,Ltd
Address: Building 11,Beishan Industrial Zone, Yantian District, Shenzhen, CHINA 518083
Email: MGI-service@mgi-tech.com, MGI_ATOPlex@mgi-tech.com
Website: en.mgi-tech.com
Tel: 4000-966-988
Version: October 2021 | MGPD113810200-01

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