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	1-5 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).

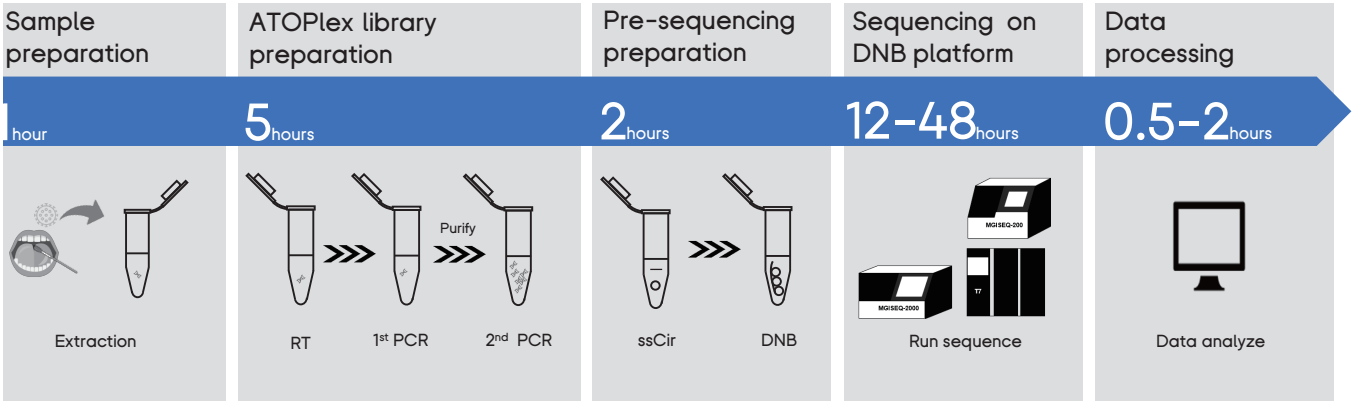

Figure1 Workflow of ATOPlex Massively Parallel Sequencing (ATOPlex MPS)

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).

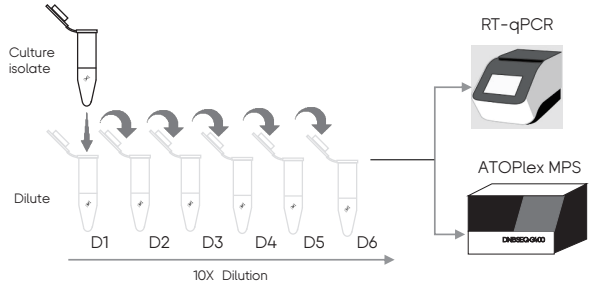

Figure 2 Workflow of subjection to direct ATOPlex MPS and RT-qPCR

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).

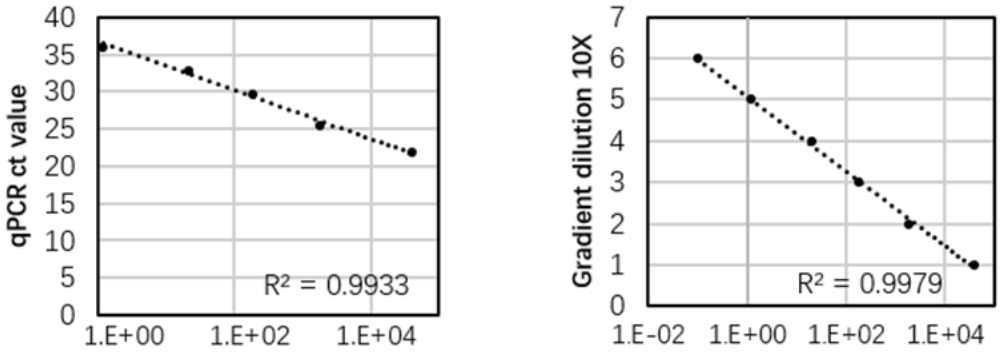

Figure 2 Quantitative Results of ATOPlex MPS. Quantify the copy number of SARS-CoV-2 using definite artificial DNA, the X-axis represents the copy number of SARS-CoV-2 relative to artificial DNA, the y-axis from the left to the right figure is the number of RT-qPCR Ct value and gradient dilution, respectively.

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
940-000183-00	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.

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