

Detection, tracing, and quantification of SARS-CoV-2 in wastewater using the ATOplex platform

MGI ATOplex Custom Platform Application Cases

Highlights

- Ultra-sensitive Detection with as Low as 10 Copies/mL Viral Loads
- >99% Full-length Genome Coverage within Challenging Samples
- Accurate Quantification by Spike-in Control

Introduction

MGI's ATOplex platform consists of the automatic design platform and standardized library preparation kits. ATOplex can be described as Auto-workflow, Trace-samples, One-tube design, and Pure-PCR technologies. It can be used for DNA, RNA and DNA methylation sequencing in multiple fields such as medicine, forensic, agriculture, DTC (Direct to customer), etc. MGI provides a total package for targeted sequencing including product design, library construction kits, automated workflow, and high-throughput sequencers, etc (Figure 1).

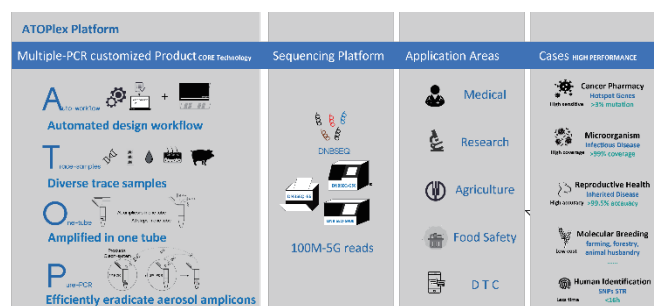


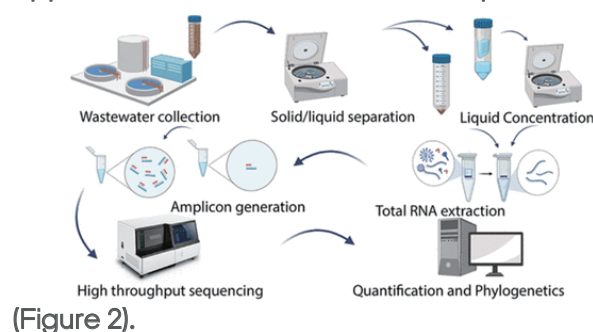
Figure 1. ATOplex platform

Case

At present, the global epidemic caused by the SARS-CoV-2 virus is seriously threatening the health of the public and economic development. Controlling the spread of the epidemic has become most important, and the early detection of pathogens is vital for prevention and control. FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

However, since many virus carriers are asymptomatic patients, there are certain limitations and lags in measuring the prevalence of the virus in the region through clinical tests. The latest research progress has shown that wastewater-based epidemiological studies (WBE) can detect the presence of viruses at an earlier time point than clinical testing. However, the low viral load in wastewater has become a major difficulty in the wide application of WBE.

Recently, the Advanced Water Management Centre from the University of Queensland, Australia and BGI Australia published a research paper in Environmental Science & Technology Letters using ATOplex RNA Library Prep Set. Combined with the DNBSEQ™* technology results show a high accuracy, low duplicate rate and less index hopping. This workflow can accurately detect, quantify, and trace the SARS-CoV-2 virus even at a very low load in urban wastewater. This is achieved with the help of high-throughput sequencing, which provides a very sensitive and scalable research protocol for the wide application of WBE in the field of public health



(Figure 2).

Experimental Method

Technical feasibility verification

A ~500 bp cDNA fragment was obtained by reverse transcription of an RNA positive control of SARS-CoV-2 virus (TWIST Biosciences) followed by ultrasonic fragmentation. The cDNA positive control was used as standards (D1-D12) after gradient dilution and was quantified using droplet digital polymerase chain reaction (ddPCR).

The quantitative standard references were sequenced using the ATOplex RNA Library Prep Set on the DNBSEQ™ sequencing platform. The subsequent re-quantitative and traceability analysis was conducted to verify the feasibility of the technical process in challenging samples.

Meanwhile, the N1 and N2 genes in the standard reference were detected and quantified by RT-QPCR, to compare the detection limit of ATOplex sequencing to conventional RT-QPCR technology.

Real sample preparation

Six untreated (influent) wastewater composite samples (named S01-S06) with a volume of 50 mL were collected from two local wastewater treatment plants (WWTPs) in Brisbane from late March to early April 2020. Total RNA extraction (RNeasy Power Microbiome Kit, Qiagen) was carried out for the solid and liquid fractions separately.

Library preparation

ATOplex RNA Library Prep Set and MGI Circularization kit were used for library preparation of the abovementioned wastewater samples. A short fragment amplicon of ~200 bp was obtained. The preparation process strictly followed the ATOplex user manual (Figure 3).

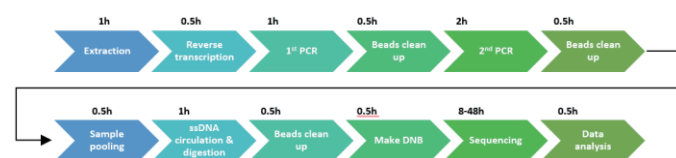


Figure 3. Workflow of virus detection and full-length genome analysis

ATOplex technology realize reverse transcription and amplification in one tube and enrich the target fragment millions of times through two rounds of PCR, to detect very low concentration of virus. Meanwhile, based on 259 amplicons that cover almost the full-length of SARS-CoV-2, effective full-length gene sequence and mutation information could be obtained for subsequent evolutionary tracing analysis.

Sequencing and bioinformatics processing

PE100 sequencing was performed on MGI's DNBSEQ-G400* platform, with a data requirement of 10M reads per sample. The obtained data were processed by using the SARS-CoV-2 ultra-high multiplex PCR technology analysis provided by MGI (https://github.com/MGI-tech-bioinformatics/SARS-CoV-2_Multi-PCR_V1.1). The reads number/ratio of SARS-CoV-2 and the corresponding virus sequence information were received, and the full-length gene sequence of the virus was assembled. Then, other open-source software was used for tracing analysis of the phylogenetic tree.

Results

ATOplex demonstrates ultra-high sensitivity and accurate quantitative performance

Regression analysis was carried out using data from both ddPCR and ATOplex analysis on the positive control dilution series (D1-D12) in GraphPad Prism.

The results showed that the SARS-CoV-2 reads number obtained by the sequencing method was highly positively correlated with viral load in the

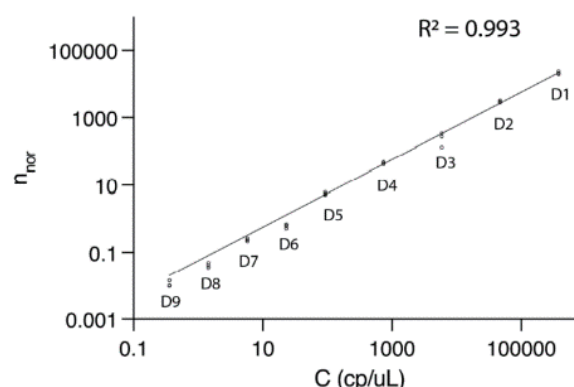


Figure 4. Linear regression analysis of s SARS-CoV-2 reads number (Y-axis) and viral load (X-axis) based on ATOplex

samples (R2=0.993), which proved that the sequencing process based on ATOplex technology can quantify the viral concentrations in the controls (Figure 4).

At the same time, by comparing the results of ATOplex sequencing with RT-QPCR, ATOplex is more sensitive than conventional RT-QPCR. ATOplex could recover 8.4% (at ≥ 30 times sequencing depth) of the viral genome at a concentration that is at least 1 order of magnitude lower than the assay limit of detection (ALOD) of RT-QPCR (D11). Although the genomic coverage with ≥ 30 times the depth in D12 was zero, SARS-CoV-2 reads were obtained. Upon comparison with the absolute viral concentration determined by ddPCR, ATOplex quantification in D12 is no longer

- Precise quantitative analysis found that the solid phase sample of wastewater contains more viruses

Figure 5. Phylogenetic tree analysis

In the past research on wastewater usually excluded the solids of the samples and only analyzed the liquid phase. In this study, the researchers used the high-throughput sequencing method combined with ATOplex technology to analyse the solid phase component as well as the liquid phase component of the wastewater samples (S01- S06).

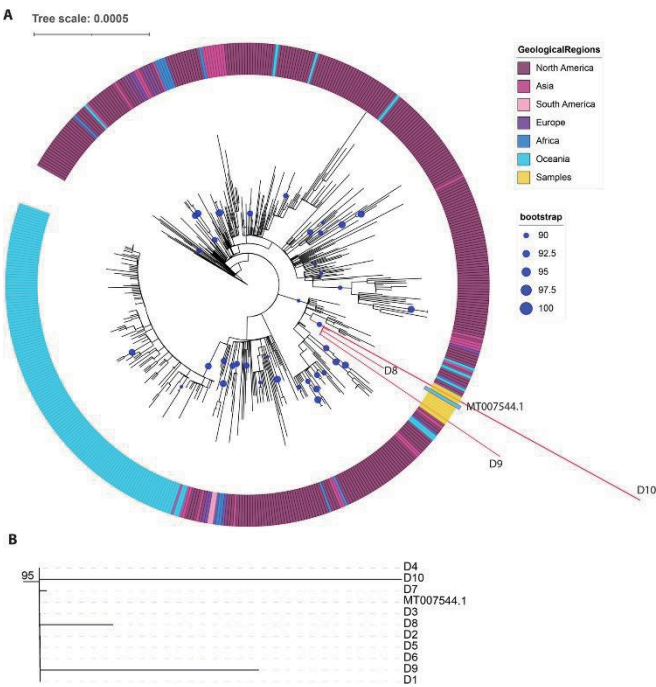
While RT-QPCR failed to detect the presence of SARS-CoV-2 in all the samples, the ATOplex high-throughput sequencing method could detected

Table1 ATOplex feasibility verification

Dilution	Absolute viral concentration (cp/μL)	ATOplex measurement (cp/μL)	Genomic coverage at ≥ 30 times the depth (%)	Mapping rate (%)	RT-QPCR measurement f or N1 (cp/μL)	RT-QPCR measurement f or N2 (cp/μL)
D1	3.8×10^5	3.8×10^5	99.8 ± 0.56	99.66	$3.4 \times 10^5 \pm 2.2 \times 10^4$	$2.8 \times 10^5 \pm 2.0 \times 10^4$
D2	4.7×10^4	5.3×10^4	99.9 ± 0.33	99.68	$4.8 \times 10^4 \pm 5.6 \times 10^3$	$4.4 \times 10^4 \pm 1.6 \times 10^3$
D3	5.9×10^3	4.3×10^3	99.9 ± 0.07	99.7	$(6.9 \pm 1.8) \times 10^3$	$5.6 \times 10^3 \pm 3.6 \times 10^2$
D4	7.4×10^2	7.9×10^2	100.0 ± 0.11	99.68	$8.3 \times 10^2 \pm 3.1 \times 10$	$8.0 \times 10^2 \pm 3.0 \times 10$
D5	9.2×10	9.9×10	99.9 ± 0.51	99.64	$1.1 \times 10^2 \pm 1.7 \times 10$	$1.0 \times 10^2 \pm 1.1 \times 10$
D6	2.3×10	1.0×10	99.0 ± 0.57	99.4	$3.5 \times 10 \pm 1.3$	$3.2 \times 10 \pm 7.1$
D7	5.8	4.2	97.7 ± 1.00	99.42	8.7 ± 2.0	9.9 ± 3.3
D8	1.4	0.7	96.9 ± 4.50	99.37	2.0 ± 0.6	0.6
D9	0.4	0.2	68.4 ± 1.67	99.39	1.3	undetected
D10	9.0×10^{-2}	7.3×10^{-2}	31.0 ± 11.35	99.36	undetected	undetected
D11	2.2×10^{-2}	2.6×10^{-2}	8.4 ± 4.25	99.32	undetected	undetected
D12	6.0×10^{-3}	6.0×10^{-5}	0 ± 0	99.4	undetected	undetected

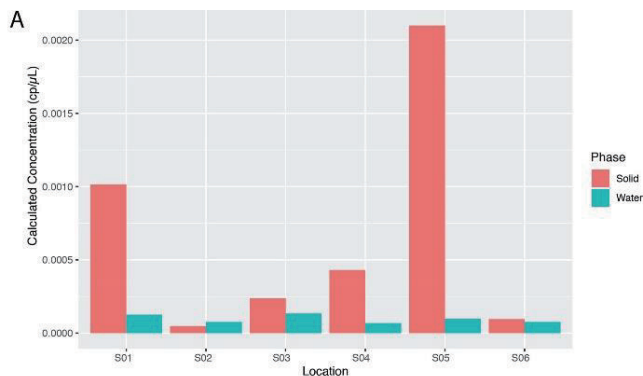
robust, but detection is still possible, and the detect limit is lower than 6 copies/mL. The mapping ratios for all dilution series samples were >99.3%, thus demonstrating the high specificity of the primer pool (Table 1).

The phylogenetic tree of the D1-D12 standard was mapped based on the sequencing results. The results showed that ATOplex sequencing achieved accurate traceability even when the virus concentration is below one quarter of the RT-QPCR detection limit (D10, 90 copies/mL). It is proved that ATOplex sequencing can recover the virus genome information even in wastewater samples to enable virus variant detection, so as to accurately and quickly trace the origin of virus variants (Figure 5).



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SARS-CoV-2 virus in each sample. In addition, the quantitative ATOplex technology showed that the virus signal in the solid phase was significantly higher than in the liquid phase. It is proven that there is a large number of viruses in urban wastewater particles, which is an important source of virus detection and surveillance (Figure 6).



Conclusion

Combining the ATOplex technology and MGI's DNBSEQ™ sequencing platform, the Advanced Water Management Centre at the University of

Queensland in Australia successfully detected the SARS-CoV-2 virus in urban wastewater. The study showed that this method can accurately quantify the virus concentration in wastewater and obtain the full-length gene sequence for subsequent phylogenetic tree analysis, which is conducive for the further application and development of WBE in public health emergencies.

The ATOplex platform developed by MGI is based on ultra-high multiplex PCR enrichment technique, which can detect multiple targets at the same time, and can achieve low-concentration virus enrichment and high-throughput sequencing. It completes virus detection, tracing, and quantification with a simple operation, and has the advantages of low cost, high efficiency, and precise traceability of viruses in the detection of environmental samples.

Reference

1. Ni, G., Lu, J., Maulani, N., Tian, W., Yang, L., Harliwong, I., Wang, Z., Mueller, J., Yang, B., Yuan, Z., Hu, S., & Guo, J. (2021). Novel Multiplexed Amplicon-Based Sequencing to Quantify SARS-CoV-2 RNA from Wastewater. Environmental Science & Technology Letters. <https://doi.org/10.1021/acs.estlett.1c00408>

Ordering information

Product Category	Product Name	Product Number
Instrument	Genetic Sequencer DNBSEQ-G400RS*	900-000170-00
Library preparation	ATOplex RNA Library Prep Set	940-000183-00
Sequencing Reagents	DNBSEQ-G400RS* High-throughput Sequencing Set (FCL PE100)	1000016950
	MGIEasy Circularization Kit	1000020570
	CPAS Barcode Primer 3 Reagent Kit (PE Sequencing)	1000020834

More information

Website: <https://www.mgi-tech.com/products/atoplex/>

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* Unless otherwise informed, all sequencers and sequencing reagents are not available in Germany, USA, Spain, UK, Hong Kong, Sweden and Belgium.

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