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).

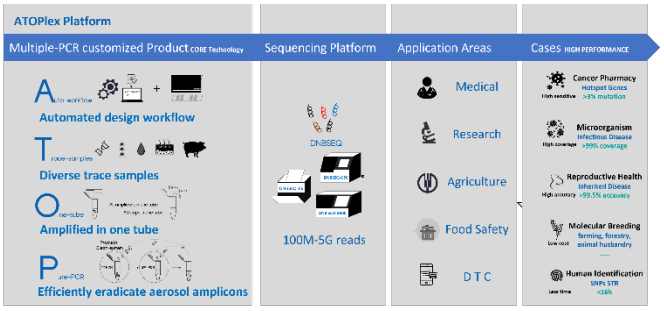
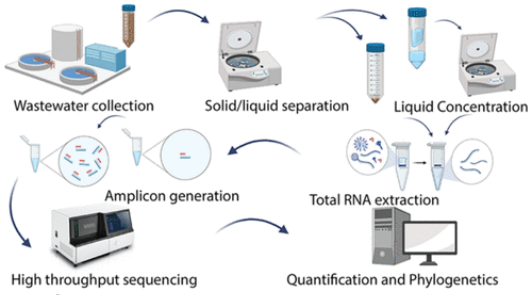


Figure 1. ATOPlex platform

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. 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).



FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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).

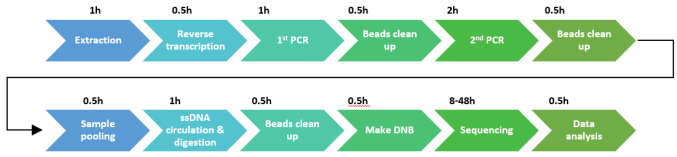


Figure 3. Workflow of virus detection and full-length genome analysis

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

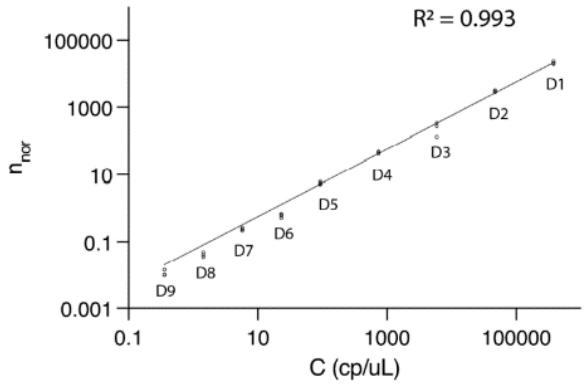


Figure 4. Linear regression analysis of s SARS-CoV-2 reads number (Y-axis) and viral load (X-axis) based on ATOPlex samples (R2=0.993), which proved that the sequencing process based on ATOPlex

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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 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).

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 for N1 (cp/μL)	RT-QPCR measurement for N2 (cp/μL)
D1	3.8 × 105	3.8 × 105	99.8 ± 0.56	99.66	3.4 × 105 ± 2.2 × 104	2.8 × 105 ± 2.0 × 104
D2	4.7 × 104	5.3 × 104	99.9 ± 0.33	99.68	4.8 × 104 ± 5.6 × 103	4.4 × 104 ± 1.6 × 103
D3	5.9 × 103	4.3 × 103	99.9 ± 0.07	99.7	(6.9 ± 1.8) × 103	5.6 × 103 ± 3.6 × 102
D4	7.4 × 102	7.9 × 102	100.0 ± 0.11	99.68	8.3 × 102 ± 3.1 × 10	8.0 × 102 ± 3.0 × 10
D5	9.2 × 10	9.9 × 10	99.9 ± 0.51	99.64	1.1 × 102 ± 1.7 × 10	1.0 × 102 ± 1.1 × 10
D6	2.3 × 10	1.0 × 10	99.0 ± 0.57	99.4	3.5 × 10 ± 1.3	3.2 × 10 ± 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

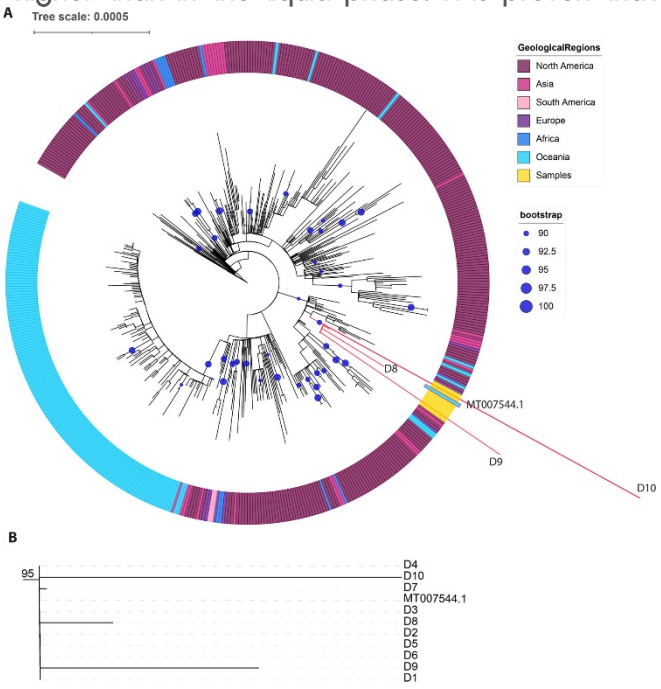
The phylogenetic tree of the D1-D12 standard was mapped based on the sequencing results. The results showed that ATOPlex sequencing achieved accurate traceability even when the virus concentration is below one quarter of the RT-QPCR detection limit (D10, 90 copies/mL). It is proved that ATOPlex sequencing 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).

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

Figure 5. Phylogenetic tree analysis

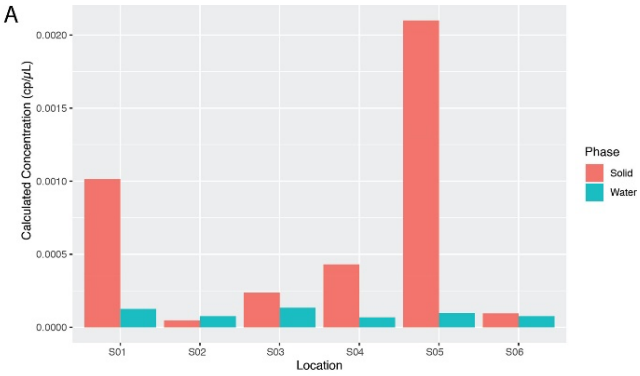
In the past research on wastewater usually excluded the solids of the samples and only analyzed the liquid phase. In this study, the researchers used the high-throughput sequencing method combined with ATOPlex technology to analyse the solid phase component as well as the liquid phase component of the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



FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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
	Circularization Kit	1000020570
	CPAS Barcode Primer 3 Reagent Kit（PE Sequencing）	1000020834

More information

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

Email: mgi_atoplex@mgi-tech.com

MGI Tech Co.,Ltd | Building 11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA, 518083 en.mgi-tech.com | MGI-service@mgi-tech.com

The copyright of this brochure is solely owned by MGI Tech Co. Ltd.. The information included in this brochure or part of, including but not limited to interior design, cover design and icons, is strictly forbidden to be reproduced or transmitted in any form, by any means (e.g. electronic, photocopying, recording, translating or otherwise) without the prior written permission by MGI Tech Co., Ltd.. All the trademarks or icons in the brochure are the intellectual property of MGI Tech Co., Ltd. and their respective producers.
Information in this brochure is updated to 2022/11/30 and only for your reference. In no event shall the brochure be regarded as warranty or commitment made by MGI Tech Co., Ltd. All rights and obligations shall subject to final executed agreement.
*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.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.