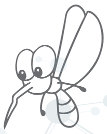


ATOPlex DENV1-4 Targeted Sequencing Package

——Accurate and powerful, providing a powerful tool for dengue virus identification, typing, and mutation detection.

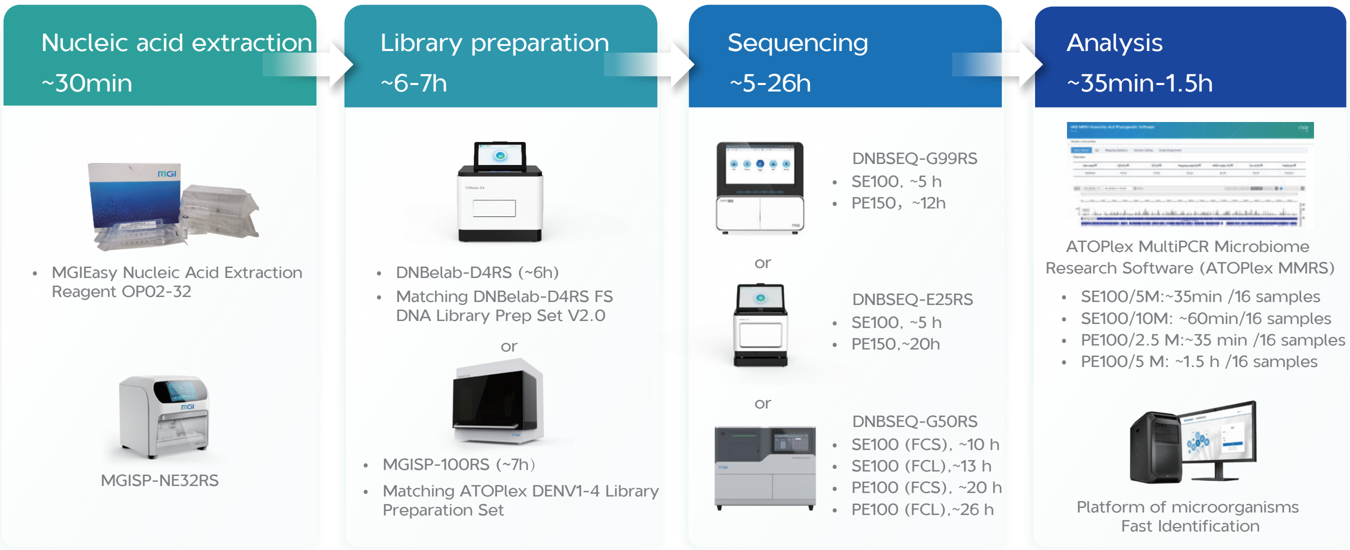
Features

- **Easy to operate**
  Incorporating reverse transcription, multiple amplification, and Fast PCR-FREE technology, library amplification can be completed in one tube with simple operation.
- **Sensitive detection**
  Suitable for detecting low viral loads, it covers DENV1-4, provides rapid software analysis, delivers accurate typing results, and offers high accuracy in mutation detection.
- **Short process**
  This sequencing package covers the entire processfrom nucleic acid extraction to analysis, and the entireprocess can be completed in about 12 h.

Background

Dengue fever is an acute vector-borne infectious disease caused by dengue virus (DENV), which is mainly transmitted by *Aedes aegypti* and *Aedes albopictus*. It occurs mostly in tropical and subtropical regions worldwide, with a higher prevalence in urban and semi-urban areas. The most severe outbreaks are found in Southeast Asia, the Western Pacific, and South America. In China, dengue fever is mainly prevalent in regions such as Guangdong, Yunnan, and Zhejiang.

ATOPlex DENV1-4 Targeted Sequencing Package, a compre hensive solution for the rapid and accurate detection of dengue virus (DENV). This innovative package offers a streamlined workflow encompassing nucleic acid extraction, library preparation, DNBSEQ™ sequencing, and data analysis, delivering results in as little as 12 hours to aid the rapid detection of dengue fever outbreaks



Nucleic acid extraction
~30min

- MGIEasy Nucleic Acid Extraction Reagent OP02-32

MGISP-NE32RS

Library preparation
~6-7h

- DNBelab-D4RS (~6h)
- Matching DNBelab-D4RS FS DNA Library Prep Set V2.0

or

- MGISP-100RS (~7h)
- Matching ATOPlex DENV1-4 Library Preparation Set

Sequencing
~5-26h

DNBSEQ-G99RS
- SE100, ~5 h
- PE150, ~12h

or

DNBSEQ-E25RS
- SE100, ~5 h
- PE150,~20h

or

DNBSEQ-G50RS
- SE100 (FCS), ~10 h
- SE100 (FCL),~13 h
- PE100 (FCS), ~20 h
- PE100 (FCL),~26 h

Analysis
~35min-1.5h

ATOPlex MultiPCR Microbiome Research Software (ATOPlex MMRS)
- SE100/5M:~35min /16 samples
- SE100/10M: ~60min/16 samples
- PE100/2.5 M:~35 min /16 samples
- PE100/5 M: ~1.5 h /16 samples

Platform of microorganisms Fast Identification

Figure 1. MGI ATOPlex DENV1-4 Targeted Sequencing Package

Product Introduction

This package includes the ATOPlex DENV1-4 Library Preparation Set, which leverages MGI's proprietary ATOPlex multiplex PCR technology, enabling easy operation and requiring minimal sample input.

Based on ATOPlex multiplex PCR technology

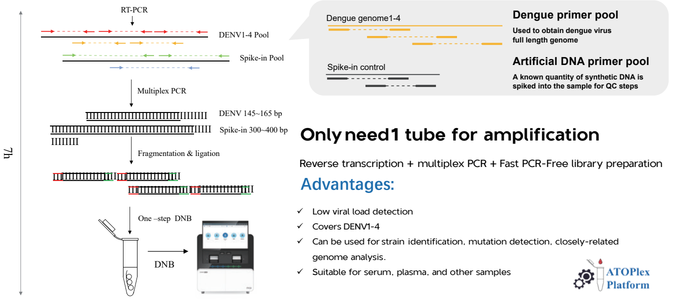


Figure 2. Library preparation principle

Table 1-1. 16 RXN library preparation set and included components

ATOPlex DENV1-4 Library Preparation Set (16 RXN) PN:940-002197-00	940-001776-00	ATOPlex DENV1-4 Panel	16 RXN
	940-000128-00	ATOPlex RNA Multiplex PCR Amplification Module	16 RXN
	940-000950-00	ATOPlex Multiplex PCR Spike-in Control	96 RXN
	940-000886-00	MGIEasy Fast PCR-FREE FS DNA Library Prep Set V2.0	16 RXN

Table 1-2. 96 RXN library preparation set and included components

ATOPlex DENV1-4 Library Preparation Set (96 RXN) PN:940-002196-00	940-001774-00	ATOPlex DENV1-4 Panel	96 RXN
	940-000127-00	ATOPlex RNA Multiplex PCR Amplification Module	96 RXN
	940-000950-00	ATOPlex Multiplex PCR Spike-in Control	96 RXN
	1000005278	MGIEasy DNA Clean Beads	8 mL
	940-000884-00	MGIEasy Fast PCR-FREE FS DNA Library Prep Set V2.0	96 RXN

It also offers flexible sequencing solutions with SE100 for faster turnaround time and PE100 for cost-effectiveness. Depending on different Ct values, different amounts of data are recommended for individual samples, and different sample loading amounts per flow cell are recommended according to different sequencing platforms, catering to diverse user needs (Table 2).

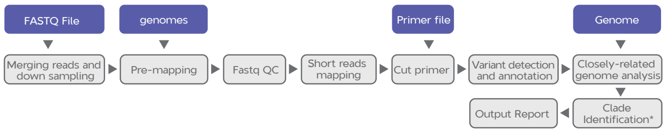
Table 2-1. SE100 solution (faster turnaround time)

CT value	⩽ 30	> 30
Reads (M)	5M	10M
DNBSEQ-E25	4 samples/FCL	2 samples/FCL
DNBSEQ-G99	16 samples/FCL	8 samples/FCL
DNBSEQ-G50	20 samples/FCS	10 samples/FCS
DNBSEQ-G50	96 samples/FCL	48 samples/FCL

Table 2-2. PE100 solution (more cost-effective)

CT value	⩽ 30	> 30
Reads (M)	2.5M	5M
DNBSEQ-E25	8 samples/FCL	4 samples/FCL
DNBSEQ-G99	32 samples/FCL	16 samples/FCL
DNBSEQ-G50	40 samples/FCS	20 samples/FCS
DNBSEQ-G50	192 samples/FCL	96 samples/FCL

Additionally, the ATOPlex MultiPCR Microbiome Research Software (ATOPlex MMRS) included in this package is a software specifically designed for the analysis of ATOPlex Multiplex PCR targeted sequencing data, used for the identification, typing, and mutation detection of target microorganisms. The software is pre-installed with reference genome information and primer sequences for four microorganisms: SARS-CoV-2, Monkeypox virus, Dengue virus, and Respiratory Syncytial Virus. Its functional modules include data quality control, sequence alignment, variant software supports the input of custom reference genome information nddetection, and analysis of closely related genomes. Additionally, the software supports the input of custom reference genome information and primer sequences for other species, enabling the above-mentioned analyses for those species.



*Clade identification: When the input species exists in the Nextclade database (which currently only includes SARS-CoV-2, monkeypox virus, and respiratory syncytial virus), strain identification will be performed on the generated consensus sequences.

Figure 3. ATOPlex MMRS analysis workflow

Table 3. Product parameters

Parameter	Content
Amplification region	Dengue full length genome (NC_001477.1/NC_001474.2/NC_001475.2/NC_002640.1)
Amplicon size	145-165 bp
Amplicon number	436
Number of tubes	1 tube
Sample type	Plasma, serum and other samples
RNA input	10 μL , >100 copies/ rxn
Automated library preparation	MGISP-100, DNBelab-D4
Sequencing	SE100+10+10 (faster turnaround time)
	PE100+10+10 (more cost-effective)
Analysis	SE100: 5 M reads/ sample (qPCR ct value⩽30); 10 M reads/ sample (qPCR ct value >30)
	PE100: 2.5 M reads/ sample (qPCR ct value⩽30); 5 M reads/ sample(qPCR ct value >30)
Coverage (%)	10 x Coverage ⩾ 98%
Detection type	SNV , INDEL
Turnaround time (from sample to report)	12~35 h

Performance

1. High-quality library preparation

Different RNA samples (DF and M) were amplified manually, and the concentration of the amplification products were ⩾4 ng/μL. Subsequently, library preparation was carried out manually or using the MGISP-100, resulting in library concentrations of ⩾0.8 ng/μL. The success rate of library preparation for all samples was 100%. DNBs were prepared with concentrations of ⩾8 ng/μL, which met the requirements for subsequent sequencing (Table 4).

Table 4-1. Amplification and library concentrations of sample DF prepared manually and by MGISP-100

Sample name	Manual/ MGISP-100	Ct value	Serotype	Input volume(μL)	Amplified product concentration (ng/μL)	Library concentration (ng/μL)
DF1	Manual	22	2	1.4	116	1.27
	MGISP-100_1	22	2	1.4	116	1.00
	MGISP-100_2	22	2	1.4	116	1.46
DF7	Manual	15	1	5	136	1.12
	MGISP-100_1	15	1	5	136	1.73
	MGISP-100_2	15	1	5	136	1.98
DF8	Manual	20	1	5	182	1.08
	MGISP-100_1	20	1	5	182	1.96
	MGISP-100_2	20	1	5	182	1.67
DF10	Manual	15	1	5	151.2	0.95
	MGISP-100_1	15	1	5	151.2	1.87
	MGISP-100_2	15	1	5	151.2	1.55

Table 4-2. Amplification and library concentrations of sample M prepared manually and by MGISP-100

Sample name	Manual/MGISP-100	Amplified product concentration(ng/μL)	Library concentration (ng/μL)
M1	Manual	102.8	2.26
	MGISP-100	102.8	4.00
M2	Manual	99.2	1.68
	MGISP-100	99.2	2.14
M3	Manual	103.6	2.26
	MGISP-100	103.6	3.68
M4	Manual	100	1.22
	MGISP-100	100	2.44

Table 4-3. DNB making results

DNB concentration (ng/μL)	Genetic Sequencer
27.4	DNBSEQ-G99ARS
28	
30.5	DNBSEQ-E25RS
37	DNBSEQ-G50RS

2. High sequencing quality

Different DNBs were loaded onto different sequencing platforms: DNBSEQ-G99ARS, DNBSEQ-E25RS, and DNBSEQ-G50RS (SE100+10+10), the Total Reads all met the requirements of more than 80M, 25M, and 100M, respectively, and the Q30 of each flow cell was greater than 95% (Table 5). At the same time, the average values of 1×Coverage(%), 10×Coverage(%), and 100×Coverage(%) of the three sequencing platforms were all greater than 99 % (Figure 4), and the sequencing results of different libraries produced by the MGISP-100 were comparable to those obtained manually. (Figure 4C , D) .

Table 5. QC metrics of different sequencing platforms

Genetic Sequencer	Chip Productivity (%)	Total Reads (M)	Q30 (%)	Split Rate (%)
DNBSEQ-G99ARS	86.26	122.48	95.24	92.3
	88.94	129.2	96.20	94.43
DNBSEQ-E25RS	95.15	29.53	96.37	95.11
DNBSEQ-G50RS	83.79	157.9	95.8	90

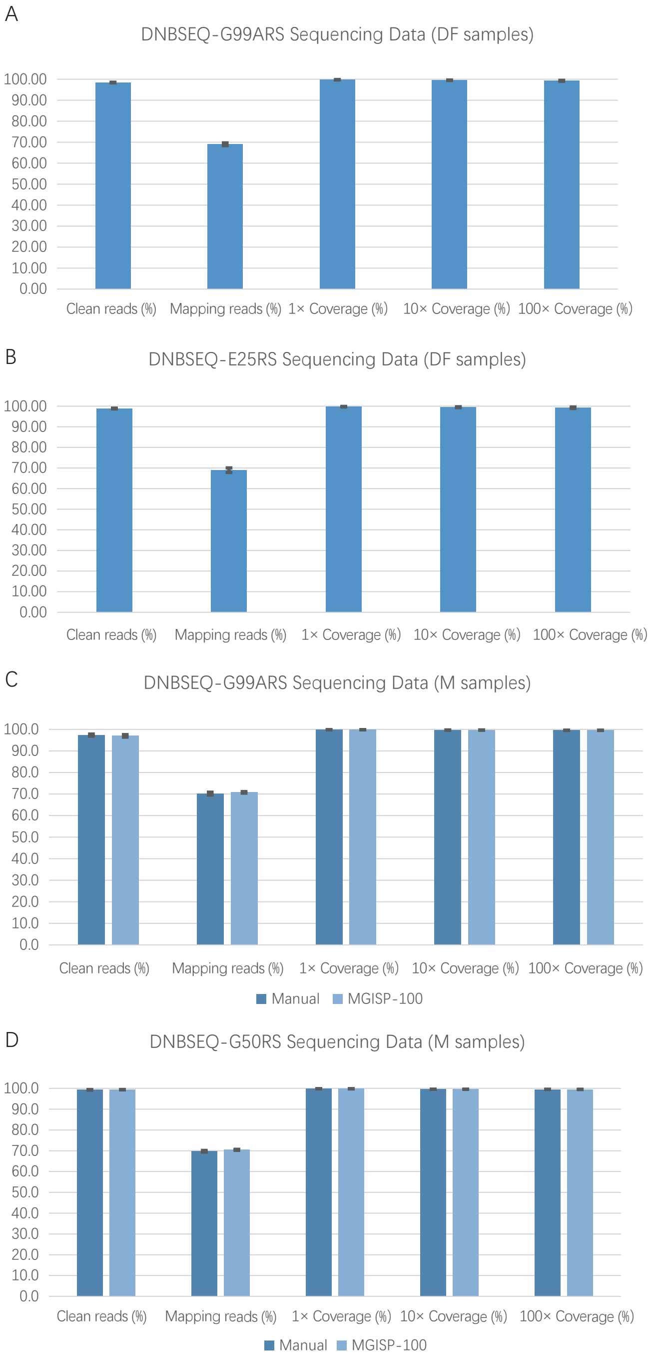


Figure 4. QC metrics of different sequencing platforms.

(A. B) DF samples were prepared by MGISP-100 and the sequencing results were obtained on DNBSEQ-G99ARS/DNBSEQ-E25 ;(C. D) M samples were prepared by manual/MGISP-100, combined with DNBSEQ-G99ARS/NBSEQ-G50RS for sequencing.

3. Accurate typing results

Further clade identification analysis revealed that the typing results of DF samples were consistent with the known results, DF1 was DENV2 , DF7 , DF8 , and DF10 were DENV1. All M samples were detected to be DENV1（Table 6）.

Table 6-1. Typing results of DF samples

Genetic sequencer	Sample	Repeat	10× Coverage (%)	Pathogen (%)	Result	Species	Expected type	Detected type
DNBSEQ-G99ARS	DF1	MGISP-100_1	99.24	90.55	Positive	Dengue	2	2
		MGISP-100_2	99.24	89.66	Positive	Dengue	2	2
	DF7	MGISP-100_1	99.82	94.59	Positive	Dengue	1	1
		MGISP-100_2	99.82	95.6	Positive	Dengue	1	1
	DF8	MGISP-100_1	99.08	94.64	Positive	Dengue	1	1
		MGISP-100_2	99.25	94.8	Positive	Dengue	1	1
	DF10	MGISP-100_1	99.82	96.91	Positive	Dengue	1	1
		MGISP-100_2	99.82	96.79	Positive	Dengue	1	1
DNBSEQ-E25RS	DF1	MGISP-100	99.24	90.18	Positive	Dengue	2	2
	DF7	MGISP-100	99.82	95.13	Positive	Dengue	1	1
	DF8	MGISP-100	99.11	94.99	Positive	Dengue	1	1
	DF10	MGISP-100	99.82	96.99	Positive	Dengue	1	1

Table 6-2. Typing results of M samples

Genetic sequencer	Sample	Repeat	10× Coverage (%)	Pathogen (%)	Result	Species	Type
DNBSEQ-G99ARS	M1	Manual	99.78	98.14	Positive	Dengue	1
		MGISP-100	99.78	98.08	Positive	Dengue	1
	M2	Manual	99.6	97.02	Positive	Dengue	1
		MGISP-100	99.6	96.49	Positive	Dengue	1
	M3	Manual	99.78	98.46	Positive	Dengue	1
		MGISP-100	99.78	98.46	Positive	Dengue	1
	M4	Manual	99.6	96.33	Positive	Dengue	1
		MGISP-100	99.6	96.22	Positive	Dengue	1
DNBSEQ-G50RS	M1	Manual	99.6	98.61	Positive	Dengue	1
		MGISP-100	99.6	98.58	Positive	Dengue	1
	M2	Manual	99.6	97.71	Positive	Dengue	1
		MGISP-100	99.6	97.21	Positive	Dengue	1
	M3	Manual	99.6	98.84	Positive	Dengue	1
		MGISP-100	99.6	98.86	Positive	Dengue	1
	M4	Manual	99.5	97.11	Positive	Dengue	1
		MGISP-100	99.5	97.12	Positive	Dengue	1

4. DNBelab-D4RS combined with different sequencing platforms can also achieve high-quality results

Utilizing DNBelab-D4RS for library preparation, DNB concentrations all exceeded 8 ng/μL. When combined with DNBSEQ-E25 and DNBSEQ-G99 sequencing platforms, the total reads surpassed the standards of 25 M and 80 M, respectively. The Q30 exceeded 85%, and the average values of 1×Coverage(%), 10×Coverage(%), and 100×Coverage(%) all surpassed 99%, reflecting the high quality of sequencing and the precision of the typing outcomes (Table 7).

Table 7. Sequencing data obtained by DNBelab-D4RS with different sequencing platforms

Sample name	Test scheme	DNB concentration (ng/μL)	Total reads (M)	Q30(%)	1× Coverage (%)	10× Coverage (%)	100× Coverage (%)	Pathogen (%)	Result	Number of SNPs	Type
A	D4+E25	26.4	27.26	87.32	99.24	99.22	99.1	96.47	Positive	775	DENV1
B	D4+G99	25.3	113.63	92.78	99.49	99.38	99.24	94.1	Positive	783	DENV2

Summary

MGI's ATOPlex DENV1-4 Targeted Sequencing Package, based on the DNBSEQ-E25/DNBSEQ-G99/MGISEQ-200 sequencing platforms, offers excellent data quality and high automation, providing robust support for the detection of dengue virus and epidemiological research.

Recommended ordering information

Category	Product Name	Product number
Genetic Sequencers	DNBSEQ-E25RS Genetic Sequencer	900-000490-00
	DNBSEQ-G99RS Genetic Sequencer	900-000561-00
	DNBSEQ-G99ARS Genetic Sequencer	900-000560-00
	DNBSEQ-G50RS Genetic Sequencer	900-000350-00
Automation Instruments	DNA Sequencing Library Preparation System MGISP-100RS	900-000070-00
	DNBelab-D4RS Digital Sample Preparation System	900-000625-00
	Automated Nucleic Acid Extractor MGISP-NE32RS	950-000019-00
Nucleic acid extraction reagents	MGIEasy Nucleic Acid Extraction Kit OP02-32 (32 RXN)	1000023774
Library preparation reagents	ATOPlex DENV1-4 Library Preparation Set (16 RXN)	940-002197-00
	ATOPlex DENV1-4 Library Preparation Set (96 RXN)	940-002196-00
	DNBelab-D4RS FS DNA Library Prep Set V2.0 DNBelab-D4RS 16 RXN/4 Cartridges	940-001198-00
	ATOPlex DENV1-4 Amplification Set (16 RXN)	940-002702-00
Sequencing reagents	DNBSEQ One-Step DNB Preparation Kit (OS-DB) (4RXN)	1000026466
	DNBSEQ-G99RS High-throughput Sequencing Kit (G99 FCL SE100/PE50)	940-001268-00
	DNBSEQ-E25RS High-throughput Sequencing Reagent Set (FCL SE100)	940-000573-00
	DNBSEQ-G50RS High-throughput Rapid Sequencing Kit (FCS SE100)	940-001626-00
	DNBSEQ-G50RS High-throughput Sequencing Kit (FCL SE100)	940-001640-00
	DNBSEQ-G99RS High-throughput Sequencing Set (G99 FCL PE150)	940-001269-00
	DNBSEQ-E25RS High-throughput Sequencing Set (FCL PE150)	940-000567-00
	DNBSEQ-G50RS High-throughput Rapid Sequencing Set(FCS PE100)	940-001618-00
	DNBSEQ-G50RS High-throughput Sequencing Set(FCL PE100)	940-001623-00
Data analysis	ATOPlex MultiPCR Microbiome Research Software Analysis Package(16 reports) ATOPlex_MMRS	970-000528-00
	ATOPlex MultiPCR Microbiome Research Software ATOPlex_MMRS	970-000518-00
	Platform of microorganisms Fast Identification(PFI)	900-000392-00

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