MGIEasy Fast PCR-FREE FS Library Prep Set V2.0

■ Introduction

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 is a specifically designed fast and flexible library prep product with high conversion efficiency to convert 25 ng - 900 ng of gDNA into a DNA library for DNBSEQ™ sequencers. The library prep set combines the DNA fragmentation, end-repair and A-tailing into one step and uses a fast purification method, to simplify the experimental process and significantly reduce library construction time. The library prep set has excellent performance in genome coverage uniformity, variation detection precision and sensitivity. It is compatible with various sample types, including blood, saliva, oral swabs, Meta DNA, virus long amplicons, and suitable for applications such as WGS, Low-pass WGS, Microbiome sequencing, Metagenomics sequencing, and virus long amplicons sequencing.

This library prep set provides up to 384 selected barcodes that enable flexible throughput options for 8 - 384 samples multiplexing. The library prep set supports equal volume pooling of libraries from the same sample type and input amount in Low-pass WGS application, eliminating the need for time-consuming library quantitation and normalization steps before pooling.

■ Highlights

● Fast library prep workflow

Combine the DNA fragmentation, end-repair and A-tailing into one step to simplify the experimental process and minimize manual operations, significantly reducing library prep time to about 1.3 h.

● Stable and highly compatible fragmented performance

High-quality fast frag enzymes and efficient adapter ligation reaction conditions, compatible with various sample types and common DNA dissolution buffers with highly uniform and consistent insert size under the same fragmentation conditions.

● Excellent performance

The PCR-free method and optimized library prep reagent and reaction conditions enhance the performance in genome coverage uniformity, variation detection precision and sensitivity.

● Broad DNA input range and various sample types

High conversion efficiency and flexible library prep workflow supports a broad DNA input range (25 ng - 900 ng) and various sample types, such as blood, saliva, oral swabs, Meta DNA, and virus long amplicons.

● Wide range of applications

Suitable for applications such as WGS, Low-pass WGS, Microbiome sequencing, Metagenomics sequencing, and virus long amplicons sequencing.

● Flexible throughput options

Up to 384 Unique Dual Barcodes enables flexible throughput for 8 - 384 samples multiplexing.

● Efficient automatable workflow

Minimal operation steps, easy to use and suitable for automated platforms MGISP-100, MGISP-960, and MGISP-Smart 8, providing flexible, efficient, and automated solutions.
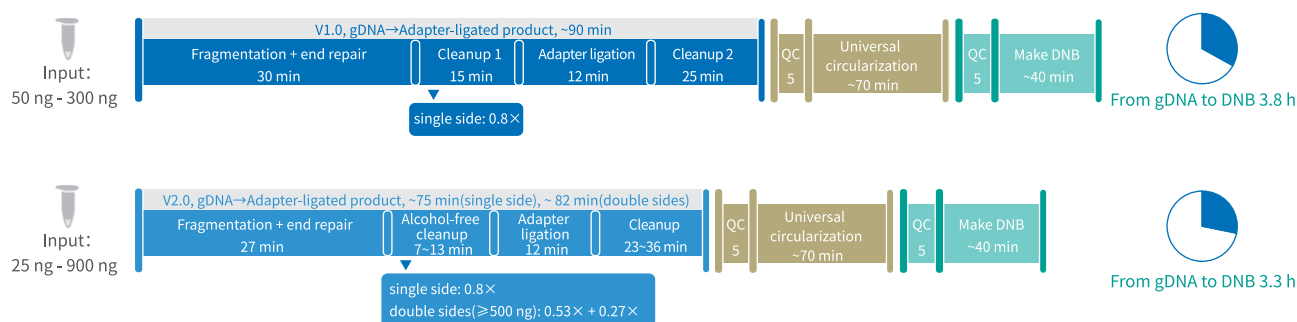
Specification

Assay Time	~ 1.3 h - 1.4 h (from gDNA to ligation library)
Hands-On Time	~ 20 min
Input Quantity	25 ng - 900 ng gDNA
Barcode	~ 384 barcodes
Sample Types	gDNA from blood, saliva, oral swabs, tissue, Microbial gDNA, Meta DNA, Virus long amplicons
Compatible Species	Human, Animal, Plant, Microbe
Applications	Human WGS, Low-pass WGS, Plants & Animals WGS, Microbial WGS, Metagenomics sequencing, Virus long amplicons sequencing
Sequencers	DNBSEQ-G400, DNBSEQ-G50, DNBSEQ-G99, DNBSEQ-E25, DNBSEQ-T7, DNBSEQ-T10×4, DNBSEQ-T20×2
Sequencing Types	SE100 / PE100 / PE150
Automation Platform	MGISP-100 (16RXN), MGISP-960 (96RXN, 192RXN), MGISP-Smart 8

■ Product Performance

Fast and flexible library perp workflow

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 is the fastest, easiest-to-use product among all MGI DNA library prep products. It combines the DNA fragmentation, end-repair and A-tailing into one step and uses a fast purification method to simplify the experimental process and minimize manual operations, significantly reduce library prep time to only 1.3 h (from gDNA to ligation library). It adapts to two circularization methods, including MGI universal circularization or MGI OneStep DNB making. Fast PCR-FREE V2.0 combined with the MGI one-step DNB kit can complete the process from gDNA to DNB in about 2 h (Fig.1).

Comparison before and after the upgrade of the universal circularization method:

Input:
50 ng - 300 ng

V1.0, gDNA→Adapter-ligated product, ~90 min

| Fragmentation + end repair 30 min | Cleanup 1 15 min | Adapter ligation 12 min | Cleanup 2 25 min | QC 5 | Universal circularization ~70 min | QC 5 | Make DNB ~40 min |

single side: 0.8×

From gDNA to DNB 3.8 h

Input:
25 ng - 900 ng

V2.0, gDNA→Adapter-ligated product, ~75 min(single side), ~ 82 min(double sides)

| Fragmentation + end repair 27 min | Alcohol-free cleanup 7~13 min | Adapter ligation 12 min | Cleanup 23~36 min | QC 5 | Universal circularization ~70 min | QC 5 | Make DNB ~40 min |

single side: 0.8×
double sides(≥500 ng): 0.53× + 0.27×

From gDNA to DNB 3.3 h

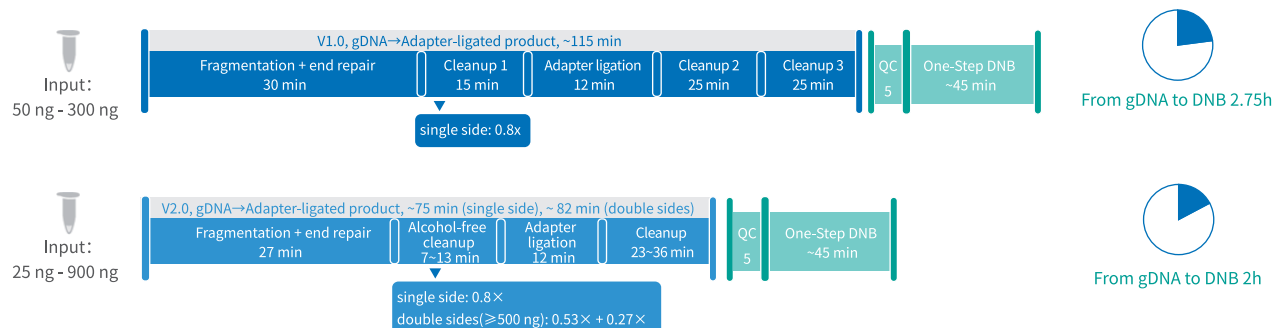## Comparison before and after the upgrade of the One-Step DNB method:

Fig. 1 The whole process from gDNA to DNB under different library preparation methods and circularization methods

Calculate the process time by manually preparing one library once. The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 is compatible with two circularization methods: 1) MGI universal circularization method. The prepared ligation library is circularized using the MGI circularization kit (MGI Cat. No. 1000020570) to obtain single-stranded circular DNA (ssCir DNA), followed by conventional DNB making by using the DNB making reagent in the MGI sequencing reagent set. The process from gDNA to DNB takes about 3.3 h. 2) MGI OneStep DNB method. Use the MGI OneStep DNB kit (MGI Cat. No. 940-000036-00*) to complete the library circularization and DNB making in one step, directly obtaining DNB. This simplifies the process from the ligation library to DNB and reduces the DNB preparation time. The process from gDNA to DNB takes about 2 h.

Excellent performance in genome coverage uniformity and variation detection

Using NA12878 standard as the test sample, PCR-free libraries were constructed using different library prep kits and PE150 sequencing was performed on MGI sequencers and another sequencing platforms. The results showed that the MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 has better genome coverage uniformity, and excellent variation detection precision and sensitivity (Fig. 2).

Fig. 2a Genome coverage under different library prep kits

Fig. 2b Variation detection under different library prep kits

Fig. 2 Sequencing data of different PCR-free library prep kits and platforms

PCR-free libraries were constructed using MGIEasy PCR-Free DNA Library Prep Set (as PCR-free), MGIEasy FS PCR-Free DNA Library Prep Set (as FS PCR-free), MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 combined with MGI OneStep DNB kit (as Fast PCR-FREE V2 with OS V2), MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 combined with MGI circularization kit (as Fast PCR-FREE V2), and competitive kit (as ILM PCR-Free). MGI PCR-free libraries were sequenced on DNBSEQ-G400 or DNBSEQ-T7. ILM PCR-Free library was sequenced on another sequencing platform.

Compatible various DNA dissolution buffers

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 uses high-quality fast frag enzymes and efficient adapter ligation reaction conditions to provide stable library construction performance. It is compatible with various sample types and common DNA dissolution buffers, and it produces highly uniform and consistent insert sizes under the same fragmentation conditions (Fig. 3a). Using the 200 ng NA12878 that dissolved in different DNA dissolution buffers as the test samples, a comparison was performed between different DNA dissolution buffers by preparing the libraries using the MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 combined with the MGI OneStep DNB kit (MGI Cat. No. 940-000036-00) and sequencing on DNBSEQ-T7 with PE150. The results showed that under the same fragmentation condition and library prep workflow, the libraries prepared from gDNA dissolved in different DNA dissolution buffers had uniform and consistent insert sizes, performed excellently and were comparable in genome coverage and variation detection (Fig. 3a, 3b, 3c).
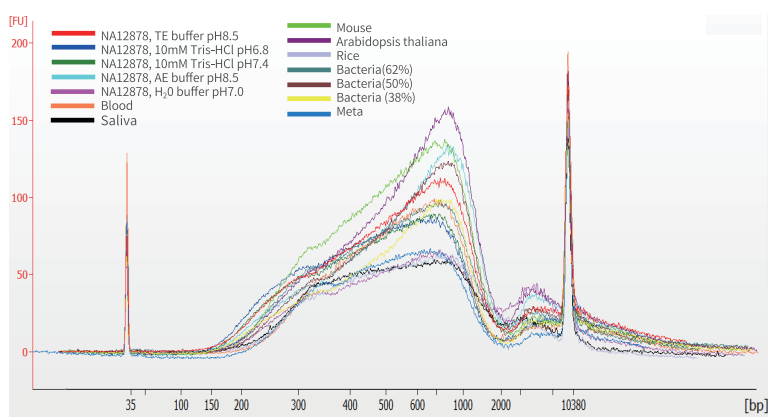

Fig. 3a Consistent insert sizes across different sample types and DNA dissolution buffers
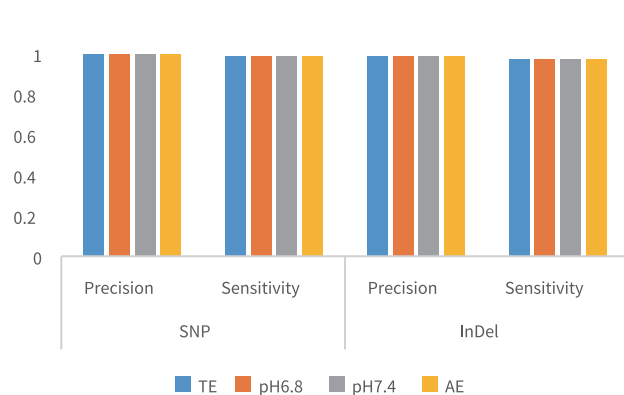

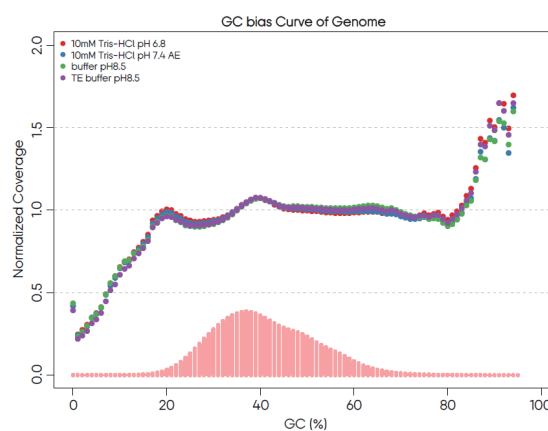Fig. 3b Variation detection under different DNA dissolution buffers

Fig. 3c Normalized coverage under different DNA dissolution buffers

Fig. 3 Performances under different DNA dissolution buffers

Easy to use, support equal volume pooling of libraries from the same sample type and input amount in low-pass WGS

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 delivers consistent library yields under the same DNA input amount, which supports equal-volume pooling of libraries from the same sample type and input amount in low-pass WGS application, eliminating the need for time-consuming library quantitation and normalization steps before pooling. The 384 selected barcodes lead to the uniformity of barcode splitting ratio to enable equal volume pooling of libraries from the same sample type and input amount.

Libraries were produced with saliva gDNA and NA12878 of the same starting amount using the MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 and MGI OneStep DNB kit (MGI Cat. No. 940-000036-00) on MGISP-960 and pooled with equal volume for making DNB and sequencing on DNBSEQ-T7 at PE100. The results showed good uniformity in the reads percentage of these libraries. The barcode split rate reached 98%.

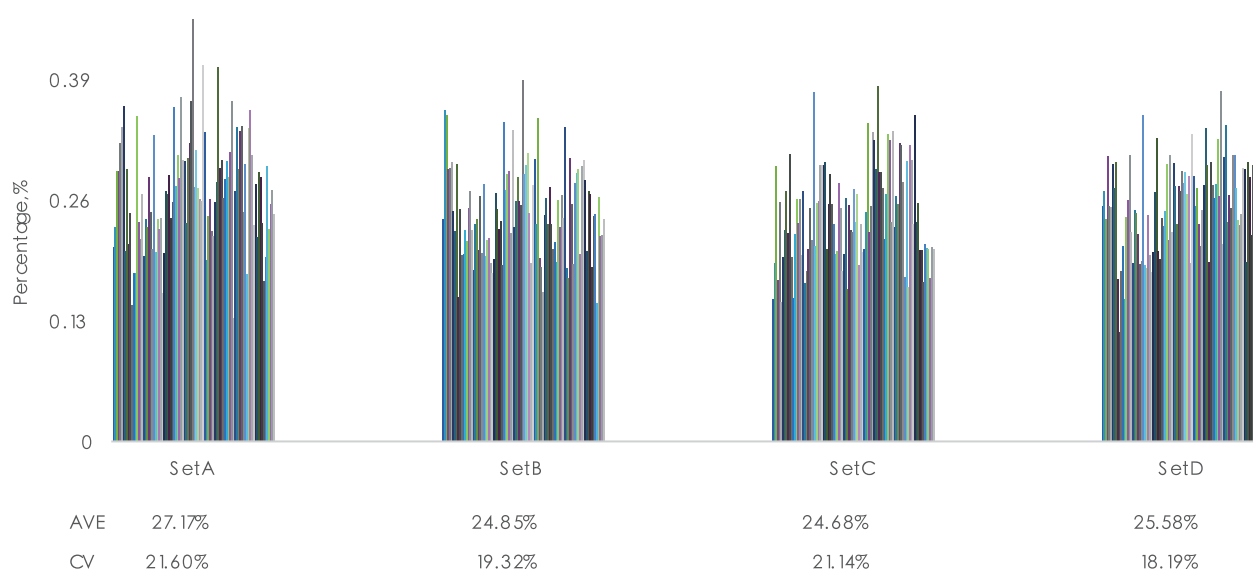|	SetA	SetB	SetC	SetD
AVE	27.17%	24.85%	24.68%	25.58%
CV	21.60%	19.32%	21.14%	18.19%

Fig. 4 The reads percentage of the different libraries which pooled with equal volume for sequencing

High quality library with high quality sequencing data in amplicon sequencing

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 is suitable for virus long amplicon sequencing. The standard samples of influenza A and B virus were used to prepare virus long amplicons with MGIEasy Respiratory Microorganisms Genome Amplification Kit (MGI Cat. No. 940-000059-00). Libraries were prepared from these virus long amplicons using the MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 and MGI OneStep DNB kit (MGI Cat. No. 1000026466) on MGISP-100 and sequenced on DNBSEQ-G99 at PE100. The sequencing data were analyzed using MGI FLuTrack software. The results showed that these libraries produced high quality sequencing data with a high Q30 rate and split rate. The coverage of 8 genomic fragments of influenza virus performed well, which is helpful for virus traceability and typing analysis.

Table 1 DNBSEQ-G99 Sequencing data

	Reads (M)	Q30 (%)	Split rate (%)
Sequencing data	97.4	96.46	96.78

Table 2 The coverage of 8 genomic fragments of influenza virus

Smaple	A-seg1	A-seg2	A-seg3	A-seg4_H1	A-seg5	A-seg6_N1	A-seg7	A-seg8
Amix-c500	99.19%	99.44%	99.82%	98.31%	98.98%	99.73%	98.34%	99.78%
A3-c1000	99.83%	99.83%	99.28%	99.61%	98.72%	98.43%	98.34%	96.97%
A3-c10000	99.83%	97.95%	99.42%	98.99%	99.30%	99.79%	98.25%	99.21%
A4-c1000	99.83%	99.15%	98.70%	98.31%	99.74%	99.11%	97.27%	97.87%
A4-c10000	99.19%	99.44%	100.00%	99.04%	99.30%	99.11%	99.61%	98.76%
A5-c500	99.83%	99.83%	99.42%	99.55%	98.72%	99.73%	99.61%	97.98%
A6-c500	99.83%	99.74%	99.28%	99.04%	99.30%	99.11%	98.25%	98.54%
NTC-1	/	/	/	/	/	/	/	/
B1-c500	100.00%	98.86%	99.87%	99.36%	100.00%	100.00%	100.00%	96.35%
B1-c1000	100.00%	98.99%	99.78%	96.49%	99.78%	96.60%	99.50%	96.35%
B1-c10000	99.46%	98.86%	99.44%	100.00%	100.00%	99.68%	99.58%	99.91%
B2-c500	100.00%	98.86%	100.00%	94.79%	100.00%	99.94%	100.00%	96.35%
B2-c1000	98.83%	98.90%	99.61%	96.17%	100.00%	100.00%	99.58%	96.35%
B2-c10000	99.83%	98.86%	99.48%	99.26%	99.51%	99.29%	100.00%	96.35%
B3-c500	99.46%	98.82%	99.26%	95.32%	100.00%	100.00%	100.00%	94.99%
NTC-2	/	/	/	/	/	/	/	/
Average *	99.65%	99.11%	99.53%	98.16%	99.53%	99.32%	99.17%	97.55%

These A samples are the standard samples of influenza A virus and these B samples are the standard samples of influenza B virus. The c500, c1000, c10000 mean 500 copies, 1000 copies, 10000 copies of the virus samples. NTC is a negative control and is not used for calculating the average.

■ Summary

The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 provides a fast, flexible, and automatable library preparation workflow and can achieve the process from gDNA to DNB in only 2 h by using MGI OneStep DNB kits. This library prep set has high conversion efficiency and flexible library prep workflow to support a broad DNA input range (25 ng - 900 ng) and various sample types, and has excellent performances in genome coverage uniformity, variation detection precision and sensitivity. This product is suitable for WGS, low-pass WGS, Microbiome sequencing, Metagenomics sequencing, and virus long amplicons sequencing. The MGIEasy Fast PCR-FREE FS Library Prep Set V2.0 can help you achieve your research goals faster and more easily.

Ordering Information

Product	Configuration	Cat No.
MGIEasy Fast PCR-FREE FS Library Prep Set V2.0	16 RXN	940-000886-00
	96 RXN	940-000884-00
	384 RXN	940-000882-00
MGIEasy Dual Barcode Circularization Kit	16 RXN	1000020570
DNBSEQ OneStep DNB Make Reagent Kit V2.0 (OS-DB)	4 RXN	940-000036-00
DNBSEQ OneStep DNB Make Reagent Kit (OS-DB)*	4 RXN	1000026466

*This kit is recommended for DNBSEQ-G99RS (PE100/SE100) and DNBSEQ-G50RS (PE100/SE100) .

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