·MGIEasy FS PCR-Free DNA Library Prep Set

Highlights

- **Good compatibility for different species fragmentation**

  Use a high-quality，low bias fragmentase for different species and input amounts shearing with the same incubation condition can get the consistent and concentrated fragment range.

- **Stable library yield**

  The optimized chemistry increases the library quality and results in a stable library yield.

- **Simple and automated workflow**

  Library construction can be completed within 3.9 hours. Compatible with automatic sample preparation systems to provide an automated workflow.

- **No amplification error accumulation**

  WGS PCR-free library preparation in combination with sequencing by the MGI DNBSEQ^{TM} platform has no amplification error accumulation, resulting in better genome fidelity.

- **Good coverage uniformity**

  The genome coverage of FS PCR-free achieved comparable performance to Covaris PCR-free. The FS PCR-free technique covers the GC-rich, promoter and repetitive regions. Significantly better than traditional WGS methods.

- **Excellent variant performance**

  The variant detection performance of FS PCR-free is similar to Covaris PCR-free, and better than traditional PCR, especially InDels.

Overview

MGIEasy FS PCR-Free DNA Library Prep Set is specifically designed for making WGS libraries without PCR amplification for the MGI DNBSEQ^{TM} sequencer. For the high-quality and low-bias fragmentation enzyme, the set could fragment 50-1000 ng gDNA. The distribution of fragmented DNA is similar to that fragmented by mechanical techniques. The set, combined with the automatic sample preparation system MGISP, could provide an automatic library preparation workflow. FS PCR-free combined with MGI DNBSEQ^{TM} sequencer, is truly PCR-free sequencing.

Product Parameters

Total Time	~3.9 hours
Hands-on-time	~30 minutes
Sample Amount	50-1000 ng gDNA
Insert Size	PE100, recommend 350 bp-475 bp； PE150, recommend 400 bp-660 bp
Sample Type	gDNA
Species Compatibility	Human, animals, plants, fungi, bacteria, metagenomics, etc.
Application	Whole genome sequencing
Platform Compatibility*	MGISEQ-2000*, MGISEQ-200*, DNBSEQ-G400*, DNBSEQ-G50*, DNBSEQ-T7*
Sequencing Strategy*	SE100, PE100, PE150, PE200

*Other sequencers and sequencing strategies are under testing, will update on MGI website sooner.

Performance

- **Good Compatibility for Different Species Fragmentation**

gDNA from five species using different input amounts, including mouse, rice, bacteria and metagenomics, each with 1000 ng gDNA and human NA12878 with 200 ng, 500 ng and 1000 ng, were fragmented by the fragmentation enzyme of MGIEasy FS PCR-Free Library Prep Set. For the different species and input amounts with the same conditions of enzyme fragmentation, the main peaks of fragmented DNA are consistent and concentrated.

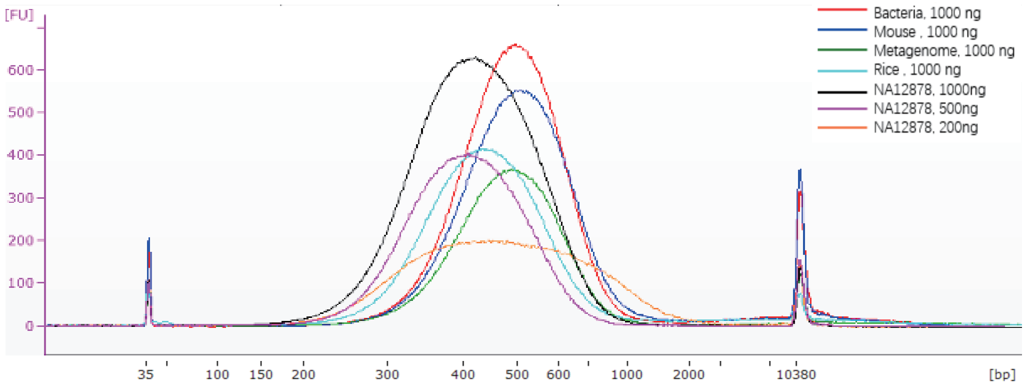

Figure 1 The library fragment size distributions are obtained from different species and a variety of DNA input.

- **Simple and Automated Workflow**

The operational steps are simple, and suitable for manual operation as well as an automated sample preparation system that could save time and improve the throughput of samples.

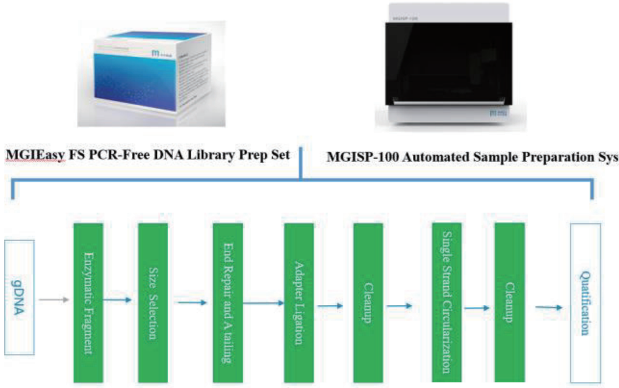

Figure 3 Automated MGI FS PCR-free workflow

- **No Amplification Error Accumulation**

Since the PCR-free kit removes PCR amplification during library preparation, the library has no PCR error or PCR bias. The MGI DNBSEQ^{TM} technology functions such that it produces every replicated copy from the original genomic fragment in a rolling circle replication process, unlike PCR which uses generated DNA copies to make more copies. MGI PCR-free, combined with DNBSEQ^{TM} technology, is the first and only PCR-free NGS workflow, resulting in better genome fidelity.

- **Good Coverage Uniformity**

FS PCR-free without PCR amplification, avoids amplification bias and results in good coverage uniformity across the genome. The coverage plots of high GC bacteria and low GC bacteria are similar to middle GC bacteria, and close to the expected normalized coverage of 1.0. This indicates the FS PCR-free has uniform GC coverage over a broad range of GC content (see Figure 4).

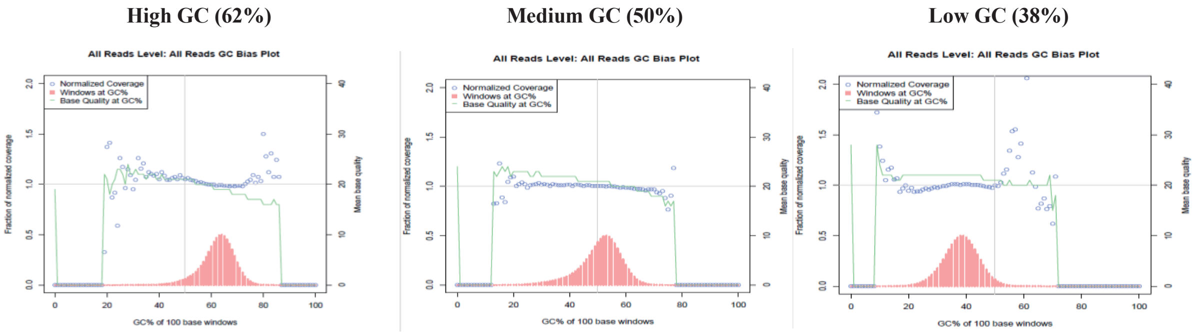

Figure 4 GC bias plot of bacteria with different genome GC content (Olsenella, 62% GC; E.Coli. , 50% GC and Bacillus megaterium, 38% GC). Libraries were prepared with MGIEasy FS PCR-Free DNA Library Prep Set from 1 μg gDNA, and sequenced on MGISEQ-2000. The plots were assessed by calculating the GC content of the reference in 100 bp bins. The expected normalized coverage of 1.0 is indicated by the horizontal grey line, and the blue dotted lines represent the normalized coverage for each library. The closer the blue dot lines near to 1.0, the better the coverage uniformity.

The sequencing depth frequency distribution of FS PCR-free is closer to covaris PCR-free, higher than FS PCR, indicating FS PCR-free provides good coverage uniformity across the genome (see figure 5).

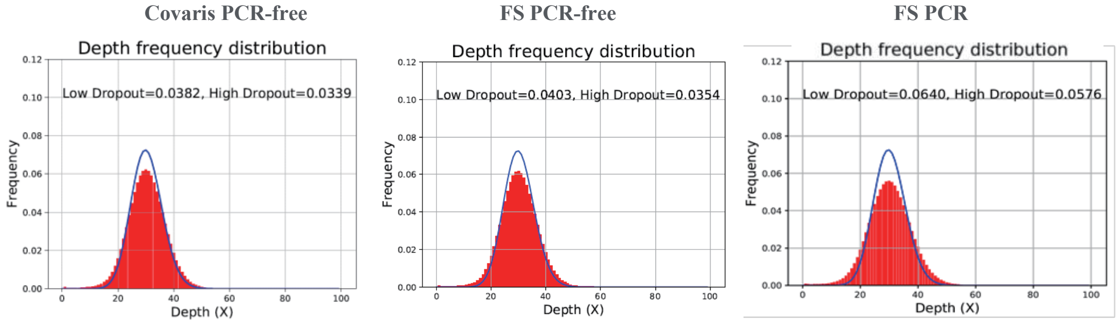

Figure 5 The plot of sequencing depth frequency distribution. Libraries were prepared using 1 μg NA12878, prepared with MGIEasy PCR-Free DNA Library Prep Set (Covaris PCR-free), MGIEasy FS PCR-Free DNA Library Prep Set (FS PCR-free), and traditional WGS library prep set (FS PCR) respectively, sequenced on MGISEQ-2000 (PE150), and analyzed at 30X data. X-axis, sequencing depth; Y-axis, sequencing depth distribution frequency that represents the percentage of a depth on the full depth. The red histogram is the actual distribution. The blue line graph is the expected distribution.

- **Excellent Variant Performance**

  The variant detection performance of FS PCR-free is similar to Covaris PCR-free, and better than traditional FS PCR, especially InDels. As shown in Figure 6 the precision and sensitivity of FS PCR-free are 99.09% and 98.75% respectively. This indicates the capability of variant detection of FS PCR-free is higher.

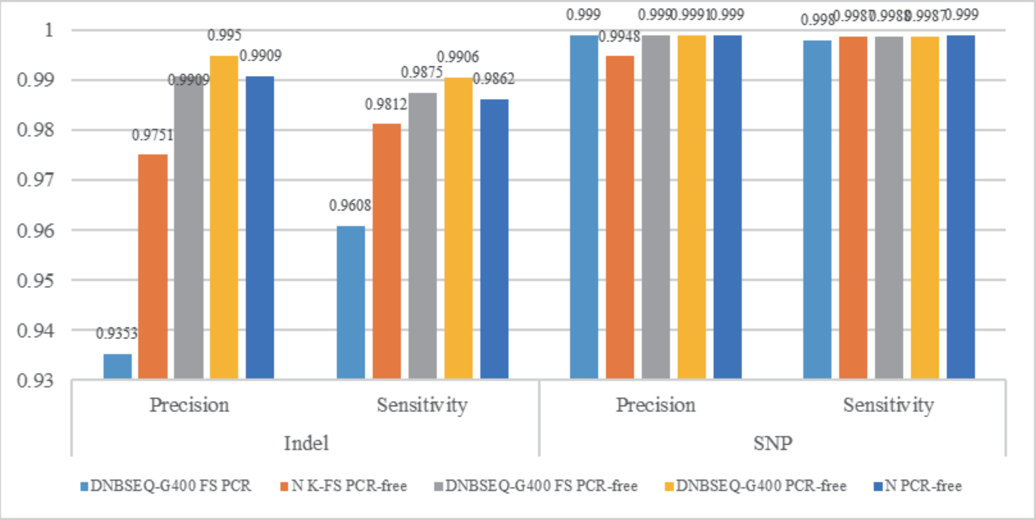

Figure 6 Comparison of the variant detection performance of FS PCR-free with different library prep kits and sequencing platform. The samples are NA12878 gDNA. Libraries were prepared with MGIEasy FS DNA Library Prep Set (DNBSEQ-G400 FS PCR)， vendor K FS Library Prep Set (with PCR-Free) （N K-FS PCR-free）， MGIEasy FS PCR-Free Library Prep Set (DNBSEQ-G400 FS PCR-free), MGIEasy PCR-Free Library Prep Set (DNBSEQ-G400 PCR-free) and vendor i PCR-Free Library Prep Kit (N PCR-free); And those libraries were sequenced on different sequencing platform, DNBSEQ-G400 (PE150) and N sequencing platform of vendor i （PE150）. The sequencing data were analyzed for variant detection performance with 30X average depth.

Summary

The MGIEasy FS PCR-Free DNA Library Prep Set is used for broad application of whole genome sequencing without PCR amplification. The set including the fragmentation enzyme is suitable to use with an automatic sample preparation system to construct libraries. By using the high-quality and low-bias fragmentation enzyme, the fragmented DNA distribution is similar to a mechanical fragmentation method. By removing PCR amplification, MGI FS PCR-free WGS has been shown to produce high-quality sequencing data with minimal error accumulation, good coverage uniformity and excellent variant detection performance, which supports whole genome research comprehensively.

Ordering Information

Product	Specification	Item Number
MGIEasy FS PCR-Free DNA Library Prep Set	16 RXN	1000013454
	96 RXN	1000013455

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

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