

## MGIEasy PCR-Free DNA Library Prep Set

### Product Highlights

- Compatible with Multiple Species Types

Compatible with human, animals, plants, bacteria, fungi etc., e.g. human (blood, saliva, fresh tissue), mice, rice, E.coli and metagenomics.

- Fast, Simple and Automated Workflow

Library preparation can be completed in 3.5 hours with size-selected DNA as input; the workflow is simple and suitable for automated library preparation without the risk of amplicon contamination.

- No Amplification Error Accumulation

By eliminating PCR amplification steps, WGS PCR-free prepared and sequenced by MGI DNBSEQ™ platform has no amplification error accumulation, resulting in better genome fidelity.

- Higher Coverage Uniformity

Compared with traditional WGS (PCR amplification), WGS PCR-free reduces GC bias and improves coverage uniformity across the genome, such as GC-rich region, promoter and repetitive region.

- Excellent Performance of Variant Detection

Compared with traditional WGS (PCR amplification), WGS PCR-free shows higher sensitivity and precision of variant detection, especially indels.

### Overview

MGIEasy PCR-Free DNA Library Prep Set is specifically designed for making WGS library without PCR amplification for MGI high-throughput sequencing platform. MGI WGS PCR-free library preparation, combined with MGI DNBSEQ sequencer, is the true PCR-free NGS workflow without PCR amplification, resulting in no error accumulation and higher data accuracy. The kit is compatible with multiple species types, such as human, animal, plant and microorganisms. Its workflow is fast and simple. Compared to traditional WGS (PCR amplification), MGI WGS PCR-free has the advantages of no error accumulation, higher coverage uniformity and higher performance of variation detection.

### Workflow

MGIEasy PCR-Free DNA Library Prep Set uses size-selected dsDNA fragments as input for NGS library preparation. After end-repair and A-tailing, adapter ligation and cleanup, the purified library product is denatured into

single-strand DNA. Circularization is performed to obtain a sequencing library dedicated to the MGI high-throughput sequencing platform.



### Product Parameters

|                        |   |
|------------------------|---|
| Total Time             | ~3.5 hours  |
| Hands-on-time          | ~30 minutes   |
| Sample Amount          | 120–200 ng fragment DNA ( recommended to 1 μg genomic DNA)  |
| Insert Size            | 350–400 bp  |
| Sample Type            | Fragment DNA  |
| Species Compatibility  | Human, animals, plants, fungi, bacteria, metagenomics, etc. |
| Application            | Whole genome sequencing                                     |
| Platform Compatibility | MGISEQ-2000, BGISEQ-500, DNBSEQ-G400                        |
| Sequencing Strategy    | PE100, PE150  |

### Product Performance

- Good Stability of Library Yield

Library preparation of selected samples from six species, including human NA12878, mouse, silkworm, rice, E.coli and metagenomics, each with 1 μg gDNA as input, result in good yields of ssCir ( single-stranded circle DNA ) library (consistently above 12 ng). This indicates that the MGIEasy PCR-Free DNA Library Prep Set produces sufficient ssCir library yield for subsequent circularization and sequencing for different species and different genome size samples.

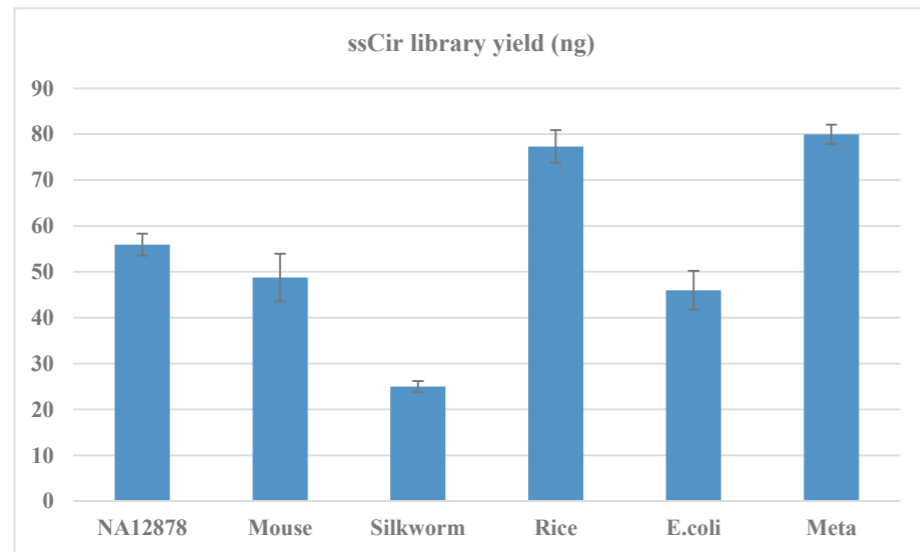


Figure 1 ssCir library yield for different species (N=3 to 6 replicates)

Libraries were prepared from NA12878 under different conditions (for example different lots and operators). The ssCir library yield is consistently higher than 12 ng. This indicates that the MGIEasy PCR-Free DNA Library Prep Set is capable of producing stable library yield under different conditions.

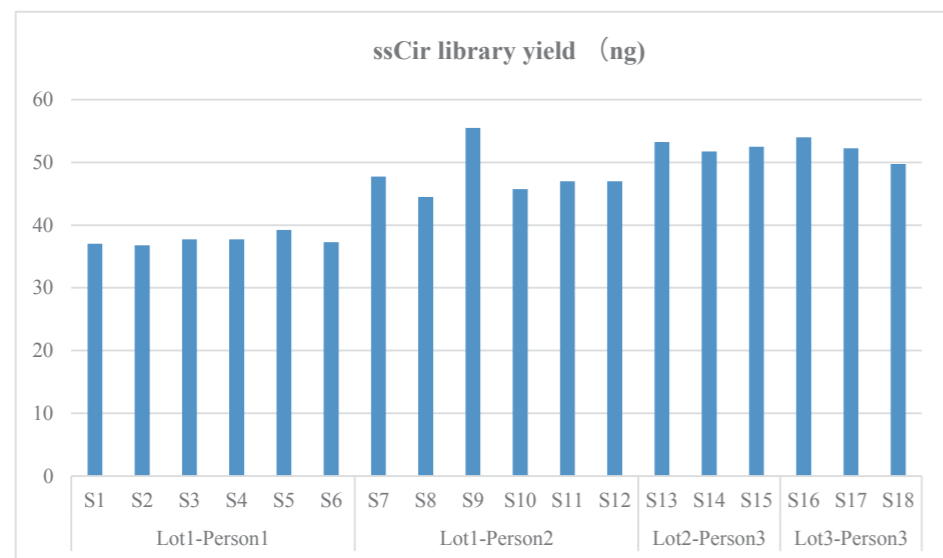


Figure 2 ssCir library yield of NA12878

### No Error Accumulation

Since our WGS PCR-free kit removes PCR amplification during library preparation, there is no PCR error or PCR bias. The MGI DNBSEQ™ technology is that every replicated copy is produced from the original genomic fragment in a rolling circle replication process, unlike in PCR where generated DNA copies are used to make more copies. MGI WGS PCR-free, combined with DNBSEQ™ technology, is the first and only PCR-free NGS workflow, resulting in better genome fidelity.

### Higher Coverage Uniformity

MGI WGS PCR-free without PCR amplification shows minimal amplification bias and higher coverage uniformity across the genome. The coverage plot of high GC bacteria and low GC bacteria, similar to middle GC bacteria, are close to the expected normalized coverage of 1.0. That indicates the MGI WGS PCR-free has uniform GC coverage over a broad range of GC contents (see Figure 3).

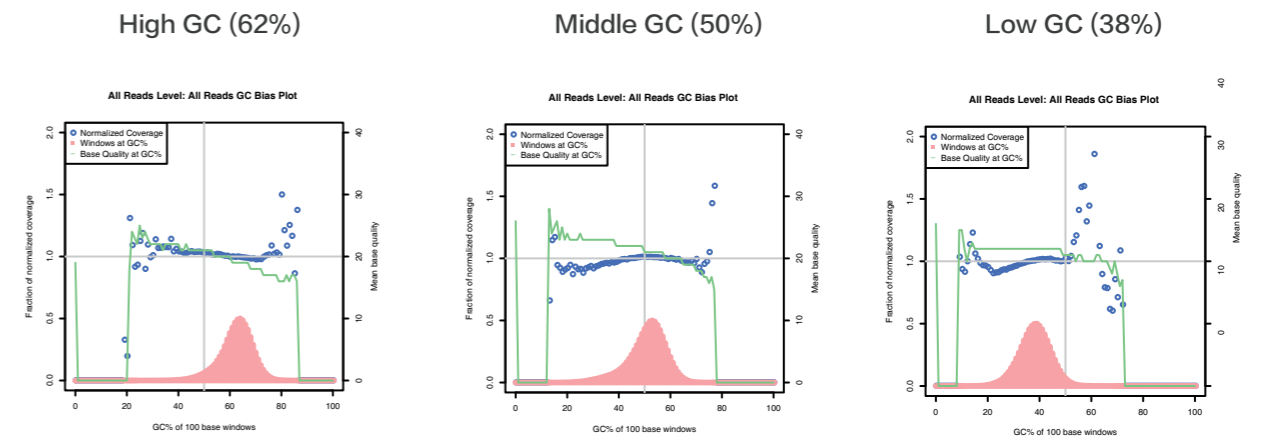


Figure 3 GC bias plot of bacteria with different genome GC content (Olsenella, 62% GC; E.Coli., 50% GC and Bacillus megaterium, 38% GC). Libraries were prepared with MGIEasy PCR-Free DNA Library Prep Set from 1 μg gDNA, and sequenced on MGISEQ-2000(PE150). The plots were assessed by calculating the GC 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.

Compared with traditional WGS (PCR amplification) (Figure 4, right, red histogram), the depth frequency distribution of MGI WGS PCR-free (Figure 4, left, red histogram) is closer to the Poisson-like depth frequency distribution (blue line for each), indicating MGI WGS PCR-free provides higher coverage uniformity across the genome.

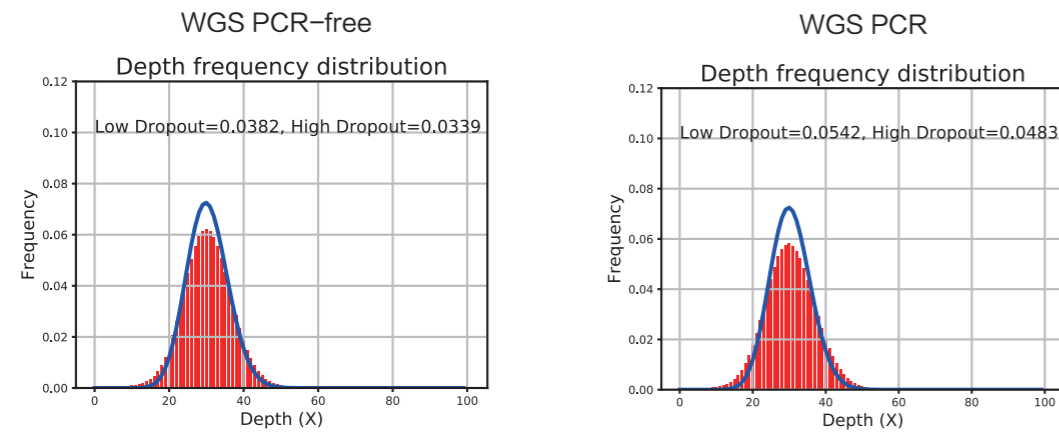


Figure 4 The plot of sequencing depth frequency distribution. Libraries were prepared using 1  $\mu$ g NA12878, prepared with MGIEasy PCR-Free DNA Library Prep Set and traditional WGS library prep set respectively, sequenced on MGISEQ-2000 (PE150), and analyzed at 30X data. X-axis, sequencing depth; 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 Performance of Variant Detection

For the NA12878, detection precision and sensitivity of indels prepared with MGIEasy PCR-Free DNA Library Prep Set were high (99.49% and 99.18%), significantly better than the performance of the WGS PCR-free and PCR of Vendor i products. Meanwhile, detection sensitivity and precision of SNP prepared with the MGIEasy PCR-Free DNA Library Prep Set were both higher than 99.9%, similar to WGS PCR-free and PCR of Vendor i products on N sequencing platform. This indicates MGI WGS PCR-free has excellent detection performance.

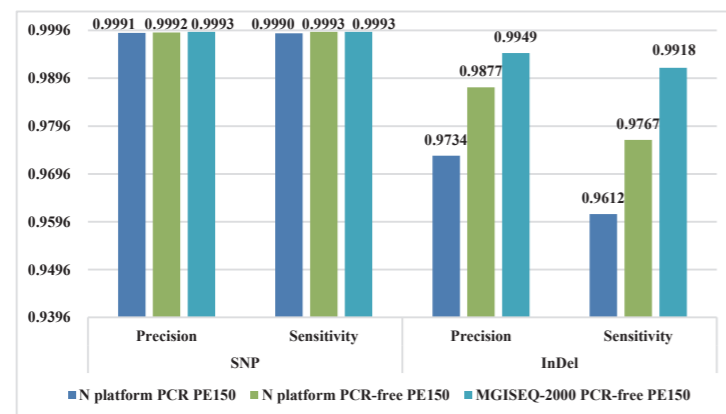


Figure 5 Comparison of variant detection performance of PCR-free WGS and PCR WGS on two sequencing platforms. Libraries were prepared using NA12878, prepared with MGIEasy PCR-Free DNA

Library Prep Set, sequenced on MGISEQ-2000 (PE150), and analyzed for variant detection performance. The results were compared with Vendor i products (T PCR-Free kit and T PCR kit) on N sequencing platform, using data downloaded from their official website.

### ■ Summary

MGIEasy PCR-Free DNA Library Prep Set is suitable for broad application of WGS. For removal of PCR amplification, MGI PCR-free WGS has been shown to produce high-quality sequencing data with minimal error accumulation, higher coverage uniformity and excellent variant detection performance, which supports whole genome research comprehensively.

### ■ Ordering Information

| Product                               | Specification | Item Number |
|---------------------------------------|---------------|-------------|
| MGIEasy PCR-Free DNA Library Prep Set | 16 RXN        | 1000013452  |
|                                       | 96 RXN        | 1000013453  |

### ■ Contact Us

MGI Tech Co., Ltd  
 Add.: Building11, Beishan Industrial Zone, Yantian District, Shenzhen, CHINA 518083  
 Email: MGI-service@genomics.cn  
 Website: www.mgitech.cn  
 Tel: 4000-966-988  
 Revision: 2019.5



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