MGIEasy Fast FS Library Prep Set V2.0 Enables Ultra-Low DNA Input Library Preparation

——Unlocking the potential application of trace samples in genomics research

This study, based on MGIEasy Fast FS Library Prep Set V2.0, accurately analyzed bacterial species abundance in 1 pg ~ 10 pg ZymoBIOMICS™ Microbial Community DNA Standard (D6305) samples with the optimized ultra-low DNA input library preparation workflow. It can realize the library preparation with as low as 1 pg DNA input, and provide a reliable data foundation for downstream high-throughput sequencing and species detection research.

Recommended application: Ultra-Low DNA Input Library Preparation

- **Breaking trace sample input limitations**

  The ultra-low input library preparation workflow breaks through sample input limitations, enabling efficient library preparation and sequencing of genomic DNA (e.g., metagenomic samples) with as low as 1 pg to 10 pg input.

- **Suitable for low-input samples**

  The kit is compatible with library preparation and sequencing of trace microbial samples and other low-input samples from scenarios such as extreme environments and ecological research.

- **High sensitivity and accuracy**

  The ultra-low input library preparation workflow can accurately detect low-abundance nucleic acid sequences, facilitating the analysis of microbial community abundance and composition in special low-input samples.

Background

Extremely low DNA input amounts remain a persistent challenge across diverse sample types in fields of biomedical research and environmental sample analysis. This difficulty exists in many scenarios such as rare animal and plant species, clinically derived micro samples like needle biopsy tissues[1] and single cells[2], as well as specialized environmental samples with microbial scarcity[3] like paleontological fossils[4], polar regions[5] and deep-sea microbial samples[6]. The genetic information contained within these samples is crucial for addressing important scientific questions including species evolution, early disease diagnosis and precision medicine, as well as the structure and function of microbial communities in ecosystems.

Traditional library preparation methods typically require high sample input amounts, making it unsuitable for research on ultra-low input samples. MGIEasy Fast FS Library Prep Set ultra-low input library preparation workflow breaks through the limitations of sample input and achieves library preparation and sequencing with sample input as low as picogram (pg) levels, unlocking the potential of trace samples in genomics research. The ultra-low input library preparation workflow could generate up to 95 ng library with as low as 1 pg of input DNA from ZymoBIOMICS™ Microbial Community DNA Standard (D6305). The data output exceeds 500M reads on DNBSEQ-G400 sequencing platform (Paired-end 150 bp, PE150), with Q30 exceeding 90%. This workflow is suitable for the analysis of microbial community abundance and composition in certain trace samples from scenarios such as extreme environments and ecological research.

Study Description

MGIEasy Fast FS Library Prep Set V2.0 provides an effective solution for ultra-low input library preparation by optimizing library preparation workflow. It enables library preparation and sequencing for small genomic gDNA (e.g., metagenomic samples) with trace sample input (1 pg ~ 10 pg). In this study, the bacterial species abundance in 1 pg ~ 10 pg ZymoBIOMICS™ Microbial Community DNA Standard samples were analyzed with the optimized ultra-low DNA input library preparation workflow and DNBSEQ-G400 sequencing platform. The results showed that ultra-low DNA input library preparation workflow can output stable species identification results even when the input DNA is as low as 1 pg, effectively improving the success rate of ultra-low input library preparation.

Materials and Methods

DNA Sample Preparation

This study carried out a performance evaluation of ultra-low DNA input library preparation workflow based on ZymoBIOMICS™ Microbial Community DNA Standard. This standard is composed of 8 different microbial DNA and 2 different yeast DNA in a certain ratio (Table 1). The DNA Standard was serially diluted and three initial input amounts (1 pg, 5 pg and 10 pg) were selected for library preparation, sequencing and bioinformatic analysis.

Library preparation and sequencing

Libraries were prepared by using the MGIEasy Fast FS Library Prep Set V2.0, which combines the fragmentation, end-repair and A-tailing into one step, simplifying the preparation process and significantly shorten the time of DNA library preparation.

Three experimental duplicates were set for each initial DNA input amounts (1 pg, 5 pg and 10 pg), library preparation was conducted following the ultra-low DNA input library preparation workflow in Table 2. For the experimental operation details, please refer to the *MGIEasy Fast FS Library Prep Set Ultra-low input Library Prep User Manual*. The purified PCR libraries were analyzed by Agilent 2100 Bioanalyzer to detect the fragment distribution and library quality. The qualified libraries were converted into single-stranded circular DNA libraries, then prepared into DNA nanoballs (DNBs) and sequenced on DNBSEQ-G400 platform with PE150.

Bioinformatic analysis

The sequencing data were analyzed by using the Platform of Microorganisms Fast Identification software (PFI) to achieve rapid and accurate microbial identification and analysis. On this platform, filtered FASTQ data are used for sequence alignment, species identification, and generating identification reports and analysis results.

Table 1. Microbial components in D6305

Species	Gram Stain	GC Content (%)	Genomic DNA (%)
Pseudomonas aeruginosa	−	66.2	12
Escherichia coli	−	46.7	12
Salmonella enterica	−	52.2	12
Lactobacillus fermentum	+	52.4	12
Enterococcus faecalis	+	37.5	12
Staphylococcus aureus	+	32.9	12
Listeria monocytogenes	+	38.0	12
Bacillus subtilis	+	43.9	12
Saccharomyces cerevisiae	Yeast	38.3	2
Cryptococcus neoformans	Yeast	48.3	2

Note: Gram strain refers to gram-negative/positive, GC Content (%) refers to the GC value of genomic DNA, and Genome DNA (%) refers to the composition and abundance of DNA of each species.

Table 2. Comparison of the universal and ultra-low input library preparation workflows for the MGIEasy Fast FS Library Prep Set V2.0

Step	Universal Library Prep workflow	Ultra-Low Input Library Prep workflow	Note
Fragmentation + End Repair + A-Tailing	22 min	30 min	Extend fragmentation time to ensures sufficient fragmentation
0.8× Bead Purification	13 min	No	Minimize sample loss during purification
Adapter Ligation	Adapter：50× Dilution Ligation mixture：30 μL	Adapter：80× Dilution Ligation mixture：36 μL	Increase the volume of the ligation mixture to ensure the efficiency of adapter ligation reactions
Post-Ligation Purification	En-TE：22 μL En-Beads：20 μL	En-TE：10 μL En-Beads：30 μL	Increase the amount of En-beads to recover as many DNA fragments as possible.
PCR	1 ng：11~14 Cycles	1 pg~10 pg：20~24 Cycles 10 pg~100 pg：18~20 Cycles 100 pg~1 ng：14~18 Cycles	Increase the number of PCR cycles to improve the yield of PCR product
Post-PCR Purification	En-TE：32 μL En-Beads：38 μL	En-TE：22 μL En-Beads：38 μL	Reduce the amount of magnetic beads used in the second-round purification
Second-round Purification	No	En-TE：22 μL En-Beads：0.8X	Further removal of primer dimers

Sample collection	Library prep & Sequencing	Bioinformatics Analysis	Result analysis
ZymoBIOMICS™ Microbial Community DNA Standard (D6305)	 MGIEasy Fast FS Library Prep Set V2.0  DNBSEO-G400 genetic sequencer	Platform of microorganisms Fast Identification software ,PFI	Compositional abundance of each strain in the standard was analyzed and compared with the theoretical compositional abundance

Results

High-quality library preparation

In this study, DNA Standard was used for library preparation with varied initial DNA input amounts (1 pg, 5 pg and 10 pg) according to ultra-low DNA input library preparation workflow. The results showed that with 1 pg, 5 pg and 10 pg of sample input, the library yields were ~95 ng, ~100 ng and ~190 ng, respectively. This result can meet the needs of downstream high-throughput sequencing, indicating the efficient utilization of trace samples (Fig.1). The Agilent 2100 Bioanalyzer results showed that the library fragment distribution at different initial input amounts is highly consistent, with an expected amplicon size and no primer dimer or other non-specific peaks (Fig.2).

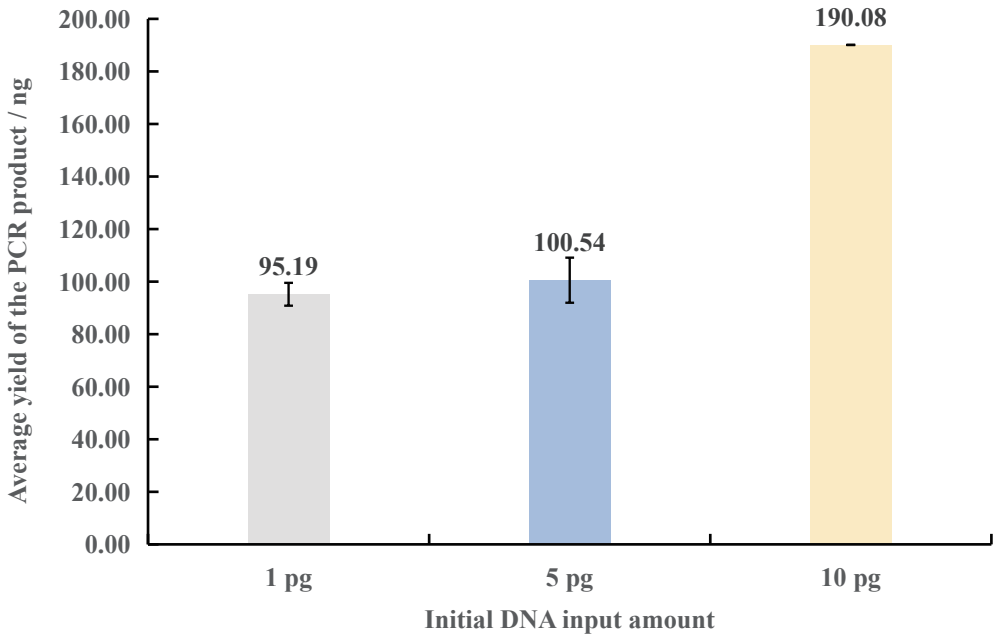

Figure 1. PCR yields for library preparation with different initial DNA input amounts. Each input amount was tested in triplicate. The number of PCR cycles for 1 pg was 22, and the number of PCR cycles for 5 pg and 10 pg was 20.

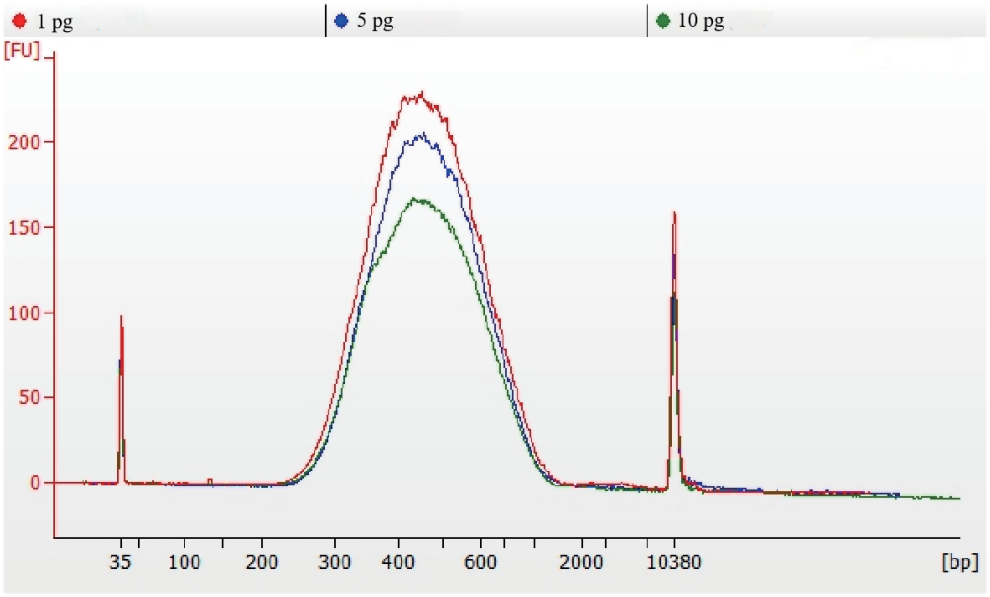

Figure 2. Agilent 2100 Bioanalyzer electropherograms of libraries with different initial DNA input amount.

Excellent sequencing quality performance

Library sequencing was performed on DNBSEQ-G400 with PE150. High-quality sequencing data was obtained with a total of 515 M reads data output, Q30 exceeding 90% and Split rate exceeding 95% (Fig.3A). From the base sequencing quality distribution diagram (Fig.3B), it showed that the mass values remain stable at each of the sequencing cycle, indicating that the high quality of sequencing data, which met the needs of downstream deep analysis.

A

QC indexes	Values
Total Reads (M) *	515.42
Q30 (%)	93.15
Split Rate (%)	98.65

*. Total Reads/Lane

B

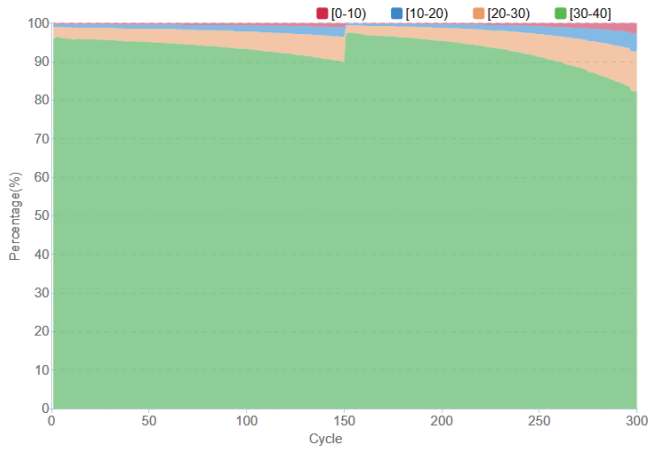

Figure 3. Sequencing quality.

High accuracy detection performance

PFI was applied for data analysis after sequencing. The species abundance analysis was performed on one replicate randomly selected from each of the 1 pg, 5 pg and 10 pg input groups. The measured data showed that the abundance of microbial composition across different initial sample input amounts was consistent as expected. (Fig.4,Table 3). For example, at the input amount of 1 pg, the relative abundances of *Pseudomonas aeruginosa, Escherichia coli, Salmonella enterica, Lactobacillus fermentum, Enterococcus faecalis, Staphylococcus aureus, Listeria monocytogenes, Bacillus subtilis, Saccharomyces cerevisiae* and *Cryptococcus neoformans* were 14.61%、11.64%、11.81%、11.02%、11.33%、11.38%、10.48%、10.65%, 1.47% and 1.38%, respectively, which were consistent with theoretical proportions. Similar results were observed at other input amounts (Table 3).

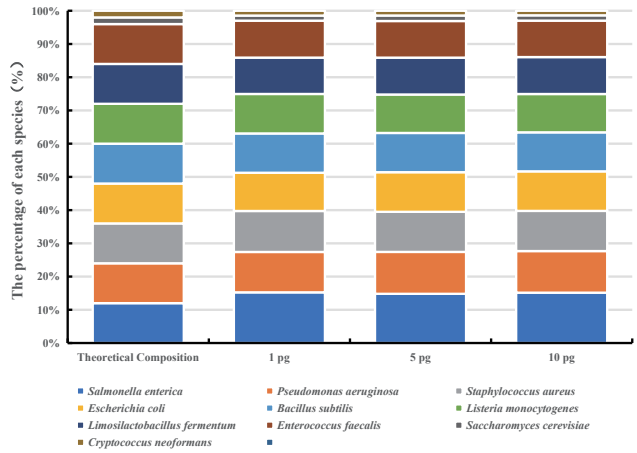

Figure 4. Species abundance of different initial DNA input amounts. The abundance of microbial composition at different initial DNA input amounts (1 pg, 5 pg, 10 pg) were close to the theoretical composition.

Spearman correlation coefficients between samples with different initial input amounts and the theoretical composition were calculated. The results showed that the correlation coefficients between samples with initial input amounts of 1 pg, 5 pg and 10 pg and the theoretical species composition were 0.9927, 0.9953 and 0.9942, respectively. Meanwhile, the correlation coefficients between samples with different initial input amounts were all as high as 0.99 (Fig.5). This indicates that the species abundance distribution patterns of samples with different initial input amounts are highly consistent, which can effectively support comparative analysis between samples with different initial input amounts. It also suggests that the ultra-low input library preparation workflow can generate stable and comparable species abundance data with as low as 1 pg to 10 pg input.

Table 3. Test results with different sample input amounts

Species	Theoretical Composition (%)	1 pg	5 pg	10 pg
Pseudomonas aeruginosa	12	14.61%	14.67%	14.87%
Escherichia coli	12	11.64%	12.49%	12.29%
Salmonella enterica	12	11.81%	11.95%	11.86%
Lactobacillus fermentum	12	11.02%	11.81%	11.68%
Enterococcus faecalis	12	11.33%	11.70%	11.52%
Staphylococcus aureus	12	11.38%	11.46%	11.32%
Listeria monocytogenes	12	10.48%	10.99%	10.91%
Bacillus subtilis	12	10.65%	10.92%	10.76%
Saccharomyces cerevisiae	2	1.47%	1.64%	1.57%
Cryptococcus neoformans	2	1.38%	1.41%	1.34%

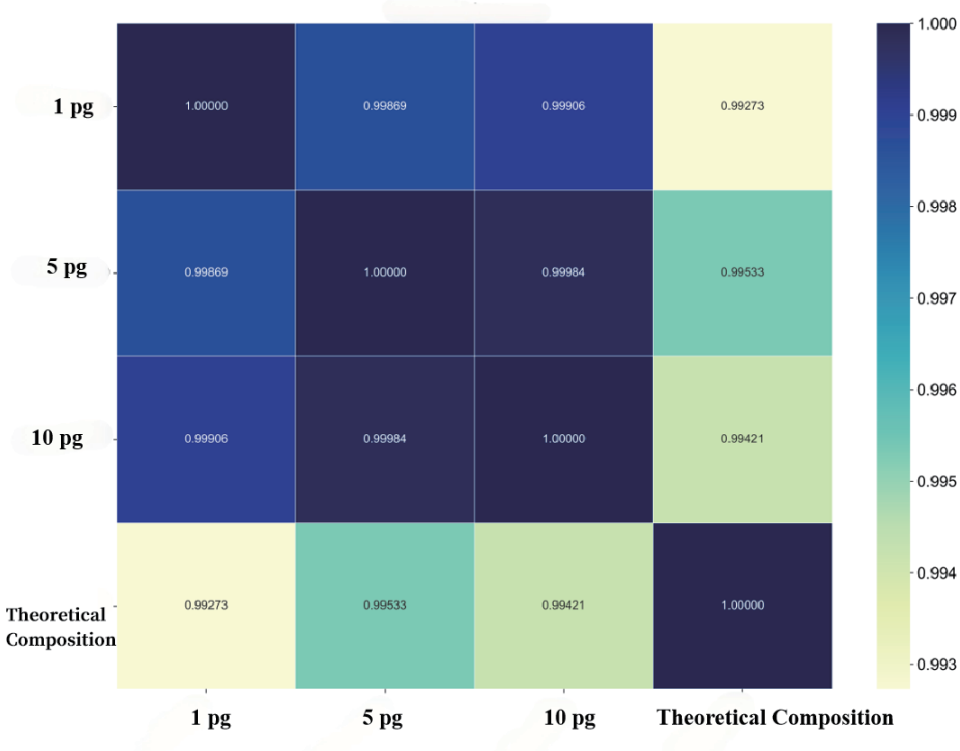

Figure 5. Spearman correlation coefficient of species abundance at different initial DNA input amounts.

Summary

MGIEasy Fast FS Library Prep Set ultra-low input library preparation workflow can effectively detect species abundance in trace Meta gDNA samples (1 pg to 10 pg). It enables efficient and rapid library preparation for trace samples, showing comprehensive performance in ultra-low input library preparation applications, and greatly improves the utilization of precious samples. In addition, it also provides a library preparation solution for the analysis of microbial community abundance and composition in certain trace samples from scenarios such as extreme environments and ecological research.

Genetic Sequencer DNBSEQ-G400

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Recommended Ordering Information

Product	Packaging specifications	Cat. NO.
MGIEasy Fast FS Library Prep Set V 2.0	16 RXN	940-002915-00
MGIEasy Fast FS Library Prep Set A V2.0	96 RXN*	940-002916-00
MGIEasy Fast FS Library Prep Set B V2.0	96 RXN**	940-002917-00
MGIEasy Fast FS Library Prep Set C V2.0	96 RXN***	940-001831-00
MGIEasy Dual Barcode Circularization Kit	16 RXN	1000020570

*: With MGIEasy UDB Primers Adapter Kit A;
**: With MGIEasy UDB Primers Adapter Kit B;
***: With MGIEasy UDB Primers Adapter Kit C;

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