APPLICATION NOTE

Automated whole genome sequencing with QIAseq® FX DNA library kit on the Fontus system.

Introduction

The challenge of metagenomics lies in capturing an accurate snapshot of diverse microbial populations. Whether working with environmental or oral samples, obtaining high yields without introducing sample-specific bias is essential for reliable downstream analysis.

QIAGEN's QIAseq® FX DNA Library Kit offers an all-enzymatic DNA fragmentation protocol for whole genome sequencing (WGS) library prep, eliminating the need for mechanical shearing. It supports low and high inputs amounts and produces high-quality libraries with low GC bias.

The Fontus™ NGS workstation by Revvity streamlines metagenomic workflows by consolidating the trusted span-8 flexibility of the JANUS™ systems with the high-throughput 96/384 array and labware handling of Sciclone™ and Zephyr™ platforms making it ideal for clinical research. This integrated technology enables seamless tube-to-plate reformatting and high-yield library preparation. It features intuitive software, vendor-qualified workflows, and integrated peripherals like thermocyclers and temperature modules.

The automated method of the QIAseq® FX DNA Library Kit on the Fontus NGS workstation offers three reactions allowing for conversion of gDNA into a whole genome library. This method reduces hands-on time and run-to-run variability.

The Fontus simplifies WGS library prep with a multi-step automated protocol: fragmentation, end-repair, adapter ligation, amplification, and cleanup. Key features include:

- Validated pause points for flexible scheduling
- Customizable parameters (batch size, fragment size, number of PCR cycles)
- Reduced consumables and operator variability

Runtime summary

Step	Description	Duration
1	FX fragmentation	56m
2	Adapter ligation and cleanup	1hr 49min
3	Amplification and cleanup	58m
Total		3hr 43m

Setup takes ~30 minutes, after which the run proceeds without user intervention (based on 48 sample run).

Key features

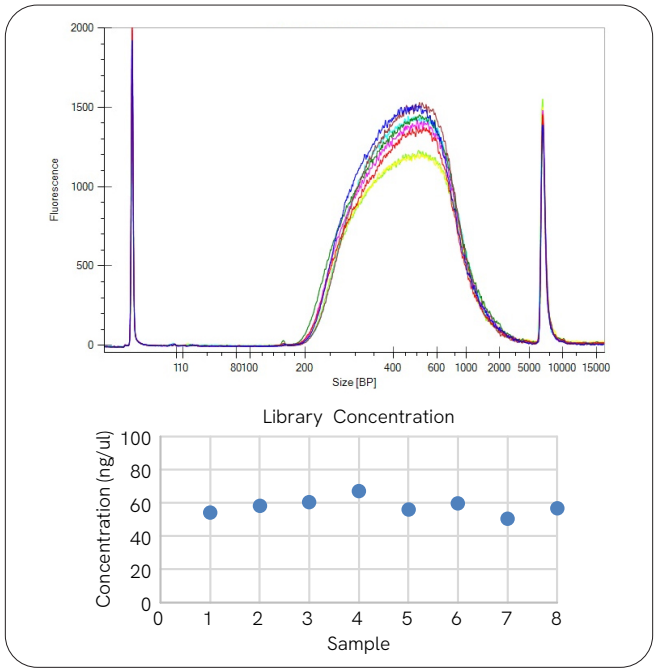
- Processes 1–96 samples
- High library complexity and uniform coverage
- 384 Unique Dual Index (UDI) adapters for multiplexing
- DNA input range: 10 ng – 1 µg
- Compatible for various sequencing instruments

Experimental design

48 Libraries were prepared from Promega G304A using QIAseq® FX DNA Library Kit and UDI Y-Adapter Kit A on the Fontus system. Each sample used 100 ng starting input. Final library concentrations were measured with the Thermo Fisher Scientific™ Qubit HS and library size was evaluated with the Revvity Labchip™ system.

Final libraries were primer dimer free with yields within the acceptable range. Libraries were normalized to 4nM using QIAseq® Library Normalizer Kit. A random selection of 8 libraries were selected for sequencing on the Illumina® Nextseq 2000 with 2 x 151 bp paired read length. FASTQ files were analyzed with QIAGEN® CLC Genomics Workbench for mapping and quality control of reads.

Results



- Library quality: 470 ± 20 bp fragments, adapter-free
- Median library quantification: 57.50 ng/ul
- Sequencing yield: 452.02 Gbp
- Q30 score: 93.60%
- Reads passing filter: 84.89%
- Median read mapping to reference: 99.12%

Conclusion

The automated workflow using QIAseq® FX DNA Library Kit on the Fontus NGS System delivers high-quality, sequencing-ready libraries, as demonstrated by the experimental results. Libraries showed optimal library profiles, free of adapter dimers with high concentrations. Sequencing metrics further demonstrate robust performance, indicating exceptional accuracy and sensitivity. The data shows consistency that is ideal for scalable whole genome sequencing applications, reducing variability and limiting human error.

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