Automation solutions for food safety surveillance with the MiSeq™ i100 Series

Highly uniform libraries and high-quality data for foodborne pathogen detection

Reduced hands-on time

Consistent, reliable performance

Comprehensive coverage of bacterial genomes

Introduction

Managing food safety is an essential part of public health. Foodborne illness related to microbial contamination from pathogenic bacteria represents a substantial threat to human health. In particular, recurring, emerging, or persisting (REP) bacterial strains, including pathogenic *E. coli*, *Listeria*, *Campylobacter*, *Shigella*, and *Salmonella,* can periodically cause acute outbreaks.[1,2]

Using laboratory methods to subtype enteric bacteria, including *Salmonella*, has been critical in identifying potential outbreaks and linking bacteria-causing illnesses to outbreak sources. In the United States, national molecular surveillance via next-generation sequencing (NGS) prevents over 250,000 illnesses from enteric bacteria every year and saves half a billion dollars in medical costs and lost productivity.[3,4]

While NGS-based whole-genome sequencing (WGS) has provided significant advantages in the speed, accuracy, and depth of information to microbiology labs, library preparation remains a bottleneck. Automating library preparation using a liquid-handling system generates uniform libraries with less hands-on time and fewer opportunities for human error.[5]

This technical note demonstrates the performance of a comprehensive NGS workflow that integrates three options for automating library preparation using Illumina DNA Prep with sequencing on the MiSeq i100 Series and analysis with DRAGEN™ software for efficient and accurate food safety surveillance (Figure 1).[6]

Methods

Samples

Thirty-two *Salmonella enterica* isolates collected as part of PulseNet routine surveillance[7] were cultured on BBL Blood Agar Base without the addition of blood (BD, Catalog no. 211037). Genomic DNA (gDNA) was extracted using the Wizard Genomic DNA Purification Kit (Promega, Catalog no. A1120) with slight modifications to the protocol for the isolation of gDNA from gram-negative bacteria.[8] Purified gDNA was quantified with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Catalog no. Q32853) before library preparation.

Library preparation

Sequencing-ready libraries were prepared in triplicate using three options for automated solutions and one manual option for Illumina DNA Prep (Illumina, Catalog no. 20060059). For library yield normalization, 100 ng of extracted gDNA per sample was used as input. For optimal insert size on the MiSeq i100 Series, an additional round of bead purification with a bead-to-sample ratio of 0.5× was included to remove short inserts.[9] The three automation solutions evaluated included the Biomek NGenius Next Generation Library Prep System (Beckman Counter, Catalog no. C62), the Biomek i7 Automated Workstation (Beckman Coulter, Catalog no. B87585), and the firefly platform (SPT Labtech) (Table 1). The

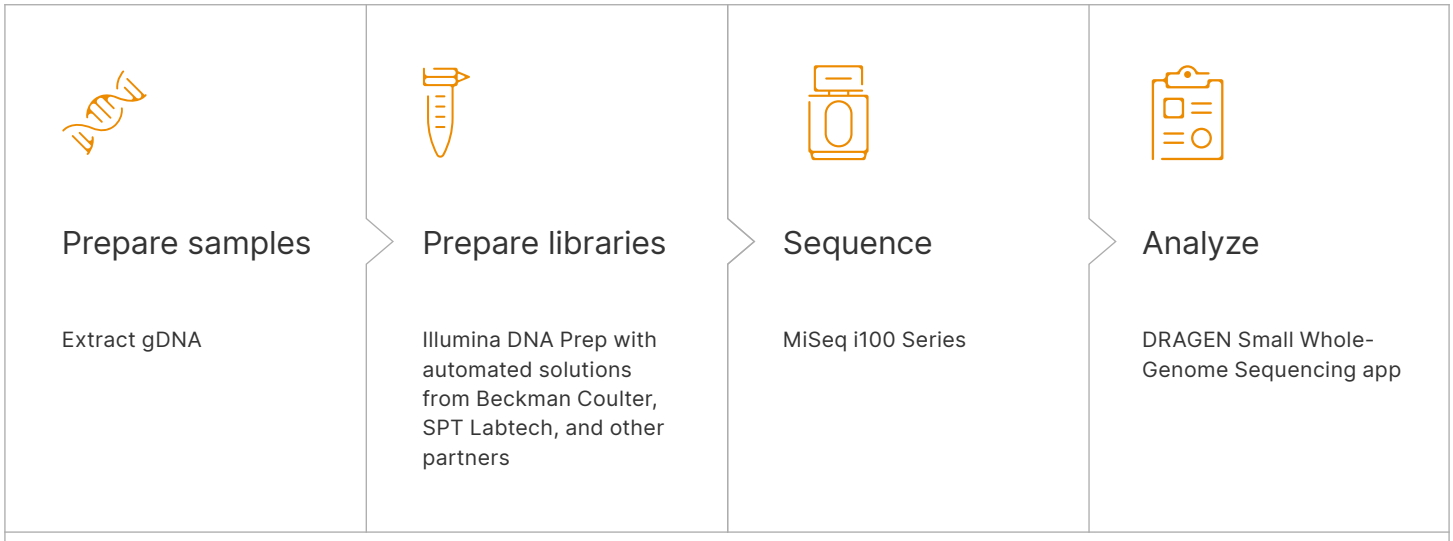

Figure 1: Comprehensive NGS workflow for *Salmonella* detection
Combine automated solutions for Illumina library preparation with sequencing on the MiSeq i100 Series and DRAGEN secondary analysis for accurate surveillance of the foodborne pathogen *Salmonella*.

quality and concentration of PCR-amplified libraries were assessed using the 4200 TapeStation System (Agilent Technologies, Catalog no. G2991BA) and the Qubit 1X dsDNA High Sensitivity (HS) Assay Kit (Thermo Fisher Scientific, Catalog no. Q33231) before pooling.

Sequencing

Prepared libraries were pooled by volume and diluted to a loading concentration of 60 pM (32 libraries/run). Sequencing was performed on the MiSeq i100 Plus System using a 25M flow cell with a run configuration of 2 × 301 bp. For larger studies, sequencing runs can be scaled up to the NextSeq™ 1000, NextSeq 2000, or NovaSeq™ 6000 Systems.

Data analysis

After sequencing was complete, data were streamed to BaseSpace™ Sequence Hub and processed with the FASTQ Toolkit (v2.2.6) to downsample the data sets to 700,000 paired-end (PE) reads. Analysis was performed using the DRAGEN Small Whole-Genome Sequencing (sWGS) app (v4.3.13) to obtain genome coverage, median depth metrics, and additional assay metrics.

Table 1: Assay times with manual and automated library preparation methods

Method[a]	Automation time	Hands-on time	Total assay time
Manual	N/A	60–90 min	~4–4.5 hr
Biomek NGenius	21 hr 45 min	~45 min	~21 hr 45 min[b]
Biomek i7	~4 hr 10 min	~20–24 min	~4 hr 34 min
SPT firefly	3 hr 8 min	~20–22 min	~3 hr 28 min

a. 96 samples per run.
b. Biomek NGenius run time to process 24 samples is 7 hr 15 min.

Results

Sequencing metrics

The MiSeq i100 Plus System generated sequencing data with an average of ≥ 96.5% of bases above Q30 and ≥ 75% of reads passing filter (PF) (Table 2). The concentration of libraries prepared with automated and manual methods was comparable (Figure 2A). The percentage of demultiplexed reads, which represents the proportion of the total number of reads that were successfully assigned to a sample based on its barcode, show comparable values with a low coefficient of variation (CV) (≤ 10%) across automated and manual methods, further indicating good quality data was obtained with all library preparation methods evaluated (Figure 2B).

Median insert sizes

Insert size is an important parameter in NGS library preparation and can be affected by tagmentation reaction conditions such as time, temperature, and library cleanup steps using Illumina Purification beads. Automated library preparation resulted in consistent median insert size that was larger than what was observed with manual library preparation (Figure 3).

Table 2: Sequencing metrics and run times with manual and automated library preparation methods

Method	Mean % Q30	% PF	Total no. of PE read PF	Sequencing run time
Manual	97.95 ± 0.40	81.09 ± 8.4	64,122,141	14 hr 13 min
Biomek NGenius	97.92 ± 0.18	77.72 ± 0.96	61,455,112	14 hr 2 min
Biomek i7	96.67 ± 0.55	78.04 ± 2.79	61,710,695	14 hr 2 min
SPT firefly	97.66 ± 0.13	75.31 ± 1.49	59,551,797	14 hr 21 min

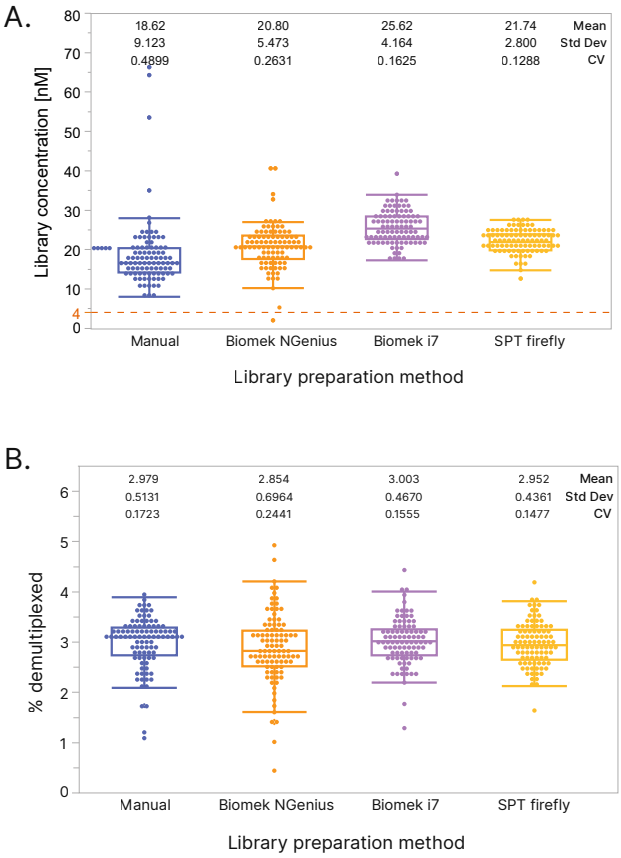

Figure 2: High-quality data with automated methods
(A) The concentrations of libraries prepared with various automated methods were comparable to manual preparation, with slightly lower coefficient of variation (CV) values. (B) Automated methods for library preparation result in high-quality sequencing data with % demultiplexed values comparable to manual library preparation.

Genome coverage

DRAGEN sWGS analysis demonstrated ≥ 90% of the *Salmonella* genome was covered at ≥ 20× coverage depth, as shown by a rolling average of coverage in a representative sample (Figure 4) and across 30 out of 33 samples assayed (Figure 5). The shorter median insert size with manual preparation (Figure 3) may explain the lower depth of coverage seen with manual preparation (Figure 4), though it still achieves comprehensive genome coverage (Figure 5).

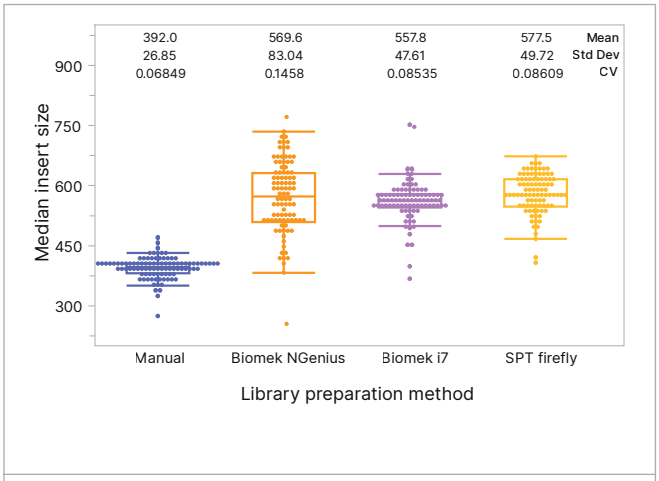

Figure 3: Consistent median insert size with automated methods
Median insert size for automated library preparation methods was consistently larger than the median insert size generated with manual library preparation.

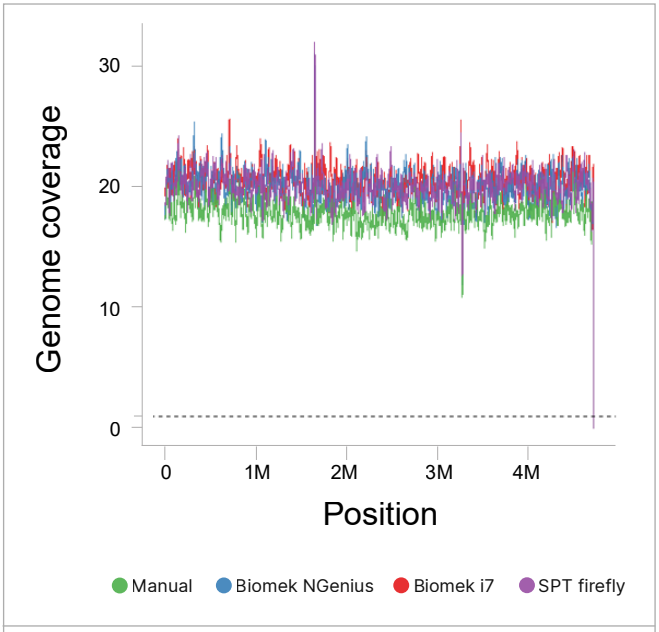

Figure 4: Genome coverage with automated methods
Automated methods for library preparation result in high-quality sequencing data, as shown by a rolling average of genome coverage in a representative sample.

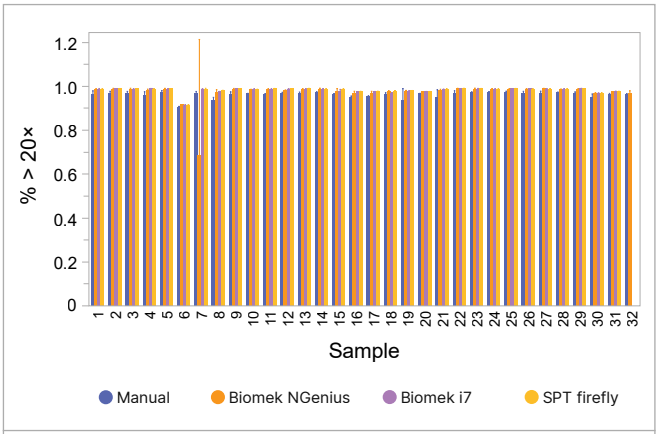

Figure 5: Comprehensive genome coverage with automated methods across samples

Automated methods for library preparation result in high-quality sequencing data with ~ 90% of the genome at ≥ 20× coverage, across all samples assayed, except for one outlier replicate (sample 7) that underperformed on the Biomek NGenius platform.

Summary

Foodborne illness related to microbial contamination from bacteria represents a substantial threat to human health. Automated methods generate uniform libraries with less hands-on time and fewer opportunities for human error. The MiSeq i100 Series can incorporate automated methods for library preparation to enable a fast, comprehensive NGS workflow that provides high-quality data for effective surveillance as part of public health efforts.

Learn more →

Illumina DNA Prep

MiSeq i100 Series

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