TECHNICAL NOTE

Automation workflow for Illumina DNA Prep with Exome 2.5 Enrichment

Minimize errors and reduce hands-on time with the Hamilton SBS STARlet platform

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Introduction

Illumina DNA Prep with Exome 2.5 Enrichment delivers efficient human whole-exome sequencing (WES) results with outstanding performance and data quality. The easy-to-use library preparation and enrichment solution is part of an end-to-end next-generation sequencing (NGS) workflow that spans from samples to reporting (Figure 1). Automated liquid-handling systems help labs achieve and maintain consistent results while driving efficiency. NGS library prep automation also allows for the rapid scaling of throughput while reducing hands-on time. Illumina Qualified Methods, developed by our automation partners with input from Illumina to ensure performance and data quality, are available on a range of automation platforms.

This technical note presents an Illumina Qualified Method for Illumina DNA Prep with Exome 2.5 Enrichment as part of an integrated NGS workflow that automates library preparation and enrichment on the Hamilton SBS STARlet Automated Liquid Handling Platform. Results demonstrate that the automated workflow generates consistent, high-quality data with less hands-on time, compared to the manual workflow.

Methods

Samples

Reference genomic DNA (gDNA) samples were obtained to evaluate the automated workflow, including Roche Reference gDNA (Sigma Aldrich, Catalog no. 11691112001) and NA12878 reference DNA (Coriell Institute for Medical Research, Catalog no. NA12878).

Library preparation

Library preparation and enrichment was performed using 50–200 ng of input gDNA and Illumina DNA Prep with Exome 2.5 Enrichment, (S) Tagmentation Set B (96 Samples, 12-plex) (Illumina, Catalog no. 20077595). Automated library preparation and enrichment was performed on the Hamilton SBS STARlet Automated Liquid Handling Platform following the Ready Workflow for Exomes Mid-Throughput by Illumina reference guide. The workflow reduces the number of user touch points and includes multiple safe stopping points (Figure 2). To compare performance, sequencing data from libraries prepared with the Hamilton SBS STARlet platform was compared to data generated from manually prepared libraries available in BaseSpace™ Sequence Hub.

Sequencing

Prepared libraries were pooled manually and sequenced on the NextSeq™ 2000 System (Illumina, Catalog no. 20038897) using the NextSeq 2000 P3 XLEAP-SBS™ Reagent Kit (300 cycles) (Illumina, Catalog no. 20100988). Sequencing was performed with a run configuration of 2 × 151 bp read length.

Data analysis

Sequencing data was analyzed using the DRAGEN™ Enrichment app version 4.2.7 in BaseSpace Sequence Hub for alignment and variant calling was carried out using the Variant Calling Assessment Tool app version 4.1.0 for comparison of variant cell sets. Emedgene™ software can be used to streamline data interpretation and reporting.

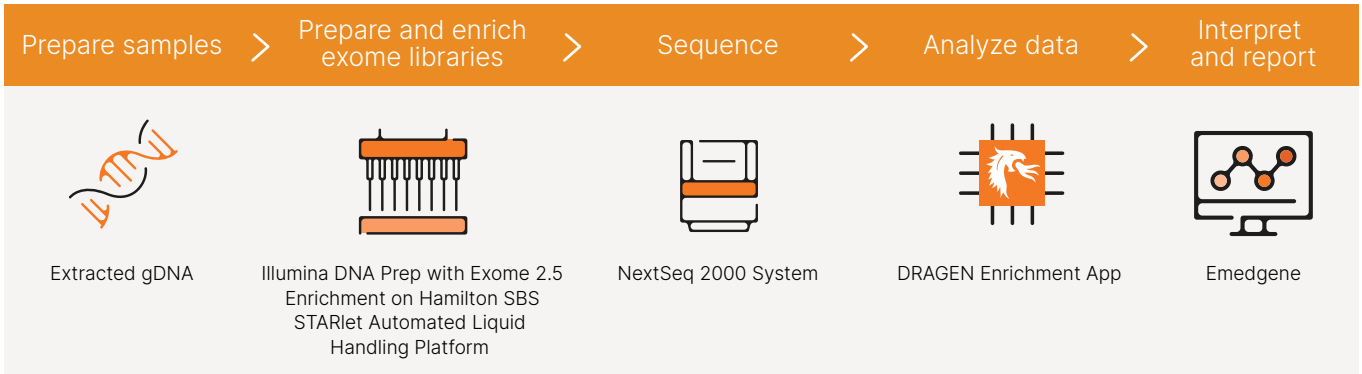

Figure 1: Integrated NGS workflow—Illumina DNA Prep with Exome 2.5 Enrichment is part of a Ready Workflow by Illumina that supports automated library preparation and enrichment, sequencing on the NextSeq 2000 System, DRAGEN secondary analysis, interpretation and reporting using Emedgene software.

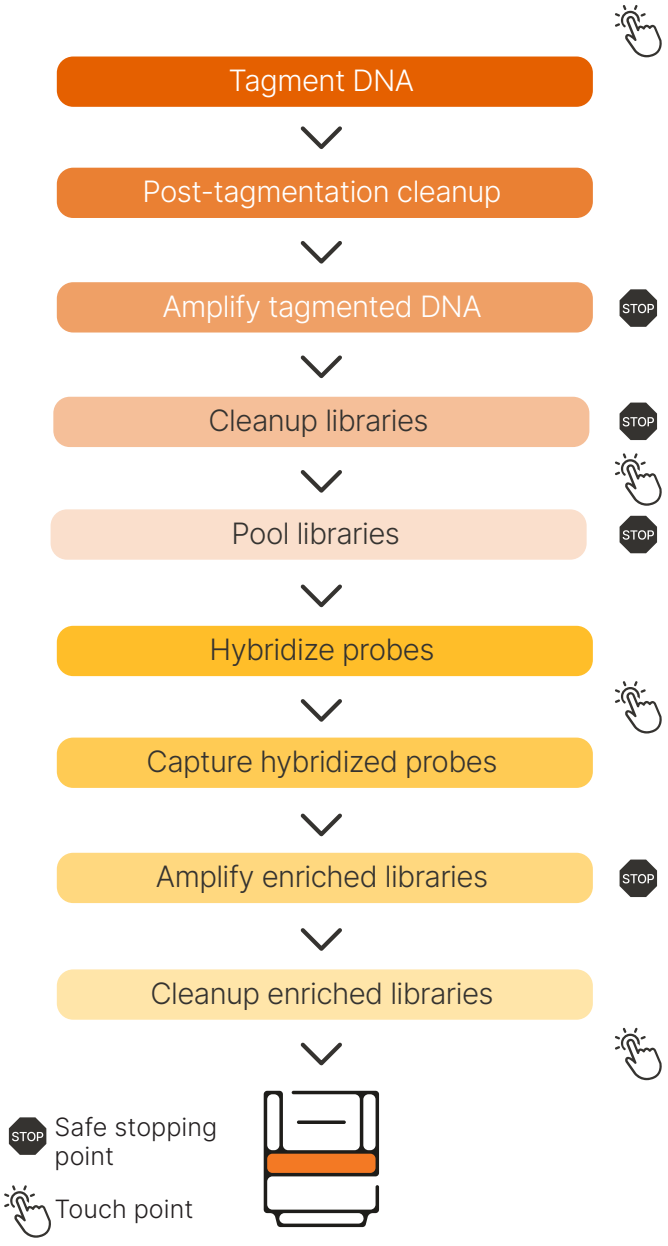

Figure 2: Automated workflow—The automated workflow for preparation and enrichment of sequencing-ready libraries features a reduced number of user touch points and includes safe stopping points throughout the protocol.

Results

Within-lab precision

Reproducibility of results was assessed for a total of 72 samples across three sequencing runs conducted at a single site (Illumina Centre, Cambridge, UK) on the same Hamilton SBS STARlet platform by the same user and sequenced on the same NextSeq 2000 System. Results show high performance with all samples meeting recommended guidelines for quality control (QC) metrics, including fragment length median, padded unique read enrichment, mean target coverage depth, and more (Figure 3).

Second-site reproducibility

The reproducibility of results was evaluated for three different Hamilton STARlet platforms across three different laboratories with three sequencing runs. All runs were conducted by the same experienced user at Site 1 (Illumina Centre, Cambridge, UK), Site 2 (Illumina Solution Centre, Evry, FR), and Site 3 (Illumina Solutions Centre, Milan, ITA). Results show that QC metrics across the runs were above the recommended guidelines for mean target coverage depth, percent target coverage at 20× and 50×, uniformity of coverage, and percent padded unique read enrichment (Figure 4). This demonstrates that results generated on multiple instruments at different sites are reproducible.

Manual versus automated performance

Variant calling was compared between the automated and manual workflows. In this study two runs of 24 replicates of Coriell NA12878 DNA input (200 ng) were processed using Illumina DNA Prep with Exome 2.5 Enrichment on the Hamilton STARlet platform. After sequencing on the NextSeq 2000 System, variant calling was performed using the DRAGEN Enrichment app followed by the Variant Calling Assessment tool (v 4.1.0). Results show comparable performance for single nucleotide variants (SNV) precision, SNV recall, insertions/deletions (indel) precision, and indel recall between the automated method and data from manually prepared libraries (Figure 5). Importantly, the automated method resulted in reduced hands-on time compared to the manual method (Table 1).

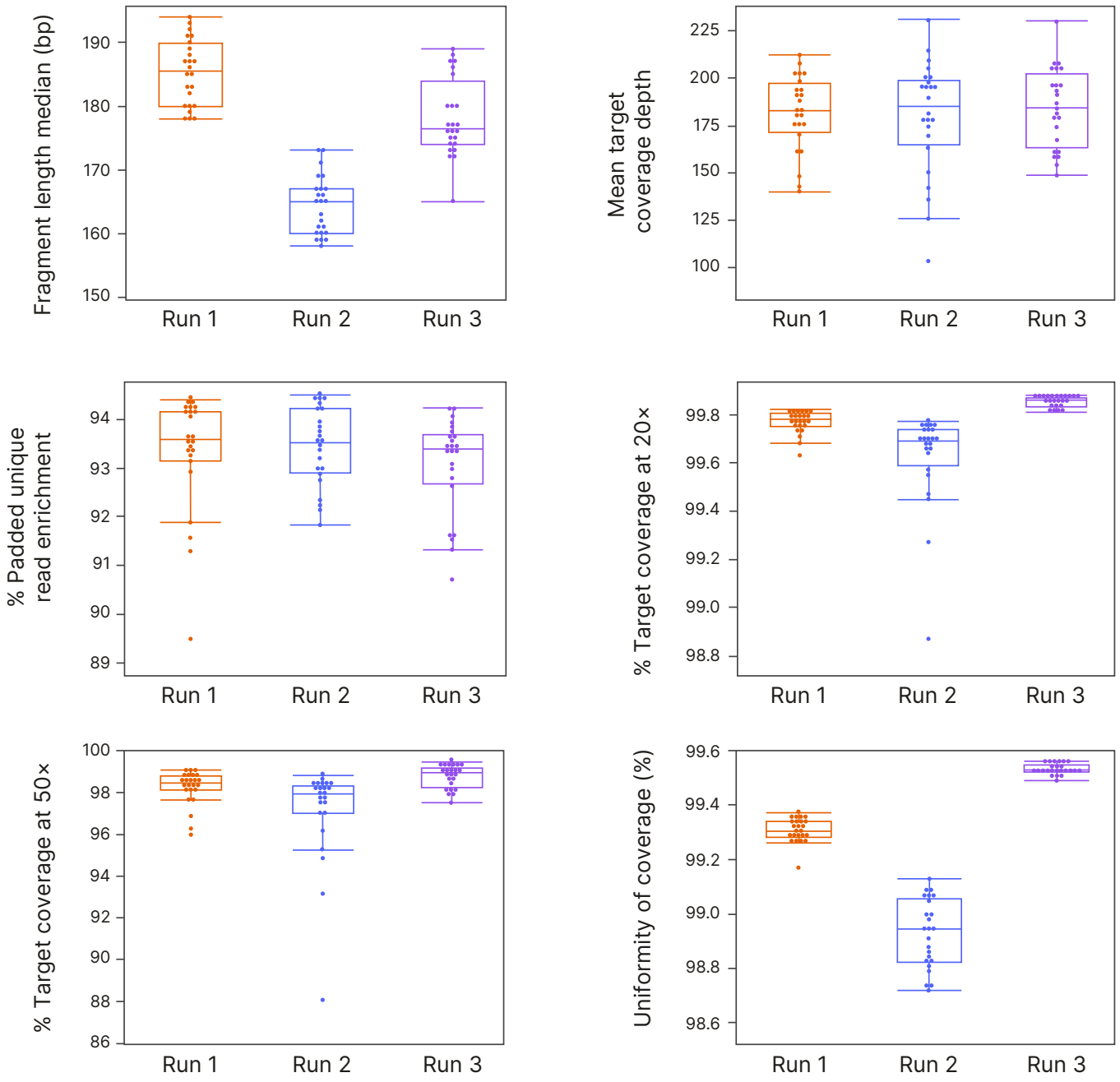

Figure 3: Within-lab precision—Sequencing metrics for 72 samples evaluated across three different runs within the same lab demonstrate high precision with all QC metrics at or above recommended guidelines.

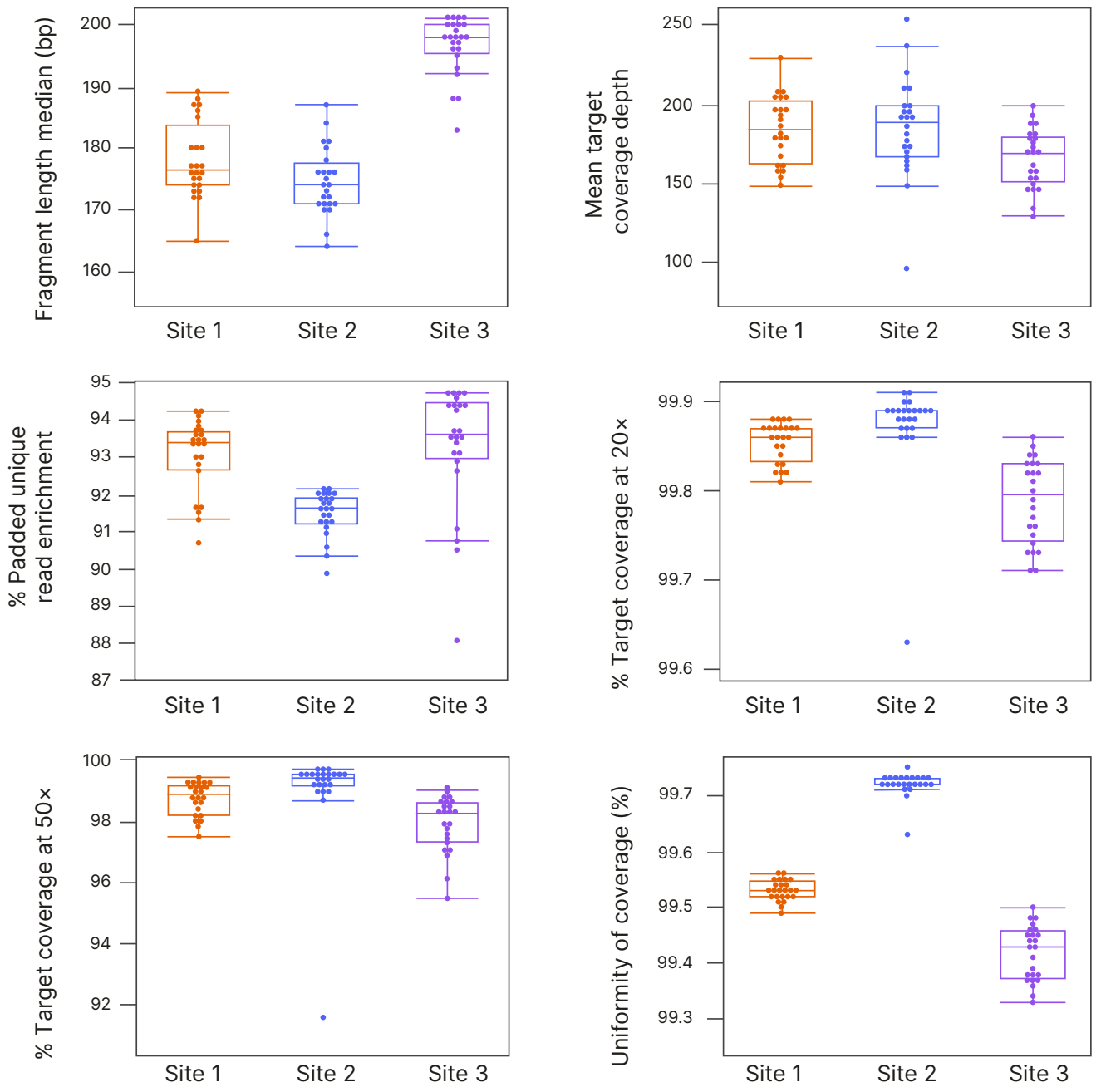

Figure 4: Reproducibility across sites—Sequencing metrics for 72 samples evaluated across three different runs at three different laboratory sites demonstrate high reproducibility with all QC metrics at or above recommended guidelines.

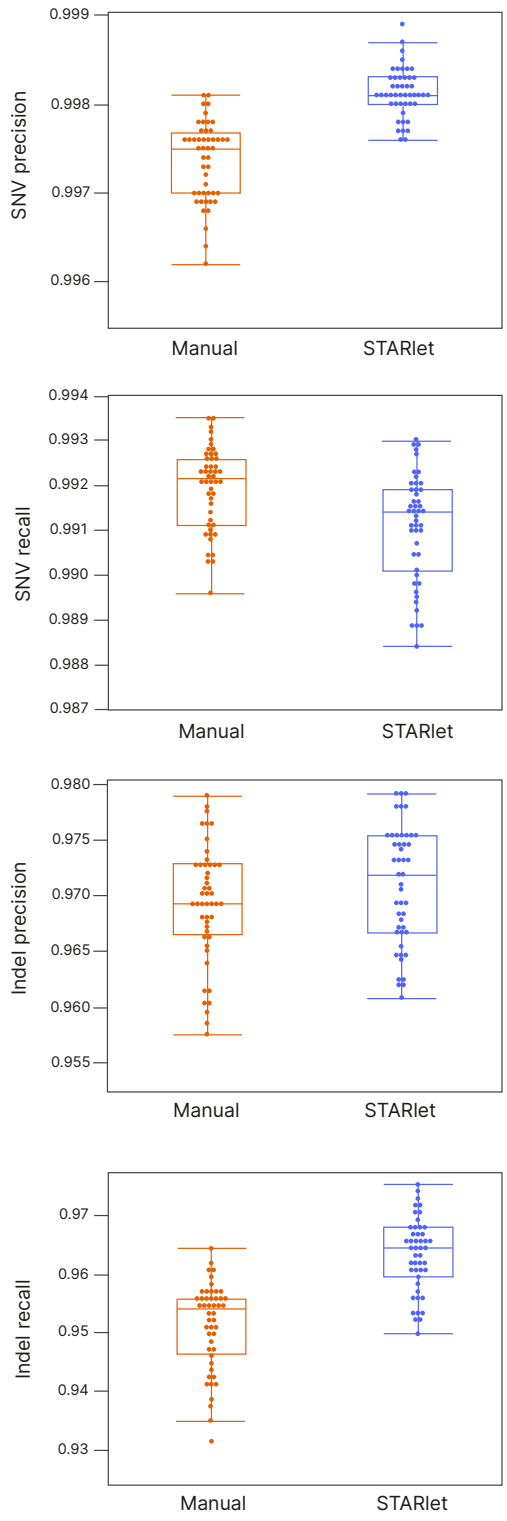

Figure 5: Concordance between manual and automated workflows—Manual and automated methods resulted in comparable performance for variant calling, as demonstrated by SNV precision, SNV recall, indel precision, and indel recall.

Table 1: Reduced hands-on time with automation

Parameter	Manual workflow[a]	Automated workflow[a]
Total time[b]	260 min	385 min
Hands-on time	105 min	80 min
Total time per library	16–22 min	16 min
Hands-on time per library	7–9 min	3.35 min

a. The manual workflow is based on a 12-sample run; the automated workflow is based on a 24-sample run.
b. Excludes hydridization time (16 hours).

Summary

Illumina DNA Prep with Exome 2.5 Enrichment is a well-designed, reliable human WES solution that is effective and efficient. Additional efficiency gains can be achieved by adopting Illumina Qualified Methods that integrate automation into the NGS workflow. This technical note demonstrates that Illumina DNA Prep with Exome 2.5 Enrichment libraries prepared with automation on the Hamilton SBS STARlet Liquid Handling Platform provide comparable performance to libraries prepared manually. Also, reproducible results are achieved with less hands-on time across multiple runs, instruments, sites, and operators when using the Illumina Qualified Method for Illumina DNA Prep with Exome 2.5 Enrichment.

Learn more

Illumina DNA Prep with Exome 2.5 Enrichment

Illumina automation solutions

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

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