

# Automation workflow for Illumina DNA Prep with Exome 2.5 Enrichment v2

Minimize errors and reduce hands-on time  
with Hamilton NGS STAR Line platforms

## Optimized automated method

Ready to deploy Illumina method  
that delivers high-quality results  
immediately

## Efficient library preparation

Streamlined workflow with  
minimal touchpoints that reduces  
opportunities for errors

## Concordant performance

High-quality, consistent library  
generation results comparable to  
manual preparation

## Introduction

Illumina DNA Prep with Exome 2.5 Enrichment v2 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 a comprehensive next-generation sequencing (NGS) workflow that spans from samples to research reporting. 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 integrated NGS workflow that features automated library preparation and enrichment for Illumina DNA Prep with Exome 2.5 Enrichment v2 (Figure 1). Two Hamilton STAR Line platforms were evaluated: the NGS STAR MOA and NGS STARlet Automated Liquid Handlers. Results demonstrate that the automated workflow performed with either platform generates consistent, high-quality data with less hands-on time, compared to the manual workflow.

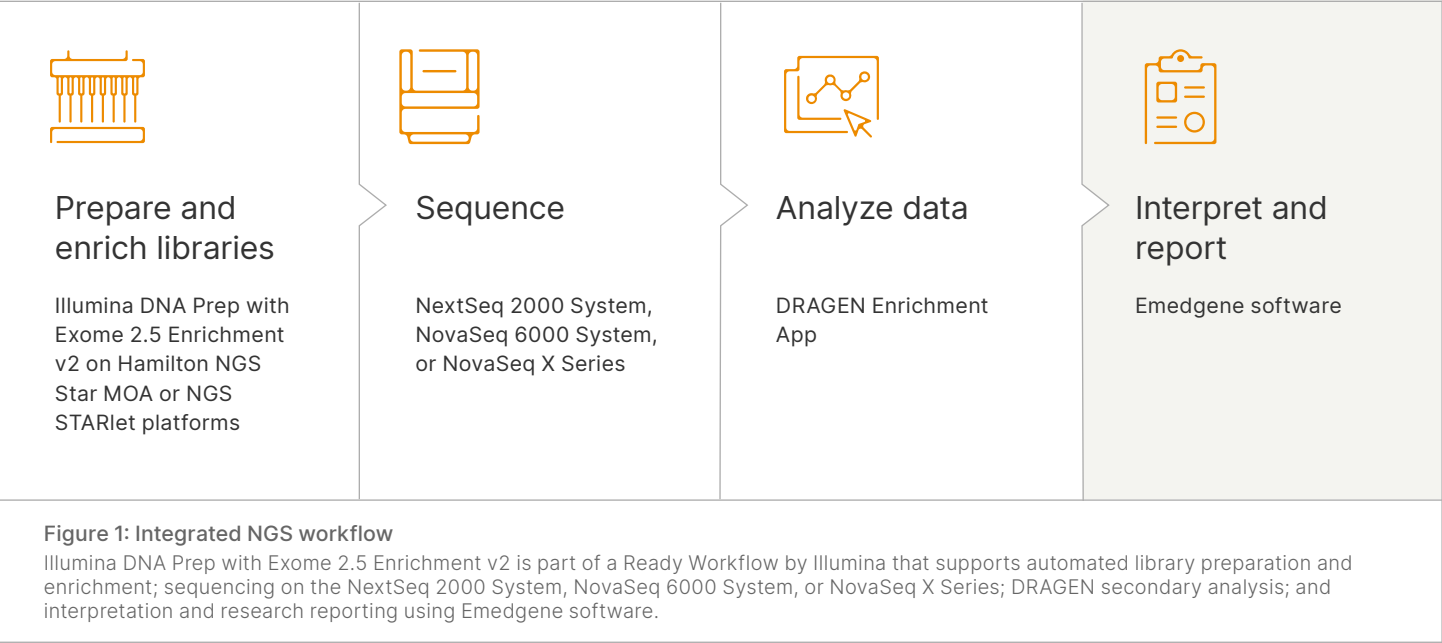
## Methods

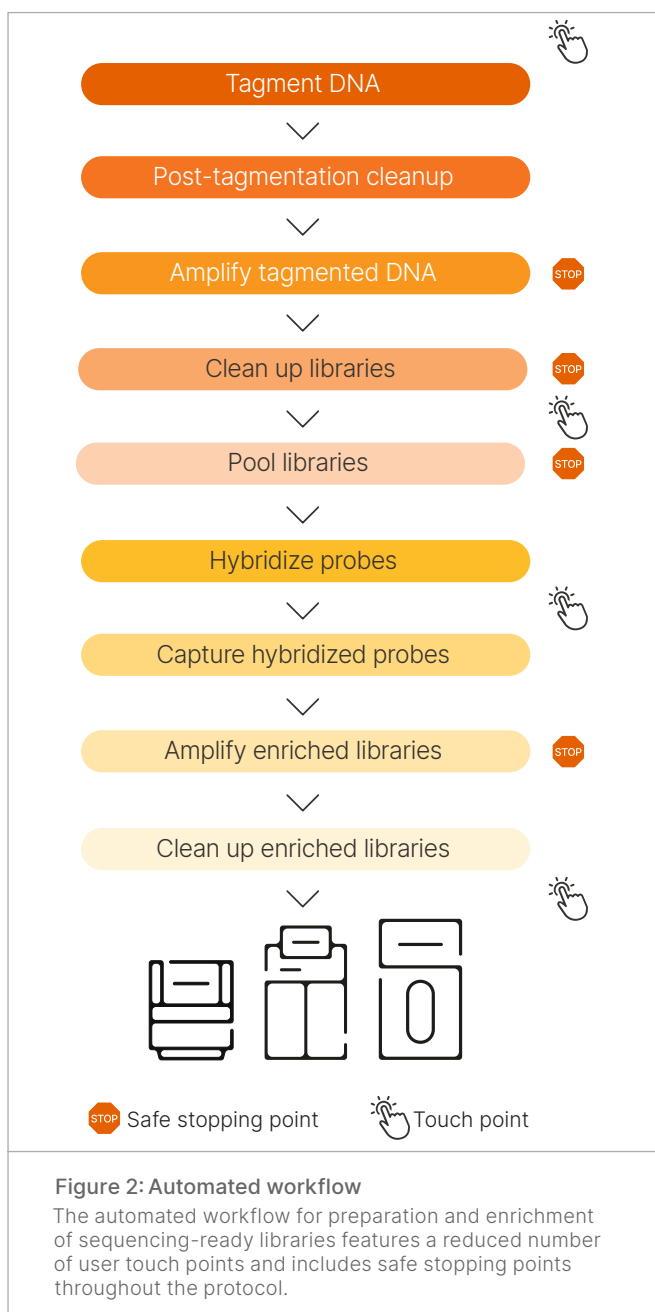
### Samples

NA12878 reference genomic DNA (gDNA) (Coriell Institute for Medical Research, Catalog no. NA12878) was obtained to evaluate the automated workflow.

### Library preparation

Library preparation and enrichment were performed using 50–1000 ng of input gDNA and Illumina DNA Prep with Exome 2.5 Enrichment v2, (S) Tagmentation Set B (96 Samples, 12-plex) (Illumina, Catalog no. 20155541). Automated library preparation and enrichment was performed on the Hamilton NGS STAR MOA Automated Liquid Handling Platform (Hamilton, Catalog no. 806800) or the Hamilton NGS STARlet Automated Liquid Handling Platform (Hamilton, Catalog no. 806691). The workflow reduces the number of user touch points and includes multiple safe stopping points (Figure 2). To compare performance, sequencing data from Illumina DNA Prep with Exome 2.5 Enrichment v2 libraries prepared with the Hamilton NGS STAR MOA and STARlet platforms were 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. 20012850) using the NextSeq 2000 P3 XLEAP-SBS™ Reagent Kit (300 cycles) (Illumina, Catalog no. 20100988) or the NovaSeq™ 6000 System (Illumina, Catalog no. 20038897) using the NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles) (Illumina, Catalog no. 20028314). 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 4.3.13 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 research reporting.

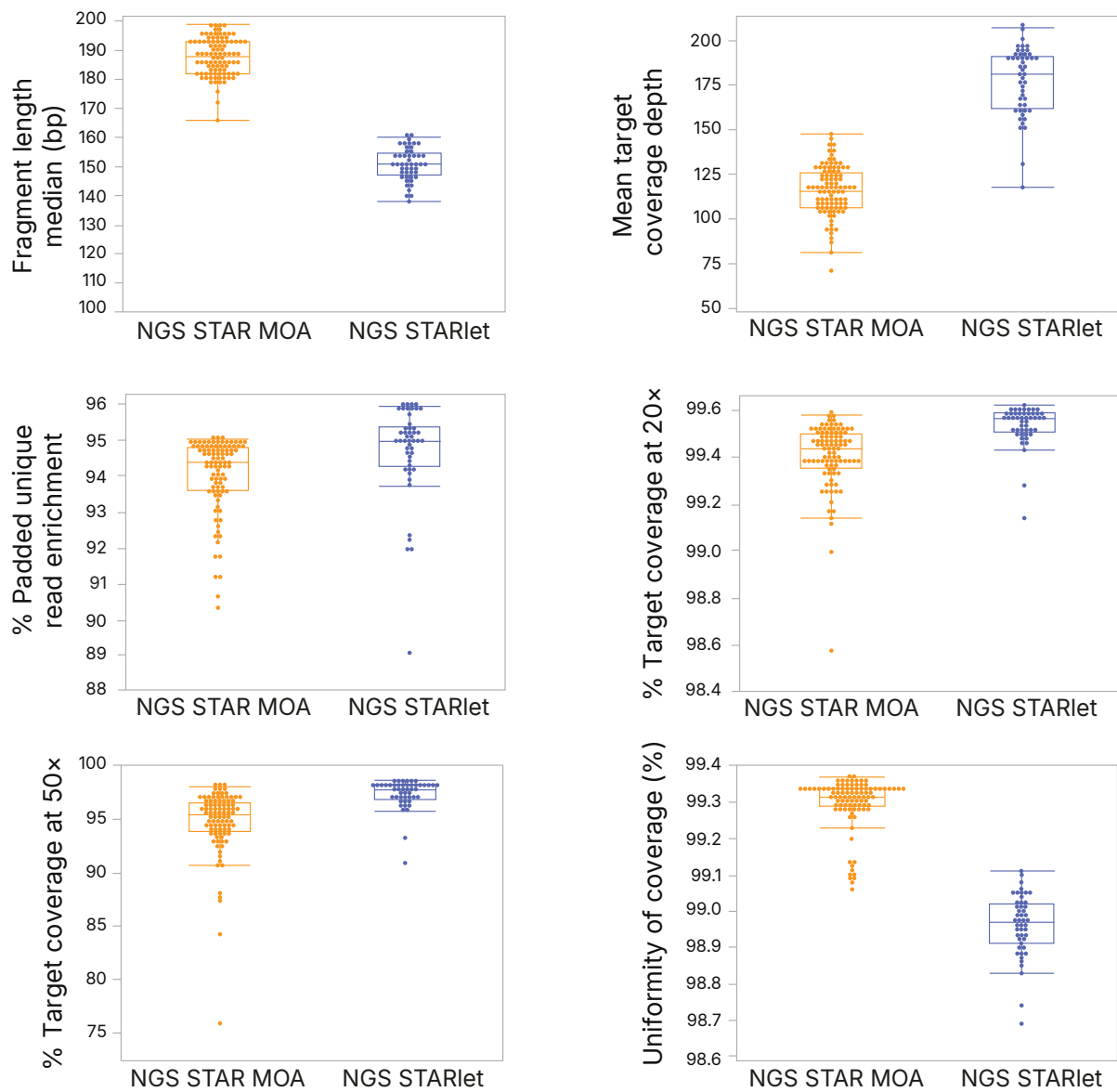
## Results

### Concordance across automation platforms

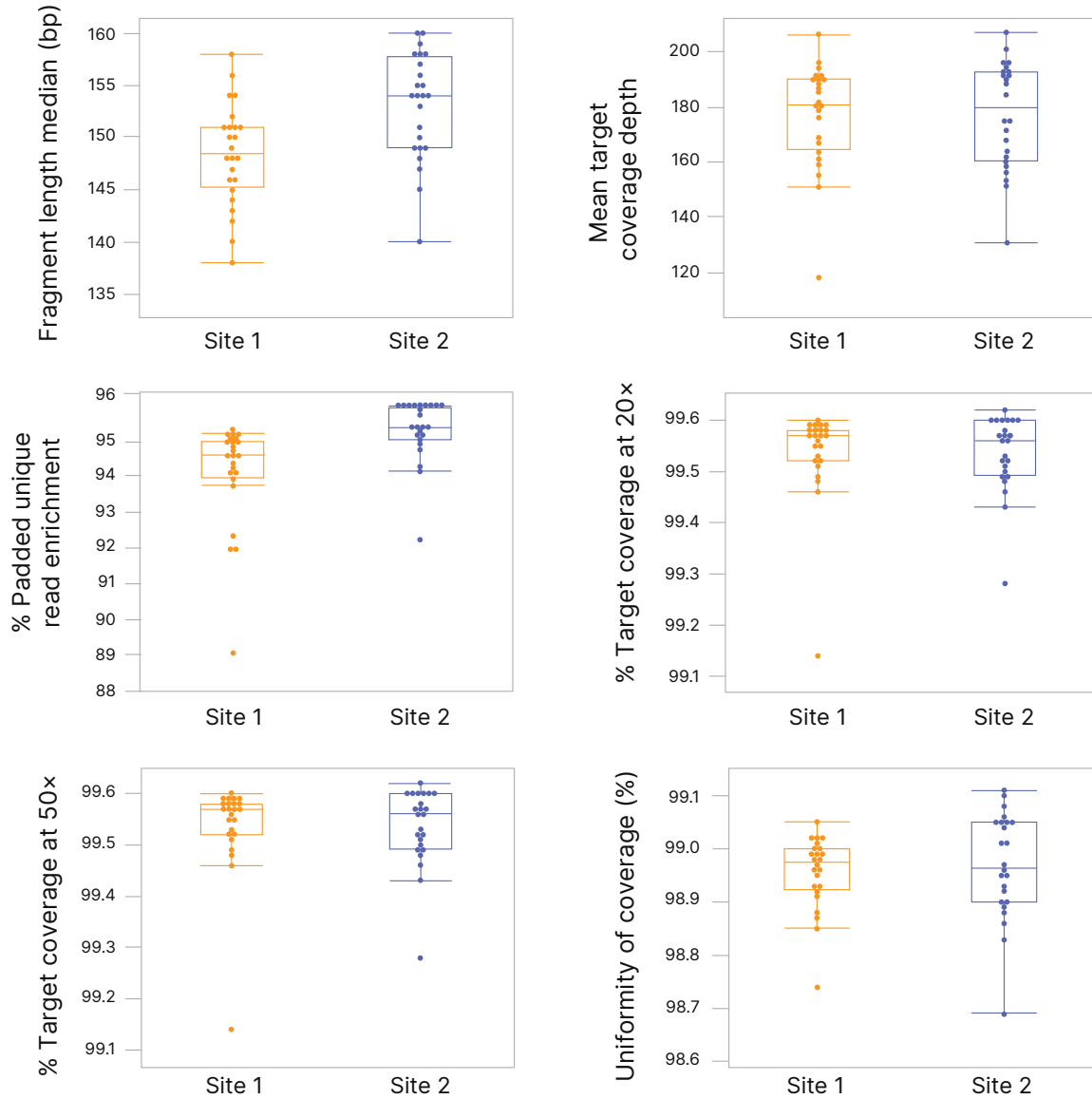
Reproducibility of results was assessed for a total of 288 samples prepared on the Hamilton NGS STAR MOA platform and 72 samples on the NGS STARlet platform. Libraries prepared on the NGS STAR MOA platform were sequenced across three sequencing runs on the same NovaSeq 6000 System by the same user. Libraries prepared on the NGS STARlet platform were sequenced across three sequencing runs on the same NextSeq 2000 System by the same user. Results show high performance with all samples prepared using both automation platforms that meet 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 across two different Hamilton NGS STARlet platforms at different laboratories with runs sequenced on separate NextSeq 2000 Sequencing Systems. All runs were conducted by the same experienced user at Site 1 (Illumina Centre, Cambridge, UK) and Site 2 (Illumina Solutions Centre, Milan, ITA). Results show that QC metrics across both 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 different NGS STARlet platforms generate reproducible results across sites and different laboratory conditions.



**Figure 3: Concordance across Hamilton STAR Line platforms**  
Sequencing metrics for triplicate runs of 96 samples evaluated on the Hamilton NGS STAR MOA platform and triplicate runs of 24 samples on the NGS STARlet platform demonstrate high reproducibility with all QC metrics at or above recommended guidelines.



**Figure 4: Reproducibility across sites**

Sequencing metrics for 48 samples evaluated across two different runs on the Hamilton NGS STARlet platform at two different laboratory sites demonstrate high reproducibility with all QC metrics at or above recommended guidelines.

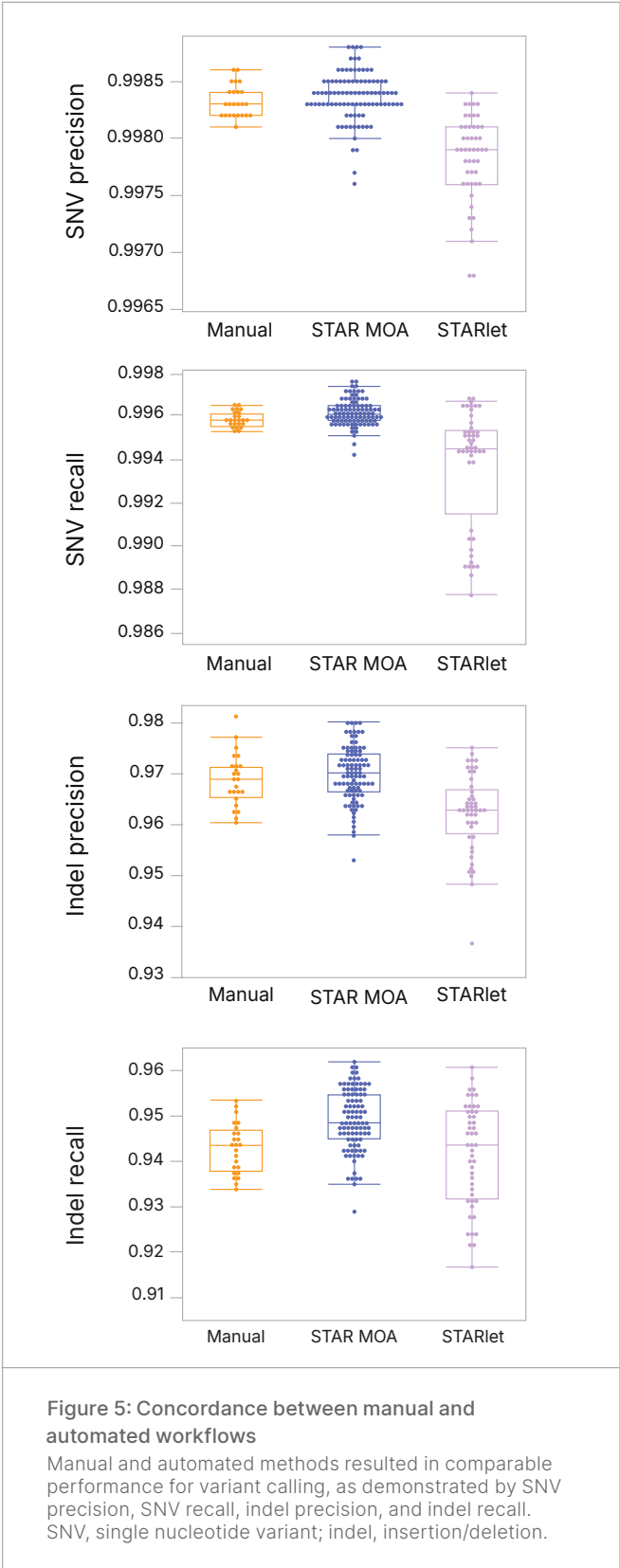
Manual versus automated performance

Variant calling was compared between the data generated using automated and manual workflows. In this study, 96 and 24 replicates of Coriell NA12878 DNA input (200 ng) were processed on the Hamilton NGS STAR MOA and NGS STARlet platforms, respectively. After sequencing, 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 variant (SNV) precision, SNV recall, insertion/deletion (indel) precision, and indel recall between the automated method using the Hamilton NGS MOA or STARlet platforms 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).

Table 1: Reduced hands-on time with automation

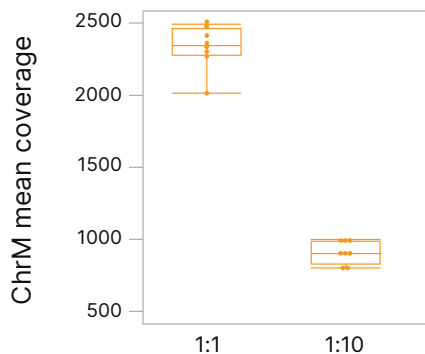
| Parameter                 | Manual workflow | Automated workflow |
|---------------------------|-----------------|--------------------|
| No. of samples per run    | 12 samples      | 24 samples         |
| Total time <sup>a</sup>   | 260 min         | 385 min            |
| Hands-on time             | 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. Excludes hybridization time (16 hours).



## Mitochondrial genome coverage

Mitochondrial genome coverage was evaluated using the Twist Bioscience for Illumina Mitochondrial Panel (Illumina, Catalog No. 20093180) as a spike-in on the Hamilton NGS STAR MOA platform. The optional custom panel feature of the automated method was enabled. Eight samples were spiked with a 1:1 dilution, and eight samples were spiked with a 1:10 dilution. Results show good performance of the Twist Bioscience for Illumina Mitochondrial Panel with high mitochondrial genome coverage (Figure 6).



**Figure 6: High mitochondrial genome coverage**

Mitochondrial genome coverage was assessed for eight samples run on the Hamilton NGS STAR MOA platform at 1:1 and 1:10 dilutions. Results show high mitochondrial genome (ChrM) coverage.

## Summary

Illumina DNA Prep with Exome 2.5 Enrichment v2 is a well-designed, reliable human WES solution. Additional efficiency gains can be achieved by adopting Illumina Partner Network and Illumina Ready Methods that integrate automation into the NGS workflow. This technical note demonstrates that Illumina DNA Prep with Exome 2.5 Enrichment v2 libraries prepared with automation on the Hamilton NGS STAR MOA and NGS STARlet Liquid Handling Platforms provide comparable performance to libraries prepared manually while significantly reducing hands-on time across runs, instruments, and operators.

**Learn more →**

[Illumina DNA Prep with Exome 2.5 Enrichment v2](#)

[Illumina automation solutions](#)



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