Application Note

Next Generation Sequencing

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An Automated Workflow for DNA Library Preparation and Target Enrichment Using Agilent SureSelect Max and the SPT Labtech firefly+ Platform

Abstract

In this study, we present NGS results obtained with the Element Biosciences AVITI system employing an automated workflow for DNA library preparation, with target enrichment achieved using Agilent SureSelect Max chemistry on the SPT Labtech firefly+ platform—a compact system integrating liquid handling and on-deck thermocycling. This flexible approach supports sequencing across multiple platforms, including Illumina, and long-read systems such as ONT and PacBio. The combination of streamlined chemistry and integrated automation enables a walkaway workflow with reduced hands-on time and improved reproducibility.

Introduction

High-quality next-generation sequencing (NGS) data depends on robust and reproducible library preparation workflows. Target enrichment plays a critical role in these workflows by selectively capturing genomic regions of interest, enabling deeper sequencing coverage while reducing overall sequencing burden. This targeted approach improves variant detection sensitivity and significantly lowers sequencing, data storage, and downstream analysis costs.

Automation further enhances reproducibility and throughput by minimizing manual variability and enabling standardized workflows across laboratories. Scalable automation platforms are particularly valuable for high-throughput environments where consistency and operational efficiency are essential.

The Agilent SureSelect Max system incorporates an accelerated hybridization chemistry that reduces workflow time while maintaining high capture efficiency and uniformity. Its modular reagent design enables flexible workflow configuration, allowing users to tailor library preparation, enrichment strategies, and sequencing platform compatibility according to experimental and operational requirements.

This application details the use of SureSelect Max for automated DNA library preparation and target enrichment using the firefly+ automation platform.

Experimental

DNA library preparation with SureSelect Max on the firefly+ system

The firefly+ protocols automate key steps in both library preparation and target enrichment based on Agilent SureSelect Max reference protocols.1 Integration of liquid handling and on-deck thermocycling enables uninterrupted execution of complex workflows in order to reduce manual intervention and potential sources of variability. The system supports:

- Flexible throughput (1–12 columns per run)
- 96-well plate format
- Both pre-capture and post-capture pooling strategies
- Simultaneous processing of multiple enrichment panels under uniform conditions

The firefly+ protocol (SureSelect Max DNA library preparation) automates the following library preparation workflow steps, after which an optional quality control (QC) can be performed:

- Fragmenting, end-repair, and 3'-dA-tailing of the DNA (Frag/A-tail)
- Ligating the adaptor
- Purifying libraries using magnetic purification beads
- Amplifying and indexing the libraries
- Purifying amplified libraries using magnetic purification beads

Target enrichment with SureSelect Max on the firefly+ system

Following library preparation, target enrichment was performed on firefly+ with SureSelect Max target enrichment parts 1 and 2. Target enrichment using SureSelect Max enables:

- High on-target read rates, improving sequencing efficiency
- Deeper coverage of regions of interest, enhancing sensitivity for low-frequency variants
- Reduced sequencing depth requirements, lowering cost and data burden
- Improved uniformity, minimizing coverage bias

The workflow is divided into two automated parts: (1) hybridization and capture and (2) post-capture amplification and cleanup. This modular workflow structure aligns with automation, enabling optimization of individual steps without affecting the entire process. The firefly+ protocols used here support both the post-capture pooling workflow and the pre-capture pooling workflow.

SureSelect Max target enrichment part 1: Automating hybridization and capture

Table 1. firefly+ protocol names and run times.

Protocol Name	firefly+ Run Times
SureSelect Max DNA library preparation	3 hours 50 minutes (96-sample run)
SureSelect Max target enrichment part 1	4 hours 35 minutes (8-pool run)
SureSelect Max target enrichment part 2	1 hour 12 minutes (8-pool run)

The first firefly+ target enrichment protocol automates the following target enrichment workflow steps:

- Hybridizing libraries to the SureSelect probe
- Preparing beads and buffers for capture
- Capturing the hybridized libraries

SureSelect Max target enrichment part 2: Automating post-capture amplification and cleanup

The second firefly+ target enrichment protocol automates the following post-capture amplification and cleanup steps:

- Amplifying the captured libraries
- Purifying the final libraries using magnetic purification beads QC

Hybridization was performed at 66 ºC; captured libraries were amplified by 10 cycles of post-capture PCR. Single captures (1-plex) and multiplex captures were tested with Agilent SureSelect XT HS Human All Exon V8 (Exome V8) and Agilent SureSelect Cancer CGP panels. Exome single captures (3x NA12878 and 3x NA24385 libraries) and CGP single captures (7x NA12878 and 7x NA24385 libraries) were performed with 1,000 ng library input into each 1-plex capture.

Multiplex captures were tested in duplicate. For multiplex captures, pre-capture pools were created with equal number of NA12878 and NA24385 libraries, with 4,000 ng total library input into each pre-capture pool. An 8-plex capture was tested with Exome V8 and a 16-plex capture was tested with SureSelect Cancer CGP.

Following the target enrichment runs on firefly+, the libraries were analyzed on the Fragment Analyzer system (Agilent, DNF-474 HS NGS Fragment Kit) and qPCR (LightCycler 480 System - Roche, KAPA Library Quantification kit) to determine sizing and concentration, respectively.

SureSelect Max DNA library preparation with enzymatic fragmentation

A 96-sample SureSelect Max DNA library preparation with enzymatic fragmentation run performed on firefly+ included 90 high-quality genomic DNA samples—45 replicates of Coriell NA12878 and 45 replicates of Coriell NA24385. Two hundred ng was used as the starting input amount. Six no-template controls (NTCs) were also included and spread across the plate.

The run included a 10-minute enzymatic fragmentation time and seven cycles of library amplification, with a run time of 3 hours and 50 minutes. The resulting libraries were then diluted 1:10 and analyzed on the Agilent Fragment Analyzer system (Agilent, DNF-474 HS NGS Fragment Kit).

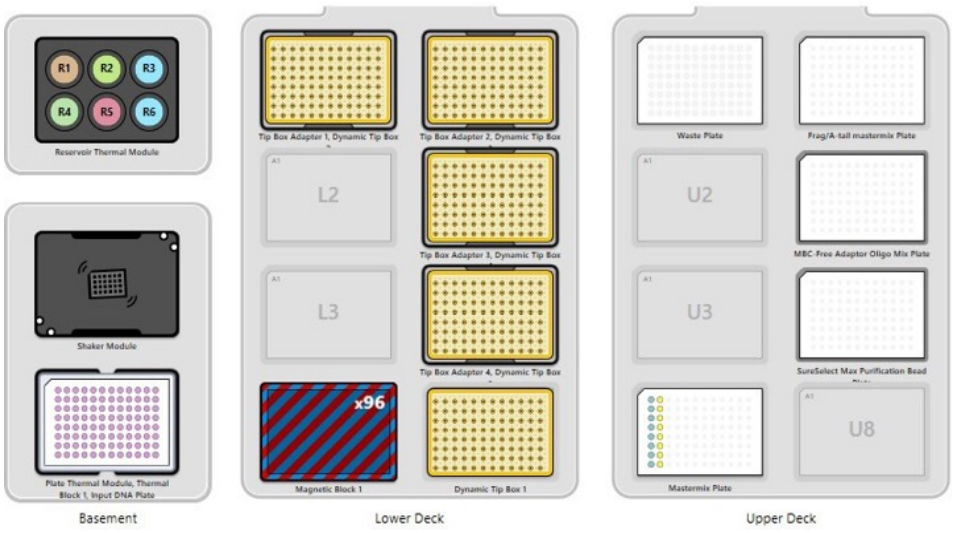

Figure 1. firefly starting deck layout for a 96-sample library preparation run. Deck layout – basement: reservoirs R1 – SureSelect Max purification beads, R2 – nuclease-free water, R3+R6 – 70% ethanol, R4 – amplification master mix, R5 – ligation master mix, plate thermal module – 96-sample thermo adapter block and input DNA plate. Lower deck: L1, L5, L6, L7 – 100 µL 96-format ATL tips on ATL 35–125 µL tip stands, L8 – 100 µL 96-format ATL tips, L4 – Alpaqua Magnum FLX 96 sample magnetic block. Upper deck: U1 – waste plate, U4 mastermix plate, U5 frag/Atail mastermix plate, U6 – MBC-free adapter oligo mix plate, U7 – SureSelect Max purification bead plate.

Results and discussion

Across the 90 DNA input samples, the Fragment Analyzer reported an average size of 341 base pairs (bp) for a region of 100 to 800 bp, with a coefficient of variation (CV) across the average sizes of 3.0%.

The resulting libraries were quantified by qPCR to determine their size-adjusted concentration (nM) using a LightCycler 480 System (Roche, KAPA Library Quantification kit). The average concentration across the plate was 4490 nM with a CV of 9.5%. No significant contamination was observed in the six NTCs; the average quantification value of 0.19 nM is just 0.0042% of the average quantification value of the positive samples.

Sequencing of the target-enriched libraries prepared on the firefly+ was performed using the AVITI Cloudbreak Freestyle sequencing kit (Element Biosciences). Table 2 and Figures 2, 3 and 4 summarize the sequencing data.

Data was down-sampled to 6 Gb (40 M 2x150 bp) for SureSelect Human All Exon [AT3.1]V8 and 0.45 Gb (3 M 2x150 bp) for CGP analysis. PCT_ SELECTED_BASES, PCT_EXC_DUPE, MEDIAN_TARGET_ COVERAGE[AT4.1], and Fold80 are reported from analysis with Picard HS Metrics (Broad Institute).

Vardict (version 1.8.3) was used for variant calling. Recall/sensitivity and precision are reported for GIAB [AT6.1] high-confidence variants in regions targeted by SureSelect exome V8. Exome V8 target regions intersected with HG001 or NA12878 cover 18,426 total variants (18,060 SNPs and 366 indels). Exome V8 target regions intersected with HG002 or NA24385 cover 18,545 total variants (18,190 SNPs and 355 indels). Recall = true positive / (true positive + false negative) and precision = true positive / (true positive + false positive).

Table 2. Key capture metrics.

Description	SSEL V8 1-Plex	SSEL V8 8-Plex	CGP 1-Plex	CGP 16-Plex
PCT_SELECTED_BASES	86.1%	87.0%	82.4%	82.7%
PCT_EXC_DUPE	4.7%	8.6%	2.0%	5.1%
MEDIAN_TARGET_COVERAGE	51	50	61	58
FOLD_80_BASE_PENALTY	1.51	1.48	1.48	1.49
Percentage of targeted bases covered by:				
...at least 10 reads	97.5%	97.5%	98.9%	98.8%
...at least 20 reads	96.2%	96.3%	97.9%	97.7%
...at least 30 reads	93.4%	93.6%	96.1%	95.7%
...at least 40 reads	88.8%	88.8%	93.1%	92.4%

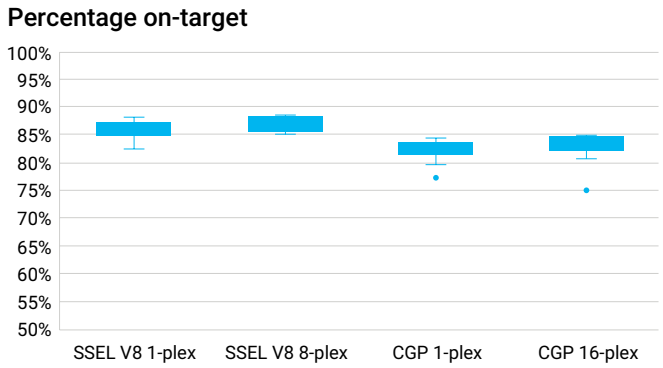

Figure 2. Percent on-target.

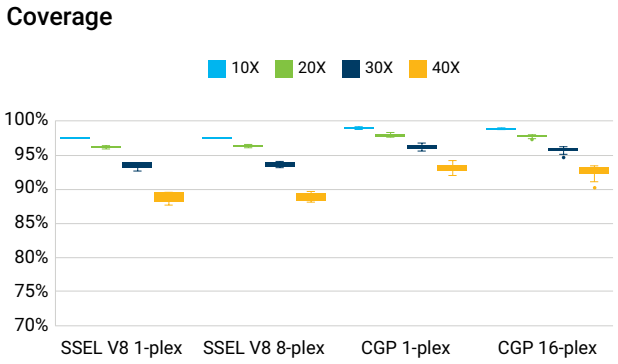

Figure 3. Base coverage at 10X, 20X, 30X, and 40X.

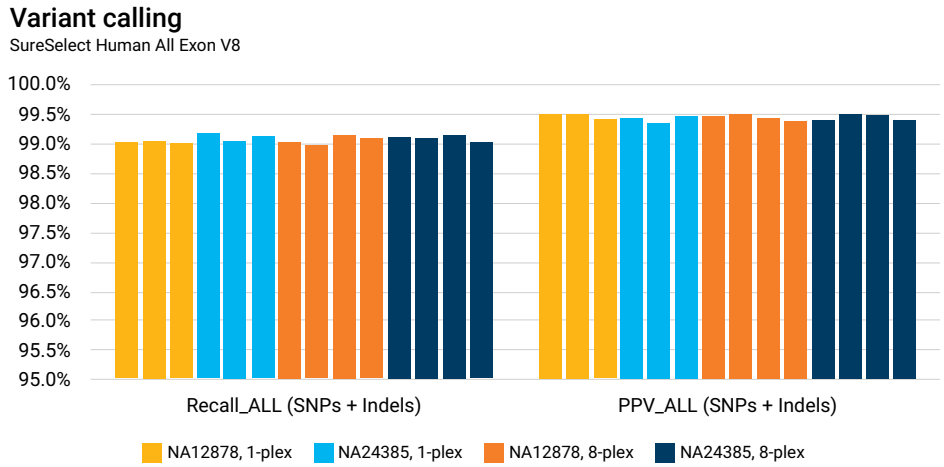

Figure 4. ≥99% recall precision for all samples in single (1-plex) and multiplex (8-plex) captures.

Conclusions

This study demonstrates that SureSelect Max DNA library preparation and target enrichment workflows can be effectively automated on the firefly+ platform to generate high-quality, reproducible sequencing libraries at scale.

Pairing SureSelect Max chemistry with firefly+ automation allows integration of liquid handling and thermocycling in a single platform, thereby reducing hands-on time, and improving reproducibility across runs and operators. Automation enabled consistent performance across a 96-sample format, with minimal variability and no detectable contamination, highlighting the robustness of the integrated workflow. Target enrichment using SureSelect Max delivered high on-target rates and uniform coverage, supporting accurate and sensitive variant detection while reducing sequencing requirements. These benefits translate into improved cost efficiency and streamlined downstream analysis.

This approach provides a scalable and flexible walkaway solution for high-throughput NGS applications, particularly where consistent performance, efficient target enrichment, and workflow standardization are critical. The modular design of the SureSelect Max system enables flexible workflow configuration, compatibility with multiple sequencing platforms, and support for both pre- and post-capture pooling strategies. This adaptability allows laboratories to optimize workflows based on throughput, sample type, and experimental goals without compromising performance.

References

1. Protocol: Agilent SureSelect Max Target Enrichment Using Fast Hybridization for NGS on the Illumina Platform (Agilent publication G9660-90000, Version A0) September 2024.

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