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A Modular Workflow with Minimal QC Steps Provides Improved Capture Performance for Intact gDNA/total RNA as well as Low Input FFPE

Nikki Liu[2], Adam Janssen[1], Mike Borns[1], Sergey Shiryaev[1], Keith Chen[1], Bilan Hsue[1], Brandyn Clark[1], Jeff Fox[1], Nedda Saremi[2], Inlora Jingga[2], Kyeong-soo Jeong[2], Khine Win[2], Gilbert Amparo[2], Neelima Mehendale[2], Jayati Ghosh[2], Cindy Hon[2], Bahram Arezi[1]

[1]Agilent Technologies, La Jolla, CA

[2]Agilent Technologies, Santa Clara, CA

Abstract

The limited availability of genomic DNA as well as its poor quality and variation in tumor fraction from sample to sample, can influence how readily NGS is applied to tumor samples. Furthermore, practical considerations such as turnaround time, workflow simplicity, pre-capture pooling capability and performance are important factors on whether NGS would be the method of choice for germline mutation detection.

Here we address these challenges by developing a modular and flexible workflow solution which is optimized for a wide range of DNA and RNA input (10–200 ng), various sample types (intact, FFPE, and cfDNA samples), standard and molecular-barcoded adaptors (dsMBC or ssMBC), different shearing methods (mechanical vs. enzymatic), sequencing chemistries (2 x 100 bp, 2 x 150 bp, or 2X250bp), optional pre-capture QC requirement, fast or overnight bait hybridization, and up to 384 unique dual sample indices to eliminate index hopping. Our enzymatic library preparation method combines fragmentation, end repair, and dA-tailing in a single step using a high volume of 50ul. This approach allows FFPE DNA samples with low concentrations to be utilized without the need for vacuum concentration, with an input capacity of up to 40 µl. As we will demonstrate, fragmentation and library preparation experiments using three bacteria of varying GC content (30%-67%) shows high coverage uniformity and minimal bias across all GC content (Fold 80 of 1.12-1.13). Optional molecular barcoding at the ligation step allows PCR errors to be filtered out by making consensus calls using MBC information from both strands (duplex MBC), hybrid (duplex MBC where info is available), single strand (single MBC) or discard MBC information (no MBC), based on the application. While both duplex and single MBC improve the accuracy of low VAF detection compared to no MBC, duplex MBC enables the most effective error correction. Finally, our novel fast hybridization buffer formulation provides maximum complexity (3-5% duplicates at 6Gb for 8 pooled exome V8 libraries) while maintaining high uniformity (Fold 80 of 1.4) and coverage (96% at 30X base coverage), specifically to address the issue of complexity loss when multiple libraries are pooled during bait hybridization.

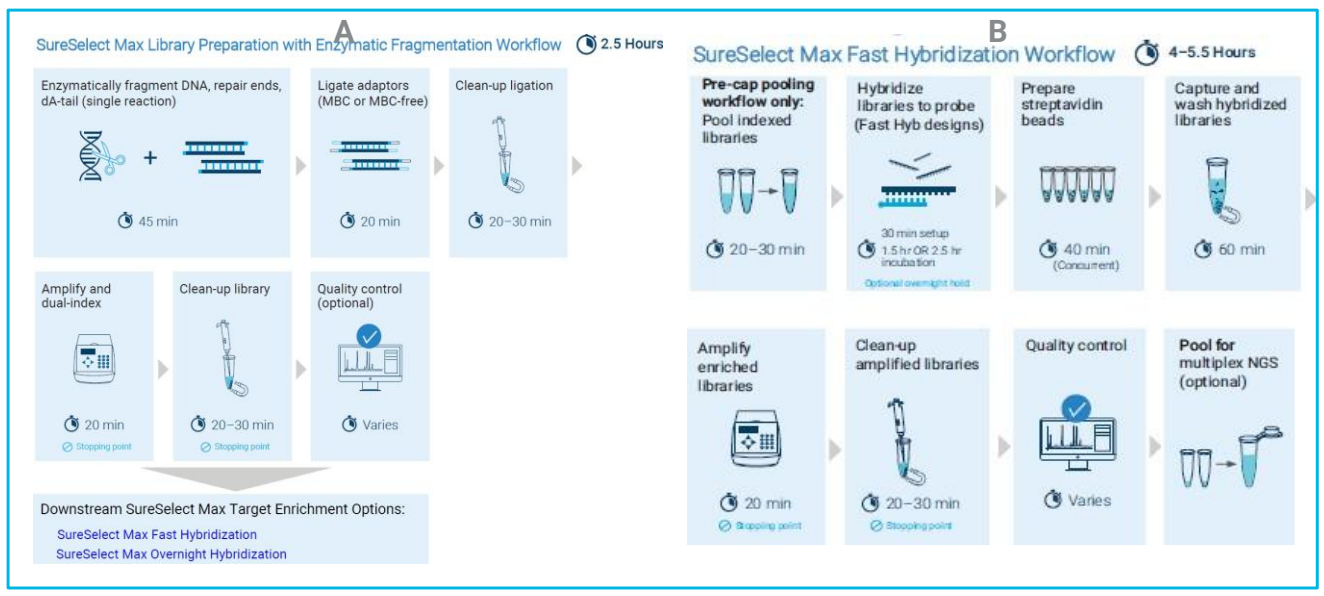

Figure 1. SureSelect Max Workflow

(A) SureSelect Max One-step Enzymatic Library Prep workflow with inputs from 10-200ng using gDNA. The streamlined workflow of one-step enzymatic fragmentation/end repair/dA-tailing, coupled with shorter ligation incubation and reduced PCR cycles, enables library preparation to be completed in under 2.5 hours.(B) SureSelect Max Fast Hyb module (new formulation that maintains higher complexity) is compatible with individual or pre-cap pooling up to 8 libraries for large baits (e.g., exome V8) or 16 libraries for smaller baits. The workflow is optimized to allow skipping of pre-cap QC. as well as extending the 1-day workflow to 2 days, if desired

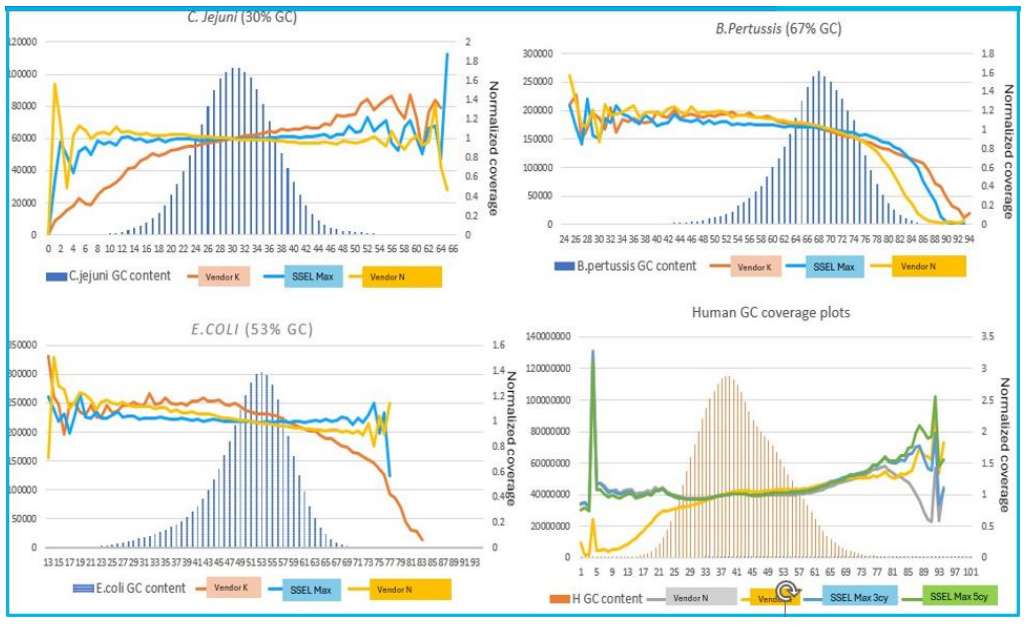

Figure 2. Uniform GC Coverage using SureSelect Max WGS with minimal PCR cycles on 3 different microbial as well as human gDNA with varying GC content.

Agilent's SureSelect Max WGS libraries were prepared in conjunction with library preps using vendors K and N. SureSelect Max shows higher coverage in high AT regions with *C. jejuni (30%GC)*/human genome and high GC regions in *E.coli (53%GC)* in comparison to vendor K. SureSelect Max outperformed vendor N at high GC regions for *B pertussis (67%GC).* Libraries were sequenced on Novaseq 6000 and reads were normalized to 310 million (2X150bp)

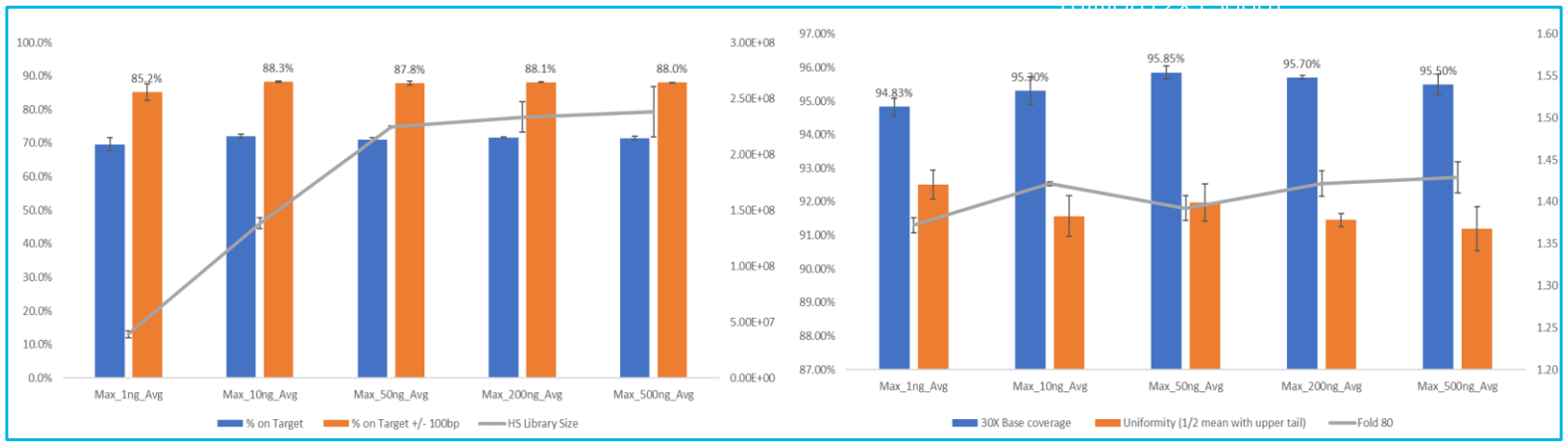

Figure 3. Robustness of SureSelect Max enrichment beyond the recommended input range.

The library preparation and target enrichment was evaluated with two replicates across various inputs from 1ng to 500ng (recommended input is from 10-200ng). % On Target +/-100bp for all samples are above 85%, with fold 80 below 1.45, for input amounts ranging from 1ng to 500ng of intact gDNA samples.The SureSelect Max Enzymatic Library Prep was employed, with Max Fast Hyb buffer using the Agilent XTHS V8 bait panel. Libraries were sequenced on Novaseq 6000 and reads were normalized to 40 million (2X150bp).

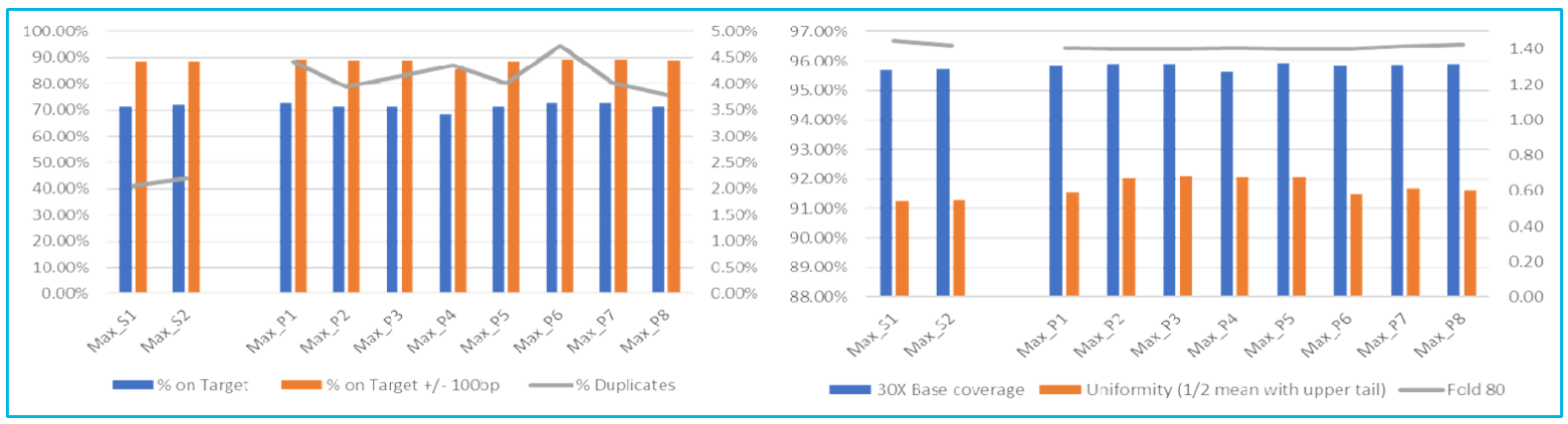

Figure 4. Reproducible performance across individual and pre-cap pooled libraries.

Library preps were performed using SureSelect Max Enzymatic library prep/fast hyb with Agilent exome V8 for both individual and pre-cap pooling samples. Two individual captures (12ul pond each, no Tapestation QC) and eight pre-cap libraries (total of 4000ng pond) were enriched with exome V8 and sequenced on NovaSeq 6000 at 2x150 condition. Reads were normalized to 40 million for analysis.

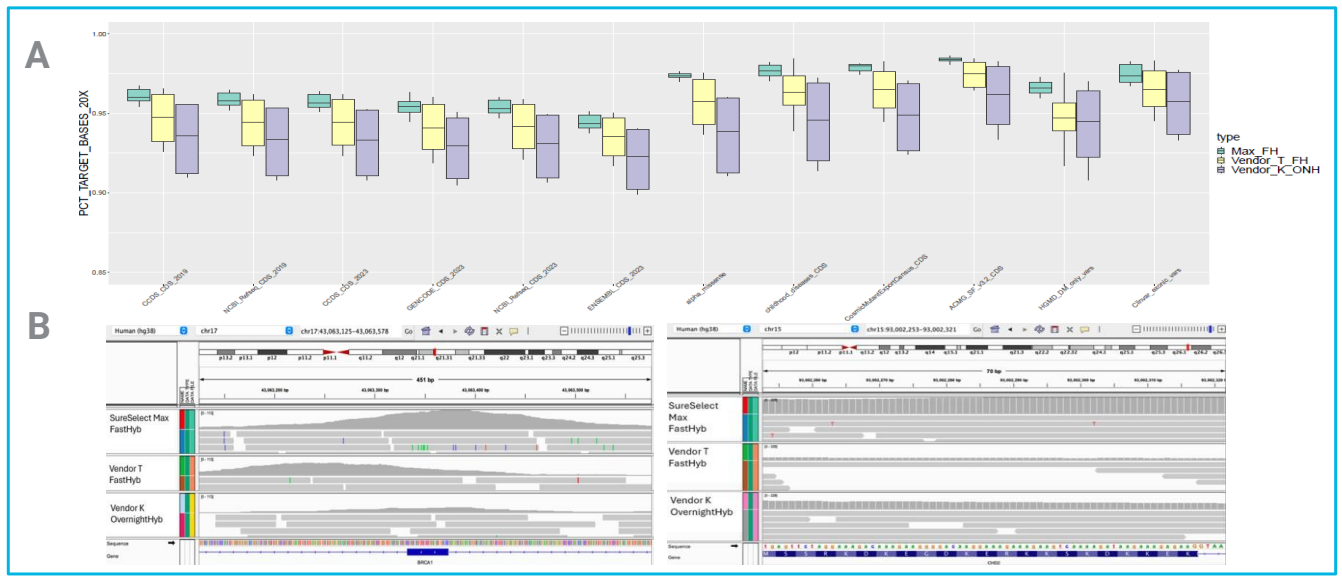

Figure 5. SureSelect Max shows highest 20X Base Coverage Against Other Vendors Mapping to Various Database

(A) SureSelect Max Library Prep with Fast Hyb shows the highest coverage for all CDS and potential clinically relevant databases with the lowest variability across tested samples when compared to vendor T Fast Hyb and vendor K Overnight Hyb. (B) IGV plots of SureSelect Max samples showing the highest coverage in important exonic regions (BRCA1 and CHD2) versus vendors T and K.

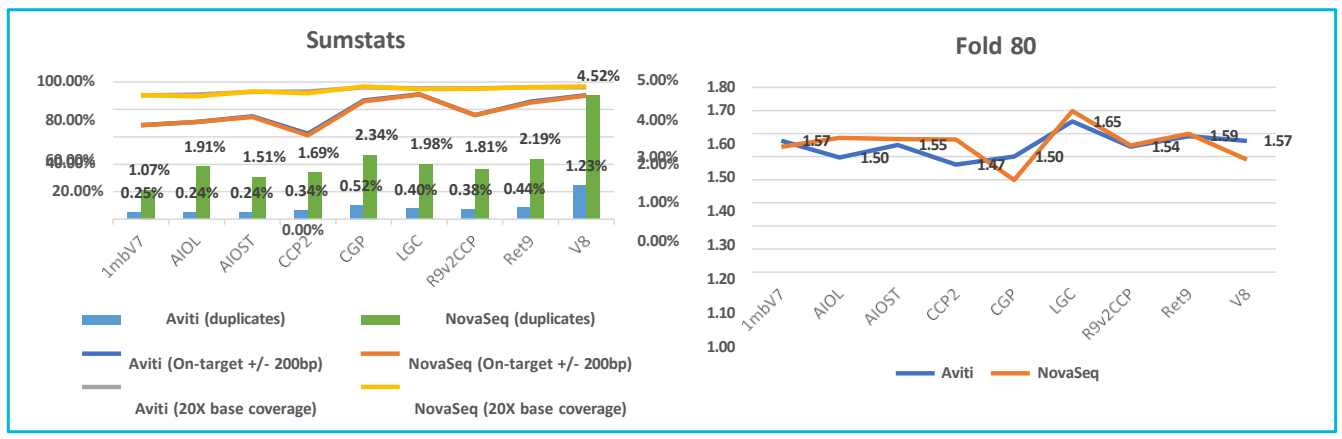

Figure 6. Compatibility of SureSelect Max library prep with Element Aviti Cloudbreak freestyle sequencing.

SureSelect Max library prep and target enrichment is compatible with the Aviti sequencer without requiring additional conversion steps. We prepared libraries using Max Enzymatic Library Prep and enriched using Max Fast hyb buffer and nine bait panels of varying GC content and sizes. Libraries were sequenced on both Illumina NovaSeq 6000 and Element Aviti Cloudbreak. Reads were normalized to 40 million reads for sumstats comparisons.

Conclusions

- SureSelect Max generates higher library complexity, deeper and more uniform coverage in both pre-cap and post cap pooling (New library prep and fast hyb chemistry)
- SureSelect Max provides a more streamlined one-step enzymatic library prep with higher fragmentation volume (50ul), and optional pre-cap QC step
- SureSelect Max is fully modularized and automation compatible, supporting both DNA and RNA, as well as low cycle WGS (for FFPE and cfDNA)

https://www.agilent.com/en/product/next-generation-sequencing

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