Streamlined and Flexible Workflow Solution Provides Improved Coverage Uniformity and High Library Complexity from Genomic DNA/Total RNA.

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Abstract

The application of NGS-based assays can be hindered by limited availability and poor quality of genomic DNA from certain sample sources. Additionally, practical factors such as turnaround time, workflow simplicity, target enrichment capability, and overall performance play crucial roles in determining whether NGS is the preferred method for conducting genotyping studies. Here we address these challenges by developing a flexible and automation-compatible workflow solution which enables WGS (PCR-free and plus PCR based on the application) and target enrichment sequencing using a single library prep. Our workflow is modularized to support a wide range of DNA and RNA input (10–500 ng), standard and in-line molecular-barcoded adaptors, enzymatic and mechanical shearing methods, sequencing chemistries (2 x 100 bp, 2 x 150 bp, or 2X250bp), fast or overnight probe 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 and allows samples with low concentrations to be utilized. We will demonstrate, using three bacteria of varying GC content (30%-67%) and human gDNA, high base coverage uniformity and minimal bias across all GC content (Fold 80 of 1.12-1.13). Finally, our novel fast hybridization buffer formulation provides maximum complexity while maintaining high uniformity and base coverage, specifically to address the issue of complexity loss when multiple libraries are pooled during bait hybridization.

Workflow Overview

Figure 1. SureSelect Max workflow

(A) SSEL Max One-step Enzymatic Library prep workflow supports inputs from 10-500ng using DNA/RNA. To allow maximum flexibility, the kit has been designed in modular format to process mechanically (not shown) or enzymatically sheared DNA, total RNA, and mRNA in either a manual or automated setting. Modules for both MBC and MBC-free adaptors can be selected based on downstream sequencing applications. Options for both WGS+PCR and WGS PCR-free workflows will soon be available.

(B) SSEL Max Fast Hyb module (optional for target enrichment) is reformulated to better maintain library complexity during hybridization and 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 can be pool minimize hands on time and cost. The workflow is also optimized to allow skipping of pre-cap QC, as well as extending the 1-day workflow to 2 days, if desired.

Uniform GC Coverage using SSEL Max WGS across full range of GC content

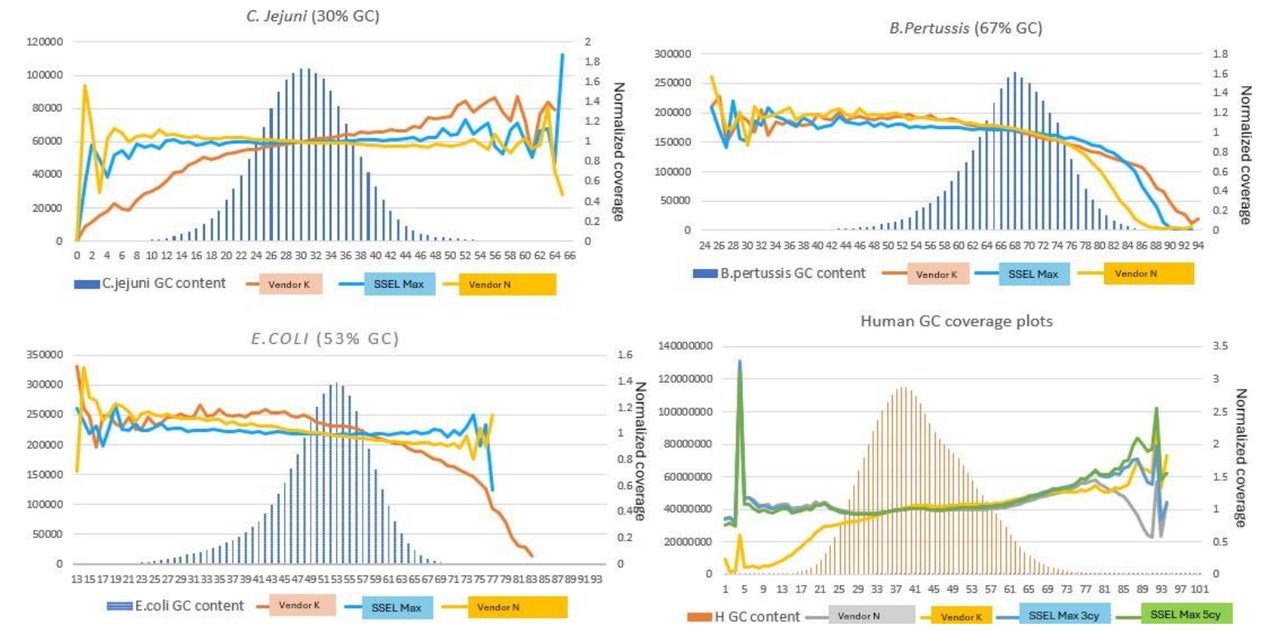

Figure 2. Agilent SureSelect Max with enzymatic fragmentation WGS libraries were prepared in conjunction with enzymatic library prep kits from vendors K and N using 100ng *C.jejuni*, *B.pertussis*, *E.coli*, and a human reference standard NA12878. Libraries were quantified with qPCR and sequenced to 25X on the NovaSeq 6000. SSEL 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. SSEL Max shows higher coverage at high GC regions for *B.pertussis* (67%GC) compared to vendor N.

SSEL Max: High base coverage, low variability, and superior PPV/recall

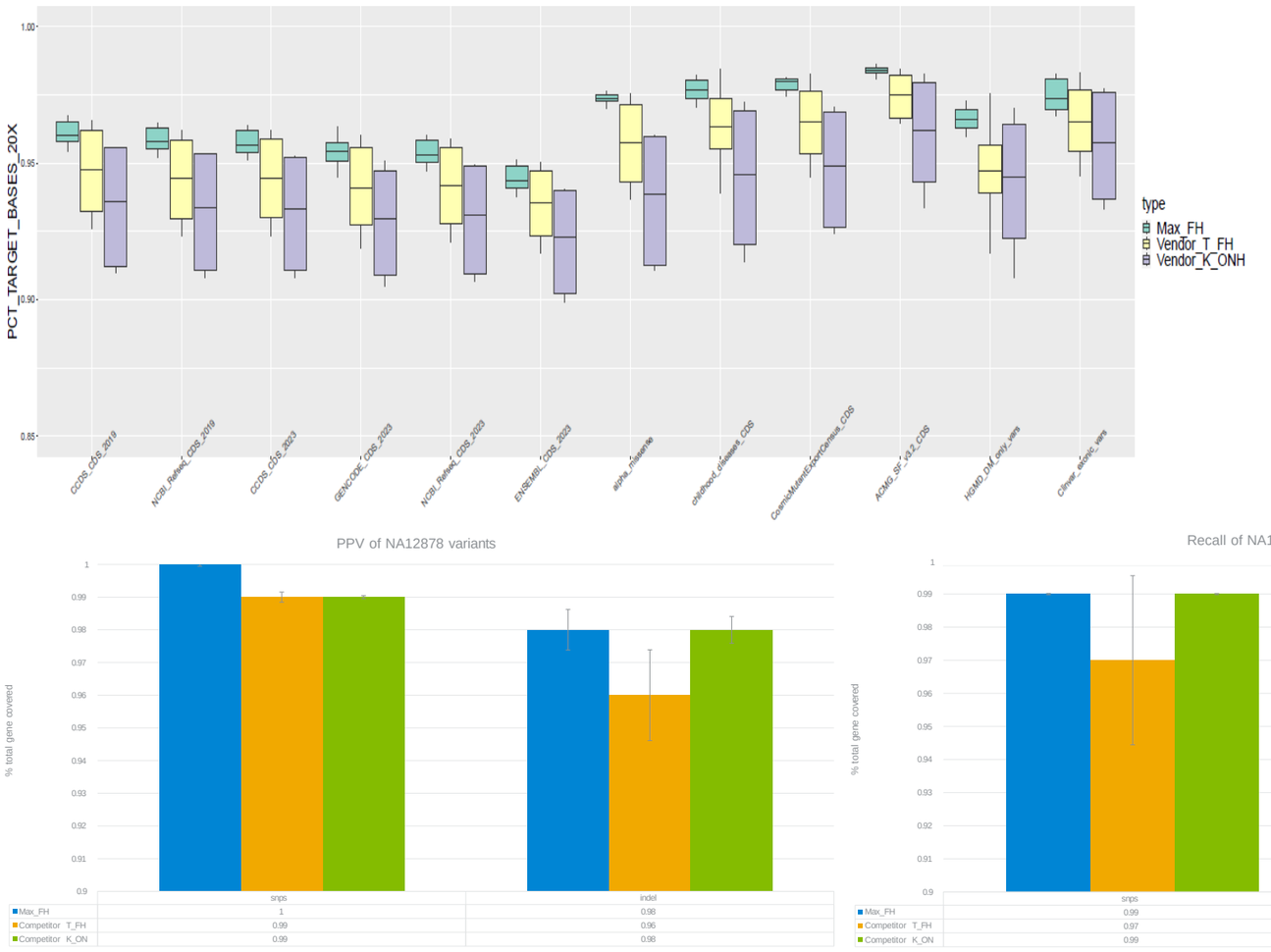

Figure 3.

(Left) SureSelect Max Library Prep with Fast Hyb shows the highest percentage of target bases at 20X in twelve commonly used databases with the lowest variability across tested samples when compared to vendor T Fast Hyb and vendor K Overnight Hyb.

(Below) PPV and Recall of a reference sample (NA12878) for snps and indels. SSEL Max with fast hyb + whole exome v8. SSEL Max is detecting ~600 more SNPs on average than vendor T.

Compatibility and performance of SSEL Max with Element AVITI Cloudbreak sequencer

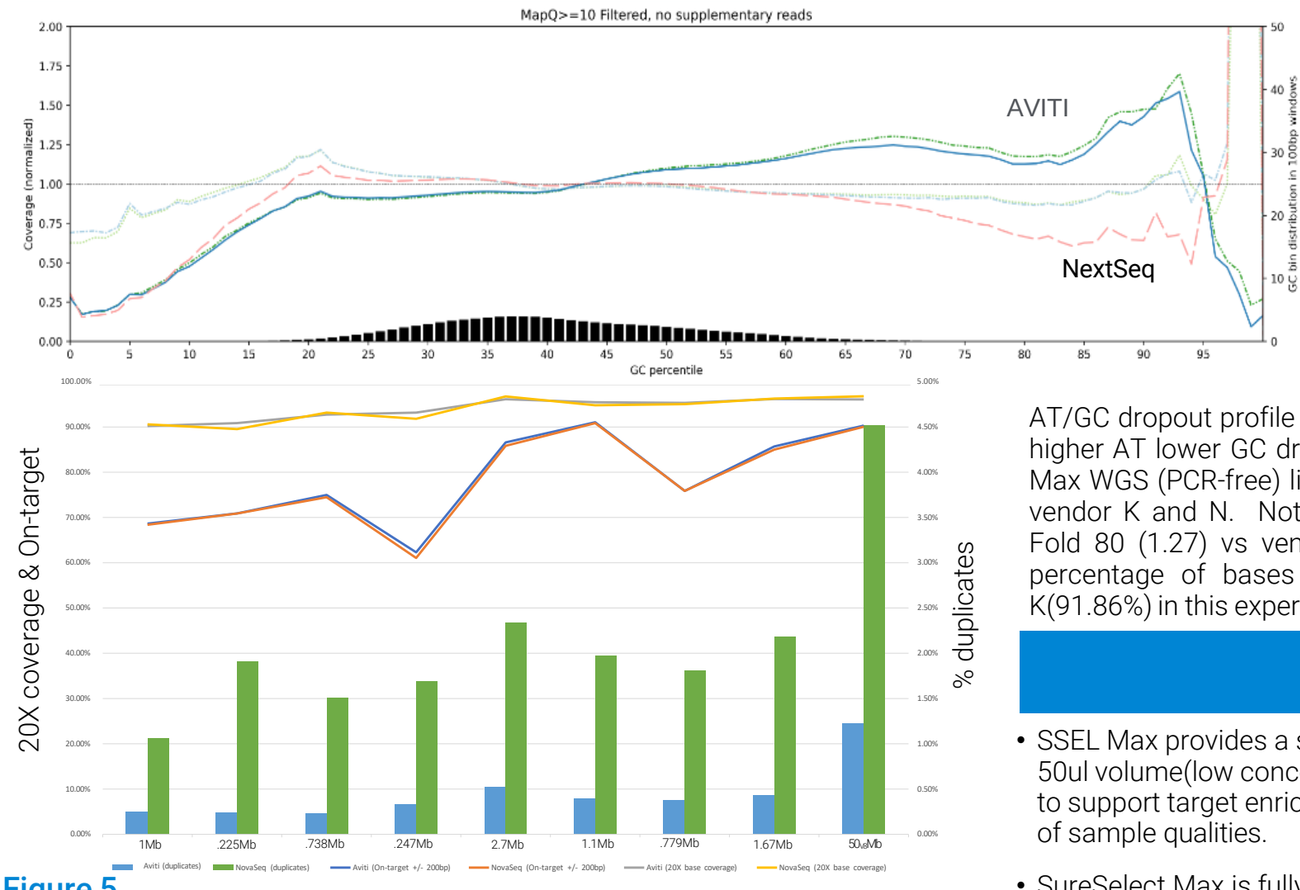

Figure 4. SSEL Max WGS (PCR-free) with enzymatic shearing libraries from 100ng human reference NA12878 were prepared along with similar PCR-free libraries from vendor K and vendor N. All libraries were then sequenced on both the Illumina NextSeq / Element AVITI and normalized to 25X for comparison.

AT/GC dropout profile for the AVITI sequencer trends towards higher AT lower GC dropout compared to the NextSeq. SSEL Max WGS (PCR-free) libraries have lower AT/CG dropout than vendor K and N. Not shown: SSEL Max libraries had lower Fold 80 (1.27) vs vendor K (1.31) and N (1.28) and higher percentage of bases covered at 10X (94.78%) vs vendor K(91.86%) in this experiment.

Figure 5.

Demonstrating compatibility and performance of SureSelect Max library prep with enzymatic shearing and the AVITI sequencing platform. 200ng human reference NA12878 was captured using SSEL Max Fast hybridization and bait panels of varying GC content and size (.2Mb-50Mb). Libraries were sequenced directly on the Element AVITI Cloudbreak(no library conversion necessary) and the Illumina NovaSeq 6000. Reads from both sequencing runs were normalized to 40 million reads for comparisons. High correlation the AVITI and NovaSeq 6000 observed foron-target % and % 20X bases. AVITI generally had lower or similar Fold 80 uniformity(not shown) across the baits tested while having lower (>2X) duplicates.

Conclusions

- SSEL Max provides a streamlined one-step enzymatic LP in 50ul volume(low concentration samples) in a modular format to support target enrichment or WGS/WES from a wide range of sample qualities.
- SureSelect Max is fully modularized and automation compatible, supporting both DNA and RNA, as well as low cycle WGS (for FFPE and ct DNA), and PCR-free WGS (coming soon!)
- SSEL Max generates deeper and more uniform coverage with out sacrificing library complexity or SNP detection in both pre-cap and post cap pooled samples.
- SSEL Max is compatible and provides support for multiple high throughput NGS sequencers.