Broadening the NGS Landscape with Watchmaker Library Prep and the Element AVITI™ System

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Introduction

High-resolution, next-generation sequencing (NGS) applications drive the need for increasingly sophisticated sample preparation workflows and sequencing solutions. The Element AVITI™ System workflow reinvents surface chemistry, base detection, and data analysis to offer a flexible and cost-effective platform for a variety of applications. Broad compatibility with standard NGS libraries provides a straightforward access point to in-house sequencing while integrated, customization-friendly software tools streamline operations.

Methods

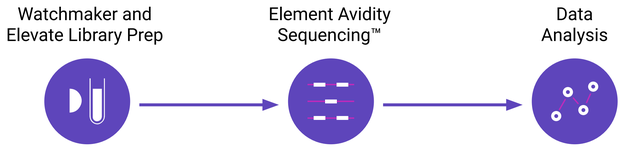

Figure 1. NGS sequencing workflow. Seamless compatibility of Watchmaker Library Prep Kits with the AVITI System.

DNA Library Prep. Libraries were constructed in duplicate from 200 ng of gDNA (NA12878, Coriell Institute) using the Watchmaker DNA Library Prep Kt with Fragmentation.

RNA Library Prep. 10 ng and 500 ng of Universal Human Reference total RNA were used as input into the Watchmaker RNA Library Prep Kit with Polaris Depletion.

Element AVITI System Sequencing. *De novo* NGS library prep for the AVITI System is accomplished using the Element Elevate™ Library Prep workflow, which introduces ligation adapter and index primers as substitutions in a standard NGS library preparation kit as demonstrated here using the Watchmaker Library Prep Kits. The Elevate workflow is comprised of two kits, the Elevate Index Plate and Adapter Kit and the Elevate Library Circularization Kit. The Index Plate itself contains 96 Unique Index Pairs for multiplexing strategies.

Analysis. For DNA libraries, summary metrics including library insert length, GC-bias, chimeras, and artifacts were derived from data downsampled to 2 million read pairs. Variant calling results were generated from data downsampled to 360 million read pairs per sample. No downsampling was performed for RNA libraries. Read pairs per library are as follows: (312M for 10 ng A; 291M for 10 ng B; 313M for 500 ng A; 247M for 500 ng B).

Simple Library Construction Workflows

Table 1. Summary of Watchmaker DNA and RNA Library Prep solutions

Product	Watchmaker DNA Library Prep Kits with Fragmentation	Watchmaker RNA Library Prep Kits with rRNA/Globin Depletion[1]
Applications and methods	WGS, WES, targeted	WTS, targeted
Recommended sample types	gDNA, FFPE, blood, saliva	Blood, FFPE, human/mouse/rat RNA
Input	<1 ng to 500 ng dsDNA	10 ng to 1000 ng total RNA
Workflow time	1 hr 30 min (PCR-free) 2 hr 10 min (with PCR)	<5 hours
Automation friendly?	Yes	Yes

[1]Pre-launch

The Element AVITI System

- Novel Avidity Sequencing™ chemistry yields exceptional data quality
- Engineering and chemistry efficiencies greatly reduce run costs
- 2 random access flow cells with flexible run start of each flow deliver up to 480 Gb combined output

Figure 2. The Element AVITI System.

The AVITI System surface chemistry is uniquely designed with its own proprietary attachment chemistry and oligonucleotide sequences for optimum sequencing accuracy and cycling efficiency.

To adapt to this surface chemistry, the AVITI System comes with both standard and *de novo* NGS library prep options to fit seamlessly into nearly any genomics applications workflow.

Scalable and Accurate DNA-Seq

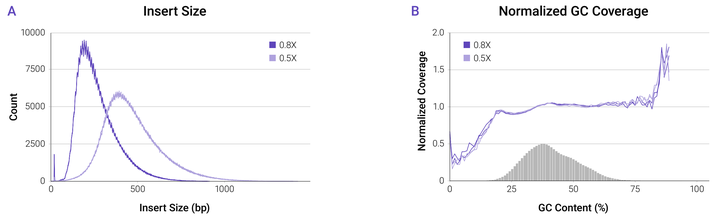

Figure 3. Library sizes are easily tailored to application-specific needs. Two sets of libraries were prepared using NA12878 gDNA Enzymatic fragmentation was performed at 30°C for 7 minutes followed by 0.8X post-ligation SPRI or 30°C for 3 minutes followed by 0.5X post-ligation SPRI. **(A)** Final library insert size histograms for mapped reads were plotted. **(B)** Consistent uniformity of GC coverage for insert-size tuned libraries ensures dependable performance across a range of applications.

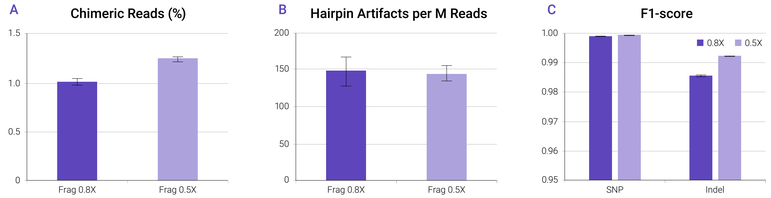

Figure 4. Reduced sequence artifacts enables accurate variant calling. **(A)** Libraries prepared with fragmentation produced low chimeric reads, on par with Covaris samples where ~1% is typical. **(B)** Furthermore, libraries had low levels of hairpin artifacts which are often associated with enzymatic fragmentation. **(C)** F1-scores for variant calling precision and recall for SNPs and Indels. Libraries with longer inserts (0.5X SPRI, see Figure 3) have higher read mapping which leads to higher variant calling accuracy, as expected.

Robust and Reproducible RNA-Seq

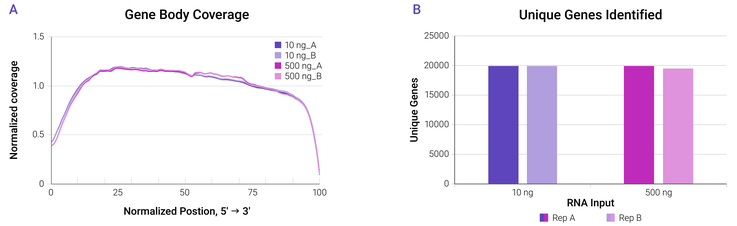

Figure 5. Highly reproducible performance. **(A)** Gene body coverage uniformity calculated for transcripts greater than 100 nt indicates even 5' to 3' coverage and excellent agreement between technical replicates and RNA input amounts. **(B)** A similar number of unique genes were identified between 10 ng and 500 ng inputs. Only genes supported by 10 or more reads were included in the analysis.

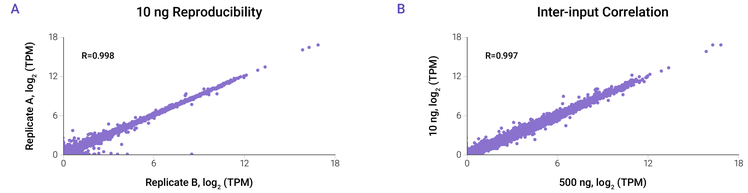

Figure 6. Robust performance with low input amounts. **(A)** Gene abundance correlation between 10 ng technical replicates. **(B)** Gene abundance correlation between averaged 10 ng and 500 ng libraries. Raw counts were converted to TPM. TPM values were $\log_2$ transformed and averaged (if applicable). Only genes with a TPM > 1 were included. Results show highly reproducible performance, as well as a high degree of agreement between high- and low-input samples.

Conclusions

- The Watchmaker DNA and RNA library prep workflows pair seamlessly with the Element AVITI System to broaden the NGS landscape for users.
- Watchmaker's DNA Library Prep Kit with Fragmentation delivers tunable insert sizes, even GC coverage uniformity, and excellent sequence accuracy with a highly scalable workflow.
- Watchmaker's RNA library prep solution enables library construction in approximately 5 hours without sacrificing data quality, even with low input amounts.
- Element's AVITI System provides sequencing application flexibility, with high accuracy and reproducibility demonstrated in both the human WGS and bulk RNA-Seq data sets.