Scalable Transposase-based Multiplexed Library Prep on the Element AVITI™ System with plexWell™

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Introduction

Microbial whole genome sequencing is a demanding application for NGS library prep and sequencing, often involving high-throughput processing of large numbers of samples that have diverse nucleotide content. plexWell™ 384 DNA Library Preparation kits offer unique advantages over other transposase methods including built in auto-normalization, a streamlined workflow and reduced GC bias for low and high GC organisms. The plexWell library prep system leverage full length dual indexed adapters during Tn5 tagging, enabling pooling of 96 samples into a single tube early in the process to streamline the workflow. The power of plexWell library prep is further enabled with the high-quality sequencing data, highly advantageous operating costs and scalability of the AVITI™ System from Element Biosciences. plexWell library pools are fully compatible with AVITI, and GC bias, insert size distribution, and duplication rates are all maintained or improved on the AVITI System.

Results

Sequencing Quality & Demultiplexing

plexWell libraries on the AVITI System produced high quality data with mean quality scores >39, robust demultiplexing, and low CV of read counts for the 384 samples (Table 1, Figure 1).

Table 1. AVITI Sequencing performance. Duplication Rate calculated at 1 M read pairs.

Reads Demultiplexed	Perfect Barcode match	≥Q30	Mean Quality	384-sample index CV	Reads Aligned	Duplication Rate, PCR	Duplication Rate, optical
98.25%	97.57%	91.50%	39.3	27.8%	97.0%	1.56%	0.11%

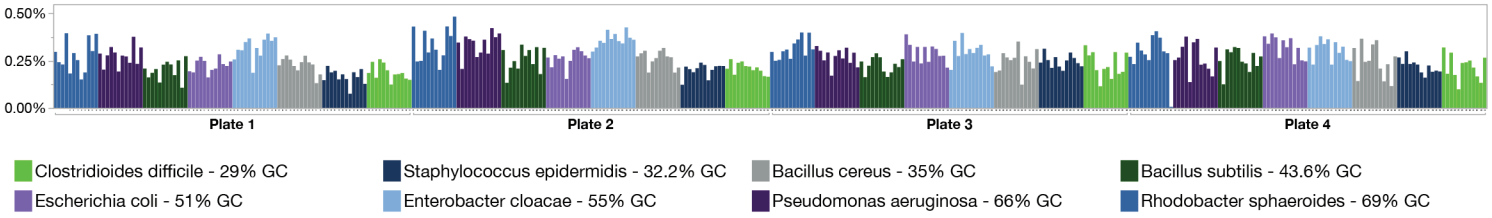

Figure 1. Read count distribution for 384 index combinations (as % of 384)

Genome Coverage

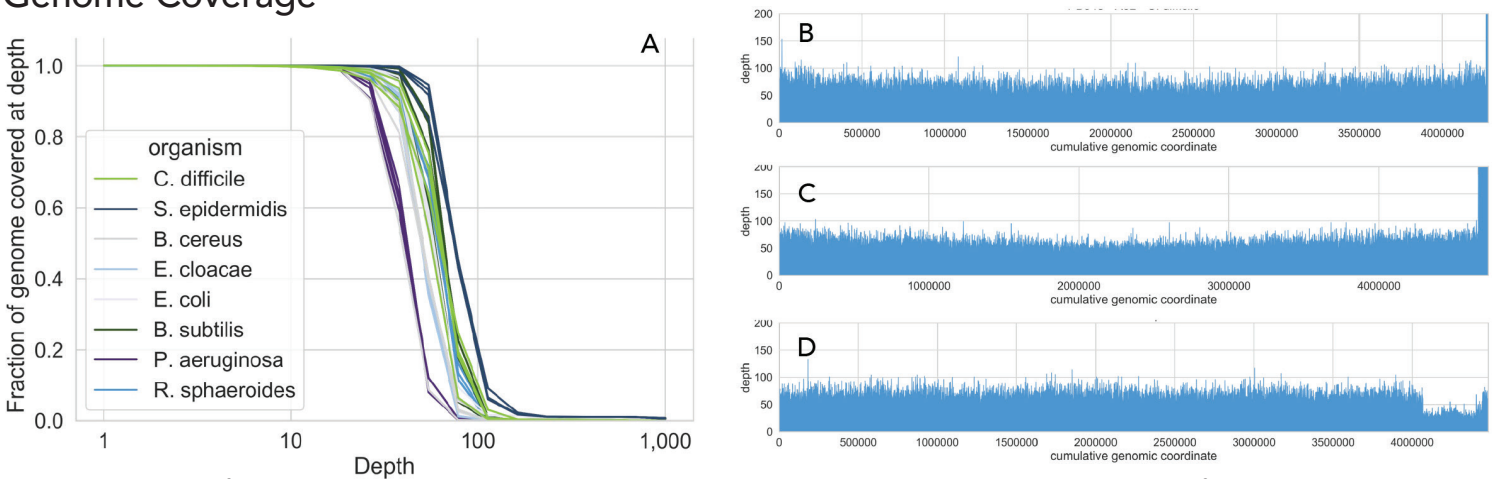

Figure 2. Fraction of the genome covered at varying depth (A). Coverage is consistent between replicates of the same genome. Even coverage maintained for all GC contents. (B-D) Coverage by genome coordinate for (B) C. difficile (29% GC), (C) E. coli (51% GC), and (D) R. sphaeroides (66% GC)

Insert Size

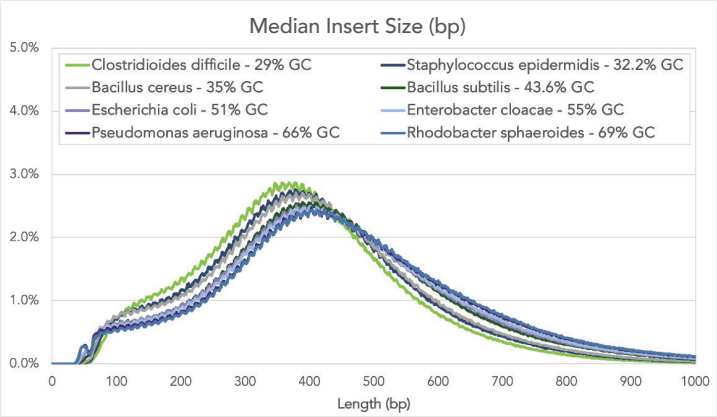

Figure 3. Insert Size distributions are consistent over varying GC content (29-66%)

GC Bias

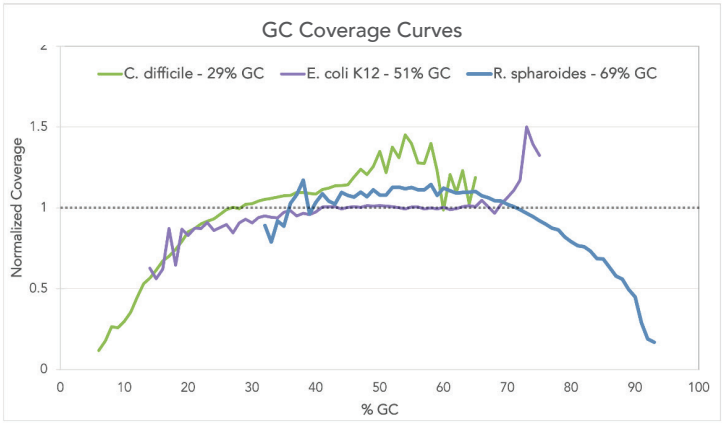

Figure 4. GC bias for low, medium and high samples are reduced compared to other transposase library preps.

Materials and Methods

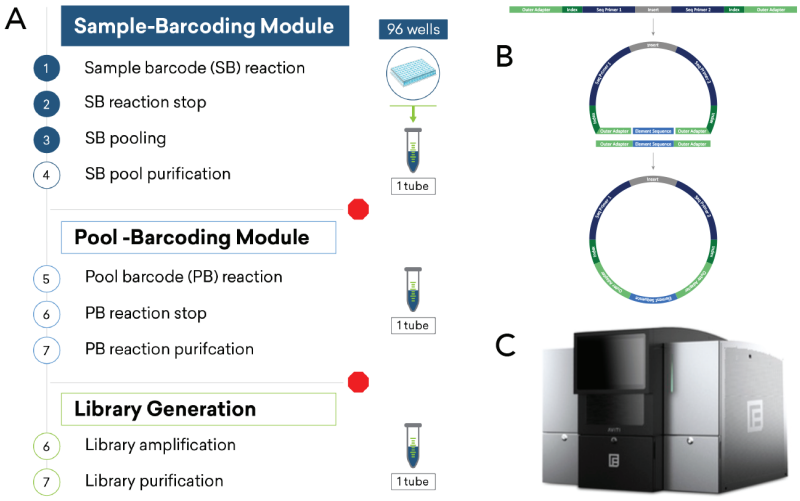

Figure 5. (**A**) plexWell 384 DNA Library Preparation Workflow Diagram. (**B**) Adept Library Compatibility Workflow. (**C**) AVITI sequencer featuring Avidity Sequencing chemistry.

Here, 384 replicates of eight different bacterial strains with GC contents between 29-69% were prepared using the plexWell 384 library preparation kit (Figure 5). Samples in each plate are first tagged with 96 unique i7 indexes (Sample Barcoding). Following this step, all 96 samples are pooled into a single tube for i5 (Pool Barcoding) tagging and all subsequent steps. Different i5 reagents are used for each plate. A total of 24 i5's are available enabling up to 2,304 samples to be multiplexed in a single lane. Following the pool barcoding reaction, library fragments are amplified and purified. The four normalized pools of 96 were combined to create a single 384-plex pool for sequencing.

Prior to sequencing on the Element Biosciences AVITI™ System, the pool of 384 underwent the Element Adept™ Library Compatibility Workflow, an easy 75-minute protocol (25 min hands on). In short, linear library is annealed to splint oligos, adding the Element surface primers. A ligation reaction then circularizes the library, and a digestion reaction removes any leftover splint oligos or linear library material without any additional amplification steps. A final bead cleanup removes any remaining small materials, salts, and enzymes.

The AVITI System is a novel sequencing technology that combines Element Avidity Sequencing™ chemistries with advances in base detection and data analysis to offer a highly flexible and cost-effective platform for a variety of genomics applications. Sequencing of plexWell 384 libraries was performed with 2 x 150 bp paired end sequencing and following demultiplexing, data was down-sampled to 1 million reads per sample for alignment & Picard analysis.

Summary

plexWell libraries are fully compatible with the Element AVITI technology. The superior performance and workflow benefits of the plexWell technology compared to other tagmentation workflows were all maintained, including a highly streamlined workflow, built-in auto-normalization, reproducible insert size, and robust coverage across a wide spectrum of GC contents. Combined, plexWell 384 library preparation and the Element AVITI System demonstrate an efficient and cost-effective workflow for a wide variety of samples and applications without sacrificing performance.