Benchmarking One-Step Library Preparation Against Tn5-Based and Fragmentation–Ligation Workflows for Microbial Whole Genome Sequencing

Sabina Gude, Michelle Rahardja, Stella Huang, Rebecca Feeley, Jenna Couture, Yanyan Liu, Maura Costello, and Jack Leonard

seqWell, Inc. Beverly, MA USA

Introduction

Microbial whole genome sequencing (WGS) is a cornerstone technology for advancing research in microbiology, biotechnology, and public health. As sequencing studies scale to hundreds or thousands of samples, library preparation has emerged as a critical bottleneck, where labor intensity, variability, and GC bias can limit throughput and data consistency. There is a growing need for simplified, robust library preparation workflows that maintain performance across diverse microbial genomes.

In this study, we performed a comparative evaluation of ExpressPlex™ Plus library prep kit, a one-step library preparation kit, against a commercially available Tn5-based library preparation method and a fragmentation and normalization-by-ligation workflow. ExpressPlex Plus employs a proprietary enzymatic formulation that combines tagmentation & library amplification into a single reaction. Libraries were prepared from eight bacterial species spanning a wide range of GC content (29–69%), using a fixed input of 10 ng, with each condition prepared in triplicate.

Libraries from each preparation method were sequenced on an Illumina NovaSeq X Plus system using 2 x 150 bp chemistry. Across all conditions tested, ExpressPlex Plus generated libraries with consistent insert size distributions, uniform genome coverage across GC extremes, and high continuity of genome assembly. In comparison, the Tn5-based workflows exhibited increased variability in coverage particularly at extreme GC content. The fragmentation/normalization-by-ligation workflow requires multistep hence increase consumables cost and labor intensity and thus do not help relieve the bottleneck.

Collectively, these results demonstrate that ExpressPlex Plus provides a scalable and reliable library preparation solution for microbial WGS, delivering robust performance relative to commonly used Tn5-based and fragmentation/normalization-by-ligation workflows and enabling efficient high-throughput sequencing applications.

Methods

- The ExpressPlex Plus library preparation kit was used to process eight bacterial strains in triplicate including gram positive and negative strains with GC contents between 29 – 66%. A yeast sample (S. cerevisiae) was also run.
- For comparison, two competitor kits were also run side-by-side with ExpressPlex Plus: an in-solution Tn5 based kit and a fragmentase plus normalization-by-ligation based kit.
- Starting DNA input for all was 30 ng of total genomic DNA.
- All libraries were sequenced on a NovaSeq X Plus (2 x 150 bp) with the goal of achieving ≥100x genome coverage per microbe.
- Following demultiplexing and alignment, assembly was done using SPAdes (v4.2.0) and Quast (v5.3.0)

Table 1. Summary of bacterial and fungal genomic DNA samples used in the study.

Organism	Genome Size (Mb)	%GC	Gram Stain
Rhodobacter sphaeroides	4.5	69	-
Pseudomonas aeruginosa	6.8	66	-
Enterobacter cloacae	5.3	55	-
Escherichia coli-K12 ATCC	4.6	51	-
Bacillus subtilis	4.2	44	+
Bacillus cereus	5.4	40	+
Saccharomyces cerevisiae	*12.1*	*38*	Yeast
Staphylococcus epidermidis	2.6	32	+
Clostridioides difficile	4.3	29	+

ExpressPlex Plus Library Preparation Workflow

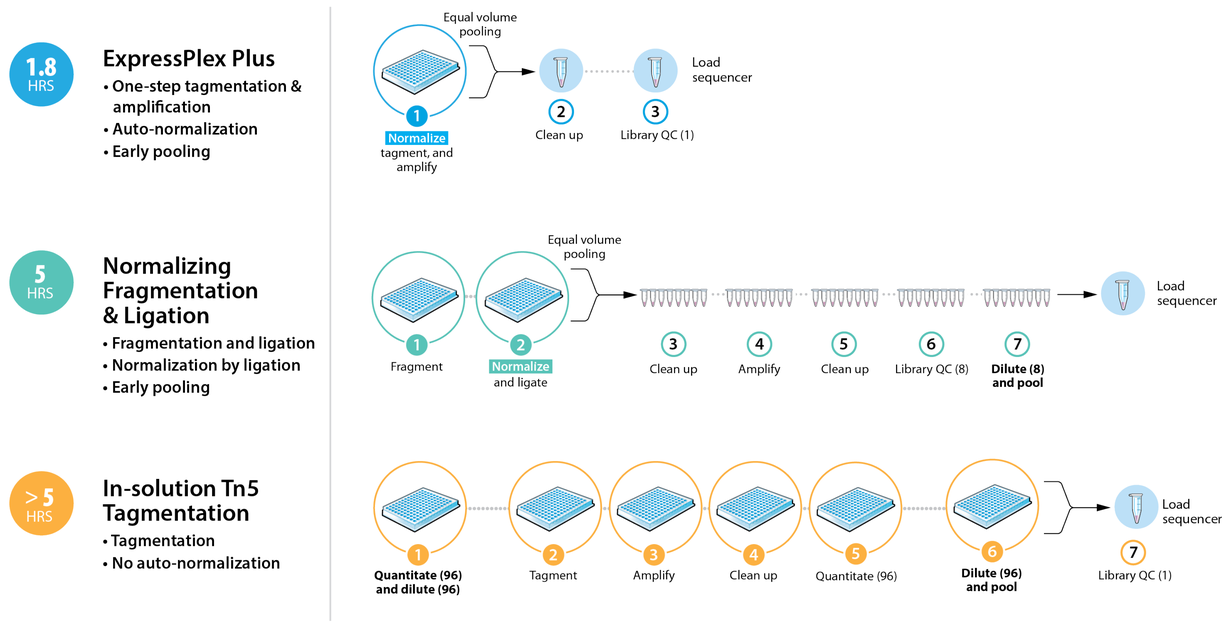

Figure 1. ExpressPlex Plus uses seqWell's next-generation TnX™ transposase that was specifically engineered for NGS library preparation. The ExpressPlex Plus library prep kits utilize a proprietary mixture of enzymes to tag input DNA with indexed adapters and amplify libraries all in a single reaction (left). Different full-length i7 indexed adapters tag the 96 DNA samples and barcoded libraries are amplified in separate wells, making for a highly efficient, one-step multiplexed library prep workflow (right). Additional index sets achieve multiplex levels >96. Samples are pooled volumetrically, purified, and converted into libraries to complete the 100-minute workflow, which includes 30 minutes of hands-on time. Built-in auto-normalization obviates the need to normalize sample input.

NovaSeq X Plus Library & Sequencing Metrics

Table 2. Sequencing performance metrics for ExpressPlex Plus versus Tn5 transposase-based and fragmentase plus normalization by ligation libraries sequenced on NovaSeq X Plus. Data was down-sampled to a target of ≥ 100x mean coverage, however not all individual libraries had sufficient depth to reach that target (reflected in lower average mean coverage values below).

Library Prep Method	Type of Organisms	# of Samples	Avg Mean Coverage	Avg Reads Needed to Reach Mean Coverage	Avg Median Insert Size (bp)	Avg Duplication Rate
ExpressPlex Plus	Bacterial	24	118	2.2 M	512	7.8%
	Fungal	3	120	6.9 M	525	15.2%
Fragmentase + Norm by Ligation	Bacterial	24	109	2.4 M	284	3.9%
	Fungal	3	53	3.5 M	297	12.2%
In-Solution Tn5 Transposase Kit	Bacterial	24	95	2.7 M	251	12.5%
	Fungal	3	43	3.2 M	213	16.0%

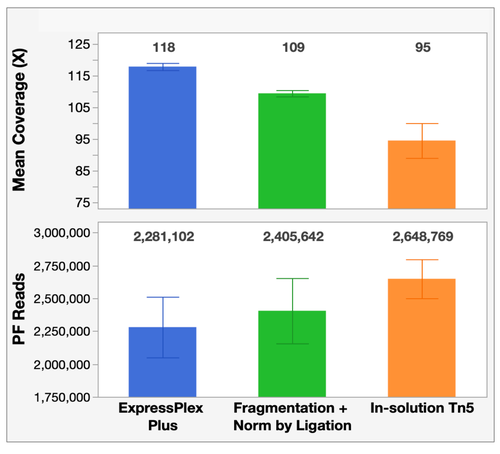

Figure 2. ExpressPlex Plus libraries achieve higher mean coverage with less reads compared to in-solution Tn5 or fragmentase plus normalization by ligation libraries. Side-by side-comparison of mean coverage achieved vs. total PF reads required to meet that coverage. ExpressPlex Plus achieves higher mean coverage with less overall reads.

Coverage and Assembly Performance of Microbial WGS

ExpressPlex Plus achieves uniform coverage across yeast as well as bacteria of varying GC content and gram statuses (Figure 3). Genome assembly quality at ~100X overall depth was also assessed (Figure 4). ExpressPlex Plus assemblies have lower contig counts and larger N50s compared to in-solution Tn5 and are much less variable in quality compared to the normalization by ligation workflow libraries.

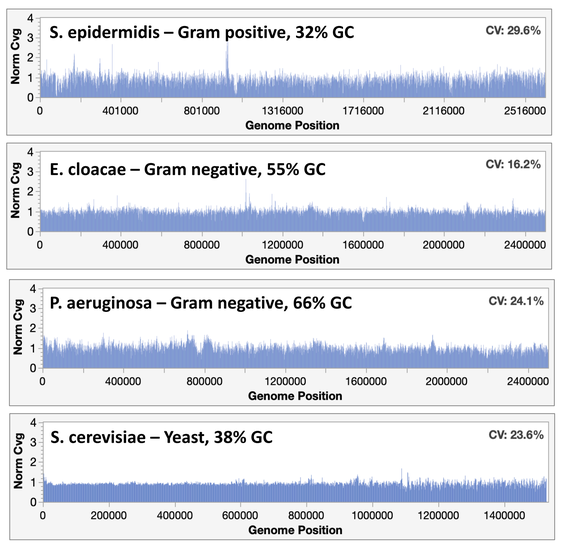

Figure 3. ExpressPlex Plus generates uniform coverage across diverse genomes with varying GC content. Normalized, deduplicated coverage (y-axis) is shown for 3 microbial and 1 fungal species with (% GC from 32% to 66%) calculated using 1,000-base genomic windows (x-axis). The %CV of coverage is also shown.

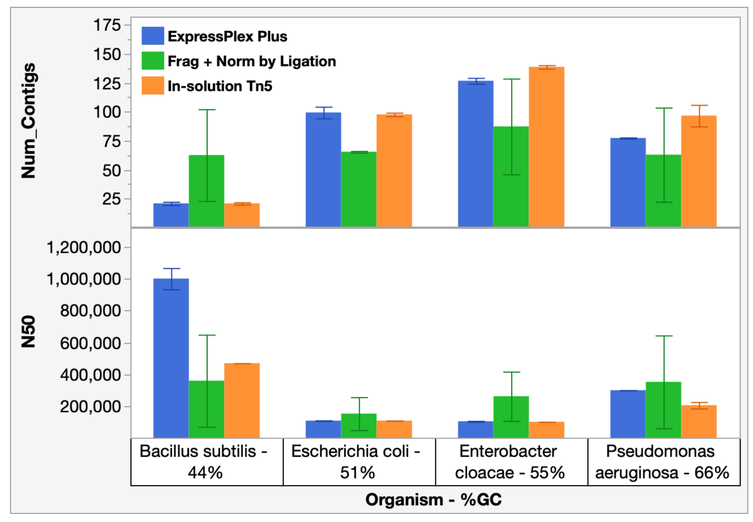

Figure 4. Bacterial assembly data. Comparison of assembly results using 4 different microbes of varying GC contents (44-66 % GC). ExpressPlex Plus (blue bars) consistently produces fewer contigs and higher N50 values than the Tn5 transposase-based method (orange bars), reflecting more contiguous assemblies. While on average the normalization by ligation libraries (green bars) had more contiguous assemblies, there was high variability in assembly quality between replicates which can be seen in the wide error bars for that method.

Summary

- The ExpressPlex Plus streamlines high-throughput microbial WGS by delivering higher mean coverage and generating longer inserts (Figure 2) across eight bacterial strains (29–69 % GC), lowering the reads required to hit target coverage while reducing hands-on time and variability.
- The ExpressPlex Plus provides a scalable, robust library prep for microbial sequencing by delivering uniform, normalized coverage across diverse GC contents of organisms (Figure 3) and high genome assembly quality by consistently producing fewer contigs with higher N50s (Figure 4).

The ExpressPlex Plus is available as an early access product. For more information, please contact earlyaccess@seqwell.com