A Novel One-step Library Preparation Method for Streamlined Microbial Whole Genome Sequencing

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Introduction

Microbial whole genome sequencing (WGS) has revolutionized the study of microorganisms, offering critical insights into ecology, biotechnology, and human health. Despite advances in sequencing platforms, library preparation remains a key bottleneck—particularly in high-throughput workflows where sample diversity and GC content variation can impact data quality and consistency. To address these challenges, streamlined and scalable library prep solutions are essential. Here, we evaluate the performance of **ExpressPlex™ 2.0 Custom High Strength**, a novel one-step library prep method designed to reduce hands-on time and technical variability in microbial WGS. We tested this kit across eight bacterial strains (GC content 29%–69%) and yeast, each prepared in triplicate. The 27-plex ExpressPlex libraries were pooled and sequenced on the NovaSeq X Plus (2 × 150 bp), achieving ≥100× coverage. Our results demonstrate that ExpressPlex 2.0 Custom High Strength enables consistent insert sizes, robust coverage across a wide GC range, and reproducible results across different Gram stain species and fungal genome. These findings support its utility as a scalable, high-throughput solution for microbial genome sequencing.

Methods

- The ExpressPlex 2.0 Custom High Strength library prep was used to processed three sets of replicates using 10 ng input of one fungal and eight bacterial strains with GC contents ranging from 29% to 69% genomes (Table 1).
- For comparison, a competitor Tn5 transposase-based kit was run side-by-side using the same input amount from each microbial species.
- The libraries prepared using ExpressPlex 2.0 Custom High Strength and Tn5-based competitor kit were sequenced on a NovaSeq X Plus (2 x 150 bp) to a coverage of ≥100x.
- Sequencing data were demultiplexed, aligned via Picard Tools, assembly was done using SPAdes (v4.2.0), and assembly metrics were collected using Quast (v5.3.0)

Table 1. Summary of bacterial and fungal genomic DNA samples assessed 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 Library Preparation Kit Workflow

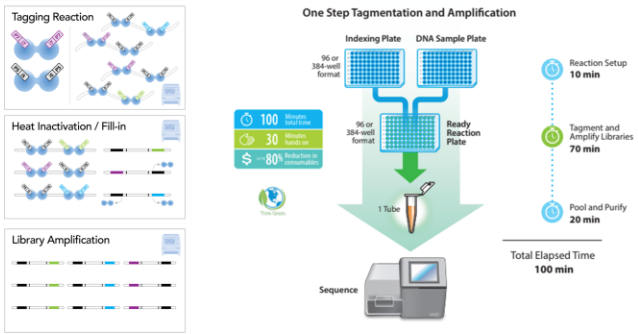

Figure 1. ExpressPlex 2.0 uses seqWell's high performance TnX™ transposase that was specifically engineered for NGS library preparation. The ExpressPlex 2.0 library prep kits utilize a proprietary mixture of enzymes to tag input DNA with indexed adapters and amplify libraries all in a single reaction (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.

Sequencing Metrics

Table 2. Sequencing-performance metrics for ExpressPlex 2.0 Custom High Strength using TnX™ versus Tn5 transposase-based libraries on the NovaSeq X Plus. All datasets were down-sampled to a target of ≥ 100x mean coverage. Samples that did not meet this threshold are reflected by their lower observed mean coverage 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 2.0 Custom High Strength	Bacterial	24	113	2.4 M	316	6.4%
	Fungal	3	75	4.9 M	216	16.7%
Tn5 Transposase-based Kit	Bacterial	24	95	2.7 M	251	12.5%
	Fungal	3	43	3.2 M	213	16.0%

The ExpressPlex 2.0 Custom High Strength yields lower duplication rate and longer inserts (Figure 2) compared to Tn5 transposase-based kit. The two characteristics combined optimize data yield per run enables researchers to sequence fewer total reads to reach their target genome coverage (Table 2).

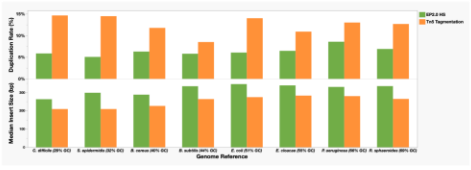

Figure 2. ExpressPlex 2.0 Custom High Strength yields lower duplication rate while producing larger library inserts versus a Tn5 transposase-based kit. Across eight bacterial genomes ranging GC content of 29-69 %, ExpressPlex 2.0 Custom High Strength libraries (green) maintain a consistently lower duplication rate (5-8%) compared to Tn5 transposase-based kit libraries (9-15 %) and yields larger median fragment sizes (260-350 bp vs. 200-280 bp).

Comprehensive Coverage of Microbial WGS

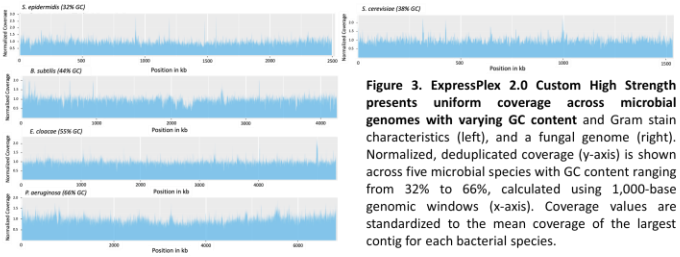

Figure 3. ExpressPlex 2.0 Custom High Strength presents uniform coverage across microbial genomes with varying GC content and Gram stain characteristics (left), and a fungal genome (right). Normalized, deduplicated coverage (y-axis) is shown across five microbial species with GC content ranging from 32% to 66%, calculated using 1,000-base genomic windows (x-axis). Coverage values are standardized to the mean coverage of the largest contig for each bacterial species.

ExpressPlex 2.0 Custom High Strength achieves uniformity of coverage across different microbes with varied GC content and different Gram stain bacterial species (Figure 3). Genome assembly quality was assessed using N50 and the number of contigs. ExpressPlex 2.0 Custom High Strength assemblies deliver both high contig counts and large N50s (Figure 4).

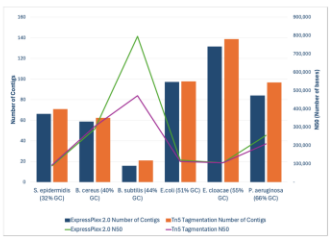

Figure 4. ExpressPlex 2.0 Custom High Strength enable higher assembly contiguity versus Tn5 transposase-based kit. Bar-and-line comparison of seven bacterial genomes (32-69 % GC) shows ExpressPlex 2.0 (blue bars/green line) consistently produces fewer contigs and higher N50 values than the Tn5 transposase-based libraries (orange bars/purple line), reflecting more contiguous assemblies. Data for *S. cerevisiae, C. difficile,* and *R. sphaeroides* were omitted because the Tn5 transposase-based kit did not reach equivalent coverage, preventing a fair comparison.

Summary and Conclusions

- The ExpressPlex 2.0 Custom High Strength streamlines high-throughput microbial WGS by delivering lower duplication rate 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 2.0 Custom High Strength 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 2.0 Custom High Strength is currently in Alpha Testing. For more information about this product or interest in joining the alpha program, please contact alpha@seqwell.com