plexWell Low Pass 384 Library Preparation Kit: Microbiome Applications

Jessica Smith, Ariele Hanek and Kerri Stellato

seqWell, Inc. 376 Hale Street, Beverly MA 01915 USA

Introduction

Most microbiome projects today utilize large-scale parallel sequencing to taxonimically and sometimes functionally characterize microorganisms in a given host/sample type. Due to the large number of samples that are often sequenced, these projects will benefit from a streamlined, scalable library preparation method. Herein we demonstrate the utility of the plex-Well technology for microbiome research using mock communities from ATCC representing species commonly found in gut, oral and skin populations.

plexWell library preparation kits create normalized multiplexed libraries without the need for individual adjustment of input DNA concentrations, significantly simplifying the complex task of high-level multiplexing. The technical foundation of plexWell is a reagent-limited initial transposition step performed on many samples in parallel, coupled to a subsequent pooled library generation step; when applied in conjunction, these two steps yield an approximately equal number of sequencer-ready library fragments from each of a potentially large collection of samples
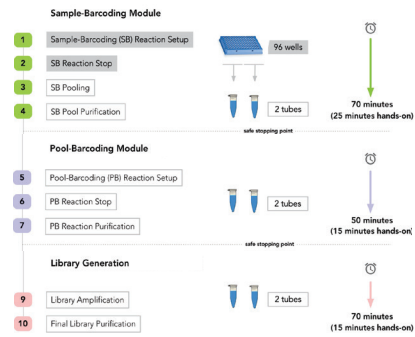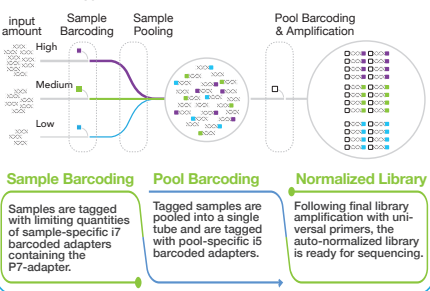
Methods

Figure 1. plexWell Workflow

Figure 2. plexWell Normalizing Iterative Barcoding Technology

Sample Barcoding
Samples are tagged with limiting quantities of sample-specific i7 barcoded adapters containing the P7-adapter.

Pool Barcoding
Tagged samples are pooled into a single tube and are tagged with pool-specific i5 barcoded adapters.

Normalized Library
Following final library amplification with universal primers, the auto-normalized library is ready for sequencing.

Synopsis

- DNA Samples
  - ATCC DNA mock community for gut, oral and skin
  - Zymo DNA mock 10 sample community
- Library Preparation
  - plexWell Low Pass 384 library preparation kit
  - Nextera XT library kit for comparison
- NextSeq paired 2x150 sequencing to >5 M pairs
- Analysis used Kraken2 with a kmer length of 35 and excluding bases with a fastq score less than 20. Species-level and genus-level relative abundances were estimated using the Baysian model implemented in Bracken2
- Factors considered
  - Reproducibility between replicates
  - Comparison to Nextera XT
  - Limit of Detection/Data

Results

Figure 3. Reproducible species identification and distribution for ATCC gut mock community in plexWell library preparation
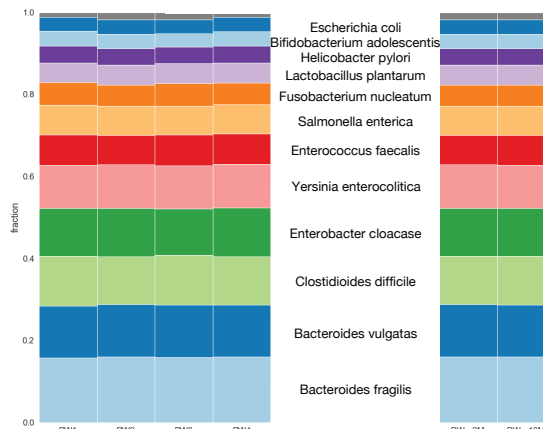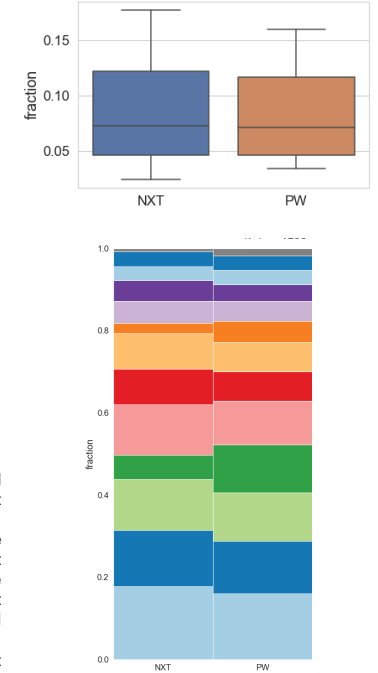

The ATCC gut DNA mock community consists of an even mixture of 12 genomic DNA prepared from fully sequenced, characterized, and authenticated bacterial species observed in normal and atypical gut microbial communities.

Analysis of the plexWell library, demonstrates all 12 species are correctly identified. The relative abundance of each species is consistent across all 4 replicates (figure 3, left side). Additionally the relative representation is preserved after downsampling 80%, demonstrating that less sequencing data is needed per sample, lowering the overall sequencing costs of microbiome survey projects.

Comparison of plexWell to Nextera XT (Figure 4) demonstrates that plexWell more closely aligns with the expected distribution from ATCC.

Figure 4. plexWell vs. Nextera XT ATCC gut mock community

Figure 5. ATCC Skin mock community

Figure 6. ATCC Oral mock community
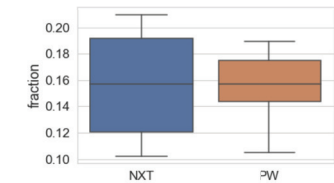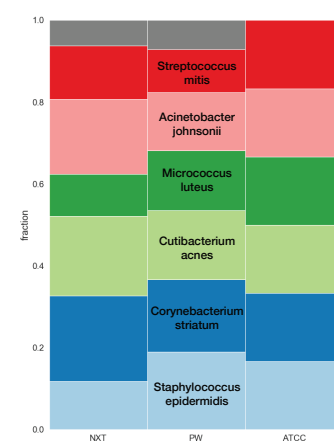
plexWell libraries better approximate the idealized ATCC data for all microbiome mock communities tested (figures 4-6). The plexWell library correctly identifies all species in the samples.

Moreover the only species identified are species known to be in the samples. Gray bars represent unmapped reads.
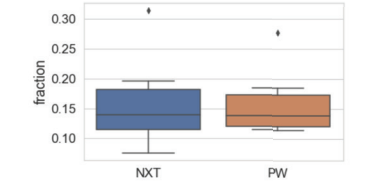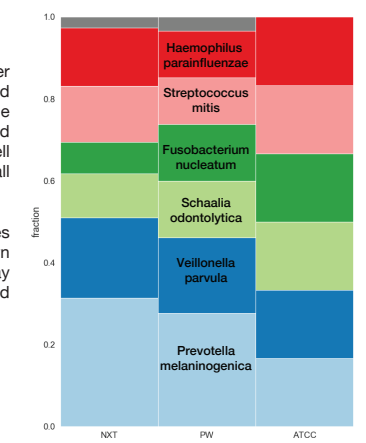
Conclusions

plexWell™ Low Pass 384 Library Preparation Kit provides a streamlined scalable reproducible solution for survey microbiome sequencing of up to 1152 samples in a single sequencing run. This analysis shows the data is reproducible for skin, oral and gut microbiome samples. The plexWell library performance outperforms Nextera XT in terms of data accuracy and ease of scalability. Spurious species were not present in the plexWell libraries.