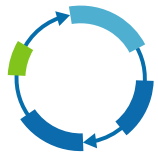
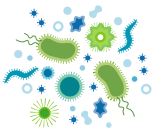
LongPlex™ Multiplexing Kit

Fragmentation and Indexing for Scalable, Long-Read Sequencing



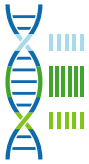
Plasmid Sequencing

Microbial and Small Genome WGS



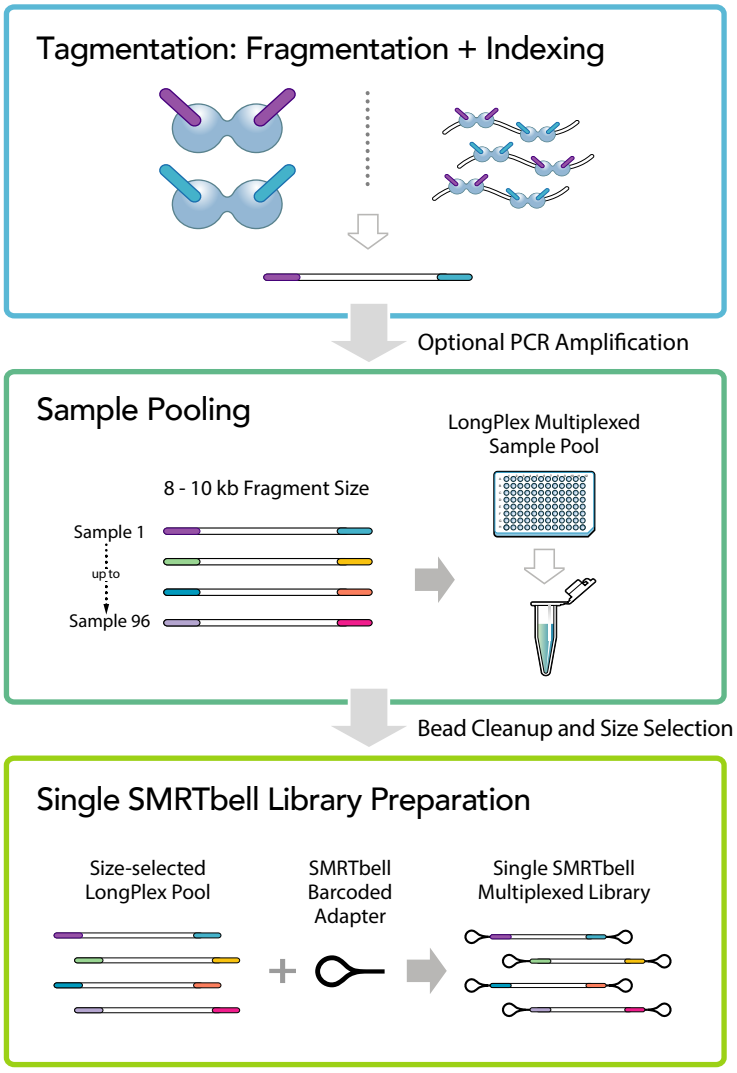
Metagenomic Sequencing

Low Pass Sequencing



Targeted Hybrid Capture

Realize the potential of your PacBio long-read sequencer



Make short work of long reads...

Use transposase-based fragmentation and sample multiplexing to unleash truly scalable, cost-efficient long-read sequencing.

- Eliminates the time and resource burden of mechanical shearing
- Early multiplexing enables more samples per flow cell
- Multiple workflow options for a variety of applications and desired fragment lengths
- Workflows compatible with PacBio SMRTbell® Prep Kit 3.0

Figure 1: DNA is fragmented and unique dual indexes (UDIs) are added using Tn5 transposase. Samples are pooled, cleaned up, and size selected. The size-selected LongPlex pool proceeds to a single PacBio SMRTbell library prep.

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LongPlex™ Multiplexing Kit

Enabling High-Throughput, Cost-Efficient Long-Read Sequencing

One-step fragmentation & indexing and early sample pooling simplify the library prep workflow and reduce the total number of required SMRTbell library preps while increasing the number of samples per SMRT Cell.

Simple workflow

No mechanical shearing. UDI-loaded transposases simultaneously fragment and index DNA.

Rapid

DNA fragmentation to size-selected, multiplexed pool in under 2 hours (30 minutes hands-on time).

Scalable

Automation-friendly method using 96 LongPlex UDIs. Early sample pooling reduces bead cleanup and QC burden.

Savings

Multiplexing prior to SMRTbell library prep reduces cost per sample without sacrificing data quality.

Flexible

PCR, PCR-free, and hybrid capture workflows to address a variety of applications.

Transposase-based DNA Fragmentation and Indexing

LongPlex consistently produces fragments of >10 kb from high quality DNA, with final Femto Pulse fragment sizes of >8 kb following SMRTbell library preparation. A LongPlex XL protocol is available to produce larger final fragment lengths.

Sample	Post LongPlex Femto size (bp)	Post SMRTbell Femto size (mean, bp)	Post SMRTbell Femto size (mean, bp)
24-Plex A	11,503	12,472	8,443
24-Plex B	10,953	11,444	9,302
24-Plex C	10,710	11,902	9,463
24-Plex D	10,156	10,112	9,490

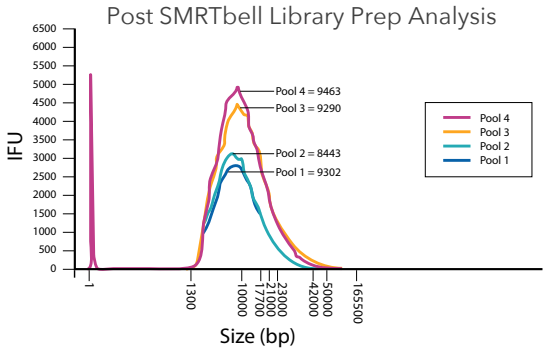

Table 1 and Figure 2: QC results of four (4) 24-plex library pools generated using the LongPlex PCR-free workflow from 8 different microbial strains. Fragment profiles were generated using the Agilent Femto Pulse with the Genomic DNA 165kb Analysis Kit. Fragment sizes ranged from 10.1 - 11.5 kb following LongPlex tagmentation. Fragment profiles of the final pool post SMRTbell library prep displayed a mean size of 10.1 – 12.4 and a mode of 8.4 – 9.5 kb.

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Microbial Whole Genome Sequencing (WGS)

- Consistent insert size across different genomes and DNA input levels
- Uniform coverage of genomes with high, medium, and low GC-contents
- Equal volume pooling prior to PacBio SMRTbell library prep - no quantification or normalization required

Organism	%GC	Genome Size (Mb)	PCR-Free Coverage	PCR-Free Max Contig (Mb)
Clostridioides difficile	29	4.3	160	4.1
Staphylococcus epidermis	32	2.6	396	2.6
Bacillus cereus	35	5.4	74	5.4
Bacillus subtilis	44	4.2	23	1.2
Escherichia coli	51	4.6	191	4.6
Enterobacter cloacae	55	5.3	136	5.3
Bordetella pertussis	67	4.0	259	4.0
Rhodobacter sphaeroides	69	4.5	209	3.2

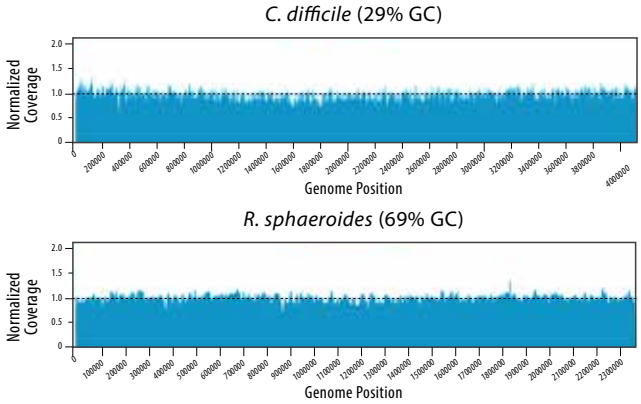

Figure 3: gDNA samples from 8 different microbial isolate strains (ATCC), which contained GC contents ranging from 29-69%, were processed in quadruplicate using the LongPlex PCR-free workflow. Post LongPlex tagging, samples were pooled into four (4) 24-plex pools for cleanup and bead-based size selection. Final LongPlex pools underwent library preparation using PacBio SMRTbell prep kit 3.0. The 4 SMRTbell libraries were pooled and sequenced using a single Revio SMRT Cell.

Metagenomic Taxonomy Profiling

- Long read lengths enable more complete, accurate assembly, and better resolution of complex genomic regions
- Increased throughput with reduced time, labor, and total sequencing costs
- Consistent read counts and sequencing quality

Sample	Total Reads	Average Read Length	Bray-Curtis Dissimilarity
Replicate 1	285,171	4482	0.124
Replicate 2	314,897	4532	0.127
Replicate 3	298,056	4492	0.124
Replicate 4	286,638	4529	0.125

% bps ≥Q40 96.9%

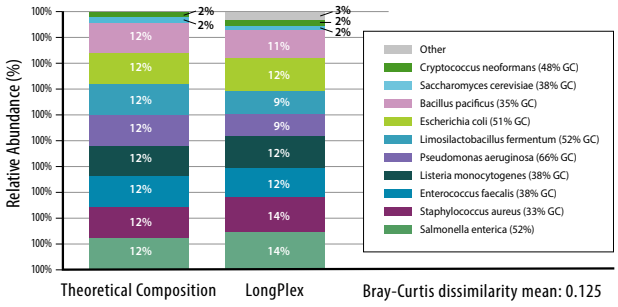

Figure 4: Taxonomic profiling of ZymoBIOMICS Microbial Community DNA Standard which consists of gDNA from pure cultures of 8 bacterial (2.99 - 6.79 Mb in size) and 2 fungal strains (12.10 and 18.90 Mb in size) with GC contents ranging from 33-66%. The theoretical composition is 12% for each bacterial strain and 2% for each fungal strain. Four sample replicates were processed using the LongPlex PCR-free workflow with a total DNA input of 285 ng. Reads were demultiplexed using customized Lima, and sequencing data were down-sampled to 200,000 random HiFi reads prior to taxonomy profiling analysis using Kraken2 with a kmer length of 35. Bracken2 species-level and genus-level relative abundances were estimated using the Baysian model implemented in Bracken2. Those reads labeled as "other" (grey) are unmapped reads.

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"[LongPlex] both fragments and adds barcodes to long fragments of DNA across 96 samples thus, enabling **higher throughput, low-cost** metagenomics preparations."

Jeremiah Minich,
Salk Institute for Biological Studies

Minich, *et al.*, Culture-independent meta-pangenomics enabled by long-read metagenomics reveals associations with pediatric undernutrition. *Cell* 188, 1–21

LongPlex Multiplexing Kit Specifications

Workflow Options	• LongPlex PCR-free • LongPlex PCR-plus • LongPlex Hybrid Capture • LongPlex XL
Sample Types*	Genomic DNA (DIN ≥8.0 recommended)
Reactions per Kit	96 reactions
DNA Input Recommended	150 - 500 ng
Indexing Method	Unique Dual Indexing (UDI)
Number of Unique Index Combinations	96
Batch Size**	1 - 96 samples
Output Fragment Size***	• Standard protocol: 5,000 - 8,500 bp • LongPlex XL protocol: >10kB
PCR Amplification	• WGS: PCR-free and PCR-plus methods available • Hybrid Capture: 8-10 PCR cycles recommended
Total Protocol Time	• PCR-free: < 2 hours (30 minutes hands-on) • PCR-plus: 3 - 4 hours including long range PCR (30 minutes hands-on)
Sequencer Compatibility	PacBio Revio™ PacBio Sequel™ II and ile

* Other sample types may be compatible. Contact seqWell support for guidance.

** Can pool up to 24 samples prior to SMRTbell library preparation. Please contact support@seqwell.com for guidance on pooling >24 samples.

*** Fragment size will depend on DNA quality, magnetic bead cleanup ratios, LongPlex workflow and QC method. Final sequencing read lengths will vary depending on downstream application.

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