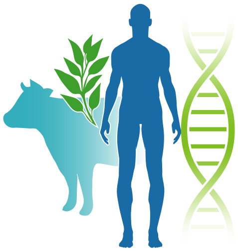
LongPlex™ Multiplexing Kit

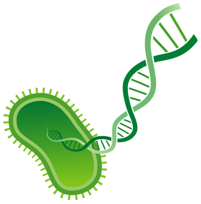
Make short work of long reads

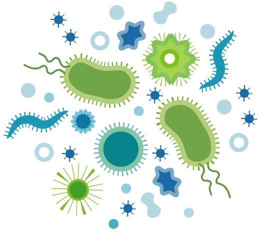
Transposase-based Long-read
Fragmentation & Multiplexing

seqWell™

LongPlex Applications

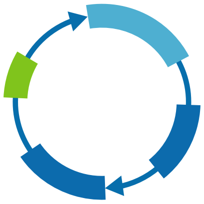

Low-Pass Whole-Genome Sequencing


Microbial & Small Genome WGS


Metagenomic Sequencing


Whole-Exome Sequencing


Targeted Hybrid Capture Sequencing


Plasmid Sequencing

Realize the full potential of your PacBio long-read sequencers by eliminating mechanical shearing

Simple, Scalable Workflow

- Transposase-based fragmentation
- Multiplex up to 96 samples
- Low number of touchpoints
- Seamlessly migrates into SMRTbell® library prep workflow

Savings

- Multiplexing prior to SMRTbell library prep significantly reduces total sequencing cost per sample
- Significant time and labor savings compared to mechanical shearing

Rapid

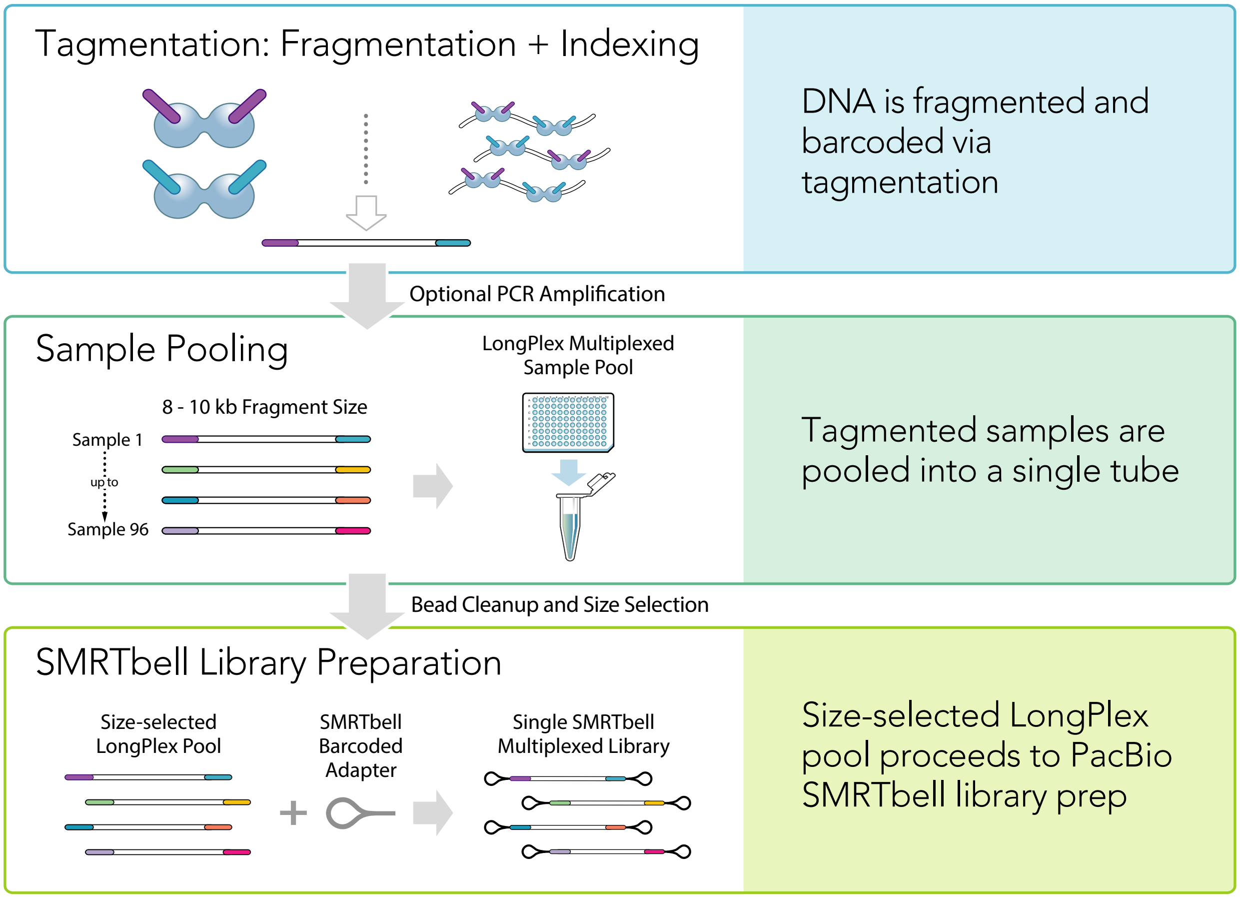
- High-throughput DNA fragmentation to multiplexed pool in < 2 hours
- Early sample pooling reduces cleanup and QC burden

Flexible

- PCR and PCR-free workflows
- Supports sequencing & hybrid capture
- LongPlex XL protocol available for longer fragment sizing

seqWell

High-Throughput, Cost-Efficient Long-Read Sequencing



Tagmentation: Fragmentation + Indexing

DNA is fragmented and barcoded via tagmentation

Optional PCR Amplification

Sample Pooling

Tagmented samples are pooled into a single tube

Bead Cleanup and Size Selection

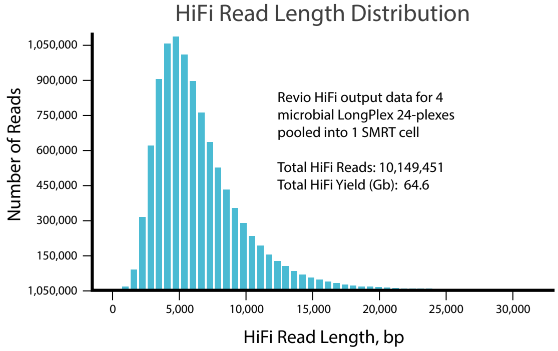
SMRTbell Library Preparation

Size-selected LongPlex pool proceeds to PacBio SMRTbell library prep

Transposase-based DNA Fragmentation and Indexing

LongPlex consistently fragments high quality DNA, to generate HiFi sequencing read lengths between 6 - 9 kb, with robust sequencing results on PacBio systems. A LongPlex XL protocol is available to produce larger final sequencing read lengths up to 15 kb.

Sample ID	HiFi Reads	HiFi Yield (Gb)	HiFi Read Length (mean, bp)	HiFi Quality (median)
24-Plex #1	2,559,646	16.8	6,562	Q46
24-Plex #2	2,099,116	13.5	6,434	Q46
24-Plex #3	1,721,057	11.0	6,401	Q46
24-Plex #4	1,785,947	11.4	6,374	Q46



Revio HiFi output data for 4 microbial LongPlex 24-plexes pooled into 1 SMRT cell

Total HiFi Reads: 10,149,451
Total HiFi Yield (Gb): 64.6

Table 1 and Figure 1: HiFi results calculated separately for each 24-plex, demonstrating consistent HiFi read lengths, data yield, and HiFi quality across the full set of 96 samples. Summary Revio HiFi output data for 96 microbial samples pooled into four (4) 24-plex library pools and sequenced together on the same Revio SMRT cell, generating a mean HiFi read length of 6.4 kb and a total HiFi data yield of 64.6 Gb.

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	%CG	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

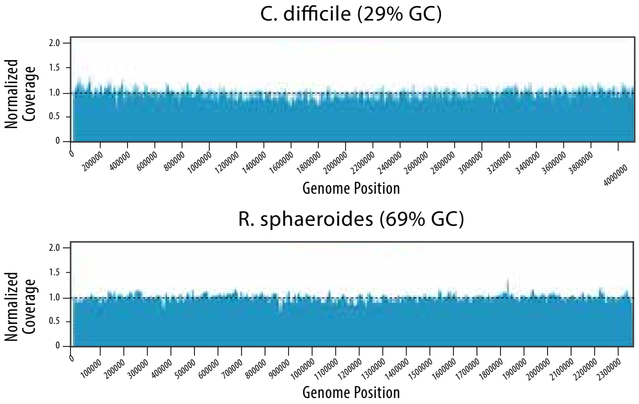


Figure 3: gDNA samples from 8 different microbial isolate strains (ATCC), which contained GC contents ranging from 29-69%, were processed in quadruplicate using the LongPlex PCR-free workflow. Post LongPlex tagging, samples were pooled into four (4) 24-plex pools for cleanup and bead-based size selection. 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	% bps ≥Q40
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%

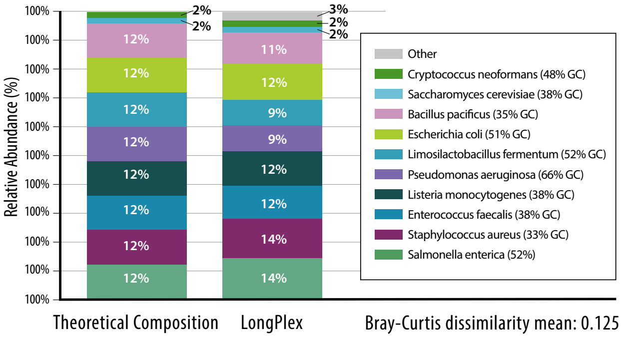
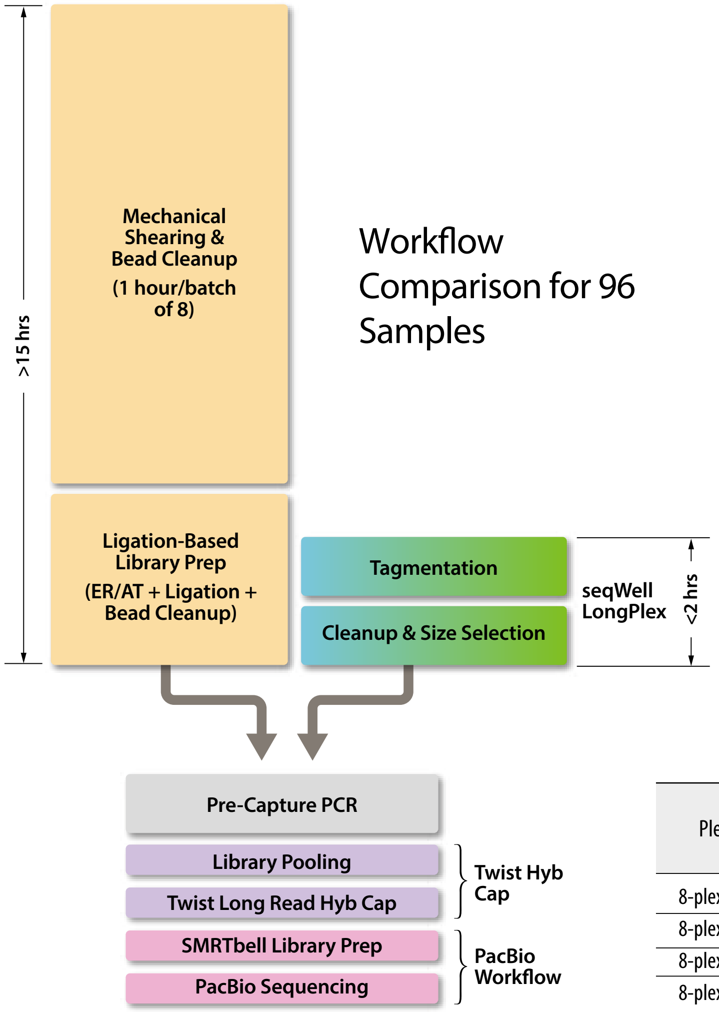


Figure 4: Taxonomic profiling of ZymoBIOMICS Microbial Community DNA Standard which consists of gDNA from pure cultures of 8 bacterial (2.99 - 6.79 Mb in size) and 2 fungal strains (12.10 and 18.90 Mb in size) with GC contents ranging from 33-66%. The theoretical composition is 12% for each bacterial 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.

Targeted Hybrid Capture

- Elimination of mechanical shearing and ligation saves >12 hours for 96 samples
- Seamless integration of size-selected LongPlex libraries into standard target enrichment protocols, including workflows for Twist Bioscience panels
- Quality and reproducibility of LongPlex chemistry produce equivalent or better target enrichment metrics ensuring you maintain uniform coverage and critical variant detection

Workflow Comparison for 96 Samples



>15 hrs

Mechanical Shearing & Bead Cleanup
(1 hour/batch of 8)

Ligation-Based Library Prep
(ER/AT + Ligation + Bead Cleanup)

Tagmentation

Cleanup & Size Selection

seqWell LongPlex

<2 hrs

Pre-Capture PCR

Library Pooling

Twist Long Read Hyb Cap

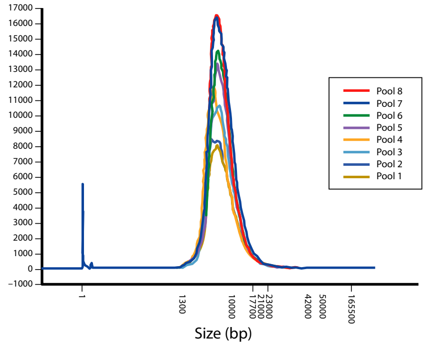
Twist Hyb Cap

SMRTbell Library Prep

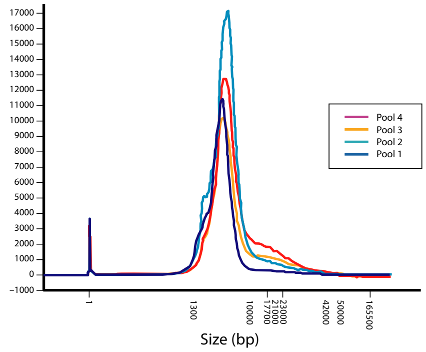
PacBio Sequencing

PacBio Workflow

Frag size post LongPlex with 9-cycle PCR ~7.5 kb



Frag size post Hybrid Capture ~6.5 kb



Plex Name	Reads Per Sample	HiFi Read Length (bp)	% Duplication	Mean Target Coverage	Fold80 Penalty
8-plex capture A	207,095	4505	18.0%	177.6	1.76
8-plex capture B	159,129	4515	13.4%	147.6	1.79
8-plex capture C	118,512	4790	9.6%	109.0	1.84
8-plex capture D	162,724	4749	12.8%	154.6	1.81

Figure 2 & Table 2: 32 samples were prepared following the LongPlex hybrid capture protocol using 250 or 500 ng human gDNA (NA12878) per sample. For hybrid capture, four 8-plex pools were prepared using a total of 4000 ng per pool. The standard Twist Bioscience protocol was followed for the PGx Long Read panel. Transposase adapter-compatible blockers (IDT) were substituted for Twist Universal Blockers. SMRTbell library prep 3.0 was run on the four 8-plex pools and sequenced on the PacBio Revio™. Fragment size of the pools before and after hybrid capture were measured on the Agilent Femto Pulse systems.

"[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., (2025). *Cell* 188, 1–21.

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 Mean Read Length***	• Standard protocol: 5,000 - 9,000 bp • LongPlex XL protocol: >10 kB
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 Vega™ PacBio Sequel™ II and ile

* Other sample types may be compatible. Contact support@seqwell.com for guidance.

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

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

Ordering Information

Catalog Number	Description	Number of Reactions
301310	LongPlex Multiplexing Kit, Set 1	96 reactions

www.seqwell.com/longplex-multiplexing/
sales@seqwell.com

Request a Quote