

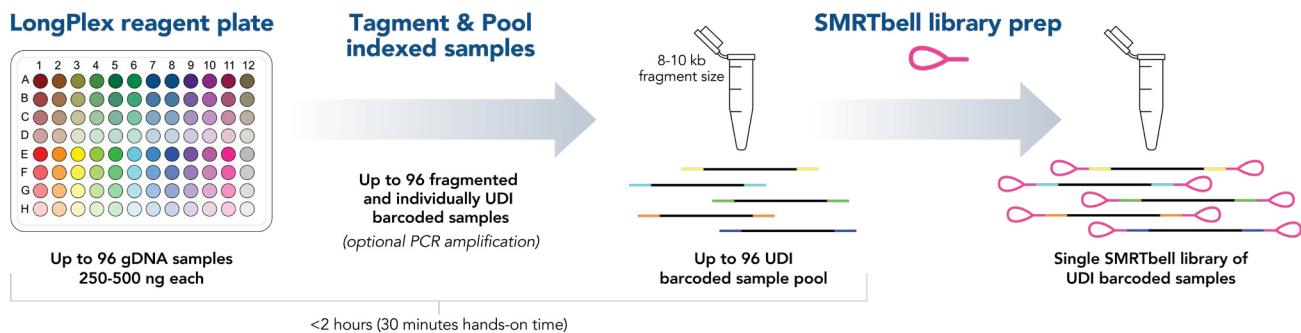
# LongPlex™ Long Fragment Multiplexing Kit

Realize the throughput and cost-effectiveness of PacBio™ HiFi sequencing using seqWell's LongPlex Long Fragment Multiplexing Kit. Its speed, simplicity, and scalability enable massive sample multiplexing that unleashes scalable long read sequencing.

## Supported Long-Read Applications:

- Microbial and small genome sequencing
- Metagenomics
- Low pass sequencing
- Targeted hybrid capture

## LongPlex Workflow Upstream of SMRTbell Library Prep

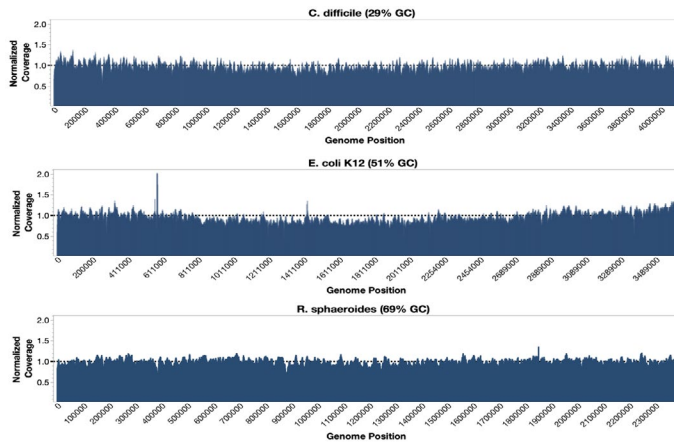


## Key Benefits: Realize the potential of your PacBio long read sequencer

- **SPEED:** Eliminate mechanical shearing using a fully scalable, enzymatic method to simultaneously fragment & tag genomic DNA resulting in typical fragment sizes ranging from 8 – 10 kb
- **SIMPLICITY:** Automation-friendly, plate-based method with total workflow performed in <2 hours (30 minutes hands-on time) upstream of SMRTbell library preparation
- **SCALABILITY:** Massive multiplexing using 96 unique dual indexes (UDI) that can be combinatorially expanded with PacBio SMRTbell indexing
- **SAVINGS:** Early sample pooling greatly reduces all-in cost per sample for long read sequencing without sacrificing data quality
- **FLEXIBILITY:** PCR-free and PCR-based protocols available to support a variety of applications and sample needs

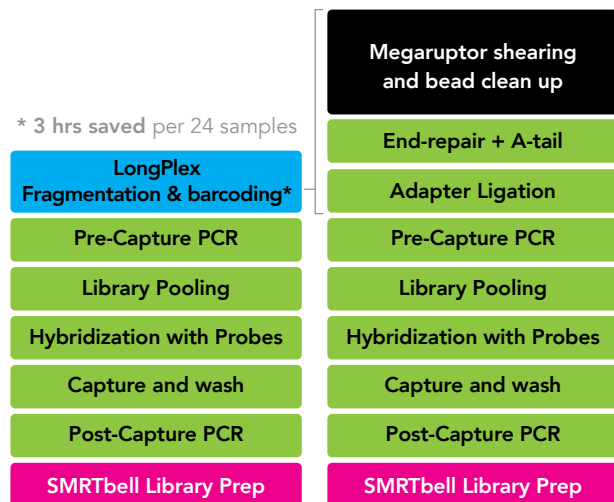
## Application: Microbial Whole Genome Sequencing

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



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

Microbial gDNA samples from 8 different microbial isolate strains (ATCC), which contained GC contents ranging from 29-69%, were processed in quadruplicate using the LongPlex Long Fragment Multiplexing Kit. Post LongPlex tagging, samples were pooled into 4 x 24-plexes for bead-based size selection followed by PacBio SMRTbell prep kit 3.0. The 4 SMRTbell libraries were pooled into a single Revio SMRT Cell.



## Application: Targeted Hybrid Capture

- Streamlined, automation-friendly workflow
- Save up to 3 hours/24 samples compared to Megaruptor fragmentation
- Equivalent data to mechanically sheared DNA
- Compatible with a variety of hybrid capture panels including Twist PGx and Dark Gene panels

| 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           |

32 samples were prepared with LongPlex using varying inputs (250-500 ng) of NA12878. Samples were pooled into 4 x 8-plexes post LongPlex, and then captured using a Twist PGx panel following manufacturer's protocol. Post capture samples underwent SMRTbell library preparation and were sequenced on the PacBio Revio.



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