LongPlex™ - Enabling Highly Scalable PacBio HiFi™ Target Capture Assays with Multiplexed Long Fragment Transposition

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Introduction

Recent improvements to long-read sequencing platforms, such as PacBio's Revio, have significantly lowered sequencing costs. However, for targeted assays requiring pooling large number of samples, significant challenges remain in efficiently preparing these libraries at scale. A major barrier is the mechanical fragmentation methods currently used. Diagenode's Megaruptor 3 instrument can take up to 3 hours per batch of 8 samples; fragmenting 96 samples could take multiple days or require the purchase of multiple costly instruments.

To address these challenges, we have developed LongPlex™, a multiplexed transposase-based kit that rapidly generates high-quality long fragment (~8 kb) libraries barcoded with unique dual indexes in an easy to automate plate-based format and without the need for separate fragmentation instrumentation. In ~3.5 hours, LongPlex libraries are ready to be pooled for hybrid capture and subsequent PacBio prep.

LongPlex™ 8 kbp Multiplexed Library Workflow

Figure 1

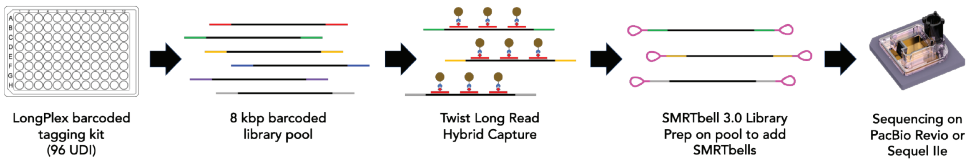


Figure 2

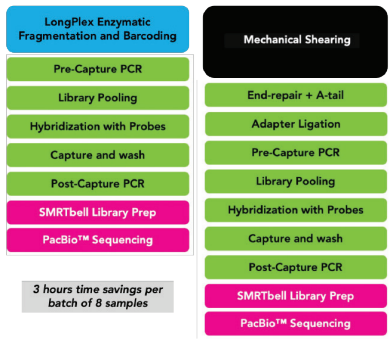


Figure 1

- LongPlex simultaneously fragments to ~8 kbp and barcodes using transposases loaded with full length UDI adapters. The starting input is 250 ng of genomic DNA (DIN ≥6.5).
- Following PCR, barcoded fragments can then be pooled for Twist long read hybrid capture.
- Finally, PacBio adapters are added using the SMRTbell 3.0 kit followed by PacBio sequencing.

Figure 2

- LongPlex fragmentation and barcoding is done in a plate based 3-step process: 15 min tag, 10 min stop, and a bead clean up.
- This replaces the mechanical shearing, end repair + A-tailing, and adapter ligation steps in the conventional workflow **saving up to 3 hours per batch of 8 samples**.

Performance in Twist PGx Long Read Panel and PacBio Revio Sequencing

Experimental design:

- Replicates of HapMap control DNAs (250 ng) were prepared with LongPlex, and 500 ng of each library was pooled for hybrid capture using Twist's PGx long read panel according to Twist's standard protocol, substituting transposase adapter specific blockers for Universal Blockers. Captured library pools were then converted to PacBio libraries using the SMRTbell 3.0 kit and sequenced on Revio.
- Following sequencing and HiFi consensus read generation with SMRTlink, data was demultiplexed on the LongPlex indexes using LIMA and hybrid capture metrics generated using a modified version of Picard CollectHSMetrics.

Figure 3 – LongPlex library sizing

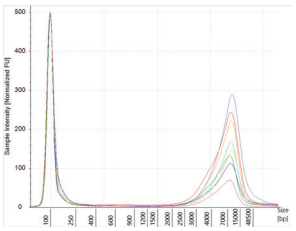
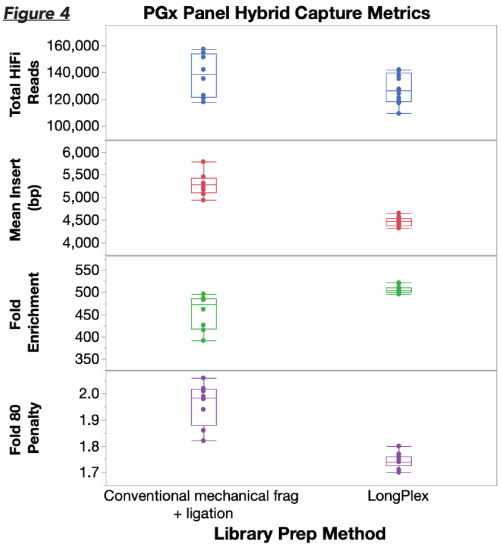


Table 1 – Library quantification data

Sample	TapeStation Peak (bp)	Conc (ng/µL)	Library Yield (ng)
NA12878 Rep 1	9432	35.9	1077.0
NA12878 Rep 2	8943	32.2	1030.4
NA12878 Rep 3	7772	42.4	1356.8
NA12878 Rep 4	8629	40.8	1305.6
NA12878 Rep 5	8762	30.1	963.2
NA12878 Rep 6	9322	31.8	1017.6
NA12878 Rep 7	7836	28.2	902.4
NA12878 Rep 8	8273	29.2	934.4

Figure 4



Results:

- LongPlex PGx panel capture data was compared to data from libraries made using conventional mechanical fragmentation and adapter ligation method per Twist's user guide – See Figure 4 at left.
- While the LongPlex libraries were slightly shorter, they achieved higher fold enrichment with similar total HiFi reads.
- Additionally, the Fold 80 Penalty scores were lower for LongPlex, indicating more even coverage of the PGx targets.
- **Combined, this shows LongPlex libraries perform robustly, with equivalent or better metrics compared to mechanically fragmented libraries.**

Summary and Conclusions

- LongPlex reagents allows for simultaneous fragmentation and barcoding to generate multiplexed long read libraries in a scalable and time saving plate-based format. Libraries performed equal to or better than conventional mechanical fragmented libraries.
- **LongPlex is currently in Early Access. For more information, please contact earlyaccess@seqwell.com**