LongPlex™ XL: An Improved Long-read Tagmentation Workflow for Cost Effective Highly Multiplexed ≥12 kb HiFi Libraries

Maura Costello, Christianto Putra, Zac Zwirko, Gavin Rush, Joe Mellor
seqWell, Inc. Beverly, MA USA

Introduction

seqWell introduced the LongPlex™ Long Fragment Multiplexing kit in 2024, utilizing transposase tagmentation to simultaneously fragment and barcode genomic DNA in a fast workflow, enabling pooling of up to 24 samples prior to PacBio® SMRTbell® library preparation, reducing the overall number of required SMRTbell preps and thus saving time and costs.

The standard LongPlex protocol is optimized to generate libraries with HiFi read lengths between 6-10 kb, ideal for long read targeted capture or for microbial and small genome applications where genomic DNA can often be degraded. However, researchers with high-quality, large molecular weight genomic DNA may wish to generate HiFi read lengths >10 kb to truly take advantage of the gigabase output of PacBio's Revio™ instrument.

To address this, we developed a modified "LongPlex XL" protocol to prepare multiplexed libraries generating HiFi read lengths ≥12 kb by incorporating PacBio's Short Read Eliminator (SRE) kit to size select fragmented and pooled LongPlex products. Performing pooled size selection prior to SMRTbell library preparation provides a significant workflow advantage and reduces time, effort, and reagent costs vs. traditional fragmentation and sizing methods.

Modified LongPlex XL Workflow

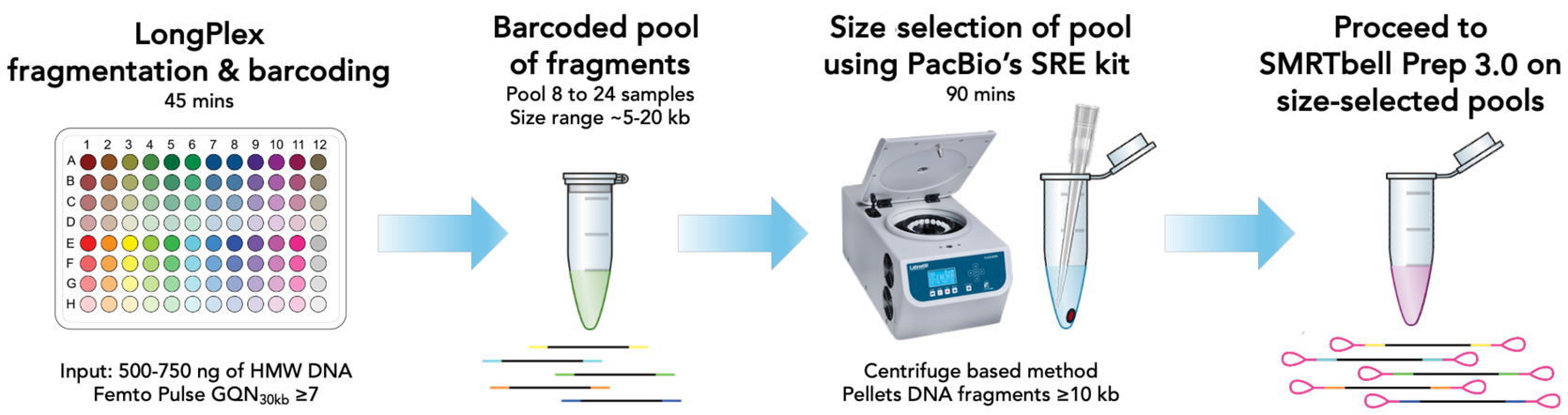

Figure 1: Overview of LongPlex XL workflow. 500-750 ng of high-quality DNA (Femto Pulse GQN_{30kb} ≥7 required) is fragmented and barcoded with the LongPlex kit using a modified tagging reaction with a reduced volume of transposase to generate longer fragments.

The barcoded samples are then pooled (8 to 24 samples per pool) and size-selected to remove fragments <10kb using PacBio's SRE kit following the standard protocol. **High-quality DNA must be used; significant sample loss will occur during SRE with degraded samples.**

Size-selected pools can proceed to the SMRTbell prep kit 3.0 and finally SMRT sequencing.

SMRT Sequencing of Libraries Prepared Using LongPlex XL Protocol

Experimental Design:

- Two sets of 8 replicates using 500 ng of high-quality human control DNA (Promega; GQN_{30kb} = 8.5) were fragmented and barcoded using the LongPlex Kit.
  - One set of 8 was fragmented using the modified "XL" protocol and SRE size selection following tagging and pooling.
  - One set of 8 was fragmented using the standard protocol without size selection.
- Both pools were then processed using the SMRTbell prep kit 3.0 to add SMRTbell adapters (total of 2 SMRTbell libraries), fragment sizes measured via Femto Pulse and sequenced on 2 different Revio SMRT cells to determine the mean HiFi read length of each pooled SMRTbell library.

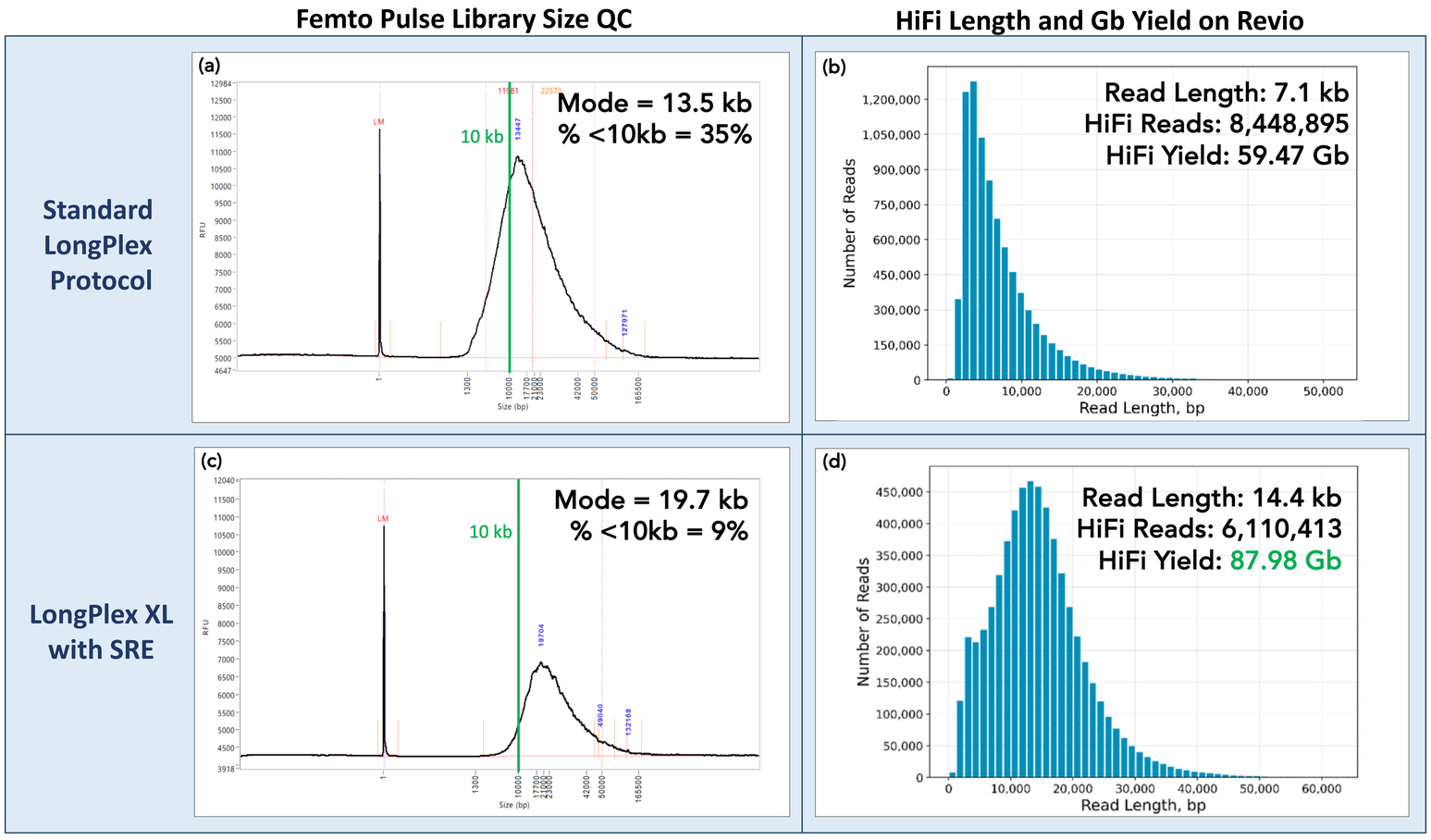

Figure 2: Comparing standard LongPlex to LongPlex XL protocols with high-quality DNA. For 8-plex pool prepared with standard LongPlex protocol: (A) Femto Pulse library fragment size QC and (B) Revio HiFi sequencing read length and yield metrics. The HiFi read length was ~7 kb with a run yield of 59 Gb.

For 8-plex pool prepared with LongPlex XL protocol (C) Femto Pulse library fragment size QC and (D) Revio HiFi sequencing read length and yield metrics. **The HiFi read length was ~14 kb with a total run yield of ~88 Gb.**

Table 1: DNA yield after each step

	Standard LongPlex	LongPlex XL
Post LongPlex 8-plex pool yield	1670 ng*	2226 ng
Post SRE yield	--	1030 ng
Post SMRTbell yield	609 ng	378 ng

* Standard LongPlex yield is expected to be lower due to an included bead size selection to deplete material <3 kb which is omitted in XL in lieu of SRE size selection.

Conclusions

The <u>modified LongPlex XL</u> protocol with SRE may be the right choice when <u>all</u> the following criteria are met:

- The application requires reads >10 kb and/or more Gb of data
- Input DNA is confirmed high-quality (**GQN_{30kb} ≥7 a must**), there is ≥500 ng per sample, and the batch size is at least 8 samples.
- Adding 90 minutes to the workflow for SRE is acceptable.
- The lab scientist is experienced with SRE, which can be a difficult method to perform due to the near invisibility of pelleted DNA following centrifugation.

The <u>standard LongPlex</u> protocol without SRE size selection would be the right choice when:

- HiFi read lengths of 6-10 kb are appropriate for the application.
- Input DNA is degraded (GQN_{30kb}<7) or <500 ng per sample.
  - Note: many microbial, microbiome, and environmental samples fall into this category.
- Adding 90 minutes to the workflow for SRE is undesirable.
- Having the ability to automate the workflow from end-to-end is desirable as SRE is a manual, centrifuged-based process.