

**PacBio**

# Enabling Long-Read Sequencing by Automating DNA Shearing and PacBio SMRTbell Library Construction on the Biomek i7 Hybrid Workstation

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**Figure 1.** Biomek i7 Hybrid Workstation.

## Introduction

The PacBio SMRTbell<sup>®</sup> prep kit 3.0 for whole genomic and metagenome libraries is a 5-step process that enables sequencing on PacBio<sup>®</sup> long-read systems Sequel II/IIe<sup>®</sup> or Revio<sup>®</sup> or Vega<sup>®</sup>. A variety of sample inputs including human, plant, animal, microbial, or metagenomic can be used. The PacBio SMRTbell prep kit 3.0 can be purchased in sets of 24 reactions. To scale the PacBio SMRTbell prep kit 3.0 up to 96 reactions a total of 4 kits should be purchased.

## Automation Spotlight

The Biomek i7 Hybrid Workstation deck for the automated DNA shearing and SMRTbell prep kit 3.0 workflow features 2 ColdPlates (QInstruments, CP96PCR1 and CPI), a BioShake (QInstruments, BS1), and an optional on-deck thermal cycler (Applied Biosystems, ATC1).

Benefits of automating DNA shearing and SMRTbell prep kit 3.0 on the Biomek i7 Hybrid Workstation include:

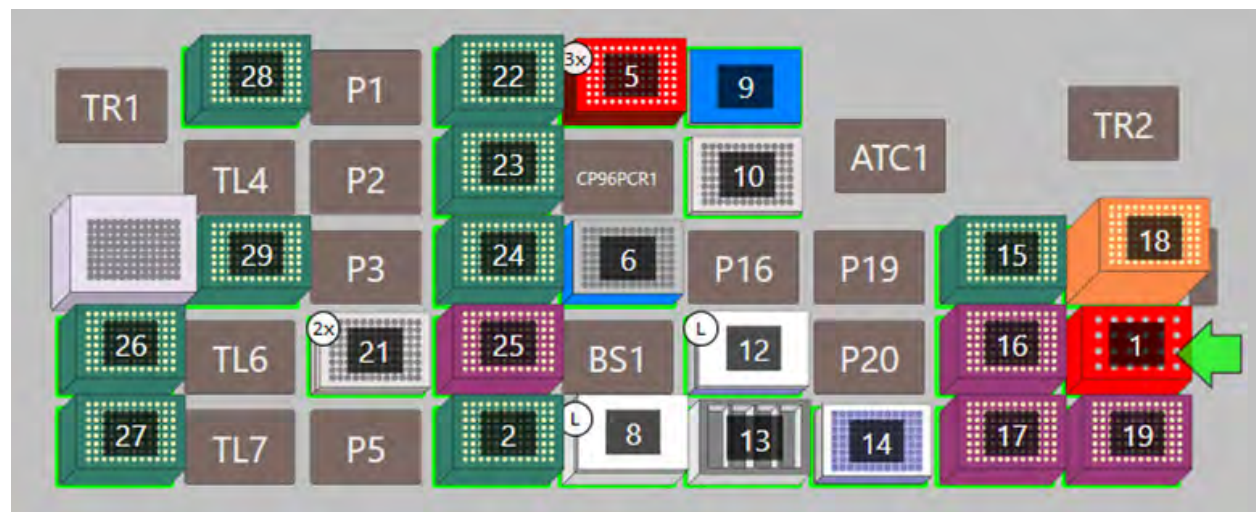
- Automated pipette shearing
- High-throughput scalable solutions for library preparation
- User-friendly Method Options Selector (MOS) interface for real-time workflow selections
- Guided labware setup (GLS) to provide runtime setup instructions, reagent volumes, and user-friendly labware and deck visuals
- Reduced hands-on time and pipetting errors

### Automated Method

Here, we present an automated solution to streamline and accelerate library construction for long-read sequencing. The automated method for DNA Shearing and SMRTbell prep kit 3.0 library construction on the Biomek i7 Hybrid Workstation can process up to 96 samples in 6 hours and features a user-friendly interface for real-time selection of workflow options. Although the PacBio SMRTbell prep kit 3.0 can be split into 5 sections, the automated workflow is processed in 4 sections to align with QC and safe stop points.



**Figure 2.** Automated workflow for pipette shearing and SMRTbell prep kit 3.0 on the Biomek i7 Hybrid Workstation. The entire workflow can be processed from start to finish in 6 hours for 96 samples.



**Figure 3.** Complete deck setup for processing 96 samples for the automated DNA pipette shearing and SMRTbell prep kit 3.0 on the Biomek i7 Hybrid Workstation.

## Method Features

### Automated Pipette Shearing of DNA

Automated DNA pipette shearing on the Biomek i7 Hybrid Workstation results in gDNA sizes comparable with Megaruptor 3. Additional devices are not required, and DNA shearing can be fully automated on the Biomek i7 Hybrid Workstation using the 1200  $\mu$ L multichannel pipetting head.

### Method Options Selector (MOS)

The automated DNA shearing and SMRTbell prep kit 3.0 method for Biomek i7 Hybrid Workstation provides an intuitive user interface, the MOS, showcasing all workflow options available at the start of method run. The workflow options are:

- Number of samples 8-96
- On-deck vs off-deck thermocycling
- Adapter tube or 96-well barcoded Adapter Plate
- Nuclease Cleanup option or SMRTbell bead (over 5 kb) or diluted AMPure beads (under 5 kb)

### Guided Labware Setup (GLS)

The GLS provides clear step-by-step instructions during the deck setup for the DNA Shearing and SMRTbell prep kit 3.0 library construction automated method. Dynamic reagent volumes and tube locations based on sample number will be displayed via visual representations to provide additional help to the operator.

### DeckOptixFinal Check (DFC)

The DFC will use instrument cameras to analyze the final instrument deck for missing or misplaced labware to reduce possible operator setup errors.

## Experimental Design

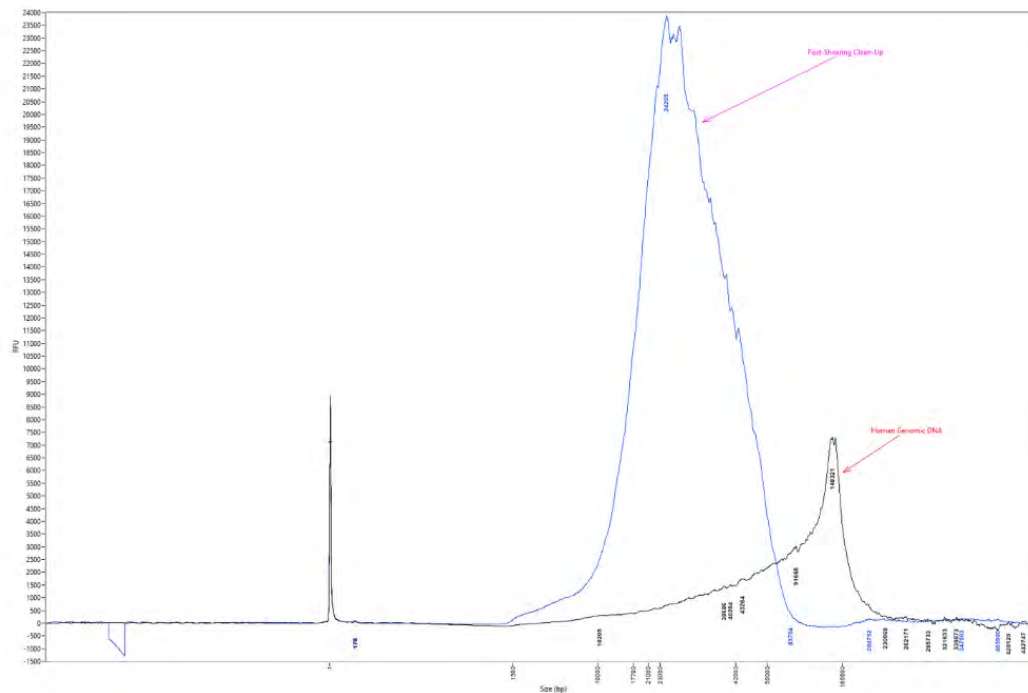
We prepared a total of eight libraries following the complete automated DNA Shearing and SMRTbell prep kit 3.0 library construction workflow on the Biomek i7 Hybrid Workstation. Four samples were commercial genomic DNA (gDNA) from Promega (G3041), and four microbial culture service samples. Despite different starting sizes of gDNA all samples were sheared with identical conditions. A total of 200  $\mu$ L of sample was used for automated pipette shearing and the SMRTbell prep kit 3.0 library construction. Additional workflow options were for use of the SMRTbell adapter tube, and an alternative bead cleanup for a library size greater than 5 kb.

After library construction, the final libraries were quantified using a Qubit fluorometer and analyzed on the Femto Pulse system to determine the size distribution of individual libraries. Using the calculated molarity values, the four gDNA samples from Promega were pooled equimolarly into one pool, and the four microbial service samples were combined into a second pool. After pooling and before sequencing each pool was quantified with Qubit and analyzed with Femto Pulse to determine sequencing settings and then sequenced using a single SMRT Cell® on a Sequel IIe sequencer according to the manufacturer's guidelines. All data analysis was performed by EMBL.

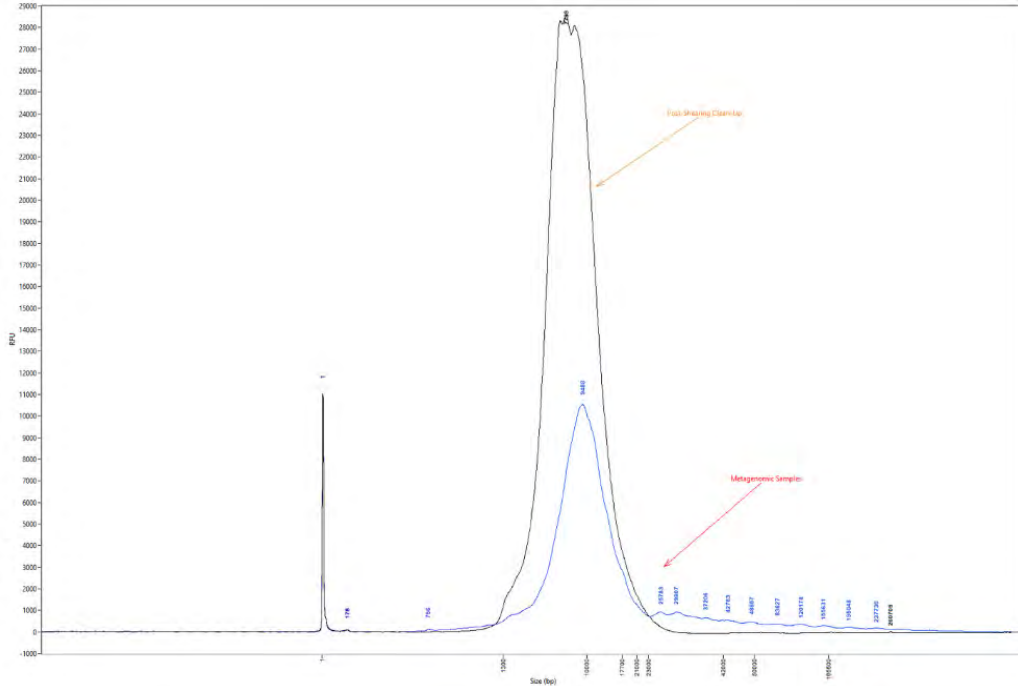
## Results

Well ID	Sample Type	Pre-Shear (kb)	Post-Shear and Clean (kb)	% Difference	Library Size (kb)	Final Conc. (ng/ $\mu$ L)
A1*	Commercial	148.3	24.2	143.9	28.9	28.6
B1	Commercial	156.7	32.8	130.8	31.1	43.2
C1	Commercial	146.1	24.7	142.2	27.8	39.2
D1	Commercial	145.7	22.8	145.9	24.9	33.8
E1	Service	15.3	15.5	1.3	16.1	56
F1	Service	20.7	16.3	23.8	16.5	39.6
G1*	Service	9.4	7.7	19.9	8.4	53.4
H1	Service	24.8	17.8	32.9	21.7	44.4

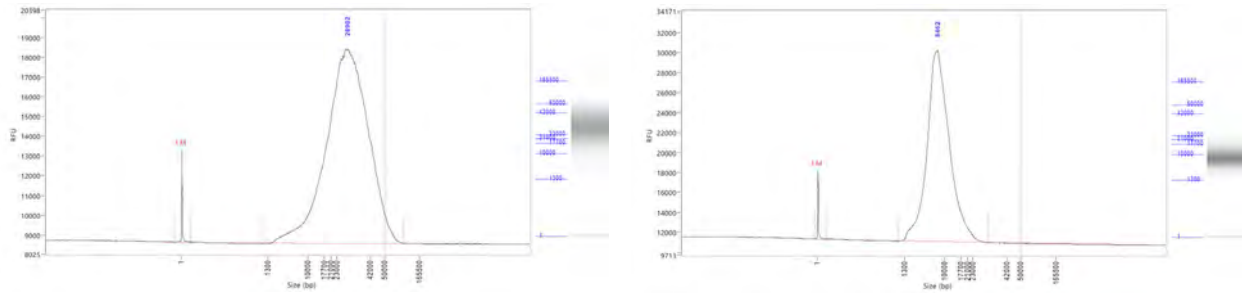
**Table 1. Comparison of library size and concentration of gDNA samples before and after automated pipette shearing and library construction.** Library size was determined by Femto Pulse and final concentration (conc.) was determined by Qubit. \*Samples have Femto Pulse traces shown in additional figures.



**Figure 4. Size distribution of commercial gDNA sample (A1) before and after automated pipette shearing.** Library size was determined by Femto Pulse and showed a 143.9% difference between pre-shearing (black line) and after automated pipette shearing and shearing cleanup (blue line).



**Figure 5. Size distribution of metagenomic service sample (G1) before and after automated pipette shearing.** Library size was determined by Femto Pulse and showed a 19.9% difference between pre-shearing (black line) and after automated pipette shearing and shearing cleanup (blue line).



**Figure 6. Size distribution of final libraries for commercial sample (A1, left) and metagenomic service sample (G1, right).** Library size was determined by Femto Pulse.

Well ID	Sample Type	Barcode Quality	HiFi Reads	HiFi Read Length (mean, bp)	HiFi Yield (bp)
A1*	Commercial	98.0	339087	15145	5135655884
B1	Commercial	98.0	252826	14444	3651994740
C1	Commercial	98.0	313665	15553	4878593012
D1	Commercial	98.0	340979	15271	5207330580
E1	Service	98.0	414750	15264	6330849158
F1	Service	98.0	545453	16404	8947760577
G1*	Service	98.0	680291	9622	6546376125
H1	Service	98.0	570452	17614	10048428745

**Table 2. Sequencing report for commercial and service libraries prepared with the automated pipette shearing and SMRTbell prep kit 3.0 Biomek i7 Hybrid Workstation method and sequenced on a Sequel IIe system.**

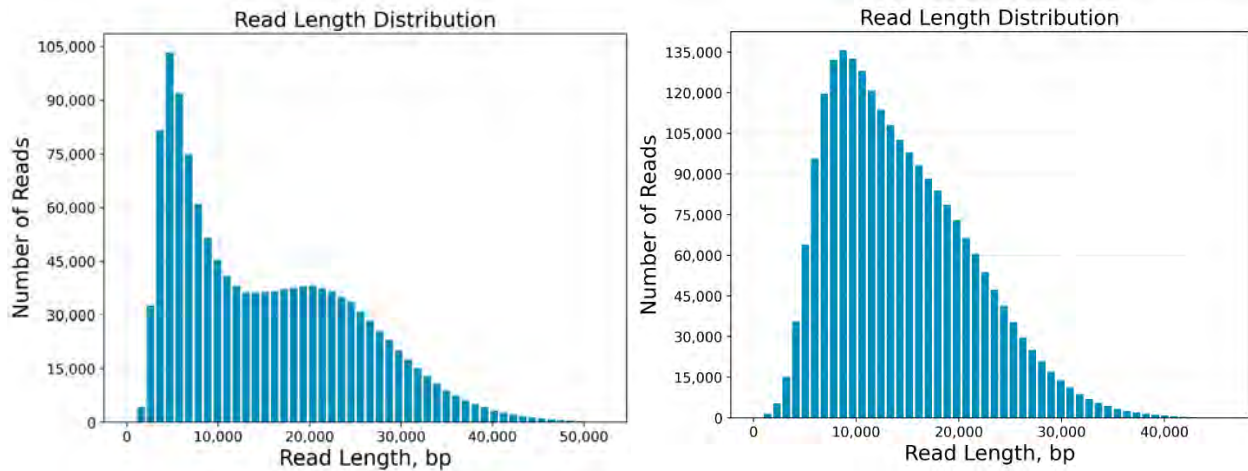


Figure 7. Read Length Distribution for commercial sequencing pool (left) and metagenomic service sequencing pool (right).

## Summary

Automating pipette shearing of DNA and SMRTbell prep kit 3.0 library construction on the Biomek i7 Hybrid Workstation delivers:

- An efficient and robust automated method for shearing DNA to sizes intended for long-read library construction.
- High-quality and high-yield libraries across commercial and service samples.

Overall, this data shows the automated workflow with pipette shearing and SMRTbell prep kit 3.0 library construction on the Biomek i7 Hybrid Workstation showcases a streamlined, versatile, and robust solution for long-read library preparation.

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