APPLICATION NOTE No. 497

HiFi Library Preparation Using PacBio® SMRTbell® Prep Kit 3.0 on epMotion®

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Introduction

The preparation of high-quality SMRTbell® libraries is essential for achieving optimal sequencing performance with PacBio® platforms. Automation of this process enhances reproducibility, minimizes hands-on time, and reduces potential sources of human error.

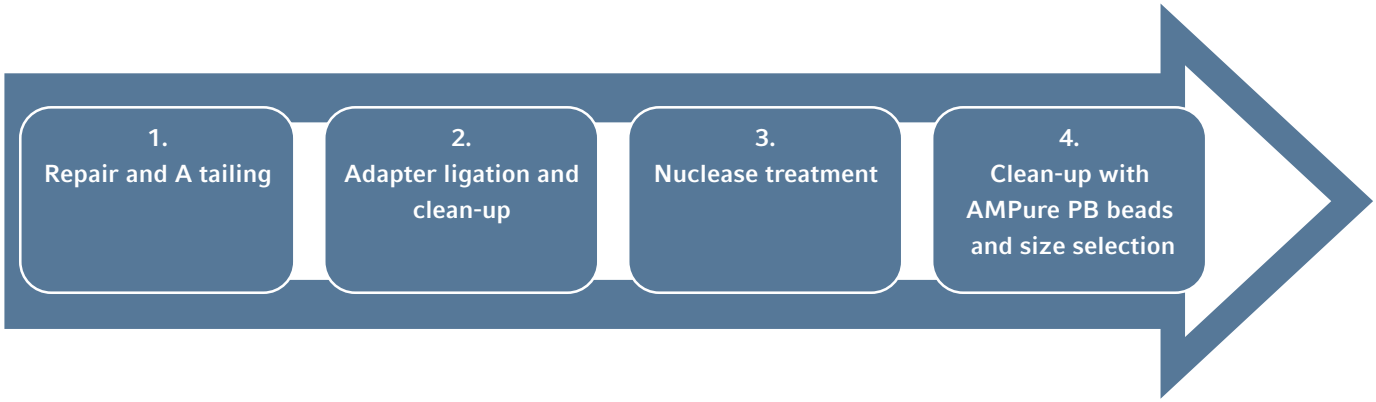
This application brief summarizes the successful implementation of the PacBio® SMRTbell® prep kit 3.0 on the Eppendorf epMotion automated liquid handling system, demonstrating high efficiency, yield, and quality of prepared libraries.

User benefits

> High Yield and Consistency – Delivers reproducible library preparation with minimal variability between runs.
> Suitable for Long-Read Sequencing – Produces high-quality libraries and enhances efficiency for laboratories using PacBio sequencing platforms.
> Reliable and Automated Solution – Seamless integration of the PacBio® SMRTbell® prep kit with the Eppendorf epMotion system ensures a standardized workflow with improved data accuracy, reliability and reproducibility.
> Time-Saving Workflow – Reduces hands-on time, allowing researchers to focus on data analysis and other tasks.

In cooperation with Pacific Biosciences Inc.

Workflow
The library preparation workflow on epMotion 5075t for processing 24 samples consisted of the following steps.



Sheared gDNA from *E. coli* (fragment size 8-10 kb) was used as the starting sample for both the manual as well as epMotion runs. The epMotion system was programmed to execute the end-repair, ligation, and bead purification steps with precision, ensuring consistent and high-quality libraries with no user intervention required during the process. The walk-away time when processing 24 samples was 250 minutes or about 4 hours and 10 minutes.

Results

Yield and Quality Assessment
The automated workflow on the epMotion system consistently produced SMRTbell® libraries with high yield and quality. The following key metrics were observed:

> **Library Yield:**
The library concentrations prepared with the SMRTbell® prep kit 3.0 on the epMotion were within the expected range. The repeatability was improved using an automated process (CV 4%) compared to a manual process (25%)

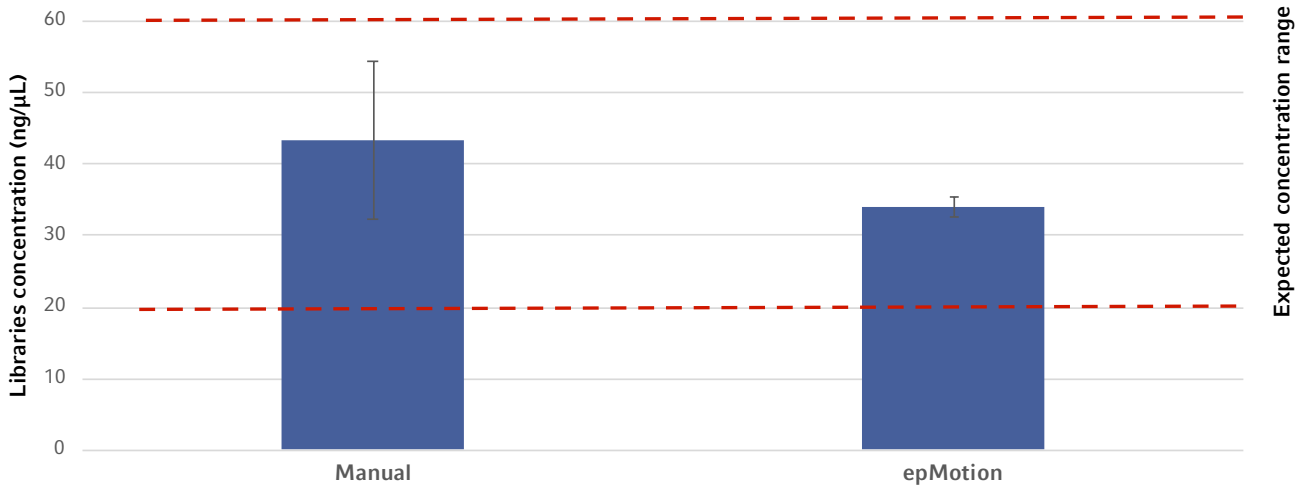
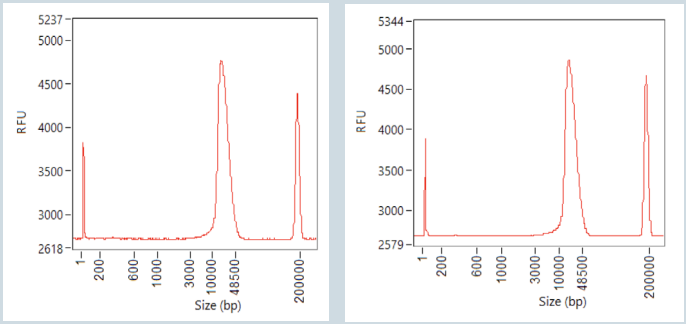


Figure 1: Libraries were generated using SMRTbell prep kit 3.0 on the epMotion. Repeatability was improved markedly with automation (CV 4%) compared to manual methods (CV 25%).

> Library fragment size:

The fragment size of the libraries was checked using the TapeStation and Femto Pulse™ (Agilent Technologies® Inc). The libraries were within the expected range (15-20 kb). Comparison of the manual workflow data and the automated runs from Tape Station showed that the quality of results when following automated workflow is not compromised.

A



B

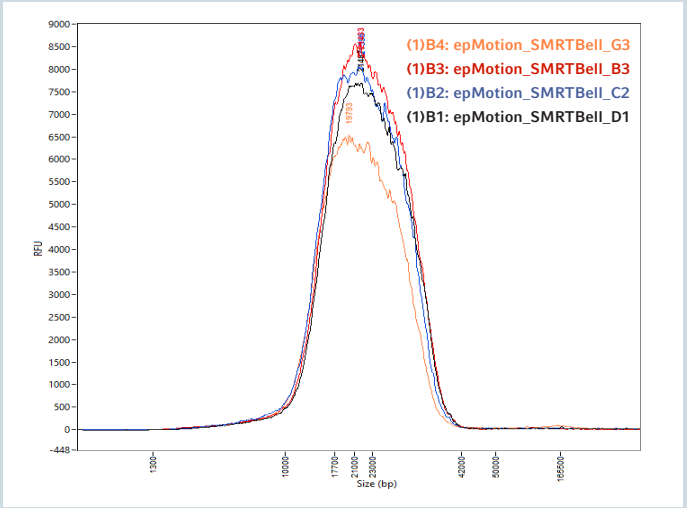


Figure 2: A) Library fragment sizes analysed by TapeStation were within the expected 15–20 kb range confirming that automated workflows maintained data quality comparable to manual methods. B) Library fragment size was also analysed by Femto Pulse™ System, confirming their high molecular weight.

> Sequencing Performance:

Initial sequencing runs demonstrated quality of the libraries prepared using the epMotion. The libraries were sequenced on a PacBio Revio® system using Revio v1 chemsitry and achieved results that met or exceeded the acceptance specifications.

		Revio sequencing using epMotion for sample prep (plate loading concentration 225pM)	Acceptance criteria – cutoff value
		Mean	
Metric	Femto Peak (kb)	21077	10000-25000
	ABC % Recovery	78.0	60
	P1 loading	0.5	0.5
	HiFi Reads (M)	4.7	1
	HiFi Yields (Gb)	87.4	80 (Revio)
	HiFi Read Length (kb)	18.8	10
	Read Quality	Q33	Q30
	Polymerase Read Length (kb)	76.5	60
	Base Rate (bp/s)	2.5	2
	SMRTBell % recovery	21	20

Table 1: Libraries prepared with epMotion demonstrated high quality, as sequencing on the PacBio Revio® system met or exceeded acceptance criteria.

Summary

Overall, this data shows the automated workflow for SMRTbell® prep kit 3.0 library construction on the Eppendorf epMotion liquid handling system showcases a high-quality, versatile, and robust solution for PacBio® library preparation.

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