PO0651

A high-throughput, low-cost automated library prep method for PacBio long-read sequencing at scale

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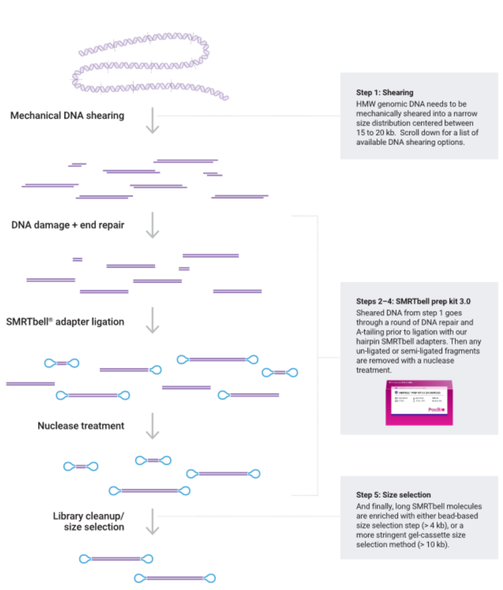
Introduction

Bottlenecks in long-read library prep workflows such as DNA shearing (aka fragmentation) and size selection are barriers to scaling long-read sequencing (LRS) independent of sequencing costs. Here we present a new automated high-throughput library prep method for PacBio native long-read sequencing that removes these bottlenecks, dramatically lowers costs, and operates in a 96-well plate format.

Library prep workflow for PacBio lrWGS



For more information on WGS with PacBio

Figure 1. DNA library prep workflow for PacBio HiFi sequencing

An automated *Short Read Eliminator* (SRE) protocol improves sample quality and HiFi read length

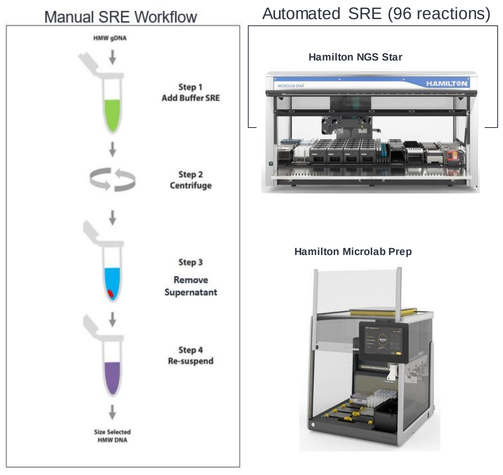


Figure 2. All pipetting steps of the manual SRE protocol were automated on the Hamilton NGS STAR MOA 96 and Microlab Prep systems. Automating the pipetting steps enables the preparation of up to 96 samples in a single run, ensures consistent results, and reduces the risk of losing the DNA pellet.

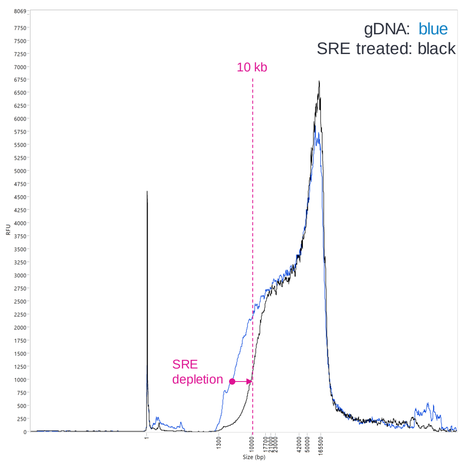


Figure 3. Agilent Femto Pulse electropherogram with original genomic DNA (blue) overlaid with the SRE treated fraction (black). DNA less than 10 kb is progressively depleted. The SRE protocol is performed using liquid handling automation

Improving the quality of the genomic DNA prior to the shearing step translates to increased HiFi read length.

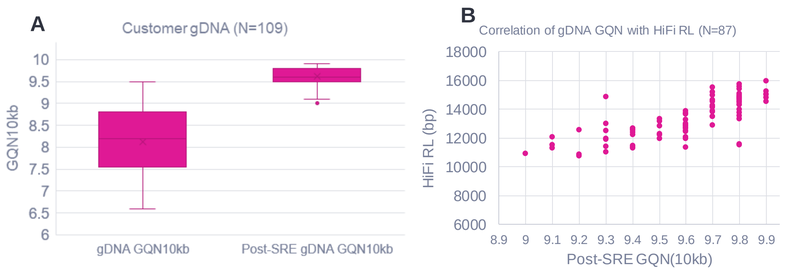


Figure 4. The genome quality number (GQN) of 109 genomic DNA samples increased from an average of 8.1 (pre-SRE) to 9.6 after SRE treatment as measured by the Agilent Femto Pulse system (A). An increase in GQN is correlated with higher HiFi read length on the Revio system. All 87 samples that were sequenced used a bead-based size selection on the library.

An automated SRE protocol improves sample quality and HiFi read length (continued)

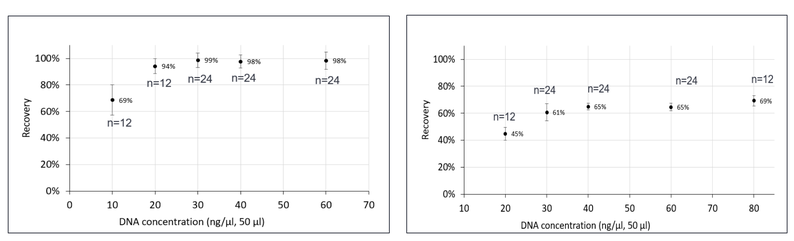
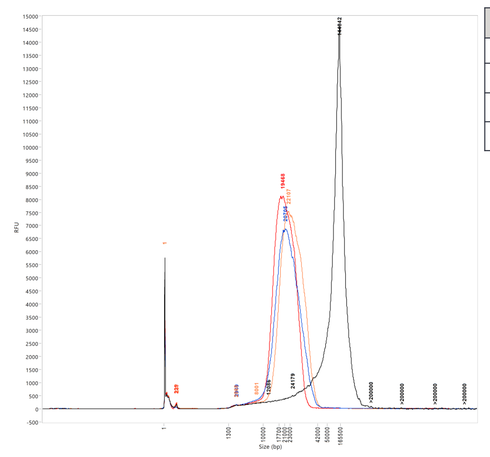


Figure 5. DNA recovery after SRE will depend on concentration and quality. High-quality samples with sufficient input will have better recoveries. User can expect an average of ≥60% recovery on most samples when using a recommended 3 µg of DNA input.

Automating DNA shearing for LRS

In addition to the SRE protocol to improve sample quality and read length, we've developed a quick, robust, and cost-effective method to shear DNA using Hamilton NGS liquid handler systems.



Femto Pulse 165 kb	Femto size (bp)
gDNA	144.8kb
Microlab Prep	19.5 kb
Megaruptor3	20.7 kb
NGS STAR MOA 96	22.1 kb

DNA shearing on Hamilton systems produces effectively equivalent size distribution profiles as compared with the Diagenode Megaruptor 3 system

Figure 5. Agilent Femto Pulse system electropherogram image of HMW gDNA (black) sheared on the Megaruptor 3 (blue), Hamilton Microlab Prep (red), and the Hamilton NGS STAR MOA 96 system. The settings used produce the size distribution recommended for HiFi sequencing with peak at ~20 kb.

Workflow for automated DNA shearing

Shear up to **96 samples** in **8 minutes** for the cost of a pipette tip. The full workflow, including preparing for shearing and concentrating the DNA sample prior to library prep, is shown below.

Quant gDNA	Prepare gDNA	Shear gDNA	QC	1X cleanup	SMRTbell prep kit 3.0
Quant using a dsDNA assay (e.g., Qubit)	Dilute and/or normalize gDNA to ≤10 ng/µL	Run shearing method on Hamilton instrument	Estimate sheared gDNA size on Agilent Femto Pulse system	Concentrate sheared gDNA for library prep	Manual or automated LP

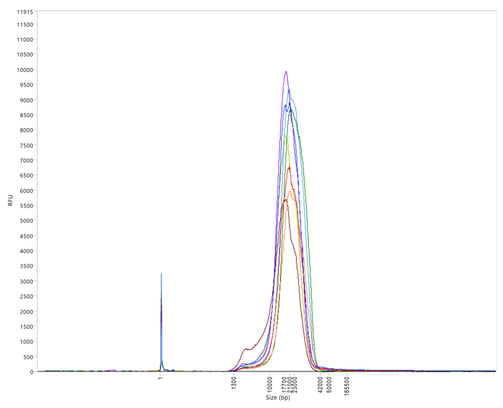
Hamilton NGS STAR

Microlab prep

PacBio developed methods for both the NGS STAR and Microlab Prep systems

Table 1. Settings for shearing DNA using Hamilton NGS liquid handler systems. These settings are exclusive to Hamilton systems and therefore may not work on other systems. To achieve the proper size distribution profile the genomic DNA must be dilute (≤10 ng/µL). If DNA is too concentrated, the shearing size distribution will be too broad, or the sample may fail to shear.

Parameter	Settings
Volume	300 µL (low TE buffer)
DNA conc.	≤10 ng/µL
No. of cycles	300
Speed	500 µL/sec
Tips	300 µL conductive filter
Liquid following	83% volume



Sample well	Femto GQN (>10kb)	Femto size (bp)
A5	9.1	17,701
B5	9.5	19,477
C5	9.3	18,335
D5	7.6	18,335
E5	9.6	20,937
F5	9.6	20,233
G5	9.4	20,156
H5	9.2	20,079

Figure 6. Agilent Femto pulse electropherogram of 8 plant genomic DNA samples, sheared (at different concentrations ≤10 ng/µL) on the Hamilton NGS STAR system. This demonstrates the method is consistent across different samples and concentrations.

Library prep automation

Table 2. PacBio qualified automated library prep protocols, including polymerase binding.

	Hamilton	Integra	Revvity	Tecan
DNA shearing for WGS protocol				
HiFi sequencing 13–22 kb	• NGS STAR • STARlet • STARv • Microlab Prep	-	-	-
WGS library prep protocol				
SMRTbell prep kit 3.0	• NGS STAR	Miro Canvas	SciClone NGSx	DreamPrep Compact
Polymerase binding protocol				
SequelII binding kit 3.2	• NGS STAR	-	-	DreamPrep Compact
Revio polymerase kit	• NGS STAR	-	-	DreamPrep Compact

Revio sequencing performance for end-to-end automated workflow

Combining the new automated SRE and shearing protocols with previously qualified SMRTbell prep kit 3.0 protocols makes for an end-to-end automated solution that precludes the need for manual, gel-based size selection.

Metric	Value
HiFi reads	6.1 M
HiFi reads yield	101.22 Gb
HiFi read length N50	18,016 bp
HiFi read quality (median)	Q34
Base quality ≥Q30 (%)	92.78%

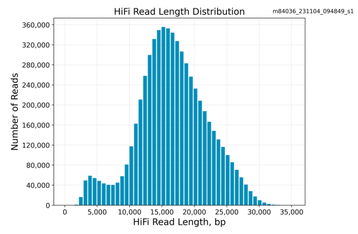


Figure 7. Revio sequencing results for human cell line DNA (HG002) prepared using automated protocols for SRE, DNA shearing, and SMRTbell library prep. Sequenced on a single Revio SMRT Cell using a 24-hour movie time.

Adding SRE improves average read lengths by >4 kb. Moreover, there is no introduction of GC bias by using SRE.

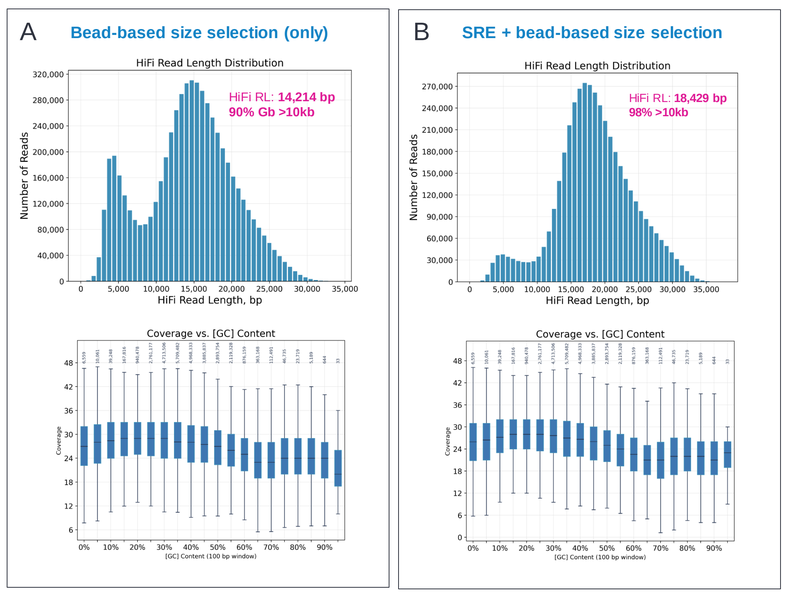


Figure 8. Revio HiFi read length for the same HG002 DNA using bead-based size selection only (A) versus an upfront SRE treatment (B). The addition of the SRE increased the mean read length by >4 kb. Also shown, HiFi read depth coverage by GC% bin.

Table 3. Assembly performance of HG002 DNA that was prepared using the automated SRE, shearing, and library prep workflow. Assembly performed using hifiasm.

Method	Coverage	Shearing	NL 50
Revio, SRE + AMPure PB	31×	NGS STAR	• Hap 1, 43 Mb • Hap 2, 46 Mb

For more animal and plant results using SRE + automated shearing, please see poster **PO0633**: ***High-throughput HMW DNA animal blood extraction and sequencing on the PacBio Revio system***.

Conclusions

The introduction and development of automated protocols for SRE and DNA shearing, combined with previously qualified automated library prep protocols, solves barriers to scaling by providing an end-to-end automated workflow. Shearing DNA using a liquid handler is a fraction of the cost of previous methods using Covaris gTUBEs or Diagenode Megaruptor 3 hydropores, while the SRE precludes the need to do any post-library gel-based size selection enabling greater labor, time, and cost savings.

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