Technical note

SIZE SELECTION OF PACBIO SMRTBELL LIBRARIES WITH THE LIGHTBENCH INSTRUMENT FROM YOURGENE HEALTH

Introduction

Precise size selection is essential for maximizing the benefits of PacBio® highly accurate long-read sequencing. The *LightBench®* from Yourgene Health uses *Ranger® Technology* to deliver accurate size selection for whole genome sequencing and includes the ability to perform fragment length analysis for additional quality control. With an automated workflow to optimize walk-away time, the *LightBench®* offers a scalable size selection solution for the preparation of SMRTbell® gDNA libraries for HiFi sequencing.

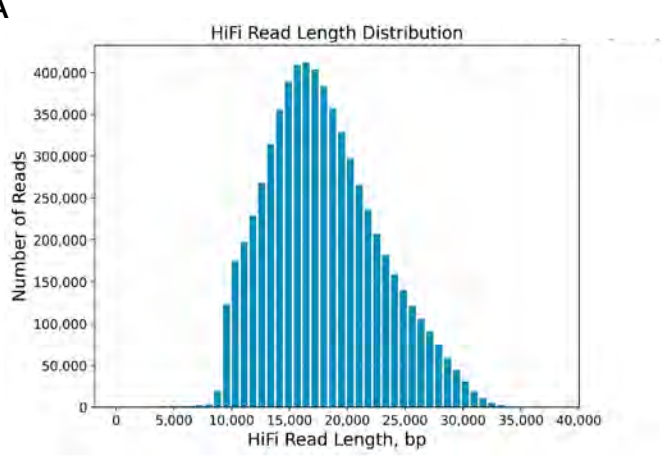
Input DNA	500 ng–2.0 µg
Throughput	12 samples
Run time	~3 hours
Hands-on time	~20 minutes
8 kb cutoff	~83% recovery*
10 kb cutoff	~67% recovery*

*Size selection recovery of SMRTbell library.

This technical note provides an overview of the size selection workflow for PacBio SMRTbell sequencing libraries with the *LightBench®* and reports experimental results demonstrating effective size selection at two fragment lengths.

In experimental benchmarking of 84 human gDNA samples from fresh blood for library preparation for HiFi long-read sequencing, the *LightBench®* achieved consistent yields for 8 kb or 10 kb cutoffs across all samples and a 0% failure rate. The 8 kb cutoff yielded an average recovery of ~83% of the input SMRTbell library while the 10 kb cutoff recovered an average of ~67%.

A



B

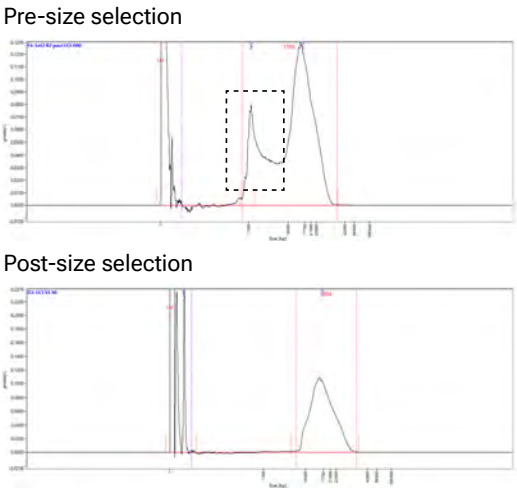


Figure 1. Representative HiFi read length distribution (A) and comparison of pre- and post-size selection on the Agilent *Femto Pulse* system (B) for 10 kb cutoff demonstrates effective removal of <10 kb fragments.

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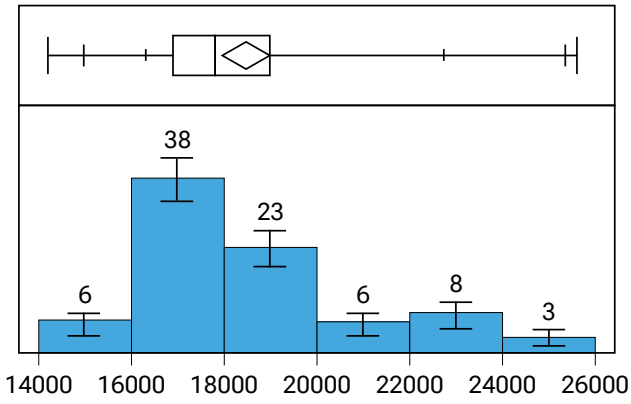

Figure 2. Mean read length distribution across 84 samples on the Revio™ system.

Following library preparation and size selection with the *LightBench®*, 84 samples were sequenced on the Revio™ platform. Mean HiFi read length was 18,517.5 bp (figure 2, max: 25,613 bp; min: 14,202 bp) and mean HiFi data yield was 101.27 Gb/SMRT® Cell (figure 3).

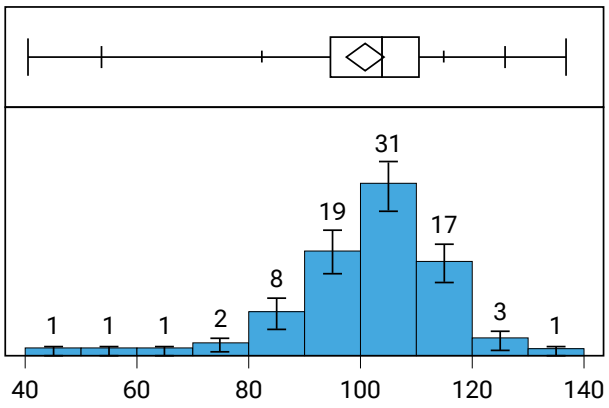

Figure 3. HiFi yield (Gb) distribution across 84 libraries on the Revio system.

These results demonstrate the *LightBench®* as an effective method for the size selection of HiFi libraries. See the workflow overview for a summary of the recommended size selection procedures and visit Yourgene Health for detailed protocol steps.

Workflow overview

Required materials and equipment

Equipment	Part number
LightBench®	CG-12500-03
Materials	
Cassette and Dual Dye Loading Buffer kit	CG-14100-13-050-31-31
Includes	
Dual Dye Loading Buffer 7 kb + 7 kb markers	CG-14000-31-31
0.5% *In-Channel Filter Size-selection* cassette	CG-10600-13-050

Workflow steps

1. Prepare the *Ranger® Technology* software setup according to Yourgene *LightBench®* recommendations.
2. Prepare the samples by combining with *Dual Dye Loading Buffer* containing the 7 kb marker (CG-14000-31-31). Mix thoroughly to homogenize and spin down to remove any air bubbles.
3. Prepare the cassette and remove excess buffer from the reservoir.
4. To load samples into the cassettes, remove recommended volume of buffer from each loading well, and dispense the full volume of aspirated samples into loading wells.
5. Allow the electrophoresis process to proceed.
6. For sample extraction, remove the cassette from the instrument and rinse the extraction wells.
7. Refill extraction wells with 1×TBE buffer and insert the *In-Channel Filter* (ICF) array into the extraction well. Fill ICF collection chambers with 1×TBE buffer.
8. Return the cassette to the *LightBench®* drawer tray and allow the extraction process to proceed. Extract targeted material when prompted by the software.
9. Size selected fractions should be bead-cleaned and assessed for suitable concentration and fragment length distribution prior to sequencing.

To learn more, visit yourgenehealth.com

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