



Technical Note

Library Prep



Preparing Libraries for PacBio® Whole Genome Sequencing for *de novo* Assembly: Quality Control and Size Selection

Introduction

Single Molecule, Real-Time (SMRT®) Sequencing uses the natural process of DNA replication to sequence long fragments of native DNA. As such, starting with high-quality, high molecular weight genomic DNA will result in longer libraries and better performance across difficult to sequence regions of the genome. This technical note is intended to give recommendations, tips and tricks for assessing and preserving the quality and size of your SMRTbell® library, and size selection procedures for libraries intended to be used with whole genome sequencing for *de novo* assembly.

Topics Covered

Library Quality Control (QC)

- Quantification
 - Qubit® fluorometer
 - NanoDrop® spectrophotometer
- Size
 - CHEF Mapper® System
 - Pippin Pulse™ System
 - FEMTO Pulse™ System

Library Size Selection

- BluePippin™ System

Library QC

Quantification

After library preparation, we recommend measuring the concentration of the DNA library present using fluorometric quantitation, for example using the Qubit fluorometer. Library preparation should remove anything other than double-stranded DNA from the sample; therefore, it is also acceptable to measure library concentration with spectrophotometric methods, e.g. using a NanoDrop device.

Size

It is important to determine the size distribution of the final purified SMRTbell library using one of the following methods for accurate input into Sample Setup when running PacBio Systems:

1. [CHEF Mapper System \(BioRad\)](#)
2. [Pippin Pulse System \(Sage Science\)](#)
3. [FEMTO Pulse System \(Advanced Analytical\)](#)

Library Size Selection

BluePippin System

The size selection protocol to be used on the BluePippin System will depend on the size distribution of the library as determined by the previous QC step.

- If the mean library size is between 30 and 100 kb, then we recommend size selecting the library with the BluePippin System using the “0.75% DF Marker U1 high-pass 30-40 kb v3” kit and cassette definition protocol.
- If the DNA distribution is shorter than 30 kb, meaning the majority of the library DNA molecules range in lengths between 10-30 kb, then we recommend size selecting the SMRTbell library on the BluePippin System using the “0.75% DF Marker S1 high-pass 15-20 kb” kit and cassette definition protocol. This lower selection cutoff will ensure suitable library yields for sequencing, but will also affect the overall read lengths and yield for the sequencing run.

For more details on operation considerations, please see the protocol details from SAGE Science for each respective kit.

Note: It is important to understand the sequencing variables affected by various size selection methods/ranges. If an abundance of library is generated, selecting the highest-attainable size cutoff will result in optimal read lengths and yield for the sequencing run. However, if library amount is limited, it may be advantageous to reduce the cutoff size to ensure enough library for sequencing the genome to the desired depth. Depending on the size of the overall library, one can see a 50% reduction in library yield after stringent size selection.

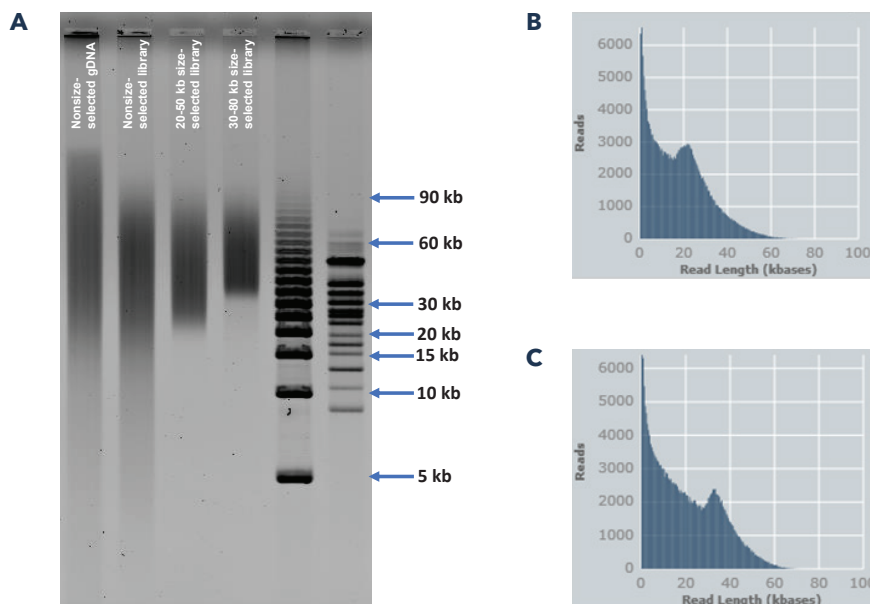


Figure 1. Library size and sequencing results.
A: Pippin Pulse gel showing non size-selected genomic DNA (gDNA) in lane 1, nonsize-selected library in lane 2, 20-50 kb size-selected library in lane 3, 30-80 kb size-selected library in lane 4, and ladders in lanes 5 and 6. **B:** Results of sequencing one SMRT Cell with the 20-50 kb size-selected library from lane 3 of A. **C:** Results of sequencing one SMRT Cell with the 30-80 kb size-selected library from lane 4 of A.

Additional Considerations for SMRTbell Library Preparation and Sequencing

- Reduce or eliminate freeze/thaw cycles of your library to prevent damage.
- **Storage:** If you are planning to sequence within ~1 week of library generation, storing the library at 4°C in Elution Buffer, which comes in PacBio sequencing kits, is recommended. For storage longer than 1 week, aliquot the library and store at -20°C.
- **Shipping:** It is recommended to ship libraries in Elution Buffer at 4°C with overnight shipping priority.