NIPPON Genetics Co.,Ltd.

# Application Note

Product Name : BluePippin (BLU0001)
Manufacturer : Sage Science
Application : The effect of size selection of PacBio long-fragment libraries (≧7 kb) (comparison of size distribution and sequencing results)

The following data were provided by the courtesy of Dr. Yasuhito Arai of Division of Cancer Genomics, National Cancer Center Research Institute,Japan.

## Method

In the previous study, sequencing without size selection resulted in an average subread length of 1,289 bp, which increased to 2,163 bp after the library was size-selected for fragments of 4 kb or larger by BluePippin (High-pass mode 4kb-50kb). (See Application Note 2013 <21>)

This time, we used BluePippin to carry out size selection for longer fragments (≧7 kb) (High-pass mode 7kb-50kb) and checked the amount of DNA recovered after BluePippin size selection.
In addition, library size distribution before and after size selection was compared to evaluate whether size selection is effective for increasing the PacBio sequencing length.

● **Sample and library preparation**
Library preparation was performed using human genomic DNA following the protocol for 20 kb SMRTbell™ library. The libraries were purified using ×0.45 AMPureXP.

● **Genomic DNA fragmentation**
g-Tube (Covaris) *centrifuged in Eppendorf-5415R centrifuge at 5,400 rpm

● **Conditions for BluePippin size selection**

| | |
|---|---|
| Sample load | : 6,210 ng/30 µL per lane |
| Gel cassette | : 0.75% gel cassette Marker S1 (BLF7510) |
| Extraction condition | : set to high-pass mode 7kb-50kb (size selection and recover 7 kb or longer) |

● **Conditions for PippinPulse pulsed-field electrophoresis**

| | |
|---|---|
| Power supply | : Pippin Pulse (PPI0200) from Sage Science, 2-50kb program 16 hr |
| Electrophoresis gel tank | : Midi plus-2 (ME1571015) from Major Science |
| Agarose | : SeaKem GOLD from Lonza, 0.75% 100 mL |
| Electrophoresis buffer | : ×0.5 KBB buffer from Sage Science |

● **Quantification of DNA recovered after BluePippin size selection**
Qubit® 2.0 Fluorometer (Life Technologies)

● **Evaluation points**
(1) DNA quantity recovered after BluePippin size selection
(2) Comparison of size distribution before and after BluePippin size selection (confirmation of size selection accuracy)
(3) Effectiveness for increasing sequencing length

**<Workflow>**

Genomic DNA extraction
↓
Fragmentation in g-Tube
↓
20 kb SMRTbell™ library preparation
↓
BluePippin size selection, recovery of ≧7 kb fragments
↓
Purification, sequencing

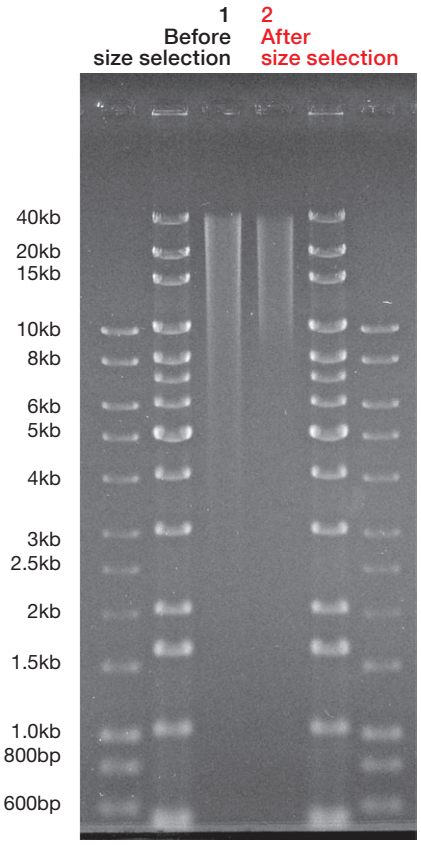
● **Sequencer**
PacBio *RSII*
(Pacific Biosciences)

**BluePippin**
Capable of performing pulsed-field electrophoresis.
Optimum for size selection of long size DNA fragments.

**PippinPulse, pulsed-field electrophoresis power supply**
Enables easy performance of pulsed-field electrophoresis.
Optimum for checking sizes of long size DNA fragments.

## Results

### Result of BluePippin size selection for ≧7 kb fragments




**Evaluation 1: Amount of DNA recovered after BluePippin size selection**

| | |
|---|---|
| BluePippin sample load | : 6,210 ng/30 μL……before size selection |
| Solution recovered | : 61 μL (used 1 μL for Qubit quantification) |
| Qubit quantification result | : 44 ng/μL |
| Total DNA recovered | : 2,684 ng/61 μL……After size selection |

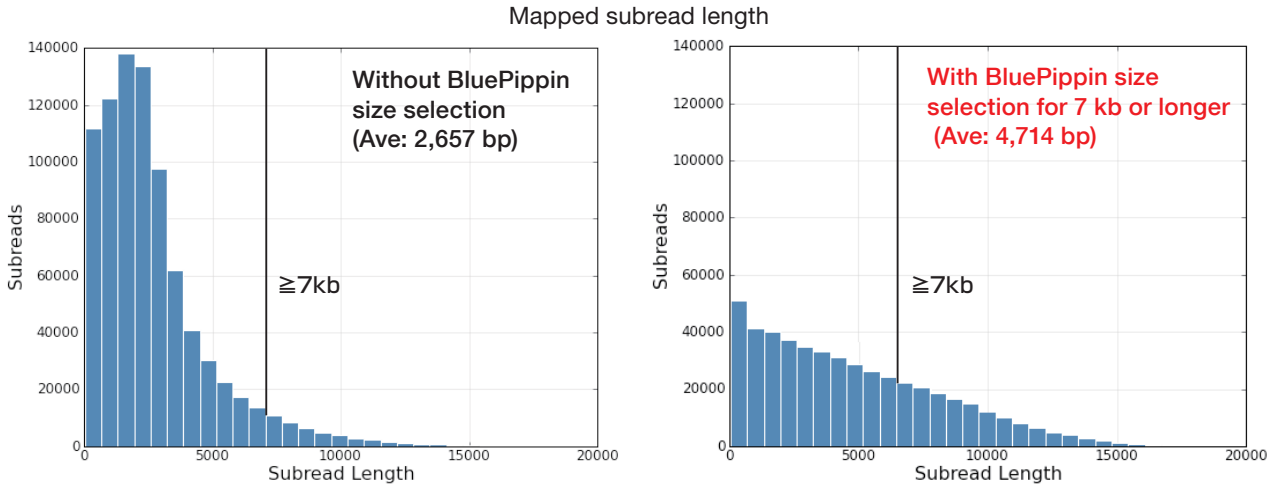
6,210 ng of sample was applied per lane in BluePippin and extracted at High-Pass mode ≧7 kb. 2,684 ng was recovered as long-chain library.

**Evaluation 2: Comparison of library size distribution before and after BluePippin size selection (confirmation of size selection accuracy)**

<PippinPulse pulsed-field electrophoresis (figure on the right)>
Lane 1 Before BluePippin size selection : around 300 ng/5μL
Lane 2 After BluePippin size selection : 220.0 ng/5μL

The result of PippinPulse pulsed-field electrophoresis (figure on the right) demonstrates that BluePippin size selection enables recovery of long libraries and effective removal of short libraries.

### Mapping data of PacBio sequences (comparison of subread length distribution)




(Analyzed by Dr. Hama of National Cancer Center Research Institute)

**Evaluation 3: Effectiveness for increasing sequencing length**

The mapped subread length was compared. When sequencing was performed without BluePippin size fractionation, the average subread length was 2,675 bp. Meanwhile, in the library size-selected by BluePippin, the average subread length increased to 4,714 bp.

**<Customer's comments>**

In order to take advantage of PacBio long reads in genome sequencing, it is crucial to prepare a library enriched for long size DNAs.
Accurate size selection by BluePippin could substantially increase the sequencing length in human genomic DNA.

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