NIPPON Genetics Co.,Ltd.

Application

# Size selection using BluePippin in library preparation with MinION

Product Name: Automated DNA size selection system BluePippin（Cat.No. BLU0001）

Manufacturer: Sage Science, Inc.

The following data is provided by the courtesy of Kazuharu Arakawa, Associate Professor, Institute for Advanced Biosciences, Keio University, Japan.

## Background

Generally in the next-generation sequencing, shorter library data tends to be acquired preferentially up to now. In this case, to prepare a library of samples separated and collected in a desired size by "size selection" is a solution for obtaining the desired data more effectively.
This application note highlights an example of results from sequencing using MinION (Oxford Nanopore Technologies) a library prepared by size-selected samples using BluePippin.

## Experimental conditions and procedures

● **Species**
One leg of adult *Nephila clavipes*

① Sample DNA extraction
Extract DNA using Qiagen Genomic-tip 20/G
（The final elution volume is 100 μL in Buffer EB（Qiagen）following precipitation of isopropanol）

② Check the size with Agilent TapeStation Genomic ScreenTape
Measure the DNA quantity using Invitrogen™ Qubit™ system

③ Using BluePippin, perform size selection of DNA samples（10 kb or more）
Gel cassette: 0.75% gel cassette Marker S1（BLF7510）
Extraction conditions: high-pass mode 0.75% DF Marker S1 High-Pass 6-10kb vs3
Input DNA quantity: 10 μg/well（30 μL）

Cat.No. BLU0001

④ Check the size with Agilent TapeStation Genomic ScreenTape
Identify the DNA quantity using Invitrogen™ Qubit™ system

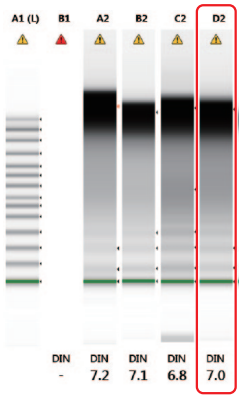
⑤ Library preparation
Library preparation kit: ONT Ligation Sequencing Kit 1D（SQK-LSK108）
Input DNA quantity: 1 μg（45 μL）
*Library preparation points（protocol changes）*
- Skip fragmentation / repair to start with end preparation
- Change the incubation time during end preparation to 15 minutes at 20 ℃, 5 minutes at 65 ℃ from 5 minutes at 20 ℃, 5 minutes at 65 ℃ which are specified in the protocol.
- Change the incubation time for Adapter Ligation to 30 minutes at room temperature from 10 minutes at room temperature.
- Change the volume of AMPure XP（Beckman-Coulter）for Adapter Ligation to 45 μL from 40 μL.

⑥ Check the size with Agilent TapeStation Genomic ScreenTape

⑦ Sequence analysis using MinION（Oxford Nanopore Technologies）

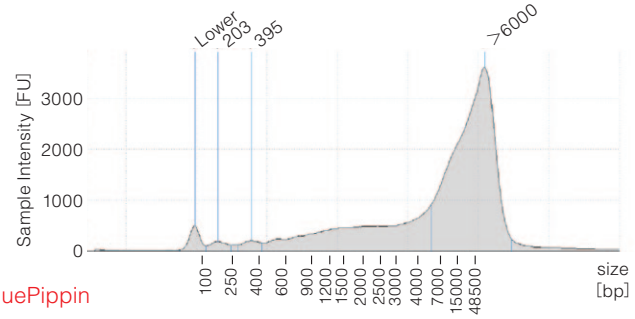
## Results

② Checking the size with Agilent TapeStation Genomic ScreenTape (before use of BluePippin)

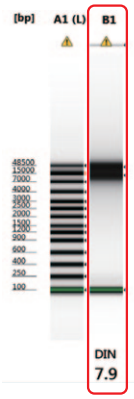



←Before use of BluePippin

| Well | DIN | Conc. [ng/μl] |
|---|---|---|
| D2 | 7 | 349 |

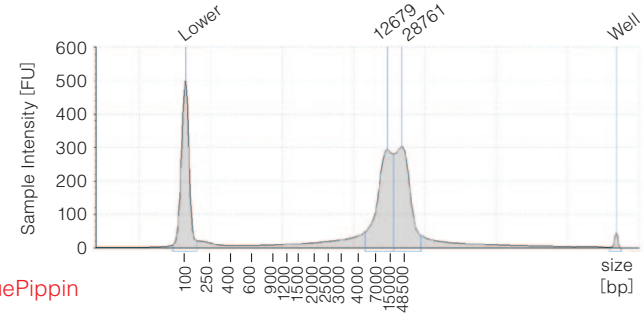



② Checking the size with Agilent TapeStation Genomic ScreenTape (after use of BluePippin)

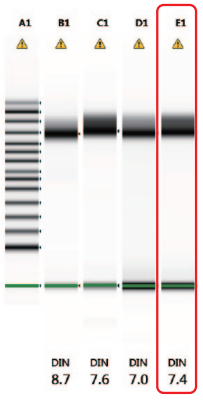



←After use of BluePippin

| Well | DIN | Conc. [ng/μl] |
|---|---|---|
| B1 | 7.9 | 26.9 |

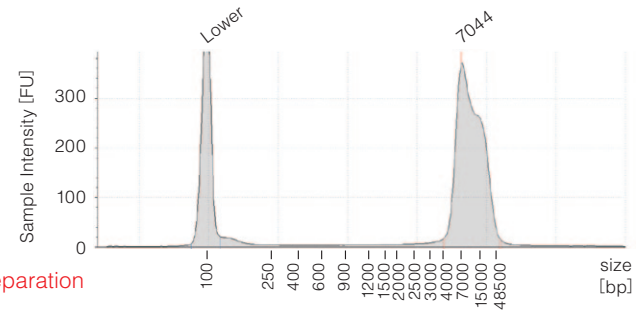



⑥ Checking the size with Agilent TapeStation Genomic ScreenTape (after library preparation)

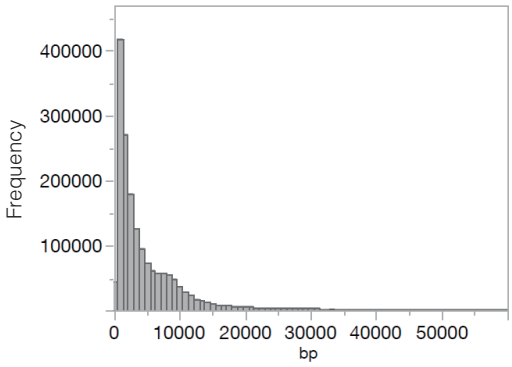



←After library preparation

| Well | DIN | Conc. [ng/μl] |
|---|---|---|
| E1 | 7.4 | 19.4 |




⑥ Sequence result




| | |
|---|---|
| Read number | 1,670,827 |
| Total read length | 7,655,872,327 |
| Average read length | 4,582 |
| Longest read | 566,198 |
| Shortest read length | 5 |
| N50 | 8,489 (#268112) |
| N90 | 2,034 (#956151) |

As a library for MinION is prepared while tethers which help DNA ends move to pores are attached, it is not possible to perform size selection after a library is prepared (tethers are detached by electrophoresis). Therefore, size selection needs to be performed before library is prepared. Meanwhile, DNA is somewhat fragmented through such steps as pipetting performed during library preparation or drying of purified AMPure XP which is performed repeatedly, which causes short DNAs to be read out in sequence data, even if size selection is performed. However, if size selection is not performed, the proportion of much shorter reads will be increased.

Customers' comments

Size selection using BluePippin allowed us to remove short reads and perform effective sequence of long-chain DNA fragments, compared to using samples not size selected (no data).



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