NIPPON Genetics Co.,Ltd.

Application Note

Product Name : BluePippin (BLU0001)
Manufacturer : Sage Science
Application : Size selection of long-chain fragments (8kb) in Nextera Mate Pair library preparation

The following data were provided by the courtesy of Dr. Yoshitoshi Ogura and Dr. Yasuhiro Gotoh of Division of Microbiology, Department of Infectious Diseases, School of Medicine, Faculty of Medicine, University of Miyazaki, Japan.

Abstract

When preparing mate-pair libraries for sequencing purposes, accurate DNA size selection is essential for successful data analysis. In this study, mate-pair libraries were prepared for six bacterial strains. We extracted 8kb fragments using BluePippin, Automated Preparative Gel Electrophoresis, and sequenced the obtained libraries.
We analyzed the data and compared the statistics from "assembly using only paired-end reads" and those from "assembly additionally using 8kb mate-pair sequences"; the latter assembly resulted in 1-9 scaffolds (≧5 kb), demonstrating a substantial improvement in assembly.

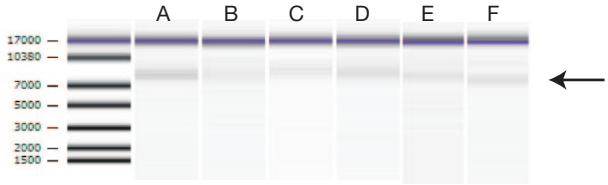
Experimental method

- **Sample DNA**
  Origin: Six bacterial strains A to F
  Purification method: QIAGEN Genomic DNA Buffer Set & Genomic-Tip 100
- **Library preparation kit**
  Nextera Mate Pair Sample Prep kit (Illumina, Inc.)
- **Assembler**
  Platanus version 1.1.4
- **Conditions for BluePippin size selection**
  Sample load : 30 µL/ lane (=3 µg/ lane)
  Extraction condition : Tight 8kb extraction (after elution and recovery, additional washing and recovery using 40µL Tween buffer)

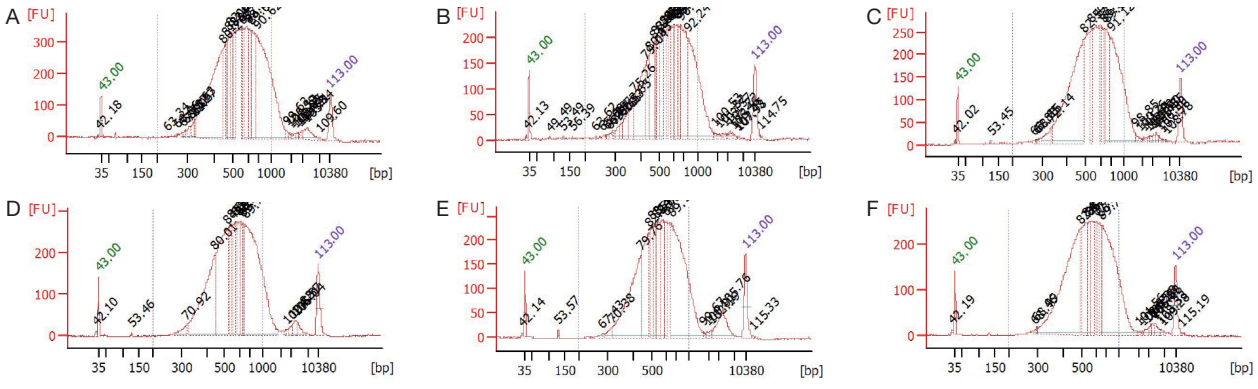
Automated Preparative Gel Electrophoresis
Capable of performing pulsed-field electrophoresis. Optimum for size selection of long-chain DNA fragments.

Results

Tagmentation was performed using Nextera Mate Pair Sample Prep kit (Illumina, Inc.), and 8kb DNA fragments were extracted using BluePippin. Fragment size was confirmed using BioAnalyzer.



Subsequent steps of circularization, fragmentation, recovery of junction sequences, TruSeq adapter ligation, etc. were performed using the Nextera Mate Pair Sample Prep kit, and the final library was checked using BioAnalyzer.



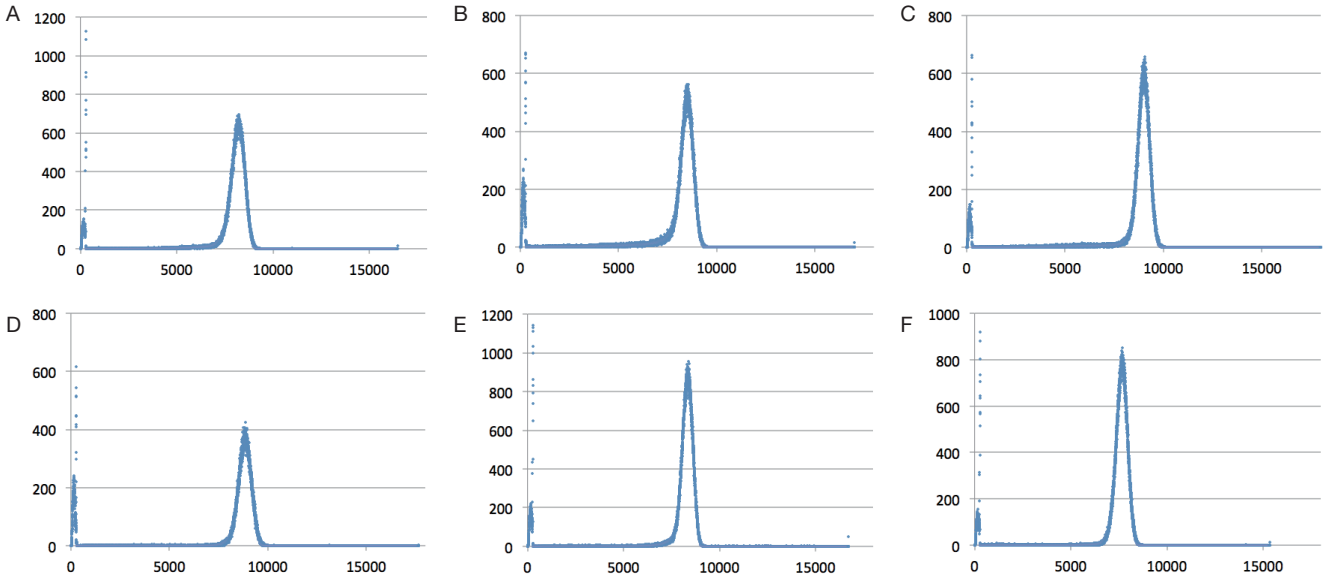
* For all samples A-F, libraries with expected size distributions (300-1,500 bp) were ultimately obtained.

Results

Comparison of statistics from "assembly using only paired-end reads" and those from "assembly additionally using 8-kb mate-pair sequences"

Sample	Paired-end only				Paired-end + 8kb Mate Pair			
	No. of scaffolds (≥ 500 bp)	N50 (bp)	Longest (bp)	Total length (bp)	No. of scaffolds (≥ 5 kb)	N50 (bp)	Longest (bp)	Total length (bp)
A	45	120,239	310,639	2,142,184	2	1,425,894	1,425,894	2,179,544
B	86	97,027	281,891	2,152,036	5	666,271	992,168	2,208,732
C	47	125,904	339,807	2,125,667	1	2,093,081	2,093,081	2,155,836
D	73	91,994	202,911	2,202,235	9	621,239	884,953	2,231,603
E	92	116,808	340,240	2,222,598	8	575,797	1,085,503	2,278,986
F	65	151,930	231,228	2,102,029	3	1,989,566	1,989,566	2,176,244

Distribution of distances between mate-pair reads after assembly



<Customer's comments>

We used BluePippin for performing size selection of Nextera Mate Pair Library.
We have been conventionally performing manual gel extraction, which was a troublesome step requiring much time and labor.
BluePippin could drastically shorten the actual operation time.
The amount of DNA extracted was rather small but was practically not a problem. Analysis of the data revealed that the use of mate pair sequences could effectively reduce the number of scaffolds.
We did not directly compare the present method with manual extraction, but we were satisfied with the results of data analysis and concluded that the present method was comparable to manual extraction.
We believe that the automated method using BluePippin is effective in terms of efficiency and accuracy.

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