Application Note
2018 〈03〉

NIPPON Genetics Co.,Ltd.

Application

DNA fragmentation by KAPA Frag enzyme stably yields target size distribution

～ cDNA fragmentation in single-cell RNA-Seq library preparation using Ion Proton ～

Product Name　KAPA Frag Kit（KK8600, KK8601, KK8602）

Manufacturer　KAPA BIOSYSTEMS

The following data was provided by courtesy of Dr. Satoshi Yamashita, Laboratory Head of Division of Epigenomics, National Cancer Center Research Institute, National Research Development Agency, Japan.

Introduction

As a pre-treatment for ligation-based library preparation, DNA need to be fragmented into appropriate size for sequencing. When there are many samples, it is difficult to optimize fragmentation conditions for individual samples in order to have them fragmented into the same size.
This note reports the use of KAPA Frag Kit as the DNA fragmentation reagent in cDNA fragmentation, performed as a pre-treatment for preparing RNA-Seq library from cDNA using the Ion Proton system. The kit stably yielded DNA fragments of the target size with little variation among different samples.

Workflow

1. cDNA purification
   Purify 2μL cDNA solution (total to 1μg cDNA) with AMPureXP
   Elute with 36μL 10mM Tris HCl Buffer (pH8.0), recover 35μL

2. DNA fragmentation by KAPA Frag Kit
   ① Preparation of enzyme reaction mixture on ice

AMPureXP-purified DNA	35 μL
10X KAPA Frag Buffer	5 μL
KAPA Frag Enzyme	10 μL
Total	50 μL

   ② Fragmentation reaction 37℃ 35 min.
   ＊condition determined by preliminary experiment
   ③ Add 5μL Stop Solution on ice, mix by pipetting

3. Purification by AMPureXP
   ① Bead purification (×1.8)

Reaction mixture	55 μL
AMPureXP	99 μL

   Thoroughly mix by pipetting, leave for 5 min, remove supernatant on magnet
   ② Washing
   Wash with 70%EtOH 200μL× twice
   ③ Elution
   Elute with 36μL 10mM Tris HCl Buffer (pH8.0), recover 35μL

4. DNA quantification by PicoGreen® and size distribution check by TapeStation

5. Library preparation

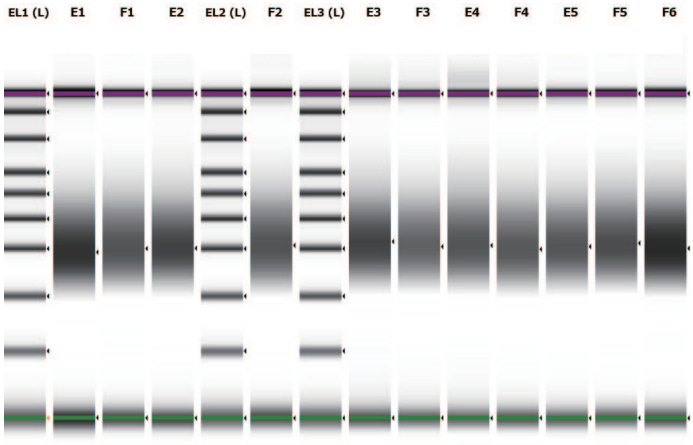
6. Sequencing by IonProton

KAPA Frag Kit

Kapa Frag Kit employs a DNA fragmentation enzyme (KAPA Frag enzyme) that has been developed for performing DNA fragmentation required for sample treatment of next-generation sequencing.
The enzyme causes little fragmentation bias (bias generated by GC content or sequence of DNA) and enables fragmentation size to be controlled by temperature and time, independent of the size or amount of input DNA.

Results

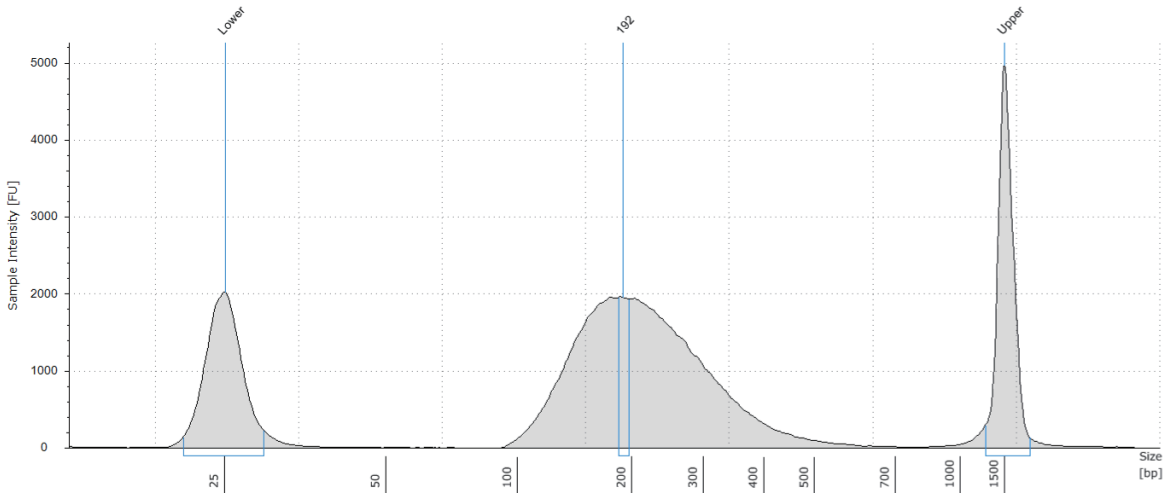
1. Size distribution of DNA fragmented by KAPA Frag Kit



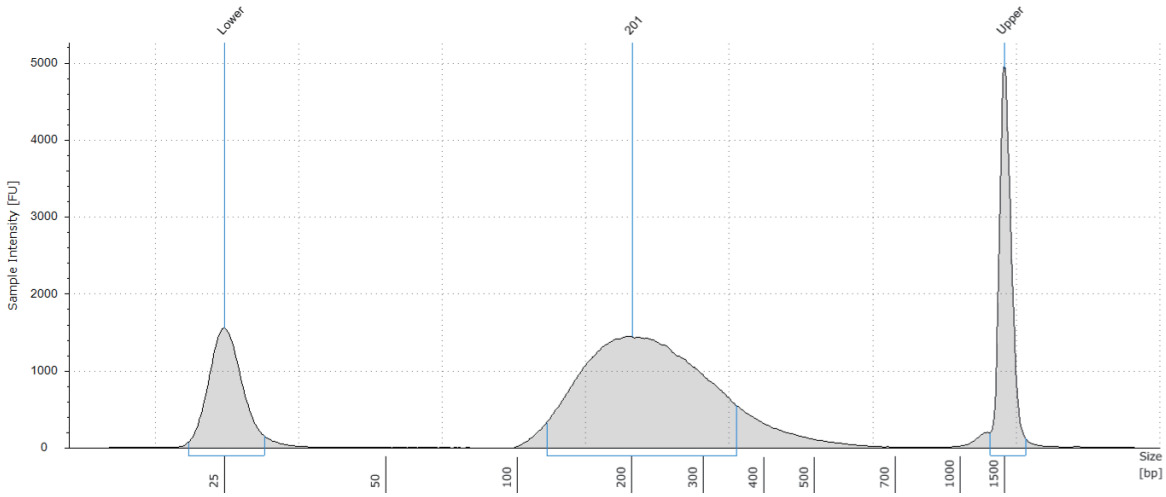
Sample Info

Well	Sample Description	Size（bp）
EL1	Electronic Ladder	
E1	KO1	192
F1	WT1	201
E2	KO2	201
EL2	Electronic Ladder	
F2	WT2	211
EL3	Electronic Ladder	
E3	KO3	224
F3	WT3	207
E4	KO4	211
F4	WT5	199
E5	KO5	207
F5	WT6	218
F6	WT7	201

E1: KO1



F1: WT1



The use of KAPA Frag Kit has been confirmed to result in fragmentation with a uniform size distribution.

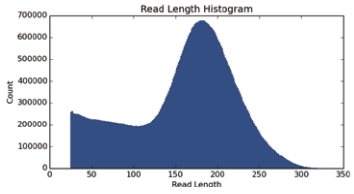
2. Sequencing results

As shown below, the sequencing results were as expected.

① Run Summary

158bp	170bp	182bp
Mean	Median	Mode

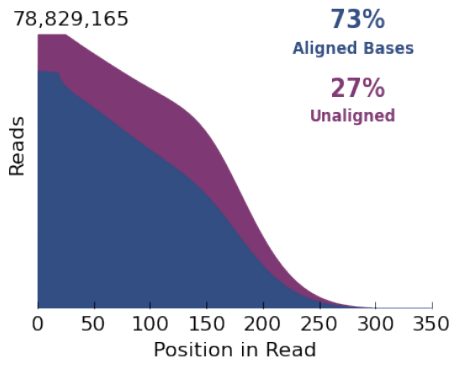
Read Length



Barcode Name	Sample	Bases	≧Q20	Reads	Mean Read Length
IonXpress_081	KO1	896,832,710	779,998,143	5,808,385	154 bp
IonXpress_082	KO2	960,770,582	835,661,464	6,097,664	158 bp
IonXpress_083	KO3	711,775,994	621,885,185	4,397,380	162 bp
IonXpress_084	KO4	916,293,475	792,425,579	5,843,541	157 bp
IonXpress_085	KO5	1,111,950,579	965,733,438	6,983,688	159 bp
IonXpress_086	KO6	804,206,585	698,752,122	5,081,347	158 bp
IonXpress_087	WT1	1,020,360,953	892,232,606	6,227,964	164 bp
IonXpress_088	WT2	1,009,453,122	879,267,105	6,048,007	167 bp
IonXpress_089	WT3	1,073,947,054	928,734,315	6,774,391	159 bp
IonXpress_090	WT4	1,078,999,073	942,141,796	6,884,147	157 bp
IonXpress_091	WT5	986,229,625	859,795,372	6,227,131	158 bp
IonXpress_092	WT6	991,663,277	860,141,966	6,169,506	161 bp
IonXpress_093	WT7	953,159,781	826,010,242	6,286,014	152 bp

② Alignment Summary

TotalAlignmentBases	AverageCoverage DepthofReference
9.17G	**3.0X**



	Count	%
Total Reads	78,829,165	–
Aligned Reads	68,225,498	86.5%
Unaligned Reads	10,603,667	13.5%

Alignment Quality

	AQ17	AQ20	Perfect
Total Number of Bases [Mbp]	6.57 G	5.64 G	4.32 G
Mean Length [bp]	134	123	99
Longest Alignment [bp]	348	341	335
Mean Coverage Depth	2.1	1.8	1.4

Customers' comments

I considered using physical fragmentation methods for performing DNA fragmentation for NGS library preparation, but with such methods, it would be difficult to obtain fragmented DNA of the same size when the original DNA samples vary in their amount or size.
I was looking for an easy, enzyme-based fragmentation method and found KAPA Frag Kit, so I tried it. It seemed that DNA samples, whether they were kilobase-long samples or entire genomes, could be fragmented by almost the same condition, so I only needed to optimize the reaction time.
I was so impressed that I could achieve uniform DNA fragments much more easily than I thought.

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