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

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°C 35 min.
      *condition determined by preliminary experiment
   ③ Add 5 µL 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 36 µL 10mM Tris HCl Buffer (pH8.0), recover 35 µL

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

<table>
<thead>
<tr>
<th>Well</th>
<th>Sample Description</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL1</td>
<td>Electronic Ladder</td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>KO1</td>
<td>192</td>
</tr>
<tr>
<td>F1</td>
<td>WT1</td>
<td>201</td>
</tr>
<tr>
<td>E2</td>
<td>KO2</td>
<td>201</td>
</tr>
<tr>
<td>EL2</td>
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<td></td>
</tr>
<tr>
<td>F2</td>
<td>WT2</td>
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</tr>
<tr>
<td>EL3</td>
<td>Electronic Ladder</td>
<td></td>
</tr>
<tr>
<td>E3</td>
<td>KO3</td>
<td>224</td>
</tr>
<tr>
<td>F3</td>
<td>WT3</td>
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<td>WT5</td>
<td>199</td>
</tr>
<tr>
<td>E5</td>
<td>KO5</td>
<td>207</td>
</tr>
<tr>
<td>F5</td>
<td>WT6</td>
<td>218</td>
</tr>
<tr>
<td>F6</td>
<td>WT7</td>
<td>201</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Read Length</th>
<th>158bp</th>
<th>170bp</th>
<th>182bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mode</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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     | 1,109,453,122 | 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

<table>
<thead>
<tr>
<th>Result</th>
<th>Position in Read</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
</tr>
</tbody>
</table>

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Alignment Quality

<table>
<thead>
<tr>
<th>AQ17</th>
<th>AQ20</th>
<th>Perfect</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.57 G</td>
<td>5.64 G</td>
<td>4.32 G</td>
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<tr>
<td>134</td>
<td>123</td>
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<tr>
<td>348</td>
<td>341</td>
<td>335</td>
</tr>
<tr>
<td>2.1</td>
<td>1.8</td>
<td>1.4</td>
</tr>
</tbody>
</table>

As shown below, the sequencing results were as expected.

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.