Customer feedback on products

<table>
<thead>
<tr>
<th>Product Name</th>
<th>KAPA Hyper Prep Kit (KK8500, KK8502)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturer</td>
<td>KAPA BIOSYSTEMS</td>
</tr>
<tr>
<td>Application</td>
<td>Sequence determination of MtDNA from the ear specimen of <em>Tragulus kanchil</em> which was immersed in ethanol for long time</td>
</tr>
</tbody>
</table>

All data in this paper was provided by the kindness of Dr. Taichi Ishige, The NODAI Genome Research Center, Tokyo University of Agriculture, Japan. We would like to express our deep appreciation for the contributions.

Introduction

DNA extracted from the sample which has been stored for a long time has generally less amount and poor quality. Here, the KAPA Hyper Prep Kit was used to be able to prepare a library from the sample having less amount and poor quality to obtain data without any problems and to report them.

Condition for experiment

Amount of the sample at the beginning: total DNA, see Table 2
Biological species: *Tragulus kanchil*
Sample material: the ear specimen of *Tragulus kanchil* in south-eastern Asia which was immersed in ethanol for 18 years
DNA extraction: QiAamp DNeasy Blood & Tissue Kit
Method for fragmentation of DNA: Covaris
Library preparation: KAPA Hyper Prep Kit for illumina
Adaptor*: KAPA Adapter Kit
Sequencer: HiSeq2500 (illumina)
*Supplementation by Nippon Genetics: At present, we recommend FastGene™ Adapter Kit (for illumina) (Cat. No. FG-NGSAD24).

Working flow for preparing the library

1. For the extraction of the genome DNA, QiAamp DNeasy Blood & Tissue Kit were used. Check of the property of DNA by agarose gel electrophoresis and measurement of the concentration by Qubit
2. Fragmentation of the genome DNA (covarix: 500bp)
3. End-repair, dA-tailing
4. Adapter-ligation
5. Purify with AMPureXP
6. Amplification of the library
7. APurify with AMPureXP
8. Size Selection by Pippin Prep
   Gel cassette: 2.0%
9. Check the distribution in the size by Agilent Bioanalyzer
10. Measurement of the concentration by Kapa Library Quantification Kits
11. Pool the library (added 1%PhiX)
12. Next generation sequencing (illumina HiSeq 2500)

Result

1. For the extraction of the genome DNA (QiAamp DNeasy Blood & Tissue Kit) is used. Check of the property of DNA by agarose gel electrophoresis and measurement of the concentration by Qubit

![Image](image)

**Figure 1.** Agarose gel electrophoresis of the extracted DNA

<table>
<thead>
<tr>
<th>name</th>
<th>Qubit (ng/μl)</th>
<th>Bio spec (ng/μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.62</td>
<td>11.2</td>
</tr>
<tr>
<td>2</td>
<td>9.8</td>
<td>12.6</td>
</tr>
<tr>
<td>3</td>
<td>too low</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 1. Extracted amount of DNA measured by Qubit

<table>
<thead>
<tr>
<th>name</th>
<th>input DNA (μl)</th>
<th>input DNA (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qubit</td>
<td>Bio spec nano</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>92.4</td>
</tr>
<tr>
<td>2</td>
<td>10.2</td>
<td>99.96</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>too low</td>
</tr>
<tr>
<td></td>
<td></td>
<td>72</td>
</tr>
</tbody>
</table>
Result

• Fragmentation of the genome DNA (covaris: 500 bp)

<table>
<thead>
<tr>
<th>name</th>
<th>Qubit (ng)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>too low</td>
</tr>
<tr>
<td>2</td>
<td>1.66</td>
</tr>
<tr>
<td>3</td>
<td>0.41</td>
</tr>
</tbody>
</table>

• Adapter-ligation

<table>
<thead>
<tr>
<th>name</th>
<th>Concentration of the stock</th>
<th>Amount to be added or one reaction (total 110 μL)</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>750nM</td>
<td>5 μL</td>
<td>34nM</td>
</tr>
<tr>
<td>2</td>
<td>15 μM</td>
<td>5 μL</td>
<td>680nM</td>
</tr>
<tr>
<td>3</td>
<td>7.5 μM</td>
<td>5 μL</td>
<td>340nM</td>
</tr>
</tbody>
</table>

• Amplification of the library

<table>
<thead>
<tr>
<th>name</th>
<th>Number of PCR cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
</tr>
</tbody>
</table>

• Size Selection by Pippin Prep

• Next generation Sequencing (illumina HiSeq 2500)

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Sample Ref</th>
<th>Index</th>
<th>Control</th>
<th>Yield (Mbases)</th>
<th>% PF</th>
<th># Reads</th>
<th>% of raw clusters per lane</th>
<th>% Perfect Index Reads</th>
<th>% One Mismatch Reads (Index)</th>
<th>% of &gt;= Q30 Bases (PF)</th>
<th>Mean Quality Score (PF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>N</td>
<td>GCCAAT</td>
<td>N</td>
<td>10,367</td>
<td>100</td>
<td>103,666,038</td>
<td>35.02</td>
<td>98.99</td>
<td>1.01</td>
<td>93.73</td>
<td>36.58</td>
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<tr>
<td>2</td>
<td>N</td>
<td>ATCACG</td>
<td>N</td>
<td>9,594</td>
<td>100</td>
<td>95,938,014</td>
<td>32.41</td>
<td>99.19</td>
<td>0.81</td>
<td>93.39</td>
<td>36.48</td>
</tr>
<tr>
<td>3</td>
<td>N</td>
<td>CTGTA</td>
<td>N</td>
<td>9,048</td>
<td>100</td>
<td>90,476,788</td>
<td>30.56</td>
<td>99.1</td>
<td>0.9</td>
<td>93.41</td>
<td>36.46</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>N75</th>
<th>N50</th>
<th>N25</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Average</th>
<th>Count</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,119</td>
<td>1,268</td>
<td>1,661</td>
<td>651</td>
<td>175,450</td>
<td>1,455</td>
<td>33,956</td>
<td>49,418,356</td>
</tr>
<tr>
<td>2</td>
<td>1,136</td>
<td>1,283</td>
<td>1,540</td>
<td>712</td>
<td>31,211</td>
<td>1,333</td>
<td>81,957</td>
<td>109,238,541</td>
</tr>
<tr>
<td>3</td>
<td>1,132</td>
<td>1,273</td>
<td>1,522</td>
<td>644</td>
<td>48,192</td>
<td>1,334</td>
<td>73,867</td>
<td>98,512,409</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Consensus length (bp)</th>
<th>total reads</th>
<th>Average coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16,356</td>
<td>29,814</td>
<td>178.13</td>
</tr>
<tr>
<td>2</td>
<td>16,329</td>
<td>52,264</td>
<td>312.95</td>
</tr>
<tr>
<td>3</td>
<td>16,312</td>
<td>34,226</td>
<td>205.3</td>
</tr>
</tbody>
</table>

• Summary

Even in the sample 3 which amount at the time of the beginning is unknown, extremely high quality read information could be obtained to be 93% or more in read of Qscore greater than 30.
Almost full length of MdNA could be determined in all samples by De Novo assembly in CLC Genomics Workbench.

<Customer's comments>

Since a library could be prepared from DNA which has extremely low concentration and is poor in its condition, we believe that this method is useful for preparing a library of ancient DNA.
Since the loss of fragmented DNA is large due to the use of Covaris, we consider that this problem has to be solved.