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Assessment of Nextera Long Mate-Pair Libraries: A Rapid, Low-Input Method for Mate-Pair Library Construction Yields Improved Assemblies

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Introduction

Long mate-pair libraries are invaluable tools for genome assembly. However, traditional methods of long mate-pair library construction require large (20 µg) quantities of DNA and several days of hands-on time. Illumina’s Nextera™ Long Mate-Pair (LMP) method is rapid and requires only 1 to 4 micrograms of input material. Here we present an initial assessment of the method for both gel-free and gel size-selected libraries using microbial, fungal, and plant samples. We observed uniform read coverage and high read uniqueness for Nextera™ LMP libraries. Assembly using ALLPATHS-LG generated low contig and scaffold numbers even with relatively low mate-pair coverage.

Methods Overview

Results

Organisms Tested

<table>
<thead>
<tr>
<th>Species</th>
<th>%GC</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phycomyces blakesleeanus</td>
<td>36%</td>
<td>Filamentous fungi</td>
</tr>
<tr>
<td>Spirochaeta smaragdinae</td>
<td>49%</td>
<td>Gram (-) microbe</td>
</tr>
<tr>
<td>Conexibacter woesei</td>
<td>73%</td>
<td>Gram (+) microbe</td>
</tr>
<tr>
<td>Cellumonas flavigena</td>
<td>74%</td>
<td>Gram (+) microbe</td>
</tr>
<tr>
<td>Suillus luteus</td>
<td>47%</td>
<td>Basidiomycete fungi</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>42%</td>
<td>Plant</td>
</tr>
</tbody>
</table>

Table 1. Initial testing organisms and their GC-content

<table>
<thead>
<tr>
<th>Organism &amp; Assembly Type</th>
<th>Scaffolds</th>
<th>Contigs</th>
<th>Scaffold L50</th>
<th>Contig L50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conexibacter woesei Frag+Traditional LMP</td>
<td>1</td>
<td>7</td>
<td>6355 Kb</td>
<td>1190 Kb</td>
</tr>
<tr>
<td>Conexibacter woesei Frag+ Nextera LMP</td>
<td>1</td>
<td>8</td>
<td>6328 Kb</td>
<td>744 Kb</td>
</tr>
<tr>
<td>Cellumonas flavigena Frag+Traditional LMP</td>
<td>8</td>
<td>48</td>
<td>4060 Kb</td>
<td>188 Kb</td>
</tr>
<tr>
<td>Cellumonas flavigena Frag+ Nextera LMP</td>
<td>4</td>
<td>27</td>
<td>3493 Kb</td>
<td>408 Kb</td>
</tr>
<tr>
<td>Suillus luteus Fragment only</td>
<td>1944</td>
<td>2113</td>
<td>57.6 Kb</td>
<td>51.3 Kb</td>
</tr>
<tr>
<td>Suillus luteus Frag+ Nextera LMP</td>
<td>397</td>
<td>1477</td>
<td>240 Kb</td>
<td>54.6 Kb</td>
</tr>
</tbody>
</table>

Table 3. ALLPATHS-LG assemblies were improved with the inclusion of Nextera LMP data compared to traditional LMP data.

Summary

- User-friendly protocol with short hands-on time
- Low template requirement compared to traditional long-mate pair methods (1µg/4 µg)
- Read uniqueness is high for Nextera LMP libraries
- Nextera LMP libraries have uniform read coverage
- Insert size doesn’t seem to have significant impact on contig N50
- ALLPATHS-LG generated low contig and scaffold numbers for microbes, even with low coverage
- Addition of Nextera LMP data generally improved assembly results

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