Sample ID: *Barron S. (B.S)*  
*Male-fair 52, 05 bp - Eve's sample*  
Date: 9/28/19

Sample Information

Lab: *Yonmend*  
Concentration: 250 ng/μL  
Amount/Volume used for preparing Library: 30 μg/118 μL

Shear the DNA

<table>
<thead>
<tr>
<th>Insert Size</th>
<th>Shearing Method</th>
<th>Hydroshar Speed Code/ Covaris S2 Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.5 μL</td>
<td><strong>Hydroshear</strong></td>
<td><strong>Standard - Assembly</strong>&lt;br&gt;SC15, 5 cycles, 150 μL</td>
</tr>
</tbody>
</table>

1. If you are using the Covaris™ S2 System:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μL)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>96% Glycerol</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>10 to 20 μg DNA</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Up to 500</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>500</strong></td>
<td></td>
</tr>
</tbody>
</table>

2. If using the HydroShear®: **some DNA does not get sheared** (Common results for Hydroshear)

<table>
<thead>
<tr>
<th>Component</th>
<th>Tube 1</th>
<th>Tube 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA (μg)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>DNA (μL)</td>
<td>59</td>
<td>59</td>
</tr>
<tr>
<td>Nuclease Free Water (μL)</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>

Purify the sheared DNA using the QIAquick Gel Extraction Kit (QIAquick spin columns have a 10-μg capacity)

This clean up removes Unsheared Genomic DNA

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (μL)</th>
<th>Product</th>
<th>Lot N°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td></td>
<td>Kit</td>
<td></td>
</tr>
<tr>
<td>Buffer QG (3X)</td>
<td></td>
<td>Buffer QG</td>
<td></td>
</tr>
<tr>
<td>Isopropanol (1X)</td>
<td></td>
<td>Buffer PE</td>
<td></td>
</tr>
<tr>
<td>Final Sample</td>
<td></td>
<td>Buffer EB</td>
<td></td>
</tr>
<tr>
<td>Per Column</td>
<td></td>
<td>Column</td>
<td></td>
</tr>
<tr>
<td>Volume per column (times)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elution</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nanodrop: ______ ng/μL - x ______ μL = ______ ng (total yield) ______ μg (total yield)

--- This step removes Unsheared DNA (Genomic DNA), so you don't waste end-repair reagents in the next step ---

Repair the DNA Ends

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume (μL)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheared DNA</td>
<td>x</td>
<td>217</td>
</tr>
<tr>
<td>10X End-It Buffer</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>End-It ATP (10 mM)</td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td>End-It dNTPs (2.5 mM)</td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td>End-It Enzyme Mix</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Variable</td>
<td>51</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>300</td>
<td>400</td>
</tr>
</tbody>
</table>

Incubate the mixture at room temperature for 30 minutes.
Verify the DNA using the QIAquick Gel Extraction Kit.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Product</th>
<th>Lot N°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>400</td>
<td>Kit</td>
<td></td>
</tr>
<tr>
<td>Buffer QG (3X)</td>
<td>1200</td>
<td>Buffer QG</td>
<td></td>
</tr>
<tr>
<td>Isopropanol (1X)</td>
<td>400</td>
<td>Buffer PE</td>
<td></td>
</tr>
<tr>
<td>Final Sample</td>
<td>2000</td>
<td>Buffer EB</td>
<td></td>
</tr>
<tr>
<td>Per Column</td>
<td>666</td>
<td>Column</td>
<td></td>
</tr>
<tr>
<td>Elution</td>
<td>180</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nanodrop: 83.75 ng/µL × 180 µL = 15,079 ng (total yield) 15 µg (total yield)

Methylation of the Genomic EcoP15I Sites

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Sample</th>
<th>Lot N°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheared, end-repaired DNA</td>
<td>x</td>
<td>180</td>
<td></td>
</tr>
<tr>
<td>10X NEBuffer 3</td>
<td>25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>100X BSA</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>EcoP15I Enzyme (10 U/µL)</td>
<td>x</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>S-adenosylmethionine (32 mM)</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Variable</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>250</td>
<td>2.50</td>
<td></td>
</tr>
</tbody>
</table>

Incubate the methylation reaction mixture at 37 °C for 2 hours or overnight in a water bath at 37 °C.

Note: Use a final concentration of at least 360 µM S-adenosylmethionine and 10 U of EcoP15I enzyme per 1 µg of end-repaired DNA. Adjust the final volume to 250 µL with nuclease-free water. The above reaction is set up for approximately 20 to 25 µg of DNA derived from 30 µg of starting input DNA. If the amount of starting input material is >30 µg, the reaction composition (based on the amount of enzyme needed) must be modified. Typically the final reaction volume is at least 10 times the enzyme volume.

Purify the methylated DNA using the QIAquick Gel Extraction Kit. — 9/29/9

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Product</th>
<th>Lot N°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>250</td>
<td>Kit</td>
<td></td>
</tr>
<tr>
<td>Buffer QG (3X)</td>
<td>750</td>
<td>Buffer QG</td>
<td></td>
</tr>
<tr>
<td>Isopropanol (1X)</td>
<td>250</td>
<td>Buffer PE</td>
<td></td>
</tr>
<tr>
<td>Final Sample</td>
<td>1250</td>
<td>Buffer EB</td>
<td></td>
</tr>
<tr>
<td>Per Column</td>
<td>625</td>
<td>Column</td>
<td></td>
</tr>
<tr>
<td>Elution</td>
<td>128</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nanodrop: 97.01 ng/µL × 128 µL = 12,417.28 ng (total yield) 12.4 µg (total yield)

Run the DNA on an E-Gel 0.8%. After separation, the fragment used was 2.6 µL = 252 ng for checking the shearing profile.

rer
**Calculation of the EcoP15I CAP Adaptors to the Methylated DNA**

Calculate how many pmoles of EcoP15I CAP adaptors (ds) are needed, first calculate the picomoles of the insert DNA based on its size as follows: targeted multiple sizes, but used 2-8-bp for calculations.

\[
(1 \text{ µg DNA}) \times (10^6 \text{ pg/g}) \times (1 \text{ pmol/µg}) \times \left( \frac{1}{2500} \text{ insert size in bp} \right) = 0.61 \text{ pmoles/µg DNA}
\]

\[
(1.24 \text{ µg DNA used}) \times (0.61 \text{ pmoles/µg DNA}) = 7.52 \text{ pmoles DNA in sample}
\]

\[
\left( \frac{7.52 \text{ pmoles DNA in sample}}{50 \text{ pmole/µL EcoP15I CAP adaptors needed}} \right) \times (100) = 25.2 \text{ # pmoles EcoP15I CAP adaptors needed}
\]

\[
\left( \frac{25.2 \text{ pmoles adaptors needed}}{50 \text{ pmole/µL EcoP15I CAP adaptors reagent}} \right) = 0.5 \text{ µL EcoP15I CAP adaptor reagent}
\]

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoP15I CAP Adaptor (ds) (50 pmole/µL)</td>
<td>X</td>
<td>15</td>
</tr>
<tr>
<td>2X NEB Quick Ligase buffer</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>NEB Quick Ligase</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>DNA</td>
<td>Y</td>
<td>12.3</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>Variable</td>
<td>4.2</td>
</tr>
<tr>
<td>Total</td>
<td>300</td>
<td>360</td>
</tr>
</tbody>
</table>

Incubate at room temperature for 10 minutes.

**Note:** If a larger reaction volume is required to incorporate all of the methylated DNA, scale up the Quick Ligase and Quick Ligase buffer. Add 1 µL of Quick Ligase per 40 µL of reaction volume. Add 1 µL of 2X NEB Quick Ligase buffer per 2 µL of reaction volume.

**Purify the DNA using the QIAquick Gel Extraction Kit.**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
<th>Product</th>
<th>Lot. N°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>500</td>
<td>Kit</td>
<td></td>
</tr>
<tr>
<td>Buffer QG (3X)</td>
<td>900</td>
<td>Buffer QG</td>
<td>0803006</td>
</tr>
<tr>
<td>Isopropanol (1X)</td>
<td>300</td>
<td>Buffer PE</td>
<td>0910412</td>
</tr>
<tr>
<td>Final Sample</td>
<td>1500</td>
<td>Buffer EB</td>
<td>1980906</td>
</tr>
<tr>
<td>Per Column</td>
<td>750</td>
<td>Column</td>
<td>0915180</td>
</tr>
<tr>
<td>Volume per column (3 times)</td>
<td>2250</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[ 2 \times 18 = 36 \times 2 = 60 \text{ mL} \]

\[ \text{Nanodrop:} \quad \frac{\text{ng/µL}}{x} \times \frac{128}{18} \text{ µL} = \text{ng (total yield)} \quad \text{µg (total yield)} \]

**Size Selection of DNA with a 0.8-1% Agarose Gel**

<table>
<thead>
<tr>
<th>Desired Insert Size</th>
<th>Percentage of Agarose Gel Needed</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>600 bp to 3000 bp</td>
<td>1.0%</td>
<td>-</td>
</tr>
<tr>
<td>3 kb to 6 kb</td>
<td>0.8%</td>
<td>-</td>
</tr>
</tbody>
</table>

\[ 0.489 = 600 \text{ mL} + 1 \times \text{TAE} \]

\[ 600 \text{ µL sample} - 6 \text{ µL 10x loading dye} = 66 \text{ µL} \]

\[ 54 \text{ µL sample} - 3.5 \text{ µL 10x } = 59.7 \text{ µL } \quad (19.8 \text{ µL/ well}) = \text{loaded all in 2 wells (expected)} \]

\[ 12.5 - 4 \text{ µL} + 20 \text{ µL of } \text{B+2.5 µL 10x } = 26.4 \text{ µL } \div 2 = 13.2 \text{ µL/p well/} \]
Extract the DNA from the agarose with the QIAquick Gel Extraction kit

- Place each gel portion into a 15-mL conical polypropylene tube that is large enough to hold 3 times the volume of the gel. If the gel piece is large, slice it up before placing it into the tube.
- Tare the balance with an empty tube of the same kind (327 mg) 3–4 times to cut

Also proceed other fragment sizes - check on the base.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>327</td>
</tr>
<tr>
<td>Buffer QG (3×)</td>
<td>681</td>
</tr>
<tr>
<td>Isopropanol (1×)</td>
<td>327</td>
</tr>
<tr>
<td>Final Sample</td>
<td>163</td>
</tr>
<tr>
<td>Per Column</td>
<td>16.35</td>
</tr>
<tr>
<td>Volume per column (3 times)</td>
<td>550</td>
</tr>
<tr>
<td>Elution</td>
<td>6.4</td>
</tr>
</tbody>
</table>

Note: Don’t forget to wash with extra 500 µl of buffer QG after loading the column with the sample

Nanodrop: \[ \frac{23.70 \text{ ng/µL} \times 64.5^2 \text{ µL}}{29.5 \text{ µL}} = 13,963 \text{ ng} \text{ (total yield)} \]

Circularize the DNA

\[ \frac{1}{2} \text{ Accura} \div \frac{1}{2} \text{ Accura} \]

29.5 µl 29.5 µl

Using Table 2-2 below, determine the concentration of DNA needed to achieve intramolecular ligation of 95% of the sample based on the insert size of your library.

<table>
<thead>
<tr>
<th>Insert Size</th>
<th>Final Concentration of DNA for Circularization</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 to 700 bp</td>
<td>4.3 ng/µL</td>
</tr>
<tr>
<td>700 to 1000 bp</td>
<td>3.75 ng/µL</td>
</tr>
<tr>
<td>1 to 2 kb</td>
<td>2.74 ng/µL</td>
</tr>
<tr>
<td>2 to 3 kb</td>
<td>2.1 ng/µL</td>
</tr>
<tr>
<td>3 to 4 kb</td>
<td>1.8 ng/µL</td>
</tr>
<tr>
<td>4 to 5 kb</td>
<td>1.6 ng/µL</td>
</tr>
<tr>
<td>5 to 6 kb</td>
<td>1.4 ng/µL</td>
</tr>
</tbody>
</table>

Based on the desired insert size, use the following table to prepare a 95% circularization reaction for every 1 µg of library. When using more than 1 µg of DNA for circularization, modify the reaction appropriately. For higher circularization efficiency, it is important to increase the amount of Quick Ligase enzyme in proportion to the volume (that is, 1 µL enzyme per 40 µL reaction volume):

\[ \frac{99.15}{699.15} = \frac{659.52}{659.52} \]

<table>
<thead>
<tr>
<th>Components</th>
<th>500 to 700 bp</th>
<th>700 to 1000 bp</th>
<th>1 to 2 kb</th>
<th>2 to 3 kb</th>
<th>3 to 4 kb</th>
<th>4 to 5 kb</th>
<th>5 to 6 kb</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
<td>1 µg</td>
</tr>
<tr>
<td>2X NEB Quick Ligase Buffer</td>
<td>117.5 µL</td>
<td>135 µL</td>
<td>182.5 µL</td>
<td>250 µL</td>
<td>280 µL</td>
<td>312.5 µL</td>
<td>360 µL</td>
<td></td>
</tr>
<tr>
<td>Internal adaptor (ds) (2pmoles/µL)</td>
<td>3.75 µL</td>
<td>2.64 µL</td>
<td>1.5 µL</td>
<td>0.9 µL</td>
<td>0.65 µL</td>
<td>0.5 µL</td>
<td>0.4 µL</td>
<td></td>
</tr>
<tr>
<td>NEB Quick Ligase</td>
<td>6 µL</td>
<td>8.75 µL</td>
<td>9 µL</td>
<td>12.5 µL</td>
<td>14 µL</td>
<td>15.6 µL</td>
<td>18 µL</td>
<td></td>
</tr>
<tr>
<td>Nuclease-Free water</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
<td>Variable</td>
</tr>
<tr>
<td>Total</td>
<td>235 µL</td>
<td>270 µL</td>
<td>365 µL</td>
<td>506 µL</td>
<td>560 µL</td>
<td>625 µL</td>
<td>720 µL</td>
<td></td>
</tr>
</tbody>
</table>

* α: Determine the quantity of nuclease-free water needed. Add the water to the sample, and then add the remaining reagents in order. Thoroughly mix all the components and finally add the enzyme to the reaction.

Incubate at room temperature for 10 minutes.
<table>
<thead>
<tr>
<th>Library</th>
<th>85 3-4 mL</th>
<th>85 4-5 mL</th>
<th>85 5-6 mL</th>
<th>85 6-7 mL</th>
<th>85 7-8 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 mL</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>0.509 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 columns</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>qC 15 mL</td>
<td>1</td>
<td>113 mL</td>
<td>1875 mL</td>
<td>1 column</td>
<td>1 column</td>
</tr>
<tr>
<td>ISO 50 mL</td>
<td>1</td>
<td>375 mL</td>
<td>1 column</td>
<td>1 column</td>
<td>1 column</td>
</tr>
<tr>
<td>Total 25 mL</td>
<td>1</td>
<td>1 column</td>
<td>1 column</td>
<td>1 column</td>
<td>1 column</td>
</tr>
<tr>
<td>= 2 columns</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1275 mL/col</td>
<td>1</td>
<td>1895 mL/col</td>
<td>1465 mL/col</td>
<td>1465 mL/col</td>
<td>1465 mL/col</td>
</tr>
<tr>
<td>÷ 650 mL</td>
<td>1</td>
<td>650 mL/col</td>
<td>650 mL/col</td>
<td>650 mL/col</td>
<td>650 mL/col</td>
</tr>
<tr>
<td>loaded 2x 650 each</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 68 mL</td>
<td>1</td>
<td>Total 68 mL</td>
<td>Total 68 mL</td>
<td>Total 68 mL</td>
<td>Total 68 mL</td>
</tr>
<tr>
<td>16.48 mL</td>
<td>21.53 mL</td>
<td>21.61 mL</td>
<td>13.83 mL</td>
<td>1398.3 mL/14 mL</td>
<td></td>
</tr>
<tr>
<td>1 x 0.64 mg/1 mL</td>
<td>1 x 3.12 mL</td>
<td>1 x 3.3 mL</td>
<td>1 x 3.8 mL</td>
<td>1 x 3.8 mL</td>
<td></td>
</tr>
<tr>
<td>1/2 Akram</td>
<td>1/2 Akram</td>
<td>1/2 Akram</td>
<td>1/2 Akram</td>
<td>1/2 Akram</td>
<td></td>
</tr>
<tr>
<td>60% 2 mL</td>
<td>58% 2 mL</td>
<td>61% 2 mL</td>
<td>30.5 mL</td>
<td>29.5 mL</td>
<td></td>
</tr>
</tbody>
</table>
Gel extraction and Purification. After Circularization

<table>
<thead>
<tr>
<th>BS 1.2-kb</th>
<th>BS 2.3-kb</th>
<th>BS 3.4-kb</th>
<th>BS 4.5-kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>365 µl</td>
<td>500 µl</td>
<td>560 µl</td>
<td>625 µl</td>
</tr>
<tr>
<td>10.95 µl</td>
<td>1500 µl</td>
<td>1680 µl</td>
<td>1825 µl</td>
</tr>
<tr>
<td>365 µl</td>
<td>500 µl</td>
<td>560 µl</td>
<td>625 µl</td>
</tr>
<tr>
<td>1825 µl</td>
<td>700 µl</td>
<td>1000 µl</td>
<td>1825 µl</td>
</tr>
<tr>
<td>2.6 times</td>
<td>3.6 times</td>
<td>4 times</td>
<td>4.5 times</td>
</tr>
<tr>
<td>2 × 32 µl</td>
<td>2 × 32 µl</td>
<td>2 × 32 µl</td>
<td>2 × 32 µl</td>
</tr>
<tr>
<td>60 µl</td>
<td>60 µl</td>
<td>60 µl</td>
<td>60 µl</td>
</tr>
</tbody>
</table>

Plasmid Safe

<table>
<thead>
<tr>
<th>Component</th>
<th>BS 1.2-kb</th>
<th>2.3-kb</th>
<th>3.4-kb</th>
<th>4.5-kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>5</td>
<td>10</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Plasmid Safe Buffer</td>
<td>10</td>
<td>10</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Master Mix</td>
<td>0.3</td>
<td>0.3</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>DNA</td>
<td>60</td>
<td>60</td>
<td>24.7</td>
<td>24.7</td>
</tr>
<tr>
<td>Water</td>
<td>24.7</td>
<td>24.7</td>
<td>60</td>
<td>24.7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Incubated 37°C for 40 minutes (Qian)
**Glycerol Gel Extraction Kit**

<table>
<thead>
<tr>
<th>Size (kb)</th>
<th>1.2</th>
<th>2.3</th>
<th>3.4</th>
<th>2.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Glycerol</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size (kb)</th>
<th>2x27u/</th>
<th>2x27u/</th>
<th>2x27u/</th>
<th>2x27u/ = 54 u/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrot</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.41 ng/u</td>
<td>3.58 ng/u</td>
<td>3.93 ng/u</td>
<td>3.22 ng/u</td>
<td></td>
</tr>
<tr>
<td>x 50</td>
<td>x 50</td>
<td>x 50</td>
<td>x 50</td>
<td></td>
</tr>
<tr>
<td>170.5 ng</td>
<td>179 ng</td>
<td>196.5 ng</td>
<td>161 ng</td>
<td></td>
</tr>
</tbody>
</table>

Continue sample prep.

**Goal:** Minimum 200 ng to continue sample preparation

---

**Eco P151 Digestion**

<table>
<thead>
<tr>
<th>Size (kb)</th>
<th>1-2 kb (1 tube)</th>
<th>2.3 kb</th>
<th>3-4 kb</th>
<th>4-6 kb (3 tubes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>471 ng DNA x 10 u x 1 = 47.1 u</td>
<td>179 x 5 = 0.895 u</td>
<td>197 x 5 = 0.985 u</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>1000</td>
<td></td>
</tr>
<tr>
<td>= 1.71 u of Eco P151</td>
<td>0.895 u enzyme</td>
<td>0.985 u enzyme</td>
<td>0.805 u enzyme</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size (kb)</th>
<th>1-2 kb</th>
<th>2.3 kb</th>
<th>3-4 kb</th>
<th>4-6 kb</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>50-48</td>
<td>50-48</td>
<td>50-48</td>
<td>50-48</td>
</tr>
<tr>
<td>10x NEB3</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>100x BSA</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sinefungin</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>10x ATP</td>
<td>0.71</td>
<td>0.89</td>
<td>0.985</td>
<td>0.805</td>
</tr>
<tr>
<td>Eco P151</td>
<td>16.29</td>
<td>19.1</td>
<td>19.1</td>
<td>19.2</td>
</tr>
<tr>
<td>Water</td>
<td>150</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

37°C / Overnight / PCR Machine
Digest circularized DNA II (4 samples
1-2 kb, 2-3 kb, 3-4 kb, 4-6 kb)

Eco 151 - Digested DNA 100
10mM Einfungin 1
10X ATP 2
Eco 7151 0.5
Total 103.5

Incubated 37°C 5 hr, 65°C 20 minutes, ice 5 minutes.

End-Repairs Klenow (4 samples)

Eco Digested DNA 103.5
dNTP Mix 1.5
DNA Polymerase, Klenow 1
Total 106

Same Tube - PCR Machine

Room Temperature - 30 minutes
Denatured 65°C - 20 minutes
Ice - 5 minutes.

Streptavidin Binding Buffer

Tris-HCl, pH 7.5 50mM

9M Tris, V1 = 0.5 (500 mM), 100
V = 0.5 ml Tris (9 M)/some Nuclease free water
Lot No. 118218

0.5 M Tris - 20 ml
5 M Sodium chloride - 400 ml - 07/10003
0.5 M EDTA - 2 ml - 08/05005
Nuclease free water - 578 ml/4000 ml
Bind Library to MyOne Ti Streptavidin Beads

1x BSA

\[
\begin{align*}
100\times \text{BSA} &= 20 \mu l \\
\text{Nuclease free water} &= 1980 \mu l \\
\text{Total} &= 2000 \mu l
\end{align*}
\]

Lot No.: 0207

Cat. No.: 656.02

Beads Lot No.: G22404

1x Quick Ligate Buffer

\[
\begin{align*}
2\times \text{Quick Ligate (NEB)} &= 1200 \mu l \\
\text{Nuclease free water} &= 1200 \mu l \\
\text{Total} &= 2400 \mu l
\end{align*}
\]

Lot No.: 0010903

Ligate P1 and P2

\[
\frac{x \times 10^6}{1} \times \frac{4}{660} \times \frac{4}{736}
\]

\[
\frac{10^6}{1,017,760} = 0.98 \text{ pmol/µg DNA}
\]

\[
\text{0.17 µg} \times \frac{1}{1} \times 30 \times \frac{1}{50} = 0.1 \text{ µl } P1/ P2
\]

\[
\frac{0.2 \text{ µl } P1}{0.2 \text{ µl } P2}
\]

2-3 h

\[
\frac{0.18 \text{ µg} \times 0.6 \times 30}{50} = 0.064 \text{ µl adapter needed.}
\]

3-4 h

\[
\frac{10^6}{660 \times 3536} = 0.16 \text{ µg} \times 0.33 \times 30
\]

\[
\text{(0.05 µl adapter)}
\]

Y = 6 h

\[
\frac{10^6}{660 \times 4536} = 0.16 \times 0.33 \times 30
\]

\[
\text{0.03 µl}
\]

0.2 µl P1 / P2 all reactions
Ligate P1/P2 Adaptors

9 reactions

<table>
<thead>
<tr>
<th>DNA Bead complex</th>
<th>97.5</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 adapter</td>
<td>0.2</td>
<td>0806011</td>
</tr>
<tr>
<td>P2 adapter</td>
<td>0.2</td>
<td>P1-0806011</td>
</tr>
<tr>
<td>Quick ligase</td>
<td>0.5</td>
<td>PZ-0806010</td>
</tr>
<tr>
<td></td>
<td>200 μl</td>
<td>98/1108</td>
</tr>
</tbody>
</table>

Wash DNA-bound beads

40 x NTE 2  
50 x 4 = 200

Water  
540 x 4 = 2160

Nick-Translation (4 samples)

<table>
<thead>
<tr>
<th>DNA Bead Complex</th>
<th>96 μl</th>
<th>Lot No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTP Mix</td>
<td>2 μl</td>
<td>86</td>
</tr>
<tr>
<td>DNA Polymerase II</td>
<td>2 μl</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 μl</td>
<td></td>
</tr>
</tbody>
</table>

16°C - 30 minutes

1x Bead Wash  
Lot No. 0805007

1x Bind Wash  
0809008
PCR Amplification

**Platinum PCR Super Mix** = 200 x 4 Samples = 800
**Library Primer 1** = 8 = 32
**Library Primer 2** = 8 = 32
**Closed Pfu** = 1 = 4
**Water** = 143 = 572
**Total** = 360 = 1440

4 tubes
- Control 96 ml / 60 ml

4 x 270 ml
- 1-2 270 ml / 30 ml
  - 3 tubes 270 ml + 30 ml
    - 3 tubes 100 ml
  - 270 ml + 30 ml
    - 3 tubes 100 ml

**PCR 9700 - Gold Block**

95°C 10 min
95°C 15 sec
62°C 15 sec
60°C 1 min
95°C 4 sec

5 cycles

Lonza Gel = loaded gel of PCR product from each sample + Controls (-)
Lanza. 5 cycles - No amplification was observed.

PCR - 5 More cycles

95°C
15 sec 62°C 15 sec

5 cycles

Lanza Gel

1. 100-4Kb ladder
2. 1-2 Kb - Positive Band
3. 2-3 Kb - Positive Band
4. 3-4 Kb - Positive Band
5. 4-6 Kb - No Band
6. 1-2 Kb - Control
7. 2-3 Kb - Control
8. 3-4 Kb - Control
9. 4-6 Kb - Control
10. Quant Ladder

PCR 9700 - Gold Block
4-6 Kb - Mate Pair

↓

PCR - 3 more cycles → Total: 13 cycles

95°C
15 sec

62°C
15 sec

60°C
4 min

4°C

3 cycles

↓

Lonza Gel

1 2 3

mate pair BANANASLUG 1099 46kb13cycles1.jpg

1. 100-4 Kb ladder
2. 4.6 Kb Positive Band
3. Quant ladder

All samples were purified according to Not Solid.
Samples were magnetic beads separated. Removed Beads

Supernatant was purified with Qiagen gel extraction kit

All samples were considered as containing 292 µL

<table>
<thead>
<tr>
<th>Sample</th>
<th>292 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>QG</td>
<td>876</td>
</tr>
<tr>
<td>2xpropo</td>
<td>292</td>
</tr>
</tbody>
</table>

\[ \text{730 µL} \]

\[ \text{730 µL per column/2 columns each sample} \]

\[ \text{1-2 µL} \]

\[ \text{2-3 µL} \]

\[ \text{3-4 µL} \]

\[ \text{4-6 µL} \]

\[ \text{1} \]

\[ \text{1} \]

\[ \text{1} \]

\[ \text{2 columns} \]

\[ \text{2 columns} \]

\[ \text{3 columns} \]

\[ \text{2 columns} \]

\[ \text{2 columns} \]

\[ \text{730 µL each} \]

\[ \text{730 µL each} \]

\[ \text{730 µL each} \]

\[ \text{730 µL each} \]

\[ \text{730 µL each} \]

\[ \text{Eluted} \]

\[ \text{Eluted} \]

\[ \text{Eluted} \]

\[ \text{Eluted} \]

\[ \text{2x15 µL=30} \]

\[ \text{2x15 µL=30} \]

\[ \text{2x15 µL=30} \]

\[ \text{2x15 µL=30} \]

\[ \text{60 µL} \]

\[ \text{60 µL} \]

\[ \text{60 µL} \]

\[ \text{60 µL} \]

\[ \text{samples were kept at -80°C} \]

\[ \text{47°C gel - Remove self ligated adapters} \]
Banana Slug - Mate Pair 9/23/19

Shearing Conditions for obtaining 5-6 kb fragments

**Instrument:** Hydroshear

**Program:** Large-Assembly 5-6 kb

1. \(5 	imes 16\) cycles 20
   - Cycle 1
   - Volume 150 ml

2. \(5 	imes 16\) cycles 25
   - Volume 150 ml

---

**DNA** - 1ug of DNA sheared - 2 tubes (each 1ug)

**Banana slug DNA** extracted 9/22/19 - 250 ng/ul

**1 ug DNA**

**Tube 1:** 4 ul Banana slug DNA

121 ul Nuclease free water (nfw)

Condition 1 - 20 cycles

**Tube 2:** 4 ul Banana slug DNA

121 ul Nuclease free water (nfw)

Condition 2 - 25 cycles

---

After shearing DNA was concentrated & purification using Qiagen gel extraction kit.

**QG = 375 ul**

**Isopropanol = 125 ul**

**Total = 625 ul**

1 column

**Eluted = 8 ul x 2 = 16 ul**
Genomic DNA = 800 ng
Bgl II (Nanodrop) = 16.98 ng/ul x 66 = 272 ng (lost = 72.8%) (started 1000 ng)
Bst II (Nanodrop) = 22.74 ng/ul x 6 = 364 ng (lost = 63.6%) (started 1000 ng)

Gel - 0.8% 0.5x TBE (EtBr)
1) 1 kb
2) Genomic DNA
3) Bananas shv II (20 cycles)
4) Bananas shv III (35 cycles)

DNA seems concentrated between 3-4 kb either
for 20-25 cycles.
Banana Slug Shearing Conditions 9/24/19

Genomic DNA = 255 ng/ul

Used sol = 2 ug

Mixed sol DNA with 117 ul H2O

125 ul (2 tubes)

HydroShear

Large Assembly

Nanodrop

150 ng
16 speed code
30 cycles

890 ng
(7.12 ng/ul)

Concentrated with Speed Vacuum - 4h

Gel - 0.8% 1X TAE + ET Br

150 ul
16 speed code
40 cycles

786 ng
(6.28 ng/ul)

P.s. Out of 2 ug, we recovered about 800 ng after shearing (No Purification performed). So, we are losing a lot of DNA during the shearing.
1) 1 kb ladder
2) 30 cycles
3) 40 cycles

RE: The Genomic DNA was not completely sheared.
(The band above 12 kb). We don't see this band anymore
after purification with Qagen kit. (This result is
expected for shearing with hydroshear).
Banana Slug - Shearing Conditions 9/24/9

Genomic DNA - 255 ng/μl

↓

Used avg. = 8 μl of DNA

Mixed 8 μl of DNA with 117 μl of nuclease free water

12 tubes

↓

Hydroshear conditions

180 μl
16 speed code
35 cycles

↓

QG = 355 μl
100% ethanol = 125 μl
Etched = 2 x 9 μl = 18 μl

↓

Large Assembly

1 μl DNA
150 μl
16 speed code
30 cycles

↓

Qagen Plasmid Gel extraction kit

↓

Qagen Plasmid Gel Extraction kit

↓

Gel: 0.8% made with 1x TBE + Ethidium Bromide (2 μl to 60 μl)

↓

1) HD ladder
2) Banana Slug Genomic DNA + 3.5 μl loading dye
3) Shearing 1 - 25 cycles (10 μl sample + 3.5 μl 6x loading dye)
4) Shearing 2 - 20 cycles
1) Genome DNA
   1 Kb ladder

2) Banana Sky 1 - 42.66 ng/ul x 18 ul = 768 ng (lost 62%)

3) Banana Sky 2 - 44.85 ng/ul x 18 ul = 807 ng (lost 60%)

DNA seems concentrated between 3-4 Ks
We might be able to collect between 11-12 Ks

There is a slight difference between the amount of DNA we lose between 25 cycles and 30 cycles (30 cycles loses less DNA)

After Qiagen purification we don't recover fragments above 12 Ks. However, what happens if we don't purify?
Hydroshear Standard Assembly - Conditions for obtaining 4-5 kbs
Standard Assembly - speed code 15, 5 cycles - 125 uL.

Tested a few samples and conditions.

1) Used Human DNA as control for shearing condition - 2 ug each sample
1.1) Sheared human DNA using large assembly for testing
     conditions to obtain fragments between 5-6 kbs
     SC 16, 25 cycles, 150 uL. (twice)
1.2) Sheared human DNA using standard assembly for obtaining
     1.2.1) 4.5 kbs - SC 15, 5 cycles, 150 uL (8040D manual) (2X)
     1.2.2) above 5 kbs - SC 19, 5 cycles, 150 uL (Hydroshear manual) (2X)

2) Used Banana Slug DNA - 2 ug each tile

   Tested Standard Assembly for obtaining:
2.1) 4.5 kbs - SC 15, 5 cycles, 150 uL (8040D manual) - 2X
2.2) above 5 kbs - SC 19, 5 cycles, 150 uL (Hydroshear manual) - 2X

   DNA - 2 ug (8 uL - Slug/Human) + 117 uL H2O

   Total = 125 uL

Washing conditions: 2 washes with solution I
2 X solution II
3 X solution III (Water)
Quantitate DNA after shearing

BS sc 15 cycles - 12.71 ng/μl = 1588.75
BS sc 19 cycles - 12.03 ng/μl = 1503.75
LA - Human sc 16 25 cycles UV 12.86 ng/μl = 1663.75
Human sc 15 cycles - 13.31 ng/μl = 1663.75
Human sc 19 cycles - 12.73 ng/μl = 1591.25

After quantitation - Speed Vacuum sample for 30 minutes (which was too much)

Run gel - 0.8% 0.5X TBE + EDTA

1) BS sc 15 5 cycles va
2) BS sc 19 5 cycles va
3) Human sc 15 5 cycles va
4) Human sc 19 5 cycles va
5) Human sc 16 25 cycles va
Gel Purify Library  10/16/09

4% Agarose Gel (LE) in 1x TAE

150 ml Gel - \( \frac{150 \times 4}{100} = 6 \) g of agarose in 1x TAE

1.2 kb = 60 \( \mu l \) + 6 \( \mu l \) 10x loading dye
2.3 kb = 60 \( \mu l \) + 6 \( \mu l \)
3.4 kb = 60 \( \mu l \) + 6 \( \mu l \)
4.6 kb = 60 \( \mu l \) + 6 \( \mu l \)

loaded 3 wells each with 20 \( \mu l \) of sample, dye mixture

2 \( \mu l \) Tracer, 25 \( \mu l \) ladder + 1 \( \mu l \) loading dye + 16 \( \mu l \) EB

Band 154-156 bp

<table>
<thead>
<tr>
<th>Gel 1</th>
<th>1st well</th>
<th>25 bp</th>
<th>1-2 1-2 1-2</th>
<th>2-3 3-3 2-3</th>
<th>25 bp</th>
<th>25 bp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>3-3 3-3 3-3</td>
<td>4-6 4-6 4-6</td>
<td>25 bp</td>
<td></td>
</tr>
</tbody>
</table>

| Gel 2  | 1st well | 25 bp | 3-4 3-4 3-4 | 4-6 4-6 4-6 | 25 bp |
Exposure 545 ms
1. 25 bp ladder
2. Blank
3
4 \( \geq 1-2 \text{ KS} \)
5
6. Blank
7
8 \( \geq 2.3 \text{ KS} \)
9
10. Blank
11. 25 bp
12. Blank

Exposure 1.9 s
Same picture as above

120 V 45 min
1X TAE
1. Blank
2. 25bp ladder
3. Blank
4. > 3-4 kb
5. 6
6. 7. Blank
7. 8
8. 9. > 4-6 kb
9. 10. 11
12. > Blank
13. 14. 25bp ladder

120V for 45 minutes
1X TAE
<table>
<thead>
<tr>
<th>AQG</th>
<th>1209 mL</th>
<th>503 mL</th>
<th>( \frac{300}{1} ) mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>300p</td>
<td>1209 mL</td>
<td>503 mL</td>
<td>( \frac{300}{1} ) mL</td>
</tr>
</tbody>
</table>

\[
\begin{array}{|c|c|c|}
\hline
& 2-3 mL & 3-4 mL & 4-6 mL \\
\hline
AQG & 0.635 g & 0.495 g & 0.638 g \\
\hline
& \frac{1905 mL}{635 mL} & \frac{1914 mL}{638 mL} & \frac{2475 mL}{3190 mL} \\
\hline
\end{array}
\]

2 columns

1007 mL per column

2 columns

1587/mL column

1237/mL column

1595/mL column

2 columns

9.1x each

1.7x each

2.15x each

2 columns

2 x 15 mL each

60 mL

-20°C

-20°C

-20°C
Akrani’s Sample - BS Mate-Pair 3x25 bp
After Circularization / Plasmid Seq.

Digestion - Eco P151

<table>
<thead>
<tr>
<th>1-2 Ks</th>
<th>2-3 Ks</th>
<th>3-4 Ks</th>
<th>4-6 Ks</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.35 ng/ul (659.8 ng)</td>
<td>17.85 ng/ul (678.3 ng)</td>
<td>17.42 ng/ul (661.9 ng)</td>
<td>21.59 ng/ul (820.42 ng)</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{Eco} & \quad \frac{659 \times 10}{100} \times \frac{1}{10} = 6.59 \mu l \\
\text{Eco} & \quad \frac{678 \times 5}{100} \times \frac{1}{10} = 3.3 \mu l \\
\text{Eco} & \quad \frac{661 \times 5}{100} \times \frac{1}{10} = 3.3 \mu l \\
\text{Eco} & \quad \frac{820 \times 5}{100} \times \frac{1}{10} = 4.1 \mu l
\end{align*}
\]

\[
\begin{align*}
\text{NHE} & \quad 38 \\
\text{VEB3} & \quad 10 \\
\text{Eco} & \quad 1 \\
\text{refr} & \quad 1 \\
\text{mM} & \quad 20 \\
\text{Eco} & \quad 6.6 \\
\text{Water} & \quad 23.4 \\
\text{Water} & \quad 100
\end{align*}
\]

\[
\frac{26.6}{100} = 26.6
\]

37° C Overnight - PCR Machine

Digestion Part II - 4 samples

\[
\begin{align*}
\text{Digested DNA} & \quad 100 \\
10 nM Sse838M & \quad 1 \\
10x ATP & \quad 2 \\
\text{Eco} & \quad 0.5 \\
\text{Eco} & \quad 103.5
\end{align*}
\]

37° C - 1h / Denature 65° C 20 minutes - PCR Machine
End - Repair - Klenow - 4 samples

Eco DNA 403.5
dNTP Mix - 1.5
Klenow - 1
Total 106

Room Temperature 30 minutes > PCR Machine
Denature 65°C for 20 minutes

Streptavidin Binding Buffer

Tris- HCl, pH 7.5 (500 mM) 30
Sodium chloride 5M 400
EDTA, 0.5M 2
Water 578
1000

DNA = 106
Strep Buffer = 200
Nuclease free water = 74
400 μl

Quick Ligation Buffer

2 x Quick ligation - 500 = 1000
NFW - 370 = 1230
600 - 2400

Dynabeads MyOne T1
Cat N: 656.02
Lot 622404

NEB buffer
448 x 2 = 896
Quick ligation Buffer
E60108-NP
Lot 4/0908
1- 2 ml

\[ 1 \mu g \text{ DNA} \times 10^6 \times \frac{1}{660} \times \frac{1}{1536} = 1 \text{ pmol/μl DNA} \]

\[ 0.66 \mu g \times \frac{1}{4} \times 20 \times 1 \times \frac{1}{50} = 0.4 \mu l \text{ P1/P2} \]

2- 3 ml

\[ 1 \mu g \text{ DNA} \times 10^6 \times \frac{1}{660} \times \frac{1}{2536} \times \frac{1}{1673760} = 0.6 \text{ pmol/μl DNA} \]

\[ 0.66 \mu g \times 30 \times 0.6 \times \frac{1}{50} = 0.35 \mu l \text{ P1/P2} \]

3- 4 ml

\[ 1 \mu g \text{ DNA} \times 10^6 \times \frac{1}{660} \times \frac{1}{3536} \times \frac{1}{2383760} = 0.42 \text{ pmol/μl DNA} \]

\[ 0.66 \mu g \times 0.43 \times 30 \times 1 \times \frac{1}{50} = 0.17 \mu l \text{ P1/P2} \]

4- 6 ml

\[ 1 \mu g \text{ DNA} \times 10^6 \times \frac{1}{660} \times \frac{1}{4536} \times \frac{1}{2993760} = 0.33 \text{ pmol/μl DNA} \]

\[ 0.82 \mu g \times 0.33 \times 30 \times 1 \times \frac{1}{50} = 0.2 \mu l \text{ P1/P2} \]
1/2 x 5 DNA Complex = 97.5
Quinea lyzase = 2.5
P1 = 0.2 μl
P2 = 0.2 μl

Room Temperature for 15 minutes

2 x 500 DNA Complex = 97.5
Quinea lyzase = 2.5
P1 = 0.2 μl
P2 = 0.2 μl

Quinea lyzae
Lot = 98/1108
E 600 Qb NP

NEB

40 x NEB 2 = 60 = 240
Water = 540 = 2160

Nico transf. DNA

DNA - bead Complex = 96
100 mM DNTP mix, 25 mM and a c
DNA Polymerase I = 2
Total = 100

Incubate 30 minutes

1 x Bead Wash Lot No. - 0903013
1 x Bind Wash Lot No. - 0902006
Amplification - Agarwala libraries

$ul\times4$ samples

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platinum PCR Master Mix</td>
<td>200</td>
</tr>
<tr>
<td>Primer 1</td>
<td>8</td>
</tr>
<tr>
<td>Primer 2</td>
<td>8</td>
</tr>
<tr>
<td>Cold PfU</td>
<td>1</td>
</tr>
<tr>
<td>Nuclease free water</td>
<td>143</td>
</tr>
</tbody>
</table>

$\frac{800}{360} = 2.22\text{ samples}$

$\frac{572}{1440} = 0.40\text{ samples}$

- $270\mu M$ MM
  - 30x - 1-2 kb
    - 3 tubes
      - 100 ul each
  - 270x - 2-3 kb
    - 3 tubes
  - 270x - 3-4 kb
    - 3 tubes
- 90x MM
  - 10 ul water
- 90x MM
  - 10 ul water

PCR 9700 - Gold 

95°C 10 min 15 sec

95°C 15 sec

62°C 15 sec

60°C 4 min

4°C∞

4 cycles

Lonzza Gel
1. Quanti Kit
2. 1-2 kb
3. 2-3 kb
4. 3-4 kb
5. 4-6 kb
6. 1-2 kb
7. 2-3 kb
8. 3-4 kb
9. 4-6 kb
p. 100-4 kb ladder
↓
No Amplification

9/13 2009

95°C
15 sec
↓
60°C
15 sec
↓
60°C
4 min
↓
4°C

⇒ Total 6 cycles
↓
Ladder Gel

⇒ Repeat PCR cycles
3 more cycles
⇒ total 9 cycles
↓
Ladder Gel

⇒ Repeat PCR cycles
4 more cycles
⇒ total 13 cycles
↓
Ladder Gel

⇒ Repeat PCR cycles
7 more cycles
⇒ total 20 cycles
↓
Stained Gel
1 - Giant ladder
2. 1-2 kb
3. 2-3 kb
4. 3-4 kb
5. 4-6 kb
6. 4-2 kb
7. 2-3 kb
8. 3-4 kb
9. 4-6 kb
10. 100-500 kb ladder

No Amplification
13 cycles

Checked only samples. No Controls

1. 100-4 KB ladder
2. 1-2 KB
3. 2.3 KB
4. 3.4 KB
5. 4-6 KB

No Amplification

1. 100-4 KB ladder
2. 1-2 KB
3. 2.3 KB
4. 3.4 KB
5. 4-6 KB

No Amplification

- Control

Stopped Sample Prep

Trouble shooting