Prepare DNA samples

1. Bring DNA sample up to 30μl with TE.
2. Bring loading solution to room temperature.
3. For each sample, combine 30μl of DNA sample with 10μl of loading solution.
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

Load Samples

1. Re-check the buffer level in the sample wells. Make sure that sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer, if necessary.
2. Remove 40μl of buffer from the first sample well, and load 40μl of sample (or DNA marker E) into that well.
   Take care not pierce the agarose with the pipette tip. There is gel on all sides and bottom of the sample well. In addition, there is an agarose “chimney” surrounding the top of the sample well that protrudes up through the cassette cover. When removing buffer, some users find it useful to immerse the pipette tip just below the surface of the buffer and follow the liquid level down with the tip as the buffer is removed. When buffer removal is completed, there will be ~30ul of buffer left in the well. When adding sample, place the tip of the pipette just below the surface of the buffer, and follow the liquid level up with the tip as the well fills. Don’t be concerned if the sample well slightly overfills. The density of the sample will allow it to sink before it can flow out of the well.
3. Repeat step 2 for the remaining four wells.

Run

1. Close the lid, go to the Main Tab, and make sure the proper protocol is loaded in the “Protocol Name” field.
2. Press “START”. The run will automatically stop when every collection is complete.

<table>
<thead>
<tr>
<th>Target (bp)</th>
<th>Time to Collect (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>51</td>
</tr>
<tr>
<td>300</td>
<td>67</td>
</tr>
<tr>
<td>600</td>
<td>100</td>
</tr>
</tbody>
</table>

Recommended Sample Load Guidelines

**Ionic strength:** The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions).

**Protein in the sample:** For best results, samples should be de-proteinized prior to loading if possible.

Maximum Load: 10 μg sheared genomic DNA
Minimum Load: low single nanograms

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Help: support@sagescience.com or call 978.922.1832
**Program a Protocol**

1. In the Pippin Prep Software, go to the Protocol Editor Tab.
2. Click "Cassette" folder, and select "2% Marker E v2".
3. Select the collection mode for each lane ("Tight" or "Range" mode), and enter the size selection parameters.
4. Enter the lane number to which the DNA marker will be loaded into the "Reference Lane" field:
5. Click the "Apply Reference to All Lanes" button:
6. The "Ref Lane" fields should contain the lane number to which the DNA marker is added.
7. Press "Save As" and name and save the protocol.

**Prepare the Cassette for Loading**

1. Dislodge bubbles from behind the elution wells. Tilt the cassette sample well side down, to release the any trapped bubbles behind the elution modules.
2. Place Cassette into the optical nest. Keep the cassette slightly tilted down so that the bubbles in the elution reservoirs don't return to the area behind the elution modules. Be sure the cassette is fully seated into the bottom of the nest to ensure proper optical alignment.
3. Remove the white tabbed adhesive strips from the cassette. Place one hand on the cassette, and hold it firmly in the nest. Grab the white tabs of the tape and pull the strips firmly and slowly toward the front of the BluePippin until they are removed.
4. Check the buffer level in the sample wells. Immediately prior to loading, sample wells should be completely filled to the top with buffer. If any wells are underfilled, top them up with additional buffer.
5. Perform the continuity test. Close the lid and press the "Test" button located in the lower right area of the Main screen. The test routine runs automatically and measures the current in each separation and elution channel. And should return a "PASS" for each separation and elution channel. The cassette temperature must be above 170C (620F).

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