**Quick Guide**

**Prepare DNA samples**

1. Bring DNA sample up to 30μl with TE.
2. Bring loading solution to room temperature.
3. For each sample, combine the 30μl 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 U1) 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 ~30μl 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.

**Collect Fractions**

1. **Remove samples using a standard 100-200μl pipette.** Samples will be in a Tris-TAPS buffer at a volume of 40μl. Samples should be suitable for amplification. Do not let samples remain in cassette overnight.

2. **Remove cassette and dispose of properly.** Do not keep used cassettes in the Pippin with the cover closed. Humidity from the cassette may cause damage to electrodes.

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**BluePippin™ Quick Guide**

**0.75% Agarose Gel Cassette**

for targets between 10kb - 18 kb

**Product Number:** BUF7510

**Software version:** v.5.9

**Cassette Definition:** 0.75% DF 10-18kb Marker U1

**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.

<table>
<thead>
<tr>
<th>Sample Load*</th>
<th>5μg sheared genomic DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum Load</td>
<td>low single nanograms</td>
</tr>
</tbody>
</table>

*Calibrations were determined using 5μg loads of genomic DNA. Adjustments to collection settings may be required to account for other amounts (up to 10μg) or input fragment distribution profile.

**Approximate Run Times for “Tight” Range Targets (hr:min)***

<table>
<thead>
<tr>
<th>Target (bp)</th>
<th>Time to Collect (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000</td>
<td>175</td>
</tr>
<tr>
<td>12,000</td>
<td>180</td>
</tr>
<tr>
<td>15,000</td>
<td>194</td>
</tr>
<tr>
<td>17,000</td>
<td>211</td>
</tr>
<tr>
<td>18,000</td>
<td>220</td>
</tr>
</tbody>
</table>

* Remaining lanes are temporarily shut off during elution, overall cassette run time may be up to 40 min. longer.

**Prepare DNA samples**

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2. Bring loading solution to room temperature.
3. For each sample, combine the 30μl DNA sample with 10μl of loading solution.
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.
Program a Protocol
1. In the BluePippin Software, go to the Protocol Editor Tab.
2. Click “Cassette” folder, and select the appropriate cassette definition.
3. Select the collection mode for each lane (usually “Tight” or “Range”), and enter the size selection parameters.
4. Determine which lane to which the DNA marker (U1) will be added and enter it into the “Reference Lane” field. In the example below, U1 is loaded in lane 1.
5. Click the “APPLY REFERENCE TO ALL LANES” button:
6. Make sure each “Ref Lane” field contains the marker lane designation:
7. Press “Save As” and name and save the protocol.

Prepare the Cassette for Loading
1. Remove the cassette from the foil packaging.
2. Inspect the levels of buffer in all buffer reservoirs. Reservoirs should be nearly full. If the buffer level in any reservoir appears less than 50% full, fill with spare buffer.
3. Inspect the gel columns. Look for obvious breakage of the agarose column in each channel. If there is obvious breakage, do not use the lane. Remaining lanes can be used.
4. Inspect for bubbles due to separation of agarose from bottom of cassette in the region used for optical detection of DNA. If a bubble is observed, do not use the lane. A bubble on the top of the gel column will not affect detection.

Calibrate the Optics with the Calibration Fixture
1. Place the calibration fixture onto the optical nest. Close the Lid.
2. Press “CALIBRATE” to launch the calibration window.
3. Enter 0.60 in the “Target I ph, mA” field. Press “CALIBRATE” button in the window, and when complete press “EXIT”.

Inspect the Gel Cassette
1. Remove the cassette from the foil packaging.
2. Inspect the levels of buffer in all buffer reservoirs. Reservoirs should be nearly full. If the buffer level in any reservoir appears less than 50% full, fill with spare buffer.
3. Inspect the gel columns. Look for obvious breakage of the agarose column in each channel. If there is obvious breakage, do not use the lane. Remaining lanes can be used.
4. Inspect for bubbles due to separation of agarose from bottom of cassette in the region used for optical detection of DNA. If a bubble is observed, do not use the lane. A bubble on the top of the gel column will not affect detection.