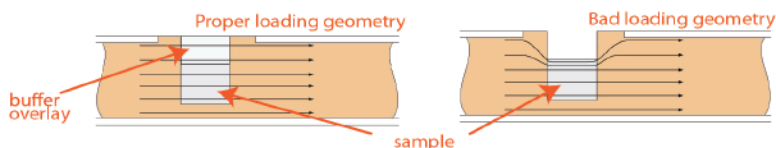


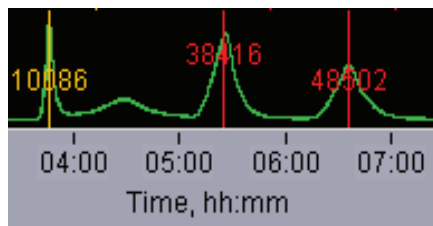
Load Samples



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 Z1) 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 displayed in the "Protocol Name" field.
2. Press "START". The run will automatically stop when every collection is complete.



DNA marker should appear as shown

Collect Fractions

1. **Remove samples using a standard 100-200µl pipette.** Samples will be in a Tris-TAPS buffer at a volume of 40ul. 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.

| Approximate Run Times | | |
|-----------------------|---------|-------------|
| Target | hr:min* | Add (min)** |
| 50kb tight | 7:50 | 15 |
| 50kb broad | 8:50 | 30 |

* time to elute one sample lane

** add time for each additional sample lane elution

BluePippin™ Quick Guide

0.75% Agarose Gel Cassette 50kb Targeted Collections

Product: BHZ7510
Software: v.6.02 CD 15
Cassette Definitions:

0.75% DF 50kb Marker Z1 - tight
0.75% DF 50kb Marker Z1 - broad

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.

Sample Load*: 5 µg sheared genomic DNA
Minimum Load: low single nanograms

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

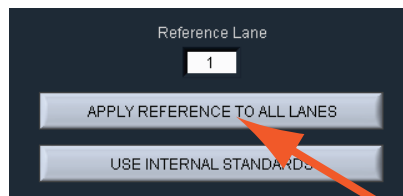
Prepare DNA samples

1. Bring DNA sample up to 30µl with TE.
2. Bring loading solution to room temperature.
3. Add 10µl of loading solution to each 30µl DNA sample.
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 the "Tight" collection mode for each lane, and enter "50000" into the "BP Target" field.
4. Determine which lane into which the DNA marker (Z1) will be loaded. Enter the lane number in the "Reference Lane" field. In the example below, Z1 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:

| | Tight | Range | Time | Peak | Ref Lane |
|---|-------|-------|------|------|----------|
| 5 | | | | | 1 |
| 4 | | | | | 1 |
| 3 | | | | | 1 |
| 2 | | | | | 1 |
| 1 | | | | | 1 |

7. Press "Save As" and name and save the protocol.

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.

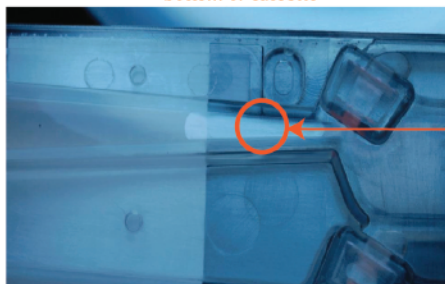
anode buffer chambers, side view



buffer reservoir on right is low fill with spare buffer

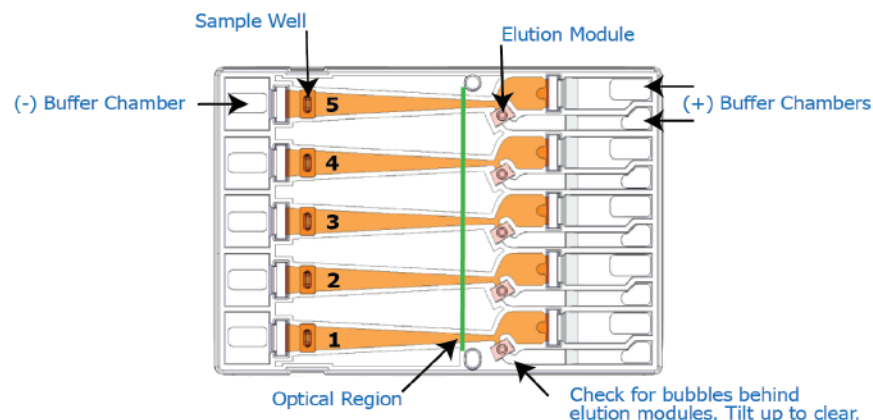
3. **Inspect the gel columns.** Look for obvious breakage of the agarose column in each channel. If there is obvious breakage, do not use the lane. Remaining lanes can be used.
4. **Inspect for bubbles due to separation of agarose gel column from bottom of cassette in the region used for optical detection of DNA.** If such bubbles are present, do not use the lane for the DNA marker. (Bubbles on the top surface of the gel column will have no effect on optical detection or purification – lanes with such bubbles can be used.)

bottom of cassette



optical region

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. **Remove buffer from all elution modules and replace with 40µl of fresh electrophoresis buffer.** Make sure that the pipette tips extend all the way to the bottom of the elution modules without sealing the elution port opening. Test tip fit using the empty rinse cassette supplied with the instrument.
5. **Seal the elution wells with the adhesive tape strips.** Tape for sealing the elution wells are supplied with cassette packaging. Place tape over the elution wells and rub firmly to fix the tape in position.
6. **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.
7. **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 17°C (62°F).