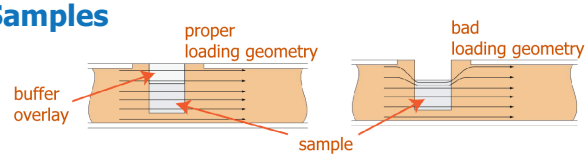
F. Load Samples

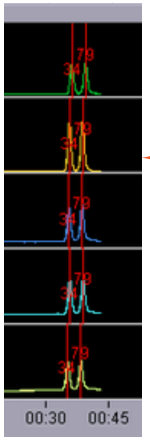


1. **Re-check the buffer level in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer if necessary.

2. **Remove 40µl of buffer from the first sample well, and load 40µl of sample into that well.** Take care not to 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. Note: If running an external marker, remove 40µl of buffer from the assigned lane's sample well, and load 40uL of the prepared Marker solution.**

G. Run



Marker V2

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.

Internal markers should appear as shown

Approximate Run Times for "Tight" Range Targets

Target (bp)	Time to Collect (min)
100	49
200	57
300	63
400	71
500	79
600	86

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

Help: support@sagescience.com or call 978.922.1832

BluePippin™ Quick Guide

2% Agarose Gel Cassette

for targets between 100-600bp

Product Number: BDF2010
Cassette Definition: 2% DF Marker V2
Cassette and Marker: 2% Agarose / Marker V2

Research Use Only

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

A. Prepare DNA samples with internal standards

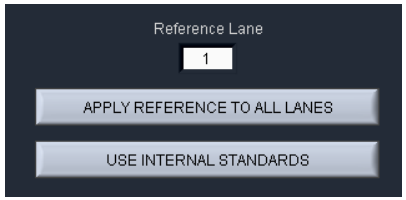
1. Bring the loading/marker mix (**Marker V2**) to room temperature.
2. Bring DNA samples up to 30µl with TE.
3. Combine each DNA sample with 10µl of loading/marker mix (**Marker V2**) (total volume/sample = 40µl).
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

(Optional) Prepare DNA samples with an external marker

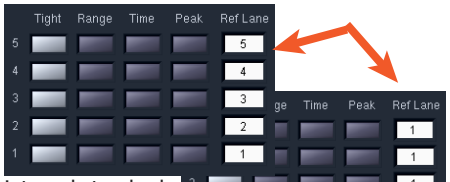
1. Bring the loading/marker mix (**Marker V2**) and **Loading Solution** tubes to room temperature.
2. In a single tube, add 30µl of TE and then add 10µl of loading/marker mix (**Marker V2**).
3. Bring DNA samples up to 30µl with TE.
4. Combine each DNA sample with 10µl of **Loading Solution** (total volume/sample = 40µl).
5. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Program a Protocol

1. In the BluePippin Software, go to the Protocol Editor Tab.
2. Click "Cassette" folder and select "**2% DF Marker V2**".
3. Select the collection mode for each lane (usually "Tight" or "Range"), and enter the size selection parameters.
4. Click the "Use Internal Standards" button or "Apply Reference to All Lanes" if using an external marker:



5. Make sure the "Ref Lane" values match the lane number that will contain markers:



internal standards

external marker in lane 1

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

C. Calibrate the Optics with the Calibration Fixture

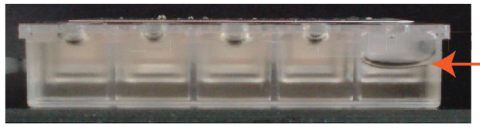
1. Place the calibration fixture onto the optical nest. Close the Lid.
2. Press "CALIBRATE" to launch the calibration window.
3. Press "CALIBRATE" button in the window, and when complete, press "EXIT".

Optical Calibration Fixture

D. 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.

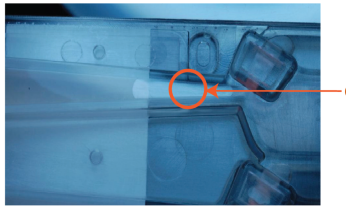
anode buffer chambers, side view



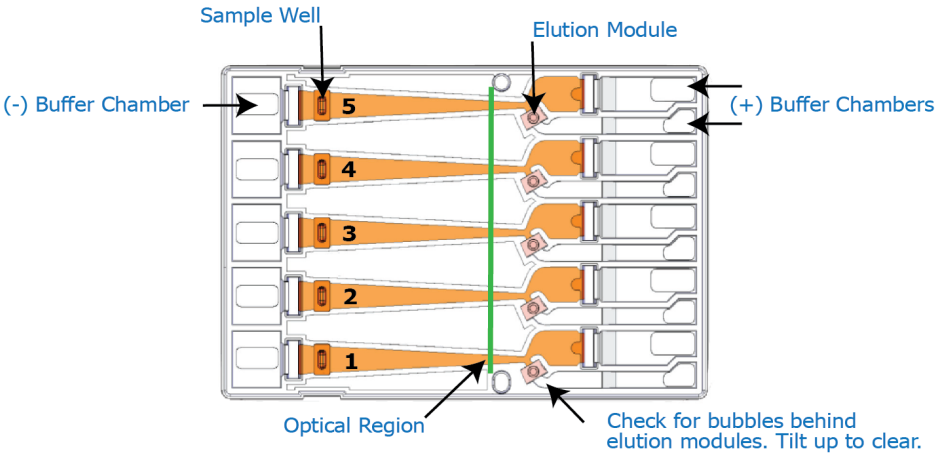
buffer reservoir on right is low
fill with spare buffer

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 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.

bottom of cassette



optical region



E. Prepare the Cassette for Loading

1. **Dislodge bubbles from behind the elution modules.** Tilt the cassette, sample well side down, to release 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 the tip fit using the empty rinse cassette supplied with the instrument.
5. **Seal the elution modules with the adhesive tape strips.** Tape for sealing the elution modules are supplied with cassette packaging. Place tape over the elution modules 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 17ºC (62ºF).

Doc# UOC-460049 RevB