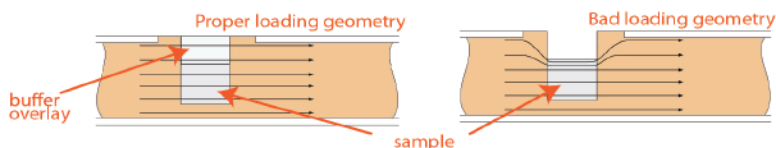


Load Samples



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.

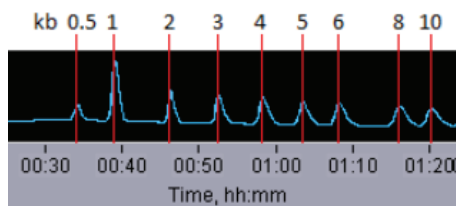
2. **Remove 40 μ l of buffer from the first sample well, and load 40 μ l of sample (or DNA marker D) into that well.**

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 \sim 30 μ l 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.



DNA markers should appear as shown

Approximate Run Times for "Tight" range Targets

Target (bp)	Time to Collect (min)
2000	62
4000	75
6000	87
8000	93

Collect Fractions

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

Help: support@sagescience.com or call 978.922.1832

Pippin PrepTM Quick Guide

0.75% Agarose Gel Cassette for targets between 2 kb - 8 kb

software v. 5.3
and higher

0.75% use marker **D**

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 μ g sheared genomic DNA
Minimum Load: low single nanograms

* DNA migration rates are sample load dependent. Cassettes have been calibrated with 5 μ g input with broad fragment distribution. Collections setting may need to be adjusted to account for other sample loads or fragment distribution profiles.



Caution! Cassettes contain Ethidium Bromide.

Prepare DNA samples

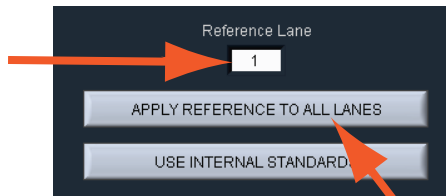
1. Bring DNA sample up to 30 μ l with TE.
2. Bring loading solution to room temperature.
3. For each sample, combine 30 μ l of DNA sample with 10 μ l of loading solution/marker mix (**labeled D**).
4. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.



© 2012 Sage Science, Inc. Pippin Prep is a trademark of Sage Science, Inc.

Program a Protocol

1. In the Pippin Prep Software, go to the Protocol Editor Tab.
2. Click "Cassette" folder, and select "**0.75% Marker D Target (tight) cuts only**".
3. Select the collection mode for each lane ("Tight" mode only), 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.

	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.

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 **0.80** in the "Target I_{ph}, mA" field. Press "CALIBRATE" button in the window, and when complete press "EXIT".



Caution! Cassettes contain Ethidium Bromide.

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.

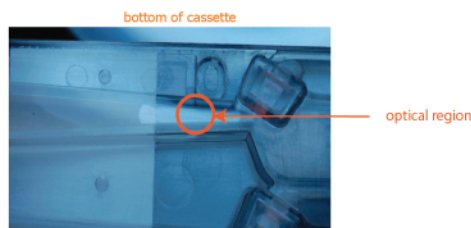
anode buffer chambers, side view



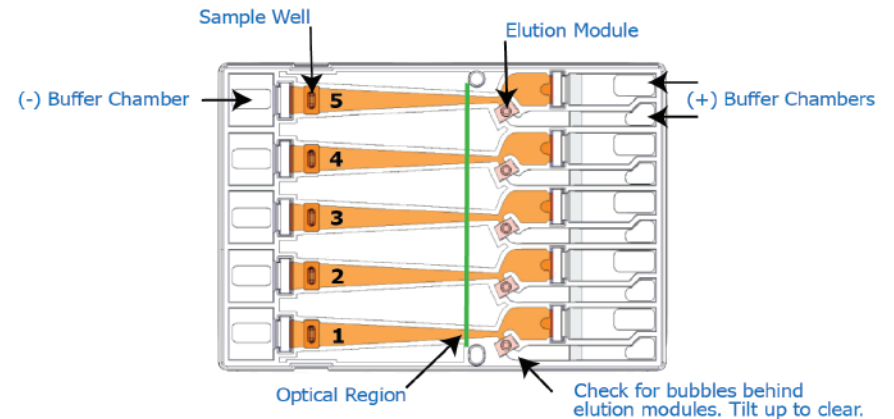
buffer reservoir on right is low fill with spare buffer

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.



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).