BluePippin™ Quick Guide

Cassette Kit# BRT-7510 and BRT-7503
Range + T Size Selection, 9-30kb

Important!: The Range + T method requires BluePippins with a Serial Number 2700 and above.

Note: For best results, DNA samples should be de-proteinized. The Range + T method is used to account for uncertainty that results from variations in DNA fragment distribution profiles. It is important to have an analysis of the input DNA profile before starting.

A. Prepare DNA samples and External Marker

1. Bring the external marker (**E2**) to room temperature.
2. Bring the loading solution to room temperature.
3. Dilute up to 5 µg of a sheared DNA sample into a final volume of 30 µl TE.
4. Add 10µl of the loading solution to each of the 30µl DNA samples.
5. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Program a Protocol

1. In the Protocol Editor Tab, Click the "Cassette" folder, and select the following cassette definition:

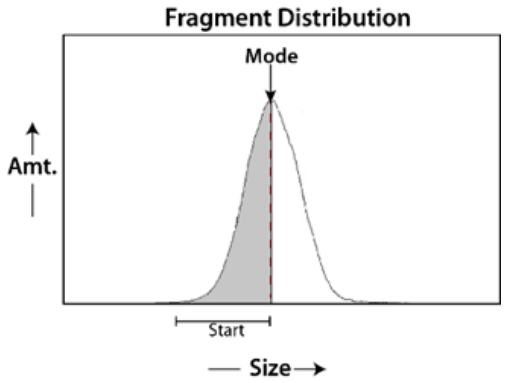
0.75% DF 9-30kb R +T Marker E2

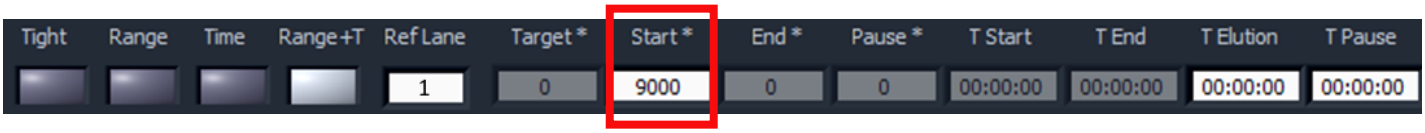
2. Determine the lane into which the DNA marker (E2) will be added and enter it in the "Reference Lane" field. In the example below, E2 will be loaded into lane 1.

3. Click the "APPLY REFERENCE TO ALL LANES" button, and click the "Range+T" mode buttons for each sample lane. The mode section of the editor should appear as shown:

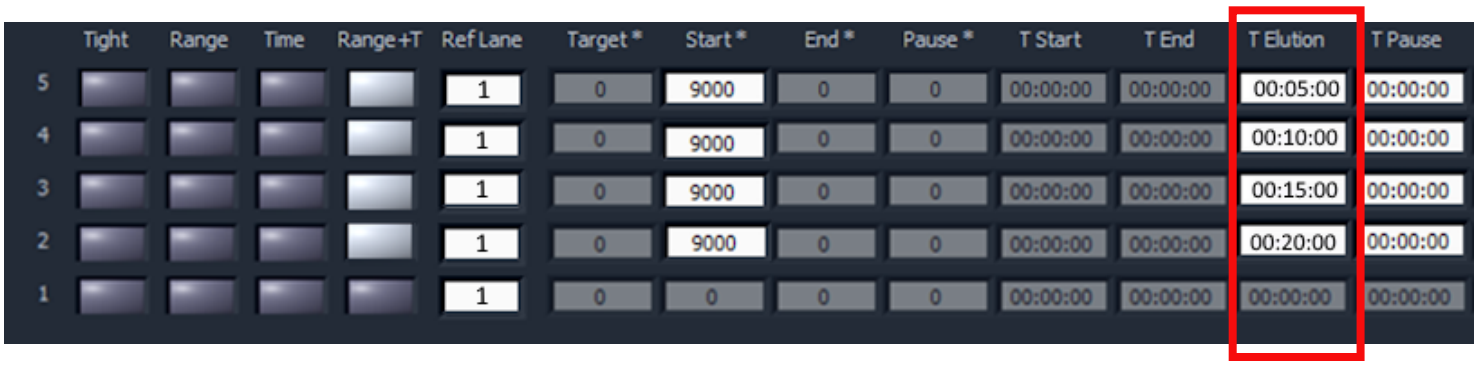
Do not select a collection mode for the lane to which the external standard has been assigned.

4. In the sample lanes, select "Range + T" mode, and enter a Start value between **9-30kb** in the "Start" field. If the fragment distribution is gaussian, or has a peak, make sure the Start value is <u>before</u> the mode of the distribution.

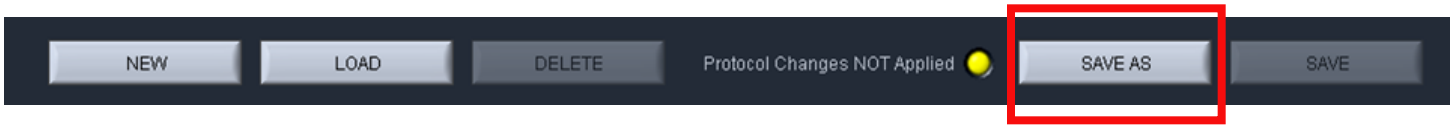




5. If the TElution time has already been determined, enter the time value, as hh:mm:ss in the "T Elution" field. Otherwise, enter times with increasing values to test for a desirable fragment range. A good guideline to begin with is 5, 10, 15 and 20 minutes. <u>Do not exceed 30 minutes.</u> Determining the best elution time may require several iterations of size selection.

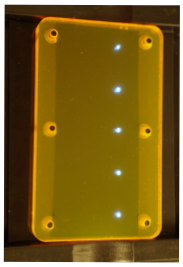


6. Press "Save As" and name and save the protocol.



C. Calibrate the Optics with the Calibration Fixture

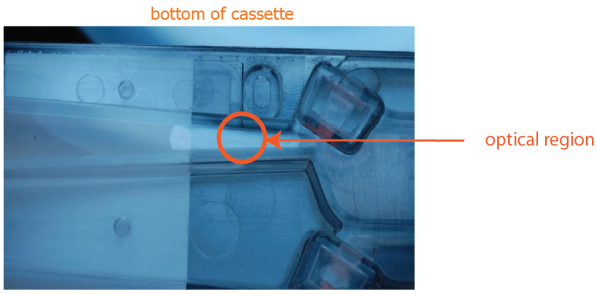
1. Place the calibration fixture onto the optical nest. Close the Lid.
2. Press "CALIBRATE" to launch the calibration window.
3. Press the "CALIBRATE" button in the window. When complete, the "Calibration Status" field will indicate "Calibration OK". Press "EXIT" to continue.

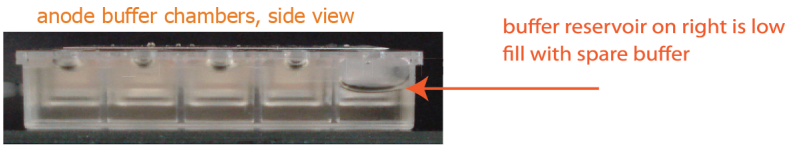


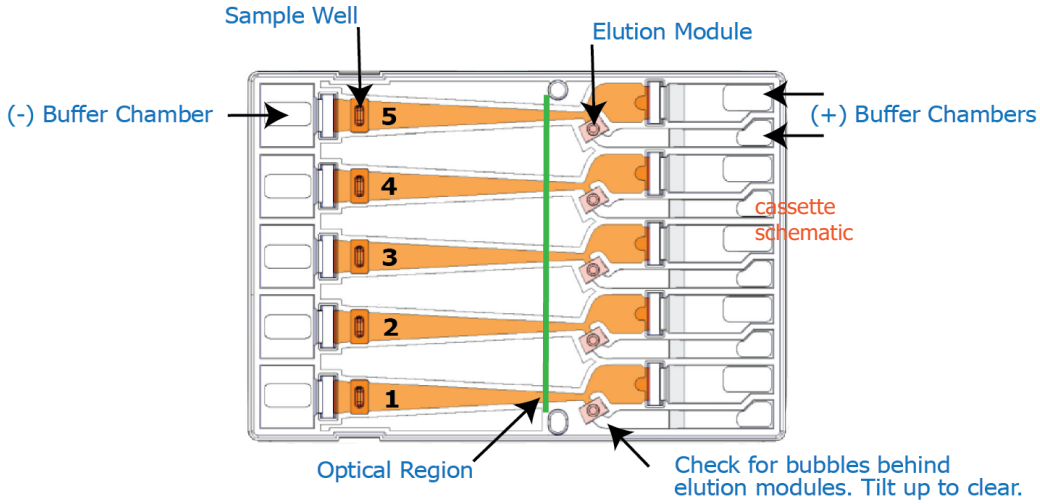
Calibration fixture

D. Inspect the Gel Cassette

1. **Remove the cassette** from the foil packaging.
2. **Inspect the gel columns.** Look for obvious breakage of the agarose column in each channel. Inspect for bubbles due to separation of agarose from the bottom of cassette (especially in the region used for optical detection). Do not use the affected lane pair if these problems are detected, the remainder of the lanes can be used.
3. **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.







E. Prepare the Cassette for Loading

1. **Dislodge bubbles from behind the elution wells.** 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 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.

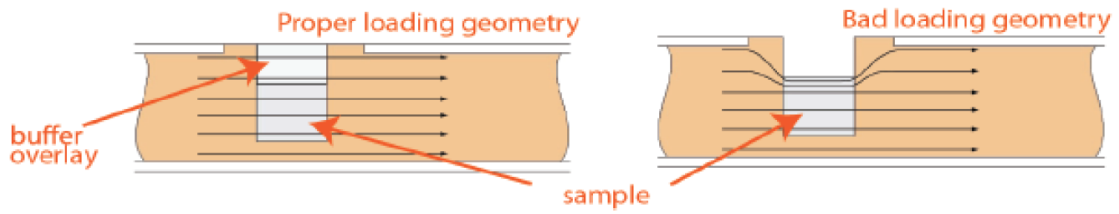
5. **Seal the elution wells with the adhesive tape strips.** Place supplied adhesive tape over the elution wells and rub firmly to fix the tape in position.

Help: support@sagescience.com or call 888.744.0144

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).

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.

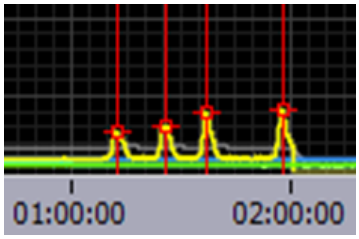
2. **Find the designated external marker lane, remove 40µl of buffer. Load 40 µl of Marker E2 into that lane. Repeat with DNA samples in the remaining sample lanes.**

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 adding sample, place tip of 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.

G. 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.



The external marker should appear as shown

0.75% DF 9-30kb R +T Marker E2

Suggested TElution Guidelines:

5, 10, 15, 20 minutes
do not not exceed 30 minutes

9-30kb Range +T Start (bp)	Time to start of elution (hh:mm)
9000	2:00
15000	3:00
16000	3:20
19000	3:40
22000	4:00
30000	5:00

H. Collect Fractions

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

2. **Remove 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 electrodes.