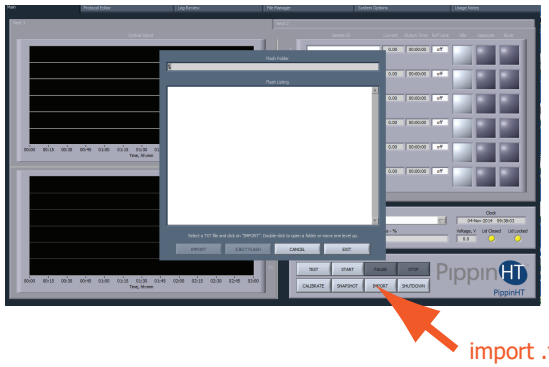
G. Enter Sample Information (Optional)

Sample information may be manually keyed, read with an external barcode reader, or imported as a text file from a USB drive. A log file name prefix may be entered in the System Options Tab.

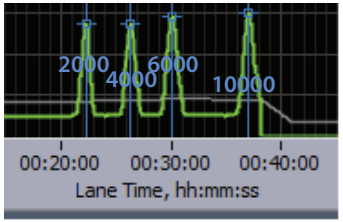


H. Run

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.

0.75% Agarose 6-10kb high-pass 75E



High Pass Threshold (bp)	Time to Collect (hr:min)
6000	1:00
10000	1:15

0.75% Agarose 15-20kb high-pass 75E

High Pass Threshold (bp)	Time to Collect (hr:min)
15000	2:20
17000	2:34
20000	2:45

The external marker 75E should appear as shown

I. Collect Fractions

1. **Remove samples using a standard 100-200µl pipette.** Samples will be in a Tris-TAPS buffer, 30µl volume.

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

Help: support@sagescience.com or call 978.922.1832

PippinHT™ Quick Guide

Product:	**HPE7510**
Cassette Definitions:	**0.75% Agarose high-pass 6-10kb 75E**
	0.75% Agarose high-pass 15-20kb 75E
Cassette and Marker:	**0.75% Agarose / Marker 75E**

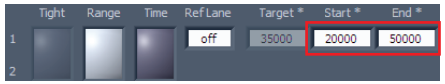
Research Use Only

A. Prepare DNA samples and Enternal Marker

1. The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions). For best results, samples should also be de-proteinized.
2. Bring DNA samples up to 20µl with TE.
3. Bring the external marker (**75E**) and the loading solution to room temperature.
4. Combine DNA samples with 5µl of the loading solution.
5. Mix DNA samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Program a Protocol

1. In the PippinHT Software, go to the Protocol Editor Tab.
2. Click the "Cassette" folder, and select the appropriate cassette definition:
   0.75% Agarose 6-10kb high-pass 75E
   0.75% Agarose 15-20kb high-pass 75E
3. Make sure the "Cassette 1" tab is selected.
4. In lane-pair 1/2, select "Range" mode, and enter a threshold value between **6-10kb** or **15-20kb** in the "Start" field. Enter **50000** in the "End" field.



5. To copy the size selection to the remainder of the lanes, press "COPY LANE 1 TO CASSETTE":

6. Using the drop-down menu, select a lane into which the external marker will be loaded. Press "APPLY TO ALL LANES".

7. Go to the lane-pair which has been selected for the external marker and press "Range". This will clear the size selection parameters, and prepare the lane-pair for loading the external marker.

8. Select the "Cassette 2" tab. To copy the "Cassette 1" size selection parameters to "Cassette 2", press "COPY CASSETTE 1 TO CASSETTE 2":

9. Press "SAVE AS" and name and save the protocol.

C. Calibrate the Optics with the Calibration Fixture

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

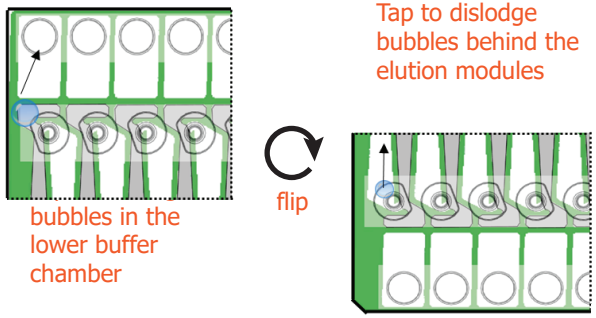
D. Inspect the Gel Cassette

1. **Remove the cassettes** 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 the 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 lane pairs can be used.

E. Prepare the Cassette for Loading

1. **Dislodge bubbles in the lower buffer chamber**. Bubbles may become trapped under a plastic shelf at the end of the gel column. Hold the cassette with the lower buffer chambers facing up and tap to dislodge any bubbles.

2. **Dislodge bubbles from behind the elution modules.** Flip the cassette so the sample wells are facing up. Tap the cassette to dislodge any bubbles that are trapped behind the elution modules.



3. **Remove the adhesive tape.** Do this by placing the cassettes onto a benchtop and firmly pulling with an even motion.

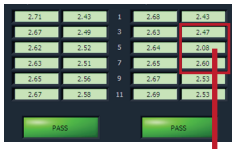
4. **Remove 150µl of buffer from each of the upper buffer chambers.** Refer to the diagram on the next page for the location of the chambers.

5. **Remove all buffer from the elution modules and replace with 30µl of spare electrophoresis buffer.**

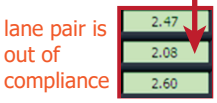
6. **Re-seal the elution modules** with the adhesive tape provided with the kit.

7. **Perform the Continuity Test.** Place the cassettes onto the nests, close the lid, and press "Test" in the Main Tab. This will launch the Continuity Test window. Press the "Test" button in the Test window.

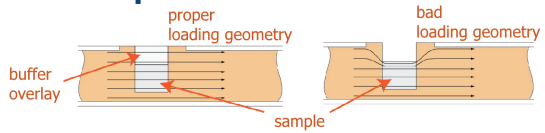
8. **A PASS/FAIL result will be reported.** If a lane pair shows a FAIL state (red), replace the buffer in the electrode ports or elution modules as indicated for that pair.



9. **If only one lane of a pair is obstructed, the pair may still indicate a PASS.** Check that the lane-pair values in each display column are within ~+/0.1 of each other. If not, replace the buffer in the affected lane pair.



F. Load Samples



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

2. **Remove 30µl of buffer from the first sample well and load 25µl of the sample (or external marker, if assigned to that lane) 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 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 wells. Make sure the external marker has been loaded into the assigned reference lane.**