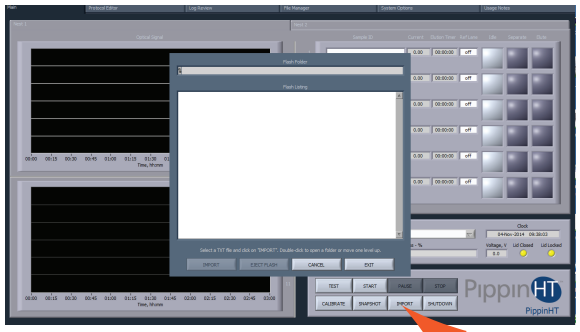
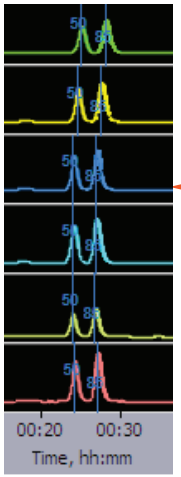
G. Enter Sample Information (Optional)

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



Marker 30G

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

Target	Time to Collect (min)
100	31
150	38
250	47

I. Collect Fractions

1. **Remove samples using a standard 100-200µl pipette.** Samples will be in a Tris-TAPS buffer, 30µl volume. Do not let samples remain in cassette overnight.
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: HTG3010
Cassette Definition: 3% Agarose 100-250bp 30G
Cassette and Marker: 3% Agarose / Marker 30G

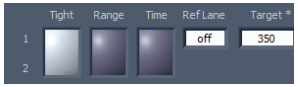
Research Use Only

A. Prepare DNA samples with Internal Standards

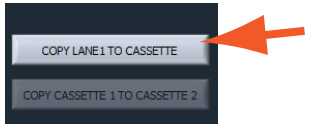
1. The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions). For best results, samples should also be de-proteinized.
2. Bring DNA sample up to 20µl with TE.
3. Bring the internal standard/loading solution (30G) to room temperature.
4. For each sample, combine the 20µl DNA sample with 5µl of the 30G solution.
5. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Program a Protocol

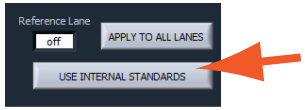
1. In the PippinHT Software, go to the Protocol Editor Tab.
2. Click the "Cassette" folder, and select the appropriate cassette definition: **3% Agarose 100-250bp 30G**.
3. Make sure the "Cassette 1" tab is selected.
4. Enter size selection parameters in lane-pair 1/2.



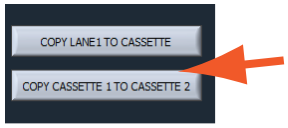
5. To copy the size selection to the remainder of the lanes, press "COPY LANE 1 TO CASSETTE":



6. Press "USE INTERNAL STANDARDS" to assign internal standards to each lane pair.



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



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

C. Calibrate the Optics with the Calibration Fixture

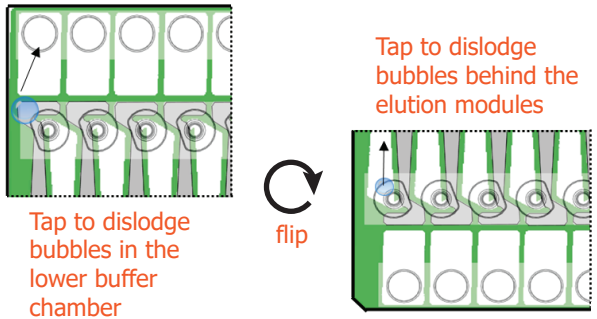
1. Place the calibration fixtures onto both optical nests. Close the Lid.
2. Press "CALIBRATE" to launch the calibration window.
3. Press the "CALIBRATE" button in the window, and when complete, press "EXIT".

D. Inspect the Gel Cassette

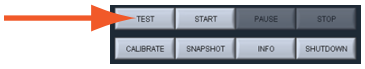
1. **Remove the cassettes** from the foil packaging.
2. **Inspect the gel columns.** Look for obvious breakage of the agarose column in each channel. Inspect for bubbles due to separation of agarose from the bottom of the cassette (especially in the region used for optical detection). Do not use the 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.

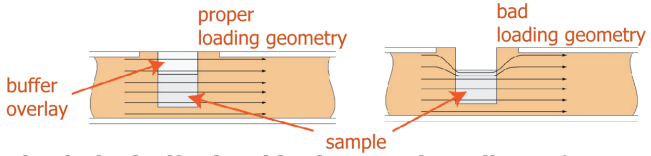


3. **Remove the adhesive tape.** Do this by placing the cassettes onto a benchtop and firmly pulling with an even motion.
4. **Remove 80µl of buffer from each of the upper buffer chambers.** Refer to the diagram on the next page for the location of the chambers.
5. **Remove all buffer from the elution 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.



8. **A PASS/FAIL screen will pop up.** 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 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 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.
3. **Repeat step 2 for the remaining eleven wells, or use a 12-channel multipipettor to load the wells at the same time.**