

H. Run



1. Close the lid, go to the Main Tab
2. Press "START". The run will automatically stop when every collection is complete.

← Internal markers should appear as shown and should appear by 20-22 min.

Total Run Time = 28 - 30 min.

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

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PippinHT Quick Guide

Cassette Kit#

PGT-1510

for use with the Agilent OnePGT Solution

A. Prepare DNA samples with Internal Standards

1. For best results, samples should be de-proteinized.
2. Bring DNA sample up to 20µl with TE.
3. Bring the internal standard/loading solution (**15C**) to room temperature.
4. For each sample, combine the 20µl DNA sample with 5µl of the **15C** solution.
5. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

B. Select the OnePGT Protocol

1. In the PippinHT Software, go to the Protocol Editor Tab.
2. Click the "Protocol" folder, and select the the following protocol from the drop down menu:

OnePGT 335-575 bp Size Selection

3. All of the relevant fields in the Protocol Editor will auto-fill.

	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause
1				1	455	335	575	0	00:00:00	00:00:00	00:00:00
2											

Auto-filled parameters in a lane-pair

C. Calibrate the Optics with the Calibration Fixture

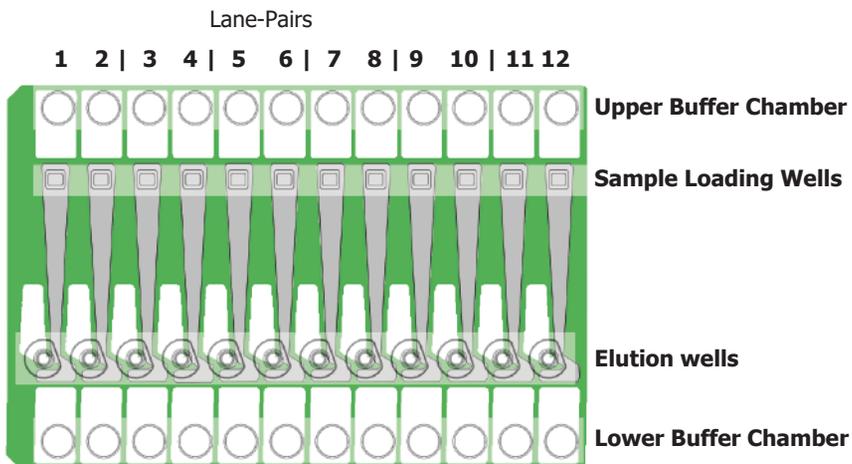
1. Place the calibration fixtures onto both optical nests. Close the Lid.
2. Press "CALIBRATE" to launch the calibration window.
3. Press the "CALIBRATE" button in the window. When complete, the "Calibration Status" field will indicate "Calibration OK". Press "EXIT" to continue.

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 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 pairs can be used.

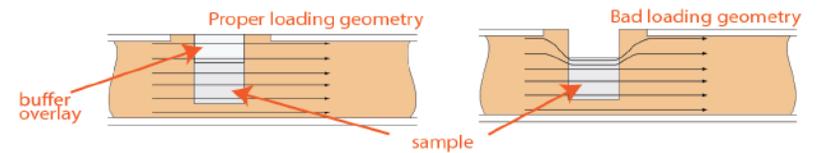
E. Prepare the Cassette for Loading

1. **Dislodge bubbles from behind the elution modules.** Do this by gently tapping the cassette while holding sideways with the elution modules facing up.
2. **Remove the adhesive tapes.** Do this by placing the cassettes onto a benchtop and an firmly pulling with an even motion.
3. **Remove 80 μ l of buffer from each of the upper buffer chambers.** Refer to the diagram below for the location of the chambers.
4. **Remove all buffer from the elution modules and replace with 30 μ l of spare electrophoresis buffer.**
5. **Re-seal the elution modules** with the adhesive tape provided with the kit.
6. **Perform the Continuity Test.** Place the cassettes onto the nests, close the lid, and press "Test" in the Main Tab.



2.

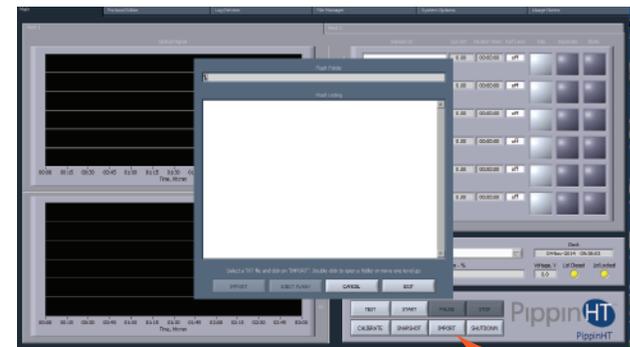
F. Load Samples



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 30 μ l of buffer from the first sample well, and load 25 μ l of sample 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 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.**

G. Enter Sample Information (Optional)

In the Main screen, sample information may be manually keyed, read with a 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.



Main Screen

import .txt file

3.