

PippinHT™ Quick Guide

Cassette Kit# HRT-7510 and HRT-7504 Range + T Size Selection, 9-30kb

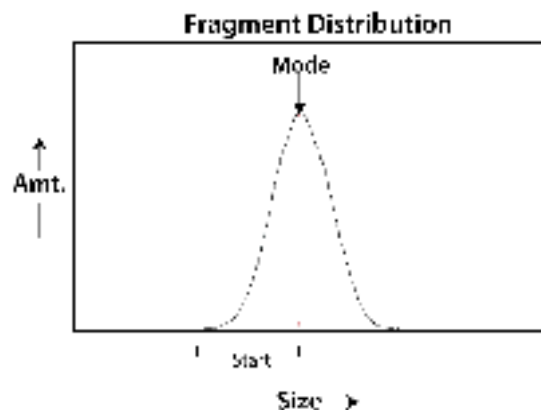
Note: For best results, DNA samples should be de-proteinized. The Range + T method is used to account for uncertainty that results from variations in DNA fragment distribution profiles. It is important to have an analysis of the the input DNA profile before starting.

A. Prepare DNA samples and External Marker

1. This protocol requires a dedicated lane-pair to run an external marker.
Prepare up to 10 samples per cassette to be run.
2. Bring the external marker (**75E**) to room temperature.
3. Bring the loading solution to room temperature.
4. Bring DNA samples up to 20µl with TE.
5. Add 5µl of the loading solution each of the 20µl DNA samples .
6. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.

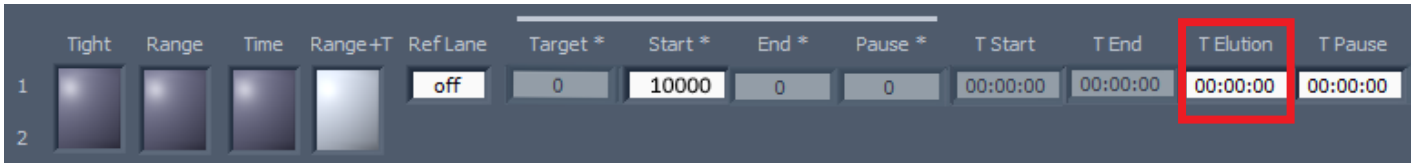
B. Program a Protocol

1. In the Protocol Editor Tab, make sure the Cassette 1 tab is selected.
2. Click the "Cassette" folder, and select the following cassette definition:
0.75% Agarose 9-30kb R +T 75E
3. In lane-pair 1/2, 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 before the mode of the distribution.

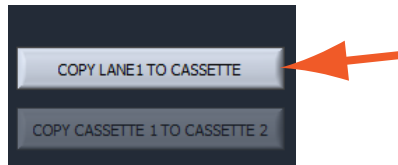


	Tight	Range	Time	Range+T	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Elution	T Pause
1					off	0	10000	0	0	00:00:00	00:00:00	00:00:00	00:00:00
2													

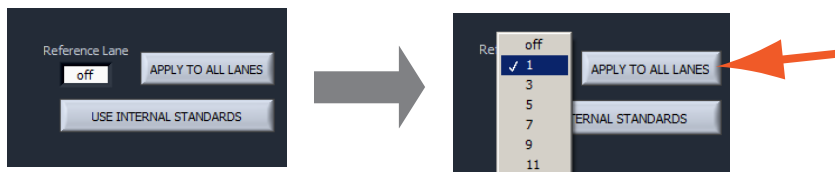
4. If the TEluion time has already been determined, enter the time value, as hh:mm:ss in the "TEluion" field. Otherwise, leave the values as zero (enter them after lanes have been copied [Step 8])



5. In the lower left the protocol screen press "COPY LANE 1 TO CASSETTE". This will copy the size selection parameters in lane-pair 1/2 to the remaining lane-pairs.



6. In the lower left-hand corner of the protocol screen, select a lane into which the external marker will be loaded using the drop-down menu. It must be an odd-numbered lane. Select "APPLY TO ALL LANES".

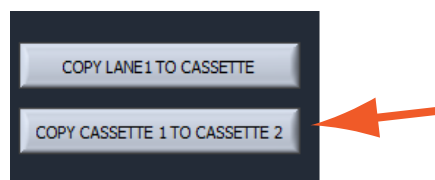


7. Go to the lane-pair which has been selected for the external marker and press "Range + T". This will clear the size selection parameters, and prepare the lane-pair for loading the external marker.



8. To begin to optimize for an elution time value, enter increasing values into the TEluion fields for each lane pair. A good guideline to start with is 5, 7.5, 10, 15 and 20 minutes. Do not exceed 30 minutes of elution. After the run, the elutions should be analyzed. Determining the best elution time may require several iterations of size selection.

9. If running two cassettes: select the cassette 2 tab. To copy the cassette 1 size selection parameters to cassette 2, press "Copy Cassette 1 to Cassette 2" (if necessary).



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

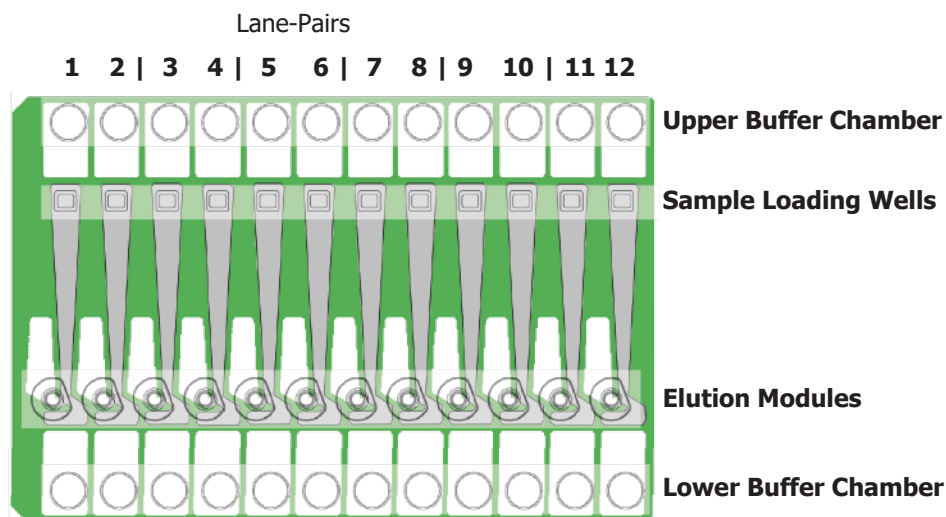
C. Calibrate the Optics with the Calibration Fixture

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. 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.

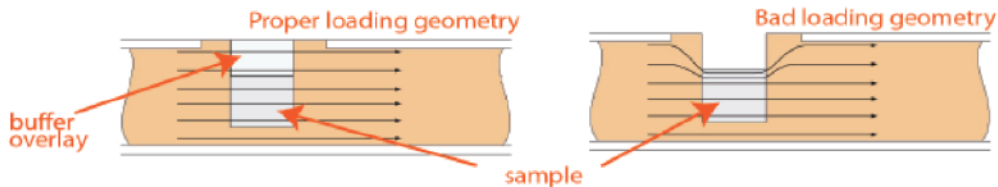
Note: Refer to the cassette schematic below for the steps that follow.



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 firmly pulling with an even motion.
3. **Remove 150µl of buffer from each of the upper buffer chambers.** Refer to the diagram above for the location of the chambers.
4. **Remove all buffer from the elution modules and replace with 30µl 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.

F. Load Samples



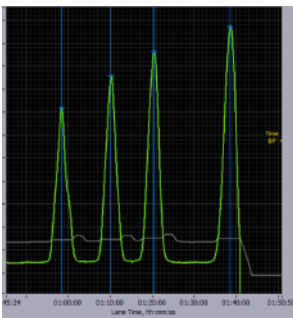
- 1. Re-check the buffer level in the sample wells.** Make sure that sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer if necessary.
- 2. Find the designated external marker lane, remove 30 μ l of buffer. Load 25 μ l of Marker 75E into that lane.** The marker must be loading into an odd-numbered lane (optically detected lane). The adjacent lane in the lane-pair is not used (the next highest lane number, i.e.1/2).
- 3. 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.
- 4. Repeat step 3 for the remaining 10 wells, or use a 12-channel multipipettor to remove buffer and load the wells simultaneously.**

G. Enter Sample Information (Optional)

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.

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.



The external marker should appear as shown

0.75% Agarose 9-30kb R +T 75E

Suggested TEIution Guidelines:

5, 7.5, 10, 15, 20 minutes
do not not exceed 30 minutes

9-30kb Range +T Start (bp)	Time to start of elution
10000	2:00
15000	2:40
17000	3:00
20000	3:15
25000	3:45
30000	4:15

I. Collect Fractions

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