

PippinHT™ Quick Guide

Product: HRT7510
Cassette Definitions: 0.75% Agarose 9-30kb R+T 75E
Cassette and Marker: 0.75% Agarose / Marker 75E

Research Use Only

Note: The ionic strength of the sample should be lower than the ionic strength of the buffer (80mM monovalent ions). For best results, DNA samples should be de-proteinized.

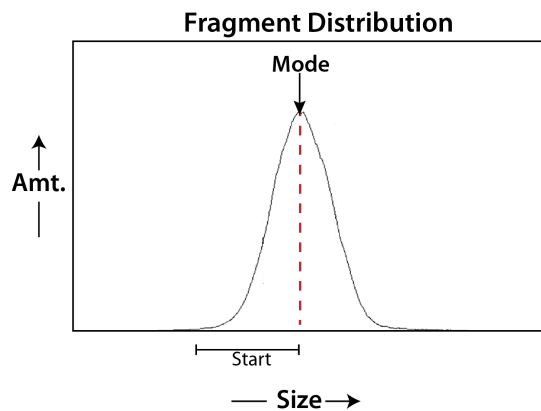
The Range + T method is used to account for uncertainty that results from variations in DNA fragment distribution profiles. **It is important to have an analysis of the input DNA profile before starting.**

A. Prepare DNA samples and External Marker

1. This protocol requires a dedicated lane-pair to run an external marker. Prepare up to 10 samples per cassette to be run.
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

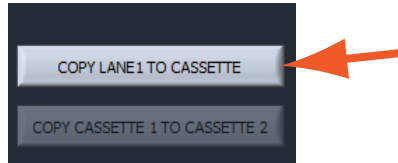
1. In the Protocol Editor Tab, make sure the Cassette 1 tab is selected.
2. Click the "Cassette" folder, and select the following cassette definition:
0.75% Agarose 9-30kb R+T 75E
3. In lane-pair 1/2, select "Range + T" mode, and enter a Start value between **9-30kb** in the "Start" field. If the fragment distribution is gaussian, or has a peak, make sure the Start value is before the mode of the distribution.



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



5. In the lower left of 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".

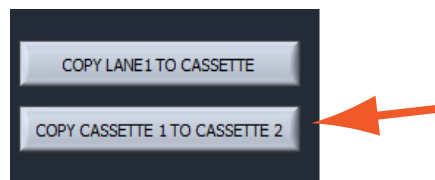


7. Go to the lane-pair which has been selected for the external marker and press "Range + T". This will clear the size selection parameters, and prepare the lane-pair for loading the external marker.



8. To begin to optimize for an elution time value, enter increasing values into the TEIution 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, and when complete, press "EXIT".

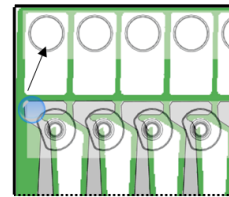
D. Inspect the Gel Cassette

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 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.

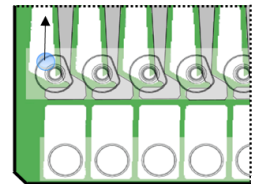
2. **Dislodge bubbles from behind the elution modules.** Flip the cassette so the sample wells are facing up. Tap the cassette to dislodge any bubbles that are trapped behind the elution modules.



Tap to dislodge bubbles in the lower buffer chamber



Tap to dislodge bubbles 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.**
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 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.

2.71	2.43	1	2.68	2.43
2.67	2.49	3	2.63	2.47
2.62	2.52	5	2.64	2.08
2.63	2.51	7	2.65	2.60
2.65	2.56	9	2.67	2.53
2.67	2.58	11	2.69	2.53

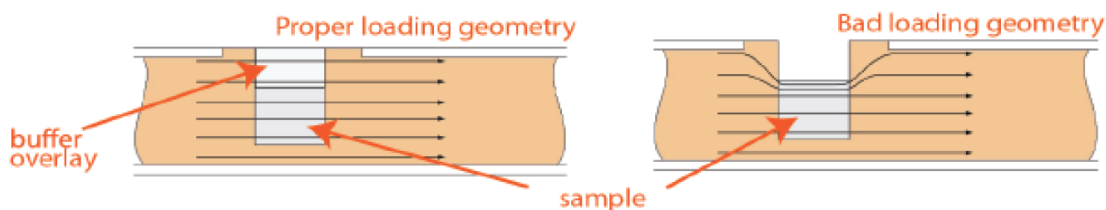
PASS PASS

2.47
2.08
2.60

lane pair is out of compliance

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 $\sim \pm 0.1$ of each other. If not, replace the buffer in the affected lane pair.

F. Load Samples



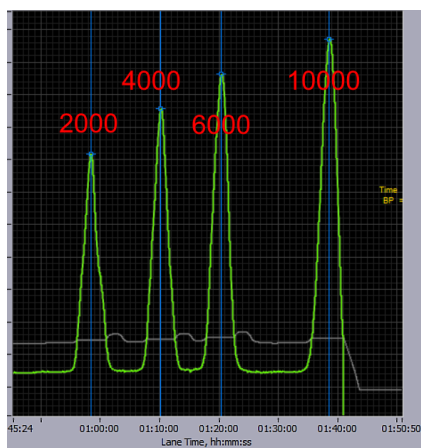
1. **Check the buffer level in the sample wells.** Make sure that the sample wells are completely full to the top with electrophoresis buffer. Fill with additional buffer if necessary.
2. **Remove 30µl of buffer from the first sample well and load 25µl of the sample (or external marker, if assigned to 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.**

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 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

The external marker 75E should appear as shown

I. Collect Fractions

1. **Remove samples using a standard 100-200µl pipette.** Samples will be in 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.