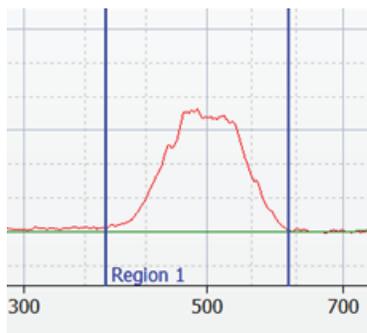
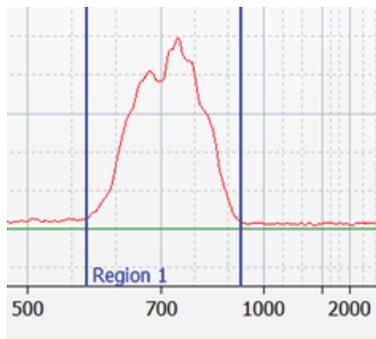


400-600 bp Range selection



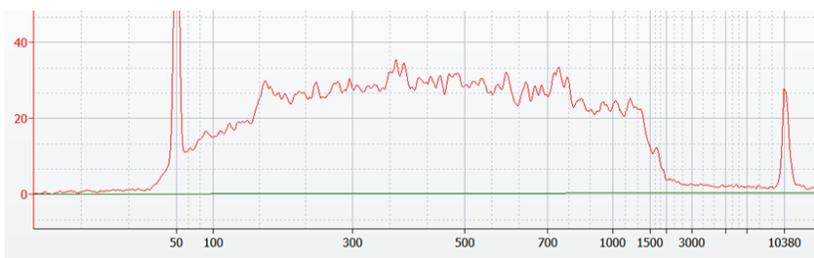
Target = 400-600 bp
Ave Size = 390-620 bp
Conc. [ng/ul] = 9.00

600-900 bp Range selection



Target = 600-900 bp
Ave Size = 589-935 bp
Conc. [ng/ul] = 11.27

Input DNA (CDH1504)



* These data are not intended to imply guaranteed results or performance. This product is intended to demonstrate that the PippinHT product is functioning as expected, and that proper operational technique is being used. Users should refer to the Operations Manual for performance specifications.

PippinHT™

Control DNA CDH1504

For validation of 1.5% agarose gel cassettes
(part nos.: HTC1510 or HTC1504)



Suite 2400, 500 Cummings Center
Beverly, MA 01915
support@sagescience.com
978.922.1834

What is Enclosed

The PippinHT systems are developed and validated using restriction digests of genomic DNA from E. coli. The DNA digestion approach has been selected such that fragment size distribution matches the useful fractionation range of the cassette without any significant peaks or gaps. Following restriction digestion, the control DNA is purified by phenol:chloroform extraction, dialyzed, and diluted into PippinHT electrophoresis buffer.

Enclosed is one tube of gDNA digest with sufficient volume for 48 sample loads (approx 1000 ul). Users should use reagents and gel cassettes supplies with 1.5% agarose gel kits (HTC1510 or HTC1504). The final DNA load amount per sample well will be 1.0 µg, which is the sample amount used for size selection calibration and ongoing quality control.

Control DNA is useful to test, refine, and troubleshoot PippinHT size fractionation protocols. It can also be used to check system performance.

To Use

1. Pipette 20 µl of gDNA digest per reaction tube.
2. Add 5 µl of internal standard to each tube (Marker 15C).
3. Mix samples thoroughly (vortex mixer). Briefly centrifuge to collect.
4. In the software protocol editor, program size selection parameters shown below, to compare with the Bioanalyzer analysis examples on the following pages.
5. Carefully follow sample load instructions outlined in the Operations Manual or cassette Quick Guide.
6. Pipette 25 µl of the gDNA/marker mix into a sample well.

QC Protocol for 1.5% Agarose Cassettes

Quality control procedures use the following size selection parameters to assess the performance of the PippinHT system (8 samples are loaded in 4 lane-pairs in the example below):

	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause	Sample ID	Template	Force LED	No.	Range Flag	
1				1	600	532	668	0	10:00:00	10:00:00	10:00:00					light	
2																	
3				3	900	800	1000	0	10:00:00	10:00:00	10:00:00					light	
4																	
5				5	500	400	600	0	10:00:00	10:00:00	10:00:00					broad	
6																	
7				7	750	600	900	0	10:00:00	10:00:00	10:00:00					broad	
8																	
9				9	0	0	0	0	10:00:00	10:00:00	10:00:00					none	
10																	
11				11	0	0	0	0	10:00:00	10:00:00	10:00:00					none	
12																	

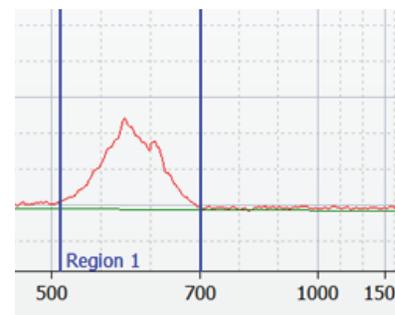
Typical Results

At the end of a run, marker peaks will be detected in each lane-pair in the Main screen of the PippinHT software interface. They will appear between 19-24 min. as shown here. :



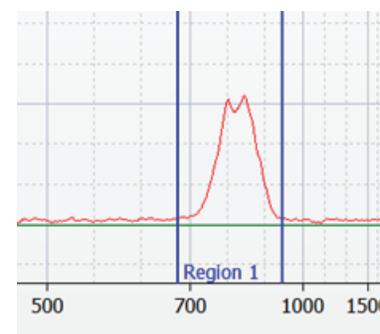
Extracted samples are run on an Agilent Bioanalyzer using a DNA 7500 chip. The analysis volume is 4 µl from a 30 µl elution volume. The following bioanalyzer results indicate typical results from QC testing:

600 bp Tight selection



Target = 600 bp
Ave Size = 606 bp
Conc. [ng/ul] = 4.86

800 bp Tight selection



Target = 800 bp
Ave Size = 822 bp
Conc. [ng/ul] = 4.18