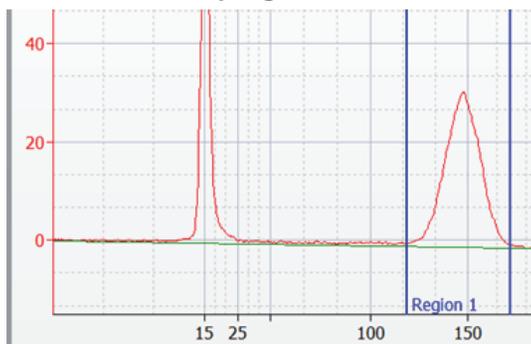


150 bp Tight selection



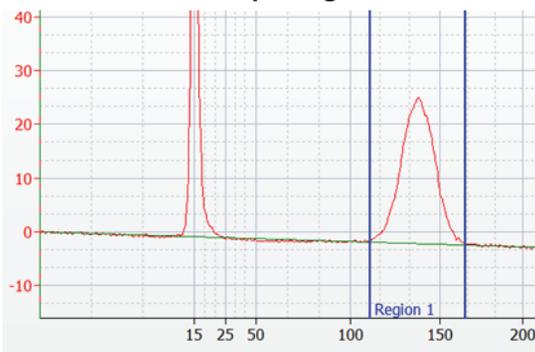
Target = 150 bp
Ave Size = 148 bp
Conc. [ng/ul] = 5.20

100-300 bp Range selection



Target = 100-300 bp
Ave Size = 94-297 bp
Conc. [ng/ul] = 41.15

110-165 bp Range selection



Target = 110-165 bp
Ave Size = 111-165 bp
Conc. [ng/ul] = 5.74

* 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 CDH3004

For validation of 3% agarose gel cassettes
(part nos.: HTC3010 or HTC3004)



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. Users should use reagents and gel cassette supplies with 3% agarose gel kits (HTC3010 or HTC3004). 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 30A).
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 3% agarose cassettes

Quality control procedures use the following size selection parameters to assess the performance of the PippinHT system:

	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause	Sample ID	Template	Force LED	On	Range Flag
1				1	90	74	106	0	00:00:00	00:00:00	00:00:00					tight
2																
3				3	110	91	129	0	00:00:00	00:00:00	00:00:00					tight
4																
5				5	150	128	172	0	00:00:00	00:00:00	00:00:00					tight
6																
7				7	200	100	300	0	00:00:00	00:00:00	00:00:00					broad
8																
9				9	138	110	165	0	00:00:00	00:00:00	00:00:00					broad
10																

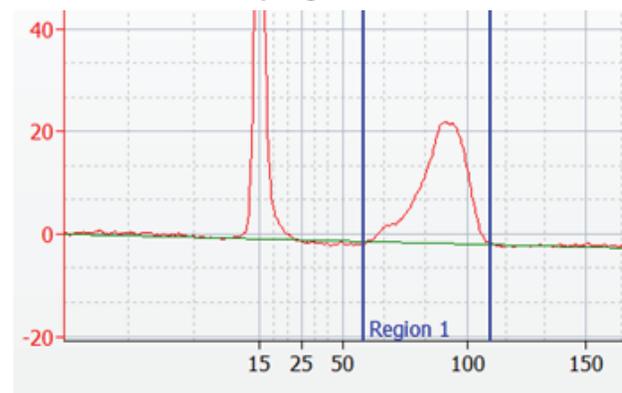
Typical Results

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 20-30 min. as shown here. :



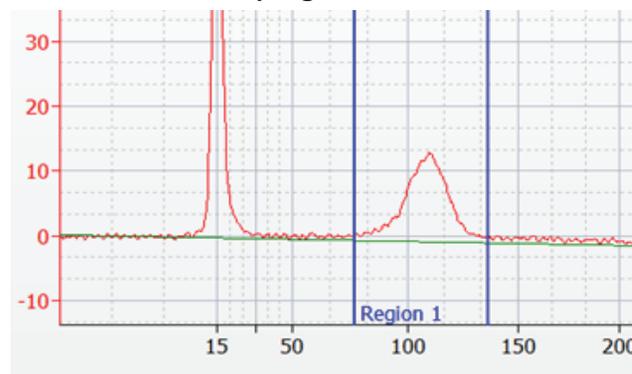
Extracted samples are run on an Agilent Bioanalyzer using a DNA 1000 chip. The analysis volume is 3 µl from a 30 µl elution volume (1:10 dilution). The following bioanalyzer results indicate typical results from QC testing:

90 bp Tight selection



Target = 90 bp
Ave Size = 89 bp
Conc. [ng/ul] = 3.70

110 bp Tight selection



Target = 110 bp
Ave Size = 108 bp
Conc. [ng/ul] = 4.60