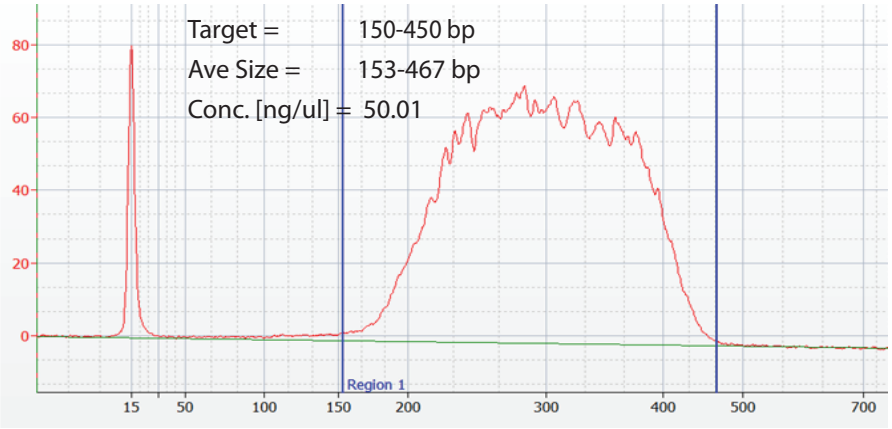
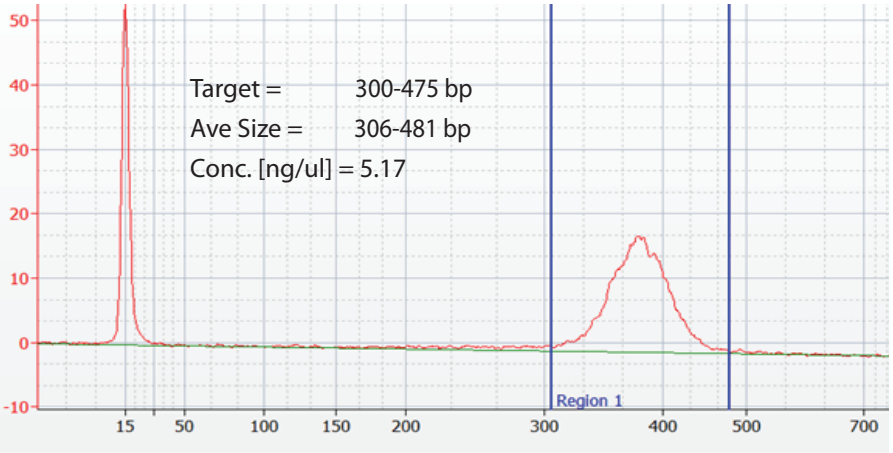
150-450 bp Range selection



300-475 bp Range selection



* 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 CDH2004

For validation of 2% agarose gel cassettes
(part nos.: HTC2010 or HTC2004)

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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 signifcant peaks or gaps. Following restriction digestion, the control DNA is purifed by phenol:chloroform extraction, dialyzed, and diluted into PippinHT electrophoresis buffer.

Enclosed is one tube of gDNA digest with suffcient volume for 48 sample loads. Users should use reagents and gel cassettes supplies with 2% agarose gel kits (HTC2010 or HTC2004). 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. Pippette 20 µl of gDNA digest per reaction tube.
2. Add 5 µl of internal standard to each tube (Marker 20B).
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. Pippette 25 µl of the gDNA/marker mix into a sample well.

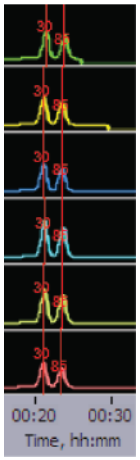
QC protocol for 2% agarose cassettes

Quality control procedures use the following size seletion parameters to assess the perfomance 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	275	215	335	0	00:00:00	00:00:00	00:00:00			tight
2														
3				3	500	430	570	0	00:00:00	00:00:00	00:00:00			tight
4														
5				5	300	150	450	0	00:00:00	00:00:00	00:00:00			broad
6														
7				7	388	300	475	0	00:00:00	00:00:00	00:00:00			broad
8														

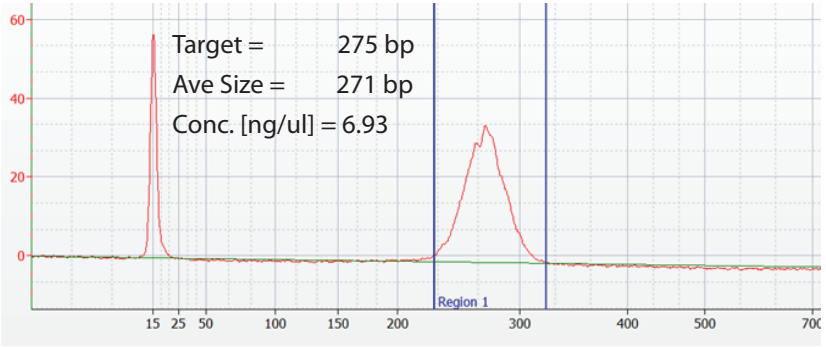
Typical Results

At the end of a run, marker peaks will 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 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:

275 bp Tight selection



500 bp Tight selection