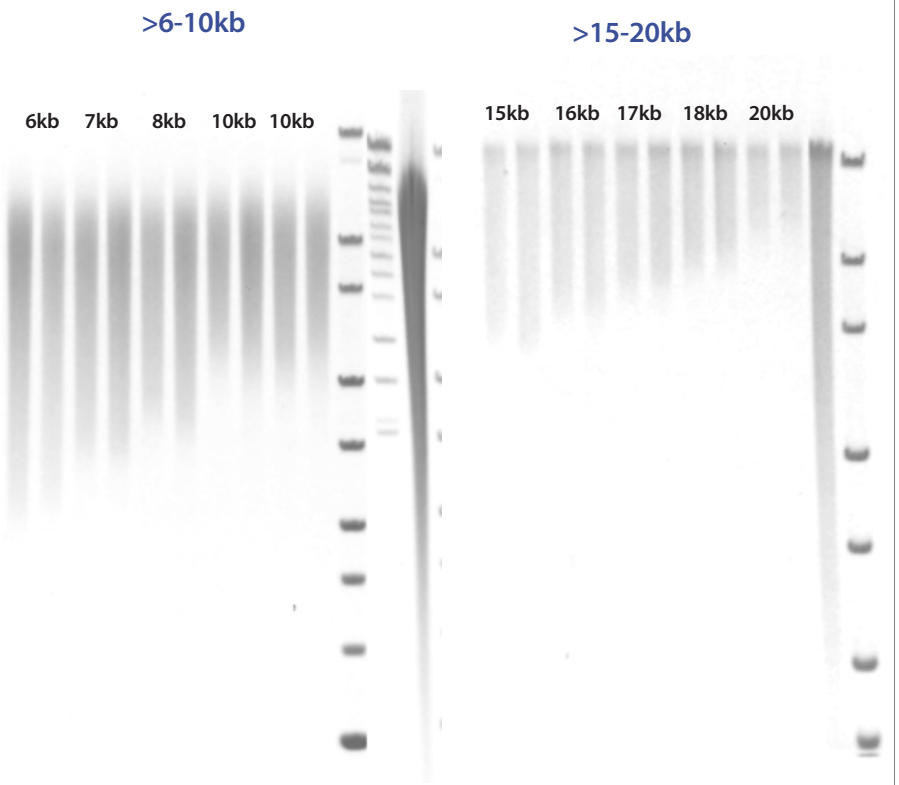
Expected Yield

Sample yield is improved if samples are allowed to equilbrate in the elution modules for **45 min** after completion of a run. Intinsic yield of DNA should be 50%.

The gel images below illustrate the type of result that the high pass protocol should provide with sheared DNA samples.



* 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 CDH7504

For validation of High-Pass Protocols

>6-10 kb and **>15-20 kb**

Use with cassette kit Nos:

HPE7510 or HPE7504

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#460065 Rev A

What is Enclosed

Control DNA for High-Pass protocols consists of a DNA ladder with 16 size markers between 0.5 - 36 kb (48 sample loads [0.75µg/25µl] in 1200µl total volume). With High-Pass protocols, users set a threshold (between 6-20kb) in the PippinHT software. DNA above that threshold will be collected, and lower molecular weight DNA will be filtered out from the genomic sample.

Using this control sample, users can familiarize themselves with the >6-10kb and >15-20kb high pass protocols on the PippinHT system.

To Use

1. Use the HPE7510 or HPE7504 (Marker 75E) agarose gel cassette .

2. Carefully follow the cassette preparation and sample load instructions that are outlined in the PippinHT Operations manual or cassette Quick Guide.

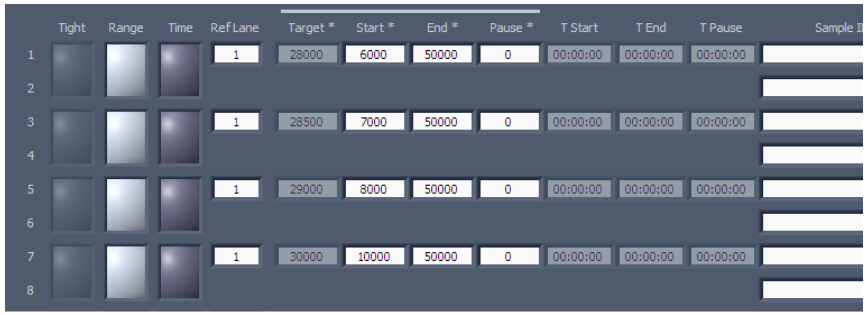
3. Load the "0.75% Agarose 6-10kb high-pass 75E" or "0.75% Agarose 15-20kb high-pass 75E" cassette definition into the PippinHT software protocol editor.

4. Enter one or more of the size selection parameters as shown below in the examples below.

5. Pippette 25µl of control DNA into a sample well or wells and load the marker (75E) into the well for the designated calibration lane.

6. Analyze the collected fractions on pulsed-field slab gel (using Pippin Pulse) for sizing, and/or Qubit® Fluorometer and Quant-iT™ HS dsDNA reagent for quanitation to assess yield.

Sample Protocol for >6-10kb High Pass (marker is in lane 1)



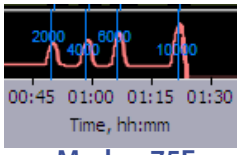
	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause	Sample ID
1				1	28000	6000	50000	0	00:00:00	00:00:00	00:00:00	
2												
3				1	28500	7000	50000	0	00:00:00	00:00:00	00:00:00	
4												
5				1	29000	8000	50000	0	00:00:00	00:00:00	00:00:00	
6												
7				1	30000	10000	50000	0	00:00:00	00:00:00	00:00:00	
8												

Sample Protocol for >15-20kb High Pass (marker is in lane 1)

	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause
1				1	32500	15000	50000	0	00:00:00	00:00:00	00:00:00
2				1	33000	16000	50000	0	00:00:00	00:00:00	00:00:00
3				1	34000	18000	50000	0	00:00:00	00:00:00	00:00:00
4				1	35000	20000	50000	0	00:00:00	00:00:00	00:00:00

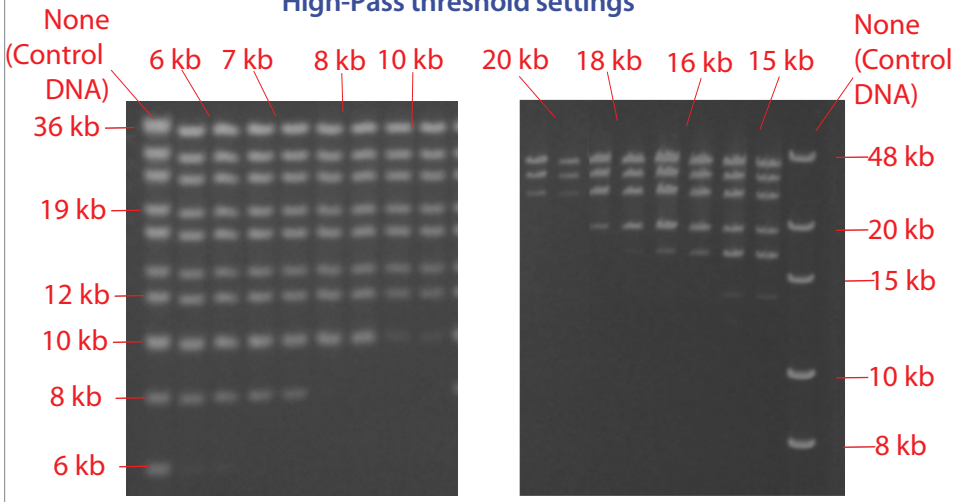
Typical Results

At the end of a run, marker peaks will be detected in each lane-pair in the Main screen of the PippinHT software interface.



Marker 75E

High-Pass threshold settings



The gel images above show expected size selections of control DNA at the thresholds set in the example protocols when compared to the non-selected marker. 10µl of the total 25µl elution was loaded on the gel.

The analytical gel was run with a Pippin Pulse using the 10-48kb pre-set protocol and run for 15 hours. 0.75% SeqKem Gold agarose