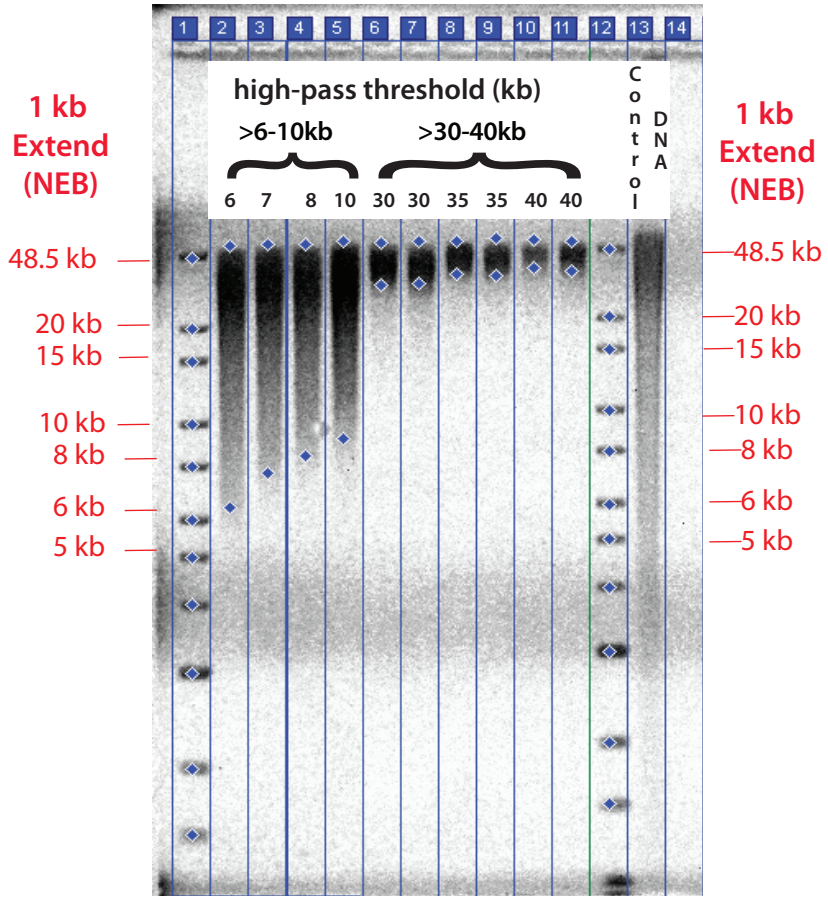
Expected Yield

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

The gel images below illustrate the type of result that the high pass protocol should provide with Control the DNA. 15µl of the total 25µl elution was loaded and run with a Pippin Pulse using the 10-48kb pre-set protocol and run for 15 hours. 0.75% SeqKem Gold agarose from Lonza was used.



* 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 CDH7604

For validation of High-Pass Protocols

> **6-10 kb** (*cassette kit*: **HPE7510 or HPE7504**)
> **30-40 kb** (*cassette kit*: **HPF7510 or HPF7504**)

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What is Enclosed

Control DNA for High-Pass protocols consists of unsheared E.coli DNA (48 sample loads [2µg/25µl] in 1200µl total volume). With High-Pass protocols, users set a threshold (between 6-10kb or 30-40) 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 >30-40kb high pass protocols on the PippinHT system.

To Use

1. Use the HPE7510 or HPE7504 (Marker 75E) casssette kit for >6-10 kb High Pass, or HPF7510 or HPF7504 (Marker 75F) agarose gel cassette kit for >30-40 kb High-Pass.

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 30-40kb high-pass 75F" 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 or 75F) 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.

Note: *This Control DNA cannot be used with the # >15-20kb High-Pass protocol.*

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

	Tight	Range	Time	Range+T	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Elution	T Pause	Sample ID Template
1					off	0	0	0	0	00:00:00	00:00:00	00:00:00	00:00:00	1
2														
3					1	28000	6000	50000	0	00:00:00	00:00:00	00:00:00	00:00:00	6kb
4														
5					1	28500	7000	50000	0	00:00:00	00:00:00	00:00:00	00:00:00	7kb
6														
7					1	29000	8000	50000	0	00:00:00	00:00:00	00:00:00	00:00:00	8kb
8														
9					1	30000	10000	50000	0	00:00:00	00:00:00	00:00:00	00:00:00	10kb
10														

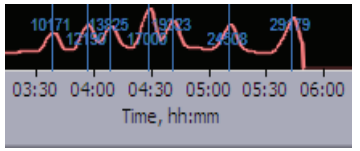
Sample Protocol for >30-40kb High Pass (marker is in lane 1)

	Tight	Range	Time	Ref Lane	Target *	Start *	End *	Pause *	T Start	T End	T Pause	Sample ID Template
1				off	0	0	0	0	00:00:00	00:00:00	00:00:00	Marker 75F
2												
3				1	40000	30000	50000	0	00:00:00	00:00:00	00:00:00	30kb
4												
5				1	42500	35000	50000	0	00:00:00	00:00:00	00:00:00	35kb
6												
7				1	45000	40000	50000	0	00:00:00	00:00:00	00:00:00	40kb
8												

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



Marker 75F

(Analytical gel on next page)