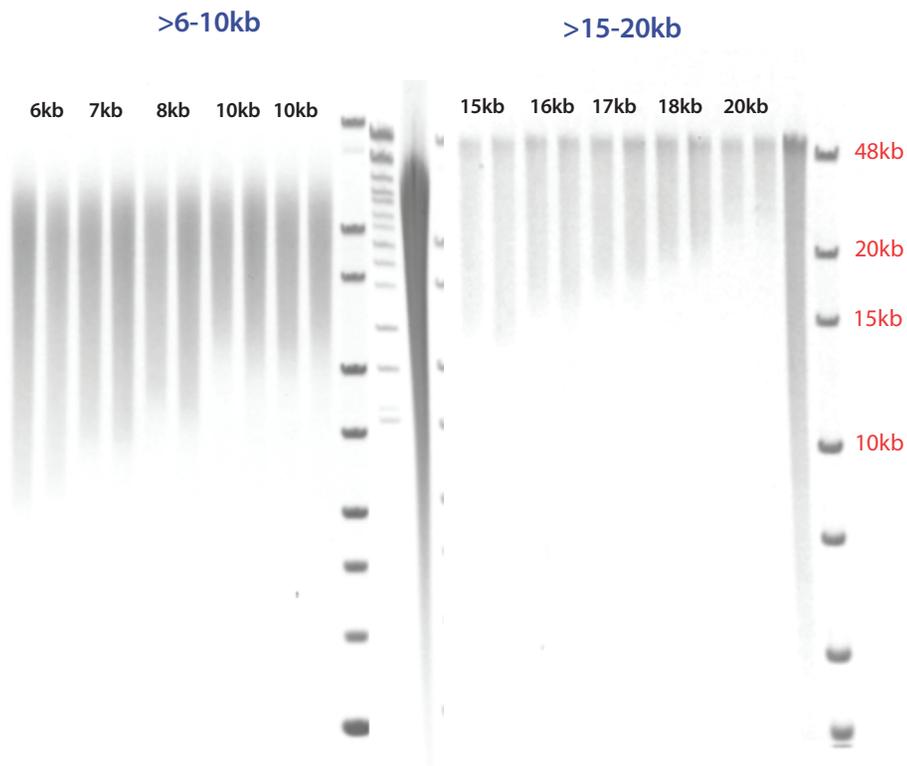


Expected Yield

Sample yield is improved if samples are allowed to equilibrate 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 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 between 6-20 kb

Use with cassette kit Nos:
HPD7510 or HPD7504
HPE7510 or HPE7504

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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 HPD7510 (Marker 75D, for >6-10kb high pass) or HPE7510 (Marker 75E, for >15-20kb high pass) 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 75D" 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. Pipette 25µl of control DNA into a sample well or wells and load the marker (75D or 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 quantitation 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 I |
|---|-------|-------|------|----------|----------|---------|-------|---------|----------|----------|----------|----------|
| 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 | | | | | | | | | | | |
| 3 | | | | 1 | 33000 | 16000 | 50000 | 0 | 00:00:00 | 00:00:00 | 00:00:00 |
| 4 | | | | | | | | | | | |
| 5 | | | | 1 | 34000 | 18000 | 50000 | 0 | 00:00:00 | 00:00:00 | 00:00:00 |
| 6 | | | | | | | | | | | |
| 7 | | | | 1 | 35000 | 20000 | 50000 | 0 | 00:00:00 | 00:00:00 | 00:00:00 |
| 8 | | | | | | | | | | | |

Typical Results

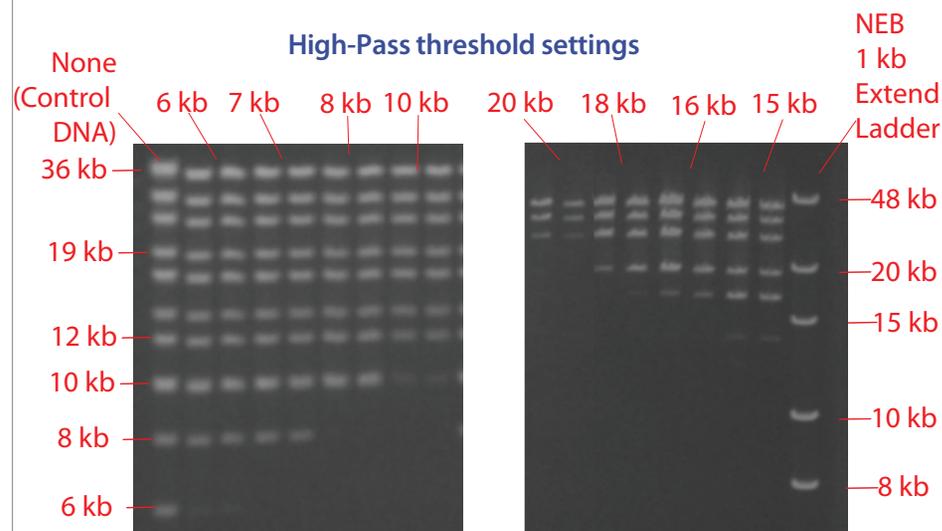
At the end of a run, marker peaks will be detected in the designated marker lane in the main screen of the PippinHT software display.



Marker 75D



Marker 75E



The gel images above shows expected size selections of control DNA CDH7504, at 6-10kb and 15-20kb thresholds, 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 from Lonza was used.