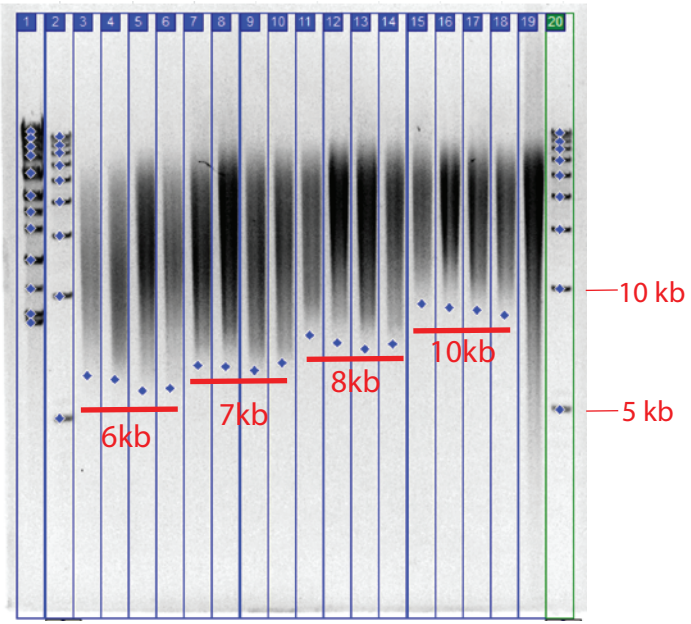
Sheared DNA Samples

The gel image below shows sheared genomic DNA samples run on the BluePippin using the "0.75% DF Marker S1 high-pass 6 -10kb vs3" cassette definition.



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Control DNA

For Testing and Validation of 0.75% Agarose Gel Cassettes

For High-Pass protocols

Product No. CHP7504

For:

BluePippin™

PAC20KB Gel Cassettes
&
BLF7510 Gel Cassettes

6-10kb DNA Threshold Settings
Version3 High-Pass Cassette Definition

sage science

What is Enclosed

Control DNA for High-Pass protocols consists of a DNA ladder with 16 size markers between 0.5 - 48 kb (16 sample loads [1.25µg/40µl] in 680µl total volume). With High-Pass protocols, users set a threshold (between 6-10kb) in the BluePippin software and all DNA above the threshold will be collected, and filtering out the lower molecular weight DNA from a genomic sample.

Using this control sample, users can familiarize themselves with the version3 BluePippin High-Pass cassette definition.

To Use

1. Use the PAC20KB (Marker S1) or BLF7510 (Marker S1) agarose gel cassette .

2. Carefully follow the cassette preparation and sample load instructions that are outlined in the BluePippin Operations manual, "High-Pass Guide for SMRTbell templates for the PacBio RS system", or BLF7510 Quick Guide.

3. Load the "0.75% DF Marker S1 high-pass 6-10kb vs3" cassette definition in the BluePippin software protocol editor and enter one or more of the size selection parameters shown below.

4. Pippette 40µl of control DNA into a sample well or wells and load marker S1 into the well for the designated calibration lane.

5. 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.

Recommended High-Pass Selection Parameters

	Tight	Range	Time	Peak	Ref Lane	BP Target	BP Start	BP End	BP Pause
5					1	28000	6000	50000	0
4					1	28500	7000	50000	0
3					1	29000	8000	50000	0
2					1	30000	10000	50000	0
1					1	0	0	0	0

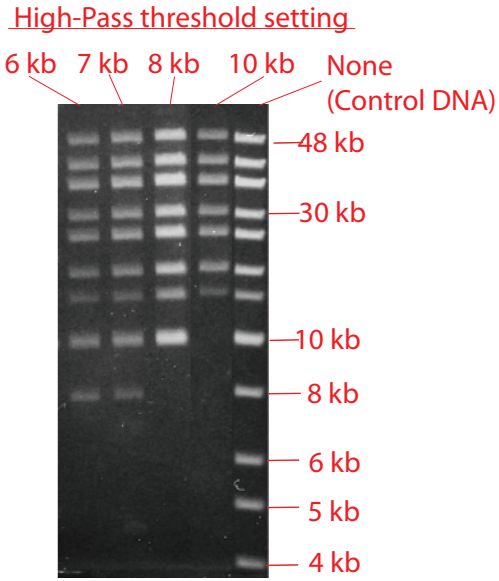
Typical Results

The S1 marker set will appear as follows in the graph display of the Main Tab of the BluePippin software:

Sizing

The gel image to right shows expected size selections of control DNA CHP7504, at the thresholds set in the previous page, when compared to the non-selected marker. 10µl of the total 40µl eution 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.



Expected Yield

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

The values to right represent expected results using the Qubit sysytem. 10 µl (of a 40µl elution volume) of size selected DNA from the Control was used. The intrinsic yield on the BluePippin is 25-50%.

High-Pass threshold setting	Qubit analysis (ng/ml)	
6 kb	350	+/-50
7 kb	300	+/-45
8 kb	275	+/-40
10 kb	225	+/-30

* *These data are not intended to imply guaranteed results or performance. This product is intended to demonstrate that the BluePippin is functioning as expected, and that proper operational technique is being used. Users should refer to the Operations Manual for performance specifications.*