10x Genomics®

Sample Preparation Demonstrated Protocol

DNA Size Selection

10x GENOMICS®

Developed in collaboration with sage science

10xGenomics.com

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Manual Part Number

CG00018 Rev D

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Customer Information and Feedback

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1. Overview

High Input Genomic DNA Length Results in Optimal Performance of the Chromium™ Genome Protocols

The Chromium Genome Protocols generate long-range information across the length of individual DNA molecules. Starting the process with High Molecular Weight (HMW) genomic DNA (gDNA) will typically result in better application performance, such as increased haplotype phase block length and ability to call structural variants. Optimal performance has been characterized on input gDNA with a mean length >50 kb.

However, gDNA samples may exist that do not meet this specification, for example:

- gDNA samples may not have been extracted using an optimized HMW extraction protocol
- gDNA samples may be old and the DNA has degraded
- HMW gDNA isolation is generally difficult for certain sample types (e.g. solid tissue)

This Demonstrated Protocol outlines the use of both the BluePippin and the PippinHT instruments for removing gDNA molecules <20 kb and DNA molecules <40 kb. Briefly, gDNA was extracted from live cultured cells (cell lines NA12878 and NCI-H228) following the HMW gDNA extraction protocol outlined in the Chromium Genome User Guides. The extracted gDNA was then mechanically sheared to degrade the DNA size to ~30-40 kb and then subject to size selection on the BluePippin or PippinHT instruments. Sizing results were verified by pulsed-field gel electrophoresis (results not shown) and DNA sequencing.

Size Selection Criteria

For best results with the BluePippin or PippinHT instruments, the starting gDNA sample must contain molecules >20 kb or >40 kb (depending on the size selection protocol used). The final yield of DNA from the BluePippin and PippinHT instruments depends on both the mass and size distribution of each sample loaded into the instrument. Yields range from 0.05% to 90% of input DNA.

The gDNA size distribution must be analyzed prior to any size selection protocol to ensure that sufficient DNA can be recovered for downstream use with the Chromium Genome Protocols. The 10x Genomics® HMW DNA QC Demonstrated Protocol should be consulted for further information. If a sample contains a very small fraction (<10%) of gDNA above the chosen size selection cutoff, final yields may be extremely low.

The recommend input amount of gDNA for size selection is 500 ng - 1 µg. The BluePippin and PippinHT instruments support lower DNA input amounts, but final size-selected DNA yields may be too low to proceed with the Chromium Genome Protocols.

2. DNA Size Selection

NOTE

These Demonstrated Protocols use operating instructions for the BluePippin and PippinHT instruments provided by Sage Science. Please visit www.sagescience.com for detailed instructions.

2.1. BluePippin Size Selection

	Removes DNA <20 kb	Removes DNA <40 kb
Gel Cassette	0.75% Agarose Gel Cassette and Marker S1	0.75% Agarose Gel Cassette and Marker U1
Gel Cassette PN	BLF7510	BUF7510
Instrument Settings		
Marker	S1	U1
Cassette Definition	0.75% Agarose Dye-Free 0.75% DF Marker S1 high-pass 15-20 kb	0.75% Agarose Dye-Free 0.75% DF Marker U1 high-pass 30-40 kb
Start BP	20,000	40,000
End BP	50,000	80,000

2.2. PippinHT Size Selection

NOTE

The 20 kb and 40 kb size selection protocols for the PippinHT instrument were demonstrated using the following gel cassettes and instrument settings. The same gel cassettes were used for both the 20 kb and 40 kb protocols to allow samples requiring different size selections to be run on a single gel cassette

	Removes DNA <20 kb	Removes DNA <40 kb
Gel Cassette	0.75% Agarose Gel Cassette and Marker 75F	0.75% Agarose Gel Cassette and Marker 75F
Gel Cassette PN	HPF7510	HPF7510
Instrument Settings		
Marker	75F	75F
Cassette Definition	0.75% Agarose 0.75% Agarose 30-40 kb high-pass 75F	0.75% Agarose 0.75% Agarose 30-40 kb high-pass 75F
Start BP	20,000	40,000
End BP	80,000	80,000

NOTE

Sage Science offers an additional protocol for the 20 kb PippinHT size selection using a different gel cassette. This protocol has not been tested or demonstrated by 10x

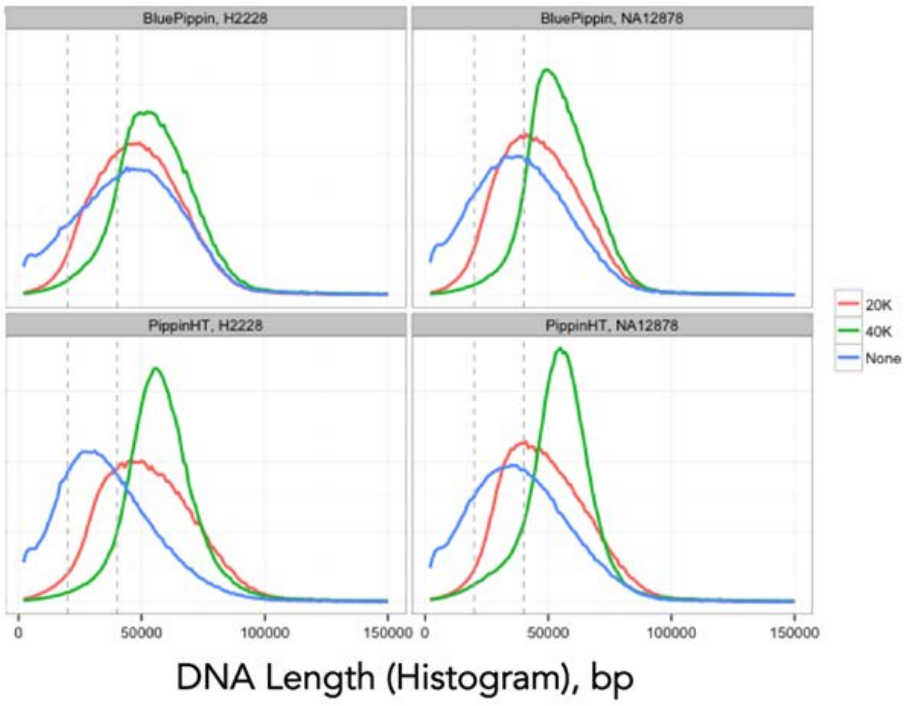
Genomics®, but is expected to work for the 20 kb size selection purpose only. This protocol can be found in Section 4.

Each of the 20 kb PippinHT protocols offers an advantage. The protocol presented in Section 2.2 is advantageous because a user can run both 20 kb and 40 kb size selections on the same cassette and during the same instrument run. However, the 20 kb PippinHT protocol outlined in Section 4 was designed by Sage Science for a maximal sample recovery.

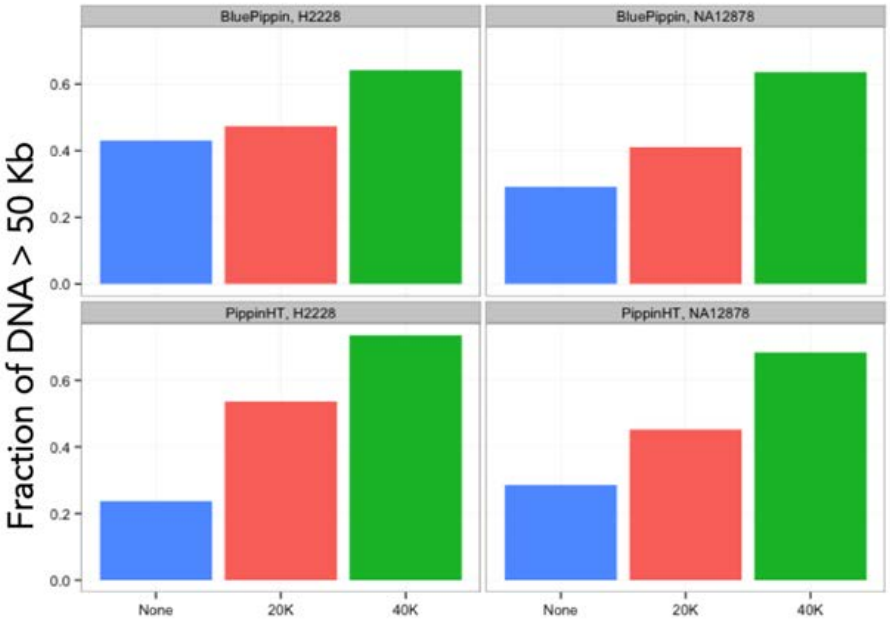
3. Results & Conclusion

NOTE

The starting degraded gDNA samples and the size selected gDNA samples were analyzed by pulsed-field gel electrophoresis (not shown) and carried through the Linked-Read sequencing workflow and sequenced on the Illumina HiSeq 4000 (see below). The results demonstrate the successful removal of gDNA molecules below the chosen cutoff values for both the BluePippin and PippinHT instruments.



Linked-Read Data - gDNA length distribution before and after size selection. Degraded gDNA samples (blue lines) from 2 different cell lines were processed using each of the 4 Size Selection Demonstrated Protocols (red and green lines). The plots above are smoothed histograms of DNA molecule length determined by DNA sequencing (x-axis = DNA size in bp). The results are concordant with DNA sizing results determined by pulsed-field gel electrophoresis (not shown). Dashed vertical lines represent 20,000 and 40,000 bp, the cutoff values of the Demonstrated Protocols.



Linked-Read Data - Improvement in the fraction of gDNA molecules > 50 kb after size selection. Degraded gDNA samples (blue bars) from 2 different cell lines were processed using each of the 4 Demonstrated Protocols (red and green bars). The 40 kb cutoff protocols yield the best results. Over 60% of the recovered DNA (post-size selection) is greater than 50 kb for all 4 DNA test samples.

NOTE

The 4 Demonstrated Protocols for the BluePippin and PippinHT instruments result in significant improvement in gDNA size distributions as determined by both pulsed-field gel electrophoresis as well as linked read DNA sequencing. Following these Demonstrated Protocols is recommended when working with degraded gDNA samples. Chromium™ Genome Protocol performance will vary depending on the quality of both the starting and the recovered gDNA samples.

4. Alternate PippinHT <20 kb Size Selection Protocol

NOTE

The protocol below has not been tested or demonstrated by 10x Genomics®, but is expected to be a low risk alternative to the demonstrated 20 kb PippinHT Size Selection protocol above. Developed and tested by Sage Science, the alternate protocol and is outlined here for convenience (below right). Differences from the Demonstrated Protocol outlined in Section 2 are highlighted in red.

	Removes DNA <20 kb (Demonstrated Protocol)	**Removes DNA <20 kb (Alternate Protocol)**
Gel Cassette	0.75% Agarose Gel Cassette and Marker 75F	0.75% Agarose Gel Cassette and Marker 75E
Gel Cassette PN	HPF7510	HPE7510
Instrument Settings		
Marker	75F	75E
Cassette Definition	0.75% Agarose 0.75% Agarose 30-40 kb high-pass 75F	0.75% Agarose 0.75% Agarose 15-20 kb high-pass 75E
Start BP	20,000	20,000
End BP	80,000	80,000