10x Genomics®

Sample Preparation Demonstrated Protocol

High Molecular Weight DNA QC

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Notices

Manual Part Number

CG00019 Rev B

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For technical information or advice, please contact our Customer Technical Support Division online at any time.

Email: techsupport@10xgenomics.com

10x Genomics

7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA

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HMW gDNA QC

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)

Due to these variables, input gDNA quality control (QC) is highly recommended to maximize Linked-Read application performance.

HMW DNA QC

Pulsed-field gel electrophoresis (PFGE) is the most accurate method available for sizing up to 5 Mb, but is costly and time consuming. Other methods are lower cost and faster, but lack accurate DNA sizing >50 kb. The table below contains comparative information on the three methods for HMW DNA QC recommended for use in combination with 10x Genomics® Protocols.

Instrument	Recommended Use	Minimum Input Requirement	Run Time	Cost
CHEF PFGE (Bio-Rad)	Sizing DNA up to 5 Mb	100 – 200 ng (>10 ng/µl)	19.5 h	High
Pippin Pulse (Sage Science)	Determines fraction of DNA >50 kb	100 – 200 ng (>10 ng/µl)	20 h	Low
TapeStation (Agilent)	Determines fraction of DNA >50 kb	10 – 20 ng (>10 ng/µl)	15 min	High

This Demonstrated Protocol outlines the use of the Bio-Rad CHEF-DR® II PFGE Instrument, Sage Science Pippin Pulse™, and the Agilent TapeStation for HMW DNA QC. To maximize Linked-Read application performance, gDNA >50 kb is acceptable, whereas gDNA >80 kb is ideal. If QC show a significant amount of gDNA <50 kb, the sample may be rescued by performing a size selection using the Sage Science Blue Pippin or PippinHT instruments. The 10x Genomics® DNA Size Selection Demonstrated Protocol should be consulted for further information.

2. Bio-Rad CHEF PFGE System

2.1. Equipment & Materials

Vendor	Material	Part Number
Bio-Rad	Bio-Rad CHEF-DR II Chiller System Pulsed Field Gel Electrophoresis	170-3725
	5 kb Ladder	170-3624
	8-48 kb Ladder	170-3707
	S. cerevisiae Size Standard	170-3605
Promega	Lambda Ladder	G3011
Invitrogen	SYBR Safe DNA Gel Stain*	S33102
	UltraPure LMP Agarose*	16520-050
	50X TAE Electrophoresis Buffer*	24710-030
Lonza	SeaKem GOLD AGAROSE 125G	50150
BioLabs Inc	Gel Loading Dye, Blue (6X)*	B7021S

*Or equivalent product from alternate vendor

2.2. QC Protocol

NOTE

Download and review the instrument instruction manual available on the manufacturer's website prior to beginning this Demonstrated Protocol.

a) Prepare the CHEF PFGE instrument according to the parameters below.

Size Range	5 – 500 kb	
Run Time	19.5 h	
Cooling Temperature	9°C	
Flow Rate	0.75 l/min	
Block Parameters	**Block 1: (b-1)**	**Block 1: (b-2)**
Volts / CM	6 V/cm	6 V/cm
Run Time	7.5 h	12 h
Initial Switch Time	1 sec	60 sec
Final Switch Time	6 sec	120 sec

b) Prepare a 1% SeaKem Gold Agarose gel in 0.5X TAE buffer and refrigerate at 4°C for >4 h (preferably overnight) before use.

c) For visualization of long DNA (>100 kb), the suggested size standards are Bio-Rad's S. cerevisiae and Promega's Lambda Ladder. Remove agarose pieces for each ladder and equilibrate in 0.5X TAE buffer for at least 10 min before use.

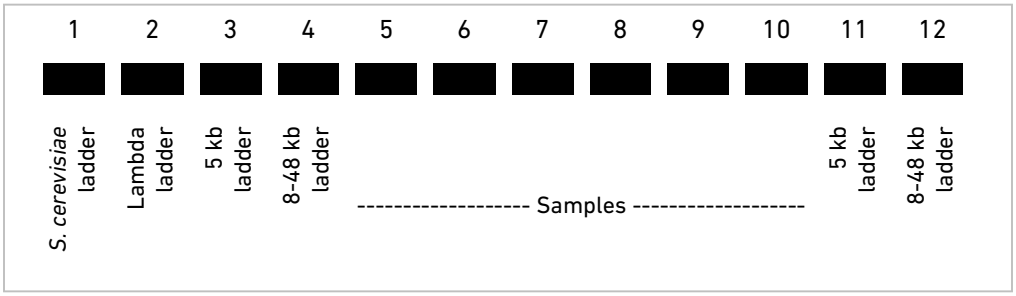
d) Prepare the 5 kb and 8-48 kb ladders by mixing each standard with the appropriate volumes of loading dye and water or 0.5X TAE buffer. The total volume should be determined by the comb used to create the wells.

e) Using wide-bore pipette tips, prepare the HMW gDNA by mixing 100-200 ng HMW gDNA sample(s) with the appropriate volumes of loading dye and water or 0.5X TAE buffer. The total volume should be determined by the comb used to create the wells.

f) Load one piece each of equilibrated S. cerevisiae and Lambda ladder size standards into the first two wells using a small spatula. Fill each well containing the size standards with molten agarose (suggested: 1% LMP agarose) in 0.5X TAE Buffer.

g) Place the gel in the CHEF instrument (pre-cooled to 9°C).

h) Using a wide-bore pipette tip, slowly pipette the prepared ladders and samples into the wells, being careful to fill each well with the appropriate volume and ensuring that material does not float out of the well. See example gel layout below.



i) Once all samples are loaded, close the top of the instrument and start the run.

j) Follow standard protocols to stain and image the gel. See the manufacturer's instruction manual for more information.

3. Sage Science Pippin Pulse Electrophoresis Power Supply System

3.1. Equipment & Materials

Vendor	Material	Part Number
Sage Science	Pippin Pulse Electrophoresis Power Supply System	15-100-135
	10X KBB Buffer	12-100-577
	Galileo 1214 Mini Gel Unit*	PGB0001
Bio-Rad	5 kb Ladder	170-3624
	8-48 kb Ladder	170-3707
	S. cerevisiae Size Standard	170-3605
Promega	Lambda Ladder	G3011
Invitrogen	SYBR Safe DNA Gel Stain*	S33102
	UltraPure LMP Agarose*	16520-050
Lonza	SeaKem GOLD AGAROSE 125G	50150
BioLabs Inc	Gel Loading Dye, Blue (6X)*	B7021S

*Or equivalent product from alternate vendor

3.2. QC Protocol

NOTE

Download and review the instrument instruction manual available on the manufacturer's website prior to beginning this Demonstrated Protocol.

a) Prepare the Pippin Pulse instrument according to the parameters below.

Protocol Name	5-150 kb
Run Time	20 h

Waveform Type	V	A	B	C	D	E	F	G
.5-15kb	100	90	36	0	0	0	0	5000
10-48kb	80	25	10	25	10	5	2	21
5-150kb	75	150	50	30	10	3	1	81

Example screenshot of the pre-set Pippin Pulse 5-150 kb parameters

b) Prepare a 0.6% SeaKem Gold Agarose gel in 0.5X KBB buffer and refrigerate at 4°C overnight before use. For visualization of long DNA (>100 kb), the suggested size standards are Bio-Rad's S. cerevisiae and Promega's Lambda Ladder. Remove agarose pieces for each ladder and equilibrate in 0.5X KBB for at least 10 min before use.

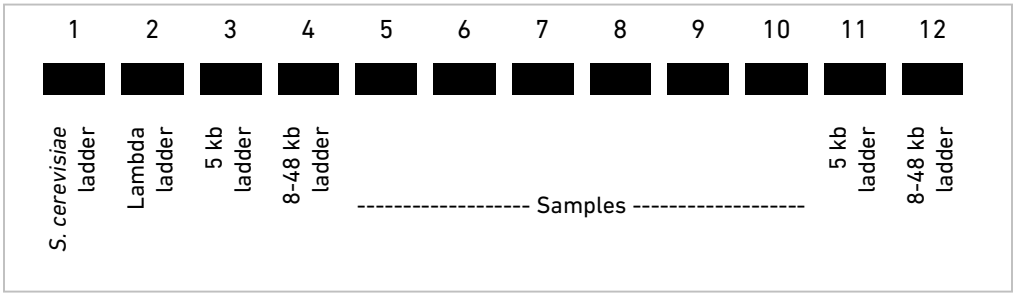
c) Prepare the 5 kb and 8-48 kb ladders by mixing each standard with the appropriate volumes of loading dye and water or 0.5X KBB buffer. The total volume should be determined by the comb used to create the wells.

d) Using a wide-bore pipette tip, prepare the HMW gDNA by mixing 100-200ng of HMW gDNA sample(s) with the appropriate volumes of loading dye and water or 0.5X KBB buffer. The total volume should be determined by the comb used to create the wells.

e) Load one piece each of equilibrated S. cerevisiae and Lambda ladder size standards into the first two wells using a small spatula. Fill each well containing the size standards with molten agarose (suggested: 1% LMP agarose) in 0.5X KBB Buffer.

f) Place the gel in the gel box instrument filled with 0.5X KBB Buffer.

g) Using a wide-bore pipette tip, slowly pipette the prepared ladders and samples into the wells, being careful to fill each well with the appropriate volume and ensuring that material does not float out of the well. See example gel layout below.



h) Once all samples have been loaded, close the top of the instrument and change the run time before starting the run. In the Pippin Pulse software:

   i. Select "5-150 kb" to highlight the row of pre-set run parameters, and change the "Target Duration, hh:mm" to 20:00 for a 20 h long run.

   ii. Select "START" to begin the run. The blue light on the power supply indicates the run is in progress.

i) Follow standard protocols to stain and image the gel. See the manufacturer's instruction manual for more information.

NOTE

This method has high run-to-run variability for sizing DNA between 50 kb and 150 kb, but this system is accurate for sizing DNA <50 kb.

4. Agilent TapeStation (2200 or 4200)

4.1. Equipment & Materials

Vendor	Material	Part Number
Agilent	4200 TapeStation System	G2991AA
	Loading Tips, 10 PK for version 4200	5067-5599
	OR	
	2200 TapeStation System	G2964AA
	Loading Tips, 10 PK for version 2200	5067-5152
Agilent	Genomic DNA ScreenTape	5067-5365
	Genomic DNA Reagents	5067-5366
	Optical tube strips (8x Strip)*	401428
	Optical tube strip caps (8x Strip)*	401425
	Optional: Needle Change Cartridge	5067-5783
	Optional: ScreenTape Rack	G2991-40007
	Optional: 96-Well Sample Plate*	5042-8502

*Or equivalent product from alternate vendor

4.2. QC Protocol

NOTE

Download and review the instrument instruction manual available on the manufacturer's website prior to beginning this Demonstrated Protocol.

a) Prepare and run the gDNA samples following the Genomic DNA Assay preparation guidelines in the Agilent 2200 or 4200 TapeStation Manual with the following modifications:

   i. Use a minimum of 10 ng of material per DNA sample for accurate results.

   ii. Use a wide-bore pipette tip, to slowly pipette mix the HMW DNA sample 5 times prior to adding TapeStation reagents.

b) In the TapeStation software, select on "Regions > Add Region."

c) In the pop-up window, enter the size range for the analysis region: 48.5 kb to 200 kb. Select "Apply to File." The analysis region will be displayed on each of the sample graphs and will be saved for future runs.

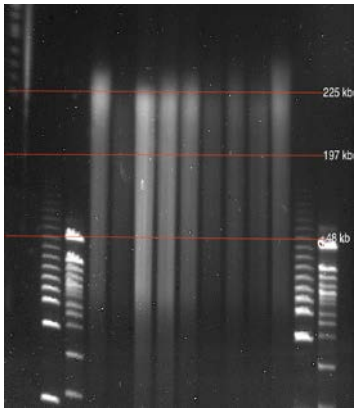
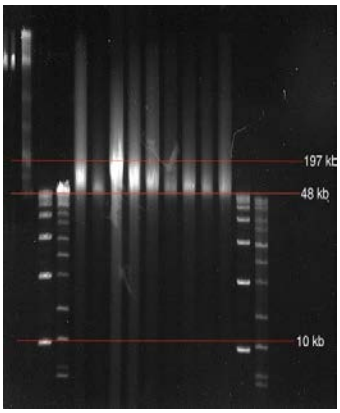
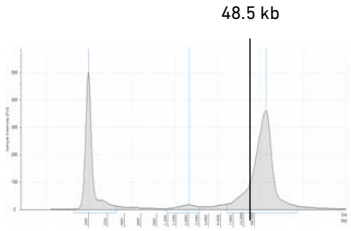
d) Record the % of DNA that is within the analysis region.

NOTE

The maximum reported DNA size is ">60,000 bp." All DNA >60,000 migrates as a single peak.

5. HMW gDNA Results, Comparison & Summary

Results

Bio-Rad CHEF PFGE Gel	Pippin Pulse Gel	Agilent TapeStation
		
Majority of gDNA >50 kb Significant population of molecules >200 kb	Majority of gDNA >50 kb	The HMW gDNA migrates to a peak “>60 kb” with ~70% of DNA >48.5 kb

Comparison

Instrument	Recommended Use	Advantages	Challenges
CHEF PFGE (Bio-Rad)	Sizing DNA up to 5 Mb	Most accurate method for sizing DNA >50 kb	High cost Long run time (20 h) DNA input >100 ng
Pippin Pulse (Sage Science)	Determines fraction of DNA >50 kb	Low cost Roughly approximate % of molecules >50 kb	Run-to-run variability Compression zone >50 kb Long run time (20 hours) DNA input >100 ng
TapeStation (Agilent)	Determines fraction of DNA >50 kb	Low DNA input (>10 ng) Short run time (15 min) Roughly approximate % of molecules >50 kb	High cost Sizing accuracy declines for molecules >50 kb

Summary

Each method for analyzing HMW gDNA has benefits and challenges. The Bio-Rad CHEF PFGE instrument is the most accurate option, while the Pippin Pulse is the most cost-effective option and the Agilent TapeStation is the lowest input and fastest turn-around time option. Each of these methods can be used to evaluate gDNA samples prior to running 10x Genomics® Protocols.