Application Note

Genomics

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Reliable Genomic DNA Quality Control with the Agilent Femto Pulse System for Optical Mapping

Authors

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Abstract

Optical mapping on the Saphyr from Bionano Genomics helps researchers make sense of complex genomic structures that next-generation sequencing (NGS) is unable to easily resolve. While powerful, the success of optical mapping on the Saphyr depends on the use of high-molecular weight genomic DNA that meets stringent quality standards. Quality control (QC) analysis of genomic DNA is complex and time-consuming. Using the Agilent Femto Pulse system, genomic DNA quality can be accurately assessed visually and with a grading scale developed to predict the performance of samples on the Saphyr.

Introduction

Genome assembly using NGS is often confounded by repeat sequences and other complex genome structures, leading to uncertainty in contig order, orientation, and gap size. Due to the limitations of sequencing technologies, many large (>1 kb) structural variations (SV) go undetected. Multiple sequencing strategies and computational models have been developed to try and address these issues but remain unable to resolve the entire genome.

Optical mapping complements genome assembly by providing a scaffold and identifying and characterizing SVs and genomic rearrangements across the whole genome. The Saphyr from Bionano Genomics is the premier optical mapping instrument on the market.

To ensure optimal performance on the Saphyr, robust quality control (QC) analysis is advised to guarantee only high-quality DNA samples are used. Confident QC of genomic DNA (gDNA) on the Agilent Femto Pulse system prior to optical mapping, provides researchers with a highly accurate method to assure each project starts with the highest quality DNA.

Saphyr

The Saphyr is Bionano Genomics' third-generation optical mapping platform for rapid, high-throughput, long-range genome mapping to construct ***de novo*** physical maps of most genomes and provides unmatched SV discovery and scaffolding capabilities. The Saphyr and Saphyr Chip combine to linearize and directly image extremely long, high molecular weight (HMW) DNA. Bionano NanoChannel arrays on the Saphyr Chip keep DNA molecules linear and intact, enabling long-range genome mapping at single-molecule resolution. Starting with highquality, HMW DNA is key to the platform's success. Long DNA molecules (from 100,000 bp to megabase pairs) are stained with a backbone dye, then labeled using a variety of enzymes with specific six or seven-base recognition motifs. Labeled single molecules are cycled through the NanoChannel arrays on the Saphyr Chips and imaged. The image data is digitized, and the digital molecules are pairwise aligned to each other to generate a ***de novo*** assembly using Bionano's included software. The new assembly can be used to detect SVs against a reference genome (or second Bionano assembly) or used as a scaffold in a genome improvement project.

- Less than 100 ng of labeled DNA will be loaded into a flow cell
- Guaranteed 640 Gb throughput per Saphyr Chip per day for human samples (320 Gb per flow cell of molecules larger than 150 kb)
- Sample to SV call or hybrid scaffolding in as little as five days

Agilent Femto Pulse system

The only parallel capillary electrophoresis instrument on the market with a pulsed-field power supply, the Agilent Femto Pulse system provides researchers with a powerful tool for the analysis of gDNA and large DNA fragments. QC analysis of gDNA and large DNA fragments includes quantification, sizing, and qualification and has never been easier or faster. In contrast to pulsed-field gel electrophoresis (PFGE), the Femto Pulse system, using pulsed-field capillary electrophoresis (PFCE), completes gDNA and large DNA fragment separations in about 1.5 hours, eliminating overnight PFGE. Conducted in parallel, the Femto Pulse system can complete 12 separations at a time, without compromising resolution or quantification.

The Femto Pulse system attains an unprecedented level of sensitivity using a specially designed detection system. This remarkable sensitivity allows researchers to use 1,000x less sample than what is required by PFGE, enabling the conservation of sample for more runs on the Saphyr.

Experimental

Initial sample preparation

Bionano Genomics provided 25 gDNA samples (Table 1) for QC analysis on the Agilent Femto Pulse system. Samples were diluted to between 200 to 500 pg/µL in 0.25x TE Rinse Buffer (Agilent Technologies, p/n DNF-497-0275) except for samples 16 to 19, which were diluted in water.

Separation on the Agilent Femto Pulse system

Samples were prepared for PFCE on the Femto Pulse system using the Agilent Genomic DNA 165 kb kit (p/n FP-1002). Samples were separated on the Femto Pulse system using the Genomic DNA 165 kb method with 70 minute PFCE.

Smear analysis

The smear analysis function in the Agilent ProSize data analysis software was used to quantify and size the gDNA smears.

Genomic Quality Number (GQN)

Scoring with GQN in the Agilent ProSize data analysis software was used to determine the suitability of gDNA samples for use on the Saphyr. A GQN with standardized size threshold of 50,000 bp ($GQN_{50\,kb}$) was employed, and all fragments < 20,000 bp were excluded from the analysis. One of three grades were assigned to samples as determined by GQNs: fail ($GQN_{50\,kb} < 3.5$), equivocal ($GQN_{50\,kb} = 3.5$ to 4.5), or pass ($GQN_{50\,kb} > 4.5$).

Results and discussion

The Femto Pulse system provided superior QC analysis of gDNA samples prior to analysis on the Saphyr; accurately predicting which samples are best suited for optical mapping. Each sample received one of three possible grades: fail ($GQN_{50\ kb}$ < 3.5), equivocal ($GQN_{50\ kb}$ = 3.5 to 4.5), or pass ($GQN_{50\ kb}$ > 4.5). These grades reflect the quality of the HMW gDNA needed for successful optical mapping on the Saphyr.

Bionano Genomics supplied 25 samples for analysis on the Femto Pulse system that were also run on the Saphyr. Of the 25 samples provided, the Femto Pulse system accurately predicted the performance of 21 samples on the Saphyr (Table 1). Three of the provided samples were classified as Equivocal by the Femto Pulse system, all three went on to perform well on the Saphyr. A single sample did not match its predicted performance on the Saphyr. Sample #5 displayed a high amount of degradation with low levels of HMW gDNA, receiving a grade of fail on the Femto Pulse system, though optical mapping was successful on the Saphyr.

	Agilent Femto Pulse System $GQN_{50\ kb}$					
Sample ID	Performance on Saphyr	Expected size**	Average GQN	Quality assessment	Agree with Saphyr	Smear size
#1 93449 Human *	Pass	1.5 Mb+	9.3	Pass	√	215,114 bp
#2 93447 Human*	Pass	300 kb	9.1	Pass	√	302,460 bp
#3 93673 Barley	Pass	120 kb	4.1	Equivocal		53,119 bp
#4 93672 Barley	Pass	250 kb	8.2	Pass	√	258,556 bp
#5 93673 Barley	Pass	90 kb	3.3	Fail		53,250 bp
#6 93605 Leopard*	Pass	2 Mb+	7.4	Pass	√	145,526 bp
#7 93589 Leopard*	Pass	2 Mb+	8.8	Pass	√	193,737 bp
#8 AP - 1 plant	Pass	1 Mb+	4.9	Pass	√	69,144 bp
#9 AP - 2 plant	Pass	1 Mb+	4.6	Pass	√	72,788 bp
#10 AP - 2 plant	Fail	60 kb	2.8	Fail	√	49,972 bp
#11 93282 deer	Pass	1 Mb+	7.0	Pass	√	136,192 bp
#12 93357 dee QI	Pass	200 kb	5.8	Pass	√	122,947 bp
#13 93357 dee SI	Pass	190 kb	5.8	Pass	√	123,357 bp
#14 93282 deer SI	Pass	145 kb	6.0	Pass	√	122,023 bp
#15 93491 human buccal SJ	Pass	160 kb	5.0	Pass	√	62,299 bp
#16 93491 human buccal CS	Pass	150 kb	5.2	Pass	√	63,776 bp
#17 93533 human buccal H_2O	Pass	140 kb	4.0	Equivocal		46,287 bp
#18 93533 human buccal PBS	Pass	260 kb	4.7	Pass	√	60,018 bp
#19 93265 BN - 06	Pass	1 Mb+	4.7	Pass	√	60,245 bp
#20 93365 BN - 12	Pass	1 Mb+	6.3	Pass	√	107,047 bp
#21 93365 BN - 13	Pass	1 Mb+	5.1	Pass	√	62,861 bp
#22 93720 frog	Pass	1.5 Mb+	9.4	Pass	√	180,181 bp
#23 93720 frog	Pass	1.5 Mb+	9.4	Pass	√	173,468 bp
#24 93585 shrimp N1	Pass	1 Mb+	8.4	Pass	√	176,167 bp
#25 93585 shrimp N4	Pass	1 Mb+	4.3	Equivocal		36,428 bp

Table 1. Summary of the analyzed samples, including sample performance on the Agilent Femto Pulse system and on the Saphyr. Samples highlighted in orange are provided as representative examples in this section. Samples marked with * had an n=2, all others n = 3. **Expected size was determined through a combination of pulsed-field gel electrophoresis and the N50 generated by Saphyr runs.

Grade: fail

Samples that receive a fail ranking are characterized by extensive degradation and fragmentation of HMW genomic DNA (Figure 1). Genomic DNA samples that fail QC analysis on the Femto Pulse system are ill-suited for further analysis on the Saphyr. These samples lack the requisite amount of HMW DNA for successful optical mapping.

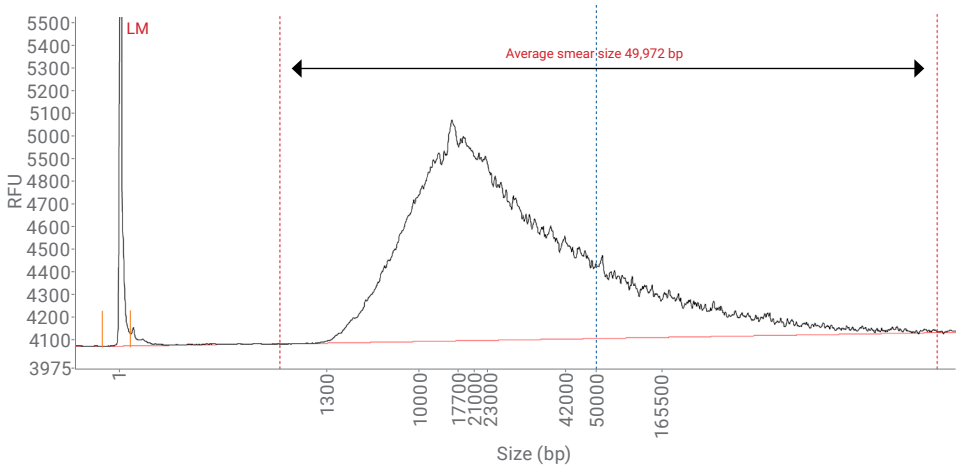

Figure 1. Separation of extracted plant genomic DNA on the Agilent Femto Pulse system using the Agilent Genomic DNA 165 kb kit. Post-separation analysis was performed using ProSize data analysis software. The sample displayed extensive degradation and fragmentation, forming a smear with an average smear size of 49,972 bp, as determined by smear analysis (indicated by the red dotted lines; range: 1 to 650 kb, n = 3). With a $GQN_{50\,kb}$ of 2.8 (blue dotted line), this sample did not meet necessary criteria for use on the Saphyr.

Grade: equivocal

Samples that receive an equivocal ranking displays moderate amounts of degradation and low amounts of higher molecular weight DNA suited for optical mapping (Figure 2). Higher amounts of degraded DNA may confound QC analysis. While these samples may prove to be of sufficient quality for optical mapping, their performance on the Saphyr cannot be predicted.

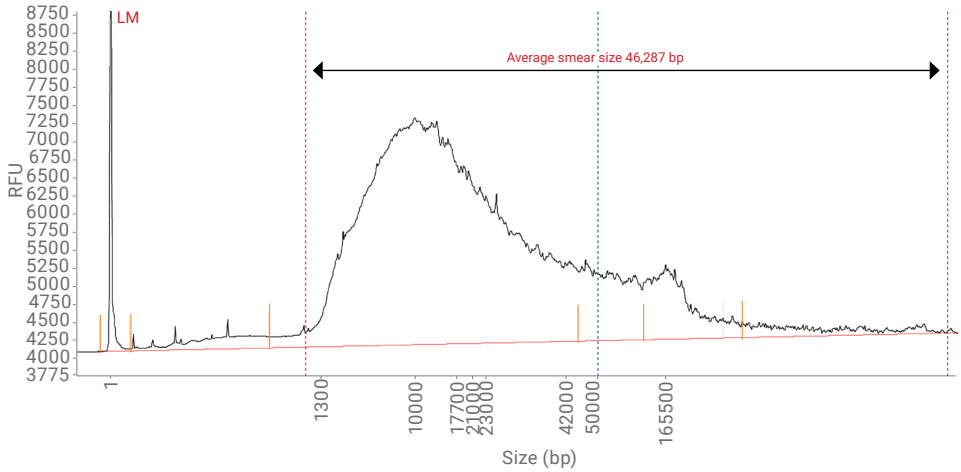

Figure 2. Separation of extracted human genomic DNA on the Agilent Femto Pulse system using the Agilent Genomic DNA 165 kb kit. Post-separation analysis was performed using ProSize data analysis software. The sample displayed moderate degradation and fragmentation, forming a smear with an average size of 46,287 bp, as determined by smear analysis (indicated by the red dotted lines; range, 1.2 to 650 kb, n = 3). With a $GQN_{50\,kb}$ of 4.0 (blue dotted line), the sample receives a grade of equivocal.

Grade: pass

Samples that receive a pass ranking are characterized by a significant amount of HMW DNA, with the possibility of low levels of degradation (Figure 3). Samples that meet these criteria are ideal for optical mapping on the Saphyr.

Conclusions

The Saphyr provides superior optical mapping of diverse sample types, shedding light on genome architecture hidden from NGS strategies. The use of HMW DNA of sufficient quality is essential to successful optical mapping. The Agilent Femto Pulse system provides unmatched QC analysis of HMW DNA. Using pulsed-field capillary electrophoresis, the Femto Pulse system completes separations of large DNA smears through 165 kb in approximately 1.5 hours. Subjective analysis of sample quality is eliminated with the GQN. Using a Standardized size threshold, the Femto Pulse system can accurately determine if a sample can be successfully run on the Saphyr.

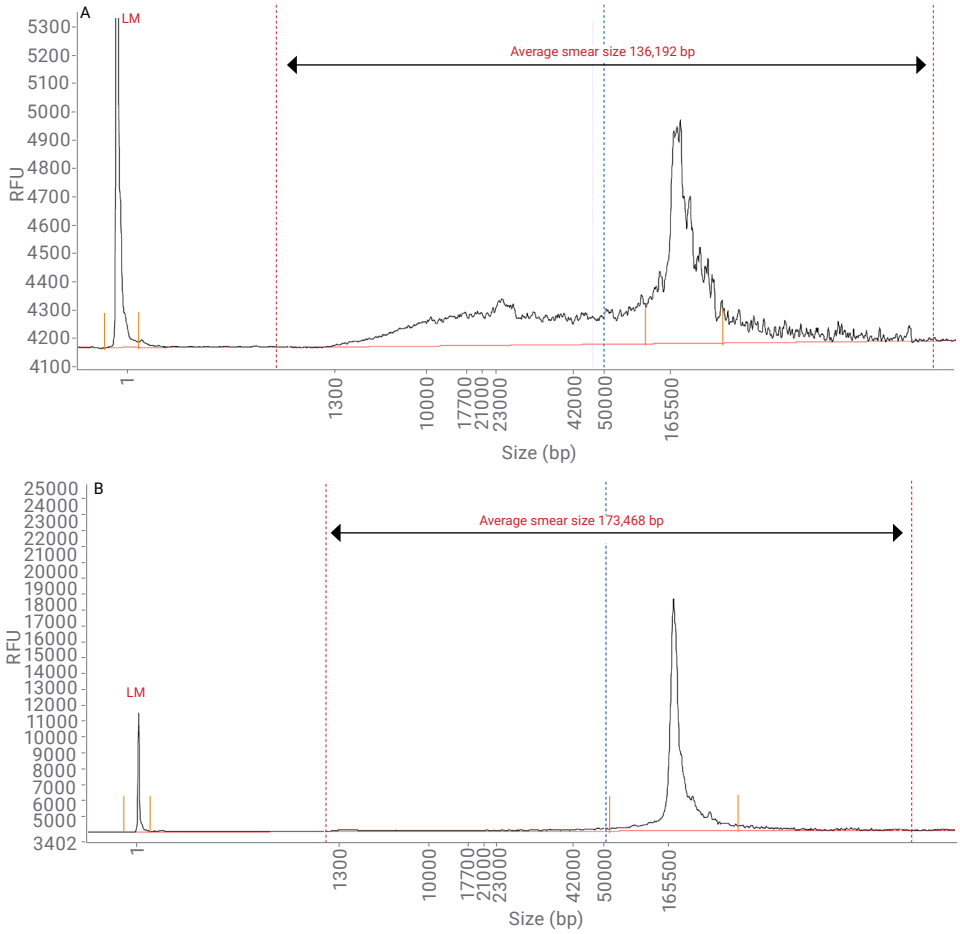

Figure 3. Separations of extracted animal genomic DNA performed on the Agilent Femto Pulse system using the Agilent Genomic DNA 165 kb kit. Post separation analysis was performed using ProSize data analysis software. A) Deer genomic DNA. Average smear size of 136,192 bp as determined by smear analysis (indicated by the red dotted lines; range: 1 to 650 kb, n = 3). With a $GQN_{50\,kb}$ of 7.0 (blue dotted line), this sample meets the criteria for confident analysis on the Saphyr. B) Frog genomic DNA. Average smear size of 173,468 bp as determined by smear analysis (indicated by red the dotted lines; range: 1.2 to 650 kb, n =3). With a $GQN_{50\,kb}$ of 9.4 (blue dotted line), this sample exceeds the criteria for accurate analysis on the Saphyr.

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Printed in the USA, May 1, 2019
5994-0885EN

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