Use of the Agilent Femto Pulse system in sample quality control of long-read sequencing and low input libraries

Kyle Luttgeharm[1] Solange Borg[2]

[1]Agilent Technologies , Ankeny, IA, U.S.A

[2]Agilent Technologies , La Jolla, CA, U.S.A.

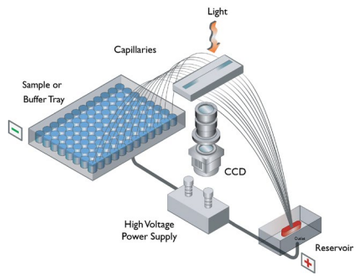
ESHG Conference
Gothenburg, Sweden
June 2019

Agilent
Trusted Answers

Introduction

Long-read sequencing and miniaturization of library preparations are becoming increasingly common as new next-generation sequencing workflows are developed. Traditional quality control methods do not provide the required sizing accuracy of DNA greater than 50 kb or the sensitivity allowing for sample conservation during the quality control assessment steps. The Agilent Femto Pulse system by Agilent Technologies works to streamline quality control by separating genomic DNA up to 165 kb in as little as 1.5 hours, down from the 16+ hours required for traditional agarose PFGE. By coupling capillary electrophoresis with optimized optics, the Femto Pulse system is 500 – 1000x more sensitive than legacy pulse field analysis. The unparalleled single cell gDNA sensitivity of the Femto Pulse system also allows for preparation of low input NGS libraries from cfDNA, RNA, and miniaturized DNA NGS libraries.

Experimental



Automated Pulsed-Field Capillary Electrophoresis

All analysis was completed using the Agilent Femto Pulse system capable of separating 12 samples in parallel with minimal hands on time. The Femto Pulse system can handle a wide variety of sample types including high molecular weight DNA, low concentration NGS libraries, and low concentration RNA samples.

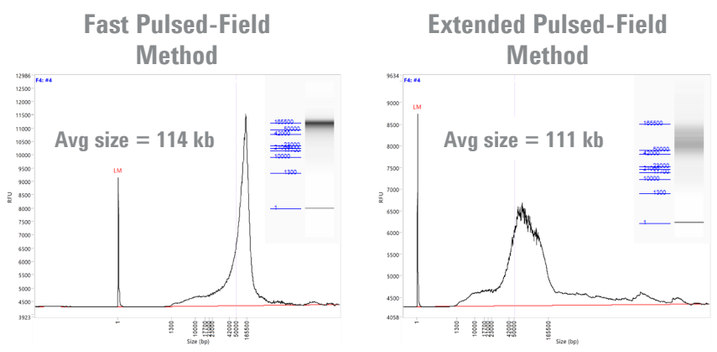
Conclusions

- Agilent Femto Pulse system allows for pulsed-field analysis of large DNA smears up to 165 kb in as little as ~1.5 hours eliminating overnight analysis steps.
- Use picograms of gDNA sample instead of the nanograms required for legacy agarose pulsed-field analysis, providing more sample for downstream applications.
- Accurate and reproducible sizing helps determine correct molarity concentrations for efficient flow cell loading, which generates high quality sequence data.
- Unattended analysis allows for programmed assays to run automatically freeing up time for additional tasks.
- Use of Ultra Sensitivity NGS and RNA kits allows for quantification and qualification of low concentration NGS smears, RNA extractions, and cfDNA extractions.

High molecular weight DNA analysis though 165 kb

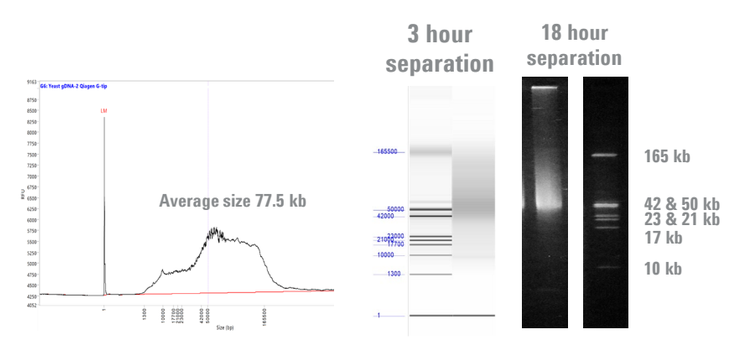
Field inversion pulsed-field electrophoresis

The Femto Pulse system uses highly optimized field inversion pulsed-field gel electrophoresis for accurate sizing of DNA smears and fragments through 165 kb, reducing the time required for the quality control of high molecular weight libraries. By controlling the time each field is engaged rapid separations of HMW DNA can be obtained.



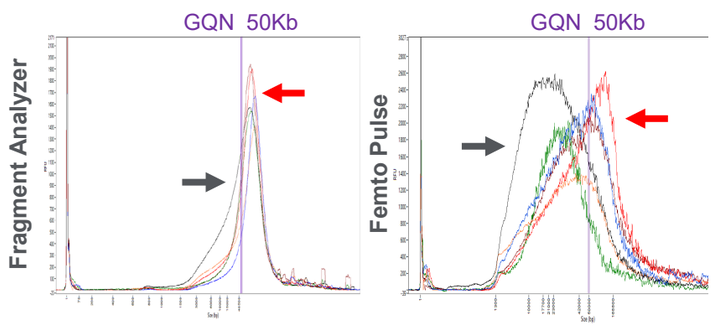
Two pre-programmed pulsed-field methods

Depending on application needs, choose between the Fast 165 kb method (~1.5 hours) for quicker time to results or the Extended 165 kb method (~3 hours) for enhanced resolution between 50 kb and 165 kb. Shown are the same sample run using each method.



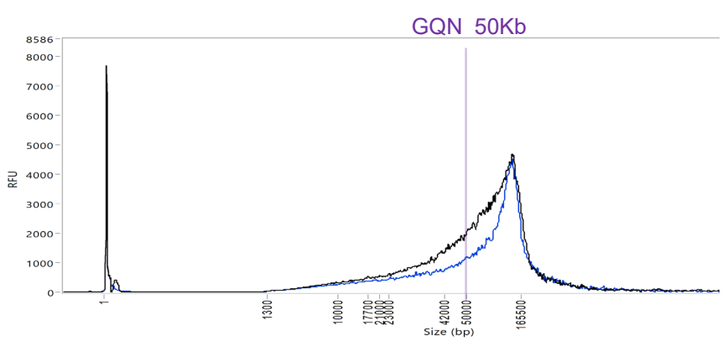
Femto Pulse system matches traditional agarose PFGE

A HMW gDNA sample was analyzed using the Femto Pulse system (Extended 165 kb method) and traditional agarose PFGE. Both samples show a peak maximum of ~50 kb.



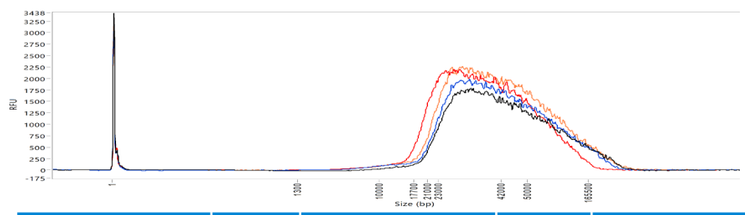
Pulsed Field electrophoresis eliminates peak compression seen with traditional direct field.

Traditional direct field electrophoresis results in compression of nucleic acid greater than ~50 kb preventing accurate sizing of large gDNA smears. Samples were analyzed on the Femto Pulse and Fragment Analyzer systems to show the difference in size distribution. Of particular interest are the red and black traces which show significant differences when run with direct field versus pulse field electrophoresis.



Genomic quality number provides objective view of data

The Genomic Quality Number (GQN) is a user-defined threshold that provides the percent of sample larger than the threshold. Shown above are two samples and a GQN threshold of 50 kb. Sample 1 (black) GQN = 5.8 and Sample 2 (blue) GQN = 6.3 indicating that 58% and 63% is greater than 50 kb respectively

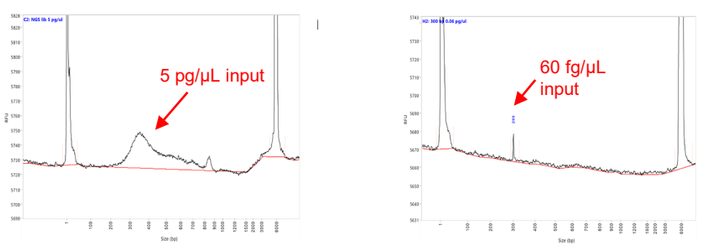


Average Smear	%CV	Average GQN 40 kb threshold	%CV	Sample Number
48,867 bp	7.4%	4.3	8.1%	24

Smear reproducibility section

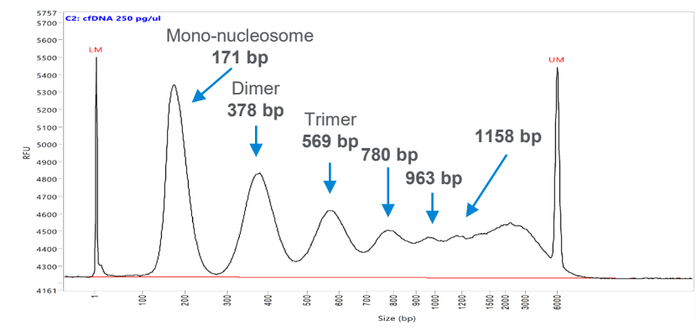
High molecular weight gDNA was analyzed across two instruments for a total of 24 data points. Shown above is an overlay of 4 representative samples. Smear analysis was performed to determine average size and the GQN was calculated with a 40 kb threshold.

Ultra sensitive nucleic acid detection



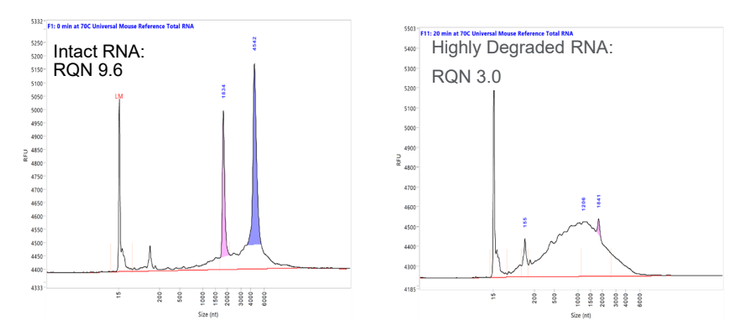
Quantify and qualify low input smears and fragments

Using optimized optics, DNA smears from 25 pg/µL – 250 pg/µL (5 pg/µL LOD) and fragments from 100 fg/µL – 5 pg/µL (50 fg/µL LOD) can be quantified. The high sensitivity of the Femto Pulse system allows for miniaturization of library preparations and conservation of precious sample.



Detect 3 major cfDNA nucleosomes and beyond

With the Ultra Sensitivity NGS kit the Femto Pulse system is able to detect the mono-, di-, and tri-nucleosome as well as additional peaks of potential cfDNA. With input concentrations as low as 8 pg/µL the Femto Pulse system allows for conservation of precious cfDNA sample.



Objectively analyze low concentration RNA

Detect total RNA as low as 2.5 pg/µL and mRNA enriched samples as low as 15 pg/µL with the Ultra Sensitivity RNA kit. The RNA Quality Number (RQN) provides objective analysis of RNA quality comparable to the RIN. When analyzing mRNA enriched samples the amount or ribosomal RNA contamination is automatically calculated.