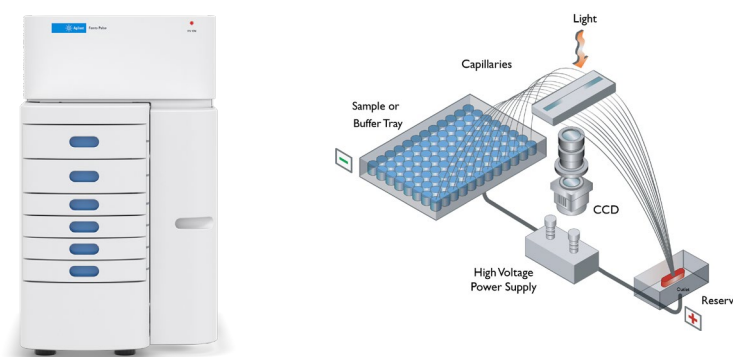


Introduction

Electrophoretic analysis of genomic DNA extractions prior to long-term storage allows biobanks to provide critical sizing and integrity information to potential customers. This information aids biobank customers during sample selection ensuring the ordered material suits their needs. Traditionally, these separations have been done using pulsed-field slab gel electrophoresis which require consumption of nanogram amounts of material and long separation times. The Agilent Femto Pulse system overcomes sample consumption and sizing limitations by combining an optimized optical system capable of detecting picogram levels of gDNA with a powerful pulsed-field power supply allowing for separations of genomic DNA through 165 kb. ProSize data analysis software provides smear analysis for average size determination along with the user defined Genomic Quality Number (GQN), aiding in the objective comparison of samples.

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. Samples are prepared in a 96-well plate with the Femto Pulse able to hold three 96-well plates for analysis of up to 288 samples without user intervention. 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

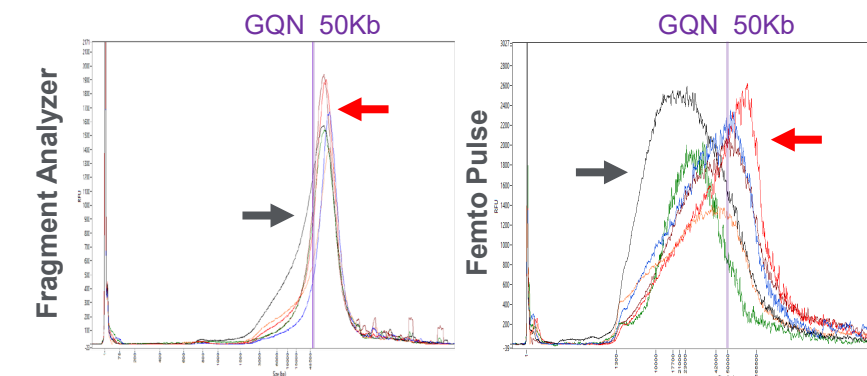
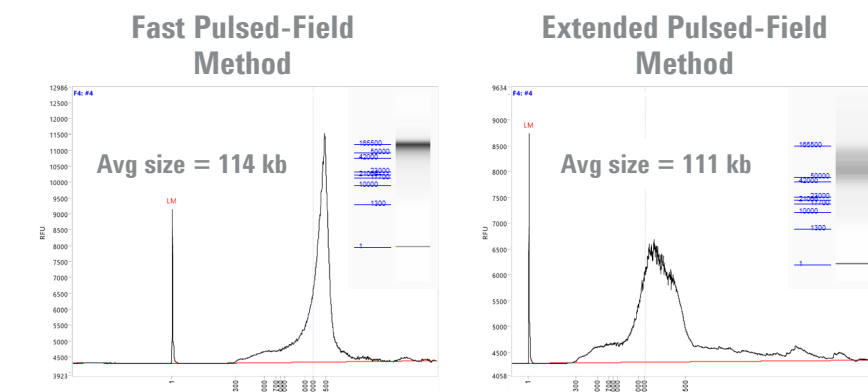
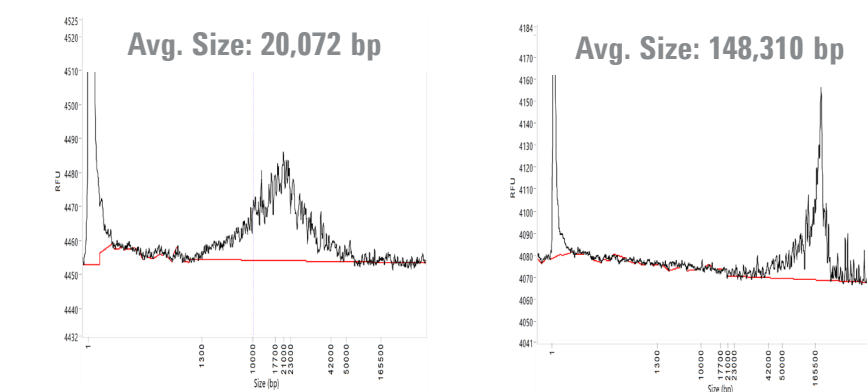
- Use picograms of gDNA sample instead of the nanograms required for legacy agarose pulsed-field analysis allowing for sample conservation and quality control analysis before storage and prior to shipment.
- Digital data and LIMS integration capabilities simplifies sample analysis, tracking, and data storage.
- Accurate and reproducible sizing eliminates ambiguity during customer selection of biobank samples.
- Analyze large gDNA smears, up to 165 kb, in as little as ~1.5 hours streamlining workflows.
- Unattended analysis allows for programmed assays to run automatically, freeing up time for additional tasks.

High molecular weight DNA analysis through 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 biobank samples. By controlling the time each field is engaged rapid separations of HMW DNA can be obtained.

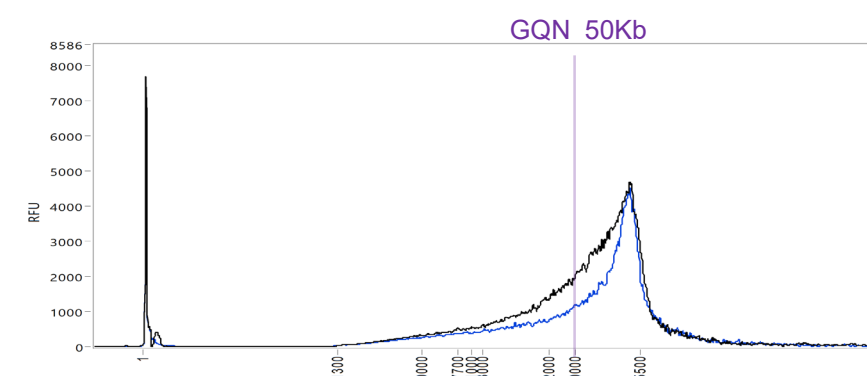


Pulsed-field electrophoresis eliminates peak compression seen with direct-field separations

Traditional direct-field electrophoresis results in compression of nucleic acids greater than ~50 kb preventing accurate sizing of HMW gDNA smears. Samples were analyzed on the Agilent Fragment Analyzer and Femto Pulse 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 pulsed-field electrophoresis.

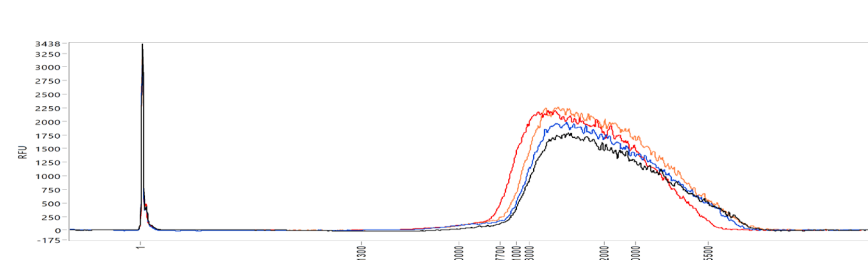
Detect a single cell worth of gDNA

One cell contains ~6pg gDNA. Highly optimized optics and gel chemistry allows for detection of gDNA down to ~2pg/μL. Shown are two commercially available gDNA samples diluted down to 2 pg/μL. With 2 μL of sample used, 4 pg total was loaded into the sample well. Low concentration gDNA detection is influenced by smear distribution, with tighter distributions having a lower limit of detection.



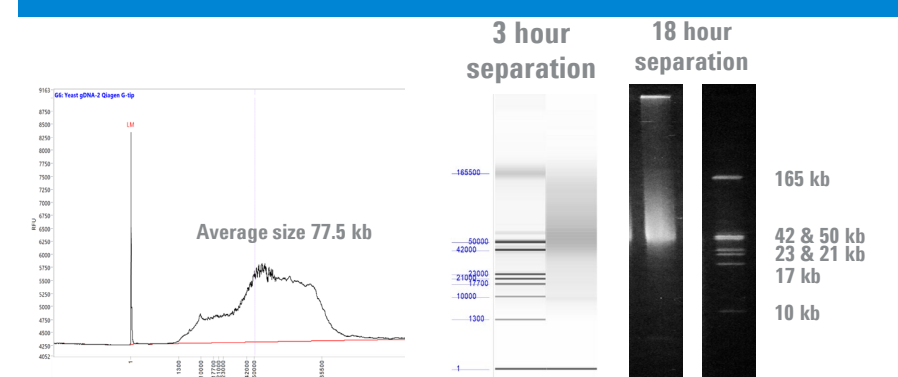
Two pre-programmed pulsed-field methods

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 is the same sample run using each method.



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

Data output format and LIMS integration

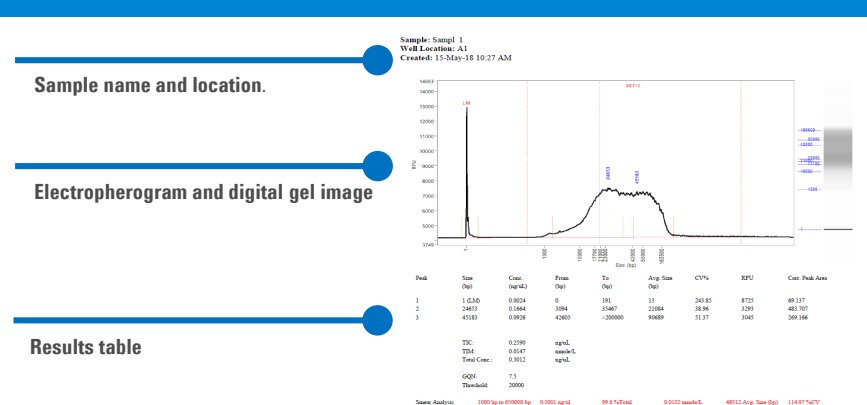


Femto Pulse system outputs an electropherogram and digital gel image matching traditional PFGE

A HMW gDNA sample was analyzed using the Femto Pulse system (Extended 165 kb method) and traditional agarose PFGE. The electropherogram and digital gel image is shown for the Femto Pulse system; an ethidium bromide stained gel is shown for traditional PFGE. Both samples show a peak maximum of ~50 kb.

Genomic quality number provides an 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 is the separation of two HMW gDNA samples. The GQN was calculated for each sample with a 50 kb threshold, sample 1 (black) GQN = 5.8 and sample 2 (blue) GQN = 6.3, indicating that 58% and 63% of the respective sample is greater than the 50 kb threshold.

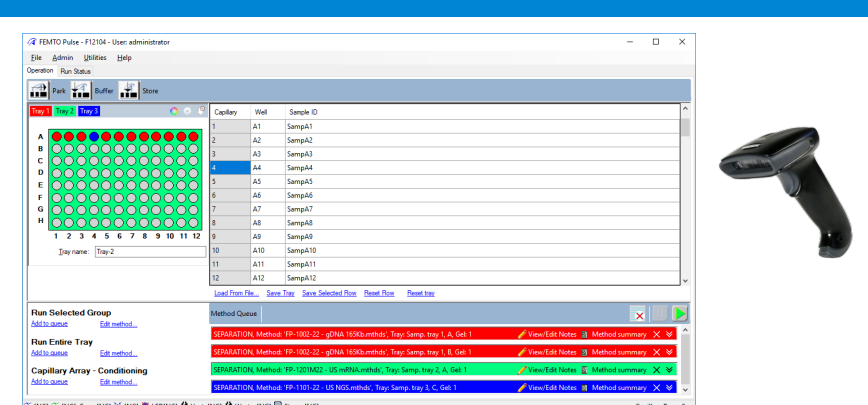


Generate easy to read PDF reports

ProSize data analysis software automatically generates easy to read PDF reports for each sample. The results table contains size information based upon the integrated peaks along with any additional analysis performed, such as smear analysis and the GQN. A digital gel image containing all samples appears at the beginning of the report.

Highly reproducible pulsed-field separations

High molecular weight gDNA was analyzed across two Femto Pulse systems for a total of 24 data points. Shown above is an overlay of 4 representative separations. Smear analysis was performed to determine average size and the GQN was calculated using a 40 kb threshold. Low %CV values demonstrate the reproducibility of the Femto Pulse system.



Seamless integration with Laboratory Information Management Systems

Sample names can be automatically populated into the Femto Pulse system controller software using a barcode reader for integration with an existing LIMS. Additionally, data can be exported in .csv format along with BMP, JPEG, or PNG files of the electropherogram and digital gel images for importing into a LIMS for data archiving.