Technical Note

Utilizing the Femto Pulse System for Assessing Ultra-Long DNA

Introduction

Pulsed-field gel electrophoresis (PFGE) is the recommended technique for evaluating the length and homogeneity of ultra-long, high molecular weight DNA (hmwDNA) for applications such as electronic genome mapping (EGM) with the Nabsys OhmX™ Platform. PFGE enables accurate resolution of DNA ranging from 10 to 1,000 kilobases (kb), encompassing the optimal EGM target size range of 50 to 500 kb. While PFGE provides an effective method for visualizing DNA integrity, access to PFGE instrumentation remains limited in many laboratories. Alternative methodologies, such as standard electrophoresis, fail to accurately resolve DNA larger than approximately 25 kb, making them ineffective for EGM applications.

The Agilent® Femto Pulse® System represents an alternative for laboratories lacking a PFGE apparatus. As with PFGE, the Femto Pulse System employs a pulsed electric field for DNA separation but differs in its gel matrix and detection system. The system uses capillary electrophoresis with a pulsed, oscillating field to separate hmwDNA on a specialized gel matrix. The DNA is subsequently quantified via fluorescence as it passes through the detection chamber. The sensitivity of detection allows less DNA (< 500 pg/sample) to be used than for PFGE (0.4 µg/sample). Additionally, the Femto Pulse matrix provides faster separation than overnight PFGE runs. Both methods allow for the parallel analysis of at least 12 samples per run.

This technical note examines the performance of PFGE and the Femto Pulse System in assessing longer DNA for EGM applications using the OhmX Platform. Our findings demonstrate that while the Femto Pulse System does not fully resolve DNA beyond 165 kb, it represents a viable alternative to PFGE for assessing DNA suitability for Nabsys EGM workflows.

Evaluation of DNA Length and Resolution

To assess the Femto Pulse System, hmwDNA was extracted from human cells using the New England Biolabs® (NEB) Monarch® HMW DNA Extraction Kit for Cells & Blood (Catalog #T3050). Different sizes of DNA were created by adjusting the shaking speed during cell lysis, as recommended by NEB (300-2,000 rpm, Figure 1). Higher shaking speeds result in shorter DNA molecules, as seen in the gel analysis. DNA samples spanning 10 to 1,000 kb were analyzed via both PFGE and the Femto Pulse System.

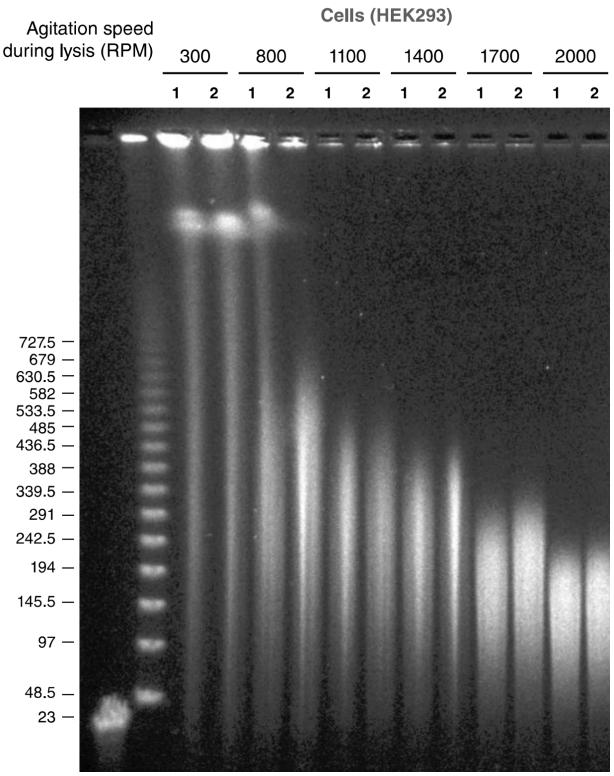

Figure 1: PFGE analysis of hmwDNA extracted from HEK293 cells using the NEB Monarch HMW DNA Extraction Kit for Cells & Blood adapted from Figure 5 of the NEB T3050 manual (https://www.neb.com/en-us/-/media/nebus/files/manuals/manualt3050.pdf). DNA size distributions were generated by varying the shaking speed during cell lysis (300–2,000 rpm). Two replicates were analyzed per shaking speed. DNA sizes are measured in kb.

The optimal size DNA for most EGM applications is 50 to 500 kb, which usually corresponds to shaking at 1,200 rpm. DNA samples (0.4 µg) run on PFGE were diluted to 250 pg/µL and run by Agilent on a Femto Pulse System using the Genomic DNA 165 kb Kit (Catalog #FP-1002-0275). Because preliminary experiments using the 70-minute separation protocol yielded suboptimal results, an extended 3-hour separation protocol was used for this analysis. The results of this protocol are shown in Figure 2.

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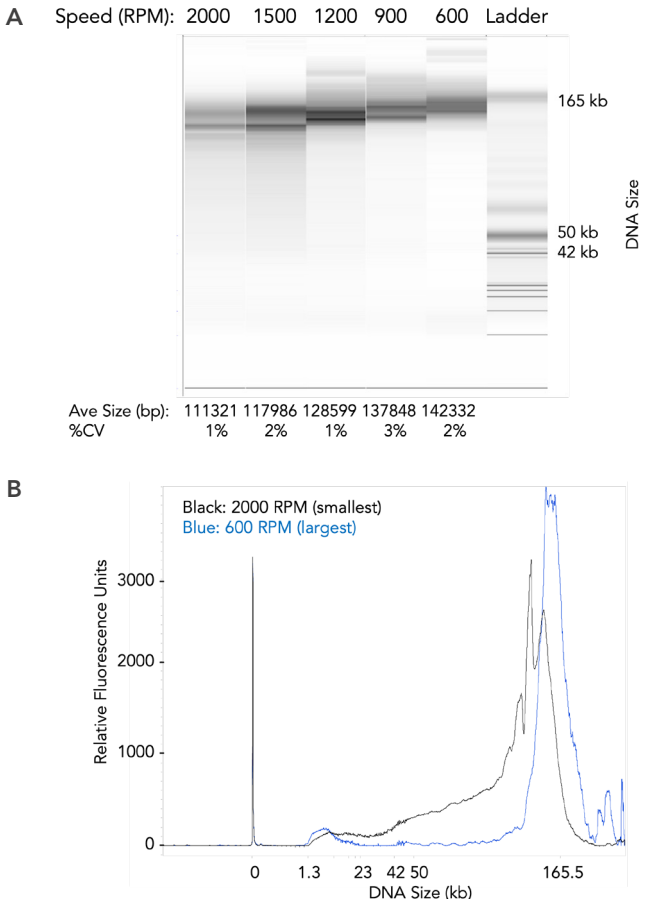

Figure 2: Femto Pulse analysis of hmwDNA using the extended 3-hour separation protocol. DNA samples extracted under different shaking speeds (600–2,000 rpm) were analyzed in triplicate, and the average size and coefficient of variation (%CV) were reported. The gel-like image (A) displays the distribution of DNA sizes, while the electropherogram (B) illustrates the size distribution profiles. Data was generated and analyzed by Agilent.

While the DNA lengths reported by the Femto Pulse System were shorter than those obtained via PFGE, the sizes of the DNA relative to one another remain the same. Importantly, PFGE is more accurate for sizing DNA above 100 kb, while the Femto Pulse System is more accurate below 100 kb. Despite underestimating DNA lengths above 100 kb, the Femto Pulse System can still serve as a proxy quality control (QC) method by relating its measurements back to PFGE. A rough cutoff for Femto Pulse-measured DNA lengths can be estimated for choosing samples for EGM analysis, with DNA samples averaging over 120 kb being more likely to yield reliable results. Additionally, relative lengths of different samples can be determined using the Femto Pulse System, aiding in the assessment of length suitability of DNA samples for EGM analysis.

Assessing DNA Homogeneity and Entanglement

In addition to DNA length, PFGE also offers valuable insights about DNA homogeneity. Entanglement is a frequent issue with ultra-long DNA samples, wherein individual DNA molecules become intertwined or knotted. The extent to which this happens depends on the DNA length, concentration, and extraction method used. To provide preliminary homogeneity assessments, an additional QC step with a NanoDrop instrument can be performed by taking readings from the top, middle, and bottom of the sample. Readings taken from a homogenous sample will vary by ≤ 5 ng/µL. While disparities in these readings indicate sample heterogeneity, uniform readings do not necessarily confirm homogeneity. When entangled DNA is analyzed using PFGE, it either fails to enter the gel matrix or exhibits streaking patterns, indicative of heterogeneity (Figure 3).

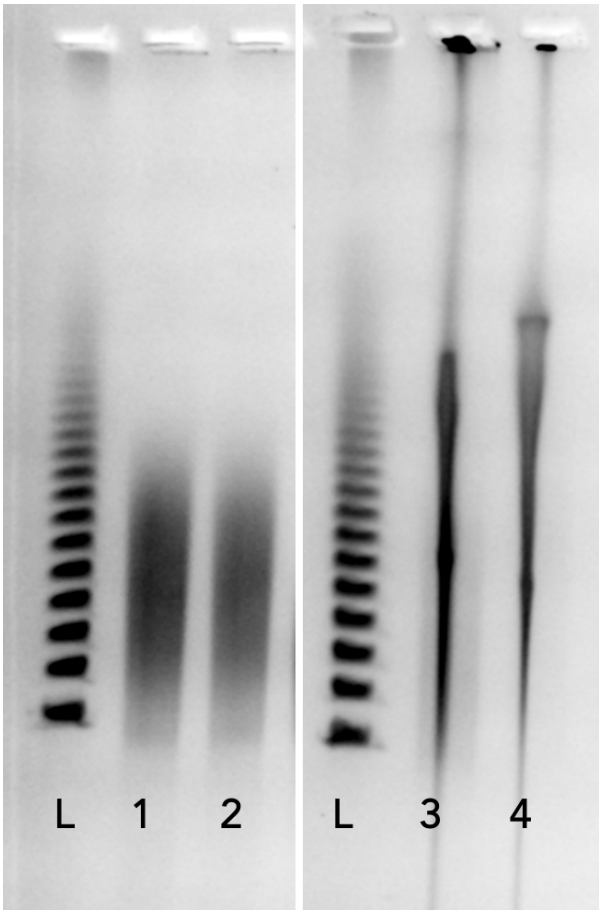

Figure 3: PFGE analysis of DNA homogeneity. Lanes labeled 'L' contain a lambda DNA ladder, while lanes 1 and 2 correspond to homogeneous DNA samples. Lanes 3 and 4 represent heterogeneous DNA samples, characterized by streaking and irregular migration patterns indicative of DNA entanglement.

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While some ultra-hmwDNA samples yield shorter DNA sizes than expected on Femto Pulse runs, it was not possible to reproducibly detect entanglement. Given that Femto Pulse samples are analyzed at significantly lower concentrations than those used in PFGE or EGM, issues of DNA entanglement and heterogeneity may be less apparent. While heterogeneity information may be incomplete, the combination of NanoDrop and Femto Pulse analyses provides significant QC data, aiding in the selection of DNA samples most likely to perform successfully in EGM.

Summary

The Femto Pulse System represents a viable alternative method to PFGE for the assessment of hmwDNA length and provides valuable insights into DNA sample quality for EGM applications using the Nabsys OhmX Platform. Its smaller sample requirement conserves valuable DNA, while its faster separation run time shortens the analysis. Although the Femto Pulse method does not fully resolve DNA sizes beyond 165 kb, it offers a rapid and high-throughput approach for relative DNA length comparisons. Despite its limitations in detecting DNA entanglement, the Femto Pulse System, when used in conjunction with NanoDrop-based QC assessments, enables the informed selection of DNA samples to perform EGM successfully using the OhmX Platform.

The Nabsys OhmX Platform delivers the highest resolution for whole genome structural variant verification and analysis to support your research. Learn more at nabsys.com/products.

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