

Megaruptor® 3 and Femto Pulse as Essential Tools for Long-Read Sequencing

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Accelerating epigenetic studies with NGS

Epigenetics is crucial for understanding how inheritance, environmental factors, and human behavior influence gene expression without any alterations to the DNA sequence itself, offering insights that can advance human health. Hologic Diagenode is a global leader in epigenetic services and solutions, with over 20 years of experience supporting research worldwide. Providing high-performance products to accelerate biomarker discovery is the cornerstone of the company's mission.

The Bioruptor® and Megaruptor® instrument lines from Hologic Diagenode provide unbiased mechanical fragmentation of DNA, chromatin, and RNA for short-read sequencing (SRS) and long-read sequencing (LRS), respectively, enabling high-quality sample preparation for epigenetic studies and sequencing applications. The Megaruptor® 3 device is a gold standard for LRS DNA fragmentation. It shears high molecular weight (HMW) DNA precisely into appropriate fragment sizes for smooth and robust high-quality sequencing library preparation.

The Megaruptor® 3 system provides crucial advantages for LRS by enabling precise control over DNA fragment size distribution. Maintaining a tight fragment size distribution is essential: it minimizes the presence of excessively long DNA fragments that can block nanopores on Oxford Nanopore Technologies flow cells, thereby enabling efficient flow cell reuse with the Flow Cell Wash Kit. At the same time, it avoids generating short DNA fragments, which can reduce read lengths and waste sequencing capacity in PacBio HiFi platforms. By consistently producing DNA fragments within the optimal size range, independent of sample origin, concentration and volume, the Megaruptor® 3 system improves read quality and yield, reduces the required DNA input, and makes LRS more cost-effective and reliable.

Development of the Megaruptor® 3 and the validation of its several shearing protocols relied significantly on accurate and precise sizing of DNA fragments, especially as different parameters are tested for varying fragment sizes. The Femto Pulse system, in conjunction with the Agilent genomic DNA 165 kb kit, has been used extensively to size large Megaruptor® 3-sheared DNA fragments. HMW DNA is separated using a pulsed-field power supply and the instrument can detect femtogram quantities of nucleic acids for low-input sample types. The Femto Pulse system is 1,000 times more sensitive and 10 times faster than traditional agarose pulsed-field gel electrophoresis (PFGE), and it separates DNA fragments as large as 165 kb in approximately 1.5 hours. This application note highlights the use of the Megaruptor® 3 and the Femto Pulse within LRS workflows.

Materials and Methods

Experimental conditions

Purified HMW human genomic DNA (gDNA) was purchased from Promega (Promega, G1471) and sheared to smaller sizes using the Megaruptor® 3 device (B06010003). A 200 µL portion of gDNA at 10 ng/µL was placed into Megaruptor® 3 hydrotubes and spun down for a few seconds using a benchtop mini-centrifuge. The tube was then plugged into a Megaruptor® 3 Shearing Kit (E07010003) and the consumables were placed into a Megaruptor® 3 8-sample cassette or 12-sample cassette (B06010020). The DNA samples were then sheared in the Megaruptor® 3 using 19 different speed settings between 1 and 59 to reach fragment sizes ranging from 6 kb to 150 kb.

Sheared DNA was diluted to a concentration of 150 to 200 pg/μl for sizing on the Agilent Femto Pulse (Agilent p/n M5330AA) system. Regular-bore pipette tips (1 mL) were used to carefully mix the dilutions to avoid uncontrolled shearing if smaller-volume pipette tips were used. Samples were separated on the Femto Pulse with the Genomic DNA 165 kb kit (Agilent p/n FP-1002-0275) according to the kit quick guide.

Data analysis

Profiles of the separated sheared and unsheared DNA samples were analyzed using the Agilent ProSize data analysis software. ProSize offers two sizing options: the peak size and the average size of the smear:

- Peak size indicates the fragment size with the highest abundance in the shearing profile, representing the most prevalent DNA fragments in the sample. However, peak size is most informative when the sample profile is perfectly symmetric, which is not always the case.
- Average size of the smear considers the entire population of DNA fragments within a user-defined region of the sample and calculates their mean size. This approach provides a more representative and consistent measure, making it better suited for reliably assessing the size of sheared DNA.

Agilent and Hologic Diagenode both highly recommend using the average size of the smear for sheared DNA sizing.

The ProSize smear analysis tool was used to determine the average smear size for each sample. For each run, ensure that the ladder peaks are correctly assigned. Manual adjustment is often required, especially for the 165 kb peak. To calculate the average smear size accurately, the smear boundaries are defined by the user and should be set to encompass the main portion of the smear as illustrated in Figure 1, excluding the tails at both ends where the DNA concentration is very low. The difference between the upper and lower smear limits defines the smear width, reflecting the distribution of sheared DNA fragments in the sample.

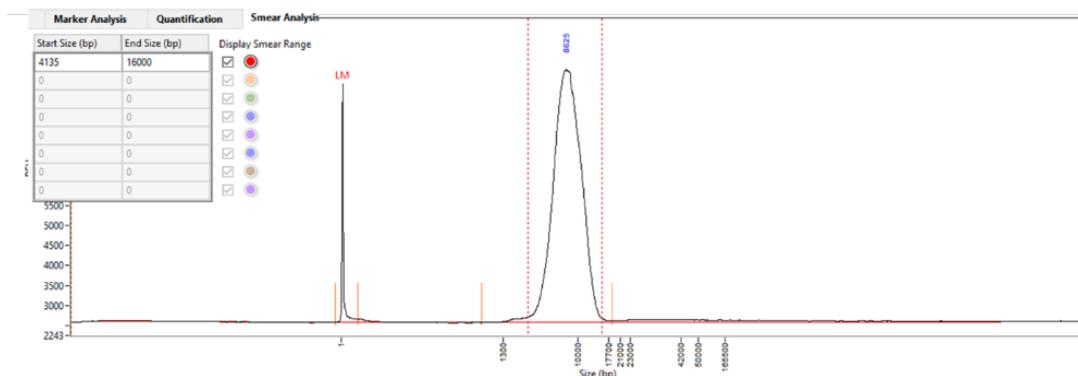


Figure 1. Electropherogram of gDNA sheared using the Megaruptor® 3 at speed setting 56 and analyzed on the Agilent Femto Pulse system. The average size is determined using user-defined smear analysis settings in the Agilent ProSize data analysis software, indicated by the red dotted lines on the electropherogram.

Results and discussion

Precise shearing of genomic DNA using the Megaruptor® 3

The Megaruptor® 3 utilizes hydrodynamic shearing technology, where DNA samples repeatedly pass through a hydropore—a surface with precisely controlled porosity. Fragmentation continues until a consistent target fragment size is achieved, determined by the precise pressure applied as the sample moves through the hydropore. The pressure attained is directly dependent upon the selected speed setting, allowing for accurate control of DNA fragment sizes.

Precise control of the speed with which the DNA sample solution passes the Megaruptor® 3 Shearing Kit hydropore enables efficient fragmentation of all HMW DNA in a sample and a very tight distribution of the produced fragments within the desired size range (Figure 2). The Femto Pulse is an ideal solution for quality control of the produced fragments.

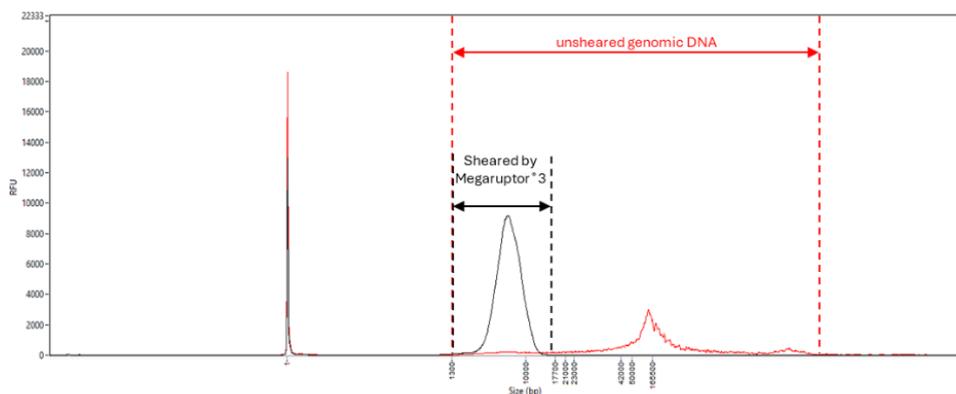


Figure 2. Electropherogram overlay of unsheared gDNA (red) and sheared gDNA (black) using the Megaruptor® 3 at speed setting 56 with the Megaruptor® 3 Shearing Kit. Both samples were analyzed with the Agilent Femto Pulse system, and the average size determined using user-defined smear analysis settings in the Agilent ProSize data analysis software, indicated by the dotted lines on the electropherogram.

The Megaruptor® 3 Shearing Kit allows consistent shearing across various DNA sample types, concentrations, and species. Risk of clogging is very low compared to alternative shearing technologies for large DNA fragments for LRS.

An alternative Megaruptor® 3 shearing consumable—the DNAFluid+ Kit (E07020001)—is available and specifically designed for highly concentrated and viscous DNA samples. It can be used as a pretreatment to reduce and standardize sample viscosity prior to fragmentation with the Shearing Kit or as a standalone solution to attain ultra-high molecular weight DNA fragments up to 150 kb.

The Megaruptor® 3 shears genomic DNA across a wide range of target fragment sizes

The Megaruptor® 3 allows for precise fragmentation of genomic DNA across a broad spectrum of fragment sizes depending on the selected speed setting (Figure 3).

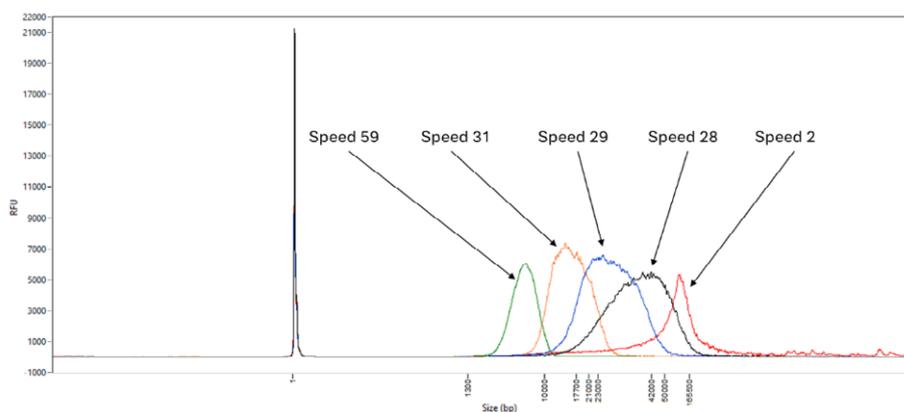


Figure 3. Electropherogram overlay of human gDNA samples sheared using different Megaruptor® 3 speed settings: 59 (green), 31 (yellow), 29 (blue), 28 (black), and 2 (red). All samples were analyzed with the Agilent Femto Pulse system, and the average size determined using user-defined smear analysis settings in the Agilent ProSize data analysis software.

Figure 4 further illustrates the ability to achieve specific DNA fragment sizes depending on the selected speed setting. The Megaruptor® 3 has a total of 65 different speed settings, which increases the fine-tuning and optimization possibilities.

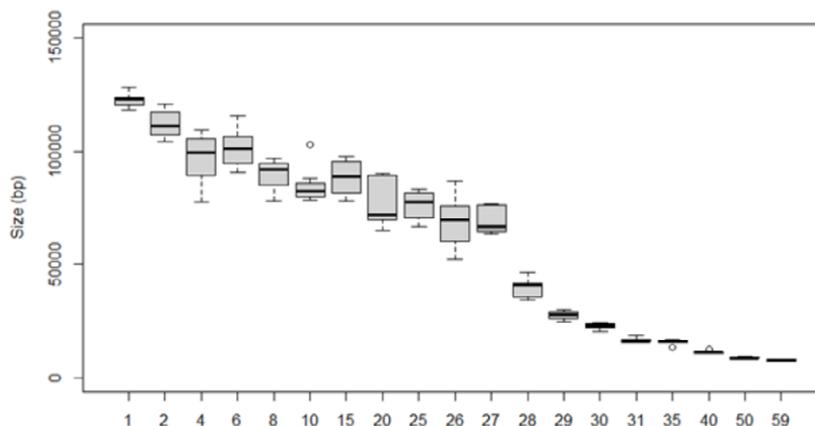
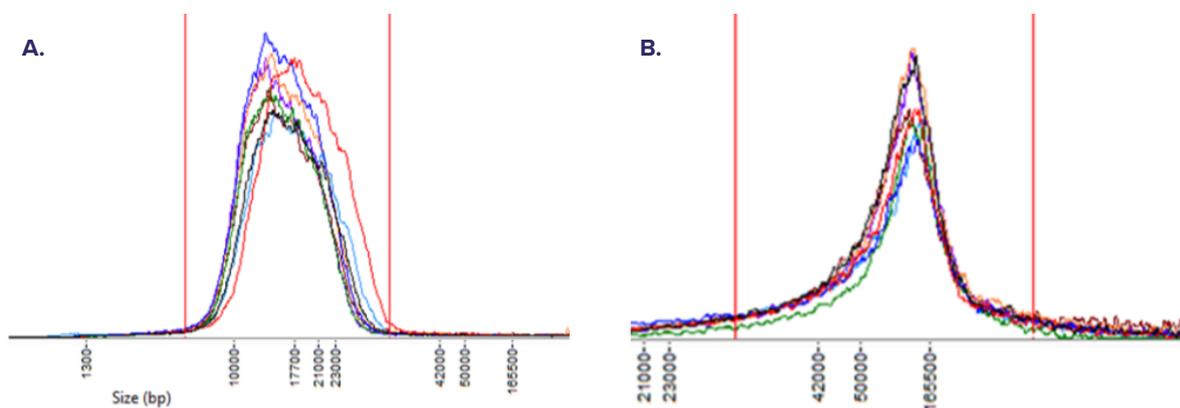


Figure 4. Box-plot graph representing the range of DNA fragment sizes obtained using 19 different Megaruptor® 3 speed settings (from a total of 65). Each box plot represents the size range of at least eight replicates using identical shearing parameters. The average smear size of each sample was determined using the Agilent Femto Pulse system and the Agilent ProSize smear analysis tool.

Megaruptor® 3 DNA shearing is highly reproducible

The Megaruptor® 3's DNA shearing performance is highly reproducible across the entire range of achievable target fragment lengths from less than 10 kb to ultralong fragments that are substantially larger than 100 kb. Figure 5 shows two experiments with eight replicates each, in which human gDNA was sheared to a target size of either 17 or 120 kb, precisely and reproducibly sized using the Femto Pulse.



| Range (bp) | Avg. (bp) | Mean (bp) | CV% |
|-----------------|-----------|---------------|------------|
| 9,000 to 40,000 | 17,115 | 16,872 | 5.9 |
| 9,000 to 40,000 | 18,897 | | |
| 9,000 to 40,000 | 16,334 | | |
| 9,000 to 40,000 | 16,484 | | |
| 9,000 to 40,000 | 16,179 | | |
| 9,000 to 40,000 | 16,404 | | |
| 9,000 to 40,000 | 15,907 | | |
| 9,000 to 40,000 | 17,659 | | |

| Range (bp) | Avg. (bp) | Mean (bp) | CV% |
|-------------------|-----------|----------------|------------|
| 23,131 to 399,275 | 120,413 | 122,320 | 2.5 |
| 23,131 to 399,275 | 119,577 | | |
| 23,131 to 399,275 | 120,465 | | |
| 23,131 to 399,275 | 126,702 | | |
| 23,131 to 399,275 | 119,641 | | |
| 23,131 to 399,275 | 126,391 | | |
| 23,131 to 399,275 | 124,452 | | |
| 23,131 to 399,275 | 120,922 | | |

See next page for figure legend →

Figure 5. Electropherogram overlays of gDNA sheared using the Megaruptor® 3 to two target sizes: A) 17 kb (speed 31, 200 µl, 10 ng/µl) on; B) 120 kb (speed 1, 200 µl, 10 ng/µl). All samples were analyzed with the Agilent Femto Pulse system, and the average size determined using user-defined smear analysis settings in the Agilent ProSize data analysis software, indicated by the red lines on the electropherograms. Tables below the shearing profiles display the average smear size for each sample, as well as the mean size and coefficient of variation (CV%).

Conclusions

The combination of the Hologic Diagenode Megaruptor® 3 and the Agilent Femto Pulse system establishes a new standard for high-quality sample preparation for LRS.

The Megaruptor® 3 delivers highly reproducible and precise fragmentation of high molecular weight genomic DNA, providing unmatched flexibility with 65 speed settings to generate a broad spectrum of fragment sizes. This precise control ensures optimal DNA fragment distributions for various sequencing platforms, minimizing issues such as nanopore blocking, and maximizing read length and throughput.

Complementing the shearing process, the Femto Pulse enables rapid, sensitive, and accurate sizing of large DNA fragments, greatly facilitating quality control and protocol optimization. Together, these technologies empower researchers to efficiently prepare DNA libraries for long-read sequencing, improving data quality, yield, and cost-effectiveness.

With robust performance across diverse sample types and concentrations, and with tools for further optimization, the Megaruptor® 3 and Femto Pulse prove essential for preparing high molecular weight DNA in advanced genomics applications. The integration of these systems into LRS workflows accelerates biomarker discovery and epigenetic studies, advancing our understanding of gene regulation and human health.

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