

Developing DNA Shearing Devices for Short- and Long-Read Sequencing at Hologic Diagenode

Accelerating epigenetic studies with NGS

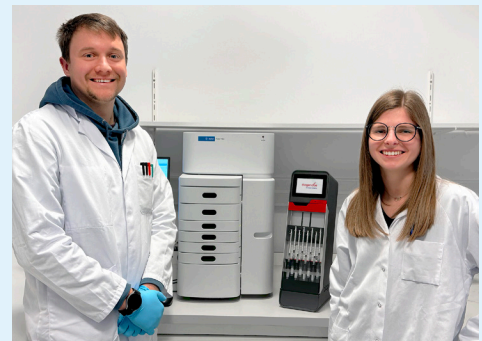
Understanding how human behavior and environmental changes impact gene expression is pivotal in epigenetics research, revealing valuable insights that can help improve the human condition. At Hologic Diagenode, a leading global provider of epigenomics services and solutions, building high-performance products to accelerate biomarker discovery is a key part of the company's mission.

One of the main focuses of Hologic Diagenode's epigenetics department, located in Belgium, is developing DNA and chromatin shearing devices for short- and long-read sequencing: the Bioruptor and Megaruptor, respectively. Dr. Wassim Lakhali, team leader in the Equipments Department, specifically works with the R&D team to optimize the DNA shearing process for long-read sequencing (LRS) and next-generation sequencing (NGS).

Creating high-quality DNA shearing devices

Sequencing larger DNA fragments offers the ability to detect more complex genomic regions, however, it's essential that DNA is effectively sheared into appropriate fragment sizes for best results. Considering this, Dr. Lakhali's team is dedicated to ensuring that every Hologic Diagenode shearing device is produced with the highest quality. To do so, the R&D team needs to work with accurately and precisely sized DNA during product development, especially as different parameters are tested for varying fragment sizes. Another critical step after device production is performing quality control (QC) to assess performance after samples are run.

Given the specific quality requirements, the team decided to use the Agilent Femto Pulse and Fragment Analyzer systems for their DNA sizing and quality analysis. Moreover, other teams at Hologic Diagenode use these systems for additional applications such as RNA, chromatin-based methylation studies, and NGS library profiling. So, system flexibility was another important factor in the team's decision.



Hologic Diagenode lab technicians Damien Polys and Caroline Lausberg with the Agilent Femto Pulse system and Hologic Diagenode's Megaruptor 3 shearing instrument.

Facing the challenge of increased workload

The success of creating high-performance shearing devices didn't come without challenges in earlier years. One of those being the number of samples to read. During that time, the lab was using the Agilent 2100 Bioanalyzer system to analyze samples during R&D activities concerning the Bioruptor shearing device for short-read sequencing (150 bp to 1 kb fragments). However, with a growing workload generating many more lanes to run, they realized that a technology upgrade would significantly improve lab productivity. The team runs several hundred DNA samples a day with the shearing devices, requiring multiple replicates and repetitions for accurate product testing, adding another layer to the complexity.

To achieve higher throughput and greater flexibility, the department decided to use the Fragment Analyzer system for their DNA fragment analysis. Once the system was integrated into the lab's workflow and suitable protocols and settings were determined, they quickly discovered how this change greatly improved workflow efficiency. Dr. Lakhali noted, "I was personally amazed by the instrument's flexibility. The way it works, the precision, the high resolution. And the fact that you can load all your samples on many plates and even remove samples while the device is running." The ability for continuous sample loading on the Fragment Analyzer enabled higher throughput, allowing the team to handle many more samples per day.

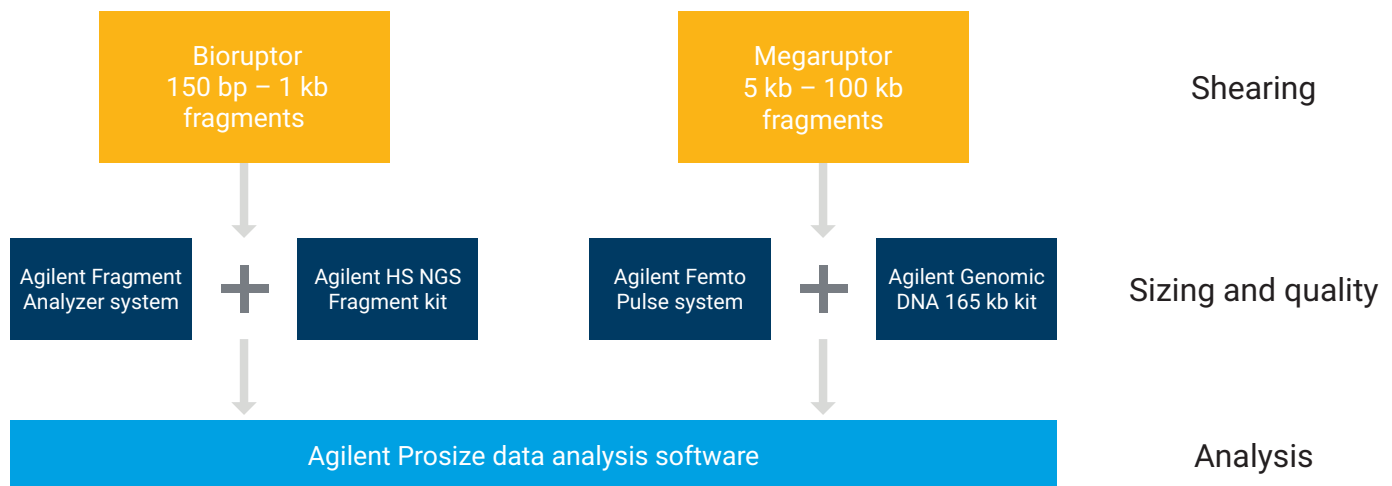
As the R&D team at Hologic Diagenode continued to achieve success in producing DNA shearing devices, as well as in other projects, even more workload growth was inevitable. A couple of years later, they switched to the Femto Pulse system for pulsed-field capillary electrophoretic analysis of their high molecular weight DNA fragments for LRS.

Scaling up for large fragment DNA sizing and analysis

One major factor driving growth in Hologic Diagenode's shearing device product line was the inclusion of large fragment sizing and analysis for LRS capabilities. Hologic Diagenode now produces the Megaruptor 3, a bespoke shearing device featuring technology assuring consistent DNA fragmentation to fragments ranging from 5 kb to over 100 kb with unparalleled tight size distribution for LRS.

Before changing to the Femto Pulse for large fragment sizing, the Fragment Analyzer system with Agilent Large Fragment kit (product number DNF-464) was used for sizing of fragments up to 48 kb, with high accuracy. Yet, as Dr. Lakhali was now working with even larger fragments, sizing accuracy was needed, prompting the switch to the Femto Pulse system. Since acquiring the instrument around two years ago, it's finally possible to not only more precisely analyze samples at 48 kb, but also up to 165 kb using the Agilent Genomic DNA 165 kb kit (product number FP-1002-0275).

Users of the Megaruptor 3 can progressively increase or decrease genomic DNA shearing by changing the sizing parameters based on the application. This could be 20, 30, 65, even 100 kb. Therefore, fragment sizing flexibility is of utmost importance. In addition, Wassim and the R&D team at Hologic Diagenode need a highly accurate system to precisely size sheared fragments and perform QC to support device development. The Femto Pulse met these needs and even helps with generating data to help build protocols for their customers.



The significance of flexibility and throughput

The epigenetics R&D team has successfully implemented large DNA fragment analysis into their daily workflow, supported by the Femto Pulse system. They described that the most beneficial features for their applications are the throughput, high resolution, flexibility, and ease of use. Dr. Lakhali highlighted the user-friendliness of the system. “It takes five to 15 minutes to prepare the instrument and then the rest is really flexible, so we can run a lot of samples.” They have multiple teams in the department that use the Femto Pulse, so the system’s flexibility is important. “The fact that we can combine the runs, change the ranking of the runs, the order of running, it’s also very great for us.”

Another key feature of both the Fragment Analyzer and Femto Pulse systems supporting the team’s work is the Agilent ProSize data analysis software. Dr. Lakhali stated that the software includes many useful options for data extraction and analysis. “We can combine samples, superimpose, compare, change the scales, and play with the baseline. It’s quite an amazing software.” Because they run so many samples daily, he mentioned that automating smear analysis using the ProSize data analysis software allows them to get more information from the samples faster, accelerating product development. “We can easily and quickly analyze hundreds of samples in a couple of hours or even less. Then we can spend more time on analyzing and interpreting the data rather than spending time on calculations.” This not only enhances lab efficiency, but also enables the ability for staff to focus on other tasks.

Continuing the success

For the team at Hologic Diagenode, being able to analyze a growing number of both small and large DNA fragments was a top priority. They were able to keep up with the growing workload by increasing throughput, flexibility, and efficiency with the help of both the Fragment Analyzer and Femto Pulse systems. Specifically, Dr. Lakhali emphasized that Femto Pulse is indispensable for labs performing long-read sequencing that spend time running pulsed-field gel electrophoresis (PFGE) only to get visual results versus quantified data. He highlighted that the system offers great ease of use, especially for users doing long-read sequencing or working with large DNA fragments. “I couldn’t imagine how our daily lives in the lab would be without the instruments, or how we would be advancing in our different projects. It came in at the perfect time.”

The Hologic Diagenode epigenetics R&D team will continue to work on developing high-performance DNA and chromatin shearing devices for customers of various sequencing applications. They will continue fine-tuning the Megaruptor’s precision for larger fragments and hope to achieve even higher throughput in their workflow. Looking to the future, Hologic Diagenode plans to also develop more solutions focused on methylation biomarkers in the effort to expand the possibilities in epigenetics research.

For more information on Hologic Diagenode's mission, visit:

www.diagenode.com

To learn more about the innovative Hologic Diagenode Megaruptor 3 technology, visit:

www.diagenode.com/en/categories/megaruptor

Explore the Agilent Fragment Analyzer and Femto Pulse systems at:

www.agilent.com/genomics/fragment-analyzer

www.agilent.com/genomics/femto-pulse

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