Agilent Case Study: Femto Pulse system

Successful Long-Read Sequencing at the University of Wisconsin Biotechnology Center

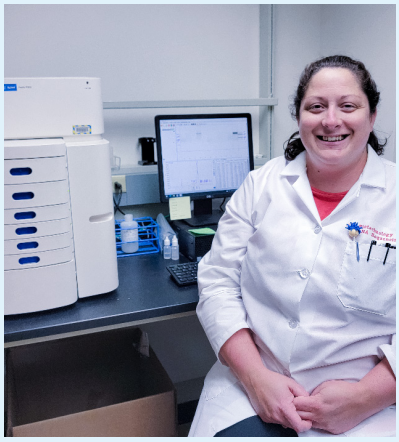
A Reputation for Excellence

Molly Zeller, M.S., Laboratory Manager, has led the University of Wisconsin Biotechnology Center (UWBC) DNA Sequencing Facility to become a key reference lab for long-read sequencing. As a core laboratory, the UWBC DNA Sequencing Facility provides analysis services for all University of Wisconsin campus labs. The team also receives project submissions from laboratories around the world. The facility offers long-read sequencing using instruments from PacBio and Oxford Nanopore Technologies, as well as next-generation sequencing (NGS) using Illumina platforms.

UWBC DNA Sequencing Facility researchers focused their time and energy on optimizing protocols and implementing stringent sample quality control (QC) procedures. The investment was worthwhile. This team now enjoys a reputation among their clientele for delivering high-quality long-read sequencing results. Even sequencing manufacturers refer their customers to the UWBC DNA Sequencing Facility for long-read sequencing services.

The Significance of Long-Read Sequencing

According to Ms. Zeller, NGS and long-read sequencing can be thought of like two different types of puzzles. Like an intricate, 1,000-piece puzzle, NGS creates shorter DNA fragments, or contigs, that can be more difficult to put together against the original genome. But long-read sequencing delivers much longer fragments and, more like a children's puzzle with larger pieces, is easier to piece together and uniquely map more of the genome. These differences matter when researchers need to identify structural variants which are more likely to be identified using long reads. Additionally, large fragments and base modifications can be uniquely mapped using long-read sequencing. "The platforms themselves are capable of detecting base modifications, such as methylation," said Ms. Zeller.



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The long-read sequencing workflows from PacBio and Oxford Nanopore are similar because they both read native DNA, and typically no PCR is performed. The only change is that adaptors are added prior to placing the libraries on the sequencers. The manner of sequence analysis differs between the two methods. PacBio technology circularizes the DNA via bell adaptors – during sequencing, as bases are added, light is emitted. "It is highly accurate consensus sequencing," said Ms. Zeller. Oxford-Nanopore technology sends linear DNA through a pore that measures electrical current as it passes through.

Specialists in Rare Samples

The UWBC DNA Sequencing Facility performs DNA extraction from hundreds of submitted samples they receive each day and sequences DNA in many forms, including amplicons and whole genomes. Uniquely, the UWBC DNA Sequencing Facility specializes in non-model organism projects even though current, off-the-shelf protocols do not work in these cases. This is one of the biggest challenges the UWBC DNA Sequencing researchers faced. Undeterred, Ms. Zeller and her team have developed innovative DNA extraction protocols for a wide variety of unique specimens, including those with low biomass. Extraction can be performed from many organisms and sample types: plants, animals, soil, water, fecal samples, saliva, and blood. Now, most of their business comes from non-model organisms. "This is probably my favorite part of the job. Whatever the sample is, we will try to get DNA out of it. We can do anything up to and including biosafety level 2 (BSL2) microorganisms," said Ms. Zeller.

Why Sample Quality Control is Important

The UWBC research team knew that PacBio and Oxford Nanopore sequencing could produce excellent, high-quality results. But when they first started working with long-read sequencing, they did not get the results they expected. And because their long-read sequencing business was growing, Ms. Zeller set out to quickly find a resolution. During a PacBio user group meeting in 2019, Ms. Zeller discussed sample quality control with other colleagues. One participant told her the key was to use the Agilent Femto Pulse system.

Once the UWBC research team implemented the Femto Pulse system, it enabled them to fine-tune their overall quality control procedures to become more stringent. Today, they use UV-vis spectrophotometry to determine sample purity, fluorimetry for DNA quantification, and the Femto Pulse system to analyze DNA fragment size. For long-read sequencing on the PacBio and Oxford-Nanopore platforms, it is important to be able to size fragments up to 200 kb. The Femto Pulse system can analyze the size of these long DNA fragments. "These are the three non-negotiable tests when performing quality control on our samples," said Ms. Zeller. And ever since they started using the Femto Pulse system as part of the QC procedure, she noted that they are getting "stellar results".

Future Perspectives

The greatest successes that the UWBC DNA Sequencing team has achieved include obtaining reproducible, high-quality results with long-read sequencing and increasing their long-read project capacity. When they started long-read sequencing in 2017, they had an Oxford Nanopore GridION and a PacBio Sequel I. Since then, they have doubled their throughput and continue to grow. Ms. Zeller and her team have upgraded to using two PacBio Sequel II sequencers, the Oxford Nanopore PromethION sequencer, as well as the Oxford Nanopore GridION sequencer.

There is a large interest in long-read sequencing, as evidenced by the increasing number of projects in the lab's queue. Thanks to researchers like Ms. Zeller and her team, the UWBC DNA Sequencing facility has become an important referral lab for long-read sequencing, not only for University of Wisconsin researchers, but for researchers all over the world.

Learn more about the Agilent Femto Pulse system at:
www.agilent.com/genomics/femto-pulse

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