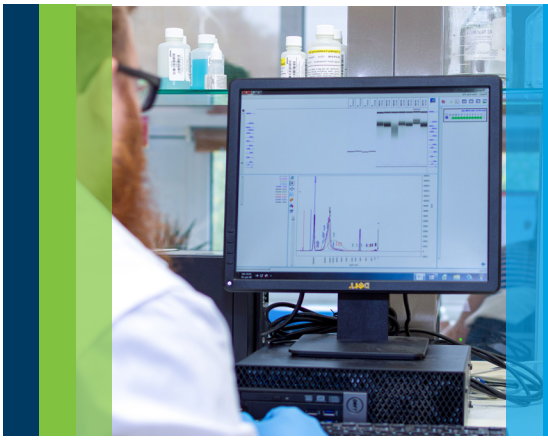


Agilent Case Study: 5200 Fragment Analyzer system

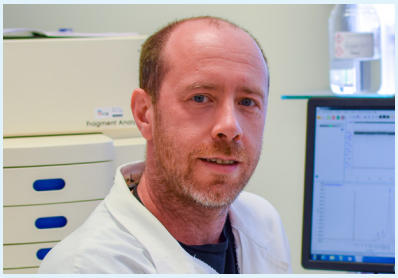
Advancing Genomic Discoveries with NSF's Comprehensive Sequencing Support

Advancing genomic studies at Neuromics Support Facility (NSF)

The advent of long-read sequencing (LRS), particularly when powered by nanopore technology, marks a significant advancement for genomic studies. By enabling the detailed analysis of long DNA and RNA fragments, this method allows scientists to explore genetic information with remarkable precision, uncovering new layers of complexity and fostering advancements in personalized medicine and beyond.

NSF, based in Belgium, is a core support facility within the University of Antwerp's Center for Molecular Neurology (CMN) and is dually affiliated with the Flemish Institute of Biotechnology (VIB). Founded in 2015 and led by Dr. Mojca Stražišar, the NSF provides dependable and flexible support for the scientific efforts of various CMN centers, with a focus on sequencing technologies. Initially specializing in Sanger, short-, and long-read next-generation sequencing (NGS), the NSF has evolved its expertise and is increasingly transitioning to nanopore sequencing using Oxford Nanopore Technologies (ONT). The facility also offers a range of auxiliary services, such as sizing using Agilent instruments, sample manipulations, and quality control (QC).

With a team of experts, the NSF explores cutting-edge technologies, assisting CMN researchers with project design and execution, method and protocol development, and workflow optimization in genomics, transcriptomics, bioinformatics, imaging, and more.



From top to bottom: Tim De Pooter, Dr. Mojca Stražišar, and Geert Joris, experts at the Center for Molecular Neurology (CMN), Long-Read Sequencing Expertise Unit

Collaborative support for genomic research

As a support facility, the NSF primarily aids neurodegenerative and neurodevelopmental research conducted within the CMN. It also collaborates with and services other research groups, commercial, and industrial partners that require the center's specialized knowledge. Specifically, their expertise ranges from sequencing and bioimaging to bioinformatics. Within sequencing, their focus is on providing long-read-sequencing support that begins at project conception and extends beyond data delivery, continuing with expert assistance in scientific data interpretation.

Agilent

Trusted Answers

"Projects ranging from long-read single-cell sequencing to pooled sequencing typically come through our facility. We assist with project conception, sample preparation, library preparation, data quality assessment, and data interpretation," explained Dr. Stražišar. The team supports a wide range of projects, sequencing samples from bacteria to humans and even plants depending on the project scope.

DNA- and RNA-based projects are central to the NSF's sequencing work. The team processes these nucleic acid samples through targeted approaches. Tim De Pooter, an expert NSF lab technician, noted, "We cover all types of samples, from extracted whole genomic DNA to prepared RNA-based libraries."

Robust QC methods for long-read sequencing

For sequencing experiments, conducting sample and library QC are essential steps in the workflow. The lab's initial QC instrument posed challenges in terms of quality score reliability, analysis time, and cost-effectiveness due to the need for different kits based on quality metric.

Shifting its focus primarily to LRS and nanopore sequencing, the team needed a more robust method for QC analysis. LRS requires flexibility to measure the varying quality metrics of DNA and RNA fragments. "In general, for quality control, we have three measurements," stated Tim. "First, for nanopore sequencing especially, we must look at the purity of the samples because this metric can interfere with experiment outcomes. Second, we use a fluorometer to determine the exact measurement of the DNA or RNA concentration. And the third, crucial measurement is the integrity of the sample." These metrics allow the team to analyze the quality of extracted nucleic acids before downstream sequencing.

The facility invested in the Agilent 5200 Fragment Analyzer system for more versatile sample QC analysis. Dr. Stražišar explained that for LRS workflows, first, QC is performed for the extracted DNA before moving to sample preparation. "Sample prep is not library prep for us. In ONT protocols, it often involves fragmentation followed by size selection." After every step, the Fragment Analyzer is used to ensure that the fragments are consistent and of expected size.

Dr. Stražišar continued, "Our collaborators are often interested in structural variants that could be significant in various pathologies. These variants are quite large, so we need fragments of a specific size to detect them properly. When performing manipulations like fragmentation or size selection, we aim to ensure high quality of the sample before proceeding to the more expensive library prep and sequencing." This process is time-consuming because it typically includes several rounds of QC, which supports optimal results. "If something goes wrong during sample prep, library prep, or sequencing, we can trace it back to pinpoint where the issue occurred."

Tim further elaborated, "For nanopore sequencing, we primarily use molarity instead of mass in library prep and flow cell loading. The mass difference between a molecule of 1 kb and one of 20 kb in molarity is significant and affects sequencing. Overloading was an issue in the past, mostly resolved with the latest chemistry. However, if we don't use molarity as a measure, we can still underload the flow cell, which impacts the final output tremendously."

Optimized QC with the 5200 Fragment Analyzer system

The 5200 Fragment Analyzer system is now the lab's preferred instrument, used for flexible projects that require analyzing both DNA and RNA. Switching from the 96-channel to the 12-channel instrument has reduced consumable usage and costs, while increasing flexibility and versatility. Tim noted, "What's really economical is that most of the consumables are interchangeable, so we only need one set, which can then be run on either Fragment Analyzer instrument."

Different kits are used based on project requirements. The main kit being the Agilent HS Large Fragment 50 kb kit for initial nucleic acid integrity analysis. After fragment size selection, additional LRS sample manipulations are performed with the standard-sensitivity Agilent Large Fragment kit. For RNA sequencing, the team uses both the Agilent HS RNA and RNA kits. "We also use the Agilent NGS Fragment kit for smaller projects, such as long-read cDNA or amplicon sequencing," said Tim.

The NSF team emphasized the kits' adaptability. "All the kits, all the consumables work in the same way," stated Dr. Stražišar. "If you know how to use one kit, you can easily adapt the specifications and ranges. It's basically just different gels, markers, and ladders." The system's design allows for easy switching between applications and efficient loading, enabling results by the next day.

Geert Joris, an NSF lab technician, said, "We are confident in the system's reliable and reproducible results. The Fragment Analyzer is ideal for our facility due to its flexibility and quick preparation time." The NSF team also noted the operational simplicity and openness of the system. The supplied Fragment Analyzer data analysis software is easy to use and allows for scoring of different quality metrics.

Future for NSF

As the NSF expands its endeavors into LRS and nanopore sequencing, the team will continue collaborating with the University of Antwerp, VIB, and external research centers. With growing project requests for sample quality and integrity analysis on the Fragment Analyzer system, the NSF remains key in helping collaborators ensure their samples meet quality standards, thereby saving time and resources.

"We will stick with long-read sequencing for a while because we are just beginning to explore its various applications," said Dr. Stražišar. "We aim to remain a small- to medium-sized support facility, offering our built-up expertise. We are selective with the projects we undertake, often choosing the more challenging ones to see if they can succeed."

Targeted sequencing applications require upfront information on sample integrity, which can be expensive and complex. Knowing the likelihood of success beforehand allows researchers to refine extraction and manipulation techniques to improve sample quality. Tim emphasized the importance of performing QC steps throughout every stage of the protocol. "Cutting corners often leads to poor libraries and results. The Fragment Analyzer plays a crucial role in ensuring quality before samples are loaded onto the sequencer."

"You have to be able to assess in advance whether any type of experiment will be successful or not," Dr. Stražišar concluded.

The team will continue focusing on DNA and RNA sequencing, although they are also keenly interested in protein sequencing. "We are patiently waiting for advancements in nanopore sequencing that will include protein sequencing. This next step will be more challenging than DNA and RNA sequencing," Dr. Stražišar explained. By embracing these challenges, the NSF is committed to driving forward the frontiers of science, fostering innovation, and contributing to the global scientific community.

www.agilent.com/genomics/fragment-analyzer

PR7001-4159

Published in the USA, April 9, 2025
5994-8300EN

Agilent
Trusted Answers