

Addressing the Challenges of RNA- and DNA-Sequencing at the Georges-François Leclerc Cancer Center

The Agilent SureSelect XT HS2 RNA kit and custom panels developed with Agilent SureDesign software helped a clinical and translational research lab make progress with formalin-fixed paraffin-embedded (FFPE) fusion sample analysis.

Attaining reliable fusion data from precious, FFPE tumor samples can be challenging, but clinical scientists at the Georges-François Leclerc Cancer Centre (CGFL) in Dijon, France, established successful methods to obtain this data. The Molecular Biology Laboratory, part of CGFL's Department of Biology and Pathology of Tumors, is focused on clinical and translational research and development, sequencing core services, and molecular diagnostics. Romain Boidot, Ph.D., Head of Somatic Genetics, Constitutional Genetics, and the Molecular Biology Laboratory, and Sandy Chevrier, M.S., Associate Laboratory Manager, have developed several custom panels for both DNA-sequencing and RNA-sequencing. Through this process, Dr. Boidot, Ms. Chevrier, and their team have also optimized lab workflows, improved sequencing depth, enhanced sample throughput, and reduced analysis turnaround times.

Custom Panel Designs Aid Cancer Identification

These clinical researchers created two panels for DNA-sequencing to investigate the somatic genetics of solid tumors, referred to as CGFL and Gyneco. CGFL was designed for analyzing target genes for lung cancer, colon cancer, melanoma, and gastrointestinal tumors (GIST). Gyneco was developed to study ovarian, breast, endometrial, and prostate cancers. These panels are used on DNA obtained from many sample types including FFPE tumor samples, plasma (cell-free DNA), cerebrospinal fluid, and pleural fluid.

The team has also created two custom panel designs for targeted RNA-sequencing of FFPE tumor samples. One panel identifies gene fusions to aid in their theranostic goals, and the second panel includes markers associated with sarcoma classifications. For constitutional genetics studies, the team designed a single panel based on over 30 genes to analyze DNA extracted from whole blood. This design is used to screen for potential predisposition to breast, ovarian, digestive tract, and prostate cancers.

The Molecular Biology Laboratory annually sequences approximately 3,000 samples, including FFPE tumors, fresh immune cells, human cancer cells, and mouse cancer cells. But the lab does much more than routine DNA-Seq and RNA-Seq, offering additional genomics services. Dr. Boidot said, "supporting our DNA sequencing core, we have routinely performed whole-exome sequencing (WES) on tumor and blood samples for more than six years." As part of their sequencing services offering, the laboratory also performs total RNA-Seq and WES, representing hundreds of samples per year. In parallel, the clinical scientists perform some ChIP-seq and small RNA-Seq experiments. Occasionally, they conduct single-cell 3' RNA-Seq and Methyl-Seq.



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Preserving Precious RNA, Enabling Rapid Reports

The Georges-François Leclerc Cancer Centre receives FFPE microbiopsies from many different pathology labs for fusion analysis. Each of these labs have their own tissue preparation processes, which contributes to sample quality heterogeneity. Furthermore, the formalin fixation and paraffin embedding processes in FFPE tissue sample preparation leads to nucleic acid degradation. Ensuring reliable fusion data from these very small samples is a key technical challenge. "Our policy is to test every sample, whatever the quality of the RNA. Variable RNA quality is another important constraint we aim to address in order to minimize invalid results," said Ms. Chevrier.

Variable RNA quality has contributed to a library failure rate of 15 to 20% and limited multiplexing capability with only six samples multiplexed on an Illumina High Output kit (150 cycles cartridge). The research team wanted to improve these parameters and contacted Agilent Technical Support to discuss their current workflow. Agilent Field Application Scientists recommended the SureSelect XT HS2 RNA kit because of its capability for improved fusion detection.

Using the SureSelect XT HS2 RNA kit in combination with Agilent SureDesign to create custom panels, the CGFL team was able to develop custom capture-based panels for the analysis of 20 genes each. The technical support team also helped Dr. Boidot and Ms. Chevrier refine their panels. "We were able to optimize our library preparation process for all the samples tested. It allowed us to successfully finalize our validation," said Ms. Chevrier.

The optimized process improved key sequencing quality metrics. "Thanks to this technology, we were able to increase the sequencing depth and to reduce the total amount of sequencing data per sample," said Ms. Chevrier. The lab now multiplexes up to 24 samples on an Illumina NextSeq 500 Mid Output Kit (300 cycles cartridge) and has reduced the library failure rate to under 5%. For these reasons, the lab uses the SureSelect XT HS2 RNA kit for all their routine targeted RNA-Seq applications.

Once the data is produced, reports must be delivered within a short time period to meet the needs of the requesting laboratory or department. "Consequently, we need analytical processes that are robust and reliable, whatever the quality of starting material, enabling a quick turnaround time," said Dr. Boidot. With the high quality and precision of the fusion data that is generated using targeted sequencing, it is easier to meet those expected timelines. "Our previous technology was deep whole-transcriptome analysis. This approach is interesting for discovery, but not in the development of potential diagnostic solutions, due to its cost and possible lack of sensitivity compared with targeted RNA sequencing," said Dr. Boidot.

Future Perspectives

With the success the CGFL team has achieved with RNA-Seq, they have already expanded to using the SureSelect XT HS2 DNA kit for constitutional investigation. Dr. Boidot said, "This will help us develop an even more robust panel, regardless of DNA sample quality."

The lab plans to upgrade their current CGFL and Gyneco DNA panels from the Agilent SureSelect XT panel to the SureSelect XT HS2 DNA kit for library preparation. They will migrate from Agilent SureSelect All Exon V6 and SureSelect XT library preparation kits to Agilent SureSelect All Exon V8, which is associated with the SureSelect XT HS2 technology. "This will allow us to use the same, most up-to-date technology for all our purposes," said Dr. Boidot.

Through their efforts to upgrade and optimize their genomics testing methods, the CGFL Molecular Biology Laboratory team has achieved their goals. This includes attaining reliable fusion data from precious FFPE samples, improving sequencing depth, increasing sample throughput, and reducing analysis turn-around times. Thanks to clinical laboratory scientists like Dr. Boidot and Ms. Chevrier, clients of the Georges-François Leclerc Cancer Centre can routinely rely on rapidly produced, robust genomics reports.

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