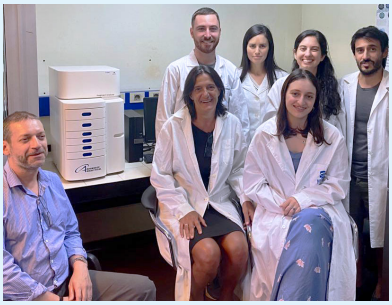
Agilent Case Study: Success with Next-Generation Technologies at the University of the Republic, Uruguay

Viral genome characterization facilitated by the Agilent 5200 Fragment Analyzer system

Viral Genome Evolution Studies at the University of the Republic, Uruguay

Yanina Panzera, Ph.D., professor at the University of the Republic (UdelaR) in Montevideo, Uruguay, has research and teaching responsibilities in the division of Evolutionary Genetics in the Department of Animal Biology. She and her team conduct studies dedicated to uncovering the evolution of viral genomes and developing novel detection methods to explore distinct genetic variants. Learning more about the mutational changes experienced in viral genomes helps to expose critical insights. In turn, these discoveries contribute to a greater understanding of the disease epidemiology, including viral origins, migration pathways, and evolutionary relationships. As Dr. Panzera mentioned, "the study of genomic changes is a basis to understanding viral population dynamics and evolution."

With the emergence of the COVID-19 outbreak, researchers and healthcare workers globally face the challenge of understanding the fast-adapting behaviors of the SARS-CoV-2 virus. Dr. Panzera and her research team use SARS-CoV-2 genetic diversity to analyze the virus' emergence and evolution.



Yanina Panzera, Ph.D. and research team

University of the Republic
Montevideo, Uruguay

NGS is a Key Technology for Viral Genomics

Performing next-generation sequencing (NGS) on viruses comes with a significant challenge. Various procedures to enrich viral genomes before NGS must be implemented due to contamination of host cell DNA, for example, polymerase chain reaction (PCR). Furthermore, with the complex steps involved in applying NGS to viruses, a quality control (QC) system is crucial to ensure high quality results.

Next-generation sequencing has been a critical technology in the team's research. Previously, testing was done in collaboration with The Pasteur Institute, a French non-profit organization, as they had the necessary equipment. But in 2018, UdelaR was able to set up its own in-house NGS platform. Now, the team has optimized their NGS workflow, from sample preparation and library generation to sequencing and data analysis, including nucleic acid quality analysis using the Agilent 5200 Fragment Analyzer system. "Our aim has always been to control the complete

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workflow," Dr. Panzera noted. One important factor driving their research success is the equipment involved in SARS-CoV-2 variant detection. Using NGS technology and parallel capillary electrophoresis with the Fragment Analyzer system, the research team successfully detected the emergence of a 12-nucleotide deletion in SARS-CoV-2, unique to patients from a single outbreak in Montevideo, Uruguay. This evidenced that the virus can retain functionality regardless of genetic material loss, a significant aspect of SARS-CoV-2 evolution.[1]

Detection of Viral Genome Mutations

Dr. Panzera noted, "we frequently use the Fragment Analyzer system, a parallel capillary electrophoresis instrument in our lab, to perform library size analysis and check library quality." She mentioned that the system was handy for detecting specific mutations within the SARS-CoV-2 genome by comparing amplicon size in viral strains. "Let's run a strain that doesn't have the deletion against a wild-type strain to see if the system is capable of distinguishing one from the other," Dr. Panzera said. They found that the instrument succeeded, solidifying their decision that it would be the right equipment for the job. "We used the Fragment Analyzer for screening genome deletions, including a huge deletion mutation of 872 nucleotides using the Fragment Analyzer.[2] This is the largest SARS-CoV-2 variant reported in the literature to date," she said. "But we also detected other smaller mutations. One of 12- and another of 68-nucleotides.[3]" The Fragment Analyzer was able to differentiate between deleted and wild-type strains from all strains with deletions that were circulating in Uruguay.

The Fragment Analyzer system was also the team's top choice for NGS library prep QC due to its precision capability to distinguish DNA fragments differing by only a few nucleotides. For these reasons, the instrument served two purposes – for NGS library QC analysis and for PCR amplicon fragment analysis as a quick screening method to help identify mutations post-NGS.

Achieving Innovation During a Pandemic

Conducting research during the height of a global pandemic presented additional challenges. During this time, our team led by Dr. Ruben Pérez, like most others worldwide, had trouble obtaining lab supplies, making it very difficult to perform the required lab techniques. For Dr. Panzera's team, they had to make the most of what resources were readily available, so establishing their own viral genomics platform was their method. In addition to thorough staff training, they have supported their research through the increased use of the Fragment Analyzer.

Recognizing the potential of the Fragment Analyzer system for viral research in her country, Dr. Panzera made it a goal to ensure that it was well-utilized. With the help of Agilent technicians, staff was trained in all potential uses, allowing the lab to capitalize on this asset. In addition, by hosting promotional seminars as encouragement for other research teams, Dr. Panzera is determined to raise awareness of the Fragment Analyzer system's value, especially while resources are limited. "We really enjoy innovation," she said, and having this resource has allowed them to expand their opportunities. She and her team are currently using it for a technical development that will enable them to characterize viral genomes at minimal cost, hoping to benefit those countries within South America with less access to supplies.

Achievements and Looking to the Future

One of these opportunities, and a remarkable achievement, was making a technology transfer with the Uruguay Ministry of Public Health, training their staff on the functionality of their own, underutilized NGS platform. The Ministry has used this knowledge to fulfill its SARS-CoV-2 sequencing program. The goal is to continue this training and technology transfer from UdelaR to the private sector and government institutions, setting the benchmark for virus detection and characterization methods.

As a professor in the Faculty of Science at UdelaR, Dr. Panzera aims to train staff in various ways, such as teaching post-graduate courses, conducting pre- and post-graduate thesis supervision, and training through internships. Her main objective is to increase knowledge of NGS and capillary electrophoresis methods and transfer this technology to as many others as possible, with the help of her dedicated team. "These technologies are here to stay and set the gold standard for methods of viral detection and characterization," she concluded.

References:

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2. Panzera, Y., Cortinas, M.N., Marandino, A., et al. Emergence and Spreading of the Largest SARS-CoV-2 Deletion in the Delta AY.20 Lineage from Uruguay. *Gene Reports* **2022**, 29, 101703. https://doi.org/10.1016/j.genrep.2022.101703

3. Panzera, Y., Calleros, L., Goñi, N., et al. Consecutive Deletions in a Unique Uruguayan SARS-CoV-2 Lineage Evidence the Genetic Variability Potential of Accessory Genes. *PLOS ONE* **2022**, 17 (2). https://doi.org/10.1371/journal.pone.0263563

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