

A powerful combination for qPCR

The Dittmer Lab at the University of North Carolina is investigating the biology of viral cancers using a Freedom EVO® 150 with an integrated LightCycler® 480 Real-Time PCR System. This versatile set-up provides automated qPCR-based analysis for viral gene and microRNA expression profiling, as well as viral load testing of clinical trial samples.



Dr Pauline Chugh

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The Dittmer Lab is part of the Department of Microbiology and Immunology and Lineberger Comprehensive Cancer Center at the University of North Carolina (UNC), and focuses on the investigation of viral cancers, such as those caused by Kaposi’s sarcoma-associated herpesvirus (KSHV). KSHV is responsible for one of the most common AIDS-associated cancers, posing a significant risk to HIV-positive or immuno-compromised individuals. The Group uses qPCR profiling to study various aspects of KSHV, as postdoctoral research associate Pauline Chugh explained: “We use three main strategies to investigate viral tumorigenesis – viral gene expression, microRNA (miRNA) expression and viral load testing of clinical samples – based on 96 primer qPCR arrays. Performing the assays manually would be very labor-intensive, and so we use automation to provide the throughput necessary for these studies.”

The Dittmer Lab relies on a Freedom EVO 150 workstation equipped with an eight-channel Liquid Handling (LiHa) Arm to ensure accurate and reliable assay plate set-up in a 384-well format. This platform is linked to a Roche LightCycler 480 Real-Time PCR System (LC480) to provide walkaway automation of qualitative and quantitative nucleic acid detection by real-time PCR. Pauline continued: “This set-up gives us the ability to perform automated analysis of up to 750 gene sequences per sample in just one day, allowing us to, for example, look at the whole miRNA library following infection with the virus. Before purchasing the Freedom EVO platform we used a basic, single channel pipetting robot, which was not capable of reliable automation on the scale required for this kind of investigation, and required manual transfer

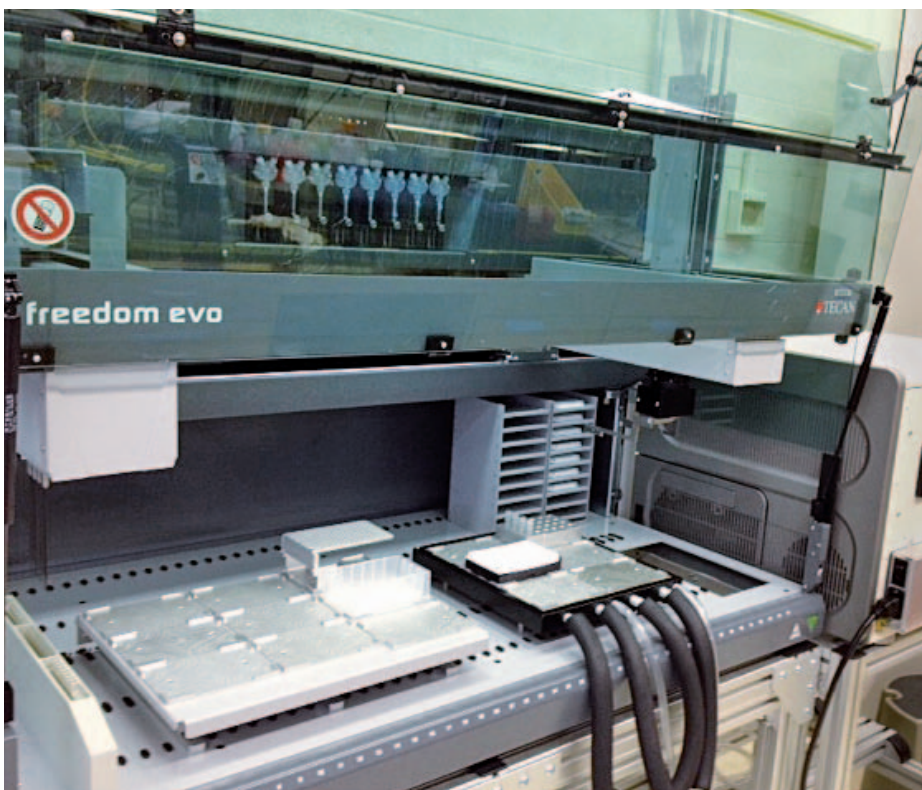
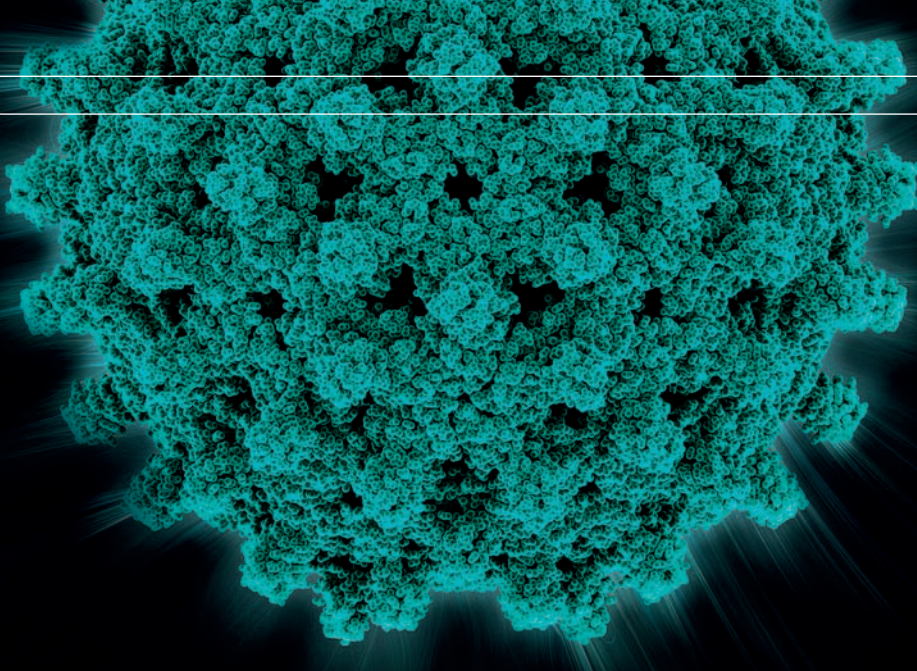


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of plates to the LC480. In contrast, we can now load 60 individual arrays – four arrays per 384-well plate – onto the Freedom EVO and leave the system running overnight. It’s basically generating data while you sleep.”

“Having the ability to multitask during working hours has also significantly improved our productivity, allowing us to perform other experiments or analyze data while the instrument is running, and having confidence in the platform’s pipetting accuracy and process security is vital to this. We have run numerous quality control tests to make sure that the results the system produces are robust, and have seen no systematic errors or issues, obtaining good standard deviations for the data.”

“The system’s accuracy and reproducibility have also allowed us to miniaturize many of our protocols, which offers significant cost savings in terms of reagents. For example, we have been able to reduce the total volume for our miRNA assays from the manufacturer-recommended 20 µl to just 6 µl. Optimizing the liquid classes has allowed us to significantly reduce the amount of very expensive miRNA primer



The Roche LC480 is linked directly to the Freedom EVO workstation

required per assay – effectively doubling the number of assays we can perform – with no loss of accuracy, and Tecan’s engineers have been instrumental in realizing these benefits. As most of our experiments use very similar methods, Tecan worked with us to create a script for one of our more complex protocols when the platform was first installed, and spent several days training us to adapt and optimize it for our other assays.”

To minimize the risk of contamination, miRNA primers are stored in Matrix SeptraSeal® tubes with pre-slit, self-sealing septum caps which can be pierced by the

LiHa Arm’s fixed tips. Pauline commented: “Although the need to wash the pipette tips thoroughly after every dispense makes the protocol longer than our methods which do not use Matrix tubes, the enhanced process security and cost savings from using lower primer volumes more than offset this, and we are still able to generate data far quicker than it can be analyzed.”

The flexibility and performance of the Freedom EVO has also led to the Dittmer Lab’s recent purchase of a second instrument equipped with a MultiChannel Arm™ 96, for use on other projects. “This platform

is intended for multiple applications, and allows us to take advantage of the benefits of automation without disrupting our ongoing work. We are also inviting members of other groups and departments to use this flexible system, encouraging new collaborations and furthering our research,” Pauline concluded.

To find out more on Tecan’s genomics solutions, visit www.tecan.com/genomics

To find out more about the Dittmer Lab, visit www.med.unc.edu/microimm/faculty/dittmerlab