Enhancing NGS sample preparation with automation

The University of North Carolina (UNC) has automated its major sample preparation protocols for next generation sequencing on a Freedom EVO® workstation, enabling more efficient processing.

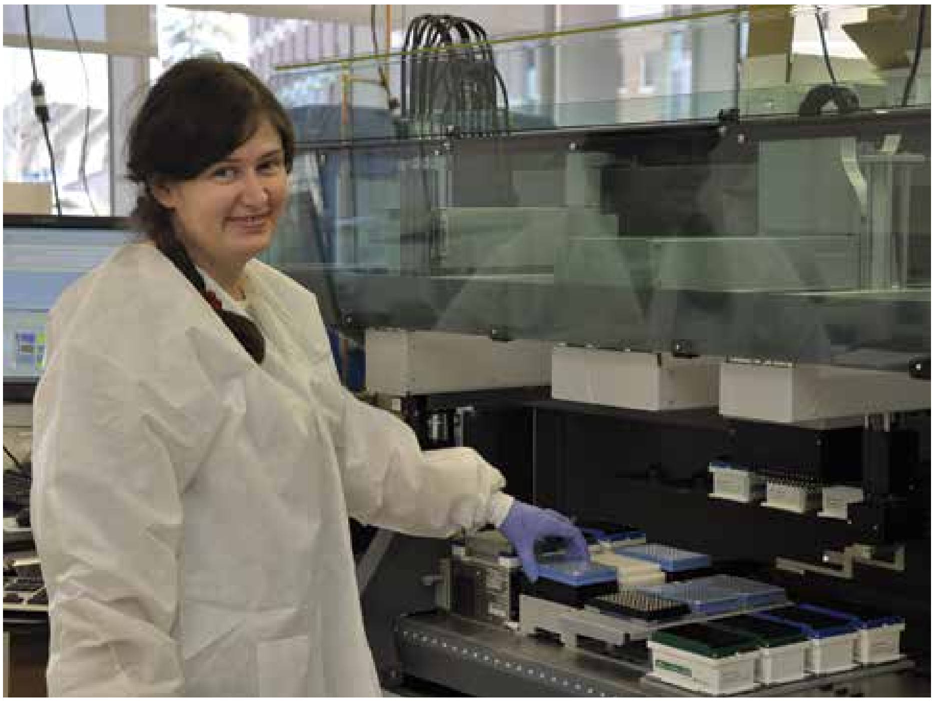
The High-Throughput Sequencing Facility
THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

The High-Throughput Sequencing Facility (HTSF), part of the Department of Genetics at the University of North Carolina, USA, is a full service sequencing facility providing state-of-the-art techniques for genetic and genomic research. The Department is involved in a number of important research projects, including The Cancer Genome Atlas (TCGA) project – a large-scale collaboration to characterize the genomic changes occurring in cancer – and UNCseq™, an internal study into the mutations triggering cancer to enable personalized treatment. UNC is also home to NCGENES (North Carolina Clinical Genomic Evaluation by NextGen Exome Sequencing), where semi-clinical studies encompassing exome capture and sequencing are undertaken.

The HTSF has turned to automation for standardization of its major sample preparation protocols, choosing a Freedom EVO platform. Piotr Mieczkowski, Research Assistant Professor and Director of the HTSF, explained: “I was recruited to the HTSF six years ago, when the UNC was investing in a next generation sequencing (NGS) laboratory. It was an exciting time for me because of my interest in different technologies; I knew that NGS was going to be one of the biggest players in bioresearch, and that it would be very important in the future. Initially, we had a single, basic genome analyzer and no automation; the focus was on learning to use the technology. Over time, more analyzers were purchased, but throughput was still not sufficient to warrant investment in automation. After a year or two, our first Illumina HiSeq® instrument was introduced, and automation became a necessity. It made perfect sense to choose Tecan; I knew from personal experience that the Freedom EVO was a flexible platform, and I was already comfortable with the programming.”

The HTSF is now developing and verifying a range of automated NGS protocols for the Freedom EVO platform in collaboration with Tecan and Illumina. The workstation is equipped with an eight-channel Air LiHa Arm, a Robotic Manipulator Arm, a temperature-controlled shaker, INHECO heat blocks with exchangeable plate adapters to accommodate 96- and 384-well microplates for thermal incubation, and a 96-position magnetic separator for bead-based assays. The joint venture began with the automation of Illumina’s Nextera® Rapid Capture kits and, with these protocols now verified, the collaboration is focusing its attention on the TruSeq™ Stranded Total RNA and TrueSeq Nano DNA kits. Piotr commented: “We have learnt a great deal from Tecan, but it is also very good for Tecan to collaborate with universities; we have a variety of different instruments and the expertise required to run them, and we know what the market trends are in terms of the chemistry that needs to be used. Working with Tecan and Illumina has been the perfect combination.”

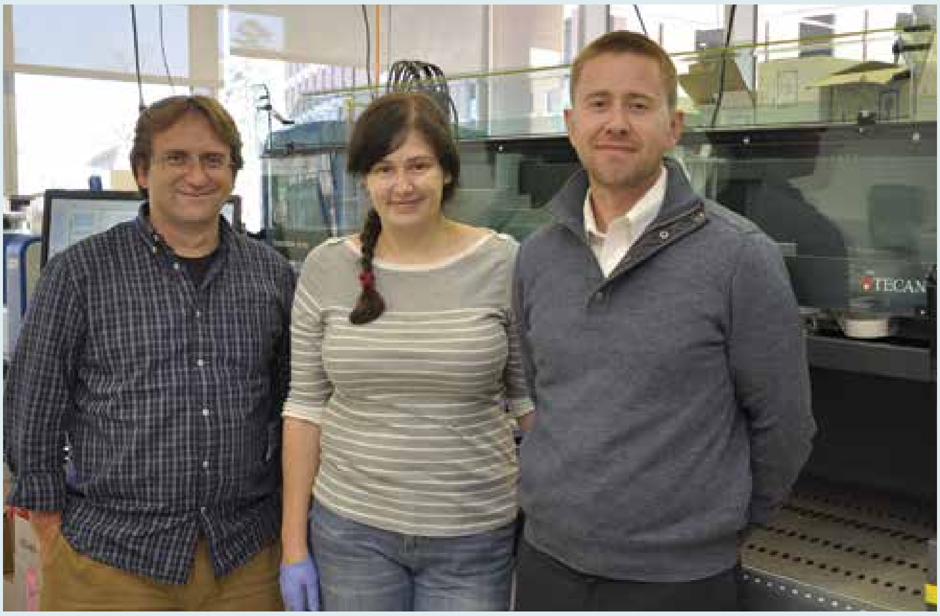
Many different types of sequencing – complete and targeted genome resequencing, small RNA sequencing, transcriptome



Ewa Malc loads a microplate onto the Freedom EVO

sequencing, ChIP-seq, bisulfite-treated DNA sequencing and metagenomic sequencing – are performed by the HTSF, and sample numbers can vary considerably, from just a few samples to perhaps 2,000 per year, depending on the project. Combining an automated liquid handling workstation with an NGS platform enables high throughput sample processing and allows more efficient data generation. The HTSF has successfully demonstrated this by automating parallel processing of up to 48 samples using Nextera Rapid Capture kits, providing an effective, robust, streamlined process for library preparation and exome enrichment. Sample throughput was increased compared to manual preparation – without any decrease in data quality – and minimal hands-on time was required, freeing staff for other tasks. But throughput is not the only issue. Reproducibility is equally important, and automation removes the likelihood of operator-to-operator variation, as Piotr commented: “For high throughput, the benefit of automation is clear. But even for low sample numbers, we prefer to use automation if possible, as it ensures every step is performed in exactly the same way every time. This is especially true in clinical laboratories, where automation needs to be oriented towards low, but reproducible, throughput.”

As sample throughput increases, so does the demand for data analysis, requiring a great deal of bioinformatics support. This may be from a single laboratory, or from a bioinformatics facility such as the UNC Center for Bioinformatics, led by Hemant Kelkar. While single labs tend to be quite specialized and oriented towards one particular application, the Center covers all applications, which is ideal for the HTSF. “This type of support is essential to our research, since we generally need a broad range of applications; as new projects get underway, we decide how the data will be analyzed and then direct the researchers to the bioinformatics group which best fits their needs,” said Piotr.



Left to right: The HTSF's Piotr Mieczkowski and Ewa Malc with Tecan's Jon Smith and the Freedom EVO system

He concluded: “Automation is becoming a critical tool, offering efficient, reproducible library preparation, and our aim is to automate all our library preparation protocols, whatever the technology platform; the Freedom EVO has proved ideal for this purpose.”

To find out more about Tecan’s NGS solutions, visit **www.tecan.com/ngs**

To learn more about the HTSF, go to **sites.google.com/site/htsfunc**

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