Sample preparation: the heart of NGS

The Albert Einstein College of Medicine is using a Freedom EVO® platform to automate sample handling and library preparation for next generation sequencing. The flexibility and throughput offered by this workstation enable straightforward processing of a wide range of genomic and epigenomic studies, providing high quality libraries for sequencing using the College's two Illumina HiSeq® 2500 analyzers.

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The Albert Einstein College of Medicine of Yeshiva University, New York, has a strong focus on research, and is dedicated to the use of innovative biochemical techniques to improve medical understanding. As part of this multidisciplinary approach, Einstein has developed a number of core genetic facilities which provide expert support and services to researchers across the entire College. David Reynolds, Director of the Genomics Core, explained: "We have had a genomics core for over 15 years, originally providing Sanger sequencing and microarray-based services, and became interested in next generation sequencing (NGS) in 2008. We quickly learned that library preparation for NGS is a very labor-intensive process, so looked at automation as a way of expanding our throughput."

"We had a good idea of what we wanted to achieve with an automated solution, and none of the existing instruments for NGS sample preparation offered us the freedom to develop our own protocols and applications; the Freedom EVO gives us the flexibility to evolve the platform as our protocols and requirements change. Dr John Greally, Director of the Center for Epigenomics at Einstein, recognized this, and was an important advocate for bringing the system into the lab."

The Genomics Core chose a Freedom EVO 200 workstation with eight-channel Liquid Handling (LiHa), Robotic Manipulator (RoMa), and Pick and Place (PnP) Arms. The system is also equipped with a PlateLoc™ Thermal Microplate Sealer (Agilent), an S2 Focused-Ultrasonicator (Covaris) and a TRobot Thermocycler (Biometra), as well as an integrated Infinite® 200 PRO multimode reader for nucleic acid quantification and a bespoke magnet for bead-based nucleic acid purification using AMPure® beads (Agencourt) in deep-well plates. This set-up – known as SARGE (Sample Automation Robot for Genomics and Epigenomics) – enables rapid automated processing of samples in a 96-well microplate format.

David continued: "Our initial goal was to automate our manual bead-based protocol using TruSeq® library kits (Illumina) for basic

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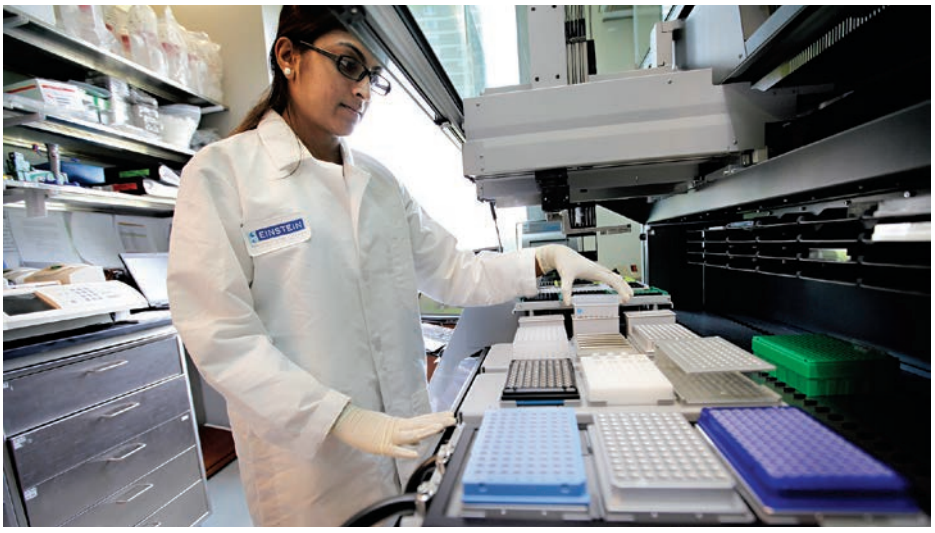
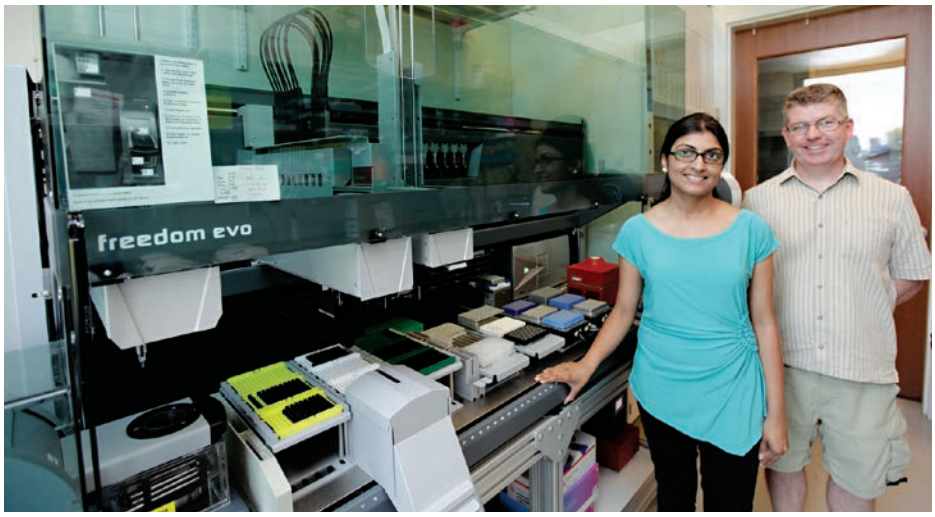


Zeineen Momin loads samples onto the Freedom EVO

DNA library preparation. Although this was less straightforward than we first expected – library kit manufacturers did not have recommendations for automation of their protocols at the time – the Tecan Integration Group (TIG) was very proactive in developing the workstation to meet our needs, modifying difficult to integrate modules and creating custom tube racks for the workdeck of the Freedom EVO."

Zeineen Momin, an R&D technician in the Genomics Core, added: "The system is very user friendly, and the software is straightforward to program. Although we did not have previous experience with Tecan instruments, we were able to start optimizing the scripts after some basic on-site training. The Helpdesk also proved very useful for immediate support if we had any issues, and we attended a Tecan training course in North Carolina to help us further optimize our protocols."

David commented: "Once we had established the initial method for generating TruSeq DNA libraries, the experience we gained made it much easier to develop new applications, and we have now developed a second workflow based on KAPA Biosystems NGS reagent kits. Creating these new scripts was extremely easy, requiring just minor changes to the liquid classes, reaction volumes and timings of the various steps to achieve excellent DNA yields. We have also moved away from whole genome sequencing – which isn't commonly performed at Einstein – to exome, amplicon, RNA and cDNA sequencing, and use KAPA kits for all of these applications. For RNA-seq we use a custom directional protocol upstream, followed by KAPA with a modified enrichment master mix, while exome-seq is performed using the Roche NimbleGen™ SeqCap™ EZ kit. This work was a collaboration with Shahina Maqbool, Director of the Epigenomics Core,



Zeineen Momin and David Reynolds with Einstein's Freedom EVO

who helps to develop protocols and ensure high quality sequencing data. We have been very happy with the results. The flexibility of our platform allows all of these applications to be set up and performed very rapidly, processing up to 48 samples per run."

"We have also been impressed by the robustness of the protocols, enabling a wide range of sample types to be used for library generation. Although we perform quality control testing on all samples before library preparation, and ask for a minimum of 1 µg of nucleic acid, we are a core facility, and researchers come to us with a wide range of starting materials. For example, we were recently supplied with yeast samples for whole genome sequencing which were supposed to be at least 1 µg. When we tested them, there was actually only 10 to 25 ng of genetic material per sample. The researcher was happy to proceed on the understanding that we might not be successful, but the library generation worked perfectly without having to change the protocol."

"We are still exploring the full capabilities of the instrument, and are constantly evolving our workflow, but the flexibility of the Freedom EVO makes it easy to adapt our scripts and update protocols. There are also several applications which are currently being performed manually that we would like to automate, and we are hoping to use TouchTools™ to enable more people to run basic scripts," said David.

Dr Greally concluded: "Massively-parallel sequencing is something of a commodity now, so we have recognized that the future lies in our ability to develop unique approaches to library production, especially in the fields of epigenomics and single cell genomics. These custom protocols, coupled with the Freedom EVO, will allow the Genomics Core to drive innovative research here at Einstein."

To find out more about Tecan's sample preparation solutions for next generation sequencing, visit **www.tecan.com/ngs**

To learn more about the Genomics Core at Einstein, go to **www.einstein.yu.edu/departments/genetics/resources/genomics-core.aspx**