Seeing is believing: harnessing the power of automation in single-cell genomics workflows

The prevalence of eye disease is rising around the world and, for most of them, there are no effective therapies available. Disorders that impair vision – such as macular degeneration or glaucoma – are a leading cause of disability and loss of an independent lifestyle in aging populations. At the other end of the spectrum, the incidence of myopia – or short-sightedness – is also on a steep incline, with up to 90 percent of teenagers being affected in some regions. Researchers in Basel are using various cutting-edge tools – including single-cell genomics – to understand the molecular mechanisms behind some of these diseases, with the aim of developing effective therapeutics.

The Institute of Molecular and Clinical Ophthalmology Basel (IOB) was established in 2018 to enable researchers and clinicians to advance our understanding of vision and its diseases, and to develop new therapies for vision loss. As part of its growth, IOB has set up a state-of-the-art single-cell genomics facility to provide guidance, support and advice to other groups at the institute wishing to use these techniques in their research. One of the aims of the department is to develop new single-cell methods to improve data quality, increase throughput and reduce costs. Simone Picelli, Platform Leader, explained: "I have been working with single-cell RNA sequencing for many years, trying to come up with new ideas or new protocols that can be automated and miniaturized to reduce the costs and make it more efficient. We have recently developed a novel workflow – FLASH-seq[1,2] – that we have also automated with Tecan's Fluent® Automation Workstation."

Single-cell RNA sequencing has transformed genomics in the last decade, with plate-based methods – such as Smart-seq2[3] – routinely being used as an alternative to water-in-oil emulsion methods. These approaches offer superior sensitivity, and the ability to provide full-length transcript information, but they typically require extensive hands-on time, leading to lower throughput and a higher cost per sample. Simone continued: "We developed FLASH-seq to address most of these issues, generating sequencing-ready libraries in a single workday while providing superior data quality.[1] We have created an automated version of the FLASH-seq workflow, where throughput is only dependent on reaction and incubation times, manual intervention is limited to the preparation of master mixes, and almost all the dispensing and clean-up steps are performed by the instruments. We have found that automating our single-cell RNA library prep has made big improvements to our throughput, data quality and reproducibility, while also reducing reagent costs and saving time."

The IOB turned to Tecan when developing the automated workflow, to ensure the liquid handling system would meet its specific needs. "I have used Tecan equipment for many years," Simone added. "I started out using a Freedom EVO®, which I really liked for its intuitive software and good amount of deck space. It was also very robust, even in my inexperienced hands at the time. When I moved to Basel, I switched to the newer Fluent Automation Workstation because, while I would have happily kept using the Freedom EVO, I could see clear advantages of moving on to the latest platform."

Almost every step of the FLASH-seq workflow has been automated – including single-cell isolation, cDNA preparation, purification, quality control and quantification, and NGS library preparation – to simplify plate handling. "We run the protocol using 384-well plates, and many of the steps are automated using the Fluent. There are several stages where liquid needs transferring or adding to every well, and this can be achieved in a single step with the 384-channel head on the Multiple Channel Arm™, so it only takes five seconds for a whole plate. This arm can pipette down to 300 nanoliter volumes for aqueous solutions, which is

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ideal for transferring DNA or primers. We also need to add index adaptors to the different cells, which we can do easily with the Fluent. There is enough space on the deck to store plates and tip boxes, which is very convenient and it allows us to process multiple plates in parallel. We also have an integrated shaker for steps which require the use of magnetic beads, as these need to be continuously agitated to stop them from settling.”

Automating single-cell RNA sequencing workflows allows researchers to minimize hands-on time, improve reproducibility and manage costs, and miniaturization can take these savings even further. Simone concluded: “I have worked with Tecan software for many years, and am comfortable developing more and more complicated scripts, as I know that the system is very reproducible and robust. This has allowed us to develop a very efficient automated FLASH-seq workflow, detecting many more genes, much more easily, compared to other published methods.”

1. Hahaut V. *et al.* Fast and highly sensitive full-length single-cell RNA sequencing using FLASH-seq. *Nat Biotechnol*, 2022, **40**, 1447-1451. doi:10.1038/s41587-022-01312-3

2. Picelli S. and Hahaut V. FLASH.seq protocol V.4. *protocols.io*, 2023. doi:10.17504/protocols.io.kxygxzkrwv8j/v4

3. Picelli S. *et al.* Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nat Methods*, 2013, **10**, 1096-1098. doi:10.1038/nmeth.2639

To find out more about Tecan’s automated NGS library preparation solutions, visit lifesciences.tecan.com/ngs-sample-preparation

For more information about the IOB single-cell genomics platform, visit www.iob.ch/research/molecular-research-center/single-cell-platform-s-picelli