Designer proteins

Researchers at the University of Montreal have benefitted from Tecan's collaboration with SciRobotics to automate DNA assembly protocols for synthetic biology. Combining a Freedom EVO® 200 with a Pickolo™ Colony-Picker and various other components, the Systems Biology and Synthetic Biology Research Unit has built a streamlined cloning workflow with increased throughput and reliability.

Synthetic biology blends molecular and systems biology with computer-based modeling and new genetic engineering methods. Part of the University of Montreal's Institute for Research in Immunology and Cancer, the Systems Biology and Synthetic Biology Research Unit – led by Professor Michael Tyers – uses systems-level interrogation of complex cellular networks to build models of cellular behavior. These models are then tested through synthetic biology approaches, allowing the team to both re-engineer natural pathways and build artificial networks that can perform novel biological functions. Almer van der Sloot, Senior Research Associate in the unit, explained: "Broadly speaking, we design and develop artificial signaling circuits and sensors, investigating how yeast and mammalian cells respond to these signals using primarily protein-based synthetic biology approaches. By combining DNA elements encoding different functionalities, we can create new molecular circuits and study their effects on cells, with a view to potential applications in biomedicine and drug discovery."

Raik Grünberg, a former Senior Research Associate in the lab, continued: "To meet these aims, we decided to implement an automated and comprehensive cloning and DNA assembly workflow on our Freedom EVO 200 workstation. Our workflow starts with manual or semi-automated design of DNA constructs and assembly primers. We then perform PCR amplification and clean-up of the template constructs, followed by Gibson

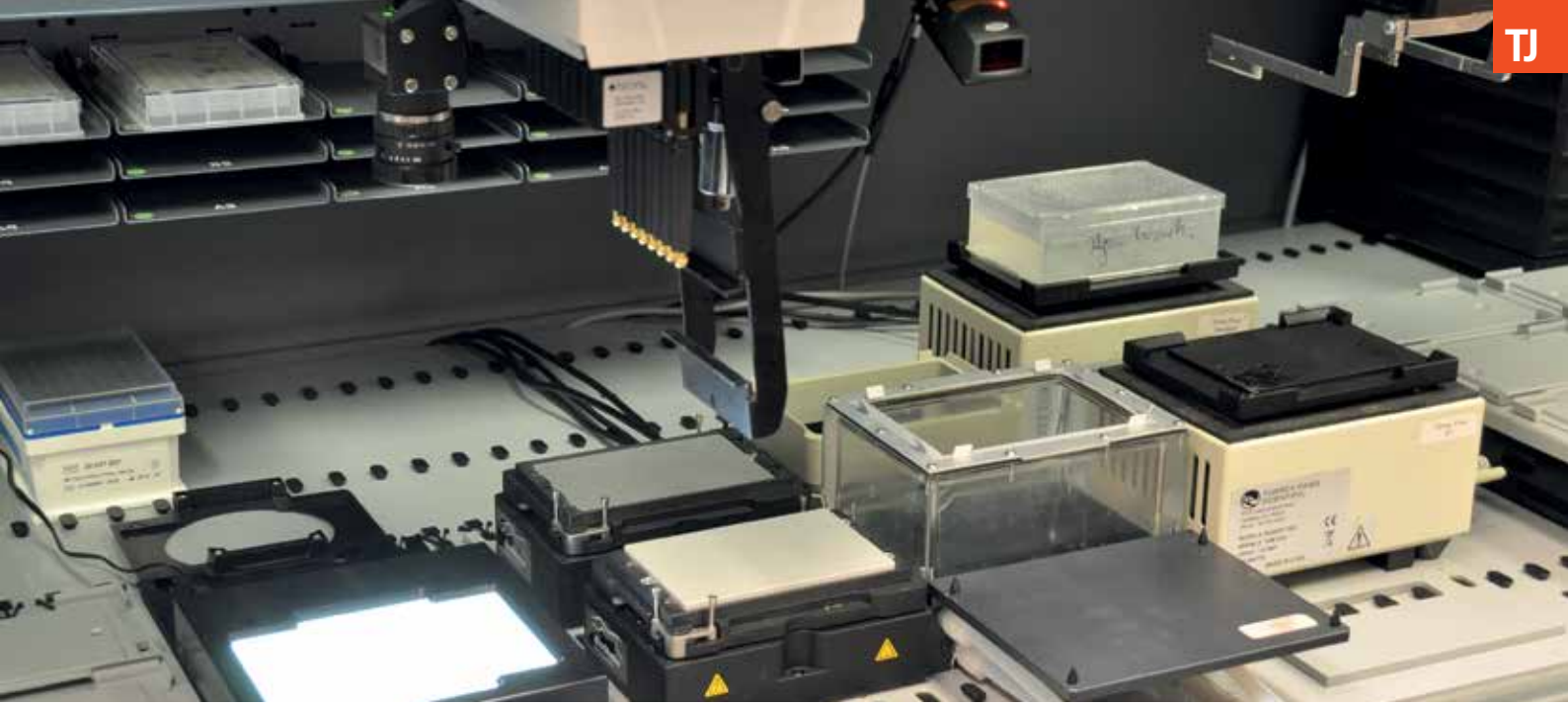
"Thanks to the scalability it offers, we can now 'think bigger' when designing our experiments."

Isothermal Assembly and transformation into *E. coli* cells. The transformed bacteria are then plated out onto agar in either 6- or 12-well plates for incubation. After the incubation, we pick and screen the colonies to verify correct assembly."

The unit's Freedom EVO workstation is configured with an eight-channel Air LiHa Arm, a RoMa Arm, two BioShake® 3000 plate shakers (Q.Instruments), a barcode reader, a Te-VacS™ vacuum separator and EchoTherm™ RIC20 Series heating/cooling units (Torrey Pines Scientific).

Importantly, the platform also has an integrated Pickolo Colony-Picker from SciRobotics. Raik continued: "Automating colony picking was critical, eliminating the need for someone to sit down and pick all those colonies by hand. The Pickolo provides a cost-effective and space-saving solution, and proved easy to integrate into our workflow. The preparation of so many agar plates turned out to be the next bottleneck, so we automated this as well. This not only speeds up plate production, it is also far more reproducible than manual procedures, ensuring that each one is reliably filled to the specified volume."

Plating out bacteria onto the agar plates is another important step which has traditionally been difficult to automate. The Freedom EVO's Air LiHa Arm has been programmed to pipette in a spiral

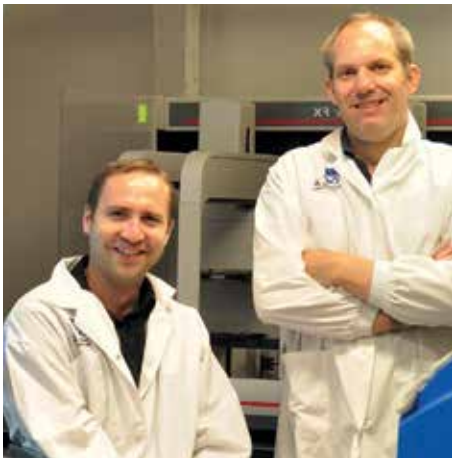


Integration of the Pickolo and other third-party devices onto the workstation proved key to automating the DNA assembly workflow

pattern across the surface of the agar in each well of the plate, spreading the bacterial cells over a large area. The team has also developed a second plate streaking strategy using SLAS-format 8-channel reservoir plates filled with agar. The *E. coli* culture is pipetted into one end of the channel by the Air LiHa, then the plate is lifted at one end by the RoMa Arm. The liquid simply flows down the channel, giving a good spread of bacterial cells on the agar surface. This is technically simpler, offering a higher throughput, but reduces the definition of individual colonies at higher cell densities compared with spiral plating. "Overall, automation has given us a dramatically higher throughput," Almer added. "We can now routinely perform 48 or even 96 DNA assembly reactions from start to finish in the time it used to take us to do about 12 manually. Importantly, automation of our cloning and DNA assembly workflow has improved accuracy and reproducibility, as the Freedom EVO workstation's advanced liquid handling capabilities eliminate pipetting or sampling mistakes."

"The Tecan team integrated all the third-party components on the Freedom EVO workstation, as well as dealing with our complicated and diverse questions during the build. Most of the workflow automation was actually performed together with Xingjian Xu, an internship student who joined the lab for one year via the UBC Vancouver co-op program, and an application expert from Tecan also visited us twice to help us learn more about the Freedom EVO's capabilities. Thanks to the scalability it offers, we can now 'think bigger' when designing our experiments.

The Freedom EVO is also used to generate consistently filled agar plates



Almer van der Sloot and Raik Grünberg

We also plan to automate protein interaction studies on this system, and have already begun integration of a ForteBio Octet® system onto the Freedom EVO for this application. Once complete, this will give us the ability to go straight from cloning to protein interaction measurements on the one platform," Raik concluded.

To learn more about Tecan's synthetic biology solutions, visit **www.tecan.com/syntheticbiology**

To read more about the Systems Biology and Synthetic Biology Research Unit, go to **www.iric.ca/en/research/principal-investigators/michael-tyers**