

Freedom EVO® in Evogene's Biolab runs 13 cloning related procedures

Automation with the Freedom EVO system and SciRobotics' Pickolo™ colony picker has revolutionized the gene cloning and colony picking workflow at Evogene's laboratory, allowing time-efficient and reliable tracking of cloning processes and enabling scientists to increase the yearly production of clones.

Evogene is a world leader in developing improved plants for the agriculture and biofuel industries through the use of plant genomics. Based in Rehovot, Israel, the Company provides a complete solution for improving plant traits, such as yield or drought tolerance, for several agricultural companies, using advanced breeding methods and its own proprietary computational genomic technology. The process of discovering and targeting new plant traits combines numerous technologies, including high throughput plant validation systems, field experiments and genomic data creation.

As part of the process, the Biolab team needs to clone and amplify several hundreds of genes of interest in a limited time span

that are then passed over to the Plant Transformation Unit for transformation into a model plant. Many of the steps involved at this stage, such as PCR amplification, DNA restriction and ligation, clone screening and sequencing, require specific clone design and a different set of restriction enzymes, as well as different sets of unique primers. All of these procedures need to be handled with the highest attention to detail; the success of every step in the process needs to be tracked and all the intermediate products need validation. Performed manually, this process is time-consuming, labor-intensive and error-prone.

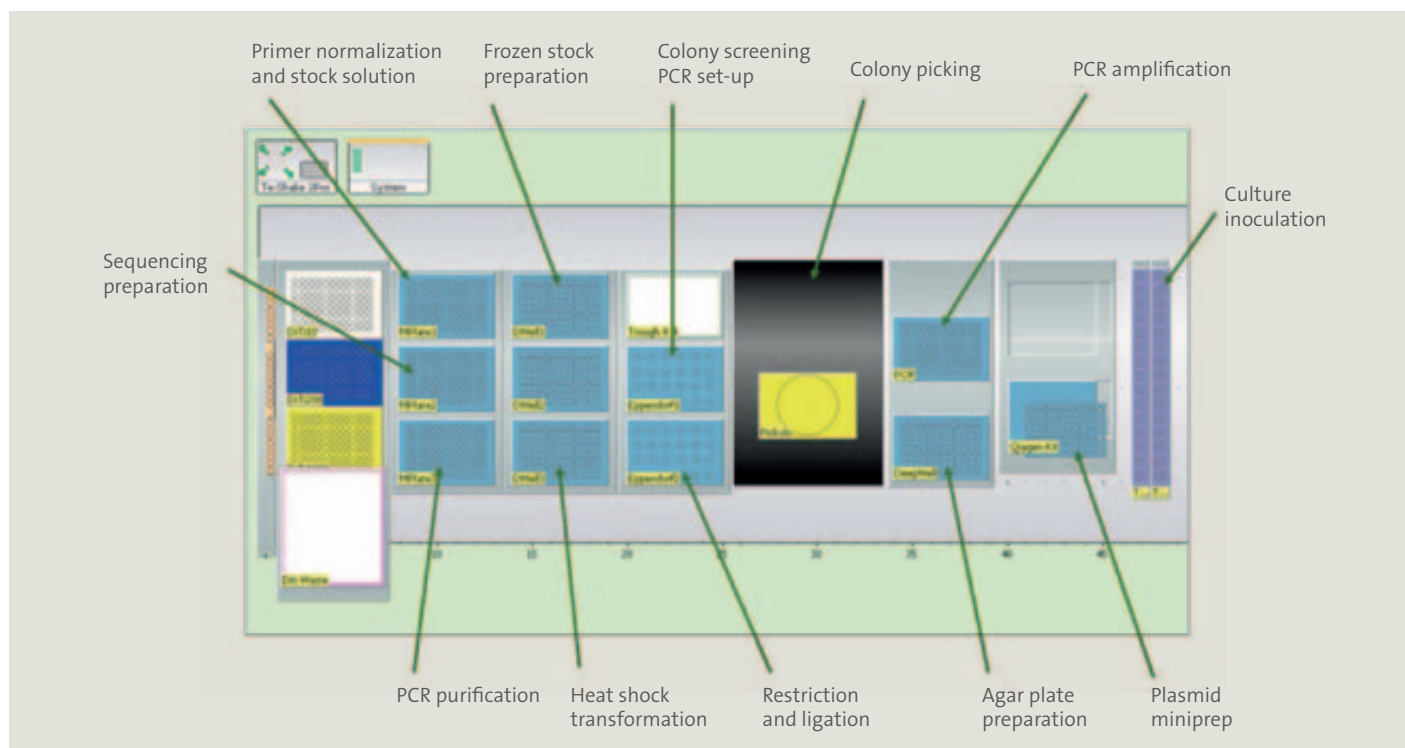
Dr Alin Sela-Brown, Head of Evogene's Biolab, explained the challenges of the manual cloning process in her lab: "The colony PCR screening days used to be a very stressful time in our laboratory. Hundreds of colonies had to be picked into hundreds of PCR reactions, each composed of a different primer set." On the same day, the colony PCR product was loaded onto a gel for electrophoresis and two positive colonies from each clone were chosen. These were then selected and inoculated into overnight cultures for plasmid purification. "To achieve this we had to 'borrow' many laboratory members performing other projects and assignments to help us with this PCR screening task and the process was very intensive."

"Due to the large amount of genes that we have to clone, we knew that a standalone picker performing only a single task would not be enough and realized that, by integrating colony picking into the liquid handling platform, Tecan offered us exactly the throughput we needed."



Dr Alin Sela-Brown (Evogene, front) and Dr Shai Kaplan (Head of R&D, SciRobotics Ltd) with the Freedom EVO workstation

The deck layout of Evogene's Freedom EVO platform



The Freedom EVO 150 system was configured with a Liquid Handling arm with eight channels, a Te-Shake™ shaker, a Te-VacS™ vacuum separation module and SciRobotics' integrated Pickolo colony picker. The system performs several tasks for the Biolab, including colony picking, primer normalization and stock dilution, PCR amplification and DNA purification, restrictions and ligations, agar plate dispensing and preparation of frozen stocks.

"Colony picking was our main process bottleneck but we also wanted to automate other main upstream and downstream applications – PCR set-up and DNA extraction – on the instrument. We were very satisfied when our Freedom EVO was able to run not just these three processes but, in fact, thirteen of them!"

"Before we had the Freedom EVO system, colony picking and PCR screening required very intense and focused days. Many people from our lab were recruited and devoted only to this task. Now, it takes just one person a few hours, leaving enough time for downstream processing and parallel activities. And the stress-free process we have with our Freedom EVO system drastically reduced the errors."

- **Primer normalization and stock dilution** – The instrument automatically normalizes the primer and dilutes for working stock according to the primers order file received with the primer plate.
- **PCR amplification** – The instrument matches the correct template with the correct primers according to the database information.
- **PCR purification** – PCR purification is performed using the Te-VacS, with purification products tracked automatically.
- **Restriction** – The amplicon is restricted with the clone-specific restriction enzymes.
- **Ligation** – The restricted amplicon of each clone is ligated to a suitable plasmid.
- **Colony screening** – A unique primer is used for each clone to allow screening.
- **Colony picking** – The Pickolo automatically identifies positive colonies.

- **Culture inoculation** – Positive colonies are inoculated to starter cultures and solid agar stock replicates and incubated overnight.
- **Freeze-stock preparation** – Glycerol freeze-stocks of positive colonies are prepared.
- **Plasmid miniprep** – The instrument performs plasmid purification using the Te-VacS with a specific kit.
- **Sequencing preparation** – The instrument prepares several sequencing reactions for each clone with its own sequencing primers and automatically prepares documentation of the sequencing plate.
- **Heat shock transformation** – The instrument performs the necessary pipetting steps of the heat shock procedure.
- **Agar plate preparation** – The instrument dispenses the agar media from a stirrer into 96-well plates.

The ability to handle any subset of clones and perform time-consuming procedures on the Freedom EVO system has provided Evogene's Biolab with the flexibility and speed it needed to respond to its growing requirements. The integration of the Freedom EVO workstation and SciRobotics' Pickolo colony picker saves time both at the bench and in experimental planning, creating an efficient and reliable cloning workflow.

To find out more on Tecan's Freedom EVO liquid handling platforms, visit www.tecan.com/freedomevo

To learn more about SciRobotics, go to www.scirobotics.com

To learn more about Evogene, go to www.evogene.com

Pickolo is a trademark of SciRobotics.