Fully integrated plating and colony picking for synthetic biology workflows

Automation of the complete workflow from cloning to PCR confirmation

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Introduction

The Institute for Research in Immunology and Cancer (IRIC) at the University of Montreal pursues a unique approach to investigate complex biological processes. Combining genomics, proteomics and bioinformatics with systems and synthetic biology, IRIC aims to identify new approaches in biomedicine and drug discovery. The Systems Biology and Synthetic Biology Research Unit – led by Professor Michael Tyers – investigates the mechanisms of cell growth and cell division, aiming to unravel interactions at both the genetic and proteomic levels. This information can then be used to re-engineer natural networks and build entirely artificial networks that can perform novel biological functions.
Molecular cloning plays an essential role in the group's research, allowing them to develop models of complex biological processes. As throughput demands increased, automation was seen as an opportunity to streamline this process considerably, while eliminating unnecessary errors and repetitions. The laboratory team established a reliable, fully automated cloning workflow on a Freedom EVO® workstation. This system performs fully automated multi-fragment DNA assembly, customized plating in ANSI/SLAS-format plates, colony picking and PCR set-up – as well as agar plate preparation – allowing the group to greatly increase the throughput of their experiments.

The ability to integrate colony picking using a Pickolo™ Colony-Picker (SciRobotics) with upstream and downstream tasks (see Figure 1) offers the laboratory a flexible solution which has significantly contributed to the success of their projects.).

Figure 1: Automated cloning workflow on the Tecan Freedom EVO workstation.

Materials and methods

Automation equipment
IRIC's Freedom EVO 200 workstation is equipped with an eight-channel Liquid Handling (LiHa) Arm and a Robotic Manipulator (RoMa) Arm. Two chilling/heating devices – EchoTherm™ RIC20XR and RIC20XT (Torrey Pines Scientific) – are used to pre-warm the agar for plating and water for wash steps respectively. The integrated Pickolo Colony-Picker includes a high resolution camera mounted on the LiHa Arm, a backlight carrier and the Pickolo software. The platform also has a Te-VacS™ vacuum station for DNA extraction and clean-up, two orbital shakers (BioShake® 3000, Q.Instruments) and a temperature-controlled incubator (MIO™), as well as microplate carriers and shelves for additional on-deck storage. A barcode reader mounted at the back of the workdeck allows full tracking of barcoded plates.

Figure 2: Freedom EVO 200 worktable overview.

Plate preparation and plating procedure
To ensure that the complete cloning workflow can be performed using ANSI/SLAS-format plates, two different methods were developed, allowing plating into either standard 6-well and 12-well culture plates (BD Falcon™, flat bottom with lids) or 8-row polypropylene (PPE) reservoir plates (Seahorse Bioscience Labware, Figure 3).

Figure 3: 8-row PPE reservoir plates were used for an alternative plating strategy.

For both plate types, the pre-boiled agar is kept at approximately 70 °C (with the heater set to 100 °C) in a single-well reservoir. The agar is then transferred to each well or lane using multiple aspirate-dispense steps with either three, four or eight tips in parallel, resulting in agar plates with highly reproducible fill volumes.

Plating into 8-row PPE reservoir plates
100 µl of bacterial suspension is pipetted into one end of each lane. The plate is then lifted at the same end using the RoMa Arm, allowing the bacterial suspension to spread along the agar surface.

Plating into 6- and 12-well plates
Implementation of a customized Freedom EVOware® LiHa firmware command allows a spiral-like motion of the tip during pipetting, as shown in Figure 4. While dispensing, the tips of the LiHa Arm move alternately in x- and y-directions, creating a square, spiral plating pattern and ensuring that the bacterial suspension is evenly spread across each well.

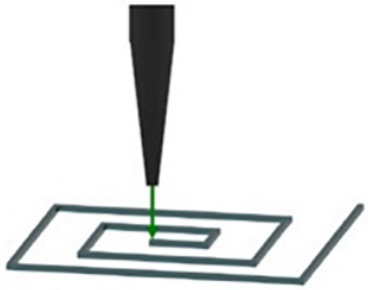

Figure 4: Graphic representation of the spiral tip movement for plating on 6- or 12-well ANSI/SLAS-format plates.

Colony picking
After plating, the culture plates were incubated externally at 37 °C for 8-16 h prior to colony picking using a customized picking profile; the agar surface was detected by conductivity, followed by a small, 0.6 mm back and forth 'scratching' movement of the pipette tip to ensure reliable transfer of sufficient bacterial material for inoculation.

Four to eight colonies were picked per well or lane, transferred to a 96-well PCR plate for PCR confirmation, and copied to a deep-well plate for storage and further analysis. After PCR, positive clones were evaluated offline via agarose gel electrophoresis.

Results and discussion

Successful preparation of agar plates
Implemetation of an automated agar plate preparation procedure results in culture plates with precisely controlled fill volume and fewer air bubbles, helping to ensure high picking accuracy for the Pickolo.

Automated plating
Two distinct methods have been developed for plating out of transformed bacteria into ANSI/SLAS-format culture plates, representing a practical alternative to the use of Petri dishes for complete automation of cloning workflows.

The plating procedure described for 8-row reservoir plates is faster, offering higher throughput for many of the laboratory's applications. In contrast, plating into 12- or, in particular, 6-well plates in a square spiral pattern offers more robust single-colony picking from cultures with very high cell densities.

Reliable picking
The flexibility of the software allowed the creation of a customized picking profile, mirroring the manual picking movement with a small sideways scratch. This ensures that enough material is transferred to both the PCR plate and the deep-well storage plate. Using this automated procedure, colonies are reliably collected, and samples can be tracked throughout the whole workflow.

Conclusion

The flexibility of the Freedom EVO platform has allowed the group to close two gaps in their automated cloning workflow – agar plate preparation and plating of bacteria onto ANSI/SLAS-format culture plates – creating a powerful and reliable, fully automated cloning solution for synthetic biology research.

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