

APPLICATION NOTE

Automation of the CRISPR- based cell line development workflow

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Introduction

An increase in demands for faster high-throughput cell-line development and better screening in the pharmaceutical space is driving research facilities and companies to automate existing labs. Though robotics has been in some laboratories for about four decades, it is more common now and aids in freeing researchers of repetitive tasks by increasing walk-away time. Simple to complex assays could be automated to reduce the workload of unskilled steps like sample handling, centrifuging, moving to racks, and liquid handling. With emerging new trends in robotics, we have better, smaller, and easy-to-program robots which could easily be incorporated into an integrated workcell solution.^{1,2}

Typical cell-line development requires the screening of tens of thousands of clones to find the best stable cells that yield high amounts of bioproducts. Here we discuss the automation of a mammalian cell-line development workflow—the automated, integrated screening of transfected cells for edits, monoclonality, and growth assessment (Figure 1). This system includes multiple instruments and a collaborative robot to perform all steps, from a single cell dispense to screening monoclonal cells and expanding them for further validation assays. This

Benefits

- Quick scale-up acceleration
- Increase in walk-away time
- Consistent processing
- Reduce redundant tasks
- Better cellular monitoring
- Dynamic workflow solutions

integrated system not only increases the throughput from low to high but also eliminates human error by boosting consistency. The cells are maintained in the best possible conditions and monitored over the weekends too, which decreases the production timeframe drastically and enables faster scalability. This automated workcell solution includes all the instruments in Figure 1. In addition, other instruments can be easily added based on the requirements or complexity of the assays.^{3,6}

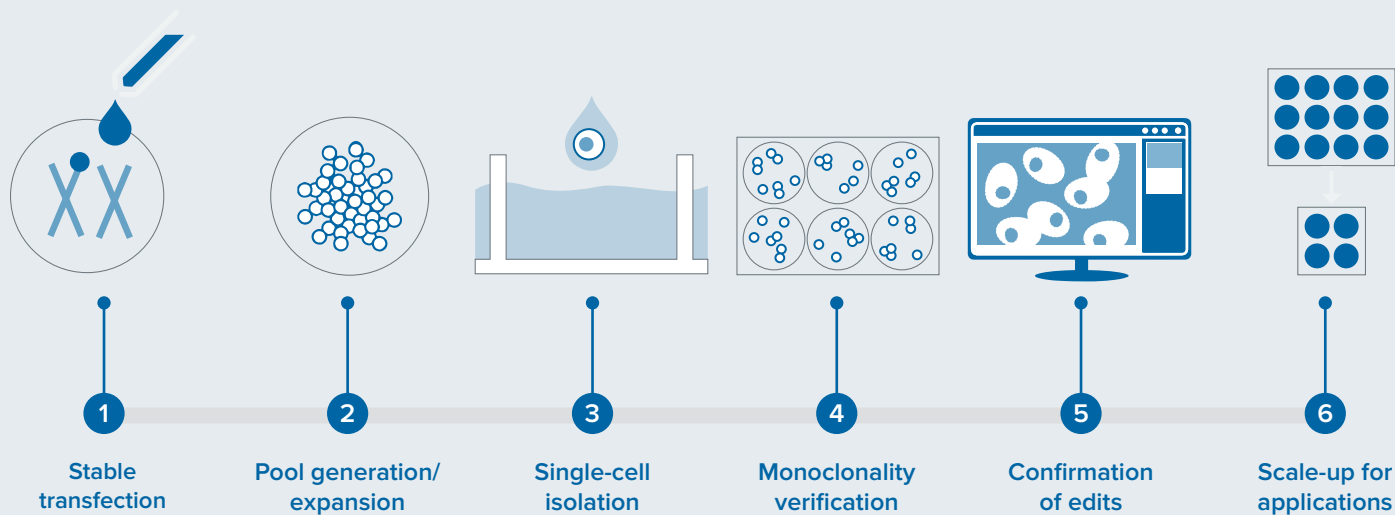
Case Study

Automated screening of CRISPR-edited cell lines for monoclonality

The integrated system included a single-cell printer, Clone Select Imager – Florescence (CSI-FL), a liquid handler, an automated incubator, hotels, barcode readers, and a collaborative robot. Using the system, the transfected cells were printed into 96 and 384 well plates, imaged for Day 0 monoclonality assessment, and monitored regularly for growth. The complex workflow using these tools increased throughput and automation of gene editing assays, screened the cells for monoclonality, and ran the endpoint assays. This solution can not only be applied to cells but also to develop CRISPR-edited organoids for various applications (Figure 1).

The instrument operations were scheduled in advance using automated laboratory scheduling software (GBG, Figure 2) to run steps of the workflow. CRISPR editing of the cells and maintenance were carried out using a liquid handler and incubator. Later these cells were single-cell

dispensed into multi-well plates for monoclonal cell-line development. These multiwell plates were imaged on Day 0 followed by imaging until Day 14 using CSI-FL (Figure 3 & Figure 4) to generate a day 0 monoclonality assurance report (Figure 1) and monitor the growth of every cell. The cells were electronically tracked and monitoring parameters were stored in plate data: cell confluence, cell number estimation, and growth curve. The instrument assesses cell growth objectively and quantitatively with compatibility for adherent or settled suspension cell types which includes diverse cell types such as CHO, HEK, hybridomas, iPSCs, and many others. Here we used CRISPR plasmids for p53 KO (Santa Cruz Bio) to develop an adherent monoclonal HEK-293 cell line through transfection. This generated cell line was further subjected to endpoint assays for edit verification [refer to app notes cited: 7–9].



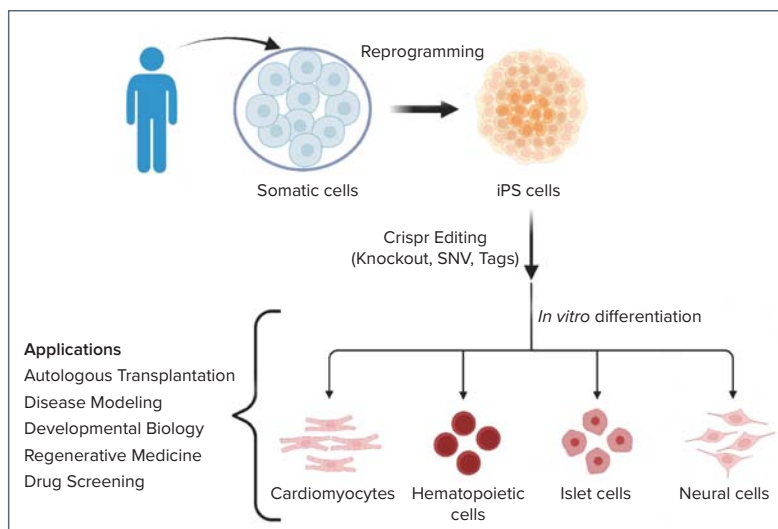
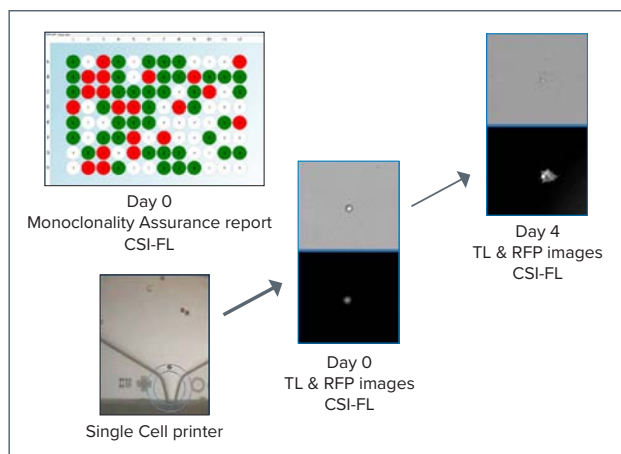
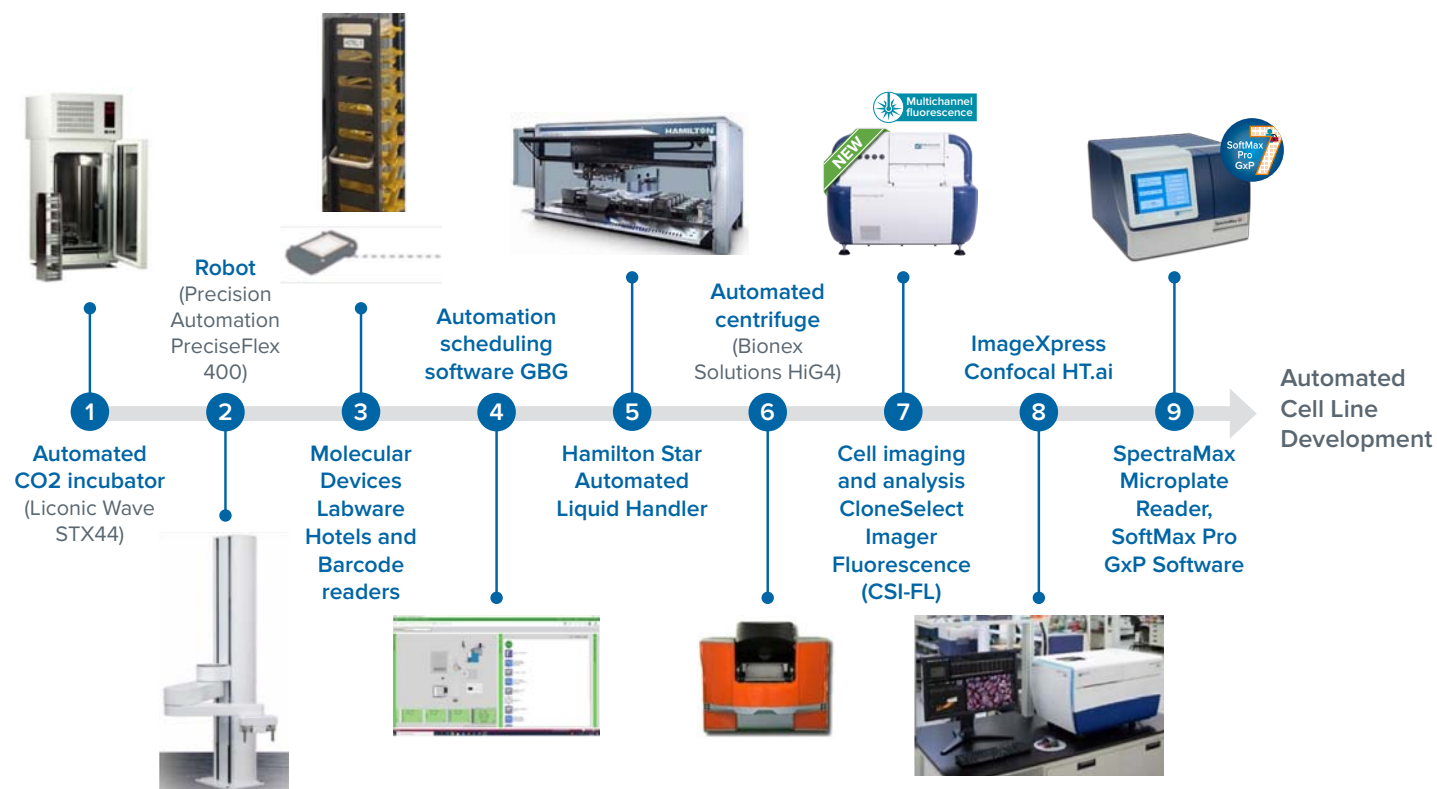


Figure 1. Workflow steps involved in a cell line development and integrated automated system for a CRISPR-edited cells/organoid screening—disease modeling. Includes various instruments and automation of different steps: the screening, and monitoring for cellular monoclonality being the most critical (performed by CSI-FL) and potential applications.



Figure 2. This virtual platform shows CSI-FL, automated incubator, liquid handler, hotel, and barcode readers. Integration of these instruments is done on a fully virtual environment (GBG) across a workstation to run an automated workflow. The devices were monitored in real-time and added or dropped off of the system based on the workflow needs.

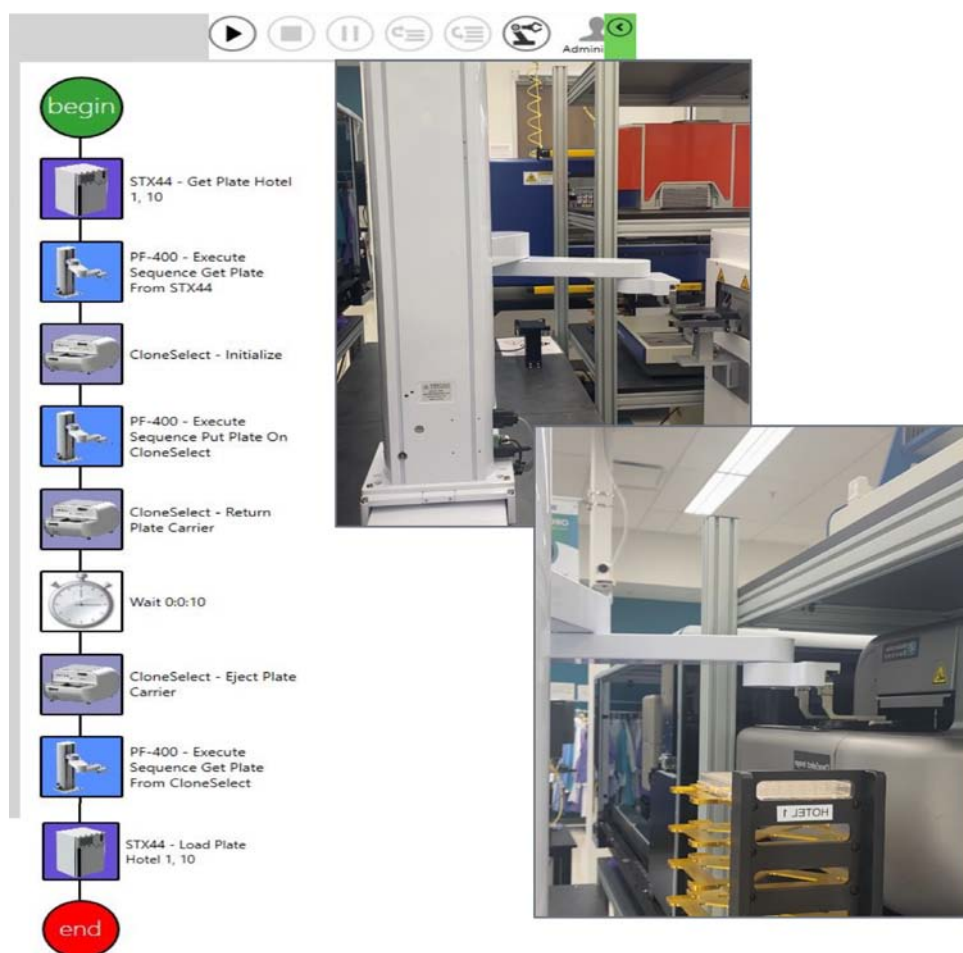


Figure 3. This sequence of steps was designed on automation software (GBG, left) to image a single cell printed 96-well plate. The path is timed/scheduled and allows imaging – incubator → imager → incubator. The top right image shows the collaborative robot picking up the 96-well plate, and the bottom right image shows the robot placing the plate in CSI-FL.

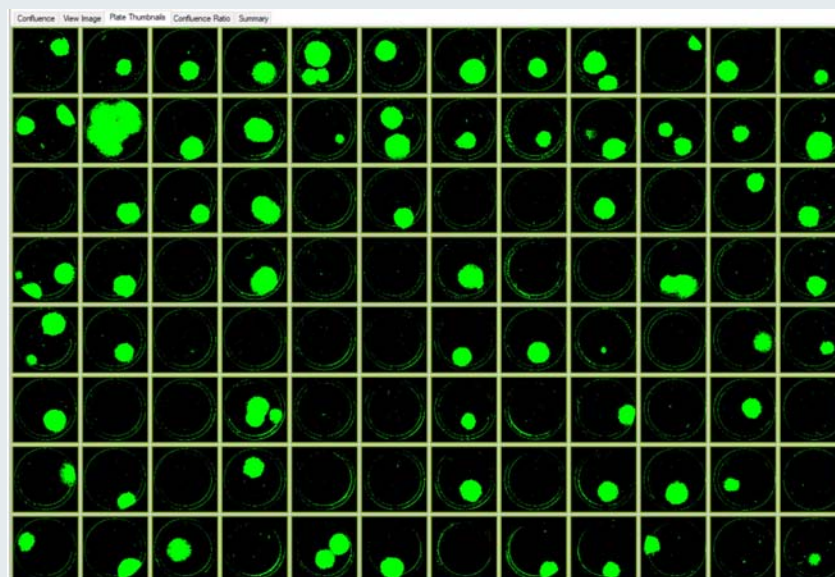


Figure 4. Images of colonies of single-cell printed CRISPR edited cells with RFP marker. This plate layout image of a 96–well Costar 3300 plate with cells on Day 9 was acquired using CSI-FL at 4X.

Summary

With automated instruments and CSI-FL, the edited cells could be imaged and tracked for monoclonality with great ease and weeks of walk-away time. The throughput, reliability, and chances of contamination were drastically changed. High-quality CRISPR-edited cell lines were obtained and used for end-point assays. In response to the ever-increasing demand to shorten timelines for cell-line development and automated solutions, we offer an automated integrated systems workflow with CSI-FL.

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