Automated workflow for screening of CRISPR-edited cell lines. High throughput screening and analysis of monoclonality.

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Summary

Gene editing allows us to manipulate DNA through deletions, additions, or other modifications using several tools. There are numerous efficient strategies to carry out these modifications with a wide range of applications in basic research, disease-fighting, and bioprocess development/bioproduct manufacturing. CRISPR (clustered regularly interspaced short palindromic repeats) associated protein 9 (Cas9) has revolutionized targeted gene editing and has application subsets of disease model development, drug development, target gene/genes functional analyses, creating transgenic organisms, chimeric protein production, and more. Gene editing and screening for the edited cells is a long and labor-intensive process; high throughput screening and/or automated processing of these edited cells would reduce the time and effort involved.

We describe a feasible automation of integrated screening of transfected cells for edits, monoclonality, and growth assessment. The integrated system included a single-cell printer, Clone Select® Imager FL, an automated incubator, a liquid handler, 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. We further discuss the automation assisted expanded CRISPR-edited cancer model cells using immunocytochemistry, ELISA, and apoptosis assay. We probe the tools for increasing throughput and automation of gene editing assays and screening the cells, as well as the development of endpoint assays using high content imaging and analysis.

In our study, we used CRISPR technology to knock down the p53 protein in Human Embryonic Kidney cells (HEK-293). We have demonstrated that this engineered cell line had greater resistance to the apoptosis-inducing compound staurosporine, in comparison to control cells, by measuring apoptosis with Annexin V assay. The workflow could be suitable for generating multiple engineered cell lines that carry a specific mutation and can facilitate the development of cell-based disease models.

Methods

HEK-293 cells from ATCC were maintained as per instructions before they underwent TP53 knockout using the Santa Cruz p53 CRISPR/Cas9 KO plasmid and p53 Homology-directed repair (HDR) plasmid following the manufacturer's instructions (Santa Cruz Biotechnology) with the TransIT-X2 delivery system (Mirus Bio) and 24h post-transfection cells were selected with puromycin. The puromycin concentration was ~1.5µg/mL for the first 72 hours and then gradually tapered to ~0.5µg/mL or regular maintenance until stable line generation.

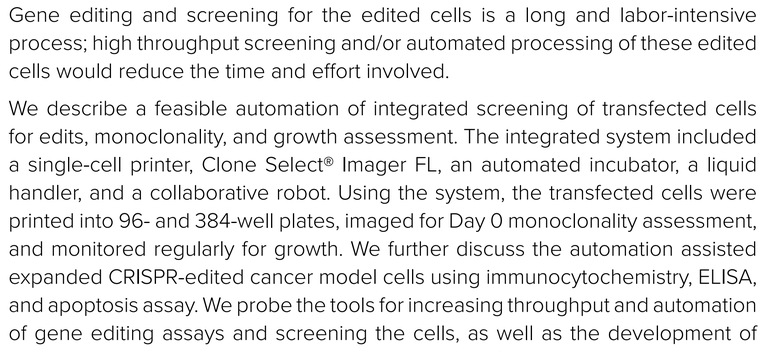

Figure 1. Cell line development workflow using CSI-FL.

This pool at 1 million cells/mL in serum-free media was used to single-cell print 96/384 well plates using Cytena F.Sight™. These plates were then monitored on day 0, followed by days 1, 4, 6, 7, and 11 to monitor growth using white light and an RFP filter of CSI-FL (4X objective). These cells were further imaged using our ImageXpress® Confocal HT.ai High-Content Imaging System to get a closer look at the cells (10X objective). Once grown enough were expanded to a 12 well plate ➔ 6 well plate ➔ 25 cm^2 flask ➔ 75 cm^2 and frozen for further experiments.

Annexin V: A 96-well plate of HEK-293 cells was exposed to staurosporine (5 µM) in 1:2 serial dilutions to induce apoptosis after 48 hours of seeding. 4 hours of Apoptosis induction later followed detection with Annexin V-FITC Apoptosis kit (ab14085) according to the manufacturer's instructions. The images were acquired using the 10X objective of the ImageXpress Confocal HT.ai and the results were analyzed using MetaXpress™ High-Content Image Acquisition and Analysis Software and SoftMax® Pro Software.

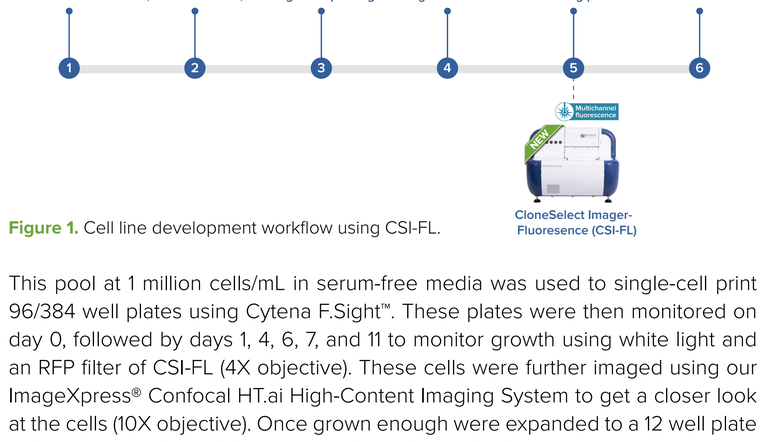

Figure 2. Cell line development and integrated automated system for CRISPR-edited cells Include various instruments and automation of different steps: the screening and monitoring for cellular monoclonality being the most critical (performed by CloneSelect™ Imager - CSI FL).

Results

Stable transfection and pool generation

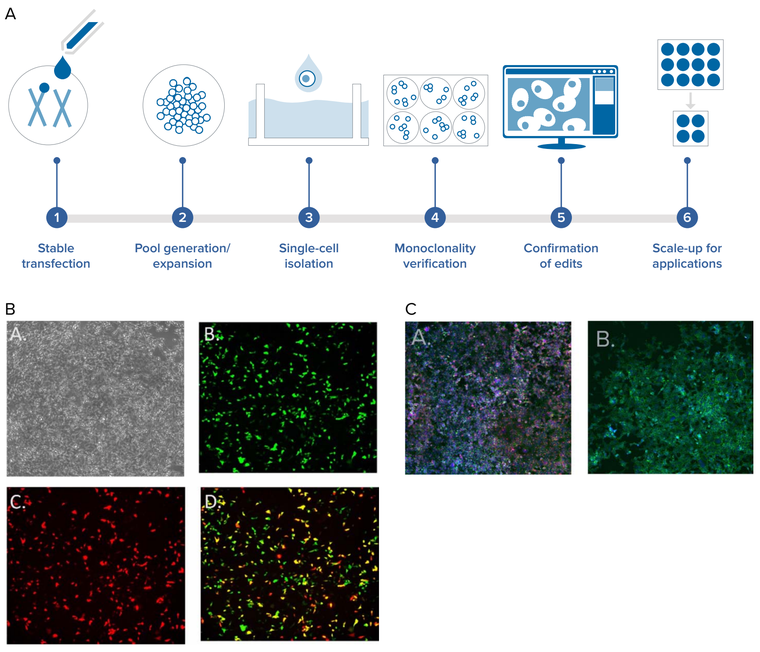

Figure 3. A. Schematic of Cell line development workflow. B. (left panel) Cell confluence and cell number estimation for each cell displayed during puromycin selection of transfected HEK-293 cells and control cells. The cell distribution is estimated by the software for TL, GFP, and RFP imaging (top number panel). ImageXpress Confocal HT.ai images of cells after transfection before puromycin screening (bottom image panel). A. TL image of transfected HEK-293 (all cells), B. FITC image (GFP expressing cells), C. Texas Red image (RFP expressing cells), and D. Overlay of B and C. C. Composite images of p53 KO HEK-293 cells (B.), has reduced p53 expression and control cells (A.) have higher p53 expression when labeled with p53 Antibody (DO-1) conjugated to Alexa Flour 647 (sc-126 AF647, Santa Cruz Biotechnology), Phalloidin 488 (1:400), Hoechst 3342 (1:1000).

Single-cell isolation and monoclonality screening

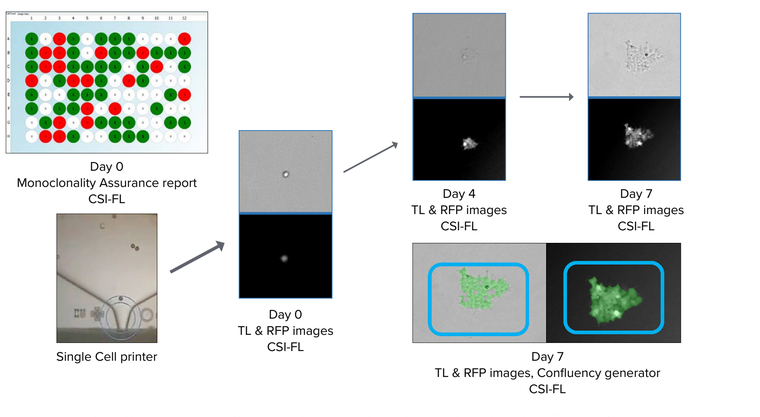

Figure 4. Day 0 monoclonality report generated (top left) using CSI-FL multi-channel imaging and Fusion software (Green – one cell detected, Grey – no cells detected, Red - more than one cell detected). Single-cell printing (SCP) and cell growth (Day 0 ➔ Day 7) representation. SCP and monitoring using CSI-FL and confluence (boxed bottom right) were generated to count the number of cells using Fusion software. Both white light and RFP images were considered.

Confirmation of monoclonality and endpoint assays for scale up

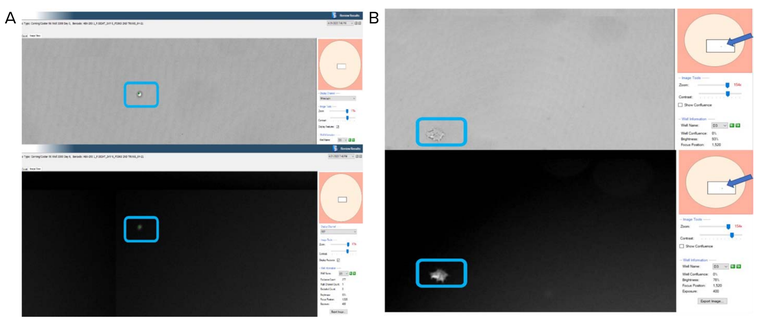

Figure 5. A. CSI-FL Fusion software white light (top) and RFP (bottom) filter image view of well D3 from plate 1 on Day 0 in figure 3. Monoclonality assurance plate report. The green dot on the cell is Fusion software confluence generation representation. B. CSI-FL Fusion software white light (top) and RFP (bottom) filter image view of well D3 from plate 1 in figure 3. Monoclonality assurance plate report, on day 6. On the right of both the images (arrowed), the well layout shows the zoomed image location (boxed) and cells in the well (green spot).

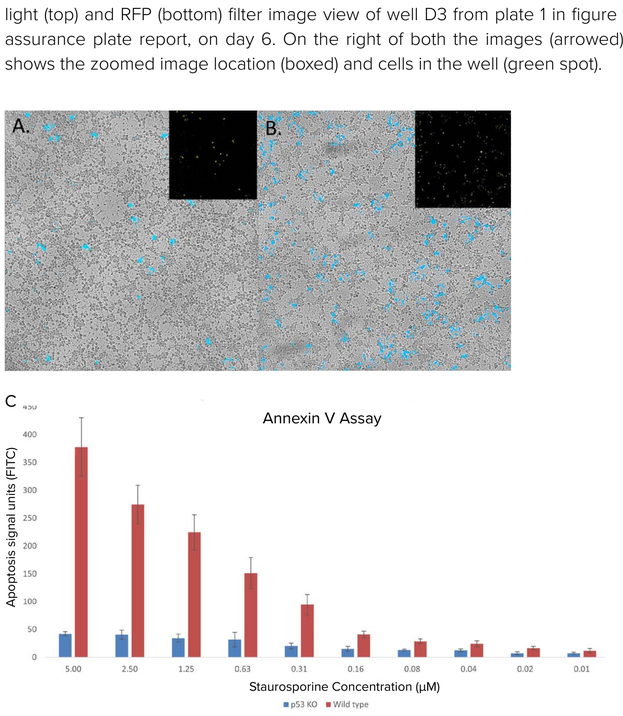

Figure 6. Cell apoptosis detected by Annexin V-FITC (Top image panel), overlay of white light, and FITC Images. A. p-53 KO cells treated with staurosporine (2.5 µM), B. Control cells treated with staurosporine (2.5 µM). Graphical representation of data obtained using ImageXpress Confocal HT.ai and analyzed by MetaXpress software (bottom) and C. the graph represents the FITC signal implying apoptotic activity in the treated cells against the concentration of staurosporine used to treat. The knockout has decreased the average apoptotic activity of the cell pool substantially on induction of apoptosis.

Results

Automation of monoclonality screening

The instrument operations could be scheduled in advance using automated laboratory scheduling software to run the steps of the workflow. CRISPR editing of the cells and maintenance could be carried out using a liquid handler and incubator. Later these cells could be dispensed into multi-well plates for monoclonal cell-line development. These multiwell plates can be imaged on Day 0 followed by imaging until Day 14 using CSI-FL (Figure 8) to generate a day 0 monoclonality assurance report (Figure 3) and monitor the growth of every cell. The cells then can be electronically tracked and monitored for parameters to be stored as plate data: cell confluence, cell number estimation, and growth curve.

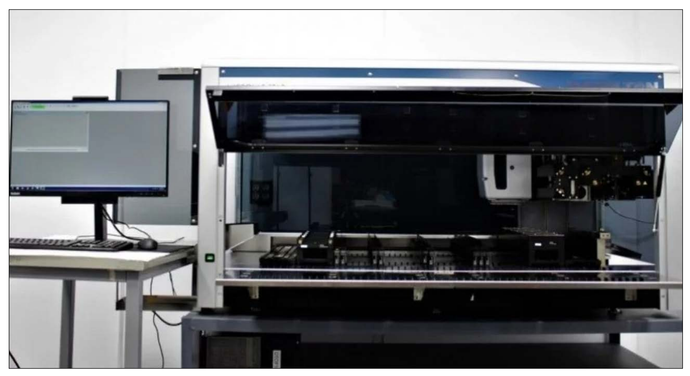

Figure 7. This platform will include a CSI-FL, an automated incubator, a liquid handler, a hotel, and barcode readers. Integration of these instruments could be done in a fully virtual environment across a workstation to run an automated workflow. The devices could be monitored in real-time and added or dropped off the system based on the workflow needs. The image on left shows a Hamilton star liquid handler.

Major components of the automated workflow

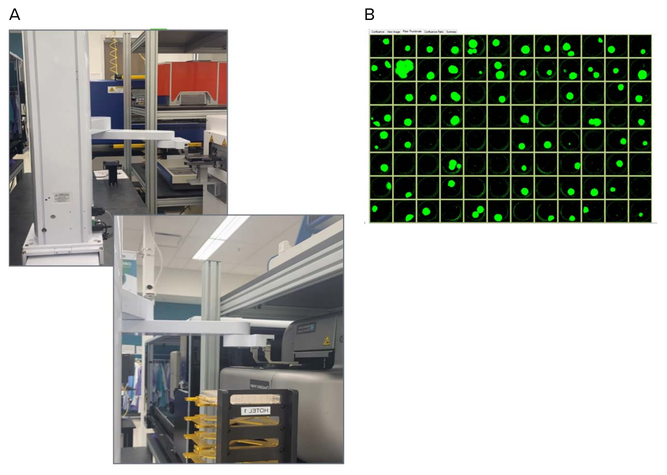

Figure 8. A. This sequence of steps could be designed on automation software to image a single-cell printed 96-well plate. The path is timed/scheduled and allows imaging – incubator ➔ imager ➔ incubator. The top left image shows the collaborative robot picking up the 96-well plate, and the bottom right image shows the robot placing the plate in CSI-FL. B. 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.

Conclusions and future directions

- The experimental study established an effective and rapid process for developing a p53 knockout HEK-293 cell pool using CRISPR-based gene editing and antibiotic selection. This was effectively carried out using our new CSI-FL. The cells were further studied using various imaging assays to assess the knockout of p53. The knockout has decreased the average apoptotic activity of the cell pool substantially on induction of apoptosis.
- CSI-FL usage established an effective and rapid process for monoclonal cell-line development. Screening of SCP printed cells and assessment for monoclonality were carried out efficiently using the CSI-FL imaging system and its analysis software, Fusion.
- 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 could be drastically changed. High-quality CRISPR-edited cell lines could be obtained and used for end-point assays.

The future direction is to navigate our way through CRISPR-edited 3D model workflows using automation.

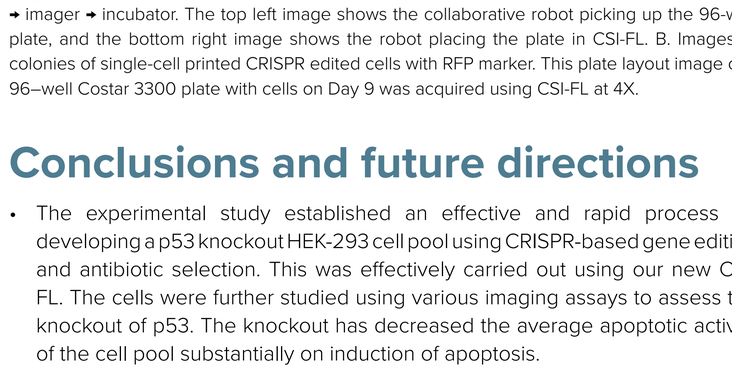

Figure 9. Illustration of CRISPR editing in patient-derived iPSCs to generate cell lines for 3D Organoids to model diseases.

MOLECULAR DEVICES

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