Unlocking Innovation from a Single Cell:

Automated Single Cell Cloning for a Golden Age of Cell Line Development

Introduction

Stable cell line development remains a foundational requirement across biologics manufacturing, disease-relevant model generation, and rapidly growing areas such as CRISPR-mediated cell engineering and iPSC–based discovery. As new genome editing methods, patient-derived systems, and high-throughput screening technologies expand, pressure continues to grow on laboratories to scale single-cell cloning capacity while maintaining cell health, accuracy, and reproducibility.

Traditional approaches to isolating monoclonal populations—particularly when working with delicate primary cells, engineered cells, or pluripotent stem cells—are slow, labor-intensive, and often rely on manual Limiting Dilution (LD) or cumbersome FACS sorting. These constraints extend development timelines and reduce the number of high-quality clones available for downstream analysis.

To address these challenges, Bio-Techne developed the Pala™ Single Cell Dispenser, a microfluidic-based system integrating flow cytometry based light scatter and fluorescence detection with gentle, low pressure (<2 PSI / 13.79 kPa) cell sorting in a compact, simple to use, bio-safety-cabinet-compatible instrument. Pala enables efficient generation of hundreds of single-cell–derived clones, supporting a broad set of applications ranging from CHO-based biologics development and stable recombinant protein production to CRISPR-edited iPSC and HEK293 **cell line engineering**, and generalizable single-cell workflows in drug discovery.

Pala's microfluidic cartridges—which maintain sterility and support rapid sample switching—require no calibration between runs. The combination of flow-cytometry-like optical detection and controlled microfluidic actuation provides FACS-style gating without the cell stress typically associated with high-pressure droplet sorting.

This application note demonstrates Pala's ability to:

- Achieve high single-cell accuracy (1 cell per well deposition).
- Support robust colony outgrowth with efficiencies comparable to or better than limiting dilution.
- Enable consistent performance across diverse cell types (CHO, iPSC, HEK293, A549).
- Maintain cell viability through gentle microfluidic handling.
- Support fluorescence-based cell selection and dispensing.

FIGURE 1.

Single Cell Deposition Accuracy: Pala vs. LD (Day One)

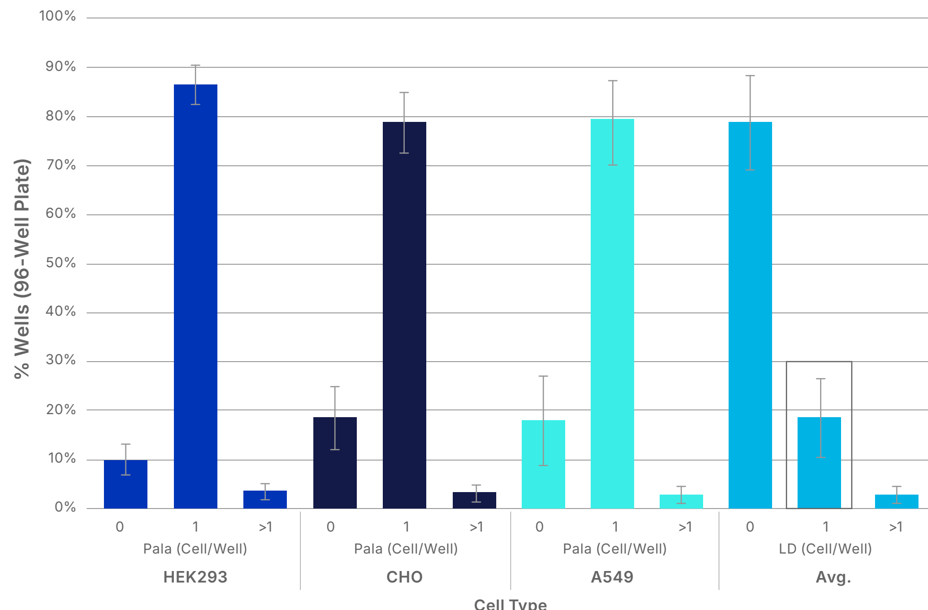

FIGURE 1. Pala single cell dispensing accuracy compared to Limiting Dilution (LD) pipetting of common immortalized cell lines utilized in commercial biologics development and manufacturing.

Materials and Methods

Single-Cell Dispensing Accuracy & Colony Outgrowth of Immortalized Cell Lines

Immortalized cells (HEK293, CHO, and A549) were cultured to ~80% confluence, counted, diluted in serum free growth medium to 10,000 cells/mL, and stained with CellTracker™ Red CMTPX (Thermo Fisher C34552). Across six Pala instruments, a total of 56 plates were dispensed in 96-well format using gates on the forward scatter by total scatter chart and the PE by forward scatter chart to best identify single, viable cells. For comparison, 14 limiting-dilution plates were generated in parallel. Plates were imaged two hours post-dispensing using the CountStar® Castor X1, enabling quantification of wells containing exactly one cell. Cell outgrowth (37 °C, 5% CO_2) was monitored at 10 days post seeding using the CountStar Castor X1. Outgrowth efficiency was determined by matching day-10 wells with corresponding day-0 images.

Single Cell Dispensing Accuracy & Colony Outgrowth of iPSCs

iPSCs were cultured to ~80% confluence, single cell dissociated with Accutase (Thermo Fisher 00-4555-56) and stained with Calcein AM (Thermo Fisher C3099) viability dye in Excellerate iPSC expansion medium (Bio-Techne CCM036) supplemented with CEPT cocktail (Bio-Techne 7991). The cells were then counted and resuspended at 10,000 cells/mL in Excellerate iPSC expansion medium supplemented with CEPT cocktail. The cells were then single cell deposited onto Cultrex ReadyBME (basement membrane extract) (Bio-Techne 3434-050-RTU) coated 96-well plates (Fisher Scientific 07-200-91) with Pala using gates on the forward scatter by total scatter chart and the FITC by forward scatter chart to best identify single, viable cells. Limiting-dilution plates were generated in parallel. Plates were imaged two hours post-dispensing using the CountStar Castor X1, enabling quantification of wells containing exactly one cell.

Following dispensing, plates were incubated for 14 days (37 °C, 5% CO_2). For the first 3 days 50uL/well Excellerate iPSC expansion medium without CEPT cocktail was added to the plates. For days 4 – 14, half of the medium volume in each well was replaced with fresh Excellerate iPSC expansion medium without CEPT cocktail. Outgrowth efficiency was determined by matching day 14 wells with corresponding day 0 images.

iPSC Stemness Marker Expression and Clone Size Determination

On day 14 the colonies are stained with anti-TRA-1-60 antibody (Thermo Fisher A25618) to assess their stemness. TRA-1-60 AF488 monoclonal antibody was added at a dilution 1:50 to each of the well that showed colony and incubated at 37°C, 5% CO_2 for 4h. The wells were then gently washed with Fluorobrite DMEM (Thermo Fisher A1896701) and imaged using the CountStar Castor X1. The clone area and the green intensity were quantitated using ImageJ version 1.54g.

FIGURE 2.

Percentage of Wells in 96-Well Plate with Single Colony

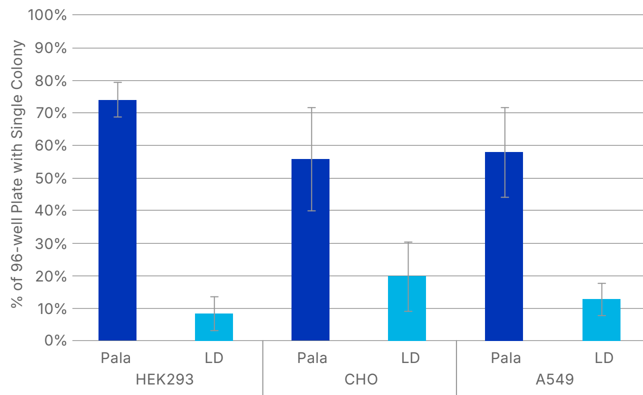

FIGURE 2. Percentage of wells in a 96-well plate containing single colonies grown for 10 days from single cells dispensed with Pala or Limiting Dilution (LD) pipetting.

FIGURE 3.

Percentage of Isolated Single Cells Growing into Colony

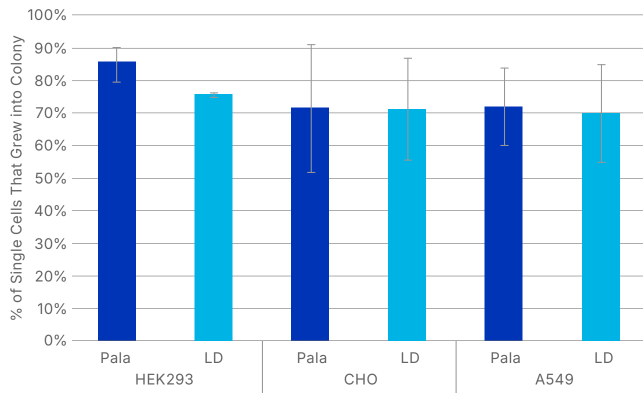

FIGURE 3. Similar percent efficiency of a single cell growing into a colony dispensed with either Pala or Limiting Dilution (LD) pipetting shows that Pala is as gentle as LD.

Results

High Single-Cell Deposition Accuracy

Across all cell types tested, Pala achieved reliable delivery of single cells into individual wells with accuracy superior to conventional limiting dilution by at least 3-4 fold (figures 1 & 4). The integrated fluorescence detection enabled clear discrimination of viable target populations, supporting robust gating and consistent selection across instruments.

FIGURE 4.1

iPSC Single Cell Deposition Accuracy

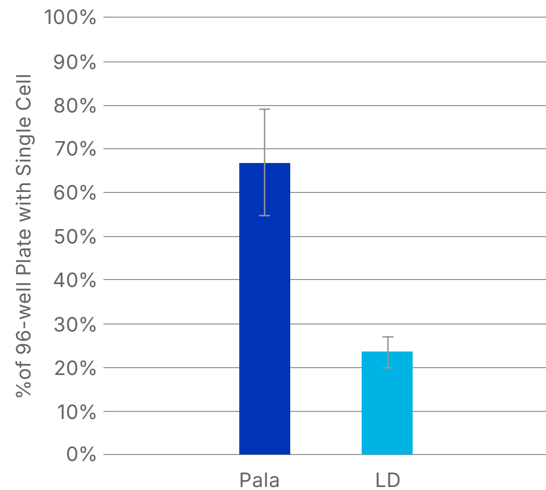

FIGURE 4.2

iPSC Single Colony Counts

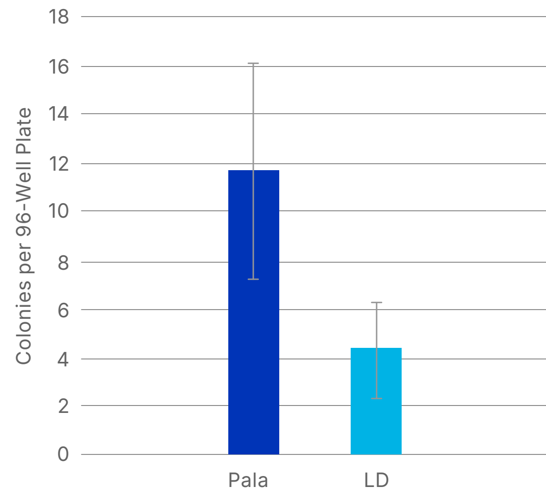

FIGURE 4. iPSC single cell dispensing accuracy and colony outgrowth of Pala dispensed cells compared to Limiting Dilution (LD) pipetting.

FIGURE 5.

Pala is Gentle on Sensitive Cell Types

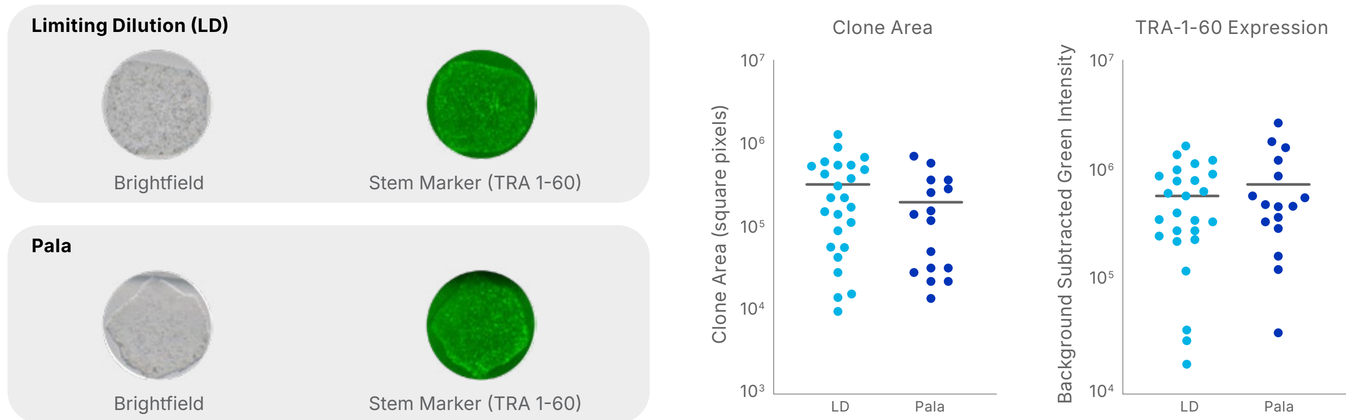

FIGURE 5. Colony size and TRA-1-60 stemness marker expression of colonies derived from Pala dispensed single cells or Limiting Dilution (LD).

Across immortalized cell lines (HEK293, CHO, A549), Pala-dispensed plates showed consistently higher number of single cell deposition per 96-well plate compared to LD (Figure 1). Colony formation efficiency, which is the proportion of single cells that grow into a colony, is similar to LD, demonstrating strong viability and robust expansion after low-pressure (<2 PSI / 13.79 kPa) microfluidic dispensing (Figure 3). However, due to the higher single cell accuracy rates with Pala, this results in 56-74% of the wells in a 96-well plate containing a single colony compared to 8-20% with LD on average (figure 2). These results confirm that Pala's gentle handling preserves cell integrity while enabling efficient generation of monoclonal lines used in biologics development and engineered cell model creation.

Induced pluripotent stem cells (iPSCs) exhibited similarly strong outgrowth, as shown in Figure 4.1 and 4.2, iPSCs exhibited high rates of single cell deposition and colony outgrowth compared to LD. Additionally, Figure 5 demonstrates that iPSC colonies maintained characteristic morphology, compactness, and stemness following single-cell dispensing—an outcome that is challenging to cells dispensed with FACS. Pala's combination of low-shear microfluidics and fluorescence-guided selection supports the recovery and expansion of viable iPSC clones suitable for CRISPR editing and disease modeling.

Application: Streamlining iPSC Gene Editing for Disease Modeling

Human induced pluripotent stem cells (iPSCs) are central to modern disease-modeling workflows, enabling researchers to introduce precise genetic modifications, generate isogenic controls, and differentiate edited clones into relevant cell types. These pipelines depend on the ability to isolate large numbers of healthy, truly monoclonal iPSC lines—yet single-cell cloning remains one of the most challenging and labor-intensive steps due to iPSCs' sensitivity to mechanical stress, shear forces, and high-pressure sorting, resulting in low yields of healthy single cell derived colonies.

Pala directly addresses this bottleneck (Figure 6). Its fast and gentle <2 PSI / 13.79 kPa microfluidic environment supports the survival of single iPSCs during the most vulnerable stages of dissociation and replating, while fluorescence-based gating enables targeted selection of successfully edited, viable cells. The platform's combination of accurate single-cell deposition, rapid plate processing, and stable recovery conditions increases the yield of healthy colonies/plate and likelihood of obtaining correctly edited clones suitable for downstream expansion.

By enabling efficient isolation of monoclonal iPSC populations, Pala streamlines CRISPR editing workflows used for generating disease-relevant models, studying genotype–phenotype relationships, and building robust stem-cell–derived systems for discovery and translational research.

Pala's accurate single-cell deposition, gentle microfluidic environment, and fluorescence-compatible detection directly address this need. The ability to process a 96-well plate in 1.5 minutes and seamlessly switch between samples without the risk of sample-to-sample cross contamination and expands cloning throughput while minimizing hands-on time. This increases the probability of capturing the optimal clone and accelerates downstream screening across a range of cell engineering and biologics development applications.

FIGURE 6.

Clonal Stem Cell Line Development Workflow

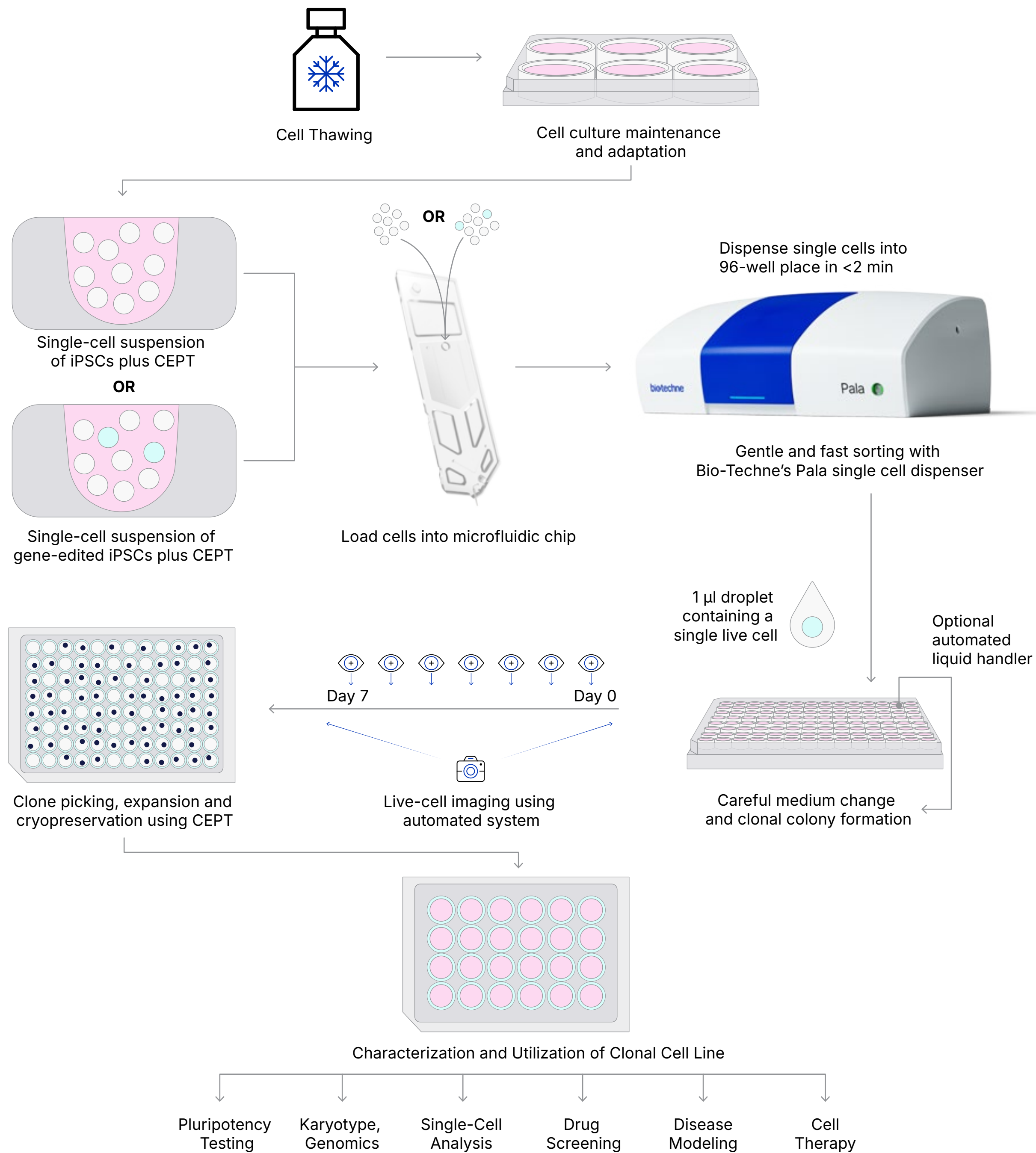

FIGURE 6. Clonal stem cell line development workflow with the Pala Single Cell Sorter and Dispenser

Conclusion

The Pala Cell Sorter and Single Cell Dispenser integrates a unique combination of speed, gentle cell handling, and flexible fluorescence detection to streamline stable **cell line development** workflows. High single-cell deposition accuracy and strong colony outgrowth demonstrate that the system maintains cell health and supports the generation of high-quality monoclonal populations.

By enabling rapid expansion of clone numbers and eliminating common bottlenecks in difficult workflows, Pala provides a practical and scalable solution for laboratories advancing diverse applications requiring creation of clonal cell lines, from biologics development, to engineered stem cell models, and next generation cell and gene therapies.

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