R&D systems by bio-techne

Single cell cloning iPSCs with the Pala Single Cell Sorter preserves colony stemness and cell viability for high-throughput cell line engineering workflows

Ahmed Shaaban, Samuel Fox, Bhamini Purandare, Francisco Ramirez, Ryan McComb, & Chris Heger

R&D Systems by Bio-Techne, San Jose, CA

Summary

- Single cell cloning of gene edited iPSCs is fundamental for generating isogenic and clonal cell lines.
- Traditional approaches to single cell cloning –particularly when working with sensitive cell types – are slow, labor-intensive, or harmful to viability.
- The Pala™ Single Cell Sorter and Dispenser integrates flow cytometry and gentle, low-pressure cell dispensing in a compact, simple-to-use instrument.
- The following study compares the utility of the Pala system against limiting dilution (LD) for isolating single iPSCs and their outgrowth into monoclonal colonies.

Introduction

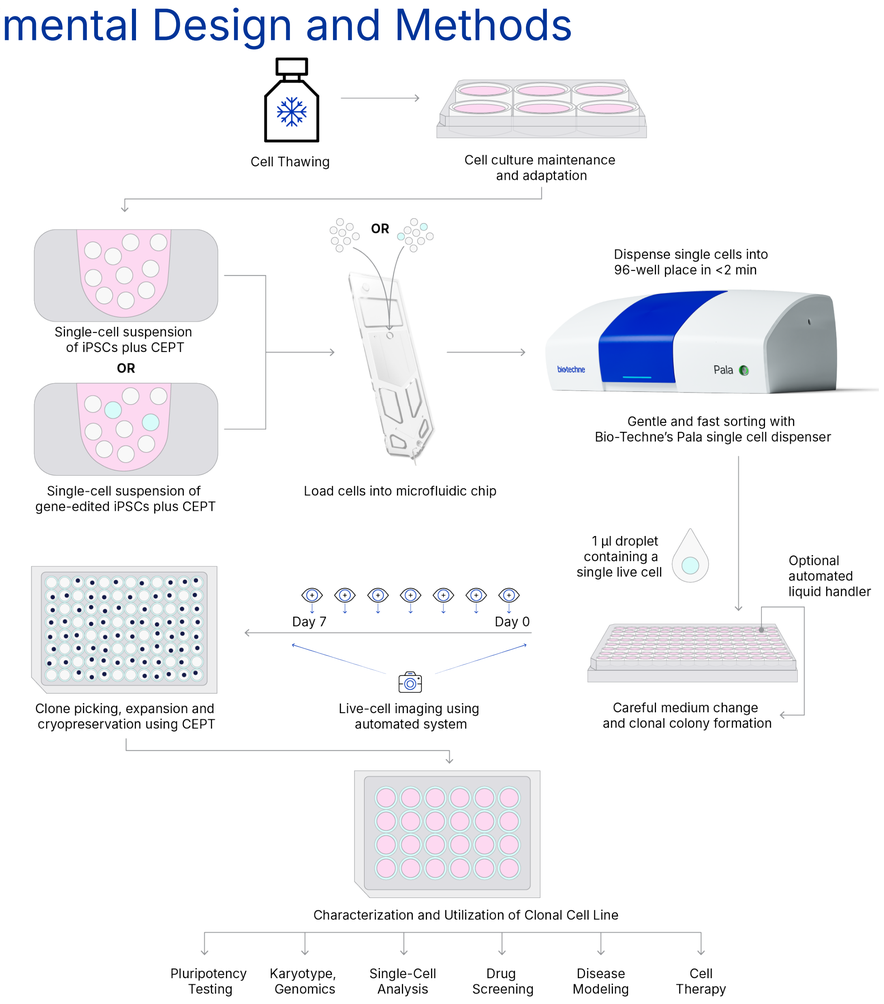
Human induced Pluripotent Stem Cells (iPSCs) paired with advanced gene editing tools give researchers the ability to develop cell models for disease paired with their isogenic corrected controls. These cell lines can further be differentiated into relevant tissue types to express relevant proteins and phenotypes for research or drug screening. Emerging iPSC derived cell therapies require correction of the disease-causing gene and/or knocking out genes implicated in host immune system rejection. The methods for designing, editing, and deriving monoclonal iPSC cell lines can take multiple months and is prone to bottlenecks and low success rates because of inefficient processes.

Single cell cloning for generating monoclonal cell lines is necessary to achieve stable cultures that provide high reproducibility in research and therapeutic outcomes. Single cell cloning remains a significant bottleneck in most cloning workflows and is commonly performed using limiting dilution (LD) or FACS sorting. These methods are inefficient, cumbersome, and difficult to scale. In this study, we compare the efficiency of single cell isolation, monoclonal colony health, and stemness marker expression to evaluate the Pala Single Cell Dispenser to LD. Furthermore, as a proof-of-concept, single cell derived colonies are generated with Pala from iPSCs with the beta-2-microglobulin gene knocked out, a component of the MHC class 1 molecules and a target of host immune rejection, to demonstrate Pala's utility in cell therapy development.

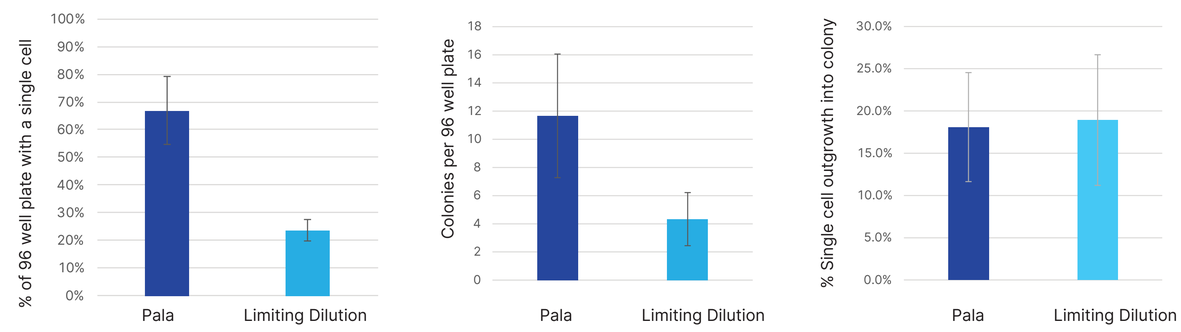
Pala Single Cell Dispenser

- Fast – Minutes to start-up and load a sample. Dispense a 96-well plate in as little as 1.5 minutes or 384-well plate in 6 minutes
- Gentle – Cells are under <2 PSI (13.8 kPa) sorting pressure
- Microfluidic cartridges – eliminates sample-to-sample carryover and contamination risk
- Fluorescence enabled – 2 excitation lasers, up to 11 fluorescent channels
- Compact – fits in a standard Bio-Safety Cabinet and weighs 35 lbs.
- Simple-to-Use – Intuitive interface and prompt guided workflow

Experimental Design and Methods

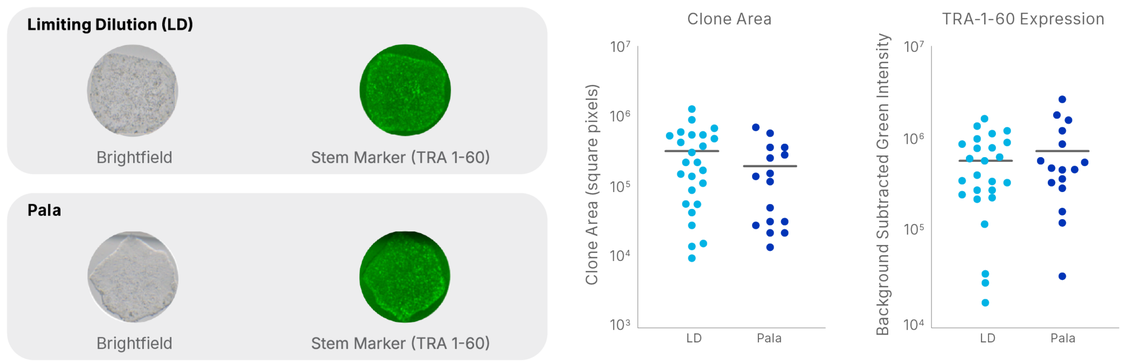


Single Cell Isolation & Outgrowth

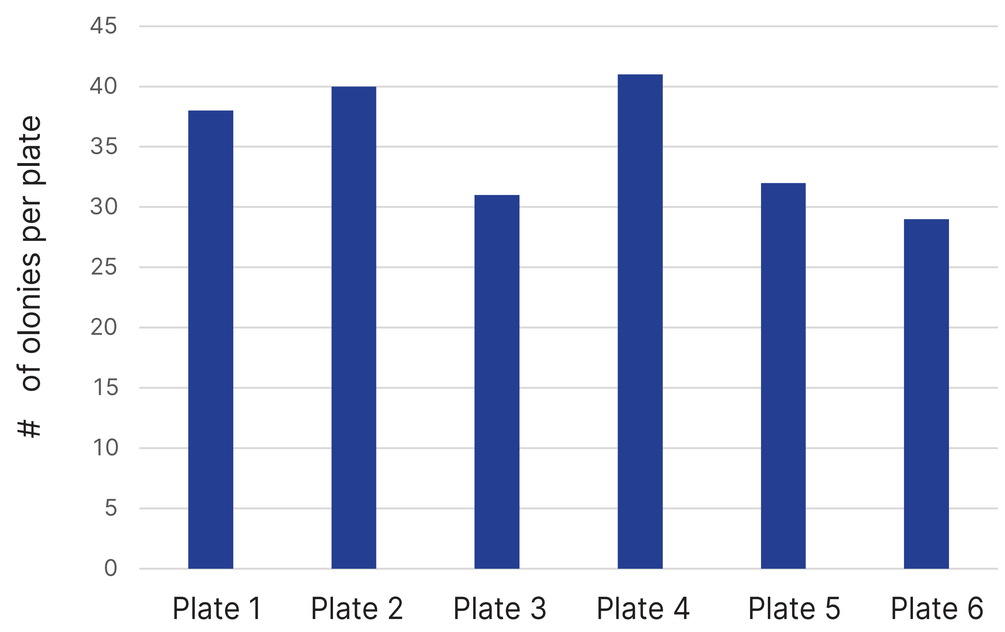


Single cell dissociated iPSC cells from one donor line was either dispensed with Pala or hand pipetted using Limiting Dilution into Cultrex ReadyBME coated 96-well plates pre-filled with Excellerate iPSC expansion medium supplemented with CEPT to enhance single cell survival. Cells were imaged 2 hours post plating to determine single cell clonality and on day 14 to assess colony outgrowth. On day 14, colonies were stained with anti-TRA-1-60 antibody and imaged with the CountStar Castor X1 imager. Images were quantified with Image J software

Colony Health & Stemness Marker Expression



iPSC Clonal Outgrowth of Beta-2-Microglobulin KO



iPSCs were electroporated with CRISPR/Cas9 targeting the β2M gene. The transfected iPSCs were cultured to ~80% confluence, single cell dissociated, and stained with Calcein AM viability dye and anti- β2M-PE antibody. Cells were resuspended at 10,000 cells/mL in Excellerate iPSC expansion medium supplemented with CEPT cocktail. Cells were then single cell deposited onto coated 96-well plates with Pala using gates on forward scatter by total scatter chart, FITC by forward scatter chart and PE by forward scatter chart to best identify single, viable, β2M KO cells. Plates were imaged two hours post-dispensing, enabling quantification of wells containing exactly one cell. Plates were incubated for 14 days (37 °C, 5% CO_2). Outgrowth efficiency was determined by matching day 14 wells with corresponding day 0 images.

Conclusion

- Pala showed high accuracy of single cells deposited per well in 96-well plates compared to limiting dilution (LD) (>65% vs. <25% on average)
- Both Pala & LD dispensed single cells showed similar proportions of single iPSCs growing into colonies demonstrating the gentle nature of Pala dispensing. However, Pala yielded as many as 2.5-3x more colonies per plate than LD.
- Pala derived colonies showed similar growth characteristics (clone area) and expressed similar levels of the TRA-1-60 stemness marker as LD indicating similar colony health and undifferentiated status.
- Pala's unique attributes of fast start-up and dispensing rates, high single cell dispensing accuracy, 2-laser fluorescence, and gentle handling provide stem cell research and cell therapy manufacturing labs the ability to scale iPSC engineering capacity without sacrificing cell health.

- Learn More about the Pala Platform