Cell Line Development – Single Cell Detection, Clonal Validation, Transfection

The process of developing a cell line to produce a specific protein or antibody involves multiple stages, all of which can be greatly aided by Celigo imaging cytometer.

Transfection
- Determine transfection efficiency
- Monitor stability of transfection

Stabilized Outgrowth
- Monitor growth in each well
- Identify confluent wells for passaging
- Increase growth with media optimization

Monoclonality
- Verify efficiency of limiting dilution, or single cell dispensing
- Identify single cell per well

Colony Outgrowth
- Confirm single colony

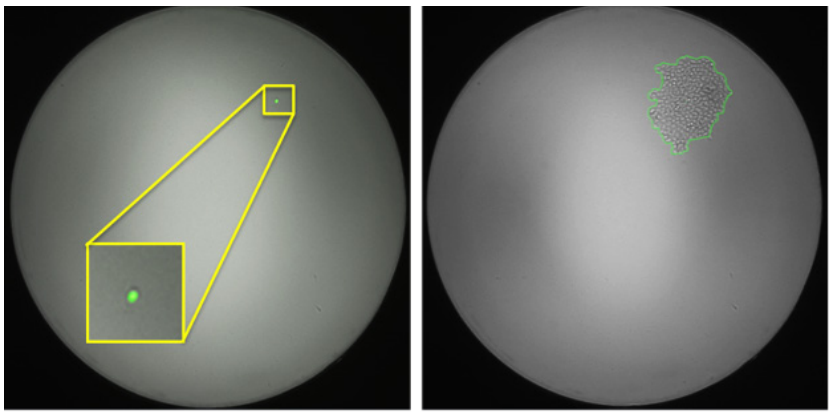
Robotic Integration

The Celigo provides an optional robotic API which can be controlled by various automation scheduling software applications. The Celigo is ready for integration with multiple automation partners and can be coupled with robotic arms, automated incubators and liquid handlers.

The Celigo can be used through the whole process of cell line development.

- Compatible with 96-, 384- and 1536- well plates.
- Identify wells with a single colony to avoid the time-consuming and manual identification of clones by eye.
- Measures colony size using bright field and aids the process of selecting wells for clonal expansion
- Automate cell line generation process with Celigo robotic integration.

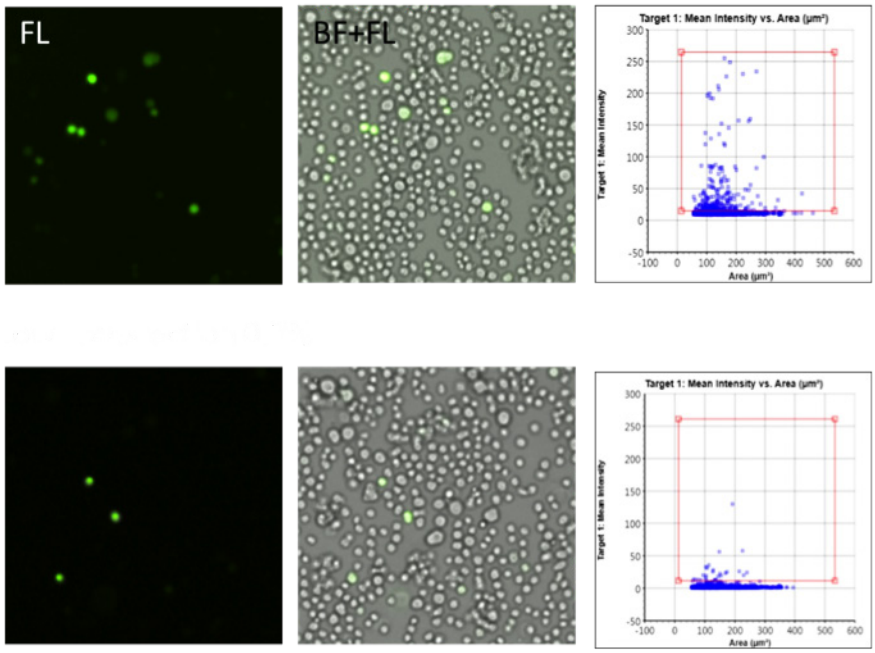
Day 0 - Single Cell **Day 7 - Single Colony**



Well with Single cell that grew into a Single colony

Transfection & Transduction Optimization

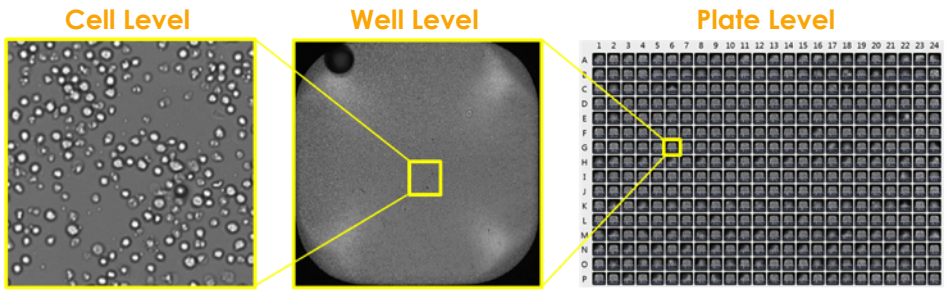
- Quickly identify optimal parameters for high-efficiency transfection
- Determine transient and stable transfection rates and evaluate antibiotic induction using live imaging



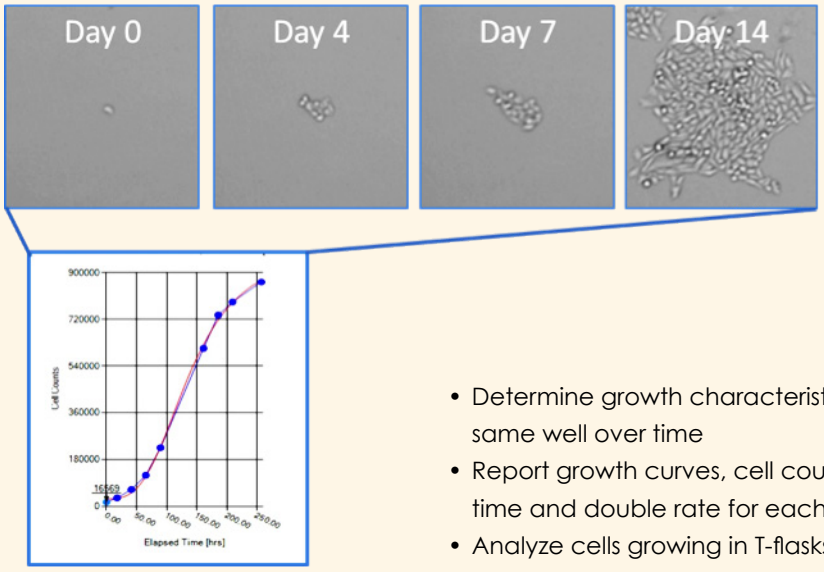
Sample 1 shows moderate transfection efficiency while sample 2 has lower efficiency.

- Monitor transfection efficiency on Celigo directly
- Acquire both bright field and green fluorescence cell images
- Identify all the cells using bright field
- Produce scatter plot for gating GFP+ cells
- Calculate % GFP+ cells automatically
- 96 or 384 wells

Cell Line Characterization



Brightfield images can be viewed from the cell, well or plate level.



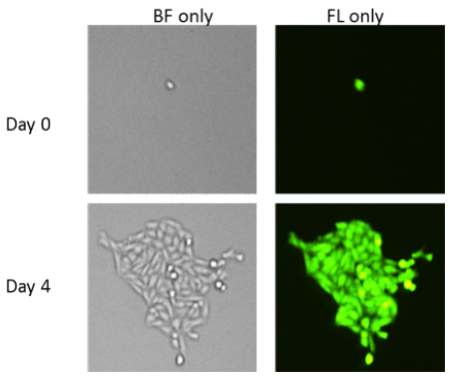
- Determine growth characteristics of cells directly from the same well over time
- Report growth curves, cell counts, confluence, doubling time and double rate for each well
- Analyze cells growing in T-flasks

Media optimization experiment using a single 384-well plate

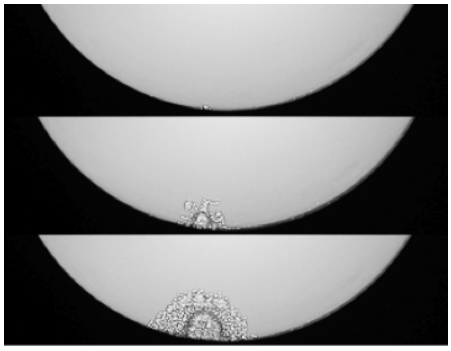
- Providing additional nutrients to media can help increase proliferation rates.
- Evaluated media supplements using an 8-parameter design of experiment (DoE) methodology, where 3 reagents were mixed in multiple combinations.
- On day 0, 10 CHO-S per well were plated with supplement combinations using a 384-well plate, which allowed 35 replicates per condition.
- Three supplement reagents were tested on one 384-well plate for n=35 per condition

Monitoring Monoclonality and Outgrowth

Adherent Cells

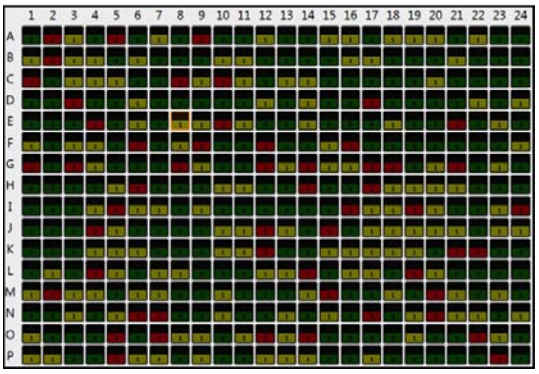


Suspension Cells

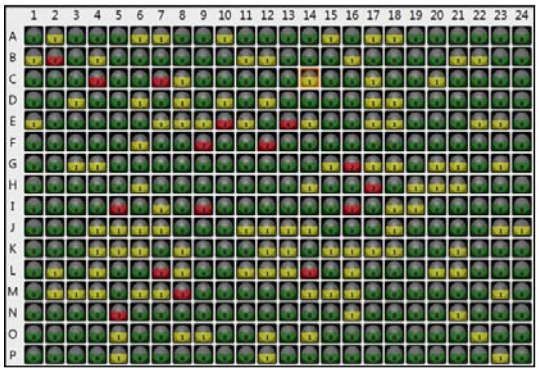


Dual channel bright field and fluorescent imaging of a single cell in a 96-well plate allows identification of candidate wells for clone development.

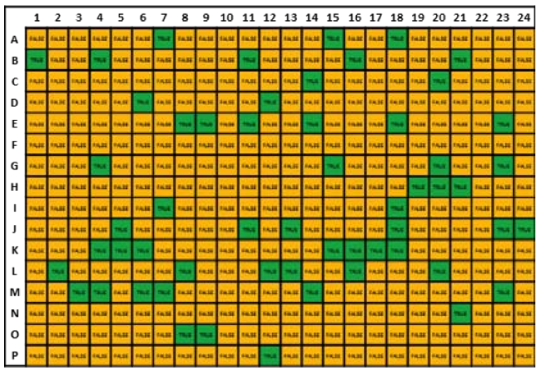
Single Colony BF Counts



Single Colony FL Counts



- Identify wells with single colony on the final day
- Identify wells with single cell on the first day
- Overlay single cell plate map with the single colony plate map to produce the heat map of single cell and single colony.



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1001263 RevA 09/14