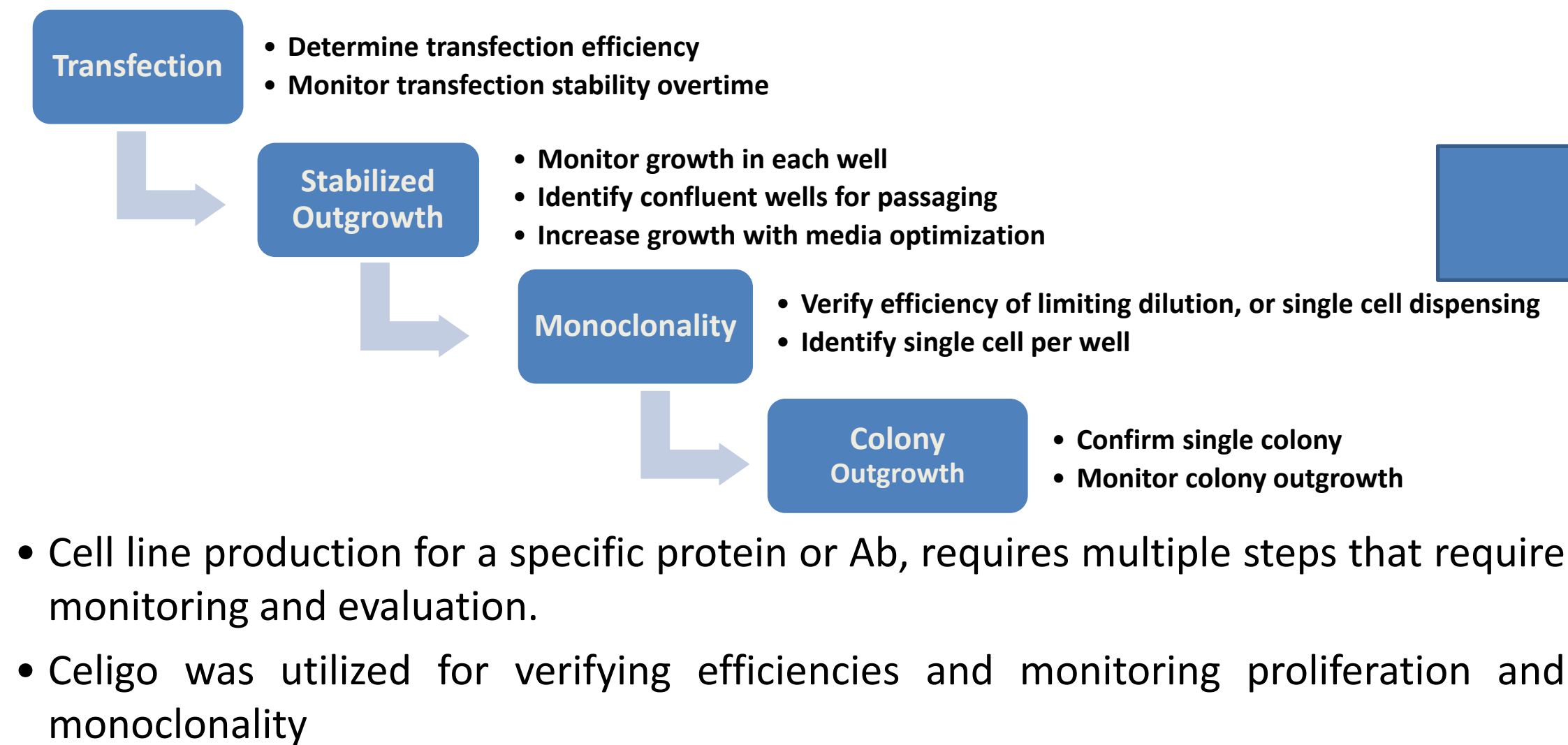


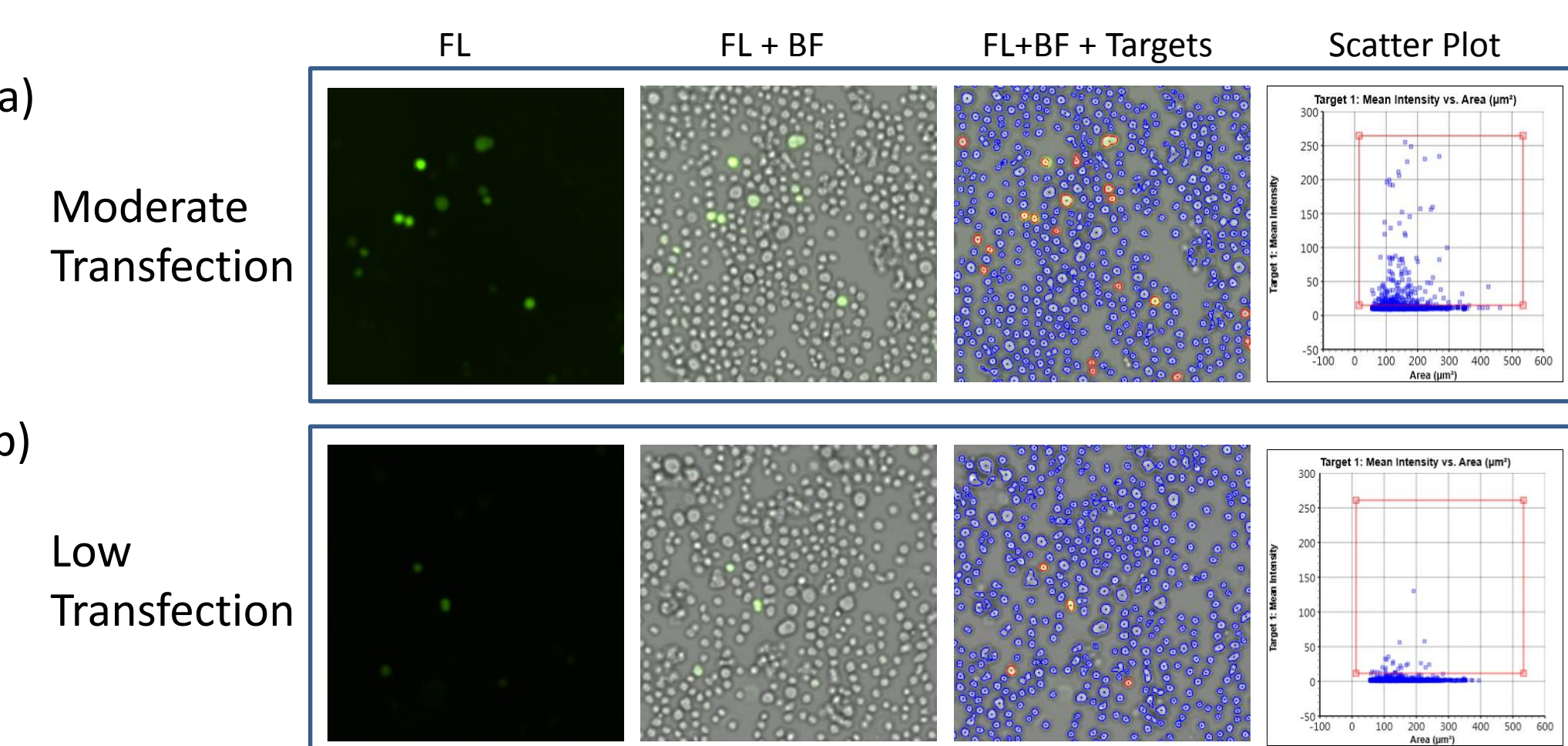
1. ABSTRACT

Development of monoclonal cell lines is essential in research and for the production of recombinant protein therapeutics. Due to the increasing number of biologic treatments, monoclonal antibody (mAb) producing cell lines are under increasing regulatory scrutiny, and therefore, ensuring monoclonality is becoming an essential step in the cell line development process. Some challenges in producing clones remain and relate to cell plating, identification and outgrowth of single cells for monoclonality. Researchers have used limiting dilution techniques and microscope visualization to ensure that populations of cells in a well were derived from a single cell. Moreover, transfected cells can exhibit slower growth rates to accommodate for the production of bio-products and require adjustments to selection reagents. Optimizing media formulations can increase the percentage of single cell clonal growth. In this study, using the CHO-S cell line, the Celigo® Imaging Cytometer was used to monitor transfection efficiency, identify passaging times, optimize media, identify single cells and track clone outgrowth. After transfection, cells were allowed to stabilize, then imaged in bright and fluorescence to monitor and evaluate transfection efficiencies. As wells grew asynchronously, brightfield imaging was used to track which wells needed passaging. As genetically modified isolated cells have decreased growth rates, design of experiment (DoE) methodologies and automated imaging were used for media formulation improvements to increase their growth and survival. Media supplements that have been shown to increase growth were tested with an 8 parameter DoE [3 levels, 3 factors, and single output response]. The results show that the use of B-27 positively promoted cell growth as compared to the control media. While the identification of single cells in brightfield is possible, using the fluorescent marker such as Cell Tracker Green or GFP to monitor the single cells was found advantageous. Wells growing a single cell were subsequently monitored for the formation of a colony, which also allowed for measurement of optimal passaging times of asynchronous clones. Overall, the Celigo Imaging Cytometer has demonstrated the utility of automation in the development and monitoring of new CHO-S based cell lines for increasing efficiency in cell line development.

2. WORKFLOW FOR CELL LINE DEVELOPMENT

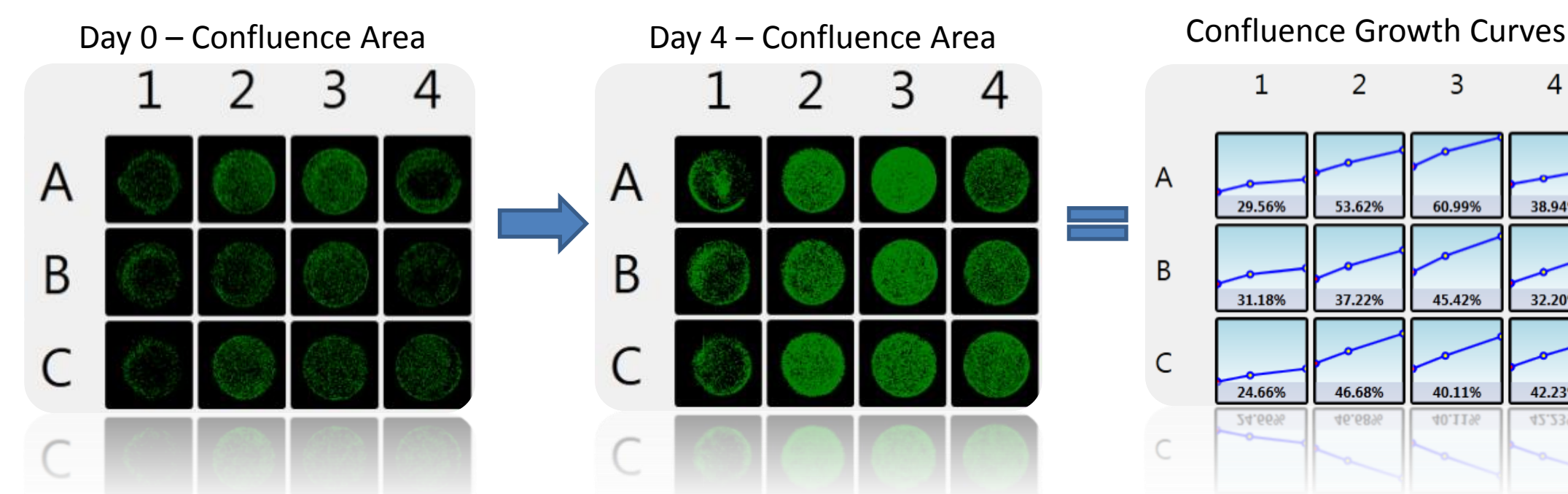


3. TRANSFECTION EFFICIENCY



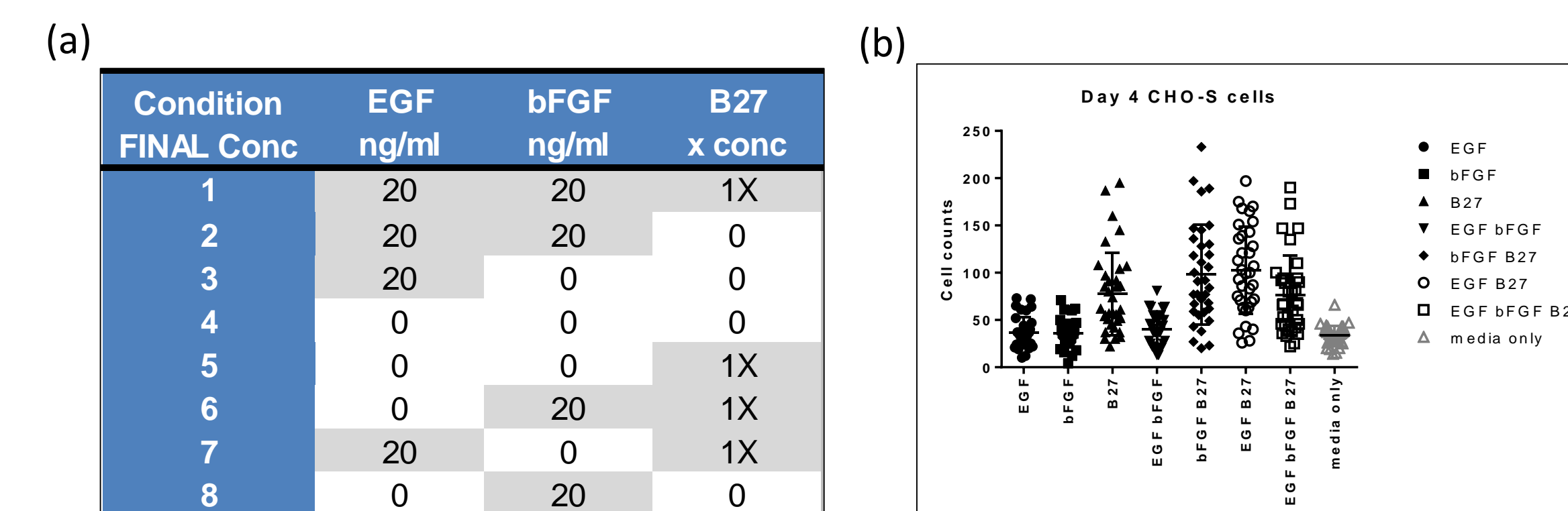
- Celigo acquires brightfield and fluorescent images of transfected cells throughout their recovery without removing them from the wells.
- Transfection efficiency is determined from plotting intensity levels from fluorescent cells detected from the images. (a) Moderate transfection 5.2% (b) low transfection 0.7%.

4. STABILIZED GROWTH



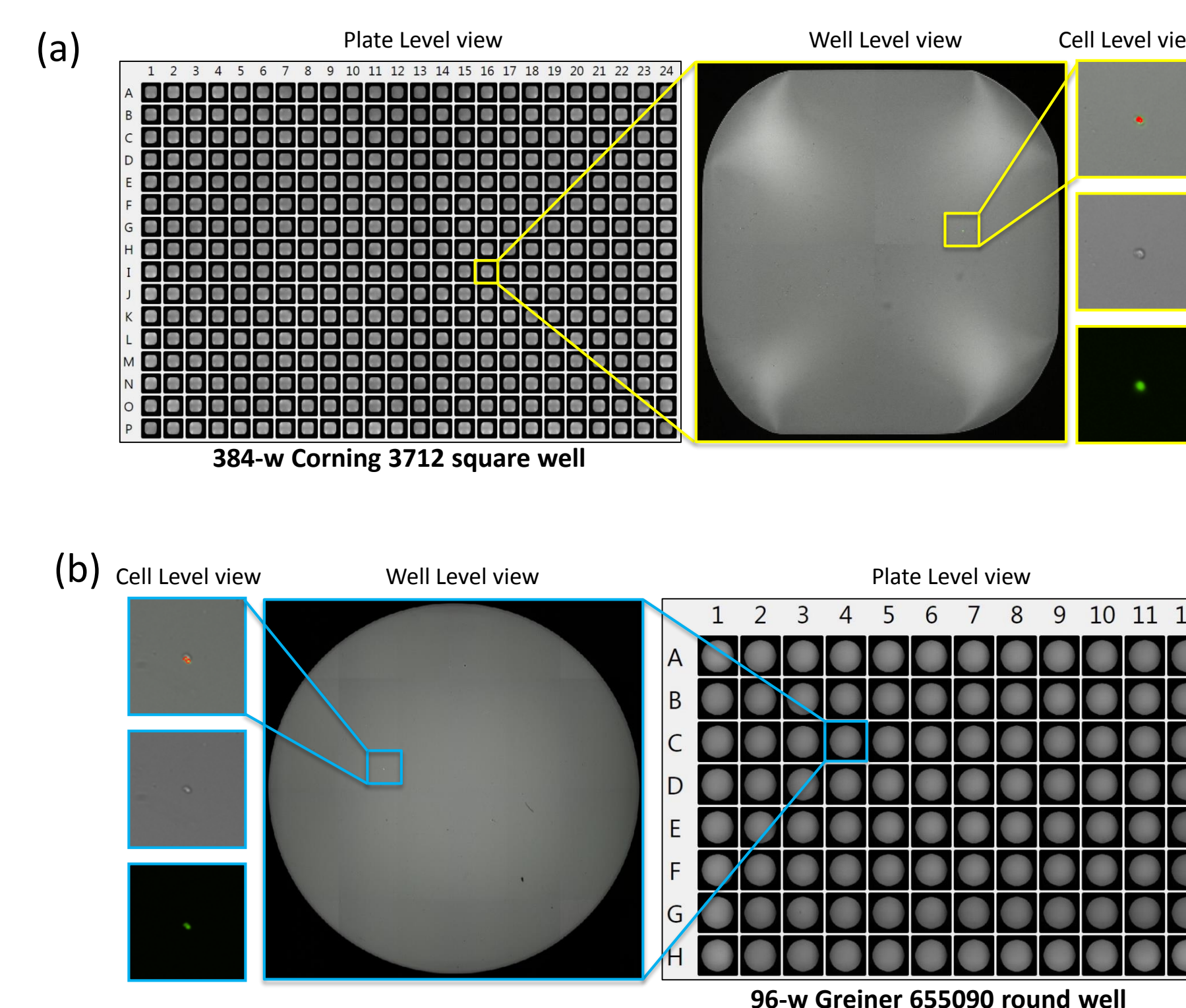
- After transfection, these genetically altered cells need time to adjust and stabilize back to a normal growth pattern.
- Plates were scanned on multiple days with the Celigo Confluence application. For easy plate level viewing, the detected areas of confluence were filled in with a green color & black background.
- Growth curves were created for each well showing variation per well. Slower growth was easily differentiated from faster growing wells, which helped determine passaging times.

5. MEDIA OPTIMIZATION



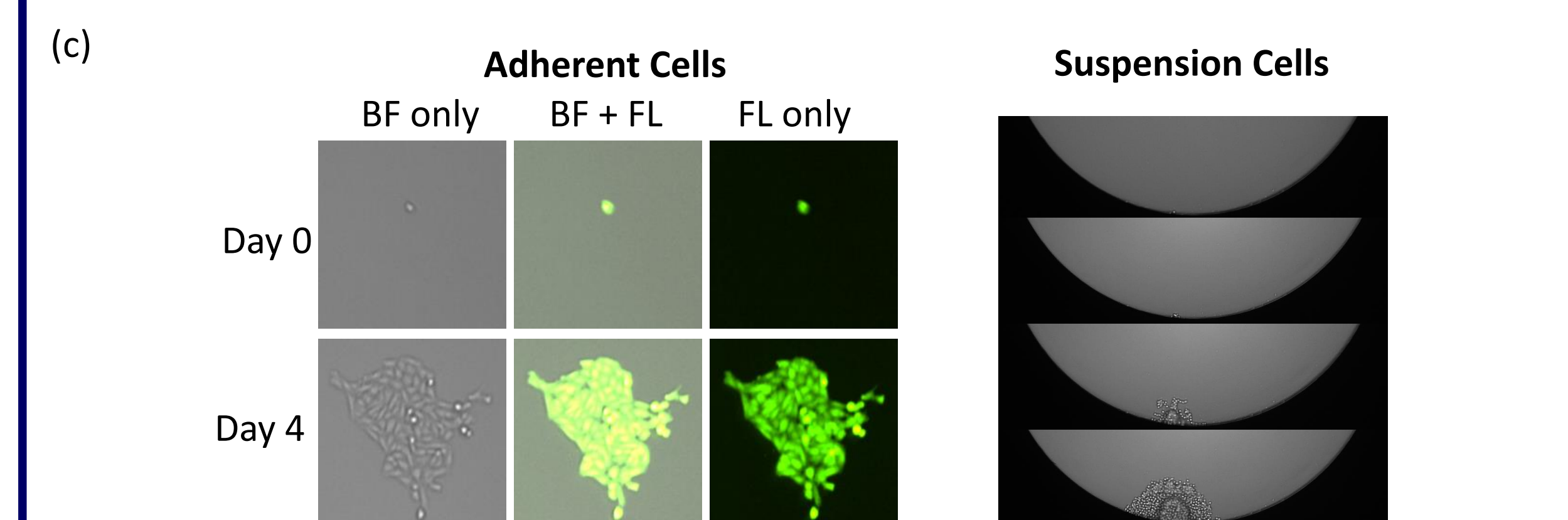
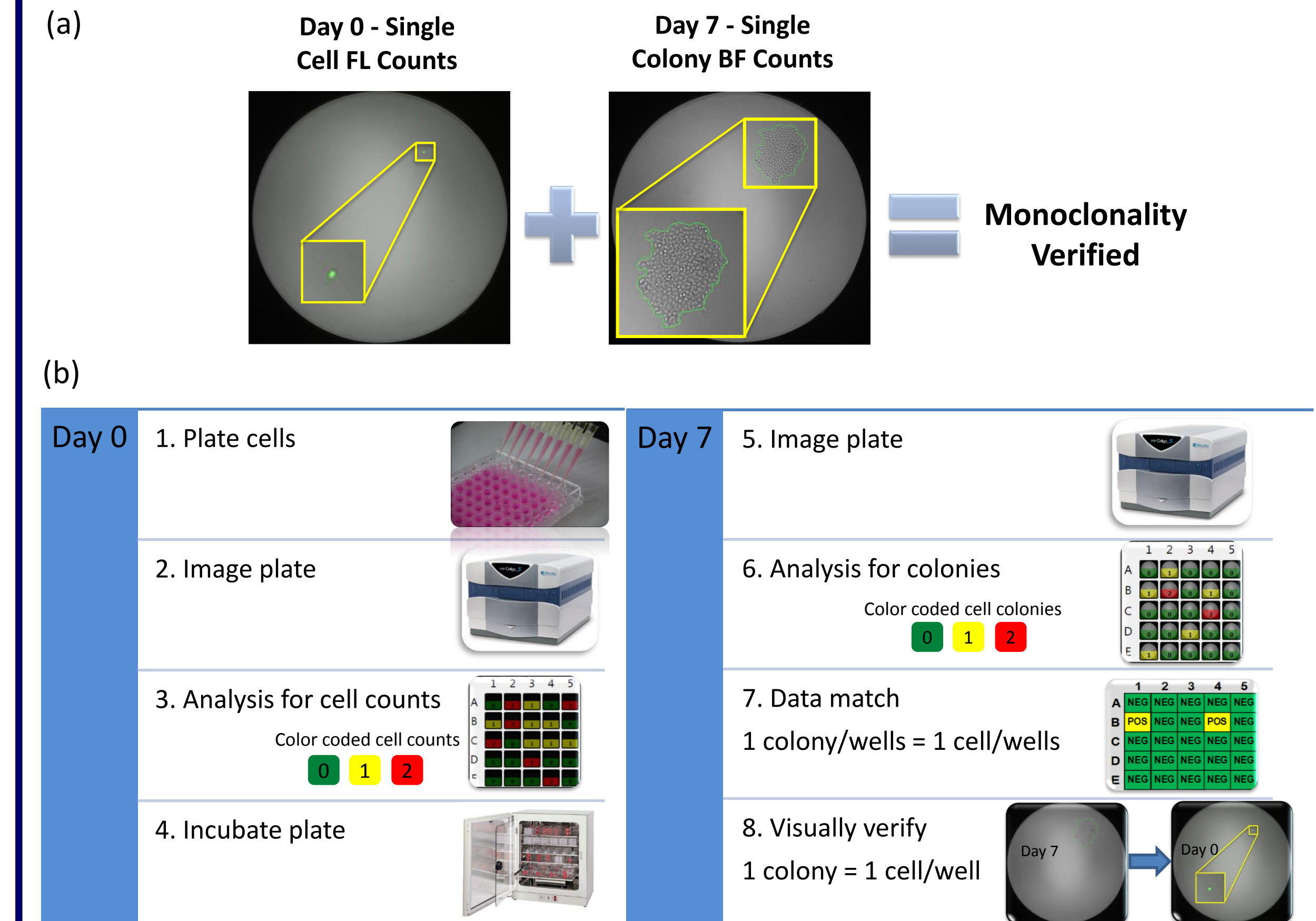
- Providing additional nutrients to media can increase proliferation rates.
- This test evaluated media supplements using an 8 parameter design of experiment (DoE) methodology where 3 reagents (EGF, bFGF, B27) were mixed in multiple combinations (a).
- On day 0, 10 CHO-S per well were plated using a 384-well plate, which allowed 35 replicates per condition. On day 4, proliferation was monitored on Celigo.
- Results showed certain combinations had increased proliferation as compared to control. The common reagent across those groups was B27 (b).

6. MULTIPLE WELL FORMATS



- Single cell detection can be performed in 384- well (a) or 96-well (b) plate format.
- Images can be visualized in brightfield and fluorescence at the cell, well, and plate level.

7. SINGLE CELL DETECTION & VERIFICATION FOR MONOCLONALITY



- Typically, monoclonality is achieved with limiting dilution of cells or single cell sorting, then allowing those wells to grow out into a colony. Visual verification is done to ensure the colony started from a single cell. With multiple plates this become laborious to do as well as track.
- In this study, ensuring and tracking monoclonality was done by imaging plates when cells are plated, and then again after a number of days when the cells have grown into a colony (a).
- This easy workflow procedure was performed using Celigo (b). Cells were plated on Day 0, imaged and analyzed for cell counts. Wells with single cells were easily visualized with a color coded map, where yellow indicated wells with a single cell. After multiple incubation days, the plates were again imaged and analyzed for colonies. Yellow coded wells were indicative of single colonies. Using logic with the data from both days, clearly mapped out which wells most likely were monoclonal. The final verification was reviewing fewer wells of those single colony wells that came from wells with a single cell.
- Images of monoclonal adherent and suspension colonies grown from single cell (c).

8. CONCLUSION

Cell line development for the production of biological products consists of many steps that require monitoring and evaluation of efficiencies. The large volume of plates that are used to successfully produce clones can be overwhelming and daunting to monitor and track. By using image-based cytometry, we were able to monitor transfection efficiency, and passaging times. As these genetically altered cells tend to have slower growth rates, using DoE we evaluated and found media supplements that increased proliferation. Additionally, we were able capture and examine images of single cells that grew into single colonies, ensuring monoclonality. Using the Celigo throughout the process of cell line development demonstrated how automation alleviated many pain points that are traditionally difficult to overcome.

