High-throughput detection of CRISPR/Cas 9 gene editing efficiency, cell proliferation/viability, and monoclonality validation using Celigo Image Cytometer

Suzanne Riches, Sun Yung, Scott Cribbes, and Leo Li-Ying Chan

[1]*Revvity Health Sciences, Inc., 360 Merrimack St., Suite 200, Lawrence, MA 01843*

1. ABSTRACT

The CRISPR/Cas 9 gene editing technology has become one of the favoured methods to generate cell lines with targeted gene knockins or knockouts. This method is highly precise and efficient, and has been adopted by many research labs within both industry and academia. In general, research experiments involving the CRISPR/Cas 9 protocol will generate many cell lines for characterisation, thus requiring a high-throughput method to measure the efficiency of the gene editing, the proliferative and healthiness of the generated cell lines, and verify cell line monoclonality. In recent years, the use of standard microwell plates for image cytometry has become increasingly popular due to the need for higher throughput. Specifically, the Celigo Image Cytometer can perform high-throughput, whole-well, cell-based assays, utilising special optics for rapid capture and analysis of brightfield and fluorescent images. Performing a 96-well, whole-well, cell-based assay using brightfield and one fluorescence channel typically requires less than 8 min.

In this work, we demonstrate the employment of the Celigo Image Cytometer to perform rapid high-throughput imaging and analysis of CRISPR/Cas 9 gene-edited cell lines. There are three major assays where speed and efficiency can be greatly improved by using the image cytometry method. Firstly, cells may be directly imaged and analysed in brightfield and fluorescence to measure gene editing efficiency. This assay may be performed without the need to trypsinize cells, as otherwise required for flow cytometry, thus disturbing their natural state. Secondly, it is critical to monitor the proliferative capability of the generated cell lines as well as their viability. Many of the generated cell lines may not proliferate due to cell death, therefore it is important to be able to perform high-throughput cell proliferation assays to characterise them. Traditionally, MTT or CellTiter-Glo® assays are performed, but can introduce uncertainties without actual cell images. In addition, enzymatic activities may be disrupted by treatments, thus generating inaccurate endpoint viability results. Finally, for therapeutic cell line generation, it is important to ensure monoclonality, therefore image cytometry was used to capture whole well images, identify single cells, and monitor outgrowth over time. This method is a significant improvement over the current method of manually inspecting every well to identify single colony outgrowth to validate monoclonality, which may have a high operator-dependent error rate.

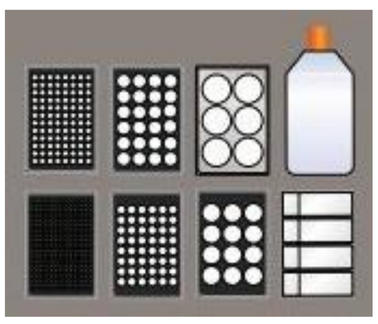
In conclusion, the use of image cytometry may alleviate many issues from traditional detection methods. The ability to quickly characterise the CRISPR-generated therapeutic cell lines can improve the efficiency in identifying potential candidates for treatment.

2. CELIGO IMAGING CYTOMETRY FOR CRISPR/CAS 9 CELL-BASED ASSAYS

- Celigo Imaging Cytometer is a plate-based cytometer that can scan the entire well of standard microplates and capture bright-field and fluorescent images.
- The captured images are analysed with the Celigo software to measure size, morphology, cell count, confluence, and fluorescent intensity.
- The measured parameters are used to generate cell proliferation kinetic data, GFP/RFP expression, tumor spheroid size change, cell cycle analysis, apoptosis, and ADCC cytotoxicity results.

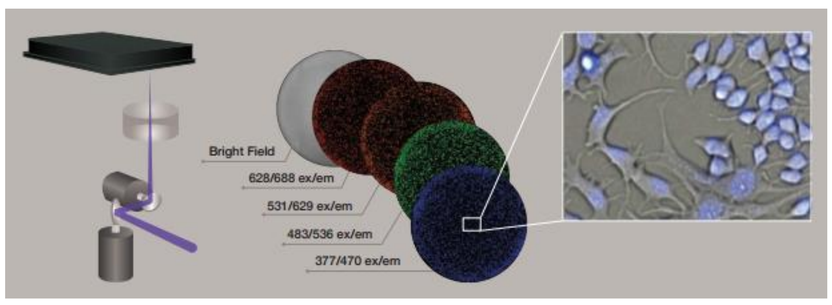
Large variety of live cell assays for all project needs

Supports all culture formats

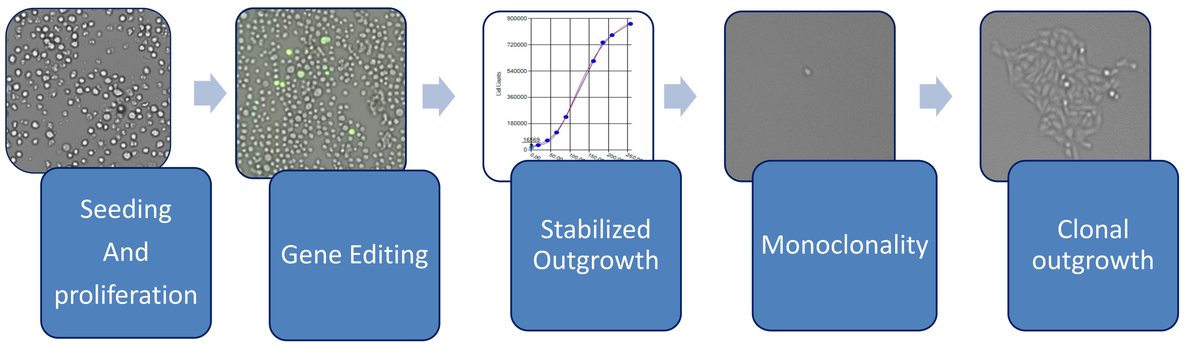


High-throughput automation ready

Best in class bright-field with four fluorescent channels

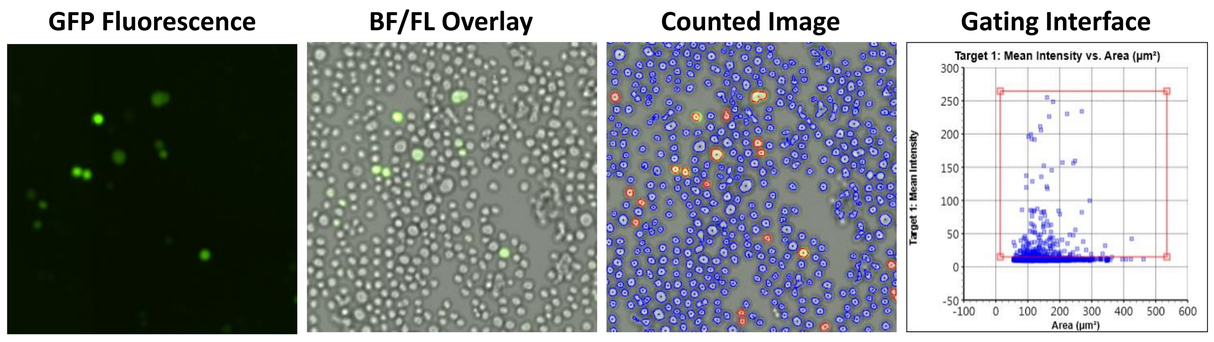


Celigo helps overcoming challenges in cell line maintenance and development

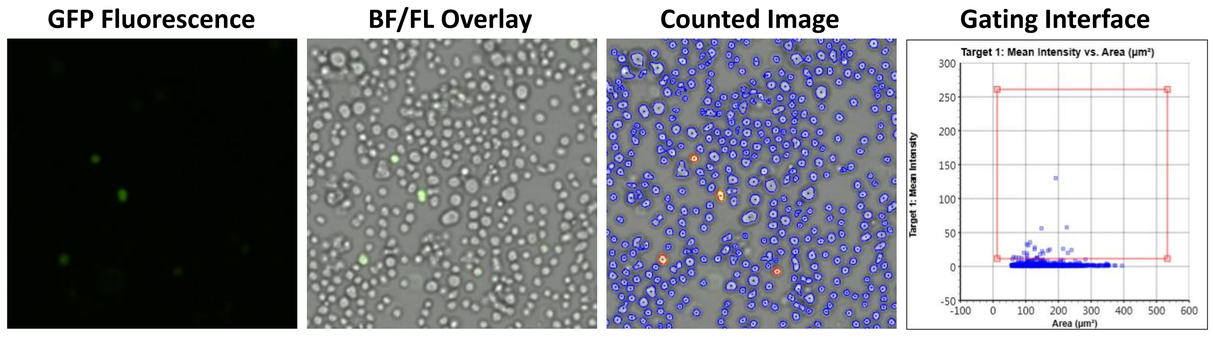


3. DETERMINING CRISPR/CAS 9 GENE EDITING EFFICIENCY

Moderate Gene Editing Efficiency 5.2%

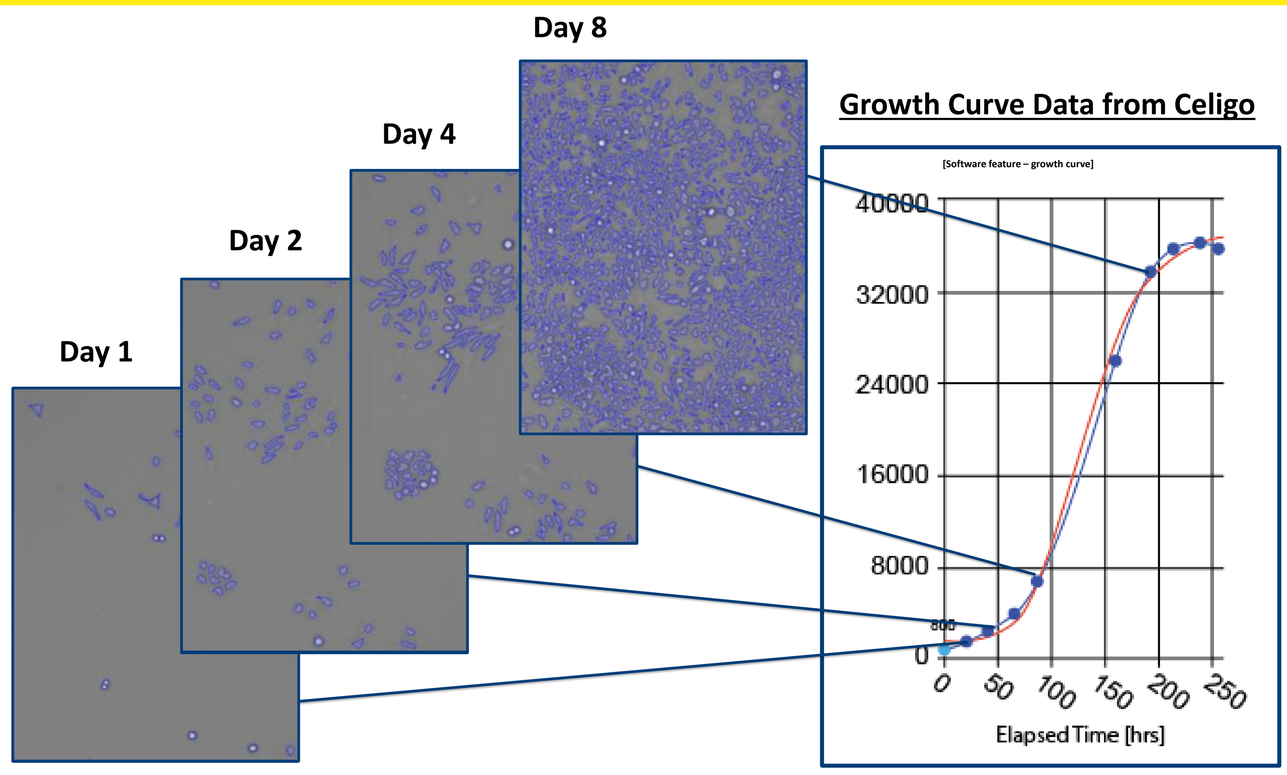


Low Gene Editing Efficiency 0.7%

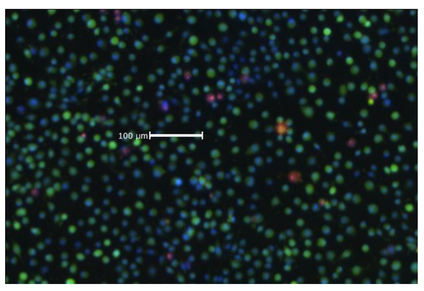


- CRISPR gene edited cells are seeded into a 96- or 384-well plate and allowed to settle
- Celigo was used to image in both bright field and green fluorescent channels
- The captured images are analysed to count the total number of cells in bright field and using the Gating Interface in the Celigo software
- The results are directly generated in the software for the percent GFP positive cells or the efficiency

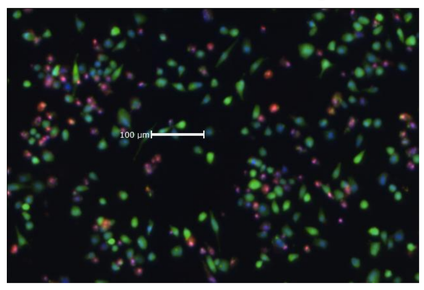
5. MEASURING CELL PROLIFERATION AND VIABILITY USING DIRECT CELL COUNTING METHOD



Raw 264.7 - Vehicle



Raw 264.7 – Drug



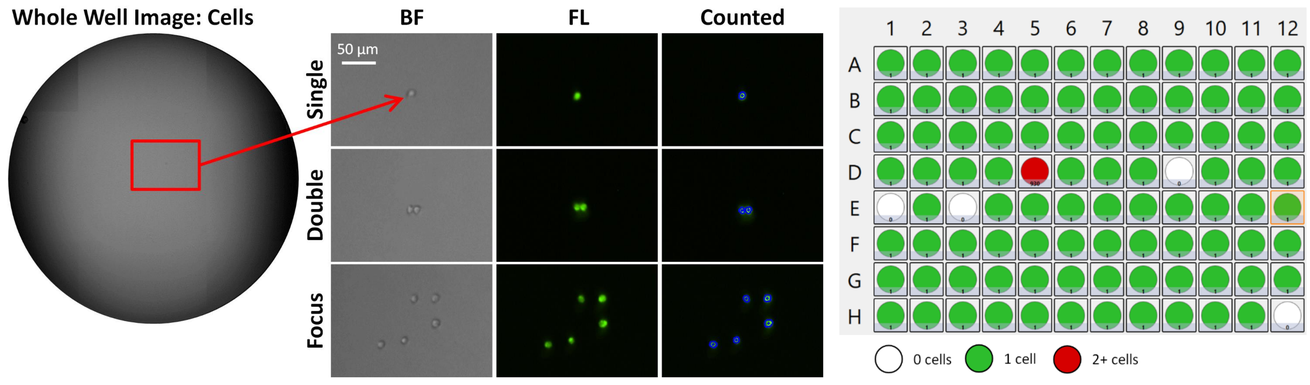
Label-free cell proliferation measurement

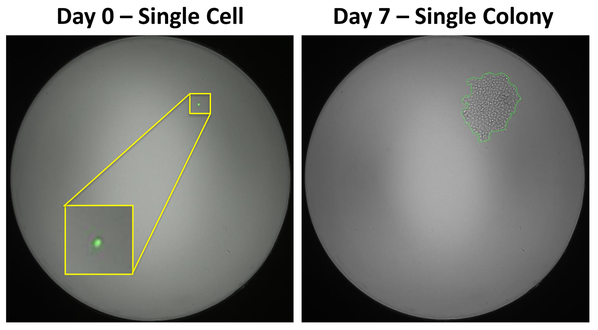
- CRISPR gene edited cells are seeded in 96- or 384-well plates and allowed to proliferate over time
- Celigo is used to capture bright field images from day 0 to day 11
- The bright field images are analysed to directly count individual cells
- The number of cells are generated for each time point and directly plotted in the Celigo software for showing growth curves
- Wells with cells not proliferating or low growth may indicate issues with the gene insertion or knockout

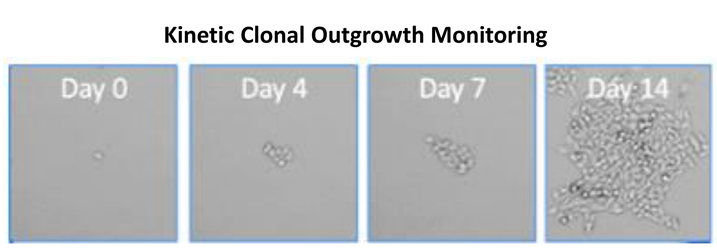
Cell viability measurement

- At the end point of the cell proliferation, fluorescent stains such as calcein AM, propidium iodide, and Hoechst are added to determine cell viability
- After staining, the Celigo is used to measure viability by identifying
  - ❑ Total number of cells with Hoechst
  - ❑ Number of metabolically active cells with calcein AM
  - ❑ Number of dead cells with propidium iodide

6. VALIDATING MONOCLONALITY AND CLONAL OUTGROWTH FOR CELL LINE GENERATION







Well with Single cell that grew into a Single colony

- Single cells can be dispensed into a 96- or 384-well plate using FACS, cell printer, or limiting dilution
- Celigo was used to scan and validate individual cell in each well, and can quickly reviewed on the heat map
- The single cell can be tracked and single colony is identified at a later time point
- Kinetic clonal outgrowth can be monitored on a daily basis

7. POSTER SUMMARY

- Celigo Image Cytometer can perform high-throughput CRISPR cell-based assays
- Imaging and analysis for 96- and 384-well plates in less than 5 min for bright field and 10 min for bright field + Fluorescence
- Currently, Celigo can be integrated with robotic automation to stream-line the entire cell line generation process
- More CRISPR cell-based assays are developed through our collaboration program