Rapid Monoclonality Verification Methods to Boost Cell Line Development

Wilson Lew, Anna Forsyth, John Philips, Alison Glaser, Natalia Lysaya
Molecular Devices, LLC, 1311 Orleans Drive, Sunnyvale, CA 94089

OVERVIEW

- Regulations for cell line development are increasingly more stringent. Providing evidence that a cell line is derived from a single cell is a crucial factor in cell line generation.
- CHO-S cells were stained in CellTracker Green CMDFA, a fluorescent dye used for monitoring cell location. The cells were then imaged on the CloneSelect Imager FL in white light and fluorescence.
- The CloneSelect Imager FL software automatically analyzed the fluorescence images to provide cell counts for each well. Users can view both the white light and fluorescence images to confirm the results.
- Single cell identification with the aid of fluorescence is more conclusive and robust, helping to meet stringent regulatory demands in cell line development.

INTRODUCTION

Limiting dilution or fluorescence activated cell sorting is typically performed to seed single cells into a well. Microscopy is then used to determine the number of cells seeded in each well and monitor cell growth. While monoclonality verification via white light imaging is possible, debris, dust, and air bubbles make it difficult and time consuming to identify single cells which may cause high value clones to be discarded.

Here we present a fluorescent method for identifying monoclonal CHO-S cells using CellTracker Green CMDFA. We incubated CHO-S cells with Cell Tracker Green CMDFA and performed limited dilution to seed single CHO-S cells into 96-well plates. The CloneSelect Imager was used to image CHO-S cells in white light and fluorescence channels. By using fluorescence to identify cells, monoclonality verification is easier and more conclusive than using white light imaging or microscopy alone.

METHODS

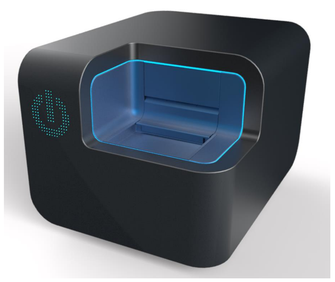
CHO-S Cell Staining with Cell Tracker Green

- CellTracker Green CMDFA (Life Technologies), a cell membrane permeable dye used to monitor cell location, was resuspended in DMSO to a concentration of 10mM and then diluted to 10µM in XP Media CHO Growth A (Part# K8860, Molecular Devices) supplemented with 4mM L-glutamine.
- CHO-S cells were initially cultured in XP Media CHO Growth A supplemented with 4mM L-glutamine and then stained with 10µM of CellTracker Green and incubated for 30 min at 37°C. After incubation, CHO-S cells were washed twice with XP Media CHO Growth A supplemented with 4mM L-glutamine.
- The stained CHO-S cells were seeded into a 96-well plate at a density of ~1 cell/well for all wells except well B2. Well B2 was seeded at a density of ~300 cells/well to facilitate setting the focus on the CloneSelect Imager FL.

White light and Fluorescence Imaging of CellTracker Green Stained CHO-S Cells

- The 96-well plates were imaged both in white light and fluorescence on the CloneSelect Imager FL. A FITC filter set was used for fluorescence imaging.

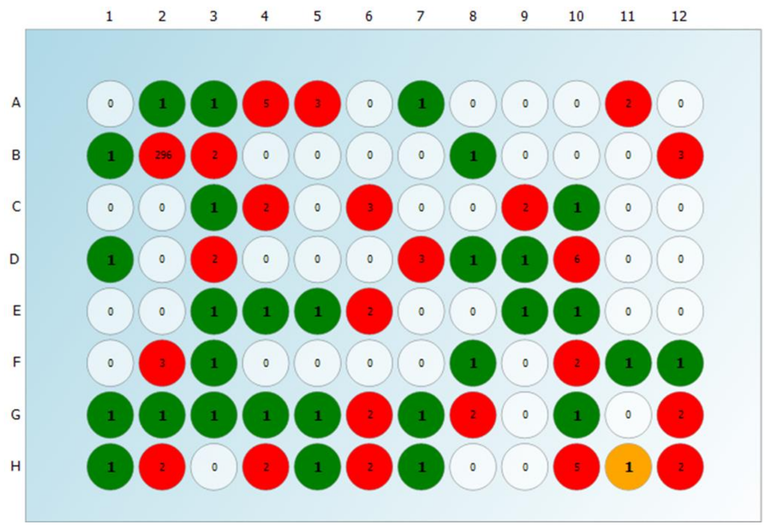
INSTRUMENT OVERVIEW



CloneSelect Imager FL

- Monoclonality analysis
- Colony forming assays
- Consistent determination of cell confluence and cell number estimation
- Automated generation of growth curves
- Suitable for adherent and settled suspension cells
- Integrable with automation allowing growth as throughput and workflows expand

CLONESELECT IMAGER FL MONOCLONALITY SOFTWARE ANALYSIS



Grey: No cells detected

Green: One cell detected with high certainty.

Orange: One cell detected with low certainty

Red: More than one cell detected

Figure 1. After a plate is imaged, the CloneSelect Imager FL software automatically generates a Cell Count plate view that displays the number of cells present in each well based on fluorescence detection. The wells are color coded for easy cell number viewing. Monoclonality certainty is determined by roundness criteria which can be adjusted to accommodate various cell lines.

EASY CELL IDENTIFICATION AND LOCATION USING THE CLONESELECT IMAGER FL WELL IMAGE VIEW

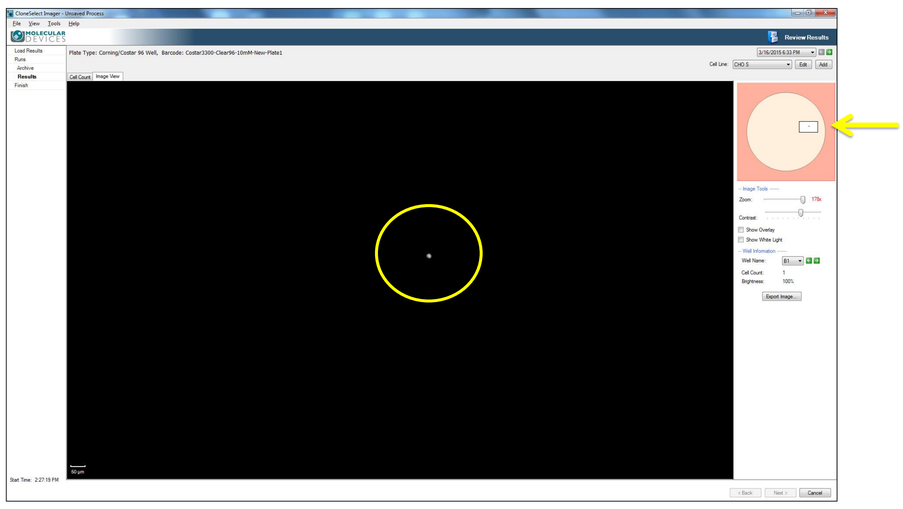


Figure 2. CloneSelect Imager FL software fluorescence image view of well B1 from the plate in Figure 1. In the middle shows the CellTracker Green fluorescence from a CHO-S cell (circled). On the top right (arrowed), there is a well layout that shows the zoomed image location (boxed) and the location of the cell in the well (red spot). The use of fluorescence and the well layout make it simple to locate the cell within the well.

WHITE LIGHT AND FLUORESCENCE IMAGING OF CELLTRACKER GREEN STAINED CHO-S CELLS

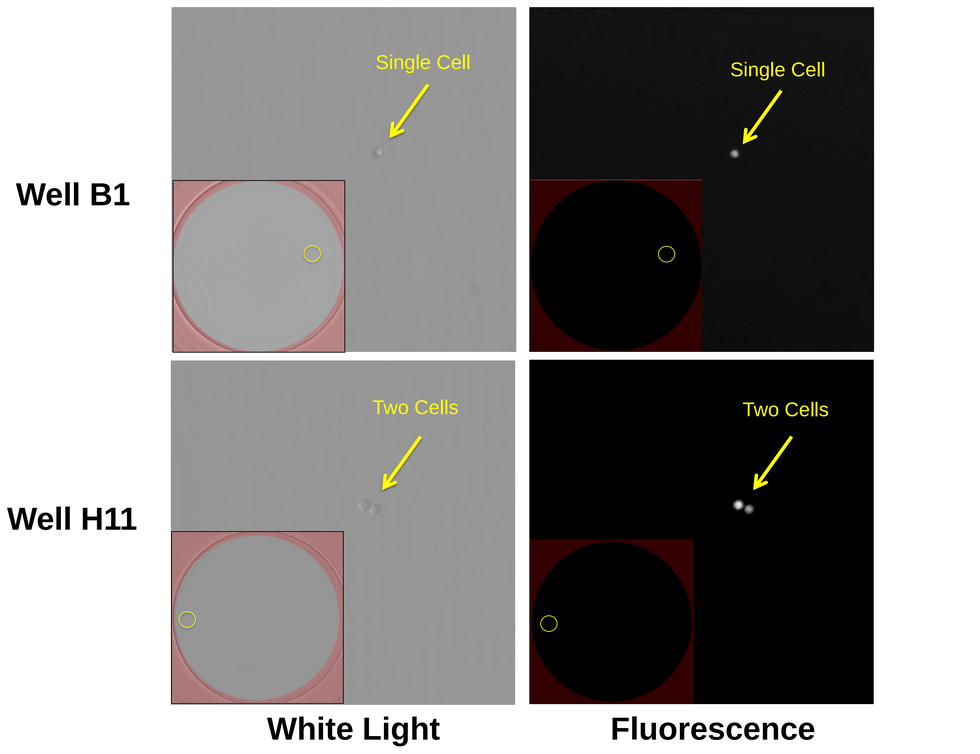


Figure 3. CloneSelect Imager FL imaging of the stained CHO-S cells from the plate in Figure 1. The whole well image is shown on the bottom left of each magnified well image. The circles on the whole well image show the location of the cells in the magnified image. The ClonePix FL software identified well B1 as having a high certainty of monoclonality and well H11 as having a low certainty of monoclonality. Because of the image resolution of the CloneSelect Imager FL, it is possible to identify two cells close to each other when looking at the white light image as seen in well H11. The cells in the image are round and have a 3D shape just as they would appear in a white light microscope making the cells easy to distinguish from debris. With the addition of a fluorescent image, confidence of monoclonality verification is enhanced. In the fluorescence images, we can clearly visualize that well H11 contains two cells while well B1 contains one cell.

CONCLUSIONS

- The use of a fluorescence cell stain provides a more reliable and conclusive method for determining monoclonality than the traditional white light method.
- The CloneSelect Imager FL and CellTracker Green staining can be used to fluorescently detect cells and determine cell number in a well for monoclonality verification. This method can be used for other fluorescence stains as well.
- The CloneSelect Imager FL provides fast, high quality white light and fluorescence imaging. The CloneSelect Imager FL software automatically performs cell count analysis on the fluorescence images making it easy to identify wells that contain a single cell.

MOLECULAR DEVICES