Assay Name: GFP Transfection Efficiency Measurement

Assay ID: Celigo_02_0018

Table of Contents

Experiment: GFP Transfection Efficiency Measurement

Purpose	To analyze transfection efficiency over a 4 day period when treated with various amounts of a transfection compound.
Current Method(s)	Manual observation using fluorescent microscope
Target Cell Type	293H cells
Experiment Plan	Plate transfected cells and measure transfection efficiency for 4 days
Hypothesis	Higher viral dosage treatments will lead to higher GFP numbers and fluorescent intensities

Celigo Setup

Plate Type	Greiner 655090 96-well black wall clear bottom
Scan Channels	Bright field + Green
Resolution	1 µm/pixel
Scan Area	Whole well
Analysis Method	Confluence Ratio: Confluence 1 + 2
Scan Frequency	Daily
Scan Time	~8 min

Assay Protocol and Plate Setup

Goal

To analyze transfection efficiency over a 4 day period when treated with various amounts of a transfection compound.

Protocol

Cell preparation

- Transfected cells were collected and plated on Day 0 following the plate map below

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		60,000 cells/well					80,000 cells/well					
C												
D												
E												
F												
G												

- After plating, the plate was centrifuged to settle the cells in the bottom of the dish, in order to image the cells in a monolayer for optimal focus
- Cells were then treated with compounds following the plate map below

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		Control	Low	High	Low	High	High	Low	High	Low	Control	
C												
D												
E												
F												
G												

- The plate was then imaged using Celigo for bright field and GFP green fluorescence
- The plate was imaged and analyzed on Day 0, 1, 2, and 3

Data Collection

1. After centrifuging the plate, the plate was scanned using the Celigo
2. The scanning parameters for 2 channels were set up, where Confluence channel 1 + 2 are GFP and bright field, respectively

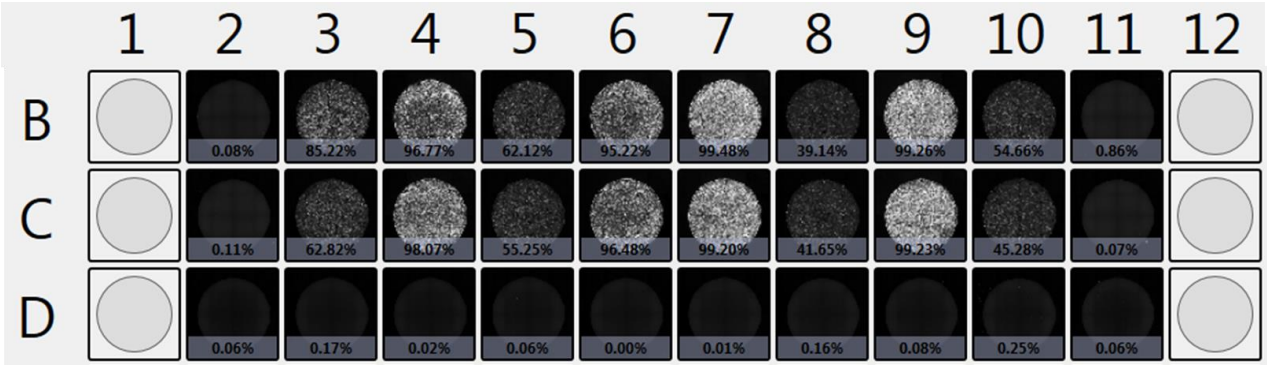
Data Analysis

- The images were analyzed to measure total area of cell coverage in bright field and in fluorescence
- The automatically calculated confluence ratio indicates GFP transfection efficiency as a percentage of the total cell area

Results

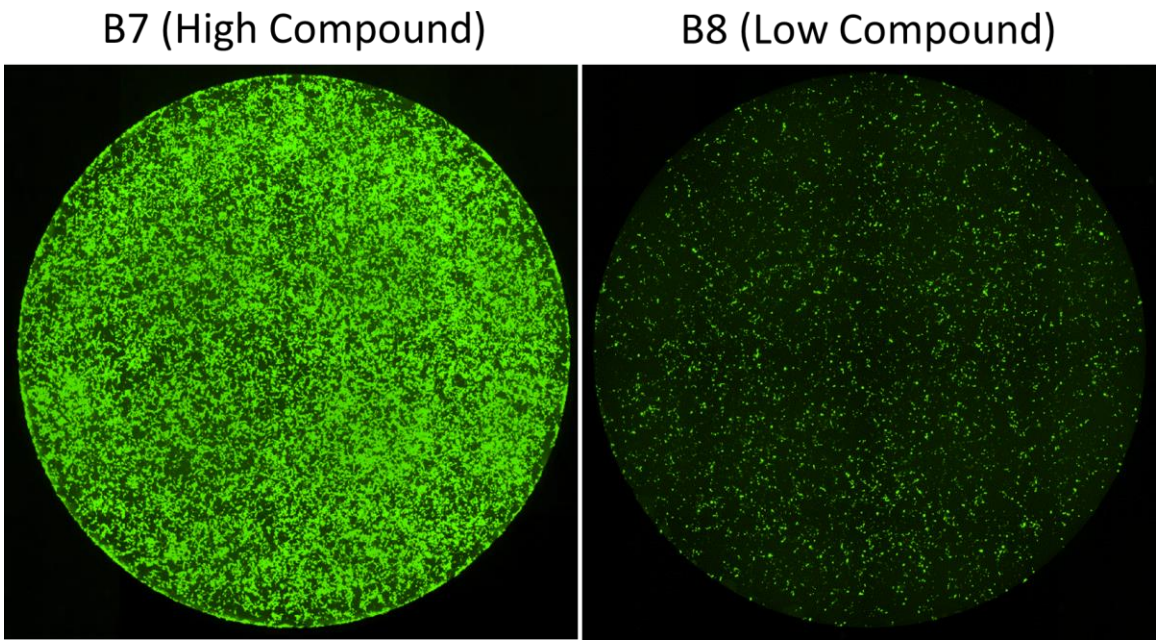
1. GFP expression fluorescent images

- The GFP fluorescent images showed differences between low and high compound treatment
- The GFP expression plate view is shown below

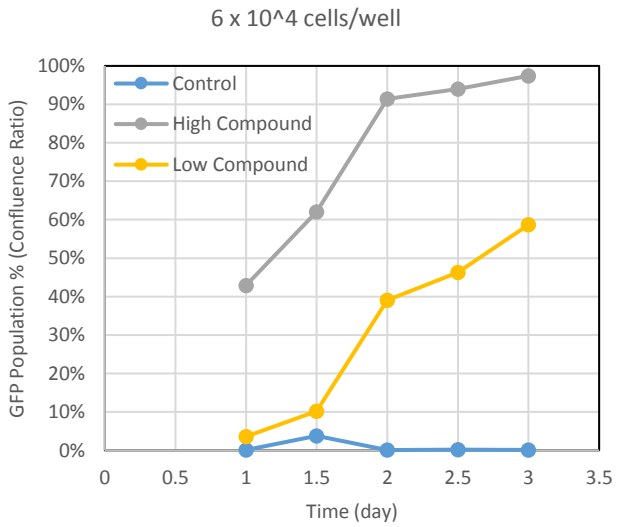


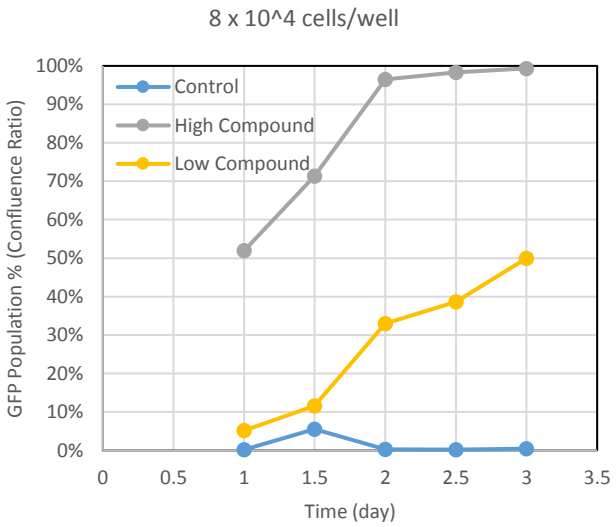
2. GFP transfection efficiency measurement results

- Whole-well fluorescent images reveal the GFP expression level for cells treated with high and low concentration of compound



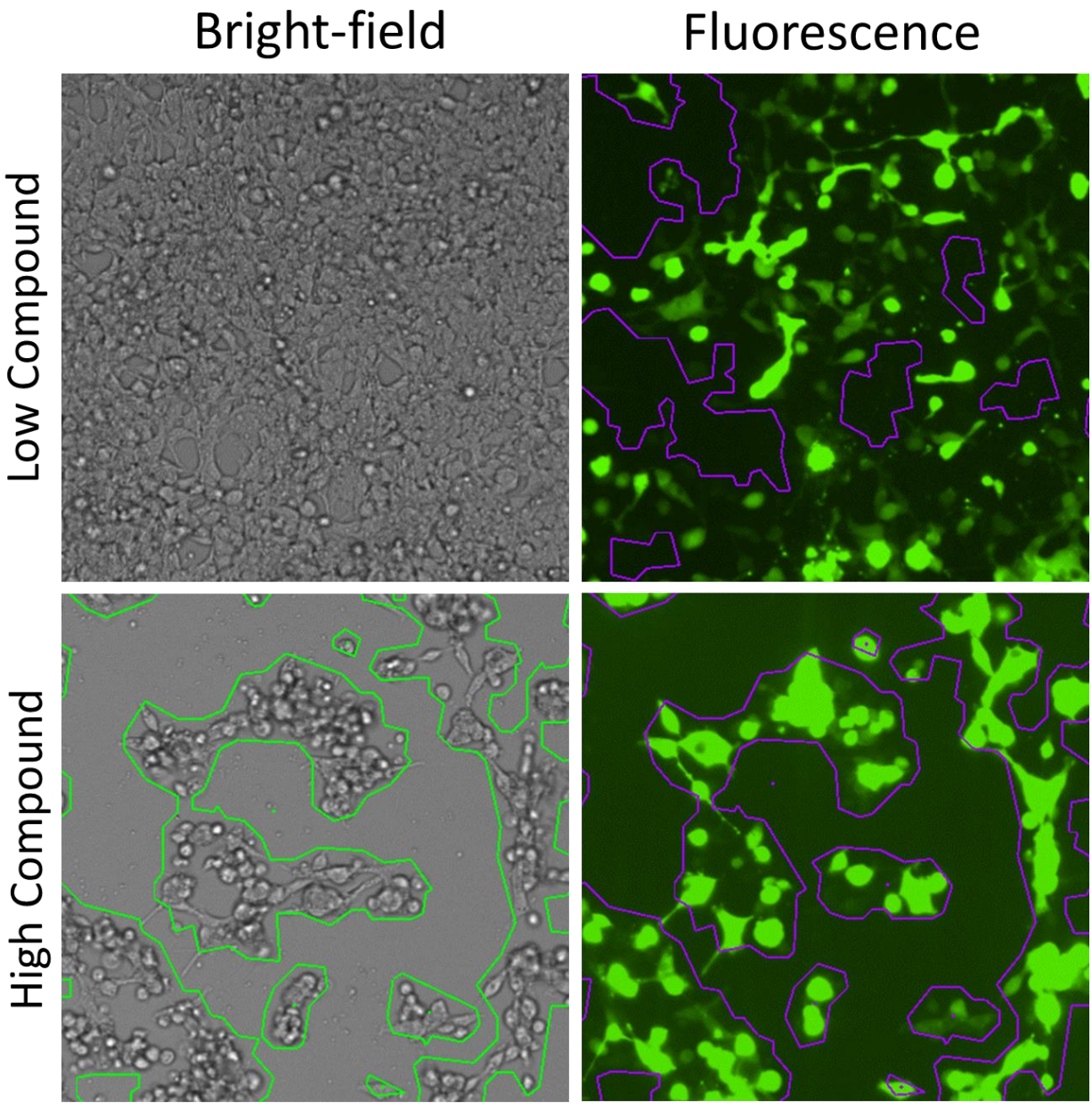
- The GFP transfection percentage for 60,000 cells/well showed no expression for the negative control, low compound treatment showed 60%, and the high compound treatment showed 100%
- The results were similar for the 80,000 cells/well

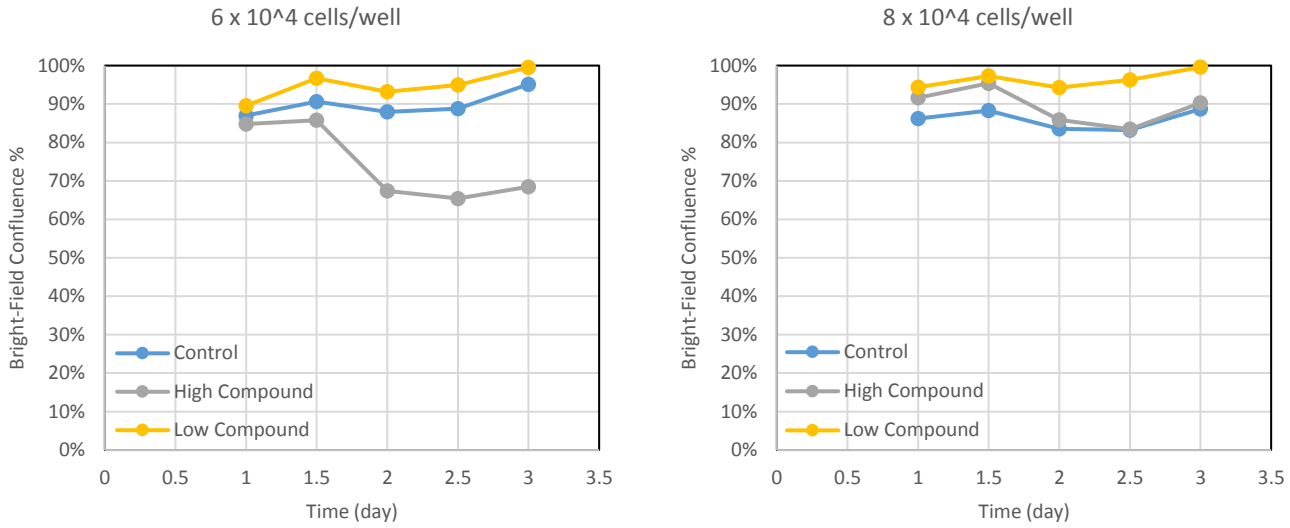




3. Bright field confluence level

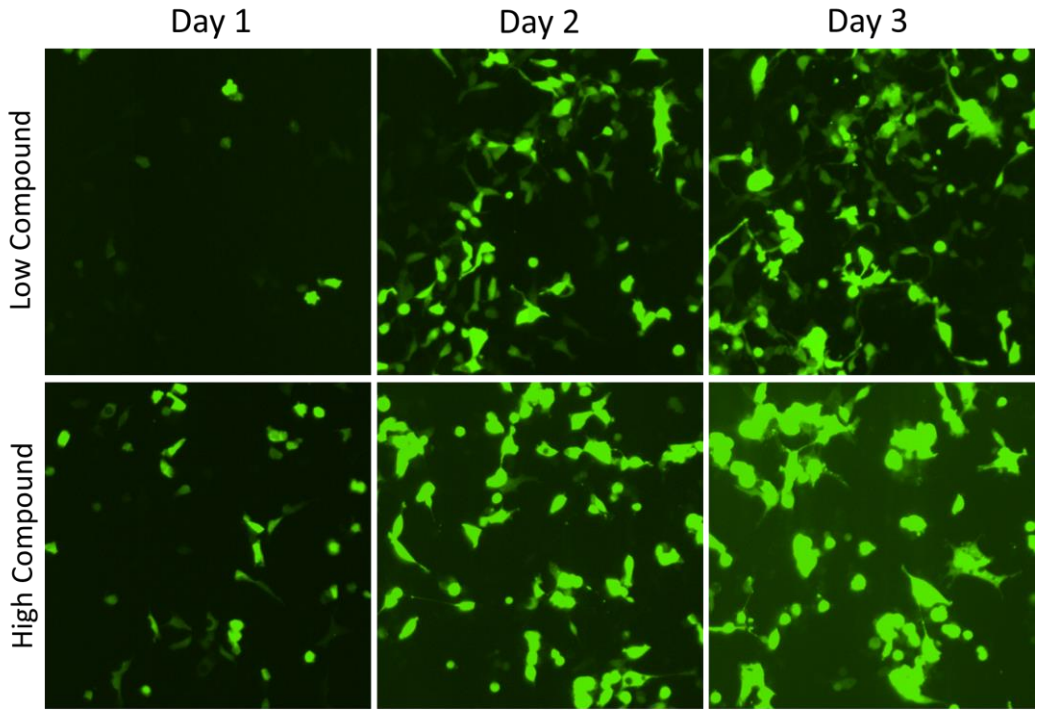
- The bright field images showed that at high compound treatment, there is reduction in cell confluency, in comparison to the low compound treatment





- At 60,000 cells/well, the high compound treatment showed a large decrease in cell confluency
- At 80,000 cells/well, the high compound treatment did not show any decrease in cell confluency

4. GFP transfection time-course images
   - Green fluorescent images showed an increase in the number of GFP-positive cells over 3 days of culture
   - The low compound treatment resulted in fewer GFP-expressing cells compared to the high compound treatment



Conclusion

- The GFP transfection efficiencies were highly dependent on the compound treatment
- At lower seeding density, the cells showed higher damage due to high compound treatment