Monitor iPSC Reprogramming, Stem Cell Pluripotency, Differentiation

Here we demonstrate the ability of the Celigo adherent cell cytometer to combine the advantages of microscope imaging and flow cytometry population analysis in a plate-based kinetic assay for the study of iPSC reprogramming.

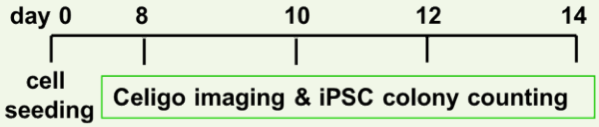
- Follow iPSC reprogramming over time without trypsinization
- Image, record and detect all the colonies in the whole well of 6-well plates
- Faster assay (7 minutes to read a 6-well plate)
- High proliferation rate of iPSC and death of feeder cell will not affect results as compared to FC analysis.

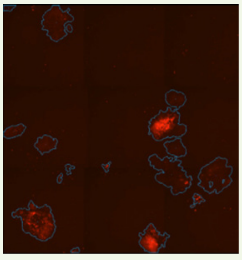
Using a transgenic MEF cell line containing mOrange OKSM and GFP Nanog we were able to track the evolution of iPSC colonies by detecting the production of orange colonies and the subsequent evolution of green colonies.

iPSC Reprogramming Experiment 1

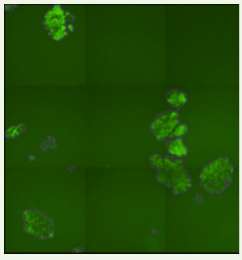
1. Plate at 5x 10^4 transgenic murine embryonic fibroblast (TMef) and 1x10^5 Wild-type (WTMef) in 6-well gelatin coated costar plate (cat # 3516).
2. Doxycyline induction of Yamanaka factors produce mOrange colonies and upon preprogramming progression colonies start to express Nanog GFP. Media is replenished every two days and contains Doxycyline to keep reprogramming on track. Nanog GFP normally appears at D8 so that is when imaging and colony counting starts.
3. To monitor the progression of reprogramming, the plates were scanned and analyzed to score total colonies and green colonies every two days from d2 –d14.

Data courtesy of Kaji Lab, MRC Centre for Regenerative Medicine, University of Edinburgh.

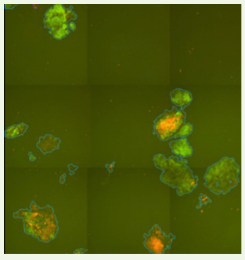




Count mOrange+ colonies

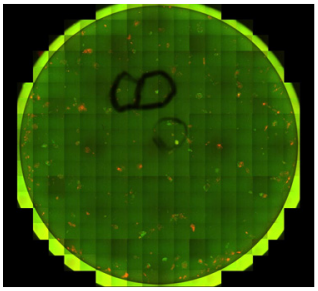


Count Nanog GFP

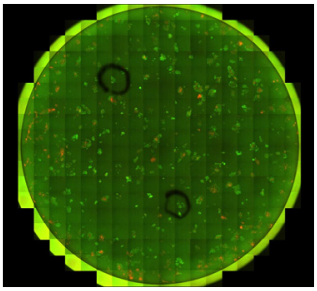


Overlay of green and red images

Level of Reprogramming



Low

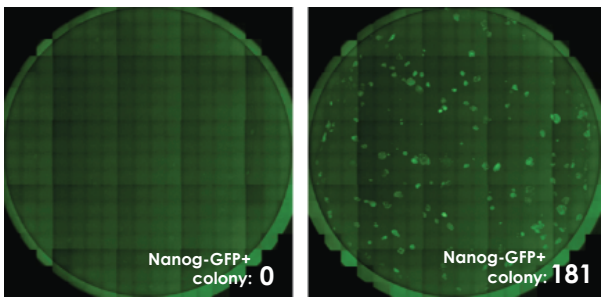


High

Reprogramming	# of Colonies	# of Orange Colonies	# of Green Colonies	% Reprogramming
Low	200	200	50	25
High	700	700	600	86

iPSC Reprogramming Experiment 2

The Celigo assay allows researchers to investigate other factors involved in the reprogramming process. Here we can observe the addition of shRNA for factor x enhances the production of Nanog-expressing colonies. From this data we can summarize that factor x acts upstream of OKSM and downstream of Nanog and may play a key role in iPSC reprogramming



Current practice in iPSC Reprogramming Analysis

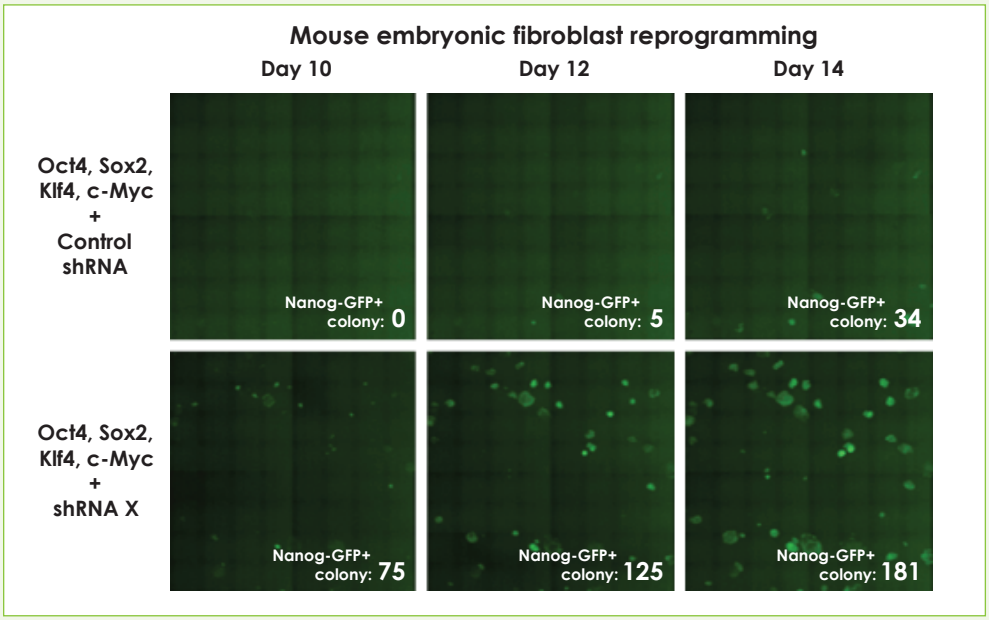
A combination of microscope and flow cytometry analysis were used. The microscope was used to visualize reprogramming. One or two representative colonies were chosen to image throughout the process. These colonies were identified using a marker pen for identification. iPSC antigen expression was then examined using flow cytometry post-trypsinization.

Disadvantage of Microscope Analysis for iPSC Reprogramming

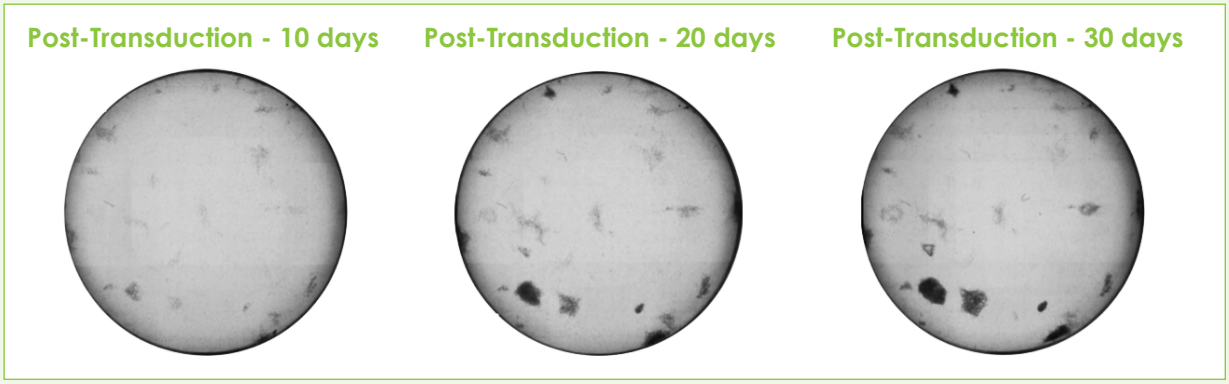
- Can only follow a small number of colonies throughout the reprogramming
- Colonies chosen to follow the reprogramming process may not be ideal examples
- Finding the same focal plane not always possible
- Images need to be exported and analyzed on a separate image processing software package.

Disadvantage of Flowcytometer Analysis for iPSC Reprogramming

- Trypsinize stem cell colonies into single cell suspension for cell population analysis does not reflect colonies with varying size and composition
- Interference from MEF cells and dead cells due to trypsinization and manipulation
- Destructive, requiring new sample for each time point

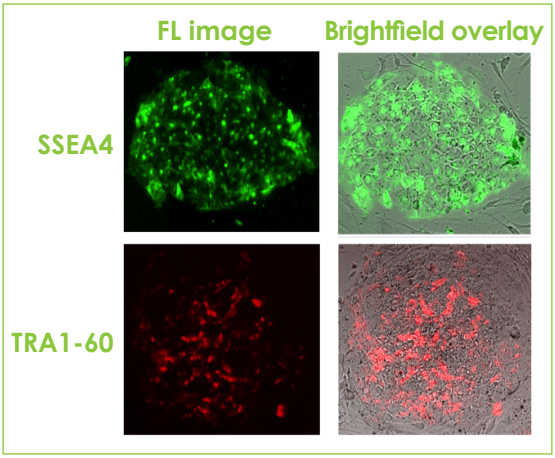


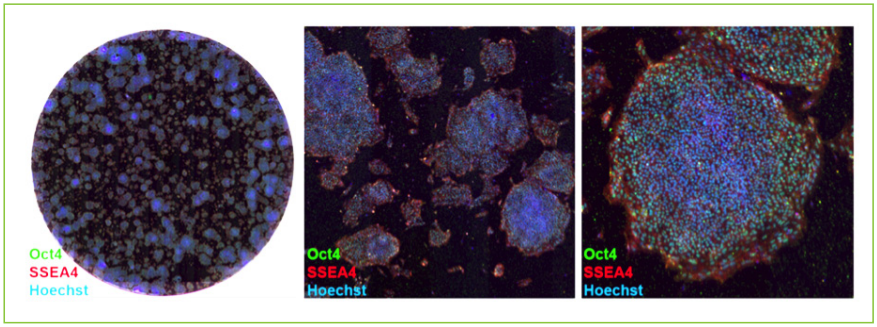
Monitor Human Fibroblasts Reprogramming Experiment Over Time



Whole-well imaging of human fibroblasts reprogramming experiments monitored in 12-well plates using the Celigo at 10 and 30 days post-transduction.

Measure stem cell pluripotency with live-cell staining





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