A software tool for the automatic, label-free selection of induced pluripotent stem cell colonies at early time points during reprogramming

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Introduction

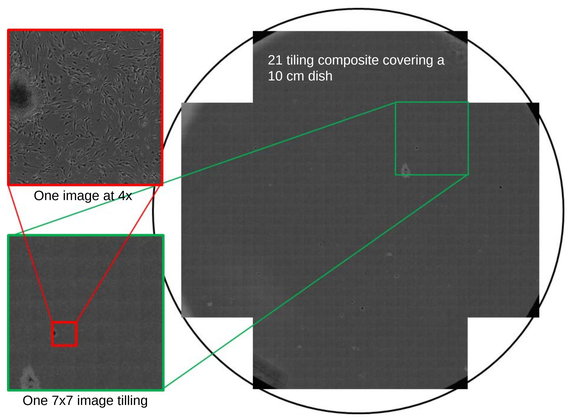
Induced pluripotent stem (iPS) cell technology is currently revolutionizing basic research and drug discovery, and poised to have major impact in health care broadly. Soon it could be routine practice to create a personalized cell bank containing your own cells for personalized drug testing, diagnostics, cell therapy, and other future applications of personalized medicine.

iPS cell technology is often used to reprogram a patient's fibroblast cells from a skin biopsy into an embryonic cell-like state where they form up into colonies. A few of these pluripotent stem cell colonies are selected, transferred to their own container and then grown into a patient-specific embryonic stem cell line. This line can be maintained or directed to differentiate into many types of mature cells. Applications include generating many cell lines from different patients for drug discovery, or for personalized drug efficacy testing or cell therapy in a clinical setting.

This reprogramming process causes the fibroblasts to change morphology, proliferate and form up into hundreds and thousands of colonies – of which only a very few have been fully and successfully reprogrammed to become embryonic cells. The task of selecting these few embryonic colonies is highly labor intensive and requires skilled stem cell scientists. As the industry looks to automate cellular reprogramming for high-throughput applications a critical problem is the lack of a method to automatically pick fully reprogrammed, true iPSC colonies at early time points in adherent cultures. Early, automated selection of iPSC colonies can greatly reduce the cost and labor of iPSC production, facilitating high-throughput deployment of iPSC technology. Selection in adherent cultures can reduce the complexity of automation systems, and therefore lower their cost and training requirement.

In a previous study, we used our kinetic image pattern recognition technology to uncover a promising, kinetic pattern of colony formation that can reliably predict the emerging colonies that will become fully reprogrammed in patient fibroblast samples undergoing reprogramming. This technology could be deployed as an automated software tool for the automatic, label free selection of iPSC colonies at early time points during reprogramming. Here we present an overview of the technology and its preliminary validation on human patient diseased and healthy neonatal fibroblasts undergoing retrovirus- and Sendai virus-mediated reprogramming. In addition we discuss the application context of the tool and possible automation protocol. The results show that our imaging software can be used with each of these reprogramming methods to select iPS colonies at early time points with high specificity.

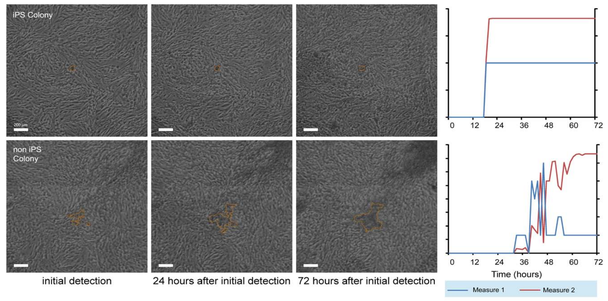
Imaging reprogramming



Our research was conducted using the Nikon BioStation CT and CL-Quant software (Nikon Corporation). The CT allows continuous imaging and incubation of the cells undergoing reprogramming. In these studies the CT generates spatially aligned images which are formed into a composite of tiled image sequences upon loading in CL-Quant covering the complete 10 cm dish. One time point consists of 21, 7x7 image tilings as shown in the figure above. The CT provides long term, whole vessel coverage enabling detection of the rare iPSC colony formation events.

In our preliminary validation, we used three patient samples, two protocols and healthy neonatal and diseased samples from Spinal Muscular Atrophy (SMA) patients. All samples were imaged once every 6 hours using phase contrast optics at 4x magnification.

Kinetic image pattern recognition



CL-Quant provides excellent image detection tools that enable quantification of colony formation in phase contrast image sequences. Using CL-Quant we are even able to quantify early events prior to formation. Measurements characterizing pre and post colony formation are assigned to colony tracks and are easily exported. Next, we generate kinetic statistics from the raw measurements, and apply a classification rule using our proprietary tools under development. The objective is to have near perfect specificity, while accepting lower sensitivity so long as at least three colonies per reprogramming run are detected.

Results

Table 1. *Colony selection can be done as early as 7.5 DPI*

Days post-infection (DPI)		DPI 7.5	DPI 10	DPI 12.5
All Detected	iPS	4	6	8
	non-iPS	556	1122	1740
Rule 1 (+0 days)	iPS	3	4	6
	non-iPS	15	37	71
Rule 2 (+2 days)	iPS	2	2	3
	non-iPS	0	0	1

We evaluated the performance of the classification rule at different time points using one fibroblast sample from an adult SMA patient, imaged every 6 hours while undergoing retrovirus - mediated reprogramming using Klf4, Oct3/4, Sox2 and c-Myc. Two rules were applied. The first used kinetic data from the first day after detection, and the 2nd rule used kinetic data up to 3 days after detection (to 10.5 DPI). At DPI 7.5 560 colonies were analyzed, and 2 iPS were selected with no false positives after the 2nd rule; the performance was the same at DPI 10 and just one false positive was detected at DPI 12.5. Here we report only the test data, no training data is reported. The results show that picking could be done reliably as early as 7.5 days DPI.

Table 2. *Improved classification rule performance under two protocols*

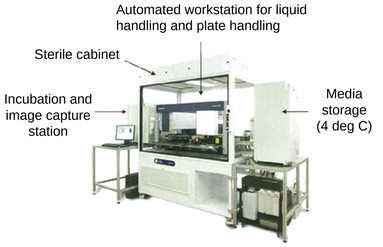
Category	non-iPS	iPS	Total count	Error count	Error rate
non-iPS	587	0	587	0	0.000
iPS	5	9	14	5	0.357
Total count	592	9	601	5	0.008
Error count	5	0	5	-	-
Error rate	0.008	0.000	0.008	-	-

In Table 2, an improved classification rule was applied to an adult SMA fibroblast patient sample and neonatal fibroblast sample, also imaged every 6 hours while undergoing Sendai virus - mediated reprogramming using Klf4, Oct3/4, Sox2 and c-Myc . A single rule was applied at DPI 7.5. 472 colonies were analyzed, resulting in 11 good iPS detections and one false positive.

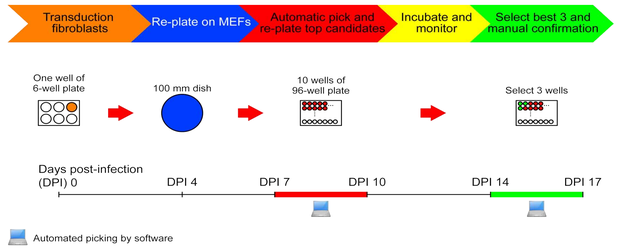
Discussion

Automated iPSC Selection

The software tool could be incorporated into a fully automated iPS cell reprogramming and expansion system. A fully automated system would include many components such as liquid and plate handling, media storage and cell incubation. Importantly a phase contrast microscope, computer and digital camera are required.

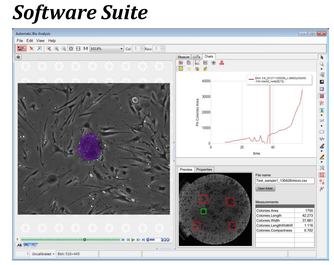


Possible Automation Protocol



The figure above illustrates an attractive protocol that could be executed once our iPSC selection tool is integrated with a fully automated picking system. After re-plating to mouse embryonic fibroblast, a 10 cm dish is imaged every 6 hours for 3 – 6 days. At that point (DPI 7 - 10), the best 10 colonies are selected automatically using the iPSC selection tool, and transferred to 10 wells of a 96 well plate where they are further imaged once daily for a week (DPI 14-17), and the final colonies are chosen automatically. This protocol is simple, requires no fluorescent markers, and picking at DPI 7-10 allows for vessel format reduction to save on reagent costs.

Software Suite



In addition to an in-line, behind-the-scenes picking module, the software could also provide an interactive user interface for colony selection. The technician could be presented with a number of colonies selected by the software, along with associated data and goodness metrics. A record of the selection criteria could be saved to assist with methodology standardization, reporting and quality enhancement best practice. In addition, a suite of tools could be provided for other stem cell tasks. For example, software modules for monitoring the culture quality of lines under expansion (i.e. to identify differentiated colonies, or merged colonies) , or other procedures such as genome editing.

Conclusion

Here we have presented the concept for an automated iPS colony selection system using kinetic image pattern recognition applied to large composite, time-lapse sequences in phase contrast optics showing adult diseased and healthy neonatal fibroblasts undergoing reprogramming using two protocols. We are currently working to validate the automated iPSC colony identification software tool on a large database of 16 patients. While our current study has one false positive, we are developing new kinetic measurements customized for characterizing colony formation. We anticipate that the new measurements will give us improved discrimination power.

In addition to an automated, in-line selection function, the software could also provide an interactive workflow to assist users in iPS colony selection and reporting, as well as quality monitoring during expansion and genome editing. We are working with stem cell core labs to identify the usage requirements for the software.

Acknowledgements

The project described was supported by Grant Number R44HL106863 from the National Heart, Lung, And Blood Institute, and the Harvard Stem Cell Institute. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Heart, Lung, And Blood Institute, the National Institutes of Health or the Harvard Stem Cell Institute.