Increase iPSC Colony Generation and Outgrowth

The innovative CellRaft® Technology is revolutionizing the field of stem cell research by removing bottlenecks in iPSC monoclonal colony generation. With the ability to grow stem cells in flask-like culture conditions prior to analyzing and automatically isolating them, this technology is unlocking new insights into the behavior and potential of these remarkable cells by enabling:

- increased clonal iPSC generation by 5X
- tracking of iPSCs from single cell to clone
- reprogram, characterize, edit, or expand on one consumable
- reduced time, consumables, materials, and labor required
- 2D and 3D applications
- the gentle, automated isolation of iPSC colonies

Common Challenges

1) iPSCs are sensitive and easily perturbed, requiring constant maintenance.
2) Poor culture conditions and cell line instability can lead to spontaneous differentiation and loss of pluripotency.
3) Generating monoclonal cell lines is incredibly challenging, with low efficiency and lack of proven clonality.
4) The labor, cost, and reagent burden associated with iPSC maintenance and workflows is incredibly high.

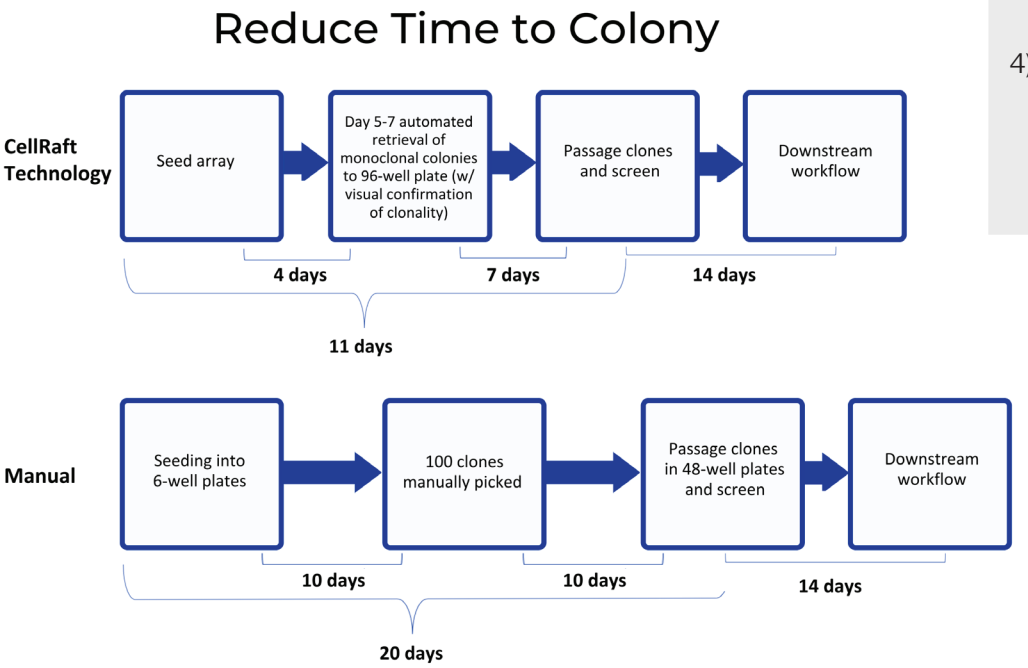


Figure 1. Compared to a manual limiting dilution process, clones were able to be passaged and screened nine days earlier using CellRaft Technology. In addition, less hands-on time was required with CellRaft Technology as the system is able to automatically retrieve colonies and move them to 96-well plates.

Save Time, Consumables, Materials, and Labor

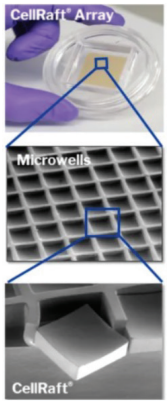
The labor, cost, and reagent burden associated with iPSC maintenance and workflows can be incredibly high. With CellRaft Technology the volume required for media and Matrigel are reduced while the number of viable colonies is increased compared to a limiting dilution method.

Efficient Scale-up for Maximum Clones

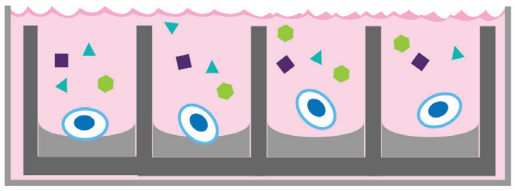
	Limiting Dilution	Single Array	% Change
# Monoclonal Colonies	100	~500	⬆ 5X
Volume Media	90ml	10.5ml	⬇88%
Volume Matrigel	66.67ul	44.44ul	⬇33%

Grow Cells in a Flask-like Culture Environment

At the heart of CellRaft Technology is the CellRaft Array which contains thousands of microwells, called CellRafts, that allow cells to be spatially separated while sharing a contiguous media volume, mimicking the growing conditions of a flask, which leads to improved viability and proliferation of single cells.



Shared Media Improves Cell Viability



Single cells are grown into colonies and then isolated at the colony stage, resulting in a higher percentage of outgrowth (Figure 2). Colonies that were derived from a single cell in the CellRaft were located using the track-and-trace features within the software for isolation, resulting in a full plate of high viable monoclonal colonies.

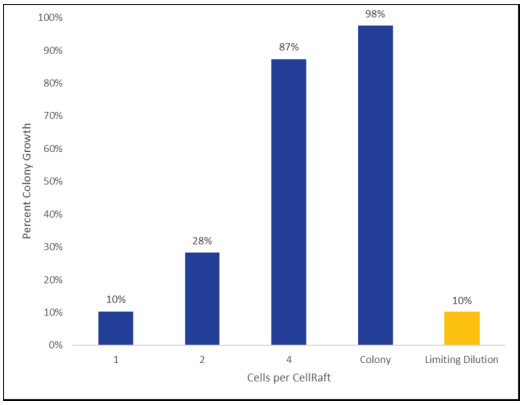


Figure 2. The CellRafts in the experiment above were isolated at different growth stages and the success rate of growth increases significantly when the cells are isolated at the colony stage.

Track and Trace from Single Cell to Clone

Using the CellRaft AIR® System, images of the CellRaft Array are taken at multiple time points as cells grow from single cells into colonies allowing for visual confirmation of clonality (Figure 3).

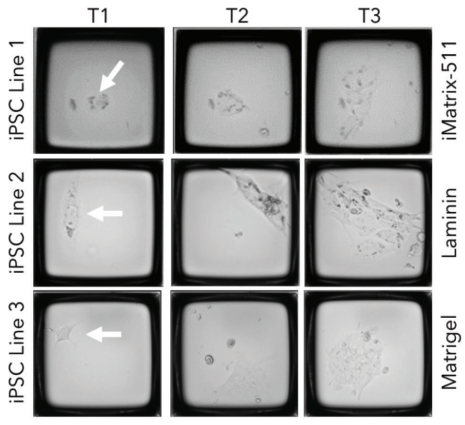


Figure 3. Three different iPSC cell lines were seeded on CellRaft Arrays on one of three coatings (iMatrix-511, h-ESC Matrigel, or Laminin-521). The arrays were serially scanned starting 4 hours post-cell seeding and every 24 hours after to monitor clone formation.

Software-guided Identification and Automated Isolation of Cells of Interest

CellRaft Cytometry™ software allows for image-based verification of single cells to ensure monoclonality and analysis of a variety of parameters over time, ranging from size and morphology to gene expression. Users can easily define the characteristics of the target cells or colonies and map them for software-guided CellRaft selection and automated isolation by the CellRaft AIR System.

After identification, the CellRafts are gently dislodged from the CellRaft Array by a release needle and retrieved from the array by a magnetic wand to transfer the desired iPSCs into 96-well plates for downstream analysis. The fully automated system enables gentle, dissociation free isolation of fully validated, intact clones for propagation without the use of fluidics.

Grow and Isolate iPSC-derived Organoids

CellRaft Technology can be used to grow and maintain hundreds of individual organoids. You can serially image the same organoid over time and phenotypically characterize organoids to identify the ones of interest for automated isolation (Figure 4).

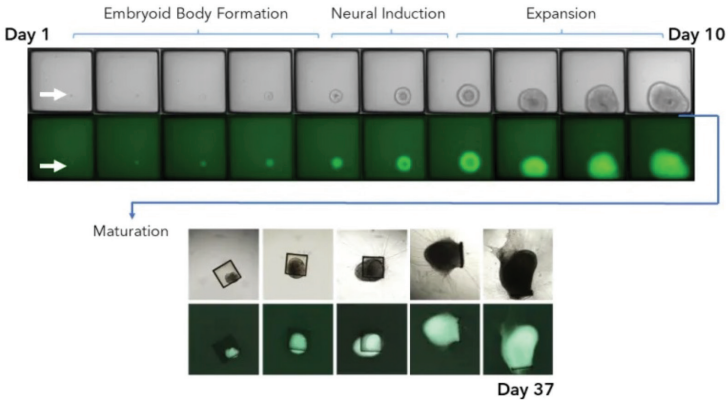
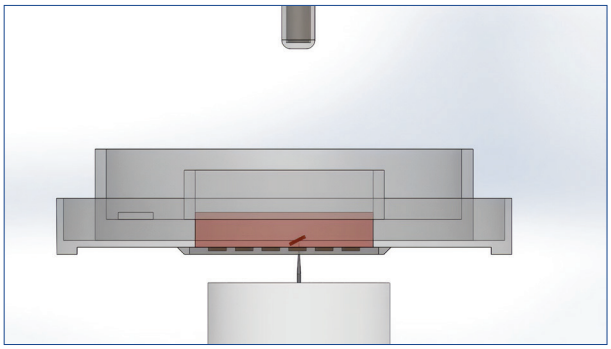
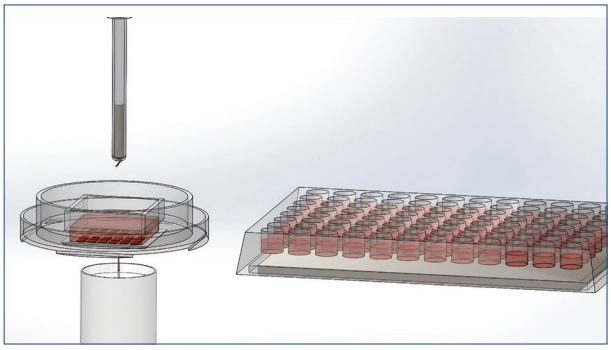


Figure 4. A single green organoid is identified at day 1. The majority of the expansion is done by day 10 and then it is isolated into a collection plate. The organoid continues to grow off of the array in the collection plate.

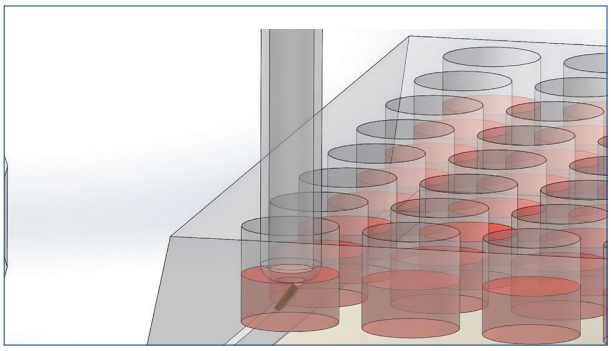
The desired microwell with cells is dislodged from the array



The wand picks up the microwell using a magnet

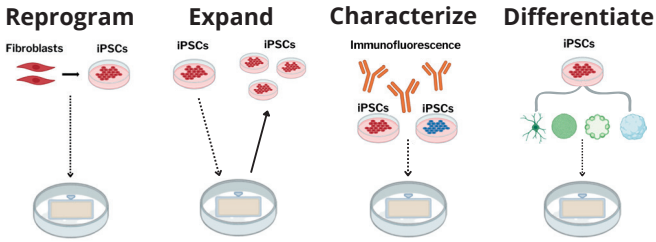


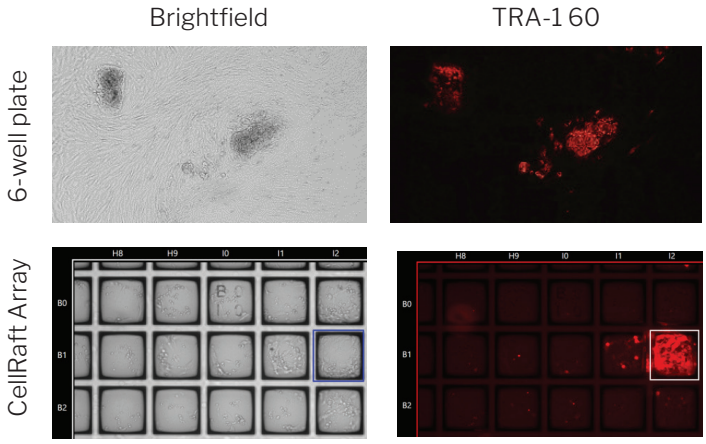
The wand places the microwell with the cells in the 96-well plate

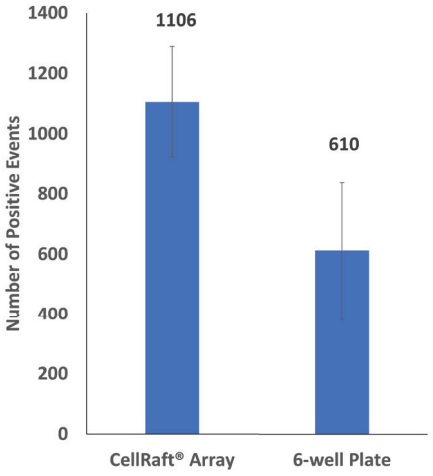


Enable All iPSC Workflows Using CellRaft Technology

- Reprogram differentiated fibroblasts into pluripotent stem cells (Figure 5)
- Expand the clonal population on the array
- Characterize for phenotyping or pluripotency
- Differentiate into 3D tissues or organoids







Automated isolation of nearly 2X the number of reprogrammed colonies

Figure 5. BJ fibroblasts were transfected using the Epi5 Episomal iPSC Reprogramming Kit and cultured for 15 days in a 200µm CellRaft Array or in a 6-well plate. On day 15, cells were live-stained using the TRA-1-60 Alexa Fluor 594 Conjugate Kit and positive events were calculated using CellRaft Cytometry for the Array or manually counted in the 6-well plate (red fluorescence).

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