

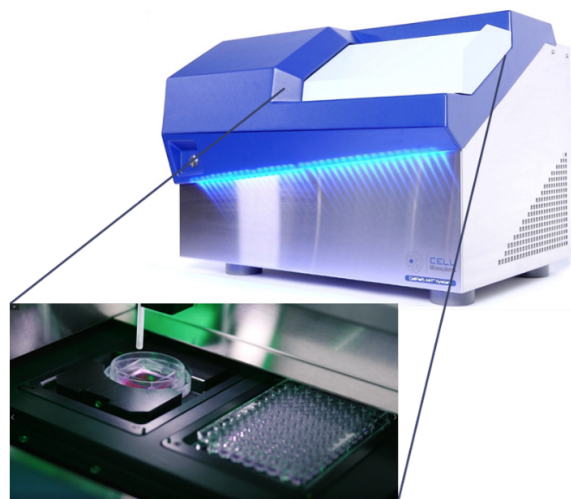
## Using the CellRaft AIR® System to Image, Identify, and Isolate Suspension Cells

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### Summary

The CellRaft® technology developed by Cell Microsystems allows for streamlined and efficient cell line development by leveraging the combined power of our CytoSort® Array consumable with our automated imaging and isolation instrument, the CellRaft AIR® System. The AIR System offers an efficient solution to an otherwise labor-intensive workflow. It supports cell health, time-course imaging for clonal verification, and automated isolation for downstream propagation, altogether providing an alternative that is more time, labor, and cost-effective for cell line development. We have extensively demonstrated that the CellRaft AIR System can accelerate workflows using adherent cell lines. Although adherent cell lines are widely used for research and discovery, suspension cells are often employed for a variety of biopharmaceutical applications, including large scale recombinant protein, vaccine, and monoclonal antibody production. There are many instruments, such as flow cytometers and droplet dispensers, that can screen suspension cells. The limitations to these technologies include impacts on cell viability, inability to interrogate small numbers of cells, and reliance on fluorescent markers or staining for cell characterization.

To demonstrate the value of the CellRaft technology for use in suspension cell line development, we have established methodologies for attaching suspension cells to the CytoSort Arrays during single cell expansion while still

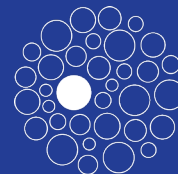


**Figure 1:** The CellRaft AIR System and CytoSort Array provide fully integrated brightfield and 3-channel fluorescent imaging, automated release and transfer, and optional stage-top incubation.

allowing the cells to expand in suspension after the clone of interest has been isolated.

### Using the CellRaft AIR System and CytoSort Array

The CellRaft AIR System and CytoSort Array offer a novel approach to single cell isolation and recovery that are ideally suited for generating monoclonal cell lines. Cells are first seeded on the CytoSort Array – a disposable microwell array consumable for culturing and imaging (Figure 1) – at an appropriate density to segment them as single cells within the microwell chambers. While the cells settle and adhere to the polystyrene CellRafts at the bottom of each microwell, they share a contiguous media reservoir, allowing for exchange of growth factors and other cell secretions that improve cell viability and



proliferation, a unique benefit not available in single cell plating methods such as limiting dilution, FACS, and droplet dispensers.

After cell attachment, the CytoSort Array is scanned and imaged in as little as 10 minutes using the CellRaft AIR System, first to identify rafts with a single cell, then periodically throughout the experiment to monitor colony formation of single cells in each segregated microwell on the array. The AIR System contains an internal microscope capable of brightfield and three-channel fluorescent imaging, and the software conveniently stores scans in a central database for each array, allowing for phenotypic analysis of cells of interest and easy tracking of colony growth over time.

CellRafts with monoclonal colonies can be selected, released from the CytoSort Array, and transferred into a 96-well plate for further expansion. A motorized needle penetrates the resealable elastomeric floor of the CytoSort Array to displace the individual CellRaft from its microwell. The CellRaft material is loaded with magnetic nanoparticles, allowing it to be retrieved with a magnetic wand. The cells remain attached to the CellRaft through the transfer, obviating trypsin or other dissociation reagents, which when combined with isolation at the small-colony stage, preserves cell viability and phenotype. The overall workflow reduces hands-on time and dramatically improves the output, providing an alternative that is more time, labor, and cost-efficient for cell line development.

### The Suspension Cell Challenge

To date, we have characterized over 40 adherent cell types on the AIR System. This workflow is straightforward for adherent cell lines ([see Cell Characterization protocol](#)), as adherent cells readily attach to the polystyrene CellRaft, divide, and remain attached during the isolation process.

Cell Line	Cell Type	Cell Source/Cat #
FreeStyle™ 293-F	Human embryonic kidney	Thermo Fisher/R79007
Mab 108	Mouse hybrid-oma/B-lymphocyte	ATCC/HB-9764
Jurkat	Human T-lymphoblast	ATCC/TIB-152
Raji	Human B lymphocyte	ATCC/CCL-86
THP-1	Human monocyte	ATCC/TIB-202

**Table 1:** Suspension Cell Lines Tested

Coating	Catalog #	Company
Poly-D-Lysine (PDL)	A3890401	Gibco
Poly-L-Lysine (PLL)	A005C	MilliporeSigma
Poly-L-Ornithine (PLO)	A004C	MilliporeSigma

**Table 2:** Coatings Tested

However, suspension cells that grow in liquid media without attaching to the tissue culture surface present a unique challenge for use on the CytoSort Arrays. Standard protocols for seeding suspension cells are compatible with the imaging capabilities of the AIR System; however, if the cells are not physically adhered to the CellRaft prior to isolation, it is difficult to maintain clonality and the cells cannot be reliably retrieved and expanded off-array. A potential solution is to employ commercially available cell coating substrates that promote cell adhesion to tissue culture surfaces. To determine whether CytoSort Arrays can be utilized to develop monoclonal suspension cell lines, we tested five suspension cell lines and three cell coating substrates (Tables 1 and 2).

### Coating CytoSort Arrays

We have previously concluded that properly coating the CellRafts with substrates such as Matrigel requires a longer exposure to the coating than is typically recommended by manufacturer datasheets. To determine optimal coating conditions for the substrates being tested to adhere suspension cells,

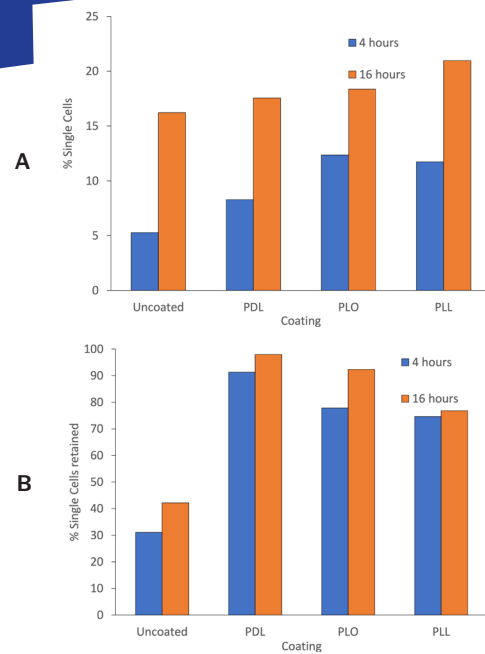
## Using the CellRaft AIR® System to Image, Identify, and Isolate Suspension Cells

CytoSort Arrays were incubated with 100ug/mL of either PDL, PLL, or PLO for four or 16 hours (for proper array preparation prior to coating, see the [CytoSort Array User Manual](#)). Jurkat cells were seeded on the coated arrays and allowed to adhere to the CellRafts, and the arrays were scanned on the AIR System at four and 24 hours post-seeding. Using the Off-the-AIR software, the total number of CellRafts containing a single cell was counted for each coating and timepoint.

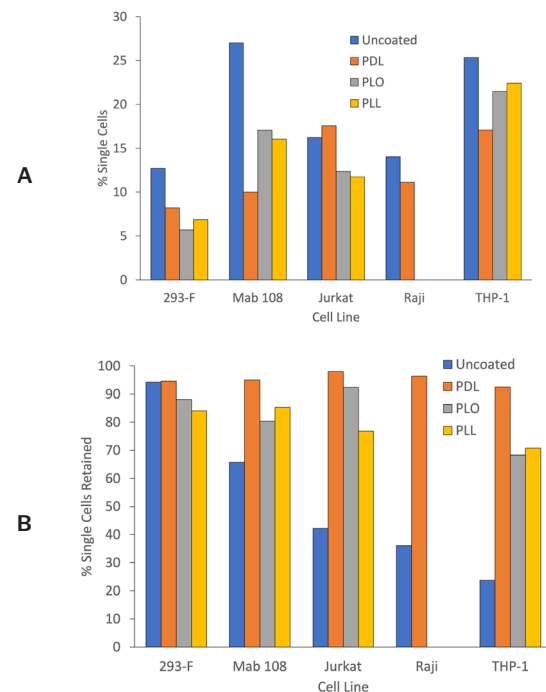
As shown in Figure 2A, the percentage of single cells on day 1 dramatically increases with longer exposure to the coatings. To determine whether the extended coating time also improves cell adhesion, the number of single cells that remained attached to the CellRaft was calculated using the 24-hour timepoint. As shown in Figure 2B, both PDL and PLO benefited from longer coating times, and the number of single cells lost at 24 hours significantly decreased. Interestingly, the percent of single cells lost in the PLL reservoirs was identical between the two coating times, suggesting that four hours of coating time is sufficient for PLL. Therefore, it is recommended that coatings be applied for no less than four hours prior to seeding suspension cells to ensure maximal cell adherence and recovery.

### Seeding suspension cells on coated CytoSort Arrays for colony propagation

After optimizing the cell coating protocols for the three candidate substrates, CytoSort® Quad Reservoir 100 x 100 um arrays were prepared for cell seeding (one array per cell line) and coated overnight (see [CytoSort Array coating protocol](#)). Since the Quad array has four individual reservoirs, each reservoir was used for a different coating (uncoated, PDL, PLO, and PLL), and 5,000 cells were seeded per reservoir (0.78 cells:CellRaft ratio). NOTE: Only PDL was shown to adhere the Raji cells to the CellRafts, so PLL and PLO<sup>3</sup> were not tested using these cells (data not



**Figure 2:** Extended coating times on the CytoSort Array improve single cell seeding efficiency and decrease cell loss after 24 hours. CytoSort Quad 100 arrays were incubated with media (uncoated), PDL, PLO, or PLL for either 4 or 16 hours at 37°C to coat the CellRafts, and 5x10<sup>3</sup> Jurkat cells were seeded in the coated reservoirs. A) For all coatings tested, there were significantly more single cells attached to the CellRafts with longer coating times, and B) the longer coating time improved single-cell retention for PDL and PLO.



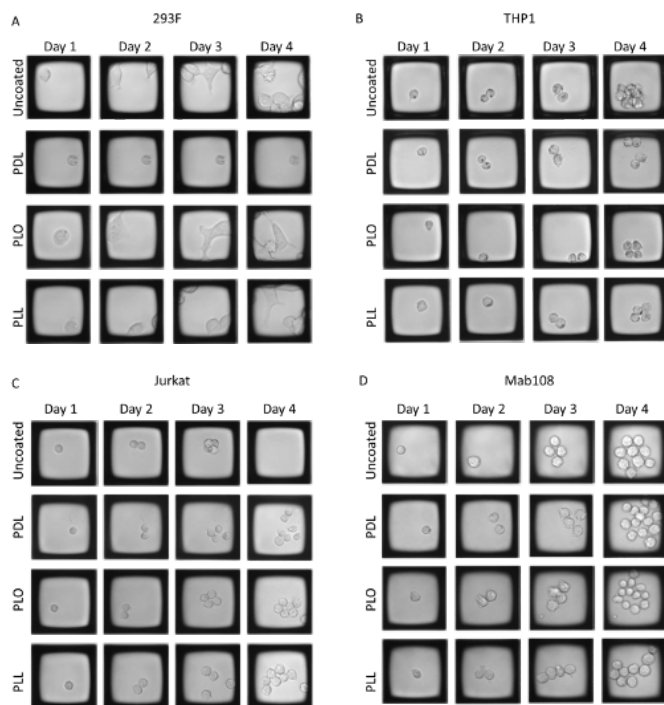
**Figure 3:** Coating CytoSort arrays improves single cell adherence. A) Suspension cells are able to adhere to CellRafts regardless of the coating at 4 hours post seeding, but B) the majority of single cells are lost after 24 hours without a coating substrate. A) CytoSort Quad 100 arrays were coated with PDL, PLO, or PLL and 5x10<sup>3</sup> cells of each cell type were seeded per reservoir. The percent of single cells attached at 4 hours was determined for each cell type and coating. B) 16 hours post-cell seeding, the CellRafts with single cells were interrogated for cell movement, and the percentage of single cells retained was determined. Coating the arrays dramatically increases suspension cell attachment to the CellRafts.

were scanned to identify single cells, and the cells were cultured for 4 days to allow single-cell derived colonies to form. As shown in Figure 3A, after four hours, single cells attach to the CellRafts regardless of the coating. However, by 24 hours post-seeding in the uncoated reservoirs, most single cells have moved and are no longer attached to their original CellRaft (Fig. 3B). All three coatings significantly improved the retention of single cells for the Mab108, Jurkat, and THP-1 cell lines, with PDL having the highest percentage of single-cells retained.

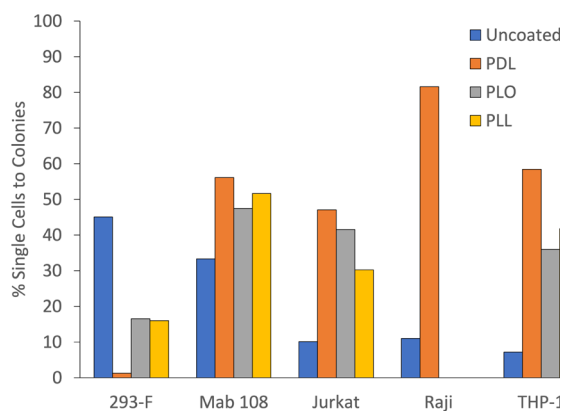
Interestingly, the 293-F cell line showed the greatest cell adhesion in the uncoated reservoirs (Fig. 3A), and the coated CellRafts appeared to negatively impact the cells (Fig. 3B). This is likely because 293-F are adapted from an adherent cell line and are better suited to attaching to an uncoated substrate than the other four lines tested. These findings highlight the importance of experimentally determining the ideal conditions for new cell lines tested on the CytoSort Arrays.

### Single-cell derived colony formation on coated CytoSort Arrays

To determine whether the coatings allowed the suspension cells to divide normally with high viability, the seeded CytoSort Arrays were maintained at 37°C in a standard tissue culture incubator for four days until 8-16 cell colonies had formed. The arrays were imaged using the AIR System every 24 hours to follow clonal formation. As shown in Figure 4, single cells were easily identifiable on day 1, and the exponential growth of the cells can be followed throughout the duration of the experiment. All of the suspension cells were able to divide, and the daughter cells remained attached to the coated CellRafts. While the 293-F were able to grow on uncoated, PLL, and PLO coated CellRafts (Fig. 3C), PDL appeared to be



**Figure 4:** Suspension cells are able to attach and divide on coated CytoSort arrays. A) 293F, B) THP1, C) Jurkat, and D) Mab108 cells were cultured for 4 days on the coated CytoSort arrays and serially imaged every 24 hours post-cell seeding. Although the suspension cells were able to attach to the uncoated rafts, there was more cell movement and the cells are clearly less attached in some lines (B,C). The suspension cells adhered strongly to the coated rafts at Day 1, and the daughter cells remained attached to the surface throughout the culture period.



**Figure 5:** Single-cell derived colonies can be generated using coated CytoSort arrays. The percentage of single cells that formed viable colonies after four days in culture was determined for each cell line and coating. Coating the arrays dramatically increases the percentage of single cells that were able to form isolatable colonies compared to the uncoated rafts.

toxic to the cells, and the cells never divided.

Using the next generation Off-the-AIR brightfield cytometric mode, serial images were assessed for each cell line and coating to determine how many single-cells formed

	Uncoated	PDL	PLO	PLL
293-F	288	5	47	55
Mab 108	451	293	405	414
Jurkat	82	413	381	317
Raji	123	725		
THP-1	91	499	386	468

**Table 3:** Number of confirmed single-cell derived colonies

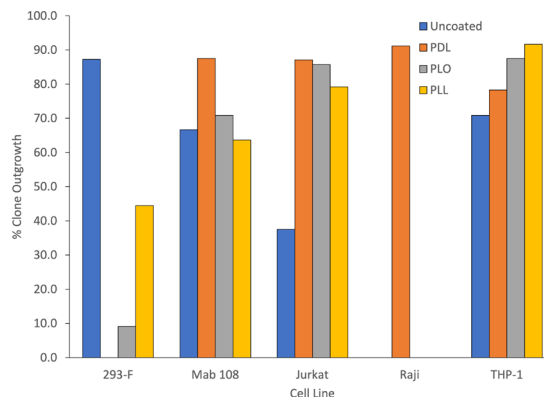
a viable colony that could be isolated for downstream analysis. The discovery: coating the arrays significantly improved the single cell colony formation. For Jurkat cells, Raji cells, THP-1 cells, and Mab109 cells, there were hundreds of viable colonies available for isolation and recovery for cell line expansion or downstream assays.

As shown in Figure 5, less than 10% of single Jurkat, Raji, and THP-1 cells seeded on uncoated CellRafts formed a monoclonal cell line. Coating the arrays drove single-cell colony formation to 30-50% for the Jurkat cells, 80% for the Raji cells, and 40-60% for the THP-1 cells. The Mab108 cells, which are loosely adherent on uncoated tissue culture plastic, had 30% single-cell derived colony formation on uncoated arrays and 50-60% on coated arrays.

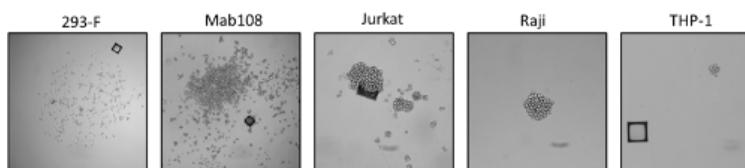
In Table 3, the total number of available single cell-derived clones for each cell type and coating are shown. For all but the 293-F, there were hundreds of viable colonies available for isolation and recovery for cell line expansion or downstream assays.

### Isolation and transfer of suspension cell colonies off array

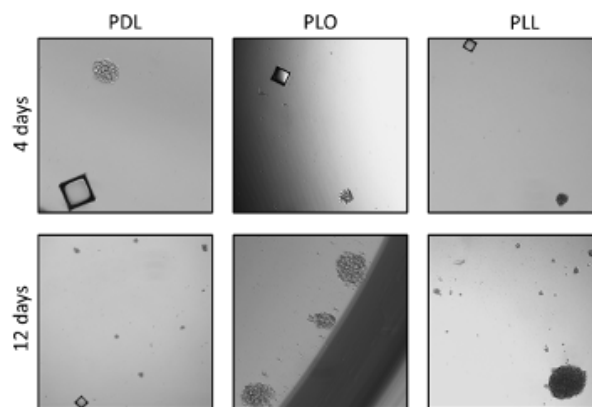
While it was encouraging that the suspension cells could be adhered to the CellRafts and propagated without detriment to cell growth or viability, additional key metrics for success in



**Figure 6:** Outgrowth of single-cell derived suspension cell clones off array in 96 well plates. After isolation of single-cell derived suspension cell clones, the wells of each 96 well plate were monitored for cell growth off raft and the formation of a viable colony for further propagation. For all cell types tested, the suspension cells were able to grow off the CellRafts and continue to expand off array.



**Figure 7:** Representative images of colony growth off CellRafts after isolation and transfer to 96 well plates. After isolation, the suspension cells were able to detach from the CellRafts and return to suspension culture in as little as 4 days post-isolation.



**Figure 8:** THP-1 cells on cell coatings grow and return to suspension off array. Four days after isolation, THP-1 cells have detached from the coated CellRafts, and by 12 days, the cells have expanded sufficiently in suspension for passaging and further characterization.

this workflow are 1) recovery of the clone off array and 2) the growth and propagation of the cells back into suspension after isolation. To determine whether adhered suspension cell clones could be successfully isolated from

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the array, identified clones from above were mapped for isolation into 96-well plates using the CellRaft AIR System.

After isolation, the cells were monitored for growth and expansion for 10 days post isolation to determine the percentage of colonies that formed off-array and returned to suspension after transfer. As shown in Figures 6 and 7, for Mab108, Jurkat, Raji, and THP-1 cells, the coatings significantly improved successful isolation and outgrowth efficiency, with 60-90% of CellRafts forming downstream colonies off array. Although the Mab108 and THP-1 cells did adhere to the uncoated rafts and continued growing off array, the Raji and Jurkat cells did not adhere to the CellRafts as tightly during isolation and the colony outgrowth percentage was lower. As before, the 293-F were not able to form viable colonies on the coated arrays, but >80% of single-cell clones grew off raft for the uncoated arrays. Importantly, as seen in Figures 7-8, all cell types tested were able to grow off of the coated CellRafts after isolation, and the cells continued to expand and grow in suspension. The 293-F were the lone exception and continued to grow adherent as expected. However, when the media in the wells was gently pipetted, the 293-F cells were able to return to suspension culture.

### Conclusions

The data presented clearly demonstrate that the CytoSort Arrays and CellRaft AIR System can easily be adapted to enable suspension cell line workflows. We were able to image, identify,

### Recommendations for Success

- Before executing critical experiments, choose the CytoSort Quad Arrays for efficient cell culture condition optimization for new cell lines.
- Serially image the CytoSort® Arrays at every doubling time to monitor single cells, growth rates, morphology, and clonality.
- Use our state-of-the-art next-generation Off-the-AIR software to identify single cells reliably, determine colony growth, and map plates for downstream workflows.
- Isolate as few as 96 CellRafts and expect up to 90% outgrowth efficiency, ensuring an abundance of viable, phenotypically characterized, clonal colonies available for downstream interrogation.

and isolate single-cell derived suspension cell clones using three different types of surface coatings in as little as one week. Using five cell lines highlights that this workflow is amenable to many types of cells and applications. Ultimately, the ability to attach suspension cells to the CellRafts prior to isolation enables upstream characterization of suspension cells using morphologic, growth, or surface marker expression data and confirms clonality to increase downstream efficiency and success.

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