CELL Microsystems®

RAFTNOTE

Cell Line Development of Sf9 Cells

Overcoming Challenges and Bottlenecks

The baculovirus expression vector system (BEVS) allows for the in vitro expression of heterologous genes in cultured insect cells and is one of the most versatile eukaryotic vector systems for producing recombinant proteins. To date, eleven BEVS-derived therapeutics have been approved for human and veterinary use[1]. The most common insect cell lines used with the BEVS include Sf9, Sf-21, Tn-368, and High-Five, with Sf9 and derivatives like Mimic Sf9 and ExpiSf9 (Thermo Fisher) being one of the most widely used.

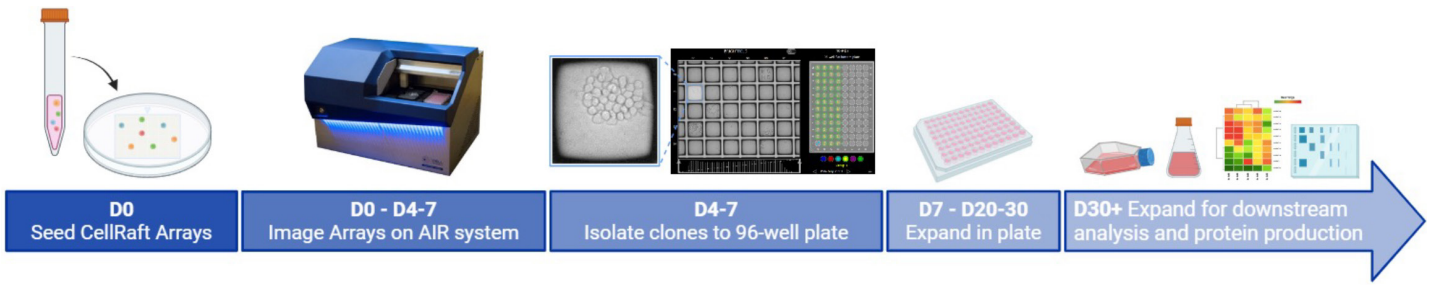
The BEVS workflow consists of the construction and purification of a recombinant baculovirus expressing a gene of interest and subsequent transfection of insect cells with the baculovirus to produce large amounts of recombinant protein. While single cell cloning is the preferred method for obtaining a stable population of production cells, this remains a significant challenge for Sf9 cells and a major bottleneck in BEVS workflows.

Insect cells are particularly susceptible to stressors like low seeding density and high sorting pressure encountered in common cell line development techniques like limiting dilution and droplet dispensing. The inability to clone these cells necessitates settling for polyclonal lines from a subset of cells in the population with a desired phenotype, resulting in unstable cell lines with unreliable protein production[2,3,4]. In this RaftNote, we demonstrate how CellRaft® Technology overcomes these limitations and provides a streamlined, automated workflow for high- efficiency single cell cloning of Sf9 cells.

Key Highlights:

1) Generating insect Sf9 clones for recombinant protein production using traditional methods of single cell dispensing is nearly impossible.
2) Culturing Sf9 cells on CellRaft® Arrays enabled clonal growth due to shared media conditions that mimic shake flask culture.
3) Hundreds of clonal Sf9 colonies could be identified and isolated using the CellRaft AIR® System, enabling resource- and time-efficient scale-up for downstream production pipelines.

Workflow



Questions this RaftNote Answers

1) What is the best way to obtain monoclonal populations of insect cells?
2) How can I increase protein production from insect cells?
3) How can I prove monoclonality of a cell line?

Experimental Design

Sf9 cells were maintained in shake flasks in Sf-900 III SFM (Thermo Fisher Cat. No. 12658019). A single cell suspension of Sf9 cells was seeded on CS-100S and CS-200S CellRaft Arrays with and without CellRaft Array seeding inserts at seeding densities of $8x10^4$ cells (100S Array) or $4x10^4$ (200S Array) cells in 700 µL fresh media or conditioned media (CM).

CM was collected from the supernatant of Sf9 cells in the exponential growth phase. The cells were allowed to settle on the Array for 4 hours after seeding, then the excess media was removed, and the Array was washed with PBS to remove cells that did not adhere; subsequently, the seeding insert was removed.

The Array was then washed again with PBS, followed by culture media, and incubated with 100% CM for 2 hours. After the 2-hour incubation, an initial scan of the Array was performed on the CellRaft AIR System.

The Array was then scanned every 24 hours to monitor viability, clonality, and growth rate. The CellRaft Cytometry™ software was used to identify rafts containing a single cell at the first scan and a colony at the final scan, and these population sets were overlayed to identify rafts containing monoclonal colonies. On the day of isolation, 96-well collection plates were pre-loaded with 100 µL of either 20% or 100% CM, and monoclonal rafts were isolated to the collection plates using the CellRaft AIR System for further expansion. Outgrowth was visually confirmed on a microscope. Four clones were expanded up to T25 flasks, and one clone was expanded up to a 125 mL shake flask.

To compare the efficiency of the CellRaft Array to a standard limiting dilution workflow, three 96-well plates were seeded with a single cell suspension at approximately 0.8 cells/well in 100% CM.

Results

Single Sf9 cells successfully proliferated on the CellRaft Array, and viability, clonality, and growth rate were tracked with daily scans on the CellRaft AIR System (Figures 1 and 2). CellRaft Cytometry identified thousands of single cells on the day of seeding and hundreds of monoclonal colonies by day 7 on each Array (Figure 3). Use of the seeding insert preserved the monoclonal integrity of cells by preventing cells from clumping on the Array borders, dissociating into suspension, and settling out of suspension into CellRafts (Figure 4). After 7 days, 65% of single cells on the 100S Array and 54% of single cells on the 200S Array had formed a colony of at least 8 cells (Figure 5). Seeding cells on the Array with higher concentrations of CM increased clonal colony growth efficiency (Figure 6A) and influenced Sf9 cell morphology. Sf9 cells seeded on the Array in 20% and 100% CM exhibited a flattened morphology while cells seeded in 0% CM retained the round morphology typically observed in suspension (Figure 6B).

Importantly, Sf9 cells isolated from the Array into 96-well collection plates continued to expand off the CellRafts. Both the day of isolation and CM concentration in the collection plate impacted outgrowth efficiency, and optimal outgrowth from both Arrays was obtained by isolating on day 7 into 100% CM. In contrast, none of the cells seeded in limiting dilution plates yielded any colonies (Figure 7A).

To demonstrate that the single-cell derived clones were capable of being expanded up to production level volumes, four clones were expanded from the 96 well plates up to T-25 flasks, and one clone was expanded up to a 125 mL shake flask (Figure 7B). Total workflow duration from seeding the CellRaft Array to outgrowth of monoclonal colonies off the CellRafts in collection plates required less than 10 days with less than 2 hours of hands-on time. Expansion to the shake flask required an additional 50 days.

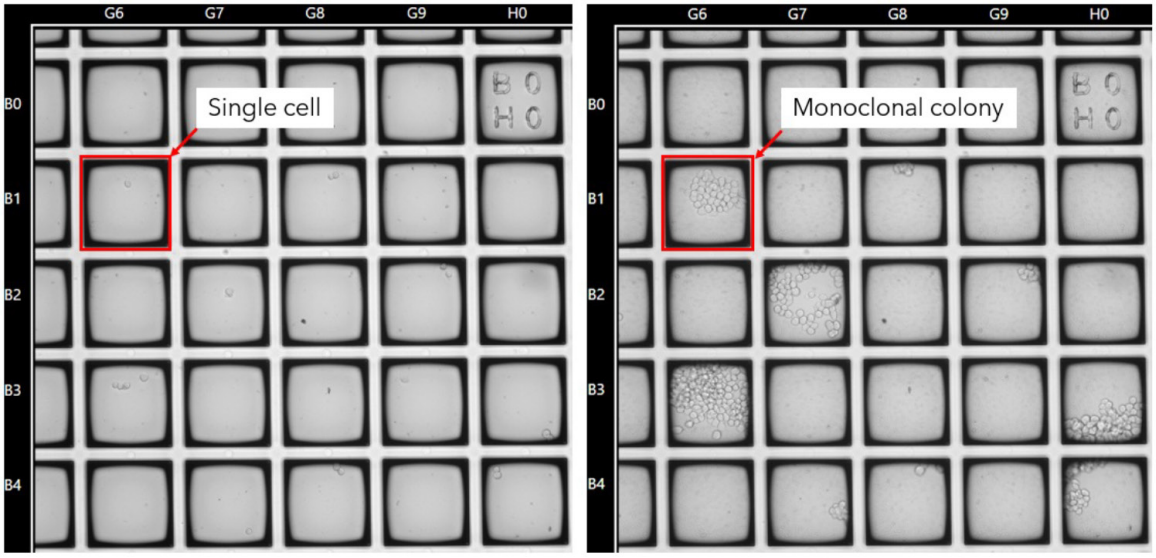


Figure 1. Field of view of a 200S CellRaft Array with a raft containing a single cell at day 0 and a monoclonal colony at day 7. Sf9 cells proliferated and maintained monoclonality on the CellRaft Array.

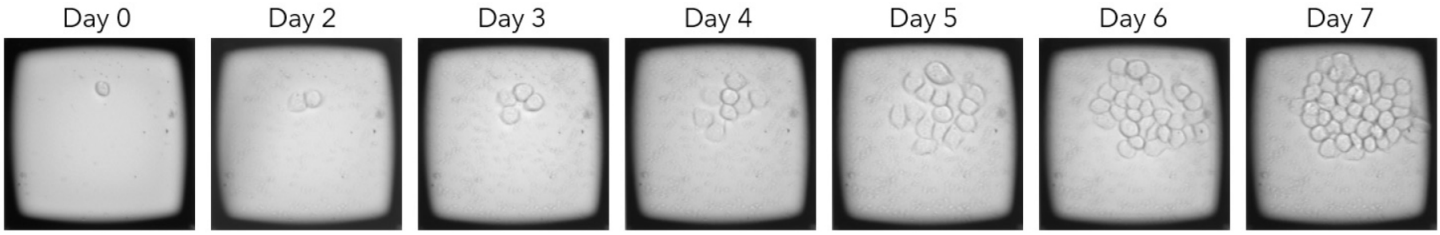


Figure 2. Proliferation of a single Sf9 cell to a monoclonal colony on a CellRaft within a CellRaft Array. An Sf9 single cell suspension was seeded on a 200S CellRaft Array on Day 0 and scanned daily for 7 days to monitor cell viability and clonality.

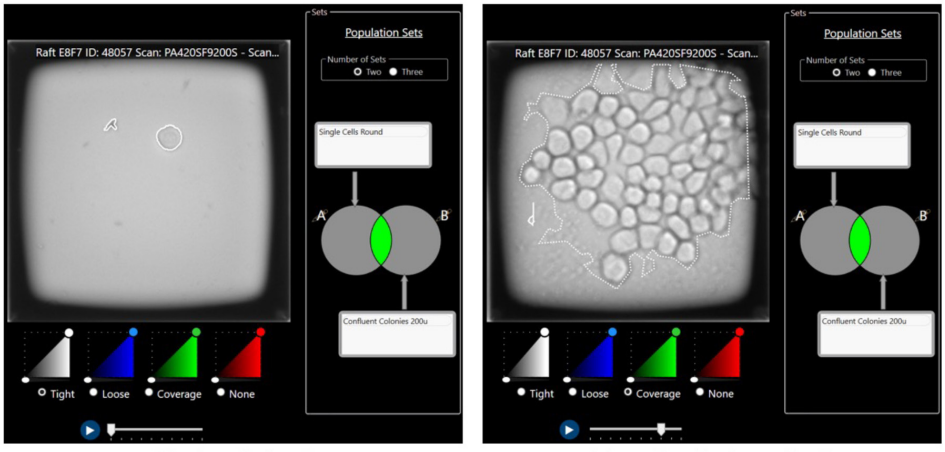


Figure 3. Representative example of identification of monoclonal colonies using CellRaft Cytometry. Two population sets, single cells on day 0 and confluent colonies on day 7, were overlayed to identify CellRafts containing monoclonal colonies.

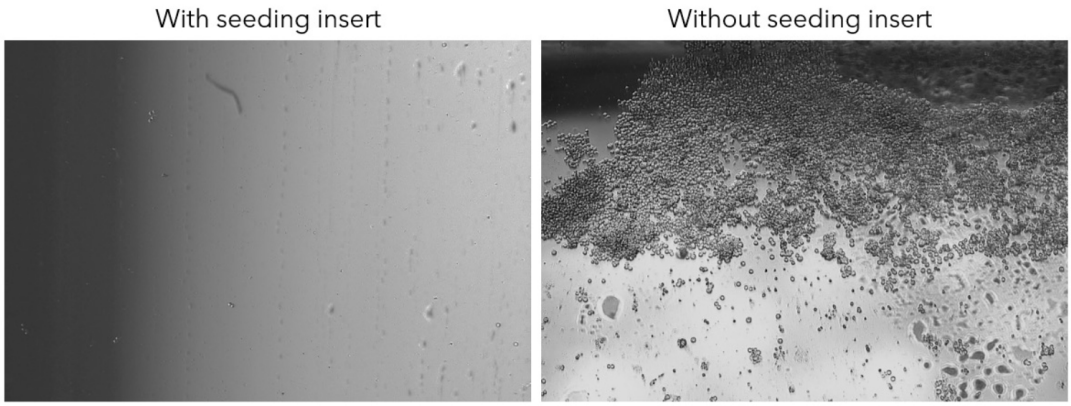


Figure 4. Array borders 4 days post-seeding with and without the seeding insert. Use of the seeding insert preserved monoclonality of cells within the CellRafts by preventing dense areas of cells on the borders from dissociating into suspension and falling into CellRafts.

Array	Cells seeded	Single cells	Clonal colonies	% Single cells formed colonies on Day 7
100S	80,000	3107	2038	65%
200S	40,000	1233	671	54%

Figure 5. Single cell and clonal colony growth of Sf9 cells on 100S and 200S CellRaft Arrays. Over 500 monoclonal colonies (≥8 cells) were available for isolation from each Array after 7 days of propagation.

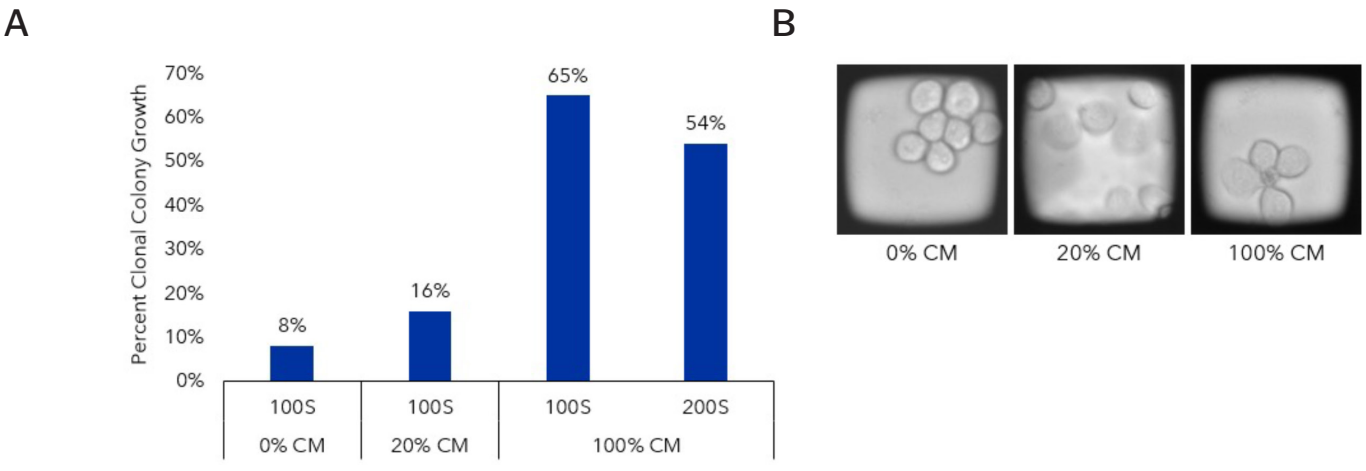


Figure 6. Effect of CM concentration on clonal colony growth efficiency and cell morphology on CellRaft Arrays. (A) Seeding cells in CM increased clonal colony growth efficiency on the Array. (B) The addition of CM caused Sf9 cells to exhibit a flattened morphology compared to the rounded morphology typically observed in suspension.

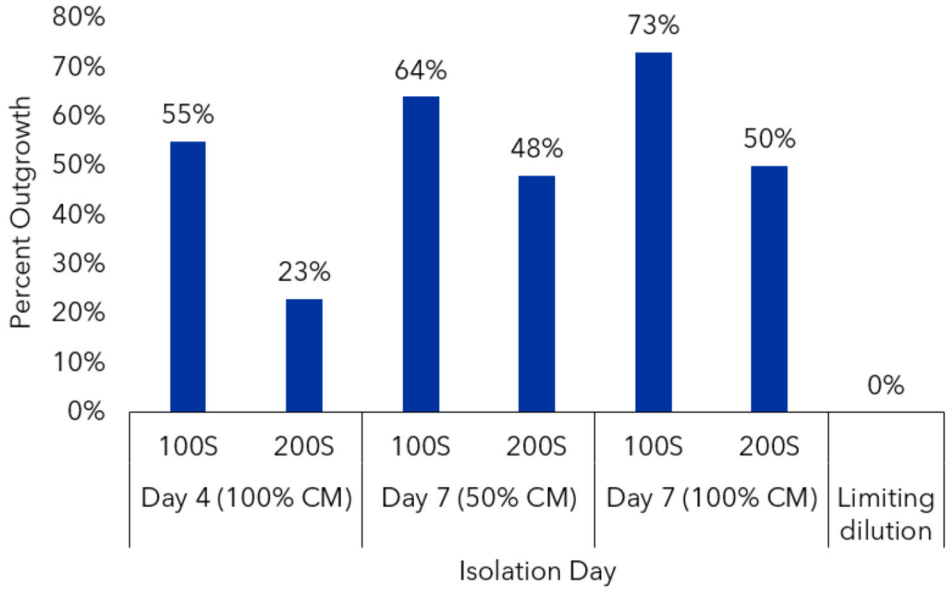


Figure 7. Expansion of Sf9 clones from the CellRaft Array and the effect of conditioned media on expansion. Isolation after 7 days into 100% CM resulted in optimal outgrowth efficiency of clones.

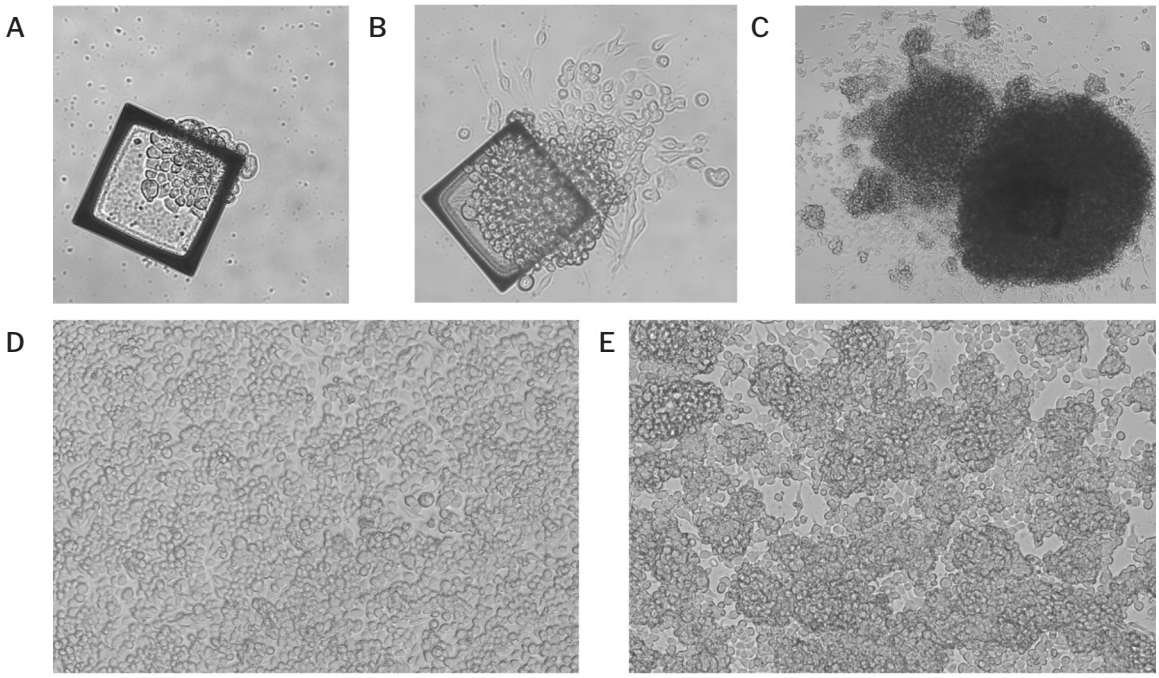


Figure 8. Expansion of an Sf9 clone off an isolated 200 µm CellRaft. A-C, Cells expanding off the CellRaft: (A) 2 days post-isolation (10X magnification), (B) 8 days post-isolation (10X magnification), and (C) 21 days post-isolation (4X magnification). D-E, (D) Expanded cells in a 6-well plate at 36 days post-isolation and (E) a T-75 flask at 53 days post-isolation (10X magnification). Cells from the confluent T-75 flask were transferred to a 125 mL shake flask and further expanded in suspension. The total workflow duration from seeding single cells on the CellRaft Array to expansion up to the shake flask was 60 days.

Discussion

Although BEVS is a versatile and widely used tool for recombinant protein production, it is limited by the challenges associated with obtaining a monoclonal population of Sf9 cells following transfection with a baculovirus. Common methods for cell line development, such as limiting dilution and droplet dispensing, demand large amounts of time and resources and often fail to generate clonal colonies as insect cells are particularly sensitive to low seeding density and high sorting pressure. Cell lines are instead derived from a subset of cells with a desired phenotype and are not truly monoclonal, resulting in a genetically unstable cell line with unreliable protein production[2,3,4].

In this application note, three limiting dilution plates of Sf9 cells failed to yield any colonies. By contrast, a single CellRaft Array yielded thousands of monoclonal colonies, each with an image-based record tracking propagation back to a single cell. The CellRaft Array workflow also significantly reduced the resources required to generate Sf9 clones, requiring less than 2 hours of hands-on time and up to 500X less media per single cell screened compared to limiting dilution. While the cells used in this application note were not fluorescent, the CellRaft AIR System's ability to image in three fluorescent channels can also replace fluorescence-activated cell sorting (FACS), which often results in post-sorting viabilities of less than 10% for fragile insect cell lines[5].

Conclusion

In this RaftNote, we demonstrated the use of CellRaft Technology for highly efficient cell line development of Sf9 cells. Propagation of monoclonal cell lines remains a significant bottleneck for the use of insect cells in recombinant protein production, leading users to rely on unstable polyclonal cell lines for protein output. Using the novel CellRaft Technology, we demonstrated the ability to propagate thousands of monoclonal colonies of Sf9 cells on just one CellRaft Array consumable, automatically isolate these colonies to collection plates using the CellRaft AIR System, and expand clonal populations up to shake flask culture.

References

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For more information on the presented data or CellRaft Technology, visit cellmicrosystems.com

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