Sf9 Single-Cell Cloning for Recombinant Protein Production *Is Possible* with CellRaft® Technology.

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Background

- The baculovirus expression vector system (BEVS) is one of the most versatile eukaryotic vector systems for recombinant protein production in insect cells. The BEVS workflow consists of the construction and subsequent transfection of insect cells with a baculovirus expressing a gene of interest. Eleven BEVS-derived therapeutics have been approved for human and veterinary use[1].
- One of the most common insect cell lines used with the BEVS is Sf9 and its derivatives, like Mimic Sf9 and ExpiSf9 (Thermo Fisher).
- Single cell cloning to obtain a stable transfected population remains a significant challenge with Sf9 cells. Insect cells are fragile to stressors like low seeding density and high sorting pressure encountered in common cloning methods like limiting dilution and droplet dispensing, leading users to derive unstable polyclonal cell lines with unreliable protein production[2,3,4].
- We hypothesize that the flask-like culture conditions of the CellRaft® Array, together with the automated imaging and isolation on the CellRaft AIR® System, will overcome the limitations of existing single cell cloning methods and enable streamlined, high-efficiency cell line development of Sf9 cells.

CellRaft Workflow

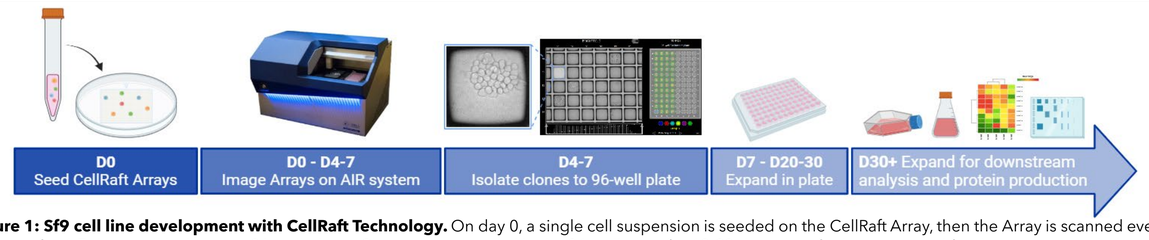

Figure 1: Sf9 cell line development with CellRaft Technology. On day 0, a single cell suspension is seeded on the CellRaft Array, then the Array is scanned every 24 hours for 4-7 days until isolation to 96-well plates. Clones are expanded in the 96-well plate for 13-23 days, then further expanded for downstream analysis and protein production. Figure created with BioRender.com.

Visual confirmation of monoclonality over time

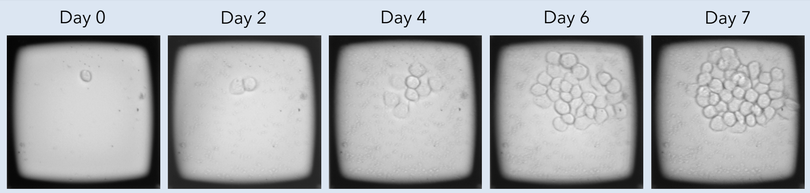

Figure 2: Representative time-course images of a monoclonal Sf9 colony proliferating on a 200 µm CellRaft. CellRaft Arrays were imaged on the CellRaft AIR System 4 hours post-seeding to identify single cells and every subsequent 24 hours to monitor clonal growth.

Software-guided clonal selection identified thousands of clonal colonies

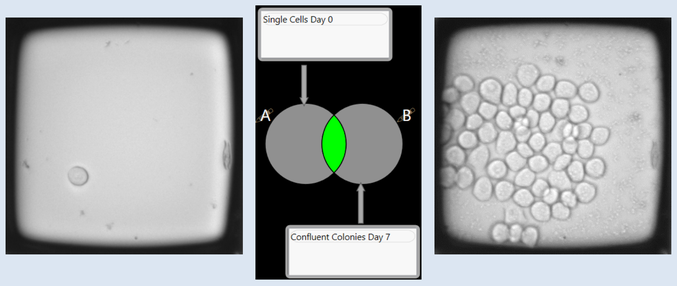

Figure 3: Representative example of identification of monoclonal colonies using CellRaft Cytometry™. Two population sets, single cells on day 0 and confluent colonies on day 7, were overlayed to identify CellRafts containing monoclonal colonies.

Thousands of single cells and monoclonal colonies were identified

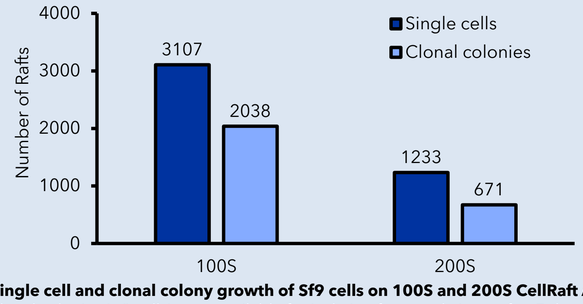

Figure 4: Single cell and clonal colony growth of Sf9 cells on 100S and 200S CellRaft Arrays. Over 500 monoclonal colonies (≥8 cells) were available for isolation from each Array after 7 days of propagation.

Conditioned media improved clonal colony growth on the CellRaft Array

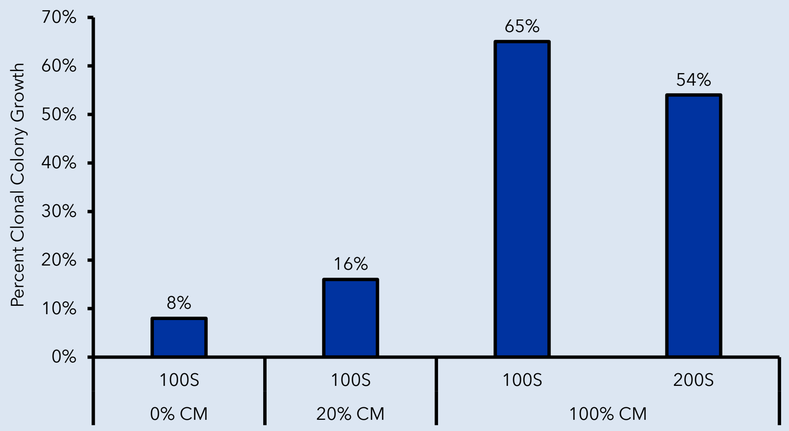

Figure 5: Effect of conditioned media (CM) composition on clonal colony growth on the CellRaft Array. Seeding single cells in 100% CM significantly increased the growth efficiency of monoclonal colonies (≥8 cells) after 7 days on the Array.

Flask-like culture on the CellRaft Array enabled clonal expansion of Sf9 cells

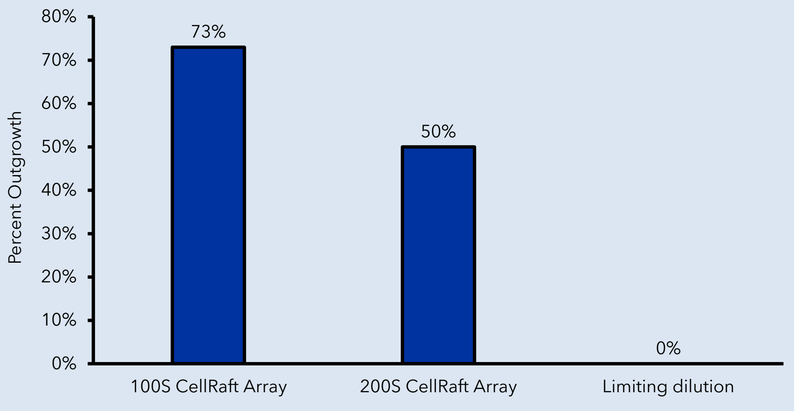

Figure 6: Clonal outgrowth efficiency from isolated CellRafts and limiting dilution. Single Sf9 cells were seeded in 100% conditioned media directly to 96-well plates for limiting dilution or propagated on CellRaft Arrays for 7 days before isolation to 96-well plates. Limiting dilution was not amenable to clonal growth, while propagating clones on the CellRaft Array before isolation to a 96-well plate yielded up to 73% clonal expansion.

Monoclonal colonies were expanded from the CellRaft Array to bulk shake flask culture

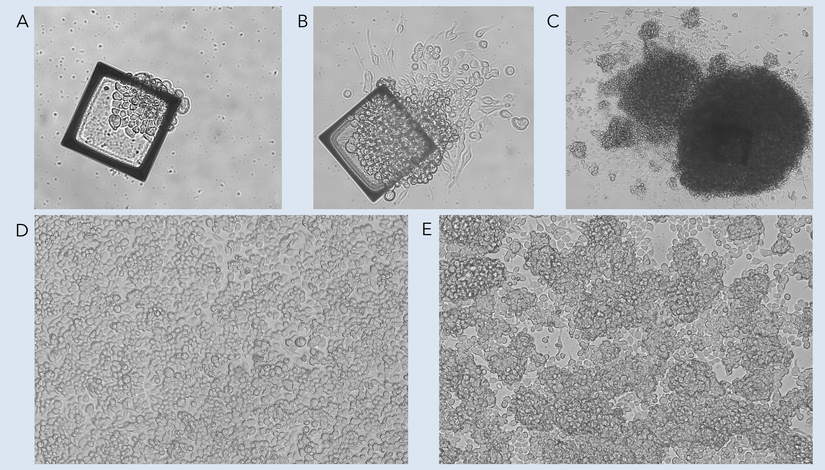

Figure 7: Expansion of an Sf9 clone off an isolated 200 µm CellRaft. A-C, Clonal cells expanding off the CellRaft (A) 2 days post-isolation (10X magnification), (B) 8 days post-isolation (10X magnification), and (C) 21 days post-isolation (4X magnification). D-E, Expanded cells (D) in a 6-well plate at 36 days post-isolation and (E) in 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. Total workflow duration from seeding single cells on the CellRaft Array to expansion up to the shake flask required 60 total days.

Summary

- Establishing monoclonal insect cell lines remains a significant challenge in recombinant protein production using baculovirus vectors, leading users to rely on unstable polyclonal cell lines with unreliable protein expression.
- The flask-like culture conditions of the CellRaft Array supported the propagation of thousands of monoclonal colonies on one cell culture consumable.
- Culturing cells on the CellRaft Array before isolation to a collection plate resulted in up to 73% outgrowth efficiency compared to 0% outgrowth efficiency with limiting dilution. Monoclonal Sf9 cell lines were successfully expanded from the CellRaft Array up to bulk suspension culture.
- The entire single cell cloning workflow, from seeding the CellRaft Array to outgrowth of monoclonal colonies off CellRafts, was completed in less than 10 days with less than 2 hours of hands-on time, with an additional 50 days required for expansion up to a 125 mL shake flask.

References

1. Hong, M. et. al., Genetic engineering of baculovirus-insect cell system to improve protein production. Front. Bioeng. Biotechnol., 2022.
2. Ma, H. et. al., The Spodoptera frugiperda Sf9 cell line is a heterogeneous population of rhabdovirus-infected and virus-negative cells: Isolation and characterization of cell clones containing rhabdovirus X-gene variants and virus-negative cell clones. Virology, 2019.
3. Brogee, P., Towards the Development of a ϕC31Int-Competent Sf9 Cell Line. UWSpace, 2018.
4. Zitzmann, J. et. al., Single-cell cloning enables the selection of more productive *Drosophila melanogaster* S2 cells for recombinant protein expression. Biotechnol. Rep., 2018.