CELL Microsystems®

APPNOTE

Restoring high-titer antibody production in a hybridoma cell line

Introduction

Hybridomas are generated through the fusion of non-proliferative primary B cells with immortalized myeloma cells, followed by a labor- and resource-intensive single cell screening and cloning process to obtain a monoclonal hybridoma cell line. While this cell line initially serves as a reliable source of mAbs, the inherent genetic instability of hybridoma cells often results in decreasing antibody yield over time. To restore high yield, hybridomas must again undergo the laborious single cell screening and cloning process, typically by plating single cells in limiting dilution and identifying high-producers via Enzyme-Linked Immunosorbent Assays (ELISAs)[1]. Depending on the extent of genetic drift, hundreds of 96-well plates may need to be screened to recover a high-producing clone and restore antibody titer in the hybridoma line.

Scientists at Tetracore, a biotechnology company offering biothreat detection kits, observed a decrease over time in yield from a hybridoma cell line used in commercial antibody production, eventually rendering the line unsuitable for continued use. We hypothesized that CellRaft® Technology, combined with immunofluorescence-based reporting of antibody secretion, could be used to identify and isolate rare, high-producing hybridoma clones, restore high antibody titer in the population, and ultimately allow Tetracore to resume use of the hybridoma line in commercial antibody production.

Key Highlights:

- Scientists at a biotechnology company observed antibody titers decreasing over time in a commercial hybridoma line, rendering the cell line unsuitable for continued use.
- Time-course imaging and immunofluorescent reporting of antibody secretion revealed that only 0.3% of the hybridoma population was secreting detectable antibody.
- Using CellRaft Technology, antibody titer was recovered to levels that qualified for commercial use in less than one month from single cell seeding to characterized cell banking.

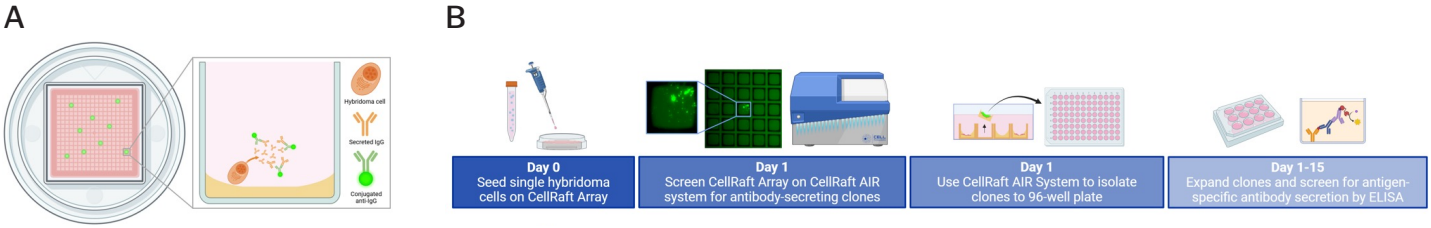

Figure 1. Screening and single cell cloning of hybridoma cells with CellRaft Technology. (A) Schematic of microwell-based antibody screening within the CellRaft Array (40,000 microwells per array). IgG antibody secreted by hybridoma cells binds a fluorophore-conjugated anti-IgG antibody, enabling fluorescent signal detection. (B) Workflow of hybridoma single cell screening and cloning using CellRaft Technology. Single hybridoma cells are seeded with a fluorophore-conjugated anti-IgG on a CellRaft Array containing 40,000 microwells, then imaged on the CellRaft AIR system. The accompanying CellRaft Cytometry image analysis software is used to identify antibody-secreting single cell clones by immunofluorescence. These clones are then automatically isolated to 96-well plates for further expansion, and expanded clones are screened by ELISA to confirm that secreted antibody is target-specific.

Experimental Design

Seeding hybridoma cells on CellRaft Array

A CellRaft Array (40,000 100x100 µm microwells) was coated overnight with poly-D-lysine to facilitate attachment of hybridoma cells. A total of 20,000 hybridoma cells secreting IgG antibody against Botulinum Toxin A Complex were seeded on the coated CellRaft Array in culture medium supplemented with a hybridoma cloning reagent and a FITC-conjugated anti-IgG-Fcγ antibody. A CellRaft seeding insert was used during cell seeding to prevent cells from attaching outside of the microwells.

Identification and automated isolation of monoclonal antibody-secreting hybridoma cells on the CellRaft Array

The CellRaft Array was imaged at 4- and 24-hours post-seeding using the CellRaft AIR® System to monitor single cell proliferation and antibody secretion. CellRaft Cytometry™ was used to identify CellRafts containing a single cell at the first scan and a green fluorescent signal at the second scan, representative of a monoclonal, antibody-secreting colony. The CellRaft AIR system was used to automatically isolate and transfer CellRafts containing these colonies to a 96-well plate, and colonies were allowed to expand for 2 weeks.

Screening of antibody-secreting clones for target-specific antibody production

Clone supernatants were screened by ELISA at a dilution of 1:1000 to verify that secreted antibody was target specific. Supernatants from two clones exhibiting the highest target-specific reactivity were pooled and antibody was purified using protein G. This antibody was screened by ELISA against previous lots of purified antibody that had either passed or failed quality control standards for use in commercial antibody production.

Results

Single hybridoma cells rapidly proliferated on the CellRaft Array, with a doubling time of approximately 12 hours. Secreted antibody appeared as a punctate fluorescent signal that remained localized to the CellRaft in which the antibody-secreting cells were adhered (Figure 2). Imaging of the CellRaft Array on the CellRaft AIR system with the CellRaft Cytometry image analysis software enabled tracking of monoclonality and fluorescence-based screening of clones for antibody secretion (Figure 3).

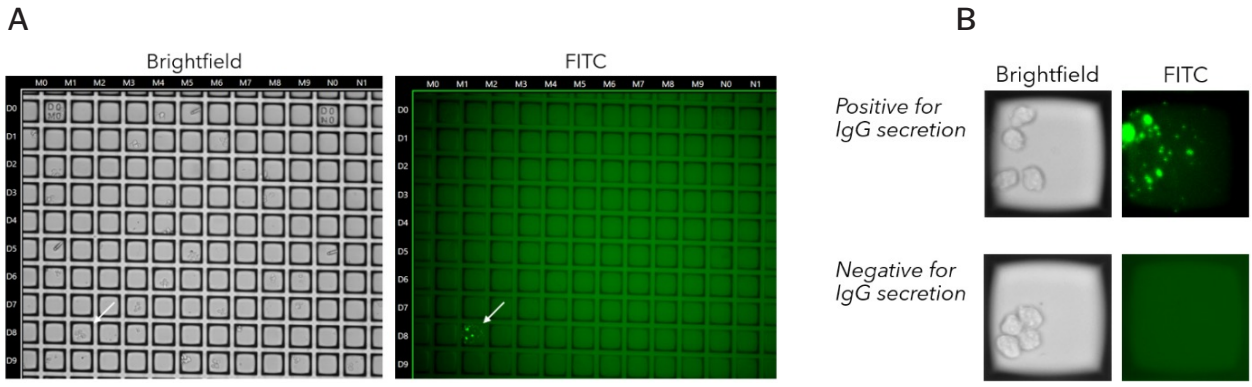

Figure 2. Immunofluorescence-based screening of monoclonal antibody secretion on the CellRaft Array.
A, A single 100x100 µm CellRaft containing an antibody-secreting hybridoma clone (white arrow) within a field of view of the CellRaft Array. **B**, Representative images of single CellRafts containing hybridoma clones positive (bright, punctate fluorescent FITC signal) or negative (no FITC signal) for antibody secretion.

Of 20,000 total cells seeded, only 68 cells were found to be secreting antibody detectable by immunofluorescence (0.3%). Of those 68 cells, 27 CellRafts were identified as monoclonal and were automatically transferred from the CellRaft Array to a 96-well collection plate using the CellRaft AIR system (Figure 4). Screening of dilute supernatants by ELISA for target-specific antibody production revealed no signal above background for the parental line and a 12- to 21-fold increase above background for all screened clones (Figure 5).

Two of the highest-producing clones were further interrogated by pooling cell supernatants and purifying antibody using protein G. Antibody purified from the CellRaft clones displayed a greater than 3-fold increase in reactivity compared to a previous antibody lot that had failed quality control standards, effectively restoring antibody titer to levels suitable for commercial use (Figure 6).

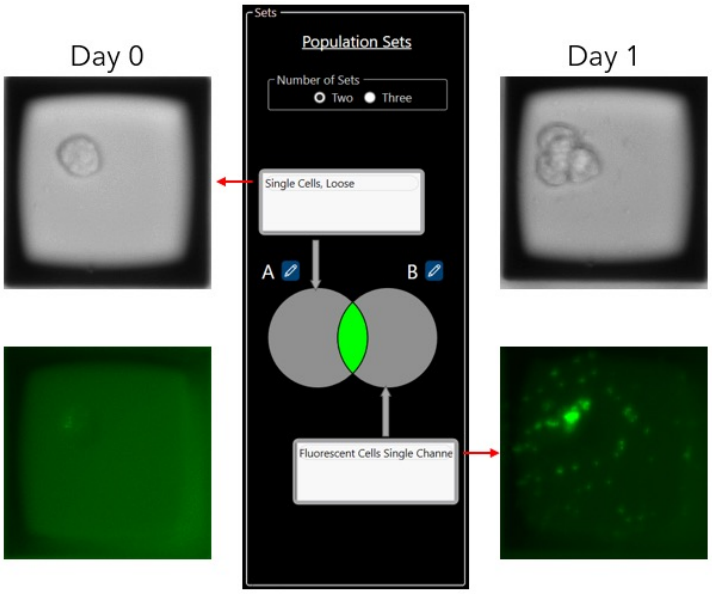

Figure 3. Use of CellRaft Cytometry to identify monoclonal, antibody-secreting colonies on the CellRaft Array. Two CellRaft Cytometry gates for identifying single cells at day 0 and green fluorescence at day 1 were overlayed to identify CellRafts containing monoclonal antibody-secreting colonies for isolation and expansion.

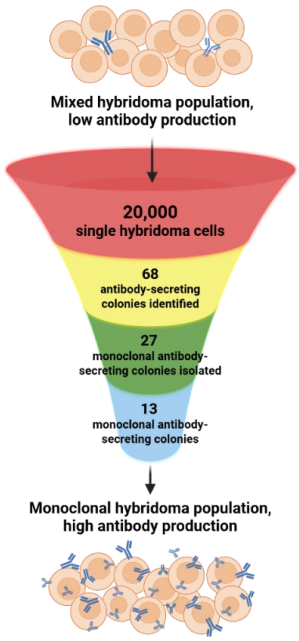

Figure 4. Screening and isolation of a mixed hybridoma population on the CellRaft Array. Only 68 out of 20,000 total cells screened were positive for antibody secretion.

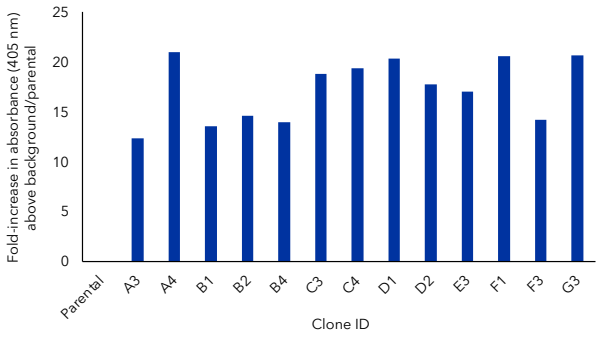

Figure 5. Fold-increase in target-specific antibody production above background/parental for CellRaft clones. Supernatants were diluted 1:1000 and screened by ELISA for target-specific antibody production. No signal above background was observed for the parental supernatant while supernatants of all tested clones exhibited a 12- to 21-fold increase above background.

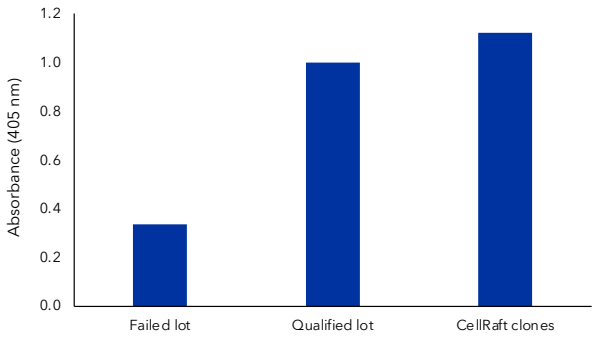

Figure 6. Screening by ELISA of purified antibody from CellRaft clones compared to previous antibody lots. Purified antibody from CellRaft clones demonstrated a greater than 3-fold increase in target-specific reactivity over previous antibody lots that had either failed or passed quality control standards. The CellRaft clones effectively restored antibody titer to levels that qualified for commercial use.

Discussion

Hybridoma technology serves as a critical tool in biomedical research, providing a reliable supply of monoclonal antibodies. However, as demonstrated in this case study with a commercial hybridoma cell line, the inherent genetic instability of hybridomas often results in decreased antibody yield over time.

Upon screening this hybridoma line for antibody secretion using CellRaft Technology, we discovered that only 0.3% of cells in the population were secreting levels of antibody detectable by immunofluorescence. By screening 20,000 hybridoma cells on a single CellRaft Array, we recovered 27 monoclonal antibody-secreting hybridoma populations for further expansion and characterization. Given the extent of genetic drift in the population, screening and single cell cloning by limiting dilution and ELISA would have required at least (7) 96-well plates to identify one antibody-secreting clone, assuming a generous single cell survival and seeding efficiency.

Screened supernatants of clones selected from the CellRaft Array demonstrated a significant fold-increase in antibody secretion above parental for all tested clones. Purified antibody from CellRaft clones revealed that antibody titer was restored to levels that qualify for commercial antibody use. The CellRaft workflow required less than one month and approximately 7 hours of hands-on time, from single cell seeding to banking of characterized, monoclonal cell lines. Screening an equivalent number of cells by limiting dilution and ELISA would consume significantly more time and resources (Table 1).

	CellRaft Technology	Limiting dilution and ELISA
Culture media volume	<25 mL	>5000 mL
Hands-on time	<8 hours	>40 hours
Total cost	<$1,200	>$10,000

Table 1. Economic advantage of hybridoma cloning and screening using CellRaft Technology compared to conventional limiting dilution and ELISA. CellRaft Technology, with a confirmatory ELISA in one 96-well plate, required minimal reagents, hands-on time, and total cost compared to a limiting dilution/ELISA workflow to recover the same number of clones.

Conclusion

In this case study, CellRaft technology was leveraged to restore antibody production in a commercial hybridoma cell line that no longer qualified for commercial use due to low antibody titer. Concurrent single-cell cloning and immunofluorescence-based detection of antibody secretion on the CellRaft Array allowed for the screening of 20,000 hybridoma cells, equivalent to screening over (200) 96-well plates by limiting dilution or single cell sorting and ELISA. Using the CellRaft Cytometry image analysis software, we identified rare colonies (0.3% of cells) that were secreting antibody and utilized the CellRaft AIR system to automatically isolate monoclonal, antibody-secreting colonies to a 96-well plate for further expansion. Screening of purified antibody from pooled CellRaft clones revealed a recovery of antibody titer to levels that qualified for commercial use. Restoration of antibody titer required less than one month from single cell seeding to banking of monoclonal, characterized cell lines, demonstrating CellRaft Technology as a powerful tool for the streamlined screening and single cell cloning of hybridoma cells.

"Before the Clone Challenge, we developed a hybridoma cell line that produced a monoclonal antibody against Botulinum Toxin A Complex, which we sold as a standalone product and as part of an ELISA kit. However, over time, the recovery of this cell line began to yield a monoclonal antibody with reduced activity against the A Complex. Consequently, we had to replace these commercial products with alternatives. After participating in the Clone Challenge, we received clones from Cell Microsystems that demonstrate significantly improved reactivity. Even a dilution of cell supernatant of 1:1,000 in the ELISA outperformed the current purified antibody 3-fold. We hope to resume selling the improved antibody and the ELISA kit commercially once again."

- Senior Scientist, Tetracore, Inc.

References

1. Page, M., Thorpe, R. (2009). Screening Hybridoma Culture Supernatants Using ELISA. In: Walker, J.M. (eds) The Protein Protocols Handbook. Springer Protocols Handbooks. Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-59745-198-7_205
2. BioRender.