Growing Challenging Clones — Case Studies

Cell Microsystems, Inc., Durham, NC USA

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Clone Challenge
Success Stories

Summary

- Here, we spotlight the remarkable journeys of the six recipients of our 2023 Clone Challenge Grant Giveaway. Delve into their unique narratives to learn about the clone challenges these researchers faced. Then, discover how the innovative capabilities of CellRaft® Technology were used to successfully navigate and overcome these obstacles.
- We selected six applicants to send us their cells, giving them access to our application team's expertise to help grow the clones needed. Using CellRaft® Technology, we seeded the cells, cultured them, and imaged them over time to form monoclonal colonies from single cells. Clones that match the desired image-based phenotypic characteristics were selected and gently isolated using our automated CellRaft AIR® System. The colonies were then expanded and shipped back to the selected applicants.

Having challenges growing single cells into colonies?
We are confident that our technology can grow colonies, even from difficult cell lines.

CellRaft Workflow

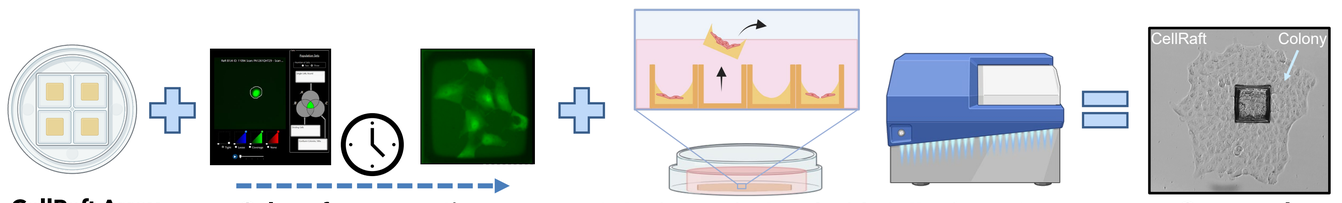


Figure 1: Cell line development with CellRaft Technology. On day 0, a single cell suspension is seeded on the CellRaft Array, then the Array is scanned every 24 hours for several days until isolation to 96-well plates. Clones are expanded in 96-well plates for downstream analysis and ultimately shipped back to the researcher for validation.

UNC Lineberger Comprehensive Cancer Center

The Challenge

Cells Became Senescent and Died

A Success Story

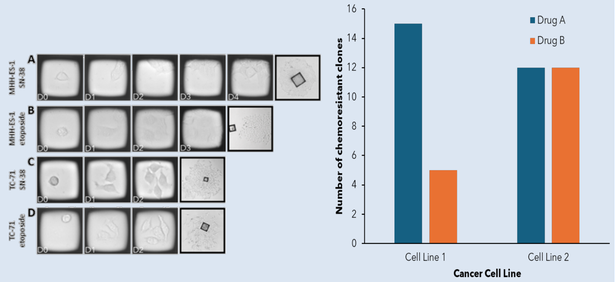
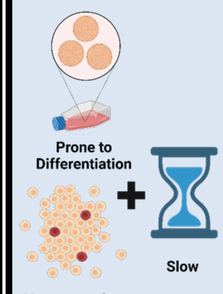


Figure 2: The Challenge: Cells went into senescence under limiting dilution. **The Success:** 43 clones were expanded and provided to the researcher in 3 weeks. The researcher identified 14-63% were still chemo-resistant after expansion.

"We recommend the CellRaft Technology for our colleagues struggling with single cell isolation….CellRaft not only speeds up this this process by providing many more clones but more importantly the unique monitoring process provided by CellRaft supplies additional information for every single clone with a higher quality of clones." - Pengda Liu, Ph. D., Associate Professor

Stanford

The Challenge



A Success Story

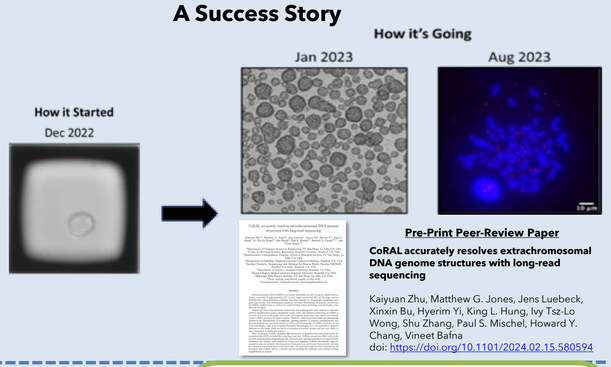
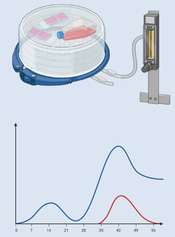


Figure 3: The Challenge: Cells were difficult to clone due to heterogeneity, slow proliferation, and prone to differentiation. **The Success:** 30 clones delivered in 1 month. Clones were confirmed by the researcher using FISH, and researchers are in the process of publishing.

"I would like to recommend Cell Raft Technology if anyone has a problem in isolating single clones, especially those difficult culturing types."
- Shu Zhang, Ph.D., Postdoctoral Scholar in the Department of Dermatology

Columbia University

The Challenge



Altered Hypoxia Response

A Success Story

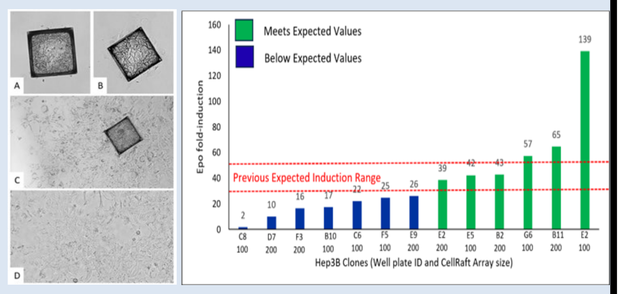
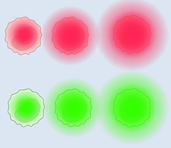


Figure 4: The Challenge: Clonal cells drifted in hypoxia response. **The Success:** 18 clones were expanded and delivered in 3 weeks. RT-qPCR of hypoxia-induced clones revealed> 30-fold induction of Epo in 6 of 13 clones tested, a phenotype previously lost from limiting dilution.

"The CellRaft Technology allowed expansion of a high percentage of colonies derived from a single Hep3B cell that faithfully retained the ability to respond to hypoxia, a critical measure of success for this procedure."
- Joseph Garcia, M.D., Ph. D. Professor of Medicine

Georgia State University

The Challenge



Varying Intensity of Edit

A Success Story

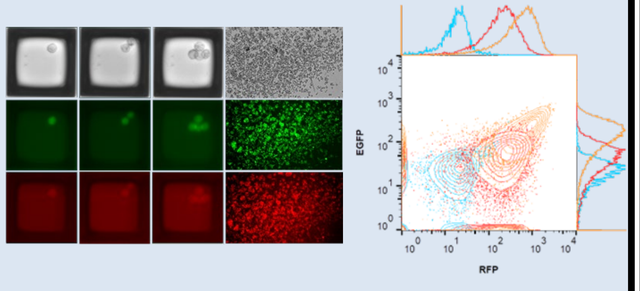
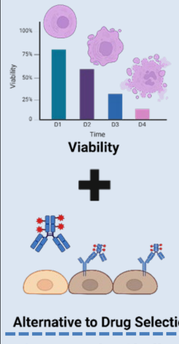


Figure 5: The Challenge: Isolating strong dual reporters. **The Success:** 26 dual monoclonal reporters expanded and delivered in 4 weeks. Colonies were verified by the researcher by genomic PCR and FACS and confirmed to have the desired edit with fluorescence.

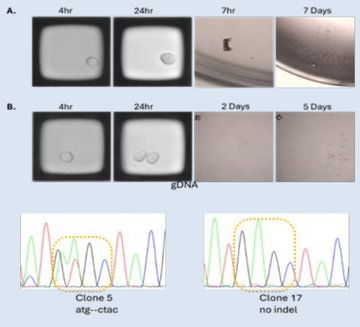
"I would recommend this technology as a viable alternative to FACS, especially when cell populations are limiting."
- Gregory Poon, Ph.D., B.Sc. Phm., Professor of Chemistry

Johns Hopkins University

The Challenge



A Success Story



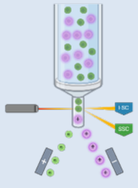
CellRaft Clones	Genotype
Clone 1	2bp deletion
Clone 2	2bp deletion
Clone 3	2bp deletion
Clone 4	2bp deletion
Clone 5	2bp deletion
Clone 6	2bp deletion
Clone 7	2bp deletion
Clone 8	2bp deletion
Clone 9	Multiple Indels
Clone 10	2bp deletion
Clone 11	2bp deletion + 1bp insertion
Clone 12	2bp deletion + 1bp insertion
Clone 13	2bp deletion
Clone 14	2bp deletion
Clone 15	Parental/unedited
Clone 16	Parental/unedited
Clone 17	Parental/unedited
Clone 18	2bp deletion
Clone 19	2bp deletion
Clone 20	2bp deletion
Clone 21	2bp deletion
Clone 22	2bp deletion

Figure 6: The Challenge: Cloning was too stressful for cells, and they could not survive selection. **The Success:** 22 clones delivered to the researcher. All 22 clones were confirmed by the researcher to be clonal and 86% contained an indel of interest.

"We employed the CellRaft Technology to help isolate clones derived from verified single cells in a cell line that was very difficult to clone by regular limiting dilution. It worked beautifully, producing clones with sequencing-verified distinct genotypes quite easily. This technology is definitely a big advance for anyone trying to isolate clones from single cells!!."
- Brian Dalton, M.D., Ph. D., Assistant Professor of Oncology

NC State University

The Challenge



FACS Failed to Detect

A Success Story

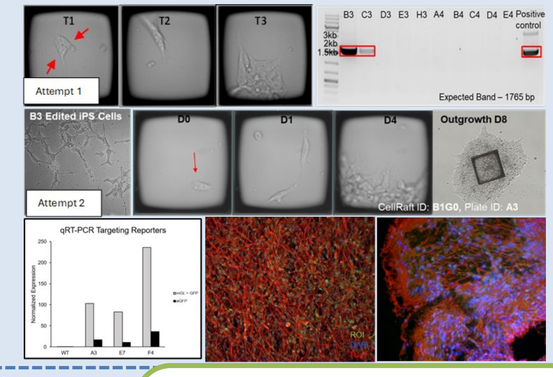


Figure 7: The Challenge: FACS failed to detect signal in cells due to the location. **The Success:** 20 pools of cells were delivered containing 2 positives that were re-screened to form 80 clones. 17 clones were screened, and all confirmed to contain reporter. Cells were then differentiated.

"I'd absolutely recommend Cell Raft Technology to others. CellRaft Technology is a game-changer for researchers tackling similar challenges in gene editing and visualization, offering real potential for advancing our understanding of cellular biology. Plus, the support and guidance from the Cell Microsystems team were invaluable."- Samantha Stuppy, Graduate Research Assistant