Overcoming Challenges in CRISPR Editing

Generating monoclonal CRISPR edited cell lines is a common pain point for scientists. Traditional cell sorting or single cell dispensing require large numbers of cells to find edits, and post-isolation cell viability and colony formation are often prohibitively low. Typically, this is due to the inability of a single cell to "condition" its cell culture environment through secreted factors and cell-to-cell crosstalk.

With the unique CellRaft® Technology, many challenges in the CRISPR workflow can be overcome.

- Improve cell viability by culturing cells in a flask-like culture environment
- Screen thousands of CRISPR edited clones in one dish
- Confirm editing and monoclonality
- Find even rare cells
- Reduce reagent and plastic use

Challenges with CRISPR Cell Line Development

1) Low cell viability post-isolation
2) Requires significant reagent and plastic use
3) Laborious manual screening and manipulation
4) Low editing efficiency
5) Multiple pieces of expensive equipment needed

	Time to Colony	Viability Rate
FACS	6-10 days	29%
CellRaft AIR	3-4 days	83%

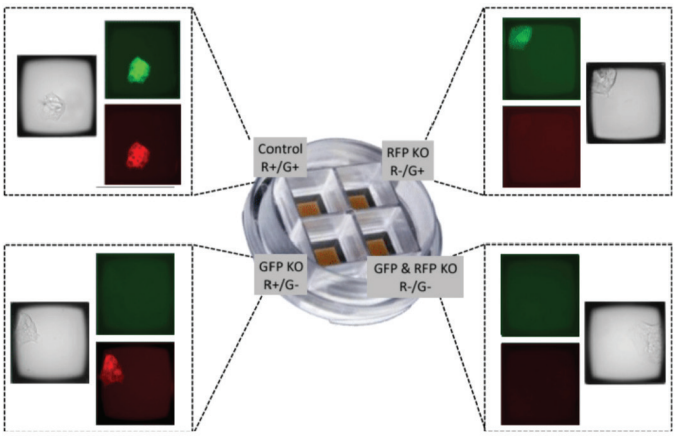

Figure 1. The CellRaft Quad Array is used to isolate clonal colonies from four distinct CRISPR gene editing processes. Using the GFP/RFP-expressing model system, all four conditions were processed in parallel to generate both experimental and control gene editing cell lines in a single cell culture consumable. Given the 500 µL volume of each Quad Array well, reagent consumption was also reduced by 50 – 100 fold.

Grow Cells in a Flask-like Culture Environment

Single cells are seeded on the CellRaft Array after introducing transgenic or CRISPR/CAS9 elements. This eliminates the need for trypsin, scraping, high-pressure fluidics, or limiting dilution. The CellRaft Array is made up of thousands of microwells called CellRafts that allow the cells to share a contiguous media volume so that while the cells are segregated, they are not “alone.”

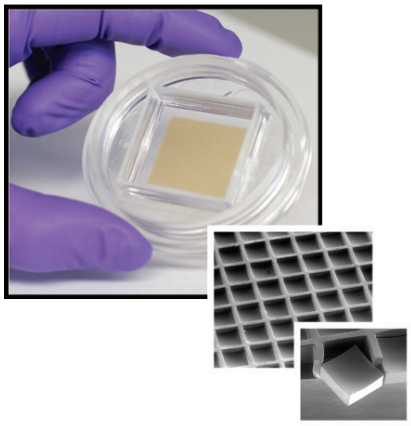

Image CellRafts at Multiple Time Points

Ensuring cells undergoing gene editing grow into clonal colonies is an essential component of the CRISPR workflow. Using the CellRaft AIR® system, single cells can be imaged and tracked over the course of colony formation. In addition, the knockout phenotypes (elimination of GFP and RFP signal) can be tracked over time which provides a unique capability for phenotypic CRISPR screening.

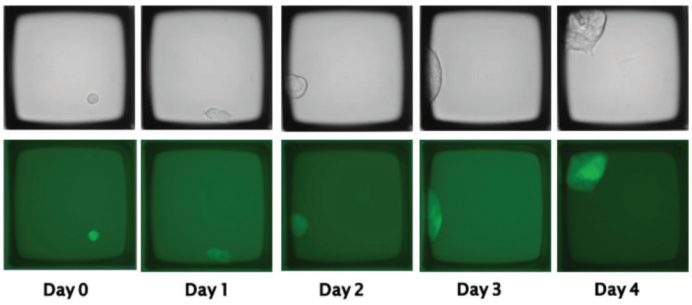

Figure 2. Cells transfected with a GFP-expressing plasmid were tracked for the growth of clonal colonies.

Software-guided Identification and Automated Isolation of Cells of Interest

CellRaft Cytometry™ software allows for image-based verification of single cells to ensure monoclonality and analysis of a variety of parameters over time, ranging from size to morphology to gene expression. Users can easily define the characteristics of the target cells or colonies and map them for software-guided CellRaft selection and automated isolation by the CellRaft AIR System.

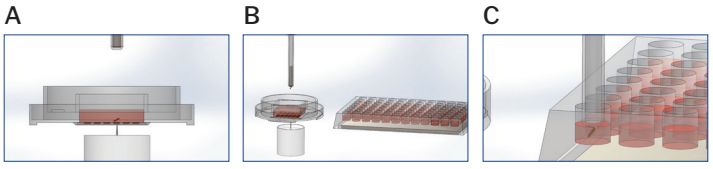

Figure 3. The desired microwell with cells is dislodged from the array (A). The wand picks up the microwell using a magnet (B). The wand places the microwell with the cells in the 96-well plate (C).

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