RaftNote

Applications and protocols for use with CellRaft™ Technology

Sorting, Cloning, and Colony Propagation for CRISPR/Cas9 Genome Editing Using the CellRaft™ System

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Abstract

Genome editing, particularly via CRISPR/Cas9 is a powerful method for investigating the role of a given gene in cellular physiology. The method relies on: 1) Reliable introduction (i.e. transfection) of genetic material into cells; 2) Identifying transfection-positive cells; 3) Selecting clones capable of growth and 4) Propagating colonies for analysis of genome edits and downstream functional assays. Here, we present optimized methods using the Cell Microsystems CytoSort Array, a microwell array with releasable single cell isolation features called CellRafts. Using the CytoSort Array, combined with the manual CellRaft System for use with inverted microscopes, we have developed a protocol which represents a more rapid and flexible approach to post-transfection cloning compared to limiting dilution or fluorescence-assisted cell sorting (FACS).

The CellRaft Technology

Cell Microsystems has developed a single cell isolation and recovery platform ideally suited for high-throughput production of genome-edited cell lines. The core technology comprises a disposable microwell array (the CytoSort™ Array; **Figure 1** - top) on which cells are seeded and imaged. To isolate single cells, a motorized needle penetrates the resealable elastomeric floor of the array to displace the individual CellRaft from its microwell. The CellRaft material is loaded with magnetic nanoparticles, allowing retrieval of the CellRaft and the attached cell, using a magnetic wand. A schematic of the operation of the CellRaft System is found at the bottom of **Figure 1**.

The CytoSort Array is also amenable to coatings, such as MatriGel, CelTak, and extracellular matrix constituents such as laminin and fibronectin,

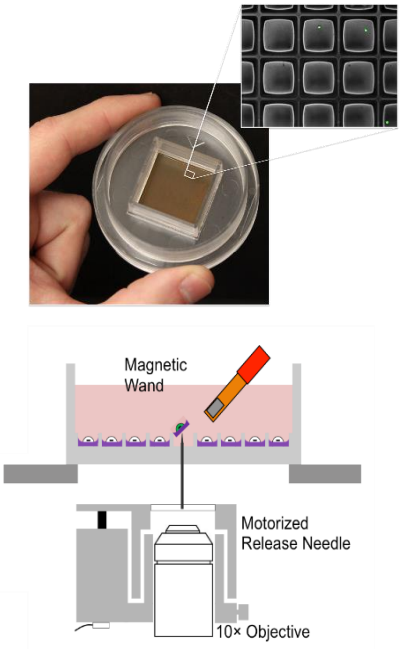

Figure 1*:* The CytoSort Array. Raft features are 200X200 microns, with about 10,000 CellRafts per array. The release needle is positioned on a collar fitted to the imaging objective. The magnetic capture wand is applied by hand to capture and transfer the CellRaft to a tube or well plate.

allowing the customization of the culture environment for various cells of interest.

The system has been used for a wide range of single cell genomics applications including RNA-Seq [1], whole genome sequencing[2] and single nucleus sequencing[3]. Variations of the technology have also been used for T-cell screening for cancer immunotherapy studies[4] and cloning for genome editing[5].

CRISPR/Cas9 Workflow using CytoSort Arrays

For the CRIPSR/Cas9 workflow described here, the CellRaft Technology replaces harmful trypsinization, stressful fluorescence-assisted cell sorting (FACS), labor-intensive limiting dilution and several re-plating/recovery steps, resulting in a method amenable to rapid, high-throughput CRISPR cell line generation.

After transfection, cells are seeded on the CytoSort Array, and the following steps are performed (see **Figure 2** workflow description): screening single cells for transfection markers (with or without antibiotic selection); monitoring single cells for clonal colony formation; release of the CellRaft-attached clonal colony for expansion culture. This method allows individual cells to recover from transfection on the CytoSort Array, which replicates standard cell culture conditions and undergo their first mitotic divisions. Once a more robust, sustainable colony has grown on the CytoSort Array, it can be isolated by releasing its CellRaft. A magnetic wand is then used to capture the CellRaft with the colony attached, and deposit it in a 96-well plate for expansion culture.

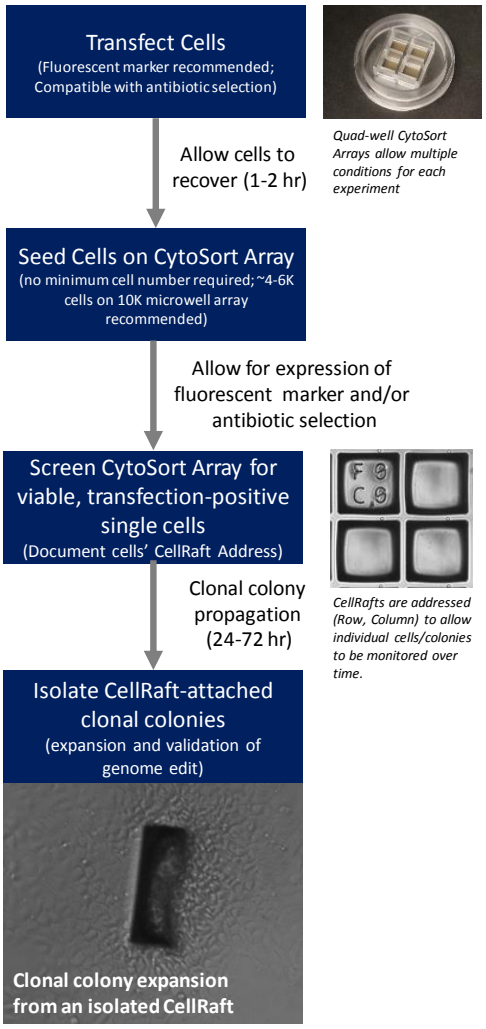

Figure 2: Workflow diagram of the CellRaft CRISPR/Cas9 genome editing method described here. The method is generally transferable to nearly any lipid-based transfection protocol. Primary murine fibroblast colonies are shown here.

Cloning using CellRaft Technology has several key benefits:

1. ***Colony clonality is assured*** by identifying the CellRafts with single cells immediately after transfection and tracking their growth into clonal colonies.
2. ***Cell viability is improved*** during the challenging post-transfection period, allowing initial colony formation on the CytoSort Array prior to transfer to a larger culture vessel.
3. ***Elimination of trypsinization, FACS and re-plating steps*** results in a less stressful process for cells and fewer hands-on steps for investigators.
4. ***Each clonal colony is individually collectable*** through its CellRaft, allowing clones to propagate at varying rates and the investigator to collect colonies from the CytoSort Array in real time.

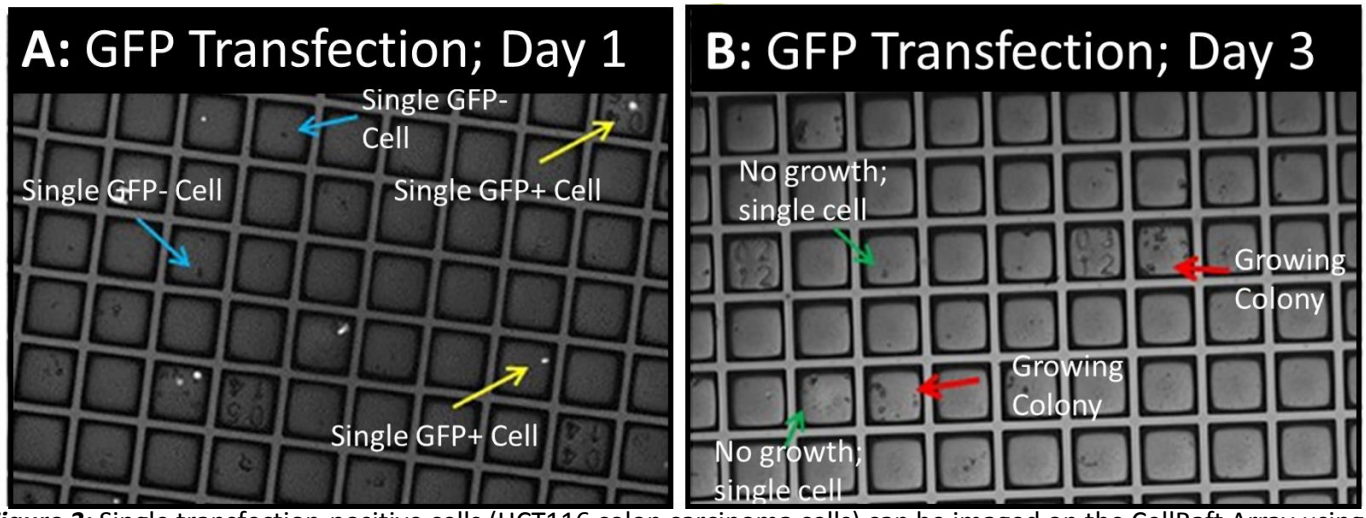

Figure 3: Single transfection-positive cells (HCT116 colon carcinoma cells) can be imaged on the CellRaft Array using virtually any standard inverted microscope. After waiting several days, single cells can be expanded into smaller colonies for subsequent isolation and longer-term expansion culture.

Single Cell Imaging and Initial Colony Formation

The use of an optical marker allows the identification of transfection positive cells on a standard microscope. Once a single cell of interest has been identified, the location of the cell on the CytoSort Array can be recorded. Each CellRaft on the array has an address which can be recorded. Over the course of several days, those locations can be re-examined for the presence of clonal colonies. Once clonal colonies of a desired size have emerged from the transfection positive single cells, the CellRaft can be released and placed in a larger culture vessel for continued clonal expansion. This process allows not only identification of transfection-positive cells, but also for initial screening of those clones capable of propagation. Figure 3 provides an example of optical identification of transfection positive cells (GFP+) on Day 1 (**Figure 3A**), and screening of propagation competent cells on Day 3 (**Figure 3B**).

Clonal colony isolation and propagation culture

Once clonal colonies have grown to a desired size the underlying CellRaft can be released with the colony still attached and placed into a 96-well plate or other culture vessel for continued propagation. **Figure 4** depicts the process for identifying a small colony, releasing the targeted CellRaft from the array, and depositing both the CellRaft and attached cell/colony into a vessel for expansion culture. A larger colony will grow from these cells with no detectable inhibition due to the CellRaft itself.

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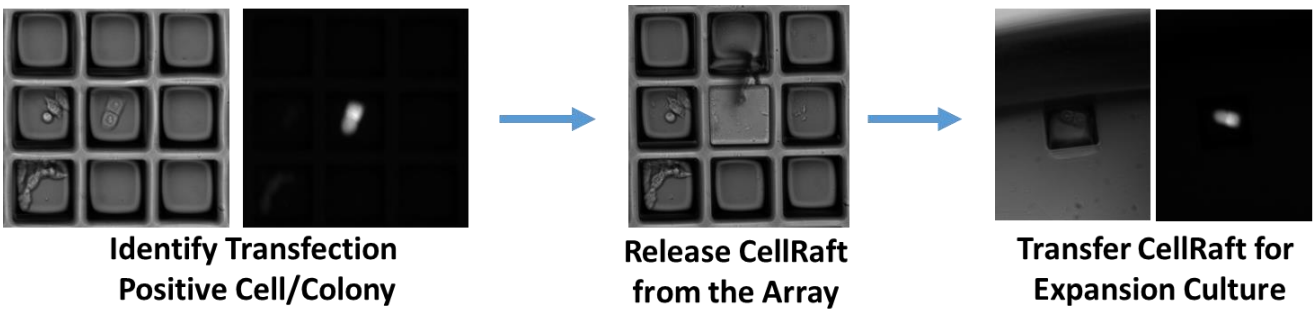

Figure 4: Process for cloning via CellRaft imaging, release and transfer to an expansion culture vessel. HCT116 colon carcinoma cells transfected with a GFP-expressing plasmid are shown here.

Literature Cited:

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