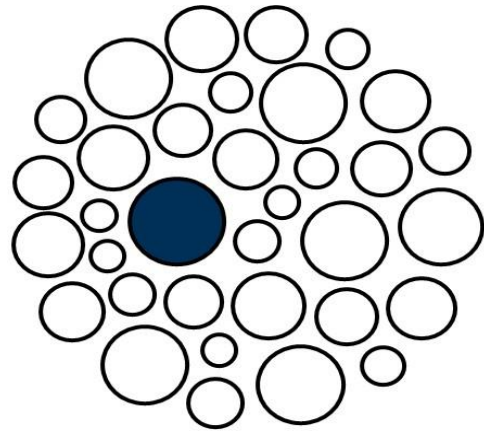


CRISPR/Cas9 Genome Editing using the CellRaft AIR™ System



CELL
Microsystems

Nick Trotta, PhD and Steve Gebhart, PhD
Cell Microsystems, Inc.

Product Applications - ntrotta@cellmicrosystems.com

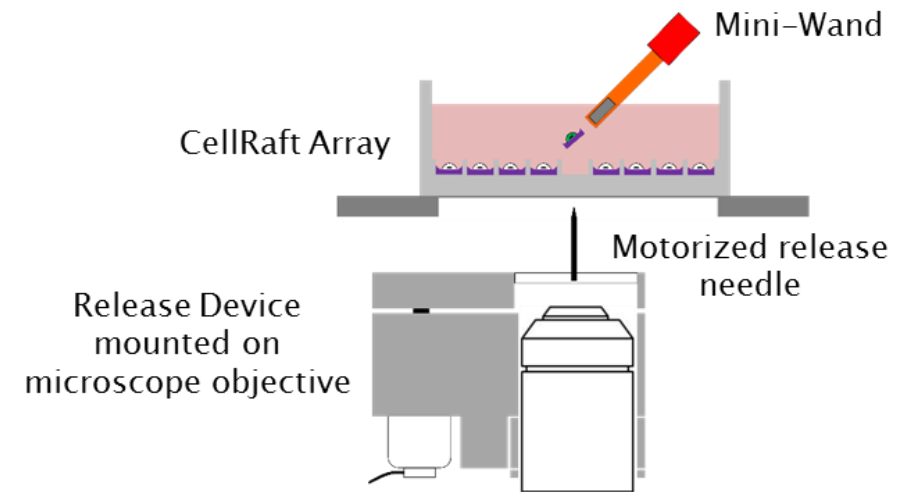
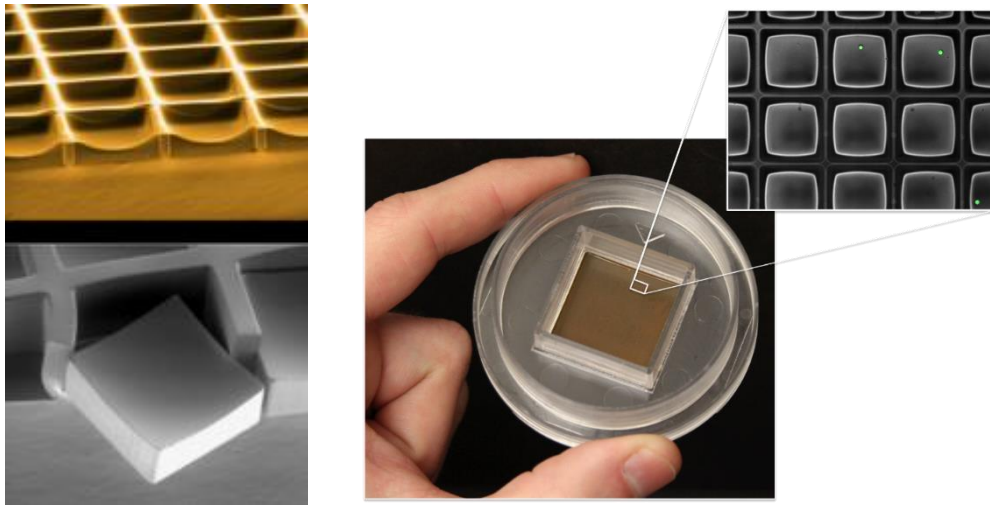
Engineering - sgebhart@cellmicrosystems.com

CellRaft Technology

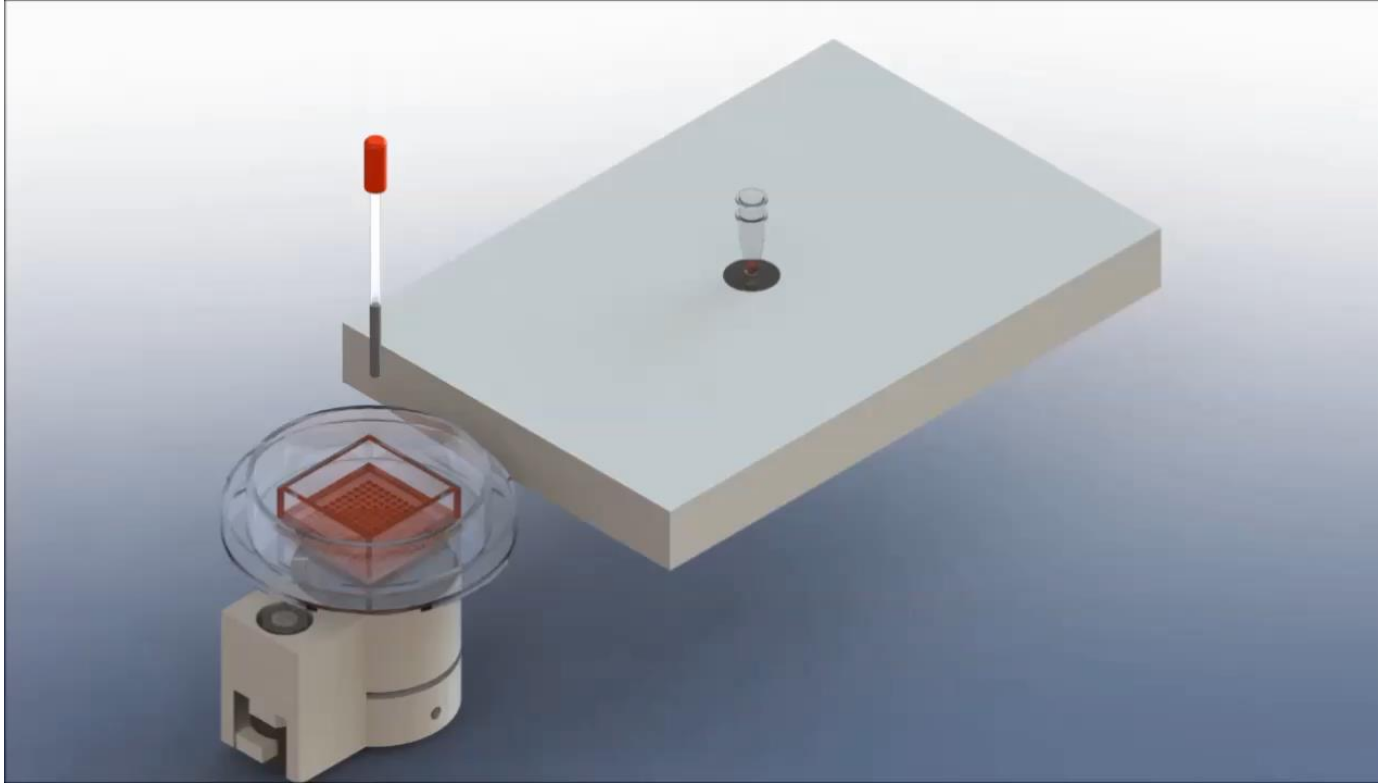
- Straightforward means of imaging, sorting and isolating single cells
- Employs a microwell array which replicates standard culture conditions
 - Eliminates minimum sample input requirements
 - Imaging-based sorting has advantages over FACS and other methods to see cell phenotypes prior to isolation
- Multiple products available tailored to specific experimental and throughput needs

CellRaft Technology - Components

- CytoSort Array
 - Microwell array
 - Releasable CellRaft in each microwell
 - 100 or 200 micron CellRafts
 - Elastomer array
 - Polystyrene CellRaft
- Release/Transfer Devices
 - Needle for release of CellRaft from array
 - Magnetic wand for CellRaft recovery and transfer to plate or tube
 - Manual and automated versions



CellRaft Technology - Operation



Video can be viewed here: <https://cellmicrosystems.com/cellraft-technology/>

CellRaft Systems

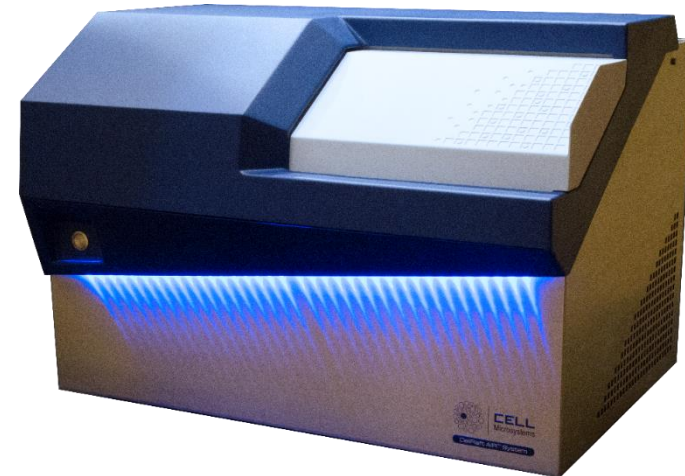
- CellRaft System – Manual

- Fits on virtually any inverted microscope
- Very flexible and cost-effective
- Compatible with any type/size of CytoSort Array
- Approximately 50 cells in 2–4 hours
- Manual collection into any vessel
- Comes with 2 CytoSort Arrays

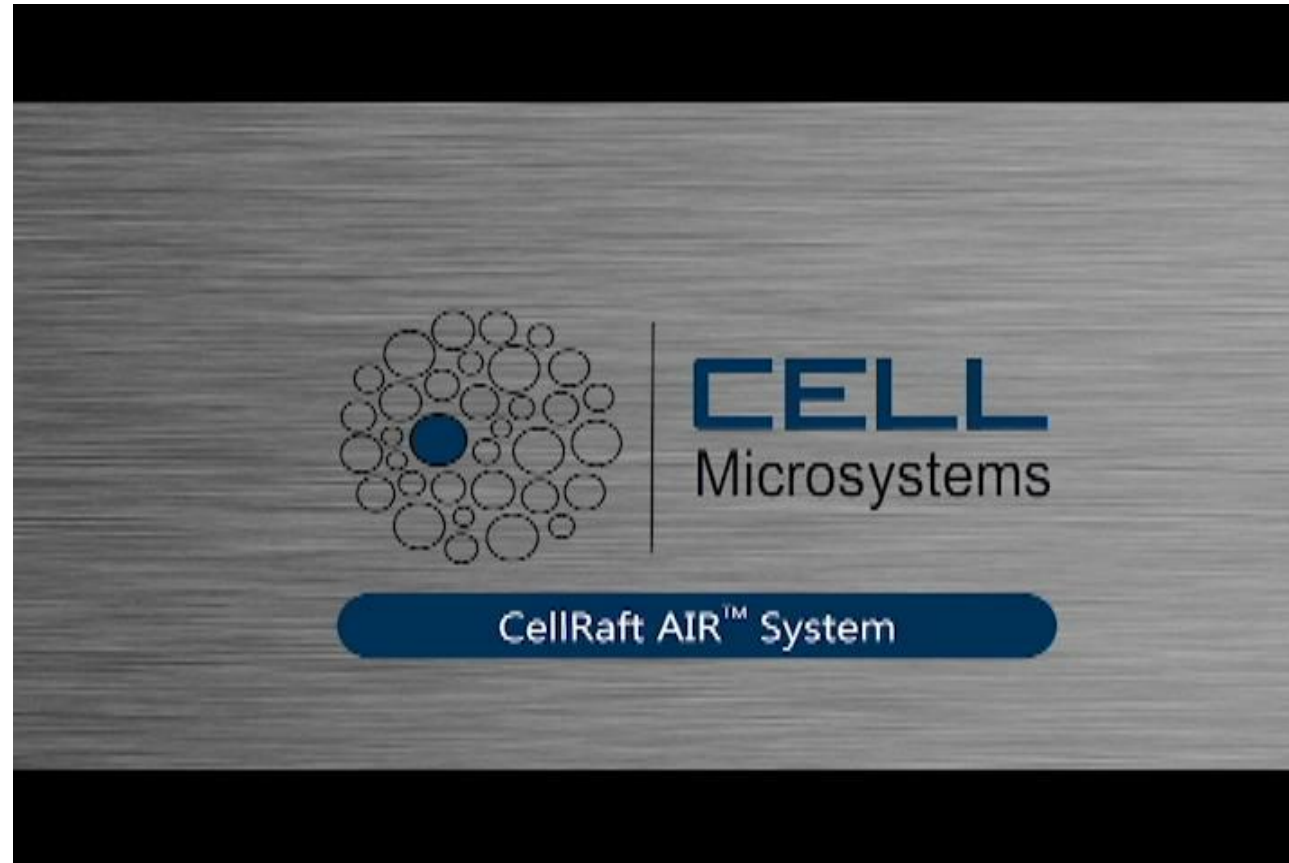


- AIR™ System – Automated

- Fully integrated microscope and release/transfer hardware
- 3-channel fluorescence
- “Real-time” or “Cytometric” sorting capabilities
- 96 cells in approximately 1.5 hr;
- 30 min for each subsequent 96 cells
- Collect into
 - 96-well plates (~150–200 μL volume)
 - PCR strip tubes (minimum 2.5 μL volume).



AIR System in Operation



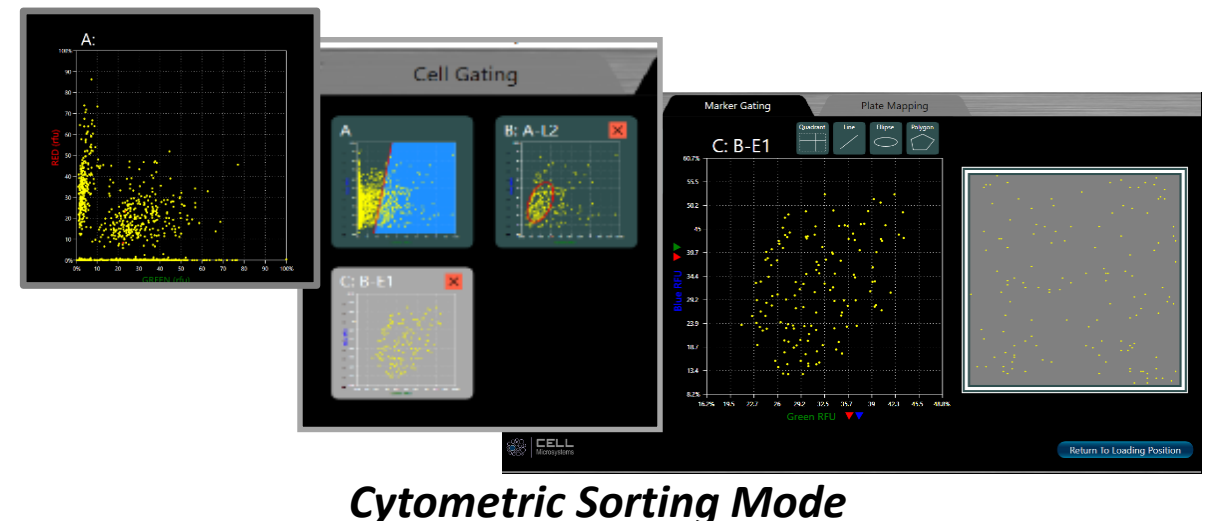
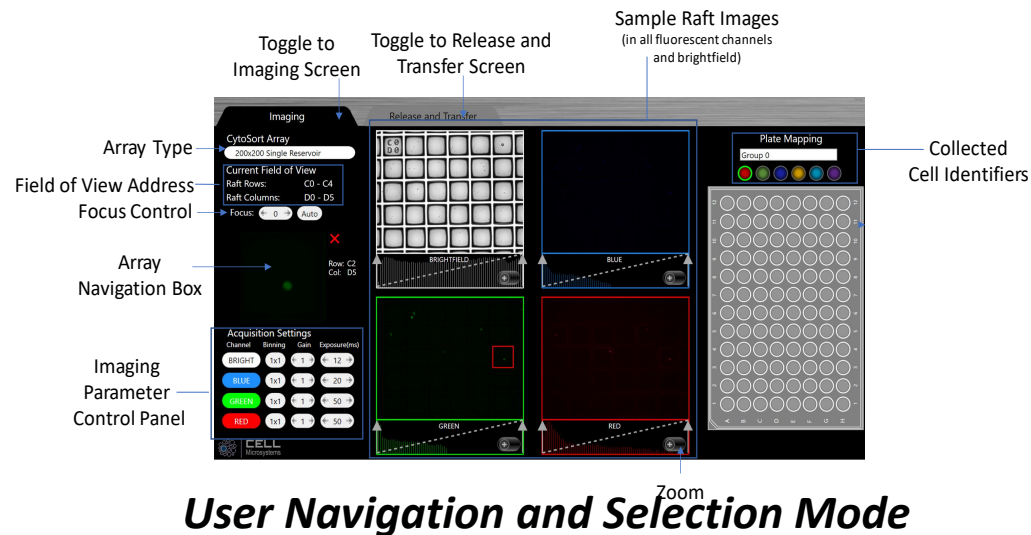
CellRaft Technology for CRISPR

- Allows imaging-based sorting of single cells and colonies
 - Seed a cell suspension on the CytoSort Array
 - Image on the AIR™ System (or your microscope)
 - Isolate cell/colony by release of a single CellRaft from the array
- Technology supports the growth of clonal colonies
 - Monitor colony growth over time and release individual colonies without disturbing the growth and viability of neighbors
 - *Laboratories observe a significant increase in efficiency by growing clones on the array prior to transferring to expansion culture*

AIR System Run Modes

- Multiple ways to conduct an experiment based on sorting requirements

Feature	User Navigation and Selection	Full Array Scan + User Selection	Full Array Scan + Cytometric Mode	Multiple Full Array Scans + User Selection
Sorting Modality	User navigates the array by mouse clicks and empirically identifies cells of interest	After full array scan user navigates the array images by mouse clicks and empirically identifies cells of interest	Fluorescent intensities plotted on XY axes; set thresholds and gates on the histogram	After full array scan user navigates the array images and movies by mouse clicks and empirically identifies cells of interest
Array Position	CytoSort Array must remain on system	Array can be removed from the AIR System after Full Array Scan concludes	Array can be removed from the AIR System after Full Array Scan concludes	Array can be removed from the AIR System after each Full Array Scan concludes
Imaging Parameters	Imaging parameters can be adjusted in real time	Set at beginning of scan	Set at beginning of scan	Set at beginning of each scan
Applications	Identify single cells for molecular analysis	Sensitive cells	Sorting of subpopulations with varying marker expression	CRISPR; Clonal colony tracking; time-course analysis



Clonal Colony Tracking

- Multiple Full Array Scans + User Selection Mode
- Once cells are seeded, software has been developed to monitor cells over time as clonal colonies grow
- Colonies can then be isolated at any time without disturbing neighboring colonies
- Multiple array scans can be viewed simultaneously to watch movies of colony growth

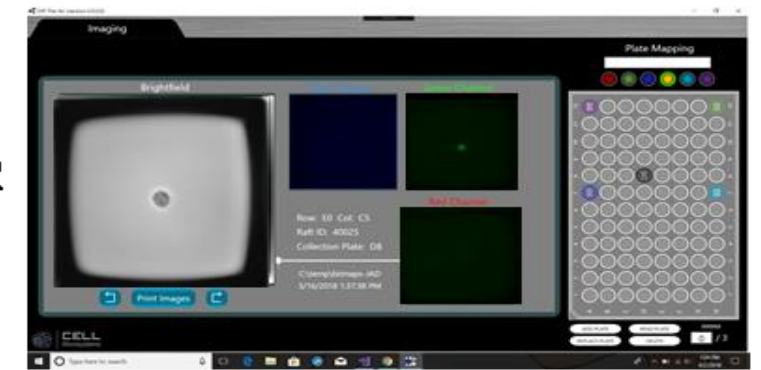
Off the AIR Software:
Database software
allows multiple full
array scans to be
viewed simultaneously

CytoSort Array: P29 Array Type: 200x200 Single Reservoir
Description: GC_TUT4KO

Full Array Scans (6 scans 3 selected)		Isolation Plate Maps (2 merged 0 isolated)		Analysis History (Under Construction)		Report Generation	
Description	Start Time	Image Folder	Channel	Binning	Gain	Exposure	
☐ P29 Rebuild	07/23/18 12:57	c:\CMSData\P29\Scan1\	N/A	N/A	N/A	N/A	
☒ Array P29 - Scan # 2	07/25/18 14:56	c:\CMSData\P29\180725-1456\	G/	1/	1/	10/	
☐ Array P29 - Scan # 3	07/27/18 12:32	c:\CMSData\P29\180727-1232\	G/	1/	1/	15/	
☐ Array P29 - Scan # 4	07/30/18 11:41	c:\CMSData\P29\180730-1141\	G/	1/	1/	10/	
☒ Array P29 - Scan # 5	07/31/18 11:23	c:\CMSData\P29\180731-1123\	G/	1/	1/	15/	
☒ Array P29 - Scan # 6	08/02/18 08:36	c:\CMSData\P29\180802-0836\	G/	1/	1/	37.5/	

Buttons: User Review and Selection, Gradual Review, Close Record, Edit Mode

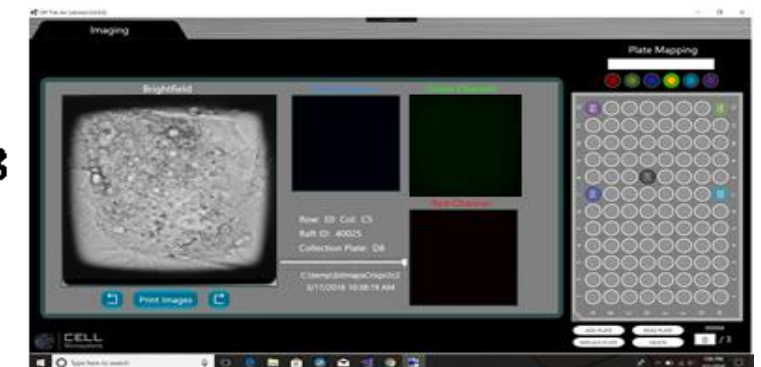
Day 2



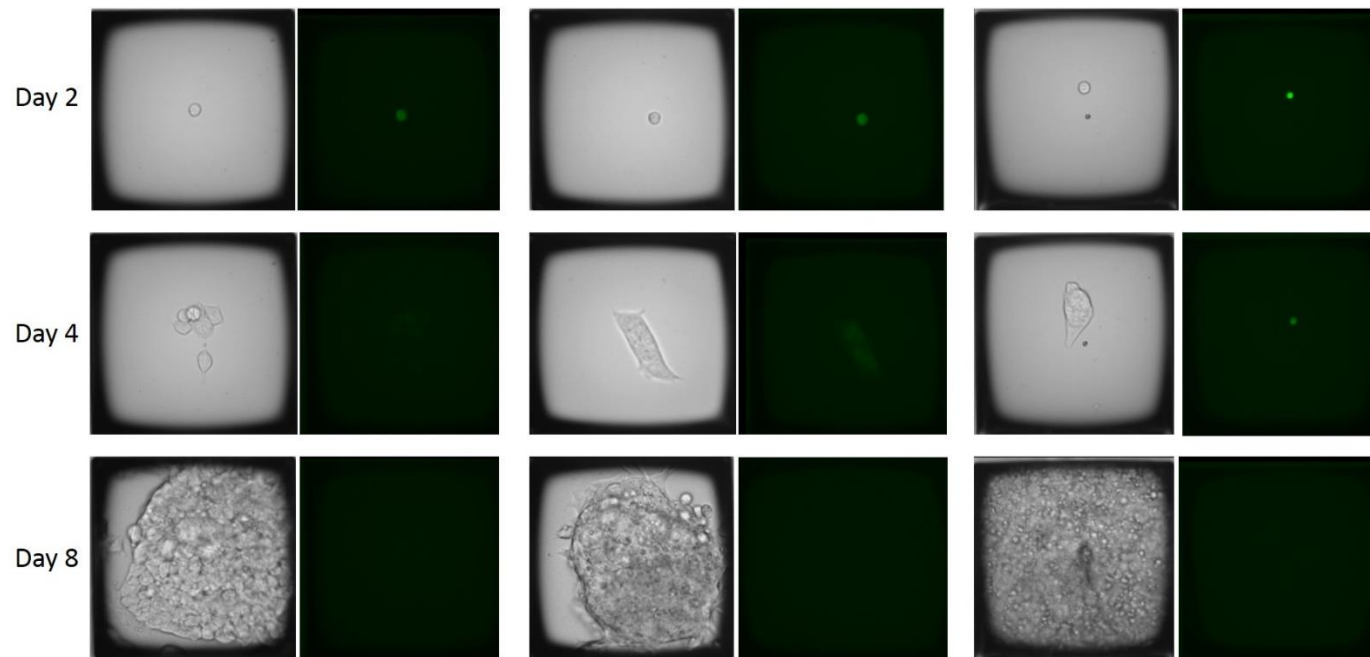
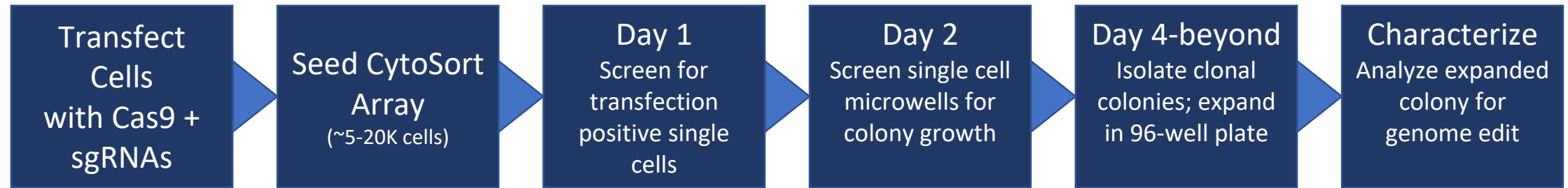
Day 4



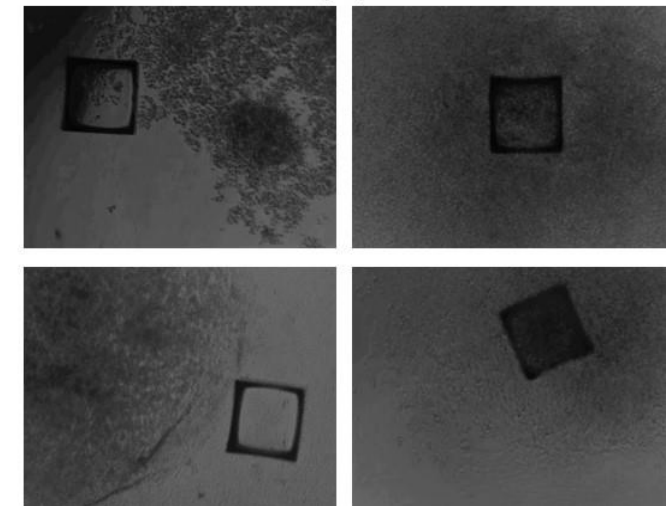
Day 8



CRISPR/cas9 Clonal Colony Propagation



Expansion culture in 96-well plate after 19 days



Double KO – TUT7 + 3'hExo

- Using the same workflow a double KO was generated
 - TUT7 – uridylates 3' of histone mRNAs
 - 3'hEXO – binds and degrades histone mRNAs at appropriate times of cell cycle
 - Two double KO cell lines generated

Allele #1 GGATCCAAGT-----GCGAGTGACTTCAGTCACCCG

Wild-type GGATCCAAGTTCATGACCTCCAGTGCGAGTGACTTCAGTCACCCG

Allele #2 GGATCCAAGTTCATGACCTT GTGCGAGTGACTTCAGTCACCCG

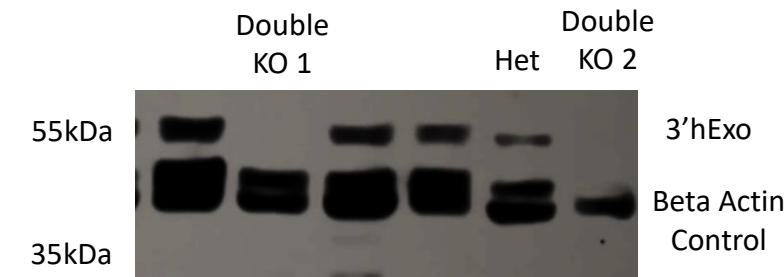
1 NT MUTATION 2 NT DELETION

CCT is the PAM site: red box shows the guide RNA

Wild-type GGATCCAAGTTCATGACCTCCAGTGCGAGTGACTTCAGTCACCCG

Both alleles GGATCCAAGTTCATGA-----AGTGCGAGTGACTTCAGTCACCCG

5 NT DELETION



3'hEXO
KO western

Data courtesy of William Marzluff, PhD; UNC Chapel Hill

Other Applications of the CellRaft Technology

- Capable of both replacing FACS and enabling novel experiments
- Single cell genomics
 - Single cell RNA-Seq (Welch et al., 2016)
 - Single cell DNA-Seq (SNP analysis; Milholland et al., 2017; Dong et al., 2017)
 - Single nucleus sequencing (Weirman et al., 2017)
- Clonal colony propagation
 - Stem cell niche co-cultures (Gracz et al., 2015)
 - Organoid culture (Williamson, et al., 2018)
- Time-course screening for imaging-based phenotypes
 - Cytotoxic T-Cell screening for cancer immunotherapy (Attayek et al., 2016)

See our website for a comprehensive list of publications:
<https://cellmicrosystems.com/publications/>

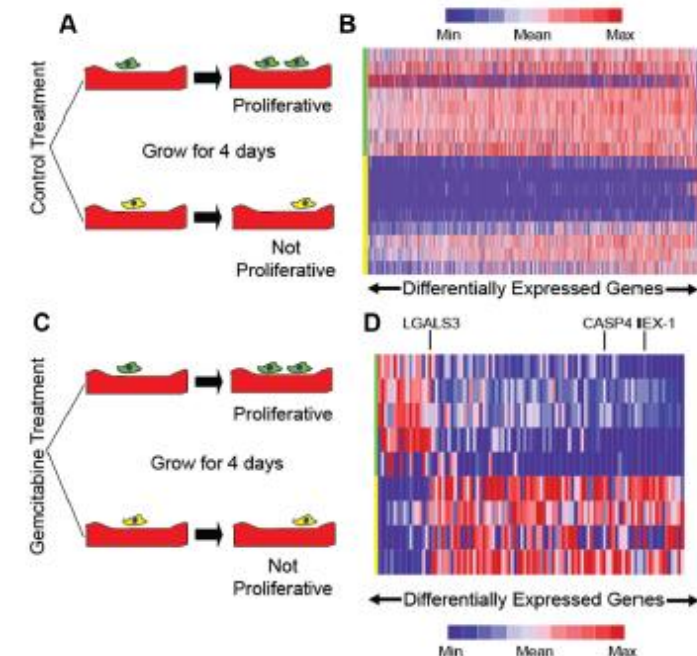
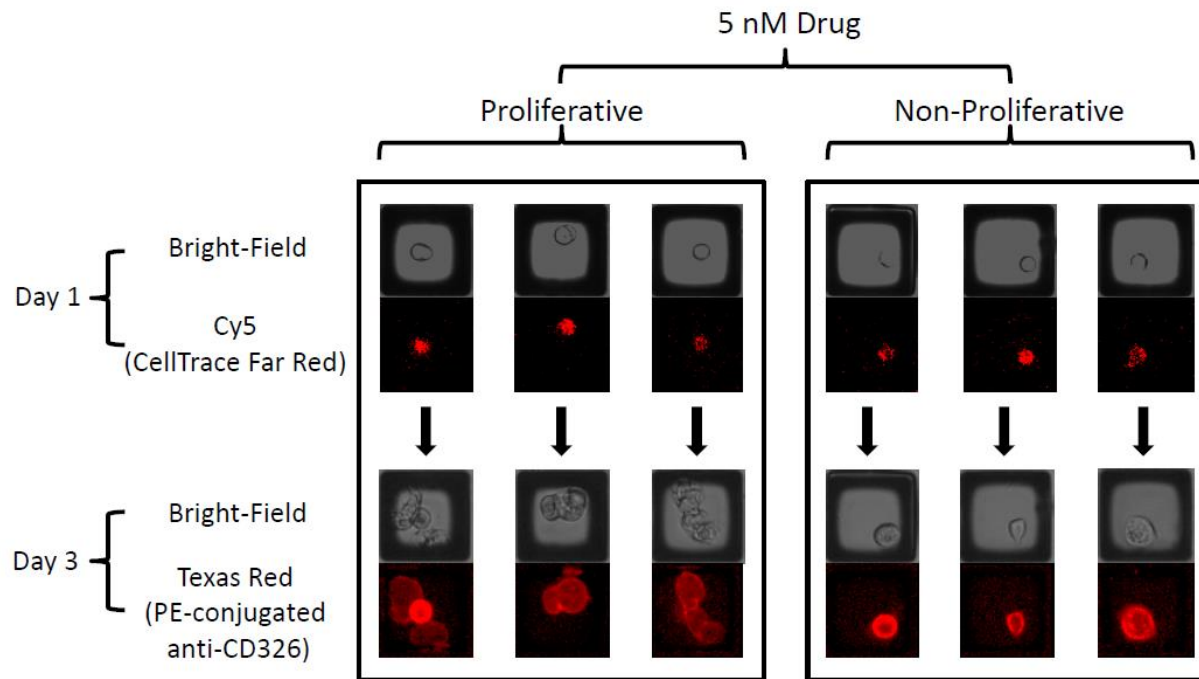
CellRaft Technology compared to FACS

- Some workflows are uniquely supported by the CellRaft Technology
- Some workflows enabled by FACS are also supported

Comparison to Flow Sorting	
No minimum input	Isolate at least 96 cells from samples with as few as 500 cells
Minimal set-up	Cell culture consumable goes into the system; no specific set-up for cell size or type
Flexible collection	Collect in 96-well plates or PCR strip tubes in as low as 2.5 μ L
Efficient	95% in 96-well plates and 90% in PCR strip tubes (2.5 μ L volume)
Dynamic Collection	Can isolate individual colonies without disturbing neighboring colonies not yet ready for collection
High Efficiency	Colonies grow beyond “critical period” prior to isolation; 85-90% colony survival compared to 10-15% from single cells from FACS

Gemcitabine sensitivity of CFPAC-1 Cells

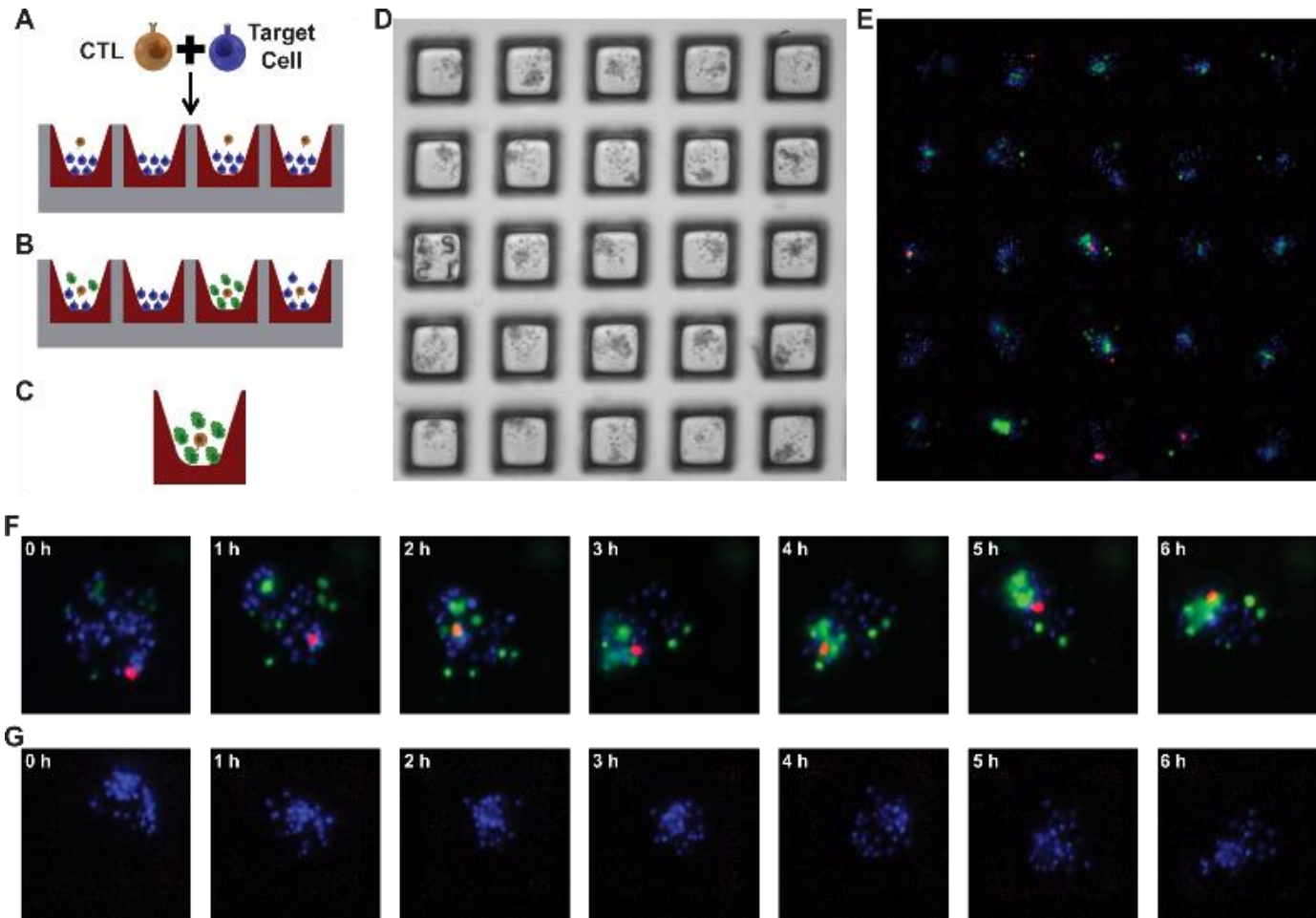
- Cells were exposed to drug which should eliminate proliferation
- Drug-treated cells which remain proliferative may be expressing drug-resistance genes



Functional Assay → Genomic Assay

Welch et al., Nucl Acid Res 2016

Cancer Immunotherapy



- Monitor cytotoxic T-cells over time for tumor cell killing
 - Kinetics can be monitored
 - T-Cells can be cloned to identify TCR sequences



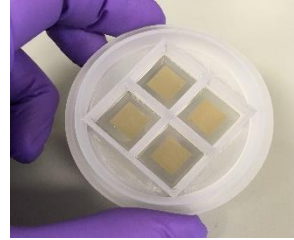
Clone	M1p Tetramer	TCR α	TCR β
CTL3-MR-B8	Positive	V19-CAISEAGTGGSYIPTF-J6	V19-CASSMFVGQPQHF-J1-5
CTL3-MR-D10	Positive	V41-CAVSVEETSGSRLTF-J58	V19-CASSFFHNNEQFF-J2-1
CTL3-MR-F9	Positive	ND	V19-CASSIRSSYEQYF-J2-7
CTL3-LD-B2	Negative	ND	ND
CTL3-LD-C4	Negative	ND	ND
CTL3-LD-C8	Negative	ND	ND
CTL3-LD-G3	Negative	ND	ND

Attayek et al. (2016) Integrative Biol. 8(12):1197-1314

New CytoSort Arrays

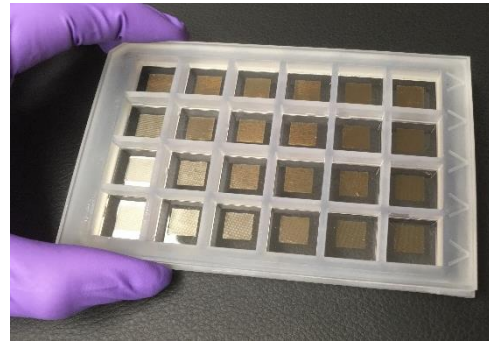
■ CytoSort-Quad

- Four reservoirs each featuring a CellRaft Array
- ~1,600 CellRaft microwells in each reservoir's array
- Seed 100–1000 cells and allow colonies to grow
- No minimum and capable of growing hundreds of clonal colonies



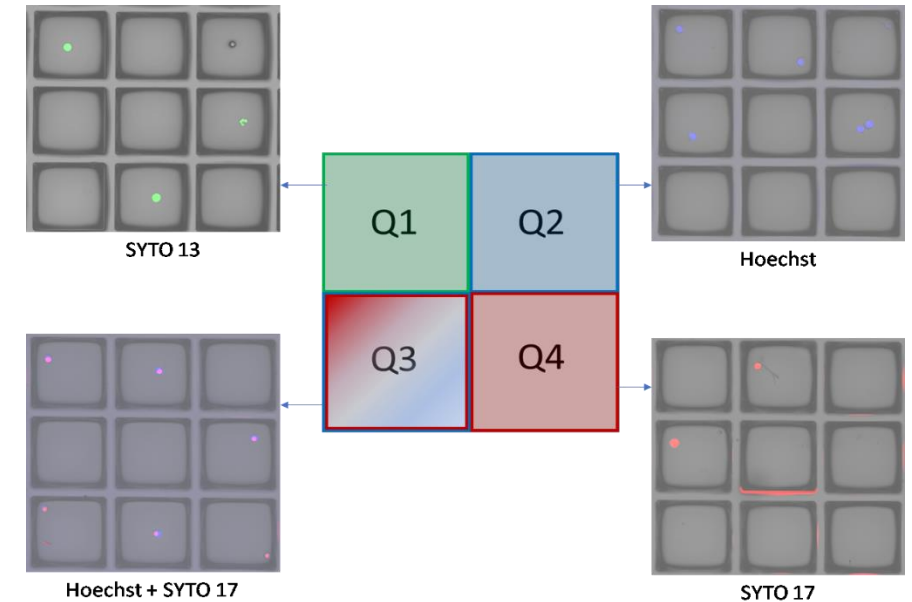
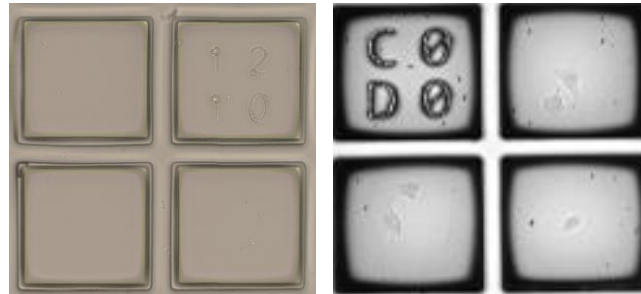
■ HexaQuad Array

- Same principle as Quad arrays
- 24 reservoirs
- Array is on SLAS–standard footprint to allow compatibility with liquid handling robots



■ High resolution imaging arrays

- Plastic of the CellRaft is replaced by a glass-like material
- Improved refractive index complementarity and less “shadowing”



- Please check our website and twitter for updates
- New RaftNotes, publications, webinar and workshop announcements
- Technology descriptions and other literature

- Fully automated
Imaging, sorting and isolation of single cells with
no minimum sample size requirement
- Application flexibility
Compatible with genomics and cloning workflows

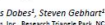
 Standard cell culture

CytoSort Arrays keep cells happy and viable, enabling cloning and reducing artifacts

Imaging-based sorting

Detailed image analysis with 3-channel fluorescence

 1 hour for 96 cells
Cells sorted into standard multi-well plates and tubes

[illegible]

Cytostar Arrays are fabricated using an elastomeric well array, filled with individual Cellofate made of thinned out culture plastic. Cytostar arrays are manufactured in two half sizes: 100x100 microns (74,000 Cellofates) which are best suited to single cell molecular analysis and 200x200 microns (15,000 Cellofates) which are best suited to some expansion culture applications. In addition, Cytostar Arrays are provided with either a single well combining all Cellofate features, or a well divided into a smaller plate for experiments requiring multiple treatments or culture conditions.

	175C	400-000	400-040
Length	175.00 mm	400.00 mm	400.00 mm
Width	175.00 mm	400.00 mm	400.00 mm
Well	Well-400	Well-400	Well-400
Area	30,625 sq. mm	160,000 sq. mm	160,000 sq. mm

Size
175 x 175, 200 x 200, 275 x 275

Weight
125 lbs

Microscope
20X Objective, Custom Autofocus, Motorized

[illegible]

www.cellmicrosystems.com

Applications and protocols for use with CellRite Technology

Imaging and Sorting Living Cells Stained with Vital Dyes on Cell Microsystems' CytoSort™ Array and Automated CellRaft AIR™ System

Jacquelyn DuVall, PhD, Nick Trotta, PhD, Rob McOellan,
M. Anwen La Dine and Steven Gebhart, PhD
Cell Microsystems, Inc. / Research Triangle Park, NC

Abstract

Applications and protocols for use with CellBrite Technology

Methods for preparing cryopreserved neural tissue samples for single nucleus sequencing (SNS) using the CellRift Technology

Mike McConnell, PhD, Ian Burbulis, PhD, Jacquelyn DuVall, PhD, Nick Trotta, PhD,
Cell Microsystems, Inc. | Research Triangle Park, NC

Single cell genomic analysis has already impacted a large field of life science fields, including tumor clonality, neuronal mosaicism and drug resistance mechanisms. These methods rely on the ability to process small amounts of DNA, ensuring that a maximum amount of high quality nucleic acid can be extracted from a given cell. Translational research however depends on the availability of large numbers of samples, such as to acquire in large numbers as fresh samples. As an alternative to single cell sequencing, single nucleus sequencing (SNS) has enabled genomic analysis of single nuclei. SNS has been used to sample cells found in various biobanks and other facilities. The CellRanger Technology uniquely enables sorting and isolation of single nuclei and downstream analysis. This review discusses optimal methods for imaging nuclei on CytoSort Arrays, and downstream molecular biology protocols for the isolation and preparation of nucleic acid prior to sequencing.

Translational Single Cell Analysis using FACS

Applications and protocols for use with CellRaft™ Technology

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

²Dept. of Biochemistry and Biophysics, University of North Carolina at Chapel Hill; Chapel Hill NC

Abstract

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 Microsystems

neural tissue
(SNS) using

mic analysis. The methods for SNS using the CellRaft Technology were in the laboratory of Mike McConnell, University of Virginia and are published.

100 milligram tissue sample

Clear Preparation
(gradient centrifugation)

Fixed and Cell-type Marker Staining

Array Coating and Seeding

Isolate Single Nuclei from