Single Cell Genetics; from Sorting to Fluidigm

John D. Mountz, MD, PhD
Director, CFCC at Shelby
jdmountz@uab.edu; 4-8909

Erik Malarkey, PhD
Single Cell Specialist at CFCC
malarkey@uab.edu; 4-0994

May 26, 2016,
The Wonderful World of Technology Series
Comprehensive Flow Cytometry Core Support

Please cite the following grant numbers in your publications if you used the Core:

NIH P30 AR048311
NIH P30 AI27667

Special Acknowledgements:

- Fluidigm/BioMark are acquired through a VA Shared Equipment Evaluation Program (Lou Dell’Italia, MD)
- FACS Index Sorting is supported by UAB CCTS (Robert Kimberly, MD)
- UAB Research Foundation for sponsoring CFCC
Topics to Discuss

• Technology of the Fluidigm C1 and BioMark HD

• Assays/Designs

• How to work with the Core to set up your Fluidigm experiments?
What does the Core have to facilitate your single cell analyses?

- **Sorting**
  - FACS ARIA II & Index sorting
  - Fluidigm C1

- **Single cell PCR and SNP**
  - IFC controllers MX (48 x 48)
  - HX (96 x 96)
  - BioMark (qPCR)
  - Singular software

All in SHEL Rm 271
The Fluidigm microfluidic technology supports genomics-based applications:

• Single cell gene expression
• High-throughput SNP genotyping
• RNA-seq
• Protein expression analysis
• Digital PCR
• Mutant detection
• More under development
What can one do with the Fluidigm C1 Single Cell Autoprep System?

Watch the C1 video here: Fluidigm C1 general concept

<table>
<thead>
<tr>
<th>Application</th>
<th>Use in combination with other modules</th>
<th>Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA expression</td>
<td>BioMark</td>
<td>Single-cell Gene Expression brochure</td>
</tr>
<tr>
<td>mRNA sequencing</td>
<td>Clontech’s SMARTer Ultra Low RNA input Kit for cDNA synthesis and Illumina’s Nextera XT Sample Preparation Kit for library preparation.</td>
<td>mRNA sequencing protocol</td>
</tr>
<tr>
<td>DNA sequencing</td>
<td>Next-Generation Sequencing</td>
<td>C1 DNA Sequencing Brochure</td>
</tr>
<tr>
<td>Chromatin accessibility (ATAC-seq)</td>
<td>Hudson Alpha</td>
<td>view protocol worksheet</td>
</tr>
</tbody>
</table>
What is done inside a Fluidigm C1 Prep?

Batches of cells are loaded in a single pipetting step.

Individuals cells are:
- Captured
- Washed
- Verified
- Lysed
- Reverse Transcribed
- Amplified

This is the first step bulk gene amplification.
# Applications enabled by the Fluidigm C1 Prep

<table>
<thead>
<tr>
<th>Specification</th>
<th>Details</th>
</tr>
</thead>
</table>
| Supported applications | Targeted gene expression  
                          Whole transcriptome analysis (mRNA sequencing)  
                          miRNA analysis  
                          DNA sequencing (see DNA Sequencing Data Sheet, PN 100-7426) |
| Sample sources         | Primary and cultured cells                                             |
| Sample input           | 200–1,000 cells                                                        |
| Average cell size      | 5–25 μm                                                                |
| Cell capture efficiency* | >90% occupancy rate with 1,000 cells input                            |
Fluidigm C1 General Workflow

- Capture
- RT
- mRNA-seq prep
- Whole genome amp

Fluidigm C1/BioMark Workflow

- Isolate & Enrich
- Pipette to C1 IFC
- Capture up to 96 single cells, Wash, stain & image
- Lyse, Preamplify and Harvest
- Pipette 96 samples to Dynamic Array IFC
- Load IFC
- Run PCR on BioMark
- Data Analysis

Chip for sorting
Chip for PCR
# Fluidigm C1 in Combination with BioMark for Gene Expression

*All carry out at the CFCC*

<table>
<thead>
<tr>
<th>Targeted Gene Expression (specific target amplification)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Workflow</strong></td>
</tr>
</tbody>
</table>
| **Supported assays** | Delta Gene™ Assays  
TaqMan® Gene Expression Assays (probe-based) |
| **Assay format** | Two-step reverse transcription and amplification |
| **Throughput** | 9,216 datapoints per run |
| **Time to results** | Cells to preamplified target: 8 hours (overnight)  
Real-time PCR: 4 hours  
Total assay time: 12 hours |
| **Hands-on time** | 70 minutes |
| **Chip-to-chip R² correlation** | 0.99 |
**Fluidigm C1 in Combination with Illumina Sequencing for RNA-seq**

**mRNA Sequencing Application**

<table>
<thead>
<tr>
<th><strong>Workflow</strong></th>
<th>Capture, stain, image, lyse, reverse transcribe, amplify, harvest, prepare library, and sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Supported platforms</strong></td>
<td>Any Illumina® sequencing system</td>
</tr>
<tr>
<td><strong>Time to results</strong></td>
<td>Cells to sequence-ready library: 13 hours (overnight)</td>
</tr>
<tr>
<td><strong>Hands-on time</strong></td>
<td>3 hours</td>
</tr>
<tr>
<td><strong>Typical sequencing performance observed from K562 and HL60 cell lines:</strong></td>
<td></td>
</tr>
<tr>
<td>cDNA concentration per cell</td>
<td>&gt;300 pg/μL</td>
</tr>
<tr>
<td>Number of detected RefSeq transcripts with &gt;10 reads mapped</td>
<td>≥4,000 (≥200,000 total single-end reads or ≥400,000 total paired-end reads)</td>
</tr>
<tr>
<td>Reads mapped to rRNA/total reads</td>
<td>≤1% rRNA/total reads</td>
</tr>
</tbody>
</table>

**Oxford Genomics Centre**

**Wellcome Trust Centre for Human Genetics**

**Carry out at the CFCC**

**Heflin Genomics Core Hudson Alpha**

---

**Diagram:**

- **Enrich**
- **Load and Capture**
- **Wash, Stain and Image**
- **Lyse, RT, and Amplify**
- **Prepare Library**
- **Sequence**
- **Analyze**

- **C1 Single-Cell Auto Prep System**
- **Any Illumina System**
The Biomark HD is compatible with multiple system components to meet a variety of application and sample throughput needs.

### IFC Controller Compatibility

<table>
<thead>
<tr>
<th>Component</th>
<th>IFC Controller MX</th>
<th>IFC Controller HX</th>
<th>IFC Controller RX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression</td>
<td>48.48 Dynamic Array IFC</td>
<td>96.96 Dynamic Array IFC</td>
<td>—</td>
</tr>
<tr>
<td>SNP genotyping</td>
<td>48.48 Dynamic Array IFC</td>
<td>96.96 Dynamic Array IFC</td>
<td>192.24 Dynamic Array IFC</td>
</tr>
<tr>
<td>Digital PCR</td>
<td>12.765 Digital Array IFC</td>
<td>48.770 Digital Array IFC</td>
<td>—</td>
</tr>
<tr>
<td>qdPCR 37K IFC</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Experiment tracking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gas pressure</td>
<td></td>
<td>Internal compressor</td>
<td></td>
</tr>
<tr>
<td>Interface</td>
<td></td>
<td>USB and ethernet</td>
<td></td>
</tr>
<tr>
<td>IFC Controller MX, HX, RX or WX software</td>
<td></td>
<td>Touchscreen interface for operating and tracking</td>
<td></td>
</tr>
<tr>
<td>Dimensions (approx.)</td>
<td>19 x 9.5 x 13 inches; 48.5 x 24 x 33 cm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**BioMark HD for Gene Expression**

1. **Prime**: prime the Dynamic Array IFC to prepare it for sample transfer
2. **Transfer**: Pipet samples and primer probe sets into the designated inlets of the IFC.
3. **Load**: Load the IFC on the controller, which prepares pressure loading of samples and assays into the reactions
4. **Run**: Run the IFC in BioMark for thermal cycling and end-point real-time fluorescence detection.
5. **Analyze**: View and analyze color-coded expression heat maps, allele maps and cluster plots for single or multiple runs with the Singular software.

[BioMark procedure watch it here](#)
Topics to Discuss

- Technology of the Fluidigm C1 and BioMark HD
- Assays/Designs
- How to work with the Core to set up your Fluidigm experiments?
Fluidigm Experiment Design

Venn Diagram:
- Chips
- Cells
- Primers
Two types of chips for gene expression

C1 chip used during the Fluidigm C1 step

Dynamic IFC chip for PCR
Chip for the Fluidigm C1 – single cell sorting, lyse, RT, pre-amp

Pipetting Map for C1 System
Pre-amplification Implementation in Chip
The C1 chip is optimized to catch your cells

- Capture: Live cells capture more efficiently than dead cells
- Lysis: Efficient for live cells, not optimized for the cross-linking that occurs during fixation
- Transcript quality: maximal for efficiently prepared live cells

Size does matter

The cell size determines which chip to use for the experiment.
Chips capture three size ranges of cells:
- 5-10 μm
- 10-17 μm
- 17-25 μm

Cell size must be measured on cells in suspension. The Core has a Nexelom Cellometer X4 Cell Counter to determine cell count/size.
Chips for IFC controller and BioMark

Dynamic Arrays For GX, GT, CNV

<table>
<thead>
<tr>
<th></th>
<th>96 x 96 array</th>
<th>48 x 48 array</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost per chip</td>
<td>~ $900 / Chip + reagent</td>
<td>~ $300 / Chip + reagent</td>
</tr>
<tr>
<td>Validation capability</td>
<td>Duplication/triplication</td>
<td>Less so</td>
</tr>
<tr>
<td>Time</td>
<td>More time for primer design and pipetting</td>
<td>Less time for primer design and pipetting</td>
</tr>
<tr>
<td>Information</td>
<td>9,216 data points</td>
<td>2,304 data points</td>
</tr>
</tbody>
</table>
Other Chip Formats for IFC controller and BioMark

Dynamic Arrays
For GX, GT, CNV

- 48.48
- 96.96
- Flex Six™ IFC

Digital Arrays
For dPCR, CNV

- 192.24
- 12.765
- 48.770

NGS library prep
Access Array 48.48

qdPCR 37K IFC
IF YOU DO NOT WANT  HIGH THROUGHPUT

FLEX SIX GENE EXPRESSION CHIP

Any 12X12 configurations

Use the chip up to 6 times

Format Flexibility

• 72 samples by 12 assays
• 12 samples by 72 assays
• Any combination of 12x12
• Or 12x12 multiple times
Fluidigm Experiment Design

Chips

Cells

Primers
## Cells

<table>
<thead>
<tr>
<th></th>
<th>Fluidigm Sort</th>
<th>FACS ARIA II</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cost</strong></td>
<td>$400/chip + reagents</td>
<td>$75 assisted sort/hr</td>
</tr>
<tr>
<td><strong>Single cell validation</strong></td>
<td>Can be checked fairly quickly under a microscope</td>
<td>In theory, it should be one cell/well, but hard to verify</td>
</tr>
<tr>
<td><strong>Easy flexibility to alter analysis template</strong></td>
<td>Yes</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Phenotype of cells</strong></td>
<td>Not inherent in the system but can be done with additional steps</td>
<td>Index sorting allows FACS determination of phenotype of each cell</td>
</tr>
</tbody>
</table>
How many cells should I load?

- Large chip: 200-300 cells/μl
- Medium chip: 250-400 cells/μl
- Small chip: 400-800 cells/μl
- In total 1000-5000 cells are loaded into the chip to capture 96

Notes on cell suspension

- Debris in cell suspension negatively effects capture
- Non-monodisperse cell suspensions will present multiple cells in capture sites.
What protocols should I use to prepare single cell suspension?

- Cell culture samples: regular PBS/trypsin harvest
- TrypLE (ThermoFisher) is a more gentle equivalent alternative
- Minimize the time between cell prep and loading on C1
- Do Not vortex the cells
- Minimize cell clumping: triturate shortly before loading
- Filter w/ cell strainers: Pluribead cell strainer (10, 20, 30 μm)
- Add 1-5% BSA for very sticky cells
Dissociation for cultured primary cells

- Freshly derived from living organisms and maintained in culture
- Limited number of divisions before entering senescence
- More sensitive than non-primary cell cultures
- General recommendation: TrypLE cell dissociation
- Alternative recommendation: Acutase “stem PRO acutase” for more gentle dissociation of sensitive cells
Enzymes for tissue dissociation: Papain, Collagenase, Elastase

Papain Dissociation system:
• Recommended for epithelial tissues, brain cells
• Worthington Biochem has recommended protocols
• Unlike many other enzymes papain works in the presence of Ca and Mg

Acutase:
• Recommended for embryonic dissociation
• Stem PRO acutase (ThermoFisher) - a marine-origin enzyme with proteolytic and collagenolytic activity for the detachment of primary and stem cell lines and tissues.
• Gentle (compared to trypsin) dissociation for sensitive cells

• Equilibration with 95%O2 and 5% CO2
• Important for the survival of tissue during dissociation
Tumor or embryonic tissues - single cell suspension prep

Combined mechanical and enzymatic dissociation:

- **Mechanical:** Gentle MACS (Mylteni) easy semi automated dissociation of solid tumor tissues into single cell suspensions

- **Enzymatic dissociation:** Collagenase efficiently removes connective tissues
Buoyancy of the single cell suspension is another factor affecting cell capture.
Cell Buoyancy Assessment

**Inadequate Buoyancy**

Cells settled on bottom of well

**Balanced Buoyancy**

Cells suspended through volume of well

Excessive buoyancy resembles inadequate buoyancy, but the cells settle at the top of the fluid volume and no cells are visible at the bottom surface.
Cell Buoyancy Testing on C1 chip

- Testing can be done on the upper wells of the C1 chip
- 10 μL per well of sample
- Make titrations from table below
- Let samples sit for 10 min and examine titrations using inverted brightfield microscope.
- The balanced buoyancy solution will have even cell distribution throughout the volume of the well.

<table>
<thead>
<tr>
<th></th>
<th>0%</th>
<th>50%</th>
<th>55%</th>
<th>60%</th>
<th>65%</th>
<th>70%</th>
<th>75%</th>
<th>80%</th>
<th>85%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells (μl)</td>
<td>10</td>
<td>5</td>
<td>5.5</td>
<td>6</td>
<td>6.5</td>
<td>7</td>
<td>7.5</td>
<td>8</td>
<td>8.5</td>
<td>9</td>
</tr>
<tr>
<td>Suspension Reagent (μl)</td>
<td>0</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td>3.5</td>
<td>3</td>
<td>2.5</td>
<td>2</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Total volume/well</td>
<td>10</td>
<td>μL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
How do I enrich the samples for C1 experiment during FACS Sorting?

FACS general guidelines:
• Fragile cells: larger nozzle and low psi.
• Increased throughput & yield or for small cells: smaller nozzle and high psi

FACS Aria setting up options:
1. High: 70 μm nozzle and 70 psi sheath pressure
2. Medium: 80 μm nozzle and 40 psi sheath pressure
3. Low: 100 μm nozzle and 20 psi sheath

The Core should help you with this but please always remind the Core of your single cell analysis attempt.
Fluidigm Experiment Design

Chips

Cells

Primers
Two types of primers/probes can be used for Regular qPCR.

### EvaGreen (≈ SYBRGreen)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SYBR® Green-based detection</th>
<th>TaqMan®-based detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Medium*</td>
<td>High</td>
</tr>
<tr>
<td>Sensitivity-low # of copies</td>
<td>Variable*</td>
<td>1-10 copies</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Medium*</td>
<td>High</td>
</tr>
<tr>
<td>Gene expression</td>
<td>Low level of quantitation</td>
<td>High level of quantitation</td>
</tr>
<tr>
<td>Applications</td>
<td>Gene expression, DNA quantitation (pathogen detection), CHiP</td>
<td>Gene expression, DNA quantitation, CHiP, SNP genotyping, Copy number variation, Pathway analysis, microRNA &amp; small RNAs, Mutation detection, Protein analysis, Multiplexing</td>
</tr>
</tbody>
</table>
The EVA Green Approach

- Denature
- Anneal/Amplification
- Binds to any dsDNA (not sequence specific)
- Heat 65-95
- Product Melts at specific Tm, Dye falls off
- Melt Curve Following PCR
EVAGreen Pros and Cons

**Pros**
- F/R primers with non-specific DNA-binding dye = A lot more economical than TaqMan
- Flexible, easy to change genes in and out

**Cons**
- Can be prone to non-specific PCR products (melt curve required)
The TaqMan Dual–labeled Probes Approach

Dual–labeled probes are designed to hybridize to a complementary region of the cDNA.

The probe is flanked by an upstream and downstream primer pair that generates a PCR product.

During PCR, when the polymerase extends the PCR product from the upstream primer, the 5' exonuclease activity of the polymerase cleaves the probe. This separates the fluorescent quencher and reporter dyes.

The increase in fluorescence intensity is proportional to the number of probe molecules that are cleaved.
TaqMan Pros and Cons

**Pros**
- One probe for each target = specificity
- Less likely to report on non-specific PCR products
- Easier to use (all in one tube)

**Cons**
- A lot more expensive
- Harder to design the primers
1. Conventional qPCR design

2. Fluidigm DELTAgene® Assays (Fluidigm-designed primers for genes/panels)
   • Minimize upfront cost of assays
   • Uses EvaGreen Chemistry
   • Amplicons designed to cross an intron
   • Assays predicted to multiplex well

For EVAGreen primers - Order primers through Invitrogen

**Purification**: Desalted
**Starting Synthesis Scale**: 25nmole
**Ship Medium**: Dry
**Normalization**: None

Calculate primers to 100 uM using DNA suspension Buffer from Teknova cat # T0221 (CFCC at Shelby has this buffer)
Topics to Discuss

• Technology of the Fluidigm C1 and BioMark HD

• Assays/Designs

• How to work with the Core to set up your Fluidigm experiments?
How to work with the Core

- The IDEA session to discuss your Fluidigm Exp
- Have all your primers ready
- Single cell sorting and cDNA preamp
- BioMark gene expression
- Singular Data analysis

-4~6 wk
-1~3 wk
-1~3 day*
+0~1 day
+1~.... day

**The IDEA session to discuss your Fluidigm Exp**
- John D. Mountz, MD, PhD
- Erik Malarkey, PhD
- Shanrun Liu, MD, PhD

**Have all your primers ready**
- Davide Botta for TaqMan primer consultation
- Shanrun Liu for EvaGreen primer design consultation
- Hui-Chen Hsu for tested EvaGreen primer list
- Enid Keyser for FACS sort

**Single cell sorting and cDNA preamp**
- Erik Malarkey, PhD

**BioMark gene expression**
- Erik Malarkey, PhD
- Shanrun Liu, MD, PhD

**Singular Data analysis**
- Erik Malarkey, PhD
- David Crossman, PhD at the Heflin Core

* cDNA can be stored for future use
<table>
<thead>
<tr>
<th>Name</th>
<th>Specialty</th>
<th>Phone</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>John D. Mountz, MD, PhD</td>
<td>General discussion</td>
<td>4–8909</td>
<td><a href="mailto:jdmountz@uab.edu">jdmountz@uab.edu</a></td>
</tr>
<tr>
<td>Erik Malarkey, PhD</td>
<td>General Fluidigm/BioMark</td>
<td>4–0994</td>
<td><a href="mailto:malarkey@uab.edu">malarkey@uab.edu</a></td>
</tr>
<tr>
<td>Shanrun Liu, MD, PhD</td>
<td>Single cell genomic analysis and EVAGreen primer design consultation</td>
<td>6–2176</td>
<td><a href="mailto:shanrun@uab.edu">shanrun@uab.edu</a></td>
</tr>
<tr>
<td>Enid Keyser</td>
<td>FACS sorting</td>
<td>4–1362</td>
<td><a href="mailto:efk@uab.edu">efk@uab.edu</a></td>
</tr>
<tr>
<td>Davide Botta, PhD</td>
<td>TaqMan Fluidigm analysis</td>
<td>5–3324</td>
<td><a href="mailto:dbotta@uab.edu">dbotta@uab.edu</a></td>
</tr>
<tr>
<td>Hui–Chen Hsu, PhD</td>
<td>EvaGreen Fluidigm primer list (~200 pairs)</td>
<td>4–8909</td>
<td><a href="mailto:rheu078@uab.edu">rheu078@uab.edu</a></td>
</tr>
</tbody>
</table>
Decision points of your Fluidigm assay

You bring the cells to sort via one of the following way
1. FACS sorting into 1xcell/well
2. Fluidigm sorting into 1xcell/well
3. FACS sorting into 1 population, then Fluidigm sorting into 1xcell/well
4. Other methods that suit your purpose.

You bring the primers to the Core:
For each Exp, one needs to bring:
1. All primers mixed in 1 tube (100 uM, combined primers, 1 ul each).
2. Individual primer set in individual tube (eg. 96 primer sets in 96 tubes, 1 ul each).
**Primers**

1. The Core does not provide any primers

2. The Core offers sequence of EVAGreen primers that has been validated.

<table>
<thead>
<tr>
<th></th>
<th>C1 Chip</th>
<th>C1 AutoPrep kit</th>
<th>96.96 chips + loading kit</th>
<th>48.48 chips + loading kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost/Chip</td>
<td>~$300</td>
<td>~$60</td>
<td>~$900</td>
<td>~$300</td>
</tr>
<tr>
<td>C1 sorting</td>
<td>~$400</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioMark gene expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total $: For 96x96 gene expression</td>
<td>~$1.300</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For 48x48 gene expression</td>
<td>~$700</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Our Experience and Tips
(96.96 Dynamic Array Assay)

- Importance to do duplicates or triplicates
- Importance to have marker genes of your population of interest.
- Clear heterogeneity exists within the same population
- Primer design is very important
- Cross reference of the results with real-time PCR
Can single cell PCR reveal something not seen by a regular bulk cell PCR analysis?

<table>
<thead>
<tr>
<th>Bulk real-time PCR results</th>
<th>Bulk Transitional T2 B cells</th>
<th>Bclxl (Activation gene)</th>
<th>Bcl3 (Anergy gene)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BXD2 (B hyperactivity mice)</td>
<td>23.2</td>
<td>37.5</td>
<td></td>
</tr>
<tr>
<td>BXD2–Il17ra KO (B anergic mice)</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

**Fluidigm/BioMark single cell results**

- **Gapdh**
- **Bclxl**
- **Bcl3**

**BXD2**

**BXD2 Il17ra KO**
Downstream Application Under Development

Heflin Genomics Core
Hudson Alpha

Novel Probes
- Tetramers
- Dendramers
- Nanoparticles
- Click-IT
- Enzymatic probes

Single Cell

BioMark HD gene expression analyzer

BioPlex suspension array system

Milenyi MACS Sorter

BD LSR II Analyzer

The HyperCyt autosampler

BD ARIA Sorter

Amnis ImageStream VA ShEEP grant supported

Fluidigm C1 Single Cell Prep

Erik Malarkey, PhD (malarkey@uab.edu)

At CFCC for Gene Expression

In partnership with other UAB Cores & Facilities

Potential applications

Genetic/Epigenetic
- Genome
- Epigenome
- ChIP-seq
- RNA-seq
- Cloning of genes

Protein
- Cytokines
- Phospho-protein
- Signalome
- Kinome
- Transcriptome

Cell
- Cloning
- Transfection
- Imaging
- Metabolome
- Functional assays

Heflin Genomics Core
Hudson Alpha
Thank you for your attention. Are you ready to take the single cell challenge now?