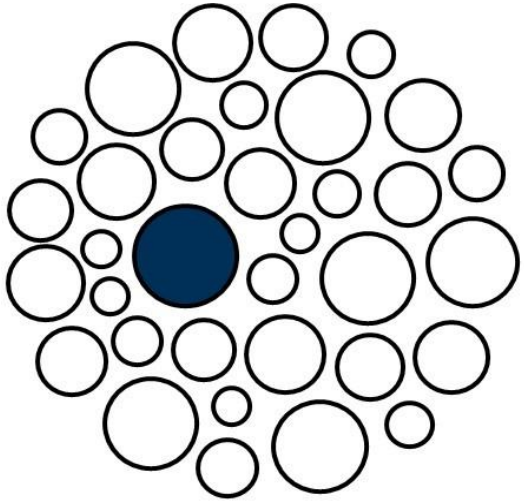
Single Nucleus Sequencing Enabled by the CellRaft Technology



CELL Microsystems

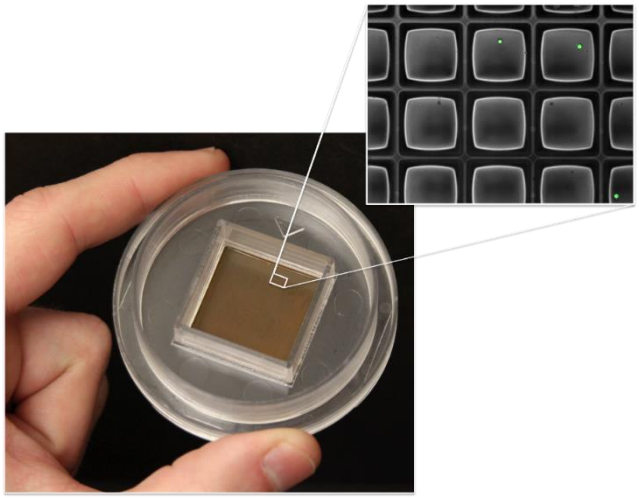
Nick Trotta, PhD
Cell Microsystems, Inc.
ntrotta@cellmicrosystems.com

CellRaft Technology

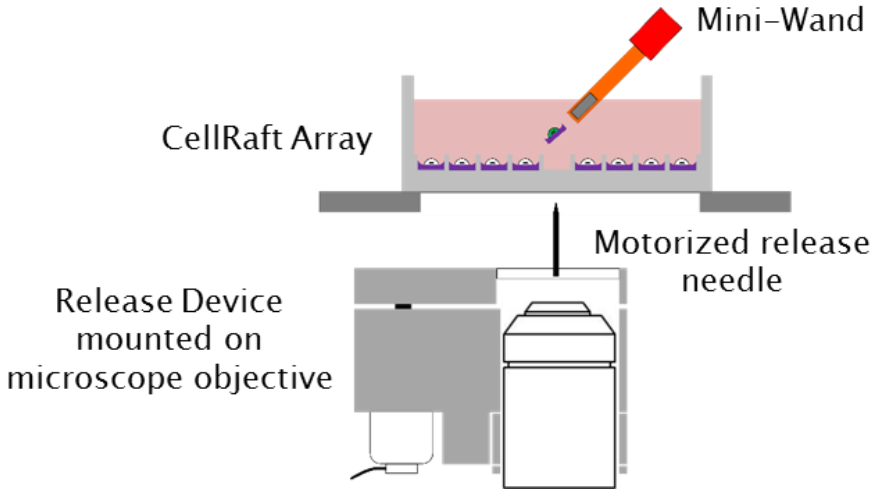
- Straightforward, “track and trace” means of imaging, sorting and isolating single cells or nuclei
- Employs a microwell array which replicates standard culture conditions – improves cell viability and reduces appearance of spurious phenotypes
- Multiple products available; each tailored to specific experimental and throughput needs

CellRaft Technology - Components

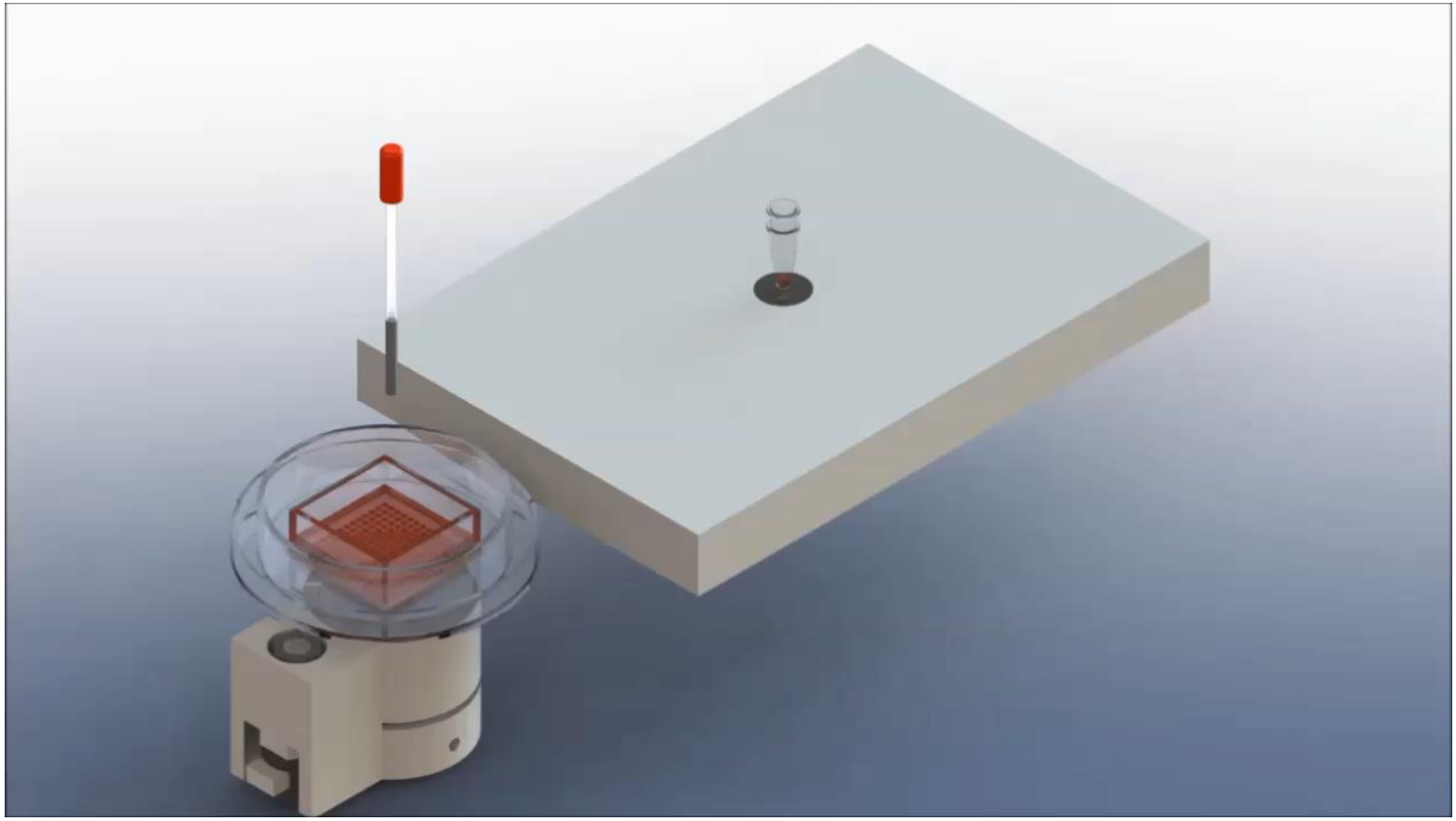
- CytoSort Array
  - Microwell array
  - Releasable CellRaft in each microwell
  - 100 or 200 micron CellRafts
  - Elastomer array
  - Polystyrene CellRaft



- 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



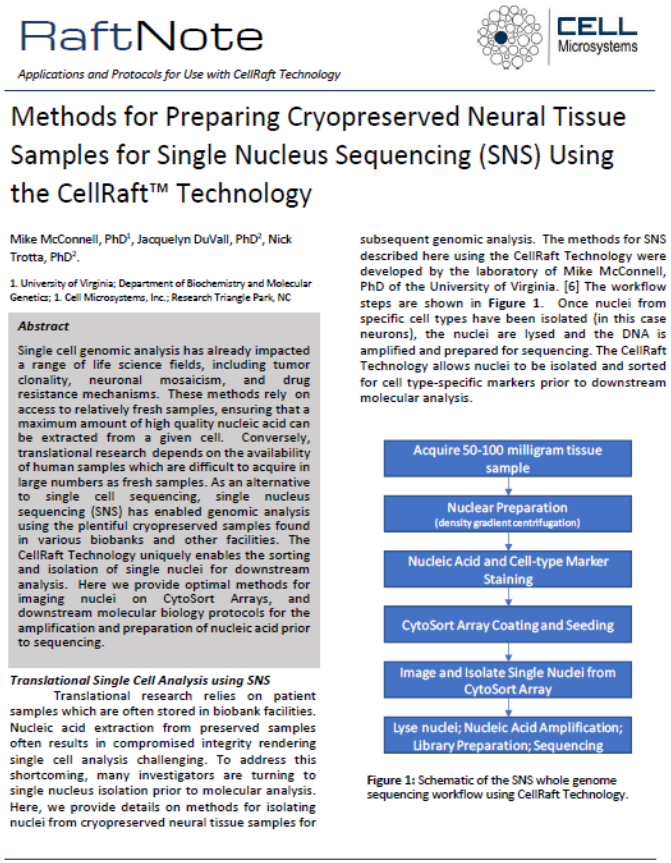
CellRaft Systems

- 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).

Contact Us

- Contact us for info and materials
  - info@cellmicrosystems.com
  - ntrotta@cellmicrosystems.com



RaftNote

CELL Microsystems

Applications and Protocols for Use with CellRaft Technology

Methods for Preparing Cryopreserved Neural Tissue Samples for Single Nucleus Sequencing (SNS) Using the CellRaft™ Technology

Mike McConnell, PhD[1], Jacquelyn DuVall, PhD[2], Nick Trotta, PhD[2].

1. University of Virginia; Department of Biochemistry and Molecular Genetics; 1. Cell Microsystems, Inc.; Research Triangle Park, NC

Abstract

Single cell genomic analysis has already impacted a range of life science fields, including tumor clonality, neuronal mosaicism, and drug resistance mechanisms. These methods rely on access to relatively fresh samples, ensuring that a maximum amount of high quality nucleic acid can be extracted from a given cell. Conversely, translational research depends on the availability of human samples which are difficult to acquire in large numbers as fresh samples. As an alternative to single cell sequencing, single nucleus sequencing (SNS) has enabled genomic analysis using the plentiful cryopreserved samples found in various biobanks and other facilities. The CellRaft Technology uniquely enables the sorting and isolation of single nuclei for downstream analysis. Here we provide optimal methods for imaging nuclei on CytoSort Arrays, and downstream molecular biology protocols for the amplification and preparation of nucleic acid prior to sequencing.

Translational Single Cell Analysis using SNS

Translational research relies on patient samples which are often stored in biobank facilities. Nucleic acid extraction from preserved samples often results in compromised integrity rendering single cell analysis challenging. To address this shortcoming, many investigators are turning to single nucleus isolation prior to molecular analysis. Here, we provide details on methods for isolating nuclei from cryopreserved neural tissue samples for subsequent genomic analysis. The methods for SNS described here using the CellRaft Technology were developed by the laboratory of Mike McConnell, PhD of the University of Virginia. [6] The workflow steps are shown in **Figure 1**. Once nuclei from specific cell types have been isolated (in this case neurons), the nuclei are lysed and the DNA is amplified and prepared for sequencing. The CellRaft Technology allows nuclei to be isolated and sorted for cell type-specific markers prior to downstream molecular analysis.

Figure 1: Schematic of the SNS whole genome sequencing workflow using CellRaft Technology.

Single Nucleus Sequencing Enabled by CellRaft Technology

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University of Virginia

School of Medicine

Center for Public Health Genomics

Center for Brain Immunology and Glia

The Robert M. Berne Cardiovascular Research Center

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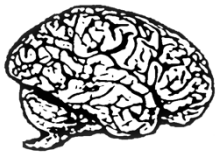
School of Medicine

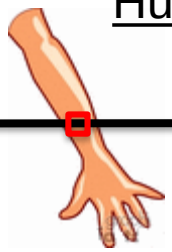
How do human genomes encode human brains?

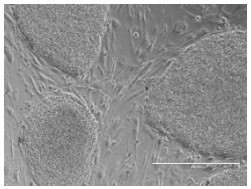


Neurological Disease
Twin Discordance (i.e. individuality)

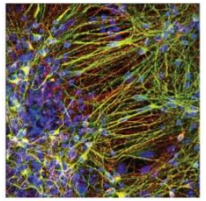
Human induced Pluripotent Stem Cells

Human Genomes in-a-dish

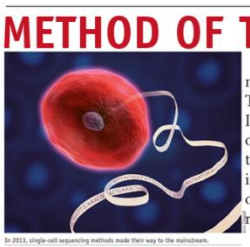




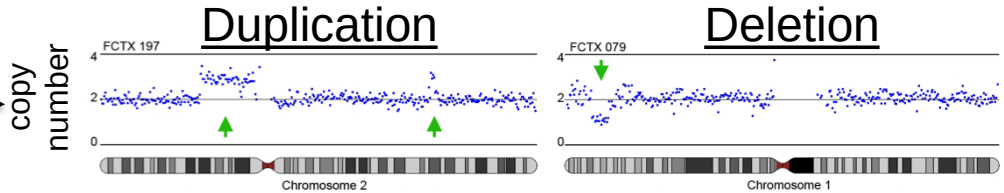
Human Neurons in-a-dish



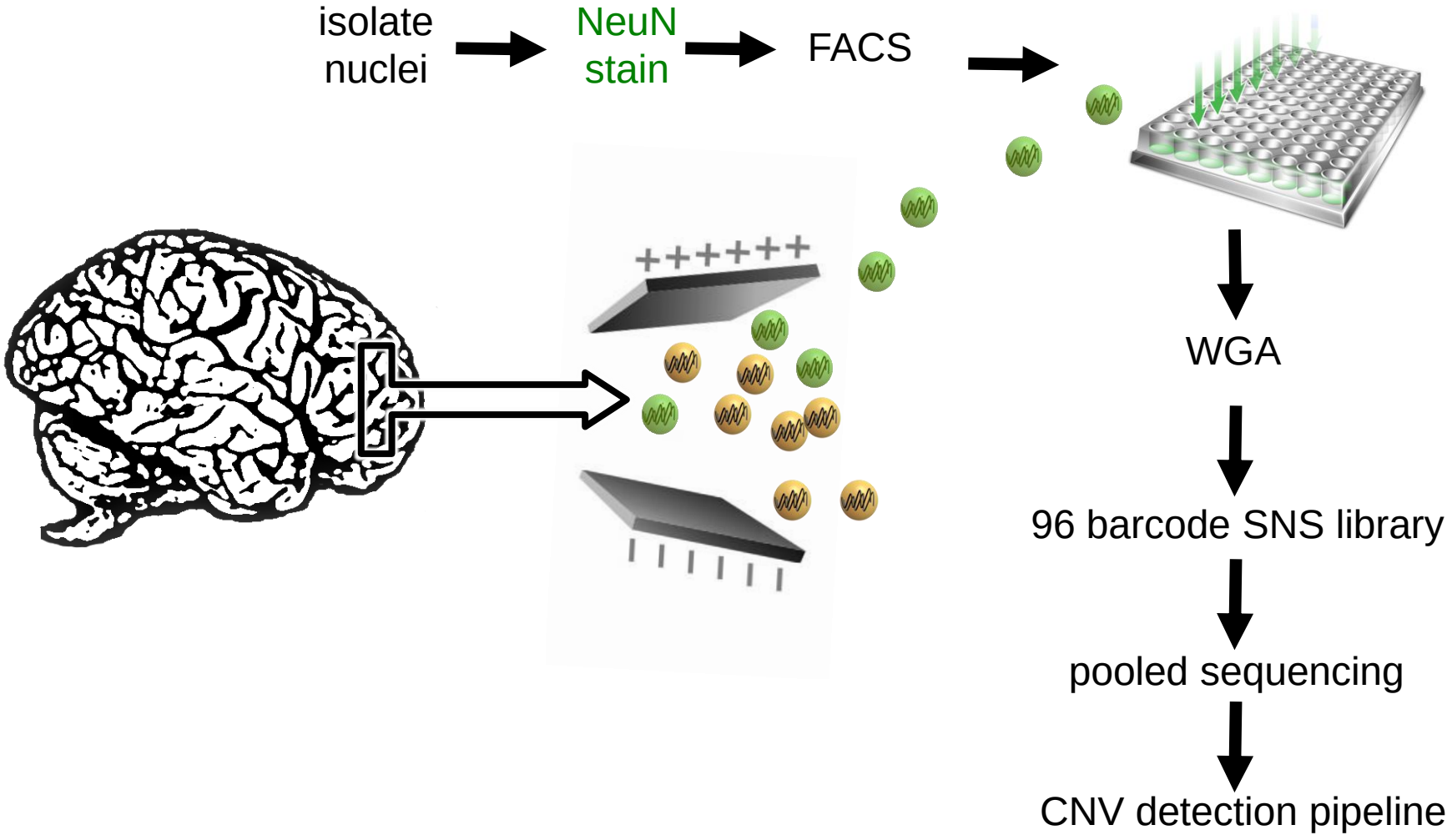
Single Cell Genomics



Mosaic Copy Number Variation in Human Neurons



McConnell, et al. (2013) *Science*



McConnell, et al. (2013) *Science*

nature medicine

Chromosome instability is common in human cleavage-stage embryos

Evelyne Vanneste[1,2,9], Thierry Voet[1,9], Cédric Le Caignec[1,3,4], Michèle Ampe[5], Peter Konings[6], Cindy Melotte[1], Sophie Debrock[2], Mustapha Amyere[7], Miikka Vikkula[7], Frans Schuit[8], Jean-Pierre Fryns[1], Geert Verbeke[5], Thomas D'Hooghe[2], Yves Moreau[6] & Joris R Vermeesch[1]

LETTER

doi:10.1038/nature09807

Tumour evolution inferred by single-cell sequencing

Nicholas Navin[1,2], Jude Kendall[1], Jennifer Troge[1], Peter Andrews[1], Linda Rodgers[1], Jeanne McIndoo[1], Kerry Cook[1], Asya Stepansky[1], Dan Levy[1], Diane Esposito[1], Lakshmi Muthuswamy[3], Alex Krasnitz[1], W. Richard McCombie[1], James Hicks[1] & Michael Wigler[1]

nature COMMUNICATIONS

ARTICLE

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DOI: 10.1038/ncomms11022 OPEN

Nuclear RNA-seq of single neurons reveals molecular signatures of activation

Benjamin Lacar[1,*], Sara B. Linker[1,*], Baptiste N. Jaeger[1,*], Suguna Rani Krishnaswami[2], Jerika J. Barron[1], Martijn J.E. Kelder[1], Sarah L. Parylak[1], Apuã C.M. Paquola[1], Pratap Venepally[2], Mark Novotny[1], Carolyn O'Connor[1], Conor Fitzpatrick[1], Jennifer A. Erwin[1], Jonathan Y. Hsu[1], David Husband[1], Michael J. McConnell[3], Roger Lasken[2] & Fred H. Gage[1]

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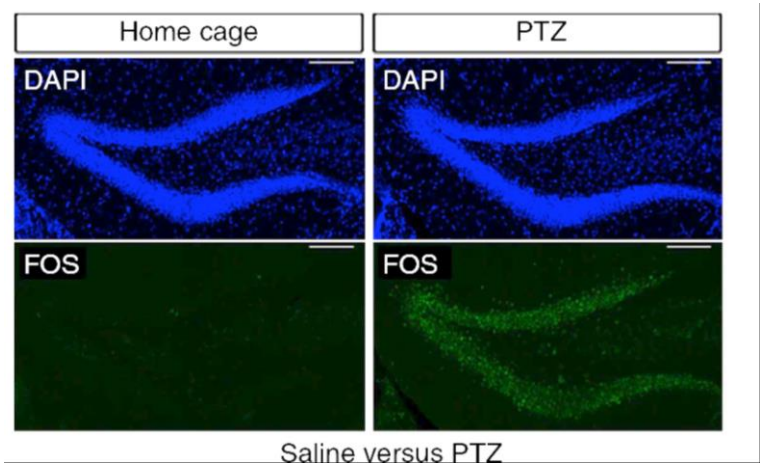
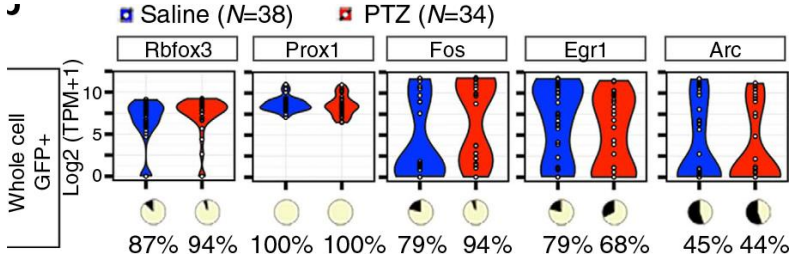
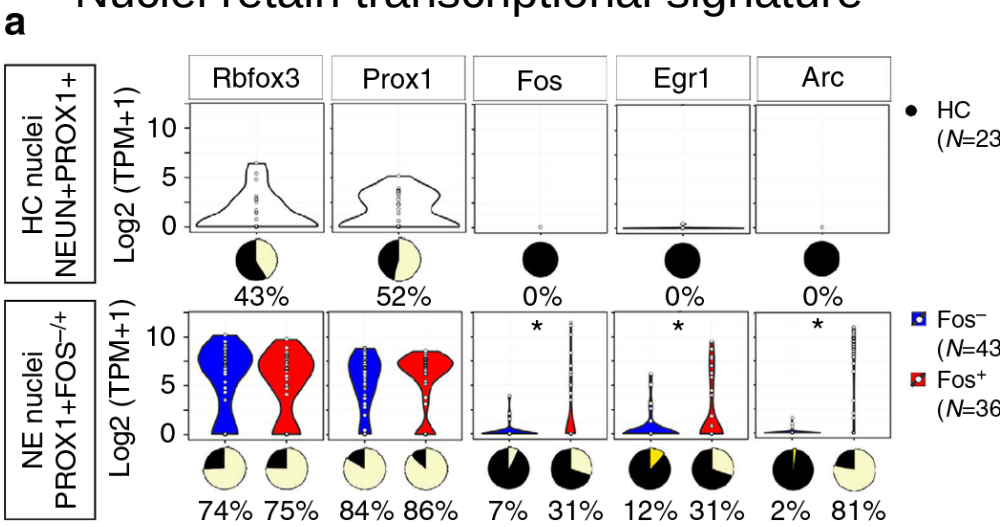
University of Virginia School of Medicine



Intact cells lose transcriptional signature



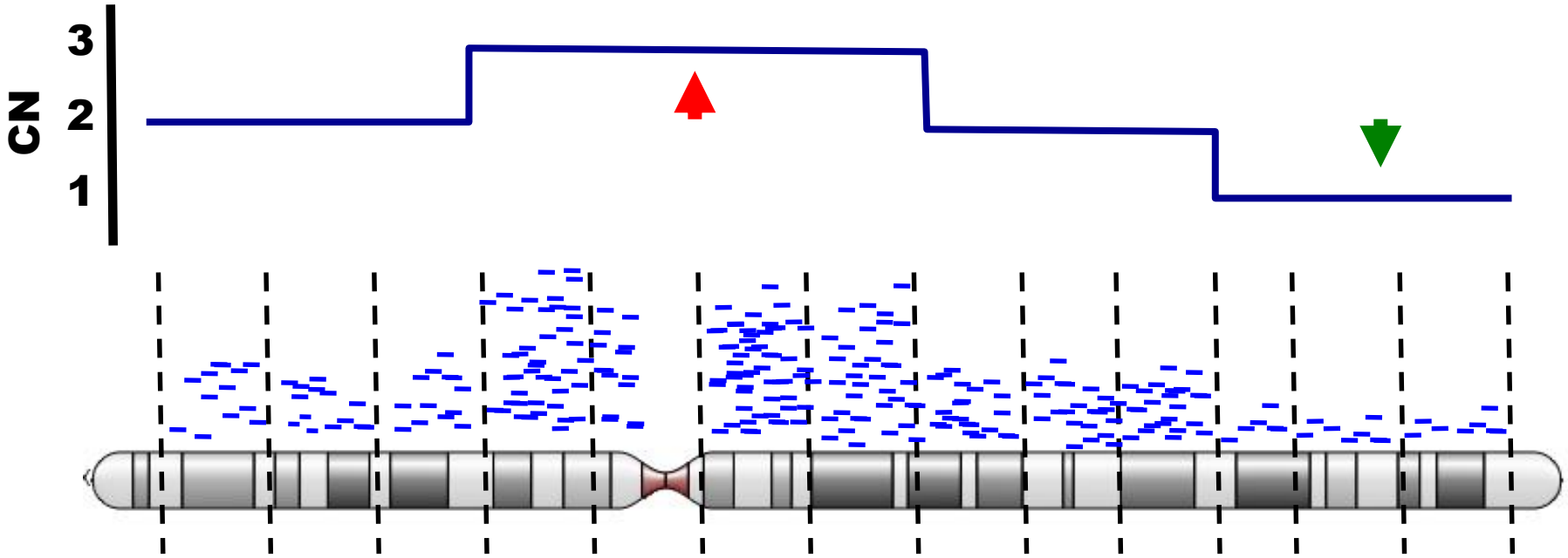
Nuclei retain transcriptional signature



Read Depth

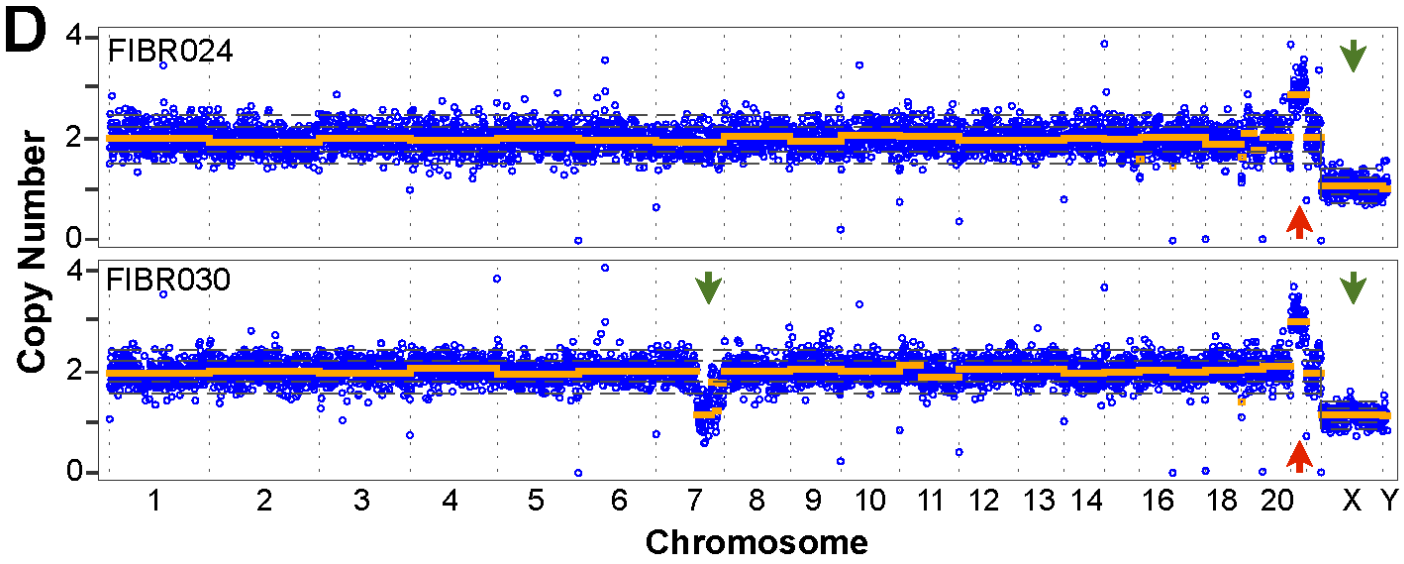
PE 50bp reads mapped to ~500kb non-overlapping bins of mapable sequence

Segmentation algorithm calls regions of copy number gain / loss based on MAD scores



(Note: not to scale)

Whole Genome Amplification (WGA) followed by single cell sequencing



Integer-like gain and loss in trisomic male fibroblasts

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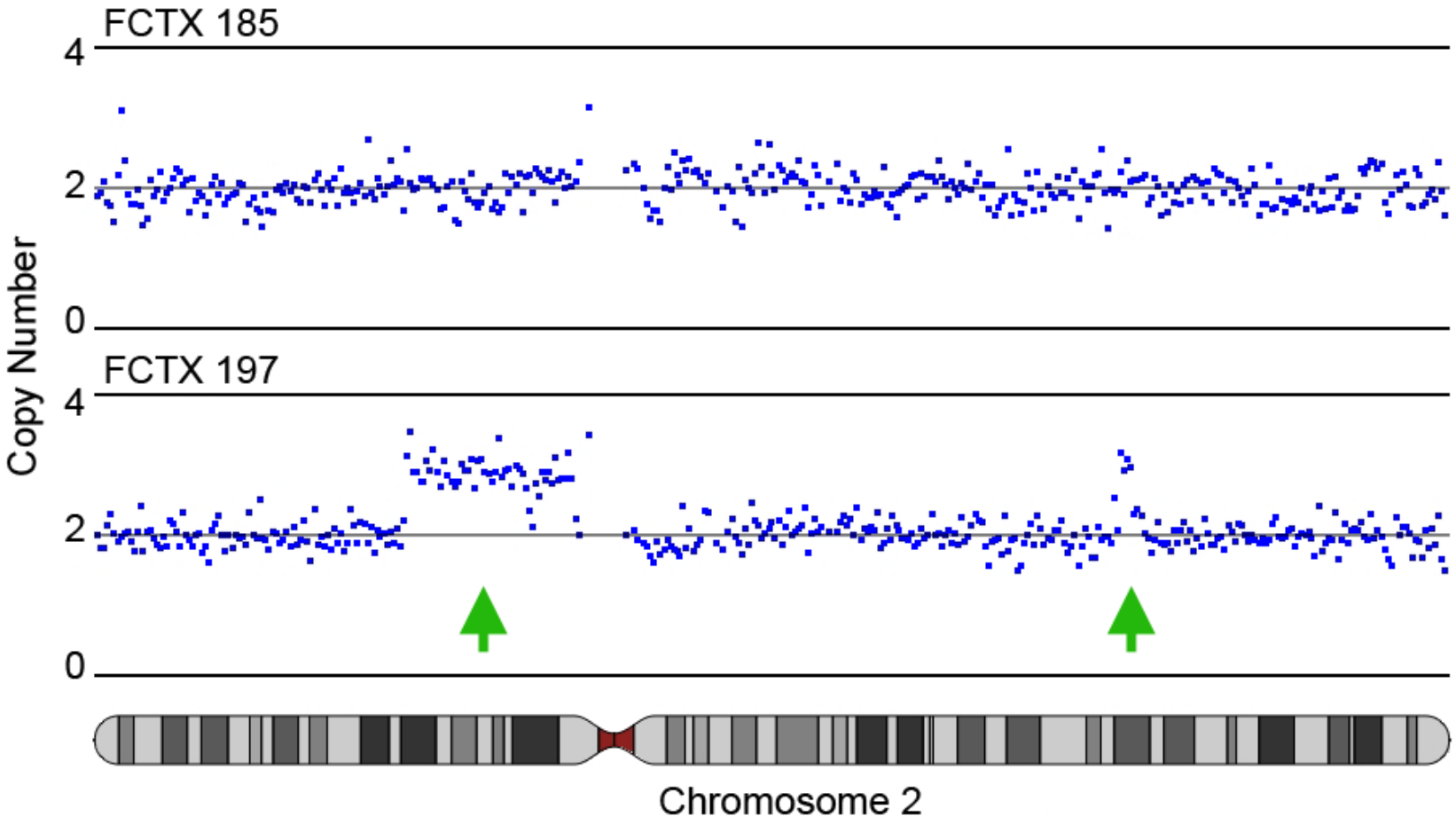
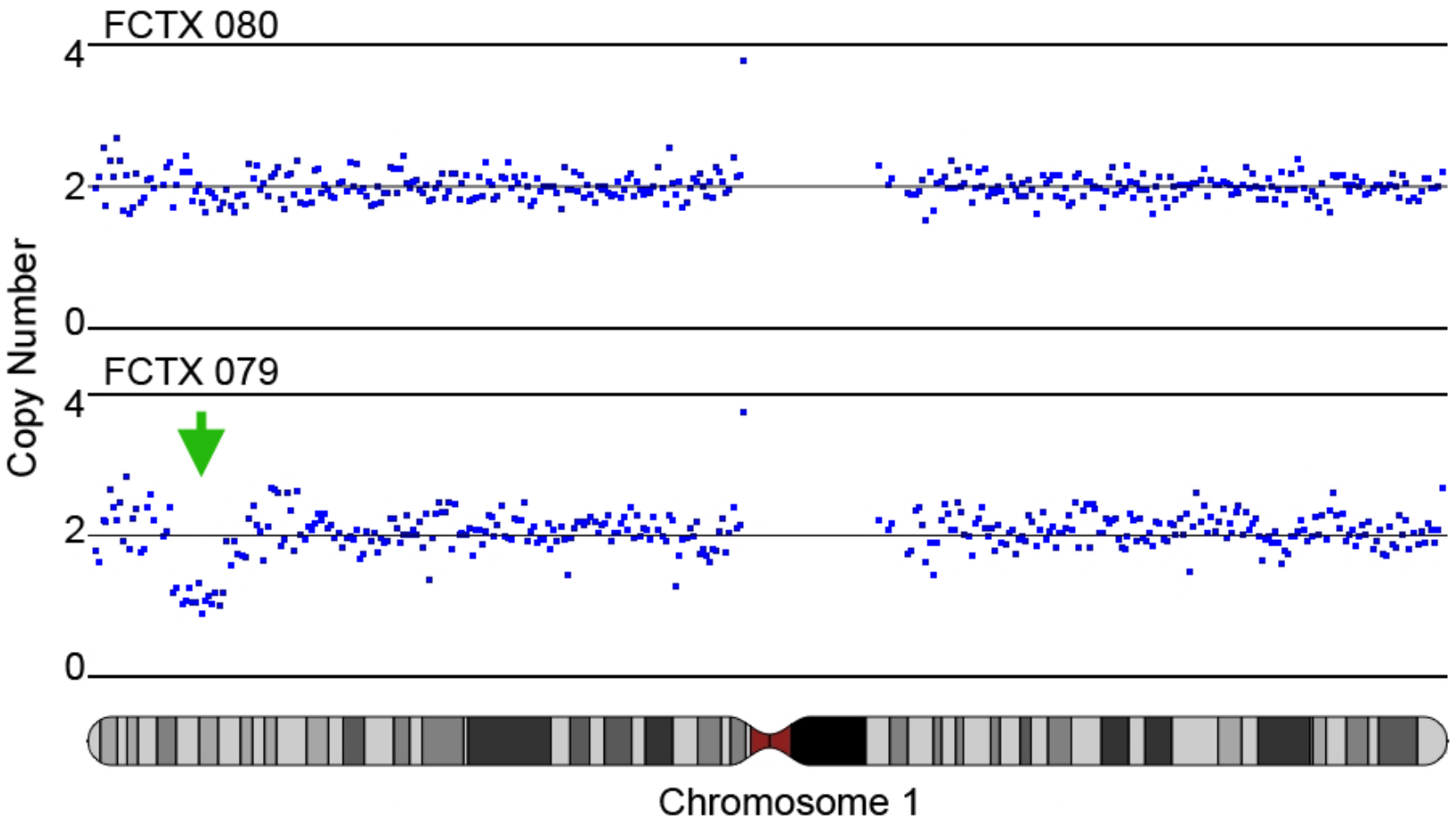
University of Virginia
School of Medicine

A Chr2 Duplication in a FCTX Neuron



McConnell, et al. (2013) *Science*

A Chr1 Deletion in a FCTX Neuron



McConnell, et al. (2013) *Science*

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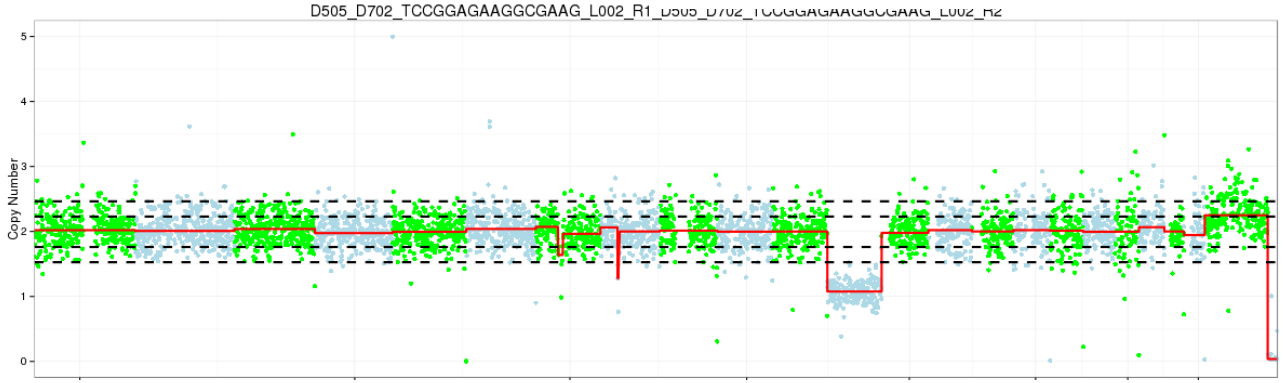
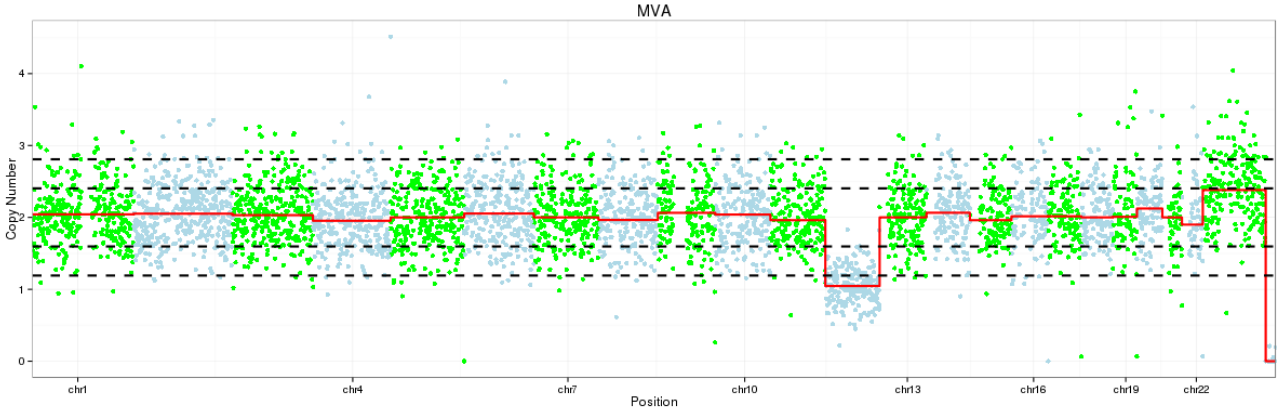
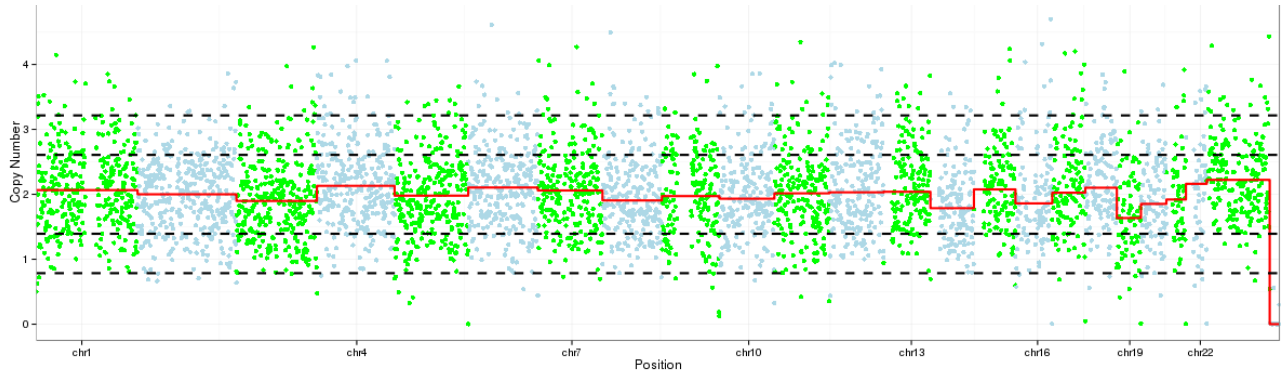
PicoPLEX
(Rubicon Genomics)



MALBAC

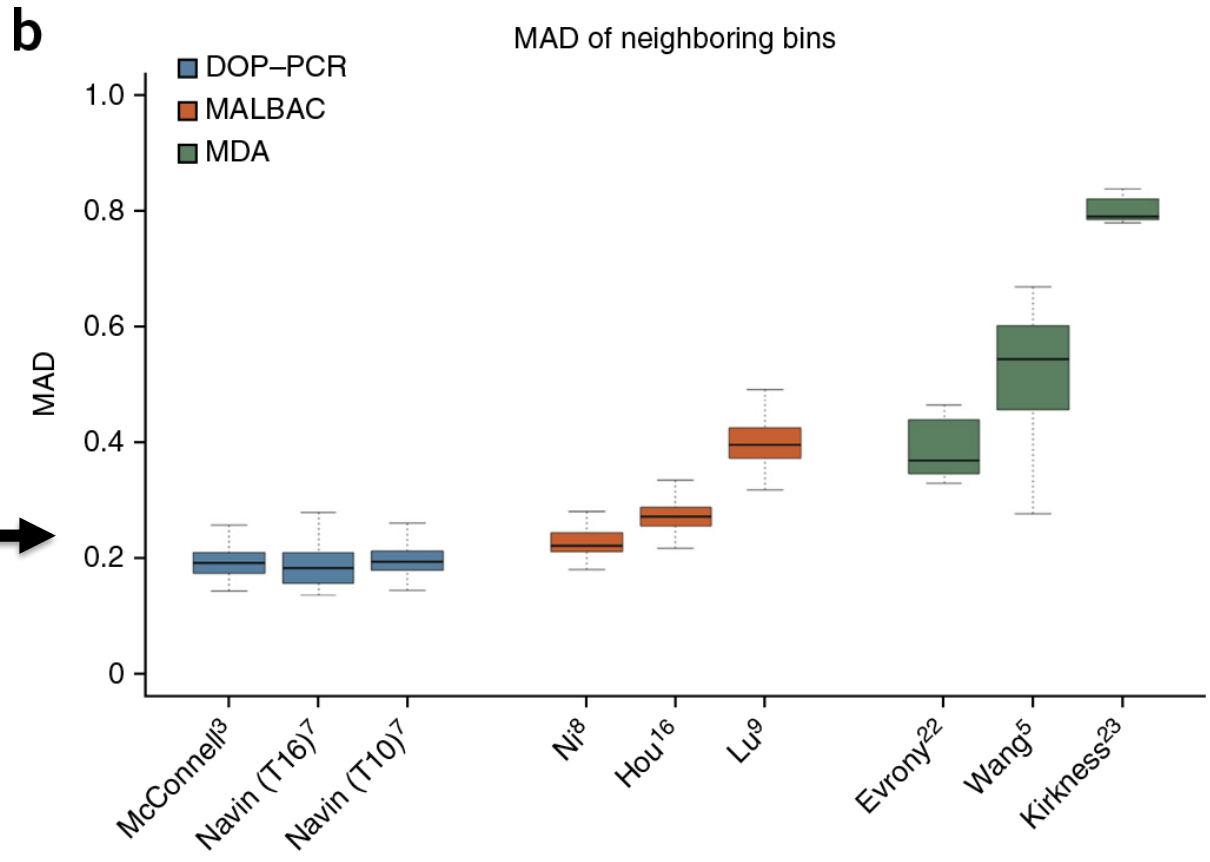


MDA



Low median absolute deviation (MAD) indicates high confidence CNV detection.
Ginkgo (CSHL)

PicoPLEX →



Garvin, et al. (2015) *Nature Methods*

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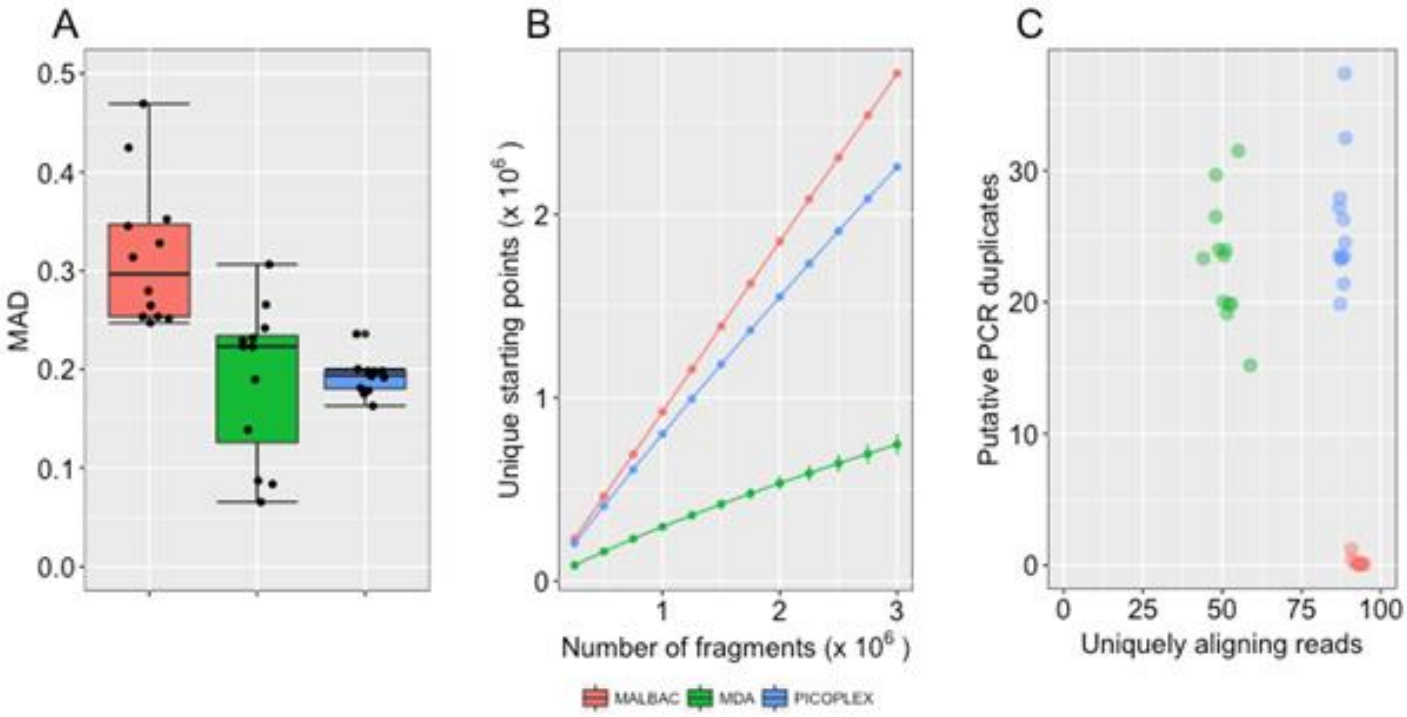

Burbulis, Ratan, McConnell et al. (in preparation)

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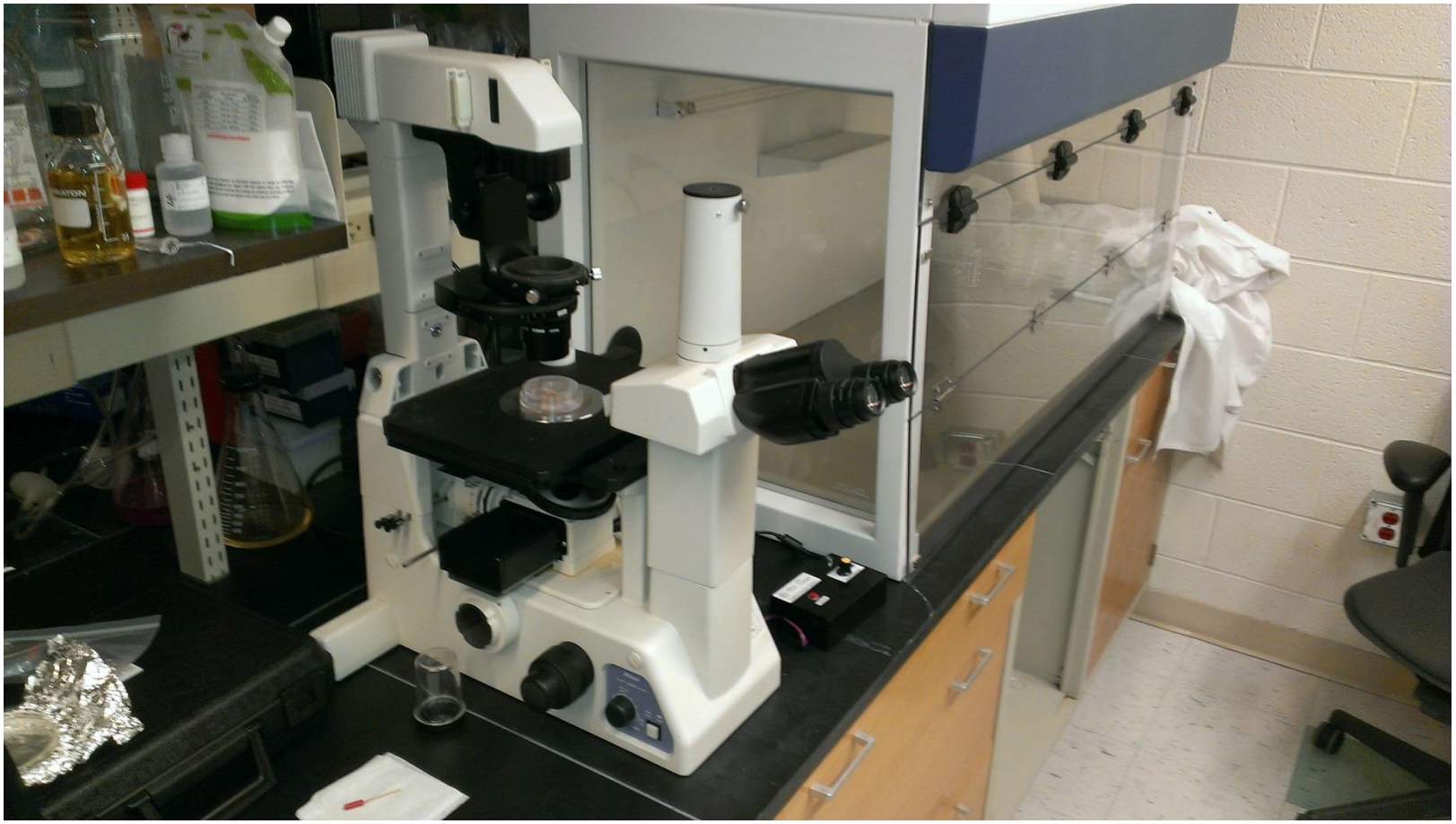

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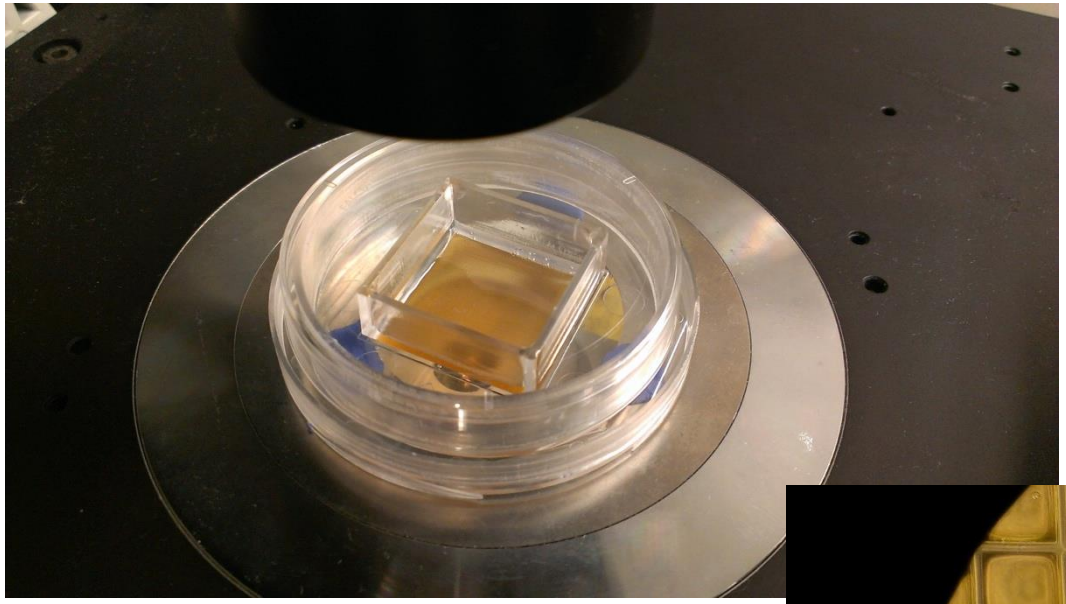

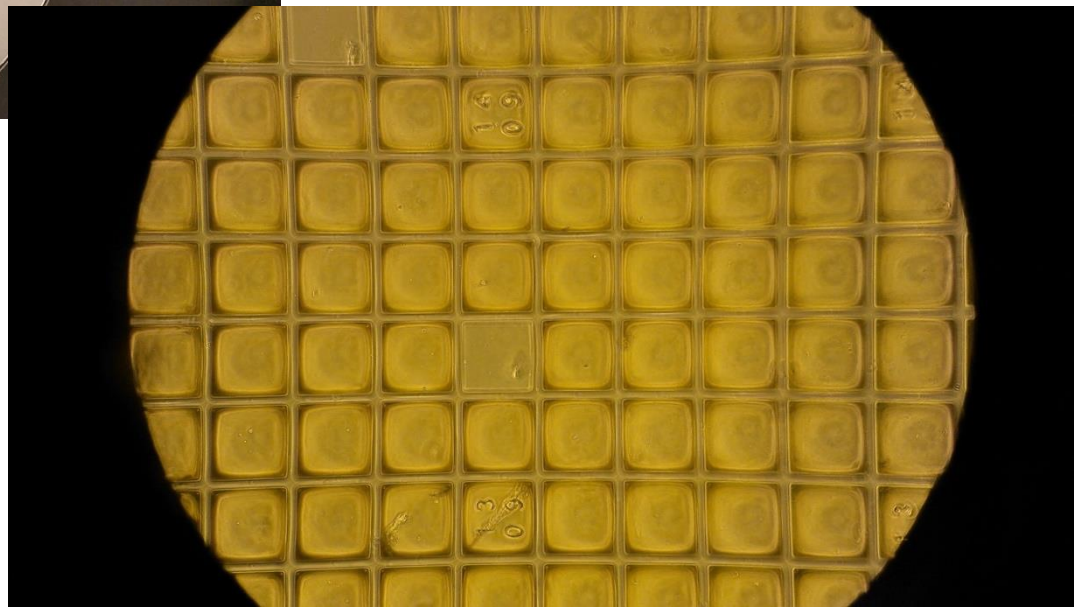

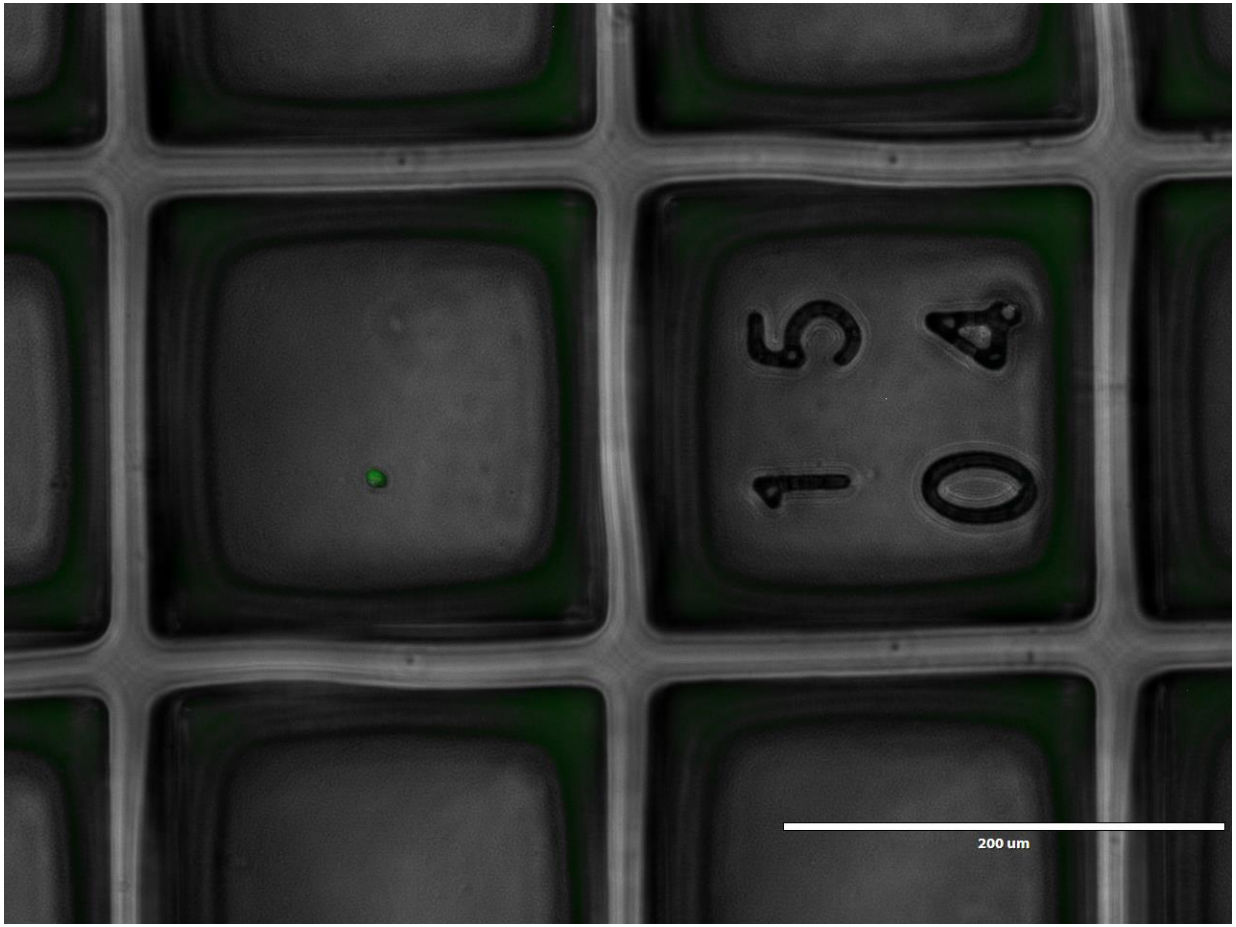
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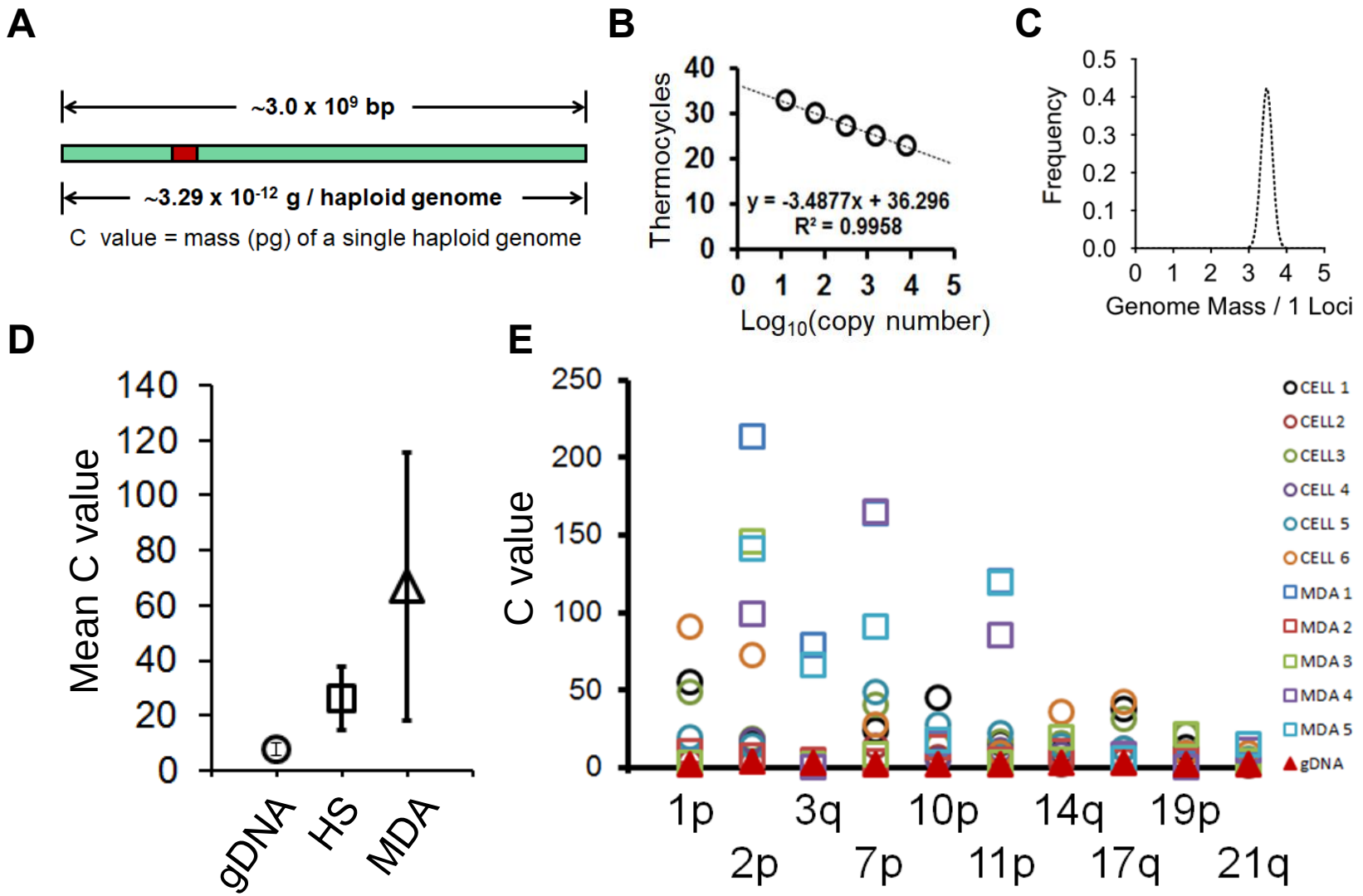
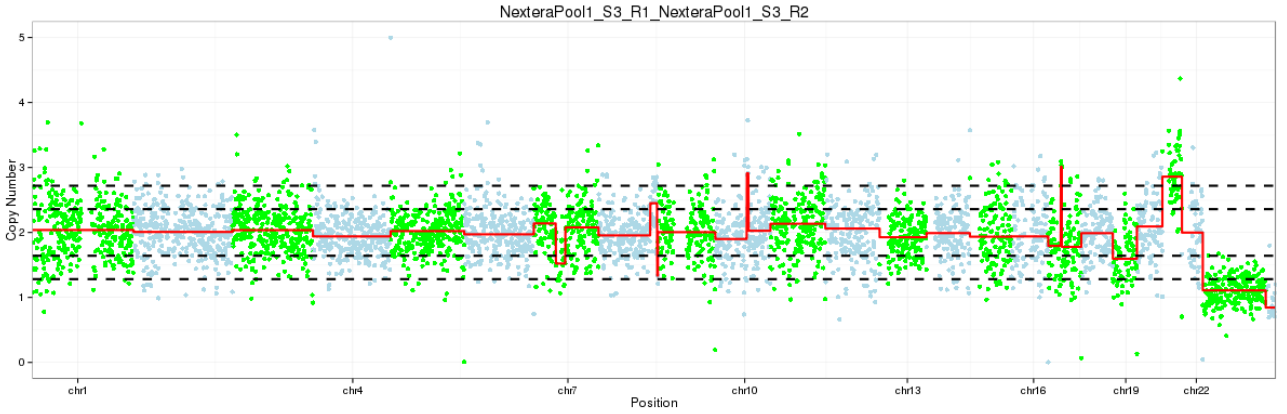
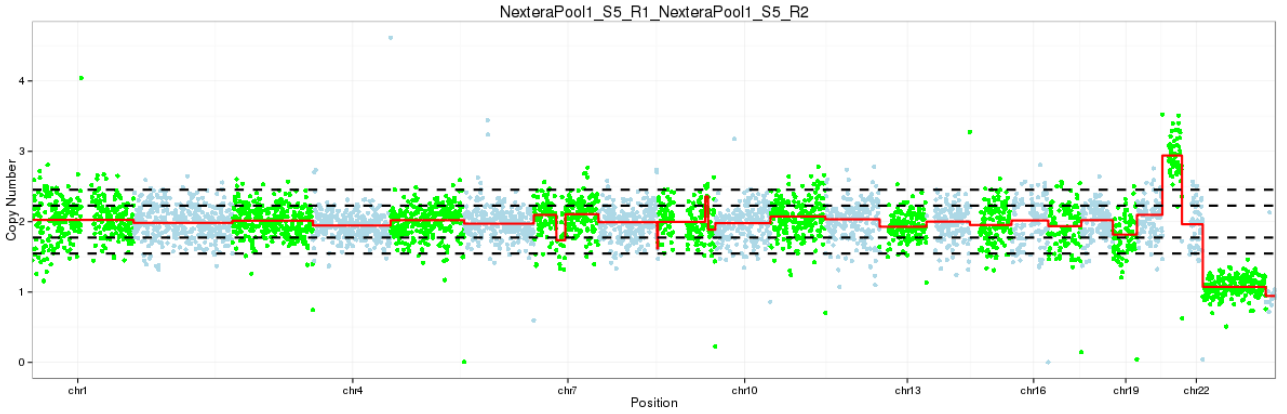


Burbulis, Ratan, McConnell et al. (in preparation)

MALBAC (Standard Buffer)



MALBAC (Burbulis Buffer)



Advantages of CellRaft Approach:

- Avoid using core facility
  small number of cells required
  rapid turnaround to next experiment
  schedule as needed

- Positive identification of specific single cells

- Preserve scarce tissue
  >50,000 cells needed to establish FACS gates

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MEETINGS & COURSES PROGRAM

Welcome | Travel & Location | Application | Sponsors & Stipends | Information | Payments | Policies

Single Cell Analysis

June 29 - July 12, 2018

Application Deadline: March 31, 2018

Instructors:

David Chenoweth, University of Pennsylvania
Michael McConnell, University of Virginia School of Medicine
Gene Yeo, University of California, San Diego

Co-Instructor:

See the roll of honor - who's taken the course in the past

The goal of this two-week course is to familiarize students with cutting-edge technologies for characterization of single cells. Modules of the course will be taught by scientists with expertise in distinct areas of single cell analysis. Topics to be covered include quantitative single cell analysis by RNAseq, genomic DNA analysis, proteomics, and metabolomics. Multiple nucleic amplification methodologies including droplet-based RNAseq, MALBAC and MDA will be employed. In addition, students will be instructed in basic bioinformatic analysis of next generation sequencing data.

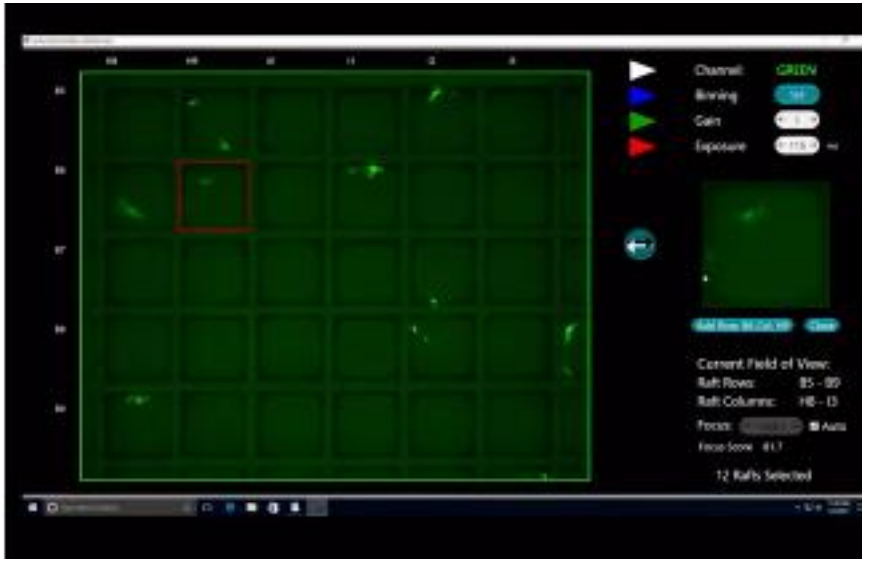
Topics:

- **Single cell genome, transcriptome, and proteome measurement**
- **Introductory next generation sequencing data analysis**
- **Photoactivatable single cell probes**
- **Single cell mass spectrometry / Soft X-ray tomography**

Speakers and Module Leaders in 2017 include:

Nancy Allbritton, University of North Carolina
Lacramioara Bintu, Stanford University
Jim Eberwine, University of Pennsylvania
Amy Herr, University of California Berkeley
Carolyn Larabell, University of California, San Francisco
Elena Romanova, University of Illinois
Stas Rubakhin, University of Illinois
Rickard Sandberg, Karolinska Institute, Sweden
Carsten Schultz, Oregon Health & Science University
Peter Sims, Columbia University
Nick Trotta, Cell Microsystems
Xiaowei Zhang, Harvard University

This course is supported with funds provided by: National Institute of General Medical Sciences, Howard Hughes Medical Institute and Helmsley Charitable Trust.



Acknowledgements

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Lise Harbom
Will Chronister
Nadine Michel
Matt Wolpert
Mark Haakenson

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Dan Weinberger (LIBD)
Kristen Brennand (Mt. Sinai)
Mark Beenhakker (UVa)
Aakrosh Ratan (UVa)
Stefan Bekiranov (UVa)

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