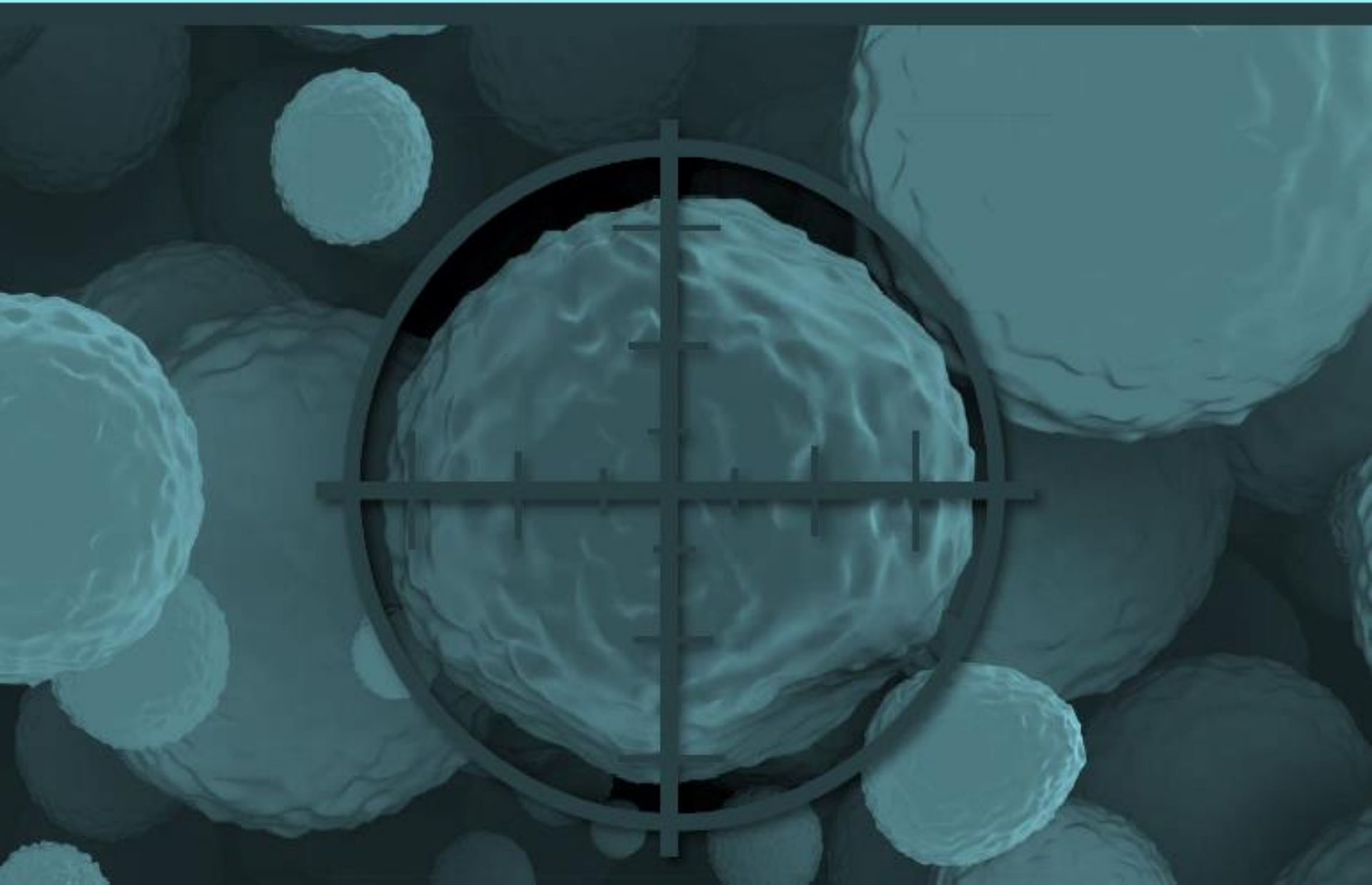
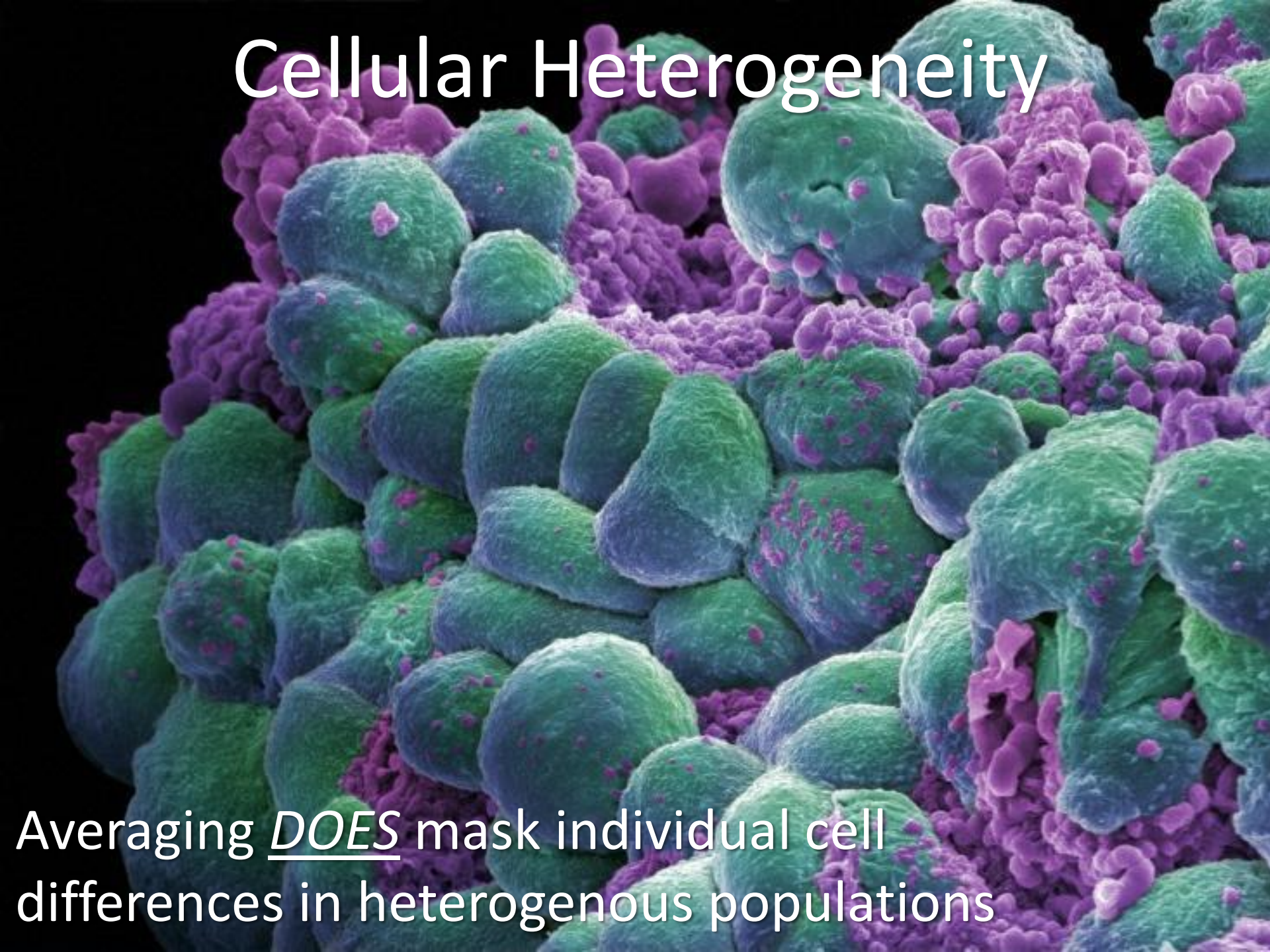
Why Single Cells Matter



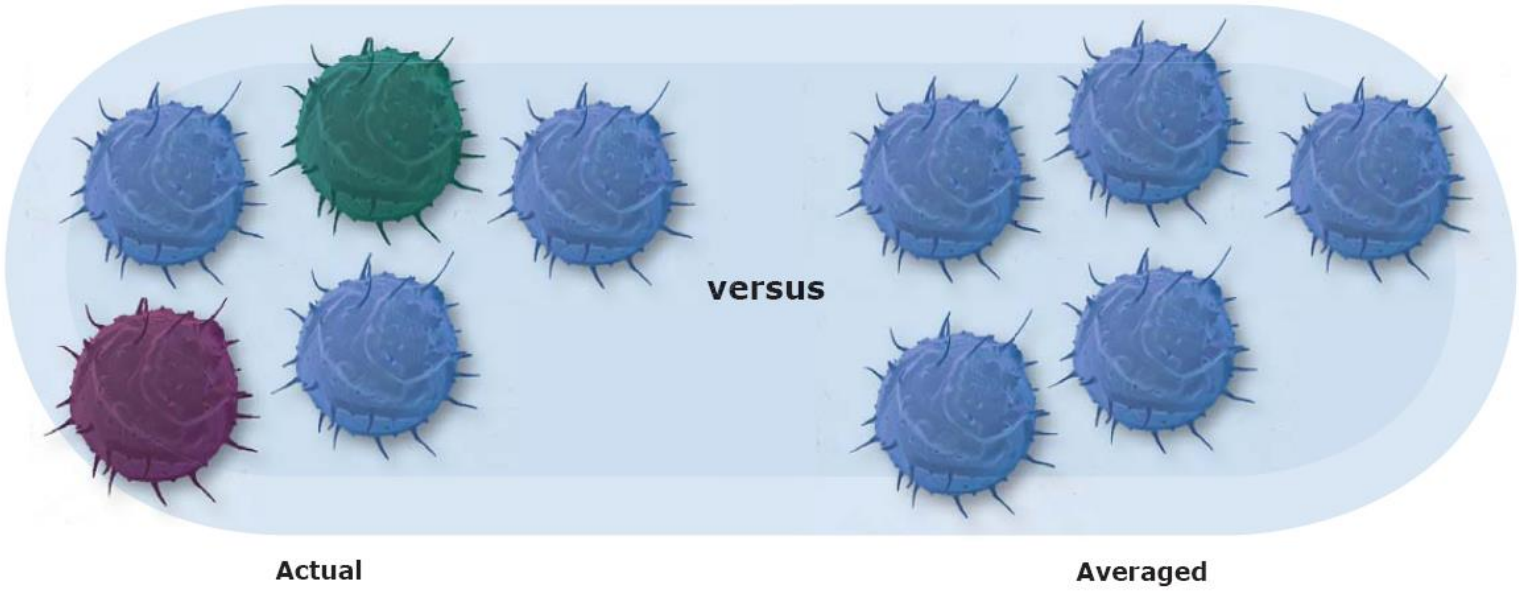
Cellular Heterogeneity



Averaging *DOES* mask individual cell differences in heterogenous populations

Single-Cell Genomics

"The Population Average is a Lie."



Single-Cell Genomics

Peer-reviewed publications have shown that samples are heterogeneous and that variations are hidden in small sub populations

Single-cell gene-expression profiling reveals qualitatively distinct CD8 T cells elicited by different gene-based vaccines

Lukas Flatz[a,b,1], Rahul Roychoudhuri[a,1], Mitsuo Honda[a,1], Abdelali Filali-Mouhim[c], Jean-Philippe Goulet[c], Nadia Kettaf[c], Min Lin[d], Mario Roederer[a], Elias K. Haddad[c,e], Rafick P. Sékaly[c,e,2], and Gary J. Nabel[a,2,3]

[a]Vaccine Research Center, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892-3005; [b]Institute for Infectious Diseases, University of Bern, CH-3010 Bern, Switzerland; [c]Laboratoire d'immunologie, Centre de Recherche du Centre Hospitalier de l'Université de Montréal, Saint-Luc, Montréal, QC, Canada H2X 1P1; [d]Fluidigm Corporation, San Francisco, CA 94080; and [e]Vaccine and Gene Therapy Institute, Port St. Lucie, FL 34987

Edited* by Ralph M. Steinman, The Rockefeller University, New York, NY, and approved February 4, 2011 (received for review September 3, 2010)

PROTOCOL

Comprehensive qPCR profiling of gene expression in single neuronal cells

Ami Citri[1,6], Zhiping P Pang[2,3,6], Thomas C Südhof[2,4], Marius Wernig[5] & Robert C Malenka[1]

[1]Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, California, USA. [2]Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, California, USA. [3]Child Health Institute of New Jersey, Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School, New Brunswick, New Jersey, USA. [4]Howard Hughes Medical Institute, Chevy Chase, Maryland, USA. [5]Department of Pathology, Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine, Stanford, California, USA. [6]These authors contributed equally to this work. Correspondence should be addressed to A.C. (citri@stanford.edu) or Z.P.P. (zpang@stanford.edu) or R.C.M. (malenka@stanford.edu).

C1 single cell RNA seq and Monocle algorithm define transcriptional dynamics of cell differentiation processes

nature biotechnology

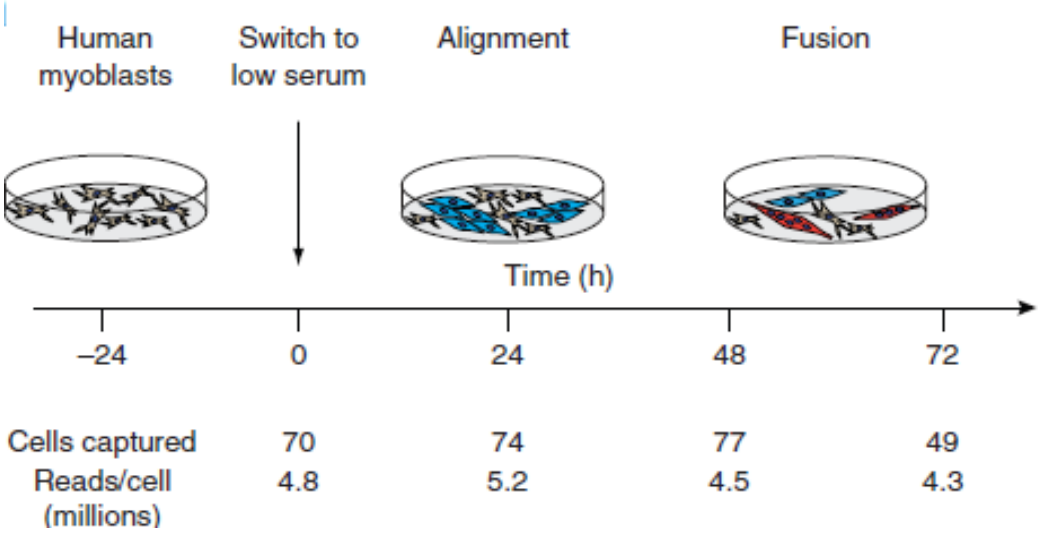
The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells

Cole Trapnell[1,2,6], Davide Cacchiarelli[1–3,6], Jonna Grimsby[2], Prapti Pokharel[2], Shuqiang Li[4], Michael Morse[1,2], Niall J Lennon[2], Kenneth J Livak[4], Tarjei S Mikkelsen[1–3] & John L Rinn[1,2,5]

[1]Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, Massachusetts, USA. [2]The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. [3]Harvard Stem Cell Institute, Harvard University, Cambridge, Massachusetts, USA. [4]Fluidigm Corporation, South San Francisco, California, USA. [5]Department of Pathology, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA. [6]These authors contributed equally to this work. Correspondence should be addressed to J.L.R. (john_rinn@harvard.edu).

Fluidigm

EXPERIMENTAL APPROACH



Primary Human Myoblasts were cultured and switched to low serum.

Single cells were captured on C1 at 24h intervals and processed with our RNA seq protocol.

Sequenced to an average depth of 4 million reads per cell.

Used Monocle to order RNA seq data of differentiating myoblasts in pseudotime.

Fluidigm

C1 mRNA seq data combined with Monocle identify key regulators of myoblats differentiation

Many of the transcription factors identified by C1 mRNA seq/ Monocle approach were not considered before to be important for muscle development.

Authors performed knockdown experiments (silencing by shRNA) on bunch of transcription factors to confirm that these factors have important role in muscle differentiation.

The results confirmed that transcription factors identified by Monocle indeed Influenced myoblasts differentiation.

SINGLE CELL GENE EXPRESSION ENABLES BETTER UNDERSTANDING OF CELLULAR REPROGRAMMING

Cell

Single-Cell Expression Analyses during Cellular Reprogramming Reveal an Early Stochastic and a Late Hierarchic Phase

Yosef Buganim,[1,7] Dina A. Faddah,[1,2,7] Albert W. Cheng,[1,3] Elena Itskovich,[1] Styliani Markoulaki,[1] Kibibi Ganz,[1] Sandy L. Klemm,[5] Alexander van Oudenaarden,[2,4,6] and Rudolf Jaenisch[1,2,*]

QUESTION

The authors wanted to understand molecular events/ genes that drive reprogramming of MEFs to iPSCs

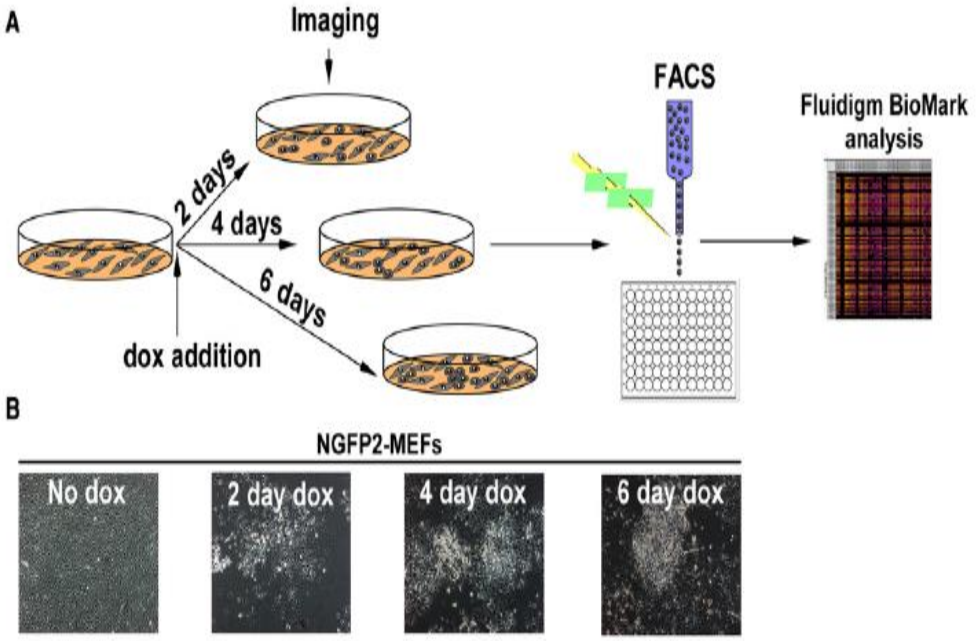
"Because the molecular changes occurring at the different stages during the reprogramming process were based upon the analysis of heterogeneous cell populations, it has not been possible to clarify the events that occur in the rare single cells that eventually form iPSCs"

During cellular reprogramming only small fractions of cells become iPSCs

WORKFLOW

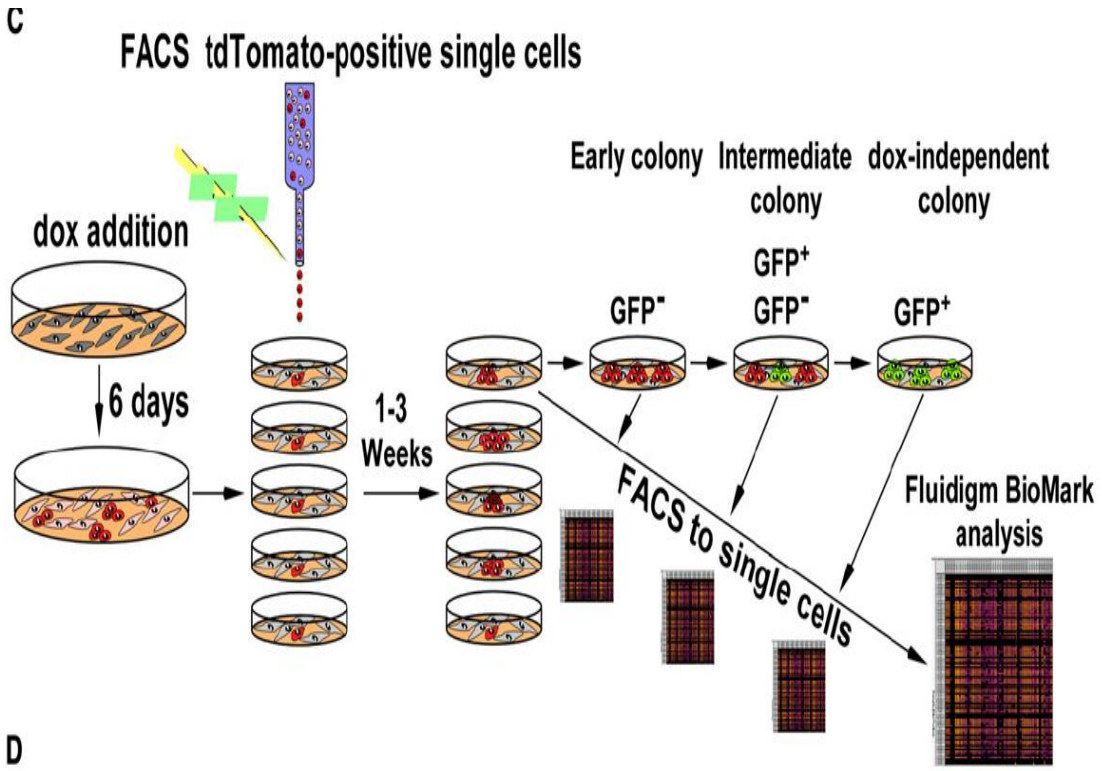
Capture the cells at early and intermediate stages of reprogramming

Thousands of single cells against 48 genes

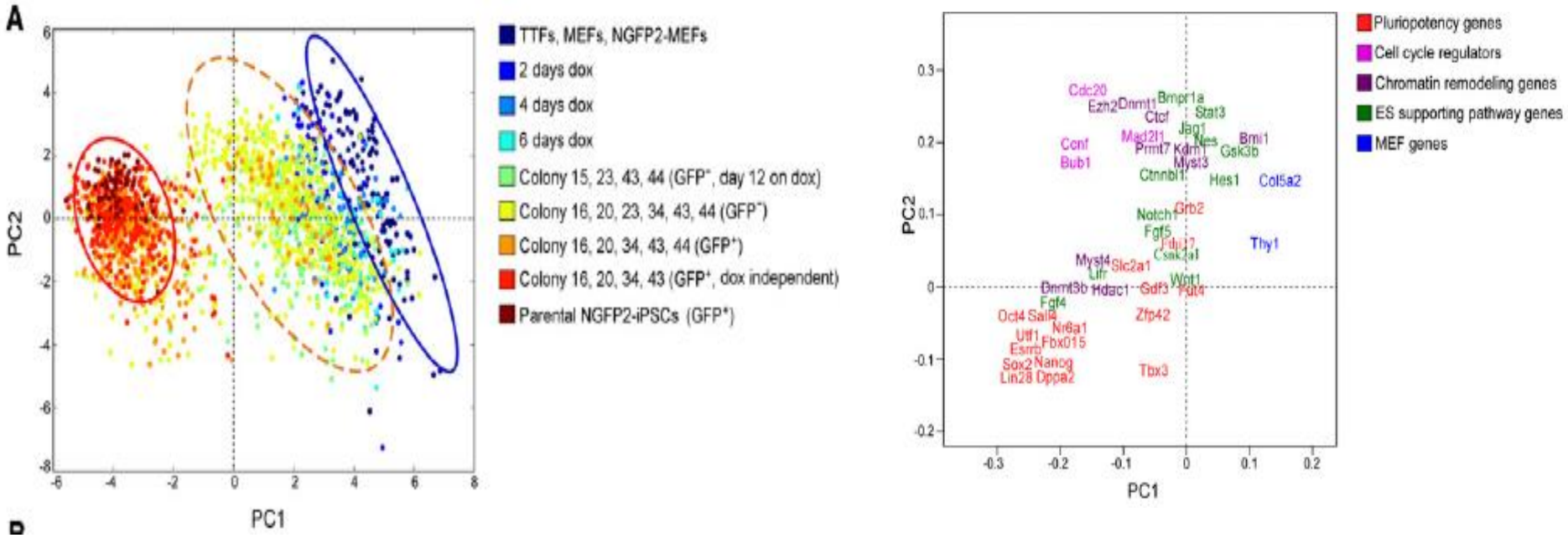


STUDYING LATER STAGES OF IPSCs FORMATION

Use the cells also from later stages of reprogramming



PRINCIPAL COMPONENT (PCA) : projection of individual cells and each of 48 genes



"To globally visualize data we used PCA- a technique that reduces dimensionality of data by finding linear combinations (in this case the number of genes) of the original data ranked by their importance"

Expression of Efssrb, Utf1, Lin28 and Dppa2 is a better predictor of cells to progress Into iPSCs than expression of previously suggested: Fbxo15, Fgf4 and Oct4

BIOLOGY VALIDATES SINGLE CELL GENE EXPRESSION SCREENING

TESTING COMBINATIONS OF KEY PLURIPOTENCY GENES THAT INDUCE REPROGRAMMING

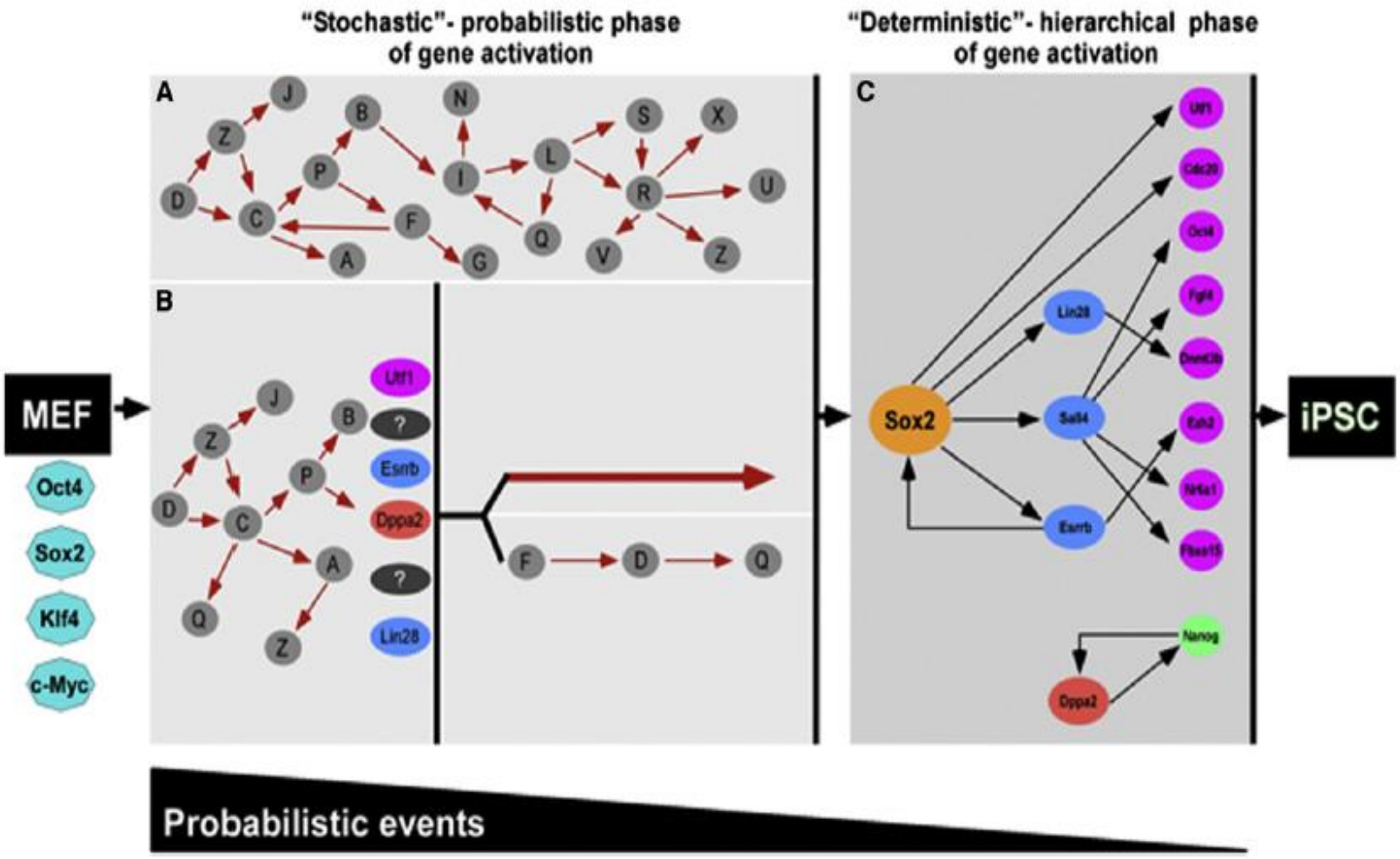
MEFS were infected with combinations of transcription factors: used different combinations of transcription factors

Excluded Exogenous Oct4, Sox2 or Nanog

Showed that various combinations of factors could induce pluripotency even in the absence of exogenous Oct4, Sox2 and Nanog

Activation of pluripotency is possible even in the absence of generic Yamanaka factors

THANKS TO SINGLE CELL GENE EXPRESSION DATA AUTHORS PROPOSE STOCHASTIC AND DETERMINISTIC REPROGRAMMING OF MEFs TO IPSCs

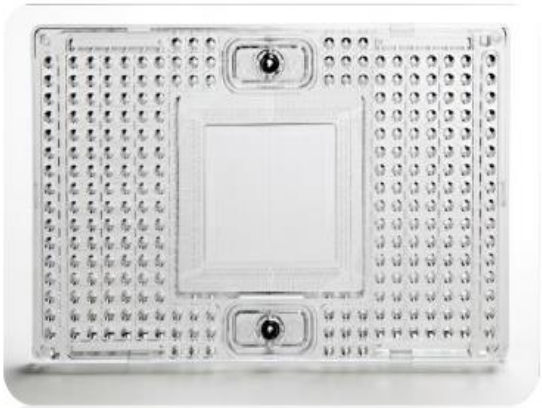


C_1™ Single-Cell AutoPrep System

by Fluidigm®

What is the C1 system?

C_1 Single-Cell AutoPrep IFC



A proprietary array for capture & highly paralleled preparation of 96 individual cells

C_1 Single-Cell AutoPrep Kit

A pre-formulated reagent kit to support cell suspension, lysis & purification*

C_1 Single-Cell AutoPrep System

A breakthrough, bench-top system that automates the isolation, lysis and pre-amplification from single cells

C_1 Single-Cell AutoPrep Workflow

Cell Suspension

↓

Capture Single Cells

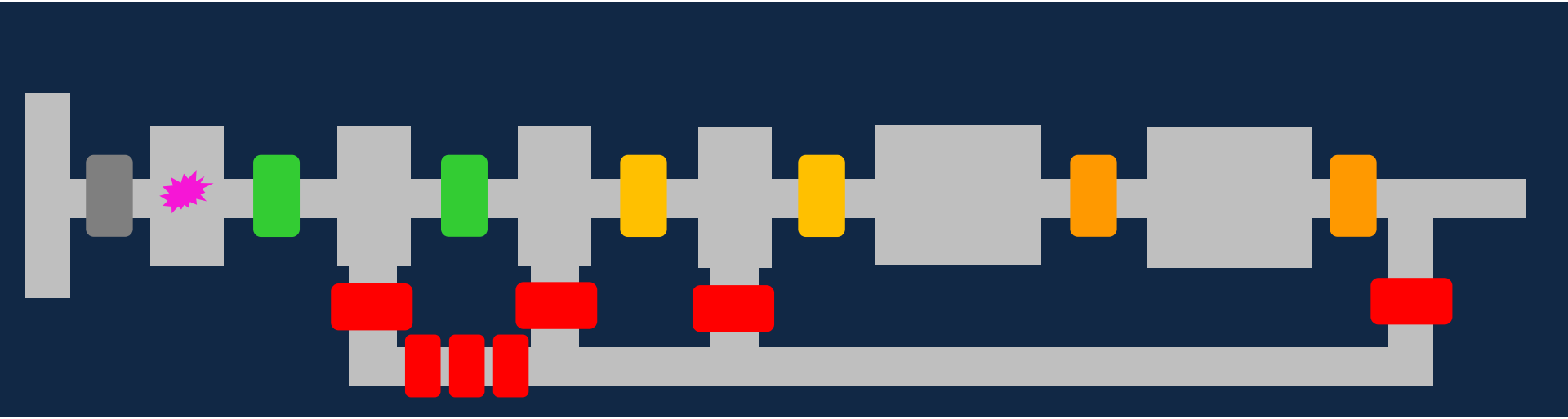
↓

Multistep chemistry
- RT-STA
- mRNA-Seq Prep
- Whole Genome Amp

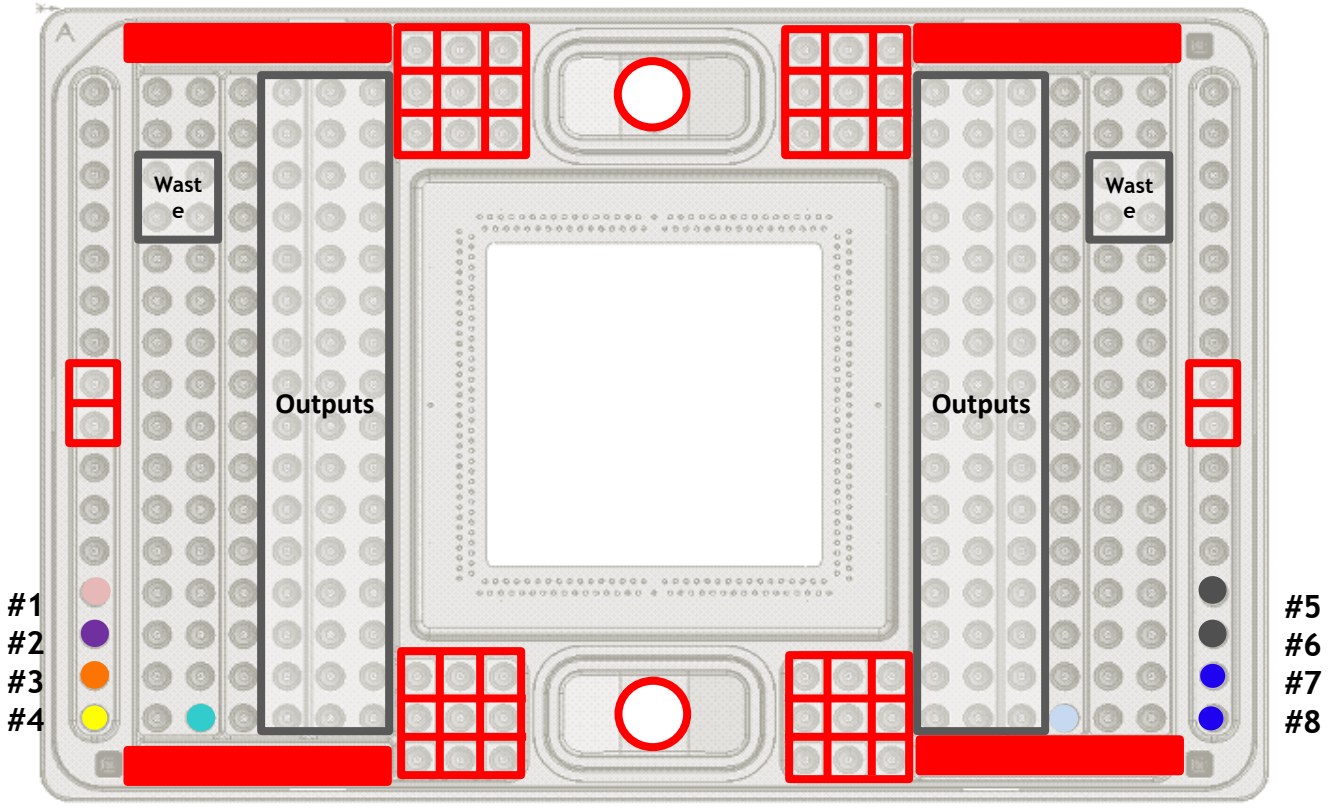
↓

Export, analyze
- qPCR (BioMark HD)
- Next Gen Sequencing

Preamplification Implementation in Chip



Pipetting Map for C1 System



C_1 Harvest Reagent

C_1 Harvest Reagent

Cell Input

Cell Outlet

C_1 Cell Wash Buffer

LIVE/DEAD Staining Solution

C_1 Preloading Reagent

C_1 Harvest Reagent

Lysis Mix

RT Mix

PreAmp Mix

C1 featured in peer reviewed publications

Stem Cell Reports

ISSCR

Article

OPEN ACCESS

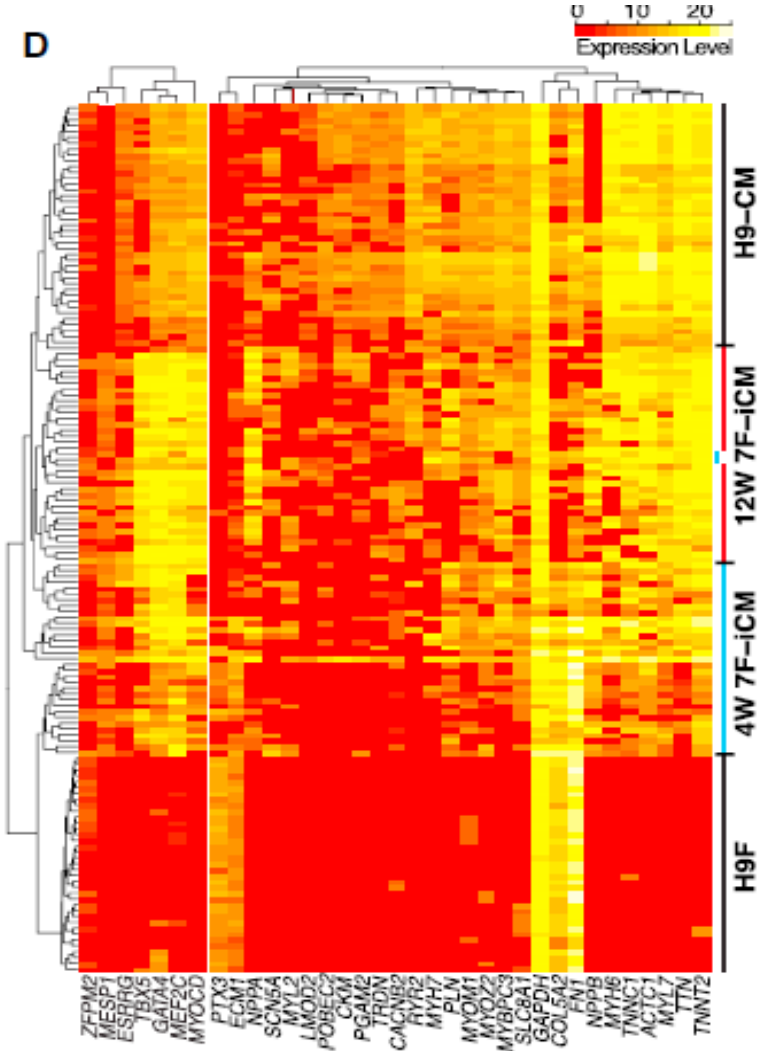
Direct Reprogramming of Human Fibroblasts toward a Cardiomyocyte-like State

Ji-Dong Fu,[1,2,3,4] Nicole R. Stone,[1,2,3,4] Lei Liu,[1] C. Ian Spencer,[1] Li Qian,[1,2,3,4,7] Yohei Hayashi,[1] Paul Delgado-Olguin,[1,8] Sheng Ding,[1,2,6] Benoit G. Bruneau,[1,2,3,5] and Deepak Srivastava[1,2,3,4,*]

[1]Gladstone Institute of Cardiovascular Disease, San Francisco, CA 94158, USA

C1 and Biomark as a tool to identify on single cell level transcription factors for transformation of fibroblasts to cardiomyocyte like cells

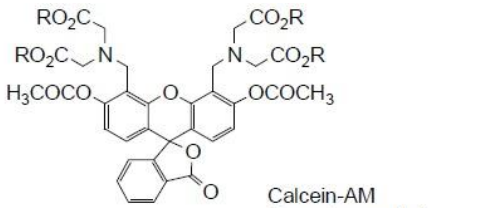
Single cell gene expression confirms essential factors for fibroblasts reprogramming

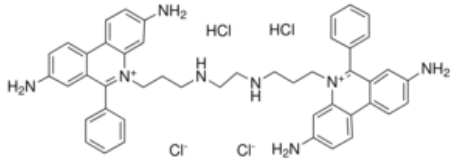


Molecular Probes™
invitrogen detection technologies

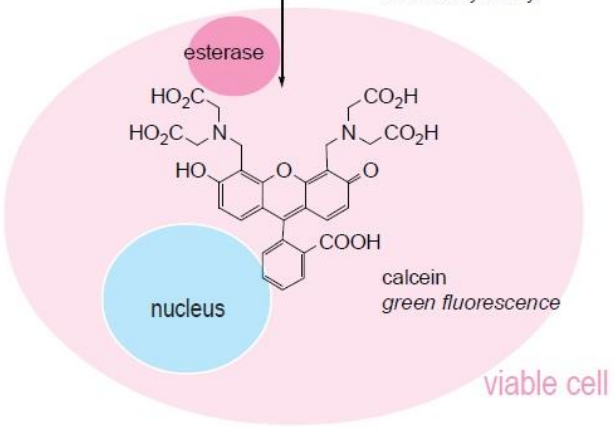
Product Information
Revised: 21–December–2005

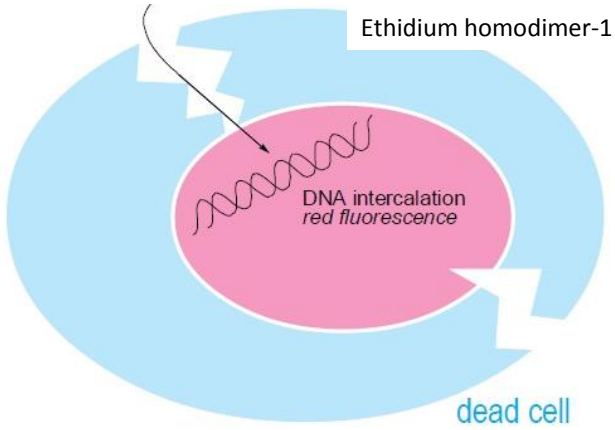
*LIVE/DEAD® Viability/Cytotoxicity Kit *for mammalian cells**



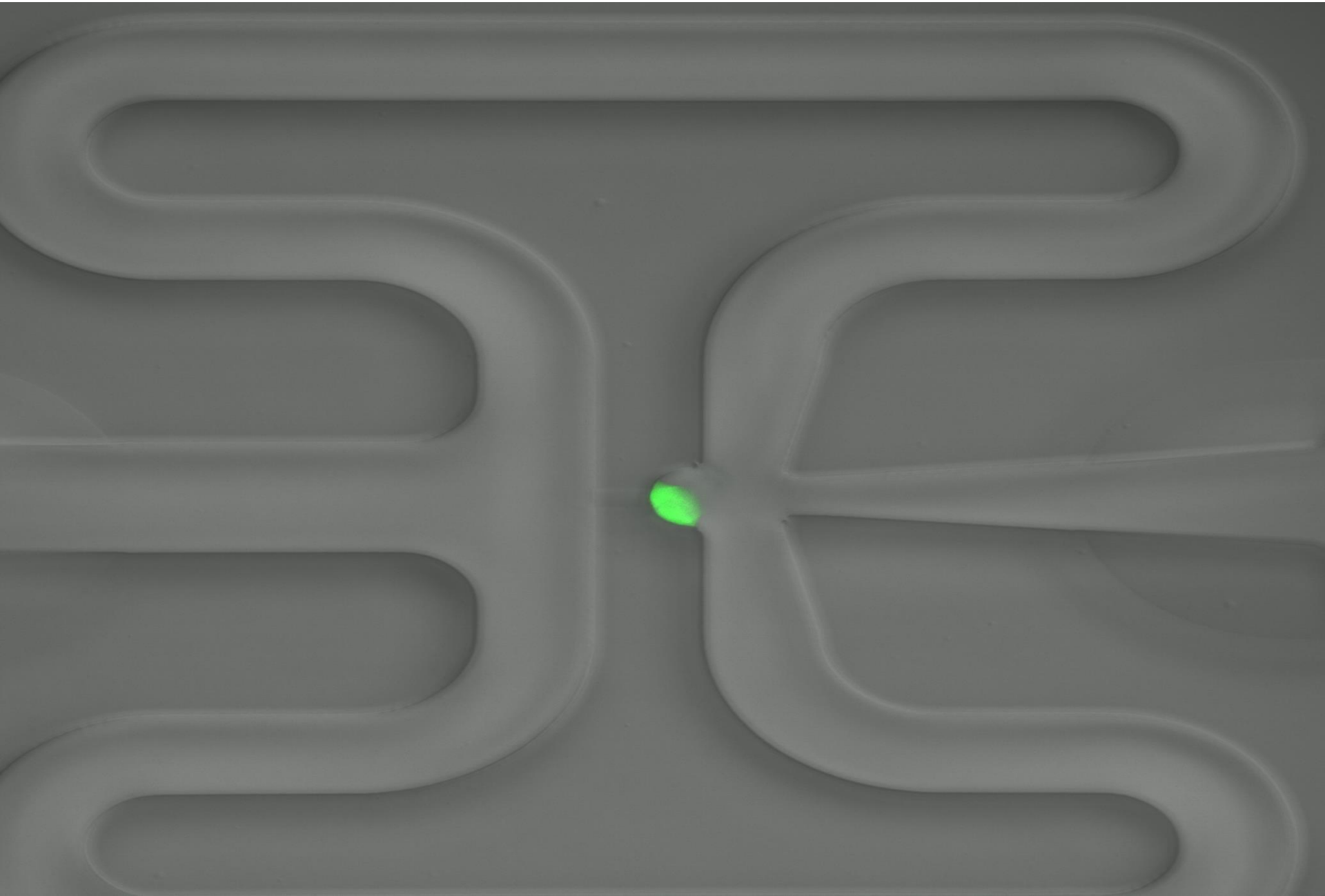


Ethidium homodimer-1

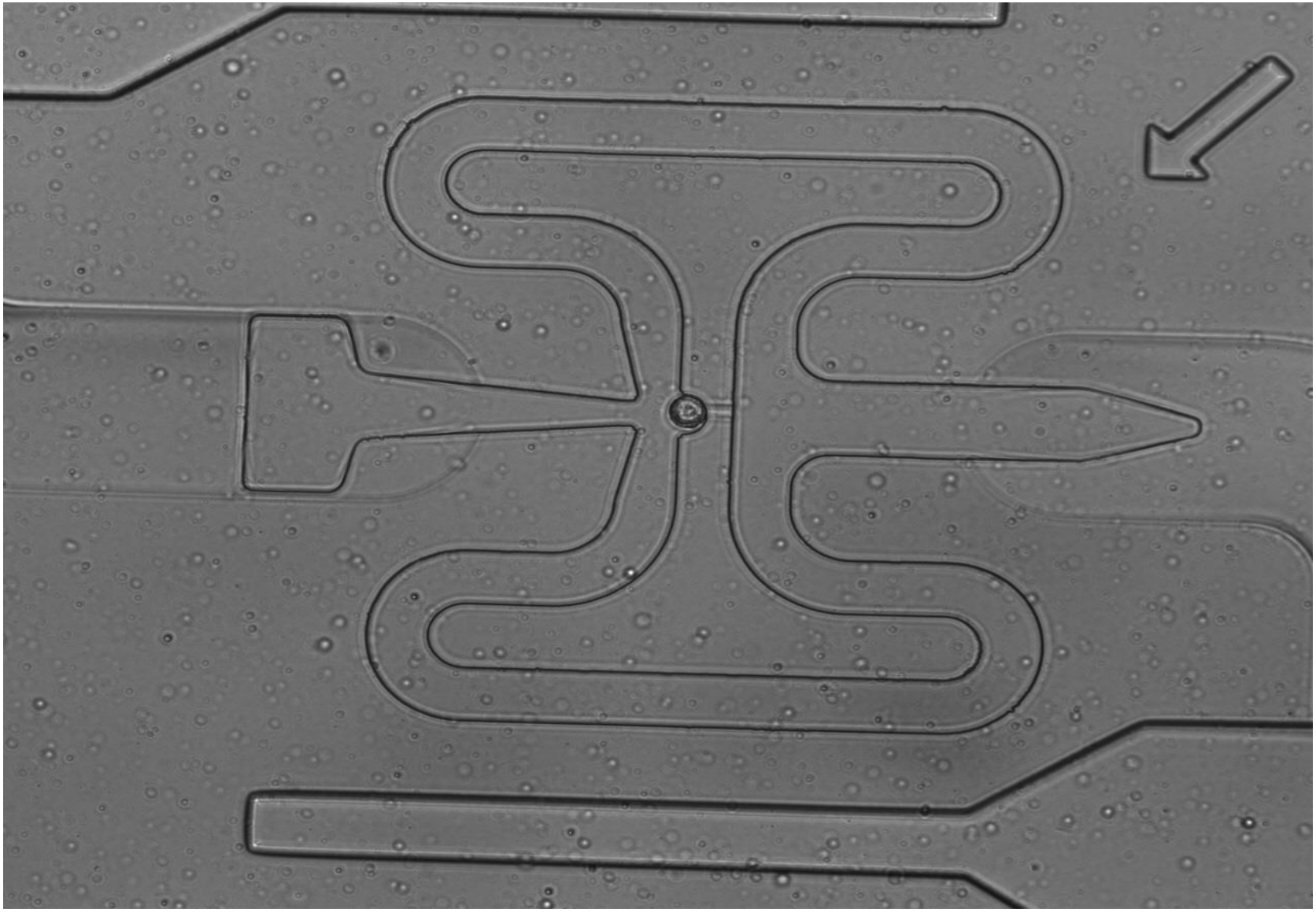




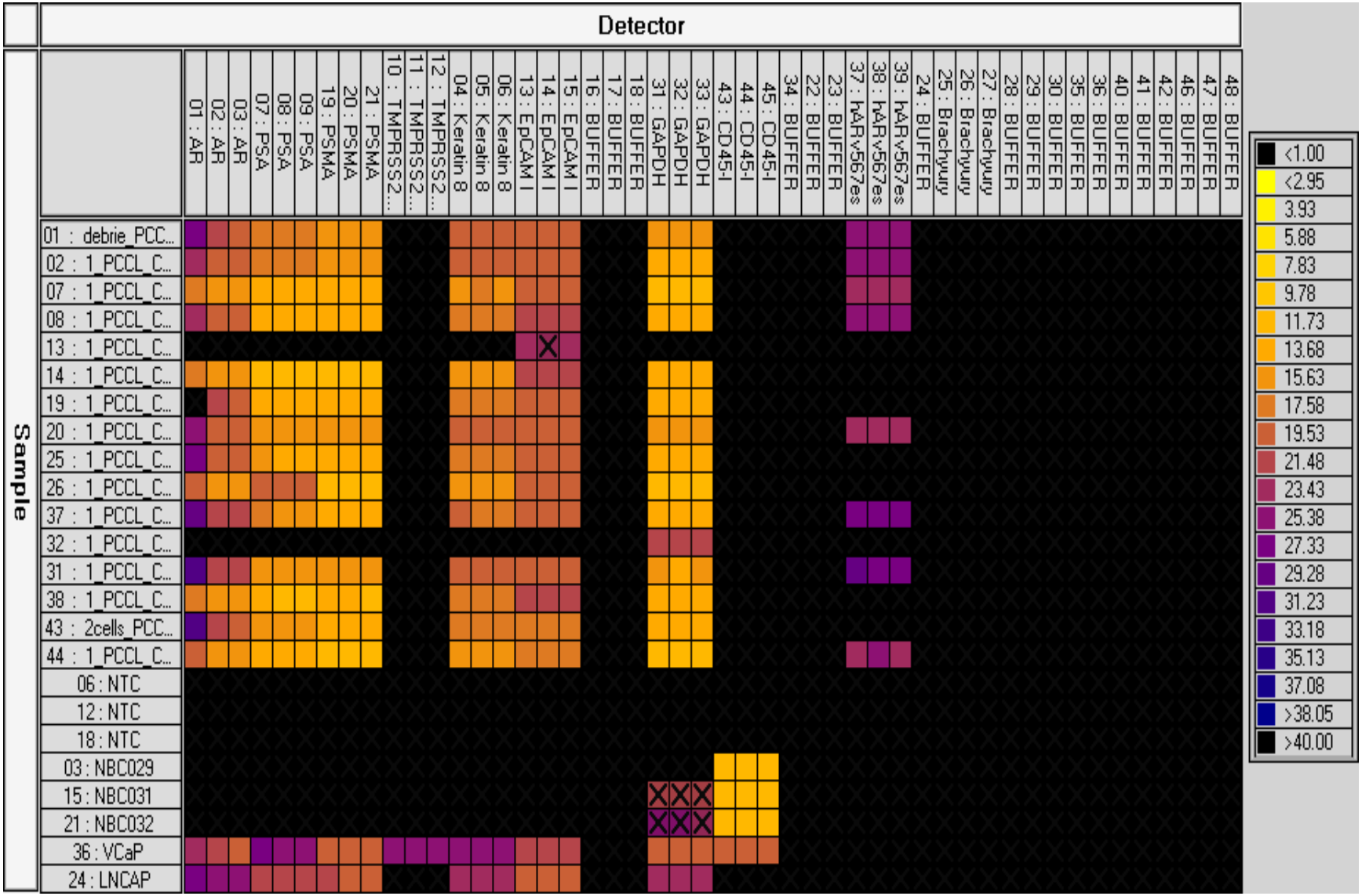
Single IPSC cell captured at the C1 nest



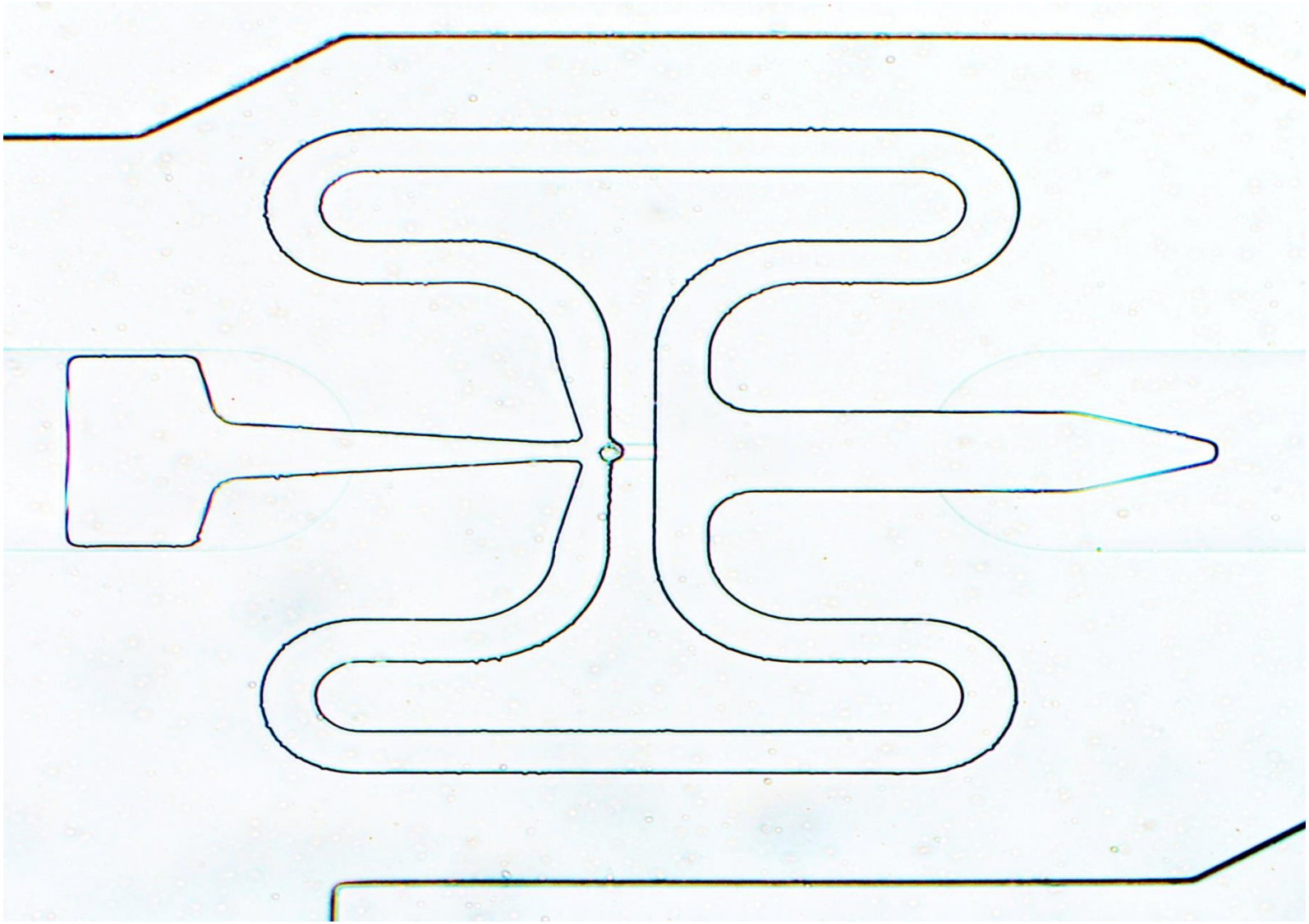
The single prostate cancer cell captured at C1 nest



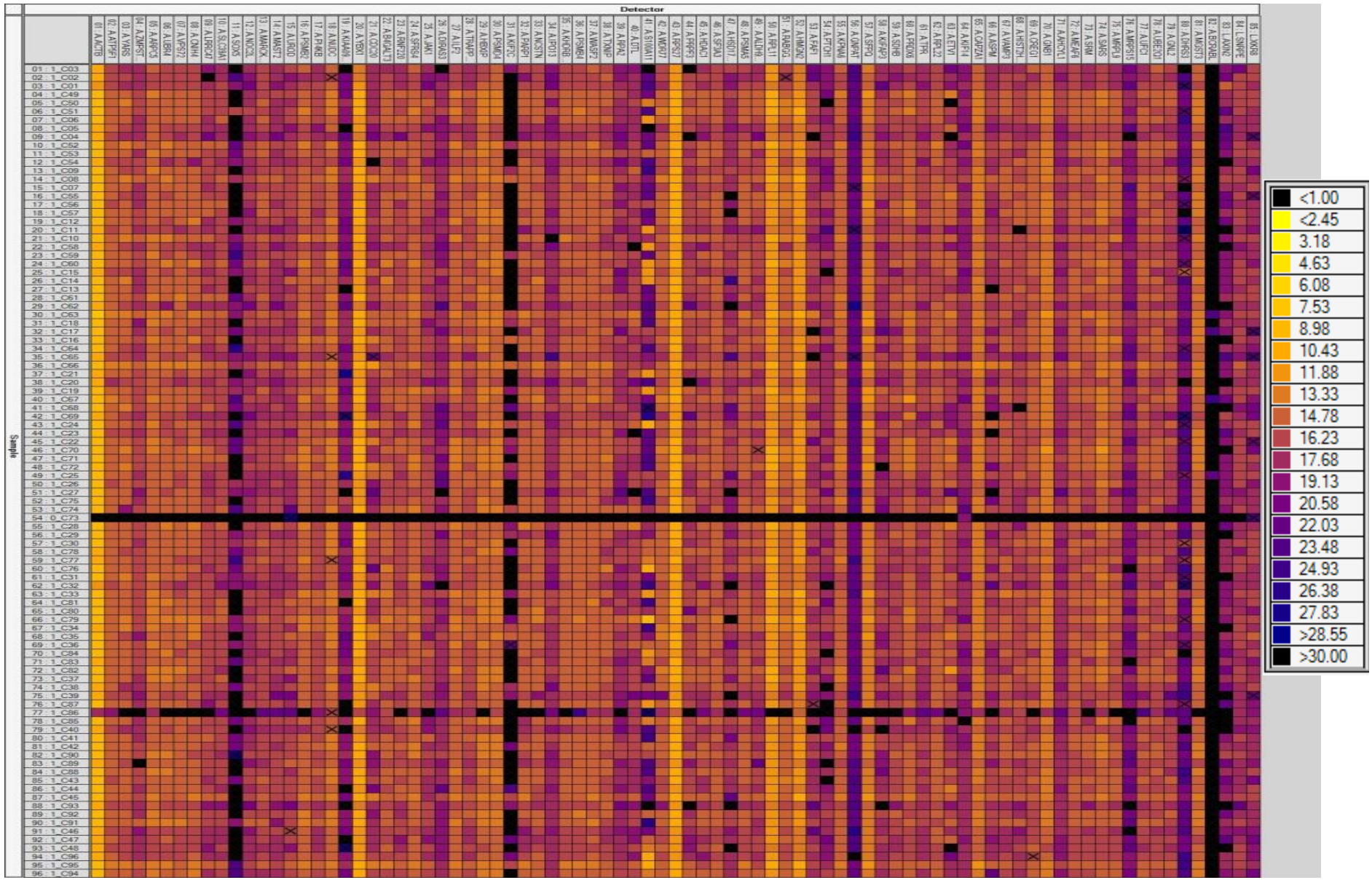
Real Time PCR from single prostate cancer cells



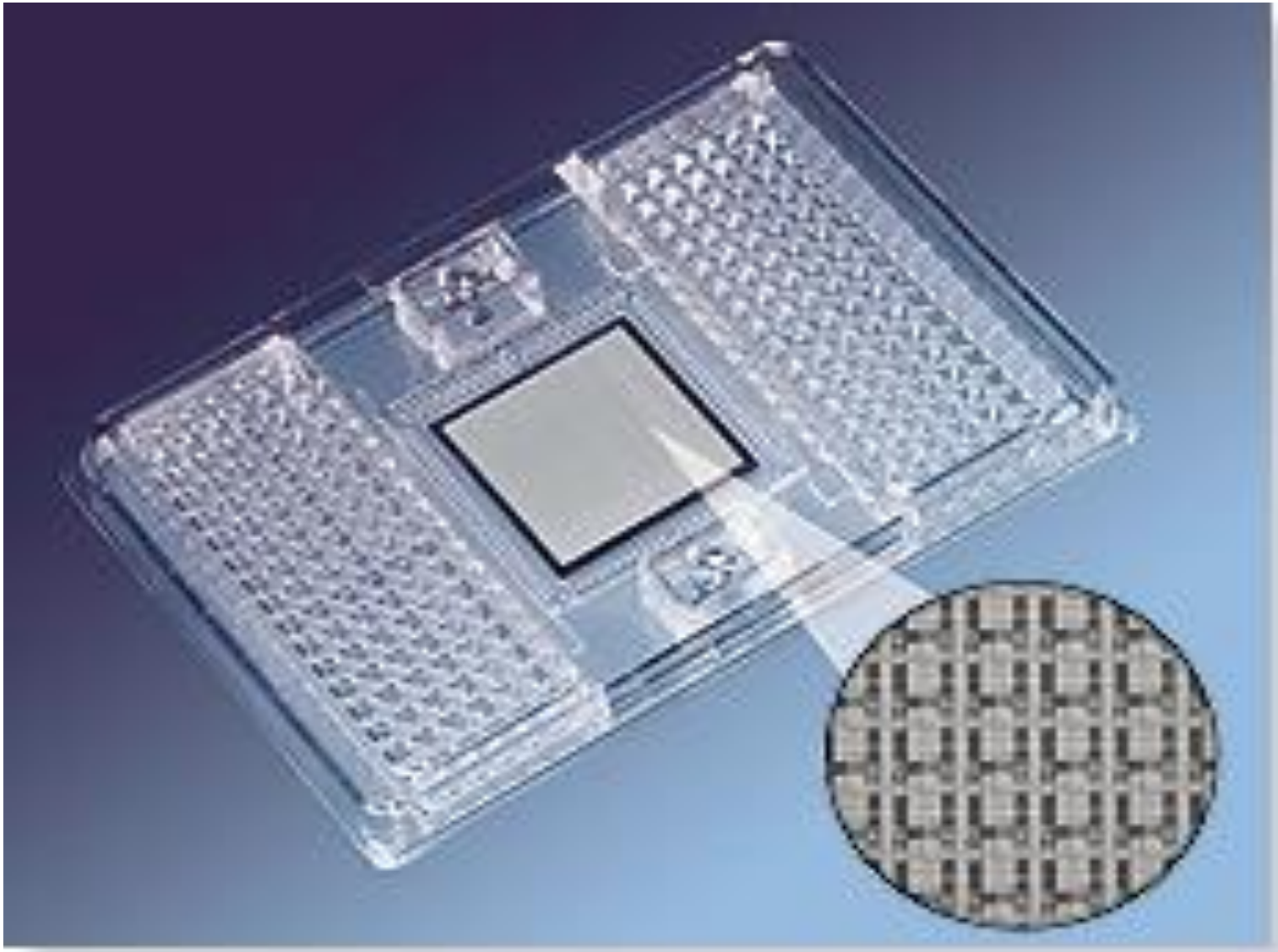
C1 capture of human neuroprogenitor cells



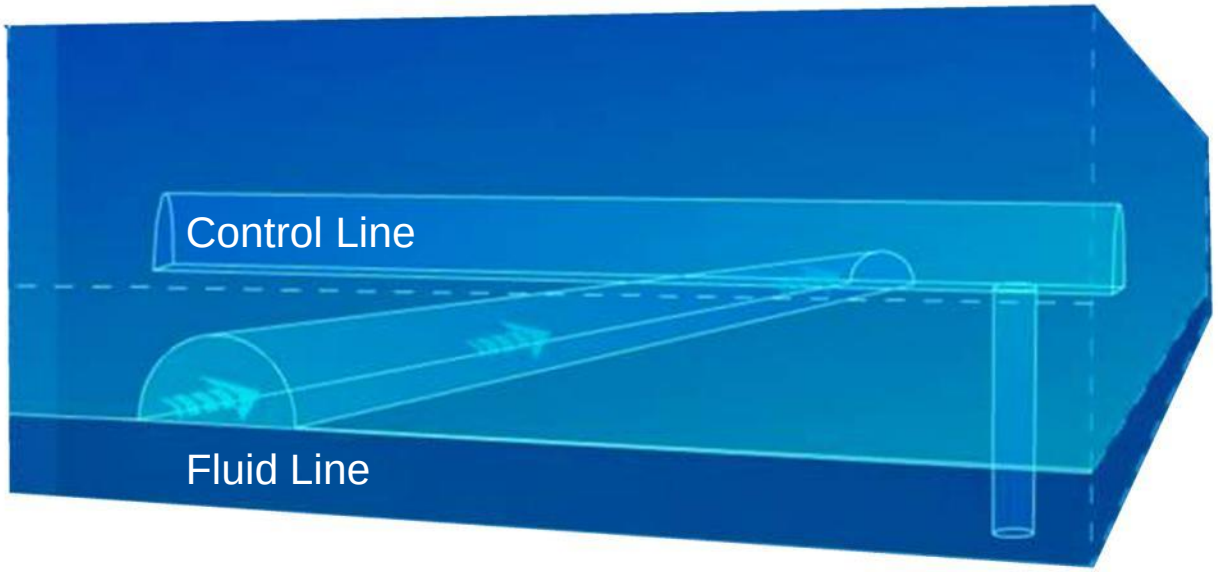
Biomark heat map results for neuroprogenitor cells

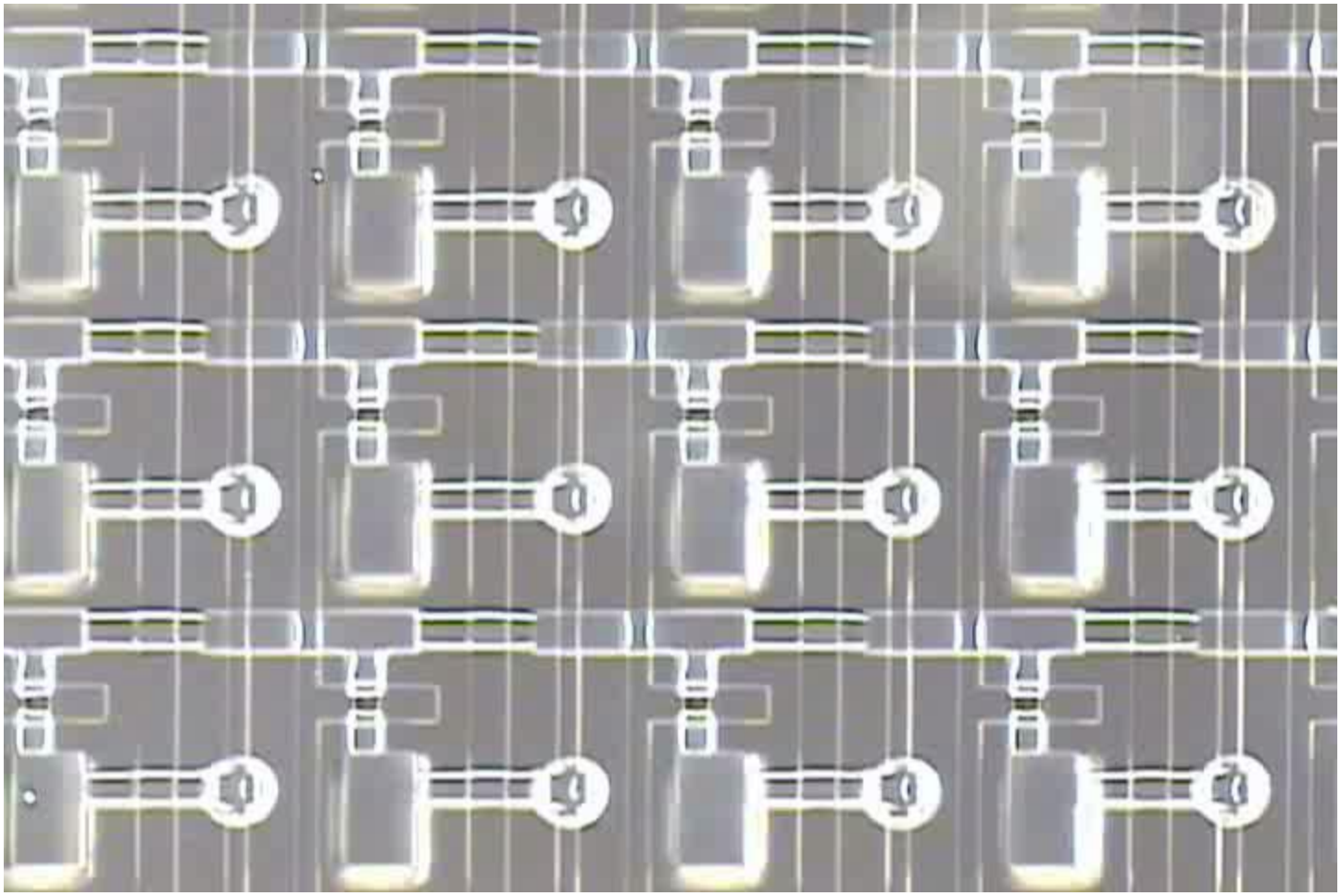


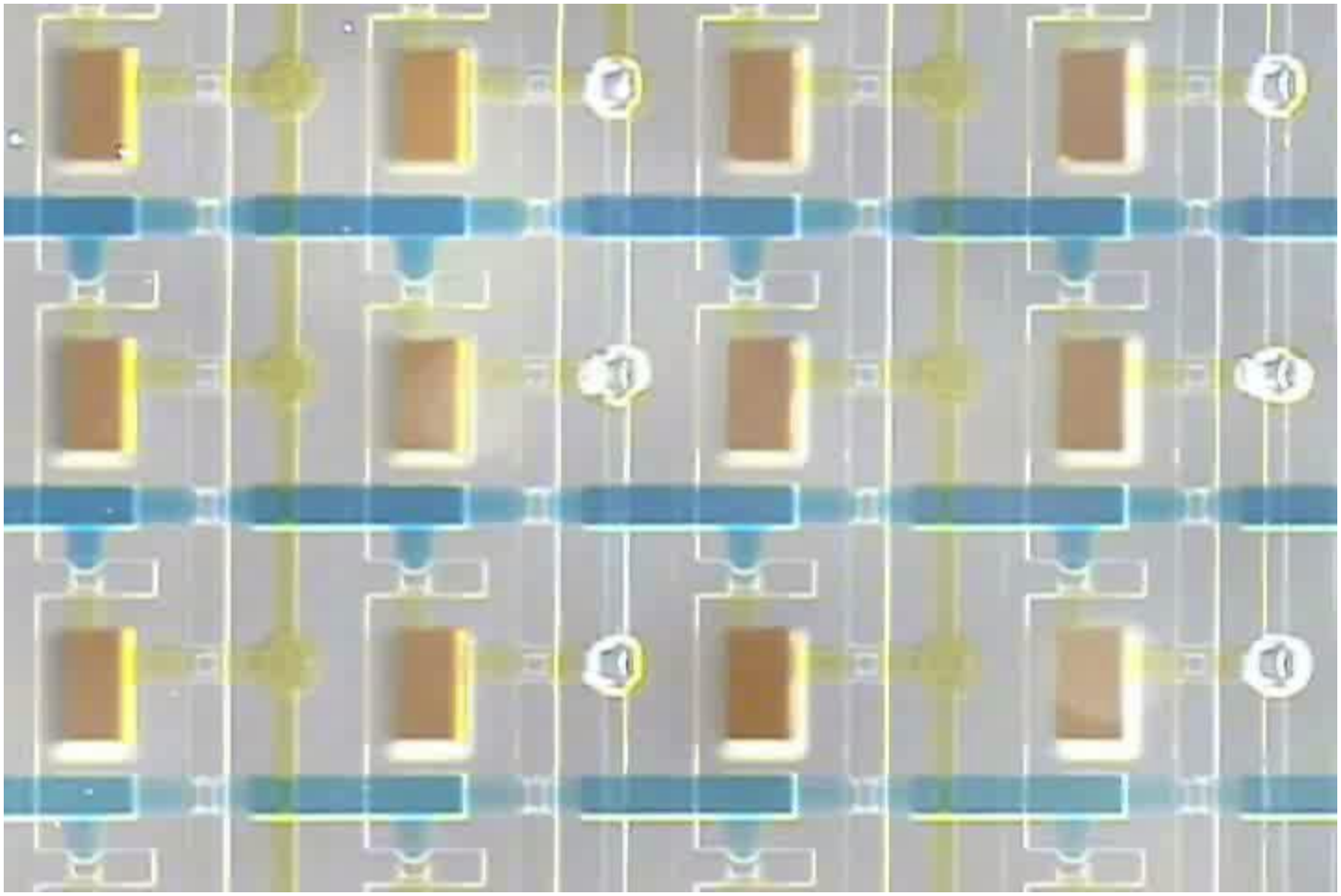

BioMark HD
FLUIDIGM
BioMark HD
FLUIDIGM
Fluidigm
Fluidigm
HX
DELL



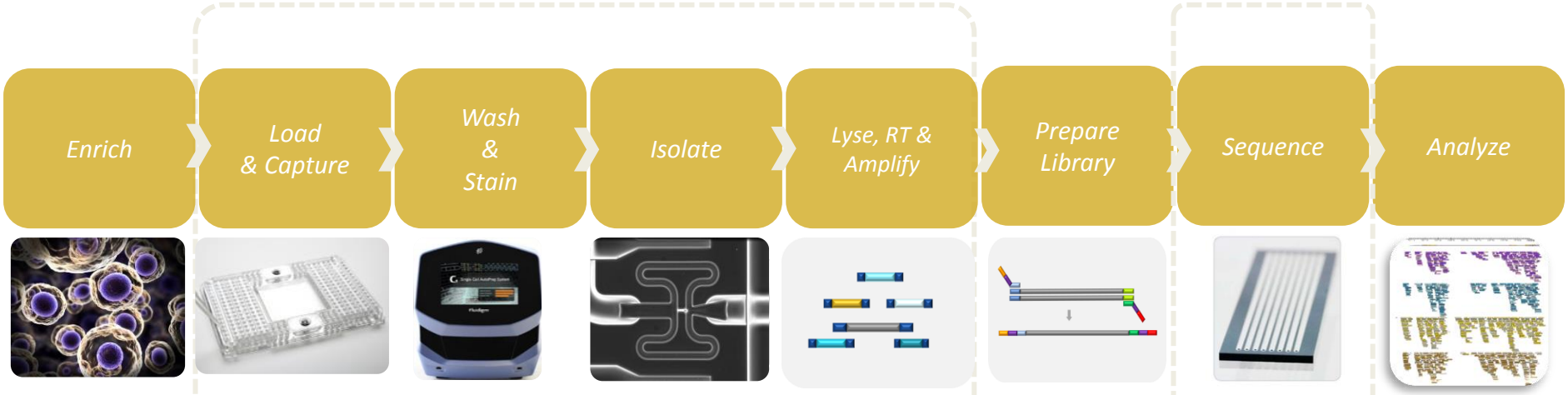
Success Built on Technology

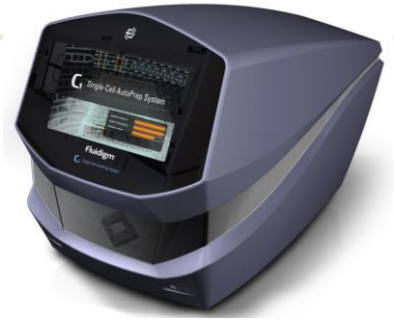






mRNA seq Workflow





C_1 Single-Cell Auto Prep System

Any Illumina System

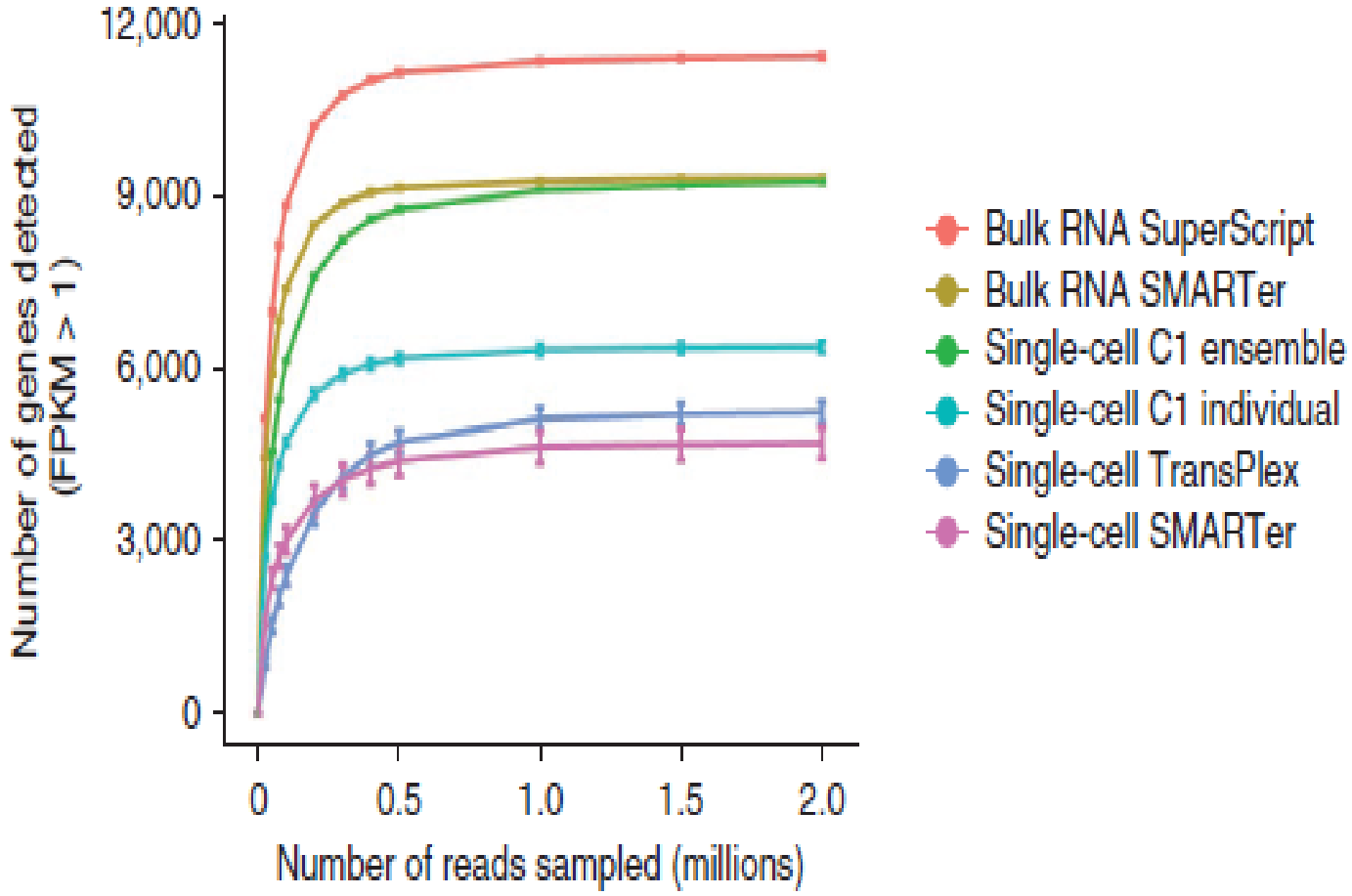
Assessing the performance of C1 mRNA seq method

NATURE METHODS

Quantitative assessment of single-cell RNA-sequencing methods

Angela R Wu[1], Norma F Neff[1], Tomer Kalisky[1,8], Piero Dalerba[2–4], Barbara Treutlein[1], Michael E Rothenberg[5], Francis M Mburu[1,6], Gary L Mantalas[1], Sopheak Sim[3], Michael F Clarke[2–4] & Stephen R Quake[1,6,7]

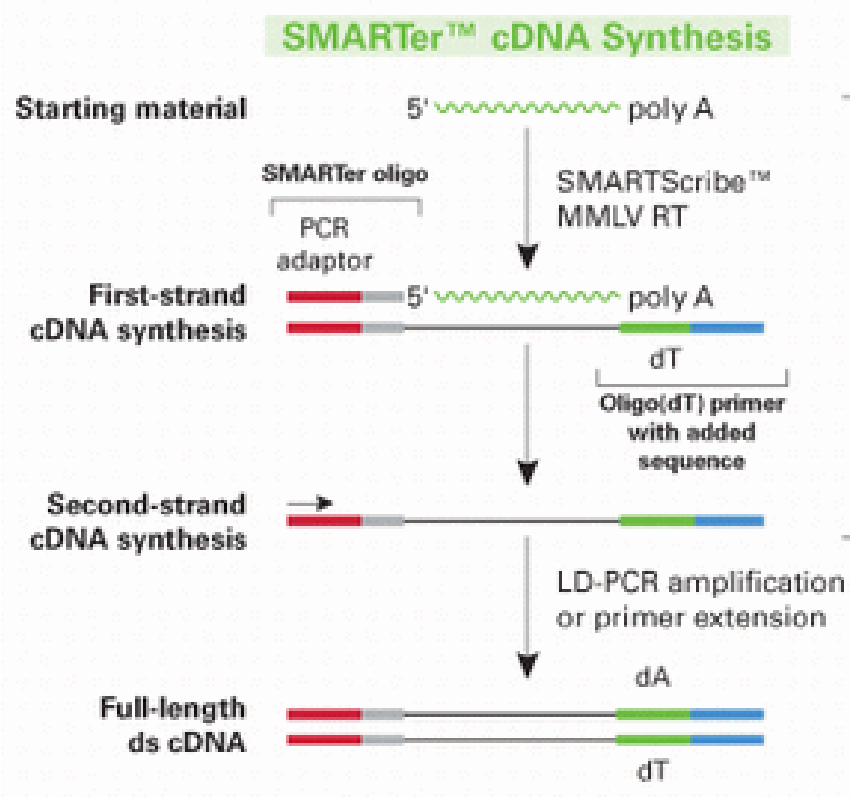
Nanoliter sample preparation improves RNA seq sensitivity



mRNA Amplification

SMARTer (Clontech)

Template-Switching Method

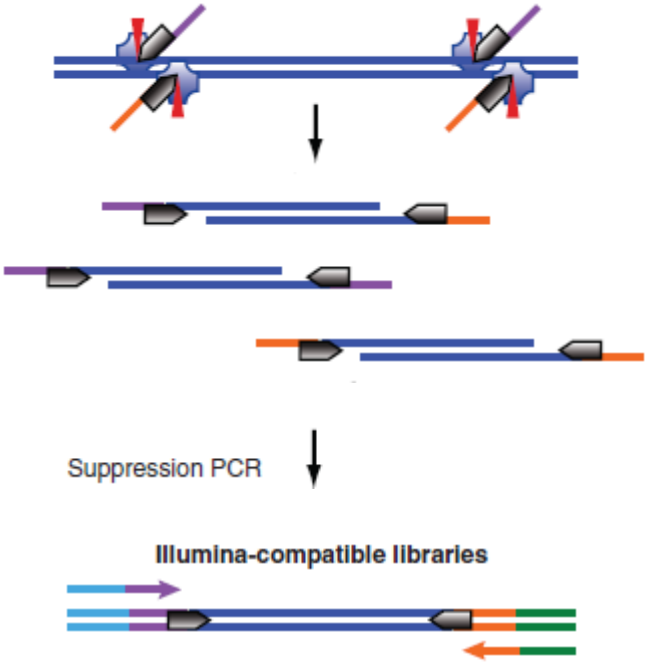


- Works directly from cell lysate
- No RNA Fragmentation
- PolyA+ RNA
- Produces long ds cDNA

Library preparation

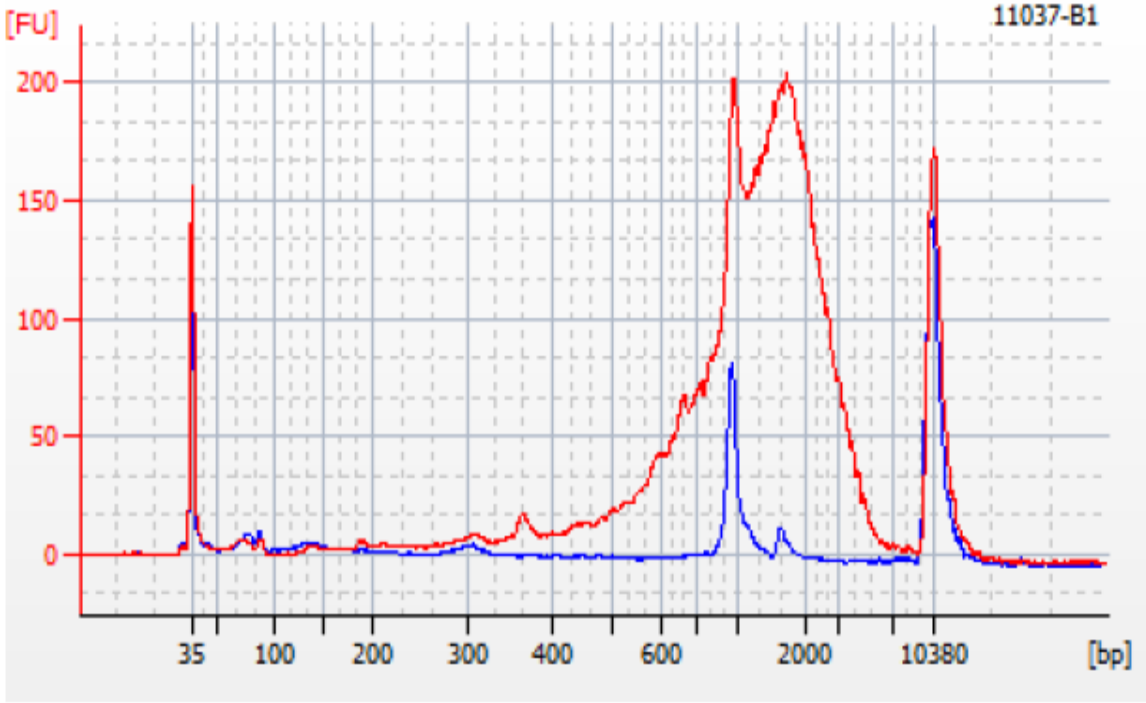
Nextera XT (Illumina)

Transposase-based Method

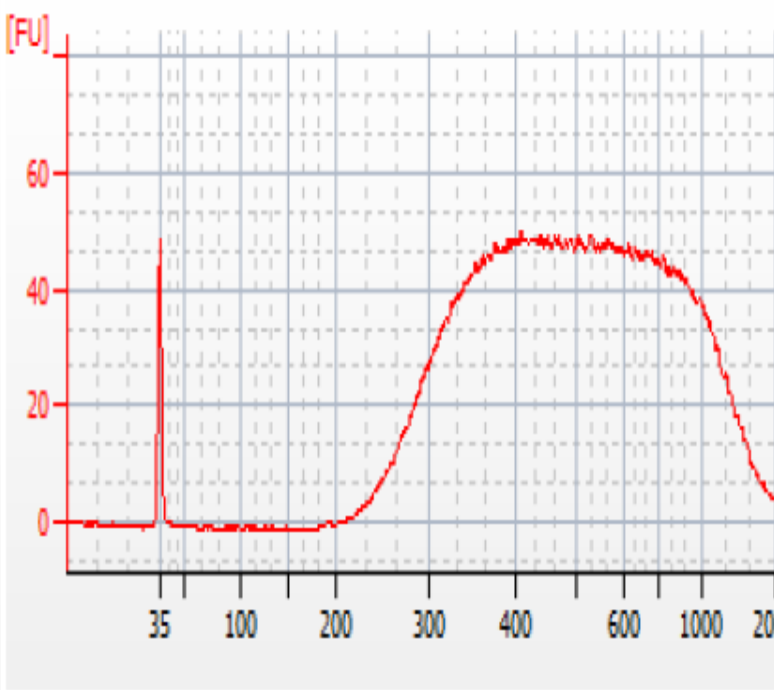


C_1 mRNA seq protocol generates, high quality f
length cDNAs

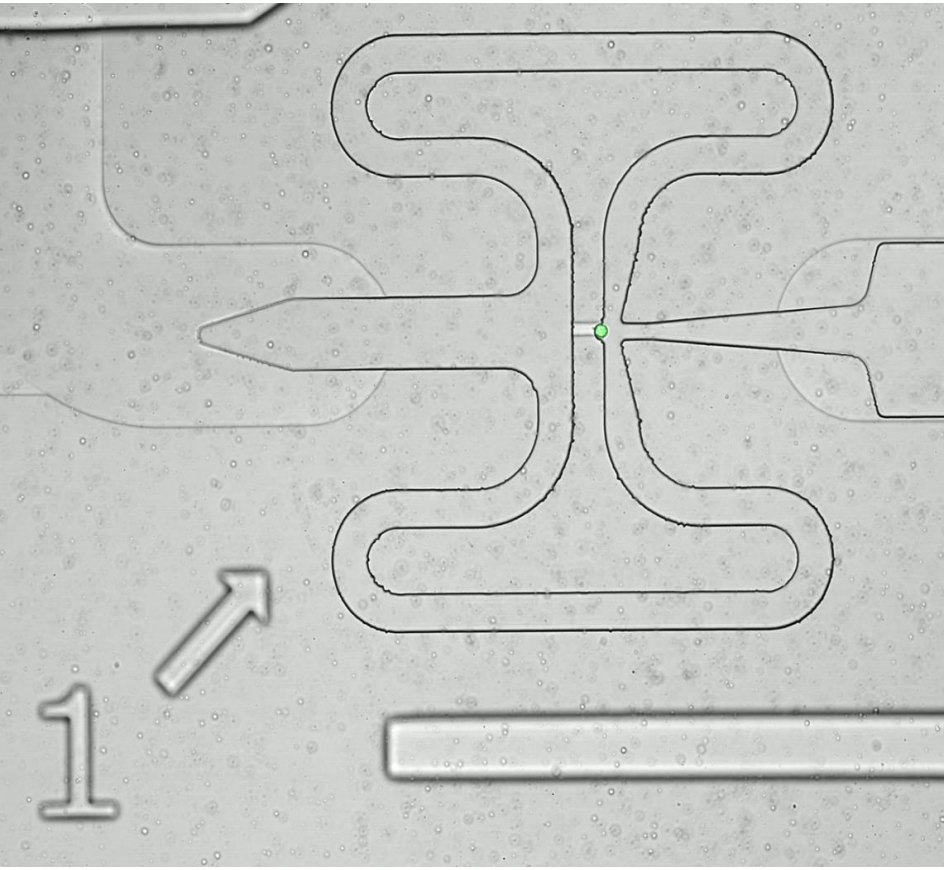
Size Distribution of cDNA after Harvest from C_1 array

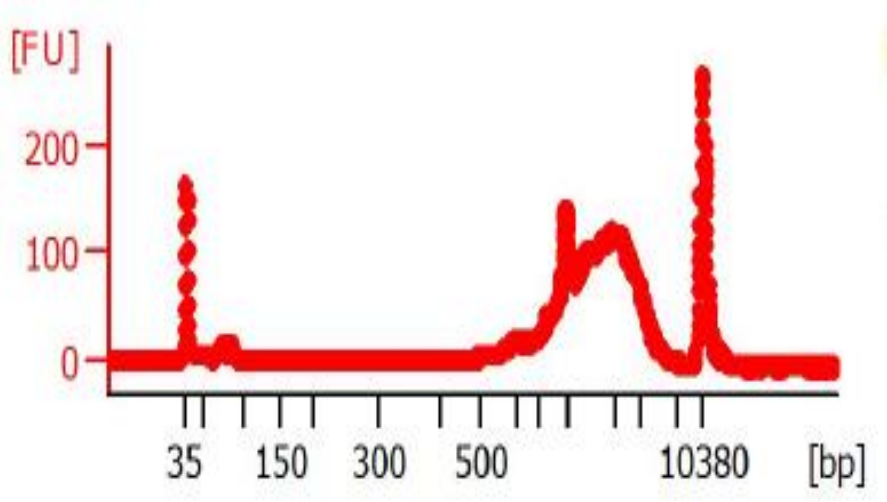


Size Distribution after Lib

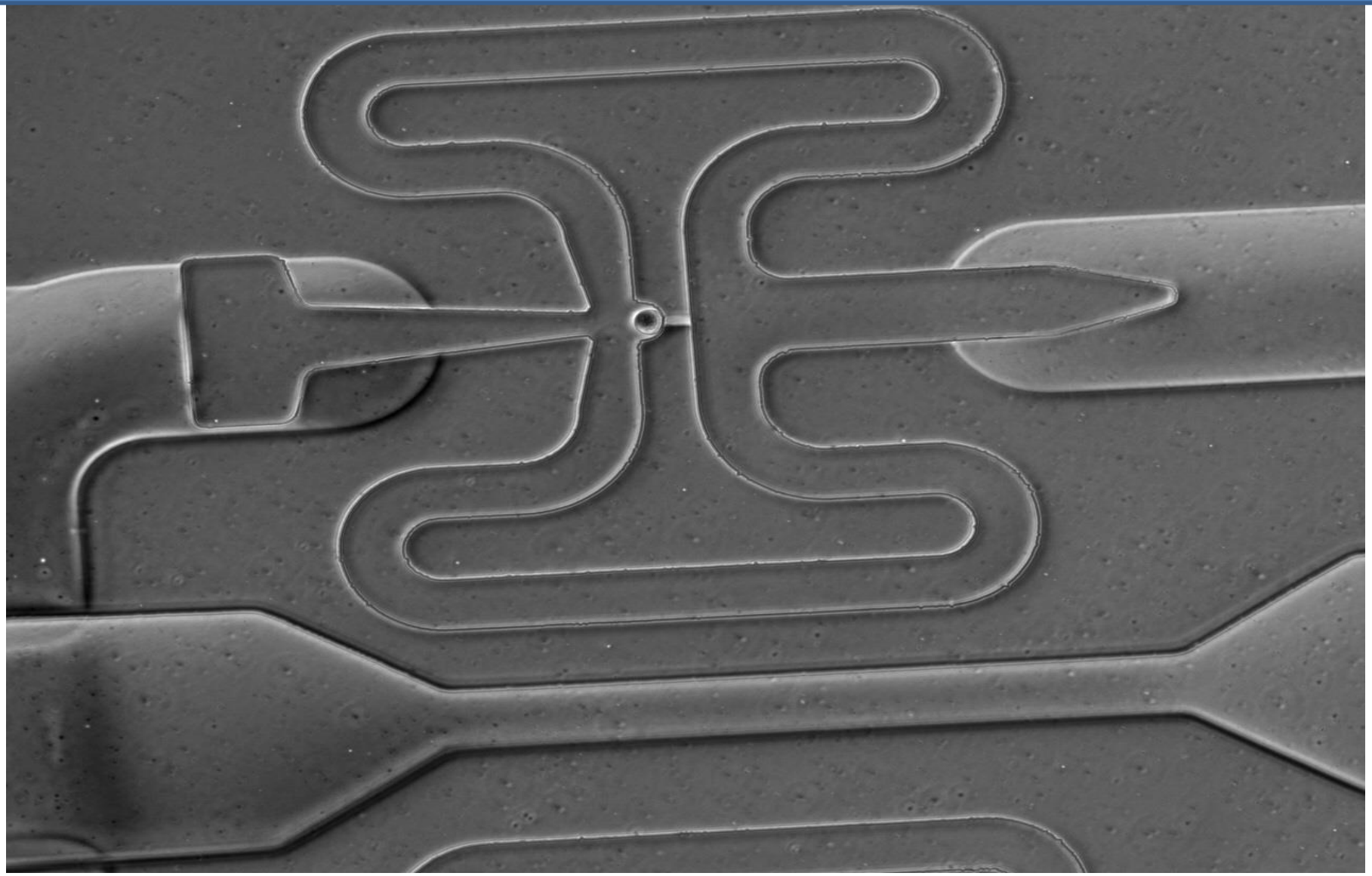


The capture of primary mice epithelial cell and generation of full length cDNA

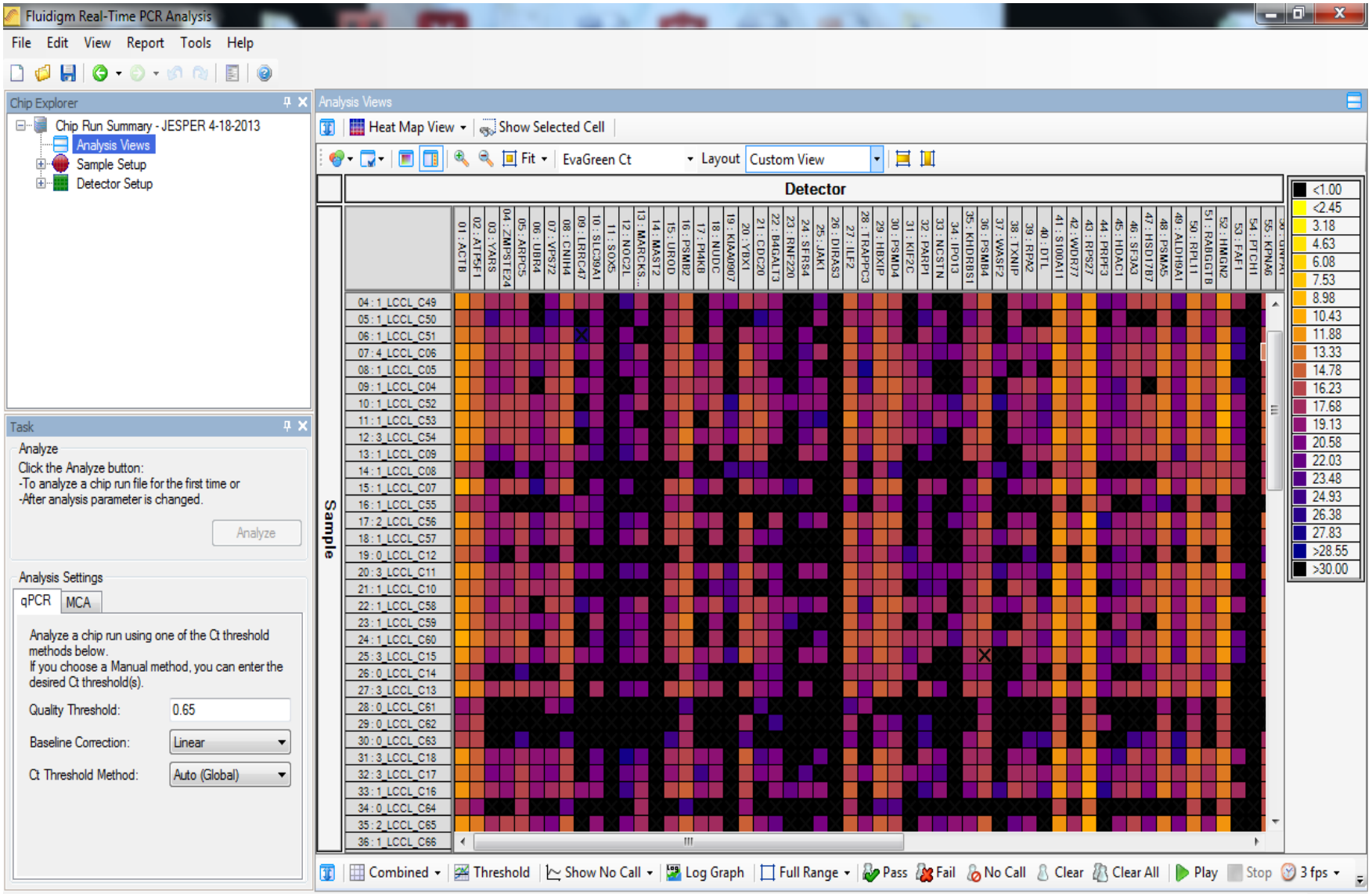




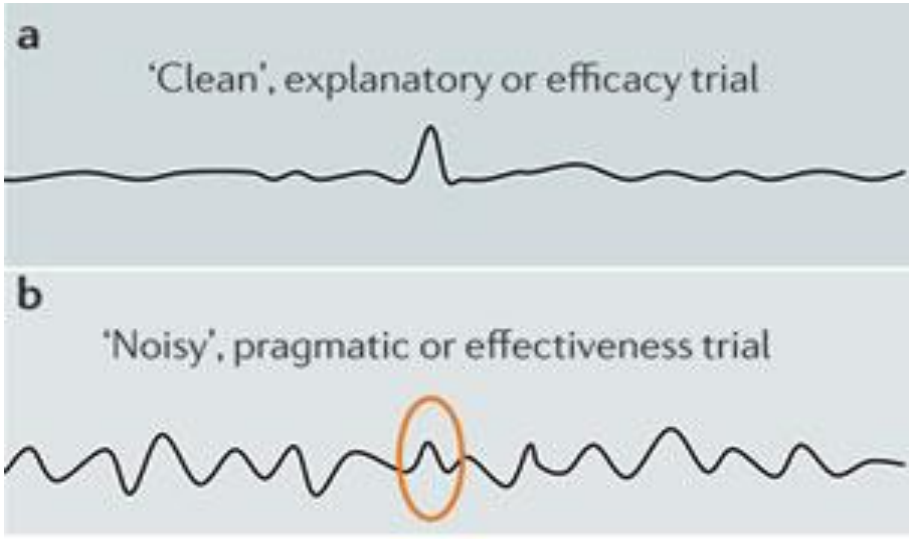
Single Liver Cancer Cell Captured at C1 Nest



The quality of full length (Smarter kit) double stranded cDNAs confirmed by Real Time PCR on Biomark HD



Reveal Hidden Variation:
C_1™ Single-Cell DNA Sequencing Workflow



Nature Reviews | Drug Discovery

Random, low-abundance mutations are currently inaccessible by standard high-throughput sequencing approaches because they cannot be distinguished from sequencing errors. One way to circumvent this problem and simultaneously account for the mutational heterogeneity within tissues is whole genome sequencing of a representative number of single cells.

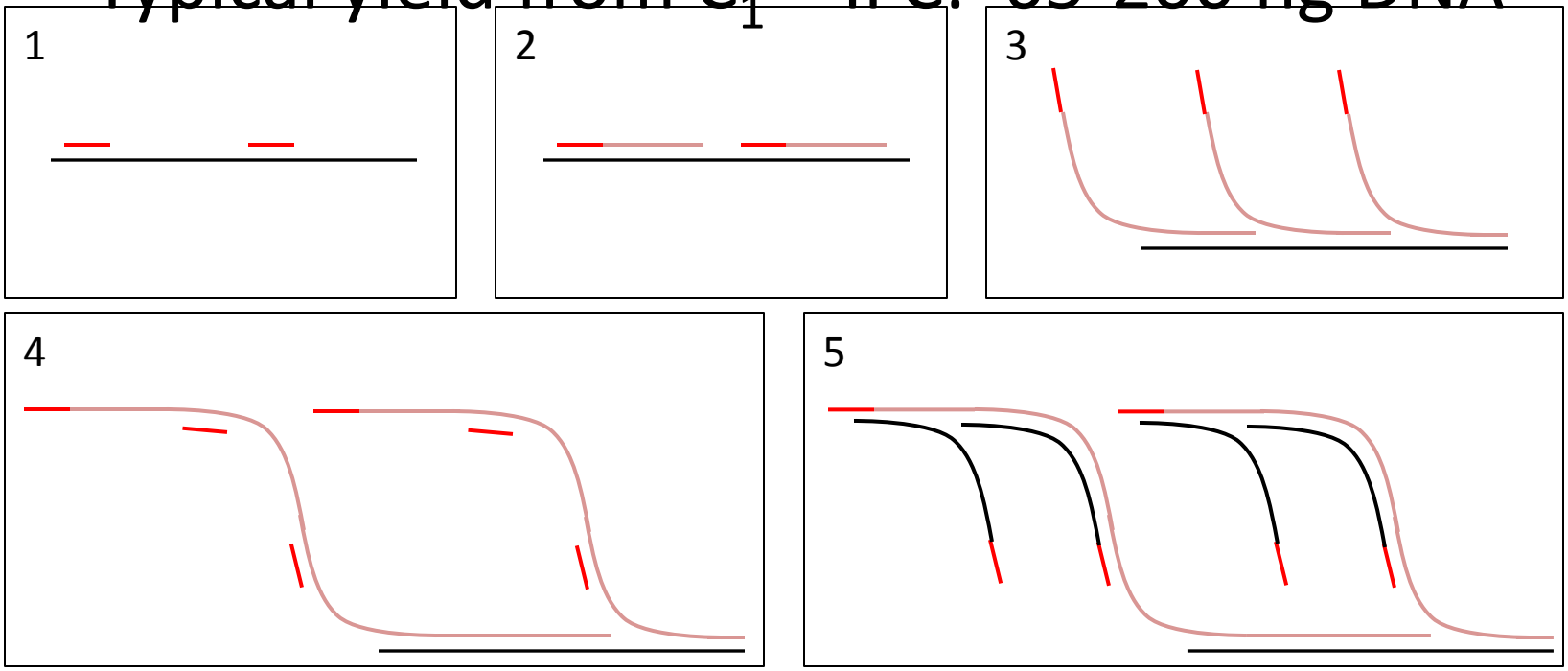
Michael Gundry, PhD.
Albert Einstein College of Medicine
Nucleic Acid Research (March 2012)

Three Major Single-Cell DNA Applications

Single-Cell Whole Genome Sequencing	Single-Cell Whole Exome Sequencing	Single-Cell Targeted Resequencing
Comprehensive approach to discover all possible somatic mutations in both **functional** and **regulatory** regions of the genome.	Faster and more cost effective alternate approach to WGS to discover causal variants in protein coding regions of the genome, most biological activity	Screen for known mutations or identify signatures that may identify disease susceptibility, progress or therapeutic impact.

Multiple Displacement Amplification

- Random hexamer primed
- Isothermal amplification
- Average product length ~12kb
- Typical yield from C_1™ IFC: 65-200 ng DNA



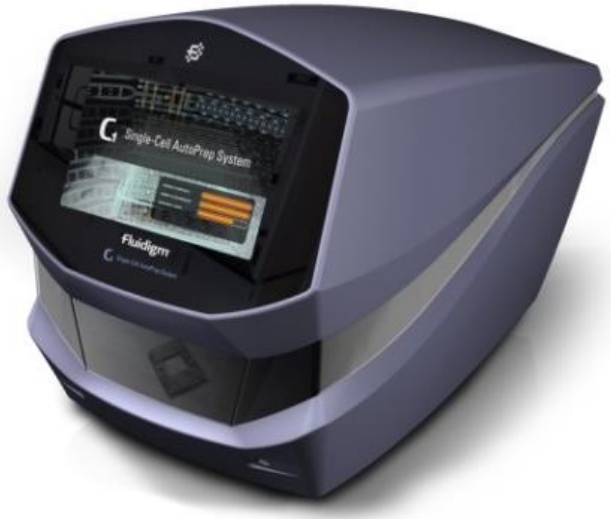
C1 DNA seq workflow for discovering genetic variants in cells

Flexible platform:

- One universal sample prep workflow for targeted & whole genome sequencing
- Enables SNP, small indel, and translocation detection

Sensitive and robust

- Single-cell sensitivity
- Broad sample compatibility



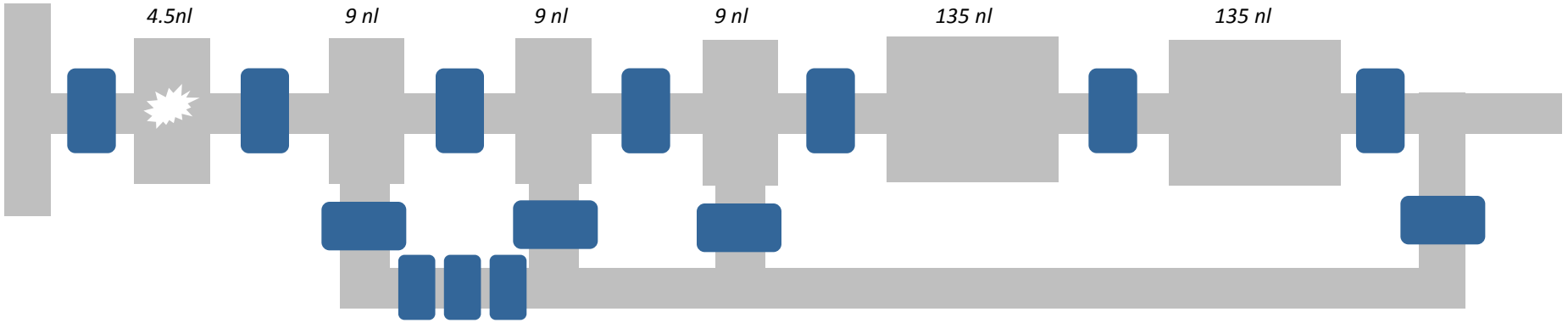
Efficient workflow:

- Easy to use
- Rapid time to results
- Multiple samples per run

Reliable data quality:

- Good genomic coverage and uniformity with low GC bias

WGA implementation on the C_1^{TM} WGA IFC



Capture & Wash → Lysis → Neutralization → MDA

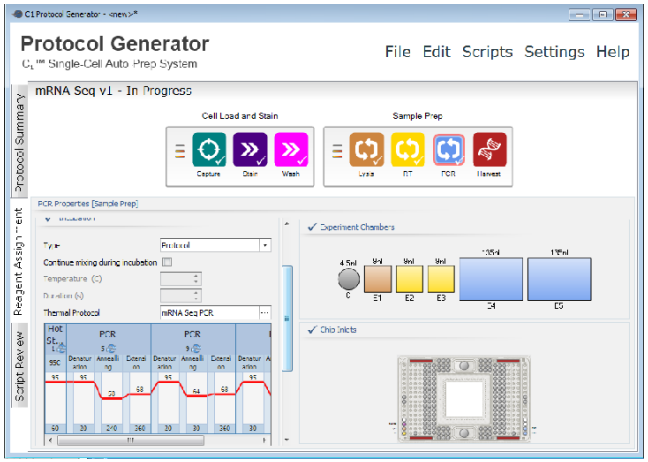
C1 PROTOCOL GENERATOR TOOL

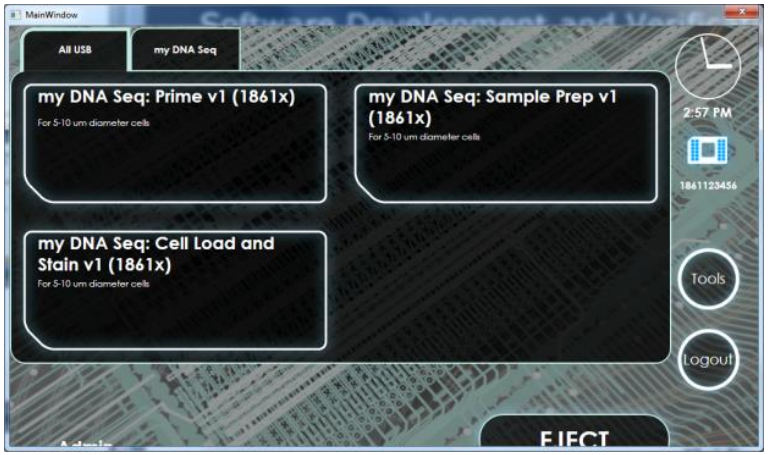
A software tool you can use to develop new protocols on C1

Define load, lyse, wash and thermal cycling paramters

C_1 Script Builder Overview

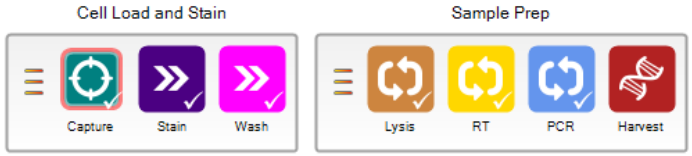
The C_1 Script Builder software is a standalone desktop software application). User will use the software to create custom scripts for C_1 Open App IFC and import them to the C_1 to run.

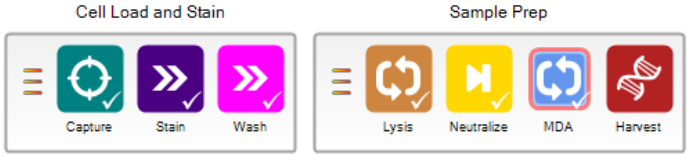




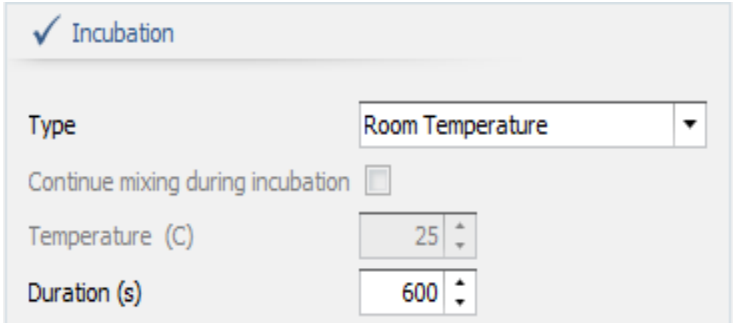
C_1 Script Builder Features

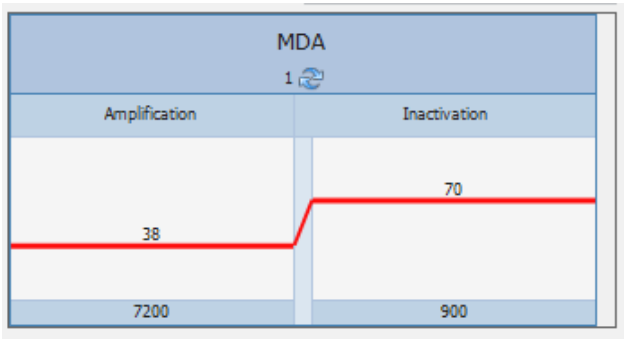
- Fluidigm provides STA, mRNA Seq, and DNA Seq templates for customer to modify:





- Users can modify reagent incubation temperature and duration (single temperature or multiple temperature steps)





- Script Builder validates the entire script workflow and estimates script lengths

nature

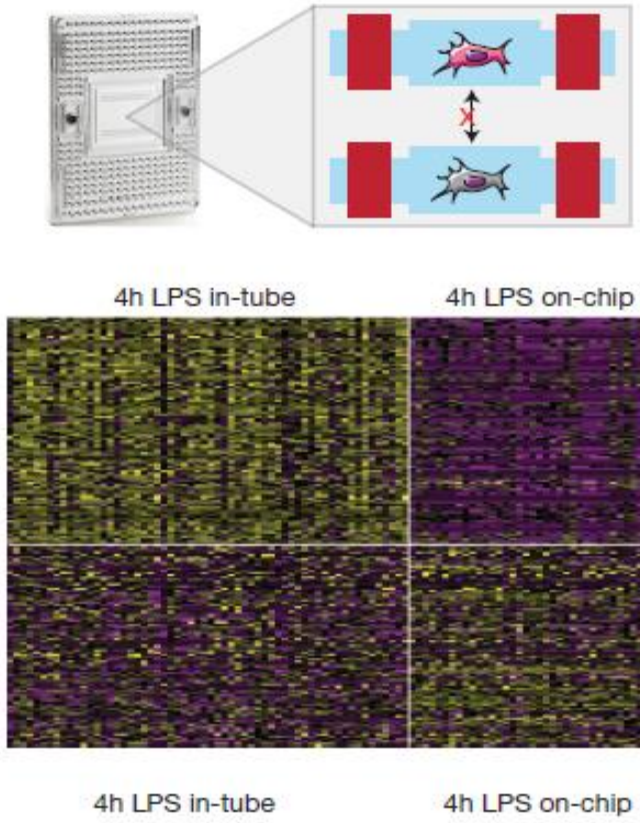
Single-cell RNA-seq reveals dynamic paracrine control of cellular variation

Alex K. Shalek[1,2,3]*, Rahul Satija[3]*, Joe Shuga[4]*, John J. Trombetta[3], Dave Gennert[3], Diana Lu[3], Peilin Chen[4], Rona S. Gertner[1,2], Jellert T. Gaublomme[1,2], Nir Yosef[3], Schraga Schwartz[3], Brian Fowler[4], Suzanne Weaver[4], Jing Wang[4], Xiaohui Wang[4], Ruihua Ding[1,2], Raktima Raychowdhury[3], Nir Friedman[5], Nir Hacohen[3,6], Hongkun Park[1,2,3], Andrew P. May[4] & Aviv Regev[3,7]

Understand gene expression in seemingly identical cells in response to stimulation

Sequenced 1770 single dendrytic cells

C1 enabled interrogation of dynamic immune response at the single cell level



After the capture , stimulated individual Cells with LPS for 4h and then continued With lysis and amplification