Single-Cell Genomics
Deep insights into complex biology
Cellular **Heterogeneity** is a Fundamental Feature of Biological Systems

- **Development**
- **Disease**
- **Adult Tissues**
Individual cells behave differently from the average of many cells.
Single-cell studies reveal new insights in cellular heterogeneity.

Profile
How many cell types or subtypes are present? What is their frequency?

Comparison
How do cell states differ by state or stage of progression

Lineage
How do cells develop? Which cells are related?
• Cell isolation and preparation
• Supports real-time PCR and NGS
• Consistent data quality
• Easy to use
• Discovery, validation and screening
The C1 IFC
Multi-step reaction architecture.

Cell capture module
Up to 5 reaction steps
Active mixing between chambers
Sample-specific input/output
Configurable reaction schemes
Single-cell gene expression analysis

C₁ Single-Cell Auto Prep System

Targeted

BioMark HD System
Single-Cell qPCR

mRNA-Seq

Any Illumina System
Systems Biology – Many modalities define biological heterogeneity

- Transcriptome
- Epigenome
- Genome
- Micronome
- Metabolome
- Lipidome
- Interactome
- Proteome

Full-length mRNA profiling
- Regulatory Heterogeneity
  - ‘Regulome’
- Lineage Heterogeneity

3’-end counting
mRNA-Seq Options for C1

Full Transcript

3’-end Counting
New subpopulations have been classified by single cell.

600 Dendritic cells
9 Subpopulations

3000 Neuronal cells
47 Subpopulations

Shalek, et al., Broad (2014)
Single-cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells

Kyu-Tae Kim\textsuperscript{1,8}, Hye Won Lee\textsuperscript{2,3,7}, Hae-Ock Lee\textsuperscript{1,6}, Sang Cheol Kim\textsuperscript{1}, Yun Jee Seo\textsuperscript{2,4}, Woosung Chung\textsuperscript{1,7}, Hye Hyeon Eum\textsuperscript{1,8}, Do-Hyun Nam\textsuperscript{2,4,7}, Junhyong Kim\textsuperscript{9,10}, Kyeung Min Joo\textsuperscript{2,5,7} and Woong-Yang Park\textsuperscript{1,6,7}

A

Xenograft Transplantation

[Pt tumor]

[LUAD]

Dissociation & Primary culture

RNA-seq
- bulk cells
- single cells

Drug treatment

RNA-seq
- survived cells
Linking cellular genotype and phenotype

Group 2 cells survived *in vitro* anti-cancer treatments
Transcriptomics is only the tip of the iceberg

RNA
Epigenetics (DNA)
DNA
Protein
miRNA

What are you missing?

Picture credit: Pegasus Vertex, Inc.
The C1 Open App Program

• Harness the flexible design of the C1 to build and share the applications you need.

C1 Script Builder
The easiest way to implement custom methods on your C1.

Script Hub
Evolve together. Get access to emerging single-cell methods.
C₁ 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
Start script building now

Open App IFC
One IFC for any application

Open App Reagent Kit
Everything you need to develop your next app
Script Builder → Script Hub

**SCRIPT BUILDER**
Method developers everywhere create innovative single-cell applications and share them on Script Hub.

**SCRIPT HUB**
The single-cell community gets free access to the latest methods and can ask for best practices from developers and other C1 users.

- Can I use FACS antibodies to stain during your RNA seq method?
- How much yield should I expect out of the C1?
- Are there certain dissociation methods you recommend for your protocol?
Script Hub
C1 – only complete solution

miRNA TaqMan
Fluidigm

DNA WG-Seq
Fluidigm

HT-mRNA Seq
Fluidigm

STRT
Sten Linnarsson Lab
Karolinska Institute

CEL-Seq
Itai Yani
Technion

STA
Fluidigm

ATAC-Seq
Will Greenleaf lab
Stanford University

CAGE-Seq
Jay Shin lab
Riken Institute
DNA-Seq for the Study of Tumor Heterogeneity

- Tumors are not clonal, they are composed of sub-clones.
- Knowledge of the sequence of molecular events leading to tumor development in many cancers is lacking.
- Understanding genetic variation between sub-clones can shed light on sub-clonal “fitness” and drug resistance.

Fully understanding genomic heterogeneity in tumors requires single-cell analysis.
Clonal evolution in breast cancer revealed by single nucleus genome sequencing

Yong Wang¹, Jill Waters¹, Marco L. Leung¹,², Anna Unruh¹, Whijae Roh¹, Xiuting Shi¹, Ken Chen³, Paul Scheet³,⁴, Selina Vattathil³,⁴, Han Liang⁵, Asha Multani⁵, Hong Zhang⁶, Rui Zhao⁶, Franziska Michor⁷, Funda Meric–Bernstam⁷ & Nicholas E. Navin¹,²,³

[Diagram of clonal evolution in breast cancer]
Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics

Charles Gwad\textsuperscript{a,b}, Winston Koh\textsuperscript{b}, and Stephen R. Quake\textsuperscript{b,1}

\textsuperscript{a}Division of Pediatric Hematology-Oncology, Department of Pediatrics, Stanford University, Palo Alto, CA 94305; and \textsuperscript{b}Departments of Bioengineering and Applied Physics, Stanford University and Howard Hughes Medical Institute, Stanford, CA 94305

<table>
<thead>
<tr>
<th>1</th>
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<tbody>
<tr>
<td>Bulk exome seq to identify heterogeneous regions</td>
<td>Single cell isolation &amp; WGA</td>
<td>Fluidigm Targeted re-sequencing</td>
<td>Reconstruction of clonal architecture</td>
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6 patients

\textasciitilde245 cells/patient
Single cell sequencing reveals clonal structure
Towards targeted combinatorial therapy for treating Cancer

- Tumors are highly heterogeneous

- Complex, combinatorial therapies for various cancers have been proposed (Prados et al. *Neuro-Oncology*, 2015)

- A better understanding of the molecular profile of these complex tumors is needed.

The Polaris system

Seamlessly integrates cell biology with molecular analysis

- Actively select and isolate targeted cells.
- Image cells to ensure phenotype and cell viability.
- Maintain and feed single cells on IFC.
- Challenge cells with a wide range of factors, including RNAs, transcription factors, bacteria, small molecules and more.
- Prepare individual cells for mRNA sequencing.
C1 IFC updates and availabilities

Fluidigm recently redesigned all 10-17um C1 IFCs to ensure higher rates of single-cell captures.

C1 chips currently available:
- 5-10um: mRNA seq, DNA seq, PreAmp, Open App
- 10-17um: mRNA seq, DNA seq, PreAmp, Open App, 800 chip for mRNA seq
- 17-25um: mRNA seq, DNA seq, PreAmp, Open App

To be tentatively released:
- 5-10um 800 chip for mRNA seq (late summer)
- 10-17um 800 chip for mRNA seq (newly optimized version late summer/early fall)
- 17-25um 800 chip for mRNA seq (2017)
Simplify the complex quest to understand and apply biology.
Maximizing flow Cell Saves time and money

<table>
<thead>
<tr>
<th># cells</th>
<th># of chips/lane</th>
<th>Read depth</th>
<th>Cost/cell</th>
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<tbody>
<tr>
<td>96</td>
<td>1 C1-96</td>
<td>2.6M/cell</td>
<td>$20.80</td>
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<tr>
<td>384 (current max)</td>
<td>4 C1-96</td>
<td>650K/cell</td>
<td>$5.20</td>
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<tr>
<td>800</td>
<td>1 C1-HT</td>
<td>315K/cell</td>
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<tr>
<td>1600</td>
<td>2 C1-HT</td>
<td>157K/cell</td>
<td>$1.25</td>
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</tbody>
</table>

* Assumptions: $2000/lane, Illumina HiSeq @ 250M reads/lane