Illumina®|Bio-Rad®
Single Cell Sequencing Solution

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The importance of single cell sequencing

“The single cell ‘omics revolution is firmly underway. Nearly every expression study worth doing will be worth doing at single cell level…” Ewan Birney, EMBL

Macosko et al, Cell: May, 2-15
Single-cell analysis – a brief history

First single-cell mRNA sequenced, Tang, et.al.

DNA-Seq of single human cancer cell, Navin, et.al.

First single-cell exome sequenced, Xu, et.al.

Single cell WGS of Neurons, Evrony, et.al.

First single-cell T-cell epigenome with HiC, Nagano, et.al.

Organ lineage tracing with SC RNA-Seq, Truettlein, et.al.

Single cell qPCR of neurons, Eberwine, et.al.

SC whole transcriptome microarrays

STRT-Seq 5' end Seq Islam, et. al.

CEL-Seq Remove PCR bias with IVT Hashimshony, et. al.

MALBAC Zong, et.al.

Incorporation of UMI's to methods Jaitin, et. al. Islam, et.al.

Drop-Seq Macosko, et.al.

SMART-Seq Full length pre-amp Ramskold, et al.

SC RNA-Seq of immune cells, Shalek, et.al.

First single-cell RNA-Seq of immune cells, Xu, et.al.

SC ATAC-Seq of human cell lines, Cusanovich, et.al.

DNA-Seq of human cell lines, Eberwine, et.al.

First whole genome amplicon sequencing of SC, Zong, et.al.

SC RNA-Seq of immune cells, Picelli, et.al.

SC ATAC-Seq of immune cells, Cusanovich, et.al.

SC RNA-Seq of SC WGS of Neurons, Shalek, et.al.
Why single cells?

1. Assess cell-to-cell heterogeneity
   - Heterogeneous tissue or tumor
   - PCA
   - Component 2
     - cell type C
     - cell type B
     - cell type A
   - Component 1

2. Map cell trajectories
   - Lineage A
   - Lineage B
   - Trajectory A
   - Trajectory B
   - t₀, t₁, t₂
   - trajectory analysis pipeline

3. Dissect transcriptional mechanics
   - Gene Transcription "off"
   - RNA Polymerase disassociated with gene
   - Gene Transcription "on"
   - RNA Polymerase bound and transcribing gene

4. Infer gene regulatory networks
   - genes
   - cells
   - Module 1
   - Module 2
   - Module 3
   - Network inference
   - low
   - high
Single cell sample prep: Low vs High-Throughput

**Highlights**
- Drop-seq enables highly parallel analysis of individual cells by RNA-seq
- Drop-seq encapsulates cells in nanoliter droplets together with DNA-barcoded beads
- Systematic evaluation of Drop-seq library quality using species mixing experiments
- Drop-seq analysis of 44,808 cells identifies 39 cell populations in the retina

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**In Brief**
Capturing single cells along with sets of uniquely barcoded primer beads together in tiny droplets enables large-scale, highly parallel single-cell transcriptomics. Applying this analysis to cells in mouse retinal tissue revealed transcriptionally distinct cell populations along with molecular markers of each type.

**Accession Numbers**
GSE63473

**Macosko et al., 2015, Cell 161, 1202–1214**
May 21, 2015
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**Hands on Time**

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<th>Cell Through-put</th>
<th>Mechanical</th>
<th>FLDM C1</th>
<th>ICELL8</th>
<th>DropSeq</th>
<th>10X</th>
<th>ddSEQ™ System</th>
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<td>Up to $10^2$</td>
<td>Up to $10^3$</td>
<td>$10^3$ to $10^4$</td>
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The Illumina | Bio-Rad Single Cell Sequencing Solution

Isolate and barcode single cells

Prepare your library

Sequence efficiently

Analyze and store data with BaseSpace Sequence Hub

**Illumina**

**SureCell™ WTA 3’ Library Prep Kit for the ddSEQ™ System**

**ddSEQ™ Single Cell Isolator**

**NextSeq**

Cloud

**Scalable, high-throughput, cost-effective NGS workflow solution**

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Encapsulate Thousands of Cells in < 5 min

Cell Barcoding Microsphere

Disposable Cartridges

Cell Suspension

ddSEQ™ Single-Cell Isolator with Droplet Digital™ Technology

Process Thousands of cells in minutes

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SureCell™ WTA 3’ Library Prep Kit for the ddSEQ™ System

- Single cell encapsulation with the ddSEQ™ Single-Cell Isolator
- Sensitive assay chemistry without pre-amplification
- Modified Nextera® Library prep

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Sequencing Power for Every Scale

Increasing system output & price

Decreasing price per GB

MiniSeq™
7.5 Gb | 25M
2x150

MiSeq®
15 Gb | 25M
2x300

NextSeq®
120 Gb | 400M
2x150

HiSeq 2500
1000 Gb | 4B
2x125

HiSeq 3000
750 Gb | 2.5B
2x150

HiSeq 4000
1500 Gb | 5B
2x150

HiSeq X® Five
1800 Gb | 6B
2x150

HiSeq X Ten
1800 Gb | 6B
2x150

Single Cell RNA-Seq

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BaseSpace® Single-Cell RNA App

- **Simple analysis set-up** for samples across multiple sequencing runs
  - Up to 96 samples per analysis
- **Easily choose analysis parameters**
  - Reference genome
  - ERCC spike-ins
  - Subsampling for QC
- **Rapid** alignment, cell and gene counting, and filtering
BaseSpace® Single-Cell RNA App

- **Per-sample reports** available in-browser and as PDF
  - Alignment quality
  - Coverage information
  - Abundant sequences
- Perform global PCA and tSNE clustering
- Cell-cycle heatmap
- All output files available for download, or as input into downstream applications
  - Includes cell-gene expression table
Detection of Genes in a Heterogeneous Population of Cells

- Two-species cell mixture (HEK293/NIH3T3) demonstrates low crosstalk and high purity

Cells: 1384
Duplets: 5.8%
Purity: 99.1%
T-SNE analysis identifies a sub-population in a heterogeneous cell mixture

Total cells: 602
mm10 cells: 558
hg19 cells: 43
Sub-population: 7%
PCA clustering of 1:1 mixture of mouse and human cells detects distinct population

HEK293 / NIH3T3 (human/mouse)  hg19 RPL13  mm10 Rpl13
Sensitivity & Reproducibility Across Cell Lines

HEK293 Genes vs. Reads Per Cell

Human Genes Detected

Mouse Genes Detected

Replicate 1 Gene Count
Replicate 2 Gene Count

R2: 0.975
NumGenes: 10,011

NIH3T3 Genes vs. Reads Per Cell

Gene Count Reproducibility

Genes Detected Across Different Cell Sizes

A20 3T3 HEK BJ

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Cell Cycle Analysis of HEK293 Cells
PBMC Cell Types

Colored based on simple threshold on single genes
Measuring a time course of gene expression during stem cell differentiation

Data courtesy of Drs. C.N. Svendsen & R.Ho
Cedars-Sinai Medical Center
Board of Governors Regenerative Medicine Institute
Summary

• The Illumina | Bio-Rad Single Cell Sequencing Solution can reproducibly partition and analyze thousands of single cells in sub-nanoliter droplets in minutes.

• Analysis of human and mouse cell line mixing experiments demonstrates the ability of this platform to distinguish cells in a heterogeneous population by gene expression profiles.

• Robust chemistry allows for a high percentage assignment of transcripts to single cell barcodes in multiple cell lines.

• Transcriptional variation can be measured in single cells by analyzing changes in cell cycle gene expression.

• High sensitivity of gene expression is detected across a number of cell types and is not impacted by cell diameter.
Thank you for your time!

Learn more @ www.bio-rad.com/ddSEQ
Additional Cell Tools for the Single-Cell RNA-Seq Solution

- Counting & visualizing cells is part of general cell culture maintenance
  - Measure cell confluency to optimize cell culture
  - Visualize cells to confirm cell health

- Optimal cell dissociation is important for the success of the ddSEQ™ System workflow
  - Visualize and prevent doublets or triplets caused by cell aggregates

- Sub population enrichment may be employed
  - Enrich for a particular sub population of cells to maximize experimental efficiency
  - Improve dissociation of tissues and difficult samples