Illumina® | Bio-Rad®
Single Cell Sequencing Solution
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Sr. Library Prep Specialist

For Research Use Only. Not for use in diagnostic procedures.
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
Why single cells?

1. Assess cell-to-cell heterogeneity

![PCA plot showing cell types A, B, and C]

2. Map cell trajectories

![Trajectory plots for Lineage A and B]

3. Dissect transcriptional mechanics

![Gene transcription on and off with RNA polymerase]

4. Infer gene regulatory networks

![Gene expression across modules with network inference]

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**illumina**
The Illumina | Bio-Rad Single Cell Sequencing Solution

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

Single Cell RNA-Seq

**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

**HiSeq X® Ten**
- 1800 Gb | 6B
- 2x150

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How much sequencing will I need?

How many genes do I want to detect per cell?

Am I looking for potentially rare cell types?

Saturation of # detected genes requires more reads

Range depends on cell type and expression levels and also the biological question you’re asking

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Sequencing cost per cell

Range depends on cell type and expression levels and also the biological question you’re asking

- How many genes do I want to detect per cell?
- Am I looking for potentially rare cell types?
- Saturation of # detected genes requires more reads

NextSeq 500

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• **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

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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%
Sensitivity & Reproducibility Across Cell Lines

**HEK293 Genes vs. Reads Per Cell**

- Human Genes Detected
- Mouse Genes Detected

**NIH3T3 Genes vs. Reads Per Cell**

- Human Genes Detected
- Mouse Genes Detected

**Gene Count Reproducibility**

- R2: 0.975
- NumGenes: 10,011

**Genes Detected Across Different Cell Sizes**

- A20
- 3T3
- HEK
- BJ

Average Cell Diameter, µm

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Thank you for your time!

Learn more @ [www.bio-rad.com/ddSEQ](http://www.bio-rad.com/ddSEQ)
The Next Step in Expression
Combining RNA-Seq and Methylation Analysis
Methylation Analysis Technology

The bang for your buck model

$5,000

$500

$50

All-in Price Per Sample

TruSeq DNA Methylation (WGBS)

Infinium® EPIC Array

TruSeq Methyl Capture

Amplicon Nextera®

Sequencing

Array

# of CpG sites
How does this technology work?

Methylation Analysis With Bisulfite Conversion

Methylation Arrays:
Identify the difference between a C and A at ~850,000 discreet sites of interest

Methylation Sequencing:
Identify the difference between a C and A in targeted regions up to 38M+ CpG sites in complete regions
Methylation Arrays or Sequencing

- Arrays are cost effective for large scale screens
- Sequencing provides deep information across CpG rich regions and can call SNPs, indels within the region covered
Methylation Technology Options

- **Array**
  - # of CpG sites: 10s
  - $50

- **Amplicon**
  - # of CpG sites: 500K
  - $500

- **TruSeq DNA Methylation (WGBS)**
  - # of CpG sites: 50M
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 2M
  - $2,000

- **Targeted Methyl Seq**
  - # of CpG sites: 5M
  - $3,000

- **Targeted Methyl Seq**
  - # of CpG sites: 1M
  - $4,000

- **Targeted Methyl Seq**
  - # of CpG sites: 20M
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 50M
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 100M
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 1B
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 5B
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 10B
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 100B
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 1T
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 10T
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 100T
  - $5,000

- **Targeted Methyl Seq**
  - # of CpG sites: 1P
  - $5,000
TruSeq Methyl Capture Workflow

Prepare Libraries
TruSeq Methyl Capture

Sequence NextSeq®
or HiSeq®

Analyze
BaseSpace

Differential methylation annotation:
- promoter: 9%
- exon: 35%
- intron: 14%
- intergenic: 41%
Sequencing Content Compared to Array

**Infinium MethylationEPIC BeadChip DMRs**
- Covered by Methyl Capture EPIC: 97% (2160)
- Unique to EPIC Array: 3% (67)

**TruSeq Methyl Capture EPIC DMRs**
- Covered by EPIC Array: 56% (8865)
- Unique to Methyl Capture EPIC: 44% (7065)

**DMR**
Differentially Methylated Region
TruSeq Methyl Capture Content

![Bar chart showing genomic regions covered by TruSeq Methyl Capture EPIC and Kit R and Kit A.](chart.png)
# Sequencing a TruSeq Methyl Capture Library

<table>
<thead>
<tr>
<th>Sequencing System</th>
<th>Samples / Run</th>
</tr>
</thead>
<tbody>
<tr>
<td>NextSeq®</td>
<td></td>
</tr>
<tr>
<td>Mid-Output Flow Cell</td>
<td>2</td>
</tr>
<tr>
<td>High-Output Flow Cell</td>
<td>8</td>
</tr>
<tr>
<td>HiSeq® 2500</td>
<td></td>
</tr>
<tr>
<td>Rapid Run Mode, Dual Flow Cell</td>
<td>10</td>
</tr>
<tr>
<td>High-Output Mode, Dual Flow Cell</td>
<td>72</td>
</tr>
<tr>
<td>HiSeq® 3000</td>
<td>Single Flow Cell</td>
</tr>
<tr>
<td>HiSeq® 4000</td>
<td>Dual Flow Cell</td>
</tr>
</tbody>
</table>
**Methylation Sequencing Analysis Workflow**

**BaseSpace Apps**

**Align Data**
- Map sequencing reads to a reference genome
- Define that reference for targeted sequencing (manifest)
- Bisulfite conversion specific aligner in MethylSeq

**Call CpG Methylation**
- Based on bisulfite conversion rate
- Calculated as % methylated
- Also uses MethylSeq

**Compare Samples**
- Differential methylation between samples with MethylKit
- Tumor/Normal, Treated/untreated, etc
Correlating Methylation with RNA-Seq Data

**MethylMix**
- Differential Methylation (array or sequencing) and matched RNA-Seq analysis
- Bioconductor Package @ bioconductor.org/packages/MethylMix/
- Demo data available from TCGA

**COHCAP**
- Differential Methylation (array or sequencing) and matched RNA-Seq analysis
- Bioconductor Package @ bioconductor.org/packages/COHCAP/
- Discussion groups online for Q&A
- Demo data included
Thank you!
Questions?