Genetics, Biomedical Computing, and Genomics Monthly Meeting –
New Technologies in the UCI Genomics Research and Technology Hub (GRT Hub)

**Single Cell Multi-omics: Parse Biosciences and 10X Genomics**

Quy Nguyen
April 4, 2023
Currently Available at GRTH—Single Cell Profiling Technologies

- **10X Genomics**
  - Chromium Single Cell Assay: Droplet-based
    - RNAseq
    - Immune cell profiling (VDJ)
    - ATACseq
    - Multiome (RNA+ATACseq)

- **Parse Biosciences**
  - Evercode Single Cell Assay: Combinatorial Barcoding
    - RNAseq
    - Immune cell profiling (VDJ)
10X Genomics
Chromium Single Cell Assay

- Next GEM (Droplet-based) Technology
  - Cells or nuclei are partitioned into individual droplet with reagents, for reverse transcription and barcoding.
10X Genomics
Droplet Generation

• Cells (or nuclei), gel beads, reagents, and oil are loaded onto the chip.
• Pumps drives the solution through the microfluidics channel to form droplet.
• ~100,000 (to 200,000; for HT) droplet are formed. ~1-10% contains single cell and gel bead.
10X Genomics
Gel Beads – Transcript Capture and Barcoding

• Gel beads contains primers for RT capture of mRNA (for ATAC; capture sequence for chromatin fragment) and other targetable species.
10X Genomics
Single Cell Multiomics – Feature Barcoding

• Feature Barcoding enable simultaneous capture of gene expression and labeled antibodies, antigens, or CRISPR guides.
10X Genomics
3’ Gene Expression Assay

• Single cell (or nuclei) transcriptome 3’ gene expression.
• Poly(A) capture of mRNA.
• Additional feature capture.
  • Antibodies, CRISPR guides, or anything with a specific oligonucleotide tag.
10X Genomics
5’ Gene Expression Assay + VDJ Profiling

• Single cell (or nuclei) transcriptome 5’ gene expression.
• Poly(A) reverse transcription of mRNA, followed by 5’ end capture.
• Capture TCR and BCR sequence.
• Additional feature capture.
  • Antibodies and antigens.
**10X Genomics**

**ATAC Assay**

- Assay for Transposase Accessible Chromatin.
- Open DNA regions fragmented and adapters added, follow by capture of DNA.
- Profile the chromatin regulatory landscape.
10X Genomics
FLEX (Fixed) RNA Assay

- Fixed single cell (or nuclei) gene expression.
- Formaldehyde fixed samples.
- Probed-based gene expression capture; for human or mouse only.
- Additional feature capture.
  - Antibodies

https://www.10xgenomics.com/support/single-cell-gene-expression-flex
10X Genomics
Multiome (Gene Expression + ATAC)

• Single nuclei gene expression and ATAC profiling.
• Epigenomics landscape and gene expression from the same nuclei.
<table>
<thead>
<tr>
<th>Species</th>
<th>Acquired Data</th>
<th>Assay size</th>
<th>Samples Status</th>
<th>Multiome</th>
</tr>
</thead>
<tbody>
<tr>
<td>3’ Gene Expression</td>
<td>Any</td>
<td>3’ transcriptome</td>
<td>500-10,000 cells /sample</td>
<td>Live cells or nuclei</td>
</tr>
<tr>
<td>3’ HT Gene Expression</td>
<td>Any</td>
<td>3’ transcriptome</td>
<td>2,000-20,000 cells /sample</td>
<td>Live cells or nuclei</td>
</tr>
<tr>
<td>5’ Gene Expression + VDJ Profiling</td>
<td>Any</td>
<td>5’ transcriptome + TCR and/or BCR sequence</td>
<td>500-10,000 cells /sample</td>
<td>Live cells or nuclei</td>
</tr>
<tr>
<td>5’ HT Gene Expression + VDJ Profiling</td>
<td>Any</td>
<td>5’ transcriptome + TCR and/or BCR sequence</td>
<td>2,000-20,000 cells /sample</td>
<td>Live cells or nuclei</td>
</tr>
<tr>
<td>ATAC</td>
<td>Any</td>
<td>Open chromatin sequence</td>
<td>500-10,000 cells /sample</td>
<td>Fresh Nuclei</td>
</tr>
<tr>
<td>FLEX (Fixed RNA)</td>
<td>Human or Mouse only</td>
<td>Probe-based gene expression</td>
<td>500-128,000 cells /sample</td>
<td>Fixed cells or nuclei</td>
</tr>
<tr>
<td>Multiome (RNA+ATAC)</td>
<td>Any</td>
<td>3’ transcriptome + open chromatin sequence</td>
<td>500-10,000 cells /sample</td>
<td>Fresh Nuclei</td>
</tr>
</tbody>
</table>
Currently Available at GRTH—Single Cell Profiling Technologies

- **10X Genomics**
  - Chromium Single Cell Assay: Droplet-based
    - RNAseq
    - Immune cell profiling (VDJ)
    - ATACseq
    - Multiome (RNA+ATACseq)

- **Parse Biosciences**
  - Evercode Single Cell Assay: Combinatorial Barcoding
    - RNAseq
    - Immune cell profiling (VDJ)
Parse Biosciences
Evercode Single Cell Assay

- Combinatorial Barcoding Technology
  - Fixed cells or nuclei undergo a series of barcoding to label transcript with a unique sequence of barcodes.
Genomics Research and Technology Hub

Parse Biosciences
Combinatorial Barcoding

- Samples are split, barcoded, and pooled over several rounds to generate cDNA that contain a unique sequence of barcodes.

- Uniquely barcode 10,000 to 1 million cells in one experiment.

https://www.parsebiosciences.com/technology
Parse Biosciences
Sample Fixation and Freezing

- Cells or nuclei are fixed and frozen using the Parse Bio Fixation kit.
- Can store frozen in -80C freezer for up to 6 months.

Parse Biosciences
Evercode WT Gene Expression Assay

• Single cell (or nuclei) gene expression.
• Poly(A) + random hexamer capture of mRNA.
• Multiplex up to 12-96 samples per run.
Parse Biosciences
Evercode TCR Assay

- Single cell (or nuclei) gene expression and T-cell receptor.
- Poly(A) + random hexamer capture of mRNA.
- Multiplex up to 12-96 samples per run.

Parse Biosciences
Evercode WT and TCR Kits

• Three different kit sizes.
  • Evercode WT Mini, WT, and WT Mega
  • Evercode TCR Mini, TCR and TCR Mega

## Parse Biosciences
### Single Cell Assay Summary

<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Acquired Data</th>
<th>Assay Size</th>
<th>Sample Status</th>
<th>Multiome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evercode WT Mini</td>
<td>Any</td>
<td>3’ transcriptome</td>
<td>10,000-20,000 cells Up to 12 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Evercode WT</td>
<td>Any</td>
<td>3’ transcriptome</td>
<td>100,000 cells Up to 48 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Evercode WT Mega</td>
<td>Any</td>
<td>3’ transcriptome</td>
<td>1,000,000 cells Up to 96 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Evercode TCR Mini</td>
<td>Any</td>
<td>3’ transcriptome + TCR Sequence</td>
<td>10,000-20,000 cells Up to 12 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Evercode TCR</td>
<td>Any</td>
<td>3’ transcriptome + TCR Sequence</td>
<td>100,000 cells Up to 48 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Evercode TCR Mega</td>
<td>Any</td>
<td>3’ transcriptome + TCR Sequence</td>
<td>1,000,000 cells Up to 96 samples</td>
<td>Fixed and Frozen cells or nuclei</td>
<td>None</td>
</tr>
<tr>
<td>Capture number</td>
<td>10X Genomics, 3' Gene Expression</td>
<td>10X Genomics, HT 3' Gene Expression</td>
<td>10X Genomics, Fixed RNA Profiling</td>
<td>Parse Biosciences, 10k Evercode WT Mini Gene Expression</td>
<td>Parse Biosciences, 100k Evercode WT Gene Expression</td>
</tr>
<tr>
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<td>-------------------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Up to ~10,000 cells per reaction. If multiplexing, up to ~30,000 cells.</td>
<td>Up to ~20,000 cells per reaction. If multiplexing, up to ~60,000 cells per reaction. *Must run a minimum of 2 reactions at once.</td>
<td>Up to ~10,000 cells per reaction. If multiplexing, up to ~128,000 cells.</td>
<td>Up to ~20,000 cells per kit/run.</td>
<td>Up to ~100,000 cells per kit/run.</td>
<td>Up to ~1,000,000 cells per kit/run.</td>
</tr>
</tbody>
</table>

**Sequencing info**
- Poly(A)-3' RNA capture.
- Poly(A)-3' RNA capture.
- Human or mouse whole gene probe capture. ~18,000-19,000 genes.
- Poly(A)-3' RNA + random hexamer capture.
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- Poly(A)-3' RNA + random hexamer capture.

**Samples status**
- Live Cells or Nuclei.
- Live Cells or Nuclei.
- PFA Fixed Cells or Nuclei. *FFPE sample prep protocol in development.
- Fixed and frozen Cells or Nuclei.
- Fixed and frozen Cells or Nuclei.
- Fixed and frozen Cells or Nuclei.

**Sample multiplexing**
- Officially supported by 10x: CellPlex (for 3' GEX only), multiplex up to 12 samples. *Additional cost required.
- Officially supported by 10x: CellPlex (for 3' GEX only), multiplex up to 12 samples. *Additional cost required.
- Officially supported by 10x: Multiplex probe set, multiplex kit for 4 or 16 samples. *Additional cost required.
- Built into Parse kit, multiplex up to 12 samples.
- Built into Parse kit, multiplex up to 48 samples.
- Built into Parse kit, multiplex up to 96 samples.

**Cells required**
- For capture of ~10,000 cells, need to load ~16,500 cells. For capture of ~30,000 cells, load ~49,500 cells.
- For capture of ~20,000 cells, need to load ~33,300 cells. For capture of ~60,000 cells, load ~115,000 cells.
- For capture of ~10,000 cells, need to load 16,500 cells. For ~128,000 capture, load 220,000,000.
- For ~10,000 cells capture kit, need to load about 35,000 cells. Can capture 20,000 cells with the same input.
- For ~100,000 cells capture kit, need to load about 350,000 cells.
- For ~1,000,000 cells capture kit, need to load about 3,500,000 cells.

**Accuracy of capture**
- Depends on accuracy of count and cell types.
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**Cost**
- Cost per cell: ~$0.23
- Cost per cell: ~$0.13
- Cost per cell: ~$0.24
- Cost per cell: ~$0.28
- Cost per cell: ~$0.11
- Cost per cell: ~$0.02

**Sequencing**
- 10,000 cells with 50,000 reads/cell = 500 million reads.
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- Depends on capture number and depth. 10,000 cells with 50,000 reads/cell = 500 million reads.
- Depends on capture number and depth. 10,000 cells with 50,000 reads/cell = 500 million reads.
- Depends on capture and depth. 100,000 cells with 50,000 reads/cell = 5 billion reads.
- Depends on capture and depth. 1,000,000 cells with 50,000 reads/cell = 50 billion reads.

**Additional cost**
- None. *Additional cost for multiplexing: CellPlex reagents and Multiplex Library prep. Feature barcode library construction $123/each (Total Seq and/or CellPlex).
- None. *Additional cost for multiplexing: CellPlex reagents and Multiplex Library prep.
- 10x Fixed RNA Sample Preparation kit. $370 for sample preparation kit, for up to 16 samples. *Additional cost for multiplexing: Multiplex Probe set.
- Parse Bio Fixation kit. $261 for fixation kit, for up to 4 samples.
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**Notes**
- Multiplet rate for: 10,000 capture is ~8%. 30,000 capture is ~24%. If super loading for ~30,000 capture, there is a higher chance of chip clog/failure.
- Multiplet rate for: 20,000 capture is ~8%. 60,000 capture is ~24%.
- Multiplet rate for: 10,000 capture is ~8%. 120,000 capture is ~6.4% for 16 probes multiplexed.
- Multiplet rate for: 10,000 capture is 2.3%.
- Multiplet rate for: 100,000 capture is 1.7%.
- Multiplet rate for: 1,000,000 capture is 3.2%.