Recommendations for Obtaining High Viable Cell Counts for Single Cell Sequencing

Heidi Monroe
April 14, 2020
Input Requirements for Single Cell Sequencing

Gene Expression-Immune Profiling-CNVA-ATAC

For high quality libraries:

- Cells should be in a single cell suspension with no clumping
- Cell suspensions should be free of debris
- Cells should have a minimum viability of 80%
- Concentration should be between 700-1200 cells/μL
Recommendations for High Cell Viability

- Gentle and slow pipetting using low retention, wide bore tips in lo bind tubes
- Proper centrifugation conditions (300rcf for most cell types)
- Wash and resuspend cells in 1X PBS (calcium and magnesium free)/0.04% BSA
- PBS/BSA can be substituted with some cell culture media
- Debris should be removed from cell suspensions
- Dead cell removal for viabilities under 70% (optional between 70-80%)
- Minimal cell handling with delivery to the GRC immediately after preparation
- Flow cytometry tends to overestimate cell counts
Single Cell QC at the GRC

- To preserve cell quality, all single cell sequencing projects are scheduled in advance
- Lowest acceptable viability will be discussed prior to submission
- Standard QC involves a cell count and viability check
- Initial QC on a test sample to determine success of your cell type/preparation
- QC on your experimental sample prior to loading on the 10X Chromium Controller
High cell viability will result in:

- Greater recovery of cells
- High fraction of reads in cells
- Cleaner data with lower background
3,000 Cell Target at 92% Viability

<table>
<thead>
<tr>
<th>Summary</th>
<th>Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>2,464</strong></td>
<td>Estimated Number of Cells</td>
</tr>
<tr>
<td><strong>44,293</strong></td>
<td>Mean Reads per Cell</td>
</tr>
<tr>
<td><strong>3,124</strong></td>
<td>Median Genes per Cell</td>
</tr>
</tbody>
</table>

### Sequencing

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Reads</td>
<td>109,138,133</td>
</tr>
<tr>
<td>Valid Barcodes</td>
<td>97.9%</td>
</tr>
<tr>
<td>Valid UMIs</td>
<td>100.0%</td>
</tr>
<tr>
<td>Sequencing Saturation</td>
<td>35.6%</td>
</tr>
<tr>
<td>Q30 Bases in Barcode</td>
<td>97.3%</td>
</tr>
<tr>
<td>Q30 Bases in RNA Read</td>
<td>87.6%</td>
</tr>
<tr>
<td>Q30 Bases in Sample Index</td>
<td>96.9%</td>
</tr>
<tr>
<td>Q30 Bases in UMI</td>
<td>96.8%</td>
</tr>
</tbody>
</table>

### Cells

- Barcode Rank Plot
- Estimated Number of Cells: 2,464
- Fraction Reads in Cells: 85.3%
- Mean Reads per Cell: 44,293
- Median Genes per Cell: 3,124
- Total Genes Detected: 17,131
- Median UMI Counts per Cell: 11,880
10,000 Cell Target at 37% Viability

**Estimated Number of Cells**
- **2,537**

**Mean Reads per Cell**
- **20,779**

**Median Genes per Cell**
- **903**

**Sequencing**
- **Number of Reads**: 52,717,555
- **Valid Barcodes**: 97.7%
- **Sequencing Saturation**: 40.1%
- **Q30 Bases in Barcode**: 97.5%
- **Q30 Bases in RNA Read**: 85.5%
- **Q30 Bases in Sample Index**: 97.1%
- **Q30 Bases in UMI**: 97.3%

**Cells**
- **100% Cells (1678-1678)**
- **UMI counts**
  - Estimated Number of Cells: 2,537
  - Fraction Reads in Cells: 39.6%
  - Mean Reads per Cell: 20,779
  - Median Genes per Cell: 903
  - Total Genes Detected: 24,831
  - Median UMI Counts per Cell: 1,018
10,000 Cell Target at 83% Viability

**Estimated Number of Cells**

| Estimated Number of Cells | 7,086 |

**Mean Reads per Cell**

| Mean Reads per Cell | 6,576 |

**Median Genes per Cell**

| Median Genes per Cell | 820 |

**Sequencing**

<table>
<thead>
<tr>
<th>Number of Reads</th>
<th>46,600,677</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Barcodes</td>
<td>97.6%</td>
</tr>
<tr>
<td>Sequencing Saturation</td>
<td>26.4%</td>
</tr>
<tr>
<td>Q30 Bases in Barcode</td>
<td>97.4%</td>
</tr>
<tr>
<td>Q30 Bases in RNA Read</td>
<td>86.4%</td>
</tr>
<tr>
<td>Q30 Bases in Sample Index</td>
<td>97.1%</td>
</tr>
<tr>
<td>Q30 Bases in UMI</td>
<td>97.3%</td>
</tr>
</tbody>
</table>

**Cells**

- **Estimated Number of Cells**: 7,086
- **Fraction Reads in Cells**: 85.1%
- **Mean Reads per Cell**: 6,576
- **Median Genes per Cell**: 820
- **Total Genes Detected**: 25,656
- **Median UMI Counts per Cell**: 1,671
Important Steps in Maintaining Cell Viability

- Gentle handling
- Buffers-PBS/0.04% BSA preferred
- Timing-Cells should be delivered quickly after preparation
- We offer dead cell removal and filtering
- The GRC is available to offer guidance and support along the way
  - Different cell types may have unique requirements
  - We offer multiple QC opportunities for optimizing cell preparation
  - We also have a Miltenyi GentleMACS Dissociator