SureCell ATAC-Seq
Library Preparation Kit

User Guide

Version 1.0.1
Bio-Rad Technical Support Department

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# Revision History

<table>
<thead>
<tr>
<th>Document</th>
<th>Date</th>
<th>Description of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>SureCell ATAC-Seq Library Preparation Kit User Guide DIR#10000106678 Ver C</td>
<td>May 2023</td>
<td>Incorporate SME edits, remove Omnition Analysis Software on the cover, update legal notices and trademark symbols, correct page numbers, fix variable entries in text and footer</td>
</tr>
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<td>March 2023</td>
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</tr>
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</tr>
</tbody>
</table>
Chapter 1 Introduction

Single-Cell ATAC-Seq (Assay for Transposase-Accessible Chromatin using sequencing) provides a novel method for mapping genome-wide chromatin accessibility for thousands of individual single cells. The simple one day workflow provides easy access to epigenetic information, including transcriptional dynamics from cell to cell. Four samples can be processed per ddSEQ™ cartridge per run.

Nuclei isolated from single cells are tagged using Tn5 transposase at regions of open chromatin in bulk. Amplification from tagged sites within individual droplets generates a library of fragments representative of the original open chromatin on a per cell basis. Barcoding within individual droplets enables the reconstruction of single cell data from the barcode sequences after sequencing.

The following graphic shows sequencing adapters in red and green.
Key Features and Applications

Features
- In-depth investigations into DNA accessibility for various applications
- Scalable to largest studies
- Single-day workflow with minimal hands-on time
- Sensitive for characterizing specific cell populations

Applications
- Identify cell types in a heterogeneous cell population using single-cell ATAC profiles
- Investigate cell-to-cell regulatory variation
- Understand epigenetic structure
- Quantify changes leading to gene expression and repression
- Provide insights into gene regulatory mechanisms underlying development, cancer heterogeneity, and drug response
- Validate scRNA-Seq studies

Data analysis is conducted using Omnition Analysis Software from Bio-Rad™. Omnition takes raw sequencing files, processes the data, and provides QC metrics, experiment report, and output files.

Additional Resources

You can find support for this protocol at bio-rad.com, including background information, brochures, additional documentation, downloads, and compatible products.
## Chapter 2 Required Consumables and Equipment

Table 1. SureCell ATAC-Seq Library Prep Kit contents

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Storage requirement</th>
<th>Associated document catalog number</th>
<th>Description</th>
<th>Qty (each)</th>
<th>Cap color</th>
</tr>
</thead>
<tbody>
<tr>
<td>12009359</td>
<td>Room temperature</td>
<td>12008720</td>
<td>SureCell dSeq M Cartridge</td>
<td>2</td>
<td>N/A</td>
</tr>
<tr>
<td>12009358</td>
<td>4° C</td>
<td>12008740</td>
<td>Priming Solution</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>4° C</td>
<td>12008761</td>
<td>Droplet Disruptor</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>4° C</td>
<td>12008762</td>
<td>Encapsulation Oil</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>4° C</td>
<td>12008754</td>
<td>ATAC Barcode Mix</td>
<td>1</td>
<td>Blue</td>
</tr>
<tr>
<td>12009357</td>
<td>–20° C</td>
<td>12008781</td>
<td>ATAC Barcode Buffer</td>
<td>1</td>
<td>Blue</td>
</tr>
<tr>
<td></td>
<td>–20° C</td>
<td>12008782</td>
<td>ATAC PCR Supermix</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>–20° C</td>
<td>12008784</td>
<td>ATAC Enhancer Enzyme</td>
<td>1</td>
<td>Red</td>
</tr>
<tr>
<td></td>
<td>–20° C</td>
<td>16005990</td>
<td>ATAC Primer Mix</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>–20° C</td>
<td>12008775</td>
<td>ATAC Tagmentation Buffer</td>
<td>1</td>
<td>Clear</td>
</tr>
<tr>
<td></td>
<td>–20° C</td>
<td>12008776</td>
<td>ATAC Tagmentation Enzyme</td>
<td>1</td>
<td>Clear</td>
</tr>
</tbody>
</table>
### Table 1. SureCell ATAC-Seq Library Prep Kit contents, continued

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Storage requirement</th>
<th>Associated document catalog number</th>
<th>Description</th>
<th>Qty (each)</th>
<th>Cap color</th>
</tr>
</thead>
<tbody>
<tr>
<td>–20°C</td>
<td></td>
<td>12008778</td>
<td>ATAC Enzyme Buffer</td>
<td>1</td>
<td>Red</td>
</tr>
<tr>
<td>–20°C</td>
<td></td>
<td>12008779</td>
<td>ATAC Enzyme</td>
<td>1</td>
<td>Red</td>
</tr>
<tr>
<td>–20°C</td>
<td></td>
<td>16005986</td>
<td>ATAC Sequencing Primer</td>
<td>1</td>
<td>Clear</td>
</tr>
</tbody>
</table>

### Table 2. Verified apparatus and equipment (not provided)

<table>
<thead>
<tr>
<th>Apparatus/equipment</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent 2100 Bioanalyzer</td>
<td>G2939B</td>
<td>Agilent</td>
</tr>
<tr>
<td>TC20 Automated Cell Counter</td>
<td>1450102</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>PX1 PCR Plate Sealer</td>
<td>1814000</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>ddSEQ Single Cell Isolator</td>
<td>12004336</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>C1000 Thermal Cycler with deepwell module or PTC Tempo Thermal Cycler (deepwell)</td>
<td>1851197 12015392</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Eppendorf ThermoMixer C</td>
<td>5382000023</td>
<td>Eppendorf</td>
</tr>
<tr>
<td>Centrifuge 5418R (rotor of 1.5 ml tube required)</td>
<td>540100137</td>
<td>Eppendorf</td>
</tr>
<tr>
<td>Centrifuge 5430 (rotor of MTP and 15 ml conical tube required)</td>
<td>022620509</td>
<td>Eppendorf</td>
</tr>
<tr>
<td>Magnetic Stand 96</td>
<td>AM10027</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>DynaMag-96 Side Skirted Magnet</td>
<td>12027</td>
<td>ThermoFisher Scientific</td>
</tr>
</tbody>
</table>
### Table 3. Verified reagents (not provided)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Digitonin (20 mg/ml or 2%)</td>
<td>G9441</td>
<td>Promega</td>
</tr>
<tr>
<td>Bovine serum albumin (cell culture grade)</td>
<td>A9418-5G</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Tris-HCL pH 7.4, 1 M</td>
<td>T2194-100 ml</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Resuspension buffer (10 mM Tris-HCl, pH 8.0, no EDTA), DNase free</td>
<td>T1173</td>
<td>Teknova</td>
</tr>
<tr>
<td>Tween 20, 10% solution</td>
<td>T0710</td>
<td>Teknova</td>
</tr>
<tr>
<td>MgCl₂, 1M</td>
<td>AM9530G</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>NaCl, 5 M</td>
<td>AM9759</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>PBS, pH 7.4</td>
<td>10010023</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>NP-40 Surfact-Amps Detergent Solution</td>
<td>28324</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>Ethanol, 80% (make fresh)</td>
<td>N/A</td>
<td>Any</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>N/A</td>
<td>Any</td>
</tr>
</tbody>
</table>

### Table 4. Verified consumables (not provided)

<table>
<thead>
<tr>
<th>Consumable</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agencourt AMPure XP</td>
<td>A63880</td>
<td>Beckman Coulter</td>
</tr>
<tr>
<td>ddPCR 96-well plates</td>
<td>12001925</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>PCR Plate Heat Seal with pierceable foil</td>
<td>1814040</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Optical Flat 8-cap strips</td>
<td>TCS0803</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Microseal F PCR Plate Sea with pierceable foil</td>
<td>MSF1001</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>Cell strainer (appropriate pore size)</td>
<td>N/A</td>
<td>Any</td>
</tr>
</tbody>
</table>
# Chapter 2 Required Consumables and Equipment

## Table 5. Commercial kits

<table>
<thead>
<tr>
<th>Commercial kit</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent High Sensitivity DNA Kit</td>
<td>5067-4626</td>
<td>Agilent</td>
</tr>
<tr>
<td>TC20 Cell Counting Kit, with trypan blue</td>
<td>14500003</td>
<td>Bio-Rad</td>
</tr>
<tr>
<td>SureCell ddSEQ Index Kit</td>
<td>12009360</td>
<td>Bio-Rad</td>
</tr>
</tbody>
</table>
# Chapter 3 SureCell ATAC-Seq Workflow

The following table provides a summary of workflow steps for this protocol.

<table>
<thead>
<tr>
<th>Step</th>
<th>Workflow procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Prepare, count, and assess viability of single-cell suspension</td>
</tr>
<tr>
<td>2</td>
<td>Use OMNI-ATAC tagmentation OR Whole-cell tagmentation</td>
</tr>
<tr>
<td>3</td>
<td>Prepare barcode and enzyme Suspension Mixes</td>
</tr>
<tr>
<td>4</td>
<td>Partition the nuclei</td>
</tr>
</tbody>
</table>
| 5    | Perform ddSEQ barcoding and sample index PCR  
*Pause point: Store at 4°C for 72 hrs, or store at −20°C long-term.* |
| 6    | Perform emulsion breakage and first PCR cleanup  
*Pause point: Store at 4°C for 72 hrs, or store at −20°C long-term.* |
| 7    | Perform second PCR  
*Pause point: Store at 4°C for 72 hrs, or store at −20°C long-term.* |
| 8    | Perform second PCR cleanup  
*Pause point: Store at 4°C for 72 hrs, or store at −20°C long-term.* |
| 9    | Perform Library QC and sequencing |
| 10   | Perform data analysis |
OMNI-ATAC Tagmentation Protocol vs. Whole-Cell Tagmentation Protocol

Isolation of nuclei from mammalian cells requires the use of detergents to disrupt the integrity of the cellular membrane. There are two protocols to choose from, depending on cell type, as follows:

- The OMNI-ATAC tagmentation protocol has been optimized for cell lines and uses a combination of harsh detergents.
- The Whole-Cell tagmentation protocol uses a combination of gentle detergents and is more suitable for fragile cells, such as blood cells and PBMCs. This protocol is not ideal for cell lines and other cell types that are more resistant to lysis.

Further optimization might be necessary to determine which tagmentation protocol is appropriate for the individual cell type or cell source depending on specific need. When working with a new cell/tissue type, it is recommended to optimize this portion of the protocol.

Table 6. Protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Cells verified by the current protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONMI-ATAC</td>
<td>HEK-293, K562, NIH-3T3, GM12878, mouse embryonic stem cells</td>
</tr>
<tr>
<td>Whole-cell</td>
<td>Peripheral blood mononuclear cells (PBMCs), bone marrow mononuclear cells</td>
</tr>
</tbody>
</table>
Key Workflow Considerations

Note the following:

- A single-cell suspension is required to start the protocol. Depending on sample type, additional optimizations might be needed for cell dissociation. See Supporting Information for more details.

- A minimum of 150,000 cells are required to start the workflow.

- Take extra precautions to minimize cell loss when performing cell preparation steps in the Cell Lysis and Tagmentation section. Cell loss at this step reduces cell numbers in the final library.

- The emulsion droplets must, without exception, be amplified in the recommended 96-well Bio-Rad plate (Bio-Rad ddPCR 96-Well Plate) and sealed with a pierceable foil PCR Plate Heat Seal (Bio-Rad #1814040) using a PX1 PCR Plate Sealer. You can perform all other steps using either the suggested PCR plate and seals or 8-strip tubes and associated caps.

- All sample ports in a cartridge must be loaded with enzyme and barcode reaction mixtures. If fewer than four samples are available, replace cells with water in the enzyme reaction mixture.

- A custom sequencing primer is provided for Read 1. You must use the custom primer in sequencing to obtain the correct cell barcode information.

**Important:** You cannot combine the custom primer with other standard Read 1 sequencing primers, as it results in poor cluster quality and loss of reads.
Cell Preparation Guidelines

Note the following:

- **Fully Dissociated Single Cells**
  
  This protocol requires cells in single-cell suspension. Cell aggregates or doublets present in the suspension significantly increase the probability of doublets or multiplets during single-cell isolation on the ddSEQ Single Cell Isolator. Dissociation, straining, washing, and quantitation might require additional optimization and specific techniques appropriate to the individual cell type or cell source.

- **Cell Viability**
  
  The presence of dead or damaged cells can reduce recovery and impact performance due to ambient DNA and cellular debris. Bio-Rad recommends starting with cells that have viability above 80%. In order to assess viability, use the Bio-Rad TC20 Automated Cell Counter (Bio-Rad #1450102) along with 0.4% trypan blue (Bio-Rad #1450022). Pipette mix trypan blue and cells at equal volumes before loading 10 µl on a cell counting slide. If viability is less than 80%, treat cells with DNase I to digest ambient DNA. For more information, see S1, Working with Low Viability Cells in Appendix A, Supporting Information.

- **Centrifugation**
  
  The sedimentation portion of this protocol requires pelleting of cells by centrifugation to remove supernatant. When placing tubes in the centrifuge during a pelleting step, note the orientation of the tube relative to the center of the centrifuge. The cells should pellet on the side of the tube farthest from the center of the centrifuge. When removing supernatant, use multiple pipeting steps. First, use a 200 or 1,000 µl pipet to remove all the volume except 20 µl. Then, remove the remaining liquid using a 20 µl pipet, taking care not to disturb the pellet.

- **Cell Counting**
  
  Accurate cell counting is critical to achieve target cell throughput and avoid cell multiplets. Bio-Rad has validated and recommended the TC20 Automated Cell Counter (Bio-Rad #1450102) for counting of cells with a round shape and a cell diameter between 6 and 50 µm. Size-based gating for automated counters or manual count might be required to avoid counting cell debris. Both viable and non-viable cells should be included in total cell count.
Best Practices

Bio-Rad recommends the following:

- Before starting, read through the complete protocol to become familiar with the workflow.
- Make sure all required equipment is properly installed and functioning before performing the workflow.
- Ensure all required materials and reagents are available before starting the protocol.
- Pause the protocol only at designated stopping points.
- Use separate areas for appropriate steps. We recommend that you perform the cell culture steps in a designated cell culture room and hood, and that pre-PCR and post-PCR steps be conducted in their respective zones.
- Avoid cross-contamination by changing pipet tips between each sample and each reaction.
- Use only specified plastic consumables when handling emulsions.
Chapter 4 Protocol

Buffer Preparation

Before starting cell preparation and tagmentation, prepare fresh buffers according to the tables in this section.

Note the following:

- If using the OMNI-ATAC protocol, prepare the buffers in Table 7 through Table 10.
- If using the Whole-Cell protocol, prepare only the buffer in Table 7.

Important: Keep all prepared buffers on ice.

Table 7. 1x PBS + 0.1% BSA (100 µl volume sufficient for 4 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration</th>
<th>Final concentration</th>
<th>Required volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS, pH 7.4</td>
<td>1x</td>
<td>1x</td>
<td>100 µl</td>
</tr>
<tr>
<td>BSA (powder)</td>
<td>N/A</td>
<td>0.1% (w/v)</td>
<td>0.1 g</td>
</tr>
</tbody>
</table>

Table 8. ATAC-RSB (10 ml volume sufficient for 4 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration, mM</th>
<th>Final concentration, mM</th>
<th>Required volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclease-free water</td>
<td>N/A</td>
<td>N/A</td>
<td>9,850</td>
</tr>
<tr>
<td>Tri-HCl, pH 7.4</td>
<td>1,000</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>NaCl</td>
<td>5,000</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1,000</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>10,000</td>
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</tbody>
</table>
### Table 9. ATAC-Lysis buffer (300 µl volume sufficient for 4 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration, mM</th>
<th>Final concentration, mM</th>
<th>Required volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC-RSB (Refer to Table 6)</td>
<td>N/A</td>
<td>N/A</td>
<td>291</td>
</tr>
<tr>
<td>NP-40</td>
<td>10</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>Tween 20</td>
<td>10</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>Digitonin*</td>
<td>1</td>
<td>0.01</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>300</td>
</tr>
</tbody>
</table>

*Digitonin is supplied at 2% in DMSO. Dilute 1:1 with nuclease-free water to make 1% stock solution and keep at –20°C (good for as many as 5 freeze thaw cycles).

### Table 10. ATAC Tween buffer (5 ml volume sufficient for 4 reactions)

<table>
<thead>
<tr>
<th>Component</th>
<th>Stock concentration, mM</th>
<th>Final concentration, mM</th>
<th>Required volume, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC-RSB (Table 6)</td>
<td>N/A</td>
<td>N/A</td>
<td>4,950</td>
</tr>
<tr>
<td>Tween 20</td>
<td>10 mM</td>
<td>0.1 mM</td>
<td>50</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td></td>
<td>5,000</td>
</tr>
</tbody>
</table>

### Cell Preparation

#### Guidelines

To start this section, you must first put the cells into solution with user-defined and optimized protocols.

Methods and materials to put K562 and NIH-3T3 cells in suspension are provided in Section S2 in [Appendix A, Supporting Information](#).

For all other cell types, including adherent or suspension cell cultures, tissues, or FACS sorted cells, you must implement your own protocols, as well as supply necessary materials to create cell suspensions.

Note the following:

- To obtain an estimate of the total number, count the cells after they are in suspension.
- Follow instructions to minimize cell loss during wash steps for samples with <1 million cells.
- Keep cells and nuclei on ice at all times when not being handled.
Cell Washing and Filtering

Complete the following steps:

1. For every cell type that will be used, chill the cell strainers on ice in their collection tubes.
   The appropriate cell strainer varies according to the cell type. For K562 and NIH-3T3 cells, 35 µm strainers are adequate.

2. In a 15 ml conical tube, centrifuge cells at 300x g for 3 min.
   If starting with <1 million cells, spin the cells in a 1.5 ml microcentrifuge tube. A chilled centrifuge is not required.

3. Discard the supernatant. Do not disturb the cell pellet. Remove all but approximately 1 ml using a serological pipet. Remove the remaining liquid using a P1000 pipet.
   If starting with <1 million cells, use a P1000 only to remove the liquid.

4. Spin down and resuspend the cells in 1 ml of cold 1x PBS + 0.1% BSA. With a P1000 pipette, break the cell clumps by pipet-mixing 10 to 15x.
   Skip Step 5 if you are starting with <1 million cells.

5. Add 9 ml of cold 1x PBS + 0.1% BSA and invert the 15 ml conical tube 3–5x.

6. Repeat Steps 2 to 5.

7. Perform a cell count measurement to verify how much buffer is needed in the next resuspension step:
   a. Pulse vortex cells at medium speed 3x, 3 sec per pulse.
   b. Using a P20 pipet, remove 10 µl of the cell suspension and load onto a Bio-Rad TC20 counting slide.
   c. Insert the slide into the TC20 Cell Counter to obtain count.

8. To obtain an approximate cell concentration of 1,000 cells/µl, spin down and resuspend the cell pellet in an appropriate volume of cold 1x PBS + 0.1% BSA.
   A range of 500–5,000 cells/µl is acceptable. Pipet-mix cells 20x.
   **Note:** For cells that will be processed through the Whole Cell Protocol, having >4,000 cells/µl, if possible, will streamline washes downstream.

9. Filter cells through the prechilled cell strainers on ice to remove residual cell aggregates.
   **Tip:** To account for dead volume in the cell strainer, filter at least 250 µl.
Cell Viability and Cell Count Measurements

Complete the following steps:

1. Pulse vortex cells at medium speed in the strainer tube 3x, 3 sec per pulse.

2. Immediately after mixing, use a P20 pipet to aspirate 10 µl from the middle of the cell suspension and transfer to a 1.5 ml tube.

3. Add 10 µl of 0.4% trypan blue and mix well by pipeting 10x.

4. Immediately pipet the stained cell mix onto Bio-Rad TC20 counting slide.

5. Insert the slide into TC20 Cell Counter to initiate measurement.

6. Record count and check that viability is at least 80% before proceeding to the next step.

   Note: If viability is <80%, refer to Section S1, Working with Low Viability Cells in Appendix A, Supporting Information.

7. To perform cell count measurements as follows, without adding trypan blue:
   a. Pulse vortex cells at medium speed in the strainer tube 3x, 3 sec per pulse.
   b. Using a P20 pipet, remove 10 µl from the middle of the cell suspension and load onto a Bio-Rad TC20 Cell Counter counting slide.
   c. Insert the slide into the TC20 Cell Counter to obtain counts.
   d. Repeat steps a–c 3x for a total of 4 counts.
   e. Take the average of all 4 counts to determine accurate cell count.
Cell Lysis and Tagmentation

Table 11. Advance preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Part number</th>
<th>Storage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Tagmentation Buffer</td>
<td>12008775</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. When thawed, keep on ice.</td>
</tr>
<tr>
<td>ATAC Tagmentation Enzyme</td>
<td>12008776</td>
<td>–20°C</td>
<td>Flick the tube to mix, then centrifuge briefly. Keep on ice.</td>
</tr>
</tbody>
</table>

OMNI-ATAC Protocol

Complete the following steps:

1. Make sure the microcentrifuge is cooled to 4°C and the thermostirxer pre-heated to 37°C before starting the protocol.

2. For each sample, transfer 150,000 of the filtered cells prepared in Cell Preparation on page 22 to a 1.5 ml Eppendorf tube. Before transferring, ensure that cells are in suspension by pulse vortexing 3x for 3–5 sec each at medium high speed.

3. Place tubes into the cooled microcentrifuge. Place all tubes with the hinge oriented upward. Spin down at 500 RCF for 5 min at 4°C.

4. Remove all supernatant, taking care not to disturb the cell pellet. If the pellet is difficult to visualize, assume it is located on the same side as the tube hinge. Keep the cell pellet on ice.

   **Important:** It is important to minimize cell loss at this step to ensure enough cells are available for the final reaction. Avoid disturbing the cell pellet; do not use a large volume pipet (for example, P1000) to remove supernatant. Use a P100 or P200 pipet to aspirate supernatant, leaving approximately 20–30 μl. Switch to a P20 pipet to remove the remaining liquid.

5. Add 50 μl of cold ATAC-Lysis buffer and pipet mix at least 15x or until the cell pellet goes back into suspension.

6. Incubate on ice for 3 min.

7. Add 1 ml of cold ATAC-Tween Buffer to each tube. Invert the tubes 3x to mix.

8. Spin down at 500 RCF for 10 min at 4°C in a fixed angle centrifuge.
9. Once centrifugation from Step 8 is finished, remove supernatant from the tube, taking care not to disturb the nuclei pellet. Keep the nuclei pellet on ice.

   **Important:** It is essential to minimize nuclei loss at this step to ensure enough nuclei are available for the final reaction. Use a P200 or P1000 pipet to remove supernatant, leaving approximately 40 μl. Switch to a P20 pipet to remove the remaining liquid.

10. Resuspend the nuclei pellet in 20 μl cold 1x PBS + 0.1% BSA. Pipet mix 10–15x or until pellet goes back into suspension. Keep on ice.

11. Count nuclei in the TC20 Automated Cell Counter.
   a. Immediately take 2.5 μl nuclei from middle of the suspension and transfer to a 1.5 ml tube.
   b. Add 7.5 μl of 1x PBS + 0.1% BSA and pipet mix 15x to thoroughly mix the diluted nuclei.
   c. Add 10 μl of 0.4% trypan blue to the diluted nuclei and mix well by pipeting 10x.
   d. Immediately pipet the stained nuclei mix onto the Bio-Rad TC20 counting slide.
   e. Insert the slide into TC20 Cell Counter to initiate measurement.
   f. Record count and ensure that viability at this step is <10% before moving to the next step.
   g. Pulse vortex nuclei suspension at medium speed 3x, 3 sec per pulse.

   **Important:** Take nuclei count measurement immediately after adding trypan blue and mixing. To improve accuracy, do not let nuclei sit in trypan blue longer than 30 sec. Make sure to include 4x dilution factor in calculation of final nuclei concentration.

12. Prepare the required number of OMNI-ATAC Tagmentation Mixes according to **Table 12**.

**Table 12. OMNI-ATAC tagmentation mix**

<table>
<thead>
<tr>
<th>Component</th>
<th>Required volume, μl (1 rxn + 10% excess)</th>
<th>Required volume, μl (4 rxn + 10% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Tagmentation Buffer</td>
<td>11.00</td>
<td>44.00</td>
</tr>
<tr>
<td>ATAC Tagmentation Enzyme</td>
<td>2.75</td>
<td>11.00</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>23.10</td>
<td>92.40</td>
</tr>
<tr>
<td>TOTAL</td>
<td>36.85</td>
<td>147.40</td>
</tr>
</tbody>
</table>

13. Mix by pulse vortexing the OMNI-ATAC Tagmentation Mix and keep on ice until you are ready to use it. For every sample, dispense 33.5 μl into a 1.5 ml Eppendorf Lo-Bind tube.
14. Add 60,000 nuclei to the pre-aliquoted OMNI-ATAC Tagmentation Mix followed by 1x PBS + 0.1% BSA to a final volume of 50 µl. Use the following equations to calculate the volume of nuclei and 1x PBS + 0.1% BSA required for each reaction.

- Volume of nuclei suspension required, µl = 60,000 nuclei/nuclei concentration determined from count (nuclei/µl)
- Volume of 1x PBS + 0.1% BSA required, µl = 16.5 µl – volume of nuclei suspension required (µl)

**Important:** Before adding nuclei to the OMNI-ATAC Tagmentation Mix, pulse vortex the nuclei suspension 3x for 3–5 sec each at medium high speed. This ensures that the nuclei are evenly distributed and that the correct number is added to the tagmentation reaction. In order to maintain correct concentrations of components in the tagmentation reaction, do not add more than 16.5 µl total volume of nuclei and 1x PBS + 0.1% BSA in the final reaction.

15. Set a P100 pipet to 30 µl and mix the Tagmentation Reaction by pipeting up and down 10x to mix.

16. Incubate at 37° C for 30 min in a ThermoMixer with 500 rpm mixing.

17. Transfer the tagmentation reaction onto ice after the 37° C incubation. Proceed immediately to the first step in Prepare Solutions for Droplet Encapsulation on page 30.
**Whole Cell Protocol**

Complete the following steps:

1. Make sure the microcentrifuge is cooled to 4°C and the thermomixer pre-heated to 37°C before starting the protocol.

2. Prepare the required number of Whole-Cell Tagmentation Mixes according to Table 13. Vortex to mix the solution and keep on ice.

### Table 13. Whole-Cell Tagmentation Mix

<table>
<thead>
<tr>
<th>Component</th>
<th>Required volume, µl (1 rxn + 10% excess)</th>
<th>Required volume, µl (4 rxn + 10% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Tagmentation Buffer</td>
<td>11.00</td>
<td>44.00</td>
</tr>
<tr>
<td>ATAC Tagmentation Enzyme</td>
<td>2.75</td>
<td>11.00</td>
</tr>
<tr>
<td>0.5% Digitonin</td>
<td>1.10</td>
<td>4.40</td>
</tr>
<tr>
<td>5% Tween 20</td>
<td>1.10</td>
<td>4.40</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>20.90</td>
<td>83.60</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>36.85</td>
<td>147.40</td>
</tr>
</tbody>
</table>

3. Ensure that filtered cells prepared in Cell Preparation on page 22 are in suspension by pulse vortexing at medium speed 3x for 3 sec each.

4. If the concentration of cells obtained in Cell Viability and Cell Count Measurements on page 24 is >3,600 cells/µl, skip to step 10. If the concentration is <3,600 cells/µl, proceed to step 5.

5. Transfer 150,000 cells to a 1.5 ml Eppendorf tube.

6. Place tubes into the cooled microcentrifuge. Place all tubes with the hinge oriented upward. Spin down at 500 RCF for 5 min at 4°C.

7. Remove all supernatant, taking care not to disturb the cell pellet. If pellet is difficult to visualize, assume it is located on the same side as the tube hinge. Keep cell pellet on ice.

   **Important:** It is important to minimize cell loss at this step to ensure enough cells are available for the final reaction. Avoid disturbing the cell pellet; do not use large volume pipet (for example, P1000) to remove supernatant. Use a P100 or P200 pipet to aspirate supernatant, leaving approximately 20–30 µl. Switch to a P20 pipet to remove the remaining liquid.
8. Resuspend cell pellet in 20 µl cold 1x PBS + 0.1% BSA. Pipet mix 10–15x or until cell pellet goes back into suspension. Keep on ice.

   a. Pulse vortex cell suspension at medium speed 3x, 3 sec per pulse.
   b. Immediately take 2.5 µl cells from middle of the suspension and transfer to a 1.5 ml tube.
   c. Add 7.5 µl of 1x PBS + 0.1% BSA and pipet mix 15x to thoroughly mix the diluted cells.
   d. Add 10 µl of 0.4% trypan blue to the diluted cells and mix well by pipeting 10x.
   e. Immediately pipet the stained cell mix onto the Bio-Rad TC20 counting slide.
   f. Insert the slide into TC20 Cell Counter to initiate measurement.
   g. Record count and ensure that viability is still > 80% as measured in Cell Preparation on page 22.

   **Tip:** Take cell count measurement immediately after adding trypan blue and mixing. To improve accuracy, do not let cells sit in trypan blue longer than 30 sec.

   **Important:** Make sure to include a 4x dilution factor in calculation of final cell concentration.

10. Mix by vortexing the Whole-Cell Tagmentation Mix and dispense 33.5 µl into a 1.5 ml Eppendorf LoBind tube.

11. Add 60,000 cells to the pre- aliquoted Whole-Cell Tagmentation Mix followed by 1x PBS + 0.1% BSA to final volume of 50 µl. Use the following equations to calculate the volume of cells and 1x PBS + 0.1% BSA required for each reaction.

   - Volume of cell suspension required, µl = 60,000 cells/cell concentration determined from count (cells/µl)
   - Volume of 1x PBS + 0.1% BSA required, µl = 16.5 µl – volume of cell suspension required (µl)

   **Important:** Before adding the cells to the Whole-Cell Tagmentation Mix, pulse vortex the cell suspension 3x for 3–5 sec each at medium high speed. This ensures that the cells are evenly distributed and that the correct number is added to the tagmentation reaction. In order to maintain correct concentrations of components in the tagmentation reaction, do not add more than 16.5 µl total volume of cells and 1x PBS + 0.1% BSA in the final reaction.

12. Set a P100 pipet to 30 µl and mix the Tagmentation Reaction by pipeting up and down 10x to mix.

13. Incubate at 37°C for 30 min in a ThermoMixer with 500 rpm mixing.

14. Transfer the tagmentation reaction on to ice after the 37°C incubation. Proceed immediately to the first step in Prepare Solutions for Droplet Encapsulation on page 30.
## Prepare Solutions for Droplet Encapsulation

### Table 14. Advance preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Cap color</th>
<th>Catalog number</th>
<th>Storage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Enzyme Buffer</td>
<td>Red</td>
<td>12008778</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. When thawed, keep on ice.</td>
</tr>
<tr>
<td>ATAC Enzyme</td>
<td>Red</td>
<td>12008779</td>
<td>–20°C</td>
<td>Flick the tube to mix, then centrifuge briefly. Keep on ice.</td>
</tr>
<tr>
<td>Enhancer Enzyme</td>
<td>Red</td>
<td>12008784</td>
<td>–20°C</td>
<td>Flick the tube to mix, then centrifuge briefly. Keep on ice.</td>
</tr>
<tr>
<td>ATAC Barcode Buffer</td>
<td>Blue</td>
<td>12008781</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. Once thawed, keep on ice.</td>
</tr>
<tr>
<td>ATAC Barcode Mix</td>
<td>Blue</td>
<td>12008754</td>
<td>4°C</td>
<td>Centrifuge briefly, then vortex vigorously to mix. Keep on ice.</td>
</tr>
<tr>
<td>SureCell ddSEQ Index Kit</td>
<td></td>
<td>12009360</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. Once thawed, keep on ice.</td>
</tr>
</tbody>
</table>
Prepare Barcode Suspension Mix

Complete the following steps:

1. Prepare the Barcode Reaction Mix according to **Table 15**. Thaw and keep all components and mixes on ice. Vortex all components immediately before addition. Make sure to also vortex the final mix after all components have been added.

   **Important**: Do not spin down the ATAC Barcode Mix after vortexing. This creates a concentration gradient affecting amount of barcode input.

   **Tip**: Pulse vortex the completed Barcode Reaction Mix 3x with 5 sec each at medium speed to thoroughly mix the solution.

**Table 15. Barcode Reaction Mix**

<table>
<thead>
<tr>
<th>Component</th>
<th>Catalog number</th>
<th>Required volume, µl (4 rxn + 10% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Barcode Buffer</td>
<td>12008781</td>
<td>33.00</td>
</tr>
<tr>
<td>ATAC Barcode Mix</td>
<td>12008754</td>
<td>55.00</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td><strong>88.00</strong></td>
</tr>
</tbody>
</table>

2. Dispense 20 µl of the Barcode Reaction Mix into alternating wells of 8-well PCR strip or 96-well PCR plate (A1, C1, E1, and G1). Leave the filled strip or plate in a cold block on ice.

   This layout facilitates cartridge loading in [Droplet Encapsulation on page 34](#).

3. To those same wells, add 5 µl of SureCell ddSEQ Sample Index, one per well, selected from 24 available indices.

   **Tip**: Use a different index for each well and record the index name for each sample. This information will be required when setting up the sequencing run.

4. Centrifuge briefly to bring contents to the bottom of the tube. The completed Barcode Suspension Mix should have a total volume of 25 µl. Keep the mix on ice.
Prepare Enzyme Suspension Mix

Complete the following steps:

1. Prepare the Enzyme Reaction Mix according to Table 16. Thaw and keep all components and mixes on ice. Vortex all components before preparing the mixes. Make sure to also vortex the final mix after all components have been added.

   **Tip:** Pulse vortex the completed Enzyme Reaction Mix 3x for 3 sec each at medium speed to thoroughly mix the solution.

Table 16. Enzyme Reaction Mix

<table>
<thead>
<tr>
<th>Component</th>
<th>Catalog number</th>
<th>Required volume, (\mu l) (4 rxn + 20% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC Enzyme Buffer</td>
<td>12008775</td>
<td>60.00</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>N/A</td>
<td>7.80</td>
</tr>
<tr>
<td>ATAC Enzyme</td>
<td>12008779</td>
<td>9.0</td>
</tr>
<tr>
<td>Enhancer Enzyme</td>
<td>12008784</td>
<td>4.80</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>81.60</td>
</tr>
</tbody>
</table>

2. Dispense 17 \(\mu l\) of the Enzyme Reaction Mix into alternating wells of 8-well PCR strip or 96-well PCR plate (B1, D1, F1 and H1). Leave the filled tube strip or plate in a cold block on ice.
3. To those same wells (B1, D1, F1 and H1), add the required volume of tagmented nuclei and water to obtain the desired cell throughput per sample. Refer to Table 17 for volumes required.

Ensure the tagmented nuclei are suspended and homogenous before adding to the Enzyme Reaction Mix. Using a P100 pipet set to 30 µl, pipet the tagmented nuclei up and down 10x and immediately aspirate from the center to remove required volume.

**Note:** Targeted cell throughput is an estimate. Actual cell throughput might vary depending on variability in cell counts, cell viability, and differential loss during upstream tagmentation.

### Table 17. Nuclei and water input volumes per sample

<table>
<thead>
<tr>
<th>Targeted cell throughput, cells</th>
<th>Tagmented nuclei, µl (from OMNI-ATAC Protocol or Whole Cell Protocol)</th>
<th>Nuclease-free water, µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty well</td>
<td>N/A</td>
<td>8.0</td>
</tr>
<tr>
<td>≤ 500</td>
<td>0.64 *</td>
<td>7.36 *</td>
</tr>
<tr>
<td>501 to 1,000</td>
<td>1.56</td>
<td>6.44</td>
</tr>
<tr>
<td>1,001 to 3,000</td>
<td>4.69</td>
<td>3.31</td>
</tr>
<tr>
<td>3,001 to 5,000</td>
<td>8.00</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* Pipeting 0.64 µl might result in error. Prior dilution of the tagmented nuclei is recommended. Dilute the tagmented nuclei by adding 2 µl of the tagmentation reaction to 6 µl of water and mix by pipeting. After mixing, add 2.56 µl of the diluted tagmented nuclei and 5.44 µl of nuclease-free water to the reaction per sample.

4. Set a P20 multichannel pipet to 20 µl and pipet up and down 5x to mix the completed Enzyme Suspension Mix. Leave the mix on ice and immediately proceed to Droplet Encapsulation on page 34.
**Droplet Encapsulation**

**Guidelines**

Note the following:

- Make sure the PX1 PCR Plate Sealer is installed to manufacturer instructions. Set the plate temperature to 180°C and the time to 5 sec.
- Make sure the ddSEQ Single-Cell Isolator is installed according to manufacturer instructions and the power indicator is lit.
- Use Rainin pipets and corresponding tips to load the cartridge. Use of other tips can negatively affect ddSEQ cartridge performance.
- Make sure that the ddSEQ cartridge is correctly inserted into the cartridge holder when loading reagents.
- ddSEQ cartridges are one-time use only. Dispose of cartridge after use applying proper laboratory protocol.
- ddSEQ Priming Solution and ddSEQ Encapsulation Oil should equilibrate to room temperature before use.
Best Practices: Loading Cartridges

**Important:** All sample chambers on the cartridge must be loaded before inserting the cartridge into the ddSEQ instrument. Confirm that the correct number of samples have been prepared in *Droplet Encapsulation on page 34* to ensure that no sample wells are left empty.

Bio-Rad recommends the following:

- Vortex or pipet mix the Barcode Suspension Mix and Enzyme Suspension Mix immediately before loading onto the cartridge.
- Press the pipet plunger only to the first stop when loading the cartridge with Barcode Mix and Enzyme Mix to avoid bubbles.
- Aspirate and dispense Encapsulation Oil slowly due to the viscosity of the solution.

Best Practices: Droplet Handling

Bio-Rad recommends the following:

- After droplet generation, immediately transfer droplets to the recommended plate (Bio-Rad ddPCR 96-well plate) chilled on ice.
- Slowly (3–5 sec count) aspirate the droplets and slowly dispense (3–5 sec count) the droplets down the side of the 96-well plate.
- When droplets are loaded onto a plate, keep plate chilled on ice when transferring to a thermal cycler.
- Avoid static generation when handling encapsulated samples:
  - Work in a clear, static-free area.
  - Do not use latex gloves when making or handling droplets.
Preparation the Cartridge

Complete the following steps:

1. Grip the cartridge by the tab and remove it from the package. Do not touch the wells or gaskets.

Fig. 1: Bio-Rad ddSEQ cartridge

2. Insert the cartridge into the cartridge holder:
   a. Lift the cartridge holder lever.
   b. Position the green gasket on the cartridge with the green stripe on the cartridge holder, insert the tab under the rails, then slide the cartridge into the holder.
   c. Check that the cartridge is fully inserted and lying flat against the bottom of the holder, then close the lever. If the lever does not close completely, reinsert the cartridge.

Fig. 2: Insert cartridge into cartridge holder
The following graphic shows an incorrect assembly:

![Incorrect Assembly](image1)

**Fig. 3: Incorrect assembly**

A) shows the cartridge tilted  
B) shows the cartridge upside down

The following graphic shows an assembled cartridge and cartridge holder:

![Assembled Cartridge and Holder](image2)

**Fig. 4: Assembled cartridge and cartridge holder**

A) Encapsulated sample output wells  
B) Barcode Suspension Mix input wells (Blue)  
C) Enzyme Suspension Mix input wells (Red)  
D) Encapsulation Oil input wells
**Priming the Cartridge**

Complete the following steps:

1. Use a P200 single-channel pipet to add 25 µl of room temperature ddSEQ Priming Solution to each well of an 8-tube strip.

2. Use a P20 multi-channel pipet to transfer 20 µl of ddSEQ Priming Solution from the 8-tube strip to each well of second row (marked A) on cartridge as shown in Fig. 5.

   **Tip:** When loading the cartridge, dispense pipet plunger only to the first stop to avoid bubbles.

3. Allow the ddSEQ Priming Solution to remain in wells for 1 min.

4. Remove all 20 µl Priming Solution in well with a P20 multichannel pipet set to 25 µl.

5. Repeat aspiration of liquid once to make sure all ddSEQ Priming Solution is removed from the wells.

   **Important:** Do not allow the ddSEQ Priming Solution to remain in wells longer than 3 min. Make sure all ddSEQ Priming solution is removed from the wells before proceeding to the next step.
Loading the Cartridge and Generating Droplets

Complete the following steps:

1. Using a multichannel P20 pipet set at 20 μl, mix the Barcode Suspension Mix in the 8-strip tubes by pipeting 10x.

2. Using a P20 single channel or multichannel pipet, prime the pipet tip by pipeting up and down 2x, pressing the pipet plunger only to the first stop.

3. When the tip is primed, aspirate 20 μl of the Barcode Suspension Mix and dispense into the bottom of the B ports (Blue).

   **Important:** Press the pipet plunger only to the first stop to avoid bubbles. Repeat until all four B ports are loaded.

   ![Barcode Suspension Mix wells](image)

   **Fig. 6: Barcode Suspension Mix wells**

4. Pulse vortex the Enzyme Suspension Mix at medium speed for 3 sec and repeat 3x to create a homogenous single nuclei suspension.

5. Using a P20 single channel or multichannel pipet, prime the pipet tip by pipeting up and down twice, pressing the pipet plunger only to the first stop. Once the tip is primed, load 20 μl of the Enzyme Suspension Mix into the bottom of the red ports, numbered 1–4.

   **Important:** Press the pipet plunger only to the first stop to avoid bubbles. Repeat until all four ports are loaded.

   ![Enzyme Suspension Mix wells](image)

   **Fig. 7: Enzyme Suspension Mix wells**
6. Pour the Encapsulation Oil into a multichannel pipet reservoir.

7. Using a P200 multichannel pipet, load 80 μl of room temperature Encapsulation Oil into each well of the bottom row of the cartridge labeled OIL.

   **Important:** Press the pipet plunger only to the first stop to avoid bubbles.

   **Note:** One bottle of Encapsulation Oil is enough for 2 cartridges. You can perform steps 6 and 7 with a single channel pipet if only using one cartridge.

8. Press the silver button on the top of the ddSEQ Single-Cell Isolator to open the instrument.

   ![Fig. 8: Bio-Rad ddSEQ Single-cell Isolator](image1)

9. Place the cartridge holder into the instrument. Make sure that the cartridge indicator light is solid green (middle light) to confirm that the cartridge holder is in the correct position. If the cartridge indicator light is not lit, reseat the cartridge holder on the magnetic plate.

   ![Fig. 9: ddSEQ cartridge loaded on ddSEQ Single-cell Isolator](image2)
10. Press the silver button on the top of the ddSEQ Single-Cell Isolator to close the instrument.

   **Note:** Droplet generation begins automatically after the ddSEQ Single-Cell Isolator door is closed and takes approximately 5 min. The droplet indicator flashes green to indicate that cell isolation is in progress. Single-cell isolation is complete when all 3 indicator lights are solid green.

11. While the cartridge is running, chill a 96-well plate by securely placing it on a chilled 96-well cooling block.

12. Once the run is complete, press the silver button on the front of the ddSEQ Single-Cell Isolator to open the instrument.

   If running another cartridge, wait for the instrument to reset (only the first light should be solid green) before loading the next cartridge.

13. Remove the cartridge holder from the ddSEQ Single-Cell Isolator. Successfully encapsulated samples appear cloudy in the output wells. Check for wells that look clear or empty, as droplet generation might have failed.

14. Use a P50 multichannel pipet set at 43 μl to gently and slowly aspirate all encapsulated sample at a slight angle from the output wells.

   **Important:** Fast or harsh pipeting will break encapsulated samples. Pipet very slowly to avoid yield loss.

![Fig. 10: Emulsion layers from output well](image)

   A) Aqueous layer
   B) Oil layer
   C) Oil and air bubbles

   **Note:** Total emulsion volume transferred to each well is 35–40 μl and approximately 5 μl of air.
15. Dispense the encapsulated sample as follows:
   a. Very slowly dispense the encapsulated sample into the corresponding column of the plate, as shown in Fig. 11. Dispensing should take approximately 5 sec.
      
      **Important:** Do not discard tips until all the encapsulated sample has been transferred to the plate. Discarding tips with sample will result in yield loss.
   
   b. Wait 5 sec for remaining sample to collect at the tip of pipette.
   
   c. Slowly dispense the remaining sample into the same column of the plate:
      
      - Sample 1 from cartridge to rows A–B of the plate
      - Sample 2 from cartridge to rows C–D of the plate
      - Sample 3 from cartridge to rows E–F of the plate
      - Sample 4 from cartridge to rows G–H of the plate

   ![Fig. 11: Transferring encapsulated samples](image)

16. Using a PX1 PCR Plate Sealer set at 180°C for 5 sec, seal plate with a pierceable foil PCR Plate Heat Seal (Bio-Rad #1814040) and promptly remove.

   **Important:** Use of other seals will lead to evaporation of sample during thermal cycling. Use only pierceable foil PCR Plate Heat Seals at this step.

   **Tip:** When transporting droplets, keep the plate chilled on ice to maintain droplet stability.

17. If you have finished processing cartridges, proceed immediately to **Barcoding and Amplification of Fragments on page 43**.

   **Tip:** When removing the cartridge from the cartridge holder, do not invert the cartridge. Dispose of cartridges according to standard laboratory procedures.
Barcoding and Amplification of Fragments

Complete the following step:

- Place the 96-well plate on the deep well C1000 Touch Thermal Cycler and proceed with the incubation protocol in Table 18 and Table 19, until amplification is complete.

**Important:** Always keep the plate in the 96-well cooling block on ice while transporting to the thermal cycler to maintain droplet integrity.

<table>
<thead>
<tr>
<th>Table 18. ATAC first amplification (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lid temperature</td>
</tr>
<tr>
<td>105°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 19. ATAC first amplification (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

This is a safe stopping point. You can store the sample at 4°C for up to 72 hr, or at −20°C for a longer period.
Emulsion Breakage and AMPure XP Cleanup

Guidelines

Note the following:

- Ensure that AMPure XP beads are at room temperature and thoroughly mixed before use.
- Prepare fresh 80% Ethanol daily for all wash steps.
- Sample wells contain separate oil and aqueous layers during this step. It is important to mix the contents at the interface of the oil and aqueous layers. When mixing, mix only in the specified layer.
- This procedure requires both a Magnetic Stand-96 (peg stand) and a DynaMag-96 Side Skirted Magnet. Both magnets are needed in this protocol and are not interchangeable. Pay careful attention to which magnet is used for each step.

Table 20. Advance preparation

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog number</th>
<th>Storage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet disruption</td>
<td>12008761</td>
<td>4 °C</td>
<td>Vortex 3–5x immediately before use to mix, then centrifuge briefly. This reagent can be kept at room temperature during use.</td>
</tr>
</tbody>
</table>

Procedure

Complete the following steps:

1. Remove the 96-well plate from the thermal cycler
   
   **Important:** Do not vortex or spin down the plate after removing it from the thermal cycler.

2. Visually examine the samples, which should all have equal volumes. Each sample has 2 distinct layers, an oil layer on the bottom and an emulsion layer on top.

3. Carefully remove the foil seal.
   
   **Tip:** If the foil seal is difficult to remove, carefully pierce openings above each sample with a pipet tip.

4. Add 20 µl of Droplet Disruptor by dispensing slowly against the side of the well above each sample.
   
   **Important:** DO NOT mix or pipet Droplet Disruptor into the sample.
5. Wait 30 sec and then add 30 µl of water by dispensing against the side of the well above each sample. DO NOT mix or pipet water into the sample.

![Fig. 12: Sample emulsion layers](image)

A) Emulsion Layer  
B) Oil Layer.


7. Using either a multichannel pipet and beads poured into a reservoir, or a P100 single channel pipet, add 50 µl of AMPure XP beads to the samples by dispensing slowly into the upper aqueous layer.

8. Using either a P50 multichannel pipet or single channel P100 pipette, set the pipet to 50 µl and pipet mix the AMPure XP beads in the aqueous layer only until the layer is evenly distributed (10–15x). After mixing, the samples have 2 distinct layers: an oil layer on the bottom of the well and a homogenous brown aqueous layer on the top.

**Tip:** To mix the AMPure XP beads with the aqueous layer, aspirate liquid from just above the interface, then lift tips and dispense near the top of the tube. Repeat until the entire aqueous layer is uniformly brown and any dark brown lines at the interface go into solution. If necessary, lift the plate to examine the quality of mix for the aqueous layer.

![Fig. 13: A lighter brown aqueous layer at the top indicates incomplete mixing](image)

![Fig. 14: Properly mixed](image)

A) Mixed aqueous layer, homogenous  
B) Oil layer
9. Incubate at room temperature for 5 min.

10. Place on a magnetic peg stand and wait 5 min.

11. Using a P200 multichannel pipet set to 200 µl, remove and discard all supernatant from each well (oil and aqueous layer), taking care not to disturb the bead pellet accumulated on the magnet.

12. Wash 2x as follows, keeping the plate on the magnetic peg stand:
   a. Add 200 µl of freshly prepared 80% ethanol to each well, pipeting the ethanol directly over the pellet.
   b. Incubate on the magnetic peg stand for 30 sec.
   c. Remove and discard all supernatant from each well.
   d. Repeat steps a–c to wash again.

13. Remove plate from magnetic peg stand and seal the plate with a Microseal F PCR Plate Seal or PCR Tube 8 Cap Strips.

14. Centrifuge at 280 RCF for 10 sec to bring down any ethanol or liquid remaining on the sides of wells.

15. Place plate on the DynaMag-96 Side Skirted Magnet and wait 30 secs.

16. Use a P20 single channel pipet to remove residual 80% ethanol in each well.

17. Air-dry on the DynaMag-96 Side Skirted Magnet until there is no remaining liquid in each well.
   **Important:** Air-dry time will vary depending on the humidity and temperature of the room, but typically takes 3–5 min. Monitor wells closely. If the magnetic bead pellet begins to crack, proceed immediately to the next step, as this is an indicator of over-drying and can lead to reduced yields.

18. Remove the sample plate from the DynaMag-96 Side Skirted Magnet.

19. Using a P50 multichannel pipet and reservoir or P100 single channel pipet, add 25 µl of Resuspension Buffer (10 mM Tris-HCl, pH 8) to each sample well first to wet each pellet. Pipet to mix, making sure all beads are resuspended.
   **Important:** Confirm that solution is homogenous. Yield loss can occur if beads are not thoroughly resuspended.

20. Incubate at room temperature for 2 min.

21. Seal the plate using a Microseal F PCR Plate Seal and centrifuge at 280 RCF for 10 sec.

22. Place on a DynaMag-96 Side Skirted Magnet and wait 2 min.
23. Using a P100 single channel pipet, combine the 2 wells for each sample by transferring 20 µl of supernatant from each sample well to a new plate or 8-tube strip, as follows. Keep the sample on the DynaMag-96 Side Skirted Magnet during this step.

- Sample 1, combine rows A–B in one well
- Sample 2, combine rows C–D in one well
- Sample 3, combine rows E–F in one well
- Sample 4, combine rows G–H in one well

**Note:** After transferring, the total volume of supernatant in each well of the new plate is 40 µl.

![Fig. 15: Combined samples in plate](image)

24. Seal plate or tubes. Vortex briefly to mix, then centrifuge using a tabletop centrifuge or plate centrifuge (500 RCF for 30 sec for plates).

This is a safe stopping point. You can store the sample at 4° C for up to 72 hr, or at –20° C for a longer period.
**Second Amplification of Barcoded Fragments**

**Table 21. Advance preparation**

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog number</th>
<th>Storage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC PCR Supermix</td>
<td>12008782</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. Once thawed, keep on ice.</td>
</tr>
<tr>
<td>ATAC Primer Mix</td>
<td>16005990</td>
<td>–20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. Once thawed, keep on ice.</td>
</tr>
</tbody>
</table>

**Procedure**

Complete the following steps:

1. Prepare the PCR master mix by adding the following components in Table 22 to a 1.5 ml tube on ice. Pulse vortex 3x at medium speed, 3 sec per pulse, to mix then centrifuge briefly.

**Table 22. PCR Master Mix**

<table>
<thead>
<tr>
<th>Component</th>
<th>Catalog number</th>
<th>Required volume, µl (4 reactions, + 10% excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATAC PCR Supermix</td>
<td>12008782</td>
<td>110.0</td>
</tr>
<tr>
<td>ATAC Primer Mix</td>
<td>16005990</td>
<td>8.8</td>
</tr>
<tr>
<td>Nuclease-free water</td>
<td>N/A</td>
<td>13.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>132</td>
</tr>
</tbody>
</table>

2. Dispense 30 µl of PCR Master Mix from step 1 into each well of a 96-well plate.
3. Add 20 µl of purified sample to each well of the plate containing the PCR Master Mix.
4. Seal the plate using PCR Tube 8-Cap Strips and vortex for 5 sec to mix.
5. Centrifuge at 500 RCF for 30 sec.
6. Place the 96-well plate on the thermal cycler and proceed with the PCR protocol specified in Table 23, Table 24, and Table 25 on page 49 until amplification is complete.
Table 23. ATAC Second amplification (1)

<table>
<thead>
<tr>
<th>Lid Temperature</th>
<th>Reaction volume</th>
<th>Run time</th>
</tr>
</thead>
<tbody>
<tr>
<td>105°C</td>
<td>50 µl</td>
<td>Device dependent</td>
</tr>
</tbody>
</table>

Table 24. ATAC Second amplification (2)

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Ramp</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98°C</td>
<td>30 sec</td>
<td>Default</td>
</tr>
<tr>
<td>2</td>
<td>98°C</td>
<td>10 sec</td>
<td>Default</td>
</tr>
<tr>
<td>3</td>
<td>55°C</td>
<td>30 sec</td>
<td>Default</td>
</tr>
<tr>
<td>4</td>
<td>72°C</td>
<td>60 sec</td>
<td>Default</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td>Go to step 2 (total number of cycles – 1. Refer to Table 25 for guidance on recommended number of cycles.)</td>
</tr>
<tr>
<td>6</td>
<td>72°C</td>
<td>5 min</td>
<td>Default</td>
</tr>
<tr>
<td>7</td>
<td>4°C</td>
<td></td>
<td>Hold</td>
</tr>
</tbody>
</table>

Table 25. Recommended cycle number

<table>
<thead>
<tr>
<th>Targeted cell throughput</th>
<th>Suggested total number of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;2,000</td>
<td>9</td>
</tr>
<tr>
<td>2,000 to 3,000</td>
<td>8</td>
</tr>
<tr>
<td>3,001 to 5,000</td>
<td>7</td>
</tr>
</tbody>
</table>

This is a safe stopping point. You can store the sample at 4°C for up to 72 hr, or at –20°C for a longer period.
Second AMPure XP Cleanup

Guidelines

Note the following:

- Ensure that AMPure XP beads are at room temperature and thoroughly mixed before use.
- Prepare fresh 80% ethanol daily for all wash steps.
- This procedure requires both a Magnetic Stand-96 (peg stand) and a DynaMag-96 Side Skirted Magnet.

**Important:** Both magnets are needed in this protocol and are not interchangeable.

Procedure

Complete the following steps:

1. Remove the 96-well plate from the thermal cycler.
2. Centrifuge plate at 500 RCF for 30 sec.
3. Carefully remove the seal.
4. Vortex the AMPure XP beads until well-dispersed.
5. Add 50 µl of AMPure XP beads to each sample well. Pipettomix, making sure that all beads are resuspended and that the mixture is homogenous.
6. Incubate at room temperature for 5 min.
7. Place on a magnetic peg stand and wait 5 min.
8. Using a P200 multichannel pipet set to 200 µl, remove and discard all supernatant from each well (~100 µl), taking care not to disturb the bead pellet accumulated on the magnet.
9. Wash 2x as follows, keeping the plate on the magnetic peg stand:
   a. Add 200 µl of freshly prepared 80% ethanol to each well.
   b. Incubate on the magnetic peg stand for 30 sec.
   c. Remove and discard all supernatant from each well.
   d. Repeat steps a–c to wash again.
10. Remove the plate from magnetic peg stand and seal the plate with a Microseal F PCR Plate Seal or PCR Tube 8-Cap Strips.
11. Centrifuge at 280 RCF for 10 sec to bring down any ethanol or liquid remaining on the sides of wells.

13. Use a P20 single channel pipet to remove residual 80% ethanol in each well.

14. Air-dry on the DynaMag-96 Side Skirted Magnet until there is no remaining liquid in each well.

   **Important:** Air-dry time varies depending on the humidity and temperature of the room, but typically takes 3–5 min. Monitor the wells closely. If the magnetic bead pellet begins to crack, proceed immediately to the next step, as this is an indicator of over-drying and can lead to reduced yields.

15. Remove the sample plate from the DynaMag-96 Side Skirted Magnet.

16. Using a P50 multichannel pipet and reservoir or P100 single channel pipet, add 22 µl of Resuspension Buffer (10 mM Tris-HCl, pH 8) to each sample well.

17. Pipet to mix, making sure all beads are resuspended.

   **Important:** Confirm that solution is homogenous. Yield loss can occur if beads are not thoroughly resuspended.

18. Incubate at room temperature for 2 min.

19. Seal the plate using Microseal F foil seals.

20. Centrifuge at 280 RCF for 10 sec.

21. Place the plate on a DynaMag-96 Side Skirted Magnet and wait 2 min.

22. Transfer 20 µl of supernatant from each sample well to a new 96-well plate or 8-tube strip.

---

**This is a safe stopping point. You can store the sample at 4° C for up to 72 hr, or at –20° C for a longer period.**
Library Assessment and Quantification

Table 26. Required consumables and equipment (not provided)

<table>
<thead>
<tr>
<th>Consumable/equipment</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agilent 2100 Bioanalyzer</td>
<td>G2939BA</td>
<td>Agilent</td>
</tr>
<tr>
<td>High Sensitivity DNA Kit</td>
<td>5067-4626</td>
<td>Agilent</td>
</tr>
</tbody>
</table>

Procedure

Complete the following steps:

1. Run 1 µl undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA chip.
2. Determine the concentration of the library using the Agilent Technology 2100 Bioanalyzer Software.
3. Select the Region Analysis tab.
4. Drag the blue region lines to capture the 200–8,000 bp region.
5. Record the final library yield in nM (molarity).

Note: Typical libraries show a broad size distribution ~250–2,000 bp and an average fragment length of 400–800 bp.

Tip: If library concentration is >30 nM, the Bioanalyzer might be saturated. Dilute libraries 1:5 with RSB and rerun chip.

Fig. 16: Library size distribution on Agilent 2100 Bioanalyzer
Sequencing Guidelines

Guidelines

Note the following:

- Use the provided ddSEQ Sequencing Primer (Bio-Rad #16005986) as the custom Read 1 primer.
- Ensure that a custom Read 1 sequencing primer is specified as part of the run during setup. This is crucial for successful single-cell sequencing.
  
  **Important:** Do not mix the ddSEQ Sequencing Primer with other standard Read 1 primers, as this will result in poor cluster quality and loss of reads.
- The ddSEQ Sequencing Primer is concentrated at 50 µM and must be diluted according to the custom sequencing primer documentation for your instrument.
- If the BaseSpace Prep Tab is not used, confirm that Illumina Experiment Manager, Version 1.13 or later, is used to set up the sequencing sample sheet.
- Version 1.13 or later has the appropriate index sequences for sample demultiplexing.
- If demultiplexing outside of BaseSpace Sequence Hub, confirm that bcl2fastq Version 2.18 or later is used for FASTQ Generation.

**Table 27. Prepare the following consumables**

<table>
<thead>
<tr>
<th>Item</th>
<th>Catalog Number</th>
<th>Storage</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ddSEQ Sequencing Primer</td>
<td>16005986</td>
<td>−20°C</td>
<td>Thaw at room temperature. Vortex vigorously to mix, and then centrifuge briefly. This reagent can be kept at room temperature during use.</td>
</tr>
</tbody>
</table>

**Custom Primer Guides**

- NextSeq System Custom Primers Guide (document #15057456)
Modify SureCell ATAC-Seq Loading Concentrations

Use this table to help you determine your loading concentration. Loading concentrations presented in this table are based on Agilent 2100 Bioanalyzer quantification and are recommended to maximize data output and retain sequencing quality. If you are quantifying with another method, you might need to optimize the loading concentration.

Table 28. Determine loading concentration

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Kit</th>
<th>Loading concentration (pM)</th>
<th>PhiX</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiSeq</td>
<td>MiSeq Reagent Kit v3 (150 cycles)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>NextSeq550</td>
<td>NextSeq High Output Kit v2.5 (150 cycles)</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>NextSeq2000</td>
<td>NextSeq 2000 P3 Reagents (200 Cycles)</td>
<td>450</td>
<td>0</td>
</tr>
<tr>
<td>NovaSeq6000</td>
<td>NovaSeq 6000 S2 Reagent Kit v1.5 (200 cycles)</td>
<td>300</td>
<td>0</td>
</tr>
</tbody>
</table>

Sequencing Run Parameters

SureCell ATAC-Seq libraries use a custom Read 1 sequencing primer and are run using paired-end sequencing with single indexing. The supported number of cycles for each read is shown in Table 29.

Note: Due to a compatibility issue, the custom Read 1 primer cannot be mixed with other Read 1 sequencing primers in the same port.

Table 29. Sequencing run parameters

<table>
<thead>
<tr>
<th>Sequencing read</th>
<th>Recommended number of cycles</th>
<th>Custom primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Read 1</td>
<td>118 cycles</td>
<td>Yes</td>
</tr>
<tr>
<td>i7 index</td>
<td>8 cycles *</td>
<td>No</td>
</tr>
<tr>
<td>i5 index</td>
<td>0 cycles</td>
<td>No</td>
</tr>
<tr>
<td>Read 2</td>
<td>40 cycles</td>
<td>No</td>
</tr>
</tbody>
</table>

* i7 index cycles are only required if combining more than one sample in the same sequencing run.
Appendix A  Supporting Information

S1: Working with Low Viability Cells

This protocol requires the input of viable, single cells in suspension in order to obtain high quality data. Using cells with viability <80% can lead to reduced cell throughput and more noise in sequencing data due to ambient DNA from dead or damaged cells.

DNase I treatment is a common method for increasing cell viability by digesting ambient DNA from dead or damaged cells.

Guidelines

Note the following:

- This protocol assumes cells are already in single-cell suspension in complete media. If using tissue, further optimizations might be needed to dissociate starting material into single cells before proceeding.

- DNase I needs divalent cations so treat cells in appropriate culture media that lacks EDTA.

Table 30. Other consumables and equipment required (not provided)

<table>
<thead>
<tr>
<th>Reagent/equipment</th>
<th>Catalog number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture media (without EDTA) compatible with cell type</td>
<td>Varies</td>
<td>Varies</td>
</tr>
<tr>
<td>DNase I</td>
<td>Any</td>
<td>Any</td>
</tr>
</tbody>
</table>

Prepare Reagents

Prepare culture medium with DNase I at a final concentration of 200 U/ml. If using powdered DNase, resuspend in Hanks Balanced Salt Solution.
Procedure

Complete the following steps:

1. Centrifuge cells at 300 RCF for 3 min at room temperature.
2. Remove all supernatant, taking care not to disturb the cell pellet.
3. Resuspend cells in 5 ml of culture medium with DNase I.
   
   Tip: If starting with <1 M cells, resuspend in 1 ml instead of 5 ml of culture medium with DNase I in a 1.5 ml tube to minimize loss of cells.
4. Incubate at 37°C for 30 min.
5. After 30 min, proceed immediately with Cell Preparation on page 22.

S2: Mixed Species Control (K562 & NIH-3T3)

These are a set of verified procedures, together with required material for preparing fresh or cryopreserved K562 (human) and NIH-3T3 (mouse) cell lines for analysis as a mixed species single-cell suspension.

This protocol can be used as a control in parallel with other samples. It also enables the assessment of doublet cells by detecting mixed-species crosstalk. Upon completion of the protocol, cells are ready for Section 2 (Cell Preparation).

Guidelines

Note the following:

- These procedures have been verified for cryopreserved or fresh K562 and NIH-3T3 cells only. Further optimizations might be required for other cell types.
- If starting from fresh cells, proceed with Section S2.1 to prepare the cells. After completion of Section S2.1, skip to Section S2.3.
- If starting from cryopreserved cells, skip to Section S2.2 to thaw and prepare cells, and then continue to Section S2.3.

Table 31. Cell lines and culture media

<table>
<thead>
<tr>
<th>Supplier</th>
<th>Cell line</th>
<th>Catalog number</th>
<th>Compatible media</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC</td>
<td>K-563 (human)</td>
<td>CCL-243</td>
<td>IMDM + 10% FBS + antibiotic</td>
</tr>
<tr>
<td>ATCC</td>
<td>NIH-3T3 (mouse)</td>
<td>CRL-1658</td>
<td>DMEM + 10% FBS + antibiotic</td>
</tr>
</tbody>
</table>
List of Materials not Included in Tables 1–3

- 37°C water bath
- Complete growth medium based on the ATCC recommendation for the respective cell type
  
  **Important:** Store at 4°C. Before cell handling and preparation, you must warm up the complete growth medium to 37°C.

- Gibco 1X PBS (catalog #10010023) + 0.1% BSA solution
  
  **Important:** Store this solution at 4°C at all times. While performing cell dissociation, store the solution on ice. If working under a hood, take out a large volume from the refrigerator right before use.

- Gibco TrypLE Express Enzyme (catalog #12604013)
  
  **Note:** This is required only if you are starting from fresh cells.

- Gibco 1X PBS (catalog #10010023),
  
  **Note:** This is required only if you are starting from fresh cells.

- Corning Life Sciences Cell Strainer CP ST 5ml 500/CS (catalog #352235), 35 µm

- Vortex mixer

- Microscope

- Hood

- Cell centrifuge

- Standard cell culture lab equipment

**S2.1: Using Fresh Cells**

**Cell Dissociation Protocol for Fresh NIH-3T3 Cells**

This protocol is optimized for cells cultured in a T75 cell culture flask.

Complete the following steps:

1. Warm the required media from the refrigerator to 37°C (approximately 30 min).
2. Remove the flask of NIH-3T3 cells from the incubator and check the cells under the microscope.
   
   **Tip:** Use cells at a confluency between 60–90%.
3. Carefully remove all of the media without touching the surface of the flask.
4. Rinse attached cells using 7 ml of 1X PBS.
5. Remove and discard all of the PBS.
6. For a regular T-75 flask, add 2.5 ml of TrypLE Express to cover the entire surface of the flask.
7. Place flask in the incubator for 3–5 min.
8. Remove the flask and check to see if cells have detached or are starting to detach.
   Important: Caution: Do not tap the flask to detach cells. Allow the protease to detach cells and dissociate them.
9. Add 7.5 ml of warm medium to the flask to neutralize the TrypLE Express.
10. Using a serological pipet, break the cell clumps by pipeting up and down 10–15x.
11. Transfer the cell suspension to a 15 ml conical tube.

**Cell Preparation for fresh K562 cells (K562)**

Complete the following steps:
1. Remove the flask of K562 cells from the incubator and check the cells under the microscope.
2. Using a serological pipet, mix cells in the flask by pipeting up and down.
3. Transfer cell suspension to a 15 ml conical tube.
4. After completing cell preparation for fresh NIH-3T3 and K562 cells, skip to Section S2.3.

**S2.2: Using Cryopreserved Cells**

Complete the following steps in parallel to prepare both K562 and NIH-3T3 cells.
1. Warm the required complete growth media from the refrigerator to 37° C.
2. Set the water bath to 37° C and ensure that it has reached the desired temperature before starting this protocol.
3. Remove a cryovial of frozen cells from liquid nitrogen storage.
   Important: Wear cryogloves, safety glasses, a lab coat, and close-toed shoes and carefully follow all instructions and precautions when handling liquid nitrogen.
4. Place the cryovial in the 37° C water bath and let it thaw for no more than 1–3 min.
5. Remove vial from the water bath when the cells are almost completely thawed and only a tiny ice crystal remains in the tube.
   Important: Do not leave the vial in the water bath for more than 5 min.
6. Add 1 ml of warmed media dropwise (1 drop per sec).
7. Mix the cells by gently pipeting 10x and transfer the entire volume to a 15 ml conical tube.
8. Rinse the cryovial with 1 ml warm complete growth media.
9. Add the rinse medium dropwise (1 drop per 3 sec) to the 15 ml conical tube while gently swirling the tube.
10. Add 9 ml of warm complete growth media slowly to the 15 ml conical tube (about 0.5 ml per sec).
11. Gently invert the conical tube 4x to mix.
12. Centrifuge cells at 300x g for 3 min. Do not use a chilled centrifuge.
13. Discard the supernatant, taking care not to disturb the cell pellet. Remove all but approximately 1 ml using a serological pipet. Remove the remaining liquid using a P1000 pipet.
14. Resuspend cell pellet in 10 ml of warm complete growth media.
15. Proceed immediately to Section S2.3.

**S2.3: K562 and NIH-3T3 Cell Preparation**

Complete the steps in Cell Preparation on page 22 and then complete the following critical additional notes and steps:

1. Ensure that two 35 µm cell strainers in their collection tubes are placed on ice at the beginning of the cell preparation procedure, one for each cell type.
2. All steps from Cell Washing and Filtering on page 23 to Cell Viability and Cell Count Measurements on page 24 are to be executed on each cell type individually. Only upon completion of Cell Viability and Cell Count Measurements are cells to be mixed.
3. After Cell Viability and Cell Count Measurements is complete, mix together an equal number of K562 and NIH-3T3 cells. For a single sample, mix 75,000 cells from each species to yield a total of 150,000 cells. For technical replicates, make additional aliquots of 150,000 mixed species cells.
4. Store all mixed species aliquots on ice.
## Appendix B Ordering Information

Table 32. Ordering information

<table>
<thead>
<tr>
<th>Catalog number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>12004336</td>
<td>ddSEQ Single Cell Isolator, which includes the instrument and associated component consumables</td>
</tr>
<tr>
<td>17004620</td>
<td>SureCell ATAC-Seq Library Prep Kit, which includes</td>
</tr>
<tr>
<td></td>
<td>▪ SureCell ATAC-Seq Reagent Box A</td>
</tr>
<tr>
<td></td>
<td>▪ SureCell ATAC-Seq Reagent Box B</td>
</tr>
<tr>
<td></td>
<td>▪ SureCell ddSEQ M Cartridges</td>
</tr>
<tr>
<td>12009360</td>
<td>SureCell ATAC-Seq Index Kit</td>
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