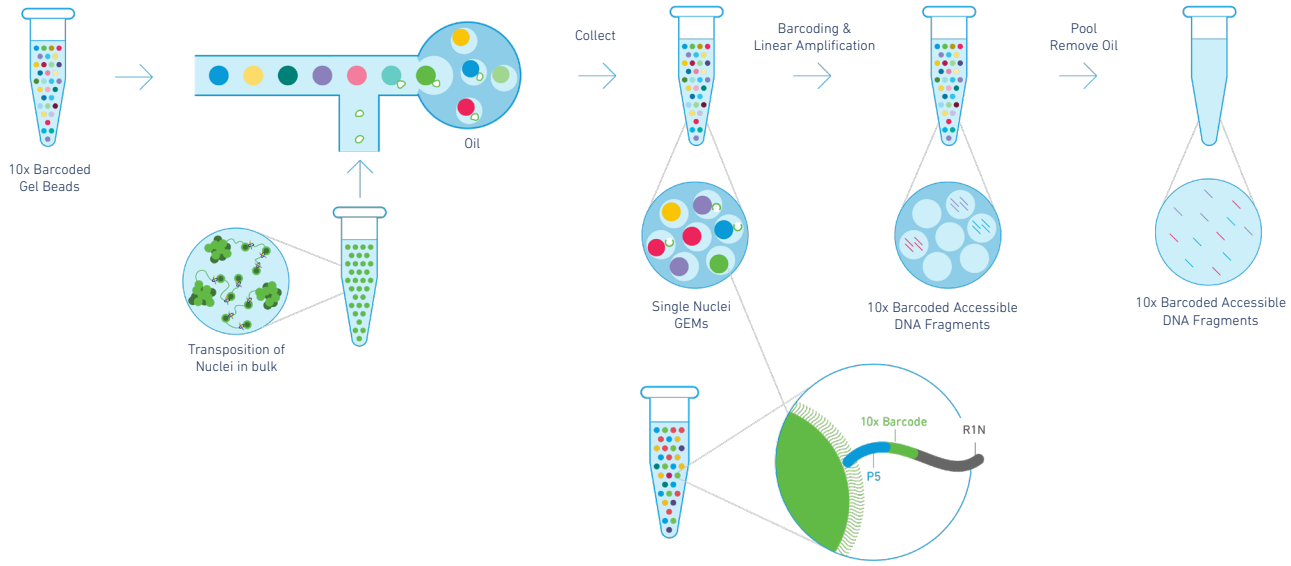
Getting Started: Single Cell ATAC

Introduction



The Chromium Single Cell ATAC Solution provides a comprehensive, scalable approach to determine the regulatory landscape of chromatin in hundreds to thousands of cells in a single sample. Nuclei suspensions are incubated in a Transposition Mix that includes a Transposase, which enters the nuclei and preferentially fragments the DNA in open regions of the chromatin. Using a microfluidic chip, nuclei are partitioned into nanoliter-scale Gel Beads-in-emulsion (GEMs). A pool of ~750,000 10x Barcodes is used to separately and uniquely index the transposed DNA of each individual nucleus. Libraries are generated and sequenced, and 10x Barcodes are used to associate individual reads back to the individual partitions, and thereby, to each individual nucleus. For more information about the assay, click here.

Sample Preparation Guidelines

Preparing clean, viable single nuclei suspension is essential for obtaining high quality single cell ATAC data.

To prepare nuclei suspension from cells (fresh or frozen), consult this Demonstrated Protocol. The protocol includes optional DNase treatment to remove ambient DNA from the samples.

To prepare nuclei suspension from tissue (fresh or frozen), consult this Demonstrated Protocol. The protocol describes nuclei isolation from embryonic mouse brain.

Counting	Nuclei Concentration	Quality
After nuclei isolation, a cell viability stain can be used for staining and counting nuclei. If debris makes nuclei counting challenging, a fluorescent dye that stains nucleic acids may enhance counting accuracy.	155-7,700 nuclei/µl Nuclei concentration should be adjusted to be within the optimal range for the targeted nuclei recovery. Use an automated cell counter or a hemocytometer with a live/dead stain to determine cell/nuclei count. Consult Guidelines for Accurate Target Cell Counts. Measuring nuclei count is critical for accurate target cell recovery, as detailed in this Technical Note.	During nuclei isolation, the outer cell membrane must be lysed without comprising the nuclear membrane to prevent nuclei leakage. A lysis timecourse is recommended to find the optimal lysis time for a sample. Good quality nuclei suspension should have intact nuclear membranes and should be free of clumps and debris.

Challenging Sample Types

Additional cleanup methods, like DNase treatment, filtering, FACS, and/or density centrifugation (listed below), may be used when isolating nuclei from complex and challenging sample types, such as tissues.

Sample Type	DNase Treatment	Filtering	FACS	Density Centrifugation
Cell	Yes	Yes	Yes	Yes
Nuclei	No	Yes	Yes, with caution	Yes
Limited Nuclei Yield	No	Yes, with a small volume filter	No	No
Abundant Nuclei Yield	No	Yes	Yes	Yes

Materials Required and Step Overview

Consult the user guide for a consumables and equipment list validated by 10x Genomics for executing the Chromium Single Cell ATAC protocol.

To enable seamless experimental planning, a breakdown of the protocol steps, along with execution times and stop & store points are listed below.
10x Genomics how-to video series provides a visual demonstration of the workflow.

	Steps		Timing	Stop & Store
2 h	**Nuclei Isolation**			
	Dependent on Cell Type		~1-2 h	
	Step 1 – Transposition			
	1.1	Prepare Transposition Mix	10 min	
	1.2	Isothermal Incubation	30 min	
4 h	**Step 2 – GEM Generation & Barcoding**			
	2.1	Prepare Master Mix	10 min	
	2.2	Load Chromium Next GEM Chip H	10 min	
	2.3	Run the Chromium Controller	18 min	
	2.4	Transfer GEMs	3 min	
	2.5	GEM Incubation	45 min	STOP 15°C ≤18 h or –20°C ≤ 1 week
	Step 3 – Post GEM Incubation Cleanup			
	3.1	Post GEM Incubation Cleanup – Dynabeads	35 min	
	3.2	Post GEM Incubation Cleanup – SPRIselect	15 min	STOP 4°C ≤ 72 h or –20°C ≤ 2 weeks
6 h	**Step 4 – Library Construction**			
	4.1	Sample Index PCR	40 min	
	4.2	Post Sample Index Double Sided Size Selection – SPRIselect	20 min	STOP 4°C ≤72 h or –20°C long-term
	4.3	Post Library Construction QC	60 min	

Sequencing

The Chromium Single Cell ATAC protocol produces Illumina®-ready sequencing libraries. The recommended library sequencing depth & run parameters are summarized in the user guide and are also available on the 10x Genomics Support website.

	Read 1	i7 Index	i5 Index	Read 2
Purpose	Transposed DNA	Sample Index	10x Barcode	Transposed DNA
Length (bp)	50	8	16	50*

*Can be 1 bp shorter to accommodate NovaSeq 100 cycle kits

Consult the Sequencing Metrics & Base Composition Technical Note for representative data generated using various sequencers validated by 10x Genomics.

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA