Mar 17, 2025

Human trigeminal samples use Chromium Next GEM Single Cell Multiome protocol

DOI
https://dx.doi.org/10.17504/protocols.io.3byl4wo4ovo5/v1

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Protocol Citation: Dongming Liang, Hao Wu 2025. Human trigeminal samples use Chromium Next GEM Single Cell Multiome protocol . **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl4wo4ovo5/v1

Protocol status: Working
We use this protocol and it's working

Created: February 21, 2025

Last Modified: March 17, 2025

Protocol Integer ID: 123165

Keywords: Human trigeminal, Chromium Next GEM Single Cell Multiome, chromium next gem single cell multiome protocol, next gem single cell multiome protocol, 10x genomics multiome assay, simultaneous profiling of gene expression, chromatin, accessible chromatin, linking open chromatin region, chromatin within the same cell, cells with similar transcriptional profile, human trigeminal sample, open chromatin region, distinct chromatin landscape, drivers of differential gene expression, differential gene expression, similar transcriptional profile, deeper mechanistic insights into dynamic gene, gene expression, single cell, comprehensive view of cellular regulation, cellular regulation, thousands of cell, rna, multiomic approach, integrating rna, same cell, dynamic gene

Abstract

The 10x Genomics Multiome assay enables simultaneous profiling of gene expression and open chromatin within the same cell across thousands of cells (ref 1-2), offering a comprehensive view of cellular regulation. By integrating RNA sequencing with assay for transposase-accessible chromatin (ATAC), this multiomic approach enhances the resolution of cell states, identifies drivers of differential gene expression, and reveals cells with similar transcriptional profiles but distinct chromatin landscapes. By linking open chromatin regions to gene expression at single-cell resolution, this technology could provide deeper mechanistic insights into dynamic gene regulatory networks of Human trigeminal normal and migraine

Materials

	Reagents	Vol. µL	5ml	final concentration
	5 M NaCl	14.6	73	73 mM NaCl
	0.5 M CaCl2	1	5	0.5mM CaCl2
	1 M MgCl2	10	50	10mM MgCl2
	1 MTris-HCl pH 7.5	5	25	5mMTris-HCl pH 7.5
	5%BSA	200	1000	1%BSA
	40 U/ul Protector RNase inhibitor	2.5	12.5	0.1U/ul Protector RNase inhibitor
	H2O	766.9	3834.5	
		1000	5000	
	10% IGPAL	20		0.2% IGPAL

Nuclei isolation

1 PIIS0896627322002288 (2).pdf 11.6MB
https://www.cell.com/neuron/pdf/S0896-6273(22)00228-8.pdf

2 *Preparation of Single Trigeminal Nuclei*

2.1 Place 100um thin tissue sections Human TG from OCT-embedded sample in a 1.5 mL Eppendorf tube.

2.2 Place thin tissue sections Human TG ganglia in homogenization buffer [73mMNaCl, 0.5mM CaCl2,
10mMMgCl2,5mMTris-HCl pH7.5, 1%BSA, 0.1U/ul Protector RNase inhibitor (Millipore Sigma 3335399001)] for 15 s on ice using chilled reagents. On ice

2.3 Transfer samples to a prechilled Douncehomogenizer in 5mL homogenization bufferfor 10 strokes with a loose pestle, then add IGPAL (Millipore Sigma 542334) to a final concentration of 0.2% and apply 5 ADDITIONAL strokes with a tight pestle. On ice

2.4 The tissue homogenate is then passed through a 30mmMACS SmartStrainerfilter (130-098-458) and diluted 1:1 with homogenization buffer. On ice

2.5 Nuclei are centrifugedat 500 g for 10 min at 4°Cand resuspended in 1ml of Resuspension buffer (1X PBS, 0.04%BSA, and0.1U/mlRNase inhibitor). 4 °C

Library preparation

3 For 10x multiome sequencing analysis of Human trigeminal, we followed the standard library preparation protocol from 10x Genomics (see below).

3.1 CG000338_ChromiumNextGEM_M... 4.7MB

Protocol references

1. Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing. https://www.cell.com/neuron/pdf/S0896-6273(22)00228-8.pdf
2. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Kits User Guide. https://www.10xgenomics.com/support/epi-multiome/documentation/steps/library-prep/chromium-next-gem-single-cell-multiome-atac-plus-gene-expression-reagent-kits-user-guide