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Title: Spatially Resolved, Integrated Single-Cell Multiomic Profiling of the Transcriptome and Epigenomic Targets in Frozen Tissue Sections

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **05/12/2026**

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#).

Current Protocol Length

Number of Steps: 19

Number of Shots: 54

Interviews

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Fatemeh S. Farassati:** We develop spatial single-cell multiome technologies to profile gene expression and regulatory epigenetic modifications within intact tissue architecture.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Fatemeh S. Farassati:** Current methods either lose spatial context or lack epigenomic resolution. This protocol integrates both, enabling spatially resolved single cell multiome profiling.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Fatemeh S. Farassati:** It enables simultaneous measurement of spatial gene expression and regulatory epigenetic modification at single-cell resolution within intact tissues.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Fatemeh S. Farassati:** Isolation of high-yield, high-quality nuclei is critical for successful spatial multiome profiling.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

1.5. **Fatemeh S. Farassati:** Functional bioinformatic analysis reveals biological significance and these insights are further enhanced by profiling additional epigenetic marks in adjacent sections.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Mayo Clinic, Rochester

Protocol

2. Dissociation of Mouse Frozen Brain Tissue and Nuclei Isolation

Demonstrator: Ting Zhang

- 2.1. To begin, examine the Trypan blue-stained coronal tissue section of fresh frozen mouse brain on the trekker tile [1]. Use tweezers to carefully place the Trekker tile on top of a Trekker O-ring (*oh-ring*) in a well of a 12-well plate placed on ice [2].
 - 2.1.1. Talent examining the Trekker tile containing blue colored tissue section.
 - 2.1.2. Talent placing the Trekker tile on a Trekker O-ring in the well of a 12-well plate placed on ice.
- 2.2. To detach the tissue from the tile, dispense 175 microliters of Invent Minute buffer A aiming at the tissue [1]. Repeat three more times to dispense a total of 700 microliters of buffer, while aiming at different parts of the tile and avoiding bead contamination [2]. Aspirate the buffer from the side of the well, then gently rinse the regions of the tile covered with tissue until it is completely detached [3].
 - 2.2.1. Talent dispensing 175 μ L Invent Minute buffer A onto the tissue.
 - 2.2.2. Talent repeating the dispensing of buffer at different parts of the tile.
 - 2.2.3. Talent aspirating the buffer from sides of the well onto the tissue until it gets detached.
- 2.3. After transferring the tile to an empty well, use a P1000 (*P-one thousand*) pipette to mechanically dissociate the nuclei from the tissue with repeated trituration until a uniform suspension is obtained [1]. Carefully transfer the nuclei suspension to a filter cartridge with a collection tube [2].
 - 2.3.1. Talent mechanically dissociating the nuclei by repeated trituration using P1000 pipette.
 - 2.3.2. Talent carefully transferring the nuclei suspension to a filter cartridge with collection tube.
- 2.4. Incubate the tube with the cap open at minus 20 degrees Celsius for 10 minutes [1]. After capping the filter cartridge, immediately centrifuge at 13000 *g* for 30 seconds in a refrigerated microcentrifuge [2].
 - 2.4.1. Talent placing the tube in -20°C refrigerator for 10 minutes.
 - 2.4.2. Talent placing the cartridge tube in a microcentrifuge.
- 2.5. Resuspend the pellet by gently pipetting ten to twenty times after discarding the filter cartridge [1]. Spin the tube in the prechilled centrifuge with swing buckets at 600 *g* for 5 minutes [2]. Carefully remove the supernatant [3] and resuspend the pellet in 200 microliters of Trekker buffer C [4].

- 2.5.1. Talent resuspending the pellet by gentle pipetting for 10-20 times.
- 2.5.2. Talent placing the tube in prechilled centrifuge with swing buckets.
- 2.5.3. Talent carefully removing the supernatant.
- 2.5.4. Talent resuspending the pellet in 200 μ L of Trekker buffer C.
- 2.6. To a 1.5-milliliter low-binding Eppendorf tube, add 1 milliliter of cold Invent Minute buffer B [1]. Carefully overlay 200 microliters of nuclei suspension by slowly expelling against the tube wall to avoid mixing of the layers [2].
 - 2.6.1. Talent adding 1 mL of cold Invent Minute buffer B to a 1.5 mL Eppendorf tube.
 - 2.6.2. Talent carefully overlaying 200 μ L of nuclei suspension by slowly expelling against the tube wall.
- 2.7. Spin the tube in the prechilled centrifuge with swing buckets at 500 *g* for ten minutes [1], then remove the milky layer and supernatant [2]. Gently resuspend the nuclei with 24 microliters of Trekker buffer C [3].
 - 2.7.1. Talent placing the tube in prechilled centrifuge with swing buckets at 500 *g*.
 - 2.7.2. Talent carefully removing the milky layer and supernatant.
 - 2.7.3. Talent resuspending the nuclei with 24 μ L of Trekker buffer C.
- 2.8. To count the nuclei, add 2 microliters of nuclei suspension to 8 microliters of Trekker buffer C [1]. Mix 10 microliters of the diluted nuclei suspension with an equal volume of acridine-orange and propidium iodide staining solution [2]. Count the nuclei according to the manufacturer's instructions [3].
 - 2.8.1. Talent adding 2 μ L of the nuclei suspension into 8 μ L of Trekker buffer C.
 - 2.8.2. Talent mixing 10 μ L of the diluted nuclei suspension with 10 μ L of AO/PI staining solution.
 - 2.8.3. Talent counting the nuclei according to the manufacturer's instructions.
- 2.9. Next, to assess the quality of isolated nuclei, add 2 microliters of nuclei suspension to 8 microliters of 4 percent trypan blue solution [1]. Examine the nuclei under a fluorescence microscope at 40X magnification, checking for intact nuclear membrane morphology and non-nuclear debris [2].
 - 2.9.1. Talent adding 2 μ L of the nuclei suspension to 8 μ L of 4% trypan blue solution.
 - 2.9.2. LAB MEDIA: Figure 2. *Video Editor: Please emphasize the left panel to show high-quality, intact nuclei.*

3. CUT&Tag Tagmentation on Isolated Nuclei

- 3.1. Prepare 100 microliters of CUT&Tag (*Cut and tag*) incubation buffer I (*one*) per reaction according to the manufacturer's protocol and place it on ice [1].
 - 3.1.1. Talent preparing CUT&Tag incubation buffer I
- 3.2. To a PCR tube on ice, add specific volumes of buffer [1], enzyme [2], and primary antibody [3], then mix gently by pipetting ten times [4]. Spin briefly and incubate the tube on an 18 rpm (*R-P-M*) rotator at room temperature for 1 hour [5]. **Note: The numbers and narration are updated for the added shot.**
 - 3.2.1. Talent adding 46.23 μL of CUT&Tag incubation buffer I and 1.33 μL of TAG enzyme.
 - 3.2.2. Talent adding 2.43 μL of anti-H3K27ac primary antibody to the tube.
Added shot: TEXT ON A PLAIN BACKGROUND

CUT&Tag incubation buffer I	46.23 μL
TAG conjugated enzyme	1.33 μL
Anti-H3K27ac primary antibody	2.43 μL
 - 3.2.3. Talent mixing the solution gently by pipetting 10 times.
 - 3.2.4. Talent placing the tube on an 18 rpm rotator at room temperature for 1 hour.
- 3.3. To incubate the isolated nuclei with antibody-TAG enzyme conjugate, spin the tube of isolated nuclei in the prechilled centrifuge with swing buckets at 500 *g* for 10 minutes [1]. Carefully aspirate the supernatant, leaving approximately 5 microliters to avoid disturbing the pellet [2]. Add 25 microliters of CUT&Tag incubation buffer I [3]. Resuspend the nuclei pellet by gently pipetting ten times [4].
 - 3.3.1. Talent placing the tube containing isolated nuclei in the prechilled centrifuge.
 - 3.3.2. Talent carefully aspirating the supernatant without disturbing the pellet.
 - 3.3.3. Talent adding 25 μL of CUT&Tag incubation buffer I.
 - 3.3.4. Talent gently pipetting ten times to resuspend the nuclear pellet.
- 3.4. Transfer the nuclei-buffer mixture to the tube containing antibody-TAG conjugate using the same pipette tip [1]. Mix gently by pipetting 10 times [2] and incubate the tube on a gentle rotator overnight at 4 degrees Celsius [3].
 - 3.4.1. Talent transferring the nuclei-buffer mixture to tube containing antibody mixture.
 - 3.4.2. Talent gently pipetting ten times to mix the two solutions.
 - 3.4.3. Talent placing the tube on gentle rotator for overnight incubation.
- 3.5. On the next day, to perform CUT&Tag tagmentation, prepare 100 microliters of 1X (*one-X*) CUT&Tag nuclei buffer per reaction and place on ice [1]. After retrieving the

nuclei-antibody mixture, add 5 microliters of CUT&Tag activator [2]. Transfer the mixture into a fresh 1.5- milliliters tube [3] and incubate for one hour at 37 degrees Celsius on the ThermoMixer at 550 rpm [4].

3.5.1. Talent preparing 1X CUT&Tag nuclei buffer on ice.

3.5.2. Talent adding 5 μ L of CUT&Tag activator to the nuclei-antibody mixture.

3.5.3. Talent transferring the mixture into fresh 1.5 mL tube.

3.5.4. Talent placing the tube on the thermomixer set at 550 rpm.

3.6. Next, add 20 microliters of CUT&Tag quench buffer [1], and gently mix the reaction by pipetting 10 times [2]. Centrifuge the tube in the prechilled centrifuge with swing buckets at 500 *g* for 10 minutes [3].

3.6.1. Talent adding 20 μ L of CUT&Tag quench buffer.

3.6.2. Talent gently mixing the reaction by pipetting 10 times.

3.6.3. Talent placing the tube in prechilled centrifuge.

3.7. Remove the supernatant, leaving approximately 5 microliters to avoid disturbing the pellet [1]. Add 100 microliters of 1X CUT&Tag nuclei buffer [2] and resuspend the nuclei by gently pipetting 10 times [3]. Spin the tube in the prechilled centrifuge with swing buckets at 500 *g* for 10 minutes [4].

3.7.1. Talent removing the supernatant without disturbing the pellet.

3.7.2. Talent adding 100 μ L of 1X CUT&Tag nuclei buffer.

3.7.3. Talent gently pipetting 10 times to resuspend the pellet.

3.7.4. Talent placing the tube in prechilled centrifuge.

3.8. Remove 85 microliters of the supernatant, leaving behind approximately 15 microliters [1]. Resuspend the nuclei gently by slowly pipetting 10 times [2]. To count the number of tagged nuclei and determine the targeting number, add 1 microliter of the tagged nuclei suspension into 9 microliters of phosphate-buffered saline [3].

3.8.1. Talent removing 85 μ L of supernatant.

3.8.2. Talent gently pipetting 10 times to resuspend the nuclei.

3.8.3. Talent adding 1 μ L of the tagged nuclei suspension to 9 μ L of phosphate-buffered saline.

3.9. Mix with 10 microliters of acridine orange-propidium iodide staining solution [1] and count the tagged nuclei as per manufacturer's instructions [2].

3.9.1. Talent mixing 10 μ L of AO/PI staining solution.

3.9.2. Talent counting the tagmented nuclei.

3.10. To obtain the desired number of captured nuclei, calculate 1.6 times the target number of nuclei [1]. Spin the tube in the prechilled centrifuge with swing buckets at 500 *g* for ten minutes [2]. Carefully remove the supernatant, leaving a final volume of 8 microliters to obtain optimal concentration of targeting tagmented nuclei required for downstream workflows ranging from single-cell Gel in-bead-emulsion, or GEM (*gem*) generation, library preparation and integrated data analysis for spatial multiomics [3].

3.10.1. Talent calculating the required number of tagmented nuclei for GEM generation.

3.10.2. Talent placing the tube in prechilled centrifuge.

3.10.3. Talent carefully removing the supernatant leaving a final volume of 8 μ L of tagmented nuclei suspension.

Results

4. Results

4.1. The CUT&Tag library analyzed by quantitative polymerase chain reaction, or qPCR (*Q-P-C-R*) to assess the enrichment of histone H-three lysine-twenty seven acetylation showed good enrichment in the constitutively active genomic region such as the promoter of the beta actin or ACTB (*A-C-T-B*) locus [1], over the promoter of repressed genomic loci such as transcription start site, or T1 (*T-one*) and intergenic locus, providing a critical quality control metric for predicting mapping outcomes [2].

4.1.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the number 67.80 in the first row corresponding to column labelled "ACTB/neg".*

4.1.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the number 0.49 in the first row corresponding to column labelled "mT1/neg".*

4.2. The generated spatial map of the individual nuclei assigned back to their spatial locations within the tissue showed a close match with the brain's anatomical features [1]. Joint analysis of spatial gene expression and histone H-three lysine-twenty seven acetylation profiles enabled the identification of major brain cell types [2] and revealed anatomically organized patterns of cellular distribution across the tissue section [3].

4.2.1. LAB MEDIA: Figure 4B. *Video editor: Please emphasize the spatial map of annotated nuclei within the graph.*

4.2.2. LAB MEDIA: Figure 4B. *Video editor: Highlight the labeled major brain cell types shown as legend on the right side of the graph.*

4.2.3. LAB MEDIA: Figure 4B. *Video editor: Highlight the grouped areas of colors spread across different regions within the graph.*

Pronunciation Guide:

1. Trypan

Pronunciation link: <https://www.merriam-webster.com/dictionary/trypan>

IPA: /'trɪpæn/

Phonetic Spelling: trip·an

2. Coronal

Pronunciation link: <https://www.merriam-webster.com/dictionary/coronal>

IPA: /kə'roʊnəl/

Phonetic Spelling: kuh·roh·nuhl

3. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkroʊˌliːtər/
Phonetic Spelling: my·kroh·lee·ter
4. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æspəˌreɪt/
Phonetic Spelling: ass·puh·rayt
5. Trituration
Pronunciation link: <https://www.merriam-webster.com/dictionary/trituration>
IPA: /ˌtrɪtʃəˈreɪʃən/
Phonetic Spelling: trich·uh·ray·shuhn
6. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentɹəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
7. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /ˌriːsəˈspend/
Phonetic Spelling: ree·suh·spend
8. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈnextənt/
Phonetic Spelling: soo·per·nayt·uhnt
9. Acridine
Pronunciation link: <https://www.merriam-webster.com/dictionary/acridine>
IPA: /'ækɹəˌdiːn/
Phonetic Spelling: ak·ruh·deen
10. Propidium
Pronunciation link: <https://www.howtopronounce.com/propidium>
IPA: /proʊˈpɪdiəm/
Phonetic Spelling: proh·pid·ee·uhm
11. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊˈresəns/
Phonetic Spelling: floor·ess·uhns
12. Morphology
Pronunciation link: <https://www.merriam-webster.com/dictionary/morphology>

IPA: /mɔr'fɑ:lədʒi/

Phonetic Spelling: mor·faal·uh·jee

13. Transposase

Pronunciation link: <https://www.howtopronounce.com/transposase>

IPA: /træn'spoʊzeɪs/

Phonetic Spelling: tran·spoh·zays

14. Conjugated

Pronunciation link: <https://www.merriam-webster.com/dictionary/conjugated>

IPA: /'kɑ:ndʒəˌɡeɪtɪd/

Phonetic Spelling: kon·juh·gay·tuhd

15. Antibody

Pronunciation link: <https://www.merriam-webster.com/dictionary/antibody>

IPA: /'æn.tɪˌbɑ:ːdi/

Phonetic Spelling: an·tee·baa·dee

16. Tagmentation

Pronunciation link: <https://www.howtopronounce.com/tagmentation>

IPA: /ˌtægmen'teɪʃən/

Phonetic Spelling: tag·men·tay·shuhn

17. ThermoMixer

Pronunciation link: <https://www.howtopronounce.com/thermomixer>

IPA: /ˌθɜ:moo'mɪksər/

Phonetic Spelling: thur·moh·mik·ser

18. Phosphate-buffered

Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>

IPA: /'fɑ:sˌfɛɪt/

Phonetic Spelling: foss·fayt

19. Multiomics

Pronunciation link: <https://www.howtopronounce.com/multiomics>

IPA: /ˌmʌlti'ɑ:mɪks/

Phonetic Spelling: mul·tee·aa·miks

20. Histone

Pronunciation link: <https://www.merriam-webster.com/dictionary/histone>

IPA: /'hɪstəʊn/

Phonetic Spelling: hiss·tohn