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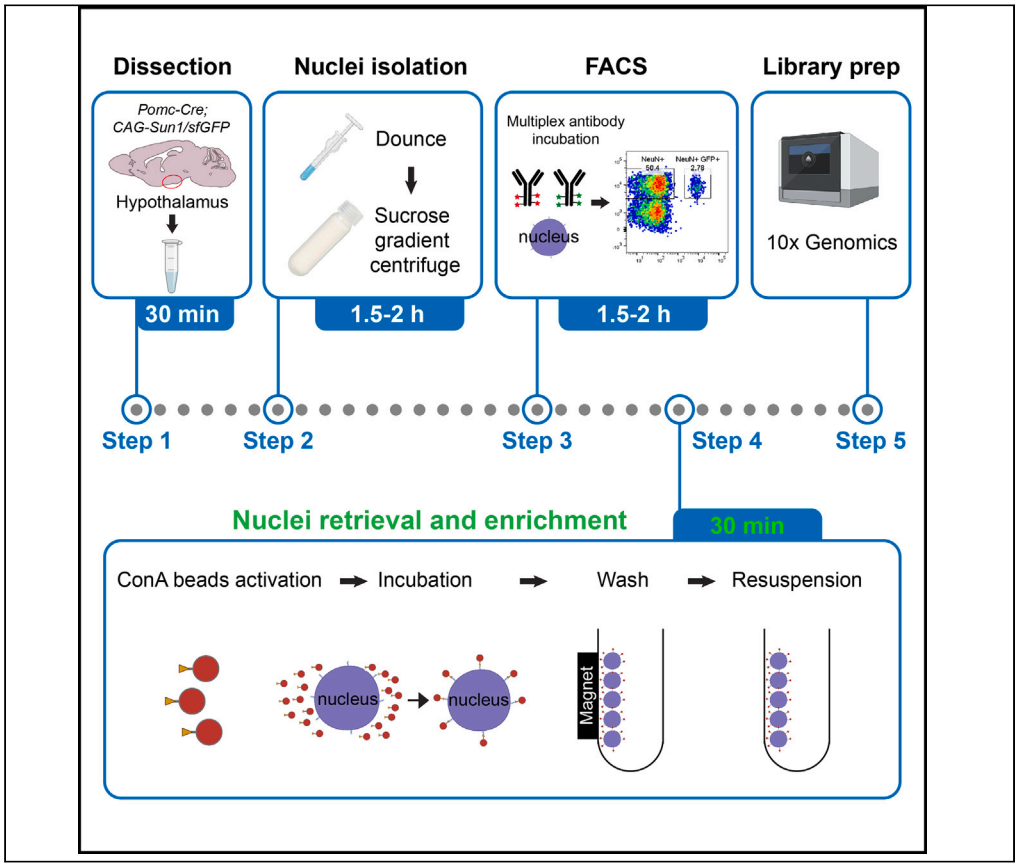
Protocol

Sample enrichment for single-nucleus sequencing using concanavalin A-conjugated magnetic beads



Single-cell/nucleus sequencing has been increasingly used to study specific cell populations. However, cells/nuclei often become diluted during isolation steps and are difficult to reconcentrate through centrifugation. Here, we present a protocol for sample enrichment using concanavalin A-conjugated magnetic beads. We describe steps for dissection, nuclei isolation, and fluorescence-activated cell soring (FACS). We then detail procedures for nuclei enrichment and library preparation. This protocol enables efficient retrieval and enrichment of cells/nuclei following FACS and integrates into existing workflows of various 10× Genomics applications.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

Li Li, Baijie Xu, Chen Liu

li2.li@utsouthwestern.edu (L.L.)
chen.liu@utsouthwestern.edu (C.L.)

Highlights

Steps to capture nuclei/cells using ConA-conjugated magnetic beads

Steps for nuclei isolation and sorting

Detailed steps for nuclei retrieval and enrichment

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Sample enrichment for single-nucleus sequencing using concanavalin A-conjugated magnetic beads

Li Li,[1,4,5,*] Baijie Xu,[1,4] and Chen Liu[1,2,3,6,*]

[1]The Hypothalamic Research Center, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX 75390, USA

[2]Department of Neuroscience, UT Southwestern Medical Center, Dallas, TX 75390, USA

[3]Peter O'Donnell Jr. Brain Institute, UT Southwestern Medical Center, Dallas, TX 75390, USA

[4]These authors contributed equally

[5]Technical contact

[6]Lead contact

*Correspondence: li2.li@utsouthwestern.edu (L.L.), chen.liu@utsouthwestern.edu (C.L.)
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SUMMARY

Single-cell/nucleus sequencing has been increasingly used to study specific cell populations. However, cells/nuclei often become diluted during isolation steps and are difficult to reconcentrate through centrifugation. Here, we present a protocol for sample enrichment using concanavalin A-conjugated magnetic beads. We describe steps for dissection, nuclei isolation, and fluorescence-activated cell sorting (FACS). We then detail procedures for nuclei enrichment and library preparation. This protocol enables efficient retrieval and enrichment of cells/nuclei following FACS and integrates into existing workflows of various 10× Genomics applications.

BEFORE YOU BEGIN

Concanavalin A (ConA) is a lectin that binds to glycoproteins on the surfaces of cells and nuclei.[1,2] Using ConA-conjugated paramagnetic beads for cell/nucleus enrichment provides several advantages. First, it enables the capture of a large number of cells or nuclei, facilitating subsequent re-suspension in a smaller volume. The increased concentration and yield reduce the need for sample pooling, thereby saving both time and cost. Second, compared to centrifugation, isolation with magnetic beads is much gentler, minimizing the loss or damage to cells/nuclei. Third, the ConA purification method seamlessly integrates into the workflows of most microfluid-based sequencing platforms including various 10× Genomics applications as well as split-pool ligation-based sequencing (SPLiT-seq).

Below, we outline the sample preparation steps for a 10× Genomics multiome (ATAC+ gene expression) study of *Pomc-Cre* expressing neurons in the hypothalamus of *Pomc-Cre;CAG-Sun1/sfGFP* mice. ConA-conjugated magnetic beads were utilized to enrich nuclei following fluorescence-activated cell sorting (FACS), enabling the retrieval of over 90% of the sorted nuclei. Additionally, the protocol incorporates several critical steps[3,4] that facilitate sample enrichment, multiplexing, and data analyses that we routinely use for single-nucleus sequencing (Figure 1). It is worth noting that we and others have successfully used this protocol to isolate nuclei from multiple brain regions. Moreover, the same protocol can also be used for purifying cells/nuclei from other tissues. Nonetheless, we recommend that researchers validate its suitability for their specific cell type.

Institutional permissions

All procedures were conducted according to the UTSW Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

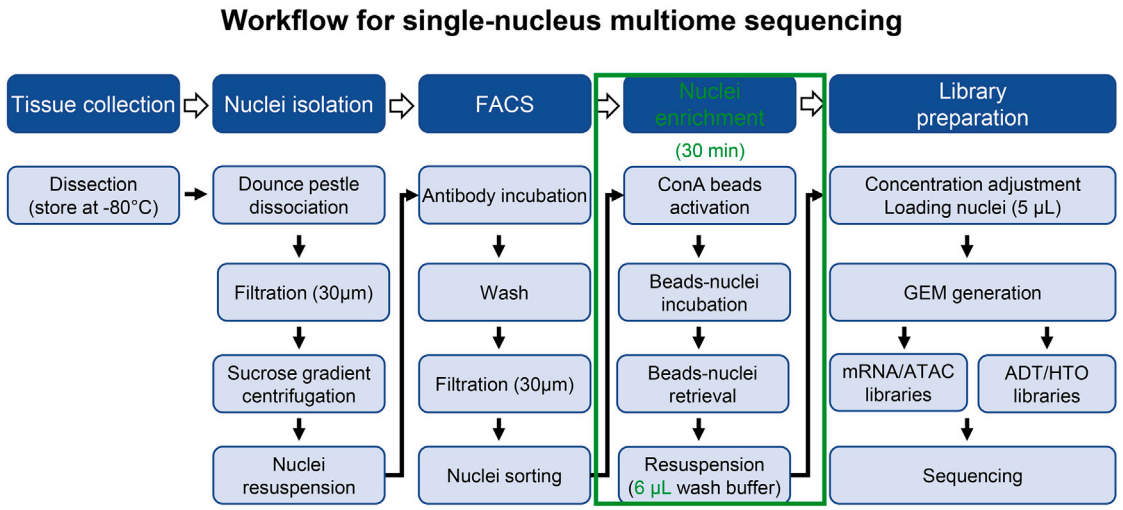

Figure 1. A flowchart for sample preparation for single-nucleus multiome sequencing

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
NeuN antibody (1:100)	BioLegend	Cat #834501
TotalSeq-A1130 anti-GFP Cite-seq Antibody (1:100)	BioLegend	Cat #900003153
TotalSeq-A0451 anti-NPC Hashtag 1 Antibody (1:200)	BioLegend	Cat #682205
TotalSeq-A0452 anti-NPC Hashtag 2 Antibody (1:200)	BioLegend	Cat #682207
TotalSeq-A0453 anti-NPC Hashtag 3 Antibody (1:200)	BioLegend	Cat #682209
TotalSeq-A0454 anti-NPC Hashtag 4 Antibody (1:200)	BioLegend	Cat #682211
Chemicals, peptides, and recombinant proteins		
1 x CUTANA Concanavalin A Conjugated Paramagnetic Beads	EpiCypher	Cat #21-1401
Tris hydrochloride solution, 1 M	Sigma-Aldrich	Cat #T2194
DTT solution	Sigma-Aldrich	Cat #646563
Magnesium chloride solution, 1 M	Sigma-Aldrich	Cat #M1028
Sodium chloride solution, 5 M	Sigma-Aldrich	Cat #59222C
BSA 10%	Sigma-Aldrich	Cat #126615
RNase inhibitor	Sigma-Aldrich	Cat #3335402001
cOmplete, EDTA-free Protease Inhibitor Cocktail Tablet	Sigma-Aldrich	Cat #11873580001
1 M HEPES solution	Sigma-Aldrich	Cat #H0887
1 M potassium chloride solution	Sigma-Aldrich	Cat #60142
0.5 M calcium chloride solution	Sigma-Aldrich	Cat #C-34006
1 M manganese(II) chloride solution	Sigma-Aldrich	Cat #M1787
DPBS	Invitrogen	Cat #14190-144
DEPC-treated H_2O	Invitrogen	Cat #AM9922
Sucrose	Fisher Chemical	Cat #S5-500
Critical commercial assays		
Single cell multiome ATAC + gene expression	10× Genomics	Cat #1000285
Chromium Next GEM Chip J single cell	10× Genomics	Cat #1000230
Deposited data		
Single cell multiome RNA + ATAC Alzheimer's disease mouse model brain coronal sections from one hemisphere over a time course	10× Genomics	https://www.10xgenomics.com/resources/datasets/multiomic-integration-neuroscience-application-note-single-cell-multiome-rna-atac-alzheimers-disease-mouse-model-brain-coronal-sections-from-one-hemisphere-over-a-time-course-1-standard

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Mouse brain nuclei isolated with Chromium Nuclei Isolation Kit, SaltyEZ protocol, and 10× complex tissue DP (CT sorted and CT unsorted)	10× Genomics	https://www.10xgenomics.com/resources/datasets/mouse-brain-nuclei-isolated-with-chromium-nuclei-isolation-kit-saltyez-protocol-and-10x-complex-tissue-dp-ct-sorted-and-ct-unsorted-1-standard
Experimental models: Organisms/strains		
Mouse: Pomc-Cre: Tg(Pomc1-cre)16Lowl/J	Jackson Laboratory	RRID: IMSR_JAX:005965
Mouse: CAG-Sun1/sfGFP B6.129-*Gt(ROSA)26Sor*$^{tm5.1(CAG-Sun1/sfGFP)Nat}$/MmbeJ	Jackson Laboratory	RRID: IMSR_JAX:030952
Software and algorithms		
Cellranger-arc	10× Genomics	https://www.10xgenomics.com
Seurat - R	GitHub	https://github.com/satijalab/seurat
Prism	GraphPad	https://www.graphpad.com
FlowJo	BD Biosciences	https://www.flowjo.com
scCustomize	GitHub	https://samuel-marsh.github.io/scCustomize/
Other		
10 µL extra-long SHARP filter tip	Thermo Fisher Scientific	Cat #P1096-FR
Nalgene Oak Ridge Polysulfone Centrifuge Tubes w/Screw Caps, 50 mL	Thermo Fisher Scientific	Cat #3115-0050PK
Magnetic separation rack, 0.2 mL tubes	EpiCypher	Cat #10-0008
Magnetic separation rack, 1.5 mL tubes	EpiCypher	Cat #10-0012
Dounce tissue grinder pestle 2 mL set	Sigma-Aldrich	Cat #P1110
Dounce tissue grinder tube 2 mL set	Sigma-Aldrich	Cat #T2690
Pre-separation filters (30 µm)	Miltenyi Biotec	Cat #130-041-407
High-speed centrifuge	Thermo Fisher Scientific	Legend RT
Swinging bucket centrifuge	Thermo Fisher Scientific	Heraeus Multifuge 3s
Fluorescent stereomicroscope	Leica	MZ10 F
Falcon 15 mL conical centrifuge tubes	Thermo Fisher Scientific	Cat #14-959-53A
Falcon 50 mL high clarity conical centrifuge tubes	Thermo Fisher Scientific	Cat #14-432-22
Unique link: https://star-methods.com/?rid=KRT648cb73306a64		

MATERIALS AND EQUIPMENT

- **2 M sucrose solution**: dissolve 342.3 g sucrose in 250 mL DEPC-treated H_2O, and then add additional H_2O until the final volume is 500 mL. The solution can be stored at 4°C for up to 1 year following filter sterilization.
- Protease inhibitor cocktail (50× conc.): dissolve 1 tablet EDTA-free protease inhibitor cocktail into 1 mL of DEPC-treated H_2O. The solution can be stored at -20°C for up to 3 months.

Sucrose Cushion buffer		
Reagent	Final concentration	Amount
2 M Sucrose	1.8 M	45 mL
1 M Tris-HCl	10 mM	0.5 mL
1 M $MgCl_2$	3 mM	0.15 mL
5 M NaCl	10 mM	0.1 mL
1 M DTT	1 mM	0.05 mL
10% BSA	0.5%	2.5 mL
RNase inhibitor (40 U/µL)	0.1 U/µL	0.125 mL
Protease inhibitor cocktail stock (50×)	1×	1 mL
DEPC-treated H_2O	N/A	0.575 mL
Total	**N/A**	**50 mL**
Sucrose Cushion buffer should be prepared fresh, filtered, and kept on ice throughout the experiment.		

Homogenization buffer		
Reagent	Final concentration	Amount
2 M Sucrose	0.32 M	8 mL
1 M Tris-HCl	10 mM	0.5 mL
1 M $MgCl_2$	3 mM	0.15 mL
5 M NaCl	10 mM	0.1 mL
1 M DTT	1 mM	0.05 mL
10% BSA	0.5%	2.5 mL
Tween 20	0.0002%	0.01 mL
RNase inhibitor (40 U/μL)	0.1 U/μL	0.125 mL
Protease inhibitor cocktail solution (50×)	1×	1 mL
DEPC-treated H_2O	N/A	37.565 mL
Total	**N/A**	**50 mL**
Homogenization buffer should be prepared fresh, filtered, and kept on ice throughout the experiment.		

Wash buffer		
Reagent	Final concentration	Amount
1 M DTT	1 mM	0.05 mL
10% BSA	0.5%	2.5 mL
Tween 20	0.0002%	0.01 mL
RNase inhibitor (40 U/μL)	0.1 U/μL	0.125 mL
Protease inhibitor cocktail solution (50×)	1×	1 mL
DPBS	N/A	46.315 mL
Total	**N/A**	**50 mL**
Wash buffer should be prepared fresh, filtered, and kept on ice throughout the experiment.		

ConA-beads Activation buffer		
Reagent	Final concentration	Amount
1 M HEPES	20 mM	1 mL
1 M KCl	10 mM	0.5 mL
0.5 M $CaCl_2$	1 mM	0.1 mL
1 M $MnCl_2$	1 mM	0.05 mL
DEPC-treated H_2O	N/A	48.35 mL
Total	**N/A**	**50 mL**
This buffer can be stored at 4°C for up to 1 year following filter sterilization.		

STEP-BY-STEP METHOD DETAILS

Tissue collection

Timing: 30 min

We use 4-week-old *Pomc-Cre;CAG-Sun1/sfGFP* mice of both sexes for this experiment. Following the protocol, we typically obtain approximately 2,000 *Pomc-Cre+* nuclei per animal. We also use nuclei isolated from two wild-type and one additional *Pomc-Cre;CAG-Sun1/sfGFP* mice as blank or single-color controls during the FACS step.

1. Perform cervical dislocation on deeply anesthetized (using 5% isoflurane) mice.
2. Quickly dissect the hypothalamus and place it under a fluorescence stereomicroscope.

Note: For a comprehensive guide on the dissection of the mouse hypothalamus, please refer to this video (jove.com/video/65674).[5]

3. Under the GFP channel, isolate the tissue containing the GFP+ *Pomc-Cre* neurons from individual *Pomc-Cre;CAG-Sun1/sfGFP* mice and promptly transfer it onto dry ice.
4. Collect the tissue from a similar region in wild-type mice without GFP fluorescence and promptly transfer it onto dry ice.

Pause point: Store the brain tissues in a −80°C freezer.

Isolating nuclei from dissected hypothalamic tissues

Timing: 1.5–2 h

5. Prepare 50 mL of Sucrose Cushion buffer in a 50 mL Falcon tube.
6. Add 8 mL of Sucrose Cushion buffer to individual 50 mL Nalgene centrifuge tubes.

Note: Keep the tubes on ice throughout the experiment.

7. Prepare 50 mL of Homogenization buffer in a 50 mL Falcon tube.

Note: Keep the tube on ice throughout the experiment.

8. Add 1 mL of Homogenization buffer into individual 2 mL Dounce tissue grinder tubes.

Note: Keep the tubes on ice throughout the experiment.

9. Power on the Sorvall RC6 Plus superspeed centrifuge and set the temperature to 4°C.
10. Place the dissected brain tissues into individual grinder tubes.

Note: Tissues from 4–5 *Pomc-Cre;CAG-Sun1/sfGFP* mice can be pooled into one grinder tube.

11. Dounce the brain tissues in individual grinder tubes 20 times using a Dounce grinder pestle.
12. Place a 30 μm pre-separation filter over a 15 mL Falcon tube.
    a. Soak the filter with 0.5 mL of Homogenization buffer.
    b. Extract approximately 1 mL of tissue homogenate from each grinder tube and filter it through the 30 μm filter into the 15 mL Falcon tube.
    c. Rinse individual grinder tubes with 1.25 mL of Homogenization buffer. Collect the liquid with a pipette and filter it through the 30 μm filter into the 15 mL Falcon tube. Repeat once.
    d. Gently and slowly transfer the filtered homogenate (~4 mL) from the 15 mL Falcon tube into individual Nalgene centrifuge tubes containing the Sucrose Cushion buffer.
13. Balance the tubes and centrifuge them at 47,000 g for 1 h at 4°C.
14. After centrifugation:
    a. Use a pipette to carefully discard the top and middle layers of the liquid (Figure 2A).
    b. Change the pipette tip and carefully discard the remaining liquid without disturbing the nuclei pellet.

Note: Do not pour the liquid.

15. Prepare 50 mL of Wash buffer in a 50 mL Falcon tube.

Note: Keep the tube on ice throughout the experiment.

16. Resuspend the nuclei with 100 μL of Wash buffer and transfer them into a new 15 mL Falcon tube.

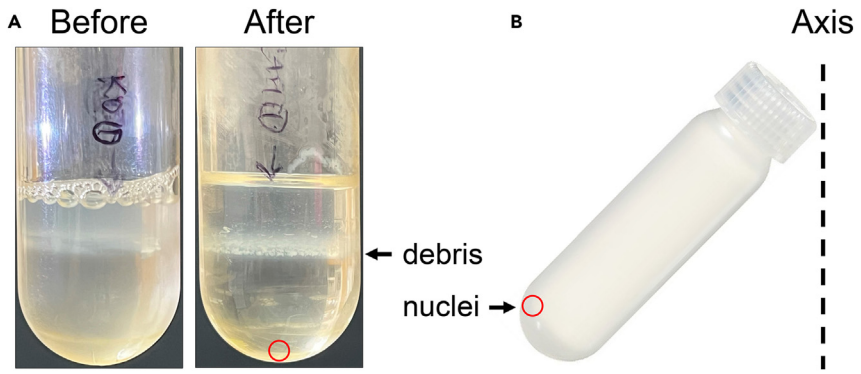

Figure 2. Nuclei isolation using sucrose gradient centrifugation

(A) Representative images showing tissue homogenate before (left) and after (right) sucrose gradient centrifugation. The white layer in the middle indicates cellular debris.

(B) It is helpful to label the expected location (red circle) of the nuclei pellet before centrifugation.

Antibody incubation (optional)

Timing: 30 min

Before conducting FACS, we incubate the nuclei isolated from *Pomc-Cre;CAG-Sun1/sfGFP* mice with several antibodies to enhance sample enrichment, enable multiplexing, and support data analyses. For example, we have discovered that FACS by GFP alone is inadequate for excluding nuclei from other cell types. This could be due to potential leakage of the *Pomc-Cre* transgene in non-neuronal cells during early development, as noted in a recent study.[6] Therefore, we employ an anti-NeuN-Alexa Fluor 647 antibody to selectively label *Pomc-Cre+* nuclei from neurons. We find that the dual color sorting approach, utilizing both Fluor 647 and GFP, improves the enrichment of neuronal nuclei while effectively reducing those from non-neuronal cells. Furthermore, we incorporate a multiplexing strategy[7] by utilizing barcoded antibodies against nuclear pore complex (NPC) proteins, which are present on the surfaces of nuclei. This multiplexing enables us to analyze nuclei from different groups, such as batch or sex, and facilitates the identification and removal of doublets (samples with barcodes from two different anti-NPC antibodies) during quality control steps.[8] Additionally, we use a barcoded anti-GFP antibody to specifically label GFP+ *Pomc-Cre* nuclei. This allows us to track and analyze this particular group during data analyses.[9]

17. Add 1 μL of anti-NeuN-Alexa Fluor 647 antibody, 0.5 μL of each barcoded anti-NPC antibody, and 1 μL of barcoded TotalSeq anti-GFP antibody to the nuclei isolated from *Pomc-Cre;CAG-Sun1/sfGFP* mice.
    a. Add 1 μL of anti-NeuN-Alexa Fluor 647 antibody to the nuclei isolated from one wild-type mouse, which will be used as a single-color (Alexa Fluor 647) control during FACS.

Note: Untreated nuclei from the other wild-type mouse and one *Pomc-Cre;CAG-Sun1/sfGFP* mouse will serve as a blank and another single-color (GFP) control, respectively.

Note: We recommend using extra-long pipette tips for this step.

18. Mix the nuclei and antibodies well and incubate the mixture on ice for 10 min.
19. Wash the nuclei by adding 3 mL Wash buffer and precipitate them by centrifugation using a swinging bucket centrifuge at 500 g for 5 min at 4°C.
20. Carefully remove the liquid using a pipette and repeat the washing step two more times.

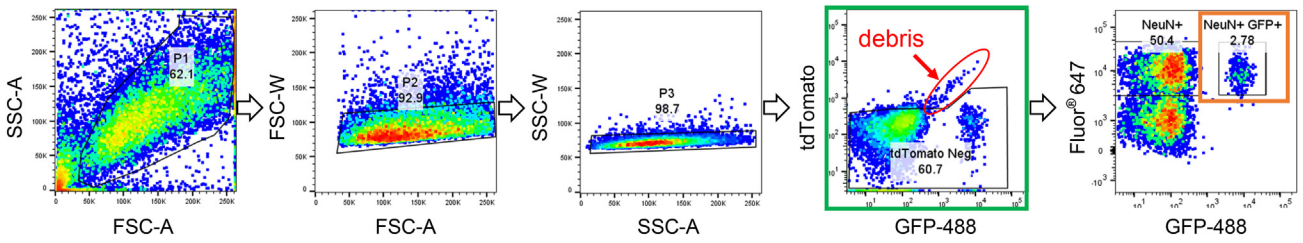

Figure 3. Gating threshold for dual-color sorting of *Pomc-Cre* neurons

Cellular debris can produce autofluorescence signals detectable across multiple fluorescent channels, such as tdTomato or DAPI. We added a negative gating step (green box) to identify and exclude debris from the collection process. Notably, the nuclei of *Pomc-Cre* neurons do not generate tdTomato fluorescence. Therefore, any fraction showing strong tdTomato signals (red oval) can be attributed to cellular debris, which we deliberately exclude from the final collection of dual-color NeuN+/GFP+ *Pomc-Cre* nuclei (orange box).

21. Resuspend nuclei with 100 μL of Wash buffer, pool all the antibody-treated nuclei from *Pomc-Cre;CAG-Sun1/sfGFP* mice, and filter them through a 30 μm filter into a BD sorting tube. Similarly, filter the nuclei designated for sorting controls and place them into separate sorting tubes.

Purifying nuclei through dual-color FACS

Timing: 1.5–2 h

22. Use the nuclei for blank, single-color (Alexa Fluor 647 or GFP) controls to set the gating threshold.

Note: Cellular debris generates autofluorescence and therefore can be mistakenly collected as nuclei during FACS. Although dyes such as DAPI are commonly used to differentiate nuclei from debris, they can intercalate with the DNA and disrupt chromatin structure, leading to lower data quality.[10] To minimize contamination, we use a negative gating strategy to identify and exclude them. Please see more details in Figure 3.

23. Sort the dual-color nuclei (Alexa Fluor 647 and GFP) into a 1.5 mL Eppendorf tube containing 100 μL of Wash buffer.

Retrieving and enriching nuclei using ConA-beads

Timing: 30 min

24. Thoroughly mix the ConA-beads solution (Figure 4A).
25. Add 11 μL of ConA-beads to a new 0.2 mL PCR tube and place it on ice.
26. Activate the ConA-beads by adding 100 μL of ConA-beads Activation buffer. Mix the solution using a pipette.
27. Incubate the solution on ice for 15 min.
28. Place the PCR tube on a magnet rack (in front of a magnet, Figure 4B). Wait for 1–2 min until the slurry clears and then remove the liquid using a pipette.
29. Repeat steps 26–28 one more time.
30. Resuspend the activated beads with 11 μL of Wash buffer and place the tube on ice.
31. Extract 10 μL of the activated beads and add them into the Eppendorf tube with the sorted nuclei.
32. Incubate on ice for 10 min.
33. Place the tube on a magnet rack (in front of a magnet) for 1–2 min until the slurry clears and then remove the liquid with a pipette.

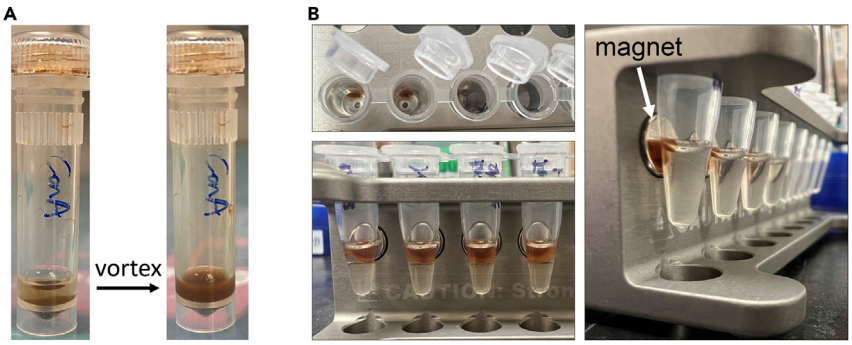

Figure 4. Retrieval and enrichment of FACS-sorted nuclei using ConA-beads

Note: Do not let the nuclei/beads dry out.

34. Resuspend the nuclei/beads with 6 μL of Wash buffer. Gently mix well on ice.
35. Use 1 μL of the nuclei/beads mixture to determine the concentration of the nuclei.

Note: Add DAPI or use GFP signals for counting nuclei.

36. Adjust the concentration to 1,000–5,000 nuclei/μL.
37. Proceed with the 10× Single-nucleus Multiome pipeline using 5 μL of purified nuclei.

EXPECTED OUTCOMES

The ConA-beads method significantly improved the retrieval of the sorted nuclei compared to conventional centrifugation techniques. With the ConA-beads, we achieved a nuclei recovery rate of 92.75% ± 7.05% (Figure 5A), whereas centrifugation yielded only 32.75 ± 8.02%. As a result, the ConA-beads method enabled us to obtain a higher concentration of nuclei (1,563 ± 120 vs. 551 ± 135 nuclei/μL) after resuspension (Figure 5B). This improvement is particularly critical considering the input constraint for the 10× Genomics Multiome Assay (a maximum of 5 μL). Thus, the ability to load more nuclei not only helps enhance data quality but also reduces the time and cost associated with sample pooling.

Additionally, we investigated the use of different amounts of ConA-beads (10 μL, 20 μL, 40 μL, and 80 μL) for nuclei retrieval and enrichment (related to step 31). We loaded various beads/nuclei

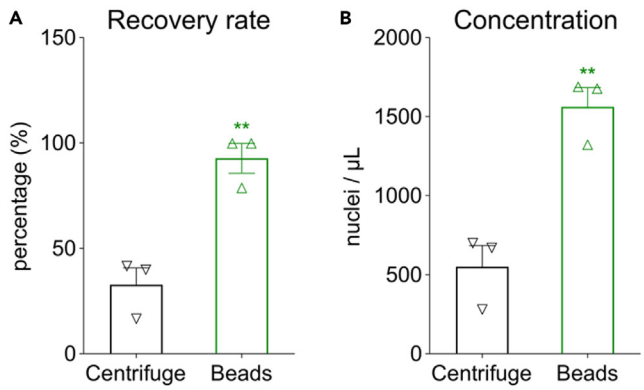

Figure 5. A comparison between the centrifuge- and ConA-beads-based methods

(A) the recovery rate of sorted nuclei.

(B) nuclei concentration after enrichment. n = 3 independent trails, unpaired t-test, **p < 0.01. Data are represented as mean ± SEM.

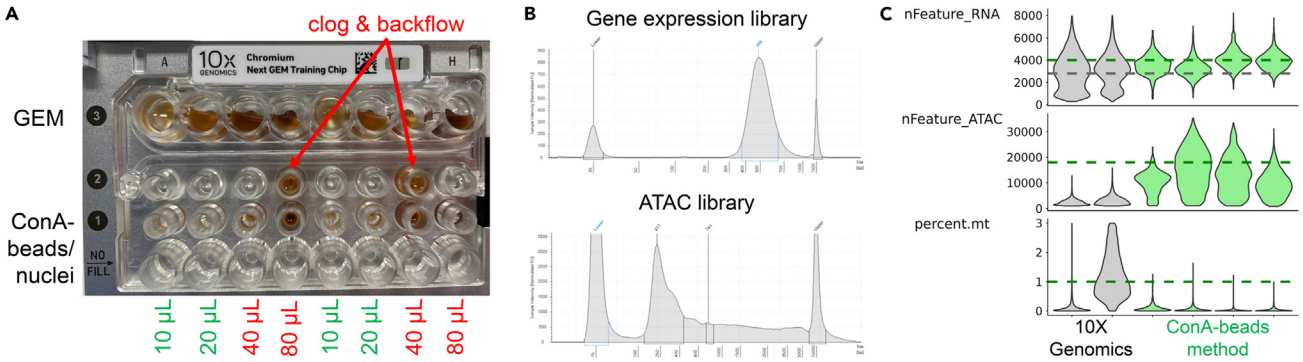

Figure 6. Generation of high-quality multiome sequencing data using the ConA-beads method

(A) GEM production using the ConA-beads/nuclei mixture. Notably, using 10 or 20 µL of ConA-beads did not cause clogging or backflow.

(B) Representative quality control data for gene expression (top) and ATAC (bottom) libraries generated using the ConA-beads method.

(C) A comparison of multiome sequencing results obtained through the standard 10× Genomics protocol and the ConA-beads method for the detection of genes (top), ATAC peaks (middle), and mitochondrial sequences (bottom). The source data for the 10× Genomics results are obtained from the manufacturer's website.

mixtures onto the 10× Genomics Next GEM Training Chip and followed the manufacturer's instructions for GEM production (Figure 6A). We found that using 10 µL or 20 µL of ConA-beads resulted in successful GEM generation without any issues of clogging or backflow. With only 10 µL ConA-beads, we were able to generate high-quality 10× Multiome mRNA and ATAC libraries (Figure 6B). Following multiome sequencing, we detected an average of 3,644 ± 185 genes and 13,547 ± 1,273 ATAC peaks per nucleus at 84% sequencing saturation, surpassing those generated using standard protocols (Figure 6C).

LIMITATIONS

It has been reported that ConA exposure can potentially influence gene expression in immune cells.[11] Moreover, the impact of ConA on other cell types, including neurons, remains to be determined. As a result, the ConA-beads approach is most suitable for purifying nuclei or fixed cells.

Additionally, we have observed that ConA-beads may lose their activity when stored at −80°C. To ensure the best results, we recommend avoiding freezing the beads/nuclei mixture. It is advisable to use freshly prepared ConA beads for each experiment to maintain their optimal performance.

TROUBLESHOOTING

Problem 1

The nuclei pellet is not visible following sucrose gradient centrifugation (related to step 14).

Potential solution

When working with small tissue samples, there might not be enough nuclei to form a sizable pellet. In such cases, it can be helpful to mark the centrifuge tube to indicate the expected location of the pellet before centrifugation (Figure 2B).

Problem 2

The nuclei yield is low after FACS.

Potential solution

This could be due to contamination from cellular debris. To address this, we recommend the following steps.

- Remove the top and middle layers as thoroughly as possible to minimize debris contamination (related to step 14).
- Dilute the sorting solution to reduce the concentration of the debris.
- Consider lowering the sorting threshold to increase the sensitivity of the process and improve the capture of nuclei.

Problem 3

GFP-negative nuclei or debris are present in the sorted nuclei.

Potential solution

We suggest the following steps:

- Ensure that the sorter is accurately calibrated to achieve optimal sorting performance.
- It is important to note that some large debris particles can have a similar size to nuclei and exhibit autofluorescence in all channels. We use a negative gating strategy (Figure 3) to identify and exclude them during the sorting process.

Problem 4

The quality of libraries is low using ConA beads.

Potential solution

To address this issue, we recommend the following steps:

- Whenever feasible, perform all procedures on ice, including the beads capture step (related to step 32). Additionally, ensure that all buffers used throughout the process contain RNase and protease inhibitors to protect the RNA from degradation.
- The ConA-beads Activation buffer contains Mn^{2+} ions, which may disrupt the activity of enzymes used for library preparation. Therefore, it is crucial to completely replace the ConA-beads Activation buffer with the Wash buffer (related to step 30) before conducting the nuclei enrichment step.
- During the washing step (related to step 33), it is important to prevent the beads from drying out.

Problem 5

Clogging during GEM generation using ConA beads.

Potential solution

Using 10 μL of ConA beads is sufficient to capture at least 50,000 nuclei, and we have not encountered any clogging problems when using this amount (related to step 31). However, as shown in Figure 6A, adding an excessive amount of ConA beads (>20 μL) may lead to blockage of microfluidic channels and subsequent backflow. Thus, we recommend using no more than 20 μL of ConA beads for nuclei enrichment to avoid any potential issues with clogging and to ensure optimal performance.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Chen Liu (chen.liu@utsouthwestern.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article includes all the data generated during this study.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

L.L., B.X., and C.L. wrote and edited the article.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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