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Slide-tags snRNA-seq Protocol (v.1.2 TAGS)

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We use this protocol and it's working

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Abstract

This protocol is for use with TAGS V9 beads, and snRNA-seq profiling of tagged nuclei on the 10x Genomics Chromium Single Cell Gene Expression 3' v3.1 platform. The extraction buffers largely derive from an earlier protocol for brain nuclei extraction: **dx.doi.org/10.17504/protocols.io.7xchpiw**, which itself is derived from an earlier study: Carter, Bean (2009) Neuron https://doi.org/10.1016/j.neuron.2009.12.011. More details on the Slide-tags protocol can be found in the preprint: https://doi.org/10.1101/2023.04.01.535228 or published paper: https://doi.org/10.1038/s41586-023-06837-4. This protocol has been verified on a variety of mouse and human tissues, but if you already have an established nuclei isolation from fresh frozen tissues protocol in your lab, you may want to perform a comparison test to ensure maximum yield and data quality. Furthermore, prior to your first Slide-tags experiment, it is recommended to perform a nuclei yield optimisation experiment (instructions can be found in the appendix).

Preparation

1 Equilibrate the following in the cryostat:
Tissue
Tissue punches
Tweezers
Brushes
Tissue chuck

2 Set a swing-bucket centrifuge that can accommodate 50 mL falcon tubes to 4 ℃.

3 Prepare the following buffers and keep on ice:
Dissociation buffer

	Molecular weight	Final concentration (mM)	for 500 mL	Cat no.
Ultrapure nuclease free water	NA	NA	500 mL	10977015 (Invitrogen)
Na2SO4	142.04	82	5.83 g	BDH9302 (VWR)
K2SO4	174.26	30	2.615 g	60528 (Sigma-Aldrich) OR BDH4618 (VWR)
Glucose	180.2	10	0.905 g	49139 (Sigma-Aldrich) OR G5802 (Teknova)
HEPES free acid	238.3	10	1.2 g	0511 (VWR)
MgCl2 (1 M)	203.31	5	2.5 mL (1 M stock)	AM9530G (Invitrogen)

This buffer was originally developed for dissociating neurons and described in: Carter BC, Bean BP. Sodium entry during action potentials of mammalian neurons: incomplete inactivation and reduced metabolic efficiency in fast-spiking neurons. Neuron. 2009;64(6):898–909. doi:10.1016/j.neuron.2009.12.011

Extraction buffer (2 mL per sample)

	Stock concentration / Molecular weight	Final concentration	For 15 mL (2 mL needed per sample)	Cat no.
Dissociation buffer	NA	NA	15 mL	-
Kollidon VA 64	45,000-70,000	1 %	0.15 g	30499398 (BASF)

100% TX-100	100 %	1 %	150 µL	T9284 (Sigma)
10% BSA*	10 %	0.04 %	60 µL	B4287 (Sigma) OR 126615 (Sigma)
NxGen RNase inhibitor †	40,000 U/mL	667 U/mL	250 µL (1 tube)	30281-2 (Lucigen)

* We have increased BSA concentrations to 0.04 % without any effect on data quality. 10x recommends up to 1 % to reduce clumping. This has not been thoroughly tested but may help if preparations show clumping.
† We have not extensively tested other RNase inhibitors, and so recommend using this part number. However, if you do choose to use an alternative, please refer to this article regarding compatibility with 10x Genomics: https://kb.10xgenomics.com/hc/en-us/articles/360049543672-Can-I-use-an-alternative-RNase-inhibitor-part-number-

Wash buffer (20 mL per sample)

	Stock concentration / Molecular weight	Final concentration	For 20 mL (20 mL needed per sample)	Cat no.
Dissociation buffer	NA	NA	20 mL	-
10 % BSA*	10 %	0.04 %	80 µL	B4287 (Sigma) OR 126615 (Sigma)
NxGen RNase inhibitor	40,000 U/mL	100 U/mL	50 µL	30281-2 (Lucigen)

* We have increased BSA concentrations to 0.04 % without any effect on data quality. 10x recommends up to 1 % to reduce clumping. This has not been thoroughly tested but may help if preparations show clumping.
† We have not extensively tested other RNase inhibitors, and so recommend using this part number. However, if you do choose to use an alternative, please refer to this article regarding compatibility with 10x Genomics: https://kb.10xgenomics.com/hc/en-us/articles/360049543672-Can-I-use-an-alternative-RNase-inhibitor-part-number-

4 1. Set up a 12-well plate on ice at a slight angle, and prepare 3 ice buckets:
- Tagging (An aliquot of DB, a slide plate with ice in one compartment and a slide on top of the ice)
- Extraction (with your extraction plate, extraction buffer, plate lid for transfer of pucks)
- Wash buffers (50 mL falcon tubes with wash buffer in for transportation to centrifuge without disturbing pellet)

5 Mount tissue in crytostat with OCT.

6 Using tweezers, prepare pucks by placing them on non-coated slides, with the puck surface facing up. Keep these at room temperature and covered with a plate lid to minimize dust/debris contamination until use.

Tagging

7 Perform diagnostic H&E/Nissl on 10/20 µm section to locate the region of interest. In some tissues, you may be able to locate the region of interest without staining the slide. Often, we also take a 20 µm prior to the section being profiled with slide-tags, for later staining. Take this section and leave in the cryostat or place on dry ice. Store at - 80 oC until required.

8 (optional) If the block of tissue is not particularly precious, you can use a biopsy punch to core-out a specific region in the tissue block.
!ENSURE you lock the cryostat wheel before performing this action.
If you are using precious (or human) tissue, this is best avoided, since it is difficult to precisely control depth. In the case of precious or human tissues, we will punch-biopsy the tissue section instead.

9 Section a 20 µm tissue section, and punch into the section on the cryostat stage if the section is larger than the puck.

10 1. Place slide-tags puck sitting on glass slide into cryostat onto the cryostat stage. Leave to equilibrate for at least 30 s.
!DO NOT put puck on ice before experiment - this will create condensation which can freeze and dislodge beads and affect tissue adherence.

11 Carefully melt the section onto puck by heating from below with your finger until the section becomes translucent.

12 Immediately transfer slide and puck onto slide plate containing ice and slide and add 5 µL (3 mm puck) OR 10 µL (5.5x5.5 puck) of DB to the tissue section. Ensure that the entire tissue section is covered, you may need to add more DB.

13 Photocleave pucks on ice for 30 seconds under UV light.

14 Incubate tissue on photocleaved puck on ice for 7.5 mins to allow diffusion.

15 After 7.5 mins, transfer the puck into 6-well plate (still on ice).

Nuclei isolation

16 Add 2 mL of extraction buffer to the well and ensure the puck is submerged in solution.

17 Gently pipette up and down 200 ul volume on the puck until tissue is **fully** released. Minimize bead loss as much as possible by pipetting orthogonally to the puck and be careful to not touch the puck surface at all. Number of pipettes necessary varies greatly by tissue, but generally 20-80 pipettes is sufficient. The puck can be inspected under a microscope (4x objective, brightfield) every ~20 pipettes or so. Ensure this is done quickly (<30 s) and time off-ice is minimized in order to prevent the sample heating which results in nuclei loss.

18 Once tissue is fully released from puck, remove puck from well with **sterilized** tweezers.

19 Finish tissue dissociation by mixing sample 10X with P1000
(can afford more pipettes if bead loss is low).

20 Transfer the dissociated sample into an empty 50 mL falcon tube

21 Wash around well 3X with 1 mL wash buffer to assure all nuclei are collected

22 Pour isolated nuclei into 50 mL falcon tube containing 20 mL wash buffer

23 Then split wash buffer - nuclei mix by pouring half of the mix back into the original 50 mL falcon tube
- If bead loss is extreme, first place dissociated sample in eppendorf, let beads settle to bottom of tube for 10 mins, then collect supernatant (leaving <50 ul in tube) and move to 20 mL wash buffer

24 Centrifuge at 600g for 10 mins at 4 °C

25 Remove supernatant and leave 500 ul pellet in each tube

26 Wet 40 µm filter with DB

27 Pool pellets by passing through 40 um filter into eppendorf to make 1 mL

28 Add 1:1000 DAPI and incubate for 10 mins
- If bead loss is extreme, let beads settle during DAPI incubation, and move supernatant to new eppendorf (leaving 50 ul beads in eppendorf)

29 Centrifuge at 200 g for 10 mins at 4C (place eppendorf in 15 mL tube with kimwipe)

30 Remove supernatant and leave 50 ul pellet

31 Count nuclei by taking 2 ul, diluting in 18 ul dissociation buffer (1:10), and loading 20 ul on hemocytometer

10X Genomics Nuclei capture, barcoding, and library preparation

32 Load 43.3 ul into 10x chromium using the protocol: Chromium Next GEM Single Cell 3' Reagent Kits v3.1(Dual Index) User Guide Rev C (Document Number: CG000315).

33 From this point on, use the 10x protocol, with the following modifications:

Step 1.4 - take all the liquid in the recovery well, this will be slightly more than 100 uL in most cases. This ensures the maximal amount of nuclei are recovered.

Step 2.2a - It is essential to use Feature cDNA Primers 2 (PN: 2000097). Failure to do so will result in loss of spatial barcodes.

Step 2.2d - For nuclei, we often add an extra cycle to the suggested number. E.g. 6000 targeted nuclei recovered = 12 + 1 = 13 cycles.

Step 2.3e - (optional) This excess supernatant can be saved at -20 oC or cleaned up. It still contains spatial barcodes so may be useful in future.

Step 4.1f - Fewer PCR cycles may be required for the spatial barcode index PCR, depending on the size of the puck used (if in doubt, set up multiple PCR reactions or a qPCR and use the minimal cycles capable of giving a viable library concentration):

- 5.5 x 5.5 mm square puck (aka MEGA): 7 cycles
- 3mm diameter circular puck : 10 cycles