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Slide-Tags using Tissue-Tape

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We use this protocol and it's working

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Abstract

Slide-tags is a method that involves tagging cellular nuclei from intact fresh frozen tissue sections with spatial barcode oligonucleotides derived from DNA-barcoded beads positioned at known locations. These tagged nuclei are then analyzed using existing single-cell methods, enhanced with spatial positioning information ([Nature 625, 101–109 \(2024\)](#)).

The traditional method of placing tissues onto DNA-barcoded pucks can be time-consuming and labor-intensive. This protocol integrates the tape transfer technique for mounting tissues ([PLoS One. 2015 Jul 16;10\(7\)](#)) with Slide-tags. The tape transfer technique allows tissues to be placed on barcoded pucks for Slide-tags, enhancing spatial placement accuracy while preserving the structural integrity of the tissue, and reducing the required time by half. The size of the tape can be chosen and adjusted to accommodate the tissue area. The barcoded single nuclei obtained through this workflow seamlessly integrate with 10x GEM generation, gene expression, and spatial barcode library generation.

Guidelines

- Keep all reagents and samples on ice to maintain RNA quality.
- Handle tissues and pucks gently to prevent damage and avoid scratches on pucks as well as tissue loss.
- Do not leave the tissue in the cryostat for more than 30 minutes. Promptly return the tissue block to freezer storage, to preserve tissue quality.
- The success of this protocol depends on maximizing nuclei yield. This can be achieved by working quickly, keeping samples on ice, and ensuring complete tissue dissociation and cell lysis.
- Slide-tags are accompanied by histology. Collect 20 μ m sections of your tissue ROI for Nissl and/or H&E staining after collecting tissue for the slide-tag puck.



Materials

- PBSAi: 5% BSA in 1x PBS + 20U/μl RNase Inhibitor (not included in the kit)
- Kit Provided Reagents:
 - Buffer A (Invent Bio, Cat. No. BN-020)
{To 800μl Buffer A, add 100μL RNase inhibitor (40U/μL)}
 - Buffer B (Invent Bio, Cat. No. BN-020)
 - Filter tube (Invent Bio, Cat. No. BN-020)
- Low Adhesion Silicon Tissue Tape [PMID: 26181725]
- 12-well plate
- UV light box
- 200 μL and 1000 μL pipette tips
- Pre-chilled centrifuge (set to 4°C)
- Pre-chilled Eppendorf tubes
- Pre-chilled Eppendorf tubes with filter
- Cryostat (CM1950, Leica or other models)
- Paintbrush
- Tweezers
- Tape roller
- C-Chip Fuchs-Rosenthal disposable haemocytometer (INCYTO, DHC-F01-5)
- Timers

Before start

Preparation

1. Reagent and Equipment Setup:

- Prepare all reagents and place them on ice.
- Set up a 12-well plate on ice [or an appropriate sized plate depending on puck size.]
- Ensure tissue is temperature-equilibrated for cryostat sectioning.
- Pre-chill the centrifuge to 4°C and keep Eppendorf tubes on ice.

2. Set-up Required:

- Paintbrush and tweezers for tissue and puck handling.
- Slide-tag puck: Leave them in the 4°C refrigerator until ready for use. Place them on a glass slide and keep them in an accessible spot outside the cryostat, with the puck facing up.

Tissue Preparation

- 1 Prepare tissue as below (*Refer to the visual guidelines provided at the end of the protocol*)
 - 1.1 Mount the tissue onto the cryostat head (use your preferred OCT mounting technique). Ensure the cryostat temperature is maintained between  -20 °C to  -30 °C to prevent any thermal challenge to the tissue.
 - 1.2 Trim the tissue to reach the region of interest (ROI).
 - 1.3 Once the ROI is exposed, affix tape to the tissue surface. Use a tape roller to smooth the tape, rolling in one direction to ensure even adhesion.
 - 1.4 Section the tissue at a thickness of 20µm the tape is still attached. (*Note: 20µm is the preferred thickness for Slide-tags*).
 - 1.5 Transfer the 'section-tape' slice to a puck. Position the section within the puck to ensure adequate coverage of the ROI.
 - 1.6 Immediately use your finger to warm the tissue, by placing it under the glass slide and on top of the tape. This lets the tissue adhere to the puck.
 - 1.7 Carefully peel the tape from the puck and the glass slide. You should find that the tissue is nicely adhered to the puck, without any tears or areas of rolling.
 - 1.8 Repeat the process of applying warmth by placing the finger tip under the glass slide
 - 1.9 Wet the tissue thoroughly with PBS and ensure that it does not dry out. (*PBS volume depends on your tissue and puck size*).
 - 1.10 Place the tissue-affixed-puck and glass slide atop a flat trough filled with ice to keep it chilled.

Photocleavage



2 UV-directed photo-cleavage and barcode diffusion:

- 2.1 Move the puck-trough setup (where the puck is placed on the glass slide, both resting flat on a trough of ice) to the UV stage.
- 2.2 While keeping the puck and glass slide on ice, center the puck under the UV beam to cover its entire surface. Then, expose the tissue within the puck area to UV light for **2 minutes**. 
- 2.3 Remove the puck-trough set up from the UV box, and incubate on ice for **7 minutes**. *[Ensure that the glass slide with the puck lies flat and remains undisturbed during this incubation step, as barcode diffusion occurs.]* 

Tissue Dissociation

- 3 Transfer the puck to an appropriate well plate:
Place a **1.2 cm** puck in a 12-well plate, a **2-4 cm** puck in a 6-well plate, and a puck larger than **4 cm** in a small petri dish.
- 3.1 Use tweezers to place the puck in the well, avoid scratching the puck surface or tissue.
- 3.2 Dissociate the Tissue:  
Pipette  800 μL of Buffer A onto the puck surface and triturate directing the buffer stream at the tissue using a  200 μL tip.
Repeat the trituration until the tissue dislodges from the puck.
(The volume of Buffer A depends on puck size: use 800 μL for a 1.2 cm puck, 1700 μL for a 2 cm puck, 3400 μL for a 3 cm puck, and 4 mL for pucks larger than 4 cm.)
- 3.3 Check under a microscope after every **15-20 trituration** until the tissue has fully dislodged from the puck and appears completely dissociated.  
- 3.4 Remove the puck from the well.

Single-Nuclei Dissociation



- 4 Triturate suspension in the well, **40-45 times** with a  1000 μL tip.  
- 4.1 Incubate for 5 minutes, then resuspend **3-5 times**. 
- 4.2 **For 1.2cm puck:** skip step 4.2 and proceed to step 4.3
For pucks >2cm: Centrifuge for **5 minutes** at  500 g and remove supernatant, leaving behind  800 μL . Resuspend the pellet. Proceed to step 4. 
- 4.3 Transfer sample to a collecting tube fitted with a filter (provided in the Invent Kit).
- 4.4 Incubate with the cap open at  -20 °C for **10 minutes**. 
- 4.5 Centrifuge at  13.000 g for **30 seconds**. 
- 4.6 Discard filter; resuspend flowthrough **10-20 times**. 
- 4.7 Centrifuge at  600 g for **5 minutes**. 
- 4.8 Remove supernatant; resuspend pellet in  0 g L PBSAi. 
- 5 **Gradient-based Separation:**
- 5.1 Overlay this nuclear suspension onto  1 mL Buffer B in a new tube.
- 5.2 Centrifuge at  1.000 g for **10 minutes**. 

- 5.3 Remove supernatant, leaving a small volume for pellet resuspension, and proceed to count nuclei and begin 10x GEM generation.

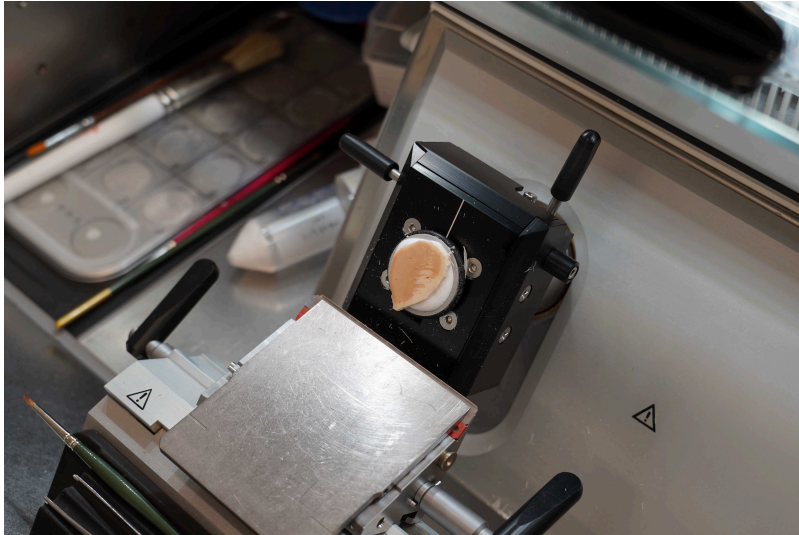


10x Genomics Workflow

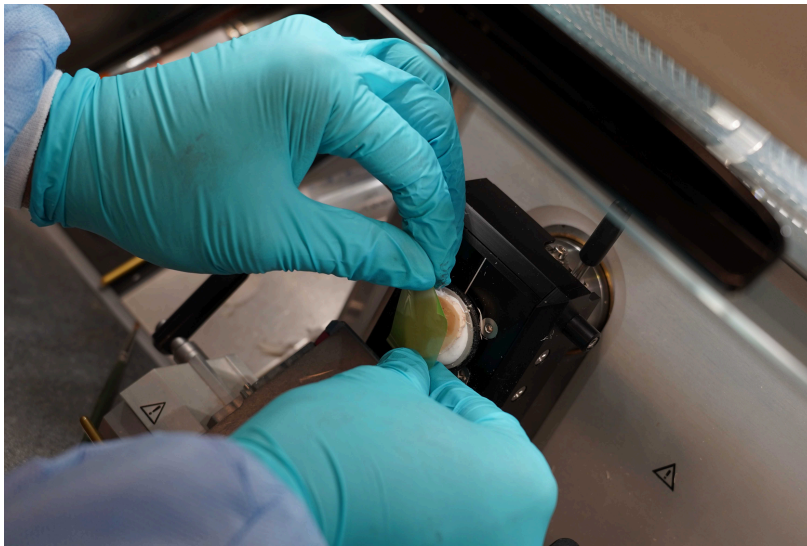
- 6 Follow 10x recommendations to construct spatial gene expression and spatial barcode libraries. (10x Genomics User Guide, CG000734, ChromiumGEM-X_SingleCell5_ReagentKitsv3_UserGuide_RevA.pdf)
- 6.1 Add Feature cDNA Primers 2 (PN: 2000097) in Step 2.2, CG000734 (This is mandatory to make a library for spatial barcodes)
- 6.2 5' Gene expression Library construction (Step 5.5d, CG000734): Use 15 cycles for index PCR step.
- 6.3 Save the supernatant removed from Step 2.3e CG000734 to make the spatial barcode library
- 6.4 Spatial Barcode Library Construction (Step 6, CG000734): Use 12 cycles for indexing.
- 6.5 In Step 6.2, perform a double-sided size selection of 0.6X followed by 1.2X. This approach effectively enriches shorter barcode library fragments while minimizing the carryover of longer gene expression library fragments.

Guidelines for tissue sectioning using tape and positioning on the puck

- 7 Visual Guides



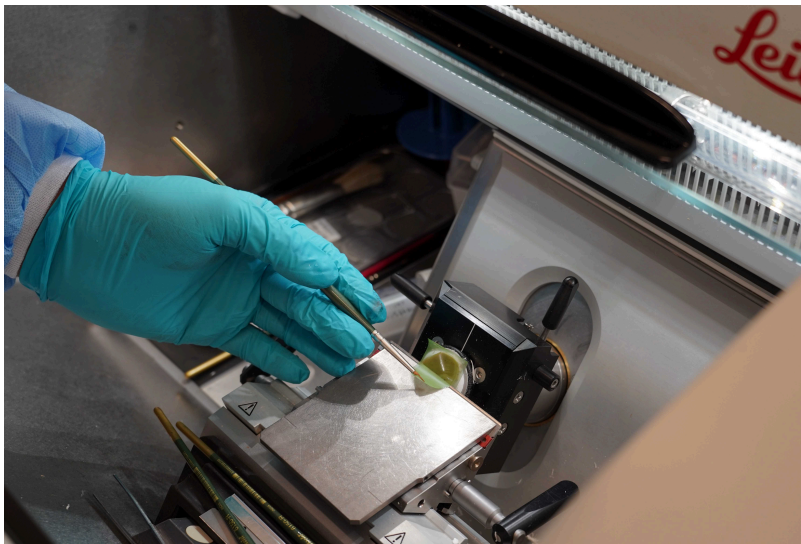
Step 1.1



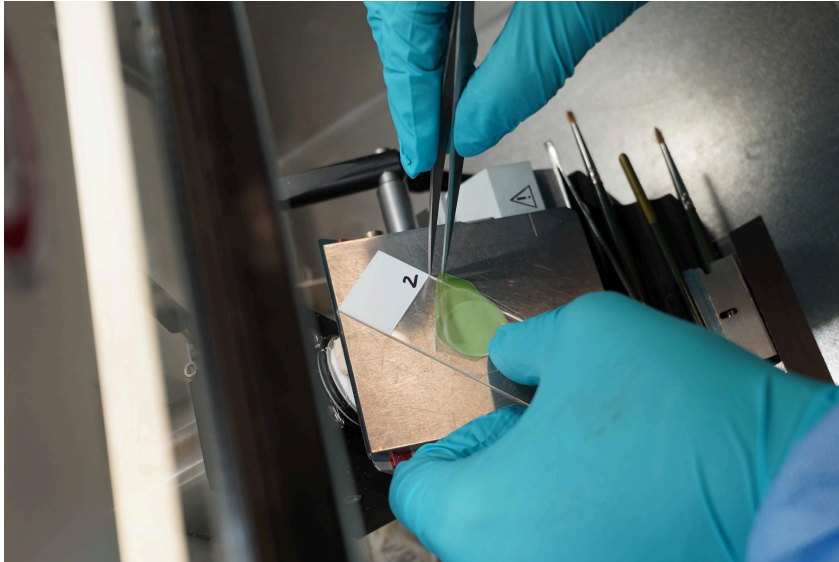
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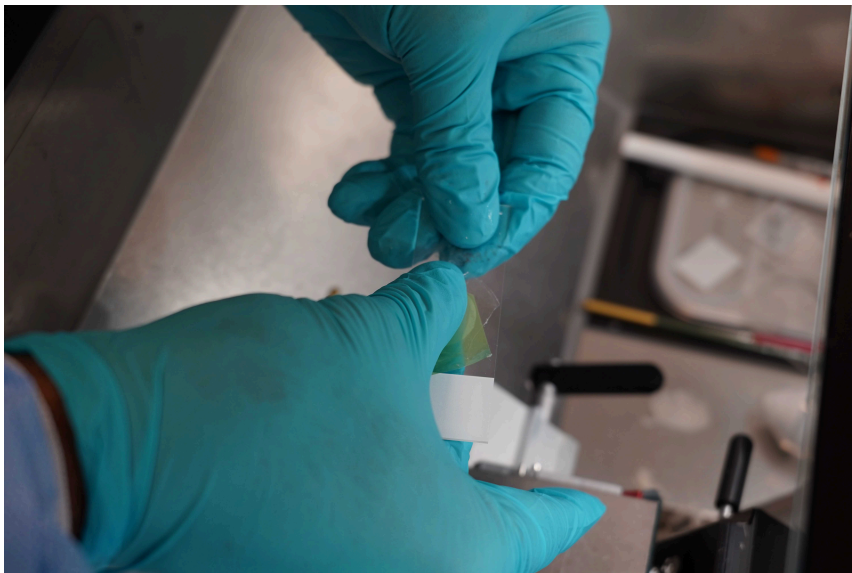
Step 1.3



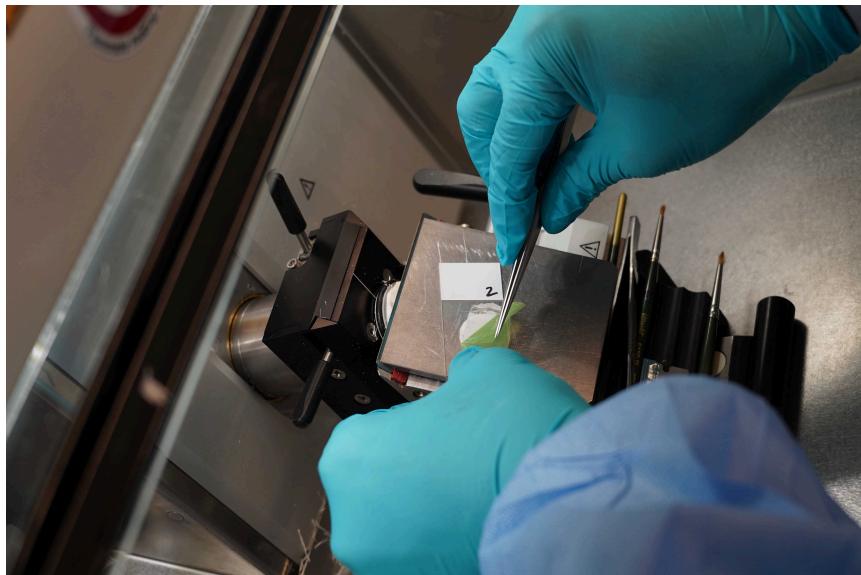
Step 1.4



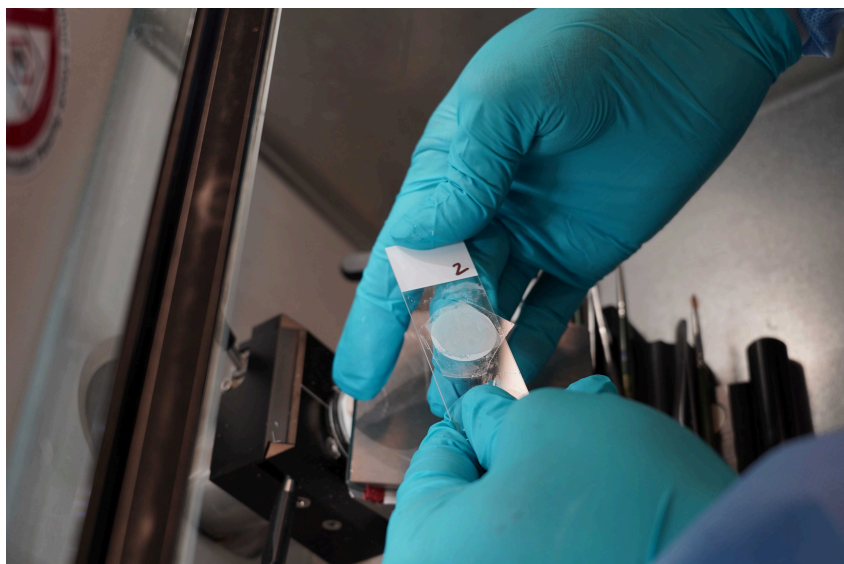
Step 1.5



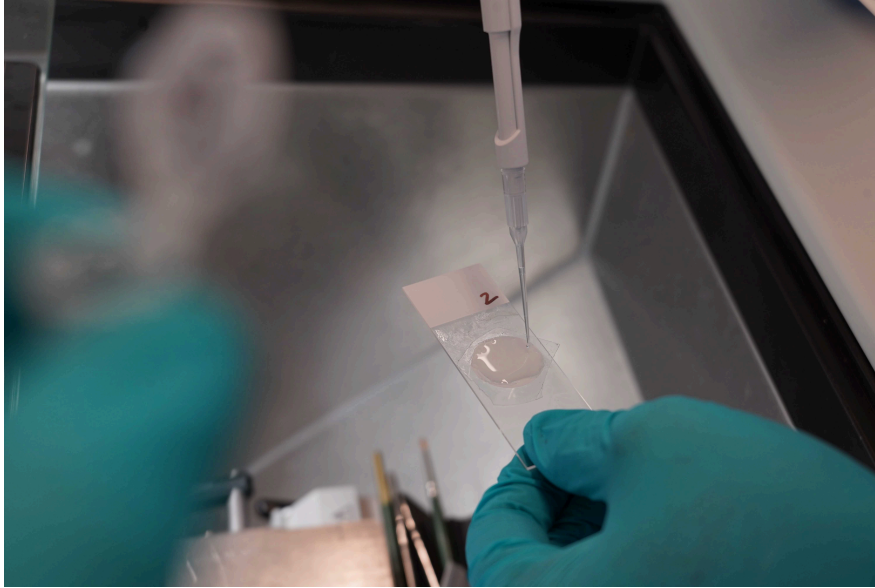
Step 1.6



Step 1.7



Step 1.8



Step 1.9

Protocol references

1. [Slide-tags enables single-nucleus barcoding for multimodal spatial genomics](#)
2. [High-Throughput Method of Whole-Brain Sectioning, Using the Tape-Transfer Technique](#)

Acknowledgements

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