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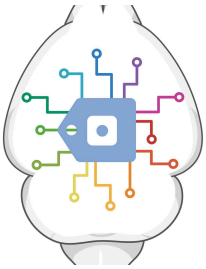
Fresh frozen tissue collection for single-cell sequencing



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Projection-TAGs

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

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Abstract

This protocol describes the process of collecting fresh frozen tissues from mouse brain and spinal cord regions of interest for single-cell sequencing experiments.

For more Projection-TAGs protocols, please visit: https://www.protocols.io/workspaces/projection-tags

Materials

Equipment and Materials

Compresstome (Precisionary Instruments, VF-310-0Z)
Microscope with ring light (Amscope, SM-1BSL-64S-V331)
BSC-PC Prechamber (Harvard Apparatus, PY2 65-0076)
Insert for BSC-PC Prechamber Tissue Slice Chamber (Harvard Apparatus, PY2 65-0608)
(insert comes with prechamber, but separate ones can be ordered if needed)
Water bath
pH sensor
Stir bar
Stir plate
Forceps
Dissecting scissors
Butterfly needle
Spring scissors
60 mL syringe
Weigh bowl
Super glue (Super Glue (Amazon), 15187)
Razor blades
35mm and 100mm petri dishes
1.5 ml DNA low bind tubes
Tissue puncher (World Precision Instruments, 504638)
Cryomold (Electron Microscopy Sciences, **62534-10**)

Reagents

Agarose (Sigma-Aldrich, A0576)

For NMDG:
KCl (Sigma-Aldrich, P5405)
NaH2PO4 (Sigma-Aldrich, M2004)
NaHCO3 (Sigma-Aldrich, S5761)
HEPES (Sigma-Aldrich, H4034)
D-Glucose (Sigma-Aldrich, G8270)
L-ascorbic acid (Sigma-Aldrich, A5960)
Thiourea (Sigma-Aldrich, T8656)
Sodium pyruvate (Sigma-Aldrich, P2256)
MgSO4 anhydrous (Sigma-Aldrich, M7506)
CaCl2 (Sigma-Aldrich, 21115)
N-acetylcysteine (Sigma-Aldrich, A7250)
InLab Storage Solution (for pH sensor) (InLab Solutions, 30111142)
HCl

Before start

All supplies, equipment, and areas must be free of PFA contamination.

Wipe down working areas and tools with 70% ethanol and RNase Zap.

Preparation of reagents

1. Ice-cold *N*-methyl-D-glucamine (NMDG)–based cutting solution:

Adjust pH to 7.3 with 12N HCl and osmolality to 300-310 mosm/kg. Keep the NMDG slicing solution chilled on ice and bubbled with 95% O_2 and 5% CO_2.

Note: NMDG-based cutting solution can be prepared the day before. Store in 4°C if prepared the day before.

	NMDG-based cutting solution	Stock	250 mL	X2
	Milli-Q H2O	-	225 mL	450 mL
	NMDG	93 mM	4.54 g	9.08 g
	KCl	2.5 mM	250 uL	500 uL
	NaH2PO4	1.25 mM	250 uL	500 uL
	NaHCO3	30 mM	0.63 g	1.26 g
	HEPES	20 mM	2.5 mL	5 mL
	D-Glucose	25 mM	2.5 mL	5 mL
	L-ascorbic acid	5 mM	0.22 g	0.44 g
	Thiourea	2 mM	0.04 g	0.08 g

Sodium pyruvate	3 mM	0.085 g	0.17 g
MgSO4 anhydrous	10 mM	1.25 mL	2.5 mL
CaCl2	0.5 mM	125 uL	250 uL
N-acetylcysteine	12 M	0.49	0.98

2. 2% agarose gel:

Use the microwave (high power/3 × 10s) to heat until fully melted, then cool down to approximately 37°C. Keep molten agarose in warm water bath at 37°C to prevent premature solidification before embedding.

2% Agarose Gel	100 mL
1 x PBS	100 mL
Agarose Powder	2 g

Set up working station

1 **Prepare compresstome**

1.1 Pre-chill compresstome chilling block on ice or in freezer.

1.2 Put the metal tube on the specimen plunger.

1.3 Glue a fresh razor blade onto the blade holder securely.

1.4 Adjust compresstome slicing parameters: section thickness 500 um, advanced speed 0.02-0.08 mm/s, oscillation amplitude 1.5-2.0 mm.

1.5 Prepare tissue slice prechamber containing NMDG-cutting solution and keep the tissue slice chamber on ice.

2 **Prepare perfusion area**

2.1 Fill a perfusing tray with ice.

2.2 Place a plastic weigh bowl on the ice. This will be the perfusion station.

2.3 Prepare perfusion supplies: forceps, scissors, spring scissors, butterfly needle, syringe.

2.4 Prepare dissecting microscope if needed.

3 **Prepare tissue collection area**

3.1 Set up a microscope.

3.2 Put a black paper under the dish for better contrast.

3.3 Place a 35mm petri dish inside a 100mm petri dish. Add 1-2 ml of ice-cold NMDG-cutting solution into the 35mm petri dish to keep the slices cold. To maintain a cold environment, place several cubes of dry ice in the 100mm dish to surround the 35mm dish.

3.4 For tissue collection, label 1.5 ml DNA low bind tubes and place in a Styrofoam box with dry ice.

Tissue slicing

4 Anesthetize mouse with ketamine cocktail.

5 When the mouse does not respond to toe pinch, perfuse mouse using ~30 mL ice-cold NMDG-based cutting solution.

6 Dissect the brain and/or spinal cord and submerge the tissues in ice-cold NMDG-based cutting solution.

7 For brain tissue, skip steps 8 and 9.

8 Cut the spinal cord to desired length and place in plastic cryomold on ice.

9 To block spinal cord, add 2% sucrose solution and wait ~ 30 s to solidify. Remove from cryomold and trim excess agarose from sides.

10 Place small amount of super glue on specimen plunger and put brain or agarose containing spinal cord on top. Wait ~ 5 s for it to dry.

11 Pull the metal tube up until the top edge reaches the top of the tissue. Slowly fill the tube with the warm agarose, ensuring the tissue is fully surrounded and centered.

12 Place the entire specimen tube in the pre-chilled chilling block. Wait ~ 15 s for agarose to solidify completely.

13 Install the solidified specimen tube into the compresstome chamber.

14 Optional: to distinguish left from right hemispheres, pierce using a 16G needle one hemisphere on a region that is far away from your region of interest.

15 Secure the blade to the compresstome.

16 Fill the slicing chamber with ice-cold NMDG-cutting solution to cover the blade and tissue.

17 Slice the tissue with section thickness set to 500 um.

18 Collect the tissue section using a fine brush or pipette tip.

19 Identify the tissue sections containing the brain and spinal cord regions of interest, and transfer them to the tissue punching area in a 35mm petri dish filled with ice-cold NMDG-cutting solution.
Tissue sections can be temporarily stored in the tissue slice prechamber containing ice-cold NMDG-cutting solution.

20 Repeat steps 17-19 to collect all tissue sections containing the brain and spinal cord regions of interest.

Tissue collection

21 Choose the tissue puncher size that best covers your region of interest.

22 Use anatomical landmarks to identify the brain and spinal cord regions of interest.

23 Punch the tissue by applying force to the puncher. Separation can be facilitated by slightly rotating the puncher.
Note: Tissue will be collected inside of the punch.

24 Place the tip of the tissue puncher to the side of the sample collection tube, and expel tissue into the tube.

25 Repeat steps 22 to 24 for all brain or spinal cord tissue sections containing the regions of interest.

26 Clean the tissue puncher tip between each sample by rinsing with 70% ethanol and nuclease-free water.

27 Store samples at -80C.