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Isolation of the Region Around Locus Coeruleus for Single Nucleus RNA Profiling

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We use this protocol and it's working

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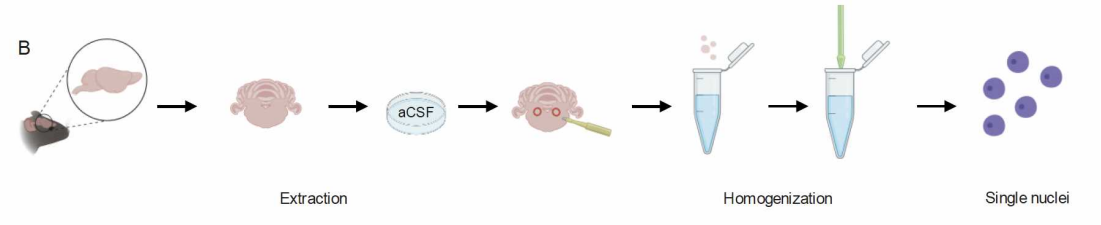
Abstract

This protocol outlines the methodology for isolating the peri-locus coeruleus (peri-LC) region in mice for subsequent single nucleus RNA profiling. Key steps include the preparation of NMDG-aCSF buffer with precise pH and osmolarity, careful euthanasia and extraction of the brain, and the use of a vibratome to obtain 300 µm thick slices. The peri-LC region is identified and isolated using a biopsy puncher, followed by immediate shock-freezing in liquid nitrogen to preserve tissue integrity. This approach facilitates high-quality RNA profiling, essential for understanding the molecular characteristics of the locus coeruleus and its surrounding regions.

1 **Tissue Isolation of the Region Around Locus Coeruleus for Single Nucleus RNA Profiling**

2



After punching peri-LC region, you can also shock freez in liquid nitrogen-

Buffer Preparation (important - steril filter and autoclave)

3 Prepare the buffer a day before, and keep it in the fridge before using

4

reagent	[c]	Mass [g]	V (taken from 1M Stock)
NMDG	96 mM	**18,74 g**	
HCl	96 mM		**5 ml**
NaHCO3	25 mM	2,10025	**30 ml**
Glucose	25 mM	**4,50**	
HEPES	20 mM	4,7662	**20 ml**
N-acetylcystein	12 mM	**0,97914**	
MgSO4-heptahydrate	10 mM	2,4647	**10 ml**
Sodium L-ascorbat (Ascorbinsäure)	5 mM	**0,99**	
Myo-Inositol (Cyclohexanhexol)	3 mM	**0,5406**	
Sodium Pyruvat	3 mM	**0,33 g – 30 ml**	
KCl	2,5 mM	0,1864	**2,5 ml**
Thiourea	2,0 mM	0,15224	**2 ml**
NaH2PO4 – monohydrate	1,25 mM	0,1724875	**1,25 ml**
CaCl2	0,5 mM	0,055495	**500 µl**
Taurine	0,01 mM	**0,00125**	
Actinomycin-d	1 µg/ml	1 mg	
TTX	0,1 µM		**50 µl**
DNQX	10 µM		**1ml**
APV	10 µM		**1ml**
Pronase (Streptomyces griseus)	1 mg/ml		

Preparation Stock Solution for NMDG- aCSF

	Stock	brauche für ACSF
1M HEPES	23, 83 g in 100 ml	20 ml
1M MgSo4 7H20	24,64 g in 100 ml	10 ml
1M NaH2Po4	3,44 g in 25 ml	1,25 ml
1M Thiourea	1,903 g in 25 ml	2 ml
1M CaCl . 2 H2O	1,837 g in 15 ml	500 µl
1M KCl	1,864 g in 25 ml	2,5 ml
1M NaHCO3	8,4005 g in 100 ml	30 ml

Stock solution to acuratly prepare working solution!

5 ~ 500ml Milli-Q-Water

6 add reagents of Table 1

7 fill up until 1000 ml

8 sterile filter

9 Bubble with 95% O2 and 5% CO2 until ph is stable (approx. 15-20 min)

10 adjust ph with HCl -> must have: 7,3-7,4 pH, 315 mOsm Can be stored at 4°C for a couple of days.

11 On the day of the experiment add freshly: + 10,0 g Trehalose+ 50 µl (0,1 µM) TTX+ 1 ml (200 µM) DNQX+ 1 ml (50 µM) APV+ 1 µg/ml actinomycin-d

Preparation for Brain Extraction

12 1. Prepare NMDG-aCSF

sterile filtered pH 7,3
→ Important between 7,3 and 7,4!! 300 mOsm after Trehalose
+ 10 mg/ 1L Trehalose
- Stir while bubbling with 5 % CO2 and 95 % O2.

for 15- 20 Min and check pH 7,3

13 Clean EVERYTHING with RNase free!

14 Prepare FALCON 3.1 Falcon with ACSF and FBS (0,5%)

14.1 250 µl FBS + 50 ml aCSF → close with Parafilm to prevent gas leakage 3.2 Falcon with ACSF and Protease (1mg/ml)

14.2 30 mg Protease + 30 ml ACSF

15 Prepare chambers for recovery of brain slices

15.1 Chamber with NMDG-aCSF (RT): Recovery of Slices

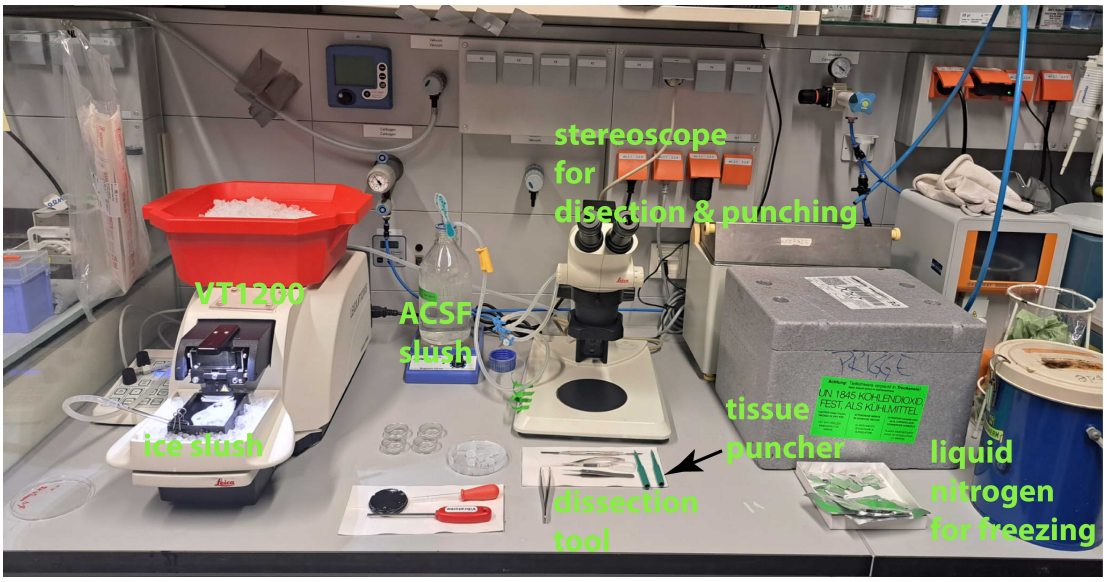
15.2 Chamber with RT NMDG-aCSF + Protease

16 Prepare Agaroseblock to assist vibrotome slicing and preventing pushing brain away during slicing
2% Agarose in Petri dish (fridge).
Cut out small block approx. 1,5 cm – 1,5 cm

17 Place Instruments on a towel for dissection and trituration → clean with RNase free

18 1. Prepare Vibratome: new blade each time!
80 Hz, 200 Frequency, Section: 300 µm (LEICA VT1200)

19



Procedure of extracting brain and cutting brain stem slices

20 Euthanize mice with 0.1 ml pento or other approved euthanizing method

21 Decapitate, remove skin, open scull (window)

22 Remove brain with spatula Block Brain (depending on region of interest (ROI)

23 Glue agarose block to platform Place and glue brain in front of agarose block

24 Place platform in vibratome, add ice cold NMDG-ACSF, bubble with 5 % CO2 und 95 % O2

25 Vibratome: 80 Hz, Amplitude 200, section 300 µm, duration 0,10 mm/s.

26 Discard brain slices until Locus coeruleus is reached

27 Place brain slices into ice-cold NMDG-ACSF bubble (5 % CO2 und 95 % O2) for 15 min Recovery

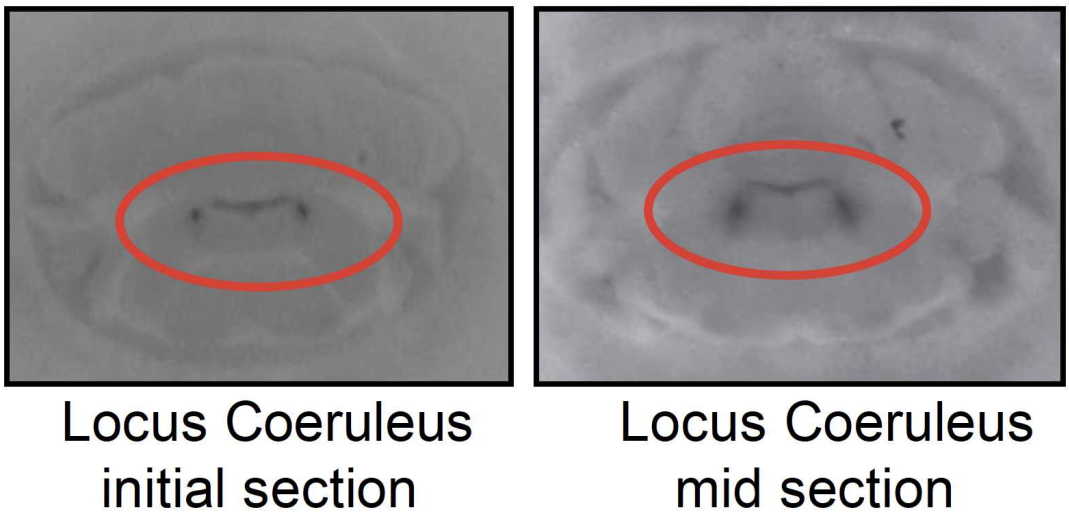
Extracting and Isolating peri-LC region

28 1. Fill Petri dish with NMDG-aCSF+FBS
Place slices with pasteur pipette into petri dish

29 **When working with fluorescence labeled cells turn off light!**

30 Punch out brain areas with biopsy puncher (1.5 mm)

31 In this image the LC is accumulating NM and iseasily visible. Landmarks such as the 4th vetricle can help you to locate LC and peri-LC region



32 After punching the peri-LC region, store the tissue in a small PCR tube and immediately shock-freeze it in liquid nitrogen. Ensure the tissue is sent to collaborators as intact tissue, **not** as isolated nuclei.

A biopsy tissue puncher with diameter of 1.5mm is recommended. We used biopuncher from Miltex (image copywrite Miltek/Tedpella https://www.tedpella.com/histo_html/miltex-plunger-punch.aspx)

33 If you immediately proceed with nuclei isolation, follow the protocol provided by the 10X Nuclei Isolation Kit (**link**)

34 Place samples on ice until further usage