Microdissection of sucrose treated brain tissues with Kuiqpick™: An optimized protocol

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Introduction

Isolation of specific subanatomical regions or single cells in the central nervous system is a delicate procedure requiring careful preparation of tissue specimen. In areas with low cell density singe cells may be collected using Kuiqpick™, while in areas with high density of cells within a cellular layer (e.g. granular cell layers of the cerebellum, dentate gyrus, or CA1-3 areas of the hippocampus) clusters consisting of the same cell types may be collected with each single acquisition step[†]. Separation of cell bodies and dendrites can also be performed in certain areas of the brain, such as the hippocampus and cerebellum.

This protocol describes the isolation of single cells, cell clusters, and subanatomical regions from sucrose treated brain slices. Sample microdissections are provided to demonstrate the effectiveness of this method.

Materials

- Kuiqpick™
- Surgical instruments
- Standard animal perfusion apparatus and setup
- Cryostat
- Standard Phosphate Buffered Saline (PBS)
- Sucrose (15-20%) in PBS filtered
- 2-methylbutane
- Dry ice
- Glass microscope slides
- Cresyl violet, toluidine blue, or hematoxylin
- Pipettor and sterile pipette tips
- Dissociation solution: 1mM EDTA, 0.05% Trypsin
- Kuiqpick™ disposable capillary units (DCU) with appropriate internal diameter (ID) for the application. ID≈20-30 µm for single cell collection. ID≈30-100 µm for subanatomical regions. Specimen preparation may be performed according to standard protocols. Described below is the preparation used for Kuiqpick™ protocol.

Tissue preparation

1. Perfuse the animal with standard PBS.
2. Remove the brain and sink in 15-20% Sucrose in PBS at +4°C overnight .
3. Flash freeze in 2-methylbutane on dry ice. If it is not for immediate use, store at -80°C .
4. Prepare cryosections from 10 to 50 µm thickness. For single cell collection with Kuiqpick™, tissues were cut at 20 µm .
5. Stain slides with 0.01% toluidine for 10 seconds. Any vital dyes may be used for staining brain sections (other dyes may be used). Avoid overstaining, as this will cause the sample to become stiff, thus difficult to microdissect .
6. Wash with standard PBS .

Microdissection with Kuiqpick™

7. Dry the back of the slide after PBS wash and place the slide on Kuiqpick™ stage. It is critical to keep the tissue slice moist at all times by adding either PBS or 15% sucrose in PBS during longer periods of microdissection. High quality RNA may be isolated from tissue samples collected within 1 hour[†] .
8. Attach a DCU to the bottom of the head unit of Kuiqpick™.
9. Under low magnification (e.g. 4X) place the tip of the DCU at the center of the crosshair in the field of view .
10. Center the tip of the DCU under 20X for microdissecting subanatomical regions or under 40X for single cell collection .
11. Calibrate the DCU tip to just above the surface of the slide adjacent to the specimen. Move the slide gently using the manual xy stage to check if the DCU tip has been lowered enough to make contact with the side of the specimen but still not contacting the slide surface. This indicates the calibration point.
12. Press the orange button on Kuiqpick™ to home the DCU to its stand by position.
13. Start with vacuum strength and pulse duration set to 2.

Bring the cell(s) of interest to the center of the crosshair and press the black button to activate acquisition. If necessary, adjust the settings incrementally by increase vacuum strength and/or pulse duration until the desired acquisition is achieved.

14. If multiple cells/regions of the same type are to be collected and combined, continue sample acquisition by locating the next cell/area of interest and repeating Step 13.
15. Once the desired cells/regions have been acquired, lift the head unit of Kuiqpick™ and remove the DCU.
16. Carefully position the tip of the DCU above a microcentrifuge tube on ice. Attach a loaded 1ml syringe to the luer of the DCU and slowly release the microdissected sample into the centrifuge tube. If there is remaining tissue inside the DCU shaft, load the attached syringe with PBS to rinse the DCU and then release the contents into the microcentrifuge tube.
    - If the samples will not be used immediately, place the microcentrifuge tubes on dry ice and then store at -80°C.
    - If the DCU is not clogged or damaged, it may be reused to collect homogeneous samples.

Results

The optimized protocol yields high quality RNA from the microdissected material. Figure 1 illustrates the microdissection of subanatomical regions from a coronal section of a mouse brain.

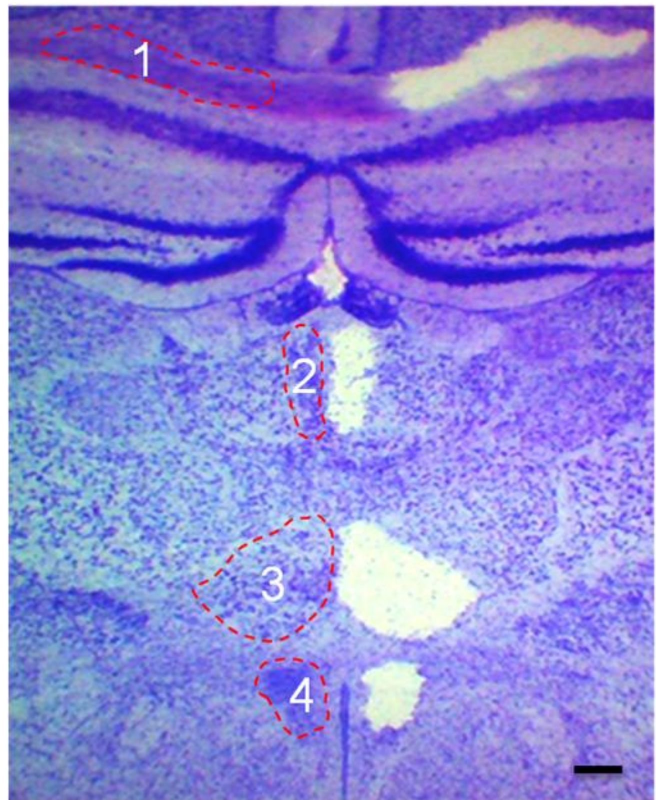

Figure 1. Unilateral microdissection of the corpus callosum (1), paratenial thalamic nucleus (2), nucleus reunions (3) and hypothalamic nucleus (4). Tissue was stained with Toluidine blue. Scale bar= 250μm .

Endnotes

† Protocol and data published in PloS One, 2012. Kudo LC, Vi N, Ma Z, Fields T, Avliyakulov NK, Haykinson MJ, Bragin A, and Karsten SL (2012) Novel Cell and Tissue Acquisition System (CTAS): Microdissection of Live and Frozen Brain Tissues. PloS One. 7(7):e41564. Epub 2012 Jul 24.

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