LCM protocol

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Background

Laser Capture Microdissection (LCM) was developed in 1996 at the National Cancer Institute as a technique that enables the isolation of small areas of tissue or single cells out of complex histological sections, in order to achieve enrichment of specific cell type populations for their subsequent analysis of their RNA, DNA, or protein profiles (Emmert-Buck et al., 1996). The protocol, presented here, is optimized for the isolation of GFP – expressing dopaminergic cells of the mouse brain (Matsushita et al., 2002; Plessy et al., 2012).

Preparation of tissue sections for LCM

Materials

Fixative (ZincFix 10x, BD Biosciences)
FALCON tubes
Embedding medium (e.g., OCT; -Neg50)
Cryomolds
Liquid Nitrogen
Isopentane
Dry-ice container
Cryostat, refrigerated to −21°C with chuck and appropriate blades
Glass slides (e.g., SuperFrost plus glass slides, Mezzle - Glasser)
Slide boxes
Desiccant (e.g. silica beads), optional
Vacuum machine, optional

Procedure

1. Fix the brain piece of interest by immersion in ice-cold Zincfix (BD Biosciences) for 6 to 8 hours.

2. Subsequently, leave the specimen in a solution of Zincfix/30% glucose, overnight at 4˚C, until it sinks to the bottom of the Falcon tube. The ratio, fixative volume to specimen volume, should be >10.

   This step cryoprotects the tissue and allows preservation of morphology.

3. Embed in –Neg50 or O.C.T. compound.

   You can omit this step, depending on your region of interest and size of the specimen.

4. Snap-freeze the brain tissue in liquid nitrogen-cooled isopentane and leave the specimen to equilibrate in a cryostat chamber at -21˚C for 1 hour before sectioning.

5. Cut 14 µm sections and mount them on plus charged Superfrost glass slides.

   Sections should be as flat as possible and without folds.

6. If not used immediately, sections can be stored either dry (with desiccant) in a vacuum or at -80˚C for up to two months.

Microdissection with the UV Laser

Materials

PALM Robot-MicroBeam System (PALM Microlaser Technology)
0.2 ml microfuge tubes with adhesive caps (PALM Adhesive Caps)
ZincFix 10x (BD Biosciences)

Procedure

1. Place the cryostat – cut sections on the slide holder of the PALM MicroBeamSystem.

2. Use the 40x objective and 1 – 2 drops of freshly prepared ZincFix over your section to visualize the fluorescent cells.

3. Select your cells, with the help of the mouse, by drawing a line around each one of them, until the tissue is wet and fluorescence is visible.

4. When the fixative has dried, activate the UV laser to make an excision along the previously drawn cell borders and subsequently allow few laser pulses to catapult the whole structure (fragmented in pieces), upwards, against gravity, into the collection cap (0.2 microfuge PALM opaque adhesiveCaps) overlying the tissue specimen (Figure 1).

   All cells are collected in 0.2 ml microfuge tubes provided with a white cap (PALM adhesive caps), filled with an inert sticky substance, which immobilizes catapulted samples instantly.

5. Monitor and control the cap with the 10x objective for the presence of microdissected material to make sure that cell selection and collection are optimal. Avoid collecting more than 1000 cells in one cap.

6. Leave microcentrifuge tubes with the collected material at RT until the end of the LCM session before proceeding to RNA extraction. Alternatively, store tubes in a box with silica beads (or other desiccant), inside vacuum for up to one week, if more samples need to be pooled together for a single RNA extraction.

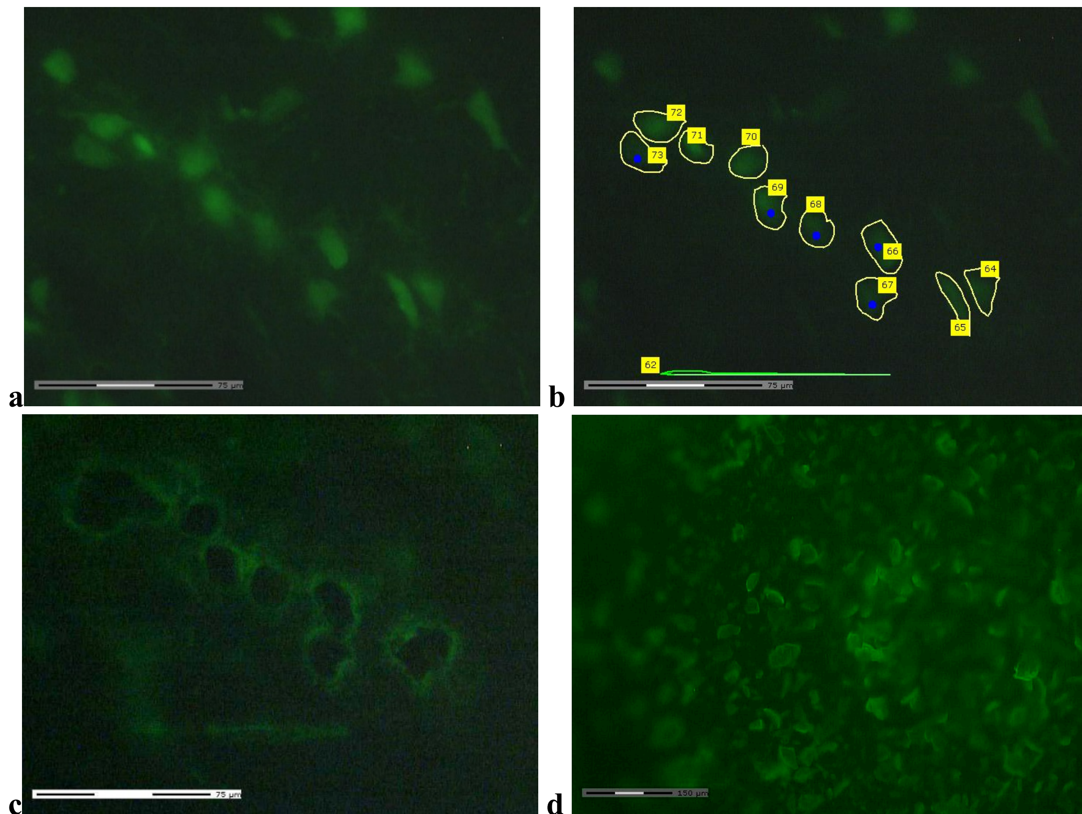

Figure 1. Zincfix fixed TH-GFP/31-21 mesencephalic sections. Several TH-GFP expressing DA cells (**a**) are marked for collection (**b**), cut by the laser and catapulted (**c**) into the cap collector for microscopic inspection (**d**).

<u>RNA extraction</u>

Materials

RNA Nanoprep kit (Stratagene)
RNA 6000 Pico Chips Agilent
Agilent Bioanalyzer

Procedure

1. To extract total RNA from collected cells, add 10 μl of lysis buffer directly onto the cells in each microcentrifuge cap. Pipette the solution up and down a few times.

2. Leave tubes in an inverted position, on ice, for 5 minutes, to allow time for cell lysis.

3. Centrifuge briefly at maximum speed to enable their collection at the bottom of the tube.

   If more samples are to be extracted as one, pool in one tube material centrifuged from more caps, and process them as one sample.

4. Perform RNA extraction with the RNA Nanoprep kit (Stratagene) according to manufacturer's instructions in an elution volume of 12 μl.

   Samples that are not extracted immediately can be kept homogenized in TRIZOL or lysis buffer at -80ºC until later processing.

5. Analyze RNA quality and yield with the RNA 6000 Pico Lab Chips (Agilent) on an Agilent 2100 Bioanalyzer.

6. Typically, the RNA quantity recovered from 1000 mouse midbrain dopaminergic cells collected by LCM ranges between 2 ng and 3 ng.

References

Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA. Laser capture microdissection. *Science* **274**, 998–1001 (1996).

Matsushita N, Okada H, Yasoshima Y, Takahashi K, Kiuchi K, Kobayashi K. Dynamics of tyrosine hydroxylase promoter activity during midbrain dopaminergic neuron development. *J Neurochem.* **82**(2), 295-304 (2002).

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