Video Article

Non-Laser Capture Microscopy Approach for the Microdissection of Discrete Mouse Brain Regions for Total RNA Isolation and Downstream Next-Generation Sequencing and Gene Expression Profiling

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URL: http://www.jove.com/video/3125/
DOI: 10.3791/3125

Keywords: Neuroscience, Issue 57, transcriptome, RNA-Seq, microdissection, total RNA, brain, mouse, microarray, RNA, RT-qPCR, gene, expression,

Date Published: 11/13/2011

Citation: Atkins, N., Miller, C.M., Owens, J.R., Turek, F.W. Non-Laser Capture Microscopy Approach for the Microdissection of Discrete Mouse Brain Regions for Total RNA Isolation and Downstream Next-Generation Sequencing and Gene Expression Profiling. J. Vis. Exp. (57), e3125, DOI : 10.3791/3125 (2011).

Abstract

As technological platforms, approaches such as next-generation sequencing, microarray, and qRT-PCR have great promise for expanding our understanding of the breadth of molecular regulation. Newer approaches such as high-resolution RNA sequencing (RNA-Seq)[1] provides new and expansive information about tissue- or state-specific expression such as relative transcript levels, alternative splicing, and micro RNAs[2-4]. Prospects for employing the RNA-Seq method in comparative whole transcriptome profiling[5] within discrete tissues or between phenotypically distinct groups of individuals affords new avenues for elucidating molecular mechanisms involved in both normal and abnormal physiological states. Recently, whole transcriptome profiling has been performed on human brain tissue, identifying gene expression differences associated with disease progression[6]. However, the use of next-generation sequencing has yet to be more widely integrated into mammalian studies.

Gene expression studies in mouse models have reported distinct profiles within various brain nuclei using laser capture microscopy (LCM) for sample excision[7,8]. While LCM affords sample collection with single-cell and discrete brain region precision, the relatively low total RNA yields from the LCM approach can be prohibitive to RNA-Seq and other profiling approaches in mouse brain tissues and may require sub-optimal sample amplification steps. Here, a protocol is presented for microdissection and total RNA extraction from discrete mouse brain regions. Set-diameter tissue corers are used to isolate 13 tissues from 750-µm serial coronal sections of an individual mouse brain. Tissue micropunch samples are immediately frozen and archived. Total RNA is obtained from the samples using magnetic bead-enabled total RNA isolation technology. Resulting RNA samples have adequate yield and quality for use in downstream expression profiling. This microdissection strategy provides a viable option to existing sample collection strategies for obtaining total RNA from discrete brain regions, opening possibilities for new gene expression discoveries.

Video Link

The video component of this article can be found at http://www.jove.com/video/3125/

Protocol

1. Sampling setup and preparation

1. Supplement Earle's balanced salt solution (EBSS) with sodium bicarbonate (0.44 g per 100 mL EBSS) and glucose (0.884 g per 100 mL EBSS)[9]. Treat the EBSS with DEPC (0.1 mL per 100 mL EBSS) for at least 12 hours at 37 °C in an autoclavable bottle or flask with a screw top.
2. Place 5 ¾" glass Pasteur pipets into a 1000 mL glass beaker, and place artist's brushes, with bristles facing upward, into a 100 mL glass graduated cylinder. Add sufficient DEPC solution (0.1 mL per 100 mL ddH_2O) to cover the pipets and brushes, respectively. Cover the beaker and graduated cylinder with aluminum foil, and incubate for at least 12 hours at 37 °C.
3. After 12-hr incubation with DEPC, heat all DEPC-treated materials to a temperature >100 °C for at least 15 minutes. Remove EBSS and cool to room temperature prior to storage at 4 °C. Pour ddH_2O away from the beaker and graduated cylinder; continue to incubate at >100 °C until materials are dry. Remove, cool to room temperature, and store for later use.
4. On the day of the experiment, treat all work surfaces with RNaseZap.
5. Place and maintain EBSS in a water bath set at 40 °C. To buffer and oxygenate the EBSS, infiltrate with 95%/5% O_2/CO_2 by placing gas tubing into a RNase-free Pasteur pipet and inserting the pipet into the EBSS. Cover the opening of the EBSS container.
6. Set up a Stoelting tissue slicer for use in serial sectioning of the brain tissue. Treat the top and bottom surfaces of the tissue slicer with RNaseZap. Attach a rectangular piece of Whatman #4 filter paper to the plexi-glass surface of the tissue chopper. Attach a double-edge razor blade to the chopper arm. Set the micrometer to zero, and adjust the minimum height of the chopper arm to allow the razor blade to cut the filter paper, but not the plexi-glass plate.
7. Gather all additional materials needed for the microdissection, including RNase-free 60x15 mm cell culture dishes, Harris Uni-Core tissue corers, 200 µL RNase-free micropipette tips for affixing to gas tubing for EBSS oxygenation of coronal brain sections during microsample punching, curved scissors, dry ice, and tubes for tissue collection. Place tubes on dry ice.

2. Brain removal and coronal sectioning

1. Place the mouse into a DecapiCone and decapitate using a rodent guillotine. Remove the head from the DecapiCone and place onto a flat surface. Using a scalpel, cut the skin down the midline of the skull starting approximately 3 mm caudal to the nostril. Laterally peel the skin flaps back to expose the cranium and the eye sockets.
2. Using curved scissors, cut both optic nerves, severing their connection to the eye. Open the cranial cavity by carefully breaking the occipital and interparietal bones and removing the dorsal parietal and frontal bones. Using a bone rongeur, break the nasal and lacrymal bones to expose the olfactory bulbs. Use curved scissors to cut the olfactory bulbs away from the rostral brain. Remove the whole brain with hindbrain intact. Place the brain into the top of a 60 x 15 mm, RNase-free Cellstar cell culture dish. Transfer to the tissue sampling work surface.
3. Use an RNase-free pipet to conservatively moisten the filter paper to the attached tissue chopper with EBSS. Wet the brain tissue with EBSS.
4. Transfer the brain to the tissue chopper and set up appropriate orientation. Note: The brain should be positioned with the ventral surface resting on the filter paper. The midline (interhemispheric fissure) should be perpendicular to the chopper blade, and the rostral edge of the brain should abut the blade.
5. Use a DEPC-treated artist's brush to hold the brain stationary by applying gentle pressure to the dorsal surface of the brain. While holding the brain stationary, lift the blade holder lever up about 4-5 cm above the dorsal tissue surface and hold it in this position. Remove the brush from the brain surface, and dial 750 µm using the micrometer. Allow the lever to drop, chopping the tissue and creating a 750 µm coronal section.
6. While the chopper blade remains stationary, use a rolling motion with the artist's brush to gently swipe the tissue section away from the blade surface. Immediately place the tissue section into the oxygenated EBSS in the petri dish. A second investigator can start on step 3.1.
7. Repeat steps 2.5 and 2.6 until serial brain sections have been obtained across the entire rostro-caudal plane.

3. Brain region microdissection

1. Adjust lighting to ensure that sample illumination will not be obstructed during tissue microdissection. Adjust the position and flow of 95%/5% mix of O_2/CO_2 to maintain a buffered, oxygenated environment for the tissue during microdissection.
2. Identify the coronal brain section that includes the region of interest. Orient the brain section flat on the bottom of the petri dish using an artist's brush. Select the tissue corer with appropriate diameter based on **Table 1** or other references. While holding the coronal tissue section in place with the artist's brush, bring the cutting tip of the tissue corer down over the surface of the tissue section.
3. While applying pressure, press the tissue corer tip through the tissue section and down firmly onto the bottom of the petri dish. Use a rolling circular motion to ensure that the region of interest has been dissociated from the surrounding tissue. Carefully lift the tissue corer away from the tissue section and liberate the micropunch, allowing it to float in the EBSS.
4. Using an artist's brush, immediately deposit the tissue micropunch into a -80 °C-stable storage tube kept on dry ice. The tissue should readily adhere to the side of the tube. Return the microsample-containing tube to the dry ice.
5. Repeat steps 3.2 - 3.4 for each brain region of interest.

4. Tissue archival

1. Once the microdissection is complete, use the VisionMate scanner system to record the respective bar codes for each sample-containing TrakMates tube. Once the 96-slot TrakMates tube rack has been filled, quickly scan the rack and immediately place the tissue samples into a -80 °C freezer until RNA extraction.

5. Total RNA extraction

1. Prepare a RNase-free workspace using RNaseZap. Prepare a sufficient amount of magnetic Bead Mix and Lysis/Binding Solution as described within the MagMAX-96 Total RNA Isolation Protocol[10].
2. Remove tissue samples from the -80 °C freezer and place the tubes directly onto dry ice.
3. Add 100 µL of the Lysis/Binding Solution to a sample-containing TrakMates tube. Attach a nuclease-free 0.5 ml tube-sized pestle to the pellet mixer and homogenize the tissue. Using a low-retention nuclease-free pipet tip, transfer the homogenized sample into a well on a round-bottom 96-well tissue culture plate. Immediately add 60 µL of 100% isopropanol to the sample using a low retention nuclease-free pipet tip, and mix by pipeting up and down 3-4 times.
4. Add 20 µL of the Bead Mix to the sample using a low retention nuclease-free pipet tip. Mix by pipeting up and down 3-4 times.
5. Repeat steps 5.3-5.4 for all tissue samples.
6. Follow the MagMAX-96 Total RNA Isolation Protocol steps starting at III.A.2 to complete the total RNA extraction. **NOTE:** Other magnetic bead-enabled total RNA isolation kits are commercially available; however, they were not examined in this study. Please refer to the manufacturer's kit manuals for the details of total RNA isolation using the respective products.
7. Measure the purity and concentration of resulting total RNA using a NanoDrop 3300 spectrophotometer or other small-volume spectrophotometer/fluorometer technologies.

6. Representative Results:

Brain Region/Nucleus	Tissue Corer Diameter (mm)	Total RNA (ng/µL)	260/280 Ratio
Frontal Cortex	2.0	27.03	1.99
Nucleus Accumbens	1.0	6.26	2.77
Striatum	1.5	27.94	2.19
Cingulate Cortex	1.5	29.05	2.07
Hypothalamus - Paraventricular Nucleus / Suprachiasmatic Nucleus (PVN/SCN)	2.0	40.34	2.03
Thalamus	2.0	34.40	2.00
Hypothalamus - Dorsomedial Nucleus / Ventromedial Nucleus (DMH/VMH)	2.0	44.83	2.05

Amygdala	1.0	10.06	1.89
Hippocampus - Ca 2/3	1.5	7.31	2.32
Hippocampus - Ca1/Dentate Gyrus	1.5	5.20	2.24
Periaqueductal Grey	1.0	48.21	2.02
Dorsal Raphe Nucleus	1.0	6.56	2.18
Reticular Formation	2.0	35.35	2.27

Table 1. Enumeration of microdissected mouse brain regions with respective optimal tissue corer diameters, representative total RNA yield and purity. The 13 microdissected brain regions, listed in rostro-caudal order, vary in size and orientation. The PVN/SCN micropunch includes the anteromedial paraventricular nucleus, portions of the periventricular nucleus, the sub-paraventricular zone and other parvocellular neurons. The thalamus micropunch includes anterior thalamic nuclei situated in the same coronal plane as the PVN/SCN micropunch region. The DMH/VMH micropunch includes accessory magnocellular nuclei and other parvocellular neurons situated between the dorsomedial nucleus and ventromedial nucleus. The amygdala sample includes both the central and basolateral subregions. The RNA yield and 260/280 ratios are single values obtained from respective microdissection samples to demonstrate readings obtained from each brain region.

Discussion

There are many critical steps in this protocol that warrant careful attention and consideration. Selection of the appropriate tissue corer diameter must be determined for removing each respective region of interest. While the tissue corer diameters presented in Table 1 are suitable for collection of the listed brain regions, each investigator should determine the appropriate-sized tissue corer for optimal collection of their respective tissue(s) of interest. Secondly, the coronal section thickness reported in this method (750 µm) was experimentally determined to be optimal for identifying the brain regions of interest (**Table 1**) in adult mice of various genetic backgrounds. Neuroanatomical landmarks are used to confirm the boundaries of respective regions of interest and do not vary between individual animals. The tissue thickness may be modified; however, selecting section thickness below 500 µm presents technical challenges that compromise the utility of this microdissection method. Lastly, the optimal length of time between animal decapitation and completion of brain sectioning is 5 minutes. By this time, all coronal brain sections should be in oxygenated EBSS media.

The use of magnetic bead technology for total RNA isolation eliminates column-based RNA extraction, which markedly reduces time required for completing this stage of sample preparation. Moreover, the use of the magnetic bead technology makes the protocol scalable; high-throughput total RNA isolation can employ commercially available magnetic particle processing system(s). In summary, the microdissection approach described here is an easily-implementable, cost-efficient, and scalable protocol that can be used across a wide array of investigations seeking to harness the power of next-generation sequencing in the analysis of transcript profiles of select mouse brain tissues.

Disclosures

No conflicts of interest declared.

Acknowledgements

This work was funded by the Defense Advanced Research Projects Agency (DARPA) and the Army Research Office (ARO), award number W911NF-10-1-006.

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