Laser capture dissection procedure: general protocol for Purkinje neurons
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June 27, 2014 version 1.0

Adult C57BL/6NCrl wild type mice are supplied by the Caltech Animal Facility.
The mouse is brought to the dissection area and euthanized via cervical dislocation.
Start the clock running to record PMI time to freeze.
The mouse is wiped down with 70% ethanol to keep hairs contained.

Brain dissection:

Using a fresh razor blade, incise the scalp from snout to lower cervical region. Then make a cross cut in the pelt of the neck at the base of the thoraco-cervical junction, being sure to cut through the muscles at the base of the neck.
Peel back the skin and the cervical muscles, exposing the cranium.
Insert tip of scissors at the magnum foramen, and cut forward towards the snout along the sagittal suture. Be sure to keep the scissor tips up, to avoid damage to the cerebellum.
Cut as far as the vibrissae on the snout, then make a cross cut through the maxilla.
Using a strong pair of serrated forceps, firmly grasp one of the separated halves of the skull and gently lift it away from the underlying brain; you should feel it break. Make additional cross cuts with the scissors on the posterior regions of the skull to help reflect the bone away from the brain. Repeat on the other side to completely expose the brain.
You may have to remove skull fragments at the posterior region of the skull with the forceps. Be careful not to damage the cerebellum.
Observe the caudal regions of the brain to see that they are free from the neck muscles. Cut with scissors as necessary.
When the brain is exposed, place a forceps gently under the frontal regions, and lift the brain gently away from the underlying base of the skull, reflecting it backwards toward the neck. You will have to sever some of the major cranial nerves, which exit on both sides, to completely dislodge it.
Sever the link between the spinal cord and the hindbrain with the scissors.
Using forceps, place the brain, dorsal side up, on a small piece of aluminum foil, then on to pulverized dry ice. Fold the foil over the brain and pile some pulverized dry ice on top to surround the brain and insure rapid freezing.
Aim for total dissection time to freeze of about 7-8 minutes.

Users choice: you can immerse your freshly dissected brain in liquid OCT sectioning medium at this point if you wish to freeze it “in block” for cryotomy. Alternatively, you can mount the frozen brain on a cryotome specimen holder with OCT later.

Laser Capture preparation :
The brain is mounted in the appropriate orientation on a specimen holder and sectioned at 20 microns in a cryostat set for -18 °C. The temperature can be varied according to local conditions to achieve smooth sectioning.

Prior to mounting, Zeiss MembraneSlides NF 1.0 PEN (order number 415190-9081-000) are treated for 30 minutes in a Stratagene UV transilluminator. This helps the sections adhere to the membrane better.
Sections are mounted on the membrane slide by pressing the room temperature slide on to the freshly cut section which is under the roll plate on the cryotome collection platform. We collect about 3 sections on the membrane. Be sure to check which side of the glass slide has the membrane.
Air dry the sections for 5 minutes at room temperature.
Stain with Pico Green DNA dye (LifeTech catalog # P7581) for 1 minute at room temperature.
Rinse 3 times for 1 minute at room temperature with solution of RNAse inhibitor (Clontech catalog # 2313A) at 1 U/uL in 1 mM DTT (Promega catalog # P1171) in RNAse-free water, by pipetting 300 uL of solution gently on to the sections on the membrane, and then aspirating with the pipet tip.
After 3 rinses and drains, tip the slide on its side to allow residual rinse to drain from the section and the membrane.
Place the slide in the dark in a Bakelite box or covered with aluminum foil, and proceed immediately to the laser capture scope. Do not store the sections.

Laser Capture Dissection

We are using a Zeiss PALM scope.
The section is mounted on the scope and visualized under 40X magnification.
The cell(s) of interest are traced with the stylus on the screen, and the command is executed to cut and catapult into a Zeiss 200 uL collection tube (catalog # 415190-9181-000).
Visualize the cut piece on the cap to be sure the catapulted fragment was retained.
Remove the collection tube from the instrument, and pipet 4.5 uL cell lysis buffer containing the 3’ CDS primer (SMARTER ultralow for Illumina Seqencing, Clontech catalog #634935) and quantitation standards (ERCC standards, Ambion, catalog # 4456740) into the cap.
Triturate with the pipet for 3 or 4 times.
Immediately close the cap into the tube, and spin down the contents in a microcentrifuge at 4°C for 30 seconds.
Immediately freeze the spun down sample on dry ice.
After samples are collected, proceed with library construction per manufacturer’s protocol (SMARTER ultralow for Illumina sequencing, Clontech catalog #634935).