## Fresh frozen tissue collection from brain sections

## for rnaseq and biochemistry

## collection of brains (in cdm)

Equipment to take to CDM:

* Isopentane (fresh bottle and waste collection bottle)
* Dissection tools (Large scissors, small scissors, fine dissecting scissors, forceps, fine spatula, fine forceps).
* Dry ice in ice foam lab ice bucket
* 50 ml beaker.
* Tubes for tissue collection, pre-labelled.
* Foil to wrap brains in.
* Mouse rectal thermometer (borrow Cembrowski labs)
1. Pour ~40ml of isopentane into 50ml beaker. Place in dry ice to chill. A piece of dry ice can be held in the isopentane with forceps to rapidly cool the solution (don’t drop in!). Check temperature with mouse rectal thermometer. **-56’C is optimal**.
2. Sacrifice mouse using isofluorane to anesthetize and scissors to rapidly decapitate.
3. Extract the brain (within 1 min) and immediately flash freeze by dropping directly into isopentane for **20 seconds**.
4. Remove brain from isopentane with plastic spoon and wrap in **pre chilled** foil.
5. To collect optic nerves, before dropping brain into isopentane hang the brain gently from the skull and cut the optic nerves where they join the brain. When brain has been removed, cut the optic nerves where they are joined to the skull and transfer to 1.5 ml Eppendorf tube with fine forceps. Dissect with care to not contaminate with non-neuronal cells from the surrounding vascular system.
	1. Place optic nerves in pre-chilled 1.5mL tube and place tube directly in dry ice to freeze optic nerves.
6. Keep brains on ice or in -80C freezer until sliced.

## Sectioning of brains on cryostat

1. Clean cryostat.
2. Set cryostat temperature to -13C.
3. Place brains in cryostat at least one hour before slicing (place brains in foils on top of the metal racks that cool the mounters).
4. Clean outside of cryostat + gloves with RNAse away before starting.
5. Change cryostat blade.
6. Cut off the cerebellum with a new blade.
7. Mount brain onto the cryostat mounters with OCT – try to use the least amount of OCT necessary. Let OCT harden completely.
8. Using the 100um trimming option, trim the brain down until you are approximately close to the beginning of the CC. Adjust so that both hemispheres are the same and the brain is properly aligned.
9. Begin collecting brain slices for your desired region/thickness.
	1. NOTES:
		1. To mount the sections onto the slides, align all the brains as indicated on the instructions next to the cryostat
		2. MUST PRECHILL THE SLIDE FOR 6 SECONDS PRIOR TO MOUNTING SLICES
			1. This is to prevent any thawing of the brain sample.
			2. Place slide on the metal rack for mounts in cryostat.
			3. Place slides in slidebox on dry ice **immediately**.
10. Collect all sections, label slides accordingly.
11. When done, clean up cryostat, RNAase away everything, and return temp to -10C.

## tissue dissections

Equipment setup:

* Prepare dissecting microscope (from tissue culture area) + microscope light.
* To keep slices frozen during dissection, place slides in half of a microscope slide box resting in dry ice (crushed dry ice keeps temperature optimal for dissection).
* Dry ice can be contained in a styrofoam box lid and mounted onto stand of microscope.
* Place frozen microscope slides to be dissected on a black square piece of paper inside the chilled microscope slide box to improve constrast/visulaization.
* All equipment is cleaned with RNAse away. All dissecting tools should be cleaned with RNAse away and then thoroughly cleaned in RNAse free water.
* Homogenize tissue with a hand held pestle (Argos Technologies – Item # RK-44468-25) in 1.5 ml Eppendorf tubes. RNAase free tubes/pestles are required if extracting RNA (Argos Technologies – Item # RK-44468-19).
* It is recommended that 2 people do this part together, one to dissect and one to homogenize the tissue quickly.

1. Prepare tubes with appropriate tissue dissociation buffer (50uL of Norgen RL buffer for RNAseq). Place on wet ice prior to dissections so RL buffer is chilled.
2. Dissections:
	1. Use tweezers, a scalpel or tissues punchers (must be pre-chilled) to dissect appropriate brain region using stereotactic atlas as a guide (http://labs.gaidi.ca/mouse-brain-atlas/). When tissue is isolated, place on tip of the 1.5 ml eppendorf pestle tip mounted in hand held pestle.
	2. Quickly place pestle into pre prepared tube with buffer and immediately homogenize the sample for 30 seconds.
	3. Spin tube briefly to bring all tissue/buffer to bottom of tube. Add the remaining volume of buffer and vortex for 30s.
	4. Place tissue on wet ice if RNA to be extracted immediately, or freeze at -80 for longer term storage.

## RNA extraction

1. Follow Norgen RNA extraction kit protocol (Total RNA Purification Micro Kit; Cat. 35300, 35350). Pre chill all reagents on wet ice.
2. Flash freeze samples at the end on dry ice.
3. Place samples in -80C until you take them to BRC to get analyzed.
	1. Take samples to BRC on dry ice so they don’t degrade.