

Brain punches

Mice sacrifices and brain punches

Material:

- Dissection kit
- Matrix (to put the brains in it and cut)
- Blades (at least 8)
- Punch pen
- Petri dishes (2 + extra if they break)
- PBS 1X (~50 mL per mice)
- Identified Eppendorfs for punches (1 per brain region per mice)
- Ice bucket, filled
- Dry ice bucket
- Cooling Eppendorf block
- Blue pad
- Gloves of you size (bring the box)
- Brown paper
- Body bags
- Keys for the perfusion room (in Manon's office)
- Atlas (if necessary)

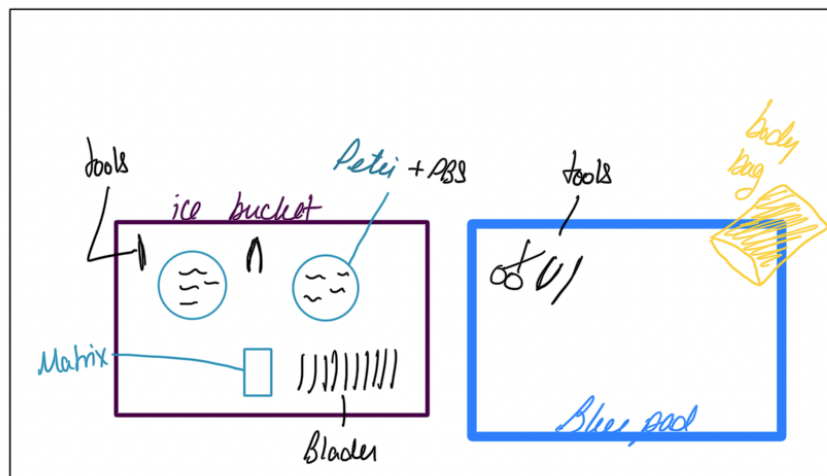
1. Identify your Eppendorfs before going down to the perfusion room.

Use the 1.5 mL Eppendorfs. You must have one Eppendorf per brain region per mouse. 2 punches, one per hemisphere, is usually collected per Eppendorf.

2. In the perfusion room, set the perfusion table as the following drawing.

Two Petri dishes are placed on the ice so that they don't move around. One Petri dish contains the washing PBS and the other is used to separate the brain slices and punch.

The blades and a few tools are in the ice to keep them cold.



3. Put the cooling Eppendorf block in the dry ice bucket.
4. The sacrifice is done by decapitation on the blue pad. The body is put in the body bag. The brain is extracted from the skull.
5. The brain is then put a few seconds in the first dish filled with fresh PBS to clean it.
6. The brain is then put in the matrix as straight as possible.

7. The blades are inserted in the matrix first at the bottom and the top of the brain to keep it steady. Then, a third blade is inserted in the matrix to cut the brain in half. The rest of the blades are added in each hole of the matrix to cut the brain in 1-mm thick slices.
8. Take all the blades out at the same time.
9. Dip the blades in the second Petri dish filled with PBS and take of the blades one by one. You can use a cold tool to help you put the brain slices in the PBS if they don't fall off by themselves.
I personally like to position the brain slices in order of front to back of the brain.
10. Select your brain slices with your region-of-interest (usually one brain slice per region).
You can use the brain atlas to help you select the brain slices.
11. Use the punch pen to punch the regions.
Doing circles helps.
Be careful to not push too hard otherwise the Petri dish will break!
12. Put your brain punch in the right Eppendorf.
13. Put the Eppendorf in the cooling Eppendorf block.
14. When you are finished:
Clean your tools with soap in the sink.
Clean the perfusion table.
The body bag goes in the freezer on the 6th floor and the used cages go in the animal facility on the 6th floor.
You can put the blue bag in another body bag and dispose of it in a yellow trash.
15. Keep the punches at -80°C until RNA extraction.