PROTOCOL-Cutting brains with the vibratome (Christian Proulx's lab)

This cutting technique is for brains that were fixed previous to the cutting experiment (usually by putting the whole mouse brain in 4% PFA for 24 hours, then putting it in fresh 1X PBS at 4°C for conservation).

Everything that is used in this protocol (PBS, brains) are kept on ice as much as possible.

1. Turn on the machine. The button is at the back-left.
2. Take your brain out of the PBS and put it on a clean paper. It needs to dry a little.
3. Take the black circular stage out of the container by unscrewing the screw in the front. You can use a screwdriver and the holes in the stage to take it out if you don't want to use your hands. Put the stage on the same clean paper. It needs to dry.
4. Use a clean blade to cut the cerebellum and the optic bulb off the brain. Put a drop of superglue in the middle of the black stage.
5. Position your brain on the superglue drop as straight as possible so that the antero-posterior axis is vertical (the drop touches the place where the cerebellum was).
6. Cover with foil for a minute. The superglue needs to dry.
7. During that time, fill the container with fresh 1X PBS. You can also annotate your wells and fill them with 1X PBS if it's not already done.
8. After a minute, make sure that the brain is well glued on the black stage. If yes, put the black stage in the container filled with PBS. Use the screwdriver to position the black stage so that the dorso-ventral axis is facing you when you are in front of the vibratome. Make sure that it is fixed by the two metal pins. Screw the screw at the front of the black stage.
9. Use the up-down pin to position the blade on top of the brain. You can click on the V-MAX button to move the stuff faster. Click again to put it back to the slower configuration.
10. Use the two-arrows button to set the blade lengths of cut on the dorso-ventral axis. Click once to set the starting position, click a second time to set the final position on the doors-ventral axis. Use the rev-forw pin to move the blade on the dorso-ventral axis. You can click on the V-MAX button to move the stuff faster. Click again to put it back to the slower configuration.
11. Set the cut thickness at 100 um. Use the + and - buttons to change the thickness.
12. Click on the SINGLE CONT button to set the configuration to SINGLE (green light must be at the top-right).
    1. In the SINGLE configuration, clicking on the START STOP button will make the blade cut only once. You have to click again on the START STOP button again to make another cut.
    2. In the CONT configuration, the vibratome makes slices continuously starting when you click on the START STOP buttom.
    3. The PAUSE buttom pauses at the position the blade is.
    4. To stop at the end of the current cut, click on the START STOP buttom.

13. Click on the START STOP button to start. Verify if the blade cuts a small piece of the brain. Click again until it cuts the brain. When it does, click on the START STOP button to stop.
14. Set the cut thickness at the thickness of your choice. Set the configuration to CONT if you want to cut the brain continuously. You can always change the cut thickness in the process of cutting according to your region(s)-of-interest.
15. Use a clean paint brush to collect your samples and put them in the wells filled with PBS.
16. When your done, unscrew the front screw of the black stage and take the black stage out of the vibratome container.
17. Use a blade to take all the brain and the superglue out of the black stage.
18. Clean your stuff.