Protocol for brain tissue processing:

1) Immunocytochemistry of brain sections

[Blocking solution contains 2% tritonX plus 1% goat serum]
- Take 500 µl of blocking solution into a new eppendorf tube, and add into it 2 µl of primary antibody against NeuN and 2 µl of antibody against GFAP. Mix by finger-tapping.

- Pipette the 500 µl of Block.sol.+antibodies into a well of the plate.

- With a brush, take one of the brain sections and transfer it into the well with Block.sol.+antibodies.

- We'll keep the antibodies incubating with the brain section for 45 mi. to 1 hour.

2) Perfusion of mice

- Inject mouse with 1 ml of anesthesia (avertin) intraperitoneally

-While waiting, prepare a 10 ml syringe with PBS, and a 20 ml syringe with 3% paraformaldehyde

- wait until the animal is fully anesthetized (the animal does not respond when pinching the toes)
- Secure down the animal into a perfusion tray.

- With scissors, open the skin around the abdomen, and cut up to the chest into the midline
- expose the rib cage out so that you can reach the heart

- cut the right atrium with the scissors (a small cut is sufficient)

-Insert the butterfly needle into the LEFT ventricle

- start applying pressure to the syringe with PBS (slowly, at a rate of 2 ml per minute)

- take the PBS syringe out of the butterfly and exchange it for a 20 ml syringe with paraformaldehyde. (Be careful that the needle does not come out of the heart)

- apply pressure t the PF syringe at a rate of 2 ml per min.

- Once the PF perfusion is complete, proceed to extract the brain out:

- cut the head from the rest of the body

- cut the skin off the skull

- carefully, cut the periphery of the skull (without damaging the brain)

- with a blunt tool, pull the brain out of the skull

1B) Continuation of the ICC

- with the brush, take the section from the antibody solution and put it into a new well with PBS
- keep it rocking for 5 minutes, and repeat this washing step twice.
- while you wait for the washings, prepare 500 µl of Block. Soln. and add to it 4 µl of fluorescein antimouse and texas red antirabbit antibodies. Finger mix.

Once you are done with the washings, put the block soln + secondary antibodies into a well, and with the brush, put the brain section there. We will incubate this for 45 min to 1 hour.

3) Sectioning the brain with a microtome or a vibratome

a) freezing microtome:

- put dry ice on the receptacle for the microtome

- make a small mound of 10% sucrose on the stage of the microtome

- place the blade on the microtome

- **very carefully** slide gently the blade to flatten the sucrose (Don't cut yourself , and don't ram the blade onto the metal!)

- once the mound is flat, place a brain on the flat, and cover it with a few drops of 10% sucrose in PBS.

- Once it is frozen, position the blade to level it with the top of the brain.

-VERY CAREFULLY, gently slide the blade to cut the brain.

- the brain section will be molten on top of the blade: pick it up with a brush and put it into a well with PBS.

b) vibratome:

- microwave 2% agarose in PBS

- with crazy glue, glue the brain to the stage of the vibratome (just one tiny drop!).

- put a few drops of molten agarose to cover the brain.

- put a tiny drop of crazy glue surrounding the agarose.

- put the blade on the vibrating head.

- set the vibratome to maximum vibrating speed and low advance speed. And start cutting.

- collect the brain sections in a well with PBS

1C) Finishing the ICC

- wash the secondary off by incubating the section in fresh PBS (three times as before)

- once you are done, take the section out of the well, lay it flat on a glass slide, and let it dry for 5 minutes

- place a 100 µl drop of glycerol on the section, and apply a thin coverglass on top (be careful not to make bubbles).

- Look at the section with the fluorescent microscopes.