Paraffin Tissue Processing

1. Immersion fix the tissue (embryonic and adult) in ice–cold Carnoy’s fixative.
2. Alternatively, perfuse the tissue with 4% PFA and immersion fix in PFA O/N.

Carnoy’s Fixative

60% Ethanol
30% Choloroform
10% Glacial Acetic acid

For 50 ml Carnoy’s

30 ml Ethanol
15 ml Chloroform
5 ml Glacial acetic acid

For 25 ml Carnoys’s

15 ml Ethanol
7.5 ml Chloroform
2.5 ml Glacial acetic acid

3. Let the tissue fix for at least 24 hrs at 4 degrees.
4. Wash the tissue in 70% ethanol (2 X 1 hr). The tissue may be stored further at 4 degrees. For PFA fixed tissue, wash the tissue in sequential washes of PBS, 30%, 50%, 70% ethanol (45 min each). The time you spend washing should roughly correspond (at least) to the time setting of the tissue processor (see below).
5. Prepare the tissue for paraffin embedding using the tissue processor. Set the program for the desire amount of time (use at least 40 min for adult cerebella).
6. Quickly remove the tissue from the processor (the tissue is held in hot paraffin at the end of the cycle) and embed in paraffin.
7. The only setting that needs to be turned “on” on the paraffin dispenser is the cold plate. All other settings are not touched.
8. Make sure you mark the orientation of the tissue before the paraffin cools.
9. Once the paraffin wax has solidified, place your tissue in the fridge for storage.