Paraffin Processing of Tissue

Material:

1. Bouin's Fixative
2. Wax (The best from Leica
3. Ethanol (70%, 95%, 100%)
4. Xylene

Procedure:

1. Dissect the embryos from pregnant mouse.
2. Cut embryo's leg or tail for genotyping. If the embryo is too small (≤ 10.5), the york sac need to be isolated for genotyping.
3. Cut off the mouse butt or make a through cut on embryo's bally; De-skin the embryo if it is older than E16.5.
4. Transfer the embryo to glass viral, label well.
5. Fix the embryo with 10-20 embryo volume of Bouin's fixative. (Note: Bouin's fixation is better than 4% PFA to preserve mouse liver. To make a slim section or get better cut, fix the embryo for over 48hours.)
6. 70% ethanol for 1 hour. (At this step, embryo tissue can last forever at 4˚C.)
7. 95% ethanol (95% ethanol) for 1 hour.
8. Absolute ethanol for 2 hour or longer.
9. First clearing agent (Xylene or substitute) 1 hour.
10. Second clearing agent (Xylene or substitute) 1 hour or until the embryo become transparent and liver is observable.
11. Transfer the embryos to plastic cassette and label the embryo with

notepaper written with pensile to avoid the dissolution of the regular markers.

12. Wax (Paraplast X-tra) at 58°C for overnight if the wax is fresh. Two steps of wax is appreciated also (First: 1hour; Second: overnight).
13. Embed the embryos into the disposable mold on Leica embedding machine. Position the embryo well within the mold with melt wax inside. Freeze the embryos on the cold plate of embedding machine.
14. Tear off the mold and attach the wax block to the plastic cassette. Trim the block to tube-shape only containing the embryo tissue. Now the block is ready for section.