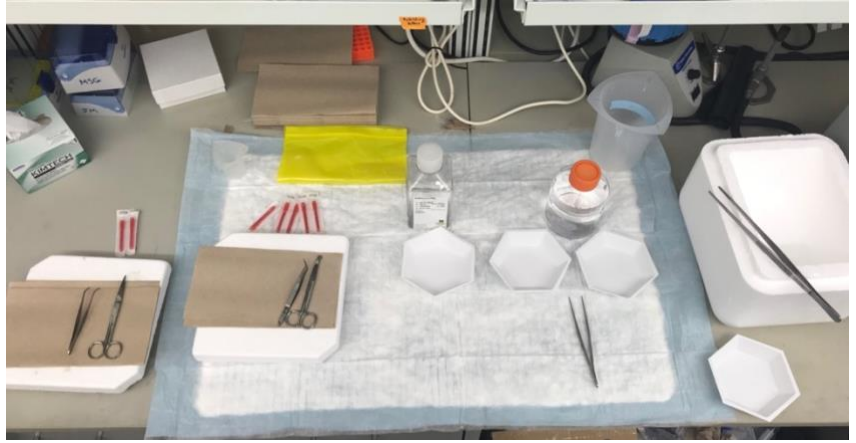


Tissue Harvest Protocol

Equipment/utensils

- Liquid nitrogen, 1 Styrofoam box to store organs
- 2 Styrofoam lids
- Dissections tool (scissors, forceps)
- Underpad Simplicity™ 23 X 36 Inch Disposable Fluff Light Absorbency
- 1L PBS
- 1L ddH₂O
- 4 weigh boats
- 2 waste containers (large and small)
- “Dead animal” bag
- Tinfoil packages for each tissue of interest typically: brain, heart, lung, liver, kidney, skeletal muscle



Protocol

- Cervical dislocate mouse and cut of head – do not perfuse or sedate animal!
 - Metabolism rapidly changes after death. Quick and efficient work is needed to preserve metabolites
- Dissect tissue of interest rapidly (the faster the better, metabolism changes by the second)
 - One person can dissect brain while another work on the internal organs and skeletal muscle
 - Typically collect in order: **heart, lung, liver, kidney, skeletal muscle**
 - **Brain** to be removed rapidly and preferably intact
 - To collect: open skull by cutting up the middle toward the eyes. Once you have reached the eyes cut down the skull to each side
 - Open skull like a window and slide closed forceps under brain to scoop out in one piece
 - LEFT Hemisphere: FFPE preserved
 - RIGHT Hemisphere: Freeze
- Drop tissue immediately into PBS then quickly wash twice in ddH₂O
- Removed excess water by gently touching tissue to paper towel
- **PRESERVING for FFPE:** Drop clean tissue into 5ml of 10% NBF (recipe: 50ml of Formaldehyde in 450ml of 1xPBS). The preferred ratio of volume of NBF:tissue is 5x-10x greater for all samples. Example, if liver from mouse-preserve in 50ml tube with 40mls of NBF because liver is larger.
 - After exactly 24 hours has passed, change to 70% ethanol. Tissue can be stored at 4C in 70% ethanol until embedding in paraffin. Sections are then cut at 4microns for MALDI-MSI analysis.
- **FREEZING:** Drop the organ into a weigh boat containing liquid nitrogen
 - After the organs are frozen in the weigh boat, store in tinfoil packages and keep in liquid nitrogen until rest of the mice haven been dissected – store organs in -80°C for later use
 - Perform dissection, washing and freezing as fast as possible. Time estimate: 4-7 min/mouse.