Harvesting skin samples for histological analysis

1. *Samples for paraffin embedding*

General:
Make sure that the volume of formalin used for fixing the tissue is 15-20x the volume of the sample. Fix your samples in 10% neutral buffered formalin (NBF) O/N at 4°C. The next day, replace the formalin with 70% EtOH and keep the sample at 4°C or room temperature.

Fill out the requisition form and email it to Histology@ucdenver.edu or drop it off at the Histology Core (P18-8403A). Leave your samples next to the Histology Request Drop Box on the Histology Core bench or in the fridge beneath the drop box labeled "Histology Core." Make sure your samples are clearly labeled matching the sample ID on the requisition form.

Back skin (E17.5-adult mice):
Within the same experiment, skin samples should be harvested from the same area of the body. Often, the back of the mouse is chosen for this purpose. If you are using back skin, take the biopsy from the middle of the back (on top of the spine) midway between the head and tail. Using a razor blade or scalpel, trim the biopsy into a rectangle, such that the long axis of the rectangle corresponds to the A-P axis of the mouse. Unless otherwise indicated, the biopsy will be sectioned along the long axis of the rectangle. If sectioned this way, your sections will provide a good view of the interfollicular epidermis as well as the hair follicles. If you prefer sectioning along a different axis, please indicate this clearly on the requisition form.

Place your rectangular piece of skin flat on a piece of Whatman filter paper or unlined index card (dermis side down). This will ensure that the skin sample will remain flat while it is fixing. Then place the paper with the skin in 10% neutral buffered formalin and proceed as above.

Embryonic skin (up to E16.5):
For embryos up to E16.5, fix the entire embryo rather than attempting to remove the skin. Carefully dissect the embryo free from extraembryonic tissues, place it in 10% NBF or Bouin's fixative, and proceed as above.

Unless otherwise indicated, embryos will be sectioned from the midline along the sagittal axis.

Ear skin:
Remove the entire ear of an adult mouse and trim the biopsy into a rectangle, such that the long axis of the rectangle is perpendicular to the head of the mouse.

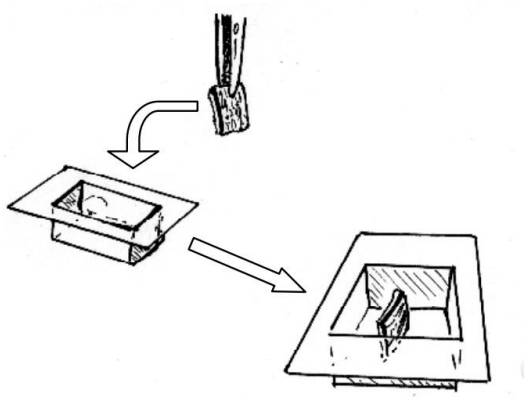
Tumors:
After dissecting out the tumor, trim the tumor to prepare a biopsy with at least one straight edge. The straight edge will be the cutting edge of the sample. If possible, the cutting edge should be perpendicular

to the skin of the mouse, such that your section will contain the most superficial as well as the most basal part of the tumor.

2. ***Samples for OCT embedding***

If you have never embedded skin in OCT, please contact Maranke Koster (Maranke.Koster@ucdenver.edu) for help with your first sample.

As a general guideline, prepare the skin sample as above (i.e. rectangular pieces of back skin, etc). Since the sample will be sectioned from the bottom of the cryomold, your sample should be placed in the cryomold accordingly. For example, rectangular pieces of back skin must be placed on the bottom of the cryomold on the edge of the long side (see figure [drawn by Irene Choi]).



Slowly fill the cryomold with OCT and place the sample in the middle of the cryomold. Once you have positioned the biopsy in OCT, make sure to remove any bubbles that may surround your tissue, as they can cause problems with cutting. You can remove the bubbles with a thin needle. Place the cryomold + OCT + biopsy on dry ice to allow the OCT to freeze (it will turn white). Once the OCT is frozen, store your samples at -80°C.

Embryos must be processed through a sucrose gradient prior to embedding in OCT. Please contact Maranke Koster for a protocol.

After embedding your samples, fill out the requisition form and email it to Histology@ucdenver.edu or drop it off in the Histology Request Drop Box on the Histology Core desk. Leave the samples in a box labeled with your initials, date and sample ID in the freezer labeled "Frozens" in Room P18-8213.

3. ***Further information***

For more information on the use of different fixatives and on processing skin tissue:

Seymour R, Ichiki T, Mikaelian I, Boggess D, Silva KA, and Sundberg JP. 2004. Necropsy Methods. In: Handbook of Experimental Animals: The Laboratory Mouse, Hedrich HJ (ed), Academic Press, London, pp 495-516.