For skin and other biopsies:

Fix flat between sponges for 24 hours in 10 volume of
- 10% buffered formalin
- Or freshly made 4% paraformaldehyde

Transfer to 70% alcohol before submitting for processing.
What are the cell / tissue types you will see in the skin?
Squamous epithelium--keratin positive
Cuboidal epithelium- around sweat glands --keratin positive
Collagen (trichrome stain will help see this better)
Sebaceous glands
Fibroblasts--vimentin
Blood vessels--CD31
Nerve bundles
Adipose tissue
Innate immune cells--CD45 etc
Keratinized Squamous Epithelium of epidermis, with underlying dermis
Keratinizing squamous epithelium of epidermis of skin

Dermis with sweat glands and sebaceous glands and hair follicles
The sebaceous gland empties its contents into the hair follicle. Bundles of arrector pili muscle (red arrows) are shown.

Bar = 250 Microns
Five strata of the epidermis:  
basale (green, oblique cut),  
spinosum (black),  
granulosum (red),  
lucidem (blue),  
corneum (yellow). Small blood vessels (long arrows) in the dermis.
Esophagus and cervix has no keratin layer
The difference in skin color between people of different pigmentation: number (quantity) of melanocytes is the same, but activity level is different (quantity and relative amounts of eumelanin and pheomelanin). This process is under hormonal control, including the MSH and ACTH peptides that are produced from the precursor proopiomelanocortin. Tyrosinase, is required for melanocytes to produce melanin from the amino acid tyrosine.
Mouse skin epidermis is usually very thin
An example of abnormally thickened mouse skin epidermis as a result of inflammation
CELL DEATH:
necrosis (occurs from the progressive degradative action of enzymes on the lethally injured cells)
apoptosis: -programmed destruction of cells during embryogenesis
- hormone dependent involution in the adult
- cell deletion in proliferating cell populations, immune cells, tumors, etc.

Sections of skin and gut are usually good controls for TUNEL assays for apoptosis
Benign tumors of skin epidermis may arise from any of the component cells:
--hair follicle tumors--trichoepitheliomas
--sweat gland tumors
--vascular tumors
--tumors arising from the supporting fibroblasts

Malignancies of the skin include:
--basal cell carcinomas--only locally invasive
--squamous carcinomas
--melanomas
--metastatic malignancies
Basal cell carcinoma cells extend below the epidermis. Note the typical nuclear palisading at the peripheral layer of the tumor.

In the histological slide, a squamous cell carcinoma has the following features:

--**dermal invasion** by abnormal cells from the epidermis
--presence of keratinization within the cells which give the cells abundant pink cytoplasm, (this may be absent in poorly differentiated type).
Normal Skin epidermis and keratin  Squamous Carcinoma, well differentiated
Features of Melanoma--"ABCD"

Asymmetry: If you could fold the lesion in two, the two halves would not match.

Border. Melanomas often have uneven or blurred borders.

Color. Melanoma typically is not one solid color; rather it contains mixed shades of tan, brown, and black. It can also show traces of red, blue, or white.

Diameter. While melanomas are usually greater than 6 millimeters (about the size of a pencil eraser) when diagnosed, they can be smaller.

If you notice a mole different from others, or which changes, itches, or bleeds even if it is smaller than 6 millimeters, you should see a dermatologist.
Location of the mammary glands in the mouse
The mammary glands are greatly modified and enlarged sweat glands. The ducts and acini (i.e. the gland parenchyma) are formed mainly from cuboidal epithelial cells, with myoepithelial cells present to aid secretion. The connective tissue contains elastic fibres and adipose tissue. Connective tissue lamellae divide the parenchyma into lobules.

In the inactive mammary gland, the interstitial spaces between lobes and ducts are filled mainly with fat. There are a few alveoli present compared to those in the lactating gland, and the ducts and lobes of the gland are smaller than when the gland is in its active phase.

During early pregnancy, the epithelial cells of the glandular tissue proliferate rapidly to form the buds which eventually enlarge to form the alveoli. As pregnancy develops, the fat and connective tissue between lobes and ducts is replaced by secretory tissue.
Mouse mammary gland stages seen as a whole mount preparation with an example of a paraffin section H&E.
Defective branching of alveolar mammary glands

KO

Wt
Defective branching of alveolar mammary glands with no production of milk postpartum
H&E of the structure of lobules in a human breast
H&E of the structure of a duct in a human breast
H&E of the carcinoma in a human breast
What are the cell / tissue types you will see in breast tissue?

Cuboidal cells---keratin positive
Adipocytes
Blood vessels
Fibroblasts
Nerve fibers
Innate immune cells
Bone and Joints

Bone has to be decalcified before it can be examined using the usual histochemical methods.

REMOVE AS MUCH MUSCLE as possible

Fix in Cal-Ex II –Fisher Cat. No. CS511-1D (buffered formalin)

but do not let them be exposed to this for more than 3 days

Fix and then decalcify in EDTA if immunostains are to be done
**Cartilage** is a type of dense connective tissue. It is composed of cells called chondrocytes which are dispersed in a firm gel-like ground substance, called the matrix.

Cartilage is avascular (contains no blood vessels) and nutrients are diffused through the matrix.

The main purpose of cartilage is to provide a framework upon which bone deposition could begin.

Another important purpose of cartilage is to provide smooth surfaces for the movement of articulating bones.

Hyaline Cartilage is the most abundant type of cartilage.

Elastic cartilage

Fibrocartilage
The matrix of cartilage acts as a barrier, preventing the entry of lymphocytes or diffusion of immunoglobulins. This property allows for the transplantation of cartilage from one individual to another without fear of tissue rejection.

Bioengineering techniques are being developed to generate new cartilage, using a cellular "scaffolding" material and cultured cells to grow artificial cartilage.
Elastic cartilage is found in the pinna of the ear and several tubes, such as the walls of the auditory and eustachian canals and larynx.

Elastic cartilage is similar to hyaline cartilage but contains elastic bundles (elastin) scattered throughout the matrix.

This provides a tissue which is stiff yet elastic.

Fibrocartilage is a specialized type of cartilage found in areas requiring tough support or great tensile strength, such as between intervertebral disks, the pubic and other symphyses, and at sites connecting tendons or ligaments to bones.
Chondrocytes develop in the perichondrium. As they mature, they are moved deeper into the cartilage. There they actually secrete the matrix that traps them. Isogenous groups (small nests of chondrocytes) result from repeated cell division.
Bone and marrow with precursor bone forming cells at different stages of development.
Elastic cartilage is found in the pinna of the ear and several tubes, such as the walls of the auditory and eustachian canals and larynx. Cartilage is present to keep the tubes permanently open.

Elastic cartilage is similar to hyaline cartilage but contains elastic bundles (elastin) scattered throughout the matrix. This provides a tissue which is stiff yet elastic.
**Periosteum.** A tissue covering the bone that brings blood and lymph vessels, as well as nerves, to it

*Compact bone* (also known as cortical bone). Dense deposits of minerals - chiefly calcium phosphate - and collagen. These are arranged in concentric circles around a central Haversian canal through which blood and lymph vessels as well as nerves pass.

*Spongy bone* (also known as trabecular or cancellous bone). The mineral deposits are arranged as a system of struts. Bone marrow fill the spaces between.

*Bone marrow.* Some bones, such as the femur, also contain a central cavity filled with bone marrow. Bone marrow contains the stem cells that give rise to all the types of blood cells.

*Epiphyseal plate.* Prior to puberty, this disk of cartilage produces more cartilage which then is converted into more bone. In this way, the bone grows lengthwise.
Compact Bone & Spongy (Cancellous Bone)

- Lacunae containing osteocytes
- Lamellae
- Canaliculi
- Osteon of compact bone
- Trabeculae of spongy bone
- Osteon
- Haversian canal
- Periosteum
- Volkmann's canal
Epiphysial Growth Plate: The epiphysial growth plate, where cartilage is removed and bone is formed, is marked by four stages:

- resting zone
- proliferative zone
- hypertrophy zone
- ossification zone

Bar = 250 Microns
The remodeling of bone requires the coordinated activity of two types of cells:

* osteoclasts: demineralize bone
* osteoblasts: secrete collagen and mineral to lay down new bone

Osteoclasts are derived from stem cells in the bone marrow - the same ones produce monocytes and macrophages. TRAP is the histochemical stain to identify them. Excess activity of osteoclasts (common after menopause in women) produces osteoporosis. The bones become weakened as cortical bone gets thinner and the spaces in spongy bone get larger.

Hormones and Bone
In addition to the role of growth hormone (GH) and sex hormones (estrogen and progesterone) in the growth of bones, the remodeling of bone is controlled by:
* parathyroid hormone (PTH). It promotes the number and activity of osteoblasts.
* calcitriol (1,25[OH]2 vitamin D3
* calcitonin and thyroid stimulating hormone (TSH), both of which inhibit the activity of osteoclasts.
* leptin

http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/B/Bone.html
Osteoclasts (under the influence of hormones, destroy bone), are multinucleated giant cells, found within bone marrow, or adjacent to bone.
Osteoblasts (build bone) are found lining bony spicules
Histo-chemistry methods

Examples: Alizarin Red and Alcian blue on cleared embryos to examine bone and cartilage carefully.

Safranin-O may also be used.
Clear tissues and stain with Alizarin Red for Bone and with Alcian Blue or Safranin-O for cartilage
A joint (the place where two bones meet) is surrounded by a capsule that protects and supports it.

The joint capsule is lined with a type of tissue called synovium, which produces synovial fluid that lubricates and nourishes joint tissues.

http://www.niams.nih.gov/hi/topics/
Osteoarthritis

Healthy knee joint

Hypertrophy and spurring of bone and erosion of cartilage

Degenerative Joint disease

http://www.umm.edu/patiented/articles/what_osteoarthritis_000035_1.htm
Common diseases of joints:
Gouty arthritis
Rheumatoid arthritis

In rheumatoid arthritis, the synovium becomes inflamed, causing warmth, redness, swelling, and pain. As the disease progresses, the inflamed synovium invades and damages the cartilage and bone of the joint. Surrounding muscles, ligaments, and tendons become weakened. Rheumatoid arthritis also can cause more generalized bone loss that may lead to osteoporosis (fragile bones that are prone to fracture).