# Mammal Skeletonizing Manual


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Illustrations by Amelia Tsai

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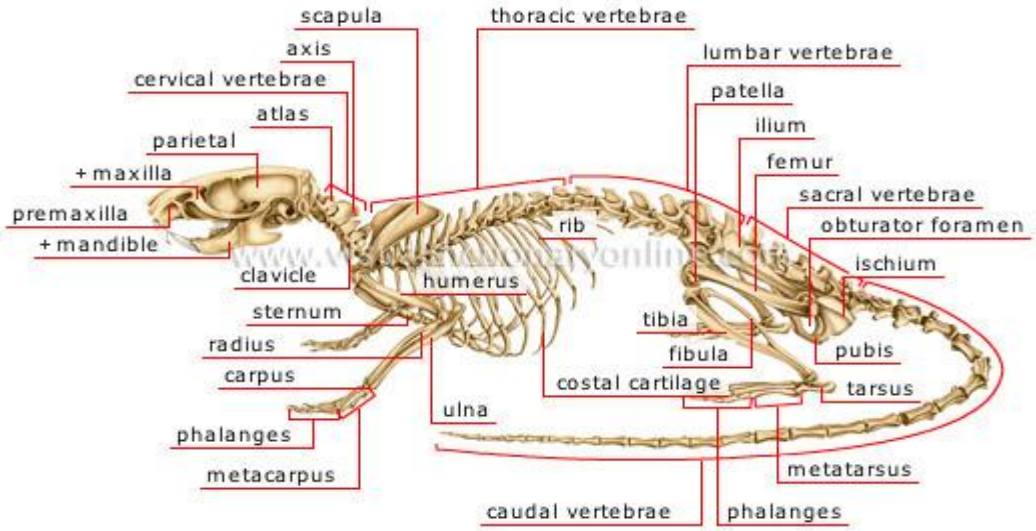
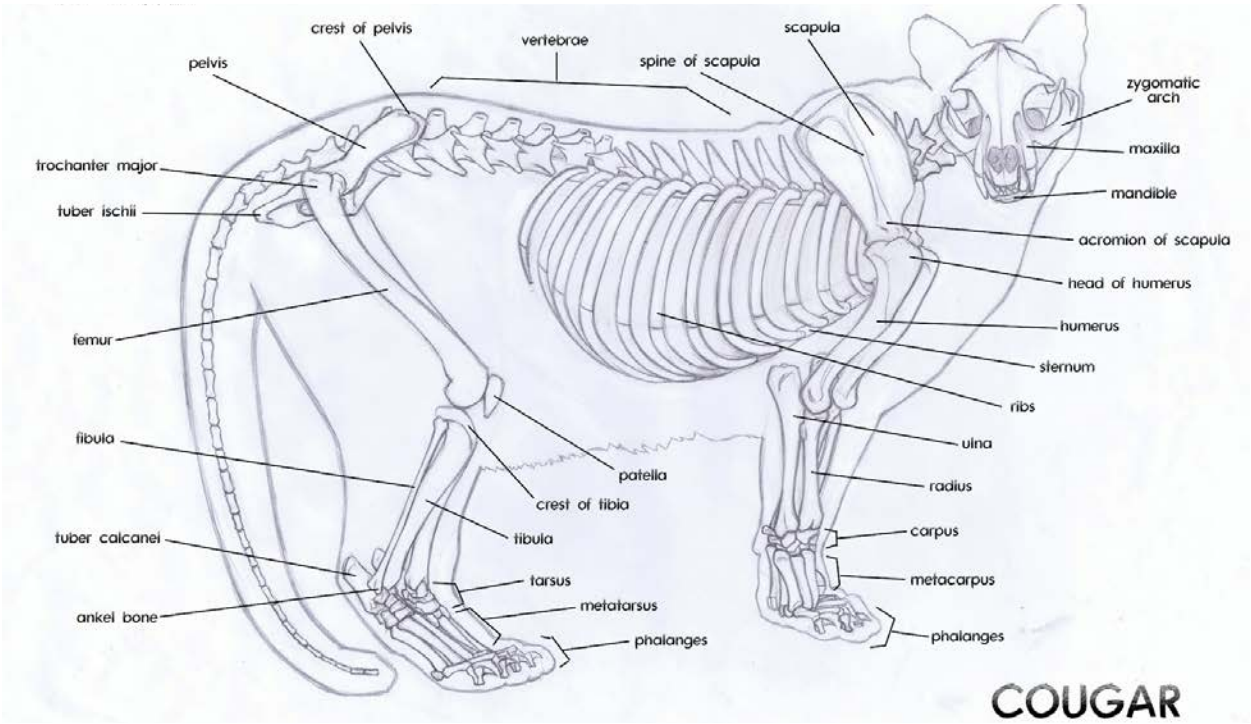
**Skeletons for Reference**

*Taken from the Merriam Webster visual dictionary*



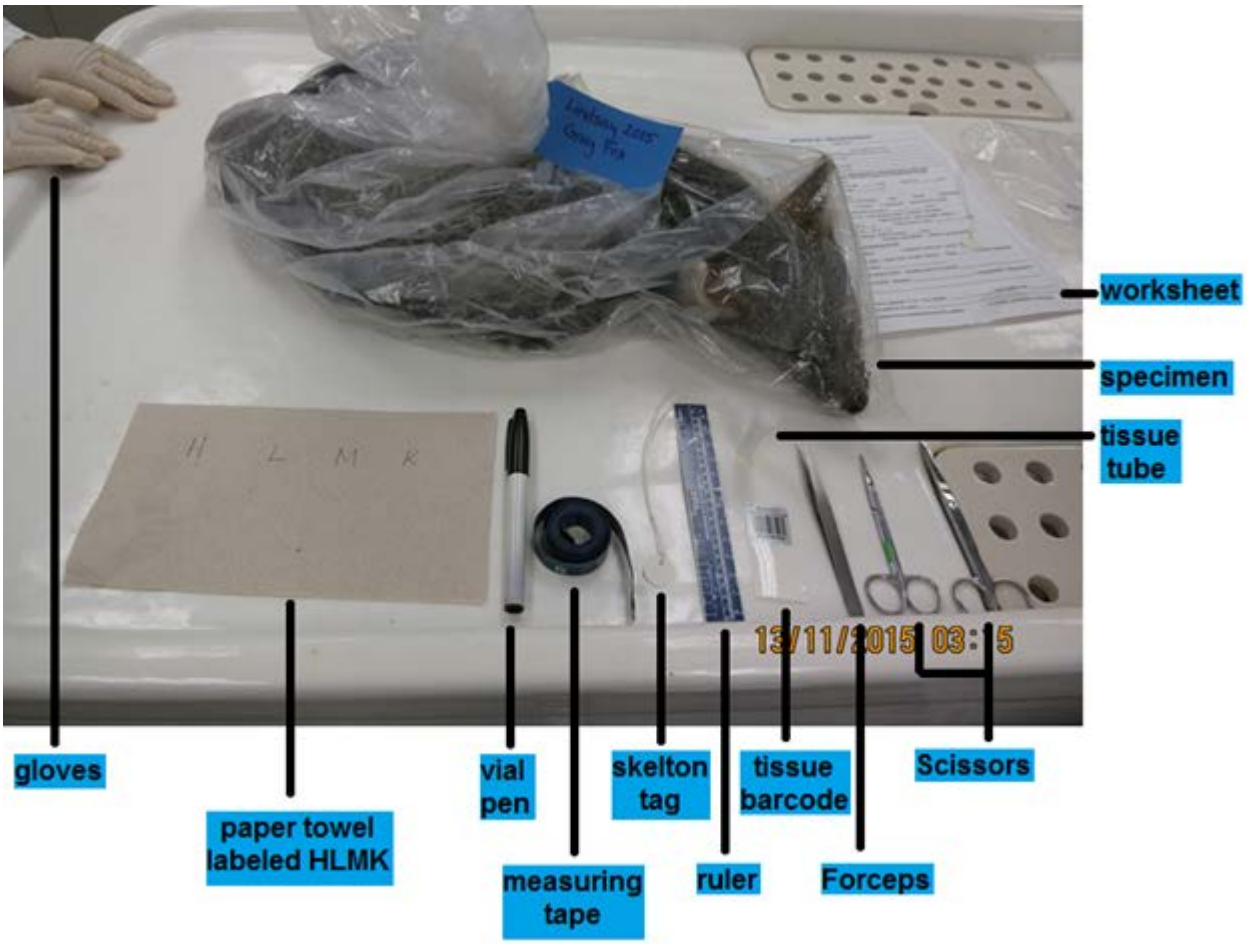



# Setting up your station

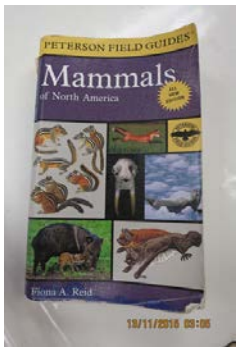
**Materials needed:**

- Mammal specimen
- Original data
- Prep lab worksheet
- Forceps
- Scissors
- Gloves
- Ruler and measuring tape
- MVZ barcode (for tissue vial)
- Tissue vial
- Round skeleton tag
- Tray (optional)
- Micron pen
- Vial pen
- Probe
- Camera*
- Sawdust (optional)
- Scale*
- Paper towel labeled H, L, M, and K
- Mammal field guide*
- Wet sample vial and fluid tag (if necessary)
- Prep Lab Catalog*
- Biohazard bin*

*only need access to these items, you do not need to keep them at your station at all times

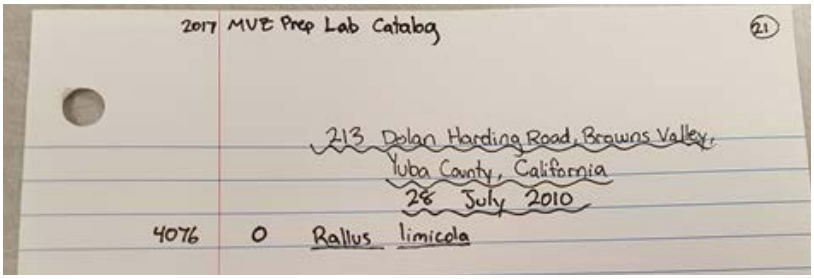


# Before you start cutting

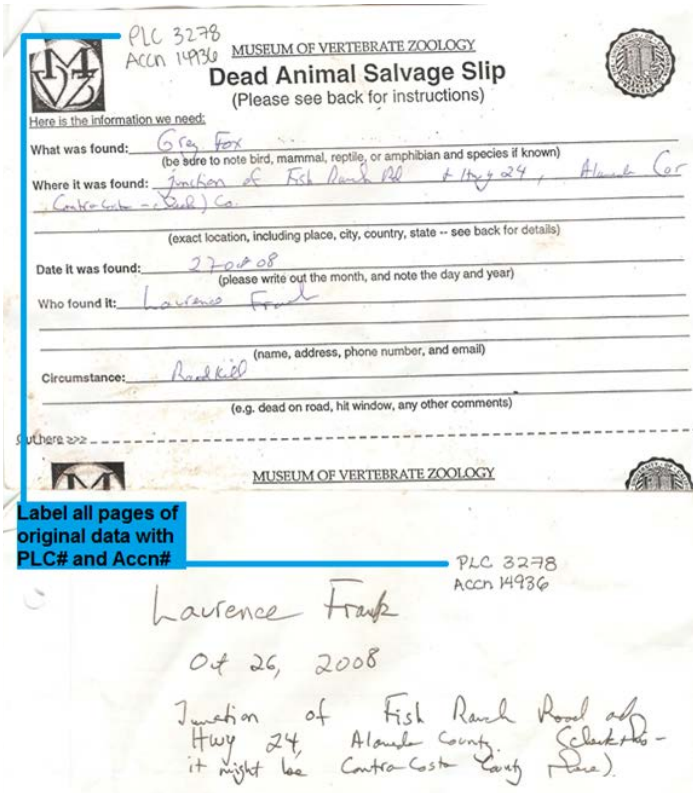



- Set up your station with all of the listed materials (or access to them)
- Determine/confirm your specimen's genus and species with the field guide. Have Terri or the class coordinator double check (**Never assume that the ID is correct**). Record this on your worksheet.
    - TIP – *use your measurements and locality info from the original data sheet to help identify*
- Fill out the top of your worksheet:
    - Accn #, received from, and other Reference # (if there is one) should be on your original data, if not, check in with Terri or the class coordinator.
        - Write the other reference # as it is on the original data (i.e. patient or admit # 213) unless it is Lindsay Wildlife which should be LWH - year - # (i.e. LWH-12-4123)
        - Donations from Lindsay say Lindsay Wildlife *Museum* since that is the larger organization but we record our specimens as received from Lindsay Wildlife *Hospital* because that is who we work with directly.
        - If "received from" is unclear, ask Terri or the class coordinator, don't guess.
    - Locality (specific to general). Include the county and state. **NO abbreviations**
        - If a state is written, we can assume that the specimen is from the United States so there is no need to write the country. If it isn't from the USA record the country as well.
        - Do not record the postal/zip code.
        - Double check a locality if you are unsure or missing some information. If there is no locality (double check with Terri or the class coordinator), write "No Data" *in pencil* both on your worksheet and in the catalog.
    - Date specimen collected
        - Date collected is written Day Month Year with no abbreviations or punctuation (i.e. 23 February 2011).
        - If we received the specimen from a rehabilitation facility (Wildcare, Lindsay Wildlife, etc.), the *date it was found or taken out of the wild/its natural habitat is the date collected*. DO NOT mix this up with the date of death or with the date it was donated to us from another facility or acquired by another institution. If there is no other date than the date of death, then, and only then, you can use that as the collected date.
        - If you have both a "date collected" and date of death, write both on your worksheet (date of death near the bottom right). Write "date collected" in your catalog entry header under locality and date of death in other original information. Example: Received from Lindsay Wildlife Hospital (LWH-12-453, date of death: 6 Mar 2012).
        - If there is no date information at all (double check with Terri or the class coordinator), write "No Data" *in pencil* on your worksheet and in the catalog.
        - DOA = Dead on arrival

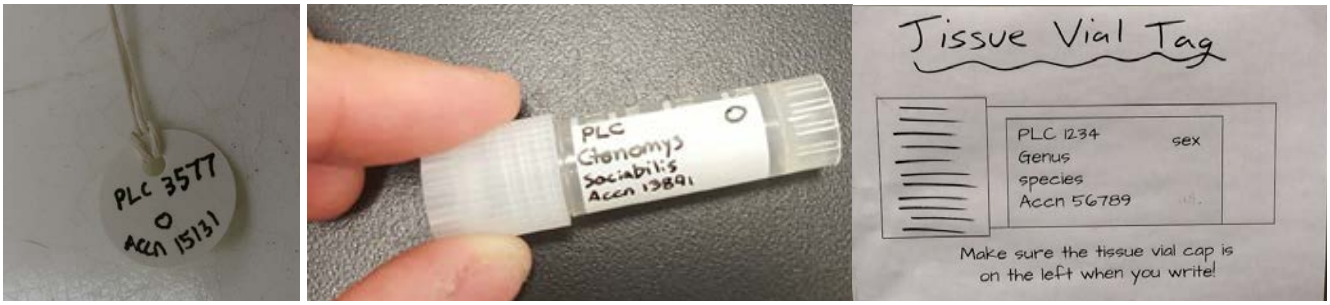
- Claim your unique PLC number
    - In the Prep Lab Catalog, write the next available number in the margin. Write as much identifying information as possible, including locality and date *collected* to avoid two people using the same PLC #. Species is also helpful.




    - Write your claimed PLC # and Accn # on ***all*** pieces of the original data (donation sheets) as well as your worksheet, tags, and vials.

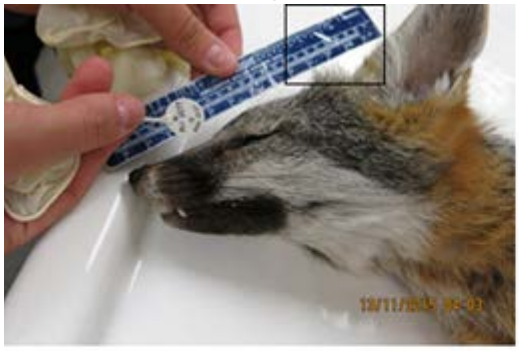
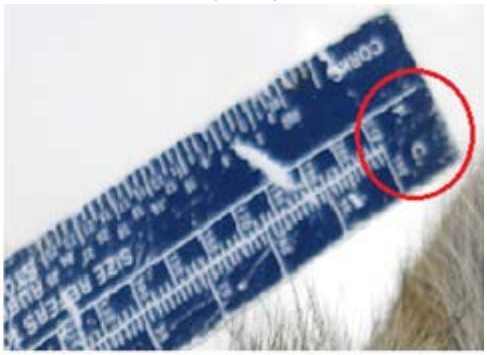
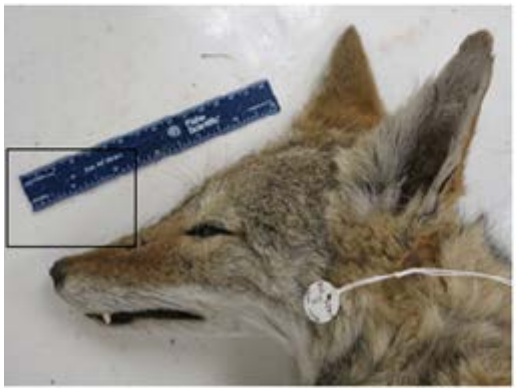



- Prepare your specimen tag: PLC #, sex, and Accn #.



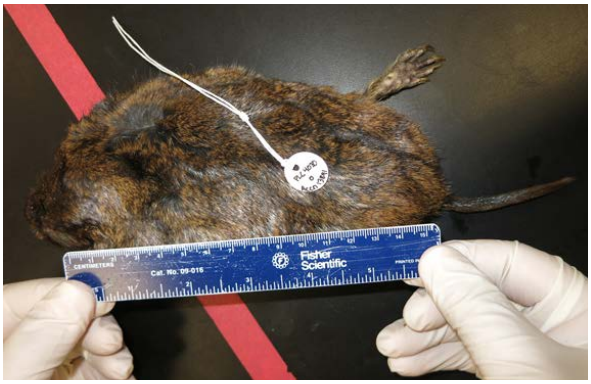

- Prepare your tissue vial: PLC #, genus, species, Accn #, sex.
    - Try to write small on the tissue vial and leave space near the bottom of the label
    - *TIP – You will need to add sex to the tag later. Sex determined externally can be a helpful indicator, but should not be recorded until confirmed with the presence of internal organs.*

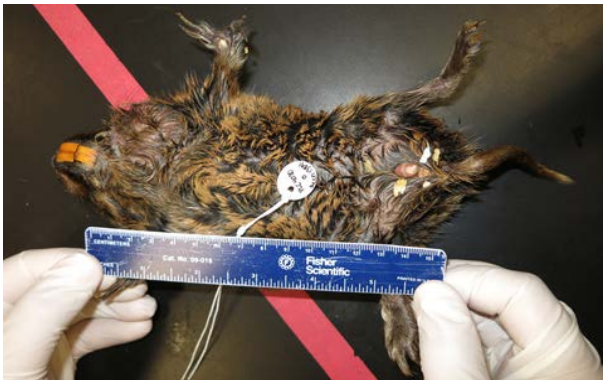
- Take photos
    - **DO NOT** wear dirty gloves while handling the camera
    - You need the trifecta in every photo: *tag, specimen, and ruler!*
        - Make sure the correct side of the ruler is showing (the side with mm on it)
        - *TIP – Take a close-up picture of the tag before taking pictures of the specimen in case the tag is unclear in the photos. However,* try to get all three items in focus.



- Checklist of pictures to take:
    - Close up of tag
        - This indicates that all following photos belong to this particular specimen
    - Dorsal (back)
        - Make sure that you do not “cut off” any part of the animal in the framing of the photo
    - Ventral (stomach)
        - Make sure that you do not “cut off” any part of the animal in the framing of the photo
    - Lateral (side)
        - Make sure that you do not “cut off” any part of the animal in the framing of the photo
    - Face (front and side view)
    - Ear
    - Tail
    - Hind foot
    - Ectoparasites (if present)
    - Close up of identifying marking (tail, face, feet, etc.), injuries or abnormalities


Fisher Scientific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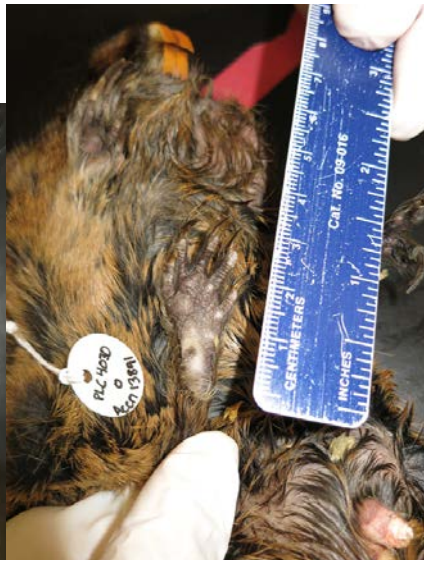
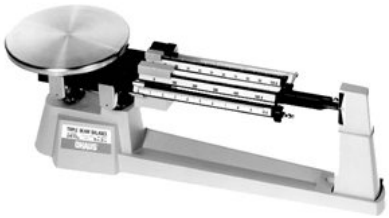
Fisher Scientific


INCHES
CENTIMETERS
PLC 4030
O

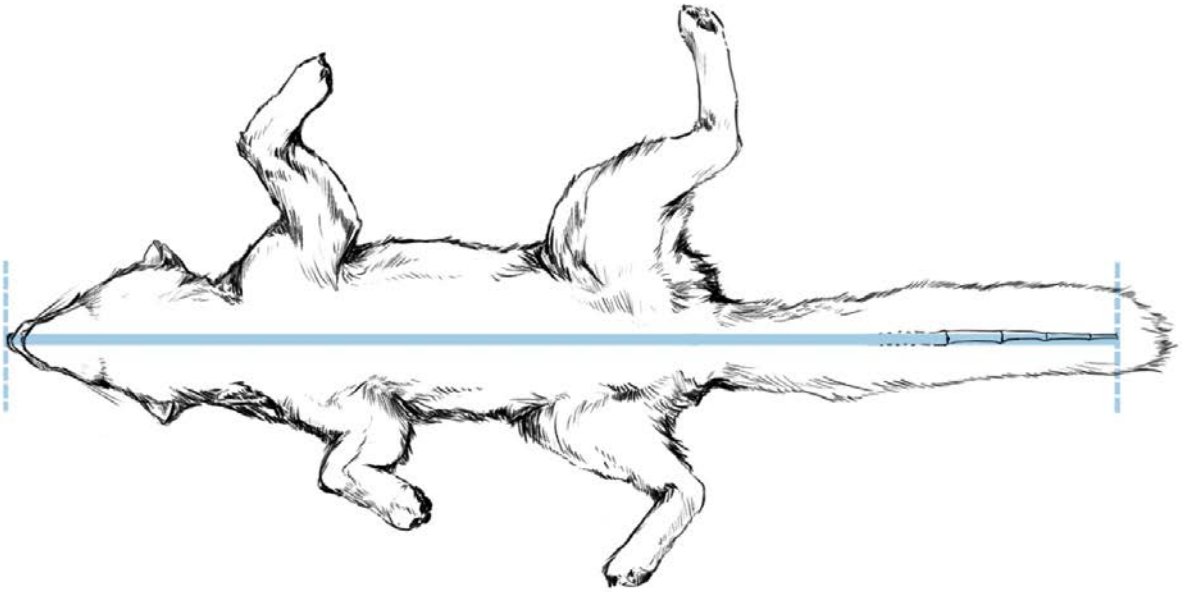

PLC 4030
O
CENTIMETERS
INCHES
Cat. No. 09-016

## External Measurements

- Weigh your specimen
    - Always weigh your specimen.
        - If original data has weight written, you can note it in observations.
    - Don't forget to zero the scale if you are using a tray or paper towel
    - Make sure you are using grams not milligrams or another measurement

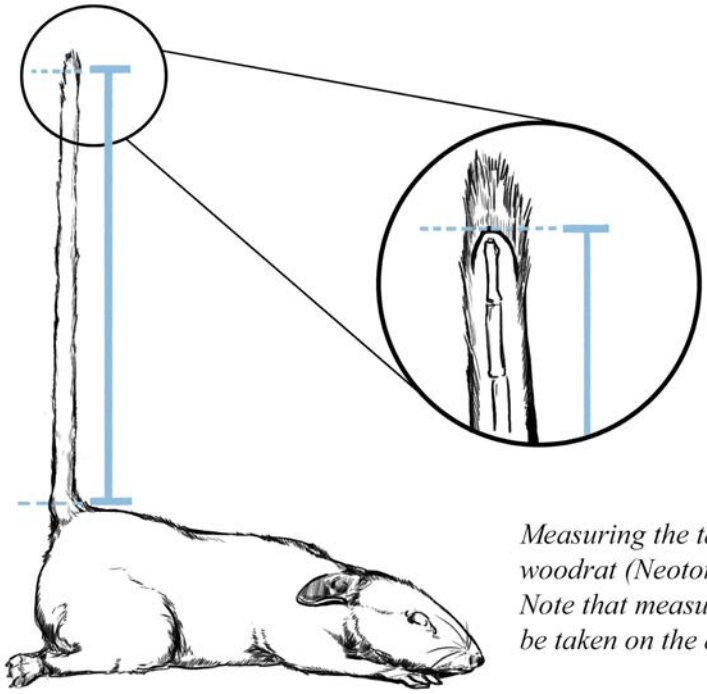


- Measure the specimen's whole length – from the nose **to the last vertebrae in the tail**
    - Stretch your specimen out! It is likely contorted from its positioning in the freezer and you want to make sure that there are no kinks in the tail and the spine is not curved.
    - Place your specimen directly on top of the ruler or tape measure
    - **DO NOT** include fur that goes past your last tail vertebrae in this measurement
    - Double check where the zero mark is on your ruler or tape measure and make sure your specimen is properly aligned with it (at the nose)



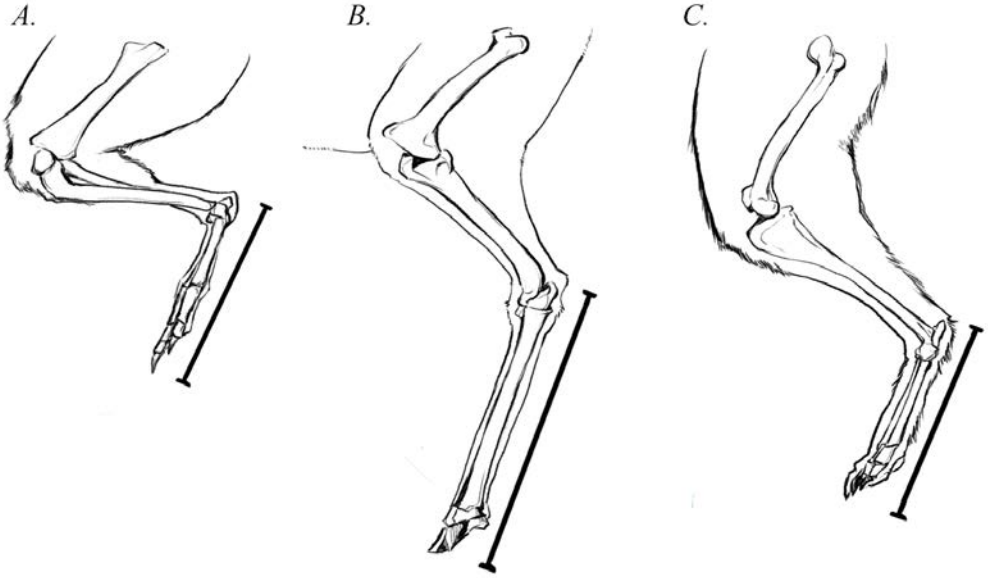
*Measuring the full length of a Urocyon cinereoargenteus (Grey fox). The specimen is laid directly on top the tape measurer, with spine straightened and ventral side facing upwards. Note that measurement begins at the tip of the snout and ends at the last vertebrae in the tail.*

- Measure the tail length – the base of the tail on the *dorsal side* to the tip of the last tail bone
    - Lay the specimen on its stomach and hold the tail upright at a 90-degree angle from the body (perpendicular).
    - **DO NOT** measure from the anus (ventral side) *or* to the end of the fur (See figure below)



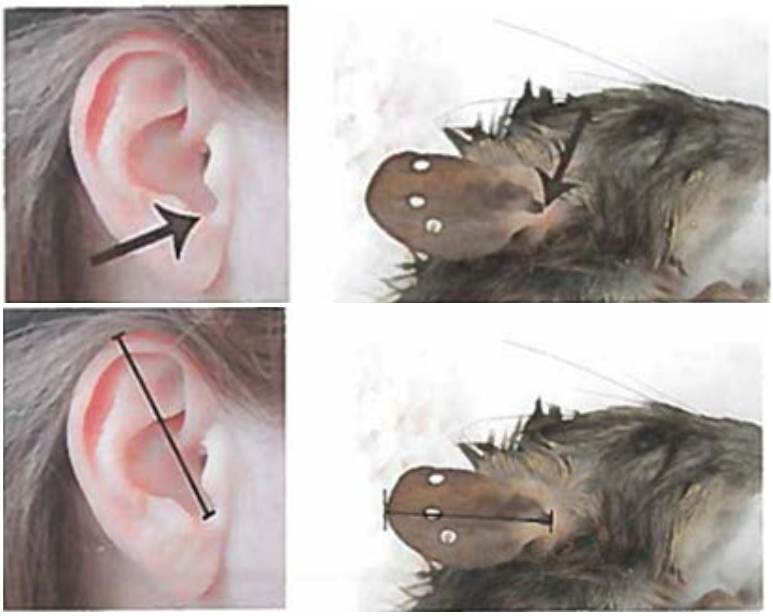
*Measuring the tail length of a woodrat (Neotoma cinerea): Note that measurement should be taken on the dorsal side of the tail*

- Measure the length of the hind foot – from the tip of the ankle joint to the tip of the longest nail
    - Uncurl the foot and toes to straighten them out and hold them flat against the ruler
    - Including the claw/nail is standard because most mammals have bones in their claws
    - The foot does not always mean only the part the animal walks on. Some mammals only walk on their toes so if they seem to have long or extra leg bones, they may just have long feet! Check with Terri or the class coordinator if you are unsure of where the ankle joint is.

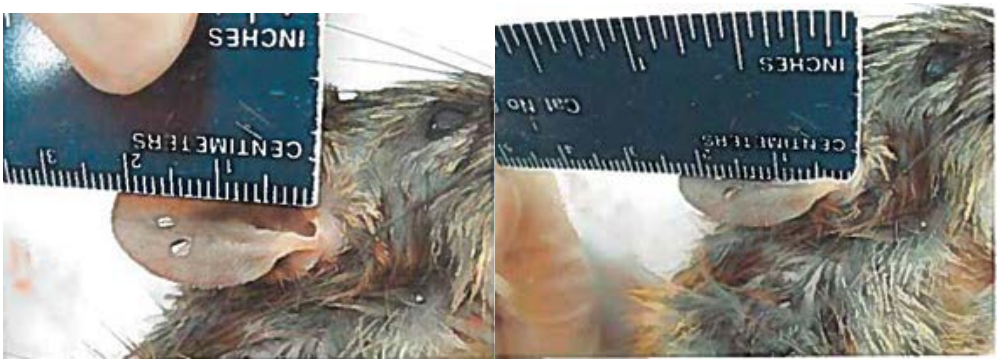



*Examples of hind foot measurements for a generic A) peromyscus, B) cervidae, and C) canidae.*

- Measure the ear length – from the base of the ear to the edge of the (pinna)
    - The pinna is the cartilaginous projecting portion of the external ear.



    - The best way to measure this is to place the base of the ruler in the notch of the ear as seen below.




**NOTE:** If the animal is missing a body part due to injury, then measure what you can and make the loss/change in your measurements very clear in your notes.

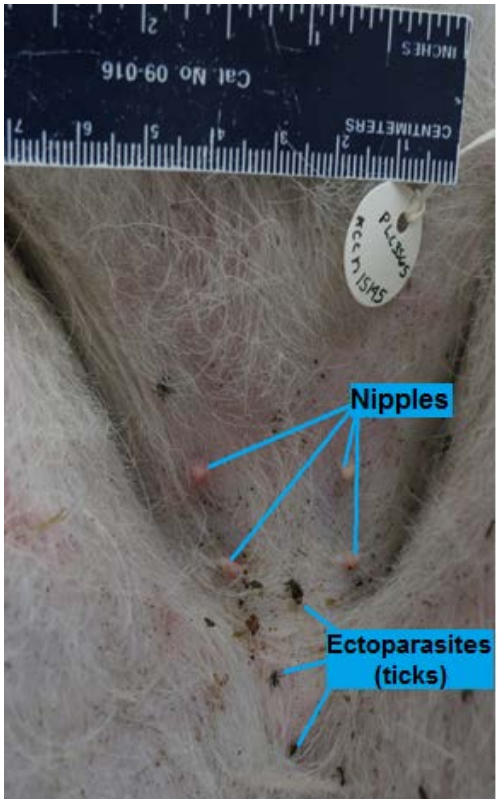
- For example if your mouse was missing its tail due to a cat attack, measure what you can and put a note in your observations. The tail length in that case would be Ø and the total weight would be affected by that as well. So although you would still record the weight, you would write in your observations that it may not be accurate due to the missing tail
- If the animal doesn't have a certain body part normally (for example some moles don't have pinnas, just ear holes) then put a zero for that measurement.

## External Observations

- Check for ectoparasites (ticks, fleas, etc.).
    - Take photos and collect any parasites in a wet sample vial with 95% Ethanol and a fluid tag.
- Count the number of nipples. Mammaries may be slight, moderate, extended, or not showing
    - *TIPS – Use the compressed air hose to search through fur if it is especially dense.*
      *– Look for nipples on the ventral side of the body, from the thorax to the abdomen.*
    - *FUN FACTS – Virginia opossums have 13 nipples, 12 in a circle like a clock and one in the middle. Pinnipeds and cetaceans have retractable nipples, an adaptation that allows these sea mammals to reduce drag while swimming.*

*Using the compressed air hose to locatenipples*

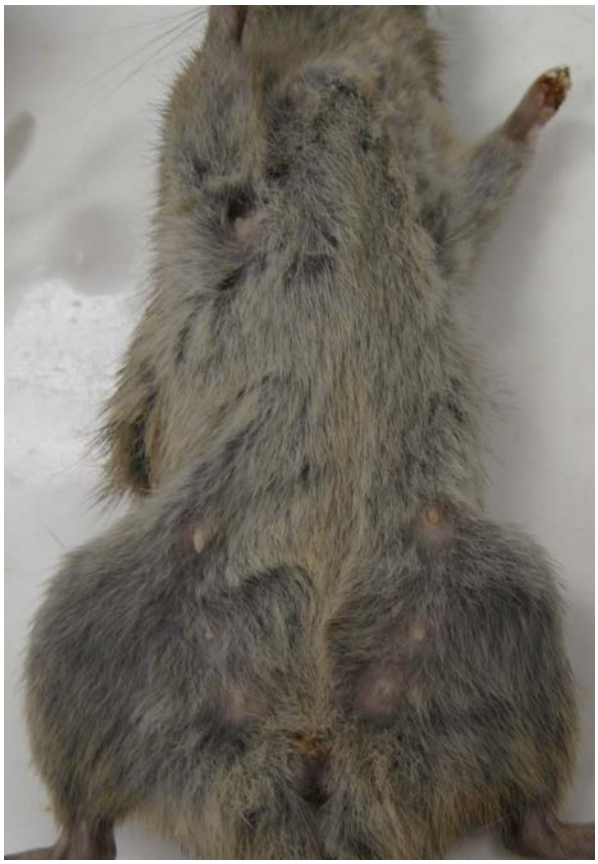
*Nipples and ectoparasites*




*NOTES: Some males have nipples too, although they often are not as pronounced. Also, just because nipples are clearly visible does not mean the mammary glands are extended. You should be able to see glands extended as they will be raised from the rest of the ventral surface.*

***You may be able to externally sex the mammals but **DO NOT** record the sex on anything (worksheet, tags, tubes, etc.) until you have seen and confirmed the gonads with the class coordinator***

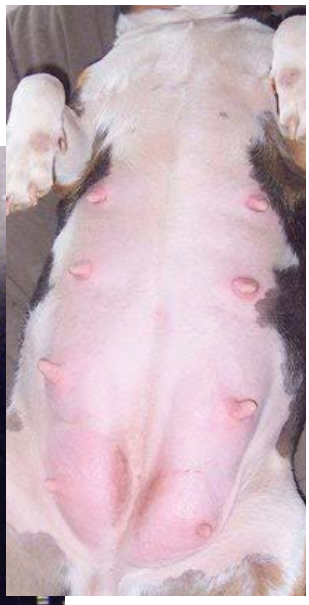
slightly extended mammaries



slightly extended mammaries (note the vaginal plug)

moderately extended mammaries


Very extended mammaries




- Check for a vagina
    - If found, describe its condition as open, plugged, or sealed by checking with a probe
        - Open is when the vaginal opening is open and can easily be probed to check.
        - Plugged or sealed can be tricky to determine due to the condition of the animals we receive and the freezing/defrosting process. Plugged means that after mating, the male left a plug that physically blocked the entrance to the female's vagina. Therefore, the probe cannot slide into the vagina easily. Sealed/ closed vaginas tends to be a condition of immature females, but also some females have closed vaginas between breeding seasons.
            - *TIP – Plugs can be crusty, waxy, goopy, or other types of consistencies, so don't remove anything that looks a bit odd or like your mammal's genital region is dirty. It might be dirt, poop, or a plug, but play it safe until you are ready to determine which it is. It should be clearer upon close inspection.*
            - NOTE – Not all female mammals have their vaginas sealed or closed between breeding seasons and not all species have males which leave

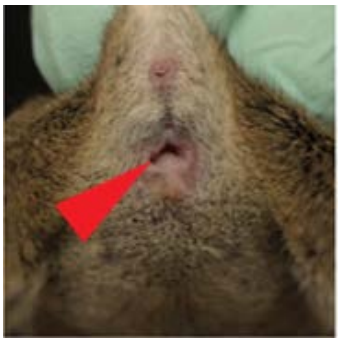
copulatory plugs behind, but they are common in rodents and some primates.
– Plug composition varies but typically they consist of densely packed sperm, mucus, seminal fluid, and enzymes that promote coagulation

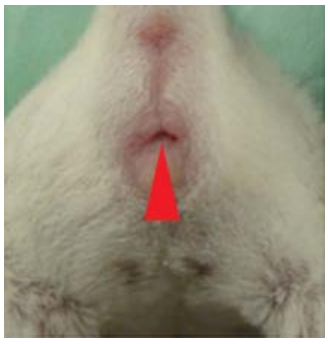
Vaginal plug of a squirrel

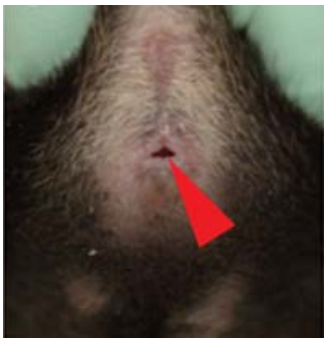
## Examples of different vaginal conditions

**The vagina is the larger hole in the pictures below, the anus is the hole closer to the tail. DO NOT confuse the anus for a vagina when sexing**

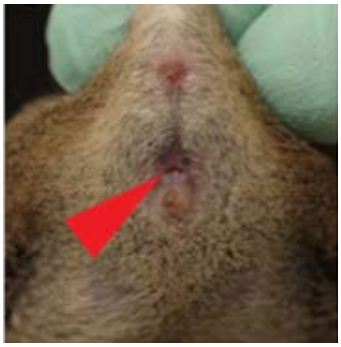
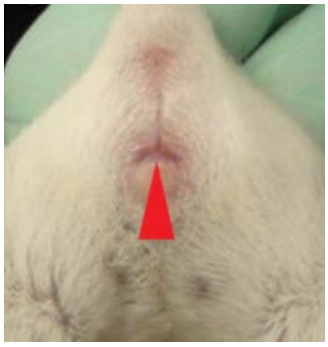
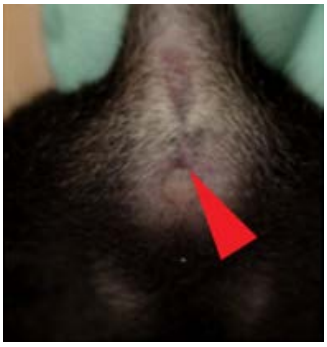
Open vaginas



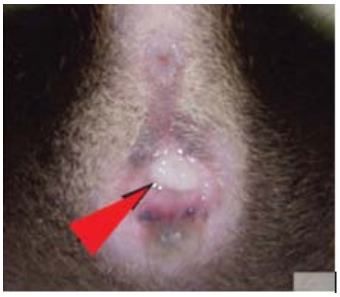
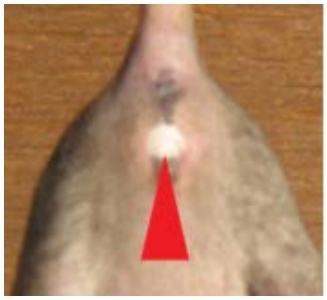
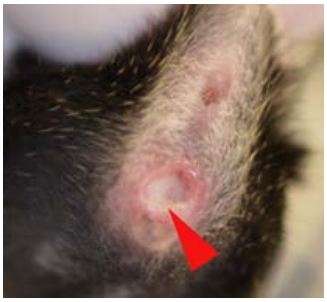




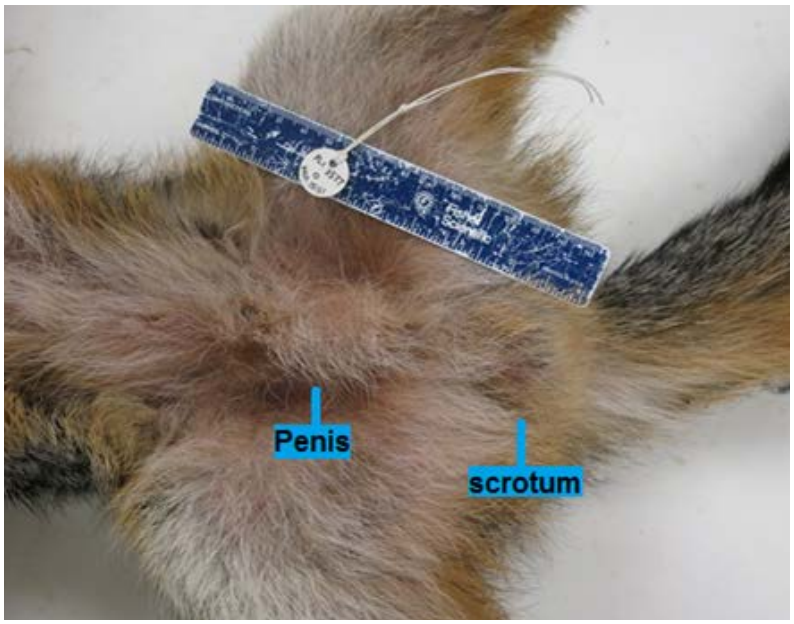
Closed vaginas

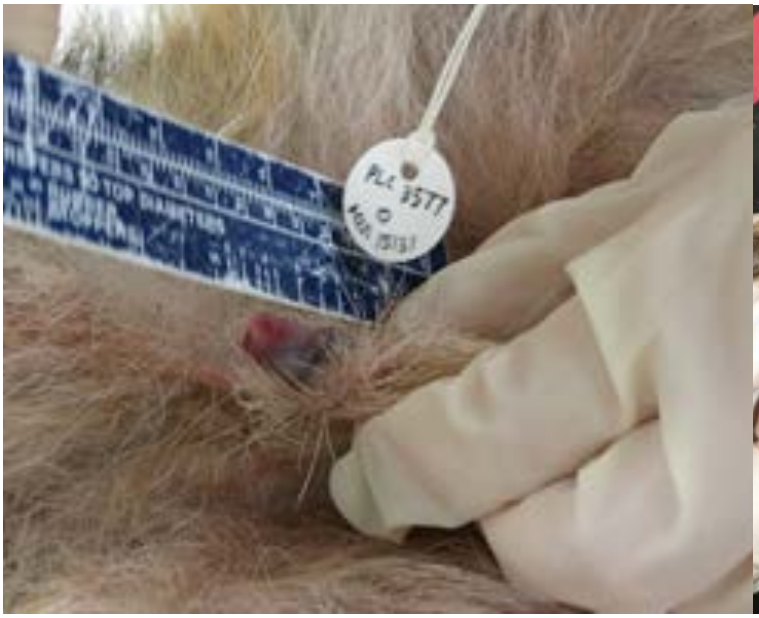
Plugged vaginas

- Check for scrotal testes
    - Scrotal testes will hang from the male's body near the lower abdomen
    - If the testes are not scrotal, it means they are abdominal and you will find them upon opening your specimen




- Check for a penis
    - Feel the abdominal region for a baculum (penis bone). You may be able to extrude the penis as seen below.
    - *NOTE – The baculum is found in the penis of many placental mammals. However, it is absent in humans, ungulates, monotremes, marsupials, hyenas, cetaceans, and more.*

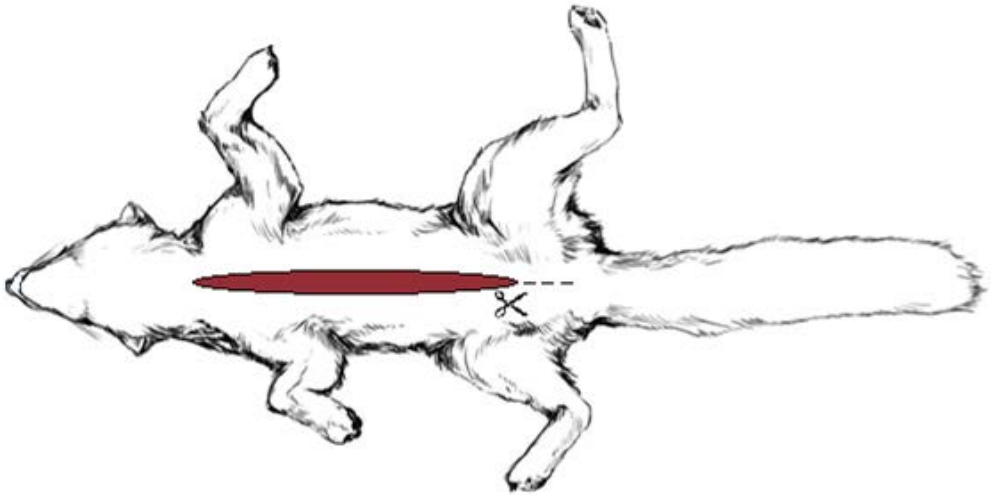



Fisher
Scientific

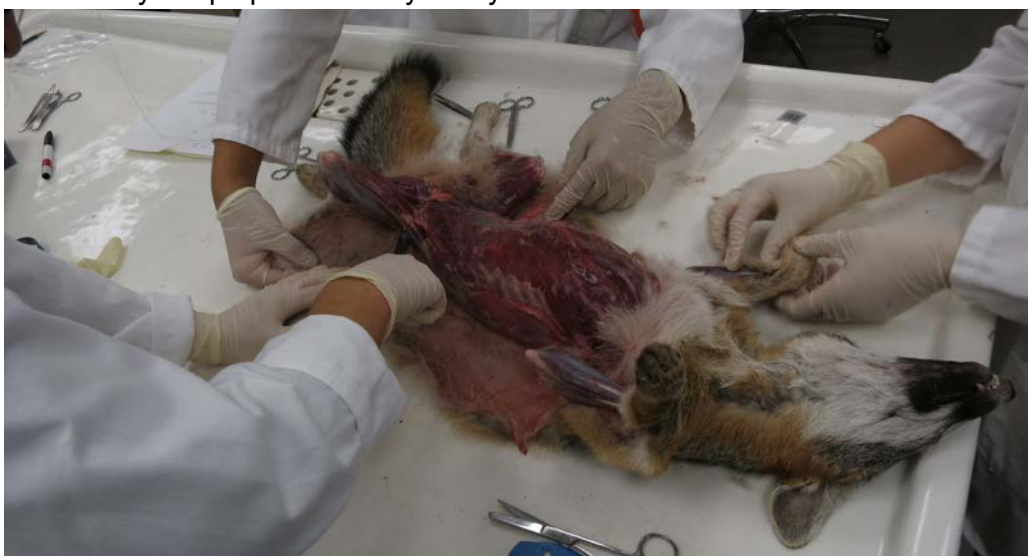
# You are ready to start skinning!

*****Check in with Terri or the class coordinator before you cut*****

- Place the animal on its back and start near the top of the rib cage (after the first few ribs) using your scissors to make an incision all the way down the animal's ventral side.
    - TIPS – *Lightly pinch and lift up the skin to avoid cutting too deep.*



- Peel and cut the skin away from the body using scissors, your hands, probes, etc.
    - Be extra careful near the abdominal cavity as the membrane covering it is much thinner than the muscle you are pulling the skin from in other areas.
    - Also be careful skinning around the genital region. If you're unsure of your specimen's sex, or it is a male with scrotal testes, you may want to leave the skin around the genital region intact until you are ready to sex it. You don't want to damage or lose anything.
    - **DO NOT** cut into the body cavity before you're ready to sex the specimen and take tissues
    - TIP – Pull the muscle away from the skin rather than the skin from the muscle so you are less likely to rip open the body cavity.



NOTE: The longer a specimen is sitting out as you prep it, the more its DNA degrades; so, you can clear the skin away from the head, tail, and feet after taking tissue samples and measuring reproductive traits.

# You are ready to cut and open!

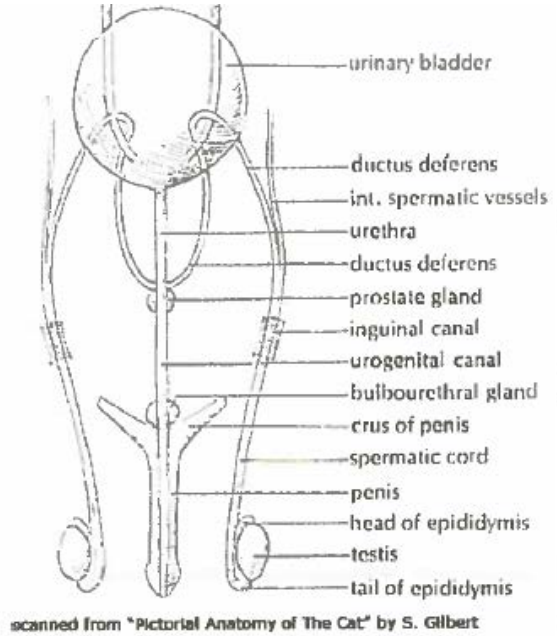
*****Check in with Terri or the class coordinator and have them cut open and sex your specimen*****

## Sex measurements

- Identify the gonads
    - Use your external sex identification to help guide you to your gonads if possible
        - If externally sexing was inconclusive or not immediately clear, be extra careful in these steps and always check for testes outside the body cavity near the lower abdomen before you open up your specimen's body cavity.
        - *TIP – When cutting in the body cavity, pull the membrane away from the body to avoid cutting into organs. For easier viewing, you may carefully cut alongside the the rib closest to the abdominal cavity but avoid breaking ribs!*
    - Take pictures of the gonads (include tag and ruler in all shots)
    - Label tag and tissue vial with the sex confirmed by your instructor

Measuring Males (testes)

- Note whether the tests are abdominal or scrotal
    - Not all males have scrotal testes, which can make it difficult to sex them externally and is a reason why we always wait for gonads to confirm sex. Sometimes immature males have undescended testes which means they are internal and abdominal as opposed to scrotal.
    - If the male has scrotal testes, you can measure them before cutting into the body cavity.
- Pick one testis, ***completely expose it***, and measure width and length.
    - **DO NOT** take testis measurements until they have been exposed completely. This means *removing the scrotal skin and any membranes that encase the testis and epididymis.* Do this is for all testes, scrotal and abdominal alike. DO NOT include the epididymis in your measurement. You can also remove the testis (after taking pictures) from the epididymis and measure it to be safe. We only measure one testis since they are almost always symmetrical. If they are clearly not, measure both and note that in your observations.
- NOTE: If the male has a baculum keep it with the rest of the skeleton. DO NOT lose it!



scanned from "Pictorial Anatomy of The Cat" by S. Gilbert

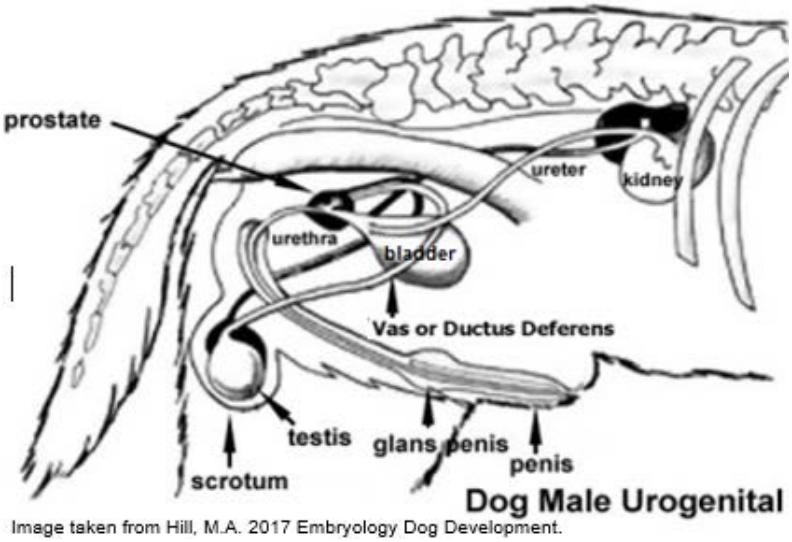
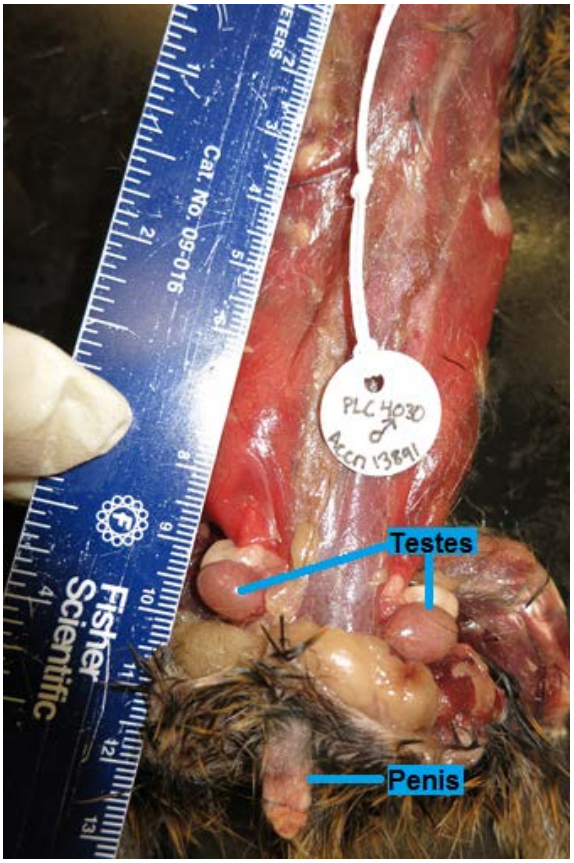


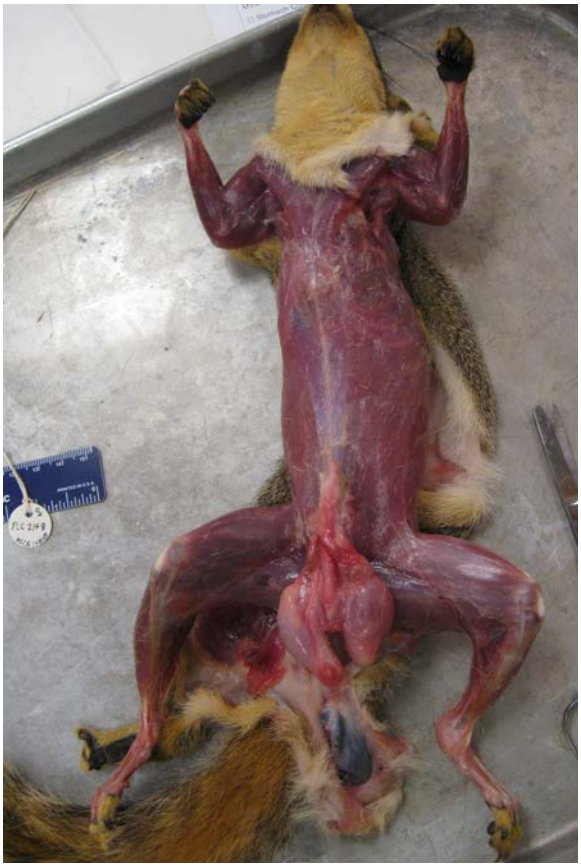
Image taken from Hill, M.A. 2017 Embryology Dog Development.

Examples of Testes

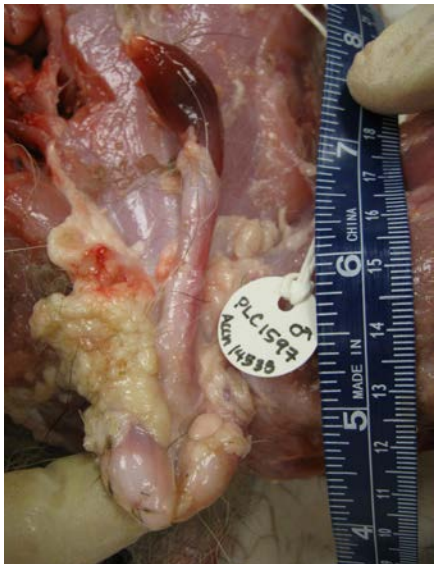
Tuco (rodent) testes and protruding penis

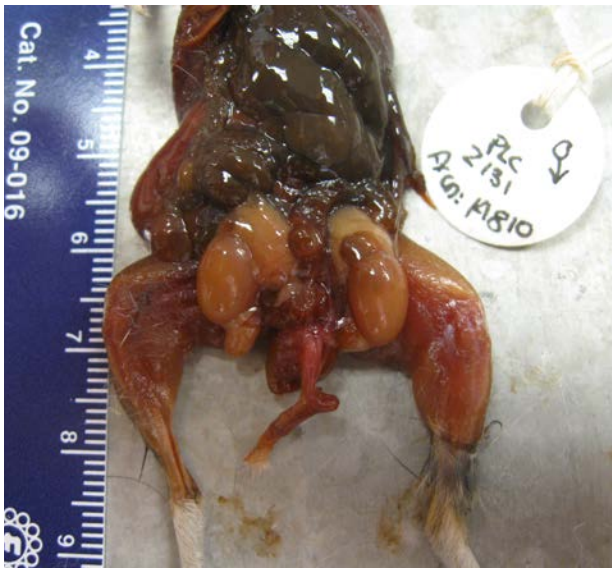



Squirrel testes and penis



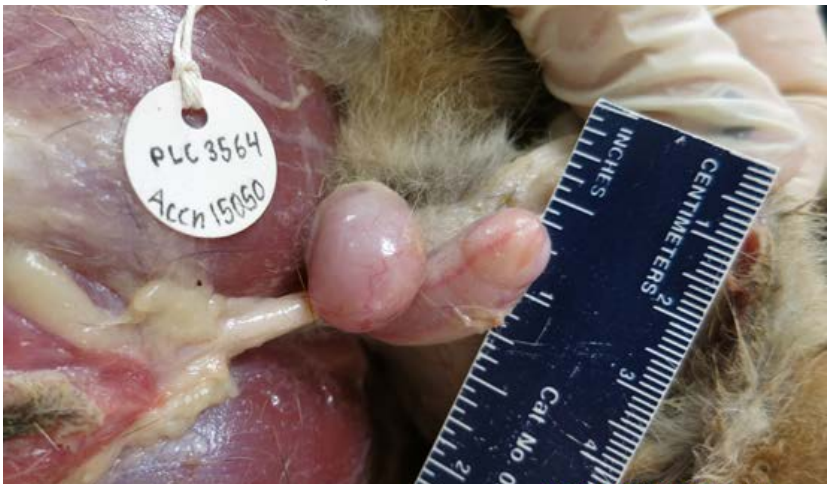
Testes and baculum in different orientations



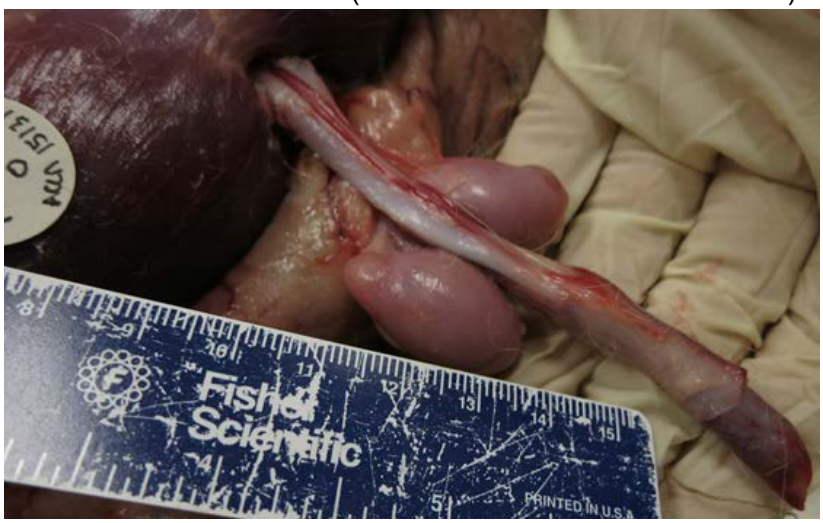



NOTE – Testes are often covered or surrounded in a layer of adipose (fat) tissue as seen above, which can make it difficult to see the testes. It must be removed before measuring the gonads. However, be careful when removing it so as to not displace or disfigure the testes

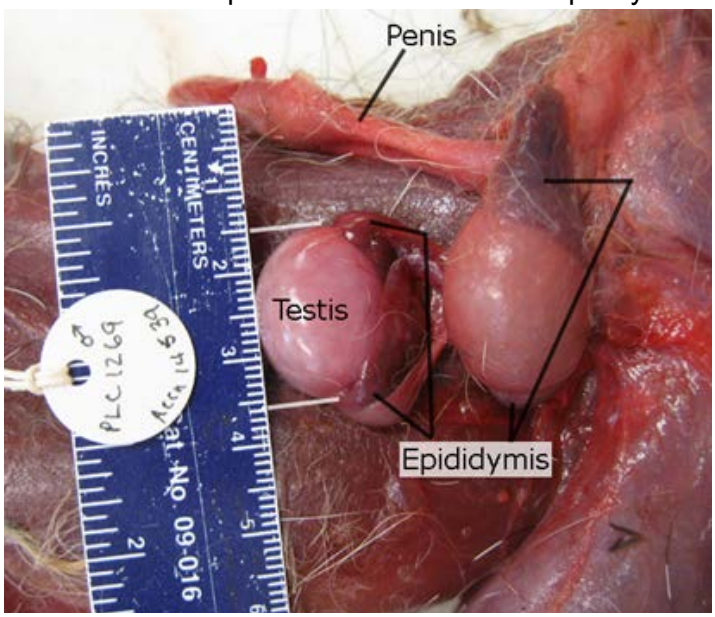
Completely exposed bobcat testes




Fox testes with baculum (testes still encased in membrane)




Measure the exposed testes without the epididymis



Measuring Females (uterus)

- Mammal uteri vary among species, but the two main types you will see are a single uterus (as in humans) or paired uterine horns (as in rodents and dogs)

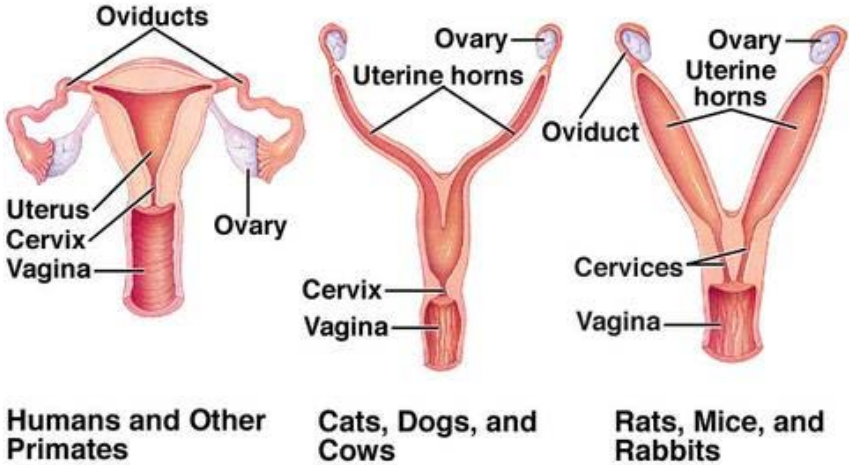
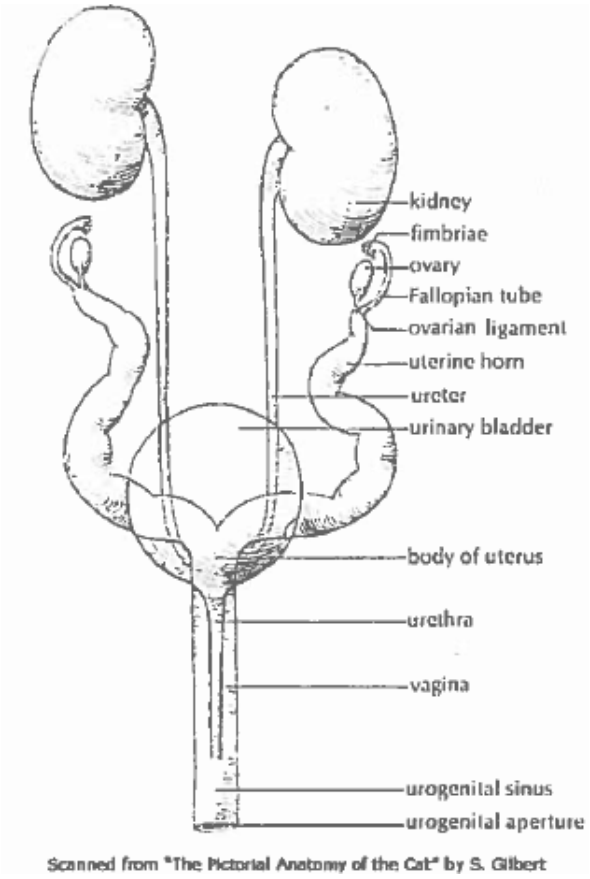
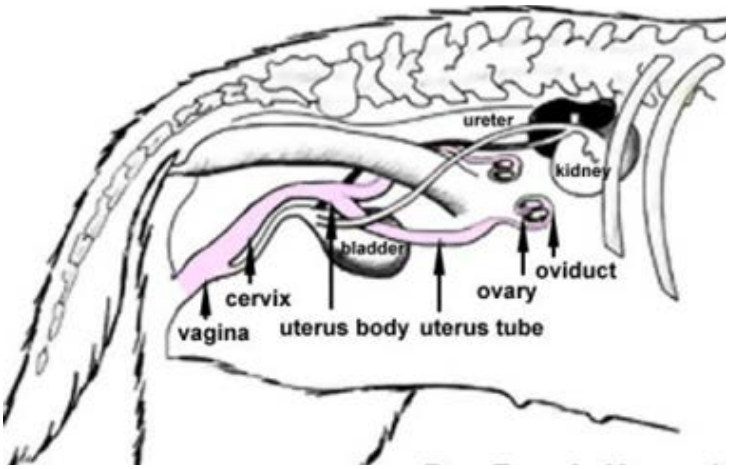



Image from the The McGraw-Hill Companies, Inc.

- The easiest way to find the uterus is to locate the bladder and where the intestines run down to the anus (the colon). The reproductive tubes should be lying between the bladder and the colon.

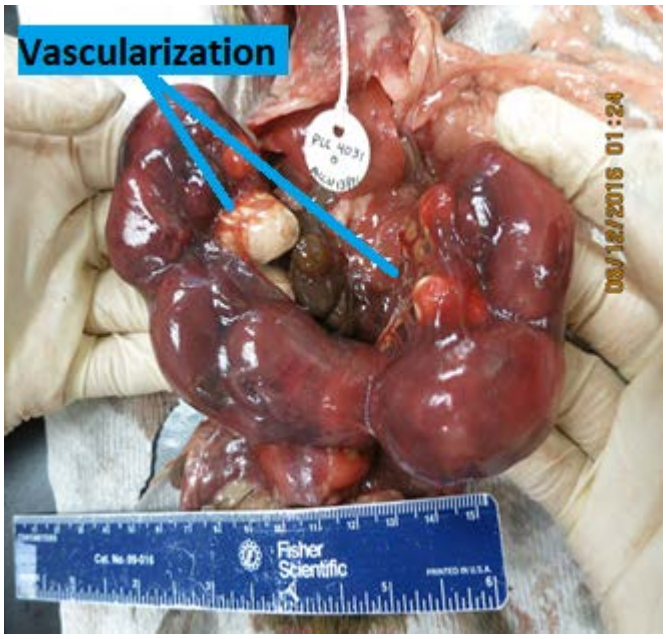


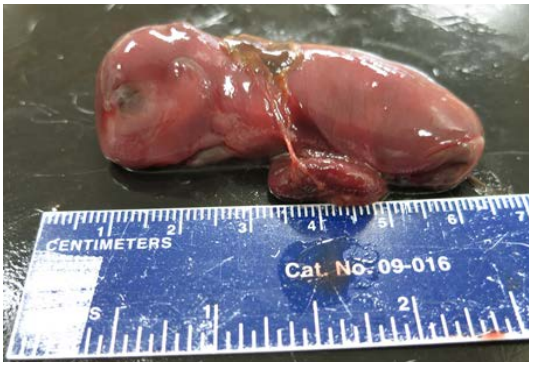



- There are two broad conditions of a female and each have different measurements
    1) The female is pregnant,
        - Count the number of embryos
        - Measure the width and length of the embryos
    2) The female is not pregnant,
        - Count the number of placental scars
        - If there are no scars, measure the width of the uterus (or uterine horns) and note whether it is floppy and vascularized or narrow and unvascularized.
- If the mammal is pregnant, count the total number of embryos in the uterus or each of the uterine horns. When measuring embryo size, you only need to measure one if there are multiple because they are typically all the same size. However, if there is a difference, choose the largest.
    - Note the vascularization in the top right picture below.

Tuco with embryos in its uterine horns


Close up of embryos

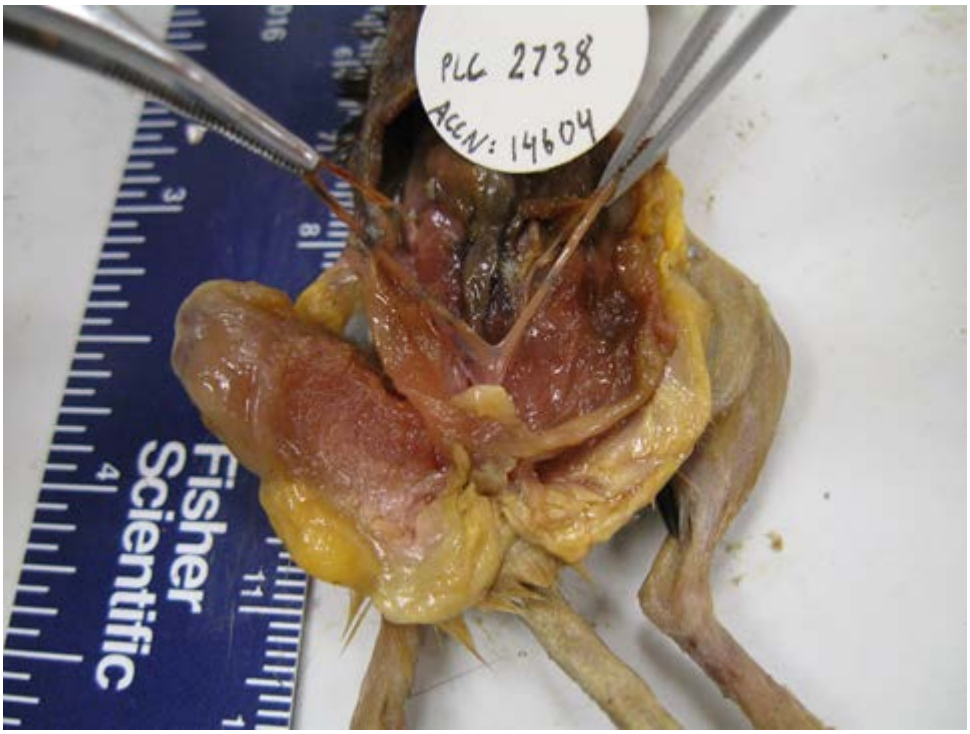


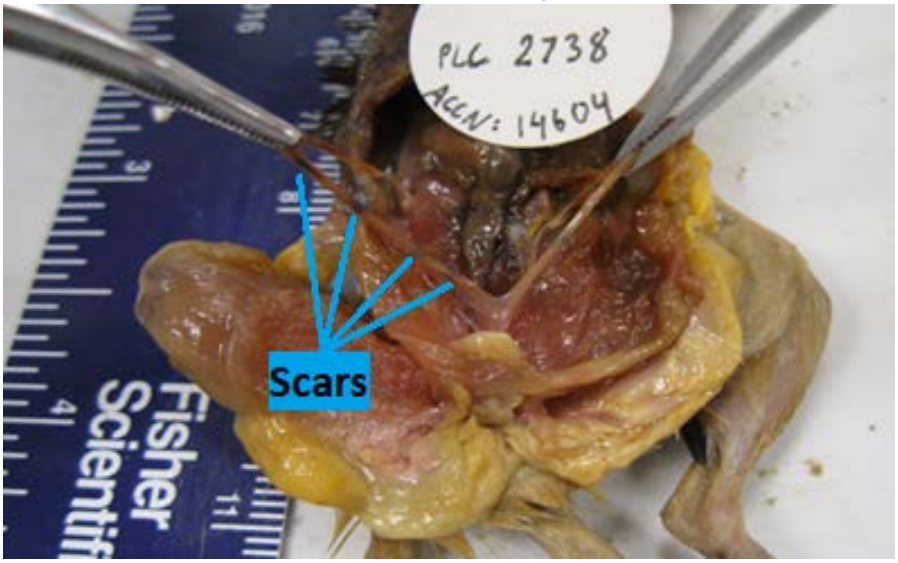

Exposed embryo ready to be measured

- Scars on the uterus are from previous pregnancies where the placenta from each embryo attached to the uterine wall. The scars appear as dark marks along the uterus.
- If there is scarring, note the number of scars on each uterus.
    - T*IP – It may be easier to remove the vagina, uteri, and ovary and lay them on a white surface, but* **take width measurements beforehand and keep track of which side is left and right**
    - NOTE – The darker the scars are, typically the more recent they are. Sometimes they can be very slight and more difficult to see so make sure to really check for any scarring.

Examples of scarred rodent uterine horns

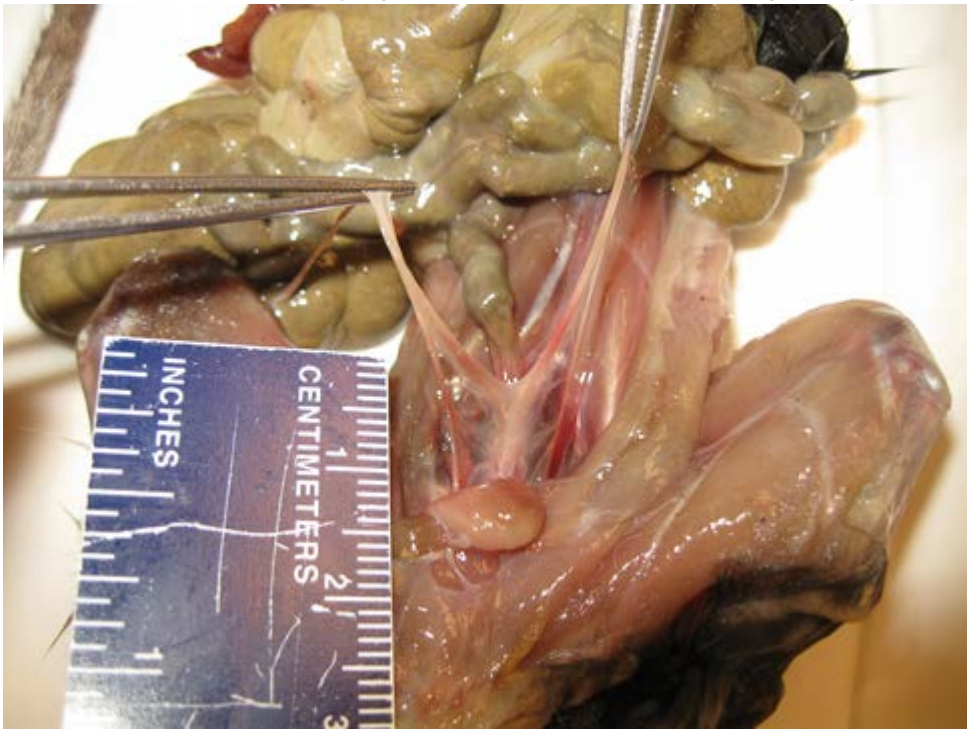



Labeled image

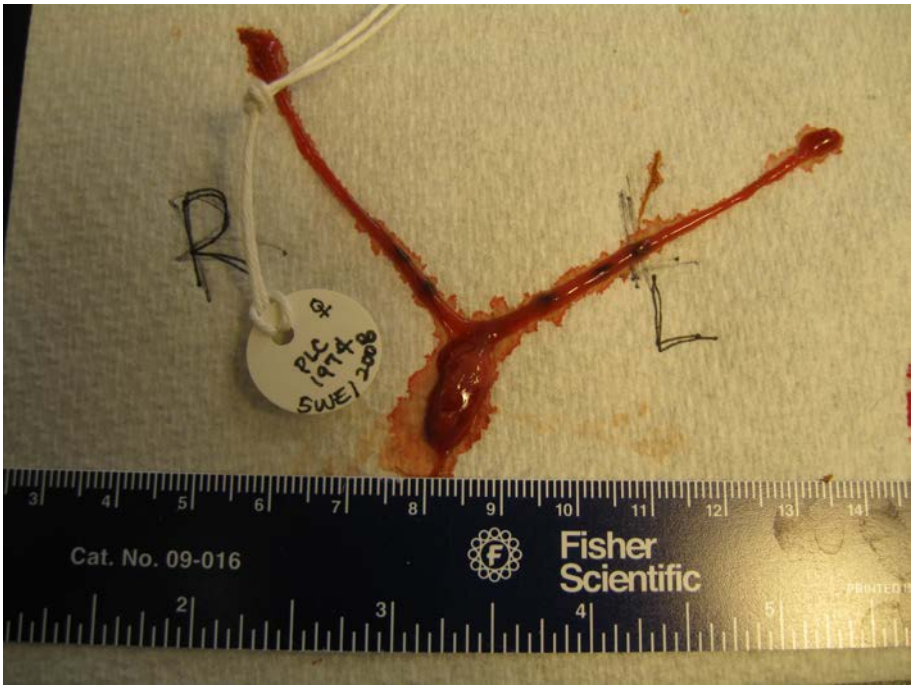


- Unscarred, narrow and unvascularized uteri are often a sign of an immature female.
    - Narrow versus floppy can be a bit difficult to differentiate between unless it is extremely one or the other. Vascularization however should not be difficult to determine. You should be able to see the blood vessels connected to the uterus if it is vascularized.

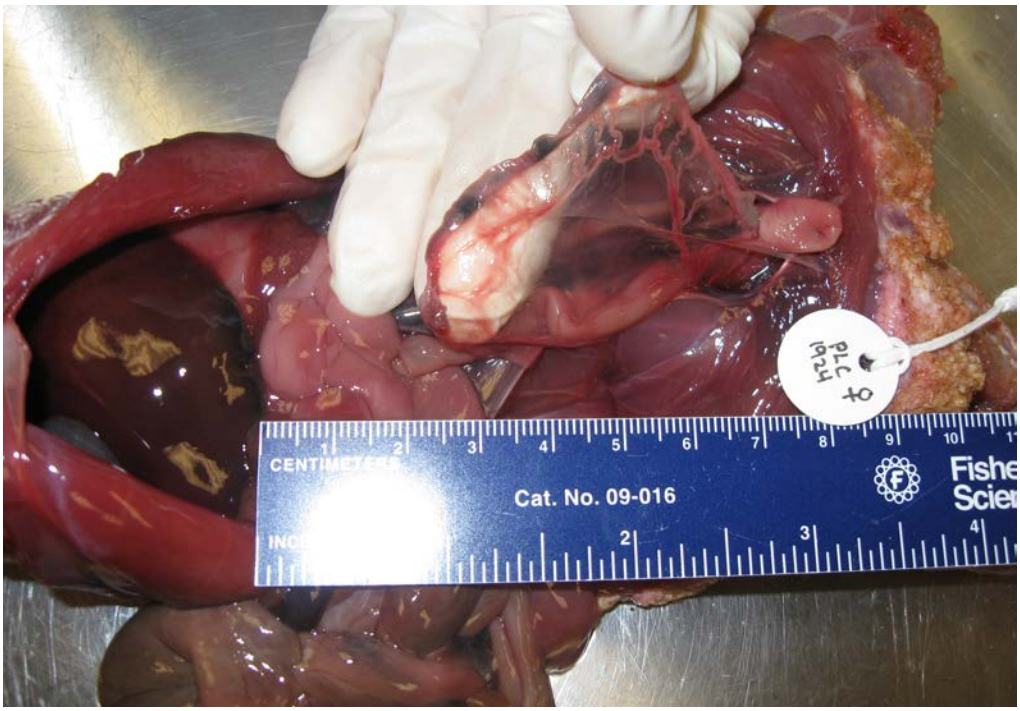
Example of narrow, unvascularized, unscarred rodent uterine horns
(You can see the bladder laying in front and the colon running along the back)

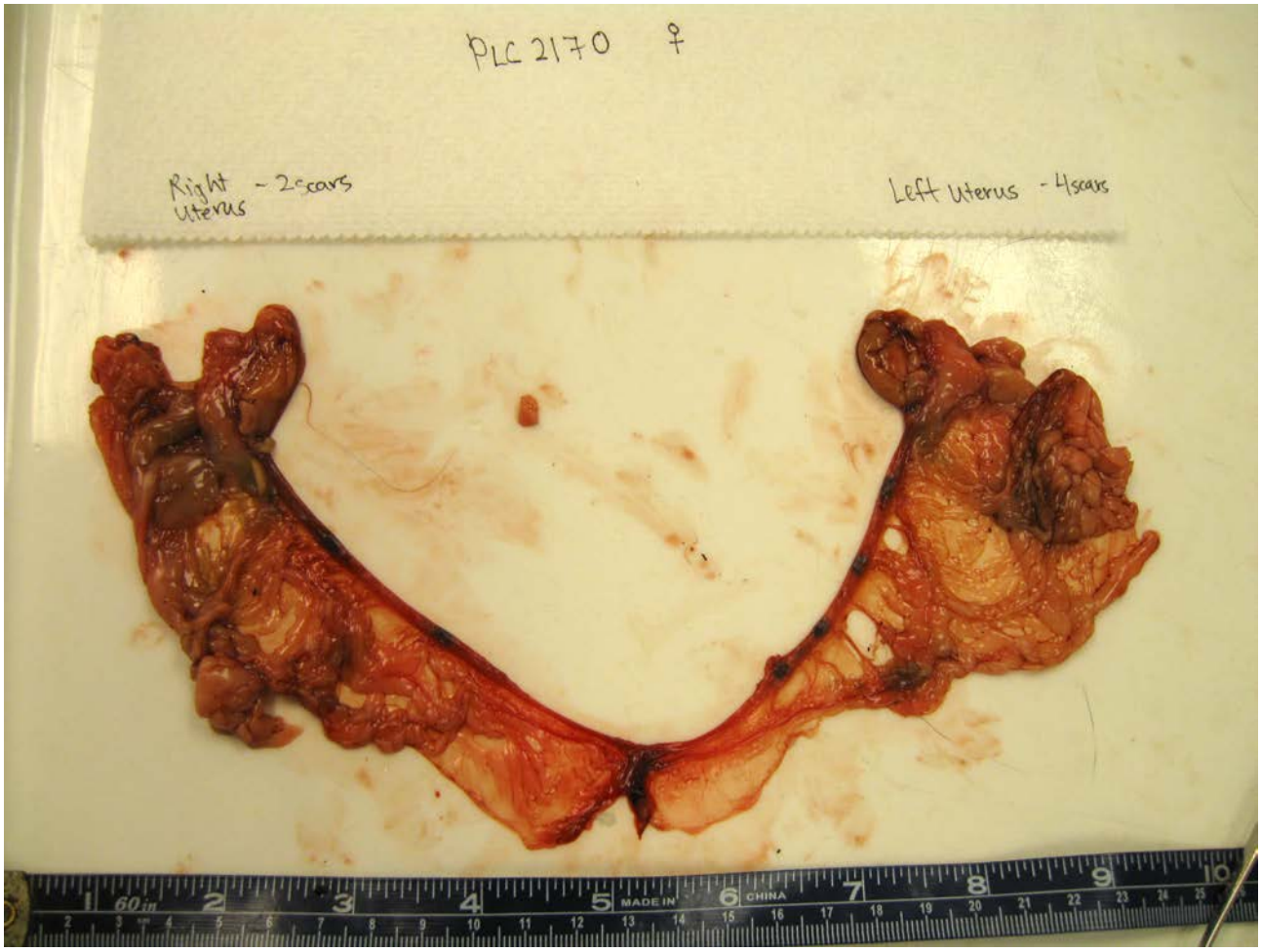



Example of narrow, unvasularized, scarred uterine horns
(note how it is labeled left and right on the paper towel)

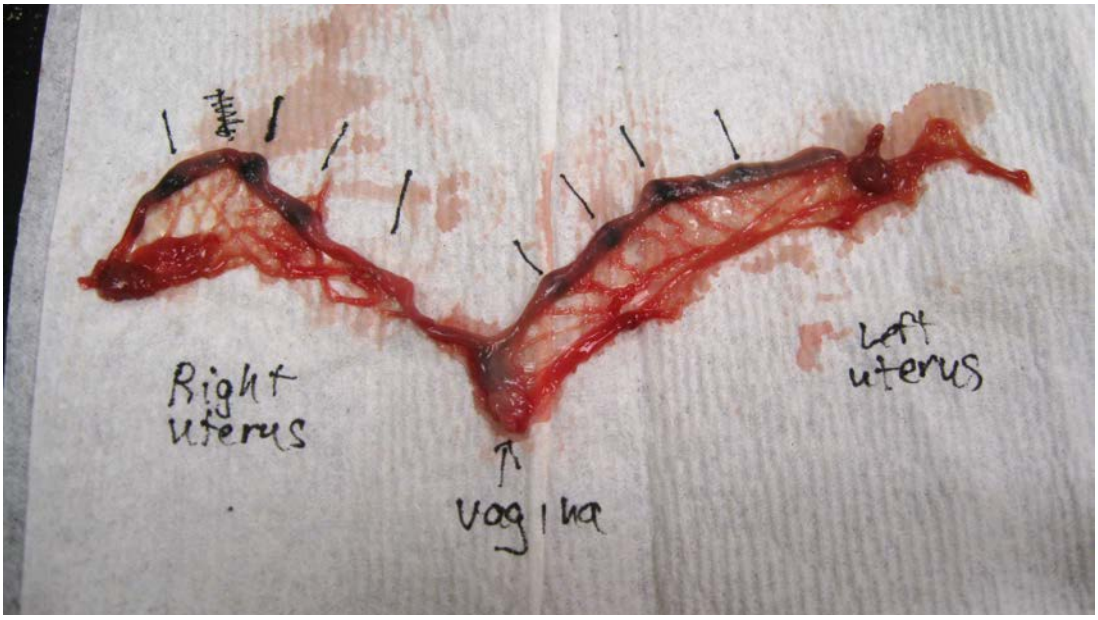


Examples of floppy, scarred, and vascularized uterine horns

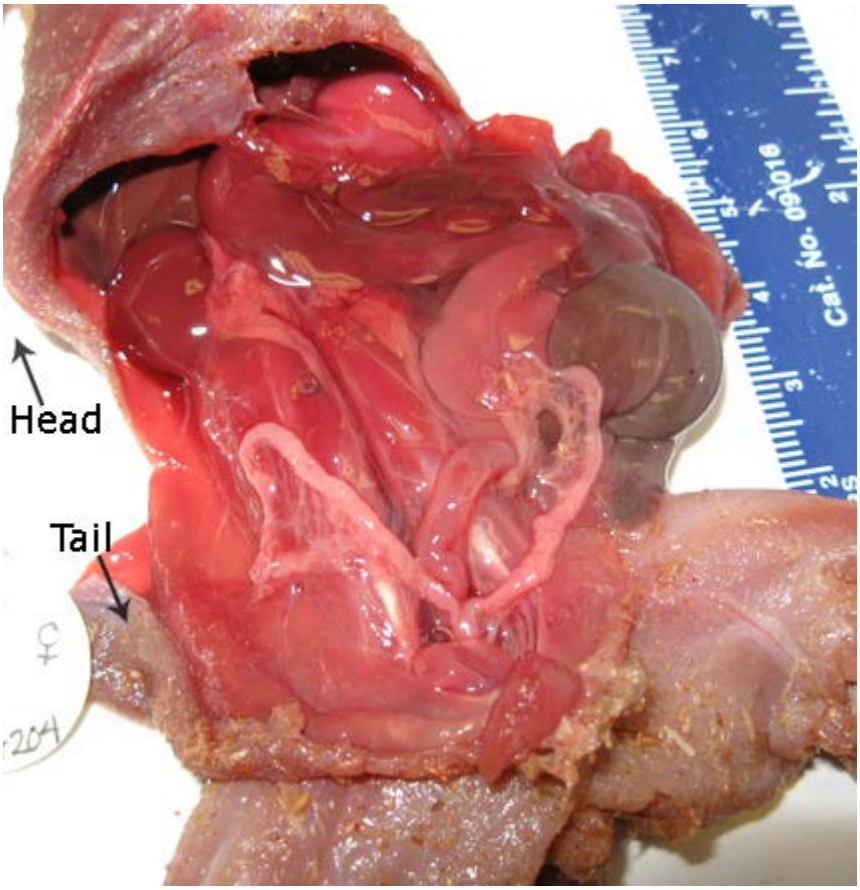


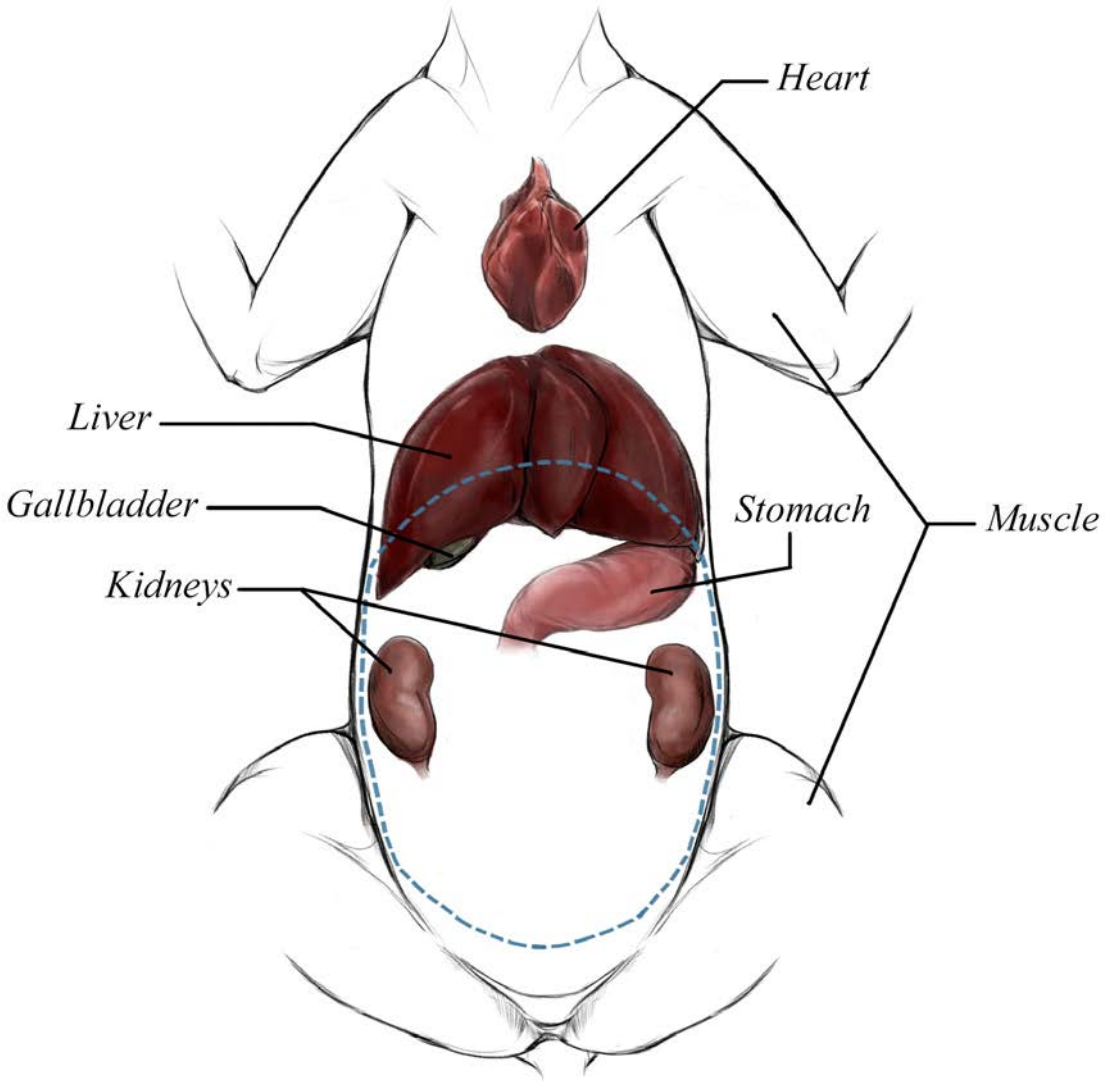

Remember the right and left sides are the animal's right and left, not yours




Check your understanding: In the photo below, find the bladder, large intestine, and the uterine horns. For the uterine horns, describe whether it's narrow or floppy, vascularized or unvascularized, and scarred or unscarred. ,



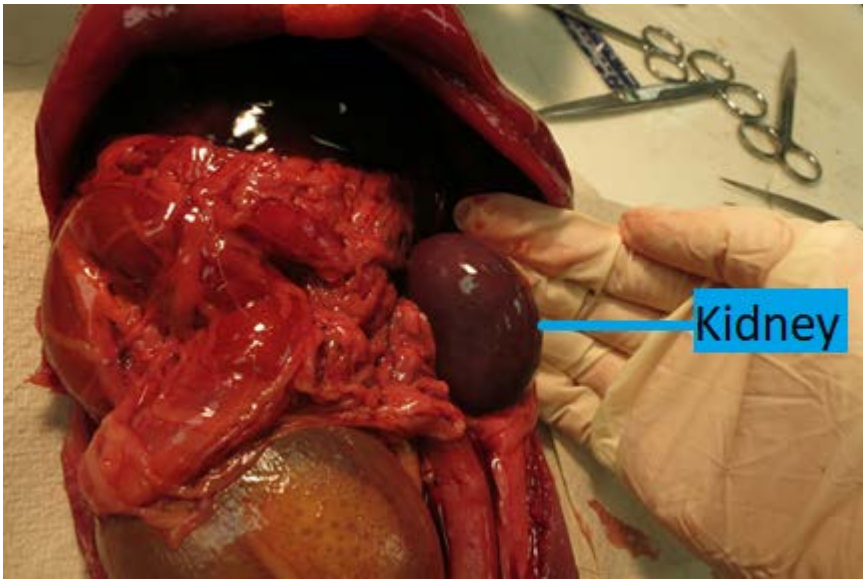
**Taking Samples**




*Simplified illustration of a grey fox showing the four tissues to be sampled, the stomach, and the gallbladder. Certain species such as rats and deer lack gallbladders. Dotted lines indicate possible cuts to expose the abdominal cavity of a larger mammal.*

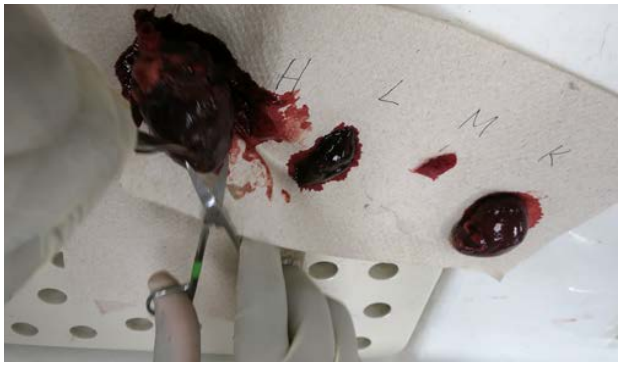
- Place all samples on your paper towel labeled HLMK before placing them in the vial
    - Be careful not to contaminate your samples with bile, feces, sawdust, or external contaminants as these could ruin the samples.
    - Fat may be present throughout the abdominal cavity but remove it from any tissues you collect and do not confuse it for internal organs. It's often interlaced with the intestines or encasing internal organs.

- Kidneys are located on the dorsal side of the body. They are situated beneath the intestines and often are bean-shaped in mammals as shown on the right side of the picture below.
    - NOTE: Kidneys often have adrenal glands directly on top or attached to the kidneys. Make sure that you are only sampling the kidney!

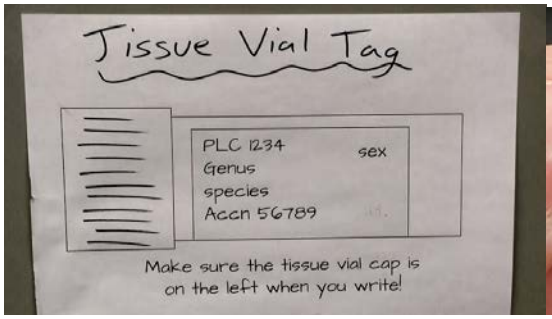


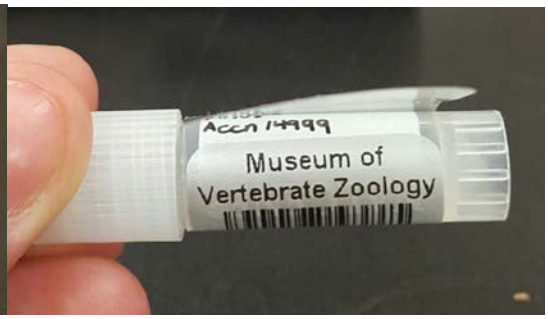

- The liver is lobed and is located on the upper edge of the abdominal cavity next to the diaphragm. Take sample of liver some distance away from the gallbladder.
    - The liver is often a slightly different, darker color (usually more burgundy) than the rest of the internal organs and muscles, but do not rely on this to be able to tell the difference.
    - Avoid the gallbladder (little green organ) at all costs as it contains bile that will quickly degrade your tissues if released. If this does happen, notify Terri, the class coordinator or a UGSI immediately and take tissue samples as quickly as possible. Taking your liver sample from the specimen's left side (your right side as you look into the body cavity) is a good way to avoid cutting into the gallbladder when sampling the liver
        - NOTE – Not all mammals have gallbladders (such as rats, horses, and giraffes).
- Pierce the diaphragm to collect a heart sample.
    - The heart often has a distinctly firm texture and can often be confirmed by the presence of coagulated blood and chambers if you cut it open.
    - *TIP – To ensure you are only collecting heart, it can be easier to pull the entire heart out and then sample it rather than trying to cut somewhat blindly up the rib cage.*
- Muscle samples can be taken from anywhere although it is typical to take it from the limbs
- Be sure to note whether your tissues are/may be degraded (if unsure, ask!)
    - NOTE: If the specimen already had an exposed/open abdominal cavity your tissues will be degraded and must be marked as such on your worksheet and in your catalog entry. However, still take all the necessary samples you can. Some areas may also appear less degraded and will serve as better samples.

- Cut the samples into pieces small enough to fit in the vial if necessary
    - NOTE: **DO NOT** fill the tissue vial to the very top. Tissues expand as they freeze and need space to do so or the vial will break.



- Place samples into the tissue vial in the following order → heart, liver, muscle, kidney.
- The tissue vial should be labeled with PLC#, genus, species, Accn#, and ***sex***. Place the barcode sticker on the vial trying not to cover any data.
    - If for some reason data will be covered, cover the Accn NOT the PLC




- Record the MVZ barcode number on your worksheet if you haven't already.
- Place the tissue vial in the chest freezer in the appropriate tissue box and write the PLC#, Accn#, and number of vials on the paper inside


- Check the stomach contents and record a description on the worksheet
    - If there is reason to save the stomach contents (such as another specimen), place them in a jar with a fluid tag and *70% ethanol* (check in before saving)
- If there are any additional parasites, record a discription on the worksheet
    - If there is reason to save the stomach contents (such as another specimen), place them in a jar with a fluid tag and *95% ethanol* (check in before saving)

# Finishing up: Clean the Specimen!

- Double check you have filled out all the information you need to/can on your worksheet
- Remove the eyeballs and any remaining skin, organs, and large chunks of tissue, including the ear cartilage, from your skeleton (the body cavity should be empty).
- Attach the skeleton tag by stringing it through the sternum and mandible
    - *TIP – If, but only if, the specimen is too big, you can use multiple tags to tag the specimen or tie a tag on just the mandible or clavicle.*

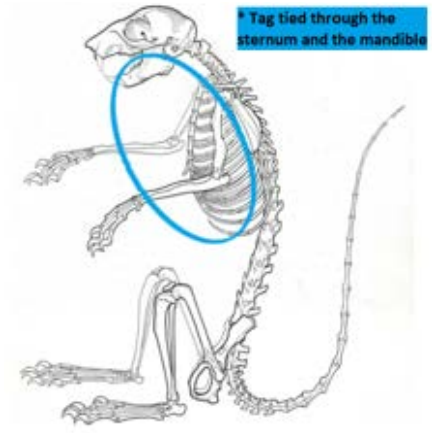



*image adapted from anatomyorgan.comi*

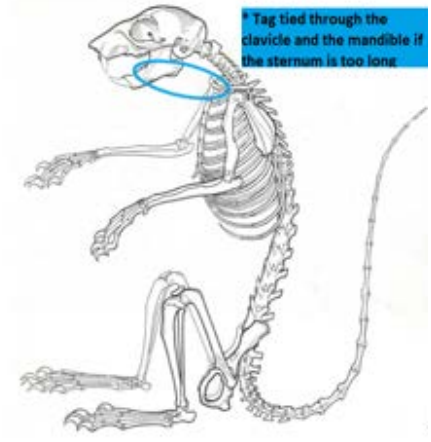
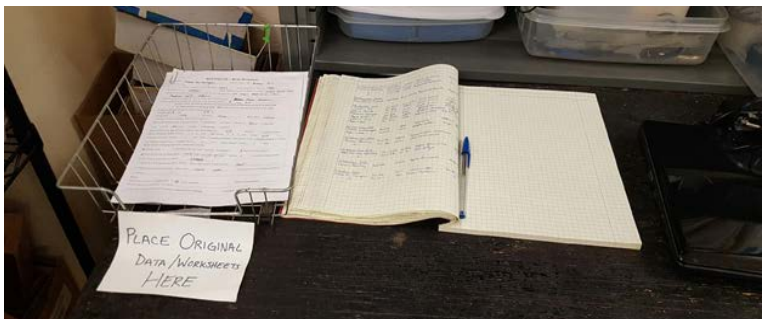



*image adapted from anatomyorgan.comi*

- If needed, place the specimen in the soak bucket.
    - Make sure the water is running slowly, the bucket is not covering the sink drain, and the strainer is over the drain (clear it out if water is pooling in the sink).
    - If the specimen is clean enough to bypass soak bucket (check in to make sure), then blow the brains using a syringe, tie it up *as small as possible* and hang it up to dry
        - Make sure the tag is visible and hanging away from the body so it doesn't stick to anything as it dries
        - If your skeleton for some reason has small or broken pieces, try to tie them to the specimen if possible. If unable to tie parts to the rest of the skeleton, consult Terri or the class coordinator on how to best group the parts together.
- Enter all the information from your worksheet into the prep lab catalog. Write neatly and pay close attention to the catalog entry examples.
    - **Have Terri, the class coordinator or a UGSI check your catalog entry before you leave.**
- Paperclip your original data to your worksheet and place it into the completed worksheet basket.
    - *TIP - Before turning in your worksheet, double check that every part of the worksheet has been completely filled out!*
    - TIP - Double check that you wrote your PLC and Accn on all original data if you haven't already done so!
- Sign out in the daily prep lab log (next to the data basket)
    - Name of preparator(s)
    - Catalog (PLC) #
    - Accession #
    - Genus and species
    - Prep and associated parts (complete skeleton)




- Clean your workstation and wash all tools and trays