Bone harvesting protocol
Please reference the Jepsen laboratory when using this protocol. Current as of 11/4/13. This protocol is subject to change. We hold no responsibility for errors in the protocol or user error that lead to personal or property damage.

Safety considerations
Please follow all federal, local, and institutional rules and regulations when using this protocol.

Materials:
Mouse
Absorbent pads
2ml Eppendorf tubes
1X Phosphate Buffered Saline (PBS)
10% Neutral Buffered Formalin (10% NBF)
Tissue cassettes (Fisher Scientific, Histocassette II)
Permanent marker
No. 2 pencil
Freezer storage boxes, cardboard or plastic
1 liter wide mouth HDPE bottles (Qorpak)
Gloves
Safety goggles
Gauze

Harvesting bones is relatively simple, but must be done without breaking them. This requires a basic understanding of anatomy to disarticulate and extract a bone without damaging the epiphyses, which can easily be sheared off in younger mice.

Prepare sample containers
1. Label cassettes for bones going to histology with the No.2 pencil. Write the animal identification number and the bone on the each cassette in two places, in case one rubs off or gets smudged.
   Never use pen or permanent marker, as the chemicals used for plastic or paraffin embedding will remove the label and you will have no way to identify your specimens! Entire research studies have been lost because of labeling with pen. Do not let this happen to you!

2. Label 2ml Eppendorf tubes for bones that will be imaged and/or used for mechanical testing using permanent marker. Write the animal identification number and bone on the top and side of each tube. See Figure 1 for an example of proper labeling of an Eppendorf tube and tissue cassette.

3. Label freezer storage boxes using permanent marker. Write the animal identification numbers (can group, i.e., B6 1-10, as opposed to B61, B62, B63…). Indicate the type of bone
   We separate bones by type, so femurs would go into one box, spines would go into a second box, etc.
4. For every 20 cassettes, fill one of the 1 liter bottles with 10% neutral buffered formalin, or a fixation agent that works with your histological processing method.
   Wear gloves and safety goggles. Bottles should be filled under a fume hood.
   Place the 1 liter bottles on an absorbent pad.
   Fill 1 liter bottle from the container of 10% NBF
   Affix a permanent label to the outside of the bottle that includes: 10% NBF (or other chemical, if used), animal identification numbers, and bone type
   Tightly close the bottle until ready to use it.
   We keep our bones separated by type, so femurs would go into one bottle, spines would go into a second bottle, etc.

5. Prepare gauze by cutting it into long pieces that will wrap around an entire bone and soaking it in PBS

**Harvest bones**

6. Sacrifice the mouse, according to your protocol and institutional guidelines. Be sure to collect the body mass of each mouse before starting dissection.

7. For bones that will be imaged or mechanically tested:
   Remove as much soft tissue from the bone as possible with gauze. If using a scalpel, be careful not to cut or scratch the bone surface.
   Wrap the bone completely in PBS-soaked gauze.
   Place the bone in the appropriately labeled Eppendorf tube. Close the tube so it is sealed.
   Place the Eppendorf tube in the appropriately labeled freezer storage box
   When done, place all freezer storage boxes in a -20°C or -40°C freezer.

   Alternatively, instead of wrapping bones in PBS-soaked gauze, place the bone in the Eppendorf tube and then fill the tube most of the way with PBS. Leave room for the PBS to expand with freezing, but make sure the entire bone is covered with PBS before freezing.

8. For bones that will be processed for histology
   Remove as much soft tissue from the bone as possible with gauze, being careful not to disrupt the periosteum.
   Place bone in the appropriately labeled histology cassette.
   Place the cassette in the appropriately labeled bottle of 10% NBF (or other fixative)
   When done, put all 1 liter bottles in an area where they are away from direct sunlight and out of heavy travel areas. Ensure any shelves used are sturdy enough to support the weight, especially if there are many bottles.