WHOLE FEMUR HISTOLOGY

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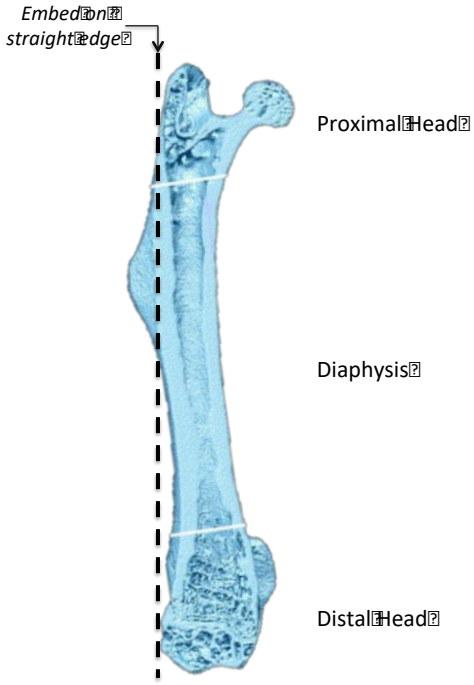
MATERIALS

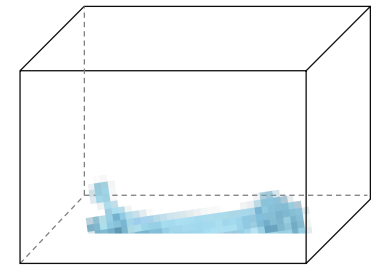
- 10% Neutral Buffered Formalin
- Shandon™ TBD-2™ Decalcifier
- Ethanol (50% & 70%)
- Dissection tools (forceps, tissue and bone scissors)
- Scalpel
- Kim wipes

PROTOCOL:

1. Douse euthanized mouse in 70% ethanol to sterilize.
2. Remove fur and skin from legs by lifting skin at the base of each leg with tweezers and cutting away skin across thigh and down to ankle. Peel skin down leg and over foot and firmly tug until it is removed.
3. Remove muscle from entire leg so that bone is completely exposed. Be very careful not to cut bone as this will compromise the sterility of the bone marrow.
4. The entire leg will be removed. To do so expose hip joint of each leg and cut above the joint, making sure to not remove the top of the femur.
5. Clean bones of any remaining muscle. *Note: Kim wipes can be used.*
6. Separate the femur from the lower leg by cutting into the knee joint and remove any remaining fragments of the hip joint. Avoid damaging the femur.
7. Place the clean femur into a labeled histology cassette and submerge in formalin for a minimum of 2 days.
8. Transfer the cassette to TBD-2 Decalcifier for 2 days. Note: To check for sufficient decalcification, test for ease of sectioning with a scalpel (no crunching sound) or whether a needle can enter the bone with minimal force. Femur can be returned to TBD-2 Decalcifier if it requires further decalcification.
9. Femurs are trimmed using a straight edge scalpel blade to create a flat surface for embedding. See figures below.
10. Transfer cassettes to 10% neutral buffered formalin for 10 minutes.
11. Transfer cassettes to 50% ethanol for 10 minutes
12. Store cassettes in 70% ethanol until ready to embed in paraffin.

Note: Due to the high granularity of bone marrow, sections should be 2 µm think instead of the standard 4 µm.





Orientation of femur embedded in paraffin block.