The GOODELL laboratory

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Title	**Bone marrow isolation, crushing technique**	
Introduction	This protocol describes the isolation of whole bone marrow from whole mice using a bone crushing technique. This protocol may be particularly useful to isolate large numbers of bone marrow cells, or to isolate bone marrow from mice for which a bone flushing technique is difficult [i.e. young or small mice].	
Materials	1. Dissecting tools, including scissors, various sized tweezers. 2. Mortar and pestle, sterilized. 3. HBSS+: Hanks Balanced Salt Solution (from Gibco) + 2% Heat-inactivated Fetal Bovine Serum + 10mM Hepes + Pen/Strep. 4. PBS and PBS+: PBS + 2% Heat-inactivated Fetal Bovine Serum. 5. Collagenase/Dispase [Roche cat# 11097113001]. 6. 50mL conical centrifuge tubes. 7. 40 micron sterile cell strainers. 8. Table top centrifuge capable of spinning falcon tubes at ~500xg. 9. Tissue culture dishes.	
Protocol		*Notes*
1.	Prepare bone marrow from mice starting by dissecting tibias, femurs and iliac crests, cleaned of all muscle and connective tissue. Place intact bones into a tissue culture dish on ice with PBS+. Continue by dissecting the arms and scapulas, again cleaning away all muscle and connective tissue. Dissect sternum and spine away from ribs, ensuring spine is also devoid of spinal cord.	*We do not typically dissect ribs, however any bones maybe used in this protocol, so long as they are dissected free from all soft tissue.*
2.	Transfer cleaned bones to sterile mortar, containing 1mL PBS+ per 3 bones.	*After trisecting the spine, we find most mice give rise to ~18 bones, so we generally use 6mL PBS+ per mouse.*

3. Using the pestle, crush bones into bone fragments,