FEMUR BONE MARROW EXTRACTION

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BACKGROUND

This protocol describes how to extract bone marrow from mouse femurs. Although the yield from femur bone marrow extractions is inferior to that of spinal extractions, it takes less time to extract bone marrow from femurs. Femur bone marrow extraction may be appropriate for applications that do not require large amounts of cells (e.g. flow cytometry) or for time- or labour-constrained experiments.

PROTOCOL

1. Under sterile conditions, isolate femurs from mice, being careful not to cut into the femur during surgical dissection.
2. Using forceps and scissors, cut away the muscle and fibrous tissue overlaying the femur to expose both ends of the femur. The red bone marrow core should be visible through the white bone. Be careful not to cut into the femur at this stage.
    a. Dissect the femur over a sterile petri dish.
    b. Fill the petri dish with PBS while keeping the petri dish lid dry.
    c. The dry petri lid dish can be used as a dry, sterile working area.
    d. The PBS-filled petri dish can be used as a pool of PBS.
    e. Periodically dip the femur into the PBS to help tissue separate from the bone.
    f. The tissue overlying the femur only needs to be dissected enough to see both ends of the femur.
3. Fill a 10mL syringe with sterile PBS and cap it with a 25G needle.
4. Uncap both ends of the femur and flush the bone marrow within into a fresh petri dish using the 25G syringe.
    a. Flip the femur after perfusing the first 5mLs of PBS through to perfuse from the other end, to ensure that all bone marrow in the femur has been extracted.
5. Using the syringe, gently pipette up and down to break the bone marrow up in the Falcon tube.
6. Filter the bone marrow-PBS solution through a 40µM nylon filter into a 50mL Falcon tube.
7. Store at 4°C until use.