Isolation of Mouse Primary Sternal Chondrocytes
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Procedure:

1) Cut out rib cage from P0-P5 pups and place into complete media (CM: DMEM + 10 % FBS + 1 % Pen/Strep (P/S))
    a. Euthanize pup by isoflourane inhalation
    b. Cut out cartilage portion of the rib cage
    c. Transfer rib cage to a well of a 6-well dish containing PBS +1% P/S
    d. Repeat a-c for remaining pups
    e. Replace PBS w/ CM and incubate rib cage overnight in 37 °C/5% CO_2 or can continue to step 2
2) Digest rib cage in 12-well dish or 15 ml conical tube:
    a. Make protease (Sigma P6911) digest mix:
        i. Per rib cage (2 ml):
            1. 2 mg/ml protease in PBS + 1% P/S
            2. Filter sterilize w/ 0.45 um filter
    b. Add 2 ml protease digest mix to each rib cage
        i. Incubate at 37 °C incubator for 15 minutes
    c. Rinse ribs 1X w/ PBS + 1% P/S
    d. Make 3 mg/ml collagenase (Sigma C6885) digest mix:
        i. Per rib cage (2 ml):
            1. 3 mg/ml collagenase in DMEM + 1% P/S
            2. Filter sterilize w/ 0.45 um filter
    e. Add 2 ml of 3 mg/ml collagenase digest mix to each rib cage
        i. Incubate at 37 °C incubator for 15 minutes
    f. Rinse each rib 3X w/ PBS + 1% P/S in 15 ml conical tube
        i. Let cartilage settle to the bottom of the tube
        ii. Aspirate PBS and soft tissue on top layer
        iii. Transfer cartilage to a well of a new 12-well plate
    g. Make 0.3 mg/ml collagenase digest mix:
        i. Per rib cage (3 ml for 12-well plate):
            1. 0.3 mg/ml collagenase in DMEM + 1% P/S
            2. Filter sterilize w/ 0.45 um filter
    h. Add 3 ml of 0.3 mg/ml collagenase digest mix to each rib cage
        i. Incubate overnight at 37 °C/5% CO_2
    i. Add 3 ml of CM to each rib cage
    j. Filter through 70 μm cell strainer onto a clean 50 ml conical tube
        i. Centrifuge at 1000 rpm for 5 min
    k. Wash 2X w/ CM
        i. Centrifuge at 1000 rpm for 5 min between washes
    l. Seed cells onto a well of a 6-well plate at 1 x 10^6 cells/well or a 60 mm dish at 5 x 10^6 cells/well in chondrogenic media (CM + 50 μg/ml ascorbic acid + 10 mM glycerol-2-phosphate)
    m. Change media the following day
    n. Expand to 100% confluency