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Musculoskeletal Cell Core
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Primary Chondrocyte Culture

** 3rd day pups are optimal for experiment **

Preparation:

- Autoclaved surgical instruments
- **DIG I**: 0.25% Collagenase II, 2.5% trypsin, in HBSS
    - Filter in hood
        1. 1.2 mL 2.5% Trypsin (ThermoFisher, 15090-046)
        2. 10.8 mL HBSS (Fisher, 21-023-CV)
        3. 30 mg Collagenase II (Worthington, LS004176)
- **DIG II**: 0.25% Collagenase II, in HBSS
    - Filter in hood

1. 10.8 mL HBSS (Fisher, 21-023-CV)
2. 30 mg Collagenase II (Worthington, LS004176)

- **Growth medium: DMEM** (ThermoFisher, 11995-065)
    - With: high glucose, sodium pyruvate, L-glutamine, phenol red
    - Without: HEPES

Add 1% Antibiotic/Antimycotic (ThermoFisher, 15240-062) and 10% FBS (ThermoFisher, 26140-079)

- **10 mg/mL Ascorbic Acid** (Sigma, A4403) in distilled water (filter in hood)

Procedure:

1. Keep pups on ice and make **DIG I**
2. Dip mouse in 70% EtOH, sac mouse, dissect ventral regions of rib cage
3. Store all of the rib cages in HBSS (iced) until last one is dissected.

**8 max/tube **

4. Under Dissecting Microscope, remove muscle and connective tissue, and store ribs in HBSS (iced) until last one is cleaned.

(Surgical instruments should be in 70% EtOH).

5. Dissociate cells in **DIG I** @ 37°C incubation for 1 hr. 15 min., with vigorous shaking every 10 min.

****STRICTLY TIME SENSITIVE ****

6. Make **DIG II** and put on ice

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7. Carefully aspirate **DIG I** solution using 25 mL Falcon, wash with HBSS and aspirate
8. Add **DIG II** and incubate at 37°C for 1 hr. 15-30 min., with vigorous shaking every 10 min.
9. Stop enzymatic reaction by adding equal amount of DMEM as stated above.
10. Strain through 70 µm cell strainer à Strain through 40 µm cell strainer
11. Count cells
12. Pellet cells by centrifugation (300xg) 5 min, room temp.
13. Aspirate → wash cells with HBSS, use pipette to break up cell clumps → spin again
14. Resuspend in appropriate amount of Bambanker freezing medium (1 mL per 1 x 106 cells), stop here and freeze cells at -
80°C, then put in liquid nitrogen storage after a day or two)
15. If plating, use growth medium + Ascorbic Acid (125 µL ascorbic acid/50 mL medium)
16. Every Monday and Friday change growth media + Ascorbic Acid

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