**Limb bud micromass culture**
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Previously described in H.-S. Seo. R. Serra, Dev Biol, 2007, 310(2):304-316
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Reagents/solutions:

1) Collagenase D, Roche Applied Sci, Cat# 11088858001
2) DPBS (1x), Mediatech, Inc., Cat# 21-031-CV
3) F12 (Ham, 1x), Invitrogen, Cat# 11765062
4) DMEM media, Mediatech, Inc., Cat# 10-017-CM
5) β-Glycerol phosphate (Sigma, #G9891)
    a. To make 10 ml of 1M β-Glycerol phosphate
        i. Add 2.1604 g of β-Glycerol phosphate to 10 ml DPBS
        ii. Filter-sterilize
        iii. Store at 4 °C
6) Ascorbic acid (Sigma, #A4544)
    a. To make 50 mg/ml of ascorbic acid
        i. Add 500 mg of ascorbic acid to 10 ml of DMEM media
        ii. Filter-sterilize
        iii. Store 1 ml aliquots (covered in foil) at -20 °C

Procedure:

1) Isolate limb buds from E11.5 day mouse embryos
2) Dissociate mesenchemal cells into single cell suspension by digesting in 300-500 μl of 1 mg/ml collagenase D (diluted from 100 mg/ml stock in sterile DPBS) for 1-2 hour in 37 °C incubator
    a. Vortex every 30 min to facilitate cell dissociation
3) Filter through 70 μm filter
4) Pellet cells by centrifugation at 1000 rpm for 5 min
5) Aspirate collagenase solution
6) Resuspend in 50 μl F12:DMEM (1:1) media supplemented with 10 % FBS and antibiotics
7) Reconstitute at density of $1x10^7$ cells/ml
8) Drop 10 μl of cell suspension into each well of a 24-well plate
9) Allow cells to attach to dish by incubating for 1 hr in 37 °C incubator
10) After incubation, flood cells with 500 μl of chondrogenic media (F12:DMEM (1:1) media supplemented with 10 % FBS and antibiotics, 50 μg/ml ascorbic acid, 10 mM β-Glycerol phosphate) **supplemented with appropriate growth factors, inhibitors, etc**.
11) Incubate cultures in 37 °C incubator for up to three days
    a. Can process cells for Alcian blue staining, immunoflourescence staining, western blotting, or real-time PCR