Karen M. Lyons Lab – UCLA
From Sotirios Tetradis Lab - UCLA
Modified on 12/7/2008

Isolation of Calvarial Osteoblasts

Procedure:

1) Cut out calvaria from P4 or P5 pups and place into CM (DMEM + 10 % FBS + 1 % Pen/Strep (P/S))
    a. Euthanize pup by isoflourane inhalation
    b. Douse pup with 70% EtOH
    c. Collect tails for genotyping
    d. Decapitate pup and cut the skin away
    e. Hold the head with curved forceps placed through the orbita, cut the calvaria loose along the edge and place in a dish with PBS (+ 1 % P/S)
        i. Cut away edges and sutures with scissors
    f. Transfer calvaria to well of a 6-well dish containing PBS (+ 1 % P/S)
    g. Repeat steps a – f for all remaining pups
    h. Replace PBS with CM and incubate calvaria 1-2 days in 37 °C/ 5 % CO_2
2) Digest calvaria:
    a. Make digest mix:
        i. per genotype (~5 calvaria):
            1. 3 mg collagenase II
            2. 1 mL trypsin (0.25 % trypsin-EDTA; Gibco)
            3. up to 5.0 ml w/ PBS + 1 % P/S)…make 3X (per digest)
            4. **filter sterilize w/ 0.22 um filter...keep on ice**

number of genotype total =	3			
	Per genotype (ul)	Final conc per genotype	x (3.1) per digest	x total number of genotype (ml)
Collagenase (100mg/ml stock)	60	6 mg	186	0.558
Trypsin (.25%)	1000	~0.05%	3100	9.3
PBS + 1 % P/S	3940		12214	36.6

5 ml total

    b. Based on genotype, pool calvaria into 50 mL conical tube containing 5 mL PBS + P/S
        i. Rinse 3X
    c. Add 4-5 mL digest mix to calvaria
        i. Tighten cap and incubate at 37 °C for 5 min
        ii. Discard supernatant
    d. Add fresh 5 mL digest mix to calvaria
        i. With loose cap, incubate at 37 °C for 15 min
        ii. Shake by hand for a few seconds
        iii. Incubate at 37 °C for an additional 15 min
        iv. Inactivate with 5 mL CM
        v. Save supernatant into new 50 ml conical tube (Incubate at 37 °C until step f is done)
    e. Add fresh 5 mL digest mix to calvaria
        i. With loose cap, incubate at 37 °C for 15 min
        ii. Shake by hand for a few seconds
        iii. Incubate at 37 °C for an additional 15 min
        iv. Inactivate with 5 mL CM
        v. Combine supernatant with that from step d.v.
    f. Spin down at 3000 rpm/5 min
    g. Aspirate supernatant
    h. Resuspend in DMEM + 10 % FBS + 1 % P/S or osteoblast differentiation media
        i. Plate onto a well of a 6-well plate or a 60 mm dish (depending on size of cell pellet)
        ii. Change media the following day
        iii. Expand to 100% confluency for 1-2 weeks
            1. Feed every 3-4 days