Primary Mammary Organoid Prep **2/7/07**

Andrew Ewald andrew.ewald@ucsf.edu

A. Put Matrigel to thaw on ice or in refrigerator
B. Sterilize dissection tools in bead sterilizer
C. Make solutions
    1. Collagenase solution
        i. Thaw insulin beforehand in 37°C water bath
        ii. Put into 50 mL tube:
            a. 47.5 mL DMEM/F12
            b. 2.5 mL (5% final) Fetal Bovine Serum (FBS)
            c. 50 µL 50 mg/mL Gentamicin
            d. 250 µL insulin (5µg/mL final; 1mg/mL stock)
        iii. Warm entire 50 mL tube in 37°C water bath
        iv. Add to warmed solution:
            a. 0.1 g trypsin
            b. 0.1 g collagenase A
        v. Shake at room temp until dissolved (~15 mins)
        vi. Filter sterilize through 0.2 micron filter
    2. Bovine Serum Albumin (BSA) solution
        i. Put into 50 mL tube:
            a. 50 mL phosphate buffered saline (PBS)
            b. 1.25 g BSA
        ii. Shake until dissolved (~15 mins)
        iii. Filter sterilize through 0.2 micron filter
    3. DMEM/F12: Put 30 mL each into two 50-mL tubes
    4. DNAse
        i. For each tissue condition to be tested, put into 15 mL tube:
            a. 4 mL DMEM/F12
            b. 40 µL DNAse (2 U/µL)
D. Collect mammary glands
    1. Spray counter surface w/ethanol
    2. Prepare dissection area: mat, dissection board & pins, sterilized dissection tools, Petri dish or slides, biohazard waste bag for mouse disposal, etc.
    3. Euthanize mouse w/CO_2, followed by cervical dislocation
    4. Spray mouse w/ethanol to keep keep fur down
    5. Collect 3's, 4's, and 5's for organoid prep; do not collect 5's for wholemounts
        i. Remember to remove lymph node from each #4 for organoid prep; leave lymph nodes in for wholemounts
E. Process mammary glands to isolate epithelium
    1. Mince mammary glands with scalpel until well chopped
    2. Transfer minced glands to collagenase solution
    3. Shake collagenase solution w/glands at 100 rpm, 37°C for 30 minutes, or until glands are relatively dispersed
    4. Spin tube in centrifuge, 1500 rpm, 10 mins, 25°C. Tube will have 3 layers: fatty layer on top, aqueous layer in middle, pellet of epithelium on bottom.
    5. **<u>Precoat all pipettes with BSA solution prior to mammary tissue contact.</u>**

Andrew Ewald andrew.ewald@ucsf.edu

6. Remove fatty layer and put in 15 mL tube. Actual # mL doesn't matter, just get all the fat. Add 5 mL DMEM/F12 to 15-mL tube with fat. Pipette up and down vigorously to disperse fatty layer.
7. Spin tube w/fatty layer at 1500 rpm, 10 mins, 25°C
8. Aspirate away supernatant and resuspend both pellets in 10 mL DMEM/F12
    i. Suggested way of doing this: aspirate supernatant from both tubes. Add 10 mL DMEM/F12 to the 50-mL tube with the original pellet. With the pipette, transfer the original pellet along with all 10 mL to the 15-mL "fatty layer" tube. Pipette up and down to disperse tissue (this is called "resuspending"); now both pellets are in 10 mL solution.
9. Spin tube w/epithelium at 1500 rpm, 10 mins, 25°C
10. Aspirate supernatant and add 4 mL DNAse solution. Shake by hand 2-5 mins
11. Add 6 mL DMEM/F12 and pipette up and down thoroughly.
12. Spin tube at 1500 rpm, 10 mins, 25°C
13. Differential centrifugation (to wash out enzymes and to separate unwanted single cells from organoids; do a **total of 4 times**)
    i. Aspirate supernatant
    ii. Resuspend pellet in 10 mL DMEM/F12
    iii. Pulse to 1500 rpm, then stop as fast as possible
14. The pellet left now should be mostly organoids.

F. **Plating**

1. Calculate desired amount of Matrigel for experiment
2. Keep Matrigel on ice.
3. Aspirate supernatant, resuspend organoids in appropriate amount of Matrigel (while in liquid form)
4. Plate Matrigel/organoid mix according to experiment
5. Incubate plates at 37°C for 20 mins
6. Add warmed growth media according to experiment, return plates to incubator

---

Basic organoid growth media formula (to make 100 mL):

- 98 mL DMEM/F12
- 1 ml Penn/Strep (100x)
- 1 ml ITS (insulin, transferrin, sodium selenite) solution (100x)

For plating purposes, take out small amounts of basic organoid media into 50 mL or 15 mL tubes and add appropriate amounts of growth factor (GF).

For **10 mL** of GF media, add _ μL:

	0.1 nM	**1 nM**	**2.5 nM**	**10 nM**
HGF	.85	8.5	21.25	85
KGF	.19	1.89	4.73	18.9
Amphiregulin	.11	1.1	2.75	11
EGF or TGFα	.06	0.6	1.5	6
bFGF (FGF2)	.17	1.72	4.3	17.2
Pleiotropin	.15	1.53	3.83	15.3

For **25 mL** of GF media, add _ μL:

0.1 nM	**1 nM**	**2.5 nM**	**10 nM**
2.13	21.25	53.13	212.5
0.47	4.73	11.81	47.25
0.28	2.75	6.88	27.5
0.15	1.5	3.75	15
0.43	4.3	10.75	43
0.38	3.83	9.56	38.25

Andrew Ewald andrew.ewald@ucsf.edu

aFGF (FGF1)	.16	1.55	3.88	15.5	0.39	3.88	9.69	38.75

Primary Mammary Organoid Prep **2/7/07**

Andrew Ewald andrew.ewald@ucsf.edu

Storage Aliquots

1. DNase: 2000 K Units per vial. Dilute at 2 units per uL, 50 uL per tube.
2. EGF: 200 ug per vial, dilute to 100 ug/mL (2 mL), then 50 uL per tube (40 tubes).
3. bFGF: 25 ug per vial, dilute to 100 ug/mL (250 uL), then 25 uL per tube (10 tubes).
4. Amphiregulin: 100 ug per vial, dilute to 100 ug/mL (1 mL), then 33 uL per tube (30 tubes).
5. HGF: 5 ug per vial, dilute to 100 ug/mL (50 uL), then 12.5 uL per tube (4 tubes).
6. KGF: 10 ug per vial, dilute to 100 ug/mL (100 uL), then 10 uL per tube (10 tubes).

Name	Manufacturer	Stock #
Trypsin	Gibco	27250-018
Matrigel, Reduced GF	Becton Dickinson	354230
Collagenase	Sigma	C5138-1G
DNAse I	Sigma	D4263
bFGF	Sigma	F0291
EGF	Sigma	E9644
ITS	Sigma	I3146
KGF	Sigma	K-1757
HGF	Sigma	H-1404
Amphiregulin, long form	R&D Systems	262-AR/CF
Fetal Bovine Serum	UCSF CCF	IC300
Insulin 1 mg/mL	UCSF CCF	CCFHK001
Gentamicin 50 mg/mL	UCSF CCF	CCFGI001
DMEM/F12	Gibco	11330
BSA	Sigma	A7906
Penn/Strep (100x)	UCSF CCF	CCGK004