Culturing immortalized human muscle cells

1. Thaw frozen cells at 37^{o}C for 1-2 minutes.
2. Resuspend cells in pre-warmed LHCN growth medium; if necessary (i.e. final dilution of frozen cells < 1:10), centrifuge cells in LHCN at 1000xg for 5 minutes at room temperature and aspirate medium. Resuspend in LHCN and seed on gelatin-coated tissue culture dishes at a concentration between ~1 to 2x10e3 cells/cm2.
3. Incubate at 37^{o}C/5% CO2.
4. Passage before cells become confluent: aspirate medium and rinse 2X with PBS; add sufficient TrypLE Express (Gibco) to cover cells and wait 3-5 minutes at RT until cells have detached. Neutralize with an equal volume of LHCN medium and count cells.
5. To freeze cells, adjust volume as required with LHCN. Mix cells with an equal volume of ice cold 2X freeze medium and aliquot to cryovials; freeze directly in liquid nitrogen vapour phase for 1 hour and transfer to permanent storage.

Differentiation of immortalized human muscle cells

1. Seed and culture cells as described above and grow until >95% confluency.
2. Aspirate growth medium, rinse with PBS and feed with differentiation medium.
3. Culture at 37oC/5% CO2 untouched. Multinucleated myotubes are usually observed within 2-3 days.

LHCN Medium (stocks for Emerson lab)

Stock	~122 ml	~245 ml	~610mL
1X MEDIUM X (4:1 DMEM/199*)	100 mL	200 mL	500 mL
FBS (Hyclone, characterized)	18.2 mL	36.4 mL	91 mL
0.03g/L Zinc Sulfate (Sigmal; 4°C)	121 uL	242 uL	604 uL
1.4 mg/mL vitamin B12 (Sigma; 4°C)	121 uL	242 uL	604 uL
55 ug/mL dexamethasome (Sigma; 4°C)	121 uL	242 uL	604 uL
2.5 ug/mL HGF (Millipore; -20°C)	121 uL	242 uL	604 uL
25 ug/mL bFGF (Millipore; -20°C)	48.2 ul	96.4 ul	241 uL
100 X Antibiotics/antimycotics	1.2 mL	2.5 mL	6 mL
1 M HEPES (add AFTER filtration)	2.4 mL	4.8 mL	12 mL

* make up from Invitrogen powdered medium; adjust final Medium X concentration to 0.088 g/l (= 0.8 mM) sodium pyruvate and 3.4 g/l sodium bicarbonate

DIFFERENTIATION MEDIUM: Filter sterilize

Stock	100 mL	200 mL	500 mL
1X Medium X	94 mL	188 mL	470 mL
Horse Serum (Hyclone)	2 mL	4 mL	10 mL
200 mM L-glutamine	1 mL	2 mL	5 mL
100X Antibiotics/Mycotics	1 mL	2 mL	5 mL
1 M HEPES (add AFTER filtration)	2 mL	4 mL	10 mL

2X freeze Medium (use ice cold)

20% DMSO (Sigma)
50% FBS (Hyclone)
30% LHCN

0.1% gelatin-coated plates: dissolve 1 g gelatin (Sigma G9391) in 1 L tissue-culture grade water; mix well and autoclave; store at room temperature. To coat dishes, add sufficient volume of 0.1% gelatin to cover surface, aspirate, and air-dry in biosafety cabinet. Store dry plates at room temperature. (Note: for certain surfaces, including permanox chamber slides, it is recommended that gelatin be allowed to sit for 1 hour to overnight at RT before aspiration.)

Chick embryo extract: (Adapted from F. Stockdale's lab protocol.) Harvest Day 12 embryos from 5 to 10 dozen fertilized White Leghorn chicken eggs (Charles River, SPF Premium Eggs), using sharp-point scissors to pierce and then cut the top off of the eggshell. Decapitate as close to the head as possible with sterile scissors, and rinse embryo bodies 2X in ice-cold HBSS. Macerate chick embryos by pushing them through a 60 ml syringe, and collect in a chilled, sterile graduated cylinder. Add an equal volume of ice-cold HBSS, transfer to a sterile beaker and stir, covered, for one hour at 4^{o}C. Centrifuge at 10,000 rpm for 1 hour at 4^{o}C and collect supernatant. (Optional: filter through 0.45 um filter system; this can be difficult depending on how clean the supernatant is, so we tend to skip this step and add an additional centrifugation step as described below.) Store aliquots in 15 mL tubes at -20^{o}C, or -80^{o}C for long-term storage. To use, place 15 mL aliquot in a room temperature water bath just until thawed, then spin for 30 minutes at 4,000 rpm at 4^{o}C to pellet any remaining contaminants from the processing. Add supernatant to medium and filter through 0.2 um filter system; aliquot leftover CEE and freeze.