Mammalian Cell Culture
Week 2

BIOE202 Spring 2016
Dr. Karin Jensen
Questions about the Module 3 assignment?

- Full assignment description is posted on the course website.
- Rubric is posted on the course website—please review and let us know if you have any questions!
Office Hours

• Dr. Jensen’s office hours this week will be Friday 1:15-2pm in DCL 3103
• Mohammad’s office hours 9-10am Friday MNTL 3261
• Yanfen’s ImageJ tutorial Monday 2/22 8-9pm 1265 DCL (for Module 4 assignment)
Questions from last lecture?
Module 2 Assignment Review

Calculating CFUs/mL of starter culture

Well F2: one 2-fold dilution, five 10-fold dilutions

\[(2 \times 10^5) \times F2\text{ culture} = \text{starter culture}\]

\[
\frac{110\text{ colonies}}{50 \mu L} \times (2 \times 10^5) \times 1000 \mu L/mL = 4.4 \times 10^8 \text{ CFUs/mL}
\]
Module 2 Assignment Review

• **Dilution**
  
  CFUs per volume/dilution
  “work forwards”
  What the relationship is relative to the starter culture

• **Dilution Factor** = Final volume / solute volume
  
  CFUs per volume*dilution factor
  “work backwards”
  What you need to multiply by to get back to the starter culture

Example: a dilution factor of 10 is a 1:10 dilution (0.1)
Module 2 Assignment Review

• Units
  – CFUs per volume of culture
  – Usually CFUs/mL

3 CFUs

110 CFUs

“Lawn”
Figure 1: Optical density of *E. coli* HB101 K-12 liquid culture decreases linearly with increasing dilution. Optical density of six serial dilutions of *E. coli* HB101 K-12 grown in LB media were measured via microplate reader at absorbance of 600nm. Starter culture is at dilution 1. Linear regression with $R^2$ value 0.998 show decreasing optical density with increasing dilution.
Figure 2: Colony forming units (CFUs) of *E.coli* HB101 K-12 on LB plates decreases linearly with increasing dilution. Nine samples of serial dilutions of *E.coli* HB101 K-12 grown in LB media were plated onto LB agar plates and grown overnight (starter culture at dilution 1). CFUs were counted and linear regression with $R^2$ value 0.941 show decreasing CFUs with increasing dilution.
The importance of preserving cells

Cells for experiments
Eggs (oocytes), embryos, and sperm
Banking tissue samples
Blood
Cord blood from newborns
Organs
Brains (Alcor Life Extension Foundation)
Upper Body (Cryonics Institute)
People?

Active area of research!

http://www.biodisem.com/blog/6635/cryopreservation-long-term
Freezing water can cause damage

A sewer access pipe is exposed in a pothole on the corner of N. Jackson and E. Mason streets in Milwaukee. The extreme cold and snow this winter has helped to create an increase in the potholes in and around the city.

By Lydia Mulvany of the Journal Sentinel

Updated: 3:26 p.m.

Milwaukee Journal Sentinel, Feb 11 2014
Ice crystals damage cells

Freeze/thaw procedures minimize ice crystal formation and the formation of pockets of highly-concentrated solutes.

http://www.freenaturepictures.com/assets/images/medres/icecrystals1.jpg
Solutions in nature!


http://www.enn.com/wildlife/article/47729
Cryoprotectants in the lab

Hydrophilic cryoprotectants sequesters water and act like antifreeze to decrease the freezing temp, also increase viscosity

Glycerol (bacterial stocks—“glycerol stocks”)

Dimethyl sulfoxide or DMSO (most cell lines)

Usually at 10%
Can penetrate many synthetic and natural membranes, including skin
Any potentially harmful substance can be carried into the circulation through the skin.
Handle with care in the presence of any toxic substances
Must be removed after the cells have been thawed
Cryopreservation process

- Freezing slowly also prevents ice crystal formation (desired 1 deg C/min)
- Storing cells at the lowest possible temperature minimizes the effects of enzymatic or chemical activities that might damage cells
Cryopreservation

Vitrification - instead of water crystallizing, the increased viscosity forms amorphous ice-“solid liquid”

Achieved with cryoprotectants and controlled cooling
Thawing Cells

- Thawing rapidly minimizes ice crystal growth and generation of solute gradients as intracellular ice melts.
- Thaw cryovials of cells in the water bath at 37 deg C.
Differentiation

• Process by which a cell changes from one cell type to another cell type

• Pluripotent stem cells can differentiate into any cell in the adult body

http://styczynski.chbe.gatech.edu/StemCells.php
Adipogenesis

Differentiation of fibroblasts to adipocytes

- Adipocytes are marked by the presence of lipid reservoirs

<table>
<thead>
<tr>
<th>Differentiation Time (days)</th>
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<td>Day 0</td>
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Function:
- Uptake, synthesis, storage and mobilization of neutral lipid
- used as fuel for cells, cushion, insulation

Location: under skin, around kidneys, in bones (adults)
Adipogenesis

How do we induce differentiation of 3T3-L1 cells?

isobutylmethylxanthine, a compound similar to caffeine

insulin, a regulator of sugar metabolism

dexamethasone, a glucocorticoid that is often used to induce differentiation

Fetal bovine serum, which contains the lipids that will accumulate in growing adipocytes
Cell Stimulation and Lysis

- Intracellular proteins can be collected from cells to analyze how they behave in response to a stimulus.
- We will be stimulating 3T3-L1 cells with a high dose of calf serum. Later this semester we will analyze the changes induced in the cell at the protein level.
RIPA Buffer

Radioimmunoprecipitation assay (RIPA) buffer

- Tris-Buffer prevents protein degradation
- NaCl-Prevents nonspecific protein interactions
- NP-40, SDS, sodium deoxycholate-detergents to extract proteins
- Protease and phosphatase inhibitor cocktail
Lysing Cells with RIPA Buffer

- Wash 1X with ice-cold PBS
- Add lysis buffer
- Scrape cells off dish into lysis buffer
- Centrifuge for 10 minutes
- Collect supernatant
  - Insoluble fraction pellets
Lab Overview

Main Lab

- 15mL conical tube:
  - growth media
  - centrifuge cell suspension

- 15mL conical tube:
  - resuspend cells in freezing media

- 1.8mL cryovial:
  - freeze cells
  - -80 °C move to LN₂ after 24 hr

Return Lab

- 6cm dish:
  - +20% CS
  - 5 minutes 37 deg C

- 6cm dish:
  - Stored at -80 deg C
Lab Practical

- **15% of your final grade**
- **Week of March 14th**
- **Trypsinizing, counting cells, and plating cells and answering questions in the presence of the TA/instructor**
- **During your normal lab time (Main or Return Lab)**
- **Electronic sign up in a few weeks**
- **You will be given a protocol for trypsinization ahead of time and can use this during the exam**
- **Practice, practice, practice!**
- **Be sure to alternate lab members in the BSC in the upcoming weeks**
Lab Practical

• Skills and concepts we will be checking:
  – Aseptic technique (handling cells, bottles, pipettes...)
  – Preparing BSC and reagents
  – Viewing cells with the microscope
  – Trypsinizing cells
  – Preparation of hemocytometer
  – Counting cells with hemocytometer
  – Calculating cells needed to plate
  – Plating cells
  – Proper disposal of wastes
  – Cleaning BSC
Lab practical details

The protocol that you may use is posted on the course website-please review! No surprises. You will take the practical with Dr. Jensen or one of the TAs.

You will be allowed 45 minutes.

Grade: technique, 6 questions, cells plated correctly

You may **not** ask questions during the practical— we can’t help you!
Schedule and logistics

• Return lab this week: Mammalian Cell Culture Week 1
• Lab next week: Mammalian Cell Culture Week 2
• Quiz next week will cover today’s lecture and Mammalian Cell Culture Week 2 handout