

# BME 174 – Module 3: Nutritional Enhancement

Spring, 2023





Module 2 Lab Report due next Monday April 10

# Module 3: Modulating nutrition in muscle cells

- April 3: Thaw muscle cells and test antioxidant treatments
- April 10: Analyze antioxidant effects on cell viability
- April 21: Seed cells with selected antioxidant treatment
- April 24: Continue culture
- May 1: Harvest, cook, and measure oxidation

Original Research Article

Engineering carotenoid production in mammalian cells for nutritionally enhanced cell-cultured foods

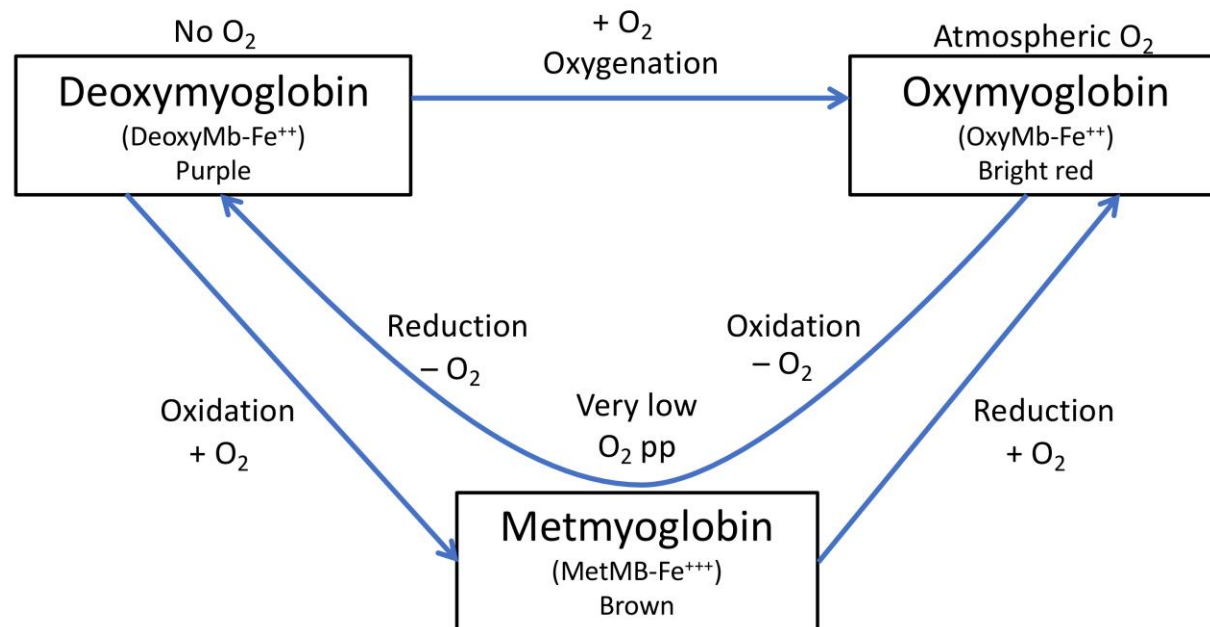
Andrew J. Stout<sup>a</sup>, Addison B. Mirliani<sup>a</sup>, Erin L. Soule-Albridge<sup>a</sup>, Julian M. Cohen<sup>a,b</sup>, David L. Kaplan<sup>a,\*</sup>

<sup>a</sup> Biomedical Engineering Department, Tissue Engineering Resource Center, Tufts University, 4 Colby St, Medford, MA, 02155, USA

<sup>b</sup> W. M. Keck Science Department, Pitzer College, 925 N Mills Ave, Claremont, CA, 91711, USA

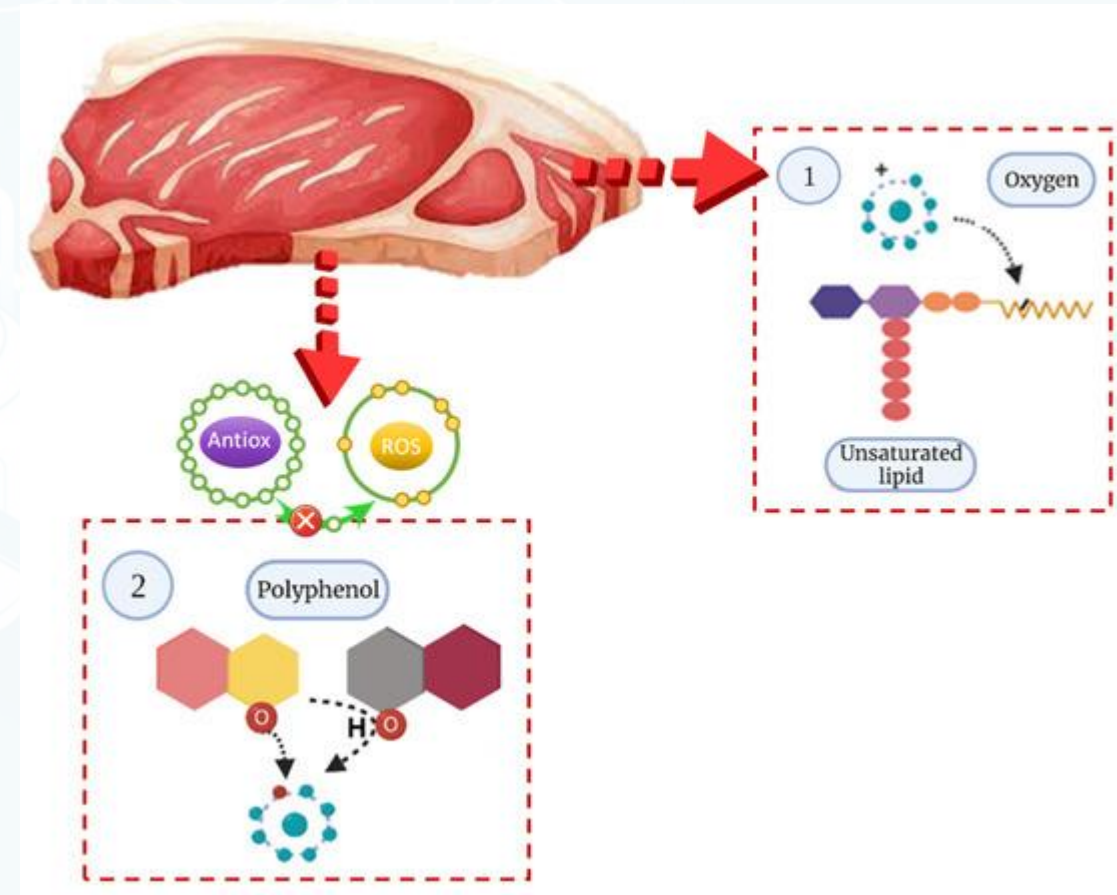
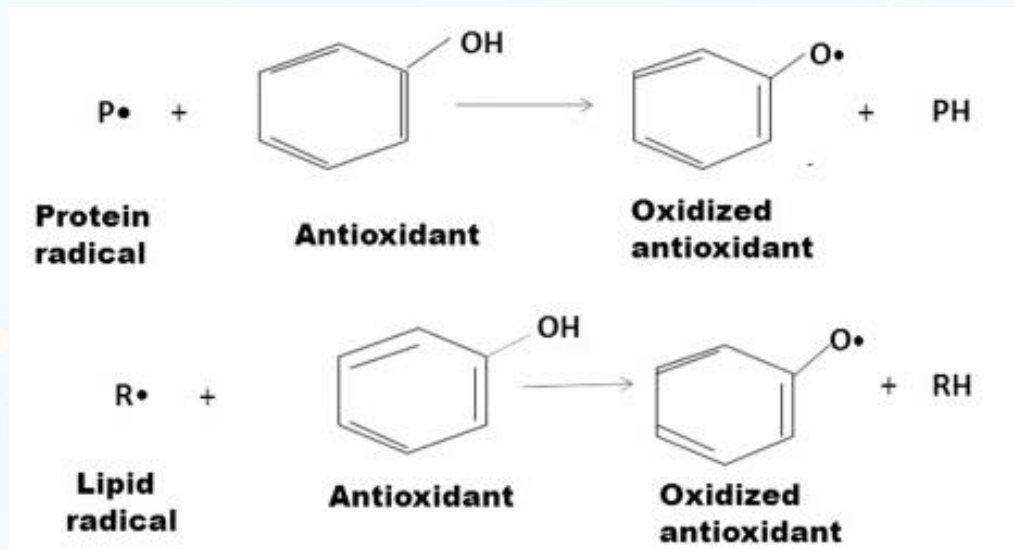
# Oxidation of Meat

- Oxidative stress → free radical reactive oxygen species and reactive nitrogen species
- Leads to discoloration, off flavors, formation of toxic compounds, etc.
- Lipid and protein oxidation are key mechanistic links between red or processed meat consumption and colorectal cancer

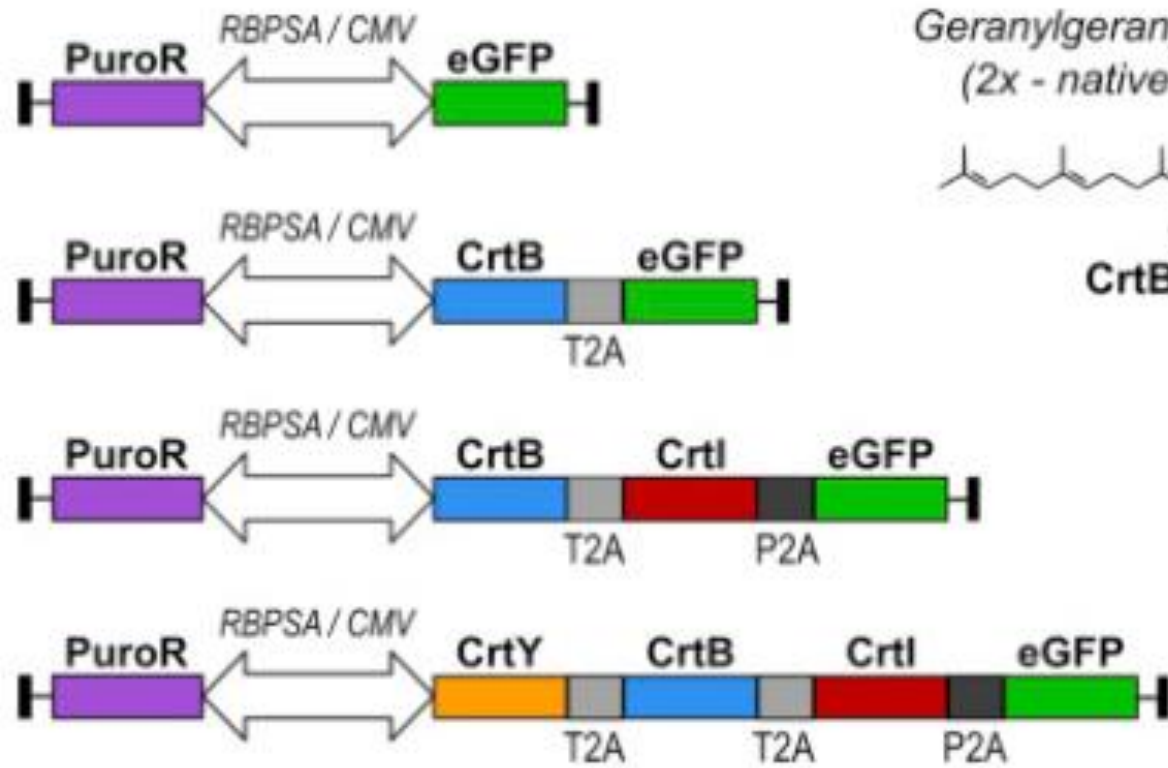


Sources: Inspired by Kropf (2003), Proc. 56<sup>th</sup> Recip. Meat Conf., 73-75 and Mancini and Hunt (2005), Meat Sci. 71: 100-121.

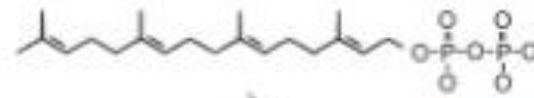
# Antioxidants prevent oxidation



# Genetic engineering of carotenoid production into C2C12 and BSCs



Geranylgeranyl pyrophosphate  
(2x - native to *Bos taurus*)



CrtB

Phytoene

CrtI

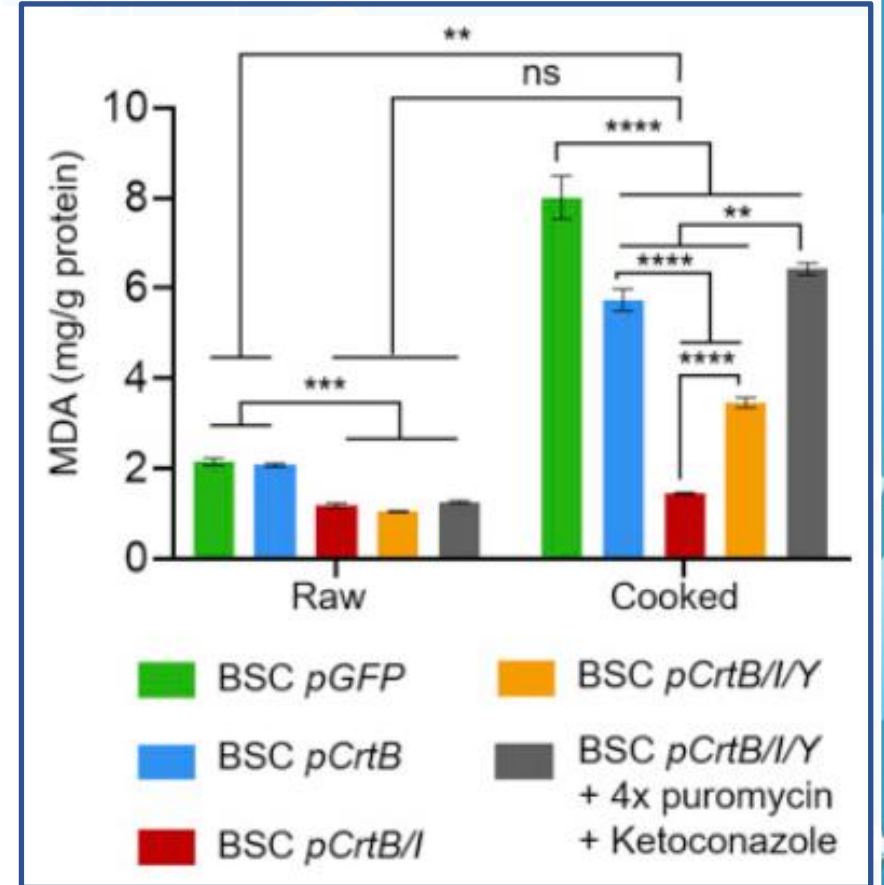
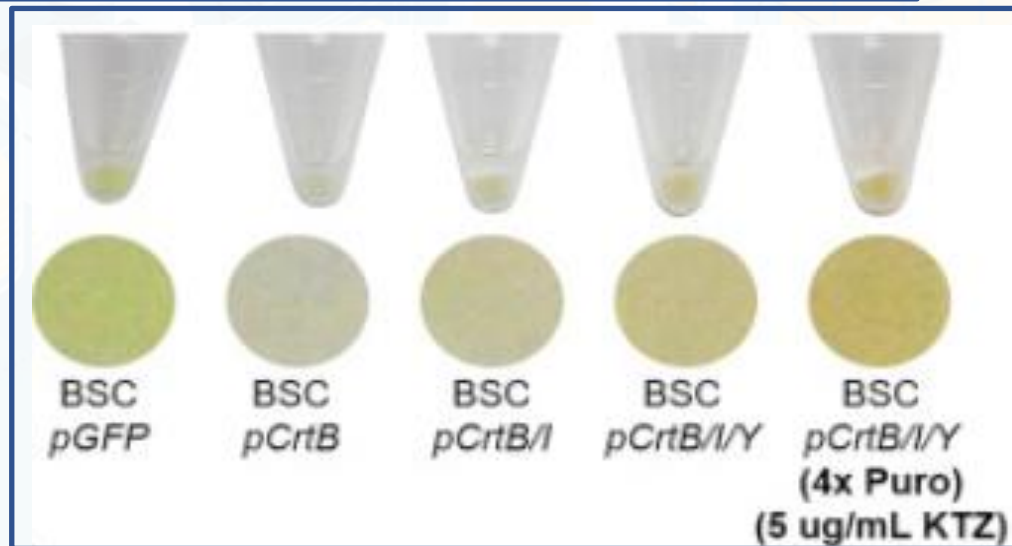
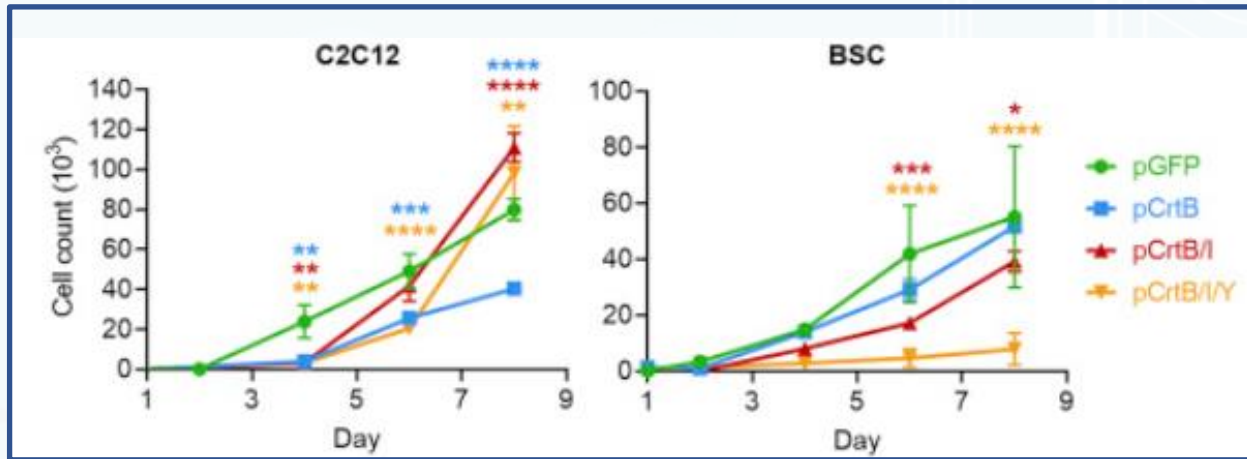
Lycopene

CrtIY

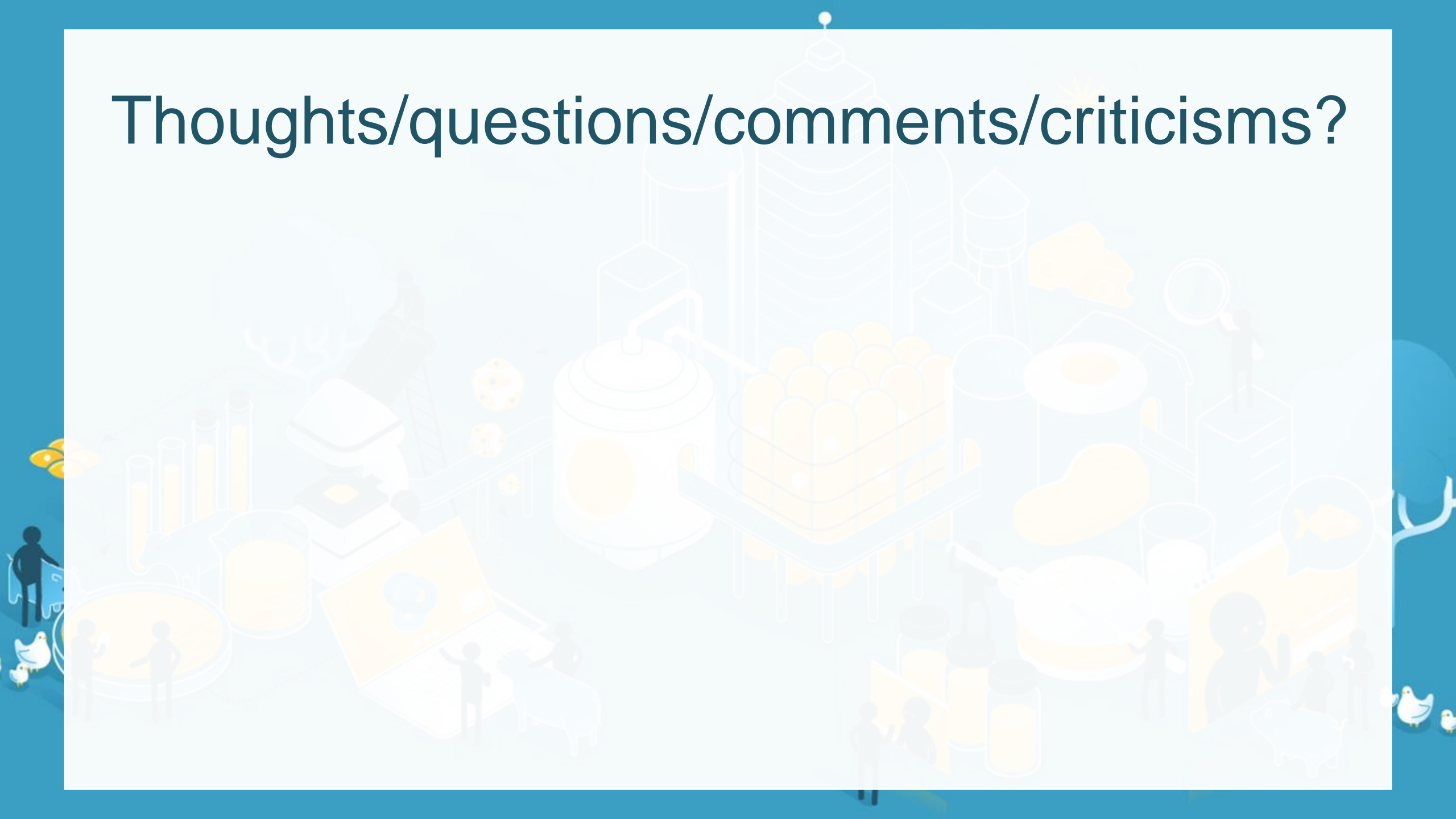
$\beta$ -carotene



# Genetic engineering of carotenoid production into C2C12 and BSCs



# Thoughts/questions/comments/criticisms?







# Today's Plan

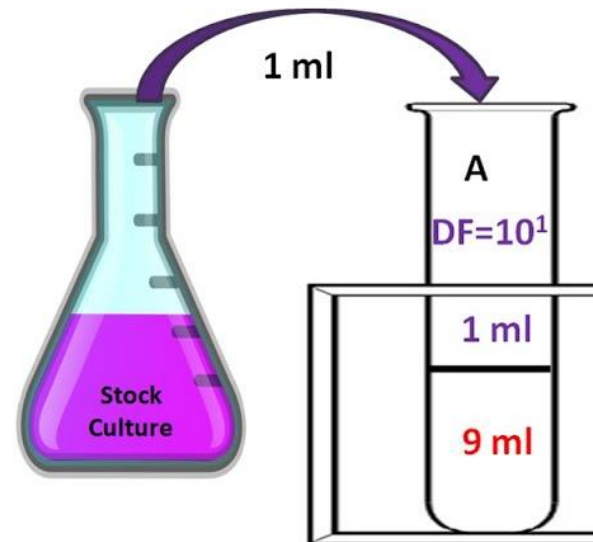
- Each group will prepare one ingredient
  - For carotenoids, make concentrated solution then sterile filter
  - For juices, pellet solids then sterile filter the supernatant
- We have thawed cells and provided you with 100,000 cells in 1 mL
- You will pick a carotenoid / juice pair and prepare at least 10 cell culture media with them
  - Test different concentrations
  - Will also have appropriate controls
- In a 48-well plate, add 200  $\mu\text{L}$  of your different media
- Then, add 1,000 (10  $\mu\text{L}$ ) cells to each well
- Feed on Wednesday and Friday for next week's viability test

# Dilution factor

?

$$\text{Dilution} = \frac{V_i}{V_f}$$

Vol. of stock transferred ( $V_i$ )  
Final Vol. (diluent vol + Stock vol) ( $V_f$ )



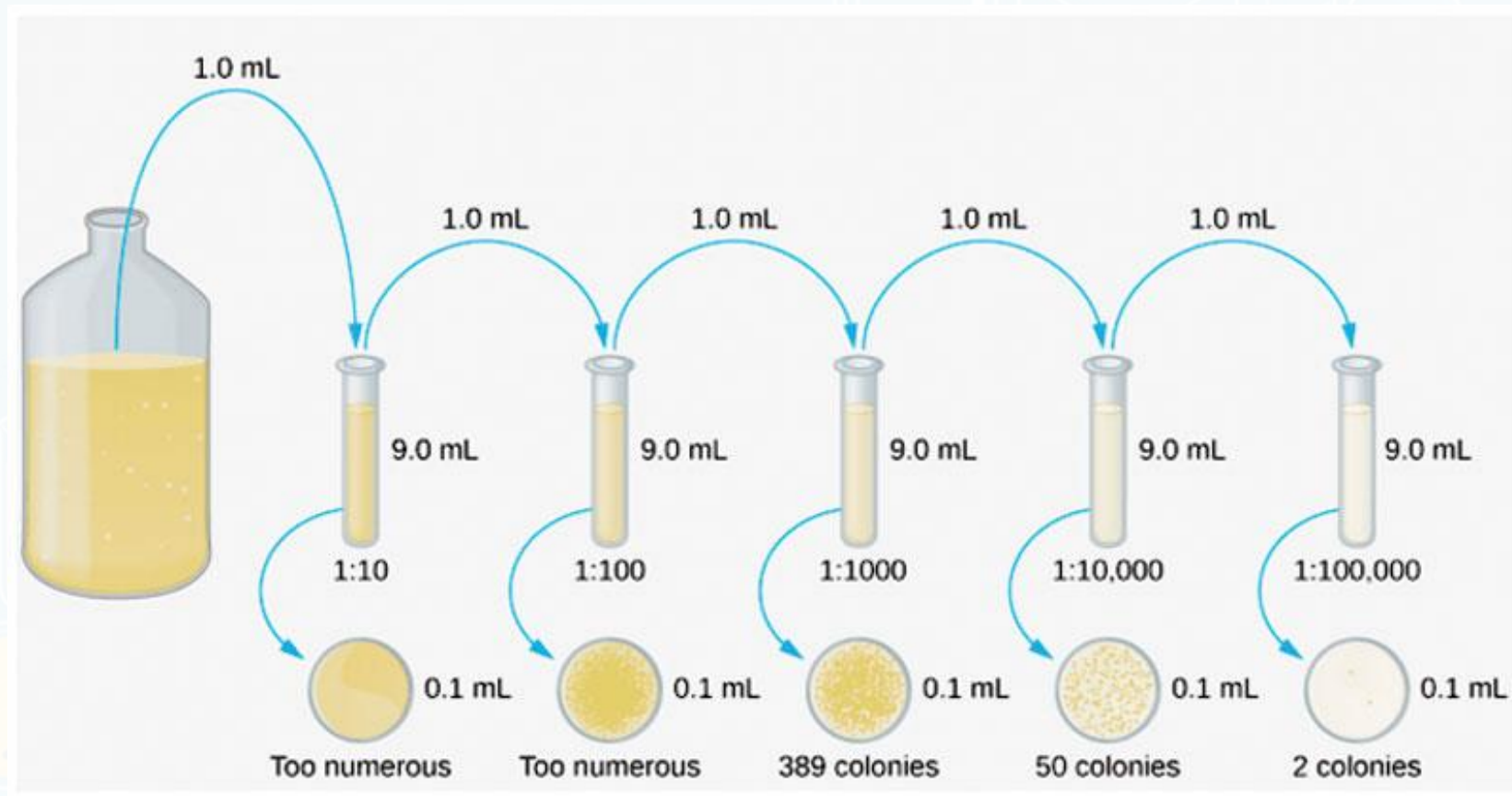
$$\text{Dilution Factor} = \frac{V_f}{V_i}$$

Reciprocal of dilution

5 minutes



# Serial dilutions



# Any Questions?

