Announcements

Quiz

Pre-lab Lecture

- Cell dilutions
- RT-PCR

Today in Lab (Mod 3 Day 4)
Announcements

• How grades are determined in 20.109
• If you went first on D2, go second today
• During down-time
  – Prep RNA area
  – Work on Module 3 report (viability analysis)
  – Other as you see fit
• RNA work: double-check today’s protocol for when to take fresh collection tube
Cell dilutions

**Case 1**

- Cells: \(1M (10^6)\)
- \(1:2\) dilution
- \(V_{\text{cells}} = 0.5\text{mL}\)
- \(V_{\text{med}} = 2.5\text{mL}\)

- Area = \(A\)
- \(V = 1\text{mL}\)

**Case 2**

- Cells: \(1M\)
- \(1:2\) dilution
- \(V_{\text{cells}} = 1\text{mL}\)
- \(V_{\text{med}} = V_{\text{total}}\)
- Area = \(2A\)
RT-PCR recap

- RT = reverse transcriptase
  - what does this enzyme do?
- Unique primer design needs
  - how to isolate transcript...
  - ... but not genomic DNA?
- RT and PCR can be done in one reaction or two
- What kinds of controls are desired?
  - amplify housekeeping gene (GAPDH, 18S rRNA) → expect to be constant across conditions
  - control for starting amount (e.g., RNA)
Module overview: 2\textsuperscript{nd} half

1. Enzymatic digestions
   - A. Pepsin 4\textdegree{}C 
   - B. Papain 60\textdegree{}C
   - 0/N
   - Test for collagen proteins (by ELISA)  
   - and for proteoglycans (with dye)  

2. EDTA-citrate dissolution
   - 1. Lyse (ALT/B. Me)
   - 2. Homogenize (reduce viscosity)
   - Guinea

Purify mRNA from cells

Prepare complete cDNAs

Next time run qPCR for CN II, CN I, and 18S RNA.

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**Normal**

Ase cell technique