

- Announcements
- Quiz
- Pre-lab Lecture
 - ❖ Cell dilutions
 - ❖ RT-PCR
 - ❖ Today in Lab (Mod 3 Day 4)

Announcements

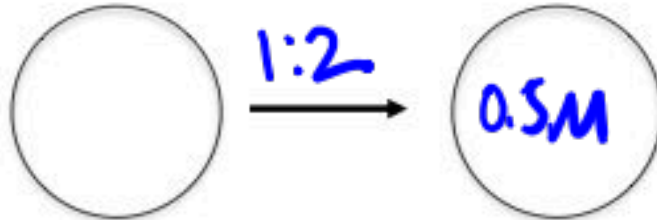
- How grades are determined in 20.109
- If you went first on D2, go second today
- During down-time
 - Prep RNA area
 - Work on Module 3 report (viability analysis)
 - Other as you see fit
- RNA work: double-check today's protocol for when to take fresh collection tube

Cell dilutions

~~1:1 = 2x dilution~~ TC convention 1:2 = 2x dilution

Case 1

Cells: $1M$
(10^6)



$$V_{\text{cells}} = 0.5 \text{ mL}$$

$$V_{\text{med}} = 2.5 \text{ mL}$$

Area = A

$V = 1 \text{ mL}$

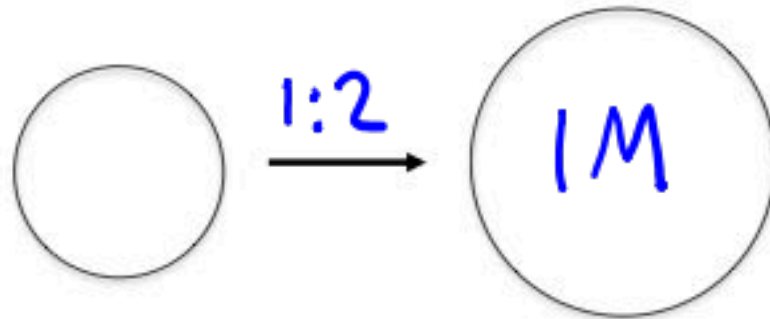


Area = A

$V = 3 \text{ mL}$

Case 2

Cells: $1M$



$$V_{\text{cells}} = 1 \text{ mL}$$

V_{med} to total

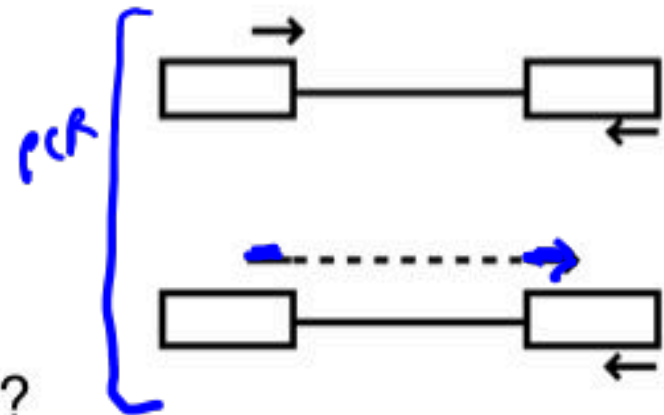
Area = A

Area = $2A$

RT-PCR recap

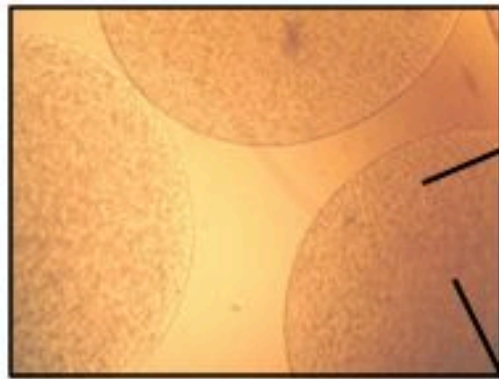
- RT = reverse transcriptase
 - what does this enzyme do?
- Unique primer design needs
 - how to isolate transcript...
 - ... but not genomic DNA?
- RT and PCR can be done in one reaction or two
- What kinds of controls are desired?

amplify housekeeping gene (GAPDH, 18S rRNA) → expect to be constant across conditions
control for starting amount (e.g., RNA)



internal co-amplified best, but complicated

Module overview: 2nd half



1. Enzymatic digestions

A pepsin 40°C
O/N

B papain 60°C
O/N

Test for collagen proteins (by ELISA) A
and for proteoglycans (with dye) B

2. RNA purification
technique

2. EDTA-citrate dissolution

① lyse (RLT/B-Me) 4 in hood

② homogenize (reduce viscosity)

Purify mRNA from cells → Prepare complete cDNAs →

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

normalization control