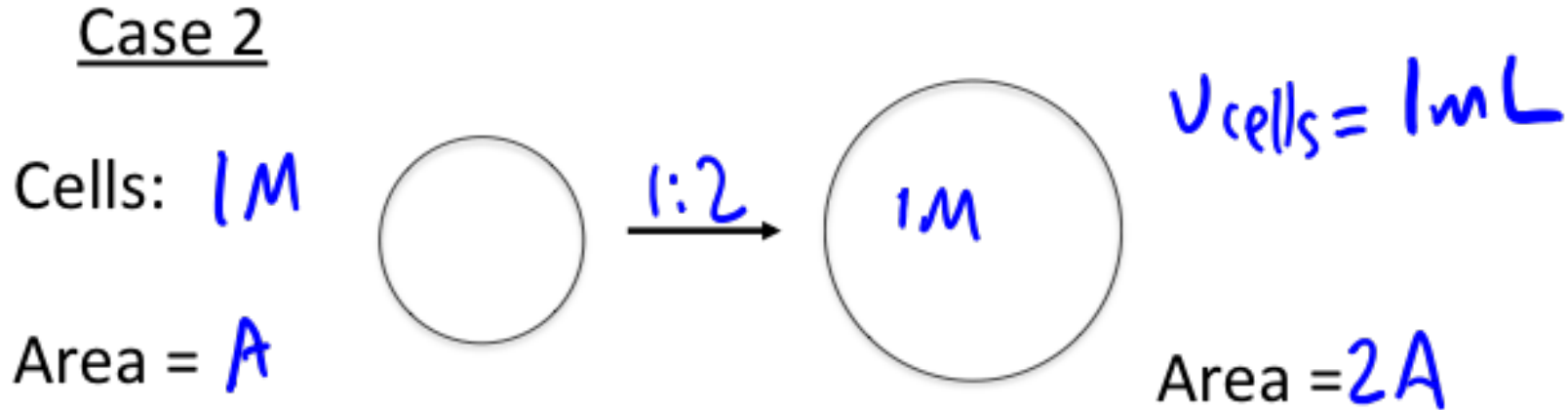
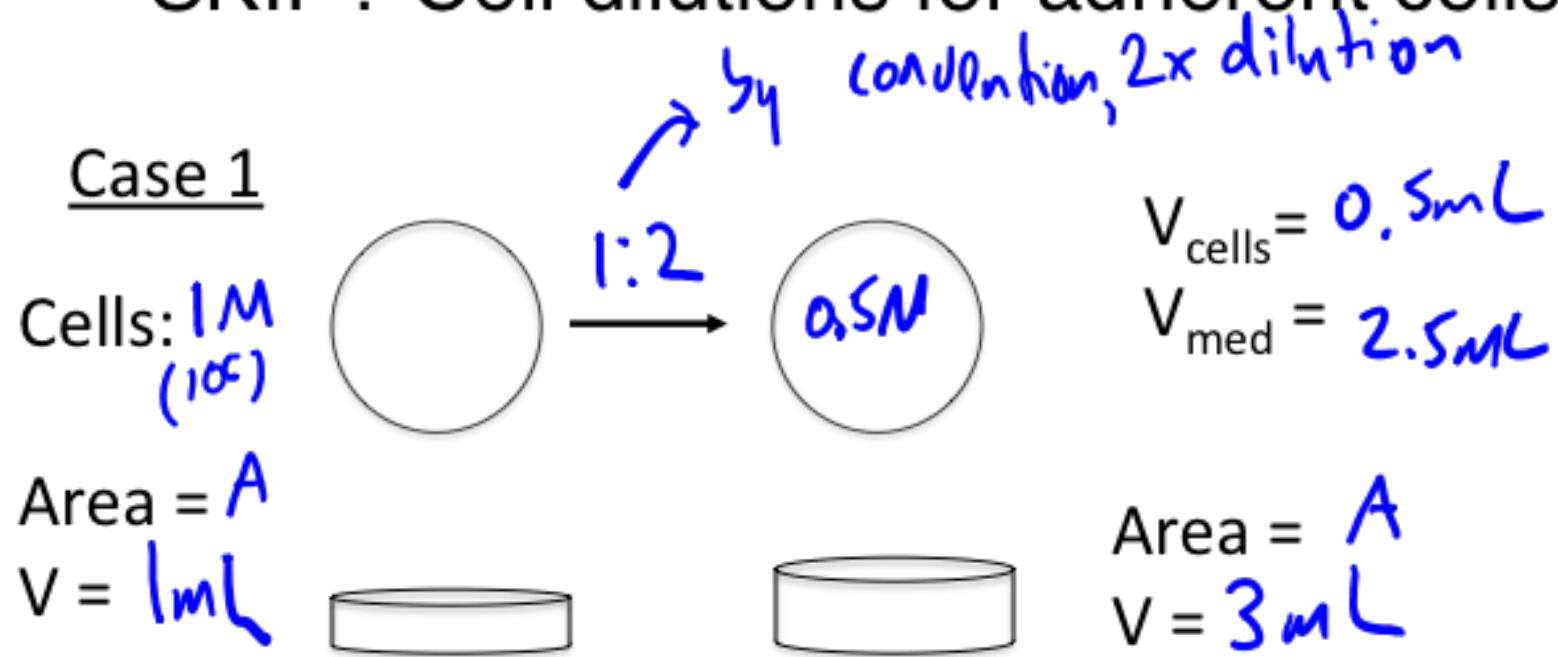


- Announcements
- Quiz (D2 and D3)
- Pre-lab Lecture
 - ❖ Cell dilutions
 - ❖ RT-PCR
 - ❖ Today in Lab (Mod 3 Day 4+)

Announcements

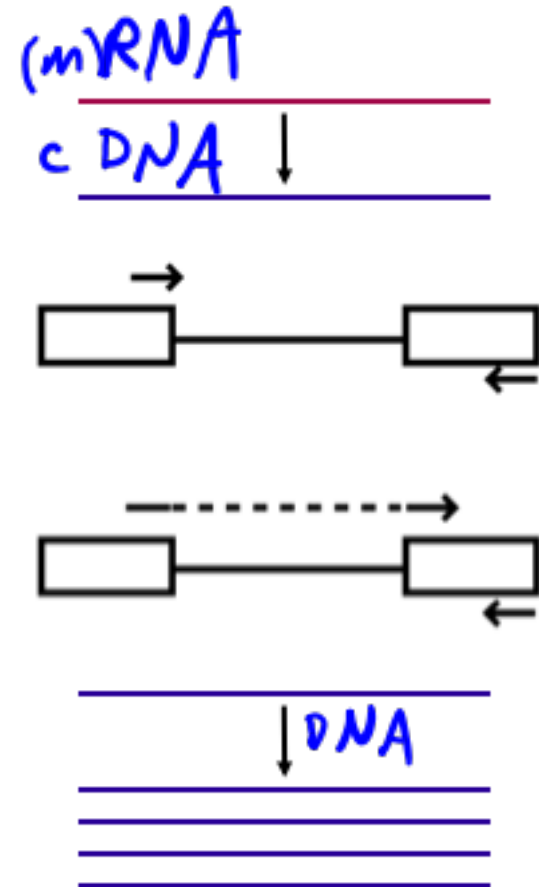
- Last notebook due today!
- Working with RNA
 - gloves on, keep area and equipment clean
 - wipe down with RNase away
 - don't toss your lysate!
 - note collection tube switches
- Before you leave today, discuss research ideas with your partner and then with Thomas
- If going to TC second, during down-time can also
 - prep RNA area
 - work on Module 3 report

SKIP? Cell dilutions for adherent cells



RT-PCR overview

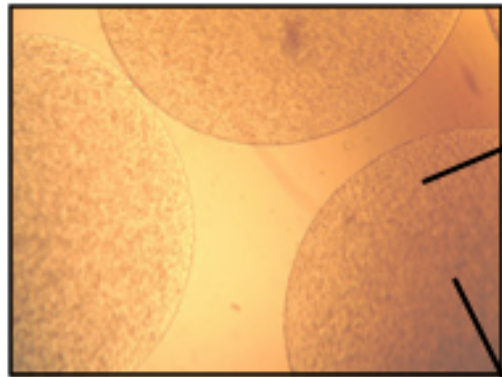
- **Goal:** determine relative gene expression levels for two culture conditions: CN II, CN I
- RT = reverse transcriptase
 - what does this enzyme do?
 - using non-specific primers – why?
- *copy entire cDNA pool at once*
- PCR: unique primer design needs
 - how to isolate transcript...
 - ... but not genomic DNA?
- What kinds of controls are desired?



RT and PCR controls

- RT step
 - no sample *contamination, nonspecific*
 - no RT *trace genomic DNA*
- PCR and analysis step
 - reference transcript: *housekeeping gene (e.g. GAPDH)*
18S rRNA
 - expected to be *constant for all conditions*
 - controls for *starting amount of RNA*
 - internal/co-amplified would be best, but complicated!

Module overview: 2nd half



1. Enzymatic digestions
pepsin @ 4°C
CN o/n →
elastase
↓
papain @ 60°C
for PA

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

2. EDTA-citrate dissolution

① lysis (RLT/β-mo) ② homogenization
↳ in hood reduce viscosity

Purify mRNA from cells → Prepare complete cDNAs →

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