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

Announcements

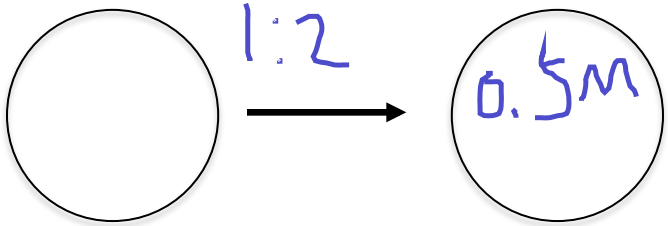
- M2 report returned today (by email)
  - 1 week to revise for up to 1 letter grade increase
  - comments from Agi: methods/results; Alan: the rest
  - review FNTs too (e.g., avoid "positive control" framing)
- If going to TC second, during down-time can
  - prep RNA area
  - work on Module 3 report (viability analysis)
  - other as you see fit
- RNA work: double-check today's protocol for when to take fresh collection tube

Cell dilutions for adherent cells

by convention, 2x dilution

Case 1

Cells: 1M (10^6)



V_{cells} = 0.5 mL

V_{med} = 2.5 mL

Area = A

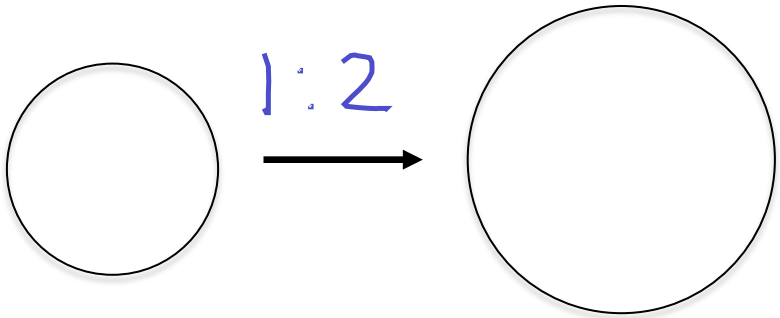
V = 1 mL

Area = A

V = 3 mL

Case 2

Cells: 1M



V_{well} = 1 mL

Area = A

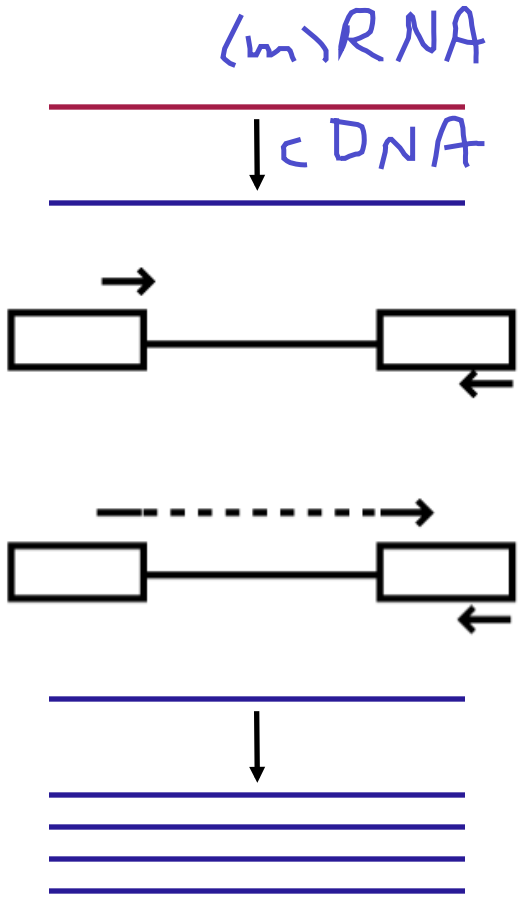
Area = 2A

RT-PCR recap and modifications

- **Goal**: determine relative gene expression levels of CN II and CN I in two culture conditions
- RT = reverse transcriptase
  - what does this enzyme do?
  - using non-specific primers – why?

copy entire cDNA pool at once

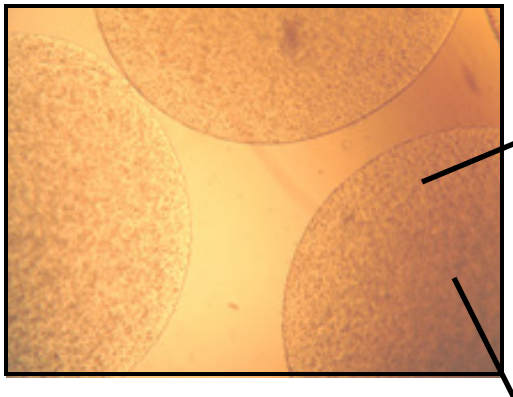
- Unique primer design needs
  - how to isolate transcript...
  - … but not genomic DNA?
- What kinds of controls are desired?



RT and PCR controls

- RT step
  - no sample non-specific contamination
  - no RT trace genomic DNA
- PCR and analysis step
  - reference transcript: housekeeping gene (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 O/N → elastase

papain @ 60°C O/N

A Test for collagen proteins (by ELISA)

B and for proteoglycans (with dye)

2. EDTA-citrate dissolution

RNase free

① lyse (RLT/β-ME) ↳ in Lood ② homogenize reduce viscosity

Purify (m)RNA from cells ⟶ Prepare complete cDNAs ⟶

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