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

Announcements

- Mod 1 report comments returned by email shortly
- No lecture T 4/29 or lab W 4/30
- But! M3D4 FNT#2 due T 4/29 during lecture hour
  - 16-220 will be open for discussion of said FNT with your partner if you wish (otherwise done in lab on M3D5)
- **Mod 3 report** due M3D7, 5 pm
  - have all D7 lab time to work in supergroups to finish
  - less formal, but clear and concise
- **Mod 3 research proposal** presentations on M3D8
- More about scope of **both** next time – rubrics on wiki

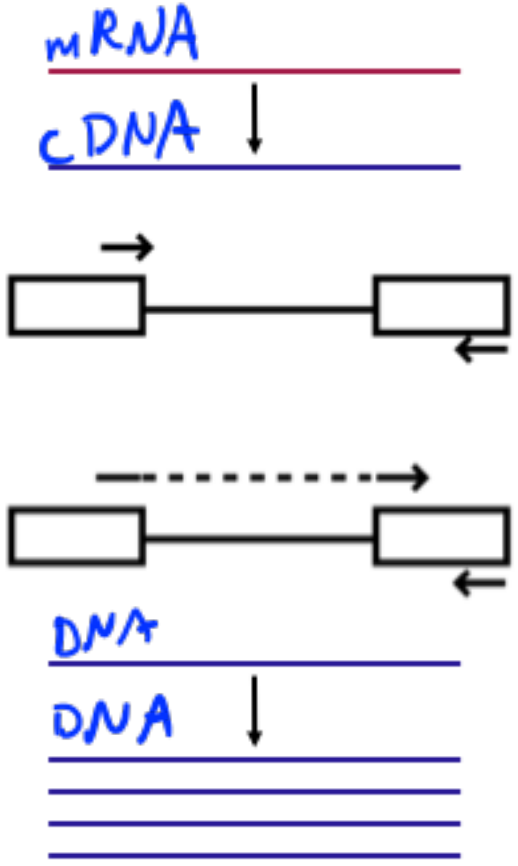
RT-PCR overview

- **Goal**: determine relative CNI and CN II gene expression levels for two culture conditions e.g., $CNI_A > CNI_B$
- RT = reverse transcriptase
  - what does this enzyme do?
  - using non-specific primers – why?

copy entire cDNA pool at once: "library"

- PCR: unique primer design needs
  - how to isolate transcript...
  - … but not genomic DNA?

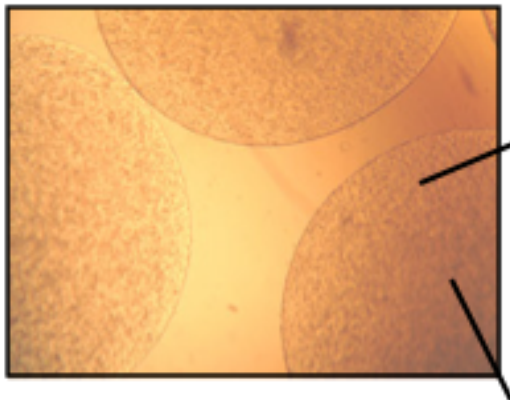
cf. M1: specific primers for CNI; CNII



RT and PCR controls

- RT step
  - no sample RNA contamination (reagents, handling)
  - no RT trace genomic DNA (or cDNA) in the sample already
- PCR and analysis step
  - reference transcript: housekeeping genes
    e.g., β-actin, GAPDH, 18S rRNA
  - expected to be constant for all conditions
  - controls for starting amount of RNA ⇒ assume RT is 100% efficient, goes to completion
  - internal/co-amplified would be best, but complicated!

Module overview: 2nd half



1. Enzymatic digestions

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

2. EDTA-citrate dissolution

↳ chelates Ca++ (our cross-linker)

Purify (m)RNA from cells → Prepare complete cDNAs — TODAY

↳ enriching for mRNA because columns >200nt

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

Today in Lab: M3D4

- If going to TC second, during down-time can
  - prep RNA area
  - work on FNT or Module 3 report
- In TC, ask for help if you have few or fragile beads
- Working with RNA
  - gloves on, keep area and equipment clean
  - wipe down equipment with RNase away
  - don't toss your lysate!
  - note collection tube switches

2nd: ROYG

~1hr in TC, ~1hr out

qPCR > ELISA (>PG)

Today in Lab: Samples