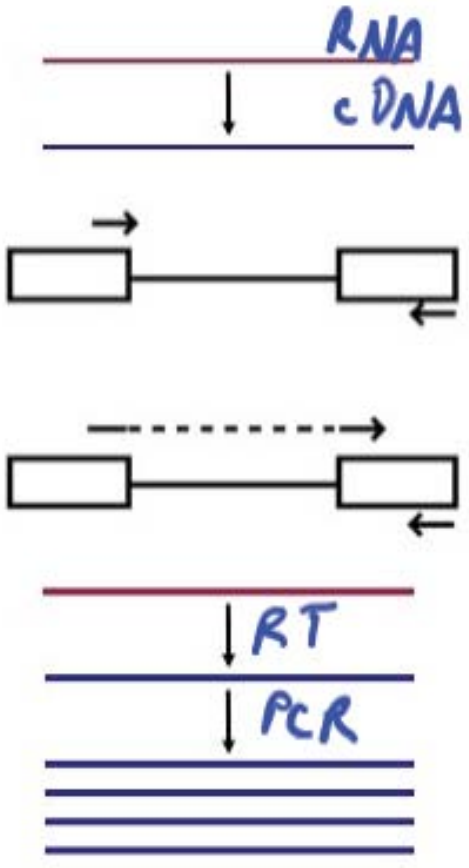
- Announcements
- Quiz
- Pre-lab Lecture
  - RT-PCR
  - Today in Lab (Mod 3 Day 4)

Announcements

- Module 2 report return
  - Revision due in 1 week
  - General comments → more during lecture 5
- During down-time today
  - Prep RNA area
  - Work on Module 3 report (viability analysis)
  - Other as you see fit

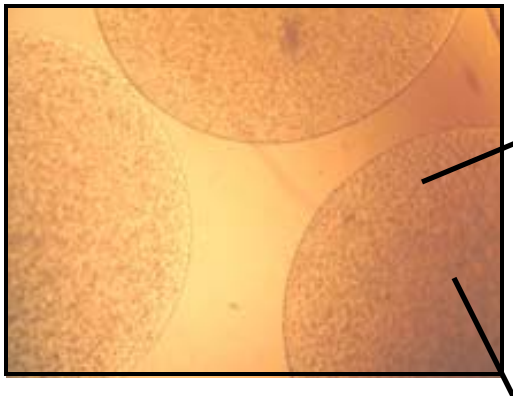
Day 4: RT-PCR recap

- 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
  - enzyme de/activation by temperature
  - hot start enzymes in general
- What kinds of controls are desired?



internal control with a co-amplified housekeeping gene → control for starting amount(s)

Module overview: 2nd half



1. Enzymatic digestion

Pepsin
4°C, O/N

Test for collagen proteins (by ELISA)

RNAse-free technique!

2. EDTA-citrate dissolution 37°C, 10'

(1) lyse (RLT+B-Me) in hood (2) homogenize (reduce viscosity)

Purify m<u>RNA</u> from cells ⟶ Amplify collagen cDNAs ⟶

Next time run a gel to compare collagen I and II transcript levels, normalized to GAPDH

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