Announcements

Quiz

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

• Grad school info session (BE Board)
  – 56-614 at 7:30 pm tonight (Tuesday)
  – With pizza

• BE seminar on adult stem cells and skeletal TE
  – 32-141 at 4 pm on Thursday
  – Dr. Rocky Truan from NIH-NIAMS (national institute of arthritis and musculoskeletal and skin diseases)

last day in TC!!
Day 4: RT-PCR

- 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 (loading)
  - no template control
Module overview: 2nd half

1. Enzymatic digestion
   - pepsin
   - 4°C for 24h
   - Test for collagen proteins (by ELISA)

2. EDTA-citrate dissolution
   - breaks Ca²⁺ X-links

Purify mRNA from cells \[ \rightarrow \] Amplify collagen cDNAs

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