Assays for transcription and protein levels

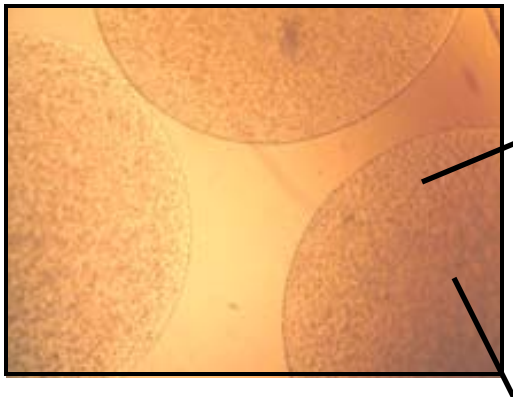
Module 3, Lecture 5

20.109 Spring 2010

Topics for Lecture 5

- Measuring protein levels
- Measuring transcript levels
- Module 2 report revision

Module overview: 2nd half



1. Enzymatic digestion

Test for collagen proteins (by ELISA)

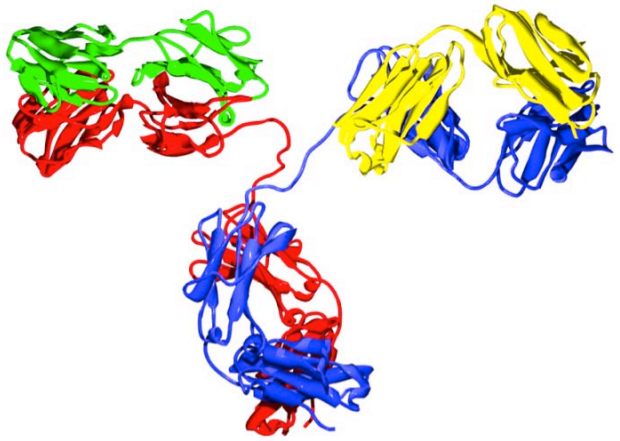
2. EDTA-citrate dissolution

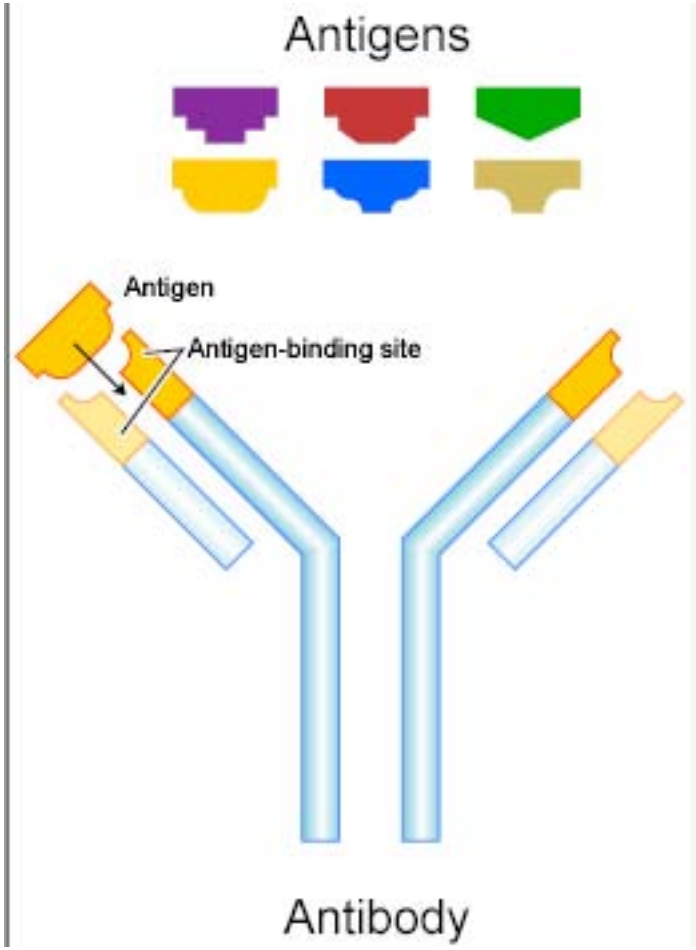
Purify mRNA from cells ⟶ Amplify collagen cDNAs ⟶

Compare collagen I and II transcript levels, normalized to GAPDH

Antibodies are specific and diverse

- Specificity
  - variable region binding, $K_D \sim$ nM
  - linear or conformational antigens
- Diversity
  - gene recombination
- Production
  - inject animal with antigen, collect blood
  - hybridomas (B cell + immortal cell)





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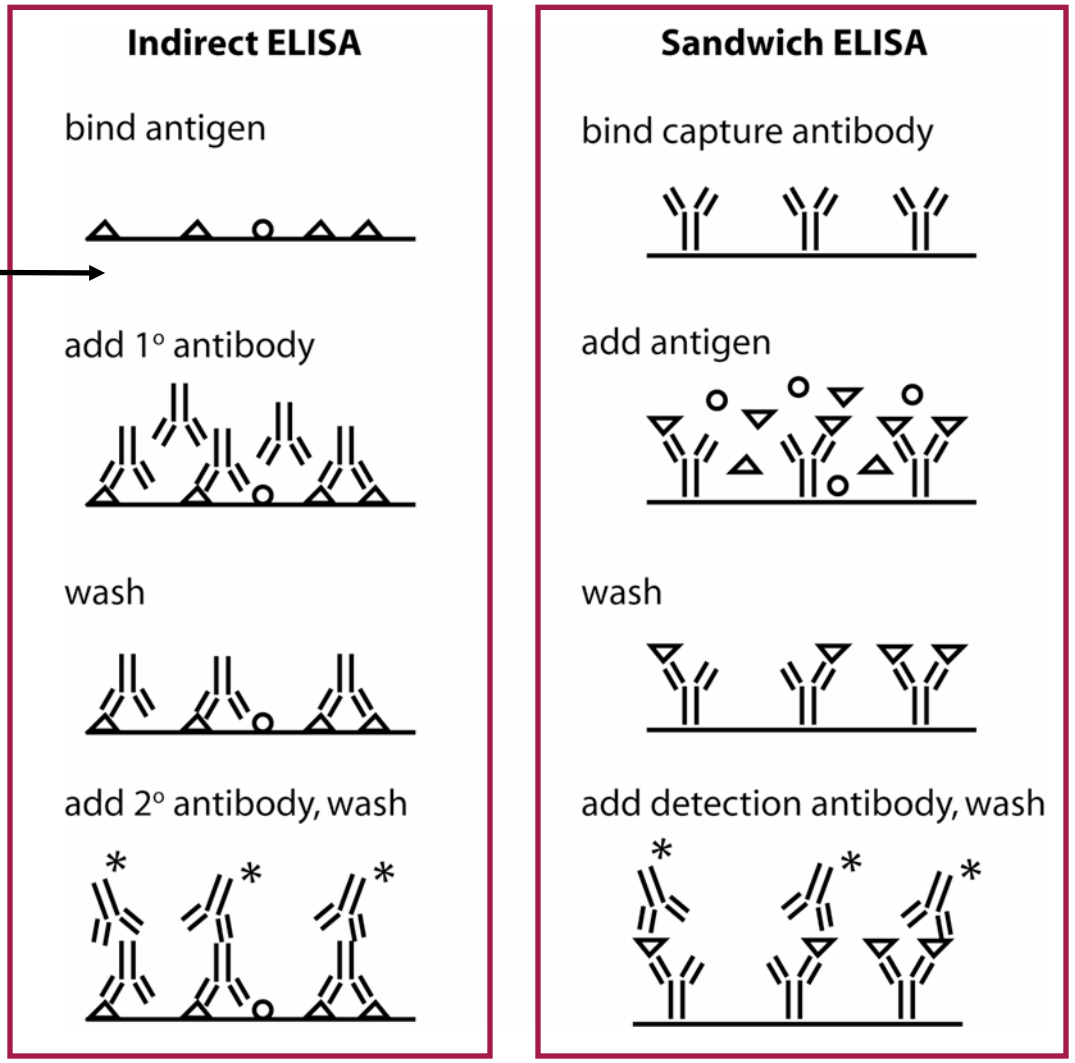
Day 5-7: protein analysis by ELISA

- ELISA: enzyme-linked immunosorbent assay
  - specific
  - sensitive
  - multiple kinds

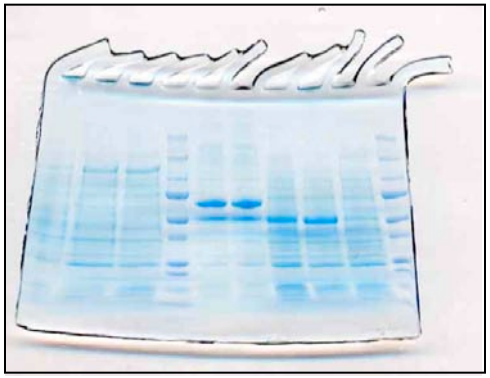
"blocking" step also needed

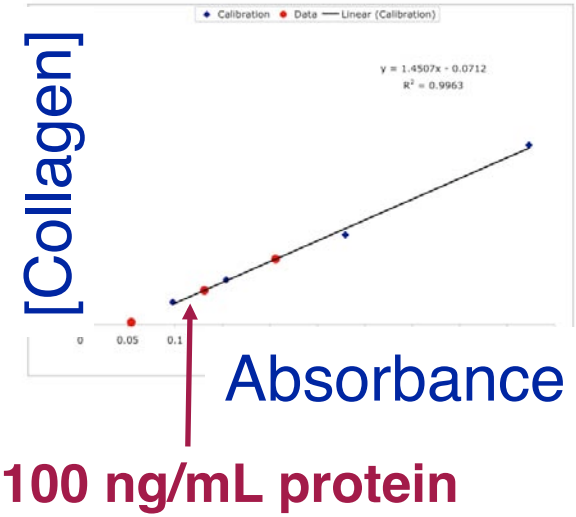
△ = protein of interest



Common protein-level assays

- PAGE
  - simple and low cost
  - Coomassie detection limit ~ 0.3-1 ug/band (2-5 ng/band for silver staining)
  - cannot distinguish two proteins of same MW
- Western blot
  - identifies specific protein
  - detection limit ~1 pg (chemiluminescent)
  - only simple for denatured proteins
- ELISA
  - detects native state proteins
  - quantitative
  - high throughput





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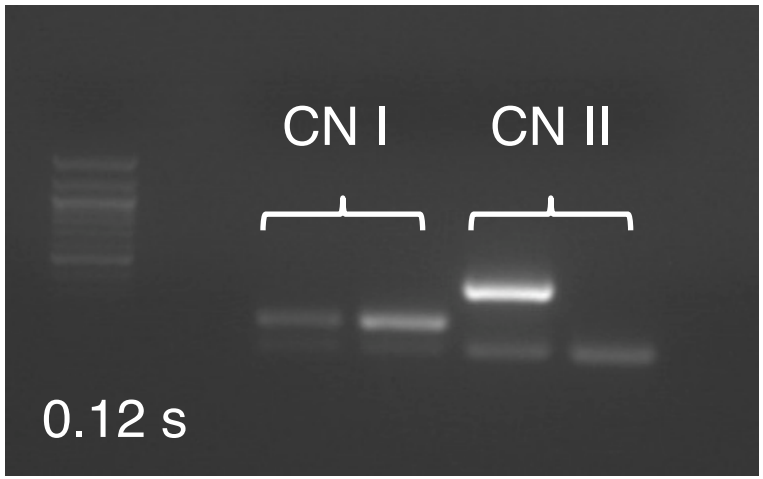
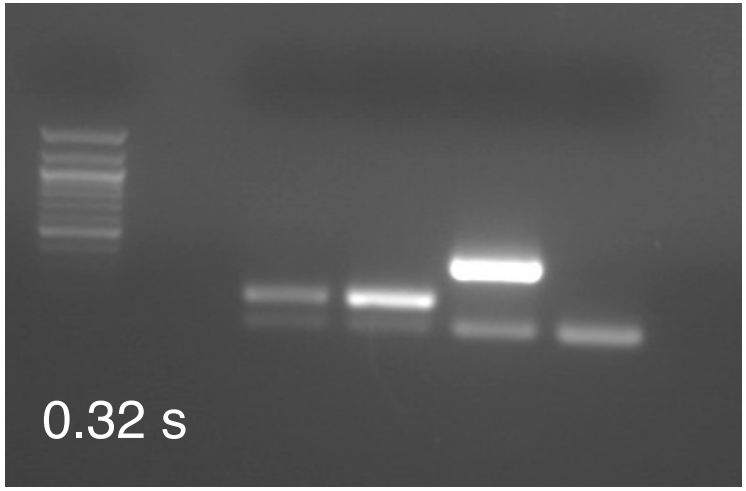
Day 4-5: transcript analysis

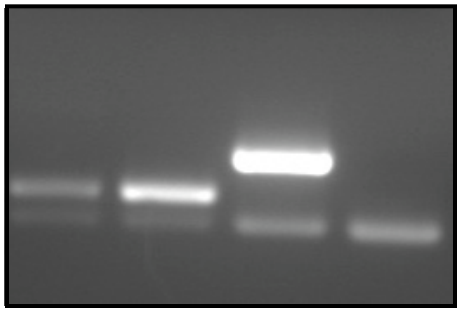
- Last time: RT-PCR
  - Collagen II + GAPDH
  - Collagen I + GAPDH
- Next: run out on a gel
- Measure band intensity/area
  - low dynamic range
  - exposure time
- Controls/references
  - GAPDH loading control
  - fresh stem cells
  - fresh chondrocytes

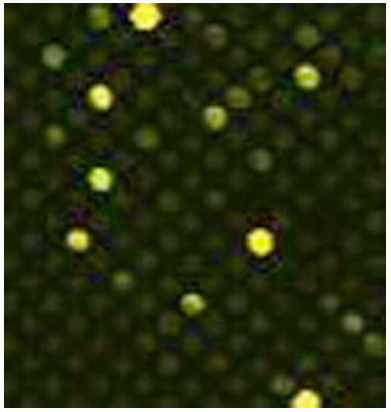




Common transcript-level assays

- RT-PCR (end-point)
  - simple, low cost
  - can be semi-quantitative
- Microarrays (end-point)
  - high cost, need specialty equipment
  - complicated and fraught analysis
  - high throughput
- q-PCR (real-time)
  - some special equipment, medium cost
  - highly quantitative
  - multiplexing potential
  - require optimization (primers)





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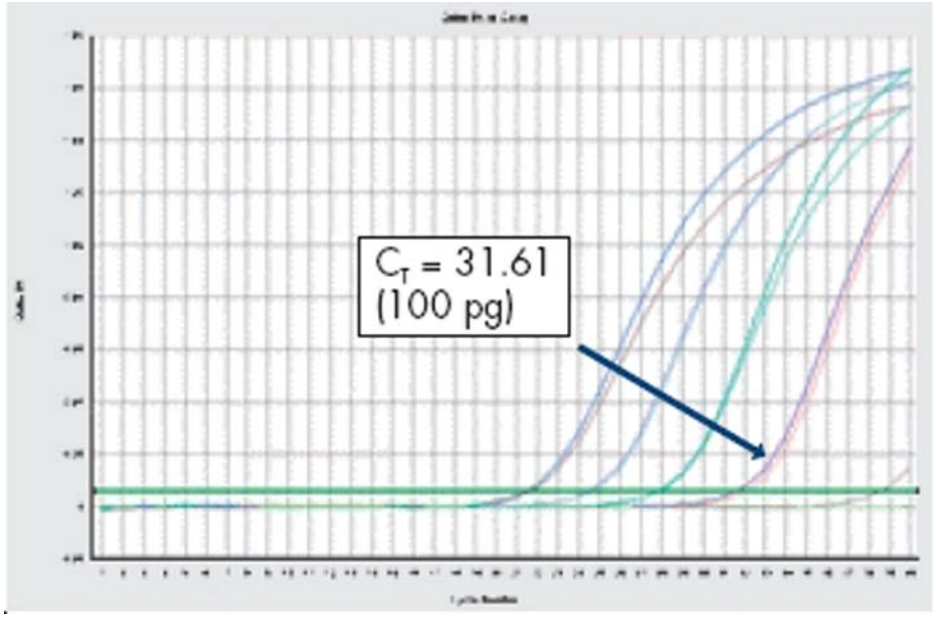
Introduction to qPCR

qiagen.com

- Real-time tracking of DNA production
- Uses probes that fluoresce
  - when bind to any DNA
  - when bind to specific DNA (FRET)
- Why does PCR plateau?
- Several analysis methods
  - threshold cycle C_T
  - relative standard curve: fold-change of a transcript (normalized)
  - efficiency-correction: compare genes
  - absolute levels by radiolabeling



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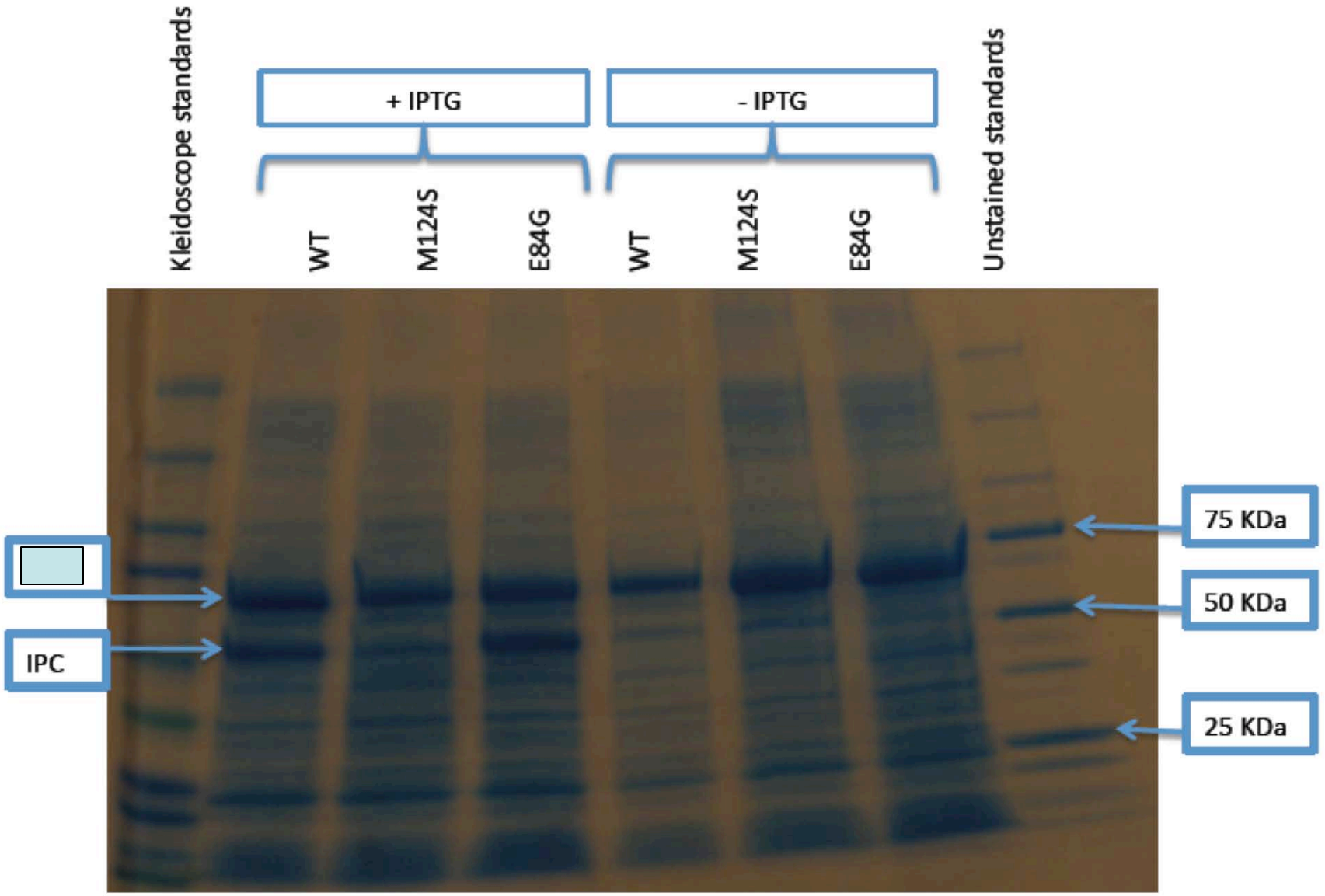
Module 2 revision: small but important points

- Words have precise meanings
  - e.g., “significantly”
- Numbers imply a claim
  - excess digits often reported
- In results, be descriptive, not jargony or methods-oriented
  - e.g., “lysis solution” vs. “BPER”
  - e.g. “aligned sequence with WT” vs. “used BLAST”
  - e.g., explain “diagnostic digest gel”
- Avoid wiki language:
  - 1) it’s plagiarism, and 2) it has a different purpose/audience than your report (most egregious e.g., “protein behavior assay”)
- Italicize enzyme names (e.g., *AccI*)

Module 2 revisions: writing and analytical examples

- Data analysis
  - Subtleties in SDS-PAGE data
- Read excerpts demonstrating
  - Appropriate abstract content
  - Sufficient narrative in a results section
  - Concise but thorough analysis
  - Effective opening for discussion section


Kleidoscope standards
+ IPTG
- IPTG
WT
M124S
E84G
WT
M124S
E84G
Unstained standards
IPC
75 KDa
50 KDa
25 KDa

Lecture 5: conclusions

- Antibodies to diverse targets (e.g., proteins) can be made and used for detection/measurement.

- Trade-offs exist (e.g., between simplicity and accuracy) for different transcript-level assays.

Next time: cartilage TE, from *in vitro* and *in vivo* models to the clinic; imaging.

20.109 Laboratory Fundamentals in Biological Engineering

Spring 2010