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, $K_D \approx \text{nM}$
  – linear or conformational antigens

• Diversity
  – gene recombination

• Production
  – inject animal with antigen, collect blood
  – hybridomas (B cell + immortal cell)
Day 5-7: protein analysis by ELISA

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

"blocking" step also needed

△ = protein of interest

**Indirect ELISA**
- bind antigen
- add 1° antibody
- wash
- add 2° antibody, wash

**Sandwich ELISA**
- bind capture antibody
- add antigen
- wash
- add detection antibody, wash
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

*Current Protocols in Cell Biology, Molecular Biology*
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)
Introduction to qPCR

• 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

Current Protocols in Cell Biology, Molecular Biology
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
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.