Cartilage TE: from *in vitro* and *in vivo* models to the clinic

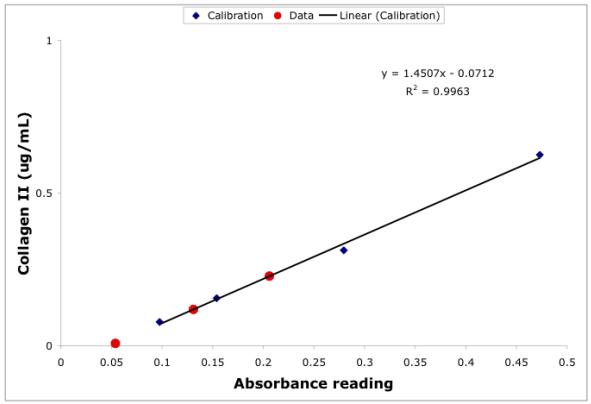
Module 3, Lecture 6

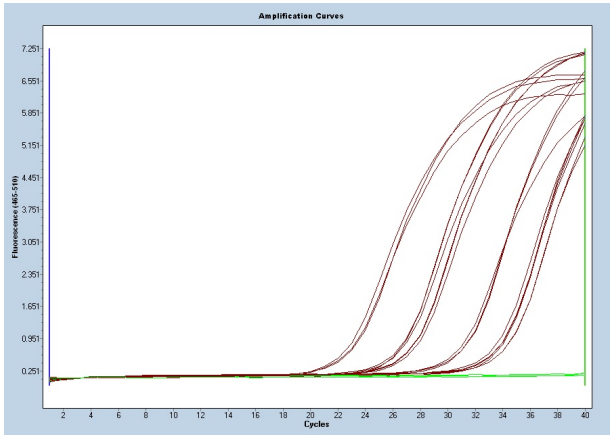
20.109 Spring 2012

Lecture 5 review

- What are some advantages of ELISA as a protein assay?

- Compare qPCR and end-point RT-PCR as gene expression assays.



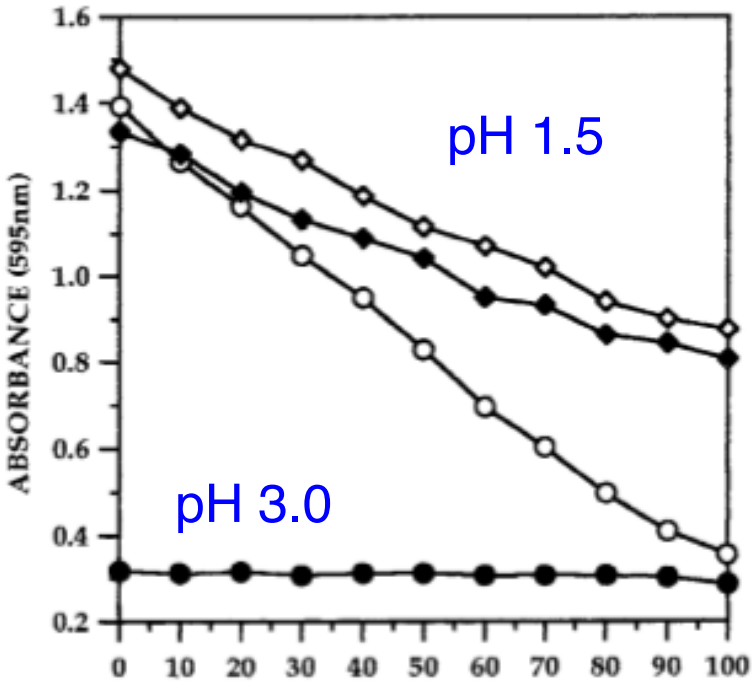


Topics for Lecture 6

- Proteoglycan assay
- qPCR analysis
- Cartilage TE *in vitro*
- Cartilage TE *in vivo*
- Cartilage TE in the clinic

Proteoglycan content assay

- DMMB cationic dye binds (-) groups on PGs
- GAG sulfates detected at pH 1.5-3.0
- Alginate carboxyls detected at pH 2.0-3.0
- Complex causes short-lived A_{595} peak reduction
- Figure shows PG standard in presence of alginate

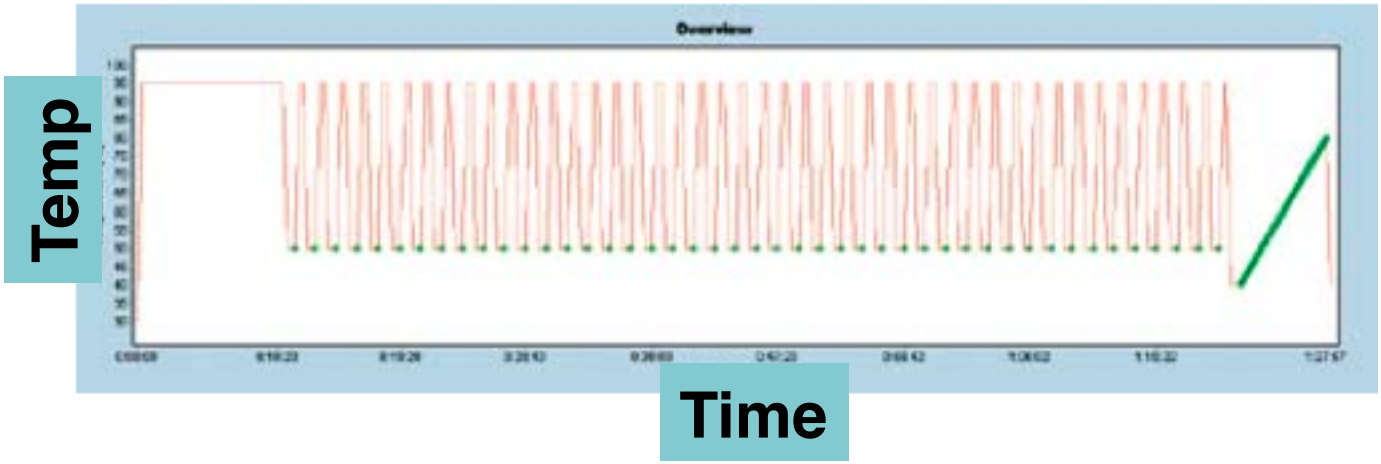


Enobakhare, et al., *Anal Biochem* **243**:189 (1996)

qPCR cycling

- Melt DNA, activate hot start enzyme, 10 min at 95 °C
- 40 PCR cycles: melt (15 sec at 95 °C); anneal/extend
- Anneal/extend <=1 min at 60 °C
  - 2-step cycling often sufficient (short products)
  - take single fluorescence snapshot at end
- Melting curve
  - slowly heat to 95 from 60 °C
  - continuously measure fluorescence

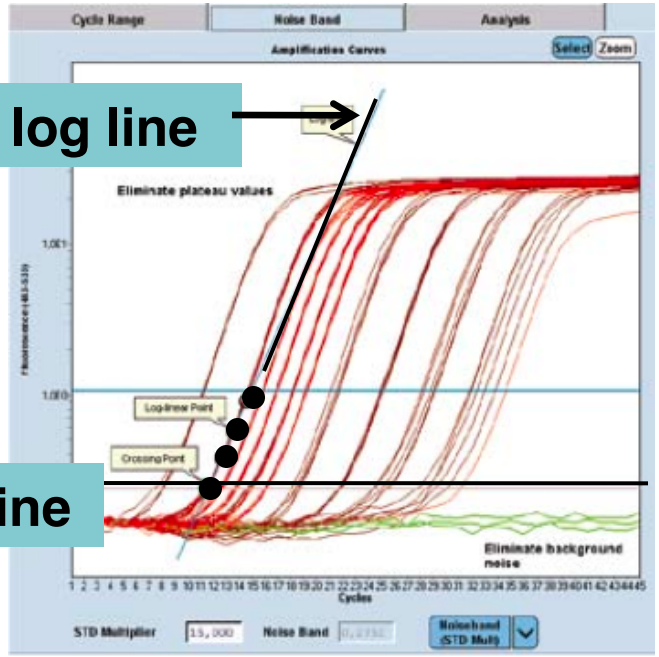
Image from Roche manual



qPCR threshold cycle C_T

- Initial cycles used to set baseline
- C_T = intensity > > background

- Two main ways to calculate C_T
- 2nd derivative maximum
  - each C_T identified by largest Δ slope
- Fit points
  - all C_Ts identified by same threshold
  - linear regression in log phase
  - recommended for our analysis type

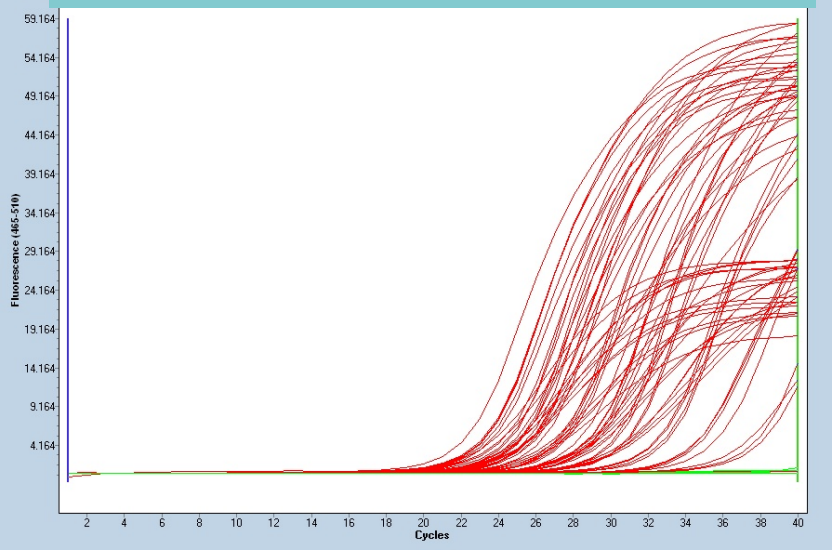


From Roche manual

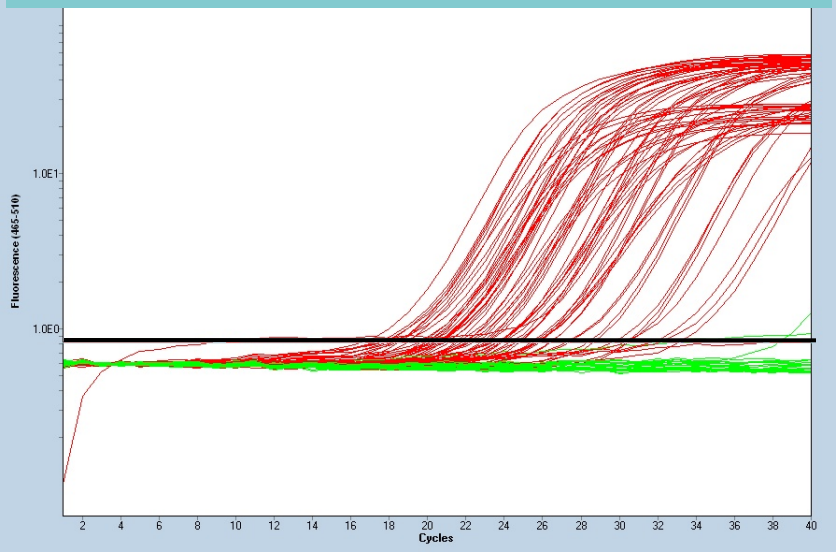
Roche, LightCycler 480 Operator's Manual, software version 1.5

qPCR amplification data

Raw fluorescence vs cycle #



Log scale with noise threshold



(S11, T/R, all)

qPCR relative expression analysis

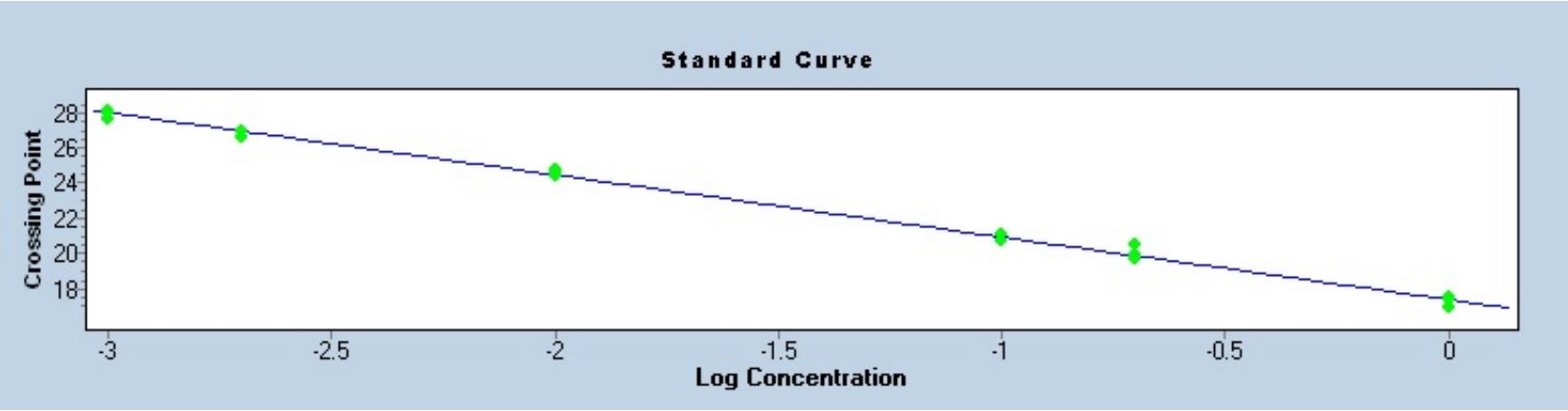
- Relative gene expression analysis
  - control for cDNA amount with reference (e.g., 18S rRNA)
  - expression change relative to a control (e.g., fresh cells)
- E is amplification efficiency for that primer set
- If E = 2, two cycle difference = 4-fold change

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta \text{CP}_{\text{target}}(control - sample)}}{(E_{\text{ref}})^{\Delta \text{CP}_{\text{ref}}(control - sample)}}$$

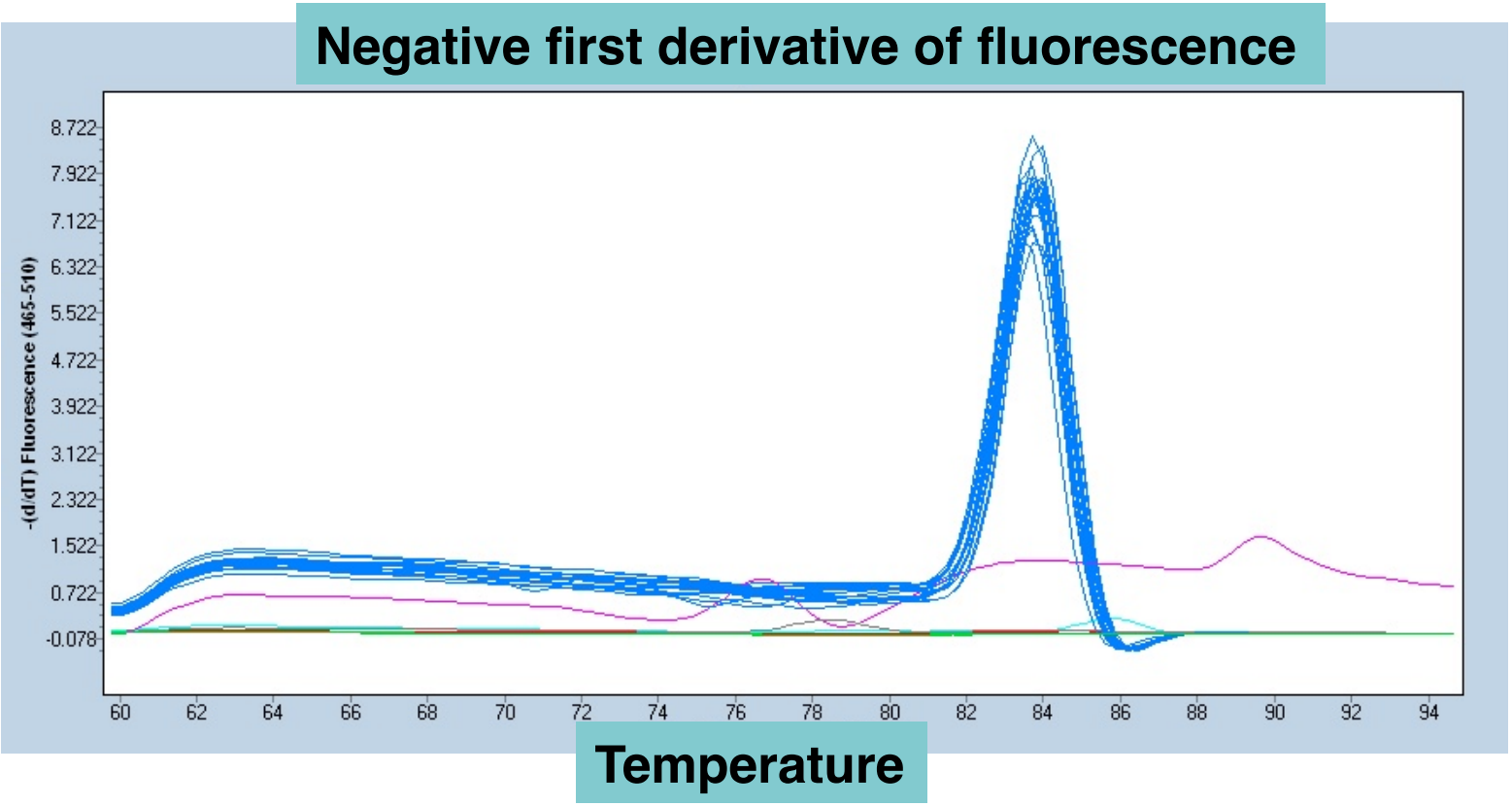
Equation 1 from M.W. Pfaffl, *Nucleic Acids Res* **29**:2002 (2001)

qPCR primer set standard curves

- Slope indicates primer amplification efficiency
  - E = 10^(-1/slope)
  - E = 2 for slope = -3.3
- Measure samples over 3-5 logs, in triplicate

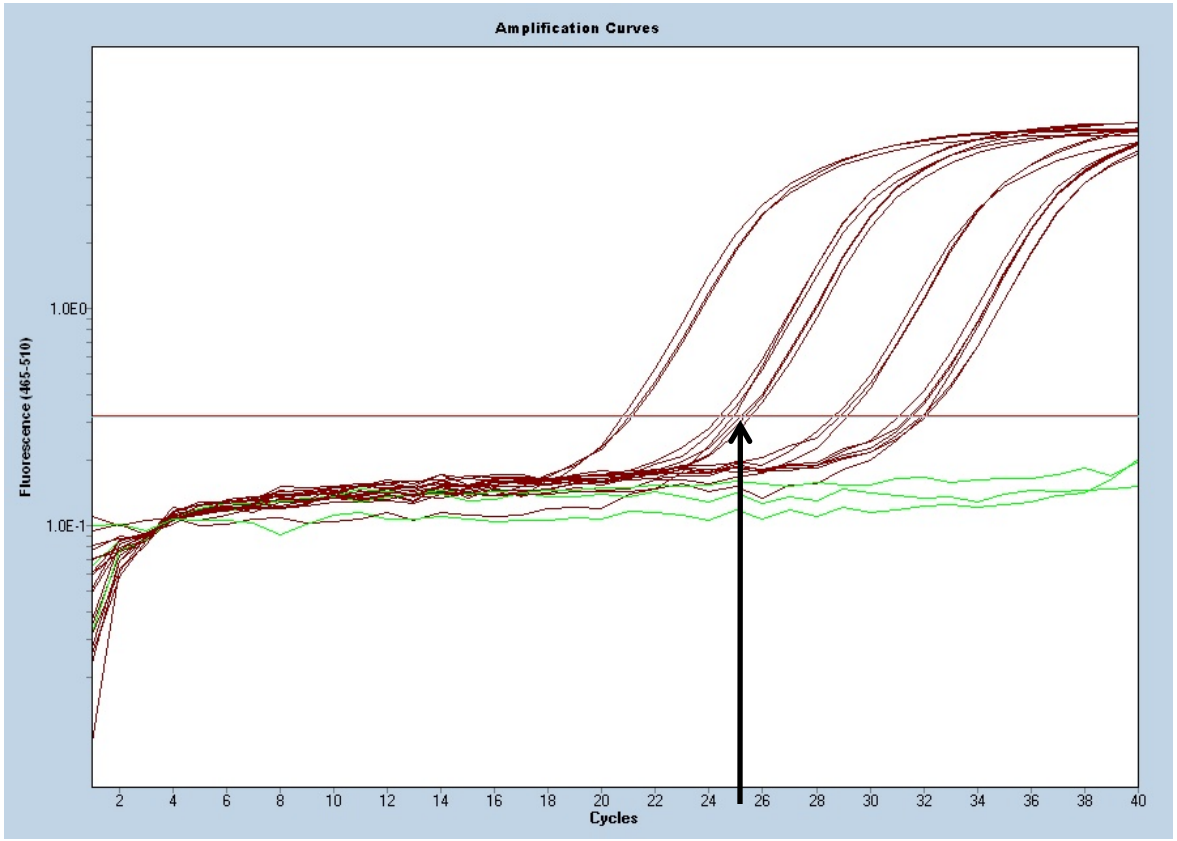


Melting curve analysis



(S12, W/F, CN II)

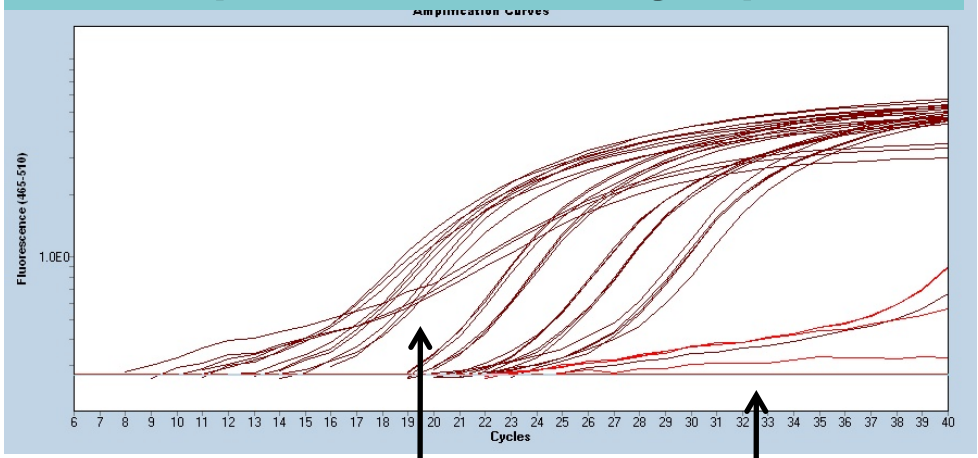
Detection limit for change



2-fold change detectable but C_T error/scatter may overlap

Optimizing primer concentration

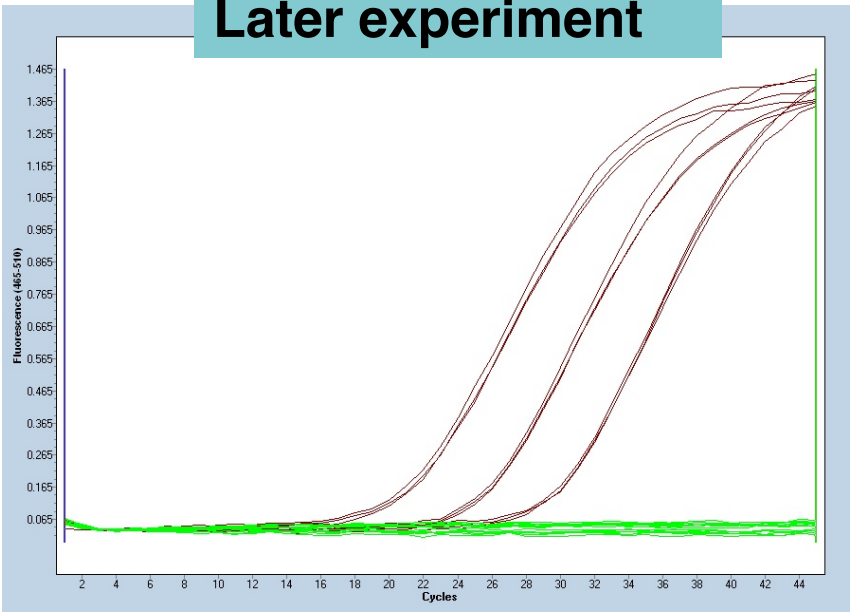
First experiment – too high [primer]



High [cDNA] sample oddly shaped

No-template controls give primer-dimer product

Later experiment



Great replicate agreement and flat controls (green)

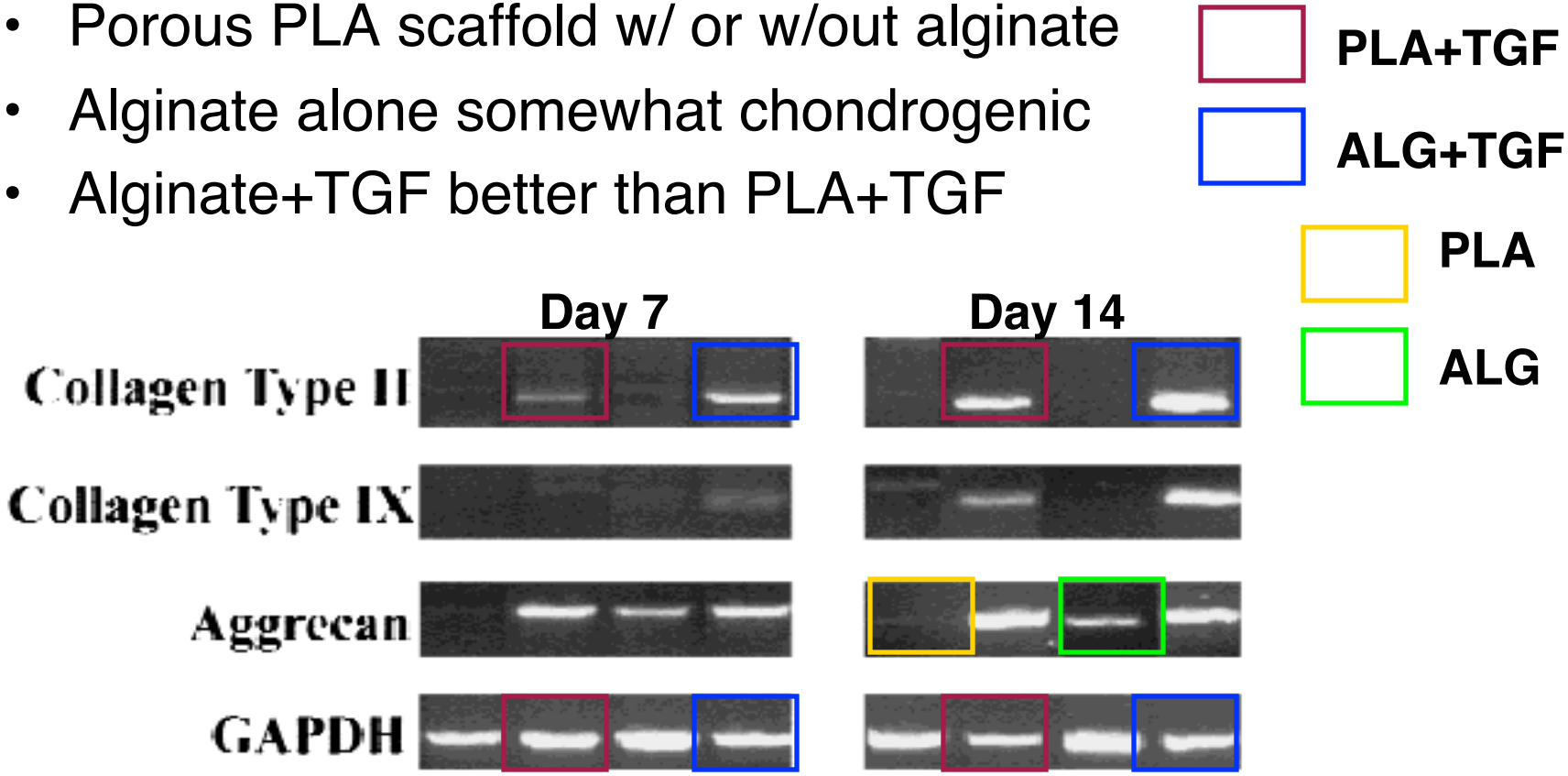
Interlude

Lecture 8: your choice of TE topics (list on board)

Which one is cuter? Tree kangaroo or human baby?

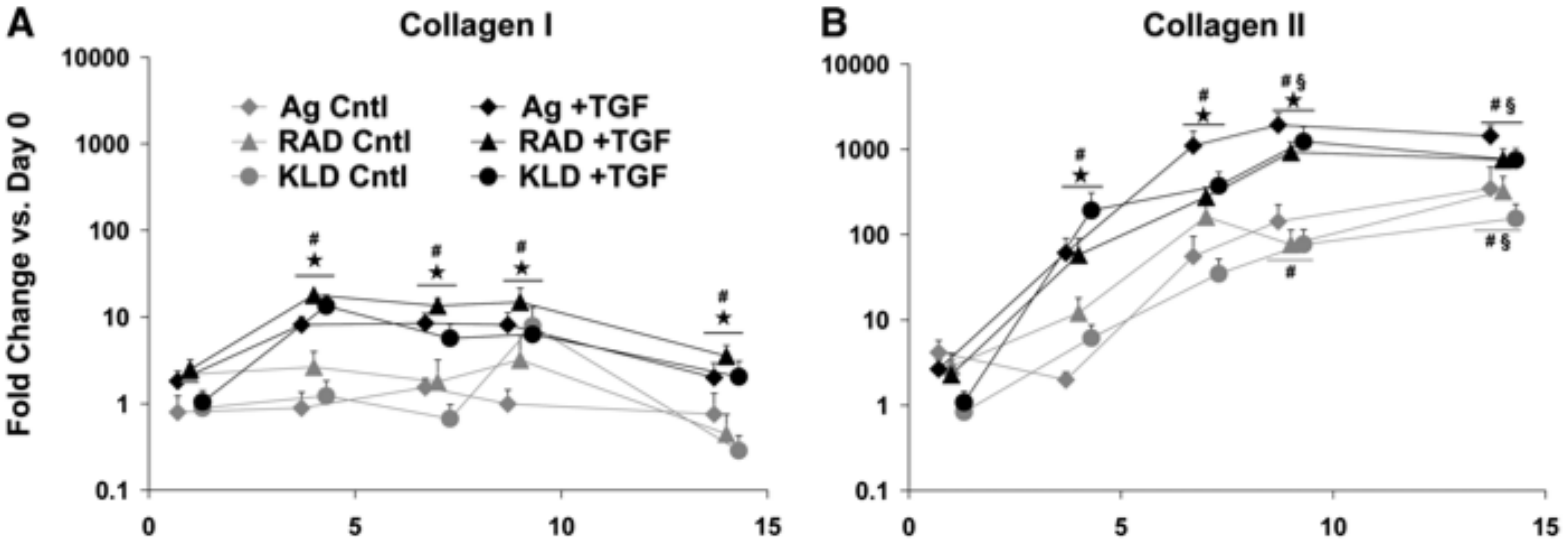
Chondrogenesis *in vitro*

- Porous PLA scaffold w/ or w/out alginate
- Alginate alone somewhat chondrogenic
- Alginate+TGF better than PLA+TGF



Caterson et al., *J Biomed Mater Res* **57**:394 (2001)

Recent Grodzinsky lab work shows merits of peptide gels



CN II expression of stem cells increases in agarose, RAD, and KLD gels over 5-10 days. Peptide gels have better proliferation, PG length.

Kopseky et al., *Tissue Eng A* **16**:465 (2010)

Scaffold-free *in vitro* cartilage TE

- Method: rotational culture of rabbit chondrocytes with no cytokines
- Results
  - mostly dynamic culture gave best results: low apoptosis, very rigid disc
  - fresh ECM made: primarily CN II and PG
  - organized architecture, similar to *in vivo*
- A scaffold-free method is inherently biocompatible
  - Any disadvantages?
  - Pros/cons of *cell*-free methods?

T. Nagai et al., *Tissue Eng* **14** (2008)

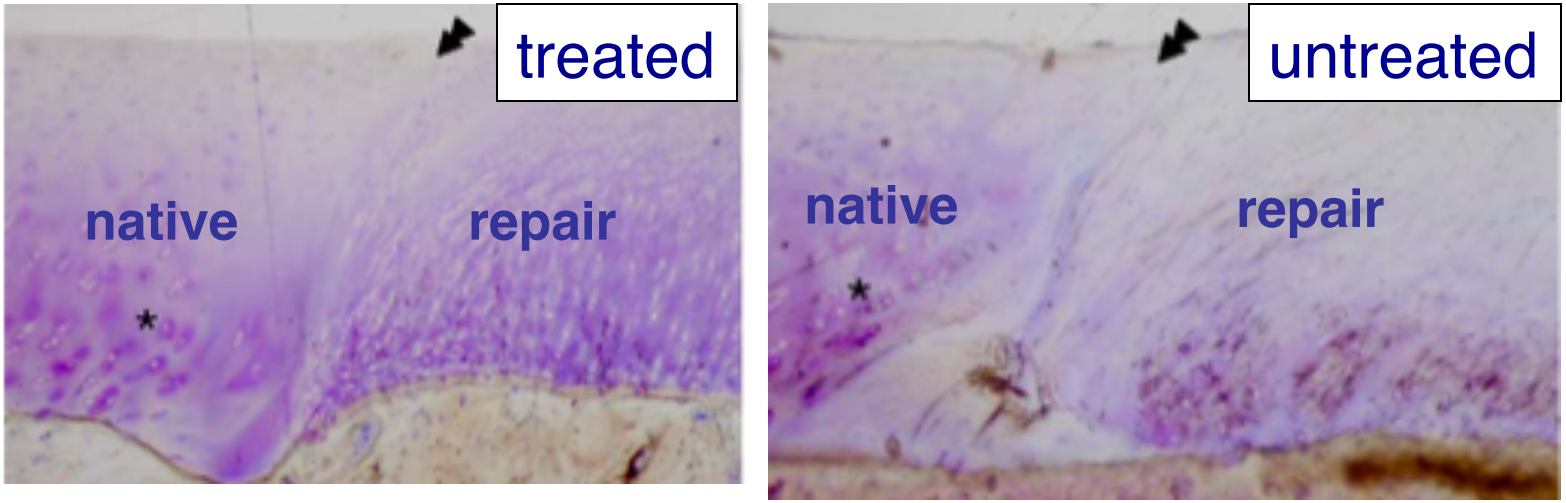
Static

Dynamic, 3 d

Dynamic, 3 w

Large animal *in vivo* model

- D. Barnewitz et al. *Biomaterials* **27**:2882 (2006)
- Biodegradable scaffold with autologous cells
- Examined horses and dissected joints after 6-12 months
- Matrix synthesis, implant integration with native tissue
- Why use a large animal model (vs. small)?



Advantages of working *in vivo*

- Ability to mimic human disease-state
- Ability to mimic therapy/surgery applied to humans
  - especially true for large animal models
- Can compare results to “gold standard” treatment
- The construct interfaces with an actual wound, the immune system, etc. - more realistic environment
- Toxicity studies more meaningful

Cartilage pathology

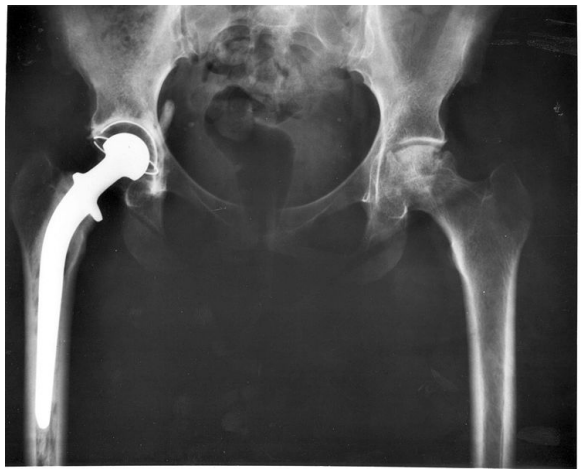
- Cartilage has little regeneration capacity – why?
- Early damage can promote later disease
- Osteoarthritis pathology
  - PG and collagen loss, PG size ↓
  - ↑ water content, ↓ strength
  - chondrocyte death
- Symptoms
  - loss of mobility
  - pain

Image from OPML at MIT: *http://web.mit.edu/cortiz/www/AFMGallery/AFMGallery.html*.

V.C. Mow, A. Ratcliffe, and S.LY. Woo, eds. *Biomechanics of Diarthrodial Joints* (Vol. I) Springer-Verlag New York Inc. 1990

Treatments for cartilage damage

- Strategy 1: enhance/provoke healing
  - biologics: hyaluronic acid, TGF-β, etc.
  - damage bone (stem cell effect)
- Strategy 2: replace tissue
  - joint replacement
    - synthetic or donated tissue
    - invasive or fiber-optic (partial)
  - cell and/or scaffold implantation
    - immature therapy
- Other/supplemental
  - mechanical, electrical stimulation
  - debridement (rid debris)



Public domain image
(Wikimedia commons)

S.W. O'Driscoll. *J Bone Joint Surg* **80**:1795 (1998)
S. Poitras, et al. *Arth Res Ther* **9**:R126 (2007)
C.M. Revell & K. A. Athanasiou. *Tissue Eng Pt B-Rev* **15**:1 (2009)

Cutting edge of treatment

- Cell-based therapies on the market (e.g., Carticel)
- Scaffold-based approaches in trials (e.g., NeoCart, INSTRUCT)

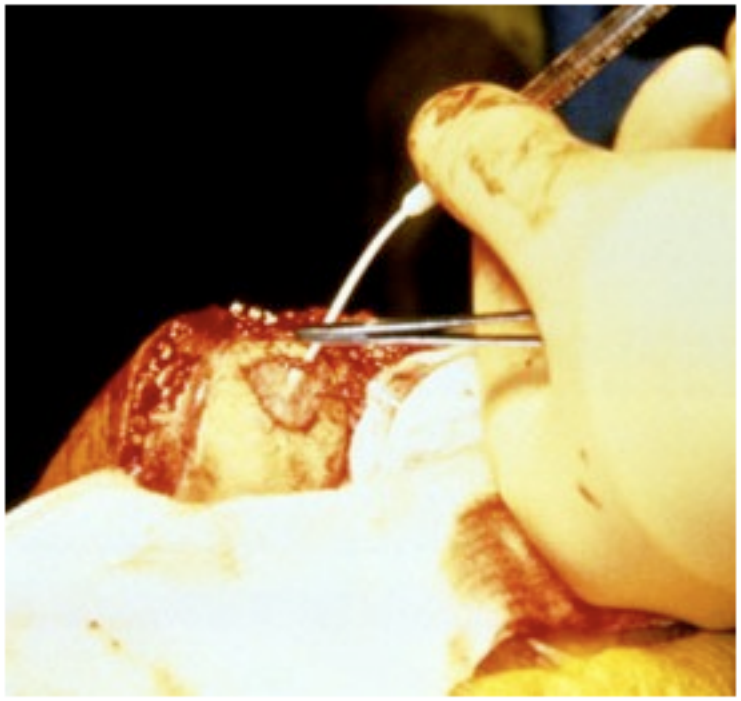


Figure 21: **Injecting Carticel under periosteal patch**

2. Tissue Production
Cells grow on a patented 3D matrix in a tissue engineering processor under conditions that simulate those in the body. >

3. NeoCart Implant
NeoCart has the characteristics of native articular cartilage.
<

Many clinical trials are ongoing

Found 246 studies with search of: cartilage

Hide studies that are not seeking new volunteers.
Hide studies with unknown recruitment status.

Rank	Status	Study
1	Recruiting	Knee Articular Cartilage Repair: Cartilage Autograft Implantation System Versus Conventional Microfracture Conditions: Other Articular Cartilage Disorders; Osteochondritis Dissecans Interventions: Procedure: Microfracture; Device: Cartilage Autograft Implantation System (CAIS)
8	Completed	Study to Compare the Efficacy and Safety of Cartistem® and Microfracture in Patients With Knee Articular Cartilage Inju Conditions: Cartilage Injury; Osteoarthritis Interventions: Biological: Cartistem; Procedure: Microfracture treatment
9	Recruiting	Chondrofix Osteochondral Allograft Prospective Study Conditions: Articular Cartilage Disorder; Degeneration; Articular Cartilage; Chronic Cartilage Injury; Acute Cartilage Injury; Defect of Articular Cartilage Intervention: Procedure: Chondrofix Osteochondral Allograft
10	Recruiting	Neocartilage Implant Phase III Trial Conditions: Articular Cartilage Disorder; Degeneration; Articular Cartilage; Chronic Cartilage Injury; Acute Cartilage Injury; Defect of Articular Cartilage Interventions: Biological: Neocartilage Implant/DeNovo® ET (Engineered Tissue Graft); Other: Microfracture
18	Recruiting	Assessment of Efficacy and Safety of 3 Different Doses of co.Don Chondrosphere to Treat Large Cartilage D Conditions: Large Articular Cartilage Lesions of the Femoral; Condyle, Trochlea, Tibia or Retropa Intervention: Drug: co.don chondrosphere®
19	Recruiting	Transplantation of Bone Marrow Stem Cells Stimulated by Proteins Scaffold to Heal Defects Articular Cartil Conditions: Osteoarthritis; Knee Arthrosis; Osteochondral Defects; Osteochondritis Dissec Intervention: Procedure: Transplantation of Bone Marrow Stem Cells Activated in Knee Arthrosis

← scaffold + own tissue

← cord blood stem cells + gel

← autologous chondrocytes, expanded

← autologous, fresh stem cells + CN scaffold

Screenshot from www.clinicaltrials.gov, May 2012

Lecture 6: conclusions

- Both *in vitro* and *in vivo* models of cartilage repair can reveal valuable insights, but have different strengths.

- Cell-based therapies have come to market for cartilage TE, and scaffold-based therapies are on the horizen.

Next time: Atissa on presenting with a partner; research proposal open discussion.

Lecture 8: special topics in TE.