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

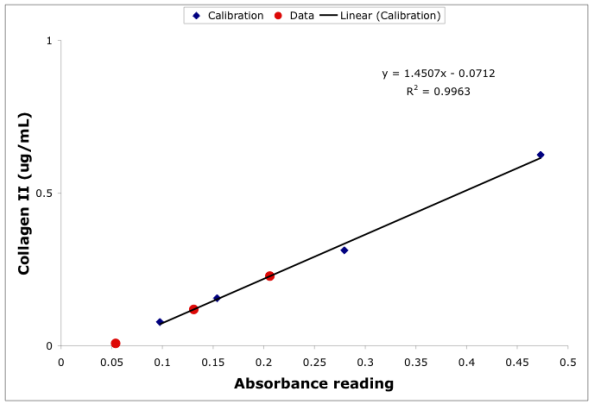
Module 3, Lecture 6

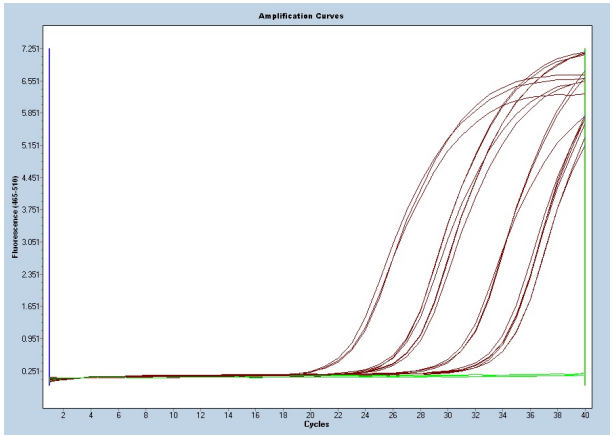
20.109 Spring 2014

Lecture 5 review

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

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



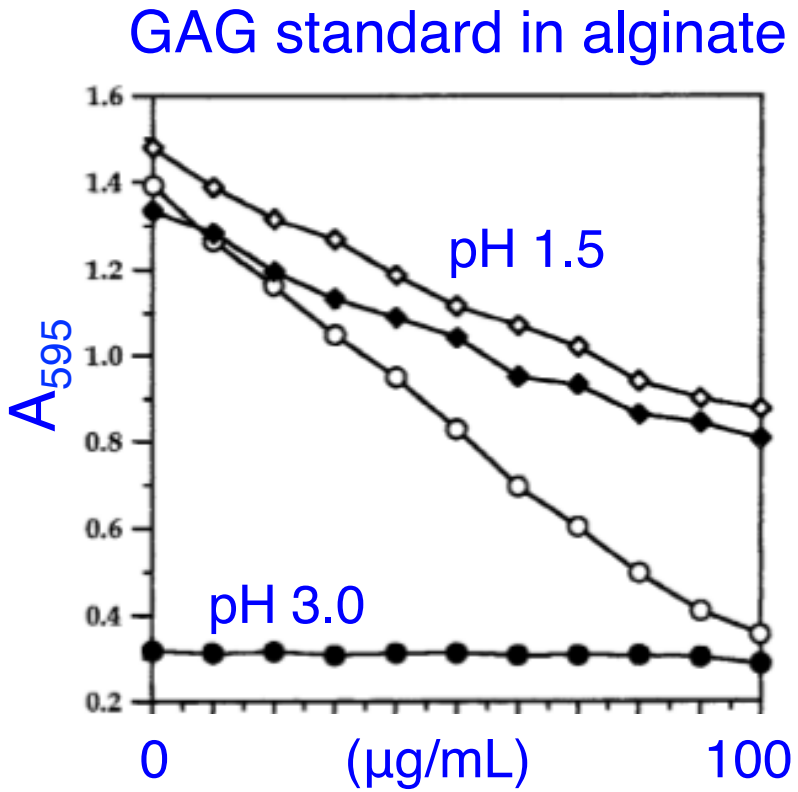


Topics for Lecture 6

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

Measuring proteoglycan content

- DMMB cationic dye binds (-) groups on PGs
- Causes A_{595} peak reduction
- GAG sulfate detection: pH 1.5-3.0
- Alginate carboxyl detection: pH 2.0-3.0
- Low pH to prefer sulfates



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

qPCR cycling parameters

- 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)
  - *single* fluorescence snapshot end of each min
- Melting curve
  - slowly heat to 95 from 60 °C
  - *continuously* measure fluorescence

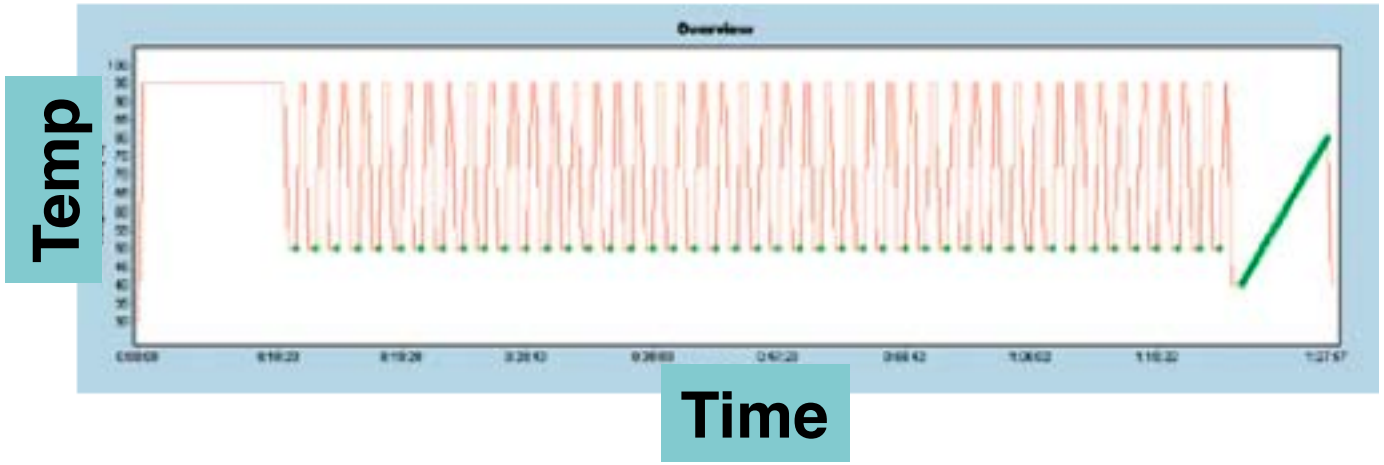
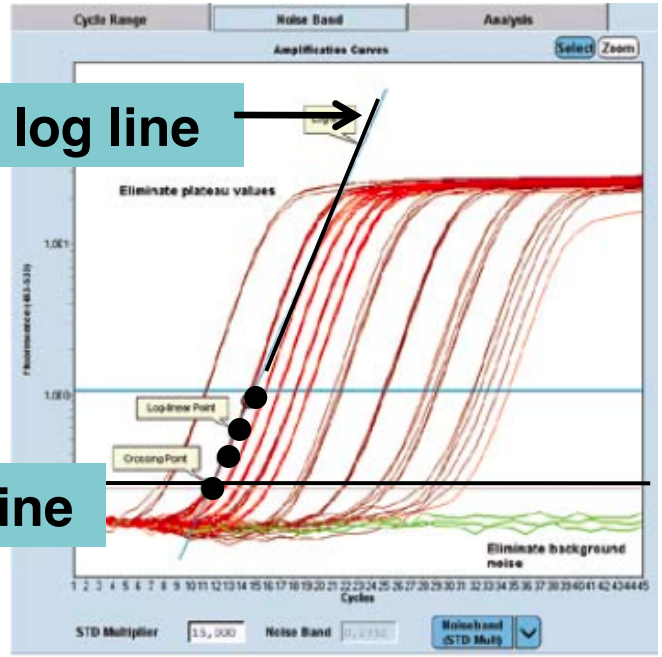


Image from Roche manual

qPCR threshold cycle C_T

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

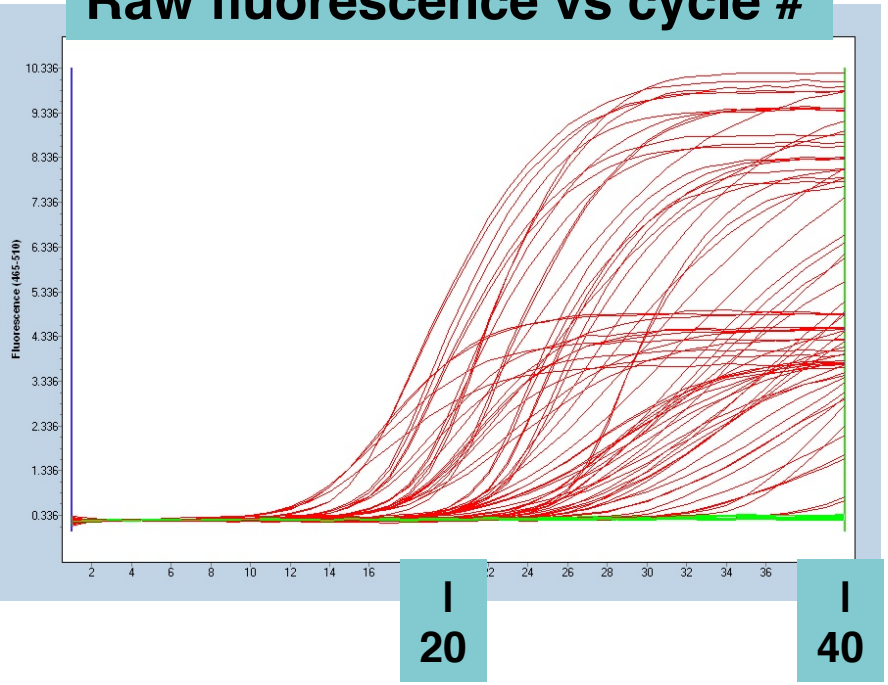
- Two main ways to calculate C_T
- 2nd derivative maximum
  - each C_T identified by largest Δ slope
- Fit points
  - all C_Ts identified by same threshold
  - linear regression in log phase
  - recommended for our analysis type



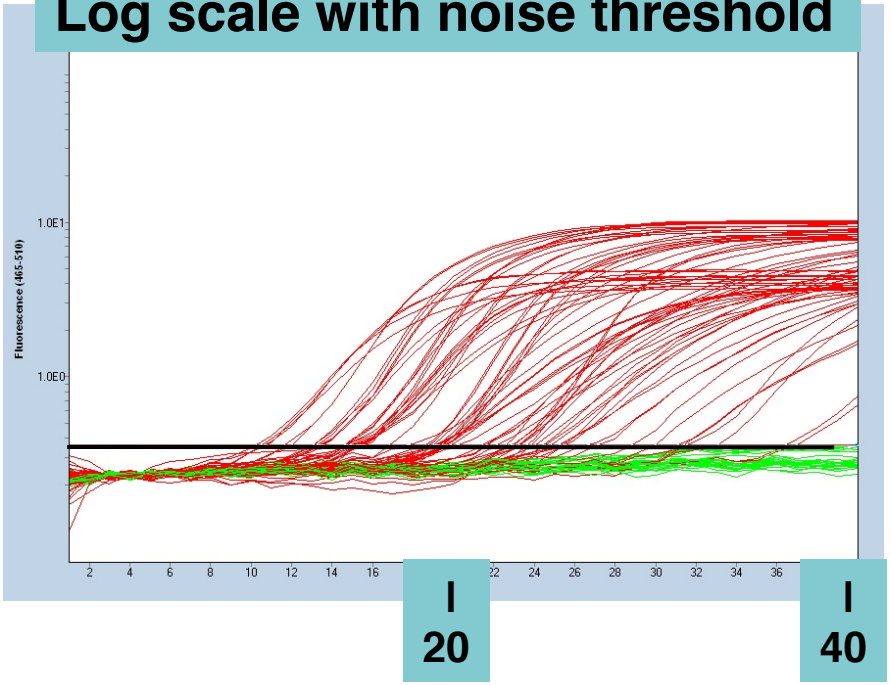
Roche, LightCycler 480 Operator's Manual, software version 1.5

qPCR amplification data

Raw fluorescence vs cycle #



Log scale with noise threshold



(S14, W/F, all)

qPCR relative expression analysis

- Relative gene expression analysis
  - control for cDNA amount with reference (e.g., 18S rDNA)
  - expression change relative to a control (e.g., fresh cells)
- E is amplification efficiency for that primer set

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

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

qPCR example analysis

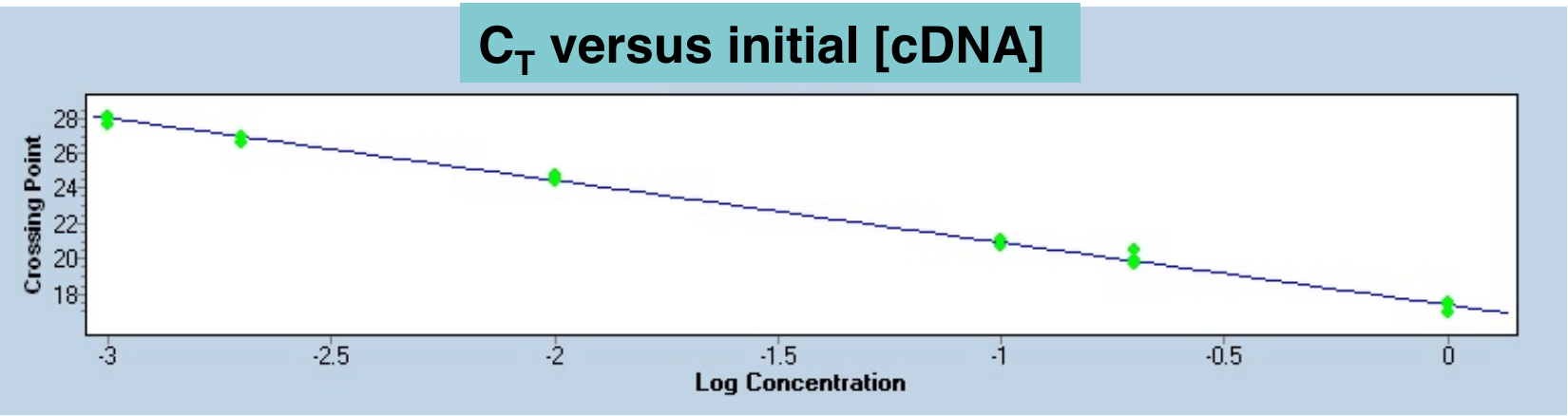
- Effect of primer efficiency
  - if E = 2, two cycle difference = 4-fold change
  - if E = 1.7, only a 3-fold change in two cycles
- Understanding signs
  - say [sample] > [control]
  - therefore CpS < CpC
  - thus exp() is positive: E^(30-20)

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

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

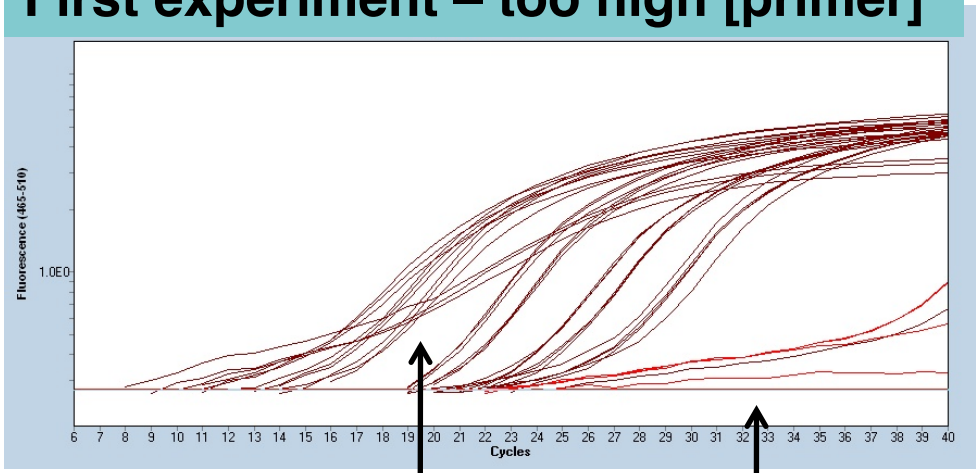
qPCR primer set standard curves

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



Optimizing primer concentration

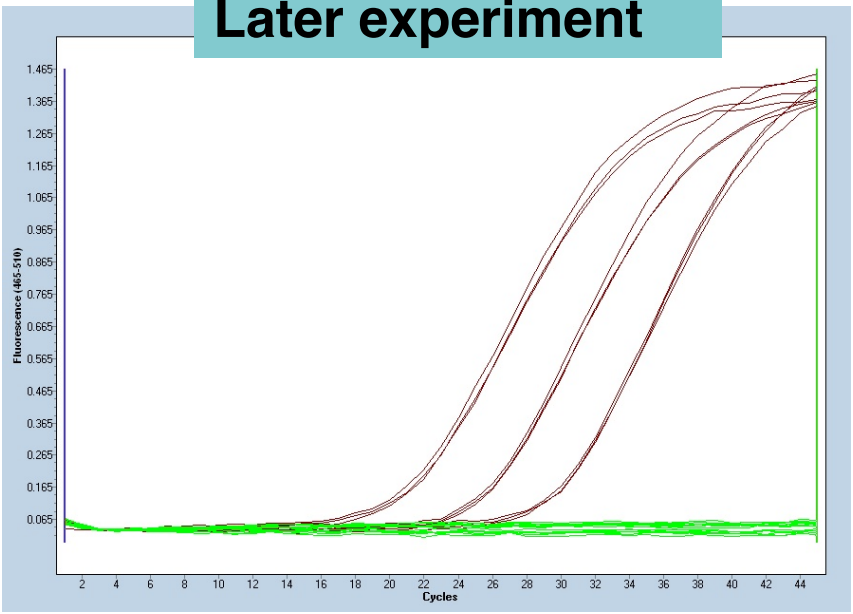
First experiment – too high [primer]



High [cDNA] sample oddly shaped

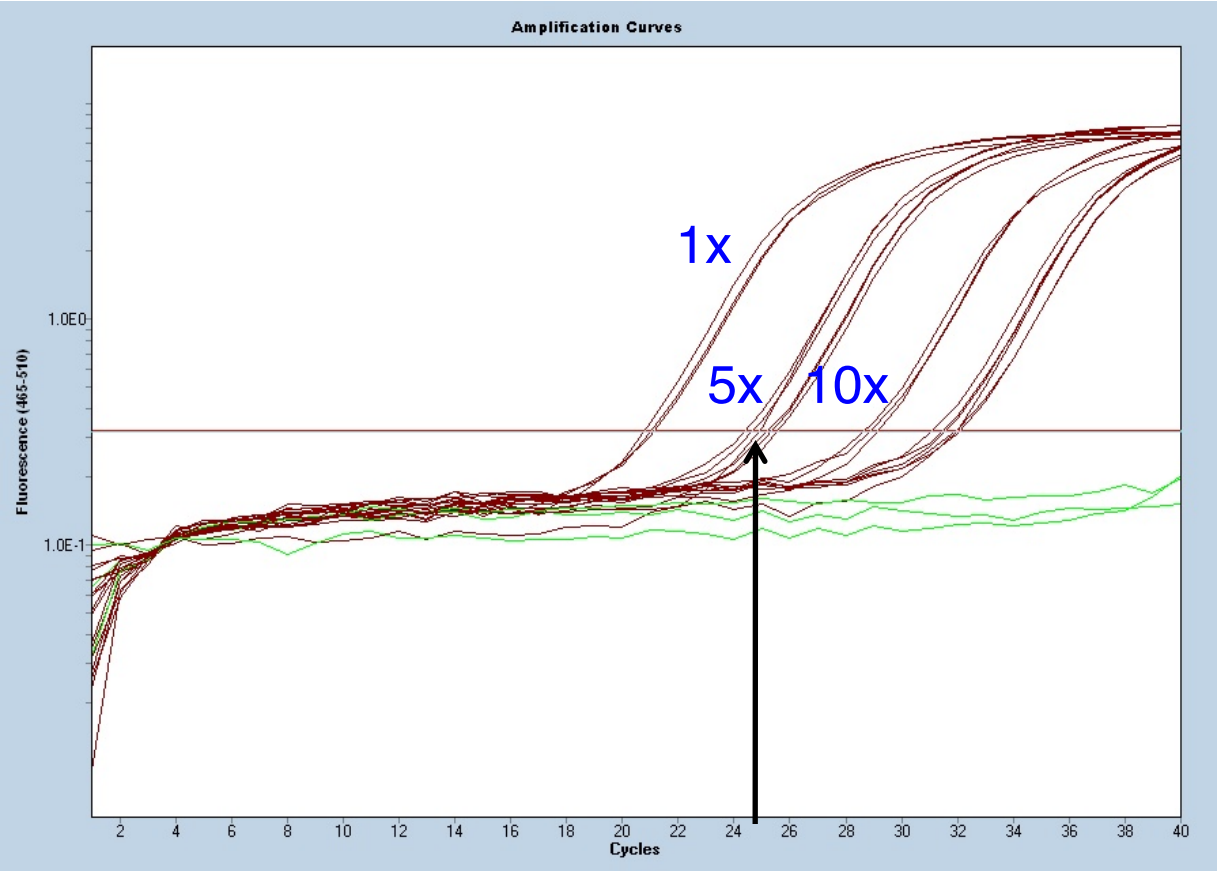
No-template controls give primer-dimer product

Later experiment



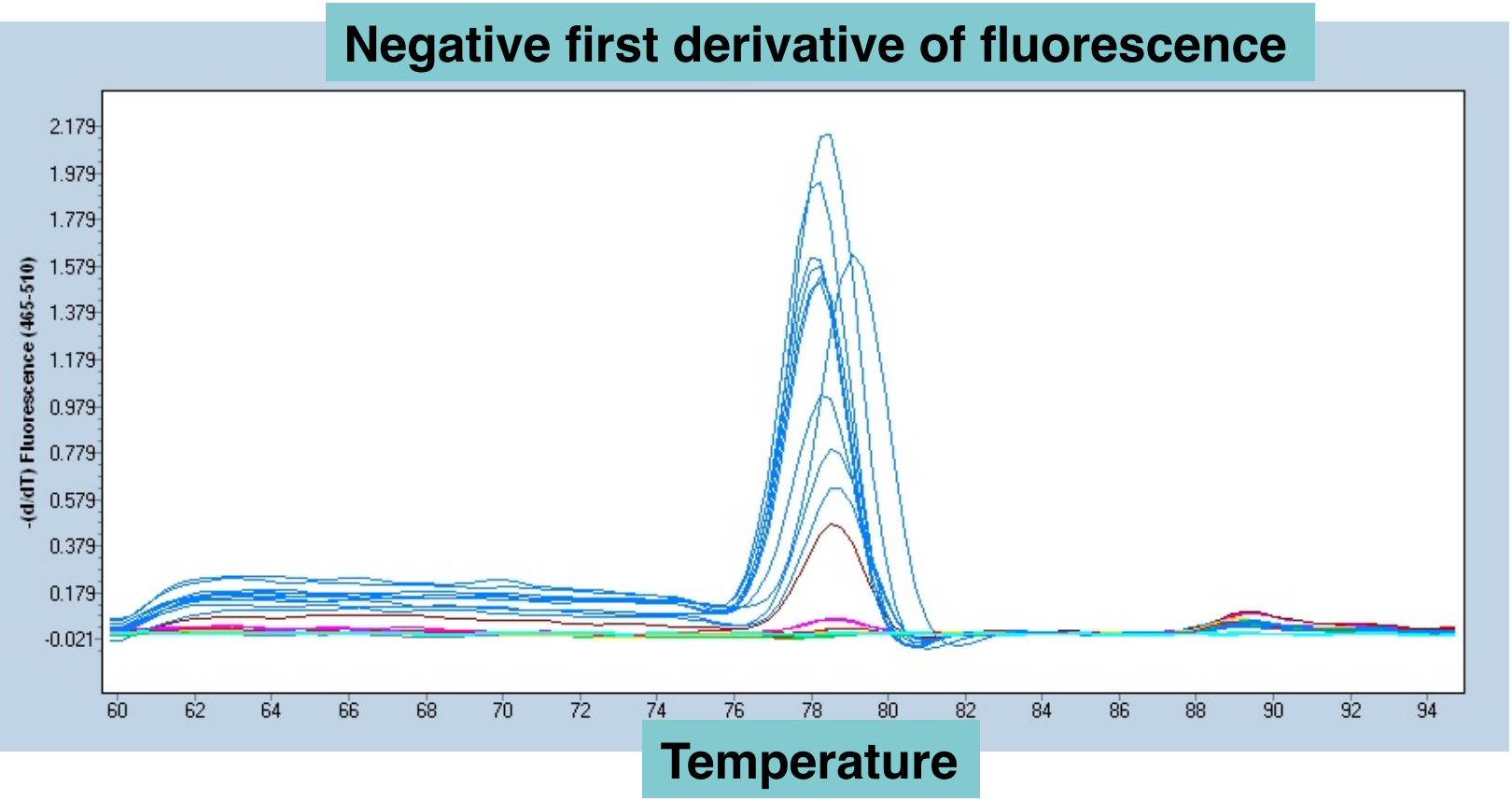
Great replicate agreement and flat controls (green)

Detection limit for change in expression is >= 2-fold



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

Melting curve analysis



(S14, T/R, CN I)

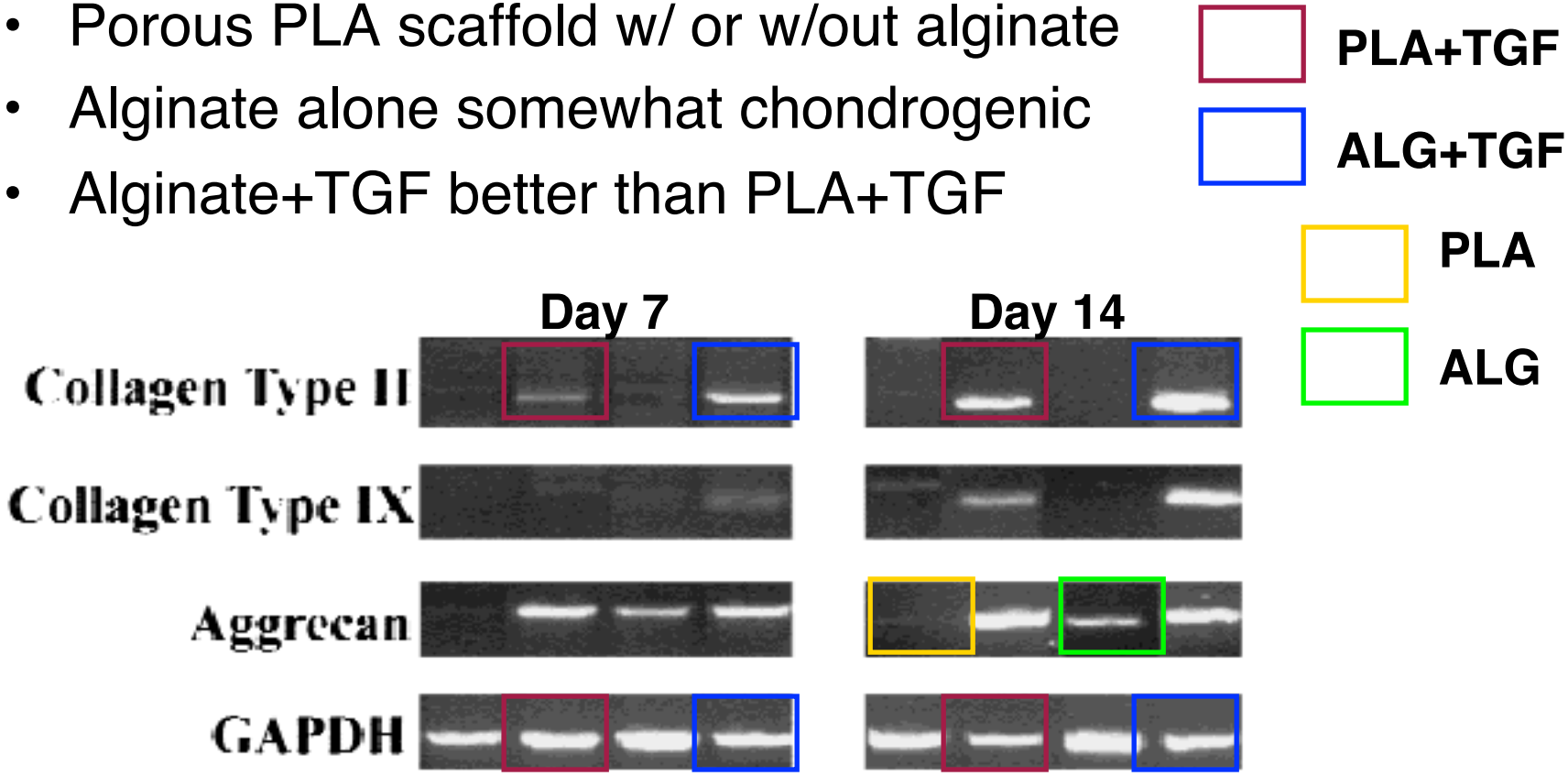
Interlude

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

Which one is cuter? Tree kangaroo or human baby?

Chondrogenesis *in vitro*

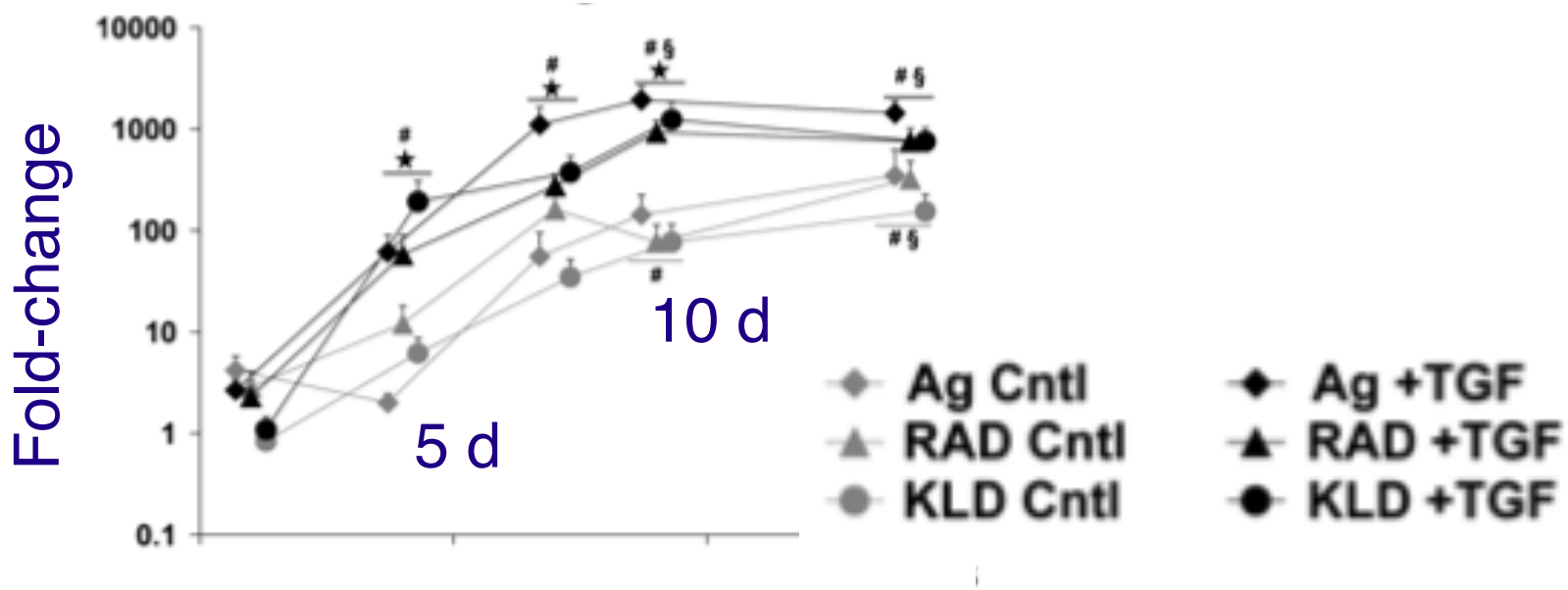
- Porous PLA scaffold w/ or w/out alginate
- Alginate alone somewhat chondrogenic
- Alginate+TGF better than PLA+TGF



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

Recent Grodzinsky lab work shows merits of synthetic peptide gels

Collagen II qPCR relative to fresh stem cells



CN II expression increase is similar among gels. *But!* Peptide gels have better proliferation, PG length.

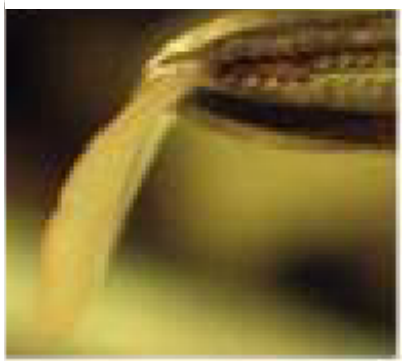
Kopseky et al., *Tissue Eng A* **16**:465 (2010)

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

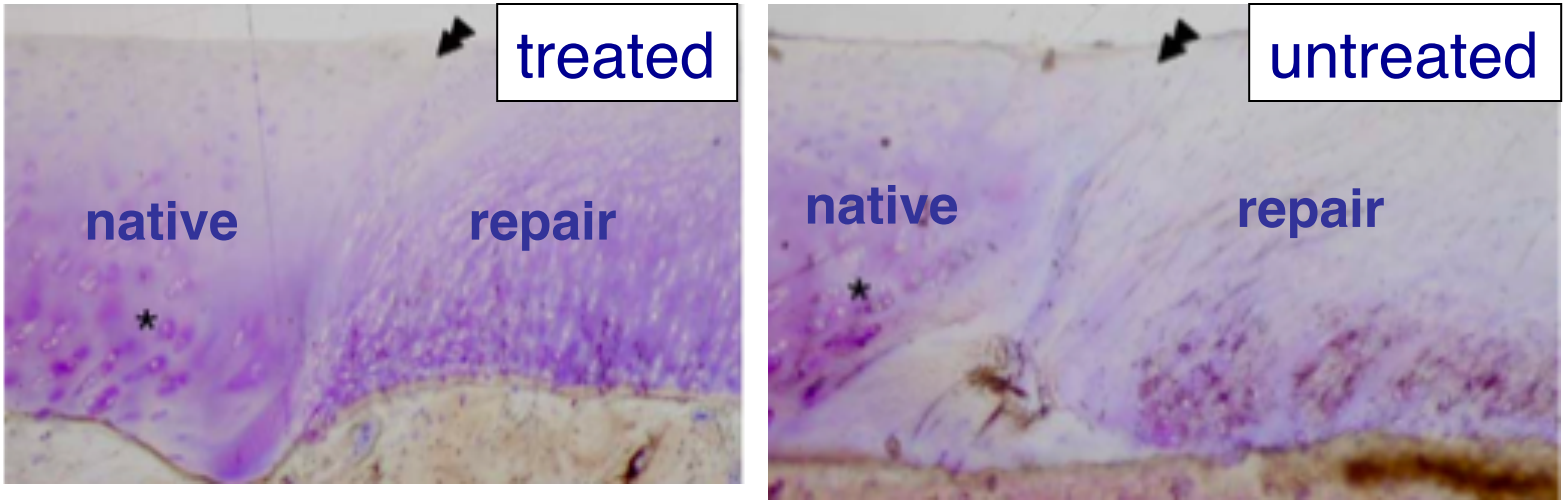


Dynamic, 3 d

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

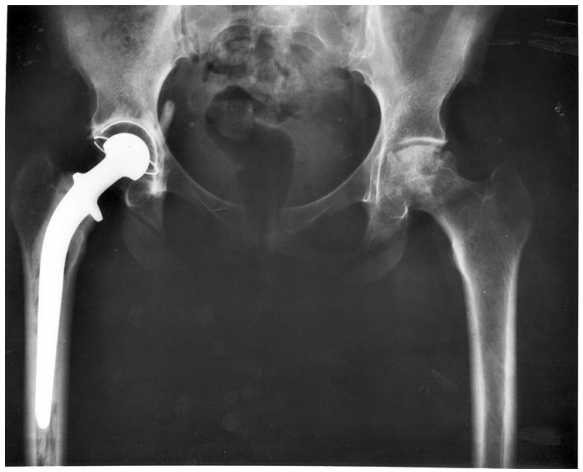
- Cartilage has little regeneration capacity – why?
- Early damage can promote later disease
- Osteoarthritis pathology
  - PG and collagen loss, PG size ↓
  - ↑ water content, ↓ strength
  - chondrocyte death
- Symptoms
  - loss of mobility
  - pain

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

- Biologics: hyaluronic acid, TGF-β, etc.
- Damage bone (stem cell influx)
- Joint replacement
  - synthetic or donated tissue
  - invasive or fiber-optic (partial)
- Cell and/or scaffold implantation
  - immature therapy
- Other/supplemental
  - mechanical, electrical stimulation
  - debridement + lavage (remove/clean)



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: Carticel (FDA), Cartistem(*)
- Scaffold-based approaches in trials and non-US(*) markets
  - e.g., NeoCart (phase III), INSTRUCT, Novocart; Hyalograft(*)

Carticel (carticel.com)

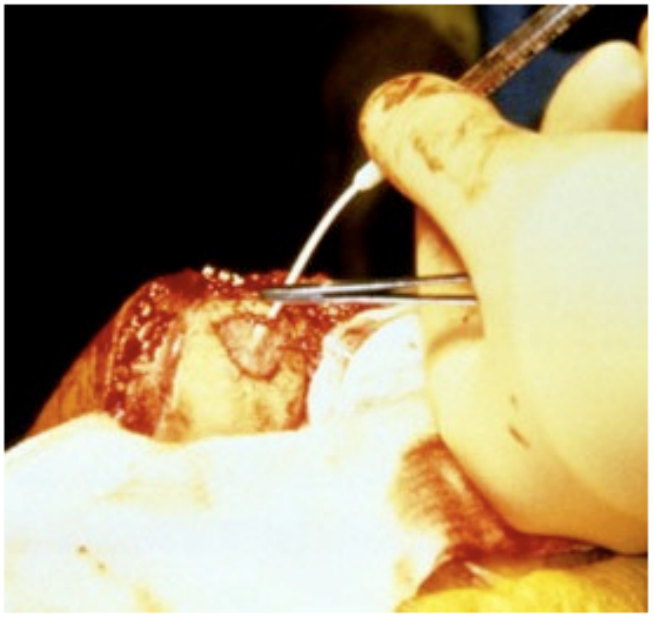


Figure 21: **Injecting Carticel under periosteal patch.**

Neocart (histogenics.com)

Own cells + CN I scaffold + bioreactor (low O_2; mech)
→ implant w/bioadhesive

Many clinical trials are ongoing

352 studies found for: cartilage (last year 295)

1	Recruiting	Knee Articular Cartilage Repair: Cartilage Autograft Implantation System Versus Conventiona **Conditions:** Other Articular Cartilage Disorders; Osteochondritis Dissecans **Interventions:** Procedure: Microfracture; Device: Cartilage Autograft Implantation Sys	← scaffold + own tissue
3	Active, not recruiting	Evaluation of an Acellular Osteochondral Graft for Cartilage LEsions Pilot Trial **Condition:** Articular Cartilage Injury **Interventions:** Device: Kensey Nash Corp. Cartilage Repair Device; Procedure: M	← degradable scaffold
4	Recruiting	Tissue Engineered Nasal Cartilage for Regeneration of Articular Cartilage **Conditions:** Cartilage Lesion; Lesion of Articular Cartilage of the Kn **Intervention:** Biological: Tissue engineered cartilage graft	← expanded nasal CDRs
6	Recruiting	A Multicenter Trial of AS902330 (Recombinant Human Fibroblast Growth Factor-18) or Placebo After Microfracture Surgery for Cartilage Injury of the Knee **Conditions:** Cartilage Repair of Knee; Microfracture Surgery of Knee **Interventions:** Drug: AS902330 (30 microgram [mcg]); Drug: AS902330 (Drug: Placebo	← drug

others: own or cord blood stem cells

Screenshot from www.clinicaltrials.gov, May 2014

Lecture 6: state of cartilage TE

- 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; reproducibility discussion.

Lecture 8: special topics in TE.