- Announcements
- Quiz (last one!)
- Pre-lab Lecture
  - DMMB assay
  - More about ELISA
  - qPCR analysis
  - Today in Lab (M3D6)

Announcements

- Lec 7: Atissa on proposal; Lec 8: special topics
- Next time: finish report + clean up; WAC survey
- Mod 3 research proposal
  - feedback on your wiki page today/tonight
- Mod 3 report
  - no separate Methods section needed: *do* state any unique conditions along the way in Results section/captions
  - required analysis: viability (incl. stats), qPCR (incl. RNA amount and cell counts), ELISA (incl. standard curves)
  - optional/if relevant analysis: PG assay; general bead, cell, and media appearance on D2, D3, and/or D4

DMMB assay

- Measure GAGs with cationic dye
- Absorbance shift due to complex A_{595} ↓
  - fades quickly! (pipet upstairs)
  - at low pH (1.5), selects for sulfates over carboxyls
  - thus a correction for alginate
  - standard curve made with chondroitin-6 sulfate
- Typically normalize to cell amount (cf DNA content in qPCR)… maybe next year

ELISA protocol

- Direct ELISA uses labeled primary antibody
- Indirect ELISA – why use a secondary antibody?
  - signal amplification
  - flexibility / efficiency (t, $) → use w/ many 1° Abs
  - but may be more cross-reactive
- Development process – what/why/how

2° Ab — AP conjugated

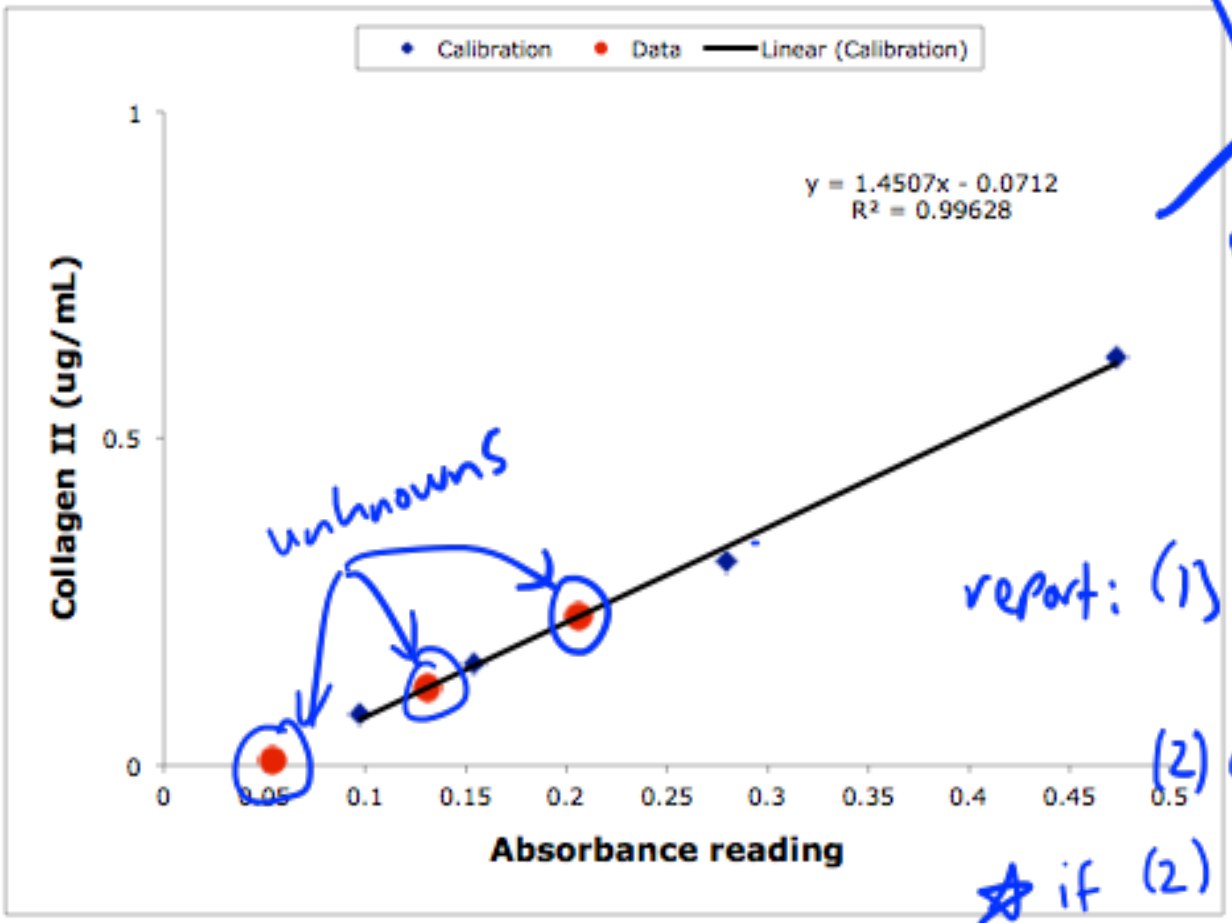
provide substrate: pNPP → colorimetric rxn. in soln; A_{420}

* development time is key ↗ detect low [protein] → long enough
  ↘ not oversaturate everything → not too long

ELISA Outcomes

Outcome	Possible Explanations
High reading in "blank" samples	• cross contamination of wells or global in PBS • skipped block step • incomplete washes
No signal at all (including standards)	• wrong or old/bad Abs • flipped plates at 1° Ab step • too high [Tween]
Saturated signal for some samples (expt'l)	too concentrated → run dilution series usually

ELISA (and PG) analysis



exclude non-linear standards/ measurements

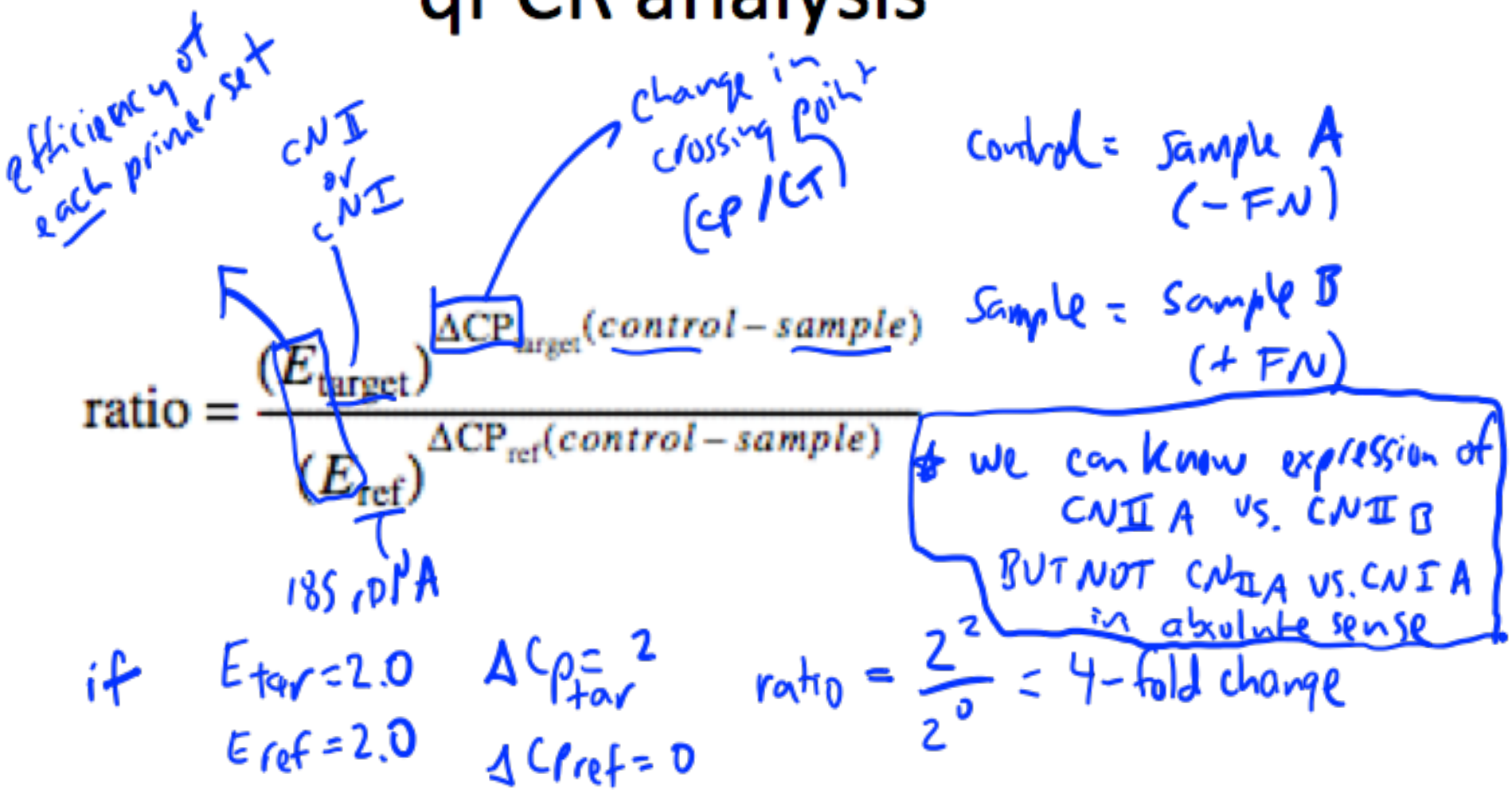
~4 usable

report: (1) CN II : I ratios

(2) absolute [CN]

★ if (2) is very low, then (1) is meaningless

qPCR analysis



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

Today in Lab (M3D6)

- Finish ELISA – includes 90 min incubation
- Meanwhile…
  - DMMB assay staggered (15-20 min of work)
  - qPCR analysis
  - finish viability analysis if you haven't already
  - cross-group research discussion
  - (optional: start clean-up)

figure out how much DEV SOLN your super-group needs!