- Announcements
- Pre-lab Lecture
  - On writing a Methods section
  - Mod 2 re/overview
  - Western Part 1
  - Today in Lab (M2D2)

Announcements

- A few points I skipped in TC
  - *why* passage? make more
    - space/nutrient/O_2 limitations
    - phenotype changes (e.g., stem cell); contact inhibition
  - why did we transfer a known volume, anyway?
    (• sense of total amount)
    - more sure that cell sticking status (on/off) isn't changing (TCPS vs PP)
- M1 primer design memo due M2D3 at 11 AM WED
  plus 1st M2 quiz (D1+D2)

Memo requirements: highlights

Motivation revise/finish

Here you aim to motivate/justify two things in about two paragraphs:

- The overall strategy you have pursued
- The specific design choices that you made

Formatting Expectations narrative

- Your main document (excluding figures) should be/have
  - .docx (preferred) or .pdf
  - 12-pt font
  - with 1-inch margins
  - **spaced at 1.5 lines**

~3pgs

Outcomes

Use this section to point out the most important findings and analysis that led to your conclusion about the future direction of your research division.

Begin by clearly describing, in both a figure(s) and text, the performance of your novel primer design. Explicitly compare this performance to your expectations. Whether or not you succeeded in designing primers superior to those with which you started, discuss the design factors that you believe had the greatest impact on primer performance.

↳ hypothesis

Be sure to establish yourself as a credible source for this information. You will be most credible if you highlight your expertise and understanding of the subtleties of the subject based upon your experimental results. Establishing credibility also requires that you appreciate and directly address any limitations in the data.

Conclusion [edit]

The purpose of this section is to help your supervisor decide whether your division merits continued funding or needs a new direction. First summarize the progress you have made, in comparison to the progress you anticipated making, in about a sentence. Next, in a few sentences, describe the next one or two experiments that you would like to pursue. (What changes would you make to your current design?) Finally, in one or two sentences, either ask for and justify continued funding for microsporidia diagnostics or suggest that the division be redirected to pursue a specific alternative target.

brainstorm
sleep on it!

Methods section exercise

sub-section heading: Detection of collagen II by ELISA

topic sentence ✓

"Enzyme linked immunosorbent assay was utilized to quantify total collagen II content. 100 µL of wash buffer ~~was added to 36 wells of a 96-well plate.~~ Wash was done 3 times. 2 µL of anti-collgen II antibody was added per well and left for 1 hr ~~on the benchtop.~~"

mostly what, only "procedural" why

with (no # @ start; composition; concentration)

fragment: make complete at RT/21-25°C

manufacturer

* conc., not volume or mass (but occ. both is ok)

* composition and/or manufacturer

* alternative OR wash buffer (100µL) was...

~~"3 EGF-stimulated, 3 TGFa-stimulated, and 3 serum starved~~

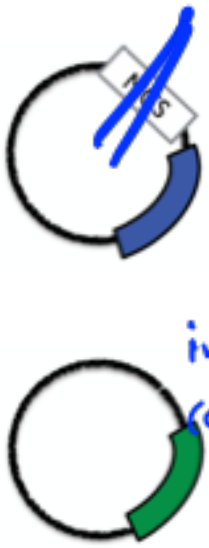
Samples were ~~added to the plate~~ prepared in quadruplicate and incubated for 2 hr."

Module 2 goal and approach – subtleties

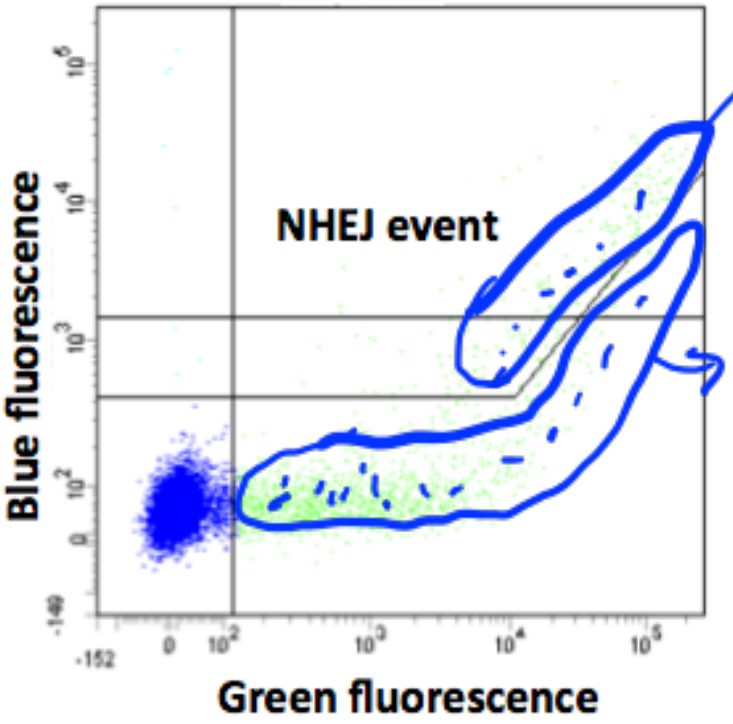
Question: How does changing DNA damage topology affect NHEJ? ← vague

How does efficiency of NHEJ vary with DNA cut topology?
(in different cell systems)

Co-transfect cells



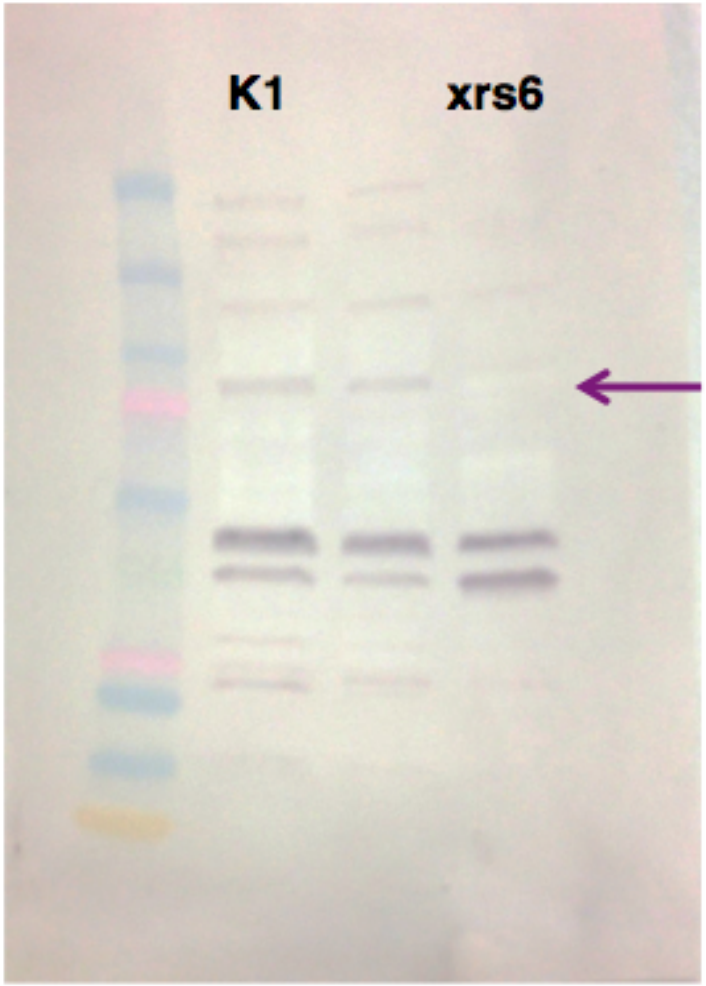
intact control



repair, not uptake, is driving difference

likely did take up GFP and BFP, but didn't repair latter

Ku80 Western – why do it?

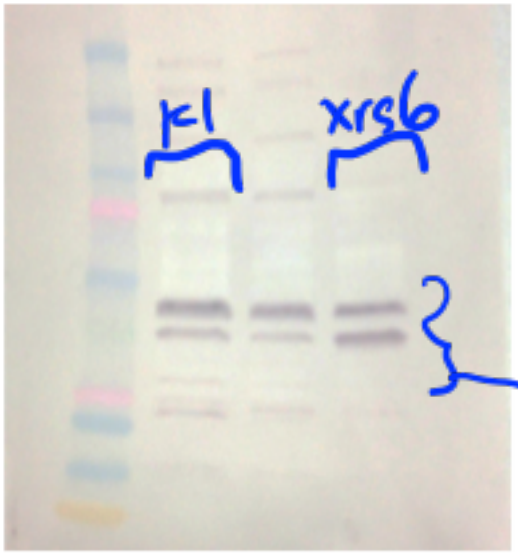


1) experimental error
- mislabelled tubes/plates

2) revert during culture
- second xrcc5 copy becomes demethylated

Preparing protein samples for Western

- Lyse cells
  - RIPA buffer: salts-- pH, osm detergents-- open up membranes (high or low strength ↓ app.)
  - protease inhibitors-- prevent degradation
- "Pre-clear" lysate and keep supernatant ↓ viscosity
- [Measure protein] – why?



- load equal amounts of protein → avoid spurious negative
- loading control even better e.g., GAPDH or actin probe

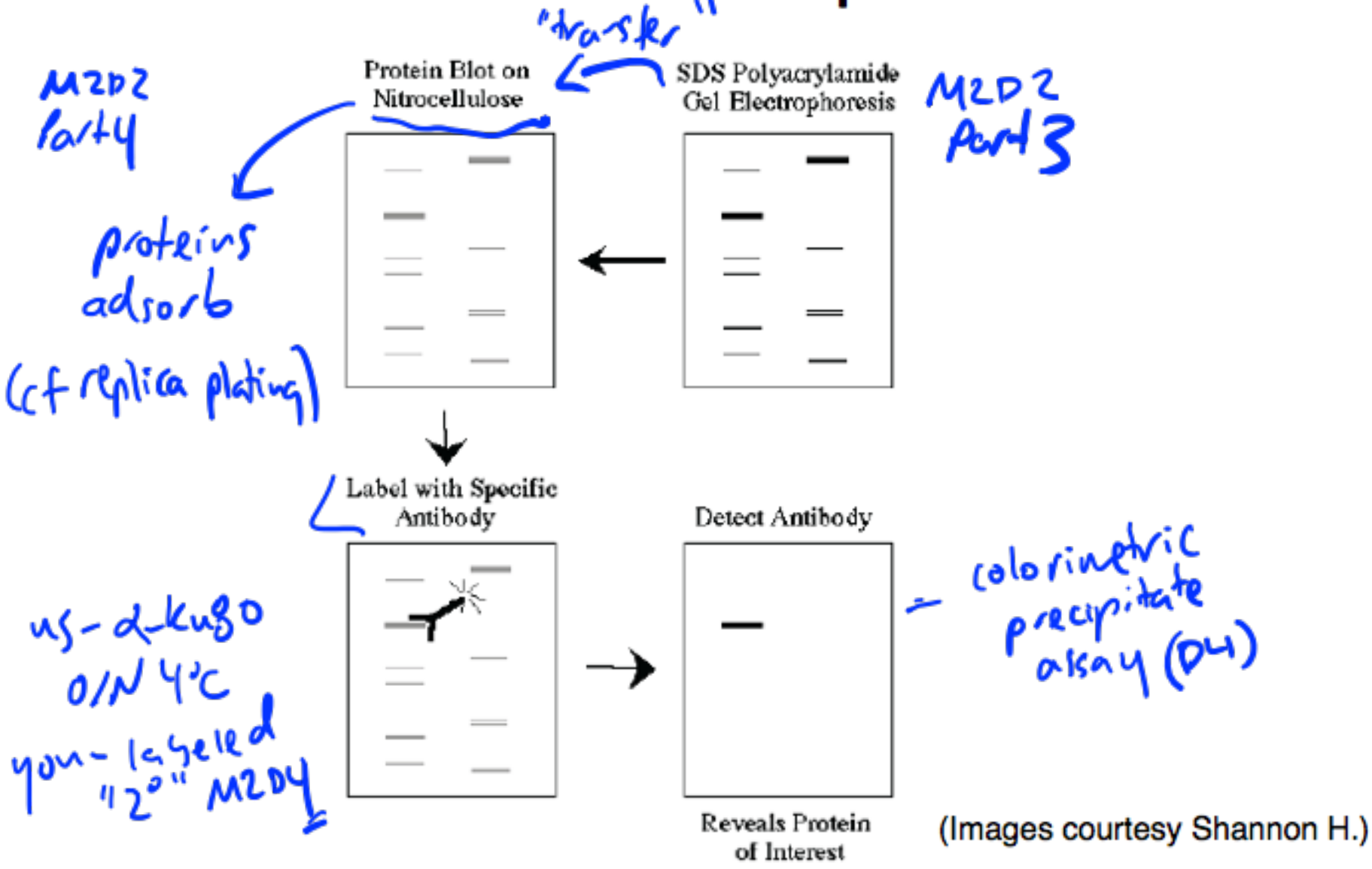
off-target bands are a start :)

Western Part 1: SDS-PAGE

acrylamide - monomer is toxic (neuro)

- Gel separates proteins based on size, shape, charge
  - make uniform
- Laemlli sample buffer
  - SDS: coat proteins w/(-) charge
  - β-Me: breaks S–S bonds
  - Boiling: denature higher-order structures
  - Laemlli has SDS, β-Me, plus glycerol, BPB dye (cf DNA gels)
- After separation
  - *can* globally stain for proteins with Coomassie – (cf E+Br)
  - or probe with antibody for *specific* protein

Western Part 2 preview

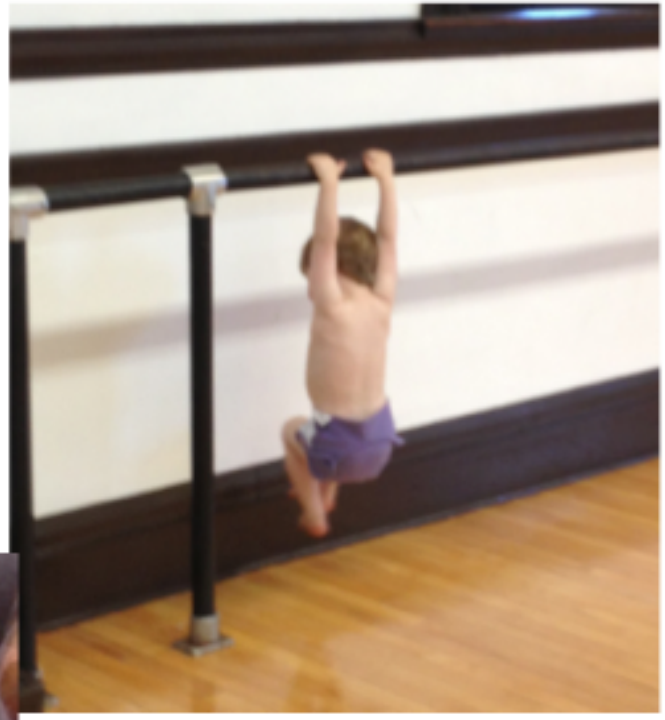


(Images courtesy Shannon H.)

Today in Lab (M2D2)

- Grab cells from incubator →
  - observe on scope, then move into main lab
- During 15 min incubation and/or spin (NOT boiling)
  - get trained on loading protein gel!
- Measure [protein]
  - mix components just before using spec
- Load gels → take a short break ☺ 30'
- Set up transfer
  - prepare a labeled box for your blot, note start time
  - we will block after 1 hour if end time is past 5 pm

PS: I wasn't kidding about the Dance Complex



← My fave: the lion song

Bonus: our indie rock album cover