M2D2: Begin WB Analysis

3/13/14

1. Pre-lab discussion — primer memo & Western blots
2. Lyse cells
3. Measure total protein concentration
4. SDS-PAGE & Transfer

Primer Design Memo — due Tuesday

Formatting Expectations

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

Directly from the wiki:

Outcomes

[edit]

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.

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.

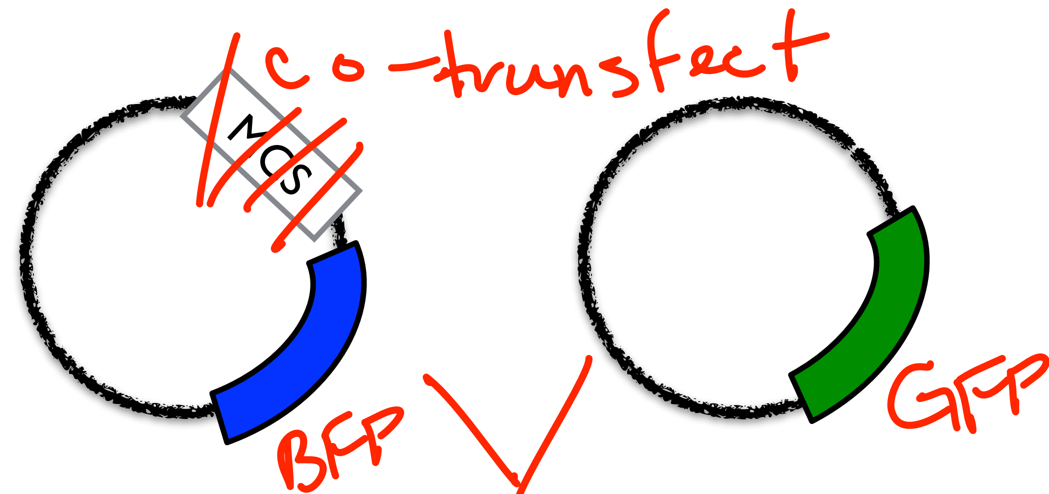
Conclusion

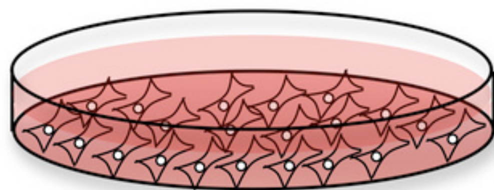
[edit]

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.

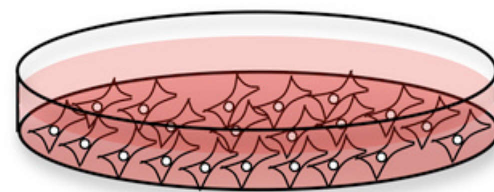
Review of Mod2 Goals:

"How does efficiency of NHEJ vary with cut topology?"



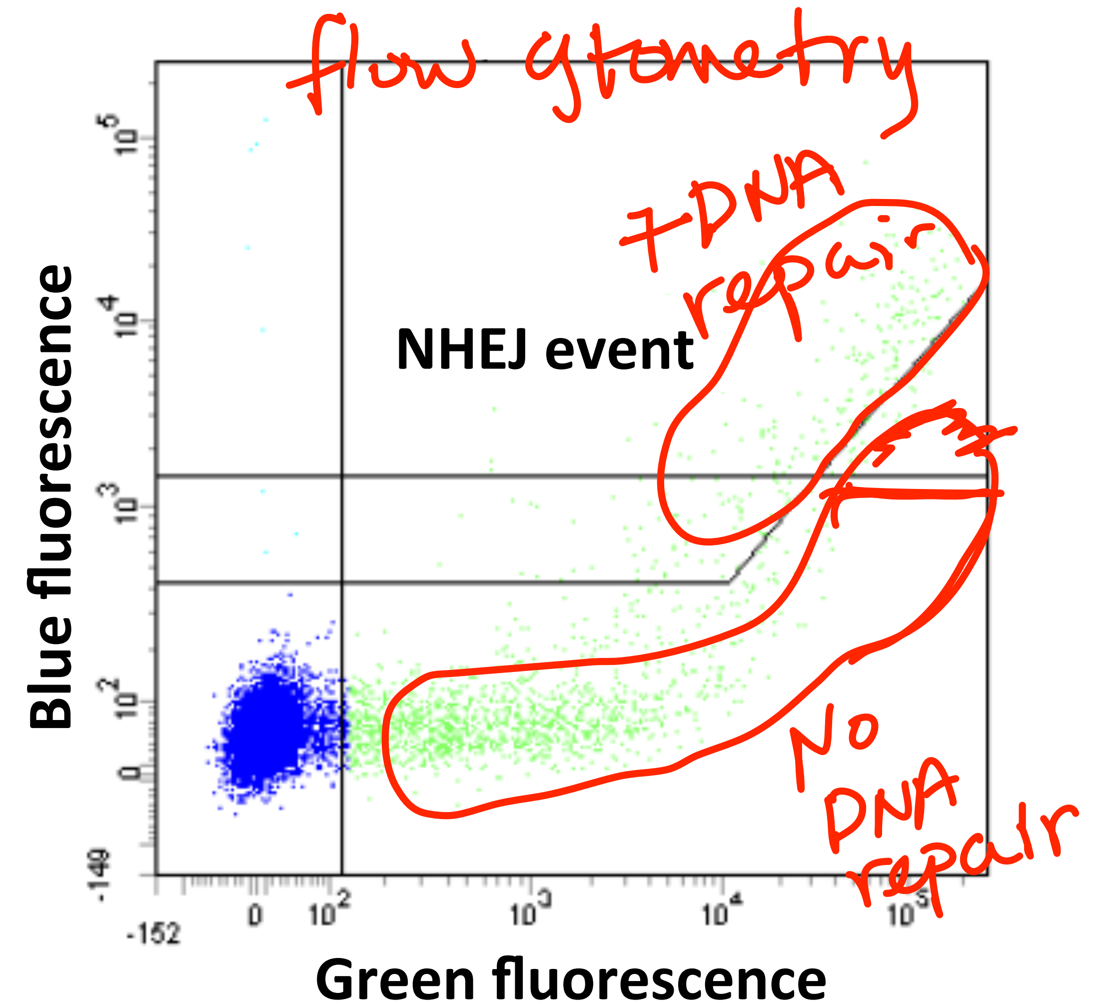


CHO-K1



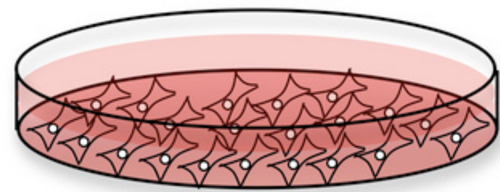
CHO-xrs6

Ku80

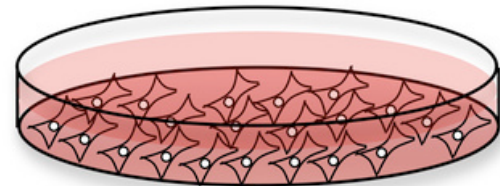


http://www.nature.com/scitable/topicpage/cell-cycle-control-by-oncogenes-and-tumor-14191459

First: validate the system.



CHO-K1 cells



CHO-xrs6 cells

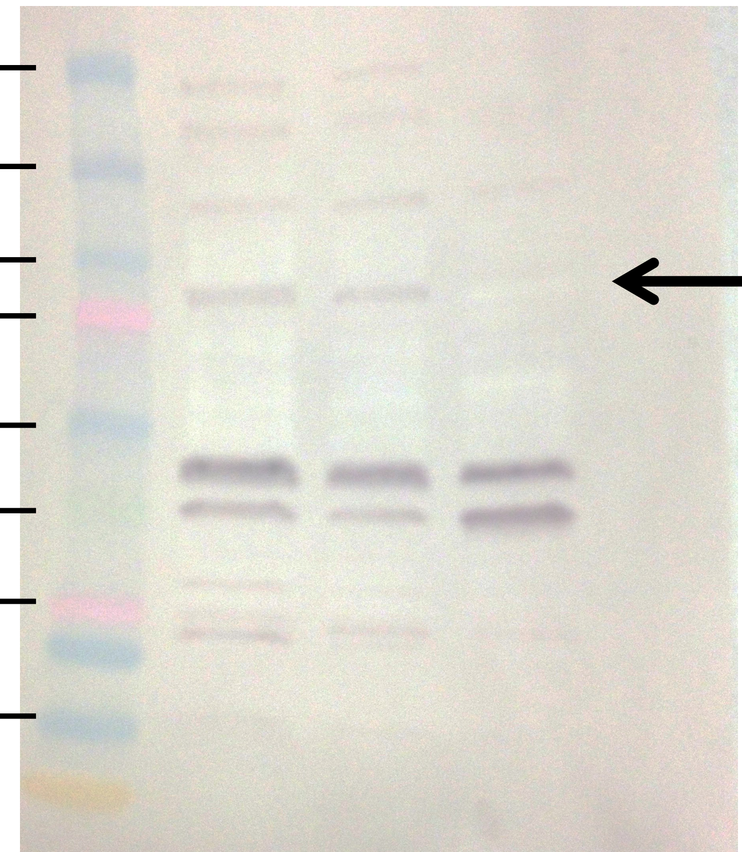
why? 1) Experimenter Error
- mislabeled tube

2) demethylation of 2nd Ku80 copy

protein biochemistry

MW (kDa)

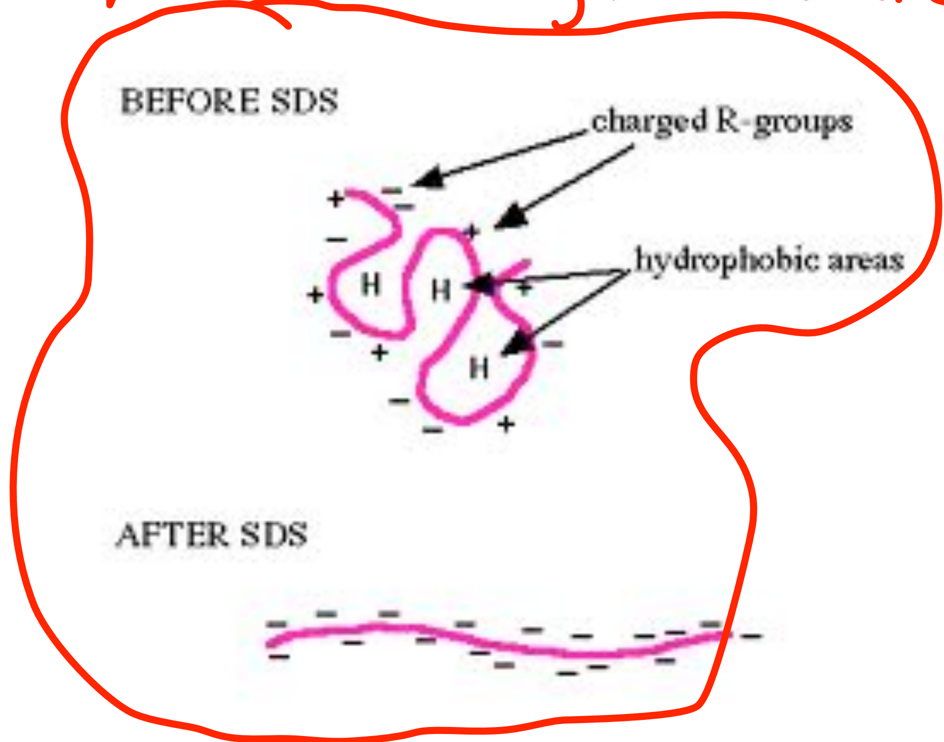
250
150
100
75
50
37
25
15



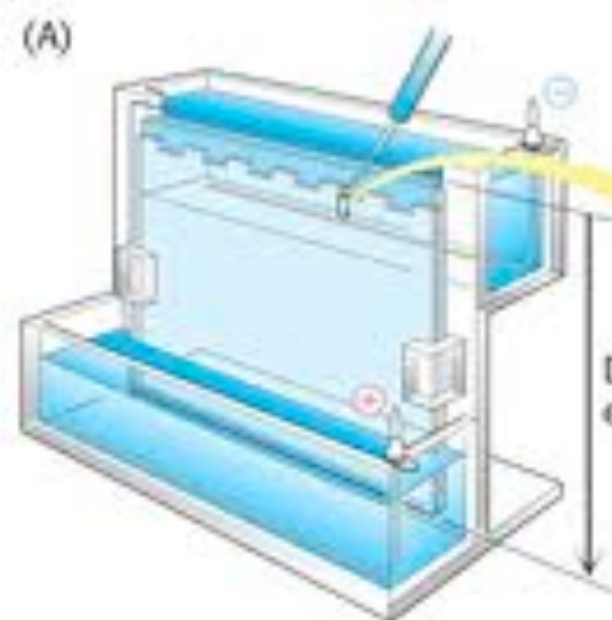
Western blot probed with α-Ku80 antibody

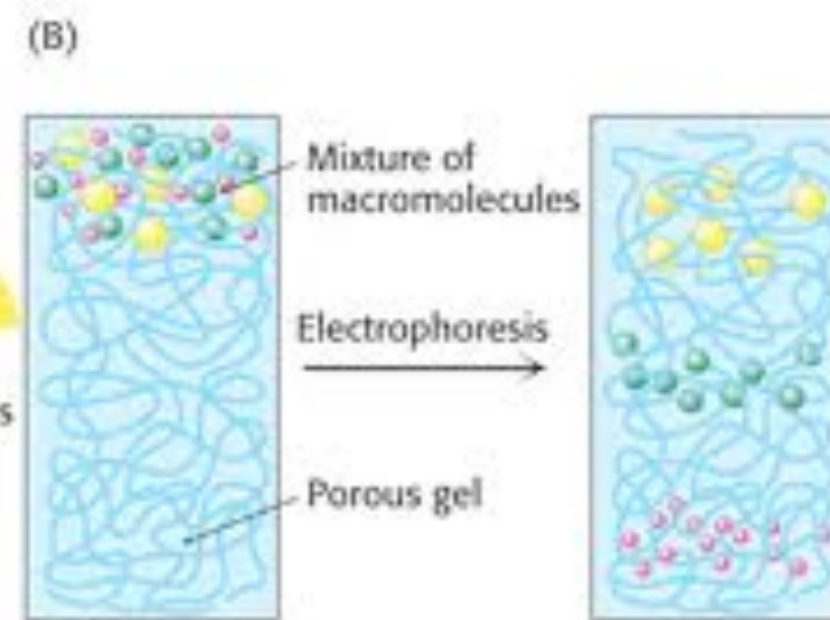
Western blot analysis: Step 1

Sodium Dodecyl Sulfate



1) Lyse cells — salts — Tris, etc pH; detergents; protease inhibitors
2) "pre-clear"
3) Keep sup
4) measure [protein]
5) Load equal amts of protein





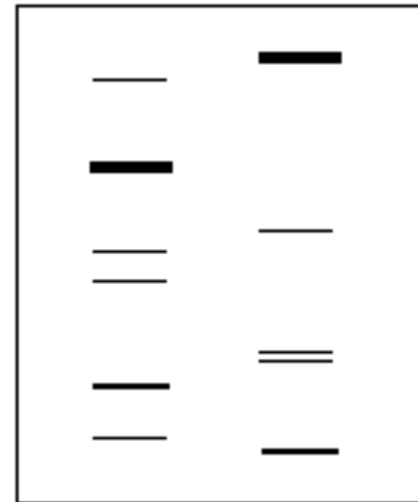
size

Western blot analysis: Step 2

'transfer'

Protein Blot on Nitrocellulose

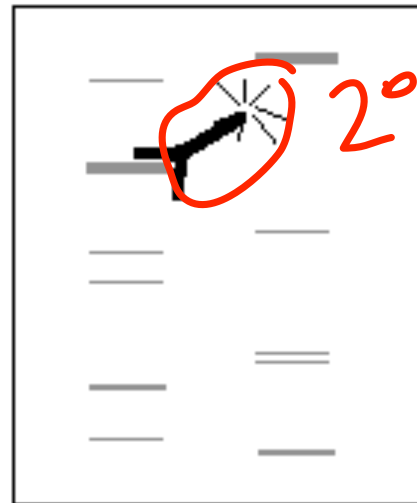
SDS Polyacrylamide Gel Electrophoresis



M2D2

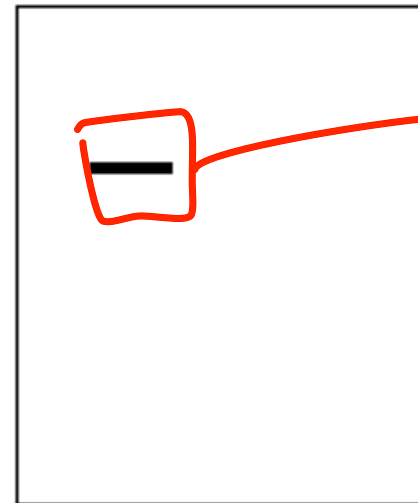
proteins adsorb very easily

Label with Specific Antibody



2°Ab

Detect Antibody



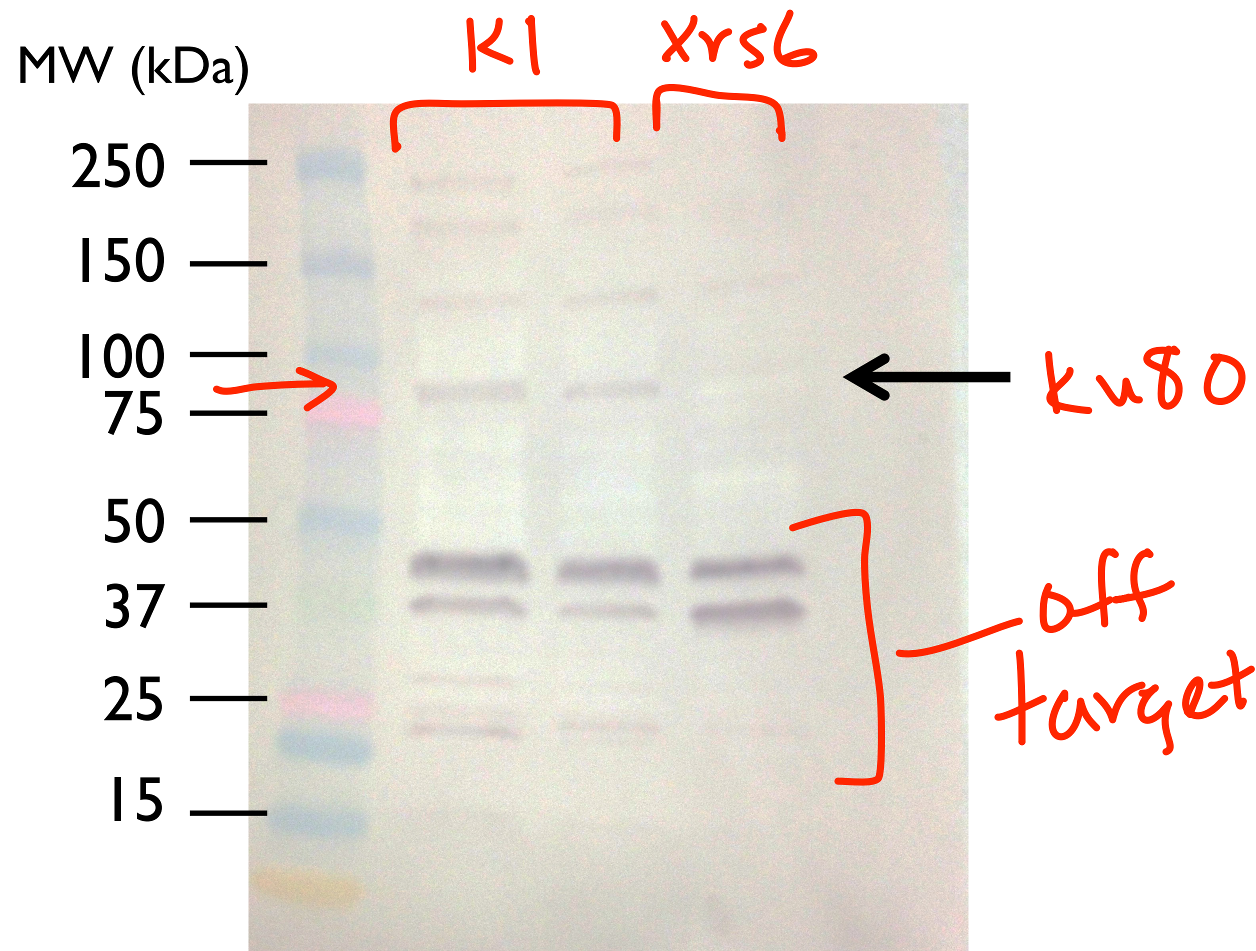
Reveals Protein of Interest

M2D4

precipitation

-probe membrane
α-Ku80
1° Ab

Western blot analysis: controls



Western blot probed with α-Ku80 antibody

1) Loading control
- probe membrane
  α-GAPDH
  α-β actin

2) Load equal amts of protein

Detection of collagen II by ELISA.

"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."

"3 EGF-stimulated, 3 TGFa-stimulated, and 3 serum starved samples were added to the plate in quadruplicate and incubated for 2 hr."

Today in Lab:

1. Lyse cells
2. Measure total protein concentration
3. SDS-PAGE & Transfer — we will block for you if we run out of time

Due on M2D3

1. Primer Design memo
2. Draft Methods section for M2D
3. Read paper about Ku80 in NSCLC (*hint: read the paper first — then draft your methods section!*)