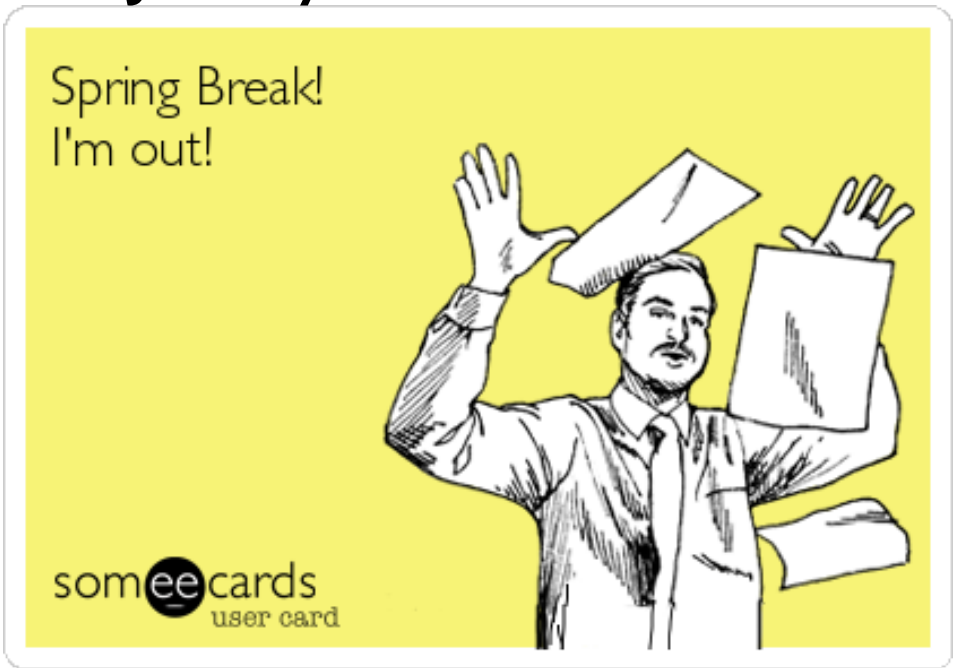
M2D3: Complete Western / Damage DNA

3/20/15

0 Lab treat

1. Pre-lab discussion: Review Western Blot(WB) & Restriction Enzyme(RE) Digest
2. Set-up RE Digest
3. 2nd step of WB
4. Gel purify cut DNA for NHEJ assay!

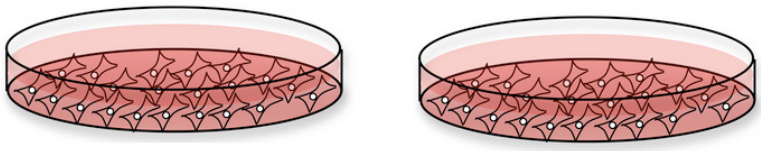
5. Spring Break!



Purpose of WB

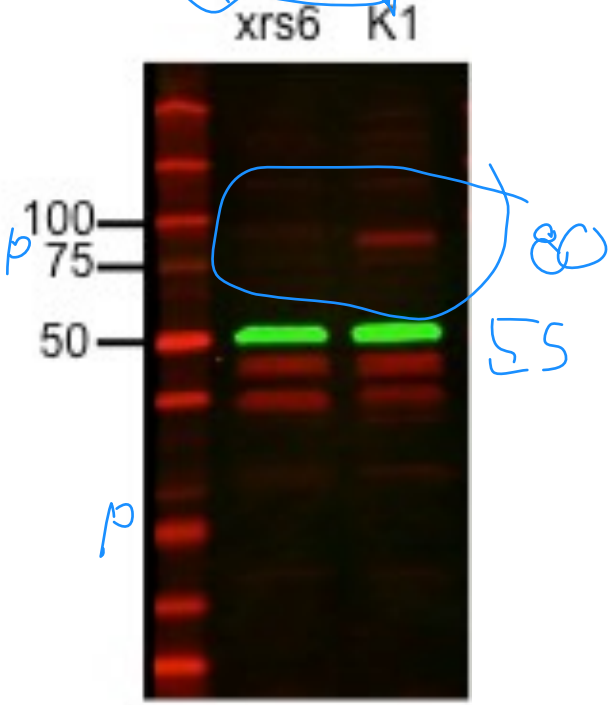
Validate the system - confirm decrease in Ku80 expression.

(+) Ku80 (-) Ku80

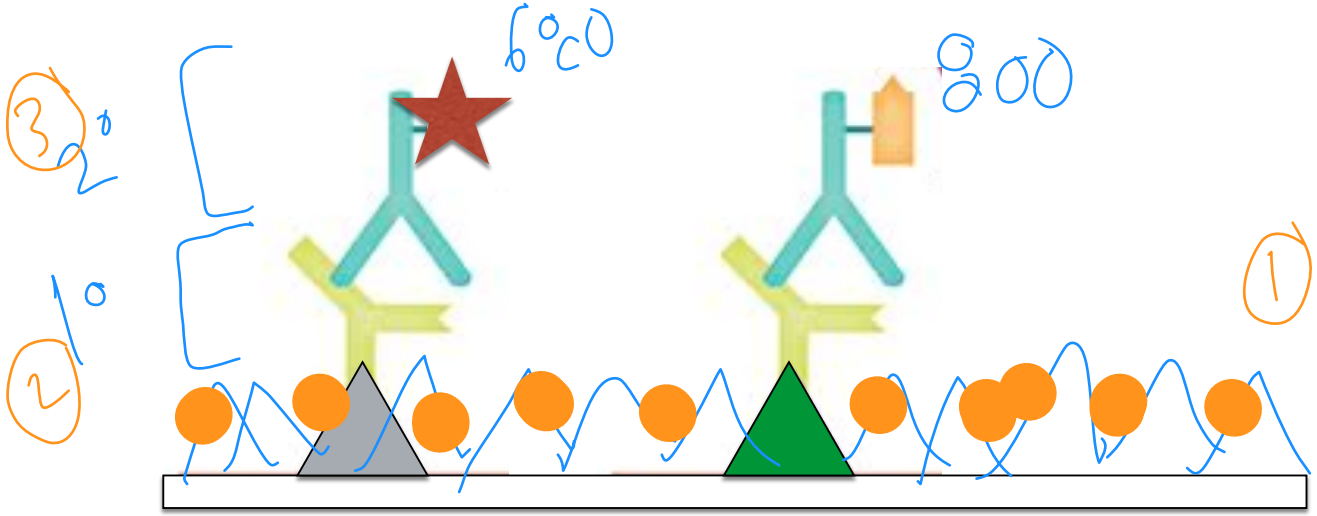


CHO-K1 cells CHO-xrs6 cells

4) LiCor



TBST. Tris Buf. Saline + 0.1% Tween (mild detergent)



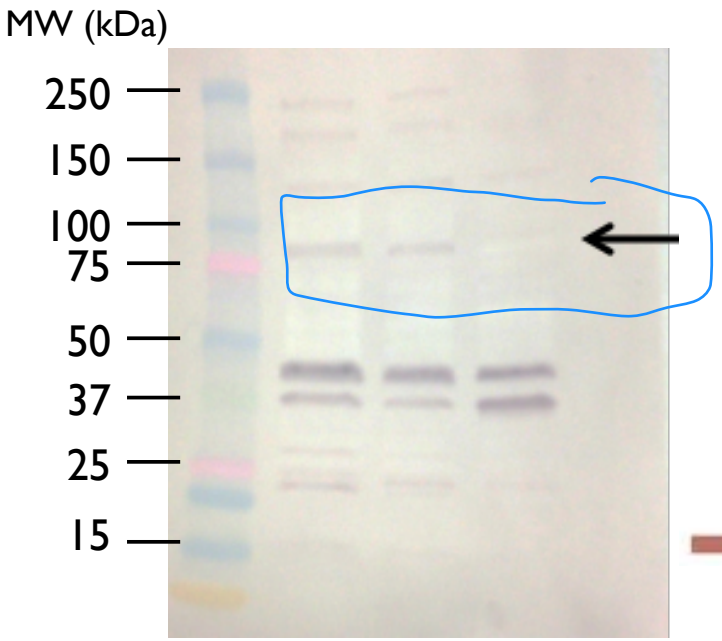
1) Block: FISH serum

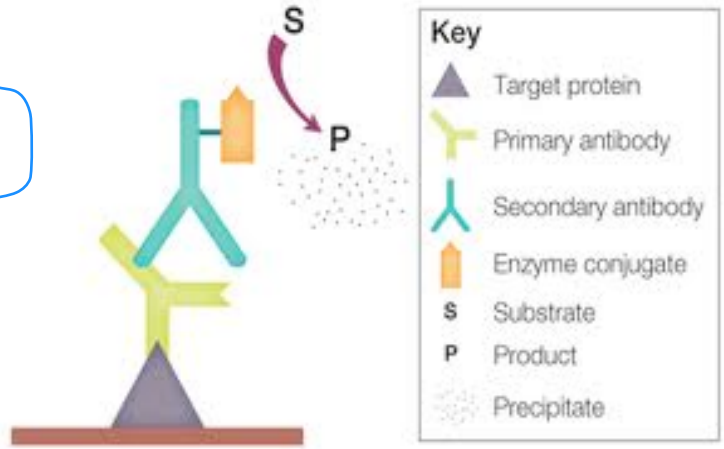
Western blot detection systems:

Colorimetric:

least sensitive

no eqip Required





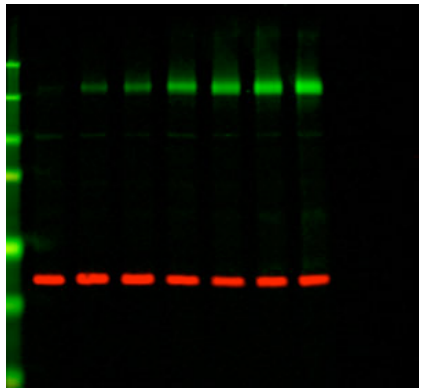
Chemiluminescent:

more sensitive

darkRoom develuper

Fluorescent:

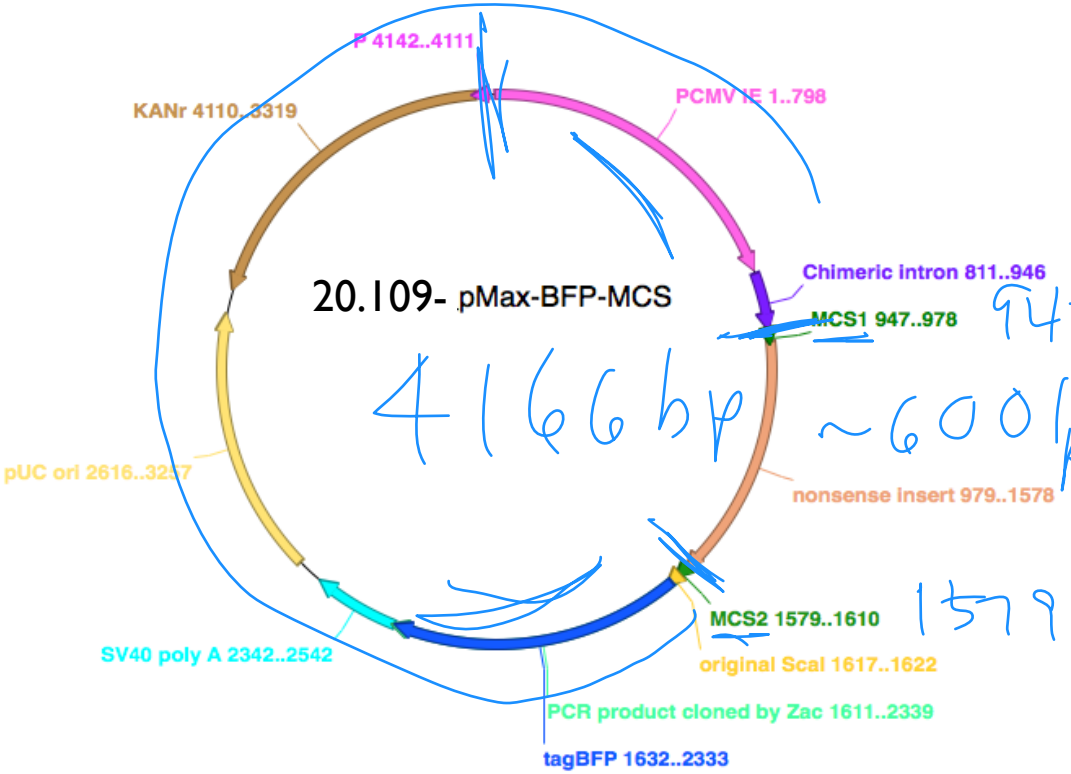
Licor

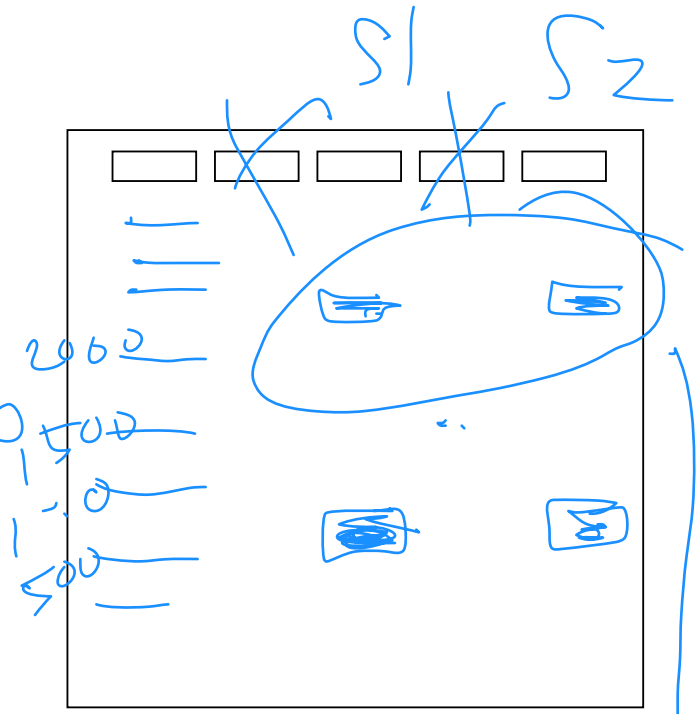


Label 2 proteins at once

Restriction Digest

Today you will prepare (and validate) your damaged DNA:





no repair = NO BFP

NHEJ = BFP

KEEP

Enzymes available in 20.109 for cut topology

Buffer - Cut Smart // BglII Onf3.1

1. Sticky ends, 5' overhangs XbaI
2. Sticky ends, 3' overhangs KpnI-HF
3. Blunt ScaI
4. Hybrid: 5' sticky upstream, blunt downstream
5. Hybrid: 3' sticky upstream, blunt downstream
6. Sticky ends with sequence mismatch
7. Sticky ends with sequence and topology mismatch

Today in Lab:

1. Set-up digest — Dilute enzyme in: BUFFER
2. Finish WB — go with Nova to imager (if time allows)
3. Gel purify digest product: Leave a space/empty lane in gel!
4. Primer Design Memo due tonight 10pm

Due after spring break (woo hoo!):

Microbiota re-write (final due SATURDAY, 4/4 5pm)

M2D4 (Wednesday April 1st)

1. Draft of methods for your Module 2 research article.
2. Figure, caption and narrative depicting your Western blot results.

Prep transfection Calculation