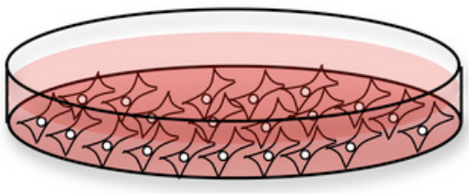
M2D4: Complete Western / Damage DNA

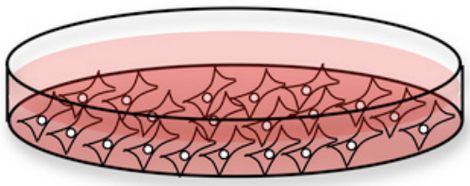
3/20/14

1. Pre-lab discussion: Review WB & RE Digest
2. Set-up RE Digest
3. 2nd step of WB
4. Gel purify cut DNA for NHEJ assay!
5. Spring Break!

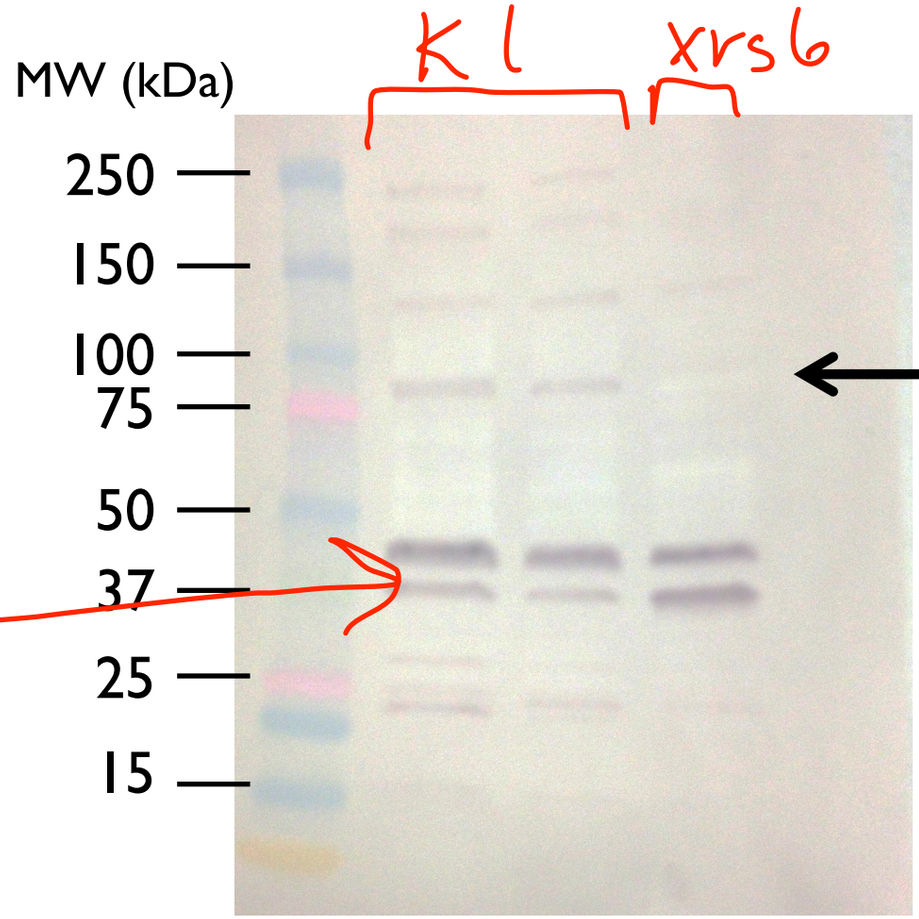
Validate the system - confirm decrease in Ku80 expression .

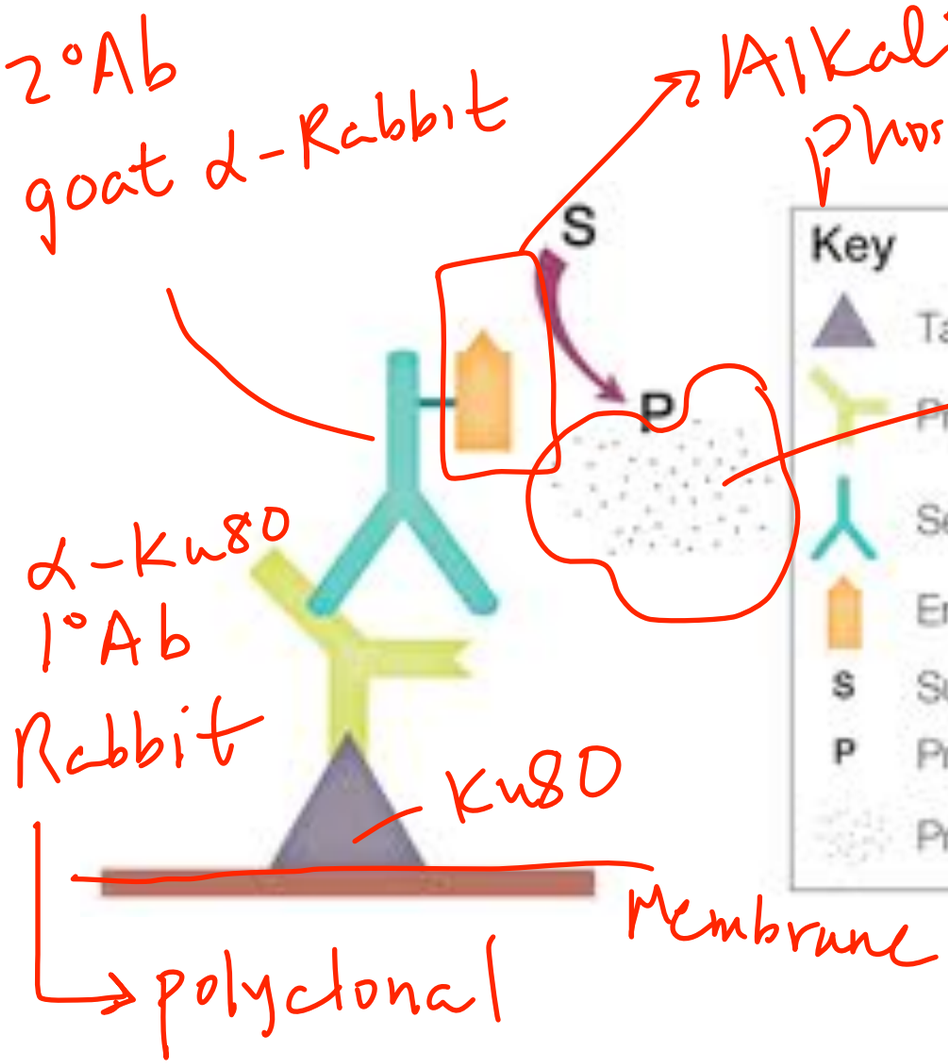


CHO-K1 cells



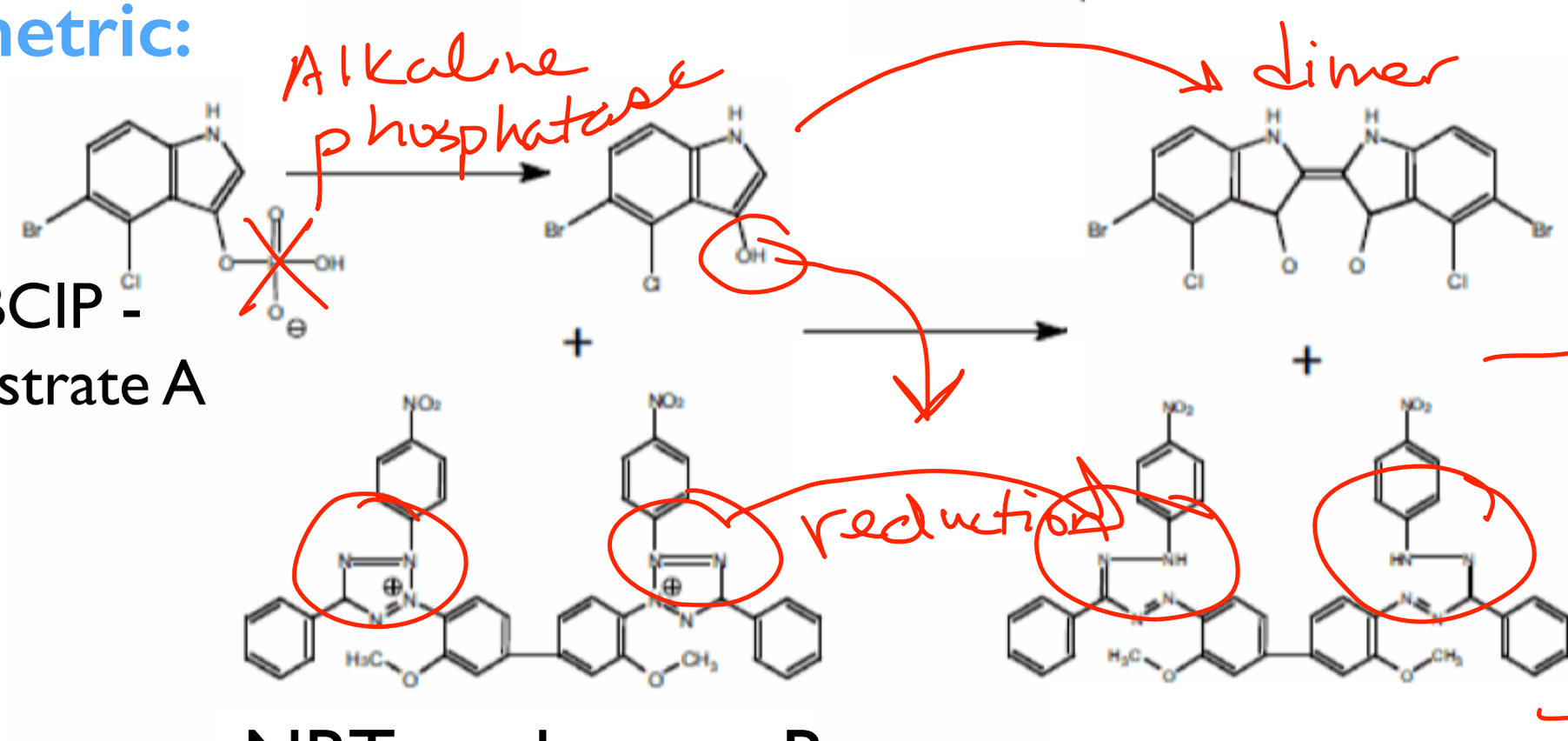
CHO-xrs6 cells





Western blot detection systems:

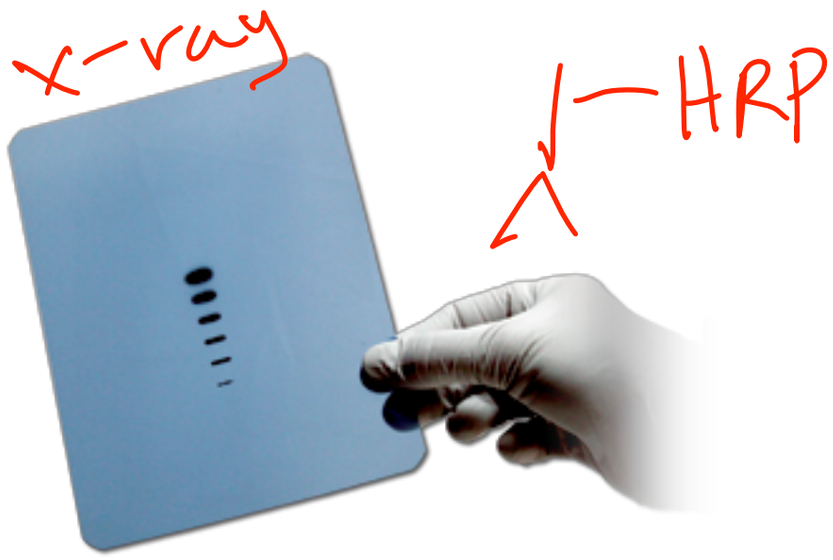
Colorimetric:



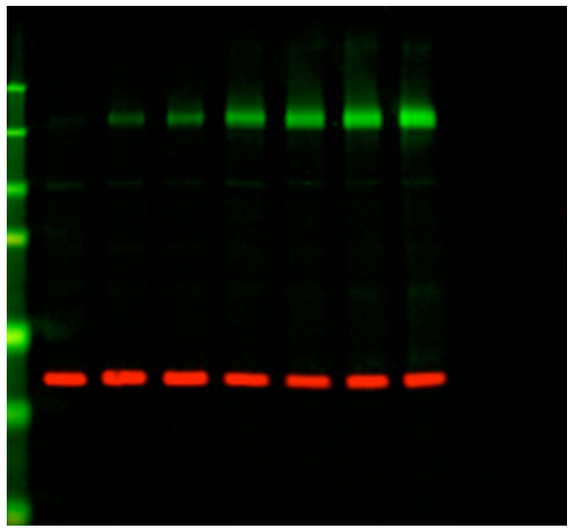
BCIP - substrate A

NBT - substrate B

Chemiluminescent:

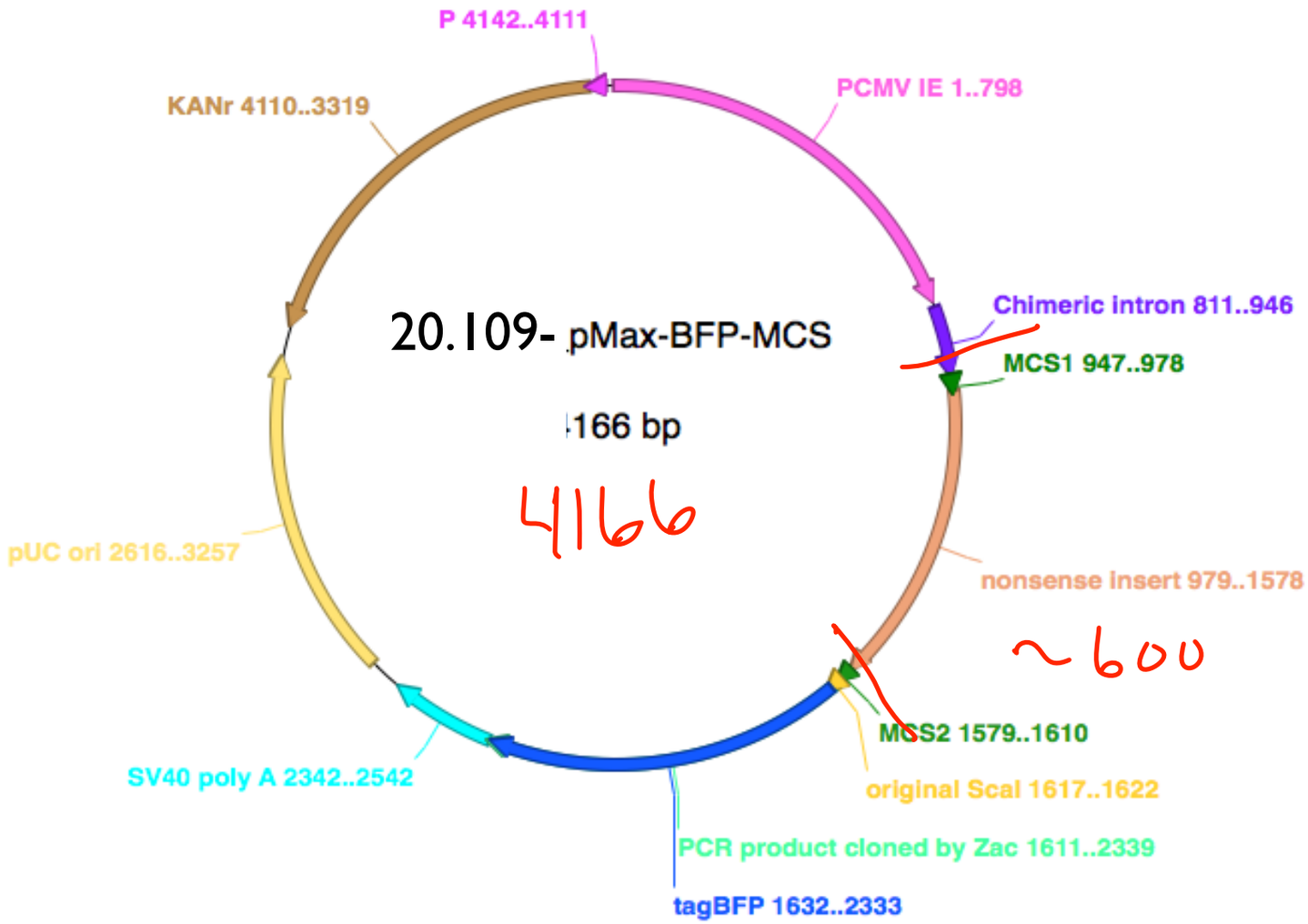


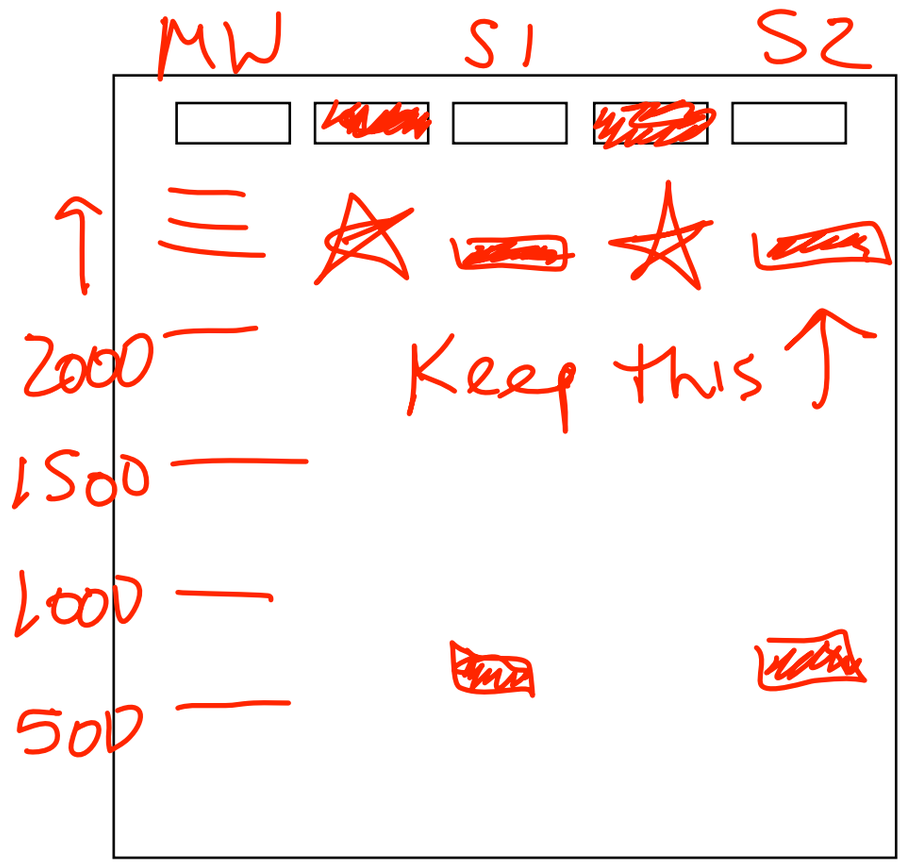
Fluorescent:



Chemical reaction pathway from sigma.com: http://www.sigmaaldrich.com/technical-documents/articles/biofiles/colorimetric-alkaline.html

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





RE Digest Calcs

$V_{e\,(full\ strength)} = 0.5\,\mu L$

$V_T = 25\,\mu L = V_{e1} + V_{e2} + V_0$ $\quad (0 \equiv \text{water, buffer, DNA})$

dilute enzyme 1:10 ($1\,\mu L\ e + 9\,\mu L$ 1x Buffer) $\Rightarrow 5\,\mu L$

$V_T - V_{e1} - V_{e2} = V_0 = 25\,\mu L - 5\,\mu L - 5\,\mu L = 15\,\mu L$

$V_0 =$ 1.5 µL 10x buffer
X µL DNA
15 − 1.5 − X = water

① 2 → $V_{e1} + V_{e2}$

② 1 → 2 V_0

③ add enzyme to V_0

Today in Lab:

1. Set-up digest — Dilute enzyme in: 1x buffer
2. Finish WB — handle with care
3. Gel purify digest product — Leave a space!

Due after spring break (woo hoo!):

1. Microbiota re-write (final due on M2D6) ✓ Friday after break
2. · Western Blot Figure + caption
3. · Cell doubling time
4. · Transfection calculations