M2D2: Begin WB Analysis + Pick Damage Conditions

3/17/15

1. Pre-lab discussion — primer memo, Western blots, NHEJ assay
2. Lyse cells
3. Measure total protein concentration
4. SDS-PAGE & Transfer
5. Investigate DNA repair sensor — pick your conditions!

Primer Design Memo — due Thursday, 10pm

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.

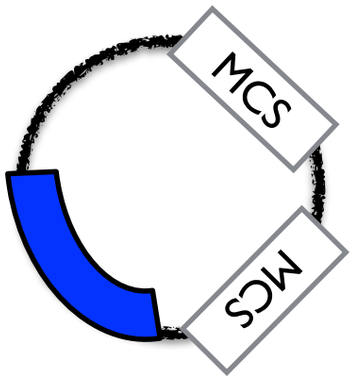
Conclusion [edit]

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 AIV Screening diagnostics or suggest that the division be redirected to pursue a specific alternative target.

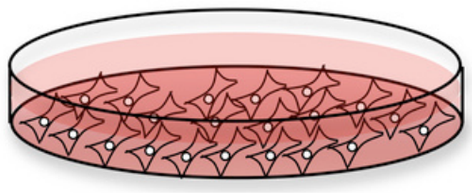
Review of Mod2 goals:

Non-technical manner:

How good is NHEJ at repairing different types of DSB?

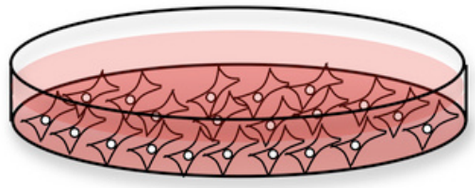


CHO-K1



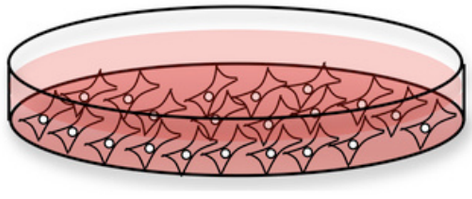
"Normal cells"

xrs6



"DNA repair-deficient cells"

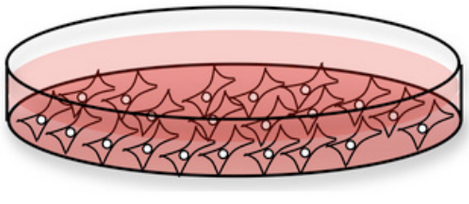
Ku-80 -/-



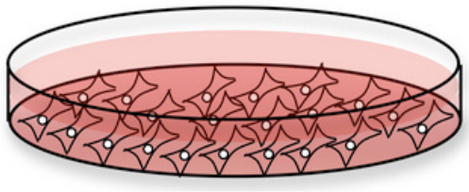
"Normal cells + inhibitor of DNA repair"

double strand breaks

First: validate the system.



CHO-K1 cells



CHO-xrs6 cells

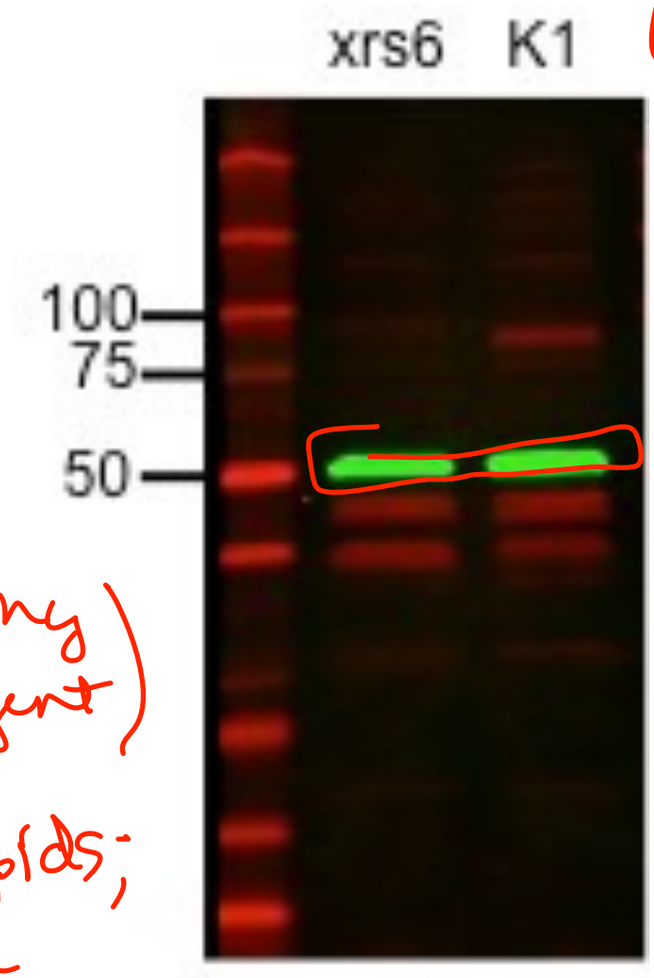
lyse

protease inhibitor cocktail

Lysis Buffer

RIPA buffer

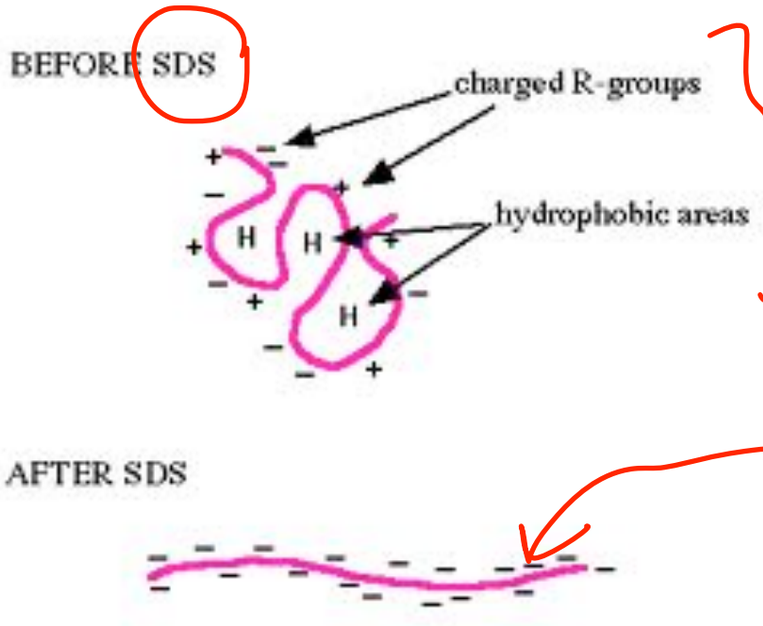
- Triton-X (detergent) strong
  - dissolve lipids; solubilize protins
- Mg^{2+}/EDTA
  - stabilize protins
- Tris-HCl → stabilize protins



Western blot probed with LiCor

Microscop detect flourscent signal FarRed
- 680nm
- 800nm

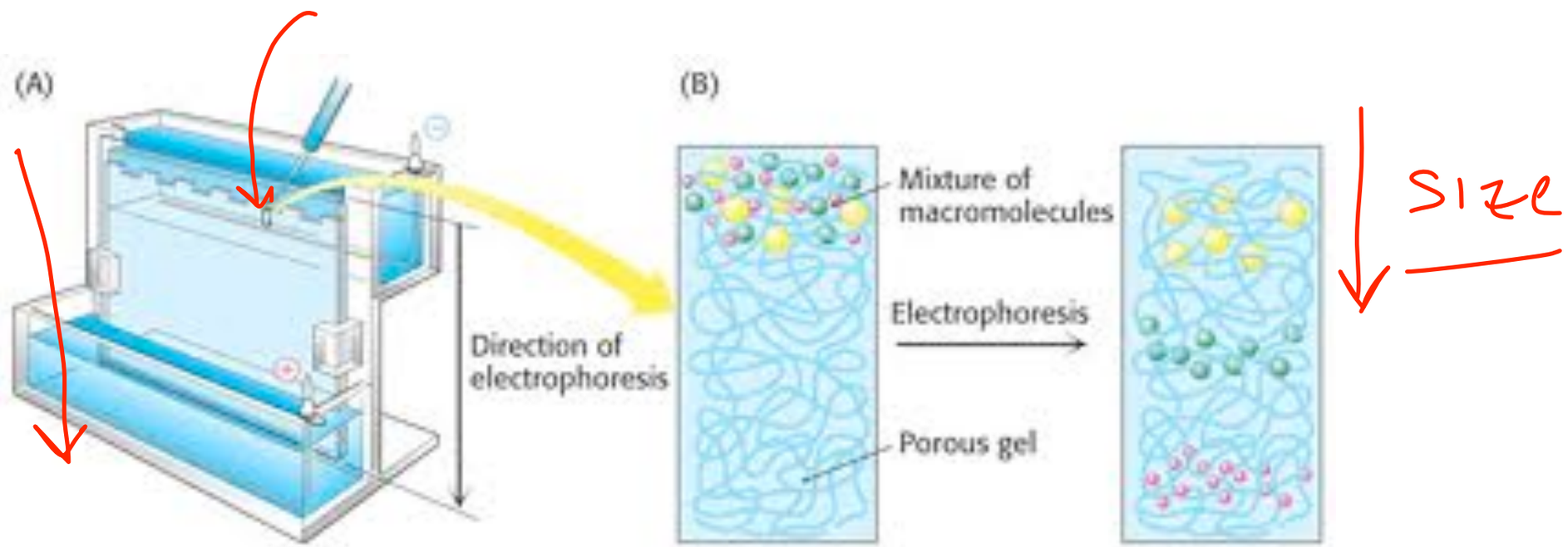
Western blot analysis: Step 1



make all proteins the same charge

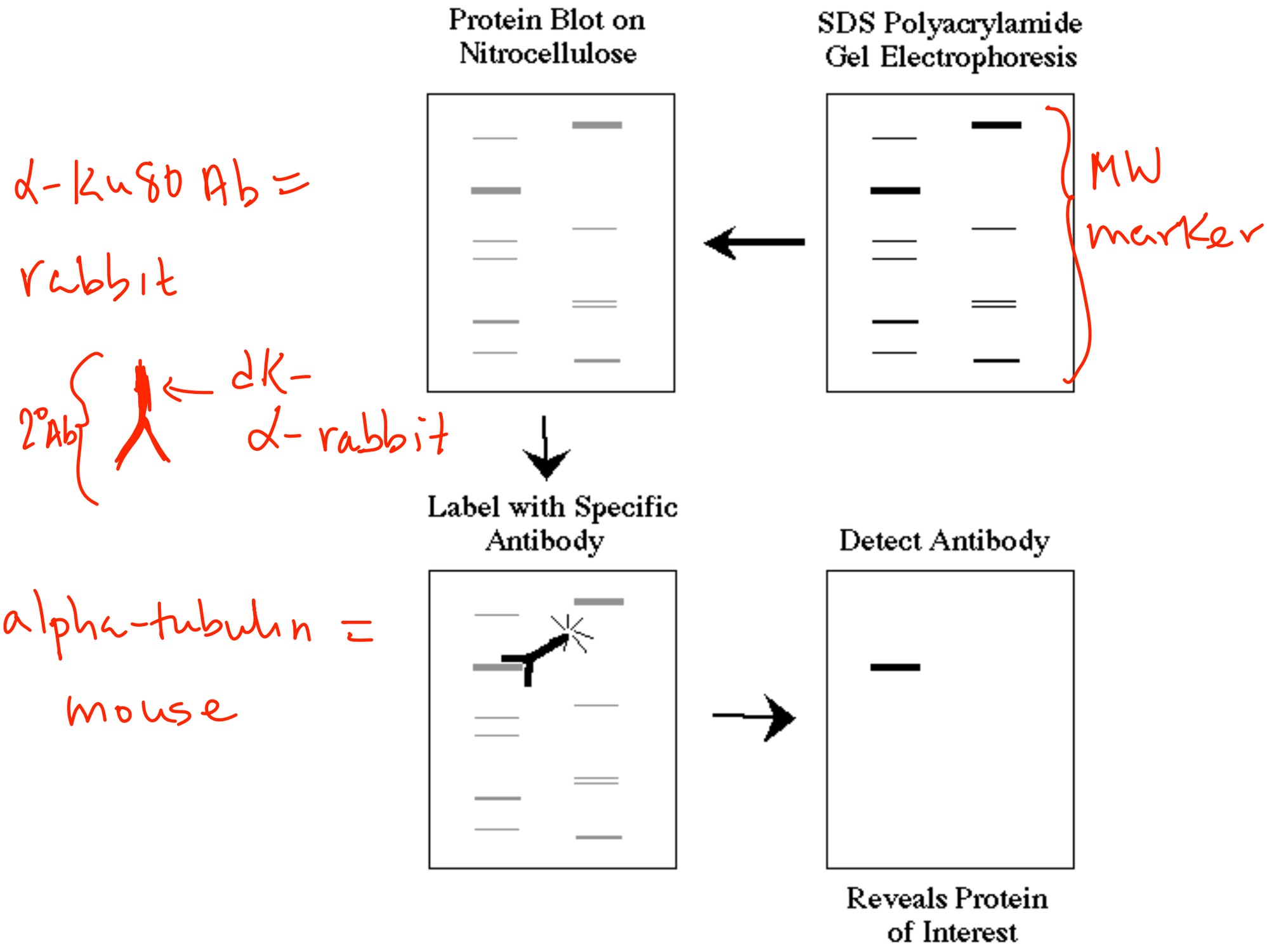
Sample buffer
- SDS
- BME - breaks Cys-Cys
- glycerol - holds in well
- bromophenol blue

4-20%



size

Western blot analysis: Step 2

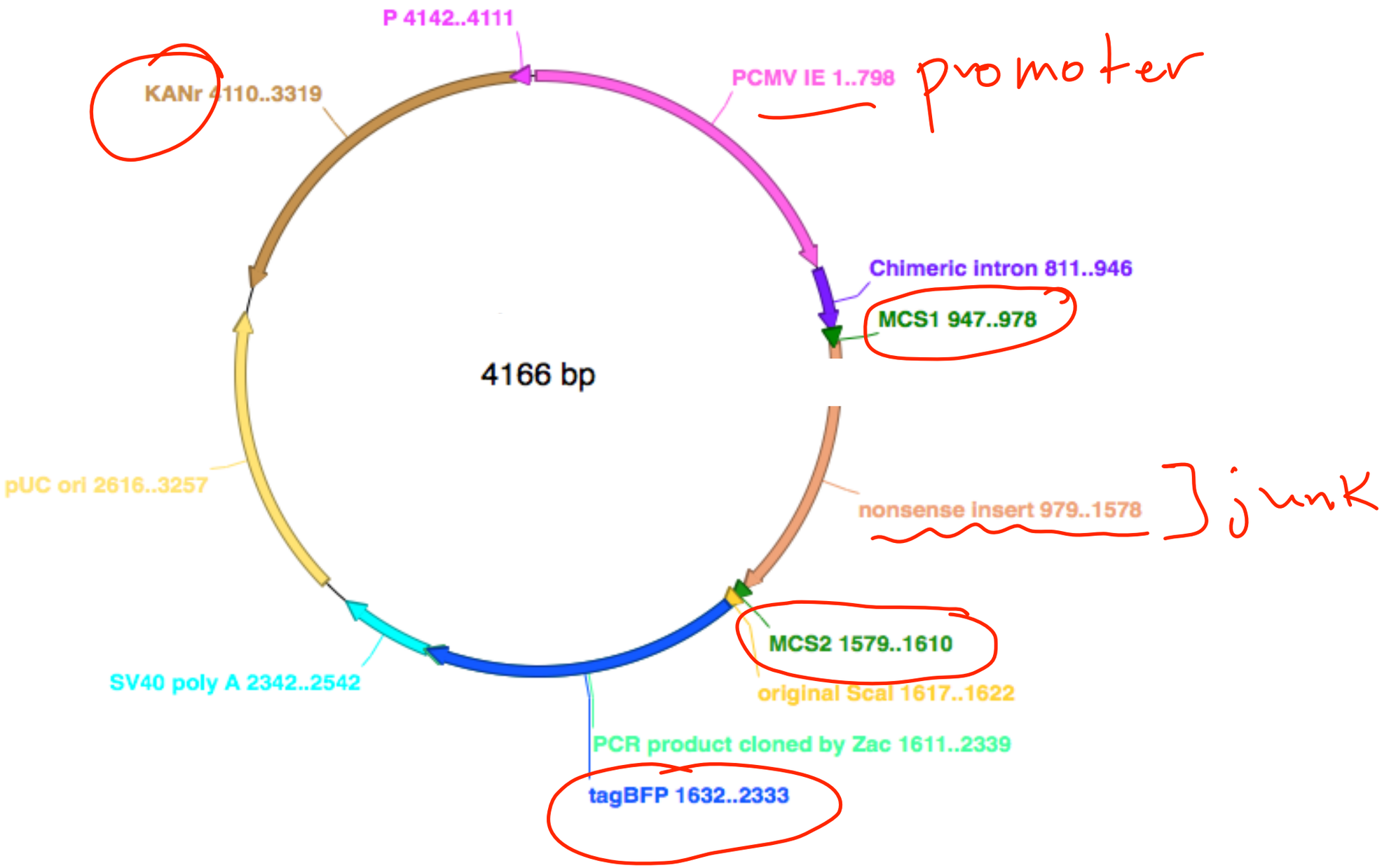


α-Ku80 Ab = rabbit

2° Ab ← α-rabbit

alpha-tubulin = mouse

Today you will build our system (virtually!)



?: How well does NHEJ rep wir

Introduction to Restriction Enzymes

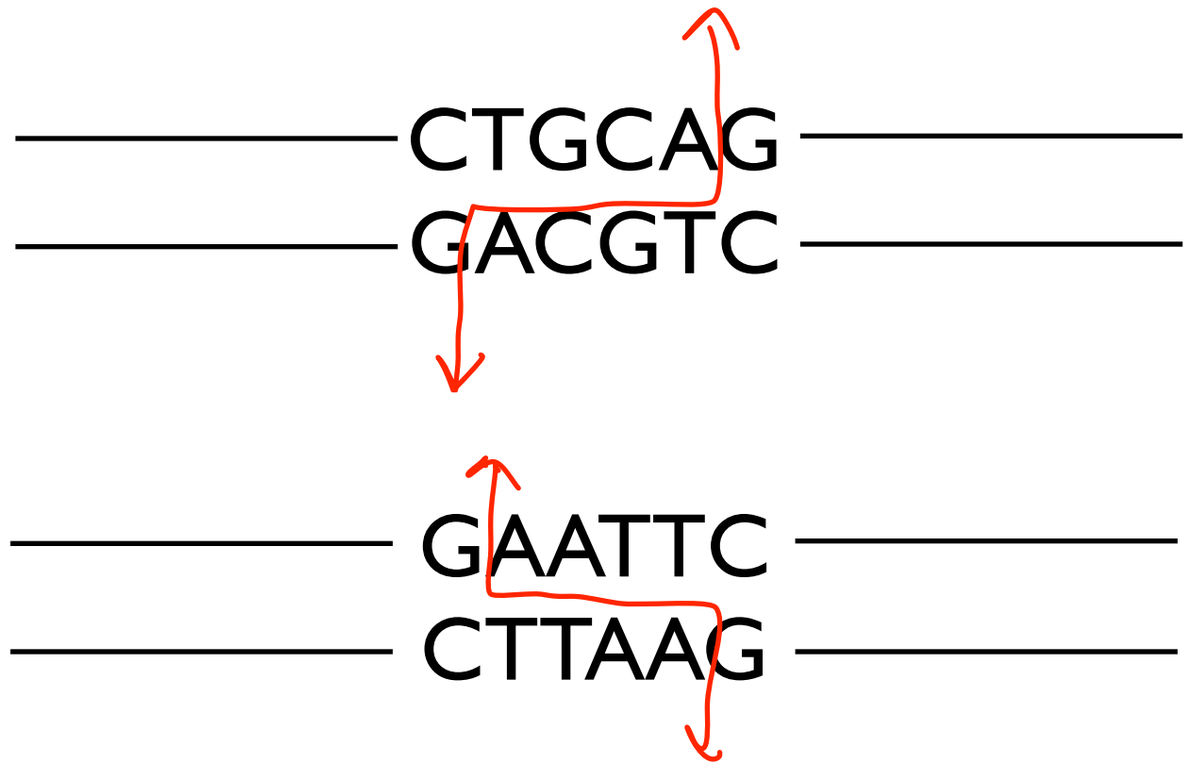
5' 3'

3' overhang

5' overhang

Possible cut topologies:

3' overhang
ex. PstI



5' overhang
ex. EcoR1

Blunt
ex. EcoRV

Our System:

Possible cut topologies:

(1)  Blunt

(2)  Blunt: 5'

(3)  3' : Blunt

(4)  different O.H.

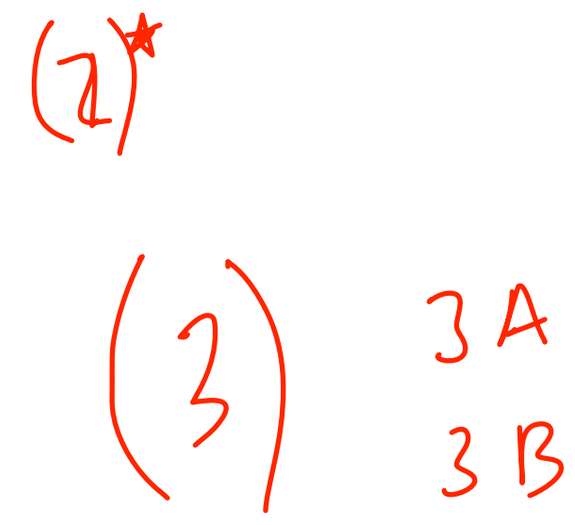
(5)  'sticky end' — 5'

" — 3'

(6) 

(7)  5' + 5'

NHEJ Hypothesis:



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
4. Re-design the NHEJ reporter

Due on M2D3

1. Primer Design memo
2. Pick damage conditions (TALK page) and set-up digest calculator (see homework section)