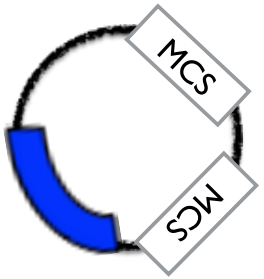
M2D2: Begin WB Analysis + Pick Damage Conditions

3/18/15

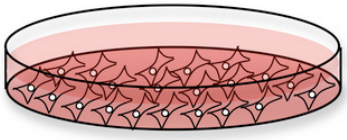
1. Pre-lab discussion — primer memo & Western blots
2. Lyse cells
3. Measure total protein concentration
4. SDS-PAGE & Transfer
5. Investigate DNA repair sensor — pick your damage conditions (add to TALK page)!

Review of Mod2 goals:

How well does NHEJ repair D.S breaks?

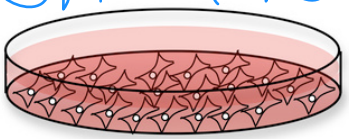


CHO-K1



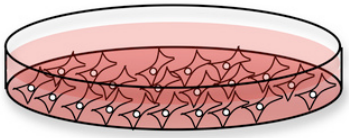
"Normal cells"

CHO xrs6



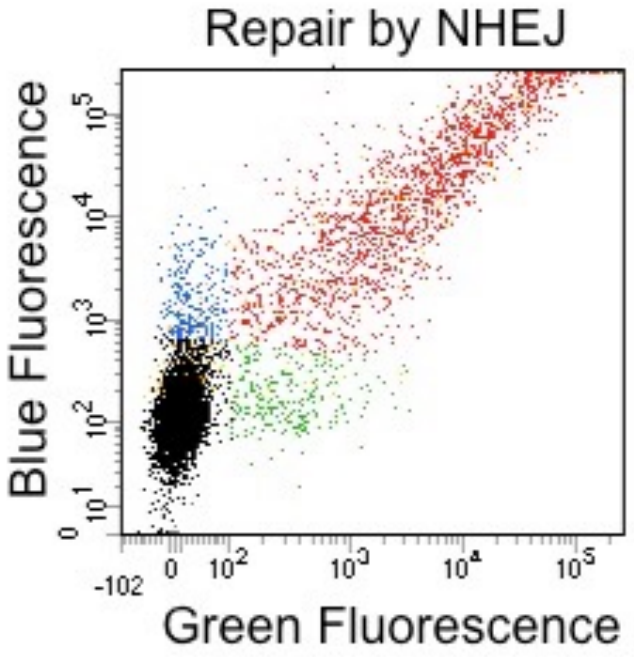
"DNA repair-deficient cells"

Ku80 def



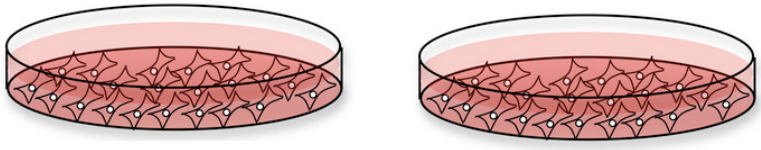
"Normal cells + inhibitor of DNA repair"

CHO + NHEJ inhib.



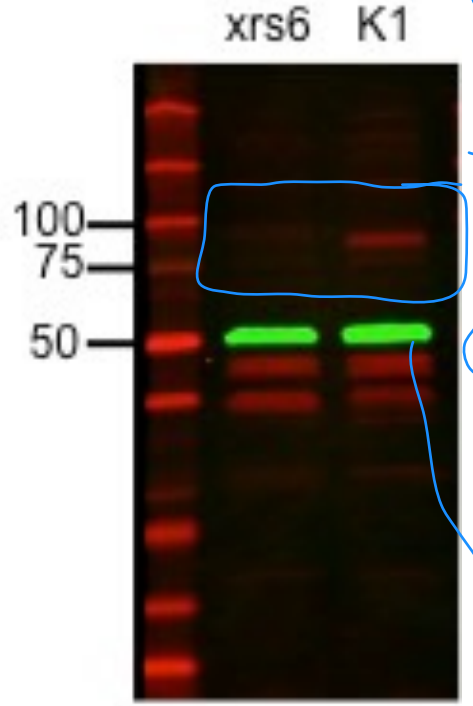
FACS

First: validate the system.



CHO-K1 cells CHO-xrs6 cells

LYSE



Western blot probed with

RIPA

- triton X (strong detergent) - dissolve lipids, soluble protein
- Mg^{2+} / EDTA. stabilize proteins stab cat protein chelator
- protease inhibitor cocktail

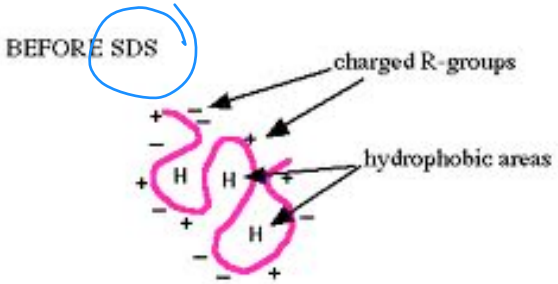
LiCor imager detect flarved

680nm

800nm

why? alpha tubulin

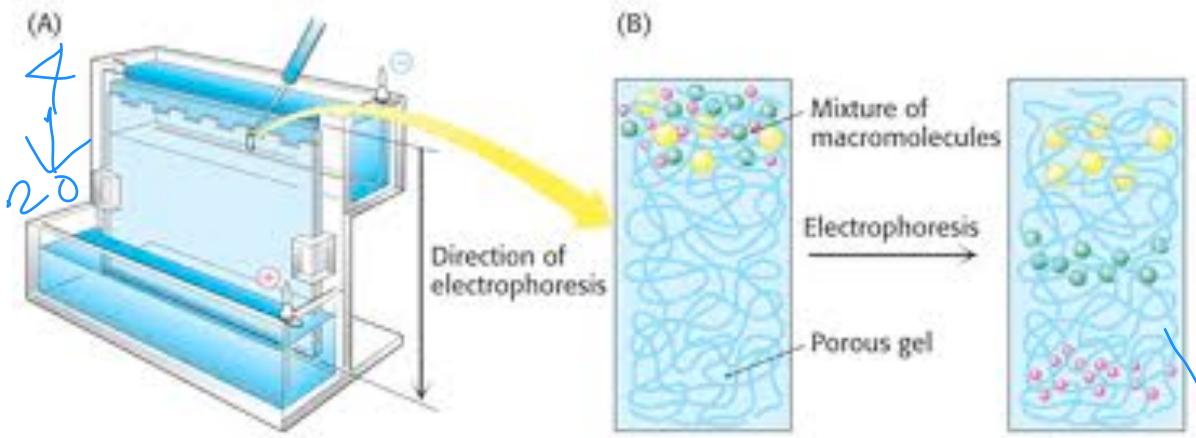
Western blot analysis: Step 1



all proteins
same charge

Sample buffer
- SDS
- BME: breaks cis-Cys
- bromophenol: size marker 3-5 kDa blue

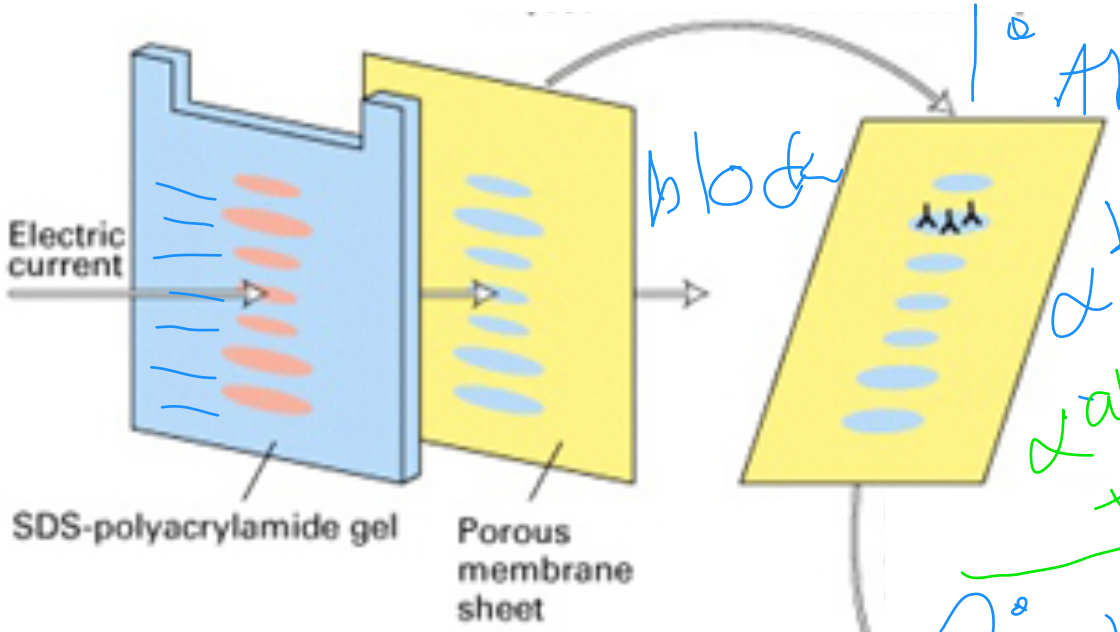
porous
4-20%



big
size
little

α = anti

Western blot analysis: Step 2



block

1° Ab

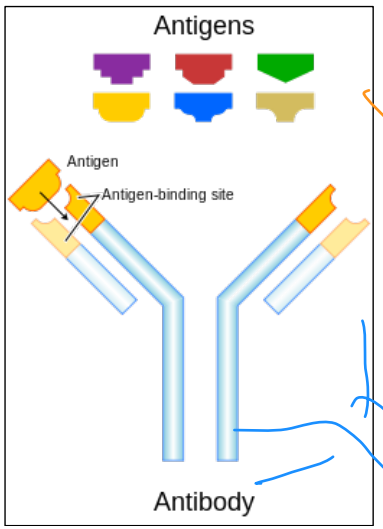
α mouse

α rabbits

alpha = mouse

2° Ab = donkey

2° α mouse 2° 680



variable

constant

α constant R rabbit

"α rabbit 2°" 800

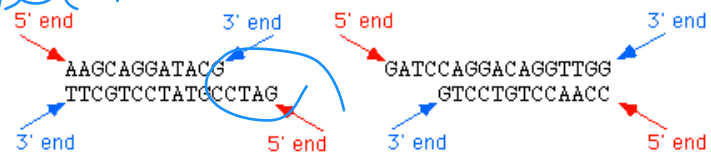
dye

bind + cut palandromes

Introduction to Restriction Enzymes

How well does NHEJ repair

Possible cut topologies:



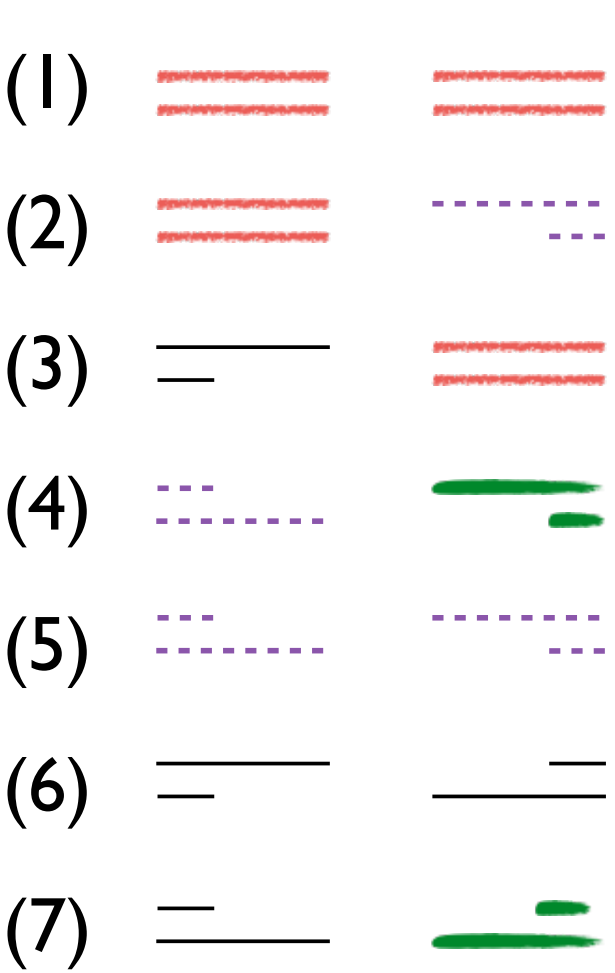
3' overhang

5' overhang

3' overhang ex. PstI	CTGCAG GACGTC
5' overhang ex. EcoR1	GAATTC CTTAAG
Blunt ex. EcoRV	GATATC CTATAG

Our System:

Possible cut topologies:



Blunt

Blunt ; 5'

3' ; Blunt

diff seq OH

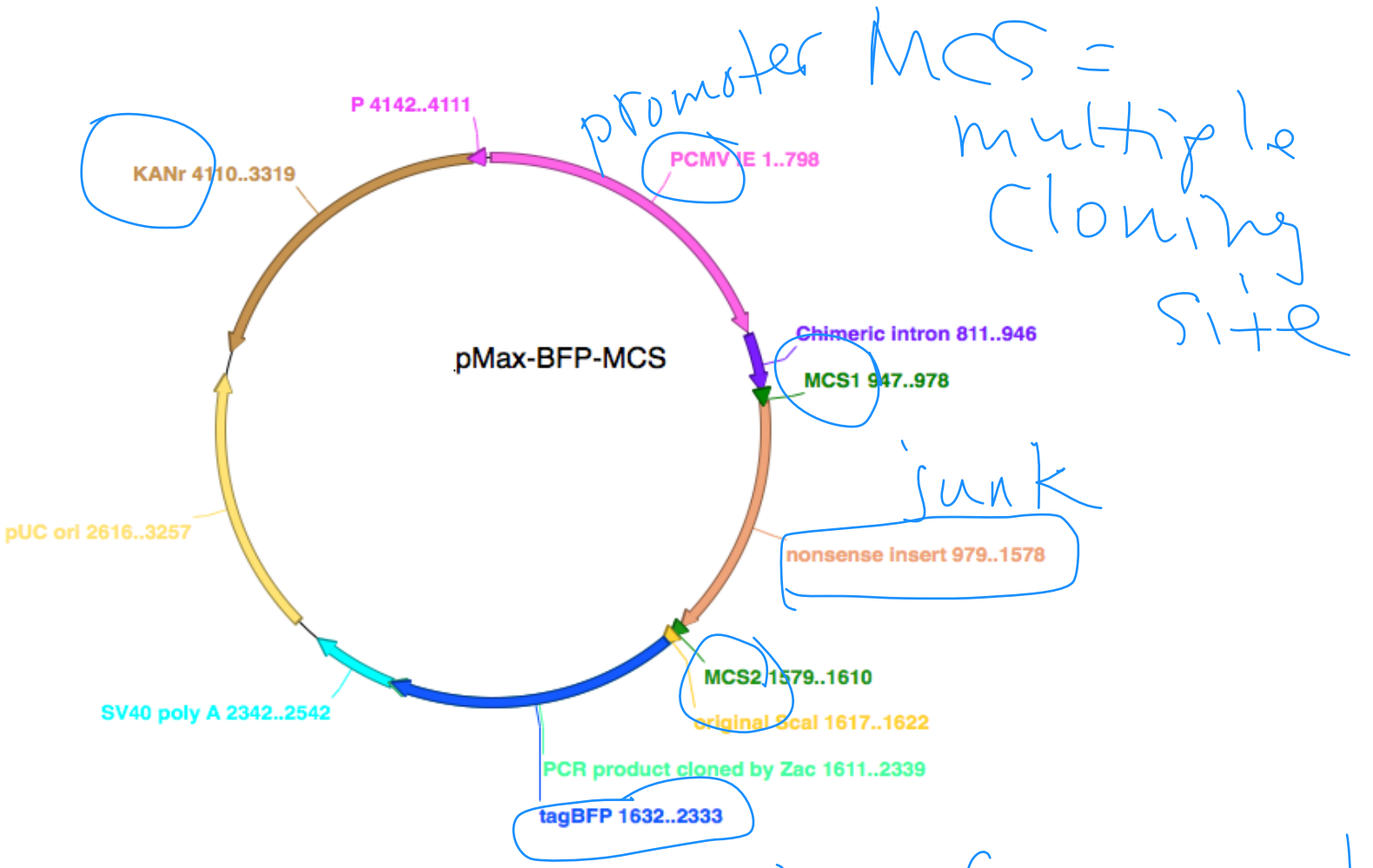
5' sticky

3' sticky

5' missing seq

NHEJ Hypothesis:

Today you will build our system (virtually!)



Today in Lab:

1. Lyse cells
2. Measure total protein concentration
3. SDS-PAGE & Transfer
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)