

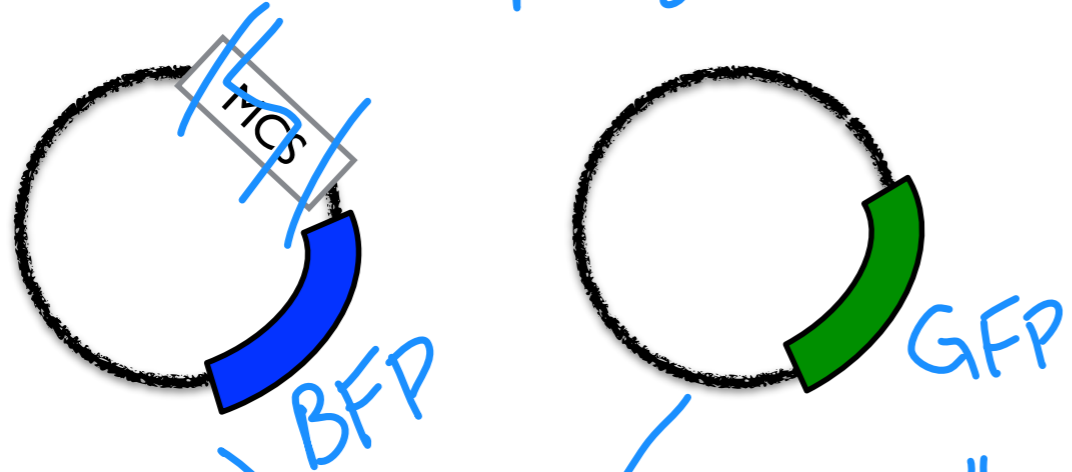
# M2D I: Introduction to cell culture

## Announcements

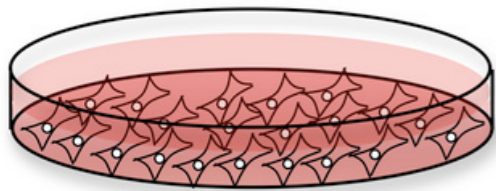
- **Module I Data Summary due Wednesday at 1 pm**
  - ★ submit to Stellar
- **Module I Primer Memo due Tuesday, March 18th**
  - All gels are posted on MID7 Talk page
- Reflection dropbox on Stellar

# Module 2 Overview — It's brand new!

6 cut topologies



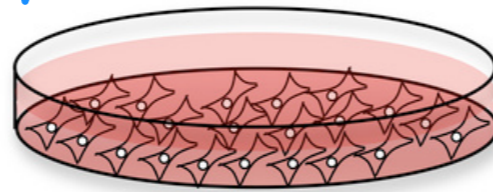
CHO cells



“Normal cells”

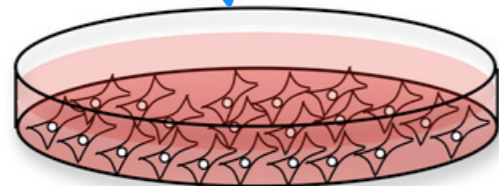
CHO-K1

“co-transfect”



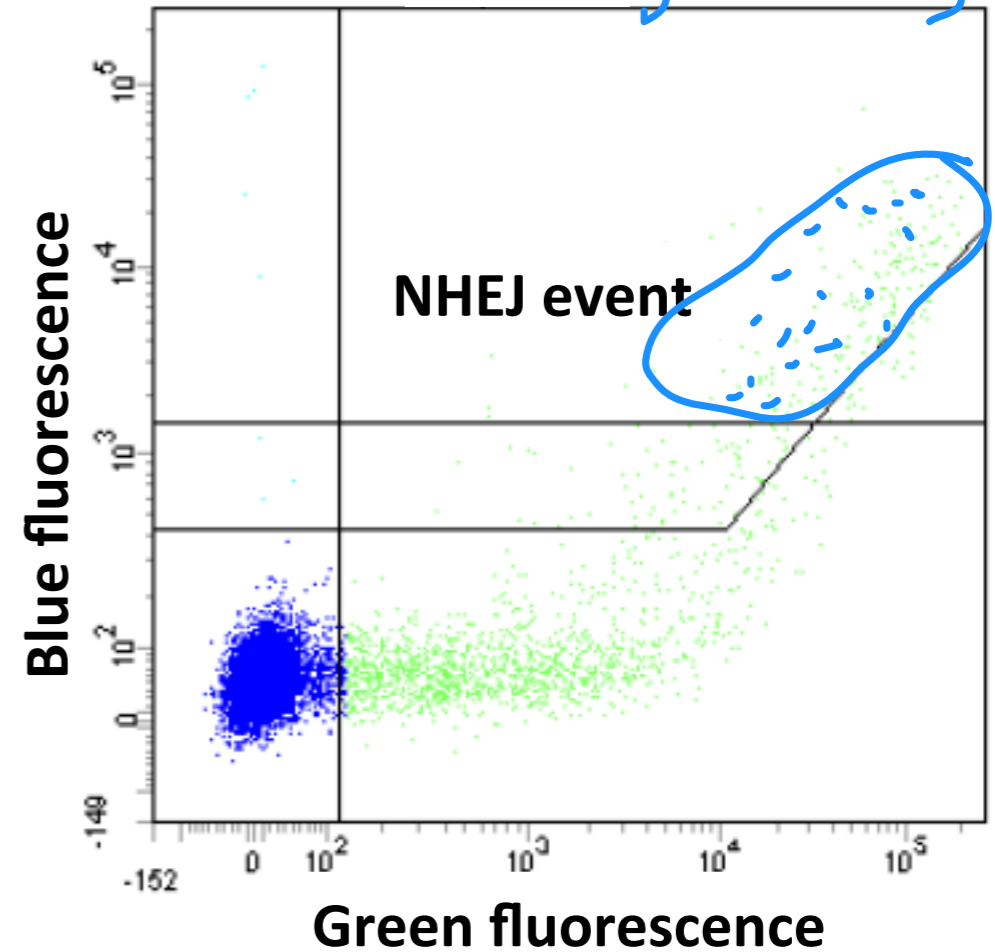
“DNA repair-deficient cells”

~~NHEJ~~ Ku80



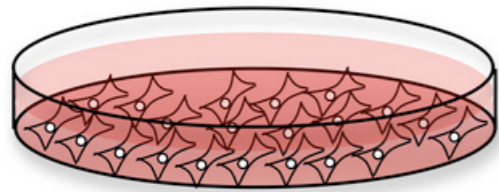
“Normal cells + inhibitor of DNA repair”

Flow cytometry

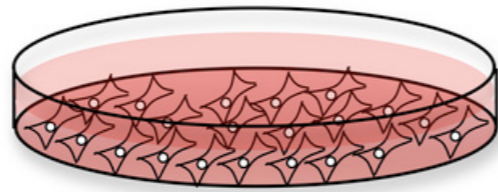


# Tools to study DNA repair: Our model system

M2D1

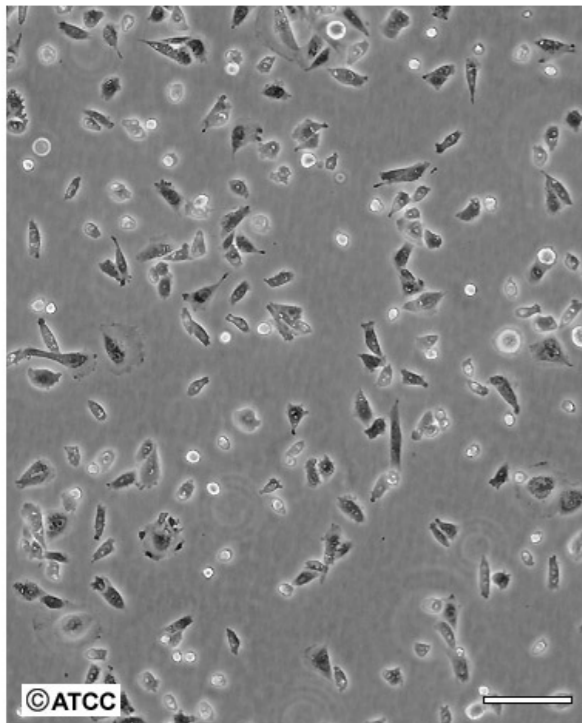


“Normal cells”  
CHO-K1



“DNA repair-deficient  
cells”  
= xrs6  
No Ku80

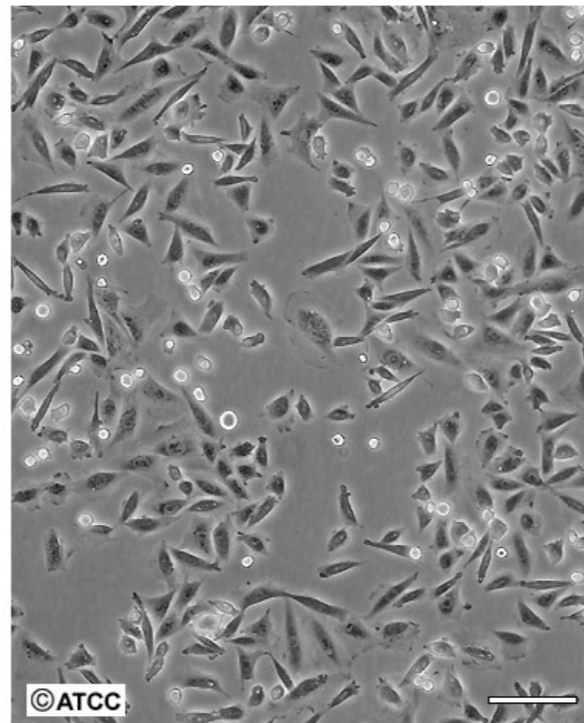
ATCC Number: CCL-61  
Designation: CHO-K1



©ATCC

Low Density

Scale Bar = 100µm



©ATCC

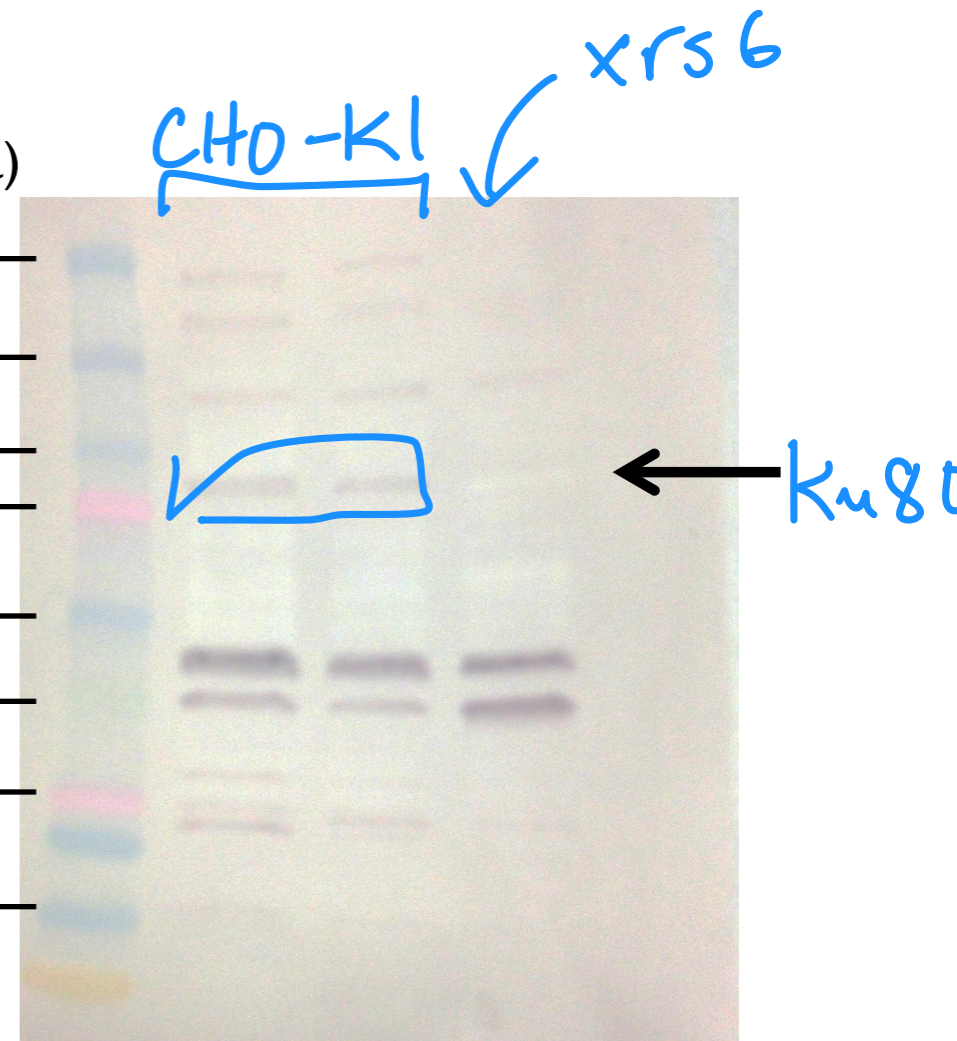
High Density

Scale Bar = 100µm

Start M2D2

MW (kDa)

250 —  
150 —  
100 —  
75 —  
50 —  
37 —  
25 —  
15 —



Western blot  
probed with  
 $\alpha$ -Ku80 antibody

# Mammalian cell culture — Tissue culture medium

What do cells need to survive?

Food(s):

1) Energy source

- 1) glucose
- 2) L-glutamine
- 3) sodium pyruvate

2) Building blocks  
a.a.

2) non-essential a.a.

3) vitamins & minerals

3) pro-life signals

FBS  
L-growth factors & cytokines  
lipids

DMEM



- Antibiotics  
- phenol red

Non-food(s):

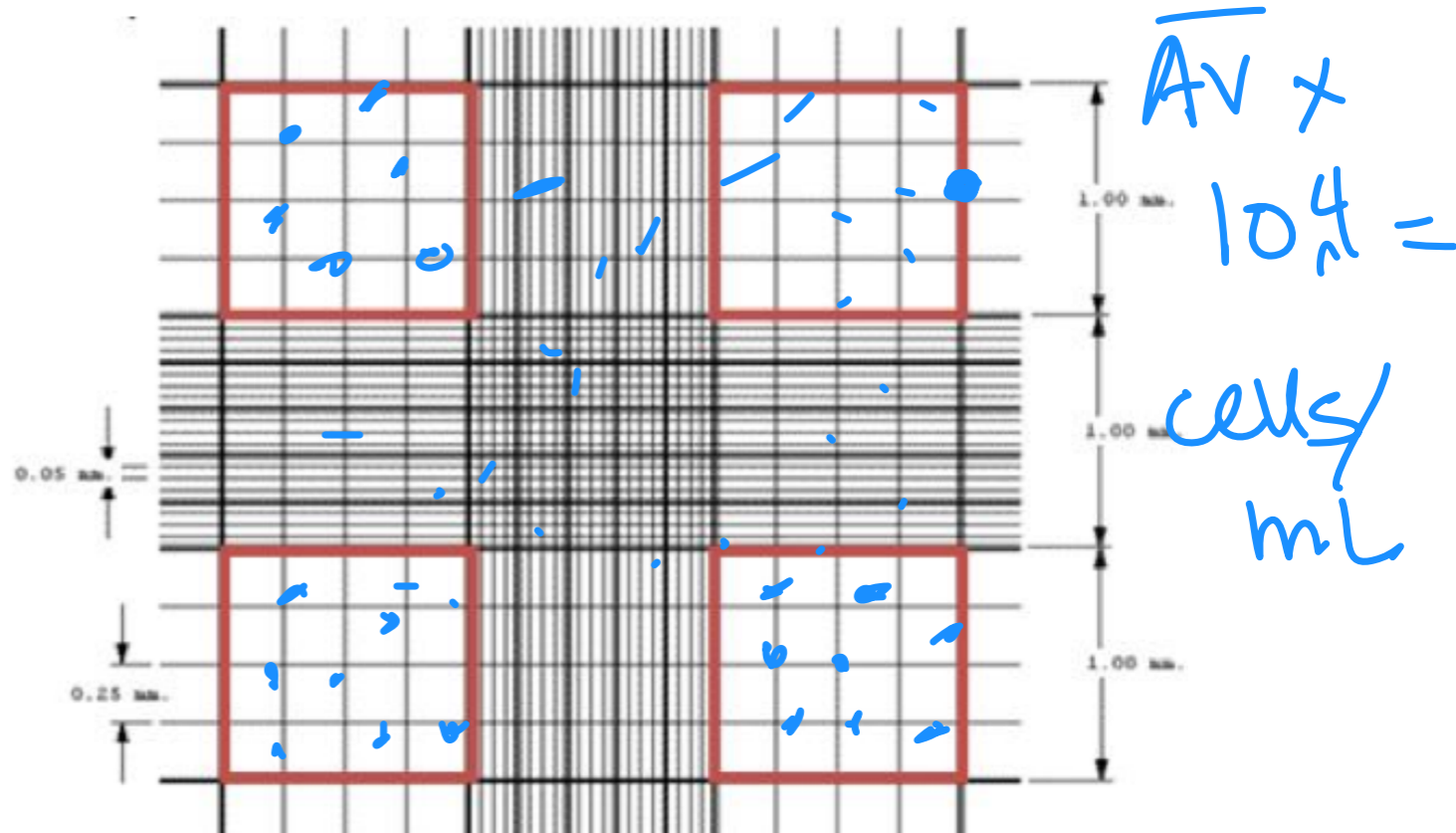
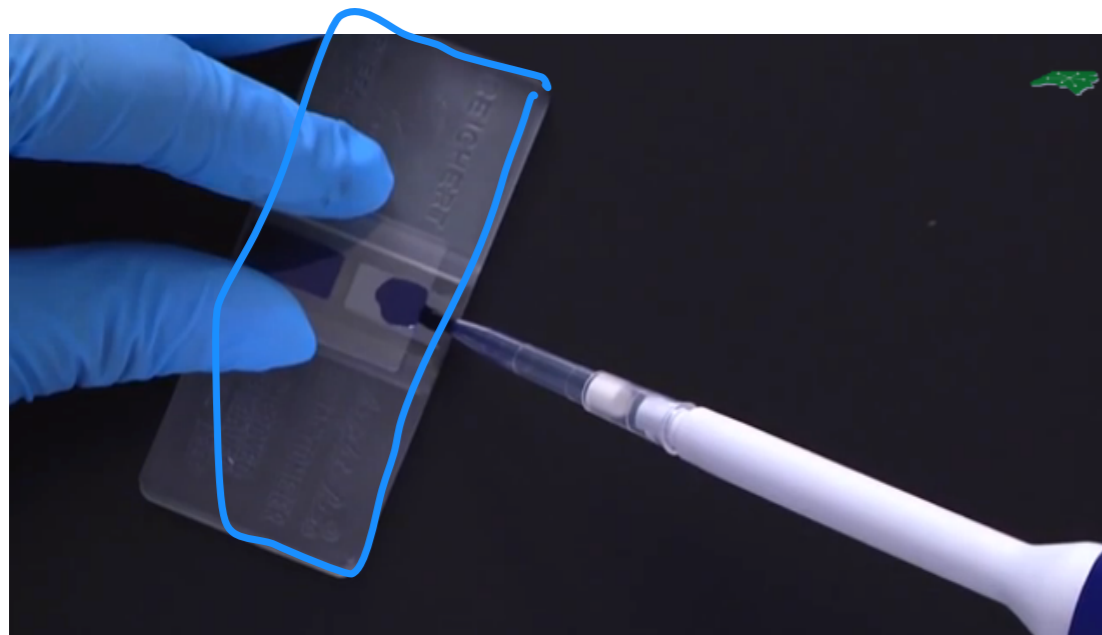


1-CHOX1

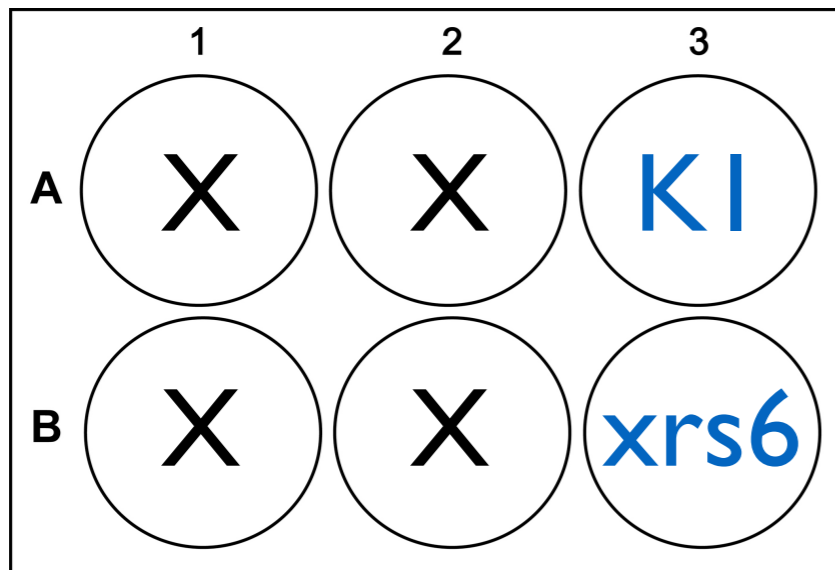
1-xrs6

# Mammalian cell culture — 'Plating' cells

Hemocytometer



200,000 cells/well



- 1) 90  $\mu$ L of cell suspension
- 2) + 10  $\mu$ L trypan blue
- 3) 10  $\mu$ L (of your 100  $\mu$ L) count

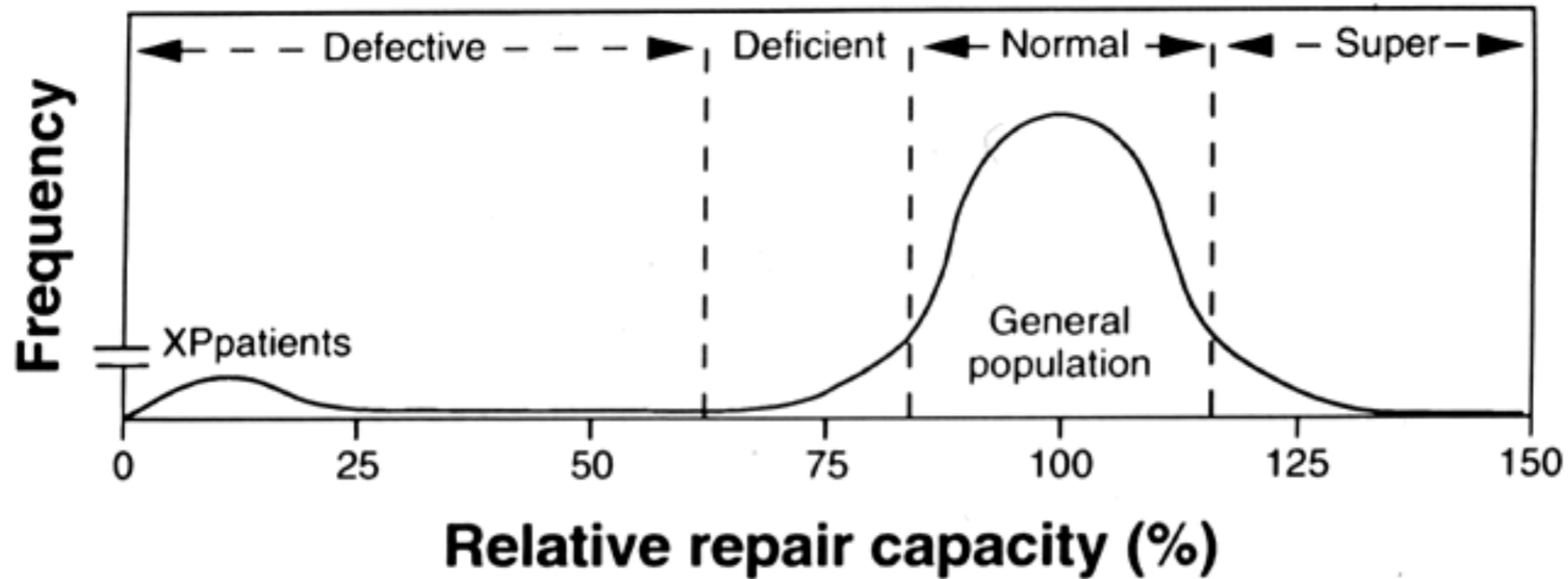
<http://www.cellsignet.com/media/templ.html>

<https://www.youtube.com/watch?v=pP0xERLUhyc>

<http://www.allcells.com/blog/how-to-count-fresh-primary-cells/>

Motivation

## Interindividual Variation in DNA Repair Capacity



Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

XP frequency =  $\sim 1:250,000$  giving a theoretical maximum of  **$\sim 28,000$  cases** worldwide with 2,000-fold increased risk

Even if just 1% of the population is relatively repair deficient, could have **tens of millions** with several-fold increased risk

# Today in lab:

★Seed cells for Western blot analysis of Ku80 expression:

Red/Orange/Yellow — in TC first

★Learn about our system:

I. Read paper from Jeggo lab

- Answer questions on wiki in your EN notebook
- This is a preview of what we'll be talking about — don't stress
- Speaking of — share your notebook with Su!

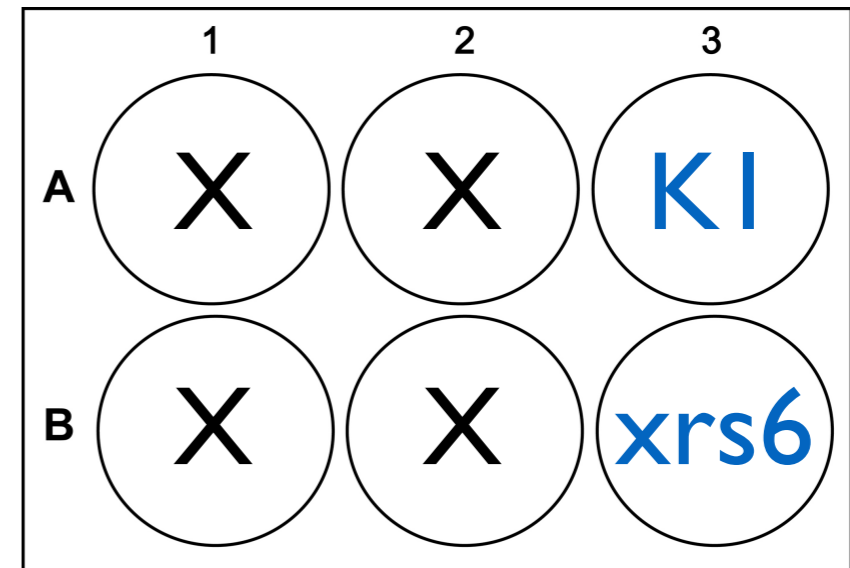
New modules are fun! Day-by-day pages may be J.I.T.

# Mammalian cell culture — ‘Splitting’ cells

1. Rinse with PBS — why?
2. Detach cells — why?
3. Count cells — why?
4. Add to new culture vessel — why?



Flask



Plate