- Announcements
- Pre-lab Lecture
  - Mod 2 overview
  - Cell/tissue culture basics
  - Today in Lab (M2D1)

Announcements

- Introducing… Su, your TA for Module 2
  - share your EN notebook with svora (MIT)

- Module 1 primer design memo due Wed, Mar 19th at 11 AM
  - all gels posted on M1D7 Talk page
  - sensitivity groups who want VC template test: tell me ASAP!
- Reflection dropbox now on Stellar
  - please submit electronic copy for posterity
  - and hard copy for immediate access
- Module 2 is brand new!
  - hence, evolving in real time
  - quiz dates long fixed; notebook dates changed recently
  - protocols etc. may be up last-minute

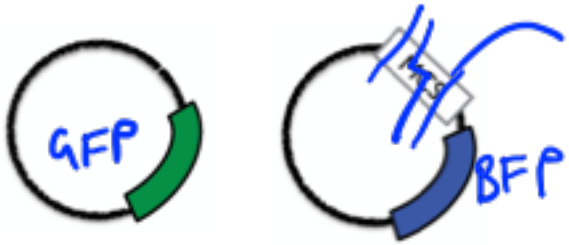
our shared adventure

Module 2 goal and approach

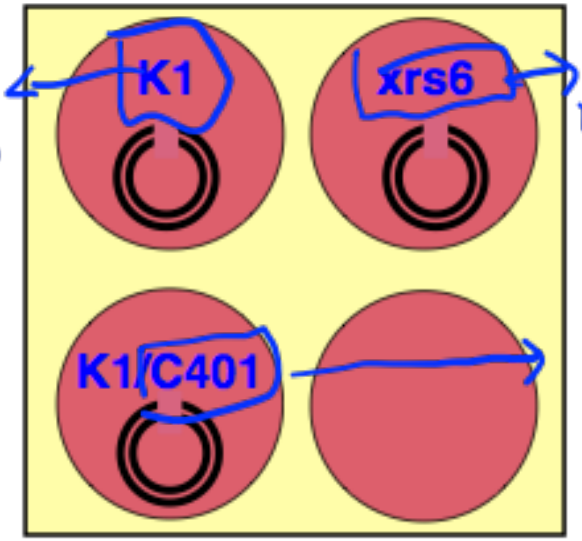
Question: How does changing DNA damage topology affect NHEJ?

Co-

Transfect cells with damaged DNA and intact control



MCS = multiple cloning site = group of restriction enzymes

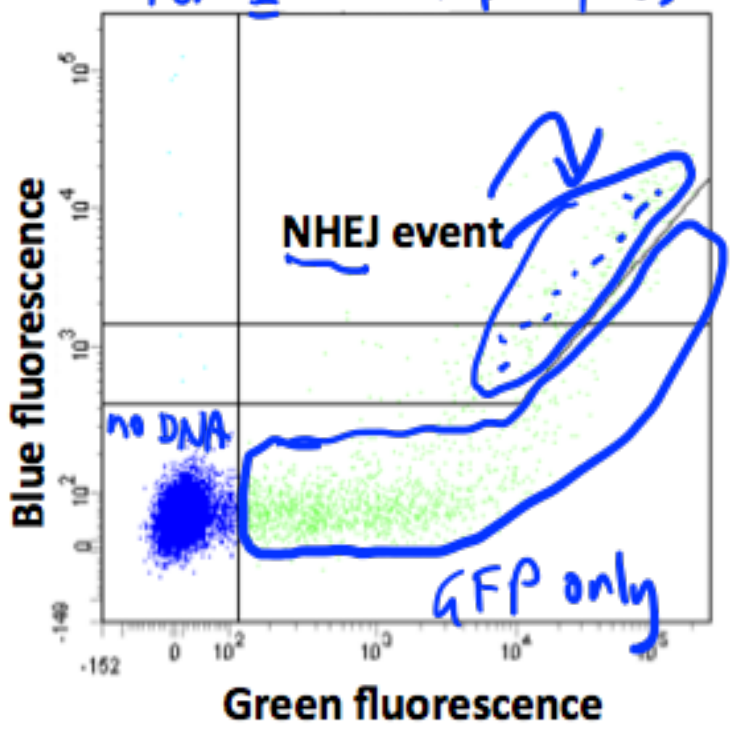


WT CHO

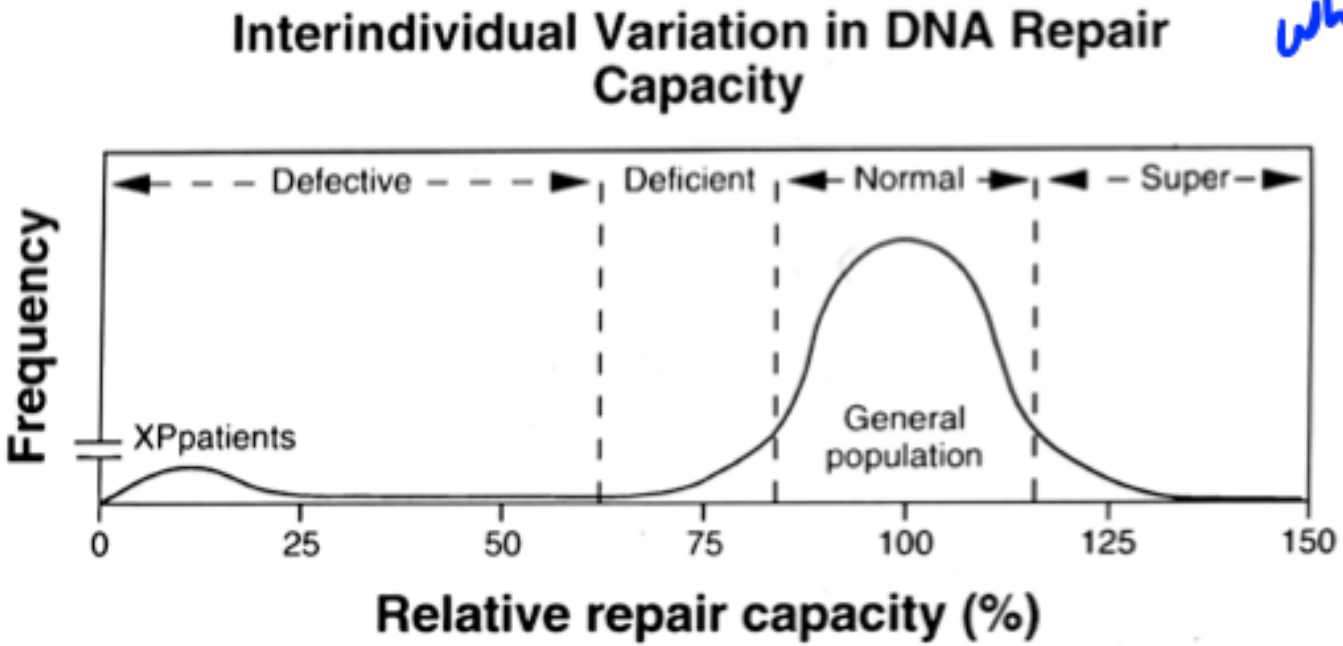
repair-deficient lack Ku80

chemical inhibitor of repair

Measure repair by flow cytometry for 6 cut topologies



Module 2 context – L. Samson



why we care about measuring DNA repair

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

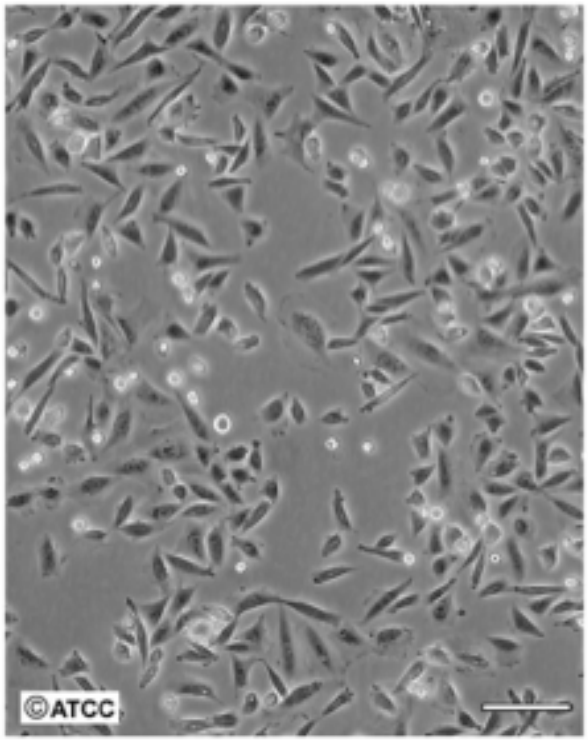
XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

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

Module 2 first steps

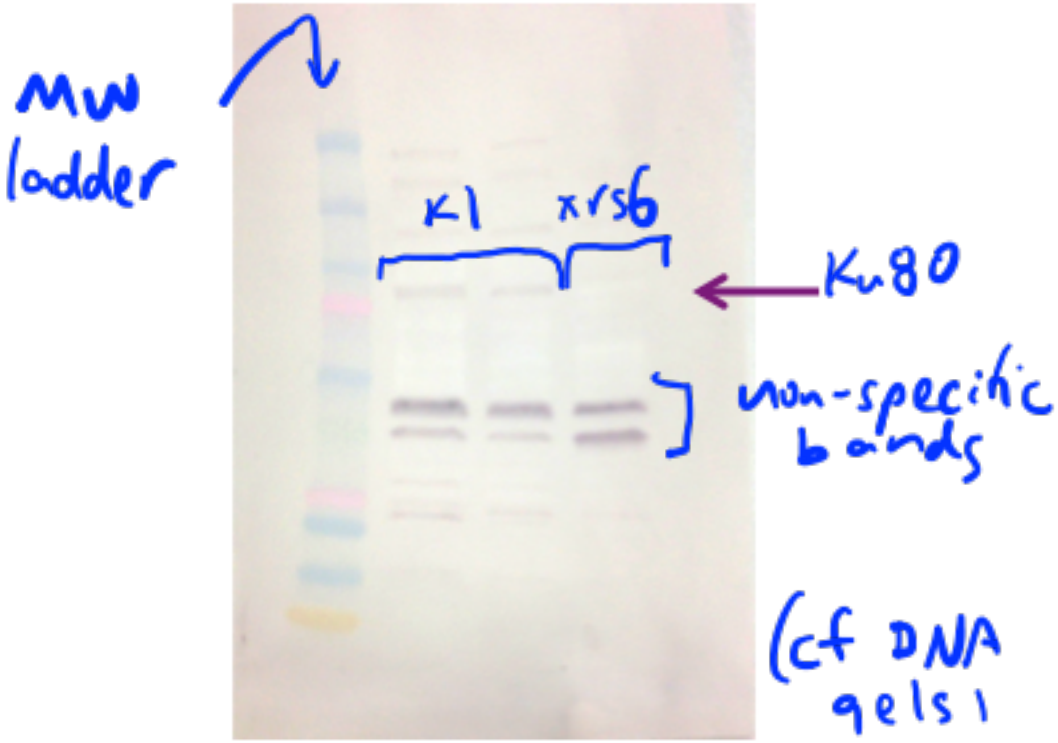
DAY 1

Plate K1 and xrs6



DAY 2 + ~~3~~ 4

Measure Ku80 levels



Tissue culture (TC) medium

- What do cells need to survive?

Food: energy source
- glucose; L-glutamine; (sodium pyruvate)

building blocks or co-factors for cell rxns
- essential amino acids
- non-essential amino acids *proline*
- vitamins, minerals, lipids

pro-life signals
- cytokines (e.g., growth factors)

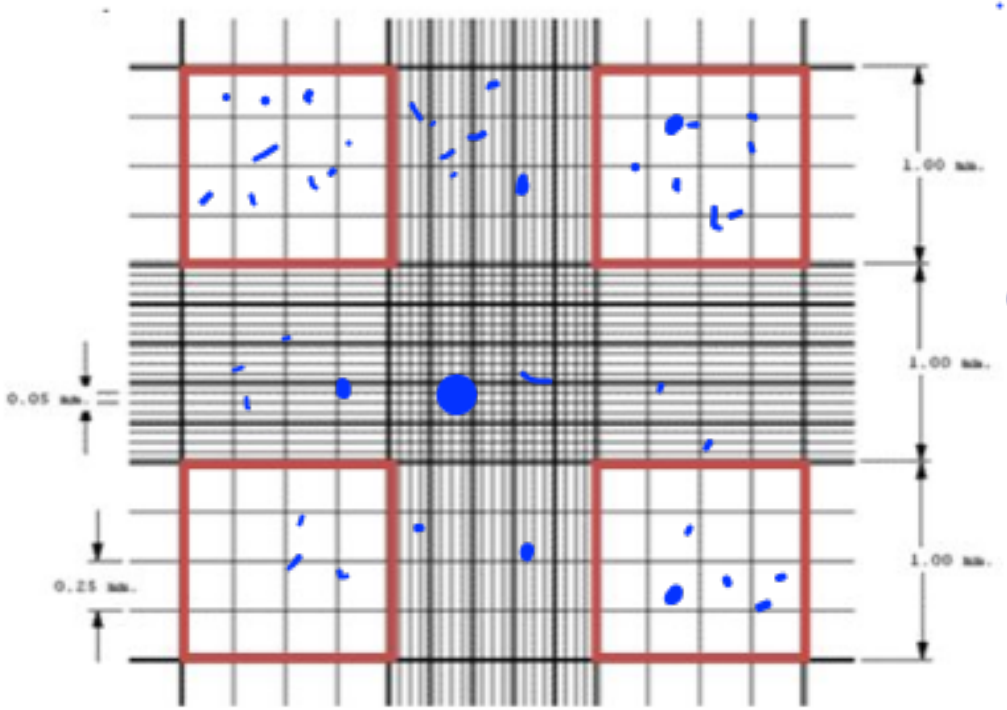
serum (~blood)

Non-food: antibiotics (P/S)
phenol red (tracks pH)

base

-make additions

Plating cells for culture



1) 90 mL cell suspension

2) + 10 mL Trypan blue

3) take 10 mL (of 100) and count

<live #> x 10^4 x dilution ratio = cells/mL

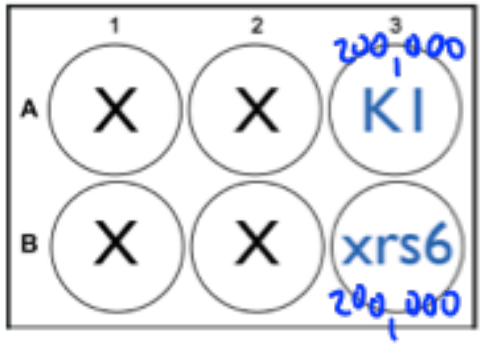


plate a known amount

Hemocytometer image: www.allcells.com/blog/how-to-count-fresh-primary-cells/
Handy well plate image from (thanks, Shannon!): www.cellsignet.com/media/templ.html

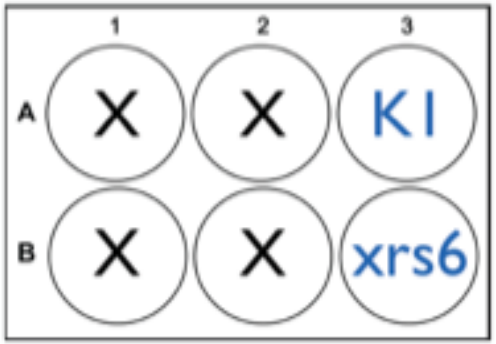
Today in Lab (M2D1)

- Seed cells for Western blot to detect Ku80 expression
  - sterile technique
  - plate known quantity
  - group 1: Blu, Wht, Org, Red
  - group 2:
- Learn about cell component of our system
  - Review ATCC page about CHO-K1
  - Read paper from Jeggo lab about CHO-xrs6
  - Take a stab at wiki questions in your notebook…
  - … but don't stress! We will cover this material together.

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

Slide from Shannon H.

http://www.sigmaaldrich.com/catalog/product/sigma/cls3815?lang=en&region=US