M2D1: Introduction to cell culture

Announcements

- Module 1 Data Summary due **Monday at 5pm**
  - ★ **submit to Stellar**
  - Blog Posts due next week.
- Primer design memos due ~~**Thursday**~~ **at 10pm**
  - Talk more on ~~Tuesday~~
- Office Hours:

Noreen(nllyell@mit.edu) Sunday 10-12pm 56-302

and Monday 2-4pm 16-429b

Shannon (skalford@mit.edu) Sunday 12-2pm 56-302

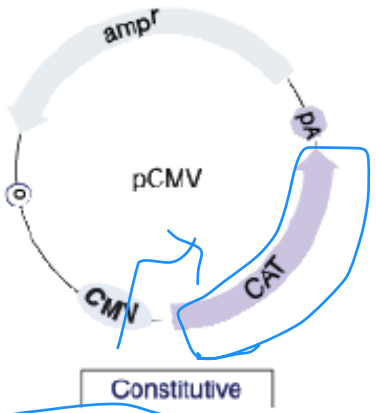
Leslie(lesliemm@mit.edu) Monday 1-2pm

Email us to meet outside of office hours!!!

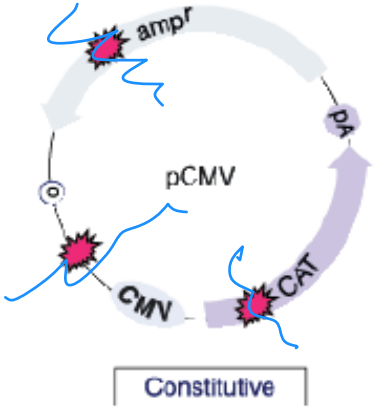
Study DNA damage repair

Reactivation of UV damaged DNA by Host cell Reactivation (HCR)

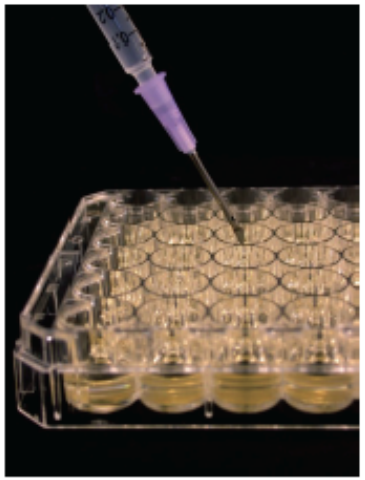
Athas & GROSSMAN
Cancer Res. 1991



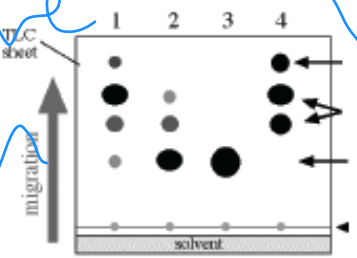
+ UV light



Transient transfection peripheral blood lymphocytes



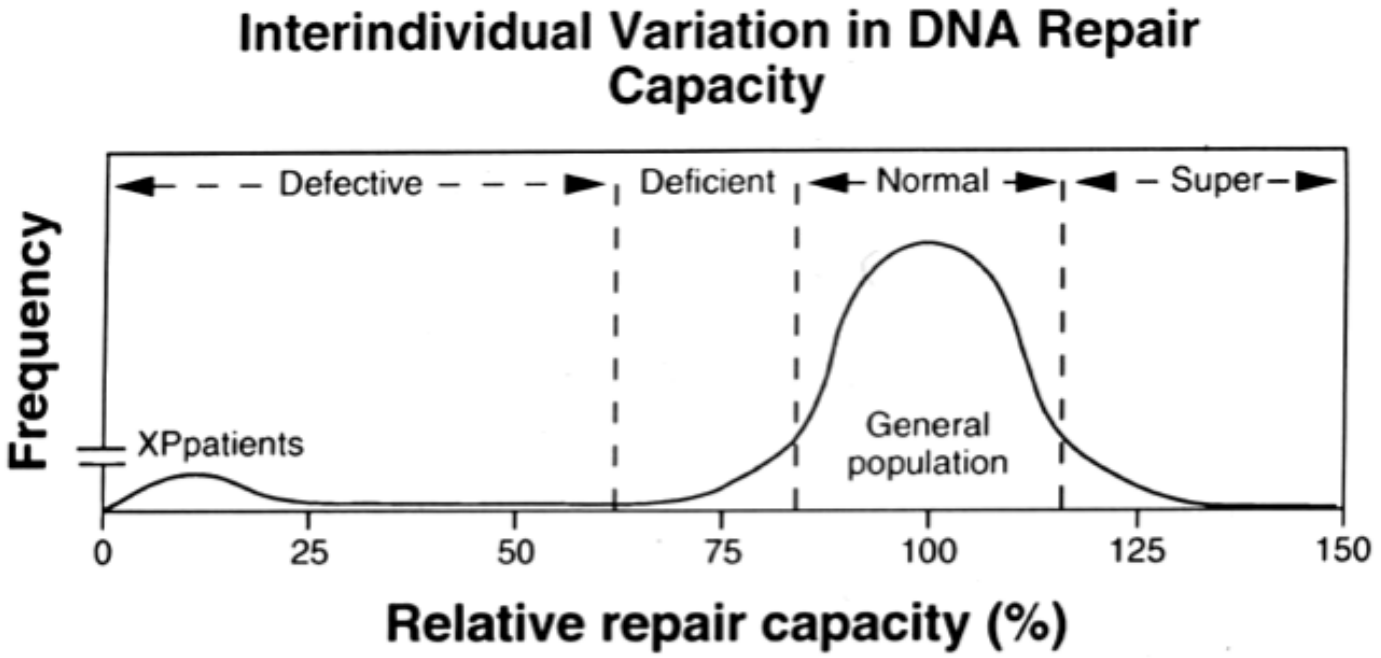
Time to repair
4 hrs



CAT Assay

measure protein = repair

Slide from M2D1 Lecture — Prof. Samson



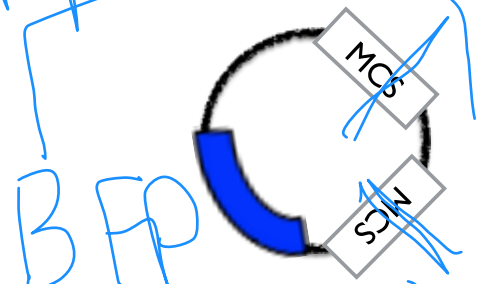
Adapted from **GROSSMAN and Wei (1995)** Clinical Chem 41: 1854-1863

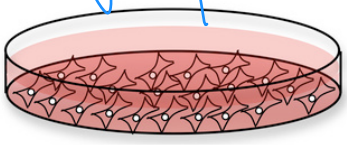
XP frequency = ~1:250,000 giving a theoretical maximum of ~28,000 cases worldwide with 2,000-fold increased risk

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

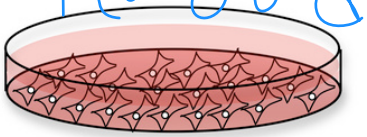
Slide from M2D1 Lecture — Prof. Samson

Module 2 Overview — Authentic Research!

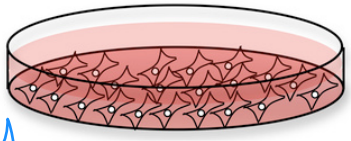




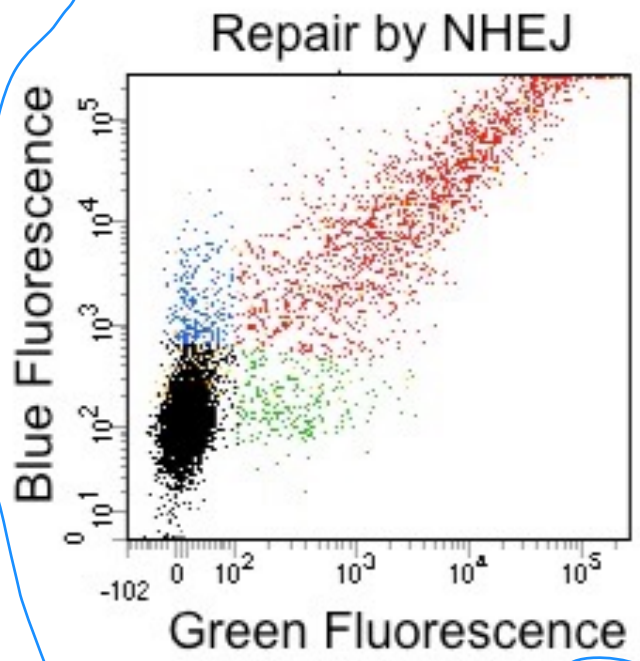
"Normal cells"



"DNA repair-deficient cells"



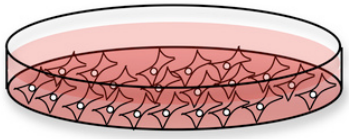
"Normal cells + inhibitor of DNA repair"



1) Validate System

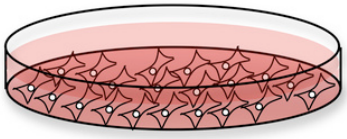
Tools to study DNA repair: Our model system

M2D1



"Normal cells"

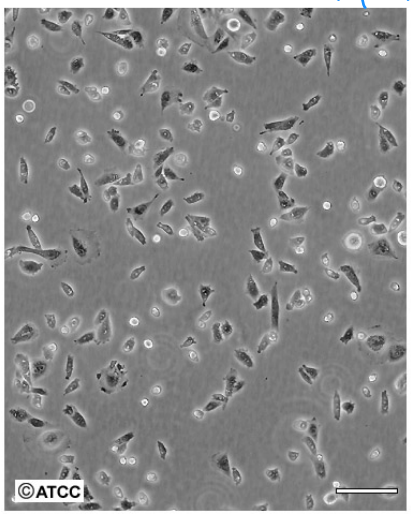
K1

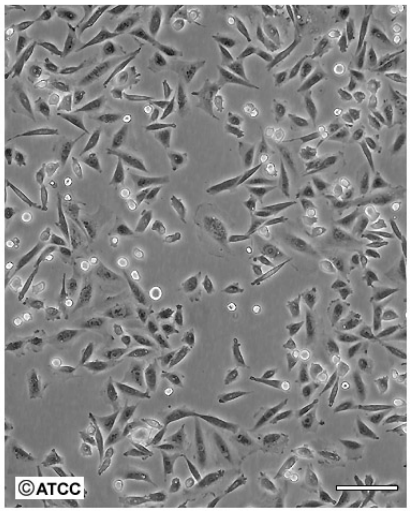


"DNA repair-deficient cells"
= xrs6
No Ku80

ATCC

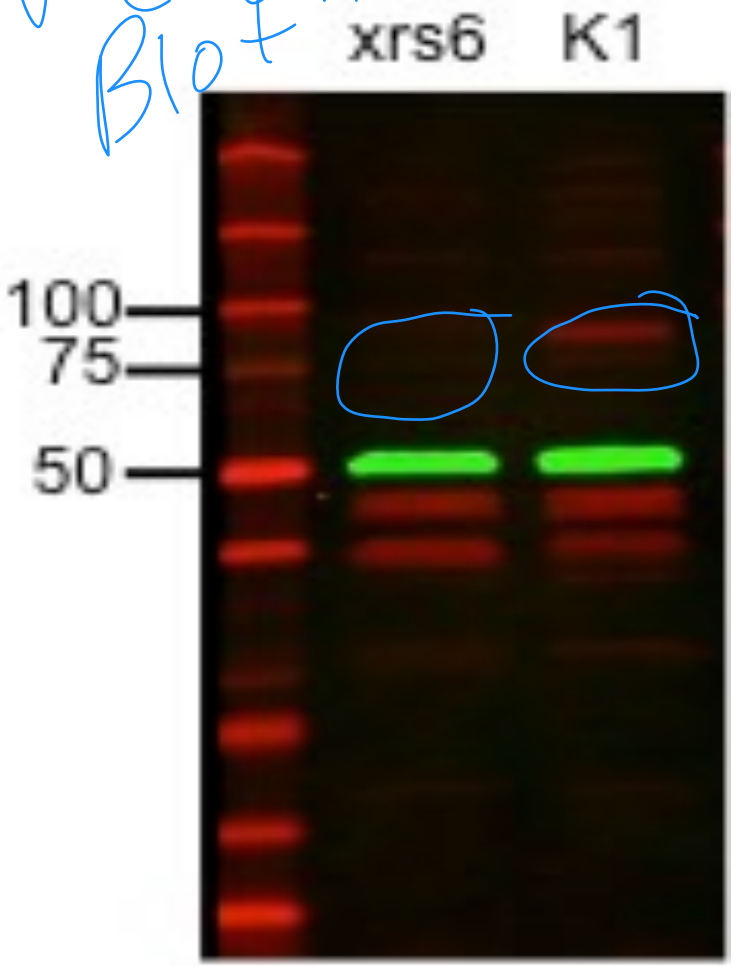
ATCC Number: **CCL-61**
Designation: **CHO-K1**





Start M2D2

Western Blot



37°C, 95% RH

Cell

Mammalian cell culture — Tissue culture medium

What do cells need to survive?

DMEM

phenol red

AA
vitamins
minerals
+glucose

pHn
7.2

Food(s):

NEAA
glutamine
Sodiumpyruvate

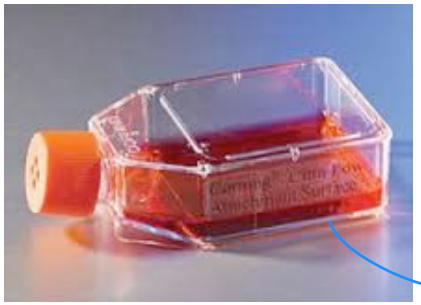
FBS
lipids
GF
cytokines

Non-food(s):

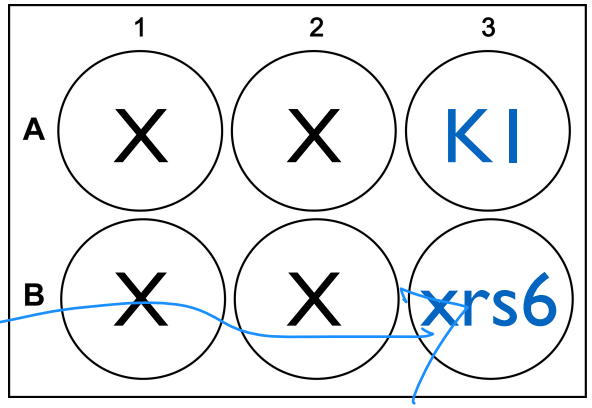
antibiotic-bacteria
antimycotics-fungus

Mammalian cell culture — 'Splitting' cells

1. Rinse with PBS — why? FBS & trypsin agents + excess protein
2. Detach cells — why? trypsin moved
3. Count cells — why? equalize #
4. Add to new culture vessel — why? Room to grow



Flask

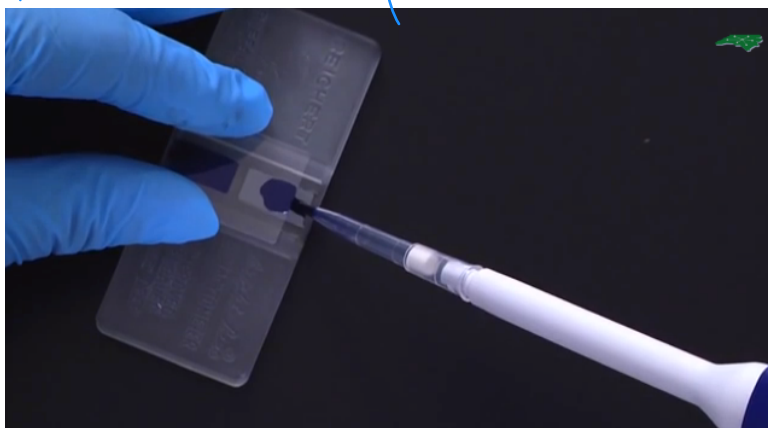


Plate

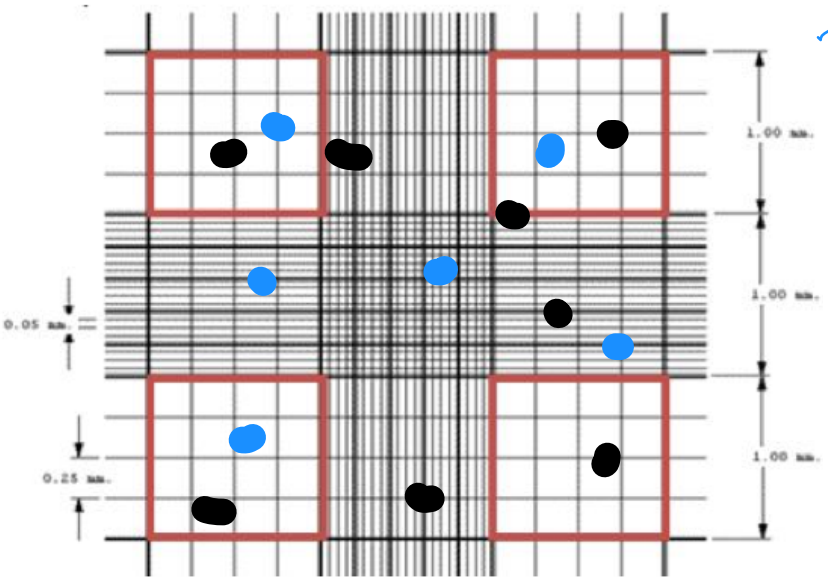
http://www.sigmaaldrich.com/catalog/product/sigma/cls3815?lang=en&region=US

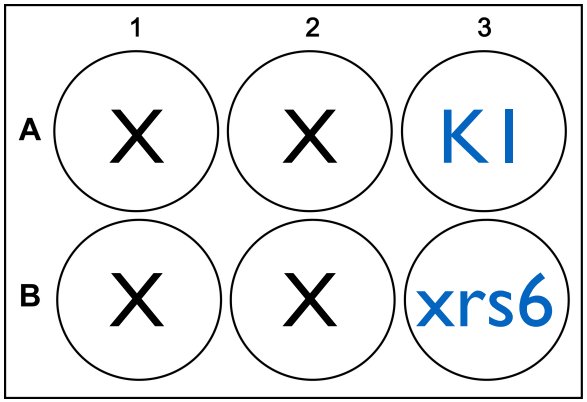
Mammalian cell culture — 'Plating' cells

hemocytometer



90 ul CHO + 10 ul trypan blue = dead





avg # $\times 10^4$ = cells/mL

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/

Today in lab:

★Seed cells for Western blot analysis of Ku80 expression:

Blue/Pink/Purple/Platinum— in TC first

★Leslie R. will be here to talk about abstracts at 2pm

★Learn about our system:

1. 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 Nova!