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:

~~R 8-10pm Simmons 528~~

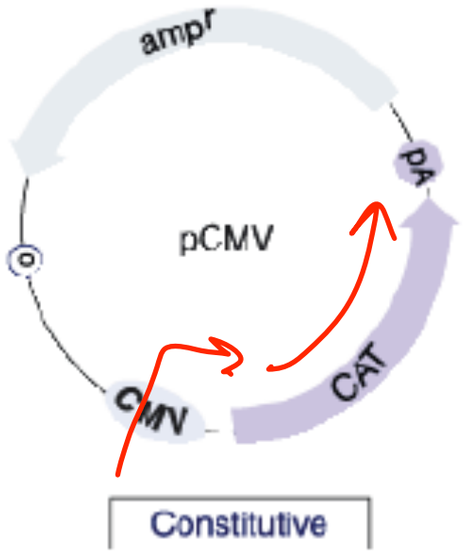
Friday 3-4pm 16-319

Sunday NOON-2pm 56-302

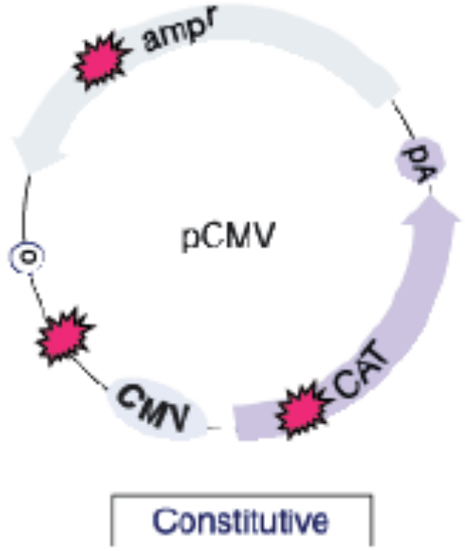
Noveen 10am-NOON Sunday

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

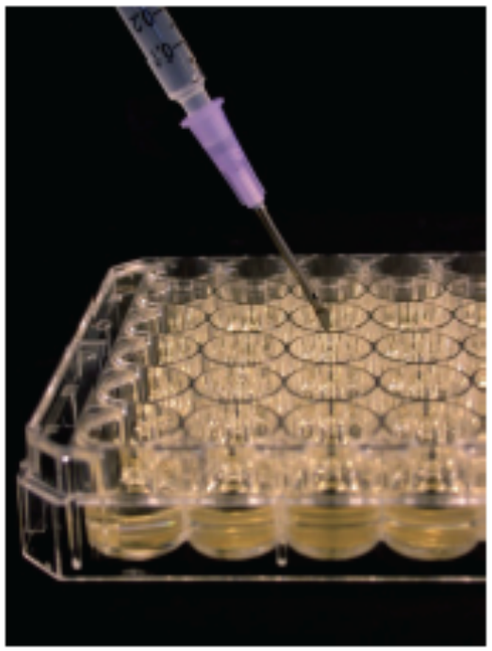
Athas & GROSSMAN
Cancer Res. 1991

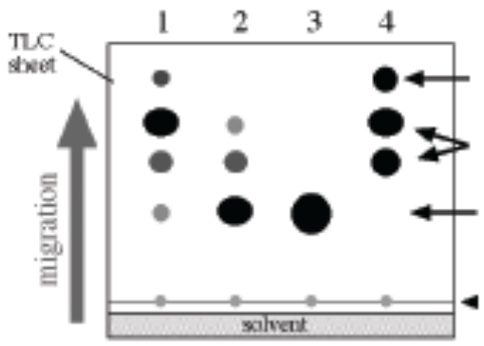


+ UV light



Transient transfection peripheral blood lymphocytes



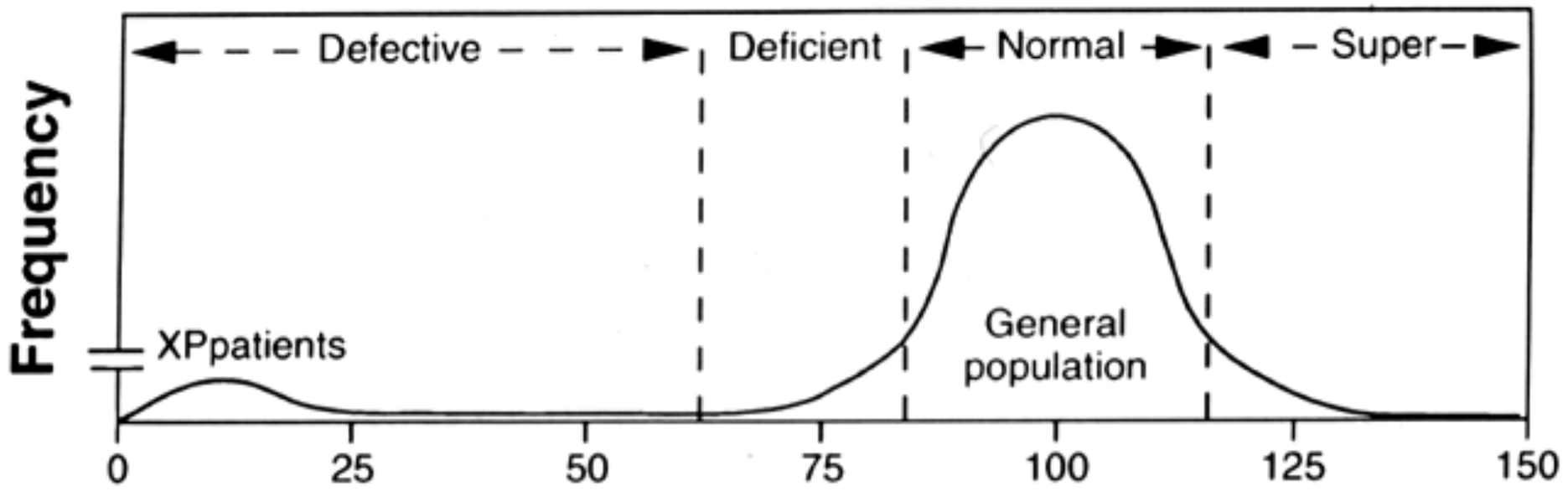


Time to repair

CAT Assay

Slide from M2D1 Lecture — Prof. Samson

Interindividual Variation in DNA Repair Capacity



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

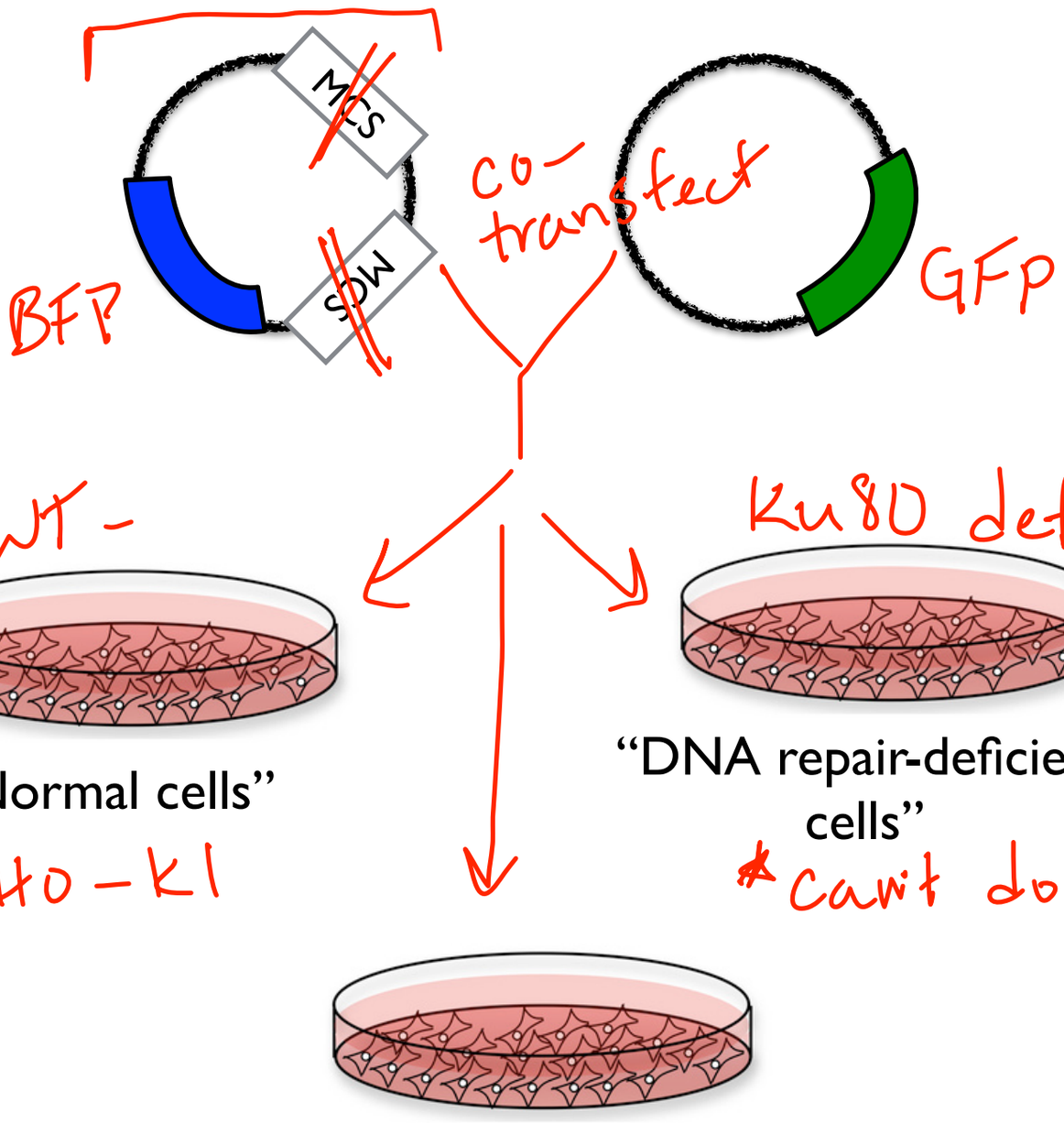
Slide from M2D1 Lecture — Prof. Samson

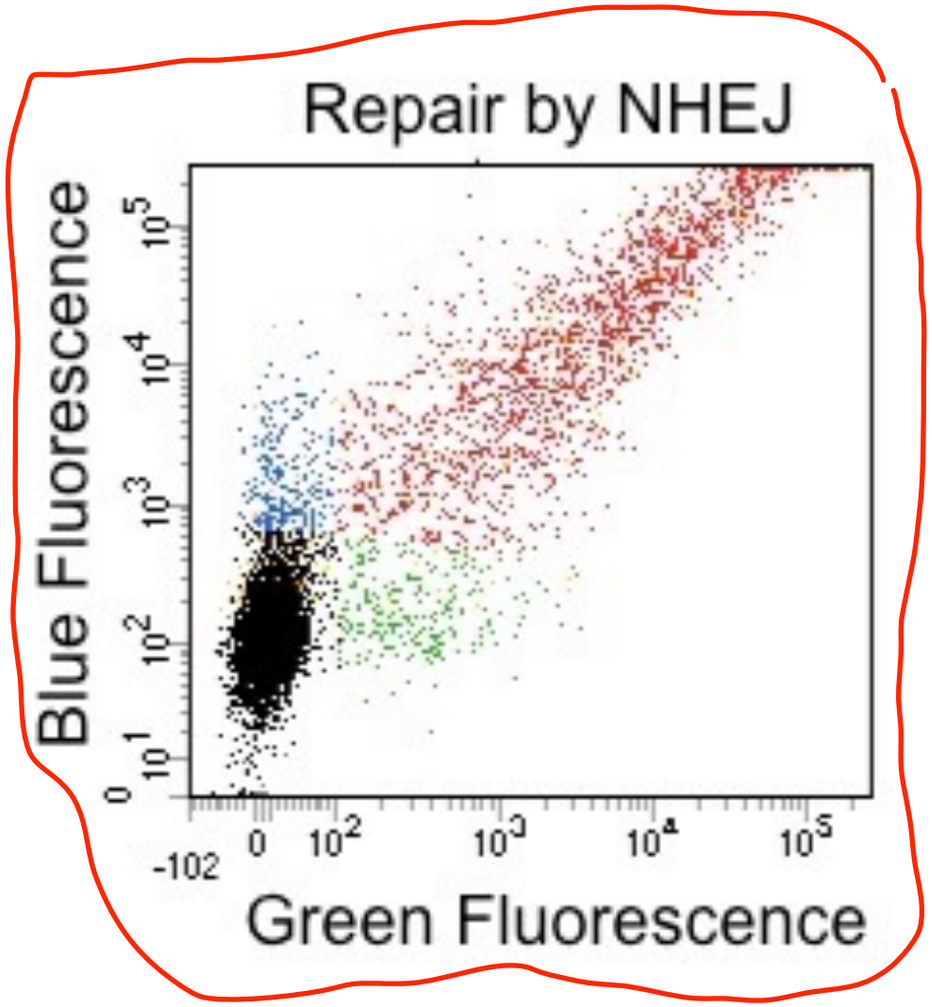
★★HELP us find a NHEJ

Module 2 Overview — Authentic Research!

DNA repair reporter

inhibitor ★★

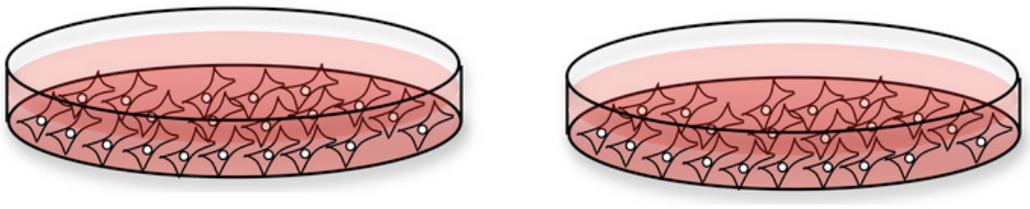




#1) Validate & our system

Tools to study DNA repair: Our model system

M2D1

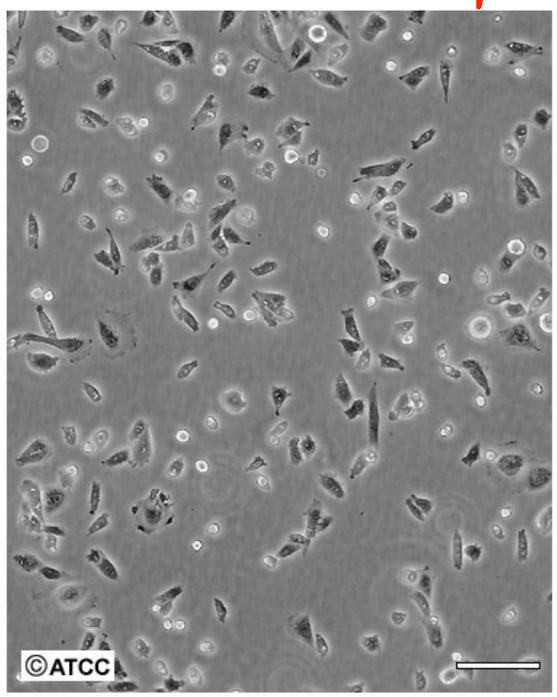


"Normal cells"

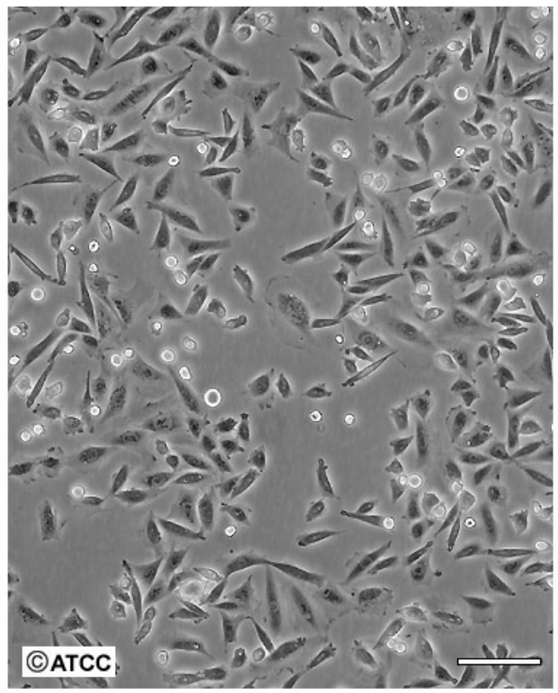
"DNA repair-deficient cells"
=
No Ku80

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

ATCC



Low Density Scale Bar = 100µm

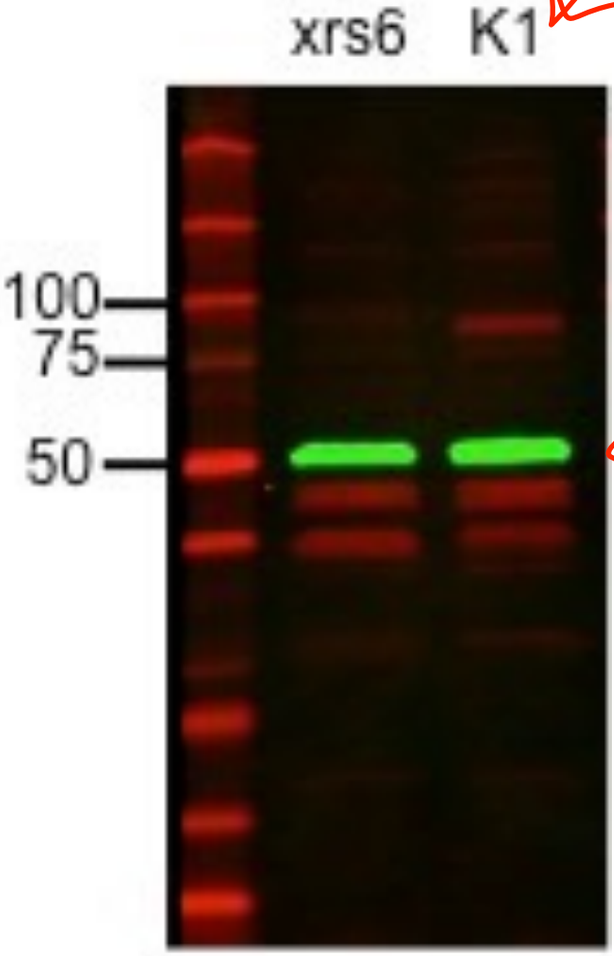


High Density Scale Bar = 100µm

Western Blot

Start M2D2

Infrared detection



Ku-80

α-tubulin

37°C → 95% R.H.

Mammalian cell culture — Tissue culture medium

Cell

What do cells need to survive?

Food(s):

(NON essential a.a)

NEAA - ↑grow

Glutamine

Sodium Pyruvate

(glucose → 2 pymate)

DMEM → A.A → vitamins → minerals

+glucose

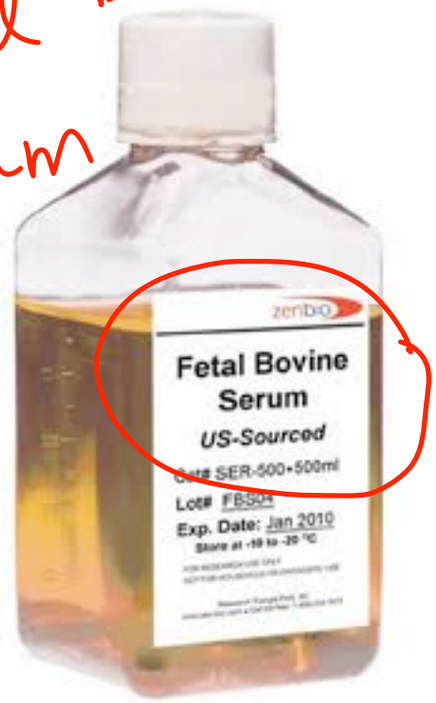
phenol red

+lipids

+growth factors

+cytokines

Fetal Bovine Serum



Non-food(s):

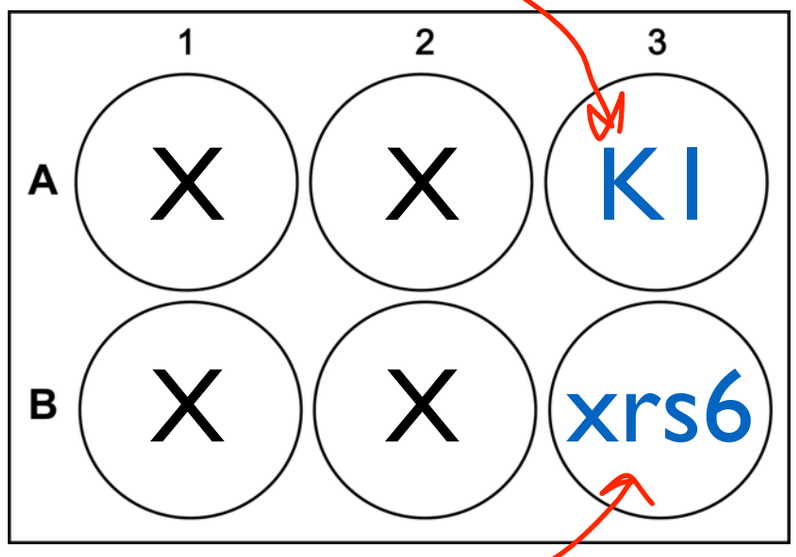
Antibiotics → prevent contamination

Antimycotic → prevents fungus

Mammalian cell culture — 'Splitting' cells

1. Rinse with PBS — why? FBS contains α-trypsin agents
2. Detach cells — why? move them 6-well plate
3. Count cells — why? - give them room to grow
   - equalize cell number
4. Add to new culture vessel — why?

Flask



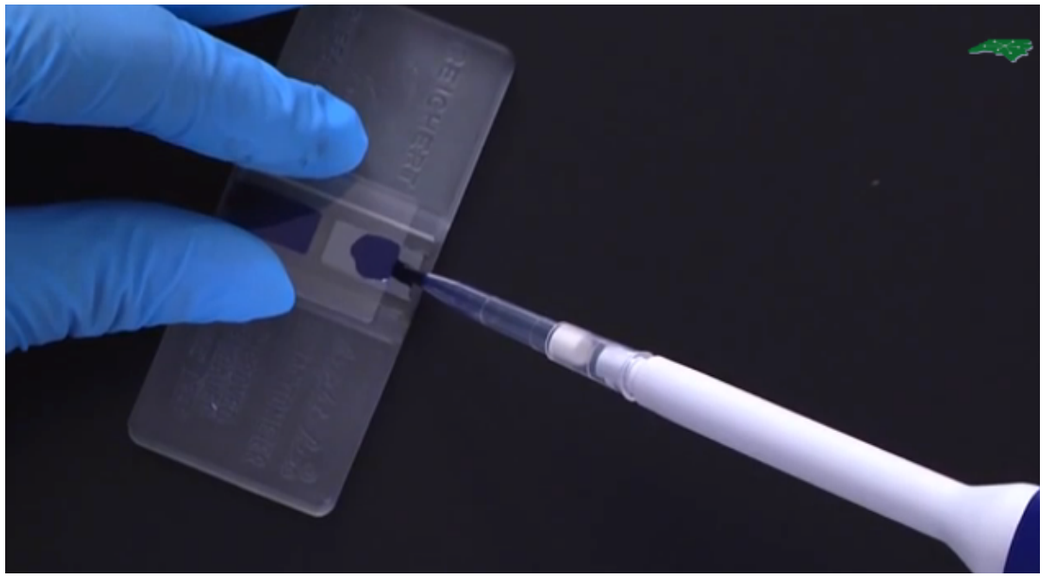
Plate

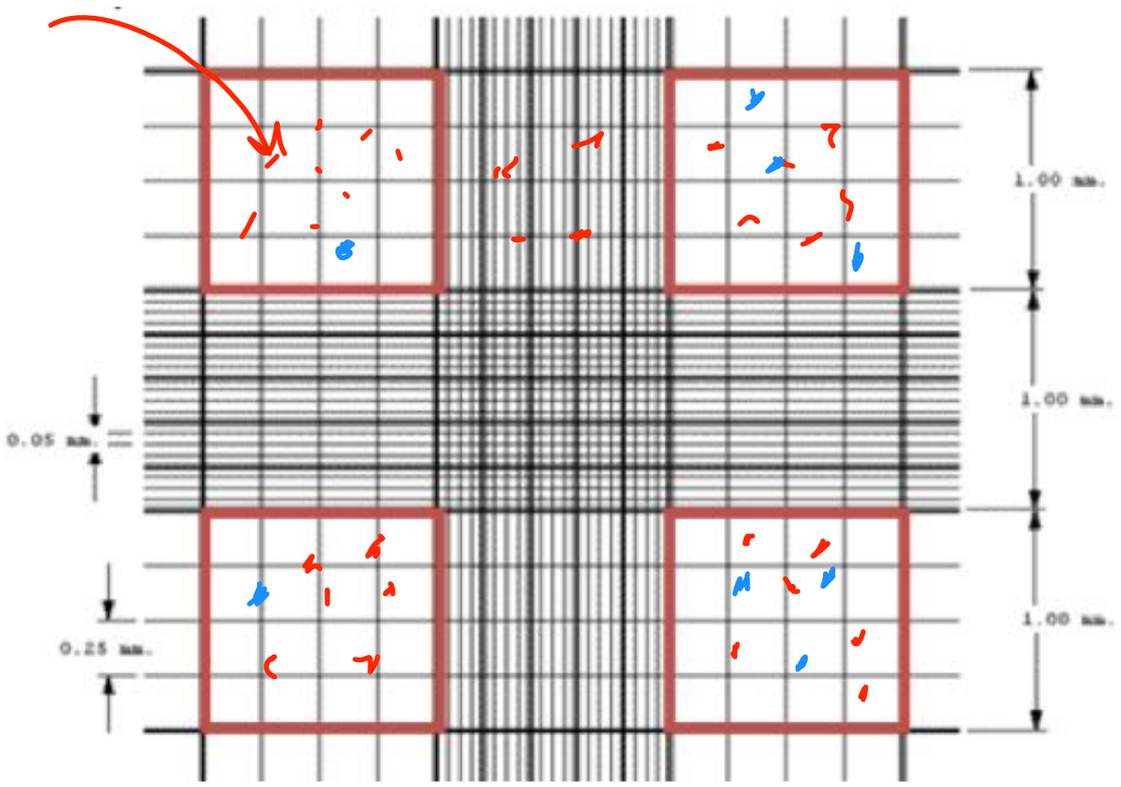
http://www.sigmaaldrich.com/catalog/product/sigma/cls3815?lang=en&region=US

Mammalian cell culture — 'Plating' cells

90 µL cell + 10 µL trypan blue

hemocytometer





	1	2	3
A	X	X	KI
B	X	X	xrs6

Av # of cells × 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:

Orange/Yellow/Green — in TC first

★Leslie here to talk about abstracts

★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!