M1D1: Prepare microwell array and practice tissue culture

09/13/16

1. Lab Orientation Quiz
2. Pre-lab Discussion
3. ½ class goes to the Tissue Culture Room
4. ½ class prepares a CometChip

Office hours

Noreen Lyell

- Mondays 1pm
- Mondays 5pm
- in 16-317

Leslie McClain

- Mondays 4pm
- Wednesdays 9am
- in 16-429b

Maxine Jonas

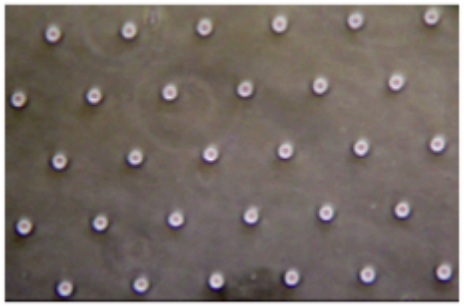
- Mondays 2pm
- Fridays 9am
- in 16-239

by appointment: nllyell@, lesliemm@, jonas_m@

M1 major assignments

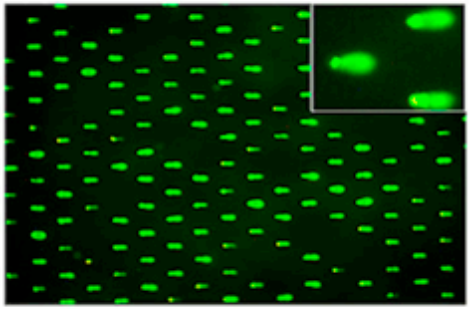
- Data summary (15%)
  - in teams, on Stellar
  - draft due 10/12, final revision due 10/24
  - bullet points, .PPTX
- Mini-presentation (10%)
  - individual, video via Gmail
  - due 10/15
- Lab quizzes (extra credit)
  - M1D3, M1D4, and M1D6
- Notebook (5% total)
  - one day will be collected and graded by Emily on M1D7
- Blog: http://be20109f16.blogspot.com/ (participation: 5% total)
  - by 10/25

Overview of
“M1: Measuring Genomic Instability”



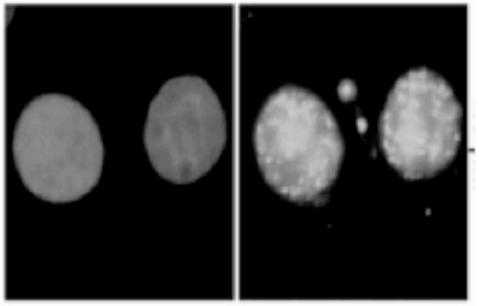
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals

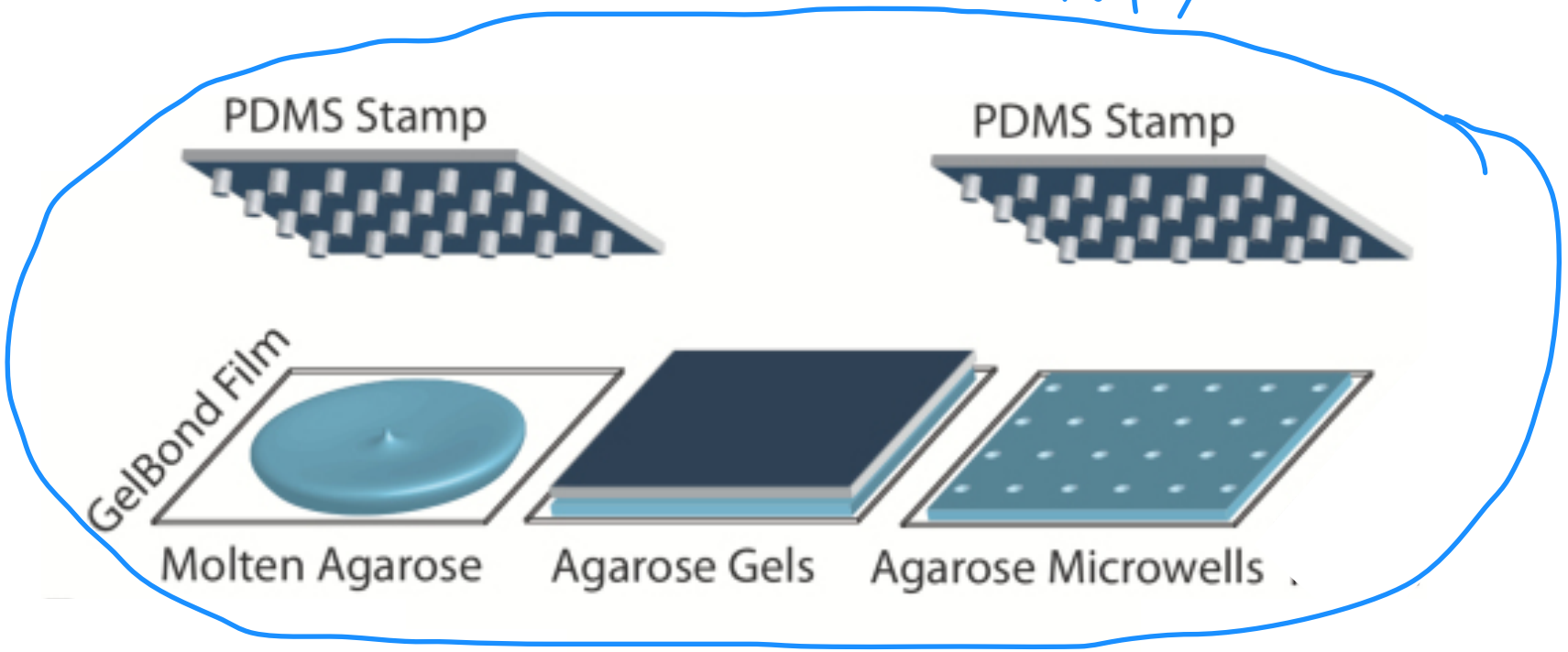


3. Use immuno-fluorescence assay to visualize DNA repair

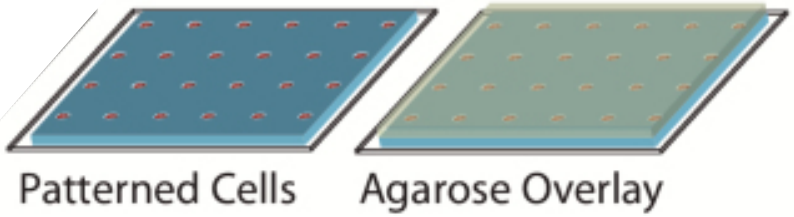
- Examine effect of H_2O_2 on DSB abundance

Creating a CometChip

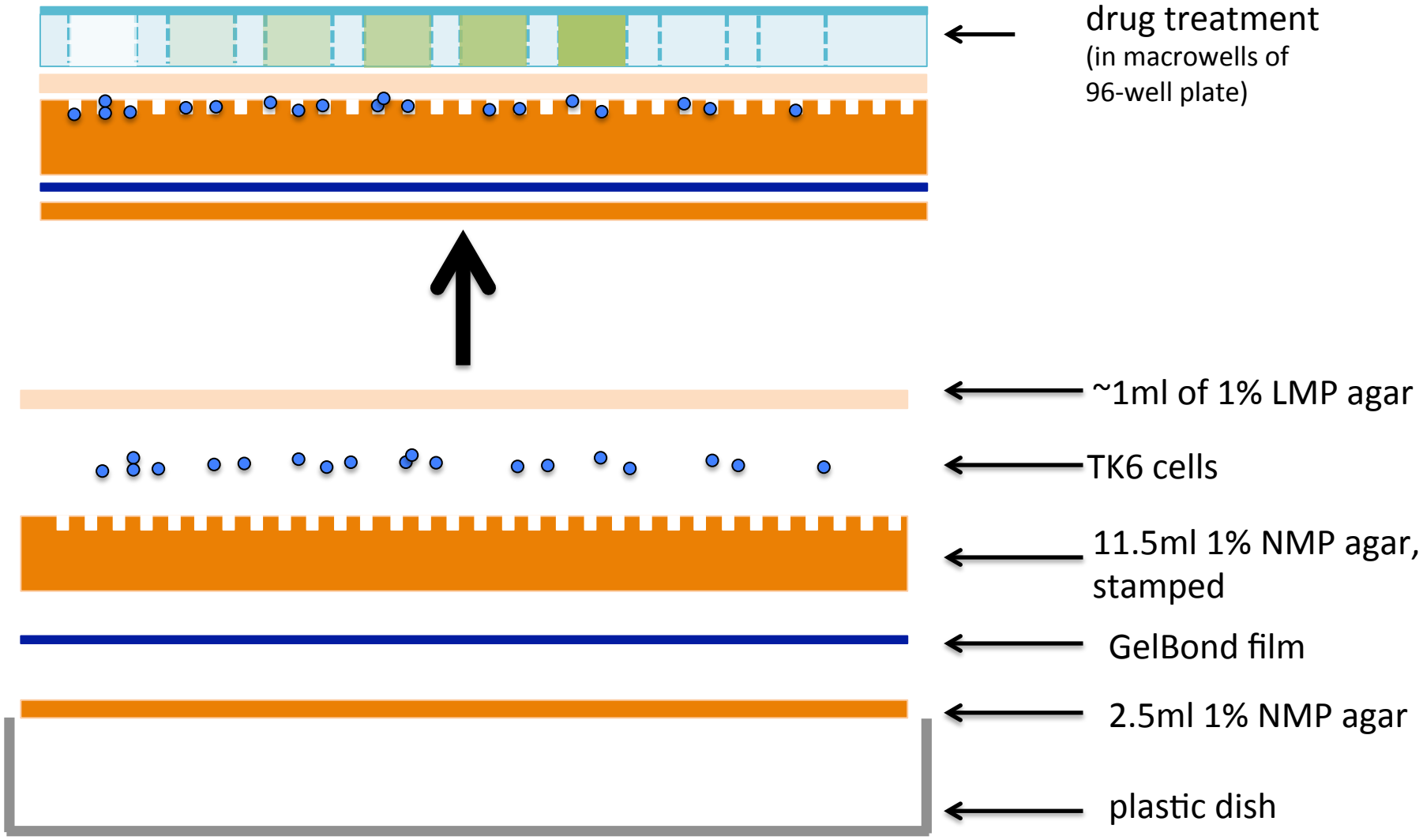
TUDAY



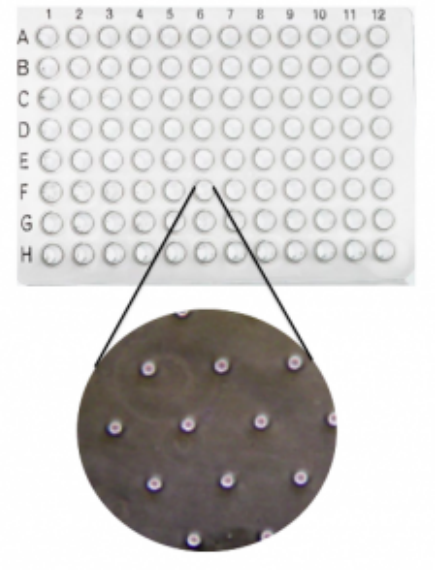
THURS

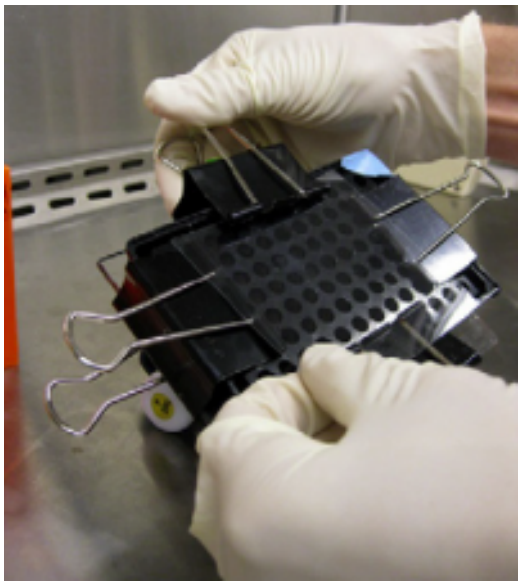


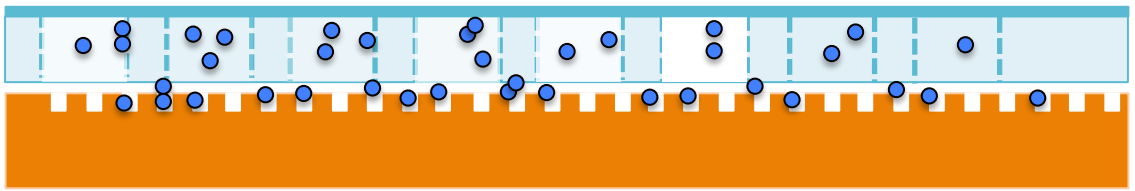
The CometChip layers



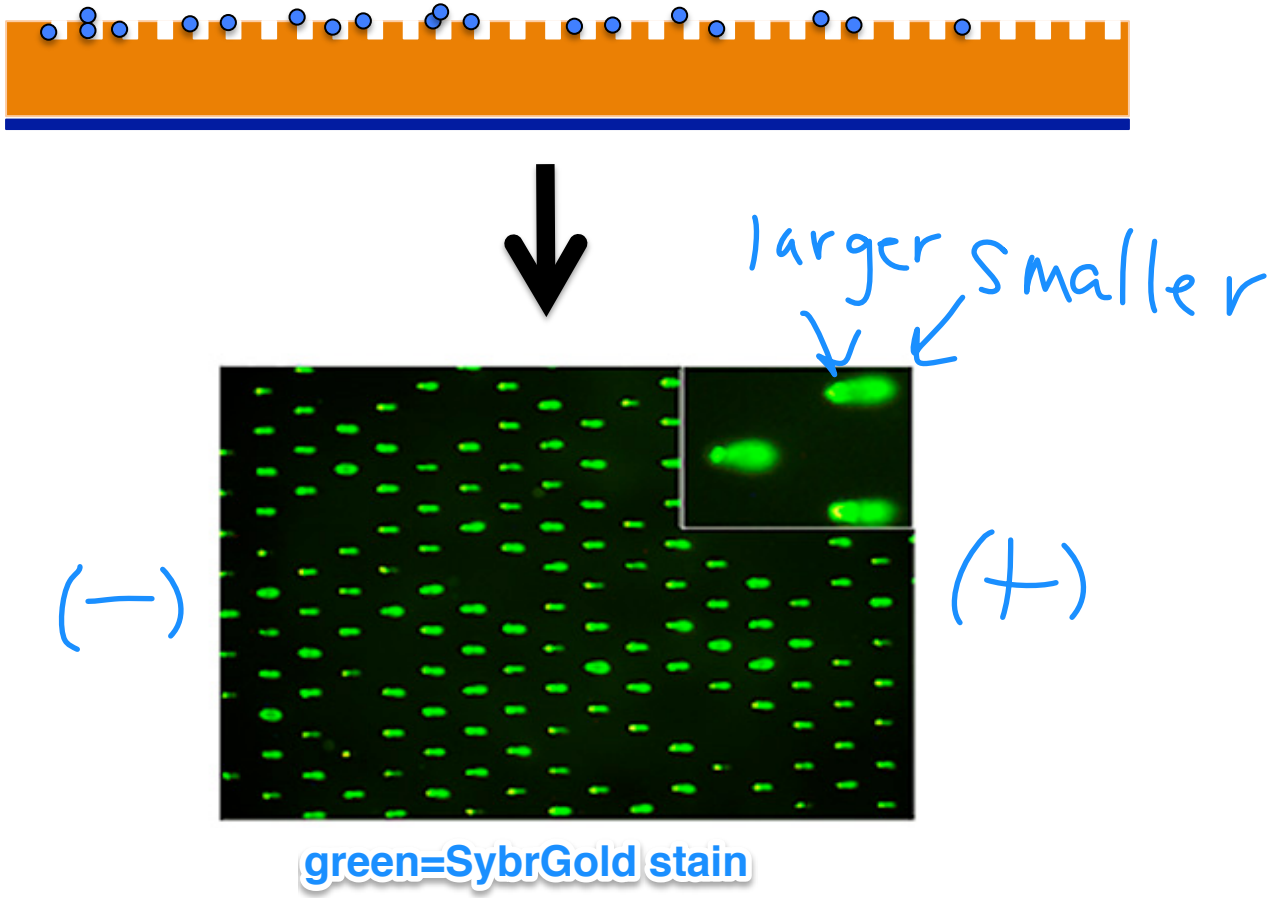
Loading the CometChip







CometChip Electrophoresis



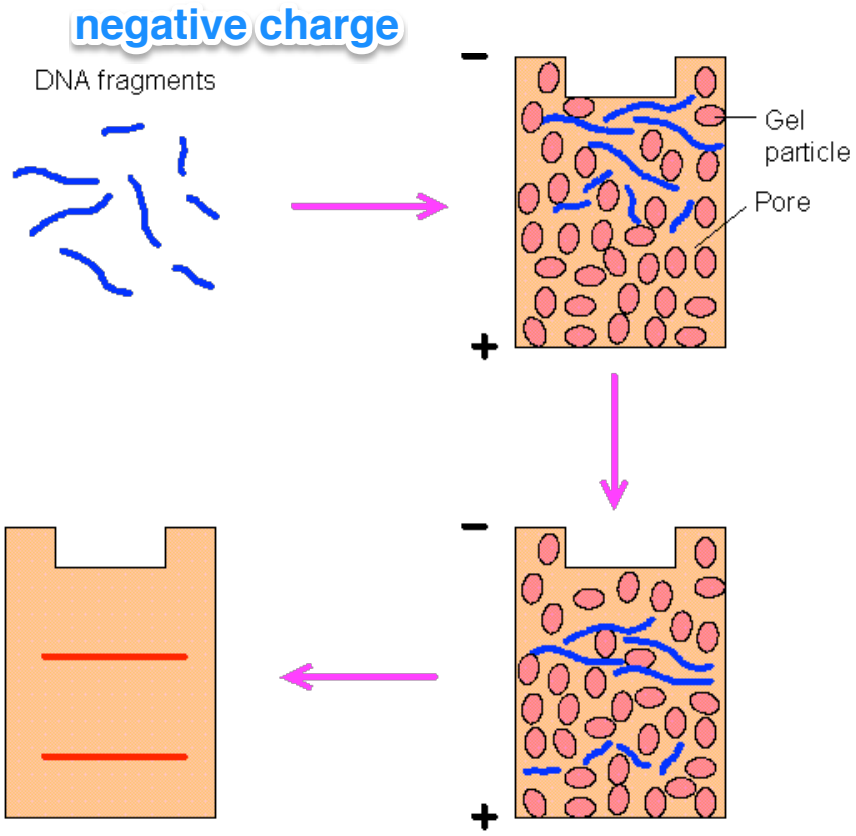
Separating DNA by gel electrophoresis

- Agarose gel electrophoresis
  – driving force:

    charge

  – separates DNA by:

    size



Visualize DNA

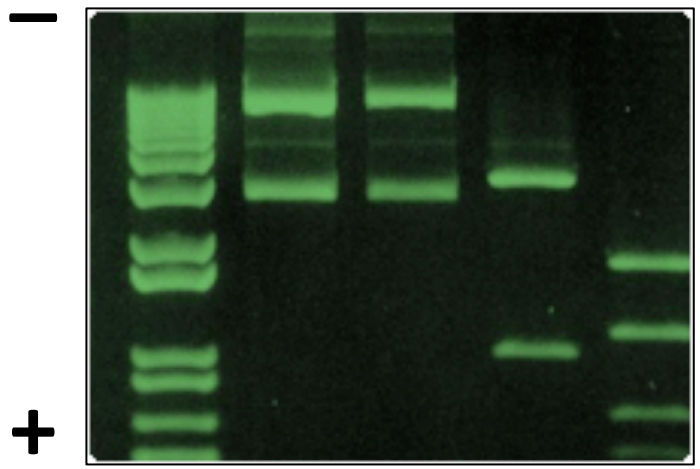
- DNA stain **SybrGold**

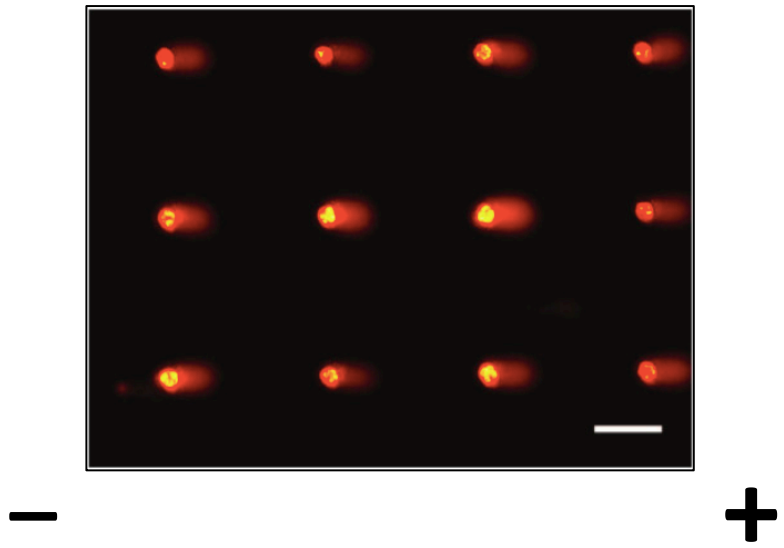
  -DNA intercalator

  -dsDNA and ssDNA

  -visualize by UV light

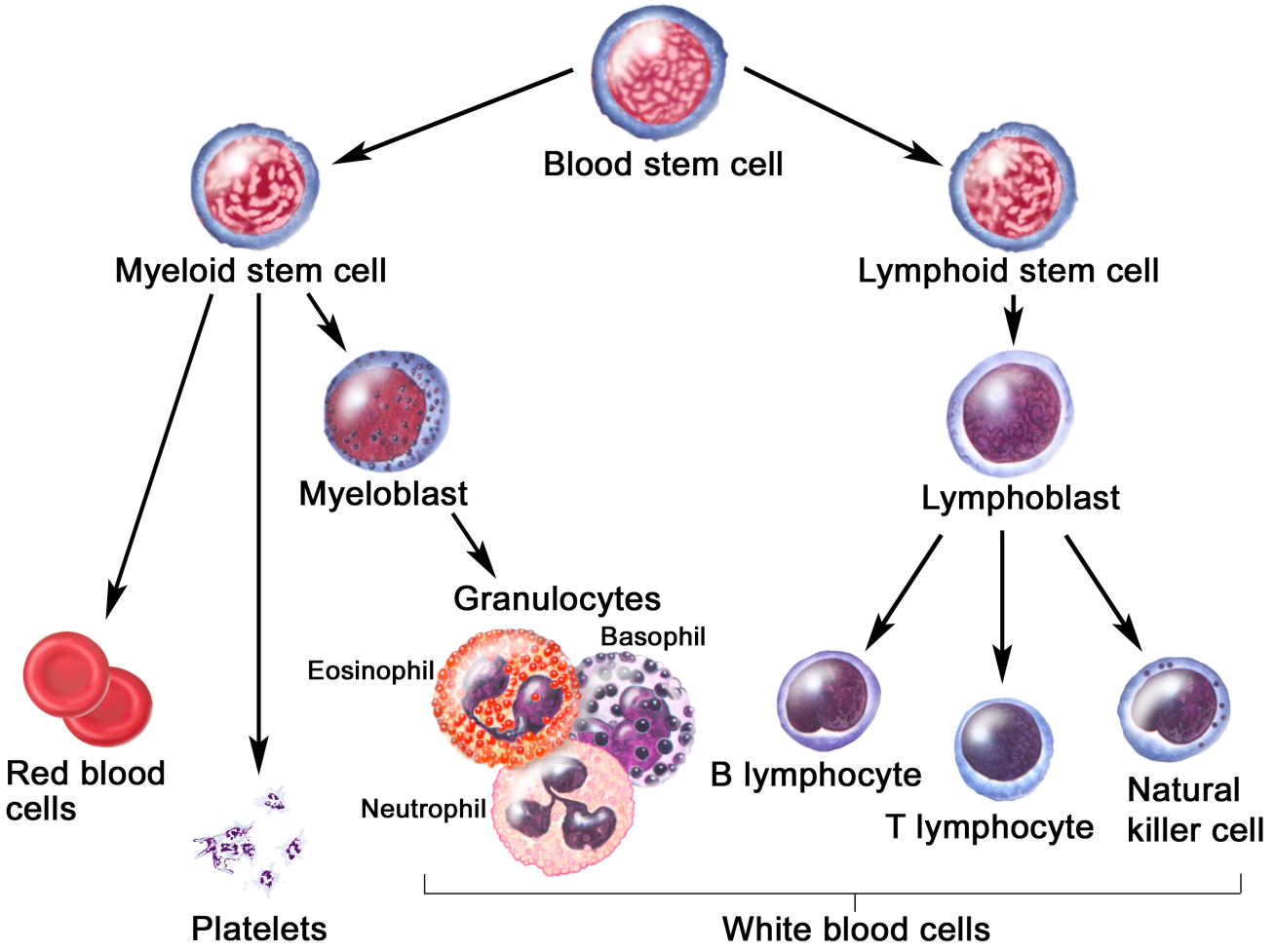
  -litte as 25pg of DNA





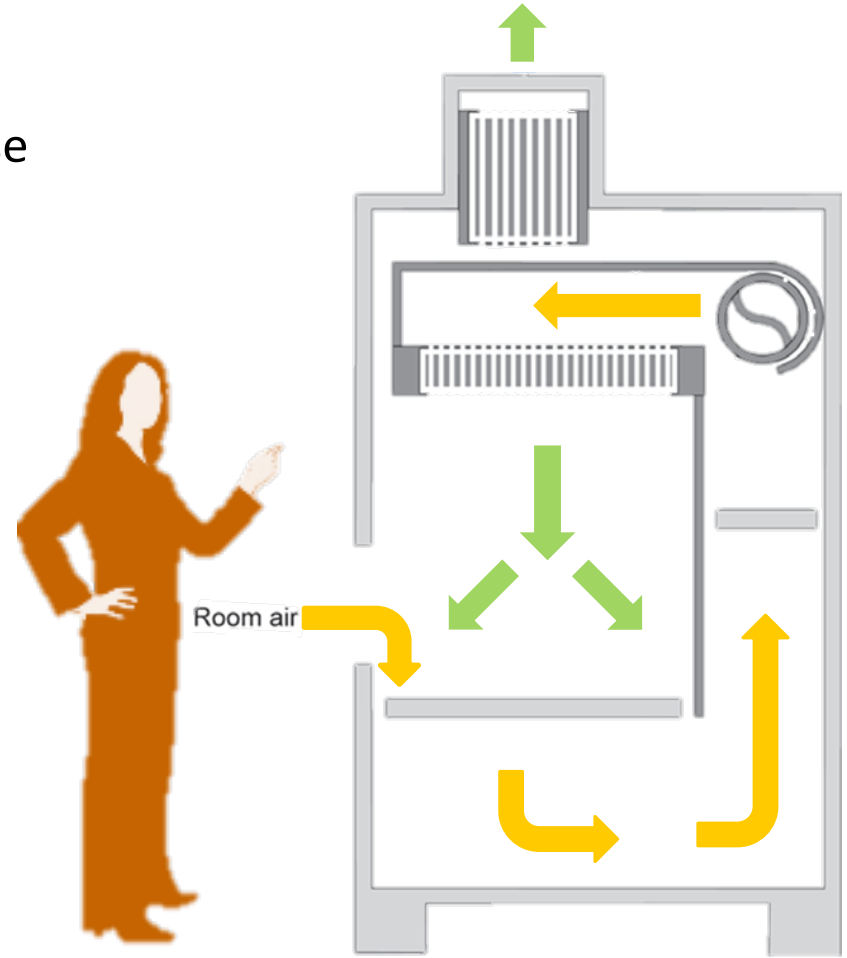
➢ Safety : wear nitrile gloves

TK6 are human lymphoblast cells



Tissue culture sterile technique

- **70% ethanol** is your BFF:
  - wipe cabinet before and after use
  - wipe everything that enters the cabinet
- **Do not disturb air flow:**
  - Do no block grille or slots
  - Minimize side-to-side arm movements
  - Work > 6” away from sash
  - Leave blower *on*
- Do not talk into incubator!
- Only open sterile media in hood



Mammalian cell culture medium

essential for growth , division and viability

Food:

- RPMI 1640 (Roswell Park Memorial Institute) **defined**

  -AAs
  -glucose
  -buffers
  -salts
  vitamins

- FBS: fetal bovine serum **undefined**

  -growth factors
  -lipids
  -cytokines
  cholestrol

Non-food:

- antibiotics:
  - penicillin
  - streptomycin

prevent bacterial growth

Splitting Cells (and other jargon!)

why?

1. Look at cells, estimate confluence

   growth rate, health

2. Count cells with hemocytometer

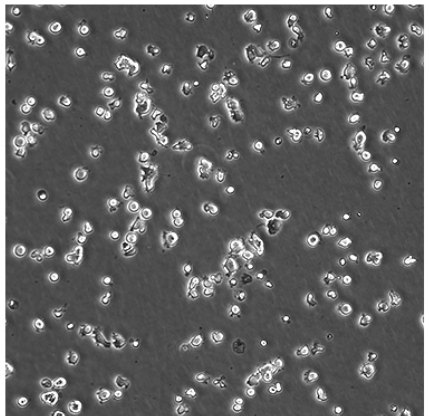
   seed specific # in a new flask

3. Seed new flask

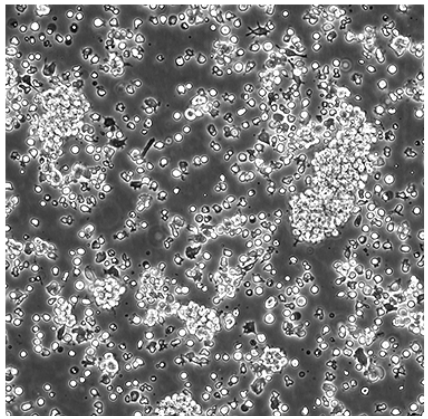
   room to divide and nutrients

Flask

Low Density

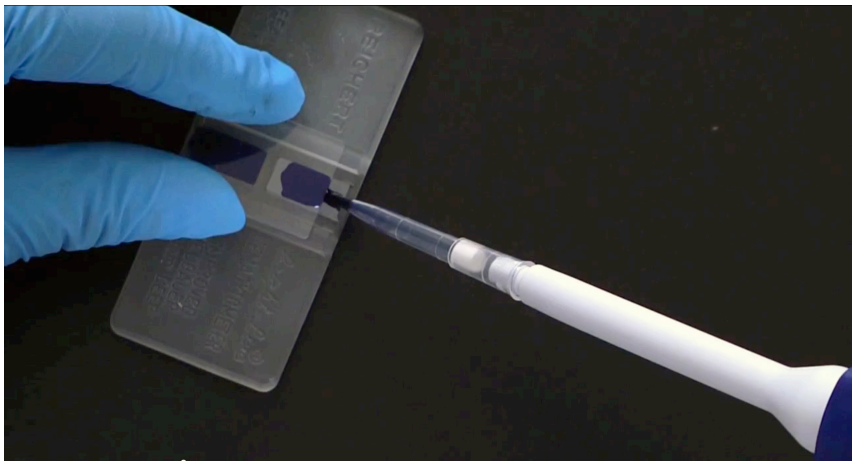


High Density

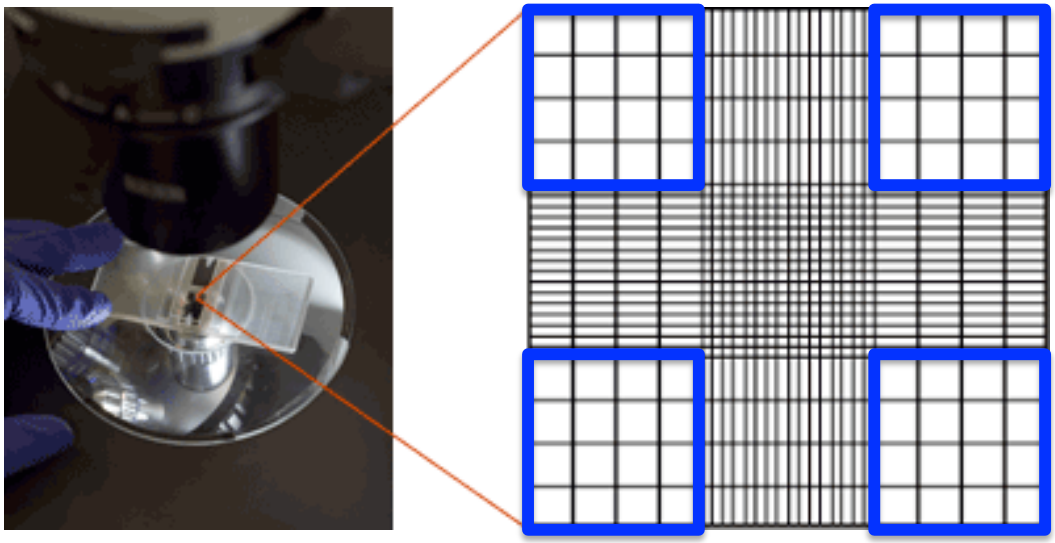


time to split!

Counting Cells



- Hemocytometer: count blood cells
- Trypan blue: -live/dead stain
  -dead cells blue
  -live cells "white" I
- # cells / mL = 10,000 x average of 4 corners



Today in lab:

1. 3 teams into tissue culture room to split TK6 cells (Blue, Pink and Purple)
2. 2 teams start preparing CometChip (Yellow and Green)
3. Make sure to keep notes in Benchling