# M2D1: Prepare cells for RNA purification

- 1. Prelab discussion
- ½ class to TC to seed cells for RNA purification
- 3. <sup>1</sup>/<sub>2</sub> group paper discussion
- 4. Work on Exercise 1 in Rstudio

Reminders:

3/9 (Sat): Extra Office Hours, 11am-6pm @ 56-302



## Tissue culture sterile technique

- 70% ethanol is the best!
  - 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 items in hood



#### Our cell line: DLD-1



- Origin: human
- From the colon of a male with colorectal adenocarcinoma
- Isolated by D.L. Dexter and associates during a period from 1977-1979

# Mammalian cell culture medium What do DLD-1 cells need to survive?







#### Defined: RPMI 1640 (Roswell Park Memorial Institute) Sats Sugar, amino acids H > phend ved > fuschin > yellow

Undefined: FBS (fetal bovine serum)
Service
Growth Factors, cytokines, lipids

#### Not for survival

- antibiotics:
  - penicillin
  - streptomycin

printer printer prevention

### Mammalian cell culture terminology

- · seeding/plating



High Density DLD-1, ~80%



## General steps for splitting cells+WHY?

- 1. Look at cells, estimate confluence nath? ready to split?
- 2. Rinse with PBS get rid of debris, protein, anti-typsin factors
- 3. Detach cells with trypsin Clear adhesions blue cells & substrate
- 4. Count cells
- 5. Seed new culture vessel



## Calculating number of cells with a Hemacytometer



- Hemacytometer, holds 10uL on each side
- Trypan blue:



# cells / mL = 10,000 x average of 4 corners  $I_{6}(4 \times 10,000$ 40,000 cells m

## R programming language

Your Workspace / Exercise_1		۰ ا	Leslie McClain
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• R is a language and free software environment

 R is popular for analysis of complex biological data
Kmd.

 Interface called Rstudio.cloud, online workspace

https://rstudio.cloud/spaces/7339/join?access\_code=C2B0D5OQ1MHPiDhX9vuTKkDn0aGpQGq0SYA9cKYn

# Documenting R analysis in your Benchling notebook

- Each lab day with a R exercise, Joe will check your progress in Benchling before you leave
- Today for Exercise 1 include:

□ Plot of 100 random numbers

□Plot line in yellow of 100 random numbers

Histogram of 100 random numbers

□Scatterplot of two animal populations per city

□Scatterplot of two animals per city without NAs

□Include 1-2 sentences that describes the differences in the scatterplots

• Feel free to make other notes in Benchling you think are important. The above list is the minimum.

# Today in lab:

- 1. Tissue Culture (TC)
  - 1<sup>st</sup>: red, orange, white, silver, blue
  - 2<sup>nd</sup>: yellow, green, pink, purple
  - Protocols printed for TC use, no need to move laptops etc.
  - Do not wear PPE in or out of TC room
- 2. Group discussion of Wei *et al.,* see wiki for guidelines
- 3. Practice data analysis in R studio Cloud

#### For M2D2

- Sign up for a Journal Club day and paper to present
- Turn in single Journal Club slide from Wei et al.

Don't forget about Mod1 assignments!

- Draft data summary due Sunday March 8<sup>th</sup> at 10pm (team)
- Mini presentation due Sunday March 15<sup>th</sup> at 10pm (individual)

# Sign up for journal club

- Pick 1 of 25 papers, or suggest your own (must be approved by instructors)
- Present M2D4 (March 18<sup>th</sup>) or M2D5 (March 20<sup>th</sup>)
- Sign up by adding your name next to paper [BCM/WF/Color]
  - first come first serve!
  - you cannot switch paper after M2D3 (March 13<sup>th</sup>)
  - only one T/R presenter and one W/F presenter per article

Slot	Day 4 (T/R)	Day 5 (T/R)	Day 4 (W/F)	Day 5 (W/F)
1				
2				
3				
4				
5				
6				
7				
8				
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#### M2D2 HW: Journal Club Slide

- Slide= Standard 4:3 powerpoint slide
- Title has a message (not just the figure / paper title)
- Don't put too much on one slide, (1 slide=1 message)
- Don't fill slide with text
- Don't include the caption from paper or a citation
- Figures from paper can be cropped or modified
- Read homework description for additional tips