M2D9:
Data analysis

04/13/2016

Today in lab

✓ Paper discussion with Prof. Leona Samson

✓ Quiz

- Stain irradiated cells
  - Look at 6-well plates in tissue culture room
  - Coomassie stain the cells   **stop at Part 2, step 5: one hour incubation**
  - Take picture on gel documentation station
  - Count colonies!

- Statistical analysis
  - Practice: compare normalized EGFP/mCherry of M059K *vs.* J cells
  - Expand to *your* M2 Results figures:

Key assignments of M2

- ✓ Journal club presentation

- ✓ Lab notebook
  - due 10pm tonight
  - M2**D1** graded by Jing (share with jgzhang@)
  - Don't forget *purpose* and *summary* sections

- • Research article (25%)
  - due 5pm on Monday, April 18
  - **use office hours to get input of your draft**

- • Blog post(s)
  - due 5pm on Tuesday, April 19
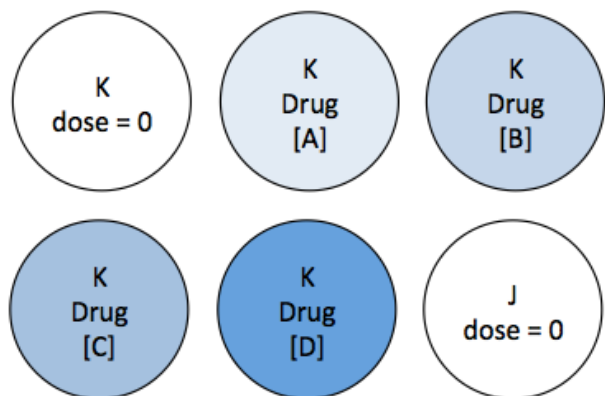 - about journal club? about second manuscript?

Extra office hours this week

- Thursday 04/14:
  - 9-11am, Maxine in 16-239
- Friday 04/15:
  - 9-11am, Maxine in 16-239
- Saturday 04/16:
  - 2-5pm, Noreen in 56-302
- Sunday 04/17:
  - 10am-12pm, Leslie in 56-302
  - 2-5pm, Noreen in 56-302
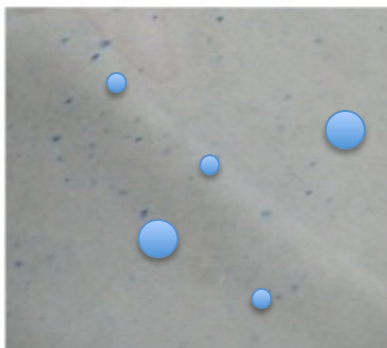
Control experiment: Did our inhibitor work?

1. Seed M059K/J cells at low density

2. After 2 days, dose response of NHEJ inhibitor around IC_{50} and expose to ionizing radiation

loperamide hydrochloride

or

DMNB

4 Gy
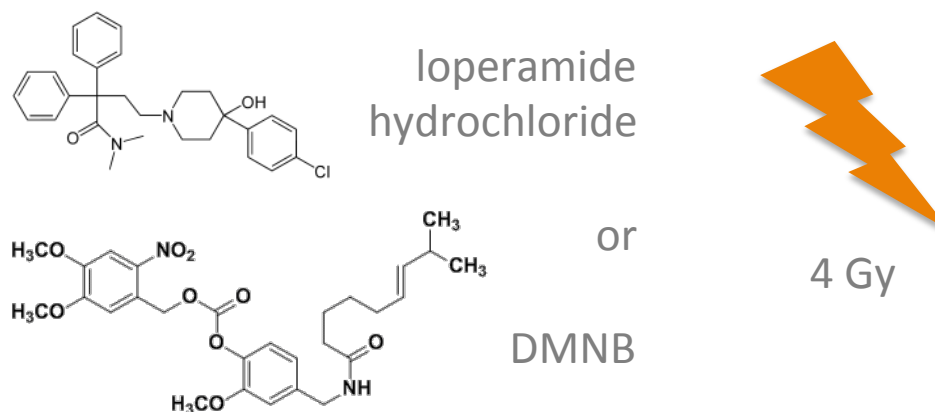
3. After 12 days, count surviving cells via colony formation assay

$$\text{surviving fraction} = \frac{\text{\# colonies with drug [X]}}{\text{\# colonies with dose} = 0}$$

Make strides on your statistical analysis!

- Practice:
  - Compare normalized EGFP/mCherry of M059K *vs.* J cells
- Expand to *your* M2 Results figures:
  - Plot your data with 95% confidence intervals

$$\bar{x} \pm \frac{t_{table} * stdev}{\sqrt{n}}$$

$$t_{table} = TINV(0.05, n-1) \quad \textbf{in Excel}$$

  - How certain are you that two populations are different?
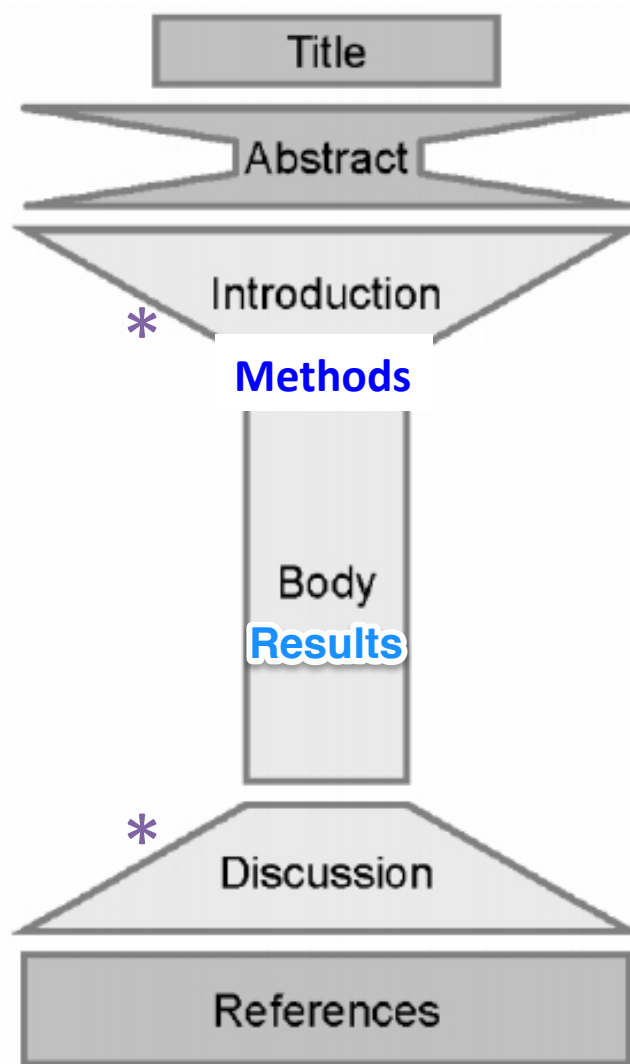
$$p = TTEST(array1, array2, 2, 3)$$

2-tailed   unequal variance (heteroscedastic ☺)

  ✧ The Student's t-test only applies to two data sets.
     Only compare two conditions at a time.
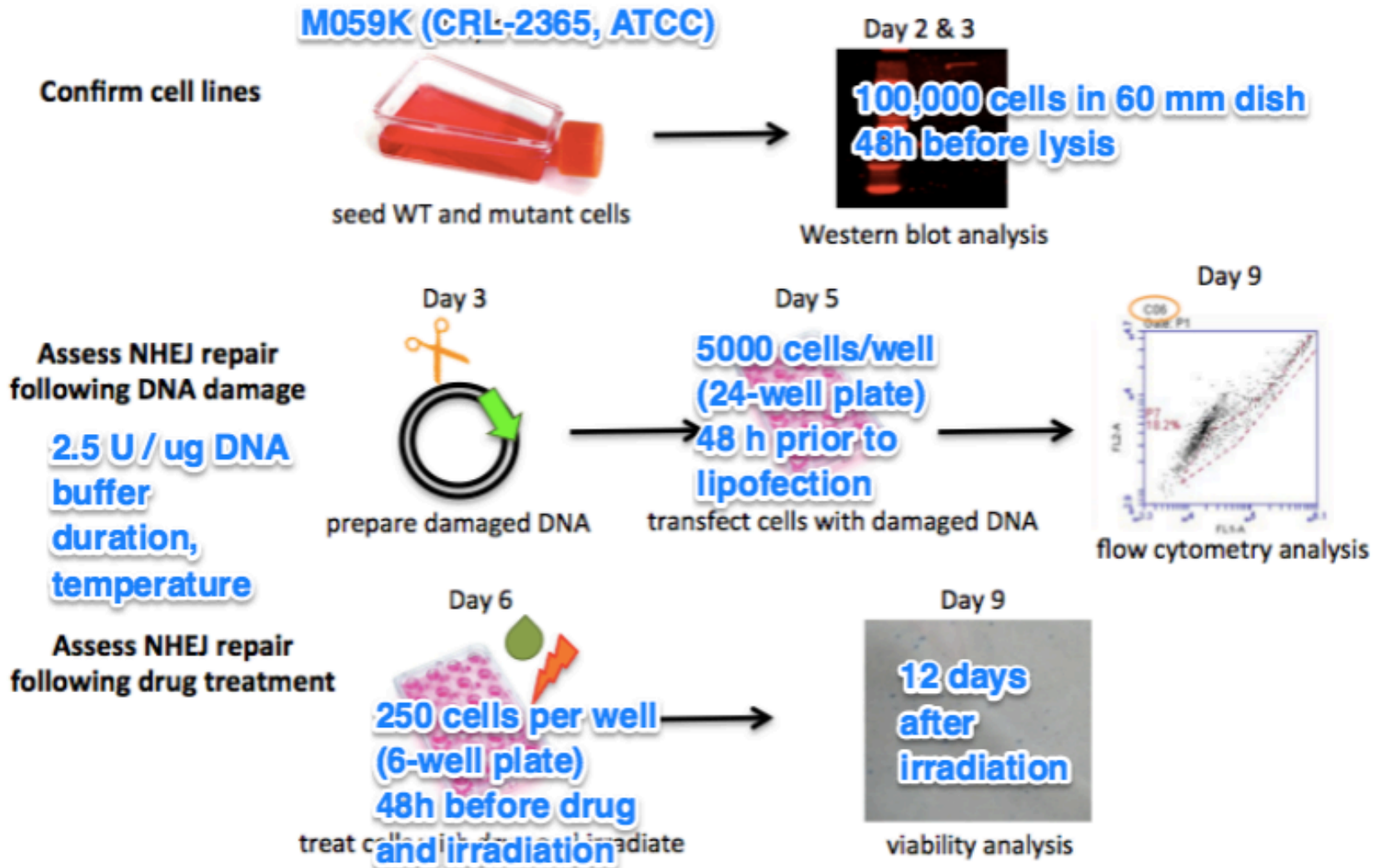
Review of manuscript architecture

What goes into the Introduction?

- General background: motivation
- Specific background: focused context
  - minimal essential information
  - define all key terms
- Knowledge gap, central question
- **Hypothesis(es)**
- Preview of results
- ... and of implications

- Don't forget references

A few words regarding the Methods

Confirm cell lines

M059K (CRL-2365, ATCC)

seed WT and mutant cells

Day 2 & 3

100,000 cells in 60 mm dish
48h before lysis

Western blot analysis

Assess NHEJ repair following DNA damage

2.5 U / ug DNA
buffer
duration,
temperature

Day 3

prepare damaged DNA

Day 5

5000 cells/well
(24-well plate)
48 h prior to
lipofection

transfect cells with damaged DNA

Day 9

flow cytometry analysis

Assess NHEJ repair following drug treatment

Day 6

250 cells per well
(6-well plate)
48h before drug
and irradiation

treat cells with drug and irradiate

Day 9

12 days
after
irradiation

viability analysis

A few words regarding the Methods

- Lipofection: citing volumes is appropriate in this case

  - *"25 μL of lipofectamine solution (1 μL LTX diluted in Opti-MEM) and 25 μL of DNA solution (250 ng pMAX-mCherry, 250 ng digested pMAX-EGFP-MCS, 0.5 μL Plus reagent diluted in Opti-MEM) were mixed, incubated 20 min at room temperature, and distributed to cells plated with 500 μL of fresh growth media.* concentration of drug *then added to "treated" wells. Control wells were transfected with intact pMAX-EGFP to represent the "maximal DNA repair" condition."*

- Flow cytometry

  - Find instrument model and excitation/emission wavelengths on wiki

- Don't forget descriptive subsection titles and introductory topic sentences

The meat of your paper

- Figures and captions
  - Decide on these first
  - Use subpanels
  - Text: limited on figure, explicit in caption
  - reasonable size
  - descriptive title
  - caption purely descriptive of image
  - intro sentence in caption
- Results
  - topic sentence
  - What you did: experiments and expectations, including controls
  - What you found:
  - summary / transition to next step

What goes into the Discussion?

- **Brief recapitulation of Results**

- Interpret

- Put in context: how does this fit with other studies?

- Highlight significance: how might this impact this/other field?

- Discuss controversial or surprising results

- Describes caveats and suggest remedy

- Conjecture (one layer only!) implications

- Propose future work, identify new questions that arise

- Follow same order as in Figures/Results

- Make sure you come back to big picture introduced in intro

- Don't overreach / overpromise!

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