M2D9: Prepare M2 Major assignments

- 1. Review Journal club presentation guidelines
- 2. Overview of Research article guidelines
- 3. Work on assignments!



Logistics for Journal club presentation

- Due date: Saturday, April 11 at 10 pm
- Review Comm Lab workshop slides!
- Completed individually
- Submission guidelines
 - Slides to Stellar
 - Video to Dropbox
- Additional assignment components:
 - Review two peer presentations and write questions
 - Meet with Noreen (and Instructors) to discuss presentation



How will you communicate *their* science?

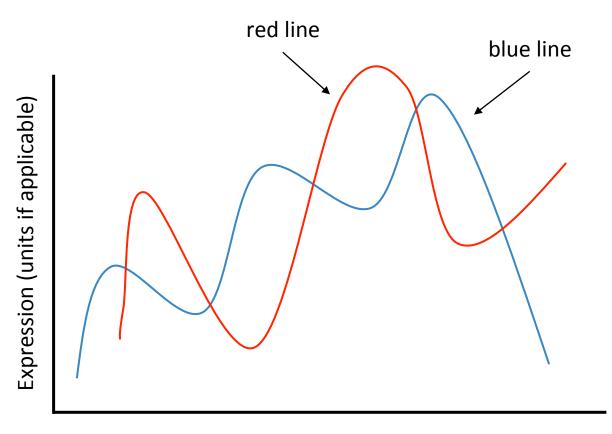
| Section | Minutes | Number of slides | DO | DON'T |
|----------------------|---------|---------------------|--|--|
| Introduction | ~2 | 2-3 | Introduce the key concepts that the audience will need to follow your presentation. Briefly state the overall scope and significance of the study what is the central question and why is it interesting? Try to summarize background material with a model slide rather than lines of text. If text is needed, bring in the details as you speak using PowerPoint animation. | Don't assume you are addressing an expert audience. Don't give more information than is absolutely needed to understand the rest of your talk. Don't put too much information on each slide. |
| Data | ~7 | 4-6 | Present the data in a logical sequence, letting each slide build upon the previous ones. Include a title for each slide. The title should be the conclusion and should be unique to the information on the slide. Make every element of your slide visible to the entire room. This means 20-point font or greater. Interpret each slide thoroughly and carefully. Point out strengths and weaknesses of the data along the way. | Don't read your talk. Similarly, do not read lists from slides. Don't put much information on each slide. Each slide should make only one point. Never say, "I know you can't read this, but". Everything on each slide should be legible. Don't be afraid to remind the audience how the data fits into the overall question |
| Summary | ~1 | 1 | Review each of your main messages.Clearly state what the study contributed to the field. | Don't repeat experimental details. |
| Question & Answer | ? | 0 | Answer the question being asked. If you are unclear about the question, ask for clarification. Respect every question and questioner. | Don't take too long with one question. If the discussion is involved, suggest meeting after the talk to discuss it more. |

How will you report their data?

- Consider how to present the main finding / conclusion using the key data from the article
 - Do not have time to show everything
- Each data slide should present a single message
 - Do not need to include all panels for every figure used
- Be mindful of slide design
 - Title line is valuable real estate, use it wisely
 - Text is okay, but only important details should be included
 - The data are the most important part of the slide, ensure labels are clear

EXAMPLE SLIDE: Blue line goes down at X time

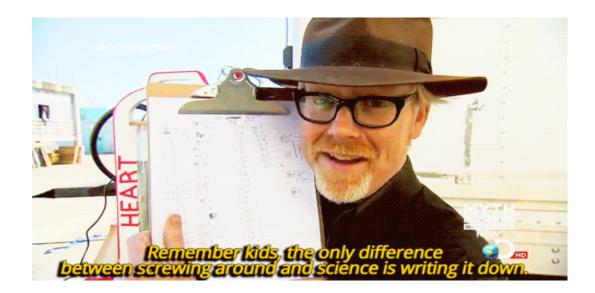
- Data represent expression of Y over time measured using method A
- Possibly something about the control(s), if applicable
- Perhaps an important note about the data that is not already stated in the title
- Transition to next slide...



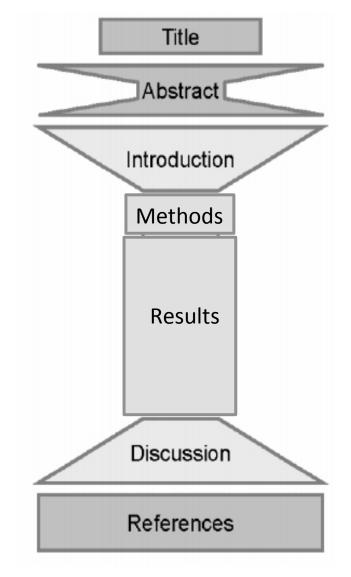
Time (units if applicable)

Logistics for Research article

- Due date: Monday, April 20 at 10 pm
- Review Comm Lab workshop slides!
- Completed individually
- Only one submission
- Formatting guidelines
 - Prepare text in word document
 - Prepare figures in powerpoint file
- Written in complete sentences and paragraphs



How will you communicate your science?



- Title & Abstract (10%)
 - First page
- Introduction (10%)
 - ~2-3 pages
- Methods (20%)
 - ~2-3 pages
- Results w/ Figures & Captions (50%)
 - ~4-5 pages
- Discussion (10%)
 - ~2-3 pages
- References
 - Last page(s)

How will you report your methods?

• You already (mostly) completed this part!

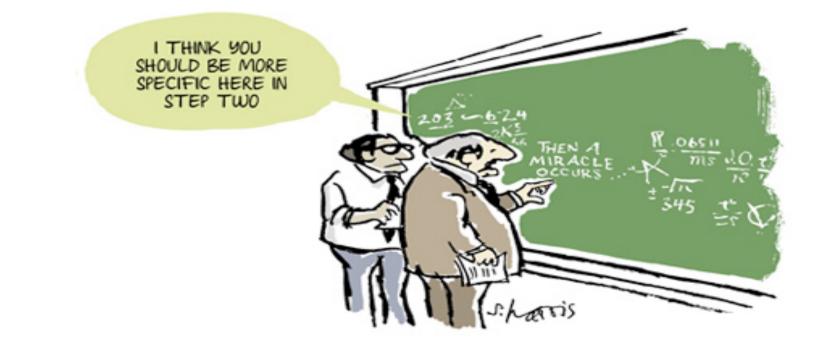


- Be sure to review the feedback from the M2D7 homework
 - Posted to Stellar
- Include the details regarding the RNA-seq analysis
 - See M2D8 prelab
- Consider the following for revisions: sub-sections, level of detail, and word choices

Methods: sub-sections

- Use sub-sections to group procedures
 - Include descriptive titles
 - Use logical, rather than chronological order
- Separate sub-sections with titles
 - Brief, but specific
- Include an introductory sentence
 - State the purpose or goal of particular method / group of procedures

Methods: level of detail



Methods: word choices

- the tube vs. the cell lysate
 - Give more informative, specific information
- combined or mixed vs. digested
 - Be precise about the procedure used
- cleaned vs. purified or isolated
 - Use the more scientific terminology
- in order to vs. to
 - Eliminate unnecessary wordiness
- avoid jargon and define all abbreviations

Be mindful of sentence structure

"<u>cell lysate was prepared by adding</u> ... and <u>proteins were separated</u> using SDS polyacrylamide gel electrophoresis (130 V for 45 min) in TGS buffer

- 1. PUT THE SUBJECT FIRST
- 2. BE SURE THE SUBJECT AND THE VERB MATCH

How will you introduce your data?

Introduction [edit]

The introduction will start on the second page. As you write your introduction, recall the idea of an hourglass structure. The information you use to set up the investigative question in your introduction should be supported by appropriate citations. **Any details you found in another researcher's work should be cited.**

The introduction provides a framework for the story you are about to tell, and thus serves two main purposes. For one, you must provide sufficient background information for a reader to understand the forthcoming results. Just as importantly, you must motivate the audience to keep reading! How? Reveal the significance of the work through connections to both prior scientific accomplishments and interesting future applications.

Most introductions are "funnel"-shaped in terms of content:

- Opening paragraph(s): most general, "big picture" paragraph(s). Here you should introduce the reader to the broader context of your
 experiment and motivate why your research is important. The best introductions tell a coherent story rather than present a dense but
 unconnected list of facts.
- Middle of introduction: "zooming in" somewhat. Once the reader has a frame for thinking about your research, you can present background information in more depth and motivation with more specificity.
- Wrapping up: most specific, a description of your particular investigation. Here you should make your overall methodology clear without getting into minute detail. In many journals, the introduction concludes with a brief preview of key findings and their implications (2-3 sentences total).

Please pay close attention to the feedback you received from the teaching faculty on your homework assignments as you prepare your introduction (as well as the rest of the report). Also, you may find that the **BE Communication Lab** is a terrific resource for providing comments on your Introduction. If the Comm Lab peer tutors (a scientifically literate audience) understand your motivation for the study -- you are in good shape!

How will you report your data?

1. Figure 1

· experimental overview / schematic illustrating the work-flow (just the key steps!) used in your research project

- 2. Figure 2 (this figure should include three panels)
 - Panel A: tables with top 5 GO terms in DLD-1 and DLD-1 + etoposide
 - · Panel B: bar graph containing the qPCR results for the genes of interest, including statistics
 - Panel C: heatmap comparing genes of interest across DLD-1 qPCR data, DLD-1 RNA-seq data, and A549 data
- 3. Figure 3 (this figure should include two panels)
 - · Panel A: plot of PCA data showing DLD-1 + etoposide and A549 + etoposide
 - · Panel B: heatmap comparing DLD-1 + etoposide and A549 + etoposide

4. Figure 4

heatmap comparing 4 GO terms

5. Figure 5

· scatterplots generated from the GO terms used in Fig. 4

The results section will account for 50% of the final grade for this assignment.

PRO TIP: USE SUB-SECTION HEADERS TO SEPARATE RESULTS TEXT ACCORDING TO FIGURES / DATA

Connect the results text to the figures!

RESULTS TEXT:

Homologous Recombination Deficiency Rationalizes the Synthetic Lethality between MSH3 and PRKDC

Given the substantial homologous recombination defect that we had observed in *MSH3*-mutant cells (Fig. 3 and Supplementary Fig. S9A), we next hypothesized that pharmacologic NHEJ abrogation through DNA-PKcs inhibition might lead to the generation of persistent unrepaired DSBs in these cells. To directly test this hypothesis, we induced DSBs in homologous recombination–proficient (H1568, HCC1359, and *Msh3^{wt/wt}* MEFs) and homologous recombination-defective (HCC44 [MSH3^{mut}]

FIGURE CAPTION:

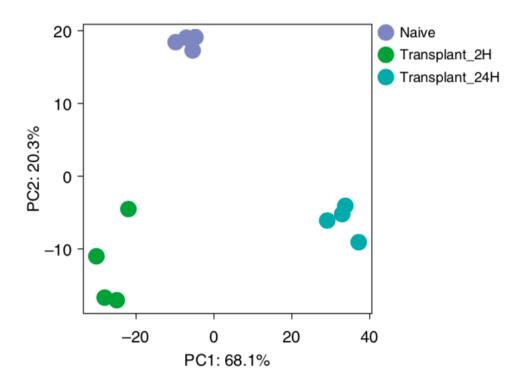
Figure 3. MSH3-mutant or MSH3-deficient cells display a robust homologous recombination defect. A, DNA (0, 4, and 72 hours) after short (1 hour) exposure to a low-dose (0.1 μmol/L) etoposide pulse. Representative in γ-H2AX or RAD51 nuclear foci: blue. 4'.6-diamidino-2-phenylindole (DAPI) counterstainl are shown for HCC44 I

What are your data?



Visualizing variability in your data

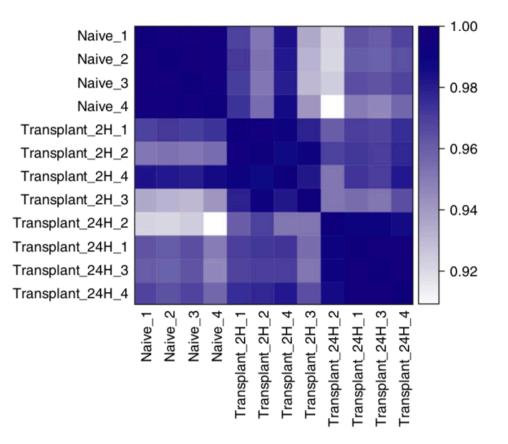
- Global overview of the data that allows researcher to assess variability and define differences between samples and groups
- Ideally, intergroup variability is greater than intragroup variability
 - What does intergroup variability represent?
 - What does intragroup variability represent?



Koch et al. (2018) A beginner's guide to analysis of RNA sequencing data. Trans Rev 59:145-157.

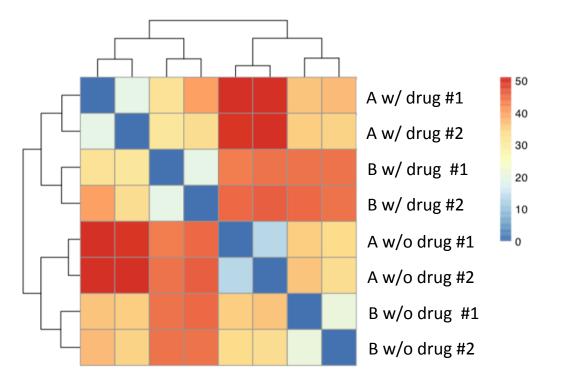
A closer look at inter / intragroup variability

- Allows researcher to assess the difference between (or distance) between samples and groups
- In this example, scale bar indicates correlation
 - Which samples are the most similar?
 - Which samples are the most different?
 - Which replicates are the most similar?
 - Which replicates are the most different?



Measuring distances between samples

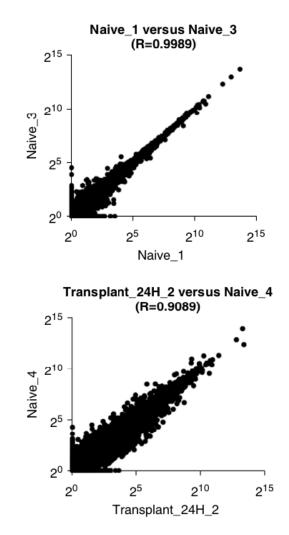
- In your analysis, Euclidean distance was used to measure the differences (or distances) in the data
 - Measures absolute distance between points in space



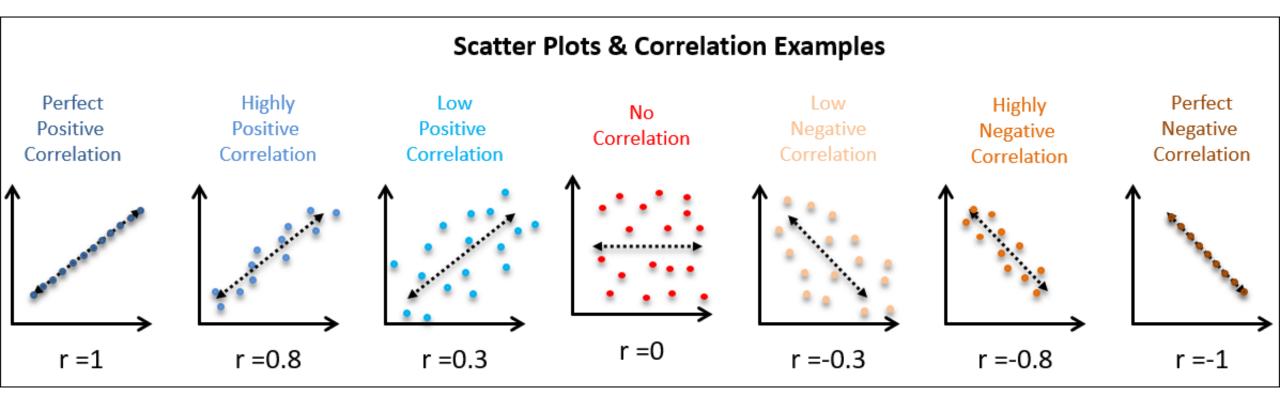
Which samples are the most similar?Which samples are the most differentWhich replicates are the most similar?Which replicates are the most different?

Visualizing correlation in your data

- Allows researcher to assess the correlation (or relationship) between samples and groups
- Correlations represented by strength and direction
 - How is strength of correlation measured?
 - How is direction of correlation determined?
 - Which scatterplot shows 'better' correlation between compared samples?



Best fit line used to represent trend in data



https://study.com/academy/lesson/scatter-plot-and-correlation-definition-example-analysis.html

How will you interpret your data?

Discussion [edit]

This section should incorporate all the good practices described in the Module 1 Data summary, but do so at a more advanced level. You will be expected to cite the broader scientific literature more thoroughly than before to inform your analysis in the discussion. You should also propose specific future experiments and otherwise show that you deeply understand the meaning and significance of your results; for example, if you have a hypothesis about why a particular transcript increased in response to etoposide treatment, consider what follow-up experiments you might try. You may also want to consider how the experiments can be improved; for example, what additional controls might be useful to include.

The purpose of the Discussion section is to interpret and contextualize your data. You should begin by reiterating the purpose of your research and your major findings. Then you might do any or all of the following: connect your findings to other research (published or that of your peers); describe any ambiguities and sources of error in the data, and suggest future experiments to resolve uncertainties; explain where you expect your work may lead, and suggest specific experiments for extending your findings; describe any conceptual or technical limitations of the research. Finally, you should explain the significance of your findings to basic science research and/or to engineering applications. As with previous sections, the discussion should have a clear organization and narrative flow, whether or not you use subsections.

The discussion will account for 10% of the final grade for this assignment.

Reporting versus Interpreting your data

RESULTS

- 1. What was the overall goal of these data?
 - State concisely as an introductory sentence.
- 2. If applicable, what was the result of your control?
 - Was it expected?
- 3. What was your result?
 - Was it expected?
- 4. What does this motivate you to do next?
 - Specifically, what experiment follows?

DISCUSSION

- 1. What evidence do you have that your result is correct or incorrect?
 - How do your controls support your data?
- 2. In sum, what do your data suggest or indicate?
 - Do your data support your hypothesis?
 Why?
- 3. What does this motivate you to do next?
 - Specifically, what is the next research question?

Be mindful of over interpreting your data

- What does it mean that samples are different / similar?
- What does it mean that genes in a GO term are enriched?
- What does it mean that down- or up-regulated genes are dominating / driving the enrichment?



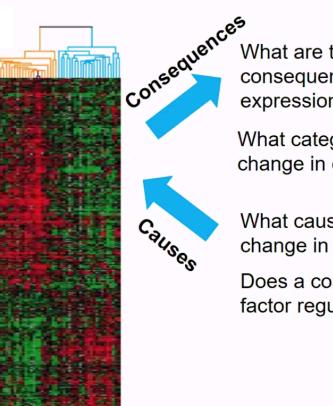
"Ralph's over-interpreting the data again."

Remember to 'tell a story' with your data

- Introduction and Discussion should match
 - Preview / Review of the key findings
- Results should be tied together with transitions
 - Figures should be connected to Results (titles should match headers)
- Discussion should integrate the results together into a cohesive takehome message
- Effective redundancy provides a broader impact
 - Each section is crafted for a particular audience, naïve or expert

Remember the lecture material!

- Which of these questions are answered by your data? How?
- Which of these questions can be answered by future experiments?
 - What experiments would answer the question?
 - How would answering this question further your research?



What are the biological consequences of the expression changes?

What categories of genes change in expression?

What causes these genes to change in expression?

Does a common transcription factor regulate them?