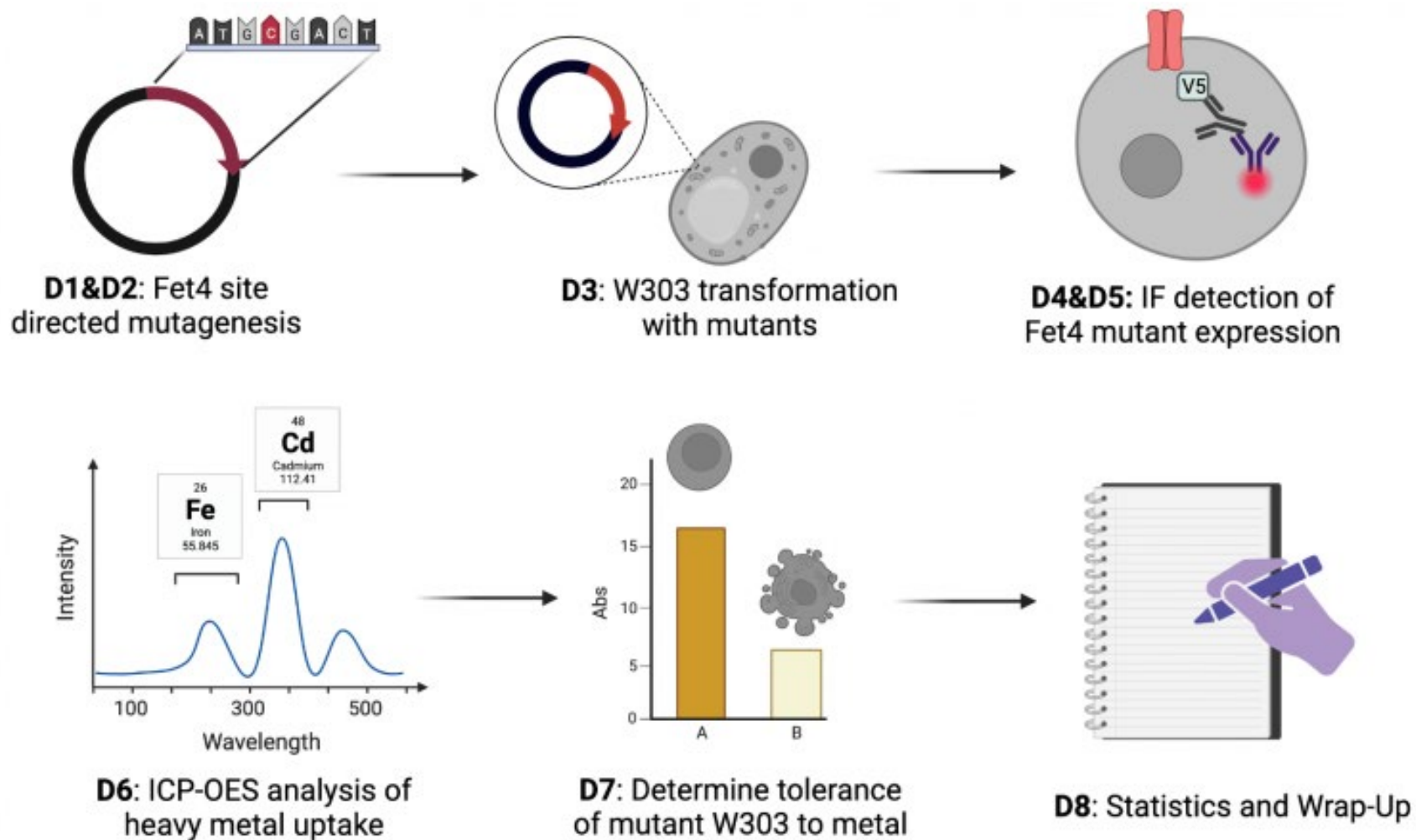# M2D4:
Determine transporter mutation and prepare expression experiment

1. Comm lab workshop
2. Prelab discussion
3. Check sequencing results
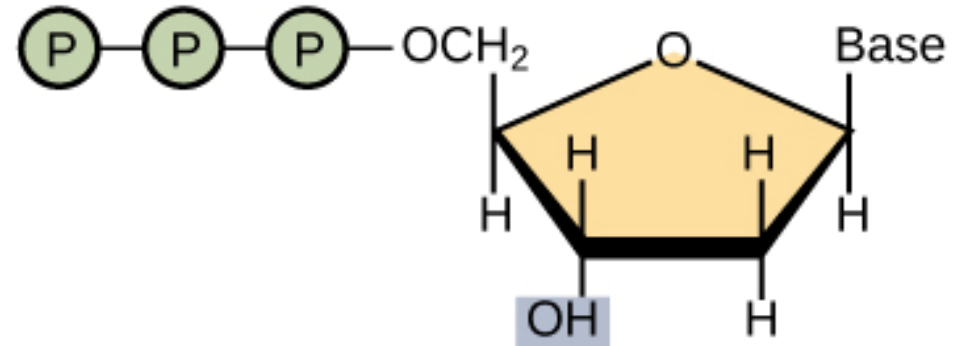4. Prepare for functional assay

Overview of Mod 2 experiments:

D1&D2: Fet4 site directed mutagenesis

D3: W303 transformation with mutants

D4&D5: IF detection of Fet4 mutant expression

D6: ICP-OES analysis of heavy metal uptake

D7: Determine tolerance of mutant W303 to metal

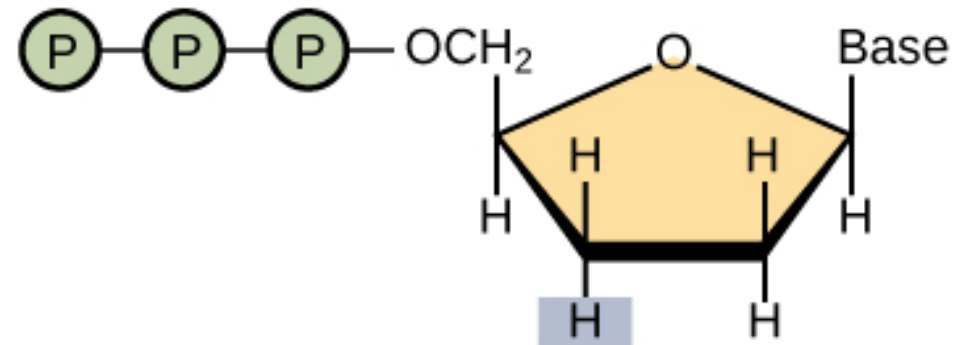**D8**: Statistics and Wrap-Up

Confirm mutation using sequencing results

- What is the difference between dNTPs and ddNTPs?

- What modification (not shown in the image to right) is made to ddNTPs used in sequencing reactions?

- How do modified ddNTPs allow for a DNA sequence to be determined?

Deoxynucleotide (dNTP)

Dideoxynucleotide (ddNTP)

Prepare for functional assay by coating slides

- For immunofluorescence staining, cells must be attached to a glass coverslip

- Many cells, including yeast cells, do not adhere well to glass

- Poly-D-lysine is a chemically synthesized extracellular matrix with net positive charge
    - Enhances electrostatic interactions between negatively-charged ions of cell membrane and positively-charged surface
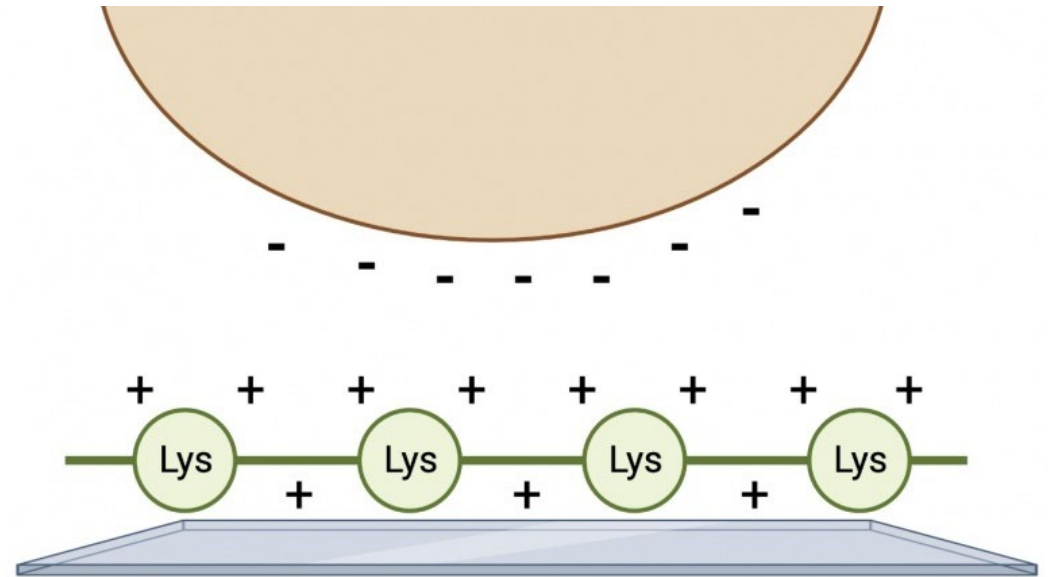
For today…

- Class will be divided for exercises:
  - Red, Orange, Yellow start on Part #2
  - Purple, Pink, Blue, Green start on Part #3
- Use extra time to get a head start on your Journal article presentation or work on your Data summary revisions!
- Review feedback from M2D3

For M2D5…

- Draft a detailed outline introduction for your Research article
- Prepare a figure using the sequencing results and draft the corresponding results text

Logistics for Journal article presentation

- Due date: by 12p on presentation date

- Review Comm Lab workshop slides!

- Completed individually

- Submission guidelines:
  - Slides to Canvas

- Additional assignment components:
  - Ask questions after peer presentations
  - Meet with Noreen to review / discuss your presentation

How will you communicate *their* science?

Format considerations [edit]

The timing provided here is a guideline for a 10-minute presentation. Your presentation may vary depending on the content.

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…
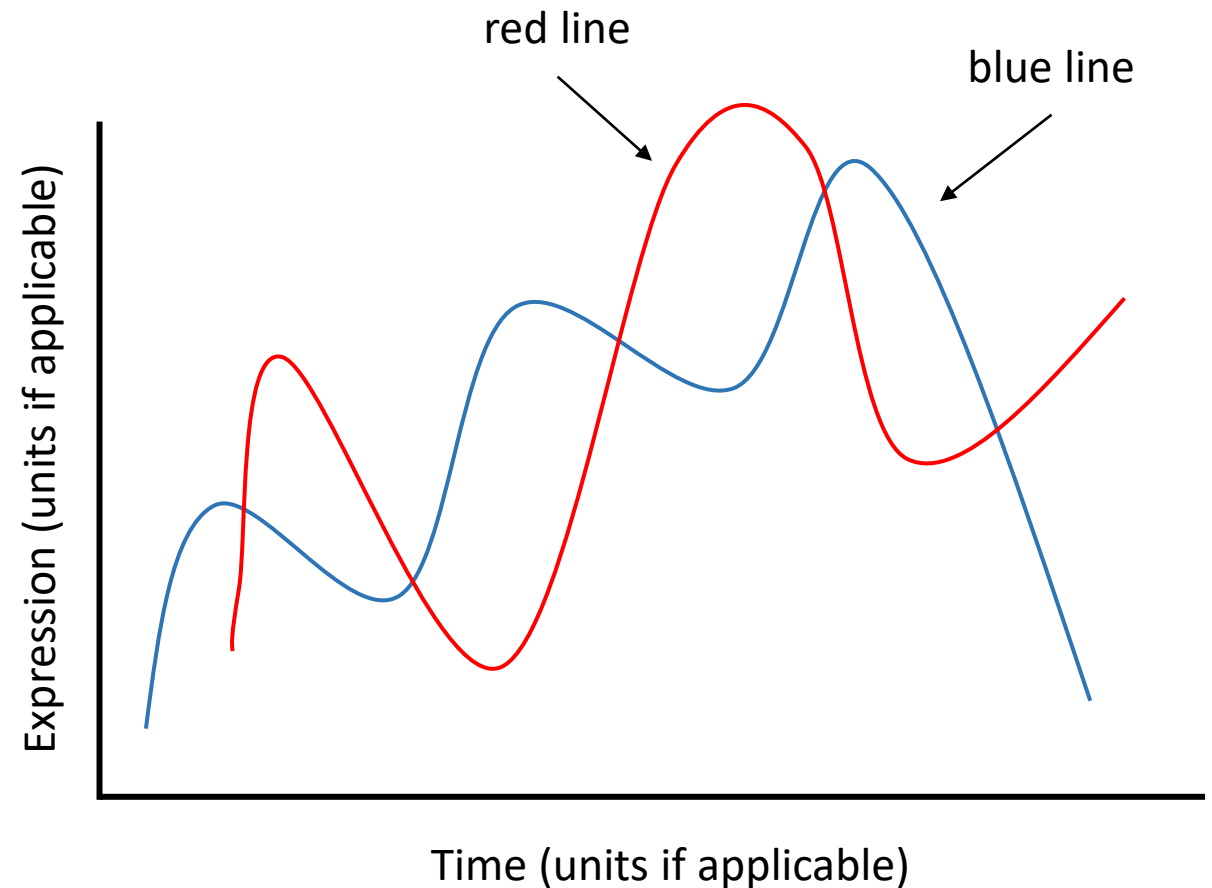
What is a figure?

- Critically think about which figures best give the take-home message

- Consider which figures are best for a visual presentation

- Omit panel labels

- What figures are you able to understand / explain?
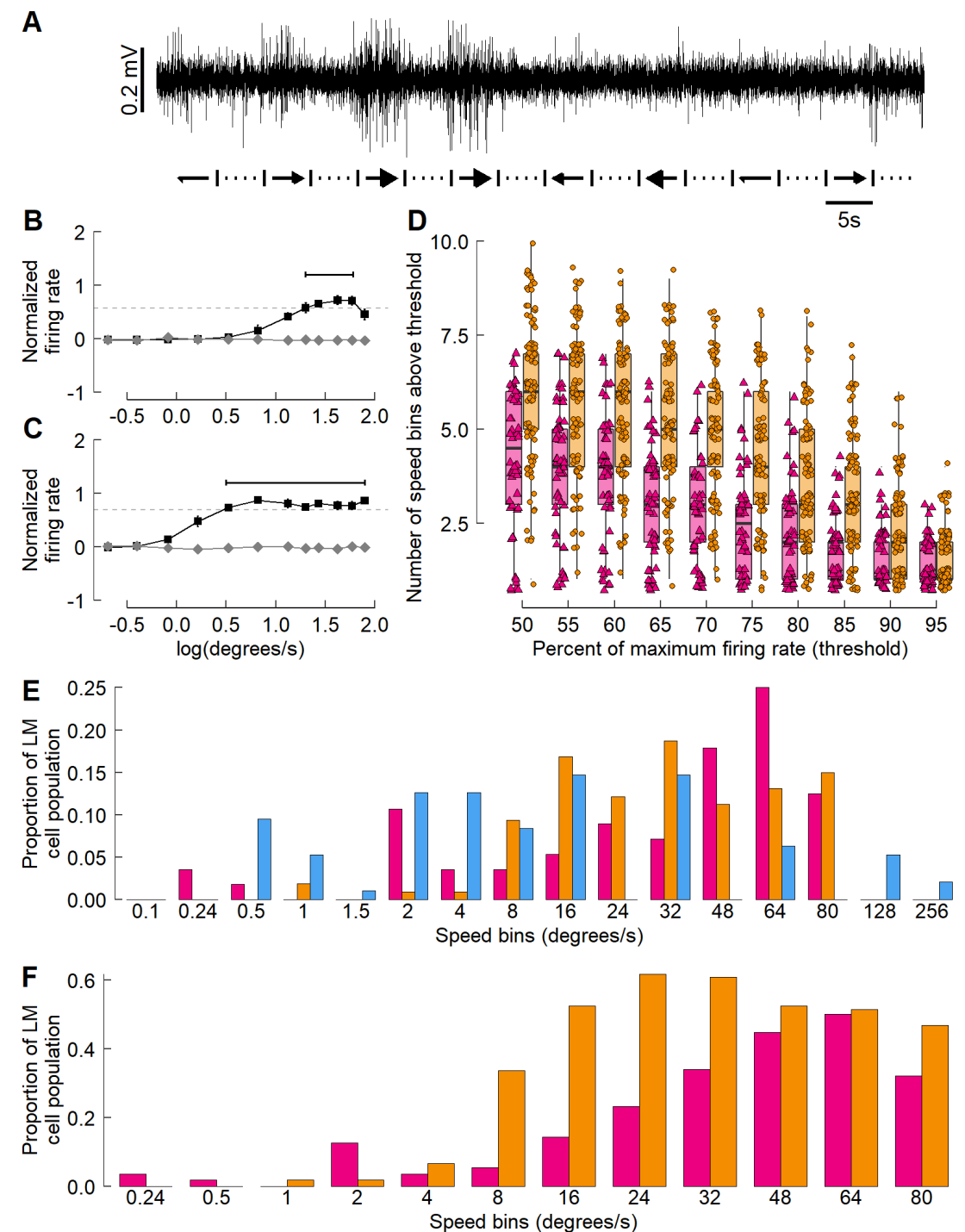
Figures can be overwhelming!

- When a complicated figure is necessary for the message, consider…
  - Using animation to layer in the information
  - Using boxes / arrows to highlight the information as it is discussed
- All color codes / labels that are shown should be explained
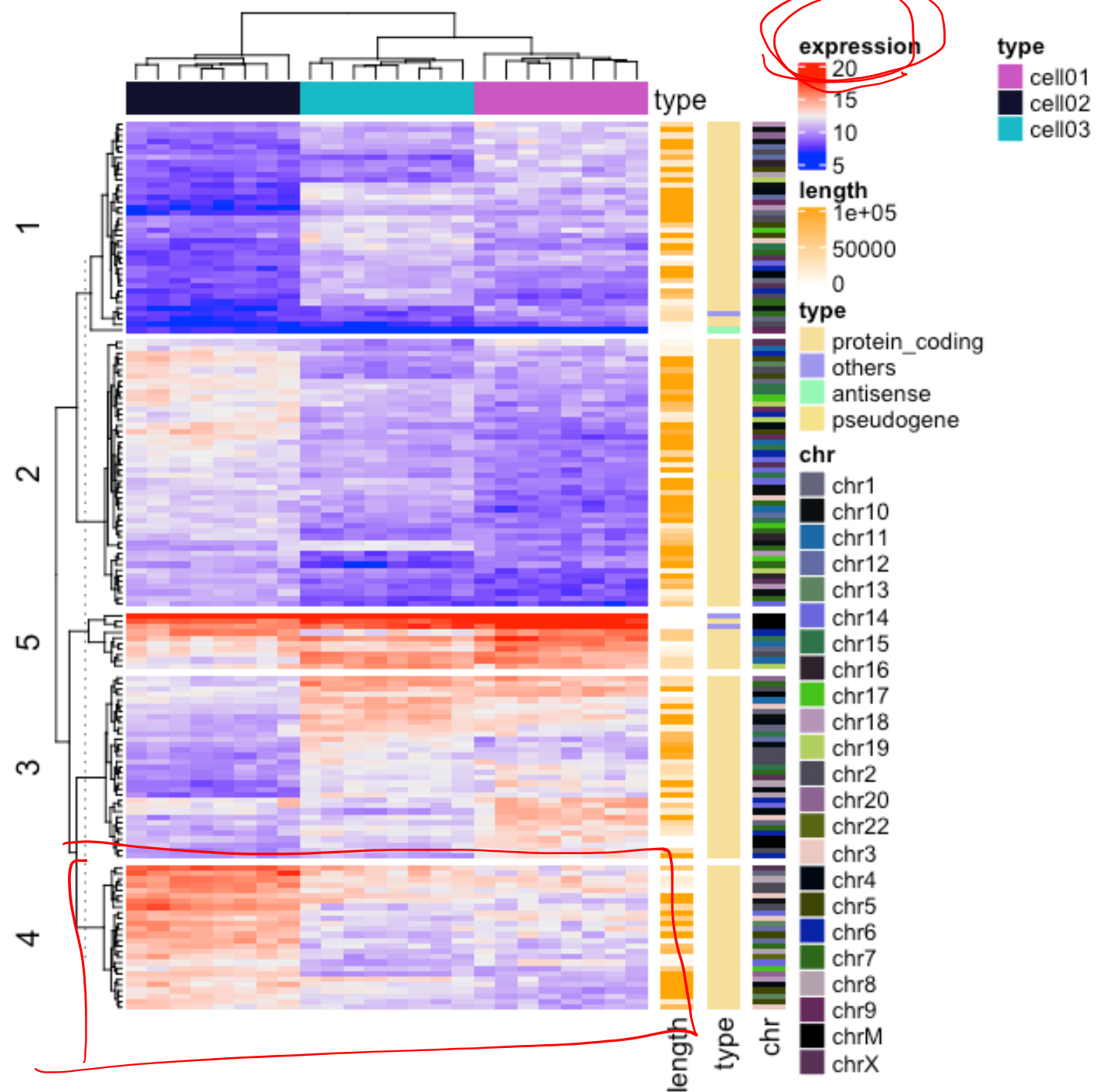
Figures may not project well!

- When an intricate / low contrast figure is necessary for the message, consider…
  - Describing each image and the key differences between the images as part of the script
  - Using clear labels on the images that define what is shown in each
- Avoid stating "this is difficult to see" or "this is better in the paper"
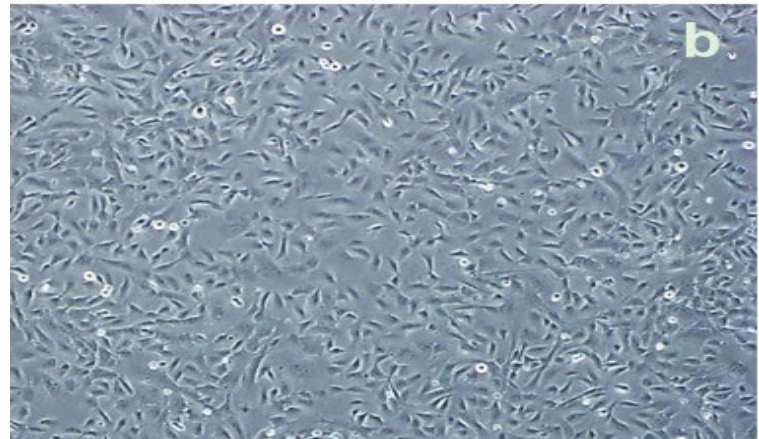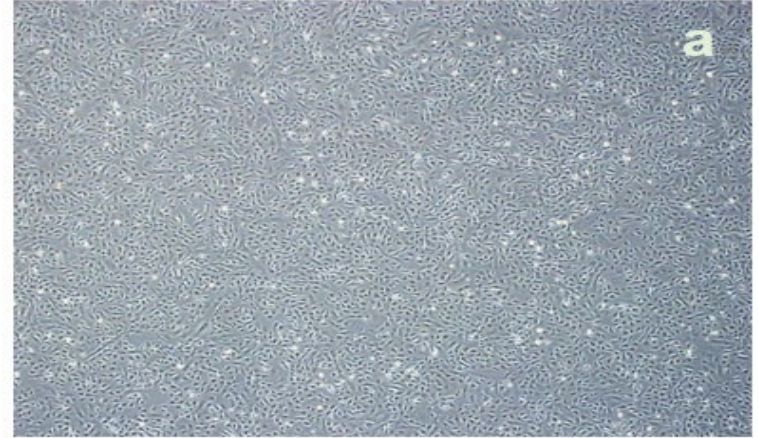
Figures may include layers of data!

- When results for controls are shown, be sure to...
  - Describe for what the result controls
  - State what is expected and how is relates to the experimental results
- Conditions and results for individual variables should be addressed