Write It Up!

A guide to Tab reports

The goal of our class this semester has been to determine if *S. pombe* orthologs of the *S. cerevisiae MET* genes are able to complement deficiencies in *S. cerevisiae met* strains. Throughout the semester, you will be collecting data and reporting that data in 5 micro-reports. At the end of the semester, the data will be compiled into a final report and a poster. The final report should be written in the form of a scientific publication, following the format of a *FEMS Yeast Research* paper. This chapter contains general and specific guidelines for preparing microreports, the final report and the poster.

The focus of each micro-report is a figure, sometimes accompanied by a table, with the experimental data. The micro-reports are scheduled so that you will receive feedback on your presentation and interpretation of the data, as well as your scientific writing, throughout the semester. You can expect to see improvement as the semester progresses. The experiments in the five micro-reports are listed below in chronological order. Figures and tables are prepared by the team. Before each microreport is due, teams will post their figure to the class data sharing site and present their figure to the class for discussion and feedback.

- 1. Identification of *met* deletion strains by their growth phenotypes on defined media containing various sulfur sources
- 2. Genotype analysis of *met* deletion strains by yeast colony PCR
- 3. Identification of yeast overexpression plasmids by restriction mapping
- 4. Transformation of *met* deletion strains with plasmids and complementation analysis of the transformed strains
- 5. Analysis of Met protein overexpression in transformed *met* deletion strains by SDS-PAGE and western blotting of cell extracts

Before proceeding, consider some general tips for good scientific writing:

- Good scientific writing is **CONCISE!** A rambling report quickly bores the reader and weakens the message of the report. Observe all page limits. Abbreviations are often helpful. With the exception of standard abbreviations, e.g. length and degrees, spell out the abbreviated term the first time that you use it and follow the term with the abbreviation in parentheses.
- Good scientific writing is **PRECISE!** Use the correct conventions for strains, genes and proteins. This class introduces you to a large number of scientific terms, which are defined in the glossary section. Use of the correct term can often prevent the need for extra words.
- Good scientific writing is **EFFECTIVE**! The same skills that produce a great essay on Henry Thoreau's *Walden* are relevant here! Use the active voice when possible. Writing should be smooth, not choppy. Avoid run-on sentences and be sure that antecedents are clear.
- Use sub-headings to divide the text into logical segments.

Micro-reports - general guidelines

Page limit: 2 PAGES, excluding figures, tables and references

Micro-reports have a condensed format. You can think of each micro-report as the equivalent of a figure or table from a scientific publication, together with the methods needed to understand the experiment and a brief analysis.

Before each micro-report is due, teams will present and discuss their data with their section. This can provide value feedback for the report. Teams are also advised to look at the results obtained by students in other sections who worked with the same strains and plasmids. Good science is reproducible. Did other students get the same results as you did? Do **NOT** reproduce the results from other sections in your report (remember the plagiarism and academic integrity rules!), but it is fine to discuss these results with proper attribution.

Micro-report organization. Include the following sections:

1. Heading

Your name Section number or TA's name (*e.g.* Jen's section)

2. *Purpose* - state the purpose of the experiment in <u>one sentence</u>.

3. Methods and Materials

The Materials and Methods (M&M) section should be written in paragraph format. Methods should NOT be written as lists of steps, as they might appear in your notebook or in a recipe. Avoid excessive detail. For example, DON'T state: "The solution was prepared by adding 5 μL of 200 mM NaCl to 95 μL of deionized water. "Instead, state: "The solution contained 10 mM NaCl." A reader who chooses to repeat your experiment may have his/her own way of preparing the solutions. The final concentrations of components are the important consideration.

If you are using a published technique, you can cite the procedure without reproducing the detailed steps. In this course, you will probably find it convenient to frequently refer to procedures in the lab manual. If you are using a commercial kit, e.g. the ZyppyTM plasmid purification kit, you can state that you followed the manufacturer's instructions. In all cases, be sure to include any modifications to the published procedure.

Rule of thumb: A good M&M section provides enough information for a trained professional to reproduce your experiments.

4. Results and Discussion

The section contains a brief narrative that guides your reader through the figures and legends that present your experimental data. **Figures** need to be clearly labeled and to be accompanied by a figure legend. The figure legend should have a title and include explanations of

the different panels or graphs in the figure. The figure legend should be placed below the figure. A well-written legend contains enough information that an expert reader can understand the experiment shown in the figure from its legend (assuming that the reader also looks at the M&M section).

Tables should have a title. Columns and rows should be clearly labeled and the units of measurement (e.g. grams/liter) should be included. When appropriate, include statistical measures of error in both figures and tables.

Report the results of all your experiments, even if you think they are incorrect. Discuss any experimental problems that you encountered in the experiment and speculate how these could have affected the results. Compare your results to those posted by other groups on the data sharing site. If your results agree, you may feel more confident about your results. Propose further experiments to resolve any remaining questions.

State your conclusions in one or two sentences.

5. Thought question

The rubric for each micro-report will include a thought question that requires you to apply your conceptual knowledge to a novel situation. **NOTE: This question is worth a significant portion of your grade for the report.**

6. References

Use the FEMS Yeast Research format for citations and references. Cite the lab manual: O'Connor CM (2014) *Investigations in Molecular Cell Biology*. Hayden-McNeill, Plymouth, MI.

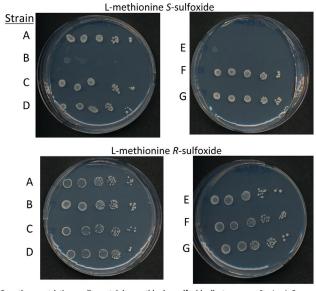
Document format:

- Reports must be typewritten
- Use 1-inch margins on all sides and double line spacing
- Use a font that generates less than 15 characters per inch

The next few pages provide specific guidelines for the five micro-reports of the semester. A rubric will be posted before each micro-report is due. The rubric will contain specific details about the grading for each micro-report. The rubric will also contain the thought question for the micro-report. It may also contain some changes and/or additions to the guidelines in this chapter. As such, the rubric should be considered to contain the definitive set of instructions.

Micro-report 1: Selective plating experiment

The figure is at the heart of every micro-report. This multi-panel figure of a spot plating experiment was prepared by students in an advanced lab class.



Growth on restrictive media containing methionine sulfoxide diastereomers Strains A-G were spot plated on SC media containing either 20 mg/L L-methionine-S-sulfoxide or 20 mg/L L-methionine-R-sulfoxide in the place of methionine. Strains A-F were constructed from the genotype met15 his3 leu2 ura3. Strains carried the additional mutations, as follows: A (none), B (msra), C (msrb), D (fmsr), E (msrc msrb), F (msra frmsr), G (msrb frmsr). Plates were incubated for 3 days at 30 degrees C.

Note the following features of the figure:

- Plates are oriented in the same direction so they can be easily compared
- Strains and media are labeled. Strain names can be used in the figure itself or indicated by a code that is defined in the legend.
- Strain names are used when the genotypes of strains are uncertain (your experiment). This figure presents results from an experiment where genotypes were known.
- The reader will need to refer to the M&M section for additional details about the media and experiment.

Specific guidelines for micro-report 1 follow.

Purpose: You have three yeast strains derived from strain, BY4742. In one sentence, what are you trying to do in this experiment?

Materials and Methods: In preparing the M&M, ask yourself "What information will an investigator need to reproduce our experiments?" Provide information on the strains and media that you used, as well as the procedures that you used for spot plating.

Strains: Include the names of your strains as well as the genotype of the BY4742 parent strain.

Media: Identify the culture media you used in the experiments. Decide on a naming convention - the same nomenclature should be used in both the figure and M&M. Reference the manual for the composition of the media, rather than including all the components here.

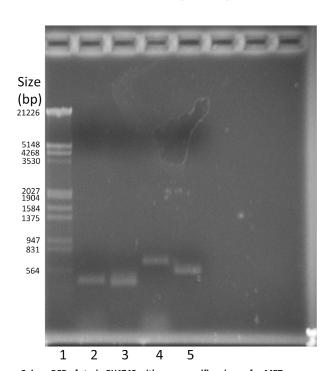
Spot plating: Someone trying to reproduce your results will need to know how your starter cultures were generated (cultures were grown overnight in YPD) and how cultures were diluted for the spot plates (e.g. a series of 1:10 dilutions in sterile water). They do NOT need to know that you transferred 10 μ L yeast culture to 90 μ L water. Readers will need to know how many microliters were used for each spot and the conditions used to incubate (time, temperature) the plates.

Results and Discussion - Your figure with the scanned plates is the focal point of this section. The R&D section tells a story of how you used your plating data to identify the *met* deletions in the YMP strains. Provide a brief narrative that guides your reader through your results and your thinking. How would deletions in your *MET* genes affect the ability of your YMP strains to use different sulfur sources? Do the data allow you to confidently identify the strains?

A <u>single</u> summary data table documenting the growth of YMP and BY4742 strains on various culture media is a good way to bring together the experimental data and your conclusions. Describe the growth of the *met* deletion strains on the various media and include your preliminary strain identifications from the experimental data.

Micro-report 2: Yeast colony PCR

In this report, you will describe the results of the yeast colony PCR experiment that you designed to identify the *met* deletions in your three YMP strains. The products of your PCR reaction will be separated on agarose gels similar to this one prepared by students in another class.



Colony PCR of strain BY4742 with gene-specific primers for *MET* genes. Gene-specific primers A and B were used for each PCR reaction. PCR products were separated by electrophoresis on 1% agarose gels. The sizes of the molecular weight standards (lane 1) are shown on the left. Primers used for the reactions correspond to *MET1* (lane 2), *MET3* (lane 3), *MET7* (lane 4) and *MET8* (lane5).

Note the following features of this figure:

- The gel is shown with the loading wells at the **top**.
- Lanes are clearly marked. In this figure, the lanes are numbered. The strains and primers used for each reaction are described in the legend. Alternatively, the lanes could be labeled with the names of strains and primers, taking care that they remain readable. Conclusions are **NOT** included in the legend.
- The sizes of the markers are indicated. (Your markers will be different.)

The reader will need to refer to the M&M for additional details on the strain genotypes and the PCR procedure.

Specific guidelines micro-report 2 follow.

Materials and Methods: Provide information on the specific strains and primers that you used, as well as the procedures for PCR and agarose gel electrophoresis.

Strains: See micro-report 1 guidelines.

Primers: In a publication, authors usually include the sequences of their PCR primers in the text or a table. You should include the names of the *MET* genes, but you do NOT need to include the actual sequences, because these are listed in Chapter 7 of the manual. Be sure to cite the manual, however. Use the correct terminology when you refer to primers. GSP-A primers are sense primers that correspond to 5'-flanking sequences of the *MET* genes. GSP-B primers are antisense primers that are complementary to ORF sequences.

PCR: The PCR methods are described in the manual. "PCR reactions were performed as described (ref.)" is adequate. If you made any modifications, however, you need to describe them *e.g.* ..."with the following modifications"....

Agarose gel electrophoresis: The PCR reaction products were analyzed on agarose gels that were stained with ethidium bromide. Describe the conditions that you used to analyze the PCR products. Include the concentration of agarose, the name of the buffer and the voltage. In very general terms describe this analysis. Refer readers to the manual for additional details.

Results and Discussion: The figure with your gel is the focal point of this section. Briefly describe your experimental design and the logic underlying your choice of primers.

As you discuss your results, remember that the bands you see on the agarose gel are **PCR products**. Size is very important in interpreting the results of a PCR experiment. As you prepared for the experiment, you calculated the sizes of PCR products that would confirm/not confirm your predictions. Did your experimental results confirm your predictions?

If your results did not confirm your predictions, you need to consider if the predictions were wrong OR if the reactions did not work. Did you see bands in any lane? If so, the PCR reagents were probably not at fault. What else might have happened?

A <u>single</u> summary data table with the predicted and observed sizes of the PCR products would be helpful. The predicted sizes should very accurate, since they are based on the actual genome sequence, which has nucleotide resolution. Your estimated sizes are much less accurate. You may be able to determine if one PCR product is smaller than another, but you will only be able to place the products within a certain size range.

Micro-report 3: Restriction mapping

Figure: The figure at the heart of this micro-report is an agarose gel containing the restriction digests of your team's plasmids. *Refer to the guidelines for Micro-report 2 to see how to set up figures with agarose gels.* The reader should be able to determine from the gel and its legend which plasmid is shown in each lane and which restriction endonuclease (RE) was used in each digest.

Materials and Methods: To reproduce your experiments, a reader will need to know which plasmids and REs that you used, how you purified the plasmids and some information about the agarose gels used for the restriction maps. When possible, refer to published procedures, noting any modifications to a published procedure. You may want to use subheadings for the M&M section.

Plasmids: The *MET* genes and homologs have been cloned into two different yeast overexpression plasmids. Use the correct nomenclature when referring to plasmids of known genotypes. Plasmid names begin with a lower case "p" and are written in normal font. For much of this report, you will need to refer to the plasmids by their number alone, since you are still trying to identify them.

Plasmid purification: There are many different methods for isolating plasmids, so inform the reader that you used the ZyppyTM Plasmid Mimiprep Kit (Zymo Research) with the modifications described in the lab manual. Include information on how you estimated plasmid DNA concentrations as well as the DNA concentration of each purified plasmid.

Restriction digests: To reproduce your experiments, the reader will want to know how much plasmid DNA you used in the digestions and which REs you used. The ratio of RE units to DNA is important for successful digests. Your reader may be using a different commercial source of REs, so also provide the number of enzyme units (U) in the reaction. Do NOT list the μ L of each component used in the reactions. Be sure to include information about the temperature and duration of the incubation.

Agarose gel electrophoresis: See the guidelines for micro-report 2.

Results and Discussion: Bring the reader through the logic for your experimental design. Why did you choose this particular RE? Refer to the bands on the gels as **restriction fragments**. Restriction fragments are the products of **RE digestions**.

Size is very important in interpreting the results of a RE digest. A single summary data table with the predicted and observed sizes of the restriction fragments should be included. Estimate the sizes of the restriction fragments by comparing their migration to the markers. Predicted sizes should very accurate, since they are based on the actual DNA sequences, which has nucleotide resolution. Your estimated sizes are much less accurate. You may be able to determine if one restriction fragment is smaller or larger than another, but you will only be

able to place the fragments within a certain size range. **NOTE:** circular plasmids run anomalously on agarose gels. Supercoiled plasmids migrate more rapidly on gels than a linear DNA molecule of the same size. Conversely, nicked (a single strand of the helix has been cleaved, producing a relaxed circle) plasmids migrate more slowly than linear DNA of the same size. Incomplete digestion can also complicate results. *Keep in mind that the number of bands on the gel is not as important as the differences in the banding patterns!*

Did your experimental results confirm your predictions? If your results did not confirm your predictions, you need to consider if the predictions were wrong OR if the reactions did not work. The undigested plasmid provides a control for interpreting the latter issue.

Micro-report 4: Complementation analysis

Figure: The figure at the heart of this micro-report is multi-panel figure showing replica plates of strains that have been transformed with overexpression plasmids. *Refer to the guidelines for Micro-report 1 to see how to set up figures with multiple panels.*

Materials and Methods: Provide information on the transformation and replica plating procedures, as well as the media used in the experiments. When possible, reference the lab manual, noting any changes that you made to procedures.

Strains and plasmids: See micro-reports 1 and 3.

Media: See micro-report 1.

Transformation: Someone trying to reproduce your results will need to know details about the transformation procedure, because transformation efficiencies vary widely and show a strong dependence on reagents and incubation conditions. You can reference the lab manual.

Replica plating: This is a standard procedure. You can also reference the lab manual.

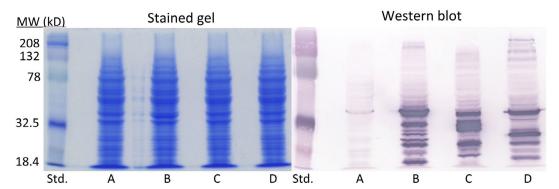
Results and Discussion: Your figure with the scanned plates is the focal point of this section. Include a single data table that with the calculated transformation efficiency with each plasmid and the ability of transformed strains (Y/N or +/-) to grow on the various replica plates. *Transformation efficiencies should be expressed in number of transformed cells/µg plasmid DNA.*

The R&D section should tell a story of how the replica plates allowed you to decide if your Met protein is conserved between the two yeast species. You may or may not have observed complementation. Failure to observe complementation is not necessarily due to experimental error. (Which plates serve as a control against experimental error?) Complementation is a functional assay that depends on both the expression of the fusion protein and the ability of the fusion protein to catalyze a reaction in Met biosynthesis. The fusion proteins have large C-terminal extensions that might affect their normal enzymatic functions. Negative results can be just as important as positive results in advancing scientific understanding.

If you did not observe complementation, discuss possible reasons that this may have happened and propose future experiments that could help to answer these questions. You may want to suggest new plasmid constructs for additional experiments. You may also want to bring in information from your BLASTP analyses. (What is the E-value?) Be sure to include enough justification that your proposed experiments will provide useful data.

Micro-report 5: SDS-PAGE and western blot analysis

In this report, you will describe the results of the SDS-PAGE and western blots that you used to analyze protein expression in your transformed cells under both repressed and induced conditions. Pay special attention to the degree to which these results confirm or contradict the results of the previous transformation/complementation report. The figure should be labeled in such a way that an experienced scientist is able to understand your results from the figure and legend alone. Many experimental details have been relegated to the M&M section. Lanes should be clearly labeled and the molecular weights of the standards should be included. (The example below is from a different class, where students used SDS-PAGE and western blots to analyze overexpression of yeast proteins expressed from pBG1805. Note that, unlike your experiments, a primary antibody to the HA epitope was used to detect proteins on western blots.)



Analysis of MSR overexpression in transformed yeast cells. Extracts were prepared from untransformed BY4741 cells (A) and from BY4741 cells transformed with pBG1805-MSRA (B), pBG1805-MSRB (C) or pBG1805-FRMSR (D), as described in the Materials and Methods. Protein overexpression was induced by incubating cells in media containing 2% galactose for 4 hr at 30 °C.. Proteins were separated on a 12% gels, which were stained with Simply Blue® (left) or used for western blots (right). Western blots were incubated with a mouse anti-HA primary antibody and a goat anti-mouse secondary antibody. The molecular weights (MWs) of the protein standards (Std.) are shown on the left.

Materials and Methods: Provide information about transformed strains, incubation conditions, preparation of cell extracts, SDS-PAGE gels and western blots. Reference published procedures when possible, noting any modifications. Subheadings may be helpful.

Transformed strains: Include the names of the strains and plasmids that you used to prepare cell extracts.

Extracts: Include information on the media and incubation times used to manipulate protein overexpression from the strains. Reference the manual for the extraction procedure, noting any modifications.

SDS-PAGE gels: Provide details about the % acrylamide of the gels, running time and voltage used for electrophoresis.

Electrophoretic transfer: Include the time and voltage used to transfer proteins from the SDS-PAGE gel to the PVDF membrane. Refer to the manual for other details.

Western blot: Include the antibodies that you used for the blot and the conditions (time, temperature) that you used for each of the incubations. Include the time that you used to detect overexpressed proteins with TMB. (This gives a some sense of the abundance of the protein in your extracts.) You do NOT need to include all the wash steps - just reference the manual.

Results and Discussion - Begin by discussing the **SDS-PAGE gel**. The SDS-PAGE gel provides a snapshot of cellular proteins and a rough comparison of protein concentrations in different extracts. The staining intensity of a band reflects its abundance in the extract.

- How did the total amount of protein compare between induced and non-induced samples? (What might this indicate about the different carbon sources?)
- Did you see any changes in individual bands on the gel? Is it possible to detect the fusion protein against the background of other proteins in the extract? Recall that cells have thousands of proteins and that a band may consist of more than one protein species.

The **western blot** allows you to detect the fusion protein against the background of other cell proteins. Include a table showing predicted and actual sizes of Met or LacZ proteins detected using the western blot technique. The sizes of the proteins are particularly important. (It is probably not possible to get an exact value of the protein sizes, because of the fuzziness of the standards on the western blots. Nonetheless, you should be able to place the proteins within a certain range.) The plasmid-encoded proteins will be larger than the naturally occurring protein because of the epitope tags encoded by the plasmid. Are the observed sizes what you expected?

Other questions to address in the Discussion:

- Did you see evidence of induction by the *GAL1* promoter on either the gel or blot?
- How did the western blot results relate to your complementation results? How do these results enrich/weaken your complementation findings?
- If you did not detect proteins on your blot, propose some explanations. Compare the samples. Do the other samples provide good positive controls for your technique?

Final lab report

The research goal of our class is to determine if the *S. pombe* orthologs of *S. cerevisiae MET* genes can complement deficiencies in *S. cerevisiae met* strains. During the semester, you have been collecting data and organizing the data into figures and tables. You will assemble these draft versions into a final report. The data hopefully form a nice story that answers your research question and/or suggests additional experiments.

The final report should be in the form of a scientific publication, which includes the numbered sections below. Unlike the mini-reports, the Results and Discussion sections in the final report should be separate. As you write, keep in mind that *good scientific writing is precise and concise!* In the professional world, journals provide an economic incentive for brevity by levying page charges on authors- the longer the report, the higher the page costs to the authors! Be careful to observe the page maximum for each section.

Experiments to include in the final report include:

- Growth of your strains on selective media
- Yeast colony PCR
- Restriction map of your overexpression plasmids
- Replica plates of transformed strains
- SDS-PAGE and western blot analysis of cell extracts
- Independent experiment (placed where most appropriate within the text)

You are welcome to cut and paste from your earlier micro-reports. *After all, it is your work and you are welcome to reproduce it!* As you do so, be well-advised to incorporate the suggestions of your TA to improve the presentation. The only data that should be included in the final report is data that your team has generated. You should NOT include data from experiments that has been posted by other students on the data sharing site.

COVER PAGE

Include the title and abstract, as well as your name and section assignment

- **1. Title** Describe your project in 150 characters or less.
- **2. Abstract** (1 paragraph, 150 words maximum) **Single-spaced**

The abstract is a very brief summary of your work that should be comprehensible to the non-expert. The abstract should include the goals of your experiments, some mention (not description) of the methods used in the experiments, a succinct summary of your experimental results, and your overall conclusions. Avoid specific details of the experiments. A reader should be able to read the abstract and understand the overall experiment and results without reading the rest of the report. *Compose the abstract AFTER the rest of the report has been completed.*

BODY OF THE REPORT - Double-spaced (Note that this is different from the abstract.)

3. Introduction (1.5-2 pages)

The introduction provides the context to your experiments. What is the goal of your experiments? How does your experiment fit in with what's already known about your topic? Has anyone done experiments similar to yours before? To answer these questions, an introduction provides the reader with relevant background information derived from the scientific literature. Discuss the function of the enzyme and its evolutionary conservation. Use the format of *FEMS Yeast Research* to insert citations into your report. *Include at least 7 citations from the scientific literature*.

In the last paragraph of the introduction, give the reader a preview of your report. Tell the reader what experimental approaches you used to answer your question. End this section with one or two sentences summarizing your conclusions.

4. Materials and Methods (2-3 pages)

During the course of the semester, you have used a variety of techniques, which have been the subject of your micro-reports. In publications, the M&M section is usually divided into subsections, often with their own subheadings, reflecting the different experiments and techniques.

The first paragraph in an M&M section often contains details about the organisms used in the experiments and their culture conditions. When yeast and other microorganisms are used for the experiments, a genotype table is often included. *Include a genotype table with table with strain and plasmid information as Table 1.* Consult Cordente *et al.* (2009) for a good example. Include the three YMP strains that you worked with at the beginning of the semester in this table. Our YMP strains were derived from strain BY4742, which has the genotype $Mat\alpha his3\Delta 1 leu2\Delta 0$ $ura3\Delta 0$. Each of the YMP strains has a met deletion caused by the insertion of a KAN^R gene. The met deletion alleles should be referenced as: met#:: KAN^R . (See Chapter 6.)

The plasmids for this report are based on pYES2.1 and pBG1805, both of which drive expression using the *GAL1* promoter. To describe these overexpression plasmids, use the format: pBG1805-*GAL1:MET#* or pYES2.1-*GAL1:Met#*

Briefly summarize the methods used in the experiments with appropriate subheadings. Feel free to cut and paste from previous micro-reports. Add additional information for your independent experiment. Remember - if you are using a published technique (or reagent), you do not need to repeat all the details in the M&M section. Instead, you may refer to the published procedure or formulation. In this course, you can refer to a protocol in the lab manual, but you need to include any modifications that you have made to the procedures or recipes.

Rule of thumb: A good M&M section should provide enough information for a trained professional to reproduce your experiments.

5. Results - (2-3 pages, not including tables and figures)

The Results section tells a story. It provides a brief narrative that guides the reader through the figures and tables with the data. Smooth transitions are important in the Results section. The reader should be able to grasp the reasoning that led from one experiment to the next. Do NOT discuss the results in any depth in this section - there is a separate discussion section in this report. Provide just enough of your conclusions that the transition to the next experiment is a logical one. For example, your selective plating data influenced your choice of primers for the yeast colony PCR experiment. (Your tables actually include some of these conclusions.) Because different kinds of experiments are frequently used in a paper, authors frequently use subheadings in the Results section.

Figures and tables from the micro-reports should be given numbers that correspond to their order of appearance in the text. **PLACE** the figures and table within the Results section **NEAR** where they are referenced. (Because of page breaks, it is not always possible to place the figure or table on the same page as it is referenced. Do NOT simply attach the tables and figures to the end of the report. The Cordente et al. (2009) paper provide examples of numbering and placement. Follow the general guidelines for micro-reports. Remember that a trained biologist should be able to understand your results by simply "looking at the pictures."

6. Discussion - 2 pages maximum

Our goal this semester was to determine if Met protein functions were conserved between *S. cerevisiae* and *S. pombe*, using complementation to analyze protein function. As you write this report, address the following issues:

- Did you observe any differences in the abilities of *S. cerevisiae* and *S. pombe* genes to complement the *met* mutation in your strain? Is your complementation data consistent with conservation?
- Reconcile the complementation data with the SDS-PAGE and western blot results. Were Met proteins of the expected size expressed in transformed cells? How does complementation relate to expression of the Met protein?
- If you did not observe complementation, was this because of technical issues or because of protein divergence between the two yeasts? How would you test this?
- How did your independent experiment confirm or extend your results?
- What additional experiments should be done to prove to the scientific community that MetXp function is/is not conserved between the two yeast species?

7. References - Single-spaced

List the references using the *FEMS Yeast Research* format, as you did in the bibliography assignment.

Poster

A good poster communicates data concisely and effectively. This poster presents the same data as your final report. Unlike the final report, which is read carefully by a single reviewer, your audience is a larger number of individuals who "visit" your poster. Your poster audience will be more diverse than your class section and they may have limited familiarity with your project. (Friends and family are welcome!) *The poster will have much less detail than your final report.* You will be able to fill in the missing details during your conversations with your visitors. Since the visitors will spend only a few minutes at your poster, so you need to communicate your results efficiently. Always consider your audience as you prepare your poster. Your visitors may provide helpful ideas and suggestions for your final report.

Follow these general guidelines:

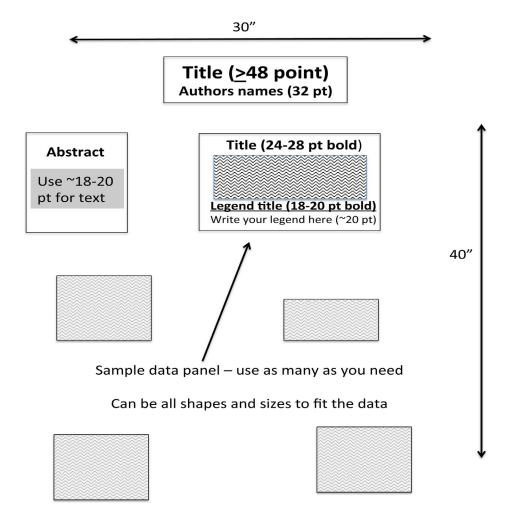
- Posters are 30" wide and 40" high. Each is mounted on an easel. Your material needs to fit within these boundaries.
- The poster presents data and background material in a **series of panels**. Each panel is mounted separately on the poster board with push pins. Panels can be mounted in either landscape or portrait orientation. You may group several panels together. *Each panel or group of panels needs to have a title in BOLD font*.
- Use large font sizes so that a viewer can read the text from a distance of 1-2 feet. Use BOLD fonts for titles. (Specific suggestions for font sizes are included below.)
- Panels with figures or tables should have a legend. The legend title should be larger or emphasized to set it aside from the text of the legend.
- Prepare the panels in a program(s) of your choice. Print the panels on regular letter paper. Include color if you wish, but this is optional.

Panels to include on your poster:

- **1. Title** This panel includes the title and the names of the authors in a smaller font. The title summarizes the content of your poster. The title MUST include the name of your gene, *e.g. MET3*. A font size of 48 pt or more for the title and a font size of 28-32 points for the authors' names are recommended.
- **2. Abstract** The title of this panel is "Abstract". "Abstract" should be in a larger and bolder font (24-28 pt) than the text (18-20 pt) of your abstract.
- **3. Background** Your visitors will not be as familiar with your project as you are. It would be a good idea to provide some information about the methionine biosynthetic pathway, as well as other information on your gene that you consider relevant.
- **4. Data panels** Use as many panels as you need to tell your story! Each data panel needs a title, figure or table, and legend. The title of a data panel often summarizes the content, *e.g.* "Plasmid identification using restriction digestion." Font sizes decrease as you move through the panel. The title of the figure legend should be smaller (18-20 pt) than the font size of the panel title (24-28pt).
- **5. Conclusion panel** Include a short list of conclusions from your work.

7. References - Use "References" or "Literature cited" as the title of this panel. List the references in *FEMS Yeast Research* format.

A mockup of a potential poster is shown below.



Organization is critical to an effective poster!

Be creative! Make your poster visually appealing!

References

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