Writing Workshop #3

• Results and Discussion

  – The examples on the following slides were all excerpted from real papers
  – These *illustrate* common problems that students encounter when drafting their Results and Discussion sections
  – When reviewing each of these examples, ask yourself whether your own paper could provoke a similar criticism.
  – How might you change your own writing to address or avoid such criticisms?
Distinguish the assay from the concept

Assaying ... promoter activity
To determine the activity of the ... promoter in the wild type and mutant strains of AN12, we performed a GUS assay.

Transcriptional Fusion Assay Analysis
GUS assays were performed in order to both reveal whether or not the promoter is transcriptionally active and to measure the promoter activity in each of the different strains if it is transcriptionally active.

In order to perform the GUS assays, constructs were made by ligating the putative promoters from the ... gene into the expression vector pAL280.
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Transcriptional Fusion Assay Analysis

Transcriptional fusion assays were performed in which the putative promoter was fused to the open reading frame of the β-glucuronidase reporter gene (gusA). Measuring GUS activity in this way would both reveal whether the promoter is transcriptionally active and measure its activity in each of the different strains.

In order to perform the assays, constructs were made by ligating the putative promoters from the … gene into the expression vector pAL280.
Don’t overuse personal pronouns

To obtain a gentamicin-resistant transposome, we isolated our transposon from pMSR1 using...
Don’t overuse personal pronouns

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Avoid lab slang

In order to determine if the transposome had really inserted at random into the genome, the plasmid rescues of all strains were sequenced...
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Huh?
In order to determine if the transposome had really inserted at random into the genome, the plasmid rescues of all strains were sequenced...

In order to determine whether the transposome had really inserted at random into the genome, we examined the sequences in the genome into which the transposons had inserted. To do this, we first recovered each of the transposons along with a portion of the adjacent genomic DNA via a plasmid rescue procedure (see Materials and Methods). Sequencing the genomic DNA recovered in this manner revealed...
Avoid lab slang

The plasmids were also sequenced using a forward primer for the transposome, and the upstream regions were BLAST compared for homology with known sequences.
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Upstream of what?

Huh?

The plasmids were also sequenced using a forward primer for the transposome, and the sequences beyond the termini of the transposons were examined via a BLAST analysis (Altschul et al., 1990) to determine whether they resembled known sequences in GenBank.
Be wary of cryptic explanations

…the plasmid integrated into the genome by homologous recombination with the nimB and ORF5468 gene. A true breeding experiment of potential knockouts showed that the rate of plasmid loss after integration is very low.
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Should I already know what this means?
Be wary of cryptic explanations

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*Either explain the “true breeding experiment” fully in Materials and Methods or
include more detail here.*
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*Either explain the “true breeding experiment” fully in Materials and Methods or include more detail here.*

…the plasmid integrated into the genome by homologous recombination with the nimB and ORF5468 gene. We tested the stability of the integrated plasmid via a true breeding experiment. In this experiment, recombinant cells were grown at the non-permissive temperature in the absence of antibiotic selection for approximately 10 generations. Following this period, aliquots from this culture were plated onto selective (LB with 5 mg/L gentamicin) or non-selective (LB) media. The ratio of the number of colonies on the selective plates to those on the non-selective plates reflected the proportion that had retained the integrated plasmid. This test of potential knockouts showed …
More cryptic statements

Transformants were successfully generated using *(the transposome)*. In the first several transformation attempts, the positive control yielded between 10 and 20 colonies, while the … negative controls yielded none.
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What positive control?
More cryptic statements

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What positive control? 
What condition are you controlling for?
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… Transformants were generated using (*the transposome*). To test whether the cells were competent to take up exogenous DNA, positive control electroporations were carried with the plasmids pEP2 or pJP10 instead of transposome, and negative controls carried out with cells alone. In the first several transformation attempts, the positive control yielded between 10 and 20 colonies, while the … negative controls yielded none.
Eliminate unnecessary lanes in gels

Fig.4. Verification of S-34, S-42 via *HindIII* and *PstI* digestion. Lane 1 contains a 1kb DNA ladder. Lane 3 and 4 contains S-34 plasmid rescue, which shows the expected 1.5 kb band between the respective restriction enzyme sites on the transposon. Similarly, lanes 5 and 6 contain the S-42 plasmid rescue, also showing the 1.5 kb band. Multiple other bands indicate presence of multiple *HindIII* sites in plasmid.
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Fig. 4. Verification of S-34, S-42 via HindIII and PstI digestion. Lane 1 contains a 1kb DNA ladder. Lane 3 and 4 contains S-34 plasmid rescue, which shows the expected 1.5 kb band between the respective restriction enzyme sites on the transposon. Similarly, lanes 5 and 6 contain the S-42 plasmid rescue, also showing the 1.5 kb band. Multiple other bands indicate presence of multiple HindIII sites in plasmid.
Eliminate unnecessary lanes in gels

**Figure 4. Plasmid Rescue of S-34, S-42.** Lane 1, 1kb DNA ladder. Plasmids recovered from transposants S-34 (lane 2) and S-42 (lane 3) were digested by *Hind*III and *Pst*I. Note, both plasmids produced the expected 1.5 kb band derived from the transposon. Additional bands indicate presence of multiple *Hind*III sites in plasmid. The similarity of these two plasmids suggest that the two transposants were clonally derived.

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Figure 4. Agarose gel electrophoresis (FspI digests of *in vitro* pCR2.1 TOPO transformants):
Lane 1: Molecular Weight Marker.
Lane 2: Colony #1, PCR transposome inserted into 1.7 kb segment of TOPO.
Lane 3: Colony #2, PCR transposome inserted into 1.1 kb segment of TOPO.
Lane 4: Digested TOPO, expected bands at 1.0 kb, 1.1 kb, 1.7 kb.
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Figure legends shouldn’t be lists

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Figure 4. *Fsp*I digests of *in vitro* pCR2.1 TOPO transposants. Whereas digestion of pCR2.1-TOPO produces fragments at 1.0 kb, 1.1 kb and 1.7 kb (lane 4), each of three separate target plasmids had suffered insertions into a different one of these fragments (lanes 2, 3 and 5) increasing the size of the respective fragments by the expected 1.9 kb. Lane 1, molecular weight marker.

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Purified PCR products were then excised from the gel, purified, and cloned separately into pCR2.1-TOPO (Figure 2). These constructs were named pTOPO_ERG12, pTOPO-ERG8, and pTOPO-MVD1, respectively, to distinguish between the genes that were cloned into each plasmid. The appropriate colonies were selected for each insert and the plasmids were extracted by miniprep. Verification of each of these pCR2.1-TOPO constructs was carried out by DNA sequencing and by various restriction enzyme digestes, as shown in Figures 3, 4 and 5.
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Eliminate unnecessary details
Eliminate unnecessary details

Way too many restriction sites

Figure 5. A plasmid of KRP9 base pairs containing the ORF of crf7. Prc is a PRT-inducible promoter, ori is the origin of replication, which is capable of replicating in AN12. SporR indicates spectinomycin resistance. KanR indicates kanamycin resistance. Lack of the lac-repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Figure 6. A plasmid of KRP1 base pairs containing the ORF of crf7. Prc is a PRT-inducible promoter, ori is the origin of replication, which is capable of replicating in AN12. SporR indicates spectinomycin resistance. KanR indicates kanamycin resistance. Lack of the lac-repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Do you need all of these restriction sites? Try to simplify these figures.

Consider combining all of these plasmid maps into one figure.
Eliminate unnecessary details

Way too many restriction sites

2 figures could be combined into one
Eliminate unnecessary details

Figure 5. **pJP10.** A plasmid of 6725 base pairs designed to express inserted genes from the trc promoter. Ptrc is an IPTG-inducible promoter. Ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. lacIq is the lac repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Figure 6. **pJP10-E.** A plasmid of 6725 base pairs containing the ORF of cbrf. Ptrc is an IPTG-inducible promoter. Ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. lacIq is the lac repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Consider combining all of these plasmid maps into one figure.
Figure 5. pJP10. A plasmid of 6725 base pairs designed to express inserted genes from the trc promoter. Ptrc is an IPTG-inducible promoter. Ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. lacIq is the lac repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Some features labeled but not defined

Figure 6. pJP10-E. A plasmid of 6725 base pairs containing the ORF of craf. Praf is a Praf-inducible promoter. ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. lacIq is the lac repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

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Some details not necessary
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Figure 6. pJP10-E. A plasmid of 6725 base pairs containing the ORF of *cry*. *Ptrc* is an IPTG-inducible promoter. *Ori* is the origin of replication, which is capable of replicating in AN12. *SpecR* indicates spectinomycin resistance. *KanR* indicates kanamycin resistance. *lacIq* is the * lac* repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

Eliminate unnecessary details...
Eliminate unnecessary details

Figure 5. pJP10 expresses inserted genes from the IPTG-inducible trc promoter (P_{trc}). NG2 ori, origin of replication, capable of replicating in AN12; SpecR, spectinomycin resistance marker; KanR, kanamycin resistance marker; lac{I}^{r}, lac repressor

Figure 6. pJP10-E. A plasmid of 6725 bp comprises the ORF of crfR. P_{trc} is a IPTG-inducible promoter. ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. *lacI* indicates the lac repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterisk indicates a restriction enzyme that cuts the plasmid only once.

do you need all of these restriction sites? try to simplify these figures.

consider combining all of these plasmid maps into one figure.

 redundant with the legend to fig 5.
Beginning the Discussion

Discussion
The sequence deviation of pFRO is not surprising because the shotgun sequencing method that was used to sequence the genome has potential holes.
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Beginning the Discussion

Discussion
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Discussion

Start by restating the hypothesis
Beginning the Discussion

Discussion
While many eukaryotes produce isoprenoids via mevalonate, very few prokaryotes use this pathway, the non-mevalonate pathway being much more common. Therefore it is not surprising to find genes encoding the entire enzymatic complement for the non-mevalonate pathway in the bacterium we studied. What is peculiar, though, is the presence of a gene encoding HMG-CoA reductase. In other organisms, this enzyme constitutes the first committed step toward isoprenoid biosynthesis via the mevalonate pathway, and the enzyme is rarely encountered in any other context. In this project we sought to determine the role of HMG-CoA reductase in this strain.

We cloned and sequenced the HMG-CoA reductase gene from the bacterial chromosome and found a small number of sequence discrepancies relative to that reported in the genome database. This sequence deviation is not surprising because…
Citing the references

The location of the \textit{ptsH} promoter is unknown, if there is a promoter for \textit{ptsH} in \textit{Rhodococcus}. In similar bacteria, such as \textit{Streptococcus salivarius}, Shine delgarno sequences have been found upstream of the \textit{ptsH} gene (Gagnon et al. 1993). Two carbon source regulated promoters for \textit{ptsH} in \textit{Streptomyces coelicolor} have also been found (Nothaft et al. 2003). Furthermore, promoters are normally found within…
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And from this example we’ve learned...what?
Citing the references

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...In similar bacteria, such as *Streptococcus salivarius*, Shine Delgarno sequences have been found upstream of the $ptsH$ gene (Gagnon et al., 1993), which enabled these researchers to identify the location of the $ptsH$ promoter in that species. A similar strategy would be helpful for identifying the location of the $ptsH$ promoter in *Rhodococcus*, had such a consensus sequence already been identified. Two carbon source regulated promoters for $ptsH$ in *Streptomyces coelicolor* have also been found (Nothaft et al., 2003). The more proximal of these two promoters was constitutively expressed, whereas the distal promoter was strongly induced by glucose. This illustrates the possibility that…
“Discussion” as “True Confessions”

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Oooh, harsh!

Such a tragedy…
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Given how little is known about the mechanism of conjugal transfer between rhodococci, any progress in this field would be welcomed. *Rhodococcus* sp. B264-1 has the ability to transfer DNA to other *Rhodococcus* strains, and it is reasonable to suspect that the genes required for this activity lie on one of the two megaplasmids that reside within B264-1. While it is clear that there is still much work to do, we have taken the first steps toward genetically tagging the elements required for conjugal transfer in *Rhodococcus* sp. B264-1…
Ending the Discussion

...Another possible explanation for the knockout growth is that over longer periods, quinones and other metabolic byproducts have diffused from the KY1 side of the plate to the 50A2 side (Figure 3c) and the cells are able to metabolize these, if poorly.
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The End
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The End

Ending with this comment makes it seem as though this issue of quinone metabolism was the most important conclusion of the research.
Ending the Discussion

...Another possible explanation for the knockout growth is that over longer periods, quinones and other metabolic byproducts have diffused from the KY1 side of the plate to the 50A2 side (Figure 3c) and the cells are able to metabolize these, if poorly.

The results we have obtained to date argue in favor of the hypothesis that nimB encodes a function that is critical for naphthalene metabolism in Rhodococcus sp. KY1. However, it is also clear that more work will be needed to confirm the precise role of this gene as well as that of the neighboring gene, ORF5468. Continued research into this area will shed important light on the degradation of aromatic hydrocarbons among rhodococci.