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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The GUS assay is a tool, not a concept

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GUS = enzyme
gusA = gene

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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

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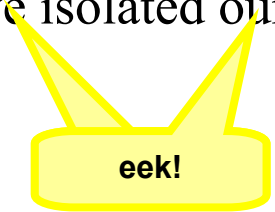

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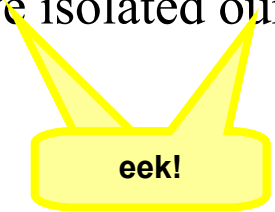

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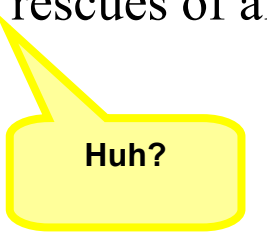

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In order to determine if the transposome had really inserted at random into the genome, the plasmid rescues of all strains were sequenced...

Huh?

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...

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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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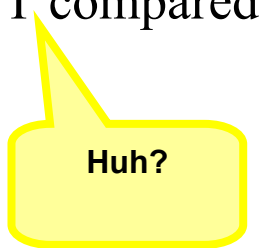

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The plasmids were also sequenced using a forward primer for the transposome, and the sequences beyond the termini of the transposons were examined via a BLAST analysis (Altschul et al., 1990) to determine whether they resembled known sequences in GenBank.

Be wary of cryptic explanations

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Should I already know what this means?

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

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…the plasmid integrated into the genome by homologous recombination with the *nim*B 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?

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

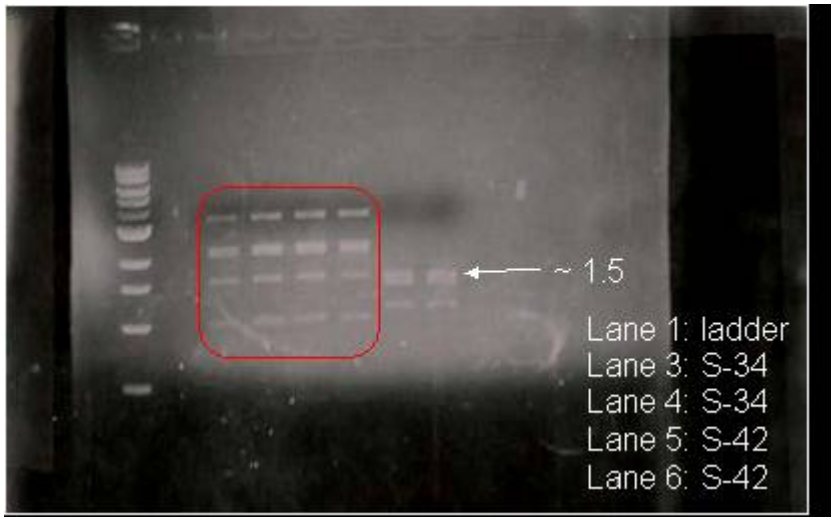

Fig.4. Verification of S-34, S-42 via *Hind*III and *Pst*1 digestion. Lane 1 contains a 1kb DNA ladder. Lane 3 and 4 contains S-34 plasmid rescue, which shows the expected 1.5 kb band between the respective restriction enzyme sites on the transposon. Similarly, lanes 5 and 6 contain the S-42 plasmid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

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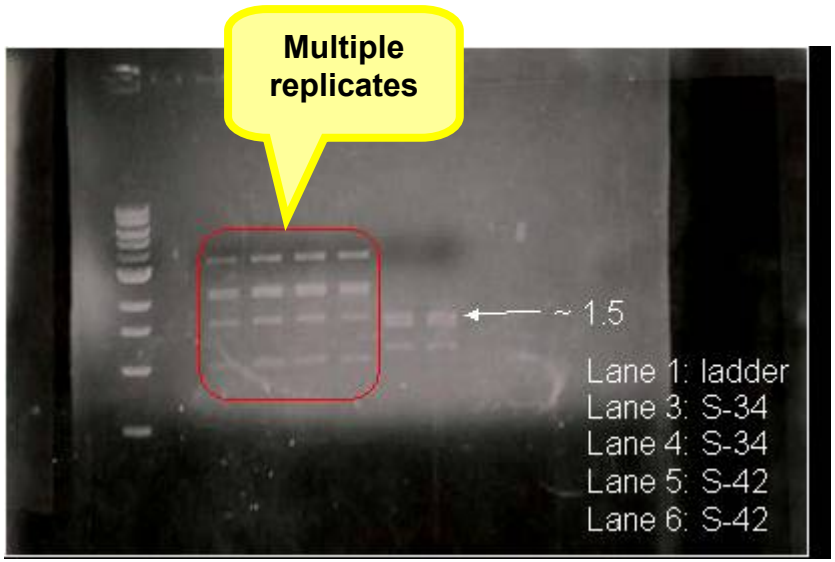

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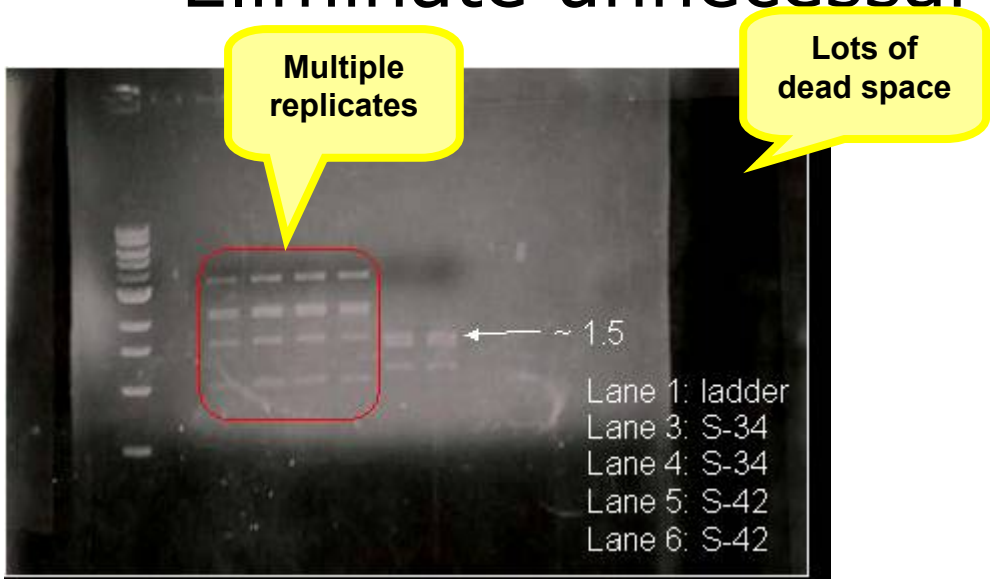

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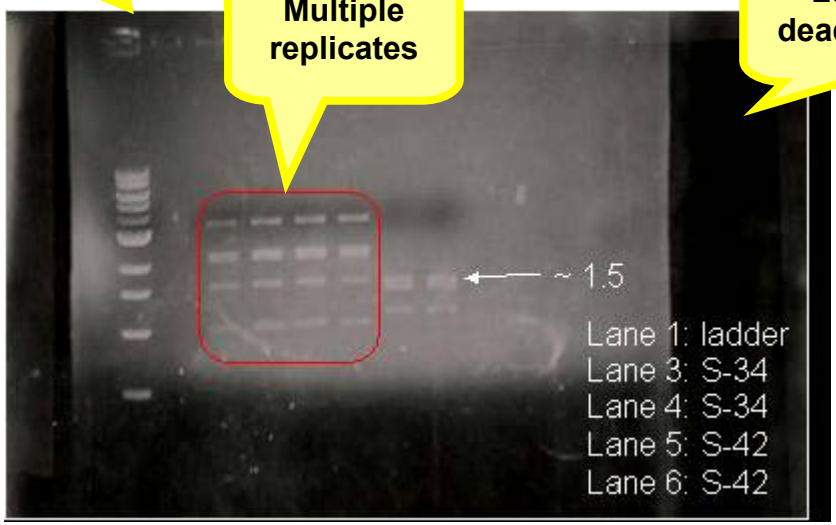

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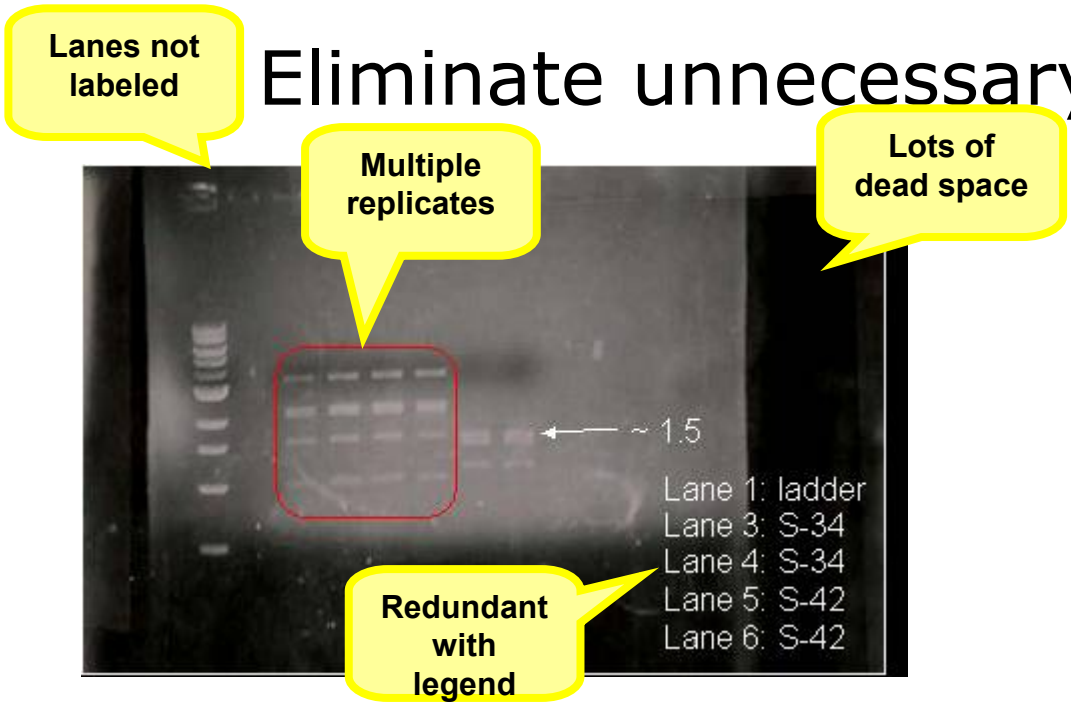

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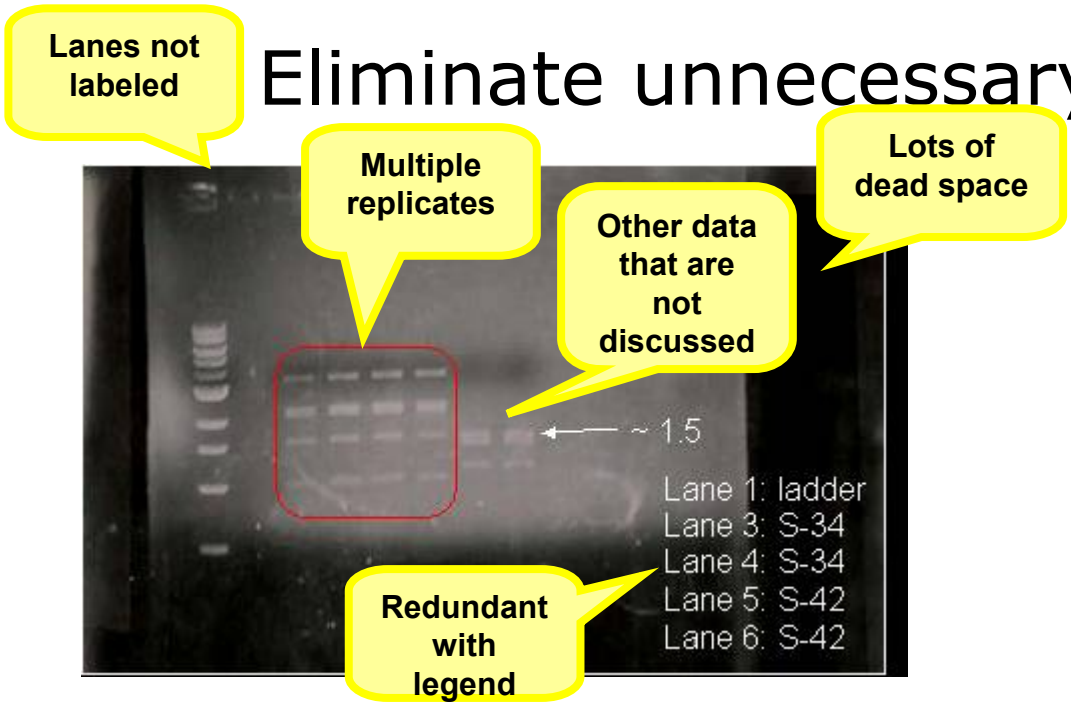

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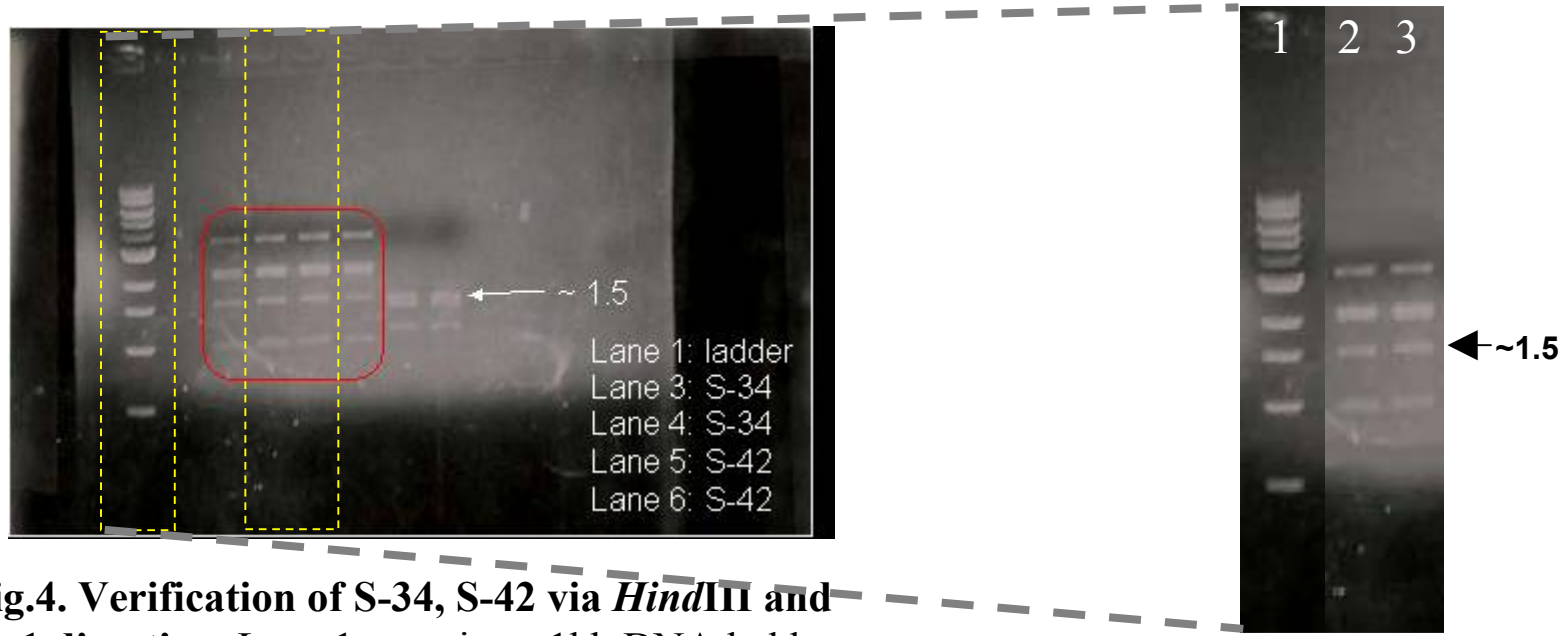

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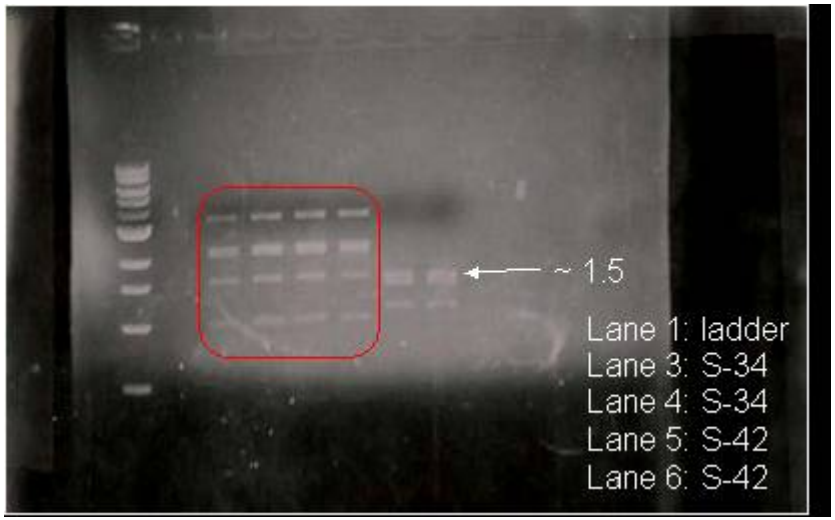

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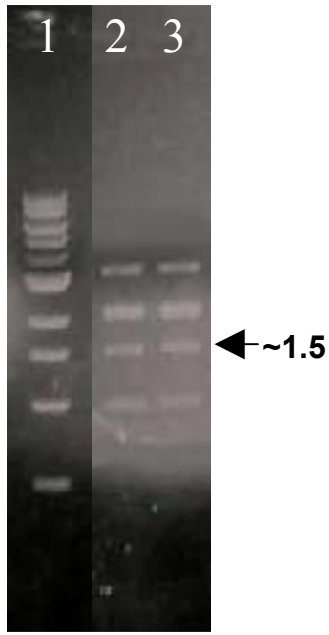

Figure 4. Plasmid Rescue of S-34, S-42. Lane 1, 1kb DNA ladder. Plasmids recovered from transposants S-34 (lane 2) and S-42 (lane 3) were digested by *Hind*III and *Pst*I. Note, both plasmids produced the expected 1.5 kb band derived from the transposon. Additional bands indicate presence of multiple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

Figure legends shouldn’t be lists

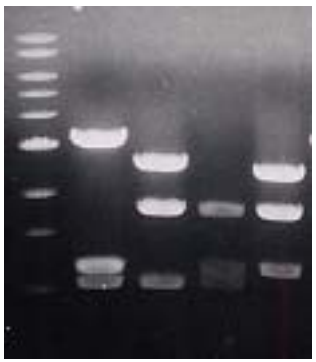

Figure 4. Agarose gel electrophoresis (FspI digests of *in vitro* pCR2.1 TOPO transformants):
Lane 1: Molecular Weight Marker.
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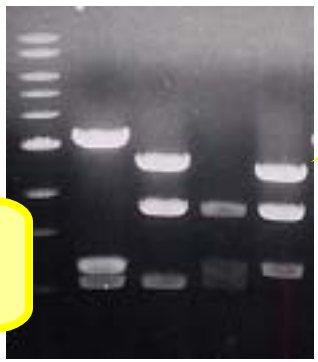

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After all this, it's not clear what main observation was supposed to be

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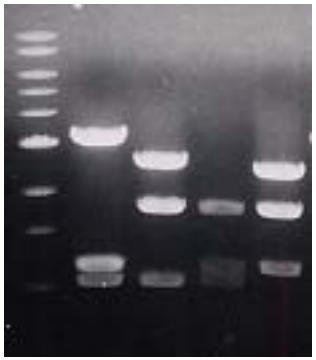

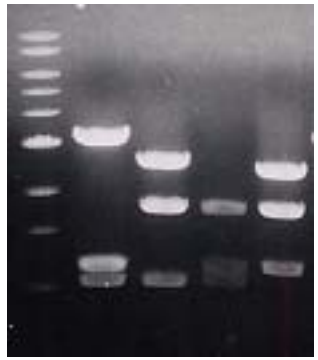

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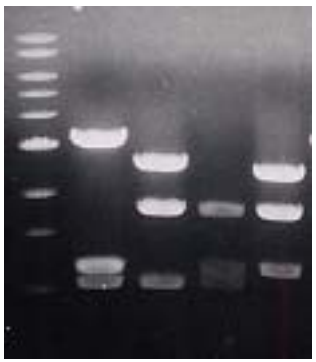

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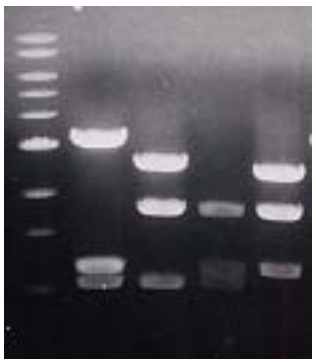

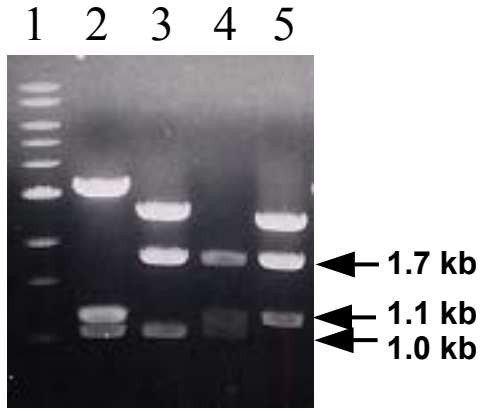

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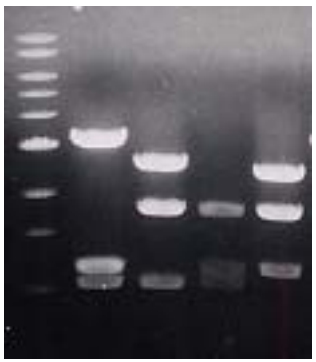

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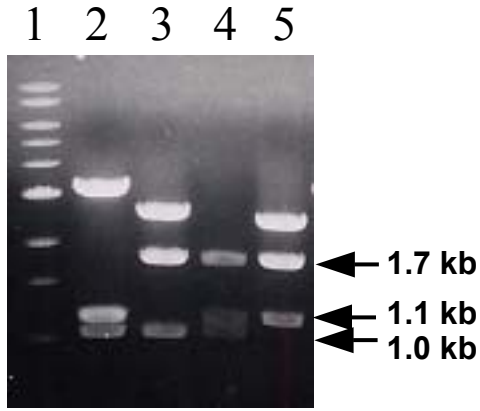

Figure 4. *Fsp*I digests of *in vitro* pCR2.1 TOPO transposants. Whereas digestion of pCR2.1-TOPO produces fragments at 1.0 kb, 1.1 kb and 1.7 kb (lane 4), each of three separate target plasmids had suffered insertions into a different one of these fragments (lanes 2, 3 and 5) increasing the size of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

Name plasmids only after you've described their successful testing

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 digests, as shown in Figures 3, 4 and 5.

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Names them…

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Names them…

THEN tests them (which implies bias in the interpretation)

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Eliminate unnecessary details

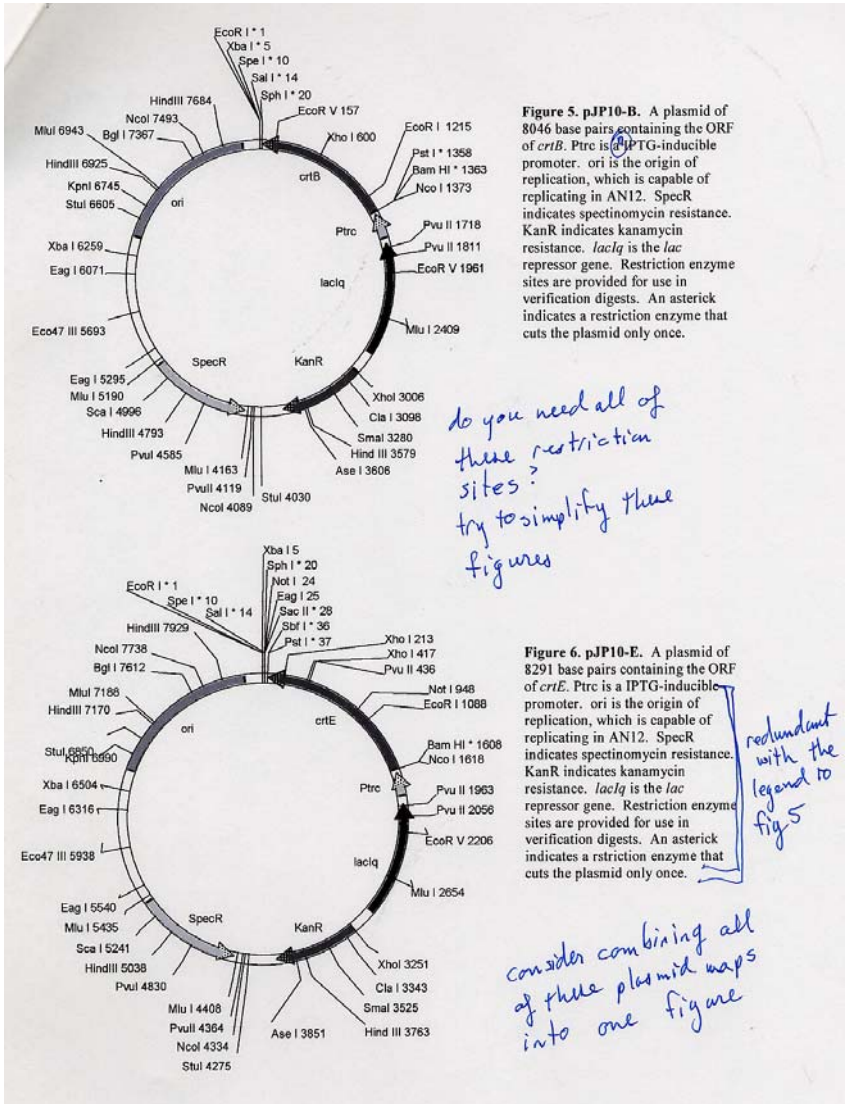


Figure 5. pJP10-B. A plasmid of 8046 base pairs containing the ORF of *crtB*. Ptrc is a IPTG-inducible promoter. ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. *lacIq* is the *lac* repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterick indicates a restriction enzyme that cuts the plasmid only once.

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

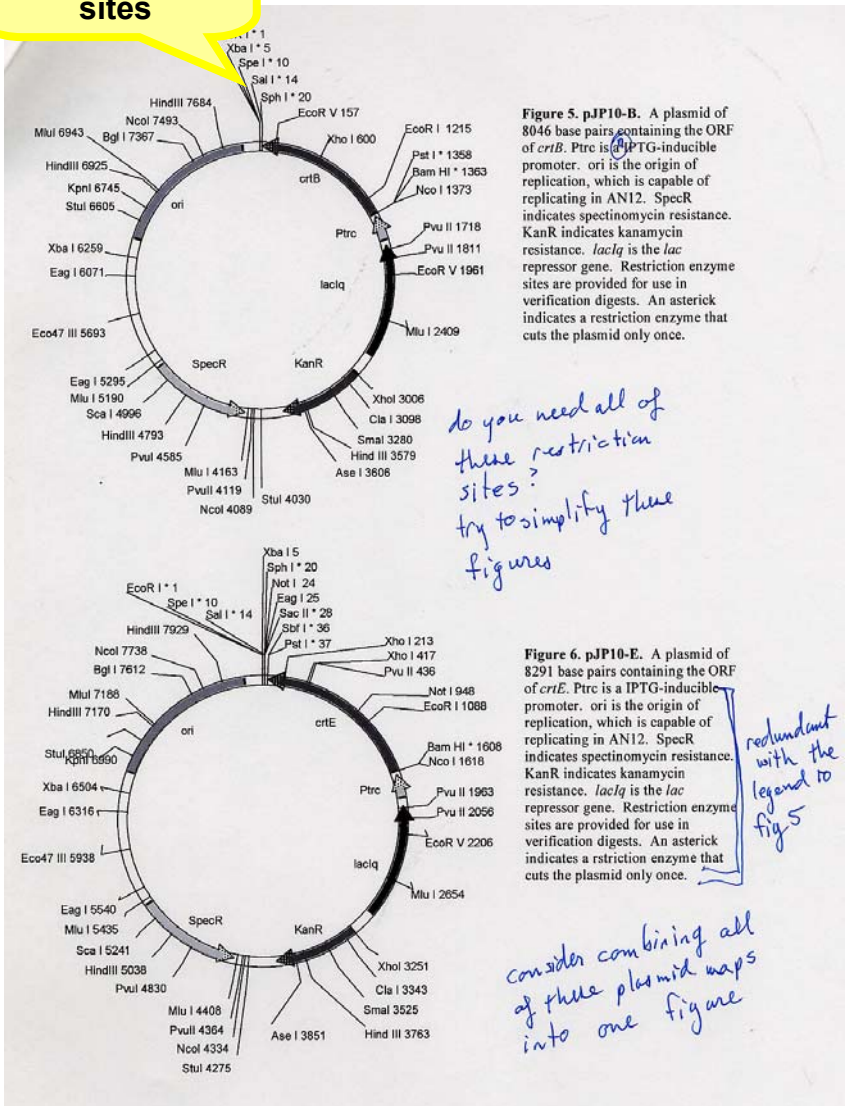
Figure 6. pJP10-E. A plasmid of 8291 base pairs containing the ORF of *crtE*. Ptrc is a IPTG-inducible promoter. ori is the origin of replication, which is capable of replicating in AN12. SpecR indicates spectinomycin resistance. KanR indicates kanamycin resistance. *lacIq* is the *lac* repressor gene. Restriction enzyme sites are provided for use in verification digests. An asterick indicates a rstriction enzyme that cuts the plasmid only once.

redundant with the legend to fig 5

consider combining all of these plasmid maps into one figure

Eliminate unnecessary details

Way too many restriction sites



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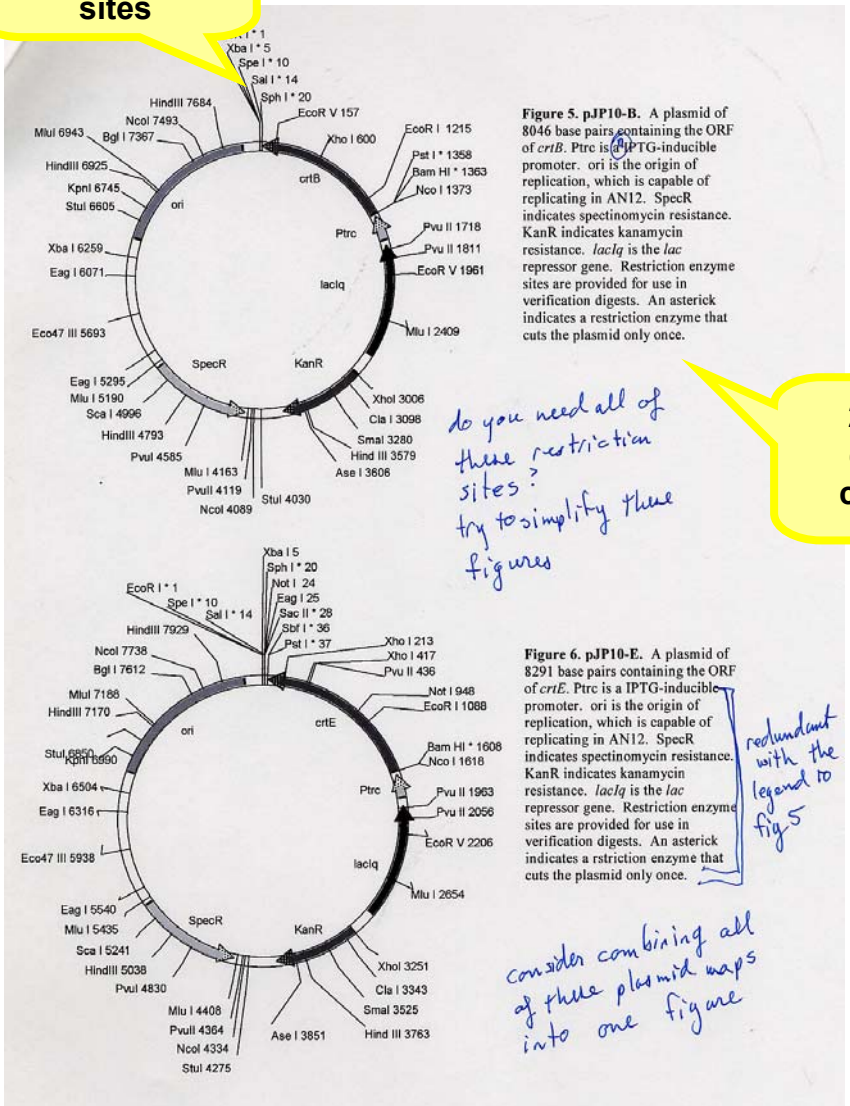

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2 figures could be combined into one

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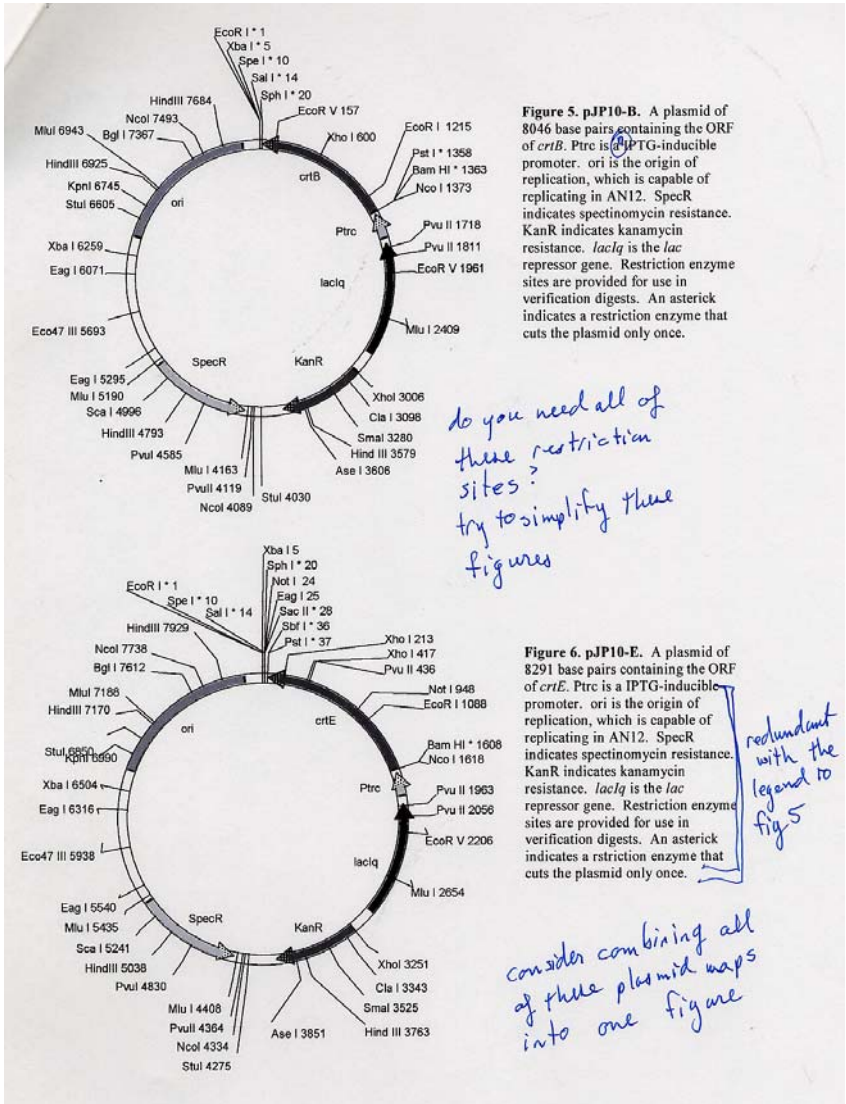

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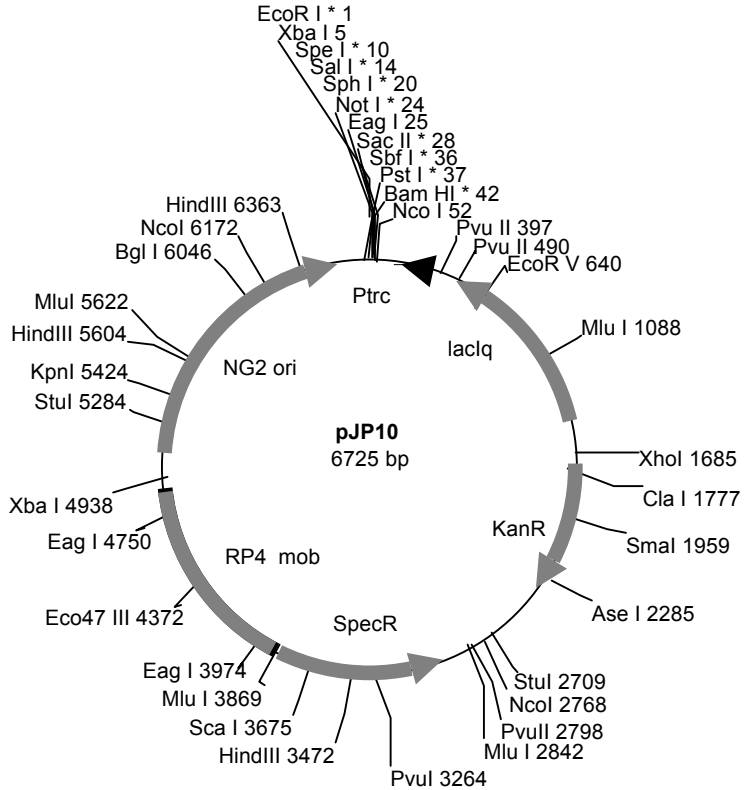

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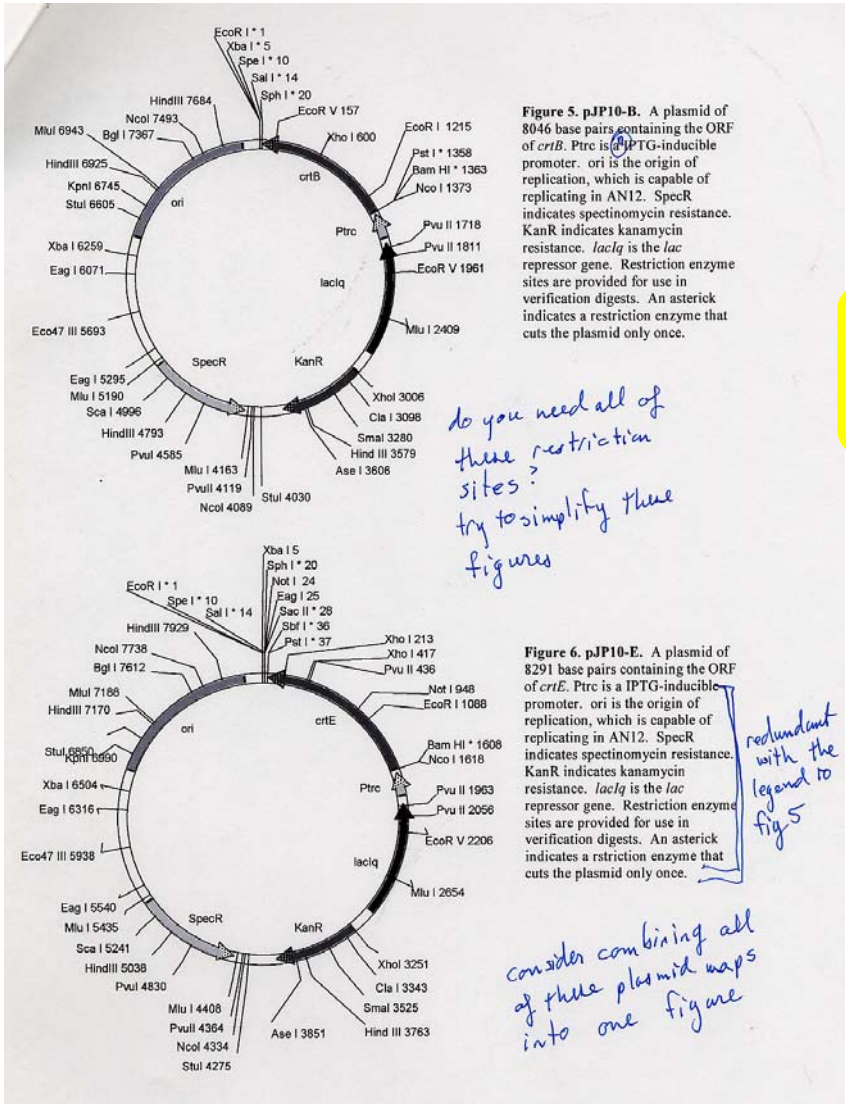

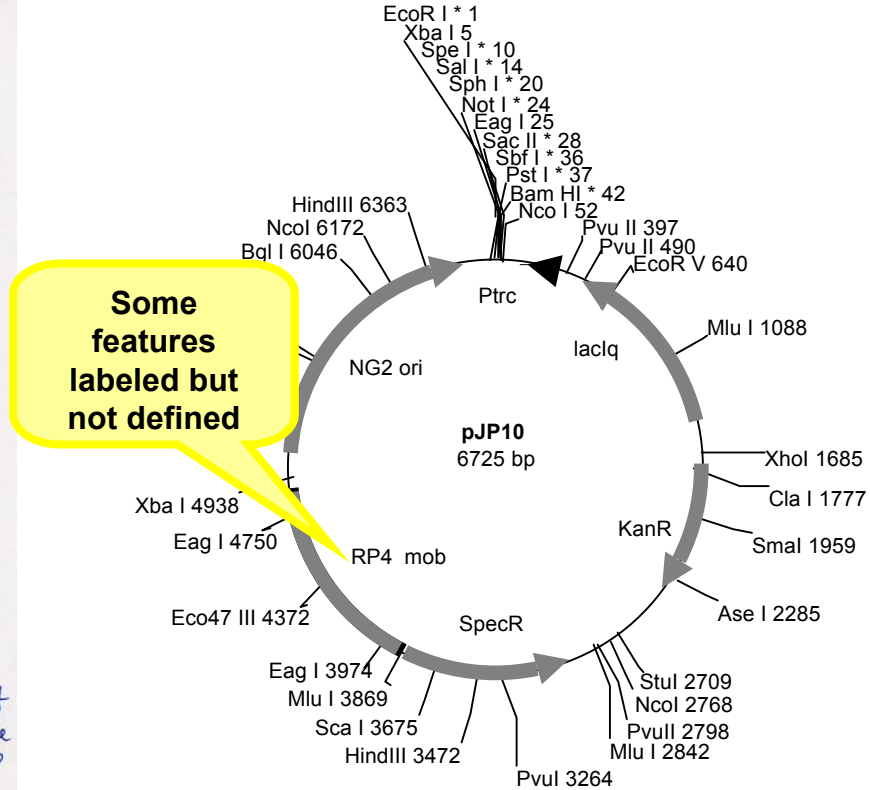

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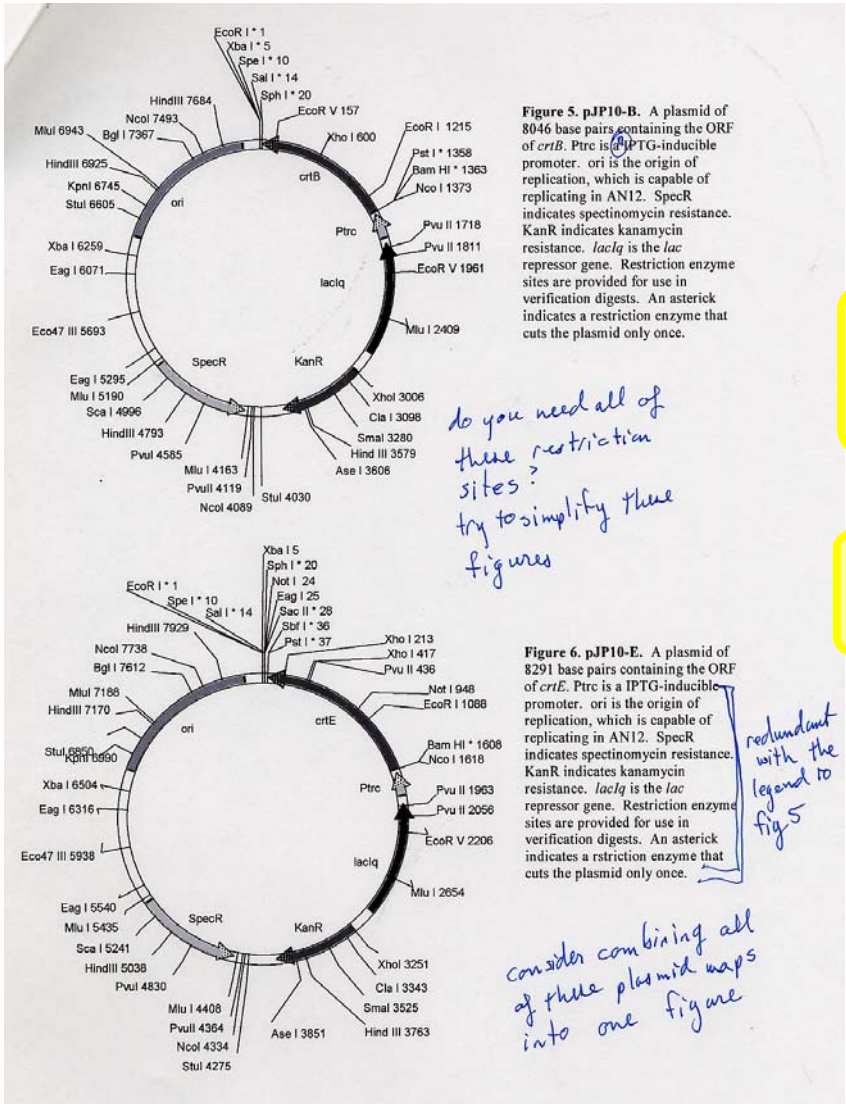

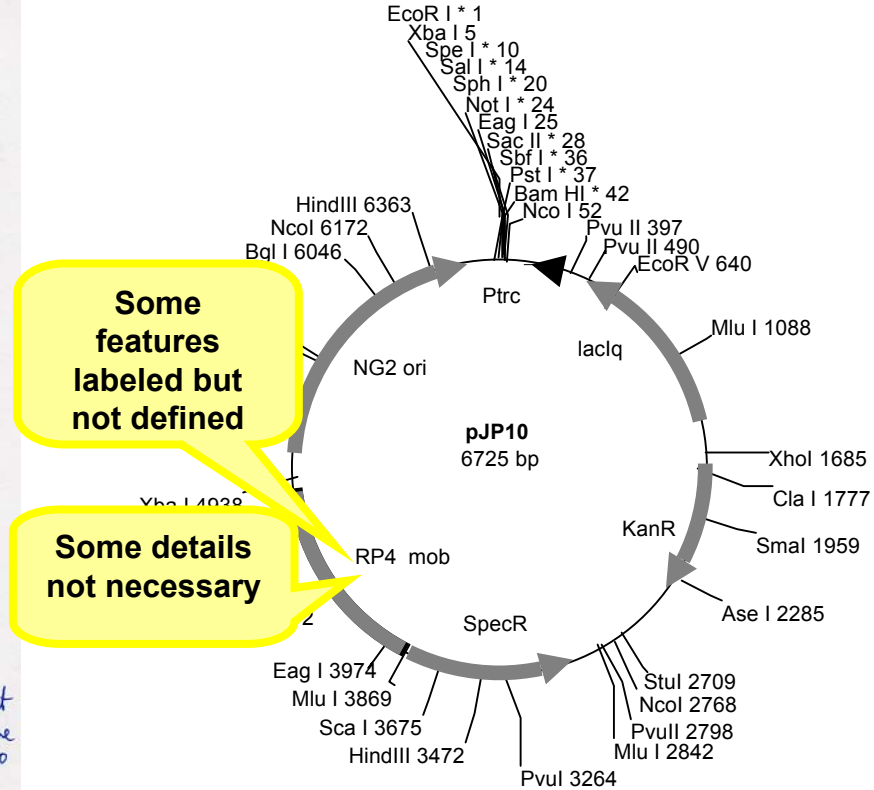

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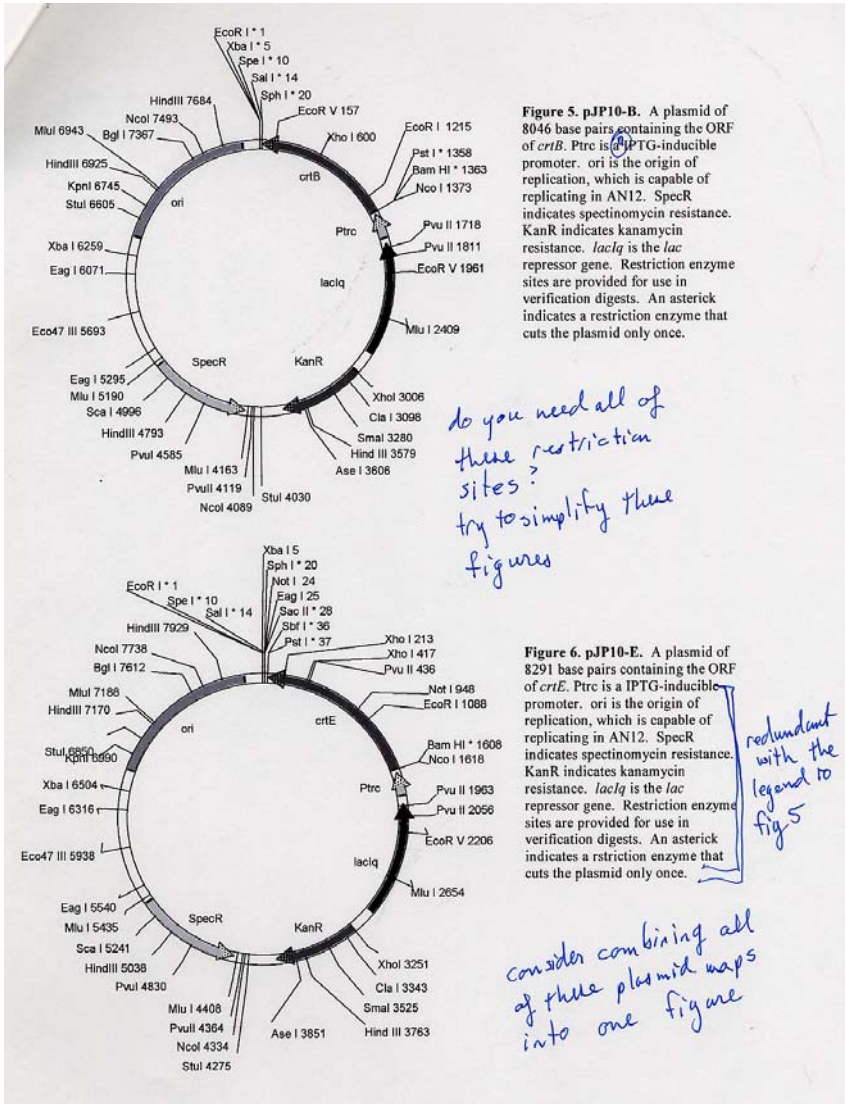

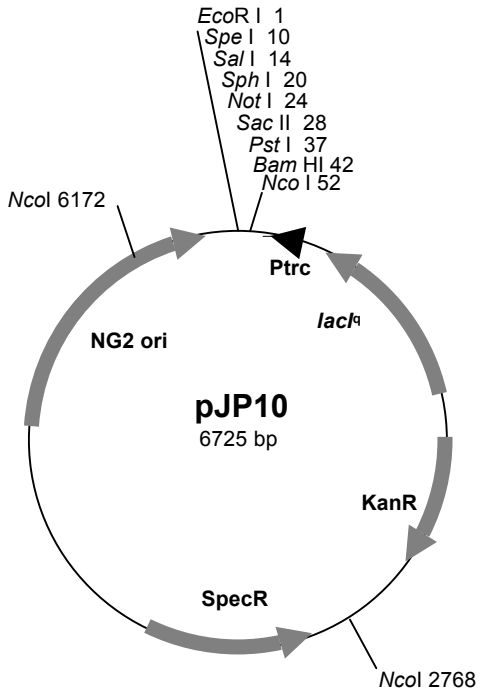

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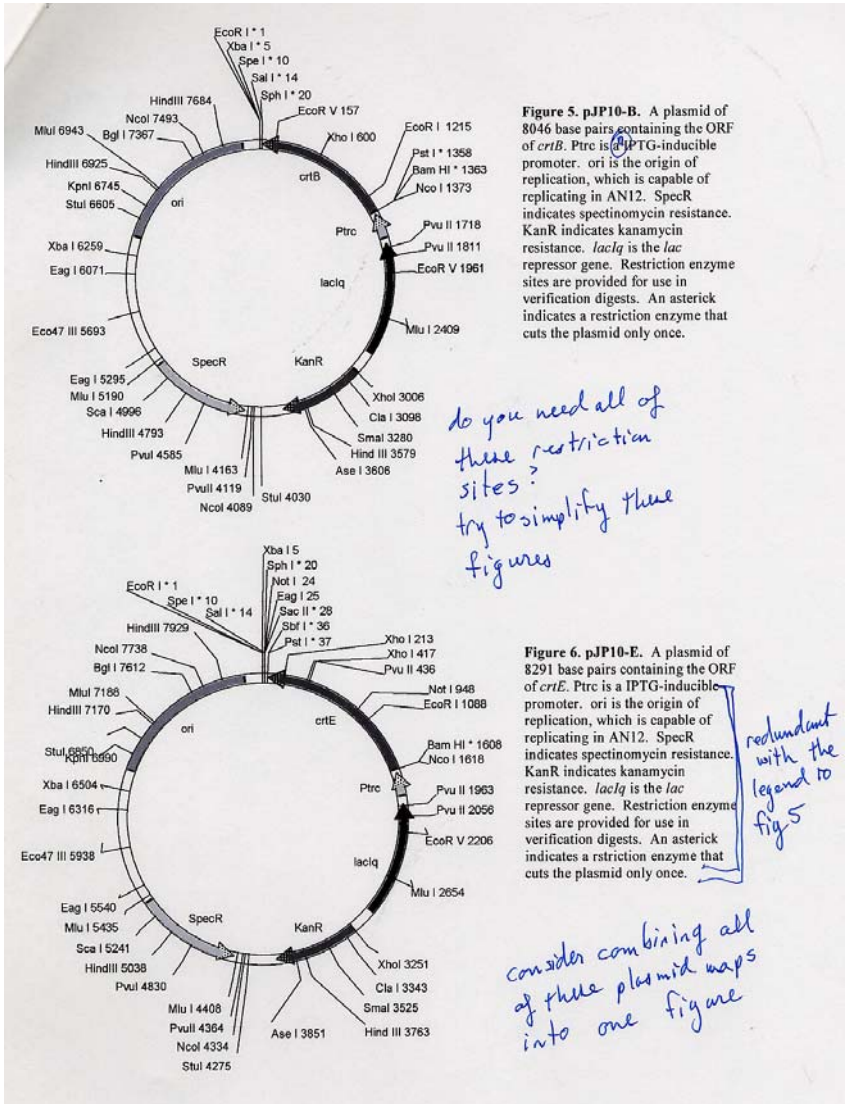

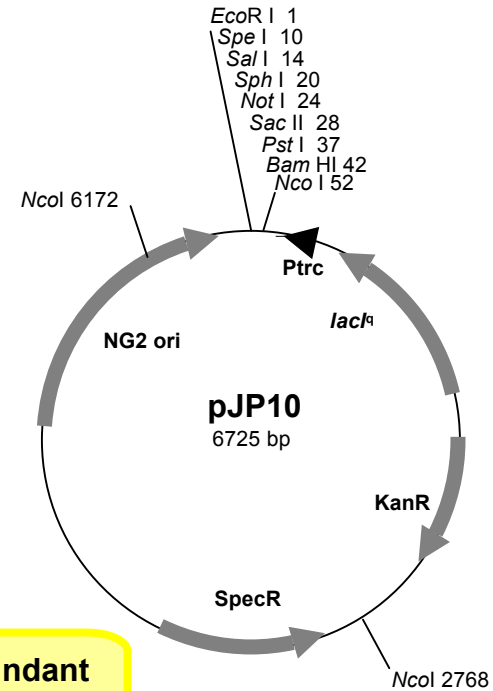

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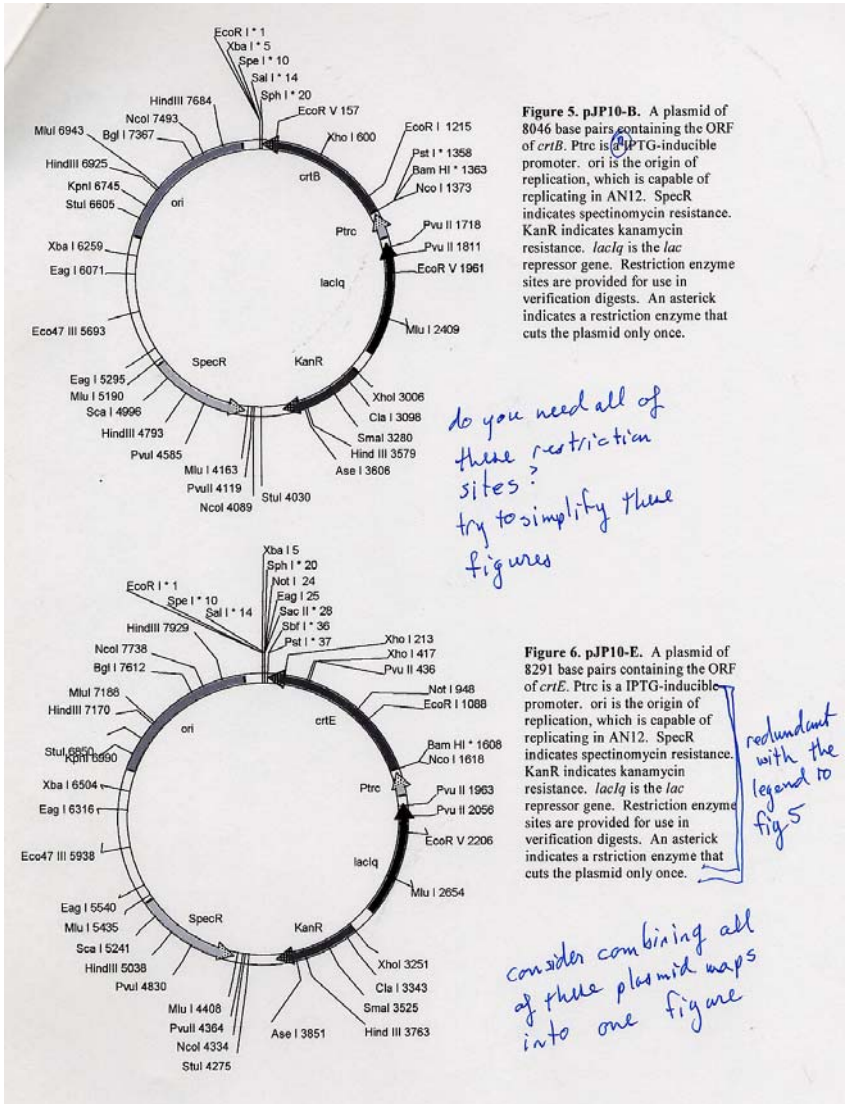

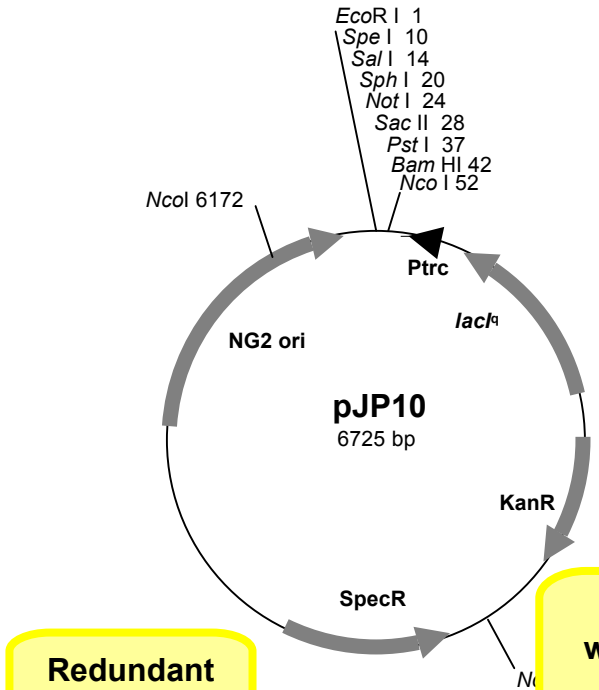

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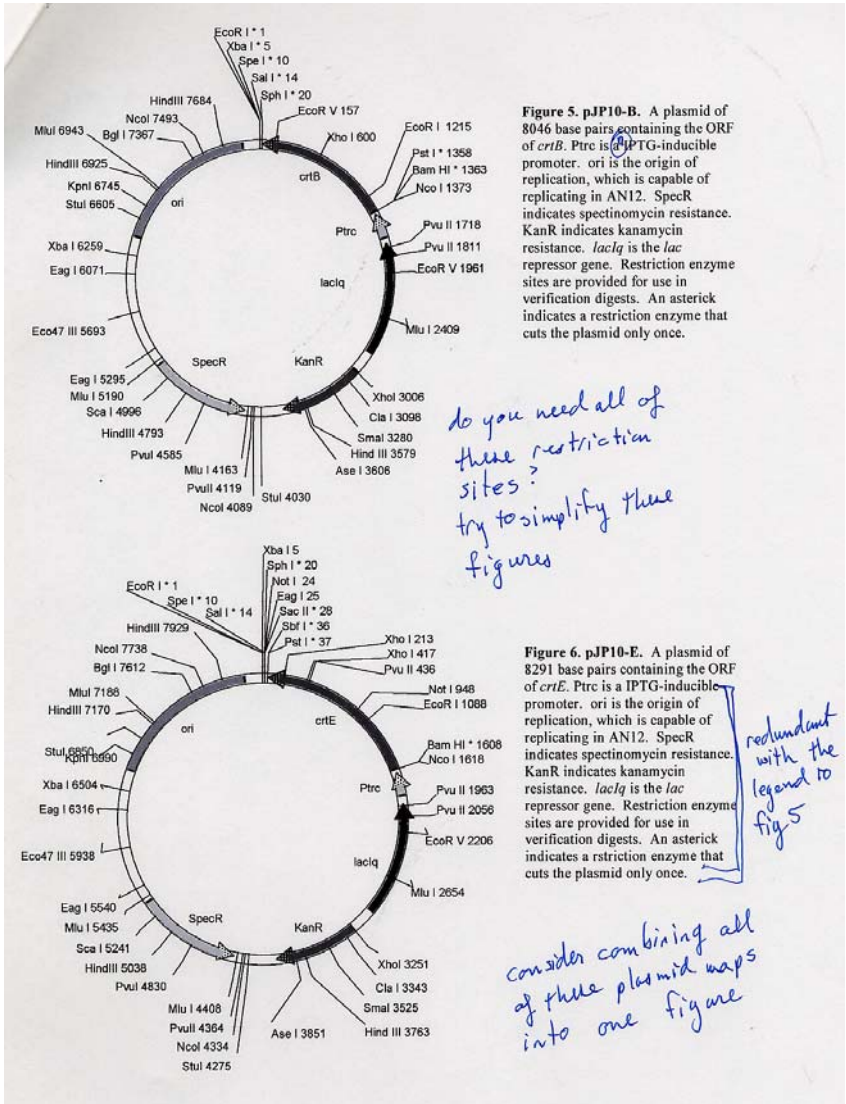

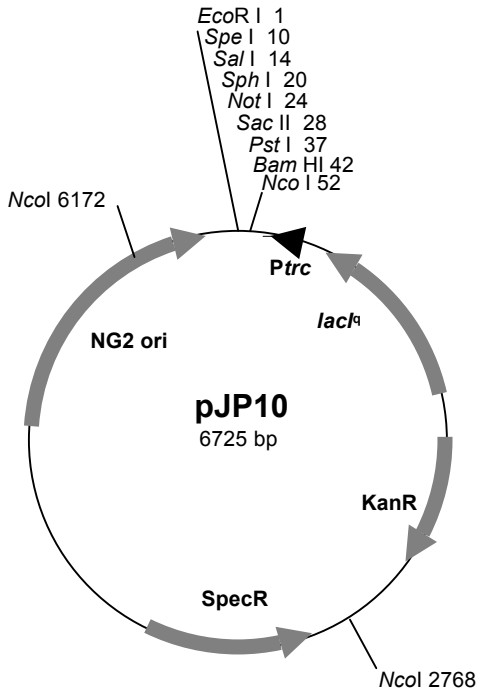

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Beginning the Discussion

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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Jumps right into the data…

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Jumps right into the data…

On a negative note, no less…

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Start by restating the hypothesis

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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 bacterrial chromosome and found a small number of sequence discrepencies relative to that reported in the genome database. This sequence deviation is not surprising because…

Citing the references

The location of the *ptsH* promoter is unknown, if there is a promoter for *ptsH* in *Rhodococcus*. In similar bacteria, such as *Streptococcus salivarius*, Shine delgarno sequences have been found upstream of the *ptsH* gene (Gagnon et al. 1993). Two carbon source regulated promoters for *ptsH* in *Streptomyces coelicolor* have also been found (Nothaft et al. 2003). Furthermore, promoters are normally found within…

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Wait! You're onto a 2nd topic and I still don't understand the 1st.

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“Discussion” as “True Confessions”

The transformation rate for B264-1 is almost so low as to be useless for the purposes of generating mutations. Given that in three months and as many different preparations of competent cells we only generated 3 transformants…

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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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Ending the Discussion

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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

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The results we have obtained to date argue in favor of the hypothesis that *nim*B 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.