**Figures/Results Questions**

***Mycobacteriophage Bxb1 integrates into the Mycobacterium smegmatis groEL1 gene***

***Kim et al., 2003***

***A Bxb1 integration-proficient plasmid vector***

Background (partially from Wikipedia)

A plasmid is a relatively small, circular piece of DNA. A **shuttle vector** is a vector (usually a plasmid) constructed so that it can propagate in two different host species. Therefore, DNA inserted into a shuttle vector can be tested or manipulated in two different cell types. The main advantage of these vectors is they can be manipulated in *E. coli* then used in a system which is more difficult or slower to use (e.g. yeast, other bacteria).

1. What did the shuttle vector constructed for this paper look like in design? Why was it the designed the way outlined in the paper? Why do they mention it is a kanamycin-resistant vector? What is kanamycin?
2. What is electroporation?
3. What do we learn from Table 1? Please only consider the top 4 lines in this Table. Be sure to point out the controls. Discuss why they included the last column in the table. What do we learn from this data?

***Identification of the M. smegmatis attachment site for Bxb1 integration***

1. What were the researchers trying to determine in the set of experiments outlined in the first paragraph?
2. Briefly outline the experiment they did in the first paragraph of this section.
3. Did they accomplish their goals? Why are they referring to Fig. 1C?
4. For Figure 2A, what were the researchers trying to learn? You may need to remind yourself what attR and attL are referring to. Also, be sure to tell us about the plasmid pAIK2. What was the experimental design? Point out the controls on the blot- are they showing what we would expect to see?
5. For Figure 2B, be sure to tell us what a Southern blot is. Provide the basic experimental design. What is on the blot, what is the probe? What do we learn from performing Southern blots? For the figure, be sure to point out the marker and controls, then the experimental results.
6. Write a brief set of conclusion bullets (3 at most?) regarding the most important points learned from this section of the paper.

***Comparison of attP, attB, attL, and attR sequences***

1. Using Figure 1A and the first paragraph of this section, describe the major features of phage/host recombination. Where does recombination take place, describe/point out some of the key features in the phage and bacterial genomes.
2. SKIP THE SECOND PARAGRAPH OF THIS SECTION – the one starting with “It is also noteworthy”.
3. For the third paragraph – starting with “Examinaton of the attP sequence” discuss the purported rationale for the inverted repeats.

***Stability of integrated plasmids***

1. Discuss the question the investigators were asking.
2. Why are they referring to figure 2A again?
3. What further experiment was performed to prove their point? Briefly describe the set-up of the experiment.
4. What do we learn by studying Figure 3? Please write-in the experiment group corresponding to the graphed lines (you will need to read the figure legend to determine this). Lastly, describe why these groups were chosen.

***Characterization of the functional attP site***

1. What was the reason for undertaking the set of experiments outlined in this section?
2. Describe the two plasmids constructed (pAIK5 and pAIK6) and the rationale for each. You may also choose to discuss the other plasmids prepared in succession and the reasons for these using Table 2.
3. Discuss the results for each of the plasmids as outlined in Table 1. Be sure to determine which data lines in the figure we should be focusing on and clearly outline the conclusions from this set of experiments.

***Compatibility of Bxb1 and L5 integration –proficient plasmids***

1. What were the investigators interested in learning more about? What was the background for this set of experiments?
2. Briefly outline the experiments that were performed, results, and what was learned. Although this was a small section of the paper, it has some very powerful implications for studying phage biology in the future.
3. Discuss the potential future relevance of the experimental result.

***Establishment of Bxb1 integration in vitro***

1. What question were the investigators trying to answer when they set-up the first set of experiments outline in paragraph 1 of this section?
2. Briefly describe the experimental system and the results using Figure 4. What were the controls used and then describe what was learned from Figure 4 – note there are several components here to describe. A suggestion is to list the items learned in bullets and then show the “proof” for each conclusion using the figure, but you may set this up how you wish.
3. For paragraph 3 of this section, what were the researchers trying to prove? Briefly describe the experimental design. Study Figure 5. What is meant by the small ‘s’ and ‘p’ on the left-hand side of the figure?