I. (12 points)

You wish to obtain transposon mutants in the enzymes involved in the biosynthesis of tryptophan in the previously underscribed gliding bacterium isolated from soil in discussion question 5.

In designing this experiment you estimate that this operon is about 0.1% of the bacterial genome. If you introduce Tn 5(neo^R) into this bacterium about what is the expected frequency of trp mutants in your isolated bacteria (selected for neo^R)? ____________ Given that the operon has 5 genes and the trp repressor is a single gene what will be the relative frequency of trp minus to trp constitute mutants (ignore the attenuator)? ____________

If you introduce Tn 5 on a defective phage into this bacterium how would you obtain trp minus mutants?
II. (10 points)

You wish to clone and express an operon containing 2 genes D and E for the biosynthesis of an antibiotic. On the line below indicate the elements required for the expression of these genes in the vector shown below. (This vector contains no sequences which will aid in the expression of your genes).

You do not need to worry about showing restriction sites, just identify each piece of DNA you need and where to put it and its function (also identify internal sequences in the operon which must be preserved).

III. (10 points)

In a cross between

<table>
<thead>
<tr>
<th>Hfr</th>
<th>pro⁺</th>
<th>leu⁺</th>
<th>lac⁺</th>
<th>Sm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>pro⁻</td>
<td>leu⁻</td>
<td>lac⁻</td>
<td>Sm²</td>
</tr>
</tbody>
</table>

What medium do you use to select for F⁻ which have become pro⁺?

What medium do you use to select for F⁻ which have become lac⁺?
III. (continued)

Does the DNA go through the pilus at conjugation?

How does a cell containing an F\(^{+}\) plasmid become an Hfr?

IV. (10 points)

In the course of mapping three genes (bio, gal and leu) you find that they are so close together that you wish to use transformation to map them. You prepare DNA from an *E. Coli* which is bio\(^{+}\), gal\(^{+}\), leu\(^{-}\) and use it to transform an *E. coli* which is bio\(^{-}\), gal\(^{-}\), leu\(^{+}\). The results shown below are obtained.

<table>
<thead>
<tr>
<th>transformants selected for</th>
<th>bio(^{+})</th>
<th>Per Cent which are gal(^{+})</th>
<th>leu(^{+})</th>
</tr>
</thead>
<tbody>
<tr>
<td>bio(^{+})</td>
<td>------------</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>gal(^{+})</td>
<td>70</td>
<td></td>
<td>80</td>
</tr>
</tbody>
</table>

What is the gene order?

What are the relative distances between the genes (numbers are not necessary)?
V. (18 points)

You wish to clone a gene for arginine biosynthesis from *Salmonella typhimurium*. You have available a wild type *S. typhimurium*, *E. Coli* which is arg-, the vector shown below & all necessary enzymes, regents, and media. Fill in the following protocol being as specific as you can.

![Diagram of vector]

1. Purify DNA from ____________________________.
2. Digest this DNA with ____________________________.
3. Purify vector DNA. Digest it with ____________________________.
4. Mix the DNAs and ____________________________.
5. Transform the product of #4 into ____________________________.
6. Plate the resulting bacteria on medium containing ____________________________ and ____________________________.
7. Which colonies have the correct cloned insert in them? ____________________________.

VI. (10 points)

![Graph of bacterial growth]

What is the doubling time of a ____________________________.

What is the doubling time of b ____________________________.

One of the cultures is in rich medium, the other is in minimal medium. Which culture is in minimal medium? ____________________________.

Why does a decrease at the end?

If these growth curves were both *E. coli* growing in the same medium but at 37\(^\circ\) and 25\(^\circ\) which one is growing at 37\(^\circ\)? ____________________________.
VII. (12 points)

You notice several of your friends' pet dogs have orange and black lesions which spread on the skin and tongue. You wish to determine if this is an infectious disease or was caused by some other mechanism such as a reaction to eating halloween candy. What do you do? (You have available guinea pigs which have similar diseases susceptibilities to dogs).

VIII. (15 points)

What is the biochemical mechanism of action of diphtheria toxin?
Why does it kill eukaryotic cells?

Why doesn't it kill Corynebacterium diphtheriae?