1. You are studying a bacterial species whose genome normally contains one gene that encodes a tryptophan tRNA. The wild-type sequence of this gene, called *trnA-trp*, is shown below. The portion of the gene that encodes the anticodon of the tRNA is boxed.

5′ GTACCTGCACTGCATGCCTAGCTAGCCCTAGCCAGCCTAGCTAGCTAGCACCAA3′
3′ CATGGACGTGACGTACGGATCGATCGGGATCGGATCGATCGATCGTGGTT5′

(a) Below is drawn the folded tryptophan-tRNA (produced from the *trnA-trp* gene) base-pairing with an mRNA containing the codon that this tryptophan-tRNA recognizes. Fill in the three boxes on the tRNA with the correct nucleotide sequence of its anticodon. Then fill in the three boxes on the mRNA (that is in the process of being translated) with the correct nucleotide sequence of the codon currently being read. Be sure to label the ends of the mRNA to show directionality.

(b) Which strand of the double-stranded *trnA-trp* gene shown at the top of the page is used as a template when the tryptophan tRNA is transcribed, the upper strand or the lower strand? Remember that tRNAs are transcribed directly from genes – there is no mRNA intermediate made during the production of a tRNA from its DNA sequence.
(c) You isolate a mutation in the \textit{trnA-trp} gene which you call \textit{trnA-trp}*. \textit{trnA-trp}* encodes a suppressor tRNA, and its sequence is shown below, with the mutation underlined. Draw the mutant tRNA that will be transcribed from the \textit{trnA-trp}* gene, using the drawing of the wild-type tryptophan trnA from part (a) as a model. Be sure to write out the sequence of the anticodon, label the ends of the tRNA to show directionality, and include the amino acid to which this tRNA would be covalently bound.

\begin{verbatim}
5’ GTACCTGCACGTGCATGCCTAGCTAGCCCTAGTCAGCTAGCTAGCGACCCAA3’
3’ CATGGACGTGACGTACGGATCGATCGGGATCAGTCGGATCGATCGATCGTGGTT5’
\end{verbatim}

(d) Which stop codon (5’-UAG-3’, 5’-UGA-3’, or 5’-UAA-3’) in an mRNA will be recognized by the mutant tRNA produced from the \textit{trnA-trp}* gene?

You are working on another project in which you are studying the gene that gives this bacterial species its wild-type “shiny” colony morphology; you name the gene \textit{dulA}. The small polypeptide DulA is the product of the \textit{dulA} gene. You isolate four mutations in the \textit{dulA} gene, and you call the mutations \textit{dulA1}, \textit{dulA2}, \textit{dulA3}, and \textit{dulA4}. Bacterial strains containing one of the mutations \textit{dulA1}, \textit{dulA3} or \textit{dulA4} have the mutant “dull” colony morphology. You are particularly intrigued by the conditional mutant \textit{dulA2} because \textit{dulA2} only gives a dull colony phenotype when in a \textit{trnA-trp} * background (that is, the \textit{dulA2} single mutant strain is shiny like wild-type).

You sequence the different wild-type and mutant forms of the \textit{dulA} gene and deduce the following information about the sequence of the \textit{dulA} mRNA. The mutations are underlined.

The mRNA produced from the wild-type \textit{dulA} gene:
\begin{verbatim}
5’ GAACUAUGGAAUACCCGUACUCAAUUGCUGCCGUAAUCUAAUUGCUUAAACG3’
\end{verbatim}

The mRNA produced from the mutant \textit{dulA1} gene:
\begin{verbatim}
5’ GAACUAUGGAAUACCCGUACUCAAUUGCUGCCGUAAUCUAAUUGCUUAAACG3’
\end{verbatim}

The mRNA produced from the mutant \textit{dulA2} gene:
\begin{verbatim}
5’ GAACUAUGGAAUACCCGUACUCAAUUGCUGCCGUAAUCUAAUUGCUUAAACG3’
\end{verbatim}
The mRNA produced from the mutant *dulA3* gene:
5’ GAACUAUGGGAAUACCAGUCUAAUCUGCCGUACUAAUUGCUUAAACG3’

The mRNA produced from the mutant *dulA4* gene:
5’ GAACUAUGGGAAUACCAGUCUAAUCUGACGUAUCUAAUUGCUUAAACG3’

(e) Fill in the chart below for each of the following bacterial strains by predicting how many amino acids long each form of the DulA polypeptide will be, and what the colony phenotype of each strain will be. Some boxes are already filled in for you correctly as examples. [**NOTE:** Assume that the suppressor tRNA is **100% efficient** when filling in this chart (but keep in mind that suppressor tRNAs are actually rather inefficient).]

<table>
<thead>
<tr>
<th>Genotype of the bacterial strain</th>
<th>Number of amino acids found in the protein product of the <em>dulA</em> gene in this strain</th>
<th>Phenotype of the bacterial colonies (dull, shiny, or “cannot conclude”)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>dulA trnA-trp</em> (wild-type)</td>
<td>10</td>
<td>shiny</td>
</tr>
<tr>
<td><em>dulA trnA-trp</em> * (single mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA1 trnA-trp</em> (single mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA1 trnA-trp</em> * (double mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA2 trnA-trp</em> (single mutant)</td>
<td></td>
<td>shiny</td>
</tr>
<tr>
<td><em>dulA2 trnA-trp</em> * (double mutant)</td>
<td></td>
<td>dull</td>
</tr>
<tr>
<td><em>dulA3 trnA-trp</em> (single mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA3 trnA-trp</em> * (double mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA4 trnA-trp</em> (single mutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>dulA4 trnA-trp</em> * (double mutant)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
2. You are studying the ability of a certain bacterial species to form biofilms, which are thick layers of bacteria that can adhere tightly to and colonize a surface. You isolate a mutation, \textit{flm1}, which results in the inability to produce biofilms.

You cannot select for the ability or inability to produce biofilms; you can only screen for it. Thus you decide to isolate a transposon insertion that is near to the \textit{flm1} mutation and that carries a gene allowing for tetracycline resistance (\textit{Tet}^r). You are given three candidate transposon insertions (Insertion One, Insertion Two, and Insertion Three) by your advisor, and you create three strains that each have a single transposon insertion and the \textit{flm1} mutation.

For your \textit{flm1} strain containing Insertion One, you grow P1 phage on this \textit{flm1} \textit{Tet}^r strain and use the resulting phage lysate to infect a wild-type strain, selecting for tetracycline resistance (\textit{Tet}^r). Among 100 \textit{Tet}^r transductants, you find that 75 can form biofilms and 25 cannot form biofilms.

For your \textit{flm1} strain containing Insertion Two, you grow P1 phage on this \textit{flm1} \textit{Tet}^r strain and use the resulting phage lysate to infect a wild-type strain, selecting for tetracycline resistance (\textit{Tet}^r). Among 100 \textit{Tet}^r transductants, you find that 25 can form biofilms and 75 cannot form biofilms.

For your \textit{flm1} strain containing Insertion Three, you grow P1 phage on this \textit{flm1} \textit{Tet}^r strain and use the resulting phage lysate to infect a wild-type strain, selecting for tetracycline resistance (\textit{Tet}^r). Among 100 \textit{Tet}^r transductants, you find that none can form biofilms.

(a) For Insertion One, we have drawn the entering DNA from the phage transduction lining up and recombining with the homologous portion of the bacterial chromosome in the recipient cell. Draw in the set of crossover events that result in the cotransduction of \textit{Tet}^r and \textit{flm1}.

(b) Express the distance between Insertion One and the \textit{flm1} mutation as a cotransduction frequency.

(c) Express the distance between Insertion Three and the \textit{flm1} mutation as a cotransduction frequency.
(d) Which insertion is physically located closer to \textit{flm1} -- Insertion One or Insertion Two?

(e) You examine the strain carrying \textit{flm1} and Insertion three more closely. You grow P1 phage on this strain and use the resulting phage lysate to infect a wild-type strain. After selecting for tetracycline resistance (Tet$^\text{r}$), you now screen 1000 Tet$^\text{r}$ transductants, and you find that none can form biofilms. Propose two possible explanations for this result.

You isolate another mutation, \textit{uvs1}, that results in sensitivity to UV irradiation. You also isolate a bacterial strain containing a transposon insertion that carries a gene allowing for kanamycin resistance. In this strain, the kanamycin resistance gene is cotransduced with \textit{uvs1} with a cotransduction frequency of 50\%. Preliminary P1 transduction experiments indicate that \textit{flm1} is linked to this same Kan$^\text{r}$ transposon insertion. You set up the following cross to map the \textit{flm} locus relative to the \textit{uvs} locus:

You grow P1 phage on a Kan$^\text{r}$ strain that contains this transposon insertion and the \textit{flm1} mutation, and use the resulting phage lysate to infect a \textit{uvs1} strain. You select for kanamycin resistance (Kan$^\text{r}$), and among 100 Kan$^\text{r}$ transductants, you find that 39 can form UV-resistant biofilms, 55 can form UV-sensitive biofilms, and 6 cannot form biofilms and are UV-resistant.

(f) Using the putative order in which \textit{uvs} is closer to the transposon than \textit{flm}, we have drawn the entering DNA from the phage transduction lining up and recombining with the homologous portion of the bacterial chromosome. Draw in the set of crossover events that yield Kan$^\text{r}$ bacteria that are UV-resistant and can form biofilms.
(g) Following the model from part (f), draw the entering DNA from the phage transduction lining up and recombining with the homologous portion of the bacterial chromosome for the **putative** order in which *uvs* is closer to the transposon than *flm*. Draw in the set of crossover events that yield Kan^R^ bacteria that are UV-sensitive and cannot form biofilms.

(h) Following the model from part (f), draw the entering DNA from the phage transduction lining up and recombining with the homologous portion of the bacterial chromosome for the **putative** order in which *flm* is closer to the transposon than *uvs* (ie. the *flm* locus is in between the transposon and the *uvs* locus). Draw in the set of crossover events that yield Kan^R^ bacteria that are UV-resistant and can form biofilms.

(i) Following the model from part (f), draw the entering DNA from the phage transduction lining up and recombining with the homologous portion of the bacterial chromosome for the **putative** order in which *flm* is closer to the transposon than *uvs*. Draw in the set of crossover events that yield Kan^R^ bacteria that are UV-sensitive and cannot form biofilms.

(j) Which putative gene order is more likely to be correct? Your choices are: *flm* is closer to the transposon than *uvs*  **OR**  *uvs* is closer to the transposon than *flm*
You isolate a triple mutant strain which you call \textit{uvs1 sup Kan}^{r}. This triple mutant contains your \textit{Kan}^{r} encoding transposon insertion linked to the \textit{uvs1} mutation, but this strain is now UV-resistant because of the \textit{sup} mutation. You grow P1 phage on this \textit{uvs1 sup Kan}^{r} strain and use the resulting phage lysate to infect a \textit{sup} single mutant strain, selecting for kanamycin resistance (\textit{Kan}^{r}). Among 100 \textit{Kan}^{r} transductants, you find that all 100 are UV-resistant.

\textbf{(k)} Can you conclude if \textit{sup} is linked to \textit{uvs1}? \textbf{If so}, state whether they are \textit{very} tightly linked, loosely linked, or unlinked.

You grow P1 phage on the \textit{uvs1 sup Kan}^{r} triple mutant strain and use the resulting phage lysate to infect a \textit{uvs1} single mutant strain, selecting for kanamycin resistance (\textit{Kan}^{r}). Among 100 \textit{Kan}^{r} transductants, you find that all 100 are UV-sensitive.

\textbf{(l)} Can you conclude if \textit{sup} is an intragenic suppressor or an extragenic suppressor of \textit{uvs1}? \textbf{If so}, state whether it is intragenic or extragenic.

3. You isolate a single mutation (\textit{genA1}) in an \textit{E. coli} \textit{gene}, \textit{genA}. \textit{E. coli} that contain the \textit{genA1} mutation have the following properties:

\begin{itemize}
\item P1 phage grown on an \textit{E. coli} strain that carries a Tn5 insertion in the chromosome (Tn5 has a gene for kanamycin resistance) will transduce wild type \textit{E. coli} to kanamycin resistance at a frequency of about \(10^{-4}\), whereas \textit{genA1} mutants are transduced to kanamycin resistance at a frequency less than \(10^{-9}\).
\item An Hfr strain that contains a Tn5 insertion as an early marker when mated to wild type \textit{E. coli} will produce kanamycin resistant exconjugants at a frequency of about \(10^{-2}\), whereas a \textit{genA1} mutant will become kanamycin resistance at a frequency less than \(10^{-9}\).
\item An \textit{E. coli} donor strain that carries an \textit{F}^{'} plasmid carrying a Tn5 insertion when mated to either wild type or a \textit{genA1} mutant will produce kanamycin resistant exconjugants at the same frequency (of about \(10^{-1}\)).
\end{itemize}

\textbf{(a)} In what cellular process does the protein product of the \textit{genA} \textit{gene} function?

\textbf{(b)} You find that \textit{genA1} \textit{E. coli} can become kanamycin resistant when transduced with \textit{int-P}_{\text{amber}} \lambda phage that carries Tn5 at the same frequency as wild type(\(~10^{5}\)). In one sentence, what does this result tell you about the process of transposition given what you know about the function of the \textit{genA} \textit{gene}?