Name_____

Student ID#_____

Microbial Genetics,
BI 410/510
Winter Quarter 2025
Exam II

1.) To try and learn more about the genes involved tryptophan biosynthesis, you decide to isolate *trp-* mutants of *E.coli*. Briefly describe how you would isolate *trp-* mutants in your screen. (4pts)

1. Plate cells on nonselective rich media (that contains tryptophan).
2. Replica plate the cells onto media plates that lack tryptophan.
3. Colonies that did not grow on the plates without tryptophan but did grow on your nonselective plates are your mutants.

2.) Does this screen involve a positive selection or a negative selection? (2pts)

Negative selection

Through your screen, you isolate 3 mutants that cannot synthesize tryptophan and name them *trp1*, *trp2*, and *trp3*. *trp1* and *trp3* are very "tight" mutations (nothing ever grows on your selective plates. However, *trp2* is leaky (after 3 days, colonies begin to appear under your selection conditions. They are just growing VERY slowly).

When you map these mutations, you find that they all three mutations map to the same 20 kb fragment on the chromosome. You wonder if all three mutations might have occurred in the same gene, so you clone the 20 kb fragment from each mutant onto a plasmid and then:

a.) You transform the plasmid containing *trp2* fragment of the chromosome into the *trp1* mutant cells. You find that the cells still **cannot** synthesize tryptophan.

b.) You next transform the plasmid containing *trp3* fragment of the chromosome into the *trp1* mutant cells. You find that the cells now **can** synthesize tryptophan.

3.) Are the *trp1* and *trp2* mutations in the same gene? WHY or WHY NOT? (4pts)

The *trp1* and *trp2* mutations are in the same gene. Even when the cells contain two copies of the chromosomal region needed for tryptophan synthesis, the cells are unable to synthesize tryptophan. Therefore, the two copies must both have a mutation that inactivates the same gene that is needed for tryptophan biosynthesis.

4.) Are the *trp1* and *trp3* mutations in the same gene? WHY or WHY NOT? (4pts)

The *trp1* and *trp3* mutations are in different genes. When the cells contain two copies of the chromosomal regions needed for tryptophan synthesis, the cells are able to synthesize tryptophan, even though neither region alone is functional. Therefore, the two copies of the *trp* region must contain mutations that inactivate different genes that are needed for tryptophan biosynthesis and the second (complementary) copy of the region is able to express a functional version of the needed gene product in each case.

To learn more about the nature of your *trp-* mutations, you decide to isolate suppressors of the *trp1 trp2* and *trp3* mutations

5.) Briefly describe how you would screen for suppressor mutations. (6pts)

1. Grow a culture of the *trp-* cells in nonselective rich media (that contains tryptophan).
2. Plate the cells onto media plates that lack tryptophan.
3. Colonies that grow on the plates lacking tryptophan are your suppressor mutants

6.) Does the suppressor screen involve a positive selection or a negative selection? (2pts)
Positive

7.) After screening more than $1*10^{12}$ cells for each mutant, you obtain the following results:

With the nonleaky *trp1* mutant, you didn't obtain any suppressor mutants
With the leaky *trp2* mutant, you obtained 30 suppressor mutants
With the nonleaky *trp3* mutant, you obtained 45 suppressor mutants

Based on the properties of the mutant and the ability to isolate suppressors, determine whether *trp1 trp2* and *trp3* mutations most likely represent a basepair change, a frameshift, or a deletion and describe WHY? (8pts)

trp1: most likely represents a deletion. Deletions are likely to inactivate the protein because they affect a large region of the protein (nonleaky) and are extremely unlikely to revert because multiple bases are missing.

trp2: most likely represents a point mutation. Point mutations would only affect a single amino acid leaving the possibility that some functionality to the protein may remain (leaky). Furthermore, the only altered a single base (at most one amino acid) making them likely to revert or change a second amino acid that suppresses the first mutation.

trp3: most likely represents a frameshift. Frameshift mutations are likely to inactivate the protein because they change all the amino acids downstream of the mutation (nonleaky), but since they only changed a single basepair, a second frameshift in the opposite direction near the initial frameshift may suppress the original mutation.

8.) An R1 plasmid maintains a copy number of about 20 copies/cell.

How would the copy number be affected by a mutation in the promoter region of the following genes or transcripts? Assume that the promoter mutation prevents any transcription from occurring at each gene. Explain your answer. (6pts)

repA: Without RepA, which is needed to initiate replication of R1 plasmids, the copy number would be reduced (to zero) and the plasmid would eventually be lost in these cells.

copA: CopA is an antisense RNA that binds to the *repA* transcript and signals it to be degraded. Without CopA, RepA expression would increase allowing replication initiation to occur more frequently and the plasmid copy number would go up.

copB: CopB is a repressor protein that binds to the repA promoter and prevents expression. Without CopB, RepA expression would increase allowing replication initiation to occur more frequently and the plasmid copy number would go up.

You have three *E. coli* strains that are all *hisA+*.
Strain 1 is an Hfr strain.
Strain 2 contains an F plasmid.
Strain 3 contains an F' that carries the *hisA+* region of the chromosome on it.

9.) Which strain(s) could transfer the *hisA+* to a recipient *E. coli that is hisA-*? Why? (3pts)
Either strain 1 or 3 can transfer the ability to synthesize histidine since an Hfr strain can transfer chromosomal sequences (and therefore the *his+* gene) and the F' plasmid contains the *his+* gene and can transfer itself to another cell.

10.) Which strain would be able to transfer *hisA+* with the highest frequency? Why? (3pts)
Strain 3 since the smaller plasmid (relative to the chromosome) transfers very rapidly. Furthermore, unlike the Hfr strain, the plasmid can replicate and perpetuate itself in the recipient. It does not require a recombination event to occur before stably establishing itself in the cell line.

11.) In which strain(s) would be the transfer of *hisA+* depend upon recombination? Why? (3pts)
Strain 1 since the Hfr transfers in a linear fragment of the chromosome. The "survival" of the fragment depends on its ability to recombine into and replicate with the cell's chromosome.

12.) A ColE1 plasmid, containing an ampicillin resistance gene, has a copy number of 5 at the time the cell divides. What would be the predicted number of cells cured per cell division if the plasmid were to sort randomly? (5pts)

Each cell division creates 2 new cells and there is a 1/2 chance that each plasmid will go into each cell, compounded by the number of plasmids...2cells x $(1/2)^{\text{number of plasmids}}$=
$2(1/2)^5$=
1/16 cells cured/ cell division

13.) If the actual cure rate is seen to be much lower, describe two general mechanisms or processes that could account for the discrepancy between the observed cure rate and your calculated cure rate. (4pts)
The plasmid may have a partitioning mechanism or plasmid addiction system.

You are trying to restore the *hisA* gene in a naturally competent bacterium *Acinetobacter calcoaceticus* that is *hisA*-. You first purify DNA from a strain of *A. calcoaceticus* that is *hisA*+. You then perform the following experiments:
a.) mix naturally competent culture of bacteria with a solution of the purified DNA that has been denatured into single stranded.
b.) mix naturally competent culture of bacteria with a solution of the purified DNA without any pretreatment.
c.) electroporate a culture of the bacteria with a solution of the purified DNA that has been denatured into single stranded.
d.) electroporate a culture of the bacteria with a solution of the purified DNA without any pretreatment.

14.) Which experiment(s) could transform the recipient to *hisA*+? Why? (4pts)
Procedure b c and d would have a chance to yield transformants because, in b, naturally competent cells bind to double stranded DNA and take it up in linear form and, in c and d, electroporation allows any form of DNA (circular, double stranded, or single stranded) to be taken up by the cell.
15.) In which experiment(s) would the ability to transform *hisA*+ depend upon recombination? Why? (4pts)
All the procedures would require recombination to form transformants since the DNA is taken up in all cases is a linear fragment of the chromosome. The "survival" of the fragment depends on its ability to recombine into and replicate with the cell's chromosome.

16.) Which experiment(s) would be likely to work if you were using purified plasmid DNA that contains the *hisA* gene on it? Why? (3pts)
Procedure c and d since circular DNA can be taken up by the cell during electroporation.

17.) You have obtained the following four mutant strains of the naturally competent bacteria *B. subtilis*. For each strain, describe what effect the mutation would most likely have on the competence of the bacteria and WHY. (6 pts)

A strain with a point mutation in the promoter of *comX* that renders the gene constitutively active:ComX is the pheromone precursor peptide that is processed and secreted by the cell. If the the pheromone were constitutively expressed, the cell would probably upregulate and express the competence genes that are needed for DNA uptake much earlier and for a longer time.
A strain with a point mutation in *comA* that renders the ComA protein constitutively active:
ComA is the transcription factor that, when activated by phosphorylation, acts as a positive transcriptional regulator that turns on the genes necessary for DNA uptake and competence. If ComA is constitutively active, cells would probably be competent most of the time.
A strain deleted for *comP*: ComP is the transmembrane receptor protein that binds to the competence pheromone to initiate the induction of the competence genes. If it is deleted, it would prevent the ability of the cell to sense and turn on the competence genes.

You have three compatible plasmids and you want to know if they are conjugative, mobilizable, or nonmobilizable.

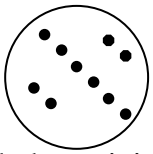
Plasmid A has an *ampR* gene (ampicillin resistance)
Plasmid B has a *kanR* gene (kanamycin resistance)
Plasmid C has a *rifR* gene (rifampicin resistance)

You use a recipient *E.coli* that is resistant to tetracycline and perform the following experiments.

i.) A culture of cells that contains plasmid A is mixed with the recipient, allowed to incubate for several hours, and then the mixture is plated on a plate containing ampicillin and tetracycline.

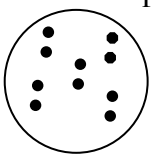
ii.) A culture of cells that contains plasmid B is mixed with the recipient, allowed to incubate for several hours, and then the mixture is plated on a plate containing kanamycin and tetracycline.

iii.) A culture of cells that contains plasmid C is mixed with the recipient, allowed to incubate for several hours, and then the mixture is plated on a plate containing rifampicin and tetracycline.

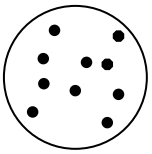


iv.) A culture of cells that contains all three plasmids, A, B and C, is mixed with the recipient, incubated for several hours, and then the mixture is spread on each of the plates shown below.

ampicillin and tetracycline



kanamycin and tetracycline



rifampicin and tetracycline

18.) Is plasmid A self-transmissible, mobilizable, or nonmobilizable? Why (3pts)
nonmobilizable. It does not transfer at all (i), even in the presence of a conjugative plasmid (iv).

19.) Is plasmid B conjugative, mobilizable, or nonmobilizable? Why (3pts)
mobilizable. It cannot transfer at by itself (ii), but it can in the presence of a conjugative plasmid (iv).

20.) Is plasmid C conjugative, mobilizable, or nonmobilizable? Why (3pts)
Conjugative. It has all the genes needed to transfer on its own by itself (iii),

21.) Which of the plasmids are likely to have the genes that encode for pilus formation? (3pts)
Plasmid C

22.) Which plasmids are likely to have the genes that encode for a relaxase or nickase? (3pts)
Plasmids B and C

23.) Which antibiotic(s) were used to counterselect against the donor cells in each experiment? (2pts)
tetracycline

An Hfr strain of *E. coli* is found to be resistant to chloramphenicol (*camR*). You decide to the map location of *camR* gene using an alternative method to see if it agrees with your previous results. Using the *camR* donor strain and an *ampR trpA- hisG- argR-* recipient, you mix the strains together and then every five minutes, you take five 1 ml samples, vortex them, and spread them on plates selecting for the indicated gene in the transconjugate.

Minutes after mixing / Set of Plates	0	5	10	15	20	25	30	35	40	45	50	55	60	70	75	80	85
Selecting for *camR*	0	0	0	0	0	0	0	0	0	0	0	5	70	550	499	512	512
Selecting for *ampR*	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn	lawn
Selecting for *trpA*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9
Selecting for *hisG*	0	45	1008	1025	1111	1234	1543	1545	1512	1552	1412	1512	1412	1541	1413	1423	1423
Selecting for *argR*	0	0	0	0	0	0	0	2	260	745	790	830	770	756	830	830	830

24.) Based on the results described above, draw the map positions for *camR ampR trpA hisG* and *argR*? (8pts)

ori-5min-hisG------30min------argR----20min----camR----20min----trpA.
----------45min----------74min----------100min----------27min

AmpR doesn't transfer because its not on the chromosome

25.) What could be added to all the plates for counterselection in this experiment? (4pts)

ampicillin