Name_____

Microbial Genetics,
BIO 410/510
2023 Exam II

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

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

2.) Does this screen involve a positive selection or a negative selection? (1pts)

Negative selection

Suppose you isolate 3 mutants. To learn more about the nature of each *his-* mutant, you decide to isolate suppressors of the *his1 his2* and *his3* mutations

3.) Briefly describe how you would screen for suppressor mutations. (4pts)

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

4.) Does the suppressor screen involve a positive selection or a negative selection? (1pts)

Positive

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

With the nonleaky *his1* mutant, you didn't obtain any suppressor mutants
With the leaky *his2* mutant, you obtained 30 suppressor mutants
With the nonleaky *his3* mutant, you obtained 45 suppressor mutants

Based on the properties of the mutant and the ability to isolate suppressors, determine whether *his1 his2* and *his3* mutations most likely represent a basepair change, a frameshift, or a deletion and describe WHY? (5pts)

his1: 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.

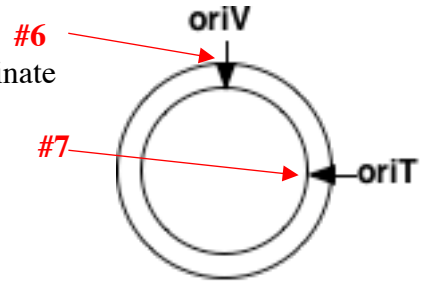
his2: most likely represents a point mutation. Point mutations would only affect a single amino acid leaving the possibility that some functionality to the protein may remain (leaky). Furthermore, the only altered a single base (at most one amino acid) making them likely to revert or change a second amino acid that suppresses the first mutation.

his3: 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.

The plasmid shown to the right has an oriV and oriT at the positions indicated, and is known to replicate bidirectionally.

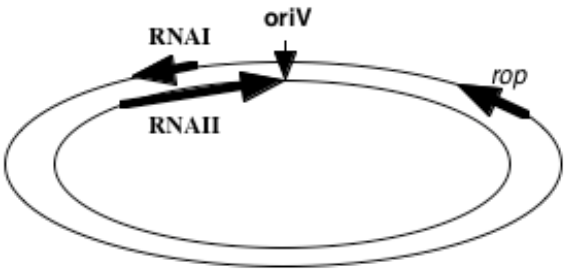
6.) Indicate where replication would be expected to begin during vegetative replication? (4pts)

7.) Indicate where replication would be expected to terminate during conjugation? (4pts)



8.) ColE1 plasmids maintain a copy number of about 16 copies/cell.

How would the copy number be effected by a mutation in the promoter region of the following genes or transcripts? Assume that the mutation prevents any transcription from occurring at each gene. Explain your answer. (8pts)

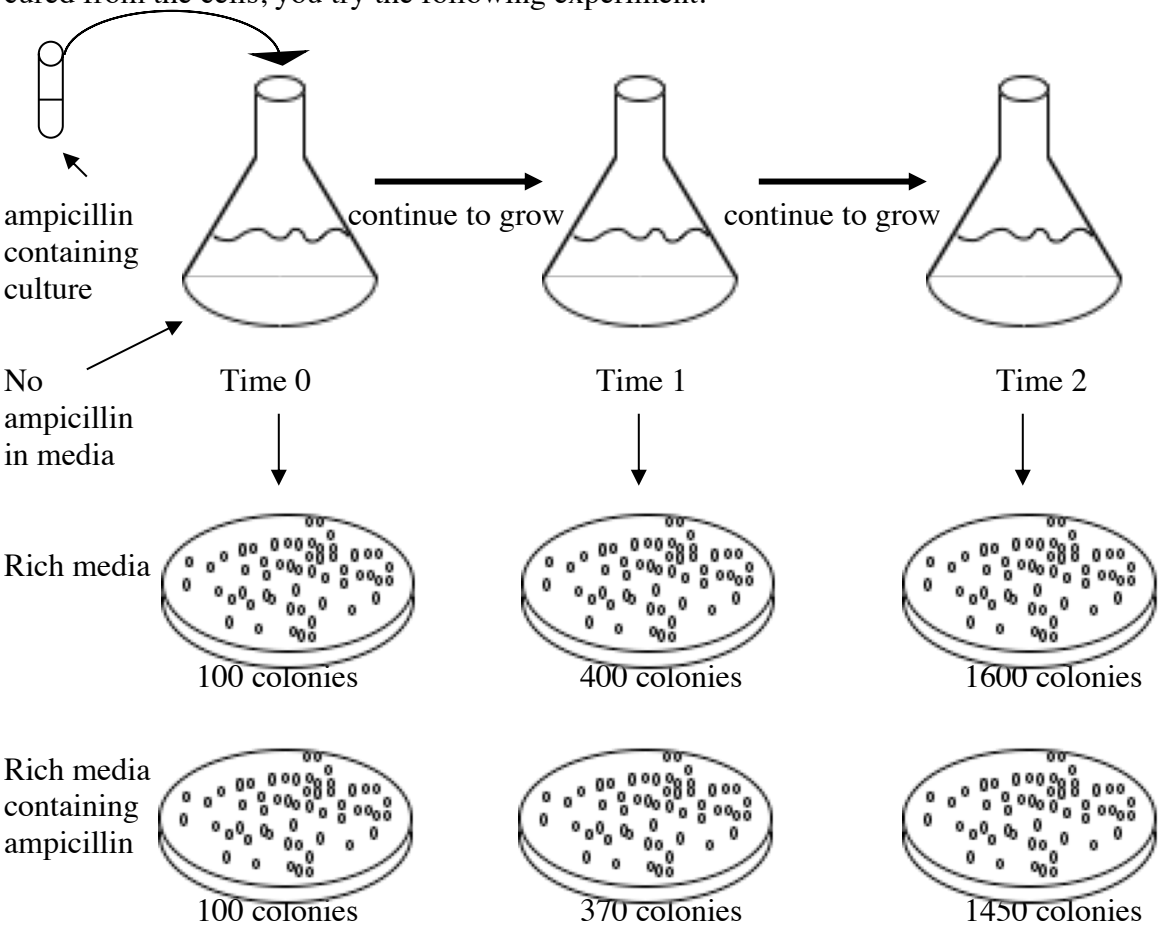


RNAI: The copy number would **increase** because the RNA I transcript hybridizes with RNAII, a transcript required for initiation, and prevents it from forming a functional initiator at the origin DNA region.

RNAII: The copy number would **decrease** (The plasmid probably could not replicate) since the RNAII is needed for nreplication initiation. The transcript hybridizes to the origin region DNA, get processed by RnaseH, and serves as a primer to initiate replication.

rop: The copy number would **increase** because the Rop protein promotes the hybridization of the inhibitor RNA I transcript to the RNAII transcript, preventing initiation from occurring.

You are using a plasmid that has an ampicillin resistance gene on it that has a copy number of 3 at the time of cell division. To measure the rate that the plasmid is cured from the cells, you try the following experiment.



9.) Based upon your results above, calculate the number of cells that are cured of the plasmid each time the cell divides. (# cells cured/ cell division) (6pts)

cured cells= total cells – cells with plasmid(ampR)

cured cells$_{T1}$= 100 – 100 = 0 cured cells$_{T2}$= 400 – 370 = 30

(cells cured/ division) = (cured$_{t2}$ - cured$_{t1}$) / (cell$_{t2}$ - cells$_{t1}$)

= (30-0)/ (400 -100)

= 0.1 cells cured/ cell division

10.) What would be the predicted number of cells cured per cell division if the plasmid were to sort randomly? (6pts)

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...

$2(1/2)^3$= 1/4 cells cured/ cell division

11.) Name two general mechanisms or processes that could account for the discrepancy between the observed cure rate in #4 and your calculated cure rate from #5. (5pts)

The plasmid may have **1.)** a partitioning mechanism that ensures each cell receives one plasmid or **2.)** a plasmid addiction mechanism that kill cells when they lose the plasmid.

12.) A cell contains two plasmids that each replicate using a ColE1 origin or replication. Are the plasmids likely to be compatible or incompatible. Why? (5pts).
Incompatible because the plasmids both utilize the same proteins to regulate when its origins of replication fire, one plasmid is likely to be replicated more frequently than the other. This may be because its origin has slightly higher affinity for the initiation proteins, it is smaller and therefore replicates faster, or just by chance, over time, each cell will not inherit equal copies of each plasmid… giving the more numberous plasmid an advantage in the next generations.

13.) What is the primary difference between conjugative plasmids and mobilizable plasmids? What genes are typically found on conjugative plasmids that are not found on mobilizable plasmids? (5pts)

Conjugative plasmids carry all the genes necessary for conjugation to occur

Mobilizable plasmids carry the genes for plasmid transfer but generally lack the genes needed for pilus formation.

Conjugative plasmids encode the genes for pilus formation/function. Mobilizable plasmids usually lack this set of gene products.

Three hospital patients recently died from pathogenic *E. coli* infections that did not respond to antibiotic treatment. After isolating the bacteria from the patients, it was found that the *E. coli* were resistant to rifampicin, chloroamphenicol, and penicillin. The pathogenic strain of *E.coli* was also unusual in that it produced an unusual red-colored capsule that was required for pathogenicity. The hospital staff sent the strain to you and asked you to determine the map location of the resistance and pathogenicity genes (the genes that make bacteria red in color). You decide that the best way to do this would be to isolate an Hfr strain of this bacteria and map the order that the genes are tranferred into an *E. coli* recipient with the following phenotype:

Recipient strain ***kanR***, ***trpB-, hisA-, gal-, rifS, camS, penS*** **and white in color.**

kan confers resistance to the kanamycin.
trpB and *hisA* are required to synthesize tryptophan and histidine,
gal is required to break down the sugar galactose

As described above, the pathogenic Hfr donor that you isolated has the following phenotype:

Hfr strain ***kanS***, ***trpB+, hisA+, gal+, rifR, camR, penR*** **and red in color.**

You mix the strains together and then every five minutes, you take 8, 1 ml samples, vortex them, and spread them on plates containing the indicated supplements and observe the following number of colonies.

Minutes after mixing / Plate supplements	0	5	10	15	20	25	30	35	40
plate#1: minimal media with glucose, tryptophan, and kanamycin	0	45	1008	1025	1111	1234	15438	13458	1423
plate#2: minimal media with glucose, histidine, and kanamycin	0	0	0	0	0	0	0	0	9
plate#3: minimal media with galactose, histidine, tryptophan, and kanamycin	0	0	0	0	5	20	100	99	104
plate#4: minimal media with glucose, histidine, tryptophan, rifampicin and kanamycin	0	0	4	255	756	723	798	745	732
plate#5: minimal media with glucose, histidine, tryptophan, chloramphenicol, and kanamycin	0	0	2	260	745	790	830	770	756
plate#6: minimal media with glucose, histidine, tryptophan, penicillin, and kanamycin	0	0	8	222	764	711	723	723	743
Plate #7: Rich media containing kanamycin	All white	All white	All white	All white	All white	All white	All white	Mostly white with a few red colonies	Mostly white with a few red colonies

14.) How did you counterselect against your donor strain? (4pts)
kanamycin was included in all the plates.

15.) List which gene(s) was being selected for in each plate (to determine if transfer of that gene had occurred)? (8pts)

plate 1	plate2	plate3	plate4	plate5	plate6	plate7
histidine	tryptophan	galactose	rifampicin	chloramphenicol	penicillin	redness

16.) What is the gene order and approximate distance between each gene? (8pts)

oriT__5min__his__5min__rif__10min__gal__15min__red__5min__trp
cam
pen

Since the red-colored capsule around the bacteria is required for the pathogenicity in this strain of *E.coli*, you decide to try and determine how many genes are involved in producing the pathogenic, red-colored capsule. You identify a single operon on the *E.coli* chromosome that is essential for the capsule to be produced. Then, you isolate 6 *E.coli* with mutations that prevent capsule formation and render the bacteria nonpathogenic. The nonpathogenic mutants are easy to identify because they produce white colonies whereas the pathogenic cells produce red colonies.

Hoping to understand more, you decide to determine how many different genes are represented by your 6 mutants. After a lot of work, you isolate 6 F' factors that each carry the operon with one of the mutations that confers resistance to phage P1.

One by one, you transfer each F' into each mutant to see if the mutations complement the phenotype. The color of the colonies formed by each transconjugate is shown.

Recipient mutant F' that carries mutant	#1	#2	#3	#4	#5	#6
#1	white					
#2	red	white				
#3	red	red	white			
#4	red	red	red	white		
#5	red	red	white	red	white	
#6	white	red	red	red	red	white

17.) Which mutations belong to the same complement group(s). At least how many genes do you suspect are involved in red-colored capsule formation? (8pts)

A (#1, #6)
B (#2)
C (#3, #5)
D (#4)

Your colleague sends you an additional mutant and asks you to determine which gene the mutations falls in. When you examine your complementation results, you obtain the following.

Recipient mutant F' that carries mutant	#1	#2	#3	#4	#5	#6	#7
#7	white	white	white	red	white	white	white

18.) What type of mutation is mutant #7 likely to be and where does the mutation map to? (2pts) #7 maps to genes A,B, and C above. This could be a large deletion that stretches over these genes
or a promoter mutation or an early frameshift if these three genes form a single operon.