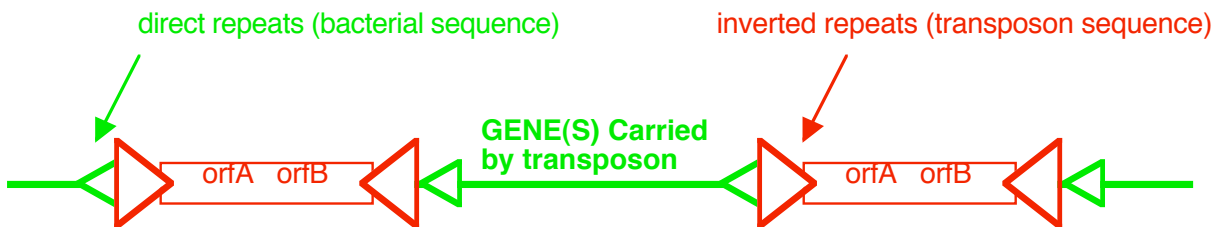


ANSWER KEY

1.) Some Insertion Sequences transpose through a Replicative mechanism of transposition. Other Insertion Sequences utilize a Cut and Paste mechanism. Describe two observations that differentiate between these two mechanisms of transposition? (4pts)

- Cut and Paste transposition does not form a cointegrate molecule as an intermediate, Replicative transposition does.
- Cut and Paste transposition does not require a resolvase activity, Replicative transposition does.
- Following Cut and Paste transposition, only the target DNA has a copy of the transposon. Following Replicative transposition, both the target and donor sequences have a copy of the transposon
- Cut and Paste transposition leaves a double strand break in the donor sequence which must then be repaired. Replicative transposition does not leave any strands unsealed.

2.) Draw the genetic map of a composite transposon inserted into a bacterial chromosome. Label the following components in your drawing: 1.) any open reading frames and 2.) any repeated sequences and their directionality. (4pts)



3.) The Holliday model, the Single Strand Invasion Model, and the Double Strand Break Repair Model of recombination all share several basic features. Describe four general steps that are shared between these recombination models. Then, name an enzyme, or enzyme complex in *E.coli* that is thought to catalyze that step in the recombination reaction (6pts)

INITIATION: RecBCD helicase nuclease: degrades from a double strand DNA end. At a chi site, RecBCD creates a 3'single strand end by preferentially degrading the 5' strand.

STRAND INVASION: RecA: binds to single stranded DNA and pairs it with homologous duplex DNA. This action catalyzes the strand invasion step of most models.

BRANCH MIGRATION: RuvAB: catalyzes branch migration of Holliday junctions.

RESOLUTION: RuvC: an endonuclease that cleaves and resolves Holliday junctions.

Name _____

4.) Phage T7 and Phage T4 utilize different mechanisms to ensure that its genes (rather than the bacterial genes) are transcribed following infection. How does this occur in each case? (4pts)

T7 encodes its own RNA polymerase which is specific for its own late promoters

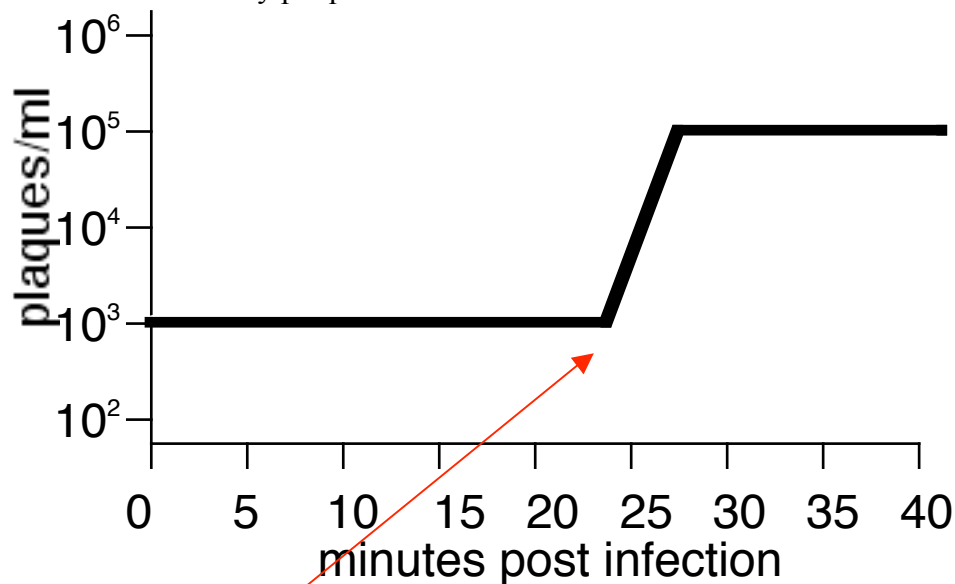
T4 encodes a sigma factor to modify the recognition of the E.coli RNA polymerase to transcribe its own genes

5.) How does Phage T4's early DNA replication differ from its late DNA replication? (3pts)

Early: Controlled bidirectional replication initiated from a unique origin

Late: Replication becomes highly recombinagenic utilizing a recombinational form of replication initiation to amplify the genetic material

Using a low MOI, you mix a solution containing a lytic phage with a culture of your bacteria and allow them to incubate for 2 minutes (time0), before washing away all the media and uninfected phage. Then, at various time intervals, you plate dilutions of your mixture on a lawn of bacteria and count how many plaques form. Your results are shown below.



6.) On average, how long is the lytic life cycle of this phage? (4pts)

~25 minutes

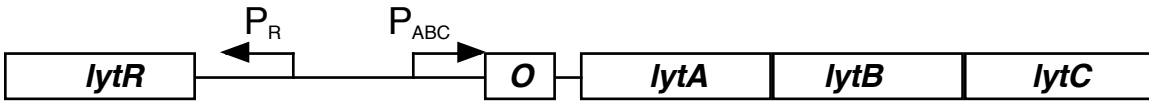
7.) On average, how many bacteriophage are produced each time a phage infects a cell? Show your work. (4pts)

10^5 phage at the end / 10^3 phage when you started =
each phage must have made an average of 100 new phage

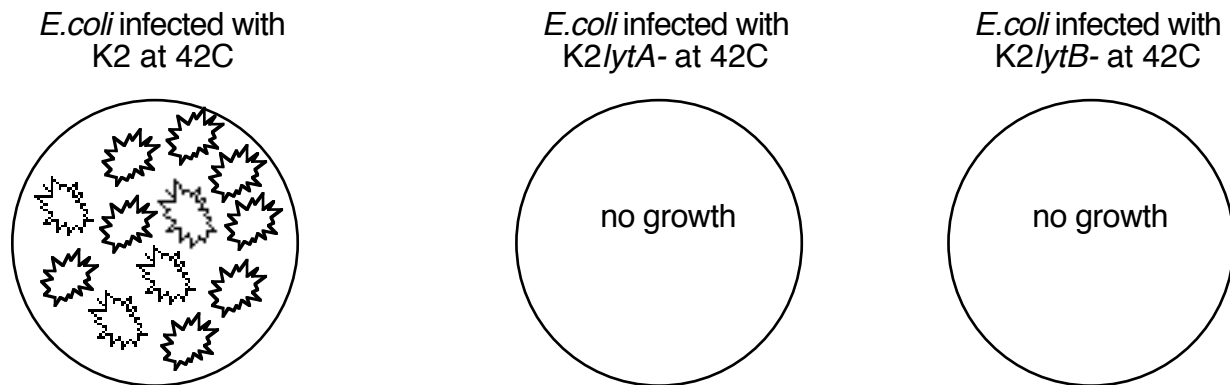
Name _____

A new bacteriophage, called K2, has been isolated from a pathogenic strain of *E. coli* that causes a severe fever in patients and, in some instances, subsequently leads to kidney failure. Since the phage appears to be associated with the pathogenicity, the CDC has recently isolated and sequenced the bacteriophage. The CDC has sent the phage to you and asked for your help in characterizing this new health threat.

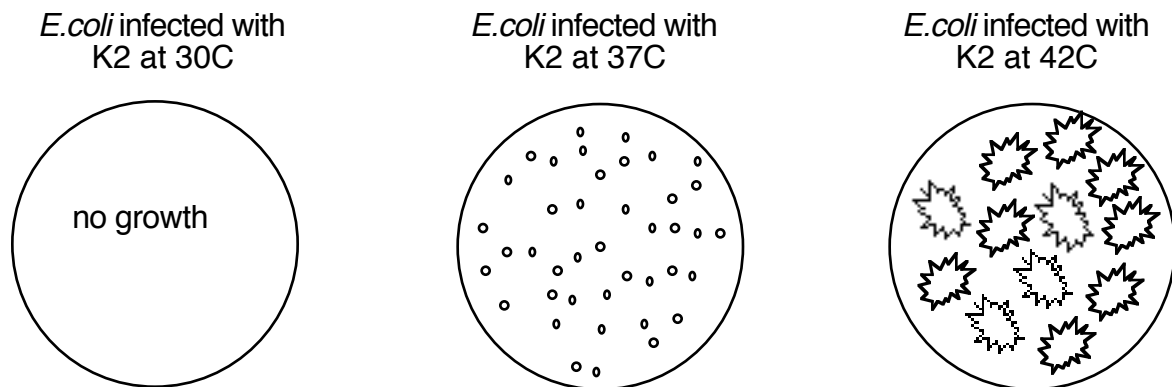
A critical region of the genome is diagrammed below.



Preliminary studies indicate that the *lytR* gene encodes a protein that regulates the expression of an operon containing the *lytA*, *lytB*, and *lytC* genes. *lytA* and *lytB* are required for the lytic life cycle of the bacteriophage, since when phage lacking these genes are allowed to infect *E. coli*, no growth is observed. The plaques observed in normal K2 phage and the lack of plaques in *lytA* and *lytB* mutants is shown below.



It is your job to characterize the regulatory region of this critical region of the phage genome. You're interested in recent reports that show that kidney failure is more likely to occur in patients that exhibit severe fevers at the initial stage of the disease than in patients that do not exhibit symptoms of fever. Based upon these reports, you decide to infect *E. coli* with the phage at different temperatures to determine if there are any differences in phage growth. The plaques formed in each case are shown below.



Name _____

8.) IF the LytR protein negatively regulates the *lytABC* operon, what would you expect to happen to *lytABC* expression when *lytR* was deleted? Why?(5pts)

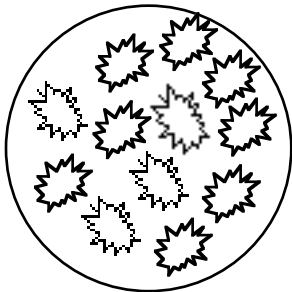
lytABC would be expressed constantly since there would be no LytR repressor to bind and shut off transcription.

9.) IF the LytR protein positively regulates the *lytABC* operon, what would you expect to happen to *lytABC* expression when *lytR* was deleted? Why? (5pts)

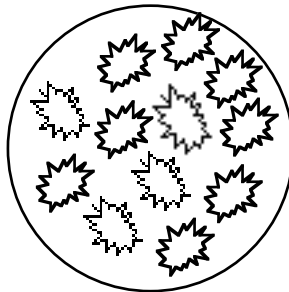
lytABC would never be expressed since there would be no LytR activator to bind and recruit transcription initiation

You construct a deletion mutant that is lacking the entire *lytR* gene. You then repeat your temperature experiment and obtain the following results

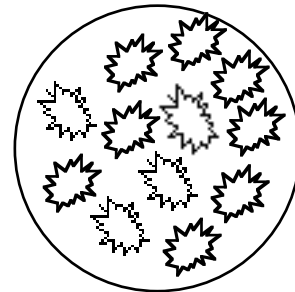
E.coli infected with K2del(*lytR*) at 30C



E.coli infected with K2del(*lytR*) at 37C



E.coli infected with K2del(*lytR*) at 42C



10.) From these results, do you suspect that LytR regulates the operon positively or negatively? Why? (5pts)

Negatively. In the absence of LytR, the phage always forms plaques. Since LytA and LytB are needed for lysis, they must be expressed in all cases, suggesting that LytR normally represses *lytABC* expression under some conditions.

11.) Other researchers have shown that the *lytR* gene is expressed constitutively. Based upon what you have learned about LytR so far, suggest a model for how the LytR protein functions to regulate *lytABC* expression at 30C? at 42C? (4pts)

Since no plaques form at 30C, LytR must be functional, so it may bind to the operator sequence and repress *lytABC* expression under these conditions.

Since plaques form at 42C, LytA and LytB must be expressed suggesting that LytR may be nonfunctional at this temperature. Perhaps LytR is temperature sensitive and denatures at 42C.

*other models are possible

Name _____

A colleague tells you that almost all of the *lytR* mutations that she has isolated behave exactly like a *lytR* deletion in the assay shown above. However, she has one mutant which she calls *lytR^{ind-}* that gives her the following results in this assay.

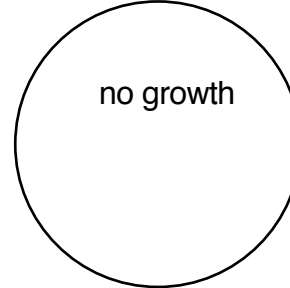
E.coli infected with
K2/*lytR^{ind-}* at 30C



E.coli infected with
K2/*lytR^{ind}* at 37C



E.coli infected with
K2/*lytR^{ind}* at 42C



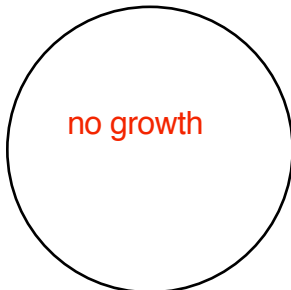
12.) What kind of mutation is *lytR^{ind}* likely to be and how does that result in the observed phenotype? (4pts) **Most mutations probably inactivate the protein...which makes sense that they exhibit phenotypes similar to the *lytR* deletion. However, since it is a rare mutation, it may have mutated a "particular" amino acid that makes it a super-repressor (Or a temperature stable repressor... a gain of function). This would be consistent with no *lytABC* expression under any conditions.**

13.) You co-infect your bacteria with a wild type K2 phage and a *lytR* deletion phage. On the plates below, CLEARLY draw what you would expect your plaques to look like on each plate?

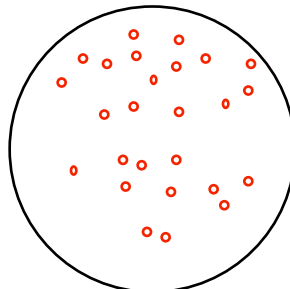
Is the *lytR* deletion dominant or recessive to wildtype? (4pts)

RECESSIVE

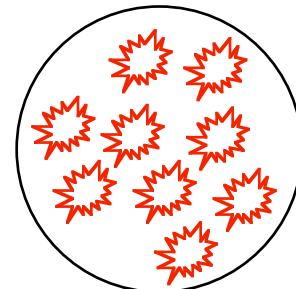
E.coli infected with
K2 at 30C



E.coli infected with
K2 at 37C



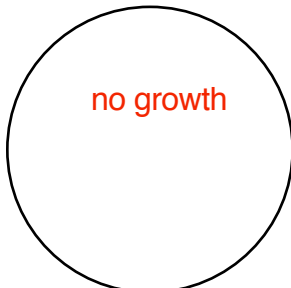
E.coli infected with
K2 at 42C



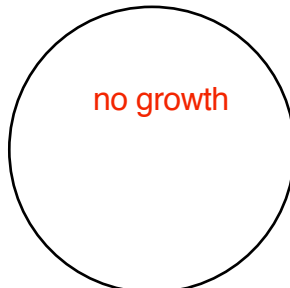
14.) You co-infect your bacteria with a wild type K2 phage and a *lytR^{ind}* phage. On the plates below, CLEARLY draw what you would expect your plaques to look like on each plate? Is

lytR^{ind} dominant or recessive to wildtype? (4pts) **DOMINANT**

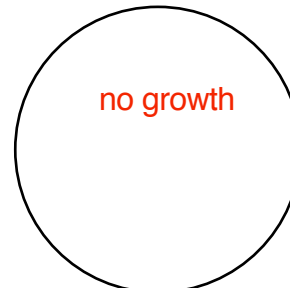
E.coli infected with
K2/*lytR^{ind-}* at 30C



E.coli infected with
K2/*lytR^{ind}* at 37C

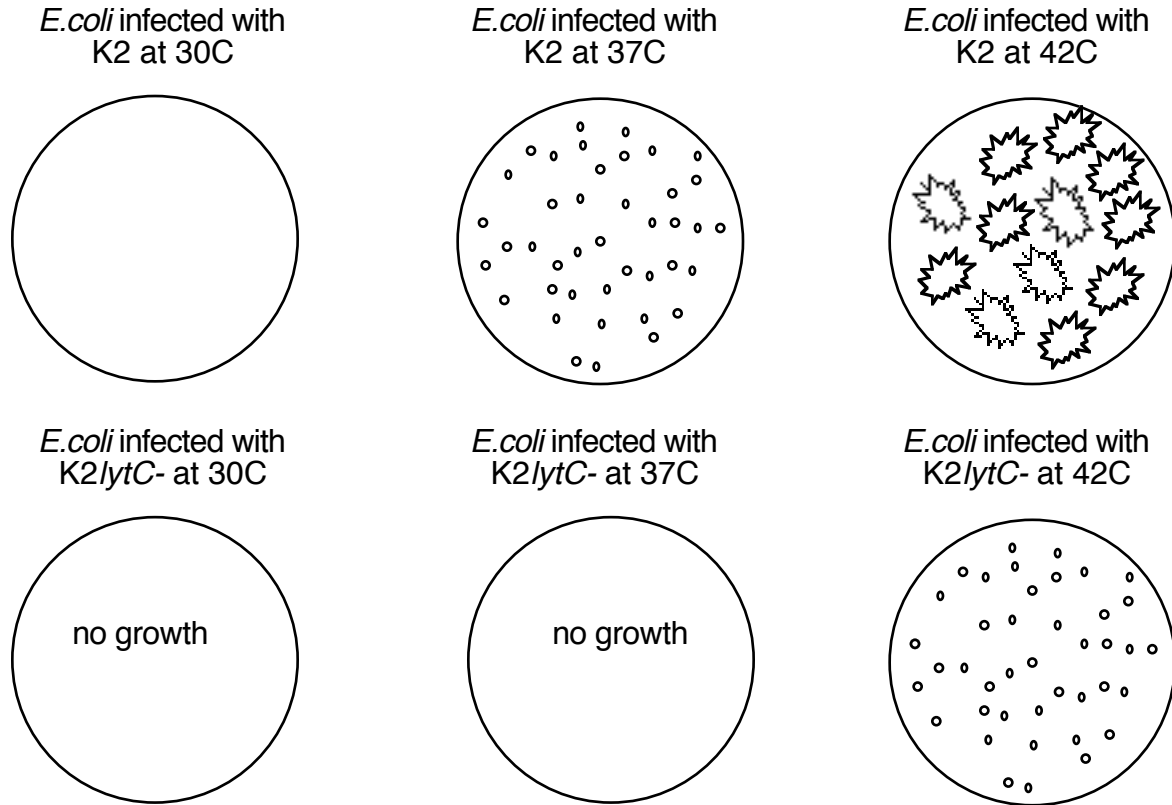


E.coli infected with
K2/*lytR^{ind}* at 42C



Name _____

lytC also seems to play a role in the lytic life cycle, however, *lytC*- mutants do not seem to be *absolutely* required for lytic growth. Although *lytC* mutants do not grow at all at 37C, they are still able to produce smaller plaques at 42C. Shown below are the results of a comparison between wild type and *lytC*- mutants in your temperature experiment.



You decide to isolate several more *lytC*- mutants.

15.) You mutagenize your phage by allowing them to grow in the presence of acridyne dyes during the infection. What temperature would you infect and plate your *E.coli* at in order to screen for *lytC* mutants? Why? (3pts)

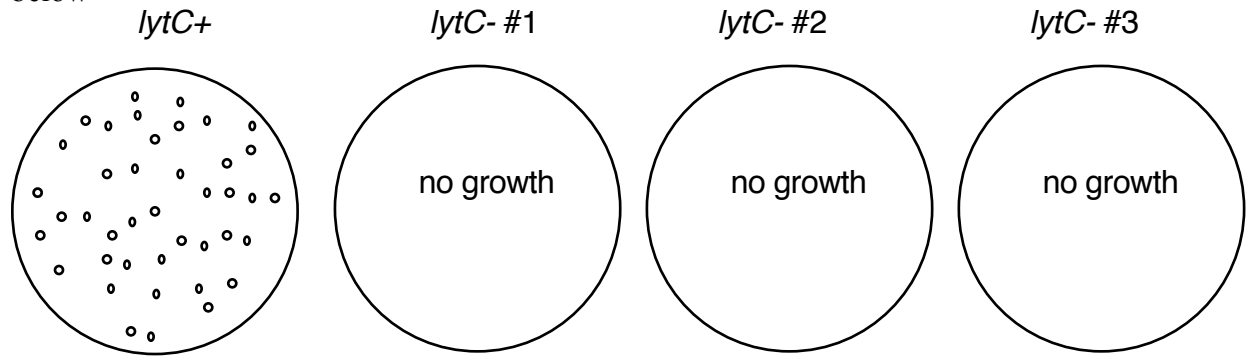
42C. That is the only temperature that a *lytC* mutant will grow.

16.) Assuming that *lytC*- suppressor mutations are extremely rare (1 mutant in 10^9 phage), briefly describe a simple method by which you could screen for suppressor mutations of your *lytC* mutants? (5pts)

Mutagenize the *lytC*- phage and then allow them to infect *E. coli* at 37C. If it can grow, a suppressor or reversion mutation probably occurred.

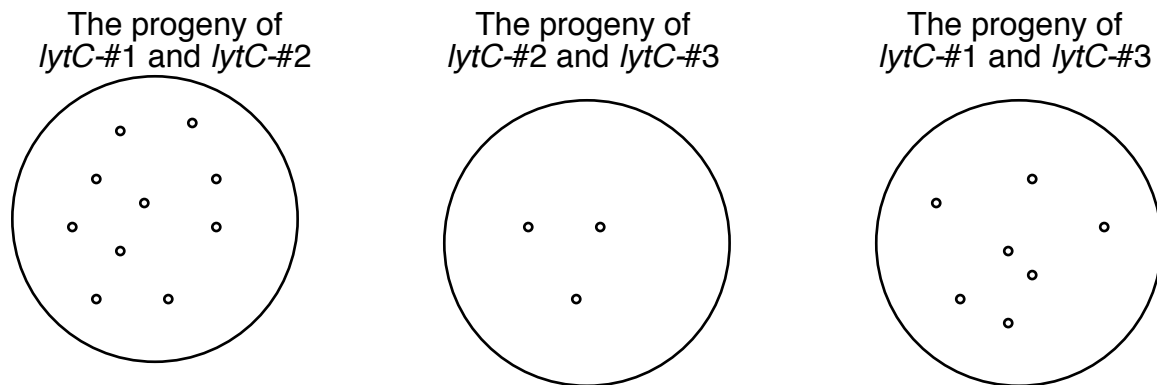
Name _____

When plated at 37C, the phenotype of the wild type and three of your *lytC* mutants is shown below



You want to map the order of these 3 mutations, so you co-infect *E. coli* with the following combinations of phage at a high MOI: mutant#1 and mutant#2; mutant#2 and mutant#3; mutant#1 and mutant#3. Then, you allow the co-infected *E. coli* to lyse and collect the lysates of the progeny phage. When you titer your lysates, you find that the solutions contain 100 phage per ml, in each case.

To look for recombinants, you use 1ml of each lysate to infect 10^9 bacteria and plate them immediately at 37C. Your results are shown below.



17.) Assuming that you plated these combinations many more times and the ratios remained the same, what is the relative distance and map order between each mutation? Show your work (8pts)

Recombination Frequency = total recombinant progeny / total progeny
 10 wt recombinants X 2 / 100 total phage = 0.2 units between #1 and #2
 3 wt recombinants X 2 / 100 total phage = 0.06 units between #2 and #3
 7 wt recombinants X 2 / 100 total phage = 0.14 units between #1 and #3

#1-----0.14 units-----#3--0.06units--#2

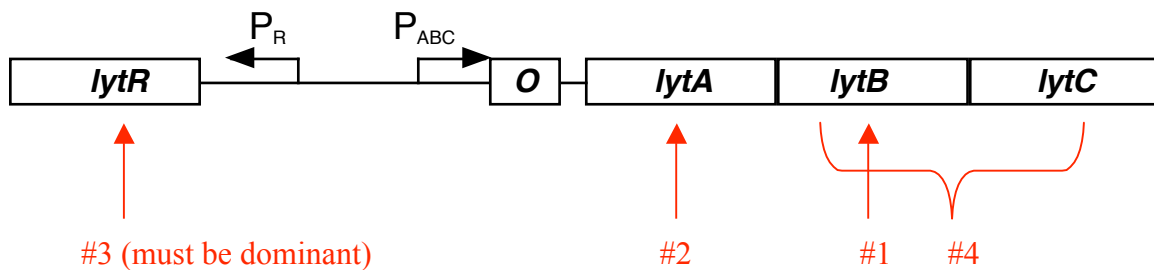
Name _____

The CDC has isolated several mutants in the *lyt* region that render the bacteriophage nonpathogenic and do not grow at 37C. They have sent you several mutants and asked you to identify what genes have mutated in each case. You decide to utilize a complementation analysis for this task and cross each mutant with other known mutations that you have in your collection.

Using a high MOI, you co-infect *E.coli* with two different mutants, as indicated in the table below, and plate them immediately after infection at 42C. Your results are as follows

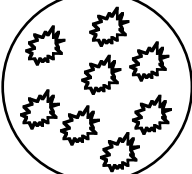
	<i>lytR</i> deletion	<i>lytA</i> -	<i>lytB</i> -	<i>lytC</i> -
Mutant #1			no growth	
Mutant #2		no growth		
Mutant #3	no growth	no growth	no growth	no growth
Mutant #4			no growth	no growth

18.) Identify what gene or genes each mutation is in on the map below? (8 pts)



Name _____

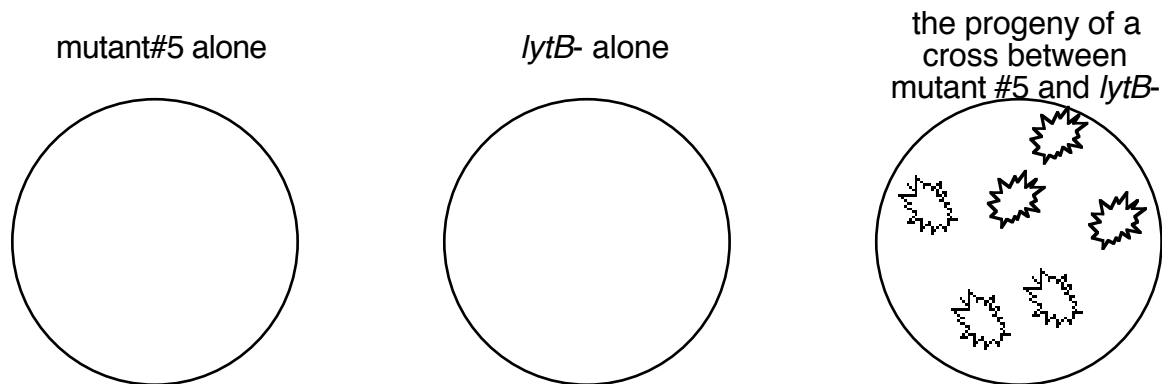
A fifth mutant has the following phenotype in your complementation analysis.

Mutant #5		no growth	no growth	no growth
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19.) Describe two possible mutations that could have occurred in mutant #5 that would produce this phenotype (4pts)

It could be a deletion encompassing *lytAB* and *C* or it could be a mutation in the operator region that prevents *lytABC* expression.

To better understand the nature of mutant #5, you perform a recombinational analysis between mutant #5 and a *lytB*⁻ mutant. You co-infect E.coli at a high MOI with both mutants and collect the progeny in a lysate. When you infect and plate the progeny at 42C, you observe the following results:



20.) What does this tell you about the type of mutation that has occurred in mutant#5? (4pts)

Since some WT recombinants formed when crossed with a *lytB* mutant, mutant #5 must still have the sequence for *lytB* present. Therefore, it could not have a large deletion in that region. This makes the mutation in the operator region more likely.

21.) In the above experiment, if you used an MOI of 5, what fraction of the bacteria would not get infected? Use the poisson expression, $P_i = (m^i e^{-m}) / i!$ and show your work. (4pts)

probability of NOT getting infected (0 phage infect) =

$$P_0 = (5^0 e^{-5}) / 0!$$

$$P_0 = (1)(.0067) / 1$$

$$P_0 = .0067 \text{ or } 0.67\% \text{ of the bacteria}$$

22.) What fraction of the bacteria will be infected by more than one phage (i.e. have a chance to recombine)? (4pts)

probability of getting infected by more than 1 phage = $1 - (P_1 + P_0)$

$$P_{>1} = 1 - ((5^1 e^{-5}) / 1! + (5^0 e^{-5}) / 0!)$$

$$P_{>1} = 1 - (.0336 + .0067)$$

$$P_{>1} = 1 - (.0403)$$

$$P_{>1} = 0.96 \text{ or } 96\% \text{ of the bacteria}$$