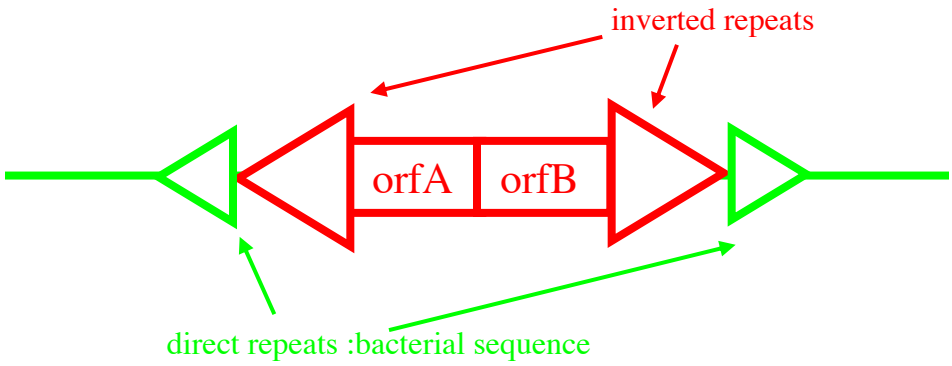
Name_____

Microbial Genetics
BI 410/510
Winter Quarter 2022
Exam III

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? (6pts)

- 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 <u>basic</u> IS element 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. (6pts)



3.) What is the difference between an IS element, composite transposon, and a noncomposite transposon? (6pts)

IS element carries no other genes than the transposase enzyme.
Composite transposons are made up of two IS elements and carry other gene or genes between them.
Noncomposite transposons carry other genes but contain only one transposable element.

4.) Thymine glycol is a common DNA lesion that is generated following the oxidation of thymine. It can either be repaired by the base excision repair pathway or the nuclotide excision repair pathway.

Briefly outline/compare the enzymatic steps that are required to repair the lesion by each pathway. (8pts)

Base Excision Repair

1.glycosylase cleaves the damaged base.
2.AP endonuclease cleaves the DNA backbone
3.polI resynthesizes the damaged region
4.ligase seals the nick

Nucleotide Excision Repair

1. UvrAB bind the lesion UvrC forms a complex and makes two incisions 12 base pairs around the lesion containing base
2. UvrD helicase displaces the damaged fragment
3. pol I resynthesizes the gap
4. ligase seals the nick

5.) Which of these repair pathways are specific for thymine glycol and which are capable of repairing other forms of DNA damage? (3 pts)

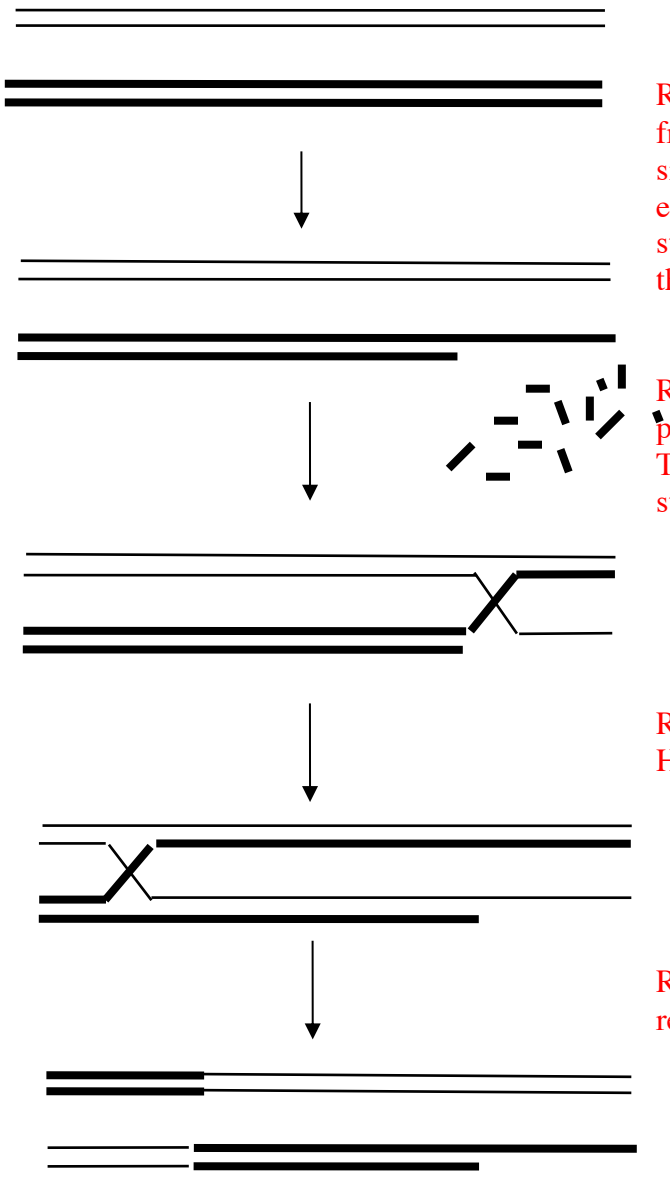
Base Excision Repair encode glycosylases specific for certain DNA lesions

Nucleotide Excision Repair can repair many forms of DNA damage by recognizing "abnormal DNA" and cutting around the lesion rather than removing the lesion itself.

6.) Describe one feature of the Replication Initiation Model that is unique from other models that we have discussed (Holliday model, Single Strand Invasion Model, and Double Strand Break Repair Model)? (5pts)

- Involves extensive replication of the recombinant molecule.
- Creates one recombinant molecule and one parental molecule that does not change (It is not recipricol)
- …Other answers were acceptable for this as well

7.) Name one gene product in *E.coli* and describe the enzymatic activity that is associated with performing each step (arrow) shown below. (10pts)



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. This is thought to be involved in the initiation step.

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

RuvAB: catalyzes branch migration of Holliday junctions.

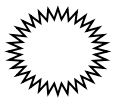
RuvC: an endonuclease that cleaves and resolves Holliday junctions.

You are working with a phage that seems to be quite similar to bacteriophage T4. Just like phage T4, this phage normally produces large, fuzzy edged plaques when plated on a lawn of *E.coli* . Also like phage T4, you are able to isolate mutants (you call these *c*- mutants) that produce smaller, clear edged plaques on *E.coli* and the *c*- mutants do not grow at all in *E.coli* lysogens of the phage lambda (*E.coli* λ). The type of plaque formed in each case is summarized below:

Use Wildtype T4 (*c*+) to infect *E.coli*

Use *c*- mutants to infect *E.coli*

Use Wildtype T4 (*c*+) to infect *E.coli* λ



Use *c*- mutants to infect *E.coli* λ

No Growth

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You decide to try and determine exactly how many genes are involved in producing the small, clear plaques and make an effort to isolate several new mutations.

8.) First you need to isolate more *c-* mutations. You mutagenize your phage by irradiating them with UV light. After you mutagenize your phage, would you infect *E.coli* or *E.coli* λ to screen for your mutants? Why? (5pts)

E.coli, because the mutants you are hoping to isolate would not grow in *E.coli* λ.

You mix two different *c-* mutations together and infect *E.coli*λ cells at a high MOI. The type of plaques produced by each combination is shown in the table below.

	***c-* #1**	***c-* #2**	***c-*#3**	***c-* #4**	***c-* #5**	***c-* #6**
***c-* #1**	**No Growth**					
***c-*#2**	**No Growth**	**No Growth**				
***c-* #3**			**No Growth**			
***c-* #4**			**No Growth**	**No Growth**		
***c-* #5**					**No Growth**	
***c-* #6**	**No Growth**	**No Growth**				**No Growth**

9.) What type of analysis is this? (2pts)

complementation analysis

10.) Which genes are in the same complementation group and how many genes are represented by the six mutants shown above? (8pts)

Group A (mut#1, mut#2, mut#6)

Group B (mut#3, mut#4)

Group C (mut#5)

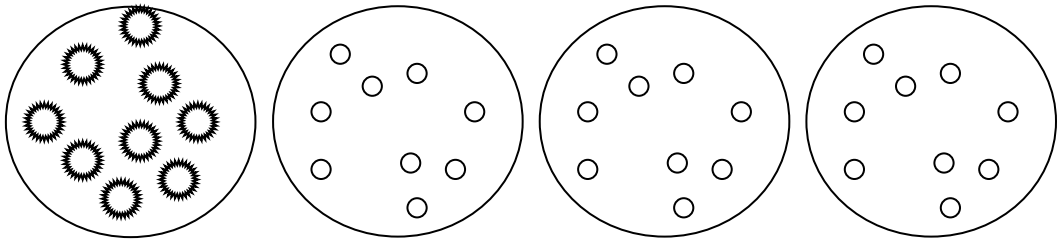
11.) How would the table above look if you infected the *E.coli* at a very low MOI? (2pts)

All combination would show "No Growth" because all the cells would be infected by only 1 mutant phage, and no complementation between mutant could occur.

You decide to map the first 3 *c*- mutants (they all seem to behave as though they are point mutations).

When allowed to infect Wildtype *E.coli*, the phage form plaques as shown below:

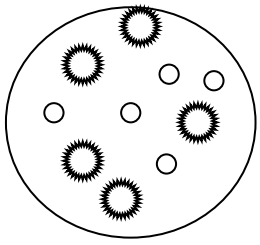
c+ mutant#1 mutant#2 mutant#3



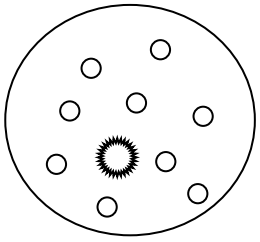
To map these mutations, you infect *E. coli* with the following combinations at a high MOI. mutant#1 and mutant#2: mutant#2 and mutant#3: mutant#1 and mutant#3. Then, you allow the co-infected *E.coli* to lyse and collect the progeny phage.

To look for recombinants, you plate the progeny of these crosses at a very low MOI on wild type *E.coli* and observe the plaques as shown below.

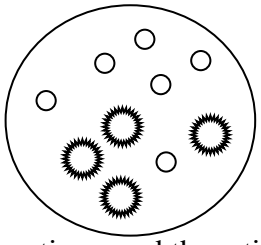
The progeny of mutant#1 and mutant#2



The progeny of mutant#2 and mutant#3



The progeny of mutant#1 and mutant#3



12.) Assuming that you plated these combinations many more times and the ratios remained the same, what is the relative distance (ie recombination frequency) between each mutation? (6pts)

5 x 2 recombinants	1 x 2 recombinants	4 x 2 recombinants
10 total progeny	10 total progeny	10 total progeny
1 unit	0.2 unit	0.8 unit
between m#1 & m#2	between m#2 & m#3	between m#1 & m#3

13.) What is the map order and relative distance between these 3 mutations? (4pts)

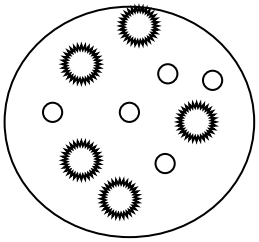
mut#1 ⟵ 0.8units ⟶ mut#3 ← 0.2units → mut#2

14.) If you plated the progeny of these crosses at a very high MOI instead, what could happen…. and how would it affect your results? (2pts)

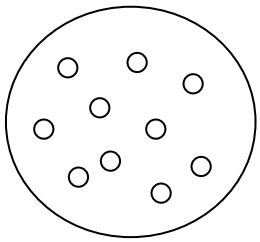
We would observe "false recombinants" because more than one phage would infect each cell giving a chance for complementation to occur. This would particularly be a problem in this case since mut#1& mut#3 and mut#2& mut#3 are able to complement each other.

You have a colleague at a nearby university who sends you a forth mutation that she isolated in her own laboratory. She asks if you will map the mutation for her, relative to your mutants. You perform the crosses and obtain the following results:

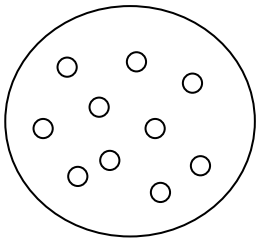
The progeny of mutant#1 and mutant#4



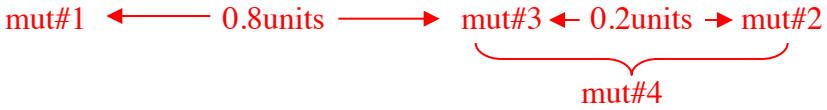
The progeny of mutant#2 and mutant#4



The progeny of mutant#3 and mutant#4



15.) Assuming that you plated these combinations many more times and the ratios remained the same, where does mutation#4 map to? (4pts)



16.) What type of mutation is mutation#4 most likely to be? (2pts)

A deletion that extends from mut#3 through mut#2

Francis Crick mutagenized the *rIIB* gene with acridine dyes to answer several fundamental questions about the genetic code.

17.) What is the unusual property of the *rIIB* gene and what is unique about the mutations produced by acridine dyes that allowed this study to work? (5pts)

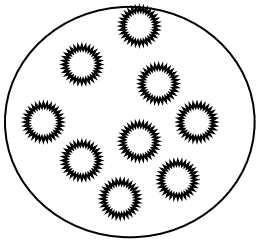
The N-terminal region of the *rIIB* gene product is not essential for proper function.
Acridine dyes primarily induce frameshift mutations.

18.) Crick first isolated an *rIIB* mutant for his study and called this FC0. Then he went on to isolate several suppressors of FC0 calling them FC1, FC2, FC3…. etc. Describe how you would screen for suppressors of FC0 mutation using *E.coli*λ? (4pts)

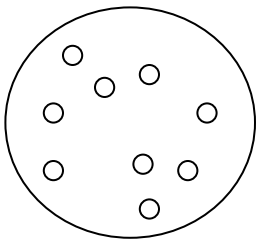
Infect and plate your mutagenized FC0 phage on *E.coli*λ. Anything that grows must also have a suppressor mutation.

To differentiate between supressors that contained second site mutations and ones that were true revertants (remember that he needed second site suppressors for his study), Crick crossed the suppressor phage with wild type (*r*+) phage and looked at the progeny.

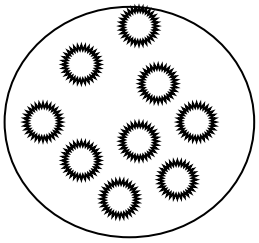
Remember that**…**



If he used wild type T4 to infect *E. coli*, he observed only large, fuzzy edged plaques.



If he used his *r*IIB mutant (FC0) to infect *E. coli*, he observed only small, clear edged plaques.



If he used any of his *r*IIB suppressors (FC1 or FC2 or FC3…etc.) to infect *E. coli*, he observed only large, fuzzy edged plaques.

19.) How should the progeny of a cross between a 2nd site suppressor phage and wild type phage differ from the progeny of a cross between a true revertant and a wild type phage? Why? (5pts)

The progeny from a cross between a second site suppressor and wild type phage should produce some recombinants that have the single mutations…and look like the original FC0 plaques.

The progeny from a cross between a true revertant (a wild type phage) and another wild type phage should produce only wild type phage.