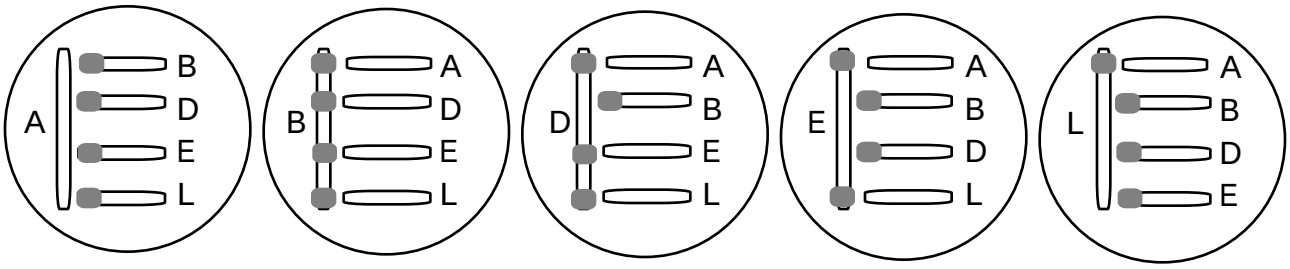
Mcbio 316: Exam 2 **Name ___ANSWER KEY____**
Spring 1999

1. [5 pts] Five different aromatic amino acid auxotrophs were isolated: *aroA*, *aroB*, *aroD*, *aroE*, and *aroL*. To determine if each of these mutations affect different steps of the aromatic amino acid biosynthesis pathway, each mutant was tested for the ability to crossfeed the other mutants. The results are shown in the figure below. The shaded arrows indicate growth and the areas that are not shaded indicate where each mutant was streaked on the plate.



Based on the above results, indicate the relative order of the steps blocked in each of the five mutants.

ANSWER: B -> D -> E -> L -> A

2. [6 pts] A series of five temperature sensitive (Ts) mutants of phage T4 were isolated and analyzed by complementation tests. The tests were performed by putting a mixture of the two phage mutants to be tested (about 10^6 of each) onto a lawn of bacteria at 40°C. The results are summarized below:

(+ = complete lysis; − = no lysis or only a few plaques in the spot)

	1	2	3	4	5
1	−	+	+	−	+
2		−	+	+	+
3			−	+	−
4				−	+
5					−

a. How many complementation groups are represented by these mutants?

ANSWER: 3 groups

b. Which mutations fall in each complementation group?

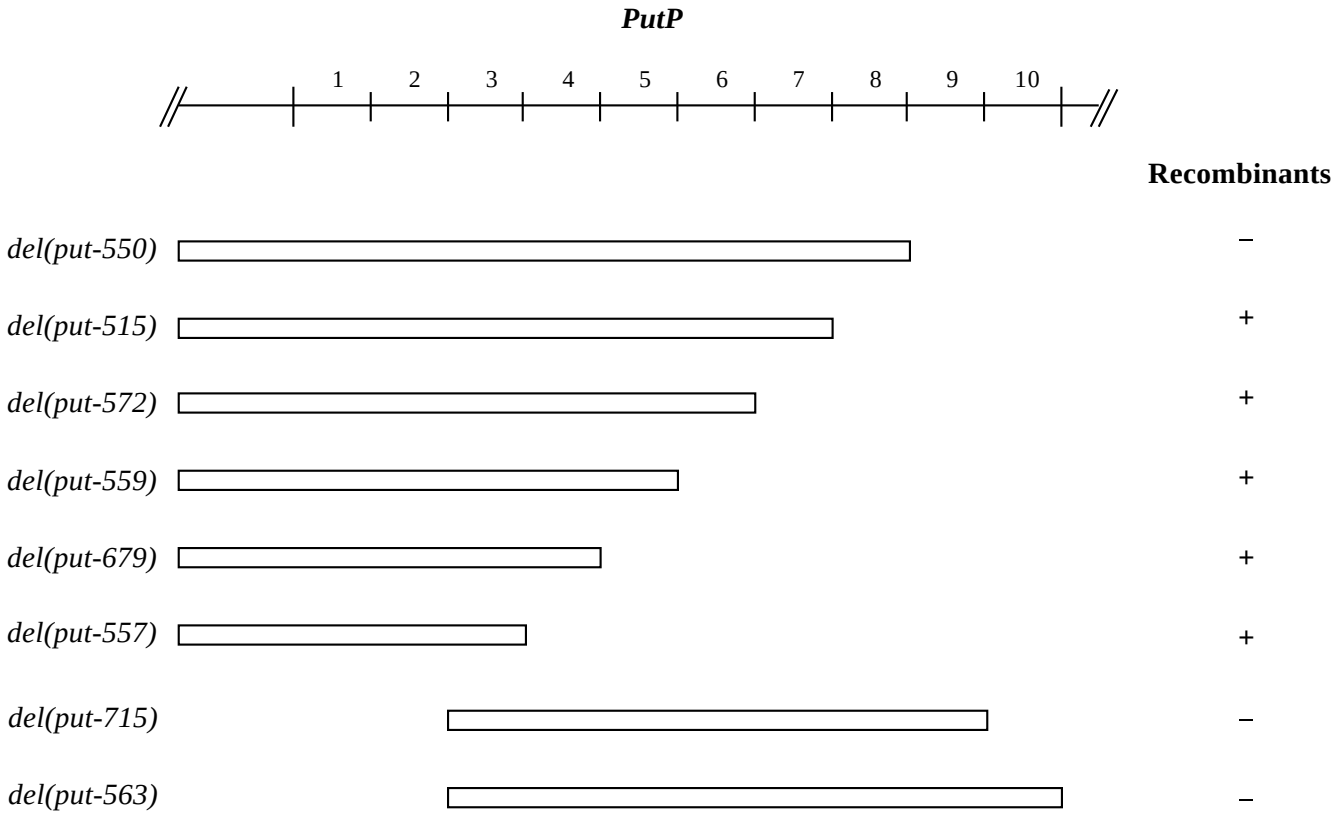
ANSWER: (i) 1 and 4; (ii) 3 and 5; (iii) 2

c. Why were Ts mutants used for this experiment? Briefly explain the rationale.

ANSWER: TS mutants are almost always due to missense mutations, thus decreasing potential confusion due to polarity.

3. [9 pts] A new *putP* mutation was mapped against a set of *putP* deletion mutations. The region removed by the deletion mutations are indicated by open boxes below the *putP* gene and the results

showing whether or not recombinants were obtained are shown to the right of each deletion. Based on these results, where does the new *putP* mutation map?



a. Based on the above results, where does the new *putP* mutation map? [Indicate map position by the numbered deletion intervals shown above the map.]

ANSWER: Interval #8

b. A second new Put$^-$ mutant was isolated that does not revert to Put$^+$ at a detectable frequency and cannot repair any of the known deletions. Based upon these results, what can you infer about the properties and location of the mutation.

ANSWER: This mutation may be a deletion. The results indicate that it removes at least part of region #3, but it could extend beyond this region.

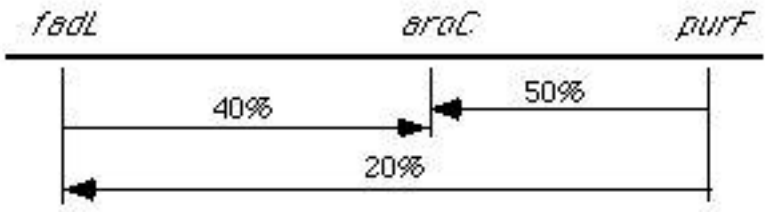
c. Suggest a genetic recombination experiment to test your idea. Indicate the donor(s) and recipient(s) and how you would select for recombinants.

ANSWER: Test for recombinational repair with a number of point mutations, including point mutations in adjacent intervals, and point mutations in region #3 that can recombine with each other. Select Put$^+$.

4. [9 pts] P22 transduction was done to map the *fadL* gene. The results of two-factor crosses between *fadL* and two linked markers, *purF* and *aroC*, are shown below: From these data, draw a linkage map of the *fadL*, *purF*, and *aroC* genes. Indicate the predicted gene order and the percent cotransduction between each gene.

Donor	Recipient	Selected marker	Recombinants	Number obtained
fadL purF$^+$	*fadL*$^+$ *purF*	*purF*$^+$	*fadL*	200
			fadL$^+$	800
fadL aroC$^+$	*fadL*$^+$ *aroC*	*aroC*$^+$	*fadL*	400
			fadL$^+$	600
aroC$^+$ *purF*	*aroC purF*$^+$	*aroC*$^+$	*purF*	500
			purF$^+$	500

ANSWER:



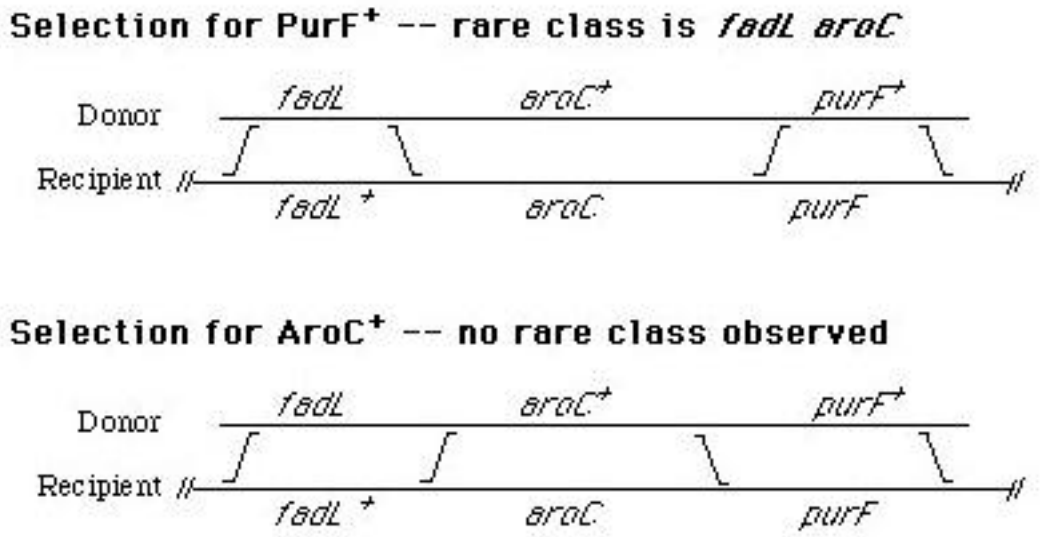
5. [9 pts] To confirm the gene order determined from two-factor crosses, the following three factor cross was done to map *fadL*. Based on these data, indicate the order of the *fadL*, *purF*, and *aroC* genes and draw the crossovers needed to explain your rationale for both of the selected phenotypes.

Donor *fadL purF*$^+$ *aroC*$^+$
Recipient *fadL*$^+$ *purF aroC*

Selected phenotype	Recombinant phenotype	Number of recombinants
PurF$^+$	*fadL*$^+$ *aroC*$^+$	180
	fadL aroC$^+$	130
	fadL$^+$ *aroC*	240
	fadL aroC	20
AroC$^+$	*fadL*$^+$ *purF*$^+$	300
	fadL purF$^+$	250

	fadL^{+} *purF*	330
	fadL purF	270

ANSWER:

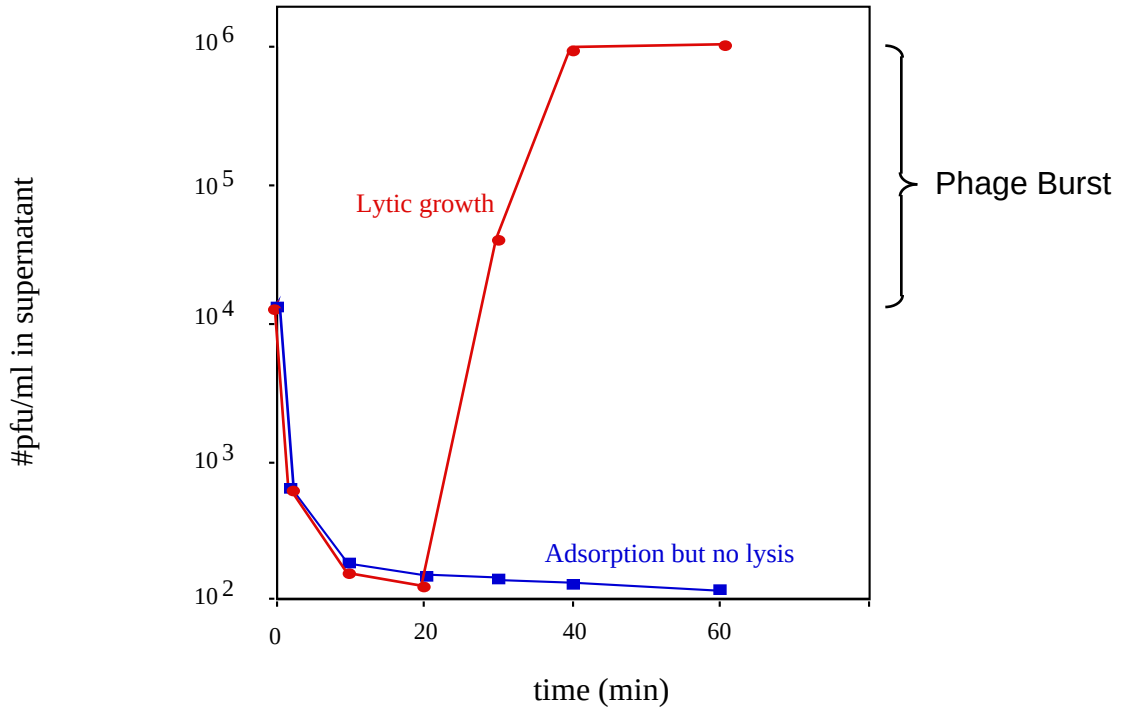


6. [6 pts] Phage Mx4 is a generalized transducing phage with a natural host range limited to *Myxococcus xanthus* strains that do not form fruiting bodies. When Mx4 infects strains of *M. xanthus* that form fruiting bodies, transductants are obtained but no plaques are formed.

a. What do these results suggest about the fate of the Mx4 in *M. xanthus* strains that form fruiting bodies?

ANSWER: Mx4 can infect and produce progeny phage in the strains that do not form fruiting bodies (red curve in figure below). In contrast, in the strains that form fruiting bodies, Mx4 can infect but cannot lyse the cells and produce progeny phage suggesting a defect in phage morphogenesis (blue curve in figure below).

b. What would be the expected results for a one-step growth curve experiment if you compared Mx4 infection of a *M. xanthus* strain that does not form fruiting bodies and a *M. xanthus* strain that forms fruiting bodies. Assuming that Mx4 has similar growth kinetics to other phage that you are familiar with, fill in the following diagram of the predicted one-step growth curves.



7. [4 pts] List two differences between generalized and specialized transducing phage.

ANSWER:

Generalized	Specialized
Transduction of any region of chromosome	**Transduction only of regions adjacent to integration site**
Only chromosomal DNA in transducing particle	**Both chromosomal and phage DNA in transducing particle**

6. [6 pts] How could you obtain a lysogen of a λ *cI*(Am) mutant?

ANSWER: (i) Infect a *sup*(Am) strain of *E. coli* and pick lysogens from the center of a turbid plaque. (ii) Coinfect the λ *cI*(Am) mutant with a lambda derivative that is *cI*$^+$ but requires some other gene product provided by the λ *cI*(Am) mutant (e.g. Int). You would have to confirm that you have a dilysogen by induction of the lysogen and demonstrating that some phage form clear plaques on a *sup*° host.

9. [6 pts] λ, 424, and 21 are all lambdoid phage. Lysogens of these phage are indicated by writing the phage in parenthesis after the bacterial strain. For example, an *E. coli* K-12 λ lysogen is indicated K-12(λ). HK22 is a newly isolated lambdoid phage isolated from pig silage in Hong Kong. When testing the growth of phage HK22 on various *E. coli* K-12 lysogens, Gottesman and coworkers noted the following results.

Infecting phage	**Lysis of strain:** K-12(λ)	K-12(424)	K-12(21)	K-12(HK22)
λ	−	+	+	−
424	+	−	+	−
21	+	+	−	+
HK22	+	+	+	−

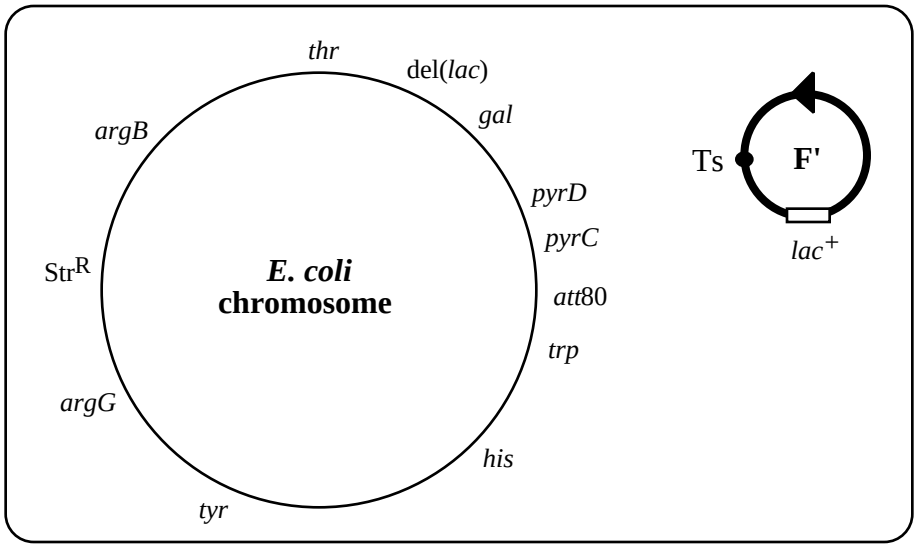
a. Why does λ grow on K-12(424) and K-12(21) but not K-12(λ)?

ANSWER: K-12(λ) produces cI which can repress incoming λ and thus it is homoimmune. The cI produced by K-12(424) and K-12(21) cannot recognize the operator sites in λ hence these lysogens are heteroimmune.

b. Suggest a plausable explanation for why λ and 424 do not grow on *E. coli* K-12(HK22).

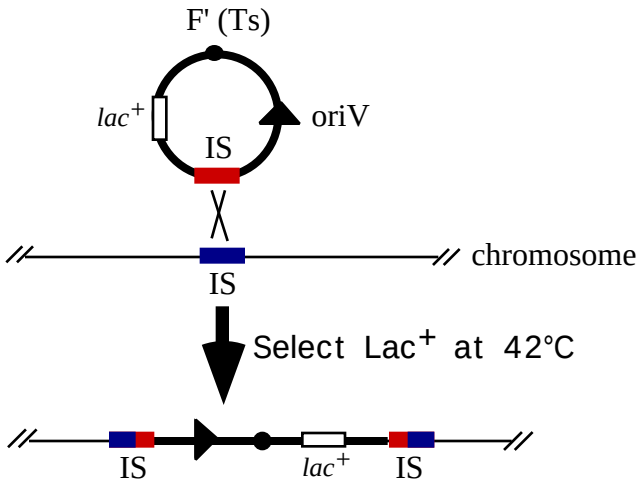
ANSWER: This cannot be a simple case of homoimmunity because it is obvious from the above table that λ and 434 are heteroimmune with each other and thus cannot both be homoimmune with HK22. Hence, these results suggest that the effect is due to superinfection exclusion due to an altered phage receptor or a restriction system produced by the HK22 lysogen.

10. [12 pts] Long before the advent of molecular cloning, Beckwith and colleagues developed a clever technique for cloning genes directly from the chromosome [Beckwith, J., E. Signer, and W. Epstein. 1966. Transposition of the *lac* region of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. 31: 393-401]. Their approach involved several steps. The first step was the isolation of an Hfr from the following *E. coli* strain with a deletion of the chromosomal region including the *lac* operon and an F' (Ts) *lac*$^+$ plasmid. The Ts mutation prevents replication of the plasmid at 42°C but not at 30°C.



a. Draw a diagram showing how you would <u>select</u> for an Hfr in this strain. Briefly describe your rationale.

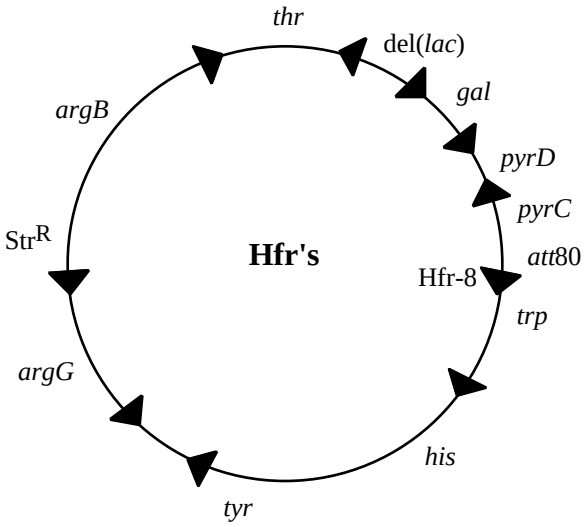
ANSWER: The F-plasmid cannot replicate at 42°C and the host is unable to use lactose due to a deletion mutation. Selection for growth on lactose as a sole carbon source at 42°C demands integration of the plasmid via homology between the IS on the plasmid and an IS on the chromosome as shown in the figure below.



b. How could you determine the relative location and orientation of the Hfr insertions?

ANSWER: Hfr mapping with an F^- auxotrophic recipient. This requires a selection for recombinants (prototrophy) and a counterselection against the donor (for example, absence of an amino acid required by the donor or antibiotic resistance such as Str^R of the recipient). The medium should allow both the selection and counterselection – for example, minimal medium with glucose as a carbon source, streptomycin, and no histidine would select for His^+ Str^R recombinants. Genes that are located proximal to the Hfr (behind the arrowhead) will be transferred early and genes that are distal to the Hfr (toward the tip of the arrowhead) will be transferred late. The relative distance from the Hfr can be determined by determining if recombinants arise after interrupted mating or by quantitating the relative frequency of recombinants that result from spontaneous breakage of mating pairs.

c. The resulting Hfr insertions were located at different positions in different cells. Although hundreds of Hfr's were mapped, the insertions only occurred at a limited number of sites (indicated by the arrowheads in the figure below). Suggest an explanation for this result.

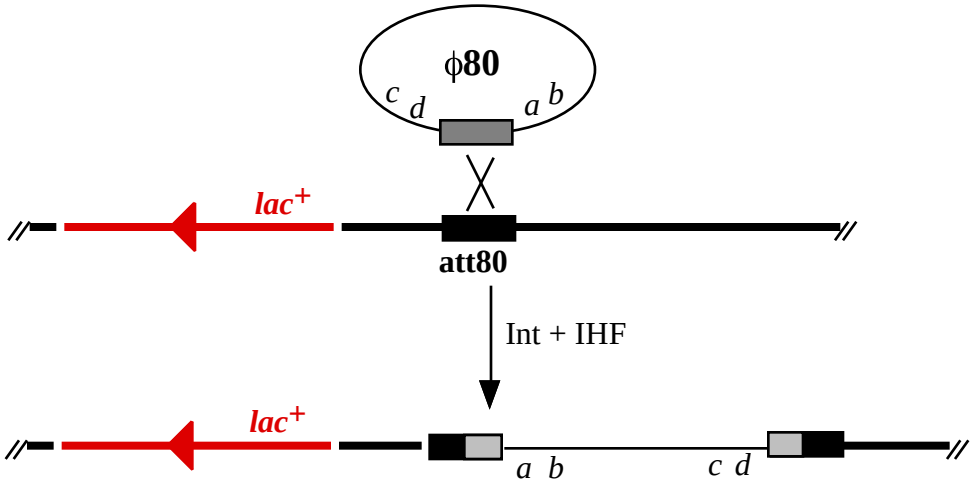


ANSWER: The F' integrates via homology with IS elements on the F-plasmid and IS elements on the chromosome. There are a limited number of sites of IS insertions on the chromosome, resulting in a limited number of Hfr sites.

11. [14 pts] The F' in Hfr-8 was inserted very close to the attachment site for the lambdoid phage Ø80. The lysis-lysogeny decision of Ø80 is essentially identical to phage λ.

a. *E. coli* Hfr-8 was infected with Ø80, and lysogens were isolated by picking bacteria from the center of a turbid plaque. Draw a diagram showing how lysogens would form after entry of the phage into a host cell and indicate the gene products required for this event.

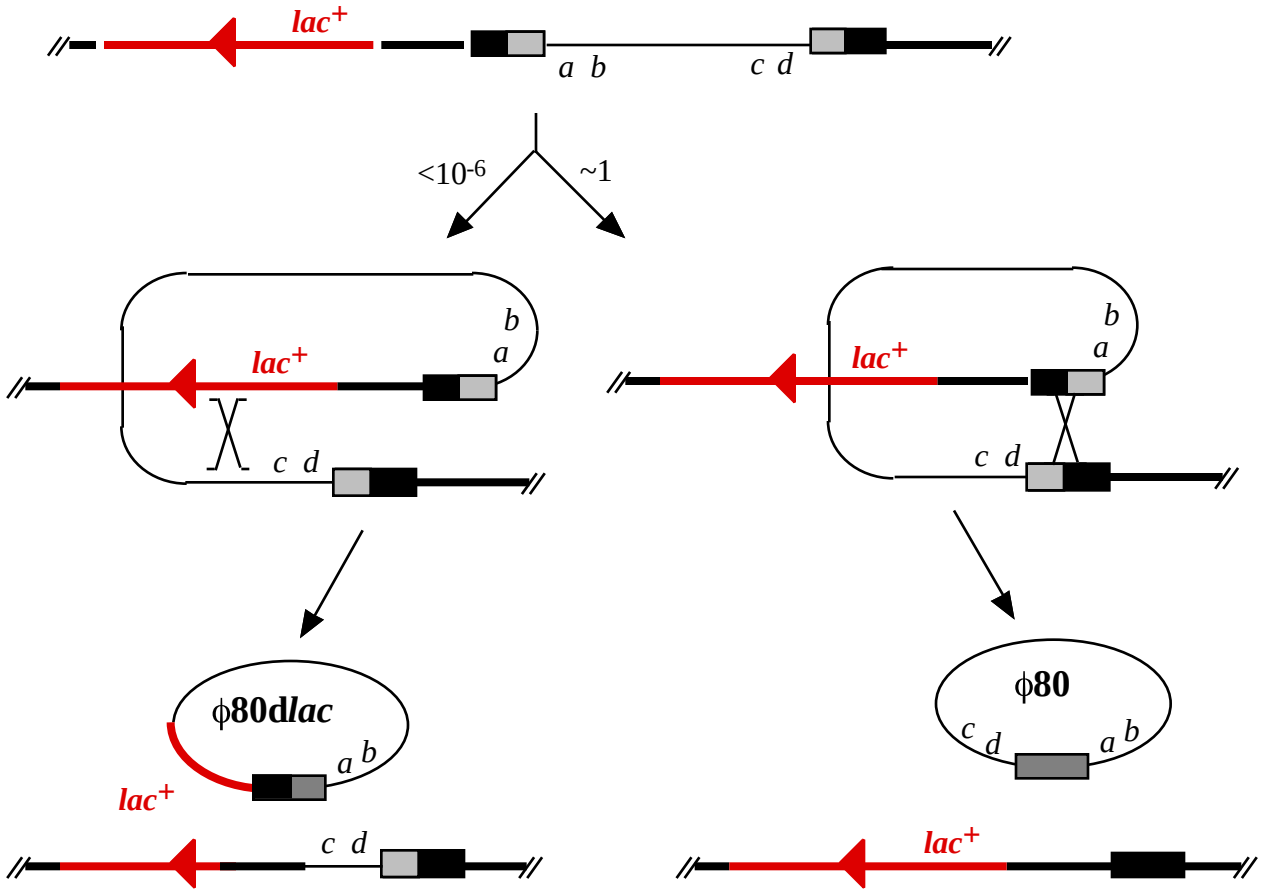
ANSWER:



b. The lysogens were induced by UV irradiation. The resulting lysate had two types of phage particles: wild-type Ø80 phage and Ø80 *lac*$^+$ specialized transducing phage. Draw a diagram

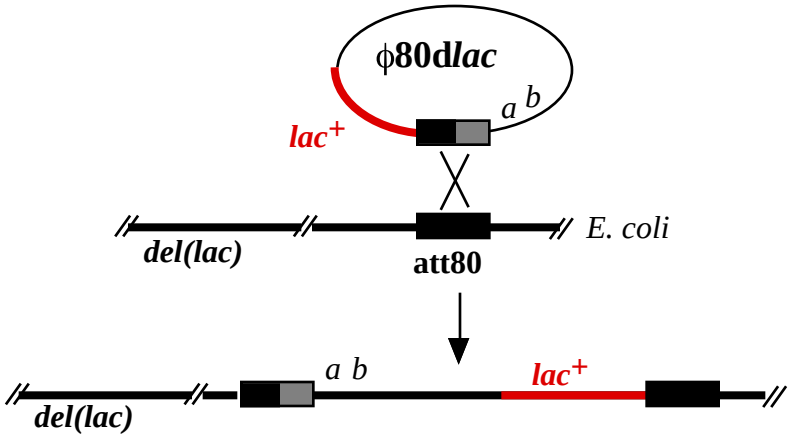
showing how each type of phage could be formed from the Ø80 lysogen. Indicate the relative frequency of each type of excision reaction.

ANSWER: Note that the phage from this lysogen would be a LFT.



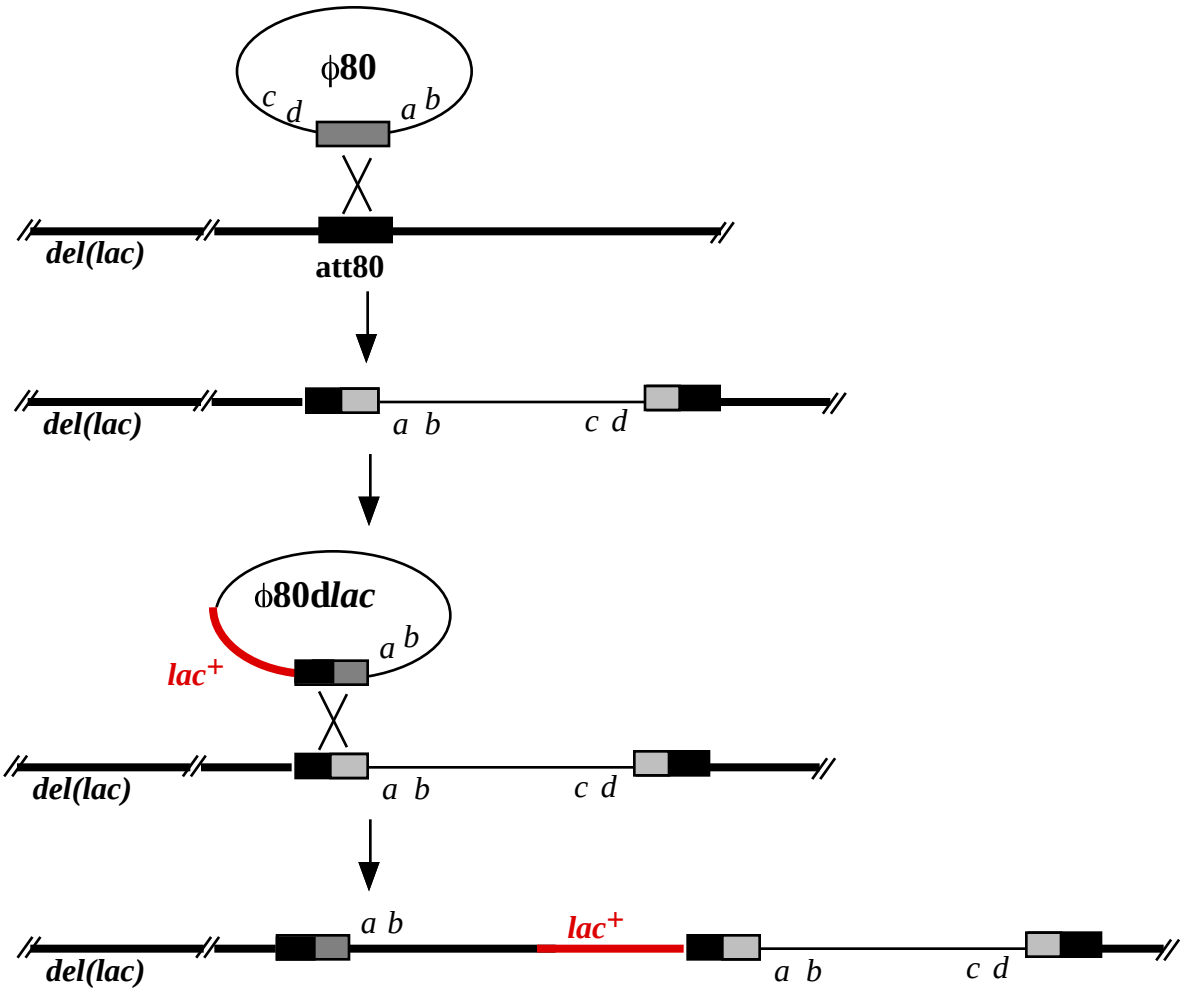
c. Draw a diagram showing how you could identify any *lac*$^+$ specialized transducing phage in the lysate.

(A) Low MOI



Note: The deletion removes the chromosomal *lac* homology, so you would not get repair of the *lac* operon by a pair of cross-overs.

(B) High MOI



d. What frequency would you expect to find the *lac*$^+$ specialized transducing phage relative to the total phage in the lysate?

ANSWER: Excision from an HFT should result in 50% specialized transducing phage. (Excision from the lysogens obtained after transduction at low MOI could occur but no viable phage would result because the phage is defective for lytic functions.)

12. [6 pts] It is possible to "cure" a strain of the plasmid pLAFR which encodes resistance to tetracycline (Tet^R) by mating in a second plasmid pPH1JI which encodes resistance to gentamycin (Gen^R).

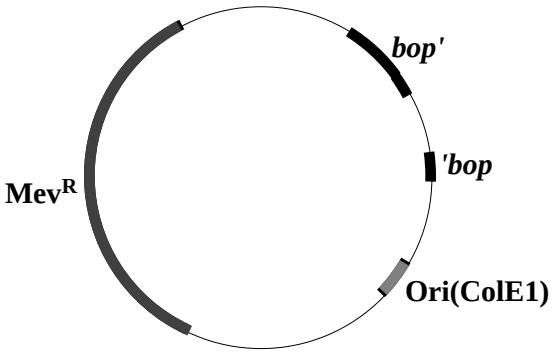
a. What does this suggest about the properties of these two plasmids?

ANSWER: The two plasmids are incompatible.

b. Would this trick work if the only selectable marker on pPH1JI was Tet^R? Briefly explain.

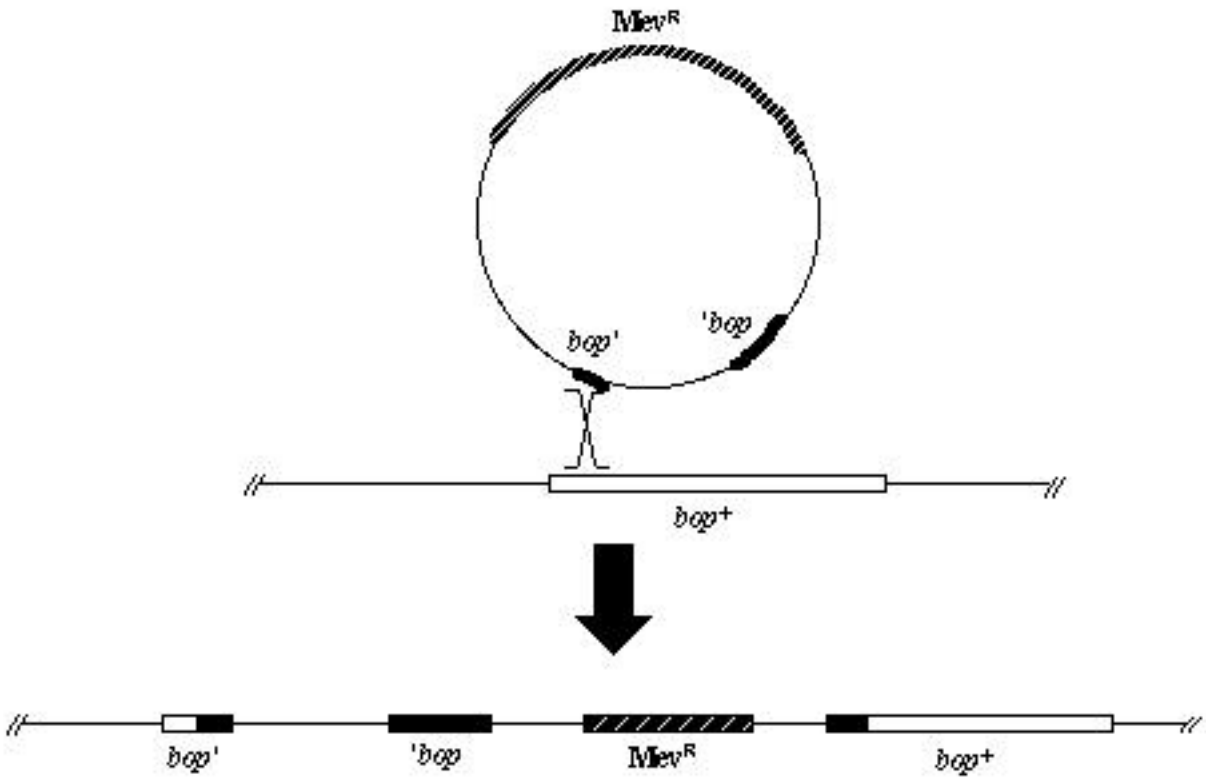
ANSWER: No, you need a selection for the incoming plasmid to kick-out the stably replicating resident plasmid.

13. [8 pts] ColE1 plasmids replicate in enteric bacteria but cannot replicate in *Halobacterium salinarium*. The *bop* gene from *H. salinarium* was cloned into a ColE1 plasmid, and an insertion mutation constructed that disrupted the plasmid encoded *bop* gene (indicated by *bop'-'bop* in the figure below). This plasmid was then transformed into *H. salinarium* with selection for resistance to the antibiotic mevinolin (Mev^R).



a. The plasmid does not have any functional replication origin and, except for the *bop* gene, the plasmid lacks any homology with the *H. salinarium* chromosome. How do the Mev^R transformants arise? Show a diagram and briefly explain your answer.

ANSWER:



b. When the resulting transformants were subsequently grown for many generations without mevinolin, some Mev^S colonies were obtained. About half of these Mev^S colonies were Bop^+ and half were Bop^-. How do the Mev^S Bop^- colonies arise? Show a diagram and briefly describe your answer.

ANSWER: