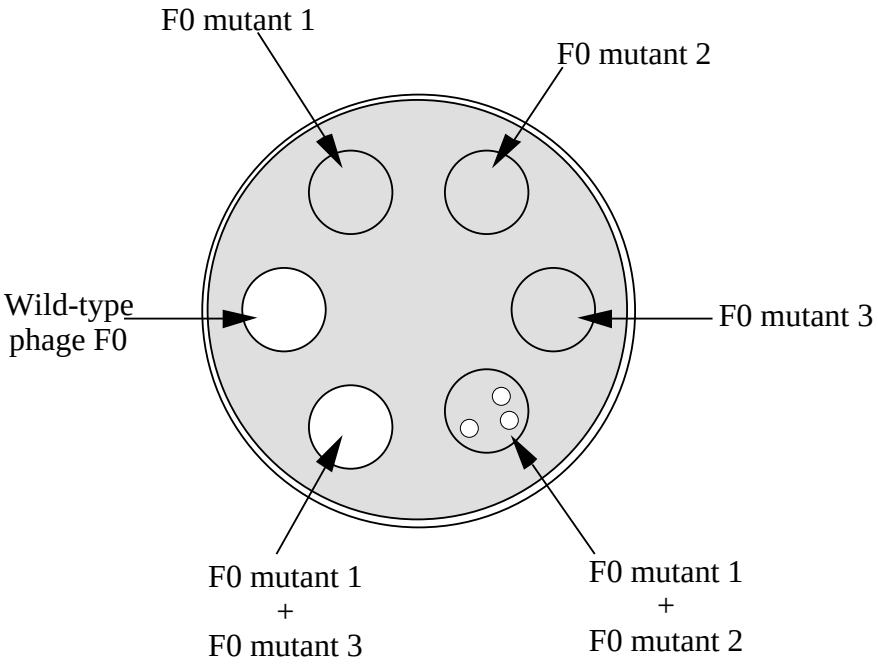
Q1. (8) F0 is a virulent phage (that is, it cannot lysogenize its host). When wild-type phage F0 phage is spotted on a lawn of *S. typhi* the cells are lysed (indicated by white spots on the bacterial lawn shown below). Three conditional mutants were isolated that prevent lysis of a nonpermissive host. All three of these mutants affect the synthesis and assembly of the phage head, a complex structure that requires proper interactions between several different proteins. To determine if these mutations affect different genes, cells were coinfected with two different mutant phage under nonpermissive conditions as shown in the figure below.



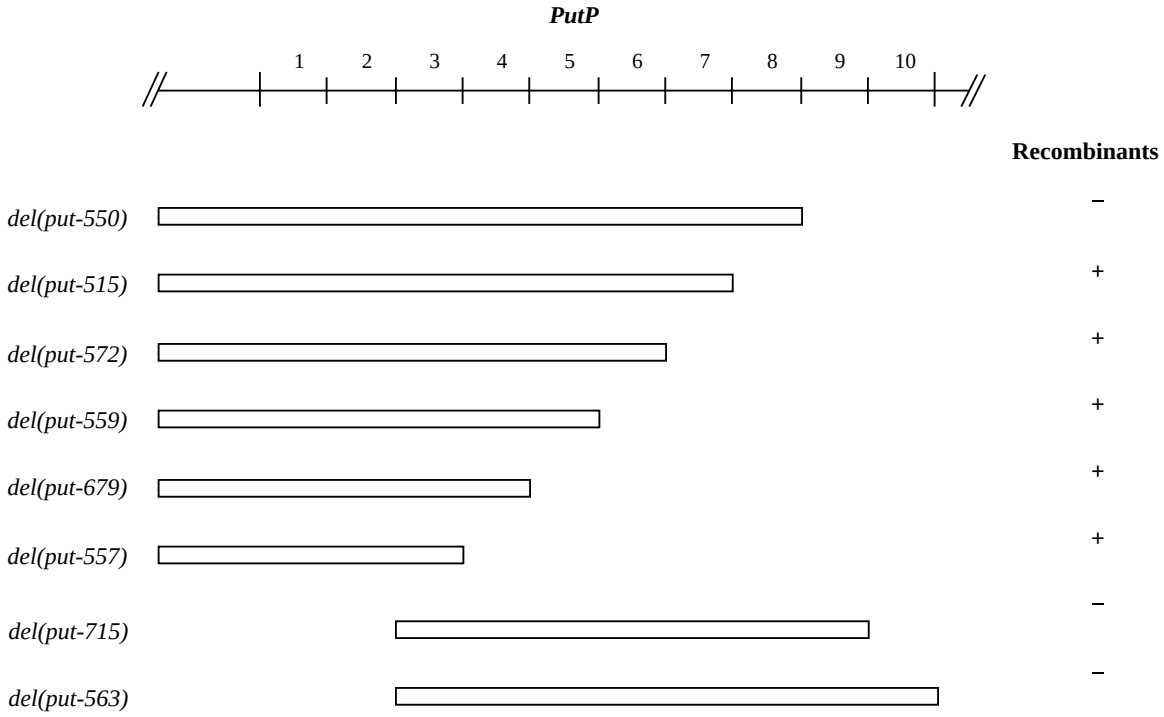
a. Do the mutations in FO-1 and FO-3 map in different genes? [Briefly explain your answer.]

The phenotype indicates that the two mutations complement each other -- thus, unless this is a rare example of intragenic complementation, then the two mutations affect different genes.

b. Why is the amount of lysis much less in the spot containing FO-1 and FO-2 compared to the spot containing FO-1 and FO-3?

As described above FO-1 and FO-3 complement each other, so every cell coinfected with both phage will be lysed. In contrast, the results indcate that FO-1 and FO-2 cannot complement each other, so the rare plaques observed are probably due to recombination between the two phage to produce wild-type phage. This requires both that the cell is coinfected with FO-1 and FO-2 and that a subsequent cross-over occurs in the region between the two mutations, and hence it is much rarer than simple complementation.

Q2. (6) A new *putP* mutation was mapped against a set of *putP* deletion mutations. The region removed by each deletion mutations is indicated by an open box below the *putP* map. The results indicating whether or not recombinants were obtained are shown to the right of each deletion.



Based on these results, which deletion interval does the new *putP* mutation map within? [Briefly explain your rationale. Note the deletion intervals are numbered on the genetic map.]

The mutation maps in deletion interval #8 because it cannot repair any deletion that removes this interval, but it can repair all the other deletions.

Q3. (10) Phage P22 HT was grown on the donor cells shown below. Using these phage lysates, two-factor crosses were done against *Salmonella typhimurium* strains with mutations in the *metA*, *aceA*, and *iclR* genes. Given the results shown in the table below, draw a genetic linkage map showing the cotransduction frequency and the predicted order of the *metA*, *aceA*, and *iclR* genes. [Show your calculations. Include arrows indicating the selected marker.]

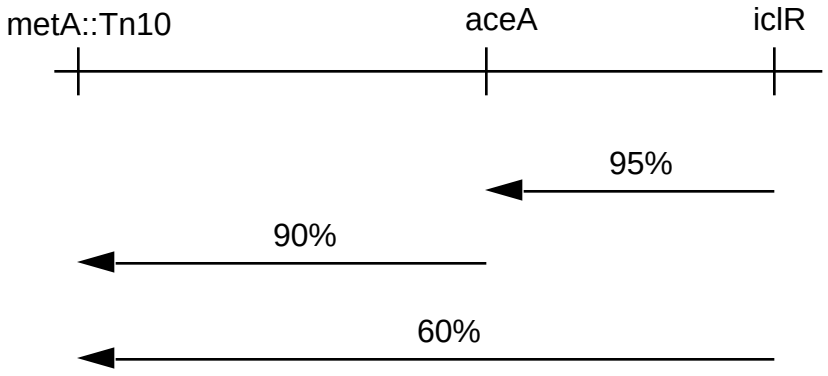
Donor	Recipient cells	Selected phenotype	Recombinants	Number obtained
metA::Tn*10* *iclR*$^-$	*metA*$^+$ *iclR*$^+$	Tet^R	IclR$^-$ IclR$^+$	60 40
aceA$^+$ *iclR*$^-$	*aceA*$^-$ *iclR*$^+$	Ace$^+$	IclR$^-$ IclR$^+$	95 5
metA::Tn*10* *aceA*$^+$	*metA*$^+$ *aceA*$^-$	Tet^R	Ace$^-$ Ace$^+$	10 90

Note that you need to determine the frequency of COINHERITANCE -- that is, the fraction of recombinants that inherited both DONOR alleles.

$$Tet^R\ iclR^-\ recombinants = \frac{60}{60 + 40} = 60\%$$

$$ace^+\ iclR^-\ recombinants = \frac{95}{95 + 5} = 95\%$$

$$Tet^R\ ace^+\ recombinants = \frac{90}{90 + 10} = 90\%$$

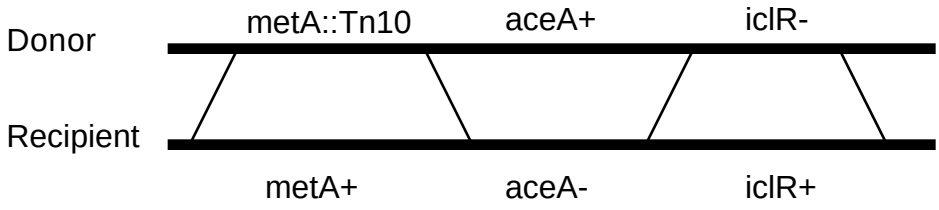


Q4. (8) To confirm the order of the *metA*, *aceA*, and *iclR* genes determined from the two-factor crosses, three-factor crosses were done. Given the results shown below, what is the order of these three genes? [Draw a diagram to show your rationale for this conclusion.]

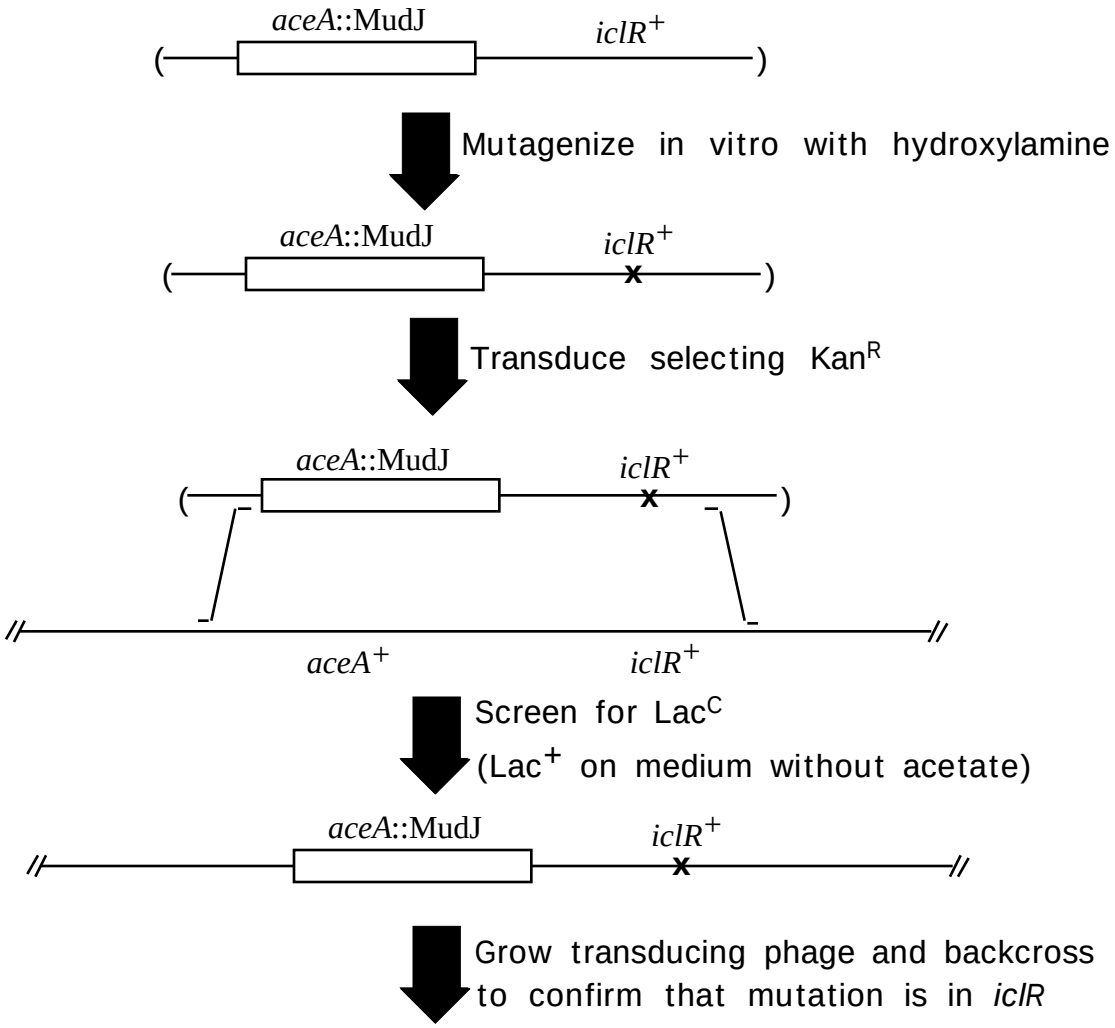
Donor	*metA*::Tn*10*	*iclR*$^-$	*ace*$^+$
Recipient	*met*$^+$	*iclR*$^+$	*ace*$^-$

Selected phenotype	Recombinants	Number obtained
Tet^R	$IclR^- Ace^+$	300
	$IclR^- Ace^-$	3
	$IclR^+ Ace^+$	30
	$IclR^+ Ace^-$	60

The rare class would require a double cross-over as shown below:



Q5. (12) The *iclR* gene encodes a repressor that turns off transcription of the *aceA* gene. Given *Salmonella typhimurium* strains with any of the mutations shown in Question #3 or Question #4, and a strain with an *aceA*::MudJ operon fusion that expresses LacZ under control of the *aceA* promoter, how would you do localized mutagenesis to obtain *iclR* mutants? [Draw a diagram showing how you would do the experiment, including the donor and recipient used and how you would identify the desired mutants. When looking for recombinants indicate whether the approach is a selection or a screen.]



❇ If the mutation is in iclR, about 90-95% of the transductants should be Lac^c and in the remaining transductants Lac should be only expressed in medium with acetate.

❇ If the mutation is in the MudJ itself then all of the transductants should be Lac^c.

Q6. (8) If a λ *cI* mutant infected a *hfl* mutant of *E. coli* would you expect the phage to grow lytically or to form a lysogen? [Explain your rationale.]

A λ *cI* mutant will grow lytically (because it lacks the repressor).

λ forms lysogens at a high frequency on a *hfl* mutant host (because cII accumulates at high levels).

The λ *cI* phenotype will be epistatic to the *hfl* phenotype (because accumulation of cII does not affect the lysis/lysogeny decision in the absence of cI) -- that is, the phage will grow lytically.

Q7. (12) Three new phages were isolated that can infect and lyse *Klebsiella aerogenes*. Phage PK1 and PK2 are temperate phage (forms turbid plaques) and phage PK3 is a lytic phage (forms clear plaques). During characterization of these phage, a clear-plaque mutant of phage PK1 (named PK1 *c1*) was also isolated.

K. aerogenes cells isolated from the center of the turbid plaques formed after infection with phage PK1 were designated *K. aerogenes* PK1. Similarly, *K. aerogenes* cells isolated from the center of the turbid plaques formed after infection with phage PK2 were designated *K. aerogenes* PK2. Growth of each of the phage on the wild-type *K. aerogenes* and the two *K. aerogenes* derivatives is shown in the following table. [Turbid indicates the phage forms turbid plaques, clear indicates the phage forms clear plaques, and none indicates the phage does not lyse the cells.]

Strain	**Lysis by phage:**			
	ØPK1	ØPK1 *c1*	ØPK2	ØPK3
K. aerogenes wild-type	turbid	clear	turbid	clear
K. aerogenes PK1	none	clear	none	clear
K. aerogenes PK2	none	none	none	clear

a. Suggest a simple explanation for the behavior of ØPK3 on each of these three strains.

ØPK3 is a lytic phage that is not homoimmune with ØPK1 or ØPK2 (that is, the repressor produced by ØPK1 or ØPK2 does not repress ØPK3)

b Suggest a simple explanation for the behavior of ØPK2 on each of these three strains.

Either the repressor produced by the ØPK1 lysogen can also repress any superinfecting ØPK2 (that is, the two phage are homoimmune) or the ØPK1 lysogen produces a superinfection exclusion system that prevents growth of ØPK2.
As expected, ØPK2 would be repressed by a ØPK2 lysogen

c. Suggest a simple explanation for the behavior of ØPK1 on each of these strains.

Either the repressor produced by the ØPK2 lysogen can also repress any superinfecting ØPK1 (that is, the two phage are homoimmune) or the ØPK2 lysogen produces a superinfection exclusion system that prevents growth of ØPK1.
As expected, ØPK1 would be repressed by a ØPK1 lysogen.

d. Suggest a plausable explanation for the behavior of ØPK1 *c1* on each of these strains.

Note that this mutant can plaque on a PK1 lysogen but not a PK2 lysogen, indicating that either there is a difference between phage entry, repression, or growth in the two lysogens. There are several possible reasons. For example: the PK2 lysogen may have a superinfection exclusion which prevents entry of the ØPK1 *c1* phage; the *c1* mutation may affect an operator site in such a way that the PK1 repressor cannot bind to the mutant operator but the PK1 repressor can still bind to the mutant operator; or the *c1* mutation may affect a PK1 protein such that it inactivates the PK1 repressor (a dominant-negative phenotype) but cannot inactivate the PK2 repressor.

Q8. (14) The *thr* operon is required for the biosynthesis of the amino acid threonine. λ specialized transducing phage were isolated which carry the *thr* operon. (The *thr* operon is not located near the normal attachment site for phage λ on the *E. coli* chromosome.) Two phage lysates, a LFT λd*thr* lysate and a HFT λd*thr* lysate, were used to transduce a *thr* mutant to Thr^+.

a. What mutation is needed in the strain used to look for λ insertions near the *thr* operon?

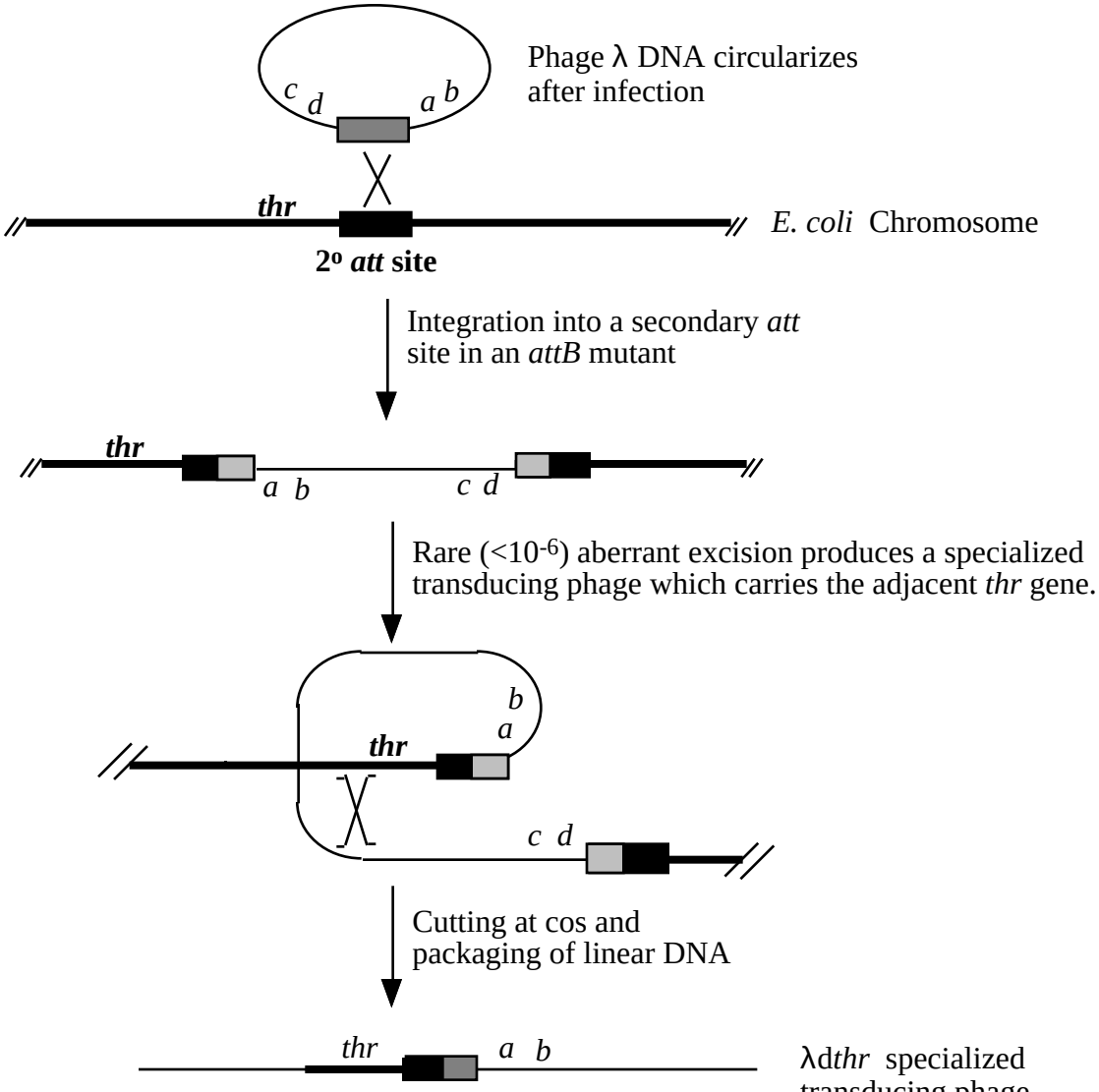
A bacterial host strain deleted for the λ attachment site (*attB*).

b. How would you select for the Thr^+ transductants?

Plate the infected cells on minimal medium without threonine.

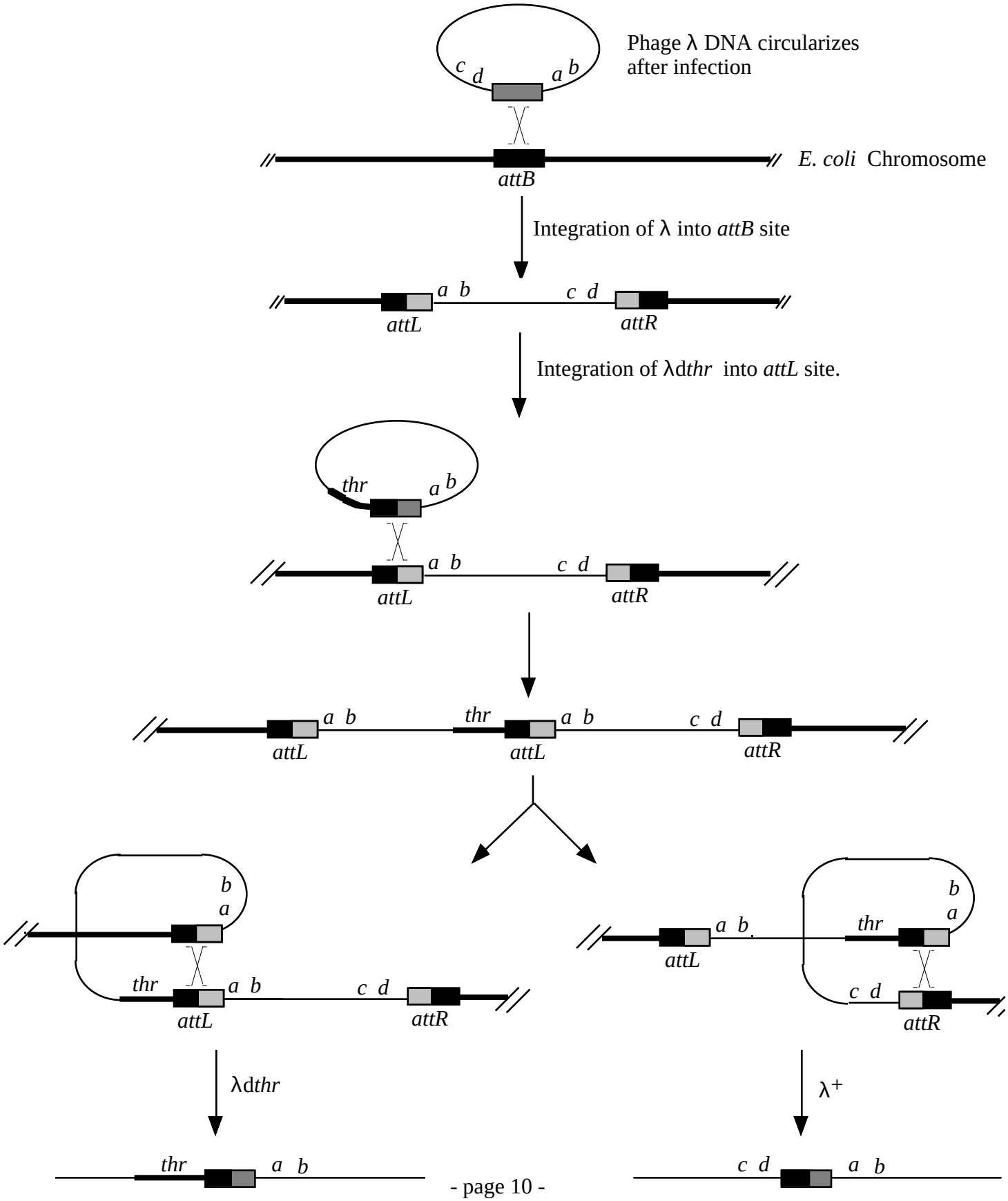
c. Given a λ insertions near the *thr* operon, how would you obtain LFT and HFT lysates? [Show a diagram of how the two types of lysates are formed.]

ISOLATION OF AN LFT LYSATE



Note that this is a low frequency transducing lysate because there is only one transducing phage produced per 10^6 normal phage.

ISOLATION OF AN HFT LYSATE



d. What is the relative frequency of Thr^+ transductants would you expect from each lysate and why?

The frequency of transduction would be proportional to the frequency of Thr^+ transducing particles in the lysate.

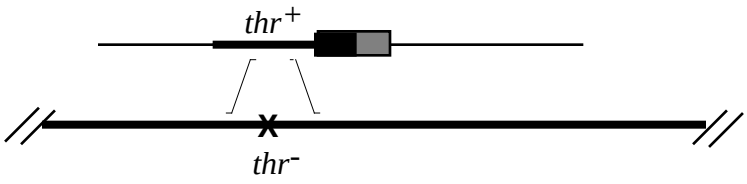
The Thr^+ transducing particles in the LFT lysate would probably be about 10^{-6} of the total phage in the population.

The Thr^+ transducing particles in the HFT lysate would be about 0.5 of the total phage in the population.

e. If the transduction was done at a high MOI, many of the resulting Thr^+ transductants would not be stable. Draw a diagram to show how the Thr^- segregrants would arise.

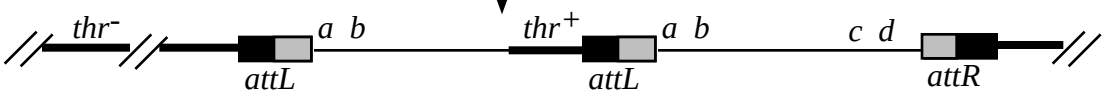
LOW MOI

At low MOI, Thr$^+$ colonies arise by homologous recombination between λ d*thr*$^+$ and chromosomal *thr*$^-$ -- the resulting colonies are stable because no duplication results.

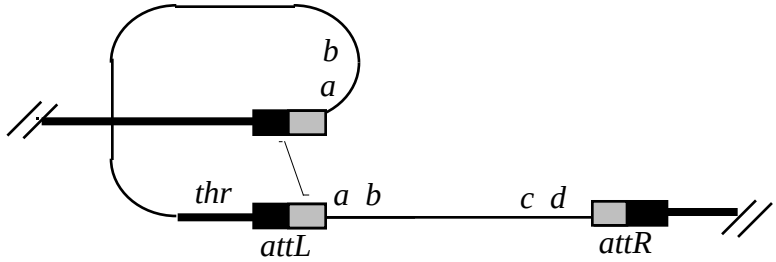


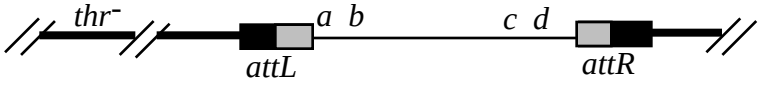
HIGH MOI

At high MOI, Thr$^+$ colonies may arise by complementation due to integration of λ d*thr*$^+$ and λ$^+$ into *attB* to form a dilysogen.

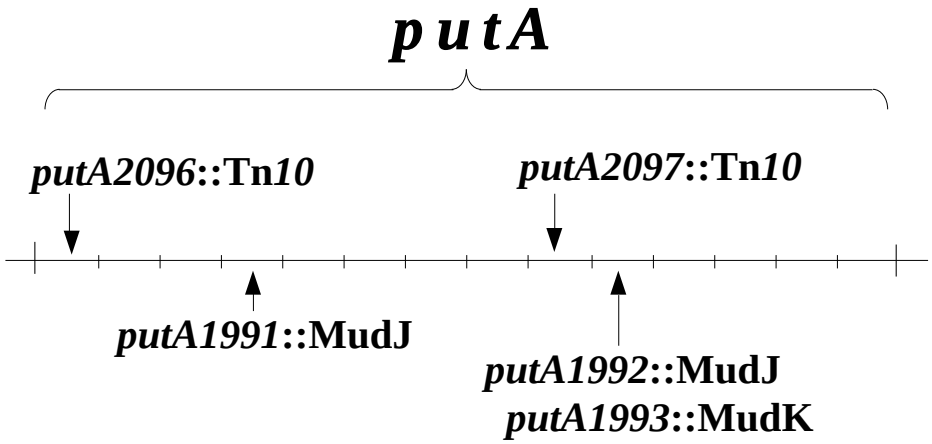


Recombination between the duplicated homologous DNA may result in segregration of *thr*$^+$ and thus loss of the Thr$^+$ phenotype.





Q9. (12) Many different mutations have been isolated in the *putA* gene of *Salmonella typhimurium*. A few of the mutations are shown on the following deletion map.



Based on the relative location of the mutants and the results shown below, fill in the blanks in the following table. [MudJ forms operon fusions and MudK forms gene fusions. The *putA1992*::MudJ and *putA1993*::MudK insertions map in the same deletion interval.]

Mutations	LacZ expression	Rationale
putA1991::MudJ	+	
putA1992::MudJ	+	
putA1993::MudK	+	
putA1991::MudJ *putA2096*::Tn*10*	–	The Tn*10* is upstream of the MudJ so it would be polar on LacZ expression
putA1992::MudJ *putA2096*::Tn10	–	The Tn*10* is upstream of the MudJ so it would be polar on LacZ expression
putA1993::MudK *putA2096*::Tn*10*	–	The Tn*10* is upstream of the MudK so it would be polar on LacZ expression
putA1991::MudJ *putA2097*::Tn*10*	+	The Tn*10* is downstream of the MudJ so it would not be polar on LacZ expression
putA1992::MudJ *putA2097*::Tn*10*	+	The Tn*10* is upstream of the MudJ so lack of polarity indicates that P_{out} is driving LacZ transcription (MudJ = operon fusions therefore *lacZ* has its own translational start sites)
putA1993::MudK *putA2097*::Tn*10*	–	The Tn*10* is upstream of the MudJ -- P_{out} would be expected to allow transcription of LacZ but it lacks necessary translational start sites (MudK = gene fusions)

Q10. (10) Using the transposon insertions shown in Question #9, how could you isolate a deletion mutation that removes the material between *putA2096*::Tn*10* and *putA2097*::Tn*10*? [Draw a diagram showing the genotype of the donor and recipient, the recombination events, the phenotype selected, and the genetic map of the final deletion mutant.]

Two simple ways of constructing the deletion are shown in the boxes below. It is necessary to indicate how you would select for any mutants used.