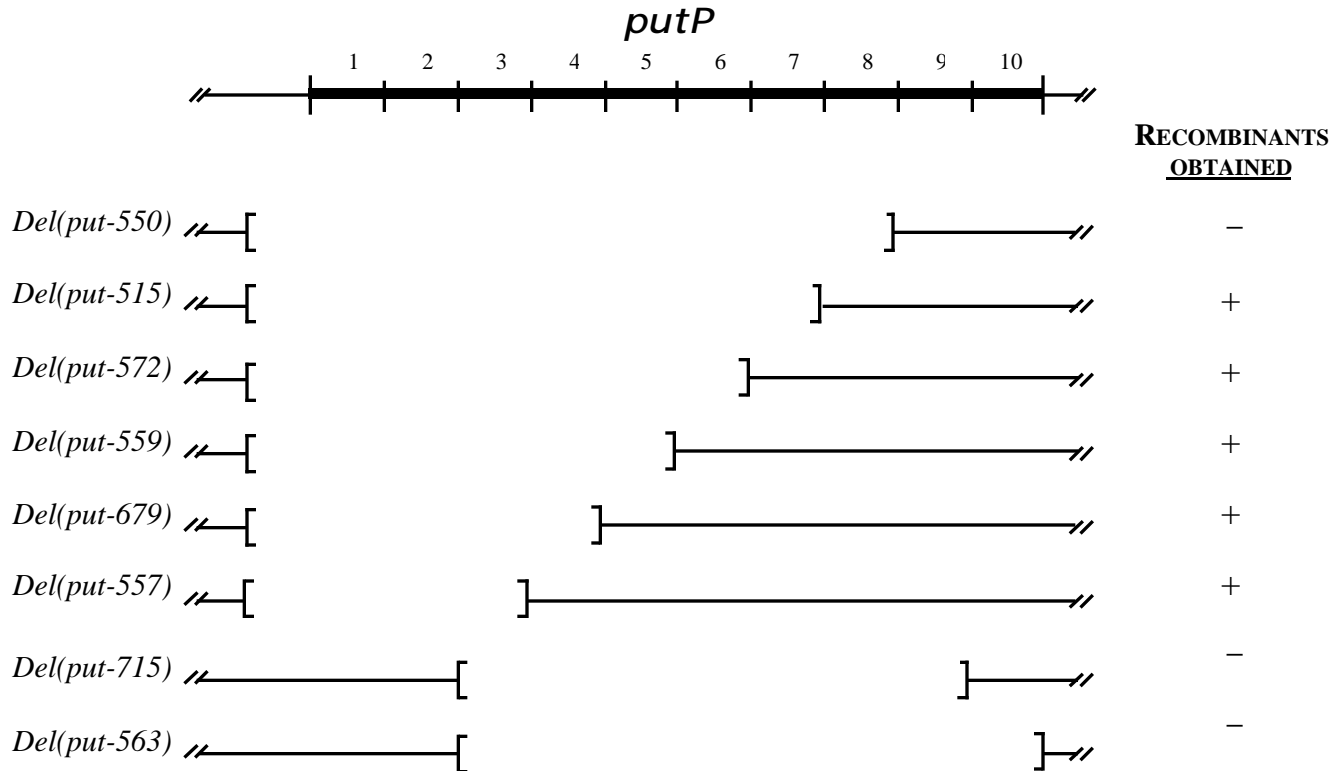


- (5) 1. A new *putP* mutation was mapped against a set of *putP* deletion mutations. The region removed by the deletion mutations are indicated by the area between the brackets below the *putP* gene. The results indicating whether or not recombinants were obtained are shown to the right of each deletion.



- a. Based on these results, where does the new *putP* mutation map?

ANSWER: The new *putP* mutation must map within deletion interval 8 because it cannot recombine with *del(put-550)*, *del(put-715)*, or *del(put-563)* which all remove region 8 but it can recombine with other deletions which remove different regions of the *putP* gene. If the mutation mapped within intervals 1-7 then *del(put-515)* would not have been able to repair it. If the mutation mapped in intervals 9-10 then *del(put-550)* would have been able to repair it.

- b. If you isolated a new *putP* mutation, how could you determine that it is a deletion mutation using strictly genetic approaches.

ANSWER: Do pairwise recombination tests with a collection point mutations or nonoverlapping deletion mutations. A mutation that fails to recombine with two other mutations that can recombine with each other, is likely to be a deletion mutation.

- (10) 2. Two-factor crosses were done to map the *nadF* mutation against the nearby markers, *lip* and *cam*. Given the results below, draw a genetic linkage map showing the most likely gene order and the coinheritance frequencies with arrows pointing toward the selected marker.

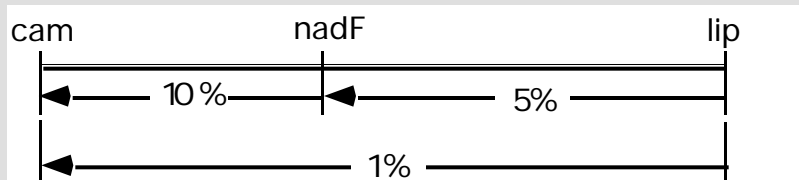
Donor	Recipient	Selected phenotype	Recombinants	Number obtained	Calculated cotransduction
<i>lip</i> ⁻ <i>nadF</i> ⁺	<i>lip</i> ⁺ <i>nadF</i> ⁻	NadF ⁺	Lip ⁻ Lip ⁺	25 475	(i)
<i>cam</i> ⁺ <i>nadF</i> ⁻	<i>cam</i> ⁻ <i>nadF</i> ⁺	Cam ⁺	NadF ⁻ NadF ⁺	40 360	(ii)
<i>lip</i> ⁺ <i>cam</i> ⁺	<i>lip</i> ⁻ <i>cam</i> ⁻	Cam ⁺	Lip ⁻ Lip ⁺	495 5	(iii)

ANSWER:

(i) $25 \div (25 + 475) = 25 \div 500 = 0.05 = 5\%$

(ii) $40 \div (40 + 360) = 40 \div 400 = 0.10 = 10\%$

(iii) $5 \div (5 + 495) = 5 \div 500 = 0.01 = 1\%$

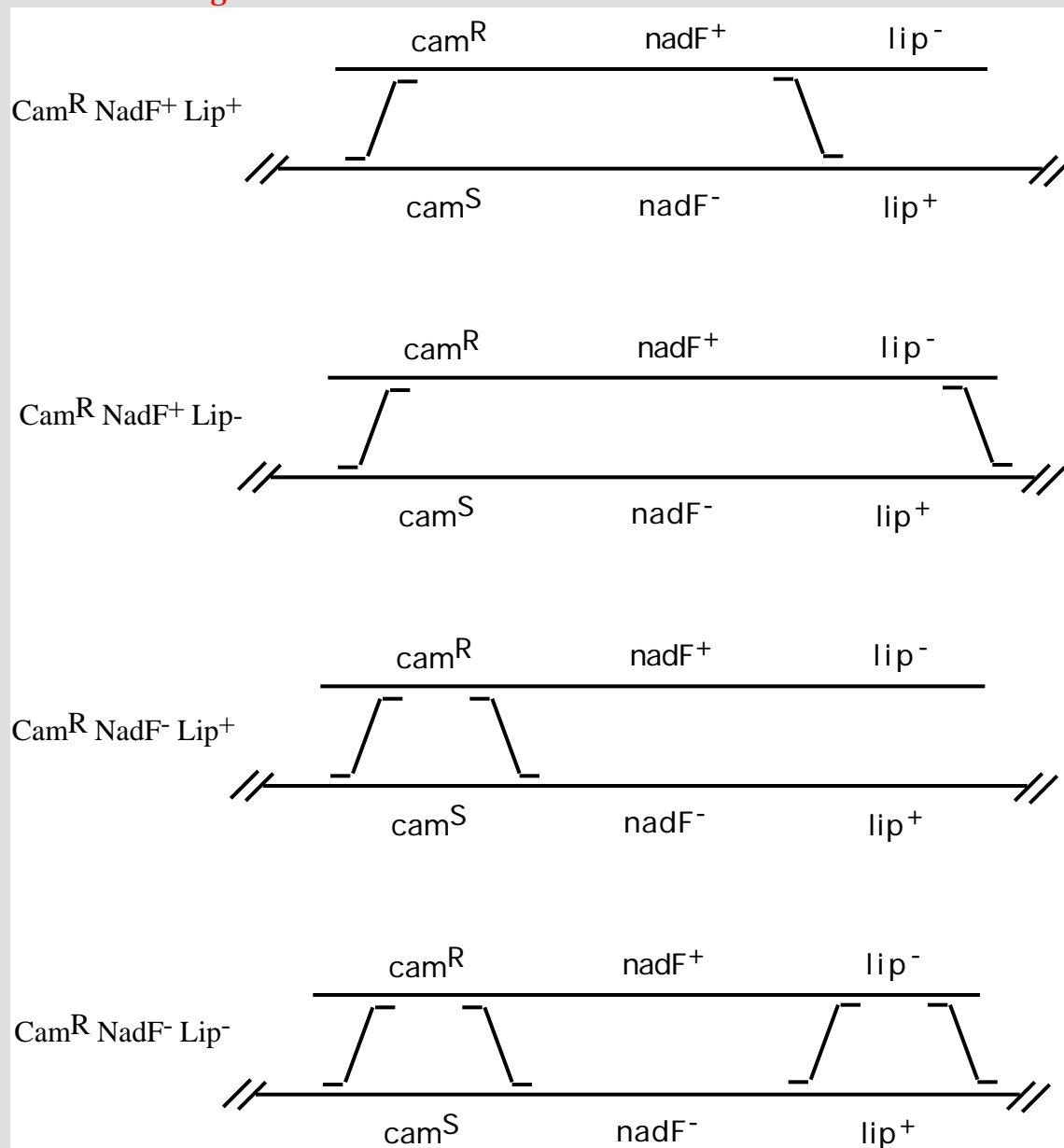


(10) 3. To confirm the order of these genes, three-factor crosses were done with a $nadF^+ lip^- cam^+$ donor and a $nadF^- lip^+ cam^-$ recipient.

a. Draw a diagram of the crossovers that yield each of the four classes of the observed recombinants.

Selected phenotype	Recombinants	Number obtained
Cam ⁺	NadF ⁺ Lip ⁺	960
	NadF ⁺ Lip ⁻	170
	NadF ⁻ Lip ⁺	8970
	NadF ⁻ Lip ⁻	0

Answer: See figure below.



- b. Given these results, what is the order of the three genes? [Briefly explain your rationale for this conclusion.]

ANSWER: The *nadF* gene is the middle marker because the rarest class of recombinants inherits the *nadF* mutation from the recipient, requiring 4 cross-over events.

- (5) 4. It is possible to eliminate the tetracycline resistant (Tet^R) plasmid pLAFR from a bacterial strain by mating in the gentamycin resistant (Gen^R) plasmid pPH1JI.

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

ANSWER: The two plasmids are probably incompatible. This means that only one of the plasmids can be stably maintained in the cell. Hence, selection for the second plasmid results in loss in the first plasmid. This process is called “curing” the strain of a plasmid. The second plasmid is called a “kick-out” plasmid.

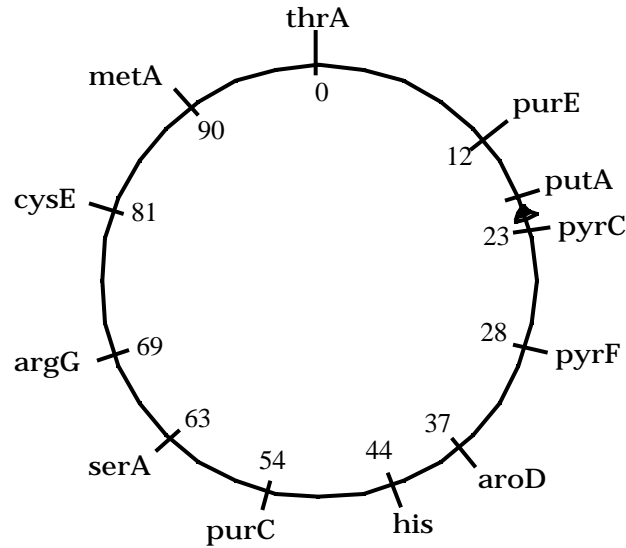
- b. Would this trick work if the only selectable marker on pPH1JI was Tet^R ? [Explain why or why not].

ANSWER: No. Because the recipient cell is already Tet^R , there is no selection for inheritance of the second plasmid. Hence, very few cells would probably be transformed and even if they were you would not be able to distinguish them from the untransformed cells.

- (5) 5. The F-plasmid can be stably maintained in both *Salmonella typhimurium* and *Escherichia coli*. However, Hfr's are formed much less frequently in *S. typhimurium* than in *E. coli*. In addition, there are many fewer insertion sites in *S. typhimurium* compared to *E. coli*. Given what you know about how Hfr's are formed, what is the most likely explanation for this difference between *S. typhimurium* and *E. coli*?

ANSWER: F integrates into the chromosome at specific IS sequences. The results suggest that there are fewer homologous IS sequences in *S. typhimurium* than in *E. coli*.

- (10) 6. A map of the *E. coli* chromosome is shown below. Given a Hfr insertion located between the *putA* gene and the *pyrC* gene as shown below, how could you isolate a F' that carries the *putA*⁺ gene? Describe any mutations in the donor or recipient needed for your experiment. [The *putA* gene product is required for growth on proline as a sole carbon source. The other genes shown are required for biosynthesis of essential metabolites.]



ANSWER: Two approaches are feasible. (i) To obtain an F' that carries the *putA*⁺ gene you could mate a Hfr *put*⁺ *metA* donor with a *put metA*⁺ *recA* recipient, selecting for Put⁺ Met⁺ transconjugants. [In this example, the counterselection was demanding methionine prototrophy, but you could also use a variety of other counterselections.] (ii) Because *putA* is a distal marker, you could do an interrupted mating and select for *putA*⁺.

(20) 7. Phage SF1 was tested for the ability to infect three strains of *E. coli*. As a control, phage grown on an *E. coli* K-12 r⁻m⁺ strain was also tested. The results are shown in the following table. ["clear" indicates that the phage produced clear plaques, "turbid" indicates that the phage produced turbid plaques, and "-" indicates that no plaques were formed].

Phage	Recipient bacteria				
	<i>E. coli</i> B		<i>E. coli</i> K-12		<i>E. coli</i> LR
	r ⁻ m ⁺	r ⁺ m ⁺	r ⁻ m ⁺	r ⁺ m ⁺	r ⁻ m ⁺
SF1	clear turbid	clear -	clear turbid	- turbid	clear -

a. What can you infer about the SF1 lifestyle based upon the type of plaques formed?

ANSWER: SF1 appears to be a lytic phage.

b. Suggest a likely explanation for the growth of each phage (SF1 and) on the *E. coli* B and *E. coli* K-12 strains. ["Growth" indicates ability to form plaques.]

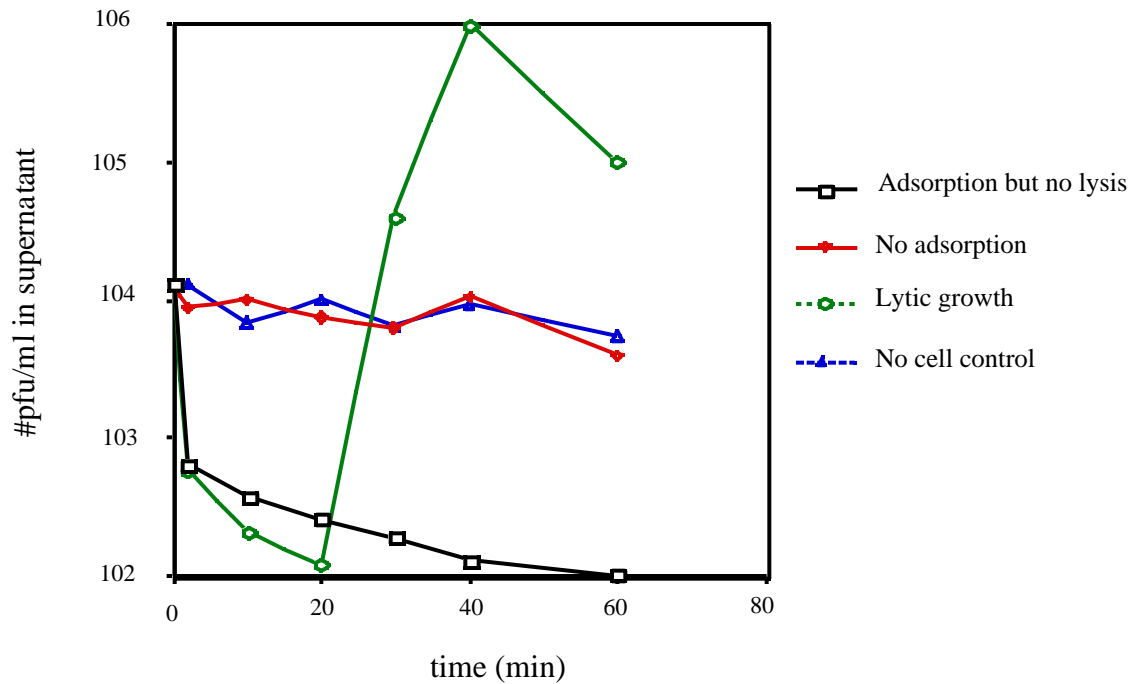
ANSWER: SF1 seems to have come from strain *E. coli* B because it is not restricted by *E. coli* B but it is restricted by *E. coli* K-12. seems to have come from strain *E. coli* K-12 because it is not restricted by *E. coli* K-12 but it is restricted by *E. coli* B.

c. Suggest two distinct, reasonable explanations why cannot grow on *E. coli* LR, but SF1 can grow on *E. coli* LR.

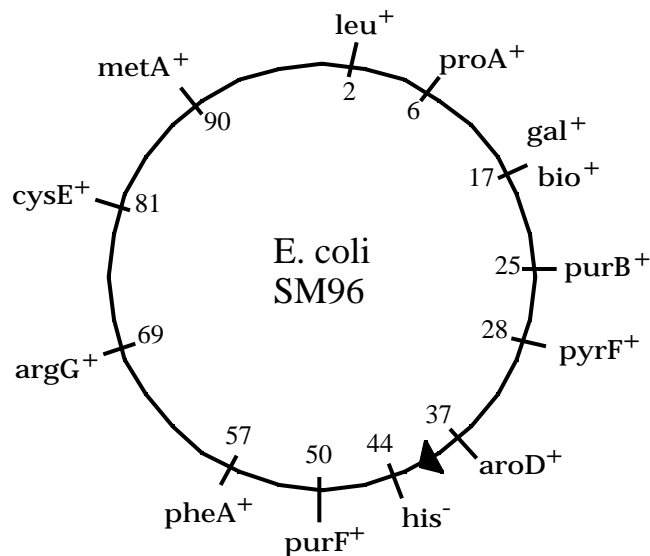
ANSWER: *E. coli* strain LR may either (i) lack a receptor for , or (ii) lack a gene product required for morphogenesis.

d. Draw the predicted results from a one-step growth curve for each of the two possibilities in the following figure. [Label the figure to indicate which curve corresponds to each explanation from 7c above.]

ANSWER: See the following curves. The green line represents the expected results for infection of wild-type cells. The blue line represents a control without added cells. The red line represents the expected results for cells lacking a phage receptor. The black lines represent the expected results for cells unable to support phage morphogenesis.



(15) 8. The *E. coli* strain SM96 is both an Hfr and a lysogen. The orientation of the Hfr is shown in the following figure. SM96 is a *his* auxotroph, but is otherwise prototrophic.



In eight separate experiments SM96 was mixed with different auxotrophic *E. coli* recipients, and the mixed cultures were plated on minimal medium without supplements. The following results were obtained:

Experiment #	Recipient mutation	# Exconjugants
1	<i>aroD</i>	+++
2	<i>pyrF</i>	+++
3	<i>purB</i>	+++
4	<i>bio</i>	++
5	<i>proA</i>	-
6	<i>leu</i>	-
7	<i>metA</i>	-
8	<i>cysE</i>	-

- a. What is the selection used in Experiment #1? [Be specific]

ANSWER: AroD+, ability to grow without aromatic amino acids, or prototrophy.

- b. What is the counterselection used in Experiment #1?

ANSWER: His+, ability to grow without histidine, or prototrophy.

- c. Suggest a likely explanation for the number of exconjugants observed from each mating experiment.

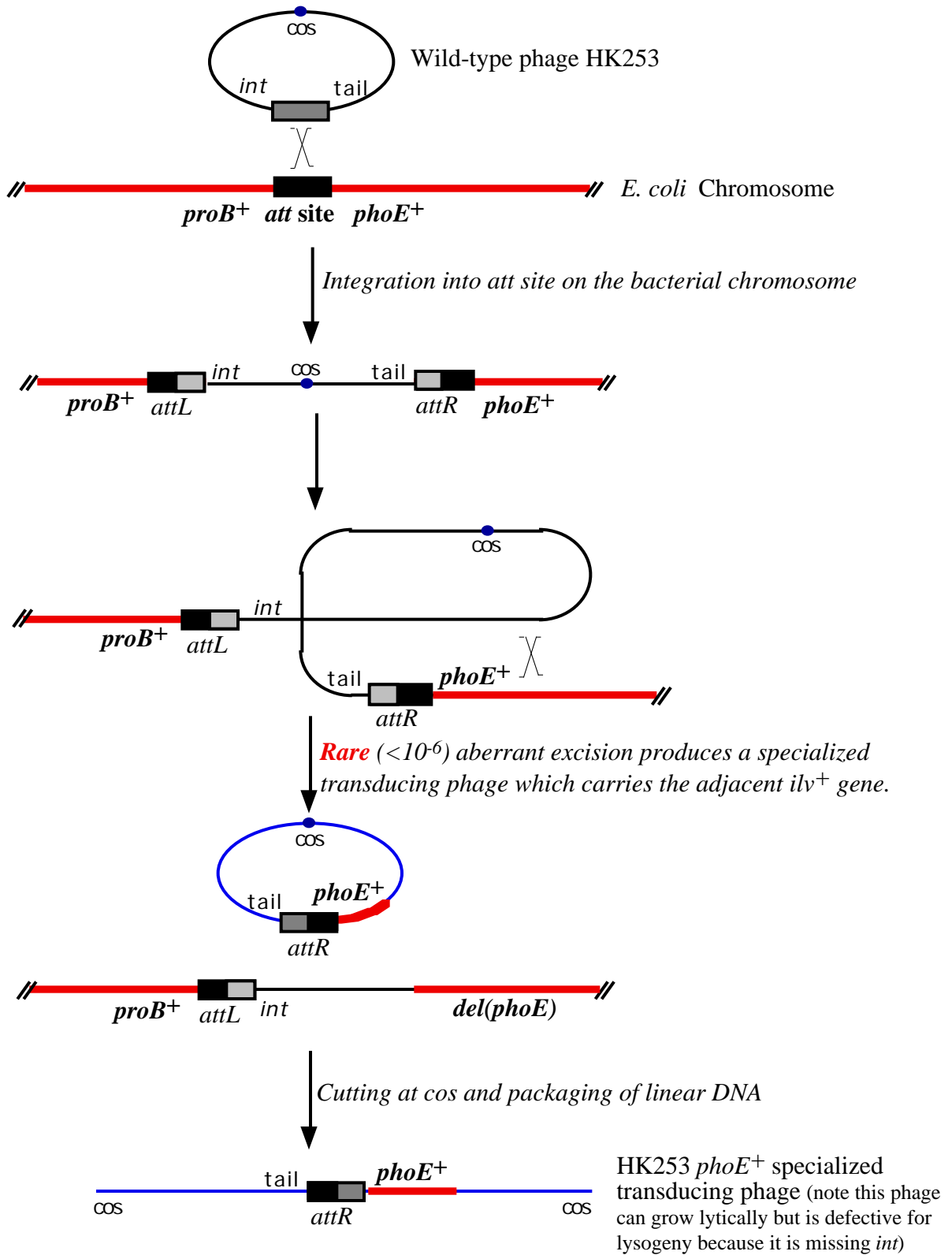
ANSWER: The donor has a lambda prophage inserted into the attachment site between the *gal* and *bio* genes on the *E. coli* chromosome. The donor remains a stable lysogen because cI protein made by the phage turns off the lytic genes. However, when the lambda prophage enters a non-lysogenic recipient cell there is no cI protein present, so the phage will be induced, resulting in lysis of the recipient cells. Hence, no recombinants will be observed for any of the markers located distal to the lambda prophage.

- (20) 9. Phage HK253 integrates into the *E. coli* chromosome between the *proB* and *phoE* genes. HK253 specialized transducing phage were isolated which carry the *phoE* gene.

- a. Draw a diagram showing how the LFT lysate could be formed following infection of *E. coli* with HK253. [Indicate the expected frequencies for any rare events.]

ANSWER: See figure below.

LFT lysate

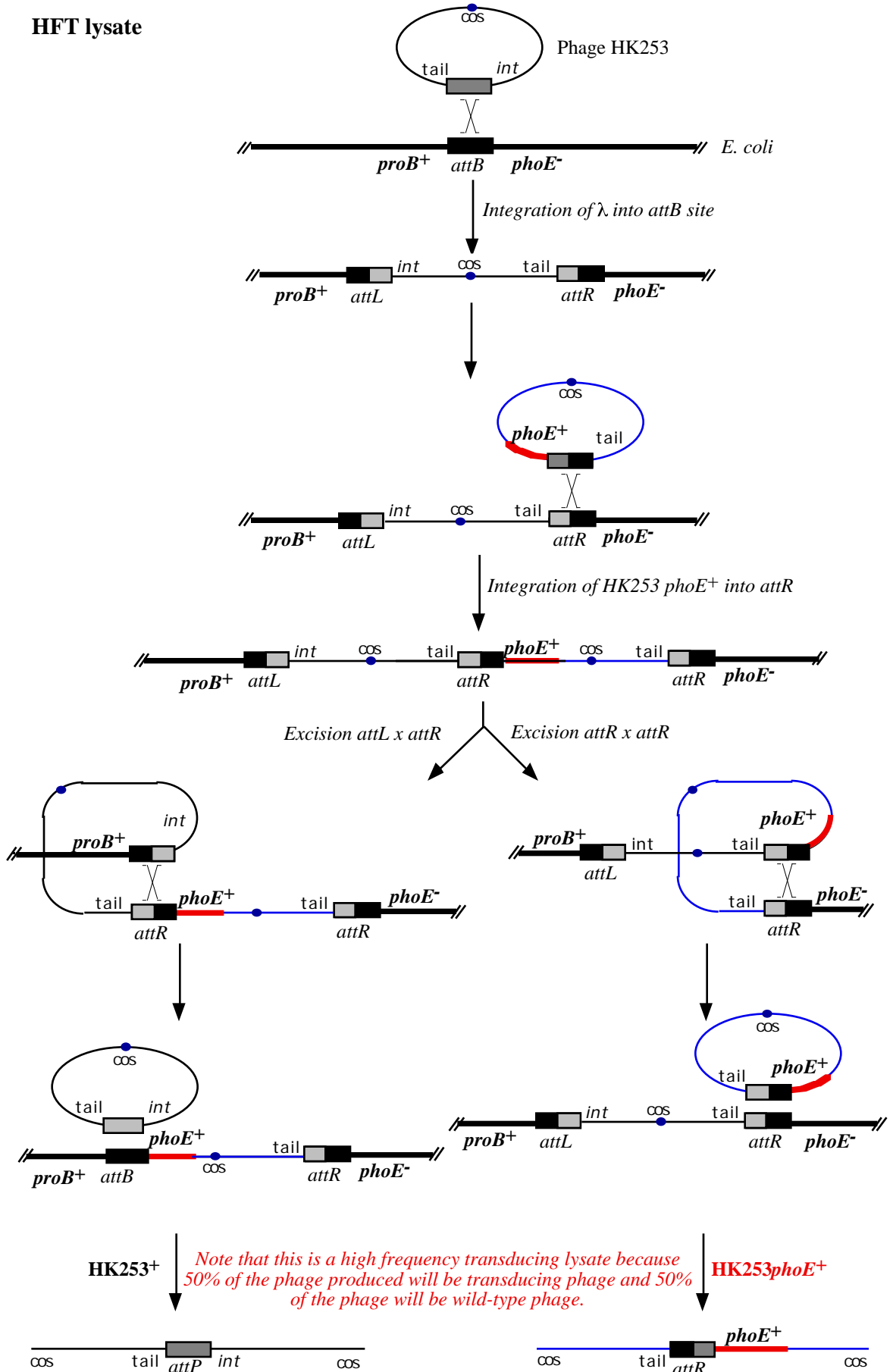


Note that this is a low frequency transducing lysate because there is only one transducing phage produced per 10^6 normal phage.

- b. Given a HK253 lysogen, how would you obtain a *phoE* HFT lysate? [Show a diagram of how the lysate would be formed.]

ANSWER: See figure below.

HFT lysate



- c. What is the relative frequency of PhoE⁺ transductants would you expect from the LFT and HFT lysates?[Briefly explain your answer.]

ANSWER: About 1/10⁶ phage in the LFT carries the *phoE*⁺ gene, while about 1/2 phage in the HFT carries the *phoE*⁺ gene.

- d. If the transduction was done at a high MOI, many of the resulting PhoE⁺ transductants would not be stable. Draw a diagram that shows how the PhoE⁺ phenotype may be lost from a bacterium without killing the host.

ANSWER: See figure below.

