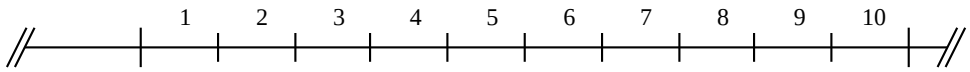
1. (12) A *putP* mutation was mapped against a set of *putP* deletion mutations. The region removed by the deletion mutations are indicated by open boxes below the *putP* gene and the results showing whether or not recombinants were obtained are shown to the right of each deletion. *Note that the first part of this question comes directly from the questions at the end of chapter 1 in the textbook and the second part comes directly from a homework question.*



	Recombinants
del(put-550)	–
del(put-515)	+
del(put-572)	+
del(put-559)	+
del(put-679)	+
del(put-557)	+
del(put-715)	–
del(put-563)	–

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.] **Deletion 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. **Deletion mutation because cannot revert (could also be a double mutant). Removes at least part of deletion interval #3**

c. Propose a genetic recombination experiment to test your idea. [Indicate the donor(s) and recipient(s) and how you would select for recombinants.] **Using potential deletion as a recipient, test recombination with donors which have point mutations in different intervals of the *putP* gene, selecting for $PutP^+$ phenotype.**

d. Some deletion intervals contain many point mutations but some deletion intervals only contain a single point mutation. List three reasons why point mutations may be much rarer in some deletion intervals than in others. **(1) Hotspots; (2) different amounts of DNA may be removed in different deletion intervals; (3) changes in amino acids at certain positions of the protein may have more drastic effects than in other regions of the protein.**

2. (9) *E. coli* K and B have different restriction and modification specificities, indicated by the subscript K or B respectively. A variety of mutations were isolated in the two restriction systems with the phenotypes: r^-m^+ or r^-m^-. The ability of λ lysates obtained from the indicated *E. coli* strains to grow on each of the indicated host strains and a merodiploid strain carrying the genes for both restriction systems is shown in the table below. [+ = lysis; - = no lysis] *Note that this question was a one-minute write from class and was posted on cyberprof.*

Phage	*E. coli* strain				
	$r_B^+m_B^+$	$r_K^+m_K^+$	$r_B^-m_B^+$	$r_K^-m_K^+$	$r_B^+m_B^+/r_K^-m_K^+$
λ ($r_B^+m_B^+$)	+	-	+	+	+
λ ($r_K^+m_K^+$)	-	+	+	+	-
λ ($r_B^-m_B^+$)	+	-	+	+	+
λ ($r_K^-m_K^+$)	-	+	+	+	-
λ ($r_B^+m_B^+/r_K^-m_K^+$)	+	+	+	+	+

a. Explain the results for the growth of each phage on each of the 5 strains.

- **$r_B^+m_B^+$ = only phage <u>modified</u> by m_B^+ can grow [λ ($r_B^+m_B^+$), λ ($r_B^-m_B^+$), λ ($r_B^+m_B^+/r_K^-m_K^+$)]**
- **$r_K^+m_K^+$ = only phage <u>modified</u> by m_K^+ can grow [λ ($r_K^+m_K^+$), λ ($r_K^-m_K^+$), λ ($r_B^+m_B^+/r_K^-m_K^+$)]**
- **$r_B^-m_B^+$ = no functional restriction for B or K so all the phage will grow**
- **$r_K^-m_K^+$ = no functional restriction for B or K so all the phage will grow**
- **$r_B^+m_B^+$ / $r_K^-m_K^+$ = <u>will</u> restrict growth of any phage not modified by m_B^+ [λ ($r_K^+m_K^+$), λ ($r_B^-m_B^+$)] ; <u>will not</u> restrict growth of phage previously modified by m_B^+ λ ($r_B^+m_B^+$), λ ($r_B^-m_B^+$), λ ($r_B^+m_B^+/r_K^-m_K^+$)]**

b. Why were no r^+m^- mutants isolated? **Such mutants would probably be lethal because the host chromosome would be degraded.**

3. (4) A lysogen of the double temperature sensitive mutant λ *cI*(Ts) *int*(Ts) was isolated at 30°C. What would happen if the lysogen was shifted to 42°C? [Explain your answer.] *Note that this question is very similar to a question to ponder I posted on cyberprof.*

- **At 42°C both cI and Int proteins are inactive.**
- **Inactivity of cI prevents repression resulting in induction of the lytic cycle.**
- **Inactivity of Int prevents excision, therefore the phage will be "locked in" and, although the host cell will ultimately die, the phage will be unable to produce progeny phage particles that can be packaged. (Remember Int is required for both integration and excision).**

4. (5) What types of suppressor(s) would you expect to obtain that would allow growth of a deletion mutation that removes the λ N gene? [Be specific. Your answer should explain the role of the N gene product and how the suppressor(s) would overcome the N^- phenotype.] *Note that this question is very similar to a question to ponder I posted on cyberprof.* **Suppression could result from rare double mutants that disrupt both the t_{R1} and t_{L1} terminators, eliminating the need for N-mediated antitermination of late gene functions.**

5. (8) Phages L5, 29, and TM4 can infect and lyse *Mycobacterium smegmatis*. Gene product 71 ("gp71") from phage L5 encodes a repressor required for maintence of lysogeny. *M. smegmatis* strains that contain a L5 lysogen were infected with two different phages as shown in the table below. Answer the following questions based upon what you know about the lysis/lysogeny decision in phage λ. *Note that this question is very similar to a supplemental question from the homework.*

Lysogen	**Lysis by phage**	
	Ø29	ØTM4
None	clear plaque	clear plaque
L5 *71*$^+$	no plaque	clear plaque
L5 *71*(Ts) at 30°C	no plaque	clear plaque
L5 *71*(Ts) at 42°C	clear plaque	clear plaque

a. Give a simple explanation for the type of plaque formed by Ø29 on each of these strains. What is this type of phage called? **Ø29 lacks its own repressor protein (thus the clear plaques on a nonlysogen) but can be repressed by the L5 71 repressor protein (thus no plaques on strains expressing functional 71 protein). Such a phage is said to be "homoimmune".**

b. Give a simple explanation for the type of plaque formed by ØTM4 on each of these strains. What is this type of phage called? **ØTM4 probably lacks regulatory sites required for repression thus it always forms clear plaques. Such a phage is said to be "virulent". (Alternatively it could be heteroimmune but lacks its own repressor protein.)**

6. (8) A. J. Clark recently isolated a phage from Hong Kong sewage that grows on *E. coli.* It acts as a generalized transducing phage on some strains of *E. coli* but it acts as a specialized transducing phage on other strains of *E. coli*.

a. How could you distinguish generalized transduction from specialized transduction using simple genetic tests? [Indicate any donor or recipient strains you would use and how you would do the experiment.] *Note that this question is very similar to a question asked on the homework.*

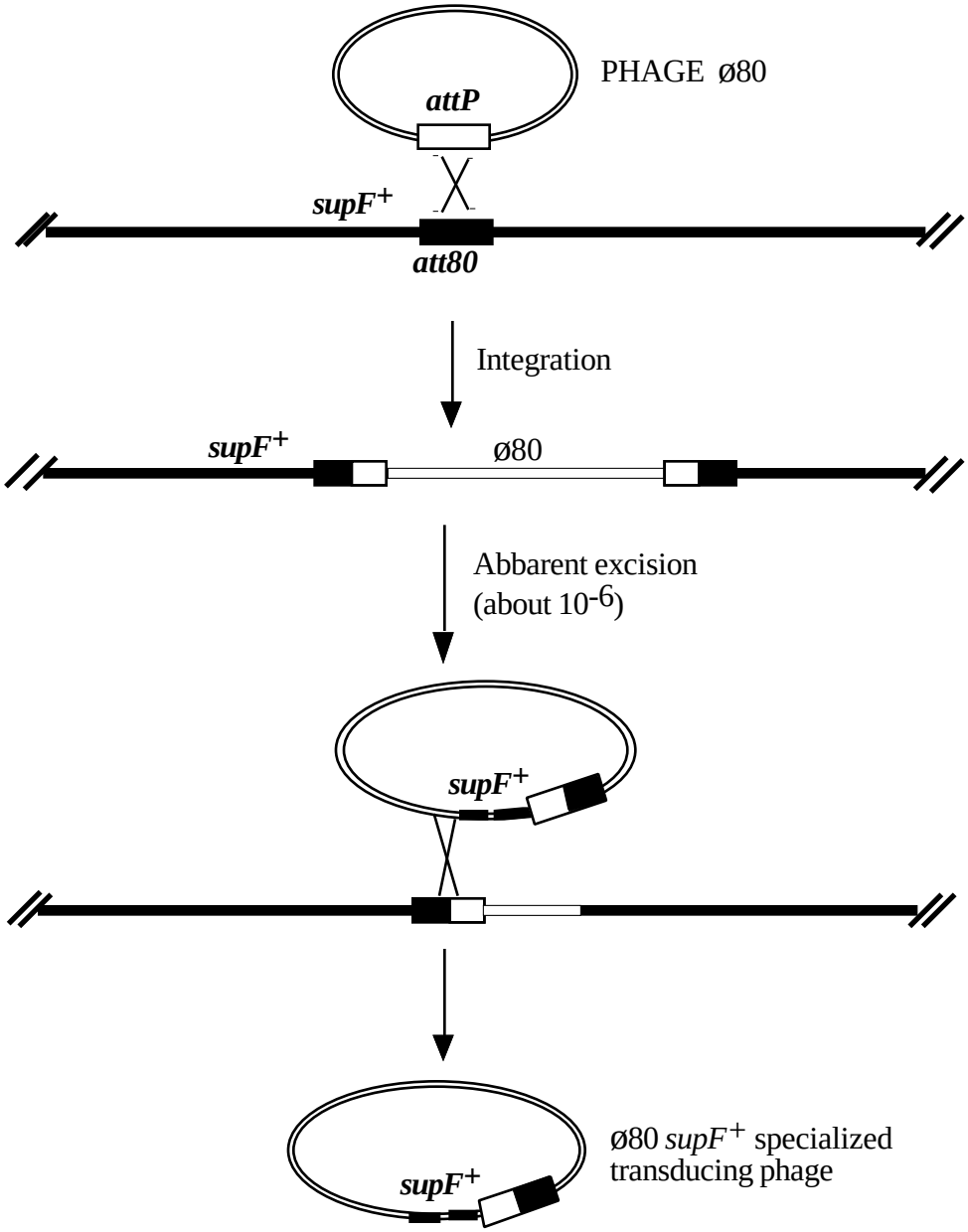
- **Generalized transduction - many different chromosomal markers will be transduced so you can try transducing several different auxotrophic recipients to prototrophy with the phage.**
- **Specialized transduction - only markers adjacent to the integrated phage will be transduced, sometimes the resulting transductants can be detected by cross-streaking after transduction.**

b. Suggest an explanation for these results.

The phage is probably unable to integrate into the chromosome of strains where it does generalized transduction. This may be due to (i) lack of a host protein that is required (e.g. IHF) or (ii) lack of an *att* site or other homology required for recombination (possibly a cryptic prophage).

7. (10) There is an attachment site for phage ø80 near the *supF* gene on the *E. coli* chromosome. The *supF* gene encodes an amber suppressor tRNA.

a. Draw a diagram showing how *supF*$^+$ specialized transducing particles could be formed following infection of a ø80^S *supF*$^+$ host. [Note the expected frequencies for any rare events.] *Note that the rationale behind this question is explained in the textbook.*

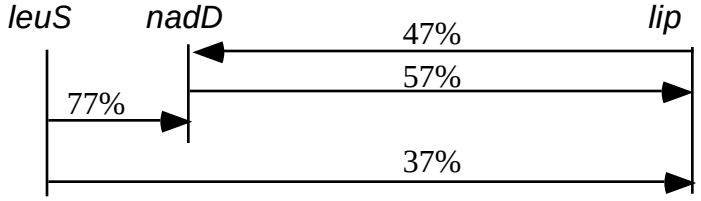


b. How would you test for *supF* specialized trans*ducing particles? [Indicate the recipient you would use and the phenotype you would test.] Note that this question asks how you would identify a sup specialized transducing phage -- that is, a phage able to suppress amber mutations.* **Transduce a strain with auxotrophic mutations caused by amber mutations -- if the phage simultaneously suppresses two different amber mutations, the phage probably carries an amber suppressor.**

8. (8) Hughes and Roth isolated a mutation (*nadD*) in a gene required for NAD biosynthesis in *Salmonella*. They did two-factor crosses with phage P22 to determine the linkage map of *nadD*relative to the *lip* and *leuS* genes. From the following two-factor cross data, draw a linkage map of the *nadD , lip and leuS* genes. [Indicate percent cotransduction and draw appropriate arrowheads to indicate crosses.] *Note that this question and the following question come directly from the questions at the end of chapter 1 in the textbook.*

Donor	Recipient	Selected phenotype	Recombinants	Number obtained
nadD$^+$ *lip*	*nadD lip*$^+$	NadD$^+$	*lip*	47
			lip$^+$	53
nadD lip$^+$	*nadD*$^+$ *lip*	Lip$^+$	*nadD*	57
			nadD$^+$	43
nadD$^+$ *leuS*	*nadD leuS*$^+$	NadD$^+$	*leuS*	77
			leuS$^+$	23
lip$^+$ *leuS*$^+$	*lip leuS*	Lip$^+$	*leuS*	63
			leuS$^+$	37

Note that the cotransduction frequency is the coinheritance of both donor alleles. The values obtained are shown in the figure below.



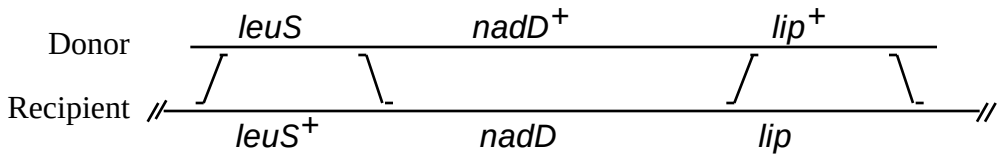
9. (8) Hughes and Roth also did three factor crosses to confirm the order of the *nadD* gene relative to the adjacent genes. Does this data agrees with the linkage map constructed from the two-factor crosses. [For each of the crosses in the table, show a drawing with the relevant crossovers and the inferred gene order.]

Donor strain	*nadD*$^+$	*lip*$^+$	*leuS*
Recipient strain	*nadD*	*lip*	*leuS*$^+$

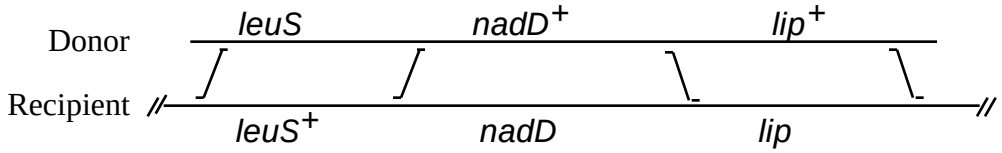
Selected phenotype	Recombinants		Number obtained
Lip$^+$	*nadD*$^+$	*leuS*	100
	nadD$^+$	*leuS*$^+$	47
	nadD	*leuS*	3
	nadD	*leuS*$^+$	150
NadD$^+$	*lip*$^+$	*leuS*	90
	lip$^+$	*leuS*$^+$	50
	lip	*leuS*	100
	lip	*leuS*$^+$	60

Note the rare classes in each cross. In the first cross there is clearly a rare class (suggesting that this class requires 4 X-overs), but in the second cross there is no class that is much rarer than the others (suggesting that they all require 2 X-overs). It is important to indicate an even number of X-overs because an odd number of X-overs would linearize the bacterial chromosome (remember that the donor is linear DNA and the recipient is circular DNA) resulting in cell death.

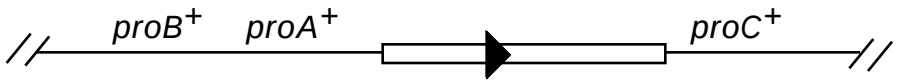
Selection for Lip$^+$ -- rare class is *nadD leuS*



Selection for NadD$^+$ -- no rare class observed



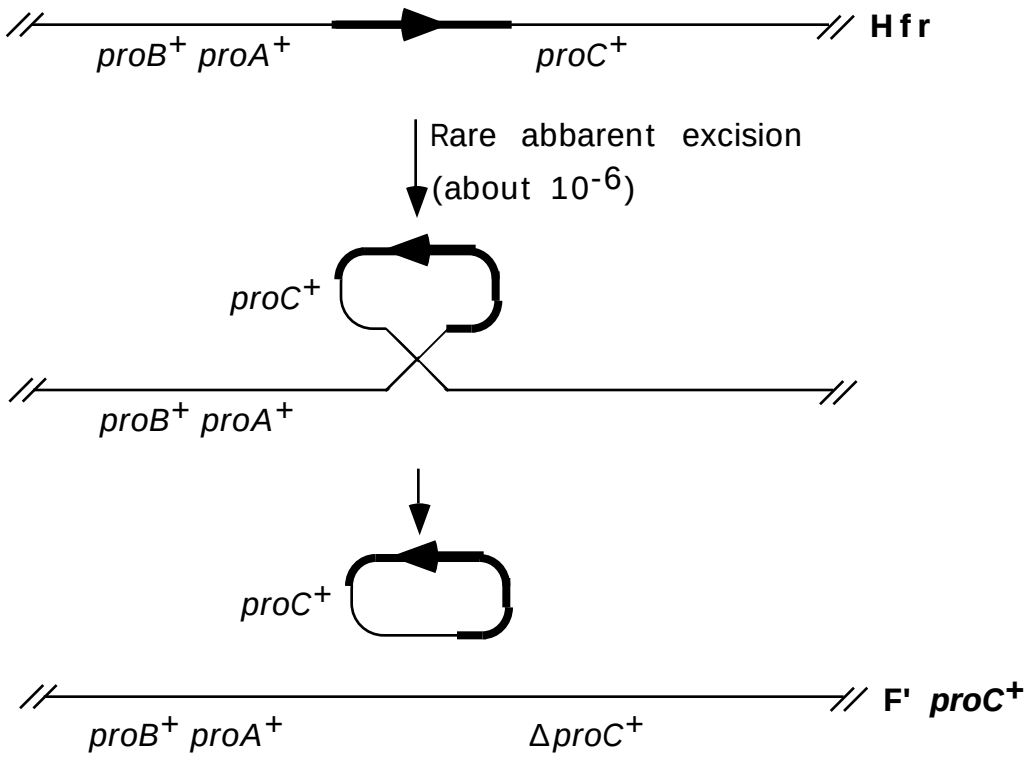
10. (10) The *proA, proB,* and *proC* genes are required for the biosynthesis of proline. You have a Str^S donor strain with a Hfr integrated between the *proA*$^+$*proB*$^+$ and *proC*$^+$ genes as shown below.



a. Given a *proB recA* Str^R recipient, how could you isolate a F' *proB*$^+$? [What medium would you use? How would you do the experiment? Explain the rationale for the isolation scheme you would use.] *Note that this question is very similar to a homework question.* **Mate with indicated recipient selecting on minimal medium without proline and with streptomycin. Because recipient is *recA*, cannot get Pro$^+$ by recombination with donor Hfr thus Pro$^+$ exconjugants are due to complementation with F' *pro*$^+$.**

b. Given the same donor strain and a *proC recA*$^+$ Str^R recipient strain, how could you isolate an F' *proC*$^+$? [What medium would you use? How would you do the experiment? Explain the rationale for the isolation scheme you would use.] **Mate with indicated recipient selecting on minimal medium without proline and with streptomycin. Recipient is *recA*$^+$ so you could get Pro$^+$ by recombination with donor Hfr, however, because *proC* is a late marker by interrupting mating you can ensure that Pro$^+$ exconjugants are due to complementation with F' *pro*$^+$.**

c. Draw a diagram showing how the F' proC$^+$ would form from this Hfr.

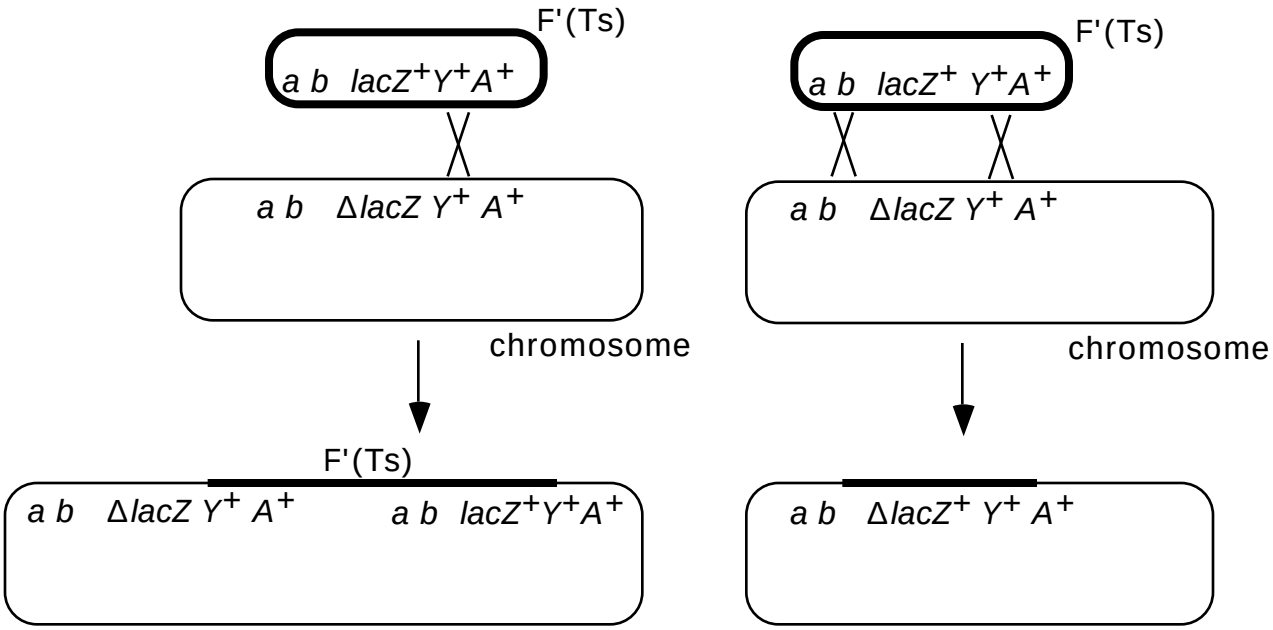


11. (10) A F'(Ts)*lacZ*$^+$*Y*$^+$*A*$^+$ plasmid has a temperature-sensitive mutation in its replication system. This plasmid was mated from a Rif^S donor into a F$^-$ Δ*lac Z* Rif^R recipient . (Rif^R indicates resistance to the antibiotic rifampicin due to a mutation in a gene encoding RNA polymerase.)

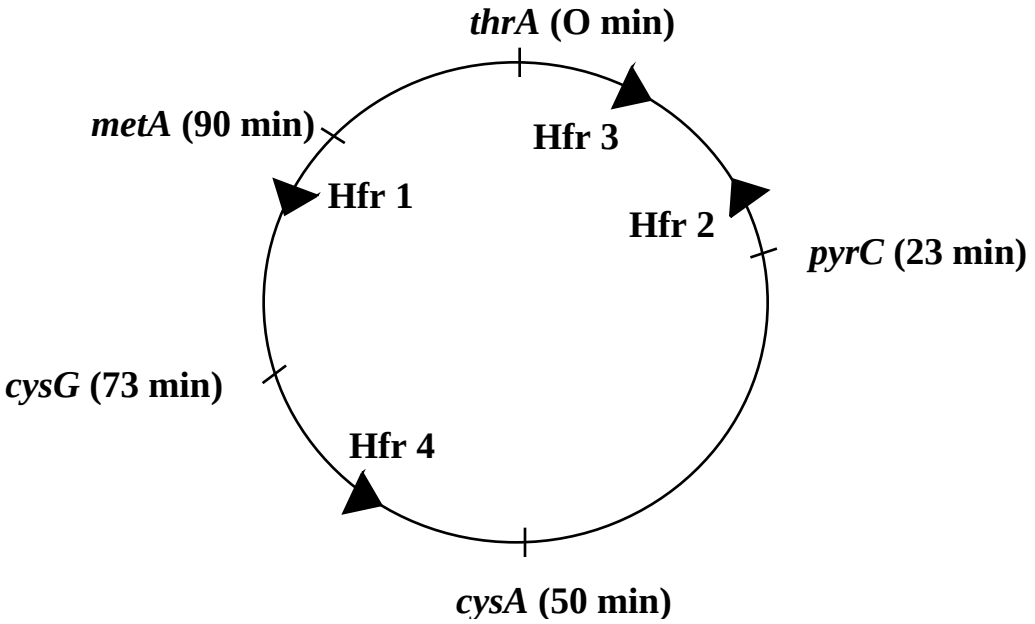
a. What medium and growth conditions would you use to isolate the exconjugants? **Minimal medium with lactose as a carbon source and rifampicin to counterselect against the donors. Mating must be done at 30°C.**

b. One of the exconjugant colonies was restreaked on MacConkey Lactose medium and incubated overnight 42°C. Describe the expected phenotype of the resulting colonies. [Explain your answer.] **The F' will segregrate rapidly at 42°C resulting in white colonies (Lac$^-$) at 42°C.**

c. A 0.1 ml aliquot of an overnight broth culture of the exconjugants was plated on minimal medium with lactose as a sole carbon source, then incubated overnight at 42°C. Rare Lac$^+$ colonies grew on these plates. Draw diagrams showing t<u>wo</u> likely explanations for this result.



12. (8) A new mutant was isolated that is Str^R and unable to use acetate as a carbon source (*ace*). To determine where the mutation maps, it was mated with the four different Str^S ace^+ Hfr donor strains shown below. [Arrowheads indicate the location and direction of transfer from each different Hfr.] *Note that this question comes from a "box" in the textbook.*



a. What is the selection for exconjugants in this experiment? **Growth on acetate as a sole C-source.**

b. What is the counterselection against the donor cells in this experiment? **Streptomycin resistance.**

c. Given the results in the following table, where does the *ace* mutation map? [Indicate the map position in minutes.] **About 95 min, in the region transferred early between Hfr#1 and Hfr#5.**

Donor strain	Ace^+ colonies
Hfr 1	1000
Hfr 2	5
Hfr 3	1000
Hfr 4	80