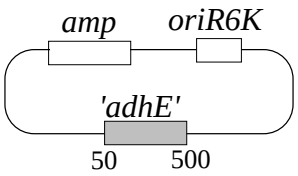
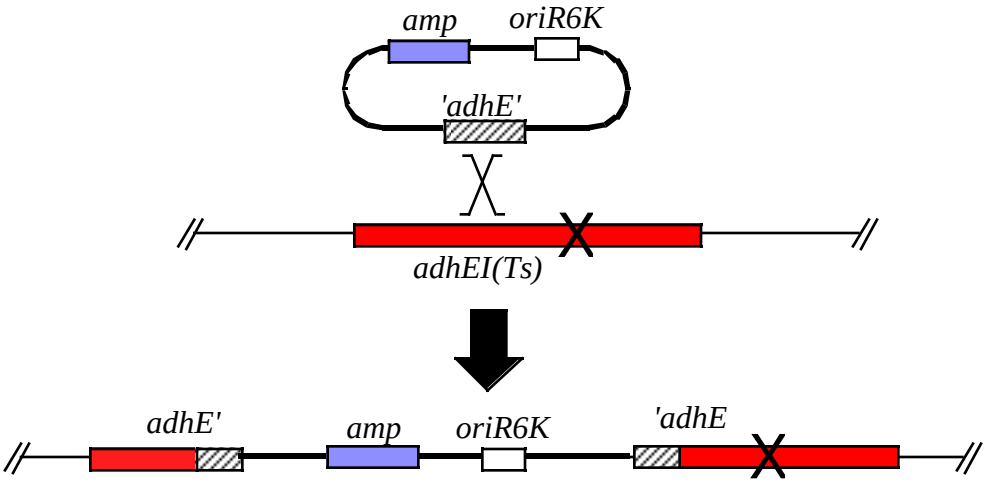
(20) **1.** The *adhE* gene is about 2000 bp. Nancy Maulen cloned a fragment corresponding to nucleotide 50 to 500 of the *Salmonella adhE* gene into the π-dependent plasmid pGP704. [pGP704 has no homology with the *S. typhimurium* chromosome.]



a. Why is this plasmid "dependent" upon the π protein?
ANSWER: The *pir* gene encodes a protein required for initiation of ori R6K replication.

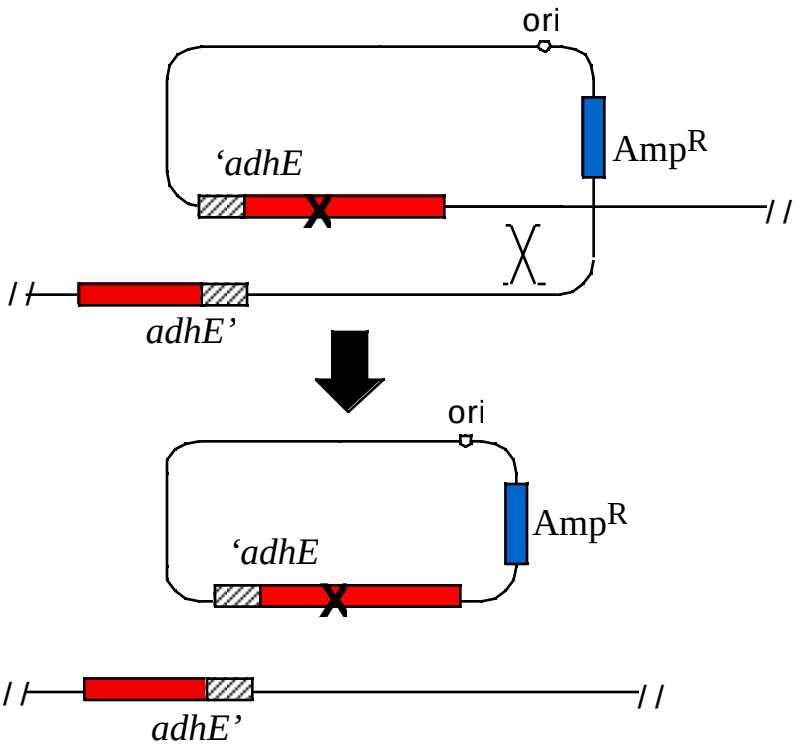
b. This plasmid was transformed into a *S. typhimurium adhE*(Ts) *pir*$^+$ strain with selection for Amp^R. What is the predicted phenotype of the resulting transformants? [Briefly explain your answer.]
ANSWER: The plasmid would replicate independently. Hence the phenotype would be Adh$^-$ at 42°C and Adh$^+$ at 30°C.

c. This plasmid was transformed into a *S. typhimurium adhE*(Ts) *pir*$^-$ strain with selection for Amp^R. What is the predicted phenotype of the resulting transformants? [Draw a diagram showing the predicted result.]
ANSWER: The plasmid would disrupt the chromosomal *adhE* gene resulting in the phenotype AdhE$^-$ Amp^R.



d. Draw a diagram showing how you could use a genetic approach to obtain a clone that carries the Adh(Ts) allele on the plasmid. [No restriction enzymes or PCR allowed.]
ANSWER: This would require rare illegitimate recombination (typically 10^{-6} frequency) as shown in the figure below. Recombination events between the *adhE* homology would simply regenerate the parental donor and recipients.

Because the product would be AdhE$^-$ due to truncation of one end of the gene, detection of the recombinant product that moved the Ts mutation onto the plasmid would requrire recombination with mutant alleles of the *adhE* gene.

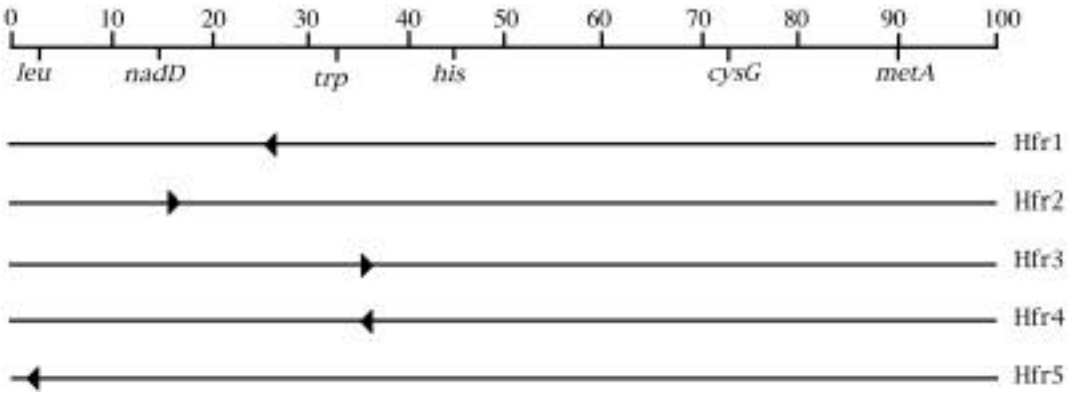


(8) **2.** It is possible to "cure" a strain of the plasmid pLAFR which encodes resistance to tetracycline (Tet^R) by mating in the plasmid pPH1JI which encodes resistance to gentamycin (Gen^R).

a. What does this suggest about the properties of these two plasmids?
ANSWER: The two plasmids are 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 approach work if the only selectable marker on pPH1JI was Tet^R? [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.

(12) **3.** A derivative of a *Salmonella* strain with a Pyr$^-$ mutation (which results in auxotrophy for uracil) was isolated. Other than this phenotype, the mutant was wild-type. To determine where the mutation mapped, it was mated with five different Hfr donor strains that are each auxotrophic for cysteine due to a *cysG* mutation. The map position and orientation of *oriT* in the Hfr donors is shown in the figure below.



a. How could you select against the recipient cells in this experiment?
ANSWER: Growth on minimal medium without uracil.

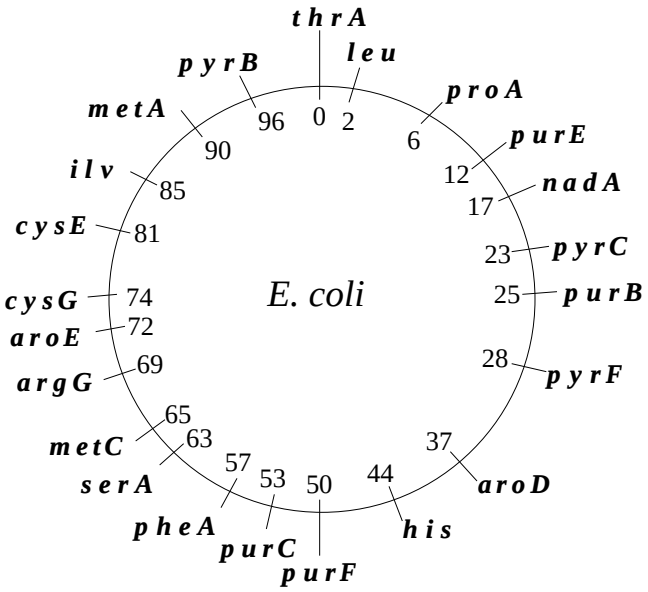
b. How could you counterselect against the donor cells in this experiment?
ANSWER: Growth on minimal medium without cysteine.

c. The results are shown in the following table. Based upon these results, what is the most likely map position of the *pyr* mutation? [Indicate the map position relative to the Hfr origins.]

Donor strain	Pyr^+ colonies
Hfr 1	490
Hfr 2	0
Hfr 3	320
Hfr 4	0
Hfr 5	50

ANSWER: The *pyr* mutation maps between oriT of Hfr 1 and oriT of Hfr3.

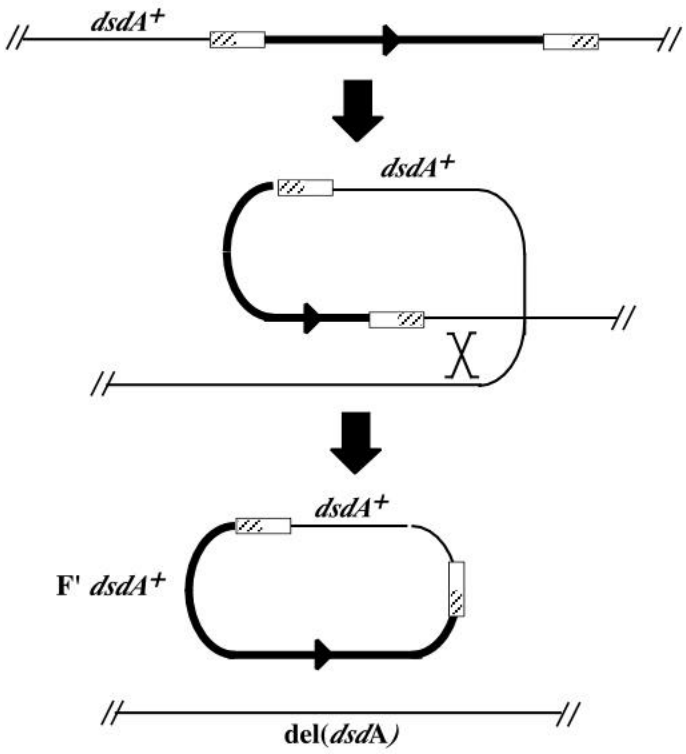
(10) **4.** The *E. coli* strain KL98 contains an F-plasmid integrated adjacent to the $dsdA^+$ gene (required for catabolism of d-serine) at about 54 min on the *E. coli* chromosome. The origin is oriented such that $dsdA^+$ is transferred into the recipient soon after the initiation of conjugation. Using the genetic map of *E. coli* shown below and assuming you have any recipient strain you need, how could you isolate an F' carrying the $dsdA^+$ gene? [Draw a diagram showing the relevant genotype of the donor and recipient, how the F' is formed, and how you would do the experiment including any media you would use. Note that mutations in any of the genes shown on the following genetic map results in auxotrophy.]



ANSWER:

YELLOW exam - Because the *dsdA*$^+$ gene will be transferred early by the Hfr as well as by the F', to ensure the phenotype is due to mating of the F' you need to select for *dsdA*$^+$ in a *dsdA recA* recipient. As a counterselection against the donor, the you could use a donor strain that is auxotrophic for a distant marker, for example a *thrA* mutant. The following diagram shows the selection for dsdA+ as a proximal marker.

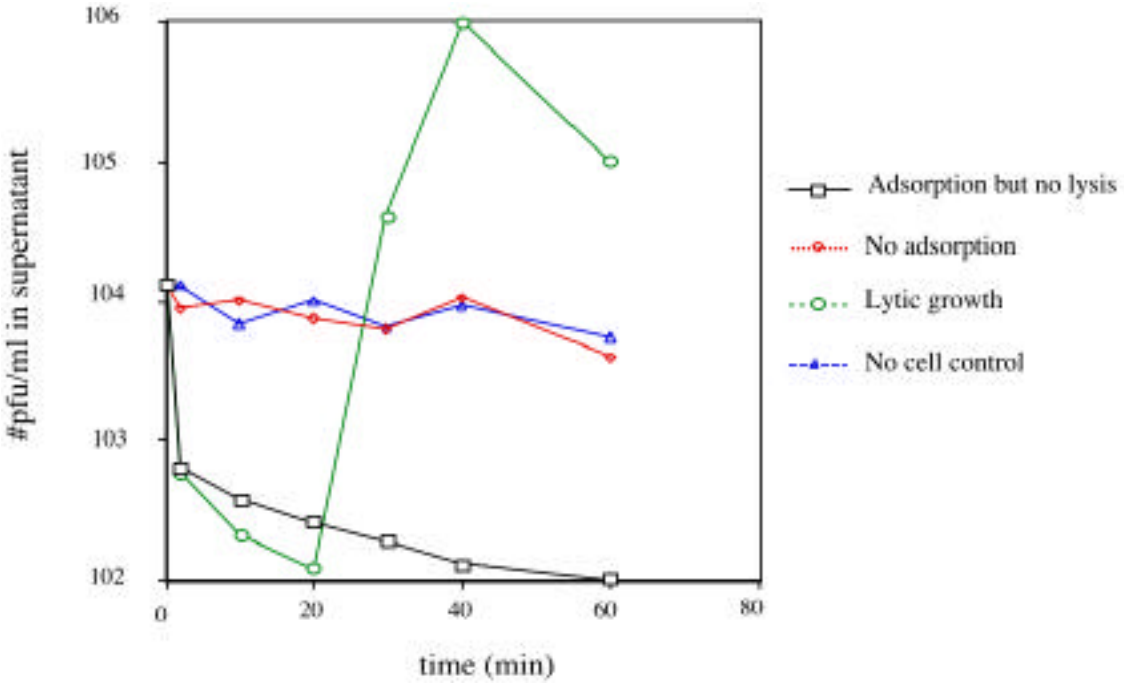
BLUE exam - To select for dsdA+ as a distal marker, the drawing would be essentially identical except that the arrowhead for oriT would be reversed.



(10) **5.** Phage P22 efficiently lyses *Salmonella typhimurium* but is unable to grow lytically on the closely related bacterium *Salmonella typhi*.

a. Suggest two possible reasons for these results.
ANSWER: One possible reason is that the phage cannot enter the cell (the one-step growth curve would look like the results shown for "no adsorption" in the figure below) and another possibility is that the phage can enter the cell but cannot develop (the one-step growth curve would look like the results shown for "adsorption but no lysis" in the figure below).

b. How would a one-step growth curve distinguish between these two possibilities? [Show a drawing of the expected results.]



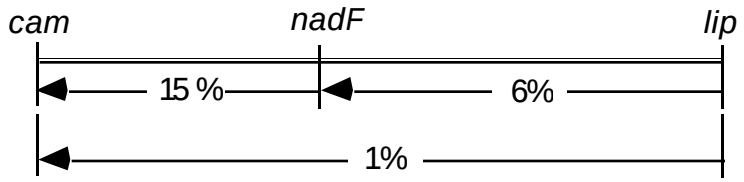
(5) **6.** Wild-type P22 cannot transduce plasmid pBR322 but P22 HT can transduce pBR322. Based upon the way these two phage package DNA, propose an explanation for this result.
ANSWER: P22 only transduces DNA with pac or good pseudopac sites while P22 HT recognizes and packages DNA fragments relatively nonspecifically. Thus, the results suggest that pBR322 does not have a pseudopac site so it cannot be transduced by P22, but because P22 HT can package the DNA "nonspecifically", pBR322 can be transduced by P22 HT.

(15) **7.** A temperature sensitive mutation was isolated in the gene encoding NAD kinase from *Salmonella*. Two factor crosses were done to map the *nadF* mutation against nearby markers. Given the results shown below, draw a genetic linkage map showing the most likely gene order and the coinheritance frequencies with arrows pointing toward the selected marker.

YELLOW exam:

Donor	Recipient	Selected phenotype	Recombinants	Number obtained	% co-tdxn
lip$^-$ *nadF*$^+$	*lip*$^+$ *nadF*$^-$	NadF$^+$	Lip$^-$ Lip$^+$	12 188	12/200 = 6%
cam$^+$ *nadF*$^-$	*cam*$^-$ *nadF*$^+$	Cam$^+$	NadF$^-$ NadF$^+$	30 170	30/200= 15%
lip$^-$ *cam*$^+$	*lip*$^+$ *cam*$^-$	Cam$^+$	Lip$^-$ Lip$^+$	2 198	2/200= 1%

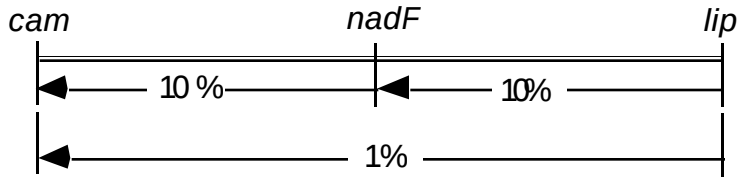
ANSWER: See figure below. Note that the arrows point toward the selected marker in the standard format used in genetic mapping and shown in practice examples.



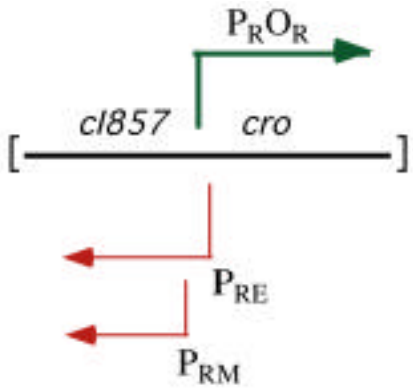
BLUE exam:

Donor	Recipient	Selected phenotype	Recombinants	Number obtained	% co-tdxn
lip$^-$ *nadF*$^+$	*lip*$^+$ *nadF*$^-$	NadF$^+$	Lip$^-$ Lip$^+$	50 450	50/500 = 10%
cam$^+$ *nadF*$^-$	*cam*$^-$ *nadF*$^+$	Cam$^+$	NadF$^-$ NadF$^+$	20 180	20/200= 10%
lip$^-$ *cam*$^+$	*lip*$^+$ *cam*$^-$	Cam$^+$	Lip$^-$ Lip$^+$	4 396	4/400= 1%

ANSWER: See figure below. Note that the arrows point toward the selected marker in the standard format used in genetic mapping and shown in practice examples.



(20) **8.** One of the first mutations isolated in the λ *cI* gene (*cI857*) is a temperature sensitive allele. The repressor is inactive at 42^o C but active at 30^o C. An *E. coli* prophage deletion strain was also isolated that deleted most of the DNA to the left and right of the immunity region as shown in the figure below. The strain contained the *cI857* allele and wild-type *cro* gene.



Note that the order of the following questions differs on the yellow and blue exams.

a. When the strain described above was infected with 100 pfu of λ^+ and plated at 42° C, 100 clear plaques were observed. Why were plaques observed and why were they clear??
ANSWER: At 42° C, the repressor is inactive. This leads constitutive expression of the *cro* gene from P_R in the lysogen. Pre-existing Cro binds to O_{R1} of the infecting phage and prevents transcription from the P_{Rm} promoter. This prevents expression of the cI gene so repressor is not made. Lysogens cannot be formed so the plaques are clear rather than turbid when lysogens are made. (This is a real experiment that lead to the concept of anti-immunity and the role of Cro in the lysogeny-lysis decision).

b. What would you predict for infection of this lysogen with 100 pfu of Phage 434 at 30° C? At 42° C?
ANSWER: You would expect about 100 turbid plaques because the lambda repressor cannot repress the O_L and O_R of the infecting phage 434. The phage would form turbid plaques because lysogens will grow in the center of the plaque just like (c).

c. When a **nonlysogenic** strain of *E. coli* is plated with 100 pfu of λ^+ phage, 100 turbid plaques are observed. What is the explanation for the turbid plaques?
ANSWER: The turbid plaques arise from lysogenic cells growing in the center of the plaque.

d. When the strain described at the top of the page was infected with 100 pfu of λ^+ and plated at 30° C, no plaques were observed. Why?
ANSWER: The repressor is active at 30° so it represses the O_L and O_{R3} of the infecting phage. This prevents expression of lytic genes so plaques are not observed.