Q1. An *E. coli* strain that is rifampicin resistant (*rif* ^{r}) and auxotrophic for arginine, histidine, proline, and tryptophan biosynthesis (*arg- his- pro- trp-*) is mixed with an Hfr donor strain that is wild-type for all of these loci (*arg+ his+ pro+ trp+ rif* ^{s}). Transconjugants that recombined the *arg*, *his*, and *trp* genes were individually selected and then screened for inheritance of the other loci. The results of this experiment are shown in the table below.

Selected marker	% Recombinant for Each Allele				
	arg	*his*	*pro*	*rif*	*trp*
arg	100	28	31	89	12
his	7	100	43	6	1
trp	29	33	30	31	100

Based on these results, draw a circular map of the *E. coli* chromosome showing the relative positions of the *arg*, *his*, *pro*, *trp* and *rif* loci, as well as the position <u>and</u> <u>orientation</u> of the Hfr origin of transfer (*oriT*). (Position the *oriT* at "12:00" on your map.)


Q2. A strain of *E. coli* contains a Tn*10* insertion within the *mogA* gene. This transposon carries a gene that confers resistance to tetracycline (Tet^r). Two other genes, *yaaH* and *htgA*, are located near *mogA* and encode protein products that are easily selected and/or identified by biochemical assay. The *E. coli* strain carrying the *mogA*::Tn*10* insertion is *yaaH+* and *htgA-*. A P1 transducing lysate is made from this strain, then used to infect another strain of *E. coli* that is *mogA+*, *htgA+*, and *yaaH-*. Transductant cells that were recombinant for a specific donated locus were then screened for the cotransduction of a second locus. The results of this experiment are shown in the table below.

Selected phenotype	Recombinants screened	Number of recombinants obtained
Tet^r	*htgA-*	75
	htgA+	25
YaaH+	*htgA-*	96
	htgA+	4
Tet^r	*yaaH+*	15
	yaaH-	85

Based on these results, draw a map showing the relative positions of the three genes. Indicate the cotansduction frequency for each pair of genes cotransduced.

Q3. The figure below represents the chromosome map of an F- *E. coli* strain carrying mutations in genes required for lactose catabolism (*lac*), tryptophan biosynthesis (*trp*), tyrosine biosynthesis (*tyr*), and isoleucine biosynthesis (*ilv*). The strain is also resistant to streptomycin due to a mutation in the *rpsL* gene. Below this are maps of several different Hfr strains, each showing the location and orientation of

its origin of transfer. (Remember that the *E. coli* map is actually <u>circular</u>, although it's represented in linear fashion here.) Each of the Hfr strains is wild-type for all of the loci indicated on the map (i.e., *lac+*, *trp+*, *tyr+*, Sr^s, and *ilv+*).
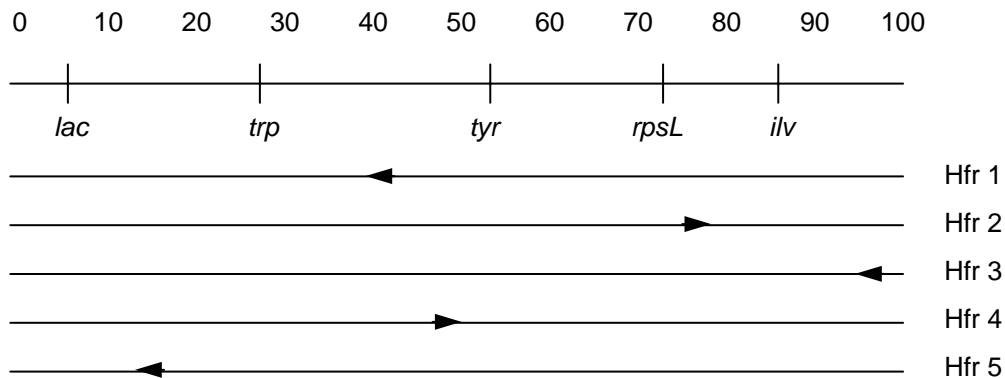
Each Hfr strain was individually mated with the F- recipient strain and cells were plated on minimal glucose (MinGlu) agar containing tyrosine, isoleucine, and streptomycin.

A. What <u>donor</u> marker or markers are being selected in this experiment?

B. Rank the five Hfr donor strains to indicate the relative numbers of transconjugants, from most to fewest, expected under this selection.

C. What medium would you use to select for transconjugants that had received both the *lac* <u>and</u> *tyr* markers from the donor cell? Which of the Hfr strains would be expected to give the highest number of transconjugants under this selection?

Q4. A P1 transducing lysate was grown on a wild-type (prototropihc) *E. coli* strain and used to infect a strain of *E. coli* that is auxotrophic for synthesis of the amino acids glutamate, proline, tyrosine, and valine (*glu-*, *pro-*, *tyr-*, *val-*). Samples of the transduced culture were spread on minimal glucose agar containing various amino acid supplements; the numbers of recombinant transductants from each of these selections is shown in the table below.

A. For each plate (1-9), list the alleles that are being <u>selected</u>.

B. Calculate the <u>percent</u> cotransduction frequency for each of the following pairs of alleles:
  glu + pro
  glu + val
  pro + tyr
  pro + val
  tyr + val

C. Draw a map showing the relative position of the four genes based on their cotransduction. Indicate on your map the cotransduction frequencies for each <u>adjacent</u> pair of genes.

D. Based on your map, what is the expected cotransduction frequency for the *glu* and *tyr* genes?

Plate #:	Amino acid supplements present:				Number of recombinants recovered
	Glu	Pro	Tyr	Val	
1	+	+	+	-	1,000

2	+	+	-	+	1,000
3	+	-	+	+	1,000
4	-	+	+	+	1,000
5	+	-	+	-	0
6	+	-	-	+	50
7	+	+	-	-	0
8	-	-	+	+	50
9	-	+	+	-	100

For the next question, use this list that describes the characteristics of the common amino acid side chains.

Hydrophobic (nonpolar): Gly, Ala, Val, Leu, Ile, Met, Phe, Trp, Cys, Pro
Polar: Ser, Thr, Asn, Gln, Tyr
Charged (**Basic**/*Acidic*): **Lys, Arg, His** / *Glu*, *Asp*

Q5. The signal sequence of the periplasmic protein β-lactamase (the enzyme responsible for resistance to Ampicillin) is shown below:

Met1 Ser2 Ile3 Gln4 His5 Phe6 Arg7 Val8 Ala9 Leu10 Ile11 Pro12 Phe13 Phe14 Ala15 Ala16 Phe17 Cys18 Leu19 Pro20 Val21 Phe22 Ala23

Part A:
If this sequence was part of a translational fusion to β-galactosidase (LacZ), please **indicate at least two single base changes** that might occur to make a mutant that would lead to a **significant defect in the targeting of the *bla-lacZ* fusion to the Sec apparatus**. (Use the codon chart in your textbook to identify appropriate changes.) Do not use nonsense mutations. Indicate the original and the final amino acids, the codon number, the starting sequence of the codon, and the mutated sequence. (Formulate your answer in the following fashion: Ser2Arg (AGU to AGA), meaning that the 2nd codon in the signal sequence, coding for Ser was changed to one coding for Arg.)

Part B:
You make a version of the *bla* gene that has one of your SS mutations included in it. The strain of *E. coli* containing this *bla* SS mutation is no longer resistant to Ampicillin, while a *bla*+ strain grows well on nutrient agar + 200 μg/ml Ampicillin at 37°C.

How would you isolate a mutation that would suppress the targeting defect of the SS*Bla mutant? Include what gene might contain the mutation and what your selection or screen would be.

Q6. Many *E. coli* strains resistant to streptomycin (Str^R) contain a mutation in the gene *rpsL*, which codes for a structural protein of the ribosome. Strains merodiploid for *rpsL*, having one copy of the Str^R allele and a second with the Str^S allele are sensitive to streptomycin.

Describe how you would isolate 50 **independent** Str^R mutants, starting with the Str^S merodiploid (*rpsL*+/*rpsL-str*^{R}). Is your strategy a selection or a screen?

Describe two classes of mutations that you would expect to come out of your Str^R isolation protocol, and what the distribution of each might be among your 50 mutants.

Q7. You discover a new drug that targets the bacterial RNA polymerase, and call it Bugicillin. Resistance to Bugicillin (Bug^R) occurs via missense mutations in the gene for the α subunit of RNA pol (*rpoA*). You observe that Bug^R strains of a species of *Shigella* (enteric bacteria, closely related to *E. coli*) cannot make

flagella or swim in liquid media, but that Bug^S strains of the same species can swim normally. You also know that the expression of genes coding for flagellar proteins is largely controlled by RNA pol with a dedicated σ factor, σ^{28}. Propose an explanation for the interference of Bug^R on flagellar expression.

ANSWERS:

Q1.

Q2.
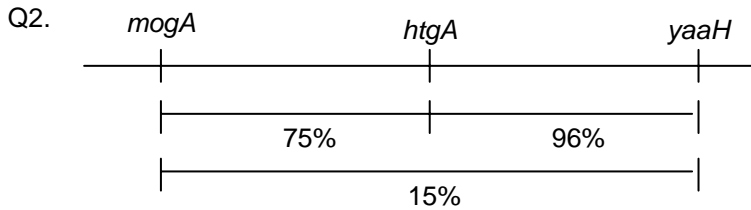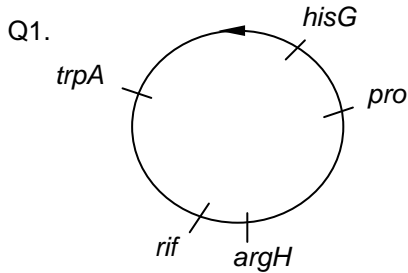
Q3. A) *trp+*

   B) Most – 5 4 3 2 1 - Least

   C) MinLac + Trp + Ile + Streptomycin (to select against donors)
      Hfr 3

Q4.   A) 1: val, 2: tyr, 3: pro, 4: glu, 5: pro + val, 6: pro + tyr, 7: tyr + val, 8: glu + pro, 9: glu + val

   B) *glu + pro* (#8): 5% (i.e., 50/1000), *glu + val* (#9): 10%, *pro + tyr* (#6): 5%, *pro + val* (#5): 0%, *tyr + val* (#7): 0%

   C)
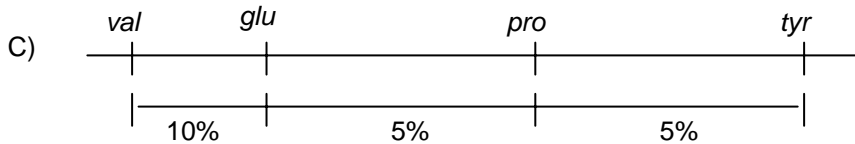
   D) 0% (The *glu* and *tyr* genes are farther apart than *pro* and *val* are from each other, and *pro* and *val* can't be cotransduced.)

Q5. Part A: There are a number of possible solutions to the problem, but you should focus on reducing the length of the hydrophobic core of the signal sequence by substituting residues with charged side chains for hydrophobic ones in the region after the short N-terminus (4-8 residues, usually has 2-4 basic residues). Examples include:

Ala15Glu (GCA to GAA), Leu10Arg (CUC to CGC), Ile11Lys (AUU to AGU), etc

Part B: Starting from strategies described in class, you could propose doing a selection for growth on ampicillin-containing medium at 37° C, thereby isolating a gain-of-function *prl* mutation in *secA, secE,* or *secY* that gives Amp^R. Any mutation that satisfies the selection will generally grow well and secret normal SS-containing proteins to the outer membrane or periplasm.

Q6. <u>Streptomycin selection</u>: Can take a plate of Strept^S cells and inoculate 50 different cultures, each with a single colony, in nutrient medium. Following growth, you should plate a large number of cells (about 10^9) from each culture onto a plate with Strept and grow at 37° C. After incubation, you should have a few colonies on at least most of the plates: choose 1 colony/plate and characterize.
There should be at least 2 classes of mutations: <u>Class 1</u> (most common, probably 80-90%) will be nonsense/frameshift mutations that knock-out synthesis of the Str^S RpsL protein (so that the strain only expresses the Str^R version of RpsL). <u>Class 2</u> should be a minority class, leading to a change in the RpsL$^+$ protein from Str^S to Str^R (so that the strain will be diploid for Str^R). This class will be relatively rare, as they are gain-of-function mutants.

Q7. The Bug^R missense mutation must change the surface of the α subunit of RNA pol so that the drug no longer binds and inhibits the activity of the polymerase. Simultaneous to altering the Bug binding site, the missense mutation must change the surface of the RNA pol polymerase so that it no longer can bind to σ^{28} and initiate transcription at flagellar promoters. This change to the *rpoA* gene is not a lethal mutation, as the RNA pol still can bind to other (essential) σ factors.