(4)   1.   Briefly compare and contrast recombination vs complementation.

Complementation = mixing of gene products, changes phenotype not genotype, no breakage/covalent rejoining of DNA
**Recombination = changes genotype, requires breakage/covalent rejoining of DNA**

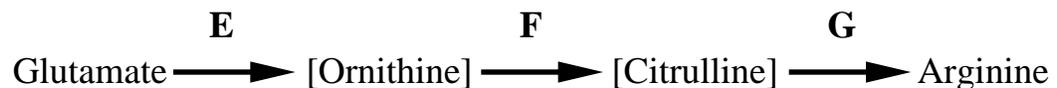(4)   2.   Briefly compare and contrast a genetic selection vs a genetic screen.

Selection = Only those cells with a specific phenotype can grow
Screen = Cells with different phenotypes (including wild-type) can grow but can distinguish phenotypes via colony color, etc

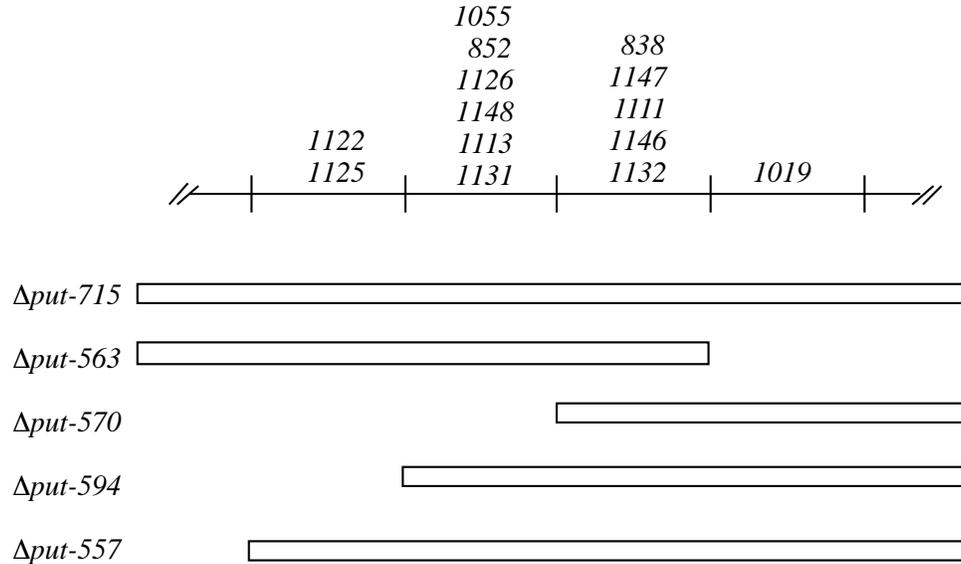(8)   3.   Three Arg⁻ mutants were isolated and crossfeeding tests were done as shown below.
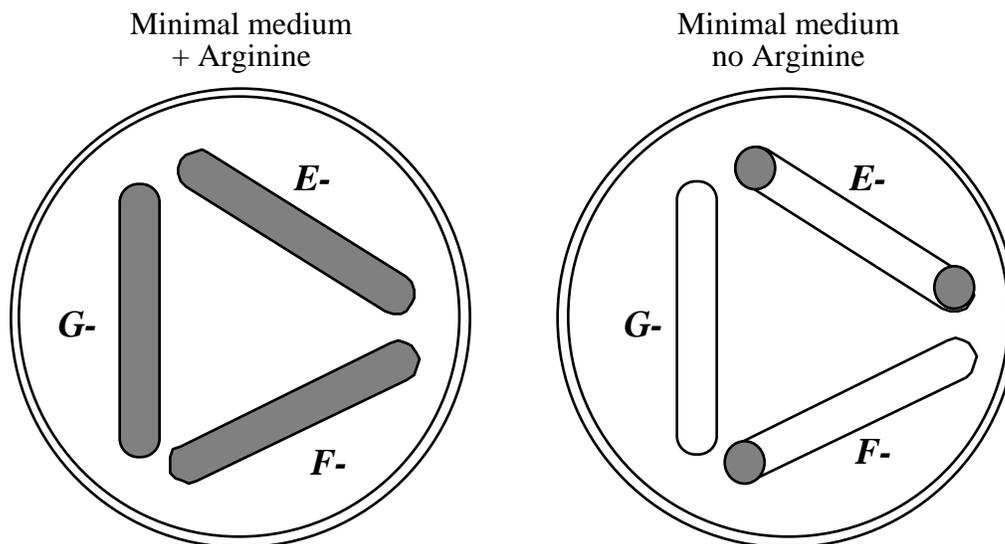
(a)   Based upon these crossfeeding results, indicate the order of the *G*, *E*, and *F* genes in the arginine biosynthesis pathway shown below.

$$\text{Glutamate} \xrightarrow{\;\;\textbf{E}\;\;} \text{[Ornithine]} \xrightarrow{\;\;\textbf{F}\;\;} \text{[Citrulline]} \xrightarrow{\;\;\textbf{G}\;\;} \text{Arginine}$$

(b)   Briefly explain your rationale for the proposed order.

F⁻ mutant accumulates <u>diffusible intermediate</u> ornithine which is excreted and can be used by E⁻ mutant. G⁻ mutant accumulates <u>diffusible intermediate</u> citrulline which excreted and can be used by F⁻ and E⁻ mutants.

(10) 4.   Proteins encoded by the *put* operon allow cells to use proline as a sole carbon or nitrogen source. A deletion map of part of the *put* operon is shown below. (The open bar shows the region deleted in the indicated mutant.)

```
                                    1055
                                     852           838
                                    1126          1147
                                    1148          1111
                        1122        1113          1146
                        1125        1131          1132        1019
        //——|———————————|——————————|————————————|———————————|——//
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Δ*put-715* [_____]

Δ*put-563* [_____]

Δ*put-570*   [_____]

Δ*put-594*   [_____]

Δ*put-557*   [_____]

(a)   A new Put⁻ mutant was isolated that <u>can</u> revert to Put⁺ but <u>cannot</u> repair any of these deletions. Based upon these results, what can you infer about the properties of the mutation. Propose a genetic <u>recombination</u> experiment to test your idea. Specify the donor(s) and recipient(s) and how you would select for recombinants.

   The new mutant can revert so it is probably NOT a deletion (i.e. it is probably a point mutant). The new mutant cannot repair any of the deletions so it probably lies within the region spanned by every deletion (that is, the interval including mutations 838, 1147, etc). A variety of experiments are also possible. For example, you could test for recombination with other point mutations in the same deletion interval, selecting for repair of the Put- phenotype -- unless every single mutation affected the exact same base pair, then the new mutation should recombine with some of these point mutations but maybe not all (note you could use the new mutant either as donor <u>or</u> recipient in these experiments). Likewise, three factor crosses with point mutations in adjacent intervals would test this idea (e.g., 1055 and 1019).

(b)   A second new Put⁻ mutant was isolated that <u>cannot</u> revert to Put⁺ and <u>cannot</u> repair any of these deletions. Based upon these results, what can you infer about the properties of the mutation. Propose a genetic <u>recombination</u> experiment to test your idea. Specify the donor(s) and recipient(s) and how you would select for recombinants.

   The new mutant can<u>not</u> revert so probably <u>is</u> a deletion (i.e. it is probably not a point mutant). (Note that many types of polar mutations including nonsense and frameshift mutations CAN revert! Furthermore, not all deletion mutations are polar.) The new mutant cannot repair any of the deletions so it probably includes the region spanned by every deletion (that is, the interval including mutations 838, 1147, etc) -- it may remove additional DNA as well, but it probably removes at least some DNA from this deletion interval. Again, a variety of experiments are possible. One good test would be to look for recombination with other point mutations located in each of the different deletion intervals, selecting for repair of the Put- phenotype -- if the mutation is a relatively large deletion, it will probably be unable to repair multiple point mutations (again you could use the new mutant either as donor <u>or</u> recipient in these experiments but it is usually a good idea to use the deletion mutant as a recipient because deletions do not undergo true reversion).

(6)   5.   In the deletion map shown in question #4, some deletion intervals contain many point mutations but some deletion intervals only contain a single point mutation. List <u>three reasons</u> why point mutations may be much rarer in some deletion intervals.

Some deletion intervals may have mutation hot spots.

Some deletion intervals may span regions of the gene product that are not very important for proper structure and function.
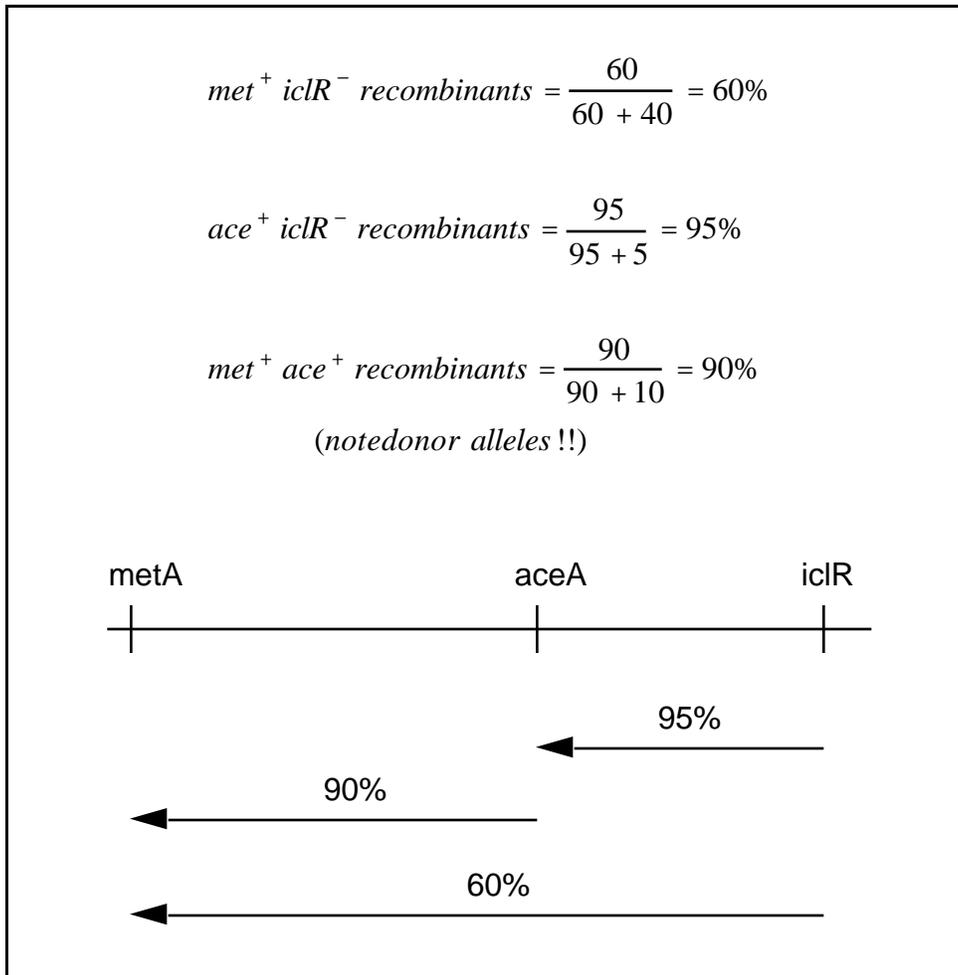
Although they are drawn as being the same size (because based on genetic tests it is not usually easy to know whether one interval is larger than another) remember that the amount of DNA deleted may be different from one interval to another -- thus, the "target size" for mutations may be larger for some deletion intervals than others.

Note that some people listed multiple reasons for hot spots or multiple reasons why mutations may not affect the gene product, but I only accepted one answer per "reason" in each of these cases.

(10)   6.   In order to map the *iclR* gene, two-factor crosses were done with the nearby genes *metA* and *aceA*. Given the results shown below, draw a genetic linkage map showing the inferred gene order and the coinheritance frequencies (include appropriate arrows).

Donor	Recipient	Selected phenotype	Recombinants	Number obtained
met$^+$ iclR$^-$	*met$^-$ iclR$^+$*	Met$^+$	IclR$^-$	60
			IclR$^+$	40
ace$^+$ iclR$^-$	*ace$^-$ iclR$^+$*	Ace$^+$	IclR$^-$	95
			IclR$^+$	5
met$^+$ ace$^+$	*met$^-$ ace$^-$*	Met$^+$	Ace$^-$	10
			Ace$^+$	90

Note that you need to determine the frequency of COINHERITANCE -- that is, the fraction of recombinants that inherited both DONOR alleles. For the three cases listed above the coinheritance frequencies and resulting map are shown below:

$$met^+ \; iclR^- \; recombinants = \frac{60}{60 + 40} = 60\%$$

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

$$met^+ \; ace^+ \; recombinants = \frac{90}{90 + 10} = 90\%$$

(*note donor alleles* !!)

metA   aceA   iclR

95%

90%

60%

(10) **7.**   To confirm the gene order of the *iclR*, *metA*, and *aceA* 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 of each cross to show your rationale for this conclusion.

	Donor	$met^+ \; iclR^- \; ace^+$
	Recipient	$met^- \; iclR^+ \; ace^-$

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

Note that you can infer the middle marker from the rare class of recombinants (the Met$^+$ IclR$^-$ Ace$^-$ recombinants). This result indicates that the Ace$^-$ allele is the middle marker in the recipient. Also note that the 4 crossovers are required to produce this rare class of recombinants. Only the crossovers yielding the rarest class of recombinants is shown below but I expected you to draw out each class of crossover event.
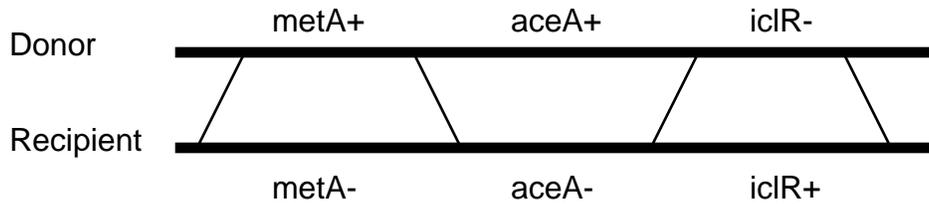
(6)   8.   Sometimes interference between closely spaced recombination events (cross-overs) results in disagreement between the gene order predicted from two-factor and three-factor crosses for very closely linked mutations. Suggest <u>two different</u> *in vivo* recombination experiments that would allow you to figure out the gene order.

DELETION MAPPING! Remember that 2- and 3-factor crosses depend upon frequencies but deletion mapping depends upon a yes or no answer.
Since the problem is that the two markers are too close together to accurately map, you could test linkage via 2-factor crosses or order by 3-factor crosses of each marker to one or more other genes which are more distant.

(6)   9.   Briefly explain the expected results for the Luria-Delbruck fluctuation test if mutants arise:

(a)   spontaneously in a population of cells before exposure to a selective agent;

Wide fluctuation in mutants from cells grown in different tubes (i.e., independent samples) relative to cells grown in the same tube. That is, there is great variance relative to the mean number of mutants.

(b)   due to adaption after exposure to the selective agent.

Relatively little fluctuation in mutants from cells grown in different tubes (i.e., independent samples) relative to cells grown in the same tube. That is, the number of mutants on each plate approximately equals the mean number of mutants.

(10) 10.   Three closely linked genes (*galK*, *galT*, and *galE*) are required for galactose utilization in *E. coli*. Four new Gal$^-$ mutants were isolated (*gal-57*, *gal-58*, *gal-59*, *gal-61*). To determine which gene each of these new mutants affected, a set of merodiploids were constructed with recessive point mutations in the *galK*, *galT*, or *galE* genes, and complementation analysis was done. The results are shown below.

	galK$^-$ galT$^+$ galE$^+$	*galK$^+$ galT$^-$ galE$^+$*	*galK$^+$ galT$^+$ galE$^-$*	Gene(s) affected?	Recessive ?
gal-57	−	+	+	K	Yes
gal-58	+	−	+	T	Yes

| gal-59 | + | + | − | E | Yes |
| gal-61 | − | − | + | K + T | No |

(a)   In the above table indicate the gene or genes that each of the new mutants affects.

(b)   In the above table indicate which of the new mutant alleles are simple recessive mutations.

(c)   Could any of the results be explained by a *cis*-dominant mutation and, if so, what would that imply about the expression of the *gal* genes or function of the *gal* gene products?

gal-61 affects expression of both galK and galT. Any one of the following answers was accepted.
Suggests that either galK and galT are downstream of galE in the same operon _and_ there is a
polar mutation in the most upstream of these two genes; galK and galT are in a separate
operon than galE _and_ there is a polar mutation in the most upstream of these two genes; this
is a deletion mutation that removes both galK and galT but does not affect galE.

(d)   Could any of the results be explained by a *trans*-dominant mutation and, if so, what would that imply about the expression of the *gal* genes or function of the *gal* gene products?

The simplest explanation is that gal-61 is a trans acting mutation that affects the proper
interactions of the galK and galT gene products. galE is not affected by this defective
protein-protein interaction.

(8)   11.   DHP is transported into the cell by proline permease and incorporated into proteins, resulting in defective proteins and subsequently cell death. Spc binds to ribosomes, inhibiting protein synthesis, and ultimately resulting in cell death. Following treatment with the intercalating agent ICR-191, the mutation rate to DHP^R is about 10^{-4} but the mutation rate to Spc^R is only about 10^{-9}. Suggest a reason for the difference in the mutation rates to DHP^R vs Spc^R and propose a <u>genetic</u> experiment to test your idea.

ICR is an intercalating agent that causes frameshift mutations.
Resistance to DHP is due to any mutation that disrupts the *putP* gene. The *putP* gene can be
disrupted by any type of mutation (including null mutations such as frameshift
**mutations) because the *putP* gene product is not essential for the cell.**
In contrast, since ribosomes are essential, such null mutations in a ribosomal gene would be
lethal. Thus, the Spc^R mutation must be due to a specific, rare missense mutations that
prevent binding of Spc to the ribosome.
The above results suggest that the Spc^R mutations found after ICR mutagenesis are not due
to the ICR mutagenesis per se, but are spontaneous base substitution mutations. If this
is true then the mutation rate to Spc^R should be the same with or without ICR, while
the rate of DHP resistance should be much higher in cells treated with ICR.
Furthermore, mutagens that stimulate base substitutions would probably produce an
increases frequency of Spc^R mutants.

(8)   12.   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. typhimurium* the cells are lysed (indicated by a white spot in the

figure below). Three conditional mutants were isolated that prevent cell lysis. 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.
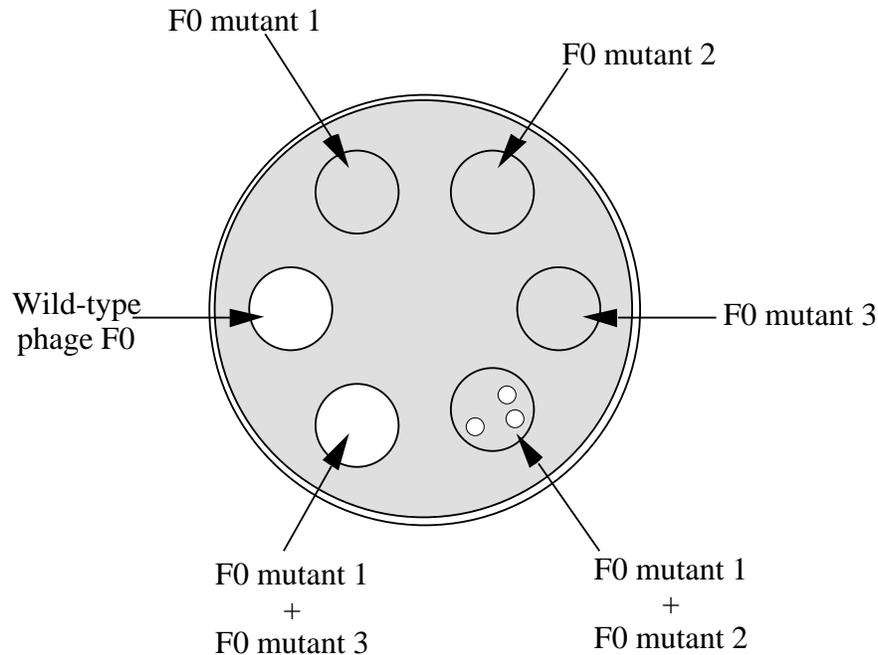
(a)　Do mutant 1 and mutant 2 map in different genes? Briefly explain your answer.

No. They do NOT complement. Complementation would result in complete clearing (i.e., lysis) of the spot.

(b)　How can you explain the rare plaques in this spot containing mutant 1 and mutant 2?

The small plaques are due to RARE <u>recombinants</u> between the two mutants that result in wild-type progeny phage. Note that the frequency of recombination is MUCH lower than the frequency of complementation seen with mutants 1 and 3. It is <u>unlikely</u> that the small plaques are revertants because no revertants are seen in the spots containing mutant 1 or mutant 2 alone.

(c)　The results suggest that mutant 2 and mutant 3 affect different complementation groups. Do these results <u>prove</u> that the two mutations affect different genes? Briefly explain your answer.

No. Different complementation groups often mean that mutants affect different genes, but they do NOT PROVE it -- <u>intragenic complementation</u> can also occur, especially in a case like this which requires many protein-protein interactions.

(10) 13.　A series of five mutants of phage T4 were isolated.   Complementation tests were performed by spotting a mixture of the two phage mutants to be tested (about 10^6 of each) onto a lawn of bacteria at either 25°C or 40°C. The results are summarized below (+ = complete lysis; _ = no lysis or only a few plaques in the spot).

25°C

	1	2	3	4	5
1	+	+	+	+	+
2		−	−	+	+
3			−	+	+
4				−	−
5					−

40°C

	1	2	3	4	5
1	−	−	−	+	+
2		−	−	+	+
3			−	+	+
4				+	+
5					−

(a) How many complementation groups do these mutants affect and which mutants are in the same complementation groups?

Two complementation groups: group I = mutants 1,2,3 and group II = mutants 4,5

(b) Describe the nature of the mutation in each mutant (for example, nonconditional, temperature sensitive, cold sensitive)?

mutant 1 = TS (temperature sensitive)
mutant 2,3,5 = nonconditional (or null would be acceptable)
mutant 4 = CS (cold sensitive)