(15) **1.** *Klebsiella aerogenes* was mutagenized with the alkylating agent Diethylsulfate (DES).

a. How could you easily determine that DES caused mutations in a variety of genes? [Describe what experiment you would do and any media you would use.]

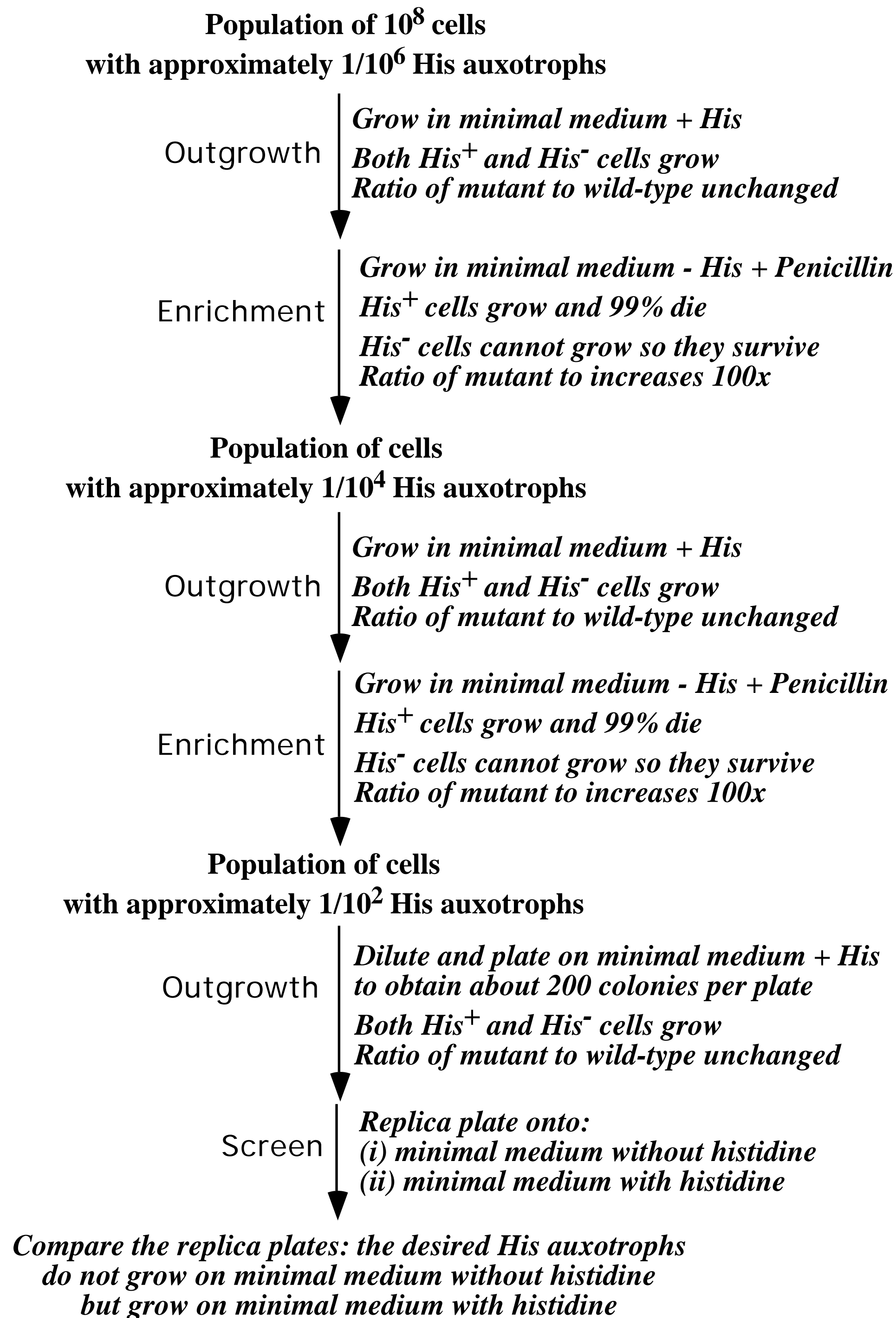
ANSWER: A simple way of demonstrating that the mutagen affects a variety of targets (vs a few hot spots) would be to look for auxotrophic mutants that different biosynthetic pathways. There are many genes that affect biosynthetic pathways so this is a large target. In addition, the test is simple because you do not need a large variety of different media to look for the desired mutants. (i) Mutagenize cells. (ii) Dilute and plate on rich medium to obtain about 200 colonies per plate. (iii) Replica plate onto minimal medium and minimal medium plus pooled supplements (see pg 72 of MCF) to identify auxotrophic mutants that affect a varitey of different pathways.

b. Is this a selection or a screen? [Briefly explain your answer.]

ANSWER: This is a genetic screen.

c. How could you do a penicillin enrichment for histidine auxotrophs? [Draw a flow diagram showing what media you would use and what the ratio of auxotrophs to prototrophs expected at each step.]

ANSWER: See flow diagram below.


Population of 10^8 cells
with approximately $1/10^6$ His auxotrophs
Outgrowth
Grow in minimal medium + His
Both His^+ and His^- cells grow
Ratio of mutant to wild-type unchanged
Enrichment
Grow in minimal medium - His + Penicillin
His^+ cells grow and 99% die
His^- cells cannot grow so they survive
Ratio of mutant to increases 100x
Population of cells
with approximately $1/10^4$ His auxotrophs
Outgrowth
Grow in minimal medium + His
Both His^+ and His^- cells grow
Ratio of mutant to wild-type unchanged
Enrichment
Grow in minimal medium - His + Penicillin
His^+ cells grow and 99% die
His^- cells cannot grow so they survive
Ratio of mutant to increases 100x
Population of cells
with approximately $1/10^2$ His auxotrophs
Outgrowth
Dilute and plate on minimal medium + His
to obtain about 200 colonies per plate
Both His^+ and His^- cells grow
Ratio of mutant to wild-type unchanged
Screen
Replica plate onto:
(i) minimal medium without histidine
(ii) minimal medium with histidine
Compare the replica plates: the desired His auxotrophs
do not grow on minimal medium without histidine
but grow on minimal medium with histidine

(10) **2.** Four independent mutations were obtained that affect the synthesis or assembly of fimbriae. The properties of the mutations are described in the table below (where + indicates that fimbriae were produced and − indicates that no fimbriae were produced).

Mutation	**Growth temperature**			
	30°C	**42°C**	**30 → 42°C**	**42 → 30°C**
fim$^+$	+	+	+	+
fim (null allele)	−	−	−	−
fim-1	−	+		
fim-2	+	−		
fim-3	−	+		
fim-4	+	−		
fim-1 fim-2	−	−	+	−
fim-1 fim-4	−	−	−	+

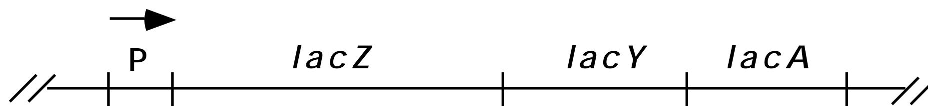
a. Based upon the above results, indicate whether each of the single mutations is a temperature sensitive (Ts) or cold sensitive (Cs) mutation.

ANSWER: ***fim-1*** **and** ***fim-3*** **are Cs mutations;** ***fim-2*** **and** ***fim-4*** **are Ts mutations.**

b. Based upon the above results, what is the predicted order of the mutant gene products in the pathway of fimbriae synthesis and assembly? [Briefly explain your answer.]

ANSWER: The results suggest that ***fim-2*** **acts before** ***fim-1*** **because the product if only made if the cells are first grown at 30°C where** ***fim-2*** **is active, then shifted to 42°C where** ***fim-1*** **is active – hence** ***fim-1*** **must convert an intermediate made by** ***fim-2*** **into the product. The results suggest that** ***fim-1*** **acts before** ***fim-4*** **because the product if only made if the cells are first grown at 42°C where** ***fim-1*** **is active, then shifted to 30°C where** ***fim-4*** **is active – hence** ***fim-4*** **must convert an intermediate made by** ***fim-1*** **into the product. Thus, the gene products probably act in the pathway in the following order:**

(10) **3.** A diagram of the *E. coli lac* operon is shown below. The three gene products can be assayed independently.



a. Amber mutations in the upstream region of the *lacZ* gene are polar on expression of the *lacY* and *lacA* genes. Draw a cartoon showing how the amber mutation prevents expression of downstream genes. [Be sure to clearly label the different steps in the process.]

ANSWER: Translation termination due to a nonsense mutation in the ***lacZ*** **gene results in disassociation of the ribosomes from the mRNA. Subsequently rho factor interacts with the naked mRNA and causes transcription termination or "polarity" which prevents transcription of downstream genes. See the cartoon under Rho dependant polarity in the section on "Chromosomes, Genes, and Proteins" on the Mcbio 316 WWW site.**

b. Would *lacY* be expressed if the *lacZ(*Am) mutation was in a strain with an amber suppressor? [Briefly explain your answer.]

ANSWER: The amber suppressor would prevent translation termination, the resulting mRNA would be coated with ribosomes, Rho factor would not bind to the mRNA, and no transcription termination would result. [This is true whether or not the lacZ gene product was functional due to insertion of an acceptable amino acid.]

(10) **4.** The *tar* gene in *E. coli* has Ala at position 19. A mutation that changes Ala-19 to Lys inactivates the Tar protein. Oosawa and Simon isolated numerous intragenic pseudorevertants of a Lys-19 mutation in the *tar* gene in *E. coli*. Several of the pseudorevertants obtained are shown in the table below.

Mutant #	Original mutation	Second-site suppressor
1	Ala-19 -> Lys-19	Lys-19 -> Gln-19
2	Ala-19 -> Lys-19	Lys-19 -> Ile-19
3	Ala-19 -> Lys-19	Lys-19 -> Thr-19
4	Ala-19 -> Lys-19	Val-17 -> Glu-17
5	Ala-19 -> Lys-19	Trp-192 -> Arg-192
6	Ala-19 -> Lys-19	Gly-271 -> Ala-271
7	Ala-19 -> Lys-19	Asp-288 -> Val-288
8	Ala-19 -> Lys-19	Thr-303 -> Ile-303

a. Based upon pseudorevertants 1-3, what can you conclude about the role of amino acid 19 in the Tar protein?

ANSWER: Insertion of a variety of different amino acids (including Ala, Gln, Ile, and Thr) at position 19 of the Tar protein does not disrupt its function. However, not all amino acids are permissive at this site. The results indicate the site may play a role in protein structure but probably do not directly affect the active site of the protein.

b. When the second site suppressors in mutants 4-8 were backcrossed into the wild-type *tar* gene with Ala-19, most of the resulting proteins were functional. What can you conclude about the allele specificity of the suppressor mutations 4-8? What does this suggest about how these mutations affect the Tar protein?

ANSWER: Since the protein remained functional when the suppressor mutation was present but the wild-type amino acid was present at position 19, the results indicate that the suppressor can function with both Lys-19 and Ala-19 (amino acids with very different properties) and hence they are <u>not</u> allele specific suppressors. This suggests that the effect of the suppressor mutation on the Tar protein is independent of the amino acid at position 19, and they probably act by causing increased protein activity (possibly by increasing stability of the protein).

(15) **5.** In order to identify the gene products required for genetic recombination, Clark screened for *E. coli* mutants that were defective for recombination. One class of mutations disrupted a gene designated *recA* and decreased recombination by 10^{-6}. A second class of mutations disrupted a gene designated *recB* and decreased recombination by 10^{-3}. He then looked for revertants of these mutations.

a. Some <u>intra</u>genic suppressors of the *recA* mutants were found to be temperature sensitive. Suggest a biological explanation for these results.

ANSWER: The temperature sensitive properties of some intragenic suppressors is a

consequence of the second amino acid substitution in the protein (i.e. a second missense mutation) that affects protein folding and stability.

b. No intergenic suppressors were found for the *recA* mutants. Suggest a biological explanation for these results.

ANSWER: Failure to find intergenic suppressors implies that there is not a simple way to suppress this reaction by changing an interacting protein or turning on a bypass pathway, indicating that this is an essential, unique step required for recombination.

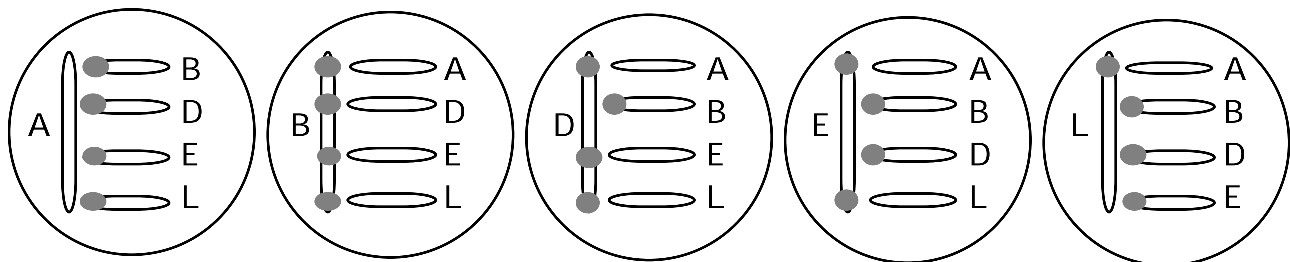
c. In contrast, intergenic suppressors were found that improve the recombination frequency of *recB* mutants, including a *recB* deletion mutant that removes most of the *recB* gene. Suggest a biological explanation for these results.

ANSWER: The results indicate that in the absence of RecB another pathway can substitute for this gene product to bypass the RecB reaction.

(5) **6.** What type of mutation(s) would be most likely to cause a negative-dominant mutant protein? Give an example of how a mutation could produce this phenotype?

ANSWER: A negative dominant mutation is most likely to be due to a missense mutation. A negative-dominant mutation could be due to an altered protein subunit that interfers with multimerization by wild-type subunits (for example, the mutant/wild-type heterodimer might be inactive). For a structural protein this would interfer with formation of the proper stucture. For an enzyme, this could either interfer with formation of the proper structure per se, or it could mess up the active site of the enzyme.

(15) **7**. Five different aromatic amino acid auxotrophs were isolated: *aroA*, *aroB*, *aroD*, *aroE*, and *aroL*. To determine if each of these mutations affect different steps of the aromatic amino acid biosynthesis pathway, each mutant was tested for the ability to crossfeed the other mutants. The results are shown in the figure below. (The shaded arrows indicate growth and the areas that are not shaded indicate where each mutant was streaked on the plate.)



a. Based on the above results, indicate which step(s) of the following pathway are blocked in each of the five mutants and BRIEFLYexplain your logic.

aroA **ANSWER: after SP (not fed by any other mutants in pathway)**

aroB **ANSWER: before DHQ (fed by all mutants later in pathway)**

aroD **ANSWER: between DHQ and DHS**

aroE **ANSWER: between DHS and S**

aroL **ANSWER: between S and SP**

b. Another Aro$^-$ mutant was isolated that behaved the same as the *aroB* mutant in crossfeeding experiments. Describe a genetic test you could do to determine if both mutations affect the same enzymatic step. [Briefly explain the experiment. No DNA sequencing or enzyme assays allowed.]

ANSWER: Note that if the mutations behave the same in crossfeeding experiments, doing additional crossfeeding experiments would not be a very good approach. Assuming that both mutations were recessive, then the best test would be an in vivo complementation test with two copies of the genes, one copy with a mutation in *aroB* and one copy with a mutation in the new Aro$^-$ mutant. This test must come with c ontrols for each mutation against itself.

c. Given two mutants that are unable to crossfeed, what results would you obtain in the experiment you described in part b if the two mutations affected the same enzymatic steps and if they affected different enzymatic steps.

ANSWER: If the two mutations affected two different steps (e.g., the one affected each arrow in the synthesis of DHQ), then the complementation test described above would allow growth without aromatic amino acids. If they affected the same step, no complementation would result.

(20) **8.** *Salmonella* Enteritidis expresses a specific fimbriae called Sef which facilitate attachment of the bacteria to macrophages during infections. A large number of mutations were isolated that prevent expression of *sef* genes, and complementation analysis was done to determine the number of genes disrupted by the mutations. For each mutation, one copy of the genes was present on the chromosome and a second copy of the genes was present on a plasmid integrated into the chromosome at a different site. The results are shown in the table below (– indicates no Sef fimbriae produced and + indicates that Sef fimbriae are produced).

Copy #1	Copy #2									
	sef-51	*sef-52*	*sef-53*	*sef-54*	*sef-55*	*sef-56*	*sef-57*	*sef-58*	*sef-59*	*sef-60*
sef^+	+	+	+	+	+	–	+	+	+	+
sef-51	–	–	–	–	–	–	–	–	–	–
sef-52		–	+	+	–	–	–	+	+	+
sef-53			–	+	+	–	+	+	–	+
sef-54				–	+	–	+	+	+	+
sef-55					–	–	–	+	+	+
sef-56						–	–	–	–	–
sef-57							–	+	+	+
sef-58								–	+	–
sef-59									–	+
sef-60										–

a. Based upon these results, how many complementation groups were detected?

ANSWER: 4 complementation groups.

b. Indicate which mutations fall into each complementation group?

ANSWER: Group #1 = *sef-52*, *sef-55*, *sef-57*; group #2 = *sef-53* and *sef-59*; group #3 = *sef-54*; group #4 = *sef-58*, *sef-60*; Note that *sef-51* and *sef-56* cannot be placed into a complementation group.

c. Suggest a likely explanation for the complementation behavior of the *sef-51* mutation. Would you expect this mutant to produce CRM? [Briefly explain your answer.]

ANSWER: *sef-51* is recessive to the wild-type in trans, indicating that this phenotype is NOT likely to be due to a *trans*-dominant negative (negative-dominant) mutant. Thus, this phenotype is likely to be due to a *cis*-acting mutation that prevents expression of all of the *sef* genes. Not likely to produce CRM because mutations in a gene resulting in cis-dominance are usually not made or truncated and thus rapidly degraded.

d. Suggest a likely explanation for the complementation behavior of the *sef-56* mutation. Would you expect this mutant to produce CRM? [Briefly explain your answer.]

ANSWER: *sef-561* is dominant to the wild-type in trans, indicating that this phenotype is likely to be due to a *trans*-dominant negative (negative-dominant) mutant gene product. Likley to produce CRM because such mutations are typically due to missense mutations.