ANSWERS TO Problem set questions from Exam 2 Unit – Mutations, Bacterial Genetics, and Bacterial Gene Regulation

Central Dogma, Mutagens and Mutations

1. The three stop codons in the genetic code are 5'UAG3', 5'UAA3', and 5'UGA3'.

(a) gln-tRNA

(b) gln-tRNA: 5'-CAA-3' ← this strand is used as a template
3'-GTT-5'

(c) mutant is: 5'-TAA-3' ← this strand is used as a template
3'-ATT-5'

2. You are studying an *E. coli* gene that encodes an enzyme of interest to you.

(a) The +1 frameshift sets the amber mutation out of frame so that the stop is not read, and the –1 frameshift after the amber mutation returns the reading frame back to the original reading frame, so that all amino acids will be as they were originally intended following the second frameshift.

(b) There must be an out-of-frame stop codon that lies in between where the –1 frameshift is placed and where the +1 frameshift is placed. This out-of-frame stop codon must be put into its reading frame by the –1 frameshift.

(c) if AT and GC equal, p(no stop for 50 codons) = (61/64)^50 = 0.0907
if AT is 40%, p(no stop for 50 codons) = (0.968)^50 = 0.179

3. One way to isolate nonsense suppressor mutations in tRNA genes is to select for the simultaneous suppression of the mutant phenotype of a strain carrying nonsense mutations in two different genes.
Explain why it would be a bad idea to start with an original strain that has an amber mutation (TAG) in the **his1** gene and an ochre mutation (TAA) in the **his2** gene.

You cannot isolate a nonsense suppressor mutation that will recognize both TAG and TAA, and you would need such a nonsense suppressor mutation to see a His+ phenotype from a strain that has a TAG mutation in his1 and a TAA mutation in his2. The only way you could ever see His+ is if you got mutations in two different tRNA genes in the same strain, such that one tRNA gene became a TAG nonsense suppressor and the other tRNA gene became a TAA nonsense suppressor. Such a double mutational event is highly unlikely.

4. Consider a phage gene that encodes the enzyme lysozyme.

(a) 1: missense, 2: frameshift or nonsense

(b) 12/1000 would be wild-type progeny

(c) There must be an out-of-frame stop codon that lies in between where the –2 frameshift is placed and where the +2 frameshift is placed. This out-of-frame stop codon must be put into its reading frame by the +2 frameshift (but not by the +1 frameshift).

(d) p (no stop for 15 codons) = (61/64)^46 = 0.11

5. You are trying to isolate mutations in the gene encoding $tRNA^{trp}$ that will produce mutant forms of this tRNA that will recognize a stop codon, as opposed to the normal trp codon.

(a) 5'-CCA-3'

(b) 5'-CCA-3'
3'-GGT-5'

(c) 5'-TCA-3'
3'-AGT-5'

5'-CTA-3'
3'-GAT-5'

5'-TTA-3'
3'-AAT-5'

(d) 5'-UCA-3'

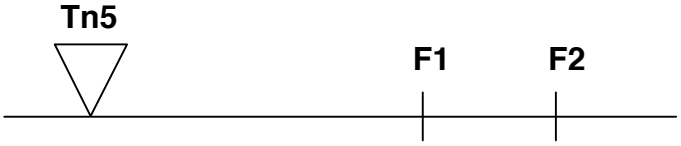
5'-CUA-3'

5'-UUA-3'

(e) UGA and UAG

Transposons and Cotransduction Mapping in bacteria (Moving DNA between bacterial cells by transduction [using phage])

1. You have isolated two *E. coli* mutants in the PyrF gene, called PyrF-1 and PyrF-2.
(a) it must be near to PyrF (within 100 kb)
(b) 30%
(c)



2. The *E. coli* **ser1** gene is required for synthesis of the amino acid serine, and strains harboring mutations in this gene will not grow unless serine is provided in the growth medium.
(a) There will still be a stop codon within ser1, which will still stop translation of the Ser1 protein, even if a second mutation is acquired within the ser1 gene. The only kind of mutation that could override a stop codon is a frameshift that puts the stop codon out of frame, but a frameshift also changes the frame of the entire rest of the protein after the stop codon.
(b) 40%
(c) it is extragenic because it is unlinked
(d) it could be intragenic
(e) yes

3. In a transduction experiment, phage P1 is grown on a bacterial host of genotype A^+ B^+ C^+ and the resulting lysate is used to infect a recipient strain of genotype A^- B^- C^-.

(a) true
(b) true
(c) true
(d) false

4. You have used mutagenesis with the chemical EMS to isolate four different *E. coli* mutants that will not grow unless the amino acid histidine is provided in the growth medium.

(a) That colony arose from a single cell that received a piece of bacterial DNA from a P1 phage that contained a Tn5 insertion that was linked to the his1 locus.

(b) 80%

(c) they are not linked, so they must be alleles of different genes

(d) make sure they are both recessive and then do a complementation test

(e) 1 and 4 are linked to each other because they both are linked to the Tn5 insertion

(f)

(g) you can't conclude either way just because they are linked

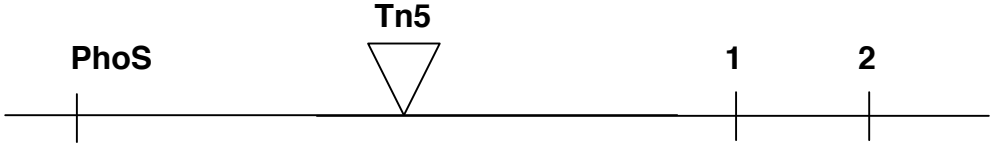
5. Wild-type *E. coli* have flagella that allow them to swim towards nutrient sources.

(a) because you cannot perform a selection for cells that receive mot DNA – the motility phenotype (or the inability to be mobile) cannot be selected for

(b) 60%

(c) PhoS and mot1 must be on different sides of the Tn5 insertion, such that both are linked to the insertion, but neither are linked (within 100 kb) of each other

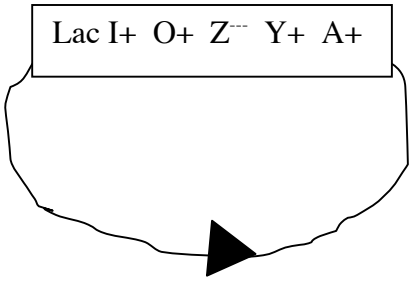
(d)



F plasmids, Hfrs, F' plasmids (Moving DNA between bacterial cells by conjugation [using mating])

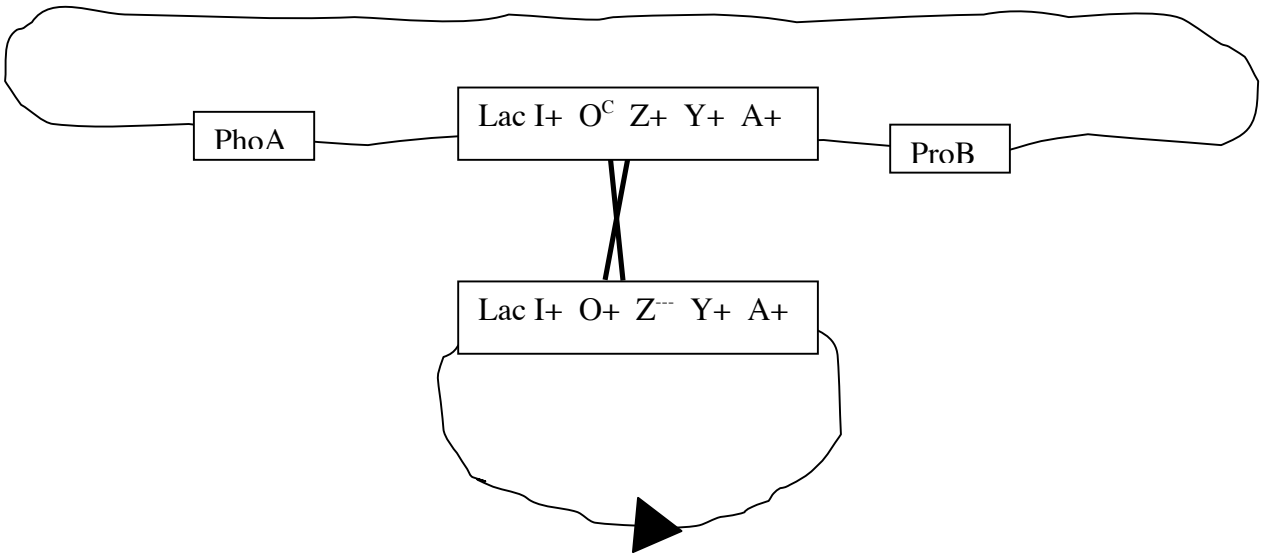
1. The region of the *E. coli* chromosome surrounding the Lac operon contains the markers PhoA — Lac I – LacZ,Y,A — ProB, in that order.

(a) the F' plasmid:



(b) early

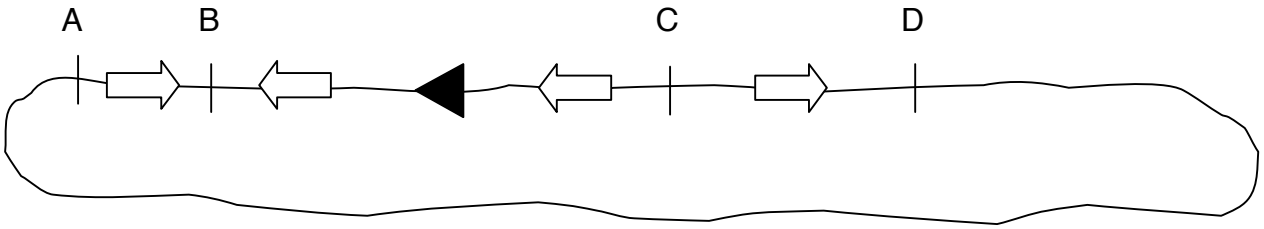
(c) recombination event between the chromosome and the F' plasmid:



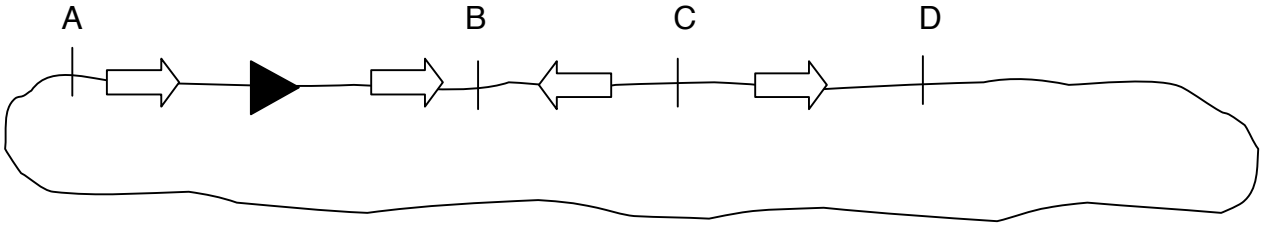
(d) complementation tests, cis/trans tests

2. The diagram below shows the F factor plasmid, and a portion of the *E. coli* chromosome that contains three different insertion sequences (IS) of the same type as that which is carried on the F plasmid.

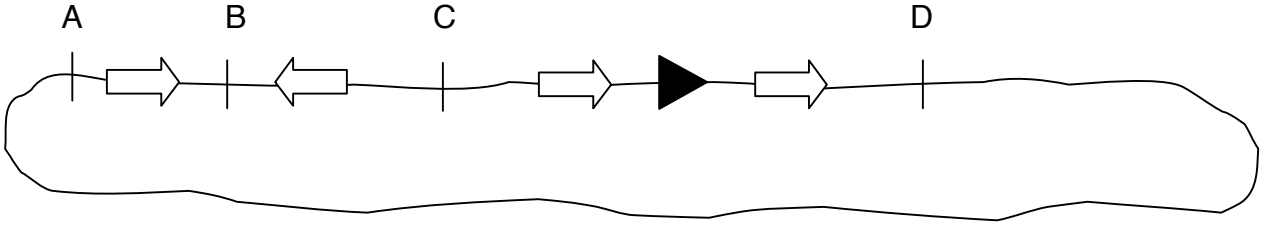
(a) 1st Hfr:



2nd Hfr:

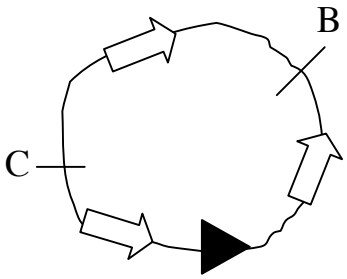


3rd Hfr:

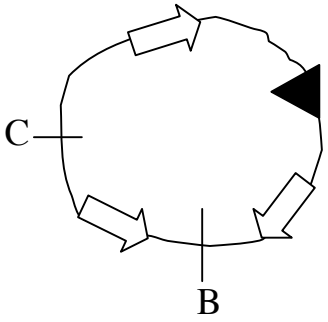


(b) 1st Hfr transfers C then D early
2nd Hfr transfers only A early
3rd Hfr transfers C then B then A early

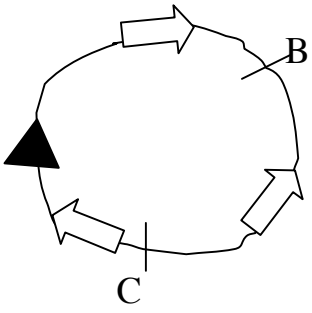
(c) 1st Hfr forms this F':



2nd Hfr forms this F':

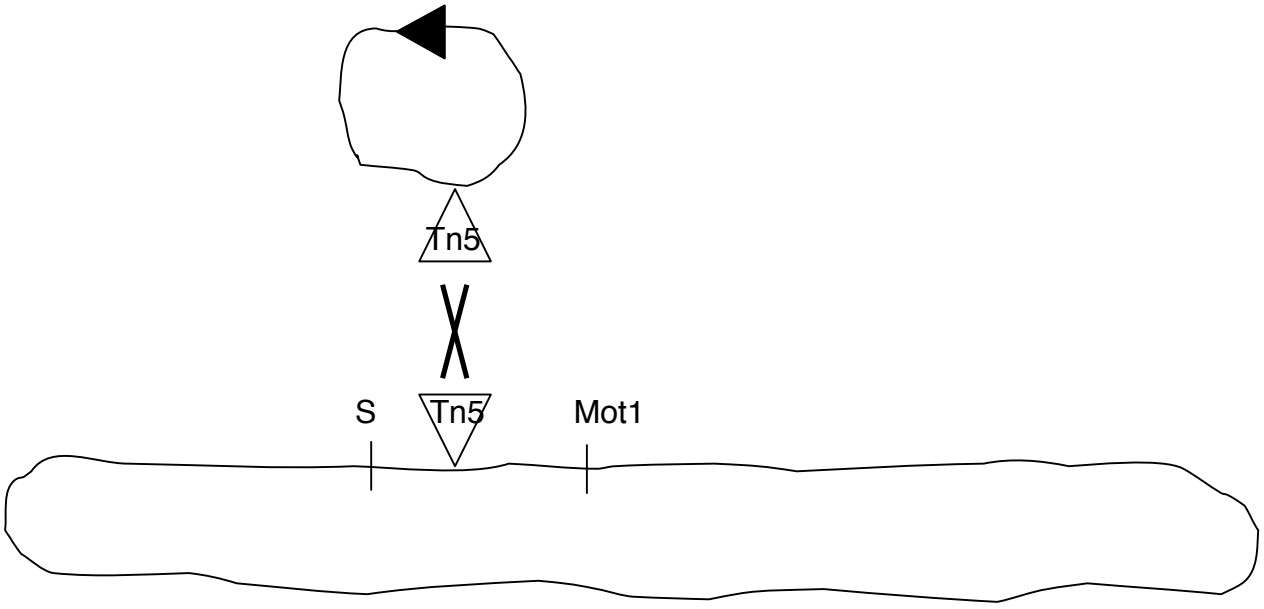


3rd Hfr forms this F':

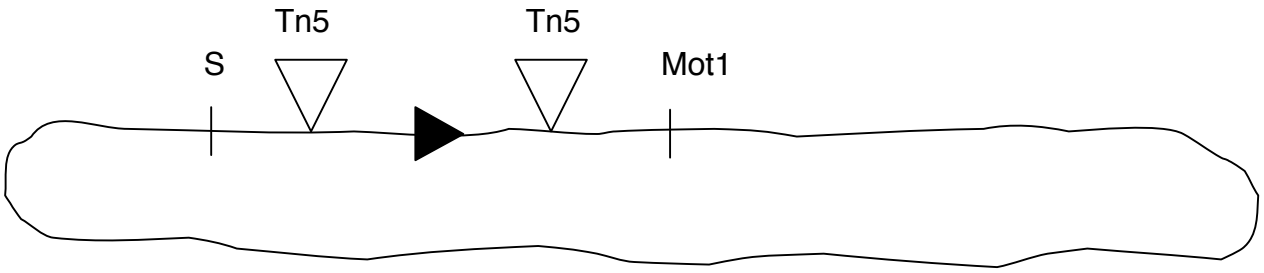


(d) 1st Hfr's F' transfers B and C
2nd Hfr's F' transfers B and C
3rd Hfr's F' transfers B and C

3. Transposons are not only useful as portable genetic markers, they can also serve as portable regions of homology for recombination.
(a) The F' recombining with the chromosome due to homology between the Tn5 insertions:

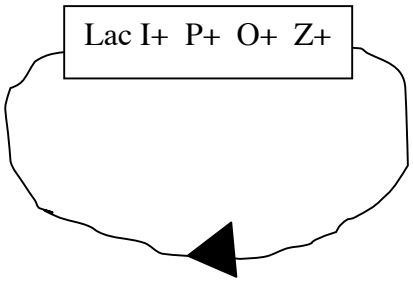


(b) The resulting Hfr:

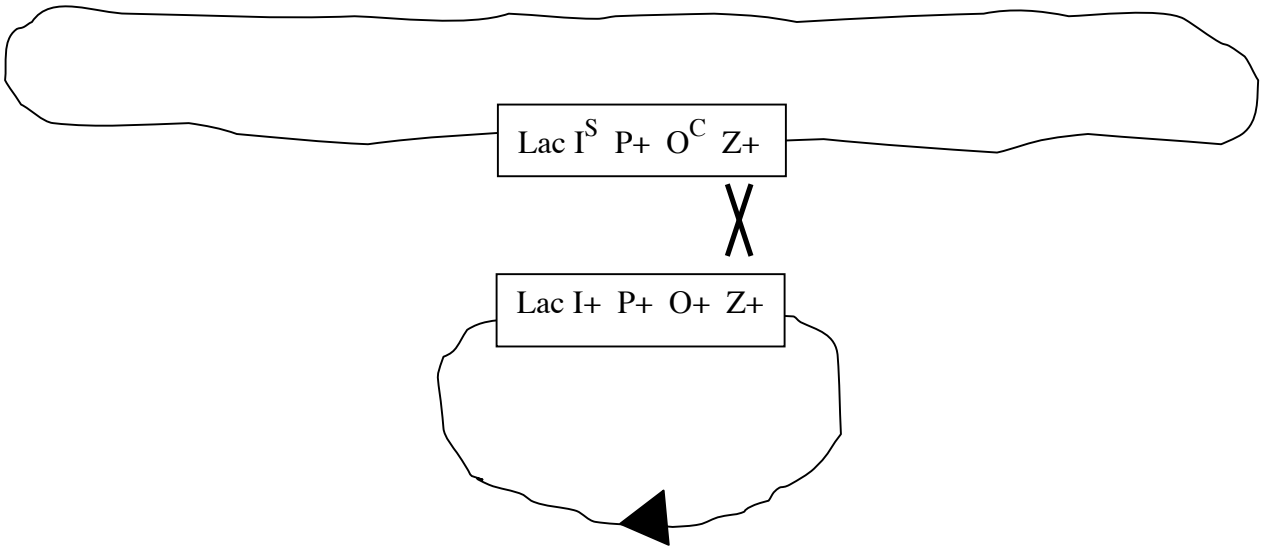


4. You are given a double mutant *E. coli* strain that you know contains an F' plasmid that carries the Lac genes, but you don't know precisely which alleles of these Lac genes are on the chromosome of the strain, or on the F' contained in the strain.
(a) the chromosome must contain LacP+ O^C Z+ and either $LacI^{---}$ or $LacI^{-d}$ or $LacI^S$ and the F' plasmid must contain LacI+ P+ O+ Z+
(b) the chromosome must contain $LacI^S$

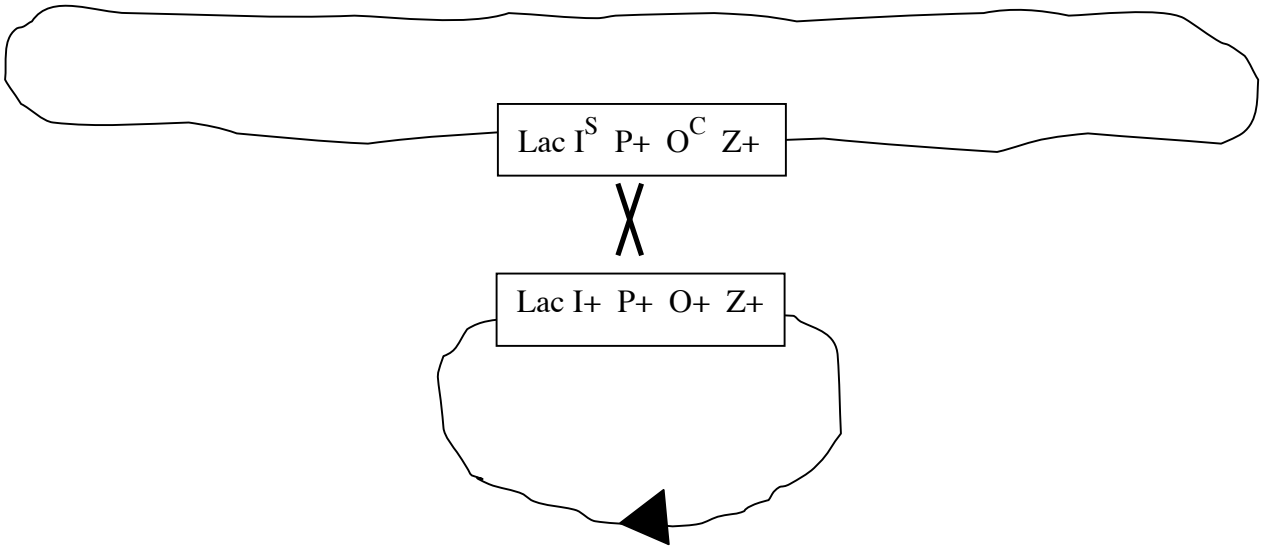
(c) the F' plasmid:



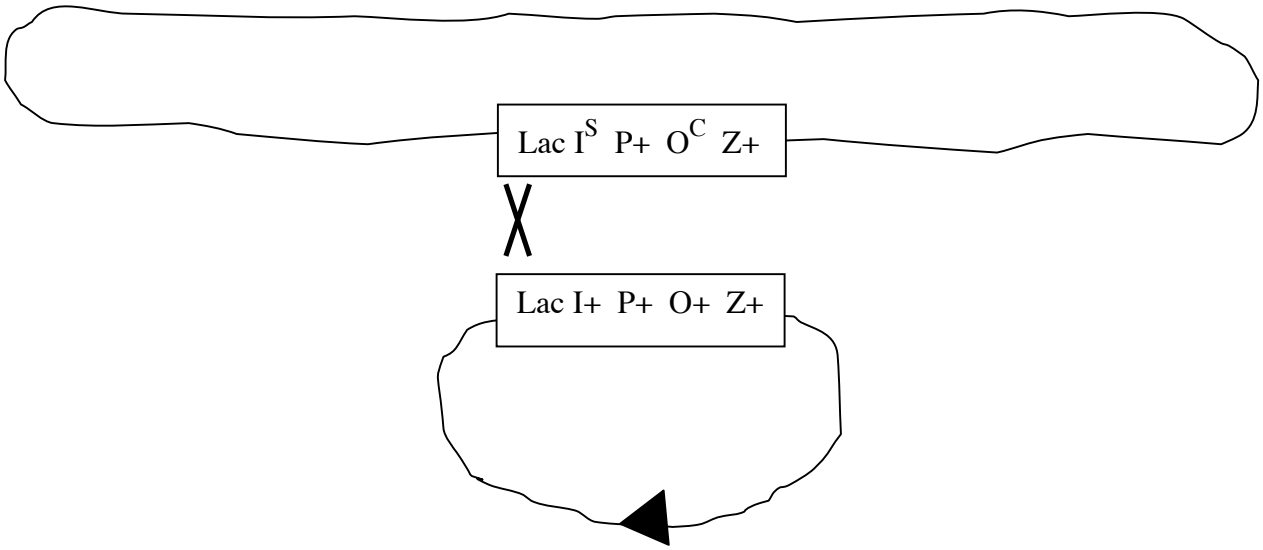
(d) The chromosome recombining with the F' to yield the first Hfr:



The chromosome recombining with the F' to yield the second Hfr:



The chromosome recombining with the F' to yield the third Hfr:



5. You are studying an interesting phenotype in a bacterial species related to *E. coli*, which is that some mutants of this species make dry, crusty looking colonies, instead of wild-type shiny colonies.

(a) There are two groups of insertions that are very close to each other, (1 and 4) and (2, 3, 5, and 6).

(b)

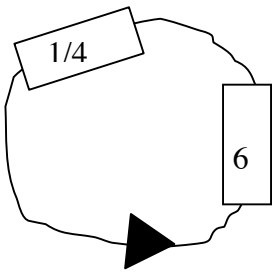
Two transposon insertions	Distance between them
1 and 4	100%
1 and 5	10%
1 and 6	24%
2 and 4	2%
2 and 5	100%
2 and 6	100%
3 and 4	15%
3 and 5	100%
3 and 6	100%

(c) These few exconjugants received an F' plasmid that contained Tn5::Kan.

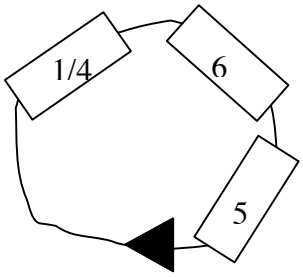
(d) It is restored by the presence of a wild-type copy of the gene that is present on the F' plasmid. This wild-type copy masks the mutant phenotype caused by the chromosomal mutation in that gene.

(e) The mating results differ because the two F' plasmids in these strains contain different genes.

F' in a:



F' in b:



(note that the origin of transfer in each F' could be oriented in the opposite direction)

(f) loss-of-function

(g) recessive

Regulation of lactose metabolism enzymes in bacteria

1. You have isolated five new *E. coli* mutants that do not properly regulate the expression of genes in the Lac operon.

(a) O^- (cis, constitutive, dominant)

(b) I^S (trans, uninducible, dominant)

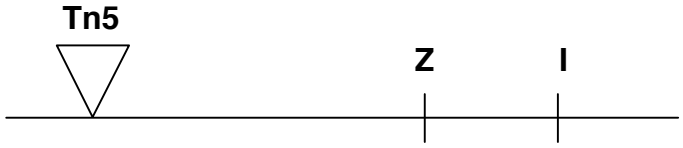
(c) I^- (trans, constitutive, recessive)

(d) I^{-d} (trans, constitutive, dominant)

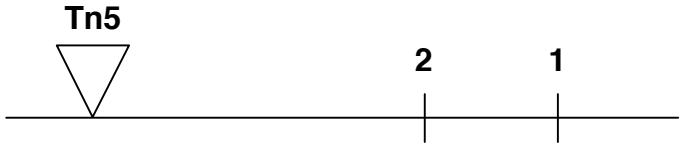
(e) P^- (cis, uninducible, recessive)

2. You have isolated an *E. coli* strain harboring a **Tn5** insertion that is linked to the **Lac** operon.

(a)



(b)



3. For the following merodiploid *E. coli* strains, determine the level ß-galactosidase expression in either the presence or absence of IPTG.

	-IPTG	+IPTG
lac I^{-d} Z^{+} / F' lac I^{+} Z^{-}	100	100
lac O^{+} Z^{-} / F' lac O^{c} Z^{+}	100	100
lac I^{+} Z^{-} Y^{-} / F' lac I^{s} Z^{+} Y^{+}	1	1
lac I^{+} O^{c} Z^{+} / F' lac I^{-d} O^{+} Z^{+}	200	200
lac I^{+} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}	101	101
lac I^{-d} O^{+} Z^{+} / F' lac I^{s} O^{+} Z^{+}	200	200
lac I^{-d} O^{c} Z^{+} / F' lac I^{s} O^{+} Z^{+}	200	200
lac I^{-d} O^{c} Z^{-} / F' lac I^{s} O^{+} Z^{+}	100	100

4. You have isolated two mutations in the *E. coli* Lac operon that cause constitutive expression of the LacZYA genes.
Classify each mutation as dominant or recessive and as cis- or trans-acting, giving the experimental result that allows you to arrive at each conclusion.

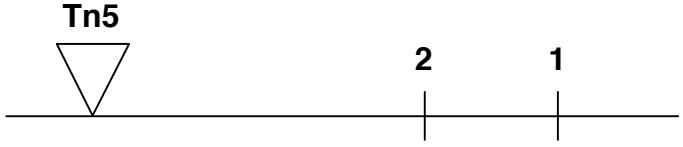
1: O^{-} (cis, constitutive, dominant)
2: I^{-d} (trans, constitutive, dominant)

5. Wild-type *E. coli* metabolizes the sugar lactose by expressing the enzyme ß-galactosidase.

(a) 18%

(b) they are not linked (distance is 0%)

(c)



(d) 3 and 1 were both recessive and in different genes

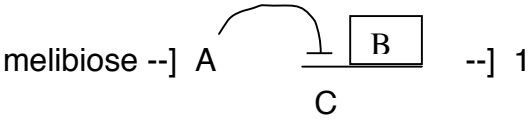
(e) either: 3 is dominant, OR 1 is dominant, OR both 3 and 1 are dominant, OR 3 and 1 were both recessive and in the same gene

Characterizing novel pathways that control the expression of bacterial genes

1. You are studying a new strain of *E. coli* that can utilize the disaccharide melibiose very efficiently.
(a) repressor, because constitutive recessive trans
(b) activator, because uninducible recessive trans
(c) melibiose –] A --] B → 1
OR
melibiose → B --] A --] 1
(d) melibiose –] A --] B → 1
(e) either an activator or a repressor, because constitutive, dominant, and trans
(f)
melibiose --] A --] B/C --] 1

(C is a coding region mutation in the B gene)

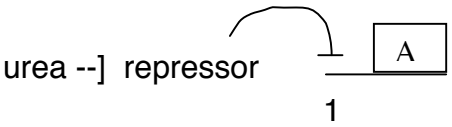
OR



(C is a non-coding region mutation in the regulatory sequences in front of the B gene)

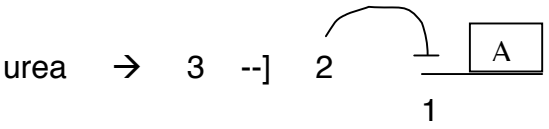
(g) superactivator, or operator⁻ (but the operator of MelB)

2. You are studying the ability of a bacterial strain to use urea as a nitrogen source.
(a) model #1: urea → 1 → A
model #2: urea --] 1 --] A
model #3:

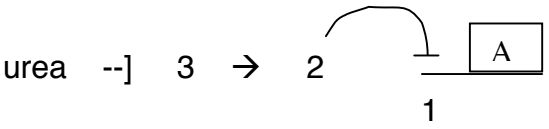


(b) models #1 and #3 still work
(c) it is an operator sequence
(d) repressor

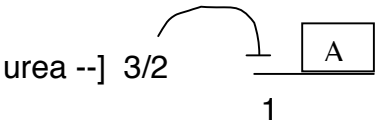
(e)



OR



(f)



3. Genes (such as the **Lac** and **Mal** genes) whose function is to metabolize compounds that can be used as energy (such as the sugars lactose and maltose) are often regulated in the sense that they are <u>induced</u> by the presence of substrate compounds.
(a) model #1: Y → A --] B → X
model #2: Y --] B --] A --] X
(b) model #1: uninducible
model #2: constitutive

4. You are studying the regulation of a new set of *E. coli* genes that are required to utilize the amino acid lysine as a source of nitrogen.

(a) 1: repressor, because constitutive, trans, recessive
2: activator, because uninducible, trans, recessive

(b) lysine –] 1 --] 2 → A
OR
lysine → 2 --] 1 --] A

(c) lysine → 2 --] 1 --] A

(d) either activator or repressor, because uninducible, trans, dominant

(e) lysine → 2 –] 1 --] 3 → A
OR
lysine → 2 –] 1 → 3--] A