Problem 1 (12 points) Gene regulation in bacteria utilizes the following terminology. 9 terms are given below (a – i), followed by six statements. Write the letter of the correct term on the line next to each statement. A single letter is sufficient. Not all answers will be used, but used answers will be used just once.

Term List

a. Positive control

b. Negative control

c. Inducible

d. Noninducible

e. Repressible

f. Constitutive

g. Operator

h. Repressor

i. Effector molecule

A. Phenotype with respect to -galactosidase synthesis for the partial diploid *I+ O+ Z+ / I– OC Z––* c. Inducible

B. Phenotype of *I+ O+ Z– / I+ OC Z+* f. constitutive

C. Phenotype of *I+ O+ Z– / I– OC Z–* d. non inducible

D. Regulatory molecule must be present at site in DNA (such as promoter) so that transcription occurs.

a. Positive control

E. Small molecules that bind to regulatory molecule, such as repressor i. effector molecule

F. Regulatory molecule that binds to operator region in DNA h. repressor

[2 points per correct line]

Problem 2: 20 points

In a theoretical operon, regions A, B, C and D represent the repressor gene, the promoter sequence, the operator sequence, and a structural gene encoding an enzyme, ***but not necessarily in that order.***

This operon is concerned with the metabolism of a theoretical effector molecule (tm). From the data in the table below, first decide if the operon is inducible or repressible in response to tm. Then assign regions A, B, C and D to the four parts of the operon.

|  |  |  |
| --- | --- | --- |
| Genotype | tm Present | tm Absent |
| A+B+C+D+ | AE | NE |
| A-B+C+D+ | AE | AE |
| A+B-C+D+ | NE | NE |
| A+B+C-D+ | IE | NE |
| A+B+C+D- | AE | AE |
| A-B+C+D+ / F’ A+B+C+D+ | AE | AE |
| A+B-C+D+ / F’ A+B+C+D+ | AE | NE |
| A+B+C-D+ / F’ A+B+C+D+ | AE + IE | NE |
| A+B+C+D- / F’ A+B+C+D+ | AE | NE |

(AE = active enzyme is produced; IE = inactive enzyme, an mRNA is transcribed and translated, but the enzyme produced is mutated and non-functional; NE = no enzyme produced at all)

Based on the data above, this system is (circle one): repressible or **inducible**

Match the gene/region to the proper description by placing the corresponding letter (A-D) on the line:

Repressor gene \_\_\_\_D\_\_\_

Promoter sequence \_\_\_\_B\_\_\_

Operator sequence \_\_\_A\_\_\_

Structural gene \_\_\_\_\_C\_\_\_\_\_

4 points for each item in red, no partial credit.

Hints for students: The promoter and structural gene are easily identified in the haploid genotypes, but you need the partial diploid to distinguish the repressor mutant from the operator mutant. So, once a student correctly sees that the promoter and structural gene are B and C, respectively, they can focus on only the partial diploids that help distinguish A and D.

Problem 3: (24 points) For the *E. coli* strains with the *lac* genotypes shown below, complete the chart using a (+) to indicate the presence of functional of beta-galactosidase and permease and a (-) to indicate lack of functional enzyme.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Lactose** | **Absent** | **Lactose** | **Present** |
| **Genotype** | **beta-galactosidase** | **permease** | **beta-galactosidase** | **permease** |
| I+ P+ O+ Z+ Y+ | - | - | + | + |
| I- P+ O+ Z+ Y+ | + | + | + | + |
| I+ P+ OC Z+ Y+ | + | + | + | + |
| I- P+ O+ Z+ Y- | + | - | + | - |
| I- P- O+ Z+ Y+ | - | - | - | - |
| I+ P+ O+ Z- Y+ / F’ I- P+ O+ Z+ Y- | - | - | + | + |
| I- P+ OC Z+ Y+ / F’ I+ P+ O+ Z- Y- | + | + | + | + |
| I- P+ O+ Z+ Y- / F’ I+ P- O+ Z- Y+ | - | - | + | - |
| I+ P- OC Z- Y+ / F’ I- P+ O+ Z+ Y- | - | - | + | - |
| I+ P+ O+ Z+ Y+ / F’ I+ P+ O+ Z+ Y+ | - | - | + | + |
| IS P+ O+ Z+ Y- / F’ I+ P+ O+ Z- Y+ | - | - | - | - |
| IS P- O+ Z- Y+ / F’ I+ P+ O+ Z+ Y+ | - | - | - | - |

0.5 points per box

Problem 4 (12 points) Consider now both the positive (CAP-mediated) and negative (lacI-mediated) regulation of the lac operon. State whether the transcription of lacZ be off, low, or high for each of the following situations:

1. lactose is present and glucose is absent (high) Off Low High
2. lactose is present, and the cell has a PCAP mutation (low) Off Low High
3. lactose is absent and glucose is present (off) Off Low High
4. both lactose and glucose are present (low) Off Low High
5. glucose is absent, and the cell has a P- mutation (off) Off Low High
6. both lactose and glucose are present, and the operon has an OC mutation (low) Off Low High

[2 points per response]

Problem 5: (20 points) Explain how the following mutations would affect the transcription of the yeast *GAL1* gene: [4 points per response, half credit available if the “bottom line” (eg, transcription would be off) is correct but the reasoning is wrong or incomplete]

a) A deletion within the *GAL4* gene that removes the N-terminal DNA binding domain of Gal4p.

If Gal4p N terminal domain is deleted, Gal4p cannot bind the UAS and cannot activate transcription of GAL1, thus GAL1 is not expressed.

b) A deletion of the entire *GAL3* gene.

If GAL3 is deleted, Gal3p cannot disrupt the interaction between Gal4p and Gal80p, and thus GAL1 transcription can never be turned on, even if galactose is present.

c) A mutation within the *GAL80* gene that blocks the ability of Gal80 protein to interact with Gal3 protein.

If Gal80p and Gal3p cannot interact, then GAL1 transcription cannot be turned on because Gal4p activation domain remains blocked by Gal80p.

d) A deletion of one of the four UAS elements upstream of the *GAL1* gene.

This will likely lead to a slight decrease in the activation of GAL1 transcription in the presence of galactose, but GAL1 will still be transcribed because the Gal4p at the other three sites will be able to activate transcription.

e) A point mutation in the *GAL1* promoter that alters the sequence of the TATA box.

GAL1 will never be transcribed. The TATA box is a core promoter element that is essential for transcription in eukaryotes.

Problem 6 (12 points) - 4 points per box

You have a cell line in your lab that contains a TRE (tetracycline responsive element, seven copies of the tetracycline operator, tetO) controlling expression of a green fluorescent protein (GFP), as shown in the diagram below:

TRE (7xTetO)

TATA

GFP

The construct above is integrated into the DNA of this laboratory cell line and is always present. There are two ways of regulating the expression of GFP in response to tetracycline in this system: by expressing the tetracycline transactivator tTA, or the reverse tetracycline transactivator rtTA.

You have made three versions of this cell line: one has only the TRE-GFP construct shown above, one has the TRE-GFP plus expresses tTA, and the third has TRE-GFP plus expresses rtTA. Unfortunately you’ve mixed up your cell flasks and don’t know which is which! You will need to add tetracycline to a sample from each flask and look for the presence or absence of GFP expression in order to figure this out.

Below are the results of the experiment you performed to identify your samples. In the right hand column, label the cell line with it’s identity:

A: TRE-GFP only

B: TRE-GFP + tTA

C: TRE-GFP + rtTA

(-) indicates no GFP expression, (+) indicates positive GFP expression

|  |  |  |  |
| --- | --- | --- | --- |
|  | no tetracycline | with tetracycline | cell line identity: (A, B or C) |
| cell line 1 | - | + | C |
| cell line 2 | - | - | A |
| cell line 3 | + | - | B |