University of Jijel

Faculty of Natural Sciences and Life

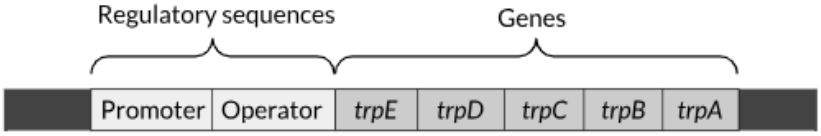
Department of Cellular and Molecular Biology

TD **Biotechnological applications of recombinant DNA (ABAR)**

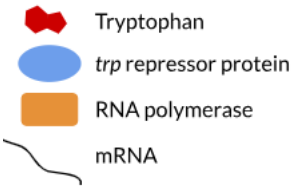
Exercise 1

1- The trp operon in *E. Coli* is a coordinately controlled set of genes involved in tryptophan biosynthesis. Tryptophan acts as a corepressor of the operon, which means it binds to and activates the trp repressor protein. This binding is more likely to occur when tryptophan is present at high levels in the cell.

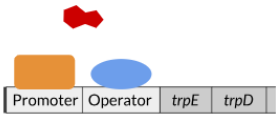
The following diagram represents the genes and regulatory sequences of the trp operon.

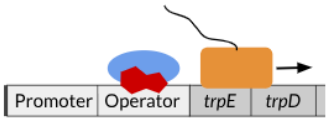


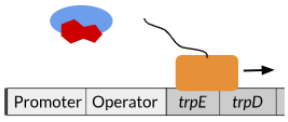
The following symbols represent the macromolecules involved in transcriptional regulation of the trp operon.

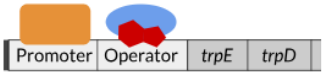


Which of the following diagrams best models the regulation of the *trp* operon when tryptophan is present at high levels?

A-


B-


C-


D-


2- In the regulation of the tryptophan (trp) operon in E. coli, …….
   a- The trp operon is typically repressed when tryptophan levels are high.
   b- The trp operon is subject to negative feedback regulation.
   c- The trp operon consists of a single structural gene.
   d- The trp operon is under positive control by the trp repressor.

3- How does the tryptophan operon halt transcription when tryptophan levels are sufficient?
   a- By preventing the binding of RNA polymerase to the promoter.
   b- By activating the trp repressor.
   c- By enhancing the transcription of the operon.

d- By promoting the binding of the trp repressor to the operator.

4- Why is the placement of the operator important in gene regulation?
   a- It directly codes for repressor proteins.
   b- The operator determines the gene's DNA sequence.
   c- Operator placement influences the timing of gene expression.
   d- Operators are involved in protein translation.

5- The tryptophan operon is regulated by ……
   a- Negative feedback
   b- Positive feedback
   c- Repression
   d- Activation

6- What is the name of the sequence responsible for regulating the trp operon through attenuation?
   a- Operator
   b- Attenuator
   c- Promoter
   d- Terminator

7- What is a result of a mutation in the lac operator that prevents release of the repressor protein?
   a- The lactose permease protein will be produced, even in the absence of lactose.
   b- Transcription of the structural genes will be blocked, even in the presence of lactose.
   c- The catabolite activator protein will be bound to the CAP site, even in the presence of glucose.
   d- Expression of the lacI gene will be repressed, even in the absence of glucose.

8- What is the primary role of the lac operon in E. coli?
   a- To metabolize glucose
   b- To transport lactose into the cell
   c- To synthesize tryptophan
   d- To metabolize lactose

9- Which component of the lac operon is responsible for positive control and activation of transcription?
   a- CAP
   b- lac operator
   c- Promoter
   d- lac repressor

10- The lac operon typically repressed…….
   a- In the absence of lactose
   b- In the presence of glucose
   c- In the absence of cAMP
   d- All at once.

11- What mechanisms prevent the lac operon from staying active indefinitely?
   a- Activation by cAMP.
   b- Repression by the lac repressor.
   c- Presence of glucose.

d- Lactose binding to the operator.

12- What are the key components of the CRISPR-Cas9 system, and what role does each component play?
   a- Cas3: Initiates DNA cleavage.
   b- gRNA: Serves as the location where Cas9 initiates DNA cleavage.
   c- tracrRNA: Binds to the target DNA.
   d- Cas9: Guides the guide RNA (gRNA) to the target sequence.
   e- Cas3: Recognizes and binds to the PAM sequence.
   f- gRNA: Directs Cas9 to the specific DNA location.
   g- Cas9: Makes precise double-stranded breaks in the target DNA.
   h- tracrRNA: Stabilizes the gRNA structure.

Exercise 2

With respect to the expression of β-galactosidase, what would be the phenotype of each of the following strains of *E. coli*?

a) I+, O+, Z+, Y+ (no glucose, no lactose)
b) I+, O+, Z-, Y+ (high glucose, high lactose)
c) I+, O+, Z+, Y+ (high glucose, no lactose)
d) I+, Oc, Z+, Y+ (no glucose, no lactose)
e) I-, O+, Z+, Y+ (no glucose, high lactose)
f) I-, O+, Z+, Y+ (high glucose, no lactose)
g) Is, O+, Z+, Y+ (no glucose, high lactose)

Exercise 3

Answers the following questions:

1- How does the CRISPRs system work?
2- What is the role of each gene in operon lactose primary purpose of using recombinant vectors in biotechnology?
3- What kind of reporter (transcriptional or translational) would you use to determine the expression pattern of GOI?
4- Researchers have developed a drug that disrupts gene expression in a parasitic species of fungi. The drug functions by preventing the complementary binding of tRNA with an mRNA molecule, what is the target of the drug? And Why?
5- How does CRISPR-Cas9 compare to other genome editing tools?
6- What kind of reporter would you use to determine the subcellular location of a protein of interest and why?
7- How does the lac repressor function to turn the lac operon "on" and "off" in response to lactose and glucose levels?
8- What kind of reporter would you use to test protein mobility between cells and why?
9- Can you cite five applications of tag reporters?
10- What is a self-processing model?

Exercise 4

1- Cite differences between immunohistochemistry and protein tag for protein localization and then expressed them in illustration.
2- In a table, present the distinctions between affinity tags and reporter tags in the production of fusion proteins discussing the different point: examples, size, objective, visualization, real time imaging, expression system compatibility, and impact on protein function.

Selma Hamimed