University of Jijel

Faculty of Natural Sciences and Life

Department of Cellular and Molecular Biology

Master 2 in Molecular and Cellular Biology

TD4 **Biotechnological applications of recombinant DNA (ABAR)**

Exercise 1

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 2

Fill in the marked cells (?) based on your understanding of the lac operon mechanism:

Glucose	Lactose	CAP binds	Repressor binds	Level of transcription
+	-			
+	+			
-	-			
-	+			

Exercise 3

Answers the following questions:

1- How does the CRISPRs system work?
2- 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?
3- What kind of reporter would you use to determine the subcellular location of a protein of interest and why?
4- What is a self-processing model?
5- What is the purpose of DNA methylation in eukaryotic cells?
6- Define the downstream processing?

7- Collect 3 examples of palindromic DNA sequences?

Exercise 4

Figure solutions to the following problem scenarios:

Scenario 1:

Your target protein shows significant degradation during purification despite using protease inhibitors. What are the major causes of protein degradation during purification? How can you identify the type of degradation occurring? Design a strategy to minimize protein degradation.

Scenario 2:

Jessica Harper, a 19-year-old female with relapsed/refractory B-cell acute lymphoblastic leukemia (B-ALL), is scheduled for CD19 CAR T-cell therapy after failing two lines of conventional chemotherapy.

Initial process

- Leukapheresis was performed successfully
- Initial T cell count: 600 cells/µL
- Viability post-collection: 94%
- Patient starts lymphodepletion protocol

During the manufacturing process, the following issues arise:

- Day 7: Poor T cell expansion (30% of expected)
- Day 10: Viability drops to 75%
- Day 14: Final product fails to meet release criteria
    - Achieved: Only 45% CAR T cells (required >70% CAR+ T cells)
    - Required viability: >80% Achieved viability: 76% (required >80%)

1-What are potential causes for poor T cell expansion?

2- How would you modify the manufacturing process for a second attempt?

Scenario 3:

The AgriGenomics Innovation Laboratory aims to develop a drought-resistant wheat variety for semi-arid agricultural regions. However, modifying the wheat genome presents multiple challenges; cite three of them. The experiment targeted genes involved in the expression of dehydration-responsive element-binding proteins, ABA biosynthesis, and stomatal regulation. After the transformation, how would you confirm successful drought tolerance?

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