Djilali Bounaama University – Khemis Miliana **Level: L3 – Microbiology**
Faculty of SNV/ST **Academic Year: 2024/2025**
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Course: Molecular Biology and Genetic Engineering (Tutorial Session)

Series No. 3

Exercise 1

1. **Explain the importance of cell lysis in the extraction of nucleic acids.**
   Hint: Discuss the different methods used to disrupt cells and release DNA or RNA.
2. **Why is it necessary to remove proteins during the extraction of nucleic acids?**
3. **What are the differences between agarose gel electrophoresis and pulsed-field gel electrophoresis (PFGE)?**
   In which situations would you use one rather than the other?
4. **Why are dyes such as ethidium bromide or SYBR Safe used in electrophoresis gels?**
   What is the difference between these stains?

Exercise 2

Imagine that you have a sample containing DNA fragments of different sizes: **500 bp, 1 kb, and 5 kb.**

- Draw an agarose gel (indicating the wells and fragments) and show where each fragment would appear after electrophoresis, assuming that the smallest fragment migrates the farthest through the gel.
- Explain why smaller fragments migrate faster than larger ones.

Exercise 3

After performing agarose gel electrophoresis, you obtain three distinct bands:
The first band is located near the well, the second in the middle of the gel, and the third near the bottom.

- Interpret the relative sizes of the DNA fragments represented by these bands.
  If you know that the fragment that migrated the farthest is **500 bp**, can you estimate the sizes of the other fragments?

Exercise 4

You have two agarose gels: one with **0.8% agarose** and another with **2% agarose**.
You have a mixture of DNA fragments of **500 bp, 1 kb, and 2 kb.**

- Explain which gel would be more suitable for separating large fragments and which would be more suitable for small fragments. Justify your answer.

Exercise 5

In agarose gel electrophoresis, buffers such as **TAE (Tris-Acetate-EDTA)** or **TBE (Tris-Borate-EDTA)** are used instead of water.

- Explain why it is necessary to use a buffer rather than water during electrophoresis.

Exercise 6

Read the following statements carefully and write **True (T)** or **False (F)**.
Correct the false ones.

1. PCR can directly amplify RNA molecules without any preliminary step.
2. The denaturation step consists of heating the DNA to separate its two strands.
3. During the annealing step, primers are degraded by Taq polymerase.
4. The extension step occurs at about 72 °C, which is the optimal temperature for Taq polymerase.
5. The role of primers is to stabilize DNA strands during denaturation.
6. Taq polymerase must be thermostable to resist the high temperatures used in PCR.
7. The melting temperature (Tm) of primers can be estimated using the formula: Tm = 4(G + C) + 2(A + T).
8. Real-time PCR (qPCR) allows monitoring and quantifying DNA amplification using fluorescent signals.
9. Multiplex PCR uses only one pair of primers to amplify several target genes at once.
10. The buffer provides Mg^{2+} ions required for the activity of DNA polymerase.

Exercise 7

A researcher wants to amplify a **1,200 bp gene** using the following conditions:

- Denaturation: 95 °C for 30 s
- Annealing: 60 °C for 30 s
- Extension: 72 °C

Questions:

1. How long should the extension step last for each cycle? (Hint: ~1 kb/min)
2. If the PCR runs for 30 cycles, how many copies of DNA are theoretically obtained from one initial molecule?
3. If one of the primers has the sequence 5'-ATGCGTAACTGGAATTCG-3', calculate its approximate Tm using the formula provided in the review.
4. Explain why incorrect annealing temperature can affect the specificity of PCR amplification.

Exercise 8

A student performs a PCR but observes **no amplification band** on the agarose gel.
Given the main components of a PCR reaction (template DNA, primers, dNTPs, polymerase, buffer, water), propose **four possible causes** of this failure and suggest **one corrective action** for each.