



Damietta University
Faculty of Science
Chemistry Department
Biochemistry Students
Level: Fourth year



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Course: Molecular Biology (2)
Course code: 402 Biochem
Degree: 90

Final exam on Molecular Biology (2)
Answer all the following questions (Exam in 4 pages)

Question 1: Choose the correct answer (33 Marks/1.5 per each)

- Which of the following is NOT a standard step in a Northern Blotting protocol?
 - RNA isolation
 - Hybridization with a nucleotide probe
 - Transfer of RNA to a nylon membrane
 - Protein gel electrophoresis.
- In a modern laboratory, which membrane is most commonly used for Western blotting due to its optimal combination of protein-binding capacity, durability, and suitability for reprobing?
 - Nylon.
 - PVDF
 - Nitrocellulose
 - Cellulose
- What is the primary factor determining sensitivity and specificity of Western Blotting?
 - Type of gel
 - Antibody quality
 - Transfer time
 - Incubation temperature
- Non-specific bands in Southern Blot → solution?
 - Decrease temperature
 - Increase temperature
 - Decrease washing
 - Increase exposure
- According to standard laboratory protocols, what is the typical length range for a labeled DNA probe used in blotting techniques?
 - 10–100 bp
 - 50–500 bp
 - 100–1000 bp
 - 1000–1500 bp
- In a two-color cDNA microarray comparing tumor tissue (red label) to normal tissue (green label), a spot appearing red indicates that gene is:
 - A tumor suppressor
 - Not expressed in either sample
 - More highly expressed in the tumor
 - An artifact or error
- In PCR, the denaturation step typically occurs at:
 - 50 - 65°C
 - 72°C
 - 94 - 98°C
 - 37°C
- The fluorescence from a microarray spot results from the binding of labeled cDNA. A yellow signal most specifically indicates that:
 - Only the green-labeled cDNA has bound
 - Only the red-labeled cDNA has bound
 - Both red- and green-labeled cDNAs have bound in roughly equal amounts
 - Neither cDNA has bound effectively
- The annealing temperature in PCR is critical for:
 - DNA denaturation
 - Primer specificity
 - Enzyme activation
 - Product labeling

- 10. The lac operon in E. coli is an example of:**
 a) Inducible gene expression
 b) Constitutive gene expression
 c) Repressible gene expression
 d) Transcriptional silencing
- 11. The purpose of PCR is to:**
 a) Sequence DNA
 b) Amplify a specific DNA sequence
 c) Digest DNA with enzymes
 d) Translate RNA into protein
- 12. The building blocks (nucleotides) added by the polymerase during PCR are called:**
 a) NTPs
 b) dNTPs
 c) rNTPs
 d) ddNTPs
- 13. Magnesium ions (Mg²⁺) in the PCR buffer are essential because they act as:**
 a) A pH buffer
 b) A detergent
 c) A cofactor for DNA polymerase
 d) A denaturing agent
- 14. The annealing temperature is primarily determined by the:**
 a) Length of the template DNA
 b) Melting temperature (T_m) of the primers
 c) Concentration of dNTPs
 d) Type of polymerase used
- 15. If you start with one DNA molecule, how many molecules will you have after 3 complete PCR cycles?**
 a) 4
 b) 8
 c) 16
 d) 32
- 16. The forward and reverse primers in a PCR should have:**
 a) Identical sequences
 b) Similar melting temperatures (T_m)
 c) Very different lengths
 d) High AT content
- 17. The fluorescent dye SYBR Green I works in qPCR by:**
 a) Binding to single-stranded DNA
 b) Intercalating into double-stranded DNA
 c) Being hydrolyzed by polymerase
 d) Binding to primer ends
- 18. COLD-PCR is used to:**
 a) Amplify DNA in the cold
 b) Enrich low-abundance mutant alleles
 c) Detect methylation
 d) Clone large fragments
- 19. Allele-Specific PCR uses primers that differ at the:**
 a) 5' end
 b) 3' end
 c) Middle
 d) Fluorescent tag
- 20. If you see no PCR product on a gel, you should first try:**
 a) Increasing the denaturation temperature
 b) Lowering the annealing temperature
 c) Adding more loading dye
 d) Running the gel longer
- 21. In a Multiplex PCR to detect 5 different pathogens, what is the most critical requirement for all primer pairs?**
 a) They must have identical lengths.
 b) They must work efficiently at the same annealing temperature.
 c) They must all have the same GC content.
 d) They must be labeled with different fluorophores.
- 22. In Gibson Assembly, overlapping ends of DNA fragments are joined. What enzyme activity is NOT part of the Gibson Assembly master mix?**
 a) 5' exonuclease
 b) DNA polymerase
 c) RNA polymerase
 d) DNA ligase

**Question 2: Put true (✓) or false (x) mark for the following Sentences:
(27 marks/ 1.5 per each)**

1. SDS-PAGE separates by shape and charge.
2. The 'parA' and 'parB' genes in a BAC vector are essential for the stable segregation of the plasmid to daughter cells during bacterial division.
3. High background solved by decreasing stringency.
4. In Next-Generation Sequencing (NGS), bridge amplification on a flow cell generates clusters where each cluster contains many different DNA sequences.
5. RNA-Seq detects low-abundance transcripts.
6. Microarrays analyze thousands of genes.
7. A key advantage of a cosmid vector over a standard plasmid is its ability to be packaged into bacteriophage lambda particles for highly efficient transduction into *E. coli*.
8. In a TaqMan assay, fluorescence increases when the probe is cleaved by the polymerase.
9. DNA microarrays used in single-cell analysis.
10. RNA-Seq completely replaced microarrays.
11. A primer's GC content should ideally be between 70-90%.
12. The typical length for PCR primers is 50-100 nucleotides.
13. mRNA separated by poly-A tail.
14. Ex vivo gene therapy modifies cells outside body.
15. Taq polymerase has proofreading (3'→5' exonuclease) activity.
16. Multiplex PCR allows amplification of multiple targets in separate tubes.
17. Oligo arrays use long fragments.
18. Assembly PCR (like Gibson Assembly) is used to join multiple DNA fragments.

Question 3: Complete the following sentences (15 Marks /1 per each)

1. Southern blotting is used to detect _____, while Northern blotting is used to detect _____.
2. The blotting technique best suited for detecting protein phosphorylation is _____ blotting.
3. Introducing a functional gene to treat a genetic disorder is known as gene _____.
4. The two most common types of membranes used for protein immobilization in Western blotting are _____ and _____.
5. Enzymes that cut DNA at specific sequences are called _____ enzymes.
6. A gene for antibiotic resistance on a vector allows for _____ of transformed cells.
7. The process of taking up foreign DNA by bacterial cells is called _____.
8. The dideoxynucleotides (ddNTPs) used in Sanger sequencing lack a _____ group at the 3' carbon.
9. The process of breaking DNA into small fragments and attaching adapters for NGS is called _____ preparation.
10. The NGS approach that sequences by adding one fluorescent nucleotide at a time is called sequencing by _____.
11. The heat-stable DNA polymerase from *Thermus aquaticus* is called _____.
12. The temperature at which 50% of primer-template duplexes dissociate is the _____ temperature (T_m).
13. The enzyme that converts RNA into cDNA is _____.
14. PCR that uses two sequential sets of primers for high specificity is called _____ PCR.
15. The chemical used to treat DNA before Methylation-Specific PCR is sodium _____.