Name: **Enrolment No:**	UPES UNIVERSITY WITH A PURPOSE

UNIVERSITY OF PETROLEUM AND ENERGY STUDIES
End Semester Examination, May 2022

Course: Instrumentation in Microbiology — **Semester: II**
Program: M.Sc. (Microbiology) — **Time: 03 hrs.**
Course Code: HSMB7015 — **Max. Marks: 100**

Instructions: Read question carefully.

SECTION A

S. No.	MCQ's /Fill in the blanks/ True & False (1.5 marks each)	**30 Marks**	**CO**
1	A researcher is trying to alter five successive base pairs in the middle of a PCR amplified DNA fragments. PCR mutagenesis is the most recommended for performing such experiment-----True/False	**1.5**	**CO3**
2	PCR needs thermal cycling, and the heat-stable (Taq) polymerases, will not denature to lose efficacy in DNA polymerization during the high temperature (95^{o}C) step.----- True/False	**1.5**	**CO3**
3	**Why are vent polymerase and Pfu more efficient than the Taq polymerase?** (a) Because of proofreading activity (b) Because of more efficient polymerase activity (c) Both a and b (d) None of the above	**1.5**	**CO3**
4	The pH at which a protein carries a net zero charge is termed as_____.	**1.5**	**CO3**
5	**What is the main purpose of polymerase chain reaction (PCR) in molecular biology?** a) Separating DNA fragments b) Amplifying DNA sequences c) Purifying DNA samples d) Analyzing protein samples	**1.5**	**CO3**
6	**Which technique uses a pH gradient to separate proteins based on their isoelectric points?** a) PCR b) Isoelectric focusing c) Centrifugation d) Gel electrophoresis	**1.5**	**CO3**
7	_____ is the first stage of the two-stage two-dimensional PAGE?	**1.5**	**CO3**
8	**Which enzyme is commonly used in polymerase chain reaction (PCR) to synthesize new DNA strands?** a) DNA polymerase b) RNA polymerase c) Reverse transcriptase d) Ligase	**1.5**	**CO3**

9	**What is the purpose of a DNA ladder in gel electrophoresis?** a) To measure the size of DNA fragments b) To amplify DNA sequences c) To separate proteins based on their isoelectric points d) To separate DNA fragments based on their size	**1.5**	**CO3**
10	**Isopycnic or equal density centrifugation is achieved in_____**	**1.5**	**CO1**
11	_____ is used for the detection of particular protein in total cell lysate.	**1.5**	**CO1**
12	**Which of the following is NOT a type of gel used in gel electrophoresis?** a) Agarose b) Polyacrylamide c) Sephadex d) Agar	**1.5**	**CO2**
13	Ultrastructure of a cell organelle can best be studied through transmission electron microscope (TEM)--True/False	**1.5**	**CO1**
14	Buoyant density centrifugation is carried out at centrifugal force of_____ for____hours.	**1.5**	**CO1**
15	Organelles can be separated from cell homogenate through density gradient centrifugation---True/False	**1.5**	**CO1**
16	**What is the purpose of a control sample in gel electrophoresis?** a) To measure the size of DNA fragments b) To amplify DNA sequences c) To separate proteins based on their isoelectric points d) To compare with experimental samples for interpretation of results	**1.5**	**CO2**
17	**What is the main advantage of isoelectric focusing over other electrophoresis techniques?** a) Higher resolution for large DNA fragments b) Ability to separate proteins based on both size and charge c) Faster results d) Lower cost	**1.5**	**CO2**
18	In SDS-PAGE, sodium dodecyl sulfate (SDS) is used to break _____ bonds of proteins.	**1.5**	**CO2**
19	What is the role of Ammonium per sulphate (APS) in SDS-PAGE?	**1.5**	**CO2**
20	What is the function of DNA loading dye in agarose gel electrophoresis?	**1.5**	**CO2**
	SECTION B (5 marks each question)		
Q	Short Answer Type Question (5 marks each) Scan and Upload 4 questions 5 marks. Word limit (100-120)	**20 Marks**	**CO**
1	Mention the difference between rate-zonal and isopycnic centrifugation.	**5**	**CO1**
2	Write down the different types of polymerase chain reaction (PCR) and their respective applications in molecular diagnostics.	**5 (2+3)**	**CO2**
3	Write different designs of Pulsed-field gel electrophoresis (PFGE). Mention the applications of PFGE.	**5 (3+2)**	**CO3**
4	Provide a comparative analysis between Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM).	**5**	**CO1**
	SECTION C 30 marks		
Q	**Two case studies 15 marks each subsections**	**30 Marks**	**CO**

1	Case Study 1 (Word limit-250-300) You are amplifying a portion of blood group antigen binding adhesin A (BabA) gene from *Helicobacter pylori* by polymerase chain reaction (PCR). The organism was isolated from the stool samples of an infected individual. After the agarose gel electrophoresis of amplified PCR products, you observed non-specific amplification or smear. **Q1:** What could be the reasons behind the observation? **Q2:** How are you going to troubleshoot the problem? **Q3:** What is primer dimer? **Q4:** What is nested PCR? **Q5:** Why bovine serum albumin (BSA) was added in a PCR reaction?	**15 (4+4+2 +3+2)**	**CO3**
2	Case Study 2 (Word limit- 250-300) Assume that you are performing an Immunofluorescence assay (IFA) to study the localization of a target protein inside a cell. Post of your sample preparation and followed by imaging through fluorescent microscopy, you have identified any one of the following problems: **A.** No fluorescence was observed. **B.** A thin outline of fluorescence on the surface of the cell was visible under microscope. **C.** Cell shape is distorted. **Q1:** What could be the reasons behind the observations of A, B and C. **(3x2)** **Q2:** Provide potential troubleshooting for each of the cases (A, B and C). **(3x3)**	**15 (6+9)**	**CO1**
	SECTION- D 20 marks		
Q	Long Answer type Questions Scan and Upload (10 marks each) Word limit 200-250	**20 Marks**	**CO**
1	You are working in a microbiology laboratory and have been asked to analyze a bacterial sample for DNA fingerprinting using pulse field gel electrophoresis (PFGE). **Q1**: List the equipment and reagents you would need for performing PFGE. **Q2**: Write down the step-by-step procedure for performing PFGE on the bacterial sample. **Q3**: Discuss the advantages and limitations of PFGE as a molecular typing method in bacterial identification.	**10 (3+4+3)**	**CO3**
2	Assume that you are analyzing the expression of your target protein in whole cell lysate of transformed bacterial cells by SDS-PAGE followed by Western Blotting using specific antibodies. Post development of your blot, you have identified any one of the following problems: **A**: Nonspecific or diffuse bands **B**: Weak or no bands **C**: High background in blot	**10 (3+6+1)**	**CO3**

	Q1: Provide inference for the observation of A, B and C **(3x1)** **Q2:** Analyze the above observations and potential troubleshooting for these. **(3x2)** **Q3:** If TEMED (tetramethylethylenediamine) is not added in SDS-PAGE gel, what could be the consequence?		