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

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

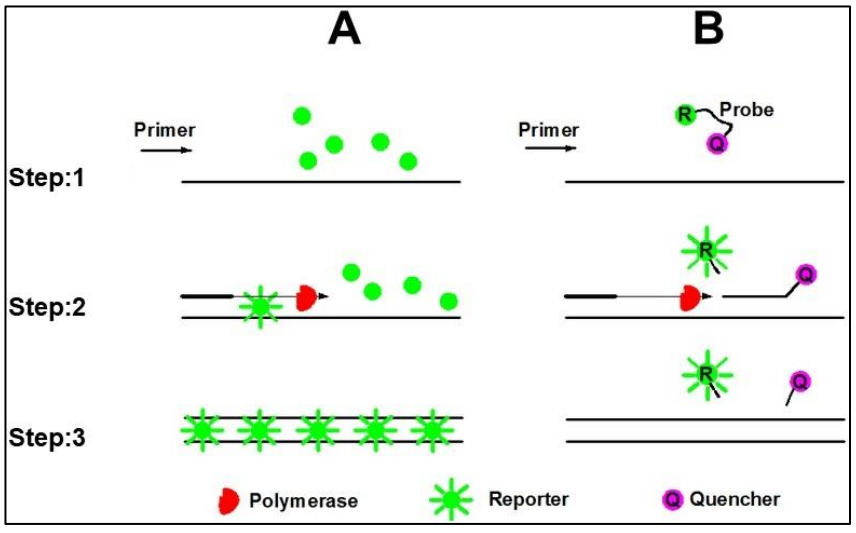
Course: Instrumentation in Microbiology — **Semester: II**
Program: M.Sc. (Microbiology) and M.Sc. (Nutrition & Dietetics) — **Time: 03 hrs.**
Course Code: HSMB7015 — **Max. Marks: 100**

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	**A hydrophobic compound will preferentially partition into an aqueous solvent. True or false?**	**1.5**	**CO3**
6	The process of passing a mobile phase through a chromatography column is called _____.	**1.5**	**CO3**
7	_____ is the first stage of the two-stage two-dimensional PAGE?	**1.5**	**CO3**
8	**Ion exchange chromatography is based on_____?**	**1.5**	**CO3**
9	**_____ is used as a carrier gas in gas chromatography?**	**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	**Time and location of DNA synthesis can be studied by means of** **a)** Extracting DNA at regular intervals from different parts **b)** Electron microscopy **c)** Carbon dating **d)** Radioactive DNA precursors	**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	**Which of these is/are the property of real-time PCR assays?** **A.** Incorporate dyes that bind double-stranded DNA. **B.** Incorporate an internal hydrolysis probe. **C.** Be performed at a single temperature with no specialized instrumentation required. **D.** Be interpreted as a plus/minus result or as a quantitative result.	**1.5**	**CO2**
17	**Which of the following is incorrect about a microarray?** **A.** It is a slide attached with a high-density array of immobilized DNA oligomers representing the entire genome of the species under study. **B.** Array of immobilized DNA oligomers cannot be cDNAs. **C.** Each oligomer is spotted on the slide and serves as a probe for binding to a unique complementary cDNA. **D.** It is the most commonly used global gene expression profiling method.	**1.5**	**CO2**
18	In SDS-PAGE, sodium dodecyl sulfate (SDS) is used to break _____ bonds of proteins.	**1.5**	**CO2**
19	**The sample is fractionated by electrophoresis through a _____. As all the proteins now have an _____ to mass ratio, they are separated on the basis of their _____.** a) Acrylamide solution, identical charge, mass b) Polyacrylamide gel, identical charge, density c) Polyacrylamide gel, identical density, mass d) Polyacrylamide gel, identical charge, mass	**1.5**	**CO2**
20	**Which of the following can prime the reverse transcriptase reaction?** A. Target sequence-specific primers. B. Random hexamers. C. Oligo dT primers. D. All of the above.	**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	Mention the working steps for TLC analysis. Write the name of two absorbents used in Stationary phase of TLC technique.	**5 (4+1)**	**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	**15 (4+4+2 +3+2)**	**CO3**

	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?		
2	Case Study 2 (Word limit- 250-300) Laser scanning confocal microscopy represents one of the most significant advances in optical microscopy ever developed, primarily because the technique enables visualization deep within both living and fixed cells and tissues and affords the ability to collect sharply defined optical sections from which three-dimensional renderings can be created. Development of modern confocal microscopes has been accelerated by new advances in computer and storage technology, laser systems, detectors, interference filters, spectral technology, and fluorophores for highly specific targets. During working with a confocal microscope, you found certain problems related with the microscope, which are provided below. Identify the reasons behind these problems with potential solutions. **1:** The bulb is on, but image cannot be seen or is dark. **2.** Image is unclear, blurred or has insufficient contrast. **3.** Image is partially obscured or unevenly illuminated. **4.** Excessive glaring. **5.** Power switch indicator does not light up.	**15 (3x5)**	**CO1**
	SECTION- D 20 marks		
Q	Long Answer type Questions Scan and Upload (10 marks each) Word limit 200-250	**20 Marks**	**CO**
1	The below figure describes the working principles of a molecular method:  **Q1:** Identify the name of the method. **Q2:** Write basic difference between strategy "A" and strategy "B". **Q2:** Mention the basic steps associated with each of the strategy. **Q4:** Mention the advantages and limitations of the method.	**10 (1+1+4 +4)**	**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 **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?	**10 (3+6+1)**	**CO3**