

MANIPAL INSTITUTE OF TECHNOLOGY MANIPAL

A Constituent Institution of Manipal University

V SEMESTER B.TECH. (BIOTECHNOLOGY ENGINEERING) END SEMESTER EXAMINATIONS, NOV/DEC 2017

SUBJECT: ANIMAL, PLANT BIOTECHNOLOGY AND BIOETHICS

[BIO3101]

REVISED CREDIT SYSTEM

Time: 3 Hours

MAX. MARKS: 50

Instructions to Candidates:

- ✤ Answer ALL the questions.
- Missing data may be suitable assumed.

1 A .	Ms Jeena is a Ph.D scholar in plant biotechnology analyzing the genomes and the barcodes. (a) She is comparing the cp genomes of wheat, rice and maize. Justify whether she can make out an evolutionary relationships among these cereals using cp genome? How? (b) She claims that the cp genome wheat contains inverted repeats. Do you agree? Justify your view. c) A recent news article reveals that DNA barcodes identified adulteration in medicinal rice plants. How these barcodes are generated? (d) Can you suggest a primer for DNA barcoding?	4
1B.	Andrews is working with maize. He realizes that some of his plants are unable to produce non-functional pollen. (a) How will you analyze this and what are your conclusions? (b) Can he correlate the effect of non-functional pollen to any genomes? If so which genome? Explain	3
1C.	Prof Wang is working with genome evolution in Duck weeds. His team finds that the C-value of DNA content in duckweed family varied nearly thirteenfold, ranging from 150 megabases (Mbp) in <i>Spirodela polyrhiza</i> to 1,881 Mbp in <i>Wolffia arrhiza</i> . (a) Why there is a wide variation in the C-Value? (b) Can he correlate genome size difference to the size of different duck weeds? Justify your reasoning (c) His project student finds that there is wide variation in the chromosome number of <i>Lemna</i> , a duck week. He approaches you for a proper reasoning for this result. How will you help him?	3
2A.	 a) Bunchy top disease of banana is a viral disease and is a reason for the financial loss of banana farmers. They do not know initially whether the saplings they purchased are infected or not. How will you provide disease free plants to them using tissue culture technology? Develop a proper plan for your idea and illustrate as a flow chart. b) Assume that you are applying for a student grant on the topic to "prepare a simple, inexpensive delivery unit of tissue culture propagated plants" by direct sowing method. Develop a proposal to this call and justify your statements. 	4

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2B.	Strychnine is a secondary metabolite from a medicinal tree <i>Strychnos nux-vomia.</i> (a) Develop a plan for <i>in vitro</i> production of strychnine (b) How will you scale up your cultures for this natural product. Illustrate with the help of a flow chart.	3
2C.	Prof. Swathi is working on <i>in vitro</i> propagation of bell pepper. She has developed methods for direct regeneration and indirect regeneration. (a) She wants to produce thousands of clones for a hybrid variety. Develop a protocol and justify (b) She realizes that there is a wide variation in the plants she has developed. What can be the reasons? Justify (c) The chromosome number of viable and flowering bell pepper is 2n=104. Can she develop a plant with 52 chromosomes? How?	3
3A.	You are a project head for an entrepreneur based on plant biotechnology. One of the research team develops a protocol for the purification of an active ingredient nick named as XAB. The team approaches for their protection of intellectual property. How you will help them?	2
3B.	 Mr. Mohan is a Biotechnology HR manager. His company is going to recruit fresh graduates. You are on the final round of his selection process. He is going to analyze you based on your inputs in the following ideas. (a) Banana is widely consumed by all kinds of people. The company wants to produce Vitamin A containing banana fruit (b) Mr Balu in his company is on a request to purchase a 50 psi helium pressure gun. Prepare a justification note for his purchase request (c) Ms Kavitha is wasting a lot of money and time in developing a transformation protocol using electroporation. Suggest her an inexpensive, simple and easy protocol for her and convince her to use the technique proposed by you. 	3
3C.	a) What do you mean by pre-treatment of culture vessels? What are the different methods by which you can achieve this? Which kind of cells (Adherent/Suspension) requires pretreated culture vessels?b) List out any two advantages of procuring cell line from culture collection centers.	3
3D	Ms. Ritu was culturing Human glioblastoma (adherent) cells in her lab. For one of her experiment she seeded (1 X 10 ⁵ Cells) in culture flask, containing media with 10% FBS. She incubated the flask in CO ₂ incubator (5% CO ₂ & 37°C). After 48 hours, complete media was evaporated from the flask. What could be possible reason for the observed problem and suggest a solution.	2
4A.	 a) Ms. Keerthi is working in cell culture lab and she is encountering problem with retrieval of cells following cryopreservation. Brief description of the problem is given below. List out all the possible reason for the observed problem and suggest an action plan for each cause. Nature of the problem Cause Action plan Poor recovery of cells following student Ms. Shruthi to establish a cell line from freshly collected foreskin biopsy sample. Suggest a most appropriate method. Justify 	4

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A Constituent Institution of Manipal University Ms. Roopa was maintaining murine cell lines in her lab. For routine culture she used to supplement media with penicillin and streptomycin. Mention any 3 4B. four disadvantages of using antibiotics in routine maintenance of cell culture. Why it is advised to supplement antibiotics in culture medium during primary culture? How antibiotics are sterilized? Mr. Sandeep isolated a natural compound Juglone, from roots of black walnut and wanted to test its efficacy against B16F1 melanoma cells. A fixed number (5×10^5) of exponentially growing cells were seeded and allowed to attach for 24 hr. Following which cells were treated with different concentrations of juglone (5, 10, 15 and 20 μ M) for 1 h. The cells without any treatment served as control. At the end of the treatment, the whole medium of control and treated groups was used to perform LDH assay. The results of assay is shon below. 35 30 3 4C. LDH Levels (U/L) 25 20 15 10 0 10 15 Juglone concentration (µL) Based on this information, answer the following questions. a) Discuss the result of LDH assay. What conclusion can be drawn from this experiment b) Comment on the principle of LDH assay. Dr. Prabhath intended to study the effect of drug (Prednisolone) in human cornel epithelial cells (hCECs). hCECs were cultured in appropriate medium (with 10% FBS) in the presence of various concentrations of drug (prednisolone) for 3 days and subjected to MTT and BrdU assays. The cells without any treatment served as control. Results of MTT assay (A) and BrdU assay (B) are shown below. 2.5 В Δ 0.4 2.0 0.3 Absorbance Absorbance 1.5 4 5A. 0.2 1.0 Drug- 4µg Drug-12µg 8µ0 4µg 12µg 8µg Control Control 0.1 0.5 Drug-Drug-Drug-0.0 0.0 Experimental Groups Experimental Groups Based on the given information answer the following questions. a) Does the drug has cytotoxic effects on human cornel epithelial cells (hCECs)? Justify b) What could be the objective of Dr. Prabhath to perform BrdU assay? Discuss the results of this experiment. c)Write a short note on priciple of BrdU assay

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5B.	Ms. Vihani intended to cryopreserve the CHO cells, thus want to count the cells before freezing. She trypsinized the CHO cells. Following detachment of the monolayer cells, she neutralized effect of trypsin by the addition of 6 mL culture medium with 10% FBS. From this cell suspension, she pipetted out 20µL and mixed with same volume (20µL) of trypan blue for cell counting. She counted both stained and unstained cells and results are shown below. $ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	3
	b) Express the number of viable cells in the cell suspension.c) If she has to seed 19,75,250 cells/cryo vial (for cryopreservation) how much volume she has to pipette out from the cell suspension?	
5C.	a) Compare and contrast trademark and trade secrets.b) Name any two regulatory agencies in United States of America for Bioethics and Biosafety.	3