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V SEMESTER B.TECH. (BIOTECHNOLOGY ENGINEERING)

END SEMESTER EXAMINATIONS, NOV/DEC 2016

SUBJECT: ANIMAL, PLANT BIOTECHNOLOGY AND BIOETHICS

[BIO3101]

REVISED CREDIT SYSTEM
(11/11/2015)

Time: 3 Hours MAX. MARKS: 50

Instructions to Candidates:

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

1A.	Solasodine is an alkaloid found in many members of the family Solanaceae. Based on this idea, (i) Develop an *in vitro* system for the production of solasodine (ii) The readings of the dry cell wt on every three days (for a culture grown upto 21 days) are 0.15, 0.30, 0.45, 0.55, 0.60, 0.62, 0.62 mg dry cell wt. The yield of solasodine is 0.0125, 0.0225, 0.0301, 0.0399, 0.0489, 0.0602, 0.0752 μg/g dry cell wt. respectively. How will you illustrate this data on your answer sheet? Use the graph sheet provided (iii) Ms Sneha wanted to locate solasodine inside a cell. Which part she is most likely to locate? Why? (iv) Assume that Mr Ketan found a plant in which leaf tissues only accumulate solasodine. If Ketan develops a culture from stem pieces, can he produce solasodine? Explain	**4**
1B.	Mr. Justin is recently appointed as an agriculture officer. He founds that in his village the availability of enough planting material (seeds) for brinjal is less. (i) He asks the regional agricultural station to find out a rapid protocol to solve this issue. What is your idea for them? (ii) The lab also developed a slower protocol in two years which uses synthetic seeds. Whether this is going to help the issue faced by farmers? Justify? (iii) What are the possibilities that farmers rejects synthetic seeds?	**3**
1C.	Wild *Oryza* species possess valuable characteristics that, if introduced into cultivated forms of rice, can increase both the resistance against pests and tolerance to environmental stress. (i) Using the tissue culture technology, develop a method for achieving this aim? (ii) Mr Arun has achieved this target by using PEG. What was the logic of using PEG? Why he has used it? (iii) Can he use influenza based viral vectors for transfer of gene to rice? Justify	**3**
2A.	Using a figure, how will you logically explain the architecture and potential use of chloroplast genome in biotechnology?	**4**
2B.	(i) What is the logic of the statement "*Agrobacterium*" as a natural genetic engineer? (ii) Can we transfer a big sized gene of ~10 MB size using this	**3**

	method? If not which method is useful for this? (iii)How is the T-DNA is transferred to plant cells?	
2C.	Mr. Varun was working in a project entitled "Accumulation of reserpine in cultured plant cells". He standardizes a method for the efficient purification of reserpine from *in vitro* cultures of *Rauwolfia serpentina.* (i) Can he patent this technique? Why? (ii) Who is the patent granting authority in India? (iii) How he can process her patent application?	**3**
3A.	Rossy, Pinky and Gincy are friends, but from different labs such as plant, microbial and animal. For their curiosity they conducted one experiment. Rossy found out that the onions in her lab are having a genome size of 1.5 X 10^{10} bp, while Pinky finds a bigger genome ie, 6.8 X 10^{11} on amoeba cells. Gincy decided to use her own cells to isolate DNA and finds that the size of the genome is 3.4 X 10^{9} bp. How will you explain these differences?	**2**
3B.	A novel virus has been discovered infecting plants with a size of 500 kb of its 150 kb are virulent regions. How will you improvise this natural process into a vehicle to deliver genes to plants? Explain	**3**
3C.	Ms. Geetha intended to monitor the expression of novel non fluorescent protein during tissue regeneration. She wanted to choose Fluorescence microscopy method for monitoring. Indicate two different methods by which she can label the novel protein by fluorescent marker. Write advantages and disadvantages of two methods which you have suggested.	**3**
3D	Dr. Girish got two samples from Kasturba Hospital, Manipal. From sample 1, he has to establish primary culture/cell line. From sample 2 (Patient specimen) he had to identify the disease. Which class of biological safety cabinet you will suggest him to process two types of samples. Justify with proper reasoning	**2**
4A.	a) Dr. Praveen wanted his student Ms. Ritika to establish a primary culture/cell line from freshly isolated mouse lung tissue. Collagen and elastin are the major composition of the lung tissue. Suggest a best suited method for tissue disaggregation with valid justification b) Ms. Navami wanted to order culture plates/flasks for the routine maintenance of cell lines. While discussing with her supervisor, he suggested her to order for pretreated culture plates/flasks. What do you mean by pre-treatment of culture plates/flasks? Mention any one method of culture plate/flasks pre-treatment. Which among these two requires pre-treated culture plates/flasks for proper growth. 1) Adherent cells 2) Suspension cells. Justify your answer	**4**
4B.	a) Name the important serum protein which helps in cell attachment b) Name the serum protein with anti-trypsin activity c) Name any two cell culture collection center	**3**
4C.	Ms. Seema has isolated curcumin — an active compound found in the rhizome (underground stem) of turmeric. This compound believed to augment cell proliferation "*in vitro"*. Suggest an assay to measure cell proliferation "*in vitro"* and write the principle of the assay.	**3**
5A.	Dr. Suresh isolated a non-fluorescent dye which he believes only stains the live cells, hence planned to utilize this dye in cell viability measurement. He wanted to compare the dye efficiency with Neutral red. In this line, mouse	**4**

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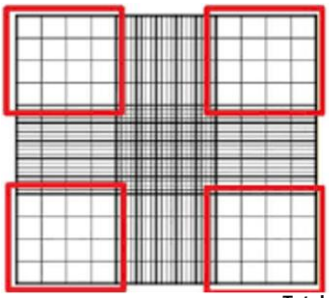
	skin fibroblast cells were trypsinized, following detachment of the monolayer cells, he neutralized effect of trypsin by the addition 4.2 mL culture medium with 10% FBS. From this cell suspension (containing fibroblast cells), he pipetted out 20µL and mixed with 20µL of isolated dye for cell counting using improved Neubauer's chamber. Another 20 µL cell suspension mixed with 20µL of Neutral red for cell counting using improved Neubauer's chamber. He counted both stained and unstained cells in both the methods separately and results are shown below.  **Improved Neubauer's Chamber (Highlighted 4 squares are used for counting)** **<u>Isolated dye method</u>** Total Stained & Unstained cells (Including 4 squares)- 350 No of stained cells- 320 (Including 4 squares) **<u>Neutral red method</u>** Total Stained & Unstained cells (Including 4 squares)- 347 No of stained cells- 318 (Including 4 squares) From the data given above answer the following questions a) Express the cell density/mL for both the methods (Neutral red & Isolated dye method) b) Calculate the number of viable cells in the cell suspension for both the methods c) Calculate the percentage of viability for both the methods. Based on the above results, do you think that the isolated non-fluorescent dye has the potential to replace/substitute neutral red for cell viability measurement?	
5B.	Dr. Sridhar isolated alkaloid "vincristine" from a medicinal plant *Catharanthus roseus*. This alkaloid compound is believed to effective against Neuroblastoma (cancer of nerve tissue) by induction of programmed cell death. However he wanted to test this hypothesis in human neuroblastoma (SH-SY5Y) cell line. He treated the human neuroblastoma cells with 10 µg of compound for 12 h. Upon treatment following assays were selected to test his hypothesis. 1. ATP bioluminescence assay 2.MTT assay 3. Annexin V-FITC & PI (dual) staining a) Which of the above mentioned assay will be more appropriate to test his hypothesis? Justify with suitable reasoning. b) Write the principle of the assay which you have suggested.	**3**
5C.	Mention five different ways of protection of Intellectual Property Right. Write a note on each method highlighting its advantages and disadvantages.	**3**

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