SEMESTER VII

Paper I **MBCR4701** Advanced Cell and Molecular Biology (100 marks)

Part A: Epigenetic regulation & Advanced Molecular Biology Techniques

Unit I: Genome Analysis: YAC-BAC-PAC; Genome walking (outline); Next-Gen Sequencing-

(6 lectures) UnitII Transcriptome Analyses: Differential display; Microarray analyses; EST analyses from (4 lectures) cDNA library (an outline) Unit III: Study of interactions within cells: Yeast two-hybrid (2 lectures) (2 lectures)

Unit IV Display: phage (outline)

Unit V Epigenetic Regulation: Concepts of chromatin and chromatin remodeling (nucleosome structure and function, higher order compaction, histone proteins) Histone modifying enzymes; chromatin remodelers ; Chromatin Techniques: Nucleosome mapping; MNase digestion; DNaseI footprinting; Indirect end-labelling; Restriction enzyme accessibility assay; Chromatin immunoprecipitation; ChIP on Chip (14 lectures)

Teachers involved:

Dr. R. Nag Chaudhuri

Recommended texts:

1) Chromatin & Gene Regulation- Bryan Turner; Chapters- 3,4,5,6,8

2) Epigenetics- C. David Allis et al; Chapters 3, 4

3) Principles of Gene Manipulation & genomics-Primrose & Twyman; Chapters- 2,5,6,11,13, 23

Part B: Cellular processes

Unit I: Post translational processes : GFP: Protein location. (8 lectures) Unit II: Protein Processing and Quality Control in the Endoplasmic Reticulum: Formation of disulfide bonds, processing of carbohydrates and folding, unfolded protein response, ERAD, protein transport in bacteria, protein import into chloroplast and mitochondrial matrix (an outline)

(12 lectures)

Unit III: Secretory pathway organelles and vesicular transport: transport assays, processing of carbohydrates, molecular mechanisms. Lysosomes and endocytic pathways. (8 lectures) Unit IV: Post transcriptional processes: Alternative splicing, Nonsense mediated decay, tmRNA, MicroRNA (siRNA, miRNA) (an outline) (8 lectures)

Teachers involved: Dr. C. Barat

Recommended texts: Molecular Cell Biology Lodish and Darnell et. al (5th edition)

Paper II

MBCR4702 Microbial Biotechnology (100 marks)

Unit I: Introduction to Food Preservation Technology: Food Preservation by use of Heat (Pasteurization and Canning), Low temperature, Radiation, Chemicals, Antioxidants, Dehydration and Osmotic pressure. (6 lectures)

Unit II: Fermentation Technology involved in production of microbiologically-fermented food products: Idli, Butter, Soy Sauce and Cheese. (4 lectures)

Unit III: Fermentation: Overall stages of fermentation process; Classification on the basis of dependence of product formation on energy metabolism; Parameters for fermentation: productivity, yield coefficients, heat production (Definition and equation only); Three-phase system of fermentation; Sterilization of gases and nutrient solution for industrial fermentation: culture media and fermentation air, batch sterilization and continuous sterilization; Scale up: definition and significance, solid substrate fermentation. (**3 lectures**)

Unit IV: Bioreactors: Basic bioreactor design, construction and operation; classification of bioreactors (conventional and specialized); design, principle of operation and applications of conventional bioreactors (batch, fed-batch and continuous); design, principle of operation, kinetics and applications of chemostat and turbidostat; specialized bioreactors (air-lift, fluidized-bed, bubble-column, pulsed-column, membrane-bioreactor, photobioreactor). **(8 lectures)**

Unit V: Kinetics of microbial growth & death, and fermentation kinetics: Growth and fermentation kinetics in batch and continuous cultures; microbial death kinetics; monitoring microbial growth in culture; analysis of mixed microbial populations. (4 lectures)

Unit VI: Downstream processing: Removal of microbial cells and solid matter, flocculation and floatation, filtration, centrifugation, cell disruptions, chromatography, extraction, crystallization and precipitation, drying. (2 lectures)

Unit VII: Introduction to Bioprocess Technology: Hydrogenation, oxidation, esterification, polymerization. (2 lectures)

Unit VIII: Enzyme Biotechnology: Sources of enzymes; Enzyme production involving isolation, purification, encapsulation and immobilization; immobilized enzymes (or whole cells) and their industrial application; representative enzymes: lipases, proteases, amylases, glucose isomerase, glucoamylase, pectinase, rennin, L-asparaginase; enzymes used in medicines and different industries. (3 lectures)

Unit IX: Use of Microbes in Industry and Agriculture: Strain development of industrially important microorganisms; Bioconversion and Biotransformation; Single Cell Protein: production and application; Single Cell Lipids; Industrial production of Chemicals: alcohol (ethanol), acids citric, acetic, gluconic and lactic), solvents (glycerol, acetone, butanol), antibiotics (penicillin,

streptomycin, tetracycline), vitamins (Riboflavin, B_{12} , β -carotene) and amino acids (L-Lysine, glutamic acid), pharmaceuticals and therapeutic agents (interferon, monoclonal antibodies, vaccines), oleo chemicals (fatty acids, glycerol, methyl-petrochemicals, perfumery chemicals); paper industry; Biosurfactants; Biofertilizers; Bioinsecticides, Biodiesel. (30 lectures)

Unit X: Recombinant Microorganisms for commercial products: Metabolic engineering for cloning and overexpression of heterologous genes; limitations in metabolic engineering; synthesis of commercial products like ascorbic acid, indigo, amino acids, antibiotics and biopolymers.

(2 lectures)

Unit XI: Brief outline of the following: Bioremediation; Bioleaching; Use of microbes in mineral benefication and oil recovery; Biofilm; Biosensors. (8 lectures)

Teachers involved:

Dr. A. Roy Choudhury (Fermentation, Downstream processing, Enzyme Biotechnology, Use of microbes in industry and agriculture, Recombinant microorganisms for commercial products, Brief outline of the following).

Prof. S. Roy (Introduction to Food Preservation Technology; Fermentation Technology involved in production of microbiologically-fermented food products; Bioreactors; Kinetics of microbial growth & death, and fermentation kinetics; Introduction to Bioprocess Technology; Use of microbes in industry and agriculture: citric acid, L-Lysine, Riboflavin, Vitamin B_{12} , antibiotics - penicillin, streptomycin, tetracycline).

Recommended texts:

- 1) Food Microbiology Frazier, Westhoff (4th Edition) Chapters 5, 6, 7, 8, 9, 10;
- Industrial Microbiology An Introduction Michael J. Waites, Neil L. Morgan, John S. Rockey, Gary Higton (2002 Indian Reprint Edition) Chapters 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15;
- 3) A Text Book of Biotechnology R. C. Dubey (2005 Reprint Edition) Chapters 14-18, 21;
- 4) Text Book of Biotechnology Dr. H.K. Das (3rd Edition) (Reference) Chapters 17, 18;
- 5) Research papers on selected topics.

<u>Paper III</u> <u>MBCR4703</u> <u>Animal Biotechnology I</u> (50 marks)

Unit I: Fertilization and assisted reproductive technology: Fertilization and its biochemical aspects, prevention of polyspermy, concept of cleavage and blastula, In vitro fertilization in human, Induced breeding in fish. (8 lectures)

Unit II: Morphogenesis: Fate maps and their generation. Gastrulation and formation of germ layers in amphibians, chick and mammals. (5 lectures)

Unit III: The Organizer concept: Spemann's experiments, embryonic induction and organization of the organizer, competence, potency and differentiation. (2 lectures)

Unit IV: Organogenesis and Development: (a) Cell aggregation in Dictyostellium.

(b) Paracrine signalling and vulva formation in *C. elegans*.

(c) Sex determination and reproductive development in *Drosophila*, mammals and reptiles.

(d) Placentation in mammals: formation and types of placenta, hormonal factors, functions of placenta.

(e) Gene regulation during development: Segmentation in *Drosophila*, homeotic gene complexes and the *Hox* genes.

(f) limb development in vertebrates.

(8 lectures)

Unit V: Regeneration and Ageing: regeneration of salamander limbs, regeneration in *Hydra*, compensatory regeneration in mammalian liver, ageing and biology of senescence. (3 lectures) **Unit VI: Neurobiology:** (a) Development of the brain, formation and differentiation of the neural tube, tissue architecture of the CNS

(b) Development of the vertebrate eye, retina, lens and cornea differentiation

(c) Neurodegenerative diseases and modern neurocognitive aspects. (6 lectures)

Unit VII: Stem Cells: embryonic and adult stem cells; extrinsic and intrinsic self renewal factors; stem cell therapies – cell based therapies, therapeutic cloning, cord blood stem cells. (4 lectures)

Teachers involved:

Dr. A. Banerji (Units II, III, IV, V, VI a, b) Dr. P. De (Units I, VI c)

Recommended texts:

1) Developmental Biology. S.F. Gilbert (8th ed.). Chapters 1, 10, 11 (Unit II, III), 2, 9, 16, 17 (Unit IV), 18 (Unit V), 12 (Unit VI a & b), 21 (Unit I)

2) Foundations of Embryology. B.M. Carlson (6th ed.). Chapters 3, 4, 5 (Unit I), 6 (Unit II), 8, 12 (Unit IV), 15 (Unit VI b).

3) The Cell – A Molecular Approach. G.M. Cooper, R.E. Hausman (5th ed.) Chapter 17 (Unit VII).

4) Neurobiology. G.G. Matthews (2nd ed.). Chapters 12, 21 (Unit VI c)

5) Relevant scientific literature (Unit VII).

MBCR4753 Animal Biotechnology Practical I (50 marks)

Unit I: Identification: (a) osteology: skull of *Bufo* sp., venomous snake, *Columba* sp., *Cavia* sp.; typical vertebrae of *Bufo* sp., *Columba* sp., *Cavia* sp.; limb bones of *Cavia* sp.

(b) non-chordate specimens: *Paramecium* sp., *Sycon* sp., *Hydra* sp., *Physalia* sp., *Taenia solium*, *Fasciola hepatica*, Ascaris sp., Ancylostoma sp., leech, Daphnia, Cyclops, Mysis, Culex sp. (female), *Anopheles* sp. (female), honey bee queen (Apis sp.), termite queen (Termes sp.), Pila sp., Achatina sp., Asterias sp., Echinus sp., sea cucumber, Balanoglossus sp.

(c) chordate specimens: Branchiostoma sp., Ascidia sp., Scoliodon sp., Catla catla, Labeo rohita, Cirrhinus mrigala, Clarias batrachus, Heteropneustes fossilis, Anabas testudineus, Hyla sp., Bufo sp., Rana sp.

Unit II: Study of life cycles: Drosophila sp., silkworm.

Unit III: Histological studies: (a) study of epithelial tissue by methylene blue staining

(b) H/E staining and identification of sections of mammalian adrenal, kidney, liver, pancreas, thyroid, testis, ovary, spleen, stomach and lung

(c) detection of carbohydrates in tissue sections by PAS method.

Unit IV: Determination of parameters of water quality: dissolved O₂, dissolved CO₂, alkalinity, hardness.

Unit V: Animal cell culture: Preparation of media, animal tissue culture.

Unit VI: Developmental biology: Observation and identification of chick embryo at 24, 48, 72 and 96 hrs incubation.

Teachers involved:

Dr. A. Banerji Dr. P. De

<u>Paper IV</u> MBCR4704

Recombinant DNA Technology II (50 marks)

Unit I: Types of PCR- colony PCR, inverse PCR, nested PCR, Ligation-mediated PCR, Real-timePCR, RACE, Primer extension, Site-directed mutagenesis by PCR(4 lectures)Unit II: cDNA Library construction and screening(4 lectures)Unit III: Eukaryotic expression systems - expression vectors in yeast (S. cerevisiae – YES, and P.pastoris – pPICZ); gene expression in animal cells - pcDNA3.1/His and pSecTag2/Hygro; viralvectors – adenoviral and retroviral (pLenti expression system).Insect expression system - baculovirus system.(6 lectures)Unit IV: Genetic manipulation of animals: knock-out mice by targeted disruption by homologous

Unit IV: Genetic manipulation of animals: knock-out mice by targeted disruption by homologous recombination in ES cells; conditional knock-out by site specific recombination (Cre/loxP system); advanced transgenic technology – inducible expression system – tet on/off system.

(6 lectures)

Unit V: Application of recombinant DNA technology - Applications of rDNA technology in molecular diagnostics, therapeutic proteins – DNA vaccines, biosynthesis of therapeutic antibodies by phage display method (through seminar presentations by students)

(8 lectures worth seminars by students) (4 classes alloted for solving problems)

Teachers involved:

Dr. R. Nag Chaudhuri (Unit I & II) Dr. U Siddhanta (Units III, IV, V)

Recommended texts:

Principles of Gene Manipulation & genomics. Primrose & Twyman (7th ed.). Chapters - 2,6 (Unit I, II) & 11, 12, 13, 15 (Unit III, IV), 26 (Unit V)

<u>MBCR4754</u> <u>Recombinant DNA Technology Practical II</u> (50 marks)

1. λ Hind III Digestion followed by ligation

2. Ni-NTA protein purification (from cell extract, SDS PAGE)

3. Transformation: pBluescript vector/ XL1 Blue i) Recombinant/ non-recombinant vector ii) Linearised/ Circular vector

4. a) Plasmid DNA preparation from recombinant vector b) Release of insert: i) Single digestion ii) Gel extraction c) PCR from purified recombinant vector

Teachers Involved:

Dr. R. Nag Chaudhuri Dr. J. Dasgupta