MAULANA ABUL KALAM AZAD UNIVERSITY OF TECHNOLOGY, WEST BENGAL

(Formerly West Bengal University of Technology)

Syllabus of B. Sc in Microbiology

(Effective from 2023-24 Academic Sessions)

Semester-V

Course Name: Recombinant DNA Technology **Code: BMMC-5301**
Credits: 3 **Total hours: 45**

Aim of the course: To acquaint students with basics of Recombinant DNA Technology

Course objectives: To impart the knowledge about different molecular tools and application, mutagenesis, genetic engineering in animals and plants

Sl	Graduate attributes	Mapped modules
CO1	To acquire comprehensive knowledge of molecular cloning tools, including restriction-modification systems, DNA-modifying enzymes, and various cloning and expression vectors.	M1
CO2	To develop technical proficiency in diverse DNA transformation and gene delivery methods, and demonstrate analytical skills in DNA, RNA, and protein analysis using molecular biology techniques	M2
CO3	To demonstrate proficiency in PCR-based techniques and DNA sequencing methods for genomic analysis and molecular diagnostics.	M3
CO4	To gain analytical competence in constructing and screening genomic and cDNA libraries and utilize advanced molecular tools for studying gene organization, expression, and genome profiling.	M4
CO5	To apply recombinant DNA techniques to develop genetically engineered organisms and bioproducts for therapeutic and agricultural purposes.	M5
CO6	To utilize genetic engineering techniques to create transgenic organisms and develop biotechnological solutions in medicine and agriculture	M6

Learning outcome

The candidates should demonstrate the comprehensive knowledge and practical skills in molecular cloning techniques, DNA amplification and sequencing in genetic engineering of animals and plants for research and biotechnological advancements They will be able to construct and screen genomic and cDNA libraries and utilize recombinant DNA technology for developing transgenic organisms and biotechnological products. Practical skills in gene

delivery methods and molecular analysis will be developed to support research and applications in genetics and biotechnology.

Module Number	Content	Total hours	% of questions	Bloom level (applicable)	Remarks, if any
THEORY					
M1	Molecular Cloning- Tools and Strategies	**12**	**26%**	**2,3**	**NA**
M2	Methods in Molecular Cloning	**10**	**23%**	**3**	**NA**
M3	DNA Amplification and DNA sequencing	**8**	**21%**	**3**	**NA**
M4	Construction and Screening of Genomic and cDNA libraries	**5**	**10%**	**3**	**NA**
M5	Random and site-directed mutagenesis	**5**	**10%**	**2,3**	**NA**
M6	Applications of Recombinant DNA Technology	**5**	**10%**	**3**	**NA**
Total Theory		**45**	**100**		

Detailed Syllabus

Module 1: Molecular Cloning- Tools and Strategies

Cloning Tools; Restriction modification systems: Types I, II and III. Mode of action, nomenclature, applications of Type II restriction enzymes in genetic engineering DNA modifying enzymes and their applications: DNA polymerases. Terminal deoxynucleotidyl transferase, kinases and phosphatases, and DNA ligases. Cloning Vectors: Definition and Properties. Plasmid vectors: pBR and pUC series Bacteriophage lambda and M13 based vectors Cosmids, BACs, YACs Use of linkers and adaptors. Expression vectors: *E. coli* lac

and T7 promoter-based vectors, yeast YIp, YEp and YCp vectors, Baculovirus based vectors, mammalian SV40-based expression vectors

(Total Hours: 12)

Module 2: Methods in Molecular Cloning

Transformation of DNA: Chemical method, Electroporation, Gene delivery: Microinjection, electroporation, biolistic method (gene gun), liposome and viral-mediated delivery, *Agrobacterium* - mediated delivery: Use of *Agrobacterium tumefaciens* and *A. rhizogenes*, Ti plasmids, Strategies for gene transfer to plant cells, Direct DNA transfer to plants, Gene targeting in plants, Use of plant viruses as episomal expression vectors.

DNA, RNA and Protein analysis: Agarose gel electrophoresis, Southern - and Northern - blotting techniques, dot blot, DNA microarray analysis, SDS-PAGE and Western blotting.

(Total Hours: 10)

Module 3: DNA Amplification and DNA sequencing

PCR: Basics of PCR, RT-PCR, Real-Time PCR, primer-design. Sanger's method of DNA Sequencing: traditional and automated sequencing. Primer walking and shotgun sequencing

(Total Hours: 8)

Module 4: Construction and Screening of Genomic and cDNA libraries

Genomic and cDNA libraries: Preparation and comparison of Genomic and cDNA library, screening of recombinants: Colony hybridization and colony PCR, Chromosome walking and chromosome jumping, reverse transcription. Genome mapping, DNA fingerprinting

(Total Hours: 5)

Module 5: Random and site-directed mutagenesis

Primer extension and PCR based methods of site directed mutagenesis, Random mutagenesis, Gene shuffling, production of chimeric proteins, Protein engineering concepts and examples (any two).

(Total Hours: 5)

Module 6: Applications of Recombinant DNA Technology

Genetic engineering in animals: Production and applications of transgenic mice, role of ES cells in gene targeting in mice, Therapeutic products produced by genetic engineering-blood proteins, human hormones, immune modulators, antisense molecules and vaccines (one example each). Gene therapy. Genetic engineering in plants: Bt transgenic - cotton, brinjal.

(Total Hours: 5)

Suggested Readings

1. Brown TA. (2010). Gene Cloning and DNA Analysis. 6th edition. Blackwell Publishing, Oxford, U.K.

2. Clark DP and Pazdernik NJ. (2009). Biotechnology: Applying the Genetic Revolution. Elsevier Academic Press, USA

3. Primrose SB and Twyman RM. (2006). Principles of Gene Manipulation and Genomics, 7th edition. Blackwell Publishing, Oxford, U.K.

4. Sambrook J and Russell D. (2001). Molecular Cloning-A Laboratory Manual. 3rd edition. Cold Spring Harbor Laboratory Press

5. Wiley JM, Sherwood LM and Woolverton CJ. (2008). Prescott, Harley and Klein's Microbiology.McGraw Hill Higher Education

6. Brown TA. (2007). Genomes-3. Garland Science Publishers 7. Primrose SB and Twyman RM

Course Name: Lab on Recombinant DNA Technology Code: BMMC-5391
Credits: 2 Total Hours: 30

1. Isolation of chromosomal DNA from plant cells.
2. Isolation of chromosomal DNA from *E.coli*.
3. Qualitative and quantitative analysis of DNA using spectrophotometer.
4. Plasmid DNA isolation.
5. Restriction digestion of DNA.
6. Making competent cells.
7. Transformation of competent cells.
8.Demonstration of PCR.

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Course Name: Immunology **Code: BMMC-5302**

Credits: 3 **Total hours:**

Aim of the course: To acquaint students with basics of Immunology

Course objectives: The goal of this course is to provide students an awareness of the immune system and its components, as well as the defense mechanisms that can create immunity and the immune system's role in the development of cancer, allergies, and autoimmune diseases. It also aims to address the Immunological Techniques and role of vaccination in immunity.

Sl	Graduate attributes	Mapped modules
CO1	The student will understand the basic ideas of both innate and adaptive immunity. Through the contributions of important scientists, the student will acquire knowledge about the evolution of immunology across time.	M1
CO2	The student will able and explain the functions of different immune cells in immunological defense as well as will understand the primary and secondary lymphoid organs' anatomy and role in immunity.	M2
CO3	The student will learn the characteristics of antigen, such as its size, complexity, and foreignness. They will be able to differentiate between entire antigens, epitopes, and haptens and will comprehend the function of adjuvants and distinguish between T-dependent and T-independent antigens.	M3
CO4	The student will understand the structure, classifications, and roles of antibodies. Using VDJ recombination, the student will investigate the genetic foundation of antibody diversity.The student will learn about the uses of chimeric and monoclonal antibodies.	M4
CO5	The student will learn the organization of MHC genes in humans and mice. They will also comprehend how MHC molecules function in the pathways that process and present antigens.	M5
CO6	The student will gain knowledge of the complement system components and their activation via classical, alternative, and lectin pathways. They will also understand the biological outcomes of complement activation, including cell lysis and inflammation.	M6
CO7	The student will understand how the immune system generates primary and secondary immune responses. They will learn the	M7

	mechanisms behind humoral and cell-mediated immunity and will explore immune tolerance and the roles of CTLs and NK cells in immune defense.	
CO8	The student will learn the causes and examples of autoimmune diseases, hypersensitivities, and immunodeficiencies and they will be introduced to immune responses in cancer, tumor antigens, and approaches to cancer immunotherapy.	M8
CO9	The student will develop an understanding of key immunological techniques such as ELISA, flow cytometry, immunoblotting, and immunofluorescence. They will also be able to interpret results from immunoassays and apply them in diagnostic and research settings.	M9
CO10	The student will understand the principles and types of vaccines, including DNA and recombinant vaccines. They will learn the differences between passive and active immunization and will recognize the role of WHO and national programs in global immunization efforts.	M10

Learning outcome: To impart basic knowledge about the following

- The fundamentals of innate and adaptive immunity, including their components and mechanisms of action.
- The structure and function of immune cells and organs that coordinate the immune response.
- The chemical nature, structure, and classification of antigens and antibodies, and their role in immune recognition.
- The genetic and molecular basis of antibody diversity and the principles behind monoclonal antibody technology.
- The organization and function of MHC (Major Histocompatibility Complex) in antigen presentation.
- The complement system pathways and their role in enhancing immune responses.
- The mechanisms involved in the generation of humoral and cell-mediated immune responses and immunological tolerance.
- The pathophysiology of immunological disorders such as autoimmunity, hypersensitivity, immunodeficiency, and the immune response in cancer.
- The principles, methodology, and applications of modern immunological techniques used in diagnostics and research.
- The concepts, types, and global relevance of vaccines, including strategies for immunization and the role of international health organizations like the WHO.

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Module Number	Content	Total hours	% of questions	Bloom level (applicable)	Remarks, if any
	THEORY				
M1	Introduction	**2**	**3**	**1, 2**	**NA**
M2	Immune Cells and Organs	**6**	**12**	**2, 4**	**NA**
M3	Antigens	**4**	**10**	**1, 2, 4**	**NA**
M4	Antibodies	**6**	**15**	**1, 2, 3**	**NA**
M5	Major Histocompatibility Complex	**4**	**10**	**1, 2, 4**	**NA**
M6	Complement System	**6**	**10**	**1, 2, 3**	**NA**
M7	Generation of Immune Response	**8**	**10**	**2, 4**	**NA**
M8	Immunological Disorders and Tumor Immunology	**8**	**10**	**1, 2, 5**	**NA**
M9	Immunological Techniques	**10**	**10**	**1, 3, 4**	**NA**
M10	Vaccines & vaccination	**6**	**10**	**2, 4, 5**	**NA**
Total Theory		**60**	**100**		

Detailed syllabus

Module 1: Introduction

Concept of Innate and Adaptive immunity; Contributions of following scientists to the development of field of immunology - Edward Jenner, Karl Landsteiner, Robert Koch, Paul Ehrlich, Elie Metchnikoff, Peter Medawar, MacFarlane Burnet, Neils K Jerne, Rodney Porter and Susumu Tonegawa

(Total Hours: 2)

Module 2: Immune Cells and Organs

Structure, Functions and Properties of: Immune Cells – Stem cell, T cell, B cell, NK cell, Macrophage, Neutrophil, Eosinophil, Basophil, Mast cell, Dendritic cell; and Immune Organs–Bone Marrow, Thymus, Lymph Node, Spleen, GALT, MALT, CALT

(Total Hours: 6)

Module 3 Antigens

Characteristics of an antigen (Foreignness, Molecular size and Heterogeneity); Haptens; Epitopes (T & B cell epitopes); T-dependent and T-independent antigens; Adjuvants

(Total Hours: 4)

Module 4 Antibodies

Structure, Types, Functions and Properties of antibodies; Antigenic determinants on Antibodies (Isotypic, allotypic, idiotypic); VDJ rearrangements; Monoclonal and Chimeric antibodies

(Total Hours: 6)

Module 5: Major Histocompatibility Complex

Organization of MHC locus (Mice & Human); Structure and Functions of MHC I & II molecules; Antigen processing and presentation (Cytosolic and Endocytic pathways)

(Total Hours: 4)

Module 6: Complement System

Components of the Complement system; Activation pathways (Classical, Alternative and Lectin pathways); Biological consequences of complement Activation

(Total Hours: 2)

Module 7: Generation of Immune Response

Primary and Secondary Immune Response; Generation of Humoral Immune Response (Plasma and Memory cells); Generation of Cell Mediated Immune Response (Self MHC restriction, T cell activation, Co- stimulatory signals); Killing Mechanisms by CTL and NK cells, Introduction to tolerance

(Total Hours: 8)

Module 8: Immunological Disorders and Tumor Immunology

Types of AutoimmModuley and Hypersensitivity with examples; Immunodeficiencies – Animal models (Nude and SCID mice), SCID, DiGeorge syndrome, Chediak- Higashi syndrome, Leukocyte adhesion deficiency, CGD; Types of tumors, tumor Antigens, causes and therapy for cancers.

(Total Hours: 8)

Module 9: Immunological Techniques

Principles of Precipitation, Agglutination, Immunodiffusion, Immunoelectrophoresis, ELISA, ELISPOT, Western blotting, Immunofluoresence, Flow cytometry, Immunoelectron microscopy

(Total Hours: 10)

Module 10: Vaccines & Vaccination

Adjuvants, cytokines, DNA vaccines, recombinant vaccines, bacterial vaccines, viral vaccines, vaccines to other infectious agents, tumor vaccines, principles of vaccination, passive & active immunization, immunization programs & role of WHO in immunization programs.

(Total Hours: 6)

Suggested Readings

1. Kuby, RA Goldsby, Thomas J. Kindt, Barbara, A. Osborne Immunology, 6th Edition, Freeman, 2002.
2. Brostoff J, Seaddin JK, Male D, Roitt IM., Clinical Immunology, 6th Edition, Gower Medical Publishing,2002.
3. Janeway et al., Immunobiology, 4th Edition, Current Biology publications, 1999.
4. Paul, Fundamental of Immunology, 4th edition, Lippencott Raven

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Course Name: Lab on Immunology **Code: BMMC-5392**

Credits: 2 **Total hours: 40**

1. Identification of human blood group
2. Perform Total Leukocyte Count of the given blood sample.
3. Perform Differential Leukocyte Count of the given blood sample.
4. Separate serum from the blood sample (demonstration).
5. To study the technique of Dot ELISA for the detection of antigen
6. To study the immunodiffusion technique by Single Radial Immunodiffusion.
7. To study the immunodiffusion technique by Ouchterlony method.
8. To study the technique of Rocket Immunoelectrophoresis for determination of the concentration of antigen in unknown sample.
9. To study the technique of Counter current electrophoresis for the determination of antigen and antibody in unknown sample
10. Separation of proteins through polyacrylamide gel electrophoresis.
11. Understanding the process of protein transfer through western blotting.