



Department of Biotechnology	LP: BT22503 Rev. No: 00
B.E/B.Tech/M.E/M.Tech : Biotechnology Regulation: 2022	Date:08.07.2024
PG Specialisation : Not Applicable	
Sub. Code / Sub. Name : BT22503/Genetic Engineering	
Unit : 1	

Unit Syllabus: BASICS OF RECOMBINANT DNA TECHNOLOGY 9

Manipulation of DNA — History of Genetic Engineering, Restriction and Modification enzymes, Prokaryotic and eukaryotic expression host systems, Introduction of recombinant DNA into host cells and selection methods.

Objective: To learn techniques for manipulating DNA, introducing recombinant DNA into host cells, and selecting transformed cells.

Session No *	Topics to be covered	Ref	Teaching Aids
1.	Fundamental Techniques of Gene Manipulation	TB1; Pg. (15-35)	LCD/BB Nebcutter
2.	Cutting DNA molecules	TB1; Pg. (36-43)	LCD/BB Nebcutter
3.	Joining DNA molecules	TB1; Pg. (44-50)	LCD/BB Snappene
4.	Cloning in Gram-negative bacteria	TB1; Pg. (179-188)	LCD/BB
5.	Cloning in Gram-positive bacteria	TB1; Pg. (189-200)	LCD/BB
6.	Cloning in <i>Saccharomyces cerevisiae</i> and other fungi	TB1; Pg. (202-212)	LCD/BB
7.	Gene transfer to animal cells	TB1; Pg. (218-249)	LCD/BB
8.	Gene transfer to plants	TB1; Pg. (277-296)	LCD/BB
9.	Genetic manipulation of animals	TB1; Pg. (251-271)	LCD/BB

Content beyond syllabus covered (if any): Innovative teaching methods such as Virtual restriction digestion using NEB cutter and Virtual Cloning using Snappene will be implemented.

* Session duration: 50 minutes



Sub. Code / Sub. Name : BT22503/Genetic Engineering

Unit : 2

Unit Syllabus : VECTORS 9

Plasmid Vectors, Bacteriophage Vectors, Cosmid Vectors, Phagemid Vectors, Fosmid Vectors, Bacterial Artificial Chromosomes (BACs), Yeast Artificial Chromosomes (YACs), Viral Vectors (Plant and Animal), Transposon Vectors, Shuttle Vectors, Expression Vectors.

Objective: To understand various vector types and their applications in genetic engineering.

Session No *	Topics to be covered	Ref	Teaching Aids
10.	Plasmid Vectors	TB1; Pg. (55-64)	LCD/BB
11.	Bacteriophage Vectors	TB1; Pg. (66-72)	LCD/BB
12.	Cosmid Vectors	TB1; Pg. (75)	LCD/BB
13.	Phagemid Vectors	TB1; Pg. (81)	LCD/BB
14.	Fosmid Vectors	Weblink-1	LCD/BB
15.	Bacterial Artificial Chromosomes (BACs)	TB1; Pg. (76-81)	LCD/BB
16.	Yeast Artificial Chromosomes (YACs)	TB1; Pg. (213-217)	LCD/BB
17.	Viral Vectors (Animal and Plant)	TB1; Pg. (238-248) TB1; Pg. (294-296)	LCD/BB
18.	Expression Vectors	TB1; Pg. (81-94)	LCD/BB

Content beyond syllabus covered (if any): Nil

* Session duration: 50 mins



Sub. Code / Sub. Name : BT22503/Genetic Engineering

Unit : 3

Unit Syllabus : GENE CLONING AND DNA LIBRARY CONSTRUCTION 9

Restriction Enzyme Cloning, Gibson Assembly, Gateway Cloning, TOPO Cloning, TA Cloning, Overlap Extension PCR, In-Fusion Cloning, Golden Gate Cloning, Homologous Recombination Cloning, Ligation independent cloning. Southern Blotting, Northern Blotting, Western Blotting, Genomic DNA library, cDNA library, Library screening techniques. Subtractive DNA hybridisation.

Objective: To master cloning techniques and DNA library construction, including screening methods.

Session No *	Topics to be covered	Ref	Teaching Aids
19.	Site specific recombinases (Cre-loxP-FLP-FRT)	TB1; Pg. (51-53)	LCD/BB
20.	TA Cloning ,TOPO Cloning	TB1; Pg. (49-50)	LCD/BB
21.	Gateway Cloning	TB1; Pg. (94)	TB1; Pg. ()
22.	Gibson Assembly	Weblink-2 Weblink-3	Internet Browser
23.	Golden Gate Cloning	Weblink-4	Internet Browser
24.	Blotting Techniques (Sothern, Northern and Western)	TB1; Pg. (18-24)	LCD/BB
25.	Genomic DNA Library	TB1; Pg. (96-102)	LCD/BB
26.	cDNA Library synthesis	TB1; Pg. (102-111)	LCD/BB
27.	cDNA Library Screening	TB1; Pg. (111-124)	LCD/BB

Content beyond syllabus covered (if any): Nil

* Session duration: 50 mins



Sub. Code / Sub. Name : BT22503/Genetic Engineering

Unit : 4

Unit Syllabus: PCR AND SITE DIRECTED MUTAGENESIS 9

Polymerase Chain Reaction (PCR), Variants of PCR - Asymmetric PCR, Digital PCR (dPCR), Fast PCR, Helicase-dependent Amplification (HDA), Hot-Start PCR, Inverse PCR, Long PCR, Loop-mediated Isothermal Amplification (LAMP), Methylation-specific PCR (MSP), Multiplex Ligation-dependent Probe Amplification (MLPA), Multiplex PCR, Nested PCR, Touchdown PCR, Overlapping Extension PCR (OE- PCR), Quantitative PCR (qPCR), Reverse Transcription PCR (RT-PCR), Site Directed Mutagenesis.

Objective: To gain proficiency in PCR and mutagenesis for precise DNA manipulation.

Session No *	Topics to be covered	Ref	Teaching Aids
28.	Polymerase Chain Reaction (PCR)	TB1; Pg. (26-28)	LCD/BB
29.	Hot Start PCR	TB1; Pg. (30)	LCD/BB
30.	Isothermal DNA amplification/Helicase Dependent PCR	Weblink-5	LCD/BB
31.	Methylation-specific PCR	Weblink-6	LCD/BB
32.	Multiplex ligation-dependent probe amplification (MLPA) PCR	Weblink-7	LCD/BB
33.	Overlap Extension PCR	Weblink-8	LCD/BB
34.	Reverse Transcription PCR (RT-PCR)	TB1; Pg. (28)	LCD/BB
35.	Real Time Quantitative PCR	TB1; Pg. (30-34)	LCD/BB
36.	Site Directed Mutagenesis.	TB1; Pg. (141-147)	LCD/BB

Content beyond syllabus covered (if any): Nil

* Session duration: 50 mins



Sub. Code / Sub. Name : BT22503/Genetic Engineering

Unit : 5

Unit Syllabus: SEQUENCING 9

Maxam and Gilbert Sequencing, Sanger Sequencing, Automated DNA sequencing, Next-generation Sequencing (NGS) - Pyrosequencing, Single-Molecule Real-Time Sequencing (SMRT), Nanopore Sequencing, Human Genome Project, Ordering the genome - Genetic maps and Physical maps. Ethical principles of Genetic engineering.

Objective: To familiarise with sequencing methods for genetic analysis and genome mapping.

Session No *	Topics to be covered	Ref	Teaching Aids
37.	Maxam and Gilbert Sequencing	Weblink-9	LCD/BB
38.	Sanger Dideoxy Sequencing	TB1; Pg. (126-130)	LCD/BB
39.	Automated DNA Sequencing	TB1; Pg. (130-132)	LCD/BB
40.	Pyrosequencing	TB1; Pg. (134-135)	LCD/BB
41.	Nanopore Sequencing	Weblink-10	LCD/BB
42.	Illumina Sequencing	Weblink-11	LCD/BB
43.	Genome Sequencing – Clone by Clone, Whole genome shotgun sequencing	TB1; Pg. (362-369)	LCD/BB
44.	Genetic Mapping and Physical mapping RFLP, STS, SNP	TB1; Pg. (346-362)	LCD/BB
45.	Ethical Principles	Weblink-12	LCD/BB

Content beyond syllabus covered (if any): Nil

* Session duration: 50 mins



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TEXTBOOKS:

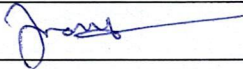
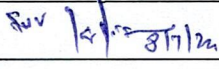
1. Primrose, Sandy B., and Twyman, Richard. Principles of Gene Manipulation and Genomics. Germany, Wiley, 2013.
2. Brown, T. A.. Gene Cloning and DNA Analysis: An Introduction. United Kingdom, Wiley, 2020.
3. Lesk, Arthur M.. Introduction to Genomics. United Kingdom, Oxford University Press, 2017.
4. Joseph Sambrook, David William Russell. Molecular Cloning: A Laboratory Manual. N.p., Cold Spring Harbor Laboratory Press.
5. Hartl, Daniel L., and Jones, Elizabeth W.. Essential Genetics: A Genomics Perspective. United Kingdom, Jones and Bartlett Publishers, 2006.


REFERENCE BOOKS:

1. Primrose, Sandy B., and Twyman, Richard. Principles of Genome Analysis and Genomics. Germany, Wiley, 2009.
2. Hartl, Daniel L., and Cochrane, Bruce. Genetics: Analysis of Genes and Genomes. United States, Jones & Bartlett Learning, 2019.
3. Carson, Sue, et al. Molecular Biology Techniques: A Classroom Laboratory Manual. United Kingdom, Elsevier Science, 2019.

WEBLINK (WL)

1. <https://www.snapgene.com/guides/gibson-assembly>
2. <https://www.neb.com/en/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/gibson-assembly>
3. <https://www.snapgene.com/guides/golden-gate-assembly>
4. <https://goldbio.com/articles/article/Common-Types-of-Cloning-Vectors>
5. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC11115679/>
6. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4611123/pdf/ACP-2004-26-5-370301.pdf>
7. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC117299/pdf/gnf056.pdf>
8. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3121328/pdf/nihms301116.pdf>
9. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC392330/>
10. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9966803/>
11. <https://www.cd-genomics.com/blog/principle-and-workflow-of-illumina-next-generation-sequencing/>
12. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7260159/>

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Remarks *: Nil		


8/7/24