SRI VENKATESWARA COLLEGE OF ENGINEERING

COURSE DELIVERY PLAN - THEORY

Department of Biotechnology	LP: BY22103
~~B.E/B.Tech/M.E~~/M.Tech : Biotechnology Regulation:2022 PG Specialisation : Biotechnology Sub. Code / Sub. Name : BY22103-RECOMBINANT DNA TECHNOLOGY Unit : I	Rev. No: 00 Date: 9/11/2022

Unit Syllabus: Basics of Molecular Tools Used in Gene Cloning **12h**

Overview of Restriction and Modification system, Different restriction enzymes, Types and examples, Methylation activity of various restriction enzymes, DAM, DCM and CpG methylase activity, Star activity of restriction enzymes, Different types of ligases used in rDNA technology, *E. coli* - DNA ligase, T4 DNA ligase, T4 RNA ligase, Other important enzymes: DNA and RNA polymerases, Reverse transcriptase, Terminal transferase, DNAses-exonuclease I, exonuclease III and Mungbean Nuclease

Objective: To enhance the knowledge of basic molecular tools used in rDNA technology

Session No *	Topics to be covered	Ref	Teaching Aids
1.	Overview of Restriction and Modification system, Different restriction enzymes, Types and examples	T1 (31-42)	PPT & BB
2.	Methylation activity of various restriction enzymes	T1(285-290)	PPT & BB
3.	DAM, DCM and CpG methylase activity, Star activity of restriction enzymes	T2 (152-165)	PPT & BB
4.	Different types of ligases used in rDNA technology	T1 (42-60)	PPT & BB
5.	*E. coli* - DNA ligase, T4 DNA ligase,	T1 (43-45) T2 (19-25)	PPT & BB
6.	T4 RNA ligase	T1 (49-60)	PPT & BB
7.	Other important enzymes: DNA and RNA polymerases	T1(48-60)	PPT & BB
8.	Reverse transcriptase	R2(35-60)	PPT & BB
9.	Terminal transferase	R2(35-60)	PPT & BB
10.	DNAses-exonuclease I	R1(17-34)	PPT & BB
11.	Exonuclease III	R3(14-19)	PPT & BB
12.	Mungbean Nuclease	R3(19-27)	PPT & BB
Content beyond syllabus covered (if any): -			

* Session duration: 50 minutes

Sub. Code / Sub. Name: BY22103-RECOMBINANT DNA TECHNOLOGY

Unit: II

Unit Syllabus: Vectors used for Gene Cloning **9h**

Introduction to cloning vectors, Plasmid biology, plasmid vectors (high copy and low copy), Phage biology, phage vectors, Cosmid vectors, Phasmid vectors, BAC vectors and YAC vectors, Yeast vectors

Objective: To relate the different types of vectors used in Gene Cloning.

Session No *	Topics to be covered	Ref	Teaching Aids
13.	Introduction to cloning vectors	T3(11-14)	PPT & BB
14.	Plasmid biology	T3(14-17)	PPT & BB
15.	Plasmid vectors (high copy and low copy)	T3(27-44) R2(181-189)	PPT & BB
16.	Phage biology, phage vectors	T2(99-104)	PPT & BB
17.	Cosmid vectors	R3(147-161)	PPT & BB
18.	Phasmid vectors	T3(169-174)	PPT & BB
19.	BAC vectors	T3(174-179)	PPT & BB
20.	YAC vectors	T3(179-183)	PPT & BB
21.	Yeast vectors	T3(183-186)	PPT & BB
Content beyond syllabus covered (if any): -			

* Session duration: 50 mins

Sub. Code / Sub. Name: BY22103-RECOMBINANT DNA TECHNOLOGY

Unit: III

Unit Syllabus: Techniques Used in Gene Cloning **9h**

Cloning after restriction digestion, Types of ligations, Blunt and Cohesive end ligation and case studies, Creation of restriction sites by PCR and cloning using linkers and adapters, cloning after homopolymer tailing, Strategies used in cloning PCR products - TA cloning, TOPO-TA cloning and ligation free cloning

Objective: To extend the existing knowledge on techniques currently used in Gene Cloning

Session No *	Topics to be covered	Ref	Teaching Aids
22.	Cloning after restriction digestion	T2 (79-86)	PPT & BB
23.	Types of ligations, Blunt and Cohesive end ligation and case studies	R3 (121-126) R2 (41-49)	PPT & BB
24.	Creation of restriction sites by PCR	T2 (86-99)	PPT & BB
25.	Cloning using linkers and adapters	T3 (69-78)	PPT & BB
26.	Cloning after homopolymer tailing	T3 (79-88) R3 (141-149)	PPT & BB
27.	Strategies used in cloning PCR products	T3 (88-96)	PPT & BB
28.	TA cloning	T3 (99-106)	PPT & BB
29.	TOPO-TA cloning	T3 (110-113)	PPT & BB
30.	Ligation free cloning	T3 (114-126) R2 (101-112)	PPT & BB
Content beyond syllabus covered (if any): -			

* Session duration: 50 mins

Sub. Code / Sub. Name: BY22103-RECOMBINANT DNA TECHNOLOGY

Unit: IV

Unit Syllabus: Sequencing of Clones and Construction of DNA Libraries 6h

DNA sequencing - Chemical & Enzymatic methods, Automated sequencing, Construction of cDNA library, Construction subtractive cDNA library, Genomic library, BAC and YAC libraries

Objective: To assess the sequencing methods of clones and methods of constructing DNA libraries

Session No *	Topics to be covered	Ref	Teaching Aids
31.	DNA sequencing	T1 (162-172)	PPT & BB
32.	Chemical & Enzymatic methods	T1 (172-177)	PPT & BB
33.	Automated sequencing	T1 (177-181)	PPT & BB
34.	Construction of cDNA library	T3 (196-201)	PPT & BB
35.	Construction subtractive cDNA library	T3 (201-212)	PPT & BB
36.	Construction of BAC and YAC libraries	T3 (212-219)	PPT & BB
Content beyond syllabus covered (if any): -			

* Session duration: 50 mins

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Sub. Code / Sub. Name: BY22103-RECOMBINANT DNA TECHNOLOGY

Unit: V

Unit Syllabus: Expression of Recombinant Proteins and its Applications **9h**

Construction of expression vectors for bacteria and yeast, Different promoters used in expression vectors, cloning of genes in the correct reading frame in the expression vector, Purification of recombinant protein using histidine tag, GST Tag, chitin-binding domain and intein with its applications, Construction of expression vectors for plants and animal cells, Bias in codon use and codon optimization

Objective: To organize the knowledge attained and to produce recombinant proteins

Session No *	Topics to be covered	Ref	Teaching Aids
37.	Construction of expression vectors for bacteria and yeast	R3 (71-94)	PPT & BB
38.	Different promoters used in expression vectors	T3 (167-189)	PPT & BB
39.	Cloning of genes in the correct reading frame in the expression vector	T3 (149-163) R2 (144-145)	PPT & BB
40.	Purification of recombinant protein using histidine tag, GST Tag	R3 (168-174)	PPT & BB
41.	Purification of recombinant protein using GST Tag	T3 (172-175)	PPT & BB
42.	Chitin-binding domain and intein with its applications	R3 (69-91) T2 (77-103)	PPT & BB
43.	Construction of expression vectors for plants	T3 (176-181)	PPT & BB
44.	Construction of expression vectors for animal cells,	T2 (146-152)	PPT & BB
45.	Bias in codon use and Codon optimization	R2 (145-162)	PPT & BB
Content beyond syllabus covered (if any): -			

* Session duration: 50 mins

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REFERENCES:

TEXTBOOKS:

1. Brown, T.A, "Gene Cloning and DNA Analysis- An Introduction, 6th edition, John Wiley & Sons, 2010.
2. Christopher Howe, "Gene Cloning and Manipulation, 2nd edition, Cambridge University Press, 2007.
3. Molecular Biotechnology, 2nd edition, S. B. Primrose, Blackwell Scientific Publishers, Oxford, 1994.

REFERENCE BOOKS:

1. Michael, R. G., Sambrook. J., "Molecular Cloning - A Laboratory Manual", 4th edition, Cold Spring Harbour Laboratory Press, 2012.
2. Milestones in Biotechnology, Classic Papers on Genetic Engineering, J. A. Davis and W. S. Reznikoff, Butterworth-Heinemann Boston 1992.
3. Route Maps in Gene Technology, M. R. Walker, and R. Rapley, Blakwell Science, Oxford, 1997.

	Prepared by	Approved by
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Date	9/11/2022	9/11/2022
Remarks *: -		

* If the same lesson plan is followed in the subsequent semester/year it should be mentioned and signed by the Faculty and the HOD