DISCIPLINE SPECIFIC CORE COURSE – (DSC-18)
FUNDAMENTALS OF RECOMBINANT DNA TECHNOLOGY

CREDIT DISTRIBUTION, ELIGIBILITY AND PRE-REQUISITES OF THE COURSE

Course title & Code	Credits	Credit distribution of the course			Eligibility criteria	Pre-requisite of the course (if any)
		Lecture	Tutorial	Practical/ Practice		
Fundamentals of Recombinant DNA Technology (BCH-DSC-603)	4	2L	0	2P	Class XII with Science and Biology	Basic course in Molecular Biology

Learning Objectives

The objective of the course is to teach the basics of theoretical and practical aspects of recombinant DNA technology and various techniques for DNA manipulation in prokaryotes and eukaryotes.

Learning outcomes

On successful completion of the course, students will be able to:

1. Perform restriction digestion of DNA samples.
2. Prepare genomic and cDNA libraries,
3. Perform basic cloning techniques to design a recombinant protein in a bacterial system.
4. Design primers for PCR, perform DNA amplification by PCR, and understand the principles of DNA sequencing.

SYLLABUS OF DSC-18

BCH-DSC-18 : FUNDAMENTALS OF RECOMBINANT DNA TECHNOLOGY
SEMESTER - VI

2.2 Course Contents

Theory (2 Credits)

Total 30 hours

Unit 1: Principles of gene cloning **(14 hours)**

Restriction and modification systems, restriction endonucleases and other enzymes used in gene cloning. Cloning vectors used in *E. coli*: plasmids pBR322, pUC, pGEM3Z. Ti-plasmid, and viral vectors (λ bacteriophage, CMV and SV40), high-capacity vectors BAC and YAC. Ligation of DNA molecules. Linkers, adapters and homopolymer tailing.

Unit 2: Selection for recombinants and clone identification (5 hours)

Uptake of DNA by cells and selection of recombinants. Making cDNA and Genomic DNA libraries. Clone identification by colony hybridization.

Unit 3: Expression of cloned genes (6 hours)

Vectors for expression of foreign genes in *E. coli,* expression cassettes: Hybrid promoters trc, tac. Challenges in producing recombinant protein in *E. coli.* Production of recombinant protein by eukaryotic cells. Fusion tags and their role in purification of recombinant proteins.

Unit 4: Polymerase chain reaction, DNA sequencing and Site Directed Mutagenesis

(5 hours)

Fundamentals of polymerase chain reaction, Types of PCR; reverse transcriptase PCR, Primer designing. DNA sequencing by Sanger's method including automated DNA sequencing, pyrosequencing. Site–directed mutagenesis (overlap extension method).

2.3 Practical (2 Credits)

Total: 60 hours

1. Isolation of plasmid DNA from *E. coli* cells.
2. Digestion of plasmid DNA with restriction enzymes.
3. Preparation of competent cells and transformation with plasmid DNA.
4. Amplification of a DNA fragment by PCR.
5. Alpha-Complementation of β−galactosidase for Blue and White selection.
6. Hyper expression of a recombinant protein (SDS PAGE).
7. Poly histidine-tagged recombinant protein and purification using Ni– affinity resin

2.4 Essential readings:

- Brown, T.A. (2016) Gene Cloning and DNA Analysis (7th ed.), Wiley-Blackwell publishing (Oxford, UK), ISBN: 978-1-4051-8173-0.
- Primrose, S.B., and Twyman, (2006) Principles of Gene Manipulation and Genomics (7th ed.), R. M., Blackwell publishing (Oxford, UK) ISBN:13: 978-1-4051-3544-3.
- Glick B.R., Pasternak, J.J. and Patten, C.L., (2010) *Molecular Biotechnology:* Principles and Applications of Recombinant DNA (4th ed.), ASM Press (Washington DC), ISBN: 978-1-55581-498-4 (HC).
- Michael R Green and J. Sambrook (2014) Molecular Cloning: A laboratory manual, (4th ed.), Cold spring Harbor laboratory press (3vol.), ISBN: 978-1-936113-42-2.

Suggested readings:

- Brown, T.A. (2007) Genomes (3rd ed.), Garland Science publishing, ISBN: ISBN 0 8153 4138 5.

3. Keywords

Genetic Engineering, cloning, Recombinant Protein expression and purification, Biotechnology.

Note: Examination scheme and mode shall be as prescribed by the Examination Branch, University of Delhi, from time to time.