

Course title: Principles of genetic engineering and recombinant DNA technology				
Course code: BBP 155		No. of credits: 3	L-T-P: 30-15-0	Learning hours: 45
Pre-requisite course code and title (if any):				
Department: Department of Biotechnology				
Course coordinator(s): Prof Anandita Singh			Course instructor(s): Prof Anandita Singh	
Contact details: asingh@terisas.ac.in				
Course type: Core			Course offered in: Semester 1	
Course description: The ability to genetically manipulate and engineer genomic sequences by precise recombination of genetic elements across organismal boundaries lies at the core of biotechnology. This foundation level core course is designed for students interested in developing a conceptual framework and technical know-how on genetic engineering methodologies. Upon successful completion of the course, students will gain an in-depth knowledge in principles of genetic manipulation and will develop an appreciation on centrality of genetic engineering in driving R&D across multiple branches of biotechnology. Students will gain proficiency in creative deployment of techniques for isolation, manipulation, novel design of genomic sequences. An introduction to properties of general DNA modifying enzymes will be given along with their applications. For example, the conceptualization, innovation, evolution and application aspects of PCR will be discussed in context to thermo-stable polymerases. An introduction to versatile and atypical modifying enzymes including non-specific endonucleases implied in new-age mutation technologies and genome engineering research will be provided. Cloning strategies will be contextualized to vector categories and applications such as plant transformation, protein expression, genomic and cDNA library construction to name a few. Host specificities and design of selection and screening strategies will be illustrated. Approaches for site-directed mutagenesis of cloned genomic fragments will be taught. Basic and advanced analytical techniques of molecular biology will not be covered in this course. To ensure coverage and sufficient depth on contemporary tools, outmoded methods no longer used has been intentionally avoided. However, students will be oriented to historical information for illustrating evolution of procedures used in contemporary biological research. Finally, an exposure will be provided to software used for <i>in-silico</i> annotation and manipulation of DNA sequences for efficient design, tracking, and management of cloning experiments in the laboratory.				
Course objectives:				
<ol style="list-style-type: none"> 1. To develop an appreciation for importance of fundamental knowledge in discovery and innovation of modern day tools and techniques of genetic engineering 2. To provide a theoretical and practical framework underlying recombinant DNA technology 3. To train and provide technical skills to students for devising broad research methodologies by creative deployment of genetic engineering techniques 				
Course contents				
Modules	Topic	L	T	P
1	Properties and applications of DNA Modifying Enzymes: Host controlled restriction modification system (Type I-IV restriction endonucleases, Isoschizomers and Neoschizomers, Homing Endonucleases); DNA Methyltransferases; Methylation Dependent Restriction Endonucleases; Exonucleases and non-specific endonucleases (Cas9 endonuclease, Structure and mis-match specific endonucleases: Fok I, FEN, Endo, Cel I and other site directed nucleases); Genome Editing with Engineered Nucleases (GEEN), Ligases; site-specific Recombinases used in	7	7	

	cloning technologies; DNA polymerases: Special case of thermo-stable DNA polymerases in context to PCR (History, concept, invention, enzymology, applications); Reverse transcriptases and expression analysis (semi-quantitative and quantitative RT-PCRs); Phosphatases and Kinases			
2	Generalised cloning strategies Host genotype specificities; classical and contemporary strategies for selection and screening; Marker and reporter genes; positive and negative selection; insertional inactivation; α -complementation; TA-cloning vectors; TOPO-TA and GATEWAY cloning vectors	3	1	
3	Vector categories and selection schemes History and evolution of plasmid and Lambda phase based vectors and their derivatives (Insertional vectors, replacement vectors, cosmids, phasmids, phagemids, <i>in-vitro</i> packaging); High-cloning capacity vectors (Virus based single stranded DNA vectors: M13, fd, f1; YACs, BACs, PACs, BIBACs); Plant transformation vectors (Binary and Conjugate), Components of Gene expression Cassettes; Protein Expression Vectors (expression systems for high level protein expression in <i>E. coli</i> and yeast, transcriptional control, inducible promoters, translational efficiency, translational initiation, elongation, codon usage), protein extraction and purification (protein purification tags, Histidine and GST tags, IMAC)	8	2	
4	DNA modifying enzymes and labelling of nucleic acid sequences End-Labeling (3'- and 5'-), Random priming and Nick translation using radioactive non-radioactive labelling techniques.	2		
5	Construction of genomic DNA libraries Procedures for partial, representative, enriched, large-insert DNA libraries in context to medium and high-capacity cloning vectors; cDNA libraries (Self-priming methods, replacement synthesis, Okayama and Berg strategy, use of Adapters/Linkers and methylation for directional cloning)	2		
6	Site Directed Mutagenesis PCR based methods for site-directed mutagenesis (Single primer methods viz. Mis-incorporation of mismatched oligos, Over-lap extension), whole plasmid single round PCR), mis-repair of mutant oligonucleotides, selection of mutant (dut/ung <i>E. coli</i> strains for SDM through uracil replacement), Ligase chain reaction	2		
7	Sequence verification: Reading electropherograms, <i>in-silico</i> analysis, plasmid mapping software for cloning designs; annotation of DNA sequence features		4	
8	Genetic manipulation to Genome Modification and Engineering: Impact of Genetic engineering on Transgenic Technology, Genome Editing: Case Studies from Biomedical research and crop biotechnology in Research and Development	6	1	
	Total	30	15	

Evaluation criteria:

1. Test 1: 30%
2. Test 2: 30%
3. Test 3: 30%
4. Assignments/Presentations: 10%

Learning outcomes:

1. Technical know-how on versatile techniques in recombinant DNA technology (Test 1-3, Assignments)
2. Understanding in application of genetic engineering techniques in basic and applied biological research (Test 1-3, Assignments)
3. Proficiency in designing and conducting experiments involving genetic manipulation (Test 1-3, Assignments)

Pedagogical Approach:

Lectures and tutorials in online or offline mode with a major emphasis on the detailed discussion of original research articles

Skill Set:

1. Isolation, manipulating, design and analysis of DNA sequences using DNA modifying enzymes
2. Designing cloning experiments using routine and specialized vectors for such applications as plant transformation, protein expression and genomic DNA library construction
3. Editing genomic sequences using site-directed mutagenesis

Employability:

1. Science Education, Research and Development, Management and Bio-services
2. Bio-pharma and Agri-biotechnology companies
3. Law firms and knowledge processing organizations (IP management consultancy)
4. Regulatory bodies and funding agencies

Materials:**Books**

1. M. R. Green, J. Sambrook. Molecular Cloning: A Laboratory Manual (Cold Spring Harbor, ed. 4, 2012).
2. M. Wink. An Introduction to Molecular Biotechnology: Molecular Fundamentals, Methods and Applications in Modern Biotechnology (Wiley, ed. 2, 2011).
3. K. Wilson, J. Walker. Principles and Techniques of Biochemistry and Molecular Biology (Cambridge University Press, ed. 7, 2010).
4. B. R. Glick, J.J. Pasternak and C.L. Pattern. Molecular Biotechnology: Principles and Applications of Recombinant DNA (ASM Press, ed. 4, 2010).
5. S. B. Primrose, R. Twyman. Principles of Gene Manipulation and Genomics (Wiley-Blackwell, ed. 7, 2006)
6. M. M. Burell. Enzymes of Molecular Biology (Humana Press, 1993)
7. H. M. Eun. Enzymology Primer for Recombinant DNA Technology (Academic Press, 2008)

Additional information (if any): The list of books suggested in the readings will only provide basic knowledge on the concepts. The actual readings will involve comprehension of prescribed Journal articles and Reviews across topics.

Representative Software (Source):

1. Gene Construction Kit® (**GCK**) (<http://www.textco.com/gene-construction-kit.php>): DNA manipulation and analysis tool, useful in plasmid mapping and restriction based cloning operations.
2. Gene Inspector® (**GI**) (<http://www.textco.com/gene-construction-kit.php>): DNA and protein sequence analysis package.
3. Vector NTI® Software (<http://www.lifetechnologies.com/in/en/home/life-science/cloning/vector-nti-software.html>): Integrated suite for sequence analysis

Student responsibilities:

1. Class attendance
2. Study of course materials as specified by the instructor
3. Self-study

Course reviewers:

The course was previously reviewed and commented on by the following experts:

1. Prof. Anil Grover

Head, Department of Plant Molecular Biology

University of Delhi, South Campus, New Delhi- 110021, India

2. Dr. Neeti Sanan Mishra

Group Leader: Plant RNAi,

International Centre for Genetic Engineering and Biotechnology

Aruna Asaf Ali Marg

New Delhi-110 067, India