Course title: Principles of genetic engineering and recombinant DNA technology			
Course code: BBP 155	**No. of credits:** 3	**L-T-P:** 21-21	**Learning hours:** 42
Pre-requisite course code and title (if any): None			
Department: Department of Biotechnology			
Course coordinator(s): Dr. Anandita Singh		**Course instructor(s):** Dr. Anandita Singh	
Contact details: asingh@teri.res.in			
Course type: Core		**Course offered in:** Semester 1	

Course description: The aim of this core-course is to acquaint the students to versatile tools and techniques employed in genetic engineering and recombinant DNA technology. A sound knowledge on methodological repertoire allows students to innovatively apply these in basic and applied fields of biological research. This course provides theoretical bases to properties and applications of versatile DNA modifying enzymes, cloning strategies, vector types, host genotype specificities for selection and screening of recombinants and/or recombinant transformants. Students will also be introduced to prominent nucleic acid labeling techniques. Introduction to various types of vectors viz. cloning, transformation, expression; and also vectors for genomic and cDNA library and whole genome sequencing will be provided. A critical appraisal of methods for site-directed mutagenesis and sequencing of cloned genomic fragments will also be covered. Finally, students will be familiarized to software permitting *in-slilico* manipulation and annotation of DNA sequences for efficient design, tracking, and management of cloning experiments in the laboratory. This course may be deemed as a foundation course serving as a platform for introduction of more advanced cutting-edge technologies that essentially are an amalgamation of basic techniques combined in diverse forms and sequence; to be introduced later in the program.

Course objectives:

1. To illustrate creative use of modern tools and techniques for manipulation and analysis of genomic sequences.
2. To expose students to application of recombinant DNA technology in biotechnological research.
3. To train students in strategizing research methodologies employing genetic engineering techniques.

Course contents

		L	T	P
1	**Defining purview of genetic engineering: Tools and techniques Properties and applications of DNA Modifying Enzymes:** Host controlled restriction modification system (Nomenclature, Type I-IV restriction endonucleases, Isoschizomers); DNA Methyltransferases; DNA polymerases; Special case of thermo-stable DNA polymerases in context to PCR (History, concept, enzymology, applications); Reverse transcriptases in context to semi-quantitative and quantitative RT-PCR.	9	5	
2	**Introduction to cloning:** Generalized cloning schemes, host genotypes specificities and applications, strategies for selection and screening (Introduction to marker and reporter genes, positive and negative selection, insertion inactivation, α complementation).	4		
3	**Types of vectors:** Plasmids; Lambda based vectors and derivatives (Insertion vectors, replacement vectors, cosmids, phasmids, phagemids, in-vitro packaging, selection schemes); high-cloning capacity vectors: single stranded DNA vectors (M13, fd, f1); YACs, BACs, PACs, BIBACs, Plant Transformation vectors Ti, Ri plasmids, Binary, Conjugate, selection schemes), Protein Expression Vectors (expression systems for high level protein expression in E.coli and yeast, transcriptional efficiency, inducible promoters, translational efficiency, translational initiation, elongation, codon usage), protein extraction and purification (protein purification tags, histidine and GST tags, IMAC).	10		
4	**Labelling and detection of nucleic acid sequences:** End-Labeling (3'- and 5'-), Random priming and Nick translation using radioactive non-radioactive labeling techniques.	2		
5	**Genomic DNA libraries** (Procedures for Partial, Representative, Enriched, Large-	4		

	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).			
6	**Nucleic acid sequencing methodologies, Dye chemistries and platforms:** Sanger's Di-deoxy Chain termination method (Use of M13 based ss DNA vectors to cycle sequencing, evolution in enzymology (Klenow, T7 polymerase, *Taq* polymerase). Autoradiography and florescence dye chemistries, slab gel based electrophoresis (semi-automated) to capillary based gel electrophoresis (automated sequencing), Interpreting electropherograms, base calling and quality scores (Phred).	3		
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 *E. coli* strains for SDM through uracil replacement), Ligase chain reaction.	3		
7	*In-silico* analysis, manipulation and annotation of DNA sequences for experimental design and efficient management of cloning experiments.		2	
	Total	**35**	**7**	

Evaluation criteria:

1. 2 minor tests 30% (each)
2. 1 major test (end semester) 40%

Learning outcomes:

1. Technical know-how on versatile techniques in recombinant DNA technology.
2. An understanding on application of genetic engineering techniques in basic and applied experimental biology.
3. Proficiency in designing and conducting experiments involving genetic manipulation.

Pedagogical Approach:

Classroom lectures and tutorials, with a major emphasis on the detailed discussion of original research articles in class.

Skill Set:

1. Manipulating DNA sequences with versatile 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 etc.
3. Editing genomic sequences using site-directed mutagenesis.
4. Employing PCR, nucleic acid hybridization and sequencing technologies for detection and diagnostics.

Employability:

Science Education, Research and Development, Management and Bio-services

1. Bio-pharma and Agri-biotechnology companies.
2. Law firms and knowledge processing organizations (IP management consultancy).
3. Regulatory bodies and funding agencies.

Materials:

Suggested readings

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., *et al.* Molecular Biotechnology: Principles & Applications of Recombinant DNA (ASM Press, ed. 4, 2009).
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, 1996).

Additional information (if any):

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.

Course reviewers:

The course has been reviewed and commented on by the following experts.

1. Dr J S Virdi, Professor, Department of Microbiology, University of Delhi South Campus, Delhi University
2. Dr Prem Jauhar, Professor of Cytogenetics, USDA-Agricultural Research Service, Northern Crop Science Laboratory, State University Station, North Dakota, USA.
3. Dr Surekha Katiyar Agarwal, Assistant Professor, Department of Plant Molecular Biology, University of Delhi South Campus.