Paper No- 12
Genetic Engineering and Biotechnology

THEORY

1. **Introduction to recombinant DNA technology (5 lectures)**
   Overview of recombinant DNA technology. Restriction and modification systems, restriction endonucleases and other enzymes used in manipulating DNA molecules, separation of DNA by gel electrophoresis. Extraction and purification of plasmid and bacteriophage DNA.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p3-5, p45-63, p25-43]

2. **Cloning vectors for prokaryotes and eukaryotes (8 lectures)**
   Plasmids and bacteriophages as vectors for gene cloning. Cloning vectors based on *E. coli* plasmids, pBR322, pUC8, pGEM3Z. Cloning vectors based on M13 and λ bacteriophage. Vectors for yeast, higher plants and animals.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p13-21, p88-117]

3. **Joining of DNA fragments (3 lectures)**
   Ligation of DNA molecules. DNA ligase, sticky ends, blunt ends, linkers and adapters. Synthetic oligonucleotides, synthesis and use.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p63-70; Molecular Biotechnology (2010) Glick et al., p98-108]

4. **Introduction of DNA into cells and selection for recombinants (5 lectures)**
   Uptake of DNA by cells, preparation of competent cells. Selection for transformed cells. Identification for recombinants - insertional inactivation, blue-white selection. Introduction of phage DNA into bacterial cells. Identification of recombinant phages. Introduction of DNA into animal cells, electroporation.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p72-86]

5. **Methods for clone identification (3 lectures)**
   The problem of selection, direct selection, marker rescue. Gene libraries, identification of a clone from gene library, colony and plaque hybridization probing, methods based on detection of the translation product of the cloned gene.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p126-146]

6. **Polymerase chain reaction (3 lectures)**
   Fundamentals of polymerase chain reaction, designing primers for PCR. Studying PCR products. Cloning PCR products. Real time PCR.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p147-154]

7. **DNA sequencing (4 lectures)**
   DNA sequencing by Sanger's method, modifications based on Sanger's method. Automated DNA sequencing. Pyrosequencing.

[Principles of Gene Manipulation and Genomics (2006) Primrose and Twyman, p126-134]

8. **Expression of cloned genes (5 lectures)**
   Vectors for expression of foreign genes in *E. coli*, cassettes and gene fusions. 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.
   [Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p225-243; Principles of Gene Manipulation and Genomics (2006) Primrose and Twyman, p81-92]

9. **Applications of genetic engineering in Biotechnology (12 lectures)**
   Site–directed mutagenesis and protein engineering. Applications in medicine, production of recombinant pharmaceuticals such as insulin, human growth hormone, factor VIII. Recombinant vaccines. Gene therapy. Applications in agriculture - plant genetic engineering, herbicide resistant crops, problems with genetically modified plants, safety concerns.
   [Molecular Biotechnology (2010) Glick, p290-326; Gene Cloning and DNA Analysis: An Introduction (2010) Brown, p245-280]

Essential Readings

1. Gene Cloning and DNA Analysis (2010) 6th ed., Brown, T.A., Wiley-Blackwell publishing (Oxford, UK), ISBN: 978-1-4051-8173-0.
2. Principles of Gene Manipulation and Genomics (2006) 7th ed., Primrose, S.B., and Twyman, R. M., Blackwell publishing (Oxford, UK) ISBN:13: 978-1-4051-3544-3.
3. Molecular Biotechnology: Principles and Applications of Recombinant DNA (2010) 4th ed., Glick B.R., Pasternak, J.J. and Patten, C.L., ASM Press (Washington DC), ISBN: 978-1-55581-498-4 (HC).

PRACTICALS

1. Isolation of plasmid DNA from *E. coli* cells.
2. Digestion of plasmid DNA with restriction enzymes.
3. Amplification of a DNA fragment by PCR.
4. Transformation of *E. coli* cells with plasmid DNA.
5. Hyper expression of poly histidine-tagged recombinant protein and purification using Ni-affinity resin.