DIPLOMA IN RECOMBINANT DNA TECHNOLOGY

(Non-Semester)

(With effect from the academic year 2013-14)

Eligibility for the Course

Candidates for admission to Diploma in Recombinant DNA Technology could posses a Bachelors degree in Zoology, Botany, Chemistry, Biochemistry, Microbiology Biotechnology/Environmental/ Animal/plant Food sciences, Dietetics & Nutrition, Bioinformatics, BE in Chemical Engineering & Biotechnology; B.Tech in Biotechnology & Bioinformatics/Nanotechnology; BDS; MBBS; B.Sc in Agri/Agri Biotechnology;B.V.Sc., B.F.Sc., .Pharm and BPT.

Duration of the Course

One year Diploma in Recombinant DNA Technology course non-semester for One Year duration

Examination

All the theory paper are of 3hours duration each for maximum of 100 marks with passing minimum of 35 marks Practical examinations are also for 3 hours duration for a maximum of 100 marks and passing minimum of 35 marks.

Question Paper Pattern

Maximum marks: 100 Time: 3 hours

Part A (5 x 3 = 15)

Five short answer questions (One question from each unit)

Part B (5 x 8 = 40)

Paragraph questions (Total questions 8, out of which answers are to be given for any five questions;

Part C (3x 15 = 45)

Total questions 5, out of which answers are to be given for any Three questions;

S.No	Theory & Practicals	Maximum Marks	Minimum Marks
1.	Recombinant DNA technology	100	35
2.	Tools and Techniques in Biotechnology	100	35
3.	Applications of Recombinant DNA technology	100	35
P1	Practical Recombinant DNA technology	100	35

Paper 1- Recombinant DNA Technology

Unit-I Characterization of Nucleic acid (DNA and RNA), Quantification, Radiolabelling of nucleic acids, labelling by primer extension, DNA sequencing: Maxam-Gilbert (Chemical) and Sanger- Nicolson (dideoxy/ enzymatic) sequencing method, Pyrosequencing.

Unit-II Restriction Enzymes: Types and uses of restriction endonuclease, classification Restriction mapping. DNA modifying enzymes: Nucleases, Polymerases, Phosphatases and ligases.

Unit-III Vectors. Plasmid vectors, Bacteriophage, expression vectors, other vectors, Construction of genomic and c-DNA libraries, Joining of DNA Fragments to vectors, cohesive and blunt end Ligation, adaptors, and linkers.

Unit-IV Principal and applications in analysis of recombinants: Principle of hybridization. Northern blotting, Southern blotting, Western blotting. Polymerase chain reaction, selection and screening of recombinants, Restriction fragments length polymorphism, RAPD, AFLP, MAP.

Unit-V Expression systems: methods of Transformation, codon optimization, host engineering. Strategies of gene delivery, in vitro translation, expression in bacteria, yeast, expression in insects and mammalian cells.

Reference

1. Principles of Gene manipulation (1994) Old R.N. and Primrose S.B.
2. From Genes to Clones (1987) Winnaeker E.L.
3. Recombinant DNA (1992) Watson J.D., Witreowski J., Gilman M. and Zooller M.
4. An Introduction to Genetic Engineering: Nicholl, D.S.T.
5. Molecular Biotechnology (1996) Pasternak
6. The Biochemistry of Nucleic acid(1996)Adam et al
7. Genetic Engineering (1998)Janke k. swtlow

Paper 2- Tools and Techniques in Biotechnology

Unit-I Microscopy: Principles and applications of simple, compound, phase-contrast and fluorescent microscopes. Electron microscopy: SEM and TEM. Confocal microscopy, Super resolution microscopy (PLAM. STROM)

Unit-II Separation Techniques: Centrifugation, Principles, type of centrifuges, density gradient centrifugation in isolation of cells, cell organelles. Fluorescence-activated cell sorting (FACS), Polyacrylamide gel electrophoresis (native and SDS), Agarose gel electrophoresis, EMSA, Blotting techniques, Immunoelectrophoresis.

Unit-III Protein- Protein Interaction: GST- pull down assay, Co-Immuno precipitation Assay, Yeast two hybrids, Mammalian Two hybrid system, FRET

Unit-IV Radio Labelling techniques: preparation of labelled biological samples. Safety measures in handling radioisotopes. RIA, non radiolabelling.

Unit V: Bioethics: Ethical issues in genetic engineering, patenting genes, cloning, genetic testing & screening; Biotechnology & social responsibility; The legal & socio-economic impact of Biotechnology; Biosafety regulatory frame work for GMOs, Use of genetically modified organisms & their release in environment, Cartagena Protocol on biosafety

Books

1. A Biologist Guide to Principle and Techniques: Willson K. and
2. An Introduction to Practical Biochemistry: Plummer D. T.
3. Thomas J.A., Fush R.L., (2002), Biotechnology & safety Assessment (), Academic press.
4. Fleming D.A., Hunt D.L., (2002), Biological safety Principles & practices (3rd) ASM Press, Washington.
5. Biotechnology- A Comprehensive treatise (Vol 12), Legal economic & ethical Dimensions VCH.
6. Sasson A, Biotechnologies & Development, UNESCO Publications. 5. Singh K, Intellectual Property Rights on Biotechnology, BCIL, New Delhi.

Paper 3- **APPLICATIONS OF RECOMBINANT DNA TECHNOLOGY**

Unit 1: Expression system and applications:

Functional analysis of genes, Genetic engineering in Yeast, Plants – Transgenic plants, Reporter gene for basic research, Genetic engineering in Animals – Transgenic animals, Uses of transgenic animals, Knockout mice

Unit 2: Gene Therapy

Introduction, Somatic gene therapy, Delivery techniques – *Ex vivo* & *In vivo*, Delivery vectors – viral & Non-viral, Germinal gene therapy, Limitations and ethical considerations.

Unit 3: Molecular Diagnosis

Introduction to molecular diagnostics, Hybridization techniques – Micro-arrays, Fluorescence *in situ* hybridization (FISH),Introduction to genetic testing, Types; Pre-implantation genetic diagnosis, Newborn screening, Prenatal diagnosis, Medical procedures – Amniocentesis, Chorionic villus sampling.

Unit 4: Forensic testing

DNA Fingerprinting, Restriction Fragment Length Polymorphism (RFLP) analysis.

Unit 5: Therapeutics

Production of monoclonal antibody, Engineered antibodies-Humanized antibodies-monoclonal antibodies for cancer diagnostics and theraphy-Immunotoxins

References:

1. Principles of Gene Manipulations 1994 by Old and Primrose Blackwell Scientific Publications.

3. Molecular Biotechnology 2nd Edition by S.B. Primrose. Blackwell Scientific Publishers, Oxford. 1994.

4. Genetic Engineering and Introduction to Gene Analysis and Exploitation in Eukaryotes by S.M. Kingsman and A.J. Kingsman, Blackwell Scientific Publications, Oxford 1998.

5. Recombinant DNA and Biotechnology: Guide for Teachers. 2nd Edition by Helen Kreuz. 2001.ASM Publications.

6. Molecular Biotechnology: Principles and Applications of Recombinant DNA. 2 nd Edition. 1998 by Bernard R. Glick and Jack J. Pastemak, ASM Publications.

Paper 4- PRACTICAL RECOMBINANT DNA TECHNOLOGY

1. Isolation of Plasmid DNA from *E.Coli* by Alkaline lysis method
2. Gene Cloning
    i. PCR Amplification
    ii. Restriction Digestion
    iii. Competent cell preparation
    iv. DNA Ligation
    v. Transformation
    vi. Screening of recombinant DNA
3. Induction of gene expression in *E.coli* , cell lysis and protein extraction
4. Visualization of proteins by SDS PAGE
5. Western blotting
6. ELISA

References:

1. DNA Cloning: A Practical Approach by D.M. Glower and B.D. Hames, IRL Press, Oxford. 1995.
2. Molecular cloning: Sambrook et al.,

PAPER –IV PRACTICAL RECOMBINANT DNA TECHNOLOGY

1. Primer designing- A computer approach.

2. Plasmid DNA Isolation from E.coli

3. Plasmid DNA Isolation from plant sources.

4. Restriction Digestion and analysis (web cutter).

5. Competent Cell preparation.

6. DNA Ligation.

7. Bacterial transformation.

8. Agarose Gel Electrophoresis.

9. Quantifying DNA by zymogram (computer approach).

10. SDS – PAGE.

References:

1. DNA cloning: A practical approach by D.M. Glower and B.D. Hames, IRL PRESS, Oxford. 1995.

2. Molecular cloning: Sambrook et al.,