UNIVERSITY OF RAJASTHAN
JAIPUR

SYLLABUS

M.SC. Biotechnology

Semester Scheme

III/IV Semester Examination 2017-2018

Dy. Registrar
(Academic)
University of Rajasthan
JAIPUR
Semester-III (2017-18)

BTH 901: Genetic Engineering

Genetic engineering tools and their applications: Restriction-modification system & enzymes, modification enzymes (methylases and other enzymes needed in genetic engineering), DNA and RNA markers. Gene Cloning Vectors- Plasmids, bacteriophages, phagemids, cosmids. Artificial chromosome vectors (YAC, BAC, MAC), CHEF analysis, virus derived vectors-SV40, M13, retroviral vectors, and general applications.

Nucleic Acid Sequencing and Amplification: Sequencing methods and their Applications- Maxim & Gilbert’s and Sanger’s methods; Pyrosequencing, Thermal PCR, Shot gun sequencing and Automated method. Nucleic Acid purification and Yield Analysis; PCR – Types and applications.


Study Gene Regulation and analysis of gene Expression: DNA transfection methods, Northern blot, Primer extension, SI mapping, RNase protection assays, Reporter assays.

Mutagenesis, Protein Engineering & Processing of Recombinant proteins - Directed Mutagenesis- Oligonucleotide with M13 DNA, PCR amplified oligonucleotide and Random mutagenesis. Protein Engineering: adding disulfide bonds, reducing number of free sulfhydrol residues, changing aminoacids, increasing and modifying enzymatic activity. Processing of Recombinant proteins: Purification and refolding, characterization of recombinant proteins, stabilization of proteins.

T-DNA and Transposon Tagging: Role of gene tagging in gene analysis, T-DNA and Transposon tagging, Identification and isolation of genes through T-DNA or transposon. Transgenic and Gene Knockout Technologies Targeted gene replacement, Chromosome engineering.

Expression Strategies for Heterologous Proteins: Vector engineering, host engineering, in vitro transcription and translation, expression in bacteria, yeast, insects and insect cells, expression in mammalian cells and plants.

Application of genetic engineering: Uses of transgenic plants and animals; production of recombinant pharmaceuticals, disease diagnoses and nanotechnology.

Suggested Laboratory Exercises

1. Growth characteristics of E. coli using plating and turbidometric methods. Growth Curve
2. Bacterial culture and antibiotic selection on media.
3. Isolation of plasmid from E. coli by alkaline lysis method and its quantification by spectrophotometer.
4. Amplification of DNA by PCR.
5. Restriction enzyme digestion of genomic DNA and plasmid DNA from E.coli and estimation of size of DNA fragments after electrophoresis using DNA markers.
6. RFLP analysis
7. RAPD analysis
8. Demonstration of DNA fingerprinting. Human Forensic
9. Restriction digestion of the plasmid and estimation of the size of various DNA fragments & Construction of Restriction digestion map.
10. Cloning of DNA fragment in a plasmid vector.
11. Transformation of the given bacterial population and selection of recombinants.
12. Co-cultivation of the plant material (e.g. leaf discs) with Agrobacterium and study GUS activity histochemically.
13. Any other practical based on the theory syllabus.

Suggested Reading:


[Signature]
Registrar
(Academic)


BTH 902: - Animal Biotechnology


Basic understanding for cell culture: Structure and organization of animal cell, Cell physiology. Primary and established cell line cultures. Biology and characterization of the cultured cells and measuring their growth.


Mammalian Cell transformation: Establishment of immortal cell lines, transfection, selection by selectable markers, gene amplification for high level protein expression. Specialized methods to transfer difficult cell types; Uses of viral vectors, Vaccinia and Baculovirus and Retrovirus in gene transfer, and use of antisense RNA and DNA in controlling gene function. Mice as the experimental material for gene introduction.

Impact of Recombinant DNA on human Genetics: Mapping and cloning human disease genes- Positional cloning, subchromosomal mapping and markers, in situ hybridization to chromosomes and RFLP.

Applications of Animal cell and Recombinant DNA technology: Cell culture based vaccines. Somatic cell genetics. Organ and histotypic cultures. Development of Transgenic animals (Mice, Cattle, Sheep, Goat, Pigs, Birds and Fish) and their uses. DNA- based diagnosis of genetic diseases. Human somatic cell gene therapy for single-gene disorders.

Suggested Laboratory Exercises.
1. Preparation of tissue culture medium and membrane filtration.
2. Preparation of single cell suspension from spleen and thymus.
3. Cell counting and cell viability.
4. Macrophage monolayer from PEC, and measurement of pathogenicity activity.
5. Trypsinization of monolayer and subculturing.
6. Cryopreservation and thawing.
8. Role of serum in cell culture.
9. Preparation metaphage chromosome from cultured cells.
10. Isolation of and demonstration of apoptosis of DNA laddering.
11. MTT assay for cell viability and growth.
12. Cell fusion with PEG.
13. Any other practical based on theory syllabus

Suggested Readings:

BTH 903:- Seminar, Scientific Writing & Presentations

Practicals through Assignments: 100

1. Writing Review of Literature: Review of the Literature/Annotated Bibliography
   Locate, analyze, summarize, and evaluate at least 3 scholarly and 3 popular sources in an area of interest and produce an Annotated Bibliography/Review of the Literature using appropriate citation style, to support the Long Article (4-6 pages).

2. Data analysis and presentation: How to present data? Which diagrams to use? What programs and data formats to use for data visualization?

3. Types of graphs (Microsoft graphics 3D): Different graphs for different purposes (poster, talk, manuscript). Students will be able to correctly identify and describe four kinds of graphs commonly used in science: pie charts, line graphs, scatter plots, and bar graphs.

4. How to give a Seminar: introduction and background information on topic. What relevant research has been performed previously? State the problem(s) that remain unanswered. State objectives and specific hypotheses you wish to test. Describe the methodology used to test your hypotheses. Present Data, Results, Discussion and Interpretations (fact vs. fiction) and explain the significance of your findings.

5. Scientific presentations/Poster/Power Point (short and long): Document Analysis Presentation (Information Literacy) Through multimedia presentation (PowerPoint or Poster Session) by analyzing selected articles and scholarly and popular science writing and journals. Use visuals. What does a good poster need? Structure of a good poster.

Suggested Reading:


can be located online at http://www.econbot.org/webmaster/docs/g&pissues/2001_spring.pdf
<table>
<thead>
<tr>
<th>Examination</th>
<th>MM:-100</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 Presentation</td>
<td>50</td>
</tr>
<tr>
<td>Poster Presentation (on Computer)</td>
<td>25</td>
</tr>
<tr>
<td>Practical Record</td>
<td>15</td>
</tr>
<tr>
<td>10 Viva-Voce</td>
<td>10</td>
</tr>
</tbody>
</table>
BTH A01:-- Entrepreneurship & Ethics


Ethical issues: introduction- causes of unethical acts, ignorance of laws, codes, policies and procedures, recognition, friendship, personal gains. Professional ethics- professional conduct. Ethical decision making, ethical dilemmas. Teaching ethical values to scientists, good laboratory practices, good manufacturing practices, laboratory accreditation.

Suggested Readings:

3. Hassan, E., Yaqub, O., Diepeveen, D. (2010). Intellectual Property and Developing Countries. A review of the literature, the RAND Corporation, 1776 Main Street, P.O. Box 2138, Santa Monica, CA 90407-2138


BTH B02: Protein Engineering


Introduction to Protein engineering: definition, basic principles; Features or characteristics of protein that can be engineered (definition and methods of study), affinity and specificity; Spectroscopic properties; Stability to changes in parameters as pH, temperature and amino acid sequence, aggregation propensities etc.

Method for Protein engineering: Rational design, Directed mutagenesis, Random mutagenesis, DNA shuffling, Evolutionary methods/directed evolution, Homology modelling, De novo enzyme engineering, strategies and case studies: Addition of disulfide bonds- T4 Lysozyme, Xylanase, Human pancreatic Ribonuclease; changing asparagine to other amino acids, reducing the number of free sulphydryl residues, increasing enzyme activity, modifying metal cofactor requirements, decreasing protease sensitivity, modifying protein specificity FokI endonuclease, Antibodies; increasing enzyme stability and specificity- altering multiple properties (Subtilisin, Peroxidase).

Computational approaches to protein engineering: sequence and 3D structure analysis, bioinformatics tools for protein study, Data mining, Ramachandran map, Mecahnism of stabilization of protein from psychrophiles and thermophiles vis-a-vis those from mesophiles; Protein design.

Application of Protein engineering: Food and detergent industry applications, Environmental challenges and protein engineering, therapeutic protein production, antibody modelling, biopolymer production, applications in Nanobiotechnology, Applications with redox proteins and enzymes, industrially important enzymes, role in gene regulation, Other new applications

Reference book

**Lab Exercise for Protein Engineering:**
1. Isolation and purification of protein
2. SDS - PAGE
3. Demonstration of Mass spectroscopy MOLDI TOF
4. Gel filtrations chromatography
5. Affinity chromatography
6. To find out capacity & nature of the given ion exchange resin
7. Effect of pH, temperature on activity or stability of protein
8. Protein structure prediction by bioinformatics
9. Protein structure prediction and classification
10. Application of Bioinformatics tools in support of protein research
11. Searching protein sequence databases
12. Protein structure visualization
13. Secondary structure prediction
14. Protein structure prediction
15. Structural prediction through homology modelling
16. Ant other exercise based on theory paper content
BTH C02:- Proteomics and Genomics

Genomics: Genome sequencing strategies and programs, new technologies for high-throughput sequencing, methods for sequence alignment and gene annotation; Approaches to analyze differential expression of genes - ESTs, SAGE, microarrays and their applications; gene tagging; gene and promoter trapping; knockout and knock-down mutants; dynamic modulation of protein structure and function, chip in chip.

Genome and Gene databases, Brief Outlook of Various Plant Genome Projects and their Outcome (Arabidopsis, Tomato, Potato, Rice), Non-coding RNAs, Transcriptomics, RNA interference and gene silencing, genome imprinting, small RNAs-biogenesis and functions, role of small RNAs in heterochromatin formation and gene silencing, tools to study methylome and histone modifications Humane and Mouse.

Proteomics: Analysis of proteins by different biochemical and biophysical procedures like CD (Circular Dichroism), NMR, UV/Visible and fluorescent spectroscopy, protein identification and analysis on ExPASy server, other protein related databases, 1-D and 2-D gel electrophoresis for proteome analysis, Sample preparation, gel resolution and staining.

Mass spectrometry based method for protein identification like PMF (protein mass fingerprinting) and LCMS, (Image analysis of 2D gels) Data acquisition, spot detection & quantitation, gel matching, data analysis, presentation, databases, conclusions; DIGE (Differential In Gel Electrophoresis), alternatives to 2-DE for protein expression analysis; Analysis of post-translational modifications and protein-protein interactions; protein chips and arrays, future directions in proteomics, scope of functional proteomics, and Protein databases, Protein-protein interaction, Interactome.

Practical Exercises:
1. Demonstration and listing of sequence retrieval online tools.
2. Demonstration and listing of sequence submission online tools.
3. Listing and demonstration of Protein and DNA Sequence Databases and their utilities.
4. Demonstration of DNA and Protein Array Technology and applications.
5. Reverse transcription-PCR to examine gene expression.
6. Real-time PCR to quantify gene expression.
7. Northern and Western Blotting analysis.
8. Demonstration of Instrumentation (MALDI/TOF, LC-MS-MS, 2DGE) by visit or audio-visual medium.
9. Protein separation techniques (Chromatography-Ion-Exchange, Gel Filtration, Affinity; Ultrafiltration, Recombinant protein separation techniques).

10. Comparison of Next-generation sequencing methods (by Chart/ poster preparation).

11. Any other exercises designed by course teacher as per the syllabus.

SUGGESTED READINGS:
2. Hammes GD (2005) Spectroscopy for the Biological Sciences; Wiley Interscience, USA.
3. Harlow and Lane D (Eds.) (1988) Antibodies – A Laboratory Manual; Cold Spring Harbor Laboratory, USA.
M.Sc. Biotechnology
Semester-IV (2017-18)

BTH X01:- Plant Biotechnology

Plant tissue culture: Principles, Concept, History of development of plant tissue culture, Concept of totipotency, PTC laboratory facilities, operation and management, General methodology. Different PTC media and their nutritional components, media preparation and sterilisation techniques, aseptic techniques and preparation of explants, histological techniques for plant tissue culture. Cryopreservation and slow growth for germplasm preservation.

Plant tissue culture technology: Shoot morphogenesis and organogenesis, rooting, hardening and field transfer; Micropropagation, production of virus free plants, callus and suspension cultures, single cell culture. Ovary, anther and microspore culture for production of haploid plants. Somatic embryogenesis, synthetic seeds and its cryopreservation. Plant tissue culture as a technique to produce novel plants, somaclonal variations. Overview of Plant Tissue Culture Applications.

Protoplast technology: Protoplast isolation, purification, viability tests, plating efficiency, culture, Somatic cell hybridization, selection of hybrid, cybrids and their regeneration.

Plant transformation (Recombinant DNA) technology: Tools and techniques, Vectors for plant transformation (Viral and Bacterial), Basic molecular characteristics of Agrobacterium, Basis of tumor and hairy-root formation, Characteristic features of vectors (Co-integrative and binary vectors, Ti, Ri plasmids, 35S and other promoters and terminators, selectable markers, reporter genes, origin of replication etc.)

Agrobacterium-mediated plant transformation: Cloning of selected gene, its integration into Agrobacterium. Agrobacterium-mediated gene transfer - mechanism of T-DNA transfer and its integration into plant genome. role of virulence gene, selection of transformed cells/
tissues, expression of the integrated gene in plants. Multiple gene transfer. Practical applications of *Agrobacterium*-mediated gene transfer.

**Direct gene transfer methods**: Particle bombardment, electroporation and micro injection. Transgenic gene incorporation, stability and expression; gene silencing. Cryopreservation and Genebanks.

**Plant Breeding**: Brief idea about conventional Plant Breeding Methods- Character identification, incorporation (hybridization), selection and release of variety; Role of **Molecular markers**: RFLP, RAPD, STS, SCAR, SSCP, AFLP in plant breeding applications. Green house and green-home technology.

**Transgenic approaches to crop improvement**: Resistant against biotic (virus, fungi, bacteria, nematode, insect, weed) and abiotic stress (salinity, drought, herbicide, cold, metals), longer shelf life. Improvement of crop yield and quality - golden rice and other developments. Extension of flower life, pigmentation and fragrance.

**Manufacture of valuable products**: Industrial applications of plant cell culture; Plant cell culture and biosynthesis of secondary products; Manufacture of - antigens, antibodies, edible vaccines, enzymes, proteins.

**Suggested Laboratory Exercises**:

1. Preparation of Stock solutions for MS medium.
2. Preparation of medium.
3. Micro propagation technique
4. Surface sterilization and Organ culture
5. Callus induction, propagation, and differentiation
7. Hardening and transfer of plants to soil.
8. Study of somatic embryogenesis.
10. Ovary culture
11. Somatic embryogenesis using appropriate explants and Preparation of synthetic seeds
12. Protoplast isolation and culture
13. Demonstration of protoplast fusion employing PEG.
15. Isolation & Identification of Sec. metabolite from Plant Cell Cultures.
16. Agrobacterium culture, selection of transformants, reporter gene (GUS) assays.
17. Any other practical based on theory syllabus

**Suggested Readings:**

BTH X002:- IPR & Biosafety


Concept of ‘prior art’
Invention in context of “prior art”; Patent databases; Searching International Databases; Country-wise patent searches (USPTO, EPO, India etc.); Analysis and report formation

Basics of Patents:-
Types of patents; Indian Patent Act 1970; Recent Amendments; Filing of a patent application; Precautions before patenting-disclosure/non-disclosure; WIPO Treaties; Budapest Treaty; PCT and Implications; Role of a Country Patent Office; Procedure for filing a PCT application

Patent filing and Infringement
Patent application- forms and guidelines, fee structure, time frames; Types of patent applications: provisional and complete specifications; PCT and convention patent applications; International patenting-requirement, procedures and costs; Financial assistance for patenting-introduction to existing schemes; Publication of patents-gazette of India, status in Europe and US Patenting by research student's, lecturers and scientists-University/organizational rules in India and abroad, credit sharing by workers, financial incentives Patent infringement- meaning, scope, litigation, case studies and examples

Biosafety
Introduction; Historical Backround; Introduction to Biological Safety Cabinets; Primary Containment for Biohazards; Biosafety Levels; Biosafety Levels of Specific Microorganisms; Recommended Biosafety Levels for Infectious Agents and Infected Animals; Biosafety Protection of New GMOs, guidelines - Government of India; Definition of GMOs & LMOs; Roles of Institutional Biosafety Committee, RCGM, GEAC etc. for GMO applications in food and agriculture; Environmental release of GMOs; Risk Analysis; Risk Assessment; Risk management and communication; Overview of National Regulations and relevant International Agreements including Cartagena Protocol.

Important Links
http://www.w3.org/IRP/
http://www.wipo.int/portal/index.html.en
http://www.ipo.co.uk/IP_convenions/patent_cooperation_treaty.html
www.patentoffice.nic.in
www.iprsumindia.org/ - 31k - Cached - Similar page
http://www.cbd.int/biosafety/background.shtml
http://www.cdc.gov/OD/ohs/symp5/jyrtex.htm
http://web.princeton.edu/sites/ehs/biosafety/biosafetypage/section3.html
BTH X003: Dissertation & Industrial Training

The project work will involve in-depth practical work on a problem suggested by the supervisor of the candidate. The evaluation of the dissertation will be done by the external examiner and carry 50 marks. The dissertation submitted by the candidate shall be evaluated by one external expert, Head of the department and supervisor of the candidate. The seminars, in-plant training and industrial visit reports will also be submitted by the candidate to the Head of the Department who will submit these to the external examiner. The examination shall be held in the department and the dissertation etc. will NOT be required to be mailed to the external examiner. The distribution of the marks will be as under:

<table>
<thead>
<tr>
<th>Component</th>
<th>Marks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissertation Report</td>
<td>50</td>
</tr>
<tr>
<td>Evaluation (PPT &amp; Viv. Voz)</td>
<td>50</td>
</tr>
</tbody>
</table>

M.M. 100
BTH A01:- VIROLOGY

Vaccines - 4 Credits

Innate Immunity;

Activation of the Innate Immunity through TLR mediated signaling; Adaptive Immunity; T and B cells in adaptive immunity; Immune response in infection; Protective immune response in bacterial; Viral and parasitic infections; Correlates of protection

Vaccination and immune response;

Appropriate and inappropriate immune response during infection: CD4+ and CD8+ memory T cells; Memory B cells; Generation and Maintenance of memory T and B cells; Dendritic cells in immune response

Adjuvants in Vaccination;

Induction of Th1 and Th2 responses by using appropriate adjuvants; Microbial, Liposomal and Microparticles as adjuvant; Chemokines and cytokines; Role of soluble mediators in vaccination; Oral immunization and mucosal Immunity

Conventional vaccines;

Bacterial vaccines; Live attenuated and inactivated vaccine; Subunit Vaccines and Toxoids; Peptide Vaccine

New Vaccine Technologies;

Rationally designed Vaccines; DNA Vaccination; Mucosal vaccination; New approaches for vaccine delivery; Engineering virus vectors for vaccination; Vaccines for specific targets; Tuberculosis Vaccine; Malaria Vaccine; HIV vaccine

Texts/References

2. Topley & Wilson’s, Microbiology and Microbial Infections Immunology Edited by Stefan H.E. Kaufmann and Michael W Steward Holder Arnold, ASM Press, 2005 or later edition.
4. Annual Review of Immunology: Relevant issues
5. Annual Review of Microbiology: Relevant issues

Suggested Projects: Based on given syllabus
BTH B02:- Applied Environmental Biotechnology

Credit: 4

Bioremediation & Phytoremediation: Biofeasibility, applications of bioremediation, Bioreduction, Phytoremediation.

Bioabsorption and Bioleaching of heavy metals: Cadmium, Lead, Mercury, Metal binding targets and organisms, Bioabsorption, Metal microbial interaction, Biomethylation of elements (Methylation of mercury and arsenic), Commercial biosorbents, bioleaching, metal precipitation, advantages and disadvantages of bioleaching.

Waste water Treatment: Biological treatment system (Oxidative ponds, aerobic and anaerobic ponds, facultative ponds, aerated ponds), Biological waster treatment, activated sludge treatment, microbial pollution in activated sludge, percolating filters, waster water treatment by biofilms. Treatment scheme of Dairy, Distillery, Tannery, Sugar, Fertilizers, Refinery, Chemical and Antibiotic waste.


PRACTICALS—CREDIT 2

Visit to
Sewage/waste water treatment plants,
Farms doing floriculture, vegetable farming,
Dairy/sugar/dye industry treatment plants
Krishivaigyanikkendras for composting
Report writing of various visits.

REFERENCES---

2. APPLIED BIOREMEDIATION AND PHYTOREMEDIATION(2004) Ed by AJAY SINGH and OWEN P WARD, SPRINGER
4. The Complete Technology Book on Biofertilizer and Organic Farming (2nd Revised Edition) [NI115] by NIIR Board,
5. The Complete Technology Book on Vermiculture and Vermicompost [NI116] by NPCS Board of Consultants and Engineers,
6. Biopesticides Handbook [NI110] by NPCS Board of Consultants & Engineers,
7. Manufacture of Biofertilizer and Organic Farming [NI132] by H. Panda,
BTH C03: - Nano Biotechnology

Introduction: History - The purple of Cassius, the Lycurgus cup, Michael Faraday and "divided metals". Richard Feynman, his theory and predictions, Moore's Law, Miniaturization of microprocessors, the story of the Damascus sword. What is "nano"? Comparison with familiar objects. From nanoscience to nanotechnology: Eric Drexler.

Nanoscience: The multidisciplinary science, Bottom-up and top-down approach of research with examples, Different types of Nanomaterials, one-dimensional: CNTs, its types and characteristics, Two-dimensional: Nanofilms, nanosheets, nanowalls.

Properties of Nanomaterials, Different types of Nanomaterials and their Size, Quantum effects. Seeing Nanomaterials: Microscopes (SEM, TEM, STM, AFM) How do the different types of microscope work with Nanomaterials. Preparation of Nanomaterials: Physical Method (hydrothermal and solvothermal) Chemical methods, Biological methods (Green synthesis using plants, microbes & other living organisms).

Nanobiotechnology: Nanomedicine, Nanocosmetics, Textiles, Nanosensors (Biological, chemical, Biosensors, Gas sensors, mechanical), Drug delivery, Cancer therapy, Tissue engineering, water purification, Lab-on-a-chip (LOC), nanocomputers, DNA computer, MRI with magnetic nanoparticles. Current trends of research in Nanobiotechnology, particularly health sciences.


Suggested Readings:

6. C M. Namocyct and C A. Minkin Nanobiotechnology. 11 2012 Wiley VCH
Suggested Exercises:
2. Green synthesis of Nanoparticles using Plant system from Leaves, fruit, callus etc.
3. Characterization of Nanoparticles using UV Visible Spectroscopy, XRD, FTIR.
4. Visualization of Nanomaterials using SEM, TEM, AFM.
5. To check the bioactivity of Nanomaterials on various pathological Fungi and Bacteria.
6. To check the effect of Nanomaterials on Plant germination parameters.