

ADIKAVI NANNAYA UNIVERSITY

CBCS/SEMESTER SYSTEM

IV SEMESTER: B.SC HUMAN GENETICS

W.E.F. 2015-16 ADMITTED BATCH

DSC 1D (Paper- IV): Recombinant DNA Technology

Unit 1 Cell Based Cloning

- 1.1 Restriction endonucleases and other enzymes used in manipulating DNA molecules
- 1.2 Cloning vectors – plasmid vectors, lambda and cosmid vectors, P1 phage vectors, YAC, BAC, M13 or phagemid vectors, expression vectors
- 1.3 Introducing recombinant DNA into recipient cells
- 1.4 DNA libraries -generation of genomic and cDNA libraries; chromosomal DNA libraries

Unit 2 Screening constructs

- 2.1 Nucleic acid hybridization-
- 2.2 Sequencing DNA- Sanger's method
- 2.3 PCR– fundamentals, designing of primers, real time PCR
- 2.4 Human- rodent somatic cell hybrids

Unit 3 Genetic and Physical Mapping of Human genome

- 3.1 Genetic Mapping – genetic polymorphism, RFLP, STR polymorphism,
- 3.2 Homozygosity mapping, linkage dis-equilibrium mapping, radiation hybrid mapping
- 3.3 Genotyping – SNPs
- 3.4 Physical mapping – assembling contigs from BAC libraries

Unit 4 Cloning Human disease genes

- 4.1 Cloning human disease genes- functional candidate gene cloning, positional candidate gene cloning
- 4.2 Detection of mutations in human genes –SSCP analysis, DGGE, chemical mismatch cleavage
- 4.3 Detection of mutation in human gene – DNA sequencing, heteroduplex analysis, protein truncation

Unit 5 Applications of rDNA technology

- 5.1 DNA fingerprinting – use of mini-satellites for DNA fingerprinting, single locus probes, STRs
- 5.2 Genetic testing – prenatal testing, neonatal screening, diagnosis of genetic disease in children after birth, pre-symptomatic testing.
- 5.3 In vivo, in vitro gene therapy; vehicles for gene therapy; gene therapy for heritable and non- heritable genetic diseases.

Practicals

1. Isolation of plasmid DNA from *E. coli* cells.
2. Digestion of plasmid DNA with restriction enzymes.
3. Estimation of size of a DNA fragment after electrophoresis using DNA markers
4. Construction of restriction digestion maps from data provided
5. Recovery of DNA from low-melting temperature agarose gel
6. Preparation of competent cells of *E. coli*
7. Transformation of competent *E. coli* cells with plasmid DNA
8. Amplification of a DNA fragment by PCR.
7. Complementation of beta-galactosidase for Blue and White selection.
8. Southern blotting
9. Western blotting.

Suggested 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).
4. Human Molecular Genetics by Sudbery.