Subject Code : CCN3P10

Core Course X (CC) – Molecular Biology and Genetic Engineering

Unit I – DNA structure, replication and repair

DNA structure: historical aspects and current concepts, melting of DNA. DNA replication: general principles, continuous and discontinuous synthesis, various modes of replication. Prokaryotic and eukaryotic DNA polymerases, structure and functions. Superhelicity in DNA, topological properties, mechanism of action of topoisomerases. Inhibitors of DNA replication (blocking precursor synthesis, nucleotide polymerization, altering DNA structure). DNA damage and repair: types of DNA damage (deamination, oxidative damage, alkylation, pyrimidine dimers). Repair pathways – methyl-directed mismatch repair, very short patch repair, nucleotide excision repair, base excision repair, recombination repair, SOS system.

Unit II – Transcription and regulation

DNA binding proteins, enhancer sequences and control of transcription. Identification of protein-binding sites on DNA. Structural features of RNA (rRNA, tRNA and mRNA) in relation to function. - Transcription: general principles - basic apparatus - types of RNA polymerases, Processing steps initiation, elongation and termination, inhibitors of RNA synthesis. Polycistronic and monocistronic RNAs. Regulation of transcription; RNA polymerases and promoter interaction, alternate sigma factors, controlled termination: attenuation and antitermination. Global regulatory responses - heat shock response - stringent response and regulation by small molecules such as ppGpp and cAMP, regulation of rRNA and tRNA synthesis. Maturation and processing of RNA: methylation, cutting and trimming of rRNA; capping, polyadenylation and splicing of mRNA ; cutting and modification of tRNA degradation system. Catalytic RNA, group I and group II intron splicing, RNase P.

Unit III – Genetic engineering Principle

Restriction and modification in bacteria *E. coli* K & B system; Restriction endonucleases type I, II, III - Ligases. Vectors – plasmids – phages, cosmids, phagemids, special vectors – broad host range, expression, integrating shuttle vectors – yeast vectors. Principles of gene cloning – □ complementation, genomic library & cDNA library – shot gun cloning – screening of recombinants – phenotypic expression of characters – colony hybridization – southern hybridization – use of antibody – Western blot – Physical mapping of the cloned gene.

Unit IV – PCR techniques

PCR technology – Gene amplification, PCR primer designing and optimization; variations in PCR (RT PCR, RACE) RAPD, RFLP and site directed mutagenesis – DNA sequencing – Manual and automated chromosome walking – DNA foot printing.

Unit V – Microbial products through genetic engineering

Cloning of human insulin, Interferon in *E. coli* – Human antibody production by rDNA technology – Vaccine production. Plant genetic engineering – Ti plasmid, CaMV vector – DNA delivery to plant protoplast – transgenic plants – cloning of endotoxins – *Cry* gene – Herbicidal resistance.

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