

Fundamentals of Molecular Microbiology (LS635A)

AS. Kharat* & SL Panwar

Unit I: Mutation & Recombination.

Mutation: Spontaneous, Induced, Adaptive. Induced; Physical, Chemical and Biological. Biological; Stress Induced, Transposon mediated mutagenesis.

Genetic Recombination: Transformation, Conjugation; F-mediated, HFR and F', Transduction; generalized and specialized transduction. Genetic mapping with genetic recombination methods.

Unit II: Modes of Genetic Engineering

Systems safeguarding DNA, mechanism of escaping restriction, modification, restriction, criteria for decision of DNA fragment to be restricted or modified, Classification of Restriction Endonucleases, their properties and specificities, applicability.

Modification Enzymes: Each of the enzyme is to be studied with reference to its source, structure, function (activity –mode of action).

An overview of vectors with reference of cloning size and their merit –demerit :.

General aspects: Natural plasmids; colE1, RSF1030, cloDF13, R6K, F, R1, EntP 307. Properties of cloning vectors.

Plasmid vectors: Construction of pBR322, negative selection or gene disruption strategies, Tet promoter, anti-Tet promoter, improved vector derivatives of pBR322 such as pUC18, pUC19.

Cloning and Expression strategies in bacteria and yeast: *Escherichia coli* expression vectors, and vectors for expression in yeast. Use of plac, ptrp, pBAD, para, pgal, phage promoters, sp6, T3, and T7 promoters in addition to lambda pR, pL, pR' promoters for expression, Codon selection, maximizing expression, hybrid promoters (ptac, ptrc.), manipulation of clones gene to achieve expression, solubilization of proteins, fusion proteins (typically translational fusion vectors, but also cover transcriptional fusion strategy) and their applications. Shuttle vectors, integrative plasmid, centromeric plasmid, autonomously replicating- episomal vectors, with reference the merit-demerits of cloning strategies.

Unit III: **Gene Expression in Prokaryotes**

Cis element and Trans Factors, Operon concept, Co-ordinated control of structural genes, the *lac*, *trp*, *ara*, *gal* operons, repressor proteins; gene /genetic system specific repressor, global regulator, operator sequences, and other DNA elements for the regulation or gene expression (regulatory sequences –DNA and RNA), attenuation mediated regulation –biosynthesis of amino acids, anti-termination mediated regulation –phage Lambda N and Q as paradigm, negative regulation, catabolite repressor –an example of positive regulation, stationary phase sigma and nitrogen fixation sigma –the *nif* genes of *Klebsiella*, regulation of nitrogen fixation in *Rhizobium*.

Unit IV: **Molecular Principles underlying microbial pathogenesis especially bacterial and fungal pathogenesis with focus on biological tools used routinely to address pathogenesis**