Bacterial Genetics

Key Words

- Genetics
- Bacterial genetics
- Genotypic & Phenotypic Variations
- Mutation & its types
  - Point mutation
  - Frame-shift mutation
  - Lethal mutation
  - Suppressor mutation
  - Mis-sense & nonsense mutation
- Mechanisms of gene transfer
  - Transformation
  - Transduction
  - Lysogenic conversion
  - Conjugation
  - Transposition (Jumping Genes)
- Bacterial Drug Resistance
- Genetic Engineering

Genetics

The term coined by British biologist William Bateson in 1906

Genetics- study of genes, their structure, function, heredity and variation

Genomics- study and analysis of nucleotide sequence of DNA

Genome- complete set of genetic information for a cell

Bacterial Genetics

- Study of heredity & gene variations seen in bacteria
- Hereditary characteristics- encoded in bacterial DNA
- Bacteria breed true & maintain characteristics from generation to generation
- Exhibit variations in particular properties in small proportion of their progeny

Bacterial Genetics

- Since 1940, principles of genetics applied to bacteria & their viruses (bacteriophages)
- Bacterial genetics- used as model to understand DNA replication, genetic characters, their changes & transfer to next generations

Molecular Biology

- Branch of biology
- Deals with formation, structure & functions of macro molecules essential for life e.g. nucleic acid and proteins
- Includes roles of these molecules in cell replication & transmission of genetic information from generation to generation

Molecular Genetics

- Concerned with analysis & manipulation of DNA using biochemical & microbiological techniques
- Helps in understanding genetic mutation that can cause certain type of diseases

Basic Principles of Molecular Biology

Central dogma of molecular biology

DNA carries genetic information

↓← transcribed

onto mRNA

↓← translated

as particular polypeptide (constitute proteins & enzymes)

↓

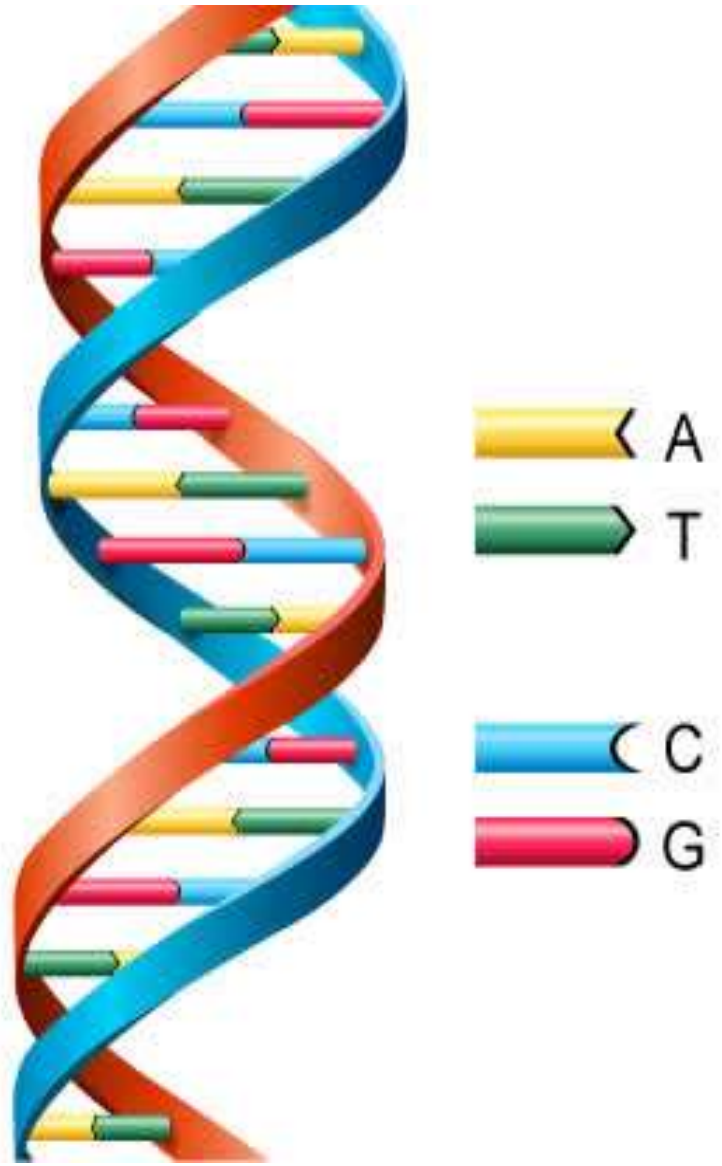
determines nature & function of cell

Molecular Biology contd.

- DNA structure
- RNA structure
- Codon
- Gene

Structure of DNA

- DNA (de-oxyribonucleic acid):- Stores information for protein synthesises
- Double Helix Structure of DNA- Watson & Crick model
- DNA- composed by 2 chains of polypeptides. Each chain has backbone of de-oxyribose sugar & phosphate residues arranged alternately

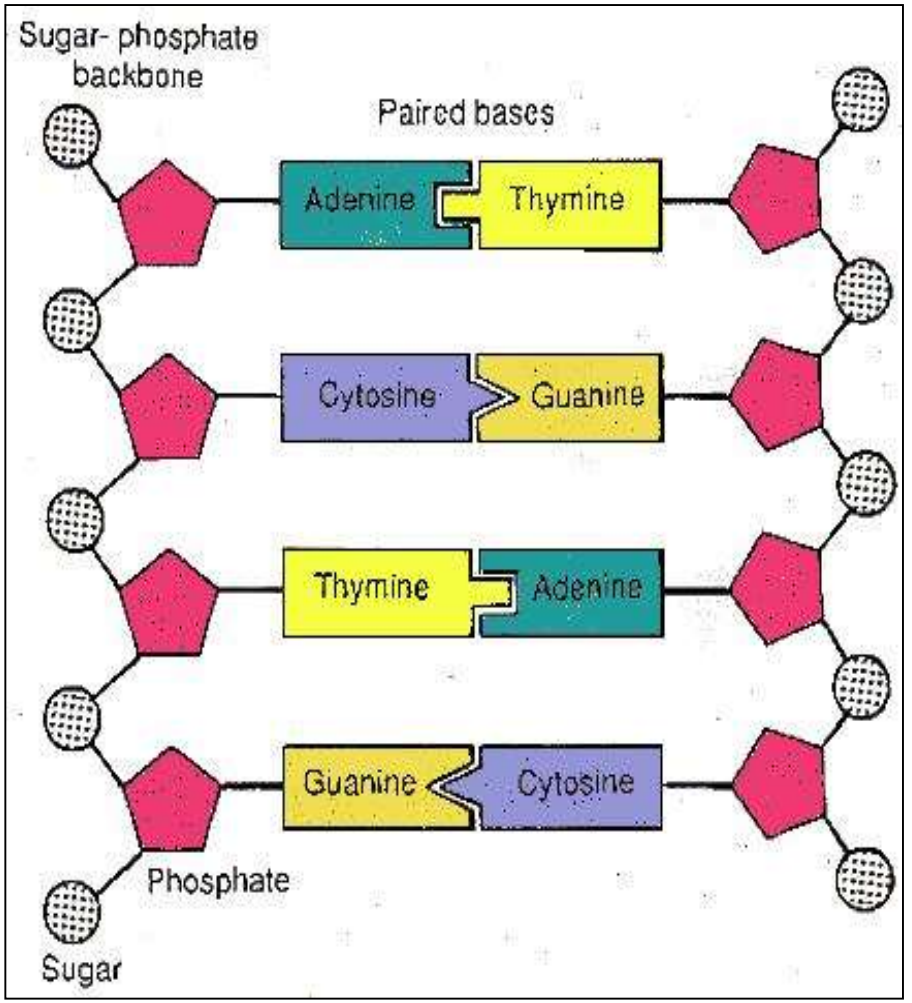


Structure of DNA contd.

4 nitrogenous bases

Adenine (A)
Guanine (G) — Purine

Thymine (T)
Cytosine (C) — Pyrimidine



Structure of RNA

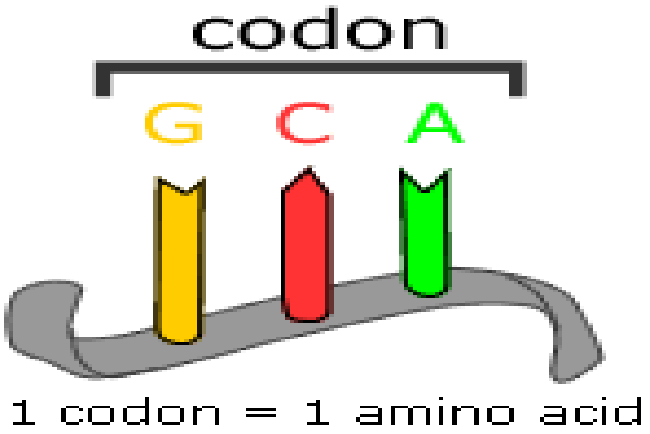
- Structurally similar to DNA, EXCEPT for 2 major differences:
  - ribose sugar
  - uracil in place of thymine

- 3 types of RNA
  - m RNA (messenger RNA)
  - t RNA (transfer RNA)
  - r RNA (ribosomal RNA)

Polypeptide Synthesis

- Genetic information- stored in DNA as *Code*
- Unit of genetic code- *codon*

Codon



- Codon- triplet
- Sequence of 3 bases
- Present on mRNA, store information of amino acid synthesis
- Genetic code- universal, specific, non-overlapping & degenerative
- 64 codons, 61- sense codon & 3- non-sense codon
- Each codon- directs production of single amino acid

Codon

- 20- amino acids, more than one codon may exist for same amino-acid e.g. AGA for Argenine & AGG, CGU, CGC, CGA CGG code for Arginine
- **Non-sense (stop)codon-** do not code for any amino-acid
- Act as punctuation marks & terminate translation- message for synthesis of polypeptide)
- e.g. UAA, UGA, UAG

Transcription & Translation

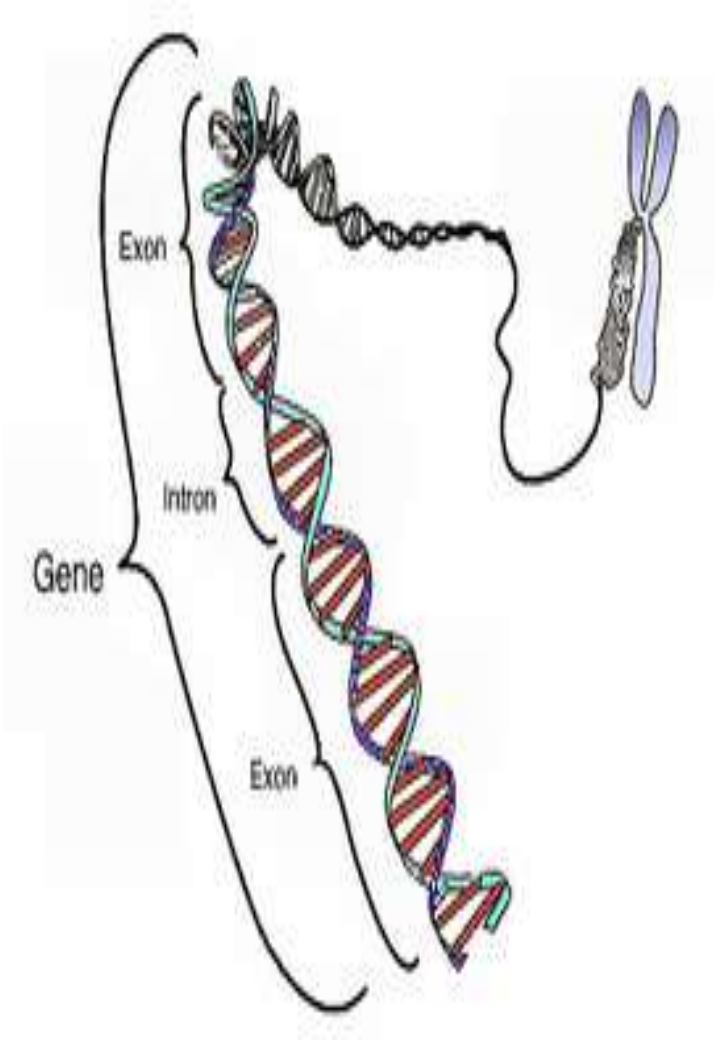
- **Transcription-** particular segment of DNA copied into RNA by RNA polymerase
  DNA acts as template for synthesis of mRNA

- **Translation-** decoding of mRNA (transcribed from DNA) by ribosome to produce a specific amino acid chain/ polypeptide, occurs in CP

Gene

- Segment of DNA
- Unit of heredity of living organism
- Gene carries codon specifying for particular polypeptide

- DNA molecule consist of large number of genes
- Each gene contains hundreds of thousands of nucleotides
- Stretches of **coded genes**- **exons**, translated into gene products
- Stretches of DNA don't appear to function as codons- **introns** (useless, non-coding intrusions)



Bacterial Chromosome

- Single, Circular, DS-DNA
  Length- 1000µ (4000kbp)
  [1kb=1000base pairs]
- Human genome- 3 million kb long

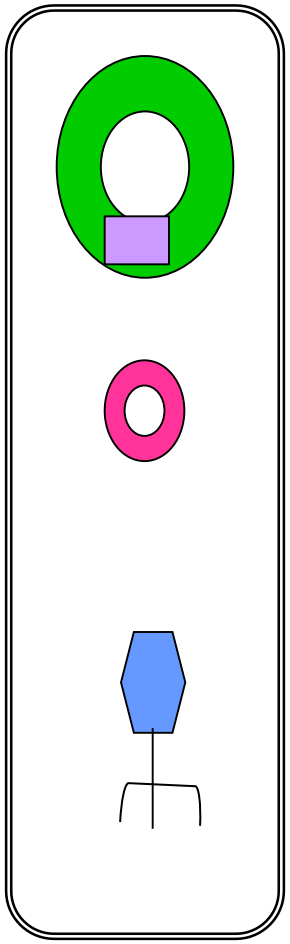
Genetic Information In Bacteria

Chromosome — Carries properties like virulence, pathogenicity & resistance

Plasmid — Extra-chromosomal genetic material present in the cytoplasm

Replicate independently

Bacteriophage — Virus infecting bacteria



Extra Chromosomal Genetic Elements

- Not essential for normal life & functioning
- Confer properties like drug resistance, toxigenicity→ leads to survival advantage under appropriate conditions

Extra Chromosomal Genetic Elements

- Plasmids
- Episomes
- Transposons

Plasmids

- Circular ds DNA molecule
- Exist free in CP of bacteria
- Capable of autonomous replication (*independent replicons*)
- Transfer genetic information from one cell to another
- Vector in genetic engineering
- Seen in yeast

Episome

- Plasmid integrate with chromosomal DNA of bacteria
- Replicate with bacterial chromosome

Plasmids- Classification

A. Based on ability to perform conjugation
   a. conjugative plasmid- ability to transfer by conjugation, (self-transmissible)
   b. non-conjugative plasmid- unable to transfer (non-transmissible)
B. Based on compatibility bet^{N} plasmids
   a. compatible plasmids- different plasmids exist in single bacterial cell if compatible
   b. incompatible plasmids-

Plasmids- Classification

C. Based on function- 5 classes

a. **Fertility/ F-plasmids**- code for expression of sex pilli, help in conjugation by forming conjugation tube

b. **Resistance plasmids**- genes code for resistance to antibiotics

c. **Col plasmids**- genes code for bacteriocin

d. **Virulence plasmids**- code for virulence factors & toxins help in bacterial pathogenesis e.g. heat labile & heat stable toxin of E.coli

e. **Metabolic plasmids**- enable the host in metabolic activities e.g. urease synthesis

Plasmid- Vector

- Plasmid- able to transfer DNA from one cell to another
- Vector in genetic engineering
- Genes- inserted artificially by recombinant technology at certain sites on plasmid
- Used in- protein synthesis, gene therapy

Gene Transfer in Bacteria

- Genetic variation- acquire new gene by mutation
- Newly acquired genes- transferred vertically to offsprings OR horizontally to other bacteria in surrounding

Genotypic and Phenotypic Variations

✪ **Genotype** – genetic constitution of cell that is transmitted to its progeny

✪ **Phenotype** – physical expression of genotype in given environment

Genotypic variation	Phenotypic variation
Sum total of gene that make up the genetic apparatus of cell	It is the physical expression of the genotype in a given environment
Hereditary includes complete genetic potential of the cell	Non heritable, cell may exhibit different situations in different environment
Stable, heritable, not influenced by environment	Reversible , non-stable and influenced by the environment & are temporary
D/t alteration in genome	Genomic alteration not occurs
e.g. mutation, gene transfer	e.g. typhoid bacillus, synthesis of beta-galactosidase enzyme by E.coli

Mutation

Spontaneous, random, undirected, heritable variation caused by an alteration in nucleotide base sequence at some point of DNA of cell

Mutation

- Natural event
- Taking place all time in all dividing cells
- Involve any gene- modify any characteristics
- Mutants- unrecognized (lethal mutation, involve minor function- unable to express)
- Appreciated- function readily observed by experiment e.g. E.coli mutant loses ability to ferment lactose

Mutation

- **Spontaneous mutation**- occur naturally in dividing cells, without adding mutagen
- **Induced mutation**- exposure of organism to mutagen (agents induce mutagenesis)
- Physical mutagens- UV rays, X-rays
- Chemical mutagens- alkylating agents, acridine dyes, 5- bromouracil, nitrous acid, benzpyrene, and 2-aminopurine
- Viruses e.g. bacteriophages

Types of Mutation

1. **Small-scale mutation**:

a. Point mutation occur at single nucleotide

b. Addition/ deletion of single nucleotide pair

2. **Large-scale mutation**:
occur in chromosomal structure

a. deletion/ addition of several nucleotide base pairs or gene duplication

Types of Mutation

Forward Mutation	Forward Mutation
A. **Substitution at single nucleotide base pair**	**B. Addition/ deletion at single or many nucleotide base pairs**
a. At DNA level	1. Frame-shift mutation
1. Transition	
2. Transversion	
b. At codon level	• **Reverse Mutation**
1. Silent mutation	1. True Reversion
2. Neutral mutation	2. Equivalent Reversion
3. Missense mutation	3. Suppressor Mutation
4. Nonsense mutation	

Point Mutation (Forward)

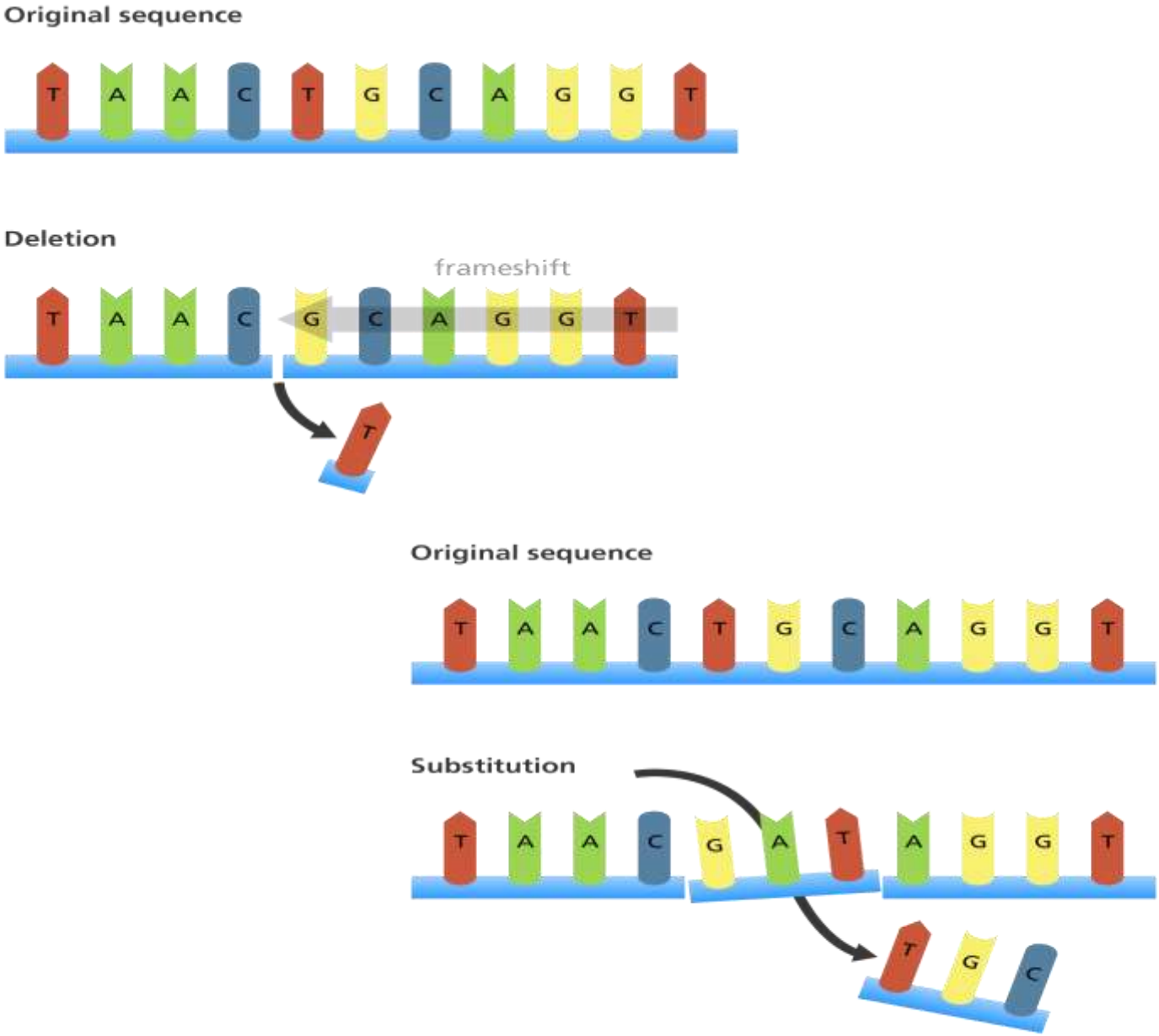
- Addition, deletion or substitution of one or more bases
- At DNA level Types-

1) Transition- Purine & pyrimidine replaced by other purine & pyrimidine e.g. A ⟷ T T ⟷ C

2) Transversion- Substitution of purine base by pyrimidine base or vice versa

A ⟷ T or C T ⟷ A or G

G ⟷ T or C C ⟷ A or G


Original sequence
T A A C T G C A G G T
Deletion
frameshift
T A A C G C A G G T
T
Original sequence
T A A C T G C A G G T
Substitution
T A A C G A T A G G T
T G C

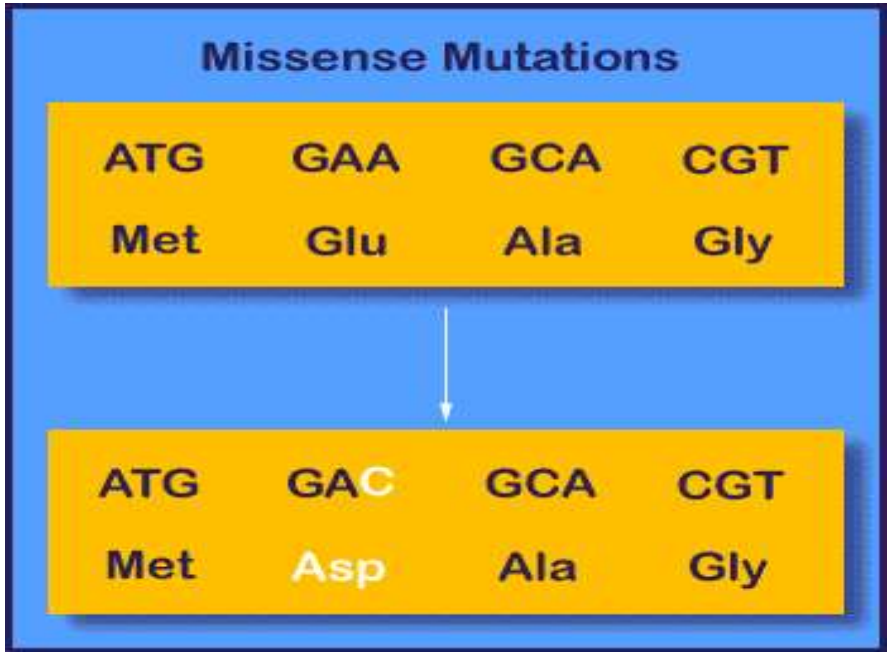
Point Mutation (Forward)

- At codon level-

1. Silent mutation- new codon codes for same amino acid e.g. AGG↔CGG codes for Arginine
2. Neutral mutation- new codon forms different but functionally equivalent amino acid e.g. AAA (Lysine) AGA (Arginine)
3. Missense mutation- new codon codes for different amino acid
4. Nonsense Mutation- new codon is stop codon- causes termination e.g. CAG (Glutamine)

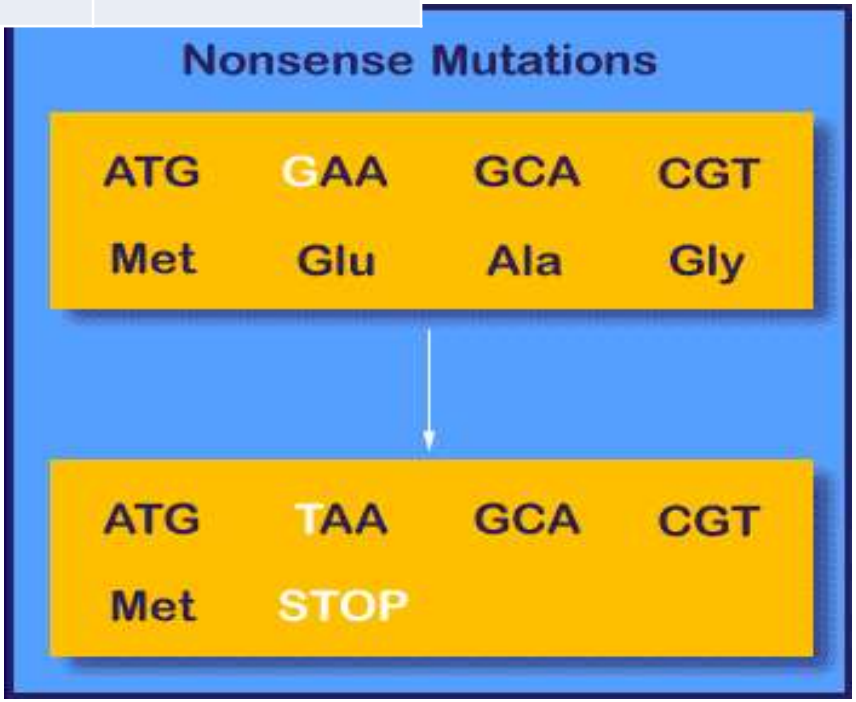
Missense mutation

ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly
	↓		
ATG	GA C	GCA	CGT
Met	Asp	Ala	Gly



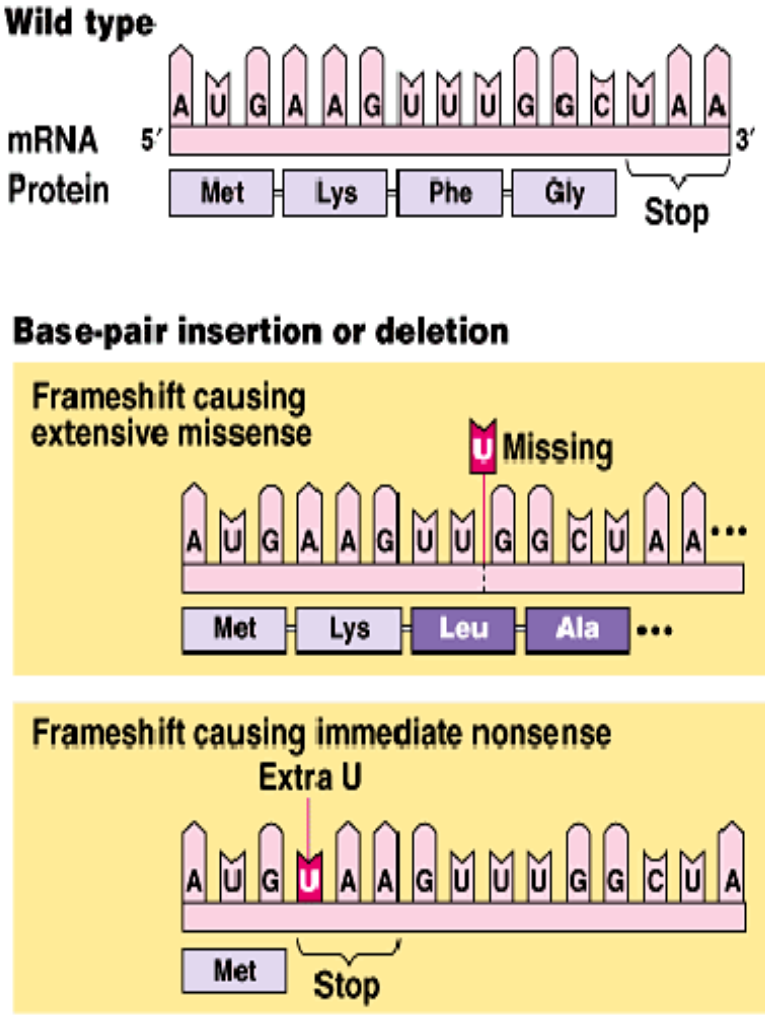
Nonsense Mutation

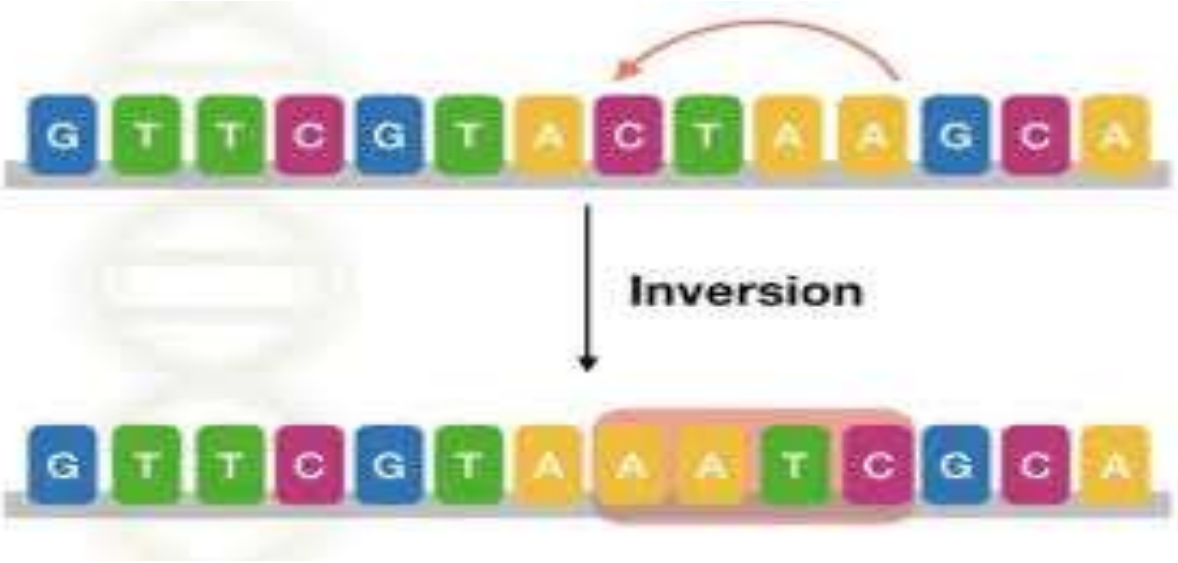
ATG	GAA	GCA	CGT
Met	Glu	Ala	Gly
	↓		
ATG	T AA	GCA	CGT
Met	STOP		

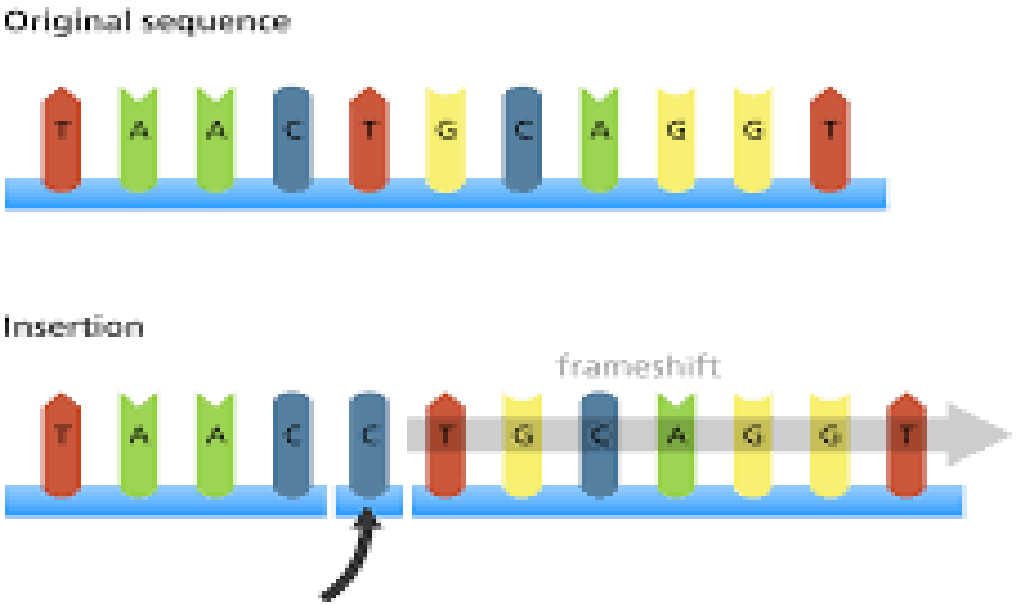


FRAME SHIFT MUTATIONS

- Caused by deletion or addition of ≥ 1 base pairs→ leads to shifting of frame resulting in incorporation of wrong amino-acid→ ends in production of inactive protein




G T T C G T A C T A A G C A
Inversion
G T T C G T A A A T C G C A


Original sequence
T A A C T G C A G G T
Insertion
frameshift
T A A C C T G C A G G T

REVERSE MUTATION

- Second mutation that nullifies effect of first mutation & results in gaining back function of wild phenotype
- **True reversion**- converts mutant nucleotide sequence back to wild-type sequence
  e.g. AAA (Lysine)→ forward mutation GAA (Glutamine)→reverse mutation AAA (Lysine)
- **Equivalent reversion**- second mutation produces different codon which codes for same amino acid of wild type sequence
  e.g. UCC (Serine)→ GAA (Cystine)→ AAA (Serine)

REVERSE MUTATION

- **Suppressor mutation**- Second mutation in different gene that reverts pheynotypic effects of already existing mutation

LETHAL MUTATION

- Mutations involve vital functions- mutants nonviable e.g Conditional Mutation
- A conditional lethal mutant- able to live under certain conditions (permissive conditions)
- Type of conditional mutant- Temperature Sensitive(ts)mutant- able to live at permissive temp. ($35^{o}C$) but not at restrictive temp.($39^{o}C$)

Effects of Mutation

- Alter drug susceptibility, Ag-structure & virulence of mutant bacteria
- Alter susceptibility of bacteria to bacteriophage, alter colony morphology, pigment production, affect ability to produce capsule/ flagella
- Development of drug resistance
- Variability in nutritional requirements, biochemical reaction, morphological features, virulence, host range

Importance of Mutation

- Drug resistance- confers survival advantage
- Development of live vaccines

Horizontal Gene Transfer

- Transformation (uptake of naked DNA)
- Transduction (thro' bacteriophage)
- Lysogenic Conversion
- Conjugation (plasmid mediated via conjugation tube)
- Transposition

Transformation (Griffith, 1928)

- Process of random uptake of free or naked DNA fragment from surrounding medium by bacterial cell and incorporation of this DNA fragment into its chromosome in heritable form

Transfer of genetic information by *free DNA* i.e. by direct uptake of donor DNA by recipient DNA

Live non-capsulated (R) pneumococci + heat killed capsulated (S) pneumococci

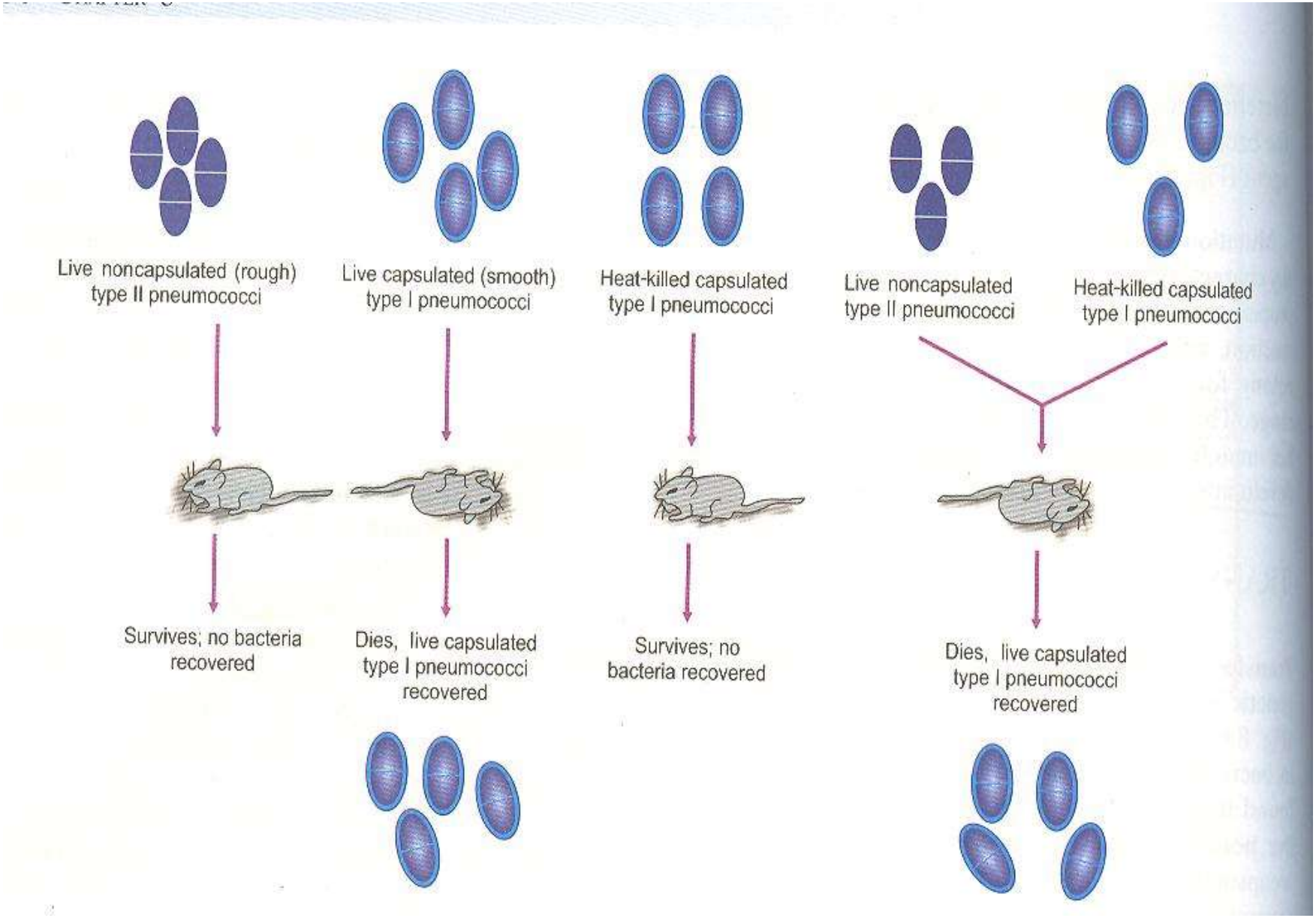
Injected into mice

↓

Death of mice

↓

- Live capsulated pneumococcus isolated from blood of mice


Live noncapsulated (rough) type II pneumococci
Live capsulated (smooth) type I pneumococci
Heat-killed capsulated type I pneumococci
Live noncapsulated type II pneumococci
Heat-killed capsulated type I pneumococci
Survives; no bacteria recovered
Dies, live capsulated type I pneumococci recovered
Survives; no bacteria recovered
Dies, live capsulated type I pneumococci recovered

Transduction

- Transmission of portion of DNA from one bacterium to another by bacteriophage

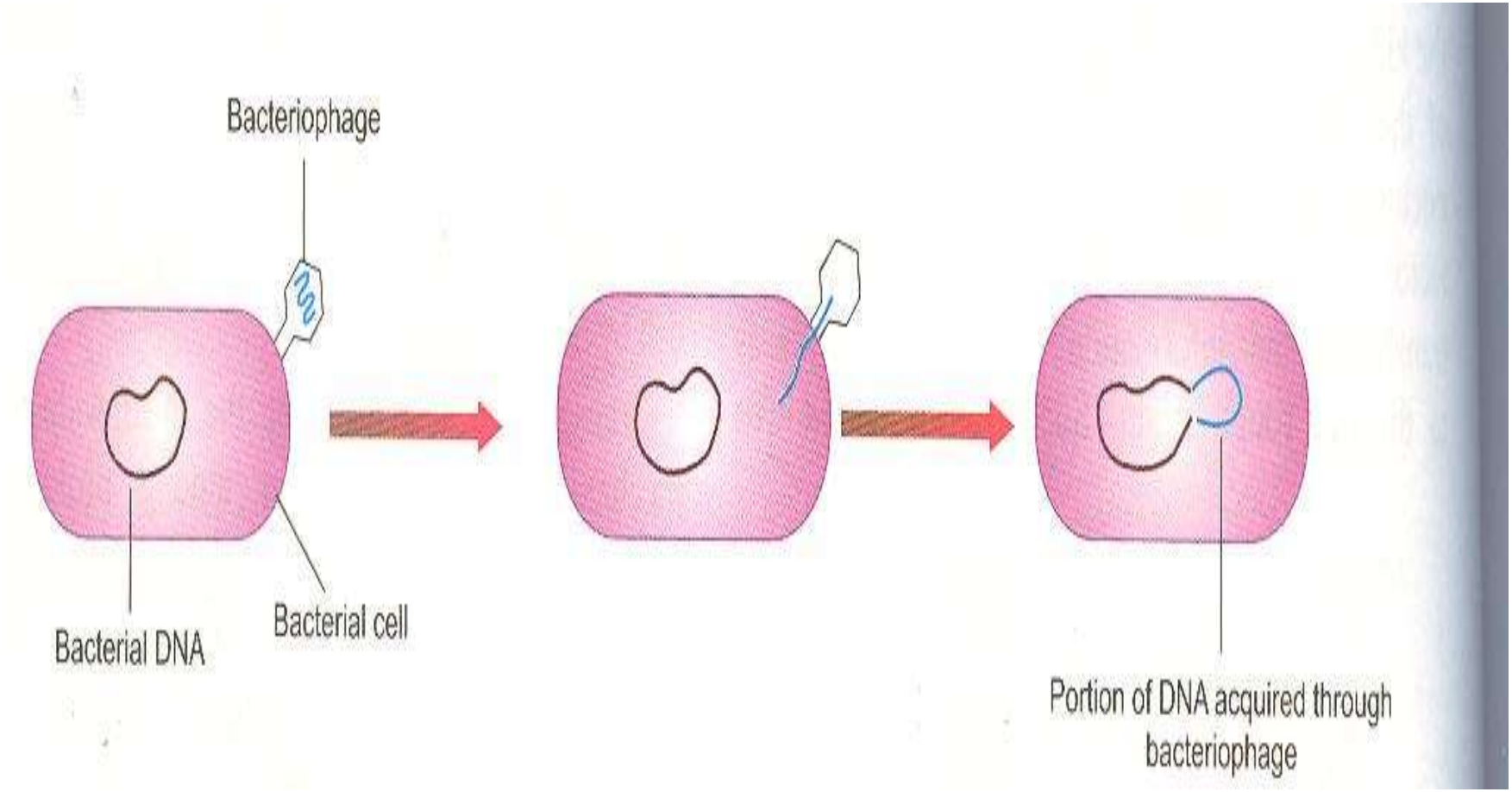
- During assembly in infected bacteria, some packaging error occur so phage particle have its own DNA & a segment of host DNA

↓

When this phage particle infect another bacteria, host DNA segment is transferred. Recipient cell acquires new characteristics coded by donor DNA

- Bacteriophage acts only as vehicle carrying bacterial genes from one cell to another

Transduction



Transduction contd.

- **Generalized transduction**:- involve any segment of donor DNA at random
- ➢ Defective assembly- instead of own DNA, host DNA incorporated into daughter bacteriophage
- **Restricted transduction**:- specific bacteriophage transduces only particular genetic trait e.g. Lambda phage of E.coli
- ➢ Defect in disintegration of lysogenic phage DNA from bacterial chromosome
- Transfer of plasmids/ episomes by transduction e.g. penicillinase plasmid- penicillin resistance in Staph.

Applications- Transduction

i) Excellent tool for genetic mapping of bacteria

ii) Proposed method of genetic engineering

a. T/t of some inborn error of metabolism

b. To correct metabolic defects in fibroblasts from galactosemic patients

Lysogenic Conversion

- Bacteriophages:- 2 types of life cycle-
  a) virulent/ lytic cycle
  b) temperate/ non-lytic/ lysogenic cycle
- In non-lytic cycle- phage DNA integrated with host (bacterial) chromosome i.e. prophage

↓

Prophage multiplies synchronously with host DNA

↓

Transferred to daughter cells (lysogeny)

- Lysogenic bacteria- bacteria containing prophage
- Prophage- additional segment of bacterial chromosome, encodes for new characteristics & transferred to daughter cells→ lysogenic/ phage conversion
- Phage DNA- responsible for bacterial virulence by coding for toxin production e.g. diphtheria toxin, cholera toxin, botulinum toxin C & D, verocytotoxin of E.coli, Streptococcus pyogenes exotoxin A & C
- Elimination of phage from toxigenic strain- renders bacterium nontoxigenic

Lysogenic conversion- contd.

- Lysogenic conversion- influences susceptibility to bacteriophages (immunity to superinfection with same or related phages)
- Lysogenic conversion- phage DNA behaves as new genetic element
- Transduction- phage acts as vehicle carrying bacterial genes

Conjugation

Described by Lederberg and Tatum (1946) in E.coli strain- K12

- Male/ donor bacterium mates/ makes physical contact with female/ recipient bacterium & transfers genetic elements/ information by forming conjugation tube

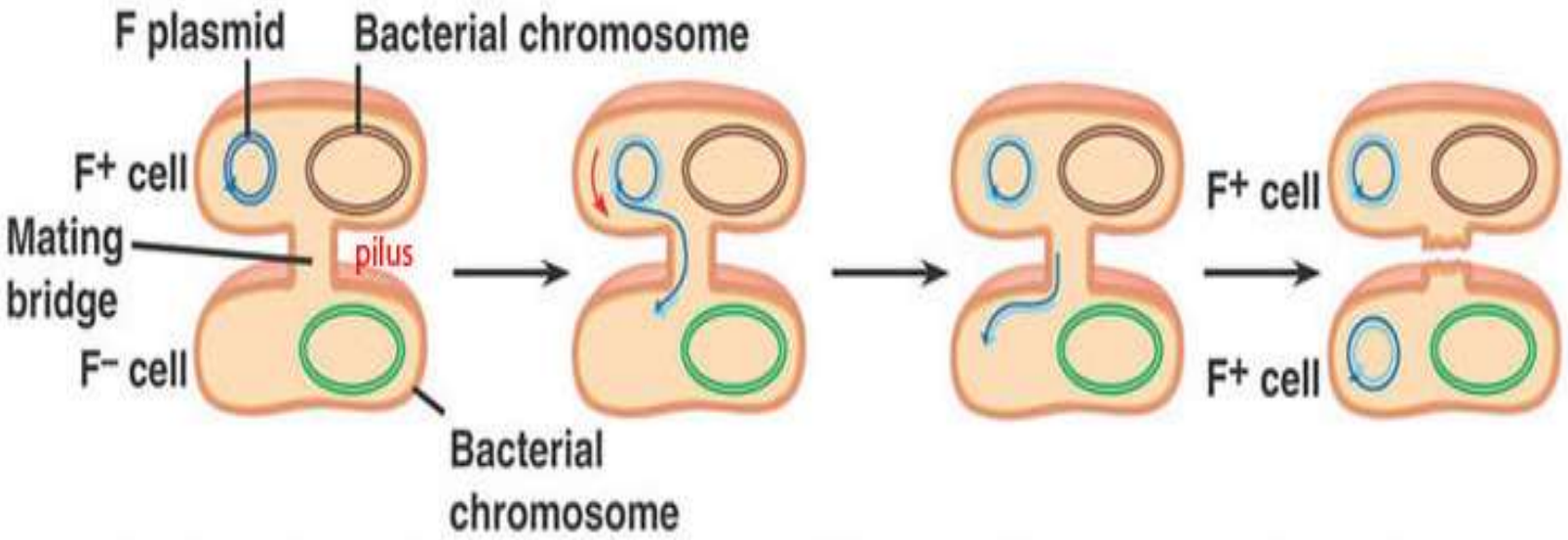
↓

Recipient bacterium becomes donor bacterium & conjugate other female cells

- Maleness- transmissible/ infectious characteristic

Process of Conjugation

- Maleness/ donor status- determined by presence of plasmid in it which codes for specialized fimbria (*sex pilus- conjugation tube*)



(a) Conjugation and transfer of an F plasmid from an F+ donor to an F− recipient

Conjugation contd.

- Plasmid responsible for conjugation- sex/ fertility factor (F-factor)
- Similar plasmids- named as ‘transfer factor’(able to conjugate)
- Transfer factors:- i) F-factor
  ii) Col-factor
  iii) R-factor

F – factor

- Transfer factor that contains genetic information necessary for synthesis of sex pilus & for self-transfer
- F^+ cells mate with F^- cells and make them F^+
- F-factor- episome, exists in 'integrated state' in some cells or inserted into host chromosome- able to tranfer chromosomal genes to recipient cells with high frequency ***(Hfr cells)***
- Conjugation with Hfr cell, F^- cell rarely become F^+ but receives genes from donor

F–factor contd.

- Conversion of F^+ into Hfr state- reversible
  When F-factor reverts from integrated state, carry some chromosomal genes
- F-factor incorporating chromosomal genes- F prime (F') factor
- When F'cell mates with recipient, it transfers along with F-factor, host genes incorporated with it
- Process of transfer of host genes thro' F' factor- **Sexduction** (resembling transduction)

Col-factor

- Plasmid- determines production of ‘colicin’
- Colicin- antibiotic like substance produced by one bacterium that inhibit other bacteria
- Similar substances- produced by other bacteria e.g. *Pseudomonas* (pyocin), *C.diphtheriae* (diphthericin)
- Named as *bacteriocin*
- Important- intra-species classification of bacteria e.g. Sh.sonnei, Ps.aeruginosa

Resistance Transfer Factoror

- Medically important plasmid- leads to spread of multiple drug resistance (MDR) in bacteria
- 1st reported by Japanese workers (1959)- transfer of MDR bet^{N} E.coli & Shigella strains

Resistance Transfer Factor

- Transfer of drug resistance by conjugation- transferable, episomal or infectious drug resistance
- Plasmid (R-factor) consists of:-

  i) RTF- responsible for conjugational transfer,

  ii) r-determinant for several antibiotics

Genetic Recombination

Along with plasmid DNA, portion of host DNA is sometimes transferred to recipient, donor DNA then combines with recipient DNA, effecting genetic recombination

Transposons (Barbara McClintock 1940)

- Bacterial genes- capable of intracellular transfer between chromosome to chromosome, plasmid to plasmid & chromosome to plasmid
- **Transposition**- process of intracellular transfer of transposons
- **Transposons**- move around genome in cut-and-paste manner- jumping genes/ mobile genetic elements
- Not self replicative, depend on chromosomal or plasmid DNA for replication

Transposition

- Transposition- transfer of genetic element from one molecule to another that has no genetic (DNA) homology
- Transposition- confer survival advantage
- Drug resistance d/t transposons- carry resist^{N} genes (r-determinants) which can easily move from one bacterium to another
- Transposition- mechanism for amplifying genetic transfer in nature

Gene transfer- Artificial methods

- **Genetic Engineering-** deliberate modification of genetic information by directly altering its nucleic acid genome

Genetic Engineering

- **Recombinant DNA technology**-
  gene coding for any desired protein is isolated from organism→ inserted into suitable vector→ cloned in such way that it can be expressed in formation of specific (desired) protein
- **Vector**- small piece of DNA, into which foreign DNA fragment can be inserted & stably maintained in organism & used for cloning
- 4 types of vectors- plasmid, bacteriophage, cosmids, artificial chromosomes (bacteria/ yeast)

Recombinant DNA Technology

- Steps:

1. Treatment with restriction enzyme
2. Southern blot- a. electrophoresis
   b. transfer to nitrocellulose membrane
   c. detection of desired gene
   d. isolation
3. Recombination with vector
4. Introduction of vector into bacteria
5. cloning

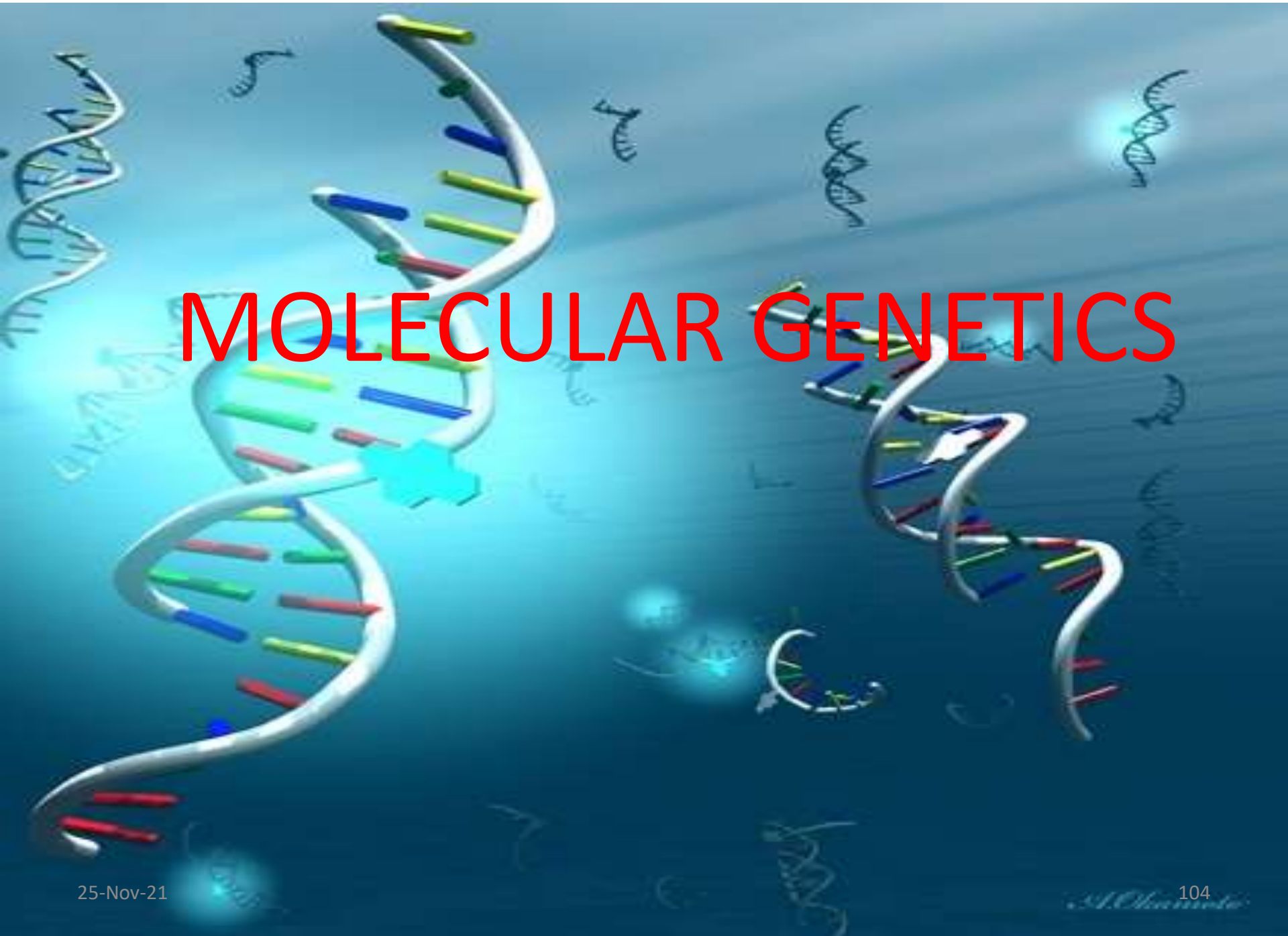
Application of Genetic Engineering

- Production of vaccines
- Production of antigens used in diagnostic kits
- Production of proteins used in therapy
- Transgenic animals
- Gene therapy

Blotting Techniques

- Blot- method of transferring DNA, RNA, proteins from gel onto carrier (nitrocellulose membrane) followed by their detection by using specific nucleic acid probes (DNA, RNA detection) or enzyme immunoassay (protein detection)
- Blotting Techniques-

a. Southern blot- DNA
b. Northern blot- RNA
c. Western blot- to detect proteins
d. Eastern blot- modification of western blot, used to analyse proteins for post-translational modifications using probes- detect lipids, carbohydrates, phosphorylation/ any other protein modifications

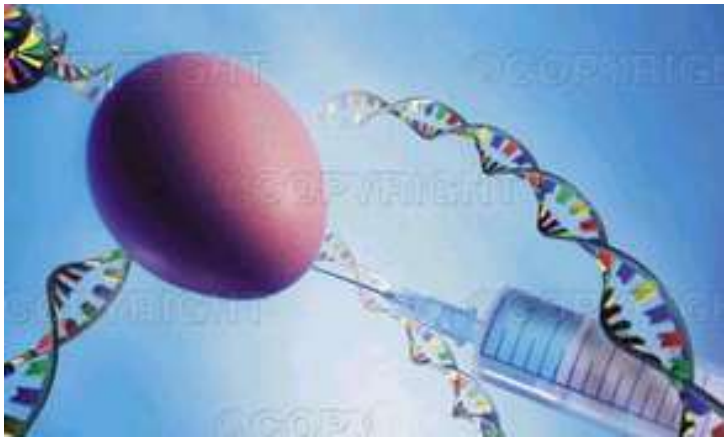

MOLECULAR GENETICS

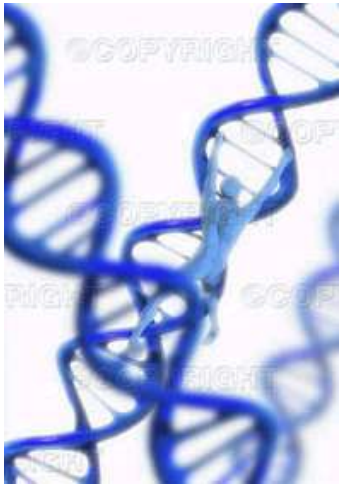
MOLECULAR GENETICS

Analysis & manipulation of DNA using biochemical and microbiological techniques

MOLECULAR GENETICS-TECHNIQUES

- Genetic Engineering
- Restriction endonucleases
- DNA probes
- Blotting techniques
- PCR



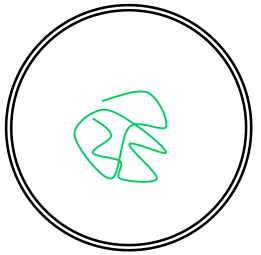


Genetic Engineering

(Recombinant DNA Technology)

Genetic Engineering

(Recombinant DNA technology)



Isolation of genes coding for any desired protein

Introduction of these genes into suitable microorganisms

Directing the microorganism for the production of specific protein

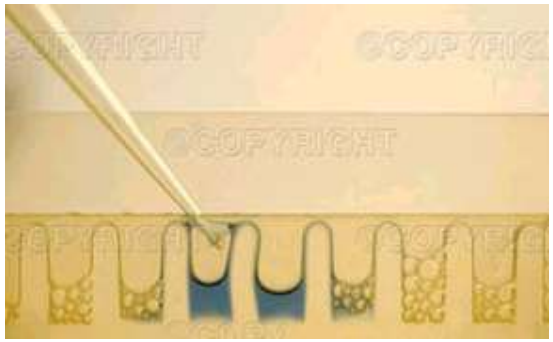
Enables the preparation of desired proteins in pure form, large quantities and at reasonable cost

Genetic Engineering- Applications

- Cloned human insulin, interferons, somatostatin, growth hormones & other biologicals
- Safer vaccines- foot & mouth disease, HBV, rabies
- Extramedical applications

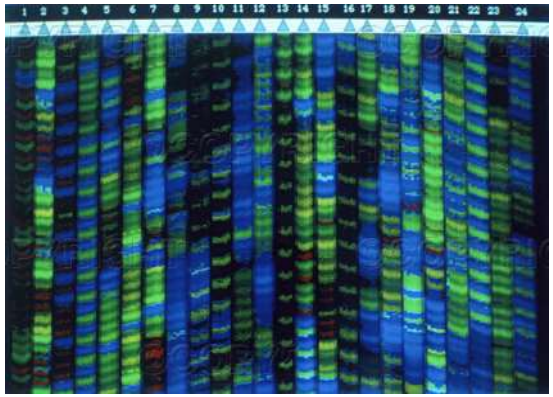
Restriction Endonucleases

Enzymes which cleave double stranded DNA at specific oligonulceotide sequences



Eco RI, Hind III, Taq I

Natural function may be destruction of foreign DNA that may enter the bacterial cells



They split DNA strands into fragments of varying lengths.

Fragments are separated by Gel electrophoresis

Stained with ethydium bromide & photographed

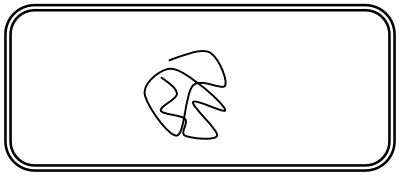
DNA Probes

- These are produced d/t some specific interact^{N} in base pairing during DNA/RNA synthesis
- DNA probes are radioactive, biotinylated or otherwise labeled copies of cloned single stranded DNA fragments containing 20-25 nucleotides containing unique nucleotide sequences
- Adv:- i) highly specific
  ii) able to detect minute quantities of DNA in the presence of other microbes in clinical specimens
  iii) recognize microbes that are difficult/ impossible to culture

DNA Probes- Applications

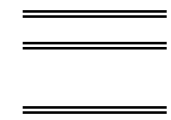
- Detection of homologous DNA by hybridisation
- Δ of infectious diseases

Blotting Technique

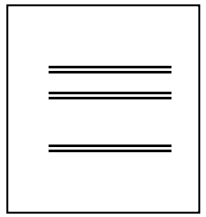


DNA fragments obtained by restriction enzyme digestion

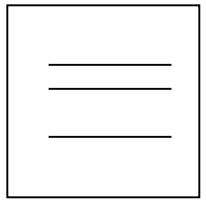
Fragments separated on gel by electrophoresis



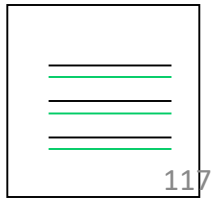
Fragments are transferred from gel to nitrocellulose or nylon membranes that bind DNA



DNA bound to membrane is denatured (converted to single strand) & treated with radioactive single strand DNA probes



Probe hybridize with homologous DNA to form radioactive double strand segments which can be detected on X ray films



Blotting Techniques contd.

- Southern blotting:- identification of DNA fragments by DNA probes (DNA hybridization)
- Northern blotting:- for the analysis of RNA by RNA probes/by DNA probes
- Westrern blotting:- identificat^{N} of proteins(Ags) by specific probes e.g. HIV proteins

Polymerase Chain Reaction(PCR)

- Rapid automated method for amplification of specific DNA sequences (genes)
- Invented by Kary B Mullis (1983)

Polymerase Chain Reaction(PCR)

- Principle- The technique is based on knowing the nucleic acid sequence for a region which for a diagnostic applicat^{N} is produced in large amounts *in vitro* from small amnt. of complex template
- Adv- i) rapid analysis,
  ii) easy automat^{N},
  iii) relative economy, &
  iv) 100% efficiency

PCR contd.

- Technique- 3 steps

i) Denaturat^{N} of sample DNA @94^{o}c

ii) Annealing of sequence sp oligonucleotide primers @50-70^{o}c

iii) Extension of the primers by DNA polymerase to form new DS-DNA

These cycles are repeated several times to get thousands of copies of the original target DNA

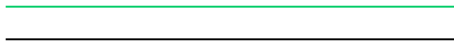
PCR- Applications

- Δ of infectious, genetic, & neoplastic diseases
- Forensic investigation
- Archeological studies of ancient specimens
- Examination of phylogenetic relationships in evolution

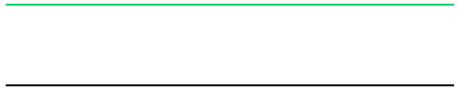
Polymerize Chain Reaction

ONE CYCLE

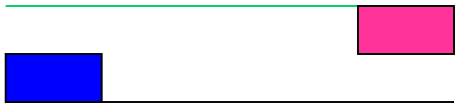
55^0 C before cycle



90^0 C DNA denaturing



55^0 C Primers anneal



72^0 C Polymerization

Extension of primers by DNA polymerase to form new double stranded DNA

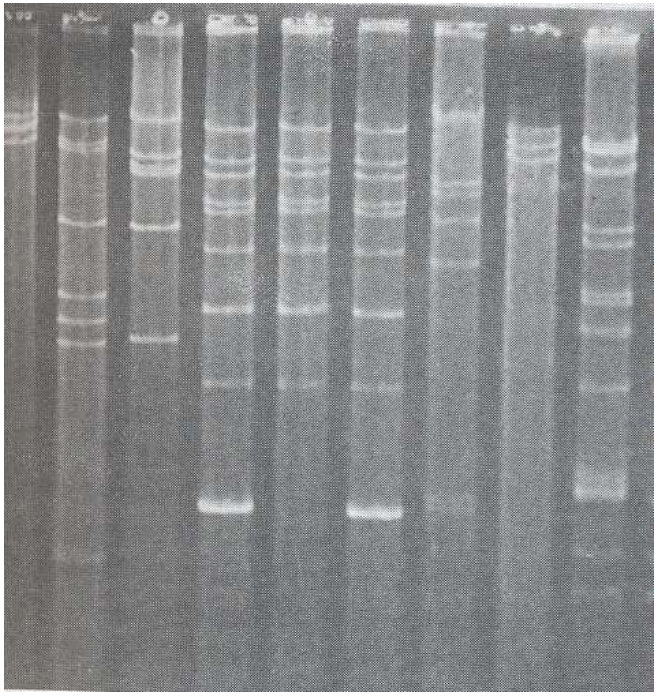
PCR contd.

The speed and ease of use, sensitivity, specificity and robustness of PCR has revolutionized molecular biology and made PCR the most widely used and powerful technique with great spectrum of research and diagnostic applications

Molecular Typing of Organisms

Methods are:

- Plasmid profile analysis
- Genomic finger printing
- PCR



Use- for identification and matching of microbial isolates for epidemiological purposes

Plasmid finger prints. Tracks 4,5, & 6 are closely related to each other