Unit-2

Short Q/Ans

→ Define vaccine ?

A vaccine is a substance that helps protect against certain diseases. vaccines contain a dead or weakened version of a microbe. It helps your immune system recognize & destroy the living microbe during a future infection.

→ Define Hormones ?

Hormone is a chemical messenger that is released in one tissue & transported in the blood stream to reach specific cells in other tissues.

→ Write a note on PCR ?

Polymerase chain reaction (PCR) is a technique used in molecular biology to amplify a single copy or a few copies of a segment of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
Sometimes, it is also referred as in-vitro gene cloning (without expression of that gene) as it is carried out in vitro.

Discovery :

PCR was invented By 'Kary Mullis' in '1983' for which he was awarded the Nobel Prize in chemistry in '1993'
Mullis wrote in Scientific American, "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents & a

source of heat."

Basic Components of PCR :

(1) DNA template -

DNA is nucleic acids which contain genetic information for development & function of live.

Polymer (DNA) of monomer units (nucleotides) containing a sugar (deoxyribose), a base & a phosphate group.

4 different nucleotides, differing in the base :
- 2 purine bases : Adenine (A), Guanine (G)
- 2 pyrimidine bases : Cytosine (C), Thymine (T)

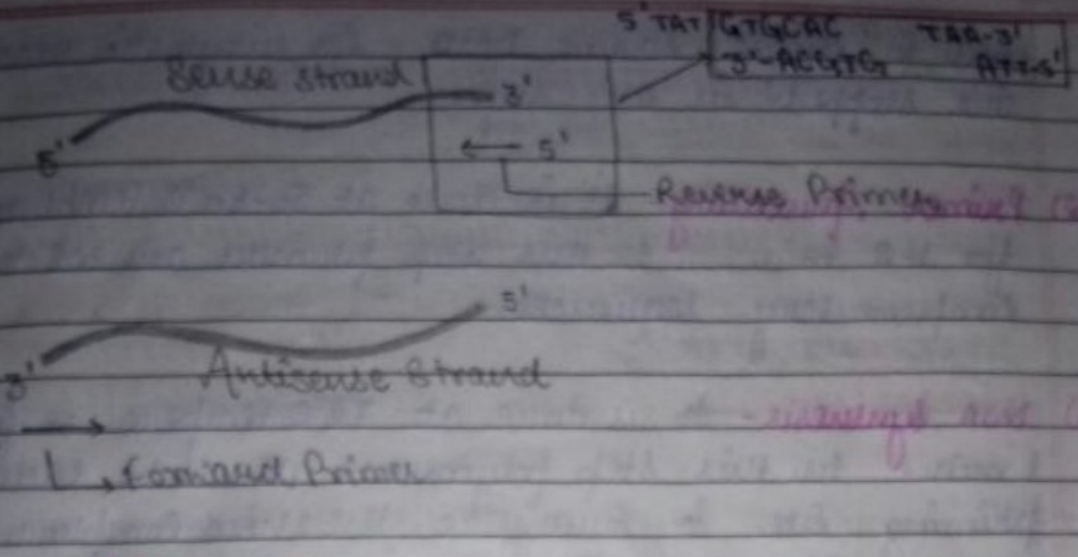
DNA is normally a double stranded molecule (double helix) held together by H-bonds b/w the bases attached to two strands.

Each type of base on one strand forms a bond with just one type of base on the other strand = Complementary base pairing.

(2) Primers -

Primer is short segments of nucleotides (usually 20 to 30 nt). It determine the beginning & end of the amplified region. one forward & one reverse primers are required for PCR.

Each complementary to one strain of the DNA template provide the specificity of amplification. They anneal to the DNA strand at the starting & ending points & supply the initiation site for synthesis of the new DNA by the polymerase.



(3) DNA polymerase –
DNA dependent DNA polymerase is used for coping the region to be amplified by extending the primer sequences. The copied primer is complimentary to original primer.

(4) Deoxynucleotide triphosphate (dNTPs) –
All types of nucleotides are 'building blocks' for new DNA strands & essential for reaction. It includes dATP, dTTP, dCTP, dGTP.

(5) Buffer, $MgCl_2$, additives –
Buffer: Provide an optimal chemical environment (pH & salt conditions) for the polymerase.
$MgCl_2$: Catalyst the rxn of the polymerase.
Additives (optional): (BSA, DMSO, formamide) they improve the amplification efficiency & specificity.

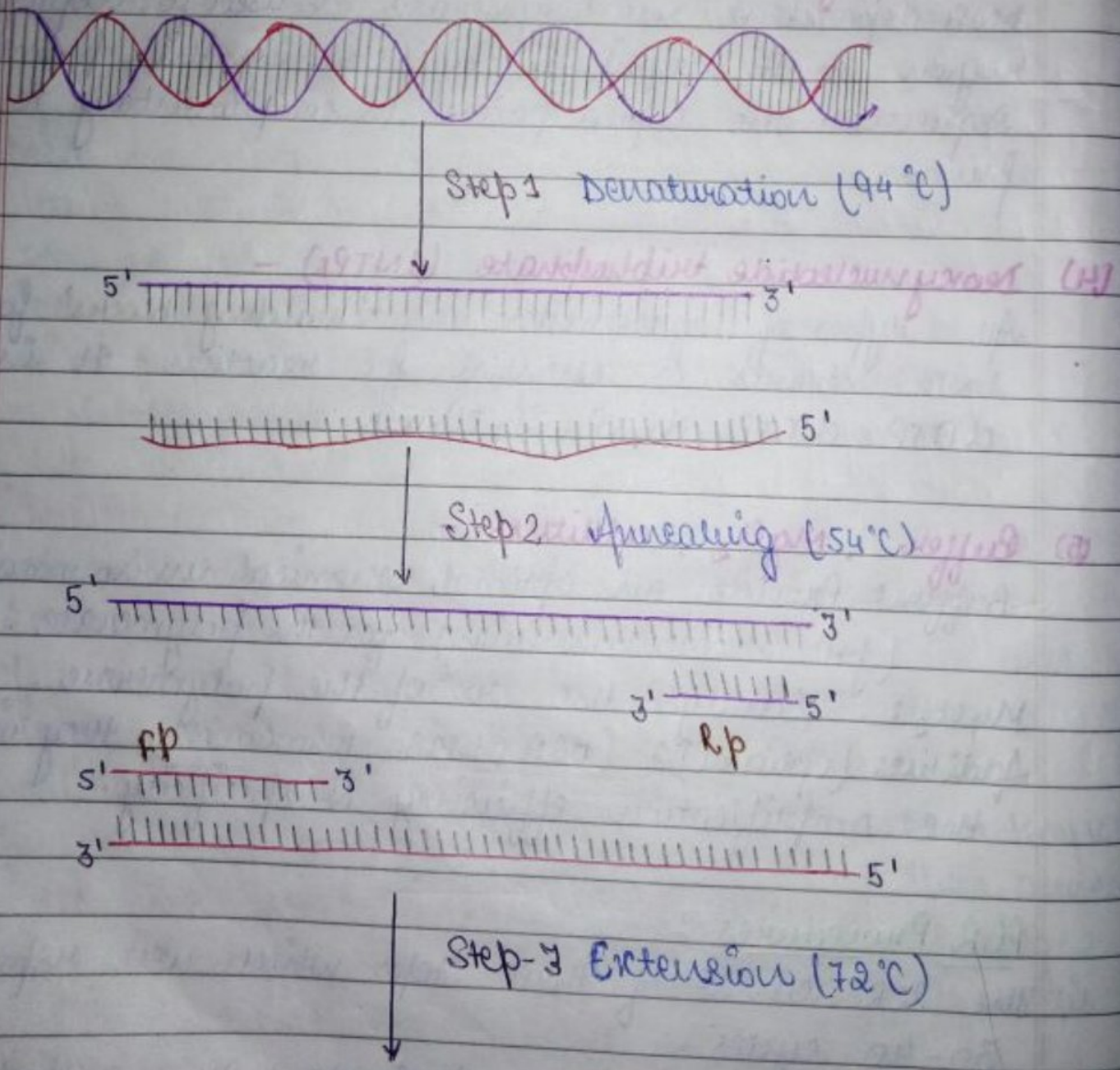
PCR Procedures:
The PCR consist of three steps which were repeated for 30-40 cycles.

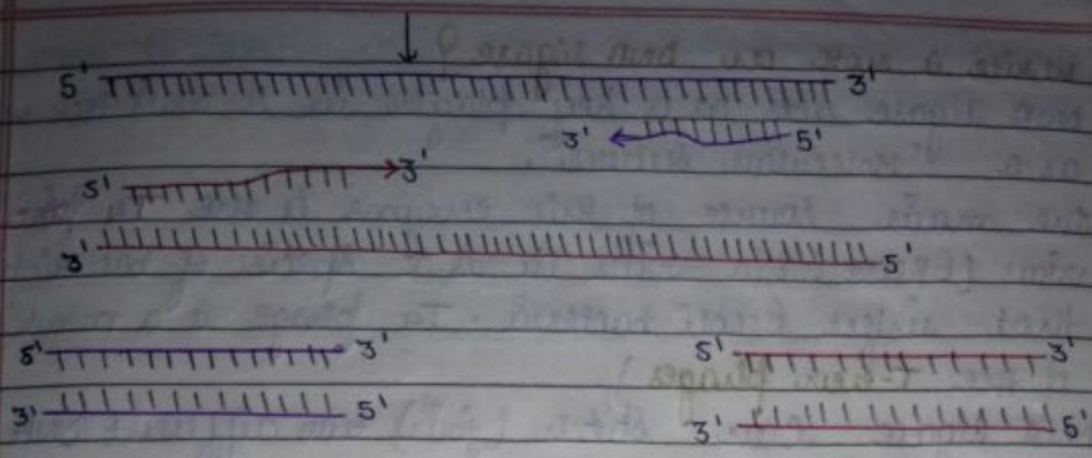
(1) Template denaturation – It is done 94-96°C temperature for 1-2 min, the parent double stranded DNA strand melts

open to single strand DNA. All enzymatic reactions are stopped in this step.

(2) **Primer Annealing**- It is done at 50-60°C temperature for 1-2 min. In this step primers anneal to their complementary sequences.

(3) **DNA Synthesis**- It is done at 72°C temperature for 1 min. In this step polymerase attach at each priming site & synthesize the new complementary DNA strand.





These 3 steps completed first cycle, the remaining cycles completed as when the first cycle is completed, the resulting DNA strands are the template for the next cycle. They doubling the no. of DNA strand duplicated in each new cycle. After 30-40 cycles, there is exponential increase of the no. of the copies of the amplified genes.

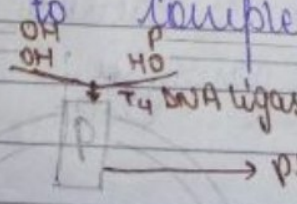
Uses of P.C.R.:

(1) for DNA amplification.
(2) for detecting mutation.
(3) for diagnosing genetic disorders.
(4) for producing in-vitro mutation.
(5) for preparing DNA for sequencing.
(6) for analysing genetic defects in single cells from human embryoes.
(7) for identifying virus & bacteria in infectious disease.
(8) for characterisation of genotypes.

→ Write a note on DNA ligase?

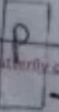
- It acts as a key player in r-DNA esterobacteria phage T4 is a bacteriophase that infects E.Coli bacteria.
- T4 phages is a membrane of the T-even phages technology as a 'Molecular Suture'.
- The main source of this enzyme is the T4 phage virus.
- DNA ligase can stitch (join) two different DNA pieces.
- Joining process requires ATP as its denies energy to construct the bond. Normally, the process is carried out at 4°C in order to reduce the kinetic energy of the molecule. This enzyme when joining two DNA pieces, forms a covalent bond b/w the 5' phosphoryl of one strand & 3' hydroxy of the adjacent strand. Henke, it catalyses the end to end joining of DNA duplex at the base paired end.

- Sealing process two phosphodiester bonds are formed by T4 DNA ligase. The association of fragments is stabilised by the formation of 3' to 5' phosphodiester linkage b/w cohesive ends. The blunt ends produced by certain restriction enzymes may hinder the sealing process. Hence, it is estimated to use ligase at a very high conc^n to complete sealing process.



→ Phosphodiester bond

Joining of 2 DNA fragments by DNA ligase!



→ Phosphodiester bond.

Long Q/Ans -

→ Write a detail note on cloning vector ?
Cloning vector is a DNA molecule in which foreign DNA can be integrated and which is further capable of replicating within host cells to produce multiple clones of it- DNA.

Characteristic :
- It should be small in size.
- It must have an origin of replication.
- It must be compatible with the host organism.
- It must possess a restriction site.
- It should bear multiple cloning site.
- Its replication property should not be compromised by the insertion of donor DNA fragment.
- To ease the identification of recombinant cell it should possess some marker gene.

Importance :
→ Cloning vectors are used as the vehicle for transporting foreign genetic material into another cell. This foreign segment of DNA is replicated & expressed using the machinery of the host organism.
→ A cloning vector facilitates amplification of a single copy DNA molecule into many copies.
→ Molecular gene cloning is difficult without the use of the cloning vectors.

History :
Herbert Boyer, Keiichi Itakura & Arthur Riggs were 3 scientist working in the Boyer's lab, University of California, where they recognized a general cloning vector.

This cloning vector had restriction sites for cloning foreign DNA & the expression of antibiotic resistance genes for the screening of recombinant / transformed cells. The first vector used for cloning purposes was pBR322, a plasmid. It was small in size, nearly 4kb & had 2 selectable markers.

Features -

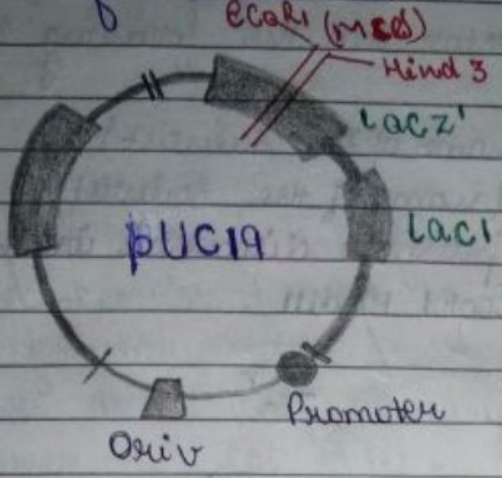
(1) Origin Of Replication (ori) -
- A specific set / sequence of nucleotides where replication initiates.
- For autonomous replication inside the host cells.
- Foreign DNA attached to ori also begins to replicate.

(2) Cloning Site -
- Point of analysis for genetic engineering.
- Vector DNA at this site is digested & foreign DNA is inserted with the aid of restriction enzymes.
- Recent works have discovered plasmids with Multiple Cloning Sites (MCS) which harbour upto 20 restriction sites.

(3) Selectable Marker -
- Gene that confers the resistance to particular antibiotics which under normal condition, is fatal for the host organism.
- Confers the host cell the property to survive & propagate in culture medium containing the particular antibiotics.

(4) Marker or Reporter Gene -
- Permits the screening of successful clones.
- Utilised extensively in blue- white selection.

(5) Inability to transfer via conjugation –
vector must not enable rDNA to escape to the natural population of bacterial cell.



Amp^R - Ampicillin Resistance gene
lacz' - Gene for α peptide of β-galactosidase

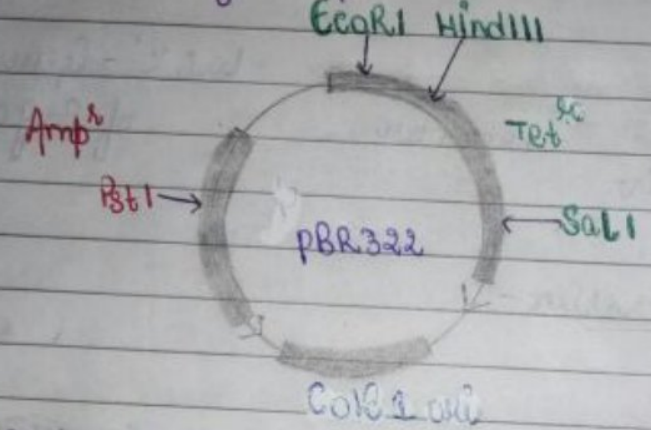
Types of cloning vector –

(1) Plasmid –

- Plasmid were the first vector to be used in gene cloning.
- They are naturally occuring & autonomously replicating extra - chromosomal double stranded circular DNA molecules. However, not all plasmids are circular in origin.
- They are present in bacteria, archaea & eukaryotes.
- The size of plasmid ranges from 1.0 Kb to 250 Kb.
- DNA inserted of up to 10 Kb can be cloned in the plasmids.
- The plasmid have high copy no. which is useful for prod" of greater yield of recombinant plasmid for subsequent experiments.
- The low copy no. plasmids are exploited under certain conditions like the cloned gene produces the protein which is toxic to the cells.
- Plasmids only encode those proteins which are essential for their own replication. These protein-encoding genes are located near the ori.

Eg - pBR322, pUC18, F plasmid, Col plasmid.

Nomenclature of plasmid cloning vector:
pBR322 cloning vector has the following elements -
P = plasmid
B = Bolivar (name of the scientist)
R = Rodriguez (name of the scientist)
322 = no. of the plasmid discovered in the lab.



Advantages:
(a) Easy to manipulate & isolate because of small size.
(b) More stable because of circular configuration.
(c) Replicate independent of the host.
(d) High copy no.
(e) Detection easy because of antibiotic-resistant genes.

Disadvantages:
(a) Large fragments cannot be cloned.
(b) Size range is only 0 to 10 Kb.
(c) Standard methods of transformation are inefficient.

(2) Bacteriophage -
- Bacteriophages or phages are viruses which infect bacterial cell.
- The most common bacteriophages utilised in gene cloning are Phage λ & M13 Phage.

- A maximum of 53 Kb DNA can be packaged into the phage.
- If the vector DNA is too small, it cannot be packaged into the phage.

Eg - Phage λ , M13 phage.

Phage λ :

- It has head, tail & tail fibres.
- Its genome consist of 48-5 Kb of DNA & 12 bp ss DNA which comprise of sticky ends at both the terminals. Since these ends are complementary, they are cohesive & also referred as Cos sites.
- Infection of λ phage requires absorption of tail fibres on the cell surface, contraction of the tail, & injection of the DNA inside the cell.

Structure of Bacteriophage λ

M13 Phage :

- These vectors are used for obtaining single - stranded copies of the cloned DNA.
- They are utilized in DNA sequencing & in-vitro mutagenesis
- M13 phages are derived from filamentous bacteriophage M13-

The genome of M13 is 6.4 Kb.
- DNA inserts of large sizes can be cloned.
- From the double-stranded inserts, pure single-stranded DNA copies are obtained.

Types of Phage vectors:
These are of 2 types of phage vectors:-

(a) Insertion vectors
These contain a particular cleavage site where the foreign DNA of upto 5-11 Kb can be inserted.

(b) Replacement vectors
The cleavage sites flank a region which contains genes not necessarily important for the host, & these genes can be deleted & replaced by the DNA insert.

Advantages of using phage vector -
(a) These ways are more efficient than plasmids for cloning large inserts.
(b) Screening of phage plaques is much more easier than identification of recombinant bacterial colonies.

(3) Phagemids or Phasemid -
- They are prepared artificially.
- Phasemid contains the f1 origin of replication from f1 phage.
- They are generally used as a cloning vector in combination with M13 phage.
- It replicates as a plasmid & gets packaged in the form of single stranded DNA in viral particles.

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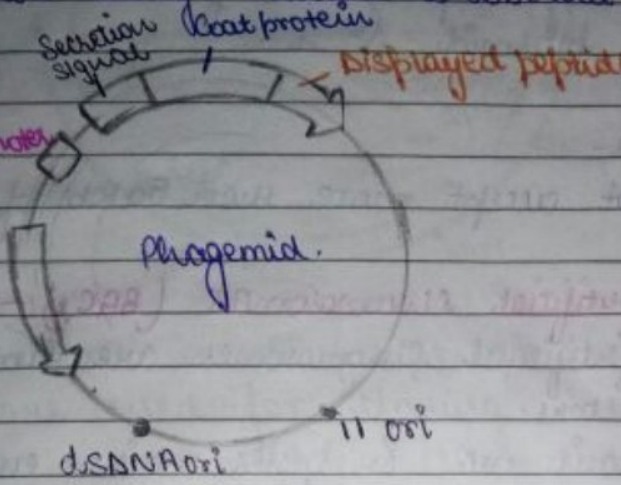
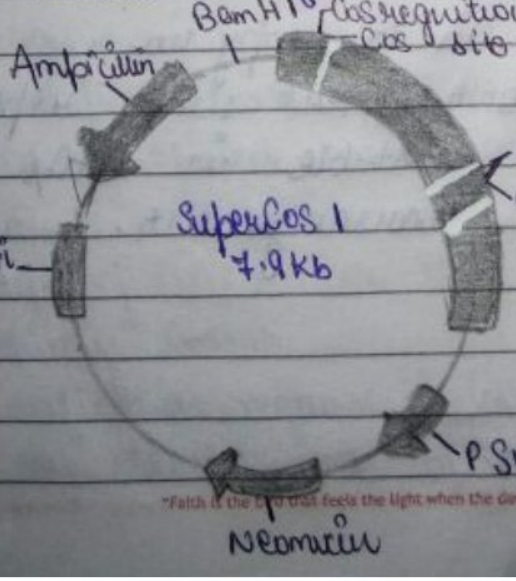
Advantages -

(a) They contain multiple cloning sites.
(b) An inducible lac gene promoter is present.
(c) Blue-white colony selection is observed.



(4) Cosmids -

- Cosmids are plasmids.
- They are capable of incorporating the bacteriophage λ DNA segment. This DNA segment contains cohesive terminal sites (Cos sites).
- Cos sites are necessary for efficient packaging of DNA into λ phage particles.
- Large DNA fragements of size varying from 25 to 45 Kb can be cloned.
- They are also packaged into λ. This permits the foreign DNA fragment or genes to be introduced into the host organism by the mechanism of transduction.



Advantages -
(a) They have high transformation efficiency & are capable of producing a large no.
(b) They can carry upto 45 Kb of insert compared to 25 Kb carried by plasmid & λ.

Disadvantages -
Cosmid cannot accept more than 50 Kb of the insert.

(5) Bacterial Artificial Chromosomes (BACs) -
- Bacterial Artificial Chromosomes are similar to E.coli plasmid vectors.
- They contain ori & genes which encode ori binding proteins. These proteins are critical for BAC replication.
- It is derived from naturally occuring f' plasmid.
- The DNA insert size varies between 150 to 350 Kb.

Advantages -
(a) They are capable of accommodating large sequences without any risk of rearrangement.
(b) BACs are frequently used for studies of genetic or infectious disorders.
(c) High yield of DNA clones is obtained.

Disadvantages -
(a) They are present in low copy no.
(b) The eukaryotic DNA insert with repetitive sequences are structurally unstable in BACs often resulting in deletion or rearrangement.

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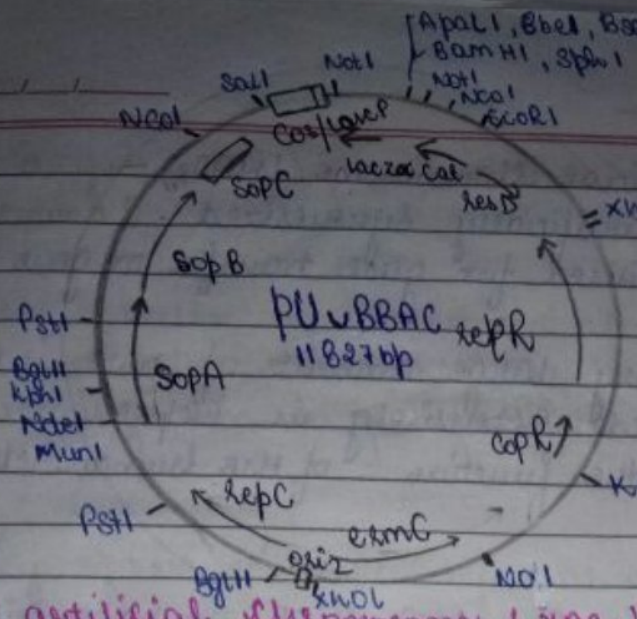


(6) Yeast artificial chromosomes (YACs) -

- A large DNA insert of upto 200 Kb can be cloned.
- They are used for cloning inside eukaryotic cells. These act as eukaryotic chromosomes inside the host eukaryotic cell
- It possesses the yeast telomere at each end.
- A yeast centromere sequence (CEN) is present which allows proper segregation during meiosis.
- The ori is bacterial in origin.
- Both yeast & bacterial cells can be used as hosts.

Advantages -

(a) A large amount of DNA can be cloned.
(b) Physical maps of large genomes like the human genome can be constructed.

Disadvantages -

(a) Overall transformation efficiency is low.
(b) The yield of cloned DNA is also low.

Advantages of BACs over YACs -

(a) Comparatively Stable (b) Easy to transform
(c) Simple purification required (d) User friendly.
(e) Aid in the development of vaccines

(7) Human Artificial Chromosome (HACs) –

- HACs are artificially synthesized.
- They are utilized for gene transfer or gene delivery into human cells.
- It can carry large amounts of DNA inserts.
- They are used extensively in expression studies and determining the function of the human chromosomes.

Advantages-

(a) No upper limit on DNA that can be cloned.
(b) It avoids the possibility of insertional mutagenesis.

(8) Retroviral Vectors –

- Retroviruses are the viruses with RNA as the genetic material.
- Retroviral vectors are used for introduction of novel or manipulated genes into animal or human cells.
- The viral RNA is converted into DNA with the help of reverse transcriptase & henceforth, efficiently integrated into host cell.
- Any gene of interest can be introduced into the retroviral genome. This gene of interest can then integrate into host cell chromosome & reside there.

Advantages -

They are widely used as a tool to study & analyze oncogenes & other human genes.

The things which matter while choosing a cloning vector are

(1) DNA insert size
(2) Size of the vector
(3) Restriction site
(4) Efficiency of cloning.

→ What do you mean by r-DNA technology? Discuss its applications?

The r-DNA technology is defined as the DNA molecules from two different source are joined & inserted into a host organism to get products useful for human use.

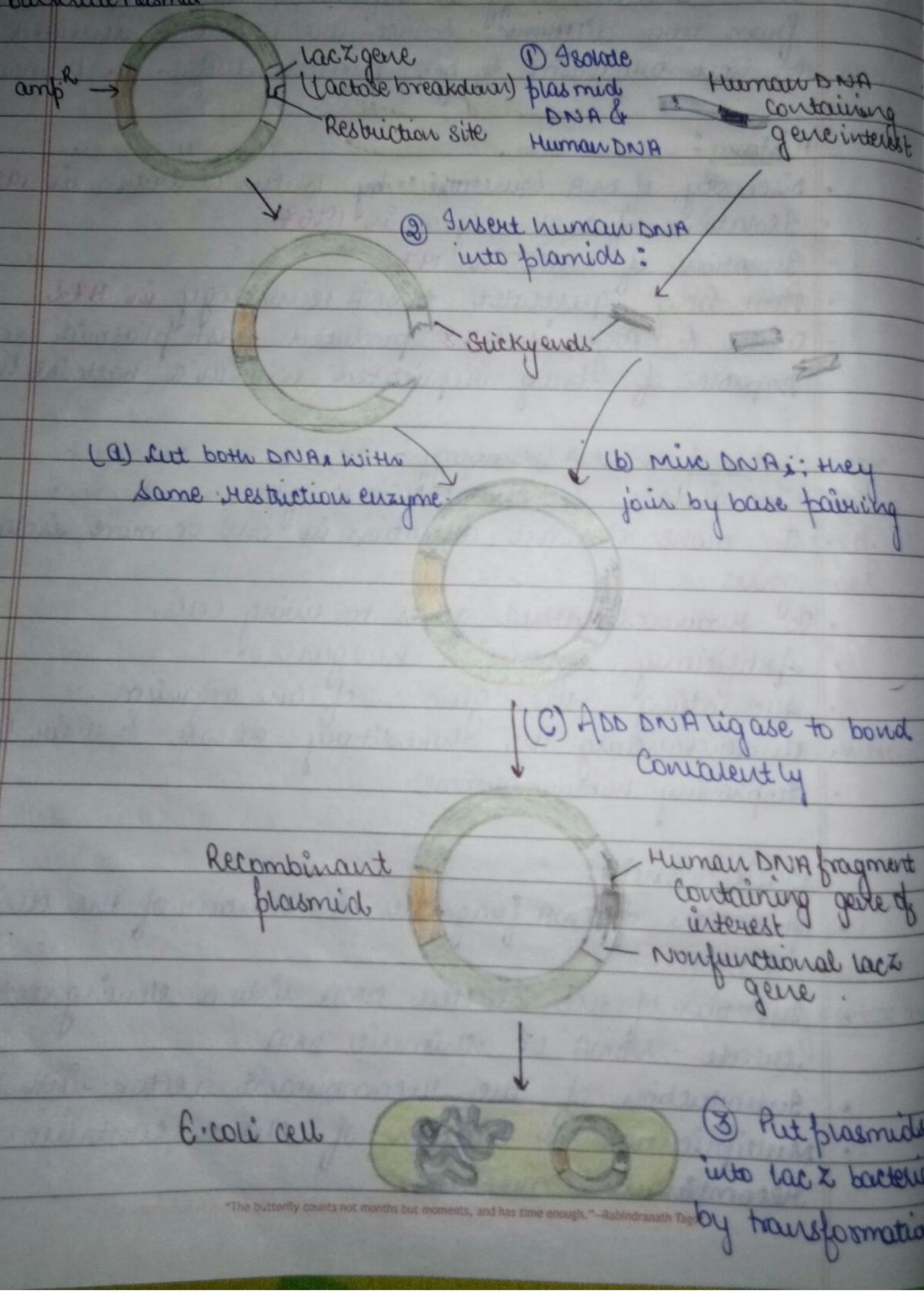
History:
- Discovery of DNA structure by Watson & Crick in 1953.
- Isolation of DNA ligase in 1967
- Isolation of REase in 1970
- Paul Berg generated r-DNA technology in 1972
- Cohen & Boyer in 1973 produced first plasmid vector capable of being replicated within a bacterial host.

Goals of r-DNA technology:
- To isolate & characterize a gene.
- To make desired alteration in one or more isolated genes.
- To returned altered genes to living cells.
- Artificially synthesize new gene.
- Alternating the genome of an organism.
- Understanding the hereditary disease & their cure.
- Improving human genome.

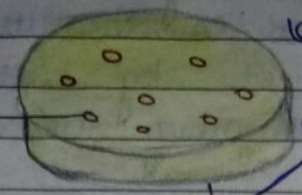
Basic Principles:
- Generation of DNA fragments & selection of the desired piece of DNA.
- Insertion of the selected DNA into a cloning vector to create rDNA or chimeric DNA.
- Introduction of the recombinant vector into host cells.
- Multiplication & selection of clones containing the recombinant molecules.

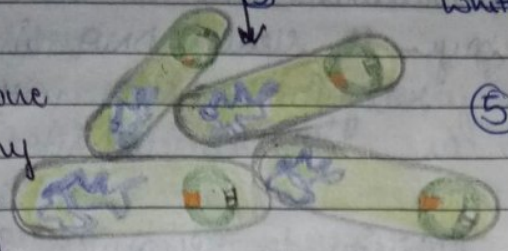
• Expression of the gene to produce the desired product.

Procedure:



④ Clone cells:
(a) Plate cells onto medium with ampicillin & x-gal
(b) Identify clones of cells containing recombinant plasmids by their ability to grow in presence of ampicillin & their white color





Bacterial clone carrying many copies of DNA human gene of interest

⑤ Identify clone carrying gene of interest

Application:

(1) Agriculture -
Growing crops of your choice, Pesticide resistance crops, Fruits with attractive colors are being grown in artificial condition.

(2) Therapeutic products -
vaccines, Growth hormones, Antibodies, vector, α-protein, Anticancer drugs.

(3) Diagnosis -
Gene therapy, CRISPR, Monitoring devices, Therapeutic strategies

(4) Energy application -
Biohydrogen, Bioethanol, Biomethanol, Biobutanol

(5) Pharmacology -
Artificial insulin production, drug delivery to target sites.

(6) Molecular diagnosis of diseases

(7) DNA finger printing.

→ What do you understand by Restriction endonuclease?

- Restriction enzymes also called restriction endonuclease is a protein produced by bacteria that cleaves DNA at specific sites along the molecule.

- Restriction endonucleases cut the DNA double helix in very precise ways. It cleaves DNA into fragments at or near specific recognition sites within the molecule known as 'restriction sites'.

- They have the capacity to recognize specific base sequence on DNA & then to cut each strand at a given place. Hence, they are called as 'molecular scissors'

EcoRI Restriction enzyme

```
...GGTCAG|AATTCGCTGA...
...CCAGT|CTTAA|GCGACT...
```

```
AATTCGCTGA...
    GCGACT...
...GGTCAG
   CCAGTCTTAA
```

Source of Restriction Enzyme -

- The natural source of restriction endonucleases is bacterial cells.
- These enzymes are called restriction enzyme because they restrict infection of certain viruses (i.e bacteriophage) by degrading the viral DNA without affecting the bacterial DNA thus, their function in the bacterial cell is to destroy foreign DNA that might enter the cell.

- The restriction enzyme recognizes the foreign DNA & cuts it at several sites along the molecule.
- Each bacterium has its own unique restriction enzyme & each enzyme recognizes only one type of sequence.

Recognition Sites -
The DNA sequences recognized by restriction enzymes are called Palindromes.
Palindromes are the base sequences that read the same on the two strands but in opposite direction.
Eg - If the sequence on one strand is GAATTC read in 5' → 3' direction, the sequence on the opposite strand is CTTAAG read in 3' → 5' direction, but when both strands read in the 5' → 3' direction the sequence is same.
The palindrome appears accordingly -
5' GAATTC 3'
3' CTTAAG 5'

How Restriction Endonucleases work :
Restriction enzymes recognize a specific sequence of nucleotides, & produce a double-stranded cut in the DNA.
These cuts are of two types :

Blunt end	Sticky end
These blunt ended fragement can be joined to any other DNA fragment with blunt ends. Enzymes useful for certain types of DNA cloning experiments -	DNA fragment with complimentary sticky ends can be combined to create new molecules which allows the creation & manipulation of DNA sequences from different sources.
CCC GGG GGG CCC	GAATTC CTTAAG

Nomenclature :
Each enzyme is named after the bacterium from which it was isolated using a naming system based on bacterial genus, species & strains.

Eg:

Derivation of the EcoR1 enzyme		
Abbreviation	Meaning	Description
E	Escherichia	Genus
Co	coli	Species
R	RY13	Strain
1	First identified	order of identification in the bacterium

Types of Restriction enzyme :
Traditionally, four types of restriction enzyme are recognized, designated I, II, III, IV, which differ primarily in structure, cleavage, site, specificity & co-factors.

(1) Type I enzymes - cleave at sites remote from a recognition site, require both ATP & S-adenosyl-L-methionine to function. multifunctional protein with both restriction & methylase activities.

(2) Type II enzymes - cleave within or at short specific distances from a recognition site, most require Mg^{+}, single function enzymes independent of methylase.

(3) Type III enzymes - cleave at sites a short distance from a recognition site, require ATP, S-adenosyl-L-methionine stimulate the reaction but it is not required, it exists as a part of a complex with modification

methylase.

(4) Type IV enzymes - Target modified DNA eg - methylated, hydroxymethylated & glucosyl-hydroxymethylated DNA

Applications:

- They are used in gene cloning & protein expression experiment.
- Restriction enzymes are used in biotechnology to cut DNA into smaller ends in order to study fragment length difference among individuals (Restriction fragment length Polymorphism - RFLP)
- Each of these methods depend on the use of agarose gel electrophoresis for separation of the DNA fragments.
- Cloning DNA molecules.
- Studying nucleotide sequence.
- Allow for the large scale prod" human insulin for diabetes using E. coli.

Some examples of Restriction enzyme:

Enzyme	Obtained from	Recognition sequence
EcoR I	Escherichia coli	5'GAATTC 3'CTTAAG
EcoR II	Escherichia coli	5'CCWGG 3'GGWCC
Bam H I	Bacillus amyloliquefaciens	5'GGATCC 3'CCTAGG
Hind III	Haemophilus influenza	5' AAGCTT 3' TTCGAA

Hormone -

→ Write in detail about, insulin, vaccines - Hepatitis B, Interferons?

Hormone - Insulin :-

Insulin is a hormone produced by β-cells of islets of Langerhans of Pancreas. Insulin is a peptide hormone

produced by pancreas & is a central regulator of carbohydrates & fat metabolism in the body.

Structure of Human Insulin :

- Chemically human insulin is small, simple protein composed of 51 amino acid sequences & has a molecular weight of 5808 Da.
- Insulin hormone is a dimer of a A-chain & a B-chain which are linked together by a disulphide bond.
- Insulin consist of two polypeptide chain :

→ Chain A - 21 amino acid long
→ Chain B - 30 amino acid long
→ Both chain are joined together by disulphide bond b/w two cysteine residue.

Production of Insulin :

⟹ The Basic steps in R-DNA technology for production of insulin :

- At first suitable vector (plasmid) is isolated from E.coli & then it is cut open by restriction enzyme.
- The gene of interest is isolated from β-cell & inserted in opened plasmid.
- Plasmid & gene of interest are recombined together by DNA ligase enzyme.
- This recombined plasmid is inserted into suitable host cells & now this recombined host cells start producing insulin hormone.

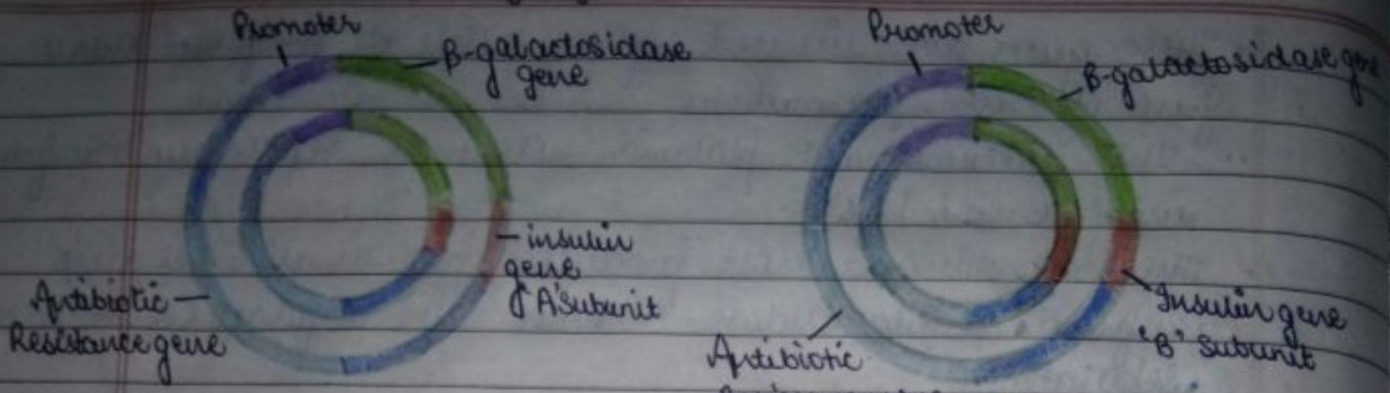
⟹ Hakura et al (1977) chemically synthesize DNA sequence of insulin for α chain A & B and separately inserted into two PBR 322 plasmid vector.

- These genes are inserted by the side of β-galactosidase gene of the plasmid.
- The recombinant plasmid were then separately transformed into E.coli host.
- The recombinant host produced pro-insulin chains i.e. fused β-galactosidase-A chain & β-galactosidase-B-chain separately.
- These pro-insulin chains A & B were separated from β-galactosidase by treatment of with cyanogen bromide.
- The detachment of pro-insulin chains from β-galactosidase is possible because an extra codon form methionine was added at N-terminal of each gene for A & B-chain.
- After detachment, A & B chains are joined in-vitro to reconstitute the native insulin by sulphonating the peptide chains with sodium disulphonate & sodium sulphite.

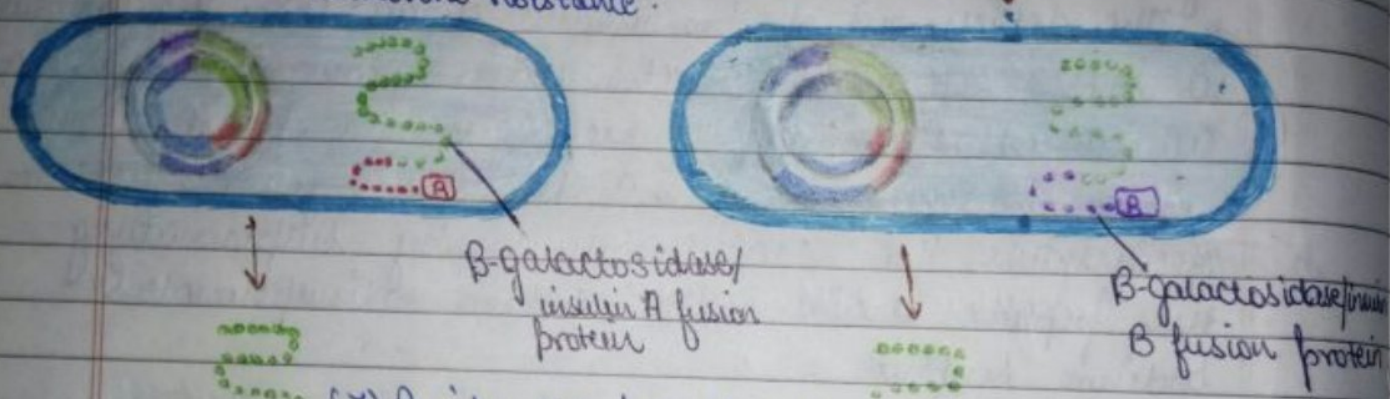
⟹ Another method of insulin production by recombinant DNA technology is designed by Gilbert & Villakomaroff:

- In this method, m-RNA for pre-pro-insulin is isolated from islets of langerhans cells.
- m-RNA is reverse transcribed to form DNA & then it is inserted into PBR 322 plamid in the middle of the gene for penicillinase.
- Then the recombinant plasmid is transformed into suitable host i.e. E.coli cell.
- The host produced penicillinase + pre-pro insulin.
- Insulin is later separated by trypsin treatment.

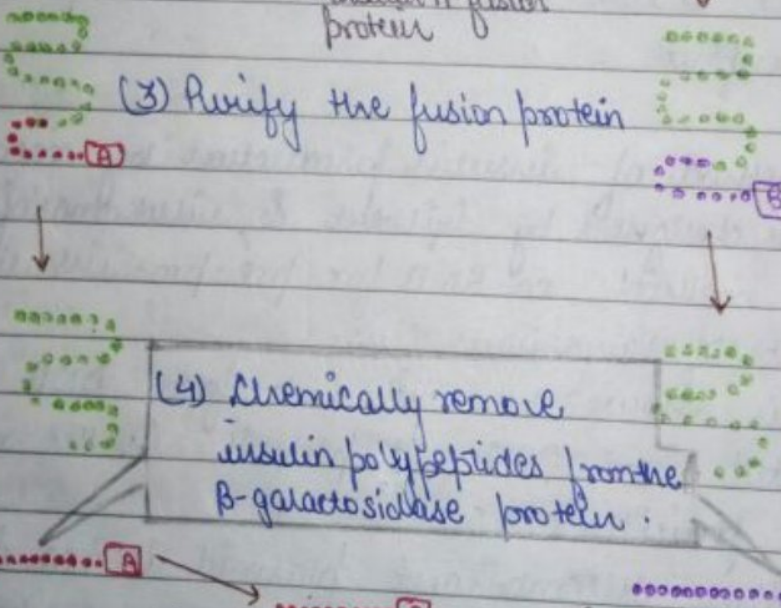
(1) Insert genes for each of the two insulin polypeptides next to a highly expressed gene for β-galactosidase 58



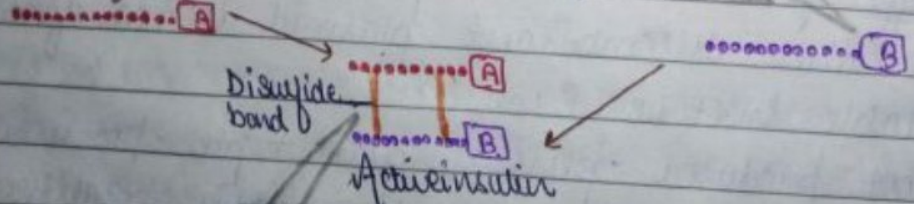
(2) Transform E.coli with recombinant expression vectors, select transgenic cells by their antibiotic resistance.



(3) Purify the fusion protein



(4) Chemically remove insulin polypeptides from the β-galactosidase protein.



(5) Combine the polypeptides to produce functional insulin

Role of insulin :
(1) uptake of glucose by tissues
(2) utilization of glucose
(3) Hypoglycemic effect
(4) Lipogenesis
(5) Anti-lipolytic effect
(6) Anti-ketogenic effect

Vaccine - Hepatitis B :-
The Recombivax HB (Merck) is a hepatitis B vaccine & one of the most significant development in r-DNA technology field. This vaccine comprises of highly specific antibodies.
Hepatitis causes a severe acute infection that ultimately results in chronic infection & permanent liver damage. It is caused by Hepatitis B virus (HBV) which is an enveloped & double stranded DNA virus. Hepatitis B can be prevented with a vaccine made using r-DNA technology.

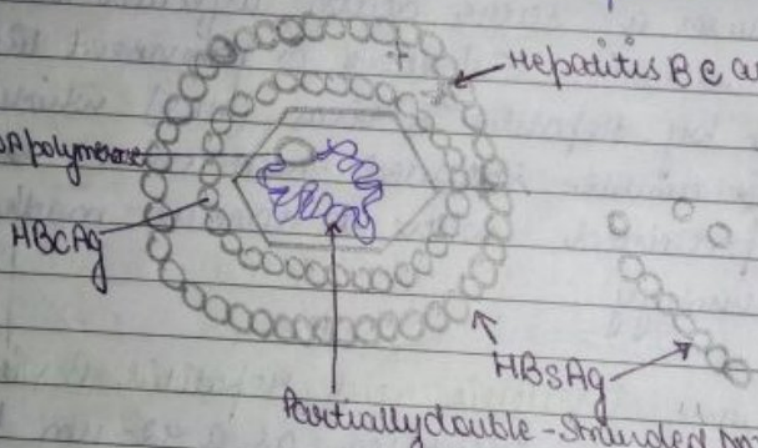
General features of nucleic acid Hepatitis B Virus :
- HB virus has been identified as a 42-nm particle containing a double stranded circular DNA molecule of about 32 Kb size.
- DNA genome has a relative molecular mass of approx. 2×10^6
- DNA cloning is an unusual feature among other viruses
- Plasma of human has been detected to have varying amount of HB antigens.

There types of antigenic marker have been recognized for the HBV infection :
(1) HBsAg : It is an surface antigen found on the viral envelope. It represents one of the earliest marks

& appears in the blood during incubation. It is found as 18-22 nm spherical or tubular form particles. Recently HBsAg gene or its subunit are used for the prodn of recombinant Hepatitis B vaccine.

(2) HBe Ag : It is the e-antigen obtained from the protein 'capsid' surrounding the DNA. It is a marker for causing active infection.

(3) HBc Ag : It is a core antigen which doesnot circulate in the blood & stimulates the prodn of the primary antibodies against HBV. These antibodies are not protective & hence donot provide immunity.



vaccine

Steps for Recombinant HB production :

Production of these genes is needed in order to get production of vaccines on a large scale.

A general procedure for the prodn of recombinant Hepatitis B vaccines are :

(1) HBs antigen producing gene is isolated from the HB virus by normal isolation

(2) A plasmid DNA is extracted from a bacterium E.coli & is cut with restricted enzyme EcoR1 forming

the plasmid vector.

(3) The isolated HBsAg producing gene is located & inserted into the bacterial plasmid vector on forming the recombinant DNA.

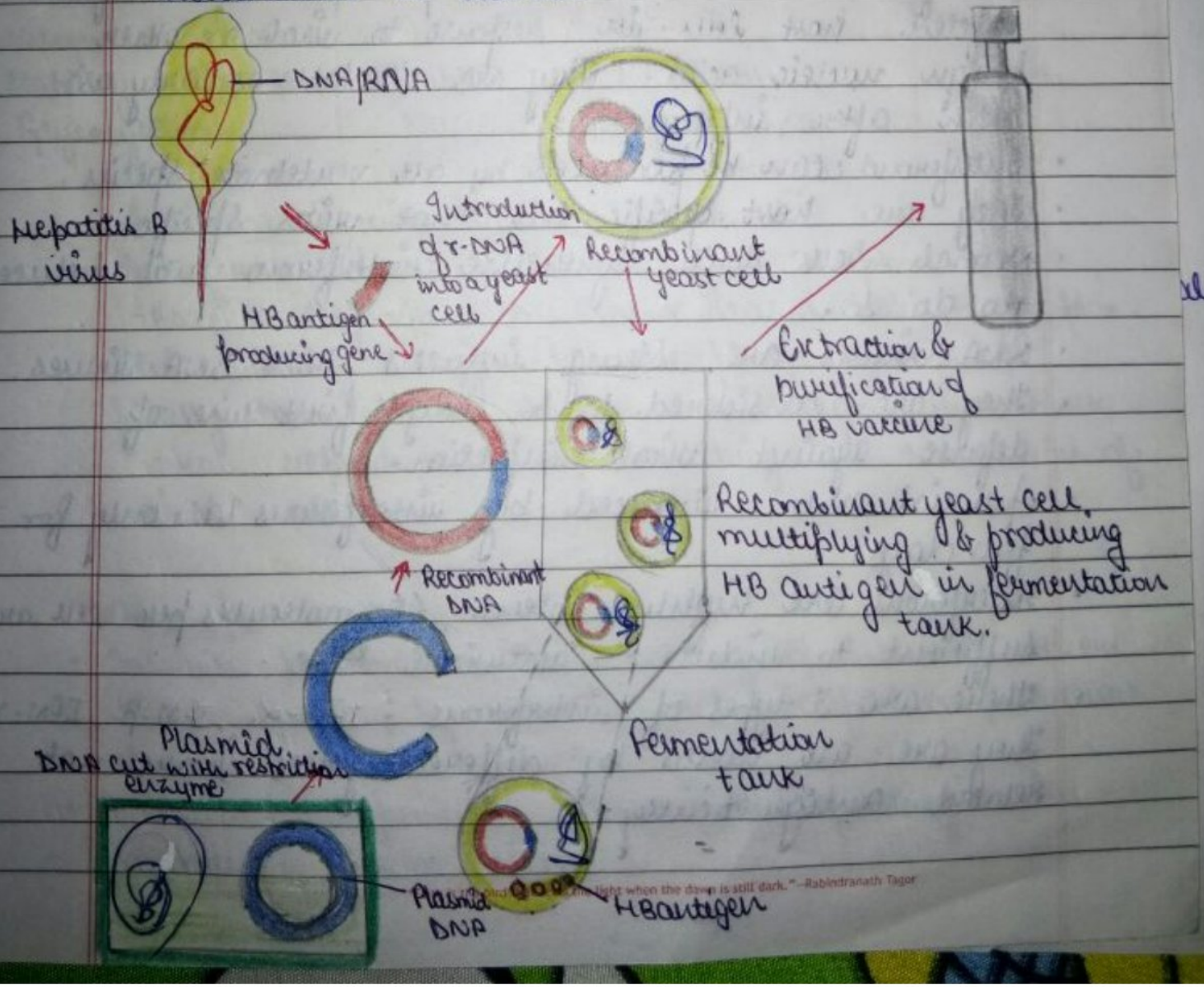
(4) The recombinant DNA, containing the target gene, is introduced into a yeast cell forming the recombinant yeast cell.

(5) The recombinant yeast cell multiplies in the fermentation tank & produces the HBsAg.

(6) After 48 hours, yeast cells are ruptured to free HBsAg. This mixture is processed for extraction of HBsAg.

(7) The HBsAg are purified.

(8) HBsAg are combined with preserving agent & other ingredients & bottled. Now it is ready for vaccination in humans.



Benefits of recombinant Hepatitis B vaccine production:
Traditional vaccines use a weakened or killed form of a virus to force the body to develop antibodies that are strong enough to combat the virus.
Using recombinant DNA technology, the vaccine uses the surface antigen of the virus that stimulates the production of protective antibodies which combat the HB virus.

Interferons :-
The name 'interferon' was given by Issacs & Lindenmann after they were recognized by their ability to interfere with viral replication.

- Interferons are natural cellular products released from infected host cells in response to viral or other foreign nucleic acids. They are detectable as early as 2 h after infection.
- Interferons can be produced by all vertebrate species.
- They are host specific but not virus specific.
- Normal cells don't synthesize interferon until induced to do so.
- RNA viruses are strong inducers than DNA viruses.
- They are considered to be body's first line of defense against viral infection.
- Antiviral state induced by interferons last only for few days.
- Interferons are highly potent, <50 molecules per cell are sufficient to induce antiviral state.
- There are 3 types of interferons : IFN-α, IFN-β, IFN-γ They are all coded by different genes & are not related antigenetically.

Properties of Interferon:

Property	α	β	γ
Present nomenclature	IFN-α	IFN-β	IFN-γ
Former designation	Leucocyte interferon (Type I interferon)	Fibroblast interferon (Type I interferon)	Immune interferon (Type II interferons)
No. of genes coding	≥ 15	1 or few	1 or few
No. of subtypes	20	2	3
Principal cell source	Leucocytes	Fibroblasts	Lymphocytes & NK cells
Inducing agent	Viruses, ds DNA	Viruses, ds DNA	Mitogens
Size (MW)	17,000	17,000	17,000
No. of amino acids	143	145	146
Introns in genes	No	No	Yes, 3
Chromosomal location	9	9	12
Stability at pH 2	Stable	Stable	Labile
Glycosylation	No	Yes	Yes

Antiviral activity of interferon:

Interferons binds to cell surface receptors, IFN α, IFN β receptor while IFN-γ has a distinct receptor. This binding triggers tyrosine phosphorylation & finally transcription. Atleast two enzymes that block viral reproduction by inhibiting the translation of viral mRNA.

Other possible mechanism:

- Inhibits penetration & uncoating of SV40 & other viruses, possibly by altering the Composition/structure of the cell membrane.
- IFN inhibits primary transcription of many virus genomes eg - SV40, HSV.

- Inhibits cell transformation by retroviruses.

Role of IFN-γ in immune regulation:

- In the presence of IL-12 & IFN-γ, Th0 cells will differentiate into Th1 cells.
- Stimulates T cytotoxic lymphocytes, neutrophils & NK cells.
- Inhibits the proliferation of Th2 cells
- Blocks the effect of IL-4 on proliferating B-cells.
- Prevents class-switching.
- Activates macrophages to kill phagocytosed microbes.
- Stimulates expression of MHC II & MHC I antigens as well as co-stimulators on antigen presenting cells.

Applications of Interferons:

- Interferon is used to treat chronic active hepatitis by HBV, HCV & condylomata accuminata by human papilloma virus.
- Patients suffering from Hairy cell leukaemia & kaposi's sarcoma may benefit from interferon treatment.
- Interferon is not thought to be very effective & may itself produce fever & vomiting.