chapter-26 Biotechnology

Q1 write short note on Biotechnology.

Answer: Biotechnology:

Meaning:- Bio mean living-organism and Technology mean Techniques or procedures or methods.

Definition:- The utilization of living organism and their processes and products for the welfare of human is called-Biotech;

Father of biotechnology:- The term Biotechnology was coined in the year 1919 by an agriculture engineer Karoly Ereky, hence he is called the father of biotechnology.

Explanation:- Biotechnology is actually the exploitation of biological processes for industrial and domestic levels. - besides this it is very important for genetic manipulation (change) of microorganisms like- E.coli, for production of antibiotics, Hormones, Vaccines, antigenic-protein, varieties of livestok, crops, wine, cheese, etc can be synthesize by the process of biological modification and genetic engineering

Genetic Engineering:- The inserting of a gene from one - organism to the DNA of another organism for the modification of its characteristics is called genetic engineering.

Q2 what is gene cloning. Explain gene cloning with the -

Answer:- Gene cloning:-

Definition:- The production of identical copies of same gene through recombinant DNA technology is called gene cloning.

Methods of gene cloning:- There are two possible methods of gene cloning — recombinant-DNA-technology and polymerase chain reaction (PCR) i.e.—

1: In vivo method:- In invivo method, copies of gene are produce by recombinant-DNA-technology.

2: In vitro method:- In invito method, many copies of gene are produce by PCR-tenchnology.

Basic steps of gene cloning:- (by Recombinant DNA technology)

Overview:- gene cloning involves the selection and isolation of desired gene and vector, formation of recombinant DNA and the transformation of a suitable host by recombinant DNA.

→ There are 5-basic steps in gene cloning such as— (ETEA-2020)

1: Gene of interest:

The gene which is to be cloned is called gene of interest or desired gene. It can be isolated or isolated from the whole DNA by following 3-methods— (ETEA-2017)

i: complementary DNA synthesis:- Gene of interest can be synthesize from mRNA by the enzyme called reverse — transcriptase. This process is called complementary DNA-technology and the DNA synthesized is complementary —

ii: Cleavage of chromosomal DNA :- Gene of interest is directly cleaved from chromosomal-DNA by using restriction endonuclease enzyme. This one is the most common method used to select and isolate gene of interest.

iii: Artificial gene synthesis :- In this process gene of interest is synthesize from specific nucleotide-sequences in the presence of various enzymes in DNA-synthesizers mechine in labotory. this method is useful for a small sized gene because it is a costly method.

2: Molecular scissors: <Restriction endonuclease>

These are special enzyme called restrication endonuclease which cut the DNA into hanging sequence. These enzymes are found naturally in bacteria. ←(ETEA-2012)

Explanation :- These are enzymes which cleave the phosphodiester bonds of both strands of duplex-DNA at a specific base-pais sequence. Every restriction enzyme recognizes a characteristic sequence of nucleotides which is known as its recognition site. most recognition sites are four to eight base-paiss long and are usually characterized by a complementary palindromic sequence- i.e- the sequence of nucleotide in one strand is completly opposite to sequence of nucleotide in other strand but can be read same from opposite-directions.

in bacteria, where they appear to serve a host-defense role because they chop up and restrict or inactivate the DNA of infecting viruses (pathogen).

Nomenclature of Restriction enzymes :- Smith and Nathans suggested the naming guidelines for restriction endonucleases in 1973. According to these guidelines, Restrication enzymes are named after host of origin. for example.

1:- EcoRI was isolated from E.coli bacterium.

2:- Hind-II and Hind-III from Haemophilus-influenzae.

3:- Xho] from xanthomonas holcicola. etc.

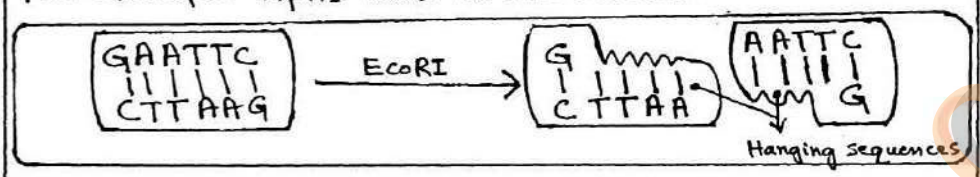
Breaking of phosphodiester bond :- (work of restriction Enz)

Restriction endonuclease cuts the phosphodiester bond in such a way that phosphate group remains with 5'-end of DNA and hydroxyl group remains with 3'-end of DNA. Every type of restriction-enzyme has its own way of making a cut in duplex-DNA. Generally, there are 3-types of cuts which can be made by different restriction enzymes i.e-

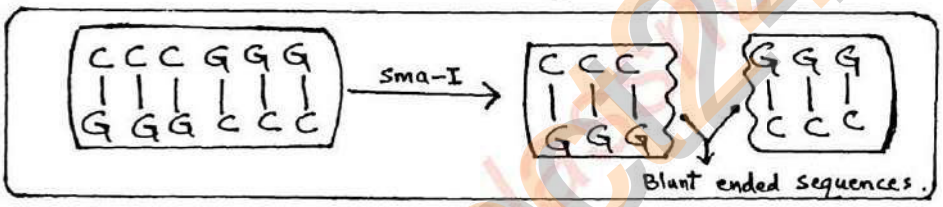
i: 5' overhange :- In this type of cut the enzyme cuts the duplex-DNA asymmetrically such that a small single-stranded segment extends or hangs from 5'-end called 5'-sticky end. e.g- BamHI cuts in this manner.

ii: 3' overhange :- In this case cut is again asymmetrical

For example KpnI cuts in this manner.



iii: Blunt cuts :- Blunts means no overhang or no sticky ends. Some enzymes make direct symmetrical cut with no sticky ends or overhangs. e.g- SmaI cut in this manner.



Mostly we use such enzymes in recombinant DNA technology which produce sticky ends. sticky-ends are also called an adhesive end because they have the power to adhere and mak bonds with other strands.

3: Vectors or molecular carriers : (ETEA-2017)

Definition :- Vectors are those agents which carrier genes of interest to expression system (host) for multiplication. It acts as vehicle. vector easily replicate in bacterial cell.

Characteristic of vector :- commonly used vector is plasmid. However, vectors should be having the following characters.

i: origin of replication :- Every vectors should have its own origin point where replication start.

marker gene. These may be antibiotic resistant gene, like Amp^R-gene (Ampicillin resistant gene). selective markers are essential for the identification of bacteria in cluster, containing recombinant plasmids or gene of interest.

iii: Multiple cloning sites or polylinker:- Each vector has at least one recognition or restriction site for restriction endonuclease. All these restriction sites are grouped into a small region of vector called as polylinker or MCS. if MCS contains many restriction sites when using different enzymes. Therefore, vector must also have MCS. (ETEA-2018)

iv: Size:- Vectors should have small desirable size so that can easily entex to bacterial cell (Host). For example the size of plasmid-cloning vector is from 0.5 to 8Kb. There are about six types of vectors commonly used in Recombinan DNA technology, each has different capacity of insert DNA size. (see in table).

Vector type	Size in Kilobase (Kb)
1- plasmid cloning vector.	0.5—8
2- Bacteriophage cloning vector.	9—25
3- cosmid (phage+plasmid)	30—45
4- YACs	250—1000
5- BACs	50—500
6- Shuttle vectors	> 1000

Types or example of vectors: common example of vectors are below:

1: Plasmid: plasmid is a circular extra-chromosomal DNA in bacteria. It is considered as good vectors because it is naturally present in bacteria and that is why it can easily replicate within the bacteria cell. It is widely used. it is more versatile. Beside this plasmid can be

2: PBR^{322}: the plasmid - PBR^{322} was one of the first multipurpose cloning vectors to be designed and constructed for the efficient cloning and selection of recombinant DNA molecules in E. coli.

4: Molecular glue or DNA Ligase: (ETEA-2020)

DNA-ligase are those enzymes which join the two ends of DNA-molecules. These are also called molecular glue.

Explanation:- DNA ligase form phosphodiester bond blw the two nucleotide and thus join the two separated - fragments of DNA. Linkage is formed between 3'-OH group at 3'-carbon and 5'-PO_4 group at carbon No-5 of the pentose sugar of adjacent nucleotides of two different DNA fragments or nicks created in a double stranded DNA. In recombinant DNA technology, DNA-ligase is used to joint two different DNA fragment that are annealed by sticky ends.

Example:- The most commonly used Ligase enzyme is the T_4-DNA-ligase produced by T_4-bacteriophage.

5: Expression System:-(Host)

A suitable organism that can act as host for the recombinant vector (e.g plasmid) to express is called expression system.

Explanation:- The expression system always depends upon the type of recombinant vector used. The most ideal - expression system is that which has - short generation time (replicates rapidly), simple genome, etc.

Example:- Best example for expression system is bacterial

Q3 Describe the proceduxe of xecombinant DNA – technology ox genetic engineexing techniques.

Answex: Recombinant DNA technology:

Definition :- The joining togethex of DNA-molecules from different organisms and insexting it into a host organism to produce new genetic combinations that are of value to science, medicine, agxiculture and industxy is known as Recombinant DNA technology.

pxocedure :- Recombinant DNA technology consists of –

1: Formation of xecombinant DNA : (ETEA-2014,2017,2019,2020)

Isolation of the gene of interest :- In the 1st – step, gene of interest is isolated and cut it into fxagments using xestxiction endonuclease to cut the identified gene fxom the DNA of – donox oxganism.

Insextion of the gene into a vectox :- now the vectox DNA digest with a suitable xestxiction endonuclease and make it linex with stickx ends. The gene of interest which is isolated is now incubated with such vectox i.e plasmid.

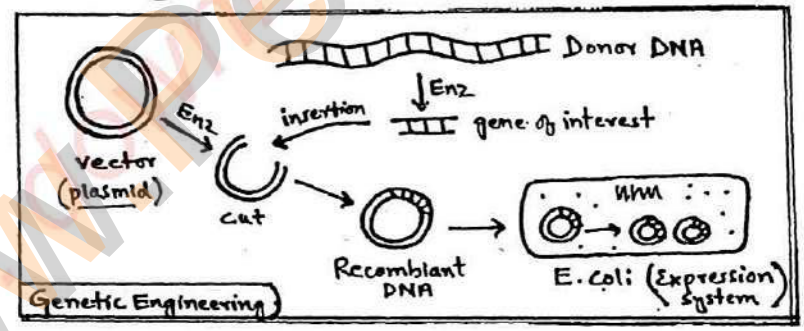
Recombinant DNA formation :- Now by using DNA-ligase enzyme the gene of interest and DNA of vectox can be joint. i.e. During incubation, the stickx ends of the two DNA stxands come closex by the base complementation and become

enzyme. This results in the formation of recombinant DNA, or vector.

2: Transformation of expression system :-

Definition :- The entry of recombinant-DNA to bacterial cell or expression system is called Transformation of rDNA.

Explanation :- Transformation can be performed if - bacterial cell (expression system) and recombinant vector (plasmid) are placed in the same medium. The bacterial-cell takes up the recombinant plasmid if bacterial cell is treated with $CaCl_2$ (calcium chloride) which increases the permeability of bacterial cell-membrane. Now as the bacterial cell divides into daughter cells, each daughter-cell with contain at least one recombinant plasmid (vector). So each daughter bacterial cell contains gene of interest which will express and - make the required product.



3: Identification of transformed clone :- The transformed cloned can be identified by adding particular antibiotic (for which resistant gene is found in plasmid) into the medium

antibiotic, so it remains alive and continues to grow - because the gene of interest is present. All the untransformed clones are killed by the antibiotic. The cloned gene can be isolated for further analysis or its protein product can be separated.

Q4 Explain the process of gene cloning with the help of PCR.

Answer: polymerase chain reaction: (PCR)- (ETEA-2020)

Definition :- The quantitative amplification (coping) of DNA with the help of enzyme (Taq-polymerase) is called PCR.

—OR— "The type of reaction which can create millions of copies of a single gene or any specific piece of DNA - quickly in a test tube is known as polymerase chain reaction".

Explanation :- polymerase chain reaction is a technique which can produce millions of copies of a single gene or part of DNA. In PCR-technique, an enzyme namely- polymerase is used. Therefore this technique is called polymerase chain - reaction technique. PCR is called chain reaction because the replication of DNA occurs repeatedly untill a large number of copies of DNA are produced.

Discovery :- This technique was first introduce in 1983 by Kary Mullis, an American biochemist, for which he has awarded Noble prize in chemistry in 1993. (ETEA-2009,2014)

1: Template DNA :- It is the original piece of DNA from which other copies of DNA are amplified (cloned or copy).

2: dNTPs :- dNTPs mean deoxyribonucleotide triphosphate. These are few nucleotides that act as raw nucleotides for synthesize of new strand. These included – dATP, dGTP, dCTP, dTTP.

3: primers :- These are 20-base sequences which are complementary to original strand. These short sequences act as foundation stone and only initiate polymerization.

4: Taq polymerase :- The polymerization of DNA occurs in the presence of enzyme – Taq-polymerase. This enzyme is obtained from bacterium called Thermus-aquaticus. This bacteria live in hot spring.

5: PCR machine :- PCR mixture (contain all the above component) is now placed in a machine called PCR-machine. (ETEA-2017)

Mechanism or proceduse of PCR :- It consists of 3-steps. i.e

1: Denaturation :- In the 1st step temperature of system was increase upto 94°C, for one minute to 5-minutes. at this temperature two strand of DNA separate from each other because the hydrogen bonds between the two strands of DNA is break down. This is called denaturation. now the double stranded DNA become single stranded DNA (ss DNA). each strand now act as template for the invitro-DNA synthesis.

2: primer Annealing :- The binding of primers – molecules on

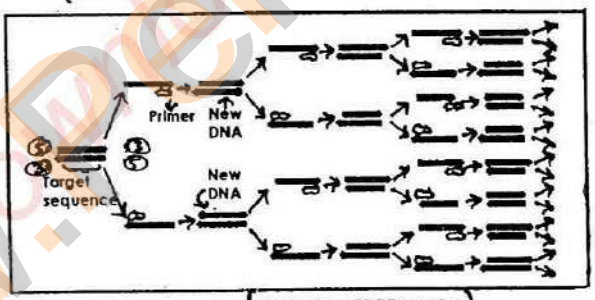
primers elongats the chain in forward direction and backward primers elongates the chain in backward direction. The temperature required for this step is 54° for only 2-min.

3: polymerization :- The formation of new strand over the template strands is called polymerization or extension. polymerization occurs under the presence of Taq-polymerase enzyme at 72° for one minute. In this process dNTps are added to synthesize new strand.

1: at the end of first-cycle, one target DNA molecule is converted into 2-molecules.

2: The second cycle immediately starts with the denaturation by heating at 94° and the cycle is repeated.

3: The number of existing DNA-molecules become double after each cycle.



Mechanism of PCR reaction

Application of PCR:

PCR is very important technique and is used in following processes.

1: PCR is used for detection of specific infectious agents like-HBV, HCV, HIV, etc.

3: PCR is very helpful in genome-analysis, production of makers for genome maping, etc.

4: One of the type of PCR is RT-PCR which can be carried out with mRNA to synthesiz cDNA (complementary DNA) from mRNA in the presence of reverse transcriptase enzyme.

5: Reactions for DNA sequencing are also simplified by PCR.

6: It is very important in criminology to find out the crime from DNA tasting. PCR is also used paternity tests.

7: PCR can be used in DNA fingerprinting.

8: Detection of mutation, embryonic diseases, etc. can also be diagnosed by PCR. 9:- used in reseach and genetics

10: Monitoring the gene in gene therapy.

11: Compare the genome of two organisms in genomic studies.

12: Time saving technique and produce thousands of copies in minutes.

Q5 write short note on genomic Library.

Answer :- Genomic Library :-

Definition :- Collection of DNA sequences in a bacterial cell or even bacteriophage clones is called Genomic Library.

Explanation :- A genomic library is a collection of independently isolated vector linked fragments derived from a single organism. It contains at least one copy of every DNA sequence

Construction of Genomic Library:

1. For genomic library a vector is required that could accommodate large size of inserts (fragmented DNA.)
2. During genomic library construction, first of all genomic DNA isolated, and then cut it into many fragments of suitable size by restriction enzyme.
3. The fragmented DNA of suitable size is now ligated in the appropriate cloning vector. Now it is called recombinant vector.
4. Finally, the recombinant vectors are transferred and maintained in organisms such as bacteria, virus or yeast.

Locating gene of interest from DNA libraries:

DNA probe: DNA probe is a sequence of nucleotides complementary to gene of interest. It is either labeled with florescent dyes or is radioactively labeled.

Explanation: if we have to identify a particular gene in genomic-library, we will have to form its probe. The probe of that desired gene is then added to the genomic library or bacterial cell. This probe will hybridize (attach) to its own complementary sequence (desired gene) and as it attaches to that sequence then we can easily identify that gene, because probe is fluorescently labeled which reflects light of particular colour or radioactively labeled which radiate particular radiations.

Q6 what is DNA sequencing. Explain the process of gene sequencing with the help of sanger's method.

Answer: DNA sequencing:

Definition:- DNA sequencing is the technique which is used to determine the exact arrangement of nucleotides along DNA strand.

Explanation:- In order to know about the exact gene structure, its expression, protein interaction and molecular mechanism of genetic diseases, it is essential to know about

1: To generate pieces of DNA of different sizes all starting from the same point but ending at different points.

2: Separation of these different sized pieces of DNA by gel electrophoresis.

3: Reading of sequence from the gel.

Methods for creating DNA fragments :- For generation of different sized DNA fragments, two different sequencing methods were developed during the late 1970. They are

1- sanger method. 2- maxam-Gibert method.

Sanger Method :- (chain termination Method)

Discovery :- This method was first develop by Frederick Sanger's in 1977 along with their collegues, for which they awarded noble prize in 1980.

Standard method :- This method is widely used and similar to natural process of DNA replication. It is now used as standard method because of its practicality.

procedure :- Sanger method has the following steps.

1: DNA is denatured to single strands, using heat because only one strand that act as a template is required in this procedure.

2: Tagged the template strand with primers of known sequence at 3'-end.

3: Furthermore, primer is either labeled with fluorescent dye or radioactive element (^{32}P) which will help us to study -

4: Once the primer is attached to the DNA, then divided the solution into four tubes and labeled that as- G, A, T, and C.

5: Add reagent to these above tubes as follows.

i: G-tube = Add all 4-dNTps + ddGTp + DNA-polymerase.

ii: A-tube = Add all 4-dNTps + ddATp + DNA-polymerase.

iii: T-tube = Add all 4-dNTps + ddTTp + DNA-polymerase.

iv: C-tube = Add all 4-dNTps + ddCTp + DNA-polymerase

6: All the tubes are same except the presence of diff; ddNTp.

7: These tubes having ddNTp are now placed in PCR-machine so that sequencing reaction can start.

8: Nucleotides (dNTps) are added in the presence of DNA-polymerase enzyme to the growing chain.

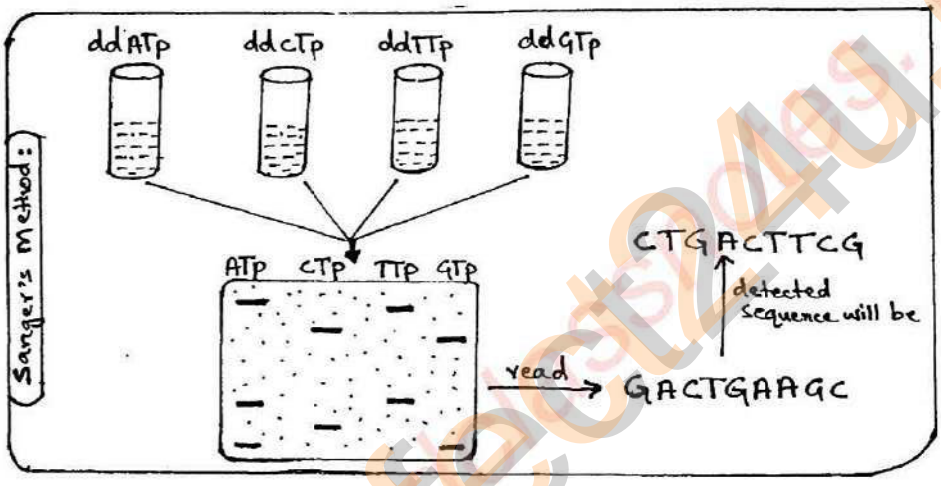
9: The new growing chain is termined when a dideoxy —-nucleotide (ddNTp) is incorporated at the place of normal nucleotide because ddNTp is chain terminating nucleotide.

10: The growing chain will terminate at different points due to different ddNTps in each tube.

11: Once these reactions are completed and double stranded DNA are formed of different sizes, then the DNA is once again denatured to single strand for electrophoresis.

12: The contents of each of the four tubes are run in separate lanes (well) on a gel in order to separate the different sized bands from each other under electric field.

is then exposed to either UV-light if primer is labeled with fluorescent dye or X-ray if primer is labeled with radioactive elements, to read the banding patterns (sequence)



Q7 Explain Gel electrophoresis in detail.

Answer:- Gel electrophoresis: (ETEA-2020)

Definition:- The movement of DNA fragments inside the gel by appling the potential difference across the terminals is called gel electrophoresis —OR— "electrophoresis is a technique of separation of charged molecules (DNA, RNA, proteins) under the influence of an electric field."

Explanation:- (Factors) separation of DNA fragments during electrophoresis depending on their net charge, size shape and applied current of electric field.

Type of gel:- During gel electrophoresis, two type of gels

1: Agarose gel :- Agarose gel is commonly used for the separation of large DNA-fragments because agarose is polymer having large holes among polymers.

2: polyacrylamide gel :- It is used for separation of small DNA-fragments because it is such polymer having small holes among polymers.

procedure of gel electrophoresis :- It consists the following steps

1: preparation of diff; size DNA fragments :- DNA fragments of different sized are prepared by applying the restriction enzyme which cuts the DNA-molecules into different size fragments.

2: preparation of gel :- Gel is semisolid-substance made from agarose. agarose is a polysaccharide obtain from sea-weed. In this process powered agarose is dissolved in boiling water and then cooled down it. This solution will be now a semisolid. This is called agarose gel.

3: Loading :- After the preparation of gel from agarose. Now we poured DNA solution (sample) to the chamber or wells. This step is called loading.

Wells :- at one end of gel slab small partial holes are made called wells. Wells are partial in sense that they are open only from upper side and closed from the other down side.

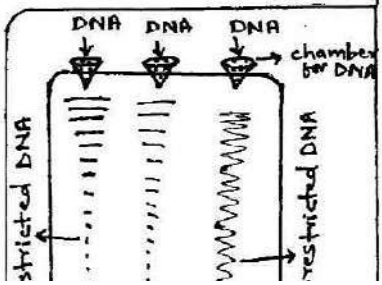
4: Establishment of electric field :- The gel slab is than suspended in a buffer solution which maintains electric field between two terminals or electrodes when potential difference

is inserted at one of the buffer and negative electrode at the other's end and potential difference is provided.

5: Separation of DNA fragments :- The current starts to flow between two electrods through buffer due to which DNA fragments also start movement toward positive electrode as DNA is negatively charged due to phosphate groups. The DNA fragments move and cover distance according to their size such that small fragments move faster and covers more distance and vice versa. The movement of DNA fragments also depends upon:— "charge of DNA fragments, number of strands (single or double), shape (circular or linear) and gel holes.

6: Reading :- when the DNA fragments stop motion, then they appear in the form of a band but they cannot be visualized because they need to be labeled with radioactive probes or fluorescent dyne. The study of bands can be done by two methods. i.e 1:- radioactive probes are added to DNA fragments. 2:- The DNA fragments are treated with fluorescent dyes which binds to DNA fragments and then bands are viewed under UV-illumination. ←(ETEA-2017)

7: Autoradiography :- after adding of radioactive probes with DNA — fragments, then nitrocellulose sheet is placed on gel slab which contains



is transformed to nitrocellulose sheet or membrane. As the bands contain radioactive probes so the nitrocellulose-sheet is then exposed to x-ray film due to which an x-ray image of bands is formed on x-ray film. The x-ray film will show some thick and some thin bands. The thick bands show high concentration of fragment while thin bands show less concentration of fragments. The sequence is then read from this sequence of bands.

Q8 How the automated DNA sequencing machine improve the quality and speed of sequencing process.

Answer :- Automated DNA-sequencing:

Introduction :- This is the process in which DNA-bases are sequenced through automatic machine. This actually through improve the quality and speed of the sequencing process.

procedure :- (principle)

1: In Automated sequencing we use the same four normal dNTPs as used in sanger method.

2: Similarly, we use ddNTPs but in this case they are not labeled radioactively rather they are tagged with particular fluorescent dyes. i.e- each ddNTP is dyed with different fluorescent dyes.

different tubes as used in sanger's method.

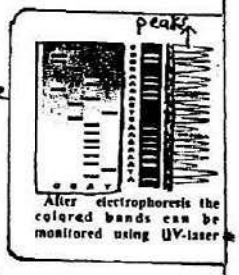
4: The DNA-replication starts and a number of fragments are formed each terminated by fluorescently dyed - ddNTPs which gives us the end nucleotides of each fragment.

5: The fragment are then separated by gel electrophoresis.

6: The gel which contains invisible banding pattern is then - exposed to UV-laser beam.

7: As the UV-falls on gel it excites fluorescently dyed ddNTPs end of each fragment which reflects characteristic colour.

8: From these colours biotechnologist knows that which ddNTp was attached here with DNA fragment.



9: No autoradiography is to be done after the process and that it why we have to no need to read the reading from gel because here the data is directly shifted to photoelectric device and then to computer screen.

10: The emission data from gel is converted into corresponding nucleotide sequence of DNA.

11: The sequence of nucleotides and nitrogenous bases as - respresented by peaks on the screen.

Q9 Explain the DNA sequencing with the help of maxam-Gilbert method -< chemical cleavage method >-

DNA-sequencing, also called as chemical cleavage method because this is based on chemical change (cleavage) of DNA at specific bases or nucleotides.

Discovery :- This was first discovered by Allan-Maxam and Walter-Gilbert in 1976-1977 for the first time.

procedure :- Maxam-Gilbert method consists of following steps

1: DNA sample is first labeled at one end with the help of enzyme-Kinase with ^{32}P-ATp which transfer radioactive--phosphorus from ATp to 5'-end of each strand of DNA. The DNA is labeled at 5'-end only.

2: Now add Dimethylsulphoxide (DMSO) to the DNA sample.

3: Heat the mixture upto 90°C (or 94°C) which result in break down of base pairs and duplex-DNA is converted into - Singal stranded DNA (SSDNA).

4: Now the two SS DNA or two strands are separated by electrophoresis

5: one strand is purified from the gel and divided into Four Samples i.e- G, A+G, T+C and C-tubes.

6: Each of which is treated with one of the cleavage reagents and modifying chemical reagent.

7: Former (ہیں) are used to cut DNA-strand at specific point and later (ہیں) cause chemical changes in the nucleo--tides.

i.e: i: in Tube-G, the cleavage reagent cut DNA strand at G-base, result small DNA fragments is formed in tube

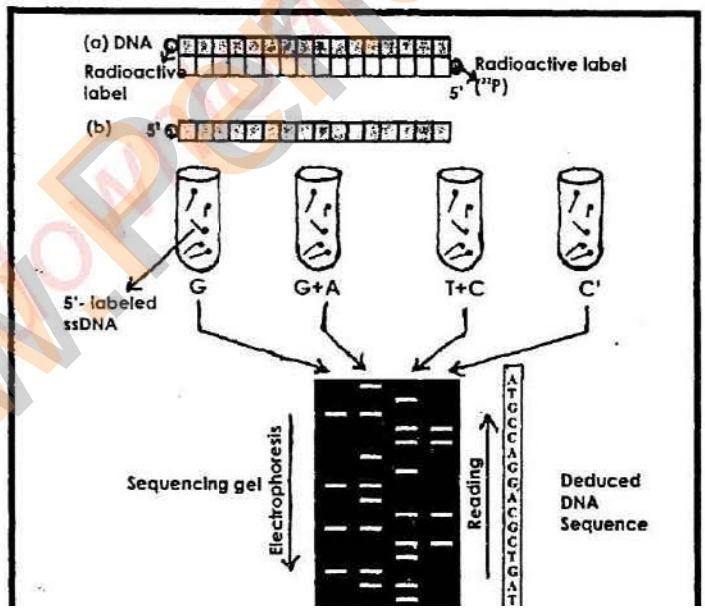
Strand at A or G-bases, result small DNA-framents are formed in tube-A+G.

iii: In tube-T+C, the cleavage reagent cut the DNA strand at T or C-bases, result small DNA fragments are formed.

iv: In tube-C, the cleavage reagent cut the DNA strand at C-base, result small DNA fragment are formed in C-tube.

8: The DNA-fragments in the four reactions or tubes are now arranged side by side in gel electrophoresis for size separation.

9: To visualize the DNA-fragments, the gel is exposed to x-ray film for autoradiograpy, yielding a series of dark bands each corresponding to radiolabelled DNA-fragment, from which the sequence may be inferred.



Q10 what is DNA analysis. Describe it procedure. (ETEA-19)

Answer:- DNA analysis :-(DNA fingerprinting or DNA profiling)

Introduction:- The analysis of DNA from samples of body tissues or fluid in order to identify individuals is called DNA analysis. every species has it own unique genetic sequences which are used for identification of individual at molecular level

Discovery:- This technique was first develop by Alec Jeffrey in 1985.

Explanation:- DNA-analysis or DNA fingerprinting is the method to find out the sequence of base in the DNA for the identification of individuals at the genetic level. DNA analysis has become a great practice for identification of paternity or maternity or criminal guilt or innocence, embryonic health or to find out diseases. Besides this DNA analysis can also help in the evolutionary history of various animals among the animal-kingdom. Thus the DNA-fingerpointing enables us to determine whether the two DNA-samples are from the same person or related persons or non related persons.

procedure:- many techniques are used for DNA-analysis but among these the most important is stand for Restriction Fragment Length polymorphis-RFLP. i.e- ←(ETEA-2016)

1: Samples collection:- First step in this process is sample collection which can be easily collected from blood, saliva, semen drops, hair-root or fossils (Extinct organism). This

2: placement of RFLP :- This is the digestion of DNA - sample by various restriction enzymes to produce fragments of different size. Keep in mind that every person has unique RFLP because the restriction site of a particular enzyme is always different in number and distribution along the length of DNA in all human being except monozygotic twins. Although 99% of human DNA-nucleatide-sequences are similar. This is only 1% difference in genome which is unique to each and every person.

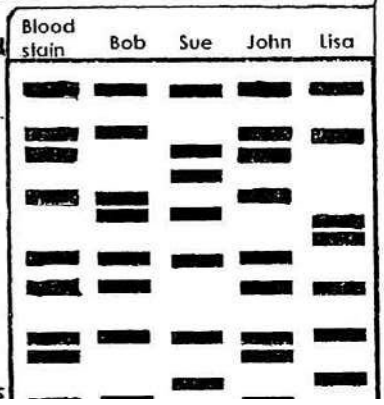
3: Separation of RFLP :- These RFLP's-fragments are now separated by gel-electrophoresis. These fragment move - according to their size and charge bearing intensity. when they stop movement, the gel is further processed for - studing banding pattern.

4: Southern blotting :- It is a routinely used method in molecular biology for the detection of specific DNA sequence in DNA-samples. It takes two steps to complete. i.e-

1:-Transfer of electrophoresis separated DNA fragments to a filter membrane.

2:- Subsequent fragment detection by probe-hybridization.

This method was discovered by British biologist Edwin Southern.



5: Autoradiography :- The excess probes

and the pattern of hybridization are now visualized on x-ray film by exposing the membrane to an x-ray source. This is called autoradiography.

Application of DNA-analysis :- DNA analysis is an important technique and it has following application.

1: Identification of crime.
2: Identification of catastrophe victims.
3: To find paternity and maternity (Biological parents)
4: Used in wild life to prevent poaching.
5: Detection of pathogen like- bacteria, viruses, etc.
6: also used in donor-recipient organ transplantation.
7: Determine pedigee for seed or livestock breeds.
8: To identify endangered and protected species.
9: It can be used to determine the evolutionary history of human population.
10: use for personal indentification.
11: use for detection of microorganism in food, water, soil, etc.

Q11 Differentiate b/w Genome map and Genetic markers.

Answer: 1: Genome Map:

Definition :- The total number of all the genes in one complete set of chromosomes (n) is called genome. where as the correct order-maping of all the genes of genome of that organism is

which guide us to reach a specific location. In the same way genome-map are used by scientists searching for specific gene in the full genome of organism.

Types of Genome Map:- Scientists use two types of genome maps to reach a gene. i.e--

i: Genetic Maps:- Genetic map provide indirect estimate of distance between the two loci of genes on chromosome.

ii: physical maps:- physical map is the estimate of true distance between two loci of genes in term of measuring base-pairs between these loci. once the number of base pair between two loci of genes is determined, one can easily determine true distance between these two genes.

2: Genetic Markers:

Definition:- A fragment of DNA which is used by scientists to identify a particular genome of individual is called genetic marker.

Explanation:- The landmarks of genetic maps are called genetic markers. Here the term marker is used for short, that is why it is used to indicate variations result from mutation at single genetic locus. genetic markers help us to identify genome of an organism so that to be differentiate from others.

location of genetic markers:-

1: genetic markers may be located within genes which code for noticable physical character such as- eye colour.

gene to deteet unique region on a chromosome. e.g RFLPs.

Importance of genetic Marker:- DNA or Genetic markers are especially used for making genetic maps when there are mutation during meiosis or formation of gametes, that lead to a high degree of variation in the DNA from individual to individual.

Example of genetic (DNA) markers:- genetic markes is - different for all humans except monozygotic twin. e.g-

1: RFLP — restriction fragment length polymorphism.
2: VNTR — variable numbers of tendon repeats polymorphism.
3: Microsatellite polymorphism.
4: SNPs — single nucleotide polymorphisms.

Q12 what is Genome analysis. Explain Human genome project.

Answer: Genome analysis:

Definition:- The complete sequencing of an organism's genome is called genome analysis.

Discovery:- This was first discovery by Fred-Sanger. He had sequenced the first bacteriophage genome for which he later won the Noble-prize.

Explanation:- Due to rapid development of genomic studies a new branch of biotechnology has emerged called genomics which deal with genome analysis. It was almost impossible until

Human Genome project: (HGP) – Human genome project is an international scientific research project based on – exploration and analysis of human genome.

Duration :– Human Genome project was started in 1990 and was completed in 2003 by the help of US deptment of energy and National institute of health in America.

Funding :– Although this project was funded initially by the US – government but later on Welcome Trust of U.K become a major partner. In later stages contribution came from Japan, France, Germany, china, etc.

1st Director :– The 1st-director of HGp was "James D. watson.

Major goals of Human genome project:

1: Identification of 2000–25000 genes in human genome or DNA.

2: Determine 3-billion base-pairs of human DNA.

3: Store information in Database form.

4: Improve tools for Data analysis.

5: Transfer of these techniques to private sectors so that – ethical, legal and social issues may arise from the research project.

Benefits of human genome project:– Human genome project is very important in molecular-medication, bioarcheology, – anthropology, evolution and migration as following–

1: Molecular medicines :– GHp improved the following–

i: Diagnosis of diseases (ii: Drug design.

V: Detection of genetic predisposstion of diseases.

2: Bioarchaeology, Anthrology, Evolution and Human migration:

1: study of lineage and germ-line mutation.

2: study of migration of different population groups based on female genetics inheritance.

3: Study of Y-chromosomal male inheritance.

4: compare evolution historical events and mutation.

Q13 what is tissue culture. Explain tissue culture in plants.

Answer:- Tissue culture:- (ETEA-2016)

Definition:- The propagation of a plant by using a plant cell or group of cells within a test tube under control circumstances is called tissue culture.

Explanation:- According to a rules of embryogenesis each cell of living organism has the potency to make other various types of cells and even the whole organism. In the same way a German botanist — Gottlieb Huberland said in 1902 that plant cells are Tottipotent i.e- each cell posses full genetic information and has the ability to make new plant of its own type by tissue culture technique.

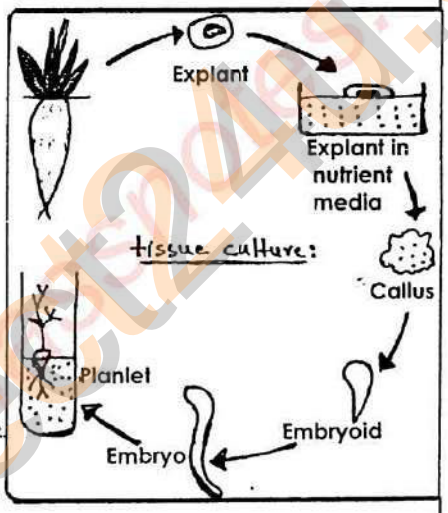
procedure:- Tissue culture consists of following steps.

1: **Explant:-** Any plant part to be used in tissue culture is -

2: Sterilization :- Sterilization mean the decontamination of explant from germs and microorganism. They are — Sterilized by using chemicals like— calcium-hypochloride, etc. The glasswares which is to be used should also be sterilized.

3: Media preparation :-

Media is the environment— like soil in nature, which will provide nutrients to explant for growth. Media usually consist of inorganic salts, vitamins, plant-hormones. Media may be solid or liquid — depending upon the need and type of culture. It must be sterilized.



4: Inoculation :- It refers to the placement of explant onto the surface of a solid or a liquid culture medium. usually — explant is planted in solid medium but sometimes directly into liquid medium particularly when cell suspension culture is — re; after placement of explant, peti plates or test— tube should be air-tight. Next, these glasswares are shifted to the growth room or incubator.

5: Development of callus :- The explant is allowed to grow into callus callus is a mass of undifferentiated loosly bounded cells, which then shift to new media for further development of

Cytokinines are equally (balance) added to media.

6: Appearance of plantlets :- As the callus grows a piece of sliced off called as plantlet or immature newly produced plant, which are then shifted to new media for further growth. In this media High concentration of cytokinins along with small amount of auxins causes shoot growth. while high concentration of auxins along with small amount of cytokinins causes root growth. The plantlets when sufficient grows then it is transferred to potting soil or even in the green house environment for complete growth.

Types of tissue culture :- Following are the types of tissue culture.

1: Callus culture :- The culturing of explant within the media when Auxin and cytokinin are equally applied is called callus culture. The explant or any plant tissue grow to callus. callus is a mass of cells.

2: Cell suspension culture :- This type of culture is usually develop from callus. The medium used in this type is always liquid.

Explanation :- cell suspension is developed from the callus when it is placed into a liquid medium and then agitated, single cells or small clumps of cells are released into the medium. These cells continues to grow and divide, producing a cell suspension culture.

Usage :- This type of culture is important in medicinal plants

suspension culture of Cinchona – Ledgeriane produce Quinine used as antimalarial drugs. Similarly Lanata produce only drug called digitoxin used for prevention of heart attack.

3: protoplast culture :- protoplast is a part of plant without cell wall. It is obtaind from mesophyll cell and then subjected to culture.

Explanation :- This type of culture is important to develop the whole plant by organogenesis or somatic embryogenesis like embryo made from somatic cells is called somatic embryogenesis. then they are exposed to UV-radiation or chemical mutagen to induce mutation which is called as somaclonal variation.

Usage :- Its main purpose is to develop Genetically modified plants.

4: Meristem culture :- The rapidly dividing cells are called meristem. These specific cells are found at the apexes of roots and shoots. This type of culture use meristem as explant.

Usage :- This culture is used for micropropogation and to obtain virus, parasite free plants. This is because all parts of plants can be infected by microbes except meristem due to the absence of vascular system in them.

5: Anther culture :- The type of culture in which pollen grains are used as explant is called anther culture.

Explanation :- mature anthers and pollens are culture in a medium. These are usually haploid cell that is why it will produce

Colchicine treatment haploid plant (n) can be made as diploid. Colchicine cause chromosomal non-disjunction i.e-chromo--somes fails to separate so the daughter cell will receive two copies of cell and hence will become diploid (2n).

Usage :- Its main purpose to develop haploid or polyploid plants.

Q14 Explain the process of animal cell culture.

Answer :- Animal cell culture :

Definition :- The culturing of animal cells in artificial media is called animal cell culture.

Explanation :- This type of cell-culture are used in recombinant DNA-technology, genetic manipulation, variety of industrial processes. They are important in production of vaccines, antibiotic, drugs, pharmaceuticals drugs, monoclonal antibiotics.

Techniques of animal cell culture :- Animal-cells are grow in a clean and well sterilized container or tube in the presence of most suitable media within a control environment circumstances. There are the following diff; types of techniques of animal cell culture.

1: primary cell culture :- A cell culture which is initiated by cells removed from an animal's organ is called primary cell-culture.

2: secondary cell culture :- When the contents of primary-culture are subjected to another culture media this is called secondary cell culture.

organ culture. native tissue are taken for animal culture within a suitable culture media to produce invivo-histological aggregation.

4: Histotypic culture :- The culture of a more defined tissue like structure is called histotypic culture. here the re-aggragation of cells occurs.

5: organotypic culture :- The recombination of different cell types to form more defined tissue or even organ is called organotypic culture.

Requirement for animal cell culture :- The essential requirements for animal cell culture are special incubator and the synthetic media i.e -

1: The incubator maintains the level of oxygen, CO_2, temperature and humidity as present in the animal's body.

2: The synthetic media consists of vitamins, amino acids and fetal serum. synthetic media are 2-types i.e

i:- Serum containing media. ii:- Serum free media.

Application of animal cell culture :-

1: production of antiviral protein or vaccines.

2: Genetic manipulation, which is easy to carry out in cell or organ culture.

3: cell fusion techniques. 4:- production of pharmacutical drugs.

5: Analysis of fetal chromosomes collected from womb.

6: production of monoclonal antibodies.

7: use of artificial skin 8:- Function of nerve cells.

9: Study of the effects of toxins and pollutants

Q15 What is transgenic organisms. Describe the advantages of transgenic bacteria:

Answer: Transgenic organisms: (ETEA-2011,2013,2020)

Definition:- Those organisms which have foreign gene are called transgenic organisms.

Explanation:- The insertion of DNA of one organism into other is called recombinant DNA technology and the resultant organisms are called genetically modified organism or genetically engineered or transgenic organism. This first transgenic organism was bacteria produced in 1973, since then many transgenic organisms such as—animals, plants and bacteria have been produced.

Transgenic bacteria:- Bacteria was first genetically modified in 1978 by Herbert-Boyer at California university and is able to produce human insulin (Humulin) by inserted human insulin gene into E.coli.

Advantages of transgenic bacteria:— produce different products.

1: Role in Biotechnology product:- Transgenic bacteria are very important because they can produce various products like—

1: Insulin for diabetes. 2:— Enzyme production

3: clotting factors for haemophilia.

4: growth hormones for dwarfism. (ETEA-2010)

5: Tissue plasminogenic activator for prevention of heart attack.

7: Bioremediation for removal of pollutants from environment especially in aquatic environment.

8: production of phenylalanine — amino acid.

2: Ecological concerns :- athough there are lots of benefits are being obtained from genetically engineered bacteria but there are some ecological concerns also associated with these bacteria. one of the main issues regarding this is the — possibility that hazardous new pathogens might be created which are then difficult to control and causes new problems.

Q16 what is transgenic plants. write its importance.

Answer :- Transgenic plants : (ETEA-2016)

Introduction :- Transgenic plants possess foreign gene in their genome. First transgenic or genetically modified plant was — Tabacco in USA and France in 1986 to be resistant to — herbicid. Beside this other plants are made resistance to pests, diseases, drugs, drought are also produce transgenically.

Method of gene transformation in plant :- There are many techniques where gene of interest is introduce into single or few plant cells which then permitted to regenerate in a — suitable culture medium. a successful tissue culture procedure result in the formation of transgenic plants.

Agriculturally improved transgenic crops :- During the last

development of transgenic plants using various techniques. As per estimates recorded in 2002, trangenic crops are cultivated worldwide on about 148-million acres or 587-million hectares land by 5.5 million farmers.

Bt or Cry genes :- This first genes available for genetic engineering of crop plants for pest-resistance were "Cry genes or Bt genes" from a bacterum — Bacillus thuringienis. Transgenic crops with Bt-genes e.g- cotton, rice, maize, potato, tomato, brinjal, cauliflower, cabbage, etc have been developed

Importance of transgenic plants:

1: Resistant against biotic stress like- pest, bacteria, virus.
2: Resistant against abiotic stress like — temperature, drought, humidity, salinity, water logging, etc.
3: Weedicide or herbicide tolerance.
4: They are insect resistant such as crops with Bt-gene.
5: Delayed fruit ripening.
6: Improved nutritional content.
7: Improved per-capita production and population growth.
8: Human Hormones formation through plants.
9: Improved the CO_2-capturing ability of plants.

Example of transgenic plants :- some of the commerically grown transgenic plants in developed counteries are —

1: Roundup Ready soybean 2: High lauric rapeseed (canola)

Q17 What is transgenic animals. write its importance.

Answer: Transgenic animals:

Definition :- Those animals which have foreign genes are called transgenic animals.

Methods for creating transgenic animals :- There are three methods for creation of transgenic animals i.e-

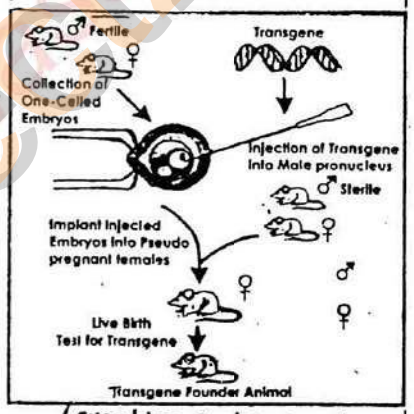
1: DNA microinjection or using gene gun.

2: Embryonic stem cell mediated gene transfer.

3: Retrovirus mediated gene transfer.

Model for transgenic animals :-

In comparsion to higher vertebrates mice have become the model animal used in the field of transgenic because of their small size, short generation time and well defined genetics as well as low cost of housing.



Creation of transgenic animal:

Importance of transgenic animals:

1: more milk and meat production.

2: Growth hormones production.

3: pharmaceutical drugs production.

4: Antithrombin-III for preventing blood clots.

5: Bovine growth hormone and antigenic protein production.

Q18 What is the role of biotechnology in health care.

Answer: Biotechnology and health care:

Introduction:- As for as biotechnology is concerned it is very important for human health care because the techniques of biotechnology have opend new doors to learn more about the human body or genome. Due to understand the molecular base of health and diseases this has lead scientists to improve methods of treating and preventing diseases. It has enabled the scientists to develop therapies and vaccines which are more safer than ever before.

Role of biotechnology in health care:-

1: Role in vaccine development:- Vaccines are antigenic proteins which produce resistant against viral diseases. They are antigenic but non-pathogenic.

Methods of vaccines preparation:- Biotechnology is used the following 3-different methods to develop vaccines.

1: Separation of a pure antigen using a specific monoclonal antibody.
2: Synthesis of an antigen with the help of a cloned gene.
3: Synthesis of peptides to be used as vaccines.

2: Role in diagnosis of diseases:- many human diseases can be diagnosed by using products of biotechnology like- Monoclonal antibodies and DNA or RNA probes i.e-

i: Monoclonal antibodies:

which are specific to only one type of antigen is called – Monoclonal antibodies.

Explanation :- The response of immune-system to any – antigen is called polyclonal. However, there are group of identical antibodies which is against only single type of – antigen. They are called monoclonal antibodies. They are a group of identical antibodies i.e– made by identical immune cells. This has become an important tool in Medicine.

Role of monoclonal antibodies :- Monoclonal antibodies – play important role in our daily life. e.g–

1: Monoclonal antibodies protect us against diseases.

2: They help to diagnose a wide variety of illness.

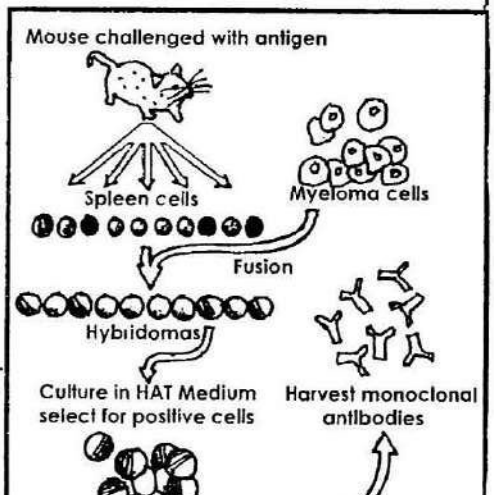
3: They can also detect the presence of drugs, viral products, bacterial products and others abnormal substance in the blood

production :-<Biosynthesis>

1: Antigen is first injected to living healthy mice. mice will be produce antibodies against that antigen.

2: then spleen cells of mice are analyzed to obtain such anti-bodies

3: Now these cells are mixed with myeloma or cancerous



the purpose of rapid division. ←(ETEA-2017)

4: They after mixing are called hybridoma cells which are - cultured and monoclonal antibodies are produced.

ii: DNA or RNA probes:

Definition :- A fluorescent and radioactive labeled fragment of DNA or RNA which is used for DNA and RNA sequence - detection and always complementary to it is called probe.

Explanation :- probe may be 100—1000 base-pairs long - fragment of DNA or RNA. probe is used to detect the DNA - sample of complementary bases. It means a specific known sequence of nucleotide (probe) is labeled with fluorescent - dyes or radioactively labeled and then it is allowed to hybridize its complementary sequence on DNA or RNA strand in a medium. Therefore, they are sucessfully used in diagnosis of viral and bacteria infections like- HBV, HCV, HIV, etc.

Diagnosis of diseases caused by protozoa and Helminthes:

monoclonal antibodies and DNA-probes are used in detection of Helminthes parasites and protozoans.

Explanation :- These monoclonal antibodies are used in blood test and it take few minutes. where as DNA-probes take hours because it is more sensitive than monoclonal antibodies. For this purpose ready made probes of herpes-virus and

Q19 Describe the gene therapy with example.

Answer: Gene therapy:

Definition :- The removal of defective gene and incorporation of effective normal gene for the treatment of a disease is called gene therapy.

Uses of gene therapy :- uses of gene therapy are as under.

1: Gene therapy is used against diseases like-cancer, Hemophilia.

2: Gene therapy is used to stimulate immune system for attacking cancer cells.

3: Gene therapy is used to produce resistance to HIV.

4: It is used in the replacement of gene that cause medically ill → health.

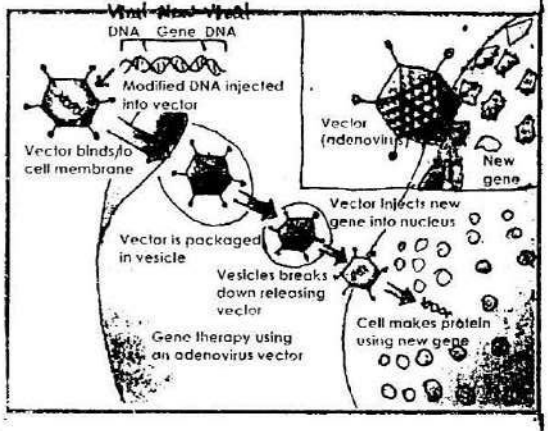
Approaches to gene therapy :- Researchers use one of - several approaches for correcting faultly genes i.e-

1: A normal gene is directly inserted within genome to - replace non-functional gene.

2: Abnormal gene can be change for a normal gene by Homo-logous recombination.

3: Abnormal gene can be repaired by reverse - mutation to its normal position.

4: particular genes are regulated like gene OFF or gene ON.



Mechanism of Gene therapy:

1: Ex-vivo :- In this type of gene therapy the genes are inserted into the cell genome outside the body is called - ex-vivo gene therapy.

2: In-vivo :- In this type of gene therapy the genes are - inserted into the cell genome inside the body called invivo therapy.

Proceduse for mechanism of gene therapy :- It is differentie-

1: Viral mediated gene delivery system :- In both above types we use a vector to deliver the therapeutic gene to patient's target cell. Target cells may be usually liver or lung-cells are infected with the viral vectors. The vector then unloads its genome containing normal genes to target cell in pathogenic manner. the generation of functional protein product from - normal gene brings the cells to normal state.

2: Non-viral gene delivery system :- Beside viral gene delivery system there are non-viral gene delivery system also where virus is not used as vector i.e -

1- The most simple method is direct introduction of therapuetic gene into target cell by microinje-tion or gene Gun. It has less common method because it can be used with certain tissues and needs large amount of DNA.

2- another non-viral approach is the creation of artificial lipid-sphere with in aqueous core called liposome. This - liposome carries normal gene to the target cell.

Example of gene therapy : cystic Fibrosis :

Introduction :- Cystic Fibrosis is an inherited disease in which mucus and sweat glands are affected. normally mucous is watery which keep the lining of organs moist and prevent them from drying out or prevent from infection. But in cystic fibrosis thick mucus is produced.

Causes of cystic Fibrosis :- causes of cystic fibrosis is the cystic fibrosis transmembrane conductance regulator gene. such normal gene encodes a protein by which the movement of salts and water is controlled in and out of cell-membrane. While in people with cystic fibrosis the such gene does not work properly and thus thick mucous produce in lining which block air ways and glands.

Symptoms of cystic Fibrosis :- Some common symptoms are-

1: Mucus membrane and sweat glands are affected.

2: lungs and digestive problems.

3: Frequently infection in mucus-membrane, etc.

Gene therapy of cystic fibrosis :- (Treatment) This disease was identified successfully by gene therapy in 1989. The discovery of this defective gene posed new possibilities of a cure and now a days the gene can be successfully replaced by normal gene in target cells such as lungs and this disease is cured in this way.

Q20 Discuss the importance of Biotechnology.

Answer: Importance of Biotechnology:

Introduction:- Biotechnology is a vast field posses many application and importance from domestic to industrial levels. During 1970 this field was emerged as a new field due to marriage of biological sciences with technology. Although it is not a pure science however it is the science of applied biological process.

Importance of Biotechnology:- Some of the important contributions of biotechnology in human welfare are given below.

1: Biochips and biological computers:- Biochip is the result of marriage of microchips business with biotechnology. In furture there is possibility of developing of biological computers. The development of biochip is a major discovery in biotechnology and many other fields like - genomics, protemics, computational biology and pharmaceuticals, etc. Biochips are able to carry out hundreds and thousands of biological reactions simultaneously. at the same time semiconductor industry has been steadly perfectly the science of micro-miniaturization. Besides this they are important in direction of biological terrorist.

2: Mycorrhiza:- This is symbiotic association between fungi and roots of higher plants like - gymnosperms. This association is very beneficial for the growth of plants. In most of the -

inoculum of mycorrhizal fungi. Now a days use of biotech-nologically produced inoculum of mycorrhizal fungi has - inhance it significance due to its multifarious role in plant growth and yield and resistance against climatic and - adaphic (soil) stress, pathogens and pests of plants.

3: Bioferstilizers :- Bioferstilizers are nutrients prepared by biological process for the growth of plants. so those - ferstilizers which contain biologically fixed nitrogen are called biofertilizer. Bioferstilizers are used to replaced the hazardous chemical fertilizers. Now a days microbial inoculants are develop and use in many counteries. Bioferstilizers are more effective than chemical ferstilizers because they supply - adequate amount of nitrogen and other nutrients to plants. Moreover, biological fixed nitrogen uses 25–30% less energy than normally done by chemical process.

4: Nanotechnology :- This is the sub-branch of biotechnology which deals with all those techniques in which matter or any - biological substance is manipulated on atomic molecular or - smaller than this level. In nanotechnology very small tiniest object at molecular level can be easily engineered. This include the manipulation of material between 1–100 nm. It also helps us to discover such nano-particles which are efficiently used to deliver drug to target cells and to diagnose diseases or-

Q21 Discuss the scope of Biotechnology:

Answer:- Scope of Biotechnology:

Introduction:- Biotechnology is one of the fastest growing field in the area of research and development. It is also called a technology of the future or technology of tomorrow. Biotechnology has many good effects on the humans and the whole universe. It has many relations with other fields also. Therefore it gives many opportunities in life.

Scope in different fields:- Students of biotechnology after completing their studies can have scope in the following fields.

1: Communications or media:- Reporting, Writing, Editing.

2: Computer science:- Database development, bioinformatics, website-developments, etc.

3: pharmaceutical companies:- Drug development.

4: Engineering:- working in bioprocess chambers, instrumentation development, Fermentation technology.

5: Research:- Example- cancer, genetically linked diseases, AIDS.

6: Diagnotic Laboratories:- Funded by public and private sectors.

7: Waste Mangement:- Bio-monitoring bodies and pollution control boards....

8: Medicine:- The medical-genetics, genetic conseling, gene-therapy and gene tasting uses biotechnological tools.

9: Bio-power plants:

10: Bio-processing industry:- e.g- enzyme technology, paper-

11: Agriculture and animal husbandry:

12: Legal field :- property, low experts, paternity and genetic tests.

13: Millitary :- pathogens identification, biological terrorism, warfare.

14: Crime and law :- Forensic sciences, crimes identification, Fingerprinting, DNA-tests of crimes, etc.

Q22 what is hazard and social or Ethical implication of using Biotechnology.

Answer: Hazard and social implication of Biotechnology:-

Biotechnology has gained shining status in modern world. It is popular for safe-purposes like-gene therapy, health care, agriculture and food production. But it has also raised - ethical, legal, social implications (ELSi). For example-

1: who should have the right to examine genes of others.?

2: How should the information obtained from the genes be used.?

Thus these ELSi will slow down the applications of biotech--nology and its benefits. However, the power of biotechnology, genetic engineering and our abilities are such that we must proceed with care and patience, irrespective of these problems.

Q23 Explain the different concerns about genetically - modified organisms. (GMO)

Answer: concerns about genetically modified organism: (GMO)

due to the cause that regarding the biosafety, ethics and issuses related to environment.

Biosafety:- United Nations has built up an informal working group on biosafety in 1991. This group prepared the voluntary code of conduct for the release of organisms to environment, due to the following considerations.

1: How to dispose of spent microbal biomass and purify the effluents from biotechnological processes. ?

2: The toxicity of the allergy associated microbial production.

3: How to deal with increasing number of antibiotic resistant pathogenic organisms ?

4: How to evalute the pathogenicity of GMOs to infect human animals and plants

5: How to prevent contamination, infection or mutation caused by processed strains.

6: The evaluation of interaction of GM-microbes with the elements of natural environment.

Q24 what is Biological Warfare.

Answer: Biological Warfare:

Definition:- Introduction of pathogenic microbes as weapons in war is called as biological warfare.

Explanation:- peoples have expressed their concerns about the possible use of genetic manipulations, Bacteria, viruses, etc for warfare purpose and military purposes even. that is

the biological weapons convention of 1972. what to never produce agents or toxin of microbes related, whatever may be their method of production, for use in wars.

Q25 what is intellectual property.

Answer: Intellectual property:

Introduction :- Intellectual property are like any other - property right which include the copyrights, trade marks, trade secrets and patents. for this purpose international property right (IPR) have been came into existance which have different types of rights granted by each country. The rights to protect this property prohibit others from making, coping, using or selling the proprietary subject matter.

For example :- Some example for intellectual property are-

1: Intellectual property of biotechnology :- In biotechnology the intellectual property covers the process of recombinant DNA-technology to produce various products.

2: Intellectual property of development of crops :- another - intellectual property is the development of crops-varieties which are protected through plant breeder's rights (PBR). The PBRs ensures that plant breeder who developed a particular variety gets the exclusive rights for maketing the variety.

End :-

Exercise

Exercise MCQs

A. *Multiple Choice Questions (MCQs).*

1. Bacteria protect themselves from viruses by fragmenting viral DNA with

a) Ligase
b) Endonuclease
c) Exonuclease
d) Gyrase

2. Transfer of DNA fragments from electrophoretic gel to a nitrocellulose sheet is done for:

a) Autoradiography
b) Primer annealing
c) Denaturation
d) Generating markers

3. The DNA fragments have sticky ends due to

a) Endonuclease
b) Unpaired bases
c) Calcium ions
d) Free methylation

4. Plasmids are used as cloning vectors for which of the following reasons?

a) Can be multiplied in culture
b) Self-replication in bacterial cells
c) Can be multiplied in laboratories with the help of enzymes
d) Replicate freely outside bacterial cells

5. Who invented the PCR technique?

a) Karry Mullis
b) Boyer
c) Sanger
d) Cohn

6. In gel electrophoresis of DNA, the different bands in the final gel form because the DNA molecules _____.

a) are from different organisms
b) have different lengths
c) have different nucleotide compositions
d) have different genes

7. In the reproductive cloning of an animal, the genome of the cloned individual comes from _____.

a) a sperm cell
b) an egg cell
c) any gamete cell
d) a body cell

8. What carries a gene from one organism into a bacteria cell?

a) plasmid
b) an electrophoresis gel
c) a restriction enzyme
d) polymerase chain reaction

9. The type of gel most commonly used for short fragment DNA electrophoresis is:

(a) agarose (b) DNA polymerase
(c) polyacrylamide (d) DNA ligase

10. Cell suspension culture of Cinchona ledgeriana produce:

(a) Quinine (b) Digitoxin
(c) Polludrin (d) Anti toxin

11. Dideoxy ribonucleoside triphosphates are used to terminate DNA synthesis at different site. Which method involves this procedure?

(a) Maxam-Gilbert's method (b) Sangar's method
(c) K.B. Mullis's Method (d) Gottlieb's method

12. The gene of choice can also be synthesized in the laboratory from mRNA, using reverse transcriptase. This DNA molecule is called:

(a) Complementary DNA (b) Replicative DNA
(c) Synthetic DNA (d) ssDNA

13. The gene of interest is joined with the sticky ends produced after cutting the plasmid with the help of another special enzyme known as:

(a) DNA ligase (b) DNA polymerase
(c) Restriction endonuclease (d) Reverse transcriptase

14. The enzyme DNA polymerase can work only in

(a) 3′→5′ direction (b) 5′→3′ direction
(c) Both the direction (d) 5′→5′ direction

15. In recombinant DNA technology a plasmid vector is cleaved by

(a) Modified DNA ligase
(b) A heated alkaline solution
(c) The same enzyme that cleave the donor DNA
(d) The different enzyme other than that cleave the donor DNA

16. The complete set of chromosomal and extrachromosomal genes of an organism is called:

(a) Genome (b) Gene pool (c) Gene bank (d) Gene library

Answer: (key)

1-b	2-a	3-b	4-b	5-a	6-b
7-d	8-a	9-c	10-a	11-b	12-a

Short Questions

Q1:- why don't the restriction enzymes destory the DNA of the organism in which they are produced.

Answer:- Restriction Enz vs host:- A bacterium is immune to its own restriction enzymes, even if it has the target sequences ordinarily targeted by them the host cell protects its own DNA from being cleaved by employing other enzymes called methylases, which methylate adenine or cytosine bases within host recognition sequences. For each of the restriction enzyme, the host cell produces a corresponding methylase that methylates and protects the host DNA from degrdation. These enzymes make up the restriction-modification systems. This is because the bacterial restriction sites are highly methylated, making them unrecognizable to the restriction enzyme.

Q2:- what are the essential features of a vector.

Answer:- see Question No-2. (write only characters of vector)

Q3:- what are monoclonal antibodies.

Answer:- see Question No-18. (write only monoclonal ant;)

Q4:- Name two conditions necessary for maintaining

Q5:- Give any two human proteins and their function which are produced biotechnologically.

Answer:- see Q-15. (write only- Role in biotechnology products)

Q6:- What are probes.

Answer:- see Q-18. (write only- DNA or RNA probes).

Q7:- what are the main aims and objective of human genome project.

Answer:- see Q-12. (write only- Human genome project).

Q8:- Differentiate b/w maxam-Gilbert method and sanger's method of gene sequencing.

Answer: Maxam-Gilbert Vs Sanger method:

1: The main difference between maxam-Gilbert and sanger sequencing is that the maxam-Gilbert sequencing is the chemical method of DNA sequencing based on the - nucleobase-specific partial chemical modification of DNA and subsequent cleavage of the DNA backbone at sites adjacent to the modified nucleotides But, on the other hand, the sanger sequencing is the chain - termination method, which interrupts elongation of DNA Sequencing by incorporating ddNTps into the sequencing.

2: Furthermore, maxam-Gilbert sequencing uses a large amount of hazardous chemical, including radioactive material and hydrazine. However, Sanger sequencing

Q9:- What is cDNA Library.

Answer:- see Question No-5.

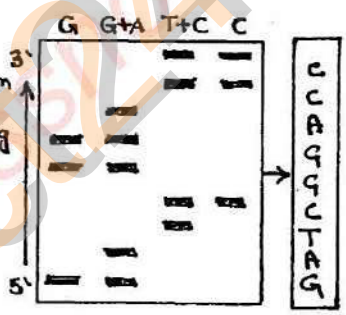
Q10:- What are the types of vector or carrier.

Answer:- see Q-2. (write only-example of vectors).

Q11:- What are the application of PCR.

Answer:- see Q-4. (write only application of PCR).

Q12:- Read carefully the diagram of gel pattern obtained through maxam-Gilbert method of gene sequencing and predicts the sequence of target DNA.



Answer:- GATCGGACC

Long Questions

Q1:- What are molecular scissors? Describe their sources and mode of action.

Answer: see question NO-2 (write only molecular scissors)

Q2:- Evaluate the process of gene sequencing with the help of Sanger's Method.

Answer: see question NO-6.

Q3:- Explain the process of gene cloning with the help of recombinant DNA technology.

Answer: see question NO-2.

Q4:- Explain the process of gene cloning with the help of PCR.

Answer: see question NO-4.

Q5:- Describe the procedure of DNA analysis.

Previous Entry Test MCQ's of Biology Chapter No -26: (Biotechnology)

2009 ETEA

1. The primers used in polymerase chain reaction has a sequence of base:
(a) 20 (b) 16 (c) 12 (d) 8

2010 ETEA

2. Tissue plasmogenic activator (TPA) is used for
(a) Treating anemia
(b) Bone marrow transplant
(c) Dissolving blood clot
(d) Treatment of cancer

3. A cloned baby Dolly was identical to the parent that.
a) Gave birth to the dolly
b) Donated reproductive cells
c) Donated somatic cell
d) Both a & b

2011 ETEA

4. Organism that contain genes from other organisms are called:
(a) Mutagenic (b) Transgenic
(c) Clones (d) Sequencing

5. A cloned baby sheep "dolly" was attributed to:
(a) Four parents (b) Three parents
(c) Two parents (d) One parent

2012 ETEA

6. Restriction enzymes are of great use in genetic engineering because:
(a) They cut DNA at a specific base level
(b) They cut DNA at serval specific levels
(c) They help in binding the pieces of DNA
(d) They are nuclease

2013 ETEA

genetic engineering is called:
(a) Transgenic (b) Probe
(c) Recombinant (d) Plasmid

8. The primer used in polymerase chain reaction has a sequence of bases:
(a) 8 (b) 12 (c) 16 (d) 20

2014 ETEA

9. Any DNA molecule having foreign DNA is Called-----
a) Mutant b) Recombinant
c) Crossing over d) All of them

2016 ETEA

10. Individuality of every person is maintained by nucleotide genome sequence difference of...
a) 1% b) 2% c) 3% d) 5%

11. Which one of the following comes into existence when bacterial plasmid naturally modified to produce it?
a) PBR 322 b) NPQ 303
c) OSR 210 d) KMG 319

12. The 1st field trial of genetically engineered plants occurred in France and USA in.
a) 1980 b) 1982 c) 1984 d) 1986

13. The tissue culture method occurs in the following sequence.
a) Sterilization → media preparation → inoculation → callus development → plantlets
b) Media preparation → sterilization → Inoculation → callus development → plantlets
c) Media preparation → inoculation → sterilization → callus development → plantlets
d) Inoculation → sterilization → media preparation → callus development → plantlets

14. For which purpose myeloma cell (Cancerous B-lymphocytes) are used in the production of monoclonal antibodies?
(a) Increased rate of cell division
(b) Immunization with antigen
(c) To avoid contamination
(d) As nutrient in media

15. Which of the following is a suitable vector to be incorporated with a large external DNA fragment?
(a) Small size vector
(b) Large size vector
(c) Large size vector with no origin of replication
(d) Small size vector with no origin of replication

16. For the location\detection of a gene in a DNA library which of the following is used?
(a) Primer (b) Probe
(c) Restriction enzyme
(d) Taq polymerase

17. Under UV illumination, DNA bands are seen in agarose due to which of the following?
(a) Agarose (b) Charge of DNA
(c) Fluorescent dye (d) Radioactive dye

18. If one of the following components is missing bacteria can not increase the number of its plasmid copies?
(a) Antibiotic resistant gene
(b) Origin of replication
(c) Cloning site (d) Ligases enzymes

19. What will happen if a vector (plasmid) is cut with a different restriction enzyme which cuts the external DNA to be incorporated in the vector (plasmid)?
(a) Ligation (b) No ligation
(c) Tight ligation (d) Cloning

using reverse transcriptase?
(a) Protein → DNA (b) RNA → DNA
(c) DNA → RNA (d) RNA → Protein

21. If the primer annealing temperature is increased to 94° C. what will happened.
a) Annealing b) Extension
c) No annealing
d) Primer dimer formation

2018 ETEA

22. All of the following acts as cloning vector except:
(a) BAC (b) YAC
(c) Cosmids (d) EcoRI

2019 ETEA

23. Recombinants contains DNA from:
(a) 2 different sources
(b) Single source (c) 2 same sources
(d) 3 same sources

24. DNA finger printing refer to
(a) Techniques used for identification of finger prints of individuals
(b) Molecular analysis of profiles of DNA samples
(c) Analysis of DNA samples using imprinting devices
(d) Both a and c

2020 ETEA

25. Which of the following is not necessary for PCR to occur?
a. dATP b. primers
c. DNA fragments d. Ribonucleotides

Key: -

1.a	2.c	3.c	4.b	5.d	6.a
7.c	8.d	9.b	10.a	11.c	12.d
13.a	14.b	15.a	16.b	17.c	18.b
19.a	20.b	21.c	22.d	23.a	24.b