CHAPTER 11-BIOTECHNOLOGY-PRINCIPLES & PROCESSES

Biotechnology is a broad area of science involving multiple disciplines designed to use living organisms or their products to perform valuable industrial or manufacturing processes or applications pertaining to human benefit.

Recombinant DNA technology:

An organism's genome contains virtually all the information necessary for its growth and development .rDNA technology creates a recombined DNA with genome of two different cells or organisms.

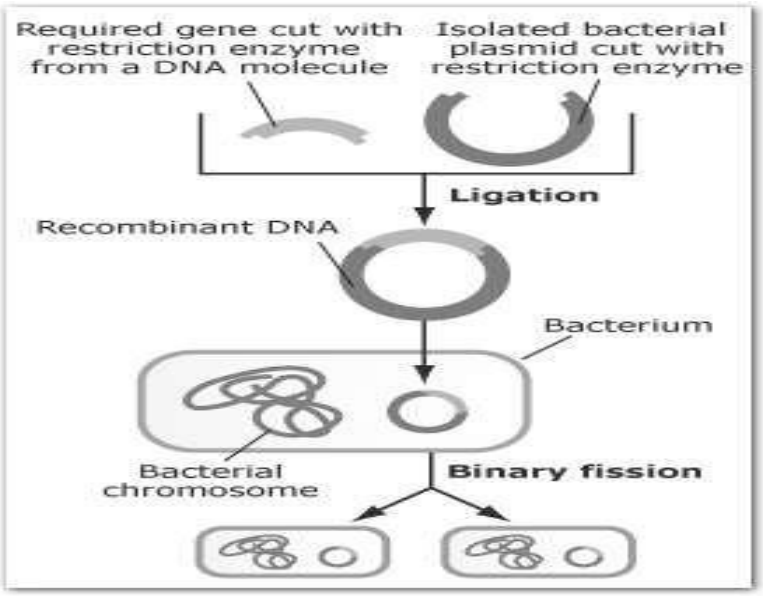
Steps in producing recombinant DNA

1. The required gene is cut from a DNA molecule using a restriction enzyme.

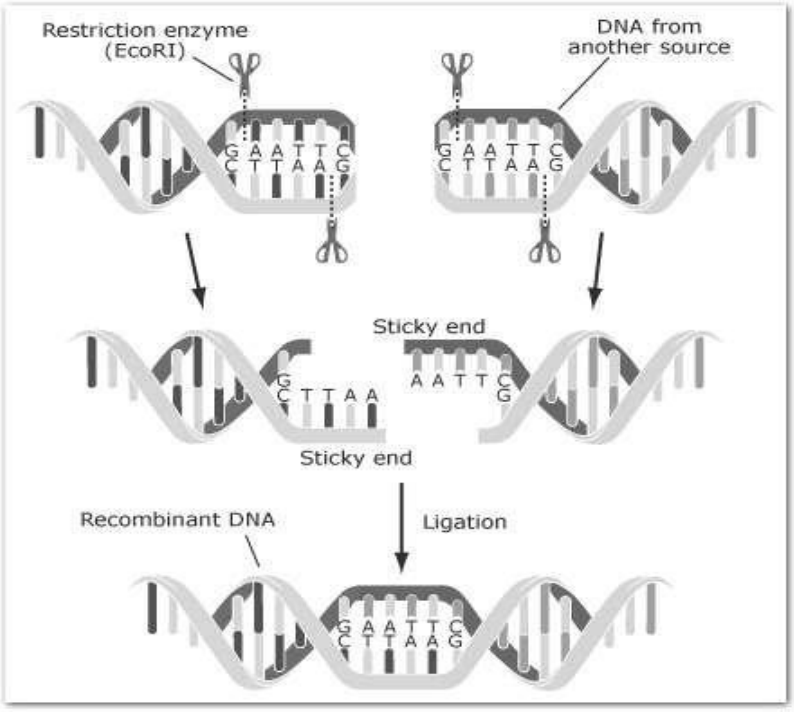
2. A bacterial plasmid is isolated and cut with the same restriction enzyme. This ensures cut ends are complementary (same base sequence) to the ends of the required gene.
3. The required gene is joined to the plasmid using the enzyme DNA ligase in a process called ligation.

4. The resulting recombinant plasmid is returned to the bacterial cell.

5. The bacteria reproduce and the required gene is cloned..



CLONING OF GENE WITH r DNA TECHNOLOGY



How do we obtain DNA and how do we manipulate DNA?

To isolate genomic DNA

1. Remove tissue from organism
2. Homogenize in lysis buffer (denatures proteins)
3. Mix with phenol/chloroform - removes proteins
4. Keep aqueous phase (contains DNA)
5. Add alcohol (ethanol or isopropanol) to precipitate DNA from solution
6. Collect DNA pellet by centrifugation
7. Dry DNA pellet and resuspend in buffer
8. Store at 4°C

Each cell (with a few exceptions) carries a copy of the DNA sequences which make up the organism's genome.

Recombinant DNA technology

Steps:

-Isolation of DNA

- Digestion using Restriction enzymes
- urification of the TARGET fragment
- Ligation with cloning vector.
- Transformation of host cell and selection of transformed cells
- Culturing for the desired product.

Introduction of recombinant DNA into host cells:

Some commonly used procedures:

1. Transformation
2. Transfection
3. Electroporation- incubation with calcium ions in alternate icy and high temperature.
4. Biolistics(gene gun)
5. *Agrobacterium* mediated gene transfer

Until 1970 there were no convenient methods available for cutting DNA into discrete, manageable fragments.

1970 - **The Beginning of the Revolution**

Discovery of a **restriction enzyme** in the bacterium *Haemophilus influenza-Hind III*

Restriction enzymes

. Restriction enzymes are endonucleases

- Bacterial enzymes .
- Different bacterial strains express different restriction enzymes.
- The names of restriction enzymes are derived from the name of the bacterial strain they are isolated from.
- Cut (hydrolyse) DNA into defined and **REPRODUCIBLE** fragments.

Basic tools of gene cloning .

- Restriction endonucleases are a natural part of the bacterial

defence system

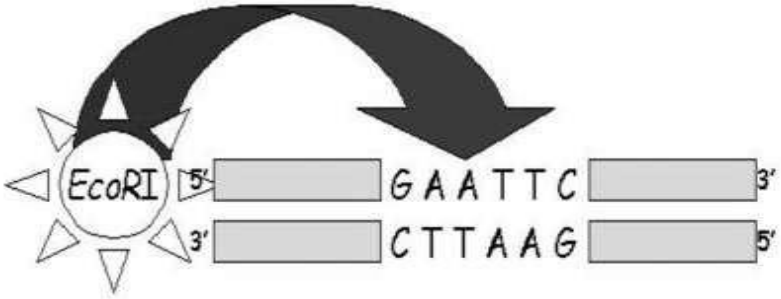
- Part of the restriction/modification system found in many bacteria
- These enzymes **RESTRICT** the ability of foreign DNA (such as bacteriophage DNA) to infect/invade the host bacterial cell by cutting it up (degrading it)
- The host DNA is **MODIFIED** by **METHYLATION** of the sequences these enzymes recognise
    - Methyl groups are added to C or A nucleotides in order to protect the bacterial host DNA from degradation by its own enzymes

Names of restriction endonucleases

Titles of restriction enzymes are derived from the first letter of the genus + the first two letters of the species of organism from which they were isolated.

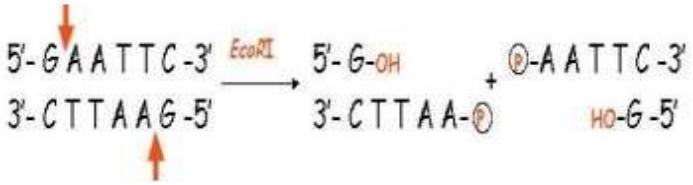
Source microorganism	Enzyme	Recognition Site	Ends produced
Arthrobacter luteus	*Alu* I	AG CT	Blunt
Bacillus amyloiquefaciens H	*Bam* HI	G GATCC	Sticky
Escherichia coli	*Eco* RI	G AATTC	Sticky
Haemophilus gallinarum	*Hga* I	GACGC(N)$_5$	Sticky
Haemophilus infulenzae	*Hind* III	A AGCTT	Sticky
Providencia stuartii 164	*Pst* I	CTGCA G	Sticky
Nocardia otitiscaviaruns	*Not* I	GC GGCCGC	Sticky
Staphylococcus aureus 3A	*Sau* 3A	GATC	Sticky
Serratia marcesans	*Sma* I	CCC GGG	Blunt
Thermus aquaticus	*Taq* I	T CGA	Sticky

Restriction enzymes recognize a specific short nucleotide sequence(PALINDROME)



This is known as a Recognition Site

The phosphodiester bond is cleaved between specific bases, one on each DNA strand



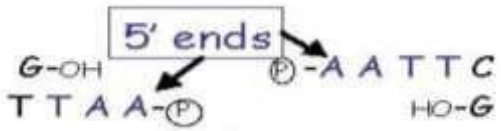
The product of each reaction is two double stranded DNA fragments

Restriction enzymes do not discriminate between DNA from different organisms

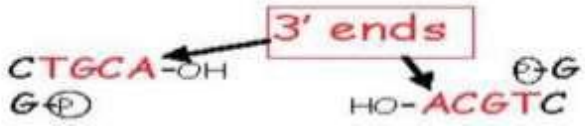
Type II - Recognise a specific target sequence in DNA, and then break the DNA (both strands), within or close to, the recognition site.Only these are used in rDNA technology as they recognize and cut DNA within a specific sequence typically consisting of 4-8 bp.e.g. *Eco*RI

Different enzymes cut at different positions and can create single stranded ends ('sticky ends')

- Some generate 5' overhangs - eg: *Eco*RI



- Some generate 3' overhangs - eg: *Pst*I



Some generate blunt ends- eg: *Sma*I

Examples of restriction enzymes and the sequences they cleave

The 'sticky' overhangs are known as COHESIVE ENDS

- The single stranded termini (or ends) can base pair (**ANNEAL**) with any complementary single stranded termini

This is the basis for **RECOMBINANT DNA TECHNOLOGY**

- Inserting foreign DNA into a cloning vector

DNA fractionation

Separation of DNA fragments in order to isolate and analyse DNA cut by restriction enzymes

Electrophoresis

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. When charged molecules are placed in an electric field, they migrate toward either the positive or negative pole according to their charge.

DNA is electrophoresed through the agarose gel from the **cathode** (negative) to the **anode**(positive) when a voltage is applied, due to the net negative charge carried on DNA.

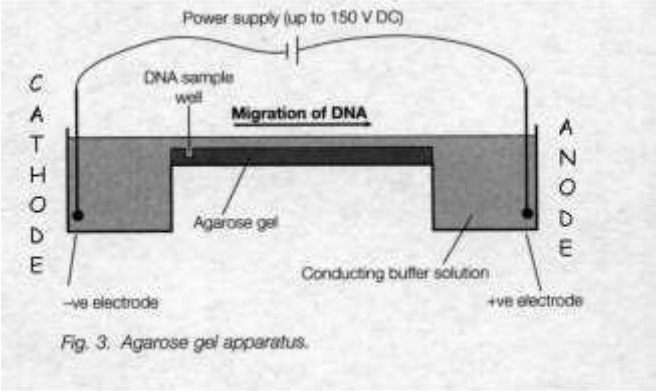
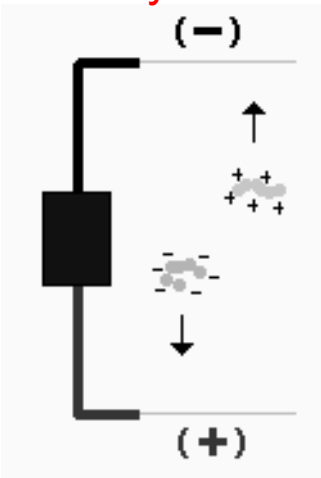


Fig. 3. Agarose gel apparatus.



When the DNA has been electrophoresed, the gel is stained in a solution containing the chemical **ethidium bromide**. This compound binds tightly to DNA and fluoresces strongly under UV light - allowing the visualisation and detection of the DNA.

Recombinant DNA: Plasmids, cloning What is DNA cloning?

DNA cloning is the isolation of a fragment or fragments of DNA from an organism and placing in a VECTOR that replicates independently of chromosomal DNA. The RECOMBINANT DNA is propagated
in a host organism; the resulting CLONES are a set of genetically identical organisms which contain the recombinant DNA

Three main purposes for cloning DNA

1) DNA sequencing
2) Protein
   production
3) Engineering
   animals/plants/proteins

Cloning and Expression Vectors

Isolated DNA is cloned into **VECTORS** for long term storage, propagation of the DNA and for production of protein from gene(s) encoded in the DNA

What are cloning vectors?

Cloning vectors are extra-chromosomal 'replicons' of DNA which can be isolated and can replicate independently of the chromosome. Vectors usually contain a **selectable marker** - a gene that allows selection of cells carrying the vector e.g. by conferring resistance to a toxin. DNA of interest can be
cloned into the vector and replicated in host cells, usually one which has been well characterised.

Commonly used vector systems

- Bacterial plasmids
- Bacteriophages
- Cosmids

- Yeast artificial chromosomes (YACs)
- Ti plasmid (plants)
- Eukaryotic viruses such as baculovirus (insect cells), SV40 virus and retroviruses.

Characteristics of a Cloning Vector

❖ **Origin of replication (ORI)**

This process marks autonomous replication in vector. Ori is a specific sequence of nucleotide in DNA from where replication starts. When foreign DNA is linked to this sequence then along with vector replication, foreign (desirable) DNA also starts replicating within host cell.

❖ **Selectable Marker**

Charecteristics of Selectable marker:

A gene whose expression allows one to identify cells that have been transforrned or transfected with a vector containing the marker gene.

A **marker gene** is used to determine if a piece of DNA has been successfully inserted into the host organism. Gene usually encoding resistance to an antibiotic. A **selectable marker** will protect the organism from a **selective agent** that would normally kill it or prevent its growth.

❖ **Restriction sites**

Allow cleavage of specific sequence by specific Restriction Endonuclease. Restriction sites in E.coli cloning vector pBR322 include HindIII , EcoRI , BamHI , SalI, PvuI, PstI, ClaI etc.

Refer NCERT text book diagram of pBR322

A Cloning Vector that Works with Plant Cells

Most commonly used plant cloning vector ***"Ti" plasmid,*** or tumor-inducing plasmid. Found in cells of the bacterium known as ***Agrobacterium tumefaciens***, normally lives in soil. Bacterium has ability to infect plants and cause a **crown gall**, or tumorous lump, to form at the site of infection.

Ti plasmid - called T DNA - separates from the plasmid and incorporates into the host cell genome. This aspect of Ti plasmid function has made it useful as a ***plant cloning vector (natural genetic engineer).*** **Plasmids** are the most commonly used vector system. Several types available for cloning of foreign DNA in the host organism *Escherichia coli*. Many *E. col*i plasmids allow the expression of proteins encoded by the cloned DNA

Bacteriophage another common vector system used for cloning DNA. These are viruses which 'infect'

E. coli. The M13 bacteriophage is a single-stranded DNA virus which replicates in *E. coli* in a double- stranded form that can be manipulated like a plasmid. It can be used to produce single-stranded DNA copies which are useful for **DNA sequencing**.

Transformation is the process by which plasmids (or other DNA) can be introduced into a cell. For *E. coli* transformation with plasmids is quite straightforward. Plasmids can be introduced by electroporation or by incubation in the presence of divalent cations (usually Ca^{2+}) and a brief heat shock (42°C) which induces the *E. coli* cells to take up the foreign DNA

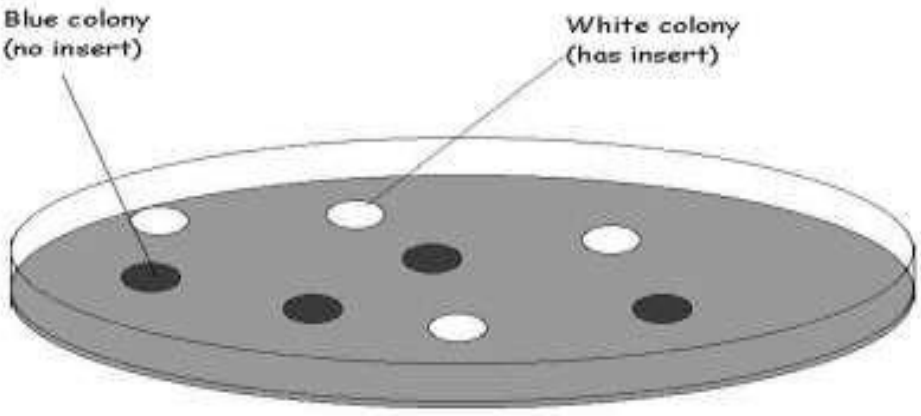
Insertional inactivation -

Subcloning a DNA fragment into an active gene (usually a marker gene whose function can be easily detected) will disrupt the function of that gene. This can be detected by looking for colonies that no longer display that phenotype.

Colour selection

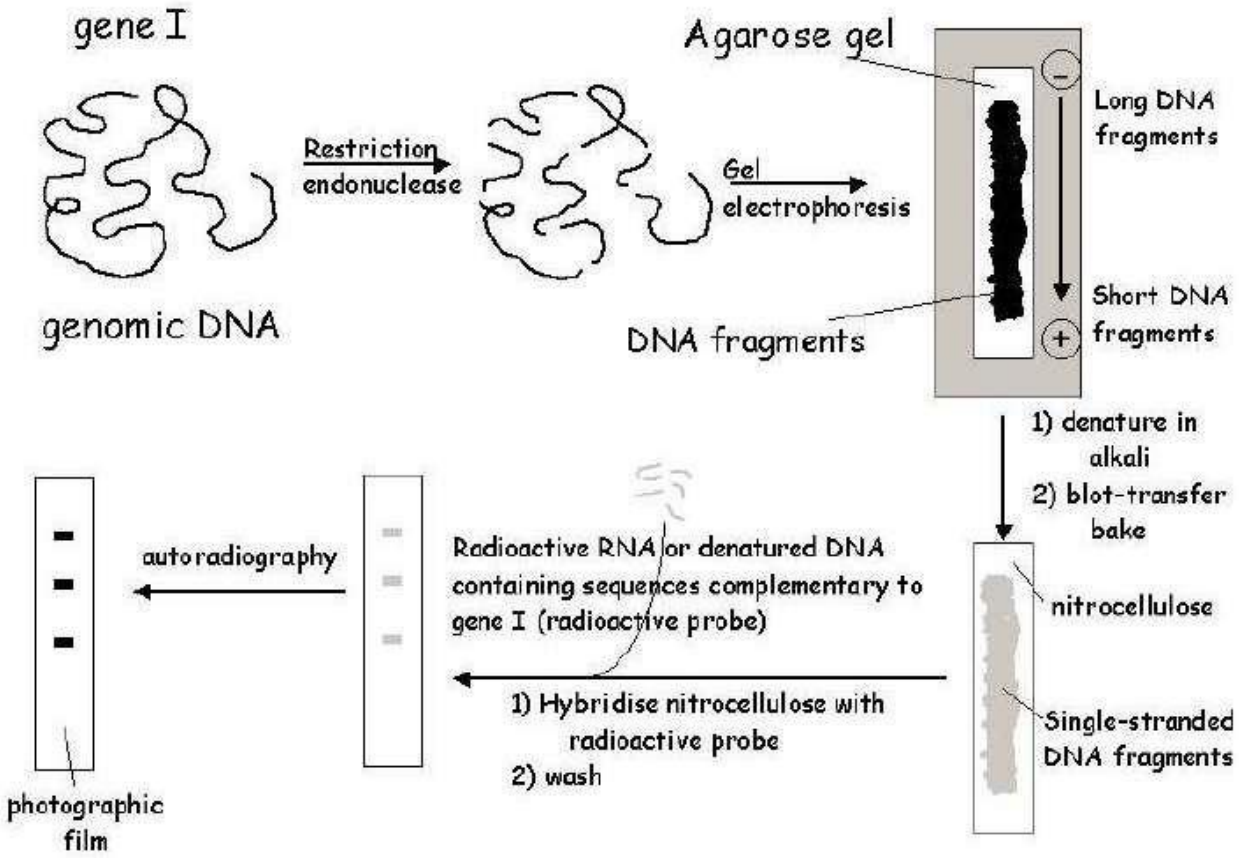
A more common method to determine which transformants contain plasmids with inserts is to use
colour selection. For *E. coli*, this involves the **lac complex** and **blue/white screening.**

Colonies carrying plasmid with no insert will be coloured **blue** whereas colonies carrying recombinant plasmid will be **white**.



For plasmids such as pBR322, which contains two antibiotic resistance genes, cloning an insert into one of these will disrupt that gene and inactivate the resistance to that antibiotic.

Electrohoresis :For separating fragments of DNA



PCR(Polymerase Chain Reaction):

PCR is a technique for the i***n vitro*** **amplification** of a desired sequence of DNA. Development of PCR won the Nobel prize for Kary Mullis and co-workers.

PCR PRINCIPLE

PCR reaction is a DNA synthesis reaction that depends on the **extension** of primers **annealed** to opposite strands of a dsDNA template that has been denatured (**melted apart**) at temperatures near boiling. By repeating the **melting**, **annealing** and **extension** steps, several copies of the original template DNA can be generated.

The amount of starting material (target) needed is very small

The **primers** are oligonucleotides **complementary** to different regions on the 2 strands of DNA template (**flanking** the region to be amplified).
he **primer** acts as a starting point for DNA synthesis. The oligo is **extended** from its 3' end by **DNA polymerase**.

PCR is a cycle of three steps:

1. **DENATURATION** - the strands of the DNA are **melted apart** by heating to 95°C
2. **ANNEALING** - the temperature is reduced to ~ 55°C to allow the primers to **anneal** to the target DNA
3. **POLYMERISATION / EXTENSION** - the temperature is changed to the optimum temperature for the DNA polymerase to catalyse **extension** of the primers, *i.e.* to copy the DNA between the primers.

The **cycle** is repeated over and over again - as many times as needed to produce a detectable amount of product.

Discovery of a thermostable DNA polymerase

The breakthrough came with the discovery of the thermostable DNA polymerase *Taq* polymerase, from the thermophilic bacterium, *Thermus aquaticus*, which lives in hot springs.

***Taq* polymerase** enzyme can resist **high temperatures** required to melt the template DNA apart without denaturation (loss of activity) and works best at **high temperatures (72°C).** This led to improved specificity & sensitivity. Annealing of primers to sites other than the target sequence is significantly reduced at the higher temperatures used for *Taq* polymerase.

Applications of PCR

1) Cloning a gene encoding a known protein
2) Amplifying 'old DNA'
3) Amplifying cloned DNA from vectors
4) Creating mutations in cloned genes

5) Rapid amplification of cDNA ends - RACE
6) Detecting bacterial or viral infection
* AIDs infection
* Tuberculosis (*Mycobacterium tuberculosis*)

7) Genetic diagnosis
a. Diagnosing inherited disorders
* Cystic fibrosis

* Muscular dystrophy
* Haemophilia A and B
* Sickle cell anemia

b. Diagnosing cancer - certain cancers are caused by specific and reproducible mutations: eg. Retinoblastoma - childhood cancer of the eye. The heritable form (germ line mutation of one of the two retinoblastoma allelles): mutation is detected in all cells. Spontaneous form: only detected in tumour tissue.

c. Blood group typing

PCR is one of the most versatile techniques invented, and has so many applications that this list could go on for quite some time.

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Downstream processing

It refers to the separation and purification of biosynthetic products, particularly pharmaceuticals, from natural sources such as animal or plant tissue or fermentation broth

Stages in Downstream Processing

Downstream processing operations are applied in order to bring a product from its natural state as a component of a tissue, cell or fermentation broth through progressive improvements in purity and concentration.

GLOSSARY:

Adult stem cells

The stem cells found in a developed organism and have the twin properties of self-renewal and differentiation. These can be obtained from fetal cord blood and bone marrow. They are multipotent in nature.

Amplification

An increase in the number of copies of a specific DNA fragment; can be in vivo or in vitro.
See also :cloning, polymerase chain reaction

Annotation

Adding pertinent information such as gene coded for, amino acid sequence, or other complementary to the database entry of raw sequence of DNA bases.

Antisense

Nucleic acid that has a sequence exactly opposite to an mRNA molecule made by the body; binds to the mRNA molecule to prevent a protein from being made.

Autoradiography

A technique that uses X-ray film to visualize radioactively labeled molecules or fragments of molecules; used in analyzing length and number of DNA fragments after they are separated by gel electrophoresis.

Bacterial artificial chromosome (BAC)

A vector used to clone DNA fragments (100 to 300 kb insert size; average, 150 kb) in *Escherichia coli* cells. Based on naturally occurring F-factor plasmid found in the bacterium *E. coli*.

Base sequence

The order of nucleotide bases in a DNA molecule; determines structure of proteins encoded by that DNA.

Bioinformatics

The science of managing and analyzing biological data using advanced computing techniques. Especially important in analyzing genomic research data.

Biotechnology

Set of biological techniques developed through basic research and now applied to research and product development. In particular, biotechnology refers to the use by industry of recombinant DNA, cell fusion, and new bioprocessing techniques.

Cancer

Diseases in which abnormal cells divide and grow unchecked. Cancer can spread from its original site to other parts of the body and can be fatal.

*See also:*hereditary cancer, sporadic cancer

Carcinogen

Something which causes cancer to occur by causing changes in a cell's DNA.

*See also:*mutagen

Carrier

An individual who possesses an unexpressed recessive trait.

cDNA library

A collection of DNA sequences that code for genes. The sequences are generated in the laboratory from mRNA sequences.

See also: messenger RNA

Clone

An exact copy made of biological material such as a DNA segment (eg. a gene or other region), a whole cell, or complete organism.

Cloning

Using specialized DNA technology to produce multiple, exact copies of a single gene or other segment of DNA to obtain enough material for further study. Process, used by researchers in the Human Genome Project, referred to as cloning DNA. Resulting cloned (copied) collections of DNA molecules constitute clone libraries. Second type of cloning exploits the natural process of cell division to make many copies of an entire cell. The genetic makeup of these cloned cells, called cell line, is identical to the original cell. Third type of cloning produces complete, genetically identical animals such as the famous Scottish sheep, Dolly.

Cloning vector

DNA molecule originating from a virus, a plasmid, or the cell of a higher organism into which another DNA fragment of appropriate size can be integrated without loss of the vector's capacity for self-replication; vectors introduce foreign DNA into host cells, where the DNA can be reproduced in large quantities. Examples are plasmids, cosmids, and yeast artificial chromosomes; vectors are often recombinant molecules containing DNA sequences from several sources.

Complementary DNA (cDNA)

DNA that is synthesized in the laboratory from a messenger RNA template.

Complementary sequence

Nucleic acid base sequence that can form a double-stranded structure with another DNA fragment by following base-pairing rules (A pairs with T and C with G). The complementary sequence to GTAC for example, is CATG.

Cosmid

Artificially constructed cloning vector containing the cos gene of phage lambda. Cosmids can be packaged in lambda phage particles for infection into *E. coli*; Permits cloning of larger DNA fragments (up to 45kb) than can be introduced into bacterial hosts in plasmid vectors.

DNA bank

A service that stores DNA extracted from blood samples or other human tissue.

DNA profiling

A PCR technique that determines the alleles present at different STR (short tandem repeat) loci within a genome in order to use DNA information to identify individuals.

DNA repair genes

Genes encoding proteins that correct errors in DNA sequencing.

DNA replication

The use of existing DNA as a template for the synthesis of new DNA strands. In humans and other eukaryotes, replication occurs in the cell nucleus.

DNA sequence

The relative order of base pairs, whether in a DNA fragment, gene, chromosome, or an entire genome.
See also: base sequence analysis

Double helix

The twisted-ladder shape that two linear strands of DNA assume when complementary nucleotides on opposing strands bond together.

Electrophoresis

A method of separating large molecules (such as DNA fragments or proteins) from a mixture of similar molecules. An electric current is passed through a medium containing the mixture, and each kind of molecule travels through the medium at a different rate, depending on its electrical charge and size. Agarose and acryl amide gels are the media commonly used for electrophoresis of proteins and nucleic acids.

Electroporation

A process using high-voltage current to make cell membranes permeable to allow the introduction of new DNA; commonly used in recombinant DNA technology.
*See also:*transfection

Embryonic stem (ES) cells

An embryonic cell having totipotency that can replicate indefinitely, transform into other types of cells, and serve as a continuous source of new cells. These cells are derived from inner cell mass of the blastocyst or the 4-8 cell stage of embryo.

Endonuclease

*See:*restriction enzyme

Exogenous DNA

DNA originating outside an organism that has been introduced into the organism.

Exon

The protein-coding DNA sequence of a gene.

*See also:*intron

Exonuclease

An enzyme that cleaves nucleotides sequentially from free ends of a linear nucleic acid substrate.

Expressed sequence tag (EST)

A short strand of DNA that is part of cDNA molecule and can act as identifier of a gene. Used in locating and mapping genes.

*See also:*cDNA, sequence tagged site

Fingerprinting

In genetics, the identification of multiple specific alleles on a person's DNA to produce a unique identifier for that person.

*See also:*forensics

Fluorescence in situ hybridization (FISH)

A Physical mapping approach that uses fluorescein tags to detect hybridization of probes with metaphase chromosomes and with the less-condensed somatic interphase chromatin.

Forensics

Use of DNA for identification. Some examples of DNA use are to establish paternity in child support cases; establish the presence of a suspect at a crime scene, and identify accident victims.

Functional genomics

Study of genes, their resulting proteins, the role played by proteins in the body's biochemical processes.

Gel electrophoresis

*See:*electrophoresis

Gene gun or particle gun: a popular and widely used direct gene transfer method for delivering foreign genes into virtually any tissues and cells or even intact seedlings.

- The foreign DNA is coated or precipitated onto the surface of minute gold or tungsten particles (1-3 µm).
- It is bombarded or shot onto the target tissue or cells using the gene gun or microprojectile gun or shot gun.
- The bombarded cells or tissues are cultured on selection medium to regenerate plants from the transformed cells.

Gene library

*See:*genomic library

Gene mapping

Determination of the relative positions of genes on a DNA molecule (chromosome or plasmid) and of the distance, in linkage units or physical units, between them.

Gene pool

All the variations of genes in a species.
*See also:*allele, gene,polymorphism

Gene therapy

Experimental procedure aimed at replacing, manipulating, or supplementing nonfunctional or misfunctioning genes with healthy genes.
*See also:*gene, inherit, somatic cell gene therapy, germ line gene therapy

Gene transfer

Incorporation of new DNA into an organism's cells, usually by a vector such as a modified virus. Used in gene therapy.
*See also:*mutation, gene therapy,vector

Genetic engineering

Altering the genetic material of cells or organisms to enable them to make new substances or perform new functions.

Genetic engineering technology

*See:*recombinant DNA technology

Genetic marker

A gene or other identifiable portion of DNA whose inheritance can be followed.
*See also:*chromosome, DNA, gene, inherit

Genetic material

*See:*genome

Genetic polymorphism

Difference in DNA sequence among individuals, groups, or populations (e.g., genes for blue eyes versus brown eyes).

Genetic screening

Testing a group of people to identify individuals at high risk of having or passing on a specific genetic disorder.

Genetic testing

Analyzing an individual's genetic material to determine predisposition to a particular health condition or to confirm a diagnosis of genetic disease.

Genome

All the genetic material in the chromosomes of a particular organism; its size is generally given as its total number of base pairs.

Genome project

Research and technology-development effort aimed at mapping and sequencing the genome of human beings and certain model organisms.
See also: Human Genome Initiative

Genomic library

A collection of clones made from a set of randomly generated overlapping DNA fragments that represent the entire genome of an organism.
Human Genome Project (HGP) Formerly titled Human Genome Initiative. *See also:* Human Genome Initiative

In vitro Studies performed outside a living organism such as in a laboratory.

In vivo

Studies carried out in living organisms.
Marker

*See:*genetic marker

Microinjection

A technique for introducing a solution of DNA into a cell using a fine microcapillary pipette or microsyringe under a phase contrast microscope to aid vision.

Microsatellite DNA

Polymorphism comprising tandem copies of usually, two-, three-, four- or five-nucleotide repeat units, also called a short tandem repeat (STR).

Phage

A virus for which the natural host is a bacterial cell.
Plasmid

Autonomously replicating extra-chromosomal circular DNA molecules, distinct from the normal bacterial genome and non essential for cell survival under nonselective conditions. Some plasmids are capable of integrating into the host genome. Number of artificially constructed plasmids are used as cloning vectors.

Polymerase, DNA or RNA

Enzyme that catalyzes the synthesis of nucleic acids on preexisting nucleic acid templates, assembling RNA from ribonucleotides or DNA from deoxyribonucleotides.
Primer

Short preexisting polynucleotide chain(generally from 17-30 nucleotides in length) to which new deoxyribonucleotides can be added by DNA polymerase.

Probe

Single-stranded DNA or RNA molecules of specific base sequence, labeled either radioactively or immunologically. Used to detect the complementary base sequence by hybridization.

Promoter

The nucleotide sequence upstream of a gene that acts as a signal for RNA polymerase binding.

Restriction fragment length polymorphism (RFLP)

Variation between individuals in DNA fragment sizes cut by specific restriction enzymes; polymorphic sequences that result in RFLPs are used as markers on both physical maps and genetic linkage maps. RFLPs are usually caused by mutation at a cutting site.

Retroviral infection

Presence of retroviral vectors, such as some viruses, which use their recombinant DNA to insert their genetic material into the chromosomes of the host's cells. The virus is then propogated by the host cell.

Satellite

Chromosomal segment that branches off from the rest of the chromosome but is still connected by a thin filament or stalk.

Sequencing

Determination of order of nucleotides (base sequences) in a DNA or RNA molecule or the order of amino acids in a protein.
The X or Y chromosome in human beings that determines the sex of an individual. Females have two X chromosomes in diploid cells; males have an X and a Y chromosome. The sex chromosomes comprise the 23rd chromosome pair in a karyotype.

Single nucleotide polymorphism (SNP)

DNA sequence variations that occur when a single nucleotide (A, T, C, or G) in the genome sequence is altered.

Single-gene disorder

Hereditary disorder caused by a mutant allele of a single gene (e.g., Duchenne muscular dystrophy, retinoblastoma, sickle cell disease).

Somatic cell

Any cell in the body except gametes and their precursors.

Transgenic

An experimentally produced organism in which DNA has been artificially introduced and incorporated into the organism's germ line.

Transposable element

A class of DNA sequences that can move from one chromosomal site to another.

Vector

DNA molecule, capable of replication in a host organism, into which a gene in inserted to construct a recombinant DNA molecule.

Yeast artificial chromosome (YAC)

Constructed from yeast DNA, it is a vector used to clone large DNA fragments.

Questions

1 Mark Questions

1) What is biotechnology?

2) Define plasmid.

3) What are molecular scissors?

4) What do you mean by recognition sequence?

5) Which enzymes act as molecular glue?

6) What is elution?

7) What are cloning vectors?

8) Name the sequence within a cloning vector from where the replication commences.

9) Mention the bacteria that acts as natural genetic engineer.

10) Name any two processes by which alien DNA is introduced into the host cell.

11) Expand the term PCR.

12) Name the microorganism from which the thermostable DNA polymerase required for PCR is obtained?

13) What is a bioreactor?

14) What are the two main processes involved in downstream processing?

15) Do eukaryotic cells have restriction endonucleases? Justify your answer.

2-marks Questions

1) Enlist the core techniques that paved the way for modern biotechnology.

2) What is gene cloning?

3) Mention the three steps involved in genetically modifying an organism.

4) Why do bacteria possesses restriction enzyme ?

5) Mention one basic difference between restriction endonucleases and exonucleases.

6) What is a palindromic sequence? Give example.

7) What are — sticky ends and —blunt ends ?

*8) Mention the role of selectable marker in cloning vector.

9) What is insertional inactivation?

10) How can you make a bacterial cell competent to take up foreign DNA ?

11) Why is *Agrobacterium*-mediated genetic transformation described as natural genetic engineering in plants?

12) Explain the contribution of *Thermus aquaticus* in the amplification of a gene of interest.

13) How can you visualize DNA on an agarose gel?

3- Marks Questions:

1) Enlist the major steps in recombinant DNA technology.

2) Mention the steps involved in the separation and isolation of DNA fragments through agarose gel electrophoresis.

3) Describe in brief the principle of DNA isolation through gel electrophoresis.

4) Highlight the salient features that are required to facilitate cloning into a vector.

5) Enumerate the major steps for isolation of DNA.

6) Draw a neat ,labeled diagram of (a) simple stirred tank bioreactor/ (b) sparged tank bioreactor. 7) What do you mean by replica plating?

8) What are the uses of embryonic stem cells?

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