Semester – II

I7MBP203 MICROBIAL TECHNOLOGY AND INTELLECTUAL PROPERTY RIGHTS 4H – 4C

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

Microbial technology is concerned with the industrial processing of materials by microorganisms to provide desirable products or serve other useful purposes. This paper emphasizes the application of biological systems to the manufacturing and service industries or the use of biological processes within the framework of technical operations and industrial production. It creates awareness on the Intellectual property rights and patenting of biotechnological processes.

OBJECTIVES

To learn the basic tools in recombinant technology
To understand the various concepts of cloning vectors
To learn the cloning strategies
To familiarize the students, with the principles of bioethical concepts
To emphasize on IPR issues and need for knowledge in patents in biotechnology

UNIT – I

Introduction to microbial technology, restriction enzymes – nomenclature – types – and its properties, isolation of DNA, plasmids and RNA. Handling and quantification of nucleic acids, radiolabelling and non radiolabelling of nucleic acids, gel electrophoresis - Blotting techniques – Southern, Northern and Western blotting techniques.

UNIT – II

Cloning vectors: Plasmid as cloning vectors - pBR322, Bacteriophage - □ , M13; Cosmid, phagemids. Yeast vector. Expression vectors. Prokaryotic hosts: *E.coli*, Eukaryotic hosts: Yeast cell. Gene cloning - basic steps, cloning construction of cDNA, selection and screening method of recombinants. Bio labeling of genes and proteins.

UNIT – III

Transgenic plants: Methodology, development of herbicide resistance plants, delayed fruit ripening, Biocontrol agents - Insecticidal toxin of BT, cry gene and baculovirus. Trangenic animals. Methodology, development of transgenic mice – its application. DNA diagnostic in medical forensics. Biosafety and Bioethics.

UNIT – IV

Discrepancies in biotechnology / chemical patenting. IPR – historical perspective – recent developments – IPR in India, IPR and the rights of farmers in developing countries. Types of IPR- Governing bodies-national and international.

UNIT – V

Patenting – fundamental requirements – patenting multicellular organisms – patenting and fundamental research. Patenting of biological materials, Product patents, conditions for patenting, Patenting of liveforms, regulating recombinant technology, Food and food ingredients. Trade secrets. How do write a patent?.

SUGGESTED READINGS

TEXT BOOKS

1. Sathyanarayana, U.(2005). *Biotechnology*. (1st ed.). Books and Allied (P) Ltd, Kolkata, India.
2. Dubey, R.C. (2002). *Text book of Biotechnology*. S. Chand and Company Ltd, New Delhi.
3. Ramawat, K.G. (2003). *Text book of Plant Biotechnology*. S. Chand and Company Ltd, New Delhi.
4. Watson, J.D., Gilman, M., and Wikowski, J., (2001). *Recombinant DNA*. (2nd ed.)., Scientific American Books. W.H. Freeman and Co. NY.
5. Verma, A., and Podila, G.K., (2005). *Biotechnological Applications of Microbes*. I.K. International Publishing House, New Delhi.

REFERENCES

1. Brown, T.A. (2001). *Gene Cloning and DNA analysis: An Introduction*. (4th ed.). Blackwell Publishing, USA.
2. Glick, B.K., and Pasternak, J.J., (2003). *Molecular Biotechnology. Principles and Applications of Recombinant DNA*. (3rd ed.). ASM Press, Washington.
3. Old, R.M., and Primrose, S.B., (2003). *Principles of Gene Manipulation*. (6th ed.). Blackwell Scientific Publication, London.
4. Primrose, S.B. (2001). *Molecular Biotechnology*. (2nd ed.). Blackwell Scientific Publishers, Oxford Press, London.
5. Winnacker, E.L. (2003). *From Genes to Clones: Introduction to Gene Technology*. (1st ed.). VCH. Weinhein, Germany.
6. Slater, A., and Scott, N., (2003). *Plant Biotechnology - The Genetic Manipulations of plants*. (2nd ed.)., Oxford University Press, New York.

DEPARTMENT OF MICROBIOLOGY
KARPAGAM ACADEMY OF HIGHER EDUCATION
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Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

I M.Sc. MICROBIOLOGY - (2017 – 2019 Batch)

MICROBIAL TECHNOLOGY & IPR (17MBP203)

SEMESTER – 2 **4H – 4C**

LECTURE PLAN

Unit I

Sl.No	Duration	Topic	Reference
1	1	Introduction to microbial technology cuttimg DNA&joining DNA	Gene Doning.T.A.Brown
2	1	Restriction Enzyme & Types	R1-3-8
3	1	Restriction Endonucleases	R1-32-38
4	1	DNA modifying enzymes & Properties	R1-32-38
5	1	Isolation of DNA	R1-36-38
6	1	Plasmid Preparation	R1-36-38
7	1	Isolation & Purification of RNA	R1-36-37
8	1	Streptometric quantification of DNA& RNA	R1-35-38
9	1	Radiolabelling & Non-Radiolabelling	R1-77-80
10	1	Blotting techniques	T1-127-130
11	1	Northern & Western Blotting	T1-127-130
12	1	Unit revision	-
		Total Hours:**12hours**	

T1-Dubey R.C.2002.Textbook of biotechnology,

R1-Brown.T.A.2001.GENE cloning & DNA analysis.An Introduction .Blackwell publishing

R1-Glick.B.K.JJ.Pasternak. 2003 Molecular biotechnology. Asm press ,Washington.D.C.

R3-Winnaker.From genes to clone VCH 2003

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SEMESTER – 2 **4H – 4C**

LECTURE PLAN

Unit II

Sl.No	Duration	Topic	Reference
1	1	Cloning vectors	R2-29-32
2	1	Plasmid as a cloning vector –Properties	R5-705-723
3	1	Types of plasmid	R3-126-132
4	1	F&col plasmid	R3-126-132
5	1	pBR 322 Structure	R3-126-133
6	1	Bacteriophage vector	R3-154-158
7	1	Cosmids	R3-148-149
8	1	Yeast vectors & Types	R3-148-149
9	1	*E.Coli* as a prokaryotic host	R3-150-152
10	1	Basic steps in gene cloning	R1-35- 50
11	1	Gene transfer & Selection	R1-45- 50
12	1	CDNA construction & synthesis	T1 -108 -112
13	1	Unit revision	
		Total hours:**13 Hours**	

R4 : Freidfielder.D.2004.Molecular biology.Narosa publishing house.

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SEMESTER – 2 **4H – 4C**

LECTURE PLAN

Unit III

Sl.No	Duration	Topic	Reference
1	1	Transfer plants	R5-524-548
2	1	Herbicide resistant plant	R5-528-548
3	1	Anti –Sense technology	R5-536-542
4	1	Delayed fruit repening	R5-540-548
5	1	Biocontrol agents	R2 292-300
6	1	Insecticidal toxin of BT toxin	R2 297-302
7	1	Cry gene & Cry protein	R2 290-302
8	1	Development of insect resistance	R2 290-302
9	1	Transgenic animals	R2-369-370
10	1	Transgenic mice	R3-192-194
11	1	Fingerprinting	R3-192-194
12	1	Biosafety & Bioethics	R2-192-194
13	1	Unit revision	
		Total hours : 13 hours	

R6- Gupta .P.K.2005.Molecular biology & Genetic engineering . Rastogi publication

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SEMESTER – 2 **4H – 4C**

LECTURE PLAN

Unit IV

Sl.No	Duration	Topic	Reference
1	1	Discrepancies in biotechnology	R2-425
2	1	Chemical patenting , ethical issues	R2-448
3	1	IPR –Historical perspective	R2-437
4	1	Recent developments	T2-557-563
5	1	IPR in India	T2-557-563
6	1	Government initiatives	T2-558-562
7	1	International implementation of farmers rights	T2 576-579
8	1	Plant breeders rights	T2 576-579
9	1	Biodiversity related issues	T2 576-579
10	1	Rights of farmers in developing countries	T2 577-579
		Unit revision	
		TOTAL HOURS : 10	

T2 : P.K .Gupta Elements of Bio-technology

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SEMESTER – 2 **4H – 4C**

LECTURE PLAN

Unit V

Sl.No	Duration	Topic	Reference
1	1	Intellectual Property Rights	R2 443-448
2	1	Types secrets ,governing	R2 447-448
3	1	Patent system ,Novelty	R2 443-448
4	1	Disclosing invention	R2 443-448
5	1	Fundamental requirement	T2 -557-563
6	1	IPR Protection	T2 -557-563
7	1	Patenting multicellular organisms	R2 -447.
8	1	Patenting biological materials	R2 -447.
9	1	Patenting of life forms	T2-580-586
10	1	Regulating recombinant technology	R2-428-431
11	1	Regulating food ingredients	R2-426
12	1	Unit revision	
		TOTAL HOURS : 12.	
	Total Hrs: 12		

T1 :. Dubey .R.C.2002 Textbook of Bio-Technology

T2 : Elements of Biotechnology.P.K.Gupta

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MICROBIAL TECHNOLOGY AND INTELLECTUAL PROPERTY RIGHTS
COURSE CODE: **17MBP203** BATCH: **2017-2019**
UNIT – 1

Introduction to Microbial technology

Recombinant DNA refers to the creation of new combinations of DNA segments that are not found together in nature. The isolation and manipulation of genes allows for more precise genetic analysis as well as practical applications in medicine, agriculture, and industry.

Isolate DNA Cut with restriction enzymes ligate into cloning vector transform recombinant DNA molecule into host cell each transformed cell will divide many, many times to form a colony of millions of cells, each of which carries the recombinant DNA molecule (DNA clone).

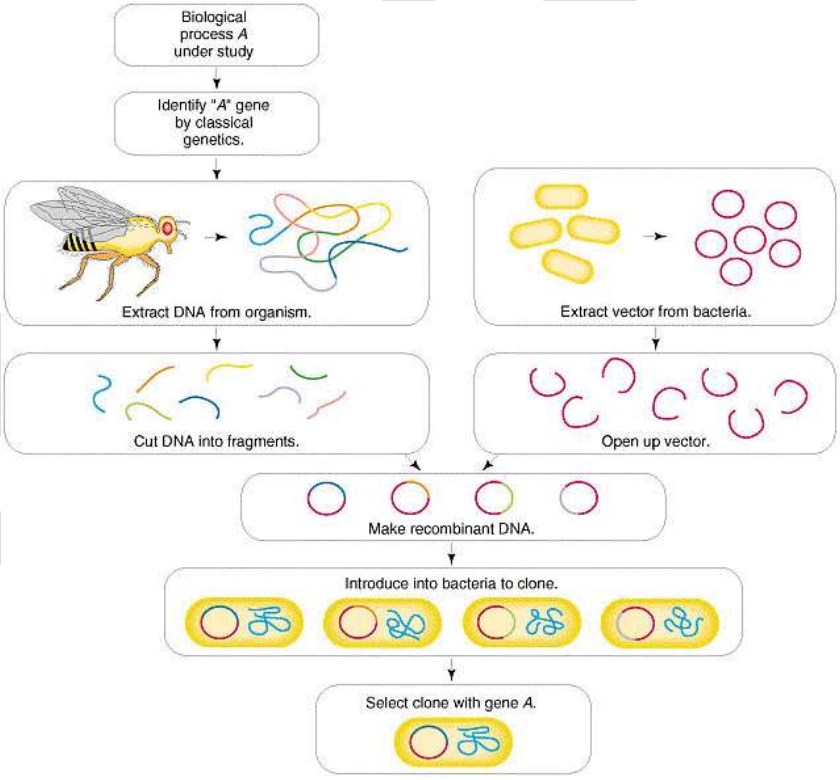


Fig: gene cloning

DNA clone = A section of DNA that has been inserted into a vector molecule and then replicated in a host cell to form many copies.

Isolating DNA

1. Crude isolation of donor (foreign) DNA is accomplished by isolating cells disrupting lipid membranes with detergents destroying proteins with phenol or proteases degrading RNAs with RNase leaving DNA at the end.

2. Crude isolation of plasmid vector DNA is accomplished by an alkaline lysis procedure or by boiling cells which removes bacterial chromosomal DNA from plasmid DNA.

3. To get purer DNA from either (1) or (2), crude DNA is

a) Fractionated on a $CsCl_2$ gradient
b) Precipitated with ethanol
c) Poured over a resin column that specifically binds DNA

Cutting DNA

1. DNA can be cut into large fragments by mechanical shearing.

2. Restriction enzymes are the scissors of molecular genetics. Restriction enzymes (RE) are endonucleases that will recognize specific nucleotide sequences in the DNA and break the DNA chain at those points. A variety of RE have been isolated and are commercially available. Most cut at specific palindromic sites in the DNA (sequence that is the same on both antiparallel DNA strands). These cuts can be a staggered which generate "sticky or overhanging ends" or a blunt which generate flush ends.

Joining DNA

Once you have isolated and cut the donor and vector DNAs, they must be joined together. The DNAs are mixed together in a tube. If both have been cut with the same RE, the ends will match up because they are sticky. DNA ligase is the glue of molecular genetics that holds the ends of the DNAs together. DNA ligase creates a phosophodiester bond between two DNA ends.

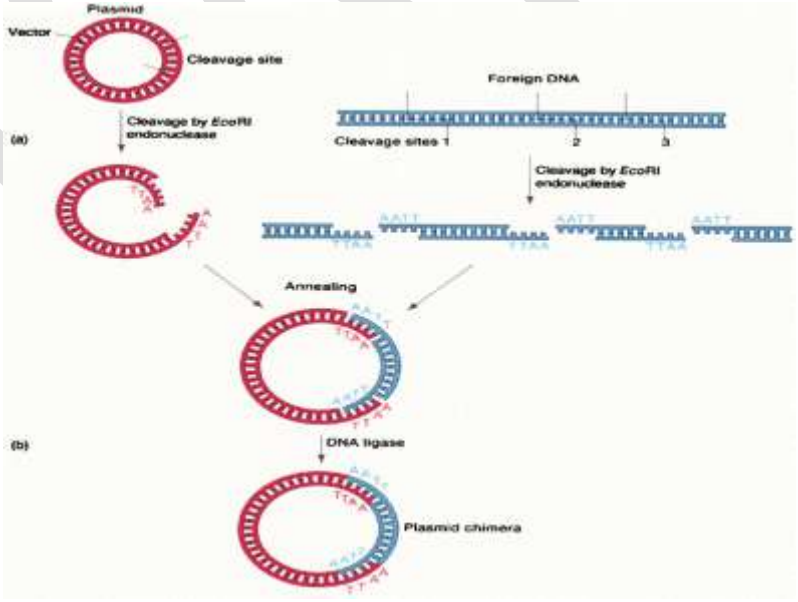


Fig: Preparation of rDNA

Amplifying the recombinant DNA

To recover large amounts of the recombinant DNA molecule, it must be amplified. This is accomplished by transforming the recombinant DNA into a bacterial host strain. (The cells are treated with $CaCl_2$ rDNA is added Cells are heat shocked at 42º C DNA goes into cell by a somewhat unknown mechanism.) Once in a cell, the recombinant DNA will be replicated. When the cell divides, the replicated recombinant molecules go to both daughter cells which themselves will divide later. Thus, the DNA is amplified.

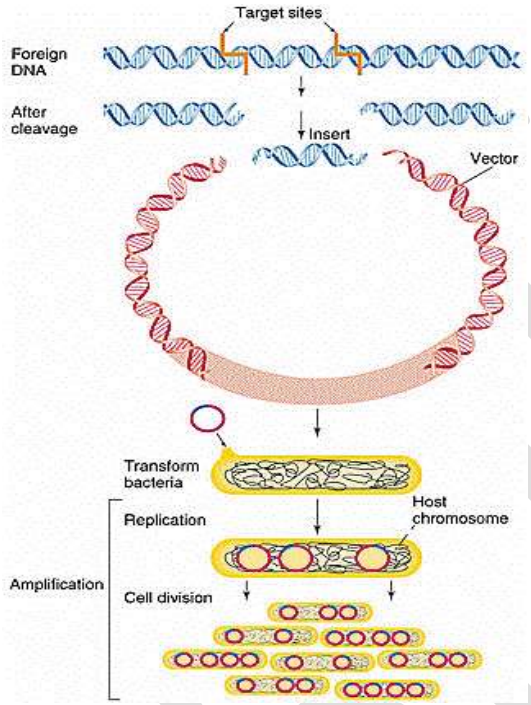


Fig: Transformed bacteria

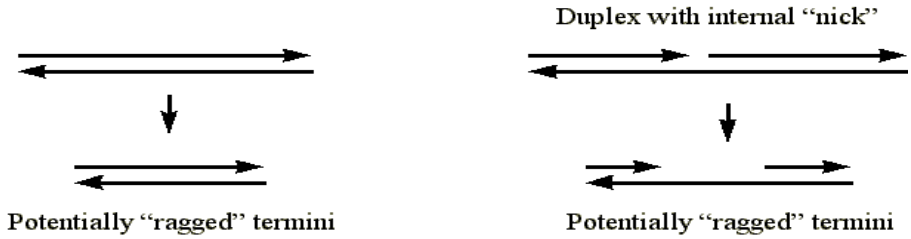
Nucleases

- **Nucleases** = enzymes that catalyze hydrolysis of the phosphodiester bonds in nucleic acids
    - Some are specific for DNA (**DNases)**
    - others specific for RNA (**RNases)**
    - still others show no specificity.
- **Exonucleases** remove nucleotides from the ends, either from the 5'- or 3'- ends.
- **Endonucleases** hydrolyze internal phosphodiester bonds.

Nuclease BAL-31

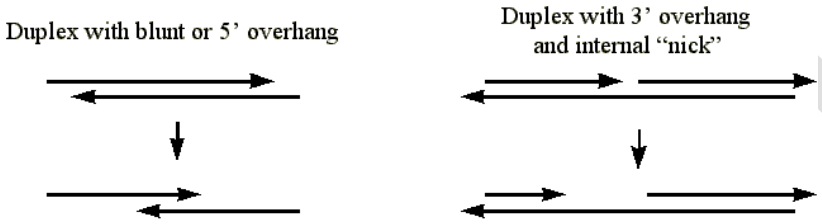
- This is an exonuclease (starts at the termini and works inward) which will degrade both 3' and 5' termini of double stranded DNA. It will not make internal cleavages ("nicks"), however, it will degrade the ends of DNA at existing internal "nicks" (which create both 3' and 5' termini).
- The degradation of termini is not coordinated, meaning that the product is not 100% blunt ended (even though the original duplex may have been blunt ended).

- Such "ragged" ends can be made blunt by filling in and chewing back by a suitable polymerase (e.g. T4 DNA polymerase). The unit definition is 1 unit is amount of enzyme required to remove 200 base pairs from each end of duplex DNA in 10 minutes at 30 °C.



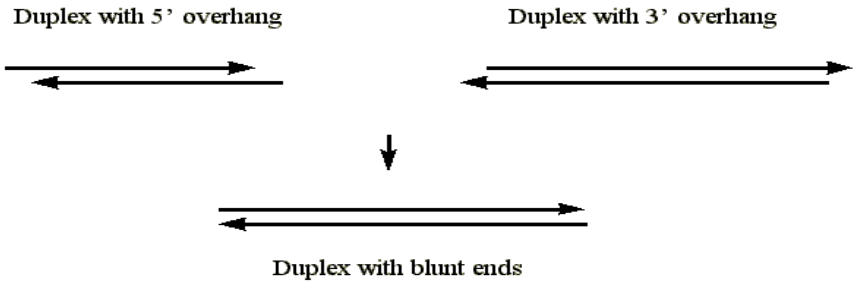
Exonuclease III

- Catalyzes the stepwise removal of nucleotides from the 3' hydroxyl termini of duplex DNA.
- The enzyme will attack the 3' hydroxyl at duplex DNA with blunt ends, with 5' overhangs, or with internal "nicks".
- Since duplex DNA is required, the enzyme **will not** digest the 3' end of duplex DNA where the termini are 3' overhangs.



Mung Bean Nuclease (isolated from mung bean sprouts)

- A single strand specific DNA and RNA endonuclease which will degrade single strand extensions from the ends of DNA and RNA leaving blunt ends.
- The single strand extensions can be either 5' or 3' extensions - both are removed and a blunt duplex is left.



Deoxyribonuclease I (DNAse I) from bovine pancrease

- This enzyme hydrolyzes duplex or single DNA strands preferentially at the phosphodiester bonds 5' to pyrimidine nucleotides
- In the presence of Mg^{2+} ion, DNAse I attacks each strand independently and produces nicks in a random fashion (useful for nick-translation)
- In the presence of Mg^{2+} ion, DNAse I cleaves both strands of DNA at approximately the same position (but leaving "ragged" ends)

Restriction enzymes

- Viruses that infect bacteria = bacteriophages (or "phages"), which consist of protein and nucleic acid.
- Some phage that grow well in one strain of bacteria are often unable to grow well in other strains of bacteria.
- Phage do not grow because their DNA molecules are cleaved and degraded by enzymes of the host bacterial cell, a defense mechanism of the host against foreign DNA.
- Degrading DNA destroys ability of phage to grow and is responsible for the pattern of growth restriction; hence the bacterial enzymes are called *restriction enzymes (restriction endonucleases).*
- Restriction enzymes, of which now more than 1000 are known, are sequence-specific. For example, *Eco*RI (from *E. coli*) is specific for the sequence (5') GAATTC in double stranded DNA.

- Restriction enzymes in a bacterial cell are just *half* of a system known as a "restriction-modification" system

  - A strain that makes a specific restriction endonuclease also makes a *DNA-modifying enzyme* with the same sequence specificity as the restriction enzyme.
  - The chemical modification of a base on *either* strand (or both) of DNA *protects* BOTH strands from cleavage by the restriction enzyme.
  - e.g., *E. coli* strains that make *Eco*RI also make **a DNA methylase,** which introduces a CH_3-group onto the 3rd base (a C) from the 5' end of each strand; with the base methylated on even one strand the host DNA is protected from the restriction enzyme, while invading viral DNA, unmethylated, is cleaved.
  - For more details look at these animations (restriction enzymes).
  - Restriction enzymes are also used in DNA fingerprinting.

Restriction modification system

Phage (or viruses) invades all types of cells. Bacteria are one favorite target. Defense mechanisms have been developed by bacteria to defend themselves from these invasions. The system they possess for this defense is the restriction-modificiation system. This system is composed of a restriction endonuclease enzyme and a methylase enzyme and each bacterial species and strain has their own combination of restriction and methylating enzymes.

Restriction enzyme - an enzyme that cuts DNA at internal phosphodiester bonds; different types exist and the most useful ones for molecular biology (Type II) are those which cleave at a specific DNA sequence

Methylase - an enzyme that adds a methyl group to a molecule; in restriction-modification systems of bacteria a methyl group is added to DNA at a specific site to protect the site from restriction endonuclease cleavage

Several different types of restriction enzymes have been found but the most useful ones for molecular biology and genetic engineering are the Type II restriction enzymes. These enzymes cut DNA at specific nucleotide sequences. For example, the enzyme EcoRI recognizes the sequence:

5' - G A A* T T C - 3'
3' - C T T *A A G - 5'

*The site of methylation protection from restriction enzyme cleavage is the 3' adenine.

This enzyme always cuts between the 5' G and A residues. But if we look at the sequence we can see that both strands will be cut and leave staggered or overlapping ends.

5' - G A A T T C - 3'
3' - C T T A A G - 5'

Not all Type II restriction enzymes generate staggered ends at the target site. Some cut and leave blunt ends. For example, the enzyme BalI.

5' - T G G C* C A - 3'
3' - A C *C G G T - 5'

is cut at the point of symmetry to produce:

5' - T G G C C A - 3'
3' - A C C G G T - 5'

(Note: * The site of methylation protection from restriction enzyme cleavage; 5' cytosine)

We began this discussion by stating that the restriction-modification system is used to protect bacteria from invasion by viral DNA molecules that may subvert the gene expression system of the bacteria to its own use. But how does this system actually work? The bacterial cell uses the restriction enzyme to cut the invading DNA of the virus at the specific recognition site of the enzyme. This prevents the virus from taking over the cellular metabolism for its own replication. But bacterial DNA will also contain sites that could be cleaved by the restriction enzyme.

How is the bacterial cell protected? This protection is offered by the action of the methylase. The methylase recognizes the same target site as the restriction enzyme and adds a methyl group to a specific nucleotide in the restriction site. Methylated sites are not substrates for the restriction enzyme. The bacterial DNA is methylated immediately following replication so it will not be a suitable substrate for restriction endonuclease cleavage. But it is unlikely that the invading viral DNA will have been methylated so it will be an appropriate target for cleavage. Thus, the viral DNA is restricted in the bacterial cell by the restriction enzyme, and the bacterial DNA is modified by the methylase and is provided protection from its own restriction enzyme.

Restriction endonucleases

One of the most significant discoveries which allowed the development of recombinant DNA technology was restriction endonuclease. It cut double stranded DNA at specific sequences, by means of which it protects bacteria from viral infection.

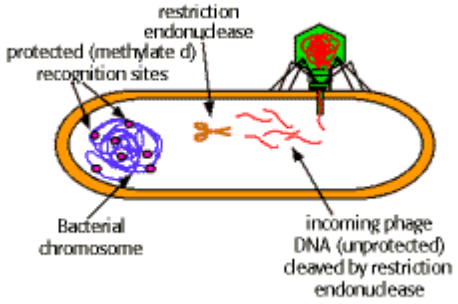


Fig: Methylation

The specific sequence upon which it acts may be palindromes i.e. a sequence which is the same when read in either direction. "Able was I ere I saw Elba".

There are three different classes of restriction endonucleases namely Type - I, Type - II, and Type - III.

TYPE - I

Type - I restriction endonucleases are single multifunctional enzymes with three different subunits. They required ATP, Mg^{2+} and S-adenosylmethionine for its activity. Their active cleavage site usually present atleast 1000 bp away from the host specificity site. There is no enzyme turnover but translocation occurs.

Example: EcoB: $TGAN_BTGCT$

TYPE - II

Type - II restriction endonucleases are most common type. The type - II enzymes recognize a particular target sequence in a duplex DNA molecule and break the polypeptide chains within, or near to, that sequence to give rise to discrete DNA fragments of defined length and sequence. Type - II recognition sequences are symmetric. Some sequences are continuous (e.g. GATC), some are interrupted (e.g. GANTC). Unlike type I, type II consists of a single polypeptide. The type - II require no cofactor other than magnesium ions. This type of restriction endonuclease found to be mostly used in recombination DNA technology.

Example: EcoRI : GAATTC

TYPE - III

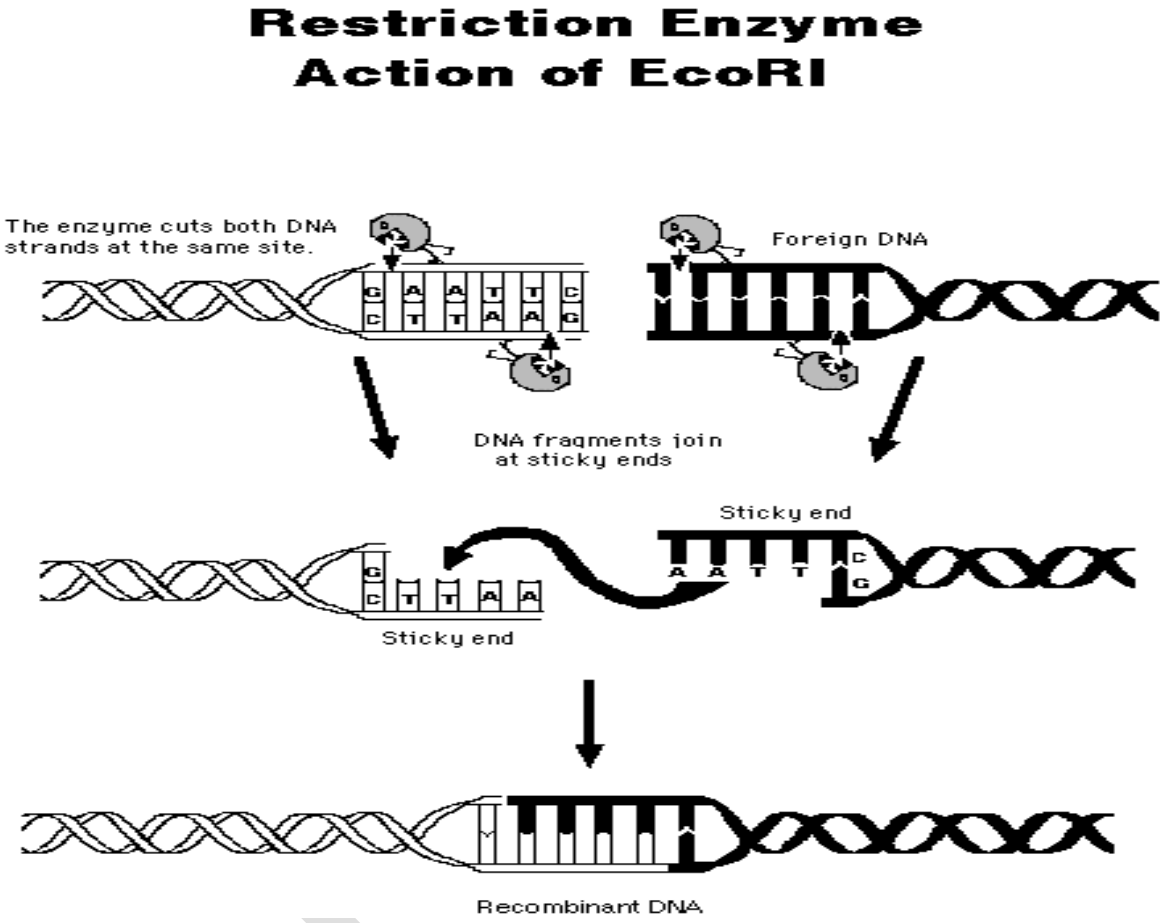
Type - III restriction systems are relatively rare and do not provide endonucleases for gene manipulation. Type - III endonuclease act as complexes of two subunits, one subunit (M subunit) responsible for site recognition and modification, the other (R subunit) responsible for nuclease action. DNA cleavage requires magnesium ions, ATP, and is stimulated by S-adenosylmethionine. The recognition sites are asymmetric and cleavage occurs by nicking one strand at a measured distance to one side of the recognition sequence. Two sites in opposite orientations are necessary to break the DNA duplex.

Example : EcoP1 : AGACC

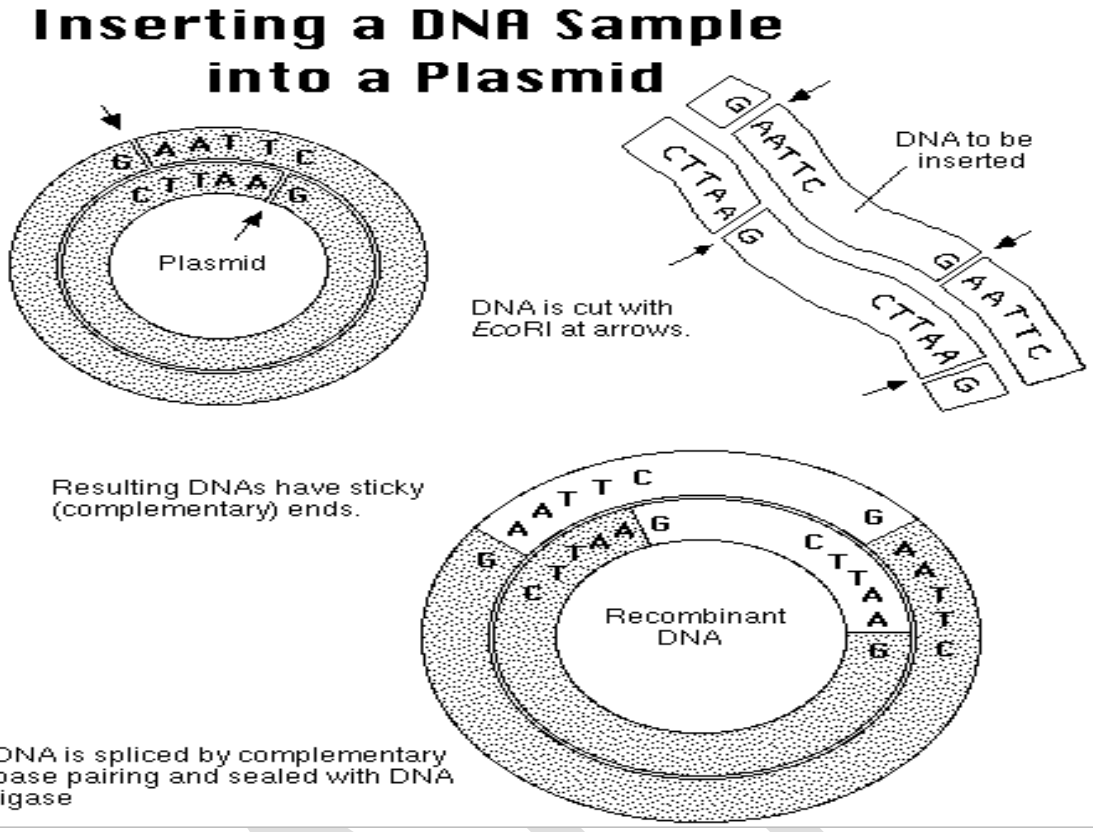
If action of two different restriction endonucleases produce same cohesive ends, then the two enzymes referred as isoenzymes. For example Bam HI and Sau3AI creates the fragments with cohesive end of "GATCC" by recognizing the sequence of GGATCC and NGATCN' respectively.

An "endonuclease" is an enzyme that cuts duplex DNA in the middle, not at an end (for exonuclease). Different species of bacteria have evolved different restriction endonucleases, each to cut foreign DNA that gets into their cells by mistake. To be cut, the DNA has to lack their own pattern of protective methylation. There are well over a hundred restriction enzymes, each cutting in a very precise way a specific base sequence of the DNA molecule.

A restriction endonuclease cuts DNA only at a specific site, usually containing 4-6 base pairs. The enzyme has to cut the DNA backbone twice, recognizing the same type of site; therefore, the site "reads" the same way backwards as forwards--a palindrome.



This "sticky ends" from two different DNA molecules can hybridize together; then the nicks are sealed using ligase. The result is recombinant DNA. When this recombinant vector is inserted into E. coli, the cell will be able to process the instructions to assemble the amino acids for insulin production. More importantly, the new instructions are passed along to the next generation of E. coli cells in the process known as gene cloning.



Nomenclature

Nomenclature of restriction of endonuclease followed the following points

1. The species name of the host organism is identified by the first letter of the genus name and the first two letters of the specific epithet to form a three-letter abbreviation in italic. For example Escherichia coli --> Eco

2. Strain or type identification is written as a subscript, e.g. Eco_k. In cases where the restriction and modification system is genetically specified by a virus or plasmid, the abbreviated species name of the host is given and the extrachromosomal element is identified by a subscript, e.g. Eco_P, Eco_R.

3. When a particular host strain has several different restriction and modification systems, these are identified by roman numerals, thus systems from Haemophilus influenza strain Rd would be Hin_dI, Hin_dII.,

4. Enzyme added with the prefix like endonuclease or methylase depending upon it is function. for example endonuclease R.Hin_dIII and modification enzyme, methylase M.Hin_dIII.

But in practice, this system of nomenclature has been simplified further:

a. Subscripts are typographically inconvenient. So, the whole abbreviation is now usually written on the line; e.g. HindIII

b. Where the context makes it clear that restriction enzymes only are involved in recombinant DNA technology, the designation endonuclease R omitted.

Name	Source Microorganism	Recognition Sequence
Bam HI	*Bacillus amyloliquefaciens*	G↓GATCC
Eco RI	*Escherichia coli* RY13	G↓AATTC
Hind III	*Haemophilus influenzae* Rd	A↓AGCTT
Not I	*Nocardia otitidis-caviarum*	GC↓GGCCGC
Pst I	*Providencia stuartii*	CTGCA↓G
Sma I	*Serratia marcescens*	CCC↓GGG

Table: Restriction enzymes

Restriction endonucleases allow the specific and reproducible fragmentation of DNA. The discovery of these enzymes allowed the development of modern recombinant DNA technology. There are about 2500 different restriction enzymes which have different specificities for cutting the DNA. Sometimes the restriction enzyme cuts straight through the DNA, cutting both strands at the same location which is referred as blunt end. Most of the time, however, restriction enzymes cut the DNA in a staggered fashion - leaving a few nucleotides of single stranded DNA extending from the cut site. These **sticky ends** or cohesive ends are key to allowing separate DNA molecules to get together. The short sticky ends actually can base pair between two DNA ends to align the two DNA molecules. Any two DNAs cut with the same restriction enzyme will have the same sticky ends and therefore can be joined. It is this ability provided by restriction enzymes that allows most of recombinant DNA techniques to work.

Restriction enzymes, also called restriction nucleases *(EcoRI in this example)* , surrounds the DNA molecule at the point it seeks*(sequence GAATTC)*. It cuts one strand of the DNA double helix at one point and the second strand at a different, complementary point (between the G and the A base). The separated pieces have single stranded "sticky-ends," which allow the complementary pieces to combine. The newly joined pieces are stabilized by DNA ligase. EcoRI, one of many restriction enzymes, is obtained from the bacteria Escherichia coli.

DNA modifying enzymes

Methylases

Just as the study of the bacterial restriction-modification sytem has provided a variety of specific endonucleases, there are also available a variety of specific DNA methylases.

- The recognition sequences of the methylases are the same as the associated endonucleases (e.g. EcoR1 methylase recognizes and methylates at the sequence "GAATTC").
- All methylases transfer the methyl group from S-adenosylmethionine (SAM) to a specific base in the recognition sequence, and SAM is an required component in the methylation reaction.

Methylation of DNA usually has the effect of protecting the DNA from the related restriction endonuclease. However, there are methylases with minimal specificity. For example, Sss I methylase will methylate cytosine residues in the sequence 5' … CG … 3'. In this case, *the methylated DNA will be protected from a wide variety of restriction endonucleases*.

Some restriction endonucleases will only cut DNA at their recognition sites if the DNA is methylated (e.g. Dpn I).
Still other restriction endonucleases will cut both methylated and non-methylated DNA at their recognition sequences (e.g. BamH I).

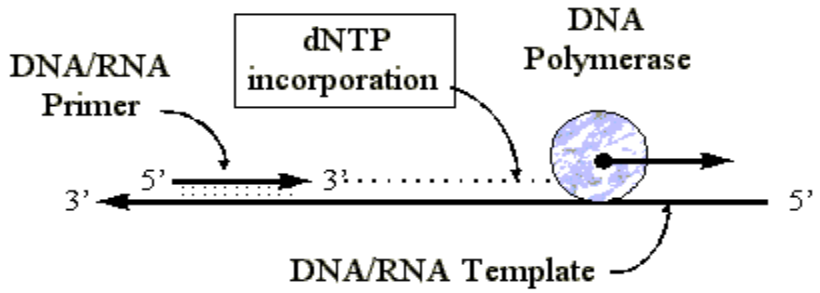
***dam* and *dcm* methylation**

- The methylase encoded by the *dam* gene (dam methylase) transfers a methyl group from SAM to the N_6 position of the adenine base in the sequence 5' … GATC … 3'.
- The methylase encoded by the *dcm* gene (dcm methylase) methylates the internal cytosine base, at the C_5 position, in the sequences 5' … CCAGG … 3' and 5' … CCTGG … 3'.
- Almost all strains of *E. coli* commonly used in cloning have a *dam+dcm+* genotype. The point here is *not* that we particularly want our DNA to be methylated, but that to make a *dam-dcm-* host someone has to mutate the bacteria and isolate the correct mutant. That apparently has not been done for a lot of bacterial strains. Probably because the *dam* and *dcm* methylation affects only a small subset of potential restriction endonucleases

DNA Polymerases

A wide variety of polymerases have been characterized and are commercially available. All DNA polymerases share two general characteristics:

1. They add nucleotides to the 3'-OH end of a primer
2. The order of the nucleotides in the nascent polynucleotide is template directed



In addition to the 5'->3' polymerase activity, polymerases can contain exonuclease activity. This exonuclease activity can proceed *either* in the 5'->3'direction, or in the 3'->5' direction.

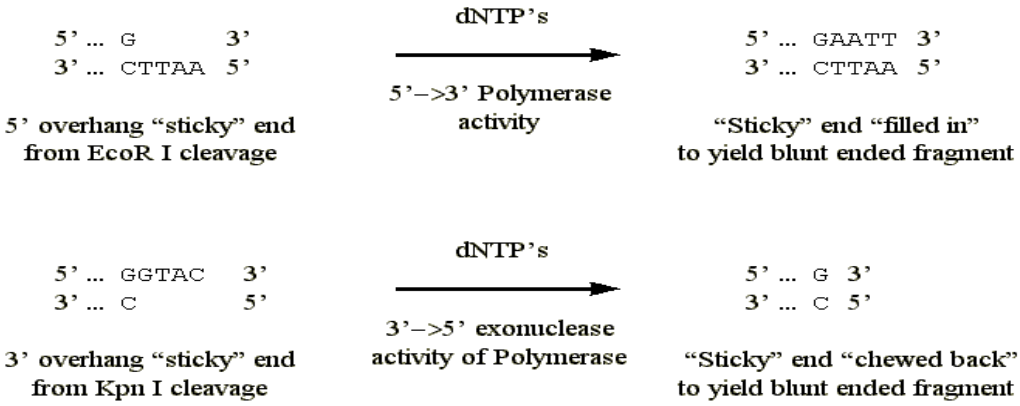
- Exonuclease activity in the 3'->5' direction allows the polymerase to correct a mistake if it incorporates an incorrect nucleotide (so called "error correction activity"). *It can also slowly degrade the 3' end of the primer*.
- Exonuclease activity in the 5'->3' direction will allow it to degrade any other hybridized primer it may encounter. Without 5'->3' exonuclease activity, obstructing primers may or may not be physically replaced, depending on the polymerase being used.

Uses of polymerases

Restriction endonucleases can yield fragments of DNA with either 3' or 5' nucleotide "overhangs".

- In the case of 5' overhangs, the 5'->3' polymerase activity can fill these in to make blunt ends.

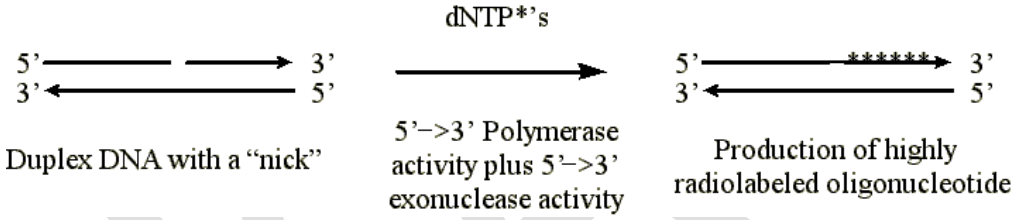
In the case of 3' overhangs, the 3'->5' exonuclease activity present in some polymerases (especially T4 DNA polymerase) can "chew back" these ends to also make blunt-ended DNA.



"Nick-translation"

This method is used to obtain highly radiolabeled single strand DNA fragments, which makes use of 5'->3' exonuclease activity present in some polymerases (*E. coli* DNA polymerase I, for example).

- In this method a DNA duplex of interest is "nicked" (i.e. one of the strands is cut; see DNAse I).
- Then DNA pol I is added along with radiolabeled nucleotides. The 5'->3' exonuclease activity chews away the 5' end at the "nick" site and the polymerase activity incorporates the radiolabeled nucleotides. The resulting polynucleotide will be highly radiolabeled and will hybridize to the DNA sequence of interest.



- Thermostable polymerases have the ability to remain functional at temperature ranges where the DNA duplex will actually "melt" and become separated. This has allowed the development of the **"Polymerase Chain Reaction"** technique (PCR), which has had a profound impact on modern Biotechnology.

Ligases

- Ligases catalyze the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini of nucleotides (potentially RNA or DNA depending on the ligase).
- In a sense, they are the opposite of restriction endonucleases, but they do not appear to be influenced by the local sequence, *per se*.
- Ligases require either rATP or NAD+ as a cofactor, and this contrasts with restriction endonucleases.

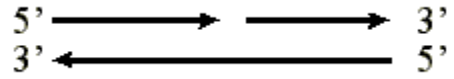
The following are different types of ligases and their characteristics.

T4 DNA ligase

- Isolated from bacteriophage T4.
- Will ligate the ends of duplex DNA or RNA.
- This enzyme will join blunt-end termini as well as ends with cohesive (complementary) overhanging ends (either 3' or 5' complementary overhangs).
- This enzyme will also repair single stranded nicks in duplex DNA, RNA or DNA/RNA duplexes. Requires ATP as a cofactor.

Taq DNA ligase

- This ligase will catalyze a phosphodiester bond between two adjacent oligonucleotides which are hybridized to a complementary DNA strand:

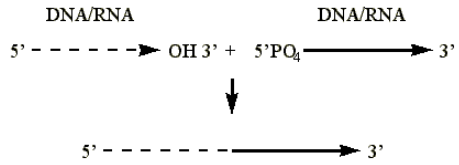


- The ligation is efficient only if the oligonucleotides hybridize perfectly with the template strand.
- The enzyme is active at relatively high temperatures (45 - 65 °C). Requires NAD+ as a cofactor.

T4 RNA ligase

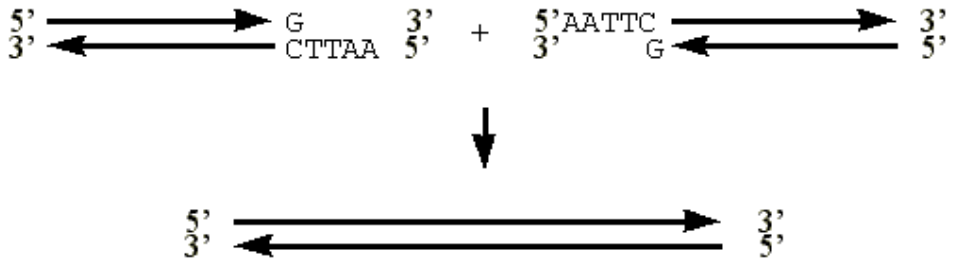
- Will catalyze formation of a phosphodiester bond between RNA/RNA oligonucleotides, RNA/DNA oligonucleotides, or DNA/DNA oligonucleotides.
- Requires ATP as a cofactor.
- This enzyme does not require a template strand.

T4 RNA ligase can be used for a variety of purposes including constructing RNA/DNA hybrid molecules.



DNA ligase (E. coli)

- Will catalyze a phosphodiester bond between duplex DNA containing cohesive ends.
- It will not efficiently ligate blund ended fragments.
- Requires NAD+ as a cofactor.

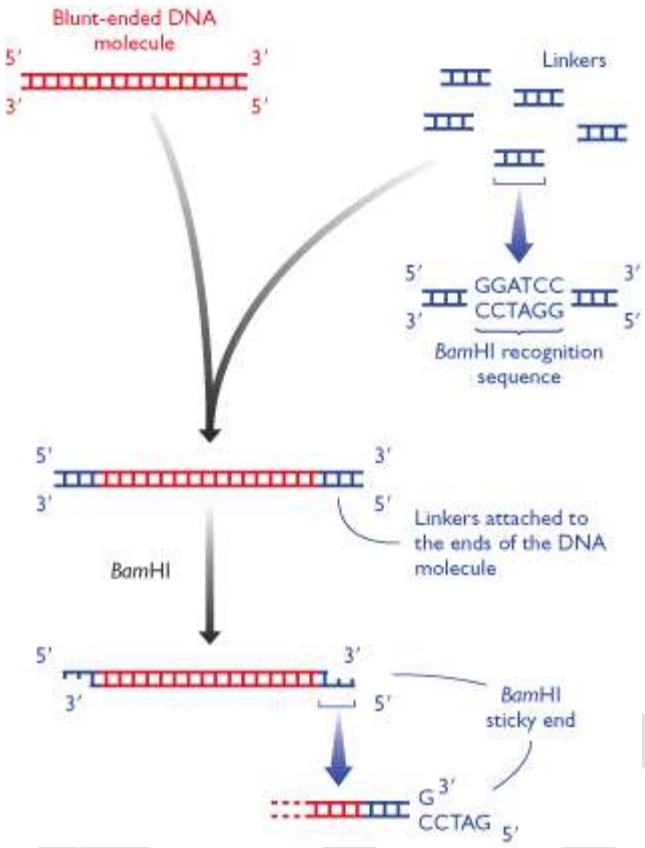


The plasmid and the foreign DNA are cut by this restriction endonuclease (EcoRI in this example) producing intermediates with sticky and complementary ends. Those two intermediates recombine by base-pairing and are linked by the action of DNA ligase. A new plasmid containing the foreign DNA as an insert is obtained. A few mismatches occur, producing an undesirable recombinant.

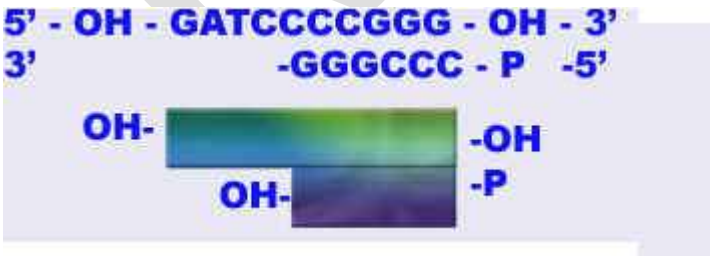
When same sticky end creating enzyme used for cleavage of vector and gene of interest, then DNA ligase seals the nick between gene of interest and vector and creates recombinant vector. Whereas when blunt end creating enzyme used then recoiling become difficult. Moreover, in both cases of using sticky end and blunt end enzymes, self coiling of vector also occurs in high rate rather than the recombination of vector and genes. These situations are overcome by using

i) Linkers
ii) Adaptors
iii) Homopolymer tailing

i) Linkers: Linker molecules are used to ligate the blunt end gene of interest with cohesive end vectors. They are normally synthesized self-complementary decameric oligonucleotiedes, which contain sites for one or more restriction endonucleases which will create sticky ends. The linker can be ligated to both ends of the foreign gene to be clones, and then treated with restriction endonuclease to produce a sticky ended fragment which can be incorporated into a vector molecule that has been cut with the same restriction endonuclease. Insertion by means of the linkers creates restriction enzyme target sites at each end of the foreign gene and so enables the foreign gene to be excised and recovered after cloning and amplification in the host bacterium.



ii) Adaptors: When linkers added to link at the end of blunt end of gene interest, then there is an possibility of joining of multiple linkers at the end. This makes some time larger genes and waste of linker molecules. This problem is overcome by using adapters. Since adapters contain only one end suitable for joining this prevents multiple coiling of adapters. Adapter is a synthetic, double stranded oligonucleotide used to attach sticky ends to a blunt ended molecule. It contain normal 5' and 3' end at blunt end and the sticky end of adapter molecule is modified in such manner that it contain OH group on both 5' and 3' ends. This is achieved by using alkaline phosphatases. In contrast to linkers, adapters contain preformed sticky ends and joining blunt ends. Because of lack of 5' phosphate group on sticky end prevents adapter polymer formation. After the adaptors have been attached the abnormal 5'OH terminus is converted to the natural 5'P form by treatment with the enzyme polynucleotide kinase, producing sticky ended fragment that can be inserted into an appropriate vector.



Eventhough adaptors prevents polymer formation, it does not prevents self ligation or recoiling of vectors during recombination reaction. This disadvantages nature of adaptors removed by using homopolymer tailing.

iii) Homopolymer Tailing: A homopolymer is simply a polymer in which all the subunits are the same. Tailing involves using the enzyme terminal deoxynucleotidyl transferase, to add a series of nucleotides on to the 3'-OH

termini of a double stranded DNA molecule. If this reaction is carried out in the presence of just one deoxynucleotide, then a homopolymer tail will be produced. In this method, gene of interest is tailed with one nucleotide and vector is tailed with a complementary base and when they are combined then only vector recombined with gene of interest. In this case recoiling of vector is mostly prevented because vector does not contain complementary ends.

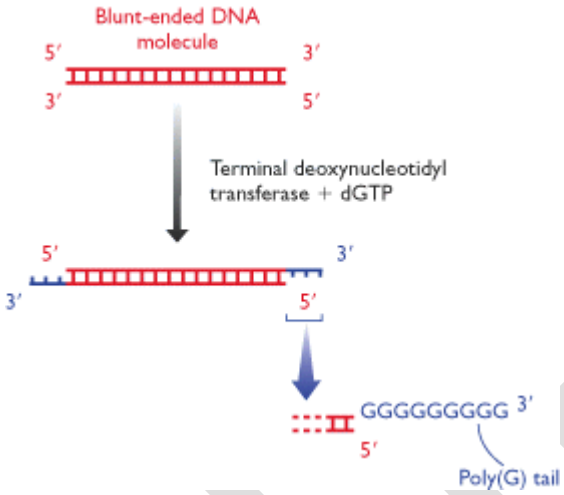


Fig: Joining of DNA

Isolation of DNA

LYSIS PROCEDURE: (SDS/Proteinase K):

1. Get the cells ready. Keep it cold, Do something physical if need be. In our case, we are working with notoriously tough tissue – i.e. that from a corn plant. We will use a mortar and pestle in the presence of liquid nitrogen to work at our sample. NOTE that in our case, this step is more about breaking open the plant cell wall so that cells can get out, rather than "lysing cells" per se.

2. Then Lyse the cells open -> to get at DNA since it is inside your cell. Make DNA easily assessible for subsequent steps. Lyse cells with **Tris**(buffering agent -> good @pH6-8).

EDTA chelates divalent cations which are necessary cofactors for DNase activity (way of shutting

down nucleases)

NaCl at physiological concentration (generally considered to be 100 – 150mM) Keeps all molecules happy (particularily proteinase k)/prevents unwanted aggregation.

SDS, nasty ionic detergent/ good at breaking membrane, general denaturant (inhibit enzyme activity). Since DNA is so robust, not really adversely affected by SDS treatment.

This essentially behaves in a similar manner to SDS.

Proteinase K->serine protease (works well at 55^{o}C), used because it is very effective and not particularily susceptible to SDS, and other denaturants such as urea. Proteinase K will chew up protein, which helps lysis in general and frees up the DNA from any protein gunk associated with it (euchromatin structures/histones, etc). Best used FRESH (*quite an important step). Incubation step generally a minimum of an hour. Although most will allow the procedure to go longer (this may be largely dependent on material being used – i.e. many procedures outline an overnight or >16hrs incubation time). I.E. mouse tails -> may want to go overnight). it's important to realize that there are many variations of the lysis procedure. Some are quicker, some are more efficient, some are more expensive, some only work in certain situations.

This purification procedure works on the principle of **"differential solubility".**

1. To your lysate, you will add an equivalent volume Phenol/Chloroform/Isoamyl alcohol. (usually at a volume ratio of 24:23:1). Phenol – organic solvent/ nucleic acids not soluble at all. Therefore, DNA/RNA will stay dissolved in aq phase. Lipids and polysaccharides preferentially go into the phenol phase. Proteins will also selectively go into phenol solution.

2. FURTHERMORE phenol also acts as a denaturant; proteins denature form aggregates and will collect at the interface.

3. **Choloroform**, also has same general attributes as phenol (as far as solvent properties) but also stabilizes the rather unstable boundary between aq and organic layers. Isoamyl alcohol also contributes to interface stability and also helps prevent frothing. Generally you do this step ~2 or 3 times. The more times you do it, the cleaner your sample (you may even note that the interphase gets cleaner and cleaner with each step). Note this procedure is very reliable and does not lose much DNA yield. This is probably why a lot of labs still like to use it.

GENOMIC DNA: DNA Precipitation.

PRECIPITATION OF DNA (alcohol and salt procedure)

You will add **Ammonium Acetate**. 0.5volume. Why? Helps in the precipitation of the DNA in EtOH. Can use NaCl, can omit entirely (dependant on concentration of DNA). Salt will help neutralize negative charge of DNA (will also sequester the solvent molecules - in this case water) Salt will also interact with water, thereby weakening it's solvating prowess. This is commonly known as "salting out" Use 100% EtOH. Time frame (show graph) EtOH generally helps because it is a much crappier solvent than something like water (which is very polar). DNA will tend to stay precipitated in >65% Ethanol.

NOTE that efficiency of EtOH precipitation is dependant on a number of things. Temp, time, amount of DNA. Can also use isopropanol for precipitation steps. RNA tends to stay soluble in this solvent (selective precipitation). which is why some people use it for this purpose. DNA will tend to stay precipitated in >50% isopropanol. Use glass pasteur pipette to spool DNA out carefully. Dip in 70% ethanol, and resuspend in TE. The glass pipette technique seems to be favoured solely for SPEED. It is a very quick way to retrieve your DNA and also wash it.

Using Agarose. Polysaccharide polymer. Used because of its ability to form pore sizes capable of resolving ~ 0.2kb to ~60kb, (200bp to 60,000bp).

Essentially, creates a big mesh of fibers that your DNA has to pass through. Electrical charge is the driving force and things will separate according to size.

DNA works well in this set-up because for things to separate in correlative manner, all DNA species generally have about the same charge density. ALSO, in our case, the DNA we are looking at has been cut with restriction enzymes - therefore all DNA fragments are predominantly linear in shape.

Loading buffer: Glycerol: thickens sample up so that it doesn't float away after you load it into the well 0.1 M EDT. A stop reagent for the assay 1% SDS help denature the RE stop the reaction. 0.1% bromophenol blue. Dye. Helps you visualize sample when loading. Will run towards +electrode. Can use it as a rough idea of where your DNA may be running (dependant on gel%)

Running buffer: Tris Borate EDTA (TBE) In this case, borate is your ion, which allows the generation of an electric field in the gel set-up. Your common alternative is TAE: Here, acetate acts as an alternative ion - is often used because it works and is much cheaper. Need 50c for 10L of TAE. But tris/borate has a significantly better buffering capacity, which means gel running is more reliable especially at high voltages (for speed), or long running times (i.e. overnight). BUT borate (when preparing the gel in microwave or oven) also forms complexes with the agarose sugar monomers/polymers. Can be a problem if using procedures to isolate band from a gel (i.e. melting of gel is required), although most band extraction kits come with chemistry to deal with this.

Visualizing the DNA: Most people now use SYBR stains Examples include SYBR SAFE,/SYBR Green I/ SYBR GOLD which are exceptionally sensitive nucleic acid gel stains with bright fluorescence when bound to dsDNA and low background in gels, making it ideal for detecting dsDNA in gels using laser scanners or standard UV transilluminators. Compared to Ethidium Bromide, these stains are generally about a hundred fold more sensitive, and less carcinogenic to boot. Stain can occur during run, or after run. Alternatively, you also have Ethidium Bromide (carcinogen), also sensitive stain that interchelates DNA (which has the added ability of slightly uncoiling it). Need to use a UV lightbox to see it – take care to use appropriate eye shielding. Some labs add EtBr into gel. Some add it after gel has run (i.e. stain with solution containing EtBr) -> adding it into the gel is much easier, but if the apparent molecular weight of closed circular DNA is particularily important to you, it may be worth adding after so that it doesn't affect its molecular weight. FOR EtBr, you should inquire at your health, safety department, as many research facilities are phasing out EtBr use.

PLASMID PREPARATION

Isolating plasmid DNA from other types of DNA (i.e. genomic) is actually very simple. In short, it usually involves a denaturing step, followed by a quick renaturing step. The IDEA is that plasmid DNA being much smaller, can renature relatively easily - consequently, once back to normal it can go into solution easily. Something like genomic DNA will have an incredibly hard time renaturing because it is simply too big and too complicated. It doesn't renature effectively and instead tangles up and precipitates out. if you think about it, you have now separated your plasmid (in solution) from your genomic (out of solution) prep. You simply have to centrifuge away the genomic pellet, and you are left with your plasmid

LYSIS METHOD (which also makes an appearance in practically all kit based plasmid prep methodologies).

Here the idea is to chemically denature and renature.

1. Need to open the cells up. NaOH and SDS. Ruptures cells, and denatures everything. Low pH

specifically breaks H bonds in dsDNA. O.K. so your test tube is now this messy mix of denatured stuff. Genomic DNA (big) -> denatured. Plasmid DNA (small) -> denatured. Proteins -> denatured. THROW IN salt that is acidic (KAc pH4.8). Salt helps in the precipitation process. Acid -> causes things to go back to neutral. DNA can renature BUT happens very quickly. Large DNA renatures as a MESS. Small DNA renatures. So, genomic DNA will precipitate out (should see a white mess), but your plasmid DNA will now be in solution.

ALTERNATIVE QUICK AND DIRTY PLASMID PREP METHOD:

Via causing the cells to lyse by using STET + lysozyme. and then the trick is to boil and then cool (this provides the denature and renature step).

RNA: Isolation and Purification

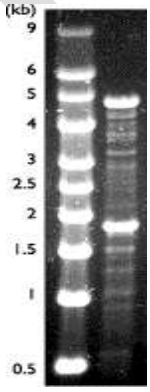
We are extracting RNA from your cheek cells using a product called Trizol.

Although this reagent is essentially a proprietory product, it is undoubtably based on the familiar Phenol+Guanidium Thiocyanate procedure. HERE, Guanidium thiocyanate is often classed as a denaturant although it is also considered a chaotropic salt (sucks water) This methods works in an analogous fashion to phenol chloroform extraction except that in this case, you also want to get your DNA to go into the phenol layer (therefore, you are left with just RNA in the the aqueous phase). This Trizol business works because RNA is still water soluble in a high molar guanidium thiocyanate solution whereas proteins and DNA is not. Consequently, the insoluble components will tend to go to the organic phase. Trizol, whilst a bit old school, is arguably still one of the best ways to get a total RNA prep from cellular material.

ALTERNATE PROCEDURES

Another way of getting RNA (at least in eukaryotes) is to utilize the fact that your RNA resides in the cytoplasm whilst the DNA resides in the nucleus (at least for eukaryotes). Consequently, an option is to first treat the cell with a "gentle" detergent to lyse the cell membrane but leave the nuclear membrane intact. Examples of common detergents used for this purpose are Triton X-100 and NP-40 (these two are almost identical). NOTE: The biochemistry and behaviour of detergents is very complicated. Consequently when dealing with a detergent, it is a good idea to follow the procedures given rather than playing around too much. Detergents have many attributes that affect their effectiveness. Detergents forming micelles don't work as well and micelle formation is very sensitive to both temperature and concentration effects which vary enormously from detergent to detergent.

CHECKING STUFF OUT ON A GEL



Running RNA on a gel has a few extra considerations to note. Firstly, most systems use a MOPS, MES buffer (getting away from Tris). In addition, RNA is usually treated with a denaturant step (i.e. + formaldehyde, or use DMSO + glyoxal), since RNA loves to form tertiary structures.

NOTE that total RNA preps look decidedly unspectacular. Normally, you see two or three very bright bands corresponding to ribosomal RNA .

Spectrophotometric quantification of DNA and RNA

Because DNA and RNA absorb ultraviolet light, with a absorption peak at 260nm wavelength, spectrophotometers are commonly used to determine the concentration of DNA in a solution. Inside a spectrophotometer, a sample is exposed to ultraviolet light at 260 nm, and a photo-detector measures the light that passes through the sample. The more light absorbed by the sample, the higher the nucleic acid concentration in the sample.

Using the Beer-Lambert law it is possible to relate the amount of light absorbed to the concentration of the absorbing molecule. At a wavelength of 260 nm, the extinction coefficient for double-stranded DNA is 50 (µg/ml)-1 cm-1; for single-stranded DNA and RNA it is 38 (µg/ml)-1 cm-1. Thus, an optical density (or "OD") of 1 corresponds to 50 µg/ml for double-stranded DNA, 38 µg/ml for single-stranded DNA and RNA. This method of calculation is valid for up to an OD of at least 2

Concentration of double stranded DNA = absorvance at 260nm * 50 * dilution (if you diluted 1 in 100 to quantify, the dilution value in this case will be 100)

Concentration of single-stranded DNA and RNA = absorvance at 260nm * 38 * dilution (if you diluted 1 in 50 to quantify, the dilution value in this case will be 50)

Migration of DNA Fragments in Agarose

Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the $\log_{10}$ of their molecular weight. In other words, if you plot the distance from the well that DNA fragments have migrated against the $\log_{10}$ of either their molecular weights or number of base pairs, a roughly straight line will appear.

Circular forms of DNA migrate in agarose distinctly differently from linear DNAs of the same mass. Typically, uncut plasmids will appear to migrate more rapidly than the same plasmid when linearized. Additionally, most preparations of uncut plasmid contain at least two topologically-different forms of DNA, corresponding to supercoiled forms and nicked circles. The image to the right shows an ethidium-stained gel with uncut plasmid in the left lane and the same plasmid linearized at a single site in the right lane.

Several additional factors have important effects on the mobility of DNA fragments in agarose gels, and can be used to your advantage in optimizing separation of DNA fragments. Chief among these factors are:

Agarose Concentration: By using gels with different concentrations of agarose, one can resolve different sizes of DNA fragments. Higher concentrations of agarose facilite separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.

The image to the right shows migration of a set of DNA fragments in three concentrations of agarose, all of which were in the same gel tray and electrophoresed at the same voltage and for identical times. Notice how the larger

fragments are much better resolved in the 0.7% gel, while the small fragments separated best in 1.5% agarose. The 1000 bp fragment is indicated in each lane.

Voltage: As the voltage applied to a gel is increased, larger fragments migrate proportionally faster that small fragments. For that reason, the best resolution of fragments larger than about 2 kb is attained by applying no more than 5 volts per cm to the gel (the cm value is the distance between the two electrodes, not the length of the gel).

Electrophoresis buffer: Several different buffers have been recommended for electrophoresis of DNA. The most commonly used for duplex DNA are TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA). DNA fragments will migrate at somewhat different rates in these two buffers due to differences in ionic strength. Buffers not only establish a pH, but provide ions to support conductivity. If you mistakenly use water instead of buffer, there will be essentially no migration of DNA in the gel! Conversely, if you use concentrated buffer (e.g. a 10X stock solution), enough heat may be generated in the gel to melt it.

Effects of ethidium bromide: Ethidium bromide is a fluorescent dye that intercalates between bases of nucleic acids and allows very convenient detection of DNA fragments in gels, as shown by all the images on this page. As described above, it can be incorporated into agarose gels, or added to samples of DNA before loading to enable visualization of the fragments within the gel. As might be expected, binding of ethidium bromide to DNA alters its mass and rigidity, and therefore its mobility

Electrophoresis of macromolecules can be carried out in solution. However, the ability to separate molecules is compromised by their diffusion. Greater resolution is achieved if electrophoresis is carried out on semi-solid supports such as polyacrylamide or agarose gels. Gels are formed by cross-linking polymers in aqueous medium. This will form a 3-dimensional meshwork which the molecules must pass through. Polyacrylamide is a common gel for proteinelectrophoresis whereas agarose is more commonly used for nucleic acids .

Agarose gels have a larger pore size than acrylamide gels and are better suited for larger macromolecules. However, either type of gel can be applied to either nucleic acids or proteins depending on the application. Gels are formed from long polymers in a cross-linked lattice (Figure). The space between the polymers are the pores. Higher concentrations of the polymer will result in smaller average pore sizes. Polyacrylamide gels are formed by covalently cross-linking acrylamide monomers with bis-acrylamide with a free radical like persulfate ($SO4\cdot$). The cross-linking of the acrylamide polymers results in 'pores' of a defined size. The total acrylamide concentration and the ratio of bis-acrylamide to acrylamide will determine the average pore size. The polyacrylamide solution is poured into a mold and polymerized. This mold can be a cylindrical tube, but is usually a 'slab' poured between two glass plates.

Since the gel is solid with respect to the mold, all molecules are forced through the gel. Smaller molecules will be able to pass through this lattice more easily resulting in larger molecules having a lower mobility than smaller molecules. In other words, the gel acts like a molecular sieve and retains the larger molecules while letting the smaller ones pass through. (This is opposite of gel filtration where the larger molecules have a higher mobility because they to not enter the gel.) Therefore, the frictional coefficient is related to how easily a protein passes through the pores of the gel and size will be the major determinant of the mobility of molecules in a gel matrix. Protein shape and other factors will still affect mobility, but to a lesser extent. Substituting size for the frictional coefficient results in:

$$\textbf{mobility} \cong \textbf{(voltage)(charge)/(size)}$$

In other words, the mobility of a protein during gel electrophoresis is primarily a function of its charge/mass ratio.

Preparation of labeled DNA probes

The two major sources of probes are previously cloned genes and synthetic oligonucleotides. In both cases a label needs to be incorporated into the probe DNA. Radioactivity is a common label, but non-radioactive probes are also available. Four methods for incorporating label into DNA probes have been

described (Box). Nick translation is an older technique that has been replaced by random priming. Random priming is the method of choice for labeling cloned DNA fragments.

Synthetic oligonucleotides are labeled using T4 nucleotide kinase.

Random priming.

In random priming (Figure) DNA is denatured by heating and mixed with hexamers of random sequence (i.e., random primers). The random primers are usually synthesized.

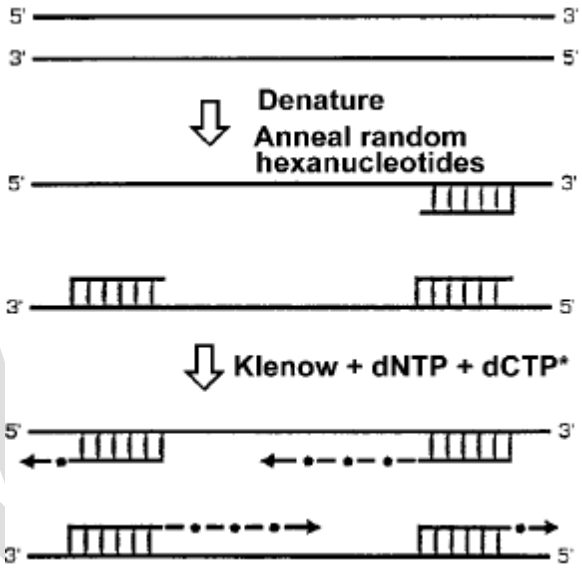


Fig: Random priming

They can also prepared from genomic DNA. A few of primers will be complementary to the probe DNA and the duplex formed between the primer and the probe DNA will serve as an initiation point for the DNA polymerase. The DNA polymerase used is **Klenow**. Klenow is the large subunit of DNA polymerase I in which the 5'→3' exonuclease activity is removed. The four dNTPs including a nucleotide containing a radioactive phosphate in the α-position are also added to the mixture. Therefore, the Klenow will make radioactive copies of the template DNA. The probe DNA is boiled immediately before use in the hybridization assay to convert the dsDNA to ssDNA.

T4 Polynucleotide Kinase

T4 polynucleotide kinase transfers the γ-PO4 from ATP to the 5'-hydroxyl of polynucleotides. It is therefore necessary to dephosphorylate the DNA with alkaline phosphatase (AP) before carrying out the phosphorylation. A disadvantage of this technique is that only one radioactive atom is incorporated per DNA strand.

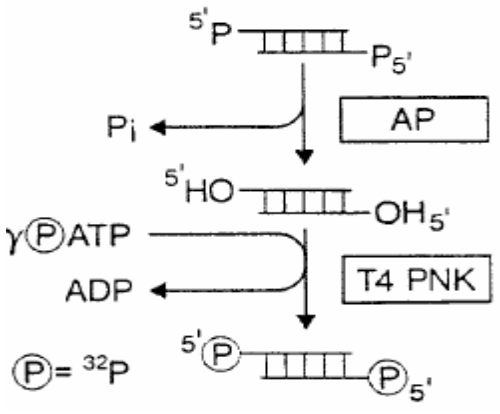


Fig: T4 Polynucleotide Kinase

However, 5'-terminal phosphorylation is widely used to label oligonucleotide probes that have been prepared synthetically. Synthetic oligonucleotides lack the 5'-phosphate and are too short for random priming. T4 kinase is also used in Maxim and Gilbert DNA sequencing and to phosphorylate (non-radioactive) synthetic linkers.

Terminal transferase

Terminal deoxynucleotide transferase (TdT) adds dNTPs to the 3-OH of either ssDNA or to 3' overhang. In the presence of Co2+ TdT will add dNTPs to the 3'-OH of either dsDNA or 5' overhangs.

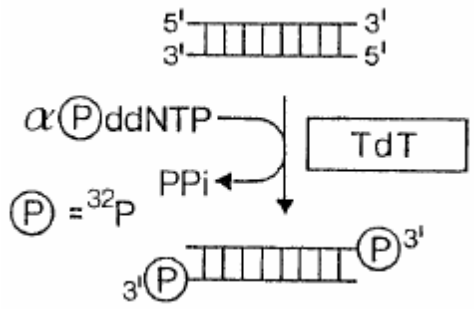


Fig:Terminal Transferase

TdT can be used to radiolabel 3' ends if radioactive nucleotides are used. A more common use, however, is to generate homopolymer tails for molecular cloning.

Non-radioactive probes

Several procedures have been devised for the detection of hybridization using non-radioactive probes (Box). All are based upon enzyme-linked systems using either alkaline phosphatase (AP) or horse-radish peroxidase (HRP). Biotinylated dNTPs can be incorporated into the probe DNA by random priming. The probe is then be detected with an enzyme-linked streptavidin. Another approach is to incorporate digoxigenin-11-(d)UTP into the DNA probe and then subsequently detected with enzymelinked antibody against the digoxigenin. A third method is to directly cross-link HRP to the DNA probe. Radioactive probes are generally more sensitive and reliable. However, non-radioactive probes can be adapted to many applications and eliminate some of the problems associated with the use of radioactivity such as waste disposal and safety issues. In addition, the use of non-radioactive probes is are particularly advantages in situation where the same probe is going to be used over a long period of time. The short half-life of 32P (14 days) necessitates that the probe be prepared on a monthly basis, whereas large amounts of a nonradioactive probe can be prepared and stored for long periods of time. Insoluble substrates, as described for Western blots, or chemiluminescent substrates can be used in association with Northern and Southern blots. Chemiluminescent substrates produce light when cleaved by the appropriate enzyme and this light is detected by autoradiography. Substrates for both alkaline phosphatase (1,2 dioxetane) and peroxidase (luminol) are available (figures). The use of chemiluminescence allows the blot to be striped and reprobed.

There are two important features of hybridization

- Hybridization reactions are specific - the probes will only bind to targets with complimentary sequence (or, in the case of antibodies, sites with the correct 3-d shape).
- Hybridization reactions will occur in the presence of large quantities of molecules similar but not identical to the target. That is, a probe can find one molecule of target in a mixture of zillions of related but non-complementary molecules.

These properties allow you to use hybridization to perform a molecular search for one DNA molecule, or one RNA molecule, or one protein molecule in a complex mixture containing many similar molecules.

Southern, Northern, and Western blots

Blots are named for the target molecule.

Southern Blot--DNA cut with restriction enzymes - probed with radioactive DNA. Northern Blot--RNA - probed with radioactive DNA or RNA. Example--used to measure angiopoietin angiopoietin expression from cDNA in Transgenicmouse. Western Blot--Protein - probed with radioactive or enzymatically-tagged antibodies.

These molecules must then be immobilized on a solid support, so that they will remain in position during probing and washing. The probe is then added, the non-specifically bound probe is removed, and the probe is detected. The place where the probe is detected corresponds to the location of the immobilized target molecule. This process is diagrammed below:

In the case of Southern, Northern, and Western blots, the initial separation of molecules is done on the basis of molecular weight, by gel electrophoresis.

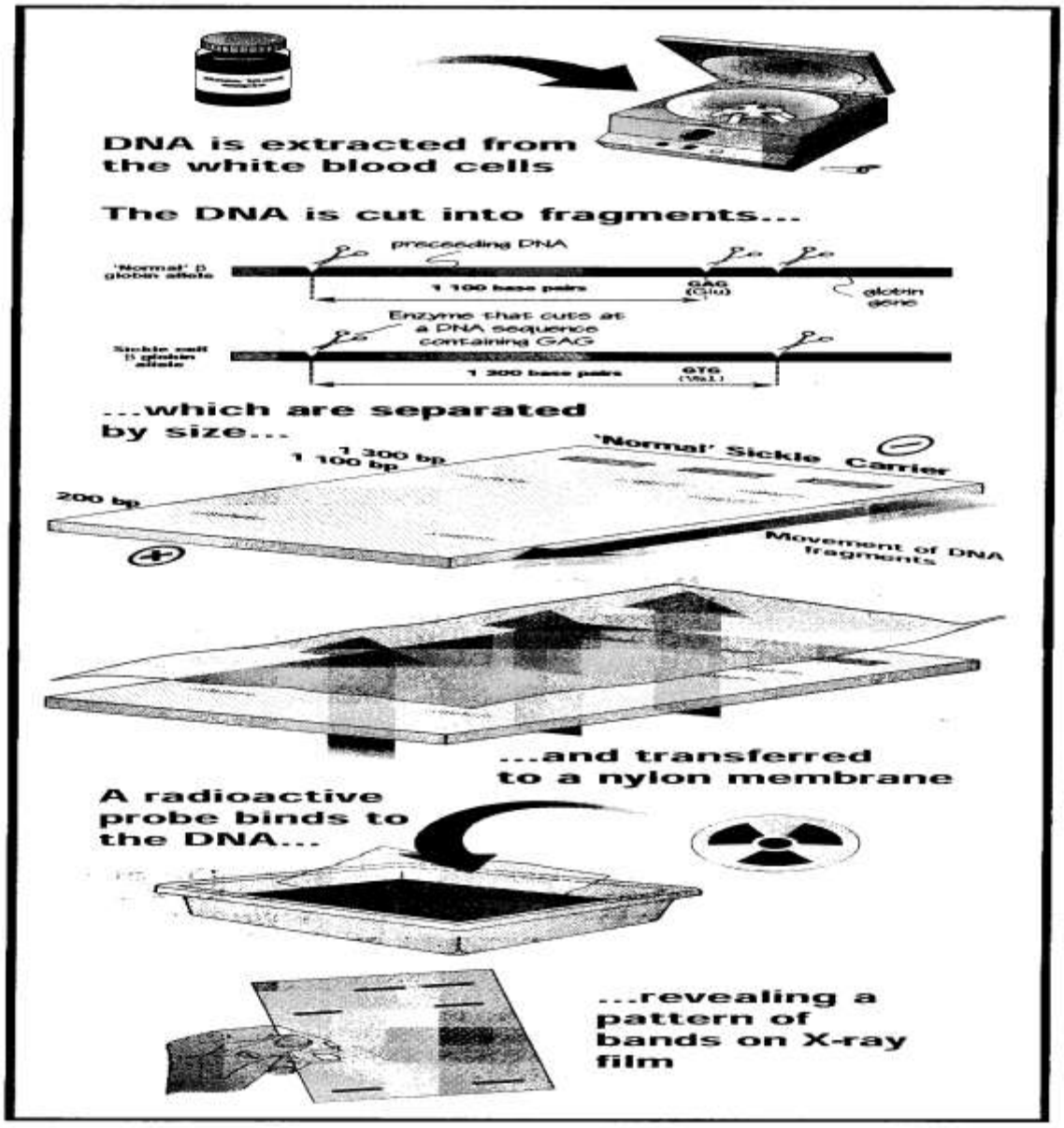
Preparing for blots

- Southern blots. DNA is first cut with restriction enzymes and the resulting double-stranded DNA fragments have an extended rod conformation without pre-treatment.
- Northern blots. Although RNA is single-stranded, RNA molecules often have small regions that can form base-paired secondary structures. To prevent this, the RNA is pre-treated with formaldehyde.
- Western blots. Proteins have extensive 2' and 3' structures and are not always negatively charged. Proteins are treated with the detergent SDS (sodium dodecyl sulfate) which removes 2' and 3' structure and coats the protein with negative charges.

Transfer to solid support. After the DNA, RNA, or protein has been separated by molecular weight, it must be transferred to a solid support before hybridization. (Hybridization does not work well in a gel.) This transfer process is called blotting and is why these hybridization techniques are called blots. Usually, the solid support is a sheet of nitrocellulose paper (sometimes called a filter because the sheets of nitrocellulose were originally used as filter paper), although other materials are sometimes used. DNA, RNA, and protein stick well to nitrocellulose in a sequence-independent manner.

After a series of treatment steps, the probe is added. The probe hybridized to the target molecules is visualized either by autoradiography or by enzyme reaction.

A southern blot involves the transfer of DNA from a gel (where it has been separated according to size) to a special type of membrane. The DNA on the member (which is in a denatured or single stranded state) is exposed to a probe. A probe is a short sequence of DNA that is complimentary to, and thus binds to, a DNA sequence of interest. Probe bound to the membrane is then visualized: this can be achieved by labelling the probe with radiation and exposing the membrane to X-ray film. A Southern Blot will usually show the alleles of VNTR's on all chromosomes, giving a complex pattern known as a DNA fingerprint.

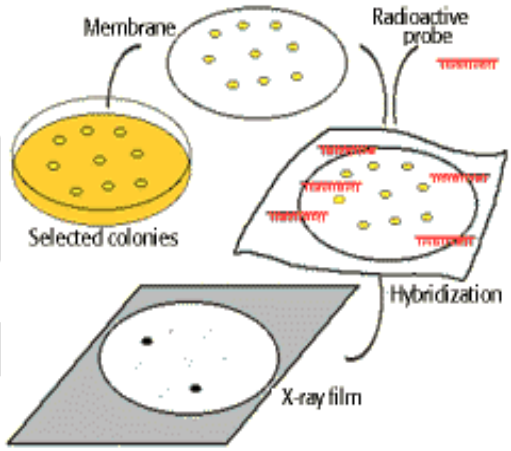

DNA is extracted from the white blood cells
The DNA is cut into fragments...
preceeding DNA
'Normal' β globin allele
1 100 base pairs
GAG (Glu)
globin gene
Enzyme that cuts at a DNA sequence containing GAG
Sickle cell β globin allele
1 300 base pairs
GTG (Val)
...which are separated by size...
1 300 bp
1 100 bp
200 bp
'Normal'
Sickle Carrier
Movement of DNA fragments
...and transferred to a nylon membrane
A radioactive probe binds to the DNA...
...revealing a pattern of bands on X-ray film

The important properties of the three blotting procedures of DNA analysis:

	Southern	Northern	Western
What is separated by molecular weight? (target)	DNA cut with restriction enzymes	RNA denatured with formaldehyde	Protein denatured with SDS
Probe	radioactive gene X DNA	radioactive gene X DNA	Antibody against protein X, labeled with radioactivity or enzyme
What do you learn from it?	Restriction map of gene X in chromosome	-how much gene X mRNA is present? -how long is gene X mRNA?	-how much protein X is present? -how big is protein X?

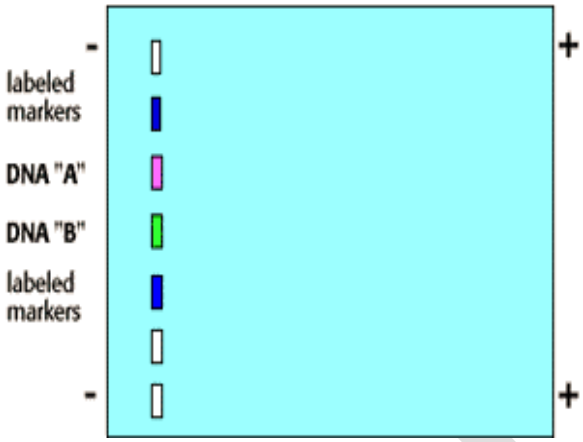
By *Insitu* hybridization technique

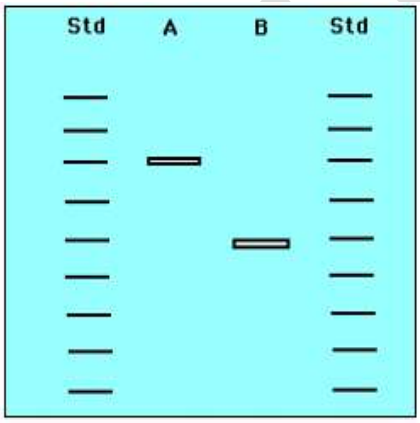
In the following scheme, bacterial containing recombinant plasmids are grown as clones. The clones are blot transferred to a membrane sheet, and the DNA present denatured and fixed onto the surface. Adding a radioactive "probe" or complementary fragment and allowing the DNA to hybridize followed by exposure to X-ray film identifies the clone containing recombinant DNA with the correct insert.

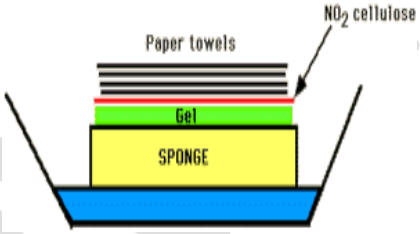


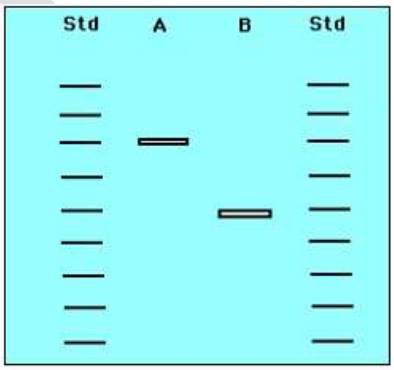
Southern blotting

DNA is taken from different colonies A and B. It is digested with restriction endonuclease. Fragments are applied onto an Agarose gel for electrophoresis. DNA has a negative charge, and in an electric field migrates towards a positive electrode. The rate of migration through a gel is proportional to the size of the fragment. DNA fragments are transferred to nitrocellulose sheets where they bind. DNA fragments are denatured and separated by gel electrophoresis. Fragments are blotted onto a sheet of nitrocellulose and fixed by heating. Blot is reacted with a radioactive probe of RNA or DNA which binds to complementary DNA. Autoradiography is used to detect radioactive fragments. The denatures fragments of DNA are fixed by baking. A radioactive probe is added. It can hybridize with a gene sequence in the DNA. The sheet is rinsed and placed next to X-ray film for autoradiography. Presence of radioactivity suggests the presence of gene of interest.


-
+
labeled markers
DNA "A"
DNA "B"
labeled markers
-
+


Std
A
B
Std


NO_2 cellulose
Paper towels
Gel
SPONGE


Std
A
B
Std

UNIT I - POSSIBLE QUESTIONS

PART B

1. How will you classify the restriction endo nuclease enzyme?
2. What is restriction endo nuclease explain with examples?
3. Briefly explain about type II restriction endonuclease enzyme with examples.
4. What are DNA modifying enzyme and discuss with suitable examples?
5. Write note on DNA polymerase and its uses.
6. What is nick translation explain with suitable examples.
7. Explain about T4 ligase enzyme and its uses.
8. How will you isolate DNA from microbial cells?
9. Describe the Southern blotting techniques?
10. Explain the techniques involved in isolation of DNA
11. Brief on DNA cloning.
12. Write note on radio labeling of nucleic acid

PART C

1. Explain in detail about blotting techniques and its application
2. Describe various gel electrophoresis method for probing a specific gene.
3. Explain Type II restriction enzymes its restriction modification system

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UNIT I

_____ are the enzymes are used to cut the target DNA fragments.	Ligases
_____ is the restriction site of E.coRI.	5' AAGCTT 3' 3' TTCGAA 5'
In southern blotting, the kind of filter paper used for blotting is	Whatmann No.1
The blotted filter paper is baked at …….	90 °C
In southern blotting, the kind of filter paper used for blotting is ……..	Whatmann No.1
._____ enzymes enable the breakage in internal phosphodiester bonds within a DNA molecule	Endonucleases
DNA ligases are isolated from	Bovine pancreas
_____ are also known as DNA ligases	Klenow fragment
Southern blotting technique helps in detecting fragments of	RNA
The southern blotting technique can be used for the	Separation of DNA
Northern blotting is used for _____	Detection of RNA
System of naming restriction enzymes was proposed by	Smith
_____ enzyme mediates Nick translation	DNA Polymerase I
Stain used in gel electrophoresis for the detection of DNA	Ethidium bromide
Western blotting involves ----------	DNA probe
…….. gel electrophoresis is used for the separation of DNA fragments	Agarose
Enzyme used to remove unannealed regions of RNA from DNA:RNA hybrids	Exonuclease III
RNA is tightly associated with	L ipids
Precipitation of RNA can be taken place by	Ethanol
Commonly used reagent in RNA extraction is	EDTA
The process by which the foreign DNA escapes host restriction is	Cloning
_____ are the enzymes are used to cut the target DNA fragments.	Ligases

Alkali treatment of DNA fragment results in the ……	Disruption
The blotted filter paper is baked at …….	90 °C
In southern blotting, the kind of filter paper used for blotting is ……..	Whatmann No.1
Western blotting is used for the identification of	DNA fragment
Solvent used in western blotting involve	SDS
Western blotting is based on the principle	Ag-Ab reaction
The Type I restriction enzyme need ……..	Methyl gps
Western blotting detects protein even at	low as 5 ug
The cutting of DNA takes place with the enzyme at _____ sites.	identification site
Site specific clearage is carried out by	Type III
……. are the enzymes are used to cut the target DNA fragments	Ligases
The cleavage site of Type III enzymes is about _____ away from the recognition site.	30 kbp
The symbol for type I restriction systems	Mod
Cofactor for type II system	mn 2+
The presence of restriction enzyme was postulated by	Werner Arber
Restriction enzymes mostly preferred for genetic engineering are of type	Type I
Use of only one single enzyme for DNA digestion during the construction of restriction map is called	Single digestion
Altering the optimal conditions for the activity of restriction enzymes as to skip some of their restriction sites during the construction of restriction maps is called	Single digestion
Two restriction enztmes which have the same recognition sequence but leave at different sites ……	Neoschizomers
The recognition / cleavage site of Type II enzymes have ___	Same site
In supercoiled DNA, if both polynucleotide strands are intact, they are describes as	CAD
CsCI2 density gradient centrifugation is to separate	nucleus
Mostly density gradient centrifugation can be used to separate supercoiled DNA from non-supercoiled DNA by using intercalating agent is	EtBr(ethidium bromide)
_____ will yield multiple copies of plasmid	Plasmid amplification
Radiolabelling of nucleic acid is done by	Horse radish peroxidose system
In vitro labeling of nucleic acid is done by	Probe preparation by PCR

Gel electrophoresis separates DNA molecules according to their	Shape
Stain used in gel electrophoresis for the detection of DNA	Ethidium bromide
DNA molecules in gel electrophoresis is labeled by radioactive isotope of	Phosphorous
Which is the effective methodology for separation of intact proteins by molecular weight	1D-SDS PAGE
The most commonly used stain for protein detection is	Methylene blue
Photochemical polymerization of polyacrylamids gel is initiated by	Riboflavin
Which is the tracking dye used in SDS-PAGE	Bromophenol blue
What is the role of SDS in SDS-PAGE	Denative the proteins
Agarose is made up of	Glucose
The polymerization of acrylamide is initiated by the addition of	Ammonium persulfate
PH of stacking gel is	7.8

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Restriction endonucleases	Methylases	Exo Nucleases
5'GAATTC 3' 3'CTTAAG 5'	5' CCCGGG 3' 3' GGGCCC 5'	5' GATC 3' 3' CTAG 5'
Aminobenzyloxymethyl	Nitrocellulose filter paper	Whatmann No.509
82 °C	100 °C	80 °C
Nitrocellulose filter paper	Aminobenzyloxymethyl	Whatmann No.509
. Exonucleases	DNaseI	S1 nucleases
Avian mycloblastosis	*E.coli* infected with phage T4	sheep
. Molecular sutures	Molecular scissors	Holo enzyme
DNA & RNA	DNA	Potein
Screening of recombinants	Denaturation of DNA	DNA sequencing
Detection of DNA	Detection of protein	Detection of plasmid
Nathans	Wilcox	Smith and Nathans
DNA Polymerase II	. DNA Polymerase III	RNA Polymerase
Crystal violet	Malachite green	Bromothymol blue
RNA probe	. Protein probe	Antibody probe
PAGE	SDS-PAGE	Agarose & SDS-PAGE
Ribonuclease T1	nuclease	Endonuclease
Amino acids	Proteins	carbohydrates
Alcohol	Formaldehyde	methane
Guanadinium Thiocyanate	NAOH	SDS
Host control restriction & modification	Sequencing	Blotting
Restriction endonucleases	Methylases	Exo Nucleases

Denaturation	depurination	none of the above
82 °C	100 °C	80 °C
Nitrocellulose filter paper	Aminobenzyloxymethyl	Whatmann No.509
RNA fragment	Antibodies	Protein
Ethanol	Chloroform	methanol
Electrophoresis	Hybridisation	Translocation
Sulfur gps	Fe+	S
low as 10 ug	less than or equal to 15 ug	less than 0.5 ug
Cleavage site	Restriction site	clear site
Type I	Type II	Type IV
Methylases	Restriction endonucleases	Exo Nucleases
24 – 26 kbp	24 – 28 kbp	40 kbp
hsd	Res	sap
ca 2+	mg 2+	fe 3+
Watson	Smith	Nathan
Type II	Type III	Type IV
Double digestion	restrictive digestion	end labeling
Double digestion	Partial digestion	End labeling
Isoschizomers	Epimers	Isomers.
Different site	Adjacent site	modified site
Open circular DNA	Covalently closed circle DNA	supercoiled DNA
DNA	aminoacid	membrane
$CsCI_2$	EtrBr- $CsCI_2$	CsNo2
Plasmid purification	plasmid denaturation	plasmid multiplication
DIG labelling system	Nick translation	Biotin-Streptavidin labelling system
Using 32 P	DIG labelling system	Horse radish peroxidose system

Size	Volume	Structure
Crystal violet	Malachite green	Bromothymol blue
Sulfur	Carbon	Iodine
2D-PAGE	Agarose gel electrophoresis	Nanospray – MS/MS
Coomassic Brilliant blue	Ethidium bromide	Crystal violet.
Vitamin C	Thymine	Vitamin A
Coomassic Brilliant blue	Ethidium bromide	Crystal violet
Separate the proteins	Stain the protein	precipitate the protein
Lactose	Agarobiose	Polyarylamide
Ammonium persulfate and TEMED	Riboflavin	NaCl
6.8	5.8	4.8

Restriction endonucleases
5’GAATTC 3’ 3’CTTAAG 5’
Nitrocellulose filter paper
80 °C
Nitrocellulose filter paper
Endonucleases
E.coli infected with phage T4
Molecular sutures
DNA
Screening of recombinants
Detection of RNA
Smith and Nathans
DNA Polymerase I
Ethidium bromide
Antibody probe
Agarose
Ribonuclease T1
Proteins
Ethanol
Guanadinium Thiocyanate
Host control restriction & modification
Restriction endonucleases

Denaturation
80 °C
Nitrocellulose filter paper
Protein
SDS
Ag-Ab reaction
Methyl gps
low as 5 ug
Restriction site
Type II
Restriction endonucleases
24 – 26 kbp
hsd
mg 2+
Nathan
Type II
Single digestion
Partial digestion
Neoschizomers
Same site
Open circular DNA
DNA
EtBr(ethidium bromide)
Plasmid amplification
Horse radish peroxidose system
DIG labelling system

Size
Ethidium bromide
Phosphorous
1D-SDS PAGE
Coomassic Brilliant blue
Riboflavin
Bromophenol blue
Denative the proteins
Agarobiose
Ammonium persulfate and TEMED
6.8

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UNIT – II

CLONING VECTORS

1. Requirements for a cloning vector

a) Should be capable of replicating in host cell
b) Should have convenient RE sites for inserting DNA of interest
c) Should have a selectable marker to indicate which host cells received recombinant DNA molecule
d) Should be small and easy to isolate.

It is usually a circular DNA, Primarily independent of the host chromosome, often found in bacterial and some other type of cells. Natural plasmids usually replicate independently of the bacterial chromosome. plasmids are capable of having gene with upto about 10 kb size. Many different types of plasmids have been found in bacteria. The most useful classification of naturally occurring plasmids is based on the main characteristic coded by the plasmid genes. The five main types of plasmid according to this classification are as follows:

a. F plasmids

Fertility plasmids carry only tra genes and have no characteristic beyond the ability to promote conjugative transfer of plasmids. e.g. F plasmid of *E.coli*

b. R plasmids

Resistance plasmids carry genes conferring on the host bacterium resistance to one or more antibacterial agents, such as chloramphenicol, ampicillin etc., this type of plasmids mainly used in recombinant DNA technology.

c. Col Plasmids

These plasmids code for colicins (proteins) which kill other bacterial growth e.g. ColE1 of E.Coli.

d. Degradative plasmids

These plasmids allow the host bacterium to metabolize unusual molecules such as toluene and salicylic acid. e.g. TOL of *Pseudomonas putida.*

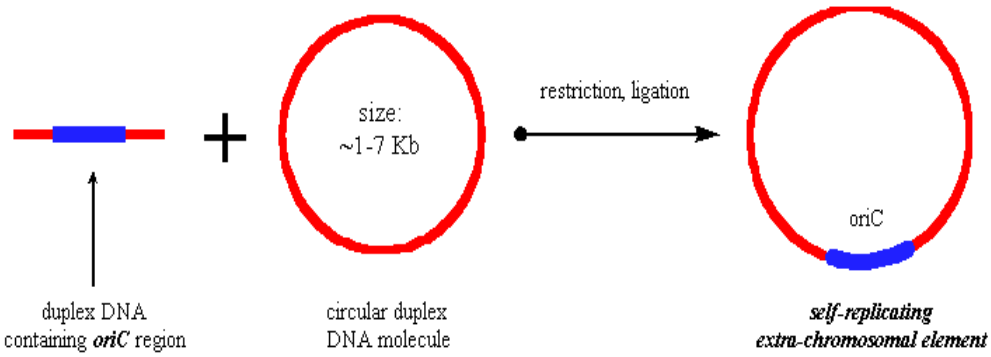
e. Virulence plasmids

They confer pathogenicity on the host bacterium e.g. Ti plasmids of *Agrobacterium tumefaciens*, which induce crown gall disease on dicotyledonous plants.

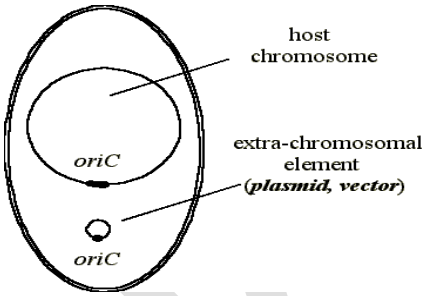
2 micron circle plasmid is an example for the plasmid which live in organism i.e. yeast other than bacteria. Two plasmid vectors that have been extensively used in genetics are pBR322 and pUC18. These vectors are derived from natural plasmids, but both have been genetically modified for convenient use as recombinant DNA vectors.

Bacterial plasmids are small, circular DNA molecules that are separate from the rest of the chromosome. They replicate independently of the bacterial chromosome. Useful for cloning DNA inserts less that 20 kb (kilobase pairs). Inserts larger than 20 kb are lost easily in the bacterial cell.

- Including an origin of replication (i.e. the *E. coli* ***oriC*** region) into a circular DNA molecule is a mechanism to have an **extrachromosomal element** in the prokaryotic cell.



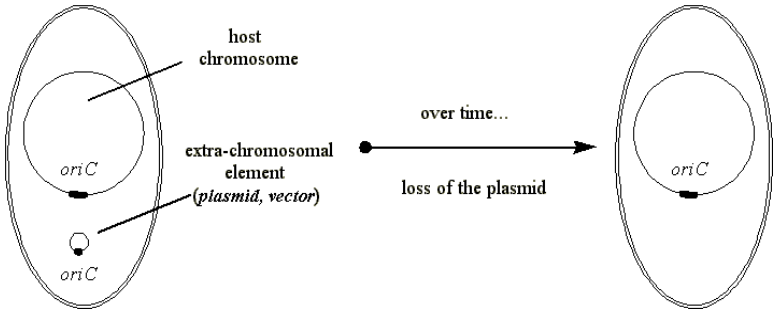
- Such an extrachromosomal element is called a ***plasmid***, or ***vector***
- The plasmid uses the host cell machinery (i.e. polymerases, helicases, dNTP's etc.) to direct replication.



- However, since the added work of replicating the extrachromosomal element is a load on a cell, it will be out-competed by other cells which do not contain the plasmid.

Since in prokaryotic cells the segregation of plasmids is a random event, daughter cells can arise which do not contain the plasmid and these grow faster (out-compete) the parent cell.

- In other words, in the absence of other pressures, after a period of time the population of cells in a culture will be those which have "lost" the plasmid.



- In organisms with more than one chromosome (eukaryotes) there are a variety of mechanisms to ensure that proper segregation of chromosomes occurs, i.e. to make sure that daughter cells contain equal numbers of all the chromosomes.

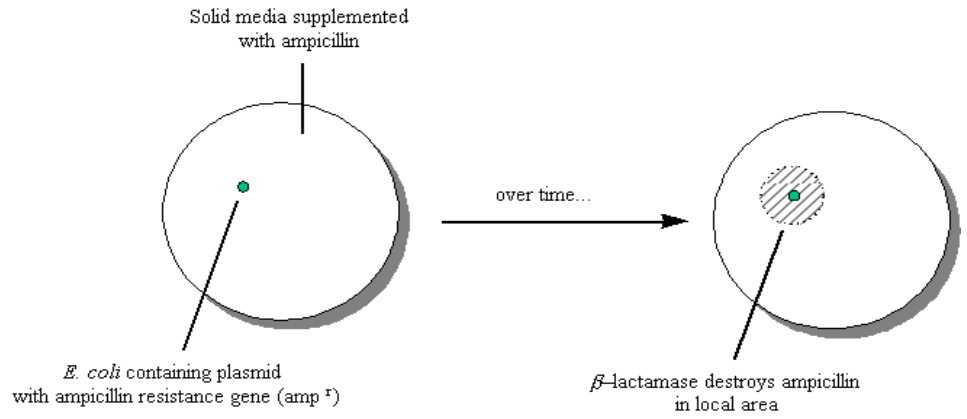
- One basic mechanism is that each chromosome contains essential genes, and if these are lost, the cell cannot survive.

Drug resistance

- By far the most common approach to the maintenance of plasmids is through the incorporation of drug resistance genes.
- These are also known as selectable markers, i.e. we can select for their presence by including *antibiotics* in the growth media.

Ampicillin

- Ampicillin binds to and inhibits a number of enzymes in the bacterial membrane that are involved in the synthesis of the *gram negative* cell wall.
- Therefore, proper cell replication cannot occur in the presence of ampicillin.
- The ampicillin resistance gene (*amp*^{r}) codes for an enzyme (beta *lactamase*) that is secreted into the periplasmic space of the bacterium where it catalyzes hydrolysis of the beta lactam ring of the ampicillin.
- Thus, the gene product of the amp^r gene destroys the antibiotic.
- Over time the ampicillin in a culture medium or petri plate may be substantially destroyed by beta lactamase.
- When this occurs, cell populations can arise which have "lost" the plasmid.



Tetracycline

- Tetracycline binds to a protein of the 30S subunit of the ribosome and inhibits ribosomal translocation along the messenger RNA which codes for the protein (i.e. the drug interferes with normal translation or production of proteins).
    - The tetracycline resistance gene (tet^r) encodes a 399 amino acid outer membrane associated protein of gram negative cells that prevents the antibiotic from entering the cell.
- Thus, this drug resistance gene does not destroy the antibiotic. Pressure will be maintained throughout the cell culture process to keep the plasmid containing the drug resistant gene.

Chloramphenicol

- Chloramphenicol binds to the ribosomal 50S subunit and inhibits protein synthesis.
- The chloramphenicol resistance gene (Cm^r) codes for a protein known at the *cat* protein.
- The *cat* protein is a tetrameric cytosolic protein that, in the presence of acetyl coenzyme A, catalyzes the formation of hydroxyl acetoxy derivatives of chloramphenicol that are unable to bind to the ribosome.

- Like with *ampicillin*, the Cm^r gene product destroys the antibiotic.
- Additionally, the expression of *cat* protein is influenced (down regulated) by the presence of glucose in the media.

Kanamycin and neomycin

- Bind to ribosomal components and inhibits protein synthesis.
- The Kan^r gene codes for a protein which is secreted into the periplasmic space and interferes with the transport of these antibiotics into the cell.
- Like tetracycline resistance, the Kan^r gene does not destroy the antibiotic.

Colicin E1

- This is a member of a general class of substances known as bacteriocins.
- *Colicin E1* causes lethal membrane changes in bacteria.
- The drug resistance gene (*cea*) codes for a protein that interferes with the action of colicin in an unknown manner.

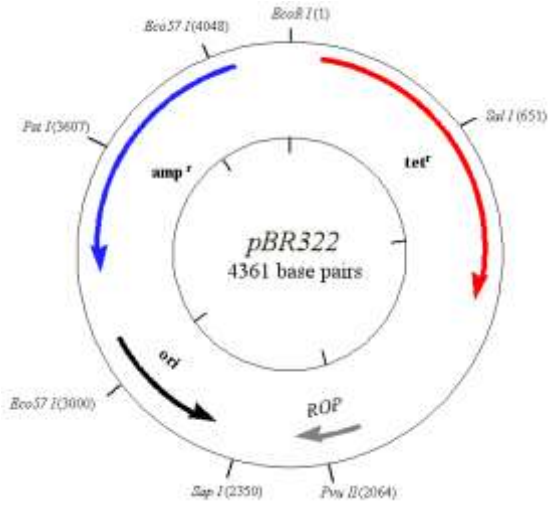
Prokaryotic plasmids

- In addition to making a plasmid by using the *E. coli OriC* region, there is a naturally occuring *E. coli* plasmid called the ColE1 plasmid
    - The ColE1 origin of replication is uni-directional (unlike *oriC*)
    - The replication from the ColE1 *ori* region does not require the associated proteins (e.g. *dnaA* protein) like *oriC,* (but it does require specific RNA molecules).
- Depending on the exact region of the ColE1 origin which is inserted into a circular dna molecule, the extrachromosomal element will be maintained with either a "low" or "high" copy number
- The rop gene near the ColE1 origin is involved in the regulation of replication.
    - If the ColE1 ori region includes this gene then the plasmid is maintained with an average copy number of 10-30 plasmids/cell. This is considered low copy number.
    - If the ColE1 ori region does not have the rop gene, then the resulting plasmid is maintained with an average copy number of 100-200 plasmids/cell. This is considered a high copy number type of plasmid.
- If the plasmid contains a gene coding for a protein (like drug resistance genes) copy number can influence the amount of such protein in the cell.

pBR322 (4.36 Kb)

- One of the original cloning plasmids.
- Constructed by ligating together:
    - the tetracycline resistance gene from plasmid pSC101
    - ColE1 and rop region from the ColE1 plasmid

- the ampicillin resistance gene from the transposon Tn3
- Contains both ampicillin and tetracycline resistance genes (markers).
- Contains unique restriction sites inside and outside of these markers.
- Contains rop region near CoE1 ori , therefore, it has a low copy number (10-30)
- Numbering begins at the unique EcoR I restriction site (GAATTC). The first 'T' in this sequence is base number "1".



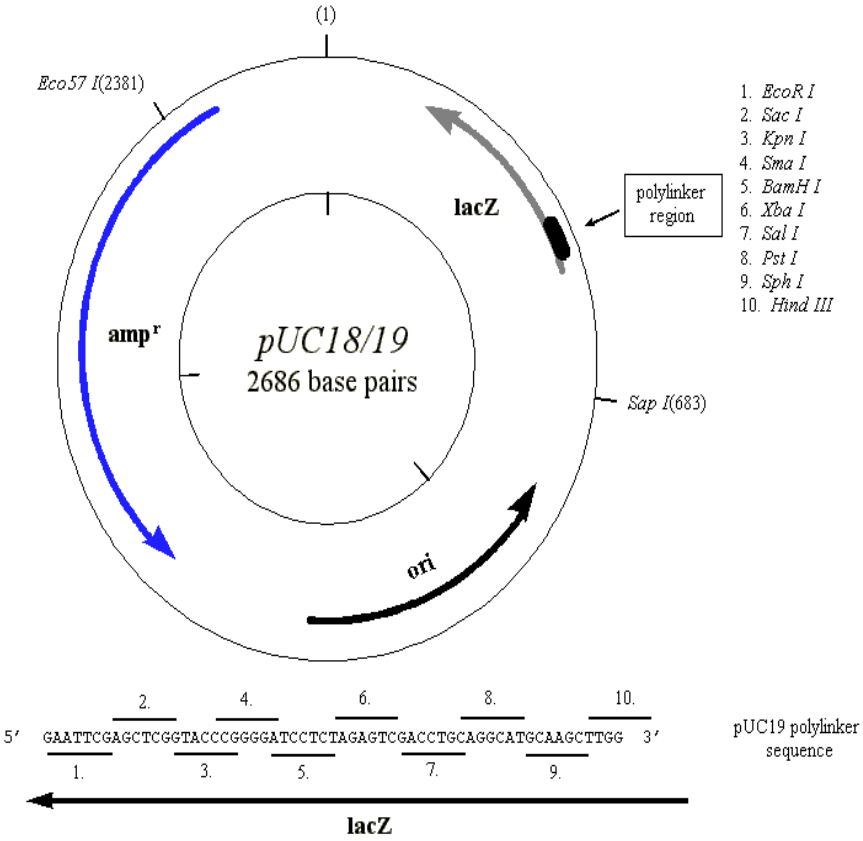
The plasmid

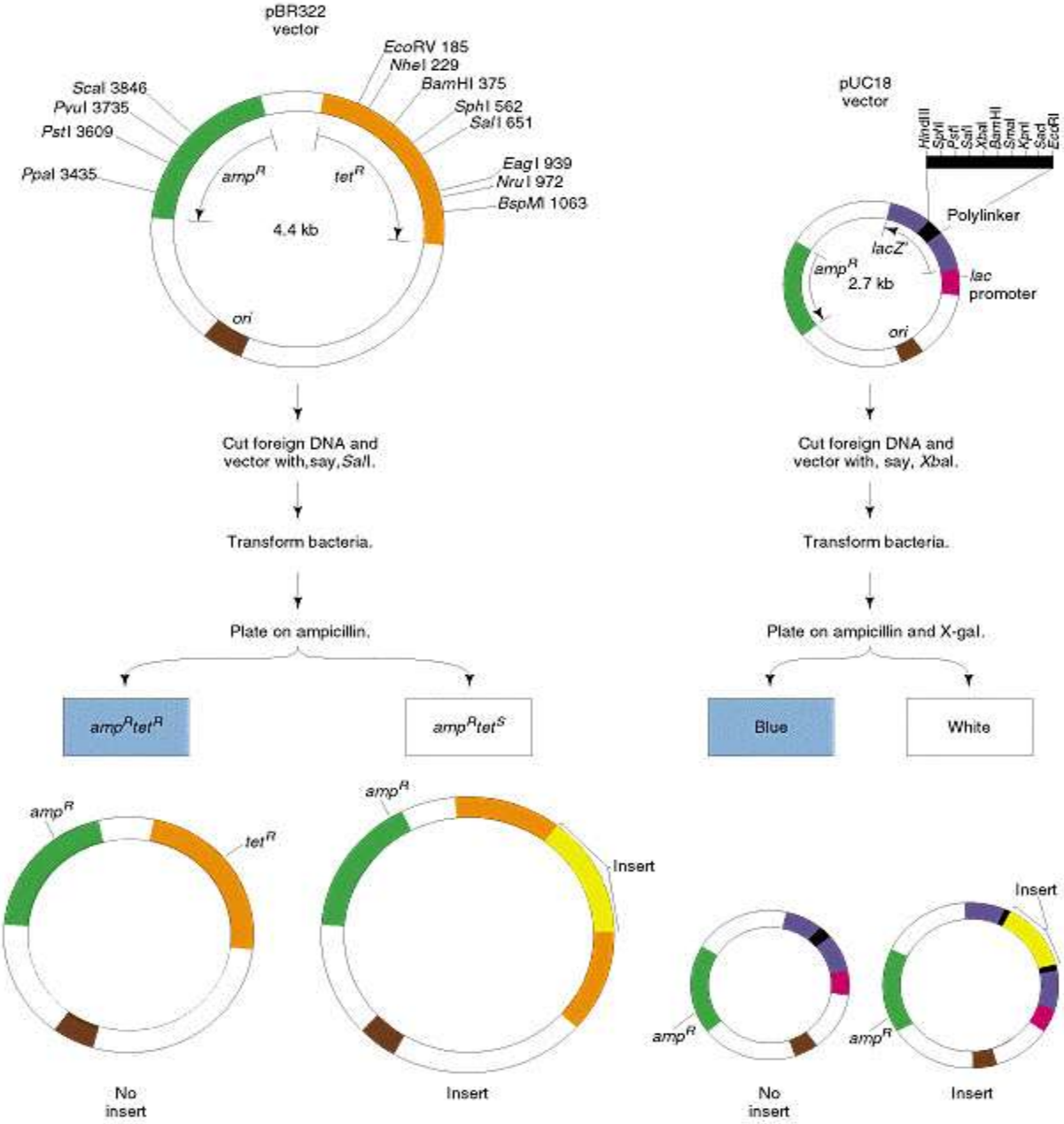
- In the center is the plasmid name (usually starts with a lower case 'p') and the size in basepairs
- the inner ring provides ticks in 1 Kilobase (Kb) intervals to give an idea of the general location of parts of the plasmid
- the arrows indicate genes, markers, ori or replication, promoters, polylinkers, transcription terminators, and other important or functional items
- the outer ring usually indicates the location of unique, or limited number (usually <3), restriction endonuclease sites. Restriction enzymes which have more than three sites will not be indicated. Be aware that enzymes which do not cut at all will also not be listed!

PUC18/19 (2.69 Kb)

- Lacks the *rop* gene near the ColE1 ori region. Thus, this plasmid tends to accumulate in high copy number (100-200).
- This vector contains only the ampicillin resistance marker.
- This vector contains a polylinker region
  - A synthetic DNA sequence which contains a clustering of unique restriction enzyme recognition sites
  - Allows DNA fragments, generated by a variety of restriction endonuclease cleavages, to be inserted into the plasmid
  - pUC18 has the polylinker in one orientation
  - pUC19 has the same polylinker, but in the opposite orientation
  - Thus, fragments with unique restriction sites on each end can be inserted in a specific orientation
  - The Pst I site in the amp^r gene was mutated to remove it. The EcoR I site at position (1) was mutated to remove it. This was done to make the restriction sites in the polylinker region unique

- This vector also contains a transcription promoter region from the lac operon, which allows foreign genes to be inserted and transcribed/translated.
    - The polylinker region is just downstream (3') to the lac promoter
    - Inserted genes can be transcribed from this promoter

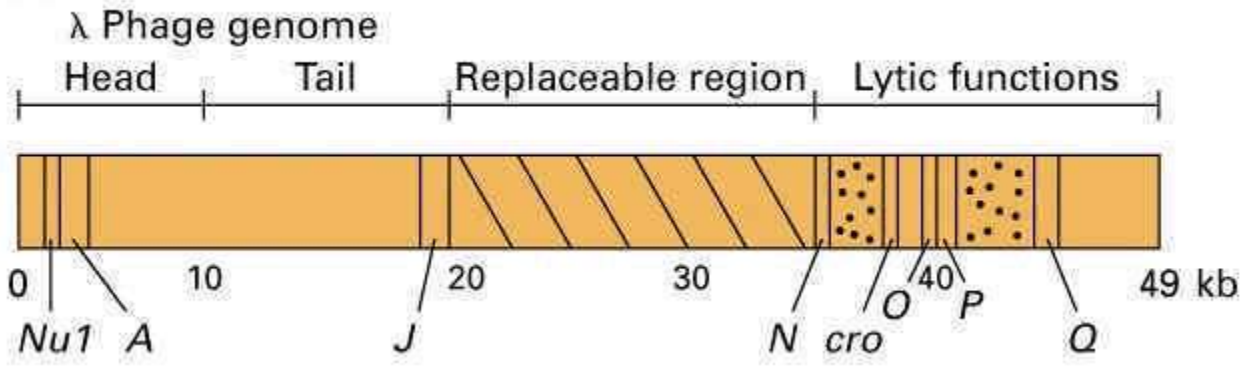




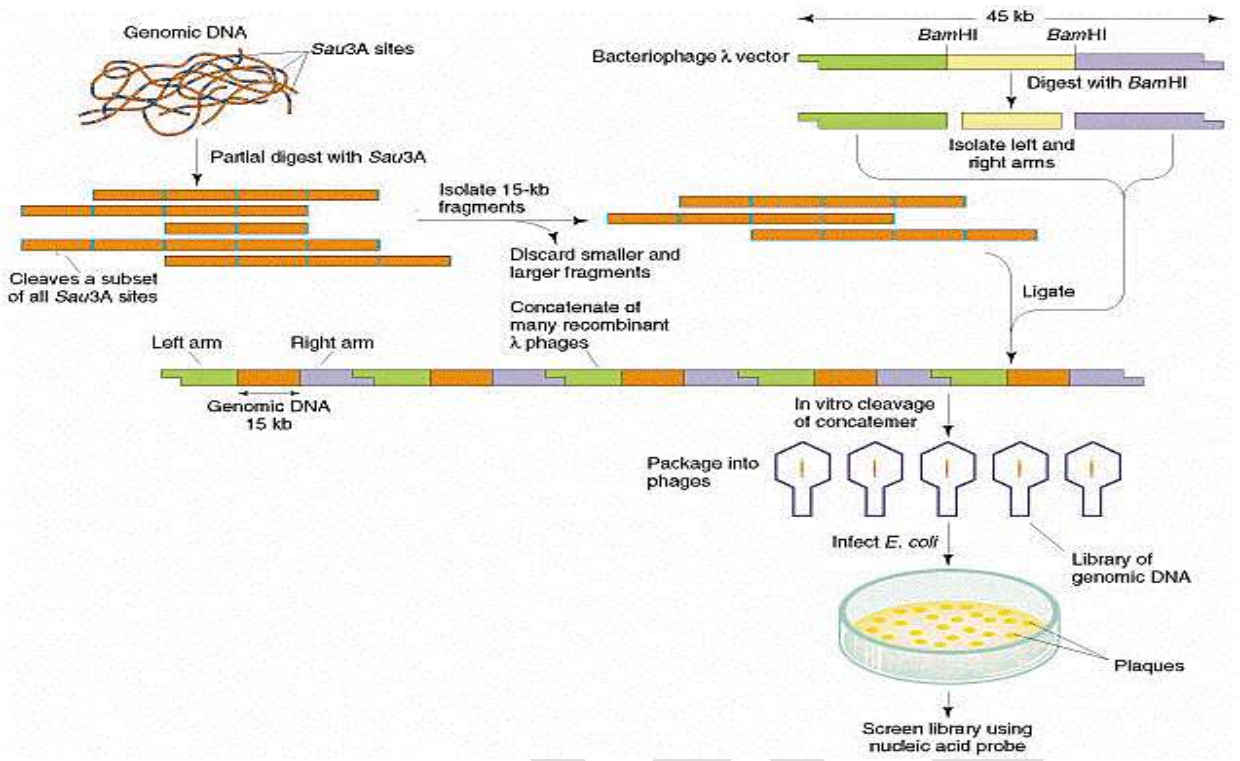
Phage vectors: Viral vectors are generally are of two types namely bacteriophage vectors and single stranded phage vectors.

Bacteriophage vectors

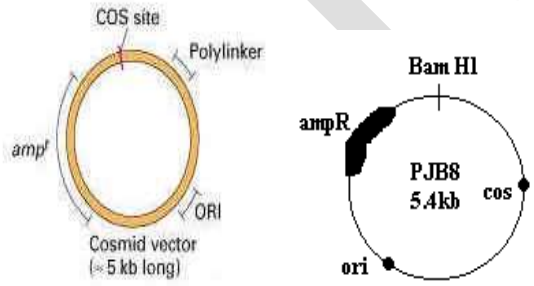
Thery are found to have several features which are advantageous as vectors. First lamda phage head capable of possessing 50kb length and central part of the phage genome is not required for replication or packaging of virus. So the central part can be utilized to insert gene of interest. Secondly, the recombinant vector packaged into phage head naturally. Third, the presence of recombinant vector can be directly selected by the lawn formation due to lytic mechanism. Finally, transforming efficiency of bacteriophage found to be greater. Bacteriophage is a virus whose host is a bacterium. Bacteriophage DNA molecules are often used as cloning vectors. These vectors can take upto 20 kb size of gene of interest.

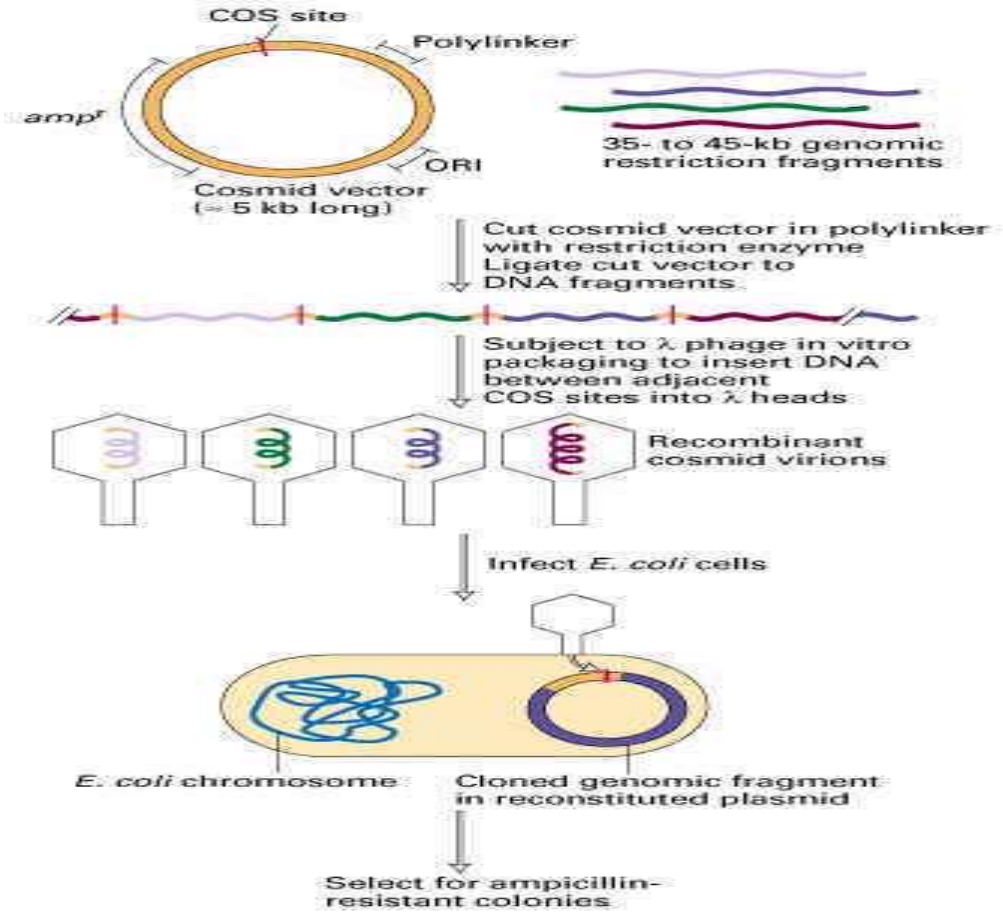


Bacteriophage lambda (45 kb) contains a central region of 15 kb that is not required for replication or formation of progeny phage in *E. coli*. Thus, lambda can be used as a cloning vector by replacing the central 15 kb with 10-15 kb of foreign DNA. This is done as follows: mix RE cut donor DNA and lambda DNA in test tube ligate use *in vitro* packaging mix that will assemble progeny phage carrying the foreign DNA infect *E. coli* with the phage to amplify.

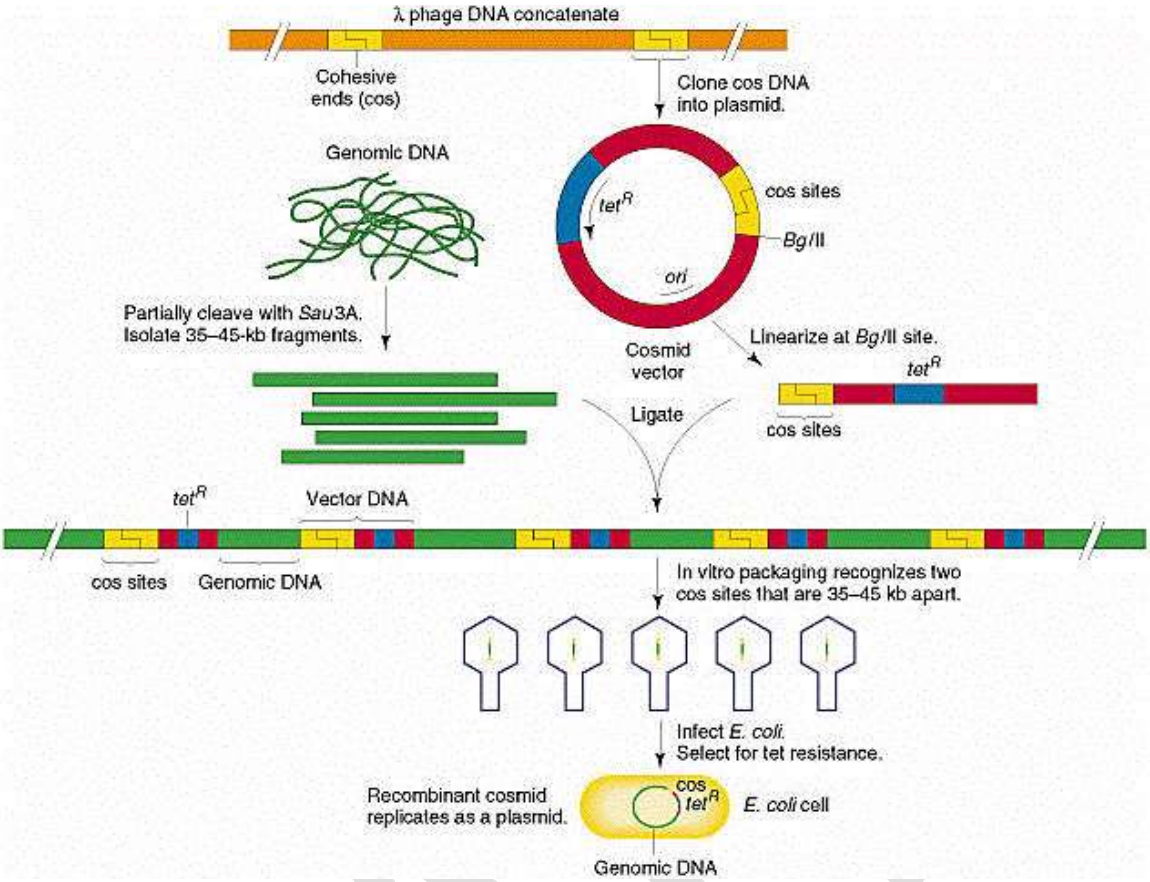


COSMIDS: It is a cloning vector consisting of the phage cos site inserted into a plasmid i.e. simply plasmid and cos sites. It is used to clone DNA fragments upto 40 kb in size. cos site is one of the cohesive, single stranded extensions present at the ends of the DNA molecules of certain strains of lamda phage. The main reason for the development of the cosmid vector is to irradiate the disadvantages of plasmids and phage vectors. Due to the formation of cosmids the low copy number property of plasmids overcome because of cos sites and rolling circle replication and the lysis of culture which is the disadvantage of phage virus overcome because the vector does not have sequence for the functional phage production. After the recombinant vector multiplied, they are packaged into virus through invitro packaging method because of the cos site in phage. Transforming efficiency increased by this package method.





Cosmids are hybrids of phages and plasmids that can carry DNA fragments up to 45 kb. They can replicate like plasmids but can be packaged like phage lambda.



Expression vectors are vectors that carry host signals that facilitate the transcription and translation of an inserted gene. They are very useful for expressing eukaryotic genes in bacteria.

The 'insert' DNA must be subcloned into the expression vector so that it is in frame with the fusion protein (or a start ATG). Most expression vectors will have a MCS with several different restriction sites. In addition, many expression vectors are designed so that variants with all three reading frames are available. Therefore, it is generally simple to choose restriction enzymes that will result in a continuous open reading frame (ORF) between the fusion protein and the foreign protein. It is also possible to shift the reading frame by cutting with restriction enzymes, filling in with Klenow, and religating (see Appendix). Digesting with an enzyme producing a 4-base overhang will result in a -1 frameshift and digesting with an enzyme producing a 2-base overhang will produce a +1 shift. In addition, stop codons can be produced by this method with certain restriction enzymes (eg., *Hin*dIII, *Spe*I).

Yeast artificial chromosomes (YACS) are yeast vectors that have been engineered to contain a centromere, telomere, origin of replication, and a selectable marker. They can carry up to 1,000 kb of DNA. Since they are maintained in yeast (a eukaryote), they are useful for cloning eukaryotic genes that contain introns. Also, eukaryotic genes are more easily expressed in a eukaryotic host such as yeast.

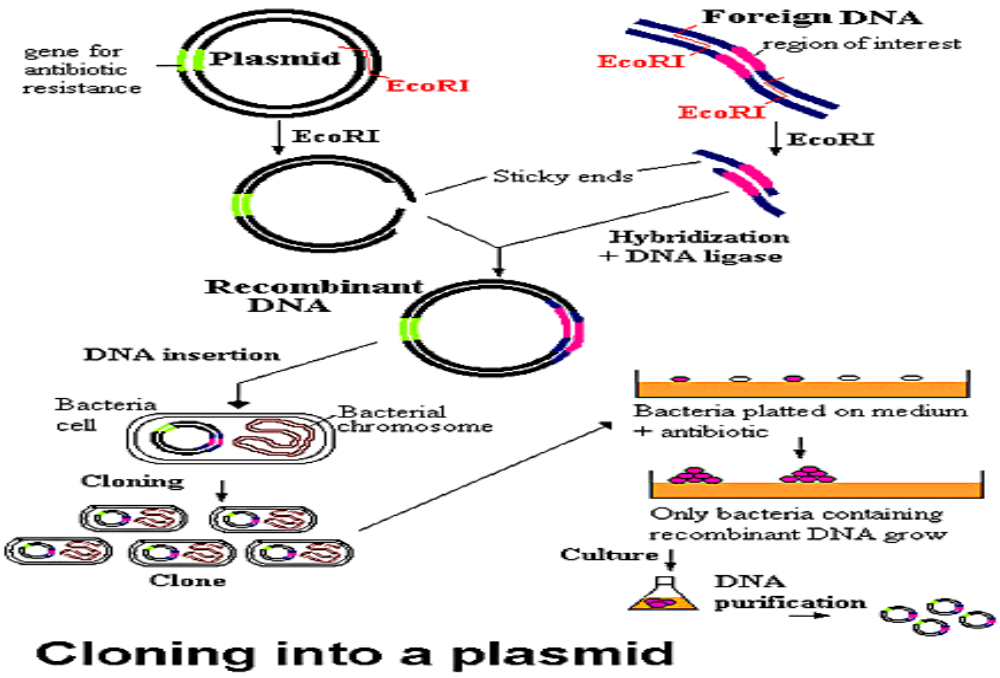
Bacterial artificial chromosomes (BACS) are bacterial plasmids derived from the F plasmid. They are capable of carrying up to 300 kb of DNA.

Basic steps in DNA cloning

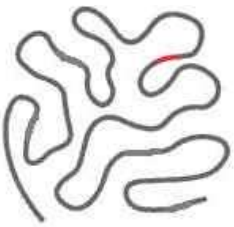
1. Isolation of gene of interest
2. Transferring gene of interest into vectors
3. Transferring recombinant vector into host cells
4. Selection of the cells with gene of interest
5. Culturing the selected clones
6. Identification of presence of gene of interest
7. Culturing the identified clone for further growth and isolation.

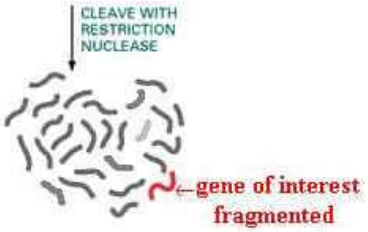
1. Isolation of gene of interest

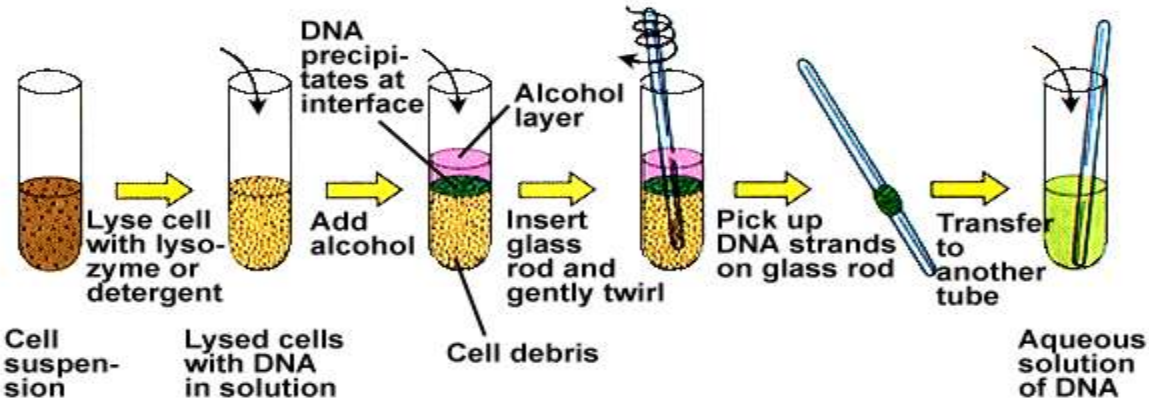
Gene of interest was first isolated. For this, initially the cells containing the gene of interest isolated and disrupted to release nucleus. From the nuclear fraction, the gene of interest released by using the restriction enzyme which posses the appropriate restriction sites at both ends of the gene of interest. After the gene of interest fragmented, they are separated by using normal isolating procedures like electrophoresis or chromatography.

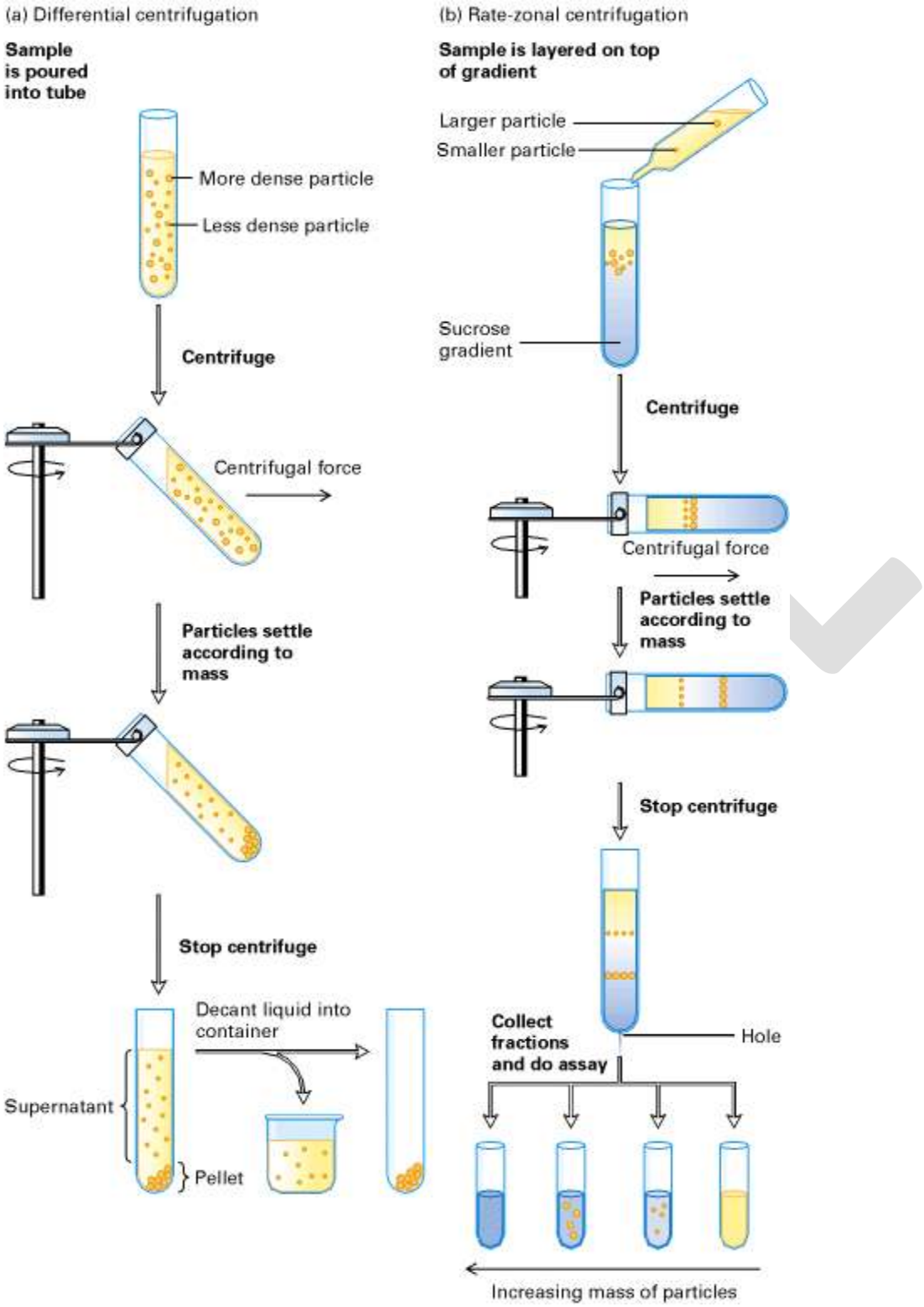


Cloning into a plasmid

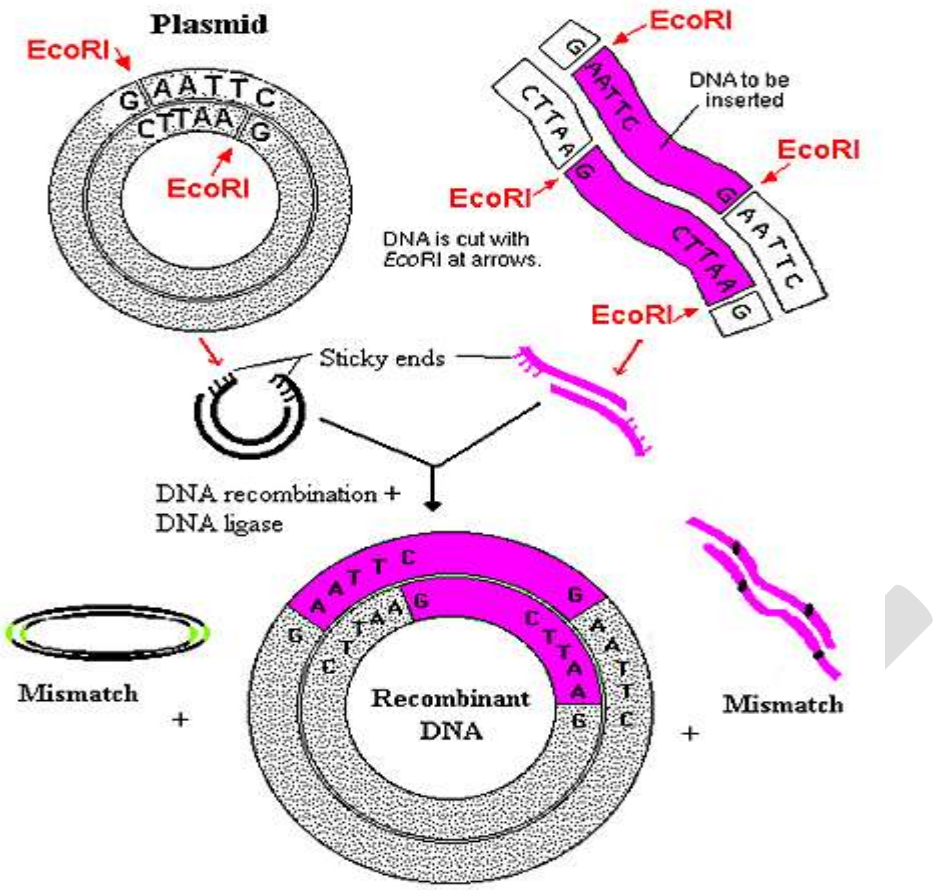



CLEAVE WITH
RESTRICTION
NUCLEASE
gene of interest
fragmented

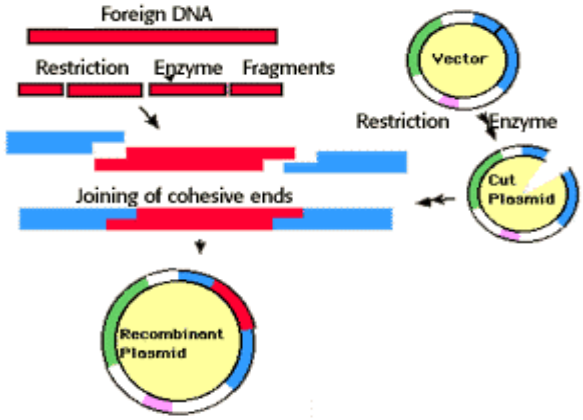

DNA
precipi-
tates at
interface
Alcohol
layer
Lyse cell
with lyso-
zyme or
detergent
Add
alcohol
Insert
glass
rod and
gently twirl
Pick up
DNA strands
on glass rod
Transfer
to
another
tube
Cell
suspen-
sion
Lysed cells
with DNA
in solution
Cell debris
Aqueous
solution
of DNA


(a) Differential centrifugation
Sample is poured into tube
More dense particle
Less dense particle
Centrifuge
Centrifugal force
Particles settle according to mass
Stop centrifuge
Decant liquid into container
Supernatant
Pellet
(b) Rate-zonal centrifugation
Sample is layered on top of gradient
Larger particle
Smaller particle
Sucrose gradient
Centrifuge
Centrifugal force
Particles settle according to mass
Stop centrifuge
Collect fractions and do assay
Hole
Increasing mass of particles

2. Transferring gene of interest into vectors



Inserting a DNA Sample into a Plasmid



Plasmid vectors are small circular molecules of double stranded DNA derived from natural plasmids that occur in bacterial cells. A piece of DNA can be inserted into a plasmid if both the circular plasmid and the source of DNA have recognition sites for the same restriction endonuclease.

The plasmid and the foreign DNA are cut by this restriction endonuclease (EcoRI in this example) producing intermediates with sticky and complementary ends. Those two intermediates recombine by base-pairing and are linked by the action of DNA ligase. A new plasmid containing the foreign DNA as an insert is obtained. A few mismatches occur, producing an undesirable recombinant.

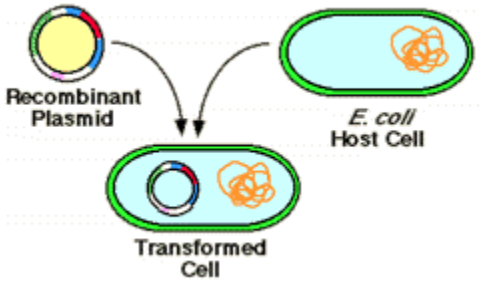
The new recombinant vector can be introduced into bacterial cells that can produce many copies of the inserted DNA. This technique is called DNA cloning.

3. Transferring recombinant vector into host cells

The process of transferring the recombinant vector into cells usually referred by three different terminologies namely transformation , transfection and transduction. Of these word transformation commonly used.

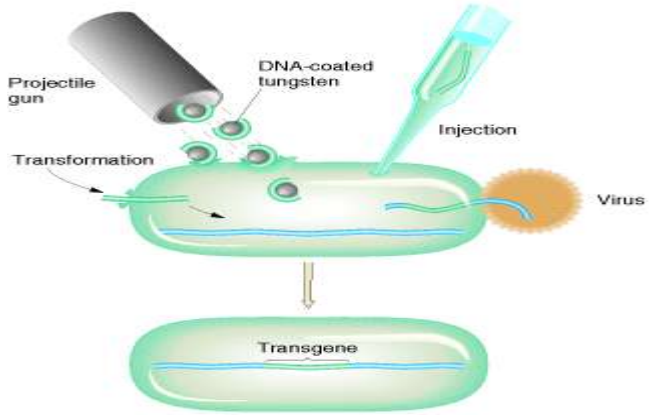
Transformation: The introduction of any DNA molecule into any living cell.
Transfection: The introduction of purified phage DNA molecules into a bacterial cell.

Transduction: The movement of genes from a bacterial donor to a bacterial recipient with the use of a phage as the vector.

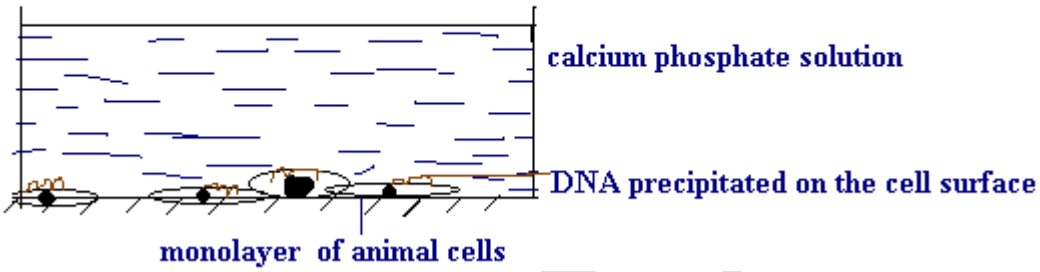


There are five different ways are available. They are as follows:

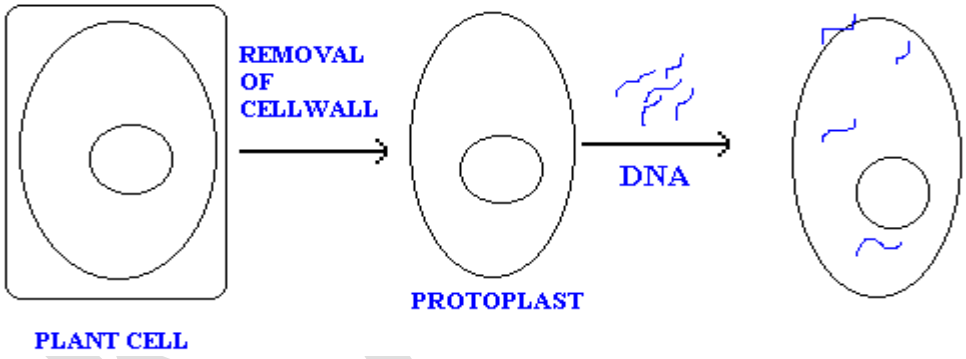
a. Precipitation
b. Protoplast fusion
c. Electroporation
d. Microinjection
e. Microprojectiles (Biolistics or gene gun)



a. Precipitation: Precipitation method involves the usage of calcium phosphate. which precipitate the DNA molecules into the surface of the host cells. Calcium plays two important role namely, first it precipitates DNA, Secondly it neutralize the charges present on the surface of cells and DNA so that they are not repelled. Once DNA in contact with the cell , they entered in to the host cells.

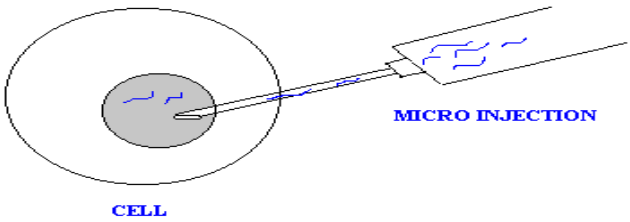


b. Protoplast fusion: In plant cells usually DNA molecules transferred in to cells when they are present in protoplast form. Because in protoplast form DNA easily taken up by the cells. Protoplast is nothing but the cell without cell wall.

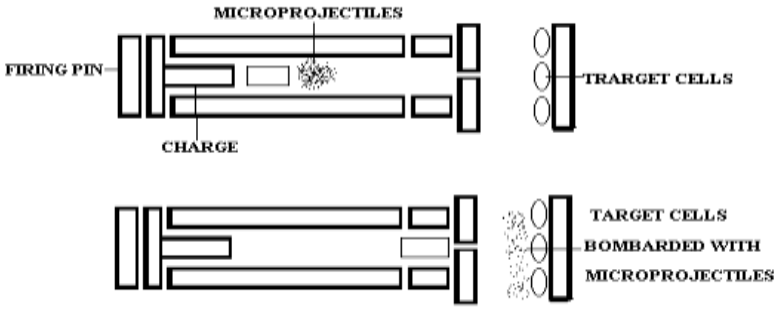


c. Electroporation: By applying short electric pulse to the cells, a small pores were created in the cell membrane. Through which DNA can be easily transferred.

d. Microinjection: It is a type of physical method because in this method a very fine pipette used to insert recombinant DNA directly into nucleus of the cells. This technique was initially applied to animal cells but has subsequently been successful with plant cells.



e. Microprojectiles: The second physical method involves bombardment of the cells with high velocity microprojectiles, usually particles of gold or tungsten that have been coated with DNA. These microprojectiles are fired at the cells from a particle gun. This unusual technique is termed biolistics and has been used with a number of different types of cell.



Of the different method of transformation, precipitation, electroporation and gene gun used when the culture cells available in large number as well as where culturing of cells is not time consuming and costly. And they are usually used to transform into bacterial culture and animal cells. Microinjection usually used to transform the recombinant vector or genome into zygote or single cell where the availability and culturing condition difficult. But in the microinjection, the transformation frequency found to be maximum compared to other methods and moreover cells found to be viable. Protoplast fusion generally used to transform recombinant vector to plant cells because other methods become inefficient due to the presence of cell wall of plants.

Production of genome library

A genomic library comprises a set of bacteria, each carrying a different small fragment of human DNA. For simplicity, cloning of just a few representative fragments (colored) is shown. In reality, all the gray DNA fragments will also be cloned.

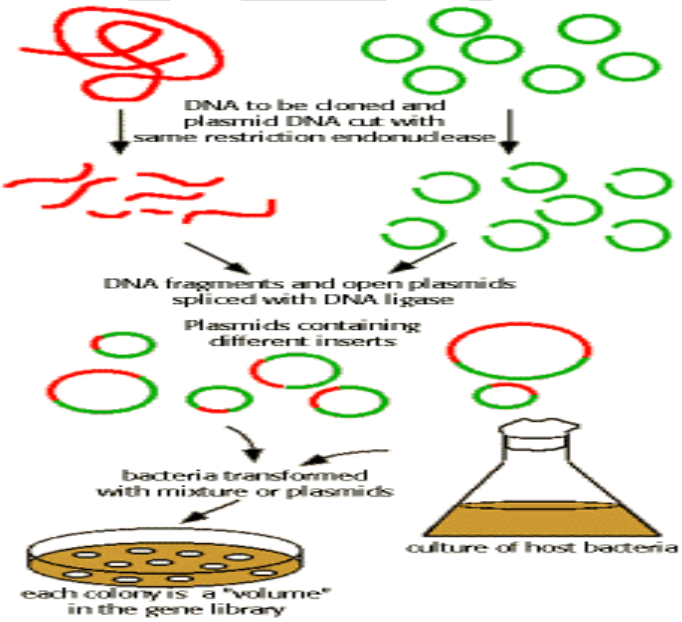


Fig: Genomic Library

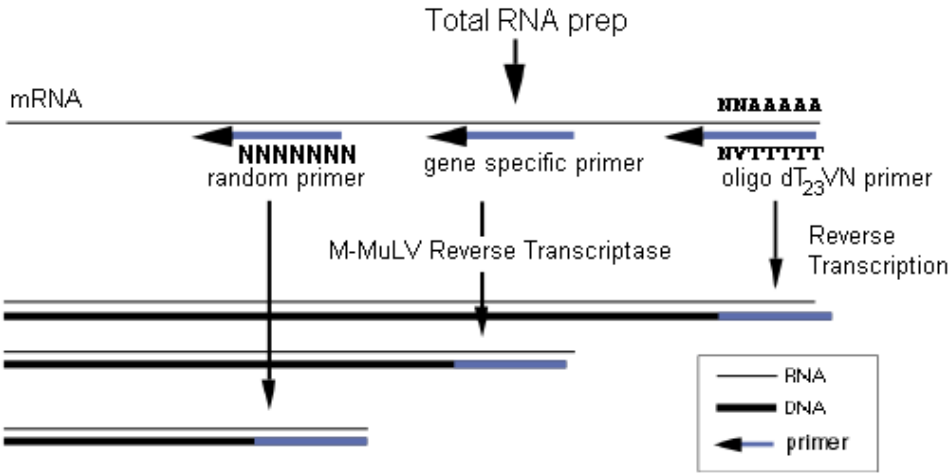
When the genomic DNA is digested by a restriction endonuclease, and all fragments cloned at random into a plasmid vector, then the majority of genetic information will be included in the mixture of bacteria. Cultures of the bacteria, with each containing only a fraction of the genome, collectively contain all the genes and are called a genome library.

cDNA synthesis

cDNA is a copy, in DNA, of sequences present in RNA (normally this means in the mature, "spliced", mRNA). We may wish to clone the cDNA. DNA is double stranded whereas mRNA is single stranded. We need to synthesise both the antisense and the sense strands.

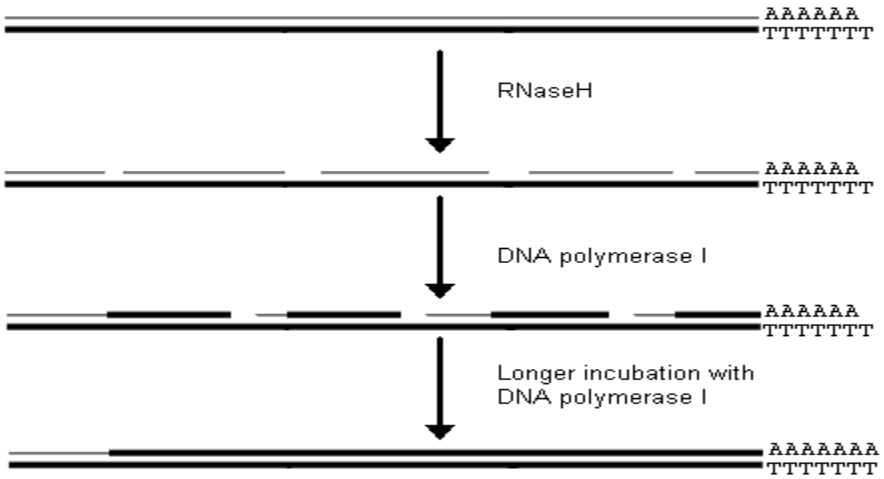
First strand synthesis

Can use oligo dT primer, random primer or gene specific primer.

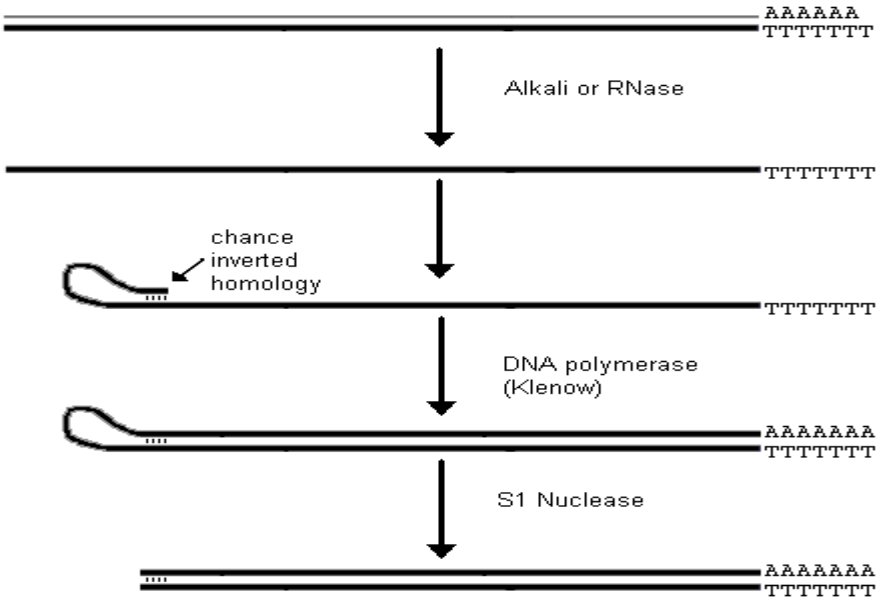


Second strand synthesis

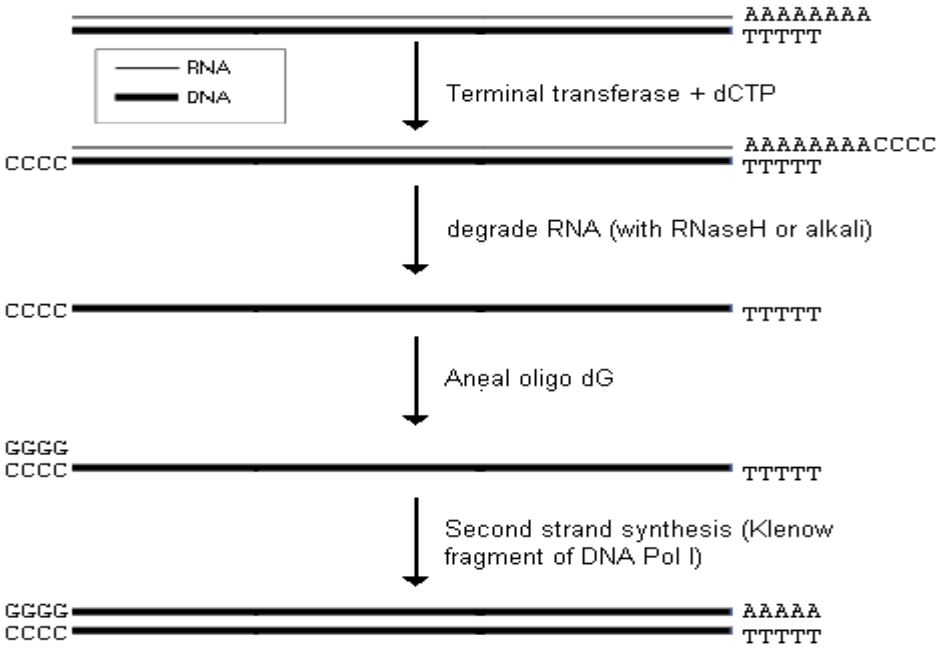
RNaseH



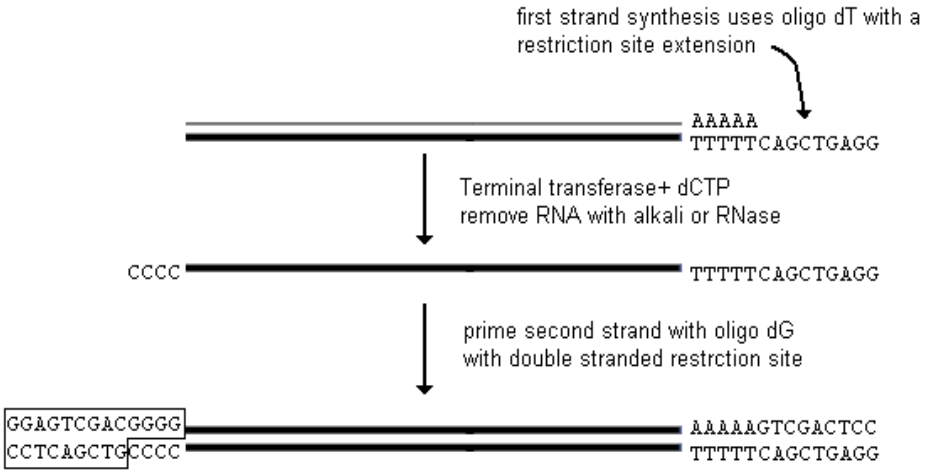
Self priming



Tailing and priming

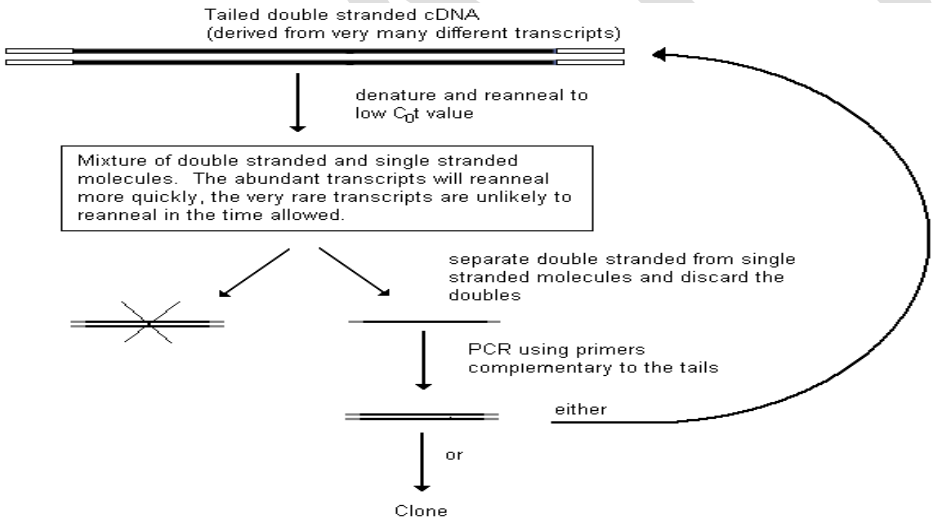


Linking and ligation

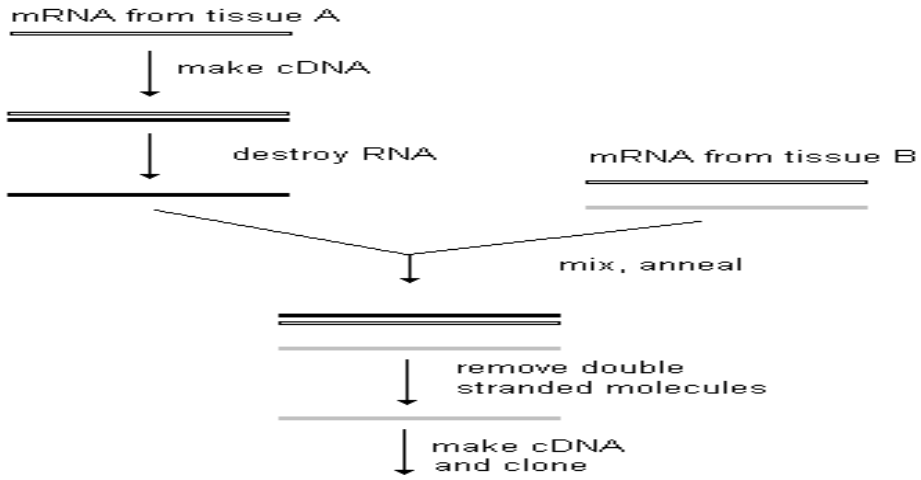


Normalized libraries

The frequency with which any one gene's transcripts are represented in a cDNA library will reflect the abundance of the mRNA in the tissue from which the cDNA was isolated. Sometimes, such as when looking for cDNAs derived from genes which are transcribed at a very low level, one might wish to construct a library in which all transcribed genes are represented equally. Such a library is said to be "normalized".

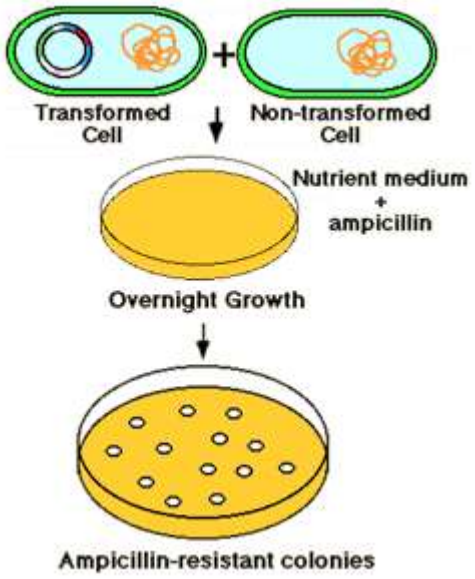


"Difference" libraries



Selection of the cells with gene of interest

Selecting transgenic cells resistant to antibiotics

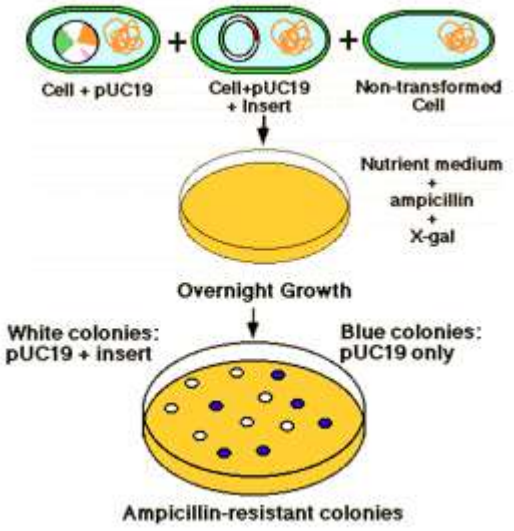


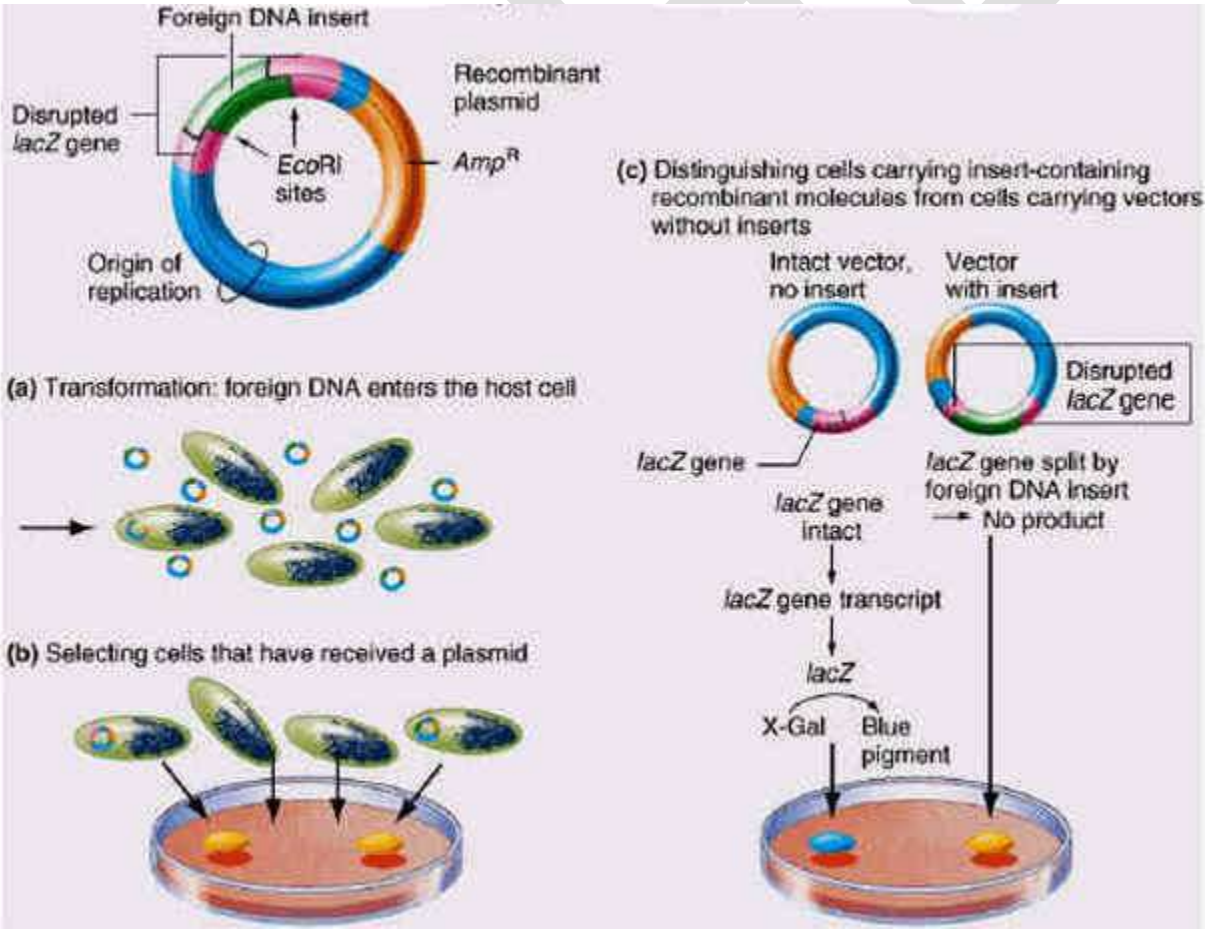
Plasmid vector contains an ampicillin resistance gene making the cell resistant to ampicillin containing medium. Growth of transformed cells (cells receiving the plasmid) can be identified on agar medium containing (e.g.) ampicillin. Thus, the cells with recombinant vector can be selected. This is a direct selection procedure.

Insertional mutagenesis identifies plasmids with DNA inserts

The plasmid vector contains another identifiable gene (e.g., a second drug resistance or an enzyme activity), with the coding sequence of this gene containing the restriction site for insertion. Insertion of the foreign DNA at this site

interrupts the reading frame of the gene and result in insertional mutagenesis. In the example shown below, the beta-galactosidase gene is inactivated. The substrate "X-gal" in the medium turns blue if the gene is intact, i.e. it makes active enzyme. White colonies in X-gal imply the presence of recombinant DNA in the plasmid.

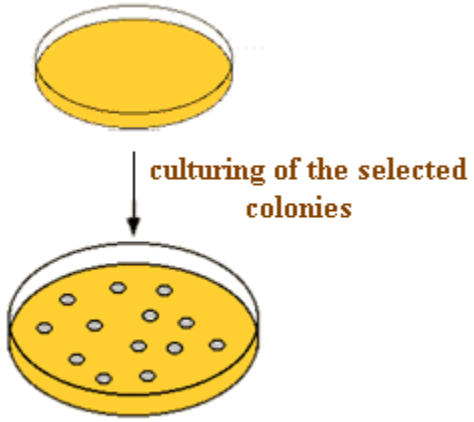




Thus by combining direct selection and insertional inactivation method used for the selection of culture with our gene of interest.

4. Culturing the selected clones

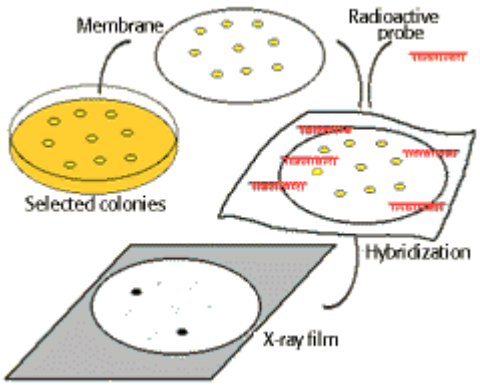
Selected colonies further cultured in a separate culture plates. This makes them to culture in good nutritional medium without any limiting factor. This culture later utilized to screen (check) the presence of the perfect nature of the gene of interest and its product.



5. Identification of presence of gene of interest

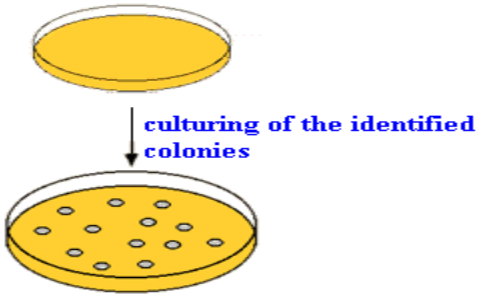
From the selected culture, the state of gene of interest screened. Screening can be carried out at the culture stage itself using replica plates. For example *insitu* hybridization method. This can also be carried out using the culture medium and cell extract. For example Blotting methods. Screening carried out either by using proteins or DNA.

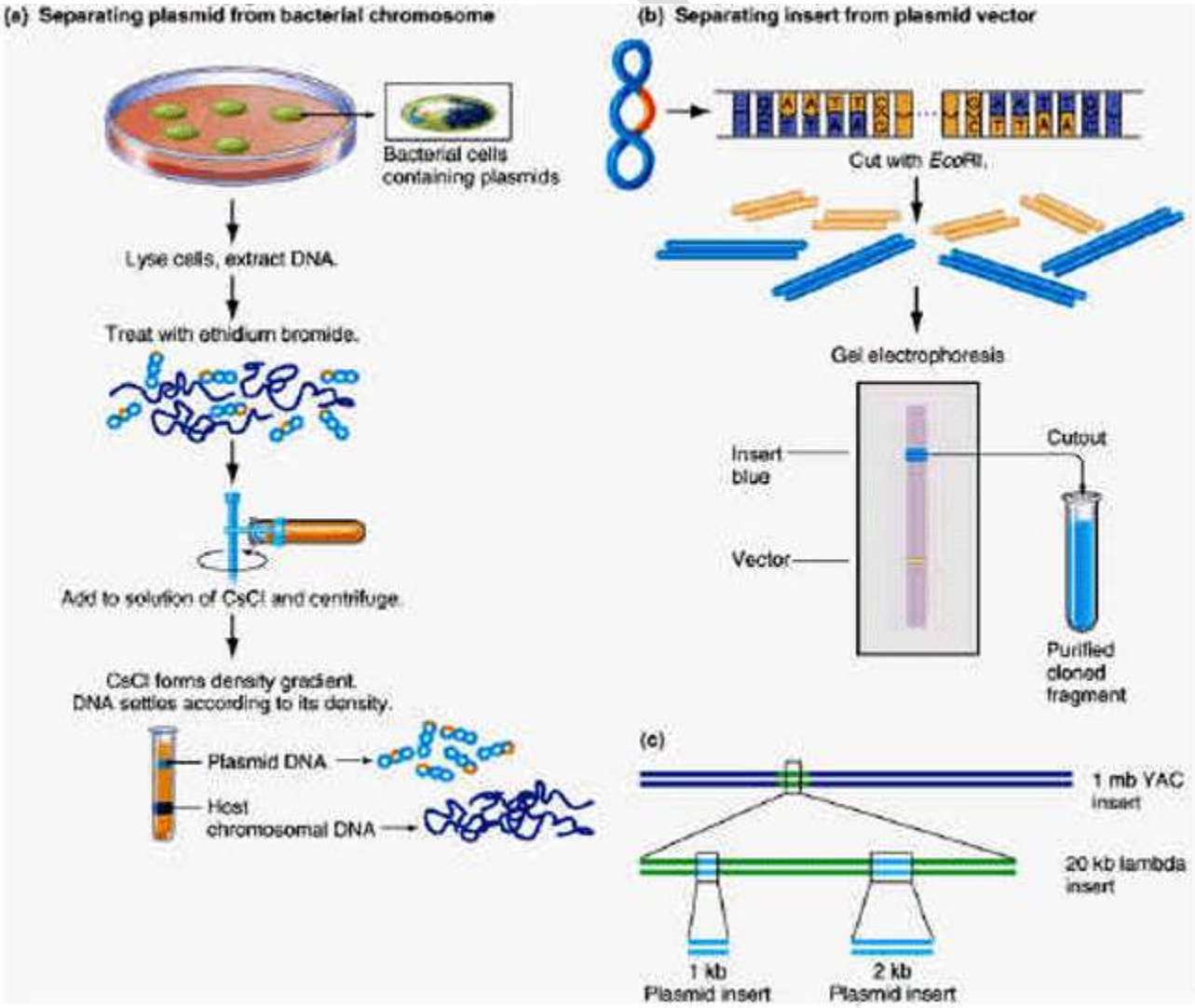
By i*nsitu* hybridization technique: In the following scheme, bacterial containing recombinant plasmids are grown as clones. The clones are blot transferred to a membrane sheet, and the DNA present denatured and fixed onto the surface. Adding a radioactive "probe" or complementary fragment and allowing the DNA to hybridize followed by exposure to X-ray film identifies the clone containing recombinant DNA with the correct insert.



6. Culturing the identified clone for further growth

After identifying the colony which was found to be containing correct gene of interest, the identified colony selected and cultured in a separate culture plates. From this culture plates gene of interest or protein was isolated and used depending upon the requirements.





UNIT II - POSSIBLE QUESTIONS

PART B

1. Explain in detail about M13 Vector

2. How pBR 322 is synthesized?

3.Write short notes on various plasmids.

4. Describe pBR322 cloning vector with suitable diagram.

5.Write notes on PUCvector.

6.Describe bacteriophage vector with diagram.

7.Write notes on cosmids with examples.

8.How will you construct the cloning vectors?

9.Write notes on expression vectors.

10.What are the steps involved in the gene cloning experiments?

11.How will you construct the cDNA library?

12. Write a note on Shuttle vectors.

PART C

1. Explain the selection and screening method of recombinants.
2. Detail account on expression vector and merits and demerits in cloning
3. Justify – *E.coli* as promising prokaryotic host

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UNIT II

The cloned genes are expressed in ----------	Periplasm
A typical example for a head and tail phage	Phage M13
What are the reasons for considering the yeast as a vector?	length
Which are the shuttle vectors?	YE ps
Which is the yeast chromosomal gene that codes for isopropyl malate dehydrozejase?	2um plasmid
Which is the one of the enzyme involved in the conversion of pyuvie acid to leucine?	Malate dehydrozenase
Origin of Replication in yeast chromosomes having 100 bp	LEU2
Bacterial plasmids carrying a yeast gene	YRPs
Phages were first discovered by	Fredrick Twort
The smallest known phage is	Phage M13
……. Can be used as an selectable marker in certain experiment to ensure the presence of plasmid present in a bacteria in a culture	DNA probe
Origin of replication in yeast chromosomes having 100 bp	Minichromosome vectors
The insertion of DNA fragment is accompanied with deletion of all or the major part of non essential region of genome, the deleted region is called	Okazaki fragment
Which plasmid is referred to as the 'work house' of gene cloning	pBR 322
The ampicillin resistant gene of pBR 322 was derived from	pSC 101
The size of pBR 322 is	4,363 bp
A derivative of pBR 322 which confers resistance to ampicillin, tetracycline and chloramphenicol	pBR 313
In Rhizobium leguminosarum, the genes for nitrogen fixation and module formations are located in	Plasmid
In EcoRI the first two letters are known as ____	Genus & specific name (species)
Bacterial plasmids carrying a yeast gene	YRPs
YAC has a approximate of …… DNA frequent	30 kbp
Which is not the difference between YAC & BAC	BAC is circular and YAC is linear
Vectors are ……..	Extrachromosomal DNA molecule

YAC differs from typical cloning vector is having	Several multiple cloning stes
Retroviral infection can be applied to introduce the gene into	fish
Which of the following is an insertion vertor	λ EHBL 4
Among these which one is the replacement vector	Charon 16A
Enzyme used to cleave the appropriate site of the λ for the insertion of vector is	Endonucleases
vector is involved in _____	cloning
restriction enzymes mostly preferred for genetic engineering are of type	blue colour
Achieving same copy by	blotting
The other name of r DNA is	Ribosomal DNA
In the method of identification of recombinants the method usually used is	Primary screening
A lactose analogue which is involved in the screening of B galactosidase	Y-gal
When X-gal added to the agar the cells of which synthesize B-galactosidase will be coloured	Yellow
Baculo virus is a	Parasite
RNA4 which encodes the ----------	Virus coat protein
Introduce the gene in to E.coli by _____	Transduction
Cells containing pBR will be resistant to …….. and sensitive to ……	Resistant to Ampicillin sensitive to tetracyclin
Bacteriophage P 1 resembles bacteriophage _____.	λ
Name the animal virus used as vectors	SV 40 Virus
SV40 vectors are grown and manipulated using _____ as the host	Plant cells
cDNA clones are ligated to suitable vector are	m13 vectorand phage vector
λ phage lacks	Icosahedral
…….. phage display system is powerful technology for engineering proteins such as functional mutant proteins and peptides	φ×174
Ti plasmid is in …… size.	~ 200 kb
The molecular weight of the cloning vector should ideally around …… kbp	100
pBR 322 is first identified and developed by	A.Chan and N.cohen
whch organism is infected by M13 bacteriophage	Pseudomonas

In pBR 322 ,322 is stands for	No of genes
ARS is …….	Autonomous replicating plasmid
Advantage of lamda phage vector	Transformation efficiency
Most commenly used plasmid vector for cloning	pBR 322
DNA ligase is synthesized from	E.coli and bacteriophage
Plasmid vector can carry upto ……kb of fragment	40kb
Baculoviruses infect	Animal cells
The digested DNA molecule are run agarose gel for	identify the change
Transfer vector is a ……vector	Shuttle Vector
Transfer vector contains __ cloning site	Single

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Recombinant cell.	Host cell	Donor cell
pHC79	lambda Vectors	pUC118
short doubling time (90 minutes)	can be grown on complex media	tough selection
PBR 322	LE u2	pUC118
pBR322	YEPs	LEU2
Isopropyl kinase	Isopropyl malate dehydrozenase	Pyruvate carboxylase
ARS	2µm plasmid	ANS
2 µm plasmid	YIPs	YAC
Edward Tautum	Flein d' Hericcle	Griffith
PUC 118	PUC 119	PHC 79
Radiolabelled RNA	Antibiotics resistant plasmid	Radiolabelled DNA.
LEU2	ARS	2um plasmid
Stuffer fragment	Coding region	Non coding region
Col El Plasmid DNA	Col El Amp Plasmid DNA	pBR 325 Plasmid DNA
Ti Plasmid	pBR 313	RSF 2124
6,600 bp	10,900 bp	5,300 bp
Col El Plasmid DNA	pBR 325	Col El Amp Plasmid DNA
DNA	Bacteriophage	RNA
Genus name	Specific name	Inventor name
2 um plasmid	YIPs	YAC
100 kbp	200 kbp	20kbp
YAC has telomere & BAC wont have	YAC has centromere and BAC wont	YAV would be bound to histone but BAC wont
protein	BAC	YAC

A centromere sequence at each end	More than one origin of replication	Tolemere sequence
mice	plant	bacteria
Charon 16A	λ GEM 12	Charon 4a.
λ ZAP II	λ ZAP II	λ EHBL 4
lyases	Ligases	Translocase
cutting	joiing	screening
green colour	colourless	greenish yellow
electrophoresis	cloning	joining
Chimeric DNA	Bactriophage	Chromosomal DNA
Replica Plating	Secondary screening	Auxano graphy
X-gal	B-galactosidase	B-galactoside permease
Red	Blue	Black
Obligate parasite	Saprophyte	Pathogen
cell wall protein	Viral core protein	Glyco protein
Particle bambrtment	Transformation	Micro injection
Resistant to methronine and sensitive to Arginine	Resistant to tetracycline and sensitive to ampicillin	Resistant Argmine and sensitive to methronine
T7	φ	T3
HIV	Rabbies	polio virus
E.coli	Bacteria	Animal cells
Yac vector	Plasmid Vector	
Base plate	Flexiblr fluid	Contractile sheath
Coliphage	M13 Phage	d)λ phage
100 kb	50 kb	150 kb
10	20	40
Elinst Berlimer	T. Bolival ond Rodrigues	Ishiwata
E.coli	Bacillus	Klebsiella

No of Restriction sites	No of base pairs	Number used to distinguish from other plasmid
Automatic replicating sequence	Automatic reproducing sequence	Autonomous reproducing sequence.
Easy to grow	low cost	Self treplication
pUC8	F plasmid	Ri plasmid
E.coli & Staphylococcus	Klebsiella & bacteriophage	Bacteriophage
8kb	20kb	75kb
Human cells	Insect cells	Plant cells
Purification	Suitable range of length of DNA.	to remove impurities
Plasmid Vector	Binary Vector	Cointegrate Vector
Multiple	Double	Triple

Recombinant cell
lambda Vectors
short doubling time (90 minutes)
YE ps
LEU2
Isopropyl malate dehydrozenase
ARS
2 μm plasmid
Fredrick Twort
Phage M13
Antibiotics resistant plasmid
ARS
Stuffer fragment
pBR 322
RSF 2124
4,363 bp
pBR 325
Plasmid
Genus & specific name (species)
2 um plasmid
30 kbp
BAC contain much larger DNA insert than a YAC
Extrachromosomal DNA molecule

Several antibiotic resistance gene
mice
Charon 16A
λ EHBL 4
Endonucleases
cloning
colourless
cloning
Chimeric DNA
Replica Plating
X-gal
Blue
Obligate parasite
Virus coat protein
Transformation
Resistant to Ampicillin sensitive to tetracyclin
λ
SV 40 Virus
E.coli
m13 vector and phage vector
Contractile sheath
M13 Phage
~ 200 kb
10
T. Bolival ond Rodrigues
E.coli

Number used to distinguish from other plasmid
Autonomous replicating plasmid
Transformation efficiency
pBR 322
E.coli and bacteriophage
8kb
Insect cells
Suitable range of length of DNA.
Shuttle Vector
Multiple

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UNIT – III

Transgenic plants and animals

Development of Glyphosate and Glufosinate Herbicide Tolerant Plants: Herbicide-tolerant (HT) crops offer farmers a vital tool in fighting weeds and are compatible with no-till methods, which help preserve topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics.

How do these herbicides work?

These herbicides target key enzymes in the plant metabolic pathway, which disrupt plant food production and eventually kill it. So how do plants elicit tolerance to herbicides? Some may have acquired the trait through selection or mutation; or more recently, plants may be modified through genetic engineering.

Why develop HT crops?

What is new is the ability to create a degree of tolerance to broad-spectrum herbicides - in particular glyphosate and glufosinate - which will control most other green plants. These two herbicides are useful for weed control and have minimal direct impact on animal life, and are not persistent. They are highly effective and among the safest of agrochemicals to use. Unfortunately, they are equally effective against crop plants.

How do Glyphosate and Glufosinate HT crops work?

1. Glyphosate-tolerant crops

Glyphosate herbicide kills plants by blocking the EPSPS enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant. One strategy is to incorporate a soil bacterium gene that produces a glyphosate-tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

2. Glufosinate-tolerant crops

Glufosinate herbicides contain the active ingredient phosphinothricin, which kills plants by blocking the enzyme responsible for nitrogen metabolism and for detoxifying ammonia, a by-product of plant metabolism. Crops modified to tolerate glufosinate contain a bacterial gene that produces an enzyme that detoxifies phosphonothricin and prevents it from doing damage.

Other methods by which crops are genetically modified to survive exposure to herbicides including: 1) producing a new protein that detoxifies the herbicide; 2) modifying the herbicide's target protein so that it will not be affected by the herbicide; or 3) producing physical or physiological barriers preventing the entry of the herbicide into the plant. The first two approaches are the most common ways scientists develop herbicide tolerant crops.

3. Safety Aspects of Herbicide Tolerance Technology

Toxicity and allergenicity: *Government regulatory agencies in several countries have ruled that crops possessing herbicide-tolerant conferring proteins do not pose any other environmental and health risks as compared to their non-GM counterparts.*

Introduced proteins are assessed for potential toxic and allergenic activity in accordance with guidelines developed by relevant international organizations. They are from sources with no history of allergenicity or toxicity; they do not resemble known toxins or allergens; and they have functions, which are well understood.

Effects on the plants

The expression of these proteins does not damage the plant's growth nor result in poorer agronomic performance compared to parental crops. Except for expression of an additional enzyme for herbicide tolerance or the alteration of an already existing enzyme, no other metabolic changes occur in the plant.

Persistence or invasiveness of crops

A major environmental concern associated with herbicide-tolerant crops is their potential to create new weeds through out crossing with wild relatives or simply by persisting in the wild themselves. This potential, however, is assessed prior to introduction and is also monitored after the crop is planted. The current scientific evidence indicates that, in the absence of herbicide applications, GM herbicide-tolerant crops are no more likely to be invasive in agricultural fields or in natural habitats than their non-GM counterparts.

The herbicide-tolerant crops currently in the market show little evidence of enhanced persistence or invasiveness.

Advantage of herbicide tolerant crops

- Excellent weed control and hence higher crop yields;
- Flexibility – possible to control weeds later in the plant's growth;
- Reduced numbers of sprays in a season;
- Reduced fuel use (because of less spraying);
- Reduced soil compaction (because of less need to go on the land to spray);
- Use of low toxicity compounds which do not remain active in the soil;
- The ability to use no-till or conservation-till systems, with consequent benefits to soil structure and organisms.

A study conducted by the American Soybean Association (ASA) on tillage frequency on soybean farms showed that significant numbers of farmers adopted the "no-tillage" or "reduced tillage" practice after planting herbicide-tolerant soybean varieties. This simple weed management approach saved over 234 million gallons of fuel and left 247 million tons of irreplaceable topsoil undisturbed.

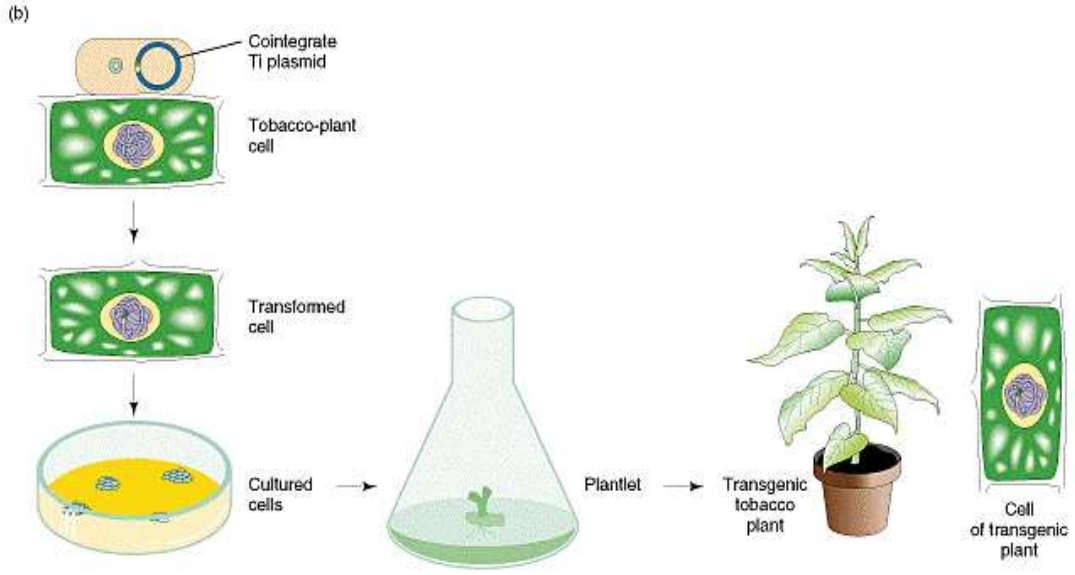
Current status of herbicide tolerance

From 1996 to 2012, herbicide- tolerant crops consistently occupied the largest planting area of biotech crops. In 2010 alone, herbicide tolerant crops occupied 89.3 million hectares or 61% of the 148 million hectares of biotech crops planted globally. The most common are the glyphosate and glufosinate tolerant varieties. The following table shows countries that have approved major HT crops for food use.

Crop	Countries
Alfalfa	*Australia; Canada; Japan; Mexico; New Zealand; Philippines; United States of America (USA)*
Argentine Canola	*Australia; Canada; China; European Union (EU); Japan; Korea, Rep.; Mexico; New Zealand; Philippines; South Africa; USA*
Cotton	*Argentina; Australia; Brazil; Canada; China; Colombia; EU; Japan; Korea, Rep.; Mexico; New Zealand; Philippines; South Africa; USA*
Flax, Linseed	*Canada; USA*
Maize	*Argentina; Australia; Brazil; Canada; China; Colombia; El Salvador; EU; Honduras; Japan; Korea, Rep.; Malaysia; Mexico; New Zealand; Philippines; Russian Federation; Singapore; South Africa; Taiwan; Thailand; USA*
Rice	*Australia; Canada; Colombia; Mexico; New Zealand; Russian Federation; USA*
Soybean	*Argentina; Australia; Bolivia; Brazil; Canada; China; Colombia; Czech Republic; EU; Japan; Korea, Rep.; Malaysia; Mexico; New Zealand; Paraguay; Philippines; Russian Federation; South Africa; Switzerland; Taiwan; Thailand; United Kingdom; USA; Uruguay*
Sugarbeet	*Australia; Canada; European Union; Japan; Korea, Rep.; Mexico; New Zealand; Philippines; Russian Federation; Singapore; USA*

Herbicide resistance

Several transgenes have been incorporated into numerous different crops for resistance or tolerance to different herbicides, including glyphosate, bromoxynil, glufosinate, and sulfonylurea. Herbicide-resistant crops, such as glyphosate-resistant wheat, can be an important part of a sustainable, cereal-based crop production system. The use of herbicide-resistant wheat along with herbicides in a no-till planting system could conserve soil and water resources, a major goal in sustainable wheat-production systems. However, several problems must be addressed before herbicide-resistant crops are incorporated into regional cropping systems, including: the potential increase in herbicide usage, problems with volunteer herbicide-resistant crops, reductions in regional biodiversity and changes in landscape attributed to an increase use of herbicides, and the transfer of herbicide-resistant genes to other plants. The use of herbicide resistant plants developed by placing foreign genes into crops is considerably more controversial than the use of herbicide-resistant plants developed through conventional crop breading programs. As an example of the latter, the herbicide tolerates, clearfield wheat has been released for use in the Northwest. Recently, Monsanto Corporation announced that it will defer the release of its Roundup Ready wheat.



Development of transgenic plant

Chemical herbicides are frequently used to control weeds. Weeds growing in the same field with crop plants can significantly reduce crop yields because the weeds compete for soil nutrients, water, and sun light. Many farmers now control weeds by spraying herbicides directly onto the crop plants. Because these herbicides generally kill only a n arrow spectrum of plants (if t hey didn't, they would kill the crop plants, too), farmers apply mixtures of multiple herbicides to control weeds after the crop has started to g row. Researcher s realized t ha t if a crop plant is genetic ally engineered to be resist an t to a b road- spectrum herbicide, weed management could b e simplified and safer chemicals could be used. It is often argued that such G E varieties reduce oil erosion, because they make adoption of soil-conserving practice s such as "no-till" easier. Resistance to synthetic herbicides has been genetically engineered into corn, soybeans, cotton, canola, sugar beets, rice, and flax. Some of these varieties are commercialized in several countries. Research is on going on many other crops. One application of this technology is that herbicide could be coated on seed from an herbicide resistant variety (for example, maize) and while the maize would germinate and thrive, weeds and parasites such as *Striga* would be killed.

Herbicide resistant crops in weed management

Several crops have been genetically modified to be resistant to non-selective herbicides. These transgenic crops contain genes that enable them to degrade the active ingredient in an herbicide, rendering it harmless. Farmers can thereby easily control weeds during the entire growing season and have more flexibility in choosing times for spraying.

Herbicide resistant crops also facilitate low or no tillage cultural practices, which many consider to be more sustainable. Another advantage is that farmers can manage weeds without turning to some of the more environmentally suspect types of herbicides.

Critics claim that in some cases, the use of herbicide resistant crops can lead to an increase in herbicide use, promote the development of herbicide resistant weeds, and damage biodiversity on the farm. Extensive ecological impact assessments have been addressing these issues.

Among the field trials conducted on herbicide resistant crops, studies in the United Kingdom have shown that different herbicides and different herbicide application practices can affect the amount of wild plants on the farm. In comparison with conventional cropping systems, weed and animal populations were negatively affected by herbicide tolerant sugar beet and rapeseed, but biodiversity was increased with the use of herbicide tolerant maize.

Currently, two herbicide resistant cropping systems are common for soybean, maize, rapeseed, and cotton.

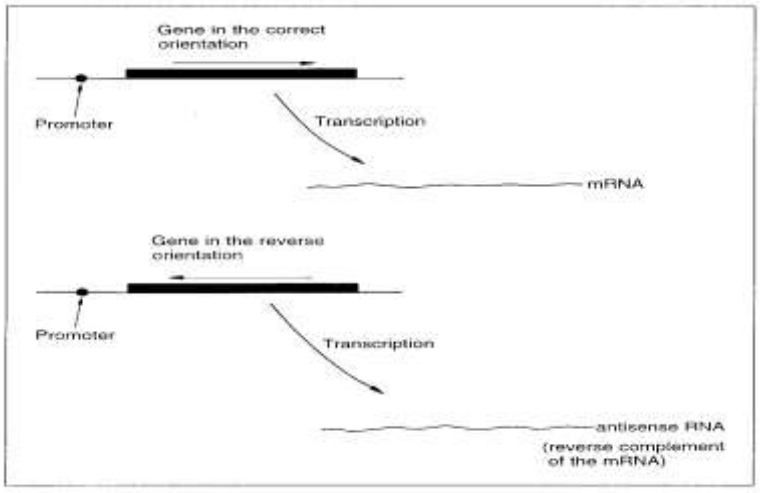
Antisense technology

In an antisense experiment the gene to be cloned is ligated into the vector in reverse orientation. This means that when the cloned 'gene' is transcribed, the RNA that is synthesized is the reverse complement of the messenger RNA (mRNA) produced from the normal version of the gene. We refer to this reverse complement as an antisense RNA, sometimes abbreviated to as RNA.

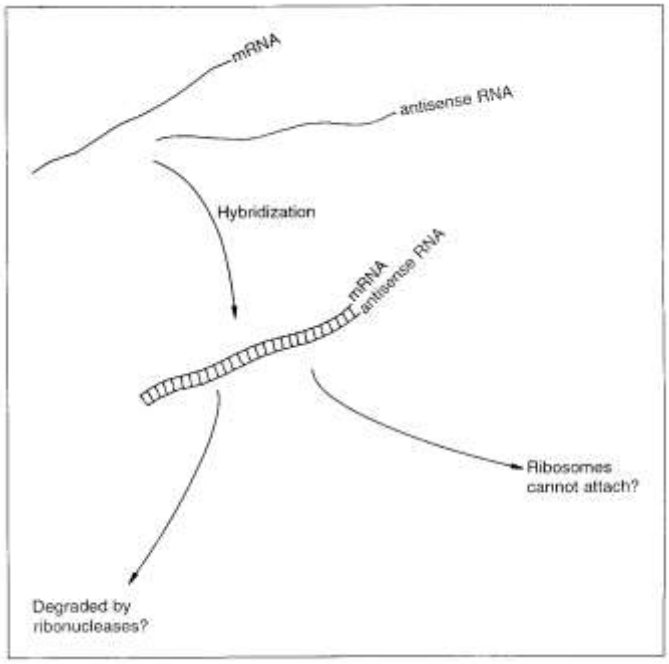
An antisense RNA can prevent synthesis of the product of the gene it is directed against. The underlying mechanism is not altogether clear, but it almost certainly involves hybridization between the antisense and sense copies of the RNA. It is possible that the block to expression arises because the resulting double-stranded RNA molecule is rapidly degraded by cellular ribonucleases, or the explanation might be that the anti-sense RNA simply prevents ribosomes from attaching to the sense strand. Whatever the mechanism, synthesis of antisense RNA in a transformed plant is an effective way of carrying out gene subtraction.

Antisense RNA and the engineering of fruit ripening in tomato

At present, commercially grown tomatoes and other soft fruits are usually picked before they are completely ripe; to allow time for the fruits to be transported to the marketplace before they begin to spoil. This is essential if the process is to be economically viable, but there is a problem in that most immature fruits do not develop their full flavor if they are removed from the plant before they are fully ripe. The result is that mass-produced tomatoes often have a bland taste, which makes them less attractive to the consumer. Two biotechnology companies - Calgene in the USA and ICI Seeds in the UK - used antisense technology as a means of genetically engineering tomato plants so that the fruit ripening process is slowed down. This enables the grower to leave the fruits on the plant until they ripen to the stage where the flavor has fully developed, there still being time to trans- port and market the crop before spoilage sets in.



Antisense RNA



Possible mechanisms for the inhibition of gene expression by antisense RNA.

The role of the polygalacturonase gene in tomato fruit ripening

The timescale for development of a fruit is measured as the number of days or weeks after flowering. In tomato, this process takes approximately 8 weeks from start to finish, with the color and flavor changes associated with ripening beginning after about 6 weeks. At about this time a number of genes involved in the later stages of ripening are switched on, including one coding for the polygalacturonase enzyme. This enzyme slowly breaks down the polygalacturonic acid component of the cell walls in the fruit peri-carp, resulting in a gradual softening. The softening

makes the fruit palatable, but if taken too far results in a squashy, spoilt tomato attractive only to students with limited financial resources. Partial inactivation of the polygalacturonase gene might increase the time between flavor development and spoilage of the fruit. How could antisense technology be used to achieve this result?

The results of the experiment were assessed in the following ways

• The presence of the antisense 'gene' in the DNA of the transformed plants was checked by Southern hybridization.

• Expression of the antisense 'gene' was measured by northern hybridiza-tion with a single-stranded DNA probe that would hybridize only to the antisense RNA.

• The effect of antisense RNA synthesis on the amount of polygalacturonase mRNA in the cells of ripening fruit was determined by northern hybridization with a second single stranded DNA probe, this one specific for the sense mRNA. These experiments showed that ripening fruit from transformed plants contained less polygalacturonase mRNA than the fruits from normal plants.

• The amounts of polygalacturonase enzyme produced in the ripening fruits of transformed plants were estimated from the intensities of the relevant bands after separation of fruit proteins by polyacrylamide gel electrophoresis, and by directly measuring the enzyme activities in the fruits. The results showed that less enzyme was synthesized in trans-formed fruits. Most importantly, the transformed fruits, although undergoing a gradual softening, could be stored for a prolonged period before beginning to spoil. This indicated that the antisense RNA had not completely inactivated the poly-galacturonase gene, but had nonetheless produced a sufficient reduction in gene expression to delay the ripening process as desired.

Biocontrol agents

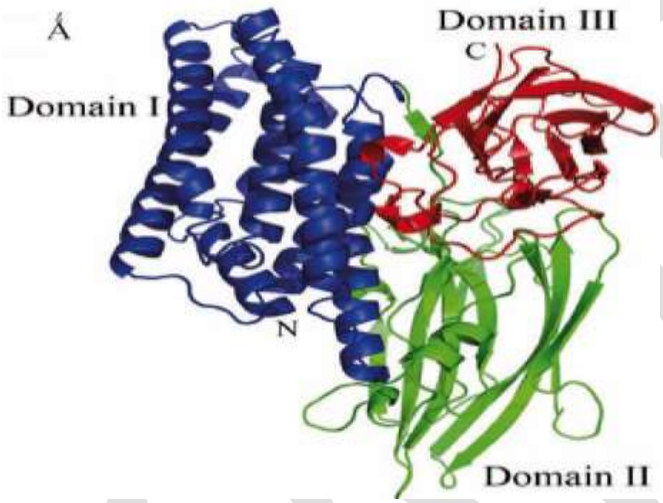
Bacillus thuringiensis is a Gram positive spore forming bacteria grouped into the *Bacillus cereus* group of *Bacilli* which produces proteinaceous insecticidal crystals during sporulation which is the distinctive feature between it and other members of the *Bacillus cereus* group. *Bacillus thuringiensis* was originally discovered in 1902 by a Japanese biologist Shigetane Ishiwatari who isolated it from diseased silkworm,

Bacillus thuringiensis readily proliferates when environmental conditions such as temperature and nutrient availability are favourable whilst the formation of spores have been shown to be triggered by internal and external factors including signals for nutrient starvation, cell density and cell cycle progression.

The life cycle of *Bt* can be divided for convenience into phases and these are Phase I: vegetative growth; Phase II: transition to sporulation; Phase III: sporulation; and Phase IV: spore maturation and cell lysis. The production of the characteristic insecticidal (Cry) proteins deposited in crystals in the mother cell have been shown to mainly start from the onset of sporulation . A number of *cry*-genes have been shown to be transcribed from two overlapping promoters BtI and BtII by RNA polymerases that contain sporulation dependent sigma factors and a mutation in the consensus region of σE has been shown to inhibit transcription from BtI and BtII promoters. It has also been shown that some *Bt* insecticidal proteins are produced and secreted into the culture medium during vegetative growth. As well as the Cry toxins *Bt* produces additional virulence factors including phospholipase C proteases and hemolysins The virulence factors are controlled by the pleiotropic regulator PlcR and it has been demonstrated that cytotoxicity of *Bt* is PlcR dependent Deletion of the *plcR* gene has been shown to result in a drastic reduction in the virulence of *Bt* in orally infected insects The production of virulence factors by *Bt* is necessary but not enough for *Bt* to be called a pathogen

but its production of proteins that have been proved beyond doubt to be independently insecticidal justifies it's name as an insect pathogen.

The insecticidal proteins in the crystalline bodies produced during sporulation have been shown to contain two types of insecticidal proteins namely Cry toxins and Cyt-toxins and there are one or more toxins produced and packaged into a single crystal or multiple crystals by a *Bt* strain. The Cry toxins acquired the mnemonic Cry from the fact that they are found in the crystal while the Cyt-toxins acquired the mnemonic Cyt because of their in vitro cytolytic activity.

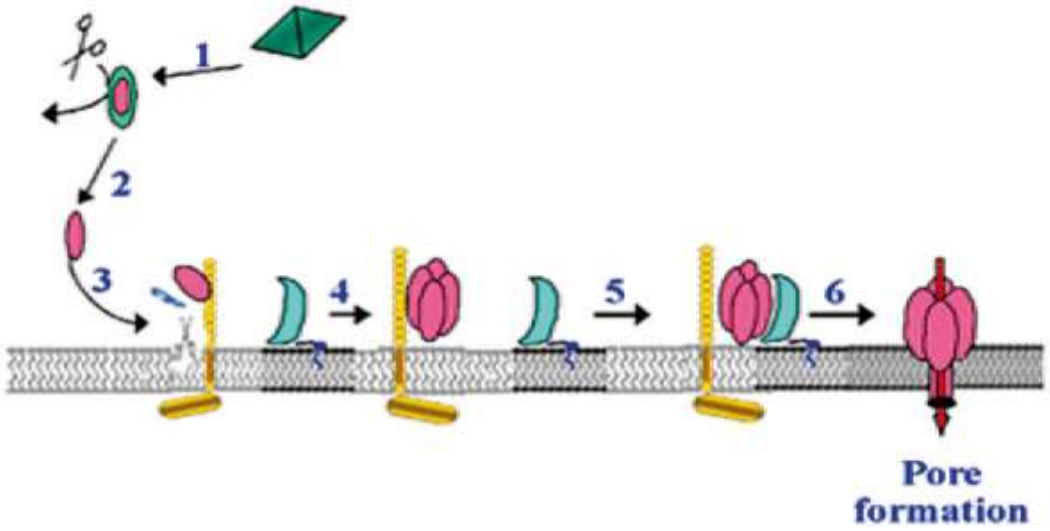


3-D crystal structure of Cry8Ea The three domains of the protein are represented with different colours with domain I coloured *blue*, domain II coloured *green* while domain III is coloured *red*.

Proposed mechanisms of action of Cry and Cyt toxins from *Bt* include pore formation in which *Bt* toxins induce cell death by forming ionic pores following insertion into the membrane, causing osmotic lysis of midgut epithelial cells in their target insect. Also, a relatively new mechanism of action of Cry toxins have been proposed which involves the activation of Mg2+-dependent signal cascade pathway that is triggered by the interaction of the monomeric 3-domain Cry toxin with the primary receptor, the cadherin protein BT-R1 The triggering of the Mg2+-dependent pathway has a knock-on effect and initiates a series of cytological events that include membrane blebbing, appearance of nuclear ghosts, and cell swelling followed by cell lysis The Mg2+-dependent signal cascade pathway activation by Cry toxins have been shown to be analogous to similar effect imposed by other pore forming toxins on their host cells when they are applied at subnanomolar concentration Though the two mechanisms of action seem to differ, with series of downstream events following on from toxin binding to receptors on target cell membranes, there is a degree of commonality in that initially the crystals have to be solubilised in vivo or *in vitro* and activated by proteases before and/or after binding to receptors such as cadherin. The midgut of lepidopteran and dipteran insects has been shown to be alkaline and this enhances the solubility of Cry toxins. Those of coleoptera are neutral or slightly acidic and in vitro solubilisation of Cry1Ba and Cry7Aa has been shown to enhance the activity of these toxins towards *Leptinotarsa decemlineata*.

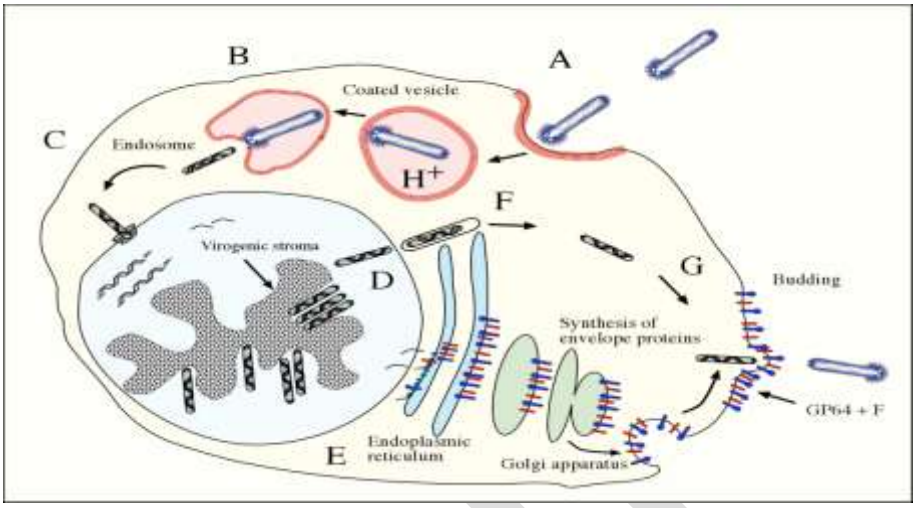
With the pore forming model, an ingested crystal toxin is solubilised in the alkaline environment of the insect's midgut releasing protoxins which are initially processed by midgut proteases. The initial cleavage of a Cry1A protoxin by the gut proteases results in the removal of the C-terminal half and about 30 amino acid residues from the N-terminal thus releasing active toxin monomers which bind to receptors such as cadherin or proteins anchored to the membrane by GPI-anchored proteins such as aminopeptidase N . The initial binding of the activated toxins to receptors is proposed to result in a conformational change which facilitates a second cleavage that removes the N-terminal helix -1, by a membrane-bound protease. The removal of helix -1 results in the formation of oligomers that are membrane insertion competent.

The binding of Cry toxins to the cadherin-like receptors have been shown to involve specific interactions of the variable loop regions in domain II and III with cadherin epitopes. The oligomerised activated toxin that is bound to membrane receptors then inserts the central hydrophobic helix -4 and 5 into the apical membrane of midgut cells causing osmotic shock, bursting of the midgut cells and finally ending in the insect death. The pore formation model as proposed by Bravo et al for Cry1A. Cyt-toxins have also been shown to effect killing of its insect targets through unspecific binding to midgut membrane lipids followed by membrane insertion which leads to pore formation and insect death. The activation of Mg2+-dependent signal cascade pathway that is triggered by the interaction of the monomeric 3-domain Cry toxin with the primary receptor, the cadherin protein has been shown to trigger a pathway involving stimulation of the stimulatory G protein -subunit and adenylyl cyclase (AC), increased cyclic adenosine monophosphate (cAMP) levels, and activation of protein kinase A (PKA). Activation of the AC/PKA signalling pathway initiates a series of cytological events that include membrane blebbing, appearance of nuclear ghosts, and cell swelling followed by cell lysis.



Model of the mode of action of Cry1A toxins. *1* Crystal toxin solubilisation, *2* Initial cleavage by gut proteases, *3* Toxin monomer binding to receptors and second cleavage bymembrane bound protease, *4* Membrane insertion-competent oligomer formation, *5* Binding of oligomeric toxin to receptors, *6* Lytic pore formation.

Viruses that cause systemic infections



Budded virus infection of a Group I virus. BV attach to receptors located in clathrin coated pits via GP64 and are endocytosed (A). The endocytic vesicle is acidified and this changes the conformation of GP64 and causes the virion envelope to fuse with the endosomal membrane releasing the nucleocapsid into the cytoplasm (B). The nucleocapsid may enter the nucleus or insert its DNA through a nuclear pore complex (C), genes are transcribed, DNA is replicated and nucleocapsids are assembled in the virogenic stroma (D). In Group I virus, at least two envelope proteins are synthesized, GP64 and F. They are likely translated in association with the endoplasmic reticulum, glycosylated and transported to and incorporated into the cytoplasmic membrane via the Golgi apparatus (E). Nucleocapsids destined to become BV exit the nucleus and are thought to transiently obtain an envelope that is lost (F). They move to the cytoplasmic membrane at the site of concentrations of GP64 and F proteins, bud through, and obtain envelopes (G).

The insect midgut

To understand the baculovirus infection cycle, a brief overview of the insect gastrointestinal tract is necessary, since this is the site of the initial infection and several major features of baculovirus biology have evolved to accommodate and exploit this unique environment (1). The insect gastrointestinal tract is composed of three sections, the fore-, midand hindgut. In Lepidoptera, the foregut is involved in facilitating the uptake, storage, and physical processing of food. It is lined with a chitin-containing cuticle that is part of the insect exoskeleton. A valve separates the foregut and midgut. The midgut is the major site of digestion of food and lacks a cuticle, but is lined with the peritrophic membrane (PM). The PM is composed of chitin, mucopolysaccharides, and proteins and it separates ingested vegetation from the midgut epithelium. It is thought that it protects the gut surface from damage caused by abrasive food material and to limit the access of microorganisms. It also allows the transfer of liquid and digested substances to the midgut epithelial cells, but prevents the passage of larger food particles. It is worn away by the passage of food and is regenerated from the epithelial cells. The most common midgut epithelial cells are columnar cells with a brush border that is adjacent to the gut lumen. Regenerative cells are present at the base of the epithelium and they replenish the columnar epithelial cells that become damaged and are sloughed into the lumen. Goblet cells are also

present and may be involved in ion transport that regulates pH. The midgut is involved in enzyme secretion and absorption of digested food and has a gradient of pH values. At the entry and exit of the midgut, the pH is near 7.0, but in the central region it can vary from 10.0 to as high as 12.0, depending on the lepidopteran species. These are among the highest pH values found in biological systems. Another Another valve separates the midgut and the hindgut. The hindgut is lined with a cuticle similar to the foregut and is involved in uptake of digested material, although to a lesser extent than the midgut.

Two types of virions

Baculoviruses have evolved to initiate infection in the insect midgut. This has led to two major features of baculoviruses; the environmentally stable but alkali-soluble occlusion body, and occluded virions that have an envelope and associated proteins that allow survival and infection in the harsh alkaline midgut environment that contains a variety of digestive enzymes. In contrast, the environment encountered within the insect has a near-neutral pH, and therefore is more benign. Upon release from occlusion bodies, the virions are called occlusionderived virus (ODV) (see below). The other virus type, budded virions (BV) has an envelope distinct from ODV that facilitates systemic infection. These types of virions differ in their efficiencies of infection for different tissues; ODV infect midgut epithelial cells up to 10,000 fold more efficiently than BV, whereas conversely, BV are up to 1,000-fold more efficient at infecting cultured cells than ODV.

Methods of application of *Bt* and Its products in agriculture

Bacillus thuringiensis and its products have been formulated into various forms for application as biological control agents. Such formulations could be solid (powdery or granulated) or liquid. Presently there are over 400 of *Bt* based formulations that has been registered in the market and most of them contain insecticidal proteins and viable spores though the spores are inactivated in some products. Formulated *Bt* products are applied directly in the form of sprays. An alternative, and highly successful, method for delivering the toxins to the target insect has been to express the toxin-encoding genes in transgenic plants.

Advantages of using *Bt p*roducts over chemical agents in agricultural practices

With their specific insecticidal effect on insect pests in the orders coleoptera (beetles and weevils) diptera (flies and mosquitoes), hymenoptera (bees and wasps) and lepidoptera (butterflies and moths) and to non-insect species such as nematodes *Bt* toxins have taken centre stage as the major biological control agent and widely preferred to chemical insecticides. Various assessments have been carried out to check for the safety of *Bt* toxins from sprays or transgenic plants to non-target species in the environment and it has been shown to be mostly environmentally friendly without significant adverse effects though there has recently been a laboratory observation that seemed to implicate a commercial *Bt aizawai* strain in the reduction of reproduction in bumblebee (*Bombus terrestris*) workers when applied at a concentration of 0.1% through sugar water and pollen. The increased popularity of biological control agents over synthetic chemicals is because of the non-selective lethal effect of the latter agents and the rapid development of resistance by insect pests to synthetic insecticides.

Insecticidal toxin of *Bacillus thuringiensis*

B. thuringiensis is a soil bacterium that produces a toxin (Bt toxin or Cry) that kills certain insects The Bt toxin or Cry is produced when the bacteria sporulates and is present in the parasporal crystal. Several different strains and subspecies of *B. thuringiensis* exist and produce different toxins that kill specific insects.

Some properties of the insecticidal toxins from B*. thuringiensis*

Strain/subsp.	Protein size	Target Insects	Cry #
Berliner	130-140 kDa	Lepidoptera	CryI
kurstaki KTP, HD1	130-140 kDa	Lepidoptera	CryI
entomocidus 6.01	130-140 kDa	Lepidoptera	CryI
aizawai 7.29	130-140 kDa	Lepidoptera	CryI
aizawai IC 1	135 kDa	Lepidoptera, Diptera	CryII
kurstaki HD-1	71 kDa	Lepidoptera, Diptera	CryII
tenebrionis (sd)	66-73 kDa	Coleoptera	CryIII
morrisoni PG14	125-145 kDa	Diptera	CryIV
israelensis	68 kDa	Diptera	CryIV

The Cry protein: mode of action

1. The Cry protein is made as an inactive protoxin
2. Conversion of the protoxin (e.g., 130 kDa) into the active toxin (e.g., 68 kDa) requires the combination of a slightly alkaline pH (7.5-8) and the action of a specific protease(s) found in the insect gut
3. The active toxin binds to protein receptors on the insect gut epithelial cell membrane
4. The toxin forms an ion channel between the cell cytoplasm and the external environment, leading to loss of cellular ATP and insect death.

Isolation and genetic engineering of Cry genes

1. The Cry (or protoxin) genes are encoded by plasmid DNA, not by chromosomal DNA in *B. thuringiensis*
2. Cry genes were expressed in *B. thuringiensis* under the control of the ptet promoter (rather than its sporulation-specific promoter) and provided increase yield
3. Constructs have also been produced to enhance toxin action and/or expand its specificity.

A potential problem with Cry: development of insect resistance

Production of hybrid Bt toxins

1. Stacking of Bt toxin genes
2. Use of Bt toxins in combination with other insecticidal proteins such as chitinase and Cyt1A
3. In plants, the planting of crop buffer zones with non-genetically engineered Bt plants to maintain an insect susceptible population.

Baculoviruses as biocontrol agents

1. Baculoviruses are rod-shaped, double stranded DNA viruses that can infect and kill a large number of different invertebrate organisms
2. Baculoviruses have limited host ranges and generally do not allow for insect resistance to develop
3. Slow killing of target insects occurs

4. In order to speed killing (enhance effectiveness), several genes can be expressed in the baculovirus including diuretic hormone, juvenile hormone esterase, Bt toxin, scorpion toxin, mite toxin, wasp toxin, and a neurotoxin .

Baculoviruses

Baculoviruses are pathogens that attack insects and other arthropods. Like some human viruses, they are usually extremely small (less than a thousandth of a millimeter across), and are composed primarily of double-stranded DNA that codes for genes needed for virus establishment and reproduction. Because this genetic material is easily destroyed by exposure to sunlight or by conditions in the host's gut, an infective baculovirus particle (*virion*) is protected by protein coat called a *polyhedron* (plural *polyhedra*: see Figs. *A, B, and C*). Most insect baculoviruses must be eaten by the host to produce an infection, which is typically fatal to the insect.

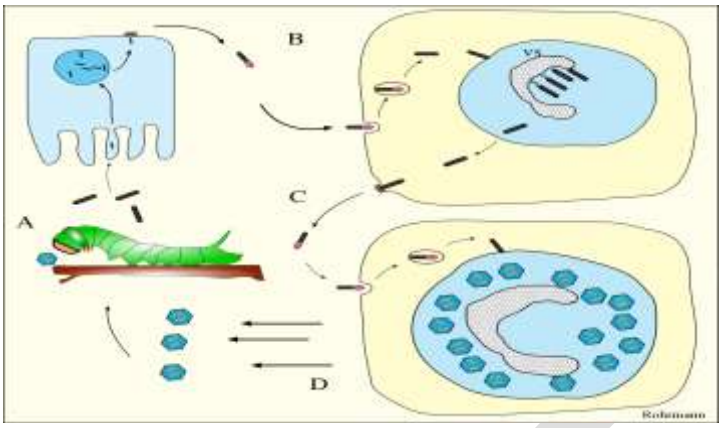
The majority of baculoviruses used as biological control agents are in the genus *Nucleopolyhedrovirus*, so "baculovirus" or "virus" will hereafter refer to nucleopolyhedroviruses. These viruses are excellent candidates for species-specific, narrow spectrum insecticidal applications. They have been shown to have no negative impacts on plants, mammals, birds, fish, or even on non-target insects. This is especially desirable when beneficial insects are being conserved to aid in an overall IPM program, or when an ecologically sensitive area is being treated. The USDA Forest Service currently uses the gypsy moth nuclear polyhedrosis virus (*LdNPV*) to aerially spray thousands of acres of forest each year.

On the other hand, the high specificity of baculoviruses is also cited as a weakness for agricultural uses, since growers may want one product to use against a variety of pests. Currently, researchers are attempting to use genetic engineering techniques to expand virus host ranges to the desired pest species. Releases of such genetically-engineered baculoviruses have been made by researchers in the U.K. and the United States and show promise, although the cost of commercial production of these agents must be reduced if they are to be competitive. Companies like Dupont, biosys, American Cyanamid, and Agrivirion (to name a few) have continued to explore the expansion and development of agricultural-use viral insecticides. Recently, biosys has released two baculovirus-based products, Spod-X for beet armyworm and Gemstar LC for tobacco budworm and cotton bollworm.

Life Cycle

Viruses are unable to reproduce without a host - they are *obligate parasites*. Baculoviruses are no exception. The cells of the host's body are taken over by the genetic message carried within each virion , and forced to produce more virus particles until the cell, and ultimately the insect, dies. Most baculoviruses cause the host insect to die in a way that will maximize the chance that other insects will come in contact with the virus and become infected in turn

Infection by baculovirus begins when an insect eats virus particles on a plant - perhaps from a sprayed treatment. The infected insect dies and "melts" or falls apart on foliage, releasing more virus. This additional infective material can infect more insects, continuing the cycle.



A life cycle of a baculovirus causing systemic infection. Occlusion bodies ingested by an insect, dissolve in the midgut and ODV are released which then infect epithelial cells (A). The virion buds out of the cell in a basal direction and initiate a systemic infection (B). Early in the systemic infection more BV are produced which spread the infection throughout the insect (C). Late in infection occluded virions are produced, and the cell then dies releasing the occlusion bodies (D). The virogenic stroma (VS) is indicated.

Relative effectiveness

It is widely acknowledged that baculoviruses can be as effective as chemical pesticides in controlling specific insect pests. However, the expense of treating a hectare of land with a baculovirus product invariably costs more than an equally efficacious chemical treament. This difference in price is due primarily to the labor intensive nature of baculovirus production. Some viruses can be produced *in vitro* (within cell cultures in the laboratory, not requiring whole, living insects). These are less expensive than those that can only be produced *in vivo*, that is, inside of living insects. The cost of rearing live hosts adds greatly to the final cost of the product. It is to be hoped that insect cell culture systems currently being developed for other uses may ultimately make viral pesticides more cost-effective.

Appearance

Insects killed by baculoviruses have a characteristic shiny-oily appearance, and are often seen hanging limply from vegetation (Fig.E). They are extremely fragile to the touch, rupturing to release fluid filled with infective virus particles. This tendency to remain attached to foliage and then rupture is an important aspect of the virus life-cycle. As discussed above, infection of other insects will only occur if they eat foliage that has been contaminated by virus-killed larvae.

It is interesting to note that most baculoviruses, unlike many other viruses, can be seen with a light microscope. The polyhedra of many viruses look like clear, irregular crystals of salt or sand when viewed at 400x or 1000x. The fluid inside a dead insect is composed largely of virus polyhedra - many billions are produced inside of one cadaver.

Habitat

Baculoviruses can be found wherever insects exist. Because rain and wind readily carry baculoviruses from place to place, it is likely that every piece of land and body of water contains some virus particles. It is widely accepted by researchers that most produce currently on the shelves is "contaminated" by baculovirus particles (Heimpel et al.,

1973). In fact, the pervasiveness of baculovirus particles, along with the results of tests performed in conjunction with registration, may be considered both indirect and direct evidence for the safety of these agents.

Pests attacked

Like most viruses, baculoviruses tend to be species or genus specific, although there are some exceptions to this rule, notably the *Autographa californica* nuclear polyhedrosis virus. Much of the genetics work currently being done to improve baculovirus-based pesticides is concentrated in the area of the virus genome controlling its host range.

Current use of baculoviruses as biological insecticides

COMMODITY	INSECT PEST	VIRUS USED	VIRUS PRODUCT
Apple, pear, walnut and plum	Codling moth	Codling moth granulosis virus	Cyd-Xe(3)
Cabbage, tomatoes, cotton, (and see pests in next column)	Cabbage moth, American bollworm, diamondback moth, potato tuber moth, and grape berry moth	Cabbage army worm nuclear polyhedrosis virus	Mamestrin*(5)
Cotton, corn, tomatoes	*Spodoptera littoralis*	*Spodoptera littoralis* nuclear polyhedrosis virus	Spodopterin*(5)
Cotton and vegetables	Tobacco budworm *Helicoverpa zea*, and Cotton bollworm *Heliothis virescens*	*Helicoverpa zea* nuclear polyhedrosis virus	Gemstar LC, Biotrol, Elcar(3)
Vegetable crops, greenhouse flowers	Beet armyworm (*Spodoptera exigua*)	*Spodoptera exigua* nuclear polyhedrosis virus	Spod-X(3)
Alfalfa and other crops	Alfalfa looper (*Autographa californica*)	*Autographa californica* nuclear polyhedrosis virus	Gusano Biological Pesticide (3)
Forest Habitat, Lumber	Douglas fir tussock moth (*Orgyia psuedotsugata*)	*Orgyia psuedotsugata* nuclear polyhedrosis virus	TM Biocontrol(2)

Pesticide compatibility

Viruses particles *per se* are generally unaffected by pesticides, although some chlorine compounds should be expected to damage or destroy viruses if applied at the same time. Baculovirus *efficacy*, however, can be altered in many ways by the effects of chemical pesticides on the host insect. A review by Jacques and Morris (1981) showed that of 10 pesticide-virus combinations, 9 resulted in an additive effect on insect mortality. However, some of the pesticides included in that review have since been banned, and this study is of limited use at present. More work is needed to explore the effectiveness of insecticide "cocktails" consisting of enviromentally friendly chemical agents and baculoviruses

Transgenic animals

A transgenic animal is one that carries a foreign gene that has been deliberately inserted into its genome. The foreign gene is constructed using recombinant DNA methodology. In addition to the gene itself, the DNA usually includes other sequences to enable it

- to be incorporated into the DNA of the host and
- to be expressed correctly by the cells of the host.

- Transgenic sheep and goats have been produced that express foreign proteins in their milk.
- Transgenic chickens are now able to synthesize human proteins in the "white" of their eggs.

These animals should eventually prove to be valuable sources of proteins for human therapy.

Two methods of producing transgenic mice are widely used

- Transforming embryonic stem cells (ES cells) growing in tissue culture with the desired DNA;
- Injecting the desired gene into the pronucleus of a fertilized mouse egg.

The embryonic stem cell method

Embryonic stem cells (ES cells) are harvested from the inner cell mass (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gametes.

Make your DNA
Using recombinant DNA methods, build molecules of DNA containing

- The gene you desire (e.g., the insulin gene);
- Vector DNA to enable the molecules to be inserted into host DNA molecules;
- Promoter and enhancer sequences to enable the gene to be expressed by host cells.

Transform ES cells in culture
Expose the cultured cells to the DNA so that some will incorporate it.

3. Select for successfully transformed cells.

4. Inject these cells into the inner cell mass (ICM) of mouse blastocysts.

5. Embryo transfer

- Prepare a pseudopregnant mouse (by mating a female mouse with a vasectomized male). The stimulus of mating elicits the hormonal changes needed to make her uterus receptive.
- Transfer the embryos into her uterus.
- Hope that they implant successfully and develop into healthy pups (no more than one-third will).

6. Test her offspring

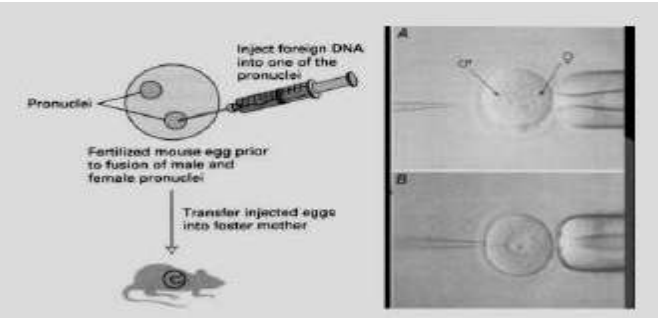
- Remove a small piece of tissue from the tail and examine its DNA for the desired gene. No more than 10–20% will have it, and they will be heterozygous for the gene.

7. Establish a transgenic strain

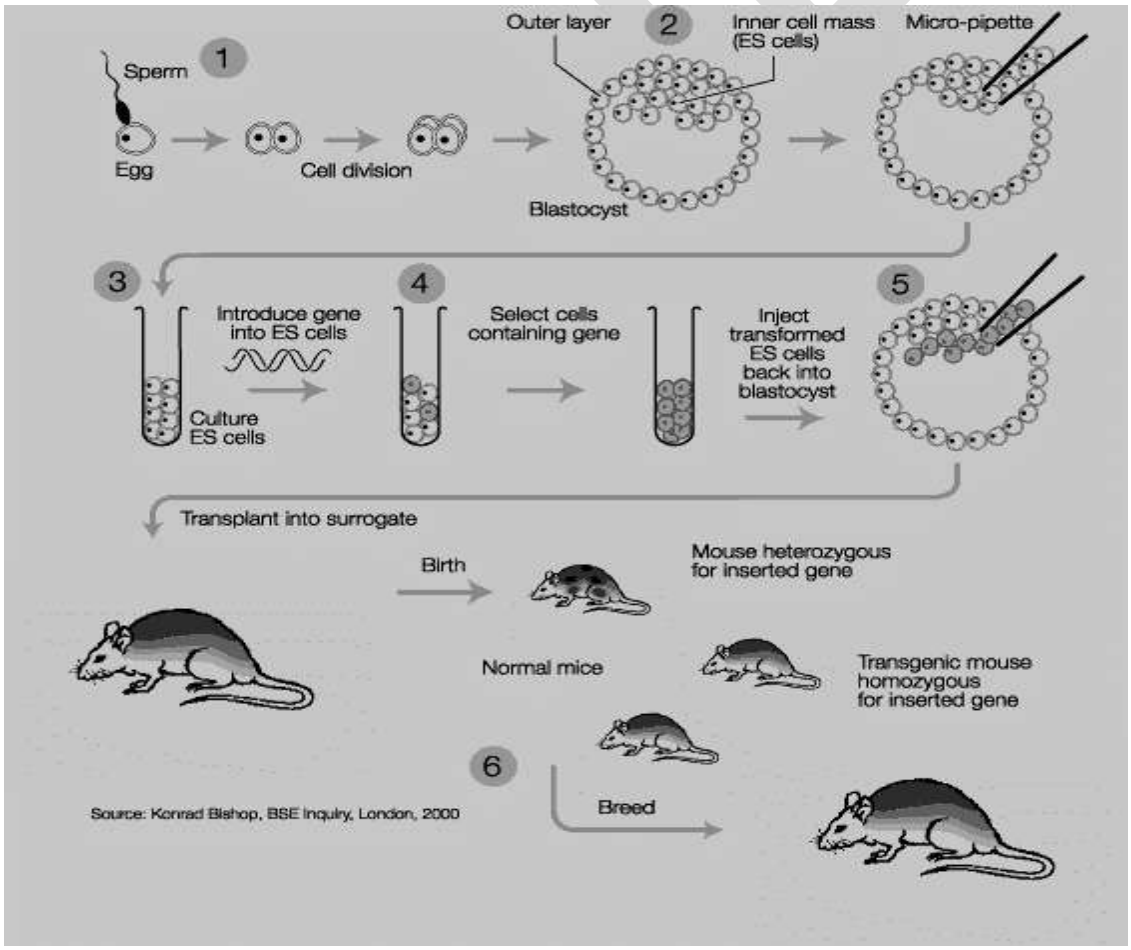
- Mate two heterozygous mice and screen their offspring for the 1 in 4 that will be homozygous for the transgene.

- Mating these will found the transgenic strain.

Injection of foreign gene in to the protonuclei



Preparation of transgenic mice



The pronuclear method

1. Prepare your DNA as in Method 1

2. Transform fertilized eggs

- Harvest freshly fertilized eggs before the sperm head has become a pronucleus.
- Inject the male pronucleus with your DNA.
- When the pronuclei have fused to form the diploid zygote nucleus, allow the zygote to divide by mitosis to form a 2-cell embryo.

3. Implant the embryos in a pseudopregnant foster mother and proceed

The giant mouse developed from a fertilized egg transformed with a recombinant DNA molecule containing:

- the gene for human growth hormone
- a strong mouse gene promoter

The levels of growth hormone in the serum of some of the transgenic mice were several hundred times higher than in control mice.

Random vs targeted gene insertion

The early vectors used for gene insertion could, and did, place the gene (from one to 200 copies of it) anywhere in the genome. However, if you know some of the DNA sequence flanking a particular gene, it is possible to design vectors that replace that gene. The replacement gene can be one that

- restores function in a mutant animal or
- knocks out the function of a particular locus.

In either case, targeted gene insertion requires

- the desired gene
- neo^r, a gene that encodes an enzyme that inactivates the antibiotic neomycin and its relatives, like the drug G418, which is lethal to mammalian cells;
- tk, a gene that encodes thymidine kinase, an enzyme that phosphorylates the nucleoside analog ganciclovir. DNA polymerase fails to discriminate against the resulting nucleotide and inserts this nonfunctional nucleotide into freshly-replicatin DNA. So ganciclovir kills cells that contain the tk gene.

Step 1

Treat culture of ES cells with preparation of vector DNA.

- Most cells fail to take up the vector; these cells will be killed if exposed to G418.
- In a few cells: the vector is inserted randomly in the genome. In random insertion, the entire vector, including the tk gene, is inserted into host DNA. These cells are resistant to G418 but killed by gancyclovir.
- In still fewer cells: homologous recombination occurs. Stretches of DNA sequence in the vector find the homologous sequences in the host genome, and the region between these homologous sequences replaces the equivalent region in the host DNA.

Step 2

Culture the mixture of cells in medium containing both G418 and ganciclovir.

- The cells (the majority) that failed to take up the vector are killed by G418.
- The cells in which the vector was inserted randomly are killed by gancyclovir (because they contain the tk gene).
- This leaves a population of cells transformed by homologous recombination (enriched several thousand fold).

Step 3

Inject these into the inner cell mass of mouse blastocysts.

Transgenic sheep and goats

Until recently, the transgenes introduced into sheep inserted randomly in the genome and often worked poorly. However, in July 2000, success at inserting a transgene into a specific gene locus was reported. The gene was the human gene for alpha1-antitrypsin**,** and two of the animals expressed large quantities of the human protein in their milk.

Sheep fibroblasts (connective tissue cells) growing in tissue culture were treated with a vector that contained these segments of DNA:

1. 2 regions homologous to the sheep COL1A1 gene. This gene encodes Type 1 collagen. (Its absence in humans causes the inherited disease osteogenesis imperfecta.)
2. This locus was chosen because fibroblasts secrete large amounts of collagen and thus one would expect the gene to be easily accessible in the chromatin.
3. A neomycin-resistance gene to aid in isolating those cells that successfully incorporated the vector. [Link to technique]
4. The human gene encoding alpha1-antitrypsin.
   Some people inherit two non- or poorly-functioning genes for this protein. Its resulting low level or absence produces the disease Alpha1-Antitrypsin Deficiency (A1AD or Alpha1). The main symptoms are damage to the lungs (and sometimes to the liver).
5. Promoter sites from the beta-lactoglobulin gene. These promote hormone-driven gene expression in milk-producing cells.
6. Binding sites for ribosomes for efficient translation of the beta-lactoglobulin mRNAs.

Successfully-transformed cells were then

- fused with enucleated sheep eggs [Link to description of the method] and
- implanted in the uterus of a ewe (female sheep).
- Several embryos survived until their birth, and two young lambs lived over a year.
- When treated with hormones, these two lambs secreted milk containing large amounts of alpha1-antitrypsin (650 µg/ml; 50 times higher than previous results using random insertion of the transgene).

On June 18, 2003, the company doing this work abandoned it because of the great expense of building a facility for purifying the protein from sheep's milk. Purification is important because even when 99.9% pure, human patients can develop antibodies against the tiny amounts of sheep proteins that remain.

However, another company, GTC Biotherapeutics, has persevered and in June of 2006 won preliminary approval to market a human protein, antithrombin, in Europe. Their protein — the first made in a transgenic animal to receive regulatory approval for human therapy — was secreted in the milk of transgenic goats.

Transgenic chickens

- grow faster than sheep and goats and large numbers can be grown in close quarters;
- synthesize several grams of protein in the "white" of their eggs.

Two methods have succeeded in producing chickens carrying and expressing foreign genes.

- Infecting embryos with a viral vector carrying
    - the human gene for a therapeutic protein
    - Promoter sequences that will respond to the signals for making proteins (e.g. lysozyme) in egg white.
- Transforming rooster sperm with a human gene and the appropriate promoters and checking for any transgenic offspring.

Preliminary results from both methods indicate that it may be possible for chickens to produce as much as 0.1 g of human protein in each egg that they lay.

Not only should this cost less than producing therapeutic proteins in culture vessels, but chickens will probably add the correct sugars to glycosylated proteins — something that E. coli cannot do.

Transgenic pigs

Transgenic pigs have also been produced by fertilizing normal eggs with sperm cells that have incorporated foreign DNA. This procedure, called sperm-mediated gene transfer (SMGT) may someday be able to produce transgenic pigs that can serve as a source of transplanted organs for humans.

Transgenic primates

In the 28 May 2009 issue of Nature, Japanese scientists reported success in creating transgenic marmosets. Marmosets are primates and thus our closest relatives (so far) to be genetically engineered. In some cases, the transgene (for green fluorescent protein) was incorporated into the germline and passed on to the animal's offspring. The hope is that these transgenic animals will provide the best model yet for studying human disease and possible therapies.

DNA diagnostic in medical forensics

Every cell of an individual carries a copy of the DNA. A cell collected from a person's skin or hair folicle contains the same DNA as from that persons heart tissue or white blood cells. Order of base pairs in the DNA of every individual is different except identical twins.

How do we distinguish one person's DNA from another?

1. We do not need to sequence the entire 3 billion base pairs of a person's DNA to distinguish it from another person's DNA.

2. Intron regions of DNA (junk DNA) contain sequences that are 20-100 bp in length that are repeated at different locations (loci) along the chromosome. CGGCTACGGCTACGGCTA (repeated 3 times at this location; at another location, it may be repeated 9 times)

3.These sequences are called Short Tandem Repeats (STRs) or VNTRs

STRs

- Each person has some STRs that were inherited from mother and some from father.
- No person has STRs that are identical to those of either parent
- The number of repeats at each loci on chromosome is highly variable in the population, ranging from 4 to 40.
- The length of the DNA after cutting the chromosome with a restriction enzyme, and its position after electrophoresis will depend on the exact number of repeats at the locus
- The uniqueness of an individual's STRs provides the scientific marker of identity known as a DNA fingerprint.
- In the United States the FBI has standardized a set of 13 STR assays (13 different locations on the chromosomes) for DNA typing, and has organized the CODIS database for forensic identification in criminal cases.
- The United States maintains the largest DNA database in the world: The Combined DNA Index System, with over 60 million records as of 2007.
-

Preparation of a DNA fingerprint

- Specimen collection
  - blood, semen, etc
  - easy to contaminate a DNA sample with DNA from other sources (bacteria, DNA of person collecting sample)
- **DNA fingerprinting is a comparative process**
  - DNA from crime scene is compared with DNA of a suspect
  - So minimum of two samples must be prepared
- **DNA extraction**
  - standardized methods have been developed
  - need to separate DNA from other cell material and debris from crime scene.
- PCR amplify STRs using target sites on chromosome
- Design primers that anneal to STR locus

Amplify all the regions of the chromosome where the STRs exist. PCR allows you to make millions of copies of the STR region from a single copy of DNA you recovered from crime scene.

- Since the # of times sequence is repeated is different for each person, fragment size will be different.
- This is done for 13 different STR sequences at this one locus
- Differences occur among individuals at each of the 13 loci on the chromosome where the STRs occur
- This allows for a lot of variation

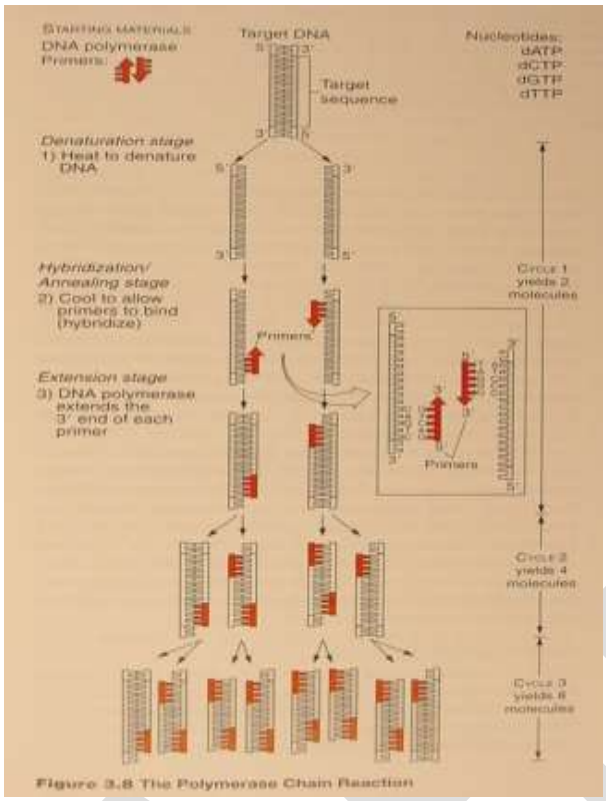


Fig: Finger printing

- Since the # of times sequence is repeated is different for each person, fragment size will be different.
- This is done for 13 different STR sequences at this one locus
- Differences occur among individuals at each of the 13 loci on the chromosome where the STRs occur
- This allows for a lot of variation

Restriction fragment length polymorphism (RFLP)

- If you do this for 13 different repeat sequences at 13 different loci on the chromosome, each person produces a different band pattern when the fragments are separated by gel electrophoresis

Dot blotting

- Because every gene amplified by PCR has the same length, we don't need to use electrophoresis to sort and separate the fragments.
- Instead, we use "blot strip" or dot blot which contains a different DNA probe that is sensitive to the sequence variability.

UNIT III - POSSIBLE QUESTIONS

PART B

1. Give short note on Ti plasmid .
2. Explain the development of transgenic mice by microinjection.
3. Explain in detail about the cry gene and its role in biocontrol.
4. Describe in details about antisense technology.
5. What are the biocontrol agents explain with examples.
6. Write short notes on methods of application of *Bt* and its products in agriculture
7. Write an essay on baculoviruses.
8. Explain the Application of finger printing.
9. Describe the Baculovirus as biocontrol agent
10. Explain - How will you achieve delayed fruit ripening using recombinant technology?
11. Write the mode of action of BT toxin.
12. Describe about biosafety and bioethics

PART C

1. Explain in detail about role of DNA diagnostics in medical forensics.
2. Explain the development of transgenic mice.
3. Detail account on development of herbicide resistant plant

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UNIT III

.----------converts the protoxin into active toxin.	Nuclease	Urease	Protease.	Lipase.
Convertion of protoxin to active toxin require both protease and ----------	Alkaline pH	Urease	Acid pH	Lipase.
Parasporal crystals sensitive to ----------	Sunlight	Chemicals	Acid pH	Lipase.
Most effective and most often utilized microbial insecticides are toxins synthesized from .	.B.amyloliquefaciens	B.thuringiensis	B.subtilis	B.licheniform
B.thuringienis subspecies kurstaki is toxic to	bugs	worms	cabbage worm	small worms
B.thuringiensis subspecies israelensis kills .	cabbage worm	black flies	lepidopteron larvae	none of the above
_____ is also known as sandeigo.	B.thuringiensis subsp israelensis	B.thuringiensis subsp kurstaki	B.thuringiensis	B.thuringiensis stubs tenebrionis
The parasporal crystal is .	not the active form	a protoxin	precursor of active toxin	all the above
The insecticidal activity of B.thuringiensis is contained within a very large structure called _____ .	parasporal crystal	parabasal crystal	perisporal crystal	sporal crystal
The subunits of the parasporal crystal can be dissociated invitro by treatment with	alcohol	ethylene	β-mercaptoethanol	.xylene

Parasporal crystals are lived in the environment	short	long	.moderately	limited
To enhance production rate for B.thuringiensis are used.	promoters active in all phase	promoters active in sporulation	promoters active in vegitation	promoters active in one phase
When tenebrionis toxin gene is transformed into B.thuringiensis subsp israelensis the transformants was toxic to .	brassicae	cabbage white butterfly	catterpiller	butterfly
The parasporal crystals of B.thuringiensis subsp israelensis shows as an insecticide	more efficacy	sinks rapidly when sprayed	sinks slowly when sprayed	medium efficacy
The alternative bacterium used for B.thuringiensis toxin is	Caulobacter crescentus	Zymomonas	A. niger	none of the above
Which one of the following can proliferate well in water surface near mosquito larvae?	Synechocystis	Synechovibrio	both a and b	Synechococcus6
Which one of the following can be used as a biocontrol agent?	Baculovirus	retrovirus	A. niger	Penicilliu m sp
Baculovirus are pathogenic to	Neuroptera	Trichoptera	Diptera	all the above
. The gene that encodes insect specific neurotoxin was produced by	Androctonus australis	Synechocystis	Caulobacter	Synechovibrio
The insect specific neurotoxin disrupts the .	normal life cycle of insect	flow of Na ions	sporulation	flow of chloride ions
When the parasporal crystal is ingested by a target insect, the protoxin is activated by .	alkaline pH	specific digestive proteases	both a and b	None of the above

The mode of action of B.thuringiensis toxins imposes certain constraints in application. They are	for killing the insect it must be ingested	insects of plant roots are less likely ingesting Bt toxin.	kills insects during a specific developmental stage	all the above
Chemical insecticides has the following disadvantage	specificity	insects become sensitive easily	beneficial insects being killed	simple degradation
The steps taken to kill insects in plant roots is	to inject Bt toxin into roots	to introduce Bt gene into cells of root	introduce Bt toxin gene into bacterial species of rhizosphere	spray the Bt toxin
Methods for biological protection of plants	transgenic plants	chemical insecticides	trimming of plants	avoid plant damage
Baculo virus is a	Parasite	Obligate parasite	Saprophyte	Pathogen
Biologiacal insecticides are usually_____ for a number of insect species.	less specific	Highly specific	resistant	highly resistant
The biological insecticides are _____to humans and other animals	hazardous	.useless	non-economical	non-hazardous
Bt toxin is safe because	persist in the environment	hazardous to mammals	does not persist in the environment	non degradable
gene transfer to animal by ----------	Transformation efficiency	microinjection	vector Ti	transduction
Site directed mutagenesis refer to	change of whole genome	changes in a single base	change of whole DNA sequence	none

The transgenic plant transformed with highly modified synthetic protoxin gene had level of expression than wild type.	10 fold	100 fold	1000 fold	10000
What is antisense RNA?	RNA molecule complementary to gene transcript (mRNA)	RNA molecule complementary to DNA	DNA molecule complementary to RNA.	Type of RNA
Sense RNA is	the translate m RNA	the translate DNA	both a and b	none
Chemical herbicides are .	No side effects	discriminate weeds from crop	persist in the environment	all of the above
The different biological manipulations that would cause a crop plant to be herbicide resistant are	overproduction of herbicide sensitive target protein	improve the ability of herbicide	resistant protein to bind to herbicide	metabolic activation of herbicide
Glyphosate is .	environment friendly	hazardous	toxic to living beings	none of the above
The EPSPS plays important role in the synthesis of amino acids in both bacteria and plants	aromatic	aliphatic	both a and b	none
Bromoxynil, a herbicide acts by inhibiting .	chlorophyll content	photosynthesis	uptake of water and nutrients	utilization of carbon dioxide
_____ can inactivate bromoxynil	nitrilase	denitrilase	salicylase	all of the above

The gene for enzyme nitrilase was isolated from .	Klebsiella ozaenae	E.coli	Pseudomonas	Staphylococcus aureus
Chemical herbicides are .	No side effects	discriminate weeds from crop	persist in the environment	disappear fm soil
. The length of the T-DNA region can vary from .	12 to 24 Kb	10 to 20 Kb	17 to 26 Kb	9 to 19 Kb
Delay of fruit ripening can be done by .	antisense RNA technology	biofarming	refrigeration	incubation
The plant growth promoter induces the experience of a number of genes involved in fruit ripening and senescence.	auxin	cytokinin	gibberellins	ethylene
Ethylene is synthesized from _____.	S- adenosyl methionine	S-thymidine methionine	S-guanosyl methionine	S-cyclosine methionine
The length of the T-DNA region can vary from .	12 to 24 Kb	10 to 20 Kb	17 to 26 Kb	9 to 19 Kb
Crown gall tumor is induced by .	E.coli	A.tumefaciens	Pseudomonas	Acinetobacter
_____ are encoded on the Ti plasmid of A.tumefaciens.	ras genes	Vir genes	coz genes	nif genes
In the T-DNA region the gene which encodes isopentenyl transferase is .	tmr gene	tms 1 gene	tms 2 gene	nif gene
_____ Permits the Ti plasmid to be stably maintained in A.tumefaciens	ori region	vir gene	opine catabolism region	Nopaline

Hairy root disease in higher plants is caused by .	A.rhizogenes	A.tumefaciens	E.coli	Bacillus
.---------- produce parasporal crystal which kills insects	B.thuringinsis	B.rhizogenes	Heliothis virescens	Autographa californica
DNA virus ---------- -----	Baculo virus	Mosaic virus	Simian virus	Satellite virus
Baculo virus particle consist of cylindrical ---------- -that surrounds viral DNA	Nucleocapsid	Nucleus	Nucleosome	Proteosome
The Cry I proteins are toxic to ----------	Diptera	Coleoptera	Lepidoptera	Both a & b
The Cry IV protein is toxic to ----------	Diptera	Coleoptera	Lepidoptera	d) Both a & b
Parasporal crystal does not usually contain the active form of the ----------	Insecticide	Pesticide	Herbicide	Both b& c
The B. thuringiensis subsp ---------- insecticidal protein is highly toxic when injested by mosquito larvae.	israelensis	kurstaki	tenebrionis	aizawai
Possible attractive host for the expression of mosquitocidal Cry genes ---- ----------	Bacillus sphacericus	B.thuringinsis	B.rhizogenes	Asticcacaulis excentricus
Protoxin is activated with in the ----------	Gut	Lungs	Respiratory tract	Stomach
Antisense therapy is .	prevents the expression of the defective gene	delivery of a remedial gene into organ	cells taken from organ after the correction it transplanted back	remedial gene is introduced into an embryo.

Testing of newborn for children for genetic disease is	parental screening	postnatal screening	antenatal screening	prenatal genetic screening
The microinjected transgene construct is in form and free of vector DNA sequences	linear and prokaryotic	Circle and prokaryotic	Circle and eukaryotic	linear and eukaryotic

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Protease
Alkaline pH
Sunlight
B.thuringiensis
cabbage worm
black flies
B.thuringiensis stubs tenebrionis
all the above
paraspora l crystal
β-mercaptoethanol

short
promoters active in all phase
cabbage white butterfly
sinks rapidly when sprayed
Caulobacter crescentus
both a and b
Baculovirus
all the above
Androctonus australis
flow of Na ions
both a and b

all the above
beneficial insects being killed
introduce Bt toxin gene into bacterial species of rhizosphere
transgenic plants
Obligate parasite
highly resistant
non-hazardous
does not persist in the environment
microinjection
changes in a single base

100 fold
RNA molecule complementary to gene transcript (mRNA)
the translatem RNA
persist in the environment.
overproduction of herbicide sensitive target protein
environment friendly
aromatic
photosynthesis
nitrilase

Klebsiella ozaenae
persist in the environment
12 to 24 Kb
antisense RNA technology
ethylene
s-adenosyl methionine.
12 to 24 Kb
A.tumefaciens.
vir genes
tmr gene
ori region.

A.rhizogenes
B.thuringinsis
Baculovirus
Nucleocapsid
Diptera
Lepidoptera
Insecticide
kurstaki
B.thuringinsis
Gut
prevents the expression of the defective gene

postnatal screening
linear and prokaryotic

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DEPARTMENT OF MICROBIOLOGY
MICROBIAL TECHNOLOGY AND INTELLECTUAL PROPERTY RIGHTS
COURSE CODE: **17MBP203** BATCH: **2017-2019**
UNIT – IV

Intellectual property rights (IPR)

One of the most important issues, due the emergence of modern biotechnology, is the legal characterization and treatment of trade related biotechnological processes and products, popularly described as intellectual property, and the rights associated with this are known as Intellectual Property Rights (IPR), or rights given to people over the creations of their minds.

Types of intellectual property rights

1. Intellectual property is intangible and include “Patents”, “Trade Secrets”, “Copyrights" “Trade marks” and “Plant Breeders' Rights”.
2. The rights to protect this property prohibit others from making, copying, using or selling the proprietary subject matter.
3. Under biotechnology, one of the most important examples of intellectual property is the processes and products, which result from the development of genetic engineering techniques through the use of restriction enzymes to create recombinant D.N.A.

Intellectual Property Rights (IPR) can be described as follows:

Patents: Granting of special exclusive rights (for trading new articles) has been a practice to encourage innovations, e.g. monopoly rights (only to inventors) were granted in some countries of Europe, as an incentive to develop new articles that would benefit the Society. Under the US Laws, a patent means selling an invention for a period of 17 years. In India, there is “Indian patent act of 1970” that allows to process patents, but no product patents for food, chemicals, drugs and pharmaceuticals. The duration of patent in India is 5 years from the date of grant of patent or 7 years from the date of filing the application, whichever is less. On December 26, 2004 the Indian government promulgated the patents (amendment) Ordinance 2004 as also the patents (Amendment) Rules, 2005 to comply with the Trade Related Intellectual Property obligations.

Before a patent can be issued, following specific conditions must be met:

- The invention must be new, (novelty) and should have utility.
- It must be inventive.
- It must be disclosed (discloses) in a way, which enables a person of normal skill to reproduce it.
- The scope of protection to be granted must be in proportion to the invention.
- It must relate to a technology, where patents are permitted (Patentable).

Trade secrets: Trade secrets often include private proprietary information or physical material that allows a definite advantage to the owner. This can be illustrated by the popular example of coca-cola brand syrup formula. Trade secrets in the area of biotechnology may include material like Hybridization conditions, Cell lines, Corporate merchandising plans, Customer lists etc. Unlike patents, trade secrets have an unlimited duration. Disclosure of a trade secret and its unauthorized use can be punished by the court of law and the owner may be allowed compensation. However, if trade secret becomes public knowledge by independent discovery or other means it is no longer protectable.

Copy rights: It involves only the expressed material (printed, painted, tape recorded, video recorded or expressed in any other form). In biotechnology, the copyright may cover the D.N.A. sequence data, which

may be published. Computer databases and photomicrograph of D.N.A. instruction manual may also become copyright material.

Trademarks: A trade mark is the word or symbol adopted and used by a manufacturer or merchant to identify his goods and distinguish them from those manufactured or sold by others.

Intellectual Property Rights are statutory rights once granted allows the creator(s) or owner(s) of the intellectual property to exclude others from exploiting the same commercially for a given period of time. It allows the creator(s)/owner(s) to have the benefits from their work when these are exploited commercially. IPR are granted to an inventor or creator, designer in lieu of the discloser of his/her knowledge.

Governing Laws in India for IPR as follows

1. Patent Act 1970
2. Trade Marks Act (1958 original) 1999
3. The Copyright Act 1957
4. The design Act 2000
5. Geographical Indication of Goods (Registration and Protection) Act 1999
6. Plant Variety and Farmers Right Protection Act 2001

What is an invention / innovation?

An invention means: a new product or process involving an inventive step and capable of industrial application. An Innovation means: The successful exploitation of new ideas in the form of a useful machinery or process, by any person, using own intellect is called as innovation. Every innovation may not be patentable invention but every invention is an innovation. All the inventions are the innovations and are patentable, but all the innovations are not the patentable inventions.

The patent system

A patent is a contract between the inventor or applicant for the patent and the state, whereby the inventor or applicant gets a monopoly from the State for a certain period in return for disclosing full details of the invention. The patent system thus ensures that information on new inventions is made available for eventual public use so as to encourage technical and economic development and discourage secrecy.

If an inventor or company has an invention, which they consider to be novel and inventive, they may apply for a patent. This may be granted only after a detailed examination by a patent office. Once the patent is granted the inventor or applicant has the sole right to make, use or sell the invention for a limited period. This period is usually twenty years. There can also be confusion about what exactly can be protected by the patent system. Patents can only be applied to inventions. These usually have an industrial dimension. An invention is normally a new product, which involves a new principle of operation or an improvement to an old principle. Alternatively it may refer to a new or improved industrial process. Things, which do not involve manufacture, are not usually considered to be inventions. For example, a new scientific theory or a new surgical procedure would not be considered to be patentable for this reason.

Novelty and inventiveness

In order to be suitable for patenting, an invention must be novel and inventive. An invention is considered to be novel if it has not been disclosed to the public at the time that the patent application was made. As long as the date of the patent application precedes any disclosure of details of the invention to the public, the invention can be validly patented. If however, details of the invention have been disclosed to the public before applying for a patent, then the invention is no longer considered to be novel in a patenting sense and it will not be possible to protect it validly through the patent system.

It is important to be aware of the danger of premature disclosure of details of an invention. Even after a patent application has been filed, details of the invention should only be disclosed as part of a planned programme of commercial exploitation. Another requirement for a valid patent is inventiveness. This means that the invention must contain an inventive step. This can be the most difficult thing to show. A patent examiner may decide that the invention is obvious i.e. that somebody knowledgeable in the subject area, when familiarised with all earlier patents or other technology in the area, would have immediately been led to the same conclusion.

Commercialization of inventions

Many inventors feel that filing a patent application is the most important and first thing they must do once they have an idea. This is rarely the case. Patenting an invention is not the only consideration and rushing to file an application may actually be the wrong thing to do first. Patents are of no value unless the commercial worth of the product or technology can be demonstrated and exploited. Many patentable inventions have failed not because they didn't work, or because they had been invented before, but because the inventor was unable to exploit them commercially. Inventing is increasingly being seen as a business. You must invest in the business if you wish to make a return, and management and marketing skills are every bit as important as technical skills. If the inventor does not have all the skills required, it may be necessary to put together a team or partnership to exploit the project or to license the invention to an existing company who already has related products. If one does successfully commercialize an invention however the rewards can be substantial. A number of successful companies' world over own patents, which protect them against, copied products home or imported. This is an important factor in present day international trade. Most other traditionally used barriers to trade are being removed in the interests of fair competition. Patents are one of the few mechanisms that companies can legally use to protect their market share. Having foreign patents also allows Irish companies to protect their products in export markets. Where a product is unsuitable for export because of distance, cost or other factors, a licensing strategy can be used. The Indian company can use the patents to license the manufacturing/marketing rights for their invention to a foreign manufacturer. In return they receive a royalty, which increases their profits. Licensing for both the home and export markets to Indian and/or foreign companies are also the appropriate strategy for inventions made by non-manufacturing companies or by universities and colleges. To succeed, an inventor does not have to have a great deal of business or technical expertise. He/she must however adopt a businesslike approach to the project. The first thing is to realize that there are several stages in the inventive process. It is vital to realize what stage one is at and what one needs to do next.

The stages of development of a successful invention are

Identification of a problem that needs to be solved.

Inventing a solution to the problem, which works.
Developing a prototype or being able to demonstrate the invention to prove how it works.
Filing a patent application to protect the invention so that it can be disclosed to other people.
Arranging the manufacturing and marketing of the invention either through one's own company or through licensing.

Each stage requires its own particular expertise and resources. It is essential that the early stages are satisfactorily completed before moving on. Experience shows that taking short cuts does not pay. For example, it is hard to get investors or potential licensees to appreciate the benefits of a particular invention if the prototype is very crude and does not work properly. Similarly there is little point in filing a patent application until one is satisfied that the invention can be shown to work. There can be some overlap between the last two stages however. If it is possible to make some progress with manufacturing and marketing without compromising the patent position, then one should do this. As mentioned elsewhere, very often the later one files the patent application the better.

Disclosing an invention

Details of an invention should not be disclosed to outsiders until such time as a patent application has been filed. However, many people make the mistake of filing patent applications too early. Because they are afraid that somebody else may invent the same thing, they file an application as quickly as possible without having any clear plan as to what they are going to do next. They then find that many months pass before they are in a position to commercially exploit the invention, and they have not left enough time to obtain the necessary finance to cover international patent filings. In general, it is better to complete the development of the invention and file the patent application when it becomes necessary to make disclosures as part of a planned program of commercial exploitation. If it is necessary to talk to technical specialists or others in order to obtain assistance during the development of the invention, this should be done on the basis of confidentiality. People should be informed that the information is strictly confidential and asked to sign a simple document undertaking not to disclose the information until given permission to do so. Adopting a proper commercialization strategy involves considering all aspects at the same time, technical, commercial and legal. At the initial stages proper attention should be given to the technical aspects, but once the patent application is filed, the commercialization should proceed as quickly as possible within the limited time scale provided by the patent system. Once an application has been filed in Ireland, applications in other countries must be made within twelve months if the best protection is to be obtained. As is explained below, an international patent program can be a very expensive business. Funding for it from either private or public sources is unlikely to be obtained unless there are definite commercial plans for the invention which are well advanced. Setting up one's own manufacturing company or identifying potential licensees and reaching agreement with them can take time. A period of longer than twelve months is usually required to complete either of these activities. Thus if one has filed ones patent application too early one will inevitably run into financial difficulties in trying to keep it going. Another reason why it can be a mistake to file too early is that development of the invention may not be completed. Designs may change during development or other inventive features may be introduced. If the patent specification has been drafted too early it may not be possible to amend it to reflect the changes made. One can end up with a patent, which does not really cover the final commercial product.

Academic research

People carrying out academic research are frequently under pressure to publish the results of their research for academic reasons. Researchers should, at all times, bear in mind the possibility of commercial results from their research. If a researcher sees a commercial application from his or her research, it would be wise to delay publication until a patent application has been filed.

Applying for a patent

The first step that people usually take in applying for a patent is to file a preliminary application in one country. When the application is filed, the date of application is recorded and this is called the "priority date". The first application can be quite basic and does not have to include a set of claims (see below). It is still an important document and specialist advice from a patent agent should be obtained in preparing it. Most countries are signatories to an international convention, which guarantees that the priority date of an invention filed in one country will be respected in other countries, provided an application is filed in the other countries within twelve months of the date of filing the first application. This is why the first document filed can be very important later.

The system of filing an application in one country initially can be of great benefit to inventors provided they have timed it correctly. It allows up to twelve months before foreign applications must be filed. During this time the inventor can assess the commercial prospects of the invention, carry out improvements on it, and arrange the necessary finance for international patenting and commercial exploitation through manufacture and sale. This period is also used to assess the market potential for the invention in various countries and to decide in which countries the expense of patenting is justified. Note though the comments earlier about the dangers of underestimating the time it takes to do these things and the dangers of filing too early.

Patent specifications

The patent system is complex, and great skill is required in reducing the principle of an invention to words, which will have legal effect. Patent agents have detailed knowledge of the complex procedures in the various foreign patent systems and work with other patent agents throughout the world to obtain patent protection for an invention in different countries. A patent specification is written in a certain format, which may not be immediately obvious to the casual reader. The specification usually contains a preamble, which describes the background to the invention. Then comes a statement of invention, which is a legal statement of the scope of the monopoly sought. This is followed by a detailed description of the invention, usually drawings or examples of how the invention is carried out. The final part of the specification includes a set of claims. These are not normally required in the preliminary application but are a vital part of the final document.

A claim in this sense has nothing to do with the conventional use of the word, and does not relate to the advantages or performance of the invention. A patent claim is where the patent agent sets out the scope or extent of the monopoly, which he claims on behalf of the inventor. In other words, one is claiming a territory of technology within which other people may not stray without infringing the patent. The scope of the patent is very important. One can imagine that a patent for a completely new type of engine would have a very broad scope whereas a patent for an improvement in one component of that engine might be quite limited in scope.

Examination

When patent specifications have been filed in the various countries the patent examiners in those countries examine them. These examiners carry out a search through previous patent specifications and other literature in order to ascertain if the invention is novel. They also look at the question of inventiveness in relation to the "prior art". As a result of the patent search, an examiner may feel that certain features of the invention have already been disclosed in previous specifications. Correspondence then ensues between the patent examiner and the patent agent until the examiner is satisfied that the claims for the patent are allowable. This can often mean an amendment or narrowing of the scope of the patent claims until the Patent Office in question is satisfied that it does not overlap the "territory of technology" claimed by previous inventors. This stage of the patenting procedure is called "prosecution" and can involve the inventor or applicant in considerable expense depending on the amount of work required to be done by the patent agent.

As part of the patent examination procedure, the specification filed by the applicant is published, usually eighteen months after the priority date. The Patent Office also publishes a list of previous patents, which were found to be of relevance in the patent search. Thus, even if an inventor has not disclosed the invention in any way up to this point, the patent system itself will make a disclosure and destroy its novelty at this time. It is for this reason that inventions once disclosed cannot be the subject of subsequent patent applications either by the inventor or by anybody else. When the Patent Office has satisfied itself concerning the scope of the claims, which are to be granted, notice of allowance of the patent will be issued and the patent will be granted. In some countries (not in Ireland) there is a period however during which interested parties may oppose the granting of the patent by lodging their grounds for opposition with the Patent Office. If no one is successful in opposing the grant of the patent, the Letters Patent Document is issued and the patent comes into force.

Infringement

If anybody attempts to make, use, or sell an invention, which is covered by a patent which is in force in a certain country, he or she may be sued in that country for infringement by the patentee. If infringement is proved, damages may be awarded to the owner of the patent. Patent litigation is notoriously expensive, and is not entered into lightly. The greater the commercial potential of an invention, the higher is the chance that the patent will be infringed or contested. The fact that a patent is granted does not automatically mean that the inventor is given full protection. A granted patent can in certain circumstances be invalid because certain information did not come to the attention of the patent examiner during the course of the examination. This could show, for example, that the invention was not in fact novel. A court decision may ultimately be needed before the inventor finds out whether he is protected or not.

IPR protection in India

Intellectual property is intangible incorporate property consisting of bundle of rights. The property imbibed from the intellectual capacity of a human brain for instant an invention, design of an article, literary or artist work, symbols / trademarks, having commercial value and the same is not available in the public domain.

Intellectual property commonly encompasses the following

1. Patent

2. Trademarks
3. Industrial Design
4. Copyright
5. Geographical Indication of Goods
6. Integrated Circuit
7. Protection of Undisclosed Information such as Trade Secrets

Patents

What is Patent?

Patent enables its owners to exclude from making, using and selling its inventions.

Term of patent: The term of patent is for twenty years (20), provided the maintenance fee is paid at the end of every year.

Territorial scope: Patent laws are territorial; a separate patent must be obtained in each country. Indian patent office protects invention only filed in India.

What is patentable?

Only inventions are patentable. An invention must be new, useful and must involve inventive steps compared to closest prior art. A new and unobvious product, process, apparatus or composition of matter will generally be patentable.

Patentability searches: Patentability search is a search for invention in hope of not finding the invention. The patentability search is an universal concept since inventions cannot be boundary constraint. But it is to be noted that the patent laws are territorial. Computer databases search is quick and relatively inexpensively. Database searches are most useful in searching sophisticated inventions, which can be described by precise, well-known terms of art. They are much less useful in searching mechanical gadget type inventions. No search will "guarantee" the patentability of any invention. The object is to make a reasonable assessment of the prospects for obtaining worthwhile patent protection. Search results are also useful in preparing a patent application.

What information is required for conducting search?
To conduct a search the description, drawings or photographs of the invention, showing how it is made, operated and used would be helpful. Further details of any known prior art; a summary of the prior art's shortcomings; an explanation of how these are overcome by the invention; a list of any other advantages of the invention; and, details of any possible variants or modifications that could be made without departing from the general concept of the invention.

Why one should go for a patent?
To enjoy the exclusive rights over the invention. If the inventor does not get the patent rights over his invention and introduce his product/process based on his invention in the market, anybody can copy his invention and exploits it commercially. To debar others from using, selling or working out his invention, the inventor must go for getting a patent.

Who can apply for a patent?

An application for obtaining a patent can be made by a true and first inventor who holds the rightful ownership in the invention due to fact that he invented the same or by any person who is an assignee/legal representative of the first and true inventor. Also a legal heir of the first and true inventor can apply for patent in case of the death of the true and first inventor.

What is not patentable invention?

1. An invention which is frivolous or which claims anything obviously contrary to whole established natural laws.
2. An invention the primary or intended use or commercial exploitation of which could be contrary to public.
3. The mere discovery of a scientific principle or the formulation of an abstract theory.
4. The mere discovery of any new property or new use for known substance or of the mere use of known process, machine or apparatus unless such known process result in a new product or employ one new reactant.
5. A substance obtained from mere admixture resulting into aggregation of properties.
6. Mere arrangement or re-arrangement or duplication of known devices each functioning independently.
7. A method of agriculture or horticulture.
8. Any process for the medicinal, surgical, curative, prophylactic or other treatment of human beings or animals.
9. Plants and animals in whole or any part in whole or any part thereof other than micro organism but including seeds, varieties and species and essentially biological processes for production or propagation o
10. A computer program per se other than its technical application to industry or combination with hardware
11. A mathematical method or business method or algorithms
12. A literary, dramatic, musical or artistic work or any other aesthetic creation whatsoever including cinematographic works and television productions.
13. A mere scheme or rule or method of performing mental act or method of playing game.
14. A presentation of information
15. Topography of integrated circuits
16. An invention which, in effect is traditional knowledge or which is an aggregation or duplication of known properties of traditionally known component or components.
17. Invention relating to atomic energy.

What is patent specification?

A patent specification discloses the details of the invention for which the patent protection is sought. The legal rights in a patent are based on the disclosures made in the specification. Specifications are of two kinds

1. Provisional: A provisional specification discloses incomplete invention or inventions requiring time to develop further. The provisional specification is filed to claim the priority date of an invention.
2. Complete: The document, containing the detailed description of invention along with the drawings and claims is called as the complete specification. Also the description regarding prior art is included in the complete specification.

What does a patent application contain?

A patent application has the following information:

1. Bibliographic: It is in structure format. It contains the title of the invention, date of filing, country of filing, inventor's name etc.

2. Background of the invention or State of the art: In this the inventor lists the state of the art available on the date of filing his invention. Here the inventor lists the shortcomings/drawbacks found in the state of the art and define his problem.

3. Description of the invention: In this the inventor describes his invention duly supported by a series of workable examples along with diagrams/charts, if needed. The invention has to be described in complete details, so that any person, who is skilled in the art, can work out the invention.

4. Claims: In the last, the inventor has to bring out a series of claims establishing his rights over the state of the art. It is this portion, upon which the protection is granted and not on the description of the invention. This has to be carefully drafted.

What is the date of priority?

The date of priority is the date on which the patent application either with provisional specification or with complete specification is filed at the patent office.

What happens to the application after filing?

Initially, a patent examiner examines the patent applications and then communicates the objections, if any, to the applicant via first examination report. The applicant has to meet up with the compliance of the patent office within specific time frame, if the applicant fails in doing so the application shall be abandoned. Otherwise the application is published in the patent gazettes issued by the patent office. The said published application is open public perusal and opposition. If there is no opposition the patent shall be granted.

How does a patent get expire?

A patent can expire in the following ways:

1. The patent has lived its full term i.e. the term specified by the patent act of the country. Generally it is 20 years from the date of filing.
2. The patentee has failed to pay the renewal fee. A patent once granted by the Government has to be maintained by paying annual renewal fee.
3. The validity of the patent has been successfully challenged by an opponent by filing an opposition either with the patent office or with the courts.

What is traditional knowledge (TK)?

The knowledge continually developed, acquired, used, practiced, transmitted and sustained by the communities/individuals through generations is called Traditional Knowledge.

In India traditional knowledge including the existing oral knowledge cannot be protected under the provisions of the existing IPR laws/acts, as mentioned herein above. However, if there is a substantial improvement in the existing traditional knowledge and if it can fulfill the requirements of the definition of the invention, then the patent application can be filed.

What is prior inform consent (PIC)?

Prior informed consent is a consent sought from the innovator and/or inventor and/or knowledge holder to develop, protect, explore, commercialized ones innovation. PIC document may be of different types each defining the scope of rights imparted to exploit the innovation.

What is a PCT?

PCT abbreviated, from the Patent Cooperation Treaty. PCT is an International treaty, which provides facility to the applicant to file a single patent application and designate the countries in which he/she wants to protect his IP rights. Thus a single patent application is filed for the purpose of an international search report and to claim the priority date in all the designated countries. After receiving the international examination report, the applicant has to file a request in each designated country to take on record his/her application and this is called national phase of a patent application. A PCT application also provides an international filing date through a single patent application. India is a member country to PCT.

UNIT IV - POSSIBLE QUESTIONS

PART B

1. Write about patents, copyrights, trade mark and trade secret.
2. What are various types of IPR?
3. What are the governing laws in India for IPR?
4. What are the patent systems in India?
5. How will you apply for a patent?
6. Describe in detail about IPR protection in India.
7. What is not patentable invention?
8. What does a patent application contain?
9. How does a patent get expire?
10. Write about chemical patenting

PART C

1. Give a brief note on patenting multicellular organisms.
2. Detail account on ethical issues of gene cloning.
3. Detail account on IPR and Present criteria for filing in National and International level

KARPAGAM ACADEMY OF EI
DEPARTMENT OF MICRO
MICROBIAL TECHNOLOGY AND INTELI
17MBP203

UNIT IV

Scienticfic theories, mathematical methods and therapeutic treatments are	Easily patentable
The legal characterization and treatment of trade related biotechnological process and products popularly described as ----------	FDA
Development of crop varities are protected through ----------	Plant Breeder's Right
According to USA ----------means grant of right to exclude others from making using or selling an invention for a 17 years period	IPR
The Indian patent Act include ----------but not ----------	Product and invention
The duration of indian patent is ----------	5 years
Before the release of genetically engineered microbial pesticide it should be approved by ----------	FDA
Grant specification and clims are the parts of	Copy right
Which is the famous convention of London	European
Which are patentable	Inventions
The first patent for living organism was awared in	1978
The Indian patent Act was formed in	1976
EMR stands for	Exclusive Market Rights
A patent should contain	Name of the inventor
Legal documents are	Pantents
Pseudomonas was patented by	TJ.C.Bose
Requirements of patents are	A New useful invention
When the Paris convention for protection of industrial property signed	1884
Paris convention is for the protection of	industrial property
How many years USPTO can issue a patent	4
IPR clasified in to	4 catagories
How many types of patents avilable	2
Duration of patent	5years

Which company has the patent for tissue plasmogen activator	Sigma
PCT came effective in India from	1999
To patent a product or process it must satisfy	3 Fundamental requirements
How many claims present in the original patent application	10
In which country Genetech applied to get patent	france
The colonical theory was proposed by	John
The colonical theory was proposed in the year	1874
The patenting of multicellular organisms constitute to raise	Ethical and social concern
Indian patent Act allow to patent	products
In USA the maximum limit of monoply is for	10years
The word Patent derived from	Latin word patere
PCT is	Patent Cooperation Treaty
PCT is an agreement for cooperation on patenting	National
What is the mode of revocation of patent	State government
Who got the patent for Psuedomonas	Robert Koch
The process of collecting biological samples for medical and scientific research is	Bioprospecting
refers to the illegal commercial development of naturally occuring biological material	Bio patenting
is the form of protection of plant related inventions	Plant Variety Protection
investigation about cell bared therapies to treat disease	Plant cell research
is done to produce pure antibodies by fusing cells	Monoclonal antibody technique
Plant patents have been granted by	American Patent Office
The patentable product involving R-DNA technology	Genes
is the patentable process involving R-DNA technology	Modifying genomic sequences
The product is kept as a safety guarded and termed as	Tread mark
protection is only form of expression of ideas	Trade mark
In which year copyright was amended	1944
In which year copyright was brought enforced	1940
.Indian copy right Act was published in	1967
In which year Copy right was amended	1994
Which symbol was used to distiguish one treade to another?	Copy right
In which year industrial design bill was passed	1940
In which year Treade mark bill was passed in India	1948

DUCATION
BIOLOGY
LECTUAL PROPERTY RIGHTS

Non patentable	Un copyrightable	long time processing
IPR	EPO	WISO
Plant biotech rights	Plant biotech regulations	Prevent breeders right
PBR	Patents	FDA
process patents and product patents	inventions and discoveries	FDA but not IPR
6 months	1 years	10 years
TRIP	GATT	EPA
IPR	Patent	intelectual right
paris	london	Budapest treaty
Discoveries	Scienticfic throries	Treatments
1988	1999	1965
1878	1960	1970
Exclusive Medical Rights	Exclusive Market Rate	External Market Rights
Name of the Patntence	Description of patent	All the three in needed
Cliams	Description	Inventions
Khorana	Dubey	Anand chakraboty
Invention	A useful product	Previously known product
1890	1883	1863
personal property	institutes property	eqiupments
2	1	3
5 catagories	2 catagories	3 catagories
3	4	5
10years	20years	1years

Non patentable
IPR
Plant Breeder's Right
Patent
process patents and product patents
5 years
EPA
Patent
paris
Inventions
1988
1970
Exclusive Market Rights
All the three in needed
Cliams
Anand chakraboty
A New useful invention
1883
industrial property
3
2 catagories
3
20years

biocon	Genetech	Genetech
1998	1997	1995
2 Fundamental requirements	5 Fundamental requirements	6Fundamental requirements
20	30	5
Japan	USA	UK
Haeckel	J.C.Bose	Anand chakraboty
1875	1876	1884
Ethical concern	social concern	trede concern
process	preparation	
5 years	17 years	2 years
paten	pantor	patentor
Patent Control Term	Public Cooperation Team	Private Cooperation Team
Local	International	State
Central government	Union territories	UN
Louis Pastuer	Dr. Chakrabarty	Edward Jenner
Bioprojects	Biogenesis	Bioprocessing
Bio piracy	Bio projects	Piracy
Plant Protection Act	Plant Patent Act	Plant Utility Act
Stem cell research	Animal tissue research	Plant enzymes
Immunoglobulins	Antigens	Antigen antibody complex
England Patent Office	Japan Patent Office	European Patent Office
Vaccines	Transposons	Base pairs
Enzyme sequences	Gene sequences	Plant genes
Trade secrete	patent	IPR
Patend	Copyright	Trade secrete
1994	1942	1960
1990	1999	2002
1957	1937	1977
1944	1984	1997
Patend	Treade mark	Treade secrete
1980	1999	1950
1978	1990	1958

Genetech
1998
3 Fundamental requirements
20
UK
Haeckel
1874
Ethical and social concern
process
17 years
Latin word patere
Patent Cooperation Treaty
International
Central government
Dr. Chakrabarty
Bioprospecting
Bio piracy
Plant Patent Act
Stem cell research
Monoclonal antibody technique
European Patent Office
Vaccines
Modifying genomic sequences
Trade secrete
Copyright
1994
1999
1957
1994
Treade mark
1999
1958

KARPAGAM ACADEMY OF HIGHER EDUCATION
DEPARTMENT OF MICROBIOLOGY
MICROBIAL TECHNOLOGY AND INTELLECTUAL PROPERTY RIGHTS
COURSE CODE: **17MBP203** BATCH: **2017-2019**
UNIT – V

Background to biotechnology and intellectual property

Since the discovery of recombinant DNA technology in the early 1970's, Biotechnology has become an important tool for many researchers and industries. Biotechnology and the inventions arising out of it have spurred the creative geniuses of many inventors and have played an important role in improving the nation's health, food supply and environment. Turning Biotechnology inventions into protected intellectual property, with concomitant Intellectual Property Rights have been taking place for over a century. For example, Louis Pasteur was granted a French patent in 1865 for yeast clones that he isolated from mixtures of yeast species. The application of technology to agriculture has helped contribute enormous increases in yield and quality. The isolation of adrenalin over a century ago from the human suprarenal gland was an important advance in the field of medicine.

Legal protection given to biotechnology

Patents are viewed as vital to protecting the commercial interests and intellectual property rights in biotechnology. Patents are limited rights based on a claim that a new technological invention has been created and fully communicated to the public. Patents can cover new products, processes that creates these new products, new processes for producing existing products and new processes generally. While patenting of a biotechnological invention it is important that it meets the 3 criteria's laid down by the TRIPs to meet patentability, namely which are new or novel, involves an inventive step or not obvious and capable of industrial application. The TRIPs gives the option of excluding certain forms of subject matter from patentability

Diagnostic, therapeutic and surgical methods for the treatment of humans or animals. Plants and animals other than microorganisms, and biological processes for the production of plants or animals other than non-biological and micro-biological processes.

However the patenting of new life forms raises arguments in favor of and against the issuance of such patents. Most recently, public debate has centered on the patenting of animals. Discussions regarding the patenting of a genetically engineered organism can involve questions relating to the environmental application of the organism, scientific questions, ethical issues and economic considerations.

The other forms of IP Protection that can be offered to biotechnology involve plant breeder's rights, trademarks, trade secrets and geographical indications.

Extent of legal protection offered by patents

The protection conferred by a patent on a biological material extends to any biological material derived from that biotechnologically invented material through propagation or multiplication and possessing the same characteristics. The protection conferred by a patent on a product containing the genetic information extends to all material in which the product is incorporated. However the protection does not extend to plant-propagating material or breeding stock

sold to a farmer by the holder of the patent or with his consent, provided that the farmer uses the biological material or livestock for his own agricultural purposes.

Where a breeder cannot acquire or exploit a plant variety right without infringing a prior patent, he can apply for a compulsory license for non-exclusive use of the invention protected by this patent, subject to payment of royalty.

Patenting of microorganisms and cells

The first successful directed insertion of recombinant DNA into a host micro-organism took place in 1973, and since then scientists realized the huge potential involved in directing cellular machinery to develop new and improved products and processes. Many of these products were micro-organisms or cells. Hence with the development of the recombinant DNA technology, the potential of patenting the living organism resulting from the technology arose.

In 1980, in the Diamond V. Chakrabarty ruling, the Supreme Court of US ruled that a living micro organism could be patented. Chakrabarty had developed a genetically modified bacterium capable of breaking down the multiple components of crude oil. Since this property was not possessed by any naturally occurring bacteria, the invention was thought to have significant value. It was held that a non-naturally occurring manufacture was a product of human ingenuity. DNA compounds having naturally occurring sequences are eligible for patenting when isolated from their natural state and when it meets the statutory criteria for patentability. Hence by a 5-4 ruling it was held that a live, human made micro-organism is a patentable subject matter under section 101 as a “manufacture” or “composition”. The fact that biotechnology was not predicted as a branch of science when the congress enacted section 101 does not arrive at the conclusion that micro-organisms are not a patentable subject matter until the congress expressly authorizes such protection.

Patenting of transgenic animals

The first animal patent was issued in April, 1988 to Harvard University for a particular type of mammal, namely the Harvard oncomouse, genetically engineered to obtain a cancer- causing gene. The oncomouse has been genetically engineered to carry a particular type of gene called as the oncogene which makes it susceptible to cancer and hence makes it ideal for cancer research. Subsequently the USPTO announced that it would consider non-naturally occurring non-human, multi-cellular organisms, including animals to be patentable subject matter under its laws.

Most of the animal patents have been granted to transgenic animals produced by recombinant DNA or genetic engineering. Transgenic animals have DNA which has been modified by adding DNA from another source other than the parental germplasm, usually from different animals or humans.

Negative impacts of patenting of transgenic animals

The major concern that arises out of patenting of transgenic animals are that transferring genes from one species to another transgresses the natural barriers between them and affects the integrity of species. Species belonging to the same group, though they may slightly vary from one region to the other based on the environmental conditions, they primarily have the same gene pool. By allowing patenting of transgenic animals, the fundamental genetic architecture is being tampered with.

Ethical issues

A number of ethical issues stem from the patenting of animals. Most of them deal with the consequences that could arise subsequent to patenting of animals while the other arguments focus on the religious, philosophical and spiritual grounds. The arguments which go against the patenting of animals are difficult to prove as many of them are factual assertions which are still to occur or to be proven. The DNA is considered to be intimately related to the species

identity and hence no part of it should be controlled for commercial interest. In case of human beings, human DNA is unique and hence possesses intrinsic value of a sacred kind. It can also be put as 'Human DNA bears the image of God' and to tamper with them and own them for commercial and economic interests would hurt the sentiments of the many. The view that plants, animals and microorganisms comprising life on earth are part of the natural world into which we are born and hence the conversion of these species, their molecules, or parts into corporate property through patent monopolies is counter to the interest of the people of the country and world, has been taken by many.

However most of the religious and ethical issues arise out of product patents which have been given to organs, cells, genes and proteins. Hence one possibility that could be accepted by such religious leaders could be the issuance of 'process patents', whereby only the process involved in the manipulation of particular genes are patented rather than the genes in itself.

Patenting of genes

Our Genes define us, as a species as well as individuals, and hence for human genes there are strong oppositions both on the religious and secular front. Patents are being granted to genes despite there being many arguments for keeping the genes in the public domain. A patent cannot be granted on a gene as it naturally occurs. Isolation of the gene is required for it to be patentable. The patent offices have treated genes as a new chemical compound and have granted "composition of matter" patents. Thus a patent granted on an isolated and purified DNA composition confers the right to exclude others from any method of using that DNA composition for upto 20 years from the date of filing. However Human Beings are not patentable as human multicellular living organisms are not a patentable subject matter under section 101.

Why are genes being patented?

Genes have been used for gene therapy though it is still in the early developmental stages. The technology used in each gene therapy will have huge commercial value in the coming future making patenting crucial. However gene therapy is not a patentable subject matter in India.

Some of the genes encode proteins that can act as therapeutic agents. (e.g.; the human growth hormone). Hence by offering patent protection to such genes, the interests of the pharmaceutical industries will be maintained. However, the pharmaceutical industries should be granted access to the genes and not the ownership. Monopolies on genes are not in the public interest.

For Biotechnological companies, gene patents are considered as value generators and enhance the value of the company in the eyes of the investors. Most of the Biotechnological companies and research institutes have created Technology Transfer Offices (TTO) so that the patents generated by their research will generate huge financial rewards.

Sequences of genes and genomics provide data for further research. The patenting of genes forces the disclosure of information instead of it being kept as a trade secret. Full and free access to genomics data is essential for academic research and owning gene patents are the best way to ensure hindrance free access to such data.

Morality and patents- is there a connection?

Law and morality are inter-connected and some areas of law require the legal adjudicators to draw on morality in considering the decision making process. The patent law especially concerning the patenting of biotechnological inventions does just this. The best example of this would be Article 53 (a) of the European Patent Convention 1973, which does not allow the grant of patents "for inventions the publication or exploitation of which would be considered

to be contrary to 'ordre public or morality". Most of the national patent laws of various countries embrace the moral standards within its ambit. When the legislatures enact patent laws, the moral standards of the community to which they belong to are one of the factors which affect their content. Moreover, the patenting system cannot be considered to be an ethically neutral concept. A system can be considered to be ethically neutral when it does not affect A's interests vis-à-vis B's interest. The whole crux of patents is to exclude others from access to information contained in the claims and hence it cannot be considered to be morally or ethically neutral. This act of excluding others to protect your interest will inevitably affect someone else's rights in some way or the other.

Patenting within the biotechnological sphere and the subsequent opposition proceedings which have been undertaken by NGO's such as the Greenpeace, which have become worried by prospects such as the patenting of life, have led courts to highlight the relationship between patents and morality. However it has to be kept in mind that the concept of morality is relative to the values prevailing in society. The decisions based on morality should not be based on what some members of the public find objectionable, but should include a detailed analysis of the effect on human health, economic impact, environmental issues and opinion of the population as a whole. The main point, however is that non-patentability would only mean that the invention is not the subject of any property rights, it can still be used and worked even if it is contrary to public interest and morality as it still lies in the public domain.

Why say no to life patenting?

The WTO has forced countries to introduce laws that allow the patenting of life forms and living organisms. In India, this was done through the Patents Act of 1970. Due to the introduction of such a monopolistic set up where biotechnological inventions can be patented, it has led to an epidemic of biopiracy and the patenting of traditional knowledge. The following are the other reasons which have been laid down which object to life form patenting.

Farmers would be obliged to pay royalties on every generation of plants and livestock they buy and reproduce for production purposes.

Breeders will no longer have free access to germplasm for developing new varieties of plants and animals. Consumers will end up paying high prices for food, medicine and other biotechnological products.

In the end, public research which is paid for by all will be privatized by a few. Food supply will be threatened by monopolistic control over genetic resources. The concept of Human rights will be threatened as human beings, and parts of their bodies will become the exclusive property of the patent holders.

Animal welfare will become a thing of the past as the patent system stimulates genetic engineering of animals for production of food and medicine no matter how they suffer. But the main objection to the patenting of life forms are that DNA is a product of nature and not a product of human ingenuity.

International implementation of farmers' rights

The Governing Body of the International Treaty must now take up the task of establishing clear guidelines for defining and implementing Farmers' Rights. An international movement for Farmers' Rights would have to tread carefully to respect the sovereignty of nations while promoting global cooperation. However, Farmers' Rights must be promoted at the international level and cannot be left only to national governments to design. If each country, under Farmers' Rights, sets up barriers to access of genetic resources, limits exchange of resources and competes to stake claims over innovations, the implications would be severe for farmers. Lack of coordination between countries and regions in framing Farmers' Rights could lead to an anticommons tragedy on a global scale. India and other developing countries are faced with a dilemma in defining and implementing Farmers' Rights. On the one hand, they are interested in acquiring IPRs and asserting ownership rights over genetic resources and farmer's innovations. On the

other hand, they must ensure access to resources for farmers and public sector institutions. An international forum to promote cooperation and useful sharing of resources is required to enable developing countries to emerge from this dilemma. The Multilateral System established under the International Treaty provides a useful starting point. The strategy of denoting a specified list of crops, or the list approach, could be effectively promoted and expanded. Not only the list of crops included but also the ways in which such resources could be opened up to the public domain should be explored. Political pressures and opposition would certainly exist, and some level of awareness building to convince parties that it is in their interest to ensure access to resources would be required. It would also not be easy to operationalize, but there could be many options to the list approach. The system should also not be reduced to a mechanism to provide free access to resources for the private sector without an onus share, as feared by some NGOs (GRAIN, 2005). The International Treaty could serve as a foundation for moving towards a development oriented definition of Farmers' Rights.

The Farmers' Rights movement has witnessed a long and chequered history. That it has taken years to establish a binding agreement may be disheartening, but the fact the momentum was not lost and the Treaty finally emerged, should be a source of inspiration. India's ability to be one of the first countries in the world to forge a national legislation on Farmers' Rights is a significant landmark. The Indian case provides important lessons for other countries in establishing Farmers' Rights, and demonstrates the complex and contentious issues that must be tackled to implement Farmers' Rights. The fact that agreement on defining and implementing Farmers' Rights has not emerged in India, even after establishing a law on Farmers' Rights, should serve as a signal internationally that establishing legislations is insufficient. A global mechanism is urgently required to promote some level of consensus on defining and implementing Farmers' Rights. Attention must now turn to the brass tacks of how to achieve Farmers' Rights. The political and strategic gains of defining Farmers' Rights as IPR type rights must be accompanied by measures to ensure economic benefits by focusing on Farmers' Rights as development rights. Farmers' Rights must also incorporate mechanisms to promote access and sharing of resources rather than only ownership rights. Farmers themselves must be seen as important stakeholders in policy making. If the global community does not face up to the challenge of unambiguously articulating Farmers' Rights, what has been achieved so far in the battle to establish Farmers' Rights may be lost. The nuts and bolts on the machinery for driving Farmers' Rights forward must now be fitted. Without proper direction, the Farmers' Rights movement itself may come to a grinding halt.

Plant breeders' and farmers' right

Plant varieties are generally protected in several countries (not in India) through plant breeders' rights (PBR) or plant variety rights. Plant Variety Production (PVP) laws granting Plant breeders' right patents of a lesser degree to whosoever claims to have discovered or developed a "new plant variety gives exclusive monopoly control over that variety".

Under the existing convention due to 'International Union for the Protection of New Varieties' (UPOV), the breeders' rights prohibit the farmer from reuse (plant back) of farm-saved seeds of a variety from his own harvest for planting another crop. Furthermore, the protected plant variety may be freely used as a plant genetic resource for the purpose of breeding other varieties.

When patent or plant breeders' rights are not available for true breeding crop varieties, plant breeders, particularly private plant breeders of countries like Germany, may feel tempted to focus their efforts on developing hybrid varieties, because hybrids do not breed true and give higher yields, no one would raise a crop from harvested seeds that will give reduced yield. Thus hybrid varieties may help in protecting intellectual property. A protected variety should be -

- New (previously not exploited commercially).
- Distinct (clearly distinguishable from all other varieties)
- Uniform (all plants of the variety should be uniform.)
- Stable (variety can be reproduced and multiplied without losing its characteristics and uniformity).

In India, new crop varieties are bred at State Agriculture Universities and at State Departments of Agriculture. Seeds of new crop varieties flew freely to farmers and to private companies and no royalty was payable. This really encouraged farmers, in the past, to grow new varieties leading to green revolution. Imposition of PBR in India will lead to following problems

1. The cost of seeds will increase.
2. There will be delay in the spread of new varieties to the farmers.
3. The benefit of new varieties will be restricted to a small segment of farmers.

Farmers' rights

It is a concept developed and adapted in FAO (Food and Agriculture Organization) as a resolution and endorsed by all member countries.

It recognizes the fact that farmers and rural communities have greatly contributed to the creation , conservation, exchange of knowledge for the utilization of genetic diversity. Therefore, it is the obligation of world community to help these farmers to carry out this task and help them in utilizing the genetic diversity available with them.

India's PVP legislation

The protection of plant variety and Farmers' Rights Act, 2001- even though it supposedly attempts to balance breeders' rights and farmers interests, essentially establishes IPR on plant varieties. Another domestic legislation regulates people's interaction with plant genetic resources in the biological diversity act of 2002. It was essentially passed with the objective of conservation of biological diversity and the equitable sharing of benefits from the use of biological resources.

World-class talent

There is no provision for patenting of plants in Indian patents act of 1970, but other countries like U.S. do have provision. Ironically, India holds patents on plants in foreign patent offices. Through the CSIR (Council of Scientific and Industrial Research) the Indian government has a plant patent (PP12426) on a novel mint plant 'Kosi' characterized by its high menthol content. CSIR also holds another U.S. patent for inventing a “novel damask rose progeny” (PP13203), ama 1, plant gene patent held by D.B.T.

International developments in plant protection

Intentionally, there is no one forum to effectively deal with the grant of patents on unlawfully acquired biological resources or traditional knowledge. Farmer groups, indigenous communities and community-based organizations have not only to lobby their government to reserve the IPR trend but also campaign against patents at the international level.

The only way to protect biological resources and their traditional knowledge (TK) is to create IPRs on them. At the world intellectual property organization (WIPO), within the intergovernmental committee on genetic resources, discussions are ongoing on traditional knowledge and folklore on designing a suitable IPR system to protect TK (traditional knowledge).

Biodiversity related issues

Biodiversity convention was held in May 1992 at Nairobi to formulate a treaty that was designed to be signed at U.N. Conference on Environment and Development (UNCED) later held in Brazil in June 1992. In this treaty, an agreement was sought by the developed countries to allow, as a matter of right, access of every country on the germplasm or biodiversity available anywhere in the world. Since tropical developing countries are far richer than temperate developed countries, such a treaty would have benefited only the developed countries. In view of this, the developing countries particularly India had rejected such a treaty because it meant to globalize the natural resources and not the benefits derived from biotechnological inventions. The developed countries wanted to privatize biotechnology through patent and other IPR. The developing countries wanted their share in biotechnology. They are gene rich and are willing to share their rich biodiversity, but they want technology transfer to be cheaper. At earth summit, Johannesburg (2002) there was agreement to share the benefits of using biodiversity with tribal people who had traditional wisdom and knowledge.

Biopiracy

Simply means smuggling of diverse forms of flora and fauna. Worldwide opposition to biological piracy is rapidly building up as more and more groups and people are becoming aware that big corporations are reaping massive profits from using the knowledge and biological resources of third world countries. Farmers and indigenous people are outraged that plants they have developed are being "hijacked" by multinational companies by having their patents by doing slight modifications in genetic resources of developing countries.

By providing documented evidence from ancient Indian texts that medicinal use of turmeric was well known in India for centuries, turmeric patent was stopped from going in the hands of multinational private companies. India has also won its battle against grant of Neem patent for its pesticide use from W. R. Grace Company after a long battle.

Can life forms be patented?

The US court in 1980 allowed to patent a life form of a bacterium *Pseudomonas* developed by an Indian scientist Dr. Chakraborty. The modified life form contained at least two stable energy generating plasmids, each of the said plasmid providing a separate hydrogen degradation pathway." The subject of the above claim was an organism, made more effective in treating oil spills by manipulating a natural *Pseudomonas*.

Oncomouse- Genetically engineered mouse, carrier of human cancer gene was protected by U.S. patent in 1988. Microorganisms such as *E .coli* in which human genes have been incorporated for production of human insulin, human growth hormone, human tissue plasminogen activator (t-PA), etc. have been recognized for patents in U.S.A. Microbial cells, engineered to produce antigens and antibodies also qualified for patents. Transgenic plants like herbicide resistant cotton, insect resistant tobacco, virus resistant potato and boll worm resistant cotton have also been patented.

Government initiatives

The government has taken steps after signing TRIPs agreement on IPR related issues in general and product patent in particular. According to reports, Rs 120 crore modernization plans of the patent offices across the country are nearing completion. The patent offices are in Delhi, Kolkata, Chennai, and Mumbai. The patent information service, Nagpur has been developed as an intellectual property training institute (IPTI).

UNIT V - POSSIBLE QUESTIONS

PART B

1. What are the legal protections given to biotechnology?
2. How will you patenting the microorganisms and cells?
3. What are the ethical issues related to genetic engineering?
4. What is the plant breeders' and farmers' right in India?
5. Write about the Conditions for patenting
6. Add the details to go for a Product patenting
7. Briefly add note on Patenting of liveforms
8. Eloberate about Trade secret in industry.
9. Write the advantages and disadvange of patenting.
10. Patenting of fundamental research.

PART C

1. Explain the regulating the use of biotechnology inventions
2. Explain the patenting of biological material
3. Describe the writing pattern of patents

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UNIT IV

---------- include the rights arising from conserving, improving and making	Agricultural act
IPR are usually limited to _____	Non-rival goods
Modern usage of the term IPR began in _____	1987
In 1994 which act was amended _____	Tread mark
In 1957 which agreement was approved for copy right act	TRIPS
The maximum limit of 17 years for monoply was given by	Paris
The meaning for patere	property
Haeckel proposed the theory of _____	Colonical
There is a 20 claims present in _____	original patent application
In 1988 the first patent was given to	Living organism
In 1883 which convention for production of industrial property was signed	Paris
Three years of patent was issued by	TRIPS
According to PPVFR Act How many rights have been given to farmers	6
_____is classified into two categories	PBR
Trademark uses symbols in _____	Intellectual committee
Geographical indications protects the quality,reputation of products originated from	Histarical area
Tade secrets protects _____ of industries	Trade information
Copyrights prevents copying and _____	Reproduction
IPR develops and protects ____resources.	Physical
For breeder's right, _____ Act is in practice.	PPR
In which year patent Act was published in India	1945
In which year patent bill was introduced to upper part of india parliament	1970
When copyright Act got TRIPS agreement	1950
When did geographical indication good bill got published	1920
Which year Act was replaced in 1999	1920
Stem cell research investigation about--- therapies to treat disease	cell bared therapies
Trade secrecy applicable rather than patents in ----------	Fermentation

Which can be protected using patents	micropropagation
Not possible to get patents for ----------	Plastic surgery
The existing patent law does not allow ----------	Process patent
In India the patent law is based on ----------	Indian Patent Act of 1970
.The main instrument of international collaboration for intellectual pro	GATT
Patents are granted by ----------	GATT
The provision of _____ enables the developing countries to paten GM crops.	TRIPS-GATT
. The TRIPS final negotiation schedule was proposed in	Jan-00
Monopoly rights were granted in -------	.USA
In USA period of patents ---------	25 years
In TRIPS, appellations of origin are covered in	Patents
. Trade Related Aspects of Intellectual Property Rights (TRIPS) is administered by	WHO
_____are the rights given to people over the creations of their minds.	Intellectual property rights
International patent protection for Biotechnology was published by	WIPO
TRIP Strands for ----------	Trade related intellectual property.
WIPO Strands for ----------	Word intellectual property organization.
. _____Comprise of private information about specific technical procedures and formulations.	copy right
. _____ is a milk clotting proteolytic enzyme that hydrolyses the K-casein protein of milk.	chymosin
---------- is the key component of the rennet	Chymosin
Milk clotting activity found in ----------	Chymosin

Mastitis is the ----------	viral infection of milk gland
EBT contaminate the ----------	Methonine
. _____ is an important example for gene piracy.	. GM *Pseudomonas*
In India, _____ is an important example biopiracy	Dalbergia
---------- Serve as a source of transplanted organs for humans.	Transgenic pigs
Trade secrete comprise the information about ----------	Specific technical procedures
Recombinant DNA technology,were known by several phasses such as	playing god
The original guide line providede by NIH was modified in need by……committee	NIH-RAC
3.earlier, most prominently used strain/host organism in RD	*klebsiella.spp*
……is responsible for the regulating the introduction of foods,drugs,pharmaceutical and medical devices into the market place	FDA
…..is an enzyme approved by FDA in making cheese	chymosin
milk clotting activity for cheese making is derived from the fourth stomach of calves and consist of mixture of substances called…..	K-casein
for cheaper industrial supply of chymosin,genes were cloned and the product was harvested from….	*E.coli k-12*
a consistent feature among the occurrence of eosinophilia myalgia syndrome(EMS),due to the consumption of large doses of aminoacid……in food supplement	Tryptophan
recombinant bovine somatotropin (BST) which also known as ……	bovine growth hormone
milk production in dairy cows was increased by……% after the injection of recombinant form of BST	20-25%
A protected hybrid crop varieties should be	old
protects confidential information of the industry with commercial value	Patent rights

PPVFR refers to	Protection of plant varities and Farmers Rights Act

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Plant breeder's act	Farmer's right	Copyright	Farmer's right
Rival goods	Imported goods	Food products	Non-rival goods
1977	1967	1955	1967
trade secrete	patent	copy right	copy right
PPVFR	IPR	PCT	TRIPS
UK	USA	India	USA
To lay open	Protection	To open	To lay open
gem theory	Theory of koch	Theory of Pasteur	Colonical
New developed patent application	patent application	IPR application	original patent application
hybride plant	plant	Fungi	Living organism
US	UK	USSR	Paris
PPVFR	IPR	USPTO	USPTO
7	10	9	9
IPR	FDA	USR	IPR
Intellectual property	Indian committee	Indian property	Intellectual property
Seasonal area	developmental area	Geographical area	Geographical area
confidential information	Machinery information	Tribunal information	confidential information
Construction	Development	Tradition	Reproduction
chemical	biological	academic	biological
PPVFR	PPVR	PPFR	PPVFR
1990	1999	2000	1999
1980	1960	1990	1970
1960	1957	1980	1957
1960	1999	2004	1999
1999	1990	1911	1911
cell therapies	Plant enzymes	Plant cell research	cell bared therapies
drugs	chemicals	invitro fertilization	Fermentation

tissue	organ culture techniques	method for reducing pathogenecity	method for reducing pathogenecity
modified plants	DNA sequences	modified Microorganisms	Plastic surgery
. Product patent	Live forms	Inventions	. Product patent
Indian Patent Act of 1911	. Indian Patent Act of 1991	. Indian Patent Act of 1811	Indian Patent Act of 1970
WIPO	OECD	Court	WIPO
WIPO	OECD	Court.	Court.
WHO	. UN	UNESCO	TRIPS-GATT
Jan-02	Jan-01	Jan-99	Jan-00
Japan	Astralia	Europe.	Europe.
12years	10 years	17 years	17 years
Trade marks	Geographical indications	Copy rights	Geographical indications
WTO	. FAO	UNDP	WTO
Human rights	Right to speech	Right to speech	Intellectual property rights
EC	.OECD	EPC.	OECD
Trademark related intellectual property.	Trend related intellectual property.	Trade related international property.	Trade related intellectual property.
World intellectual property organization	Word intellectual property origin	World intellectual property origin	World intellectual property organization
trade secret	trade mark	patent	trade secret
.renin	both	Casein	chymosin
Lipin	Casein	Rennin	Chymosin
Lipin	Casein	Ptotein	.Chymosin

Parasitic infection of milk gland	Fungal infection of milk gland	Bacterial infection of milk gland	Bacterial infection of milk gland
Lipin	Alanin	Tryptopha n	Tryptophan
Pentacliplandr a brazzeana	*Clostridiu m*	*Bacillus*	*Pentaclipl andra brazzeana*
Neem	Ginger	Onion	Neem
Transgenic rabbits	Transgenic goats	Transgeni c sheep	Transgenic pigs
symbol	particular product	document.	Specific technical procedures
manipulation of life	man made evolution	all the above	all the above
GEO's	FDA	none of the above	NIH-RAC
E.coli k-12	*E.coli*	*Pseudomon as aeruginosa*	*E.coli k-12*
WHO	NIH	none of the above	FDA
tryptophan	streptomyci n	Penicillin	chymosin
rennet	chymosin	proteolytic enzyme	rennet
Pseudomonas aeruginosa	*klebsiella.sp p*	B.thuringie nsis	*E.coli k-12*
leucine	isoleucine	lysine	tryptophan
bovine releasing hormone	bovine serum	none of the above	bovine growth hormone
30-35%	.40-45%	20-45%	20-25%
New	Not uniform	Moderate	New
Patent Control Term	Trade marks	Trade secrets	Trade secrets

Production of plant varities and Farmers Rights Act	Preparation of plant varities and Farmers Rights Act	Proper plant varities and Farmers Rights Act

Protection of plant varities and Farmers Rights Act

CIA I

Reg. No. : ----------

[17MBP203]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act, 1956)

Eachanari post, Coimbatore, Tamil Nadu, India -641 021

M.Sc. DEGREE FIRST INTERNAL EXAMINATION, JANUARUY 2018

SECOND SEMESTER

MICROBIAL TECHNOLOGY AND INTELLECTUAL PROPERTY RIGHTS

Time: 2.00 hours **Maximum: 50marks**

PART A – (20 x 1 = 20 marks)

1. The cloned genes are expressed in ----------
   a. Periplasm b. Recombinant cell
   c. Host cell d. Donor cell
2. ---------- allow the host bacterium to metabolize unusual molecules.
   a. plasmid b. virulence plasmid
   c. Degradative plasmid d. F plasmid
3. _____ enzyme mediates Nick translation
   a. DNA Polymerase I b. DNA Polymerase II
   c. DNA Polymerase III d. RNA Polymerase
4. Enzyme used to cleave the appropriate site of the λ for the insertion of vector is
   a. Endonucleases b. lyases
   c. Ligases d. Translocase
5. System of naming restriction enzymes was proposed by
   a. Smith b. Smith and Nathans
   c. Nathans d. Wilcox
6. Cofactor for type II system ----------
   a.mn 2+ b.ca 2+
   c. mg 2+ d.mg 2+
7. Alkali treatment of DNA fragment results in the ----------
   a. Disruption b. Depurination
   c. Denaturation d. Formation
8. The blotted filter paper is baked at ----------
   a. 90 °C b. 82 °C
   c. 100°C d. 80 °C
9. A lactose analogue which is involved in the screening of B galactosidase
   a. Y-gal b. Replica Plating
   c. Secondary screening d. Auxanography
10. pBR 322 constructed by ----------
    a. Bolivar and Rodriguez b. john collins
    c. Watson and Crick d. Nathan and smith
11. Ti plasmid is a large plasmid greater than ----------
    a. 200kb b. 300kb
    c. 100 kb d. 150 kb
12. ---------- have prokaryotic and eukaryotic origin of replication
    a. Ri plasmid b. Ti plasmid
    c. Col plasmid d. shuttle vector

13. Restriction enzyme cut in the middle of the recognization sequence resulting in -----
    a. blunt end b. sticky end
    c. cohesive end d. cross end
14. Stain used in gel electrophoresis for the detection of DNA
    a. Ethidium bromide b. Crystal violet
    c. Malachite green d. Bromothymol blue
15. Introduce the gene in to *E.coli* by _____
    a. Transduction b. Particle bambrtment
    c. Micro injection d. Transformation
16. Advantage of lamda phage vector
    a. Transformation efficiency b. Easy to grow
    c. low cost d. Self replication
17. Ti plasmid is in …… size.
    a. ~ 200 kb b. 100 kb
    c. 50 kb d. 150 kb
18. The smallest known phage is
    a. Phage M13 b. PUC 118
    c. PUC 119 d. PHC 79
19. Northern blotting is used for _____
    a. Detection of RNA b. Detection of DNA
    c. Detection of protein d. Detection of plasmid
20. The southern blotting technique can be used for the
    a. Separation of DNA b. Screening of recombinants
    c. Denaturation of DNA d. DNA sequencing

PART B – (3 x 2 = 6 marks)

Answer all the questions

21. Write the difference between the northern blotting and western blotting technique
22. Define expression vector and give example.
23. How will you quantify the DNA.

PART C – (3 x 8 = 24 marks)

Answer all questions

24. a. Describe the host controlled restriction phenomena.

    Or

    b. Explain the techniques involved in isolation of plasmid DNA

25. a. Describe briefly about the pBR 322 vector.

    Or

    b. Give a brief note on construction of cDNA.

26. a. Explain the selection and screening method of recombinants

    Or

    b. Give short note on cosmids and its application.