جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

تطبيقات الهندسة الوراثية

المرحلة الرابعة

الفصل الثاني

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2022-2021

Application of genetic engineering

Lec.1

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Bacteriophage

• These are the viruses that specifically infect bacteria and during infection inject the phage DNA into the host cell where it undergoes replication.

• The phages are simple in structure and consist of DNA molecule having several genes for phage replication which is surrounded by a capsid made up of proteins.

Types of phages

• On the basis of structure

1- Head and Tail phages: e.g. λ phage

2- Filamentous phages: e.g. M13

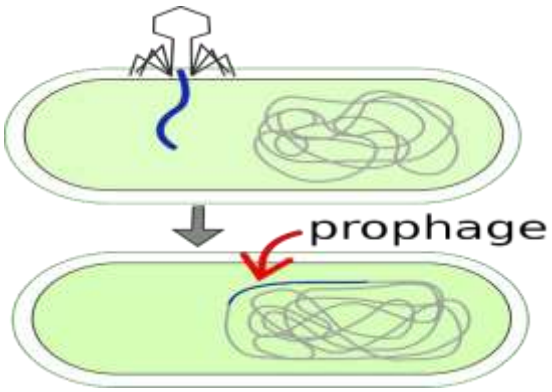
• On the basis of phage infection

cycle 1- Lytic Phage:

- The infection cycle is completed very quickly and the release of new phage particles is associated with the lysis of the host cell

2- Lysogenic phage:

- The phage DNA gets integrated into the bacterial DNA known as ROPHAGE and after many cell divisions released by the lysis of the host cell e.g. λ phage.
- Some phages do not form prophages and the new phage particles are continuously assembled and released from the host cell without the lysis of the host cell e.g. M13 phage.



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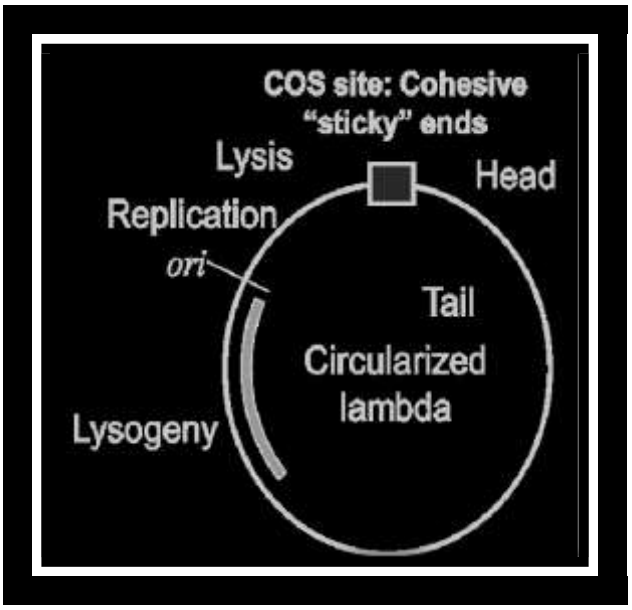
Structure of λ Phage

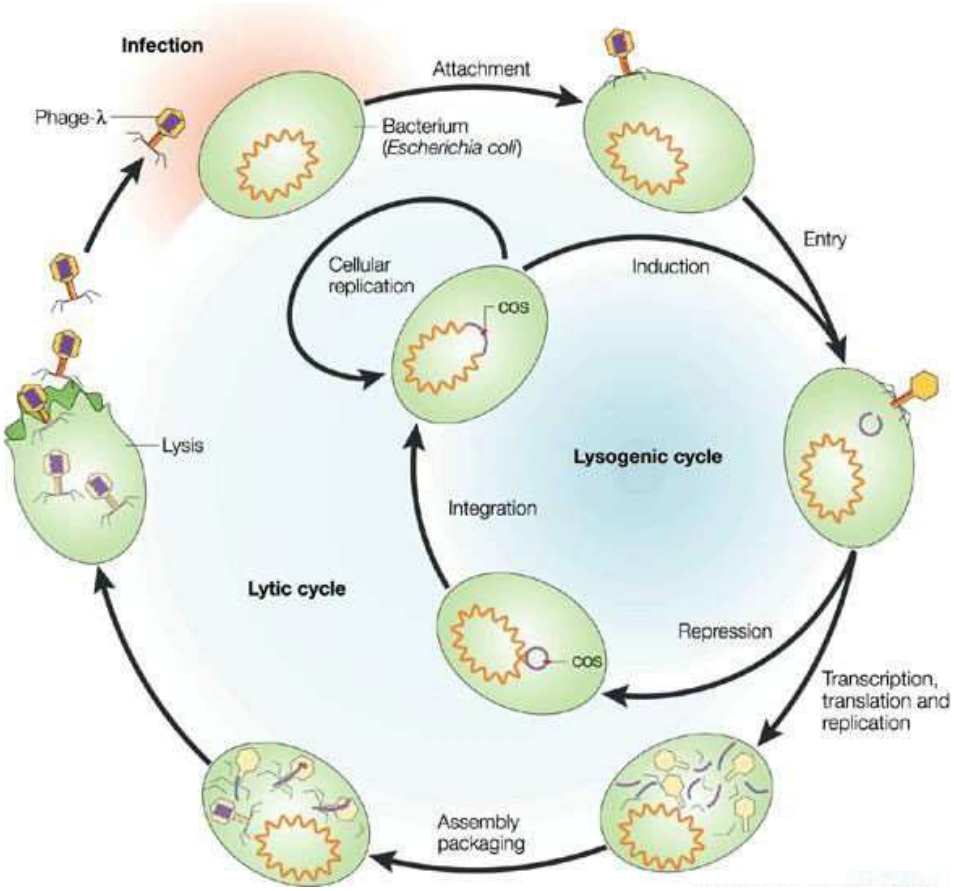
- Cos site: At the ends ss- complementary region "cohesive or sticky" ends--- circulation after infection
- Left Arm: Structural genes for head and tail
- Central Region: genes for lysogenic growth and recombination/insertion of genome into bacterial genome
- Right Arm: genes involved in DNA replication and lytic grow
- Packaging efficiency 78%-105% of the lambda genome.

Left arm (16 kb) **Cohesive end (cos)** **Head and tail genes**	**Non- essential region (15kbp)** **(recombination and lysogeny)**	**right arm (18 kb)** **Cohesive end (cos)** **metabolism genes**

Structure of λ Phage (49kb)



Lytic and lysogenic of life cycle of λ Phage



Why use λ Phage as Cloning Vector?

1- "Head and Tail" phage, very well-studied

2- Large, linear genome of ~49.0 kb

3- Two lifestyle modes

– Lytic: replicative mode

– Lysogenic: latent mode

4- It has a large size genome (49kb) and only 3kb new DNA can be inserted because if the size of the molecule is more than 52kb then it cannot be packaged into the head of the phage.(insertion vector)

5- For the construction of the cloning vector, the non-essential region (between positions 20 and 35) in the genome of the phage was removed

which decreased the size of the molecule by 15kb. Thus, up to 18kb of new DNA can be inserted (replacement vector)

6- The phage has more than one recognition sequence for almost all the restriction endonucleases. So the use of any restriction enzyme will break the phage DNA into number of small fragments.

7- Despite these disadvantages, λ phages are used to clone large DNA molecules.

8- the linear double stranded DNA molecule has a stretch of 12 nucleotides at its either ends which act as sticky ends or cohesive ends (cos sites)

9- Engineered version of bacteriophage λ (infects E. coli).

10- Central region of the λ chromosome (linear) is cut with a restriction enzyme and foreign DNA is inserted.

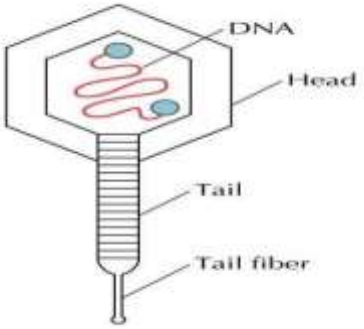
11- DNA is packaged in phage heads to form virus particles.

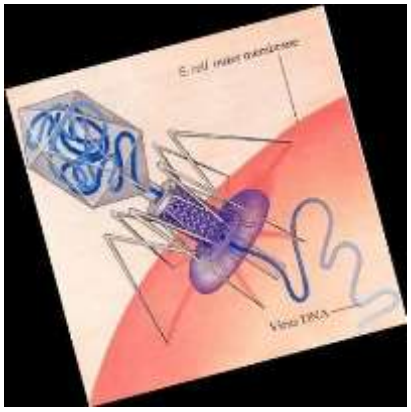
12- Phages with both ends of the λ chromosome and a 37-52 kb insert replicate by infecting E. coli.

13- Phages replicate using E. coli and the lytic cycle.

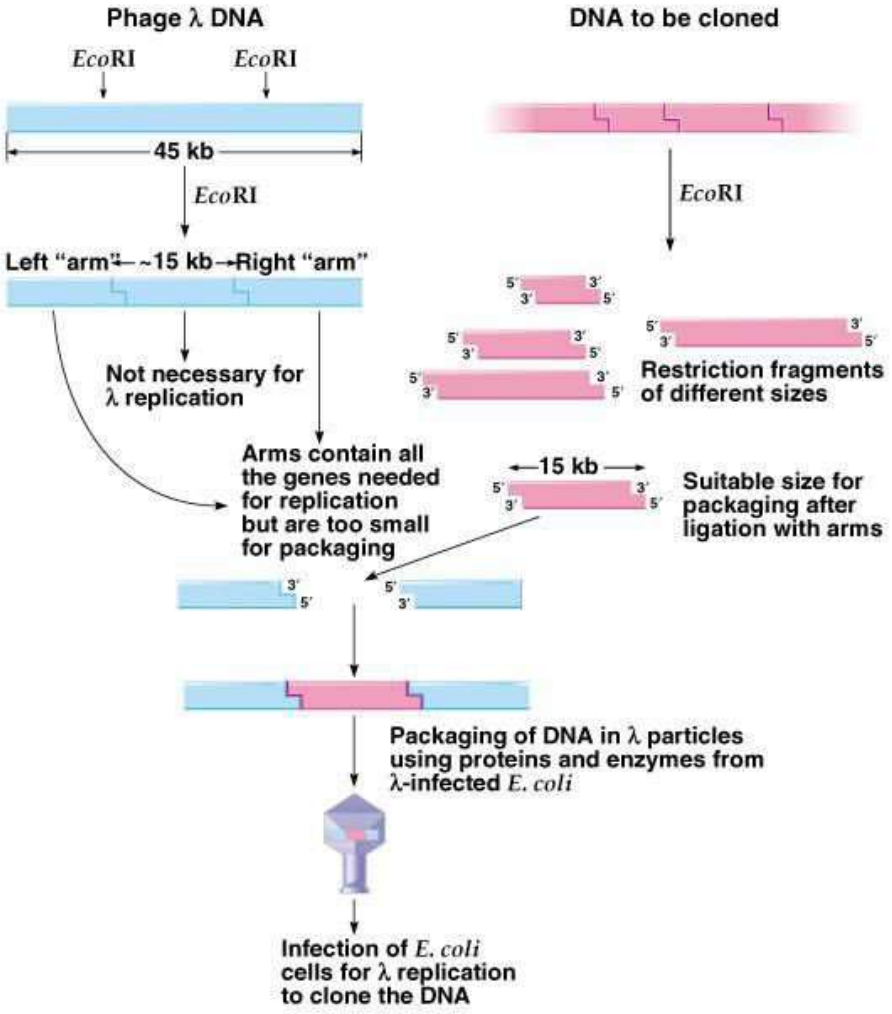
14- Produces large quantities of 37-52 kb cloned DNA.

15- Like plasmid vectors, large number of restriction sites available; phage l cloning vectors are useful for larger DNA fragments than pUC19 plasmid vectors.





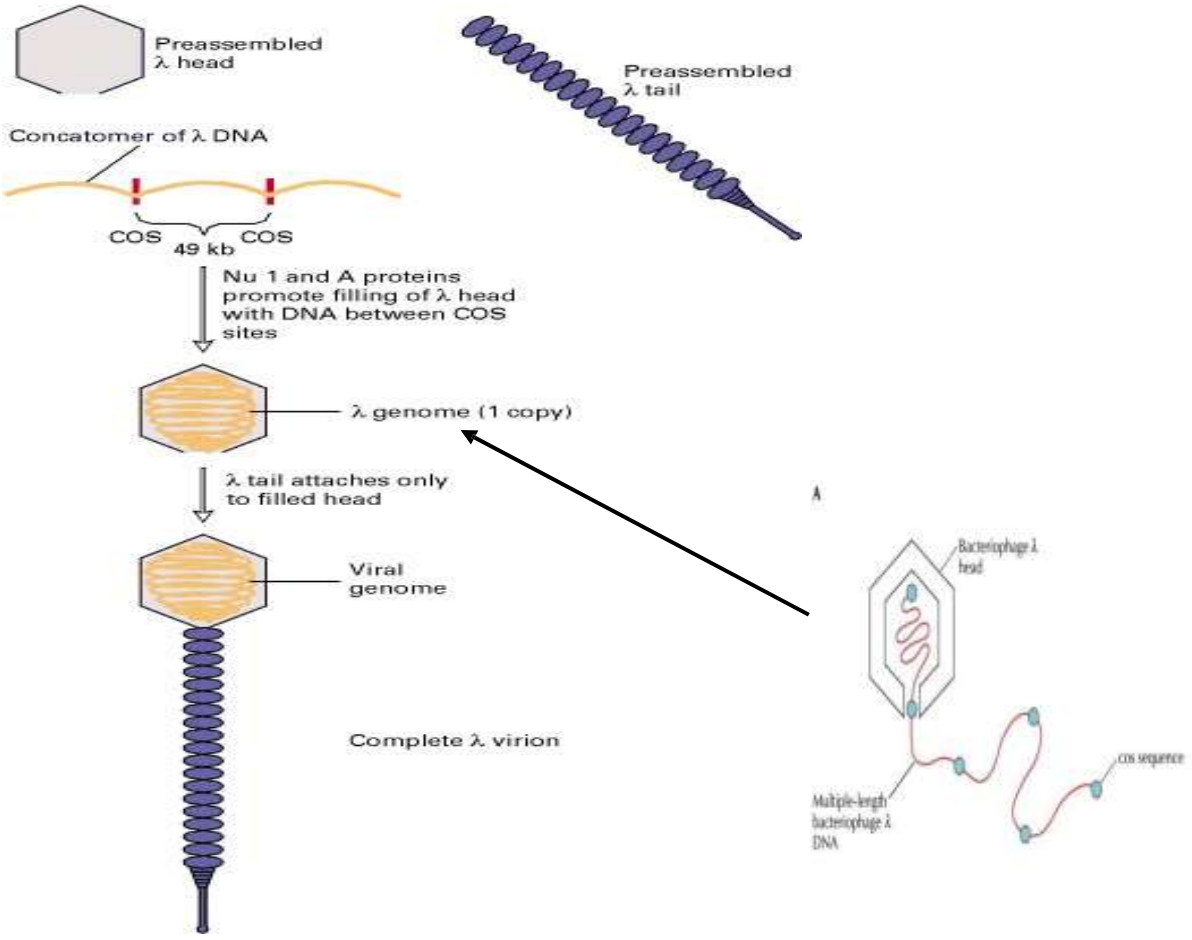
How can clone DNA in λ Phage?



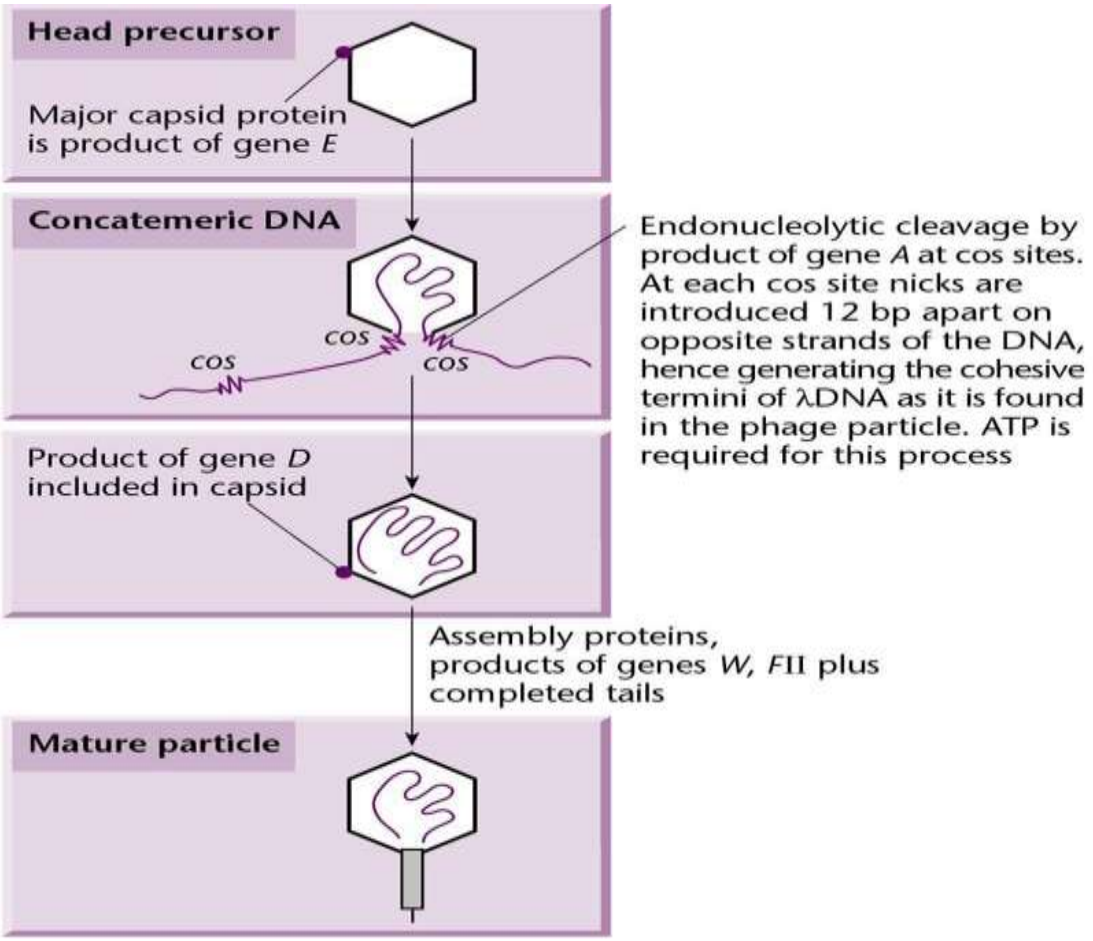
But bacterial transformation with recombinant lambda phages is very ineffective

In Vitro Packaging

DNA can be packaged into phage particle in vitro



What are the main genes of phage for in vitro packaging?



Why prefer Lambda phage as cloning vector instead of plasmid ?

1- The infection process is about thousand times more efficient than transformation with plasmid vectors.

2- 10^6 tansformed colonies per microgram of plasmid vector while 10^9 plaques per microgram of recombinant Lambda vector

Insertion phage vectors

• In these vectors in non-essential region can inset foreign DNA. The vector possesses one unique restriction site and only 3kb new DNA can

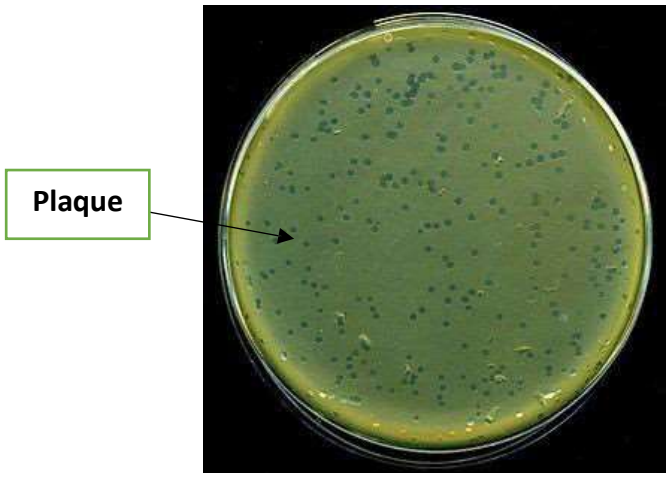
be inserted because if the size of the molecule is more than 52kb then it cannot be packaged into the head of the phage.

Replacement phage vectors

• This vector has two recognition sites for the restriction endonuclease used for cloning.

• These sites replace the segment of DNA (Stuffer fragment) from the vector genome by the DNA to be cloned.

• These vectors can carry large pieces of DNA than insertion vectors from (15 - 18)kb new DNA can be add because if the size of the molecule is more than 52kb then it cannot be packaged into the head of the phage.



Uses of Lambda Bacteriophages:

Lambda phage cloning vectors:

1- For gene cloning of large DNA fragments (eukaryotic genes)

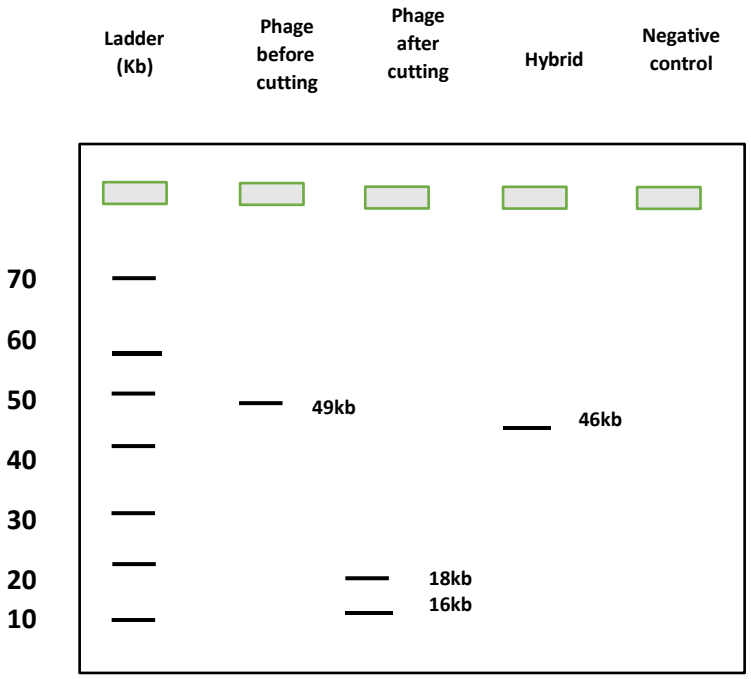
2- Excellent selection capability (stuffer stuff)

3- Clone lots of precisely-sized DNA fragments for library construction

Example 1: in cloning experiment you have a foreign DNA (12 kb) Which cloning vector can use and how can prove that the DNA inserted in cloning vector?

Answer:

1- We use replacement phage because the foreign DNA 12kb in size
2- We draw a diagram of gel to prove the DNA inserted in phage



Example 2: in cloning experiment you have a cloning vectors as below:

1- PBR322
2- λ Phage

Which of them you use if the molecular size of the gene (9.5kb) and why?

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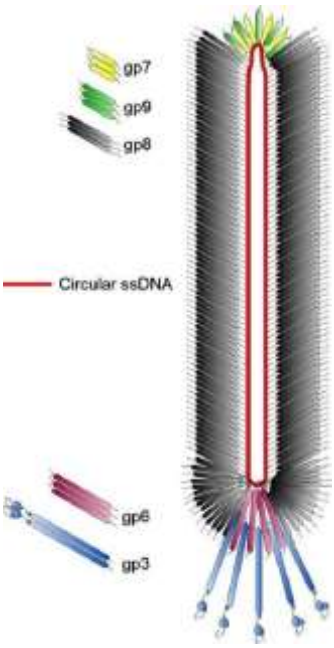
M13 Phage (filamentous phage)

It is 6407 base of nucleotides in length, circular and consists of a single stranded DNA molecule and **is used as a cloning vector because**

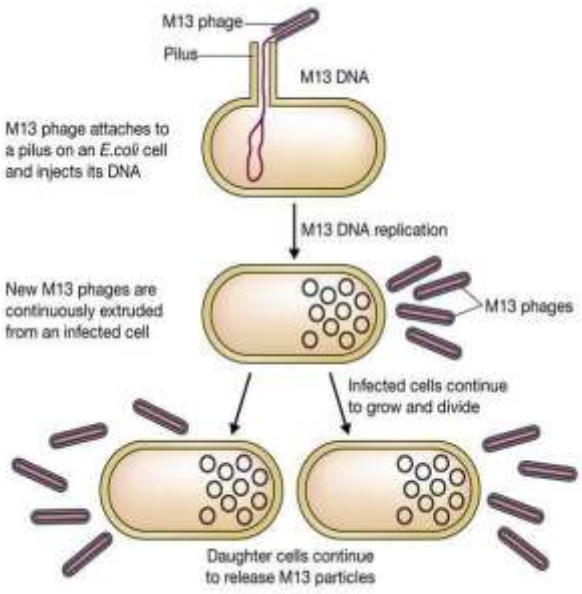
1- It is less than 10kb in size.
2- The double stranded **replicative form** of the genome acts like a plasmid.
3- It is easily prepared from the culture of infected E. coli cells.
4- Single-stranded, circular genome, 6.4 kb
5- Infect only F+ bacteria, using pilus F- coded
6- Can clone pieces of DNA up to 6X the M13 genome size (36 kb) but the larger the DNA, the less stable
7- Consists of ten closely packed genes for replication of the phage.
8- Sometimes M13 named fd phage or f1 phage

Useful for or advantages of M13

1– Sequencing
2– Site-directed mutagenesis
3– Any other technique that requires single stranded DNA

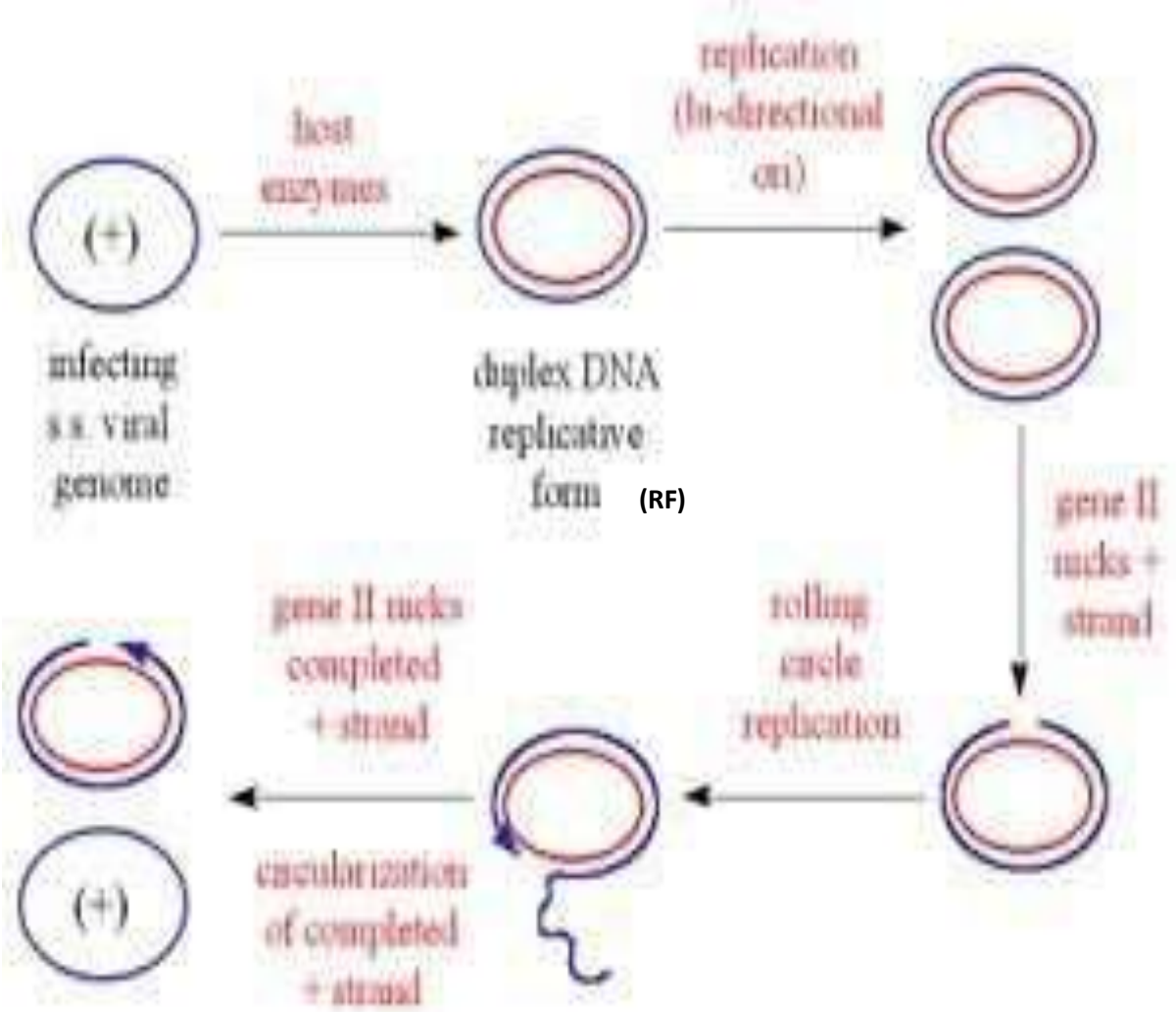


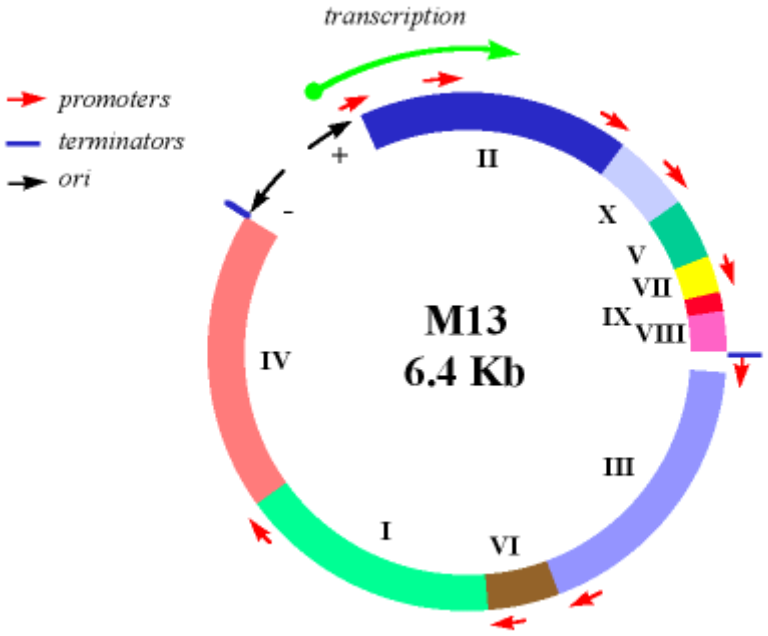
Structure of M13



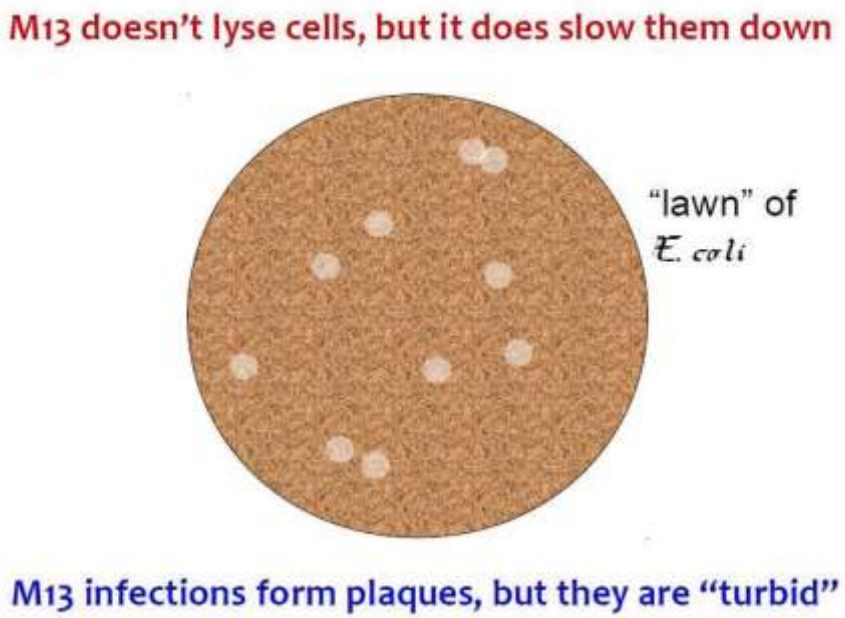
(M13 life cycle: the infection cycle of M13)

Replication of bacteriophage M13 DNA in infected bacteria (inside bacteria)





Genome of M13 (have 10 genes)



Q / make a comparison between lambda phage and M13

Application of genetic engineering

Lec.3

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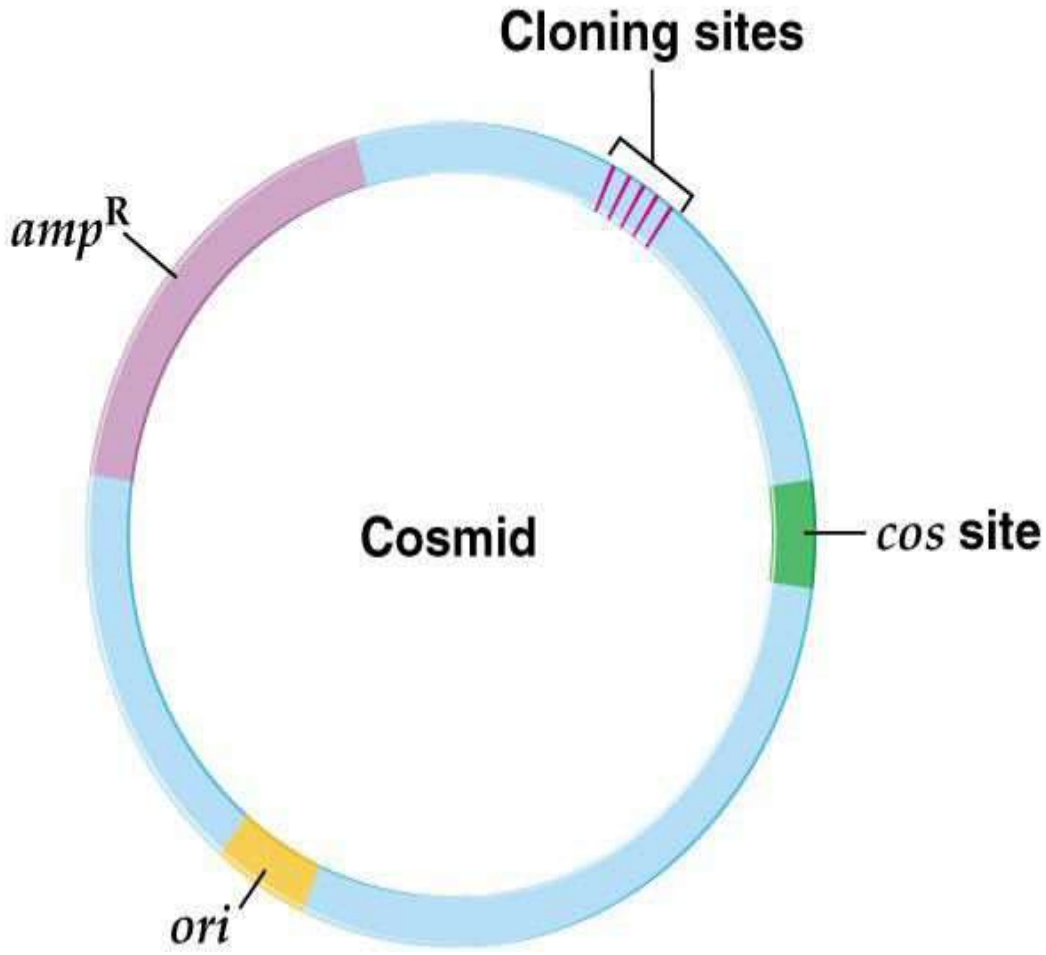
Cosmids

1- A cosmid is a type of hybrid plasmid that contains a Lambda phage cos sequence.
2- Cosmids (cos sites + plasmid = cosmids) DNA sequences are originally from the lambda phage.
3- They are often used as a cloning vector in genetic engineering.
4- Cosmids can be used to build genomic libraries.
5- Cosmids can contain 37 to 52 (normally 45) kb of DNA, limits based on the normal bacteriophage packaging size.
6- They frequently also contain a gene for selection such as antibiotic resistance, so that the transformed cells can be identified by plating on a medium containing the antibiotic. Those cells which did not take up the cosmid would be unable to grow.
7- Unlike plasmids, they can also be packaged in phage capsids, which allows the foreign genes to be transferred into or between cells by transduction.
8- Plasmids become unstable after a certain amount of DNA has been inserted into them, because their increased size is more conducive to recombination.

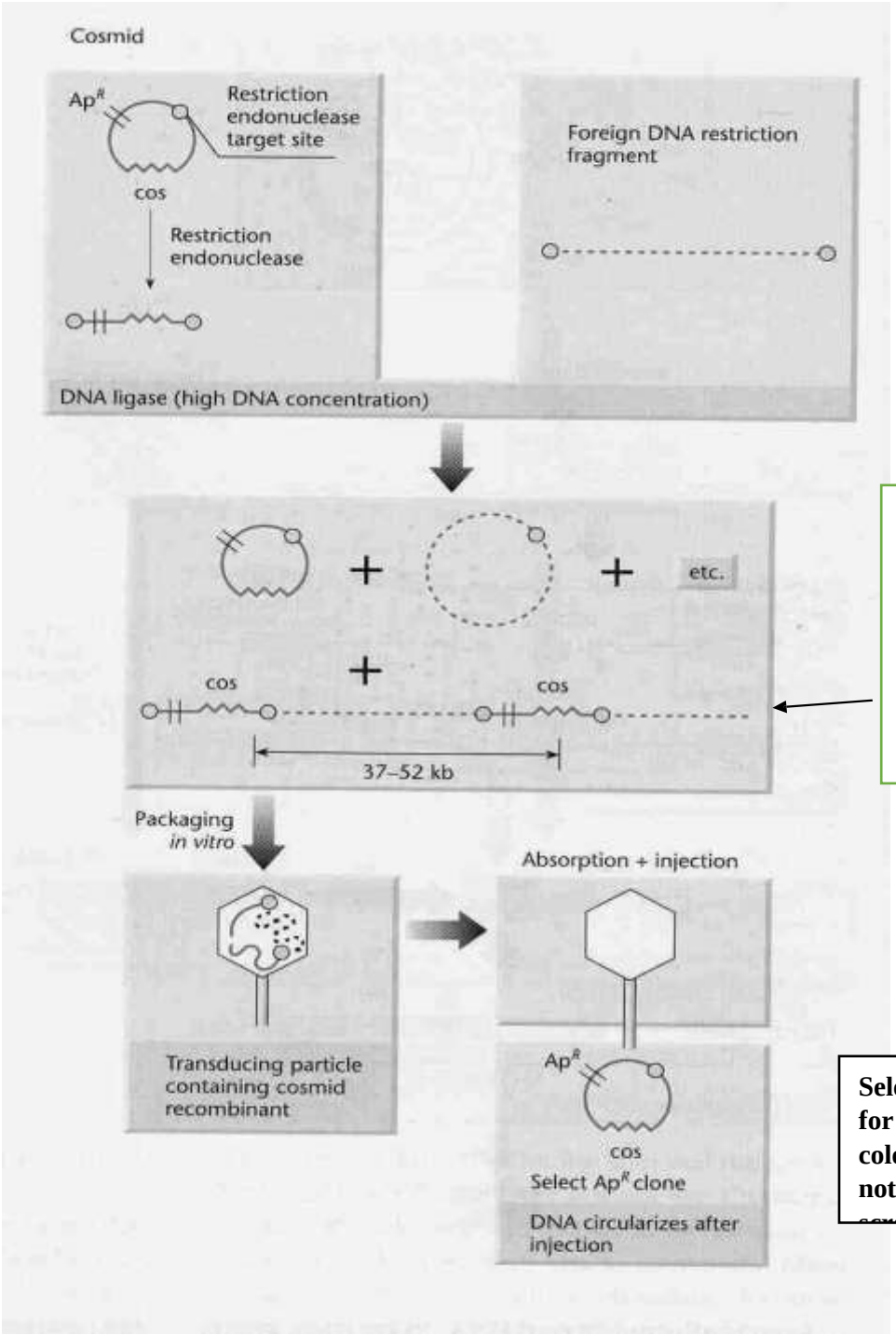
To circumvent this, phage transduction is used instead. This is made possible by the cohesive ends, also known as cos sites. In this way, they are similar to using the lambda phage as a vector, except all the lambda genes have been deleted with the exception of the cos sequence

9- Features of both plasmid and lambda phage cloning vectors.
10- Circular.
11- Do not occur naturally
12- Origin (ori) sequence for E. coli.
13- Selectable marker, e.g. amp^R.
14- Restriction sites (for cloning).
15- Contain Phage λ (lambda) cos sites which permits packaging into λphage heads and therefore introduction to E. coli cells.
16- Packaging only occurs with 37-52 kb fragments (selection for large fragments)
17- Useful for 37-52 kb and packaged DNA is inserted into cells and then replicates as a very large plasmid



Genome of cosmid

Cloning in cosmid



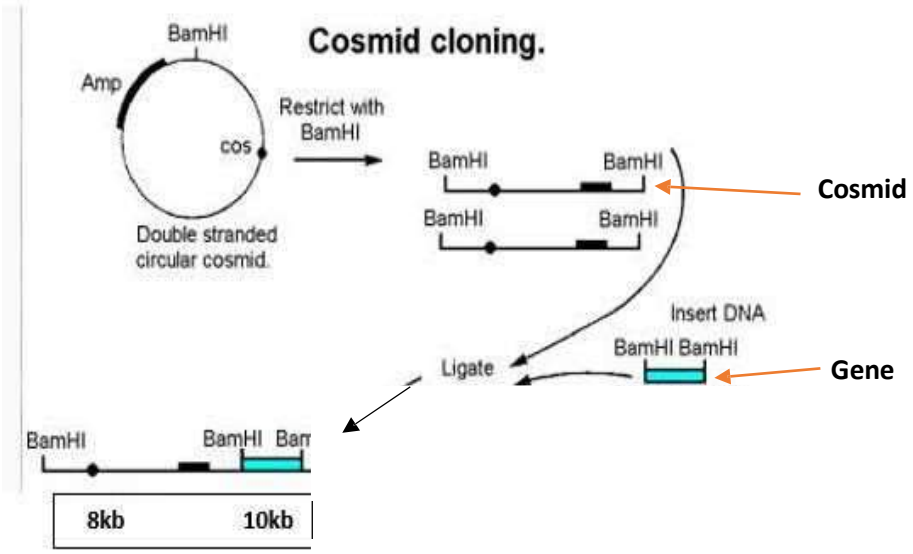
Desired ligation Products-- These are packaged Instead of transformation, (Desired ligation products are packaged and

Selection for colonies, not screening

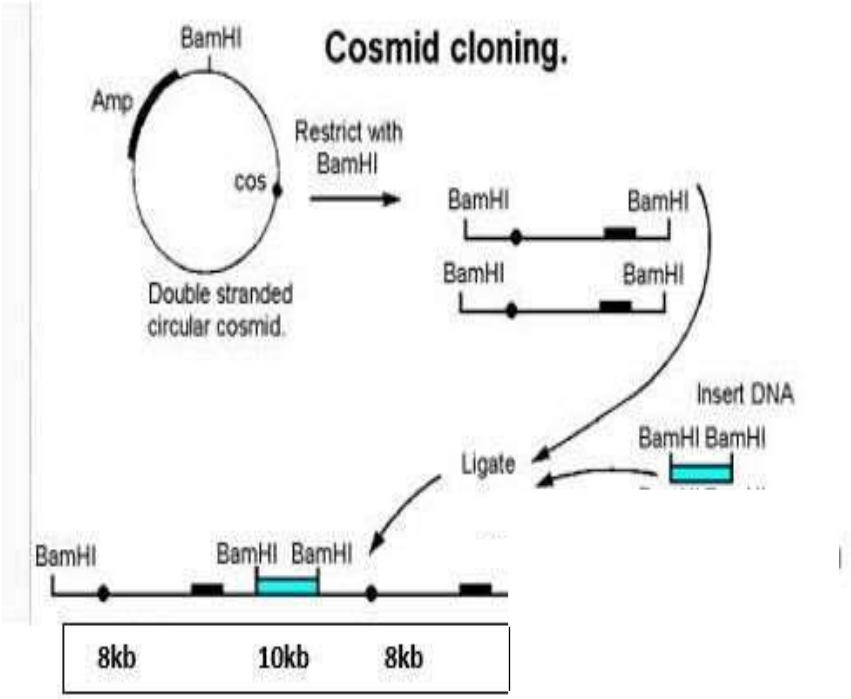
Example:- In a cloning experiment, the gene of interest molecular size (10kb) was ligated with cloning vector (cosmid (8kb)) to get DNA hybrid. Give all probabilities and draw a gel pattern to illustrate how to monitor this cloning experiment.

Answer:

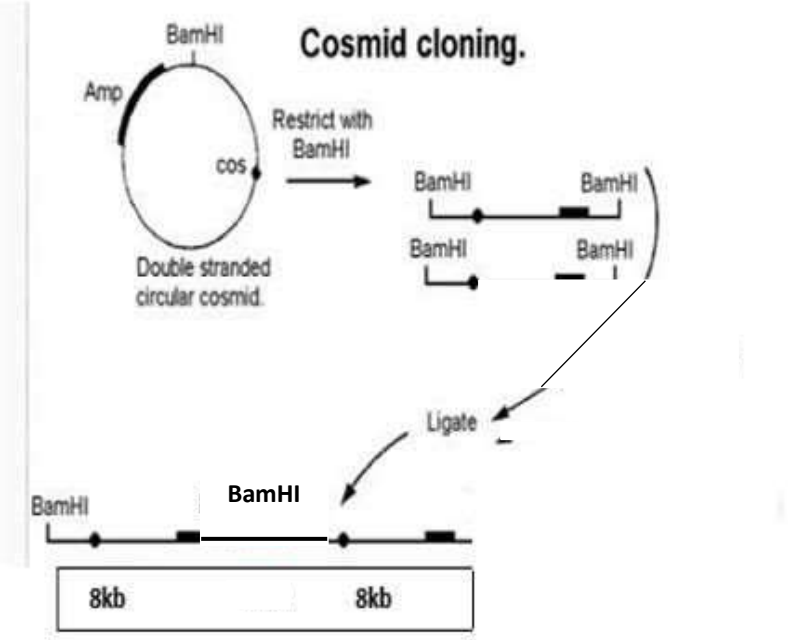
1- **Possible 1 (cosmid +gene)=8+10=18kb**



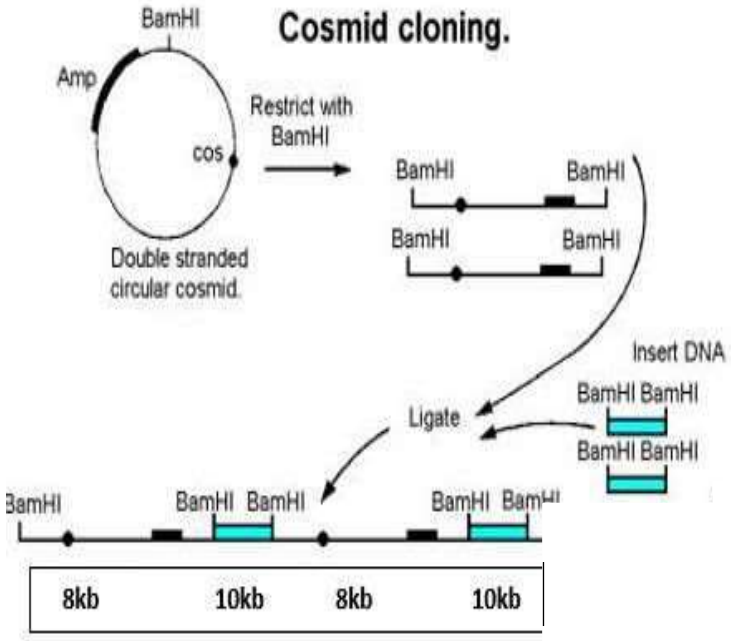
2-possible 2 (cosmid+gene+cosmid)=8+10+8=26kb



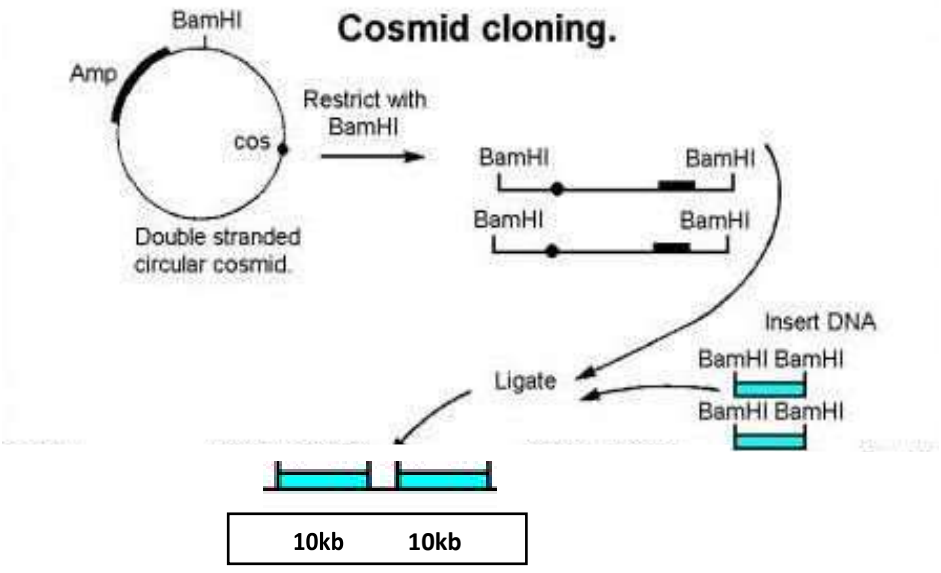
3- Possible 3 (cosmid + cosmid) =8+8=16kb



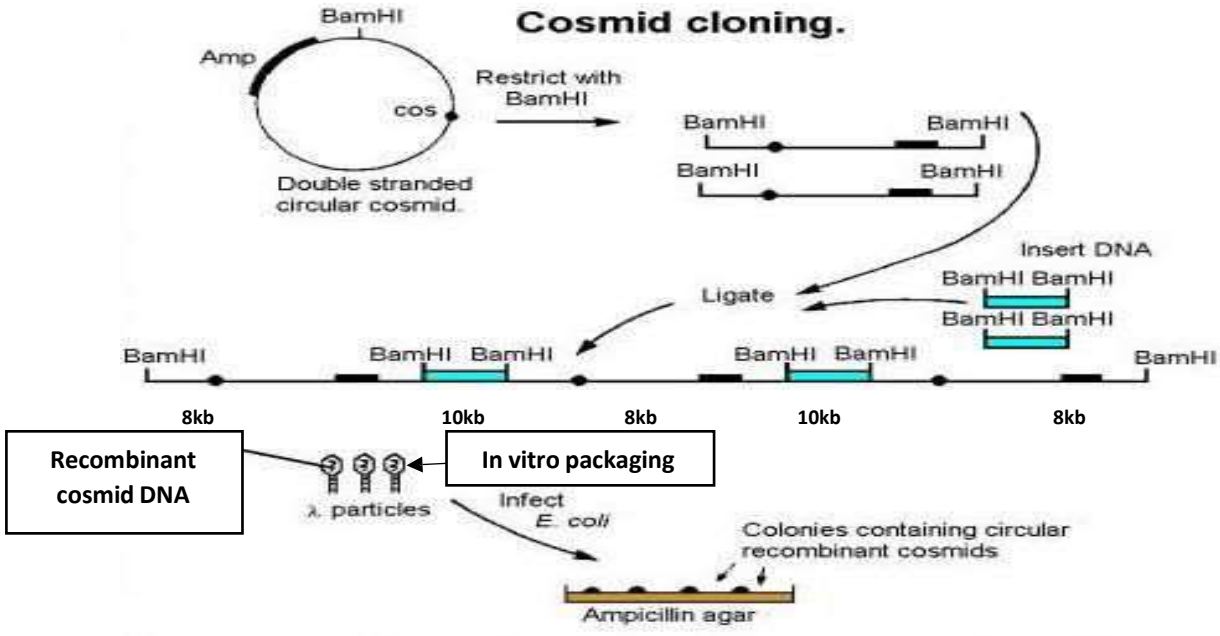
4- possible 4 (cosmid +gene +cosmid +gene)=(2 cosmid +2 gene)= 8+10+8+10= 36kb



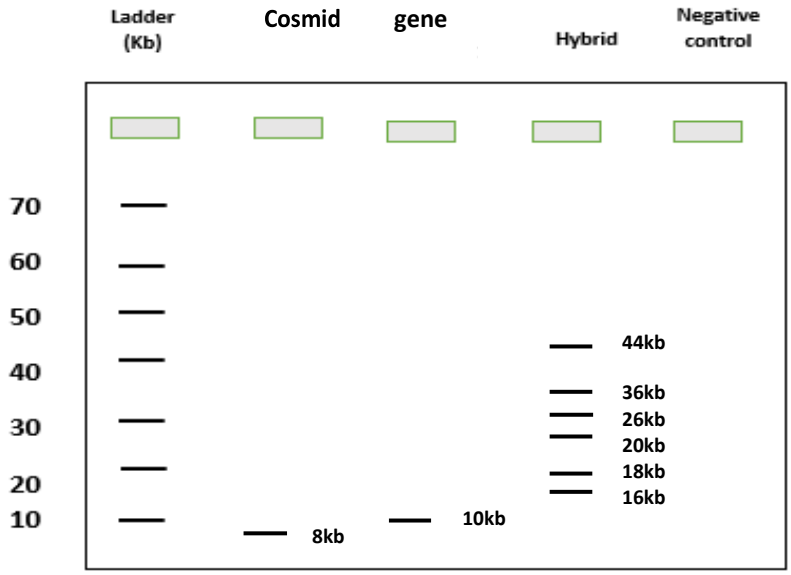
5- Possible 5 (gene +gene) = 10+10 =20kb



6- possible 6 (cosmid +gene+cosmid+gene+cosmid)=8+10+8+10+8=44kb



Draw a gel and show all possibilities



Question/ Estimate the limits of cosmid if the molecular size of the cloned gene (20 kb)

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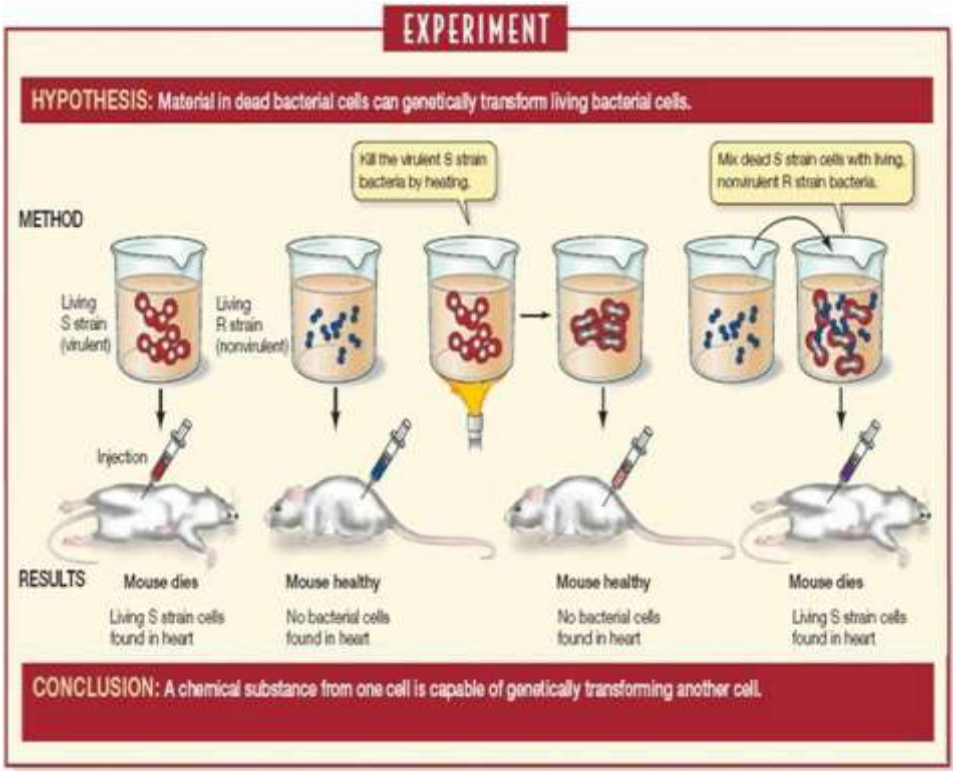
Transformation

Transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane.

Transformation in bacteria was first demonstrated in 1928 by the Frederick Griffith.

Griffith was interested in determining whether injections of heat-killed bacteria could be used to vaccinate mice against pneumonia. However, he discovered that a non-virulent strain of ***Streptococcus pneumoniae*** could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent.



In 1944 this "transforming principle" was identified as being genetic by Avery, MacLeod, and McCarty. They isolated DNA from a virulent strain of *S. pneumoniae* and using just this DNA were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation" (Avery-MacLeod-McCarty experiment) The results of Avery et al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other methods of genetic transfer (conjugation in 1947 and transduction in 1953) by Lederberg that Avery's experiments were accepted.

In 1970, Mandel and Higa showed that *E. coli* may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution. Two years later in 1972, Cohen, Chang and Hsu showed that $CaCl_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Hanahan.

Features of host cell for entering foreign DNA:

1- Must be Host sd R` (Resistance to all restriction enzymes to avoid foreign DNA from degradation)
2- Must be sensitive to antibiotics
3- F- (don't have F plasmid to avoid competition)
4- Non pathogenic
5- Don't have recombinase enzymes (Rec-)

*****(Recombinase enzymes catalyse site specific recombination events within DNA)

6- Must be competent to receive foreign DNA example (E.coli MM294)

Natural transformation in G - bacteria:

What happen to DNA inside and outside bacteria (surrounding environment) in natural transformation?

Only ssDNA will enter the bacteria through the pores of membrane and other one of DNA will degrade outside it. Inside bacteria the ssDNA either bind to bacterial chromosome in homologous or heterologous sequence and called (homologous recombination or heterologous recombination) or stay in cytoplasm (naked DNA) or degrade. When it incorporate with bacterial chromosome this called Rec+ (contain recombinase enzyme) and if not bind to bacterial chromosome it's called Rec- (don't contain recombinase enzyme).

Recombination hotspots: are regions in a genome that exhibit elevated rates of recombination or mutation.

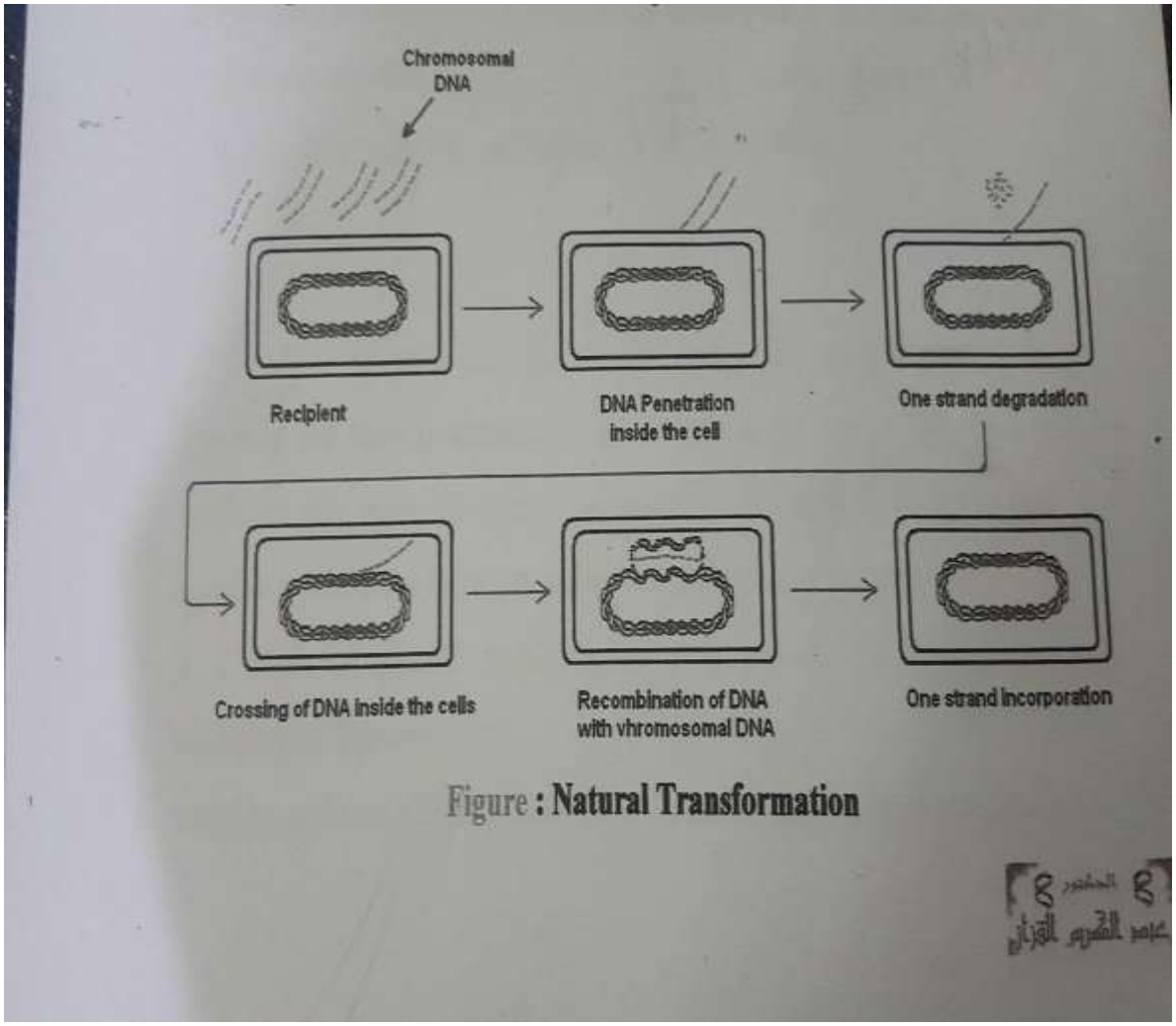


Figure : Natural Transformation

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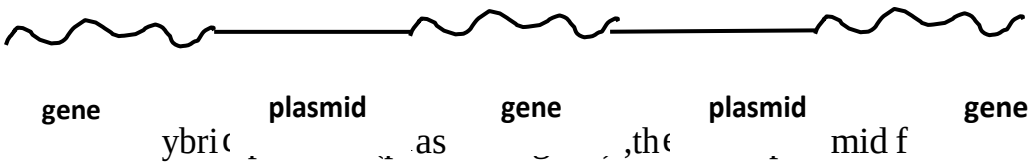
Natural transformation in (G+) bacteria *Bacillus subtillus*:

G+ bacteria differ from G- bacteria that's the cell wall contain peptidoglycan layer so it's difficult for foreign DNA to enter the cell wall in comparison with G- bacteria . The chromosome is easy to enter the cell because its linear but plasmid is circular so its difficult to enter the cell:

Methods of transformation in *Bacillus subtillus*

1- Make plasmid linear (convert CCC plasmid to linear). many linear plasmids and gene of interest join together then enter to B. subtillis



ybri as ,th mid f *ubtillis* then make the hybrid linear and then enter the bacteria . in bacteria contain the same plasmid so the plasmid will bind to the similar sequence in the hybrid except the gene and then all will replicate.

3- Protoplast transformation (chemical method)

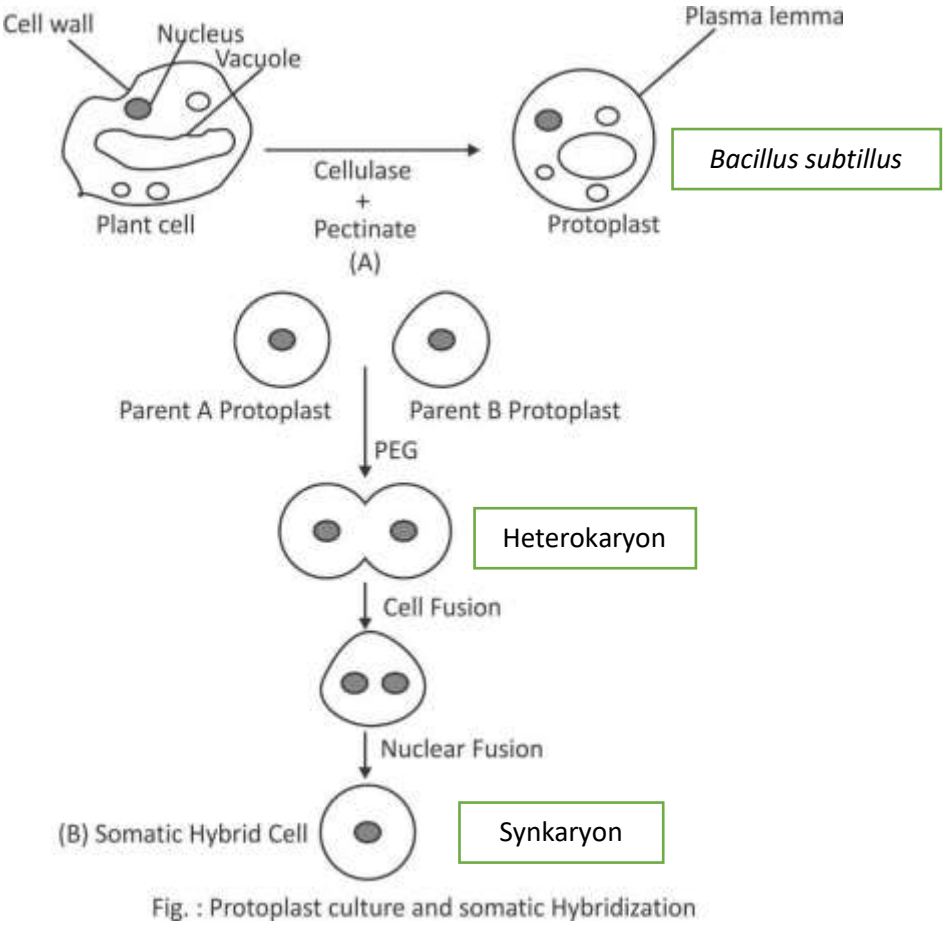


Fig. : Protoplast culture and somatic Hybridization

PEG: (chemical substance) poly ethylene glycol its fusion agent

Microinjection (physical method)

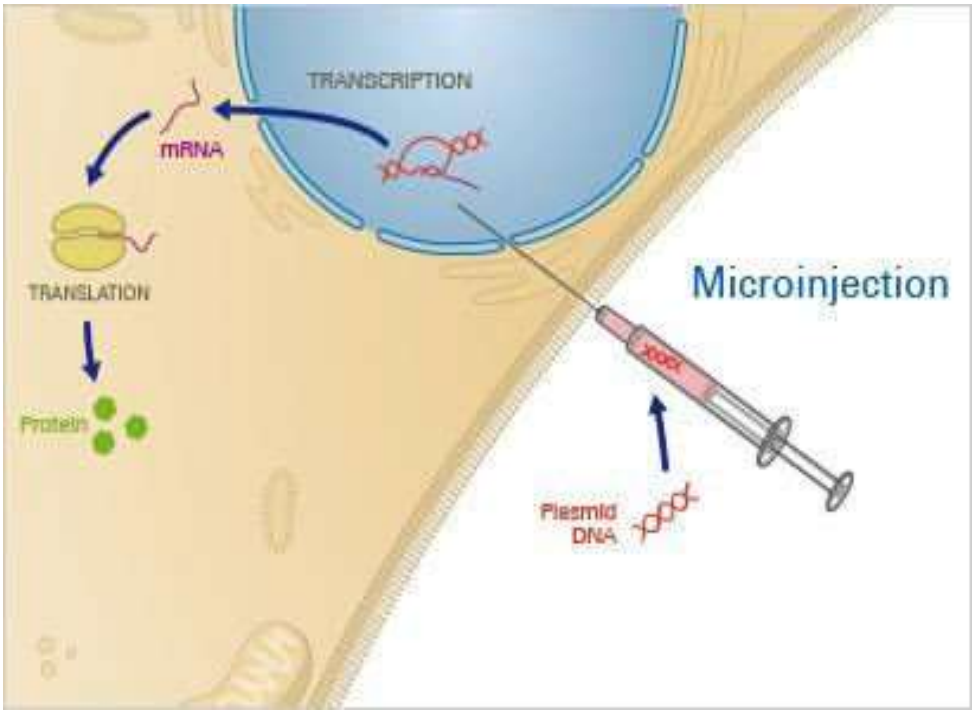
Applications:

Microinjection allows for the efficient transfer of controlled nucleotide amounts into the nucleus of a specific target cell.

Principle:

With this physical method, the target cell is positioned under a microscope and being fixed by a pipette. The nucleotide solution is then directly injected into the

cytoplasm and/or the nucleus using a fine glass capillary needle. Once within the nucleus, the nucleotide can immediately integrate into the endogenous DNA.



Application of genetic engineering

Lec.6

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Screening or selection of recombinant clones

Recombinant clones: Clone containing recombinant DNA molecules

Recombinant DNA: cloning vector + gene of interest

After the introduction of recombinant DNA into the host cells, it is essential to identify those cells which received rDNA molecule - screening (or) selection.

The vector or foreign DNA present in the recombinant cells expresses certain characters or traits, while non-recombinants do not express the traits.

Following the methods for screening or selection of recombinant clones.

1- **Direct selection of recombinants**
2- **Indirect selection of recombinants**

Direct selection of recombinants

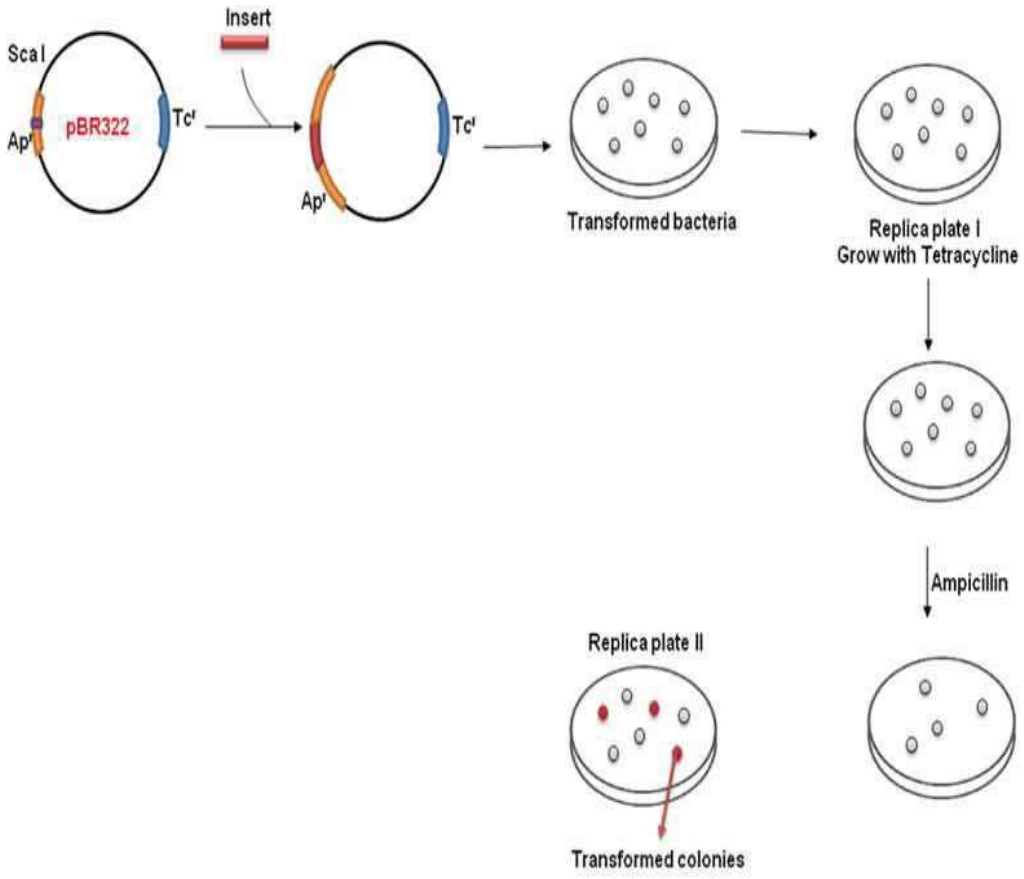
1- **Insertional inactivation**

Insertional inactivation is the inactivation of a gene upon insertion of another gene in its place or within its coding sequence.

This helps in selection of recombinant colonies in rDNA technology.

a- Insertional Inactivation of antibiotic resistance gene

Bacterial plasmid PBR322 has two antibiotic resistance gene, Apr and Tc. If a gene fragment will be cloned in ScaI, it will disrupt the Apr gene. As a result, the clone will be ampicillin sensitive and Tcr, whereas the original plasmid will be Apr and Tcr.

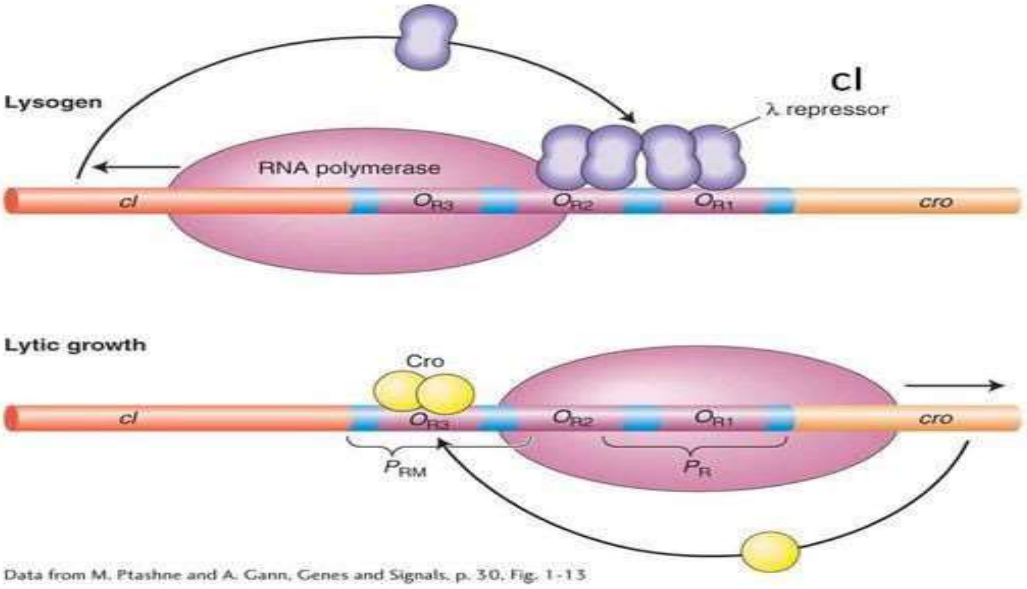


b- Insertional inactivation of cI gene

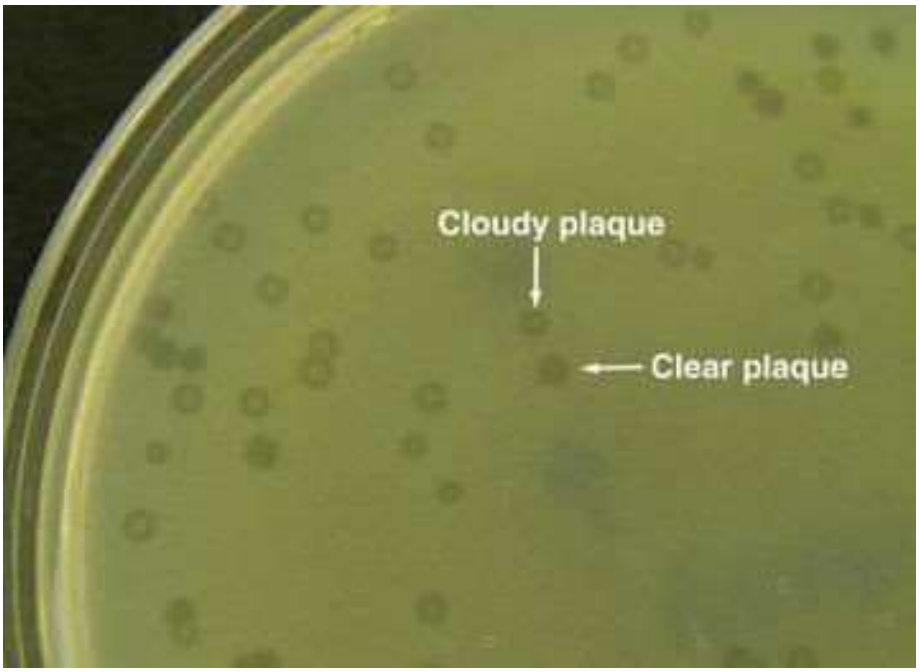
CI repressor: is a transcription inhibitor of bacteriophage Lambda. Also known as Lambda Repressor, cI is responsible for maintaining the lysogenic life cycle of phage Lambda.

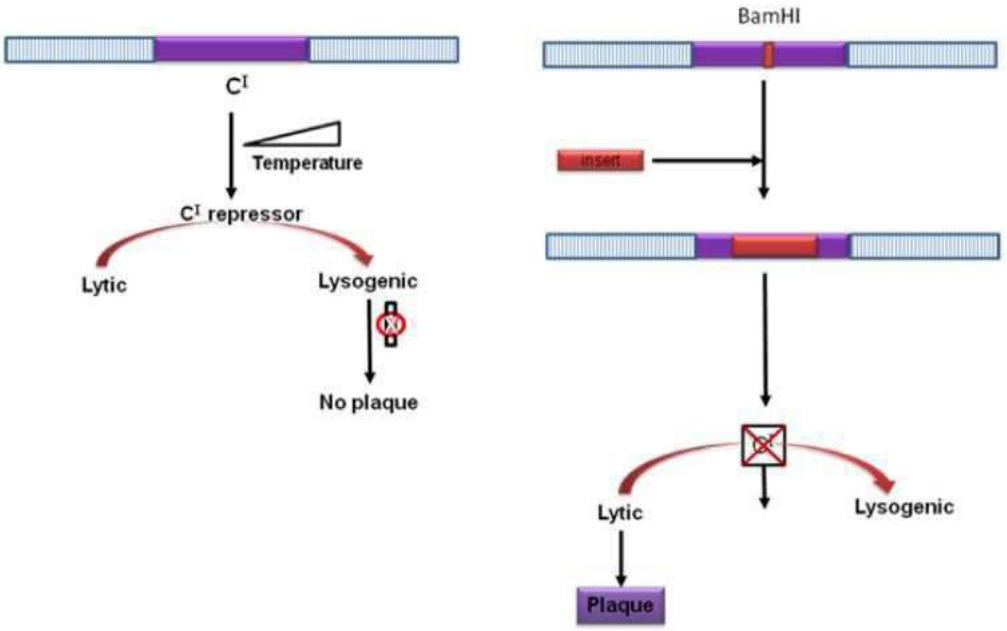
During an infection cycle, virus undergoes a lytic and lysogenic stages. The cI gene encodes for cI repressor which is responsible for the formation of lysogens. In the presence of functional cI, the plaques contains un lysed host cells and has a turbid appearance where as in the absence it will clear. This feature can be used to screen the clone to detect functional cI (absence of clone) or absence of cI

(presence of insert).



Data from M. Ptashne and A. Gann, Genes and Signals, p. 30, Fig. 1-13





2-**Blue white screening**

It is a powerful method for screening recombinants.

1– In this method a reporter gene lacZ is inserted in the vector (encodes β-galactosidase).

2– β-galactosidase breaks a synthetic substrate, X-gal into an insoluble blue coloured product.

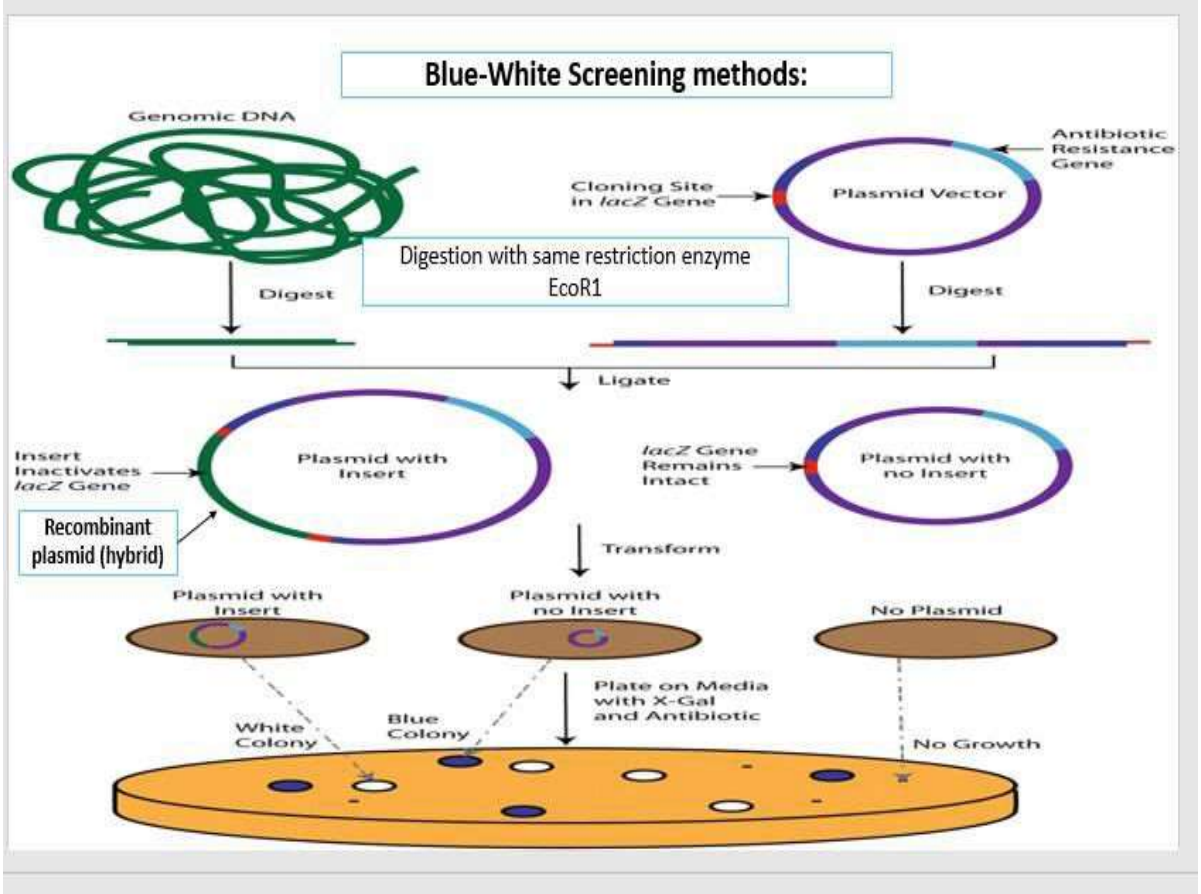
3– If a foreign gene is inserted into lacZ, this gene will be inactivated; therefore no blue colour will develop.

4– The host cells containing recombinant will form white coloured substrate on the medium containing X-gal.

5– The host cells containing non recombinants will turn blue in colour.

6– On the basis of colony colour the recombinants can be selected.

Figure 9.8 Blue-white screening on medium with ampicillin, X-gal, and IPTG. Blue colonies contain nonrecombinant plasmids. White colonies contain recombinant plasmids and can be isolated directly from this plate.



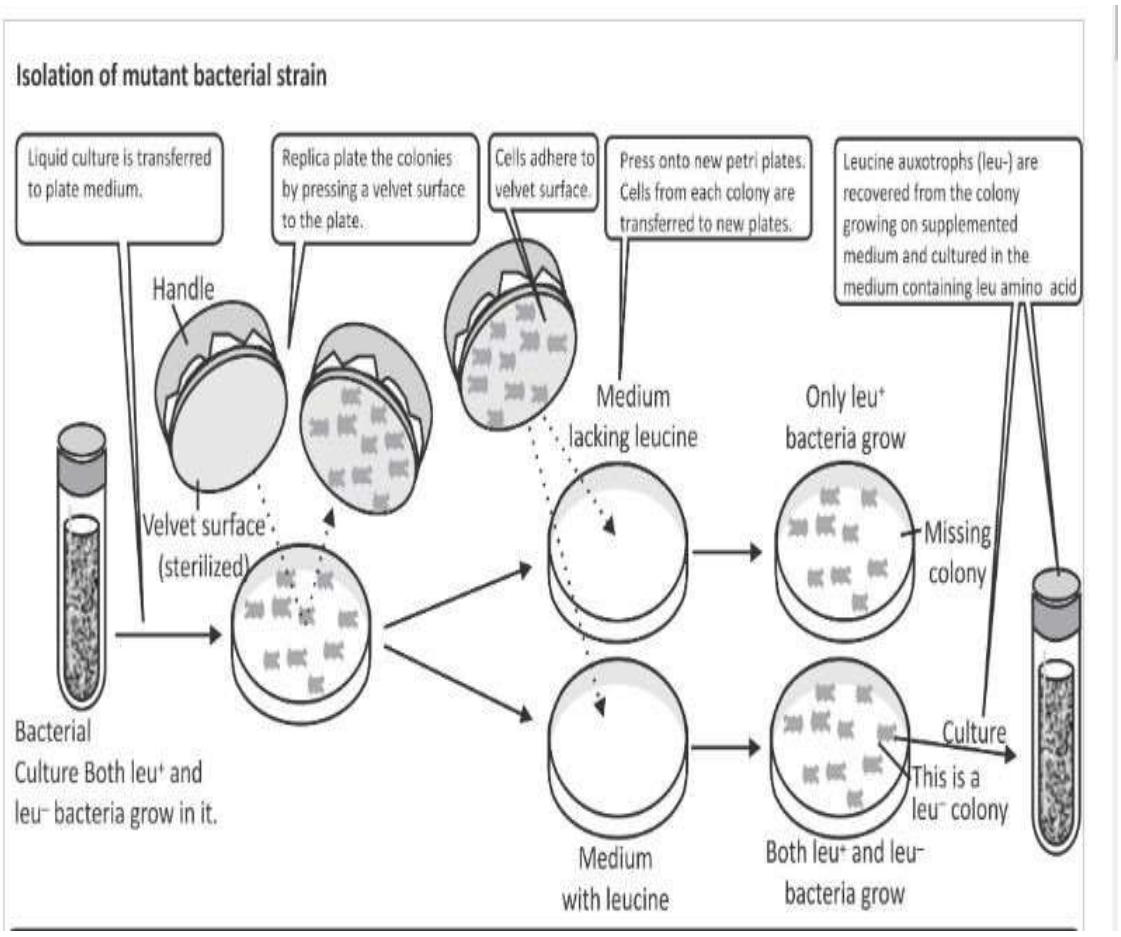
3- Auxotrophic mutant

Auxotroph = mutant that cannot grow on minimal medium, requires certain supplement(s).

Auxotrophs are microorganisms that are unable to synthesize an essential nutrient because of a gene mutation.

Example: leu- auxotroph, can't grow without added leucine

Auxotrophic strains have many uses in genetics. Researchers often use auxotrophic strains as hosts for plasmid transformation. The plasmids used for transformation carry functional gene that is defective in the host strain, making it possible to select transformants by their ability to grow on media lacking the essential nutrient.



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Screening or selection of recombinant clones

Indirect selection of recombinants

1- Immunological method or Broomet and Gilbert 1978 method

Antibodies are used to identify the colonies developed that synthesize antigens encoded by the foreign DNA present in plasmids of the bacterial clones.

1) Replica plating
2) Lysis of cells using chloroform vapour/ high temp.
3) Making gentle contact with a solid support (polyvinyl sheet).
4) Detection of antigen antibody complex by incubating the polyvinyl sheet with a radio labellled second antibody.
5) The antibodies which do not react are washed off.
6) The determination of antigen antibody complex is determined by passing through x- ray

Application of genetic engineering

Lec.7

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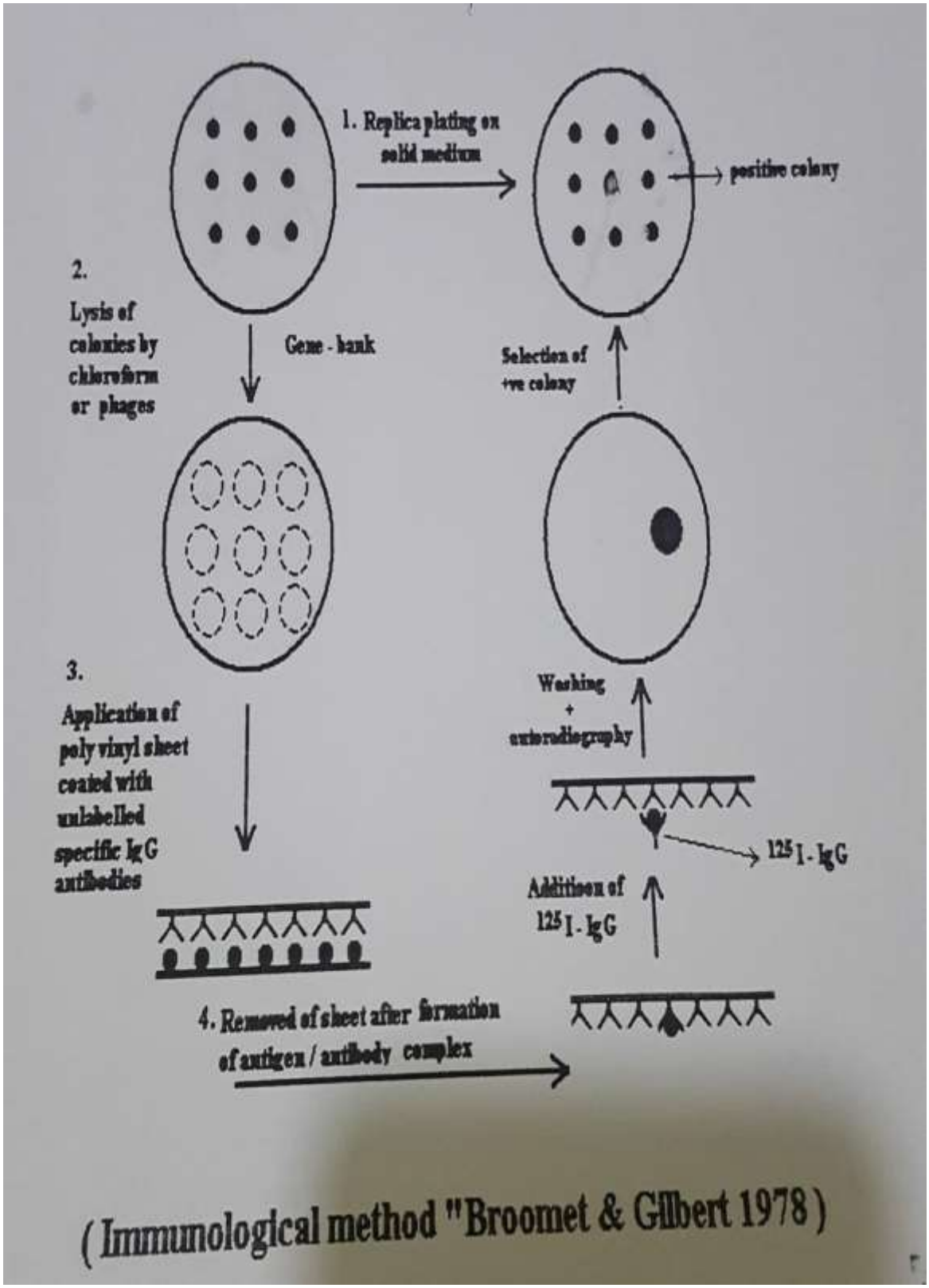


2- **Colony hybridization method or *In situ* hybridization method or Grunstein – Hogness method**.

Colony blot hybridization is applied to DNA or RNA released from blotted microbial colonies.

1– The microbial colonies are transferred (blotted) to a membrane.

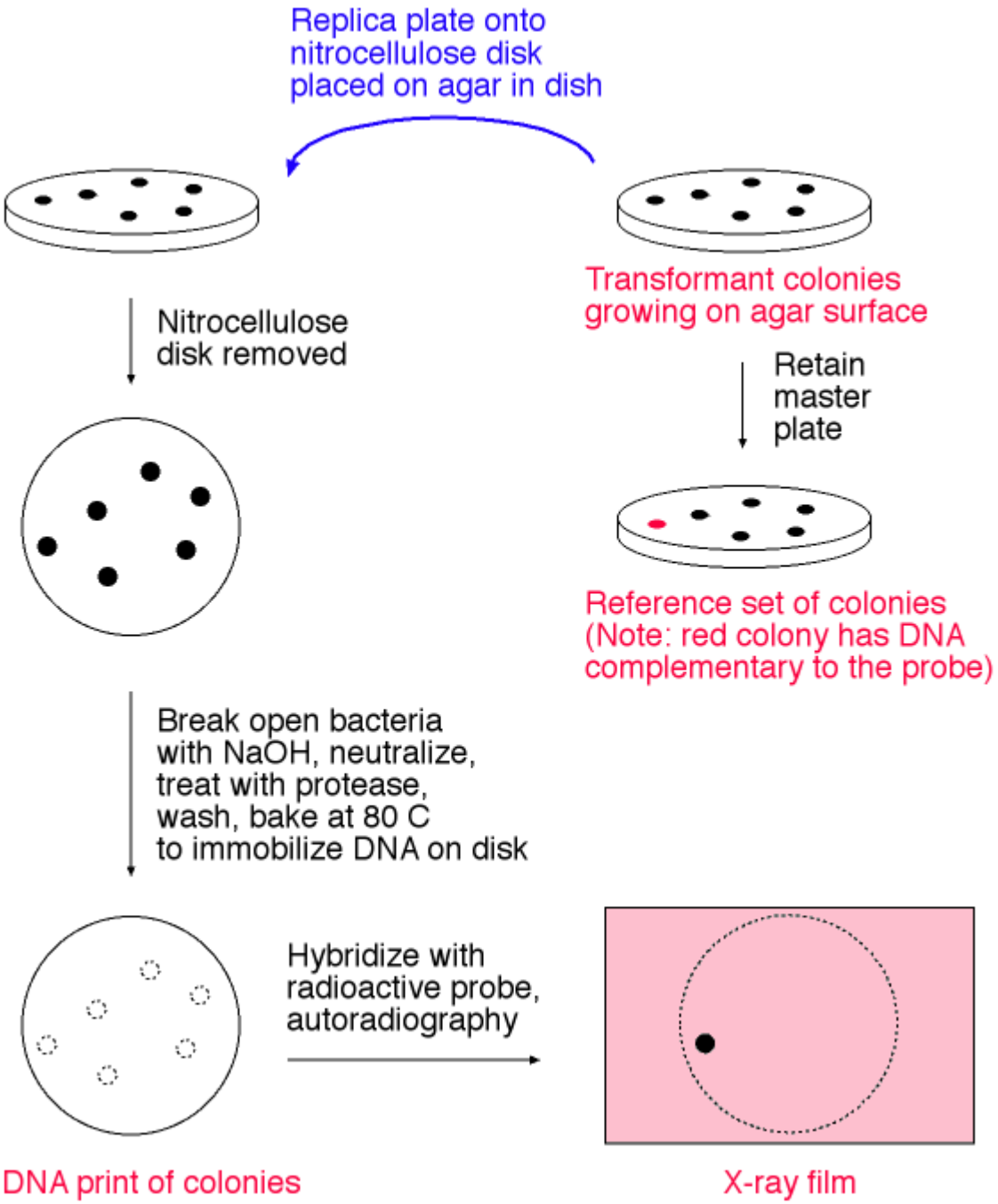
2– The cells are lysed in place to release the nucleic acids.

3– The RNA or DNA (after denaturation) is fixed to the filter and hybridized with a labelled probe.

4– Blocking reagent may be added prior to the probe to prevent unspecific binding.

5– Excess probe is washed away and the membrane is visualized by UV or autoradiography.

5– Colony blot hybridization can be used for screening clones or bacterial isolates.


Replica plate onto nitrocellulose disk placed on agar in dish
Transformant colonies growing on agar surface
Nitrocellulose disk removed
Retain master plate
Reference set of colonies (Note: red colony has DNA complementary to the probe)
Break open bacteria with NaOH, neutralize, treat with protease, wash, bake at 80 C to immobilize DNA on disk
Hybridize with radioactive probe, autoradiography
DNA print of colonies
X-ray film

Application of genetic engineering

Lec.8

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Applications of genetic engineering:-

1- Genetic engineering applications in medicine

Genetic engineering has been gaining importance over the last few years and it will become more important in the current century as genetic diseases become more prevalent and agricultural area is reduced. Genetic engineering plays significant role in the production of medicines. Microorganisms and plant based substances are now being manipulated to produce large amount of useful drugs, vaccines, enzymes and hormones at low costs.

1- Gene therapy

Gene therapy by which healthy genes can be inserted directly into a person with malfunctioning genes is perhaps the most revolutionary and most promising aspect of genetic engineering. The use of gene therapy has been approved in more than 400 clinical trials for diseases such as cystic fibres emphysema, muscular dystrophy, and adenosine deaminase deficiency. Gene therapy may someday be exploited to cure hereditary human diseases like haemophilia and cystic fibrosis which are caused by missing or defective genes. In one type of gene therapy new functional genes are inserted by genetically engineered viruses into the cells of people who are unable to produce certain hormones or proteins for normal body functions.

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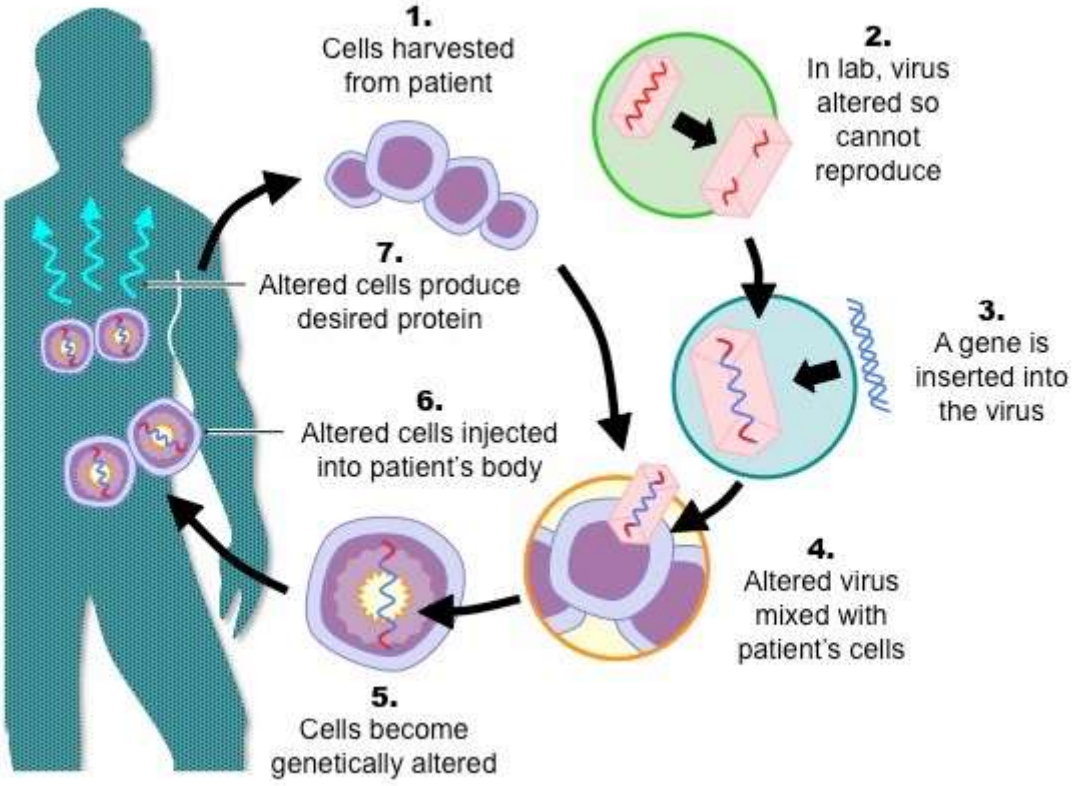
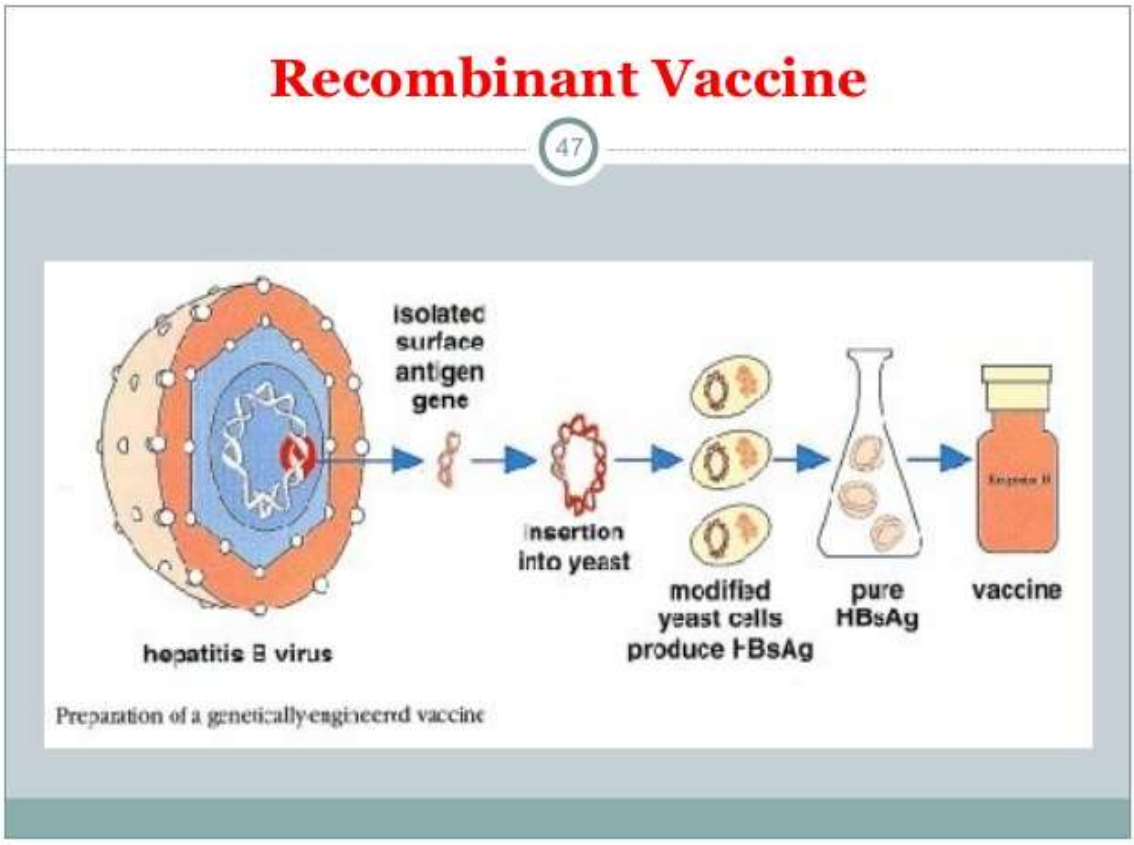


1- **Vaccines**:

Recombinant DNA Technology is also used in production of vaccines against diseases. A vaccine contains a form of an infectious organism that does not cause severe disease but does cause immune system of body to form protective antibodies against infective organism. Vaccines are prepared by isolating antigen or protein present on the surface of viral particles.

When a person is vaccinate against viral disease, antigens produce antibodies that acts against the viral proteins and inactivate them. With recombinant DNA technology, scientists have been able to transfer the genes for some viral sheath proteins to vaccinia virus which was used against small pox.

Vaccines produced by gene cloning are contamination free and safe because they contain only coat proteins against which antibodies are made. A few vaccines are being produced by gene cloning, e.g., vaccines against viral hepatitis influenza, herpes simplex virus, and virus induced foot and mouth disease in animals.

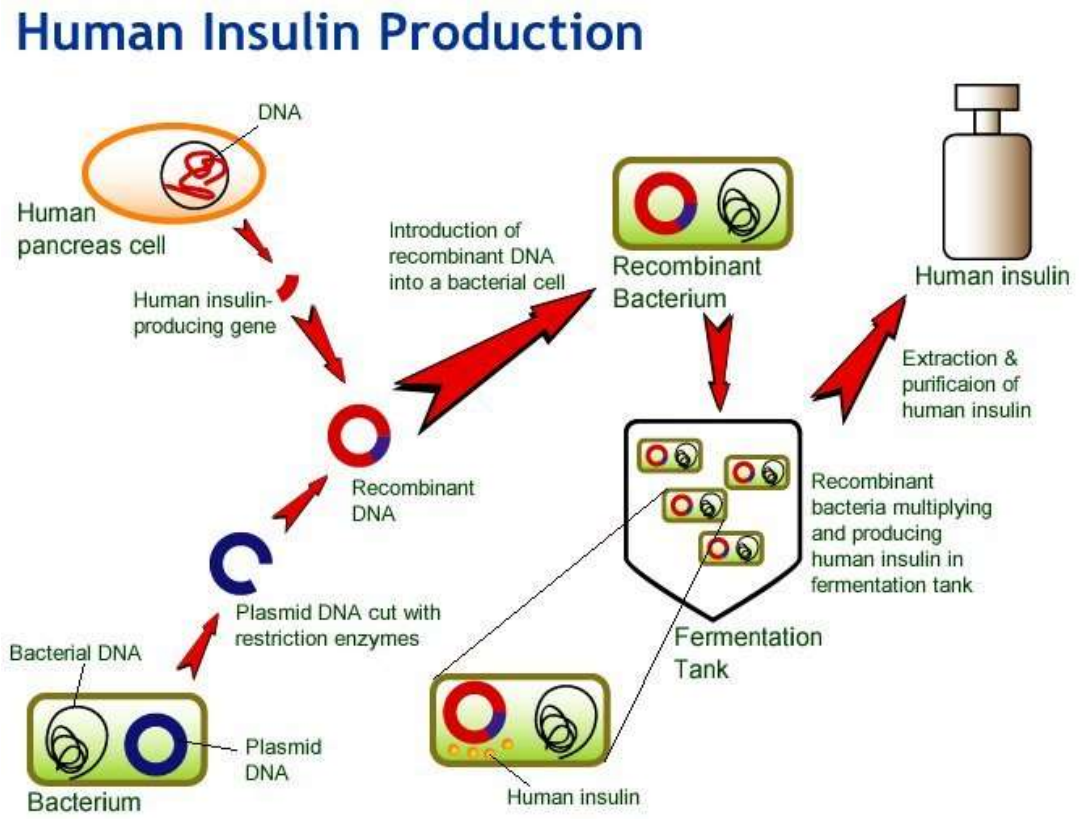


2- **Hormones**:

Until recently the hormone insulin was extracted only in limited quantities from pancreas of cows and pigs. The process was not only costly but the hormone sometimes caused allergic reactions in some patients of diabetes.

The commercial production of insulin was started in 1982 through biogenetic or recombinant DNA technology and the medical use of hormone insulin was approved by food and drug administration (FDA) of USA in 1982.

The human insulin gene has been cloned in large quantities in bacterium E. coli which could be used for synthesis of insulin. Genetically engineered insulin is commercially available as humilin.



3- **Somatostatin:**

A fourteen amino acid polypeptide hormone synthesized by hypothalamus was obtained only in a small quantity from a human cadavers. Somatostatin used as a drug for certain growth related abnormalities appears to be species specific and the polypeptide obtained from other mammals has no effect on human, hence its extraction from hypothalamus of cadavers.

Genetic engineering technique has helped in chemical synthesis of gene which is joined to the pBR 322 plasmid DNA and cloned into a bacterium. The transformed bacterium is converted into somatostatin synthesising factory.

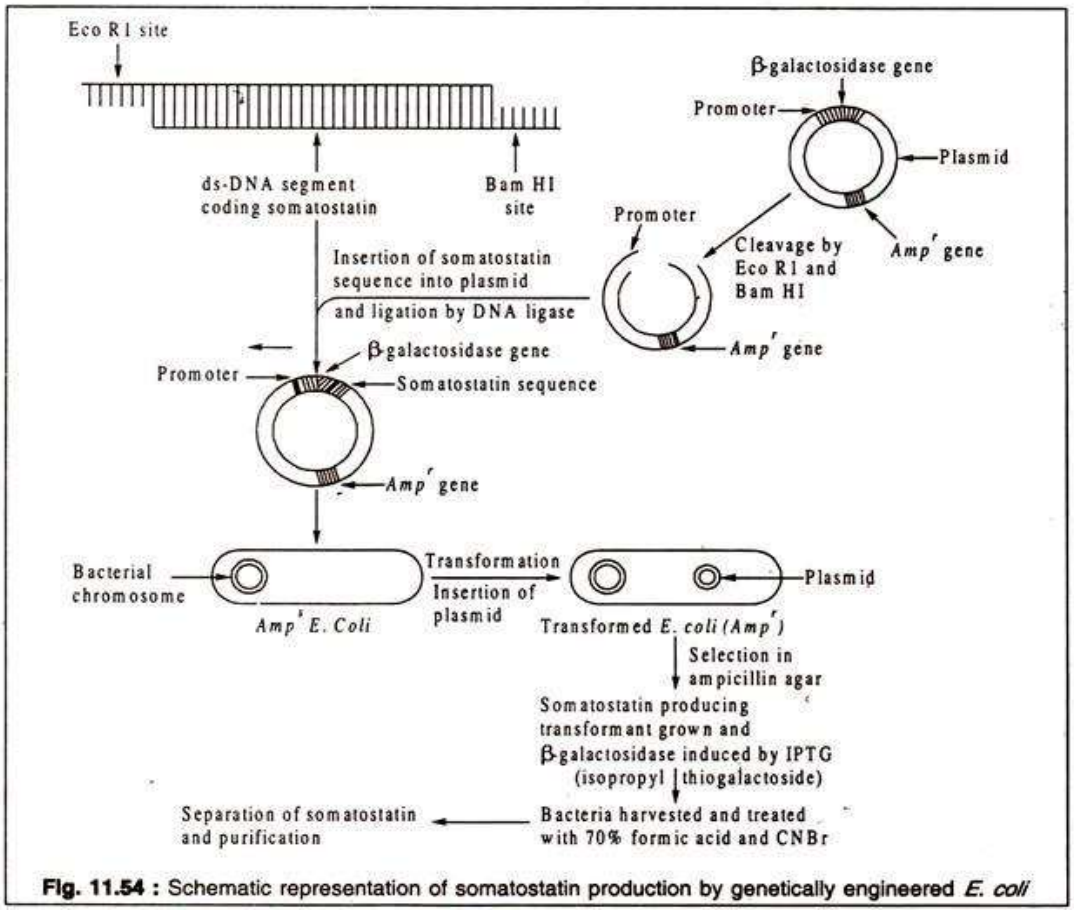


Fig. 11.54 : Schematic representation of somatostatin production by genetically engineered *E. coli*

Application of genetic engineering

Lec.9

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Applications of genetic engineering:-

2- Genetic engineering applications in agriculture

An important application of recombinant DNA technology is to alter the genotype of crop plants to make them more productive, nutritious, and rich in proteins, disease resistant, and less fertilizer consuming. Recombinant DNA technology and tissue culture techniques can produce high yielding cereals, pulses and vegetable crops.

Some plants have been genetically programmed to yield high protein grains that could show resistance to heat, moisture and diseases.

Some plants may even develop their own fertilizers some have been genetically transformed to make their own insecticides. Through genetic engineering some varieties have been produced that could directly fix atmospheric nitrogen and thus there is no dependence on fertilizers.

Scientists have developed transgenic potato, tobacco, cotton, corn, strawberry, rape seeds that are resistant to insect pests and certain weedicides.

Bacterium, *Bacillus thurenginesis* produces a protein which is toxic to insects. Using the techniques of genetic engineering, the gene coding for this toxic protein called Bt gene has been isolated from bacterium and engineered into tomato and tobacco plants. Such transgenic plants showed nee to tobacco horn worms and tomato fruit worms. These genotypes are awaiting release in USA.

There are certain genetically evolved weed killers which are not specific to weeds alone but kill useful crops also. Glyphosate is a commonly used weed killer which simply inhibits a particular essential enzyme in weeds and other crop plants. A target gene of glyphosate is present in bacterium *salmonella typhimurium*. A mutant of S. typhimurium is resistant to glyphosate.

The mutant gene was cloned to E. coli and then recloned to Agrobacterium tumifaciens through its Ti Plasmid. Infection of plants with Ti plasmid containing

glyphosate resistant gene has yielded crops such as cotton, tabacco maize, all of which are resistant to glyphosate.

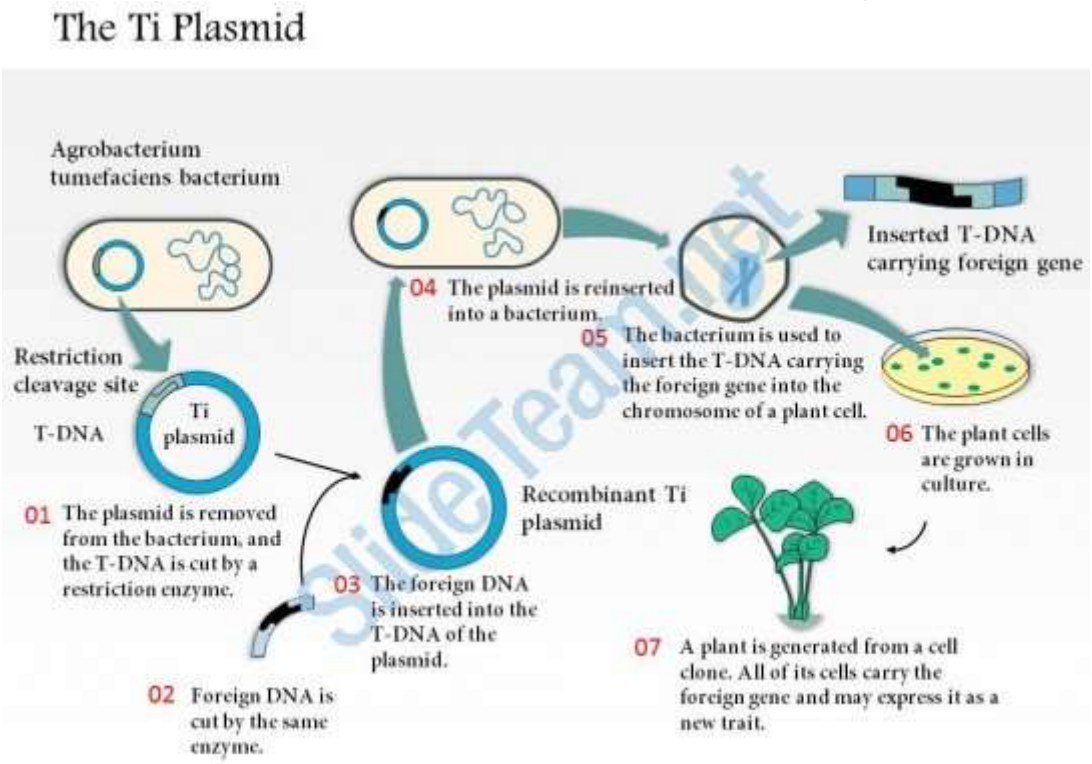
This makes possible to spray the crop fields with glyphosate which will kill the weeds only and the genetically modified crops with resistant genes remain unaffected.

Recently Calogene, a biotech company, has isolated a bacterial gene that detoxifies; side effects of herbicides. Transgenic tobacco plants resistant to T MV mosaic virus and tomato i resistant to Golden mosaic virus have been developed by transferring virus coat protein genes »susceptible plants. These are yet to be released.

The gene transfer technology can also play significant role in producing new and improved variety of timber trees.

Several species of microorganisms have been produced that can degrade toxic chemicals and could be used for killing harmful pathogens and insect pests.

For using genetic engineering techniques for transfer of foreign genes into host plant cells, a number of genes have already been cloned and complete libraries of DNA and mt DNA of pea are now known.



Applications of genetic engineering:-

3- Application of genetic engineering in industry

Genetically designed bacteria are put into use for generating industrial chemicals. A variety of organic chemicals can be synthesised at large scale with the help of genetically engineered microorganisms. Glucose can be synthesised from sucrose with the help of enzymes obtained from genetically modified organisms.

Now-a-days with the help of genetic engineering strains of bacteria and cyanobacteria have been developed which can synthesize ammonia at large scale that can be used in manufacture of fertilisers at much cheaper costs. Microbes are being developed which will help in conversion of Cellulose to sugar and from sugar to ethanol.

Recombinant DNA technology can also be used to monitor the degradation of garbage, petroleum products, naphthalene and other industrial wastes.

For example bacterium *pseudomonas fluorescens* genetically altered by transfer of light producing enzyme called luciferase found in bacterium *vibrio fischeri*, produces light proportionate to the amount of its breaking down activity of naphthalene which provides way to monitor the efficiency of the process.

Application of genetic engineering

Lec.10

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Applications of genetic engineering:-

4- Forensic genetics

Forensic laboratories will receive material that has been recovered from scenes of crime, and reference samples from both suspects and victims. The role of forensic genetics within the investigative process is to compare samples recovered from crime scenes with suspects, resulting in a report that can be presented in court or intelligence that may inform an enquiry (Figure 1.1). Several stages are involved with the analysis of genetic evidence (Figure 1.2). In some organizations one person will be responsible for collecting the evidence, the biological and genetic analysis of samples, and ultimately presenting the results to a court of law. However, the trend in many larger organizations is for individuals to be responsible for only a very specific task within the process, such as the extraction of DNA from the evidential material or the analysis and interpretation of DNA profiles that have been generated by other scientists.

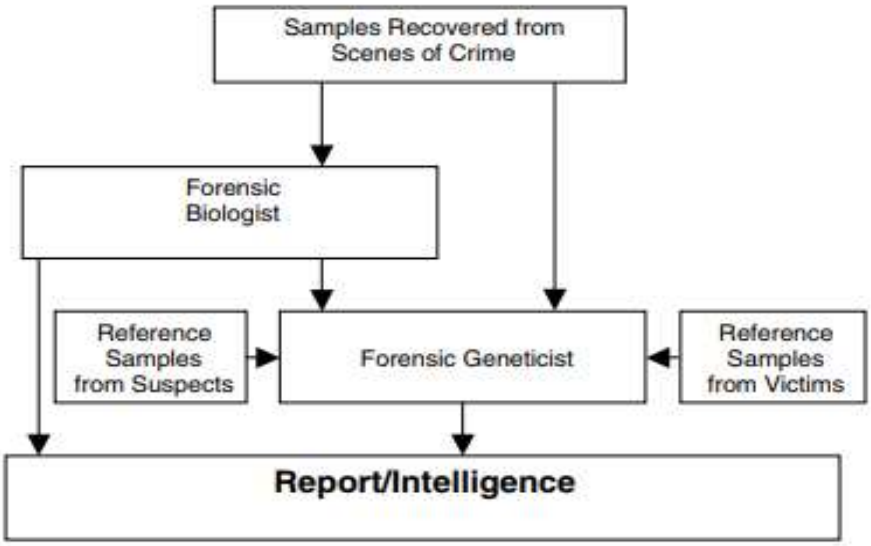

Figure 1.1 The role of the forensic geneticist is to assess whether samples recovered from a crime scene match to a suspect. Reference samples are provided from suspects and also victims of crime

A brief history of forensic genetics

Application of genetic engineering

Lec.10

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In 1900 Karl Landsteiner described the ABO blood grouping system and observed that individuals could be placed into different groups based on their blood type. This was the first step in the development of forensic haemogenetics. In 1915 Leone Lattes published a book describing the use of ABO typing to resolve a paternity case and by 1931 the absorption–inhibition ABO typing technique that became standard in forensic laboratories had been developed. Following on from this, numerous blood group markers and soluble blood serum protein markers were characterized and could be analysed in combination to produce highly discriminatory profiles. The serological techniques were a powerful tool but were limited in many forensic cases by the amount of biological material that was required to provide highly discriminating results. Proteins are also prone to degradation on exposure to the environment. In the 1960s and 1970s, developments in molecular biology, including restriction enzymes, Sanger sequencing, and Southern blotting, enabled scientists to examine DNA sequences. By 1978, DNA polymorphisms could be detected using Southern blotting and in 1980 the analysis of the first highly polymorphic locus was reported. It was not until September 1984 that Alec Jeffreys realized the potential forensic application of the variable number tandem repeat (VNTR) loci he had been studying. The technique developed by Jeffreys entailed extracting DNA and cutting it with a restriction enzyme, before carrying out agarose gel electrophoresis, Southern blotting and probe hybridization to detect the polymorphic loci.

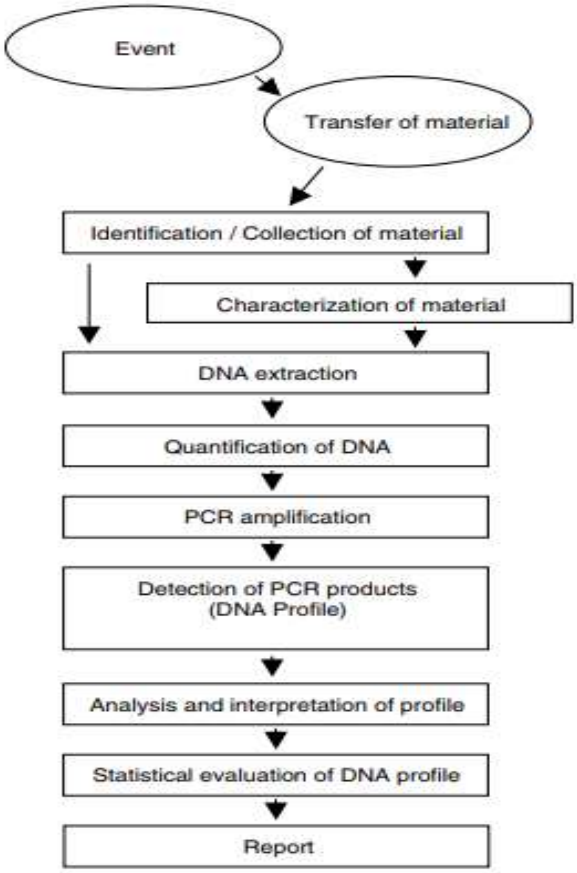

Figure 1.2 Processes involved in generating a DNA profile following a crime. Some types of material, in particular blood and semen, are often characterized before DNA is extracted

The end result was a series of black bands on X-ray film (Figure 1.3). VNTR analysis was a powerful tool but suffered from several limitations: a relatively large amount of DNA was required; it would not work with degraded DNA; comparison between laboratories was difficult; and the analysis was time consuming. A critical development in the history of forensic genetics came with the advent of a process that can amplify specific regions of DNA – the polymerase chain reaction (PCR). The PCR process was conceptualised in 1983 by Kary Mullis, a chemist working for the Cetus

Corporation in the USA.

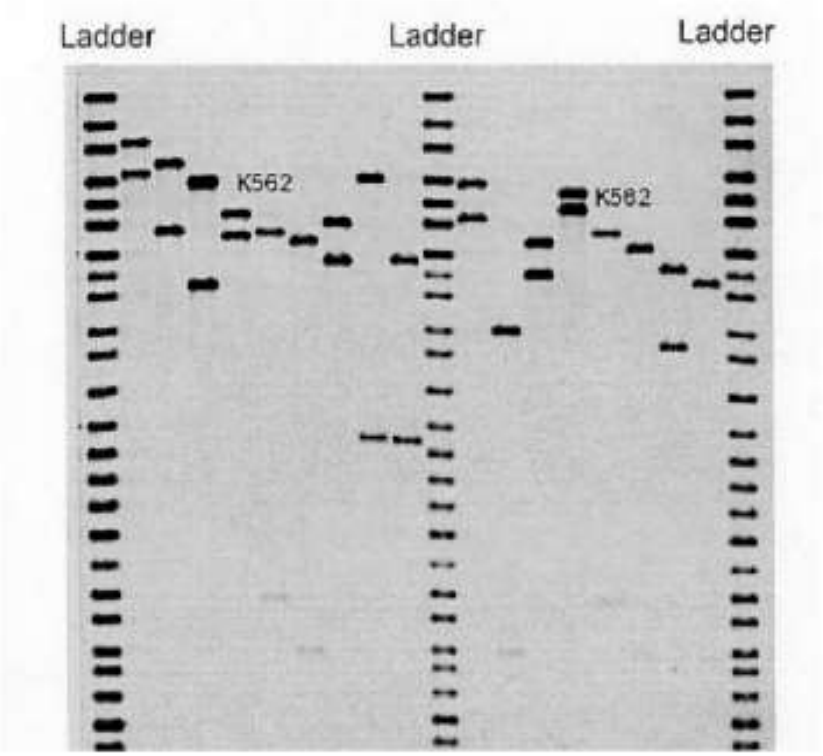

Figure 1.3 VNTR analysis using a single locus probe: ladders were run alongside the tested samples that allowed the size of the DNA fragments to be estimated. A control sample labelled K562 is analysed along with the tested samples

Kary Mullis was awarded the Nobel Prize for Chemistry in 1993. The PCR increased the sensitivity of DNA analysis to the point where DNA profiles could be generated from just a few cells, reduced the time required to produce a profile, could be used with degraded DNA and allowed just about any polymorphism in the genome to be analysed. The first application of PCR in a forensic case involved the analysis of single nucleotide polymorphisms in the DQα locus. This was soon followed by the analysis of short tandem repeats (STRs) which are currently the most commonly used genetic markers in forensic science. The rapid development of technology for analysing DNA includes advances in DNA extraction and quantification methodology, the development of commercial PCR based typing kits and equipment for detecting DNA polymorphisms. In addition to technical advances, another important part of the development of DNA profiling that has had an impact on the whole field of forensic science is quality control.

Application of genetic engineering

Lec.10

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