BIYANI GIRLS COLLEGE
First Internal Examination (2019-20)
M.Sc. Final Year (BT)
Paper-Genetic Engineering

SET-A

Time: 1:30 Hours **MM-30**

Attempt all the questions

1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**
   i. Define genetically modified organisms?

Ans. Genetically modified organisms (GMOs) are living organisms whose genetic material has been artificially manipulated in a laboratory through genetic engineering. This creates combinations of plant, animal, bacteria, and virus genes that do not occur in nature or through traditional crossbreeding methods.

   ii. Give any 2 applications of genetic engineering?

Ans. 1. In medicine, genetic engineering has been used to mass-produce insulin, human growth hormones, follistim (for treating infertility), human albumin, monoclonal antibodies, antihemophilic factors, vaccines, and many other drugs.

2. In research, organisms are genetically engineered to discover the functions of certain genes.
   iii. What is restriction modification system?

Ans. 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.

As the sequences recognized by the restriction enzymes are very short, the bacterium itself will almost certainly contain some within its genome. In order to prevent destruction of its own DNA by the restriction enzymes, methyl groups are added. These modifications must not interfere with the DNA base-pairing, and therefore, usually only a few specific bases are modified on each strand.

iv. What is Taq polymerase?

Ans. Taq polymerase is a thermostable DNA polymerase I named after the thermophilic bacterium Thermus aquaticus from which it was originally isolated by Chien et al. in 1976. Its name is often abbreviated to Taq Pol or simply Taq.

v. Define Host cell?

Ans. A host is the organism that is modified in a genetic engineering experiment. The vector is the vehicle used to transfer genetic material into a host organism.

vi. Define phagemid?

Ans. A phagemid is a plasmid that contains an f1 origin of replication from an f1 phage. It can be used as a type of cloning vector in combination with filamentous phage M13. A phagemid can be replicated as a plasmid, and also be packaged as single stranded DNA in viral particles.

vii. What do you understand by tools of genetic engineering?

Ans. Genetic engineering is the process of using gene cloning and other genetic manipulations to achieve isolate specific genes and use it for research and other purposes.

Important Molecular Tools In Genetic Engineering

1. Polymerase Chain Reaction (PCR)
2. Restriction Enzymes (Molecular Scissor)
3. Gel Electrophoresis
4. DNA Ligase

5. Plasmids
6. Transformation/Transduction
7. Identifying Transgenic Organisms.

viii. What are the properties of a good vector?

Ans. The most basic characteristics of vectors are:

1. The vector needs to be a DNA molecule so that it can be cloned with the gene of interest.
2. The vector needs to have unique restriction sites. If it possesses too many restriction sites then it would be fragmented into several pieces.
3. The vector needs to have a selectable marker. The selectable markers are required to screen out transformants, non-transformants, recombinants, and non-recombinants from the colonies. Example: tetRtetR, ampRampR, auxotrophic mutants etc.
4. The vector should have Ori site from where replication can begin.
5. The vector should be able to replicate into the host system and have a high copy number. The main motto of the cloning is to get high copy number.

3. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. Explain about λ phage based vectors?

Ans. Enterobacteria phage λ (lambda phage, coliphage λ) is a bacterial virus, or bacteriophage, that infects the bacterial species Escherichia coli. This virus is temperate and may reside within the genome of its host through lysogeny.

Lambda phage consists of a virus particle including a head (also known as a capsid), tail and tail fibers. The head contains the phage's double-stranded circular DNA genome. The phage particle recognizes and binds to its host, E. coli, causing DNA in the head of the phage to be ejected through the tail into the cytoplasm of the bacterial cell. Usually, a "lytic cycle" ensues, where the lambda DNA is replicated many times and the genes for head, tail and lysis proteins are expressed. This leads to assembly of multiple new phage particles within the cell and subsequent

cell lysis, releasing the cell contents, including virions that have been assembled, into the environment. However, under certain conditions, the phage DNA may integrate itself into the host cell chromosome in the lysogenic pathway. In this state, the λ DNA is called a prophage and stays resident within the host's genome without apparent harm to the host. The host can be termed a lysogen when a prophage is present.

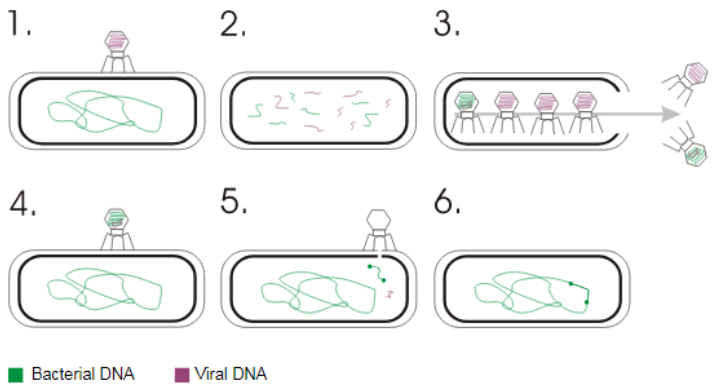
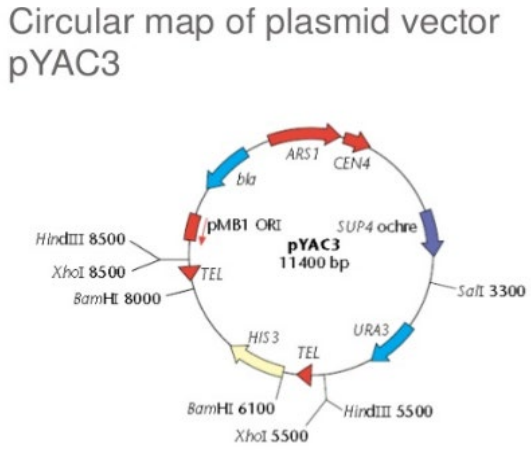


Figure: **Transduction**: Transduction is the process by which DNA is transferred from one bacterium to another by a virus.It also refers to the process whereby foreign DNA is introduced into another cell via a viral vector.

ii. Describe about Yeast Artificial Chromosomes (YAC)?

Ans. Yeast artificial chromosomes (YACs) are the products of a recombinant DNA cloning methodology to isolate and propagate very large segments of DNA in a yeast host (Burke et al., 1987). Yeast artificial chromosomes (YACs) provide the largest insert capacity of any cloning system. This system, developed by Burke and Olson in 1987, supports the propagation of exogenous DNA segments hundreds of kilobases in length. YACs representing contiguous stretches of genomic DNA (YAC contigs) have provided a physical map framework for the human, mouse, and even *Arabidopsis* genomes.



The YAC vector itself provides the essential elements for propagation of DNA as a chromosome in the yeast *Saccharomyces cerevisiae*. These elements include a yeast centromere, two functional telomeres, and auxotrophic markers for selection of the YAC in an appropriate yeast host. A problem encountered in constructing and using YAC libraries is that they typically

contain clones that are chimeric, i.e., contain DNA in a single clone from different locations in the genome.

4. **Long Answer Questions (7 Marks each 2x7= 14 Marks)**

   i. Comments on following:

   a) Restriction Enzymes **4**

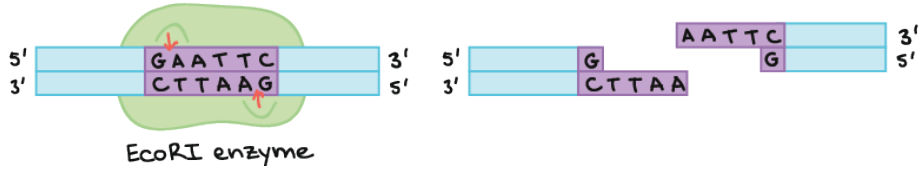
Ans. Restriction enzymes are found in bacteria (and other prokaryotes). They recognize and bind to specific sequences of DNA, called restriction sites. Each restriction enzyme recognizes just one or a few restriction sites. When it finds its target sequence, a restriction enzyme will make a double-stranded cut in the DNA molecule. Typically, the cut is at or near the restriction site and occurs in a tidy, predictable pattern.

As an example of how a restriction enzyme recognizes and cuts at a DNA sequence, let's consider *Eco*RI, a common restriction enzyme used in labs. *Eco*RI cuts at the following site:

5'-...GAATTC...-3' 3'-...CTTAAG...-5'

EcoRI site

When EcoRI recognizes and cuts this site, it always does so in a very specific pattern that produces ends with single-stranded DNA "overhangs":



An *Eco*RI enzyme binds to an *Eco*RI site in a piece of DNA and makes a cut on both strands of the DNA. The pattern of the cut is:

5'-...G|AATTC...-3' 3'-...CTTAA|G...-5'

Thus, it produces an overhang of 5'-AATT-3' on each end of the cut DNA.

If another piece of DNA has matching overhangs (for instance, because it has also been cut by EcoRI), the overhangs can stick together by complementary base pairing. For this reason, enzymes that leave single-stranded overhangs are said to produce **sticky ends**. Sticky ends are helpful in cloning because they hold two pieces of DNA together so they can be linked by DNA ligase.

b) Linkers and Adaptors **3**

Ans. Linkers and adaptors are the DNA molecules which help in the modifications of cut ends of DNA fragments. These can be joined to the cut ends and hence produce modifications as desired.

Both are short, chemically synthesized, double stranded DNA sequences. Linkers have (within them) one or more restriction endonuclease sites and adaptors have one or both sticky ends. Different types of linkers and adaptors are used for different purposes.

Linkers contain target sites for the action of one or more restriction enzymes. They can be ligated to the blunt ends of foreign DNA or vector DNA (Fig. 5a). Then they undergo a treatment with a specific restriction endonuclease to produce cohesive ends of DNA fragments EcoRI-linker is a common example of frequently used linkers.

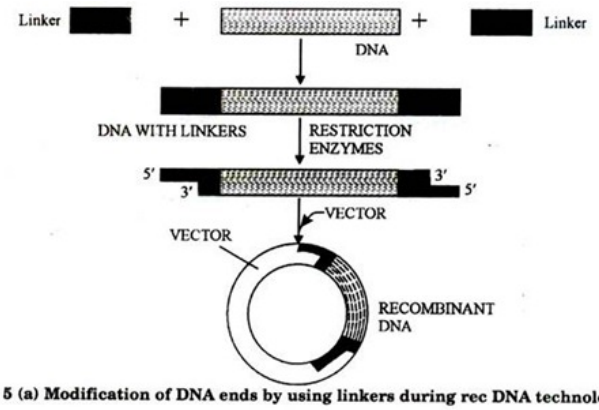


Fig. 5 (a) Modification of DNA ends by using linkers during rec DNA technology.

Adaptors are the chemically Synthesized molecules which have pre-formed cohesive ends (Fig. 5b). Adaptors are employed for end modification in cases where the recognition site for restriction endonuclease enzyme is present within the foreign DNA.

The foreign DNA is ligated with adaptor on both ends. This new molecule, so formed, is then phosphorylated at the 5′-terminii. Finally foreign DNA modified with adaptors is integrated into the vector DNA to form the recombinant DNA molecule.

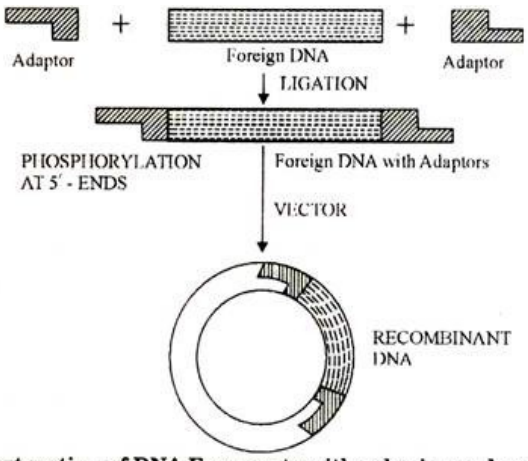


Fig. 5. (b). Construction of DNA Fragments with cohesive ends using Adaptors

ii. Explain about the Agrobacterium mediated gene transfer mechanism?

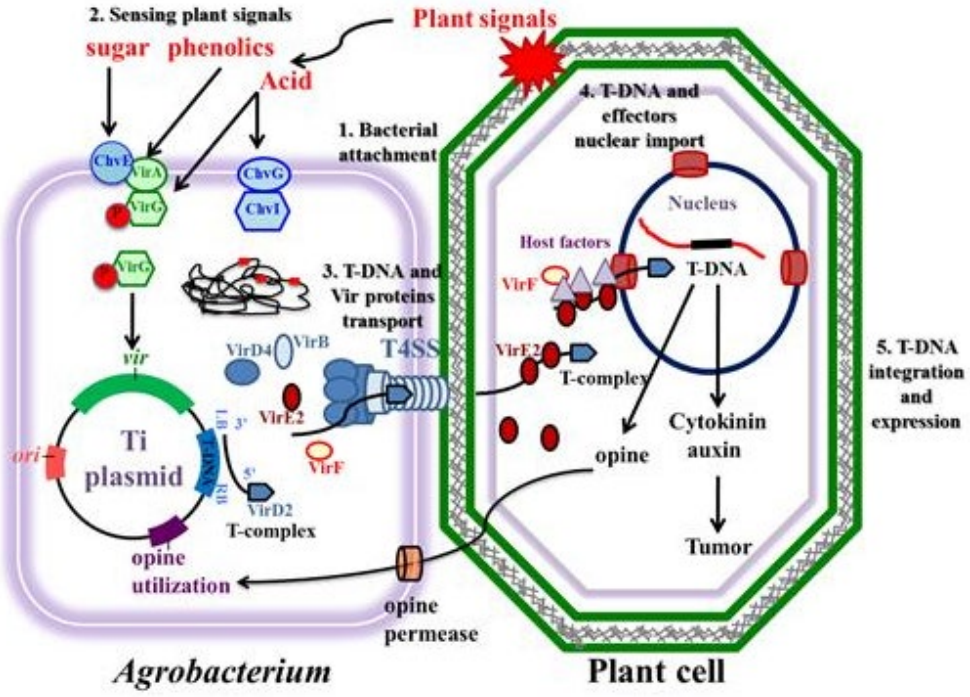
Ans. The A. tumefaciens-mediated plant genetic transformation process requires the presence of two genetic components located on the bacterial Ti-plasmid. The first essential component is the T-DNA, defined by conserved 25-base pair imperfect repeats at the ends of the T-region called border sequences. The second is the virulence (vir) region, which is composed of at least seven major loci (virA, virB, virC, virD, virE, virF, and virG) encoding components of the bacterial protein machinery mediating T-DNA processing and transfer. The VirA and VirG proteins are two-component regulators that activate the expression of other vir genes on the Ti-plasmid. The VirB, VirC, VirD, VirE and perhaps VirF are involved in the processing, transfer, and integration of the T-DNA from A. tumefaciens into a plant cell. Figure 1 shows the major steps of the Agrobacterium-mediated plant transformation process. The current knowledge and recent findings regarding the key events of Agrobacterium-mediated plant transformation process are reviewed in the following sections. Table 1 summarizes the major transformation steps and known plant factors participating in these steps.

Major steps of the *Agrobacterium tumefaciens*-mediated plant transformation process.

(1) Attachment of *A. tumefaciens* to the plant cells. (2) Sensing plant signals by *A. tumefaciens* and regulation of virulence genes in bacteria following transduction of the sensed signals. (3) Generation and transport of T-DNA and virulence proteins from the bacterial cells

into plant cells. (4) Nuclear import of T-DNA and effector proteins in the plant cells. (5) T-DNA integration and expression in the plant genome.

Figure 1.



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Paper-Genetic Engineering

SET-B

Time: 1:30 Hours **MM-30**

Attempt all the questions

1. **Short Answers Questions (1 Marks Each; 1x8= 8 Marks)**

   i. Define Cosmids?

Ans. A cosmid is a type of hybrid plasmid that contains a Lambda phage cos sequence. Cosmids (cos sites + plasmid = cosmids) DNA sequences are originally from the lambda phage. They are often used as a cloning vector in genetic engineering. Cosmids can be used to build genomic libraries.

   ii. Define pBR322?

Ans. pBR322 is a plasmid and was one of the first widely used E. coli cloning vectors. Created in 1977 in the laboratory of Herbert Boyer at the University of California, San Francisco, it was named after Francisco Bolivar Zapata, the postdoctoral researcher who constructed it. pBR322 is 4361 base pairs in length and has two antibiotic resistance genes – the gene *bla* encoding the ampicillin resistance (Amp^R) protein, and the gene *tetA* encoding the tetracycline resistance (Tet^R) protein. It contains the origin of replication of pMB1, and the *rop* gene, which encodes a restrictor of plasmid copy number. The plasmid has unique restriction sites for more than forty restriction enzymes.

   iii. What is function of T4 polynucleotide kinase?

Ans. T4 Polynucleotide Kinase catalyzes the transfer of the γ-phosphate from ATP to the 5′-terminus of polynucleotides or to mononucleotides bearing a 5′-hydroxyl group. The enzyme

may be used to phosphorylate RNA, DNA and synthetic oligonucleotides prior to subsequent manipulations such as ligation and cloning.

iv. Define Reverse Transcription?

Ans. The reverse of normal transcription, occurring in some RNA viruses, in which a sequence of nucleotides is copied from an RNA template during the synthesis of a molecule of DNA. he enzyme that makes the DNA copy is called **reverse transcriptase** and is found in retroviruses, such as the human immunodeficiency virus (HIV).

v. Define BAC?

Ans. Bacterial Artificial Chromosome - A bacterial artificial chromosome is a DNA construct, based on a functional fertility plasmid, used for transforming and cloning in bacteria, usually E. coli. F-plasmids play a crucial role because they contain partition genes that promote the even distribution of plasmids after bacterial cell division.

vi. What vector is used for gene sequencing purpose and why?

Ans. M-13 phage DNA is used for gene sequencing purpose because it is a single stranded DNA sequence so one strand of target gene can be inserted in it and can be sequenced easily.

vii. What is the role of DNA polymerase in genetic engineering?

Ans. DNA polymerase is a specific class of enzyme found in all living organisms. Its main purpose is to replicate DNA and to help in the repair and maintenance of DNA. The enzyme is critical to the transmission of genetic information from generation to generation.

viii. What are the properties of a good host cell?

Ans. The following points highlight the seven main characteristics of a good host cell.

1. Can allow the easy entry of the recombinant DNA easily into the cell.

2. Should not destroy the recombinant DNA as a foreign DNA and degrade it.

3. Can stably maintain the recombinant DNA.

4. The transformed host must not independently sustain outside the laboratory.

5. Should be easy to maintain and handle.

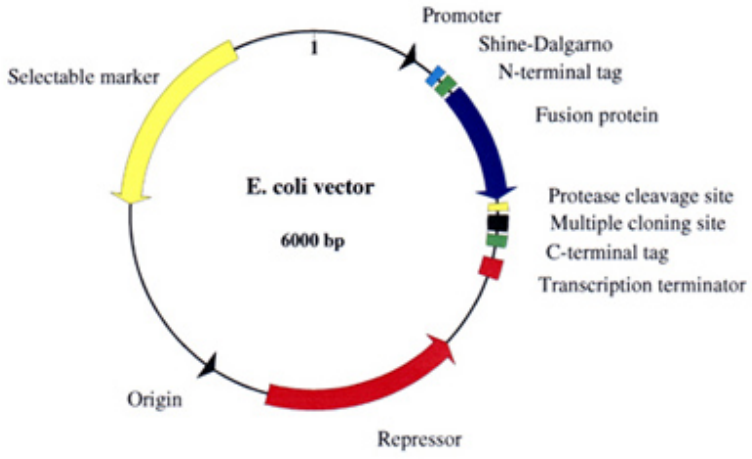
6. Should be available as a wide variety of genetically defined strains.

7. Should accept a range of vectors.

2. Medium Answer Questions (4 Marks each 2x4= 8 Marks)

i. Explain about E.coli based vectors?

Ans. The basic architecture of an E. coli expression vector is shown in the figure below and contains the following features:



There are many types of cloning vectors, but the most commonly used ones are genetically engineered plasmids. Cloning is generally first performed using *Escherichia coli*, and cloning vectors in *E. coli* include plasmids, bacteriophages (such as phage λ), cosmids, and bacterial artificial chromosomes (BACs). Some DNA, however, cannot be stably maintained in *E. coli*, for example very large DNA fragments, and other organisms such as yeast may be used. Cloning vectors in yeast include yeast artificial chromosomes (YACs).

Plasmids are autonomously replicating circular extra-chromosomal DNA. They are the standard cloning vectors and the ones most commonly used. Most general plasmids may be used to clone DNA insert of up to 15 kb in size. One of the earliest commonly used cloning vectors is the pBR322 plasmid. Other cloning vectors include the pUC series of plasmids, and a large

number of different cloning plasmid vectors are available. Many plasmids have high copy number, for example pUC19 which has a copy number of 500-700 copies per cell, and high copy number is useful as it produces greater yield of recombinant plasmid for subsequent manipulation. However low-copy-number plasmids may be preferably used in certain circumstances, for example, when the protein from the cloned gene is toxic to the cells.

ii. Describe about in vitro packaging of λ phage vectors?

Ans. In vitro packaging:

Lambda vectors are one of the most commonly used vectors nowadays. The packaging of the lambda DNA having the gene of interest ligated can be transferred into the phage head artificially i.e. in vitro packaging. This is carried out in two different ways: Single-strain packaging system and Two-strain packaging system.

Single-strain packaging system:

The researchers have developed certain strains of the lambda phage with defective cos-site gene and infected with the E. coli strain.

Cos-sites are a particular sequence which is identified by the gene A endonuclease. The phage DNA in the cytosol of the bacteria is circular and replicates by rolling circle mechanism. In this mechanism, the new linear DNA is continuously displaced which consist of cos-sites. The cos sites are the sequences where the gene A endonuclease (different bacteriophage may have different gene coading for endonuclease) cleave. This would separate the catenae phage DNA. Hence, defective cos sites would prevent the packaging of the phage DNA into the phage head.

The proteins which are produced are isolated from the culture and added to the tube which consists of recombinant lambda DNA. The assembly process of proteins and DNA to form new phage is performed in vitro. As only one strain of the bacteriophage was used it is called single strained packaging system.

Two-strain packaging system:

In this method, we use two different strains of lambda. One lambda DNA has defective genes coding for defective proteins protein D & A and the other lambda DNA has mutated protein E.

(i) The E protein, which is the major component of the phage head; mutants that are defective in this gene are unable to assemble the preheads, and therefore accumulate the other, unassembled, components of the phage particle as well as the other proteins involved in packaging.

(ii) The D and A proteins, which are involved in the packaging process itself. Mutants that are defective in these genes are able to produce the preheads, but will not package DNA. This results in the accumulation of empty preheads.

This would prevent the assembly of the phage in the bacterial system. Protein extracts from both the culture are isolated and mixed together with the recombinant phage DNA. Mixing these two extracts allows in vitro complementation and results in the ability to assemble mature phage particles. Thus, the former lambda phage would provide a functional protein B and the later phage would provide a functional protein A. Hence, the assembly of the protein coat and the recombinant phage DNA into the system takes place.

3. **Long Answer Questions (7 Marks each 2x7= 14 Marks)**
    i. Give a detail note on tools of genetic engineering?

Ans. Important Molecular Tools In Genetic Engineering

Here is a list of a genetic engineer's molecular tools/enzymes most commonly used in genetic engineering experiments:

1. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction (PCR) is the process of replicating multiple copies of the genes of interest. The discovery of thermostable DNA polymerases, such as *Taq* Polymerase, has made it possible to manipulate DNA replication in the laboratory. It amplifies the quantities of DNA segments. Primers are used to identify the gene of interest and replicate them. These copies can then be separated and purified using gel electrophoresis.

2. Restriction Enzymes (Molecular Scissor)

The discovery of enzymes known as restriction endonucleases has been essential to protein engineering. Based on the nucleotide sequence, these enzymes cut DNA at specific locations.

DNA cut with a restriction enzyme produces many smaller fragments, of varying sizes. These can be separated using gel electrophoresis or chromatography.

3. Gel Electrophoresis

Purifying DNA from a cell culture, or cutting it using restriction enzymes wouldn't be of much use if we couldn't visualize the DNA. Gel electrophoresis helps visualize the size and type of DNA extracted using PCR and restriction enzymes. It is also used to detect DNA inserts and knockouts.

4. DNA Ligase

DNA ligase can create covalent bonds between nucleotide chains. This is done to create recombinant strands, or close a circular strand that has been cut by restriction enzymes. The enzymes DNA polymerase I and polynucleotide kinase are also important for filling in gaps or phosphorylating the 5′ ends, respectively.

5. Plasmids

Plasmids are small, circular pieces of DNA that are not part of the bacterial genome, but are capable of self-replication. It is used as vectors to transport genes between microorganisms. Once the gene of interest has been amplified with PCR, the gene and plasmid are cut by restriction enzymes and ligated together. The resulting combination is known as arecombinant DNA. Viral (bacteriophage) DNA can also be used as a vector, as can cosmids which are recombinant plasmids containing bacteriophage genes.

6. Transformation/Transduction

Transformation is the process of transferring genetic material in a vector, such as a plasmid, into host cells. The host cells are exposed to an environmental change, such as electroporation, which makes them "competent" or temporarily permeable to the vector. The larger the plasmid, the lower the efficiency with which it is taken up by cells.

Larger DNA segments are more easily cloned using bacteriophage, retrovirus or other viral vectors or cosmids in a method called **Transduction**. Phage or viral vectors are often used in

regenerative medicine but may cause insertion of DNA in parts of our chromosomes where we don't want it, causing complications and even cancer.

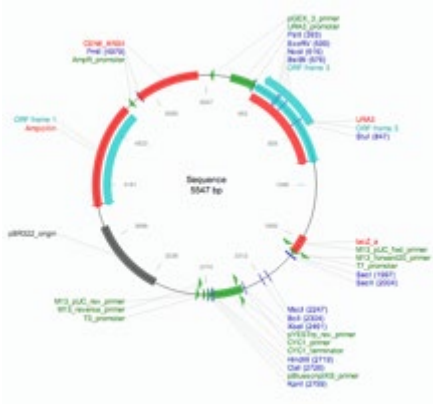
7. Identifying Transgenic Organisms

Not all cells will take up DNA during transformation. Therefore, it is essential to identify the cells that undergone transformation and those that have not. Generally, plasmids carry genes for antibiotic resistance and transgenic cells can be selected based on expression of those genes and their ability to grow on media containing that antibiotic. Alternative methods of selection depend on the presence of other reporter proteins such as the x-gal/*lacZ* system, or green fluorescence protein, which allow selection based on color and fluorescence, respectively.

ii. Explain about the yeast based vectors in detail and why yeast DNA is beneficial as vector with compare to bacterial & viral DNA?

Ans. Yeast are eukaryotes and thus contain complex internal cell structures similar to those of plants and animals. Unlike bacteria, yeast can post-translationally modify proteins yet they still share many of the same technical advantages that come with working with prokaryotes. This includes but is not limited to: rapid growth, ease of replica plating and mutant isolation, a well-defined genetic system, and a highly versatile DNA transformation system. Unlike most other microorganisms, yeast have both a stable haploid and diploid state which is useful for genetic analysis, as well as an efficient mechanism of homologous recombination to facilitate simple gene replacement/mutation. Yeast expression plasmids used in the lab typically contain all the necessary components to allow shuttling between *E. coli* and yeast cells. To be useful in the lab, the vectors must contain a yeast-specific origin of replication (ORI) and a means of selection in yeast cells, in addition to the bacterial ORI and antibiotic selection markers.

The specific ORI elements included within a yeast vector determine how the plasmid is replicated and maintained within the yeast cell. These elements control not only the number of plasmids found in each cell, but also whether the plasmid gets integrated into the host DNA or is independently replicated as an episome. *The four main types of yeast plasmids are defined below*:



- ***Yeast Integrating plasmids (YIp)***: These plasmids lack an ORI and must be integrated directly into the host chromosome via homologous recombination.

- ***Yeast Replicating plasmids (YRp)***: These vectors contain an Autonomously Replicating Sequence (ARS) derived from the yeast chromosome. As the name suggests, these vectors can replicate independently of the yeast chromosome; however, they tend to be unstable and may be lost during budding.

- ***Yeast Centromere plasmids (YCp)***: These are considered low copy vectors and incorporate part of an ARS along with part of a centromere sequence (CEN). These vectors replicate as though they are small independent chromosomes and are thus typically found as a single copy. Unlike the ARS vectors, CEN vectors are stable without integration.

- ***Yeast Episomal plasmids (YEp)***: These are most similar to bacterial plasmids and are considered "high copy". A fragment from the 2 micron circle (a natural yeast plasmid) allows for 50+ copies to stably propogate per cell. The copy number of these vectors can also be controlled if specific regulatable elements are included.

Plasmids for use in *S. pombe*, on the other hand, do not require a well defined ORI. Instead, the size and A-T content of the DNA (apparently independent of a known specific sequence) dictate the replication of these vectors. *S. pombe* plasmids oftentimes utilize an ARS to aid in high transformation efficiency; however, this region does not necessarily promote replication.

Selection markers for yeast

Historically, scientists have utilized auxotrophic selection rather than antibiotic selection when working with yeast, due to high rates of spontaneously occuring resistant mutants and the

insensitivity of yeast strains to some antibiotics. Auxotrophy is defined as the inability of an organism to synthesize a particular organic compound required for its growth. Many auxotrophic strains of yeast exist which can be easily maintained when grown on media containing the missing nutrient. Scientists can exploit these host mutations by including a copy of a functional gene which complements the host's auxotrophy. When grown on media NOT containing the nutrient, the host cells will die unless they have incorporated the plasmid carrying the required gene.

The table below lists some of the most commonly used selection markers in yeast and provides the element needed to overcome the auxotrophy as well as additional uses for said element. This link provides a more extensive list of yeast auxotrophic markers and includes the associated references.

Considerations when using auxotrophic selection

Of course, there are some drawbacks to using auxotrophic markers as a means of selection:

1. A specific selection marker needs to be used with a yeast strain deficient in that compound. Therefore known auxotrophic strain/ selection element pairs must be utilized or a new combination needs to be created in advance of the experiment.

2. The marker provided by the plasmid may be expressed at higher than normal physiological levels due to high copy numbers. This creates a potential metabolic burden on the yeast cells.

3. Some phenotypes may be altered due to the presence of the selection marker at non-physiological levels.

Scientists have tried varied approaches to combat these issues. One method to reduce the amount of marker gene expression is to use a partially defective promoter to drive expression of the selection marker. This reduces the amount of gene product present in the cell, thus allowing the yeast to maintain higher copy numbers. Additionally, improvements in antibiotic selection have made utilizing the more traditional drug selection methods feasible in yeast as a complement or alternative to using auxotrophic markers.