Cloning Vectors

UNIVERSITY OF DELHI

Lesson developed under NME-ICT

Course: Botany

Subject: Plant Biotechnology

National Coordinator: Prof. S.C. Bhatla

Lesson: Cloning Vectors

Lesson Developer: Dr. Vibha G. Checker

College / Department: Botany Department, Kirori Mal College, University of Delhi

Reviewer: Prof. Suresh Chand, Professor and Head, School of Life Sciences, Devi Ahilya Vishwavidyalaya, Indore.

Language editor: Ms. Manisha Sharma

Department: Department of Genetics

Editor: Dr Rama Sisodia

Institute of Lifelong Learning, University of Delhi

Cloning Vectors

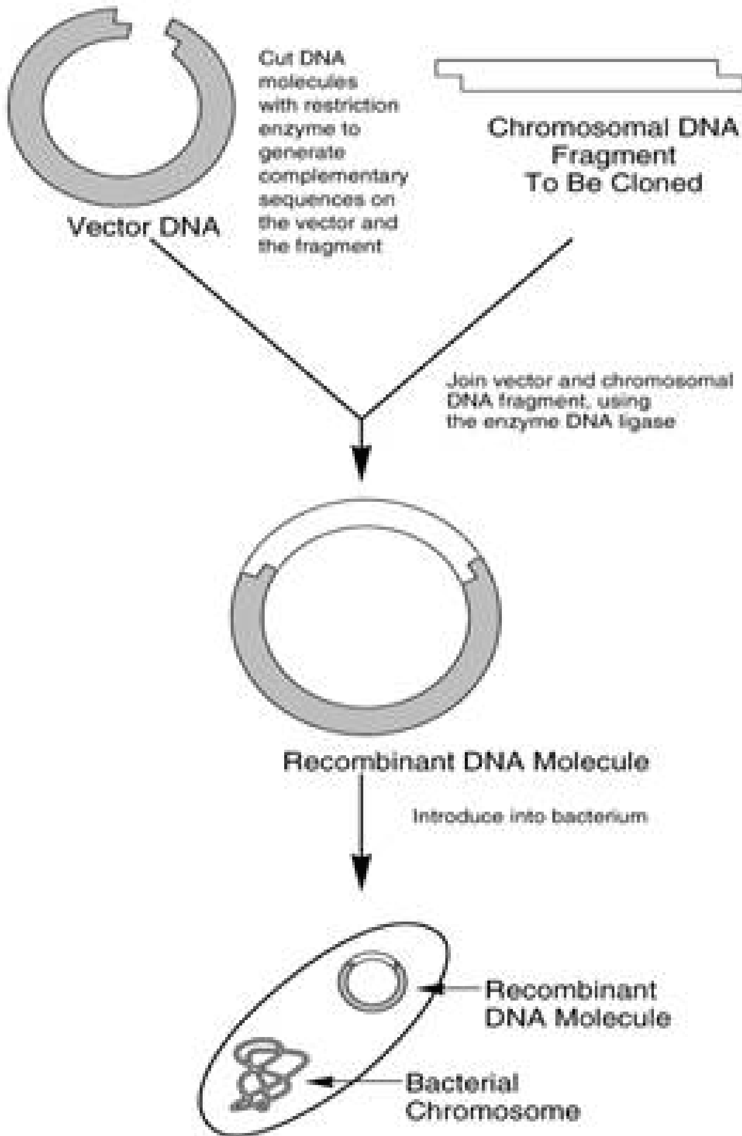
Table of Contents

Chapter – Cloning Vectors

- Introduction
  - Plasmids as Cloning Vectors
    - ✓ Purpose build plasmid vectors: pBR322 and pUC series
      - pBR322
      - pUC series
    - ✓ Ti Plasmid
    - ✓ Bacterial Artificial Chromosomes (BACs)
  - Bacteriophages as vectors
    - ✓ Lambda (λ) Phage
    - λ Insertion vectors
    - λ Replacement or Substitution vectors
    - ✓ M13 Phage
  - Phagemids/ Phasmids
  - Cosmids
  - Shuttle vectors
  - Yeast artificial chromosome
  - P1 artificial chromosome
  - Mouse artificial chromosome
  - Human artificial chromosome
  - Expression vectors
- Summary
- Exercise
- Glossary
- References
- Links for animations

Introduction

Gene manipulation has revolutionized research. 'Vector'in essence refers to a delivery agent/vehicle/carrier. Cloning vector is a small molecule of DNA that can carry foreign DNA into ahost cell and is capable of self-replication.



Source: http://genomicscience.energy.gov.

Different types of cloning vectors are available, each vector designed to perform a specific function.

Choice of the right cloning vector is an important aspect of cloning. There are certain essential properties which a cloning vector must possess:

Cloning Vectors

1. It should be able to replicate itself and the DNA segment it carries independently.
2. It should be of small size, so that it is easy to manipulate.
3. It should be easy to recover from the host cell.
4. It should be easily transferred from one cell to another by simple methods.
5. It should contain several unique restriction enzyme sites. These sites are present only once and clustered in one locationand are known as multiple cloning sites or polylinker. Restriction enzyme site is cleaved with a restriction enzyme to open the cloning vector without disrupting any other region. Insert DNA fragment is digested with the same enzyme and ligated with the vector.
6. There should be easy ways to detect their presence in host organisms. Bacterial cloning plasmids carry an antibiotic resistance gene to distinguish the host cells that carry vectors from the host cells that do not contain vectors.
7. There should be easy ways to detect the presence of inserted DNA.

The first recombinant DNA
Video: Stanley Cohen and Herbert Boyer's historic experiment used techniques to cut and paste DNA to create the first custom-made organism containing recombined or "recombinant" DNA. Source: http://www.dnalc.org/view/15915-The-first-recombinant-DNA.html

Plasmids as Cloning Vectors

Animation: Plasmids as cloning vector

Source: http://www.dnalc.org/view/15476-Mechanism-of-Recombination-3D-animation-with-with-basic-narration.html

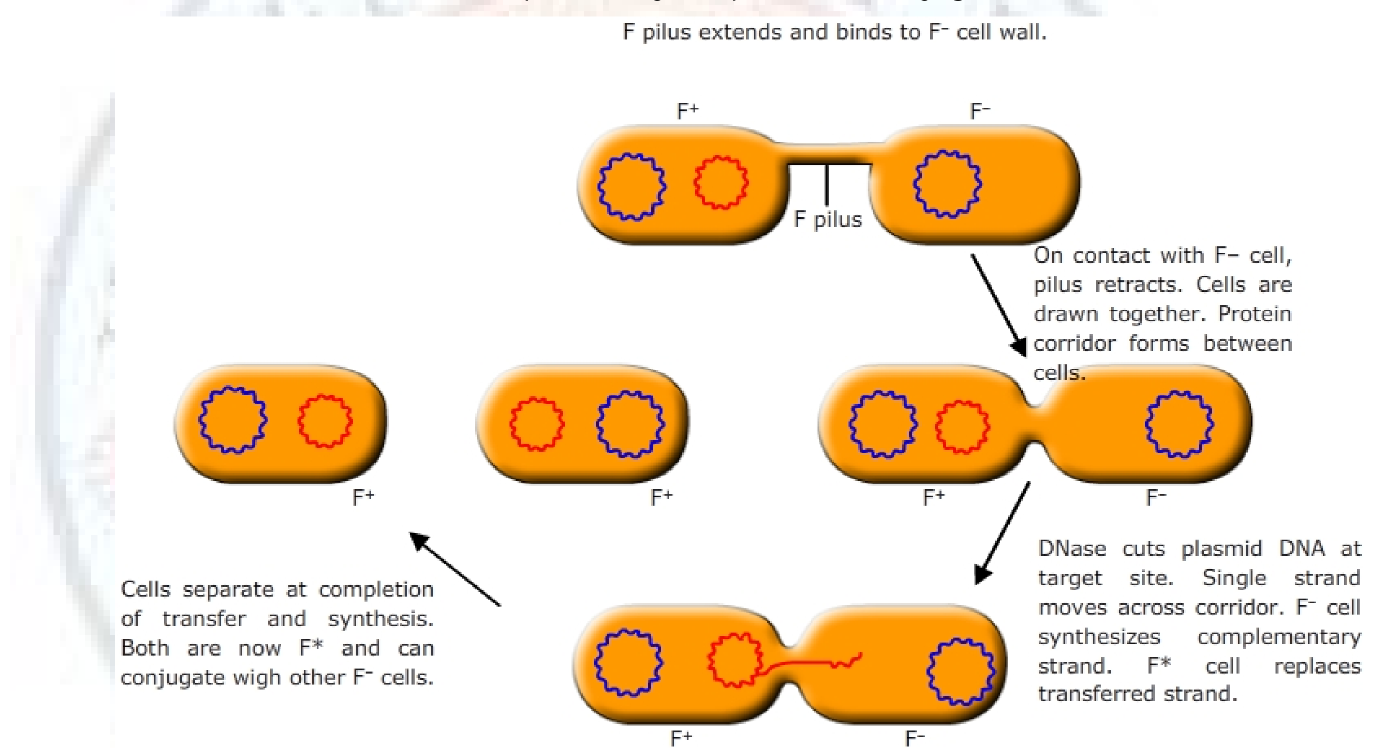
Source: http://upload.wikimedia.org/wikipedia/commons/thumb/c/cf/Plasmid_%28english%29.svg/1280px-Plasmid_%28english%29.svg.png

Cloning Vectors

Plasmids are circular double stranded 2.5 to 5 Kb long DNA molecules, present in bacteria that replicate autonomously from the host chromosome and are inherited in extra-chromosomal state from one generation to the other. They are widespread throughout the prokaryotes and are capable of carrying 15 Kbof foreign DNA. Most of the bacterial plasmids fulfill first four requirements of a cloning vector.

Five types of plasmids are:

1. Fertility plasmids or F plasmids – These are conjugative plasmids that allow F^+ bacterial cells that carry this plasmid to transfer the plasmid at a high frequency to F^- bacterial cells that lack this plasmid by the process of conjugation.



2. Resistance plasmids or R plasmids – These are also conjugative plasmids that contain genes for resistance against antibiotics, metal ions, ultraviolet radiation and bacteriophages.

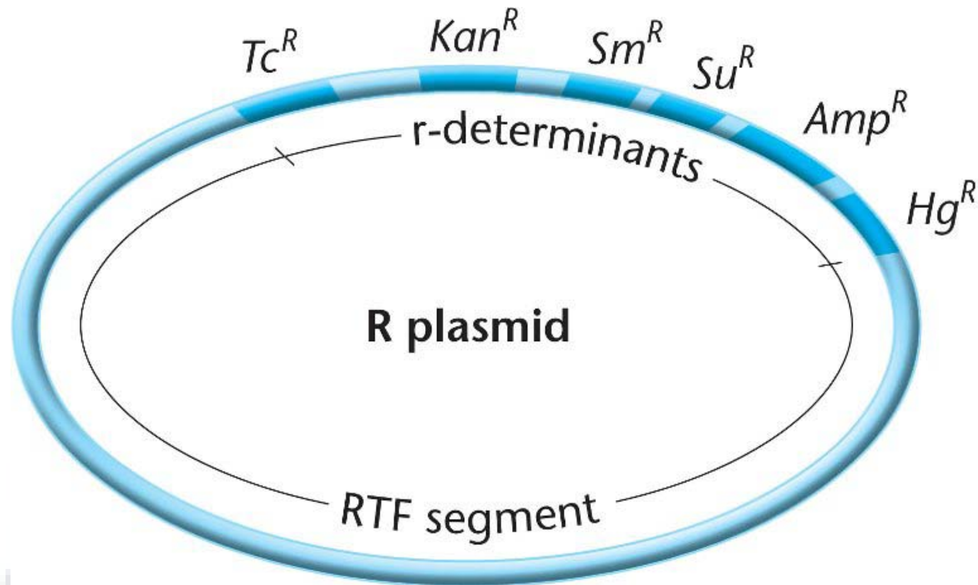


Figure: An **R plasmid** consists of a resistance transfer factor (**RTF**), which enables **conjugation**, and one or more **r-determinants**: genes conferring resistance to antibiotics.

The **r-determinants** in this diagram are Tc, tetracycline; Kan, kanamycin; Sm, streptomycin; Su, sulfonamide; Amp, ampicillin; and Hg, mercury.

Source: http://bio3400.nicerweb.com/Locked/media/ch06/plasmid.html

3. Col plasmids – These plasmids carry genes that code for bacteriocins, proteins required to kill other bacteria.
4. Degradative plasmids – These plasmids contain genes that degrade chemicals and aid in digestion of those toxins, such as toluene and salicylic acid.
5. Virulence plasmids – These plasmids are required by the bacterium for pathogenicity, the ability of the bacterium to cause disease in a host organism.

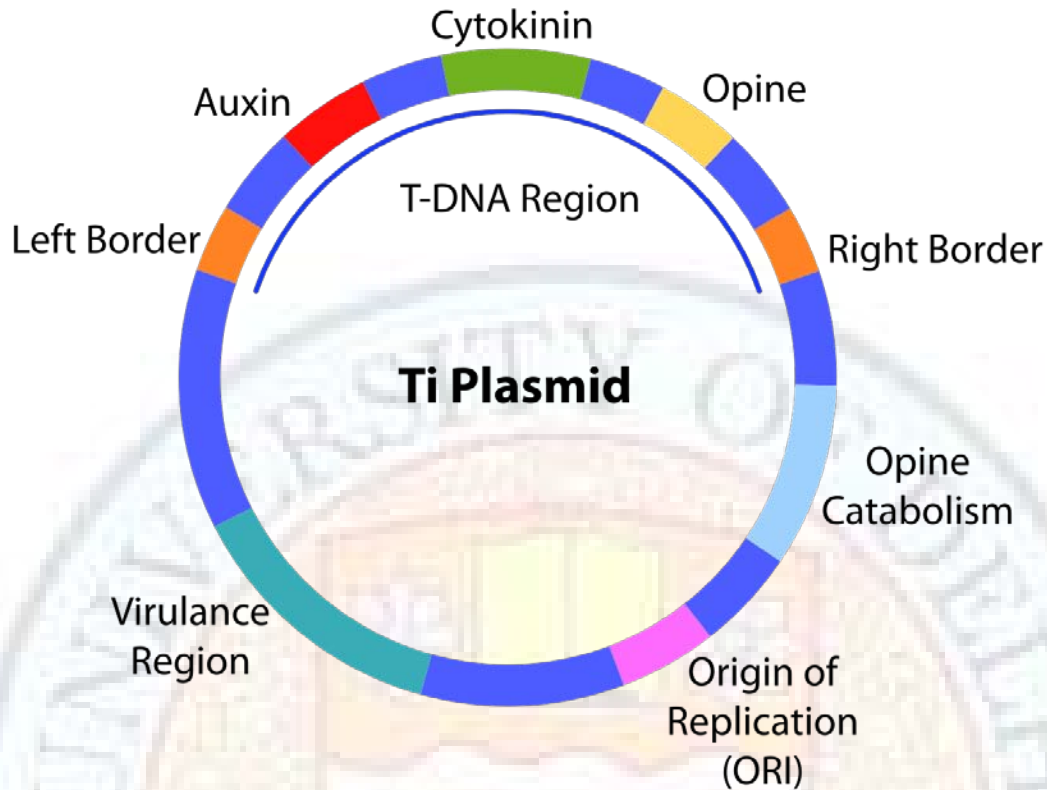


Figure: Ti plasmid contains virulence region that is responsible for its infectivity.

Source: http://upload.wikimedia.org/wikipedia/commons/thumb/d/d1/Ti_plasmid.svg/1280px-Ti_plasmid.svg.png

Earlier cloning experiments used natural *in vivo*plasmids, i.e.ColE1 and pSC101. ColE1 is a small circular plasmid known for production of 57kD toxic protein known as colicin E1. These natural plasmids are of large size, possess stringent replication control and lack marker genes. Further studies oncloning led to the construction of synthetic plasmids to meet other requirements of a vector and to enhance the delivery of gene of interest. The first successful plasmid was pSC101.

Basic properties of plasmids are:

1. Plasmids vary in their copy number in each bacterial cell. Some plasmids occur in just one/few copies and are known as low copy plasmids whereas others exist in multiple copies per cell and are known as high copy plasmids. The number of copies of plasmid in each bacterial cell depends on type of replicon present in it. Replicon contains essential regulatory elements and origin of replication or *ori*. Plasmids carrying pMB1 or colicin E1 (colE1) replicons are under stringent control and maintain low copies (15-20) in each

bacterial cell. pMB1 or colE1 replicons have been modified extensively over the years to increase their copy number. These high copy plasmids like pBR322 are more useful for cloning purposes as higher yield of insert DNA can be obtained from higher amount of plasmid DNA. Low copy plasmids like pSC101 are typically used when genes toxic to the host bacteria are cloned as higher copies will be lethal. They are also used for construction of bacterial artificial chromosome (BAC).

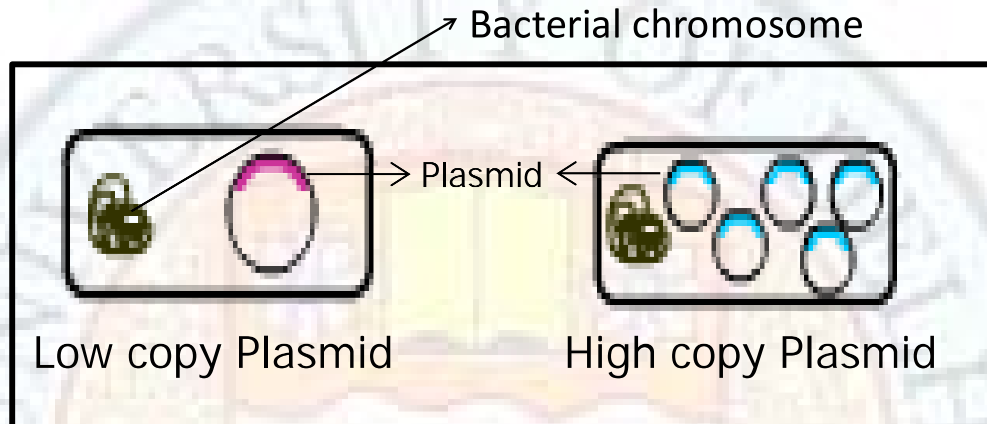


Figure: Diagrammatic representation of bacterial cells showing high and low copy number of plasmids.
Source: Author

2. Some plasmids have narrow host range as they can replicate in restricted number of bacterial species, for e.g. pBR322, pUC18 are restricted to *E. coli* and closely related species only. Some plasmids can replicate in large number of bacterial species and are known as broad range plasmids for e.g. IncP of *E.coli*. These broad range plasmids are a useful tool in bacterial mapping studies.

3.Plasmids can be introduced easily into bacterial cells by a process called transformation. Transformed bacteria are grown on agar plate and bacterial colonies derived from individual cells that have taken up recombinant plasmid are grown. Transformation is generally inefficient as plasmids are stably established in small population of cells only. These plasmids also carry selectable markers which allow rare transformants to be selected with ease.

Animation: DNA transformation

Source: http://www.dnalc.org/view/15916-DNA-transformation.html

4. Plasmids often carry genes that confer antibiotic resistance to the host organism. The antibioticresistance genes encoded by plasmid DNA is an advantageous traitand used inthe construction of vectors. These genes providean easy means of selecting cells containing the plasmid. By growing colonies on agar plate containing antibiotic it is possible to isolate bacteria that have taken up plasmid during transformation as only resistant bacteria will be able to grow.These markers confer the cells containing vector, the ability to grow in presence of antibiotics (like ampicillin, kanamycin, tetracycline etc.). Bacteria carrying parental plasmid are unable to form colonies under selective conditions. For example, if the cloning vector carries a kanamycin resistance gene (kan^r), it will confer resistance to the *E. coli* cells against kanamycin if the transformation process was successful. Therefore it is possible to isolate bacteria that have taken up plasmid during transformation as only resistant bacteria will be able to grow.

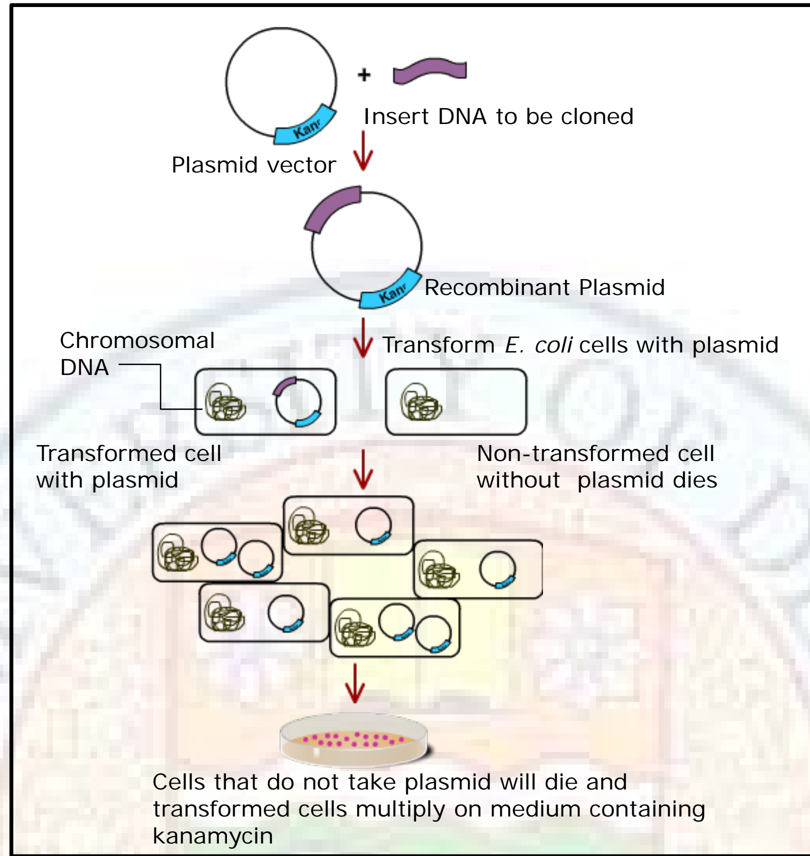


Figure: Antibiotic selection gene in plasmid provides an easy means of selecting cells containing the plasmid.

Source: Author

5. Most plasmid vectors contain multiple cloning sites (MCS) or polylinker.This allows the investigator to cleave the plasmid at one place without disrupting plasmid's replication genes. Multiple cloning sites are sometimes present in between a gene which becomes non-functional when a DNA insert is cloned into the vector. Insertional inactivation of an antibiotic resistance gene permits simple selection of the desired clone. In some cases, plasmid vectors carry two different antibiotic resistance genes. Foreign DNA is cloned into one of the antibiotic resistance gene. Recombinant host cells will be sensitive to one antibiotic and resistant to the other.

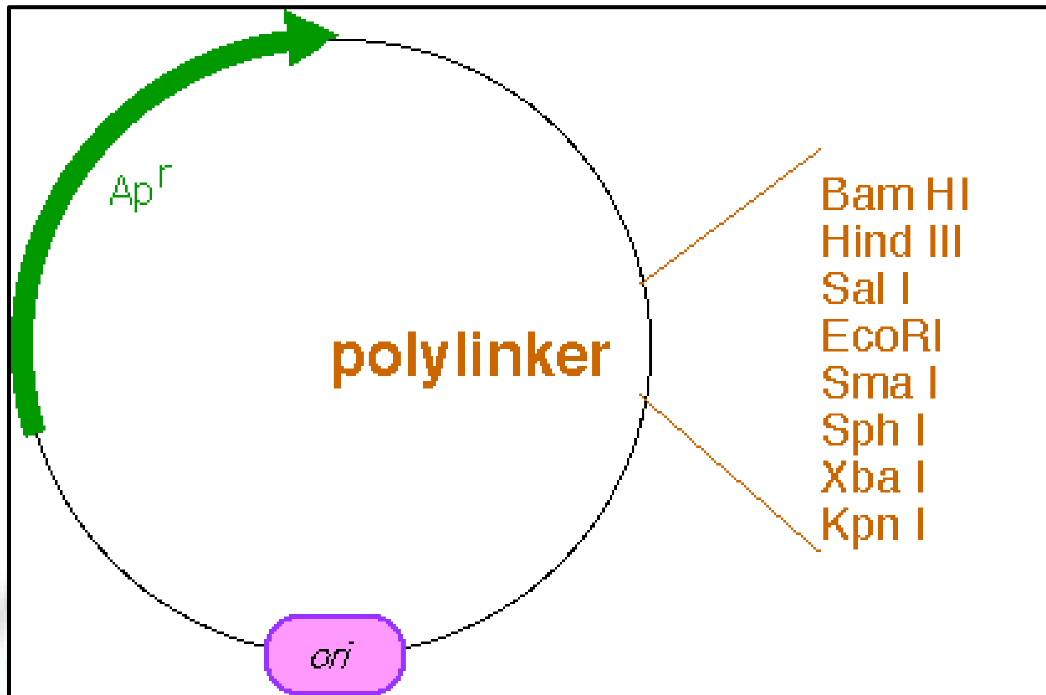


Figure: Diagrammatic representation of a plasmid vector showing origin of replication (ori), selectable marker (Ap^r) and a polylinker.

Source: http://www.uic.edu/classes/phar/phar331/lecture6/

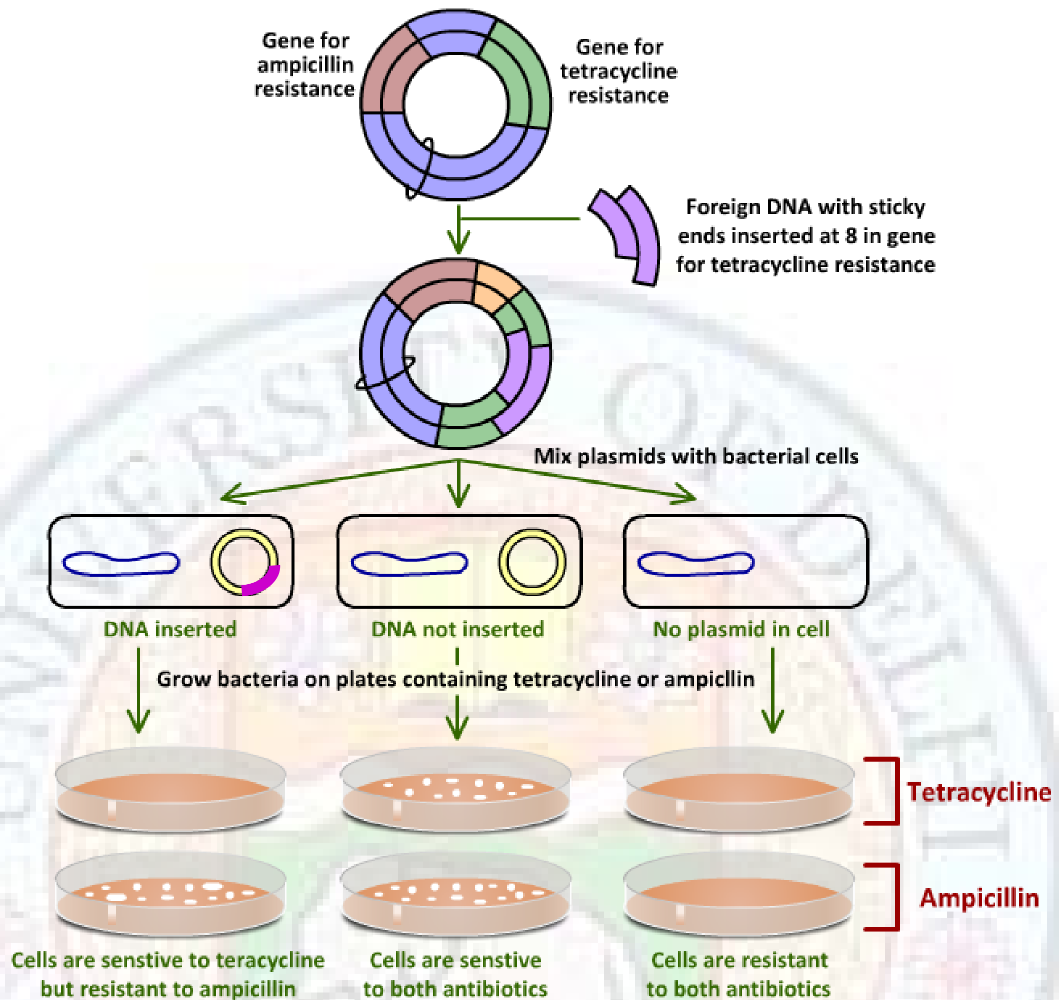


Figure:A plasmid vector with two genes for antibiotic resistance facilitates isolation of recombinants by insertional inactivation of one of the antibiotic resistance gene.

Source: author

6. Another simple method for detection of the desired clone is blue-white selection based on interruption of *lacZ* gene which is under the control of the *lac* promoter. The bacterial plasmid codes for N-terminal α fragment of β-galactosidase gene and bacterial chromosome has C-terminal ω-fragment.If both the fragments form proteins, they combine to form functional β-galactosidase.This is known as alpha complementation. If foreign DNA is inserted into N-terminal α fragment of β-galactosidase present in plasmid, encoded subunit is not synthesized and β-galactosidase is not produced. The expression of a *lacZ* gene in the vector could be induced by using lactose analog IPTG (isopropyl-ß-D-thiogalactopyranoside), resulting in formation of β-galactosidase. Its presence can be

detected by use of synthetic,soluble, colorlesssubstrate X-gal(5-bromo-4-chloro-3-indolyl-β-D-galactoside), which is hydrolyzed to yield an insoluble, blue colored product (5,5'-dibromo-4,4'-dichloro indigo). In bacterial cells containing the plasmid with an insert, functional β-galactosidase cannot be formed as *lacZ* gene is destroyed. These cells remain white even after incubation with IPTG and X-gal. Cells harboring functional β-galactosidase are without insert, and are blue in color.

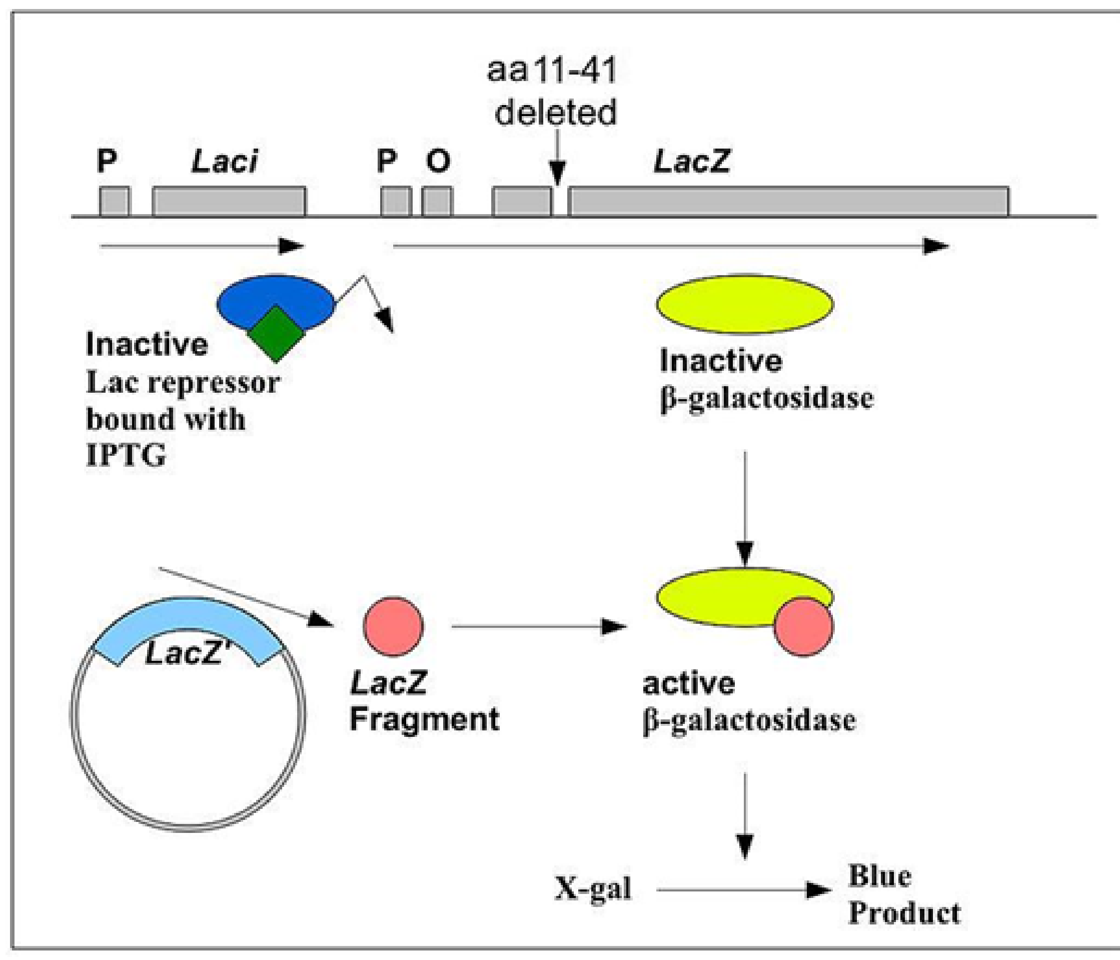
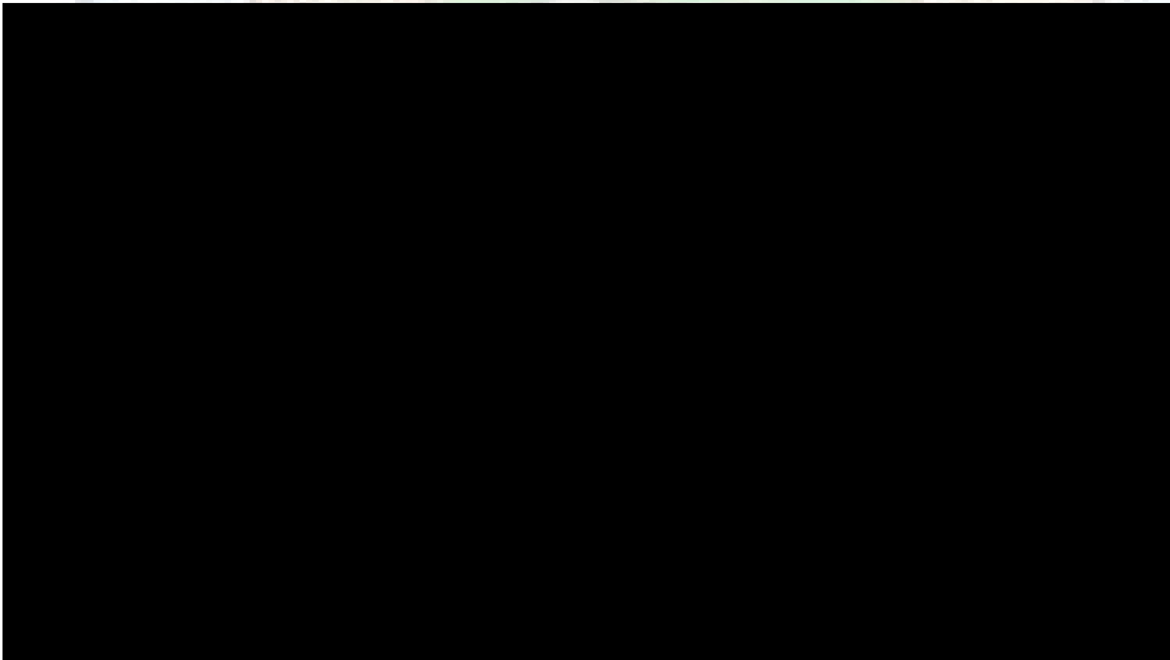


Figure: Schematic representation showing the molecular mechanism of blue white selection.

Source: http://en.wikipedia.org/wiki/File:Blue_white_assay_Ecoli.jpg

Figure: An LB agar plate showing the result of a blue white screen.

Source: http://en.wikipedia.org/wiki/Blue_white_screen



Video: Blue and white screening

Cloning Vectors

Source: https://www.youtube.com/watch?v=MvcG5ViJlVg

Purposebuild plasmid vectors: pBR322 and pUC series

Researchers have developed different purpose build plasmid vectors by modifying naturally occurring plasmids.

- **pBR322**

One of the most commonly used and versatile plasmidswas developed by Francisco Bolivar and Rodriguez in 1977 and is named eponymously as pBR322 (p stands for plasmid, B for Bolivar and R for Rodriguez).This plasmid has been completely sequenced and is therefore widely used as a cloning vector. Exact length of every inserted fragment can be calculated.

1. It has a small size of 4.361 Kb, facilitating its easy entry into cells. Entry of a vector becomes less successful as vector size increases.
2. The plasmid contains: (i) replicon, pMB1 (a close relative of ColE1) for replication of plasmid, (ii) the *rop* gene, encoding for the Rop protein, which promotes formation of stable RNA I – RNA II complex from the unstable complex and decreases copy number, (iii) the *bla* gene, encoding β-lactamase, that provides resistance to ampicillin, (iv) the tet^R gene, that confers tetracycline resistance.
3. There are over 40 enzymes with unique restriction sites on the pBR322 genome. Plasmid can be cleaved at these sites and DNA fragments can be inserted. Genes that conferresistance to antibiotics i.e., tetracycline (tet^R) and ampicillin (amp^R), allow the identification of cells containing theintact plasmid or a recombinant version of the plasmid.There are 11 restriction sites that lie within tet^R gene. 6 restriction sites are inside amp^R gene. Insertion of DNA segment within the tet^Roramp^R site inactivates the gene conferring tetracycline or ampicillin resistance. Host cells without the vector are sensitive to both antibiotics. Transformed cells containing pBR322 with insertion in tet^R gene are sensitive to tetracycline but resistant to ampicillin. Transformed cells containing pBR322 with insertion in amp^R gene are sensitive ampicillin, but resistant to tetracycline. Insertional inactivation of antibiotic resistance gene allows the recombinants to be readily selected. Cells containing pBR322 lacking the DNA insert are resistant to both antibiotics.
4. It has been widely used as a model system for the study of prokaryotic transcription and translation.

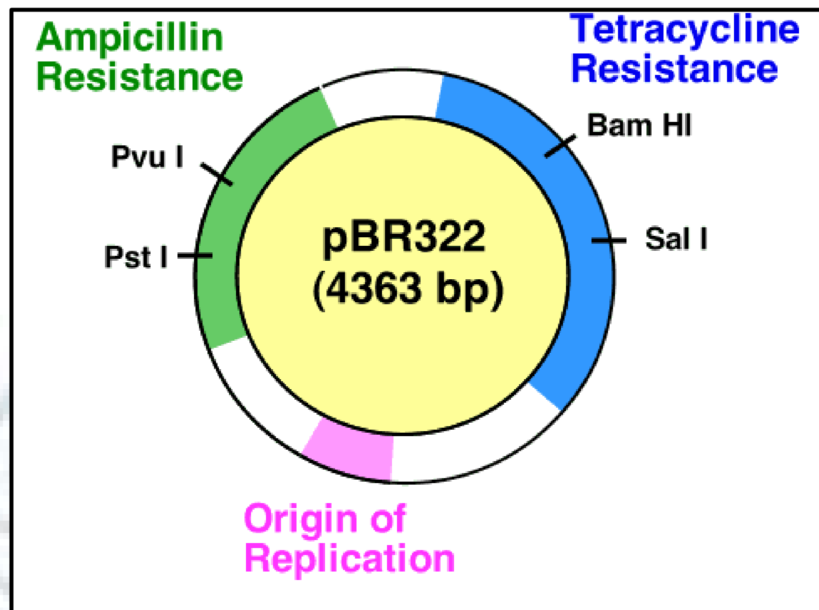


Figure: Vector map of pBR322.

Source: http://kiterunner.alliant.wikispaces.net/vectors

- **pUC series**

1. pUC series vectors were derived from plasmid pBR322 by Messing and co-workersto fulfill special cloning requirements.These plasmid vectors are called so because initial work on this vector was carried in the University of Californiaand prefix "p" stands for "plasmid".
2. pUC vectors are small, high copy number plasmids, with 500-700 copies per cell.
3. The circular double stranded DNA of pUC18/19 has 2,686 base pairs.
4. pUC18/19 plasmids contain: (i) the pMB1 replicon for replication of the plasmid. Lack of the *rop* gene and a single point mutation in the replicon rep of pMB1 results in high copy number of pUC plasmids in comparison to pBR322 (ii) the *bla* gene coding for β-lactamase, conferring ampicillin resistance. This gene differs from that of pBR322 plasmid by two point mutations. (3) *bla* gene is fused with *E. colilacZ′* gene which consists of CAP protein binding site, promoter plac, operator, lac repressor binding site and the 5′ terminal region including 146 codons that code for deleted version of the gene *lacZ* encoding β-galactosidase. Codons 6–7 of *lacZ* are replaced

by the multiple cloning site (MCS) region, where various recognition sites for many restriction enzymes are present.

5. pUC18 and puC19 are similar except that they contain multiple cloning sites (MCS) arranged in opposite orientations.
6. The pUC vectors are the first plasmid vectors to contain polylinker or multiple cloning sites (MCS). The MCS increases the number of potential cloning strategies by extending the range of enzymes. It provides extra flexibility during cloning procedure by allowing foreign DNA to be inserted at any of several restriction sites. The potential drawback of polylinker is inability to use insertional inactivation of antibiotic resistance to screen for recombinants.
7. Functional *lacZ* gene in the pUC vectors allows the researcher to differentiate between recombinant and parental host bacteria by visual screeningof bacterial colonies on media containing the lactose analogue, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galacto-pyranoside). This fragment is capable of intra-allelic (α) complementation with a defective form of β-galactosidase synthesized by the host genome. In the presence of IPTG, bacteria can synthesize both the fragments of the enzyme and form blue colonies on media containing X-Gal.β-galactosidase can hydrolyze X-Gal producing a blue colored substance and colonies that carry the plasmid are therefore blue colored. Multiple cloning site is located on the plasmid immediately downstream to the start codon of *lacZ gene*. Insertion of foreign DNA into the multiple cloning site disrupts the N-terminal fragment of β-galactosidase resulting in its inability to form blue color and therefore white colonies are formed.

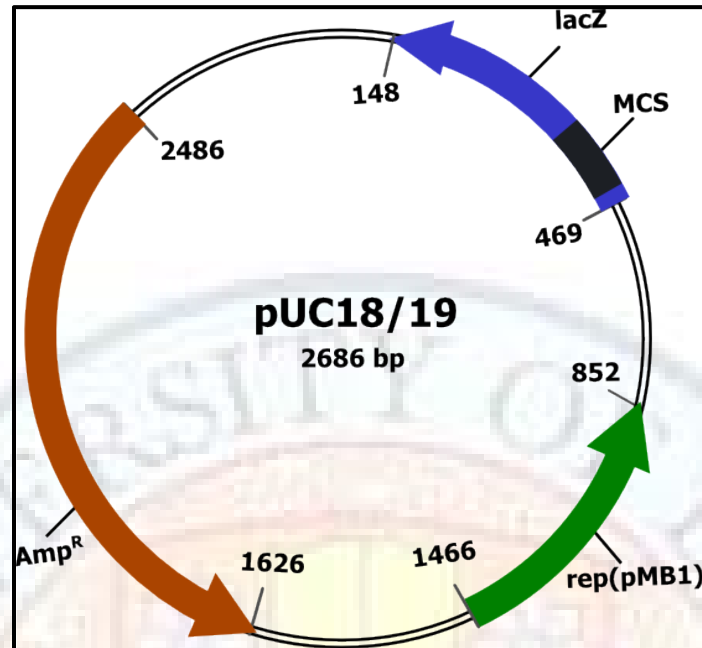


Figure: Vector map of pUC18/19.

Source: http://clipartist.info/RSS/openclipart.org/2011/April/02 Saturday/plasmid_vector_puc_1819.svg.html (cc)

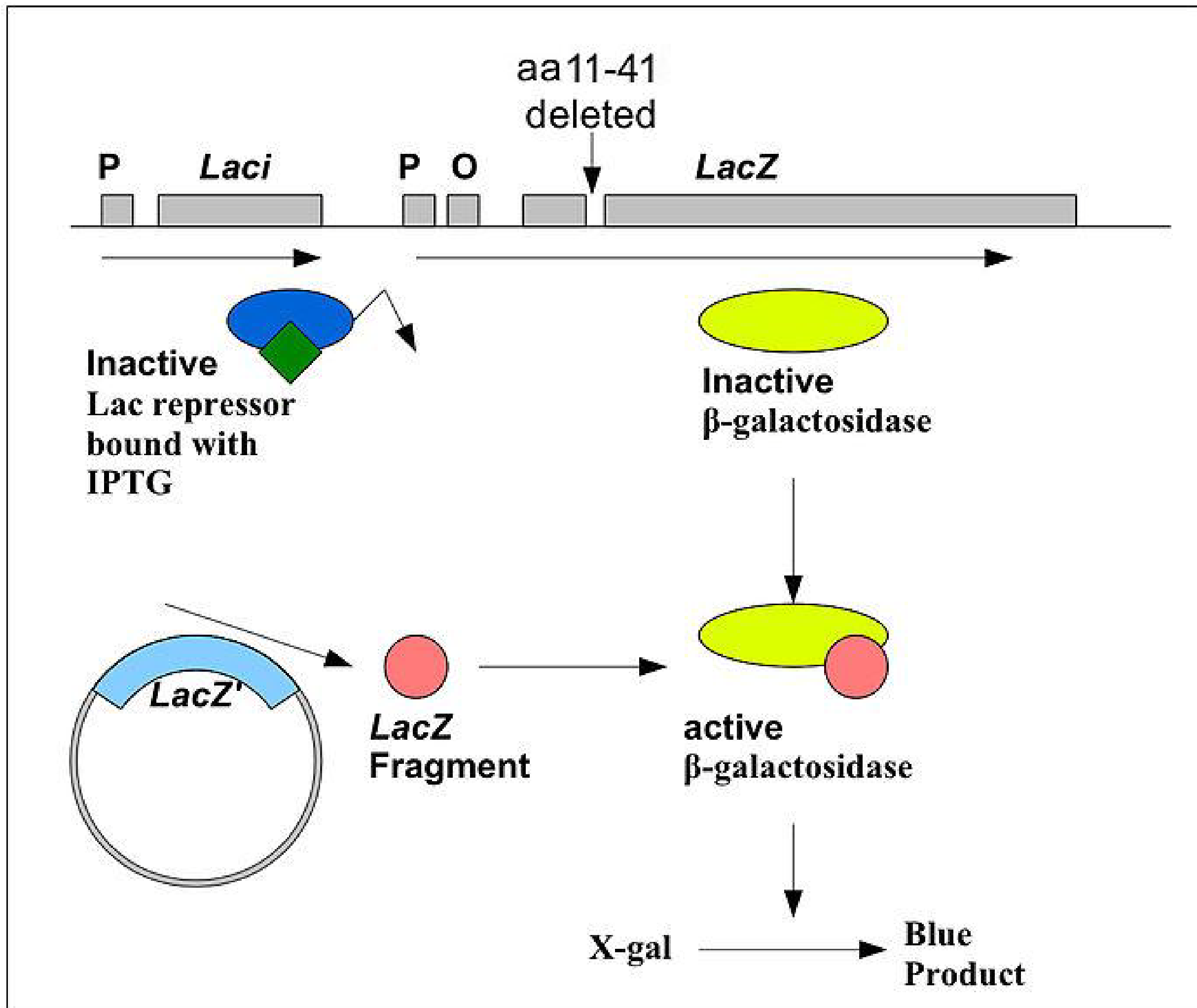


Figure: A schematic representation of the Blue-white assay, used to screen for recombinant vectors

Source: https://en.wikipedia.org/wiki/Blue_white_screen

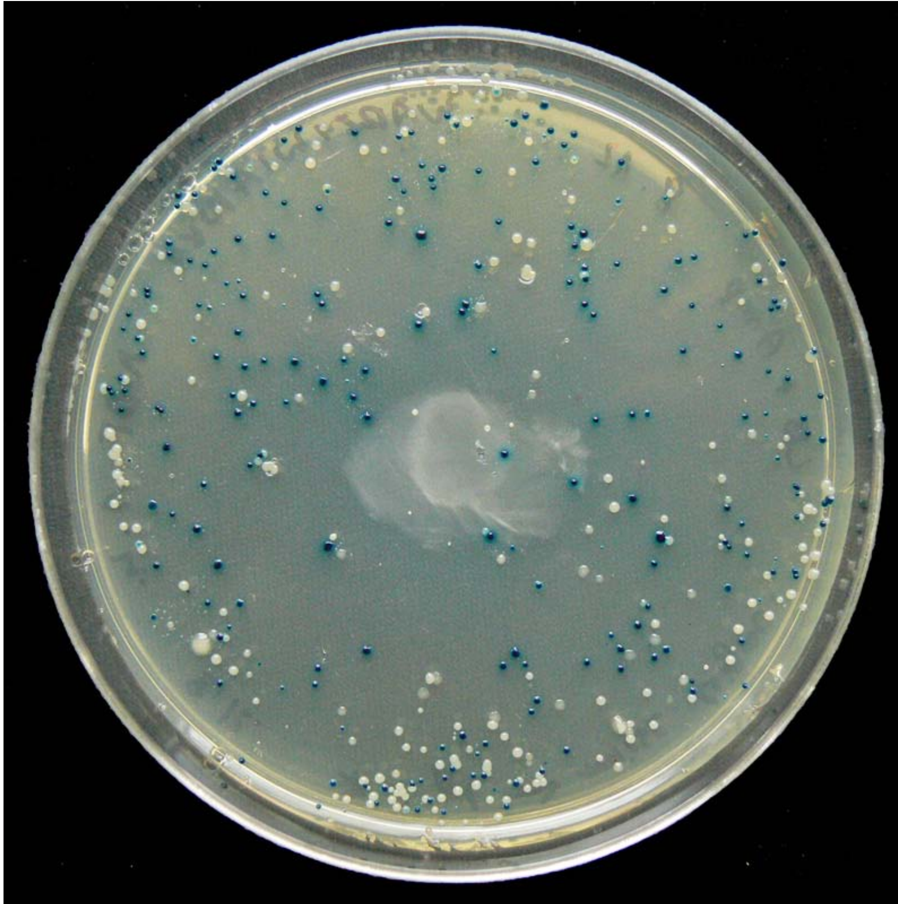


Figure: An LB agar plate showing the result of a blue white screen.
source: https://en.wikipedia.org/wiki/Blue_white_screen#mediaviewer/File:Blue-white_test.jpg

Ti Plasmid

Ti (tumor-inducing) plasmid based vectors are high efficiency vectors and were developed with the aim of introducing genes into plants. *Agrobacterium tumefaciens* causes crown gall disease in plants in which cells grow in anundifferentiated and uncontrolled manner to form a tumor. This gram-negative soil bacterium possesses tumor-causing ability because of the presence of a large (206.479 Kb) double-stranded circular extra chromosomal element called as Ti plasmid. It bears 196 genes including one structural RNA and encodes 195 proteins. This plasmid has a 20 Kb T-DNA region which can be transferred to the plant cell and is integrated into the plant DNA. It is also stably transmitted through divisions of meiosis and mitosis. This transfer of T-DNA from *Agrobacterium* to plant cells depends on 25 bp repeated sequences at both ends of the T-DNA known as left and right border repeats

and on virulence (*vir*) genes which are grouped into operons. The T-DNA carries enzymes that convert plant metabolites to hormones cytokinin and auxin, which stimulate tumor formation. It also carries genes for opine synthesis. Opines serve as a food for the bacterium and are synthesized in higher concentrations in tumors cells.Researchers engineered this Ti plasmid by disrupting these tumor causing and opine synthesis genes and replaced them with selectable marker genes conferring resistance to antibiotics. Ti plasmid based vectors were constructed with the T-DNA carrying following components: (i)*ori* for origin of replication, allowing the plasmid to replicate; (ii)Right border sequencewhich is necessary for transfer of Ti plasmid into plant genome; (iii)A multiple cloning site to ease the insertion of gene of interest into the region between T-DNA border sequences.

Recombinant genes can then be integrated into the T-DNA of the Ti plasmid, and the plasmid can be used to infect plant cells. The infected cells are placed on a culture medium containing growth factors and the selectable antibiotic. Only the cells harboring T-DNA can grow in the presence of the antibiotic.

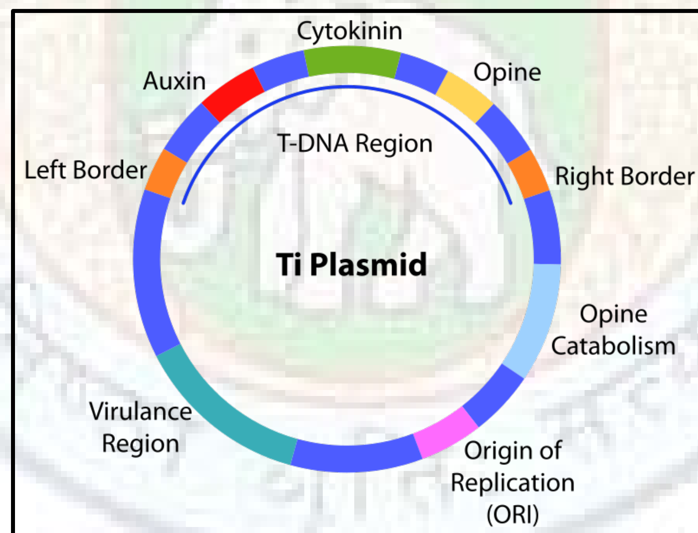


Figure: The structure of Ti Plasmid.

Source: http://en.wikipedia.org/wiki/Ti_plasmid

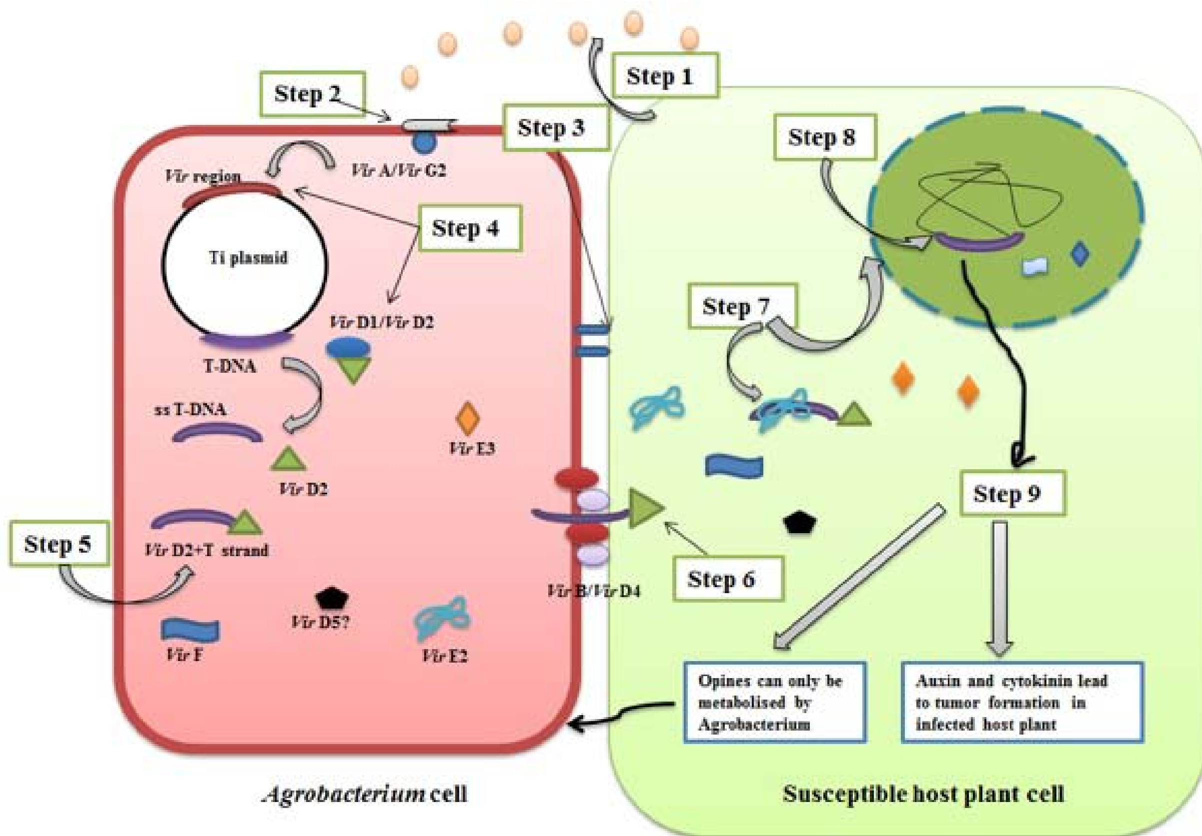


Figure: Schematic representation of *Agrobacterium*- mediated transformation. Step 1: Production of signal molecules form wounded plant cell; Step 2: Recognition of signal molecules by bacterial receptors; Step 3: Attachment of *Agrobacterium* to plant cell; Step 4: Activation of *Vir* proteins which process ss-TDNA; Step 5: Formation of immature T-complex; Step 6: T-DNA transfer; Step 7: Assembly of mature T-complex and Nuclear transport; Step 8: Random T-DNA integration in the plant genome; Step 9: Expression of bacterial genes and synthesis of bacterial proteins.

Source: http://nptel.ac.in/courses/102103013/25

Bacterial Artificial Chromosomes (BACs)

Bacterial artificial chromosomes were created fromnatural plasmids of *E. coli* called the fertilityfactor (F factor). Studies on F factor revealed that these circular molecules are capable of replication in bacteria and can accommodate millions of base pairs. Hiroaki Shizuya turned parts of F factor into a vector in 1992. These vectors are customized for the cloning of very long segments (~ 100to 300 Kb) of DNA. They generally include a very stable origin of replication (*ori*) that maintains the plasmid at one or two copies per cell and

selectable markers conferring resistance to the antibiotic chloramphenicol. BAC vectors werealso modified to incorporate *lacZ* gene to allow easy identification of recombinants. Gene *sacB* encoding levansucrase was also incorporated into BAC vectors. The protein levansucrase turns sucrose into levan, a toxic product for bacteria. Bacteria cultured on media containing sucrose will die if sacB is intact as in non-recombinant cells. If the vector carries an insert DNA, sacB is disrupted and is unable to produce levansucrase, and the bacteria can survive in the media containing sucrose. The large circular recombinant DNAs are transferred to the host bacteria by electroporation. As the bacterial cell will grow, BACs will replicate and can be isolated.

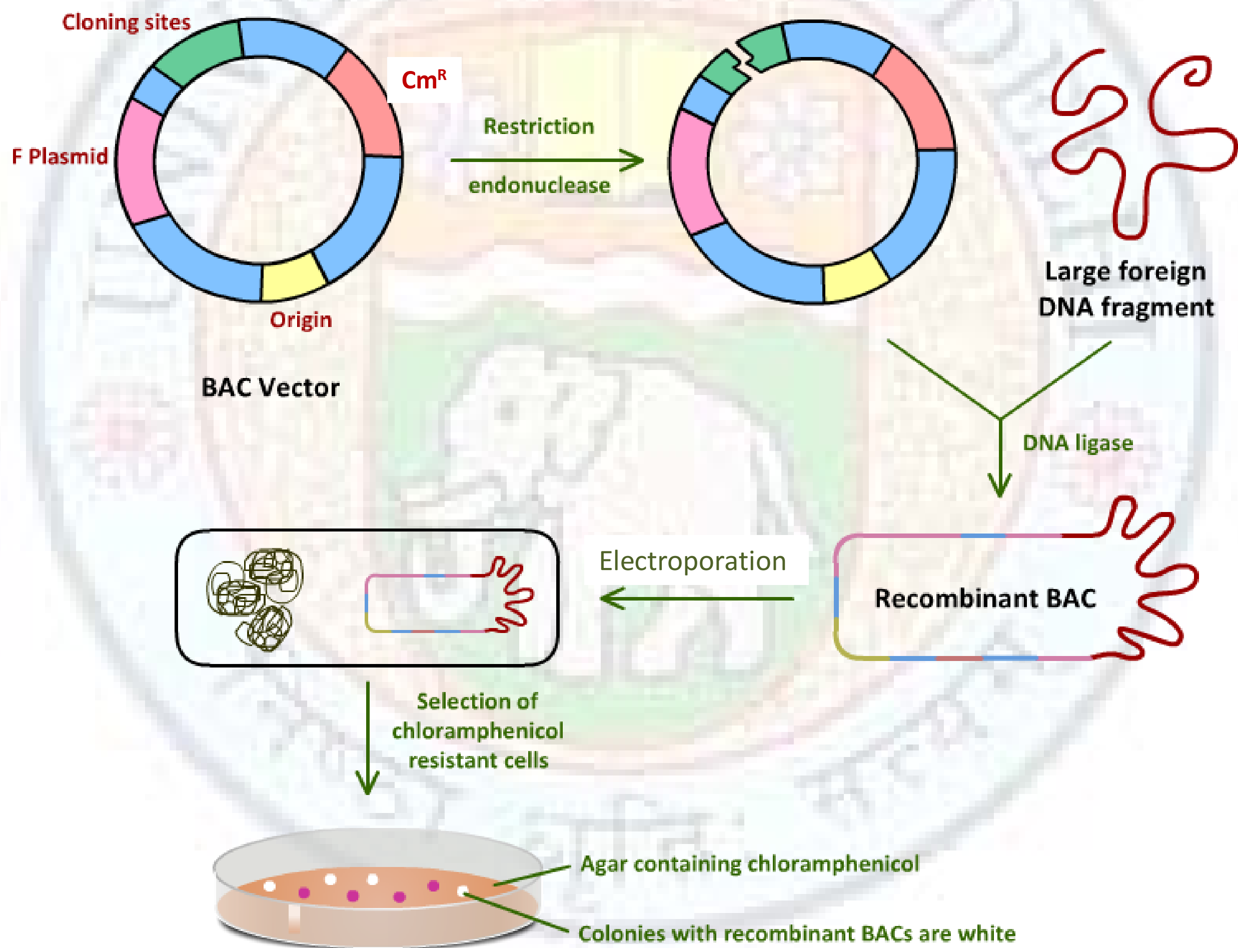


Figure: BACs as cloning vectors.

Source: author

BACs are useful for cloning larger genes including the upstream regulatory regionscontrolling gene expression. These are used in genome sequencing projects to clone several genes togethersince severalthousands of base pairs can be cloned into the BAC vector. BACs donot undergo recombination in the host cell and are helpful tools in physical mapping studies of genomes. These vectors are also utilized for cloning infectious DNA and RNA pathogens and development of vaccines.BACs are not suitable for cloning DNA sequences toxic to *E. coli* and AT-rich DNA fragments.

Bacteriophages as vectors

Max Delbruck, in the 1940s laid foundations for the study of bacteriophages. Bacteriophages or phages are viral genomes that attack bacteria. Structurally bacteriophages can be tailless, with head and tail or filamentous. Phage genome which can be singleor double-stranded DNA or RNA is encapsulated in an icosahedral protein shell known as head or capsid, in tailless or tailed forms.

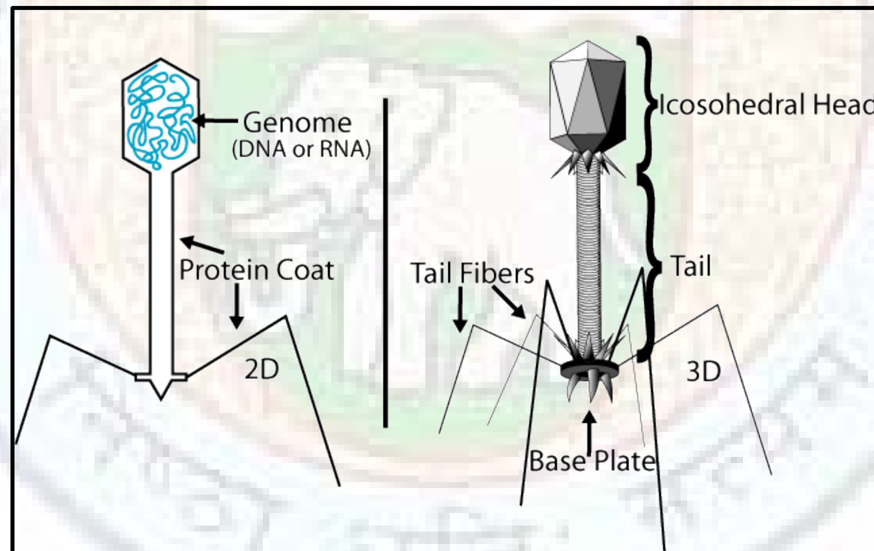


Figure: Structure of a typical bacteriophage.
Source: http://www.newworldencyclopedia.org/entry/virus (cc)

Phages may be designated as either temperate orvirulent, depending on their life cycles. When a phage enters a bacterial cell it can produce more phages of its kind and kill the bacterial cell. This is called the lytic growth cycle of a phage. It can also integrate into the

chromosome of host and remain in a quiescent state without killing the bacterial cell. This is the lysogenicgrowth cycle of a phage. Virulent phages exhibit only the lytic life cycle. Temperate phages exhibit both lysogenic life cycles and lytic cycle. Bacteriophages can be used as vectors as larger non-viral DNA segments can be packaged in the virus particle. Phage vectors infect cells more efficiently than plasmids transform the cells, therefore yield of clones with phage vectors is usually more than the yield of plasmid transformed cells.

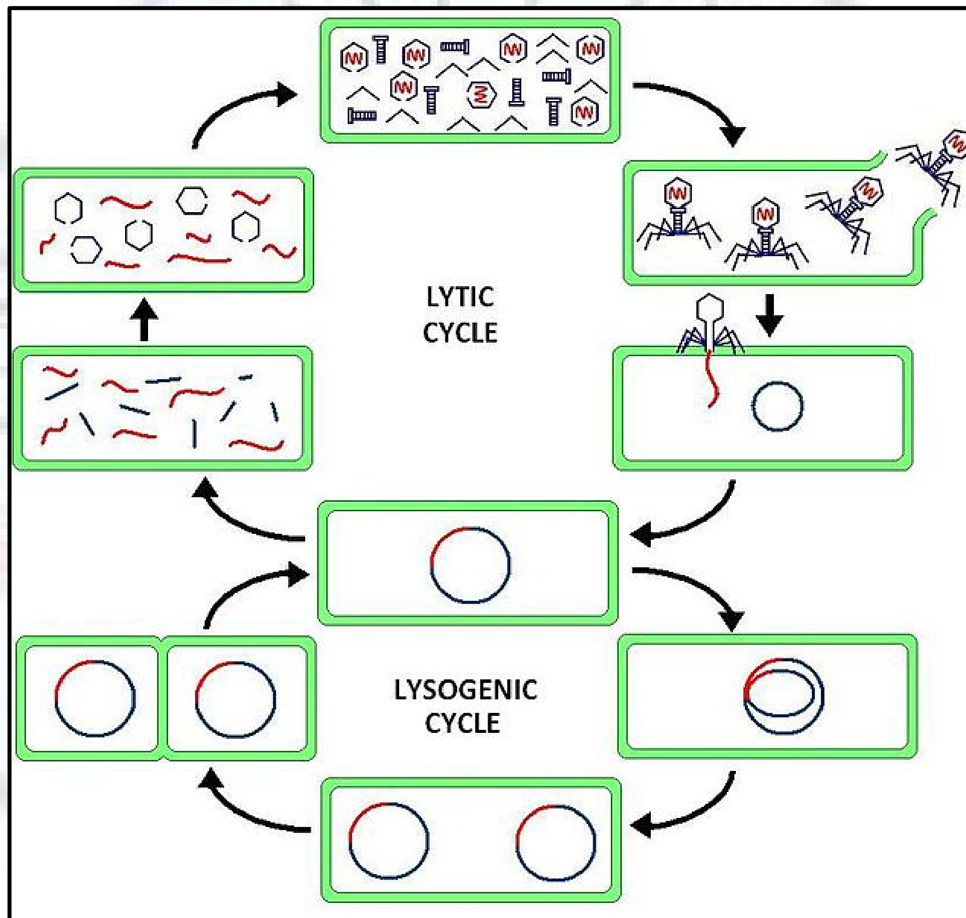


Figure: Diagrammatic representation of lytic and lysogenic cycles of bacteriophage.

Source: http://commons.wikimedia.org/wiki/File:Phage2.JPG (cc)

Lambda (λ) Phage

Lambda bacteriophages are the most important and widely used vectors having linear genomes. Wild-type lambda phages have 48.5 Kb linear double-stranded DNA

genomehaving 73 ORFs (open reading frames). The linear genomebears short (12 bp) single-stranded sequencesat its ends known as cos siteswhich are complementary to each other. These cohesive or 'sticky' endsenablecircularization of the λgenome after infection during packaging. Phage vectors bind to receptors on the bacterial surface. This is known as adsorption. The phage DNA is injected into the cell and thephage life cycle can start. The phage genome circularizes and the phage can initiate either the lytic or lysogenic cycle, depending on nutritional and metabolic status of the host cell. The ratio of phage to bacteria during adsorption also influences the lytic or lysogenic growth cycle.Specific environmental changes can alsotrigger the lysis of host cell.

Important features contributing to the utility of λ phages as vectors are:

1. Only two-thirdsof the genome is essential and about one-third of the genome is non-essential which can be discarded and replaced with foreign DNA. The middle region of the lambda genome is dispensable as it controls the lysogenic properties of the phage, therefore this region can be deleted without disrupting the functions required for the lytic growth cycle. This region is substituted with the polylinker.
2. The packaging mechanism in λ phage facilitates insertion of recombinant DNA as packaging into infectious phage particles is possible only if DNA is between 40 to 53 Kb long. λ bacteriophage vectors can be digested to release three fragments, of which two fragments harbor essential genes comprising ~ 30 Kb. The third middle fragment can be discarded when additional DNA is inserted for cloning, producing viable phage particles. Therefore bacteriophage vectors allow the insertion of DNA fragments of up to 23 Kb. Once the bacteriophage fragments are ligated to insert DNA fragments of appropriate size, the resulting recombinant DNAs can be packaged into phage particles. This requires adding packaged phage to crude bacterial cell extracts containing all the proteins required to assemble a complete phage, also known as*in vitro*packaging. Nearly all infective viable phage assemblies contain a foreign DNA fragment. They can be subsequently transferred into *E. coli* cells in an efficient manner. Additional advantage of using these modified phages as vectors is that they can enter bacteria much more easily than plasmids. One of the major limitations of λ vectors is that the head packaging imposes a physical limitation on the amount of DNA that can be incorporated into it during phage assembly. This restricts the size of foreign DNA fragments that can be cloned into λ phage. During packaging, infectious phage particles can be constituted from DNA that is approximately between 40 to 53 Kb long. Thus, a wild-type phage genome can

accommodate only around 23 Kb of cloned foreign DNA. This drawback has been taken care of by careful construction of vectors to accommodate fragments of DNA that are close to the theoretical maximum limit for the particular vector.

λ vectors can be of two types: (1) insertion vectors and (2) replacement or substitution vectors.

- **λInsertion vectors**

As the name suggests, in these vectors no phage DNA is removed and foreign DNA fragments are inserted. They have a single recognition site for one or more restriction enzymes that cuts it only once. The foreign DNA fragments are inserted into this site in theλgenome. Since phage DNA is not removed there is a limit on the size of insert that can be cloned in this vector. They are generally utilized for construction of cDNA libraries from eukaryotic mRNA sequences. λt10 and Charon16A are examples of λinsertion vectors.

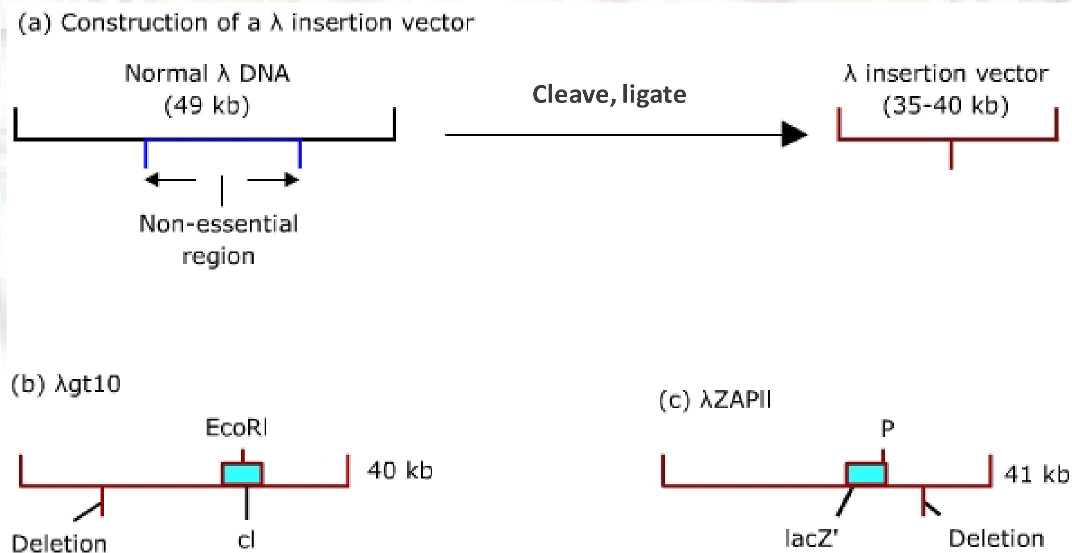


Figure: Diagrammatic representation of construction of λInsertion vectors. P represents polylinker in the lacZ′ gene of λZAPII, that contains unique restriction sites for SacI, NotI, XbaI, SpeI, EcoRI and XhoI.

Source: Ms. Manisha Sharma

- **λ Replacement or Substitution vectors**

Insertion vectors have restricted scope for cloning large pieces of DNA. Therefore, replacement vectors were developed in which a middle dispensable 'filler' fragmentis discarded and replaced with the insert DNA.These vectors contain restriction sites flanking the non-essential region of phage. They can accommodate larger inserts than insertion vectors. The middle region which is replaced by foreign DNA usually contains a gene that makes the phage non-viable in an appropriate bacterial host. Therefore vector molecules which do not contain foreign DNA can be selected against vector molecules containing foreign DNA, which will be viable.They are generally utilized for construction of genomic libraries. EMBL4 and Charon40 are examples of λreplacement vectors.

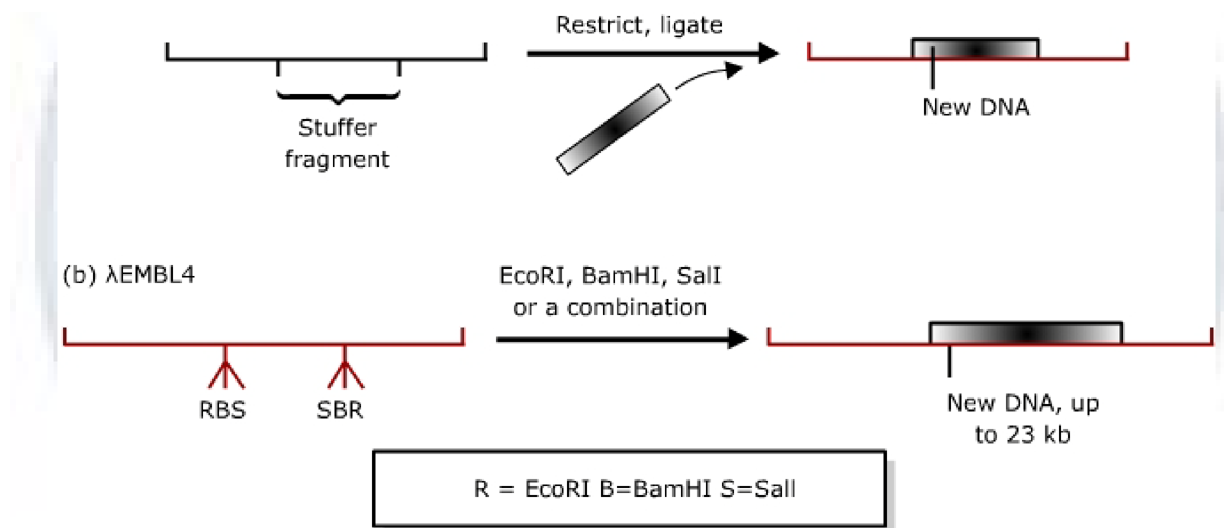


Figure: Diagrammatic representation of λreplacement vectors.

Source: Ms. Manisha Sharma

M13 Phage

M13 phage, afilamentous virus is 9 nm wide and 900 nm long and has a smaller genome than λ phage. Its genome is a single-stranded circular DNA. The 6.4 Kb single-stranded circular DNA of phage is encapsulated by 2,710 identical protein subunits.

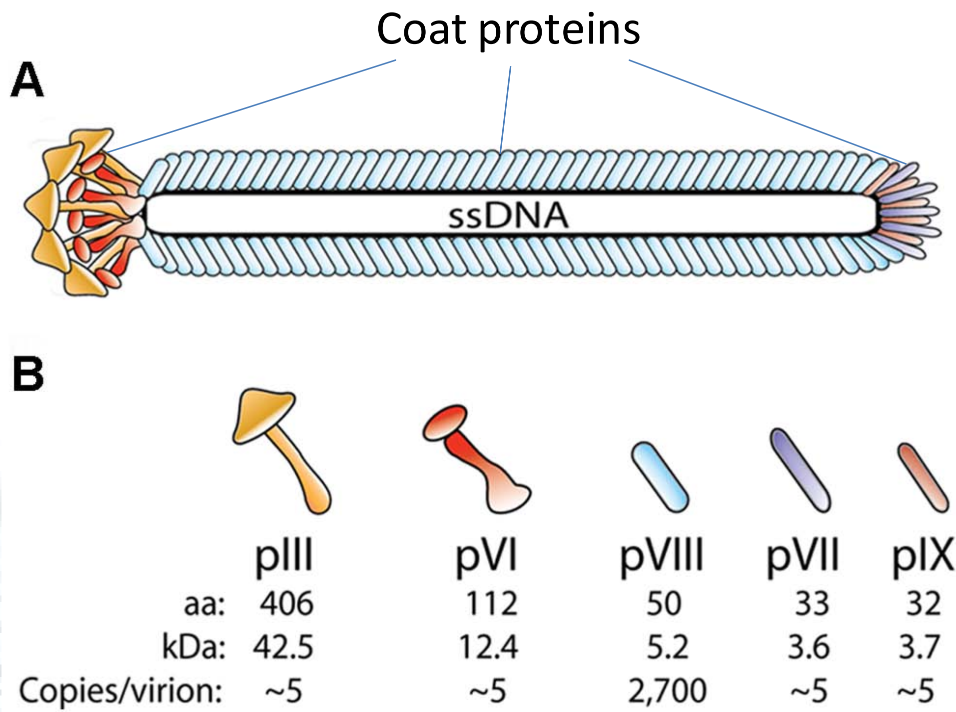
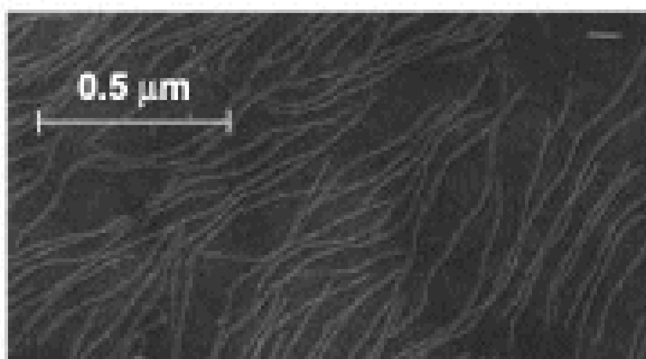
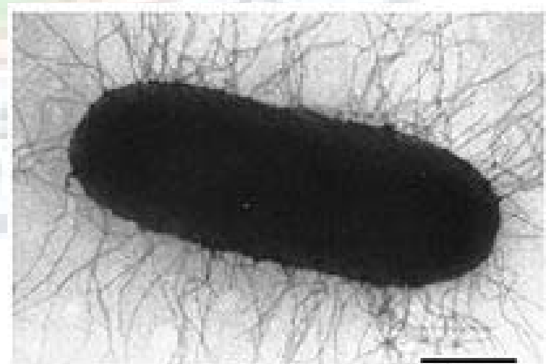


Figure: Diagrammatic representation of the wild type (wt) filamentous M13 Phage.(A) The wt virion is made up of five structural proteins that coat a single stranded DNA genome of about 6.4 kb. (B) In the wt phage there are about 2,700 copies of pVIII and approximately 3–5 copies each of the four proteins pIII, pVI, pVII and pIX, which are found at each tip of the virion. The virion size depends on the genome size at approximately 2.3 nucleotides per pVIII, and hence the length of the particle changes as a function of genome length.

Source: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017433(CC)



a



b

Figure: Electron micrographs of (a) highly purified preparation of M13 bacteriophage (b) an *E. coli* bacterium shedding M13 phages.

Source: http://www.answers.com/topic/virus, http://openwetware.org/wiki/20.109%28F11%29:_Mod_3_Day_1_Growth_of_phage_materials (cc)

Phage M13 can enter*E. coli* through the bacterial sex pilus, which is a protein appendage allowing the transfer of DNA between bacteria. Phage genome enters the bacterial cell where single stranded phage genome is converted into an intermediate circular double-stranded replicative form. This replicative form (RF) contains (+) and (-) strands and is essentially similar to plasmid. It acts as a template for replication of the single-stranded genome of the virus particle [the (+) strand] by rolling circle replication.The + strand is then packaged into new virus particles. Thousands of progeny M13 phages are produced per generation. M13 phageis a non-lytic virus as it does not kill its bacterial host and therefore large quantities of M13 phages can be cultured andharvested easily.An M13 phagecan be isolated and manipulatedas a vector for cloning in a plasmid like manner. The circular double stranded replicative form DNA is digested at a single site by restriction endonucleases. The cut is made in the polylinker region of the phage. The double-stranded insert DNA fragment is produced by digestion with the samerestriction enzyme. Insert DNA is then ligated to the digested vector. The foreign DNA can be inserted in any orientations as both ends of DNA molecules are same. Therefore, half of the newly synthesized (+) strands packaged into virusparticles contain one of the strands of the insert DNA, while the other half will contain the other strand. Infection of *E. coli* bya single phage will produce large amount of single-stranded M13 DNA containing the same strand of the insert DNA. Instead of bacterial colonies, phage plaques will be formed and infected bacteria can be easily isolated from them. M13 phage harbors *lacZ'* gene allowing blue/white selection system for screening of recombinants. Single-stranded DNA cloned into M13 phage can be subjected to sequence analysis.

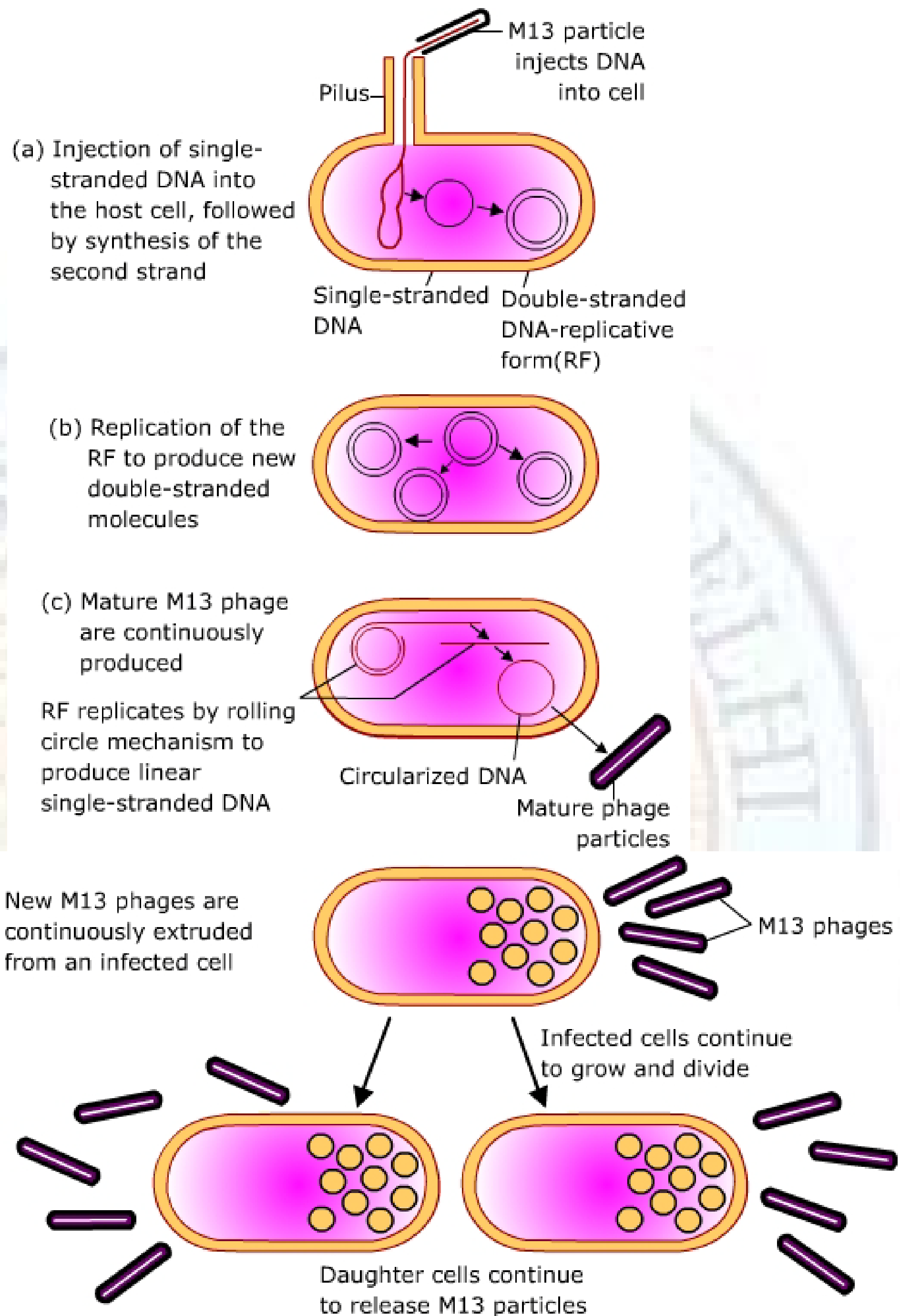


Figure: Diagrammatic representation of the infection cycle of M13 bacteriophage depictingdifferent types of DNA replication that occur.
Source: Ms. Manisha Sharma

M13 phage has several advantages as a vector:

- The replicative form of M13 phage can be isolated and manipulated as a vector for cloning in a plasmid like manner.
- The use of vectors like M13, occurring in a single-stranded formis very useful in large scale sequencing projects since cloning, amplification and strand separation steps can be performed in combination.These are also used in labeling experiments, site-directed mutagenesis and phage display libraries where a foreign peptide is fused in frame to a gene coding for one of the coat proteins.
- Another advantage of M13 phage is that it contains multiple cloning sites which are similar to pUC18 and pUC19 so that genes cloned in pUC18 and pUC19 can be sub-cloned at the same sites in M13 phages.
- M13 phages do not contain any non-essential genes. The 6.4 Kb genome of phage is used very efficiently and only 507 bp intergenic region is available for insertion of foreign DNA. Polylinker and *lacZ* peptide sequence are also inserted into this region.

M13 phages also have certain disadvantages like:

- These vectors are not suitable for long-term propagation of recombinant DNA as inserts longer than 1 Kb cannot be stably maintained.
- Though there are no packaging constraints but the cloning efficiency decreases when foreign DNA fragments longer than 1.5 Kb are inserted.

Phagemids/ Phasmids

Both plasmids and bacteriophages have certain advantages over each other as well as disadvantages.Therefore phagemids/phasmids were constructed to maintain advantages of both vectors. They are plasmid vectors with M13 origin of replication (pUC18 or pUC19 or pBR322 + M13 *ori*). They can be replicated in the host cells like plasmids maintaining double stranded replication and high copy number so that large amount of foreign DNA can be recovered. They can also package their single stranded DNA in phage particles because of the presence of M13 origin of replication. Phagemids can be introduced into bacterial host cells by transformation or electroporation. A positive selection marker is used to select bacteria containing the phagemid. Fragments of several kilobases of DNA in length can be isolated in single stranded form from the phagemids. They are also utilized for sequencing purposes and for generating templates for site-directed mutagenesis.λZAP is an example of phagemid.

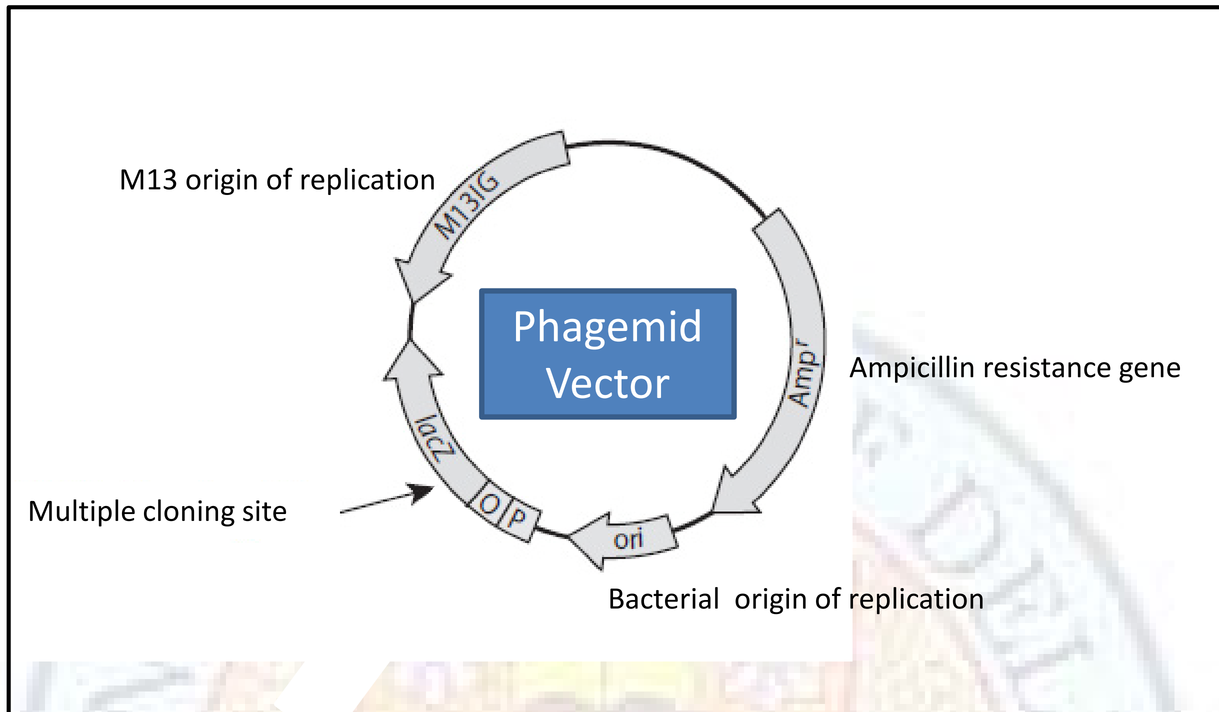


Figure: A phagemid vector.

Source: http://www.creative-biogene.com/pTV118N-phagemid-vector-VPT4036-1232966-76.html

Cosmids

The efficiency of cloning decreases in plasmids as fragment size increases and the length of non-essential region in λ phage vectors limits the length of inserted DNA in them. The requirement for accommodating larger fragments of genomic DNA led to the development of cosmid vectors by Collins and Hohn in the late 1970s. *In vitro* packaging in λ phage vectorsis sequence independent, butis dependent on the *cos* sites separated by DNA ofpackagable size. This feature has been exploited for the constructionof cosmid vectors. The term cosmid includes 'cos' from cos sites and 'mid' from plasmid vectors as these are plasmid vectors containing cos sites of λ phage vectors. Cosmids are circular double-stranded DNA molecules containing a prokaryotic origin of replication, a selection marker, cloning sites for restriction endonucleases and λ cos sites.They are small (4-6 Kb) and can hold insert DNA fragments up to 47 Kb in length. Cosmid vectors are used for cloning in a manner essentially similar to plasmid vectors and can clone larger DNA fragments than plasmid or phage. Insert DNA is ligated between two cos sites using restriction

endonucleases.DNA is packaged *in vitro* and introduced into *E. coli*. Since they lack phage genes, they act as plasmids when transferred into *E. coli*. Inside the bacterial cell, the cosmid circularizes and replicates as a plasmid maintaining 15-20 copies per cell. Yeast genome was sequenced using cosmid vectors.The cosmids require tedious working protocols for cloning and screening. Another disadvantage of cosmids is that their enormous insert size can result in recombination within the cloned sequences. But once desired recombinant clone is identified, isolation of DNA is easy.

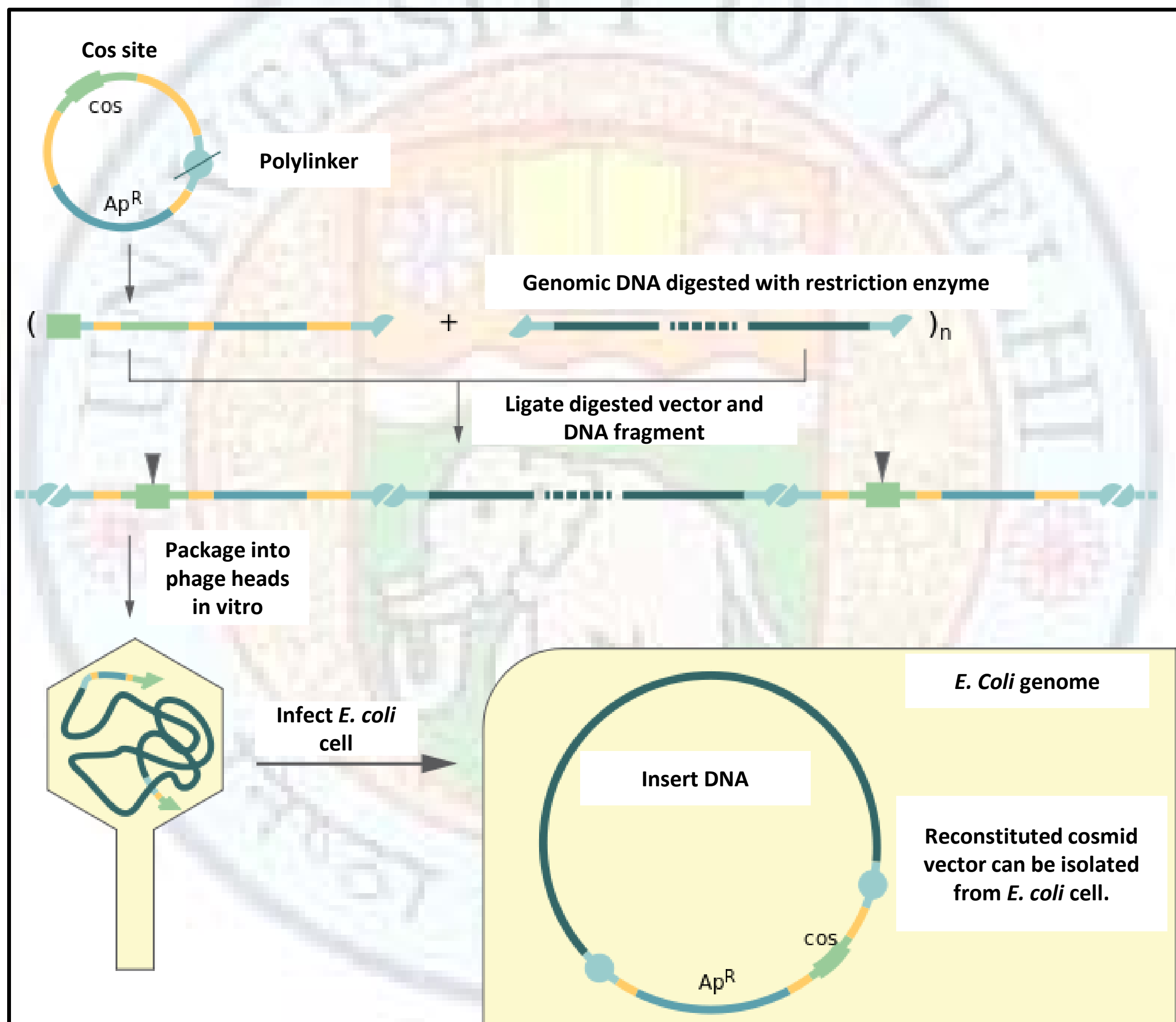


Figure: Cloning in a cosmid vector.

Source: http://commons.wikimedia.org/wiki/File:Cosmid.svg (cc)

Shuttle Vectors

They are plasmid vectors that can replicate autonomously in two different hosts.They are also known as bifunctional vectors.Shuttle vectors are plasmids with the ability of replicating and transferring ("shuttling") between two different host organisms. They can also shuttle between a prokaryote *(E. coli)* and a eukaryote (yeast). These vectors possess two origins of replication, each origin unique for each host organismand different selection marker indicating transformed host cells containing the vector.

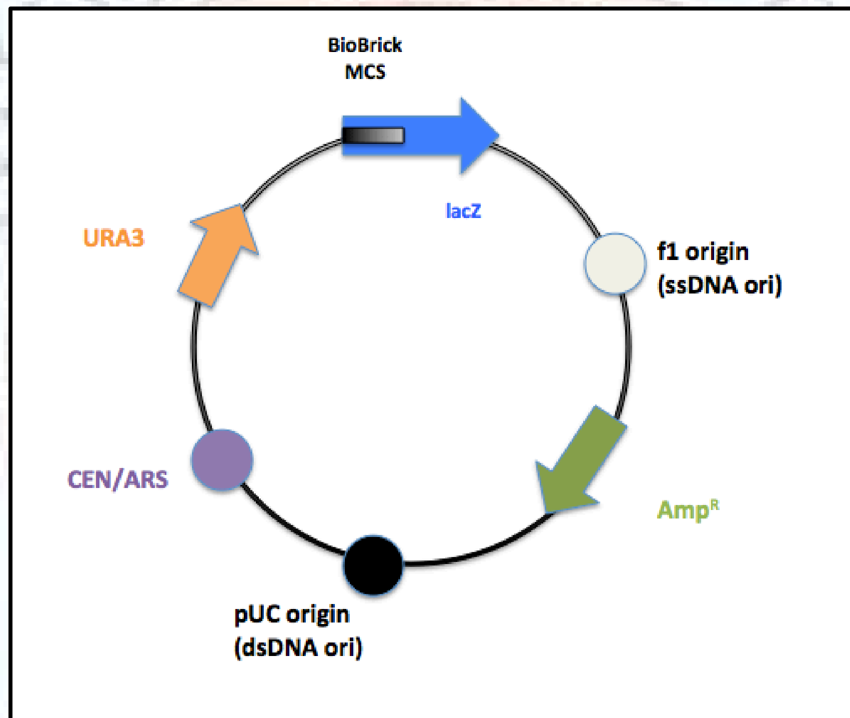


Figure: A shuttle vector.

Source: http://2011.igem.org/Team:Johns_Hopkins/Project/Vector (cc)

Eukaryotic genes can be cloned in bacterial hosts using the shuttle vectors and theexpression of cloned genes can be analyzed in appropriate eukaryotic hosts.Initially cloning and amplification of inserted DNA is done in *E. coli* and then the shuttle vector is transferred to a eukaryotic host to study the expression of gene of interest. The most commonly used shuttle vector is yeast shuttle vector. These vectors are therefore used for

functional complementation assay of screening a yeast genomic library in which a functional protein complements a recessive mutation. Functional complementation is tested for yeast genes cloned in plasmid vector and therefore shuttle vectors are used to facilitate screening of recombinant plasmids. It contains essential elements to allow cloning of yeast DNA fragments in *E. coli*. Additionally, it contains origin for DNA replication in yeast (ARS, autonomously replicating sequence), yeast centromere (CEN) to allow faithful segregation of plasmid during yeast cell division and a yeast gene encoding a selectable marker URA3 for orotidine-5′phosphate decarboxylase, an enzyme which is required for the synthesis of uracil. Yeast gene sequences are partially cleaved to release overlapping restriction fragments. Shuttle vector is cleaved with the same restriction enzyme within the multiple cloning site to produce sticky ends complementary to the gene sequence. Vector is transformed to *E. coli* cells, and cells that grow after selection for ampicillin resistance contain single type of yeast cDNA fragment.

Yeast Artificial Chromosomes (YACs)

YACs are the largest capacity yeast vectors that can accommodate megabase rangeforeign DNA fragments within yeast cells. The capacity of YACs to harbor foreign DNA is much more (between 0.2 and 2.0 Mb) than other vectors discussed so far. A YAC vector includes a cloning region containing one or more restriction sites present only once in the vector, a yeast centromere sequence (*CEN*) to allow regulated segregation during mitosis, a yeast telomere (*TEL*) at each end of chromosome, yeast origin of replication sequence (*ARS* – autonomously replicating sequence) which allows the vector to propagate in a yeast cell, a bacterial origin of replication (*ori*) which allows the circular version of the parental vector to replicate in *E. coli* and a selection marker for bacteria as well as yeast. Yeast ARS doesnot function in bacteria and bacterial *ori* will not function in yeast. Even, bacterial andeukaryotic promoters are variable, implying that the bacterialRNA polymerase is unable to transcribe the yeast selection genes namely *URA3* (involved in uracil biosynthesis) and *TRP1* gene (involved in tryptophan biosynthesis), and therefore yeastselection markers will function only in yeast. Similarly yeast RNA polymeraseII cannot transcribe the antibiotic resistance gene of bacteria. The circular YAC is digested with two restriction endonucleases, one cleaves in the multiple cloningsite and other restriction enzyme cuts betweenthe two *telomeres* producingright and left arms. Insert DNA is digested with thesame restriction enzyme which was used to cleave the YAC multiplecloning site. Insert DNA is ligated to the two arms of YAC and the recombinantmolecules are transformed into yeast. The transformants can be selected for selection markers to ensure that recombinant

YACscontain both the left and right arms. They are helpful in creating physical maps of larger genomes like human genome. There are certain disadvantages of YAC vectors. Though the principle of cloning in YAC is similar to plasmid or cosmid, but the process of cloning is too complicated to carry out.It is difficult to isolate YAC DNA from host cell because of its similar size to host chromosome. Even higher yields of YAC DNA cannot be obtained from host cells.YAC vectors sometimes accept two or more different DNA fragments, creating a chimeric YAC. Additionally insert DNA is frequently modified or deleted by the host cell.YACs harbor very large sized insert, which is prone to breakage resulting in rearrangement and recombination with other DNA in the host cell. These alterations in the genome create problems during assembly of the genome.

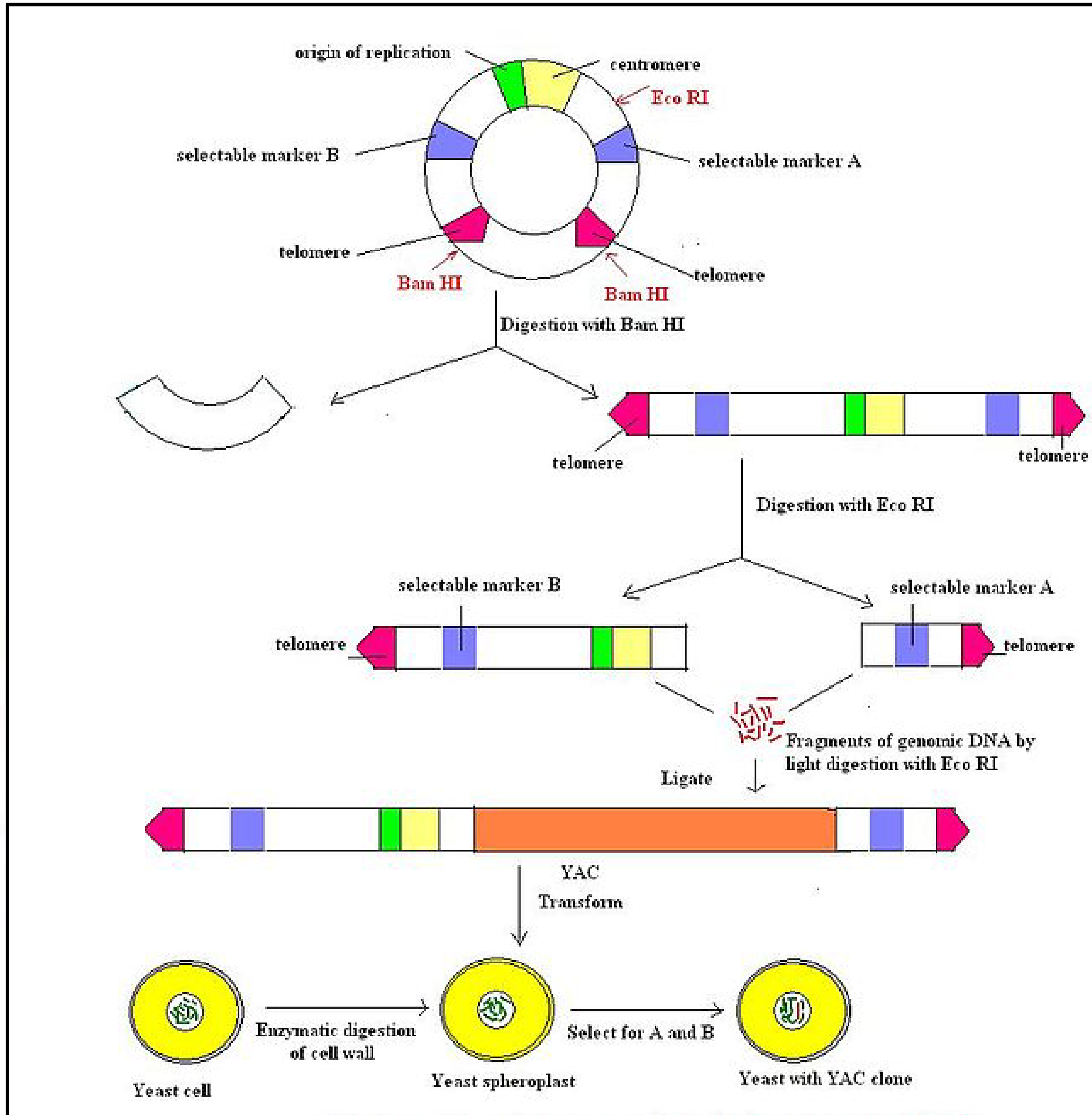


Figure: Cloning in a YAC library.

Source: http://en.wikibooks.org/wiki/Structural_Biochemistry/DNA_recombinant_techniques/Artificial_Chromosomes/Yeast_Artificial_Chromosomes_(YAC)#mediaviewer/File:Construction_of_a_YAC_chem114A.jpg(CC)

PAC (P1-derived artificial chromosome)

These vectors are constructed using DNA of P1bacteriophages.P1 bacteriophages have a much larger circular genome than phage and large genomic DNA fragments between 100 to 300 Kb in size can be cloned in this vector. These vectors were developedto overcome the problems associated with cosmid or phages. These vectors contain essential replication components of P1phage incorporated into plasmid. P1 is a temperate phage and therefore harbors two replication regions, one to control lytic growth cycle and other to maintain the plasmid during non-lytic growth cycle. After transfer to*E.coli*, bacteriophage P1 either expresses lytic growth cycle, producing 100-200 new bacteriophage particles and killing the infected bacterium or enters the non-lytic growth cycle. Transformation of PAC is simpler than transformation of YAC.

MAC (Mouse artificial chromosomes)

Mouse artificial chromosomes (MACs) were constructed from native mouse chromosomes. These vectors drive exogenous gene expression in cultured cells and transgenic animals.They can be maintained stably in mouse cells through germline transmission. These artificial chromosomes contain acceptor sites into which foreign genes can be inserted.

HAC (Human artificial chromosomes)

Human artificial chromosome (HAC) is a vector to clone full-length human genes. These vectors were synthesized in 1997. A cell containing HAC has an extra small chromosome in addition to normal chromosomes.HACs are constructed either by engineering natural chromosomes and introducing genetic material into them or by *denovo*synthesis of new chromosomes. These vectors provide an efficient system for transfer and expression of complete human genes of any size. These vectors lack copy number control and have large cloning capacity. HACs segregate freely from the host chromosomes therefore insertional mutants(insertion of transferred genetic material to host chromosome) cannot arise. HACs are preferable to othervectors like YACs and BACs. The transfer efficiency of intact HACs to the cells is quite low.

Expression Vectors

Cloning vector is a small molecule of DNA that can carry foreign DNA into a host cell and is capable of self-replication. It does not necessarily help to express a protein which the

inserted foreign DNA encodes in the cell. Expression vectors are constructed specifically to express the protein in the host cell.

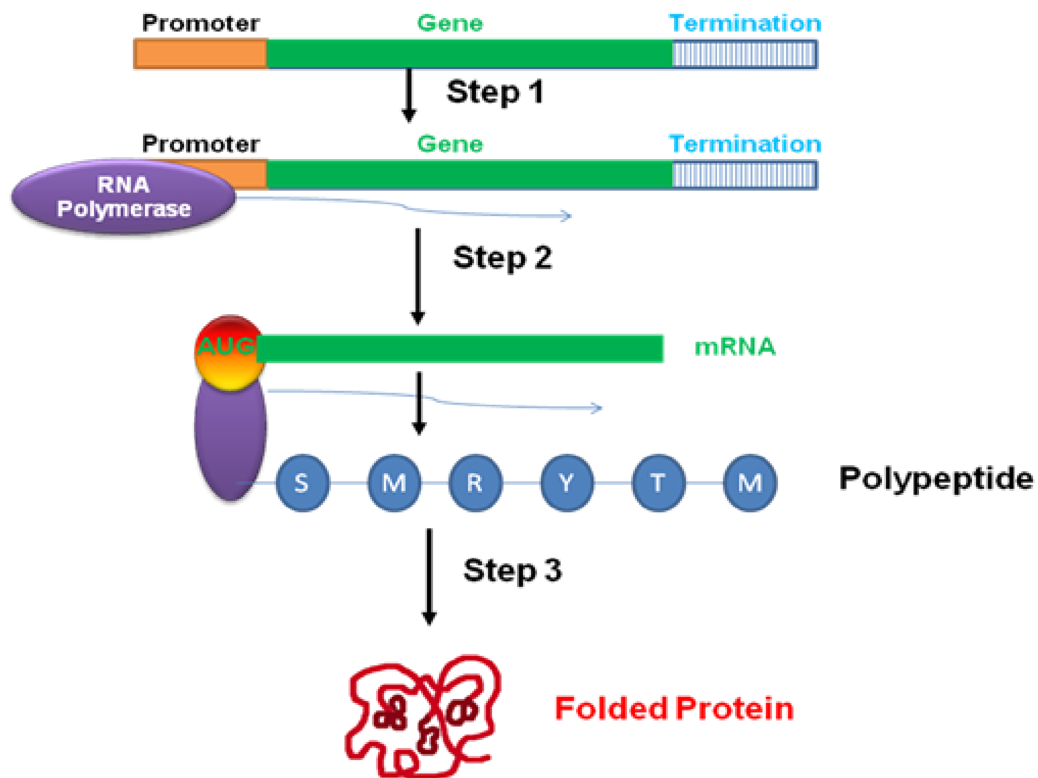


Figure: Steps in brief summary of protein synthesis in an *E.coli* expression system
Source: http://nptel.ac.in/courses/102103045/24

Prokaryotic vectors are useful for producing recombinant heterologous proteins from cloned eukaryotic complementary DNAs. However, sometimes eukaryotic proteins synthesized in bacteria either lack biological activity or are unstable. Quite a number of times, bacterial compounds that are either toxic or cause increase in body temperature in humans and animals contaminate the final product. Many proteins require post–translational modifications to be functional and therefore, prokaryotic organisms are unable to produce authentic version of eukaryotic proteins. To overcome these problems, researchers have developed eukaryotic expression system in yeast, insects, and mammalian cells, each with its own merits and demerits. These expression systems are useful for production of uncontaminated therapeutic agents for animals or humans, stable biologically active proteins for structural, biophysical and biochemical studies, and large quantities of proteins for industrial purposes.They have been used for production of insulin, enzymes, growth hormones, antibiotics, vaccines, antibodies. They are the basic tools of transgenic research

to produce transgenic plants and animals for eg. golden rice, insect resistant plants, glofish.The usage of expression system depends on the quality and quantity of recombinant protein that is produced, the cost of production and purification and ease of use. Unfortunately there is no single eukaryotic host cell that can produce functional protein from every cloned gene.

A basic eukaryotic expression vector system has all the elements of a cloning vector (an origin of replication, a selectable vector, multiple cloning site). Additionally it possesses a strong inducible eukaryotic promoter to drive the transcription of cloned gene, eukaryotic transcriptional stop signal, translational start sequence like ribosome binding site, Shine dalgarno sequence for expression in prokaryotes/ kozak consensus for expression in eukaryotes, a translation termination sequence, a sequence to add multiple adenines to mRNA (polyadenylation), a purification tag to ease out purification process.

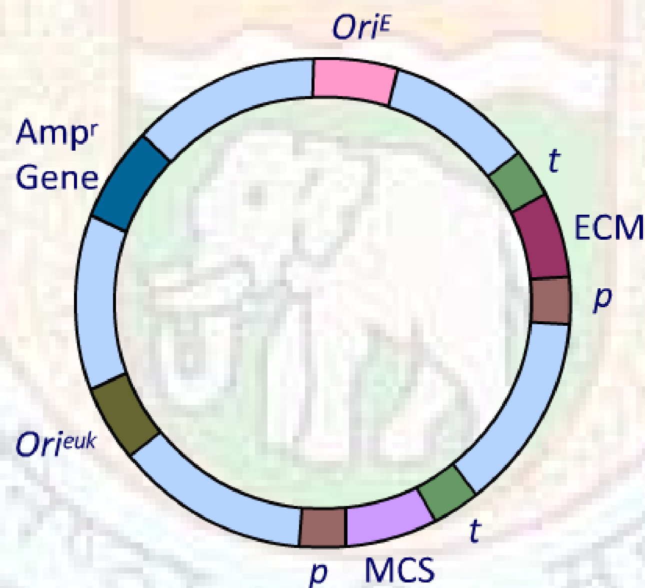


Figure: Generalized eukaryotic expression vector. The major features of a eukaryotic expression vector are a eukaryotic transcription unit with a promoter (p), a multiple cloning site (MCS) for a gene of interest and a DNA segment with termination and polyadenylation signals (t) ; a eukaryotic selectable marker (ESM).

Source: Author

Yeast expression systems

Cloning Vectors

Common yeast offers several advantages for use as a host cell for expression of eukaryotic cloned genes. Firstly it is a single celled organism and its genetics and physiology are well understood. It can be easily cultured. Several tightly regulatable, strong inducible promoters have earlier been isolated from yeast. It is also capable of carrying out many post translational modifications.

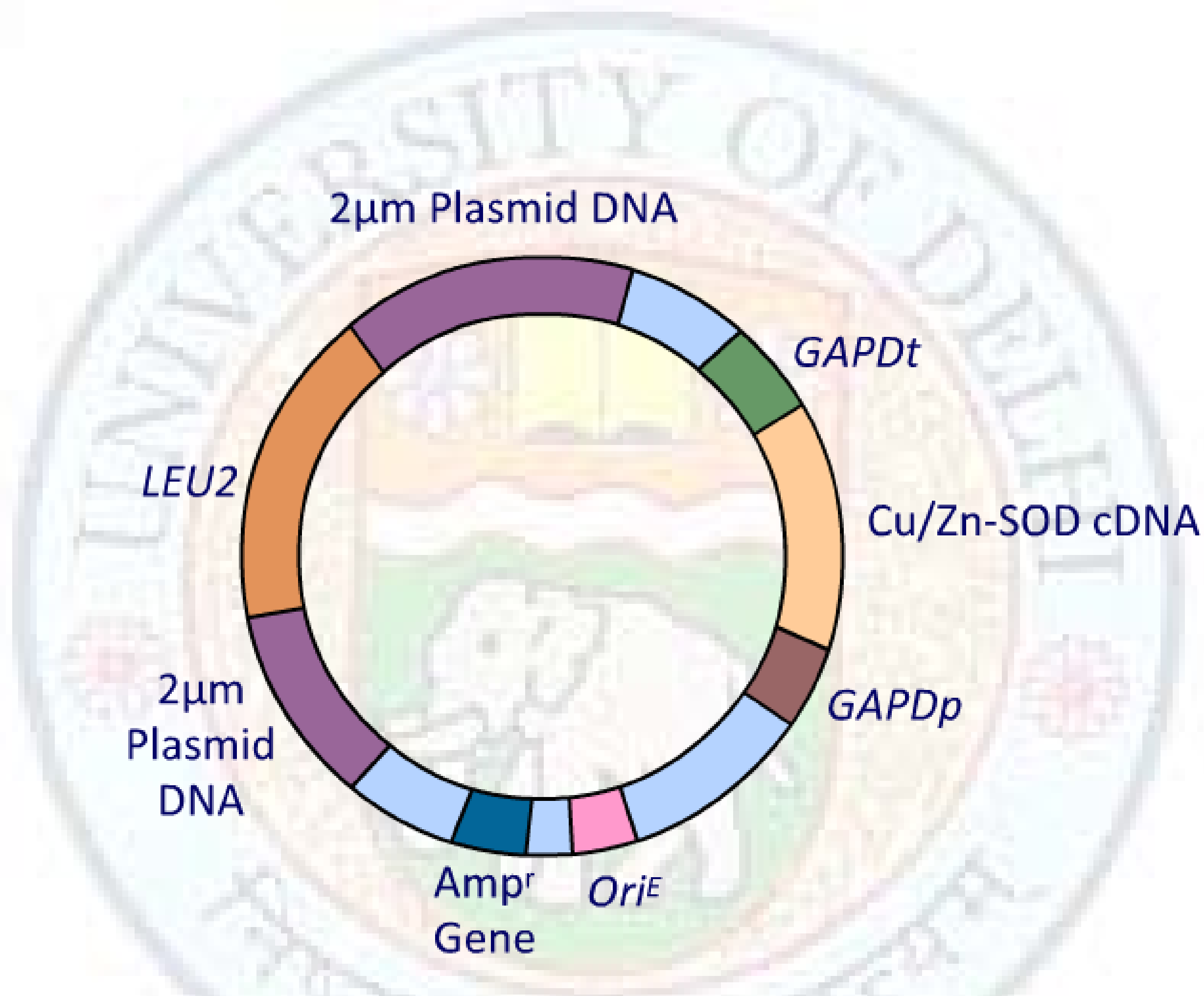


Figure: *S.cerevisiae* expression vector. The cDNA for human Cu/Zn SOD was cloned between the promoter (GAPDp) and termination-polyadenylation sequence(GAPDt) of the *S.cerevisiae* glyceraldehyde phosphate dehydrogenase gene. The LEU2 gene that was cloned between segments of the yeast2μM plasmid DNA. The ampicillin resistance gene (Amp^r) and the *E.coli* origin of replication (ori ^{E}) are derived from plasmid pBR322.

Source: Author

Yeast secrets very few proteins and therefore product protein can be readily purified. Lastly, it is recognized as safe organism by US food and drug administration. Many recombinant proteins have been produced by yeast expression system including vaccines (Hepatitis B virus surface antigen, HIV-1 envelope protein), human therapeutic agents (insulin, proinsulin, human growth factor, platelet derived growth factor, hirudin etc). However, with yeast as the host cell, recombinant heterologous proteins are hyperglycosylated and sometimes yields are low. To overcome these problems, methylotrophic yeast *Pichia pastoris* was developed because of the low occurrence of hyperglycosylation, the ease of obtaining higher yield and strong response of promoter.

Baculovirus infected insect cells

A non-fungal eukaryotic expression system producing biologically active proteins is AcMNPV baculovirus that infects insect cells.

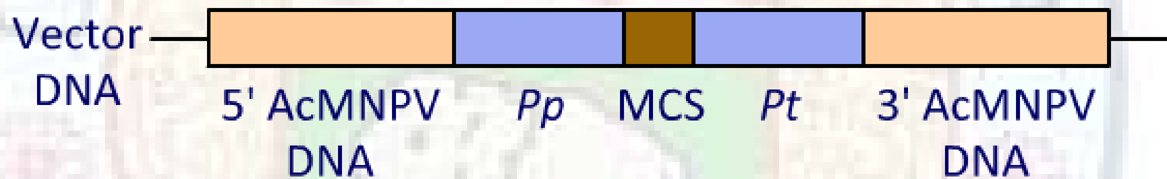


Figure: Organization of the expression unit of a baculovirus (AvMNPV) transfer vector. The gene of interest is inserted into the multiple cloning site (MCS) that lies between the polyhedron gene promoter (Pp) and the polyhedron gene transcription termination (Pt) sequences. The AcMNPV DNA upstream from the polyhedron promoter (5′ AcMNPV DNA) and downstream from the polyhedron transcription termination sequence (3′ AcMNPV DNA) provides sequences for integeration of the expression unit by homologous recombination into the AcMNPV genome.

Source: Author

Insect cells can carry out better post-translational modifications than yeast and carry better machinery for folding of proteins. Large amounts of recombinant heterologous proteins can be produced by infecting insect cells with baculovirus. The disadvantage of using insect cells is higher cost and time required to obtain purified protein.

Mammalian expression vectors

Mammalian cells lines for example HEK (human embryonal kidney) or CHO (Chinese hamster ovary) can be used for expression of proteins which need proper post-translational modifications. The standard eukaryotic expression vector system has two origins of replication, one eukaryotic usually derived from an animal virus and other prokaryotic from *E.coli.*, a prokaryotic and an eukaryotic selectable marker gene, elements regulating transcription of gene from animal viruses or mammalian genes.

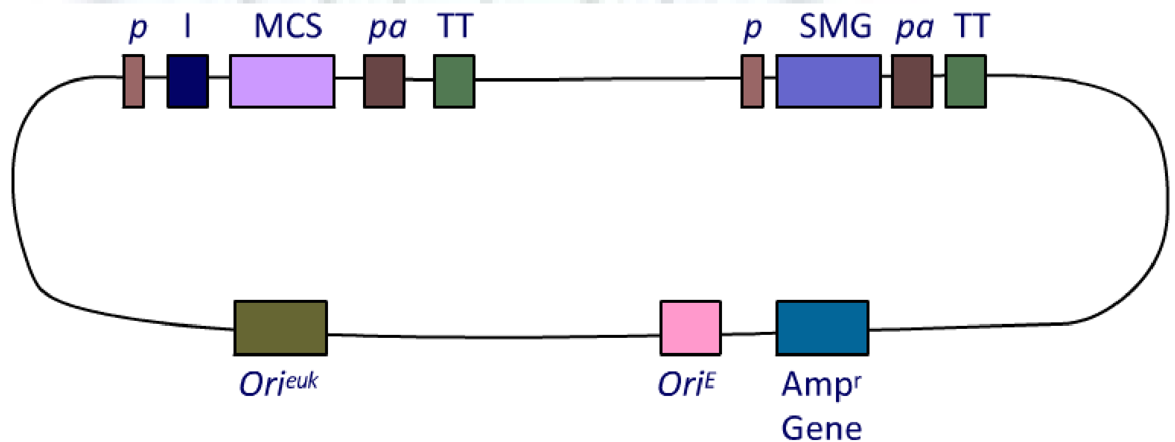


Figure: Generalized mammalian expression vector. The multiple cloning site (MCS) and selectable marker gene (SMG) are under the contro; of eukaryotic promoter (p) , polyadenylation (pa) and termination of transcription (TT) sequences. An intron (I) enhances the production of heterologous protein. Propagation of the vector in *E.coli* and mammalian cell depends on the origin of replication ori^{E} and ori^{euk} respectively. The ampicillin gene (AMP^{r}) is used for selecting transformed *E.coli* .

Source: Author

Various strategies have been developed for *invivo* assembly of proteins with two different subunits. These include *two vector expression system*, in which each vector harbors one cloned subunit of a protein dimer. After cotransfection, functional forms of both the subunits are synthesized. *Two gene expression vector system* include two different genes on the same vector. These cloned genes are in the same vector, but are under the control of different eukaryotic promoter, polyadenylation and termination of transcription sequence. Bicistronic expression vector has a single transcription unit with the two genes separated by an IRES.

Cloning Vectors

Summary

1. Many different types of vectors are constructed by researchers.A vector is a DNA molecule that is capable of replication in a host organism, and can act as a carrier molecule for the transfer of genes into the host. Choice of vectordepends on the sizes of the foreign DNA fragments to be cloned. Cloning vectors contain many of the same features: ability to self-replicate, multiple cloning site, an origin of replicationand a selectable marker.
2. Common vectors includeplasmids, bacteriophages, cosmids, YACs and BACs, PAC, MAC and HAC, each with theirown pros and cons.
3. Plasmids are circular double stranded 2.5 to 5 Kb long DNA molecules, present in bacteria that replicate autonomously from the host chromosome and are inherited in extra-chromosomal state from one generation to the other. They contain origin of replication, multiple cloning site, and a selectable maker gene.Insertional inactivation of antibiotic resistance gene and blue-white selection allows the detection of host cells containing recombinant plasmids.pBR322, pUC series and Ti plasmid are purpose build plasmid vectors.
4. Bacterial artificial chromosomes were created from natural plasmids of *E. coli* called the fertility factor (F factor) to accommodate millions of base pairs.
5. Bacteriophages can be used as vectors as larger non-viral DNA segments can be packaged in the virus particle. Lambda bacterio*phages* are most important and widely used vectors having linear genomes. The packaging mechanism in phage facilitates insertion of recombinant DNA. λ vectors can be of two types : (1) insertion vectors and (2) replacement or substitution vectors. M13, a filamentous phage with circular genome occurs in single-stranded form and is a very useful vector in large scale sequencing projects.
6. Phagemids are plasmid vectors with M13 origin of replication (pUC18 or pUC19 + M13 *ori*).
7. Cosmids are plasmid vectors containing cos sites of λ phage vectors.
8. Shuttle vectors are plasmid vectors that can replicate autonomously in two different hosts.
9. YACs are the largest capacity yeast vectors that can accommodate megabase range foreign DNA fragments within yeast cells.
10. PAC vectors are constructed using DNA of P1 bacteriophage.
11. MACs were constructed from native mouse chromosomes.

12. HAC is a vector to clone full-length human genes, constructed either by engineering natural chromosomes and introducing genetic material into them or by *denovo* synthesis of new chromosomes.
13. Expression vectors are constructed specifically to express the protein in the host cell.

Exercise

1. What features are required in all vectors used to propagate cloned DNA?
2. Name natural plasmids.
3. Discuss basic properties of a plasmid vector.
4. Define the following terms: (i) Insertional inactivation, (ii) Polylinker, (iii) Selectable marker, (iv) cos site, (v) T-DNA.
5. The lacZ gene is sometimes included in a cloning vector. What does it encode?
6. Name a detectable enzyme that is not produced if the gene is interrupted by an insert?
7. Name the selectable marker present in pBR322.
8. Give reasons for:
   a) Shuttle vectors are viable in two different species.
   b) Vectors must confer a selectable advantage to the cell (e.g., antibiotic resistance tag).
   c) Plasmids are suitable for cloning.
   d) The packaging mechanism in phage facilitates insertion of recombinant DNA.
9. What advantages do BACs and YACs provideover plasmids as cloning vectors?
10. List the disadvantages of using YACs as cloning vectors?
11. Which property of lambda phages is utilized for construction of cosmids? How are they better than plasmids and phages?
12. What are artificial chromosomes? Briefly explain human artificial chromosome and mouse artificial chromosome.
13. Differentiate between:
    a) Cosmids and phagemids
    b) λ insertion and λ replacement vectors
    c) BAC and YAC
    d) pBR322 and pUC18
    e) λ phage and M13 phage
    f) Plasmid and phage
    g) Cloning vector and expression vector

14. Write short notes on:
    a) Ti Plasmid
    b) BAC
    c) Shuttle vectors
    d) Lambda phage
    e) PAC
    f) Expression vector

Glossary

Agrobacterium tumefaciens: *Agrobacterium tumefactions*is a gram negative soil bacterium that can cause crown gall disease in which cells grow in an undifferentiated and uncontrolled manner to form a tumor.

Artificial chromosomes: They are segments of chromosomes containing inserts that harbor almost entire genome of an organism in a limited number of clones.

BAC: Bacterial artificial chromosomes arecreated from natural plasmids of *E. coli* called the fertility factor (F factor) and are capable of accommodating millions of base pairs.

Bacteriophage: They are also called phages and are viruses that infect bacteria.

β-galactosidase: A bacterial protein, encoded by the *lacZ* gene. It is the basis of blue white selection of recombinants.

Chromosome: A circular DNA molecule containing the organism's genome in prokaryotes. In eukaryotes, it is a thread like structure visible during mitosis and meiosis and composed of linear DNA molecule complexed with RNA and proteins.

Clone: Identical copies of a molecule, cells, or organisms.

Cloning: The production of identical copies of a DNA molecule by transformation and replication in a suitable host.

Cosmid: It is a circular double stranded DNA containing a prokaryotic origin of replication, a selection marker, cloning sites for restriction endonucleases and λ cos site.

Crown gall disease: Undifferentiated and uncontrolled growth of tissue caused by the Ti plasmid of *Agrobacterium tumefaciens*.

Electroporation:A transformation technique that uses an electric pulse to move polar vector molecules across the plasma membrane into the host cell.

F factor: The fertility plasmid of bacterial cells that carries *tra* genes to confer the ability to act as a donor in conjugation.

Lambda: The linear bacteriophage genome bears short (12 bp) single-stranded sequences at its ends known as cos sites which are complementary to each other and enable circularization. It isused for vector construction for the generation of insertion and replacement vectors.

Phagemid: They are plasmid vectors with M13 origin of replication.

Plaque: A clear zone in an opaque lawn of bacteria because of growth and reproduction of phage.

Plasmid: A double stranded extra chromosomal circular DNA molecule that replicates autonomously of the host chromosome.

Polylinker: It is also known as multiple cloning site and is a segment of DNA that has been constructed to contain sites for multiple restriction enzymes.

Restriction endonuclease: A bacterial enzyme that recognizes and cleaves double stranded DNA within or near specific nucleotide sequences called restriction sites.

Restriction site: A DNA sequence, within or near to which restriction endonuclease binds and cleaves the DNA.

Shuttle vector:A cloning vector that can replicate in two or more host organisms.

T-DNA: Region of Ti plasmid of *Agrobacterium tumefaciens* which can be transferred to the plant cell and is integrated into the plant DNA.

Ti plasmid:A plasmid from *Agrobacterium tumefaciens,* capable of causing crown gall diseaseand used as a vector to transfer foreign DNA to plant cells.

Transformation: A process by which genetic information is transferred in the form of exogenous DNA (as a heritable change) into a cell from its surrounding through cell membranes.

Vector: It is a double-stranded DNA molecule which can replicate autonomously in a host cell and into which a foreign DNA segment can be inserted. In essence it isa delivery agent/vehicle.

X-gal:A chromogenic substrate for ß-galactosidase and yields a blue-colored product on cleavage.

YAC: A cloning vector in the form of a yeast artificial chromosome, for cloning large DNA fragments synthesized using chromosomal components includinga centromere region (CEN), origin of replication (ARS),and marker genes from yeast.

References

1. Snustad, D.P. and Simmons, M.J. (2010). Principles of Genetics. John Wiley and Sons, U.K. 5th edition.
2. Klug, W.S., Cummings, M.R., Spencer, C.A. (2009). Concepts of Genetics. Benjamin Cummings, U.S.A. 9th edition.
3. http://faculty.tru.ca/dnelson/courses/biol335/335notes/3recdna/2-vectors/plasmid%20vectors/recDNA4a.html

Links for animations

Agrobacterium mediated transformation

Minliang Guo, Xiaowei Bian, Xiao Wu and Meixia Wu (2011). Agrobacterium-Mediated Genetic Transformation: History and Progress, Genetic Transformation, Prof. MarÃ-a Alvarez (Ed.), ISBN: 978-953-307-364-4, InTech, DOI: 10.5772/22026. Available from: http://www.intechopen.com/books/genetic-transformation/agrobacterium-mediated-genetic-transformation-history-and-progress

Construction of plasmid vectors:

http://www.youtube.com/watch?v=GDIOE9TveCI

Use of T-plasmid as a cloning vector:

http://www.youtube.com/watch?v=-20MY3nGU1M&feature=kp

Yeast vector

http://2011.igem.org/Team:Johns_Hopkins/Project/Vector#