DOWNLOAD PDF MOLECULAR CLONING OF RECOMBINANT DNA

Chapter 1 : Molecular cloning - Wikipedia

Mobilization and Reassembly of Genetic Information documents the proceedings of the Miami Winter Symposium, sponsored by the Department of Biochemistry, University of Miami School of Medicine, Miami, Florida, January

Bring fact-checked results to the top of your browser search. Creating the clone The steps in cloning are as follows. DNA is extracted from the organism under study and is cut into small fragments of a size suitable for cloning. Most often this is achieved by cleaving the DNA with a restriction enzyme. Restriction enzymes are extracted from several different species and strains of bacteria, in which they act as defense mechanisms against viruses. The most useful restriction enzymes make staggered cuts; that is, they leave a single-stranded overhang at the site of cleavage. These overhangs are very useful in cloning because the unpaired nucleotide s will pair with other overhangs made using the same restriction enzyme. So, if the donor DNA and the vector DNA are both cut with the same enzyme, there is a strong possibility that the donor fragments and the cut vector will splice together because of the complementary overhangs. The resulting molecule is called recombinant DNA. It is recombinant in the sense that it is composed of DNA from two different sources. The next step in the cloning process is to cut the vector with the same restriction enzyme used to cut the donor DNA. Vectors have target sites for many different restriction enzymes, but the most convenient ones are those that occur only once in the vector molecule. This is because the restriction enzyme then merely opens up the vector ring, creating a space for the insertion of the donor DNA segment. Of course, several types of unions are possible: Recombinant DNA associations form spontaneously in the above manner, but these associations are not stable because, although the ends are paired, the sugar- phosphate backbone of the DNA has not been sealed. This is accomplished by the application of an enzyme called DNA ligase , which seals the two segments, forming a continuous and stable double helix. The mixture should now contain a population of vectors each containing a different donor insert. This solution is mixed with live bacterial cells that have been specially treated to make their cells more permeable to DNA. Recombinant molecules enter living cells in a process called transformation. Usually, only a single recombinant molecule will enter any individual bacterial cell. Once inside, the recombinant DNA molecule replicates like any other plasmid DNA molecule, and many copies are subsequently produced. Furthermore, when the bacterial cell divides, all of the daughter cells receive the recombinant plasmid, which again replicates in each daughter cell. The original mixture of transformed bacterial cells is spread out on the surface of a growth medium in a flat dish Petri dish so that the cells are separated from one another. These individual cells are invisible to the naked eye, but as each cell undergoes successive rounds of cell division , visible colonies form. Each colony is a cell clone, but it is also a DNA clone because the recombinant vector has now been amplified by replication during every round of cell division. Thus, the Petri dish, which may contain many hundreds of distinct colonies, represents a large number of clones of different DNA fragments. This collection of clones is called a DNA library. By considering the size of the donor genome and the average size of the inserts in the recombinant DNA molecule, a researcher can calculate the number of clones needed to encompass the entire donor genome, or, in other words, the number of clones needed to constitute a genomic library. Another type of library is a cDNA library. A cDNA library represents a sampling of the transcribed genes, whereas a genomic library includes untranscribed regions. A cDNA library represents a collection of only the genes that are encoded into proteins by an organism. Both genomic and cDNA libraries are made without regard to obtaining functional cloned donor fragments. Genomic clones do not necessarily contain full-length copies of genes. Furthermore, genomic DNA from eukaryote s cells or organisms that have a nucleus contains introns, which are regions of DNA that are not translated into protein and cannot be processed by bacterial cells. This means that even full-sized genes are not translated in their entirety. In addition, eukaryotic regulatory signals are different from those used by prokaryotes cells or organisms lacking internal membranesâ€”i. However, it is possible to produce expression libraries by slicing cDNA inserts immediately adjacent to a bacterial promoter region on the vector; in these expression libraries, eukaryotic proteins are made in bacterial cells, which allows several important technological applications that are discussed below in DNA sequencing. Several bacterial viruses

have also been used as vectors. The most commonly used is the lambda phage. The central part of the lambda genome is not essential for the virus to replicate in Escherichia coli, so this can be excised using an appropriate restriction enzyme, and inserts from donor DNA can be spliced into the gap. In fact, when the phage repackages DNA into its protein capsule, it includes only DNA fragments the same length of the normal phage genome. Vectors are chosen depending on the total amount of DNA that must be included in a library. Cosmids are engineered vectors that are hybrid s of plasmid and phage lambda; however, they can carry larger inserts than either pUC plasmids plasmids engineered to produce a very high number of DNA copies but that can accommodate only small inserts or lambda phage alone. In yeast a eukaryotic organism a YAC behaves like a yeast chromosome and segregates properly into daughter cells. These vectors can carry the largest inserts of all and are used extensively in cloning large genomes such as the human genome.

Chapter 2 : DNA cloning and recombinant DNA (video) | Khan Academy

The success of recombinant DNA technology, by which microbial cells can be engineered to produce foreign proteins, relies on the faithful reading of the corresponding genes by bacterial cell machinery, and has fueled most of the recent advances in modern molecular biology.

The DNA of interest can then be propagated in a foreign host cell. This technology has been around since the s, and it has become a common practice in molecular biology labs today. Cloning is frequently employed to amplify DNA fragments containing genes, an essential step in their subsequent analysis. How Does Molecular Cloning Work? Cloning of any DNA sequence involves the introduction of a foreign piece of DNA into an extrachromosomal element cloning vector of an organism which then produces copies of the vector as it replicates itself, thereby amplifying the DNA of interest. The whole process can be summarized in the following steps: A cloning vector is simply a DNA molecule that can be inserted into a host cell and replicates inside the host bacteria or yeast , producing many copies of itself and the insert DNA. All cloning vectors contain - A sequence that allows for the propagation of itself in the host. An insertion site for the foreign DNA - also called a multiple cloning site that can be cut by several restriction enzymes. A method for selection of the host cells that contain the insert DNA of interest. This is most often done through the use of selectable markers for drug resistance. Types of Cloning Vectors: Plasmids - extrachromosomal circular DNA that autonomously replicates itself in a bacterial cell. Plasmids can be high or low copy numbers with an insert limit of about 10, base pairs 10 Kb Phage - viruses that infect bacteria usually derivatives of lambda bacteriophage. They are normally linear DNA molecules with regions that can be replaced without disrupting their ability to direct their replication by their bacterial hosts - insert limit of about 20 Kb Cosmids - circular extrachromosomal elements that combine elements of plasmids and phage - insert limit of Kb. Applications - amplifying 16s RNA genes to identify individual microbial species create cDNA libraries to determine what genes are being expressed at a particular time create genomic libraries in order to sequence organisms create multiple clone libraries in order to conduct metagenomic studies How to Clone DNA Fragments - Hide Diagram of the cloning vector puc 19 from dwb. Preparation of DNA fragments for cloning is frequently achieved by means of PCR, but it may also be accomplished by restriction enzyme digestion and sometimes fractionation by gel electrophoresis. Ligation - Subsequently, a ligation procedure is employed whereby the amplified fragment is inserted into a vector. The vector which is frequently circular is linearised by means of restriction enzymes, and incubated with the fragment of interest under appropriate conditions with an enzyme called DNA ligase. Transfection - Following ligation the vector with the insert of interest is transfected into cells. Most commonly electroporation is employed, although a number of alternative techniques are available, such as chemical sensitization of cells. Screening and Selection - Finally, the transfected cells are cultured. As the aforementioned procedures are of particularly low efficiency, there is need to identify the cell colonies that have been successfully transfected with the vector construct containing the desired insertion sequence. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected, to grow. Conformation - Nevertheless, these selection steps do not absolutely guarantee that the DNA insert is present in the cells obtained. Further investigation of the resulting colonies is required to confirm that cloning was successful.

Chapter 3 : Molecular Genetics, Recombinant DNA, & Genomic Technology | Basicmedical Key

In DNA cloning, recombinant DNA molecules are formed in vitro by inserting DNA fragments of interest into vector DNA molecules. The recombinant DNA molecules are then introduced into host cells, where they replicate, producing large numbers of recombinant DNA molecules that include the fragment of DNA originally linked to the vector.

Molecular cloning Molecular cloning is the laboratory process used to create recombinant DNA. There are two fundamental differences between the methods. The other difference is that cloning involves cutting and pasting DNA sequences, while PCR amplifies by copying an existing sequence. Vectors are generally derived from plasmids or viruses , and represent relatively small segments of DNA that contain necessary genetic signals for replication, as well as additional elements for convenience in inserting foreign DNA, identifying cells that contain recombinant DNA, and, where appropriate, expressing the foreign DNA. The choice of vector for molecular cloning depends on the choice of host organism, the size of the DNA to be cloned, and whether and how the foreign DNA is to be expressed. In standard cloning protocols, the cloning of any DNA fragment essentially involves seven steps: Protein production Following transplantation into the host organism, the foreign DNA contained within the recombinant DNA construct may or may not be expressed. That is, the DNA may simply be replicated without expression, or it may be transcribed and translated and a recombinant protein is produced. In addition, changes may be needed to the coding sequences as well, to optimize translation, make the protein soluble, direct the recombinant protein to the proper cellular or extracellular location, and stabilize the protein from degradation. That is, their appearance, behavior and metabolism are usually unchanged, and the only way to demonstrate the presence of recombinant sequences is to examine the DNA itself, typically using a polymerase chain reaction PCR test. In some cases, recombinant DNA can have deleterious effects even if it is not expressed. In some cases, researchers use this phenomenon to " knock out " genes to determine their biological function and importance. This can happen, for example, when a recombinant DNA fragment containing an active promoter becomes located next to a previously silent host cell gene, or when a host cell gene that functions to restrain gene expression undergoes insertional inactivation by recombinant DNA. Uses[edit] Recombinant DNA is widely used in biotechnology , medicine and research. In addition, organisms that have been manipulated using recombinant DNA technology, as well as products derived from those organisms, have found their way into many farms, supermarkets , home medicine cabinets , and even pet shops, such as those that sell GloFish and other genetically modified animals. The most common application of recombinant DNA is in basic research, in which the technology is important to most current work in the biological and biomedical sciences. Recombinant proteins are widely used as reagents in laboratory experiments and to generate antibody probes for examining protein synthesis within cells and organisms. Recombinant chymosin Found in rennet , chymosin is an enzyme required to manufacture cheese. It was the first genetically engineered food additive used commercially. Traditionally, processors obtained chymosin from rennet, a preparation derived from the fourth stomach of milk-fed calves. Scientists engineered a non-pathogenic strain K of E. This microbiologically produced recombinant enzyme, identical structurally to the calf derived enzyme, costs less and is produced in abundant quantities. A variety of different recombinant insulin preparations are in widespread use. This unsafe practice led to some patients developing Creutzfeldtâ€”Jakob disease. Recombinant HGH eliminated this problem, and is now used therapeutically. DrugBank entry Recombinant hepatitis B vaccine Hepatitis B infection is controlled through the use of a recombinant hepatitis B vaccine, which contains a form of the hepatitis B virus surface antigen that is produced in yeast cells. The development of the recombinant subunit vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as polio virus , cannot be grown in vitro. Insect-resistant crops Bacillus thuringeiensis is a bacterium that naturally produces a protein Bt toxin with insecticidal properties. Recently, plants have been developed that express a recombinant form of the bacterial protein, which may effectively control some insect predators. Environmental issues associated with the use of these transgenic crops have not been fully resolved. Cohen professor at Stanford University ; this patent was awarded in At the Asilomar Conference on Recombinant DNA , these concerns were discussed

and a voluntary moratorium on recombinant DNA research was initiated for experiments that were considered particularly risky. Today, recombinant DNA molecules and recombinant proteins are usually not regarded as dangerous. However, concerns remain about some organisms that express recombinant DNA, particularly when they leave the laboratory and are introduced into the environment or food chain. These concerns are discussed in the articles on genetically modified organisms and genetically modified food controversies. Furthermore, their are concerns about the bi-products in biopharmaceutical production, where recombinant DNA result in specific protein products.

Chapter 4 : Overview: DNA cloning (article) | Khan Academy

Molecular cloning generally uses DNA sequences from two different organisms: the species that is the source of the DNA to be cloned, and the species that will serve as the living host for replication of the recombinant DNA. Molecular cloning methods are central to many contemporary areas of modern biology and medicine.

The method entails clipping the desired segment out of the surrounding DNA and copying it millions of times. The success of recombinant DNA technology, by which microbial cells can be engineered to produce foreign proteins, relies on the faithful reading of the corresponding genes by bacterial cell machinery, and has fueled most of the recent advances in modern molecular biology. During the last twenty years, studies of cloned DNA sequences have given us a detailed knowledge of gene structure and organization, and have provided clues to the regulatory pathways by which the cell controls gene expression in the multiple cell types comprising the basic vertebrate body plan. Genetic engineering, by which an organism can be modified to include new genes designed with desired characteristics, is now routine practice in basic research laboratories. It has provided the means to produce large amounts of highly purified normal and mutant proteins for detailed analysis of their function in the organism. Recent advances in this technology have also changed the course of medical research. Exciting new approaches are being developed to exploit the enormous potential of recombinant DNA research in the analysis of genetic disorders. The new ability to manipulate human genetic material has opened radically new avenues for diagnosis and treatment, and has far-reaching consequences for the future of medicine. Cloning DNA Molecular cloning provides a means to exploit the rapid growth of bacterial cells for producing large amounts of identical DNA fragments, which alone have no capacity to reproduce themselves. The fragment of DNA to be amplified is first inserted into a cloning vector. The most popular vectors currently in use consist of either small circular DNA molecules plasmids or bacterial viruses phage. The vectors contain genetic information that allows bacterial DNA replication machinery to copy them. After insertion of the foreign DNA, the plasmid or phage vector is re-introduced into a bacterial cell. The growing bacterial culture replicates the foreign DNA, along with the vector, in hundreds of copies per cell. This process yields multiple, identical clones of the original recombinant molecule. It is easy to harvest vectors from the bacterial culture, and release the amplified foreign DNA fragments with the same restriction enzyme used to insert the original DNA fragment into the vector Figure 4, top. The power of molecular cloning is remarkable: For analysis of long stretches of DNA, eukaryotic vectors that can grow in yeast have been developed which can hold megabases of foreign DNA. These vectors mimic yeast chromosomal structure, so that they are replicated along with the native yeast chromosomes every time a yeast cell divides. Yeast Artifical Chromosomes, or YACs, are often the only way to clone extremely large genes including huge introns all in one continuous piece. YACs also provide a way to propagate DNA in a eukaryotic cell, where DNA modification, an important part of the eukaryotic genetic regulatory machinery, is more likely to be retained more on this later. YACs are increasingly useful in the many Genome Projects underway, as we aim to understand the metastructure of chromosomes, where the placement and arrangement of genes within the "junk" DNA surrounding them may hold as yet undiscovered regulatory information for packaging and accessibility. The plasmid vector brown is prepared to accept the isolated genomic DNA fragment by cutting the circular plasmid DNA at a single site with the same restriction enzyme, generating sticky ends which are complementary to the sticky ends of the genomic DNA fragment. The cut genomic DNA and the linearized plasmid are mixed together in the presence of a ligase enzyme, which rejoins the bonds in the DNA backbone on each side of the plasmid-genomic DNA junction. This recombinant DNA molecule is then introduced into bacteria which are able to take up plasmid DNA, and then replicate the plasmid as the culture grows.

Chapter 5 : Recombinant DNA | Biology Biological Principles

Molecular cloning is a set of techniques used to insert recombinant DNA from a prokaryotic or eukaryotic source into a replicating vehicle such as plasmids or viral vectors. Cloning refers to making numerous copies of a DNA fragment of interest, such as a gene.

Sinauer Associates ; Search term Recombinant DNA Classical experiments in molecular biology were strikingly successful in developing our fundamental concepts of the nature and expression of genes. Since these studies were based primarily on genetic analysis, their success depended largely on the choice of simple, rapidly replicating organisms such as bacteria and viruses as models. It was not clear, however, how these fundamental principles could be extended to provide a molecular understanding of the complexities of eukaryotic cells , since the genomes of most eukaryotes e. In the early s, the possibility of studying such genomes at the molecular level seemed daunting. In particular, there appeared to be no way in which individual genes could be isolated and studied. This obstacle to the progress of molecular biology was overcome by the development of recombinant DNA technology, which provided scientists with the ability to isolate, sequence, and manipulate individual genes derived from any type of cell. The application of recombinant DNA has thus enabled detailed molecular studies of the structure and function of eukaryotic genes, thereby revolutionizing our understanding of cell biology. Restriction Endonucleases The first step in the development of recombinant DNA technology was the characterization of restriction endonucleases â€" enzymes that cleave DNA at specific sequences. These enzymes were identified in bacteria, where they apparently provide a defense against the entry of foreign DNA e. Bacteria have a variety of restriction endonucleases that cleave DNA at more than a hundred distinct recognition sites, each of which consists of a specific sequence of four to eight base pairs examples are given in Table 3. Since restriction endonucleases digest DNA at specific sequences, they can be used to cleave a DNA molecule at unique sites. These fragments can be separated according to size by gel electrophoresisâ€"a common method in which molecules are separated based on the rates of their migration in an electric field. A gel, usually formed from agarose or polyacrylamide, is placed between two buffer compartments containing electrodes. Nucleic acids are negatively charged because of their phosphate backbone , so they migrate toward the positive electrode. The gel acts like a sieve, selectively retarding the movement of larger molecules. Smaller molecules therefore move through the gel more rapidly, allowing a mixture of nucleic acids to be separated on the basis of size. These fragments are then separated by electrophoresis in an agarose gel. The DNA fragments migrate toward the positive more The locations of cleavage sites for multiple different restriction endonucleases can be used to generate detailed restriction maps of DNA molecules, such as viral genomes Figure 3. In addition, individual DNA fragments produced by restriction endonuclease digestion can be isolated following electrophoresis for further studyâ€"including determination of their DNA sequence. The DNAs of many viruses have been characterized by this approach. Restriction endonuclease digestion alone, however, does not provide sufficient resolution for the analysis of larger DNA molecules, such as cellular genomes. However, restriction endonuclease digestion of larger genomes yields quite different results. Such a large number of fragments cannot be separated from one another, so agarose gel electrophoresis of EcoRI-digested human DNA yields a continuous smear rather than a discrete pattern of DNA fragments. Because it is impossible to isolate single restriction fragments from such digests, restriction endonuclease digestion alone does not yield a source of homogeneous DNA suitable for further analysis. Quantities of such purified DNA fragments, however, can be obtained through molecular cloning. Large quantities of the inserted DNA can be obtained if the recombinant molecule is allowed to replicate in an appropriate host. These recombinant molecules can then be introduced into E. The DNA of these phages can then be isolated, yielding large quantities of recombinant molecules containing a single fragment of human DNA. Moreover, the fragment can be easily isolated from the rest of the vector DNA by restriction endonuclease digestion and gel electrophoresis, allowing a pure fragment of human DNA to be analyzed and further manipulated. The resulting recombinant molecule is then introduced into E. The DNA fragments used to create recombinant molecules are usually

generated by digestion with restriction endonucleases. Many of these enzymes cleave their recognition sequences at staggered sites, leaving overhanging or cohesive single-stranded tails that can associate with each other by complementary base pairing Figure 3. The association between such paired complementary ends can be established permanently by treatment with DNA ligase , an enzyme that seals breaks in DNA strands see Chapter 5. Thus, two different fragments of DNA e. Vector and insert DNAs are digested with a restriction endonuclease such as EcoRI , which cleaves at staggered sites leaving overhanging single-stranded tails. Vector and insert DNAs can then associate by complementary base more The fragments of DNA that can be cloned are not limited to those that terminate in restriction endonuclease cleavage sites. Linkers are short oligonucleotides that can be readily obtained by chemical synthesis, allowing virtually any fragment of DNA to be prepared for ligation to a vector. Since eukaryotic genes are usually interrupted by noncoding sequences introns; see Chapter 4 , which are removed from mRNA by splicing, the ability to clone cDNA as well as genomic DNA has been critical for understanding gene structure and function. Vectors for Recombinant DNA Depending on the size of the insert DNA and the purpose of the experiment, many different types of cloning vectors can be used for the generation of recombinant molecules. The basic vector systems used for the isolation and propagation of cloned DNAs are reviewed here. Other vectors developed for the expression of cloned DNAs and the introduction of recombinant molecules into eukaryotic cells are discussed in subsequent sections. DNA inserts can be as large as about 15 kb and still yield a recombinant genome that can be packaged into phage particles. The phage particles are then used to infect cultures of E. Since each recombinant phage forms a single plaque, recombinants carrying unique inserts of human DNA can be isolated. In addition, recombinant phages containing particular genes of interest can be identified by nucleic acid hybridization or other screening methods, as discussed in the next section. The vector contains a restriction site e. In addition, cos sites cohesive ends , which are required for packaging DNA into phage particles, are present on both more Plasmid vectors Figure 3. Plasmids are small circular DNA molecules that can replicate independentlyâ€”without being associated with chromosomal DNAâ€”in bacteria. In addition, plasmid vectors carry genes that confer resistance to antibiotics e. The vector is a small circular molecule that contains an origin of replication ori , a gene conferring resistance to ampicillin Ampr , and a restriction site e. To be cloned into a plasmid vector , a fragment of the insert DNA is ligated to an appropriate restriction site in the vector and the recombinant molecule is used to transform E. Antibiotic-resistant colonies, which contain plasmid DNA, are selected. Such plasmid-containing bacteria can then be grown in large quantities and their DNA extracted. The small circular plasmid DNA molecules, of which there are often hundreds of copies per cell, can be separated from the bacterial chromosomal DNA; the result is purified plasmid DNA that is suitable for analysis of the cloned insert. Cosmid and yeast artificial chromosome YAC vectors can be used for this purpose. Cosmid vectors Figure 3. In addition, cosmids contain origins of replication and the genes for antibiotic resistance that are characteristic of plasmids, so they are able to replicate as plasmids in bacterial cells. Even larger fragments of DNA hundreds of kilobases can be cloned in YAC vectors, which replicate as chromosomes in yeast cells. These vectors are particularly useful for chromosome mapping studies, as discussed in Chapter 4. Large fragments of insert DNA approximately 45 kb are ligated to a cloning site e. DNA Sequencing Molecular cloning allows the isolation of individual fragments of DNA in quantities suitable for detailed characterization, including the determination of nucleotide sequence. Indeed, determination of the nucleotide sequences of many genes has elucidated not only the structure of their protein products, but also the properties of DNA sequences that regulate gene expression. Furthermore, the coding sequences of novel genes are frequently related to those of previously studied genes, and the functions of newly isolated genes can often be correctly deduced on the basis of such sequence similarities. Current methods of DNA sequencing are both rapid and accurate, and determining the sequence of several kilobases of DNA is a straightforward task for most molecular biology laboratories. Thus, it is now far easier to clone and sequence DNA than it is to determine the amino acid sequence of a protein. Since the nucleotide sequence of a gene can be readily translated into the amino acid sequence of its encoded protein, the easiest way of determining protein sequence is the sequencing of a cloned gene. DNA synthesis is initiated from a primer that has been labeled at one end with a radioisotope. Four separate reactions are run, each including one dideoxynucleotide either A, C, G, or T

in addition to its normal counterpart. Thus, a series of labeled DNA molecules is generated, each terminating at the base represented by the dideoxynucleotide in each reaction. These fragments of DNA are then separated according to size by gel electrophoresis and detected by exposure of the gel to X-ray film autoradiography. The size of each fragment is determined by its terminal dideoxynucleotide, so the DNA sequence corresponds to the order of fragments read from the gel. These molecules are incorporated normally into more Large-scale DNA sequencing is frequently performed using automated systems, which use fluorescence-labeled primers in dideoxynucleotide sequencing reactions Figure 3. As the newly syntehsized DNA strands are electrophoresed through a gel, they pass through a laser beam that excites the fluorescent label. The resulting emitted light is then detected by a photomultiplier, and a computer collects and analyzes the data. This type of automated DNA sequencing has enabled the large-scale analysis required for determination of the complete genome sequences of bacteria, yeast, C. Four separate sequencing reactions are performed, each containing one chain-terminating dideoxynucleotide and a primer labeled with a distinct fluorescent tag. The products are then pooled and subjected to gel electrophoresis. Expression of Cloned Genes In addition to enabling determination of the nucleotide sequences of genesâ€”and hence the amino acid sequences of their protein productsâ€”molecular cloning has provided new approaches to obtaining large amounts of proteins for structural and functional characterization. Many proteins of interest are present at only low levels in eukaryotic cells and therefore cannot be purified in significant amounts by conventional biochemical techniques. Given a cloned gene , however, this problem can be solved by the engineering of vectors that lead to high levels of gene expression in either bacteria or eukaryotic cells. To express a eukaryotic gene in E. Purifying the protein encoded by the cloned gene in quantities suitable for detailed biochemical or structural studies is then a straightforward matter. Expression vectors contain promoter sequences pro that direct transcription of inserted DNA in bacteria and sequences required for binding of mRNA to bacterial ribosomes Shine-Delgarno [SD] sequences. It is frequently useful to express high levels of a cloned gene in eukaryotic cells , rather than in bacteria. This mode of expression may be important, for example, to ensure that posttranslational modifications of the protein such as addition of carbohydrates or lipids occur normally. Such protein expression in eukaryotic cells can be achieved, as in E. One system frequently used for protein expression in eukaryotic cells is infection of insect cells by baculovirus vectors, which direct very high levels of expression of genes inserted in place of a viral structural protein. Alternatively, high levels of protein expression can be achieved using appropriate vectors in mammalian cells. Expression of cloned genes in yeast is particularly useful because simple methods of yeast genetics can be employed to identify proteins that interact with other cloned proteins or with specific DNA sequences. An alternative method to isolating large amounts of a single DNA molecule is the polymerase chain reaction PCR , which was developed by Kary Mullis in The number of DNA molecules increases exponentially, doubling with each round of replication, so a substantial quantity of DNA can be obtained from a small number of initial template copies. For example, a single DNA molecule amplified through 30 cycles of replication would theoretically yield approximately 1 billion progeny molecules. Single DNA molecules can thus be amplified to yield readily detectable quantities of DNA that can be isolated by molecular cloning or further analyzed directly by restriction endonuclease digestion or nucleotide sequencing. A specific region of DNA can be amplified from such a mixture, provided that the nucleotide sequence surrounding the region is known so that primers can be designed to initiate DNA synthesis at the desired point.

Chapter 6 : DNA Cloning with Plasmid Vectors - Molecular Cell Biology - NCBI Bookshelf

Molecular cloning, a term that has come to mean the creation of recombinant DNA molecules, has spurred progress throughout the life sciences. Beginning in the s, with the discovery of restriction endonucleases - enzymes that selectively and specifically cut molecules of DNA - recombinant DNA technology has seen exponential growth in both application and sophistication, yielding.

Outline the process of molecular cloning of a gene or segment of DNA Describe appropriate methods for cloning eukaryotic genes Compare and contrast the vectors and procedures used for creating genetically modified bacteria, plants and animals Describe genome editing with CRISPR-Cas9, including what is required for targeting and how target genes can be modified Many of our drugs, much of our food, and even our clothing are now produced using recombinant DNA technology. Instead of depending on random mutation, and either natural or artificial selection, we now have the ability to directly manipulate the genes of organisms to create new proteins and new capabilities in our domesticated bacteria, fungi, plants and animals. Recombinant DNA requires 3 key molecular tools: Cutting DNA at specific sites â€" most often performed by enzymes called restriction endonucleases restriction enzymes. The plasmid containing the inserted DNA segment will replicate in host cells. Molecular cloning of a foreign DNA fragment into a plasmic vector for incorporation into a bacterial cell. The bacterial host replicates the DNA to high copy number. The details of screening using amp and X-gal will not be covered in this course. Cloning eukaryotic genes Molecular cloning of eukaryotic genes is often either unfeasible or undesirable, or both, because they contain numerous, large introns. Plasmid vectors have a practical size limit of less than 10 kilo-base pairs kbp , and PCR is also difficult beyond about 10 kb. The mRNA, lacking introns, is a compact version of a eukaryotic gene that retains all of the protein coding information. The enzyme reverse transcriptase can be used, along with an oligo-dT primer that is complementary to the polyA tail, to synthesize a complementary DNA cDNA molecule. Adapters with restriction endonuclease sites or PCR primer sequences can be ligated to the ends of the completed cDNA to facilitate cloning into plasmids or amplification by PCR. Original illustration by J. The point of creating GMOs is usually to alter their traits, most often so they express a new gene. Plasmid vectors therefore have the cloning site within a second antibiotic resistance gene or within the lacZ gene encodes beta-galactosidase. Insertion of a foreign DNA segment will disrupt the gene. These colonies stay white. Blue colonies are discarded, and white colonies are picked for further testing. The lac promoter provides a means to regulate transcription, and protein coding sequences in the inserted DNA can be expressed as a fusion protein, containing the first few amino acids of the E. Expression of foreign genes in eukaryotes Vectors for expression of foreign genes in eukaryotic cells must provide appropriate eukaryotic promoters upstream of the cloning site, for transcription by the eukaryotic host cell, as well as downstream polyadenylation and transcription termination signals. For single-celled organisms such as yeast, and cultured cells, bacterial plasmids containing foreign genes can be transformed into the cells. For multicellular organisms, the delivery of genes into the cells of the organism poses special challenges and requires special vectors and delivery methods. We describe these challenges for one application, human gene therapy, in the next section. Gene therapy Gene therapy poses a special challenge in delivering recombinant DNA into host cells. Recombinant DNA technology can readily clone a functional copy of a defective gene and insert it into a vector with the correct regulatory sequences. But how can we deliver this functional gene into the cells of a person who has already been born? The most promising techniques use viruses. Viruses evolved to be highly efficient at delivering their own genetic information into host cells. Replacing the viral replication genes with a therapeutic human gene eliminates the ability of the virus to replicate, while co-opting the viral infection mechanism to deliver the therapeutic gene into the nuclei of host cells. Genome editing One technology developed in recent years, and being widely adopted in research labs around the world, is CRISPR-Cas9 technology, and variants. This technology enables researchers to delete, add, or replace particular bits of DNA in a cell. Human genome editing may be less controversial than human genetic modification, because no non-human DNA is added. Also, J-Lo is producing a TV series titled with the name of this technique. In

essence, Cas9 is a protein that cuts DNA. Whereas restriction endonucleases cut DNA at fixed sites, Cas9 is programmable. So in any organism where the genome sequence is known, scientists can make an sgRNA to target a particular DNA sequence in the genome, and cut it. By providing Cas9 protein, sgRNA, and a homologous template DNA that includes a desired change, scientists have successfully made precise changes in genomes of many kinds of cells and organisms, including cultured human cells. A gene combining this target sequence and a tracrRNA gene sequence is put into an expression plasmid along with the Cas9 protein coding gene. Genome engineering with zinc-finger nucleases. Genetics Society of America, , 4 , pp Put it all together: In class we will discuss how these concepts are applied to current gene therapy methods undergoing research and development.

Chapter 7 : Recombinant DNA | Fundamentals of Biology | Biology | MIT OpenCourseWare

The basic strategy in molecular cloning is to insert a DNA fragment of interest (e.g., a segment of human DNA) into a DNA molecule (called a vector) that is capable of independent replication in a host cell.

Search term Section 7. In the case of DNA , this is feasible for relatively short molecules such as the genomes of small viruses. But genomes of even the simplest cells are much too large to directly analyze in detail at the molecular level. The problem is compounded for complex organisms. Cleavage of human DNA with restriction enzymes that produce about one cut for every base pairs yields some 2 million fragments, far too many to separate from each other directly. With these methods virtually any gene can be purified, its sequence determined, and the functional regions of the sequence explored by altering it in planned ways and reintroducing the DNA into cells and into whole organisms. When a single recombinant DNA molecule, composed of a vector plus an inserted DNA fragment, is introduced into a host cell, the inserted DNA is reproduced along with the vector, producing large numbers of recombinant DNA molecules that include the fragment of DNA originally linked to the vector. Two types of vectors are most commonly used: In this section, the general procedure for cloning DNA fragments in E. These extrachromosomal DNAs, which occur naturally in bacteria, yeast, and some higher eukaryotic cells, exist in a parasitic or symbiotic relationship with their host cell. Plasmids range in size from a few thousand base pairs to more than kilobases kb. During cell division, at least one copy of the plasmid DNA is segregated to each daughter cell, assuring continued propagation of the plasmid through successive generations of the host cell. For example, some bacterial plasmids encode enzymes that inactivate antibiotics. Such drug-resistance plasmids have become a major problem in the treatment of a number of common bacterial pathogens. As antibiotic use became widespread, plasmids containing several drug-resistance genes evolved, making their host cells resistant to a variety of different antibiotics simultaneously. Such transfer can result in the rapid spread of drug-resistance plasmids, expanding the number of antibiotic-resistant bacteria in an environment such as a hospital. Coping with the spread of drug-resistance plasmids is an important challenge for modern medicine. Generally, these plasmids have been engineered to optimize their use as vectors in DNA cloning. Most plasmid vectors contain little more than the essential nucleotide sequences required for their use in DNA cloning: Diagram of a simple cloning vector derived from a plasmid, a circular, double-stranded DNA molecule that can replicate within an E. Host-cell enzymes bind to ORI, initiating replication of the circular plasmid. The parental strands are shown in blue, and newly synthesized daughter strands are shown in red. Once DNA replication more Selection of Transformed Cells In , O. This process involved the genetic alteration of a bacterial cell by the uptake of DNA isolated from a genetically different bacterium and its recombination with the host-cell genome. Their experiments provided the first evidence that DNA is the genetic material. Later studies showed that such genetic alteration of a recipient cell can result from the uptake of exogenous extrachromosomal DNA e. The term transformation is used to denote the genetic alteration of a cell caused by the uptake and expression of foreign DNA regardless of the mechanism involved. Note that transformation has a second meaning defined in Chapter 6, namely, the process by which normal cells with a finite life span in culture are converted into continuously growing cells similar to cancer cells. The phenomenon of transformation permits plasmid vectors to be introduced into and expressed by E. In order to be useful in DNA cloning , however, a plasmid vector must contain a selectable gene , most commonly a drug-resistance gene encoding an enzyme that inactivates a specific antibiotic. After plasmid vectors are incubated with E. The ability to select transformed cells is critical to DNA cloning by plasmid vector technology because the transformation of E. Exposure of cells to high concentrations of certain divalent cations, however, makes a small fraction of cells permeable to foreign DNA by a mechanism that is not understood. In a typical procedure, E. Each competent cell incorporates a single plasmid DNA molecule, which carries an antibiotic-resistance gene. When the treated cells are plated on a petri dish of nutrient agar containing the antibiotic, only the rare transformed cells containing the antibiotic-resistance gene on the plasmid vector will survive. All the plasmids in such a colony of selected transformed cells are descended from the single plasmid taken up by the cell that established the

colony. When such a recombinant plasmid transforms an E. The inserted DNA is replicated along with the rest of the plasmid DNA and segregates to daughter cells as the colony grows. In this way, the initial fragment of DNA is replicated in the colony of cells into a large number of identical copies. Since all the cells in a colony arise from a single transformed parental cell, they constitute a clone of cells. The initial fragment of DNA inserted into the parental plasmid is referred to as cloned DNA, since it can be isolated from the clone of cells. Figure General procedure for cloning a DNA fragment in a plasmid vector. Although not indicated by color, the plasmid contains a replication origin and ampicillin-resistance gene. Uptake of plasmids by E. DNA cloning allows fragments of DNA with a particular nucleotide sequence to be isolated from a complex mixture of fragments with many different sequences. As a simple example, assume you have a solution containing four different types of DNA fragments, each with a unique sequence Figure Each fragment type is individually inserted into a plasmid vector. The resulting mixture of recombinant plasmids is incubated with E. Since each colony that develops arose from a single cell that took up a single plasmid, all the cells in a colony harbor the identical type of plasmid characterized by the DNA fragment inserted into it. As a result, copies of the DNA fragments in the initial mixture are isolated from one another in the separate bacterial colonies. DNA cloning thus is a powerful, yet simple method for purifying a particular DNA fragment from a complex mixture of fragments and producing large numbers of the fragment of interest. Isolation of DNA fragments from a mixture by cloning in a plasmid vector. Four distinct DNA fragments, depicted in different colors, are inserted into plasmid cloning vectors, yielding a mixture of recombinant plasmids each containing a single DNA fragment. As noted in the introduction, restriction enzymes and DNA ligases are utilized to produce such recombinant DNA molecules. Restriction enzymes are bacterial enzymes that recognize specific 4- to 8-bp sequences, called restriction sites, and then cleave both DNA strands at this site. Since these enzymes cleave DNA within the molecule, they are also called restriction endonucleases to distinguish them from exonucleases, which digest nucleic acids from an end. Because the DNA isolated from an individual organism has a specific sequence, restriction enzymes cut the DNA into a reproducible set of fragments called restriction fragments Figure

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Molecular cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules and to direct their replication within host blog.quintoapp.com use of the.

Beginning in the s, with the discovery of restriction endonucleases â€" enzymes that selectively and specifically cut molecules of DNA â€" recombinant DNA technology has seen exponential growth in both application and sophistication, yielding increasingly powerful tools for DNA manipulation. Cloning genes is now so simple and efficient that it has become a standard laboratory technique. This has led to an explosion in the understanding of gene function in recent decades. Emerging technologies promise even greater possibilities, such as enabling researchers to seamlessly stitch together multiple DNA fragments and transform the resulting plasmids into bacteria, in under two hours, or the use of swappable gene cassettes, which can be easily moved between different constructs, to maximize speed and flexibility. In the near future, molecular cloning will likely see the emergence of a new paradigm, with synthetic biology techniques that will enable in vitro chemical synthesis of any in silico-specified DNA construct. These advances should enable faster construction and iteration of DNA clones, accelerating the development of gene therapy vectors, recombinant protein production processes and new vaccines. Rebecca Tirabassi, Bitesize Bio. Introduction Molecular cloning refers to the isolation of a DNA sequence from any species often a gene , and its insertion into a vector for propagation, without alteration of the original DNA sequence. The clones can also be manipulated and mutated in vitro to alter the expression and function of the protein. The basic cloning workflow includes four steps: Isolation of target DNA fragments often referred to as inserts Ligation of inserts into an appropriate cloning vector, creating recombinant molecules e. A summary of the discoveries that comprise traditional molecular cloning is described in the following pages. Recombinant DNA technology first emerged in the late s, with the discovery of enzymes that could specifically cut and join double-stranded DNA molecules. But the nature of the factor was not discovered until , when Arber and Linn succeeded in isolating an enzyme, termed a restriction factor, that selectively cut exogenous DNA, but not bacterial DNA 3. These studies also identified a methylase enzyme that protected the bacterial DNA from restriction enzymes. The full power of restriction enzymes was not realized until restriction enzymes and gel electrophoresis were used to map the Simian Virus 40 SV40 genome 5. The cleaved DNA can then be ligated to a plasmid vector possessing compatible ends. Much like the discovery of enzymes that cut DNA, the discovery of an enzyme that could join DNA was preceded by earlier, salient observations. In the early s, two groups discovered that genetic recombination could occur though the breakage and ligation of DNA molecules 6,7 , closely followed by the observation that linear bacteriophage DNA is rapidly converted to covalently closed circles after infection of the host 8. Just two years later, five groups independently isolated DNA ligases and demonstrated their ability to assemble two pieces of DNA These studies pioneered the concept that, because of the universal nature of DNA, DNA from any species could be joined together. Recombinant DNA technology would be severely limited, and molecular cloning impossible, without the means to propagate and isolate the newly constructed DNA molecule. The ability to transform bacteria, or induce the uptake, incorporation and expression of foreign genetic material, was first demonstrated by Griffith when he transformed a non-lethal strain of bacteria into a lethal strain by mixing the non-lethal strain with heat-inactivated lethal bacteria In the same year, Avery, Macleod and McCarty demonstrated that DNA, and not protein, was responsible for inducing the lethal phenotype Initially, it was believed that the common bacterial laboratory strain, E. Cohen applied this principle, in , when he pioneered the transformation of bacteria with plasmids to confer antibiotic resistance on the bacteria Building on the Groundwork While scientists had discovered and applied all of the basic principles for creating and propagating recombinant DNA in bacteria, the process was inefficient. Restriction enzyme preparations were unreliable due to non-standardized purification procedures, plasmids for cloning were cumbersome, difficult to work with and limited in number, and experiments were limited by the amount of insert DNA that could be isolated. Research over the next few decades led to improvements in the techniques and tools available for molecular cloning. Development of the first standardized vector. In , they

described the first vector designed for cloning purposes, pBR Vectors with on-board screening and higher yields. Although antibiotic selection prevented non-transformed bacteria from growing, plasmids that re-ligated without insert DNA fragments self-ligation could still confer antibiotic resistance on bacteria. Therefore, finding the correct bacterial clones containing the desired recombinant DNA molecule could be time-consuming. Vieira and Messing devised a screening tool to identify bacterial colonies containing plasmids with DNA inserts. When bacteria were plated on the correct media, white colonies contained plasmids with inserts, while blue colonies contained plasmids with no inserts. Early work with restriction enzymes was hampered by the purity of the enzyme preparation and a lack of understanding of the buffer requirements for each enzyme. In , New England Biolabs NEB became the first company to commercialize restriction enzymes produced from a recombinant source. This enabled higher yields, improved purity, lot-to-lot consistency and lower pricing. NEB currently supplies over of these specificities. NEB was also one of the first companies to develop a standardized four-buffer system, and to characterize all of its enzyme activities in this buffer system. This led to a better understanding of how to conduct a double digest, or the digestion of DNA with two enzymes sim-ultaneously. With the advent of commercially available restriction enzyme libraries with known sequence specificities, restriction enzymes became a powerful tool for screening potential recombinant DNA clones. Vector and insert preparation. Cloning efficiency and versatility were also improved by the development of different techniques for preparing vectors prior to ligation. It was soon discovered that treatment of vectors with Calf-Intestinal Phosphatase CIP dephosphorylated DNA ends and prevented self-ligation of the vector, increasing recovery of plasmids with insert The CIP enzyme proved difficult to inactivate, and any residual activity led to dephosphorylation of insert DNA and inhibition of the ligation reaction. The discovery of the heat-labile alkaline phosphatases, such as recombinant Shrimp Alkaline Phosphatase rSAP and Antarctic Phosphatase AP both sold by NEB , decreased the steps and time involved, as a simple shift in temperature inactivates the enzyme prior to the ligation step DNA sequencing was developed in the late s when two competing methods were devised. The Sanger method quickly became automated, and the first automatic sequencers were sold in The ability to determine the sequence of a stretch of DNA enhanced the reliability and versatility of molecular cloning. Once cloned, scientists could sequence clones to definitively identify the correct recombinant molecule, identify new genes or mutations in genes, and easily design oligonucleotides based on the known sequence for additional experiments. The impact of the polymerase chain reaction. One of the problems in molecular cloning in the early years was obtaining enough insert DNA to clone into the vector. In , Mullis devised a technique that solved this problem and revolutionized molecular cloning He amplified a stretch of target DNA by using opposing primers to amplify both complementary strands of DNA, simultaneously. Through cycles of denaturation, annealing and polymerization, he showed he could exponentially amplify a single copy of DNA. The polymerase chain reaction, or PCR, made it possible to amplify and clone genes from previously inadequate quantities of DNA. Cloning of PCR products. The advent of PCR meant that researchers could now clone genes and DNA segments with limited knowledge of amplicon sequence. However, there was little consensus as to the optimal method of PCR product preparation for efficient ligation into cloning vectors. Several different methods were initially used for cloning PCR products. The simplest, and still the most common, method for cloning PCR products is through the introduction of restriction sites onto the ends of the PCR product This allows for direct, directional cloning of the insert into the vector after restriction digestion. Blunt-ended cloning was developed to directly ligate PCR products generated by polymerases that produced blunt ends, or inserts engineered to have restriction sites that left blunt ends once the insert was digested. This was useful in cloning DNA fragments that did not contain restriction sites compatible with the vector The vector and insert are then mixed, denatured and annealed, allowing hybridization of the insert to the vector. Overlap extension PCR enabled researchers to piece together large genes that could not easily be amplified by traditional PCR methods. Overlap extension PCR was also used to introduce mutations into gene sequences Overview of PCR Development of specialized cloning techniques. In an effort to further improve the efficiency of molecular cloning, several specialized tools and techniques were developed that exploited the properties of unique enzymes. The PCR product can be easily ligated into a vector that has been cut and engineered to contain

single T residues on each strand. When mixed together, the vector and insert anneal through the long stretch of compatible ends. The length of the compatible ends is sufficient to hold the molecule together in the absence of ligase, even during transformation. Once transformed, the gaps are repaired in vivo. There are several different commercially available products for LIC. USER cloning was first developed in the early s as a restriction enzyme- and ligase-independent cloning method Many new, elegant technologies allow for the assembly of multiple DNA fragments in a one-tube reaction. The advantages of these technologies are that they are standardized, seamless and mostly sequence independent. Then, the DNA fragments to be assembled adjacent to one another are engineered to contain blocks of complementary sequences that will be ligated together. These could be compatible cohesive ends, such as those used for Gibson Assembly, or regions containing recognition sites for site-specific recombinases Gateway. The enzyme used for DNA ligation will recognize and assemble each set of compatible regions, creating a single, contiguous DNA molecule in one reaction. DNA synthesis is an area of synthetic biology that is currently revolutionizing recombinant DNA technology. Although a complete gene was first synthesized in vitro in 40 , DNA synthesis of large DNA molecules did not become a reality until the early s, when researchers began synthesizing whole genomes in vitro 41, These early experiments took years to complete, but technology is accelerating the ability to synthesize large DNA molecules. Conclusion In the last 40 years, molecular cloning has progressed from arduously isolating and piecing together two pieces of DNA, followed by intensive screening of potential clones, to seamlessly assembling up to 10 DNA fragments with remarkable efficiency in just a few hours, or designing DNA molecules in silico and synthesizing them in vitro. Together, all of these technologies give molecular biologists an astonishingly powerful toolbox for exploring, manipulating and harnessing DNA, that will further broaden the horizons of science. Among the possibilities are the development of safer recombinant proteins for the treatment of diseases, enhancement of gene therapy 43 , and quicker production, validation and release of new vaccines But ultimately, the potential is constrained only by our imaginations. Rebecca Tirabassi is an Assistant Editor at Bitesizebio.

Chapter 9 : Recombinant DNA - Wikipedia

DNA cloning is a molecular biology technique that makes many identical copies of a piece of DNA, such as a gene. In a typical cloning experiment, a target gene is inserted into a circular piece of DNA called a plasmid.

Translational Control Molecular Cloning DNA replication involves the copying of each strand of the double helix to give a pair of daughter strands. Replication begins at a specific sequence, called the origin. After initiation begins at an origin sequence, all sequences are replicated no matter what their information. This principle leads to the idea of molecular cloning, or recombinant DNA. Cloning enables the production of a single DNA sequence in large quantities. A recombinant DNA consists of two parts: Vectors supply replication functionsâ€"the origin sequences to the recombinant DNA molecule. After it joins to a vector, any passenger sequence can be replicated. The process of joining the vector and passenger DNAs is called ligation. DNA ligase carries out ligation by using ATP energy to make the phosphodiester bond between the vector and passenger. After they are ligated to a vector, it is possible to make an essentially unlimited amount of the passenger sequence. Plasmid vectors Plasmids are circular DNAs that are capable of independent replication. Many naturally occurring bacteria contain plasmids; plasmid vectors are derived from naturally occurring plasmids. Plasmid vectors have several properties. First, they contain single restriction sites for several enzymes. Cleaving with one of these enzymes generates a single, linear form of the plasmid. This feature helps to ensure efficient ligation, because every ligation product will contain the entire vector sequence. Secondly, vectors are made to contain selectable genetic markers so that cells which contain the vector can be propagated. Figure 1 Thirdly, a means to detect which cells have only the plasmid vector as opposed to the recombinant product must exist. This determination is usually accomplished by a mechanism called insertional inactivation. The idea of insertional inactivation is that inserting passenger DNA into a gene interrupts the sequence of the gene, thereby inactivating it. When cells containing just the vector are grown in the presence of an artificial substrate related to lactose, the colonies turn blue, because active enzyme is made. On the other hand, when the restriction site has a piece of foreign DNA inserted into it, the gene cannot make an active protein fragment because the DNA sequence interrupts the coding sequence of the gene. As a result, colonies of the bacteria that contain cloned foreign DNA appear whitish. The bacteria that are present in the colony can be grown separately, and standard biochemical procedures easily isolate the recombinant DNA they contain. After a recombinant plasmid has been formed in the laboratory, it must be replicated. This process begins with the growth of the cell containing the recombinant plasmid. Plasmids are usually transferred to new hosts by transformation. Transformation is the addition of naked foreign DNA into a recipient bacterium. First, growth in the presence of the antibiotic ensures that each bacterial colony contains a plasmid, while the color of the colony identifies the plasmids that contain inserted DNA. Finally, the growth of the transformed bacterial colony amplifies the clones, that is, makes more copies of the plasmid. A collection of different cloned DNAs is called a library. The number of independent sequences in a library is called its complexity; the more complex the library, the greater number of independent sequences it contains. Other types of cloning vectors Viruses that infect bacteria are called bacteriophages. Native bacteriophage have been formed into vectors that can also be used for cloning. Bacteriophage vectors have three advantages over plasmids. First, they can carry significantly more foreign DNA than can plasmids, which are limited to about 5, base pairs 5 kilobases of foreign DNA. The larger the insert, the fewer independent clones are required to have a reasonable chance of identifying any single gene or sequence in the collection of cloned DNA. Secondly, the virus particles of bacteriophage vectors can accept DNA only of a narrow size range. This means that DNA can be preselected so that each recombinant virus will contain only a single foreign sequence. This property is a consequence of the fact that DNA whether native or foreign must be packaged into a protein coat to be infectious. Finally, bacteriophage infection can be a very efficient process, with nearly percent of the packaged virus particles leading to a productive infection. In contrast, cells take up only one of , plasmid molecules in a transformation procedure. Bacteriophage clones are amplified by repeated cycles of infection and growth.