BACTERIOPHAGE AND ITS VECTORS pdf

1: Phages | Everything about Bacteriophage

Bacteriophage lambda vectors were developed because several observations were made that suggested that they could complete their life cycles even if foreign DNA was inserted into a portion of its genome.

Open in a separate window AAV: Tissue-Specific Promoter This group of promoters is active and mediates transgene expression in only specific tissues. Several tissue-specific promoters that target tumors of a single origin were characterized and used in cancer gene therapy [10]. Examples include the ovarian-specific promoter to target ovarian cancer [11], the albumin promoter to target hepatocellular carcinoma [12], and the thyroglobulin promoter for thyroid carcinomas [13]. The tyrosine kinase promoter has been used, both in vitro and in vivo, to target melanomas [14]. The prostate-specific antigen PSA promoter was used in targeting prostate cancer. PSA is predominantly expressed in prostate cells due to transcriptional activation. Both in cell culture and in vivo, PSA promoter has been previously shown to express the herpes simplex virus thymidine kinase HSVtk suicide gene in PSA-positive prostate cancer cells and prostate tumors, respectively; however, no transgene expression was observed in cells that do not express PSA [27]. Although these promoters proved efficient to deliver transgene expression in tumor cells, their activity, in both normal, as well as tumor cells, is deemed to be a major drawback. Tumor-Specific Promoter Tumor-specific promoters constitute an ideal choice for targeted cancer gene therapy in order to direct the expression of therapeutic genes, as they have been shown to be highly active in tumor cells while having little or no activity in normal cells. Based on their characteristics, tumor-specific promoters have been subdivided into four groups [10]: Cancer-Specific Promoters Cancer-specific promoters, such as the promoter of the telomerase gene, are active specifically in malignant cells and have the great potential in cancer gene therapy to target a wide variety of tumors. The promoters of these two subunits are highly active in telomerase-positive cells, such as tumor and fetal cells. Thus, these two promoters have been individually used in many targeted cancer gene therapy studies to drive therapeutic gene expression, demonstrating enhanced killing of telomerase-positive cells. Although the hTR or hTERT promoters have been broadly utilized for transcriptional regulation of therapeutic genes, they still have some limitations in clinical use as they possess low activity and some potential toxicity to certain normal cells has also been reported [15]. Tumor-Type-Specific Promoter Tumor-type-specific promoters are the promoters of oncofetal genes that are often overexpressed in certain types of tumors and are silent in normal tissues. This promoter was extensively used in different vector systems to selectively deliver various therapeutic genes, such as cytosine deaminase or HSVtk expression in CEA-positive cells, and the results demonstrated significant tumor growth suppression or regression, with no toxicity to liver and other normal organs, following prodrug 5-fluorocytosine or ganciclovir GCV administration, respectively [30]. Although these promoters mediate transgene expression in tumor tissues, and may, therefore, be good candidates for transcriptional targeting in cancer gene therapy, their application still remains limited since they cannot be administered for a variety of tumors. Tumor Vasculature-Related Promoters The genes encoding this group of promoters are overexpressed in proliferating endothelia tumor microvasculature. In addition, the promoters of genes encoding Flt-1, vascular endothelial growth factor receptor 1, and human preproendothelin-1 have also been used in gene therapy vector systems and proved efficient to drive transgene expression in the vasculature of tumors and metastases [16]. While the usage of these promoters in targeted cancer gene therapy proved efficient to deliver transgene expression in the tumor vasculature, they still have limitations as some of these promoters were shown to be active in small vessels and upregulated in injured vessels as well [31]. Tumor Microenvironment-Related Promoters Tumor microenvironment-related promoters belong to the genes that are upregulated in response to the tumor microenvironment and physiology. Compared to normal cells, tumor cells demonstrate high growth rate and an increased glucose metabolism. Recently, the heat shock genes, such as the gene of the Glucose regulated protein 78 Grp78 , have gained increasing interest in targeted cancer gene therapy because of their activation in a wide variety of tumors. The activity of its promoter and its ability to drive transgene expression within areas of tumor hypoxia, which are highly resistant to current forms of treatment, makes it even a more attractive promoter to

use in targeted cancer gene therapy. Indeed, therapeutic transgene expression driven by this promoter is induced in response to insufficient blood supply and tumor necrosis and reached high levels leading to complete tumor eradication in preclinical models [32]. For cancer targeting gene therapy, the Grp78 promoter seems to be an ideal promoter to restrict expression of the therapeutic gene within the tumor tissue, and is, therefore, worthwhile a critical evaluation. Grp78 as an Endogenous Macromolecule in Cancer Grp78 gene encodes a kDa protein Grp78 that acts as an endoplasmic reticulum ER stress response chaperone. Despite this homology, Grp78 is not induced by heat stress and it also primarily localizes in the endoplasmic reticulum ER lumen to function as a molecular chaperone in an ATP-dependent manner. Recently, however, this protein was shown to be present in the cytoplasm and expressed on the cell surface membranes; thus, it can be utilized as a biomarker for stressed cells, such as in tumors [33]. Also known as immunoglobulin heavy chain binding protein BiP , Grp78 orchestrates unfolded protein response UPR by binding to unfolded, misfolded, and incorrectly glycosylated proteins in the ER lumen. In eukaryotic cells, when the protein production exceeds the folding capacity of the ER, the misfolded proteins elicit UPR. Under normal conditions, Grp78 remains bound to three transmembrane sensor proteins: As the threshold of the incorrectly folded protein load exceeds a certain level, Grp78 dissociates from the sensors and binds these proteins. This dissociation process leads to activation of a signaling cascade, UPR. The outcome is a decrease in biosynthetic burden of the ER by desensitizing the cells to ER stress and ultimately upregulating pro-survival genes via transcriptional activation in the nucleus, such as Grp78 promoter to elevate Grp78 expression [33]. If, however, the ER homeostasis cannot be re-established, the UPR induces programed cell death [34]. Tumor microenvironment and lack of pace of neovascularization and angiogenesis result in an uncontrolled production of mutant and misfolded ER proteins that lead to the accumulation of unfolded or misfolded ER proteins, which subsequently trigger the UPR. Thus, while Grp78 expression remains low in major adult organs, such as brain, heart, and lung, it is highly upregulated in transformed cells and in several tumors, such as glioblastoma GBM , breast cancer, and prostate cancer [35], and in the endothelia of tumor vasculature [36]. This overexpression of Grp78 has been shown to correlate with an enhanced tumor recurrence risk, tumor grade, and decreased survival rate in cancer patients [34 , 37]. Grp78 plays a major role in cancer cell survival by activating the pro-survival pathway. In addition to primary tumors, Grp78 levels are highly induced in metastasis and assist secondary tumor survival by maintaining neovascularization [36]. Pyrko and colleagues demonstrated a positive correlation between Grp78 overexpression and glioblastoma GBM cell proliferation rate by knockdown of Grp78 in GBM cells that led to a reduced proliferation [38]. In addition to tumor cell survival and proliferation, cytoplasmic Grp78 plays an important role in blocking the apoptosis of stressed cells by binding and inhibiting activation of caspases-7 and Grp78 Promoter in Cancer Gene Therapy The use of Grp78 as a promoter in cancer gene therapy was first proposed by the group of Lee [25]. A retroviral vector construct carrying the HSVtk transgene was used to infect tumor cells and subsequently implanted into mice. Administration of GCV, along with HSVtk expression, driven by Grp78 promoter, was shown to suppress and eradicate murine and human breast tumor xenografts in mice [32]. Moreover, another group assessed the efficacy of HSVtk under the control of Grp78 promoter in gastroesophageal junction and gastric adenocarcinomas cells and reported significant cell death in vitro and tumor regression in vivo following GCV treatment [39]. In transgenic mice, the LacZ transgene, driven by the rat Grp78 promoter, showed high transgene expression in cancer cells, while remaining inactive in major adult organs [32]. In addition, Grp78 gene transcription can increase over time [40] because, unlike viral promoters, such as CMV, mammalian promoters are not silenced in eukaryotic cells, thus, resulting in stronger and long-term transgene expression from the vector. Ligand-Directed Targeting Ensures Specific and Efficient Transgene Delivery Progress in tumor vascular targeting has provided a platform to target agents safely, efficiently and selectively in tumorigenic tissues. The use of in vivo phage display screenings has significantly contributed to the identification of such target receptors in the affected tumor endothelium of animal models [41]. As angiogenesis is vital for tumor progression, targeting these tumor-specific and tumor associated endothelial cell-specific receptor molecules holds the potential for ligand-directed targeting in cancer gene therapy Table 2 [42]. Viral vectors can be engineered to display homing peptides on their surface in order to target specific

receptor-bearing cell types within a host. This method of targeting ensures that the vector only infects the cells bearing the receptor, while leaving non-receptor-bearing cells untouched. Table 2 List of receptors used in targeted cancer therapy [42].

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2: Cloning vector - Wikipedia

Bacteriophage vectors 1. BACTERIOPHAGE VECTORS 2. BACTERIOPHAGE Virus that infect bacteria is known as bacteriophage. It was discovered by www.enganchecubano.com in Great Britian () and Felix d' Herelle in France(). D' Herelle coined the term bacteriophage meaning 'bacterial eater' to describe the agent's bacteriocidal activity.

The following points highlight the six main types of cloning vectors. Bacteriophages as Vectors 3. Cosmids as Vectors 4. Phagemids as Vectors 5. The first vector that was developed for gene cloning was plasmids which are versatile and thus widely used. Their replication depends on the same enzymes that replicate the chromosome of the host cells, and they are distributed to daughter cells along with the host chromosome during cell-division. Plasmids range in size from a few thousand base pairs to more than kilo-bases 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. Many naturally occurring plasmids contain genes that help the host cells. For example, some bacterial plasmids encode enzymes that inactivate antibiotics. Therefore, a bacterial cell containing such plasmid is resistant to the antibiotic and can replicate in an environment containing the antibiotic, whereas the same type of bacterium lacking the drug-resistant plasmid is killed. Such property of plasmid is exploited in their use as cloning vectors. These plasmids are widely used as cloning vectors. A single plasmid may not possess all the characteristics required for easy DNA cloning. An MCS increases the number of potential cloning strategies available by extending the range of enzymes that can be used to generate a restriction fragment suitable for cloning. By combining them within an MCS, the sites are made contiguous, so that any two sites within it can be cloned simultaneously without excising vector sequences. Examples of some plasmids: In cloning experiments, the pBR group of plasmids is the most widely used cloning vectors. Among these, pBR has been completely sequenced through modification of earlier plasmids of E. The pBR plasmid is bp in length and has an origin of replication ori that has derived from a plasmid related to naturally occurring plasmid ColEI. It also possesses genes conferring resistance to antibiotics, e. There are over 10 enzymes with unique cleavage sites on the pBR genome Fig. The target sites of these enzymes lie within the tetracyclin resistant gene tetr and there are sites for further two ClaI and Hindlll within the promoter of that gene. Six unique restriction sites lie within the ampr gene. Thus, cloning in pBR with the aid of any one of these enzymes will result in insertional inactivation of either the ampr or the tetr markers. However, cloning in the other unique sites does not permit the easy selection of recombinants, because neither of the antibiotic resistance determinants is inactivated. Thus, this site is excellent for cloning by the homopolymer tailing method as described earlier. However, in some recombinants tetr is retained or even increased. This is due to the presence of Hindlll site within the promoter rather than the coding sequence. Another vector pBR was derived from pBR by deletion of nucleotides between to to reduce the size of this vector. This eliminated nucleotide sequences were also known to interfere with the expression of the cloned DNA in eukaryotic cells. However, pBR plasmids also possess the two antibiotic resistance genes, ampr and tetr. Therefore, the insert bearing plasmids can be selected by their ability to grow in a medium containing only one of two antibiotics and by their failure to grow in a medium having both the antibiotics. The plasmids, on the other hand, containing no DNA insert, will be able to grow in media containing one or both the antibiotics. These plasmids possess several advantages as a vector for cloning like: Within the lac region a large number of restriction enzyme sites have been engineered, called a multiple cloning poly-linker site. When pUC18 having no inserts are transformed into host bacterial cells, through the action of lac Z- gene, bacterial host cells will produce blue colonies. Some phages are widely used as cloning vectors. But for cloning of larger pieces of DNA e. However, in order to optimize the insertion capacity, phage DNA itself may be modified according to the purpose. Several phages used commonly in DNA cloning are described below in brief. The wild-type A virus particle or virion contains a 50 kb liner double-stranded DNA as its genome which is packaged within a protein coat. Due to this cohesive properties such sequence is called the cos sequence Fig. Once inside the bacterial cell, the cos sequences base-pair and sealing of the nicks by cellular ligases results in the formation of a double-stranded circular DNA. In the lytic cycle, the viral DNA

replicates, initially bi-directionally, and subsequently by a rolling circle model resulting in linear multi-mers of the virion. The lambda genome possesses a gene att which has a homolog in the E. In this condition the phage DNA is called provirus and the host cell as lysogen. In the lytic state, the cro gene dominates, causing the repression of cl. Normally, the lysogenic state is favoured and the phage genome is replicated along the host chromosomal DNA. In this way several important cloning vectors have been developed by modifying the lambda phage genome. Using this strategy, foreign DNA up to 23 kb in length can be cloned in such vectors. These two vectors are so designed that a central non-essential part of about 44kb long can be replaced by a foreign DNA. This central part can be replaced by foreign DNA of kb long. These two vectors have poly-linkers with reverse orders of restriction sites with respect to each other. These vectors are used for making cDNA libraries. The insertion of foreign DNA inactivates cl gene and generates a clâ€" phage. It allows screening of recombinant phages. Further, in the E. Like plasmids, these cosmids perpetuate in bacteria and do not carry the genes for lytic development. However, the disadvantage is its inability to accept more than kbp of DNA Fig. Phagemid vectors are prepared artificially like cosmid by combining the features of phages with plasmids, as the name suggests. It is derived from the plasmid pUC19 and is bp long. Vectors for cloning large DNA fragments are often required, because human and other mammalian genomes are very large. These vectors have various cloning capacity Table 9. Most vectors that are used for cloning DNA in bacterial cells contain high to moderate number of replicons. Such phenomenon is common in case of DNA inserts of eukaryotic origin where repetitive sequences occur frequently. Therefore, it is very difficult to clone and maintain large DNA in bacterial cells. One such vector is prepared from the E. Some bacteriophages have relatively large genomes and thereby can accommodate larger DNA fragments. The P1 cloning vectors are prepared from components of P1 phase that are included in a circular plasmid. This recombinant PI phage is then allowed to adsorb to a suitable host, following which the recombinant PI DNA is injected into the cell, circularized and are amplified Fig. Use of another bacteriophage T4 in vitro packaging system with P1 vectors enabled recovery of inserts up to kb in size. Construction of yeast artificial chromomes YACs provides more advantages over cloning in bacterial cells. It can clone very large DNA fragments. For cloning experiments a circular YAC is cut with one restriction enzyme that cuts in the multiple cloning sites and another restriction enzyme that cuts between the two TELs. In this way, the left and right arms are produced. High molecular weight DNA is ligated to the two arms. The resulting YAC cannot be transfected directly into yeast cells. Instead, yeast cells have to be treated in such a way so as to remove the external cell wall. The resulting yeast spheroplasts can accept exogenous fragments but are osmotically unstable and need to be embedded in agar. The overall transformation efficiency is very low and the yield of cloned DNA is also low about one copy per cell. Nevertheless, the capacity to clone large exogenous DNA fragments up to 2 Mb has made YACs a vital tool in creating physical maps of large genomes such as the human genome. These can be prepared by PCR-based methods. Besides these can be obtained using vectors based on certain bacteriophages whose genomes assume a single-stranded DNA at some stages in their life-cycle. It is a filamentous bacteriophage and its genomes consist of single-stranded circular DNA of about 6. The genomes are enclosed in a protein coat forming a long filamentous form. Following adsorption to the host E. After sometime, one of the phage genome products switches the DNA synthesis towards the production of single-stranded DNA. These single-stranded DNA then migrates to the cell membrane and are enclosed in a protein coat. M13 vectors are produced based upon the double-stranded replicative form having multiple cloning sites for generation of double- stranded recombinant DNA circles. In addition to M13 vectors, phagemid vectors can also be used for preparing single- stranded DNA. In such cases, appropriate expression signals need to be provided by the cloning system. However, if the cloned gene is to be expressed across the prokaryotic- eukaryotic boundary, then the gene may also have to be modified. Therefore, different mechanisms are used in prokaryotes and eukaryotes for the translation machinery to identify the start codon. Finally, as the transcription of gene is largely dependent upon the activity of its promoter, an easily assayable reporter gene is often fused just behind the promoter of the gene being investigated to determine the amount of the reporter gene product being formed under a particular condition. These information, can thus be used to understand the expression patterns of the promoter and hence the gene under investigation in a better way. Except tryptophan and methionine, all amino

acids are encoded by two or more codons. In most organisms, not all codons coding for one amino acid are used equally; there is a bias towards using one or more codons instead of others. This is called codon bias.

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3: Cloning Vectors used in Recombinant DNA Technology: 3 Cloning Vectors

A cloning vector is a small piece of DNA, taken from a virus, a plasmid, or the cell of a higher organism, that can be stably maintained in an organism, and into which a foreign DNA fragment can be inserted for cloning purposes.

Others may have additional features specific to their use. For reason of ease and convenience, cloning is often performed using E. Thus, the cloning vectors used often have elements necessary for their propagation and maintenance in E. The ColE1 origin of replication is found in many plasmids. Some vectors also include elements that allow them to be maintained in another organism in addition to E. Cloning site[edit] All cloning vectors have features that allow a gene to be conveniently inserted into the vector or removed from it. This may be a multiple cloning site MCS or polylinker, which contains many unique restriction sites. The restriction sites in the MCS are first cleaved by restriction enzymes, then a PCR -amplified target gene also digested with the same enzymes is ligated into the vectors using DNA ligase. The target DNA sequence can be inserted into the vector in a specific direction if so desired. The restriction sites may be further used for sub-cloning into another vector if necessary. Antibiotic resistance is often used as marker, an example being the beta-lactamase gene, which confers resistance to the penicillin group of beta-lactam antibiotics like ampicillin. Some vectors contain two selectable markers, for example the plasmid pACYC has both ampicillin and kanamycin resistance gene. Auxotrophic selection markers that allow an auxotrophic organism to grow in minimal growth medium may also be used; examples of these are LEU2 and URA3 which are used with their corresponding auxotrophic strains of yeast. Reporter gene[edit] Reporter genes are used in some cloning vectors to facilitate the screening of successful clones by using features of these genes that allow successful clone to be easily identified. Examples of fusion partners that may be used for screening are the green fluorescent protein GFP and luciferase. Elements for expression[edit] Main article: Expression vector A cloning vector need not contain suitable elements for the expression of a cloned target gene, such as a promoter and ribosomal binding site RBS , many however do, and may then work as an expression vector. The target DNA may be inserted into a site that is under the control of a particular promoter necessary for the expression of the target gene in the chosen host. Where the promoter is present, the expression of the gene is preferably tightly controlled and inducible so that proteins are only produced when required. Some commonly used promoters are the T7 and lac promoters. The presence of a promoter is necessary when screening techniques such as blue-white selection are used. Cloning vectors without promoter and RBS for the cloned DNA sequence are sometimes used, for example when cloning genes whose products are toxic to E. Promoter and RBS for the cloned DNA sequence are also unnecessary when first making a genomic or cDNA library of clones since the cloned genes are normally subcloned into a more appropriate expression vector if their expression is required. Some vectors are designed for transcription only with no heterologous protein expressed, for example for in vitro mRNA production. These vectors are called transcription vectors. They may lack the sequences necessary for polyadenylation and termination, therefore may not be used for protein production. Types of cloning vectors[edit] A large number of cloning vectors are available, and choosing the vector may depend a number of factors, such as the size of the insert, copy number and cloning method. Large insert may not be stably maintained in a general cloning vector, especially for those with a high copy number, therefore cloning large fragments may require more specialized cloning vector. Plasmid vector Plasmids are autonomously replicating circular extra-chromosomal DNA. They are the standard cloning vectors and the ones most commonly used. Most general plasmids may be used to clone DNA insert of up to 15 kb in size. One of the earliest commonly used cloning vectors is the pBR plasmid. Other cloning vectors include the pUC series of plasmids, and a large number of different cloning plasmid vectors are available. Many plasmids have high copy number, for example pUC19 which has a copy number of copies per cell, [14] and high copy number is useful as it produces greater yield of recombinant plasmid for subsequent manipulation. However low-copy-number plasmids may be preferably used in certain circumstances, for example, when the protein from the cloned gene is toxic to the cells. These are called phagemid , and examples are the pBluescript series of cloning vectors. Insertion vectors contain a unique cleavage site whereby foreign DNA with size of 5â€”11

kb may be inserted. In replacement vectors, the cleavage sites flank a region containing genes not essential for the lytic cycle, and this region may be deleted and replaced by the DNA insert in the cloning process, and a larger sized DNA of 8â€”24 kb may be inserted. This property can be used for selection - vector without insert may be too small, therefore only vectors with insert may be selected for propagation. It is normally used to clone large DNA fragments between 28 and 45 Kb. BACs are maintained in E. Yeast artificial chromosome[edit] Insert of up to 3, kb may be carried by yeast artificial chromosome. Human artificial chromosome[edit] Human artificial chromosome may be potentially useful as a gene transfer vectors for gene delivery into human cells, and a tool for expression studies and determining human chromosome function. It can carry very large DNA fragment there is no upper limit on size for practical purposes , therefore it does not have the problem of limited cloning capacity of other vectors, and it also avoids possible insertional mutagenesis caused by integration into host chromosomes by viral vector. White colonies may contain an insert in the plasmid it carries, while the blue ones are unsuccessful clones. Blue white screen Many general purpose vectors such as pUC19 usually include a system for detecting the presence of a cloned DNA fragment, based on the loss of an easily scored phenotype. The most widely used is the gene coding for E. If X-gal is included in the selective agar plates, transformant colonies are generally blue in the case of a vector with no inserted DNA and white in the case of a vector containing a fragment of cloned DNA.

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4: Lambda phage - Wikipedia

Taking advantage of the limited range of genome size (78 to % of the wild-type size) for its efficient packaging, an array of vectors has been devised to accommodate inserts of a wide size range, the limit being 24 kbp in Charon

Selection of binders is a complex process depending on multiple variables and experimental conditions, making time and resources the main limiting factors for successful biopannings. We currently offer varied options for display on the N-terminal side of the full-length gene III protein with the PelB leader peptide either using a phagemid system or phage vectors. The phagemids are best suited for the display of antibodies such as scFv and Fab fragments but can also be used to display peptides at a low valency; phage vectors produce a multivalent display and are best suited for peptides and scFv. This short guide will help you with the choice of the vector that best suits your project. Multiple tags and cloning sites. It uses a classical backbone with the high-copy number origin of replication pMB1, the f1 origin for packaging, a copy of LacI to insure proper repression of the lac promoter followed by a strong transcription terminator to prevent undesirable and toxic expression during cloning Fig. This configuration has been suggested to insure reliable cloning of antibody variable genes and improve library stability during screening 1. This vector is recommended for the display of scFv and is best suited when a step of recloning is anticipated for the expression of the insert in a soluble form. A 19 amino acid-long glycine-rich linker connects the cloning site to the first amino acid of the gene III protein. The absence of amber codon and the resistance to proteolysis of this linker result in robust display Fig. More on pADLb is available here. Schematic representation of the cloning site. The amount of scFv on the phage shows little dependence on the helper phage, but is strongly influenced by the bacterial strain used to prepare the phage particles lower panel B. Blots made with a gene III protein antibody. All vectors possess a HIS tag for purification and varied tags for detection; direct secretion in the periplasm is obtained on non-amber suppressive bacterial strains and allows for direct binding analysis without a step of recloning. Excessive expression results in toxicity Fig. Preparation of virions will also require a helper phage; we recommend CM13, which can be found here. Comparative analysis of pADLc display. Similar results were obtained through multiple experiments. These include a strong RBS, a proteolysis-resistant linker and no amber codon. Amber codons are only partially suppressed in vivo, resulting in limited synthesis of full-length pIII fusions required for incorporation in the phage structure and display. The pADL phagemid vector was designed for applications requiring the highest levels of display such as peptide phage libraries. Display can be further enhanced by the use of the pIII-defective helper phage M13KO7d3; more on this helper can be found here. More details on the cloning site can be found here for pADL Phage vectors are best suited to display peptides thanks to their inherent multivalency but are also very effective in displaying antibodies 7. We have engineered the phage vector fUSE5 4 by exchanging the tetracycline cassette with a kanamycin resistance gene and replaced the natural gene III leader peptide with the PelB leader Fig. The display of scFvs by fADL-1 is intense and multivalent see here. H istorically, the vector fUSE5 was used to build the first peptide library 4. The fd-tet vector contains a tetracycline cassette near the minus-strand origin that imposes a rate-limiting growth on all clones with the benefit of more stable libraries 3. Since then, many libraries either peptides or antibodies 5 have been build with phage vectors derived from fd-tet. We found that not only scFv are very-well displayed by fd-tet derivatives but with very limited polyphage formation to the contrary of virions produced with helper phage defective in gene III protein, a classical means to improve antibody display 6. Sequence of fADL-1 cloning site. The buyer is NOT granted a license to a use Product for human or animal therapeutic, diagnostic, or prophylactic purposes, b act as reseller or distributor of Product, or c resell, distribute, or transfer Product without modification under any name. Antibody Design Labs does not warrant that the use or sale of Product, the use thereof in combination with other products, or the use of Product in the operation of any process will not infringe the claims of any United States or other patent s. If the buyer is not willing to accept the limitations of this license, without modification, buyer may refuse this license by returning Product unopened and unused. By keeping or using Product, buyer agrees to be bound by the terms of this license. Reliable cloning of functional antibody variable domains from hybridomas and spleen cell

repertoires employing a reengineered phage display system. J Immunol Methods, 1 , 35â€” A new filamentous phage cloning vector: Gene, 9 , â€” Corruption of phage display libraries by target-unrelated clones: Searching for peptide ligands with an epitope library. Comparison of fusion phage libraries displaying VH or single-chain Fv antibody fragments derived from the antibody repertoire of a vaccinated melanoma patient as a source of melanoma-specific targeting molecules. A helper phage to improve single-chain antibody presentation in phage display. Phage versus phagemid libraries for generation of human monoclonal antibodies. PUB â€” Rev Please send comments to info abdesignlabs. The reuse or reproduction of any of the information, design or layout contained in this web site without the permission of Antibody Design Labs is prohibited.

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5: 6 Main Types of Cloning Vectors | Biotechnology

Charon 34 and Charon These vectors will accept fragments kb (kilobases) long. M13 as cloning vector for DNA sequencing. M13 is a filamentous bacteriophage of E. coli and contains a kb long single stranded circular DNA.

The limitation of this vector is the size of DNA that can be introduced into the cell by transformation. This presents problems when you are trying to create a genomic library of a large genome such as with plants. A genomic library contains all of the DNA found in the cell of the plant or any organism. If you digest plant DNA to completion with a restriction enzyme, ligate those fragments into a plasmid vector and transform bacterial cells, only a portion of those fragments will be represented in the final transformation products. If a gene of interest is located on a large fragment then you will not be able to isolate that gene from a plasmid library. But what can be done to increase the probability of obtaining a clone which contains the entire gene. First you need to use a vector that can accept large fragments of DNA. Examples of these are bacteriophage and cosmid vectors and more recently yeast artificial chromosomes. Bacteriophage lambda vectors were developed because several observations were made that suggested that they could complete their life cycles even if foreign DNA was inserted into a portion of its genome. This suggested that certain regions of the virus were not essential. This is from 37 to 53 kb in length. Two important developments suggested that lambda may be suitable as a cloning vector. First it was determined that the gene products between the J and N genes could be removed and the life cycle could be completed. Second, restriction enzyme sites could be eliminated which permitted the development of a vector with a single site for insertion of foreign DNA. Two types of vectors have been developed: One important concern when cloning with lambda vectors is that you want to maximize the number of resulting phage particles that contain foreign DNA. Or said another way you want to minimize the number of wild type particles. One approach is through spi selection. This refers to sensitivity to P2 interference. Thus only those particles from which the stuffer has been replaced can grow well in a P2 lysogen bacterial cell. Ligate the DNA to the arms of the vector. Package the DNA into phage particles using premade mixes. Screen and then amplify. Store the library for future use as a plate lysate.

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6: Bacteriophage Vectors for www.enganchecubano.com | Genetics

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DNA damage by UV leading to onset of lytic development - continues as above until cell lysis e. The presence of plaques and their appearance is a phenotypic trait allowing visual identification. Role of the terminase gene A product Distance between cos sites must be more than 37 kb and less than 52 kb. Then attachement of the tail and lysis of the cell. The switch from early to late replication requires the gam product. CI is the major l repressor, which is the only l product present in the lysogenic state. It also activates PRM, its own repression maintenance promoter. Since there is no CI at the time of infection, another promoter is used to provide the initial product PRE repression establishment. Environmental conditions have a major impact on the decision between lysogeny and lysis. If there is sufficient CI from the competition for promoters and repression , lysogeny can occur the cell becomes immune against superinfection therefore the cI region is also called imm. Phage is a related lysogenic phage. The imm regions of l and are exchangeable, but are not cross-reactive. Hence, a phage can infect a l lysogen, and vice versa. An interesting mutant of cI is cI The latter encodes a temperature-sensitive repressor, which remains active at 32C but is inactive at 42C. This often allows in vivo control by a temperature shift. See also expression vectors, vector for positive selection, etc. Spi the sensitivity to P2 inhibition or interference is a phenotype used for positive selection. Phage l is unable to grow in an E. Packaging of phage l DNA. Maturation of infective particles. Most efficient transformation procedure before the advent of electroporation. Following problems had to be solved before l vector could be developed and used a size limits. But there is sufficient possibility space for deletions. The 5 EcoRI sites were eliminated by random mutagenesis. The excisable segment is termed stuffer. However, a substantial fraction of the cells continues to grow lysogeny , and this causes the turbid background of the plaque space. Only if all cells follow the lytic route i. The first mutants that were obtained, and which fell into 3 complementation groups, were therefore named cI, cII and cIII. The gam gene is required for the switch from Q to s replication early to late replication. However, it is also responsible for the fact that that l is unable to develop in a strain which is lysogenized by phage P2. Sensitivity to phage P2 inhibition or interference. The latter property is exploited as a positive selection system for recombinants. In such case, the host cell must possess other properties to enable the conversion of the DNA of Q-circles to concatemeres. Hence the use of l vector - host cell combinations is a rather specialized field.

BACTERIOPHAGE AND ITS VECTORS pdf

7: Biology Lecture Notes - Lambda Vectors

Cancer gene therapy expanded and reached its pinnacle in research in the last decade. Both viral and non-viral vectors have entered clinical trials, and significant successes have been achieved. However, a systemic administration of a vector, illustrating safe, efficient, and targeted gene delivery.

Bacteriophage Vectors for E. The cloning of single genes is usually best carried out using plasmids, since the insert will rarely be larger than about 2 kb. However, for cloning of larger pieces of DNA e. Large DNA molecules can be injected in host bacterial cell by viral particles bacteriophages. At its ends are the cos cohesive sites, which consist of 12 bp cohesive ends. The cos ends allow the DNA to be circularized in the host cell. For the cloning of large DNA fragments, up to about 20 kb, much of the nonessential lambda DNA is removed and replaced by the insert desired or ta. The recombinant DNA is then packaged within viral particles in vitro, and these are allowed to infect bacterial cells E. Once inside the bacterial cells, the recombinant viral DNA is replicated. All the genes needed for normal lytic growth are still present in the DNA and so multiplication of the virus takes place by cycles of cell lysis and infection of surrounding cells. It gives rise to plaques of lysed bacterial cells on a background, or lawn, of bacterial cells. Cloned DNA can be recovered from the viruses in these plaques. Lambda replacement vectors contain a restriction site for phage propagation in suitable bacterial host. Remaining part of the lambda genome is removed and is replaced by foreign DNA. Ligation is performed at a ratio of arms to target DNA that favours the formation of very long concatemers. The concatemers are multiple length copies with multiple replication complexes and forks. In each of concatemers, vector and target molecules are interspersed. In most replacement vectors, the internal region that is replaced by the target contains a gene that renders the phage inviable dead in an appropriate E. Recombinant phage in which the internal region is replaced by target DNA is viable and are mostly used for cloning eukaryotic DNA fragments. When cloning into an insertion vector, the phage DNA is cleaved with a restriction enzyme that cuts it only once, and the target is inserted into this site No phage DNA is removed, therefore, much smaller sized target DNA can be inserted. This allows selection against non-recombinant phage. It includes Ff class of filamentous phages, including strains f1, fd and M13, which infect E. These Ff virions are long and thin and contain a closed loop of single- stranded DNA. Because the phages readily accept inserts of foreign DNA and they supply one strand of that DNA in an easily isolated form, vectors based on Ff phages have become standard choice of biotechnology. The M13 is a filamentous bacteriophage of E. It is nm long and 6 nm wide. It has a protein coat the capsid which is made up of three kinds of capsomeres. This filamentous single stranded DNA phage infects the bacterial cell by adsorbing to and entering through a pilius. The M 13 phage particles contain a 6. After infection of a sensitive E. Further M13 phages do not lyse the host cells to release progeny phage and bacterial DNA from the ruptured bacterial host cells like lambda phage during the lytic cycle. Infected bacterial cells will continue to grow and extrude thousands of progeny virus particles i. Since the virus particles are very small in comparison of the host bacteria, the host cells can be removed by low-speed centrifugation. The virus particles can then be collected from the supernatant suspension by high-speed centrifugation and their single-strand DNA molecules can be isolated by single phenol-chloroform extractions. Packaging of single strands of phage DNA in progeny phage provides a neat biological purification of single-strand DNA. Importantly, this statement holds true for a foreign gene cloned in the viral chromosome just as for the phage genes themselves. This property of M13 phase has been exploited for its use as a vector. Lastly, phage M13 is not used as a primary vector to clone new DNA targets, but fragments are normally sub-cloned into M13RF using standard plasmid methods when the single-stranded form of a fragment is required. Genetic organisation of wild type M13 bacteriophage: The DNA molecule of M13 phage is single-stranded and circular. It is bases long having 10 closely packed genes. All these genes are essential for the replication of the phages. There is a segment of base-long intergenic sequence IS which contains origin of replication OR. The IS containing region of viral genome is manipulated for cloning without disrupting the origin of replication. Hence, the wild M13 phage has limited use in gene cloning experiments. The size of the phage particle is decided by the size of the phage DNA. Upto six times

the normal length of M13 phage DNA can be packaged. Construction of M13 based vectors: In M13 phage DNA, the intergenic sequence is the only region which can be manipulated for gene cloning. As this region has only two restriction sites Asa I and Ava II , wild type phage is not an efficient vector. However, the intergenic sequence can be modified to introduce additional restriction sites. A few such vectors are discussed below: This step produces blue plaques on X-gal agar plates. The lac Z gene does not have any restriction site. If the second G residue is substituted by an A residue this sequence becomes an Eco R1 site, i. This type of conversion is done by in vitro mutagenesis. Now this phage is called M13 mp2. This can be tested by X-gal agar where the plaque gives a blue colour. Gene lac Z of E. The M13 mp2 is the simplest cloning vector derived from M13 phage. This insertion inactivates lac Z gene and is called insertional inactivation. Such recombinant phages fail to produce blue plaques on X-gal agar, instead, they produce clear plaques. The M13 mp7 is a derivative of MB mp2. Foreign DNA with the corresponding sticky ends is inserted to produce recombinant M13 mp7. Thus, M13 mp7 is a more complex vector having four possible insertional sites. Insertion of foreign DNA tends to inactivate the lac Z gene and the production of galactosidase enzyme is prevented. This is shown by the formation of clear plaques on X-gal agar by the recombinant phage DNA. The hybrid vectors contain components from both plasmids and phage chromosomes. These vectors replicate in E. The helper phage is a mutant that replicates its own DNA inefficiently, but provides viral replication enzymes and structural proteins for the production of plasmid DNA molecules that are packaged in phage coats. Thus, if a foreign DNA is inserted into a specific restriction site in both vectors, one vector will package the complementary strand of the gene. Therefore, both strands of the gene can be isolated, sequenced, subjected to site-specific mutagenesis, and so on. Vieira and , to distinguish from earlier members i. The pUC vectors were derived from an early plasmid cloning vector called pBR by a series of direct modifications. They are present as small, supercoiled, covalently closed circular DNA. Thus, only bacteria harbouring a plasmid will grow on medium containing the antibiotic amplicillin. They carry high copy number, up to copies per bacterium. Thus, there would be large yields of DNA from small cell cultures. They carry a polycloning region which have a variety of restriction enzyme cleavage sites. Thus many different types of restriction fragments can be inserted without modification. Thus, colonies harbouring plasmids with foreign DNA inserts can be distinguished from those carrying plasmids with no insert by a simple colour test. The lac Z gene is under the control of the lac promoter. They carry a plasmid origin of replication. They carry a phage M13 origin of replication. The polycloning regions are present in pUC and pUC in opposite orientations. Thus, if pUC packages one strand of a cloned gene, pUC will package the complement strand. Different members of the pUC vectors series contain different, but related, sets of restriction enzyme cleavage sites. The polycloning regions of pUC and pUC contain 10 clustered restriction enzyme cleavage sites. Some of these sites are substrates for two or more different restriction enzymes. The utility of pUC is greatly increased by a simple colour test that allows one of distinguish cells harbouring plasmids with foreign DNA inserts from those harbouring plasmid with no insert. So the pEMBL 8 molecules are also converted into single-stranded DNA molecules and these will be released as infective phage particles. This will act as a helper phage by providing necessary enzymes and phage coat proteins. Hence this plasmid vector has the following advantages of pUC8 plasmids and that of the M13 phages: The phage particles containing the recombinant pEMBL 8 genome are as efficient as the wild type M13 phages in their ability to infect E.

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8: Plant Genome Organization and Structure

Although progress has been made in improving these viral vectors, it is essential to investigate alternatives such as prokaryotic viruses (i.e., bacteriophage) and nonviral vectors [69,70]. This discussion focuses on the application of filamentous bacteriophage to mediate gene delivery.

Anatomy[edit] Bacteriophage lambda virion schematic. Protein names and their copy numbers in the virion particle are shown. The presence of the L and M proteins in the virion is still unclear. The whole particle consists of 12â€”14 different proteins with more than protein molecules total and one DNA molecule located in the phage head. However, it is still not entirely clear whether the L and M proteins are part of the virion. The cos site circularizes the DNA in the host cytoplasm. In its circular form, the phage genome, therefore, is 48, base pairs in length. See section below for details. It must instead use an existing pathway to invade the host cell, having evolved the tip of its tail to interact with a specific pore to allow entry of its DNA to the hosts. Bacteriophage Lambda binds to an E. The J protein interacts with the maltose outer membrane porin the product of the lamB gene of E. The linear phage genome is injected through the outer membrane. The DNA passes through the mannose permease complex in the inner membrane [7] encoded by the manXYZ genes and immediately circularises using the cos sites, base G-C-rich cohesive "sticky ends". Lambda phage DNA injection into the cell membrane using Mannose PTS permease a sugar transporting system as a mechanism of entry into the cytoplasm. Host DNA gyrase puts negative supercoils in the circular chromosome, causing A-T-rich regions to unwind and drive transcription. At first, these express the N and cro genes, producing N, Cro and a short inactive protein. N binds to the two Nut N utilisation sites, one in the N gene in the PL reading frame, and one in the cro gene in the PR reading frame. The N protein is an antiterminator , and functions to extend the reading frames to which it is bound. When RNA polymerase transcribes these regions, it recruits the N and forms a complex with several host Nus proteins. This complex skips through most termination sequences. This inhibition can induce a bacteriostatic state, which favours lysogeny. Low temperature, starvation of the cells and high multiplicity of infection MOI are known to favor lysogeny see later discussion. N antitermination[edit] N Antitermination requires the assembly of a large ribonucleoprotein complex to effectively prolong the anti-termination process, without the full complex the RNA polymerase is able to bypass only a single terminator [9] This occurs without the N protein interacting with the DNA; the protein instead binds to the freshly transcribed mRNA. Nut sites contain 3 conserved "boxes," of which only BoxB is essential. When transcribed, each sequence forms a hairpin loop structure that the N protein can bind to. The entire complex including the bound Nut site on the mRNA continues transcription, and can skip through termination sequences. Lytic life cycle[edit] Main article: Lytic cycle This is the lifecycle that the phage follows following most infections, where the cII protein does not reach a high enough concentration due to degradation, so does not activate its promoters. Cro dominates the repressor site see "Repressor" section , repressing synthesis from the PRM promoter which is a promoter of the lysogenic cycle. The O and P proteins initiate replication of the phage chromosome see "Lytic Replication". Q, another antiterminator , binds to Qut sites. Structural proteins and phage genomes self-assemble into new phage particles. Products of the lysis genes S,R, Rz and Rz1 cause cell lysis. S is a holin , a small membrane protein that, at a time determined by the sequence of the protein, suddenly makes holes in the membrane. R is an endolysin , an enzyme that escapes through the S holes and cleaves the cell wall. Rz and Rz1 are membrane proteins that form a complex that somehow destroys the outer membrane, after the endolysin has degraded the cell wall. For wild-type lambda, lysis occurs at about 50 minutes after the start of infection and releases around virions. Rightward transcription[edit] Rightward transcription expresses the O, P and Q genes. This is initiated at the ori site located in the O gene. O protein binds the ori site, and P protein binds the DnaB subunit of the host replication machinery as well as binding O. This effectively commandeers the host DNA polymerase. Soon, the phage switches to a rolling circle replication similar to that used by phage M Note that this does not release single copies of the phage genome but rather one long molecule with many copies of the genome: These concatemers are cleaved at their cos sites as they are packaged. Note that NusA can stimulate the activity of the Q protein.

Q binds to RNA polymerase in Qut sites and the resulting complex can ignore terminators, however the mechanism is very different; the Q protein first associates with a DNA sequence rather than an mRNA sequence. Part of the Qut site resembles the Pribnow box , causing the holoenzyme to pause. The head and tail genes are transcribed and the corresponding proteins self-assemble. Leftward transcription[edit] Leftward transcription expresses the gam, red, xis, and int genes. Gam and red proteins are involved in recombination. Int and xis are integration and excision proteins vital to lysogeny. This results initially in the excision of any inserted genomes from the host genome. Higher concentrations of xis than int result in no insertion or excision of phage genomes, the evolutionarily favoured action - leaving any pre-insterted phage genomes inserted so reducing competition and preventing the insertion of the phage genome into the genome of a doomed host. Lysogenic or lysenogenic life cycle[edit] Main article: Lysogenic cycle The lysogenic lifecycle begins once the cI protein reaches a high enough concentration to activate its promoters, after a small number of infections. This is expressed, turning on cI repressor production. The PI promoter expresses the int gene, resulting in high concentrations of Int protein. Elevated levels of int much higher than that of xis result in the insertion of the lambda genome into the hosts genome see diagram. The genome remains inserted into the host genome in a dormant state. The prophage is duplicated with every subsequent cell division of the host. The phage genes expressed in this dormant state code for proteins that repress expression of other phage genes such as the structural and lysis genes in order to prevent entry into the lytic cycle. These repressive proteins are broken down when the host cell is under stress, resulting in the expression of the repressed phage genes. Stress can be from starvation , poisons like antibiotics , or other factors that can damage or destroy the host. In response to stress, the activated prophage is excised from the DNA of the host cell by one of the newly expressed gene products and enters its lytic pathway. The integration itself is a sequential exchange see genetic recombination via a Holliday junction and requires both the phage protein Int and the bacterial protein IHF integration host factor. Lysogeny is maintained solely by cI. It is therefore the only protein expressed by lysogenic phage. This is coordinated by the PL and PR operators. Both operators have three binding sites for cI: When it is present at a much higher concentration, it also binds to OR3, inhibiting transcription from PRM, thus regulating its own levels in a negative feedback loop. The presence of cI causes immunity to superinfection by other lambda phages, as it will inhibit their PL and PR promoters. Induction[edit] Transcriptional state of the PRM and PR promoter regions during a lysogenic state vs induced, early lytic state. The classic induction of a lysogen involved irradiating the infected cells with UV light. Any situation where a lysogen undergoes DNA damage or the SOS response of the host is otherwise stimulated leads to induction. The host cell, containing a dormant phage genome, experiences DNA damage due to a high stress environment, and starts to undergo the SOS response. RecA a cellular protein detects DNA damage and becomes activated. This is because cI mimics the structure of LexA at the autocleavage site. Cleaved cI can no longer dimerise, and loses its affinity for DNA binding. The PR and PL promoters are no longer repressed and switch on, and the cell returns to the lytic sequence of expression events note that cII is not stable in cells undergoing the SOS response. There is however one notable difference. LexA expression leads to inhibition of various genes including LexA. Control of phage genome excision in induction[edit] The phage genome is still inserted in the host genome and needs excision for DNA replication to occur. The sib section beyond the normal PL promoter transcript is, however, no longer included in this reading frame see diagram. The new intact transcript has one copy of both xis and int, so approximately equal concentrations of xis and int proteins are produced. Equal concentrations of xis and int result in the excision of the inserted genome from the host genome for replication and later phage production. Multiplicity reactivation and prophage reactivation[edit] Multiplicity reactivation MR is the process by which multiple viral genomes, each containing inactivating genome damage, interact within an infected cell to form a viable viral genome. Repressor[edit] Protein interactions that lead to either Lytic or Lysogenic cycles for Lambda phage The repressor found in the phage lambda is a notable example of the level of control possible over gene expression by a very simple system.

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9: Bacteriophage-Derived Vectors for Targeted Cancer Gene Therapy

The lytic cycle where the phage releases its genome into the bacyerial cell and uses host machinery and enzymes to replicate its genome, produce the protein capsid, tail and other parts of a phage and them assembles them to form several daughter phages. Finally the bacterial cell is lysed to release the phage particles.

Many types or kinds of cloning vectors are used for the transfer of gene of interest into the desired tissue or cell. Some of the examples for vectors used in recombinant DNA technology are plasmids, phagemids, cosmids, shuttle vectors and much more In other words vectors are used as vehicle to carry gene of interest. Several types of vectors are being constructed in laboratory; each vector has got different molecular properties as well as cloning capacity. Some of the examples for vectors used in recombinant DNA technology are plasmids, phagemids, cosmids, shuttle vectors and much more. Plasmid as Cloning Vector: Plasmids are extra chromosomal circular double stranded DNA, present in bacterial cells. Plasmid are used as cloning vectors, include ori site or origin of replication, needed for replication in the bacterial cells. For an example E. Ori site for replication. Selectable marker genes, such as antibiotic resistance gene c. Unique restriction sites, so that the restriction enzymes can be used to cut the plasmid and DNA of interest can be inserted into the plasmid. The features of this pUC19 plasmids are as follows: High copy number, as high as nearly hundred copies per bacterial cell. This helps in getting good yield of cloned DNA in short duration. It also has got selectable marker as ampicillin resistance gene. This plasmid also got a cluster of unique restriction sites known as polylinkers or in other words this plasmid has got multiple cloning sites. The polylinkers or multiple cloning sites are also a part of lacZ galactosidase gene. When DNA of interest is cloned into the polylinkers lacZ is disrupted, this prevents the complementation from occurring. DNA of interest is inserted into a cloning vector using restriction enzyme and then ligating it with enzyme ligase. DNA of interest is also cut using the same restriction enzyme. Resulting plasmids then transformed into E. Then these bacterial cells are grown on media contain ampicillin and Xgal. Ampicillin resistance is resulted from pUC19 sequence, Blue colonies are resulted from the rejoined plasmids, and white colonies will result from transformed bacterial cells with recombinant plasmids. Many different types of cloning vectors are designed in the laboratory and they are commercially available for use. These vectors have different array of unique restriction sites in the polylinker site. Many plasmid cloning vectors are designed and available for many prokaryotic organisms as well as for eukaryotic organisms. But the plasmid cloning vectors which carry more than 5 to 10 kb gene of interest are often unstable. Therefore size of the gene of interest is limited in plasmid cloning vectors. Gene of interest is inserted using restriction digestion and also DNA ligation method. When this ligated DNA is mixed with phage proteins, this leads to the assembling of phage head and DNA is also packed to form virus particles. Only viral particles with both the phage chromosome arms and also with proper central segment kb are able to replicate by infecting the E. Many types of phage cloning vectors are available with varying features like an expanded array of restriction sites and much more.

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