DOWNLOAD PDF RESTRICTION ENDONUCLEASES (NUCLEIC ACIDS AND MOLECULAR BIOLOGY)

Chapter 1 : Restriction Endonucleases | NEB

The present book deals with all aspects of restriction endonucleases including nomenclature, diversity, evolution, genetics, structure and function, mechanism of target site location and DNA recognition, enzymology, protein design, and provides a description of the history of the discovery of and the research on restriction enzymes.

Advanced Search Abstract PvuRts1I is a prototype for a larger family of restriction endonucleases that cleave DNA containing 5-hydroxymethylcytosine 5hmC or 5-glucosylhydroxymethylcytosine 5ghmC , but not 5-methylcytosine 5mC or cytosine. Here, we report a crystal structure of the enzyme at 2. Although the protein has been crystallized in the absence of DNA, the structure is very informative. Surprisingly, fluorescence changes indicative of base flipping are not observed when PvuRts1I is added to DNA substrates containing pyrrolocytosine in place of 5hmC 5ghmC. Despite this caveat, the structure suggests a model for PvuRts1I activity and presents opportunities for protein engineering to alter the enzyme properties for biotechnological applications. There are also structural data about 5mC specific enzymes: The presence of 5-hydroxymethylcytosine 5hmC in phage 11 and mammalian DNA has been known for a long time although the initial estimates for the amount of 5hmC in mammalian DNA were too high. Much recent research was triggered by the identification of the function of TET ten-eleven translocation proteins as 5mC oxidizing enzymes 12â€”13 , and the role of 5hmC as a demethylation intermediate 14â€”15 , epigenetic mark 16 and diagnostic marker in cancer Other proteins, such as MBD3, which binds to 5hmC according to some 21 but not other 18 studies, are homologous to structurally characterized 5mC binding proteins and therefore their possible interactions with 5hmC can be deduced It is also still not understood how the presence of the 5hmC base can trigger an enzymatic reaction. The endonuclease PvuRts1I from Proteus vulgaris strain has been reported to be a dimer 22 like most endonucleases that catalyze double strand breaks. Cleavage is most efficient when two 5hmC 5ghmC bases are present in opposite DNA strands approximately 22 bases apart from each other Some double strand cleavage can also be observed when there is only a single 5hmC 5ghmC. The potential applications of 5hmC sensitive sequencing have triggered the search for PvuRts1I homologs that exhibit desirable properties for biotechnological use. This search has led to the identification of a whole family of enzymes, which differ slightly in the distance requirement for the modified bases In contrast to PvuRts1I, some of them such as AbaSI show a preference for 5ghmC over 5hmC 25 , which can be exploited in sequencing by postglucosylation of 5hmC with phage T4 glucosyltransferase. As 5hmC is much rarer than 5mC in animal genomes 26 , very high discrimination stringency is required for biotechnological use. First, it would be desirable to design an enzyme fully dependent on a single modified site only, which should make a double strand break on one or both sides of the modified base. Second, it would be useful to improve the stringency of 5hmC versus 5mC discrimination. Here, we report the crystal structure of PvuRts1I at 2. Site-directed mutagenesis experiments confirm the importance of predicted key residues in the structure. Based on the combined crystallographic and biochemical data, we suggest a structural explanation for why PvuRts1I requires 5hmC or 5ghmC bases in opposite strands at a distance of just over 20 base pairs for the introduction of a double strand break approximately halfway between the modified bases. Mutants of pvuRts1I were generated in the construct for the N-terminally tagged protein variant using the QuikChange protocol The strain was transformed with plasmids coding for the N- or C-terminally tagged versions of the PvuRts1I protein. Expression of the selenomethionine version of PvuRts1I with N-terminal tag was done in methionine auxotrophic BL DE3 cells in defined media lacking methionine and supplemented with selenomethionine The variant proteins were obtained according to the protocol for the wild-type. In the absence of suitable models for molecular replacement, experimental phasing was required. Therefore, we grew crystals of the selenomethionine variant of the protein containing four selenium atoms not counting the initiator methionine upstream of the histidine tag , which turned out to be better than the wild-type protein crystals and diffracted to 2. Diffraction data were collected at a wavelength of 0. The structure was solved by

the single anomalous diffraction SAD method. The SHELXE program 31 was then used to generate an experimental electron density map by a combination of phasing and density modification steps. All the mutants and the wild-type protein were loaded on the SDS gel to show the equal concentration of the proteins. The proteins were purified by affinity and size exclusion chromatographies. The purified recombinant wild-type proteins with tags on either end, but not controls with changes to important residues, were active against T4 phage DNA, which is known to contain a large number of 5ghmC bases at various distances to each other. Although protein activities were at least qualitatively in agreement with the literature data, the variant of PvuRts1I with N-terminal hexahistidine tag had some other unexpected properties, at least in our hands. While PvuRts1I should be a dimer also in the absence of DNA 22 , size exclusion chromatography with the N-terminally tagged, but not the C-terminally tagged, variant of the enzyme suggested a slightly lower than expected molecular mass. Despite these undesirable features of the N-terminally tagged PvuRts1I, we continued work with this variant of the protein, because it yielded well-diffracting crystals, at least in the absence of DNA. Crystallization and structure determination Crystallization of PvuRts1I was attempted either in the absence of DNA or with oligonucleotides containing two 5hmC bases at the appropriate distance. All these experiments did not yield any diffracting crystals. We concluded that PvuRts1I might have a flexible substrate binding site, and because we knew that the enzyme accepted 5ghmC containing DNA, we tried crystallization in the presence of large amounts of glucose. This proved crucial for crystallization success. Crystals belonged to space group P4 1 2 1 2, contained one molecule of PvuRts1I in the asymmetric unit and diffracted up to 2. The structure was solved by the SAD method using a crystal of the selenomethionine version of the protein. We therefore conclude that the N-terminal part of the enzyme residues 1â€” harbors the nuclease activity and henceforth refer to it as the catalytic domain. As SRA domains recognize modified bases by flipping them out of the DNA stack into a pocket of the domain 6â€”8 , the PvuRts1I could also be a nucleotide flipping enzyme. View large Download slide PvuRts1I domains and homologs. Core elements of the fold are in bright and additional elements in faint color. D Alignment of the amino acid sequences of PvuRts1I catalytic core and homologs. E Alignment of the SRA domain sequences in the pocket region. Metal ions are not present in the active site because crystals were grown in the absence of divalent metal ions.

Chapter 2 : DNA Restriction & Nucleic Acid Analysis | Molecular Biology

Part a Role of Restriction Enzymes & Enzyme Digestion. This is Part A, Role of Restriction Enzymes & Enzyme Digestion, under the module topic, DNA Restriction & Nucleic Acid Analysis.

Content Tutorial University of Calgary Biotechnology Training Centre Restriction Enzymes and Cloning This section on cloning is included with the basics module as it is a necessary basic for research laboratories, but may be required from time to time in clinical labs when something really interesting is found in the molecular lab. Cloning of a gene sequence or part is also required to have stable templates for the production of probes, or to save that interesting PCR product. Long term storage of PCR products without cloning them, leaves them susceptible to degradation by bacterial exonucleases enzymes that digest DNA from the ends. Restriction enzymes are the backbone reagents of cloning, but are used in clinical applications associated with fingerprinting â€" genetic identity, epidemiology, and in preparation for blotting for other applications. This is a step that essentially cuts DNA into little bits. We will see later the application of this to identification DNA fingerprinting. The molecular scissors are called restriction endonucleases. Restriction endonucleases are enzymes that cleave DNA. They are derived from bacteria where they function in cleaving foreign DNA and thus protecting the integrity of the host bacteria a bacterial immune system. These enzymes recognize specific base sequences in double stranded DNA. The sites on the DNA molecule recognized by the enzymes are called restriction sites. The various enzymes are named for the bacteria from which they have been derived. Eco is derived from Escherichia coli and Hin from Haemophilus influenzae, for example. You will note that the first panel above the cut leaves no overhang and is called a blunt cut leaving blunt ends. Any blunt end can be rejoined to any other blunt end â€" something to remember when cloning PCR products. Enzymes may be chosen that will cleave on either side of a desired DNA sequence. In these cases, cut sites have been predetermined by finding the sequence of nucleotides that encode a specific protein or characteristic. In other cases, various endonucleases will be tried. Once the DNA has undergone restriction digestion, it may be used to recombine with any other piece of DNA that has the complementary ends, regardless of the source of that DNA. This allows insertion of a segment of DNA into a plasmid that has been cleaved with the same enzyme s. View the two animations provided: View the video Interviews provided on the website, their titles are listed below: This topic part has three sections: Electrophoresis Techniques Usually prior to blotting the DNA, RNA, or protein molecules for further studies and detection, nucleic acids are separated based on size or mass by electrophoresis. In protein electrophoresis for molecular biology, proteins are separated by mass as charge is made negative by the pH of the buffer used. This technique will be explored in module III. As there are no issues with charge, the only difference with the molecules position in the matrix following electrophoresis is the length of the sequence. Thus it is possible to determine the length of a piece of DNA or RNA or mass of a protein by its position in a gel relative to a standard marker. This type of analysis is important in epidemiology, forensics and in just checking up on your PCR reaction. For proteins many techniques are available to determine function as well as mass. Two major types of matrix are used for electrophoresis; agarose gel or acrylamide gel. Agarose is of superior optical clarity compared to microbiology agar, but is handled in similar ways. Acrylamide is a crosslinked polymer that enables the use of small amounts of sample, separation of very similar pieces sequencing gels for example and are able to take a great deal of heat during the electrophoresis run. Buffers are added to the gels when they are made. Usually pictures or digital images are used for determination of the size of the bands in the gel. Agarose Gel Electrophoresis Electrophoresis is a method whereby charged molecules in solution migrate in response to an electric field. Because of the phosphate backbone in DNA molecules, they have a net negative charge and migrate toward the anode positive pole in an electric field. Their rate of migration or mobility is related to the strength of the field, the size of the molecule, as well as the medium gel in which they are migrating. For separation of DNA molecules, electrophoresis is often carried out in a horizontal apparatus containing a gel made of agarose.

Agarose is a highly purified polysaccharide derived from agar seaweed that is not contaminated with charged material. It comes in powder form and is dissolved by boiling in aqueous solutions. The gel is stable and will not dissolve again until raised back to boiling temperatures. The pore size of the agarose is adjusted by changing the concentration of the agarose in the gel. The higher the concentration of agarose, the smaller the gel pore size. Working concentrations are usually in the range of 0. The migration of molecules in agarose is size-dependent and allows separation of molecules up to about 20 kilobases kb in size. The smaller the molecule, the more rapidly it will be able to pass through the pores in the agarose in its migration toward the positive pole. Thus, in a mixture of DNA molecules of different sizes, the shortest fragments will migrate the most rapidly, while the largest will be retarded to the greatest extent by the pores in the agarose and will migrate the most slowly. The best separation is achieved after experimenting with variations in the concentration of agarose and the separation time in the electric field. The DNA sample to be analyzed is first mixed with a glycerol-dye solution. The glycerol in the sample makes it more dense than the running buffer so that it can be applied into the sample slot of a submerged gel in an electrophoretic chamber without diffusing away prior to application of electrical current. The inclusion of the dye bromophenol blue or others helps to make the sample visible during application and provides a marker to help track the progress of the electrophoretic run because it is also negatively charged and migrates toward the positive pole at a rate similar to that of small DNA molecules. A DNA ladder that contains DNA molecules of defined length is also included as one of the samples in one of the lanes of the gel to provide an internal marker of DNA fragment sizes and to provide an indication of how well molecules have separated in the gel. Care must be taken to wear a UV resistant shield, goggles or glasses when viewing gels on a UV box to avoid damage to your eyes. In addition, ethidium bromide is a carcinogen and mutagen and care must be taken to avoid skin contact with ethidium bromide- containing solutions. Always wear latex gloves when working with DNA samples during electrophoretic procedures to prevent contact with ethidium bromide in the gel or running buffer. Other dyes include SYBR green or gold, which are much safer to use and have similar binding characteristics as ethidium bromide, but in some circumstances, are not quite as sensitive in gels. To confirm the identity of a PCR or RT-PCR product, the agarose gel from above can be subjected to the same non-amplification techniques blotting as will be presented in Module 4. Acrylamide Gel Electrophoresis In some instances, higher resolution gels are required as for separation of small DNA fragments, ssDNA fragments or in manual sequencing, and this can be done using the cross-linked polymer acrylamide. Extreme sensitivity is obtained by staining acrylamide gels with a silver stain. Such gels can become permanent records as well by drying the gel between sheets of dialysis membrane or onto filter paper. The addition of urea to acrylamide gels will ensure that ssDNA single-stranded DNA runs according to size rather than shape due to base-pairing within a strand secondary structure like tRNA. We will explore the applications of acrylamide gel electrophoresis in greater detail in Module III. The agarose gel electrophoresis technique can also be used to analyze Polymerase Chain Reaction PCR products which will be discussed in detail in module subset II-b. They are derived from bacteria where they function in cleaving foreign DNA and thus protecting the integrity of the host bacteria. Eco is derived from Escherichia coli and Hin from Haemophilus influenzae. In these cases, cut-sites have been predetermined by finding the sequence of nucleotides that encode a specific protein or characteristic mutation. In other cases, various endonucleases will be tried to obtain the desired resolution of a fingerprint; generally bands. One thing about RFLP â€" it may be followed by gel electrophoresis and staining with ethidium bromide exactly the same as a post-PCR gel would be done, and then the gel is recorded by taking a picture. Gel documentation systems that use band-finding software are able to analyze the bands for size and pattern. The banding patterns can then be used for relationship or phylogenetic analysis. View the first 2D animation provided: Gel Electrophoresis, to learn about the mechanism of gel electrophoresis. You will view the sequencing animations and interview section in the next module. This animation depicts how restriction enzymes can cut DNA at specific sites, producing fragments of DNA at varying lengths that can be analyzed by separating the digested DNA by agarose gel electrophoresis and analyzing the varying fragment sizes. In

the virtual lab you will apply the gel electrophoresis technique to samples of DNA that you have been provided with. The DNA samples have been cut by restriction enzymes through a restriction enzyme digestion reaction. Using gel electrophoresis you will separate the fragments and analyze your results by comparing your unknown sample sizes to known standard sizes to assist in calculating the sizes of the unknown samples. You will complete the second part of this virtual lab now and you will complete the first part later on in Module Subset II-d. Screening for the sickle-cell gene Sickle cell anemia is a genetic disease in which both genes in the patient encode the amino acid valine Val in the sixth position of the beta chain betaS of the hemoglobin molecule. The only difference between the two genes is the substitution of a T for an A in the middle position of codon 6. Can you label the pedigree whether with who is a carrier, normal or afflicted individual? Two probes were used: They provide a built-in ruler for measuring the exact distance that each fragment travels. None of his bands matches the bands found in the semen. Is suspect 1 guilty? Why or why not? What can you do to prove your point. This material may note be reproduced in whole or part without written permission from the Biotechnology Training Centre, University of Calgary, Hospital Dr. The case study focuses on the molecular techniques of the Polymerase Chain Reaction and Gel Electrophoresis and demonstrates their real-life medical applications for studying human genetics and diseases.

DOWNLOAD PDF RESTRICTION ENDONUCLEASES (NUCLEIC ACIDS AND MOLECULAR BIOLOGY)

Chapter 3 : Restriction Enzymes | Biology OER

The sequence-specific DNA cleavage activity of restriction endonucleases (REases), combined with other enzymatic activities that amplify and ligate nucleic acids, have enabled modern molecular biology.

The techniques used to study molecular biology of nucleic acids are: Use of restriction endonucleases 3. Isolation of specific segments of DNA 6. Cloning DNA in plasmid vectors 8. Vector DNA can be introduced into host organisms by transformation 9. Libraries of DNA molecules can be created by cloning and a few others. Use of restriction endonucleases: Which can be used to identify specific DNA molecules. Isolation of specific segments of DNA: Which is used for separation of specific segments of DNA from much larger DNA molecules followed by their selective amplification? Hence the DNA can be sequenced. This is the ability to construct recombinant DNA molecules and maintain them in cells. This process, however, involves a vector. Cloning DNA in plasmid vectors: In it for example plasmid vector has a unique recognition site for EcoRI. Treatment with the appropriate restriction enzyme would line size the plasmid. Vector DNA can be introduced into host organisms by transformation: Transformation is a process in which host organism can take up DNA from its environment. However, transformation is a relatively inefficient process. Libraries of DNA molecules can be created by cloning: Different types of libraries are made using insert DNA from different sources. To enrich for coding sequences in the library a cDNA library is used. These fragments can be ligated into the vector. Hybridization can be used to identify a specific clone in a DNA library: The process by which a labelled DNA probe is used to screen a library is known as colony hybridization. Use of Chemically synthesized oligonucleotides: DNA polymerase can use the oligonucleotide as a primer and elongate it in a 5 to 3 direction to generate an extended region of double stranded DNA. It should be noted that to begin within the first step of the PCR the DNA template is denatured by heating and annealed or attached with synthetic oligonucleotide primers. DNA polymerase is added. It is accompanied heat and repeat. Finally DNA will increase in abundance with each subsequent cycle of the chain reaction. Nested sets of DNA fragments of several nucleotide sequences: Nested sets of DNA molecules are created by two ways. However, this chemical procedure is no longer in wide use. The second procedure chain-terminating nucleotides are used. The modern automatic sequencing machines called sequenators are based on this technology. Shotgun sequencing a bacterial genome: The bacterial species Hemophilus influenza was the first organism to have a complete genome sequence and assembly. The genome of this bacterium is composed of 1. The H influenza genome was randomly sheared into many random fragments with an average size of 1 kb. DNA was prepared from individual recombinant DNA colonies and sequenced separately on Sequenators employing dideoxy method. This method is known as shotgun sequencing. Use of shotgun strategy for partial assembly of large genome sequences: Sophisticated computer programmes have been prepared which assemble the short sequences from random shotgun DNAs into larger contiguous sequences known as contigs. Short contigs are assembled into larger scaffolds which are typically 1 â€” 2 Mb long. Use of paired-end strategy for the assembly of large genome scaffolds: Special cloning vector known as BAC or bacterial artificial chromosome. The use of BACs is used for the assignment of multiple contigs into a single scaffold of several megabases. Genome â€” wide analysis: A variety of bioinformatics tools are required to identify genes and know the genetic composition of complex genomes. A limitation of current gene finder programme is the failure to identify promoters. Therefore, most important method for validating predicted protein coding gives and identifying those missed by current gene finder programme is the use of cDNA sequence data cDNAs are produced by reverse transcription from mature mRNAs and thus represent bonafide or real exon sequences. A noted finding of comparative genome analysis is the high degree of syntery i. Searching a genome or many genomes, for all the predicted protein sequences which are related to be called query sequence.

Chapter 4 : Restriction enzyme - Wikipedia

Restriction enzymes, also known as restriction endonucleases, have played a key role in the development of recombinant DNA technology. These have been found in microorganisms tested and are known to cut double-stranded DNA to yield restriction fragments.

Published online Apr This article has been cited by other articles in PMC. Abstract Restriction endonucleases are the basic tools of molecular biology. Many restriction endonucleases show relaxed sequence recognition, called star activity, as an inherent property under various digestion conditions including the optimal ones. To quantify this property we propose the concept of the Fidelity Index FI , which is defined as the ratio of the maximum enzyme amount showing no star activity to the minimum amount needed for complete digestion at the cognate recognition site for any particular restriction endonuclease. Fidelity indices for a large number of restriction endonucleases are reported here. The FI provides a practical guideline for the use of restriction endonucleases and defines a fundamental property by which restriction endonucleases can be characterized. They are ubiquitously present among prokaryotic organisms 5 , where they form part of restriction-modification systems, which usually consist of an endonuclease and a methyltransferase. The cognate methyltransferase minimally methylates the same specific sequence that its paired endonuclease recognizes and renders the modified DNA resistant to cleavage by the endonuclease so that the host DNA can be properly protected. However, when there is an invasion of foreign DNA, in particular during bacteriophage infection, the foreign DNA will be degraded before it can be completely methylated. This is the major biological function of the restriction-modification system, protecting the host from bacteriophages 6. Other functions have also been suggested, such as involvement in recombination and transposition 7â€”9. After the discovery of the sequence-specific nature of the first REase 10 , 11 , it did not take long for people to find that certain REases cleave sequences which were similar, but not identical, to their defined recognition sequences under nonoptimal conditions 12 , This relaxed specificity was termed star activity. Many REases with star activity have been reported: Experimentally, it has been found that the following general conditions may increase star activity: It has been suggested that water-mediated interactions between the REase and DNA are the key differences between specific complexes and star complexes 17 , 38 , 65 , but the actual situation may be even more complicated and remains to be clarified. Star activity is not desirable for most REase applications; because it is normally weak and the sites of cleavage are not well defined, the products from star activity are much less predictable than the cognate activity. In conventional cloning, both the vector and the insert are cleaved by the same pair of REases, generating compatible ends for ligation. On the other hand, star activity can introduce undesirable cuts in the vector and the insert at extra sites perhaps destroying either one or both and leading to low yield of correctly ligated products. In cases such as forensic applications, where a certain DNA substrate needs to be cleaved by a REase to generate a unique fingerprint, star activity will destroy existing band s and generate new band s , complicating the analysis. Avoiding star activity is also critical in applications such as strand displacement amplification 66 and serial analysis of gene expression Though star activity can be significantly enhanced in many abnormal circumstances, for some enzymes, it can occur under normal reaction conditions. For those enzymes with apparent star activity, uncertainties in the substrate amount, substrate quality and the exact amount of restriction endonuclease can lead to unpredictable results. Furthermore, the activity of restriction endonucleases may change during storage and manipulation, and therefore reaction completeness and the effects of star activity may vary. For some enzymes, the restriction digestion reaction may not reach completion before star activity is observed. Nevertheless, in some cases star activity is advantageous; in the years when there were limited numbers of REases available, certain REases were deliberately used under star conditions so that virtual new specificities were obtained. In another case, the star activity of BamHI was used to generate unidirectional deletion vectors Even though the concept of the star activity of a REase has been known for over 30 years, no systematic study on star activity has yet

been published, largely due to the absence of a quantitative definition and the lack of sufficiently high concentrations of restriction endonucleases to allow a systematic analysis. Here we propose a quantitative definition of star activity, which we call the Fidelity Index FI. It is the ratio of the highest amount of a REase showing no star activity during digestion HNS to the lowest amount needed for complete digestion on cognate sites LCC. Similar to the unit definition of the restriction endonuclease, FI is measured on specific DNA substrates under specified conditions, mostly identical to the unit definition conditions. The factors that could alter the effective FI are also surveyed. It must be emphasized that while star activity is an intrinsic physical property of REases, it is not an all-or-none phenomenon and does not necessarily require unusual digestion conditions to be observed. For some REases, a very high concentration of the enzyme can exist in the reaction and still have no star activity, while with others, adding a slight excess or even the minimum amount to achieve complete digestion is enough to reveal star activity for others. Ipswich, MA, USA NEB as stock solutions of the highest available concentration, usually significantly higher than the commercially available standard concentration. Because of the need for high and accurate concentration of the enzymes, we compared only a few of them from other suppliers to confirm that our results are not an artifact of our preparation procedures. All reactions have the same glycerol concentration in the standard FI determination. The exact components for the diluents are as following: Three microliters of the enzyme solution were then mixed with 0. Each commercial REase supplier has their own buffer system; however, many are comparable to the buffer systems tested here. The reactions were carried out in a well microplate Corning flat bottom assay plate with low evaporation lid. All components were at the same concentrations as used for REase unit determination which specifies a different volume: This glycerol concentration was chosen because it is the highest suggested amount from most restriction endonuclease suppliers and represents the likely worst-case scenario in which to observe unwanted star activity. After electrophoresis, a picture of the gel was taken using a UV imager Bio-Rad. The photograph was deliberately overexposed a little to reveal subtle weak bands; some bands will thus be saturated but the major bands were still clearly resolved. The gel picture was taken with a white background to increase contrast. For each set of reactions, the FI was calculated based on the dilution of the lane first showing no star activity compared to the last lane showing no partial digestion. For BamHI, the following substrates were chosen: The buffer N2 was chosen to provide the clearest demonstration of star activity. All substrates were from New England Biolabs, Inc. Each reaction contained 0. The FIs were measured individually under each of the different conditions. The following REases have been tested: Each lane contains the same amount of DNA 0. The amount of enzyme is reduced as a 2-fold serial dilution across the gel. At the far right, there is very little enzyme, so little or no DNA is digested. As the amount increases toward the left, partially digested fragments start to appear, which eventually become fully digested when the amount of the enzyme reaches a critical point. At this point, all bands that can only be generated by this specific REase cleaving at its designated recognition sequence reach their highest intensities, and no other bands are present. This gives rise to the unique cleavage pattern that can be predicted based on the DNA sequence of the substrate and the recognition sequence of the enzyme. This pattern remains as the REase amount continues increasing until the star activity of the enzyme begins to appear. The amount of the REase for the lane that immediately precedes the initial star activity lane will be considered the highest amount showing no star activity HNS. Once star activity appears, the normal cleavage band s is further cleaved into smaller fragments, visible as a weakening of the normal band s and the appearance of a new star activity band s.

Chapter 5 : Restriction Enzymes: Types and Sites | Nucleic Acids

Restriction Endonucleases: Molecular Cloning and Beyond Return to Restriction Endonucleases The sequence-specific DNA cleavage activity of restriction endonucleases (REases), combined with other enzymatic activities that amplify and ligate nucleic acids, have enabled modern molecular biology.

In this article we will discuss about: Subject-Matter of Restriction Enzymes 2. Nomenclature of Restriction Enzymes 3. Subject-Matter of Restriction Enzymes: Restriction enzymes, also known as restriction endonucleases, have played a key role in the development of recombinant DNA technology. These have been found in microorganisms tested and are known to cut double-stranded DNA to yield restriction fragments. The first observations on the existence of restriction enzymes was made by Arber and Dussoix in , and proposed model to explain the restriction phenomenon. Their views on restriction enzymes affirmed that certain bacterial strains contained endonucleases able to cleave unprotected DNA. In addition, several other strains contained a modification system responsible for protecting their own DNA. Some of the observations were made by W. Arber and his associates while studying the efficiency of plating of the bacteriophage lambda on different strains of Escherichia coli. They even demonstrated that restriction endonucleases were able to cleave DNA from other strains while exempting that of the original strains. Thus, invading foreign DNA in bacteria that has not been correctly methylated will be degraded. In , Smith, Wilcox and Kelly have characterized and purified restriction enzymes and elucidated their recognition and cleavage site of a more useful restriction enzyme, Hind II. Nomenclature of Restriction Enzymes: The discovery of a large number of restriction enzymes led to the systematic assignment of uniform nomenclature, proposed initially by Smith and Nathens. The first letter code is always a genus name and written in capital and the following letters are represented by species name. The subsequent letter designates the strain, and roman numbers indicate different endonucleases from the same organism. Types of Restriction Systems: Three types of restriction-modification systems are recognised. Type II system produce the well-known restriction enzymes and they are most useful in molecular biology applications. Sites of Restriction Enzymes: Restriction enzymes recognize specific sites of different lengths and base composition. The typical restriction enzyme Type II site is an exact palindrome of 4, 5, 6, 7 or 8 base pair. Thus, as long as the same polarity exists recognition sites generally read the same on both strands. Such sequences are often described as palindromes. Some other restriction enzymes do not require a palindrome for site recognition at the typically cut DNA and one side of the recognition site. Most enzymes will not cut DNA methylated on one or both strands of their recognition site, although a few require methylation in order to cut DNA. The number and size of the fragments generated by a restriction enzyme depend on the frequency of occurrence of the restriction site in the DNA to be cut. Construction of genomic mapping requires cutting of DNA into larger fragments by eight base cutters. Six base cutters are used for cloning into specific regions of plasmids. Enzymes with the same recognition sequence do not necessarily cut at the same position of restriction site. Various factors can influence cutting of the DNA. The most important are methylation, structure of the substrate and the nature of buffer employed. Restriction enzyme will generally not cut molecules which are methylated at recognition site. Methylation takes place at other position within the recognition site may fail to affect cleavage. Cleavage by restriction enzyme can generate a number of different ends. These are called as sticky ends. Restriction system recognizing palindromic sequence typically cut within the recognition site not only produces single strand protruded ends but also produce blunt ends with no protruding bases. Purification of Restriction Enzymes: Purification of Type II enzymes involves analysis followed by high speed centrifugation. Further purification is achieved when the DNA binding protsins are bound to DNA cellulose and subsequently eluted with gradient of increasing concentration. The eluted fractions are incubated with standard DNA such as plasmid or phage DNA, and then confirmed by gel electrophoresis for the determination of restriction digestion activity. It takes 3 to 4 days for the purification of 5, to , units of enzyme from 45 to 50 gm of bacteria.

Chapter 6 : Restriction Enzymes

Endonucleases are enzymes that can hydrolyze the nucleic acid polymer by breaking the phosphodiester bond between the phosphate and the pentose on the nucleic acid backbone. This is a very strong covalent bond while the weaker hydrogen bonds maintain their interactions and double strandedness.

Return to Restriction Endonucleases The sequence-specific DNA cleavage activity of restriction endonucleases REases , combined with other enzymatic activities that amplify and ligate nucleic acids, have enabled modern molecular biology. After more than half a century of research and development, the applications of REases have evolved from the cloning of exogenous DNA and genome mapping to more sophisticated applications, such as the identification and mapping of epigenetic modifications and the high-throughput assembly of combinatorial libraries. Furthermore, the discovery and engineering of nicking endonucleases NEases has opened the door to techniques such as isothermal amplification of DNA among others. In this review, we will examine the major breakthroughs of REase research, applications of REases and NEases in various areas of biological research and novel technologies for assembling large DNA molecules. The first REases discovered recognized specific DNA sequences, but cut at variable distances away from their recognition sequence Type I and, thus were of little use in DNA manipulation. Soon after, the discovery and purification of REases that recognized and cut at specific sites Type II REases allowed scientists to perform precise manipulations of DNA in vitro, such as the cloning of exogenous genes and creation of efficient cloning vectors. The development of gene cloning vectors and selection methodologies enabled the cloning of REases. Cloning not only allowed the production of large quantities of highly purified enzymes, but also made the engineering of REases possible. Engineering Improved Performance Cleavage activity at non-cognate sites i. Of those, some exhibit star activity under sub-optimal reaction conditions, while others have a very narrow range of enzyme units that completely digest a given amount of substrate without exhibiting star activity 4. Through intensive research, scientists at NEB began engineering restriction enzymes that exhibit minimal, if any, star activity with extended reaction times and at high enzyme concentrations. This represented an excellent opportunity to engineer altered sequence specificity into the REase. As an added advantage, the sharing of the TRD between the REase and MTase activities resulted in an equivalent change in MTase activity for any change in target sequence cleavage specificity, protecting the new target site from cleavage in recombinant host cells. Through bioinformatics analysis of homologous protein sequences, scientists at NEB identified the amino acid residues that recognized specific bases within the target sequences and created MmeI mutants with altered sequence specificities 5. Rational design of MmeI mutants and homologs unlocked the potential for the creation of REases with hundreds of new sequence specificities. Engineering Nicking Endonucleases Basic research involving REases led to surprising findings about the seemingly straightforward mechanism of cleavage. Prototypical Type IIP REases normally act as homodimers, with each of the monomers nicking half of the palindromic site. These properties have been exploited to create strand-specific nicking enzymes NEases for more information about nicking enzymes, see review in 6. Using this methodology, Stanley Cohen and his colleagues incorporated exogenous DNA into natural plasmids to create the vehicle for cloning-plasmid vectors that self-propagate in E. These became the backbone of many present-day vectors, and enabled the cloning of DNA for the study and production of recombinant proteins. Restriction enzymes are also useful as post-cloning confirmatory tools, to ensure that insertions have taken place correctly. DNA fragments can also be moved from one vector into another by digesting with REases and ligating to compatible ends of the target vector. In vitro DNA Assembly Technologies Synthetic biology is a rapidly growing field, in which defined components are used to create biological systems for the study of biological processes and the creation of useful biological devices Both approaches allow for the parallel and seamless assembly of multiple DNA fragments without resorting to non-standard bases. They also require multiple cloning cycles to create a working biological system. The advantages of such an arrangement are

three-fold: The net result is the ordered and seamless assembly of DNA fragments in one reaction. The accuracy of the assembly is dependent on the length of the overhang sequences. The downside of these Type IIS REase-based methods is that the small number of overhanging bases can lead to the mis-ligation of fragments with similar overhang sequences It is also necessary to verify that the Type IIS REase sites used are not present in the fragments for the assembly of the expected product. Nonetheless, Golden Gate Assembly is a robust technology that generates multiple site-directed mutations 22 and assembles multiple DNA fragments 23, As open source methods and reagents have become increasingly available see www. Upon cleavage, the overhanging sequences of the adjoining fragments anneal to each other. Multiple pieces of DNA can be cleaved and ligated simultaneously. Gibson, of the J. Craig Venter Institute, described a robust exonuclease-based method to assembly DNA seamlessly and in the correct order. The reaction is carried out under isothermal conditions using three enzymatic activities: Applying this methodology, the In combination with in vivo assembly in yeast, Gibson Assembly was used to synthesize the 1. The synthesized genome was transplanted to a M. Other applications of Gibson Assembly include the introduction of multiple mutations, assembly of plasmid vectors from chemically synthesized oligonucleotides, and creating combinatorial libraries of genes and pathways. Gibson Assembly Workflow Gibson Assembly employs three enzymatic activities in a single-tube reaction: The polymerase activity then fills in the gaps on the annealed regions. The overlapping sequence of adjoining fragments is much longer than those used in Golden Gate Assembly, and therefore results in a higher percentage of correct assemblies. It has been widely used in cancer research to identify mutations and study gene expression. NlaIII is instrumental as an anchoring enzyme, because of its unique property of recognizing a 4-bp sequence CATG and creating a 4 nucleotide overhang of the same sequence. The use of Type IIS enzymes as tagging enzymes that cleave further and further away from the recognition sequence allows for the higher information content of SAGE analyses e. Chromosome conformation capture 3C and derivative methods allow the mapping of the spatial organizations of genomes in unprecedentedly high resolution and throughput REases plays an indispensible role in creating the compatible ends of the DNA cross-linked to its interacting proteins, such that spatially associated sequences can be ligated and, hence, identified through high-throughput sequencing. ApeKI was also used to generate the DNA library for a genotyping-by-sequencing technology for the study of sequence diversity of maize Because the nicking site is regenerated, repeated nicking-extension cycles result in amplification of specific single-stranded segments of the sample DNA without the need for thermocycling. NEases greatly streamline the workflow of such applications and open the door to applications that cannot be achieved by REases. Nicking-based DNA amplification had also been incorporated into molecular beacon technologies to amplify signal By ligating adaptors containing nicking sites to the ends of blunt-ended DNA, the simultaneous actions of the NEase s and strand-displacing DNA polymerase can quickly amplify a specific fragment of dsDNA Amplification by nicking-extension cycling is amenable to multiplexing and can potentially achieve a higher fidelity than PCR. Innovative applications of nicking enzymes include the generation of reporter plasmids with modified bases or structures 42 and the creation of a DNA motor that transports a DNA cargo without added energy A review of NEases and their applications has been published elsewhere 6. There is, however, no control over the integration site. The potential to generate new recognition specificity in the MmeI family REases, the engineering of more NEases and the discovery of ever more modification-specific REases continues to create new tools for DNA manipulation and epigenome analysis. Cell Probes 10, Learn about Restriction Endonucleases.

Chapter 7 : Restriction Endonucleases - Google Books

A restriction enzyme or restriction endonuclease is an enzyme that cleaves DNA into fragments at or near specific recognition sites within the molecule known as restriction sites. [1] [2] [3] Restrictions enzymes are one class of the broader endonuclease group of enzymes.

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This article continues the series of Surveys and Summaries on restriction endonucleases (REases) begun this year in Nucleic Acids Research. Here we discuss 'Type II' REases, the kind used for DNA analysis and cloning. We focus on their biochemistry: what they are, what they do, and how they do.

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Endonucleases can begin to degrade at specific internal sites in a nucleic acid strand or molecule, reducing it to smaller and smaller fragments. A few exonucleases and endonucleases degrade only single-stranded DNA.