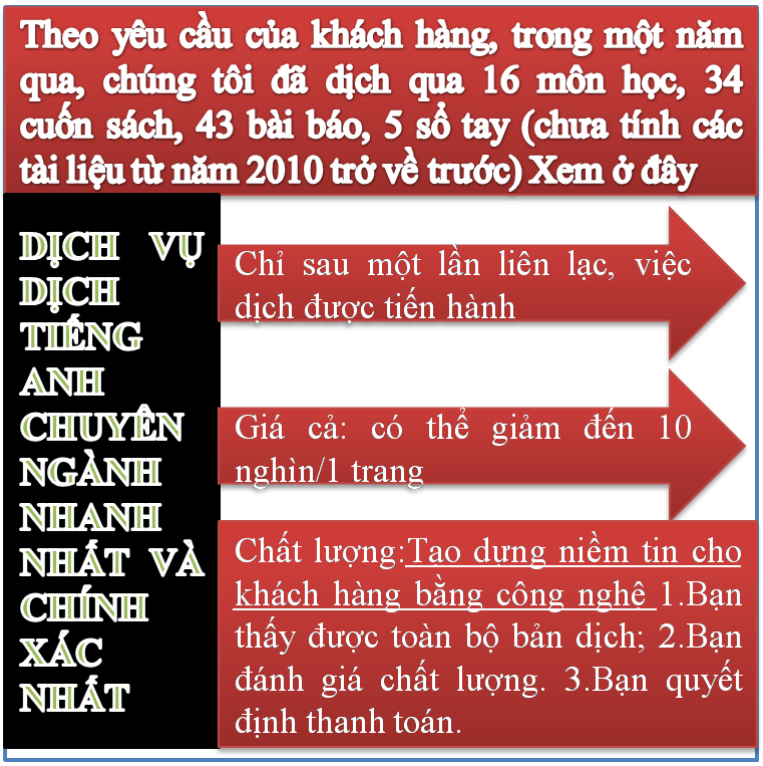

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Review: Molecular Biology of Transgenic Animals

ABSTRACT The amazing pace of discovery within the field of molecular biology has expanded the understanding of as well as the potential for genetic engineering in laboratory and domestic animals. Recently, specific techniques have been perfected, including recombinant DNA, genetic cloning, analysis of gene expression, and the sequencing of nucleic acids and preteins. These molecular techniques combined with methods allowing transfer of the recombinant genes into living cells and fertilized eggs advent the production of transgenic mice and other animals, the application of which is unlimited.

Key Words: DNA, Recombinant, Transgenic, Molecular

Introduction

The extraordinary impact of molecular biology and recombinant DNA technology on basic research, agriculture, and medicine has revolutionized our lives with the past half-century. In **1944,** DNA was merely a suggestion. In **1952,** it became a hypothetical model, and **10 yr** later sex determination was defined. The first recombinant DNA clones were made in **1972,** and by the end of the decade scientists were synthesizing and modifjing genes and transferring them into recipient cells. This development allowed scientists to create new phenotypes in cultured cells, animals,

Đánh giá: Sinh học phân tử của động vật chuyển gen

TÓM TẮT Sự phát triển vượt bậc của các nghiên cứu trong lĩnh vực sinh học phân tử đã mở rộng sự hiểu biết cũng như tiềm năng cho kỹ thuật di truyền đối với động vật nuôi trong phòng thí nghiệm và trong nhà. Gần đây, các kỹ thuật đặc biệt đã được hoàn thiện, bao gồm cả DNA tái tổ hợp, nhân bản vô tính, phân tích biểu hiện gen, và sự sắp xếp axit nucleic và preteins. Sự phối hợp giữa các kỹ thuật phân tử này với các phương pháp cho phép chuyển các gene tái tổ hợp vào trong tế bào sống và trứng được thụ tinh đã dẫn tới sự ra đời của chuột và các loài động vật biến đổi gene khác, và ứng dụng của chúng là không giới hạn.

Từ khóa: DNA, Tái tổ hợp, Biến đổi gen, Phân tử

Giới thiệu

Tác động to lớn của sinh học phân tử và công nghệ DNA tái tổ hợp đối với nghiên cứu cơ bản, nông nghiệp và y học đã cách mạng hóa cuộc sống của chúng ta trong nửa thế kỷ qua. Vào năm **1944**, DNA đơn thuần chỉ là một ý tưởng đề suất. Đến năm **1952**, nó đã trở thành một mô hình giả định, và **10 năm** sau đó việc xác định giới tính đã thành hiện thực. Các dòng DNA tái tổ hợp đầu tiên đã được tạo ra vào năm **1972**, và vào cuối thập kỷ này các nhà khoa học đã tổng hợp, biến đổi gene và chuyển chúng vào các tế bào nhận. Sự phát triển này đã cho phép các nhà khoa học tạo ra các kiểu hình mới trong tế bào nuôi,

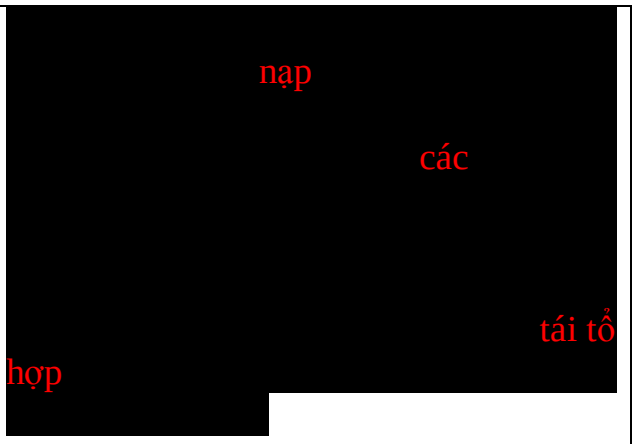
and humans. Today, several experimental clinical therapies are based on recombinant DNA to alter and(or) supplement genetic defects in serious diseases (Anderson, **1992).**	động vật và con người. Ngày nay, một số phương pháp điều trị lâm sàng thử nghiệm dựa trên DNA tái tổ hợp để thay đổi và (hoặc) bổ sung khuyết tật di truyền trong nhiều bệnh nghiêm trọng (Anderson, **1992**).
The purpose of this review is to provide a basic understanding **of** the molecular biology (procedures, techniques, and molecular jargon) that facilitates the construction and diagnosis of transgenic animals. Molecular biology combines techniques and expertise from biochemistry, genetics, cell biology, developmental biology, and microbiology to form an umbrella discipline that provides the tools and concepts necessary to create transgenic animals. Biotechnology provides techniques needed for the creation of transgenic animals, including recombinant DNA, transfer of cloned genes into living cells, injection of cloned genes into fertilized eggs, and molecular methods used to analyze the transgenics.	bài báo tổng quan chuyên ngành về chuyển vào
Genetic Manipulation The term “Genetic manipulation” is used to encompass the insertion of a piece of nucleic acid, usuallyDNA, into a small replicating unit, called a vector, which is derived from **a** virus or plasmid of prokaryoticor eukaryotic origin. The latter facilitates incorporationof the foreign DNA into the recipient cellular genome (chromosomes) to ensure continued propagation. The advent of three basic molecular techniquesmakes genetic manipulation possible: **1)**	đề cập đến (DNA ngoại lai) bộ gen của tế bào nhận

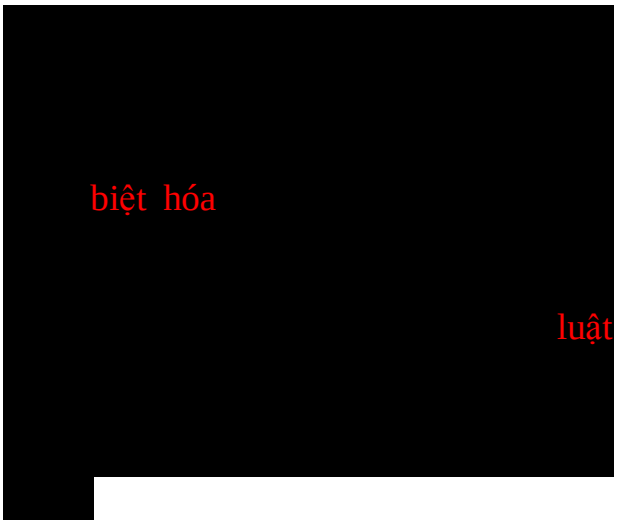
transfer of DNA into ***E. coli***, called transformation (Cosloy andOishi, **1978;** Roberts, **1984); 2)** restriction enzymesthat cut DNA at phosphodiester linkages between specific nucleotides and the ligation or joining of twoDNA molecules (Smith and Nathans, **1973);** and **3)**methods that monitor the recombinant moleculesthroughout manipulation steps (Southern, **1979).**

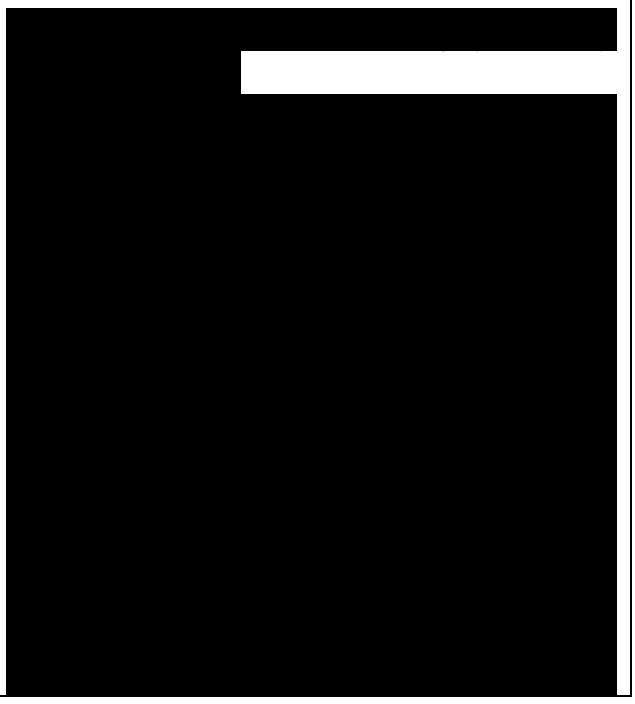
Gene manipulation assumes that the exogenous gene will function normally inside the recipient cell or organism (i.e., that it will replicate throughout cycles of cell division and differentiation and that the gene product will be made). The success of gene transfer depends on a basic understanding of the structure and regulation of eukaryotic and prokaryotic genes in the molecular steps of DNA replication, transcription, and translation.

Prokaryotic and Eukaryotic Gene Structure

The contrast of the structure and function of prokaryotic and eukaryotic genes (Figure 1) is an essential part **of** the successful cloning and expression of eukaryotic genes. The revelation of the structure and functional control of prokaryotic and eukaryotic genes continues at an astonishing speed in modern biology. The basic functional gene such as the *Lac2* operon in *E. coli* is multicistronal: three proteins, B-galactosidase, permease, and transacetylase, are expressed from a single transcriptional unit (Shapiro etal.,





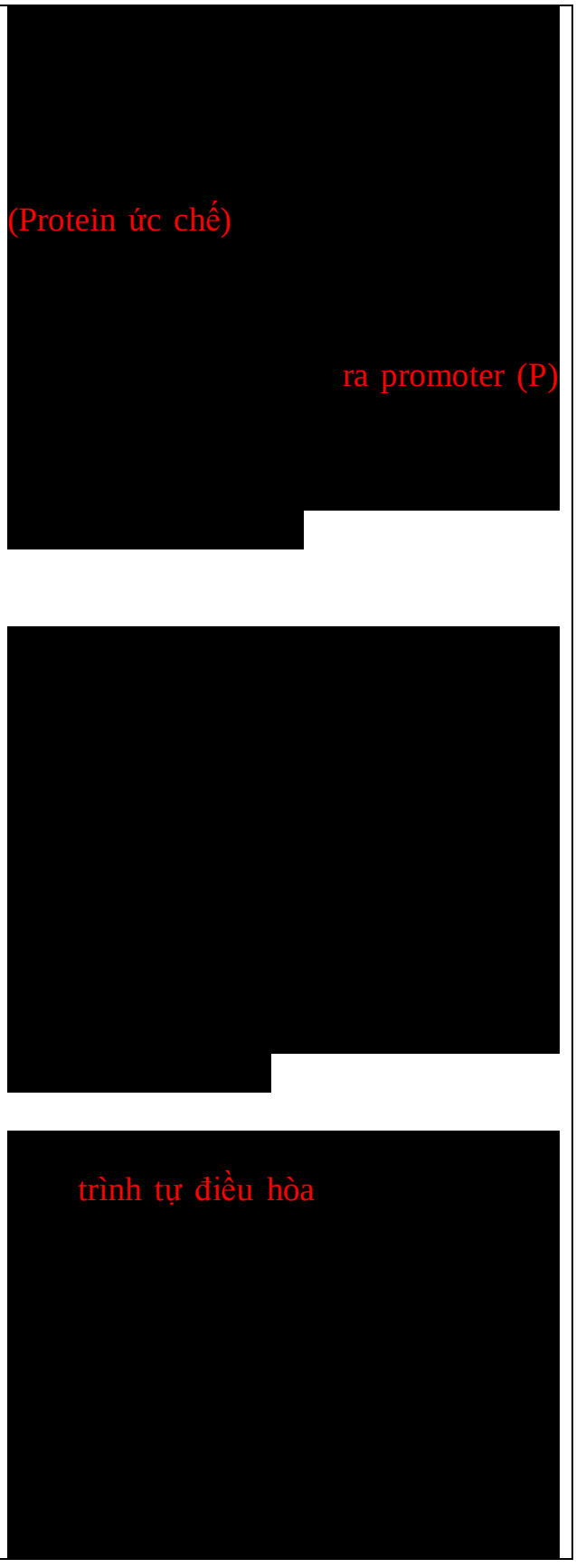


1969). The gene contains a positive and negative regulatory sequence ***5'*** to the transcription unit that controls the on or off nature of the gene in response to the environment of the cell. When the bacterium is fed glucose the ***Lac*** 2 gene product, B-galactosidase, is not needed, and therefore the repressor protein (i) binds to the operator DNA sequence ***(0)*** and inhibits the binding of the DNA polymerase to the gene to consequently inhibit transcription. When the bacterium is fed lactose and no glucose, the organism must bring the disaccharide into the cell by making a permease, then produce the enzyme B-galactosidase to cut the disaccharide into galactose and glucose, which are then metabolized by the glycolytic pathway to fuel the cell. Two important features of prokaryotic gene expression are illustrated in this example: 1) the regulatory sequences are ***cis*** (adjacent DNA sequences ***5'*** to the transcription unit) and **2)** the appropriate RNA polymerase transcribes the entire transcription unit even though there are three genes (to be translated into three separate proteins) within the unit as one continuous transcript. The prokaryotic cell has no segregation of molecular function and no distinct nucleus, and therefore the translation of the mRNA begins concomitantly with the transcription event. This means there is no processing of the transcript: it is translated according to start and stop codons sequentially.	trình tự điều hòa dương và âm của đơn vị phiên mã trình tự tạo ra các trình tự điều hòa với khác bản phiên mã

Figure 1. Structure and function of prokaryotic and eukaryotic genes. The Lac 2 gene illustrates the regulation, structure, and function of the typical prokaryotic gene. The repressor protein encoded by gene (i) binds to the operator sequence (0) and blocks the transcription of the gene. Induction removes the I protein from the 0 sequence, allowing the polymeraseto recognize the promoter (P) and transcribe thedownstream sequence. The mRNA transcript is immediately translated into the three functional proteins encoded by the Lac 2 operon.

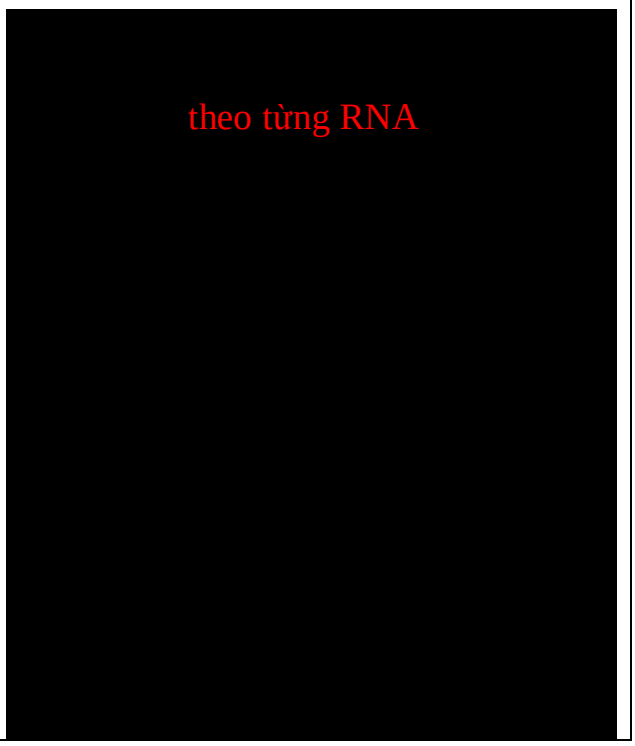
The B-globin gene illustrates the regulation, structure, and function of the typical class I1 eukaryotic gene. The promoter /P) signals the downsteam transcription by the RNA polymerase. The initial transcript is subsequently modified by addition of a 5' cap, removal of the introns by splicing, and addition of a polyadenylated tail at the 3' end. The mRNA is transported to the cytoplasm and translated by ribosomes into one protein product, betaglobin.

In contrast, eukaryotic genes contain more complex regulatory sequences and multiple processing steps. Genes that produce mRNA (messenger RNA) for subsequent translation into protein process the initial transcript in three stages, capping of the ***5'*** terminus, removal of introns by splicing, and polyadenylation of the **3'** terminus (Abelson, 1979; Maniatis et al., 1987). Additionally, the eukaryotic



transcription and processing events are separated from translation by the nuclear membrane. A classic example of an eukaryotic gene is beta-globin, shown in Figure 1. The regulatory regions are ***5'*** defined sequences and located at a specific distance from the start of the transcription unit. Some genes contain internal regulatory sequences, especially genes with specified differentiation functions. The regulatory regions are called promoters and enhancers. The promoter contains a minimum of two conserved sequences that are specifically recognized by proteins called transcription factors. The protein-DNA complex formed between the transcription factor and the DNA promoter elements forms a traffic signal for the RNA polymerase. The polymerase recognizes the signal and transcribes the sequence (Johnson and McKnight, 1989).

Sequential modifications are made as independent events following synthesis of the RNA by RNA polymerase. The ***5'***end of the transcript is modified by the addition of a 7-methylguanosine ***5'4'***phosphodiester-linked nucleotide to the first base of the transcript to create a "cap"structure. This structure regulates passage of the transcript from the nucleus to the cytoplasm and helps the ribosome identify the AUG start codon for translation (Shatkin, 1976). Sequences within the transcript not retained in the coding sequence of the mRNA are called "introns," or intervening sequences, and are removed by a process of splicing at specific



nucleotide signal sequences that identify the boundaries of the expressed sequences, exons, and the removed intron sequences. The removal of introns reduces the size of the primary transcript significantly (Padgett et al., 1984).

Finally, the 3' end of the transcript is modified in most mRNAs by the excision of the 3' end and the addition of a polyadenylated tail by a separate enzyme polyadenylase. The 3' poly(A) tail increases the stability of the mRNA in the cytoplasm and allows more protein to be made per mRNA (Aviv and Leder, 1972). The 5' cap seems to be ubiquitous among mRNAs; most are spliced and polyadenylated, but these latter two modifications are optional (e.g., they occur in some genes but not in all). In summary, the processed eukaryotic mRNA is presented to the translational apparatus as a contiguous monocistronic message due to precise excision of introns through splicing.

Recombinant DNA

The term "recombinant DNA" refers to a series of techniques that allow two distinct molecules of DNA to be specifically joined (recombined) into a new molecule. The initial two DNA molecules are 1) the source of desired genetic information, a eukaryotic viral or cellular gene, and 2) a recipient cloning vector, usually a virus or plasmid molecule. The discovery of restriction enzymes (Smith and Nathans, 1973) opened the door to the specific cutting of DNA molecules. These

chuyên biệt

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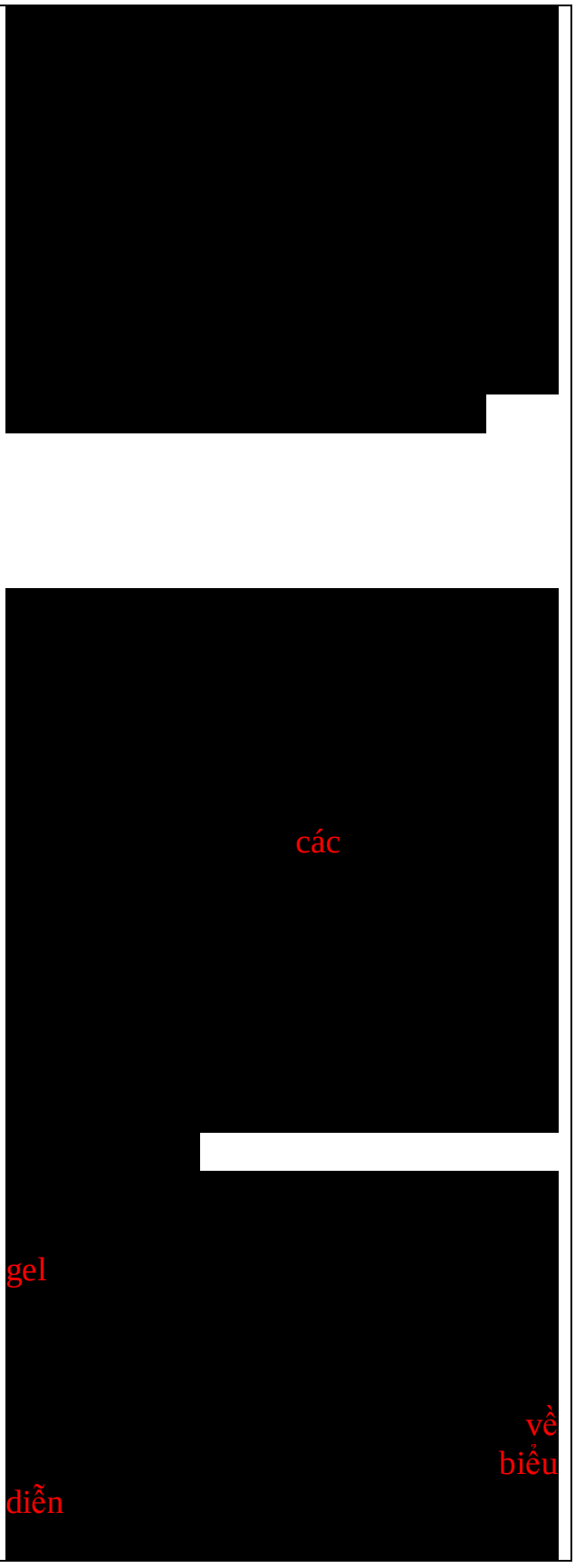
tạo

tùy ý

enzymes recognize a specific sequence of nucleotides and cleave the phosphodiester backbone of the DNA between two specific bases within the sequence. For example, a restriction enzyme from E. coli, EcoRI, recognizes 5'GAATTCS' and cuts between the first two bases (GA) of the sequence, producing two pieces: 5'----G and AA?TC---3'. Hundreds of restriction enzymes are available commercially, and the sales catalogues provide restriction maps of many plasmids as well as the recognition sequence for each enzyme. Restriction maps are circular or linear maps containing the position of each restriction enzyme cleavage site relative to each other in 5'-3' orientation or in a clock orientation that represents the topography of the DNA molecule.	[redacted] [redacted] đã có mặt trên thị trường phân cắt của đối với nhau [redacted]
An example of a cloning vector is the pBR322 plasmid, which replicates independent of the bacterial chromosome in E. coli. The plasmid is 4,362 base pairs long, contains an origin of DNA replication, and two antibiotic resistance genes, one for tetracycline (Tet- R) and one for ampicillin (Amp-R). The entire sequence of the plasmid is published (Backman and Boyer, 1983). The position and number of cuts that specific restriction enzymes make in the plasmid are known and represented as a restriction map of the plasmid (Sutcliffe, 1978). Identification of restriction sites within the plasmid allows scientists to open the plasmid at desired position(s), remove part of the plasmid DNA, and insert another	[redacted] trình tự bố đã biết được biểu diễn dưới dạng [redacted]

sequence of their choice. The two pieces of DNA are sealed with a ligase enzyme. The closed molecule may be larger than the original plasmid, up to 10 kb, without harming the amplification step in the bacterial host cells. Often the foreign DNA is inserted in place of either the Amp-R or the Tet-R gene so that recombinant DNA plasmids can be distinguished from non-recombinants (Cohen et al., 1973). For example, when the foreign DNA is inserted in place of the Tet-R gene, the resulting recombinant is Tet-sensitive (Tet-S) and Amp-R.

The recombinant DNA is transferred into E. coli using a transformation technique that uses calcium ions to alter bacterial membrane permeability (Cosloy and Oishi, 1978). Growth of E. coli then supports many cycles of plasmid DNA replication, resulting in amplified copies of the recombinant molecules. The cells are harvested, the DNA is extracted, and the lowmolecular- weight plasmid DNA is separated from the host cell high-molecular-weight DNA by differential centrifugation. Amplified recombinant DNA is purified and redissolved in Tris buffer. A sample of the DNA is then analyzed by restriction digestion and gel electrophoresis to identify the size of the added fragment and to confirm the recombination event expected by the experimental design (Southern, 1979; Okayama and Berg, 1982). The general concept of creating a gene clone is shown in Figure 2. The plasmid is cut with two different restriction enzymes,



PstI and EcoRI, and the same two enzymes are used to insert the foreign gene (gene X). The ligation of the two pieces of DNA will occur at the homologous restriction sites and insert the foreign gene into the plasmid vector for further amplification and purification. The presence of a eukaryotic promoter and polyadenylation signal in the vector will help ensure expression of the gene in eukaryotic cells.

Isolation of a Desired Gene

Myriad strategies exist for cloning specific genes or isolating a random group of genes from which specific ones can be chosen. Two fundamental strategies allow the creation of a cDNA library and a genomic library. The cDNA library consists of mRNA isolation from a cell type of interest and reverse transcription of the mRNA into double-stranded, complementary DNA. Therefore, the collection of sequences obtained represents the complementary DNA of the mRNA known as the cDNA library. The genomic library is created by restriction digestion of genomic DNA followed by cloning the randomized fragments into a plasmid vector. In both scenarios, the collection of genetic fragments are recombined with vector DNA, closed by ligation, and transformed into host bacteria for growth and amplification. The bacteria are plated on agar and individual colonies are analyzed for recombinant clones of interest. Some molecules will be present in many colonies and some in

only one. Therefore, the next step is to create a detection method to screen the clones for the desired sequence (Hanahan and Meselson, 1980; Berg, 1981).

Generation of a Probe

The polymerase chain reaction (PCR) has become one of the most popular methods for synthesis of a specific sequence of DNA with conjugated chemiluminescent, avidin, or radioactive P32 backbone that serves as a marker for the sequence. The singlestranded DNA synthesized is complementary to some sequence within the desired gene. The PCR method allows the use of a primer oligonucleotide of 20 to 30 bases bordering the target sequence. The template DNA is separated by heat and the primers are added. Each primer base-pairs with the complementary strand of the template. The Taq polymerase, isolated from Thrmus aquaticus, is unique for its heat stability, optimal temperature being 72°C.

liên hợp

đóng vai trò là những tác nhân đánh dấu của trình tự

The Taq polymerase then catalyzes the synthesis of the complementary DNA for each of the two strands, beginning with the primer and continuing to the end of the template. At the end of one synthesis cycle, the temperature is raised to 94°C for a few seconds to allow strand separation and each single strand then becomes template for another round of synthesis. These cycles continue producing hundreds of thousands of copies of the desired sequence (e.g., 30 cycles produces 268,435,456 double-stranded target molecules) (Mullis and Fuloona, 1987).

quá trình một trong hai template (bản mẫu, tiêu bản) phân tử tổng hợp mạch kép

Screening Clones

Screening is an experiment in which recombinant DNA molecules created in the random library are examined for specific genes or gene products of interest. The colony lift assay is a technique in which a replica of the bacterial colonies is made onto a filter. The filter is then placed in lysis buffer to release the protein and nucleic acid in a colony-like circle on the filter. Further analysis employs specific antibody detection of the expressed protein by immunological detection assays or molecular hybridization of the desired sequence to a specific probe, as described below.

cần tái tổ hợp chuyển khuẩn lạc chất đệm ly giải phát hiện kháng thể đặc trưng biểu hiện phát hiện dò chuyên biệt

Molecular hybridization uses a DNA probe synthesized with 32P-labeled nucleotides so that the singlestranded sequence is radioactive (Grunstein and

dò các nu-clê – ô – tít 32P trình tự mạch đơn

Hogness, 1975; Rigby et al., 1977). The creation of a probe requires some information about the gene of interest. This can be obtained from related gene sequence data using a conserved sequence, or from the amino acid sequence of the protein product, or from another source of data about the gene of interest. The hybridization reaction consists of denaturing the DNA from the individual clones using heat or alkaline buffer so that the double-stranded DNA separates. The probe (defined radioactive sequence) is added and allowed to anneal with its complementary sequence. Unreacted molecules are removed by washing, and a piece of x-ray film is added directly to the filter containing the hybridization reaction. The x-ray film is developed to reveal a dark spot over the colony containing the hybridization reaction, thereby identifying the colony with the desired sequence. A colony is then isolated from the original plate and amplified and the DNA is purified. If several colonies react with the probe, each one should be grown to amplify each of the recombinant DNA clones. Subsequent analysis may reveal that several of the first clones isolated were only parts of a gene of interest (Roberts et al., 1979).	dò từ riêng biệt đệm có tính mạch kép dò trình tự tấm Sau được khuếch đại và ADN được làm sạch dò (khuếch đại) phân lập
If the nucleotide sequence is not known, thereby making the synthesis of a DNA probe unreasonable, then the alternative is to identify the gene product by screening for specific protein products.	chúng ta không biết chính điều này làm cho việc tổng hợp đầu dò DNA bất khả thi

In this case, an expression vector is helpful, because the eukaryotic promoter that regulates transcription in the normal host may not be operative or even present.

Therefore, an expression vector containing a prokaryotic promoter and a restriction site for insertion of the DNA fragment is used to ensure expression in the host bacterial cells. Often the cloning vector is a chimera with a prokaryotic promoter such as Lac Z promoter and also contains a poly-A signal at the 3' end. Between the start site and the 3' poly-A signal is a unique restriction site where the plasmid is opened and the foreign DNA inserted. Another library is made, and the colonies are plated onto agar and lifted onto nitrocellulose filter paper for analysis. The lysed bacteria are then probed using antibody to detect the protein of interest. The colonies that react with the antibody are then isolated and amplified and the DNAis studied further (Broome and Gilbert, 1978).

DNA Sequence Analysis

The clones isolated may now be sequenced using the methods of Maxam and Gilbert (1977) or Sanger et al. (1977). Each procedure requires purified DNA fragments whose positions are known within the context of a restriction map. Location of each fragment within the restriction map will allow alignment of each sequence to the proper neighboring fragments. Computer programs assist in this alignment. Gen-Bank and other DNA sequence data

banks are available for identification of promoter elements, transcription start sites, and the most likely amino acid translation product from the cloned sequence as well as for searching for similar sequences already reported.

giải mã amin

trình tự nhân dòng

Gene Transfer into Eukaryotic Cells

The sequenced clones obtained by the recombinant DNA techniques are transferred into eukaryotic cells to determine their expression by one of several methods: DNA transfection (Graham and van der Eb, 19731, electroporation (Shigekawa and Dower, 19881, lipofection (Felgner et al., 1987), microinjection (Diacumakos, 19731, retrovirus vector infection (Eglitis and Anderson, 1988), or infection with other virus vectors (Moss et al., 1990).

đã được xác định

vector

DNA transfection is a standard protocol involving the precipitation of DNA in a calcium ionic buffer (CaP04) onto the surface of the recipient cells. The cells then endocytose the DNA and transport it to the nucleus. Several rearrangements may occur in the transport process and the experimenter has little control over the condition or stability of the DNA inside the recipient cell nucleus. Transfection of several hundred copies of the gene into several million cells increases the chance of stable integration of an intact gene and subsequent expression (Scangos et al.,

sát nhập ổn định

1981). Electroporation uses an electric shock to open the plasma membrane, which allows DNA in the solution to enter cells and subsequently be transported to the nucleus for integration. Electroporation is often chosen over the transfection protocol for cells that grow in suspension or for primary cells that are sensitive to the precipitate. Lipofection is based on the adherence of DNA to a hydrophobic lipid and subsequent fusion to the recipient cell plasma membrane. Transfection, electroporation, and lipofection only facilitate the DNA across the plasma membrane and rely on intracellular transport for nuclear relocation. The advantage of these protocols is the ease of manipulation and the ability to transfer DNA into millions of cells, increasing the frequency of obtaining a positive result. Microinjection, in contrast, is more difficult and requires expensive equipment, but it allows the direct injection of a small amount of DNA directly into the nucleus of the desired cells. The caveat is that only several hundred cells are usually injected and, if the frequency of positive expression is low, the microinjection procedure becomes tedious and expensive.

Figure 2. Construction of an expression vector. The foreign gene designated Gene X is obtained by endonuclease restriction enzyme double digestion with PstZ and EcoRI. Likewise, the pBR322 plasmid DNA is cut with PstI and EcoRI and the two pieces of DNA are ligated with T4 ligase. The resulting

[redacted]

được ưa chuộng hơn so với phương pháp chuyển nạp

[redacted]

kết hợp

[redacted]

plasma của

[redacted]

[redacted]

nhờ quá trình tiêu hủy kép

[redacted]

recombinant DNA molecule contains the plasmid origin (Ori] to control replication of the DNA inside the host E. coli, a Tet-R, Amp4 phenotype and the elements necessary for eukaryotic expression, including a second Ori sequence, eukaryotic promoter (PI, and poly-A signal (A)n.

Co-Transfection with Marker Genes

Dominant selectable marker genes are commercially available. They are often co transfected with the desired non-selectable gene. A complex of largemolecular- weight DNA forms in the precipitate in the Cap04 buffer, forming a "transgenome," which is a combination of the gene of interest, the selection gene, and the excess carrier DNA (Mulligan and Berg, 1980). The dominant selectable gene encoding a bacterial antibiotic-resistant protein for neomycin is called NE0 and is selected for in eukaryotic cells with an aminoglycoside antibiotic, G418. Cells expressing the prokaryotic NE0 gene continue to grow because the gene product is neomycin phosphotransferase, which inactivates the G418. Co transfection of gene X with NE0 followed by selection in G418 allows selection of cells that express the transfected DNA. The pSV2NEO gene (Mulligan and Berg, 1980; Southern and Berg, 1982) was constructed to maintain the pBR322 origin of replication for amplification in E. coli, and regulatory sequences, a promoter and poly-A signal sequence, from SV40. The NE0 gene was inserted at a unique restriction site within the pBR Tet-R gene between

promoter

đồng

điều hòa

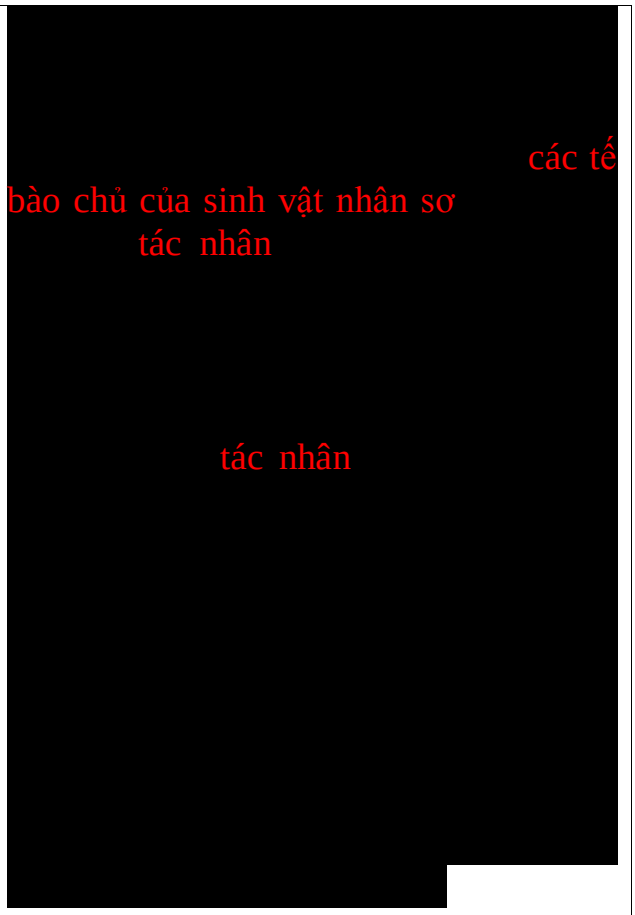
trình tự

vị trí

the SV40 promoter and the poly-A signal sequences. The resulting plasmid is called an expression vector because it contains regulatory elements for expression of the gene in eukaryotic cells as well as amplification of the plasmid in the prokaryotic host cells (Figure 2). Several selectable markers are available, each of which encodes an enzyme essential to a metabolic step inhibited by selective metabolites in the growth medium. Two examples of selectable markers are hygromycin-Bphophotransferase (HPH), an enzyme that inactivates Hygromycin-B, an inhibitor of protein synthesis, and thymidine kinase (TK), an enzyme in thymidylate synthesis without which cells grown in medium containing hypoxanthine, aminopterin, thymidine (HAT) can only replicate when the TK enzyme is expressed.

Functional Genetic Analysis

To determine the function and expression of the gene of interest requires the detection of the mRNA or protein product. Northern blots are one way to determine the presence of the mRNA from the gene (Alwine et al., 1977). The RNA is extracted from the cells, separated on agarose gels by electrophoresis, and blot-transferred to a nitrocellulose filter. The filter containing the RNA is hybridized to the cDNA probe containing the 32P-nucleotide sequence prepared earlier to identify the clone of choice. The hybridization reaction is washed to remove nonspecific probe, exposed to x-ray film, and developed to reveal the



dark bands of hybridization between RNA and cDNA. The size of the band is identified by the migration distance of the RNA on the gel relative to size markers in another lane of the same gel.

Western blots are similar except that they contain the isolated proteins from the cells, separated by gelelectrophoresis and blotted by electrophoretic transferto nitrocellulose paper. The reaction between desired proteins and antibody are identified by a secondary antibody conjugated to avidin-biotin, alkaline phosphatase, horseradish peroxidase, or a radioactively labeled secondary antibody (e.g., 12510dine). Either system is effective for the identification of the protein of interest. If the clones isolated do not produce mRNA or protein of the gene expected, then the entire gene with regulatory regions may not be present. To reisolate the intact gene requires further cloning.

Southern Hybridization

The complementarity between a cloned DNA sequence and the genomic DNA region is determined using Southern hybridization (Southern, 1975). This technique involves isolation of genomic DNA, separation by gel electrophoresis, transfer to nitrocellulose paper, and subsequent hybridization with the probe used to isolate the clones. Radioactive, single-stranded DNA probe anneals with the complementary sequences in the genomic DNA and makes a dark band on x-ray film due to the

được phân
lập từ điện di
gel
thể
thể liên
thể
phân lập
cùng với
các vùng điều hòa

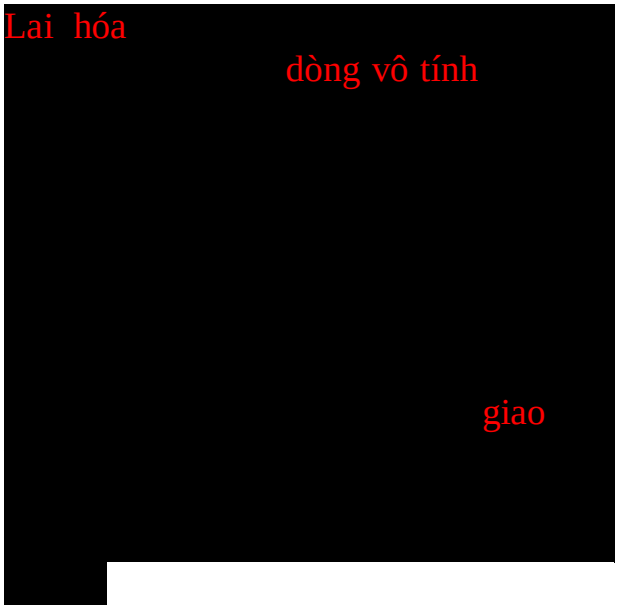
Lai hóa

lai hóa
phân
các dòng vô tính dò mạch
khung

radioactive phosphates in the backbone of the DNA probe. Southern hybridization between genomic DNA of the original source and the clone of interest allows detection of a genomic piece of DNA homologous to the sequence isolated, and if the hybridizing genomic band is larger than the cloned fragment, the chance of isolating the intact gene is increased. The larger genomic DNA is eluted from the agarose and cloned in the same vector or one suitable to the size of the new fragment. Repeat the gene transfer and subsequent steps in functional analysis to determine whether a functional and intact gene is cloned.

In Vitro Mutagenesis

Functional genetic analysis requires the ability to change the DNA sequence and examine the effect the single or multiple base changes have on the genetic expression of the cloned gene. Mutants are defined as altered phenotypic expression in classical genetics. Recombinant DNA techniques including sequence analysis allow detailed detection of subtle changes, silent mutants wherein the phenotype remains the same as wild type but the genetic sequence is altered (mutant), and detailed investigation of altered phenotypes produced by intentional sequence alteration. The new approach, called reverse genetics, encompasses the creation of mutants in a test tube by altering the genetic sequence of a cloned gene and then testing the phenotype produced. Mutations can be created by



deletion of regions between restriction sites within a clone, insertion of a base or segment at restriction sites, and the substitution of one or more bases at specific sequence sites (site-directed mutagenesis) (Smith, 1985).

Figure 3. Transgenic mouse scheme. The fertile male and hyperovulated females are mated 12 h before collection of embryos. One of the two nuclei of the fertilized embryo is microinjected with the foreign DNA and then reimplanted into a pseudopregnant foster mother. The newborn pups are tested by extraction of DNA from a small tail clip for DNA analysis. Animals positive for the foreign gene are designated founder transgenics.

Transgenics

Following isolation of the cloned gene in an appropriate expression vector, and evidence that the gene is functional in eukaryotic cells, a transgenic animal is made by microinjection of the cloned DNA into the fertilized ova (eggs) of a donor animal. The essential steps in producing a transgenic mouse are illustrated in Figure 3. Donor females are superovulated and mated with syngeneic males. The fertilized eggs are surgically removed and placed in buffer for microinjection. The procedure involves the use of an inverted phase-contrast microscope equipped with differential interference contrast (DI C) optics to reveal the two pronuclei, a pipette to support the target cell, and an injecting pipette loaded with the desired DNA. The injecting pipette is inserted

các vị trí trình tự
Gây đột biến

cùng gen

cùng với quang học tương phản
giao thoa vi sai

into the pronucleus and no more than 2 fL of foreign DNA is injected. The pronucleus swells slightly, indicating the successful microinjection of DNA. After microinjection of 100 eggs is completed, they are surgically reimplanted into the oviduct of a foster mother, who gives birth to the new litter. In mice, the survival rate after manipulation and reimplantation is about lo%, and of the survivors, the number that retain the injected DNA is about 40%. In other species (e.g., rabbits, sheep, and pigs), the frequency is 10 to 1,000 times less (Hammer et al., 1985; Purse1 et al., 1989). The identity of the transgenic is determined by extraction of DNA from a tail clip of the newborns and analysis by Southern hybridization using the cDNA probe created to detect the foreign gene. Positive hybridization of DNA extracted from the tail-clip indicates that the gene has integrated in the one-cell stage and has been faithklly replicated along with the endogenous chromosomes of the embryo throughout development and is putatively in each cell of the newborn. Transgenic animals are defined as possessing a foreign DNA sequence in each cell throughout development; however, this does not ensure that the foreign DNA is expressed. A newborn transgenic animal is termed a founder animal, which when mated with a normal mouse produce F1 generations that retain the foreign DNA in Mendelian fashion (Gordon andRuddle, 1983; Brinster et al., 1985; Hogan et al., 1986; Palmiter and Brinster, 1986).	của lai hóa dò thường được cho là nằm trình tự

Integration of the microinjected DNA occurs at random positions in the chromosome of the recipient nucleus. Therefore, unless the DNA is constructed to target a specific chromosomal locus (Thomas et al., 19861, the gene will be the third allele or a new allele in the transgenic animal. If the gene functions normally (like an endogenous gene), expression will occur in a tissue-specific fashion. For example, transgenic mice containing the human insulin gene express insulin in discrete clusters of endocrine cells and not in other tissues. The rat insulin genetic regulatory region was cloned upstream (5') of the coding region of SV40 large T antigen. T antigen was expressed according to the insulin promoter/enhancer. Only beta cells of the endocrine pancreas, and not surrounding exocrine cells, expressed T antigen in the transgenic mice. Islet tumors consisted of proliferating beta cells also expressing the SV40 oncogene, T antigen (Hanahan, 1985; Selden et al., 1986).

Current and Future Applications

The use of transgenic animals is limited only in the human imagination. Transgenic animals have provided a valuable tool for expanded research on the structure and functional relationships of genes, developmental control, differentiation, and the genetic

[redacted]

[redacted]

vùng mã hóa của SV 40

nội tiết tuyến tụy chứ không phải xung quanh các tế bào ngoại tiết

Các khối u dạng đảo

gen gây ung thư

[redacted]

Các ứng dụng hiện tại và tương lai

Việc sử dụng động vật biến đổi gen chỉ bó hẹp trong tưởng tượng của con người. Động vật biến đổi gen trở thành công cụ có giá trị cho những nghiên cứu mở rộng về cấu trúc và các mối quan hệ chức năng của các gen, kiểm soát phát triển, phân biệt và cơ sở di truyền của

basis of diseases (Camper, 1987; DePamphilis et al., 1988; Hanahan, 1988, 1989, 1991; Gordon, 1989; Jaenisch, 1989; Westphal and Gruss, 1989). Use of transgenic domestic animals for food production, creation of vaccines for many species, and production of pharmaceutical products continues to develop at a rapid pace (Clark et al., 1987). The creation of transgenic animals other than mice should continue to produce exciting frontiers in biology, and the success rate will predictably increase as more genetic and developmental biology is revealed (Church, 1987). The induction of predictable proteins in lactation by genetically inserted genes in transgenic pigs is an example of the progress in the area of species other than mice (Shamay et al., 1992). The future of the planet Earth and the species that inhabit it can be significantly affected by these technologies. The hope is that scientists will use the technologies wisely to the benefit of all (Thompson, 1988; Seidel, 1989; Berkowitz, 1990; OConnor, 1991).	các loại bệnh (Camper, 1987; DePamphilis et al., 1988; Hanahan, 1988, 1989, 1991; Gordon, 1989; Jaenisch, 1989; Westphal ADN Gruss, 1989). Hoạt động sử dụng các động vật biến đổi gen trong nước để sản xuất thức ăn, vắc xin cho các loài và dược phẩm tiếp tục phát triển với tốc độ nhanh chóng (Clark et al., 1987). Việc tạo ra các động vật biến đổi gen khác bên cạnh chuột sẽ tiếp tục tạo ra những ngành sinh học mới và tỷ lệ thành công sẽ có thể tăng khi sinh học phát triển và gen được khám phá (Church, 1987). Việc công bố các loại protein trong sữa thông qua các gen được đưa vào những cá thể lợn biến đổi gen chính là minh chứng cho thấy sự tiến bộ trong lĩnh vực này ở các loài khác, không chỉ có chuột (Shamay et al., 1992). Những công nghệ này có thể tác động lớn đến tương lai của trái đất và các sinh vật sống ở đó. Hi vọng rằng các nhà khoa học sẽ sử dụng những công nghệ này một cách thông thái để đem lại lợi ích cho cộng đồng(Thompson, 1988; Seidel, 1989; Berkowitz, 1990; OConnor, 1991).