CLONING AND SEQUENCING EXPLORER SERIES pdf

1: Cloning - Wikipedia

The Cloning and Sequencing Explorer Series allows students to apply molecular biology skills and techniques to complete a real-world research project, potentially encouraging them to pursue a career in research.

Are any of my sequences primarily cloning vector? Individual Clone Sequences Do my individual sequences align to give me a single long sequence? Are there discrepancies between my reads? What is the protein sequence of my clone? A Once activated- change your passwords! Active for 2 months. Convention rates sequences by the number of basecalls that have quality values of 20 or higher- a Q20 value. Salvia folder Look at data Go back to folder report Click: Scroll down to sequence alignments Query: You have cloned, sequenced and annotated a novel gene. You could now submit this to GenBank. Find the column labeled Q These data correspond to the number of bases in each read that have a quality value of 20 or greater. Click the column labeled Q20 to sort the data so that the lower quality data appear low numbers of Q20 bases at the top of the table. If there are more than 25 rows in your folder, click all to view them on one screen. Click one of the chromatogram labels where the Q20 value is greater than zero to view the Chromatogram Read report. Notice the graph of the quality values. The dotted line marks where the quality value is equal to Do you see many quality values above 20? Did many bases need to be trimmed? Do the same with a high Q20 value. In list of homologous sequences describe each field see below In Sequence alignment point out query vs subject seq and sequence coordinates Then have class look at the four seqs and decide which GAPC gene Ask whether there is a possibility that same clone may match different genes- and why? An accession number is the unique identifier given to a DNA sequence when it is submitted to a database. It can also refer to a submitted protein sequence. In this case since the Arabidopsis genome has been completely sequenced the top accession number refers to the chromosome. The description refers to the source of the matching sequence. Again since the entire Arabidopsis genome is in the database these matches are to a complete chromosome sequence. The max score comes from the block of aligned sequence that had the highest score. Because the blastn score is about twice the number of matching nucleotides, it is possible to estimate that the maximum score of for the top sequence represents either matching bases or a longer region that contains gaps. The total score is obtained by adding the scores from the region of the query sequence that matches any region on the sequence chromosomal in this case in the database. In this example, the total score is not very helpful because it represents the total from all the matching regions on a single chromosome. Since both chromosomes 1 and 3 of Arabidopsis contain multiple copies of the GAPDH genes, this score is not informative. The query coverage corresponds to the fraction of the entire query sequence that is matched by parts of the subject sequence. The E value can have such a large range that it is reported as a power of 10 expressed as an exponent; for example, e-2 means 10â€”2. For each subject sequence or match in the database, the E value represents the number of equally good sequence matches to the query sequence that would be expected in a database of the same size containing random sequences. When E values are below 1 they can be translated to the probability that two sequences will match to the same extent. This would mean that with an E value of 0. While low E values are good, high E values suggest that it is possible to find an equally good match by random chance. In the top row of this example, the E value is 8 x 10â€” This means that there is a one in 1. In other words, a match like this is not likely to occur by random chance. Two additional factors have a strong influence on E values: Max ident maximum identity: This column shows the block of sequence that has the highest percentage of matching bases. This is a good example of how short sequences can give a good match that is not meaningful. In this example then max ident is not a very useful statistic. The final column in the blastn alignment table contains links to other databases that are identified in a key above the table on the BLAST results page. In this example, there are no links to other databases and for this analysis these links will probably not be useful Sequence G the poor quality sequence does not match anything- possible this is just intronic sequences and therefore we do not expect it to match since only the exons will be homologous between species or the sequence quality is too poor to match. Thus you just cut and paste the sequence into this page. This is an iterative process. Good quality data makes this much easier and takes much less time to process. We did this

previously for the individual sequences. Now we are doing this for the entire clone which should give us more confidence in the result since we are looking a for a much longer match. Thus by comparing our genomic DNA to a database of mRNA sequences we can see which parts of the genomic sequence are not present in the mRNA and therefore must be intronic.

CLONING AND SEQUENCING EXPLORER SERIES pdf

2: Cloning and Sequencing your PCR Product

The complete Cloning and Sequencing Explorer series is a week series of laboratory activities in which students clone and analyze a portion of the GAPC gene. Activities range from DNA extraction to computer-based sequence analysis.

Natural cloning[edit] Cloning is a natural form of reproduction that has allowed life forms to spread for hundreds of millions of years. It is the reproduction method used by plants , fungi , and bacteria , and is also the way that clonal colonies reproduce themselves. Molecular cloning Molecular cloning refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes , but it can also be used to amplify any DNA sequence such as promoters , non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic fingerprinting to large scale protein production. Occasionally, the term cloning is misleadingly used to refer to the identification of the chromosomal location of a gene associated with a particular phenotype of interest, such as in positional cloning. In practice, localization of the gene to a chromosome or genomic region does not necessarily enable one to isolate or amplify the relevant genomic sequence. To amplify any DNA sequence in a living organism, that sequence must be linked to an origin of replication , which is a sequence of DNA capable of directing the propagation of itself and any linked sequence. However, a number of other features are needed, and a variety of specialised cloning vectors small piece of DNA into which a foreign DNA fragment can be inserted exist that allow protein production , affinity tagging , single stranded RNA or DNA production and a host of other molecular biology tools. Subsequently, a ligation procedure is used where the amplified fragment is inserted into a vector piece of DNA. The vector which is frequently circular is linearised using restriction enzymes , and incubated with the fragment of interest under appropriate conditions with an enzyme called DNA ligase. Following ligation the vector with the insert of interest is transfected into cells. A number of alternative techniques are available, such as chemical sensitivation of cells, electroporation , optical injection and biolistics. Finally, the transfected cells are cultured. As the aforementioned procedures are of particularly low efficiency, there is a need to identify the cells that have been successfully transfected with the vector construct containing the desired insertion sequence in the required orientation. Modern cloning vectors include selectable antibiotic resistance markers, which allow only cells in which the vector has been transfected, to grow. Nevertheless, these selection steps do not absolutely guarantee that the DNA insert is present in the cells obtained. Further investigation of the resulting colonies must be required to confirm that cloning was successful. Cloning unicellular organisms[edit] Cloning cell-line colonies using cloning rings Cloning a cell means to derive a population of cells from a single cell. In the case of unicellular organisms such as bacteria and yeast, this process is remarkably simple and essentially only requires the inoculation of the appropriate medium. However, in the case of cell cultures from multi-cellular organisms, cell cloning is an arduous task as these cells will not readily grow in standard media. A useful tissue culture technique used to clone distinct lineages of cell lines involves the use of cloning rings cylinders. At an early growth stage when colonies consist of only a few cells, sterile polystyrene rings cloning rings , which have been dipped in grease, are placed over an individual colony and a small amount of trypsin is added. Cloned cells are collected from inside the ring and transferred to a new vessel for further growth. Cloning stem cells[edit] Main article: Somatic-cell nuclear transfer Somatic-cell nuclear transfer , known as SCNT, can also be used to create embryos for research or therapeutic purposes. The most likely purpose for this is to produce embryos for use in stem cell research. This process is also called "research cloning" or "therapeutic cloning. While a clonal human blastocyst has been created, stem cell lines are yet to be isolated from a clonal source. The process begins by removing the nucleus containing the DNA from an egg cell and inserting a nucleus from the adult cell to be cloned. The reprogrammed cell begins to develop into an embryo because the egg reacts with the transferred nucleus. The embryo will become genetically identical to the patient. This process can either add or delete specific genomes of farm animals. The first step is to collect the somatic cells from the animal that will be cloned. The somatic cells could be used immediately or stored in the laboratory for later use. Once this has been done, the somatic nucleus can be inserted into an egg

cytoplasm. The grouped somatic cell and egg cytoplasm are then introduced to an electrical current. The successfully developed embryos are then placed in surrogate recipients, such as a cow or sheep in the case of farm animals. It successfully cloned sheep, cattle, goats, and pigs. Another benefit is SCNT is seen as a solution to clone endangered species that are on the verge of going extinct. For example, the cloned sheep Dolly was born after eggs were used for SCNT, which created 29 viable embryos. Only three of these embryos survived until birth, and only one survived to adulthood. The biochemistry involved in reprogramming the differentiated somatic cell nucleus and activating the recipient egg was also far from being well understood. However, by researchers were reporting cloning success rates of seven to eight out of ten [15] and in , a Korean Company Sooam Biotech was reported to be producing cloned embryos per day. The resulting hybrid cells retain those mitochondrial structures which originally belonged to the egg. As a consequence, clones such as Dolly that are born from SCNT are not perfect copies of the donor of the nucleus. Asexual reproduction , Cuttings plants , and vegetative reproduction Organism cloning also called reproductive cloning refers to the procedure of creating a new multicellular organism, genetically identical to another. In essence this form of cloning is an asexual method of reproduction, where fertilization or inter-gamete contact does not take place. Asexual reproduction is a naturally occurring phenomenon in many species, including most plants and some insects. Scientists have made some major achievements with cloning, including the asexual reproduction of sheep and cows. There is a lot of ethical debate over whether or not cloning should be used. However, cloning, or asexual propagation, [17] has been common practice in the horticultural world for hundreds of years. Propagating plants from cuttings , such as grape vines, is an ancient form of cloning For the use of cloning in viticulture, see Propagation of grapevines. The term clone is used in horticulture to refer to descendants of a single plant which were produced by vegetative reproduction or apomixis. Many horticultural plant cultivars are clones, having been derived from a single individual, multiplied by some process other than sexual reproduction. Other examples are potato and banana. Many trees , shrubs , vines , ferns and other herbaceous perennials form clonal colonies naturally. Parts of an individual plant may become detached by fragmentation and grow on to become separate clonal individuals. A common example is in the vegetative reproduction of moss and liverwort gametophyte clones by means of gemmae. Some vascular plants e. Parthenogenesis[edit] Clonal derivation exists in nature in some animal species and is referred to as parthenogenesis reproduction of an organism by itself without a mate. This is an asexual form of reproduction that is only found in females of some insects, crustaceans, nematodes, [20] fish for example the hammerhead shark [21] , the Komodo dragon [21] and lizards. The growth and development occurs without fertilization by a male. In plants, parthenogenesis means the development of an embryo from an unfertilized egg cell, and is a component process of apomixis. In species that use the XY sex-determination system , the offspring will always be female. An example is the little fire ant Wasmannia auropunctata , which is native to Central and South America but has spread throughout many tropical environments. Artificial cloning of organisms[edit] Artificial cloning of organisms may also be called reproductive cloning. First steps[edit] Hans Spemann , a German embryologist was awarded a Nobel Prize in Physiology or Medicine in for his discovery of the effect now known as embryonic induction, exercised by various parts of the embryo, that directs the development of groups of cells into particular tissues and organs. In he and his student, Hilde Mangold , were the first to perform somatic-cell nuclear transfer using amphibian embryos â€” one of the first steps towards cloning. This process entails the transfer of a nucleus from a donor adult cell somatic cell to an egg from which the nucleus has been removed, or to a cell from a blastocyst from which the nucleus has been removed. Such clones are not strictly identical since the somatic cells may contain mutations in their nuclear DNA. This may have important implications for cross-species nuclear transfer in which nuclear-mitochondrial incompatibilities may lead to death. Artificial embryo splitting or embryo twinning, a technique that creates monozygotic twins from a single embryo, is not considered in the same fashion as other methods of cloning. During that procedure, a donor embryo is split in two distinct embryos, that can then be transferred via embryo transfer. It is optimally performed at the 6- to 8-cell stage, where it can be used as an expansion of IVF to increase the number of available embryos. Dolly the sheep[edit] The taxidermied body of Dolly the sheep Dolly clone Dolly , a Finn-Dorset ewe , was the first mammal to have been successfully cloned from an adult somatic cell. Dolly

was formed by taking a cell from the udder of her 6-year old biological mother. It took attempts before an embryo was successful. She was born on 5 July but not announced to the world until 22 February Before this demonstration, it had been shown by John Gurdon that nuclei from differentiated cells could give rise to an entire organism after transplantation into an enucleated egg. The first mammalian cloning resulting in Dolly the sheep had a success rate of 29 embryos per fertilized eggs, which produced three lambs at birth, one of which lived. In a bovine experiment involving 70 cloned calves, one-third of the calves died young. The first successfully cloned horse, Prometea , took attempts. Notably, although the first[clarification needed] clones were frogs, no adult cloned frog has yet been produced from a somatic adult nucleus donor cell. There were early claims that Dolly the sheep had pathologies resembling accelerated aging. This idea that the nuclei have not irreversibly aged was shown in to be true for mice. List of animals that have been cloned The modern cloning techniques involving nuclear transfer have been successfully performed on several species. King had successfully cloned northern leopard frogs: He published the findings in a Chinese science journal. Marked the first mammal being cloned from early embryonic cells by Steen Willadsen. Megan and Morag [38] cloned from differentiated embryonic cells in June and Dolly the sheep from a somatic cell in Tetra January from embryo splitting and not nuclear transfer. More akin to artificial formation of twins. Alpha and Beta males, and Brazil [45] Cat: Ralph , the first cloned rat [47] Mule: Idaho Gem , a john mule born 4 May , was the first horse-family clone. Prometea , a Haflinger female born 28 May , was the first horse clone. Snuwolf and Snuwolffy , the first two cloned female wolves Samrupa was the first cloned water buffalo.

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3: Cloning and Sequencing Explorer Series - [PPT Powerpoint]

Sequence Data Analysis Tools Sequence data storage and analysis tools (iFinch and Finch TV) Sequence comparison algorithm (NCBI BLAST) Sequence Assembly (CAP3) mRNA sequence prediction (BLAST and manual) Protein sequence prediction (EMBL-EBI EMBOSS Transeq) 6 Advanced Preparation â€¢ Practice with iFinch using the guest accounthighly recommended!

Molecular cloning is the process of of making multiple copies of a molecule. Gene cloning is a set of experimental methods in molecular biology that are used to assemble recombinant DNA molecules which can replicate and expand within host organisms. Extrachromosomal genetic element also made of a circular DNA molecule. Host cell lack Ampicilin resistant gene Ampr and cannot grow without the introduced plasmid in media with the antibiotic. Selection for inserted gene fragment: Plasmid express lac Z gene which is disrupted by the insertion of DNA fragment. Termination and PolyA site 8. Needs of to be a gene that is expressed in all plants: How do we identify and detect a specific sequence in a genome? Specificity How do we identify and detect a specific sequence in a genome? Specificity The corn genome is 5. Identifying a bp sequence in a genome would be like finding a section of this tape measure only 4 feet long! How Big Is 5. Amplification To be visible on an agarose gel, need around 10 ng DNA for fluorescent stain or around 25ng for FastBlast. How many molecules do we need to be able to see them? Done by using size exclusion column chromatography. In size exclusion chromatography small molecules like proteins, primers, and nucleotides, get trapped inside the chromatography beads while large molecules, like DNA fragments, are too large to enter the beads and pass through the column into the microcentrifuge tube. PCR Cleaning Step-by-Step procedure Resuspend the resin in the column by votexing 5 seconds Remove the Cap, snap of the tip and place the column in a 2. Place the column in a clean 1. Apply the sample ul to the top center of the column bed. Spin the column for 1minutes at rpm Save the purified sample which is in the bottom of the 1. Properly dispose the used column. Thus, once the plasmid DNA has been isolated, a restriction digestion reaction will be performed to determine the size of the insert. This polymerase functions at 70oC but not at lower temperatures, so it is not necessary to inactivate this enzyme after use. How to design a primer? Optimization of PCR conditions? Variation within PCR product shows variation. Why use GapDH While its glycolytic function conversion of D-glyceraldehydephosphate to 1,3-bisphosphoglycerate is well-known, recent evidence suggests that it is a highly versatile molecule that plays several diverse roles in living systems.

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4: Isolating, Cloning, and Sequencing DNA - Molecular Biology of the Cell - NCBI Bookshelf

1 Cloning and Sequencing Explorer Series 2 Instructors Stan Hitomi Coordinator - Math & Science Principal - Alamo School San Ramon Valley Unified School District Danville, CA Kirk Brown Lead Instructor, Edward Teller Education Center Science Chair, Tracy High School and Delta College, Tracy, CA Bio-Rad Curriculum and Training Specialists.

Please read the instruction manual carefully before starting. Invitrogen has changed the kit several times in the last year. Please note that the kits no longer contain b-mercaptoethanol or stop solution but now contain a salt solution which is added to the cloning reaction. Be sure to include a final extension step of 7 to 30 minutes during PCR. Longer PCR products will need longer extension times. Otherwise you may get a lot of clones that only have your primers as inserts. The cloning kit has 2 parts: Handle the competent cells with care. Thaw them on ice and only thaw them when you are ready to use them. If they warm up they will not transform. The SOC is very rich medium. Do not use it if it is cloudy or has anything growing in it. Return it to the freezer as soon as possible. Before you start the transformation you need to do several things: The X-gal is in the sequencing box on the top shelf of the freezer at the end of the lab. Amp plates are made by the kitchen and are in the coldroom at the end of the lab. You may want 2 plates for each transformation. To ensure a good density of colonies you can plate 50ul on one plate and ul on the other. Dry the plates in the 37C incubator with the lids ajar for at least 15 minutes. Spread 40ul of X-gal on each plate and keep at 37C for at least 30 minutes. Leave them there until you are ready to plate. The new manuals have some very vague instructions so here are some suggestions: Most people use a total of 20ng. Incubate at room temperature for minutes. Twenty minutes works well. People have found that 30 minutes is sufficient. Incubation is done in the roller drum in the 37 degree incubator. The TOPO kit contains a very useful quick reference card to use while you are transforming. A card is taped to the front of the enzyme freezer. Please return it when you are through. You should do 2 controls: It should give you very few colonies. The vector should not be able to self-ligate. See instructions on page 10 of the manual. After plating, incubate the plates overnight at 37 degrees. Remove them the next morning and store at 4C until ready to pick colonies. The PCR method is faster and easier but occasionally people have found that it does not work well on their construct. It uses strips of 8 column filters and processes 48 samples at a time, so it is best to do your samples in multiples of 8. PCR method Decide how many sequences you want to do. You will need 20ul for each clone. Taq 6ul Non-commercial Total ul 2 Fill enough 0. Pick a white colony with a 10ul pipet tip. Place the tip on the end of your P10 pipettor and expel the colony into 80ul of water by pipeting up and down 5 times. Pre-heat to 94C for 4 minutes, then do 20 cycles of 92C for 1 min, 50C for 1 min, 72C for 1 min. It is not necessary to run all your samples. A few of each type will do. If you have a large number of samples, use the Qiaquick-8 PCR purification kit and the vacuum manifold. However, a few notes: Place an ISC well plate on the block and the top of the manifold holding the columns over the plate. Make sure the columns and the wells are aligned and that the tip of the column is below the top of the well. Add 80ul of elution buffer to the center of the column. Let stand for 5 minutes and then turn on vacuum for 5 minutes. Cover with a microseal film for storage. Use either 14 ml round-bottom Falcon tubes cat or 15ml conical centrifuge tubes with the blue tops. You must poke holes in the tops of the conical tubes for aeration. Grow overnight in the 37C incubator roller-drum for hours. If you grow them longer the Amp may go off. Spin down cells at rpm for 10 minutes in the Beckman Allegra tabletop centrifuge. If you are not processing them immediately, keep the cells at 4C and spin them down just before processing. After spinning, aspirate off the supernatant or carefully pour off and blot with a kimwipe. There are 2 ways to process your minipreps. Use the Qiaprep spin miniprep kit for small numbers of samples. Follow the directions in the handbook. For a large number of samples, use the Qiaprep 8 Turbo miniprep kit with the manifold. There are 12 steps in the protocol and below you will find notes to go with many of the steps: Add buffers P1, P2 and N3 using an eppendorf repeat pipettor and the appropriate combitip. The P1 buffer stored at 4C contains RNase. Be careful not to slop it around the lab. Resuspend the pellet in the hood at the center of the lab. You will find a white microcentrifuge tube rack in the hood. The easiest way to resuspend the pellet is to run the bottom of the tube along the rack until no clumps are visible in the solution. P2 buffer contains

NaOH. Do not wait much longer than 5 minutes before adding the N3 neutralizing buffer or you may irreversibly denature your DNA. The turbofilters, clear with a dark blue stripe clear the lysates and go in the slots in the lid of the manifold. Fill any unused slots with plexiglass blanks stored on the bottom of the manifold. The qiaprep strips light blue bind the DNA and go in the plexiglass strip holder which is placed in the base of the manifold. Place the lid on the base. Unfortunately the lid can go on in 2 orientations and one will cause misalignment. Maximum vacuum should not be used for this step. Adjust the regulator to mbars before applying vacuum to the manifold. Re-adjust the regulator to mbars before passing the lysate through the qiaprep column. Maximum vacuum is used for the rest of the steps. You need to get rid of all the PE buffer before proceeding. Blot the nozzles of the strips with a large kimwipe. Put several layers of large kimwipes on the bench and rap the lid sharply until no more droplets appear. Change the kimwipes when necessary. Ignore directions in 11a and 11b. To elute your DNA, place the plexiglass block in the bottom of the manifold. Add ul EB buffer. Let stand 5 minutes before applying vacuum. After eluting sample cover with microseal film for storage. Sequencing There are several facilities you can use. I will list them in order of preference. They think 30 samples are a lot. However, not really much slower than the other places. Amounts of DNA and primer required: They call primers they provide " standard" and ones you provide "custom". For some reason they ask for different amounts of template to use with the 2 types of primers. Good quality sequences, reasonably fast turn-around time.

CLONING AND SEQUENCING EXPLORER SERIES pdf

5: PPT - Cloning and Sequencing Explorer Series PowerPoint Presentation - ID

** Cloning and Sequencing Explorer Series é²•æž—è•£ å¨„ å†› æ–¹ ç‘œ Related knowledge Molecular cloning Plasmid/vector DNA sequencing technology DNA sequence analysis Molecular Cloning Overview Cloning refers to the production of multiple copies.*

Restriction nucleases produce DNA fragments that can be easily joined together. Fragments with the same cohesive ends can readily join by complementary base-pairing between their cohesive ends, as illustrated. The two DNA fragments that join in this example more Gel Electrophoresis Separates DNA Molecules of Different Sizes The length and purity of DNA molecules can be accurately determined by the same types of gel electrophoresis methods that have proved so useful in the analysis of proteins. The procedure is actually simpler than for proteins: For DNA fragments less than nucleotides long, specially designed polyacrylamide gels allow separation of molecules that differ in length by as little as a single nucleotide Figure A. The pores in polyacrylamide gels, however, are too small to permit very large DNA molecules to pass; to separate these by size, the much more porous gels formed by dilute solutions of agarose a polysaccharide isolated from seaweed are used Figure B. These DNA separation methods are widely used for both analytical and preparative purposes. Figure Gel electrophoresis techniques for separating DNA molecules by size. In the three examples shown, electrophoresis is from top to bottom, so that the largestâ€”and thus slowest-movingâ€”DNA molecules are near the top of the gel. In A a polyacrylamide more A variation of agarose gel electrophoresis, called pulsed-field gel electrophoresis, makes it possible to separate even extremely long DNA molecules. Ordinary gel electrophoresis fails to separate such molecules because the steady electric field stretches them out so that they travel end-first through the gel in snakelike configurations at a rate that is independent of their length. In pulsed-field gel electrophoresis, by contrast, the direction of the electric field is changed periodically, which forces the molecules to reorient before continuing to move snakelike through the gel. This reorientation takes much more time for larger molecules, so that longer molecules move more slowly than shorter ones. As a consequence, even entire bacterial or yeast chromosomes separate into discrete bands in pulsed-field gels and so can be sorted and identified on the basis of their size Figure C. Although a typical mammalian chromosome of base pairs is too large to be sorted even in this way, large segments of these chromosomes are readily separated and identified if the chromosomal DNA is first cut with a restriction nuclease selected to recognize sequences that occur only rarely once every 10, or more nucleotide pairs. One sensitive method of staining DNA is to expose it to the dye ethidium bromide, which fluoresces under ultraviolet light when it is bound to DNA see Figures B , C. In the first method a DNA polymerase copies the DNA in the presence of nucleotides that are either radioactive usually labeled with 32P or chemically tagged Figure A. Because only one 32P atom is incorporated by the kinase into each DNA strand, the DNA molecules labeled in this way are often not radioactive enough to be used as DNA probes; because they are labeled at only one end, however, they have been invaluable for other applications including DNA footprinting , as we see shortly. Figure Methods for labeling DNA molecules in vitro. Today, radioactive labeling methods are being replaced by labeling with molecules that can be detected chemically or through fluorescence. To produce such nonradioactive DNA molecules, specially modified nucleotide precursors are used Figure C. A DNA molecule made in this way is allowed to bind to its complementary DNA sequence by hybridization , as discussed in the next section , and is then detected with an antibody or other ligand that specifically recognizes its modified side chain see Figure Here, six different DNA probes have been used to mark the location of their respective nucleotide sequences on human chromosome 5 at metaphase. The probes have been chemically labeled and detected with fluorescent antibodies. Both copies of chromosome more This process, called DNA denaturation , was for many years thought to be irreversible. These specific hybridization reactions are widely used to detect and characterize specific nucleotide sequences in both RNA and DNA molecules. Single-stranded DNA molecules used to detect complementary sequences are known as probes ; these molecules, which carry radioactive or chemical markers to facilitate their detection, can be anywhere from fifteen to thousands of nucleotides long. Hybridization reactions using DNA probes are so sensitive and

selective that they can detect complementary sequences present at a concentration as low as one molecule per cell. The same technique can be used to search for related but nonidentical genes. To find a gene of interest in an organism whose genome has not yet been sequenced, for example, a portion of a known gene can be used as a probe Figure Figure Different hybridization conditions allow less than perfect DNA matching. When only an identical match with a DNA probe is desired, the hybridization reaction is kept just a few degrees below the temperature at which a perfect DNA helix denatures in the more In somewhat more elaborate procedures the DNA probe is treated with specific nucleases after the hybridization is complete, to determine the exact regions of the DNA probe that have paired with cellular RNA molecules. One can thereby determine the start and stop sites for RNA transcription, as well as the precise boundaries of the intron and exon sequences in a gene Figure Figure The use of nucleic acid hybridization to determine the region of a cloned DNA fragment that is present in an mRNA molecule. The method shown requires a nuclease that cuts the DNA chain only where it is not base-paired to a complementary RNA chain. Comparing this expressed sequence with the sequence of the whole gene reveals where the introns lie. We have seen that genes are switched on and off as a cell encounters new signals in its environment. The hybridization of DNA probes to cellular RNAs allows one to determine whether or not a particular gene is being transcribed; moreover, when the expression of a gene changes, one can determine whether the change is due to transcriptional or posttranscriptional controls see Figure These tests of gene expression were initially performed with one DNA probe at a time. DNA microarrays now allow the simultaneous monitoring of hundreds or thousands of genes at a time, as we discuss later. Hybridization methods are in such wide use in cell biology today that it is difficult to imagine how we could study gene structure and expression without them. Northern and Southern Blotting Facilitate Hybridization with Electrophoretically Separated Nucleic Acid Molecules DNA probes are often used to detect, in a complex mixture of nucleic acids, only those molecules with sequences that are complementary to all or part of the probe. Gel electrophoresis can be used to fractionate the many different RNA or DNA molecules in a crude mixture according to their size before the hybridization reaction is performed; if molecules of only one or a few sizes become labeled with the probe, one can be certain that the hybridization was indeed specific. Moreover, the size information obtained can be invaluable in itself. An example illustrates this point. Suppose that one wishes to determine the nature of the defect in a mutant mouse that produces abnormally low amounts of albumin, a protein that liver cells normally secrete into the blood in large amounts. First, one collects identical samples of liver tissue from mutant and normal mice the latter serving as controls and disrupts the cells in a strong detergent to inactivate cellular nucleases that might otherwise degrade the nucleic acids. Then one separates the DNA from the RNA by their different solubilities in alcohols and degrades any contaminating nucleic acid of the unwanted type by treatment with a highly specific enzyme â€”either an RNase or a DNase. First, the intact mRNA molecules purified from mutant and control liver cells are fractionated on the basis of their sizes into a series of bands by gel electrophoresis. The paper is then incubated in a solution containing a labeled DNA probe whose sequence corresponds to part of the template strand that produces albumin mRNA. The RNA molecules that hybridize to the labeled DNA probe on the paper because they are complementary to part of the normal albumin gene sequence are then located by detecting the bound probe by autoradiography or by chemical means Figure The size of the RNA molecules in each band that binds the probe can be determined by reference to bands of RNA molecules of known sizes RNA standards that are electrophoresed side by side with the experimental sample. In this way one might discover that liver cells from the mutant mice make albumin RNA in normal amounts and of normal size; alternatively, albumin RNA of normal size might be detected in greatly reduced amounts. Another possibility is that the mutant albumin RNA molecules might be abnormally short and therefore move unusually quickly through the gel; in this case the gel blot could be retested with a series of shorter DNA probes, each corresponding to small portions of the gene, to reveal which part of the normal RNA is missing. In this example, the DNA probe is detected by its radioactivity. DNA probes detected by chemical or fluorescence methods are also widely used see Figure A A mixture of more Isolated DNA is first cut into readily separable fragments with restriction nucleases. The double-stranded fragments are then separated on the basis of size by gel electrophoresis, and those complementary to a DNA probe are identified by blotting

and hybridization, as just described for RNA see Figure To characterize the structure of the albumin gene in the mutant mice, an albumin-specific DNA probe would be used to construct a detailed restriction map of the genome in the region of the albumin gene. From this map one could determine if the albumin gene has been rearranged in the defective animalsâ€”for example, by the deletion or the insertion of a short DNA sequence; most single base changes, however, could not be detected in this way. Hybridization Techniques Locate Specific Nucleic Acid Sequences in Cells or on Chromosomes Nucleic acids, no less than other macromolecules, occupy precise positions in cells and tissues, and a great deal of potential information is lost when these molecules are extracted by homogenization. For this reason, techniques have been developed in which nucleic acid probes are used in much the same way as labeled antibodies to locate specific nucleic acid sequences in situ, a procedure called in situ hybridization. Labeled nucleic acid probes can be hybridized to chromosomes that have been exposed briefly to a very high pH to disrupt their DNA base pairs. The chromosomal regions that bind the probe during the hybridization step are then visualized. Originally, this technique was developed with highly radioactive DNA probes, which were detected by auto-radiography. The spatial resolution of the technique, however, can be greatly improved by labeling the DNA probes chemically Figure instead of radioactively, as described earlier. In situ hybridization methods have also been developed that reveal the distribution of specific RNA molecules in cells in tissues. In this case the tissues are not exposed to a high pH , so the chromosomal DNA remains double-stranded and cannot bind the probe. Instead the tissue is gently fixed so that its RNA is retained in an exposed form that can hybridize when the tissue is incubated with a complementary DNA or RNA probe. In this way the patterns of differential gene expression can be observed in tissues, and the location of specific RNAs can be determined in cells Figure In the Drosophila embryo, for example, such patterns have provided new insights into the mechanisms that create distinctions between cells in different positions during development described in Chapter Figure A Expression pattern of deltaC in the early zebrafish embryo. This gene codes for a ligand in the Notch signaling pathway discussed in Chapter 15 , and the pattern shown here reflects its role in the development of somitesâ€”the future segments more In cell biology, the term DNA cloning is used in two senses. In one sense it literally refers to the act of making many identical copies of a DNA molecule â€”the amplification of a particular DNA sequence. DNA cloning in its most general sense can be accomplished in several ways. The simplest involves inserting a particular fragment of DNA into the purified DNA genome of a self-replicating genetic elementâ€”generally a virus or a plasmid. A DNA fragment containing a human gene , for example, can be joined in a test tube to the chromosome of a bacterial virus, and the new recombinant DNA molecule can then be introduced into a bacterial cell. Starting with only one such recombinant DNA molecule that infects a single cell, the normal replication mechanisms of the virus can produce more than identical virus DNA molecules in less than a day, thereby amplifying the amount of the inserted human DNA fragment by the same factor. A virus or plasmid used in this way is known as a cloning vector , and the DNA propagated by insertion into it is said to have been cloned. To isolate a specific gene , one often begins by constructing a DNA library â€”a comprehensive collection of cloned DNA fragments from a cell, tissue, or organism. This library includes one hopes at least one fragment that contains the gene of interest. Libraries can be constructed with either a virus or a plasmid vector and are generally housed in a population of bacterial cells. The principles underlying the methods used for cloning genes are the same for either type of cloning vector , although the details may differ. Today most cloning is performed with plasmid vectors. The plasmid vectors most widely used for gene cloning are small circular molecules of double-stranded DNA derived from larger plasmids that occur naturally in bacterial cells. They generally account for only a minor fraction of the total host bacterial cell DNA, but they can easily be separated owing to their small size from chromosomal DNA molecules, which are large and precipitate as a pellet upon centrifugation. For use as cloning vectors, the purified plasmid DNA circles are first cut with a restriction nuclease to create linear DNA molecules. The cellular DNA to be used in constructing the library is cut with the same restriction nuclease, and the resulting restriction fragments including those containing the gene to be cloned are then added to the cut plasmids and annealed via their cohesive ends to form recombinant DNA circles. The plasmid is cut open with a restriction nuclease in this case one that produces cohesive ends and is mixed with the DNA fragment to be cloned which

has been prepared more In the next step in preparing the library, the recombinant DNA circles are introduced into bacterial cells that have been made transiently permeable to DNA; such cells are said to be transfected with the plasmids. As these cells grow and divide, doubling in number every 30 minutes, the recombinant plasmids also replicate to produce an enormous number of copies of DNA circles containing the foreign DNA Figure Many bacterial plasmids carry genes for antibiotic resistance, a property that can be exploited to select those cells that have been successfully transfected; if the bacteria are grown in the presence of the antibiotic, only cells containing plasmids will survive. Each original bacterial cell that was initially transfected contains, in general, a different foreign DNA insert; this insert is inherited by all of the progeny cells of that bacterium, which together form a small colony in a culture dish.

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