Lecture 33-34

Biotechnology

The DNA Toolbox:

Sequencing of the genomes of more than 7,000 species was under way since 2010. DNA sequencing has depended on advancements in technology, starting with making recombinant DNA. In recombinant DNA, nucleotide sequences from two different sources, often two species, are combined in vitro into the same DNA molecule.

Recombinant DNA Technology:

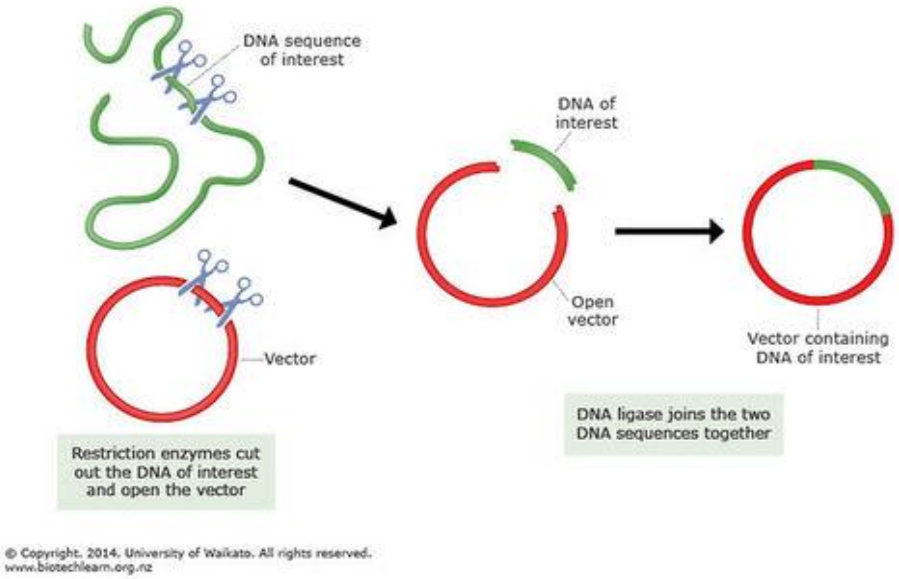
Joining together of DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that value to science, medicine, agriculture, and industry. Methods for making recombinant DNA are central to genetic engineering, the direct manipulation of genes for practical purposes. DNA technology has revolutionized biotechnology, the manipulation of organisms or their genetic components. An example of DNA technology is the microarray, a measurement of gene expression.

DNA Cloning:

The production of exact copies (clones) of a particular gene or DNA sequence using genetic engineering techniques. To work directly with specific genes, scientists prepare well-defined segments of DNA in identical copies, a process called DNA cloning.
Bacterial plasmids are just small, circular DNA molecules that copy themselves separately from the other bacterial DNA material. Plasmids are ideal to use in cloning for two reasons: they are very versatile and can carry just about any gene, but they also get passed from one generation of bacteria to the next so they carry that gene on down the line.

After isolation, the scientist will treat both the plasmid and the gene of interest with an enzyme that cuts the DNA, called a restriction enzyme. These enzymes get their name from their role in nature - to restrict invading DNA from entering bacterial cells by cutting up the foreign DNA. This invading DNA may come from other organisms or even viruses, so it's important to keep them out.
The restriction enzyme cuts the plasmid in one place so that it creates an area that the target DNA can bind to. The target gene is cut out of its original DNA strand so that just the gene of interest is attached to the plasmid for cloning. After cutting both the target DNA and the plasmid, the two are linked together with an enzyme called DNA ligase. This pasting process results in a recombinant DNA plasmid, which is a single DNA molecule combined from two different sources of DNA. It is literally 'recombined,' hence the name 'recombinant.'
After the two DNA pieces have been pasted together, the plasmid is inserted into a bacterial cell,

which will allow the bacteria to replicate and produce plasmid 'babies' that are identical to the 'parent' plasmid.



Transformation:

Genetic modification of a cell by the uptake and incorporation of exogenous DNA is called transformation. Transformation occurs naturally in some species of bacteria, but it can also be affected by artificial means in other cells.
Before transformation, bacteria are treated with a chemical called calcium chloride, which causes water to enter into the cells and makes them swell. These swollen bacteria are then known as competent bacteria.
Next, plasmid DNA (containing the foreign DNA) is mixed with the competent bacteria and the solution is heated. The plasmid DNA enters the bacteria through small pores created in the cell membranes. Once in the host cell, the plasmid DNA is copied many times by the bacteria's own DNA replicating machinery.

Gene Cloning in a Bacterial Plasmid:

In gene cloning, the original plasmid is called a cloning vector. A DNA molecule that carries foreign DNA into a host cell, replicates inside a bacterial (or yeast) cell and produces many copies of itself and the foreign DNA is a cloning vector. The following three features are common in all clones.

1. Sequences that permit the propagation of itself in bacteria or yeast.
2. A cloning site to insert foreign DNA; the most versatile vectors contain a site that can be cut by many restriction enzymes.

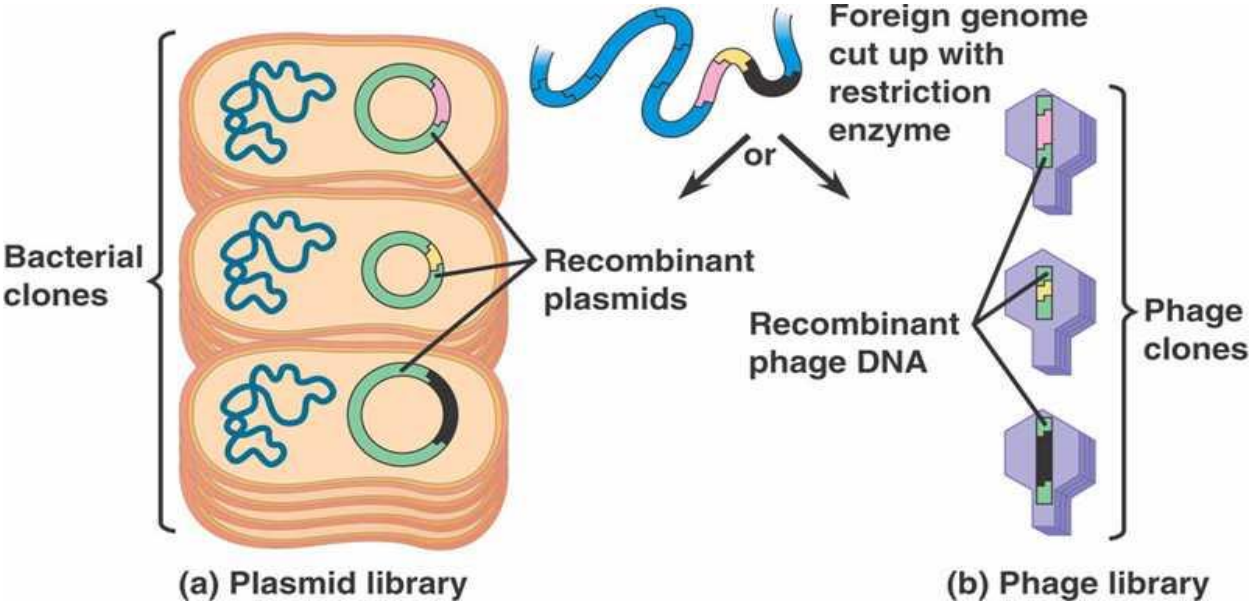
3. A method of selecting for bacteria yeast containing a vector with foreign DNA. Usually accomplished by selectable markers for drug resistance.

Producing Competent Cells:

Several steps are required to clone the hummingbird β-globin gene in a bacterial plasmid. The humming bird genomic DNA and a bacterial plasmid are isolated. Both humming bird genomic DNA and bacterial plasmids are cut with the same restriction enzyme. The fragments are mixed, and DNA ligase is added to bond the fragment sticky ends. Some recombinant plasmids now contain humming bird DNA. The DNA mixture is added to bacteria that have been genetically engineered to accept it. The bacteria are plated on a type of agar that selects for the bacteria with recombinant plasmids. This results in the cloning of many hummingbird DNA fragments, including the β-globin gene.

Preparing Genomic Libraries:

A genomic library is a collection of the total genomic DNA from a single organism. The DNA is stored in a population of identical vectors, each containing a different insert of DNA. In order to construct a genomic library, the organism's DNA is extracted from cells and then digested with a restriction enzyme to cut the DNA into fragments of a specific size. The fragments are then inserted into the vector using DNA ligase. Then the vector DNA can be taken up by a host organism. A genomic library that is made using bacteria is the collection of recombinant vector clones. A genomic library that is made using bacteriophages is stored as a collection of phage clones.



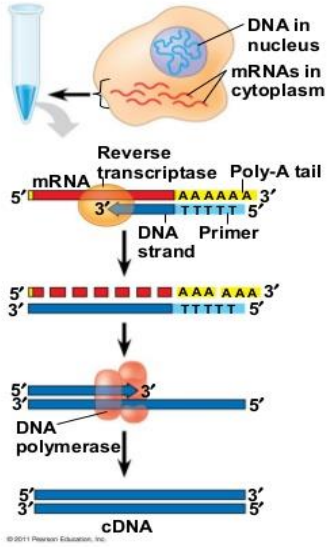
Bacterial Artificial Chromosome:

A bacterial artificial chromosome (BAC) is an engineered DNA molecule used to clone DNA sequences in bacterial cells for example, E. coli. BACs are often used in connection with DNA sequencing. Segments of an organism's DNA, ranging from 100,000 to about 300,000 base pairs, can be inserted into BACs. The BACs, with their inserted DNA, are then taken up by bacterial cells. As the bacterial cells grow and divide, they amplify the BAC DNA, which can then be isolated and used in sequencing DNA. BACs are another type of vector used in DNA library construction.

Making complementary DNA

1. CDNA refers to complementary DNA. CDNA is known to be synthesized, or manufactured from an mRNA or messenger RNA template. It is synthesized in a reaction that is catalyzed by the reverse transcriptase and DNA polymerase enzymes. Essential to note is that cDNA is usually used to clone eukaryotic genes in prokaryotes.
2. Scientists usually use cDNA when they want to express s certain protein in a cell that does not normally express such a protein. This process is referred to as heterologous expression. The expression of such a protein will be done by transferring the cDNA that codes for that protein to the recipient cell. Also, essential to note is that cDNA can also be produced by retroviruses like Simian Immunodeficiency Virus, HIV-1 and HIV-2 among others. Once the cDNA is created from such viruses, it is integrated into the genome of the host, where it goes on to create a provirus.
3. Research shows that when a protein is being synthesized, a gene's DNA is transcribed into an mRNA, which is then translated into a protein. Genes are usually divided into eukaryotic and prokaryotic genes. The only difference between these genes is that the eukaryotic genes contain introns instead of extrons that are contained in the prokaryotic genes.
4. Introns are not coding sequences, while extrons are DNA coding systems. During the transcription of the proteins, all intron RNA are cut from the primary RNA and the remaining piece is sliced back to become an mRNA. In other words, the mRNA is formed after all introns are removed from the primary RNA. Once formed, the mRNA is then translated into an amino acid and comprises a newly formed protein.

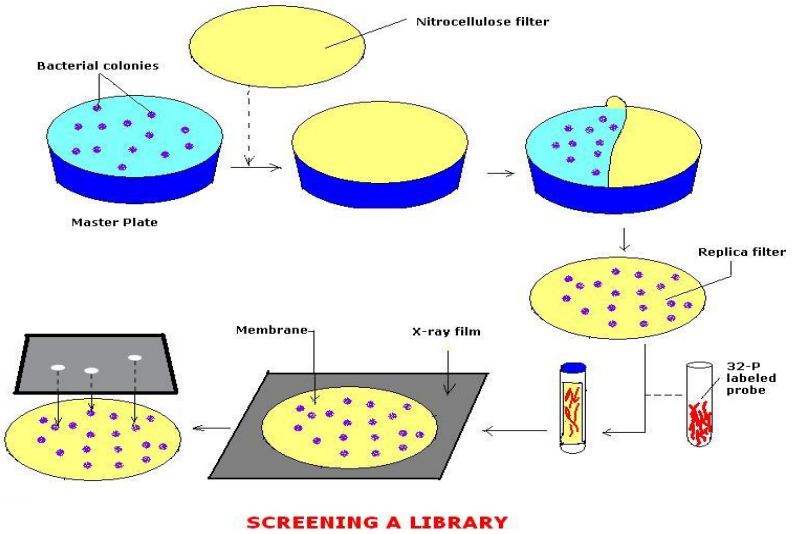
Figure 20.6 Making complementary DNA (cDNA) from eukaryotic genes.



Screening a Library

One of the key elements required to identify a gene during cloning is a probe. A probe is normally a cloned piece of DNA that contains a portion of the sequence for which you are searching. You typically will make the probe radioactive and add it to a solution. Filters containing immobilized clones are then bathed in the solution. The principal behind this step is that the probe will bind to any clone containing sequences similar to those found on the probe. This binding step is called hybridization.
Following steps involved for screening a library.

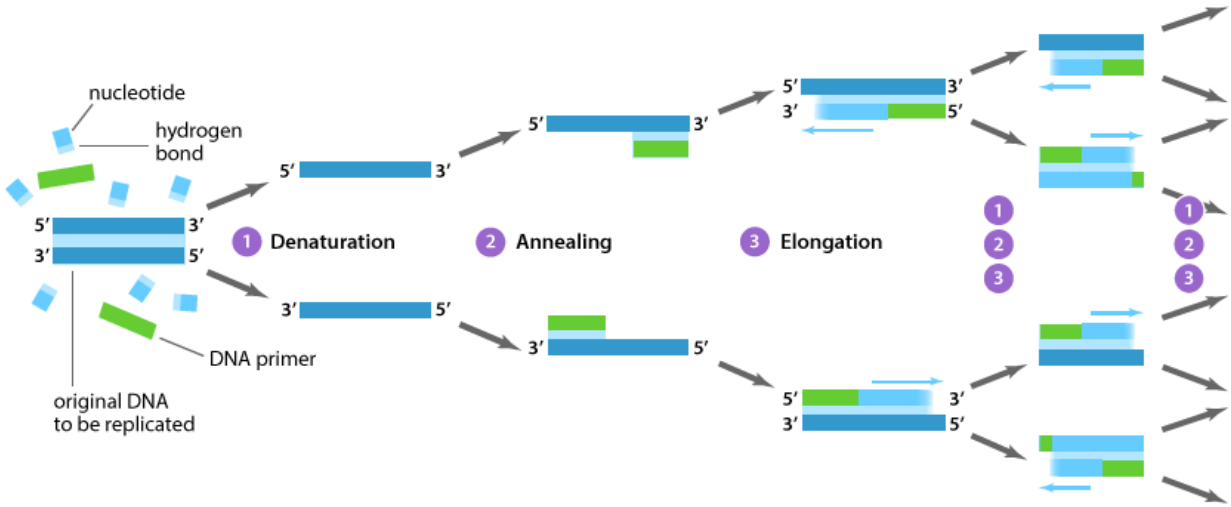
1. Immobilize members of the library onto a nylon membrane and denature them so that they are single-stranded.
2. Prepare a radiolabelled probe and denature it to make it single-stranded.
3. Hybridize the probe to the library of clones.
4. Wash the excess probe and expose an X-ray film.
5. Isolate the positive clone and analyze.



SCREENING A LIBRARY

Polymerase Chain Reaction

The polymerase chain reaction (PCR) can produce many copies of a specific target segment of DNA.A three step cycle heating, cooling, and replication brings about a chain reaction that produces an exponentially growing population of identical DNA molecules The key to PCR is an unusual, heat-stable DNA polymerase called Taq-polymerase.



DNA Sequencing

DNA Sequencing determining the order of the four chemical building blocks - called "bases" - that makes up the DNA molecule. The sequence tells scientists the kind of genetic information that is carried in a particular DNA segment. For example, scientists can use sequence information to determine which stretches of DNA contain genes and which stretches carry regulatory instructions, turning genes on or off. In addition, and importantly, sequence data can highlight changes in a gene that may cause disease.

In the DNA double helix, the four chemical bases always bond with the same partner to form "base pairs." Adenine (A) always pairs with thymine (T); cytosine (C) always pairs with guanine (G). This pairing is the basis for the mechanism by which DNA molecules are copied when cells divide, and the pairing also underlies the methods by which most DNA sequencing experiments are done. The human genome contains about 3 billion base pairs that spell out the instructions for making and maintaining a human being.

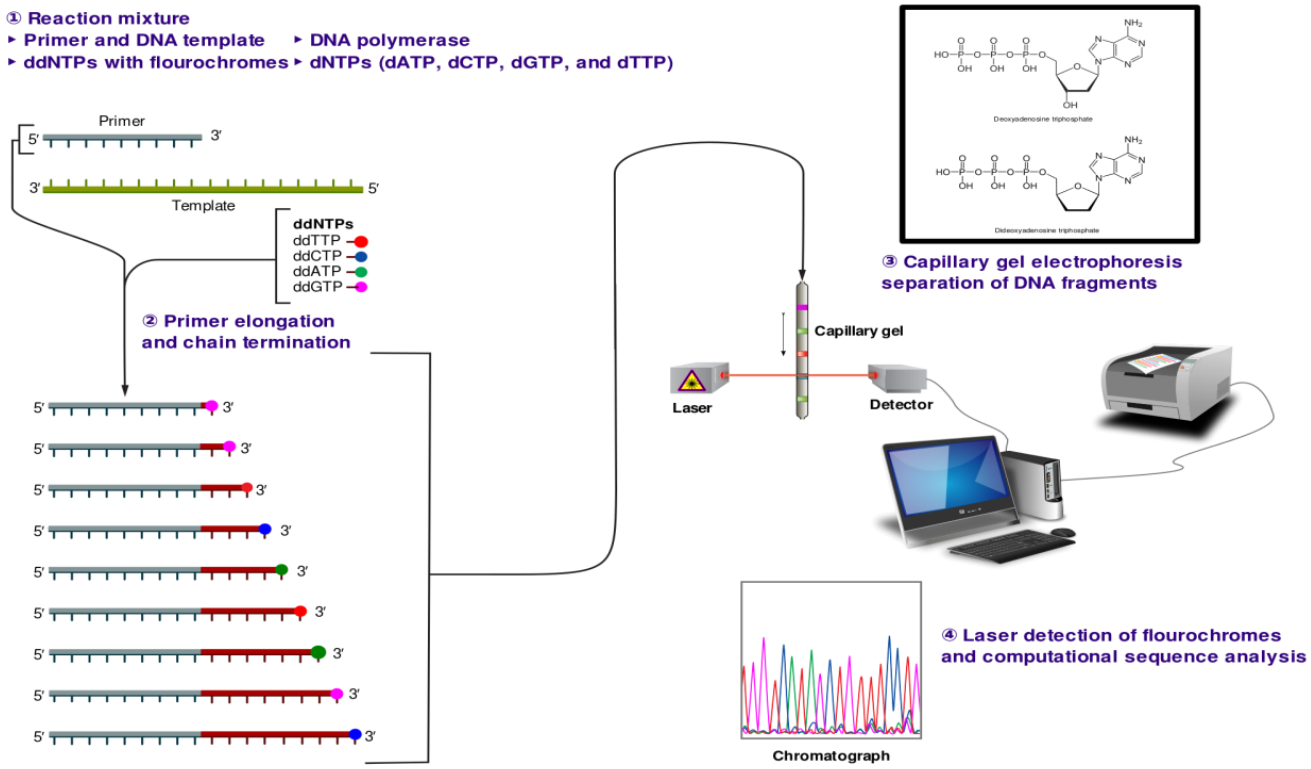
Sanger sequencing

1. Sanger sequencing is a method of DNA sequencing based on the selective incorporation of chain-terminating dideoxynucleotides by DNA polymerase during in vitro DNA replication. The classical chain-termination method requires a single-stranded DNA template, a DNA primer, a DNA polymerase, normal deoxynucleosidetriphosphates (dNTPs), and modified di-deoxynucleosidetriphosphates (ddNTPs), the latter of which terminate DNA strand elongation. These chain-terminating nucleotides lack a 3'-OH group required for the formation of a phosphodiester bond between two nucleotides, causing DNA polymerase to cease extension of DNA when a modified dNTP is incorporated. The ddNTPs may be radioactively or fluorescently labeled for detection in automated sequencing machines.

2. The DNA sample is divided into four separate sequencing reactions, containing all four of the standard deoxynucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase. To each reaction is added only one of the four dideoxynucleotides (ddATP, ddGTP, ddCTP, or ddTTP), while the other added nucleotides are ordinary ones.

3. The dideoxynucleotide is added to be approximately 100-fold lower in concentration than the corresponding dinucleotide (e.g. 0.005mM ddATP: 0.5mM dATP) allowing for enough fragments to be produced while still transcribing the complete sequence. Putting it in a more sensible order, four separate reactions are needed in this process to test all four ddNTPs. Following rounds of template DNA extension from the bound primer, the resulting DNA fragments are heat denatured and separated by size using gel electrophoresis. In the original publication of 1977, the formation of base-paired loops of ssDNA was a cause of serious difficulty in resolving bands at some locations. This is frequently performed using a denaturing

polyacrylamide-urea gel with each of the four reactions run in one of four individual lanes (lanes A, T, G, and C). The DNA bands may then be visualized by autoradiography or UV light and the DNA sequence can be directly read off the X-ray film or gel image.



Applications

Diagnosis and Treatment of Diseases

Scientists can diagnose many human genetic disorders using PCR and sequence-specific primers.
1. Then amplified product is sequenced to look for the
Disease-causing mutation.
2. SNPs may be associated with a disease-causing mutation
3. SNPs may also be correlated with increased risks for conditions such as heart disease or certain types of cancer.

Forensic Evidence and Genetic Profiles

1. An individual's unique DNA sequence, or genetic profile, can be obtained by analysis of tissue or body fluids.
2. DNA testing can identify individuals with a high degree of certainty.
3. Genetic profiles can be analyzed using RFLP analysis by Southern blotting.

Short Tandem Repeats

Even more sensitive is the use of genetic markers called short tandem repeats (STRs).
1. These are variations in the number of repeats of specific DNA sequences
2. PCR and gel electrophoresis are used to amplify and then identify STRs of different lengths
3. The probability that two people who are not identical twins have the same STR markers is Exceptionally small.

Agricultural Applications

1 .DNA technology is being used to improve agricultural productivity and food quality
2. Genetic engineering of transgenic animals speeds up the selective breeding process.
3. Beneficial genes can be transferred between varieties or species.

Genetically Modified Organisms

1. Most public concern about possible hazards centers on genetically modified (GM) organisms Used as food.
2. Some are concerned about the creation of "super weeds" from the transfer of genes from GM crops to their wild relatives.
3. Other worries include the possibility that transgenic protein products might cause allergic Reactions.

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