

Chapter 20: Biotechnology

1. Define recombinant DNA, biotechnology, and genetic engineering.

Recombinant DNA is formed when segments of DNA from two different sources, often different species, are combined *in vitro*. Biotechnology is the manipulation of organisms or their components to make useful products. This includes genetic engineering, the direct manipulation of genes for practical purposes.

2. What is a plasmid?

In 1935, Stanley crystallized the tobacco mosaic virus (TMV), confirming Beijerinck's concept. Subsequently, many viruses could be seen with an electron microscope.

3. What is gene cloning?

Gene cloning is the production of multiple copies of a single gene.

4. Explain the steps in gene cloning.

First, the gene is inserted into a plasmid, which is then put into a bacterial cell. Next, the host cell is grown in culture to form a clone of cells containing the "cloned" gene of interest. This is used for basic research and various applications, such as heart attack therapy and toxic waste cleanup.

5. Explain the steps involved in forming recombinant DNA. ✍

First, a restriction enzyme cuts the sugar-phosphate backbones at specific points on the restriction site. Next, a DNA fragment from another source is added. Base pairing of sticky ends produces various combinations. Finally, DNA ligase seals the strands.

6. What is a cloning vector?

Wild-type *Neurospora* has modest food requirements. It can grow in the laboratory on a simple solution of inorganic salts, glucose, and the vitamin biotin, incorporated into agar, a support medium. From this minimal medium, the mold cells use their metabolic pathways to produce all the other molecules they need. Mutating the wild type allowed Beadle and Tatum to identify metabolic defects.

7a. Explain why the plasmid is engineered with *amp^R* and *lacZ*.

The gene *amp^R* makes *E. coli* cells resistant to the antibiotic ampicillin, while *lacZ* encodes the enzyme β -galactosidase, which hydrolyzes lactose. This enzyme can also hydrolyze a similar synthetic molecule called X-gal to form a blue product. The plasmid contains only one copy of the restriction site recognized by the restriction enzyme, and that site is within the *lacZ* gene. Both the plasmid and the hummingbird DNA are cut with the same restriction enzyme before the fragments are mixed together, allowing base pairing between their complementary sticky ends as their sugar-phosphate backbones are covalently bonded by DNA ligase. The DNA mixture is then added to bacteria that have a mutation in the *lacZ* gene on their own chromosome, making them unable to hydrolyze lactose or X-gal.

7b. After transformation has occurred, why are some colonies blue?

Under suitable experimental conditions, the cells take up foreign DNA by transformation. Plating out all the bacteria on solid nutrient medium containing ampicillin allows researchers to distinguish the cells that have taken up plasmids, whether recombinant or not, from the other cells. Only cells with a plasmid will reproduce under these conditions because only they have the *amp^R* gene conferring resistance to the ampicillin in the medium. Moreover, the presence of X-gal in the medium allows researchers to distinguish bacteria colonies with recombinant plasmids from those with nonrecombinant plasmids. Colonies containing nonrecombinant plasmids have the *lacZ* gene intact and will produce functional β -galactosidase. These colonies will be blue because the enzyme hydrolyzes the X-gal in the medium, forming a blue product. In contrast, no functional β -galactosidase is produced in colonies containing recombinant plasmids with foreign DNA inserted into the *lacZ* gene; these colonies will therefore be white.

8a. What is the purpose of a genomic library?

The complete set of plasmid-containing cell clones, each carrying copies of a particular segment from the initial genome, is referred to as a genomic library. Each “plasmid clone” in the library is like a book containing specific information. Historically, certain bacteriophages have also been used as cloning vectors for making genomic libraries. Another type of vectors widely used in library construction are bacterial artificial chromosomes (BACs), large plasmids that are trimmed down so they contain just the genes necessary to ensure replication. While a standard plasmid can carry a DNA insert no larger than 10,000 base pairs (10 kb), a BAC can carry an insert of 100 to 300 kb!

8b. How are bacterial artificial libraries and cDNA libraries formed?

To create a library using complementary DNA, researchers modify the cDNA by adding restriction enzyme recognition sequences at each end. Then the cDNA is inserted into vector DNA in a manner similar to the insertion of genomic DNA fragments. The extracted mRNA is a mixture of all the mRNA molecules in the original cells, transcribed from many different genes. Therefore, the cDNAs that are cloned make up a cDNA library containing a collection of genes. However, a cDNA library represents only part of the genome – only the subset of genes that were transcribed in the cells from which the mRNA was isolated.

9. Explain how nucleic acid hybridization will help researchers find the piece of DNA that holds their gene of interest.

Researchers can detect the hummingbird’s β -globin gene’s DNA by its ability to base-pair with a complementary sequence on another nucleic acid molecule, using nucleic acid hybridization. The complementary molecule, a short, single-stranded nucleic acid that can be either RNA or DNA, is called a nucleic acid probe. If at least part of the nucleotide sequence of the gene of interest is known, a probe complementary to it can be synthesized. Each probe molecule, which will hydrogen-bond specifically to a complementary sequence in the desired gene, is labeled with a radioactive isotope, a fluorescent tag, or another molecule so it can be tracked. The clones in the hummingbird genomic library are stored in a multiwell plate. If a few cells from each well are transferred to a defined location on a membrane made of nylon or nitrocellulose, a large number of clones can be screened simultaneously for the presence of DNA complementary to the DNA probe.

10. Describe how a radioactively labeled nucleic acid probe can locate the gene of interest on a multiwell plate.

Plate by plate, cells from each well, representing one clone, are transferred to a defined spot on a special nylon membrane. The nylon membrane is treated to break open the cells and denature their DNA; the resulting single-stranded DNA molecules stick to the membrane. The membrane is then incubated in a solution of radioactive probe molecules complementary to the gene of interest. Because the DNA immobilized on the membrane is single-stranded, the single-stranded probe can base-pair with any complementary DNA on the membrane. Excess DNA is then rinsed off. Next, the membrane is laid under photographic film, allowing any radioactive areas to expose the film. Black spots on the film correspond to the locations on the membrane of DNA that has hybridized to the probe. Each spot can be traced back to the original well containing the bacterial clone that holds the gene of interest.

11. What are two problems with bacterial gene expression systems?

Getting a cloned eukaryotic gene to function in bacterial host cells can be difficult because certain aspects of gene expression are different in eukaryotes and bacteria. To overcome differences in promoters and other DNA control sequences, scientists usually employ an expression vector, a cloning vector that contains a highly active bacterial promoter just upstream of the restriction site where the eukaryotic gene can be inserted into the correct reading frame. Another problem with expressing cloned eukaryotic genes in bacteria is the presence of noncoding regions (introns) in most eukaryotic gene very long and unwieldy, and they prevent correct expression of the gene by bacterial cells, which do not have RNA-splicing machinery. This problem can be surmounted by using a cDNA form of the gene, which includes only the exons.

12. Explain the three initial steps that occur in cycle 1 of the polymerase chain reaction (PCR).

First, the genomic DNA is briefly heated to separate the DNA strands via denaturation. Then, through annealing, the genomic DNA is cooled to allow primers to form hydrogen bonds with ends of the target sequence. Finally, in extension, the DNA polymerase adds nucleotides to the 3’ end of each primer. Cycle 1 yields two molecules. After three cycles, two molecules match the target sequence *exactly*.

13. How many molecules will be produced by four PCR cycles?

After 33 cycles, over 1 billion molecules match the target sequence!

14. What is gel electrophoresis?

Gel electrophoresis is a technique used to separate nucleic acids or proteins that differ in size or electrical charge.

15. *Why is the DNA sample in gel electrophoresis always loaded at the cathode (negative) end of the power source?*

The gel acts as a molecular sieve: because nucleic acid molecules carry negative charges on their phosphate groups, they all travel toward the positive pole in an electric field. As they move, the thicket of agarose fibers impedes longer molecules more than it does shorter ones, separating them by length. Thus, a mixture of linear DNA molecules is separated into bands, each consisting of many thousands of DNA molecules of the same length.

16. *Explain why shorter DNA molecules travel farther down the gel than larger molecules.*

As nucleic acids travel farther down the gel, the thicket of agarose fibers impedes longer molecules more than it does shorter ones, separating them by length.

18. *A patient who is a carrier for sickle-cell anemia would have a gel electrophoresis pattern showing four bands. Add this pattern to your gel in #17 and explain why the gel shows a four-band pattern.*

The normal β -globin allele contains two sites within the coding sequence recognized by the *DdeI* restrictive enzyme. The sickle-cell allele lacks one of these sites as the mutation destroys one of the *DdeI* restriction sites within the gene. A person who has sickle-cell disease is homozygous for the mutant allele, revealing two bands, while a person who does not carry the disease or suffer from it is homozygous for the normal allele, revealing three bands. Thus, a person who carries the disease is heterozygous, revealing a band pattern that combines the band patterns of the mutant and normal alleles, resulting in four bands since the top bands in the mutant and the normal band patterns overlap.

19. *What is the purpose of a Southern blot?*

A classic method called Southern blotting allows us to detect just those bands that include parts of the β -globin gene. The principle is the same as in nucleic acid hybridization for screening bacterial clones. In Southern blotting, the probe is usually a radioactively or otherwise labeled single-stranded DNA molecule that is complementary to the gene of interest.

20. *What two techniques discussed earlier in this chapter are used in performing a Southern blot?*

Southern blotting combines gel electrophoresis and nucleic acid hybridization.

21a. *Why does a dideoxyribonucleotide terminate a growing DNA strand?*

The dideoxy chain termination method for sequencing DNA synthesizes a set of DNA strands complementary to the original DNA fragment. Each strand starts with the same primer and ends with a dideoxyribonucleotide (ddNTP), a modified nucleotide. Incorporation of a ddNTP terminates a growing DNA strand because it lacks a 3' -OH group, the site for attachment of the next nucleotide.

21b. *Why are the four nucleotides in DNA each labeled with a different color of fluorescent tag?*

In the set of DNA strands synthesized, each nucleotide position along the original sequence is represented by strands ending at that point with the complementary ddNTP. Because each type of ddNTP is tagged with a distinct fluorescent label, the identity of the ending nucleotides of the new strands, and ultimately the entire original sequence, can be determined.

21c. *Explain the four steps of DNA microarray assays.*

First, the mRNA molecules from a tissue sample are isolated. Next, cDNA is made by reverse transcription, using fluorescently labeled nucleotides. Then, the cDNA mixture is applied to a microarray, a microscope slide on which copies of single-stranded DNA fragments from the organism's genes are fixed, with a different gene in each spot. The cDNA hybridizes with any complementary DNA on the microarray. Finally, excess cDNA is rinsed off and the microarray is scanned for fluorescence. Each fluorescent spot represents a gene expressed in the tissue sample.

22. *Explain how microarrays are used in understanding patterns of gene expression in normal and cancerous tissue.*

Genome-wide expression studies are made possible by DNA microarray assays. A DNA microarray consists of tiny amounts of a large number of single-stranded DNA fragments representing different genes fixed to a glass slide in a tightly spaced array, or grid. These fragments, which ideally represent all the genes of an organism, are tested for hybridization with cDNA molecules that have been prepared from the mRNAs in particular cells of interest and labeled with fluorescent dyes. Comparing patterns of gene expression in breast cancer tumors and non-cancerous breast tissue has already resulted in more informed and effective treatment protocols.