

- Describe how gene therapy works.

Check Your Understanding

- What part of the cell contains the genetic material?
- What are the base pairing rules for DNA?

Introduction

Since Mendel's time, there have been rapid advances in the understanding of genetics. As scientists understand better how DNA works, they can develop technologies that allow us to reveal the genetic secrets encoded in our DNA and even alter an organism's DNA. Genetic engineering (or *biotechnology* or *DNA technology*) has helped us better understand and predict the inheritance of genetic diseases, produce new medicines, and even produce new food products. DNA technology has also made an impact on fighting crime. Because DNA is unique to an individual, the DNA in just a few hairs at a crime scene can help identify a criminal. This technology, known as DNA fingerprinting, has also helped innocent imprisoned people to appeal their case and clear their names. DNA technology has revolutionized not only criminal justice, but also many other aspects of our lives.

Recombinant DNA

Recombinant DNA is the combination of DNA from two different sources. It is useful in gene cloning and in identifying the function of a gene, as well as producing useful proteins. Human insulin for treating diabetes has been produced through recombinant DNA methods. In this process, a gene of interest (or piece of DNA of interest) is placed into a host cell, such as a bacterium, so the gene can be copied (and cloned) and the protein that results from that gene can be produced.

To place the gene of interest into a host cell, a **vector**, or carrier molecule, is needed to carry foreign DNA into the host cell. Bacteria have small accessory rings of DNA in the cytoplasm, called **plasmids**. When putting foreign DNA into a bacterium (a host cell), the plasmids are often used as a vector. Viruses can also be used as vectors.

The first step of making recombinant DNA involves a **restriction enzyme** that cuts the vector and the foreign (exogenous) DNA. Restriction enzymes cut DNA at specific sequences, such as GAATTC as shown in **Figure 1**. There are more than 3000 known restriction enzymes, most cutting the DNA at a unique sequence. This reaction results in the plasmid opening up a gap with “sticky ends”, which can attach with the complementary base pairs on the sticky ends of the foreign DNA. Then the enzyme **DNA ligase** seals the foreign DNA in its new place inside the plasmid. These altered plasmids are introduced back into the bacteria, a process called **transformation**. The bacteria will express the foreign gene.



Figure 1: Restriction enzymes cut DNA at specific sequences, in this example the sequence “GAATTC”. The enzyme cuts between the G and A on each strand, producing overhanging “sticky ends.”

(Source: http://commons.wikimedia.org/wiki/Image:Restriction_enzyme.jpg, License: Public Domain)

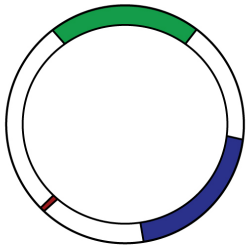


Figure 2: A plasmid can be used to introduce a foreign (or exogenous) gene (blue) into bacteria. The other segment of foreign DNA (green) encodes for antibiotic resistance, which allows for selection of the transformed bacteria by growing them on plates containing antibiotic. Only the bacteria that have not picked up the plasmid will die from the antibiotic.

(Source: http://commons.wikimedia.org/wiki/Image:Example_plasmid.png, License: GNU-FD)

One application of recombinant DNA technology is producing the protein insulin, which is needed to treat diabetes. Previously, insulin had been extracted from the pancreases of animals. Through recombinant DNA technology, bacteria were created that carry the human gene which codes for the production of insulin. These bacteria become tiny factories that produce this protein.

Cloning

Cloning is the process of creating an exact replica of an organism. The clone's DNA is exactly the same as the parent's DNA. Bacteria and plants have long been able to clone themselves through processes of asexual reproduction. In animals, however, cloning does not happen naturally.

Animals can now be cloned in a laboratory, however. In 1997, a sheep named Dolly was the first mammal ever to be successfully cloned. The process of producing an animal like Dolly starts with a single cell from the animal that is going to be cloned. In the case of Dolly, cells from the mammary glands were taken from the adult that was to be cloned. These cells are called **somatic**, meaning they come from the body and are not gametes like sperm or egg. Remember that somatic cells have a diploid number of chromosomes. Next, the nucleus was removed from this cell. The nucleus was placed in a donor egg that had already had the nucleus removed. The new cell then divided after the stimulation of an electric shock, and development proceeded normally just as if the embryo had formed naturally. The resulting embryo was implanted in a surrogate mother sheep, where it continued its development.

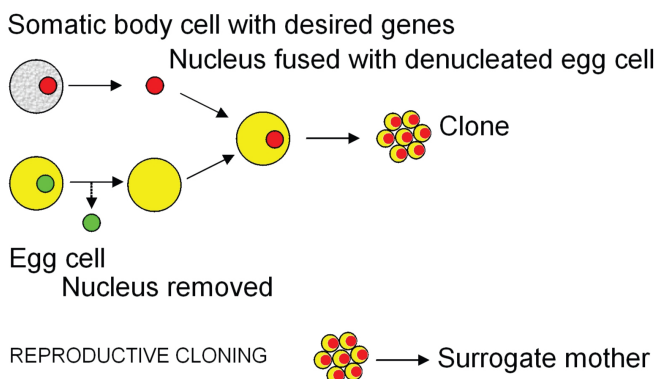


Figure 3: To clone an animal, a nucleus from the animal's cells are fused with an egg cell (in which the nucleus has been removed) from a donor.

(Source: http://commons.wikimedia.org/wiki/Image:Cloning_diagram_english.png, License: GNU-FD)

Cloning is not always successful, though. Most of the time, this cloning process does not result in a healthy adult animal. The process has to be repeated many times until it works. In fact, 277 tries were needed to produce Dolly. This high failure rate is one reason that human cloning is banned in the United States. In order to produce a cloned human, many attempts would result in the surrogate mothers experiencing miscarriages, stillbirths, or deformities in the infant. There are also many additional ethical considerations related to human cloning.

Human Genome Project

A person's genome is all of his or her genetic information; in other words, the human genome is all the information that makes us human. The **Human Genome Project** was an international effort to sequence all 3 billion base pairs that make up our DNA and to identify within this code the over 20,000 human genes. Scientists also completed a chromosome map, identifying where the genes are located on each of the chromosomes. The Human Genome Project was completed in 2003. Though the Human Genome Project is finished, analysis of the data will continue for many years.

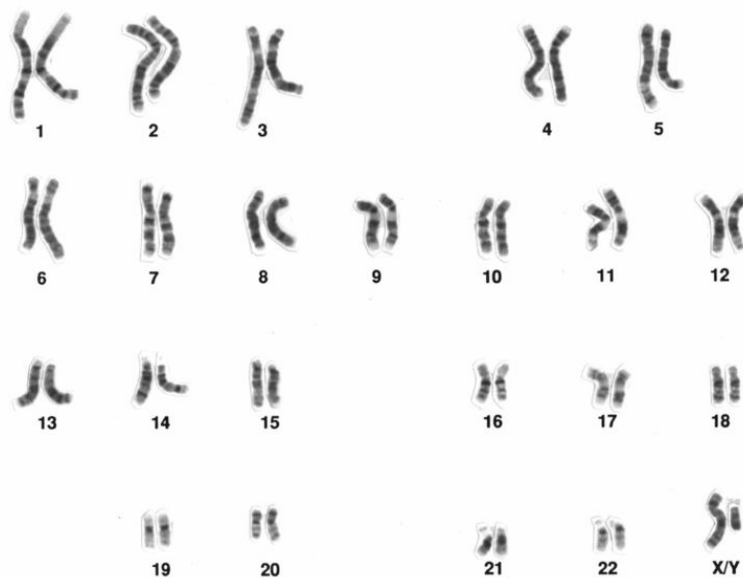


Figure 4: To complete the Human Genome Project, all 23 pairs of chromosomes in the human body were sequenced. Each chromosome contains thousands of genes. This is a karyotype, a visual representation of an individual's chromosomes lined up by size.

(Source: http://commons.wikimedia.org/wiki/Image:Human_male_karyotype_high_resolution.jpg, License: Public Domain)

There are many exciting applications of the Human Genome Project. The genetic basis for many diseases can be more easily determined, and now there are tests for over 1,000 genetic disorders. The National Institutes of Health, the United States government's premiere biomedical research community, is also looking for ways to reduce the costs of sequencing so that people can have a map of their individual genome. Although some disorders are caused by a single gene, many other illnesses are caused by a combination of several genes and a person's lifestyle. Analysis of your own genome could determine if you are at risk for specific diseases. Knowing you might be genetically prone to a certain disease would allow you to better seek preventive lifestyle changes and medical screenings.

A **genetic map** shows the location (or **loci**) of a gene on a chromosome. Genetic maps are important tools to help researchers understand genes and genetic diseases. Knowing where genes are in relation to other genes and knowing the order of genes on a chromosome is an important aspect of human genetics. The

frequency of recombination (crossing-over during prophase I of meiosis) allows geneticists to estimate the distance between loci. Because crossing-over occurs relatively rarely at any location along the chromosome, the frequency of recombination between two locations depends on their distance. The farther apart genes are on the same chromosome, the more likely there is to be a cross-over event between them. The likelihood of a cross-over event between two closely located genes (said to be **linked**) is small.

Gene Therapy

Gene therapy is the insertion of genes into a person's cells to cure a genetic disorder. There are two main types of gene therapy; one done inside the body and one done outside the body. In *ex vivo* gene therapy, done outside the body, cells are removed from the patient and the proper gene is inserted using a virus as a vector. Then the modified cells are placed back into the patient. One of the first uses of this type of gene therapy was in the treatment of a young girl with a rare genetic disease, Adenosine deaminase deficiency, or ADA deficiency. People with this disorder are missing the ADA enzyme, which breaks down a toxin called deoxyadenosine. If the toxin is not broken down, it accumulates and destroys immune cells. As a result, individuals with ADA deficiency do not have a healthy immune system to fight off infections. In the gene therapy treatment for this disorder, bone marrow stem cells were taken from the girl's body and the missing gene was inserted in these cells outside the body. Then the modified cells were put back into her bloodstream. This treatment proved sufficient to restore the function of her immune system, but only with continual repeated treatments.

During *in vivo* gene therapy, done inside the body, the vector with the gene of interest is introduced directly into the patient and taken up by the patient's cells. The vector is inserted where the gene product is needed. For example, cystic fibrosis gene therapy is targeted at the respiratory system, so a solution with the vector can be sprayed into the patient's nose. Recently *in vivo* gene therapy was also used to partially restore the vision of three young adults with a rare type of retinal disease that is congenital, meaning present at birth.

Lesson Summary

- Using recombinant DNA technology, a foreign gene can be inserted into an organism's DNA.
- Cloning of mammals is still being perfected, but several cloned animals have been created by implanting the nucleus of a somatic cell into a cell in which the nucleus has been removed.
- The Human Genome Project produced a genetic map of all the human chromosomes and determined the sequence of every base pair in our DNA.
- Gene therapy involves treating an illness caused by a defective gene through the use of a vector to integrate a normal copy of the gene into the patient.

Review Questions

1. What is the enzyme used to cut DNA at specific points? **(Intermediate)**
2. What is the term for all the genetic information of the human species? **(Intermediate)**
3. What are the rings of accessory DNA in bacteria that are often used as a vector in genetic engineering? **(Beginning)**
4. What is the term for producing identical copies of an organism? **(Beginning)**
5. Can gene therapy cure a disease caused by a virus? **(Challenging)**
6. What is the vehicle used to introduce foreign DNA into an organism? **(Beginning)**
7. What is one disease that genetic therapy can help treat? **(Intermediate)**
8. What supplies the cytoplasm of the clone's cells during the cloning of an organism? **(Challenging)**
9. What is one application of recombinant DNA technology? **(Intermediate)**

10. Is gene therapy for ADA deficiency a permanent fix? (**Intermediate**)

Further Reading / Supplemental Links

- http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml
- <http://history.nih.gov/exhibits/genetics/sect4.htm>
- <http://learn.genetics.utah.edu/units/disorders/whataregd/ada/>
- <http://www.lifesitenews.com/ldn/2007/nov/07112003.html>
- <http://www.le.ac.uk/ge/genie/vgec/sc/genomics.html>
- http://en.wikipedia.org/wiki/Recombinant_DNA
- http://www.hhmi.org/biointeractive/vlabs/transgenic_fly/index.html
- <http://www.groundreport.com/World/Scientists-to-clone-rhino>

Vocabulary

cloning	Creating an identical copy of an individual with the same genes.
DNA ligase	Enzyme that joins DNA fragments together.
gene therapy	Treatment that provides a new gene to replace a defective gene; potentially "cures" a genetic disease.
human genome project	International effort to sequence all the base pairs in human DNA.
plasmid	An accessory circle of DNA in bacteria.
recombinant DNA	DNA formed by the combination of DNA from two different sources, such as placing a human gene into a bacterial plasmid.
somatic cell	A body cell; not a gamete.
transformation	The process by which bacteria pick up foreign DNA and incorporate it in their genome.
vector	A vehicle, such as a plasmid, used to transfer foreign DNA into an organism.

Review Answers

1. restriction enzyme
2. the human genome
3. plasmids
4. cloning
5. No, gene therapy can only cure a genetic disorder.
6. a vector (such as a plasmid)
7. cystic fibrosis, ADA deficiency, etc.
8. donor egg
9. insulin production

10 No, treatments have to be repeated throughout the person's lifetime.

Points to Consider

Next we begin to discuss evolution, the change in species over time.

- Fossils provide evidence of evolution, but what is a fossil?
- If two animals are similar in structure, would you guess they are closely related? Why or why not?