CHAPTER 18

Genetics Ahead

Reflecting Questions

- How are human genetic conditions diagnosed and treated?
- How can scientists change the structure of DNA to produce new kinds of organisms?
- What are some of the benefits and risks of genetic engineering?

Parents wait to hear if their unborn child has inherited a deadly gene. A jury decides whether a young man is guilty of an assault. One researcher determines the precise nucleotide sequence of a gene associated with cold tolerance in fish, while another checks to see if a cloning experiment has been successful. The pattern of DNA bands known as a DNA fingerprint, shown here glowing under ultraviolet light, could hold the answer to any of these questions.

Every year, genetic technologies play a greater role in our society. As medical scientists learn more about the structure of DNA and its role in heredity and development, they can develop new methods to diagnose, prevent and treat genetic disorders. DNA analysis has also become a powerful tool in the courtroom, where it can prove innocence or guilt beyond a shadow of a doubt. Recent studies of the human genome promise revolutionary changes to medicine and the pharmaceutical industry. But can we know too much about each other's genetic makeup? Would you want your friends or your employer to know what genes you carry? If a company analyzes your DNA, who owns the resulting information? Each advance in genetic analysis brings a new set of moral and legal questions.

New technologies are also enabling scientists to alter an organism's DNA and to mix the DNA of different organisms — even organisms from different kingdoms. What happens, for example, when you mix bacterial, plant, and human DNA? If you follow the right recipe, you might create the corn plant shown here, which was engineered to produce a human antibody that fights cancer. Such "plantibodies" are only one example of the uses of a growing number of genetically engineered organisms. These organisms offer society benefits in such fields as medicine, agriculture, and the environment, but they may also come with significant risks.

In this chapter, you will examine some of the tools of genetic analysis and genetic engineering. You will examine the processes involved in genetic technologies to see how genetic material can be deliberately manipulated by researchers, and you will tackle some of the difficult moral and ethical questions that arise from activities such as gene therapy, DNA sequencing, genetic engineering, and cloning.

Genetic engineering can transform a corn plant into a source of human antibodies.

Chapter Contents

18.1 Diagnosis and Treatment of Genetic Disorders

OUTCOMES

- **Describe techniques used in prenatal diagnosis of genetic disorders including amniocentesis, fetoscopy and blood tests.**
- **Explain the significance of genetic markers.**
- **Describe different means of treating genetic disorders including surgery, environmental control, and gene therapy.**
- **Discuss some of the social and ethical concerns associated with gene therapy.**

Throughout history, regardless of culture or country, expectant parents have eagerly anticipated the arrival of a new human being. For the nine months a fetus develops within the womb, its parents wondered how it would look and behave. Yet at the moment of birth, concerns about appearance and personality quickly became secondary to concerns about general health and well-being.

Until recently, details about the health of a developing baby could not be known. Today, with new research and technology, the health of the child in the womb need not be a mystery. Information can be gathered not only during development of the fetus — it can even be predicted before conception.

Genetic Counselling

Couples who have a family history of a genetic disorder or have a child with a heritable disorder may want to consult a genetic counsellor before the conception of their next child. A genetic counsellor is a medical professional who gathers detailed information through interviews, blood tests, and discussions with geneticists. The counsellor then constructs family pedigrees. Using these, together with evidence of biochemical disorders and simple Mendelian genetics, the counsellor can predict the probability of the next child having a genetic disorder. A genetic counsellor communicates the level of risk to the parents so they can make their own decision about whether to conceive another child.

Diagnosis

Preimplantation Diagnosis

Preimplantation diagnosis is available to parents who have a high risk of conceiving a child with a heritable disorder. Sperm and eggs of prospective parents are brought together in a growth medium inside a glass dish. Several eggs are fertilized and begin to divide by mitosis. After two days, each fertilized egg has developed into a ball of eight identical cells, as shown in Figure 18.1.

Because all the cells in the ball have the same genes, one cell can be removed and a karyotype produced while the remaining cells continue to divide. Couples who have a family history of genetic disorders such as cystic fibrosis and Duchenne muscular dystrophy have used this technique to determine whether a disorder has been inherited. If it has not, the ball of cells can be placed inside the uterus so the "test tube baby" can continue its development.

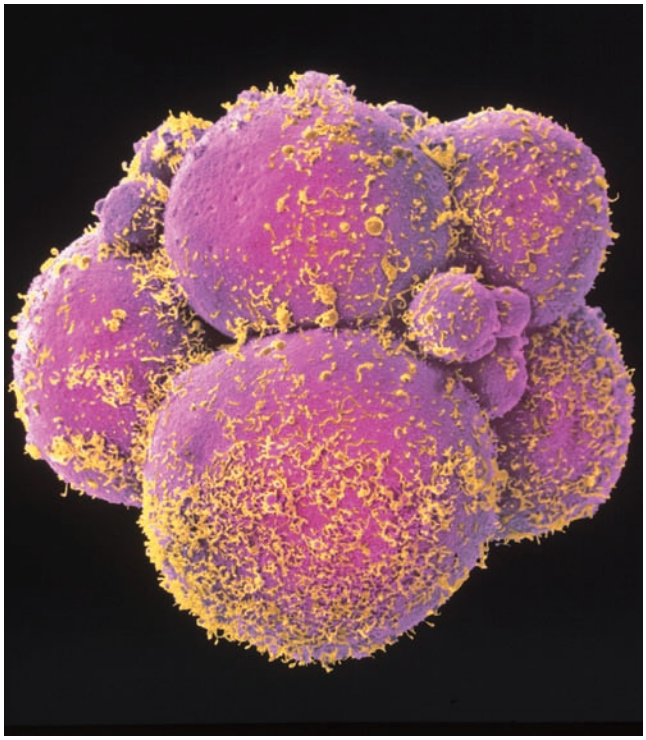

Figure 18.1 The ball of dividing cells shown in this micrograph can be implanted in the uterus to become a healthy full-term baby.

Prenatal Diagnosis

If a woman has already conceived, several tests can be done to diagnose for heritable disorders. As you saw in Chapter 14, an ultrasound provides an image of the developing fetus. During an ultrasound, sound waves beyond the limit of human hearing are sent through the amniotic fluid. The waves bounce off the developing fetus and are used to create a black and white, cross-sectional image of the fetus. This image can be studied for the presence of physical abnormalities such as a missing limb, malformed heart, or cleft palate. Many other genetic conditions, however, can only be identified by collecting a sample of blood or other tissue from the developing fetus.

Karyotyping is a tool commonly used in prenatal diagnosis to identify chromosomal disorders such as Down's syndrome. (see Chapter 16, section 16.4 for a review of karyotypes). The risk of having a baby with Down's syndrome increases if the mother is over the age of 40. To determine whether her developing fetus is affected or not, a woman may choose to have an **amniocentesis**. Throughout its development, the growing fetus is suspended in a fluid-filled membrane inside the uterus called the **amniotic sac**. As the fetus moves and grows inside this amniotic sac, some of its cells are sloughed off and become suspended in the **amniotic fluid**. A sample of this fluid will yield enough cells to create a karyotype that can be used to search for trisomies such as Down syndrome.

After a woman decides on amniocentesis, an ultrasound is used to determine the exact location of the fetus in the uterus. When the position of the fetus has been determined, a long thin needle is used to withdraw a small sample of the amniotic fluid (see Figure 18.2). The extracted fluid is placed in a special nutrient-rich medium and the cells are allowed to multiply for several weeks, until there are enough fetal cells to get a good picture of all the chromosomes and create a karyotype.

Due to the potential risk of injury to the fetus, an amniocentesis cannot be done before the fourteenth week of pregnancy. After that, it may take weeks to obtain the results. A woman interested in obtaining results sooner may opt for a procedure called **chorionic villi sampling (CVS)**. Around the ninth week of pregnancy, cells can be removed from a membranous sac called the chorion. The **chorion** is a tissue that surrounds the amniotic sac housing the fetus. It is one of the tissues that make up the placenta, an intricately branched structure that connects the mother's blood with the fetal blood. Because the chorion is made of fetal cells, it also contains genetic information about the fetus. The removed cells are grown in a special medium, after which a karyotype allows a diagnosis to be made.

Fetoscopy enables direct observation of the fetus, as shown in Figure 18.3. An **endoscope**, essentially a long tube with a camera on one end, is inserted into a small incision made in the mother's abdomen. The clear view of the fetus it provides lets medical professionals safely perform various procedures directly inside the womb. For example, excess fluid surrounding the brain can be removed, and fetal blood transfusions can be performed.

Fetoscopy also provides a safe way to collect blood samples from the fetus. Blood samples can

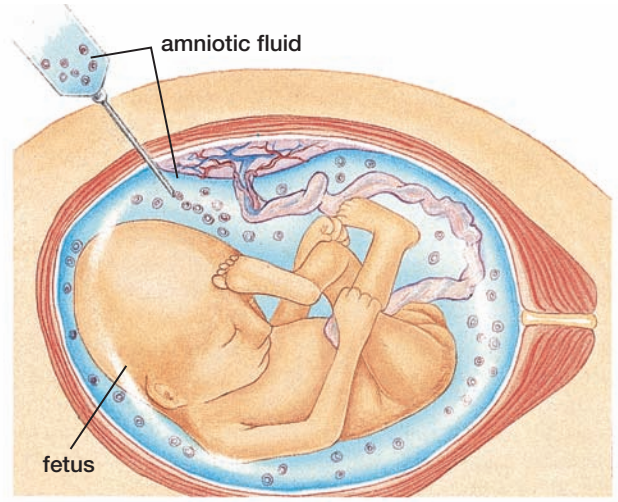

Figure 18.2 In amniocentesis, amniotic fluid is withdrawn from the amniotic sac, after which the fetal cells are cultured and studied.

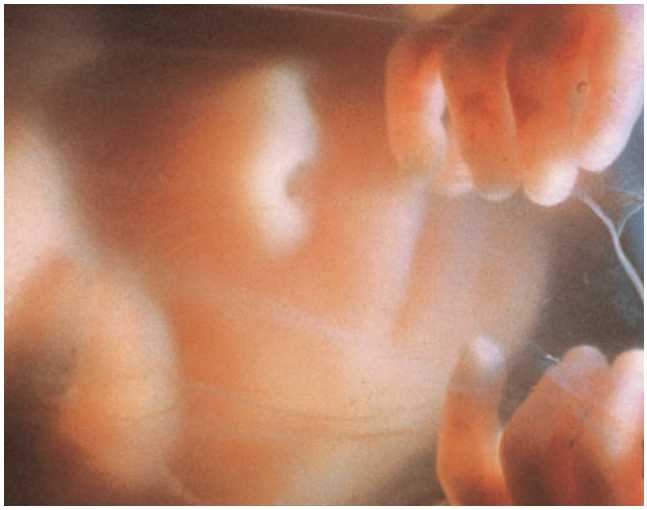

Figure 18.3 Fetoscopy provided this actual view of an 18-week-old fetus inside the womb.

be used to create a karyotype or to test for a number of genetic conditions. Technicians can use blood samples to identify the blood type of the fetus, which can be a concern in certain cases where the fetus has a blood type that triggers an immune response in the mother. Blood tests can also be used to identify genetic blood disorders such as sickle cell disease.

Genetic Markers

A **genetic marker** is any characteristic that provides information about an organism's genome. For example, in the case of Mendel's pea plants discussed in Chapter 16, white flowers were a genetic marker indicating the homozygous recessive genotype rr. As researchers learn more about the genes associated with particular disorders, genetic markers are also being identified at the molecular level within DNA.

There are two general types of DNA markers: a *linked marker* is a known sequence of nucleotides that is located close to the gene that causes the disorder. A linked marker will usually indicate the presence of the gene. A *gene-specific marker* is a sequence of DNA that is part of the gene itself. These markers always indicate the presence of the gene causing the disorder.

A DNA marker can be found using a probe that consists of a nucleic acid sequence that is complementary to the marker sequence, along with a radioactive or fluorescent chemical tag. When DNA containing the genetic marker is mixed with a solution containing the nucleic acid probe, the tagged probe forms a base pair with the marker sequence. In this way, researchers can verify the presence of the gene of interest.

Canadians in Biology

Chromosomal Abnormalities in Sperm

We know that women's eggs deteriorate as women age, but what about men's sperm? Do they also deteriorate with age? If men who are diagnosed with testicular cancer receive chemotherapy, will their sperm be affected? Do sperm have a greater-than-normal proportion of chromosomal abnormalities (in either their structure or number) after being stored in sperm banks at very low temperatures? Do men who smoke have more defective sperm than men who do not? Do infertile men, who now have the option of using reproductive technologies, run a higher risk of having unhealthy offspring?

Dr. Renee Martin

Dr. Renee Martin, a professor with the department of Medical Genetics at the University of Calgary, has researched these questions. When Dr. Martin began her medical career 20 years ago, fertility studies focussed almost entirely on women even though half of the genetic material in human embryos is contributed by men. Consequently, Dr. Martin decided to study the causes of chromosomal abnormalities in humans with an emphasis on the abnormalities that could be traced to sperm. After all, if we are ever to prevent the profound problems people sometimes inherit as a result of chromosomal abnormalities, we need to determine what causes those abnormalities.

New Molecular Technologies at Work

Dr. Martin's lab, which uses new molecular technologies to study sperm chromosomes and the genes on them, was the first in the world to demonstrate that a large proportion of some men's sperm can have abnormal chromosomes. For instance, men exposed to radiotherapy and chemotherapy during cancer treatment have more chromosomal abnormalities in their sperm. Some of these chromosomal abnormalities result in children who are born with abnormal chromosomes. Elevated levels of sperm with chromosomal abnormalities are also associated with infertile men, who can now take advantage of a new reproductive technology technique called Intracytoplasmic Sperm Injection (ICSI). ICSI is used when natural fertilization cannot take place due to, for example, low sperm count or poor motility (locomotion). During ICSI, sperm is injected directly into an egg in a test tube.

Among the new molecular techniques at the disposal of Dr. Martin's lab are those involving Fluorescence in situ

Treatment

Genetic Screening and Prevention

If no previous diagnosis has been made, a genetic disorder can be detected at birth. Through routine blood tests using special biochemical procedures, disorders such as phenylketonuria can be detected early enough for parents and medical professionals to carry out preventive measures. With a special diet, for instance, children with phenylketonuria can grow to lead normal lives. Genetic screening and prevention has lowered the incidence of phenylketonuria in the North American population dramatically in recent years.

Surgery

Some genetic conditions can be treated through surgery. Babies born with **cleft palates** have a vertical groove in their upper lips and into the roofs of their mouths. Reconstructive surgery can solve this condition.

Environmental Control

Sometimes the only treatment is to minimize the effects of the symptoms. For example, individuals affected with albinism lack the pigment melanin, which offers protection from the mutagenic effects

WEB LINK

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Use the internet to learn more about genetic testing. Go to the web site above and click on **Web Links**. Use the information you have gathered to answer these questions: What types of tissues are required for a genetic test? Which disorders can be diagnosed through genetic testing? How accurate are the results of the tests? Should insurance companies have access to this type of information?

Hybridization (FISH) and Polymerization Chain Reactions (PCR). FISH uses DNA probes tagged with fluorescent molecules to detect both simple and complex chromosomal re-arrangements (such as aberrations and abnormalities) over small areas of a chromosome. The fluorescent molecules are activated by light, and they emit light of different wavelengths (and therefore different colours). Using FISH, the structure and behaviour of different chromosomes can be studied. PCR, a technique created by Kary Mullis in 1984, creates multiple copies of a specific sequence of nucleotides within a segment of DNA. You will learn more about this technique on pages 614–615.

A Career in Genetics

A career in genetics was not one of Dr. Martin's original goals. Although she enjoyed science courses in high school, her focus was on physical education and dance. At one point, however, she had to spend a week in hospital. When she returned to school, her Grade 11 science teacher informed her that she had missed the opportunity to learn about DNA, a very important topic in the course. After the teacher explained DNA's role as a blueprint for genetic inheritance and information exchange within the body, Dr. Martin found herself intrigued and fascinated by, to use her own words, the "beauty and simplicity of it." After one year pursuing a bachelor's degree in Physical Education at the University of Toronto, she switched fields and eventually received her bachelor's degree in Science with honours (Zoology) from the University of British Columbia. Continuing in her studies, she then earned her Ph.D. in Medical Genetics from UBC by studying chromosomal abnormalities that result in too few or too many chromosomes. Examples of conditions caused by such abnormalities are Down syndrome and Turner syndrome, which are known as trisomy 21 and monosomy, respectively.

During her career, Dr. Martin has published many research papers that investigate whether age, smoking, prolonged exposure to pesticides, or cryopreservation (low-temperature storage or cryogenics) produce chromosomal abnormalities in sperm. She has also studied the ethical issues involved in assisted reproductive technologies; and the chromosomal abnormalities in sperm before, during, and after cancer treatment involving chemotherapy and radiotherapy. She has been invited to address many international conferences and symposiums on fertility and genetics in Europe, the United States, Scandinavia, the Middle East, Australia, India, and Canada. She has also served as president of the Canadian College of Medical Geneticists and the Canadian Society of Andrology and Fertility.

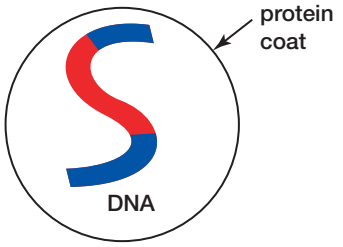
What Dr. Martin enjoys most about her job is the flexibility it gives her to plan her day as she chooses, which occasionally allows her to schedule an exercise session or a volunteer activity at her children's school. She also loves the opportunity it gives her to travel and meet with other researchers from around the world. About the only unwelcome side of her work is the continuous need to apply for research grants. Even in this activity she finds a silver lining, as it encourages her to focus clearly on her research goals.

of direct sunlight. Since there is no medical treatment for albinism, these individuals must limit their exposure to direct sunlight.

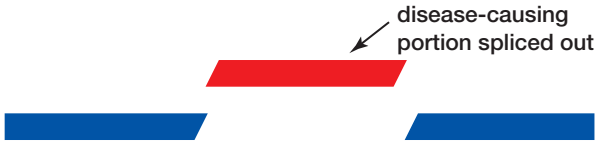
Gene Therapy

Gene therapy is a medical procedure in which normal or modified genes are transferred into the defective cells of an individual. In theory, the transferred genes will allow the recipient's cells to begin functioning normally by giving them the instructions for synthesizing the missing polypeptides, thus reversing the symptoms of the genetic disorder. In 1992, for example, a 30-year-old Québec woman made genetic history. She had already had a heart attack by age 16 and a bypass operation at age 26. Both of her brothers had died of heart attacks in their early twenties. The woman and her siblings all had a genetic condition that resulted in an abnormally high level of cholesterol in the blood.

Amid controversy about the risks and benefits of gene therapy, the woman agreed to participate in a painful and unproven procedure. The procedure involved removing about 15% of the woman's liver. The liver cells were allowed to multiply in a special medium until a sufficient number of cells had been created. In the meantime, the healthy human gene that she lacked was inserted into the genome of a harmless virus, as shown in Figure 18.4. The modified virus was added to the multiplying liver cells, where it injected its own genetic material into her cells. The harmless virus followed its normal infection cycle and injected its genetic material into the liver cells along with the healthy human gene (see Figure 18.5).



A The intact virus is made up of a protein coat containing a strand of DNA.



B The viral DNA is isolated and the disease-causing portion of the viral genome (red) is spliced out. Genes coding for the enzymes that allow the virus to insert its DNA into the genome (blue) of its host cell are left intact.

C A working human gene (green) is inserted into the viral genome. The modified viruses are then cultured with human cells. Some of the viruses will transfer the new gene into the cells' genome.

Figure 18.4 **Some viruses can be modified and used as vectors to carry new genes into a human cell. The human immunodeficiency virus (HIV) — one of the deadliest viruses known — has the potential to be a very powerful viral vector because of its ability to infect many different types of cells.**

Billions of infected liver cells were then injected into the woman's large hepatic portal vein, which transports blood directly to the liver. Some of the cells became a part of the woman's liver and began behaving as healthy cells by breaking down excess cholesterol. After two years, about 5% of the

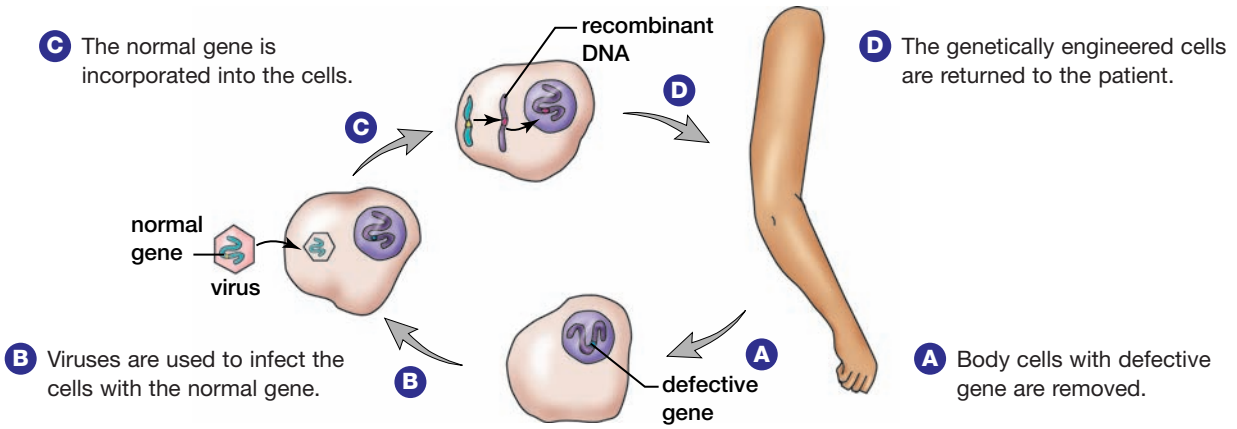


Figure 18.5 **In gene therapy, body cells with the defective gene are removed and infected with a harmless virus carrying the normal gene. The virus injects the normal gene into the cells, after which they are returned to the body.**

woman's liver cells were functioning normally. Her blood cholesterol level had dropped about 20% and her arteries were not clogged. At a press conference later, the woman announced that she was enjoying good health and an active lifestyle. Although her cholesterol level remained twice as high as normal, it was significantly lower than it would have been without the gene therapy.

To date, gene therapy has not produced any cures for genetic disorders. Many clinical trials involving both animals and human patients are under way, however. Some of these studies are aimed at finding alternatives to the use of viruses to deliver new genetic material into living cells. Although viruses work very well in many respects, their protein coat can trigger a severe — even fatal — immune response in some patients.

BIO FACT

It is estimated that 3000 to 4000 disorders are directly caused by defective genes that we inherit. We now know that these mutated genes influence how we respond to common diseases such as cancer, heart disease, and diabetes. These common diseases are believed to be caused by complex interactions between these altered genes and unhealthy lifestyle and environmental factors.

Another subject of current research is the challenge of ensuring that new genetic information can be expressed correctly in the target cells. Recall that some genes and regulatory sequences can interact with others. This means that a gene inserted in the wrong place in the genome may not work properly. Even worse, it may interfere with the function of other genes elsewhere in the genome. For these reasons, gene therapy has not yet been approved for general medical use on human patients.

All gene therapy techniques that have been the subject of medical trials to date have focused on *somatic gene therapy*. Somatic gene therapy involves modifying the genes in the patient's somatic cells. If the therapy is successful the patient may be treated, but could still pass the genetic disorder on to his or her children. *Germ line therapy*, on the other hand, would involve altering the DNA of the patient's sperm or egg cells. While this raises the possibility of eliminating inherited diseases, the risk of unforeseen long-term impacts is significant. No germ line therapy research has yet been approved in Canada.

Limits to Diagnosis and Treatment

Some genetic disorders are relatively easy to diagnose or predict. Chromosomal disorders such as Down syndrome and Turner syndrome, for example, can be readily diagnosed from a karyotype. Similarly, the likelihood of an individual being born with hemophilia or Huntington disease can be predicted using pedigree information, and genetic markers can help an individual find out if he or she carries a specific gene linked to a disorder. For many genetic disorders, however, the picture is not so clear.

Consider the case of Alzheimer's disease. This form of dementia begins with mild forgetfulness and progresses to a severe loss of memory, language, and conceptual skills. Individuals who suffer from Alzheimer's eventually forget how to perform simple tasks, no longer recognize their family members, and require constant care. The brains of people who have died from Alzheimer's show abnormalities including tangles and clumps of nerve fibres.

Figure 18.6 Alzheimer's disease is the most common form of dementia in people over age 65.

There are two general types of Alzheimer's disease. One, known as Familial Alzheimer's Disease or FAD, follows an autosomal dominant inheritance pattern. This form can strike people as early as the age of 40. By far the most common form of Alzheimer's disease, however, is known as Sporadic Alzheimer's Disorder or SAD. SAD, which affects people over the age of 60, accounts for about 90% of all Alzheimer's cases. Research indicates that this form of the disorder is associated

with a gene known as EpoA. EpoA is located on chromosome 19 and is involved in the synthesis of a protein that carries cholesterol in the blood. EpoA has multiple alleles — one of which appears to help prevent Alzheimer's, and one of which appears to increase the risk of Alzheimer's. In addition, a number of other genes on different chromosomes may be involved in regulating the action of EpoA. Environmental factors may also affect the expression of this gene.

All of this means that a genetic test for the sporadic form of Alzheimer's can deliver nothing more than a "maybe" response. That is, even if an individual carries the EpoA gene, he or she may not develop the disorder. It also means that this disorder is not a good candidate for gene therapy. Gene therapy works best in cases where a single gene is malfunctioning. In a case such as SAD, the interactions are so complex that even inserting a new form of the EpoA gene may not help treat the disorder.

Ethical Issues

Although the field of gene therapy is still very new, extensive debate is already under way about some of the moral and ethical issues at stake. For example, who should be allowed to decide whether a particular condition is a genetic disorder? Should parents be allowed to "design" their babies so that they carry certain genes and not others? Imagine that a woman who is congenitally blind, but who has a very happy and fulfilling life, is told that her unborn baby also carries the gene for blindness. She is advised to have the fetus treated to correct this condition. How might she react? Would it be wrong for her to choose not to treat the baby? Or imagine that a treatment of gene therapy is available that can increase your IQ. Would you want to take the treatment? What might happen to society if the treatment was available, but very expensive?

In the rest of this chapter you will learn more about the genetic engineering techniques used to sequence, analyze, and alter DNA. You will also explore some more of the difficult questions that accompany our increasing understanding of genetics.

WEB LINK

www.mcgrawhill.ca/links/atlbiology

Gene therapy techniques may make it possible to alter the function of particular genes to either treat disease or change people in other ways. Should parents be allowed to select the eye colour of their children? Should a healthy person who is naturally short be treated to increase his or her height? Go to the web site above, and click on **Web Links** to find out more about some of the current legal and ethical issues raised by gene therapy research. With a partner, select one issue for discussion and prepare a five-minute classroom debate in which you each argue a different position on the best way to resolve the issue.

SECTION REVIEW

1. Describe how an amniocentesis is done and the circumstances under which it would be recommended.
2. What is the advantage of sampling cells from the chorion?
3. Using a flowchart, describe the steps involved in gene therapy.
4. If an Rh– woman is expecting a child who is Rh+, the mother's antibodies will destroy the blood cells of the fetus. The developing child will therefore be in danger of developing anemia. Which prenatal procedure could be used to treat the fetus?
5. In groups of three, discuss the qualities you would expect to find in a genetics counsellor if your child was diagnosed with a genetic disorder.
6. Identify two ethical dilemmas that arise from our ability to detect genetic diseases.
7. Give an example of a genetic marker found in humans.
8. Describe three different kinds of tests that could be performed on fetal blood to identify genetic disorders. What kind of information does each test provide?
9. Give three reasons that the probability of an individual developing sporadic Alzheimer's disease is difficult to predict.
10. You are working in a lab that is trying to find a gene associated with stunted growth in mice. You know that the gene contains the sequence GGCATTATCCG on the sense strand of DNA. Use diagrams to show how you could use a nucleic acid probe to determine what chromosome carries this gene. If you did not know the DNA sequence, what other steps could you take to find the gene?
11. What features of a genetic disorder could make it a good candidate for treatment with gene therapy? Describe some of the limitations of gene therapy.

18.2 The Sequence of Life

OUTCOMES

- **Demonstrate an understanding of the basic tools used in genetic engineering, including restriction enzymes and recombinant DNA.**
- **Discuss the significance and the initial findings of the Human Genome Project.**
- **Analyze the social risks and benefits associated with the Human Genome Project.**

In 1977, a new era in genetic engineering was launched by English biochemist Frederick Sanger (shown in Figure 18.7) and his colleagues. Sanger and his team worked out the complete nucleotide sequence in the DNA of the virus known as phage θX174. This breakthrough enabled researchers to compare the exact sequence of the 5386 nucleotide bases in the virus with the polypeptide products of the virus's nine genes. As they studied the DNA sequence, the researchers made some new discoveries about the organization of genetic material. For example, from the fact that one of the genes of this virus is located entirely within the coding sequence of another, longer gene, they learned that genes can overlap. On a broader level, the work of Sanger and his colleagues opened the door to genome sequencing as a way to better understand the genetics of living cells.

The work of Sanger's team relied on three important developments. The first was the discovery of a way to break a strand of DNA at specific sites along its nucleotide sequence. The second was the development of a process for copying or amplifying DNA, which made it possible to prepare large samples of identical DNA fragments for analysis. The third development was the improvement of methods for sorting and analyzing DNA molecules. Although the techniques involved have been refined dramatically in the years since Sanger's discovery, these processes remain the basis of much of our genetic technology today. You will learn more about these processes in the following pages.

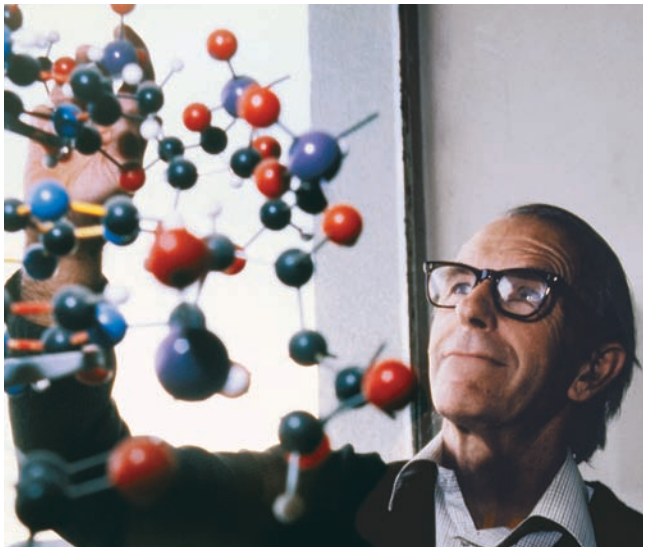


Figure 18.7 Frederick Sanger is one of only four people who have twice been awarded the Nobel prize in chemistry. He won the award in 1958 for his work on identifying the structure of proteins, and again in 1980 for his development of a technique for sequencing DNA.

Restriction Endonucleases

In order to defend themselves against infection by foreign DNA, most prokaryotic organisms manufacture a family of enzymes known as **restriction endonucleases**. Restriction endonucleases recognize a specific short sequence of nucleotides (the target sequence) on a strand of DNA and cut the strand at a particular point within that sequence. This point is known as a **restriction site**. Restriction sites occur by chance in one or more locations in almost any fragment of DNA.

Figure 18.8 illustrates the results of a typical restriction endonuclease reaction. Many different endonucleases have been isolated, each recognizing a different sequence. Two key characteristics have made them useful to genetic researchers.

- **Specificity** The cuts made by an endonuclease are specific and predictable — that is, the same enzyme will cut a particular strand of DNA (such as a plasmid or chromosome) the same way each time, producing an identical set of smaller pieces. These smaller pieces are called **restriction fragments**.
- **Staggered cuts** Most restriction endonucleases produce a staggered cut that leaves a few unpaired nucleotides remaining on a single strand at each end of the restriction fragment. These short

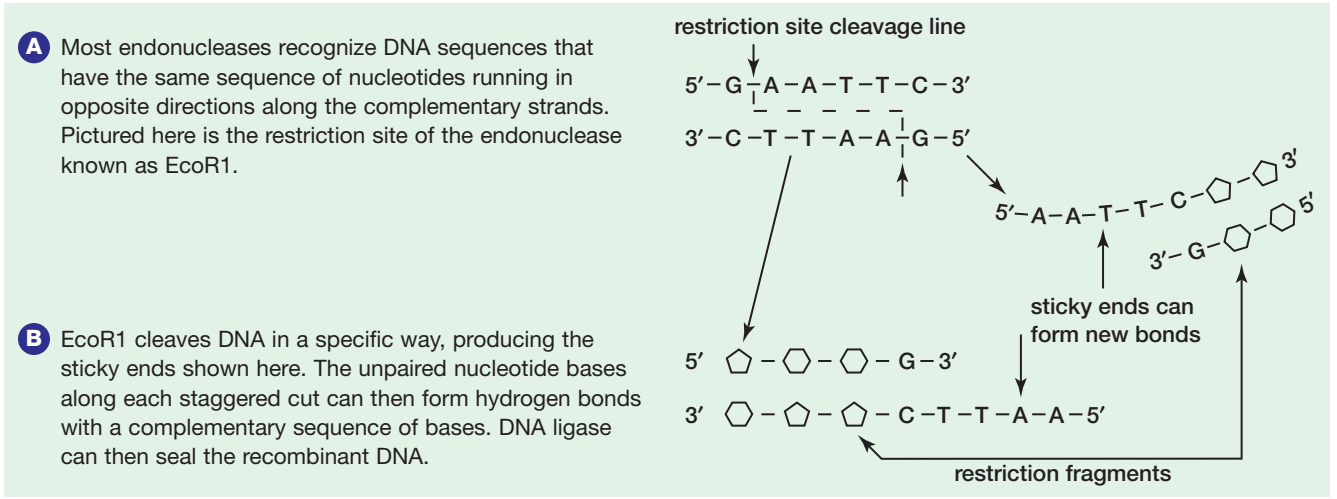


Figure 18.8 Typical restriction endonuclease reactions result in sticky ends.

sequences, often referred to as **sticky ends**, can then form base pairs with other short strands having a complementary sequence. For example, they can form a base pair with another restriction fragment produced by the action of the same enzyme on a different strand of DNA. DNA ligase can then seal the gap in each strand in the new DNA molecule. In this way, researchers can produce **recombinant DNA** by joining DNA from two different sources.

Not all endonucleases produce sticky ends. Sticky ends can make binding and recombination easier, but they can also limit the uses to which endonucleases can be put. For some purposes, researchers use endonucleases that cleave the DNA molecule in a way that produces a blunt cut.

DNA Amplification

The process of generating a large sample of a target DNA sequence from a single gene or DNA fragment is called **DNA amplification**. Two different methods, as discussed below, are used by researchers.

Cloning Using a Bacterial Vector

Cloning using a bacterial vector relies on the action of restriction endonucleases. When a target sample of DNA is treated with an endonuclease, it is broken into a specific pattern of restriction fragments based on the location of the nucleotide sequences recognized by the enzyme. These fragments are then spliced (via their complementary sticky ends) into bacterial plasmids that have been cleaved by the same endonuclease. The result is a molecule of recombinant DNA.

The first recombinant DNA was created in 1973 by the American team of Stanley Cohen and Herbert Boyer. They used the process illustrated in Figure 18.9 to splice a gene from a toad into a bacterial plasmid.

The recombinant plasmid can then be returned to a bacterial cell. As the cell multiplies, it replicates the plasmids containing the foreign DNA. In this way, millions of copies of the DNA fragment can be produced. The plasmid here serves as a **cloning vector**, the term used to describe a molecule that replicates foreign DNA within a cell.

This cloning method is still in use today as a means of amplifying larger DNA sequences. For short fragments of up to a few thousand base pairs, however, a second and much faster method has since been developed.

BIO FACT

Different bacteria produce different endonucleases. A bacterium protects its own DNA from its particular endonuclease by chemically modifying nucleotides in the target sequence of its DNA so that the endonuclease cannot bind to them.

Polymerase Chain Reaction

The **polymerase chain reaction (PCR)** is an almost entirely automated method of replicating DNA that allows researchers to target and amplify a very specific sequence within a DNA sample. It was developed by American researcher Kary Mullis in 1986 and earned him the Nobel prize.

The PCR process, shown in Figure 18.10, relies on the action of DNA polymerase, the enzyme responsible for replicating DNA. First, the sample

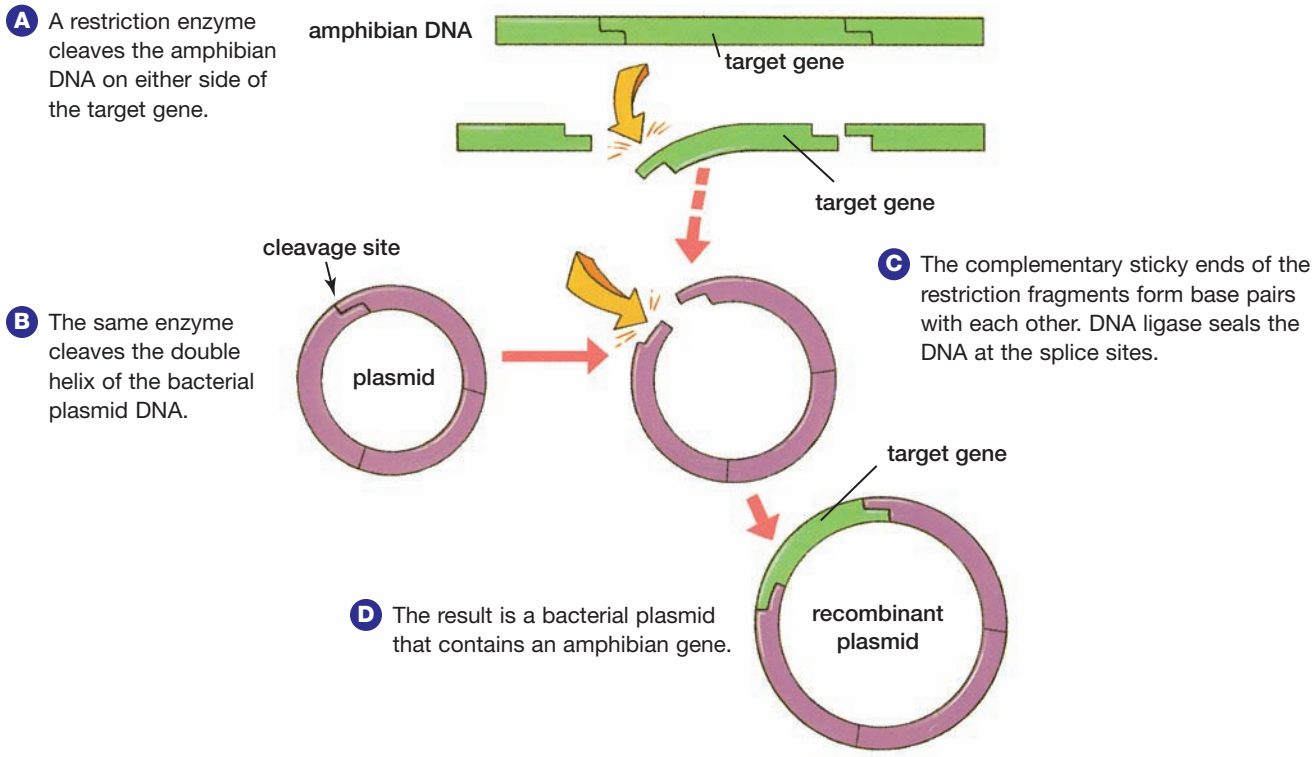


Figure 18.9 Using the bacterial vector cloning process, Stanley Cohen and Herbert Boyer developed a bacterial plasmid containing recombinant DNA.

DNA fragment is placed in a solution along with nucleotides and primers. The solution is heated to break the hydrogen bonds between base pairs, which causes the DNA double helix to open. Next, the solution is cooled. Heat-resistant DNA polymerase is added and replication begins. In just over a minute, both DNA strands are replicated, resulting in two copies of the original target sequence. The cycle then repeats itself. Because the process uses a special heat-resistant form of DNA polymerase, it is not necessary to add new enzymes after each heating stage. Each cycle doubles the amount of target DNA in the sample, so the polymerase chain reaction can quickly generate billions of copies of a DNA sequence for analysis.

Sorting DNA Fragments

The third breakthrough that made Sanger's work possible was the development of a process called gel electrophoresis. **Gel electrophoresis** is used to separate molecules according to their mass and electrical charge. This process enables fragments of DNA to be separated so they can be analyzed.

In this process, which is illustrated in Figure 18.11, a solution containing DNA fragments is applied at one end of a gel. The gel is then subjected to an electric current, which causes

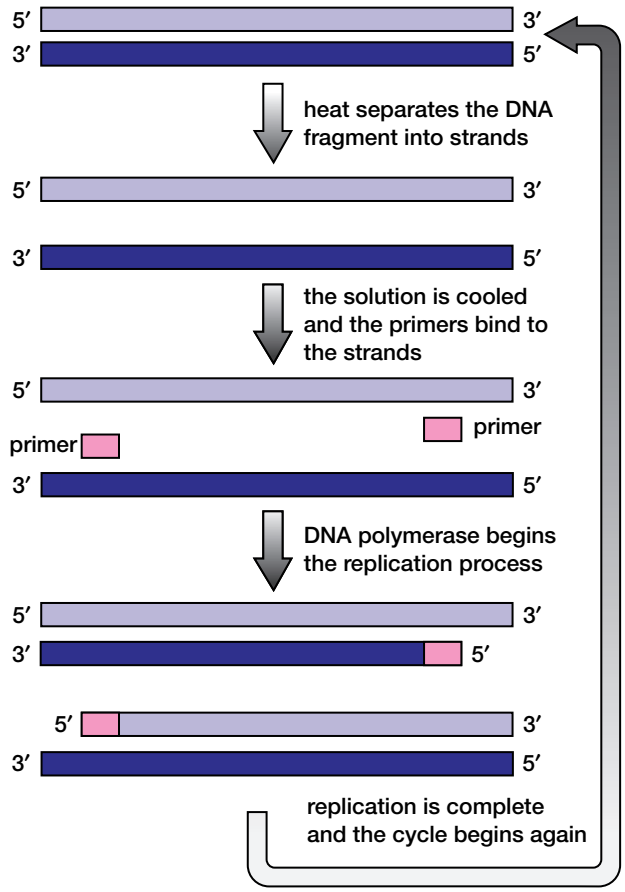


Figure 18.10 The polymerase chain reaction process is now almost entirely automated.

the ends of the gel to become polarized. Being acidic, DNA has a negative charge. Therefore, the fragments tend to move toward the gel's positive end, with the smaller fragments moving more quickly. After a period of time, the fragments separate into a pattern of bands. This pattern is called a **DNA fingerprint**. One of the developments that made Sanger's work possible in 1977 was the refinement of electrophoresis to the point that DNA fragments could be separated if they differed in length by as little as a single nucleotide.

Analyzing DNA

The three processes described above — the use of restriction enzymes, DNA amplification, and gel electrophoresis — can be used in a number of ways to help researchers analyze and compare DNA samples. For example, investigators at a crime scene might find a single hair attached to a hair follicle. The DNA from this hair follicle can be amplified using DNA cloning or PCR to produce billions of copies of the sample DNA molecules. When a sample of the DNA is then cut with a restriction enzyme and run on a gel, the pattern of bands can be compared with the DNA fingerprint of the suspect. Since no two people (other than identical twins) have the same DNA pattern, a DNA fingerprint match is very strong evidence that the suspect was present at the crime scene.

In the same way, DNA fingerprint evidence can be used to solve disputes over parentage. Because a child's DNA is inherited equally from both parents, the child's DNA fingerprint will show some matches with the DNA fingerprint of each parent. A comparison of the DNA fingerprints of different people can help researchers identify the relationships among them.

WEB LINK

www.mcgrawhill.ca/links/atlbiology

To view an animation on restriction endonucleases and an interactive exploration on DNA electrophoresis gel results, go to the web site above and click on **Electronic Learning Partner**.

Sequencing DNA

The same processes used to prepare DNA from different samples for comparison and analysis also play a role in determining the nucleotide sequence of a single DNA fragment. The process used to sequence DNA is known as **chain termination** sequencing. It relies on a modified form of the polymerase chain reaction.

In the chain termination reaction, the replicated section of DNA is synthesized in a series of small fragments rather than in one strand. The nucleotide that ends each fragment is tagged with a radioactive or fluorescent marker. When the fragments are run on a gel, the resulting fingerprint shows which fragments end with G nucleotides, which end with C, and so on. This means that it is possible to see, for example, that a DNA fragment 10 nucleotides long ends with a G — which means that G occupies the 10^{th} nucleotide position in the strand. When all the fragments are analyzed together, a researcher

THINKING LAB

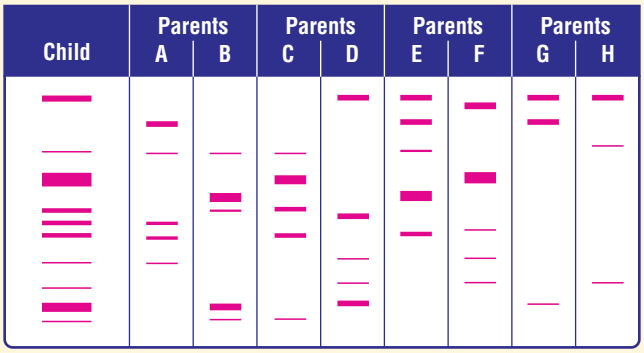
Reading a DNA Fingerprint

Background

After samples of DNA have been prepared in the laboratory, segments are stained and prepared in a special gel. These samples can then be compared with other samples to solve a crime or provide answers in other situations, such as a question of paternity (determining the father of a child). You know from your study of heredity earlier in this Unit that a child's DNA is a combination of the DNA of his or her parents. Can you analyze these DNA samples to determine the child's parents?

You Try It

1. Which parental DNA matches the child's? How did you decide this?
2. Try to determine the percentage of the father's DNA that matches the child's. Can you do the same for the mother's DNA? Explain this.
3. Describe other situations where DNA fingerprinting might be useful.



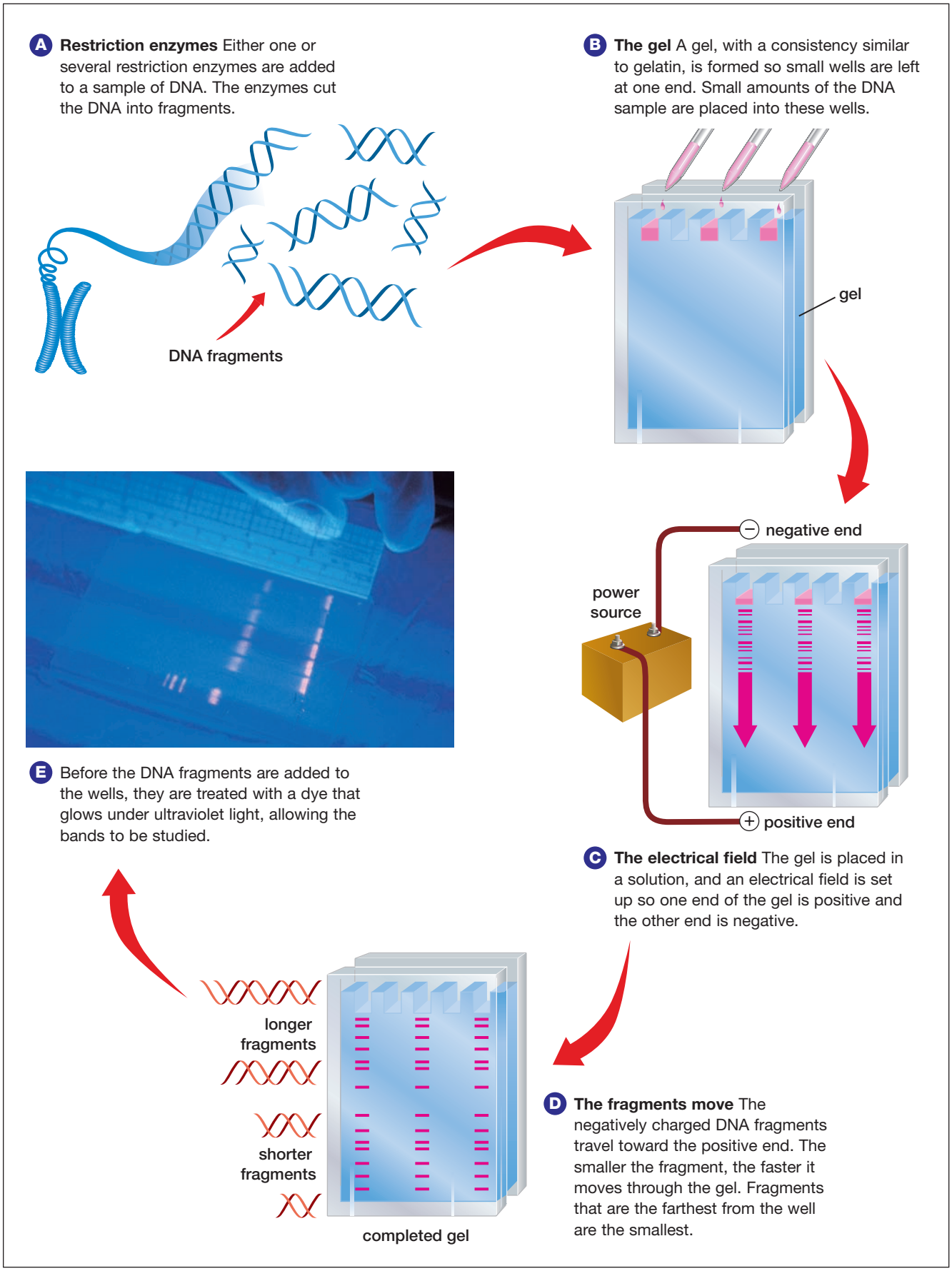


Figure 18.11 Gel electrophoresis

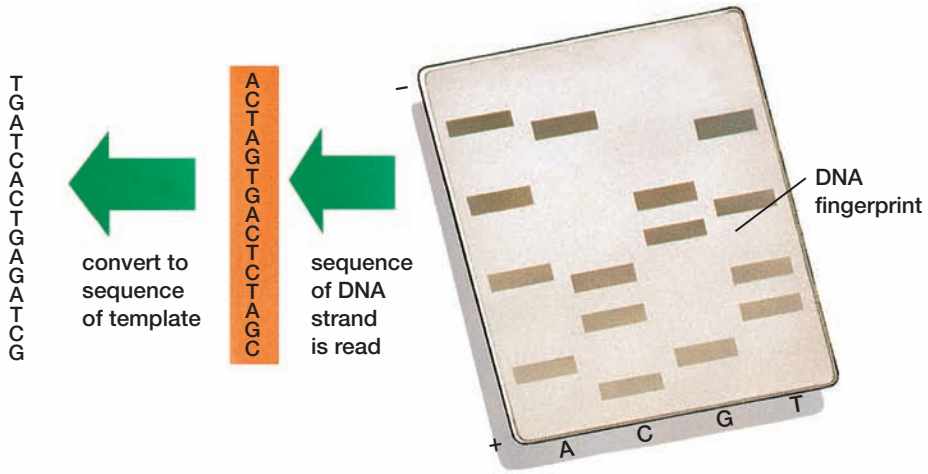


Figure 18.12 When DNA fragments from a chain termination reaction are run on a gel electrophoresis, the gel can be read from the positive end to the negative end to determine the nucleotide sequence of the original DNA strand.

can read the gel to determine the nucleotide sequence of the original DNA strand. This step usually involves the use of an automated DNA sequencer, which speeds up the reading process.

The chain termination reaction can be used to sequence DNA samples of a few thousand base pairs in a single reaction. But even the small genome of a virus contains many thousands of nucleotides, and the genome of a mammal is billions of nucleotides long. Because of this, for many years the main barrier to sequencing the DNA of eukaryotic organisms was the sheer size of the genomes involved. It was not until the late 1990s that advances in technology and computing software finally made it possible to sample and analyze enormous amounts of DNA relatively quickly.

The Human Genome Project

A complete draft of the human genome was first published in February 2001, making it the first mammalian genome to be sequenced. This landmark achievement, announced around the world at press conferences like the one shown in Figure 18.13, was the culmination of the work of thousands of researchers from laboratories around the world in a joint effort known as the Human Genome Project.

The **Human Genome Project (HGP)** determined the sequence of the three billion base pairs that make up the human genome. Among the project's immediate findings was the discovery that the DNA of all humans (*Homo sapiens*) is more than 99.9% identical. Put another way, this means that all the differences among individuals across humanity result from variations in fewer than one in 1000 nucleotides in each individual's genome.

One of the most surprising initial findings was that the human genome contains only about 35,000 genes, rather than the 100,000 genes that scientists had anticipated. Since the human body produces over 100,000 different proteins, this means that, on average, each human gene is capable of synthesizing three different polypeptides. In turn, this indicates that the DNA sequence alone is not the only factor that guides the development of complex organisms. The initial findings of the Human Genome Project confirm that the complex interactions between DNA and proteins will be a key area of genetic research in the years ahead.

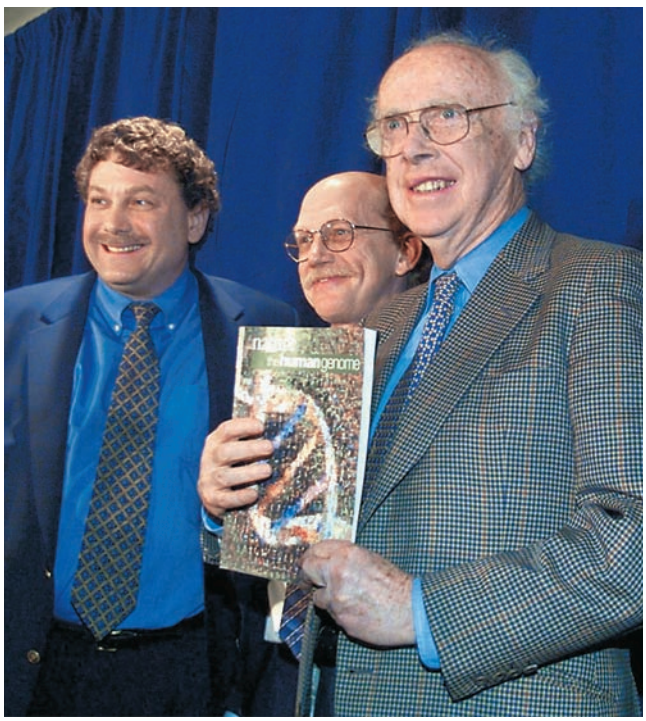


Figure 18.13 In February 2001, lead Human Genome Project researchers announced the release of the first draft of the complete human genome.

Over the long term, the information from the sequencing of the human genome will provide

geneticists with a better understanding of the relationship between the molecular structure of human genes and the biological mechanisms of gene function. Among other things, human genome sequencing will allow researchers to pinpoint specific nucleotide sequences that are involved in gene expression. Some of the potential benefits of these discoveries include better ways to assess an individual's risk of developing a disease, better ways to prevent disorders, and the development of new drugs and other treatments that are precisely tailored to an individual's personal genetic make-up.

In addition, comparing the human genome with the genomes of other species offers opportunities to learn more about the processes of development in living organisms. Such studies provide a significant foundation for further research.

New Knowledge, New Problems

Genetic research initiatives such as the Human Genome Project offer a number of potential benefits. At the same time, these advances raise significant legal and ethical issues. For example, should your employer or your life insurance company have the right to demand information about your genetic predisposition to disease? Should a company that has worked out the DNA sequence of a gene associated with breast cancer be allowed to prevent other companies from using this information to develop genetic screening mechanisms or treatments? In short, who should have access to genetic information, and for what purposes?

Or consider the following situation that has arisen in Newfoundland and Labrador in recent years. Most of the current population of Newfoundland and Labrador traces its heritage to a relatively small group of settlers who lived in stable, isolated communities. Over the generations, this has produced a strong *founder's effect*. That is, the province's gene pool has remained relatively undiluted and has a number of unique genetic characteristics. For example, Newfoundland and Labrador have a relatively high incidence of several rare genetic disorders including Bardet Biedl Syndrome, which leads to blindness.

This unique gene pool is a valuable resource for companies doing research on genetics. Such companies have collected DNA samples from Newfoundland and Labrador families and use these samples to develop genetic tests and treatments that are worth millions in the pharmaceutical

THINKING LAB

How Will We Deal with Bio-ethical Dilemmas?

Background

Gardner syndrome is a genetic disorder caused by a mutated gene on chromosome 5 that makes affected individuals more susceptible to colon cancer. It is an autosomal dominant condition in which hundreds of abnormal growths (polyps) form within the colon. Some of these polyps can become malignant, leading to cancer of the colon. Children of affected individuals have a 50% chance of carrying the disorder. Cancer can occur in adolescence or as late as in the person's seventies.

When a 38-year-old father of three sons became extremely ill, his doctor diagnosed him as having Gardner syndrome. Malignant polyps indicated that removal of the entire colon was necessary. Twenty years earlier, the man's mother had died from colon cancer. To help his sons avoid such a fate, the father requested that they be genetically tested. The tests, which the doctor indicated were 99% accurate, revealed that the eldest son had inherited the defective gene. The son graduated from college and found employment. The employer requested a physical examination for health insurance purposes, following which the son's medical history became available to the health insurance company. The employer, who would pay the health care premiums, was told by the health insurance company that the son's overall premium rate would have to be greatly increased because he was a "high risk" employee.

You Try It

In small groups, discuss and complete the following:

1. Identify the bio-ethical dilemma and write it as a question.
2. What information is required to make this decision?
3. Who will be affected by this decision?
4. What values (yours as well as others') are involved in the decision-making process?
5. Give at least three solutions to the problem that members of your group suggested.
6. Which of these solutions would you choose? Indicate the values that led you to this solution.
7. How will this solution affect the people involved in this problem?
8. Would you be willing to have the consequences of this decision applied to you? Why or why not?

market. But who owns this information? There have been complaints about companies selling DNA information to other companies without permission from the individuals who provided the samples. Some people are also angry that the companies have not shared the results of their research with the people of Newfoundland and Labrador. Many people argue that this unique DNA is a natural resource that belongs to the people of the province, and that companies should not have the right to treat it as their private property. On the other hand, if companies cannot earn a profit from their research then there is little incentive for them to invest in genetic studies. Where does the balance lie? Is knowledge about genes and DNA sequences a commodity that can be sold, or is it a public resource that belongs to all humanity?

In addition to their contributions to human health and medicine, the genetic engineering techniques discussed in this section are being applied to the study of the genomes of other living organisms. As you will see in the rest of this chapter, these techniques are finding new applications — and raising new issues — in agriculture, industry, and environmental protection.

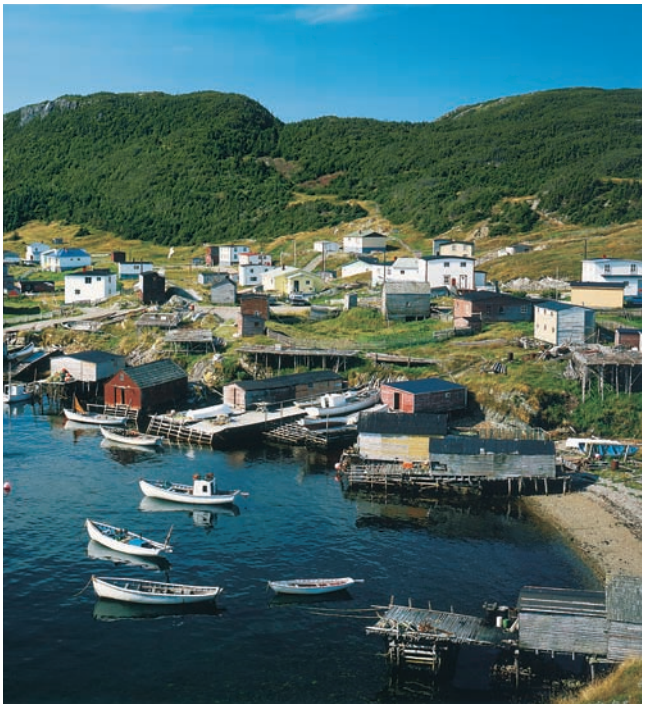
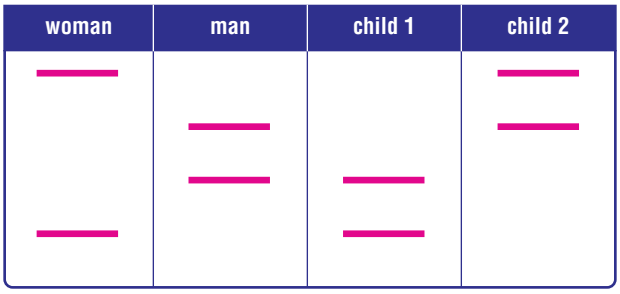


Figure 18.14 For generations, people in Newfoundland and Labrador have lived in small, isolated communities such as New Bonaventure. The result is a unique genome that is in high demand by private genetics research corporations around the world — but who owns this genetic information?

SECTION REVIEW

1. Identify three basic tools commonly used in genetic engineering.
2. Describe the contributions of the following people to the field of genetic engineering:
   (a) Sanger
   (b) Cohen and Boyer
3. Compare and contrast cloning using a bacterial vector and the polymerase chain reaction as methods of amplifying samples of DNA. When would you choose to use the bacterial cloning method?
4. Describe the steps involved in creating recombinant DNA. Indicate which enzymes are required at each step.
5. Explain why DNA fragments separate in a gel electrophoresis.
6. The DNA strand CCTAGGTCA is subjected to a chain termination reaction and the resulting fragments are run through a gel electrophoresis. Draw a diagram of the sequencing gel.
7. At a party, you tell a new acquaintance that you intend to become a molecular geneticist and study the structure of viral genomes. He says, "don't you think it would be more useful to study human DNA?" How would you respond?
8. The DNA fingerprint below shows the results of DNA analysis performed on a man, a woman, and two children. Based on this evidence, what can you conclude about the relationship among the children and each of the adults?



9. A government proposes establishing a database that contains the DNA fingerprints of every citizen. Discuss some of the potential benefits and risks to society of this initiative.
10. Discuss the advantages and disadvantages of mapping the human genome. Describe an ethical dilemma that is associated with the Human Genome Project.

18.3 The Chimera: From Legend to Lab

OUTCOMES

- **Demonstrate an understanding of genetic engineering techniques used to develop genetically modified plants and animals.**
- **Distinguish between artificial selection and genetic engineering.**
- **Describe some of the agricultural, industrial and medical applications of genetic engineering research and technology.**
- **Analyze the risks and benefits associated with the development of genetically modified foods and organisms.**

The chimera is described in Greek mythology as a fire-breathing monster with the head and shoulders of a lion, the body of a goat, and a serpent for a tail (see Figure 18.15). Today, geneticists often use the term "chimera" to describe genetically engineered organisms that contain genes from unrelated species. The name may prove very fitting. The mythical chimera combined the strengths of many different animals to produce one creature that was exceptionally powerful. But this chimera was also frightening — it breathed fire and could be ferocious. In the same way, modern genetic chimeras bring together elements of different genomes in ways that can produce important social benefits. These new chimeras and the genetic technologies that create them also pose some disturbing risks; consequently, many people consider them to be dangerous.

Figure 18.15 The chimera is a mythical beast that combines parts of a lion, goat, serpent, and dragon.

As you saw in the last section, the first chimeric organism was created in 1973 when Stanley Cohen and Herbert Boyer successfully developed a bacterial plasmid that could express an amphibian gene. The work initiated by Cohen and Boyer remains the foundation of much of the genetic engineering done today.

Inserting Animal Genes into Bacterial Cells

All mammals produce a growth hormone called **somatotropin**. When cows are treated with high levels of this hormone they grow bigger, develop larger udders, and produce more milk than they normally would. In 1990, the gene coding for this hormone in cattle (bovine somatotropin, or BST) was successfully cloned and inserted into a bacterial vector using recombinant DNA technology. Produced on a commercial scale, the resulting hormone became the first **transgenic**, or genetically engineered, product approved for agricultural use in North America.

To insert a mammalian gene into a prokaryotic cell, two basic requirements must be met. First, researchers must isolate the target mammalian gene from the genome as a whole. To do this, researchers first create recombinant plasmids using DNA from the target genome, and then use nucleic acid probes to identify those plasmids that contain the specific gene of interest (see section 18.1 for a review of nucleic acid probes).

The next challenge is to ensure that the eukaryotic gene can be correctly expressed by the bacterial cell. Recall from Chapter 17 that eukaryote DNA contains introns, while prokaryote DNA does not. This means that bacteria do not have the enzymes required to splice out introns from mRNA before it is translated into proteins.

WEB LINK

www.mcgrawhill.ca/links/atlbiology
To view animations on recombinant plasmids in bacteria, go to the web site above and click on **Electronic Learning Partner**.

The solution to this problem has been to develop artificial eukaryotic genes that do not contain introns. Figure 18.16 shows how this is done. Researchers first isolate finished mRNA from the cytoplasm of a eukaryotic cell. The mRNA is then placed in a solution with an enzyme called **reverse transcriptase**, which creates a DNA strand complementary to the mRNA strand. This DNA strand is then isolated and added to a solution containing DNA polymerase, which synthesizes another complementary DNA strand. The result is a double-stranded molecule of DNA containing only the coding portions of the eukaryotic gene. This synthetic molecule is called **copy DNA** or **cDNA**.

Another solution to both of these problems is to use eukaryotic cells as cloning vectors. Yeast cells are often used for this purpose, since they can be cultured easily. Some yeast cells also contain plasmids, so similar techniques can be used to insert recombinant DNA into the cloning vector.

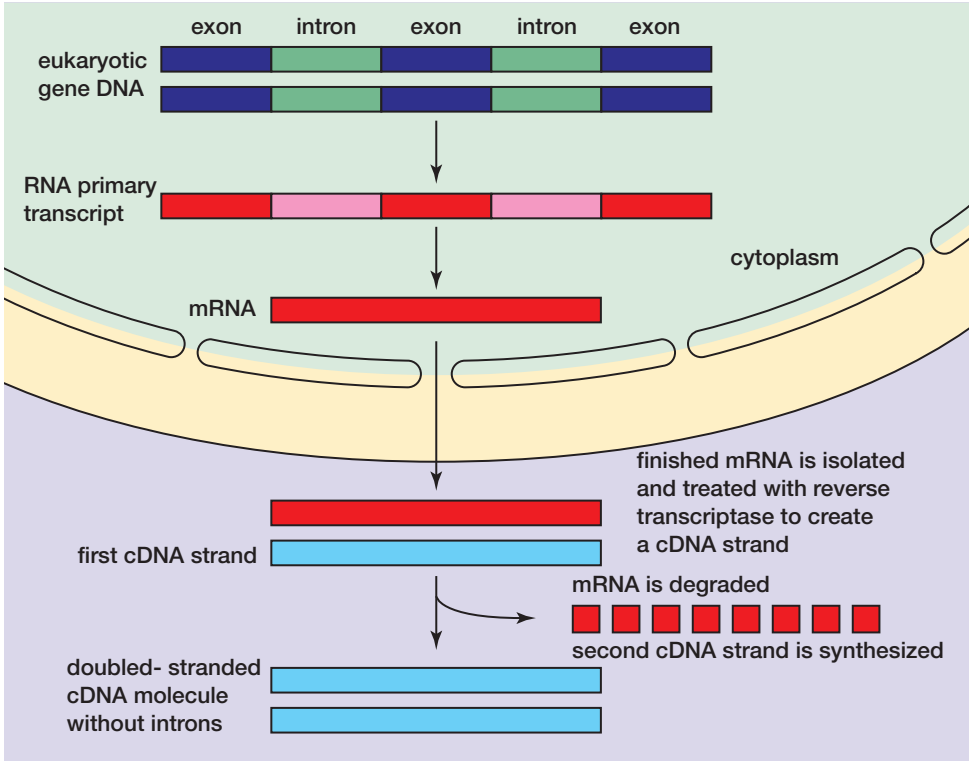
Inserting DNA into Plant or Animal Cells

In some cases, only plant or animal cells will contain all the enzymes necessary to correctly manufacture a desired protein. Such cells can be grown in cultures to serve as cloning vectors. However, because these cells are more difficult to culture, it is harder to insert foreign DNA into them.

Several methods have been developed to address this challenge. In some cases, bacterial plasmids can be used to "infect" a plant cell by inserting plasmid DNA into the cell genome. In other cases, researchers must rely on techniques that open pores in the membrane of the cell nucleus. One such method is a "DNA particle gun," developed by American researcher John Sanford. This device, pictured in Figure 18.17, can fire DNA-coated microscopic particles directly into plant cells and their nuclei. Once the fragments of DNA are in the nucleus, there is a chance that these fragments will be taken up by the host cell's chromosomes.

Putting Genetic Technologies to Use

The new strains of organisms being developed through genetic technologies are examined by government agencies to determine their benefits and risks before they are approved for commercial use. Different countries often take different approaches to these decisions. For example, after studies found no evidence that milk from bovine somatotropin-treated cows posed any risk to human consumers, the commercial use of genetically



A When a eukaryotic gene is transcribed, the initial transcript contains both exons and introns. The introns are spliced out from the pre-mRNA before the transcript leaves the cell nucleus.

B The finished mRNA can be used as a template to synthesize a new strand of DNA. Reverse transcriptase, an enzyme found in some viruses, is used to create a single cDNA strand from an RNA template.

C The single strand of DNA then becomes a template for the synthesis of its own complementary strand. The result is a double-stranded cDNA molecule containing only the coding sequences of the gene.

Figure 18.16 A molecule of cDNA contains no introns. Therefore, this molecule can be correctly expressed by a bacterial host.

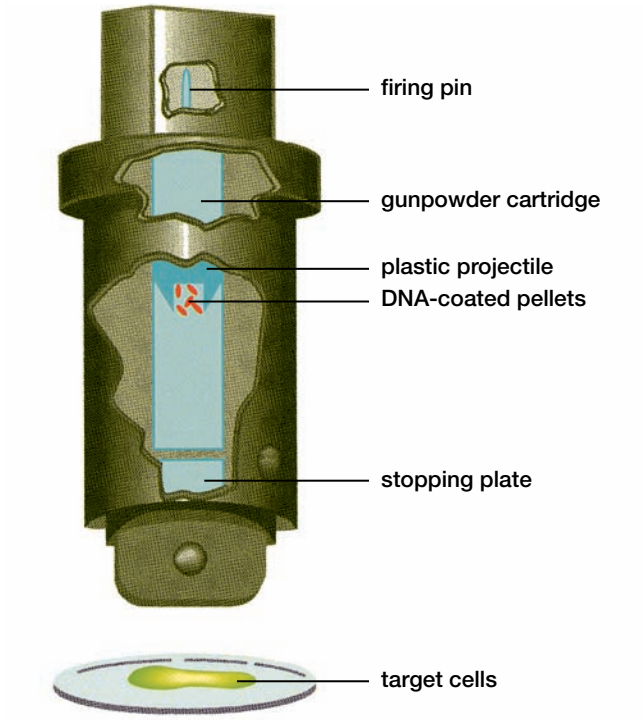


Figure 18.17 Sanford's DNA particle gun fires microscopic metal pellets coated with foreign DNA. The pellets are attached to a plastic projectile that is shot through the gun chamber. When the projectile hits the stopping plate, the pellets are torn off and carried into the plant cells. The foreign DNA may then be incorporated into the plant DNA.

engineered BST was approved in the United States in 1994. Canada, in contrast, decided in 2000 not to approve the drug for use. This decision reflected concern about the effects such hormone treatments might have on the health of cows. As this example illustrates, the potential benefits of the application of genetic engineering technologies and transgenic organisms must always be considered against the risks they may present to human health, the well-being of plant or animal stocks, and the environment. The need for standards and criteria in this area will only increase as transgenic options become increasingly available in various fields.

In the next few pages you will see how genetic engineering technologies are being put to use in a variety of fields including agriculture, medicine, and environmental protection.

Herbicide-resistant Corn

Crop plants containing recombinant DNA now account for over half of the production of corn and Canola™ in North America. Over 50 types of genetically modified crop plants have been approved for use in Canada. One example is herbicide-resistant corn. Geneticists working with a private corporation isolated and cloned a bacterial gene that provides resistance to the chemical glyphosate, an active ingredient in certain herbicides. They coated fine particles of gold with DNA fragments containing the bacterial gene and then fired the particles at a suspension of corn germ cells. Some of the cells took up the DNA. After screening for the right recombinants, the geneticists were able to grow corn that expressed the bacterial gene.

When these corn plants are grown as crops, as shown in Figure 18.18, farmers can apply herbicides to control weeds without damaging the corn. Fewer weeds means increased corn production. Corn containing this bacterial gene was approved for use in Canada in 2001, after the Canadian government concluded that the use of the transgenic corn did not present a risk to human health.

Figure 18.18 Genetically engineered plants can result in increased crop productivity.

Human Insulin

In 1982, a human insulin synthesized by transgenic bacteria was approved for medical use in the United States. This was the first example of a genetically engineered pharmaceutical product. Until that date, insulin (which is important for the treatment of diabetes) was extracted from cows and pigs that had been brought to market. Although the insulin of these livestock animals is very similar to human insulin, many patients suffered from allergic reactions. Consequently, an American pharmaceutical company developed a process for inserting the human gene for insulin into bacteria. The resulting transgenic bacteria provided a ready source of high volumes of human insulin. This

ready source, in turn, lowered the cost of treatment and also reduced the number of side effects. Since that time, bacteria have been used as vectors for producing other pharmaceutical products.

Bioremediation: PCB-eating Bacteria

Among the by-products of a number of industrial processes, particularly those in the electronics industry, are polychlorinated biphenyls or PCBs. These highly toxic, environmentally persistent compounds can build up in soil and accumulate in the food chain, thereby presenting a risk to animal and human populations around the world. Cleaning up PCB-contaminated sites is difficult and costly. In response to this problem, a number of biotechnology companies have been experimenting with the development of recombinant bacteria that can degrade PCBs into harmless compounds. One technique is to locate bacteria that naturally contain genes coding for enzymes that break down PCBs and then transfer these genes into micro-organisms that can survive and reproduce well in soil. Multiple copies of these genes can be inserted into the host genome in order to increase the rate of PCB-degrading enzyme production.

The use of living cells to perform environmental remediation tasks has become known as **bioremediation**. Other examples of bioremediation include bacteria that have been designed to clean

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Using Genes to Clean Up the Environment

How would you clean up millions of litres of oil spilled onto a gravel beach, or locate explosives buried in a minefield? Such difficult, dangerous, and costly tasks can now be made easier and safer with the help of genetically modified organisms.

Oil and explosives such as trinitrotoluene (TNT) are only two of the unlikely materials that can be used as raw materials by one organism or another. Bacteria in particular are able to use a huge variety of chemicals as a source of energy. Just as some insects can feed on leaves that are toxic to other insects, some bacteria can thrive on chemicals that would poison most organisms. For example, there are bacteria that can absorb phenol, cyanide, sulfur, and polychlorinated biphenyls (PCBs). These bacteria use enzymes to break down the chemical bonds in these molecules, much as we use enzymes in our stomachs to break down complex carbohydrates in our food into simpler glucose molecules.

Bacteria have lived on Earth for billions of years, and some are adapted to survive in Earth's most extreme natural environments — places that are acidic, radioactive, or that contain heavy metals. It is not so surprising, then, to find that certain of their species live in polluted areas around landfill sites, chemical factories, oil refineries, and mines. After collecting and culturing these organisms, scientists can identify their pollutant-breaking enzymes and the genes that encode them. They can then isolate the best clones and attempt either to improve the efficiency of their enzymes or to transfer their genes into other organisms in order to use them in bioremediation efforts.

Plants That Fight Soil Pollution

You may have heard that spinach is good for you because it contains iron. In fact, many plants contain metals, which they selectively absorb from the soil through their roots. For example, various members of the cabbage family (*Cruciferae*) absorb a long list of metals ranging from arsenic to mercury and zinc.

The key to plants' abilities to absorb metals is found in a group of proteins called metallothioneins. These proteins contain large numbers of atoms that readily bond onto metals. Depending on its particular structure, a metallothionein molecule may selectively bond to one particular metal. This bonding enables the species which express the protein to accumulate that metal in concentrations 30 to 1000 times greater than its concentration in the surrounding soil. In some examples, the plant may have a metal content of as much as 30 percent of the total dry mass of the plant's roots.

To be practical as a method of removing toxic metals from soil, plants must not only absorb these metals but also grow quickly in a range of different conditions and be easy to harvest. (If the plants are not removed, the metals will simply return to the soil when the plants die and decompose.) Through genetic engineering, scientists can add genes coding for metallothionein production to any plants that have these other desired properties. The result is a cheap, non-polluting way to remove or stabilize toxic metals that might otherwise be leached out of the soil into watercourses.

Can Transgenic Organisms Locate Toxins and Land Mines?

Genetically modified organisms called biosensors may soon play a role in the detection and monitoring of dangerous materials that cannot be discovered easily,

up oil spills, filter air from factory smokestacks, or remove heavy metals from water.

Improved Nutrition

Millions of people worldwide suffer from malnutrition because they lack access to both sufficient food and balanced diets. Malnutrition can, in turn, lead to disease. Inadequate vitamin A, for example, is associated with vision problems, a weakened immune system, fatigue, dry skin, and joint pain. These symptoms affect hundreds of thousands of people in many Asian countries, where the diet consists chiefly of rice. To the potential benefit of these people, a Swiss company has recently developed a genetically modified strain of rice known as golden rice. This rice has been genetically engineered to produce beta-carotene, a vitamin A precursor. It also contains higher amounts of iron than regular rice. Golden rice is now being offered as a staple part of the food aid delivered to many developing countries, in the hope that its higher nutrient levels will help reduce the incidence of disorders linked to vitamin A and iron deficiencies.

Weighing the Risks

Products such as golden rice, shown in Figure 18.19, have been marketed as demonstrating the benefits of genetic engineering. However, many organizations

safely, or economically by other means. For example, the standard procedure for determining if harmful toxins are leaching into the ground or water supplies is to periodically take and analyze soil and water samples. Similarly, the standard procedure for locating buried, plastic-housed land mines is for very brave individuals to search the area equipped with what is, sometimes, nothing more sophisticated than a long stick. The former procedure is an expensive, time-consuming, and labour-intensive task. The latter is a high-stakes gamble that can quickly maim its practitioner or curtail his or her life. How much more convenient would it be to have organisms living on dangerous materials sites that would unwittingly signal the location of pollutants or land mines?

Land mines buried by the millions kill or maim hundreds of civilians each year in countries where wars once raged. Plant biosensors may someday reveal their locations, allowing them to be safely detonated or removed.

How can you get an organism to send such a signal? One method is to add a gene for light production. Bioluminescence is the light produced by fireflies, glow-worms, some fungi, and many marine organisms. For example, a protein from a species of Pacific jellyfish (*Aequorea victoria*) fluoresces green when excited by blue or UV light. Genetic engineers can splice the gene that codes for this protein into a bacterium, linking it to a bacterial gene that responds to the presence of a certain toxin. When the genetically modified bacteria grow in an area containing this toxin, they will glow.

The glow of these bacteria may help identify toxins on an exposed surface, but what about those buried underground? For these applications, plants have the decided advantage of being larger and more easily tracked. One possible application of plant biosensors is to detect buried land mines containing TNT or other explosives. Historical areas of conflict such as Afghanistan, Angola, Cambodia, and the Falkland Islands are littered with millions of land mines, essentially making it impossible to farm large areas of otherwise arable land. Many land-mine casings are also plastic and thus cannot be located by metal detectors. However, some bacteria have gene promoters that are activated by TNT. These promoters can be linked to the green fluorescing genes described above and added to small, rapidly growing plants, the seeds of which could then be spread from the air over land mine sites. In time, the plant roots would spread out in the soil, absorb traces of explosives, and transport them to the leaves, which would fluoresce. Scientists could then map the location of buried explosives and decide on the best means of deactivating them.

Follow-up

Based on what you know or can find out about the characteristics of different organisms, suggest some possible combinations of genes that could be used for bioremediation or biomonitoring (using organisms to detect pollution).

and consumer groups argue that these benefits are outweighed by a variety of risks. In the case of golden rice, recent studies have shown that the amount of additional vitamin A offered by a regular daily serving may be as little as eight percent of an individual's daily requirement. Thus, these servings may not contribute much toward the goal of reducing the incidence of vitamin deficiency in those countries receiving food aid. The work undertaken to develop the rice has also consumed many millions of dollars that could have been spent on other, perhaps more meaningful forms of aid to developing countries. Given these questionable results, will the investment in genetically engineered foods prove worthwhile? Answers to questions such as this are part of the challenge involved in determining the advantages and disadvantages of genetic engineering technologies.

Figure 18.19 Genetically engineered foods such as golden rice can provide extra nutrition to people in countries receiving food aid, but at what long-term cost?

In Canada, proposals for the use of transgenic products are reviewed by government agencies such as Health Canada and the Canadian Food Inspection Agency. In deciding whether or not to approve such products for use in Canada, these agencies consider a number of criteria. These criteria include the potential social, economic, and environmental costs and benefits; the process by which the product was developed (including the source of genetic material); the biological characteristics of the transgenic product as compared with the natural variety; and the potential health effects, including the possibility that the product might contain toxins or allergens.

WEB LINK

www.mcgrawhill.ca/links/atlbiology

Each year, the Canadian government receives many applications for permission to market transgenic organisms. Use the Internet to research one application that was submitted to the government this year. Go to the web site above, and click on **Web Links**. How was this product developed? What issues does the Canadian government consider in deciding whether or not to approve a transgenic product? Prepare a brief report describing the product, its potential benefits and risks, and how you think the Canadian government should respond to the application.

Despite this review process, many organizations (including consumer advocacy groups and environmental groups) have opposed the use of a number of transgenic organisms. Among their concerns have been the following potential risks.

- **Environmental threats** The use of herbicide-resistant crops may encourage farmers to use higher levels of herbicides. This practice can lead to greater leaching of herbicides into water supplies and neighbouring ecosystems. Additionally, some recent studies suggest that genes can spread accidentally from genetically engineered organisms to wild organisms, thus posing a threat to biodiversity. There is also a risk that herbicide-resistant crop plants could crossbreed with related natural plants, thereby producing "superweeds" that would be very difficult to control. In the same way, the development of pest-resistant plants could eventually lead to the development of "superbugs" that would be immune to certain pesticides.
- **Health effects** Many consumer groups argue that simply not enough is known about the long-term effects of transgenic products. Some believe that the consumption of transgenic products may be having effects that do not show up in the studies conducted by researchers to date. Others point to the problem of ensuring that the use of genetically modified crop plants complies with government regulations. In the fall of 2000, for example, one biotechnology company was forced to recall stocks of a pesticide-resistant corn that had been approved only for use as animal feed,

but was found in human food products, such as taco shells. This incident prompted many consumer groups to argue for tighter controls on the approval of transgenic agricultural products.

- **Social and economic issues** Advocates of genetically modified crops argue that these crops will help to alleviate world hunger. Their opponents argue that world hunger is the result of unequal food distribution, not food shortages, and that harvests of transgenic crops will not address this issue. Also, since transgenic organisms are primarily developed by private companies, many people fear the control of world food supplies could become concentrated in corporate hands. Other groups argue that the cultivation of transgenic crops favours large farms over small-scale or family farms, and that it increases farmer dependence on the corporations holding the patents on the organisms.

In addition to these issues, the treatment of living organisms as commodities to be manipulated, patented, and sold raises questions about how humans view their role in the world and their relationship to their environment. The potential benefits of genetic engineering must constantly be judged against a background of numerous social concerns, economic concerns, potential health risks, spiritual beliefs, and potential environmental risks.

Transforming Animal DNA

The quagga (*Equus quagga quagga*) shown in Figure 18.19 was a horse-like creature that lived in southern Africa until it was hunted to extinction in the late 1800s. More than 100 years after the last known quagga died, geneticists isolated quagga DNA from dried blood samples found in preserved quagga skins. Using DNA fingerprinting and sequencing techniques, they discovered that the quagga was a subspecies of the African zebra.

Today, researchers hope to recreate a quagga-like animal through a project of **artificial selection**. That is, they are breeding zebras that carry genetic traits similar to those of the extinct quagga. Over a period of generations, these traits may be enhanced. As you saw in section 16.1, similar artificial selection practices have been undertaken by humans since the earliest days of civilization to produce breeds of animals and plants showing desired characteristics.

But why are researchers on the quagga project relying on selective breeding rather than turning to genetic engineering techniques? Why do they not simply insert quagga DNA into a zebra genome, in the same way that DNA from one plant can be transferred to another?

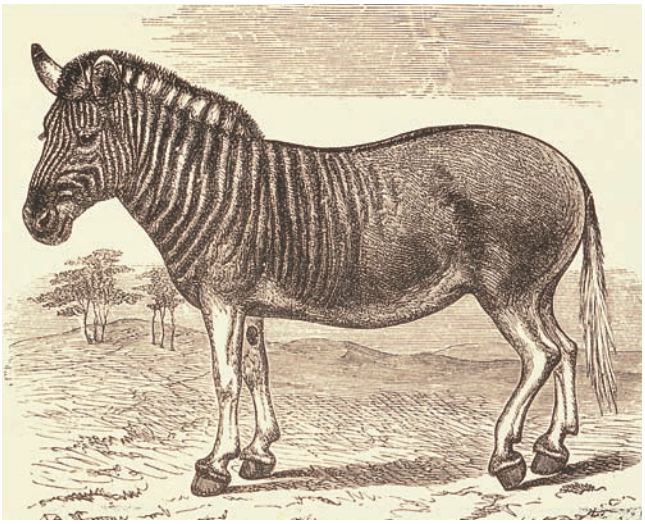


Figure 18.20 Can genetic engineering help to bring back extinct species such as this quagga?

The researchers' choice of procedure hints at some of the difficulties involved in manipulating the genomes of animals. It is much more difficult to insert foreign DNA into an animal cell than into a plant cell. One of the main reasons for this is the dissimilar process of differentiation that takes place in plant and animal cells. **Differentiation** is the process by which certain portions of a genome are activated or silenced to enable a cell to take on the specific structure and function of a given tissue. Differentiation is not permanent in most plant cells. This means, for example, that root cells taken from a fully grown plant can be cultured to produce an entirely new plant. In most animals, in contrast, once a cell has differentiated into a specialized tissue, it usually will be unable to give rise to other types of cells. As this cell differentiates, some portions of its DNA become permanently activated or repressed, making it very difficult to insert foreign DNA into the cell in a way that will allow that DNA to be expressed. In the following pages, you will explore two different fields of research that involve inserting and expressing foreign DNA in animal cells.

Cloning Animals

Organisms that are genetically identical are said to be **clones** of one another. A group of plants that have arisen through asexual reproduction from a single parent are clones. Identical twins, which form when a single zygote develops into two fetuses, are clones that arise naturally in animal populations.

In recent years, researchers have developed laboratory techniques for cloning animals.

The first experiments in this regard date back to the 1950s. At that time, American biologists Robert Briggs and Thomas King transplanted nuclei from frog embryos and tadpoles into frog egg cells whose nuclei had been removed. When Briggs and King took the transplanted nuclei from the cells of very early embryos, they found that many of the eggs developed into tadpoles. When they took the nuclei from the cells of tadpoles, however, they discovered that very few of the eggs developed. Further, even when the eggs did develop into apparently normal tadpoles, the tadpoles never developed into adult frogs. These results gave support to the idea that differentiated cells could not be used to create clones. Many researchers concluded that the process of differentiation in animal cells meant that animal cloning from adult tissue would always be impossible.

Just some 40 years later, however, researchers began achieving that seemingly impossible goal. In the early 1990s, for example, mice were cloned by using the nuclei of cells taken from mouse embryos. More recently, an even more remarkable achievement signalled the discovery that differentiation in adult animal cells was not always irreversible.

In a country known for its extensive sheep herds, why would the birth of a lamb be headline news? If the country was Scotland and the year 1997, it would be because the lamb was Dolly, the first mammal to apparently be successfully cloned using cells taken from an adult donor. Ian Wilmut and his colleagues produced Dolly (shown in Figure 18.21) using genetic information taken from the udder cells of an adult sheep.

In order for these differentiated cells to be cultured to produce a viable embryo, the process of cellular differentiation had to be reversed. Figure 18.21 on the following page illustrates the main steps by which this was accomplished. First, Wilmut collected unfertilized egg cells from a donor sheep and removed the nuclei from these cells. Then, from a second donor animal, he removed a sample of udder cells. The udder cells were cultured in a special medium that stopped the cell cycle during the G phase. (For a review of the cell cycle, see Chapter 14.) The nuclei from these cells were then transplanted into the egg cells. When the resulting cells were cultured, a few began to divide. These early embryos were then implanted into the uterus of a third sheep that acted as a surrogate mother. One of these embryos developed into a lamb. After the birth of the lamb, now named Dolly, DNA tests confirmed that this animal was genetically identical to the sheep from which the udder cells were taken.

Since the birth of Dolly, other teams have cloned a number of other animals using cells from adult donors. As scientists study how these cloned animals develop, however, evidence is mounting that a number of problems may be associated with animal cloning. Dolly, for example, has shown signs of premature aging. Researchers working with other cloned animals have reported problems associated with gene expression.

Figure 18.21 Dolly, the first mammal cloned using cells from an adult donor, was born in Scotland in 1997.

Human Cloning

In late 2001, a team of scientists at an American research facility announced the first success at cloning human cells. The research team, led by Jose Cibelli, Robert Lanza, and Michael West, used two different techniques to clone human cells. First, using the cloning process developed by Wilmut to produce Dolly, the team obtained cloned human embryonic cells that survived long enough to divide several times. In a separate procedure, they induced human egg cells to divide, and were thus successful in producing a multicellular human blastula.

Researchers involved in human cloning distinguish between therapeutic cloning and reproductive cloning. **Therapeutic cloning** is the culturing of human cells for use in treating medical disorders. **Reproductive cloning** is the development of a cloned human embryo for the purpose of

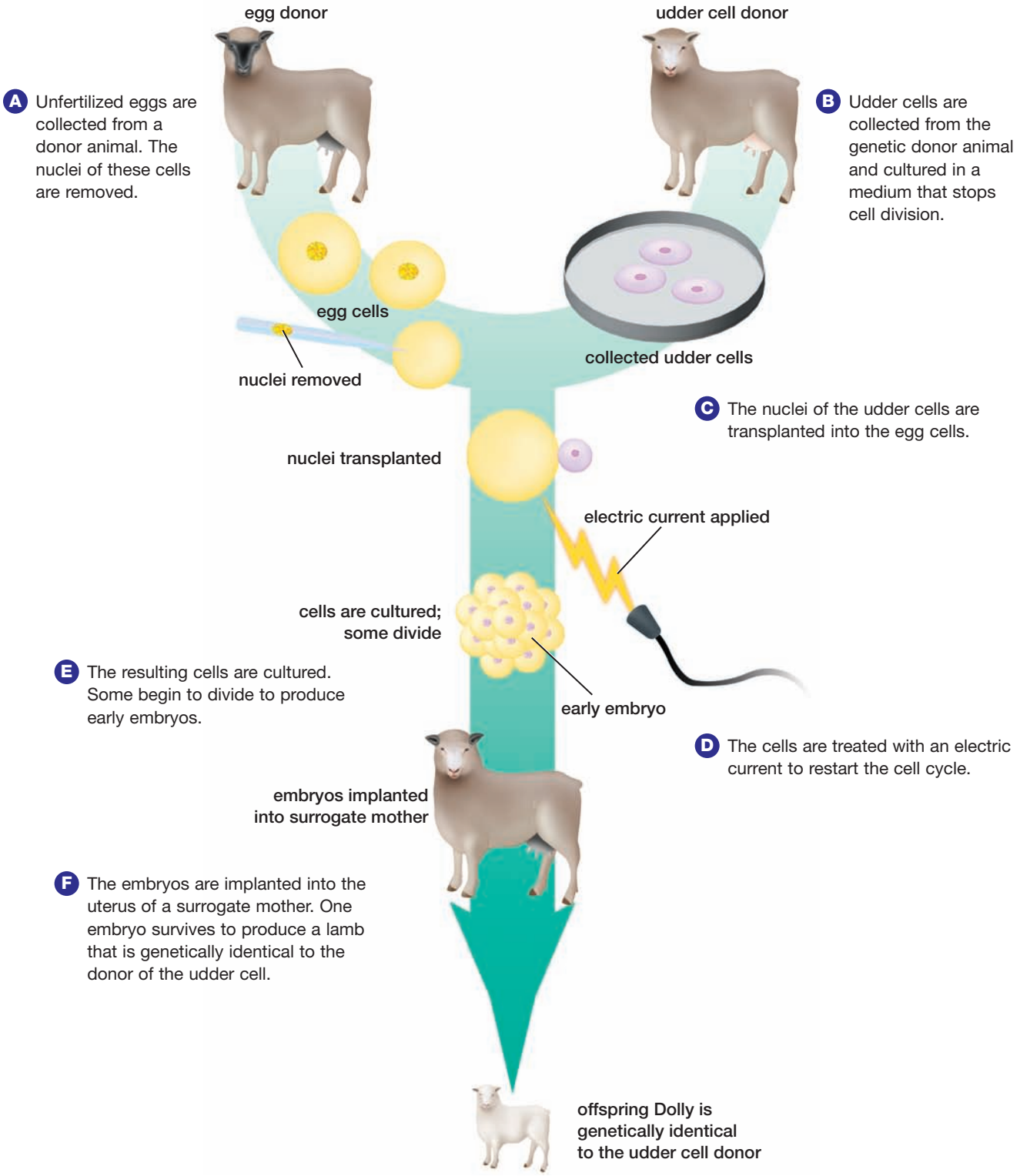


Figure 18.22 In order to produce a clone from an adult animal, geneticists must first find a way to reverse the process of cellular differentiation. In the case of Dolly, researchers stopped the cell cycle in the udder cells before inserting their nuclei into the egg cells. This procedure allowed the DNA in the differentiated udder cell nuclei to regain their potential to generate other types of cells.

creating a cloned human being. In either case, the potential benefits of these processes must be weighed against significant legal, moral, and ethical issues. Proponents of therapeutic cloning argue that this field holds the promise of eventually eliminating all human disease. On the other hand, all means of cloning animals and humans known to date involve the artificial creation and deliberate destruction of hundreds of embryos. These cloning technologies have the potential to change society's definitions of life and individuality, and will continue to be hotly debated in the years ahead.

Transgenic Animals

In recent years, populations of wild fish have dropped dramatically all over coastal North America. This has led to concerns about the sustainability of many commercial fisheries, and to a growing interest in fish farming as an alternative source of seafood products. Many aquaculture operations farm Atlantic salmon, as these fish are relatively easy to raise in confined spaces and are in high demand from consumers. In Atlantic Canada, however, fish farmers face a significant challenge. In the winter, ocean temperatures regularly drop below freezing, and Atlantic salmon cannot withstand such cold. An aquaculture research corporation based in Prince Edward Island was the first to respond to this challenge by using genetic engineering to create new varieties of salmon. The company has developed two different transgenic salmon varieties. One produces its own antifreeze, while the other grows up to ten times faster than normal fish.

Figure 18.23 Supporters of the use of transgenic salmon in fish farms say that the genetically modified fish could help take pressure off wild stocks. Opponents worry that escaped transgenic fish could spell ecological disaster.

To develop the cold-tolerant fish, researchers first isolated an "antifreeze" gene from a species of fish that survives in below-freezing ocean temperatures. The gene codes for a protein that protects the fish tissues from the cold. The team then used microscopic needles to inject strands of DNA carrying the gene into the nuclei of fertilized Atlantic salmon eggs. In some cases, the gene was incorporated into the fish genome and correctly expressed. From these fish the research team has developed a breeding population that carries the antifreeze gene.

The development of the fast-growing fish used a similar technique but required an extra step. In normal salmon, growth rate is controlled by the central nervous system through a series of feedback loops. This means that expression of the gene coding for growth hormone is governed by signals from the brain stem. Once the research team had isolated a gene for growth hormone from a fast-growing salmon species, they had to modify the mechanisms that control gene expression. By altering the nucleotide sequences that play a part in the regulation of gene expression, the researchers developed a form of the gene that is not subject to normal controls by the CNS. As a result, growth hormone is produced at much higher levels. The transgenic fish grow three to six times faster than the wild type. The advantage, for fish farmers, is that the fish could reach market size one year earlier than normal fish.

This research has sparked a storm of controversy. Supporters of the use of transgenic salmon in fish farms argue that the technology is nothing more than accelerated artificial selection, since it simply transfers a gene from one fish to another (and in the case of the growth hormone, from one breed of salmon to another). They maintain the technology brings no added risk to consumers. They also point to the potential for restoring wild fish stocks and for addressing world hunger that could be realized through fish farming.

On the other hand, the use of transgenic fish raises a number of concerns about consumer safety and ecological impacts. The microinjection technique means that the new DNA could be integrated in different places throughout the host fish chromosome, with unknown effects. In addition, the mechanisms involved in gene expression are very complex and are only beginning to be understood, which means that the manipulation of gene control systems could have unforeseen impacts on the transgenic fish, and possibly on

Medicinal Pigs and Other Clones

Every year, the lives of thousands of Canadians are saved by organ transplants. Thousands more, however, wait months or years before a suitable organ is found. As a result, each year hundreds of people on organ transplant waiting lists die before they can be treated. Even among those who receive transplanted organs, hundreds die due to organ rejection. Most of those who survive must take drugs to suppress their immune systems. Although this treatment enables their bodies to tolerate the transplanted organs, it also leaves them vulnerable to infections. Because of genetic engineering, there are now several potential avenues through which these problems may eventually be overcome.

Organs from Pigs

Pigs grow quickly and are easy to raise, and their organs are similar in size and structure to human organs. As you learned in Chapter 00, these facts have led researchers to consider pigs as a potential ready supply of organs for transplantation into human patients. So far, however, cross-species transplantation (also known as xenotransplantation) has had very limited success, because an antigen produced by animal cells usually triggers a serious immune response in humans that leads to organ rejection. Genetic researchers are exploring two different ways to avoid this result.

Genetic engineering can increase the chance of successful organ transplants from pigs to humans, but the prospect remains controversial.

In January 2002, researchers announced they had successfully developed and cloned "knock-out" piglets. These piglets were genetically modified to "knock out," or deactivate, the antigen gene in their cells. To create a knock-out pig, researchers isolate the antigen gene from the pig's genome and deactivate it by inserting a mutation into its DNA sequence. Next, they insert the modified DNA into a vector and culture the vector with the pig's cells. In some cases, the vector will insert the deactivated gene into the cultured cells. A screening process then identifies the knock-out cells, some of which are cultured into viable embryos.

Another possible way to avoid cross-species rejection is to insert a human gene into the pig genome to make the pig cells produce a human antigen instead of a pig antigen. The resulting transgenic pig embryos, along with the knock-out pig embryos described above, could then be cloned to produce a stock of pigs whose organs could be harvested.

The prospect of using pigs as a source of organs for transplantation has led to considerable debate. Many scientists are concerned about the risk of transferring diseases from pigs to humans. Others maintain it is unethical to create new kinds of animals purely for the purpose of harvesting their organs. Concerns such as these have led some researchers to look to human therapeutic cloning instead.

Become Your Own Organ Donor

Imagine you need a new heart. Your surgeon asks you for a DNA sample. From this sample a heart is grown that is genetically identical to your own, thereby eliminating both the wait for an appropriate donor and the risk of organ rejection. How could this be accomplished? The procedure would involve inserting your DNA into an enucleated cell to create an embryo — your own identical twin. This embryo would be cultured for about two weeks, then destroyed in order to collect the stem cells needed to grow your new heart.

In spite of the benefits, the practice of culturing human embryos with the object of destroying them is viewed by many as immoral and unethical. It also violates the tenets of many religions. Opponents of human therapeutic cloning also point to potential risks. Cloned animals have a higher than average rate of mortality and deformity, while those cloned using DNA from adult donors appear to age prematurely. Dolly the sheep, for example, became arthritic at the very early age of five. Such evidence suggests that organs produced through human therapeutic cloning may not function properly.

Successful therapeutic cloning for medical purposes may be years away. In the interim, governments around the world will be faced with the challenge of developing laws to guide genetic research and its applications in this rapidly changing field.

Follow-up

Different countries have enacted very different laws relating to cross-species transplantation and therapeutic cloning for transplantation purposes. Research the laws in Canada and one other country to see how they differ.

consumers. Many groups have also expressed concerns about what could happen if a population of transgenic fish escaped into the wild. Could the faster-growing fish outcompete and endanger wild stocks? What happens when transgenic fish and wild fish interbreed? How might a population of transgenic fish disrupt the fine balances of the marine ecosystem?

These questions become even more pressing as gene technologies allow researchers to produce transgenic animals that incorporate genes from very different species. In every case, the potential benefits must be weighed against potential immediate and long-term risks.

The speed at which new genetic engineering technologies are developed and applied sometimes makes it seem as though scientists have a very good understanding of how genes work. In reality, as researchers learn more about the structure and function of genetic information in living organisms, the complexity of the interactions among DNA, proteins, and the environment is only starting to become apparent. More than a century after the discovery of DNA, exploration into the field of molecular genetics is only just beginning.

SECTION REVIEW

1. List the key steps involved in the development of bacteria that produce bovine somatotropin. In terms of the application of genetic technologies, what is the significance of the hormone produced by these bacteria?

2. Describe how cDNA is used to overcome one of the challenges involved in expressing eukaryotic genes in bacterial vectors.

3. Under what conditions would you use a eukaryotic vector rather than a prokaryotic vector to express a mammalian gene?

4. Using a computer, develop a table or flowchart that compares the main features of two different processes that can be used to insert bacterial genes into a plant cell.

5. Explain how the process of cellular differentiation affects methods of developing transgenic animals.

6. Decide whether Dolly could be described as the identical twin of the genetic donor sheep and give your reasons. What implications might your answer have on society if human cloning is someday allowed?

7. In the cloning process that was used to create Dolly, what was the purpose of treating the egg cells containing the transplanted udder nuclei with an electric current?

8. Researchers in Prince Edward Island have created a transgenic fish that withstands below-freezing ocean temperatures. Could a similar project be used to create a human being who can withstand below-freezing temperatures? Explain.

9. With a partner or in a small group, brainstorm the benefits and risks of human cloning. Then write a report describing your own thoughts on whether human cloning is a good idea, and what restrictions should apply.

10. A dog breeder wants to develop a breed of dog whose fur colour changes with the season. He establishes a partnership with a furrier who breeds stoats (an animal whose coat is white in the winter and brown in the summer).

(a) What steps would the two researchers have to take to develop a breed of transgenic dog?

(b) What are the potential benefits and risks associated with this project?

11. Some companies that produce transgenic crop plants forbid farmers from saving the seeds from their crops in order to replant the transgenic organisms. Instead, the farmers must purchase new seeds each year. Write a brief report explaining some of the advantages and disadvantages of this policy. If you were a researcher working for one of these companies, what policy would you recommend?

Chapter Summary

Briefly explain each of the following points.

- Prenatal diagnoses can be used to determine some genetic conditions and disorders. (18.1)
- Certain inherited disorders can be treated and their effects lessened to improve the quality of life of affected individuals. (18.1)
- Gene therapy is being tested as one way to reverse some of the effects of genetic disorders. (18.1)
- The prediction and treatment of genetic disorders raises social and ethical issues. (18.1)
- A restriction endonuclease breaks a DNA strand in a predictable fashion. (18.2)
- Gel electrophoresis can be used to generate a DNA fingerprint. (18.2)
- Expressing a plant gene in a bacterial host presents a different set of challenges than those posed by expressing a bacterial gene in a plant cell host. (18.3)
- It is easier to clone a plant than an animal. (18.3)
- Genetically engineered organisms could have both positive and negative effects on society and the environment. (18.3)

Language of Biology

Write a sentence including each of the following words or terms. Use any six terms in a concept map to show your understanding of how they are related.

- amniocentesis
- amniotic sac
- amniotic fluid
- chorionic villi sampling (CVS)
- fetoscopy
- endoscope
- genetic marker
- cleft palate
- gene therapy
- restriction endonucleases
- restriction site
- restriction fragments
- sticky ends
- recombinant DNA
- DNA amplification
- cloning vector
- polymerase chain reaction (PCR)
- gel electrophoresis
- DNA fingerprint
- chain termination sequencing
- Human Genome Project (HGP)
- somatotropin
- transgenic
- reverse transcriptase
- copy DNA (cDNA)
- bioremediation
- artificial selection
- differentiation
- therapeutic cloning
- reproductive cloning

UNDERSTANDING CONCEPTS

1. Which diagnostic tool is used to detect nondisjunctions?
2. When a blood sample is collected from a fetus, what different tests might be performed to test for the presence of genetic disorders? What information can these tests provide?
3. What is the advantage of using chorionic sampling over amniocentesis for prenatal diagnosis of chromosomal abnormalities?
4. What is a genetic marker? In humans, could brown eyes be a genetic marker? Explain.
5. Explain how a nucleic acid probe can be used to find a molecular genetic marker.
6. Explain the difference between somatic gene therapy and germ line therapy. Which one is currently the subject of medical trials?
7. What factors make an individual's risk of developing Alzheimer's disease difficult to predict?
8. Describe the contributions of the following researchers to the field of genetic engineering:
   (a) Frederick Sanger
   (b) Stanley Cohen and Herbert Boyer
   (c) John Sanford
   (d) Kary Mullis
9. What key features make a restriction endonuclease a useful tool in genetic engineering?
10. Describe how a bacterial vector can be used to amplify a sample of DNA. When would you choose to use this method rather than the polymerase chain reaction?
11. Explain why DNA fragments migrate in a gel electrophoresis. Which fragments migrate furthest?
12. Will cells from your liver and your brain have the same DNA fingerprint? Explain.
13. Describe two important findings of the Human Genome Project.
14. An electric current is sometimes used as part of the process to insert new DNA into a eukaryotic genome. Explain what the current does and why this is necessary.

15. Explain why the dissimilar processes of cellular differentiation in plant and animal cells make it more difficult to clone animals than plants.

16. Describe the process used to develop a recombinant bacterial cell. Give three examples of how these bacteria can provide medical, environmental or economic benefits.

17. What is copy DNA (cDNA)? How is it made, and what purpose does it serve?

18. Three different adult sheep were involved in the cloning process that led to the birth of the lamb Dolly. What were their roles? Which one was Dolly's clone?

19. What role can viruses play in gene therapy? Which characteristics of viruses make them good candidates for this role?

20. Compare and contrast artificial selection and genetic engineering as ways of developing animals that have desired characteristics.

21. Distinguish between therapeutic cloning and reproductive cloning.

22. Outline the steps involved in the work by researchers in Prince Edward Island to create a fish that grows faster than normal.

INQUIRY

23. Imagine you are a genetics counselor working with two healthy couples. Couple A has a child who has cystic fibrosis. Cystic fibrosis is caused by a recessive allele carried on Chromosome 7. Couple B has a child with Down syndrome.

(a) What is the probability that Couple A will have a second child with cystic fibrosis? What procedures would you recommend to the couple?

(b) What is the probability that Couple B will have a second child with Down syndrome? What procedures would you recommend to this couple?

(c) In assessing the probabilities, would you consider the age of the couples? Explain.

24. You are given two plasmids, one from each of two bacterial species. One contains gene A and the other contains gene B. You wish to create a single plasmid containing both genes.

(a) Draw a diagram to illustrate the steps you would take to produce this recombinant plasmid.

(b) What other plasmids will result from this procedure?

25. You are studying a plant cell that produces a protein with the amino acid sequence Met-Phe-Pro-Arg-Trp. Design a procedure that would enable you to locate the gene for this protein within the genome of the plant cell.

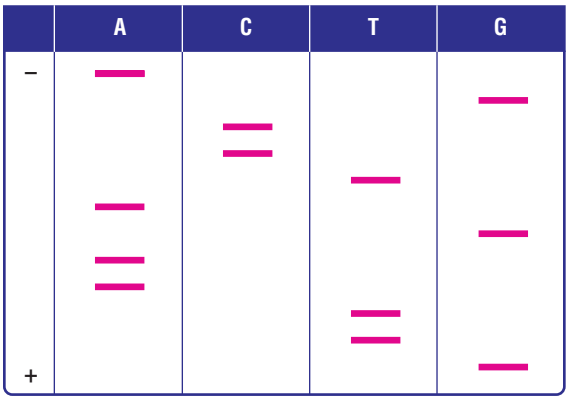
26. A molecular biologist involved in gene therapy research develops a viral vector by inserting a human gene into a viral genome. In clinical trials the vector successfully binds to target cells, but later analysis shows that none of the target cells have incorporated the new human gene into their DNA.

(a) What might have gone wrong?

(b) How could the biologist confirm the problem?

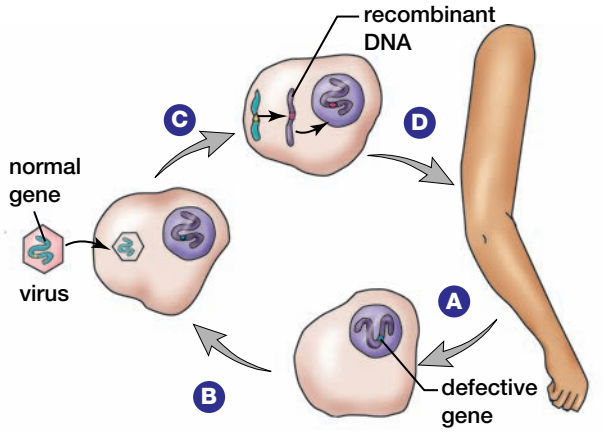
(c) What steps could the biologist take to correct the problem?

27. This illustration shows the electrophoretic gel pattern that resulted from a chain termination sequencing process. What is the nucleotide sequence of the original DNA sample?



COMMUNICATING

28. Identify the therapy shown in the illustration and describe the events indicated by each letter.



29. A representative of a genetics research corporation comes to your community and makes a presentation. The representative says that DNA samples collected from local families could help the company develop new treatments for rare genetic disorders.

(a) What main points is the corporation representative likely to emphasize?

(b) What issues or concerns might local families raise in response to the presentation?

30. In a short report, discuss the risks and benefits associated with human germ-line therapy. Conclude with a recommendation on whether or not this kind of therapy should continue to be banned in Canada.

31. Working with a partner, debate the statement, "The benefits of farming genetically engineered crops outweigh the risks." Then write a short report identifying what you thought were the three best points made by each side in the debate.

32. Imagine that scientists discover a gene associated with high IQ. They then develop a form of gene therapy that can insert this gene into the genome of a fetus.

(a) Working with a partner or in a small group, brainstorm what you think might be some of the social effects of this discovery. What if the treatment had the side effect of increasing the risk of mental disorders? Record your ideas in a list or concept map.

(b) Write a brief report to describe what you think are some steps that could be taken now to prepare society for this kind of discovery in the future.

MAKING CONNECTIONS

33. You are a genetics counselor advising a young couple who are expecting a baby. The man wants the fetus to receive prenatal tests to check for genetic disorders, while the woman does not.

(a) What kind of arguments is the man likely to make? What kind of arguments is the woman likely to make?

(b) As the counselor, what information would you provide? How would you advise this couple?

34. Some groups argue that all food products containing genetically modified ingredients should be clearly labeled. Others argue that this labeling will harm producers, and that GMO foods have been demonstrated to be safe. What labeling policy would you propose? Explain your reasons.

35. You are a senior official in a government health department. You must decide how to allocate $100 million in genetics research funding among the following three areas: development of transgenic crops; somatic cell gene therapy; therapeutic cloning of human cells. How much funding will you allocate to each area? Justify your decision.

36. In 1997, the United Nations declared that "Practices which are contrary to human dignity, such as reproductive cloning of human beings, shall not be permitted." In a short report, discuss why the United Nations considers human cloning to be a practice contrary to human dignity. In your summary, add your own reasons for why you agree or disagree with the United Nations' position.

UNIT 6 REVIEW

UNDERSTANDING CONCEPTS

True-False

In your notebook, indicate whether each statement is true or false. Correct each false statement.

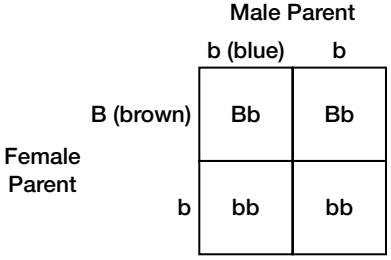
1. The phenotype refers to the genetic make-up of an organism.
2. If the genes for two given characteristics are found on the same chromosome, then the genes are linked.
3. If a mother has blue eyes (bb) and a father has brown eyes (Bb), then the probability their child will have blue eyes is 50%.
4. A chromosome is made up of smaller units called genes.
5. The ABO blood group in humans is an example of multiple allele inheritance.
6. If both parents possess the genotype AaBb, they can produce nine different genotypes in the F_1 generation.
7. In DNA, adenine always pairs with guanine.
8. The two main steps in gene expression are transcription and replication.
9. A nucleotide insertion causes a frameshift mutation.
10. A restriction endonuclease breaks DNA into random fragments.

Multiple Choice

In your notebook, write the letter of the best answer for each of the following questions. (Only four choices are provided because of the nature of the concepts in the unit.)

11. Mendel's law of dominance states that
    - **(a)** in an organism heterozygous for a trait, only one of the two alleles is expressed
    - **(b)** two alleles that determine a characteristic will separate when sex cells form
    - **(c)** an allele that is expressed is recessive
    - **(d)** the inheritance of a particular allele is not affected by the inheritance of other alleles

For questions 12 to 14, refer to the diagram.



12. What is the colour of the female parent's eyes?
    - **(a)** brown
    - **(b)** blue
    - **(c)** one brown, one blue
    - **(d)** cannot be determined
13. What would be the expected ratio of eye colour among the offspring?
    - **(a)** 2 brown : 3 blue
    - **(b)** 1 brown : 2 blue
    - **(c)** 1 brown : 1 blue
    - **(d)** 2 brown : 1 blue
14. If the parents produce four offspring, how many will probably be homozygous for brown eyes?
    - **(a)** none
    - **(b)** 1
    - **(c)** 2
    - **(d)** 3
15. Due to the similar behaviour of genes and chromosomes, we can conclude that
    - **(a)** genes and chromosomes perform similar functions
    - **(b)** chromosomes pairs separate during meiosis
    - **(c)** genes linked on the same chromosome are sometimes separated
    - **(d)** genes are located on chromosomes
16. Traits that exhibit continuous variation, such as height in humans, are generally controlled by
    - **(a)** a single dominant gene
    - **(b)** co-dominance
    - **(c)** a single recessive gene
    - **(d)** multiple genes
17. Friederich Miescher is known for being the first scientist to
    - **(a)** isolate nuclein from white blood cells
    - **(b)** show the link between nuclein and Mendel's "factors of inheritance"
    - **(c)** distinguish between DNA and RNA
    - **(d)** propose that DNA is the material of heredity
18. In a strand of DNA, each nitrogenous base forms a hydrogen bond with
    - **(a)** a phosphate group
    - **(b)** a sugar
    - **(c)** a phosphate bridge
    - **(d)** another nitrogenous base
19. Which of the following bases is not found in DNA?
    - **(a)** adenine
    - **(b)** uracil
    - **(c)** thymine
    - **(d)** cytosine
20. If the sequence of bases in one strand of DNA is AATCGG, what is the sequence in the complementary strand?
    - **(a)** TTGCAA
    - **(b)** TTAGCC
    - **(c)** UUAGCC
    - **(d)** TTUGCC

21. Which of the following is not a characteristic of the genetic code?

(a) redundancy **(c)** ambiguity
(b) universality **(d)** continuity

22. A stretch of DNA that can move from one location in the genome to another is called

(a) a transposon
(b) a pseudogene
(c) a chemical mutagen
(d) a physical mutagen

23. Stanley Cohen and Herbert Boyer are known for being the first research team to

(a) sequence a bacterial plasmid
(b) sequence the genome of a virus
(c) clone an animal
(d) create recombinant DNA

24. The polymerase chain reaction allows researchers to

(a) break DNA into small fragments for analysis
(b) read a DNA fingerprint
(c) amplify a sample of DNA
(d) bind DNA from two different sources

25. cDNA is sometimes used to carry eukaryotic genes into a bacterial cell because

(a) the genetic code of bacteria is different
(b) ordinary DNA may not be integrated in the right place in the genome
(c) bacteria cannot remove introns from mRNA
(d) bacteria cannot remove exons from mRNA

26. The field of research that aims to generate new human organs from tissue samples is called

(a) gene therapy
(b) therapeutic cloning
(c) reproductive cloning
(d) transgenics

Short Answer

In your notebook, write a sentence or a short paragraph to answer each of the following questions.

27. **(a)** Who was Gregor Mendel?
(b) Identify three reasons why pea plants were an excellent choice for his study of genetic inheritance.

28. Describe the phenotypes and genotypes when two organisms that are heterozygous for a trait are crossed.

29. How would Mendel explain why certain phenotypes and genotypes are not always expressed in offspring?

30. In a cross of purple-flowered and white-flowered plants, the F_1 generation were all purple. A cross of two F_1 plants resulted in a F_2 generation with the following numbers of phenotypes: 28 purple, 52 lavender (light purple), 19 white.

(a) What were the F_1 generation genotypes? Explain.
(b) Use a Punnett square to show how the results observed in the F_2 generation were obtained.
(c) Identify and explain the type of inheritance this cross reveals.

31. A probationary nurse mixed up the identification bracelets of three newborn babies. The blood types of the parents and babies were obtained in an attempt to identify the parents of each baby.

Parent	Blood type	Baby	Blood type
Mr. Smith	AB	Baby X	O
Mr. Rene	O	Baby Y	A
Mr. Sharetsky	A	Baby Z	AB
Mrs. Smith	O		
Mrs. Rene	O		
Mrs. Sharetsky	B		

(a) Which baby belongs to which set of parents?
(b) Using your knowledge of genetics, explain how you arrived at your answer.

32. What type of vision can be expected in the offspring if the parents have normal colour vision but the father of each parent is colour-blind? Why?

33. Explain how Chargaff's findings with respect to the composition of DNA provided evidence for the role of DNA in heredity.

34. Draw a DNA molecule that has the nucleotide sequence ATTCTGGC along one strand. Label the 5′ and 3′ ends.

35. Compare and contrast the structure of DNA and RNA.

36. Explain how elongation of a daughter DNA strand takes place

(a) in the direction of the movement of the replicating fork
(b) in the direction opposite to the movement of the replicating fork

37. Explain how telomeres could play a role in determining the life span of a eukaryotic cell.

38. Identify the function(s) of each of the following enzymes.

(a) DNA polymerase **(c)** Helicase

(b) DNA ligase **(d)** RNA primase

Use the genetic code table on page 000 to answer questions 43–45

39. What amino acid is associated with each of the following codons?

(a) GCC **(c)** ACU

(b) UUU

40. What features of the genetic code help to protect the cell from the effects of some mutations? Give specific examples using information from the table.

41. What anticodon sequences are associated with each of the following amino acids?

(a) leucine **(c)** glycine

(b) tyrosine

42. The antisense strand of a gene has the following nucleotide sequence: ATGTTTGCCTGGCCATGA. What is the amino acid sequence of the polypeptide product of this gene?

43. Describe the process of mRNA synthesis from the start of transcription to the point at which the mRNA enters the cytoplasm of a eukaryotic cell.

44. Prepare a diagram illustrating the elongation cycle of translation. Include a brief written description of each step in the cycle. How does a stop codon end the cycle?

45. Copy and complete the following table.

Nucleic Acid	Structure	Function
DNA		
mRNA		
rRNA		
tRNA		

46. A plant cell is subjected to a high level of UV radiation.

(a) What effect could this exposure produce in the DNA of this cell?

(b) What might be the effect on its daughter cells?

47. Identify and describe three different ways that a genetic disease can be detected before birth.

48. What purpose do viruses serve in gene therapy? What risk is associated with the use of viruses?

49. Describe three of the basic tools of genetic engineering. Provide an example of how they can be used together in DNA analysis.

50. What is Dolly's genetic relationship to

(a) the udder cell donor sheep?

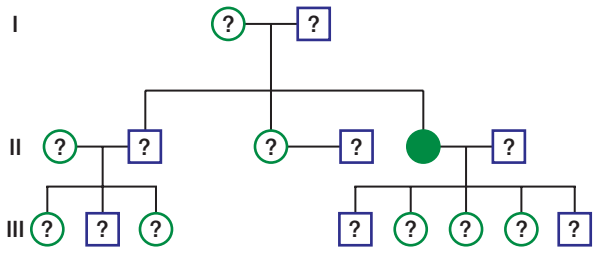
(b) the egg cell donor sheep?

(c) the surrogate mother sheep?

51. Provide an example of a product of artificial selection. Describe the process that might have led to the creation of this organism.

INQUIRY

52. In humans, the allele for normal hearing (H) is dominant over the recessive allele for congenital deafness. The trait is not sex-linked. Interpret the pedigree shown here in order to answer the following questions.



(a) Explain how it is possible for individual II-3 to be congenitally deaf if neither parent had the condition.

(b) Are boys more likely than girls to inherit the condition? Explain.

(c) What is the probability that female III-1 will be congenitally deaf? Assume that person II-1 is not a carrier for the trait.

53. Genetic information is encoded in the sequence of nucleotides in DNA. Suppose a specific nucleotide sequence on one strand of the DNA molecule is responsible for the structure of hemoglobin. Do you think the complementary sequence of nucleotides on the opposite strand also encodes useful information? Explain. Describe a method you could use to test your idea.

54. In foxes, a pair of alleles, P and p, interact as follows: PP is lethal, usually during the embryonic stage; Pp produces platinum-coloured fur, and pp produces silver foxes. Could a fox breeder establish a true-breeding variety of platinum foxes? Explain.

55. You collect a sample of DNA from a mouse cell for analysis.

(a) Complete the following table to show the base composition of this DNA sample.

Base	A	T	G	C
Percentage		29		

(b) In a separate experiment, you determine the base composition of each strand of the DNA molecule. You then collect a sample of the mRNA transcribed from this DNA. You discover that this mRNA has a very different base composition from the antisense strand of the DNA. Is this the result you would expect? Explain.

56. You plan to run a gel electrophoresis on DNA from human chromosome 22. You isolate this chromosome from one subject and then amplify it to produce millions of copies. You then create three different DNA samples, as follows:

Sample A: millions of copies of chromosome 22 that have been broken into random fragments

Sample B: millions of copies of chromosome 22 that have been treated with a restriction endonuclease

Sample C: millions of copies of intact chromosome 22

What pattern would you expect to see on your gel for each of these three samples? Explain.

COMMUNICATING

57. A couple has three children, two of whom have hemophilia. As their genetic counsellor, what would you tell them about the probability of their next child not having hemophilia? Explain.

58. Today, we are making advances in genetics at a tremendous rate. Do you think there should be limits on how this new knowledge is used? Explain.

59. Explain why the addition or deletion of three base pairs would not have the same effect on a gene as the addition or deletion of one base pair. Use an illustration to explain your answer.

60. Create a diagram you could use to explain the distinction between genetic recombination and recombinant DNA.

61. Your community is hosting a series of public information meetings about health issues. The objective of the series is to teach people about the science behind healthy lifestyle choices. You are asked to make a 10-minute presentation on the topic "DNA and mutations."

(a) Write a one- or two-sentence key message you would want your audience to remember.

(b) Outline your presentation under five main headings, beginning with "Introduction" and ending with "Conclusion."

(c) Under each heading list three points you would want to cover and describe each in a few sentences.

MAKING CONNECTIONS

62. When Mendel performed his experiments, he worked with many individual plants that produced thousands of offspring. Do you think Mendel would have obtained the same results if he worked with only 20 or 30 plants? Explain.

63. Some species of animals, such as the northern right whale, have had their populations reduced to an extremely small number. As a result, their genetic diversity has been significantly reduced. Why might a lack of genetic diversity threaten a species? Could a lack of genetic diversity threaten a small, isolated human population as well? Explain.

64. With the development of new medical treatments for genetic diseases, more people with these diseases are surviving to reproductive age. What will happen to the number of recessive alleles in the human population as a result of this? Explain.

65. The product rule can be used to predict the phenotypic and genotypic ratios of offspring. Give an example of another circumstance, outside the field of genetics, in which you could use the product rule to predict an outcome.

66. A team of researchers announces they have developed a strain of bacteria that expresses a human growth hormone.

(a) What social and environmental issues might be raised by this announcement?

(b) Would the issues be the same if the researchers had announced the discovery of a way to insert a bacterial growth hormone gene into a human patient? Explain.