16 STUDYING AND MANIPULATING GENOMES

Golden Rice, or Frankenfood?

Not too long ago, the World Health Organization made a conservative estimate that 124 million children around the world show vitamin A deficiencies. Their skin, eyes, and mucous membranes are dry and vulnerable to infection. They do not grow and develop as they should, and they show signs of mental impairment. Each year at least a million die of malnutrition, and about 350,000 end up permanently blind.

Ingo Potrykus and Peter Beyer wanted to help. As they knew, beta-carotene is a yellow pigment in all plant leaves, and it also is a precursor for vitamin A. These geneticists borrowed three genes from garden daffodils (*Narcissus pseudonarcissus*) and a bacterium, and transferred them to rice plants. The plants transcribed the genes and did something they could not do before. They made betacarotene not only in their leaves but also in their *seeds* the grains of Golden Rice (Figure 16.1).

Why rice? Rice is the main food for 3 billion people in impoverished countries. There, the poor cannot afford leafy vegetables and other sources of beta-carotene. Getting beta-carotene into rice grains would be the least costly way to deliver the vitamin to those who need it the most, but doing so was beyond the scope of conventional breeding practices. Research continues, and the amount of beta-carotene in SGR1, a more recent version of Golden Rice, is twenty-three times higher than the prototype.

No one wants children to suffer or die. However, many people oppose the idea of genetically modified (GM) foods, including golden rice. Possibly they are unaware of the history of agrarian societies, because it is not as if our ancestors were twiddling their green thumbs. For thousands of years, their artificial selection practices coaxed new plants and new breeds of cattle, cats, dogs, and birds from wild ancestral stocks. Meatier turkeys, huge watermelons, big juicy corn kernels from puny hard ones—the list goes on (Figure 16.1).

And we are newcomers at this! During the 3.8 billion years before we even made our entrance, nature busily conducted uncountable numbers of genetic experiments by way of mutation, crossing over, and gene transfers between species. These processes introduced changes in the molecular messages of inheritance, and today we see their outcomes in the sweep of life's diversity.

Perhaps the unsettling thing about the more recent human-directed changes is that the pace has picked up, hugely. We are getting much better at tinkering with the genetics of many organisms. We do this for pure research and for useful, practical applications.



Figure 16.1 Where one genetic engineering success story started: (**a**) Researchers transferred genetic information from ordinary daffodils into rice plants, which then used it to stockpile beta-carotene in their seeds—rice grains. (**b**) Two successive generations of Golden Rice compared with grains from a regular rice plant at lower left. *Facing page*, an artificial selection success story—a big kernel from a modern strain of corn next to tiny kernels of an ancestral corn species discovered in a prehistoric cave in Mexico.

IMPACTS, ISSUES



Watch the video online!

For instance, many crop plants, including corn, beets, and potatoes, have been modified. They are now widely planted. They are less temperamental about their living conditions than rice plants are, and they have not run rampant through ecosystems. After a decade-long study in the United Kingdom, researchers concluded that the new crop plants being monitored were doing no harm. Throughout Arizona, farmers grow cotton plants that are genetically engineered for pest resistance. The plantings have not put the environment at risk and might even be less disruptive compared to current agricultural practices. University of Arizona entomologist Bruce Tabashnik, who is monitoring cotton fields, notes that farmers have cut applications of chemical pesticides by 75 percent.

Take stock of how far you have come in this unit. You started with cell division mechanisms that allow parents to pass on DNA to new generations. You moved to the chromosomal and molecular basis of inheritance, then on to gene controls that guide life's continuity. The sequence parallels the history of genetics. And now, you have arrived at the point in time where geneticists hold molecular keys to the kingdom of inheritance. What they are unlocking is already having impact on life in the biosphere.



MAKING RECOMBINANT DNA

Researchers routinely make recombinant DNA molecules. They use restriction enzymes to isolate, cut, and join gene regions from DNA of different species. They use plasmids and other vectors to insert the recombinant molecule into target cells. Section 16.1

ISOLATING AND AMPLIFYING DNA FRAGMENTS

Researchers isolate and make many copies of genes that interest them. PCR is now the gene amplification method of choice. The genes are copied in amounts large enough for research and practical applications. Section 16.2

DECIPHERING DNA FRAGMENTS

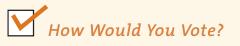
Sequencing methods reveal the linear order of bases in a sample of DNA. Automated methods complete the task with impressive speed. Sections 16.3, 16.4

MAPPING AND ANALYZING WHOLE GENOMES

Genomics is concerned with mapping and sequencing of the genomes of humans and other species. Comparative genomics yields evidence of evolutionary relationships among groups of organisms. Section 16.5

USING THE NEW TECHNOLOGIES

Genetic engineering results in transgenic organisms, which incorporate genes from another species. With gene therapy, a mutated or altered gene is isolated, modified, and copied. Copies are inserted back into the individual to cover the gene's function. The new technologies raise social, legal, ecological, and ethical questions. Sections 16.6–16.10



Nutritional labeling is required on all packaged food in the United States, but genetically modified food products may be sold without labeling. Should food distributors be required to label all products made from genetically modified plants or livestock? See BiologyNow for details, then vote online.



This chapter builds on earlier explanations of the molecular structure of DNA (Sections 3.7, 13.2), and DNA replication and DNA repair (13.4). You may wish to review quickly the nature of mRNA transcript processing (14.1) and controls over gene transcription (14.1). You will come across more uses for radioisotopes (2.2) and fluorescent light (6.6). You will be reminded of why it is useful to know about membrane proteins (5.2). You will see why the lactose operon is not necessarily of obscure interest (15.4).

MAKING RECOMBINANT DNA

16.1 A Molecular Toolkit

LINKS TO SECTIONS 13.1, 13.3, 14.1 Analysis of genes starts with manipulation of DNA. With molecular tools, researchers can cut DNA from different sources, then splice the fragments together.

THE SCISSORS: RESTRICTION ENZYMES

In 1970, Hamilton Smith was studying viral infection of *Haemophilus influenzae*. This bacterium protects itself from infection by cutting up viral DNA before it can get inserted into the bacterial chromosome. Smith and his colleagues isolated one of the bacterial enzymes that cuts viral DNA. It was the first known **restriction enzyme**. In time, several hundred strains of bacteria and a few eukaryotic cells yielded thousands more.

A restriction enzyme cuts double-stranded DNA at a specific base sequence between four and eight base pairs in length. Most of these recognition sites contain the same nucleotide sequence, in the $5' \rightarrow 3'$ direction, on both strands of the DNA. For instance, the enzyme

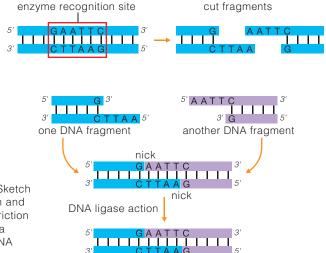
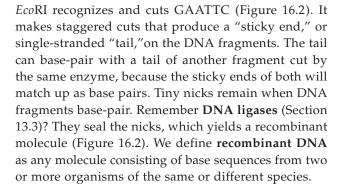


Figure 16.2 Sketch of the formation and splicing of restriction fragments into a recombinant DNA molecule.



CLONING VECTORS

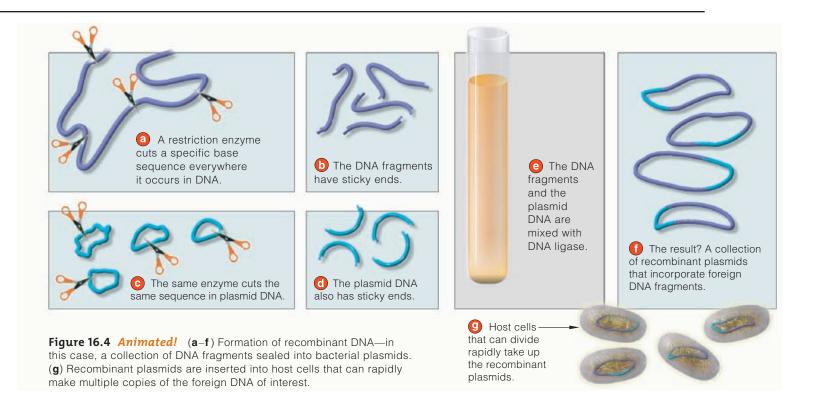
Bacterial cells, recall, have only one chromosome—a circular DNA molecule. But many also have plasmids. A **plasmid** is a small circle of extra DNA with just a few genes (Figure 16.3*a*). It gets replicated along with the bacterial chromosome. Bacteria normally can live without plasmids. Even so, certain plasmid genes are useful, as when they confer resistance to antibiotics.

Under favorable conditions, bacteria divide often, so huge populations of genetically identical cells form swiftly. Before each division, replication enzymes copy chromosomal DNA *and* plasmid DNA, in some cases repeatedly. This gave researchers the idea of inserting DNA fragments into a plasmid to see if a bacterial cell would replicate them right along with the plasmid.

A plasmid that has accepted foreign DNA and can slip into a host bacterium, yeast, or some other cell is a **cloning vector**. Most vectors have been engineered to incorporate multiple cloning sites, which are unique restriction enzyme sequences in one part of the vector (Figure 16.3). As you will see, cloning vectors contain genes that help researchers identify which cells take them up. Viruses also are used as cloning vectors.

> P T7 promoter Acc65/ Acc65/ Acc65/ Acc7/ BarrHi BarrHi

Figure 16.3 (a) Plasmids (*arrows*) from a ruptured *Escherichia coli* cell. (b) A commercially available cloning vector. Its useful restriction enzyme sites are listed at right. This vector includes antibiotic resistance genes (*blue*) and the bacterial *lacZ* gene (*red*). Researchers can check for the expression of these genes as a way to identify the bacterial cells that take up recombinant molecules.



A cell that takes up a cloning vector may give rise to a huge population of descendant cells, each with an identical copy of the vector and the foreign DNA inserted into it. Collectively, the identical cells hold many "cloned" copies of the foreign DNA.

Such DNA cloning is a tool that helps researchers amplify and harvest unlimited amounts of particular DNA fragments for their studies (Figure 16.4).

cDNA CLONING

Remember those introns in eukaryotic DNA (Section 14.1)? Bacterial cells cannot remove introns from RNA, as eukaryotic cells do. That is why researchers often use mature mRNA transcripts. The introns already have been removed, and protein-coding sequences and a few sequences that are identifiable signals are left. Researchers also may use mRNA to study gene expression, because the cells that are actively using a gene obviously contain mRNA transcribed from it.

Restriction enzymes will not cut single-stranded molecules, so they will not cleave mRNA (which is single-stranded). However, mRNA can be cloned if it is first transcribed—in reverse. Replication enzymes isolated from viruses or bacterial cells can transcribe mRNA inside a test tube. **Reverse transcriptase** is one viral enzyme that can catalyze the bonding of free nucleotides into one strand of *complementary* DNA, or **cDNA** on an mRNA template (Figure 16.5). Base pairs

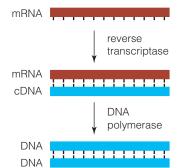


Figure 16.5 How to make cDNA. Reverse transcriptase catalyzes the assembly of a single DNA strand on an mRNA template, forming an mRNA-cDNA hybrid molecule. Next, DNA polymerase replaces the mRNA with another DNA strand. The result is doublestranded DNA.

of cDNA get hydrogen-bonded to those of mRNA, forming a hybrid molecule. Next, DNA polymerase is added to the mix. It strips RNA bases from the hybrid molecule while it copies the first strand of cDNA into a second strand. The result, a double-stranded DNA copy of the original mRNA, may be used for cloning.

Molecular biologists manipulate DNA and RNA. Restriction enzymes cut DNA from individuals of different species or the same species. DNA ligases glue the fragments into plasmids.

A recombinant plasmid is a cloning vector. It can slip into bacteria, yeast, or other cells that divide rapidly. The host cells make multiple, identical copies of the foreign DNA.

Reverse transcriptase, a viral enzyme, uses a single strand of mRNA as the template to make cDNA for cloning.



16.2 From Haystacks to Needles

LINKS TO SECTIONS 2.1, 10.1, 13.2 A genome, recall, is all the DNA in a haploid number of the chromosomes that characterize a species. To study or modify any gene, researchers must first find it among thousands of others in the genome, and it's like searching for a needle in a haystack. Once found, it must be copied many times to make enough material for experiments.

ISOLATING GENES

A **gene library** is a collection of host cells that house different cloned fragments of DNA. We call the cloned fragments of an entire genome a *genomic* library. By contrast, a *cDNA library* is derived from mRNA.

How can a single gene of interest be isolated from thousands or millions of others in a library of clones? Clones that have the gene are mixed up with others that do not. Researchers might decide to use a **probe** to find the gene. Probes are short stretches of DNA that are complementary to a gene of interest and that are tagged with a label, such as a radioisotope, that devices can detect (Section 2.1). Probes base-pair with DNA in a gene region, then researchers pinpoint the gene by detecting the label on the probe. Any base pairing between DNA (or RNA) from more than one source is known as **nucleic acid hybridization**.

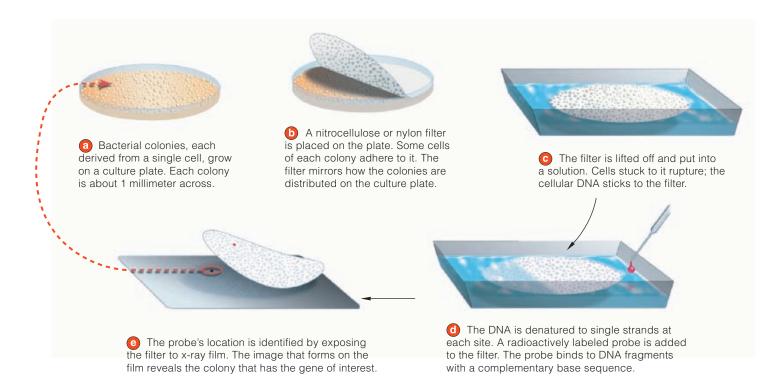
How do researchers make a probe? If they already know the gene sequence of interest, they can use it to

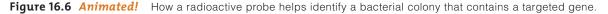
design and assemble a **primer**, or a short stretch of synthetic, single-stranded DNA. If the sequence is not known, they can use DNA that was already isolated from the same gene in a closely related species. Even if the probe is not an exact match, it might still tag the gene by base-pairing with part of it.

Figure 16.6 shows steps of one probe hybridization technique. Bacterial cells containing a gene library are spread out on the surface of a solid growth medium, usually enriched agar, in a petri dish. Individual cells undergo repeated divisions, which result in colonies of millions of genetically identical bacterial cells.

When you press a piece of nylon or nitrocellulose filter on top of the petri dish, some cells from each colony stick to it. They mirror the distribution of all colonies on the dish. Soaking the filter in an alkaline solution ruptures the cells, which releases their DNA. The solution also denatures DNA—which separates into single strands that stick to the filter in the spots where the colonies were. When the probe is washed over the filter, it hybridizes with (sticks to) only the DNA with the targeted sequence.

The hybridized probe can be detected with x-ray film or computerized imaging devices. Its position on the film pinpoints the position of the original colony on the petri dish. Cells from that colony alone can be cultured to isolate the cloned gene of interest.





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Figure 16.7 *Animated!* Two rounds of the polymerase chain reaction, or PCR. A bacterium, *Thermus aquaticus*, is the source for the *Taq* polymerase. Thirty or more cycles of PCR may yield a billionfold increase in the number of starting DNA molecules that serve as templates.

BIG-TIME AMPLIFICATION ---- PCR

Researchers may replicate a gene, or part of it, with **PCR** (*Polymerase Chain Reaction*). PCR uses primers and a heat-tolerant polymerase for a hot–cold cycled reaction that replicates targeted DNA fragments. The technique can replicate the fragments by a billionfold. It can transform one needle in a haystack, that one-in-a-million DNA fragment, into a huge stack of needles with a little hay in it.

Figure 16.7 shows the reaction steps. The primers are designed to base-pair with particular nucleotide sequences on either end of the fragment of interest. Usually they are between ten and thirty bases long.

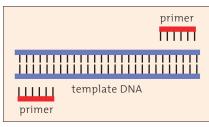
In a PCR reaction, researchers mix primers, DNA polymerase, nucleotides, and the DNA that will serve as a template for replication. Then they expose the mixture to cycles of high and low temperatures that are repeated again and again. At high temperature, the two strands of a DNA double helix separate. When the mixture is cooled, some of the primers hybridize with the DNA template.

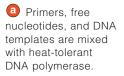
The elevated temperatures required to separate the DNA strands destroy typical DNA polymerases. But the heat-tolerant DNA polymerase employed for PCR reactions is from *Thermus aquaticus*, a bacterium that lives in hot springs (Chapter 21). Like all other DNA polymerases, it recognizes primers bound to DNA as places to start synthesis. The temperature is raised to the optimum for this enzyme (72°C). Then synthesis occurs along the DNA template until the temperature cycles up and the DNA strands are separated again.

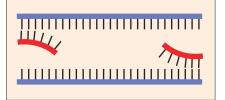
When the temperature cycles down, the primers rehybridize, and the reactions run once more. With each round of temperature cycling, the number of copies of targeted DNA can double. PCR quickly and exponentially amplifies even a tiny bit of DNA.

Probes may be used to help identify one particular gene among many in gene libraries.

The polymerase chain reaction (PCR) is a method of rapidly and exponentially amplifying the number of particular DNA fragments.



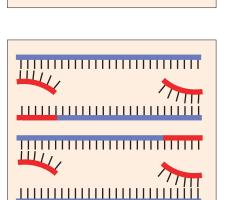


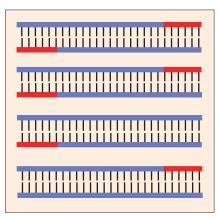


is heated, the DNA denatures. When it is cooled, some primers hydrogen-bond to the DNA templates.

b When the mixture

• *Taq* polymerase uses the primers to initiate synthesis. The DNA templates are copied. The first round of PCR is completed.





 The mixture is heated again. This denatures all the DNA into single strands. When the mixture is cooled, some of the primers hydrogenbond to the DNA.

• Taq polymerase uses the primers to initiate synthesis, copying the DNA. The second round of PCR is complete. Each successive round of synthesis can double the number of DNA molecules. DECIPHERING DNA FRAGMENTS

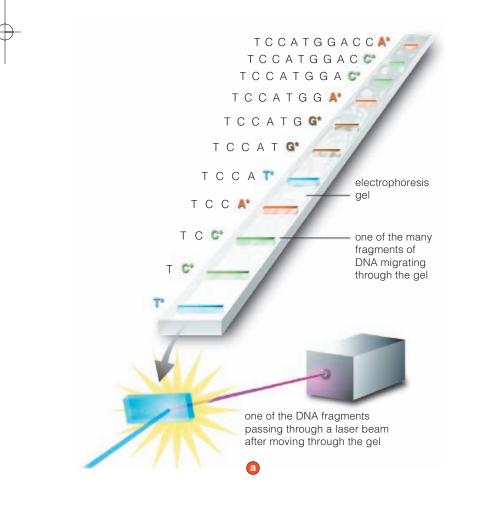
16.3 Automated DNA Sequencing

LINK TO SECTION 6.6 Sequencing reveals the order of nucleotides in DNA. This technique uses DNA polymerase to partially replicate a DNA template. Automated techniques have largely replaced manual methods.

Automated DNA sequencing can reveal the sequence of a stretch of cloned or PCR-amplified DNA in just a few hours. Researchers use four standard nucleotides (T, C, A, and G). They also use four modified versions, which we represent here as **T**^{*}, **C**^{*}, **A**^{*}, and **G**^{*}. Each form of modified nucleotide has been labeled with a pigment that will fluoresce a certain color when a laser beam hits it. Each will halt strand assembly.



Researchers mix all eight kinds of nucleotides with a single-stranded DNA template, a primer, and DNA polymerase. The polymerase uses the primer to copy the template DNA into new strands of DNA. One by one, it adds nucleotides in the order dictated by the sequence of the DNA template (Figure 16.8*a*). Every time, the polymerase randomly attaches a standard *or* a modified nucleotide to the DNA template. When one of the modified nucleotides covalently bonds to the



forming DNA strand, no more can be added. After enough time passes, there will be some new strands that stop at each base in the DNA template sequence.

Eventually the mixture holds millions of copies of DNA fragments, all fluorescent-tagged on one end. These fragments are separated by **gel electrophoresis**, a technique that sorts fragments as they move through a semisolid slab (of polyacrylamide) in response to an electric field.

Depending on their lengths, the fragments migrate at different rates through the gel. The gel hinders the migration of longer ones more than shorter ones. By analogy, elephants running through the forest in India cannot move between the trees as fast as tigers can.

The shortest fragments migrate fastest and are first to arrive at the end of the gel. The longest fragment is last. Fragments of the same length move through the gel at the same speed, and they gather into bands.

A laser beam shines on each band when it passes through the end of the gel. The modified nucleotides attached to the fragments fluoresce in response to the light, and the sequencer detects and records the color of each band. Because each color designates one of the four particular nucleotides, the order of colored bands reveals the DNA sequence. The machine itself rapidly assembles the sequence data.

Figure 16.8*b* shows the partial results from one run through an automated DNA sequencer. Each peak in the tracing represents the detection of one fluorescent color as the fragments reached the end of the gel. The sequence is shown beneath the graph line.

DNA sequencing rapidly reveals the order of nucleotides

in a cloned or amplified DNA fragment.

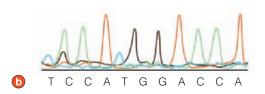


Figure 16.8 *Animated!* Automated DNA sequencing. (a) Researchers synthesize DNA fragments by using a template and fluorescent nucleotides. Gel electrophoresis sorts out the fragments by length. (b) The order of the fluorescent bands that appear in the gel is detected by the sequencer. That order indicates the template DNA sequence. Today, researchers throughout the world use sequence databases that can be accessed via the Internet.

16.4 Analyzing DNA Fingerprints

Except for identical twins, no two people have exactly the same sequence of bases in their DNA. One individual can be distinguished from all others on the basis of this molecular fingerprint.

Each human has a unique set of fingerprints. In addition, like other sexually reproducing species, each also has a **DNA fingerprint**—a unique array of DNA sequences that are inherited from parents in a Mendelian pattern. More than 99 percent of the DNA is the same in all humans, but the other fraction of 1 percent is unique to each individual. Some of these unique stretches of DNA are sprinkled through the human genome as **tandem repeats**—many copies of the same short base sequences, positioned one after the other along a DNA molecule.

For example, one person's DNA might contain four repeats of the bases TTTTC in a certain location. Another person's DNA might have them repeated fifteen times in the same location. One person might have ten repeats of CGG, and another might have fifteen. Such repetitive sequences slip spontaneously into DNA during replication, and their numbers grow or shrink over time. The mutation rate is relatively high in these regions.

DNA *fingerprinting* reveals differences in the tandem repeats among individuals. A restriction enzyme cuts their DNA into an assortment of fragments. The sizes of those fragments are unique to the individual. They reveal genetic differences between individuals, and they can be detected as RFLPs (*Restriction Fragment Length Polymorphisms*).

The fragments can be subjected to gel electrophoresis to form distinct bands according to their length. The

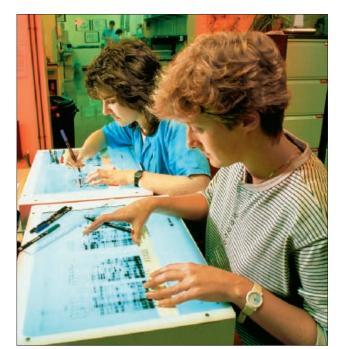
banding pattern of genomic DNA fragments is the DNA fingerprint unique to the individual. For all practical purposes, it is identical only between identical twins. The odds of two unrelated people sharing an identical DNA fingerprint are 1 in 3,000,000,000.

PCR can be used to amplify tandem-repeat regions. Again, differences in the size of DNA fragments amplified by this technique can be detected by gel electrophoresis. A few drops of blood, semen, or cells from a hair follicle at a crime scene or on a suspect's clothing yield enough DNA to amplify with PCR, and then generate a fingerprint.

DNA fingerprints help forensic scientists identify criminals, victims, and innocent suspects. Figure 16.9 shows some tandem repeat RFLPs that were separated by gel electrophoresis. Those samples of DNA had been taken from seven people and from a bloodstain left at a crime scene. One of the DNA fingerprints matched.

Defense attorneys initially challenged the use of DNA fingerprinting as evidence in court. Today, however, the procedure has been firmly established as accurate and unambiguous. DNA fingerprinting is routinely submitted as evidence in disputes over paternity, and it is being widely used to convict the guilty and to exonerate the innocent. At this writing, DNA evidence has helped release well over 100 innocent people from prison.

DNA fingerprint analysis has even wider application. For instance, it confirmed that human bones exhumed from a shallow pit in Siberia belonged to five individuals of the Russian imperial family, all shot to death in secrecy in 1918. More recently, it was used to identify the remains of those who died in the World Trade Center on September 11, 2001.



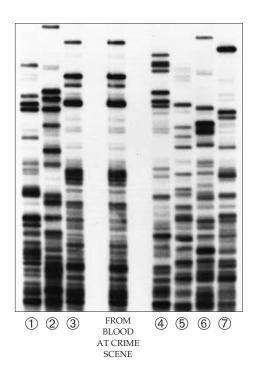


Figure 16.9 One case of a damning comparison of the DNA fingerprints from a bloodstain left behind at a crime scene and from blood samples of seven suspects (the series of circled numbers).

Can you point out which of the seven DNA fingerprints is an exact match?



16.5 The Rise of Genomics

LINKS TO SECTIONS 3.7, 13.2, 14.5 The potential benefits of sequencing and analyzing the thousands of genes in the genome of selected organisms—say, the human genome—soon became apparent. Automated gene sequencing techniques were developed in response.

THE HUMAN GENOME PROJECT

By 1986, scientists were arguing about sequencing the 3 billion bases of the human genome. Many insisted that benefits for medicine and pure research would be incalculable. Others insisted that the mapping would divert funds from other work that was more urgent and had a better chance of success.

Automated sequencing had just been invented, as had PCR, the polymerase chain reaction. At the time, both techniques were cumbersome, expensive, and far from standardized, but many sensed their potential. Waiting for faster methods seemed the most efficient approach to sequencing the human genome—but who would decide when the technology was fast enough?

Several independent organizations launched their own versions of the Human Genome Project. Walter Gilbert started one company and declared he would sequence and patent the human genome. In 1988, the National Institutes of Health (NIH) annexed the entire Human Genome Project by hiring James Watson as its head and providing 200 million dollars per year to researchers. A public consortium formed between the NIH and institutions working on different versions of the project. Watson set aside 3 percent of the funding for studies into ethical and social issues arising from the research. He then resigned in 1992 because of a disagreement with the NIH about patenting partial gene sequences. Francis Collins replaced him in 1993.

Amid ongoing squabbles over patent issues, Craig Venter started Celera Genomics (Figure 16.10). Venter cheekily declared that his new company would be the first to finish and patent the genome sequence. This prompted the public consortium to move its gene sequencing efforts into high gear.

Sequencing of the human genome was officially completed in 2003—fifty years after the discovery of the structure of DNA. About 99 percent of the coding regions in human DNA have been deciphered with a high degree of accuracy. A number of other genomes also have been fully sequenced.

What do we do with this vast amount of data? The next step is to investigate questions about precisely what each sequence means—what the genes do, what the control mechanisms are, and how they operate.

At this writing, 19,438 are confirmed as genes, and another 2,188 are probably genes. This does not mean that geneticists have learned what the genes encode.

Among the bizarre discoveries: Protein-encoding genes make up less than 2 percent of our genome. Millions of transposable elements repeated over and over make up more than half of it. There are almost as many *pseudogenes*—inactivated, nonfunctional copies of genes—as there are genes!

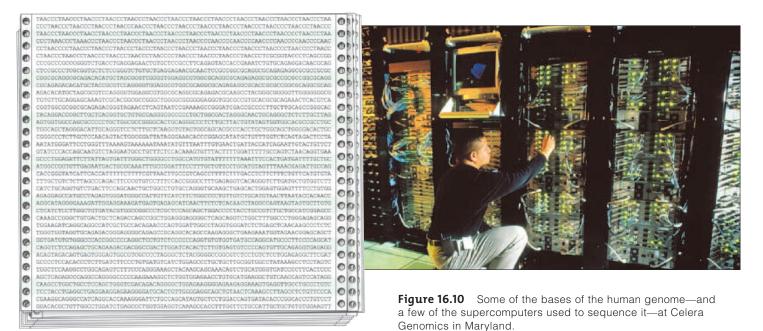


Figure 16.11 Complete yeast genome array on a DNA chip about 19 millimeters (3/4 inch) across. *Green* spots pinpoint genes that are active during fermentation. *Red* pinpoints the genes used in aerobic respiration, and *yellow*, the ones that are active in both pathways.

GENOMICS

Research into genomes of humans and other species has converged into a new research field—genomics. *Structural* genomics focuses on actual mapping and sequencing of the genomes of individuals. *Comparative* genomics sifts through the maps for similarities and differences that point to evolutionary connections.

Comparative genomics has practical applications as well as potential for research. The basic premise is that the genomes of all existing organisms are derived from common ancestors. For instance, pathogens share some conserved genes with human hosts even though they are only remotely related. Shared gene sequences, how they are organized, and where they differ might hold essential clues to where our immune defenses against pathogens are strongest or the most vulnerable.

Genomics has potential for **human gene therapy** the transfer of one or more normal or modified genes into a person's body cells to correct a genetic defect or boost resistance to disease. However, even though the human genome is fully sequenced, it still is not easy to manipulate within the context of a living individual.

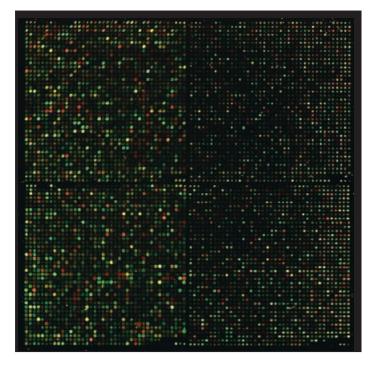
Today, experimenters use stripped-down viruses as vectors that inject genes into human cells. Some gene therapies deliver modified cells into a patient's tissue. In many cases, therapies make a patient's symptoms subside even when the modified cells are producing just a small amount of a required protein.

A caveat: No one can yet predict whether a virusinjected gene will be delivered to the right tissues and whether cellular mechanisms will maintain it.

DNA CHIPS

Analysis of genomes is now advancing at a stunning pace. Researchers pinpoint which genes are silent and which are being expressed with the use of **DNA chips**. These are microarrays of thousands of gene sequences representing a large subset of an entire genome—all stamped onto a glass plate that is about the size of a small business card.

A cDNA probe is built by using mRNA from, say, cells of a cancer patient. The free nucleotides used to



synthesize the complementary strand of DNA have been labeled with a fluorescent pigment. Only genes that are expressed at the time the cells are harvested are making mRNA, so those genes alone make up the resulting probe population. The labeled probe is then incubated along with a chip made from genomic DNA. Wherever the probe binds with complementary base sequences on the chip, there will be a spot that glows under fluorescent light. Analysis of which spots on the chip are glowing reveals which of the thousands of genes inside the cells are active and which are not.

DNA chips are being used to compare different gene expression patterns between cells. Examples are yeasts grown in the presence and absence of oxygen, and different types of cells from the same multicelled individual. RNA from one set of cells is transformed into green fluorescent cDNA, and RNA from the other set into red fluorescent cDNA. The cDNAs are mixed and incubated with a genomic DNA chip. Green or red fluorescence indicates expression of genes in the different cell types. Yellow is a mixture of both red and green, and it indicates that both genes were being expressed at the same time in a cell (Figure 16.11).

In genomics, automated gene sequencing, the use of DNA chips, and other techniques let researchers rapidly evaluate and compare genome-spanning expression patterns.

USING THE NEW TECHNOLOGIES

16.6 Genetic Engineering

Genetic engineering is the deliberate modification of an individual's genome. Genes from another species may be transferred to an individual. Conversely, the individual may have its own genes isolated, modified and copied, and then receive copies of the modified genes.

Genetic engineering started with bacterial species, so consider them first. The kinds that take up plasmids are now widely used in basic research, agriculture, medicine, and industry. Plasmids, again, function as vectors for transferring fragments of foreign or modified DNA into an organism.

For instance, like you, bacterial cells have the metabolic machinery to make complex organic compounds. Genetically engineered types can be employed to transcribe genes that have been transferred to plasmids



and synthesize desired proteins. Immense populations do this; they make useful amounts of medically valued proteins in huge stainless steel vats. *E. coli* cells were the first to transcribe and translate synthetic genes for human insulin. Their descendants were the first large-scale, cost-effective bacterial factory for proteins. In addition to insulin, vats of microbes churn out human somatotropin (growth hormone), hemoglobin, blood-clotting factors, interferon, and a variety of drugs and vaccines that we have come to depend upon.

Certain bacteria also hold potential for industry and for cleaning up environmental messes—that is, for *environmental remediation*. In nature, they break down organic wastes as part of their metabolic activities and help cycle nutrients through ecosystems. Modified types digest crude oil into less harmful compounds. When sprayed on oil spills, as from a shipwrecked supertanker, they can help mop up oil. Other species sponge up excess phosphates, heavy metals, and other pollutants, even radioactive wastes.

Genetic engineering refers to the directed alteration of an individual's genome. Microbes were the first targets. In some cases, DNA is transferred between individuals of different species, the outcome being a transgenic

In other cases, genes or gene regions from an individual are isolated, modified, then copied and inserted into the same individual.

16.7 Designer Plants

Think back on those Golden Rice plants described in the chapter introduction. They are a prime example of genetic engineering that can produce valuable transgenic plants. There is some urgency surrounding much of this work, as you will now read.

As crop production expands to keep pace with human population growth, it puts unavoidable pressure on ecosystems everywhere. Irrigation leaves mineral and salt residues in soils. Tilled soil erodes, taking topsoil with it. Runoff clogs rivers, and fertilizer in it causes algae to grow so much that fish suffocate. Pesticides harm humans, other animals, and beneficial insects.

Pressured to produce more food at lower cost and with less damage to the environment, some farmers are turning to genetically engineered crop plants.

Cotton plants with a built-in insecticide gene kill only the insects that eat it, so farmers that grow them are not required to use as many pesticides. Certain transgenic tomato plants can grow, develop, and bear fruit in salty soils that would wither other plants. They also absorb and store excess salt in their leaves, thus purifying saline soil for future crops.

The cotton plants in Figure 16.12*a* were genetically engineered for resistance to a relatively short-lived herbicide. Spraying fields with this herbicide will kill all weeds—but not the engineered cotton plants. As you read in the chapter's introduction, the practice means that farmers can use reduced amounts of less toxic chemicals. They do not have to till the soil as much to control weeds, so river-clogging runoff can be reduced. As another example, Figure 16.12*b* shows transgenic aspen seedlings that grow well and do not make as much lignin. Lignin-deficient trees are better for making paper and other forest products.

Engineering plant cells starts with vectors that can carry genes into plant cells. *Agrobacterium tumefaciens* is a bacterial species that infects eudicots, including beans, peas, potatoes, and other major crops plants. Genes in its plasmids cause tumors to form on these plants; hence the name Ti plasmid (*Tumor-inducing*). Researchers use the Ti plasmid to transfer foreign or modified genes into plants.

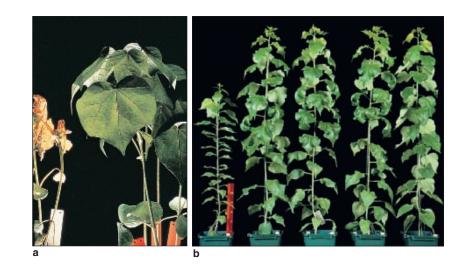
Researchers excise the tumor-inducing genes, then insert a desired gene into the plasmid (Figure 16.13). Some plant cells cultured with the modified plasmid may take it up. Whole plants may be regenerated.

Modified *A. tumefaciens* bacteria deliver genes into monocots that also are food sources, including wheat, corn, and rice. Researchers can even transfer genes into plants by way of electric shocks, chemicals, and blasts of microscopic particles coated with DNA.

organism.

Figure 16.12 (a) *Left,* control cotton plant. *Right,* cotton plant genetically engineered for herbicide resistance. Both plants were sprayed with a weed killer that is widely applied in cotton fields.

(**b**) Control plant (*left*) and four genetically engineered aspen seedlings. Vincent Chiang and coworkers suppressed a control gene involved in a lignin biosynthetic pathway. The modified plants synthesized normal lignin, but not as much. Lignin synthesis dropped by as much as 45 percent—yet cellulose production increased 15 percent. Root, stem, and leaf growth were greatly enhanced. Plant structure did not suffer. Wood harvested from such trees might make it easier to manufacture paper and some clean-burning fuels, such as ethanol. Lignin, a tough polymer, strengthens secondary cell walls of plants. Before paper can be made from wood, the lignin must be chemically extracted.



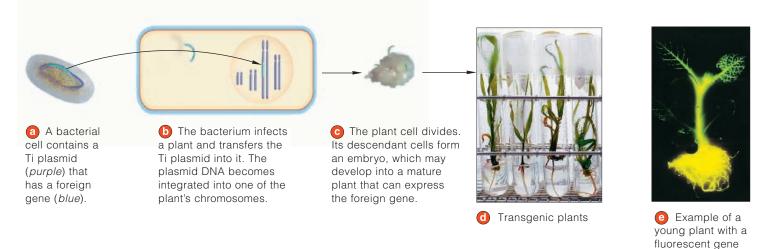


Figure 16.13 *Animated!* (**a**–**d**) Ti plasmid transfer of an *Agrobacterium tumefaciens* gene to a plant cell. (**e**) A transgenic plant expressing a firefly gene for the enzyme luciferase.

Consider another compelling reason for modifying plant species: The food supply for most of the human population is extremely vulnerable. Farmers usually want to plant crops that give them the highest yields. Over time, genetically similar varieties have replaced the more diverse, older varieties. However, genetic uniformity makes food crops far more vulnerable to many pathogenic fungi, viruses, and bacteria.

That is why botanists comb the world for seeds of the older, diverse varieties of plants and of the wild ancestors of potatoes, corn, and other crop plants. They send their prizes—seeds with genes of a plant's lineage—to **seed banks**. These safe storage facilities are designed to preserve genetic diversity. They are now being tapped by genetic engineers as well as by traditional plant breeders. Crop vulnerability is a huge problem. At one time, *Southern corn leaf blight* destroyed much of the United States corn crop. All of the plants carried the gene that conferred susceptibility to the fungal pathogen. Ever since that devastating epidemic, seed companies have been much more attentive to offering genetically diverse corn seeds. They tap seed banks, the treasure houses of plant genes.

product.

Transgenic plants help farmers grow crops more efficiently and with less impact on the environment.

Genetic engineers as well as traditional plant breeders are tapping seed banks, which are safe storage facilities designed to preserve genetic diversity of plants.



16.8 Biotech Barnyards

LINKS TO SECTIONS 5.2, 15.3 Laboratory mice were the first mammals to be genetically engineered. Today, featherless chickens, drug-producing goats, and transgenic pigs are part of the biotech barnyard.

TRANSGENIC ANIMALS

Traditional cross-breeding practices have produced unusual animals, including the featherless chicken in Figure 16.14. Now transgenic types are on the scene. The first ones arrived in 1982. Researchers isolated a gene for human somatotropin (growth hormone) and inserted it into a plasmid. They injected copies of the recombinant plasmids into fertilized mouse eggs that were later implanted into female mice. A third of the offspring of the surrogate mothers grew much larger than their littermates (Figure 16.15). The rat gene had become integrated into the host DNA and was being expressed in the transgenic mice.

Transgenic animals are used routinely for medical research. The functions of many gene products and how they can be controlled have been discovered by inactivating genes in "knockout mice" and analyzing the effect on phenotype (Section 15.3). Strains of mice, genetically modified mice to be susceptible to human diseases, help researchers study both the diseases and potential cures without experimenting on humans.

Genetically engineered animals also are sources of medically valued proteins. As a few examples, goats synthesize quantities of CFTR protein to treat cystic fibrosis and TPA protein to counter the bad effects of heart attacks. Rabbits make human interleukin-2, a protein that triggers divisions of immune cells called T lymphocytes. Cattle, too, may soon produce human



Figure 16.15 Evidence of a successful gene transfer. Two ten-week-old mouse littermates. *Left*, This one weighed 29 grams. *Right*, This one weighed 44 grams. It grew from a fertilized egg into which a gene for human somatotropin had been inserted.

collagen, which can be used to repair cartilage, bone, and skin. Goats make spider silk protein that might be used to make bullet-proof vests, medical supplies, and equipment for use in space. Different goats make human antithrombin, which is used to treat people with blood-clotting disorders (Figure 16.14*b*).

Genetic engineers have developed pigs that make environmentally friendlier manure. They have made freeze-resistant salmon, low-fat pigs, heftier sheep, and cows that are resistant to mad cow disease. Within a few years, they may give us allergen-free cats.



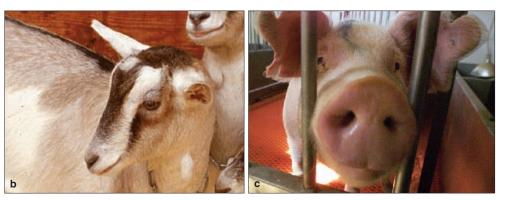


Figure 16.14 Genetically modified animals. (**a**) Featherless chicken developed by traditional cross-breeding methods in Israel. Such chickens survive in hot deserts where cooling systems are not an option. Chicken farmers in the United States have lost millions of feathered chickens in extremely hot weather. (**b**) Mira, a goat transgenic for human antithrombin III, an anticlotting factor. (**c**) Inquisitive transgenic pig at the Virginia Tech Swine Research facility.

Tinkering with the genetics of animals for the sake of human convenience does raise ethical questions. However, transgenic animal research may be viewed as an extension of thousands of years of acceptable barnyard breeding practices. Techniques have changed, but not the intent. Humans continue to have a vested interest in improving livestock.

KNOCKOUT CELLS AND ORGAN FACTORIES

Each year, about 75,000 people are on waiting lists for an organ transplant, but human donors are in short supply. There is talk of harvesting organs from pigs (Figure 16.14*c*), because pig organs function a lot like ours do. Transferring an organ from one species into another is called **xenotransplantation**.

The human immune system battles anything that it recognizes as "nonself." It rejects a pig organ at once, owing to a glycoprotein on the plasma membrane of cells that make up the blood vessels in pig organs. Antibodies circulating in human blood swiftly latch on to the sugar component and call for a response. In less than a few hours, blood inside the vessels coagulates massively and dooms the transplant. Drugs suppress this immune response, but a side effect is serious: the drugs make organ recipients vulnerable to infections.

Pig DNA contains two copies of *Ggta1*, the gene for an enzyme that catalyzes a key step in biosynthesis of alpha-1,3-galactose. This is the pig sugar that human antibodies recognize. Researchers have knocked out both copies of the *Ggta1* gene in transgenic piglets. Without the gene product, and the sugar, a pig tissue or organ may be less prone to rejection by the human immune system. Tissues and organs from such animals could help millions of people, including the ones with organs that have been severely damaged as a result of diabetes and Parkinson's disease.

Critics of xenotransplantation are concerned that, among other things, pig-human transplants would invite pig viruses to cross a species barrier and infect humans, perhaps catastrophically. Their concerns are not unfounded. In 1918, an influenza pandemic killed twenty million people worldwide. It originated with a swine flu virus—in pigs.

16.9 Safety Issues

Many years have passed since the first transfer of foreign DNA into a plasmid. That transfer ignited an ongoing debate about potential dangers of transgenic organisms entering the environment before rigorous testing.

In 1972, Paul Berg and his associates were the first to make recombinant DNA. Researchers knew that DNA was not toxic, but they could not predict what would happen every time they fused genetic material from different organisms into the recombinant molecules. Would they accidentally make superpathogens? Could they create a new form of life by the fusion of DNA from two normally harmless organisms? What if their creation escaped from the laboratory and transformed other organisms in the natural environment?

In a remarkably quick and responsible display of self-regulation, scientists reached a consensus on the safety guidelines for DNA research. Adopted at once by the NIH, their guidelines listed precautions for laboratory procedures. They covered the design and use of host organisms that could survive only under the narrow range of conditions inside the laboratory. Researchers stopped using DNA from pathogenic or toxic organisms for recombination experiments until proper containment facilities were developed.

As added precautions, "fail-safe" genes are now built into genetically engineered bacteria. They remain silent unless the bacteria escape and are exposed to environmental conditions—whereupon the genes get activated, with lethal results for the cell. Suppose that the package has a *hok* gene next to a promoter of the lactose operon (Section 15.4). Sugars are plentiful in the environment. If they were to activate the *hok* gene in a bacterial cell that escaped, the gene's product would destroy membrane function and the escapee.

Even so, does Murphy's law also apply to genetic engineering? As with any human endeavor, things can go wrong. After rabbits started taking over much of Australia, researchers were tinkering with a rabbitkilling virus in a containment laboratory on an island. Maybe the virus escaped in flying insects. However it happened, the virus is out and about, and killing lots of rabbits (Section 46.10). It is an example of why researchers are expected to expect the unexpected.

Genetic engineering started more than two decades ago. The kinds of animals being sought are beyond the scope of traditional breeding practices. Pigs engineered as donors for human organs are among the more startling cases. Rigorous safety guidelines for DNA research have been in place for decades in the United States. They have been adopted by the NIH, and researchers are expected to comply with their stringent standards. USING THE NEW TECHNOLOGIES

16.10 Modified Humans?

We as a society continue to work our way through the ethical implications of applying the new DNA technologies. Even as we are weighing the risks and benefits, however, the manipulation of individual genomes has begun.

WHO GETS WELL?

Human gene therapy is often cited as one of the most compelling reasons for embracing the new research. We already have identified more than 15,500 genetic disorders. Many are rare in the population at large. Collectively, however, they show up in 3 to 5 percent of all newborns, and they cause 20 to 30 percent of all infant deaths every year. They account for about half of mentally impaired patients and nearly a fourth of all hospital admissions. They contribute to many agerelated disorders that await all of us.

Rhys Evans, shown below, was born with a severe immune deficiency known as SCID-X1, which stems from mutations in gene *IL2RG*. Children affected by this disorder can live only in germ-free isolation tents, a "bubble," because they cannot fight infections.

In 1998, doctors withdrew stem cells from the bone marrow of eleven SCID-X1 boys. Stem cells, recall, are forerunners of other cell types, including white blood cells of the immune system. The doctors used a virus to insert nonmutated copies of *IL2RG* into each boy's stem cells, which they then infused back into his bone marrow. Months later, ten of the boys left isolation tents for good; gene therapy had successfully repaired their immune system. Since then, other gene therapy trials have freed many other SCID-X1 patients from life in a bubble. Rhys Evans is one of them.

In 2002, to the shock of researchers, two boys from the 1998 trial developed leukemia and one died. The researchers had anticipated that any cancer related to

the therapy would be extremely rare. The very gene targeted to do the repair work—*IL2RG*— may be a problem, especially when combined with the viral vector that delivered the gene into stem cells. One other child who took part in a gene therapy experiment for SCID-X1 has developed leukemia. That it developed at all is evidence that our understanding of the human genome lags behind our ability to modify it.

WHO GETS ENHANCED?

When all is said and done, the idea of using human gene therapy to cure genetic disorders seems like a socially acceptable goal to most of us. Now see if your comfort level can move CONNECTIONS

one step further. Would it also be acceptable to modify genes of some individual who falls within the normal range if he or she simply would like to minimize or enhance a particular trait?

We have already crossed the threshold of a brave new world. Researchers who are adept at transferring genes have already engineered strains of mice with enhanced memory and improved learning abilities. Perhaps their work is a beacon to those whose very lives have been turned upside down by Alzheimer's disease. Perhaps it draws others who are enchanted with the idea of simply getting more brain power.

The idea of selecting the most desired human traits is referred to as *eugenic engineering*. Yet who decides which forms of traits are most desirable? Realistically, cures for many severe but rare genetic disorders will not happen, because the payback for research is not financially attractive. Eugenics, however, might turn a profit. Just how much would potential parents pay to engineer tall or blue-eyed or fair-skinned children? Would it be okay to engineer "superhumans" with breathtaking strength or intelligence? How about an injection that would help you lose that extra weight and keep it off permanently? Where exactly is the line between interesting and abhorrent?

In a survey conducted in the United States, more than 40 percent of those interviewed said it would be fine to use gene therapy to make smarter and cuter babies. In one poll of British parents, 18 percent would be willing to use genetic enhancement to keep their child from being aggressive, and 10 percent would use it to keep a child from growing up to be homosexual.

Some argue that we must never alter the DNA of anything. The concern is that we just do not have the wisdom to bring about any genetic changes without causing irreparable damage to ourselves and nature.

One is reminded of our peculiar human tendency to leap before we look. And yet, something about the human experience gave us the capacity to imagine wings of our own making, a capacity that carried us to the frontiers of space. It gave one individual the dream of enhancing the rice plant genome to keep millions of children from going blind.

In this brave new world, two questions are before you: Should we be more cautious, because the risk takers may go too far? And what do we stand to lose if risks are not taken?

Be engaged; our understanding of the meaning of the human genome is changing even as you read this.

