7.5

Genetic Engineering

Aspen trees can reproduce by sprouting new, identical trees from their roots.

CAN YOU EXPLAIN IT?

Many organisms, such as jellyfish, fluoresce when exposed to particular wavelengths of light. Fluorescence occurs when an organism absorbs light and then emits it, appearing to glow. Fluorescent zebrafish can be purchased to enjoy in your aquarium. There's just one catch: zebrafish don't naturally fluoresce.

FIGURE 1: Zebrafish were genetically altered to fluoresce in many colors.

Gather Evidence As you explore the lesson, gather evidence to explain how a gene from one organism be inserted into the genome of an unrelated organism?

Fluorescent zebrafish are the result of decades of scientific research. Researchers at the National University of Singapore studying the green fluorescent protein (GFP) that causes fluorescence in jellyfish inserted the GFP-coding gene into zebrafish, resulting in a zebrafish that emitted green light.

The U.S. Food and Drug Administration (FDA) approved the sale of fluorescent zebrafish as pets in the United States. The FDA decided not to regulate the altered zebrafish because they were not intended to be part of the food supply. In addition, there was no research to suggest that the fluorescent strains would be more harmful to the environment than the original strains in case of accidental release.

Predict What does it mean to change the genome of an organism? Is this fluorescent zebrafish a new type of animal?

Isolating Genes

Huntington's disease causes nerve cells in the brain to break down. The onset of Huntington's often begins midlife, with no physical hints of the disease before symptoms arise. For those who have a parent with Huntington's disease, a Punnett square or pedigree analysis may provide a probability of having the disease, but not a definitive diagnosis. For Huntington's and many other diseases, genetic material can be tested to determine whether a person has, or is a carrier of, a specific disease.

Gather Evidence Would you undergo tests to determine your likelihood of having certain diseases? Why or why not? If you did, what would you want to happen to your genetic information? Should it be shared with scientific researchers, your health insurer, or your future employers? Explain your reasoning.

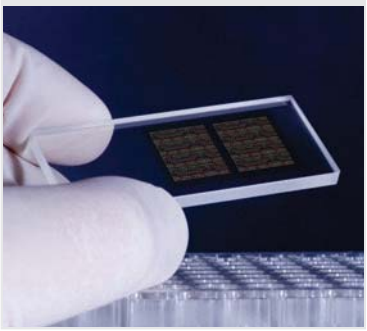
Genetic Testing

Genetic testing is the analysis of a person's DNA to determine the risk of having or passing on a genetic disorder. Geneticists test for abnormalities in genetic material, from entire chromosomes down to individual genes. It is also possible to test for proteins that indicate a particular disease. Since proteins reflect the DNA patterns of genes, this is an indirect method of testing genetic material. Genetic testing is a powerful tool to screen for genetic disorders. However, not all diseases can be found through genetic testing.

Analyze Why can't genetic testing identify all diseases? How does inheriting cystic fibrosis differ from developing cardiovascular disease due to poor diet and exercise?

There are thousands of genetic tests available, each targeting a specific gene or genomic region. DNA microarrays are tools that allow scientists to study many genes, or their expression, at once. A microarray is a small chip that is dotted with all of the genes being studied. The genes are laid out in a grid pattern. Each block of the grid is so small that a one-square-inch chip can hold thousands of genes. Microarrays, such as the one shown in Figure 2, help researchers find which genes are expressed in which tissues, and under what conditions.

FIGURE 2: DNA microarrays are used in genetic testing.



Collaborate In a group, discuss the benefits, risks, and limitations of genetic testing. Why is it important to identify carriers of a genetic disease? How should genetic information be used and safeguarded?

Replicating Genes

Genetic tests are useful for genes that have been linked to a disease, but identifying specific genes that cause disease is not simple. Scientists spend years finding genes that are associated with a particular disease among the 20,000–25,000 genes in the human genome. Small quantities of target sequences collected from patients must be amplified many times to produce the amount needed for testing. The invention of the polymerase chain reaction (PCR) was a turning point, making it possible to obtain the large amounts of DNA needed for genetic testing in hours instead of days.

FIGURE 3: The steps of the polymerase chain reaction.

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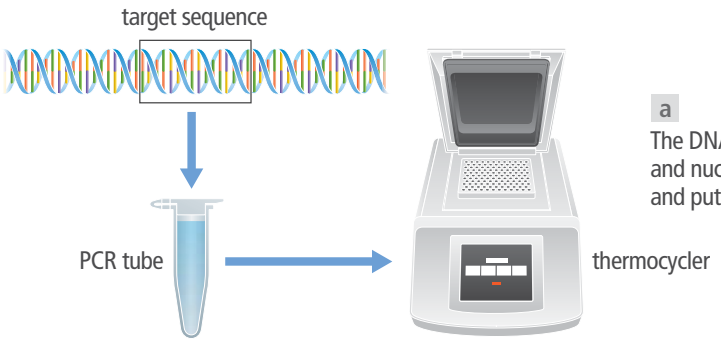
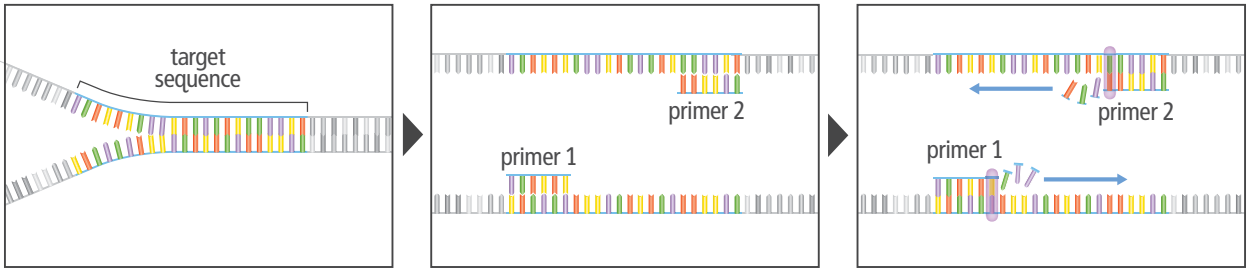


a The DNA sample, primers, DNA polymerase, and nucleotides are placed in the PCR tube and put in the thermocycler.

Cycle 1

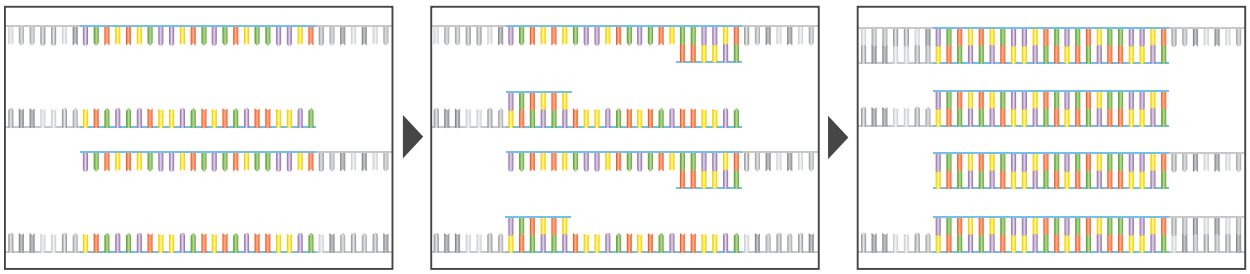


b Separating
The temperature is raised to 95 °C (203 °F) to separate the DNA strands.

c Binding
The temperature is cooled to 55 °C (131 °F), and the primers bind to the DNA strands.

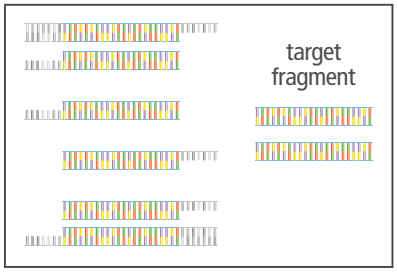
d Copying
The temperature is heated to 72 °C (152 °F). DNA polymerase locates the primers and begins synthesizing a complementary strand. It continues to synthesize the DNA strand until it reaches the end of the strand.

Cycle 2 The same three steps occur in Cycle 2 and each subsequent cycle: separating, binding, and copying.



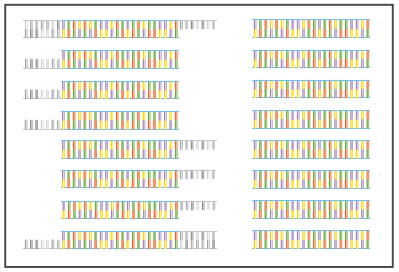
Separating **Binding** **Copying**

Cycle 3



At the end of Cycle 3, fragments that include only the target DNA have been synthesized.

Cycle 4



By the end of Cycle 4, eight target fragments have been synthesized.

Cycle 30

After 30 cycles, more than a billion fragments have been synthesized.

Figure 3a shows the beginning of a PCR run. DNA is extracted from cell nuclei and added to a PCR tube, along with primers, DNA polymerase, and nucleotides. The tube is placed inside a thermocycler, which automatically regulates the temperature of the solution.

The polymerase chain reaction occurs in three steps:

Separating The thermocycler heats the sample until the complementary strands of DNA separate (Figure 3b). Separation occurs around 95 °C (203 °F).

Binding The thermocycler then cools to around 55 °C (131 °F) (Figure 3c), and primers bind to the separated DNA strands. Primers are short nucleotide segments that allow a specific type of DNA polymerase to attach to the DNA strands. Two primers are required for each reaction. One primer attaches to the beginning of the target segment on one strand of DNA. The other primer attaches to the beginning of the target segment on the complementary strand of DNA.

Copying The thermocycler heats to 72 °C (162 °F) (Figure 3d). At this temperature, DNA polymerase attaches to the primer segments and begins adding complementary nucleotides. The free nucleotides added to the solution act as building materials for the new strands of DNA. DNA synthesis continues until the DNA polymerase reaches the end of the strand and detaches. A complementary strand of DNA is produced, and the first PCR cycle is complete.

Collaborate With a partner, take turns explaining and modeling how the three steps of PCR produce DNA sequences. While you walk your partner through the steps, explain the significance of the following terms: *DNA polymerase*, *nucleotides*, *primers*, *DNA separation*, *primer binding*, *DNA synthesis*, and *thermocycler*. Then, your partner explains and models the process to you. Continue to take turns until both of you feel comfortable with the steps of PCR.

The cycle is repeated a second time. The thermocycler heats to 95 °C and the DNA strands separate. The thermocycler cools to 55 °C and primers bind to the target sites. Finally, the thermocycler heats to 72 °C. DNA polymerase attaches to primer segments and synthesizes a complementary strand of DNA using the free nucleotides.

The thermocycler continues to heat and cool the solution automatically. The first fragment of the target DNA sequence is synthesized after the third cycle. More than one billion fragments of target DNA are synthesized after thirty cycles. PCR cycles continue until an adequate amount of the target DNA is produced.

Analyze Why is it necessary to keep changing the temperature in the PCR process? Use evidence to support your claim.

The polymerase chain reaction was invented by Kary Mullis in 1983, who shared the Nobel Prize in Chemistry in 1993. This invention solved two problems Mullis was facing. First, his lab was trying to create a new use for the oligonucleotides, or short DNA segments, they produced. PCR uses oligonucleotides as primers. Second, genetic testing and other DNA-related tests took weeks to perform. PCR greatly decreased the time required to amplify a DNA sample.

Explain Describe the relationship between genetic testing and the polymerase chain reaction. How has the PCR technique made genetic testing possible on a large scale?

Patterns

DNA replication produces a complementary strand of DNA, while PCR amplifies a target section of DNA by copying just that section. How else are DNA replication and PCR similar? How are they different?

Cloning and Engineering

FIGURE 4: These cereal plants can grow in soil with little water.

As the world's population increases, so does the demand for food. Long periods of drought in many areas of the world threaten food production because many commercial crops are not adapted to dry climates. To maintain food production as land becomes drier, scientists engineered plants that are drought resistant.

Gather Evidence Other strategies for growing food in dry climates include water conservation, sustainable farming practices, and improved fertilizers. Make a list of possible criteria for evaluating drought-resistant crops along with the other solutions.

Cloning Organisms

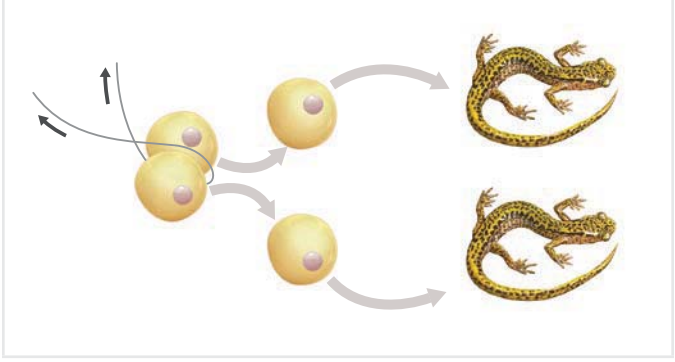
FIGURE 5: Some plants produce "pups," or genetically identical offspring.

Many plants produce genetically identical offspring, or clones, through asexual reproduction. Humans have cloned plants for thousands of years by taking cuttings from one plant and planting them, producing clones. When the offspring, or "pup," of a spider plant, shown in Figure 5, is planted, a genetically identical plant grows. Humans clone plants with desirable traits, such as bigger or more flavorful fruit. Eventually these traits appear more often in the new population.

Bacteria produce clones through binary fission, a type of asexual reproduction. In binary fission, a bacterial chromosome is replicated. The cell splits into two daughter cells that are genetically identical to the mother cell. Making clones ensures beneficial traits, such as resistance to antibiotics, spread quickly in a bacterial population.

Cloning has a low success rate in more complex organisms, such as vertebrate animals. Advances in genetic engineering, though have made it possible to produce artificial mammalian clones. The sections below describe breakthroughs in cloning.

FIGURE 6: The embryo twinning process.



Embryo Twinning

In 1903, Hans Spemann separated the cells of two-celled salamander embryos. The separated cells continued to develop normally, resulting in two salamanders (Figure 6). Spemann determined that vertebrates can be "twinned" to form identical organisms. This experiment showed that embryonic cells have a full set of genetic material. So, each cell has the potential to grow into a complete organism.

Nuclear Transfer

Cloning mammals involves replacing the nucleus of an unfertilized egg with the nucleus of a cell from the animal that is being cloned. The egg cell is implanted into a surrogate mother to develop as it would during a normal pregnancy. The resulting offspring is a clone. Some of the milestones in nuclear transfer are shown in Figure 7.

FIGURE 7: Milestones in the advancement of cloning techniques.

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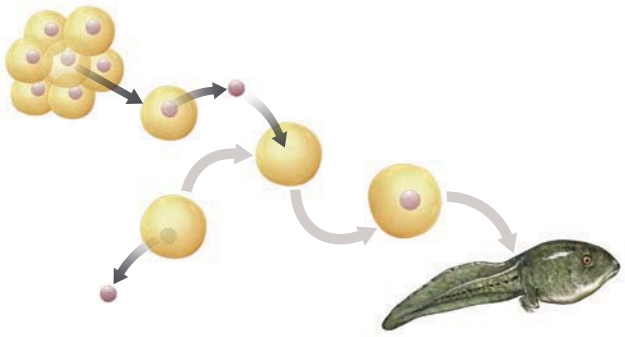
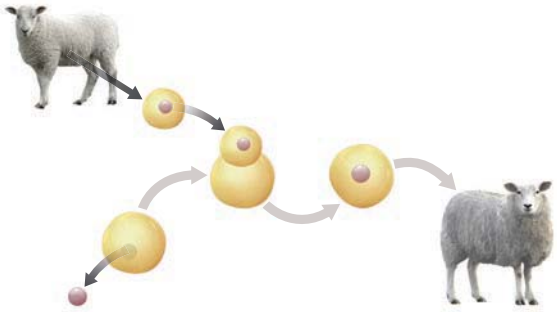


a Embryonic cell nuclear transfer



b Somatic cell nuclear transfer

In 1952, Robert Briggs and Thomas King performed the first successful nuclear transfer (Figure 7a). The nucleus from an embryonic frog cell was inserted into an egg cell with its nucleus removed. The egg cell then developed into a tadpole. This experiment demonstrated that nuclear transfer could be used to clone organisms.

Scientists later adapted nuclear transfer methods to produce clones of other animals, including mammals. Further research led to new techniques which allowed the use of other cell types as nuclear donors, eliminating the need to use embryos.

In 1996, Dolly the sheep became the first mammal cloned from an adult somatic, or body, cell (Figure 7b). Somatic cells are differentiated, so many genes not necessary for the cell's function are deactivated. These genes must be reactivated for cloning to succeed. Of 277 attempts in this experiment, only Dolly survived.

Cloning After Dolly

Milestones in cloning after Dolly include cloning primates, producing sheep from genetically engineered cells, cloning endangered animals, and creating stem cells from somatic cell nuclear transfer. New advances in cloning have raised ethical concerns, such as concerns regarding human cloning.

Pet cloning is one of these advancements. Several companies offer cloning services that will produce an exact genetic copy of a pet. Though they are genetically identical, these animals often look and act differently than the original pet.

Gather Evidence Why is a clone not an exact copy of a donor animal? Consider the effect of genetics and environmental conditions. Use evidence to support your answer.

FIGURE 8: A cloned puppy with the genetic father.

Cloning Ethics

Henrietta Lacks died of cervical cancer in 1951. Before she died, a researcher took a sample of her tumor. From this sample, scientists made the first "immortal" cell line, named HeLa for the first two letters of Henrietta's first and last names. Unlike other cells, HeLa cells did not die when cultured in the lab. The cells divided indefinitely, providing a never-ending source of cells for scientific research. From the polio vaccine and cloning to AIDS research and experiments in space, HeLa cells have been a cornerstone of science for more than half a century.

FIGURE 9: Henrietta Lacks

Most of this research took place without the knowledge or permission of Henrietta Lacks or her family. This raises the issue of cloning ethics. Ethics are principles that set standards of right or wrong for a person or group. As advances in genetics continue, discussions about ethics and treatment of genetic material become more important.

Language Arts Connection Further research the story of Henrietta Lacks. Should individuals have control over their genetic material? How would you feel if your genetic material was taken without permission? Use evidence to support your claims.

Engineering Genes

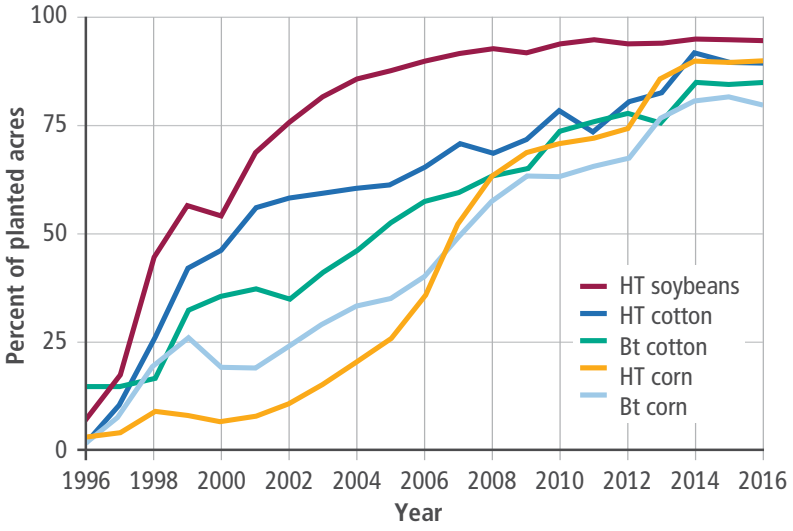
Genetic engineering is the process of altering the genetic material of an organism, changing its traits or introducing a new, desirable trait. Once a desirable trait has been successfully inserted into a genome, the new genome—and trait—can be passed on to future generations using cloning. An organism with one or more genes from another organism inserted into its genome is called a transgenic organism.

Genetically modified (GM) crops are becoming more widely used by farmers. If a farmer plants clones of GM crops, then he or she knows the desired trait is present in the entire population. However, this would also decrease genetic diversity, a necessary feature for a robust and flexible population.

Analyze Compare the risks versus the benefits of using cloned, GM plants instead of GM plants propagated through sexual reproduction.

Adoption of Genetically Engineered Crops in the U.S., 1996-2016

FIGURE 10: The usage of genetically engineered crops in the United States.



Sources: USDA, Economic Research Service using data from Fernandez-Comejo and McBride (2002) for the years 1996–99 and USDA, National Agricultural Statistics Service, *June Agricultural Survey* for the years 2000–16.

In the early 1990s, the FDA approved genetically engineered plants for human consumption in the United States. Insect resistance and herbicide resistance are among the most common genetic modifications in crops, as shown in Figure 12. Much of the genetically modified corn produced is fed to livestock, but GM corn does appear in the human food supply as ingredients such as high-fructose corn syrup and corn starch. No long-term studies have found negative side effects from eating GM plants.

Genetic Engineering in Bacteria

Recombinant DNA technology, combining the genes from more than one organism, is a key element of genetic engineering. The organisms can be from the same species or different species. One method of producing recombinant DNA is to add foreign DNA to a plasmid. In bacteria, a plasmid is a small, circular segment of DNA that is separate from the bacterial chromosome. The foreign DNA that is inserted into the plasmid is then expressed by the bacteria.

Bacteria naturally recombine their DNA by absorbing plasmids from the environment or by exchanging plasmids between two bacteria. There can be multiple plasmids within a bacterium, and each one is able to replicate independently from the bacterial chromosome. Genetically modified bacteria are able to produce antibiotics, insulin, therapeutic proteins, and other types of proteins.

Imagine foreign DNA containing a gene for producing human insulin is inserted into a plasmid. Because plasmids self-replicate, numerous copies of a plasmid can exist within a bacterium. Plasmids are shared with daughter cells during binary fission, and bacteria divide at relatively fast speeds. A handful of bacteria with a plasmid coding for human insulin can quickly become a manufacturing center for a protein.

Collaborate Genetically engineering bacteria to produce drugs can be cheaper than producing the drugs in a lab. Discuss the impacts cheaper drugs may have on society and science.

FIGURE 11: Bacterial plasmid

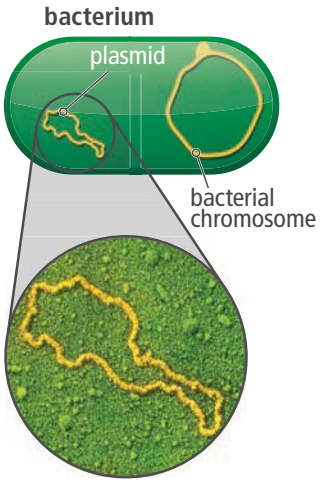


FIGURE 12: Recombinant DNA.

Engineering

Editing Genes with CRISPR

Genetically engineering organisms requires the ability to cut DNA strands in specific places. Precisely cutting DNA can be difficult, time-consuming, and costly work. To solve this problem, genetic engineers needed to find an easier, faster, and cheaper method for precisely cutting DNA.

As it turns out, bacteria use a mechanism for precise DNA cuts called CRISPR, named for the clustered regularly interspaced palindromic repeats (CRISPRs) in bacterial DNA. These repeated sequences surround segments of viral DNA that bacteria have been exposed to. An enzyme uses the information in this viral library to target and cut viral DNA, preventing viral replication.

CRISPR is exciting for genetic engineers because it provides a very precise method for cutting DNA at a specific point. Cutting DNA easily and accurately simplifies the process of replacing defective genes with functional genes. This is one of the more difficult tasks in gene therapy, but one with the greatest potential benefits to humans. New ways to apply the CRISPR system to scientific problems are still being discovered. As with most genetic advances, the excitement surrounding the prospective benefits of CRISPR is tempered by the ethical concerns raised by such a powerful gene-editing tool.

Gather Evidence In what ways do you think CRISPR can advance the field of genetic engineering? What concerns do you think people might have about CRISPR?

Genetic Engineering in Plants

One of the most common methods for genetic modification in plants is the use of bacterial plasmids. A gene for a desired trait is inserted into a plasmid, and the plasmid is added to a plant cell. When the plant cell is infected, the recombinant DNA is inserted directly into the plant genome, modifying the plant. The plant expresses the bacterial DNA as well as its own.

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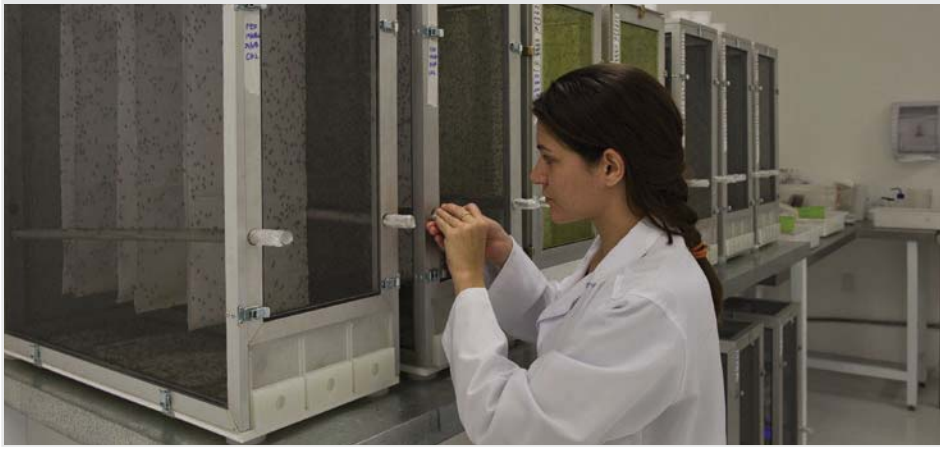
Hands-On Lab

Modeling Genetic Engineering
Simulate the techniques used by genetic engineers to modify genes in humans using recombinant DNA technology.

Genetic Engineering in Animals

Animal models of human diseases are valuable tools in medical research. These models allow scientists to study the disease process, from the genetic basis of a disease to how it responds to chemical substances. Through genetic engineering, scientists have been able to develop more and better models to study disease.

FIGURE 13: A scientist studies genetically modified mosquitoes.



Model Draw a flow chart that demonstrates how genetically engineering mosquitoes can reduce the risk of illness in humans.

Consider the use of genetically modified mosquitoes to prevent the spread of disease. Mosquitoes act as vectors for many diseases. A vector carries foreign DNA into another cell or organism. One species, *Aedes aegypti*, is known to transmit the viruses for yellow fever, chikungunya, dengue, and Zika. Dengue is one of the leading causes of illness and death in tropical and subtropical regions. There is no vaccine for dengue, and the best way to minimize dengue cases is to minimize bites from infected mosquitoes.

To solve this problem, scientists engineered mosquitoes so they required a human-made drug to survive. When modified male mosquitoes are released into wild populations, they breed with wild females, passing the drug-dependency gene to their offspring. The affected males die soon after breeding, and any offspring die before maturity without access to the drug. Several field trials demonstrated that release of mosquitoes modified in this way can effectively control mosquito populations.

The possibility of unintended effects is a big constraint to this solution. The potential unintended effects of releasing genetically engineered mosquitoes into the wild is not fully understood. There may be tradeoffs for scientists and society between the risks of unintended effects and the benefits of smaller mosquito populations.

Explain There typically are tradeoffs when selecting a solution to a problem. Genetically engineered crops may be able to help farmers produce greater yields, but a tradeoff is the reduction in the genetic variation in crops, making crops more susceptible to disease. What other tradeoffs exist for this solution?

Engineering

Genetically Engineering Salmon

Demand for Atlantic salmon has increased, and wild populations of salmon have decreased, mainly due to overfishing and other environmental impacts. Struggling wild populations suggest that commercial fishing is not sustainable at current rates. Farm-raised Atlantic salmon is an alternative to wild-caught fish and reduces pressure on wild populations by providing a reliable salmon source. Salmon can be farmed in ocean pens or in land-based facilities. Some farm-raised salmon are bred for advantageous traits, such as disease resistance.

Farming has drawbacks, though. Farms require space and resources to feed, house, and maintain the fish as they grow. The salmon typically take 28-36 months to reach market weight, and production costs can drive up the price.

FIGURE 14: A normal Atlantic salmon compared to a GM Atlantic salmon of the same age.

Analyze Define the engineering problem outlined for salmon production. What are the important criteria? What constraints might exist for a solution that reduces the stress on wild-caught salmon and on farmers raising these fish?

To solve some of the problems with farming salmon, scientists produced genetically engineered Atlantic salmon. They inserting a growth hormone from Chinook salmon and a promoter from ocean pout into the genome. The promoter allows the growth hormone to be active all year, instead of only part of the year as in normal salmon.

Transgenic Atlantic salmon grow to twice the size of normal Atlantic salmon in the same amount of time. This decreases the time to market weight to as few as 18 months, compared to up to 36 months in normal salmon. So, farmers are able to grow and sell more salmon in a given time period. There are also environmental benefits such as decreased usage and contamination of water resources. The genetically modified salmon are raised in land-based facilities with pollution management and water recycling systems. Genetically modified salmon reduce the impact on wild populations and aquatic ecosystems.

There is still public resistance to eating genetically modified organisms. This represents a social challenge to the success of farming genetically engineered salmon. One of the biggest environmental concerns is the possibility that a GM individual may escape and breed with wild individuals. This could introduce the modified gene into wild populations through any offspring produced, with unknown long-term effects on wild Atlantic salmon or other species.

Explain Design a decision matrix and use it to analyze criteria for the use of commercial fishing, normal salmon farming, and GM salmon farming in meeting the demand for salmon. Weight the criteria on a scale of 0 to 5. What is the best solution based on your criteria? Are there any problems with this solution that can be anticipated and avoided?

Further Applications of Genetic Engineering

Collaborate With a partner, discuss the benefits and risks of transgenic mosquitoes for humans and ecosystems.

New technologies can have unforeseen impacts on society and the environment. The positive effects of controlling mosquito populations with transgenic mosquitoes are clear: reduced illness and death due to infections from mosquito-borne viruses. There are also negative effects to this solution, though, that may be hard to believe. Mosquitoes may be pests for humans, but they are a food source for other animals.

Impacts on Conservation

In the future, ecosystems may undergo rapid change due to climate change, habitat destruction, and human influence. Populations may be forced to adapt or move to new habitats to survive. This is a problem because natural selection, the mechanism by which populations adapt, is not a rapid process and works over many generations. Scientists are looking for ways to help threatened species.

FIGURE 15: The ‘i’iwi.

Hawaii had no mosquitoes until the early 1800s when a whaling vessel carrying water from Mexico brought them to the islands. Today, avian malaria, carried by these invasive mosquitoes, has decimated the native bird population. The ‘i’iwi, or Hawaiian honeycreeper, and other birds native to Hawaii are going extinct. Many scientists think the only way to save these birds is to wipe out the mosquito population. Scientists are considering releasing GM mosquitoes that will die prematurely, reducing the mosquito population and hopefully saving Hawaii’s native birds.

For species threatened by climate change or low genetic diversity, scientists are investigating a process known as facilitated adaptation. Facilitated adaptation involves humans guiding adaptations in threatened populations by changing the species’ genome. Advantageous genes can be added to a genome through hybridization, selective breeding, or genetic engineering using recombinant DNA technology. For example, scientists are considering inserting genes from species that can tolerate higher temperatures into different species suffering from global warming.

One drawback of facilitated adaptation is the possibility of unintended effects related to changing genomes that have evolved over millions of years. Scientists may be able to identify the main function of a gene, but they cannot determine all the ways a gene interacts with the rest of the genome. Loss of function, or an unintended new function, may occur by changing an organism’s genome. Facilitated adaptation could also lead to an unintended loss of genetic diversity. If the genetically engineered individuals are much more successful than normal individuals, that single gene could become widespread in the population.

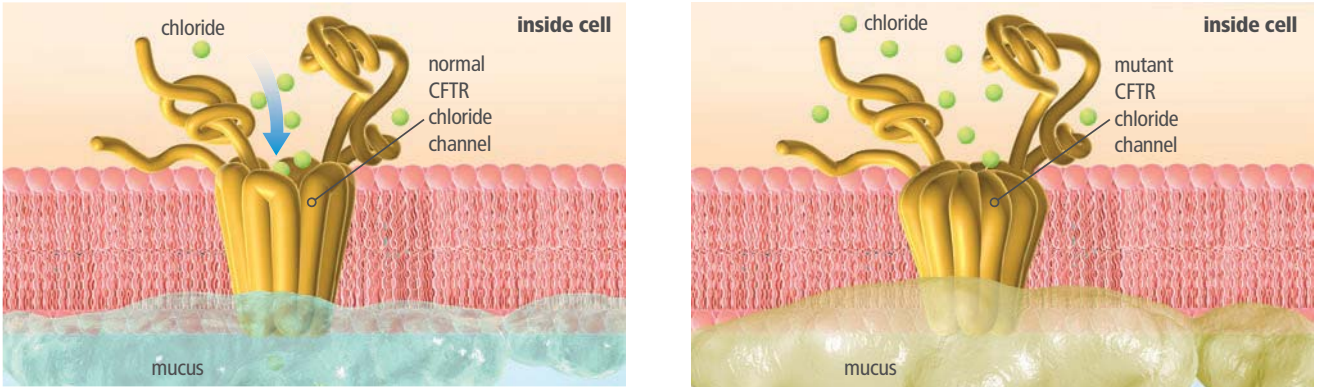
Engineering Define a problem facing conservation. Explain what role genetic engineering could play in solving that problem. Use evidence to support your claims.

Gene Therapy

Gene therapy uses genetic engineering to treat or prevent the genetic basis of disease. A common gene therapy technique uses a delivery mechanism, or vector, such as a bacterium or virus, to deliver a new gene to target cells. Once the gene enters the cells, the new DNA is transcribed and the new protein is expressed.

Not all diseases are good candidates for gene therapy. For example, a disease caused by the interaction of multiple genes is not a good candidate because the necessary modification of genes would be too complex. Also, if the genetic basis for a disease is not understood, it is not a good candidate. Scientists need to know which gene to modify to combat the disease. If the biology of the disorder is not understood, the disease is also not a good gene therapy candidate. Finally, if there is no way to get new genetic information to affected cells, the disease is not a good candidate for gene therapy.

FIGURE 16: Cystic fibrosis is caused by a mutated CFTR protein.



a Normal CFTR protein

b Mutated CFTR protein

Cystic fibrosis (CF) is an inherited disease that affects the respiratory and digestive systems. Airways and some organs are naturally lined and protected by a layer of mucus. Cystic fibrosis causes abnormal, sticky mucus secretions in these areas. Symptoms include coughing and wheezing, digestive problems, and increased probability of infections. The most common cause of death in untreated CF patients is a fatal lung infection.

The protein that regulates mucus secretion in the respiratory, reproductive, and digestive systems is encoded by the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene. A normal version of the *CFTR* gene produces a protein that acts as a channel to move chloride ions across the cell membrane in mucus-producing cells. This helps regulate the water content of surrounding tissues, leading to normal, moist mucus. A mutated gene leads to disruption of the chloride channels, lowering the water content of nearby cells. This causes the thick, sticky mucus characteristic of cystic fibrosis.

Cellular functions are highly related to the structure of DNA. In the case of cystic fibrosis, the change in the DNA sequence of the mutated *CFTR* gene results in a different amino acid sequence in the CFTR protein. Typically, a phenylalanine amino acid is missing from the protein sequence. When this protein is expressed, the abnormal structure leads to a loss of protein function.

As shown in Figure 16a, with normal CFTR function, chloride ions move across the cell membrane and congregate on the outside of the cell, making an ionic gradient. The hypertonic solution outside of the cell attracts more water and maintains mucus of a normal consistency. A healthy, watery mucus layer traps particulates and bacteria before they can harm the cell. The cilia of the cell are free to move and sweep away the foreign matter.

Gather Evidence Does cystic fibrosis meet the criteria to be considered for gene therapy? Use evidence to support your claims.

In a person affected by cystic fibrosis, the irregular protein produced by the mutated *CFTR* gene cannot transport chloride ions across the cell membrane, as shown in Figure 16b. This loss of protein function results in a higher concentration of chloride and sodium ions inside the cell and a lower concentration of these ions outside of the cell. The hypotonic solution causes water to move into the cell, drying out the mucus layer. The thick, sticky mucus prevents the cilia from moving and clearing debris. The increased presence of debris and pathogens causes increased infections in individuals with cystic fibrosis.

Engineering

Developing Approaches to Gene Therapy

The problems gene therapy attempts to solve are broad and span many kinds of diseases, from genetic immune disorders to cancers. Many different approaches are required to solve these problems. To alleviate respiratory symptoms of cystic fibrosis (CF), for example, scientists need to deliver a functioning copy of the *CFTR* gene to lung cells. However, it is hard to access and modify every lung cell. A solution to this problem is to deliver the gene therapy through an aerosol that patients inhale. Affected cells that receive a functioning copy of the gene will begin to show normal gene expression, which alleviates the symptoms of cystic fibrosis.

Analyze A loss-of-function mutation results in a mutated protein that does not function correctly. How could gene therapy treat this type of genetic disorder?

Gene therapy is not always this straightforward. For example, some mutations produce a dominant-negative protein. This type of mutated protein does not do its job correctly and also blocks normal proteins from functioning. Simply delivering a working copy of the gene to affected cells won't work because the dominant-negative protein would still block the function of normal proteins. A solution to this problem is to "silence," or turn off, the mutated gene so that no protein is produced. Huntington's disease produces a dominant-negative protein and is a promising candidate for gene-silencing therapies.

Gene therapy relies on many different biotechnologies. Without genetic testing, it would be harder to determine which patients would benefit from gene therapy. The genes required for insertion into affected cells are produced through PCR. Without the rapid amplification of DNA through PCR, gene therapies would take much longer to produce. CRISPR is a relatively new tool, but it is already affecting gene therapy by making it easier to cut and edit DNA segments of a mutated gene.

Explain Think back to the fluorescent zebrafish from the beginning of this lesson. Using this example, explain some implications of being able to edit genes. Where do you think science will go from here?

Language Arts Connection

Knockout Mice

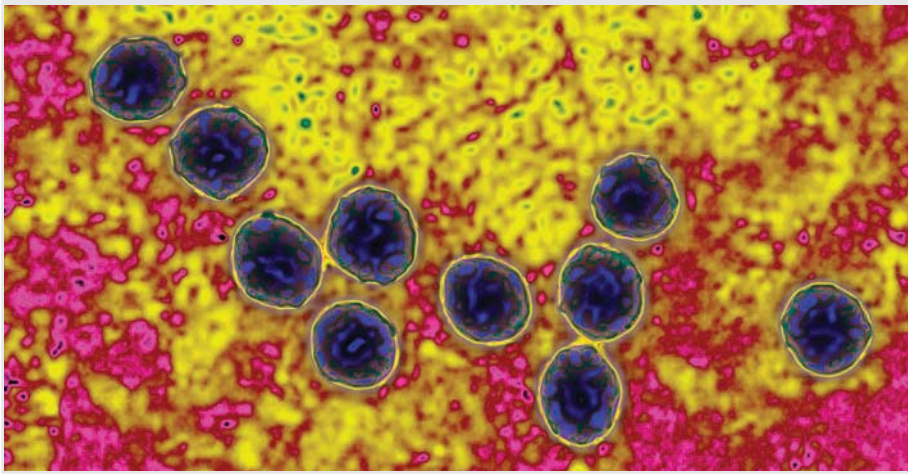
Knockout mice have a gene of interest knocked out, which means the gene is turned off. Knockout mice are often used in genetic engineering, allowing researchers to study structure and function in gene expression. Many knockout mice are named for the gene that has been deactivated. For example, the p53 knockout mouse does not have the *p53* gene, which produces a protein that stops tumor growths. This line of mice is susceptible to cancer. Other mice have genes knocked out that affect obesity, anxiety, and other traits.

Knockout mice have been used in thousands of experiments studying many different diseases. Recently, knockout mice helped scientists confirm the link between Zika infections in pregnant women and birth defects.

The Zika virus was identified in humans in 1952. The first major outbreak of Zika occurred in 2007. Another major outbreak occurred in 2016 and scientists started to study the effects of Zika infections in more detail. Of particular interest were reports that the Zika virus causes microcephaly, a birth defect characterized by a small head and abnormal brain development. Scientists needed to learn more about the link between Zika and birth defects to accurately advise the public on the risks of Zika infections.

Mice are not ideal models for testing the effects of Zika because the mouse immune system prevents a sustained Zika infection. To solve this problem, a group of scientists knocked out a key immune system gene. When the gene was not expressed, the Zika virus could replicate within pregnant mice. None of the fetuses survived, but scientists did find concentrations of the Zika virus in the placenta that were 1000 times higher than the concentration of Zika in the mother's blood. The placenta is responsible for supplying blood to the fetus. A high viral concentration in the placenta supports the hypothesis that Zika affects the placenta, thereby harming the fetus.

FIGURE 17: The Zika Virus



The Zika virus was also found in the heads of the fetuses. This suggests that Zika directly affects brain development. Scientists have continued the Zika research using knockout mice and other techniques, and there is now a confirmed link between Zika and birth defects in humans.

Knockout mice provide a valuable model for studying the effects of gene expression, but there are limitations. Some genes behave differently in mice than in humans. A knocked-out gene may fail to produce a response in mice when the gene is known to cause a response in humans. Or the gene may cause a different response in humans than is seen in mice. These constraints must be considered when developing or selecting a knockout mouse model for an experiment. Knockout mice are imperfect models in these cases, though they still may provide some information about the function of genes.

Language Arts Connection

Answer the following questions in your Evidence Notebook. Use evidence from the text to support your answers:

1. What happens when a gene is knocked out in a mouse?
2. How are structure and function related in the development of knockout mice?
3. What is a limitation of using knockout mice for disease models?

LABELING GMOs | MODELING GENETIC ENGINEERING | BACK FROM EXTINCTION
Go online to choose one of these other paths.

Lesson Self-Check

CAN YOU EXPLAIN IT?

FIGURE 18: Genetically modified zebrafish.

Fluorescent zebrafish are genetically modified. Originally, fluorescent color genes from jellyfish and sea anemones were inserted into zebrafish eggs. The color genes became part of the zebrafish DNA. It is now a heritable trait that is passed to offspring. Current generations of fluorescent zebrafish are born, not modified, but their roots lie in genetic modification.

Explain Refer to the notes in your Evidence Notebook to answer the following questions:

1. How can a gene from one organism be inserted into the genome of an unrelated organism?
2. Does genetically altering an organism make a new species?
3. What are the implications of genetic engineering?

The green fluorescent protein (GFP) is used for more than creating glow-in-the-dark pets. When a GFP sequence is added to a gene, the translated protein will include the green fluorescent protein, which glows. This glowing tag allows scientists to track the protein in the organism. Knowing where, when, and how often a protein is made is important for understanding what abnormal expression of a protein looks like. GFP has been modified to produce a range of colors. The different colors are used by scientists to track multiple proteins at the same time.

Research performed using GFP tags includes exploring cell behavior during embryonic development, monitoring cell death during apoptosis, and studying insulin cells in the pancreas. Processes that are difficult to monitor directly, such as the growth of a neuron or tumor, can be tracked using GFP-tagged proteins.

CHECKPOINTS

Check Your Understanding

1. What is the difference between genetic engineering and cloning?
   - **a.** Genetic engineering is governed by an international ethics committee. Cloning does not have any formal ethics oversight.
   - **b.** Genetic engineering uses PCR and CRISPR. Cloning does not use PCR or CRISPR.
   - **c.** Genetic engineering focuses on changing an organism's genome, while cloning focuses on exactly copying genetic material.
   - **d.** Genetic engineering refers to gene manipulation in humans. Cloning refers to gene manipulation in all other species.

2. Place the elements in order to model how mosquito populations can be controlled using genetic engineering.
   - **a.** affected males and affected offspring die
   - **b.** insertion of gene into mosquito embryo
   - **c.** release of affected male mosquitos to the wild
   - **d.** development of drug-dependency gene
   - **e.** breeding of genetically modified mosquitoes
   - **f.** affected males mate with wild females

3. What would happen if a thermocycler malfunctioned during a PCR run and never heated the solution?
   - **a.** The DNA polymerase used to separate the DNA strands would not be activated.
   - **b.** The PCR would proceed at a slower rate.
   - **c.** The primers would not bind to the target DNA sites, and DNA synthesis would not occur.
   - **d.** The DNA strands would never separate, and the PCR would never begin.

4. Which of the following is not a criterion for a disease being a good candidate for gene therapy?
   - **a.** genetic information can be distributed to new cells
   - **b.** biology is understood
   - **c.** genetic basis of disease is identified
   - **d.** controlled by one gene up to a handful of genes
   - **e.** none of the above

5. How does the mutated CFTR protein contribute to cystic fibrosis?
   - **a.** The mutation prevents the channel protein from moving chloride across the membrane, resulting in a thick, sticky mucus.
   - **b.** The mutation prevents the channel protein from moving sodium across the membrane, resulting in a thick, sticky mucus.
   - **c.** The mutation causes the channel protein to produce the sticky, thick mucus.
   - **d.** The mutation attracts more mucus to the channel protein.

6. How is CRISPR used in genetic engineering?
   - **a.** to clone cells
   - **b.** to cut DNA
   - **c.** to insert foreign DNA in a chromosome
   - **d.** to test for genetic conditions

MAKE YOUR OWN STUDY GUIDE

In your Evidence Notebook, design a study guide that supports the main ideas from this lesson:

Genetic engineering is used to solve many societal and environmental problems, but there are benefits and risks associated with genetic engineering.

The ethical considerations of cloning and genetic engineering are complex. Scientists must balance scientific progress with the concerns of the public.

Remember to include the following information in your study guide:

- Use examples that model main ideas.
- Record explanations for the phenomena you investigated.
- Use evidence to support your explanations. Your support can include drawings, data, graphs, laboratory conclusions, and other evidence recorded throughout the lesson.

Consider how genetics, engineering, technology, and society influence and affect each other.