Chapter 10

Biotechnology

10.1 Lesson 10.1: DNA Technology

Lesson Objectives

- What is meant by DNA technology?
- What is the Human Genome Project?
- Describe the goals of the Human Genome Project.
- Describe gene cloning and the processes involved.
- What is PCR?
- Describe the processes involved in PCR.

Introduction

Is it really possible to clone people? Another question is, should we clone people? Are scientific fantasies, such as depicted on TV shows such as *Star Trek* or in the movie *GATTACA*, actually a possibility? Who can really say? How, really, will science affect our future? The answers partially lie in the field of biotechnology.

Biotechnology is technology based on biological applications. These applications are increasingly used in medicine, agriculture and food science. Biotechnology combines many features of biology, including genetics, molecular biology, biochemistry, embryology, and cell biology. Many aspects of biotechnology center around DNA and its applications, otherwise known as DNA technology. We could devote a whole textbook to current applications of biotechnology; in this chapter, however, we will focus on the applications towards medicine and the extension into the forensic sciences. First, though, we need to understand DNA technology.

DNA Technology

What is DNA technology? Is it using and manipulating DNA to help people? Is it using DNA to make better medicines and individualized treatments? Is it analyzing DNA to determine predispositions to genetic diseases? The answers to these questions, and many more, is yes. And the answers to many of these issues begin with the Human Genome Project.

The Human Genome Project

If we are all 99.9% genetically identical, what makes us different? How does that 0.1% make us tall or short, light or dark, develop cancer or not? To understand that 0.1%, we also need to understand the other 99.9%. Understanding the human genome is the goal of **The Human Genome Project (HGP)**. This project, publicly funded by the United States Department of Energy (DOE) (**Figure** 10.1); and the National Human Genome Research Institute (NHGRI), part of the National Institutes of Health (NIH), may be one of the landmark scientific events of our lifetime. *Our Molecular Selves* video discusses the human genome, and is available at http://www.genome.gov/25520211.

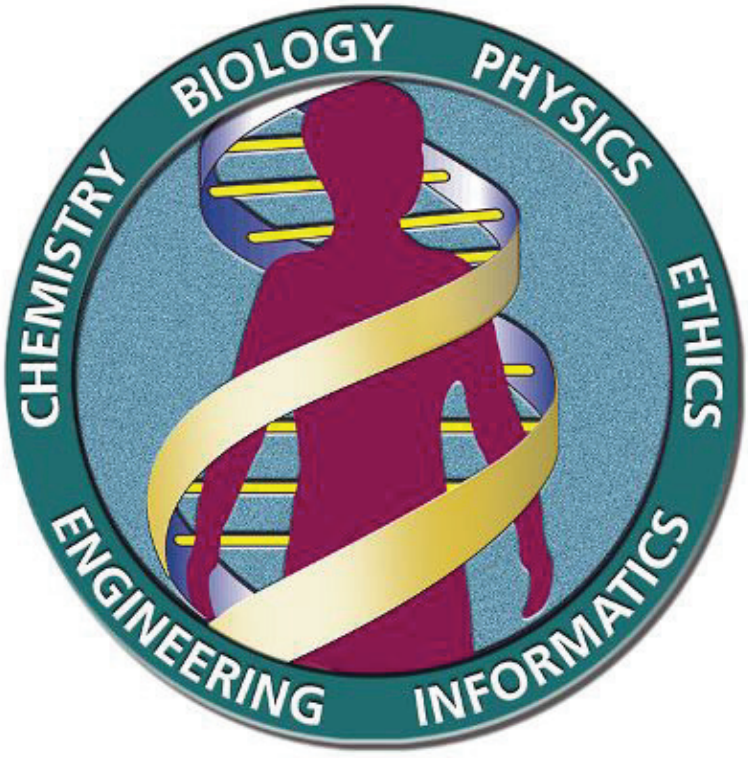

Figure 10.1: The Human Genome Project logo of the DOE. (1)

The goal of the HGP is to understand the genetic make-up of the human species by deter-

mining the DNA sequence of the human genome (**Figure** 10.2);and the genome of a few model organisms. However, it is not just determining the 3 billion bases; it is understanding what they mean. Today, all 3 billion base pairs have been sequenced, and the genes in that sequence are in the process of being identified and characterized. A preliminary estimate of the number of genes in the human genome is around 22,000 to 23,000.

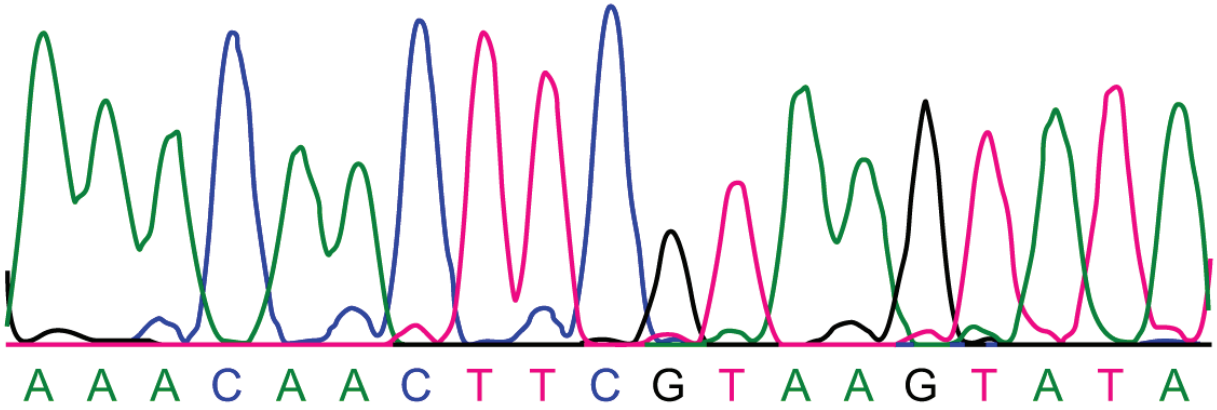

Figure 10.2: A depiction of DNA sequence analysis. Note the 4 colors utilized, each representing a separate base. (6)

The sequence of the human DNA is stored in databases available to anyone on the Internet. The U.S. National Center for Biotechnology Information (NCBI), part of the NIH, as well as comparable organizations in Europe and Japan, maintain the genomic sequences in a database known as **Genbank**. Protein sequences are also maintained in this database. The sequences in these databases are the combined sequences of anonymous donors, and as such do not yet address the individual differences that make us unique. However, the known sequence does lay the foundation to identify the unique differences among all of us. Most of the currently identified variations among individuals will be **single nucleotide polymorphisms**, or SNPs. A **SNP** (pronounced "snip") is a DNA sequence variation occurring at a single nucleotide in the genome. For example, two sequenced DNA fragments from different individuals, GGATCTA to GGATTTA, contain a difference in a single nucleotide. If this base change occurs in a gene, the base change then results in two alleles: the C allele and the T allele. Remember an allele is an alternative form of a gene. Almost all common SNPs have only two alleles. The effect of these SNPs on protein structure and function, and any effect on the resulting phenotype, is an extensive field of study.

Gene Cloning

You probably have heard of cloning. Whereas cloning of humans has many ethical issues associated with it, the cloning of genes has been ongoing for well over 30 years, with cloning

of animals occurring more recently. **Gene cloning**, also known as molecular cloning, refers to the process of isolating a DNA sequence of interest for the purpose of making multiple copies of it. The identical copies are clones. In 1973, Stanley Cohen and Herbert Boyer developed techniques to make recombinant DNA, a form of artificial DNA.

Recombinant DNA is engineered through the combination of two or more DNA strands, combining DNA sequences which would not normally occur together. In other words, selected DNA (or the DNA of "interest") is inserted into an existing organismal genome, such as a bacterial plasmid DNA or some other sort of vector. The recombinant DNA can then be inserted into another cell, such as a bacterial cell, for amplification and possibly production of the resulting protein. This process is called **transformation**, the genetic alteration of a cell resulting from the uptake, incorporation, and expression of foreign genetic material. Recombinant DNA technology was made possible by the discovery of restriction endonucleases.

Restriction Enzyme Digestion and Ligation

In the classical **restriction enzyme** digestion and ligation cloning protocols, cloning of any DNA fragment essentially involves four steps:

1. isolation of the DNA of interest (or target DNA)
2. ligation
3. transfection (or transformation)
4. a screening/selection procedure.

For an overview of cloning, see http://www.hhmi.org/biointeractive/media/DNAi_genetic_eng-sm.mov.

Isolation of DNA

Initially, the DNA fragment to be cloned needs to be isolated. This DNA of interest may be a gene, part of a gene, a promoter, or another segment of DNA, and is frequently isolated by the Polymerase Chain Reaction (PCR) or restriction enzyme digestion. A **restriction enzyme** (or **restriction endonuclease**) is an enzyme that cuts double-stranded DNA at a specific sequence (**Table** 10.1). The enzyme makes two incisions, one through each strand of the double helix, without damaging the nitrogenous bases. This produces either overlapping ends (also known as sticky ends) or blunt ends.

Table 10.1:

A.	$G^{\downarrow}AATTC$	B.	$CCC^{\downarrow}GGG$
	$CTTAA_{\uparrow}G$		$GGG_{\uparrow}CCC$

A. *Eco*RI digestion produces overlapping "sticky" ends: The enzyme cleaves between the G and A on both strands. B. *Sma*II restriction enzyme cleavage produces "blunt" ends. The enzyme cleaves between the G and C on both strands.

(*Source: Created by:* Doug Wilkin, *License:* CC-BY-SA)

The 1978 Nobel Prize in Medicine was awarded to Daniel Nathans and Hamilton Smith for the discovery of restriction endonucleases. The first practical use of their work was the manipulation of *E. coli* bacteria to produce human insulin for diabetics.

Ligation

Once the DNA of interest is isolated, a ligation procedure is necessary to insert the amplified fragment into a vector to produce the recombinant DNA molecule. Restriction fragments (or a fragment and a plasmid/vector) can be spliced together, provided their ends are complementary. Blunt end ligation is also possible.

The **plasmid** or vector (which is usually circular) is digested with restriction enzymes, opening up the vector to allow insertion of the target DNA. The two DNAs are then incubated with **DNA ligase**, an enzyme that can attach together strands of DNA with double strand breaks. This produces the recombinant DNA molecule. **Figure** 10.3 depicts a plasmid with two additional segments of DNA ligated into the plasmid, producing the recombinant DNA molecule. **Figure** 10.4 depicts DNA before and after ligation.

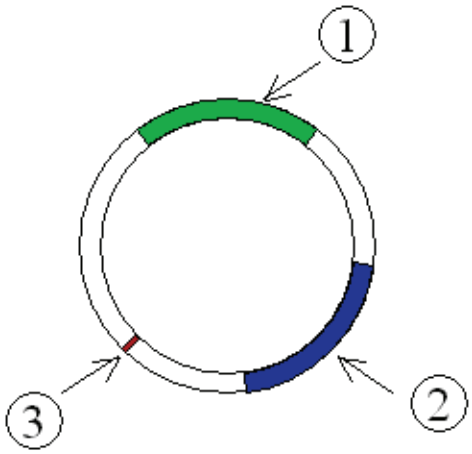

Figure 10.3: This image shows a line drawing of a plasmid. The plasmid is drawn as two concentric circles that are very close together, with two large segments and one small segment depicted. The two large segments (1 and 2) indicate antibiotic resistances usually used in a screening procedure, and the small segment (3) indicates an origin of replication. The resulting DNA is a recombinant DNA molecule. (10)

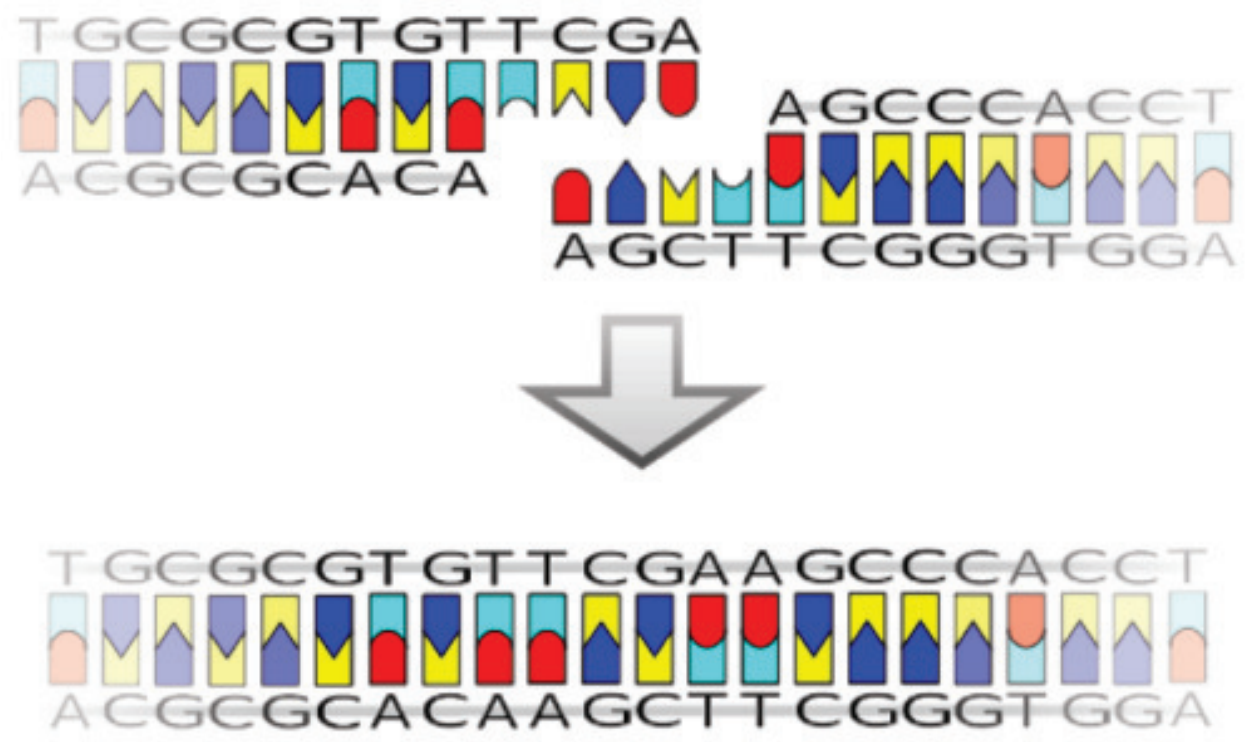

Figure 10.4: Sticky ends produced by restriction enzyme digestion can be joined with the enzyme DNA ligase. (3)

Transfection and Selection

Following ligation, the recombinant DNA is placed into a host cell, usually bacterial, in a process called **transfection** or **transformation**. Finally, the transfected cells are cultured. Many of these cultures may not contain a plasmid with the target DNA as the transfection process is not usually 100% successful, so the appropriate cultures with the DNA of interest must be selected. Many plasmids/vectors include selectable markers - usually some sort of antibiotic resistance (**Figure** 10.3). When cultures are grown in the presence of an antibiotic, only bacteria transfected with the vector containing resistance to that antibiotic should grow. However, these selection procedures do not guarantee that the DNA of insert is present in the cells. Further analysis of the resulting colonies is required to confirm that cloning was successful. This may be accomplished by means of a process known as PCR (see below) or restriction fragment analysis, both of which need to be followed by gel electrophoresis and/or DNA sequencing (DNA sequence analysis).

DNA sequence analysis (the analysis of the order of the nitrogenous bases that make up the DNA), PCR, or restriction fragment analysis will all determine if the plasmid/vector contains the insert. Restriction fragment analysis is digestion of isolated plasmid/vector DNA with restriction enzymes. If the isolated DNA contains the target DNA, that fragment will be excised by the restriction enzyme digestion. Gel electrophoresis will separate DNA molecules based on size and charge. Examples are shown in **Figure** 10.5.

Gel Electrophoresis

Gel electrophoresis is an analytical technique used to separate DNA fragments by size and charge. Notice in **Figure** 10.5 that the "gels" are rectangular in shape. The gels are made of a gelatin-like material of either agarose or polyacrylamide. An electric field, with a positive charge applied at one end of the gel, and a negative charge at the other end, forces the fragments to migrate through the gel. DNA molecules migrate from negative to positive charges due to the net negative charge of the phosphate groups in the DNA backbone. Longer molecules migrate more slowly through the gel matrix. After the separation is completed, DNA fragments of different lengths can be visualized using a fluorescent dye specific for DNA, such as ethidium bromide. The resulting stained gel shows bands correspond to DNA molecules of different lengths, which also correspond to different molecular weights. Band size is usually determined by comparison to DNA ladders containing DNA fragments of known length. Gel electrophoresis can also be used to separate RNA molecules and proteins.

The Polymerase Chain Reaction

The **Polymerase Chain Reaction** (PCR) is used to amplify specific regions of a DNA strand millions of times. A region may be a number of loci, a single gene, a part of a gene, or a non-coding sequence. This technique produces a useful quantity of DNA for analysis,

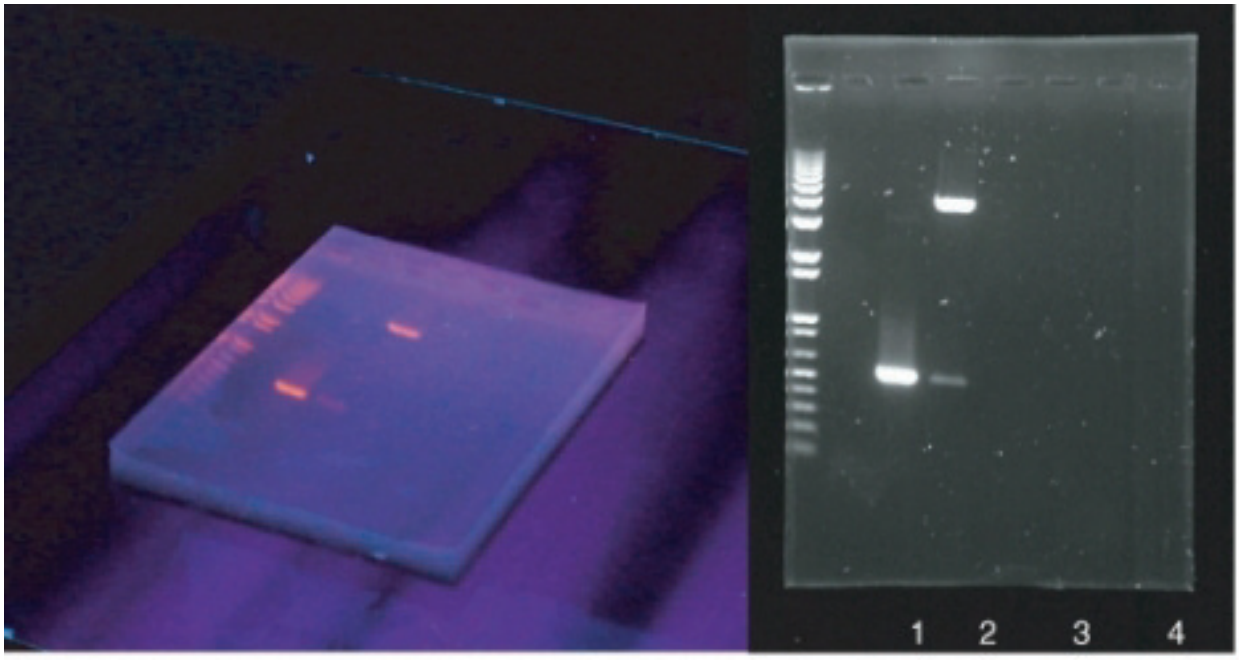

Figure 10.5: Agarose gel following agarose gel electrophoresis on UV light box: In the gel with UV illumination (left), the ethidium bromide stained DNA glows pink; Right, photo of a gel. Far left: DNA ladder of fragments of known length. Lane 1: A PCR product of just over 500 bases. Lane 2: Restriction digest showing the 500 base fragment cut from a 4.5 kb plasmid vector. (4)

be it medical, forensic or some other form of analysis. Amplification of DNA from as little as a single cell is possible. Whole genome amplification is also possible.

PCR utilizes a heat stable DNA polymerase, **Taq polymerase**, named after the thermophilic bacterium *Thermus aquaticus,* from which it was originally isolated. *T. aquaticus* is a bacterium that lives in hot springs and hydrothermal vents, and Taq polymerase is able to withstand the high temperatures required to denature DNA during PCR (discussed below). Taq polymerase's optimum temperature for activity is between 75°C and 80°C. Recently other DNA polymerases have also been used for PCR.

A basic PCR involves a series of repeating cycles involving three main steps (see **Figure** 10.6):

1. denaturation of the double stranded DNA
2. annealing of specific oligonucleotide primers
3. extension of the primers to amplify the region of DNA of interest

These steps will be discussed in additional detail below.

The oligonucleotide primers are single stranded pieces of DNA that correspond to the 5' and 3' ends of the DNA region to be amplified. These primers will anneal to the corresponding segment of denatured DNA. Taq Polymerase, in the presence of free deoxynucleotide triphosphates (dNTPs), will extend the primers to create double stranded DNA. After many cycles of denaturation, annealing and extension, the region between the two primers will be amplified.

The PCR is commonly carried out in a thermal cycler, a machine that automatically allows heating and cooling of the reactions to control the temperature required at each reaction step (see below). The PCR usually consists of a series of about 30 to 35 cycles. Most commonly, PCR is carried out in three repeating steps, with some modifications for the first and last step.

PCR is usually performed in small tubes or wells in a tray, each often beginning with the complete genome of the species being studied. As only a specific sequence from that genome is of interest, the sequence specific primers are targeted to that sequence. PCR is done with all the building blocks necessary to create DNA: template DNA, primers, dNTPs, and a polymerase.

The three basic steps of PCR (**Figure** 10.6) are:

- Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94 - 98°C for 30 to 60 seconds. It disrupts the hydrogen bonds between complementary bases of the DNA strands, yielding single strands of DNA.
- Annealing step: The reaction temperature is lowered to 50-65°C for 30 to 60 seconds, allowing annealing of the primers to the single-stranded DNA template. Stable hydrogen bonds form between the DNA strand (the template) and the primers when the

primer sequence very closely matches the complementary template sequence. Primers are usually 17 - 22 nucleotides long and are carefully designed to bind to only one site in the genome. The polymerase binds to the primer-template hybrid and begins DNA synthesis.
- Extension step: A temperature of around 72°C is used for this step, which is close to the optimum temperature of Taq polymerase. At this step the Taq polymerase extends the primer by adding dNTPs, using one DNA strand as a template to create a the other (new) DNA strand. The extension time depends on the length of the DNA fragment to be amplified. As a standard, at its optimum temperature, the DNA polymerase will polymerize a thousand bases in one minute.

Utilizing the PCR, DNA can be amplified millions of times to generate quantities of DNA that can be used for a number of purposes. These include the use of DNA for prenatal or genetic testing, such as testing for a specific mutation. PCR has revolutionized the fields of biotechnology, human genetics, and a number of other sciences. Many of the applications will be discussed in the following lesson. PCR was developed in 1983 by Kary Mullis. Due to the importance of this process and the significance it has had on scientific research, Dr. Mullis was awarded the Nobel Prize in Chemistry in 1993, just 10 years after his discovery.

To say that PCR, molecular cloning and the Human Genome Project has revolutionized biology and medicine would be an understatement. These efforts have led to numerous accolades, including Nobel prizes, and more may follow. Some of the ways that these discoveries have shaped our lives are the focus of the next lesson.

Lesson Summary

- Biotechnology is technology based on biological applications, combining many features of Biology including genetics, molecular biology, biochemistry, embryology, and cell biology.
- The goal of the Human Genome Project is to understand the genetic make-up of the human species by determining the DNA sequence of the human genome and the genome of a few model organisms.
- Gene cloning, also known as molecular cloning, refers to the process of isolating a DNA sequence of interest for the purpose of making multiple copies of it (cloning).
- Classic gene cloning involves the following steps:

1. Restriction enzyme digestion and ligation
2. Isolation of DNA
3. Ligation
4. Transfection and Selection
5. Gel electrophoresis

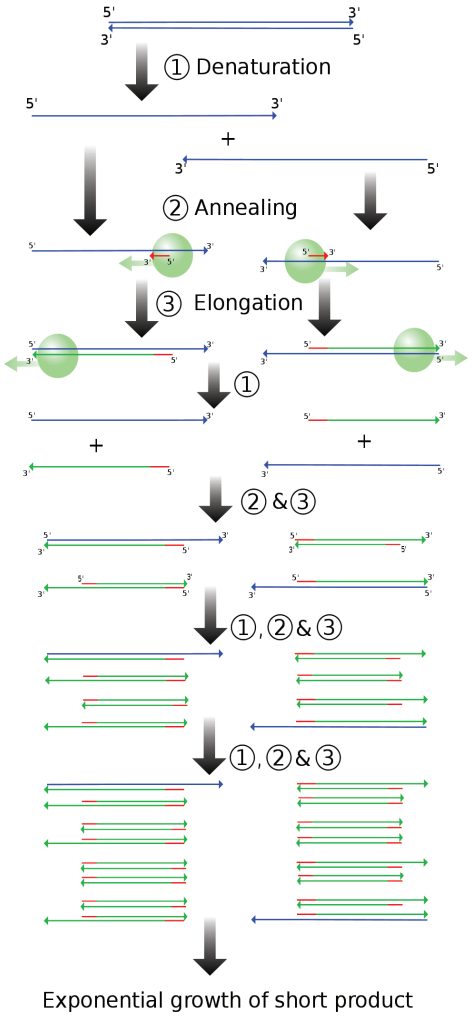

Figure 10.6: PCR: A repeating cycle of denaturation (1), annealing (2), and extension (3). Notice that initially there is a double strand of DNA, and after denaturation, the DNA is single stranded. In the annealing step (2), single stranded primers bind. These primers are extended by Taq Polymerase, represented by the green ball (3). (5)

- The Polymerase Chain Reaction (PCR) is used to amplify millions of times specific regions of a DNA strand. This can be a number of loci, a single gene, a part of a gene, or a non-coding sequence.
- PCR usually involves the following steps:

1. Denaturation step
2. Annealing step
3. Extension step

Review Questions

1. Why are biotechnology and DNA technology considered the same?
2. What are the goals of the Human Genome Project?
3. Is the DNA sequence information generated by the HGP available to anyone, and if so, how?
4. How are gene cloning and recombinant DNA related?
5. Describe the process of gene cloning.
6. How does gel electrophoresis analyze DNA?
7. What is PCR?
8. What allows PCR to be done at high temperatures?
9. Describe the PCR process.

Further Reading / Supplemental Links

- http://www.genome.gov/HGP
- http://www.ornl.gov/sci/techresources/Human_Genome/home.shtml
- http://biotech.about.com/od/cloning/tp/DNAcloning.htm
- http://croptechnology.unl.edu/viewLesson.cgi?LessonID=957884601
- http://www.pcrstation.com/
- http://nobelprize.org/educational_games/chemistry/pcr/index.html
- http://en.wikipedia.org
- http://www.vtaide.com/png/cloning.htm

Vocabulary

biotechnology Technology based on biological applications.

gel electrophoresis An analytical technique used to separate DNA fragments by size and charge.

Genbank The U.S. National Center for Biotechnology Information, part of the National Institutes of Health, which maintains the genomic sequences in a database.

gene cloning The process of isolating a DNA sequence of interest for the purpose of making multiple copies of it.

The Human Genome Project A project to understand the genetic make-up of the human species by determining the DNA sequence of the human genome and the genome of a few model organisms.

plasmid (or vector) A small circular piece of DNA that carries the recombinant DNA into a host organism for cloning.

polymerase chain reaction (PCR) A repeating series of cycles used to amplify millions of times specific regions of a DNA strand.

recombinant DNA Engineered through the combination of two or more DNA strands that combine DNA sequences which would not normally occur together.

restriction enzyme (or restriction endonuclease) An enzyme that cuts double-stranded DNA.

Taq polymerase Named after the thermophilic bacterium *Thermus aquaticus* from which it was originally isolated, is the heat-stable polymerase used in the PCR reaction.

Points to Consider

- The Human Genome Project, gene cloning, and PCR are some of the most remarkable scientific achievements of the recent past. But how can these milestones make our lives better?
- Medicine and food science are just two of the categories that benefit from biotechnology. Speculate on how our lives are made better by these achievements.

10.2 Lesson 10.2: Biotechnology

Lesson Objectives

- Describe various applications of biotechnology as related to medicine, agriculture and forensic science.

- How is DNA technology related to genetic testing and prenatal diagnosis?
- Why is biotechnology so important in agriculture?
- Why is DNA analysis the most important tool of the forensic scientist?
- Describe forensic STR analysis.
- Discuss some of the ELSI associated with biotechnology.

Introduction

Scientists have sequenced a consensus version of the human genome. Now what? Do we know what all the genes are or what they do? Not yet. Do we know what phenotypes are associated with mutations in the genes? For many genes, or even most genes, we do not. Do we even know exactly how many genes we have? Not exactly. And we are far away from knowing what makes us all unique. So how does this information help us? The Human Genome Project has been labeled a landmark scientific event. But what can we do with this information?

There are many applications of genetic information, including applications in medicine and agriculture. The applications of genetics to forensic science have become one of the most important aspects of the criminal justice system. And of course, these applications raise many ethical questions. These applications and questions will be the focus of this lesson.

Applications of DNA Technology: Medicine

As discussed in the first lesson of this chapter, the Human Genome Project has opened up many applications to take advantage of what we know about our genome in order to help us. Many of these applications are medically related. Others will be legally related. And yet still other uses of DNA technology include those in agriculture and the food sciences.

Understanding and curing genetic diseases is the ultimate goal of human geneticists. As discussed in the Human Genetics chapter, gene therapy is the insertion of a new gene into an individual's cells and tissues to treat a disease, replacing a mutant disease-causing allele with a normal, non-mutant allele. Of course, the findings of the Human Genome Project are significant in determining the disease-causing alleles.

In the 1920s, there was no known way to produce insulin, which was needed by people to remove excess sugar from the bloodstream. People with diabetes either lack insulin, produce low levels of insulin, or are resistant to insulin, and thus they may need external insulin to control blood glucose levels. This problem was solved, at least temporarily, when it was found that insulin from a pig's pancreas could be used in humans. This method was the primary solution for diabetes until recently. The problem with insulin production was raised again: there were not enough pigs to provide the quantities of insulin needed. Scientists needed to devise another way. This led to one of the biggest breakthroughs in recombinant DNA technology: the cloning of the human insulin gene.

By methods discussed in the first lesson in this chapter, the specific gene sequence that codes for human insulin was introduced into the bacteria *E. coli*. The transformed gene altered the genetic makeup of the bacterial cells, such that in a 24 hour period, billions of *E. coli* containing the human insulin gene resulted, producing human insulin to be administered to patients.

Though the production of human insulin by recombinant DNA procedures is an extremely significant event, many other aspects of DNA technology are beginning to become reality. In medicine, modern biotechnology provides significant applications in such areas as pharmacogenomics, genetic testing (and prenatal diagnosis), and gene therapy. These applications use our knowledge of biology to improve our health and our lives. Many of these medical applications are based on the findings of the Human Genome Project.

Pharmacogenomics

Currently, millions of individuals with high cholesterol take a similar type of drug. You may know of people who take a medicine to help with their cholesterol levels. However, these drugs probably work slightly differently in many of those people. In some, it lowers their cholesterol significantly; in others it may lower it only moderately; and in some, it may have no effect at all. Why the difference? Because of the genetic background of all people. Pharmacogenomics, a combination of pharmacology and genomics (the study of the genome) that refers to the study of the relationship between pharmaceuticals and genetics, may explain and simplify this problem.

Pharmacogenomics is the study of how the genetic inheritance of an individual affects his or her body's response to drugs. In other words, pharmacogenomics will lead to the design and production of drugs that are adapted to each person's genetic makeup.

Pharmacogenomics will result in the following benefits:

1. Development of tailor-made medicines. Using pharmacogenomics, pharmaceutical companies will be able to create drugs based on the proteins, enzymes and RNA molecules that are associated with specific genes and diseases. These tailor-made drugs promise not only to maximize the beneficial effects of the medicine, but also to decrease damage to nearby healthy cells.
2. More accurate methods of determining appropriate drug dosages. Knowing a patient's genetics will enable doctors to determine how well his or her body can process and metabolize a medicine. This will allow doctors to prescribe the proper levels of the medicine, allowing the medicine to have optimal results.
3. Improvements in the drug discovery and approval process. Once the genes and proteins associated with a disease are known, the discovery of new medicines will be made easier using these genes and proteins as targets for the medicine. In addition to creating much more beneficial medicines, this could significantly shorten the drug discovery process.

4. Better vaccines. Safer vaccines can be designed and produced by organisms transformed with DNA sequences from an antigen. These vaccines will trigger the immune response without the risks of infection. They will be capable of being engineered to carry several strains of pathogen at once, combining several vaccines into one.

Genetic Testing and Prenatal Diagnosis

Let's propose a hypothetical situation: unfortunately, your family is predisposed to develop a genetic disease. You and your spouse want to have a baby, but you want to know the likelihood of the child developing the disease.

This scenario could happen to anyone. As we learn more and more about disease causing genes, it will become easier to test for mutations in those genes. Currently, is there any way to determine if a baby will develop a disease due to a known mutation? Is it possible to screen for a mutation in a developing baby? Yes.

Genetic testing involves the direct examination of DNA sequences. A scientist scans, by any number of methods, a patient's DNA for mutated sequences. Genetic testing can be used to:

- Diagnose a disease.
- Confirm a diagnosis.
- Provide information about the course of a disease.
- Confirm the existence of a disease.
- Predict the risk of future development of a disease in otherwise healthy individuals or their children.
- Identify carriers (unaffected individuals who are heterozygous for a recessive disease gene).
- Perform prenatal diagnostic screening.
- Perform newborn screening.

Consultations with human geneticists and genetic counselors are an important first step in genetic testing. They will most likely prescribe some sort of prenatal screening (see the *Human Genetics* chapter). **Prenatal screening** (also known as **prenatal diagnosis** or testing) is the testing for diseases or conditions in a fetus or embryo before it is born. Methods may involve amniocentesis or chorionic villus sampling to remove fetal cells. DNA can be isolated from these cells and analyzed. If the mutation that results in the phenotype is known, that specific mutation can be tested, either through restriction fragment length polymorphism analysis or, more likely, through PCR and DNA sequence analysis. As it is the baby's DNA that is being analyzed, the analysis will determine if the developing baby will have the mutation and develop the phenotype, or not have the mutation. Parents can then be informed of the probability of the baby developing the disease.

In human genetics, **preimplantation genetic diagnosis** (PIGD) is genetic analysis performed on embryos prior to implantation. PIGD is considered an alternative to prenatal diagnosis. Its main advantage is that it avoids selective pregnancy termination, as the method makes it highly likely that the baby will be free of the disease in question. In PIGD, *in vitro* fertilization is used to obtain embryos for analysis. DNA is isolated from developing embryos prior to implantation, and specific genetic loci are screened for mutations, usually using PCR based analysis. Embryos that lack the specific mutation can then be implanted into the mother, thereby guaranteeing that the developing baby will not have the specific mutation analyzed for (and thus not have the disease associated with that mutation).

Applications of DNA Technology: Agriculture

Biotechnology has many other useful applications besides those that are medically related. Many of these are in agriculture and food science. These include the development of **transgenic crops** - the placement of genes into plants to give the crop a beneficial trait. Benefits include:

- Improved yield from crops.
- Reduced vulnerability of crops to environmental stresses.
- Increased nutritional qualities of food crops.
- Improved taste, texture or appearance of food.
- Reduced dependence on fertilizers, pesticides and other agrochemicals.
- Production of vaccines.

Improved Yield from Crops

Using biotechnology techniques, one or two genes may be transferred into a crop to give a new trait to that crop. This is done in the hope of increasing its yield. However, these increases in yield have proved to be difficult to achieve. Current genetic engineering techniques work best for single gene effects - that is traits inherited in a simple Mendelian fashion. Many of the genetic characteristics associated with crop yield, such as enhanced growth, are controlled by a large number of genes, each of which just has a slight effect on the overall yield. There is, therefore, still much research, including genetic research, to be done in this area.

Reduced Vulnerability to Environmental Stresses

Crops are obviously dependent on environmental conditions. Drought can destroy crop yields, as can too much rain or floods. But what if crops could be developed to withstand these harsh conditions? Biotechnology will allow the development of crops containing genes that will enable them to withstand biotic and abiotic stresses. For example, drought and excessively salty soil are two significant factors affecting crop productivity. But there are

crops that can withstand these harsh conditions. Why? Probably because of that plant's genetics. So biotechnologists are studying plants that can cope with these extreme conditions, trying to identify and isolate the genes that control these beneficial traits. The genes could then be transferred into more desirable crops, with the hope of producing the same phenotypes in those crops.

Thale cress (**Figure** 10.7), a species of *Arabidopsis (Arabidopsis thaliana)*, is a tiny weed that is often used for plant research because it is very easy to grow and its genome has been extensively characterized. Scientists have identified a gene from this plant, At-DBF2, that confers resistance to some environmental stresses. When this gene is inserted into tomato and tobacco cells, the cells were able to withstand environmental stresses like salt, drought, cold and heat far better than ordinary cells. If these preliminary results prove successful in larger trials, then At-DBF2 genes could help in engineering crops that can better withstand harsh environments. Researchers have also created transgenic rice plants that are resistant to rice yellow mottle virus (RYMV). In Africa, this virus destroys much of the rice crops and makes the surviving plants more susceptible to fungal infections.

Figure 10.7: Thale cress. (11)

Increased Nutritional Qualities of Crops

Maybe you've heard over and over that eating beans is good for you. True? Well, maybe. But what if it were possible to increase the nutritional qualities of food? One would think that would be beneficial to society. So, can biotechnology be used to do just that? Scientists

are working on modifying proteins in foods to increase their nutritional qualities. Also, proteins in legumes and cereals may be transformed to provide all the amino acids needed by human beings for a balanced diet.

Improved Taste, Texture or Appearance of Food

Have you ever gone to the grocery store, bought some fruit and never gotten around to eating it? Maybe you haven't, but I bet your parents have. Modern biotechnology can be used to slow down the process of spoilage so that fruit can ripen longer on the plant and then be transported to the consumer with a still reasonable shelf life. This is extremely important in parts of the world where time from harvest to the consumer may be longer than in other areas. In addition to improving the taste, texture and appearance of fruit, it will also extend the usable life of the fruit. As the world population grows and grows, this may become a fairly important issue. Extending the life of fruit can expand the market for farmers in developing countries due to the reduction in spoilage. This has successfully been demonstrated in the tomato. The first genetically modified food product was a tomato which was transformed to delay its ripening. Researchers in Indonesia, Malaysia, Thailand, Philippines and Vietnam are currently working on delayed-ripening papayas.

Reduced Dependence on Fertilizers, Pesticides and Other Agrochemicals

There is growing concern regarding the use of pesticides in agriculture. Therefore, many of the current commercial applications of modern biotechnology in agriculture are focused on reducing the dependence of farmers on these chemicals. For example, *Bacillus thuringiensis* (Bt) is a soil bacterium that produces a protein that can act as an insecticide, known as the **Bt toxin**. But it is a protein, not a foreign chemical. Could this protein be used in crops instead of pesticides? Traditionally, an insecticidal spray has been produced from these bacteria. As a spray, the Bt toxin is in an inactive state and requires digestion by an insect to become active and have any effect. Crop plants have now been engineered to contain and express the genes for the Bt toxin, which they produce in its active form. When an insect ingests the transgenic crop, it stops feeding and soon thereafter dies as a result of the Bt toxin binding to its gut wall. Bt corn is now commercially available in a number of countries to control corn borer (a lepidopteran insect like moths and butterflies), which is otherwise controlled by insecticidal spraying.

In addition to insects, weeds have also been a menace to farmers - just ask anyone with a garden how much they hate weeds. They can quickly compete for water and nutrients needed by other plants. Sure, farmers can use herbicides to kill weeds, but do these chemicals also harm the crops? Can biotechnology help with this issue? Some crops have also been genetically engineered to acquire tolerance to the herbicides - allowing the crops to grow, but killing the weeds. But the lack of cost effective herbicides with a broad range of activity - that

Figure 10.8: Kenyans examining genetically modified insect resistant transgenic Bt corn. (12)

do not harm crops - is a problem in weed management. Multiple applications of numerous herbicides are routinely needed to control the wide range of weeds that are harmful to crops. And at times these herbicides are being used as a preventive measure – that is, spraying to prevent weeds from developing rather than spraying after weeds form. So these chemicals are being added to crops. This practice is followed by mechanical and/or hand weeding to control weeds that are not controlled by the chemicals. Crops that are tolerant of herbicides would obviously be a tremendous benefit to farmers (**Figure** 10.8). The introduction of herbicide tolerant crops has the potential to reduce the number of chemicals needed during a growing season, thereby increasing crop yield due to improved weed management and decreased harm to the crops.

In 2001, 626,000 square kilometers of transgenic crops were planted. Seventy-seven percent of the transgenic crops were developed for herbicide tolerance in soybean, corn, and cotton, 15% were Bt crops for insect resistance, and 8% were developed with genes for both insect resistance and herbicide tolerance in cotton and corn.

Production of Vaccines in Crop Plants

Many little children hate shots. And many children in parts of the world do not even have access to vaccines. But what if these vaccines were available in an edible form? Modern biotechnology is increasingly being applied for novel uses other than food. Banana trees and tomato plants have been genetically engineered to produce vaccines in their fruit. If future clinical trials prove successful, the advantages of edible vaccines would be enormous, especially for developing countries. The transgenic plants could be grown locally and cheaply. Edible vaccines would not require the use of syringes, which, in addition to being unpleasant, can be a source of infections if contaminated.

Applications of DNA Technology: Animal Cloning

DNA technology has proved very beneficial to humans. **Transgenic animals** are animals that have incorporated a gene from another species into their genome (**Figure** 10.9). They are used as experimental models to perform phenotypic tests with genes whose function is unknown, or to generate animals that are susceptible to certain compounds or stresses for testing purposes. Other applications include the production of human hormones, such as insulin. Many times these animals are rodents, such as mice, or fruit flies (*Drosophila melanogaster*). Fruit flies are extremely useful as genetic models to study the effects of genetic changes on development.

But transgenic animals just have one novel gene. What about a whole new genome? It could be argued that human cloning is one of the techniques of modern biotechnology. It involves the removal of the nucleus from one cell and its placement in an unfertilized egg cell whose nucleus has either been deactivated or removed. Theoretically this would result

Figure 10.9: GloFish: the first genetically modified animal to be sold as a pet. GloFish are transgenic zebrafish transfected with a natural fluorescence gene. (9)

in an individual genetically identical to the donor. Of course, there are many ethical issues associated with human cloning. But animal cloning is arguably a different story.

In February 1997, Ian Wilmut and his colleagues at the Roslin Institute announced the successful cloning of a sheep named Dolly from the mammary glands of an adult female (**Figure** 10.10). Dolly was the first mammal to be cloned from an adult somatic cell. The cloning of Dolly made it apparent to many that the techniques used to produce her could someday be used to clone human beings. This resulted in tremendous controversy because of its ethical implications. After cloning was successfully demonstrated by Dolly's creators, many other large mammals, including horses and bulls, were cloned. Cloning is now considered a promising tool for preserving endangered species.

Figure 10.10: Dolly the sheep and her first-born lamb Bonnie. Dolly was the first large mammal to be cloned. This picture shows that a cloned animal can perform many, if not all, of the same functions as a non-cloned animal. (2)

In animal cloning, the nucleus from a somatic cell is inserted into an egg cell in which the nucleus has been removed. The resulting cell is cultivated and after a few divisions, the egg cell is placed into a uterus where it is allowed to develop into a fetus that is genetically

identical to the donor of the original nucleus (**Figure** 10.11). For an animation of cloning, see http://www.dnalc.org/resources/animations/cloning101.html.

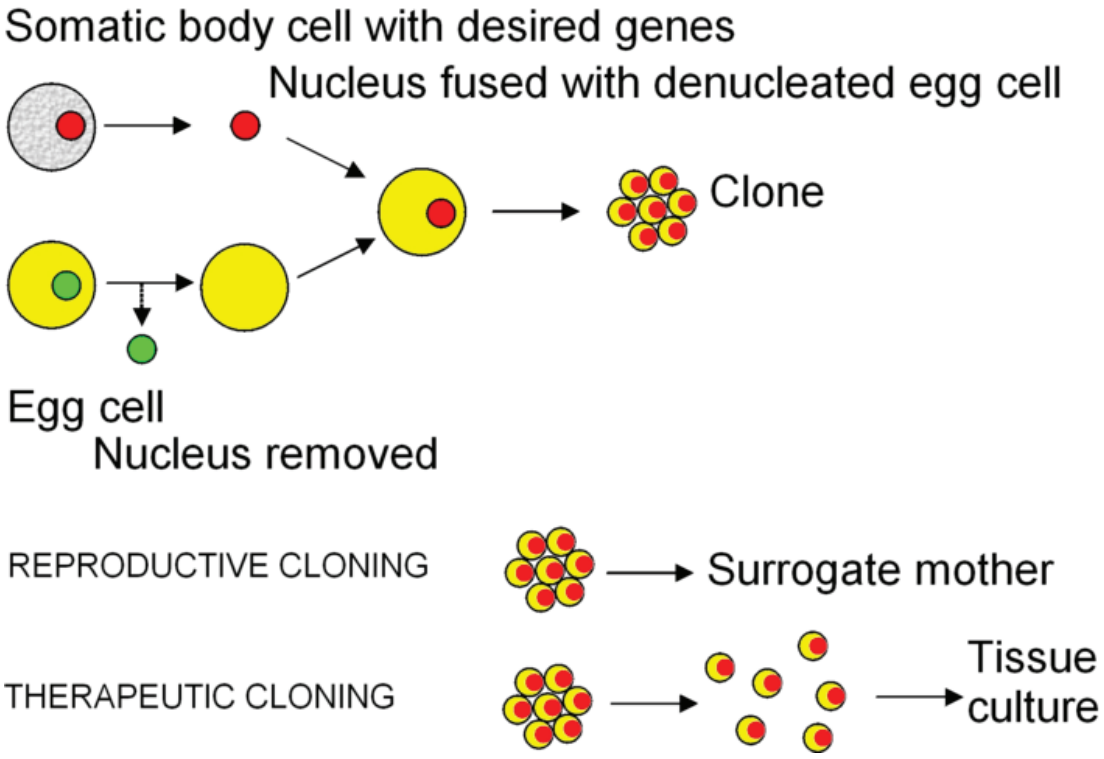

Figure 10.11: Reproductive cloning: The nucleus is removed from a somatic cell and fused with a denucleated egg cell. The resulting cell may develop into a colony of cloned cells, which is placed into a surrogate mother. In therapeutic cloning, the resulting cells are grown in tissue culture; an animal is not produced, but genetically identical cells are produced. (13)

Applications of DNA Technology: Forensic DNA Analysis

You know that DNA can be used to distinguish individuals from each other. You may have heard that DNA can also be used to match evidence and suspects and help solve crimes. This is demonstrated on shows like *CSI: Crime Scene Investigation*. But how is this done? How is a "genetic fingerprint," a DNA pattern unique to each individual (except identical twins) created? **Genetic fingerprinting**, or **DNA fingerprinting**, distinguishes between individuals of the same species using only samples of their DNA. DNA fingerprinting has thus become one of the most powerful tools of the forensic scientist, enabling law enforcement personnel to match biological evidence from crime scenes to suspects. As any two humans have the majority of their DNA sequence in common, those sequences which demonstrate high variability must be analyzed. This DNA analysis was first developed using DNA hybridization techniques, but now is almost exclusively PCR-based.

DNA fingerprinting was developed by Sir Alec Jeffreys in 1985. Genetic fingerprinting exploits highly variable repeating sequences. Two categories of these sequences are microsatellites and minisatellites. **Microsatellites**, also known as **short tandem repeats** (STRs), consist of adjacent repeating units of 2 - 10 bases in length, for example $(GATC)_n$, where GATC is a tetranucleotide (4 base) repeat and n refers to the number of repeats. It is the number of repeating units at a given locus that is variable. An **STR profile** can be created for any individual by analyzing a series of STRs (**Figure** 10.12). Two unrelated humans will be unlikely to have the same numbers of repeats at a given locus.

In STR profiling, PCR is used to obtain enough DNA to then detect the number of repeats at 13 specific loci. PCR products are separated by gel or capillary electrophoresis. By examining enough STR loci and counting how many repeats of a specific STR sequence there are at a given locus, it is possible to create a unique genetic profile of an individual. STR analysis has become the prevalent analysis method for determining genetic profiles in forensic cases. It is possible to establish a match that is extremely unlikely to have arisen by coincidence, except in the case of identical twins, who will have identical genetic profiles. The polymorphisms (different in the number of repeats) displayed at each STR region will be shared by approximately 5 - 20% of individuals. When analyzing STRs at multiple loci, such as the 13 STRs analyzed in forensic DNA analysis, it is the unique combinations of these polymorphisms in an individual that makes this method unmatched as an identification tool. The more STR regions that are analyzed in an individual the more discriminating the test becomes.

Capillary electrophoresis is similar to gel electrophoresis but uses a capillary tube filled with the gelatin material.

Genetic fingerprinting is used in forensic science to match suspects to samples of blood, hair, saliva or semen, or other sources of DNA. It has also led to several exonerations of formerly convicted suspects. Genetic fingerprinting is also used for identifying human remains, testing for paternity, matching organ donors, studying populations of wild animals, and establishing the province or composition of foods. It has also been used to generate hypotheses on the pattern of the human migration.

In the United States, DNA fingerprint profiles generated from the 13 STR loci are stored in **CODIS**, The Combined DNA Index System, maintained by the Federal Bureau of Investigation. As of 2007, CODIS maintained over 4.5 million profiles. Profiles maintained in CODIS are compiled from both suspects and evidence, and therefore are used to help solve criminal cases. Profiles of missing persons are also maintained in CODIS. The true power of STR analysis is in its statistical power of discrimination. Because the 13 loci are independently assorted, the laws of probabilities can be applied. This means that if someone has the genotype of ABC at three independent loci, then the probability of having that specific genotype is the probability of having type A times the probability of having type B times the probability of having type C. This has resulted in the ability to generate match probabilities of 1 in a quintillion (1 with 18 zeros after it) or more, that is, the chance of two samples matching by coincidence is greater than the number of people on the planet, or the number

of people that have ever lived!

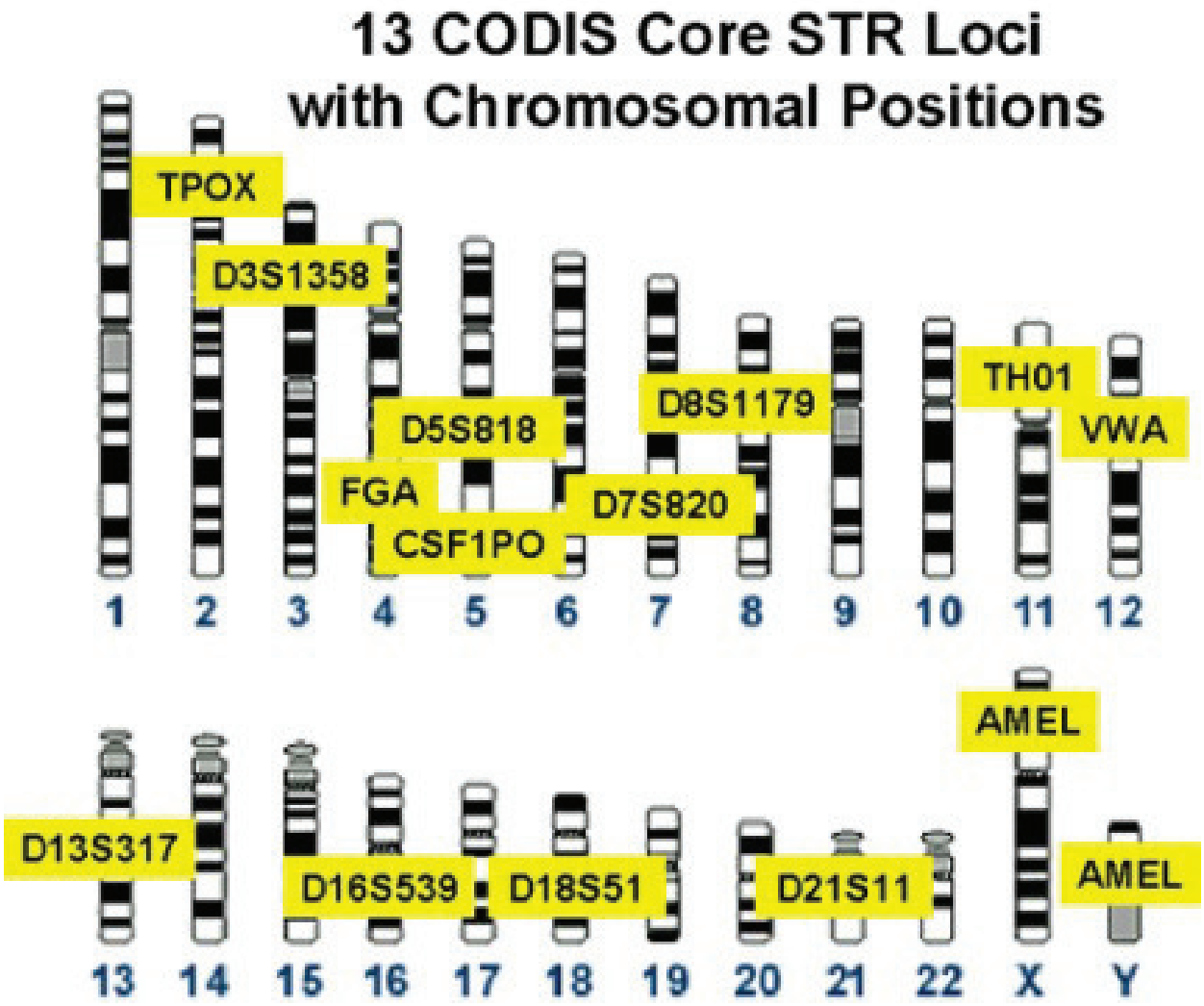

Figure 10.12: The CODIS loci analyzed by STR analysis. Notice they are spread over 14 chromosomes, and that two are on the X and Y chromosomes. (8)

The development of PCR has enabled STR analysis to become the method of choice for DNA identification. Prior to PCR, other methods were utilized. These include restriction fragment length polymorphism (RFLP) analysis and Southern blot analysis.

RFLP Analysis: Restriction Fragment Length Polymorphism

Prior to the development of PCR, restriction enzyme digestion of DNA followed by Southern blot analysis was used for DNA fingerprinting. This analysis is based on the polymorphic nature of restriction enzyme sites among different individuals, hence **restriction fragment**

length polymorphisms are formed after digestion of DNA with these enzymes. A **Southern blot**, named after its inventor Edwin Southern, is a method used to check for the presence of a specific DNA sequence in a DNA sample. Once an individual's DNA is digested with a specific restriction enzyme, the resulting fragments are analyzed by Southern blot analysis. These fragments will produce a specific pattern for that individual. Southern blotting is also used for other molecular biology procedures, including gene identification and isolation. Other blotting methods that employ similar principles have been developed. These include the western blot and northern blot. These procedures analyze proteins and RNA respectively.

RFLP and Southern blot analysis involved several steps:

1. First, the DNA being analyzed is cut into different-sized pieces using restriction enzymes.
2. The resulting DNA fragments are separated by gel electrophoresis.
3. Next, an alkaline solution or heat is applied to the gel so that the DNA denatures and separates into single strands.
4. Nitrocellulose paper is pressed evenly against the gel and then baked so the DNA is permanently attached to it. The DNA is now ready to be analyzed using a radioactive single-stranded DNA probe in a hybridization reaction.
5. After hybridization, excess probe is washed from the membrane, and the pattern of hybridization is visualized on X-ray film by autoradiography (**Figure** 10.13).

Hybridization is when two genetic sequences bind together because of the hydrogen bonds that form between the base pairs. To make hybridization work, the radioactive probe has to be denatured so that it is single-stranded. The denatured probe and the Southern blot are incubated together, allowing the probe to bind to the corresponding fragment on the Southern blot. The probe will bond to the denatured DNA wherever it finds a fit. Hybridization of a probe made to a variable segment of DNA will produce a DNA fingerprint pattern specific for an individual. This procedure has a number of steps and is very labor intensive. PCR-based methods are much simpler.

Ethical, Legal, and Social Issues

Imagine someone analyzes part of your DNA. Who controls that information? What if your health insurance company found out you were predisposed to develop a devastating genetic disease. Might they decide to cancel your insurance?

Privacy issues concerning genetic information is a growing issue in this day and age, especially among those who donate DNA for large-scale sequence-variation studies. Other concerns have been to anticipate how the resulting data may affect concepts of race and ethnicity; identify potential uses (or misuses) of genetic data in workplaces, schools, and courts; identify

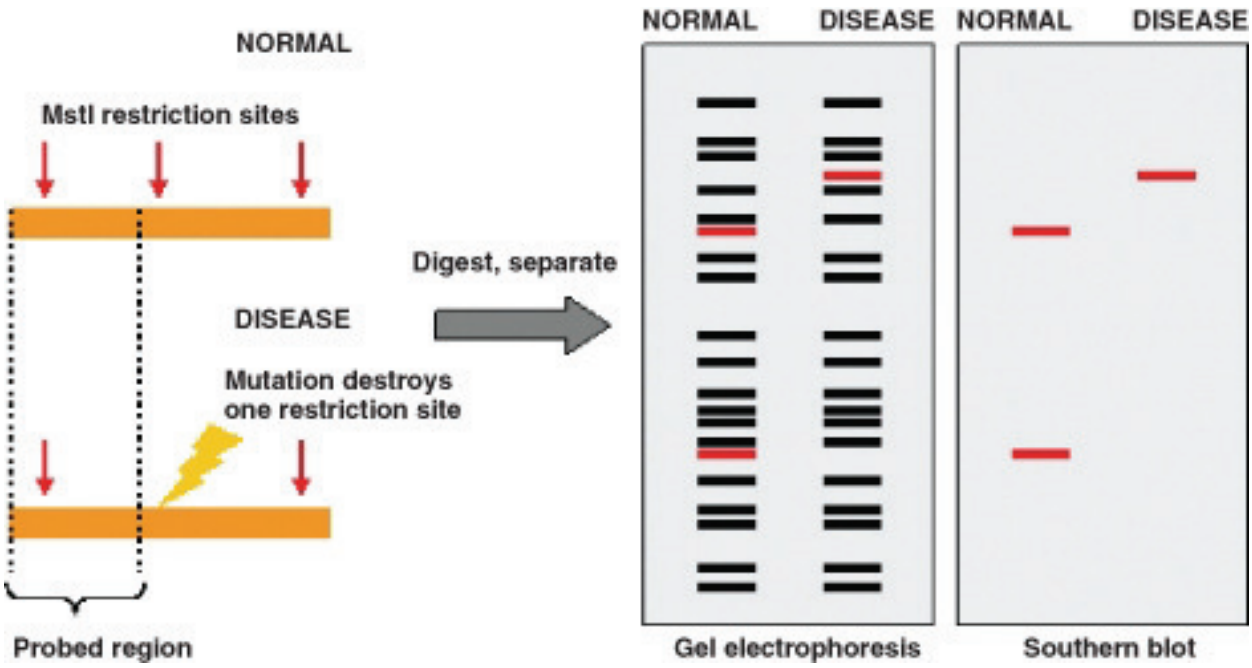

Figure 10.13: Mutations can create or abolish restriction enzyme (RE) recognition sites, thus affecting quantities and length of DNA fragments resulting from RE digestion. (7)

commercial uses; and foresee impacts of genetic advances on the concepts of humanity and personal responsibility.

ELSI stands for Ethical, Legal and Social Issues. It's a term associated with the Human Genome project. This project didn't only have the goal to identify all the approximately 20,000 – 24,000 genes in the human genome, but also to address the ELSI that might arise from the project. The U.S. Department of Energy (DOE) and the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH) devoted 3% to 5% of their annual human genome research budget toward studying ethical, legal, and social issues surrounding the availability of your genetic information. This represents the world's largest bioethics program and has become a model for ELSI programs around the world.

Rapid advances in DNA-based research, human genetics, and their applications have resulted in new and complex ethical and legal issues for society. ELSI programs that identify and address these implications have been an integral part of the Human Genome Project since its inception. These programs have resulted in a body of work that promotes education and helps guide the conduct of genetic research and the development of related medical and public policies.

ELSI programs address the following issues, among others:

- Privacy and confidentiality issues concerning personal genetic information.

- The fairness in the use of personal genetic information by insurers, employers, courts, schools, adoption agencies, and the military, among others.
- The psychological impact and stigmatization due to an individual's genetic differences.
- Clinical issues. These include the education of doctors and other health service providers, patients, and the general public in the capabilities and uses of genetic information, and the scientific/medical limitations of genetic testing. Clinical issues also include the implementation of standards and quality-control measures in genetic testing procedures.
- Reproductive issues. These include adequate informed consent for complex and potentially controversial procedures, and the use of genetic information in making decisions concerning reproductive options.
- Uncertainties associated with genetic testing. The current and future uncertainties associated with testing for susceptibilities to a genetic condition raise many ethical issues, as does the testing for predisposition to a complex condition (such as heart disease) linked to multiple genes and gene-environment interactions.
- Health and environmental issues concerning genetically modified foods and microbes.
- Commercialization of genetic products including property rights, such as patents and copyrights, and issues concerning the accessibility to genetic data and materials.

Biotechnology will have a tremendous impact on our future - of this there is no doubt. Is society entering some dangerous areas? Well, many of these issues have never been analyzed until now. With the discovery of countless amounts of genetic information and the development of its applications, many questions need to be addressed.

- Who should have access to personal genetic information, and how will it be used?
- Who owns and controls genetic information?
- How does personal genetic information affect an individual and society's perceptions of that individual?
- How does genomic information affect members of minority communities?
- How reliable and useful is fetal genetic testing?
- How will genetic tests be evaluated and regulated for accuracy, reliability, and utility?
- How do we prepare the public to make informed choices?
- Should testing be performed when no treatment is available?
- Should parents have the right to have their minor children tested for adult-onset diseases?
- Are genetic tests reliable and interpretable by the medical community?
- Where is the line between medical treatment and enhancement?
- Are genetically modified foods and other products safe for humans and the environment?
- How will these technologies affect developing nations' dependence on the West?
- Who owns genes and other pieces of DNA?
- Will patenting DNA sequences limit their accessibility and development into useful products?

Are scientific fantasies, such as those depicted on TV shows such as *Star Trek* or in the movie *GATTACA*, a possibility? Who can really say? How, really, will biotechnology affect our future? It seems as if the possibilities are endless.

Lesson Summary

- In medicine, modern biotechnology provides significant applications in such areas as pharmacogenomics, genetic testing (prenatal diagnosis), and gene therapy.
- Pharmacogenomics, the combination of pharmacology and genomics, is the study of the relationship between pharmaceuticals and genetics.
- Pharmacogenomics will result in the following benefits:

1. Development of tailor-made medicines.
2. More accurate methods of determining appropriate drug dosages.
3. Improvements in the drug discovery and approval process.
4. Better vaccines.

- Genetic testing involves the direct examination of DNA sequences.
- Genetic testing can be used to: diagnose a disease; confirm a diagnosis; provide prognostic information about the course of a disease; confirm the existence of a disease; predict the risk of future development of a disease in otherwise healthy individuals or their children; screen for carriers (unaffected individuals who are heterozygous for a disease gene); perform prenatal diagnostic screening; and perform newborn screening.
- Biotechnology in agriculture includes the development of transgenic crops - the placement of genes into plants to give the crop a beneficial trait. Benefits include improved yield from crops, reduced vulnerability of crops to environmental stresses, increased nutritional qualities of food crops, improved taste, texture or appearance of food, reduced dependence on fertilizers, pesticides and other agrochemicals, and production of vaccines.
- Transgenic animals are animals that have incorporated a gene from another species into their genome. They are used as experimental models to perform phenotypic tests with genes whose function is unknown, or to generate animals that are susceptible to certain compounds or stresses for testing purposes. Other applications include the production of human hormones, such as insulin.
- Animal cloning is the generation of genetically identical animals using DNA from a donor animal, not a gamete. Dolly, a sheep, was the first mammal to be cloned from an adult somatic cell.
- Genetic fingerprinting, or DNA fingerprinting, distinguishes between individuals of the same species using only samples of their DNA. DNA fingerprinting has thus become one of the most powerful tools of the forensic scientist, enabling law enforcement personnel to match biological evidence from crime scenes to suspects.

- ELSI stands for Ethical, Legal and Social Issues. This is a term associated with the Human Genome project. Rapid advances in DNA-based research, human genetics, and their applications have resulted in new and complex ethical and legal issues for society. ELSI programs that identify and address these implications have been an integral part of the Human Genome Project since its inception. These programs have resulted in a body of work that promotes education and helps guide the conduct of genetic research and the development of related medical and public policies.

Review Questions

1. List applications of DNA technology.
2. List how DNA technology is used in agriculture.
3. How is DNA technology used in medicine?
4. What are some of the benefits of pharmacogenomics?
5. Describe how pharmacogenomics will result in specialty medicines.
6. What are potential uses of genetic testing?
7. Describe how DNA technology can improve yield from crops.
8. Describe how DNA technology can be used to reduce vulnerability to environmental stresses. Why is this important? State an example.
9. What is the difference between a transgenic animal and a cloned animal?
10. Who was Dolly? Why was she important?
11. What is a DNA fingerprint and how is it used?
12. What is STR profiling?
13. Describe why ELSI programs are important.
14. List some ELSI issues.

Further Reading / Supplemental Links

- http://www.genome.gov
- http://www.dna.gov/basics/http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml
- http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml
- http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml
- http://www.ama-assn.org/ama/pub/category/2306.html
- http://www.americanheart.org/presenter.jhtml?identifier=4566
- http://www.ift.org/cms/
- http://www.pub.ac.za/projects/dnakits.html
- http://www.fda.gov/cvm/CloningRA_FAQConsumers.htm
- http://www.aavs.org/animalcloning_overview.html
- http://www.FBI.gov

- http://www.ornl.gov/sci/techresources/Human_Genome/elsi/forensics.shtml
- http://www.dna.gov/basics/analysishistory/
- http://www.genome.gov/ELSI/
- http://www.lbl.gov/Education/ELSI/
- http://en.wikipedia.org/wiki/Main_Page

Vocabulary

CODIS The Combined DNA Index System, is maintained by the Federal Bureau of Investigation and stores DNA profiles.

ELSI Ethical, Legal and Social Issues. This term is associated with the Human Genome Project.

genetic fingerprinting (DNA fingerprinting) Creates a unique DNA pattern that distinguishes between individuals of the same species using only samples of their DNA.

genetic testing The direct examination of DNA sequences for mutated sequence.

microsatellites (short tandem repeats) Adjacent repeating units of 2 - 10 bases in length, for example $(GATC)_n$, where GATC is a tetranucleotide repeat and n refers to the number of repeats.

pharmacogenomics The combination of pharmacology and genomics, is the study of the relationship between pharmaceuticals and genetics. It is the study of how the genetic inheritance of an individual affects his or her body's response to drugs.

preimplantation genetic diagnosis (PGD) Genetic analysis performed on embryos prior to implantation.

prenatal diagnosis (prenatal screening) Testing for diseases or conditions in a fetus or embryo before it is born. Methods may involve amniocentesis or chorionic villus sampling to remove fetal cells.

restriction fragment length polymorphism (RFLP) Analysis that analyzes the differences between restriction enzyme sites.

southern blot Named after its inventor Edwin Southern, is a method used to check for the presence of a specific DNA sequence in a DNA sample.

STR profiling Analyzes 13 STR loci to create a DNA profile utilized in forensic analysis.

transgenic animals Animals that have incorporated a gene from another species into their genome.

transgenic crops The result of placement of genes into plants to give the crop a beneficial trait.

Points to Consider

We have spent the past few chapters discussing genetics, molecular biology, and their implications. These are implicitly related to evolution.

- Can you hypothesize on the relationship between genetics and evolution?
- Why is an understanding of the principals of DNA and inheritance essential to understand evolution?

Image Sources

(1) *The Human Genome Project logo of the DOE.*. Public Domain.

(2) The Roslin Institute. http://en.wikipedia.org/wiki/Image:Dolly_the_sheep2-thumb.jpg.

(3) http://commons.wikimedia.org/wiki/Image:Ligation.svg. GNU-FDL.

(4) *http://en.wikipedia.org/wiki/File:Agarosegelphoto.jpg*. GNU-FDL.

(5) http://commons.wikimedia.org/wiki/Image:PCR.svg. GNU-FDL.

(6) http://commons.wikimedia.org/wiki/Image:DNA_sequence.png. Public Domain.

(7) http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechRFLP.shtml. Public Domain.

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