Name _____
Date _____ Period_____

MI End of Course Exam Review (Other Teachers & Coerper)
49 MC

Understandings 1.1

- Medical interventions help maintain health and homeostasis in the body.
- A variety of methods can be used to detect and/or identify infectious agents.

Essential Questions

- What is a medical intervention?
- What are the main categories of interventions that function to maintain human health?
- How do scientists gather evidence during the potential outbreak of an infectious disease?
- What is bioinformatics?
- How can DNA sequences be used to identify disease pathogens?
- What is an antibody?
- How do antibodies identify and inactivate antigens?
- How can the ELISA assay be used to detect disease?
- Why is it important for doctors to know the concentration of disease antigen present in a patient's system?
- What steps do scientists take to diagnose, treat, and prevent future spread of a disease outbreak?

Unit.Lesson	Approximate Percent
1.1	11%
1.2	7%
1.3	7%
1.4	8%
2.1	8%
2.2	6%
3.1	8%
3.2	7%
3.3	8%
3.4	7%
4.1	12%
4.2	5%
4.3	3%
4.4	3%

Understandings 1.2

- Antibiotics disrupt the pathways that bacteria use to survive.
- Bacterial cells use multiple pathways to gain resistance to antibiotics.
- Overuse and misuse of antibiotics will promote the selection of resistant bacteria.

Essential Questions

- How do antibiotics work to fight bacterial infections?
- What methods do bacteria use to share antibiotic resistant genes?
- What actions are humans taking that are contributing to bacteria becoming resistant to commonly used antibiotics?

Understandings 1.3

- Problems with one or more structures within the ear cause various types of hearing loss.
- There are a variety of interventions available to help people with hearing loss.

Essential Questions

- How do frequency and amplitude affect how humans interpret sound?
- What causes different types of hearing loss?
- How is hearing loss diagnosed?
- What interventions are available for patients with hearing loss?
- What are the bioethical concerns related to the use of cochlear implant technology?

Understandings 1.4

- Vaccines are medical interventions that activate the immune system to recognize a disease antigen and produce antibodies necessary to defend the body.
- Vaccines can be produced in the laboratory by various methods, including recombinant DNA techniques.
- Epidemiologists are dedicated medical professionals at the heart of the public health field who monitor the health of human populations, search for patterns in the development of both infectious and chronic illnesses, assist in outbreak investigations, and design disease treatment and prevention strategies.

Essential Questions

- What is vaccination?

- How does a vaccine activate the body's immune system?
- How has vaccination impacted disease trends in our country?
- What methods are used to produce vaccines in the laboratory?
- What is recombinant DNA technology?
- What are the molecular tools used to assemble recombinant DNA?
- How can recombinant DNA and bacterial cells be used to produce vaccines?
- How can engineered plasmids be inserted into bacterial cells?
- What is epidemiology?
- How can epidemiologists assist with the detection, prevention, and treatment of both chronic and infectious disease?

Unit 1 Cram Sheet

Lesson 1.1: The Mystery Infection

In Unit 1, we were first introduced to the meaning of "medical intervention." Remember that a medical intervention is anything that is used to treat, prevent, cure, or relieve the symptoms of human suffering whether it is caused by a disease, accident, or something as simple as hygiene. Officially, a medical intervention is a measure to improve health or alter the course of an illness and can be used to prevent, diagnose, and treat disease. Medical interventions can be broken down into categories and grouped together. Some of the categories include: genetics, pharmacology, diagnostics, surgery, immunology, medical devices, and rehabilitation. There are many other categories used to group medical interventions, mostly for the purposes of comparing remedies used for patients.

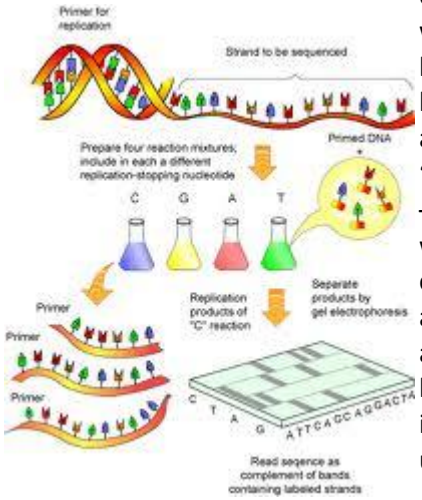
Once we learned about medical interventions, you were introduced to the Smith family for the first time. Remember Sue? She, her roommates, and several other friends came down with a mysterious illness, and we then focused on the methods that were used to discover what was causing the symptoms, come up with a diagnosis, perform tests to confirm the diagnosis, and treatments for the disease. This is a routine often used in healthcare settings: someone comes to a healthcare provider complaining of something wrong, and the healthcare professional must figure out the problem and the best course of action to get the patient back to a healthy (or more healthy) state. In Sue's case, an outbreak was suspected, so it was also our job to determine whether or not the disease had spread, and how to manage it if it had.

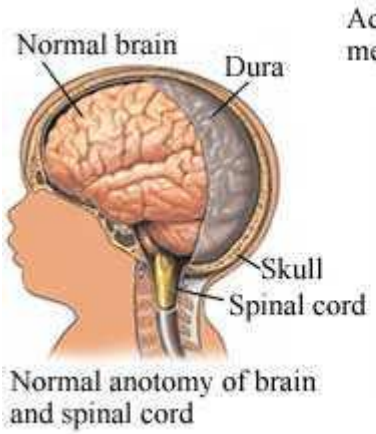
To figure out what was wrong with Sue and her friends, we completed the first step of a medical investigation: linking symptoms together and tying them to suspected disease-causing agents. It may be helpful to go back to activity 1.1.2 and to examine the symptoms that all patients shared. Client issues that can be measured and recorded are typically referred to as signs – temperature, heart rate, blood pressure, rash, swollen glands, etc. – while problems the patient reports are considered symptoms – tiredness, sore throat, nausea, etc. These signs and symptoms are the ultimate clues that are used to find out what is making patients sick so that the all-important job of making patients better can be performed. The symptoms we discovered could all be caused by several different pathogens, so steps had to be taken to determine WHICH infectious agent was making Sue and her friends sick.

We also had to figure out the root cause of the infection. In the case of a disease outbreak, finding "Patient 0," the first person infected at a site, can help medical professionals to determine what the disease was, how it spread, and who was likely exposed so that they, too, can get help.

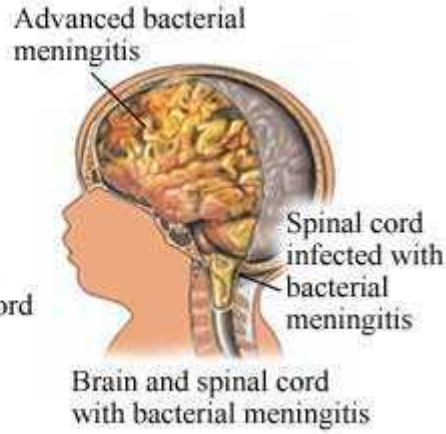
OK – so, we had sick people. We thought we knew what was wrong with them: bacterial meningitis. What has to be the next step? Yep – we need to begin trying to confirm this diagnosis. You don't want to give treatment to people who aren't sick for all kinds of reason: costs, the development of antibiotic resistance, making a misdiagnosis and having your patient get sicker, etc. – so it is important to know FOR SURE what is responsible for any disease, and especially an outbreak. We used two separate procedures to determine if our patients actually had meningitis, with the first being an application of bioinformatics (Bioinformatics, the collection, classification, storage, and analysis of biochemical and biological information using computers, can be used to identify disease pathogens) through the use of a program called BLAST. Essentially, this is a form of DNA sequencing – scanning the DNA of something to figure out what the heck it is. The first step was isolating the disease-causing agent. In the case of meningitis, a disease that causes bacteria to build up

in the meninges of the brain and in the spinal fluid, a sample of cerebrospinal fluid (CSF) is taken by doing a spinal tap. The CSF is then processed to separate human components from disease-causing agents. For bacterial infections, this is done through "plating" the CSF, which will allow any bacteria growing in the CSF – where there should be NO bacteria EVER – to grow outside the human body. If bacteria grow, that's your first sign that something is really, really wrong. Once bacteria are grown, they are lysed (blown up) and their DNA is isolated. This DNA is amplified, then run through a machine that completes DNA sequencing. The machine "reads" the DNA of whatever goes through it and produces something people can see – a long string of the letters ATC and G. This sequence can then be input into BLAST, where the DNA sequences of millions of genes and whole organisms are stored. BLAST compares the DNA sequences input into it to its large database. It then tells whoever asks the identity of the agent the DNA belonged to. In this case, the procedure was able to reveal that several people did, indeed, have bacterial meningitis. Others, however, did not, and we learned that some of Sue's other friends had the diseases infectious mononucleosis, Strep throat, and influenza. It may be helpful for you to look up the effects of all four of these diseases, as they will not be discussed here.





Normal anotomy of brain and spinal cord

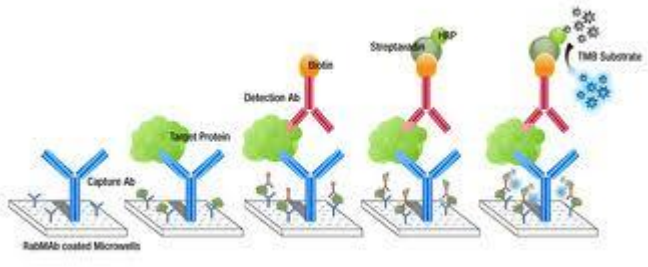


Brain and spinal cord with bacterial meningitis

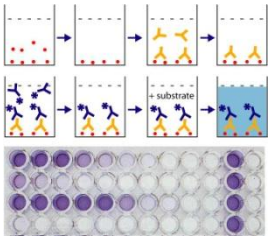
Because meningitis is such a serious disease, we also did a second test that helped us confirm whether our clients were sick with it (or not). This test also revealed HOW sick the patients were. Sounding familiar? Hopefully, the ELISA test is in your mind right about now. ELISA stands for Enzyme-linked Immunosorbant Assay. This is a test that takes advantage of some of the body's natural immune responses to identify the presence of illness. Understanding this test requires you to know the difference between an antigen and an antibody. An antigen is really a type of protein found on the outside of every living cell (and virus!). Antigens are surface markers that cells use to identify each other. It's how your body knows that your body cells are truly yours, and they are how your body identifies cells and viruses that aren't yours. Antigens on the outside of a bacteria are very different than the antigens on your own cells. Because of this, your body's immune system cells (white blood cells) are able to identify them and mount a defense against them, hopefully killing those pathogens before they can make you sick or kill you. If a pathogen is able to get through your non-specific defenses (skin, mucous, urine, etc.) designed to keep things OUT of your body, then more specific defenses are activated. One of these defenses is antibodies. These are produced by a type of leukocyte called a B lymphocyte. The job of antibodies is to attach to foreign antigens. By attaching, those foreign antigens are neutralized. That attachment also signals other types of leukocytes (T lymphocytes) to come in and destroy whatever the antibody is attached to. So, antibodies attach to antigens. That is the principle behind an ELISA.

The ELISA test is based a very simple concept: color changes mean a positive result, with stronger colors meaning more of whatever you're testing for is present. It is found in pregnancy tests, rapid strep test, and drug tests as well as being used to test for antigens from all kinds of infectious agents including bacteria, viruses, and worms. The ELISA test begins with a pre-treated tray full of small wells. These wells are pre-coated with antibodies for the pathogen being studied (in this case meningitis). The serum of patients is then added to these wells. If the serum (CSF for meningitis) contains the bacteria *Neisseria meningiditis*, the antigens on the outside of those cells will be bound to the antibodies in the wells, trapping the antigens on the wells using antigen-antibody interactions. So, now the antigens are trapped, but that doesn't show us anything. The ELISA test relies on a visible color change, and nothing is visible yet. To make this test something with visible results, MORE antibodies are added. First, something called a primary antibody is added. The role of this is to latch on to the antigen, forming a platform on which a secondary antigen with an enzyme attached can be added. This is the next step – adding the second antigen which is linked to an enzyme. Finally, a substrate is added that the enzyme responds to. The enzyme acts on the substrate and causes a color change. IF a color change occurs, it means

that the antigen (the infection) is present. This is a qualitative result, meaning it is something that is simply observed (hot/cold, soft/hard, clear/blue). Qualitative results are not measurable. The intensity of the color can be used to find out the degree of infection, with the darker color meaning a greater infection. This is actually measurable (quantitative) if you have created a serial dilution to compare it to. A serial dilution is something that is created and used to compare the results of an ELISA to. It involves beginning with a known concentration of antigen (say 100 ng/mL) and diluting it (watering it down). It involves placing that 100 ng/mL sample in a well, transferring part of it to a new well with a set amount of water. If you use equal parts water and antigen, 100 becomes 50. In the next well, it's repeated

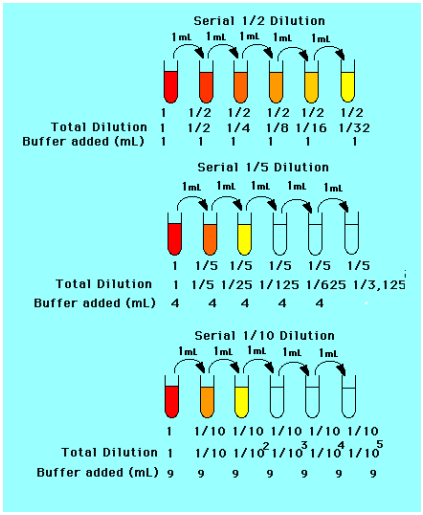


to reduce the amount of antigen to 25, then 12.5, then 6.25, and so on. When the antibodies and substrate are added to this a series of colors, from darker to lighter, is created. These samples have KNOWN amounts of antigen. Patient samples can be compared to this to determine how much of the infectious agent is in the body, which is often extremely useful in determining how aggressive to be with treatment. It can help doctors to determine how much antibiotic to give, how long care will be needed, and how it is that permanent damage will result. In our scenario with Sue, this test revealed which patients actually had meningitis, and who had the greatest concentrations of the antigen. Knowing that makes it possible to make a reasonable assumption about who got the disease first – whoever had the most antigen was probably the first person infected.



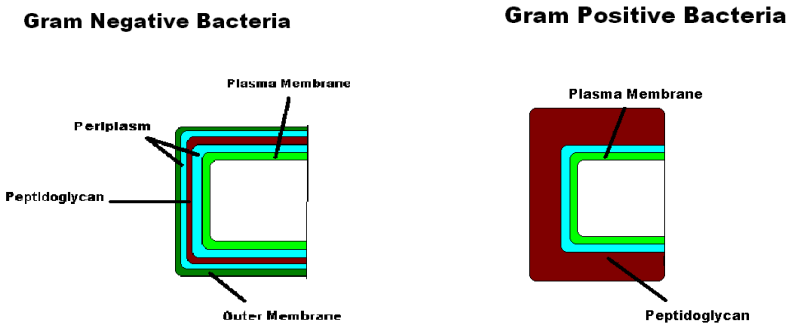
Lesson 1.2: Antibiotic Treatment

Now that we know for sure that Sue has meningitis, and we know which of her friends have the same infection, what's next? If you're thinking we need to treat the infection, you're right! Bacterial infections are treated with antibiotics.



We know that antibiotics kill bacteria, but why? Understanding that requires knowing what bacteria are and how they work. A couple years back, we discussed how most bacteria are either gram positive or gram negative. Gram positive bacteria have a very thick cell wall made mostly of peptidoglycan, while gram negative bacteria have a much thinner cell wall. Sometimes, this influences how they respond to antibiotics.

Bacteria are classified into two main groups, Gram positive bacteria and Gram negative bacteria, distinguished by the structure of their cell walls.



Gram Negative Bacteria

- The cell wall contains multiple layers, including a thin layer of *peptidoglycan*.
- The outside layer is called the outer membrane, which is made of a lipid bilayer whose outside is composed of lipopolysaccharides called *endotoxins*.
- The outer membrane serves as a barrier to the passage of most molecules and contains specialized proteins, called *porins*, which allow certain molecules to pass through the membrane.
- The region between the plasma membrane and the outer membrane is called the *periplasm* and is filled with a gel-like fluid and proteins involved in a variety of cellular activities.
- The Gram-stained cell is pinkish-red.

Gram Positive Bacteria

- The cell wall contains a thick layer of peptidoglycan and teichoic acids. There is approximately twenty times more peptidoglycan than the Gram negative bacteria.
- There is no outer membrane present.
- There are no porins present.
- The Gram-stained cell is purple.

Below, the chart lists some of the major components of bacterial cells and their functions.

Cellular Part:	Description:
Nucleoid	Gel-like region within the cytoplasm containing the single, circular, double-stranded DNA molecule. This chromosomal DNA is *supercoiled*, meaning tightly packed into a twisted form. The DNA contains all of the genetic information necessary for normal functioning of the cell.
Plasmids	Circular double-stranded DNA molecules. They are typically 0.1% to 10% of the size of the chromosomal DNA and only carry a few to several hundred genes. A single bacterial cell can carry multiple plasmids. Normal functioning of a bacterial cell is not dependent on the genetic information contained in a plasmid, but the DNA often codes for proteins that are advantageous to the cell. For example, plasmids might contain the information coding for the proteins that enable the cell to destroy or be immune to certain antibiotics. Plasmids can be transferred from one bacterial cell to another bacterial cell.
Ribosomes	Structures involved in protein synthesis. They facilitate the joining of amino acids.
Cell Wall	Rigid barrier that surrounds the cell, keeping the contents from bursting out. *Peptidoglycan* provides the rigidity for the cell wall.
Plasma Membrane (cell or cytoplasmic membrane)	Semipermeable membrane that surrounds the cytoplasm of the cell. This phospholipid bilayer is embedded with proteins that act as a barrier between the cytoplasm and the outside environment.
Capsule	A distinct and gelatinous layer, called *glycocalyx*, enveloping the cell. This layer enables the bacterial cell to adhere to specific surfaces and sometimes protects bacterial cells from human immune systems.
Flagella	Protein appendages that are anchored in the membrane and protrude out from the surface. The flagella spin like propellers, moving the bacterial cell forward.
Pili	Filamentous appendages which are similar in structure to flagella, but function in a different manner. Some pili enable the bacterial cell to attach to a specific surface (these pili are called *fimbriae*). Other pili are involved in *conjugation*, a mechanism of DNA transfer from one bacterial cell to another (these pili are called *sex pilus*).
Endotoxins	Lipopolysaccharide molecules that make-up the outer leaflet of the outer membrane of Gram negative bacteria. Endotoxins are different from *exotoxins*, which are proteins synthesized by both Gram negative and Gram positive bacteria and function as potent toxins.

So – we have bacteria parts, but what does that have to do with getting rid of an infection? What does that have to do with how infections are treated with antibiotics? Great questions! Antibiotics work by disrupting the pathways that bacteria use to survive. This may mean stopping the bacteria from reproducing, inhibiting protein synthesis, or disrupting the cell wall. Different antibiotics work in different ways – which is good because not all antibiotics work on all bacteria.

Mechanisms of Action of Antibiotics

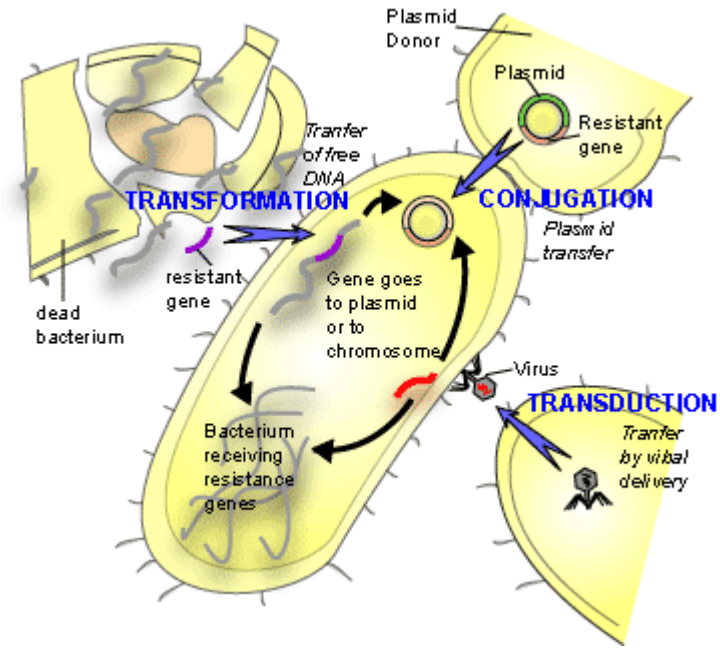
A number of bacterial processes, including the synthesis of bacterial cell walls, proteins, metabolic pathways, and the integrity of the cytoplasmic membrane, are the targets of most antibacterial drugs. The following table outlines the mode of action for some of the main classes of antibiotics:

Antibacterial Medication:	Mode of Action:
β-Lactam Antibiotics	Irreversibly inhibit enzymes involved in the final steps of cell wall synthesis. The enzymes inhibited by these drugs mediate the formation of the peptide bridges between adjacent

	strands of peptidoglycan. These drugs vary in their spectrum of activity; some are more active against Gram positive bacteria; whereas, others are more active against Gram negative bacteria.
Tetracyclines	Reversibly bind to the 30S ribosomal subunit, blocking the attachment of tRNA to the ribosome and preventing the continuation of protein synthesis. They are effective against certain Gram positive and Gram negative bacteria.
Fluoroquinolones	Inhibit one or more of a group of enzymes called *topoisomerases*, which maintain the supercoiling of the chromosomal DNA within the bacterial cells. The inhibition of these enzymes prevents essential cell processes. The fluoroquinolones are active against a wide variety of bacteria, including both Gram positive and Gram negative bacteria.
Sulfonamides	Inhibit the growth of many Gram positive and Gram negative bacteria. They are structurally similar to paraminobenzoic acid (PABA), a substrate in the pathway for folic acid biosynthesis. Because of this similarity, the enzyme that normally binds with PABA preferentially binds with the sulfonamide drugs, resulting in its competitive inhibition. Human cells are not affected by these drugs because they lack this enzyme.

A good resource on antibiotic mechanism of action is the Howard Hughes tutorial found at:
http://www.hhmi.org/biointeractive/Antibiotics_Attack/pw_1.html

At this point, we know that antibiotics work to stop bacterial infections from spreading. So, then, why not give them for everything? Why not take them all the time to prevent bacterial infections. The truth is, healthcare professionals used to do this regularly. It was standard practice to take antibiotics any time a patient even suspected an infection. Antibiotics were prescribed for everything, and doctors only delved further into a disease if the antibiotics didn't seem to help. This practice has ended in modern times because we have learned that bacteria are capable of evolving and becoming immune to antibiotics. They are able to become antibiotic resistant. Often, this begins with one bacterium that develops a mutation. This mutation may give it a stronger cell wall that can resist B-lactam antibiotics. It may mutate the way proteins are produced, so that tetracyclines don't work. No matter, the mutations that randomly develop cause the bacteria to evolve into something that an antibiotic doesn't work on. That bacteria then grows and divides, and suddenly there are even MORE bacteria with the same mutation. These divide, and lead to an infection with an antibiotic-resistant strain of bacteria that is more difficult to kill, requiring better and stronger – and possibly completely new – bacteria. As if that isn't bad enough, there's another concern: bacteria are able to share the plasmids that contain antibiotic-resistant genes. There are three methods that are commonly used: transduction, transformation, and conjugation. Conjugation (bacteria sex) is the most common method. Here, two bacteria – which do not even have to belong to the same species – link their pili, forming a bridge between the two bacteria. A plasmid can be exchanged over this bridge, allowing a bacteria with antibiotic resistance to give the gene for that resistance to another (previously susceptible) bacteria. Plasmids carrying antibiotic-resistant genes can also simply be scavenged from a dead bacterial cell through the process of transformation. Additionally, resistance can be "delivered" to bacteria using some sort of vector through the process of transduction.



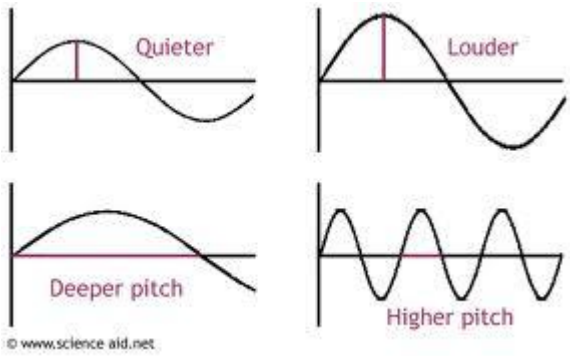
When bacteria gain antibiotic-resistance, treating them becomes a whole lot more complicated. There's no predicting when this will happen. There's no predicting how it will spread. We simply know that it does. We also know that not every infection will respond to an antibiotic in the same way, and there is no way to know whether the population of pathogenic bacteria you are treating will be controlled more or less quickly by the antibiotic than expected. Also, bacterial populations vary and the presence and number of antibiotic resistant bacteria will depend on the genetic variability in the population.

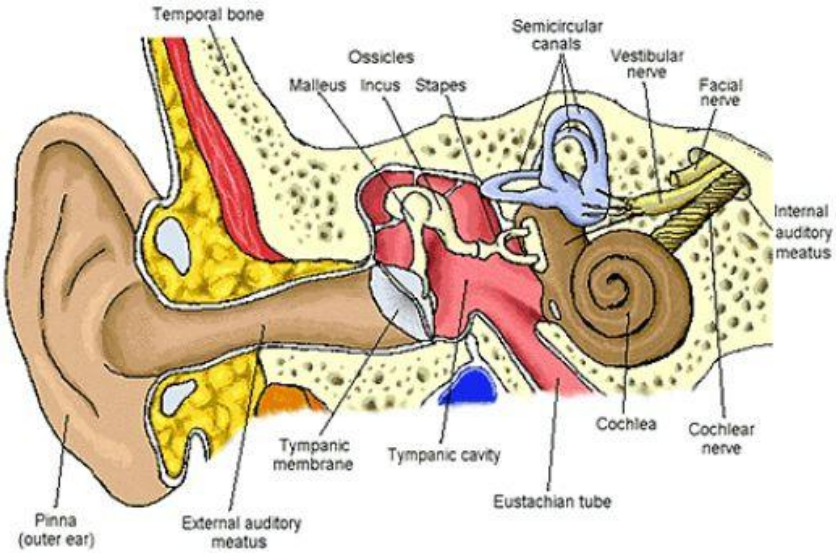
Because of antibiotic resistance, there is growing concern for the ways that we use antibiotics today. As stated earlier, it is less common for healthcare providers to give antibiotics as a preventative measure. It is less common for people to take them unnecessarily as we become more educated. However, they're used excessively elsewhere – for example, when raising livestock. Antibiotics are added to chicken and cow feed to prevent the animals from getting sick before sale. These antibiotics can stay in the meat and be passed to us – as can resistant bacteria in the meat. America is one of the few countries where this practice is used, and it is certainly likely to contribute to the development of antibiotic resistant strains of bacteria. Overprescription and overuse of antibiotics NOW is probably going to cause extremely scary problems in the future.

Lesson 1.3: Aftermath – Hearing Loss

Well – let's get beyond the antibiotic scare, and back to poor Sue Smith's story. Sue had meningitis, got it diagnosed, and got it treated. Her life was saved, but not before the bacteria caused some permanent damage. Sue has hearing loss, which was diagnosed by an audiologist. Hearing loss affects millions of people in the United States. Hearing loss can drastically impact a person's ability to communicate. Therefore, a lot of time and money has been invested into research to develop interventions to treat hearing loss. Although the degree of hearing loss varies from individual to individual, there are only three types of hearing loss: sensorineural hearing loss, conductive hearing loss, and mixed hearing loss. Hearing loss has many causes and in many cases can even be prevented.

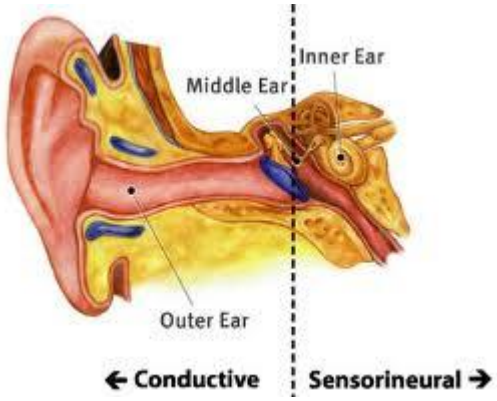
Before we talk about the three types of hearing loss, you need to know the parts of the ear and their functions as well as some basic information about sound. We will begin with sound. You may recall that sound cannot travel through a vacuum. It must travel through something – air, water,or even bone. There are a few major aspects of sound: intensity (loudness) which is measured in decibels, This has a profound effect on hearing, as listening to loud sounds for prolonged periods can have a permanent impact of hearing – and not in a good way. Two other aspects of sound are frequency and amplitude. These two terms deal with the waves that sound produces. Frequency is the number of sound waves that cross a point in a certain amount of times. Sounds with the highest frequency produce more waves to pass a point, and sound higher in pitch. (Think of nails on chalkboard compared to thunder – the nails produce packed waves and the thunder produces very wide waves.) Pitch is the way we perceive frequency. (Nails vs. thunder – very different sounds) Amplitude deals with how high the waves are, which is what we perceive as loudness.



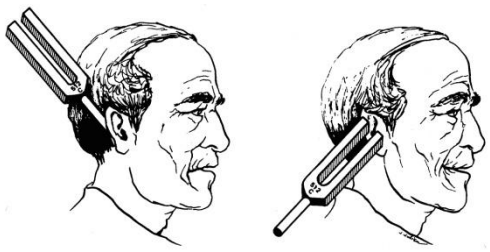


So, pitch is caused by how close together waves are (frequency) while intensity is determined by how tall the waves are (amplitude). This influences hearing in a couple ways. The human ears are designed to detect sounds in a set range of pitches and frequencies. Detecting this sound involves the ear. Sound is collected in the outer shell of the ear, called the pinna. This sound travels in air through the auditory canal until it reaches the tympanic membrane (the eardrum). Sound causes the tympanic membrane to vibrate. When the tympanic membrane vibrates and converts sound to mechanical waves, causing the ossicles (earbones) to vibrate. The malleus vibrates, causing the incus to vibrate, causing the stapes to vibrate. The stapes hits the oval window as it vibrates, pushing on the fluid inside the cochlea to vibrate in the form of a fluid wave. This vibration travels through the cochlea, stimulating the sensory hair cells, which are incredibly sensitive. Their stimulation results in a signal passing to the cochlear nerve, which sends a signal to the brain so sounds can be interpreted.

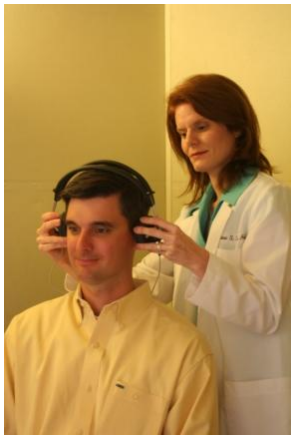
While the primary job of the ear is hearing , it also plays a role in balance. This involves the vestibule of the ear, which houses the semicircular canals. This is a set of three tubes that give you the ability to sense up, down, and sideways. Body position shifts fluids around in this area, allowing you to sense your position in space when the signals produced by the nerves in the canal send signals via the vestibular nerve to the brain. Also in the ear is the eustacian tube, which is there to maintain pressure within the inside and outside of the ear. If pressure is different, sound doesn't travel right!

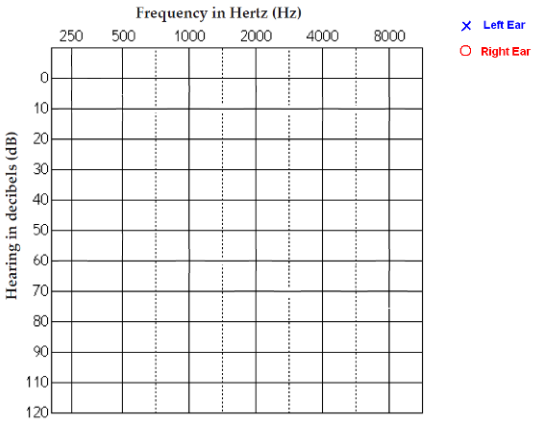


At this point we've discussed sound and the parts of the ear. These both play a role in the different types of hearing loss mentioned earlier: sensorineural hearing loss, conductive hearing loss, and mixed hearing loss. Conductive hearing loss is caused by damage to the wave-carrying portions of the ear: the pinna, the auditory canal, the tympanic membrane, or the ossicles. This type of hearing loss usually involves a reduction in sound level or the ability to hear faint sounds. This type of hearing loss can often be corrected medically or surgically. It can be caused by a loss of the outer ear, damage to the tympanic membrane, or damage to the ossicles. With sensorineural hearing loss, there is damage to the cochlea (inner ear) or the auditory nerve. In many cases, it cannot be corrected. It can be caused by repeated exposure to loud noises, an extremely loud noise one time, or aging of the cochlea. Mixed hearing loss is a combination of both. In all cases, patients (like Sue) might benefit from a hearing aid. Hearing aids amplify sounds, making them louder to the person with a device in their ear and allowing them to hear better. Another option – for sensorineural hearing loss, anyway – is a cochlear implant. This is a small device inserted surgically in two phases, with a wire placed in the cochlea to do the job of hair cells and direct sound waves from the fluid in the cochlea to the auditory nerve, and with an external implant (on the head) to pick up sounds from around the patient. Because this procedure is very expensive, may result in complete hearing loss, and is offensive to the deaf community as an infringement on their lifestyle, it remains somewhat controversial.



Earlier, it was stated that Sue suffered from hearing loss as a result of her meningitis. How is this tested for? There are actually several types of tests that can be performed. The Rinne test involves using a timer and a tuning fork to determine the difference between conductive and sensorineural hearing. Sensorineural hearing is tested by placing the handle of a tuning fork that has been hit on a table and is humming against the mastoid process on the skull and listening until the sound goes away while timing the length of time the patient can hear. When the sound is no longer heard, with no delay, the tuning fork is flipped and the pronged end is placed in front of the ear, with the patient listening again. Air conduction (conductive hearing) is checked in this way, with the tester then noting the time elapsed. If hearing is normal, the air conduction will be heard twice as long as bone conduction. If there is conductive hearing loss, bone conduction is heard longer or as long as air conduction. A speech in noise test can be done for some types of sensorineural hearing loss. This involves listening to speech with a background static of varying types and determining how well the patient is able to detect actual speech under those circumstances. If there is sensorineural hearing loss, hearing the speech will be incredibly difficult or not possible. Finally, there are audiograms, which detect both sensorineural and conductive hearing loss. Audiograms are made during a pure tone test. This involves using an audiometer to measure hearing sensitivity. The test will begin by playing a series of beeps or tones at a distinct frequency. Every time the subject hears the beep, they raise a finger or push a button or raise their hand. The tone will continue to get softer and softer until it can no longer be heard – determining the threshold for the patient for a certain frequency. The test is then repeated at other frequencies between 250 and 8000 Hz. An audiogram records thresholds, which can be used to detect where hearing loss exists at different frequencies. The thresholds are recorded on a graph, called an *audiogram*, with the frequencies on the x-axis and the hearing thresholds in decibels on the y-axis. The thresholds for the right ear are represented with a red circle and the thresholds for the left ear are represented with a blue 'X.' The following diagram is an example of a blank audiogram.

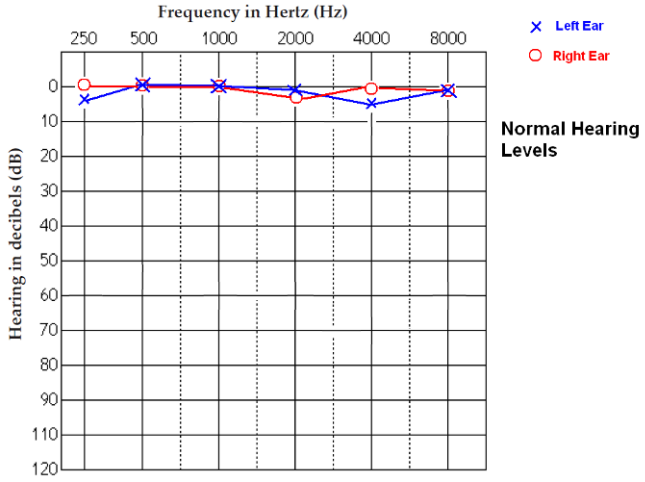




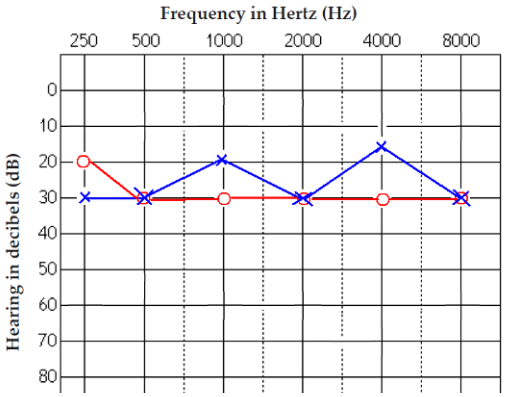
The Xs and Os are connected with lines to help keep track of the hearing levels across the different pitches. Hearing levels are often described in a progression of loss: normal hearing, mild hearing loss, moderate hearing loss, moderately severe hearing loss, severe hearing loss, and profound hearing loss. The following chart shows the ranges for each type of hearing level.

Normal Hearing	0-20 dB
Mild Hearing Loss	21-40 dB
Moderate Hearing Loss	41-55 dB
Moderate to Severe Hearing Loss	56-70 dB
Severe Hearing Loss	71-90 dB
Profound Hearing Loss	>90 dB

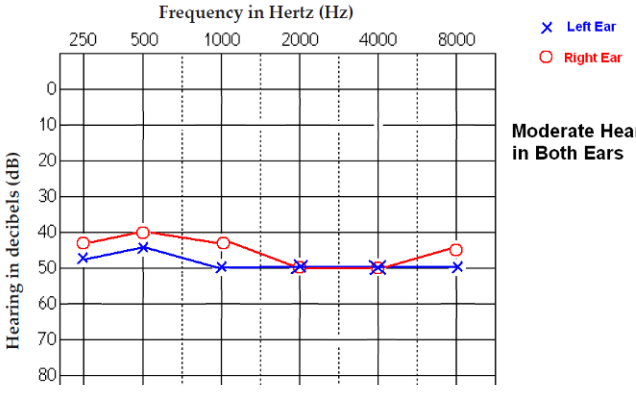
The figures below show example audiograms for different levels of sensorineural hearing loss in both ears.



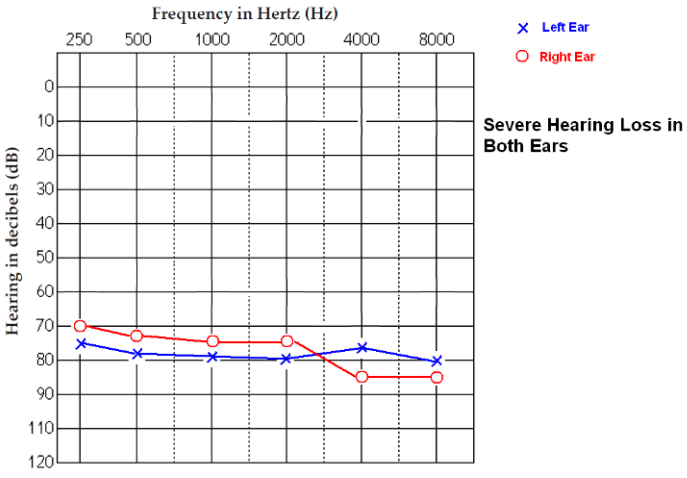
Normal Hearing Levels



Mild Hearing Loss in Both Ears

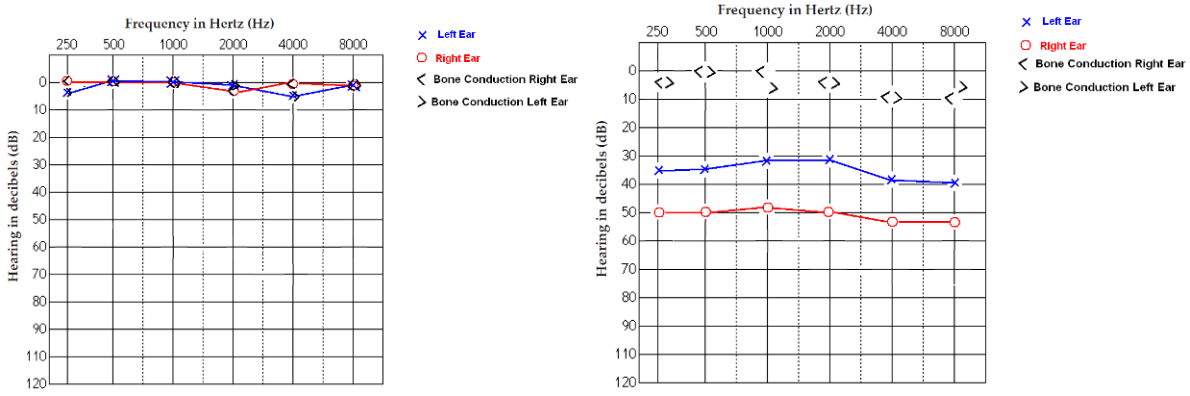


Moderate Hearing Los in Both Ears



Severe Hearing Loss in Both Ears

Conductive hearing loss can also be represented in an audiogram. The air conduction levels are represented as Xs and Os and the bone conduction levels are represented as **<** and **>**. Because conductive hearing loss is due to problems with the middle ear, hearing levels are better with bone conduction than with air conduction. Conductive hearing loss is therefore represented when bone conduction is at least 10 decibels better than air conduction, after it has been determined with a version of the Rinne test. Below are two examples of audiograms. The audiogram on the left shows no hearing loss and the audiogram on the right shows conductive hearing loss.



Lesson 1.4: Vaccination

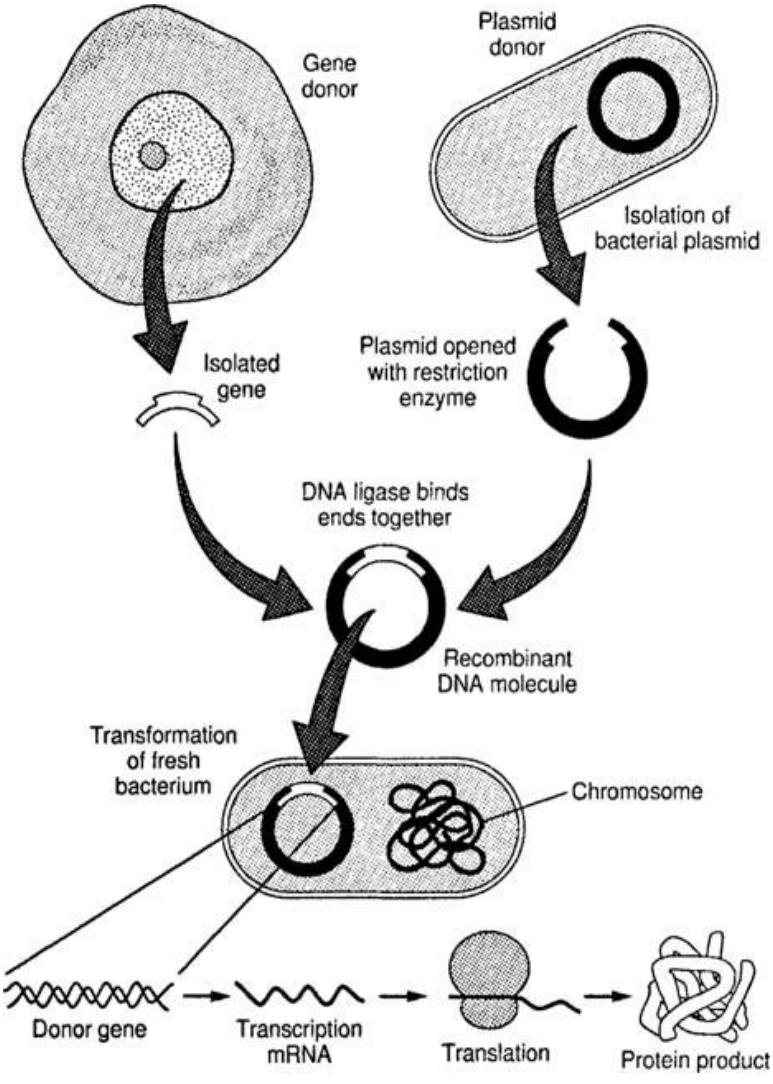
We've spent a great deal of time discussing infections, how they are diagnosed and confirmed, how they are treated, and some of the serious consequences that they can have. The last thing that we need to look at is how it is possible to prevent getting an infectious disease in the first place. How is that possible? How do we keep ourselves from getting sick? No – the answer is not "live in a bubble". Rather, the trick is to convince our bodies that we've *already* had the infection that we're trying to prevent getting. This is done through a process referred to as vaccination.

A vaccination is an injection of dead, weakened, or modified pathogens into the body. Their presence in the body activates the immune system, which responds to the substances within the vaccine the same way it responds to any other infection: by activating lymphocytes, producing antibodies, and then remembering that disease for a very long time so you don't get it again. Antigens in the material contained in the vaccine cause the body to produce antibodies. A specialized type of lymphocyte referred to as a memory cell will remain long after the "infection" is cleared out, and will be able to rapidly produce antibodies when you are exposed to the true infection, thus keeping you from ever getting sick. Vaccinations have been used to reduce the incidence of several types of disease. It has eliminated smallpox and polio, in this country and many others; it keeps us from getting the flu; it is even used to protect us from certain types of cancer, such as HPV.

As insane as it sounds, there are 6 methods used to create vaccines, and we will discuss each of them here. We will begin with the "similar-pathogen" vaccine, which is used to make a vaccination for polio. Here, you find a virus similar to the one you want to protect against (as cowpox is similar to smallpox), isolate the virus, and inject it "live" into the person being inoculated. Smallpox and cowpox are similar enough that protection against one provides protection against the other – they are similar pathogens! Another option is an attenuated virus, as is used to protect against the measles virus. This is also a live vaccine. It involves altering the virus enough that it i s weakened in the human body. In the case of measles, the virus is adapted to grow in cold environments. The human body is warm enough that cold-loving viruses don't do well, so the body has time to make antibodies before an infection sets in. After antibodies are present in the body, you are

protected from the normal measles as well as the weakened version. A killed vaccine is what we use to protect against polio. Here – you guessed it – the virus is killed with heat, radiation, or some other means, then injected dead into your body. The dead virus produces a weak response in the body – not enough for true immunity to set in, which is why boosters are often required. Which shots have you had that required boosters? A Toxoid vaccine is created for pathogens like tetanus. Here, the goal is to expose the body to the toxins a pathogen produces, rather than to the pathogen itself. Tetanus is caused by toxins produced by the bacteria *Clostridium tetani*. Toxins are extracted from the pathogenic organism (the bacteria in this case) and are neutralized so the body isn't harmed by them. Neutralization can involve chemicals like formaldehyde or aluminum salts. After neutralization, you are injected with the toxin, and the body produces a response. Like with dead viruses, boosters are also required. A subunit vaccine is made for hepatitis B. A subunit vaccine consists of nothing more than a portion of a pathogen - a chunk. A specific "chunk" of virus is chosen for vaccination, and the body recognizes that "chunk" on a pathogen when it encounters it. FINALLY, there is a vaccine called a Naked-DNA vaccine, which is currently being developed to use in an HIV vaccine. Here, a single gene (which will produce a protein) is selected for vaccination .This gene is amplified and placed into a vector of double-stranded DNA. This DNA is injected into a bacteria, the bacteria grow and are lysed, and the DNA is extracted for injection into the human.

The last thing we need to go over is how recombinant DNA technology is being used to create vaccinations. This is briefly discussed above in the naked DNA vaccination for HIV described above. We just need to add a few more details. Recombinant DNA technology involves modifying DNA by adding or removing genes, placing this modified DNA into an organism, and letting that organism replicate. It begins by selection of a gene of interest. This gene is removed from the organism it belongs to by isolating its DNA, then using restriction enzymes to "cut out" that particular section of DNA, which is then amplified (copies are made). The genes are then ligated into double-stranded DNA. Remember that to ligate it is to seal it in, as though it had been glued in place and is now a permanent part of the DNA. This DNA, often double-stranded, circular, and referred to as a plasmid, is pretty useless outside of a living cell. To get beyond that, the DNA is put into a cell using a chemical or electrical shock that makes the bacteria porous enough for the plasmid to enter. Heat shock is then used to seal the cell up again, with the plasmid inside. Once that plasmid is inside a bacterium, the bacteria produces more of that plasmid, incorporating that DNA and making copies of it before the cell divides. Soon, colonies of this modified bacteria live, containing the recombinant DNA we wanted. This DNA can be extracted from the bacteria after they have been killed, and used for the purpose of vaccination, with the DNA injected into the person who needs the vaccine.



Everything we have discussed: studying symptoms of disease, detecting disease, making diagnoses, administering treatments, studying the after-effects, and finding ways to prevent diseases from happening all together, are the jobs of an epidemiologist. Epidemiology is the study of disease, and epidemiologists are dedicated medical professionals at the heart of the public health field, monitor the health of populations and search for patterns in disease. They may assist in outbreak investigations or they may examine lifestyle factors and their relationship to chronic illnesses such as heart

disease, diabetes, and cancer. Whether in the field, in a lab, or in an office, epidemiologists play a crucial role in maintaining human health.

Understandings 2.1

- Genetic testing is the use of molecular methods to determine if someone has a genetic disorder, will develop one, or is a carrier of a genetic illness and involves sampling a person's DNA and examining the chromosomes or genes for abnormalities.
- Genetic counseling can help a family understand the risks of having a child with a genetic disorder, the medical facts about an already diagnosed condition, and other information necessary for a person or a couple to make decisions suitable to their cultural, religious, and moral beliefs.
- Proper prenatal care and monitoring of the fetus are vital to maternal and child health during a pregnancy.

Essential Questions

- What is genetic testing?
- What are the duties of a genetic counselor?
- What is the goal of PCR?
- What are the steps of the PCR process?
- What is the relationship between phenotype and genotype?
- What are SNPs?
- How can restriction enzymes and electrophoresis be used to identify SNPs and determine genotype?
- What medical interventions and lifestyle modifications can help a pregnant woman have a healthy pregnancy?
- What can amniocentesis and chorionic villus sampling tell a couple about their developing fetus?

Understandings 2.2

- Gene therapy is a type of disease treatment in which faulty genes are replaced by functional copies.
- Advances in reproductive technology open many moral, ethical, and scientific debates.

Essential Questions

- How can genetic diseases be cured if scientists could replace faulty genes?
- What vectors can be used to transfer DNA to human cells?
- How might gene therapy open the door to genetic enhancement?
- What medical interventions are available for couples who would like to choose the gender of their child?
- Should parents be able to design their children?
- What is the difference between reproductive cloning and therapeutic cloning?
- What are some of the ethical dilemmas surrounding current and future reproductive technology?

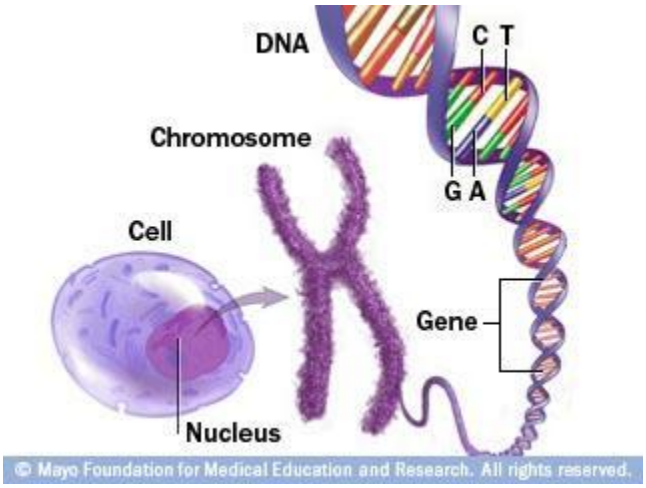
MI Unit 2 Cram Sheet

Lesson 2.1: Genetic Testing and Screening

In Unit 1, we were introduced to the Smith family for the first time. Sue, the college-age daughter of James and Judy Smith, contracted bacterial meningitis and we worked towards treating her, handling the long-term effects (hearing loss) and studied how future infections by pathogens can be prevented. In Unit 2, we find out some new and exciting information for the Smith family – Judy Smith is pregnant again. Because Judy is a bit older than when her first two children were born, there are some additional risks in the form of an increased chance of inherited diseases. In Unit 2, we looked at a new category of diseases – diseases a person is born with (inherited diseases) rather than those they "catch." We also look at genetic screening and testing, the value of screening and examining DNA, the importance of prenatal care, and the future of genetic technology.

Current technology allows us to look past the surface of our cells and to understand their inner workings, their most important part – DNA. DNA can now be isolated from cells and "picked apart" to reveal disease. Genetic testing can be used to diagnose disease before a child is even born. We can test ourselves for diseases and learn the likelihood of passing them on to children. **Genetic testing** is the use of molecular methods (DNA sequencing with BLAST, karyotyping, etc.) to determine if someone has a genetic disorder, will develop one, or is a carrier of a genetic illness. It involves sampling a person's DNA and examining the chromosomes or genes for abnormalities.

Genes, Chromosomes, and DNA



A bit of review: a chromosome is tightly coiled DNA. The human body contains 23 pairs of chromosomes: 22 pairs of autosomes and one pair of sex chromosomes. These chromosomes are inherited from your parents, and from the moment of conception (fertilization) they are your genetic code – your DNA. The chromosomes are typically only visible during cell division – the rest of the time, DNA is a jumbled mess that is invisible with a light microscope. This **DNA**, which forms **chromosomes**, holds **genes**. Genes are the coding sections of DNA, and their job is to provide the instructions for building proteins. Your body is composed of proteins. They are the workers of your body and are essentially responsible for every trait you have: hair color, eye color, blood type, skin color, and diseases you have. Chromosomes themselves can be the cause of disease, as can defective genes.

In short, too many chromosomes – bad. Not enough chromosomes – bad. Inheriting defective DNA (bad gene) – also bad.

Genetic Testing Overview

By now, you probably realize that there are all kinds of things that can go wrong when a human being is created. It's a wonder more of us don't have things wrong. Because of the possibilities that exist for problems, many people have a strong desire to know whether they have diseases, could pass them to children, or if their unborn children have a disease. That is what genetic testing is all about – using DNA to help people find out what they want (and sometimes need) to know.

Genetic testing is often performed by a genetic counselor. A **genetic counselor** is a trained professional who helps individuals and families understand and adjust to a genetic diagnosis or the possibility of having a hereditary disorder. Genetic counselors interpret family history information and educate patients and professionals about genetic diseases. As specialized counselors, these professionals help patients and families understand genetic testing options and the implications of undergoing genetic testing. In addition, genetic counselors address psychosocial and ethical issues associated with a genetic disorder and/or a genetic test result. As members of a health care team, genetic counselors serve as educators to their patients, to physicians, other health care providers, as well as to society. Genetic counseling can help a family understand the risks of having a child with a genetic disorder, the medical facts about an already diagnosed condition, and other information necessary for a person or couple to make decisions suitable to their cultural, religious, and moral beliefs. To keep things simple, they help with the testing and provide information people need to make informed choices.

Types of Genetic Disorders

Genetic testing reveals whether or not a DNA-based problem is present. These genetic disorders are caused by abnormalities in an individual's genetic material. We talked about four different types of genetic disorders: single-gene,

multifactorial, chromosomal, and mitochondrial. You may remember these – if so, feel free to skip ahead!

A **single-gene disorder** is a change or mutation in one gene. Sickle cell anemia and cystic fibrosis are good examples of these. Single-gene disorders may be classified as autosomal dominant, autosomal recessive, or sex-linked. A dominant trait is one where one copy of a gene passed to a child causes an effect in the child – like dwarfism or Huntington's disease. A recessive trait (sickle cell and cystic fibrosis) is one where a child must inherit the defective gene from both parents in order to express the trait. If the child only gets one copy, he or she is a carrier of the trait, but will not show it. A sex-linked trait is one that is passed on the sex chromosomes (the X or the Y). Remember that if a child inherits two x chromosomes, they're a girl. If a child gets an X and a Y (only dad can give a Y) the child is a boy. Sometimes, these X's and Y's contain defects. If a child inherits the defective chromosome, they are likely to express the trait. Sex-linked traits are a little confusing for some people because the rules are different for boys or girls. An x-linked trait is passed on the x chromosome. Because girls have two x chromosomes, they must inherit two defective x's to show an x-linked trait. If they only get 1, it's no big deal because they have a normal x to perform all the functions of the x chromosome. If a boy gets a defective x chromosome, though, they automatically have whatever bad trait was carried on that chromosome. This is because they only have one x chromosome, so there's no backup to perform x-related functions. This is why disorders like colorblindness, duchenne muscular dystrophy and hemophilia are much more common in males than females.

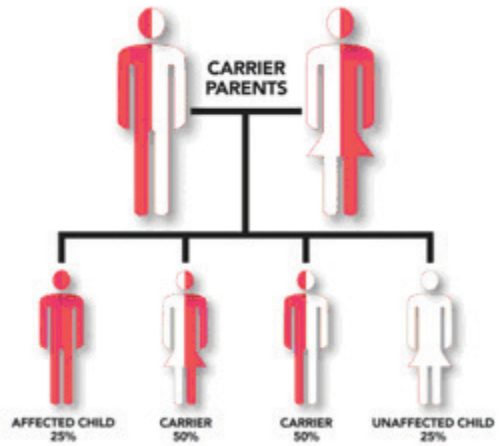
Let's look at another type of inherited disorder now: **multifactorial disorders**. These are caused by multiple bad genes AND the environment in combination. Breast cancer is an example of this. People are more prone to breast cancer if they have certain forms of certain genes, but they are not guaranteed to inherit that disease. Their chances go up a lot if they make certain lifestyle choices like alcohol use or the use of deodorant. So, both the genes and the environment play a role in multifactorial diseases. Current research is suggesting that MOST common chronic illnesses (diabetes, alzheimer's, dementia, high blood pressure, etc.) are multifactorial.

Mitochondrial disorders are fairly rare, and are caused by mutations in the DNA of mitochondria. If the mitochondria are defective, the body have a difficult time making ATP, which is needed to fuel all cell processes. These are ONLY passed from mother to child. Leber's hereditary optic neuropathy is an example of this.

Chromosomal disorders involve inheriting either not enough chromosomes or extras. This happens when either a sperm or egg are made with the wrong number of chromosomes. Diseases where you inherit extra chromosomes include Down's syndrome. Down's Syndrome is also known as Trisomy 21. This is because a person with Down's syndrome has inherited an extra copy of chromosome 21. Tri- means three, and these people have three copies of a chromosome when they are only supposed to have two. You have probably met a person with Down's syndrome at some point. You know that the condition causes them to have very distinctive traits. These traits are caused by that trisomy. The extra DNA makes extra proteins, and this is what causes the unique physical features and internal problems seen in someone with Down's syndrome. These disorders are easily revealed with a **karyotype**, a picture of the chromosomes where they have been paired based on size, banding pattern, and centromere position, then arranged from biggest to smallest.

Types of Genetic Screening

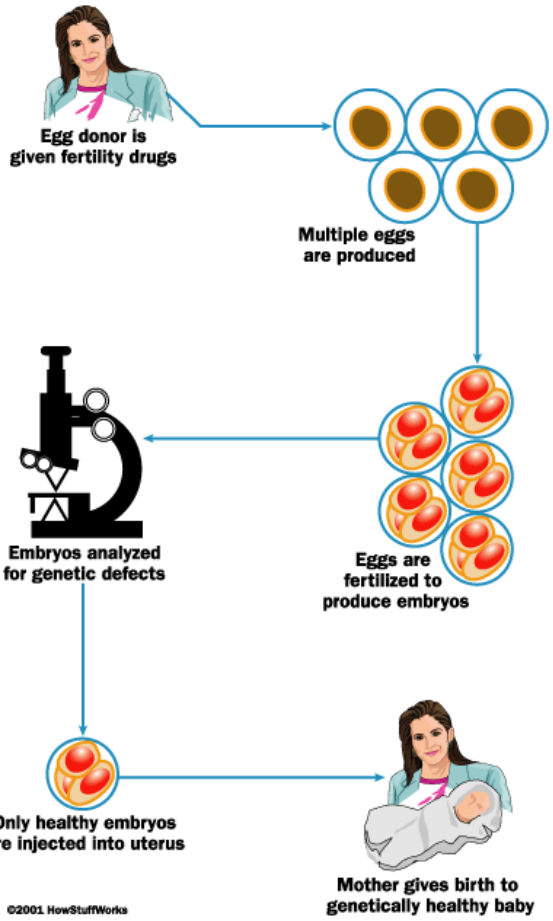
It should be clear by now that there are all kinds of genetic disorders. Because of this, there are people out there who want or need to know if they carry these diseases, can pass them on to children, or have diseases themselves. There are several types of genetic testing and screening used to provide people with that information: Carrier screening, preimplantation genetic diagnosis, fetal screening/prenatal diagnosis, and newborn screening.



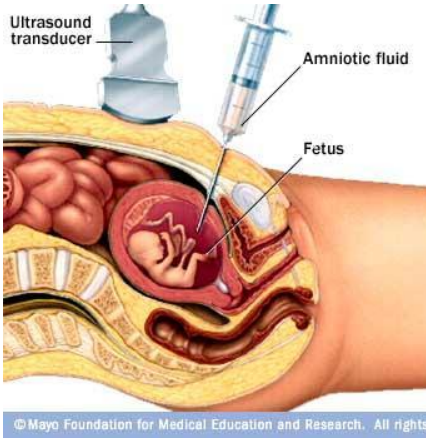
Carrier screening is a test that is typically done on adult couples who are considering having children, and want to determine if those children could inherit any diseases. Most of the time, there is a family history of something like cystic fibrosis or Tay Sachs disease in the family that the couple wants to ensure they won't pass to the child. Remember that a carrier is someone who holds a bad gene, but doesn't show it. This process is fairly simple: a blood sample is drawn, the DNA is extracted and amplified using PCR, and the DNA undergoes testing for the disease(s) they are concerned about. This may involve DNA sequencing or gel electrophoresis – sometimes both.

Preimplantation Genetic Diagnosis (PGD) is a bit different. This procedure is often used by people with known autosomal dominant or sex-

linked conditions that they do not want to pass on to their children. Here, eggs and sperm are harvested from prospective parents. The eggs are fertilized by the sperm in vitro (in a petri dish) and the embryos are allowed to develop to the 8-cell stage. After the embryos are that big, one single cell from each embryo is removed. The DNA is extracted from that one cell, amplified, and tested for the presence of the trait the parents do not want. Healthy embryos are selected and implanted in the mother for development. This technology has several ethical dilemmas surrounding it – remember designer babies???



Fetal Screening/Prenatal diagnosis is performed on fetuses while they are still in utero (inside mommy). Amniocentesis or chorionic villus sampling are used to extract cells from the fetus for testing. **Amniocentesis** involves inserting a large needle through the abdomen and into the uterus, where amniotic fluid (the fluid surrounding and protecting the baby) is removed. This fluid contains cells shed from the baby: skin cells, cells from the lining of the small intestine, or cells from the bladder. The cells in this fluid provide the DNA needed to perform genetic testing. Typically, this procedure requires the use of ultrasound to locate the baby. It is normally performed after the baby is 14 weeks old. **Chorionic villus sampling**, on the other hand, can be done earlier. Here, chorionic villus cells are removed from the placenta. This is done by inserting a needle vaginally and directing that needle to the placenta. A small sample of those cells – which are identical to the cells inside the baby – are removed and used for testing. Just like with amniocentesis, ultrasound is used to locate the baby as well as the placenta so the procedure can be done safely. Both procedures carry some risk of miscarriage.



Newborn screening is the testing of infants shortly after birth. A small sample of blood is taken from the baby, and DNA is isolated from it for testing purposes. Newborn screening is often used to test for inherited diseases if the parents choose not to implement measures that complete this testing while the baby is still in utero. Some don't want to risk miscarriage, and test their babies after birth instead. There are certain newborn screenings that are done automatically for most babies: for African Americans, sickle cell is commonly tested for; for Caucasians, cystic fibrosis may be tested for; for Ashkenazi Jews, Tay Sachs is tested for. This testing allows parents to take measures to give their children the best lives possible if a disease is present, and to start treating early.

Getting Enough DNA for Testing Purposes

Several times this section, we have brought up a key task that is part of genetic testing: amplifying DNA by PCR. Here, we will take some time to review that procedure.

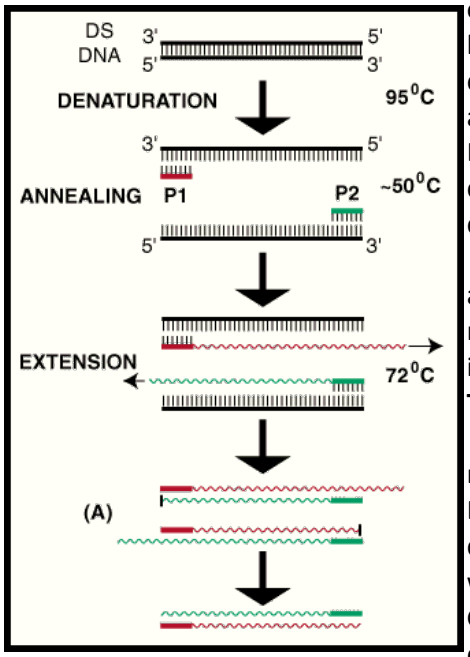
PCR stands for the polymerase chain reaction. This is a laboratory procedure that produces multiple copies of a specific DNA sequence. This can be a copy of a single gene, a large segment of DNA, or the entire genome of an individual. PCR is a three step process that usually takes place in a thermal cycler (a PCR machine – the purple thingy). Three "ingredients" are added to a sample of DNA so that copies can be made: Taq polymerase, DNA primers, and DNA nucleotides. The Taq polymerase and DNA nucleotides are included in a little pellet called a PCR bead, while the primer needed for the specific genes being tested for is added to it. The three ingredients are discussed in the paragraphs below as PCR is reviewed.

The first step of PCR is known as **denaturation**. The temperature in the thermal cycler cranks up to 95 degrees C – nearly boiling. The high temperatures break up the hydrogen bonds that hold the double-stranded DNA together. Think of a zipper being completely unzipped, with the two halves falling away from each other. Denaturation is required so that new DNA can be "grown".

The second step of PCR is called **annealing**. The thermal cycler cools to 55 degrees C, and the DNA primers which

were added to the DNA mixture early on with the bead are ready to do their job. Think of annealing as gluing. In this stage, the DNA primers (short sequences of DNA that target the beginning of the section of DNA being copied) bind to the section of DNA that scientists wish to copy. The primer is there so that the DNA is "primed" (readied) for copying. It tells Taq polymerase, described below, what section of DNA it should copy.

Finally, **extension** occurs. The temperature here is 72 degrees C, and requires both Taq polymerase and DNA nucleotides. You may remember talking about Taq polymerase in class. This is an enzyme that originated in the bacteria of hot springs, so they are able to survive the hot temperature used in PCR. In bacteria, Taq polymerase is used to copy bacterial DNA before bacterial cells divide. Scientists use this polymerase to copy DNA during PCR. Taq polymerase attaches to the DNA at the site of the primer. After attaching, it flows down the DNA strand, adding complementary nucleotides to the DNA so that it becomes double-stranded. When Taq polymerase is done doing its job, there are two double-stranded pieces of DNA made from the original one.



This three-step process repeats over and over. Each time it occurs, the amount of DNA doubles. This exponential growth of DNA allows lots of DNA to be made really really quickly. Within an hour and a half, 1 copy of DNA can be turned into more than 2 billion.

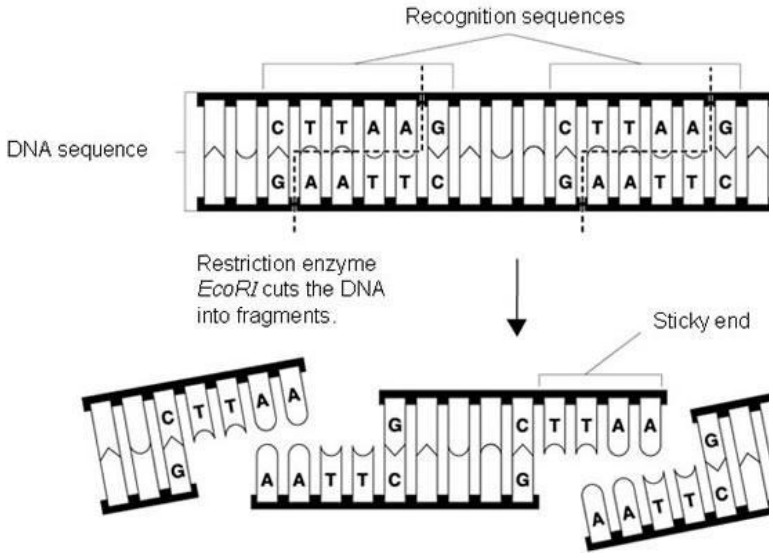
Testing for Disease

Genetic testing is not complete when DNA copies have been made. PCR makes DNA, but another process is required to use it to diagnose disease. Diagnosis of disease requires healthcare professionals to look inside cells and decode the message buried in the sequence of nucleotides. The genotype, what is written in our DNA, predicts phenotype, what we see as a result of that code. Genotype is the genetic code for the traits we have – eye color, dimples, or diseases. Testing for these traits can be done with the process of gel electrophoresis. When starting from scratch, this can be a fairly complicated process. It is described in the text that follows.

The process begins with the isolation of DNA. Cells are taken from somewhere (blood, saliva, cheek swabbing) and the cells are lysed (blown up). The blown up cells and their contents are all mixed together, so a new procedure is used to separate the DNA from the cell waste. This is centrifugation. Centrifugation (fast spinning) separates the heavy cell components from the from other waste products (plasma, spit, etc.). At the end of the process, a small pellet of cell parts – including DNA – can be found at the bottom of the spun tube, while the supernatant (fluid on top of the pellet) is merely waste that can be discarded. To that tiny tube, a small amount of Chelex is added. Chelex forces the DNA to separate itself from the remainder of the cell waste in the tube, leaving the DNA floating in fluid. After this happens, the supernatant (which in this case contains the desired DNA) is moved to a new tube, while the pellet full of cell garbage is discarded. So far… get cells → blow up cells → spin cells → dump waste → add Chelex → move DNA-holding supernatant to new tube

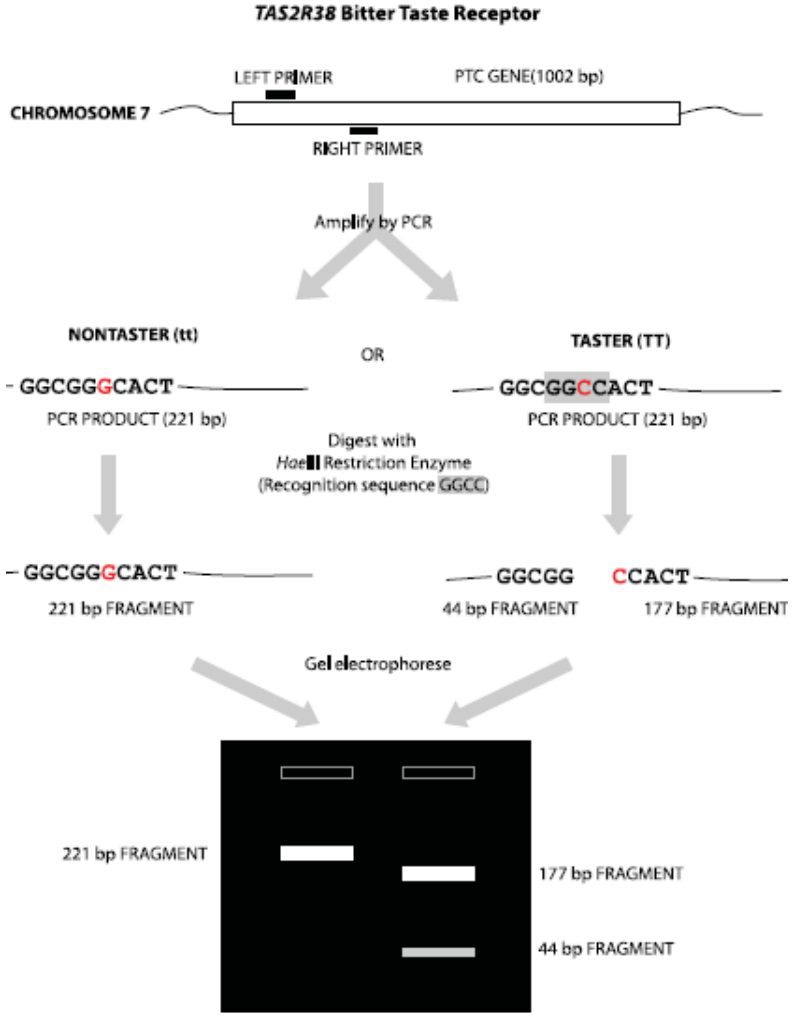
Now that we have a small sample of DNA, it must be amplified with PCR. As this process is described in the previous section, I'm not going to repeat it again here. Just know the section of DNA carrying the gene(s) of interest will be copied with PCR.

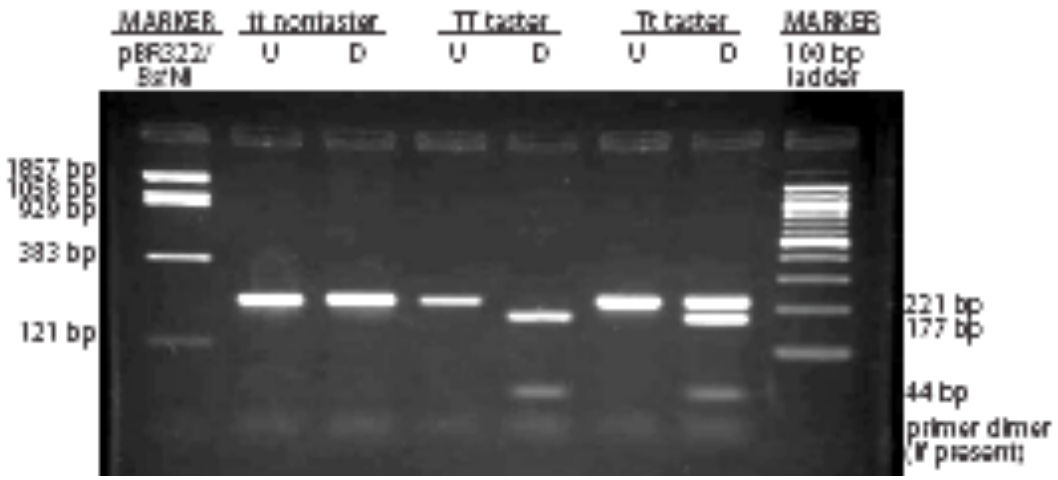
After the section of DNA we want has been copied, we next need a way to find out if people have the "bad" version of it or not. It is important to do something so that the different versions of the gene can be distinguished. This is done with restriction enzymes. Restriction enzymes are molecular scissors that recognize specific DNA sequences and cut the nucleotide strands. This allows identification of the single nucleotide polymorphisms, or SNPs. Single nucleotide polymorphisms are tiny differences in the DNA of individuals that make them unique. They are 1-



nucleotide differences in the DNA. SNPs can be the reason for disease, so being able to identify them is incredibly useful for scientists. This is one method used to detect the "bad" genes that cause some diseases. Let's try to make this simpler. Restriction enzymes are used to cut DNA. They are "picky" enzymes whose only job is to seek out the specific DNA they recognize and cut it. If scientists know the sequence of DNA they are testing, they can use restriction enzymes to cut it into fragments to reveal SNPs. Restriction enzymes may cut one version of the gene, but not another. They may also cause cuts in different places, creating different fragments of DNA that can be used to determine what version of the gene an individual has.

So, we have DNA. We've made copies. We've cut the copies. What's next? We have to be able to SEE the results. The best way to do this is with gel electrophoresis. Here, an agarose gel is prepared and placed into a buffer solution. The gel contains wells at one end to which DNA is added. Markers (standard fragments of known lengths) are added first. Following this are the samples from the patient or patients being tested. After the DNA is placed into the wells in the agarose gel, it is charged with an electrical current that separates the fragments. This occurs because DNA has a slightly negative charge. Due to the placement of the agarose gel in the buffer, DNA is pulled based on attractive forces to the positive end of the electrophoresis chamber. The pulling separates the DNA fragments, which are stained after the process is completed, and can then be read. Part of this involves determining the size of the DNA fragments. To do this, the "known" bands of the markers are used as a comparison for the sizes of the other fragments. The sizes are used to figure out which gene versions (Alleles) a person has inherited. The gel results can be used to determine which version of a gene a patient has, revealing their SNPs and answering many questions about the presence of disease or the ability to pass disease on to children. The end result of this entire process is a gel that reveals the genotype of the individual being tested. It may reveal that a person is the carrier of a disease, is affected by a disease, or that he/she has nothing to worry about. Remember that genotype determines phenotype, the physical characteristics of an individual. If genotype says a person carries a disease, it means they hold the gene, but aren't affected themselves. If genotype says a person has two copies of a disease gene, they have a disease.





Healthy Pregnancy and a Healthy Baby

Recall that at the beginning of this, we revealed that Judy Smith was pregnant with an oopsie baby. Genetic testing was used to determine whether or not that baby had some sort of disease. Judy Smith underwent an amniocentesis, then chromosomal analysis with a karyotype. She and her husband did not undergo carrier screening, although that option was likely made available to them. The karyotype revealed that her baby was fine. Still, the fact that the baby was genetically normal did not mean that nothing could go wrong. If a woman is not careful during pregnancy, a baby that is perfectly healthy genetically can be born with life-long complications.

Throughout the pregnancy, Judy's health and that of her son will be monitored. Maternal health will affect the health of the baby. The first trimester of pregnancy is especially critical, as all major body systems are formed during this time, and outside agents like alcohol, cigarette smoke, and drugs can have a drastic effect. Thankfully, Judy is smart enough to avoid these substances, but she still needs to monitor her eating habits, consume enough folate, and take her prenatal vitamins. Throughout pregnancy, chemical substances can have a nasty effect on a baby and should be avoided. Exercise and a healthy diet ensure that both mom and baby have what they need to stay healthy.

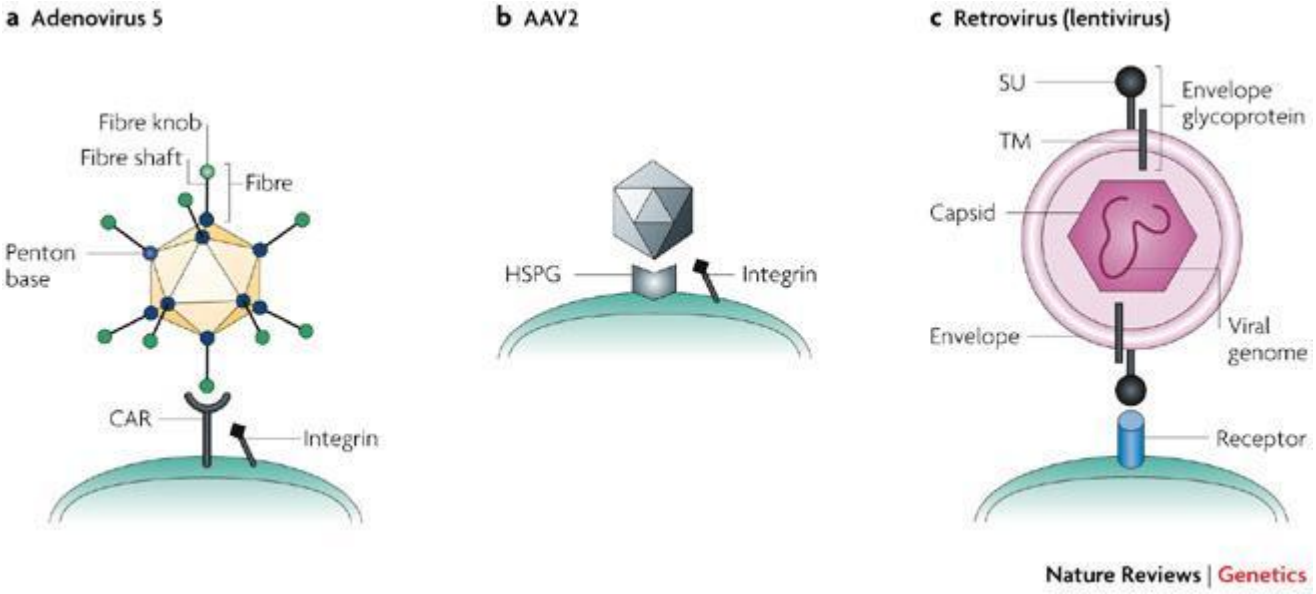
Lesson 2.2: Our Genetic Future

A Brief Introduction

Given how much we are able to do NOW when it comes to understanding DNA and its effects, it is not difficult to see that the future may reveal some amazing changes. Understanding genetics will alter the way doctors and scientists treat disease, as well as the way humans reproduce. Think about it: what if we could correct genetic diseases in children before they were born? What if we could ensure that future children would never have a major health problem like sickle cell or cystic fibrosis? What if we could make people healthier, smarter, and longer-living? These are all distinct possibilities, and will likely be topics of high interest in your lifetime. We will discuss them here.

Gene Therapy

Gene therapy is a type of disease treatment in which faulty genes are replaced by functional copies. It involves replacing "bad" genes with "good" ones, or flipping genetic switches that are making things go wrong. Gene therapy has the potential to eradicate certain inherited diseases by providing people affected with diseases with normal genes that will produce what the body needs.



How does this technology work? Gene therapy involves using vectors to deliver healthy genes to affected cells. This method is still highly experimental in most cases, although it has been used to successfully treat certain diseases in humans. There are several types of vectors: retroviruses, adenoviruses, adeno-associated viruses, herpes simplex viruses, liposomes, and naked DNA. You may have noticed that many vectors are viruses. This is because the entire existence of viruses involves finding a specific cell types and delivering genetic material to it. Given that this is the goal of gene therapy, using viruses makes sense! Regardless of the type of vector used, they all have the same purpose: getting unaffected genes to people carrying a specific disease. This involves trying to deliver DNA to the right type of cell, not to all cells. Consider someone with an inherited form of blindness. This could result from a defect in the protein meant to form the retina. If a person doesn't have a retina, they don't see. If, in theory, a vector could be used to deliver normal "retina DNA" to the eye, it might produce a retina and give a person vision. It would not be necessary to deliver retina

DNA to the brain – our brains don't need retinas. So, that is one problem we are working to solve: finding a way to get vectors to deliver DNA to target cells. Another problem is complete integration. It might be possible to get DNA to the right type of cell, and to get that DNA into the cell, but what if the DNA is not incorporated into the genome? If the retina DNA doesn't become part of the rest of DNA, a retina will never be produced. Additionally, that DNA has to be part of that cell – and every other future cell it divides into – for the life of the person. Can you imagine being given vision for the first time in your life, the having the cells that made it possible die and going back to being blind? A method of fixing that new DNA permanently into the DNA of the patient is important. Finally, we need to make sure that the DNA is fixed in a safe spot. If, in the process of incorporating Retina DNA, the DNA responsible for producing the retina disrupts another eyeball process? Suddenly, there's a retina, but no eyeball to put it in! Though this example may be a bit silly, it should illustrate the idea that, right now, gene therapy is far from perfect. Still, it has a lot of potential for curing illnesses and conditions that are seen as incurable right now.

Designer Babies

There is a dark side to this technology. If we can fix blindness, what else can we do? Could we create the perfect athlete? The perfect student? The perfect soldier? Gene therapy involves tweaking genes, and there is the potential for this to be abused in the process of genetic enhancement. Remember talking about designer babies? How far is too far when it comes to gene therapy?

Current Applications of Reproductive Technologies

Current technologies allow parents to make certain selections for their babies. It is possible to choose babies of a certain gender, and to do testing to choose babies who do NOT carry diseases of concern to parents. It is easy to see that choosing gender and healthy babies could be taken too far. If someone has enough money, what else might they choose in a child? If a parent wants to choose the gender of their child, it's possible right now. There is a procedure called sperm sorting that involves taking a sperm sample and centrifuging it. Sperm carrying an X chromosome are heavier, so sink during centrifugation, while sperm carrying a Y chromosome end up on top. By "skimming" from the top or the bottom and completing artificial insemination or in vitro fertilization, a boy or girl baby can be selected. There is also the possibility of preimplantation genetic diagnosis, which can reveal baby gender as well as tested defects. Since preimplantation genetic diagnosis was discussed earlier, we won't repeat the discussion here.

Future Applications of Reproductive Technology

Do you remember discussing Dolly the sheep, the first mammal to be cloned? It's likely she won't be the last. How long do you think it will be before we hear about the first cloned human? Reproductive cloning is cloning to make a copy of an individual. It is cloning in the most traditional sense of the word. This is very different from therapeutic cloning, which involves making a clone of a certain body part, like the kidney or the arm. One day, technology may allow a person with failing kidneys to have a sample of their DNA taken, and a brand new, healthy kidney to be grown. One day, rather than a prosthetic, we might be able to just grown an amputee a new arm. Therapeutic cloning is cloning for healing purposes rather than reproductive ones. Again, the question we need to ask ourselves is "How far is too far? Where does healing end and toying with nature begin?"

A final review

The focus of Unit 2 has been diagnosis and future prevention of inherited diseases. Parts of the puzzle are already in place: we know the human genome, we know what many genes do and their role in disease. We are beginning to learn the methods needed to change the behavior of damaged genes. In the future, this could lead to a world very different than the one we live in today.

Understandings 3.1

- Cancer is a term used for more than 100 different diseases in which cell regulation genes are mutated causing the cells to reproduce out of control.
- X-rays, CT scans, and MRI scans are used to create pictures of the inside of the body to diagnose and treat many disorders.
- Scientists use DNA microarray technology to determine the differences in gene expression between different tissue samples.

Essential Questions

- What fundamental characteristics do all cancers have in common?
- In what ways are diagnostic imaging technologies used to diagnose and treat disorders?

- What do DNA microarrays measure?
- How is DNA microarray technology used to determine the differences in gene expression between different tissue samples?
- How are the similarities of gene expression patterns between different individuals calculated?

Understandings 3.2

- Behavioral, biological, environmental, and genetic risk factors increase the chance that a person will develop cancer.
- Experiments are designed to find answers to testable questions.
- Molecular diagnostic tests can be used to detect inherited genetic mutations associated with certain cancers and can be used to predict risk for developing those cancers.
- Viruses are linked to certain types of cancer.
- Routine cancer screenings can prevent certain types of cancer or can increase the chance that cancer is detected at an early stage when treatment is more effective.

Essential Questions

- In what ways do different risk factors increase the chance that a person will develop cancer?
- How can lifestyle changes reduce the risk for developing cancer?
- How can molecular tests be used to detect inherited genetic mutations associated with certain cancers?
- How can viruses lead to cancer?
- What is the importance of routine cancer screenings?

Understandings 3.3

- Various methods are used to treat cancer.
- Various biomedical science disciplines and professionals help patients cope with cancer or the side effects of cancer treatment.
- Experiments are designed to find answers to testable questions.

Essential Questions

- What can a cancer patient receiving chemotherapy and/or radiation therapy expect during treatment?
- How is biofeedback therapy used to help patients improve their health or manage pain?
- In what ways do artificial limbs allow patients who have suffered from the loss of a limb regain lost function?
- How do advances in technology allow for the development of artificial limbs that look and move like actual human limbs?
- How do physical and occupational therapists help patients with disabilities or patients recovering from surgery or injury?

Understandings 3.4

- The field of pharmacogenetics investigates how genetic variations correlate with responses to specific medication and strives to develop medical treatments tailored to the individual.
- Nanotechnology is a field of science that can be applied to health and medicine.
- Clinical trials are biomedical or health-related research studies that investigate how a new medicine or treatment works in human beings.

Essential Questions

- Why do some drugs affect individuals in different ways?
- How can information in our genes affect how our bodies interact with certain medications?
- How are clinical trials set up to ensure all data collected is valid and that all human subjects are treated ethically?
- How might Nanomedicine change the future of medicine?

MI Unit 3 Cram Sheet

Introduction

In Unit 3, our focus is cancer. In this unit, we learn that Mike Smith, son of James and Judy, has osteosarcoma, a bone cancer that often affects teenagers. As Mike goes through the process of dealing with his disease, we learn what cancer is, risk factors for it, how it occurs, why it occurs, how it is diagnosed, how it spreads, how it is treated and prevented, and how people recover from cancer during rehabilitation. We also examine how new medications, prosthetics, and nanotechnology may affect the future of our battle against cancer.

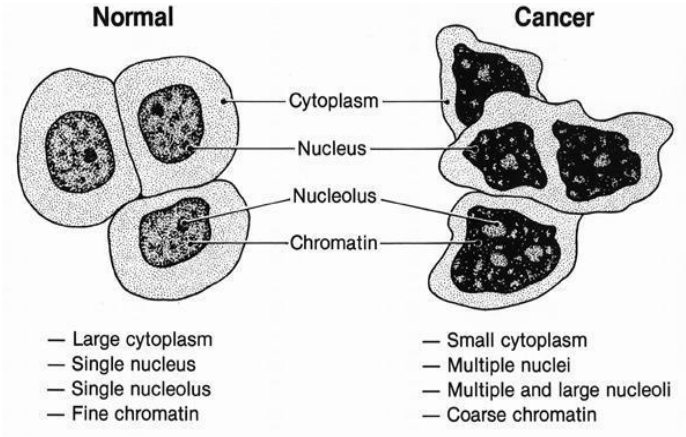
Lesson 3.1 – Detecting Cancer

Face the Facts

Cancer is the second leading cause of death in the United States, second only to heart disease. Half of all men and one third of all women in the US will develop cancer during their lifetimes. Is it any wonder that there is so much focus on studying it? Cancer is a term that is technically used to describe more than 100 different diseases. It affects different cells and people in different ways. In spite of the variety, all cancer cells share one important characteristic: they are abnormal. Cancer cells are abnormal cells in which the processes that regulate normal cell division are damaged.

So, what makes cancer cells different? Within a given healthy tissues, there is a certain uniformity to the cells. They have similar parts and similar functions. They have the same numbers of nuclei, similar shapes, and similar sizes. They are regular. Cancer cells are anything but. They can have multiple nuclei, abnormal numbers of chromosomes, irregular sizes and shapes, and may appear to grow on top of one another. Cancer cells are different. They are abnormal. In modern times, the question has been: why? What makes cancer cells behave abnormally? In normal cells, a cell cycle is followed in which cells live, grow, divide, and die - all timed out accurately to ensure the safety and health of the organism. This regulated life cycle is not present in cancer. In all cancers, genes that would normally regulate cell behavior are mutated. This causes cancerous cells to reproduce out of control.

Cancer is not that complicated. That is why it works. Here are the facts: Cancer can affect any tissue or organ of the body. Early detection and treatment often lead to a better prognosis. Incidence of cancer increases with age. Personal actions such as smoking, alcohol consumption, sun exposure, and diet can increase the risk of cancer. Many options exist for treating cancer, including chemotherapy, radiation, surgery, and stem cell/bone marrow transplants. Cancer can spread or metastasize to other areas of the body. A family history of cancer can put us at increased risk of cancer. Though these facts don't tell the whole story, they do paint a picture of who can get cancer - everyone - and how it can be treated . . . or not.



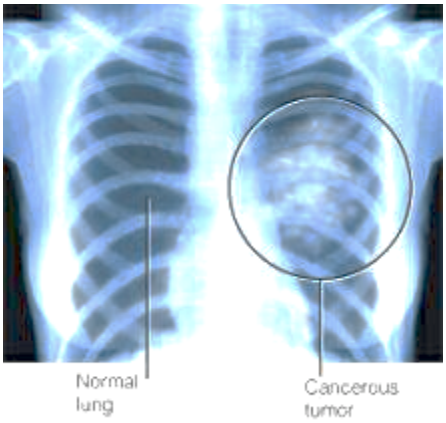
Common Tools for Detecting Cancer

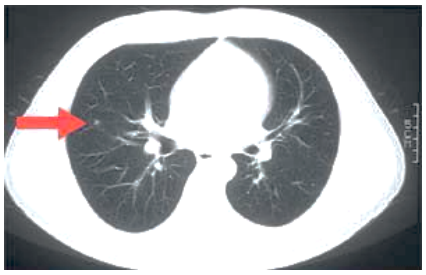
Most cancers are initially recognized when signs or symptoms appear. Perhaps a woman notices an unusual lump in a breast, or a male has a difficult time urinating. Perhaps someone just doesn't "feel right". If nothing simpler explains things, cancer can be considered a possibility. And, if your healthcare provider suspects cancer, it can be further investigated through medical tests including X-rays, CT scans, and MRI scans. Biopsies are also used to make the definitive determination as to whether cancer is present. It is important for you to understand how the different technologies can be used, so let's take some time to go over them.

X-rays, CT scans, and MRIs are used to create pictures of the inside of the body to diagnose and treat many disorders.

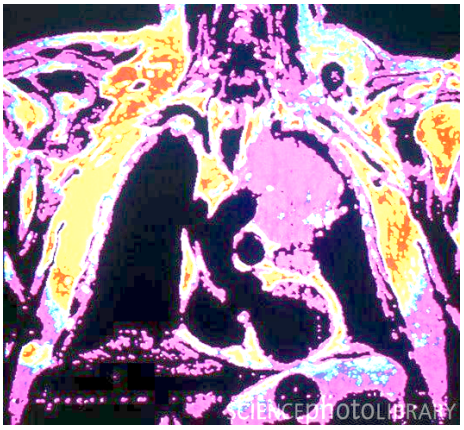
X-rays are a noninvasive medical test used to produce images of the inside of the body to help diagnose medical conditions. X-rays are a form of electromagnetic radiation that is sent through the body in the form of photons. These rays pass easily through soft tissues, which are hardly visible at all when examined on an x-ray film, but are absorbed by hard tissues like bones, making them appear on a film when a picture is taken as the x-rays are passed through a targeted section of the body. Because of this, X-rays are often used to provide images of the chest or broken bones. Structure containing lots of air are less dense, so will appear black or dark gray, while more dense structures appear gray

to white. It is possible to use this to examine soft tissues when a contrast media or metal is added. These are special dyes used to highlight areas of the body and make them appear white. This technology is limited, however, because the images are two-dimensional. Additionally, the ionizing radiation used to create the images increases the risk of certain cancers as well as increasing health risks for the fetus if the test is done on pregnant women.

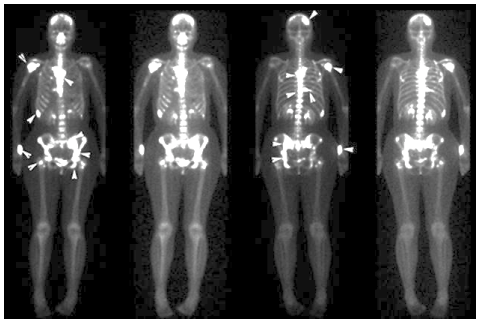




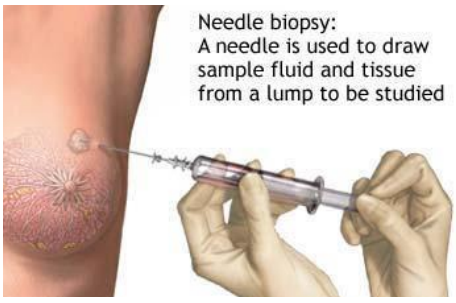
CT scans are a specialized type of X-ray. They are noninvasive, and are used to produce images of the inside of the body. In a CT scan, the patient lies down and an X-ray tube rotates around the patient taking pictures from many different angles which a computer collects. The results are translated into images that look like a "slice" of the person, or cross-sections. CT scans are more sensitive and can be used to detect disease in the soft body tissues. They can also produce images of internal organs which are impossible to see with an x-ray. They are often used to examine the chest, abdomen, pelvis, spine, and other skeletal structures. They can image bone, soft tissue, and blood vessels at the same time, and are safe to use on patients with implanted medical devices like pacemakers. Using contrast media makes it possible to see large amounts of detail, but may produce an allergic reaction. Again, ionizing radiation is used, which can increase cancer risk.



MRIs do not use x-rays. In an MRI, or magnetic resonance imaging scan, the patient lies down in a cylinder that is a very large magnet. The computer sends radio waves through the body and collects the signal that is emitted from the hydrogen atoms in the cells. Detailed images are produced with this technology of the body's soft tissues, unlike CT scans and X-rays, which are better for seeing hard tissues. A computer collects the data and forms images. MRIs provide much more details in very fine soft tissue than CT scans. The images produced are cross-sections, either going down the body in the transverse plane or in the sagittal plane. This technology can be used to examine the brain, spine, joints, abdomen, blood vessels, and the pelvis. This is very safe unless the body contains something that would be attracted by a magnet.



A bone scan is somewhat different. This is a noninvasive medical test used to produce images of the bones that help diagnose and track several types of bone disease. This is a nuclear imaging test that produces 2-D images of the body, and is very useful for detecting skeletal abnormalities thanks to the use of tracers (radionuclides) that are injected into the body before the bone scan is completed. Really, they're just x-rays with some radioactive material in the body, and white areas are places where high amounts of metabolic activity is taking place. These "hot spots" are present in areas that are irritated by problems like arthritis, or where there are cancerous cells rapidly growing.



Biopsies are done to test for nearly every type of cancer. This test involves removing a small sample of tissue from the body where cancer is suspected. (If lung cancer is suspected, a sample of lung tissue is removed.) Once the tissue is removed, a few tests are performed. The cells are "cultured" (grown in a petri dish) and examined for abnormalities. Remember how cancer cells differ from normal cells? Cancerous cells can have irregular numbers of nuclei, irregular numbers of chromosomes, irregular cell shapes, unusually large size, and abnormal cell membranes. Additionally, these cancerous cells lose "contact inhibition", which is supposed to stop cells from growing when there is a layer of them on a petri dish. Cancerous cells will continue to grow, stacking on top of each other. They will also continue to grow and divide long past their scheduled death time, whereas normal cells reach a set number of cell divisions and then die in order to protect the integrity (normal form) of the organism. It has also been noted that cancerous cells can grow in media that has less nutrition than normal tissues - meaning that they can grow in conditions that will kill normal cells. Biopsied tissue is used

to test for these things - if cells are exhibiting any of these abnormalities, then that tissue is likely cancerous and forms of treatment need to be determined.

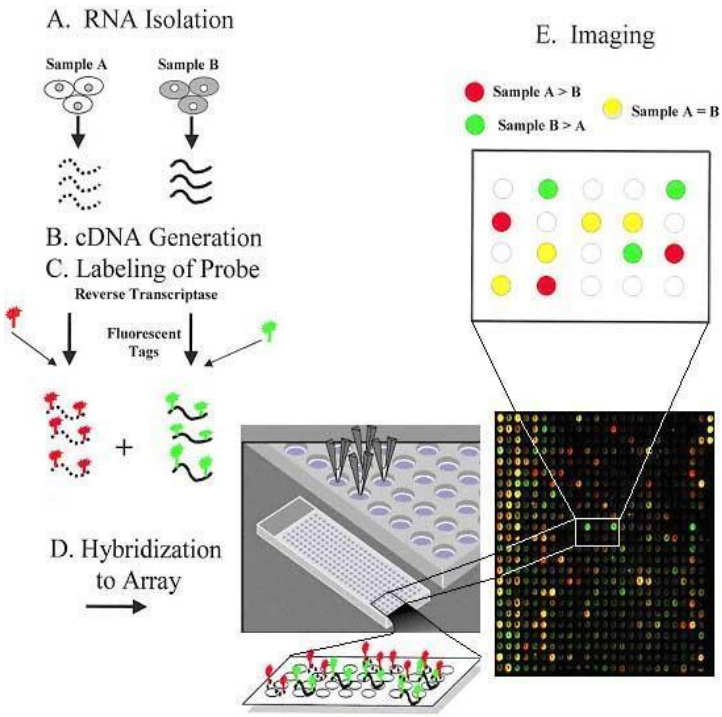
Detecting Genes Involved in Cancer

Scientists have discovered that one of the differences between healthy cells and cancer cells is which genes are turned on in each. Scientists can compare the gene expression patterns between healthy and cancer cells through the use of DNA microarray technology.

Every cell in the human body contains the same 20,000 or so genes (with the exception of red blood cells, which contain no DNA). However, not every gene is active in each cell. Within cells, NECESSARY genes are turned on so that the cell can function within the type of tissue to which it belongs. (Cells of skin tissue will have different genes active than cells of brain tissue.) The gene for melanin (a protein that gives your skin color) is only active, or turned on, in skin cells. The gene for myosin is only turned on in muscle cells.

Think back to our discussions of the central dogma of biology: DNA → RNA → proteins. We discussed how DNA is transcribed to make a copy called RNA, which acts as a blueprint for the things the body needs to make. This blueprint is used to make proteins in a process called translation. During transcription, mRNA (messenger RNA) is produced. This is the blueprint for forming proteins, and is only made when certain proteins are needed with a cell. This RNA is created using a section of the DNA called a gene. Within any given cell, we can FIND this mRNA. If mRNA is present in a cell, we can use it to figure out what genes produced it. Basically, the presence of a set type of mRNA (there's a different hunk for every gene the DNA contains) means certain genes are turned "on" and working within that cell. Therefore, if mRNA is produced from a particular gene, scientists can infer that this gene is turned on within the cell. If mRNA is not produced from a particular gene, scientists can infer that this gene is turned off within the cell. This is the premise behind the microarray - it lets us see what genes are "on" and "off" in different tissue types, including cancerous and normal tissue. This means that scientists use DNA microarrays to scan multiple genes (sometimes even thousands at a time) to quantitatively measure the gene expression for each of these genes.

DNA microarrays are glass, plastic, or silicon slides that have been spotted with thousands of short segments of DNA. These short segments of DNA are single-stranded and each contains a portion of a gene of interest to the scientist. The following steps outline the process used to develop a DNA microarray slide:

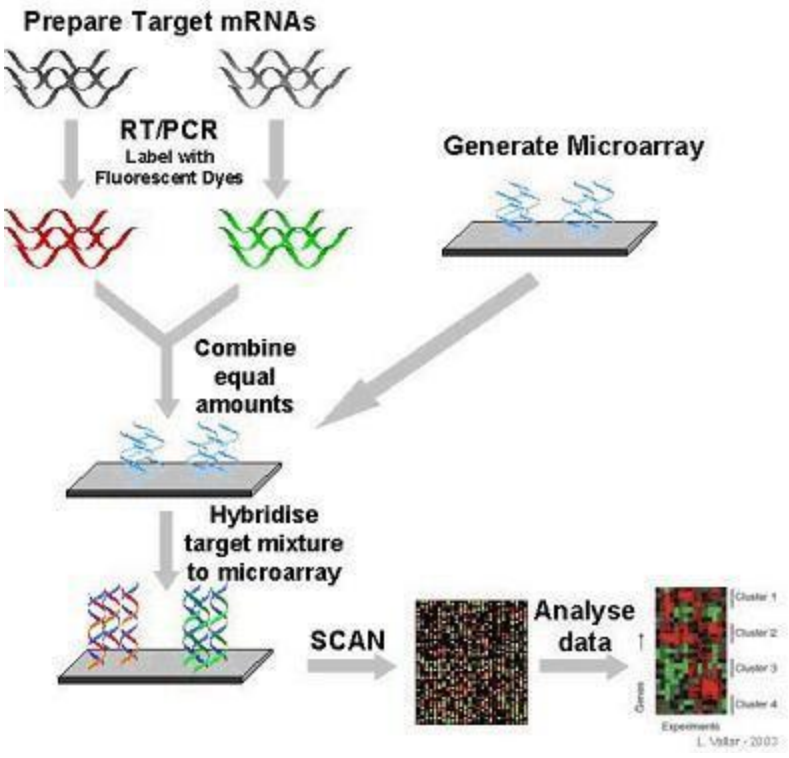


- A gene thought to be involved in a particular type of cancer is located within the human genome sequence. (the portion of the gene of interest is located)
- Primers are designed to run PCR reactions that will make copies of the portion of the gene of interest.
- The double-stranded DNA from each DNA copy is separated into single strands.
- Microscopic droplets of each single-stranded DNA sample are placed onto a specific spot on the microarray slide.
- The first four steps are followed to produce single-stranded DNA samples for each gene of interest the researcher wants to investigate. These samples are spotted in ordered rows and columns on the microarray slide.
- Computers are used to keep track of all the gene spots on the microarray and ensure that each spot contains equal amounts of DNA.

Once the microarray slide is created, it can be used for a microarray experiment. This begins with the experimenters collecting normal tissue and malignant tissue from a patients. These are then processed to separate the DNA, proteins, and RNA. This involves lysing the cells, separating the RNA using centrifugation, then pulling out the mRNA specifically.

When we discussed this in class, we viewed an animation that showed that mRNA (unlike rRNA and tRNA) contains a section of nucleotides known as the poly-A tail. This is unique to mRNA, and is how we trap the mRNA for use. Fluid samples containing an RNA mixture are run over special beads that contain long chains of Ts. These T-chains trap and bind the poly-A tails, giving a sample of pure mRNA attached to the bead. Then, the bead is washed into a separate container to give a pure fluid sample of mRNA. For ever mRNA in the sample, a cDNA (complementary DNA) strand must be created that is fluorescent. mRNA from normal tissues is used to create fluorescent green cDNA using PCR, while cancerous tissue is used to make fluorescent red cDNA. Once the samples are made, they are added to the microarray slide, where the cDNA kind of splashes around looking for sites to bind to. For every molecule of cDNA, there is a matching spot of single-stranded DNA on the microarray. When the two find each other, they base pair and hybridize together, and become bonded. Anything that does not bond is washed off, then the microarray is put through a scanner that picks up the fluorescent dye in the cDNA. The scanner looks for red glow and looks for green glow, sending this information to a computer. The computer then combines the images resulting in varied shades of red, green, as well as yellow. This gives data about gene expression in healthy tissue and cancer tissue from the same patient. A saturated red color shows a gene is highly expressed (cranked way up) in cancerous cells. A saturated green color indicates that a gene is underexpressed (turned down or off) in cancerous tissue. A saturated yellow color indicates a gene is highly expressed in both the healthy cells and the cancerous ones.

What does this mean? Essentially, cancer cells and normal cells might have different genes turned on, or they may be producing proteins at different rates. DNA microarrays measure these differences by measuring the amount of mRNA for genes that is present in a cell sample, and comparing those results between healthy and cancerous tissues. If the gene behavior can be determined for both cancerous and normal cells, there may be a way to "switch" cancer cells so they behave like normal cells again. Then, no more cancer! This isn't a perfect system at the moment, but it has a great deal of potential. At the moment, we are using this technology to learn about how cancers behave, learning what genes are "off" and "on" and "hyperactive" and "hypoactive" in a cancer cell versus a normal cell. Rather than observing reds and greens and yellows and trying to describe the differences subjectively, colors are assigned numbers we call ratios and compared to each other to determine gene expression rations. These differences can even be calculated mathematically. Visit http://www.hhmi.org/biointeractive/genomics/microarray_analyzing/01.html to find out how this works. Beware: detecting similarities of gene expression patterns between different individuals involves statistical analysis.



Lesson 3.2: Reducing Cancer Risk

We've spent some time now going over how cancer is detected, but most people would rather know how to prevent cancer. Sadly, there is no way at this time to guarantee that you won't get cancer, but there are some things you can do to reduce your chances of acquiring different types of cancer. A large part of this involves assessing your own personal risk factors.

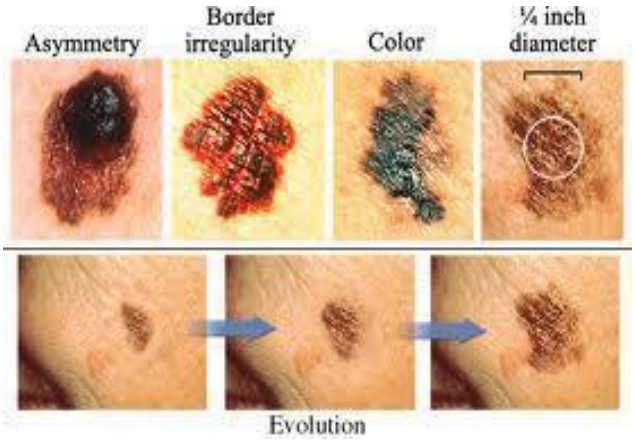
Risk Factors and Simple Prevention

We discussed four different classes of risk factors: behavioral risk factors, biological risk factors, environmental risk factors, and genetic risk factors. Behavioral risk factors are behaviors that you can change, such as smoking. Environmental risk factors are toxins found in your surrounding environment that increase your cancer risk, such as radon, air pollution, second hand smoke, and asbestos. Biological risk factors are physical characteristics, such as gender, race, and age. And finally, genetic risk factors relate to genes inherited from your parents, such as the BRCA1 and BRCA2 genes we talked about. The thing that all of these risk factors have in common is that they alter the DNA in our cells. These changes in DNA, when not repaired, potentially lead to the mutations that cause cancer.

There are ways to limit your risk factors and decrease your chances of cancer. Life-style changes are the easiest and cheapest way to keep healthy and reduce cancer risk. Avoid toxins, don't smoke or drink large quantities of alcohol, make healthy choices – applying common sense to your health can have a big impact. Biologic and genetic risk factors are harder to manage, but sometimes awareness of the risk and careful monitoring for signs of cancer is enough. Knowing that biology and genetics can cause cancer, and knowing what cancers are in your family, can help you target cancer screenings you should be doing as well as help you learn what warning signs you need to worry about.

As we discussed ways to prevent cancer, we focused for a time on skin cancer. Remember that skin cancer is caused by exposure to UV photons that damage the DNA in skin cells. UV rays have mutagenic properties, meaning they are capable of causing changes to the DNA of the cells that get exposed to it. THe longer you spend in the sun or in UV light, the more of your cells - including those of deeper skin layers - are exposed to that UV and at risk for changing. Prolonged exposure, in particular, increases your risk of DNA mutations that result in cancer. After sun exposure, your skin cells are supposed to use repair processes and transcription factors like p53 to fix any damage that occurred. Remember p53? It does several things, including producing proteins that stops cell cycle; activating transcription of repair proteins, and inducing apoptosis to truly damaged cells. This is supposed to correct damages created by UV (and other problems). However, with more exposure there is more mutation, and not all those changes can be corrected. If those changes are drastic enough, and the damaged cells aren't destroyed, cancer can be the result.

Skin cancer is the most common type of cancer in the US, and its incidence continues to increase. We looked at how skin cancer can be prevented, which involves very simple things like wearing protective clothing and gear and using sun block that protects against UVA and UVB rays. We also examined the ABCDE guide for skin cancer self-exams to do a self-check for melanoma, the most dangerous type of skin cancer. Remember that A is for asymmetry, B for irregular borders (not circular), C is for unusual color, D is for a diameter above 6 mm, and E is for evolution, or change of the mole over time. We ended our discussion of skin cancer by doing an experiment involving wild type (normal) and mutant yeast to find out what UV does to cells and how effective different forms of protection are.



Cancer Screenings

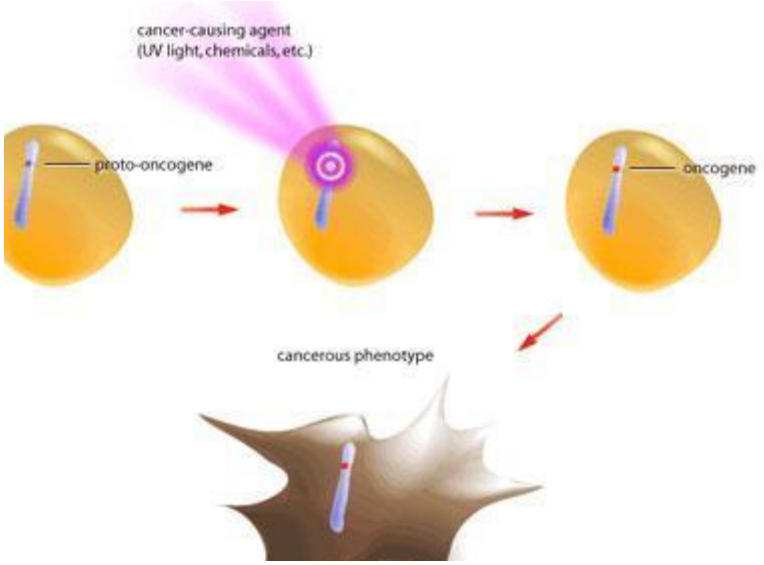
Part of preventing cancer involves cancer screenings. A cancer screening is a test that is performed to check for the presence of cancer. For females, this may involve pap smears and mammograms; for males, it involves prostate exams. Put simply, the hope behind screenings is to detect cancer early if it is present so it can be treated; the earlier cancer is detected, the better the chances get for survival.

Normal Cells and Cancer Cells

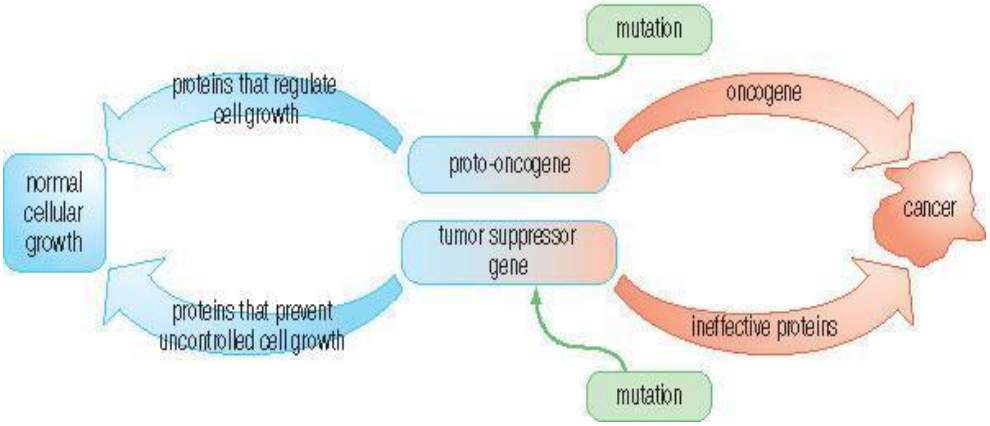
When we were first discussing cancer, one of the topics that came up was how the body prevents cancerous cells from forming under most circumstances, and what can go wrong in this process to cause cancer. Remember that all healthy cells are regulated (controlled) by something called the cell cycle. This is the process by which every cell lives its life. A cell is born from another cell. It grows, it performs processes that keep it alive, it divides and it dies. During the growth, division, and scheduled death phases are checkpoint stations, places where enzymes, transcription factors, and other things are supposed to check the progress of the cell and make sure abnormalities haven't developed. These checkpoints ensure that only healthy, normal cells are allowed to progress and divide. However, damage to the cell can result in damaged checkpoints, which can cause abnormal cells to grow and proliferate without correction/apoptosis . . . these abnormal cells are cancer. The cell cycle regulates the cell's entire life cycle. It is when something causes this process to go wrong that cancer can occur.

Sometimes, mutations result in damage to the cells, their DNA, and/or their checkpoints, which leads to changes in the process of cell division. Chemicals, UV, age, etc. can cause changes at the DNA (gene) level – or people can just be born with the wrong genes. There are three types of genes that must be discussed when studying cancer: proto-oncogenes, oncogenes, and tumor suppressor genes. A tumor suppressor gene is a gene that does what its name suggests: suppresses cancer. Tumor suppressor genes work inside cells to stop the growth and division of abnormal (tumor-causing) cells. If they become abnormal, these genes work to correct the problem. If that is not possible, these genes then trigger apoptosis, or cell death. These genes signal cells to kill themselves, sacrificing themselves for the

good of the body. Sometimes, though, these signals get ignored because of something else going wrong in the cell. Another protective force in cells is the transcription factor p53, which was discussed earlier.



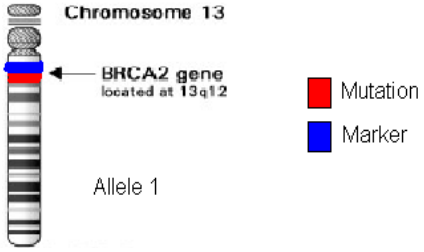
Proto-oncogenes are a group of genes that cause normal cells to become cancerous when they are mutated. The mutated version of a proto-oncogene is called an oncogene. Often, proto-oncogenes encode proteins that function to stimulate cell division, inhibit cell differentiation, and halt cell death. All of these processes are important for normal human development and for the maintenance of tissues and organs. Oncogenes, however, typically exhibit increased production of these proteins, thus leading to increased cell division, decreased cell differentiation, and inhibition of cell death; taken together, these phenotypes define cancer cells. Put simply, proto-oncogenes can become mutated, becoming oncogenes that make cells cancerous. If that happens, tumor suppressor genes may not be able to do their jobs properly and cancer can develop.



Breast cancer is something that can develop because of gene abnormalities. BRCA1 and BRCA2 are genes active in breast cells that act as proto-oncogenes. The normal version of this particular allele is thought to act as a tumor suppressor gene. In some people, however, this gene has mutated and exists as an oncogene that may cause cancer. This increases their chances of getting cancer. For a normal woman, the chances of breast cancer are 10%, while in someone with a BRCA mutation the risk increases to about 80%. There are several tests that can identify this gene mutation, including DNA sequencing and marker analysis. Because you have studied DNA sequencing in another unit, we will focus on marker analysis, which can detect the presence of the abnormal gene if it exists in the individual so preventative measures can be taken.

Marker Analysis

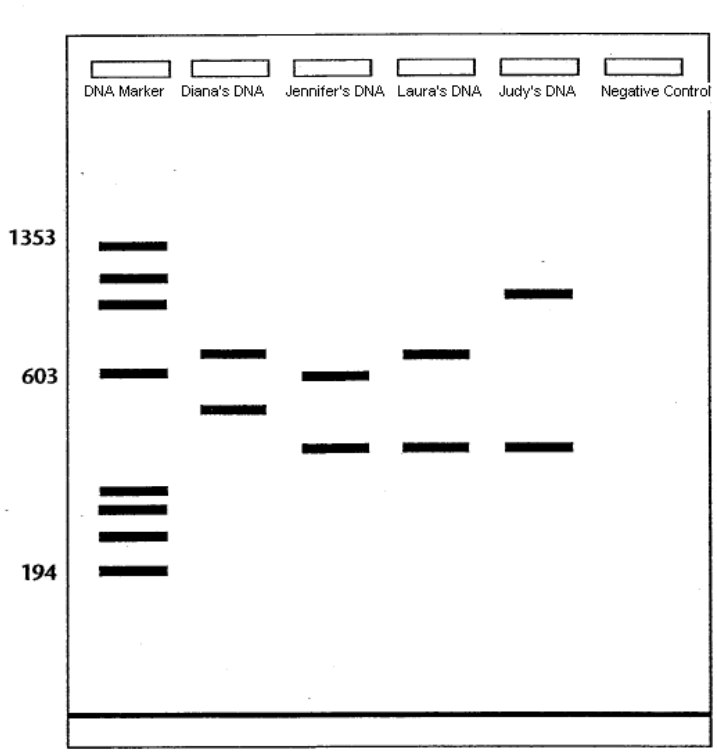
When we discussed breast cancer, you were introduced to a few new terms. First, we talked about the BRCA1 and BRCA2 genes. These are two genes commonly found in people who develop hereditary cancer. In a healthy individual, these two genes are what are known as tumor suppressor genes. In someone with a mutation, however, these genes don't do their job and tumors are more likely to develop, drastically increasing the chances of developing breast cancer. The presence of the mutated form of either gene causes a greater risk of breast cancer, so if it runs in someone's family it may be a good idea for them to get checked. The BRCA2 gene is accompanied by a section of DNA consisting of a series of short tandem repeats, or STRs. These small "chunks" of DNA have a repeating pattern (such as AATCGG) that repeats a variable number of times right next to the BRCA2 gene. Variations in the number of STRs creates different versions of the BRCA allele, most of which code for the normal proto-oncogene that helps protect from cancer. Some version of the allele, however, are mutated - not protective - and increase the risk of cancer. By detecting the "bad" allele versions (the BRCA2 mutation) people can determine whether they need to take extra precautions. This is particularly important for those with a family history of breast cancer.



It is currently possible to complete a test called marker analysis to find out your chances of developing certain types of cancer, like breast cancer. This is a

newer form of cancer screening that involves checking out your DNA and examining it to determine the chances of developing disease. Getting marker analysis performed is a fairly simple process involving gel electrophoresis. *Marker Analysis* is a technique where the gene mutation is analyzed using a genetic marker instead of directly analyzing the gene itself. A genetic marker is a short sequence of DNA associated with a particular gene or trait with a known location on a chromosome. The genetic markers used in marker analysis are short DNA sequences called *Short Tandem Repeats* (abbreviated STRs and also called *microsatellites*). An STR is a region of DNA composed of a short sequence of nucleotides repeated many times. The number of repeated sequences in a given STR varies from person to person. The alternate forms of a given STR correspond with different alleles. Most STRs occur in gene introns (non-coding regions of DNA), so the variation in the number of repeats does not usually affect gene function, but we can use STRs to differentiate between different alleles. Because pieces of DNA that are near each other on a chromosome tend to be inherited together, an STR that is located on chromosome 13 next to the known BRCA2 mutation can be used as the genetic marker for this case. The diagram below shows the relationship between the gene of interest and the genetic marker:

In order to test Judy and her family members for the BRCA2 mutation, DNA is extracted from each family member. The region of DNA containing the STR which is going to be used as the genetic marker for this mutation is amplified using PCR. The amplified DNA will then be run on a gel using gel electrophoresis. Because different alleles have a different number of repeats present in the STR, gel electrophoresis will separate different alleles based on the number of repeats present. The more repeats present in an STR, the longer the DNA fragment will be. The shorter DNA fragments will migrate the farthest down the gel.



If a family member is known to have a BRCA2 mutation, it is possible to simply compare the alleles between members. Everyone has two copies of the BRCA2 gene (one from mmo and one from dad) and if alleles are similar between an affected individual and an unknown individual it is possible to identify affected family members simply by looking. However, that is not always sufficient, as multiple alleles can be involved in traits such as cancer, with different ones having varying levels of risk.

In order to identify alleles, DNA marker analysis is necessary. Below are the steps:

- Start with the well called DNA Markers. Markers are DNA fragments of KNOWN sizes. Identify EACH band and calculate its Rf value by getting two numbers:
    - A) the distance between the bottom of the first well and the bottom of the “reference line” - the line at the bottom of the gel (you’ll be using this value for multiple calculations)[[in millimeters]]
    - B) measuring the distance between the bottom of the first well and the bottom of each band [[in millimeters]]
- For each band, divide B/A (smaller # / reference #) to calculate the Rf values for each band.
- On log paper, plot those values with Rf value on the X axis and fragment length on the Y axis.
- Create a line of best fit by drawing a line in the place that best touches or comes close to as many of your plotted points as possible
- For each remaining well, measure the distance between the bottom of the well and the bottom of each band; calculate their Rf values.
- Use these values to locate the fragment lengths on the graph you have created; this tells you the fragment size of each band.
- Identify alleles that are normal and abnormal using a results table that will tell you which allele versions

individuals have, as well as which are causing the mutations

Please refer to activity 3.2.3 for additional information on how to complete marker analysis. The activity page will walk you through the entire process.

Cancer and Viruses

Another way to prevent getting certain types of cancer is to avoid the viral infections that lead to them. Yes, viruses can cause cancer. Cervical cancer is a great example of this, as more than 80% of cases are caused by an infection with the HPV virus, which is transmitted from males to females during intercourse. Certain types of liver cancer and Hepatitis are also linked to viruses - the HBV and HCV viruses, specifically. Additionally, the EBV/Epstein Barr/Mono/Kissing Disease virus has been linked to several forms of cancer. Vaccination (where possible) can prevent these types of cancers. If an individual is immune to specific cancer-causing viruses, they can not infect the individual, mutate the DNA, and cause cancer. That is the reason vaccines like the Hepatitis B, C and Gardasil exist. You should have notes on this topic with activity 3.2.4 if you feel like you need additional information

Because of the link between cancer and viruses, virologists can play a significant role in reducing the risk of several types of cancer. Virologists can identify cancer-causing viruses and work towards developing the vaccinations that will reduce those infections. In this way, virologists are working to cure cancer.

We ended lesson 3.2 with some information on routine cancer screenings and their importance. You should have created a timeline for yourself and the cancer screenings you will need in your lifetime. Please refer to that, particularly for the cancer screenings shared between men and women, such as lung cancer screening, colorectal cancer screening, and skin cancer screening.

Lesson 3.3: Treating Cancer

The focus of this section was treatments available for cancer patients as well as the therapies available to help patients cope with the pain associated with treatment. In this lesson, we looked at chemotherapy, radiation therapy, biofeedback therapy, prosthetics, and physical and occupational therapy.

Radiation Therapy and Chemotherapy

When we were talking about radiation therapy and chemotherapy, we had a few different focuses. First, we talked about the jobs of these two forms of therapy. Clearly, both have the goal of helping an individual battle cancer effectively. Both work to destroy cancer cells by stopping or slowing their growth. We also talked about how both treatments can cause negative side effects to the patient. So, if both of these types of treatment are working to battle cancer, how are they different?

If a mass of cancerous tissue is found, the first step is to remove it (though sometimes steps are taken to shrink them before they are removed, so this order is not ALWAYs the case). Radiation therapy is then used to target and kill leftover cancer cells in the area where the mass was found. It works to “clean” cancerous material out of the area where the tumor was found. It is considered a “local” treatment, meaning it only affects the area where the tumor was located. It can be used to kill a tumor without surgery in some cases. Radiation works with a beam of high-energy rays that destroy or slow the growth of cancer cells. These treatments may be either external or internal. The most commonly used method is a machine outside the body that “beams” the rays to the site of the tumor, often from multiple directions at once for a rapid, high-intensity, specifically aimed treatment. It is also possible to insert radiation pellets near the cancerous site. Because this is a local treatment, the side effects are usually seen only in the area where the cancerous tissue was found. These may include soreness, tenderness, skin changes that look like burns, and fatigue. Oddly, many radiation patients also report nausea, even if radiation is taking place nowhere near the abdomen.

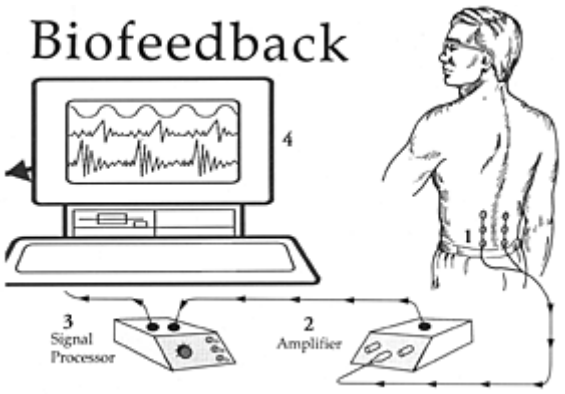
Chemotherapy works differently. This is a systemic treatment that is designed to destroy any cancer cells that may have metastasized and spread into nearby tissues or further. Chemotherapy drugs are inserted directly into the bloodstream and travel throughout the body. This treatment is given in cycles with recovery periods in between. Because chemotherapy drugs are traveling throughout the body, the side effects are seen in more places. These may include nausea, vomiting, mouth sores, hair loss, fatigue, and change in appetite. Chemotherapy targets rapidly dividing cells, affecting their ability to function, metabolize chemicals, or by altering DNA. Because cancer cells are nearly always fast-growing, chemotherapy can be a highly effective treatment for many types of cancer, particularly if that cancer has metastasized to multiple regions of the body. However, its systemic nature also results in body-wide side effects to ANY rapidly-growing cells, not just those that are cancerous. This results in skin changes (it thins, tears/bruises easily/may have lesions), mouth sores, gum tenderness, bone marrow suppression (resulting in anemia and a higher risk of infection), hair loss, and more issues with fatigue.

It is very common to use these two drugs for the same patient because they work differently. While radiation

targets the site of the tumor, chemotherapy targets any cancerous cells in the entire body. In both cases, it is not just cancerous cells that are affected, but the costs are far outweighed by the benefit – removing cancer from a patient.

Biofeedback Therapy

Both chemo and radiation therapy can cause a great deal of discomfort and pain for clients – as can other conditions. Because of this, we talked about a treatment that can be performed with patients that does not involve giving more drugs: biofeedback. Biofeedback is a technique used to make unconscious or involuntary bodily processes (like heartbeat or brain waves) perceptible to the senses in order to manipulate them by conscious control. In other words, it involves learning how your body responds to a stimulus like pain so that you can change those responses. It can help people to beat back their pain and cope with life on less drugs – a major plus to people already undergoing something as drastic as chemotherapy.



When we discussed biofeedback, it was to see how techniques such as yoga, meditation, chanting, counting, etc can change involuntary responses like heart rate, respiration rate and body temperature. Sometimes, those same techniques can help a patient to manage pain, and can be incredibly valuable tools for handling problems and stress.

Amputation and Prosthetics

Unfortunately, there are times when radiation and chemotherapy are unable to eradicate cancer. When that happens in a bone, that can mean that the only way to get rid of the cancer is to get rid of the affected limb by performing an amputation.

During an amputation procedure, wherever possible bone is removed and the remaining tissues reshaped to form a well-rounded stump that can be outfitted with a prosthetic. Though nothing can replace an arm or a leg, a prosthetic device can allow patients more freedom and independence than they would have without it. Prosthetics are designed to fit around the stump created during amputation, and each one has to be specially fitted.

It used to be that prosthetics were very basic, and had little maneuverability. They weren't very useful. Today, however, technology allows muscles of the back, chest, and abdomen to "talk" to prosthetics and to allow actual movement. This means people are able to do tasks that they could not do before this technology. The myoelectric arm uses signals picked up from muscles to control certain movements on the device. For example, flexion of a chest muscle might cause the elbow to bend, while a twist of the deltoid might cause the "wrist" to twist so something can be held. Though not perfect, this is a major step in the development of technology to make lives better for those who lose limbs to war, injury, or cancer. Technology is further advancing to allow prosthetics basic "feelings", to give the mind better control, and to improve their functionality as well as their appearance.

Physical and Occupational Therapy

If a limb is lost to cancer or anything else, part of the recovery process involves learning how to live, function, and work without that limb. Here, physical and occupational therapy is usually necessary to learn techniques to handle such a major change in lifestyle.

The job of a physical therapist is often to build strength as well as gross and fine motor skill. To help an amputee, a physical therapist would strengthen what remains of the limb so function is not completely lost, would help with strengthening other body parts to compensate for the missing one, and would train the patient in using whatever assistive devices/prosthetics would be needed. They help to bring functionality back to the patient.

An occupational therapist would help the person relearn ADLs, or activities of daily living. If a right-handed person loses that arm to cancer, an occupation therapist would help them relearn how to eat, how to use a zipper or buttons, how to groom themselves, etc. with the remaining limb. They would help the patient to perform the tasks that they want or need to perform to be a happy and productive member of society.

<u>Lesson 3.4: Building a Better Cancer Treatment</u>

So far, we have discussed risk factors for cancer, cancer prevention, why cancer happens, how it is treated, and the aftermath. Our last lesson in Unit 3 was all about new cancer treatments: pharmacogenetics and nanomedicine.

Pharmacogenetics is the study of the role that an individual's genetic make-up plays in how well a medicine works, as well as what side effects are likely. Each year, many people die or are affected by adverse reactions to prescribed medications. The chemotherapy drug azathioprine, which is prescribed to patients with *acute lymphoblastic*

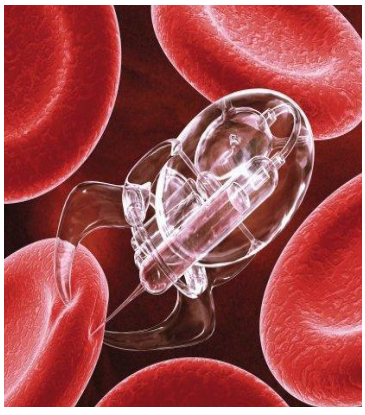
leukemia (ALL), is made of a compound called a *thiopurine*. Thiopurines work by interfering with DNA replication and, therefore, stop cancer cells from growing and spreading. Scientists have determined that an enzyme produced by the body called *thiopurine methyltransferase* (TPMT) is involved in the metabolism and breakdown of thiopurines. ALL patients need enough of the drug to keep the cancer cells from replic ating, but too much of the drug can cause damage to healthy tissue. Excess thiopurine can be deactivated with the help of the TPMT enzyme. However, if TPMT enzyme levels are low, dangerous thiopurines can build up in the body and cause awful side effects. A patient's SNP profile correlates with his or her ability to tolerate chemotherapy with azathioprine. This means that understanding a patient's SNP profile will allow doctors to predict how a patient will react to a particular drug.

SNPs can cause changes in enzymes that metabolize certain drugs in the body. Do you remember what SNP stands for? Single nucleotide polymorphism. But what is that? A single nucleotide polymorphism is a variation in one nucleotide in the sequence of DNA. It is the difference between the two strands of DNA below:

SNPs are variety within the human genome, and are responsible for some of our individual traits. And remember - they can be inherited as part of a haplotype - a group of genes close together on chromosomes that are inherited together from a parent. Inheriting a haplotype containing SNPs for the TPMT enzyme from one parent results in slightly decreased TPMT levels, but an ability to metabolize the drug. Inheriting a "defective" haplotype from both parents results in a total inability to make TPMT enzyme. The trait that causes lack of TMPT enzyme, making thiopurines so dangerous, is caused by two SNPs within a gene. If a person has the "standard" version of both nucleotides within the gene, they respond wonderfully to thiopurine. If one of the nucleotides is wrong, the medication works, but with some minor side effects. If, however, a person has both SNPs, they do not have adequate TMPT enzyme and thiopurine can cause major sickness and even death.

So why talk about this? Pharmacogenetics studies these properties of individuals to find out which medications will be most effective for people. Knowing this information, we can avoid giving ineffective medications or poisons to people, and cater pharmacology to the individual. We can create personalized medicines that will be more effective for treating diseases. Though in its early stages, this technology holds great promise for future advances in medication administration.

Nanotechnology is another newer technology that may change how we give medications. Nano- means 10^-9 m in size – really, really, really tiny. These are particles that are smaller than a cell, and in some cases smaller than a virus. Nanotechnology in medicine involves using tiny particles to treat disease. For example, nanoparticles have been created that seek out certain markers found only in cancer cells. The particles bind to those markers and then release just enough medicine to kill that cancerous cell. Can you see the potential? Rather than using medications that sicken the entire body, it may soon be possible to take a shot of nanomedicine that will find any cancer cells and destroy them while having no other effect on the body. Nanotechnology offers promise in the fight against cancer and is likely to revolutionize cancer prevention, diagnosis, and treatment. Other areas being looked at include developing nanoparticles for tumor imaging and molecular profiling of cancer biomarkers.



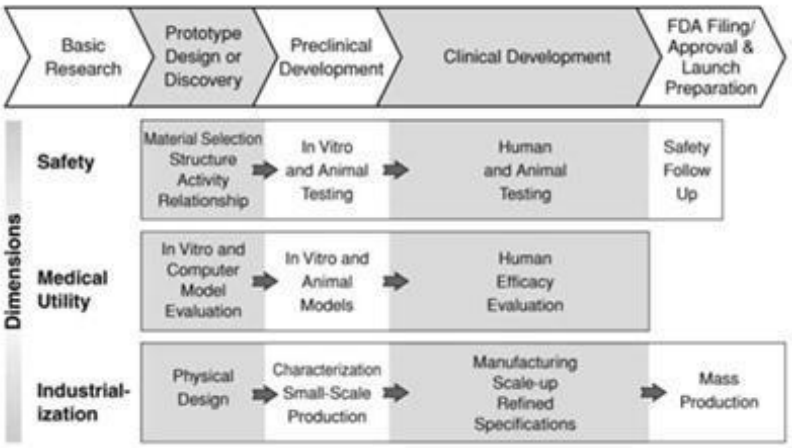
Clinical Trials

We ended Unit 3 with a discussion of clinical trials. Remember that a clinical trial is a testing phase for a potential therapeutic agent (drug, vaccine, etc.). It is a controlled experiment designed to test how well a treatment

works against diseases like cancer. There are several types of clinical trials, but most use the same phases of testing.

Because you have detailed notes on clinical trials, I am not going to include lots of information here. You will want to review the following:

- Phases of clinical trials
- Controlled study
- Single blind study
- Double blind study
- Placebo
- Phases of clinical trials



Understandings 4.1

- The methods used to diagnose and treat diabetes have changed dramatically over the last 200 years, including the use and production of insulin.
- Recombinant DNA technology allows scientists to custom-design bacteria that can produce a variety of important protein products, including insulin.
- Amino acid interactions affect the structure and function of proteins.
- Proteins in a mixture can be separated by various laboratory techniques.
- Numerous biomedical professionals assist with the production, distribution, and marketing of a new pharmaceutical or bioengineered product.

Essential Questions

- What role does insulin play in diabetes?
- How has the diagnosis and treatment for diabetes changed in the last 200 years?
- How can bacterial plasmids be used to produce proteins such as insulin?
- What is *bacterial transformation*?
- How can you gauge the success of a transformation experiment?
- How does amino acid structure relate to the overall shape of a protein?
- What is *chromatography*?
- How can chromatography be used to separate proteins?
- How can electrophoresis be used to check the purity of a protein sample?
- What is *SDS-PAGE*?
- How does protein electrophoresis differ from DNA electrophoresis?
- What biomedical professionals are involved in all stages of producing and manufacturing a protein product?
- How does a cover letter differ from a resume?

Understandings 4.2

- When the kidneys are not functioning properly, they will not filter adequately. Harmful waste products such as urea, creatinine, and blood urea nitrogen build up in the blood stream, which causes the body to make fewer red blood cells due to the lack of the hormone erythropoietin.
- Dialysis is an artificial process that removes waste products and excess water from the blood when the kidneys can no longer function.

Essential Questions

- What is End Stage Renal Disease (ESRD)?
- How is ESRD diagnosed?
- What are the treatment options or medical interventions for patients with ESRD?
- How does dialysis work?

Understandings 4.3

- Deciding who receives donated organs is not always a clear-cut issue and involves many difficult decisions guided by federal policies.
- In organ transplantation, the organ donor and recipient need to have compatible blood and tissue types.
- Organ transplant surgery is a complex procedure involving various surgical techniques and a variety of biomedical science professionals.

Essential Questions

- What (or who) decides who should receive a donated organ?
- How are organ donors and recipient matched?
- What general surgical techniques are necessary for a live donor kidney transplant?
- What are the roles of the various members of the surgical transplant team?
- How does a heart transplant compare to a kidney transplant?

Understandings 4.4

- A variety of tissues and organs can be transplanted from one person to another.
- Scientific research is investigating the possibility of replacing damaged organs and tissues using xenotransplantation and tissue engineering.
- Advancing medical knowledge and technology will enable scientists to enhance the human body.
- Scientists need to make sure that what they present is accurate and is communicated in a way that keeps interest and focus.

Essential Questions

- What parts of the human body can be replaced?
- What are the benefits and risks of using xenotransplantation and tissue engineering for replacement organs?
- What are the ethical considerations for xenotransplantation and tissue engineering?
- How can the human body be remodeled or enhanced to create a "super" human?
- What role do medical interventions play in the prevention, diagnosis, and treatment of disease?

Unit 4 Cram Sheet

Introduction

In Unit 4, we are introduced to one of the most unfortunate of the Smith family's relatives: Diana Jones, the sister of Judy Smith. Diana is a Type II Diabetic who now has her diabetes under control because she has learned to regulate her sugar with an insulin pump. That was not always the case, and Diana's past choices will have a huge impact on her future.

Lesson 4.1: Manufacturing Human Proteins

Because we are focusing on Diana Jones, a diabetic, our unit began with a study of the hormone insulin. Insulin is a protein that diabetics often use to regulate their blood sugar. In some forms of diabetes, the body does not make insulin, so sugar remains in the blood stream, causing damage to surrounding tissues. A diabetic who cannot make their own insulin must inject insulin from another source. Prior to the development of human insulin, people used to extract insulin from cow pancreases. Because this was not HUMAN insulin, it was less effective. Today, we have the technology to create human insulin, which means people have a safer source of insulin that is more effective.

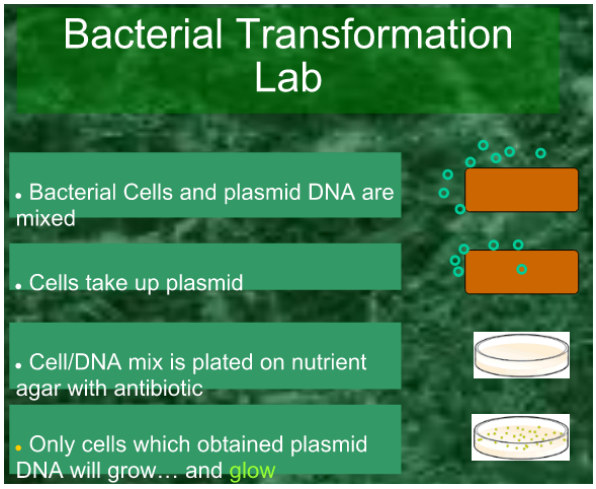
As crazy as it sounds, this insulin is produced by bacteria. In Lesson 4.1, the big portion of the activity was learning how this happens. Though we created a glow-in-the-dark protein instead of insulin, the process is the same. It is outlined below.

Bacterial Transformation

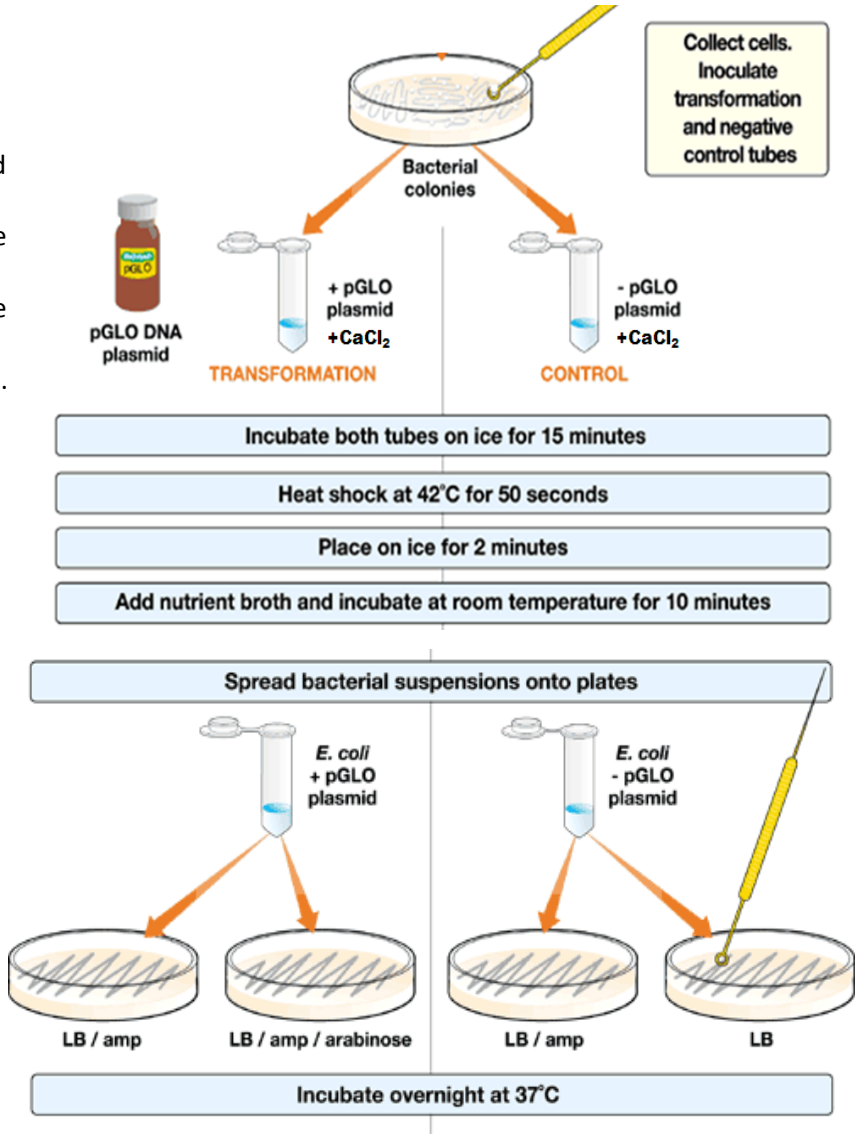
Creating a human protein must begin with finding something to "grow" that protein. The easiest living organism to use for this purpose is bacteria. This involves using recombinant DNA technology to custom-design bacteria that can produce the proteins we want. This process has several steps, which will be discussed next.

Remember that a typical bacteria may contain plasmids: small, circular strands of DNA that replicate when the bacteria do and often contain antibiotic resistance (think rubber band made of genes). These plasmids can be modified

by people through genetic engineering to contain other traits. To modify a plasmid, it is first sequenced so we know its composition. After its sequence is known, restriction enzymes are selected that will cut specific sequences of the plasmid DNA. After cutting with restriction enzymes, the plasmids are like a rubber band that has been cut with scissors. Either end of the cut contains "stick ends" - unpaired nucleotides that are able to bind to other, matching nucleotides. The gene to be added to the plasmids is then mixed with the cut plasmids. The sticky ends of the plasmid and the desired gene fragment bind, and are sealed together by DNA ligase. This creates a newly sealed plasmid that contains more DNA than the original. That extra DNA contains the gene for the protein we want the bacteria to produce. To make it simple, plasmids are changed to contain new genes, so they can produce human proteins. These modified plasmids will then be added to bacteria.



When choosing a bacterium for bacterial transformation, a simple, non-infectious bacteria is preferred - something like E. coli. The E. coli and the modified plasmid are mixed together in a vial and subjected to a form of shock. The bacteria are chilled in calcium chloride. This calcium chloride neutralizes the negative charge of DNA phosphate and phospholipids in the cell membrane, making it possible for plasmids to enter the bacterial cells. The cold slows cell membranes down and makes them more responsive to the calcium chloride. The bacteria then undergo heat-shock, which makes the cell membranes more permeable (more full of holes) that the plasmids can slip through. The two together - calcium chloride and heat shock - are critical in allowing the plasmids to slip into the bacteria, completing transformation. The bacteria are then allowed to recover in some nutrient broth, and the plasmids are sealed inside them. At this point, bacterial transformation is complete, and the bacterial cells are "plated" to allow the transformed cells to grow, reproduce, and make more of themselves. The plasmids used for transformation contain a gene for antibiotic resistance, and the bacteria are plated on agar containing a specific antibiotic. This prevents untransformed bacteria from growing, to ensure that bacteria used in later steps of the procedure actually contain the human protein desired.



Protein Purification

We now have bacteria producing human proteins, but we are not done. In order to make those human proteins useful to people, we have to get them OUT of the bacteria. This is done by killing the bacteria and using a series of steps to extract the useful human proteins from the dead cell waste. The steps are described below.

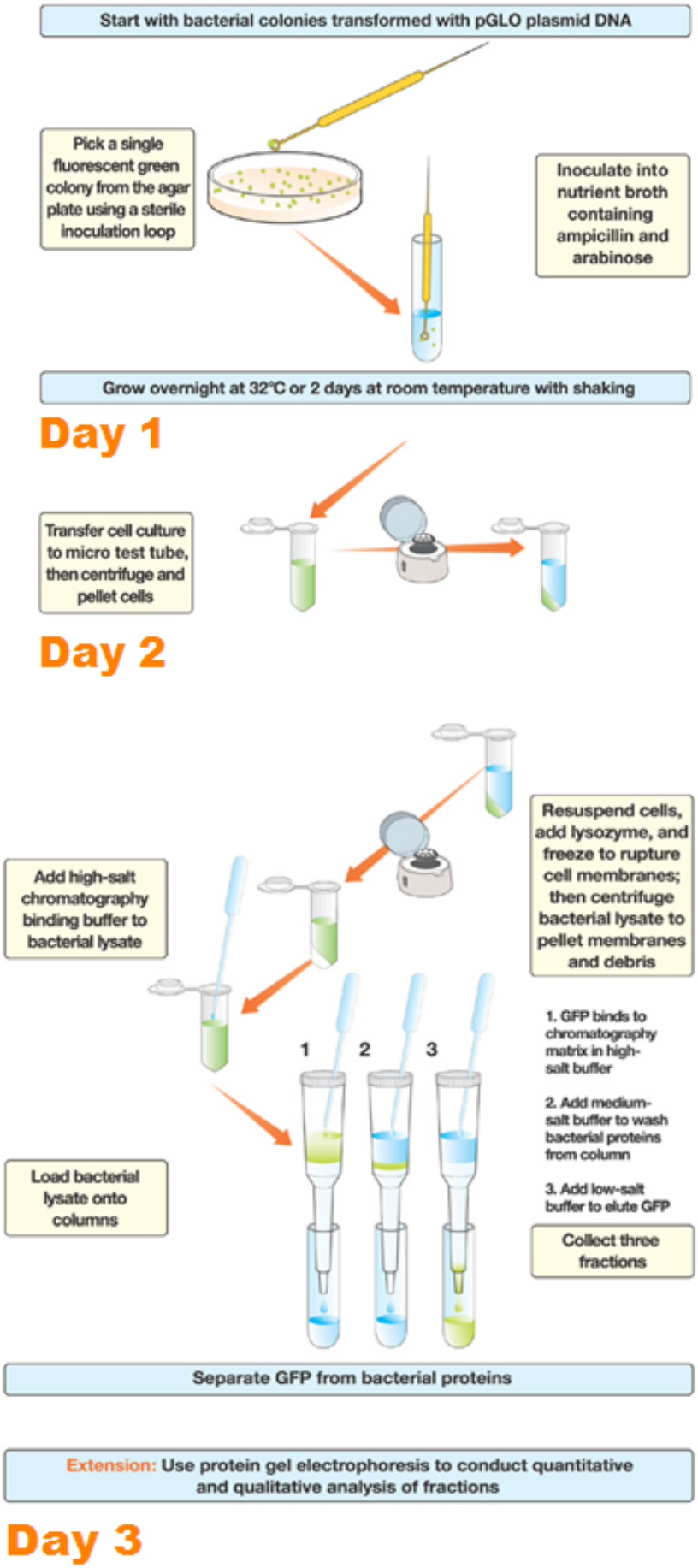
The bacteria growing on a petri dish should contain the human protein of interest, since the gene holding the

human protein coding also holds a code for protein resistance. A single, well-developed colony of these bacteria must first be transferred from a petri dish to a small vial. They are then grown overnight, then centrifuged to force the bacteria down into a pellet at the bottom of a microcentrifuge tube.

Once spun down, the pellet is resuspended in something called lysozyme, which ruptures the cell membranes. The cells are recentrifuged to help pellet the cell "waste" while the proteins remain in the fluid. Since we are trying to extract the proteins at this point, this fluid is saved.

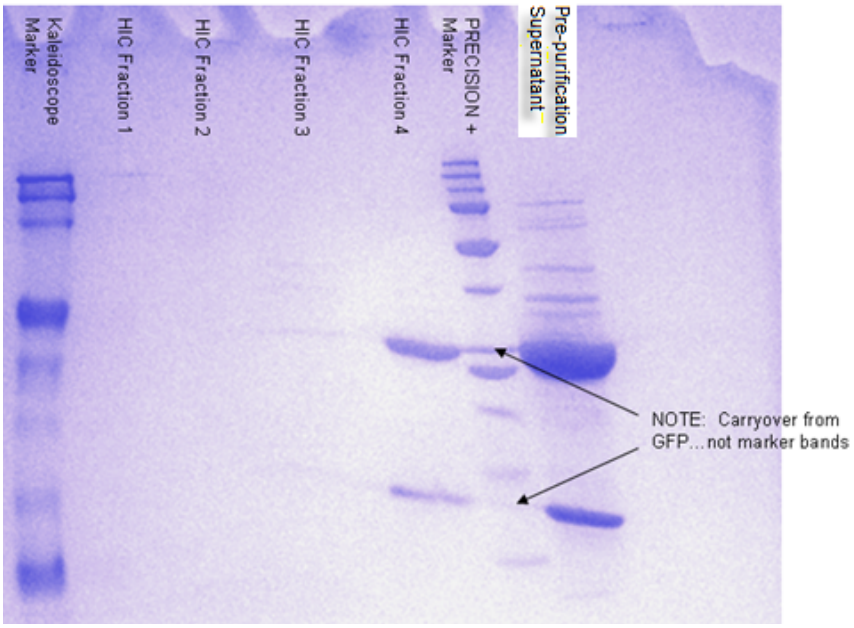
To the protein-laden fluid, we add a binding buffer. This binds to the proteins in solution. This fluid is then run through a chromatography column for the first time. The first time the fluid is run through the tube with binding buffer, all the proteins will become bound up in the pellets within the chromatography column. We then added a slightly salty buffer, which forced unwanted (hydrophilic) proteins out of the chromatography column to be collected in a second test tube. This left just the desired proteins (which are hydrophobic) bound up in the pellets within the chromatography tube. It separated them from the remaining proteins the cell produced. At this point, all the human proteins created by the bacteria are trapped within the chromatography tube. A final solution, a low-salt buffer, is added to the chromatography column. As it trickles through, it separates the hydrophobic GFP proteins from the column, and they wash into a third test tube, where the desired proteins are finally captured. Please note: this discussion is about hydrophobic human proteins . . . the steps would be slightly different if we were talking about hydrophilic human proteins or some other form. Because GFP (the glowy protein) we extracted was hydrophobic, we have focused our discussion on that sort of protein.

At this point, the third tube should hold the desired protein and nothing but the desired protein. Technically, creating and extracting the protein is complete. However, if you are making a human product, quality and purity MUST be checked before it can be sold.



Purity Confirmation with Gel Electrophoresis

The final step in creating human proteins is checking that purity using something called vertical electrophoresis, a form of gel electrophoresis. In this case, running buffer, fluid from each of the test tubes, and two forms of protein markers are run on a super-skinny acrylic gel. Separation occurs as it does for all forms of electrophoresis, with an electrical current performing the separation and larger fragments moving slower/traveling less far than smaller fragments. Below is a picture of the results. Note that in this case, the protein was NOT pure. If this were being created for humans, another attempt would need to be made to get to the proper level of purity.



Lesson 4.2: Organ Failure

So, we now know how to make human proteins. The procedure just discussed is how many human proteins are made today, including the human protein insulin. Insulin, you may recall, is commonly administered to people with diabetes in order to regulate their sugar levels. People who use insulin are called diabetics. Unregulated diabetes can cause many problems, starting with diabetic symptoms: frequent urination, constant thirst, rapid weight loss, etc. Complications can then begin to build up. These complications include diabetic retinopathy (vision loss), diabetic neuropathy (nerve damage), fatigue, etc. Finally, kidney function can be affected. Unregulated diabetes can lead to something called kidney failure, where the kidneys shut down and are unable to work properly. This means that blood does not get filtered properly, toxins build up in the blood, and the body becomes poisoned. It will lead to death if not treated properly.

Test:	Result(s):
Blood Urea Nitrogen Levels	60 mg/dL
Blood Creatine Levels	2.8 mg/dL
Blood Potassium Levels	7.1 mEq/L
Red Blood Cell Count	3.6 million cells/mcL
Glomerular Filtration Rate (GFR)	13 mL/min
Urinalysis	Presence of red blood cells Presence of white blood cells High levels of albumin (300 mg/dL)
Blood Pressure	140/90 mmHg
EKG	Normal

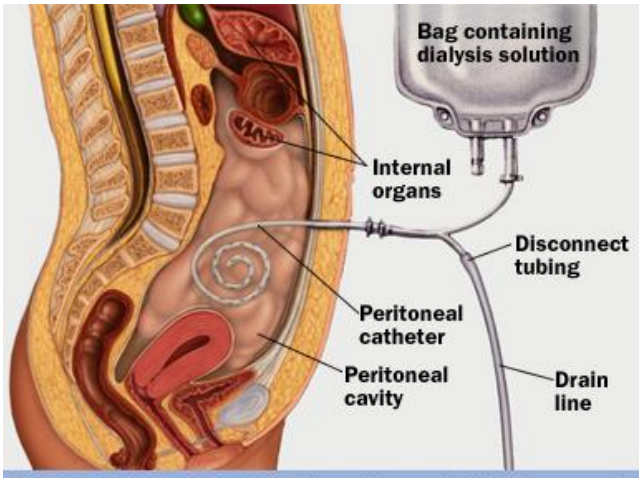
In Lesson 4.2, we met Diana Jones, the sister of Judy Smith. Diana was a long-term diabetic who, in the past, did not manage her diabetes well. Because of this, permanent damage to her kidneys occurred, and we learned that Diana was facing a diagnosis of ESRD, or end-stage renal disease. Her kidneys had failed. This disease is diagnosed using a few different tests, whose results are shown below for Diana:

All these different tests offer clues to the diagnosis of ESRD, which is confirmed with the kidney-based tests: GFR and Urinalysis. If the kidneys fail, what options are available.

Though a kidney transplant is the best option for people with ESRD, it is not always possible. While waiting for a transplant (or when a transplant is not an option) people can undergo dialysis. Dialysis is an artificial process that removes waste products and excess water from the blood when the kidneys can no longer function.

Two options of dialysis are available for people with kidney failure: hemodialysis and peritoneal dialysis. Hemodialysis involves using a machine to filter the blood outside the body. A person is hooked up to an intravenous line that removes their blood a little bit at a time, filters it, and pushes it back into the body. Review this procedure at http://www.mayoclinic.com/health/hemodialysis/MY00281 (this website has multiple pages that are all useful - don't just use the one called "Definition." The second option, peritoneal dialysis, is a bit different. Here, the body's own

peritoneum (the fatty membrane covering the intestines, is used to filter the blood and collect wastes. Review this procedure at http://www.mayoclinic.com/health/peritoneal-dialysis/MY00282. (Note that, like the other page, there's lots of information not on this first page. Use the menu on the left side of the screen for additional information.)



So, Diana has ESRD, and is temporarily using dialysis to extend her life. Without this treatment, her own blood would poison her. Diana knows this needs to be a temporary treatment, and thankfully she has lots of family willing to donate a kidney for her.

Lesson 4.3: Transplant

The reality is, there are far more people in need of organs than there are willing donors. Some facts to keep in mind: A name is added to the national transplant waiting list every 13 minutes. It is estimated that more than 70 people's lives are saved by organ transplantation each day. Unfortunately, because there are more people in need of an organ transplant than there are organ donations, it is estimated that almost 20 people die each day waiting for a donated organ. Organ donation and allocation are strictly regulated by federal guidelines which ensure that organs are distributed in a fair and equitable manner. The first step in allocating donated organs is to match the recipient with the donor. The donor and recipient not only need to have compatible blood types but also need to have compatible tissue types. Currently, the average national wait time for a donated kidney is longer than three years. There are more than 78,000 people in the United States presently waiting for a kidney transplant. When possible, family remains the best option for kidney transplants. They are more likely to be matches, and getting a donation from a relative allows people in need of kidneys to bypass the long wait time and get kidneys sooner.

We began by discussing the rules and regulations surrounding organ donation. It became clear that deciding who receives donated organs is not always a clear-cut issue and involves many difficult decisions guided by federal policies. The final decision as to who receives an organ involves multiple policies outlined by NOTA (the National Organ Transplant Act) and OPTN (the Organ Procurement and Transplantation Network).

The National Organ Transplant Act

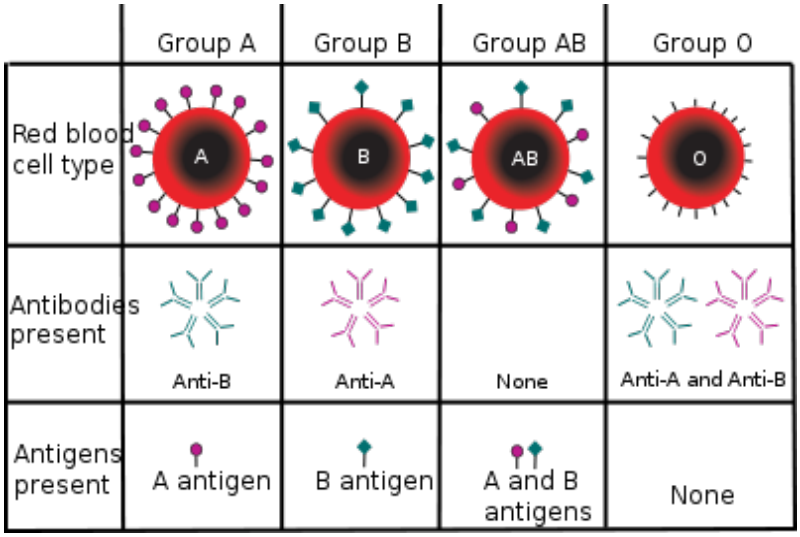
- Outlaws the sale of human organs.
- Specifies that the OPTN establish medical criteria for organ allocation including compatibility of the donor and the recipient and medical urgency (medical urgency only considered for heart, liver, and intestine transplantations).
- Excludes social criteria such as celebrity status, wealth, or prison status, from medical criteria which are not permitted in consideration of organ allocation.
- Specifies that a candidate for organ transplantation whose HIV positive, but who is in an asymptomatic state should not necessarily be excluded from candidacy for organ transplantation, but should be advised that he or she may be at increased risk of morbidity and mortality because of immunosuppressive therapy.

The OPTN Allocates Organs based on the following criteria:

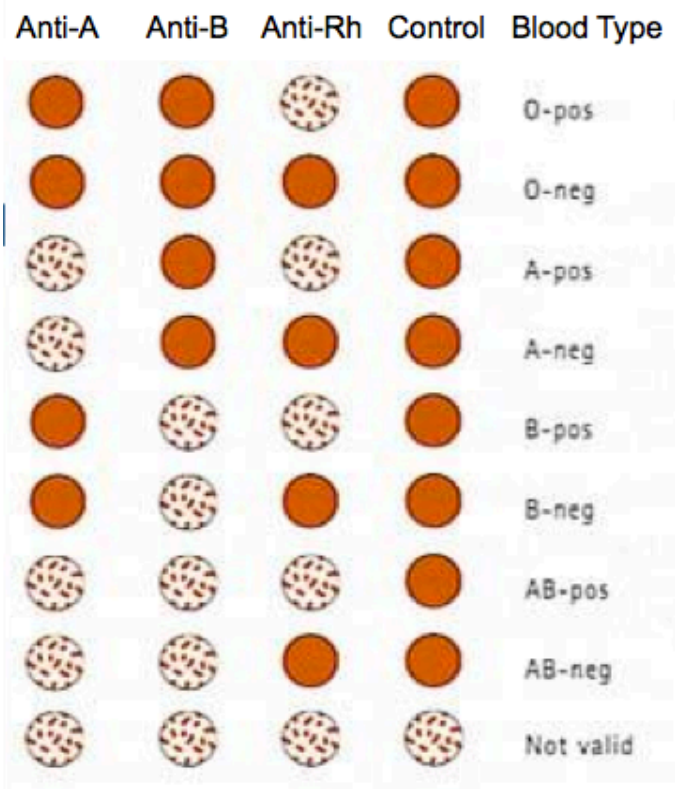
- Compatibility of the donor and recipient.
- Time on a waiting list.
- Geographical proximity between donor and recipient.
- Age of recipient (preference given to children).

These rules and criteria seek to reduce the subjective reasons people might select one recipient over another. This needs to be an emotionless, fair process based on need. Again, that isn't always easy, and may not seem fair. After being placed on the waiting list, people waiting for kidneys have certain tests performed so that a likely match can be found either from the donor banks for from a family member. Donors receive many of the same tests. These will be discussed next. One of the first – and simplest – tests when finding an organ match is blood typing, which works to find out the blood type of both potential donors and recipients. There are four common blood types: A, B, AB, and O. Blood is also classified as positive or negative based on Rh factors. Blood type and Rh factor simply discuss antigens on the outside of cells. Blood cells labeled "A+" contain both A antigens and Rh antigens. Someone who is "A-" contains A antigens, but not Rh factor. The following charts summarize blood types:

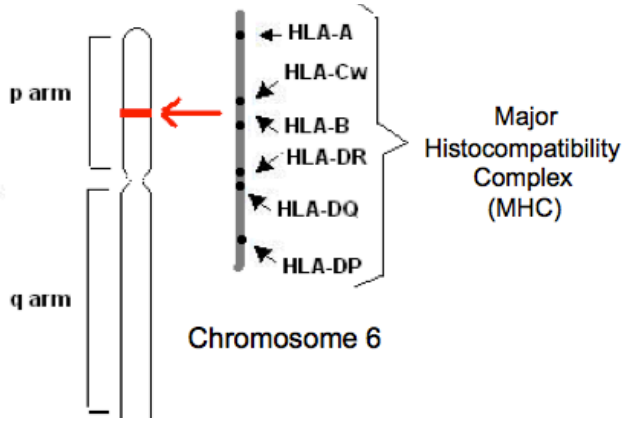
Blood Type	Genotype		Can Receive Blood From:
A	$i^A i$ $i^A i^A$	AA AO	A or O
B	$i^B i$ $i^B i^B$	BB BO	B or O
AB	$i^A i^B$	AB	A, B, AB, O
O	$i\,i$	OO	O



Blood tests are a quick, easy, cheap way to determine people who are not good donors. If donor and potential recipient do not have the same blood type, the recipient cannot get a kidney from that donor. People who are blood type matches then have further testing done. Keep in mind that the test works by checking for agglutination – clumping of blood. A person's blood is mixed with "Anti-serum" for different blood types. Type "A" blood mixed with "Anti-A" serum will agglutinate, meaning that the person has A antigens. The same is true for Type B blood – it will agglutinate with Anti-A and Anti-B serum, meaning both A and B antigens are present. This test takes advantage of blood's habit of agglutinating. This means nothing bad – it just is a way of finding out what types of antigens are present on blood cells. Agglutination with a certain "anti-serum" means that whatever the serum was testing for is present. In the picture below, agglutination (spotty sections) show which traits different blood types have. Note that Anti-D is the same as Anti-RH, and determines whether blood is + or -.



Blood typing allows elimination of people who are NOT good donors for people in need of transplant. If blood types don't match, it is highly unlikely that other, more important cell markers won't match, either. People who do match then undergo further testing. After blood typing testing is completed, the next test completed is HLA typing. First, a bit of background: Everyone has several antigens located on the surface of his/her leukocytes (white blood cells). One of the most important groups of antigens is called the HLA (Human Leukocyte Antigen) group. The HLA antigens are responsible for stimulating the immune response to recognize tissue as "self" or "non-self". It tells white blood cells which tissues belong to the body and should be left alone, and which should be attacked as foreign materials. These antigens are controlled by a set of genes on chromosome 6 called the Major Histocompatibility Complex (MHC).



HLA typing involves testing for the presence of different versions of this gene. There are multiple different versions of this gene broken down into two classes: Class I and Class II. Each class holds several types: HLA-A (there are multiple versions), HLA-B (lots of types) and HLA-Cw (lots of types). Class II holds HLA-DR, HLA-DQ, and HLA-DP – and again, multiple different

types. For a transplant to be successful, as many of these need to match as possible. The more that match, the more likely that the recipients body will not reject the transplanted organ. For this reason, tissue typing is an essential step performed prior to transplantation.

HLA typing is performed today using molecular techniques. The DNA is isolated, amplified with PCR, and sequenced to figure out which alleles are present. Again, potential donors and the recipients are tested, with the best match possible being found.

Once a match is found in this manner, there is yet another test done on the person receiving an organ: Antibody screening. This test is also known as a Panel Reactive Antibody (PRA) screening. Here, a small amount of the organ recipient's serum is mixed with cells from 60 different individuals in separate vials. This determines how many different HLA antibodies a person has in his or her blood, and how likely a person is to reject a transplanted organ. The more antibodies present in the blood, the more the body will fight a foreign organ. Ideally, PRA testing should result in the person's serum reacting less than 50% of the time with the blood of the 60 individuals. When patient serum is mixed with the random samples, agglutination is interpreted as a positive result. Agglutination means the body's serum reacted, and this is not a good sign if it occurs in more than 50% of samples. It means the body will likely reject a transplanted organ fairly quickly.

Finally, there is one more test performed before a transplant can occur: the crossmatch test. Here, the donor's white blood cells are mixed with the serum of the recipient. Hopefully, agglutination will NOT occur. This final check is to determine if the recipients body will attack the donated organ. Agglutination means a transplant will not be successful, and is considered a + crossmatch test. If this occurs, a new donor must be found. If, however, there is no agglutination, the crossmatch test is considered negative, and the transplant will happen. The crossmatch test is usually done early, and again right before surgery. It is absolutely critical that no reaction occur, as it means the body will reject the donated kidney very quickly.

So, testing is completed, and now it's time for transplant surgery. This is really a two-step procedure: kidney must be removed from the donor, and surgically implanted in the recipient. Kidney removal from the donor is done laparoscopically, a surgery that is much less invasive (causes less damage) than traditional methods. *Laparoscopy* is a minimally invasive surgical technique that uses small cylindrical tubes, called *trocars*, to enter the abdominal cavity. The trocars allow entry of a fiber-optic video camera, called a *laparoscope*, to view the inside of the abdominal cavity. Smaller trocars are used to allow entry of the instruments necessary to perform the surgery. Laparoscopic nephrectomies, compared to traditional open nephrectomies, require less pain medication, shorter hospital stays, and a shorter time before the patient can return to work. Physicians hope that the change in the surgery required to donate a kidney will encourage more people to become donors.

Immediately after the kidney is removed from the donor, it is transported to an operating room where the recipient is waiting, already unconscious and prepped for surgery. The recipient is more completely opened, and the kidney implanted without the old kidneys being removed. Here, the new kidney is attached to the iliac or femoral artery in your lower abdomen, and its one ureter is directly attached to the bladder. The body is stitched up, and sent to recovery. If all goes well, at the end of surgery the recipient has a functional, working kidney and can lead a more normal life, free of dialysis. Visit http://www.mayoclinic.com/health/kidney-transplant/MY00792 for additional information.

We ended this lesson by going over the different careers involved in transplant: anesthesiologist, transplant surgeon, surgical (perioperative) nurse, and pharmacist. Be sure to review these careers. They will not be discussed here. Something to consider: a heart transplant is a different procedure than a kidney transplant. How would it differ?

Lesson 4.4: Building a Better Body

We ended unit four with a study of how the body can be built better. We discussed tissue engineering, xenotransplantation, and bionics. Xenotransplantation, the transplantation of living tissues or organs from one species to another; and tissue engineering, the replacement of damaged organs or tissues with engineered organs or tissues created in the laboratory, are two of the technologies likely to become used in the future to create/engineer/procure organs for human use. Currently, these technologies have limited use – we use valves from cows and pigs for transplant, and grow human skin and bladders through tissue engineering. However, the future holds a great deal of promise for these two technologies.

Bionics is like a fusion of human and machine, replacing parts with machines that have similar or different functions. When we discussed prosthetics and the myoelectric arm, we were looking at one form of bionics. It is also being used to give some vision back to the blind through the use of electrodes, cameras, and sensors. Again, right now not much is done with this technology, but more will be done in the future.