DNA technology

All of the genetic information or DNA in a cell is its genome. Huge doesn't even begin to describe the size of the human genome. It's so big that it can be overwhelming, even to computers. We are usually only interested in studying one or a few genes, not the entire genome. For example, maybe we just want to study a single gene that determines eye color. It's like a needle in the haystack. We just want the needle. To study a single gene, we can create **recombinant DNA** in the lab. This allows us to study the effects of a gene in another organism.

First, the gene needs to hitch a ride to get where it needs to go. Well, a single gene cannot enter a completely different organism on its own, either. It needs a vehicle to get there. What is a gene's vehicle of choice? A bacterial plasmid, of course.

Bacteria have a large, circular chromosome and several smaller **plasmids**, which don't have many genes. Plasmids are very independent; they don't rely on the chromosome to replicate.

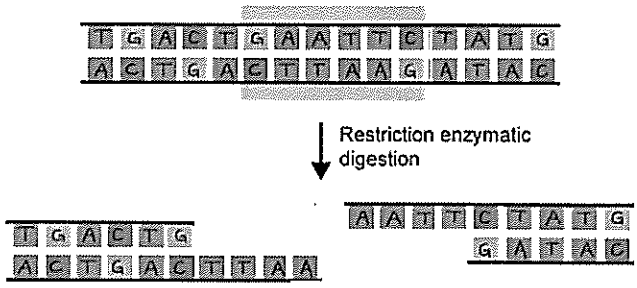
In a nutshell, we can take the gene we are studying and put it into a plasmid. When it is used to make recombinant DNA, the plasmid is called a **cloning vector**. It is called recombinant because it contains a combination of DNA from two different sources. The plasmid has all of the bells and whistles to make it easy to insert DNA.

We then put the recombinant DNA into bacteria, which reproduce quickly. The plasmid is replicated so each new bacterium will have the plasmid. Lots of plasmids are made and can be used to make protein encoded by the gene, gene sequencing, or other applications. Now that we've drawn the big picture, it's time jump into the nitty gritty. How, exactly, do scientists make recombinant DNA?

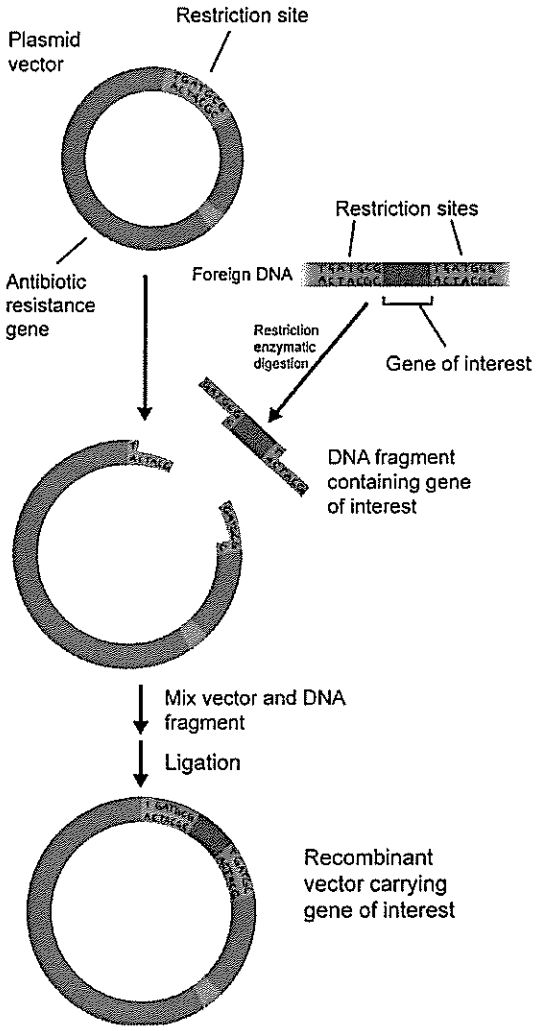
In order to clone a gene into a plasmid, both need to be cut. This is where the DNA scissors, a.k.a. **restriction enzymes**, come in. Restriction enzymes were discovered in bacteria to cut up who use them to cut foreign DNA into pieces as a protection mechanism.

The beauty of restriction enzymes is that they cut at very precise sequences of DNA called **restriction sites**. The fragments that result are called...**restriction fragments**. Why don't restriction enzymes chop up a bacterium's own DNA. It's because their DNA contains methyl groups ($-CH_3$) that have been added to adenine and cytosine bases at the sequences recognized by the enzymes. Smart move!

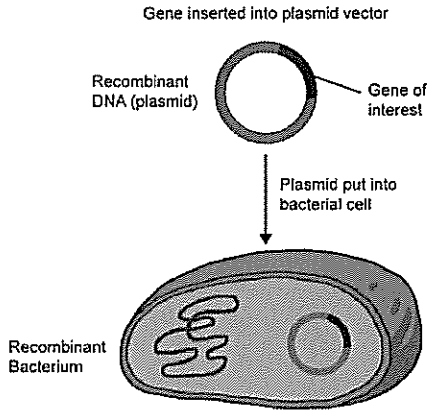
Both the plasmid and the piece of DNA to be cloned into the plasmid have to be cut by the same restriction enzyme. This generates fragments that have "**sticky ends**". The enzyme cuts through the double helix in a way that the strands separate leaving a bit of an overhang. Restriction sites are palindromic – they are identical when read forward and backward. For example, the restriction site for the enzyme *E.Cor1* is read "GAATTC" from left to right (top) and "GAATTC" from right to left (bottom).



The overhang of the sticky ends of the newly cut plasmid and gene of interest can be glued together by an enzyme called **DNA ligase**. DNA Ligase (also used in bonding together Okazaki fragments) acts as a molecular glue and creates complimentary base pairing between the sticky ends.



The recombinant DNA or plasmid can be introduced into bacteria such as *E. coli* and allowed to replicate. A technique called **electroporation** that creates temporary pores in the cell membrane is commonly used. An electrical zap is briefly applied to the bacteria creating holes through which the recombinant DNA can enter. We used a different technique called DNA transformation. The goal of both techniques is to have a small population of bacteria take up the plasmid from the environment.



This all sounds great, but how can we be sure that any of the bacteria have the recombinant DNA? The plasmid is usually engineered to contain an antibiotic resistance gene (we used ampicillin^R). When an antibiotic is added to the medium that the bacteria grows in, only the bacteria that have taken up the plasmid containing the resistance gene to that antibiotic will survive. All of the other remaining bacteria will die. At this point, the bacteria will continue to reproduce. Each new bacterium will contain the same plasmid – creating a clone of bacteria all with the recombinant plasmid DNA. Next, the plasmid DNA can be isolated and purified from the bacteria.

It's always good to get confirmation that what we have is what we think we have. The quick check we usually begin with is to cut the purified recombinant DNA with restriction enzymes and check their sizes using electrophoresis.

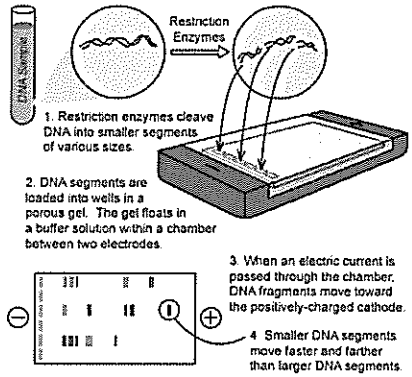
Cutting the recombinant DNA with the same restriction enzymes we used to make the expression vector in the first place should give us the pieces we started with. We can get a map of the plasmid we used with the restriction sites found in the plasmid. We also know the sequence of the DNA we are inserting. Using these tools, we can determine which restriction enzymes to use and how many and what size fragments should be produced.

It would be nice if we could actually see the DNA, but we can't. Something has to be used to visualize the fragments and to determine their size. Next up: electrophoresis.

In DNA **electrophoresis**, a chemical called agarose is used to form a gel that contains pores of uniform size. We can apply a current to the gel and place the negative electrode near the top of the gel where the DNA is loaded and the positive electrode near the bottom of the gel. The negative charges on the phosphate groups of the DNA backbone will be attracted to the positive charge - pulling the DNA down the gel towards the positive electrode.

The smaller fragments are the speediest while the larger fragments take their good old time traveling through the agarose. A dye that interacts with the DNA is added which allows visualization of the DNA bands. We also run DNA of known size on the gel (called a DNA ladder), so the size of the unknown sample fragments can be determined by comparison. This method does not provide any information on whether the sequence is the desired one or not. It just helps us determine the size of the fragment.

Figure S-2: Gel Electrophoresis

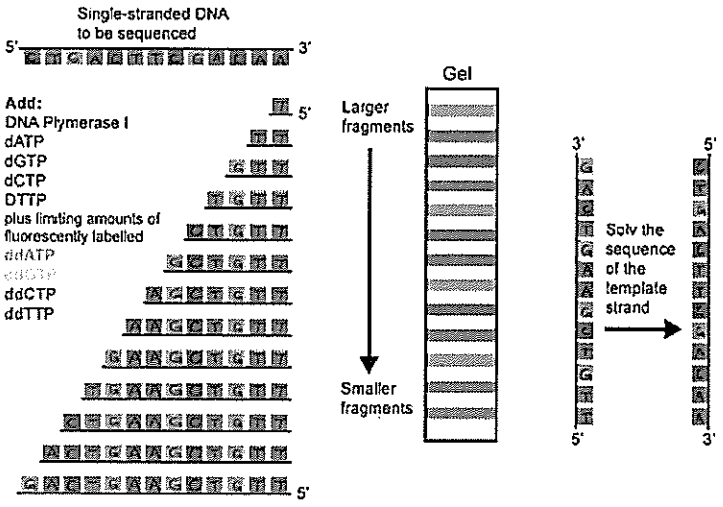


DNA sequencing can be used to determine the actual sequence of A's, G's, C's and T's. DNA sequencing has come a long way, and it is now quite simple to obtain the DNA sequence of your beloved recombinant DNA. Take a look at the evolution of DNA sequencing.

The original sequencing method was dideoxy chain termination. The principle is straightforward. Double stranded DNA is heated and the strands are separated or denatured. A short sequence of nucleotides called a primer is used to bind to a known sequence near the 3' end of the DNA. DNA polymerase, the four normal deoxynucleotides; dNTPs (dATP, dCTP, dGTP, and dTTP), and the primers are added to the tube. An altered dNTP of each type (AGCT) called a dideoxynucleotide with a fluorescent tag (a separate color for each of the AGC & Ts) are added to the tube as well. These altered dideoxynucleotides, when incorporated, stop the DNA chain from growing. They are incorporated randomly. The result? Lots and lots of DNA fragments of various sizes, each with a fluorescent tag on its 3' end.

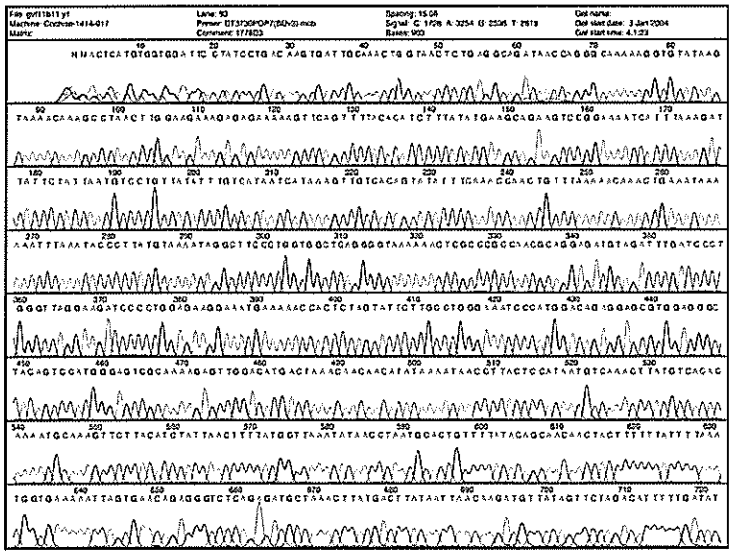
What should we do with all of these fragments? They can be separated based on their size through a gel matrix. We use a gel called polyacrylamide that has tiny pores. The smallest fragments move the quickest. The largest move the slowest (just like Gel Electrophoresis).

An instrument that detects fluorescence travels up the gel reading the dideoxynucleotides, and voilà, there's the sequence. It's actually the sequence of the complementary strand, but the original sequence can be determined from this info.



Sounds pretty tedious, doesn't it? It is. Thankfully, DNA sequencing has evolved. Now, it's easier and cheaper than ever to have DNA sequenced.

Chain termination has given rise to dangling a single-strand of DNA on a surface. Think of it as the worm when you go fishing. The dangling piece of DNA looks yummy to its complementary bases. They are added and can be detected by a machine. Much simpler. The sequence can literally be read from the print out of data.



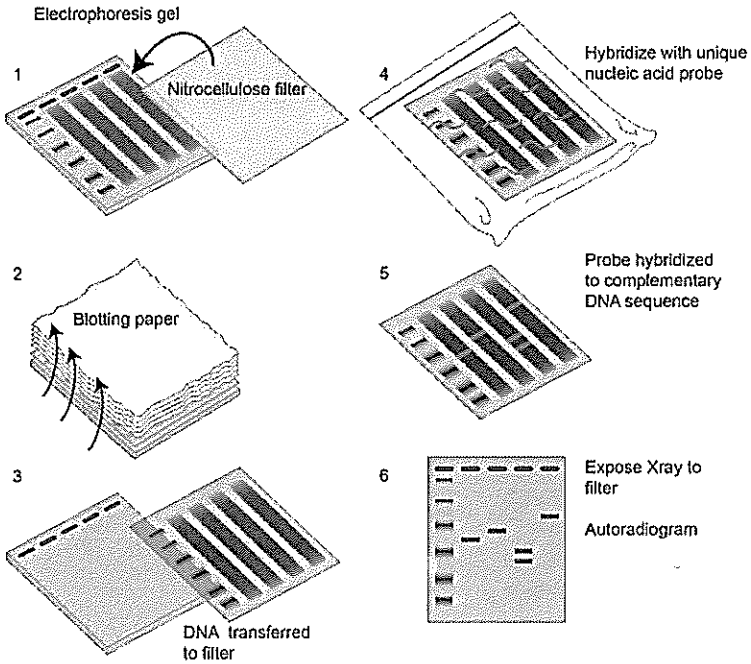
GENE EXPRESSION

We have to use DNA technology to clone the gene we want to study. It's a necessary evil. After the gene is cloned, we can get to the good stuff and look at different gene sequences and expression. We can use the following tools and techniques to get to the bottom of gene expression.

Restriction fragment length polymorphism (RFLP; pronounced "rif lip") is used to distinguish the difference in sequence between alleles for a gene. Sometimes these differences are located within a sequence recognized by a restriction enzyme. If this is the case, we can cut the DNA with a restriction enzyme and then do DNA electrophoresis. Different patterns of DNA pieces on the gel would indicate a rif lip.

A **Southern blot** (named for the scientist Edwin Southern) can be performed to look for the presence of a specific DNA sequence. In this technique, we treat different DNA samples with restriction enzymes and then use gel electrophoresis to separate the fragments. The DNA is transferred from the gel to a piece of paper-like material called nitrocellulose. This is the blot part.

We make a short sequence of DNA. This sequence is called a probe and is labeled or tagged in some way so it can be detected. It is incubated in solution with the nitrocellulose. The single-stranded probe binds its mate, or its complementary DNA. Its tag or label can then be detected.



What if we want to know if a particular gene is being transcribed into mRNA? One of the experiments that we can do is a relative of the Southern blot, called the **Northern blot**. Clever,right? The principle of the Northern blot is similar to the Southern blot.

In a Northern blot, we perform electrophoresis of mRNA. We then transfer the mRNA on the gel to nitrocellulose and use an mRNA probe to find its mate. While Northern blots are still useful, there is another more sensitive and quantitative technique to detect mRNA, called **reverse transcriptase-polymerase chain reaction (RT-PCR).**We're getting a little ahead of ourselves. Before we can discuss RT-PCR, we have to talk about PCR. **Polymerase chain reaction (PCR)** is used to amplify a DNA sequence. In fact, it can make billions of copies in just a few hours!

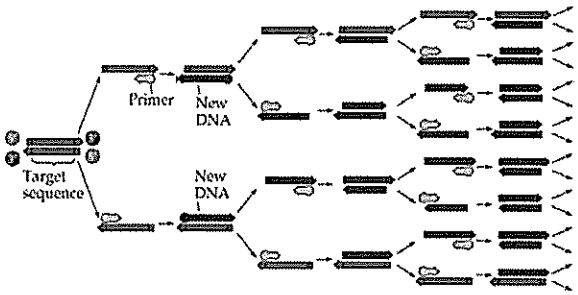
PCR consists of three steps per cycle:

Denaturation: Temperature is raised. Double stranded DNA doesn't like this, and the two strands separate.

Annealing: Short sequences complementary to the DNA sequences on opposite ends of a gene, or primers, bind to the DNA.

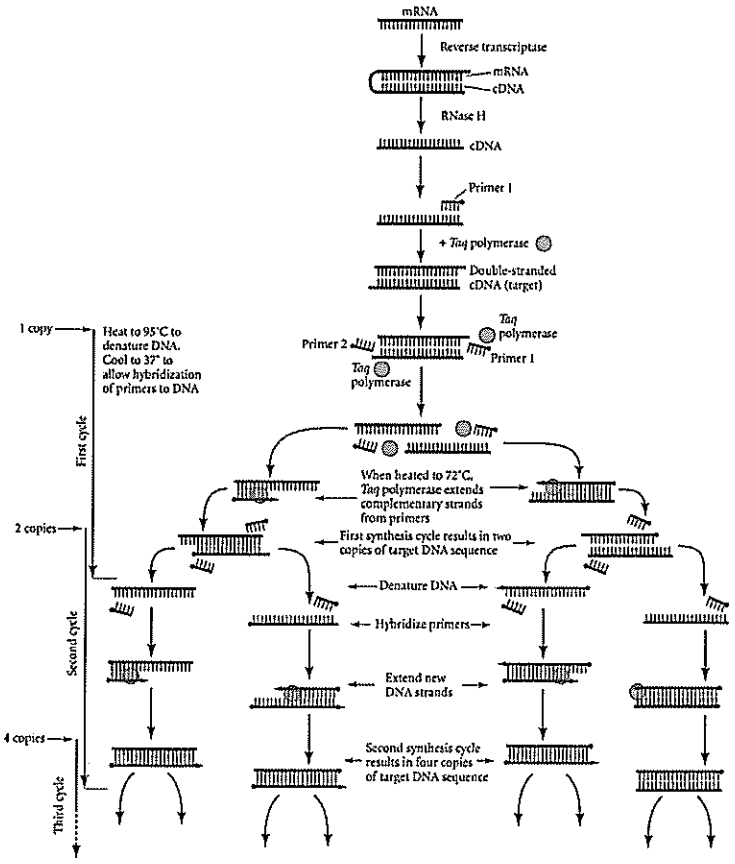
Extension: Enter the king of all DNA polymerases, Taq polymerase. Taq adds complementary bases to the 3' end of the primers.

PCR-DNA synthesis cycle



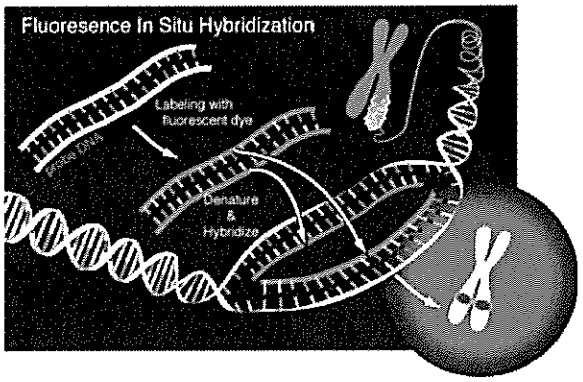
The number of molecules formed is equal to 2^n where n is equal to the number of cycles. The bottom line is this: you want amplification of your piece of DNA? PCR is your method. Several millions of copies of the same DNA sequence can be made in 24 hours. Once made, there is a sufficient enough quantity to use for other tests.

Now we can talk about RT-PCR. This technique serves the same purpose as a Southern blot, but requires smaller amounts of starting material. The mRNA is incubated with an enzyme called reverse transcriptase (RT). RT creates complementary DNA (cDNA) from mRNA. The rest is the same as normal PCR. The DNA is amplified and fragments can be separated by electrophoresis on a gel.



Yet another method to detect mRNAs is **Fluorescent in situ hybridization (FISH).** In situ hybridization recognizes the location of a particular mRNA. We can make a labeled (usually fluorescent) probe complementary to an mRNA that will recognize and bind to this sequence wherever it is located. One of the great features of this technique is the colorful data that we get out of it. FISH identifies chromosomal abnormalities, aids in gene mapping, toxicological studies, analysis of chromosomal structural aberrations and ploidy determination. FISH is used to identify the presence and location of a region of DNA or RNA within chromosome or tissue preparations which means you can view a segment or entire chromosome with your own eyes. The advantage is that it is a less labor-intensive method for confirming the presence of a DNA segment than other conventional methods like Southern blotting

Procedure:
Denature the DNA and fluorescent probe
Mix to allow for hybridization
Examine under a microscope



We've discussed Southern and ever so aptly named Northern blots. What about Western and Eastern blots? Well, if we want to go a step further than RT-PCR or in situ hybridization and look for the presence of a particular protein, we can do a Western blot, which is also called an immunoblot.

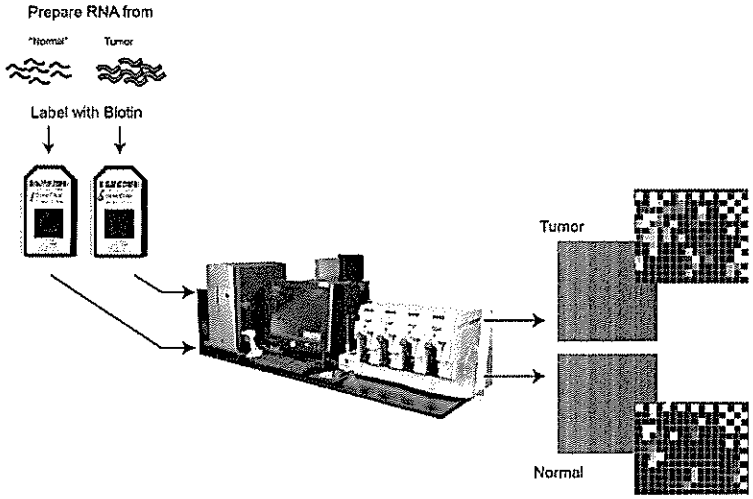
We take a sample extract from cells and incubate it in a buffer that causes the proteins to unfold (or "denature"). We then separate the samples by polyacrylamide gel electrophoresis that also involves sodium dodecyl sulfate, or SDS. SDS helps to further denature the proteins in the sample so they can be separated by size.

After the gel is finished running, the proteins are transferred to nitrocellulose. Sound familiar? After the transfer, we incubate the blot with an antibody that is specific only for the protein of interest.

One of the newest and perhaps most powerful techniques that can be used to analyze gene expression is the superman of all techniques, the **DNA microarray**. Often called gene chips or DNA chips, these microarrays contain representative sequences of several thousand genes.

We can collect mRNA from cells and use it to make fluorescently tagged cDNA. The cDNA is then applied to the microarray. Base pairing between the DNA probe sequence on the array and the cDNA would be a match. We use a special machine to scan the chip. The brightness of the fluorescent spot where there is a match correlates with the level of expression.

Aside from the fact that it's just really cool to screen thousands of genes in a single experiment, why would we want to perform microarray analysis of gene expression? Assume we want to examine the difference in gene expression between normal and tumor cells. We would isolate the RNA from both types of cells and subject it to microarray analysis.



The result will show you how the pattern of gene expression is different between the two cell types. Some genes might show higher or lower levels of expression in cancer cells compared to normal cells.

Warning: DNA microarray analysis will give you so much data that your eyes just might glaze over. Your biggest challenge will become where to actually begin sifting through all the data.

https://www.youtube.com/watch?v=o87QGg2Ggxs (clip from X-files: Scully doing a Southern Blot)

GENE FUNCTION

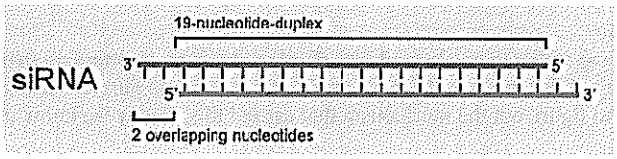
DNA technology and gene expression are important, but we all know the proteins deserve all of the credit. They are the ones actually doing the work. To see what a gene actually does, scientists can manipulate the expression of gene products or proteins and see what happens in the absence of that gene.

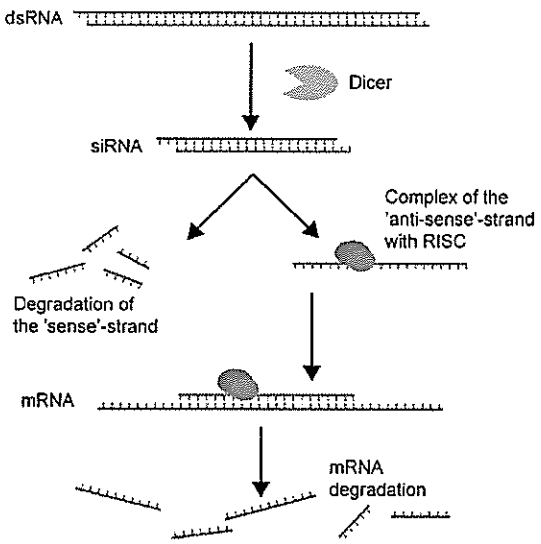
One of the ways a gene can be disrupted or turned off is through **in vitro mutagenesis**. In this technique, a cloned gene is mutated in the lab and is then put into the organism. This engineered DNA is able to knock out the normal gene so only the mutated gene is expressed. The scientist can see what happens and determine a likely function.

Many knock-out organisms have been produced, but the process takes a lot of time, and is not guaranteed to work. Over the past decade, **RNA interference (RNAi)** has been exploited to temporarily silence gene expression.

In RNAi, double-stranded RNA is cut in pieces that are 19-21 nucleotides in length by an enzyme called **Dicer**. These little pieces are called **siRNAs** or short interfering RNAs.

siRNAs are incorporated into a protein complex called **RISC** (RNA-induced silencing complex) and then hunt for the mRNA in the cell with the matching sequence for degradation. The anti-sense, or complementary, siRNA strand binds to the mRNA sequence. This sends red flags to the cells and calls for the mRNA to be destroyed. If no mRNA for a particular gene exists, then no protein can be made, and the effects can be observed.





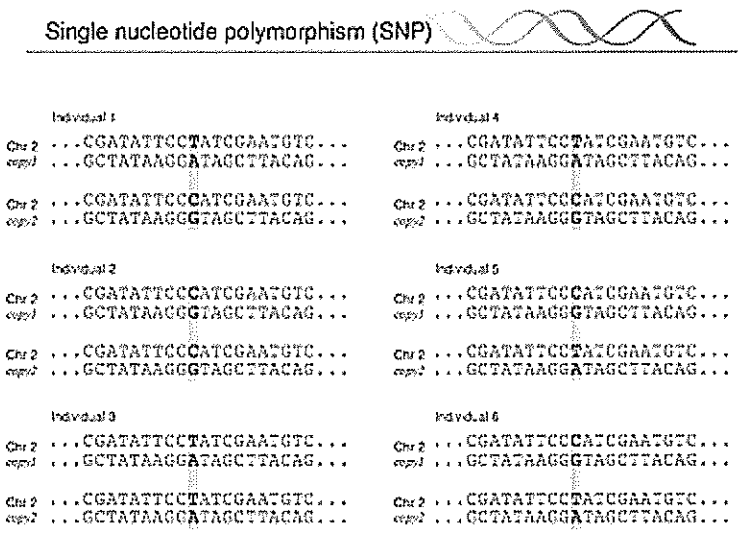
The effects of RNAi are only temporary. The mRNA is considered to be "knocked down" rather than "knocked out." Using this boxing analogy, if someone is knocked down they can get back up. Eventually, expression of the mRNA continues. However, if a gene is knocked out, no mRNA will ever be made.

Note that both in vitro mutagenesis and RNA interference are usually done in model organisms such as mice and fruit flies, as well as cultured cell lines. It would be unethical to do these tests in humans. Who would ever volunteer for something like that, anyway?

If you want to search for the genetic cause of a disease, you can look for genetic markers in the sequences of normal individuals vs. those with the disease. Genetic markers are what give rise to alleles. They are sequences in the DNA that vary in the population.

A **single nucleotide polymorphism** (**SNP** and pronounced snip; snip, snip, snip) is a variation in one nucleotide that occurs in at least 1% of the population. A SNP is usually located every 100-300 bases. They can affect how a person reacts to the environment, medication, or so on.

Scientists have been creating a map of SNPs. These maps allow conditions that are due to multiple genes to be more easily identified. Also, the human genome is so large that mapping SNPs can aid in the organization of all that data. They can serve as mile markers on the extensive roadmap that is the human genome.



[1]In 1990, Rich Jorgensen, a molecular geneticist was trying to create a vibrant purple petunia by inserting an extra copy of the gene that codes for the purple color. He thought an additional copy of the gene would make the flower even more purple. Makes sense, right? Well, the flower turned out white! It took almost ten years to figure out the mechanism that caused this. Today we know that the mRNA was targeted by RNA interference

ORGANISM CLONING

When most people think of cloning, they usually think of Dolly the sheep, who in 1996 became the first mammal to be cloned from an adult cell[2]. Unfortunately, Dolly didn't live to a ripe old age, but her birth was an achievement and scientists have learned a lot through the process.

Breeding selected organisms for their desired traits has been going on for thousands of years. More recently, think back to good ol' Gregor Mendel and his peas. His controlled, selective breeding of pea plants was a form of genetic manipulation.

To start, here's a little terminology to make all of this a little easier to understand. A **differentiated** cell has reached its final destination and is specialized. It could be a skin cell, a liver cell, or a stinger cell in a bee. Some differentiated cells can become dedifferentiated and then coaxed in the lab to become a different type of cell. These cells are called **totipotent**.

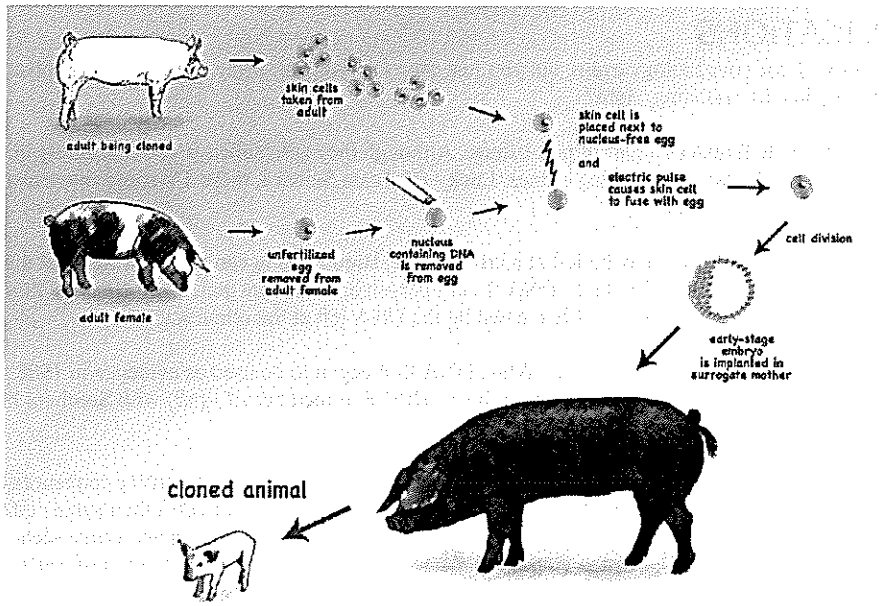
You've probably heard of **stem cells**, if not through science classes, then through the media. Stem cells are cells that are not differentiated. They have the potential to become any type of cell. That's why they are like the holy grail of genetics.
Embryonic stem cells are **pluripotent**. They can become any type of cell. Adult stem cells cannot become all types of cells, but can give rise to many cell types. For this reason, embryonic stem cells are highly coveted. Their use does not come without difficult ethical issues, as we will discuss later.

Recently, scientists have learned how to convert differentiated cells into pluripotent cells. These cells are **induced pluripotent cells (iPS)**.

Now that we have the terminology down, on to cloning we go. The first plant to be cloned from a single cell was a carrot. Yep, Bugs Bunny would be proud. Cells were taken from a carrot's roots by Charles Steward and his students in the 1950s and cultured in the lab. These cells eventually produced a plant. This showed that somehow adult plant cells could dedifferentiate and then result in all cell types of the plant. Pretty slick, Dr. Steward.

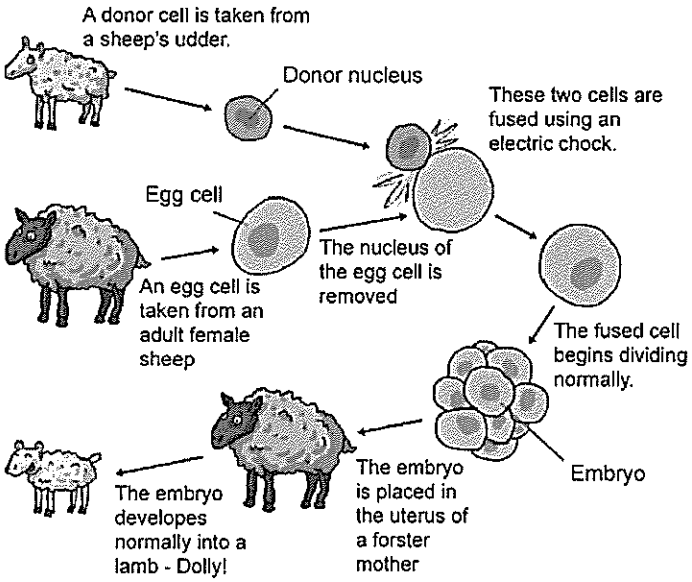
This method is used by many industries. Think about the lumber industry. What if we discovered a particular tree that was resistant to disease and could grow very tall and thick, and also enjoyed long walks on the beach? This could be a big money maker. We could take a sample and remove individual cells to grow in the lab. These cells can produce seedlings that can be transplanted into the ground. What's the end result of this hard work? Several trees genetically identical to the original tree.

Animal cloning is a little different, and a little more complex. Animals cannot be cloned using the technique for plant cells because usually differentiated animal cells cannot be grown in culture. Instead, scientists use **nuclear transplantion**. Yep, it's just as it sounds. You've heard of heart and kidney transplants. In this case, the nucleus of a differentiated cell is inserted into a fertilized or unfertilized egg cell where the nucleus has been removed (or **enucleated**). The fertilized egg divides several times in the lab and become an embryo. The embryo is then implanted into a surrogate. The resulting animal is genetically identical to the organism whose nucleus was transplanted.



This technique was used to clone Dolly the sheep. Dolly was a super big deal. Scientists did not know whether or not an already differentiated cell could be used to clone an entire organism. A differentiated cell is already programmed to do its job. Could it direct development from the earliest stages?

The answer was yes, and Dolly was proof. Researchers developed a technique to dedifferentiate cells from six-year-old sheep udder tissue (mammary cells) in the lab. They then transferred these cells to sheep egg cells in which the nuclei had been removed. The cells were allowed to divide in the lab and eventually the embryos were implanted into surrogate female sheep. Dolly was the only live lamb born.



This achievement was monumental, but problems were soon discovered with Dolly. The normal lifespan of sheep is about twelve years. At the age of six, Dolly suffered from conditions normally seen in much older sheep and was euthanized. Scientists think poor Dolly's health problem could be because the nucleus used to clone Dolly was from a six year old sheep. If that doesn't belong in a sci-fi movie, we don't know what does.

Scientists have noticed that many cloned animals are prone to health issues. A closer look at the transplanted nuclei of several organisms suggests that they are not fully dedifferentiated. This could explain the premature aging and susceptibility to disease shown by cloned animals.

Cloning animals may be beneficial to the agricultural business, but what about human cloning? Scientists aren't as interested in creating another human being as generating stem cells from human embryos. Remember, embryonic stem cells can become any type of cell. They are not differentiated yet. These cells can be studied to better understand how a cell becomes differentiated. It is thought that they hold great promise for the treatment of medical conditions.

FORENSIC APPLICATIONS

You really should finish studying for your biology exam, but you just can't tear yourself away from the latest episode of CSI. After all, you will learn a little about biology just by watching, right?

CSI and similar programs have made forensics a part of pop culture. Although they aren't always 100% accurate in their portrayal, DNA analysis forensics has been immensely useful in solving crimes. Lucky for Team Science, these TV shows have also been useful in altering the stereotype of white-coat-wearing scientists.

If investigators are lucky, blood or bodily fluid may be left at a crime scene. Blood typing can be performed and may eliminate a suspect, but blood types are nowhere near as unique as an individual's DNA. Every person has a unique genetic profile, and this fact can be exploited to confirm or rule out a suspect when compared to the profile created by the DNA left at the crime scene.

As DNA technologies improve, so do forensic applications. When DNA first began to be used as evidence in 1988, forensic scientists combined the techniques of restriction fragment length polymorphisms (RFLP, remember rif lips?) with Southern blot to look for similarities in DNA sequences.

An even more sensitive technique has been developed using **short tandem repeats**. These are repetitive sequences of DNA that are highly variable between people. PCR primers are used to amplify a particular STR, and the PCR product is then subjected to electrophoresis. The size of the product indicates the number of repeats and a comparison can be made between suspect and crime scene evidence. The beauty of this technique is due to PCR. Remember PCR is used to amplify DNA sequences, so only a small amount of starting material is needed.

MEDICAL APPLICATIONS

Some of the most immediate benefits of biotechnology can be seen in the medical field. Advances in biotechnology can be extended to the detection of pathogens or medical diagnoses, gene therapy, and the pharmaceutical industry.

D^3: Detection and Diagnosis of Disease

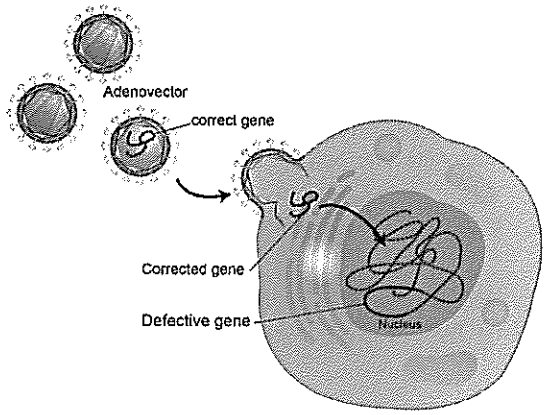
Imaging you have flu symptoms and decide to hit up the doctor. The doctor suspects you may have the H1N1 flu virus strain. There is a rapid test that the doctor performs and concludes that you do have H1N1. RT-PCR analysis can also be performed on a sample to diagnosis the virus. Using the same procedure described earlier, primers have been designed for a specific H1N1 mRNA. Detection of H1N1 mRNA levels using RT-PCR is more sensitive than a rapid test. RT-PCR can also be used to detect HIV, SARS, Dengue Virus, and mumps, just to name a few.

If the gene sequence that contributes to a genetic condition is known, it should be able to be identified by PCR. Now that we know the sequence of the human genome, scientists have been able to link a particular disease to a gene or set of genes. Sickle cell anemia, cystic fibrosis and hemophilia are just a few conditions where the gene has been identified. PCR primers specific for these genes can be used to identify and amplify those genes. This can occur before symptoms of the diseases are even noticed.

Gene Therapy

Genes may be changed such that they result in a protein that is unable to do its job. Knowing the original sequence of the gene, scientists have been devising methods to deliver the original sequence of the gene back into the cells. This is gene therapy.

While it sounds great and makes sense biologically, gene therapy has faced many obstacles. First of all, how do you get the gene into the cells? Usually, viral vectors are the vehicles of choice. In the example below, an adenovirus vector is used to deliver the correct or normal gene into the cell.



This method was used in trials in 2000 to treat ten children with severe combined immunodeficiency. David Vetter, otherwise known as the "Bubble Boy," had this condition. Nine children showed significant improvement, but three children later developed leukemia, which killed one child.

Some other issues with gene therapy still need to be hammered out. Currently, the effect is not long-lasting. The correct gene needs to be incorporated into the genome so that its protein is continually being produced.

Gene therapy introduces something foreign into human cells. This can send a red flag to the immune system. The immune system has a great memory making it difficult to administer several treatments without an immune response.

The viral vectors used may also be problematic in targeting only certain cells. Also, they may be toxic.Finally, many diseases result from problems in several genes. Gene therapy can only target single genes.

Pharmaceuticals

The DNA sequence of a gene can be used to determine the amino acid sequence of its corresponding protein. This information, as well as experiments performed in the lab, can be used to determine the 3D structure of the protein or molecule, and you won't even need your 3D glasses. Knowing how the structure of the protein relates to its function allows scientists to don their lab coats and head into the lab to engineer similar proteins or small molecules. These can then be screened for desired effects.

Selective Estrogen Receptor Modulators, or SERMs, are examples of molecules that have been produced to bind to estrogen receptor. Knowing the structure of estrogen and its receptor, scientists have been able to generate other molecules that can bind estrogen receptor. They then can select for molecules that function as estrogens or anti-estrogens.

Earlier we discussed gene cloning. This technique can be used to produce large amounts of protein. The gene coding for a protein of interest can be cloned into an expression vector and transferred to host organism such as bacteria, insects, and so on. The vector is expressed, and boatloads of protein can be made. The insulin diabetics use to regulate their blood sugar is produced in this way.

Whole animals have been created that express a gene that is often from another species. These are transgenic animals. They are also called genetically modified organisms, which we will talk more about it a bit.

In order to make a transgenic organism, an egg from a species is removed and fertilized in vitro. The gene of interest is cloned and injected into nuclei of fertilized eggs in hopes the transgene will be integrated into the genome. The transgenic fertilized eggs are then implanted in a surrogate. The end result is a transgenic animal.

Transgenic goats and chickens have been produced this way. The protein product of the transgene is found in the milk and the egg, respectively. The proteins can then be isolated and purified.

COMMON MISTAKES

DNA Technology

DNA will migrate to the positive electrode due to its negatively charged backbone. This is usually the red colored electrode, so remember, "run to red."

Electro this, electro that, electro what? Both electrophoresis and electroporation use electricity or a current to do their jobs. Electrophoresis separates nucleic acids based on size and charge and electroporation creates pores so DNA can enter a cell.

Gene Expression

With the compass naming system, it's easy to forget exactly what a Southern, Northern and Western blot actually look for and how you would choose which technique to use. Just remember this: **S**ally **D**onned **N**ancy's **M**eticulous **W**hite **P**ants (Southern = DNA, Northern = mRNA, Western = protein).

Gene Function

You might think that only the anti-sense is necessary for RNA interference, but, in fact, the buddy system is strictly enforced in RNAi. Double-stranded siRNA is essential for RNAi to occur.

Organism Cloning

Differentiated or dedifferentiated? Struggling to understand these terms that sound so much alike? Remember that in order to become dedifferentiated, cells have to be differentiated first. The prefix "de-" means something has been taken away.

Forensic Applications

Forensic scientists do not actually look at the differences in the sequence of nucleotides between the DNA left at a crime scene and the suspects. They analyze the number of repeats of small DNA sequences.

Medical Applications

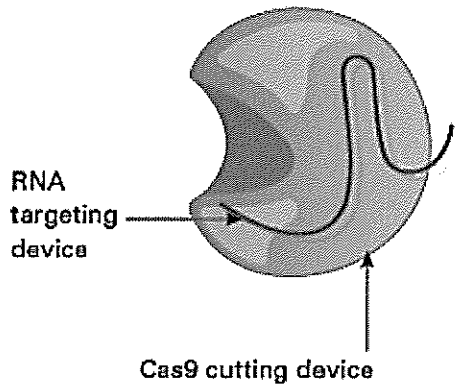
Genes cannot be delivered to human cells alone in gene therapy. They first have to be cloned into an efficient vehicle first - a viral vector that can deliver the gene to the target cells and incorporate the gene into their genome.

Recognition for CRISPR Gene-Editing Tool

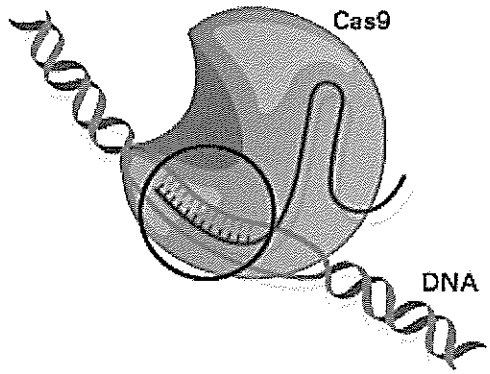
Posted by Emily Carlson on December 17, 2015
Post a Comment | View Comments (3) ↓

The CRISPR gene-editing tool was recognized today by *Science* magazine as its "breakthrough of the year." We support a number of researchers working in this exciting area and have featured it on this blog. To learn more about this exceptionally promising new method, see below for our illustrated explanation of the CRISPR system and its possible applications.

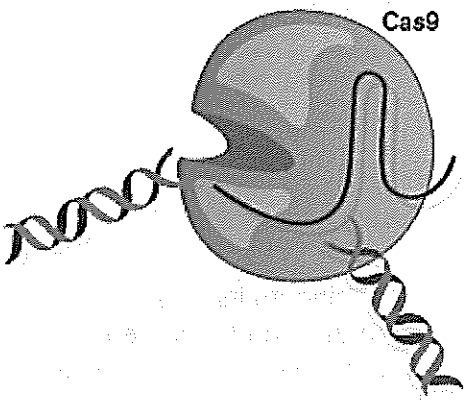
How the CRISPR System Works



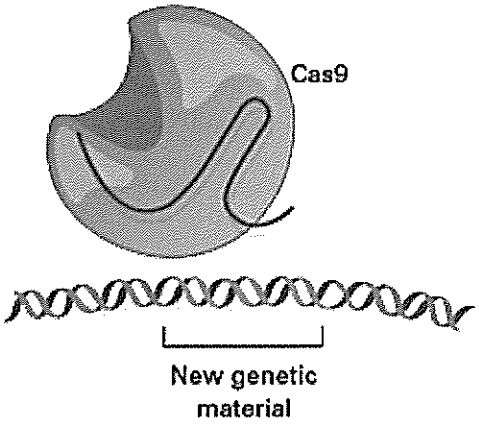
The CRISPR system has two components joined together: a finely tuned targeting device (a small strand of RNA programmed to look for a specific DNA sequence) and a strong cutting device (an enzyme called Cas9 that can cut through a double strand of DNA).



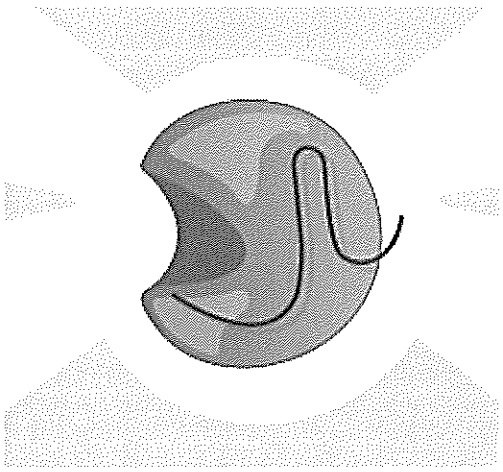
Once inserted into a cell, the CRISPR machine locates the target DNA sequence. CRISPR's RNA recognizes and binds to the target DNA (circled in black).



The Cas9 enzyme cuts both strands of the DNA.



Researchers can introduce new genetic material, which the cell automatically incorporates into the gap when it repairs the broken DNA.



Among its many possible applications, the CRISPR system could be used to:

- Add a new gene that enables the organism to produce a pharmaceutical product (a biotechnology technique).
- Help treat genetic diseases.
- Create tailor-made model organisms to study human diseases.
- Help produce replacements for damaged or diseased tissues and organs.