Who's Your Daddy?

1. **Engage: Crime Scene video:** Crime Lab Uses DNA to Solve Property Crimes in San Diego County.
   http://www.youtube.com/watch?v=dXYztbkMXwU

Watch the clip and then have groups discuss and be ready to share with the class as a whole the following questions:
Why must a person usually have a criminal record to be identified with DNA evidence? What sources of DNA might be left at a property crime such as a burglary or theft from a home? Why might drying or freezing the DNA be important? How is the DNA evidence used to identify a criminal and convict them of a crime? Is everyone's DNA different?

Exploration/Explain:

We know we can cut paper, or string with scissors, but can we cut things we cannot see, like molecules in air, atoms, or DNA? DNA carries the genetic information that determines the features that we show. This information is stored in the DNA of every cell and "tells" each cell to do. The DNA in our cells is composed of the building blocks called nucleotides. These nucleotides are in a specific sequence in every one of our cells, and each person has a different sequence in their cells. **If we could cut the DNA at a specific sequence or series of nucleotides, how might that be useful? How might we find a particular gene on the long strand of DNA in someone's cells?**
Enzymes are special proteins that are very specific in what they do. They only work on a specific substance under specific conditions. Some enzymes called restriction enzymes can cut DNA at a specific combination of nitrogen bases. Because each organism's DNA has differences in the sequence of the nitrogen bases (ATGC's) we get different cuts in the DNA using restriction enzymes and different lengths of the pieces that result. You will be working with DNA nitrogen base sequences and a pair of scissors which will act like a restriction enzyme we will call "Gee Cuts" that cuts only where the code on the DNA is GGCC and CCGG (see Diagram #1 below). For each patient's DNA you will start reading through the DNA sequence until you find a GGCC. Cut between the G's and C's to produce smaller pieces of DNA. Be sure to keep each patient's DNA and genetic disease DNA separated as you go so as not to get them mixed up. You will then have DNA fragments of different lengths in piles for each DNA sample. For each of the patient's DNA run them (make a dark line) on the gel sheet (data sheet #1) according to the number of base pairs in each fragment you cut out according to the base pair ladder on the left. So if the section had 19 base pairs (38 individual nucleotides) make a vertical line just below the 20 location on the patient's lane. If there are more than one fragment of DNA with the same number of bases make another line right next to your first one to make the line thicker and darker (in a real gel the band will appear darker). Continue doing this for each fragment for that patient. Patient number 1 has been done for you. Mark the location of all fragments of each of the patient's DNA, then "run" the genetic disease DNA in the last lane.

Discuss and answer the following Questions:
Compare the genetic disease DNA to the patient's DNA. **Which patient's DNA matches the bands of the genetic disease DNA? Write a conclusion explaining who's DNA matched the genetic disease DNA and what helped you make that conclusion. Answer the**

following questions. How would it be beneficial to cut the DNA with more enzymes? Do families have some of the same DNA? Do people have some of the same combination of nucleotides (ATGC) (do we all share some traits)? If there are genes for some diseases, does the presence of a genetic disease gene mean the patient will get the disease? Why would we call this a DNA fingerprinting?

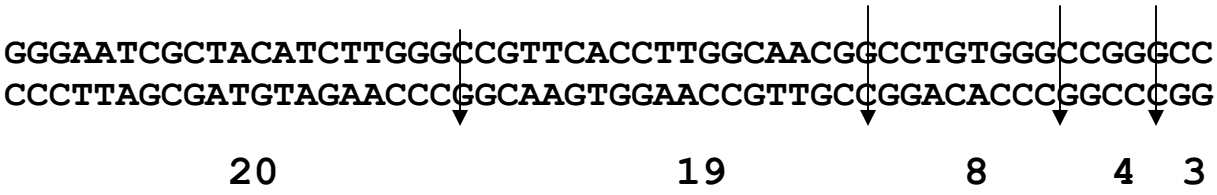
Gel Sheet
Data Sheet #1

	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
Base Pair Ladder	Patient 1	Patient 2	Patient 3	Patient 4	Genetic Disease DNA
25					
20					
15					
10					
5					
1					

Conclusions:

Diagram #1

PATIENT 1



PATIENT 2

GGGAACGTCGGGCCACCGTGGCCGTCGTGGCCATCGTGGCCTGACCACAACCCG
CCCTTGCAGCCCGGTGGCACCGGCAGCACCGGTAGCACCGGACTGGTGTTGGGC

PATIENT 3

GGGTCGTGGCCCTGACCCTTCGGCCGTCGGGCCTGGCCGGCTTCTTTCGAGGCG
CCCAGCACCGGGACTGGGAAGCCGGCAGCCCGGACCGGCCGAAGAAAGCTCCGC

PATIENT 4

GGGATCGGGGTTCCCATCGTCCGGGCCGGCCTTTCGGCCGTCTGAGGCCTTTTT
CCCTAGCCCCAAGGGTAGCCGGCCCGGCCGGAAAGCCGGCAGACTCCGGAAAAA

Genetic Disease DNA

AGGTCGTGGCCCTGACCCTTCGGCCGTCGGGCCTGGCCGGCTTCTTTCGAGGCT
TCCAGCACCGGGACTGGGAAGCCGGCAGCCCGGACCGGCCGAAGAAAGCTCCGA

Elaboration/Evaluation:

Restriction Enzyme Analysis of DNA

We learned that DNA could be cut into smaller pieces with restriction enzymes even though we cannot see DNA or the cutting process. These restriction enzymes cleave or cut DNA in a very specific fashion. They cleave the DNA at the recognition sequence and nowhere else. A common use for restriction enzymes is to generate a "fingerprint" of a particular DNA sample. Because restriction enzymes cut DNA at specific locations, they can be used to generate a "DNA fingerprint" and look for a particular gene.

In a group of 3-5 discuss and answer the following questions: If you know the sequence of a gene, and you know the pattern that a restriction enzyme cuts the DNA, how could you use this technique to "search" for a gene? Are there genes that we would want to look for? What could you do with it if you found a gene?

A restriction enzyme reaction contains the DNA to be analyzed, a restriction enzyme, and a specific restriction enzyme buffer mix. This buffer mix contains substances that help to maintain constant conditions like ph, salinity, and magnesium concentration. Most commercial restriction enzymes have activities at 10-20 units /ul. A "unit" is usually defined as the optimum amount of enzyme needed to digest (cut) 1 ug of DNA in one hour in a 50 ul reaction. You should use 10-20 units (1 ul) of restriction enzyme per reaction with a total volume of 50 ul. The DNA will be at a concentration of .5 ug/ul and the buffer will be at 10x or ten times the concentration you want for your reaction. The enzyme will be diluted with distilled water to get your final desired volume of 50ul. **How much DNA will you have to add to get 1ug? Why do you add the buffer to the solution? How much distilled water will you need to add to make the volume of your solution 50ul total. Work with your group to come up with the answers and then raise your hand to have it checked before proceeding.** Once your amounts have been approved, carefully put the DNA, buffer, distilled water, and restriction enzyme into the microtube using a miropipetter (change tips each time you change solutions and use care not to contaminate stock solutions). Pool the reagents in the bottom of the tube by tapping it on your desk with the lid closed. Your sample is now ready to be incubated.

Once you have your restriction digest set up it will need to incubate at 37^{o}C for 1.5 hours. The sample will then be stored in the refrigerator overnight and run on a gel using electrophoresis the next day.

You will add loading dye to your sample (to allow you to see how things are moving through the gel) and place your sample in a well on the agarose gel. Pool the sample in the bottom of the tube and add 4ul of 6X loading dye to your reaction tube. Be sure that the loading dye mixes with the DNA sample at the bottom of the tube by gently swirling but not shaking it. Load 12 ul of the sample into a well at the end of the agarose gel near the black electrode being careful to prevent spilling out of the well. Once all the samples are loaded the electrophoresis unit will be connected to the power supply and the DNA sample will migrate through the gel for about 30 minutes or until the loading dye has moved approximately 2/3 of the gel. When complete the power is disconnected and the gel is carefully removed from the buffer solution. The gel is very fragile so be careful not to break it. The gel now needs to be stained to visualize the DNA fragments. Place the gel in the staining container and let it sit for 5-7 minutes. Place the gel into a zip lock bag or in plastic wrap and destained overnight in the

refrigerator in a small amount of water. The destaining will allow you to see the DNA on a light box.

Diagram your gel with the sizes of DNA on a blank sheet of paper, and answer the following questions.

How will the different pieces of DNA travel through the gel? Why is it important to have the DNA "run to the red" (negative) electrode on the gel (What charge must the DNA have)? Explain how different sizes of DNA travel through the gel? Using the DNA ladder as a guide, determine the sizes of DNA fragments that you have on your gel. How could you get a specific size of DNA fragment by itself for further testing or experimentation?

Even further:

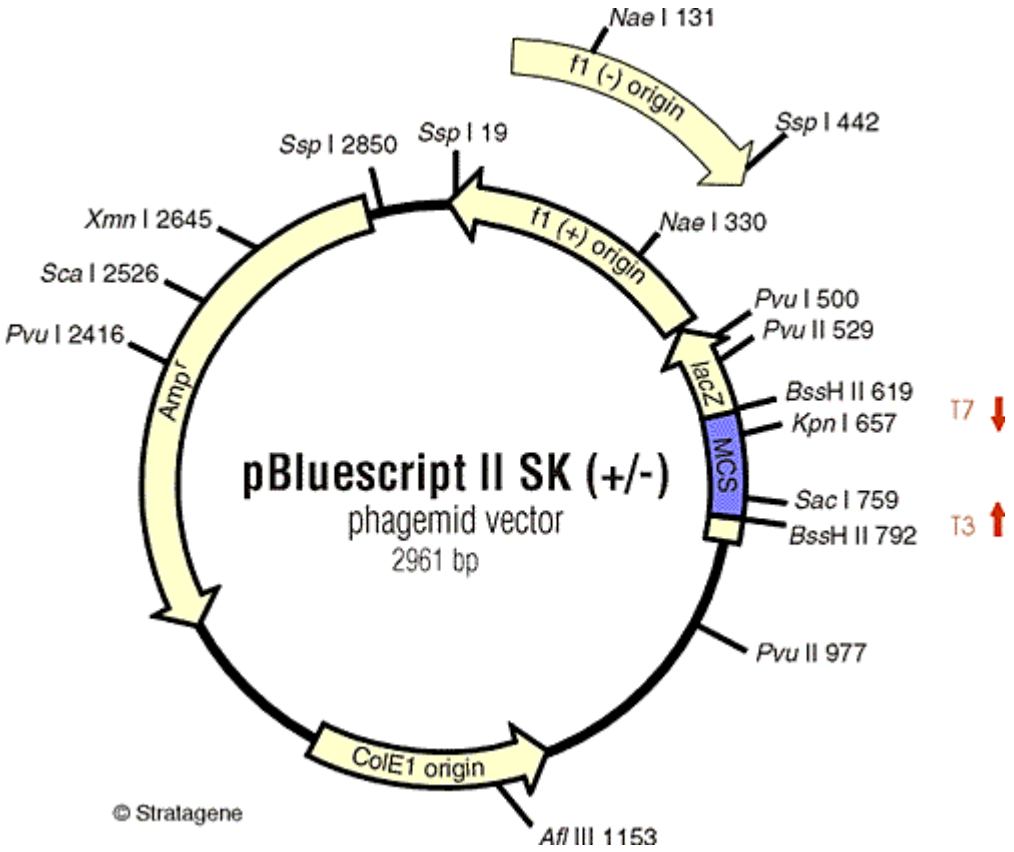
With the Human Genome Project having completed the sequencing of the entire contents of the human chromosomes one can go to the internet and find the genetic sequence for all of the known genes. These genes can then be removed from human DNA and studied or placed into circular bacterial DNA called plasmids. A plasmid is a circular piece of bacterial DNA that has known locations where restriction enzymes cut it. These locations are 3-4 letters and a roman numeral such as SCA I. Some enzymes cut at only one location, while others at more than one location on the bacterial plasmid. It may also have genes that give it resistance to antibiotics (AMPr ampicilin resistance), color changes when genes are inserted (lacZ causes white color unless the gene is disrupted and then the bacteria turn blue, as well as other genes. **Use the attached plasmid map for pBluescript II SK (+/-) to propose an enzyme or enzymes to cut the plasmid in two places to remove or disrupt (cut a part out so the gene no longer functions) the gene that causes the blue coloration (lacZ gene) of the bacteria. How large of a piece of DNA will you cut out? How will you be sure that you have cut out the correct piece of DNA? Will cutting the plasmid DNA ring cause it to move differently through a gel as a long strand than the original ring? How could you separate the DNA that has been cut out from the rest of the plasmid if you did gel electrophoresis?**

Still More:

Suggest a procedure to place a gene from a human into a bacterial plasmid to produce a substance that humans might need for a medical condition. You may develop a procedure to fix a defective gene, remove a section of a gene, or insert a new gene that is missing. Choose a genetic disorder and do some research on its mode of operation and the genetic basis of the disorder or disease. Your group will give a brief description of the disorder and then prescribe a method in detail for correcting the DNA of a person afflicted with the disorder or disease.

The new problem of the CSI effect, real jurors now evaluate what they hear in court with what they have seen or heard on television. View the following video and answer the question:
http://www.youtube.com/watch?v=EQUE8IeVmpA&feature=related

Why is this a problem for Forensic Investigators, and why must courts, judges, and attorneys deal with this new phenomenon?



The letters and numbers around the circular DNA diagram indicate locations where different restriction enzymes cut the plasmid. For instance at location 529 and 977 the enzyme Pvu II cuts the plasmid, and at location 2526 the enzyme Sca I cuts the plasmid. The total size of the vector is 2961 nitrogen base pairs long. MCS is the multiple cloning site where genes can be added into the plasmid vector to disrupt the production of the lacZ blue coloring of the resulting bacteria. Amp^r is a gene for an antibiotic resistance that allows the pBluescript bacteria to survive when ampicillin antibiotic is present. This allows scientists to prevent foreign bacteria from growing.