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Assay Fundamentals: Gel Electrophoresis Lab

Lesson plan for grades 9-12
Length of lesson: At least 2 full class days (minimum of 55 min class periods)
Adapted by: Mohammad Kamyab Javanmardi, Environmental Science Institute, 6/22/13
Authored by: nespal.cpes.peachnet.edu

SOURCES AND RESOURCES:

- Experiment Outline
  *nespal.cpes.peachnet.edu/edout/DNA**Electrophoresis**.pdf*
- How Pregnancy Tests Work
  http://chemistry.about.com/od/chemistryfaqs/f/pregnancytest.htm
- Sample Gel Electrophoresis Experiment
  http://depts.noctrl.edu/biology/resource/handbook/gel.pdf
- Virtual Gel Lab Guide
  http://learn.genetics.utah.edu/content/labs/gel/

POTENTIAL CONCEPTS TEKS ADDRESSED THROUGH THIS LESSON:

§112.34. Biology, Grade 9-12: 1A, 1G
§112.34. Biology, Grade 9-12: 6B, 6H
§112.34. Biology, Grade 9-12: 9C, 9A
§112.38. Integrated Physics and Chemistry, Grade 9-12: 5C

PERFORMANCE OBJECTIVES:

Students will be able to:

- Explain basics of how biochemical assays work and why they are useful
- Load the wells of a Gel Electrophoresis
- Identify the various components of a Gel Electrophoresis

NOTE TO TEACHERS: Due to the complexity of this lab, the EXPLAIN and ELABORATE sections are written with emphasis for the teacher to lead the discussions for the students. The worksheet accompanying this Lab is intended to supply the teacher an assessment basis for determining that students are absorbing concepts pertaining to biochemical assays.

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MATERIALS (per group of two or four):

Kit Provides:	Teacher Provides:
2.5 g Agarose powder	
100 ml 10X TBE buffer	Distilled water
Small-range micropipettors	Microwave/oven
Small-range micropipette tips	Flask, for preparing agarose
Oven mitts	Ziploc bags (sandwich size)
7 Microcentrifuge tubes of 1x loading dye	Beakers
7 Microcentrifuge tube racks	
4 Electrophoresis units (including casting tray, 2 combs, and 2 running trays)	
DNA samples (if running 14 or 4 or fewer gels)	DNA samples (if running >14 gels - see ordering information below)
FastBlast stain (1 ml concentrate)	

Gel Electrophoresis Lab Worksheet (1 per student) (PROVIDED AT THE END OF THIS FILE)
Assay Fundamentals Powerpoint Presentation (PROVIDED AT THE END OF THIS FILE)

CONCEPTS:

Nucleotides: a single molecule consisting of one of the nitrogenous bases (A,T,C,G) linked to a phosphate group.

Codons: a group of 3 nucleotides. Codons are responsible for the synthesis of particular proteins

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Genetic Assay: An assay that is used for the purposes of genetic studies. This includes assaying for DNA fragment length, reactivity, sequencing, etc.

Gel Electrophoresis: A particular genetic assay that utilizes electrical current to arrange DNA fragments based on length

Base Pairs: A pair of matching nucleotides (A matches with T while C matches with G...)

BACKGROUND:

The state of the human body is a constantly changing. Humans not only grow and mature physically but also chemically. At one period in a child's life, their body might be releasing a certain set of chemical signals and hormones. It is possible, that years later, an entirely new variety of hormones might be prevalent throughout an adult’s system causing various changes. One important question is: Can we measure and quantify these changes on a regular basis?

Here’s why the previous question is important: Just as a feverish person has their temperature taken regularly, a relatively healthy individual could possibly be examined on a daily basis. Such actions would serve as a form of preventative care; a situation where a bacteria, genetic mutation (such as those involved in cancer), or a virus can be detected before indications of illness are present. Imagine having a machine that could scan you every morning and inform you of any health related issues and how to resolve them. With such technologies, self-diagnosis would become universal in its application. Importantly, these ideas sprung from the development of early assays.

Assays are tests intended for the purpose of detecting a target biochemical molecule via the use of enzymes and dyes. These experiments have been used throughout medical science. Pregnancy tests are perhaps the most common examples of assays that are readily available for public use. Using some kind of a textured/carved surface, testing samples are placed in small wells. Once the samples are in the wells, a reaction occurs in which the sample travels through various pores or canals to indicate reactivity with particular antibodies or enzymes.

One of the more brilliant assays is the gel electrophoresis that is used for genetics research as well as crime-scene investigations. An agarose gel medium and electrical current are used to separate DNA samples based on their base pair length. By having various samples adjacent to each other, one can visually compare the gel runs in each lane to see if there is a match. Charts can even be devised to determine the approximate base pair length of the different strands located in each band based on the distance they displace from the well (see slide 30 of Dr. Ellington’s *Hot Science - Cool Talks* PowerPoint presentation; linked in Sources and Resources).

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Assays are becoming ever more abundant, affordable, and are capable of examining more things in support of medical people's care.

PREPARATION:

- The 10x TBE buffer has to be diluted to a 1x. A 1000 ml sample of 1x buffer should be sufficient for the experimental needs of a 7-group class. The dilution can be done by adding 100 ml of the 10X buffer to 900 ml of distilled water. This sample will be used to dissolve the agarose and to fill the electrophoresis boxes.

- Agarose gel: Make a 1% solution with the 1x TBE running buffer (EX: 1g/100ml 1x TBE running buffer)- *don't use water because the DNA will not separate properly*. 50Ml of solution will prepare 2 small gels, so 250 ml of solution (2.5 agarose in 250 ml 1x TBE buffer) should be more sufficient for the 7 groups. Dissolve the agarose by heating it in the microwave for a minute or two on medium power (remove and swirl the solution after each minute of heating). The container in which the agarose is heated should be twice the volume of the agarose sample (if heating 300 ml then use a 600 ml container). Once the agarose has fully melted into solution, you will not be able to see any "floaties" (or chunks) in the buffer. Remember, the agarose must stay warm before pouring into the tray to prevent it from solidifying early (a water bath set to 60°C can accomplish this).

Additional Suggestions:

- The TBE buffer within each electrophoresis box can be reused for other class periods.
- To save time, the teacher might want to pour the gels for the students beforehand.
- Manage the class time based off of the fact that an ideal gel run takes 45 minutes. The instructor will then have to time the gels and remove them for staining.
- Staining: Each group of students should label their Ziploc bag. Have the stain diluted to a 1x concentration by taking 1 ml of stain and diluting it with 99 ml of water. Place the gel in a Ziploc bag with sufficient stain to cover the gel and leave it till the next day. Then, dump out the stain and analyze the gel without removing it from the bag.
- Clean and rinse the supplies.

ENGAGE: (NOTE: Slide numbers reference the Assay Fundamentals PowerPoint Presentation in Sources and Resources section)

Teacher: DNA, our genetic code, acts as the blueprint for every feature that we have. These billions of **nucleotides** and hundreds of thousands of **codons** determine not only our physical characteristics, but also our biochemical composition (slide 2). Geneticists developed hundreds of different techniques and methods to better understand how these simple nucleotide combinations could translate and create something as complicated and intricate as a human being. Perhaps one of the most useful techniques that they used was genetic assays.

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<u>Teacher Asks</u>: Before we continue to talk about **genetic assays**, can anyone explain what an assay is?

<u>Teacher</u>: Biochemical assays can be best described as investigative laboratory procedures that are intended to detect or quantify something about a specific target molecule. These tests can be used to determine the size of the molecules, the charge of the molecules, the reactivity of the molecules, or even the presence of the desired substance in a sample (slide 3). Considering that molecules and chemicals can only be understood on the microscopic scale, assays allow scientists to visually interpret desired information about the target molecule.

To better understand what assays are and how they work, let's use a pregnancy test as our example (slide 4). Pregnancy tests are inexpensive and readily available nearly everywhere we go. Amazingly, these simple assays accomplish what once took weeks and lots of money to do. Before the development of the first pregnancy test, women would have to wait until visible signs of pregnancy or would be told by their physicians. Home pregnancy tests allow women to determine if they are pregnant after 3 weeks. This assay, like many others, uses a very technically manufactured slide with wells in it (slide 5). These wells contain antibodies and chemical indicators that react with a particular hormone called human chorionic gonadotropin (hCG). This hormone is emitted by the placenta after fertilization (initiation of pregnancy). Thus, the use of a urine or blood sample could be used to react in the wells to indicate pregnancy (slide 6).

Let us now look at a specific form of assay.**<u>EXPLORE</u>:**

<u>Teacher</u>: When studying genetics, one will come to realize that manipulating and physically studying DNA or RNA is no simple task. There have been many different techniques developed in order to not only sequence the **base pairs**, but also to locate and measure target portions of the strand (slide 7). For this activity, we will be exploring and experimenting with a type of assay called a **gel electrophoresis**.

* Teacher may want to play the "Virtual Gel Lab Guide" (see resource section) or have students watch it on computers to help make the experimental procedures clear.

<u>Teacher Does</u>: Hand out the "gel electrophoresis lab" worksheet to each student and have lab materials ready on the students' desks (*see Preparation for specific instructions*).

<u>Teacher Says</u>: "In this lab, each of you has a sample of "mystery" DNA/dye that you need to identify. You will also have 3 "reference" DNA/dye that you will use to identify the mystery sample."

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In order to successfully complete this lab, each group must follow the directions exactly and not hesitate to ask for help when it is needed. Make sure that you all read each step in the procedure completely before following through with what the step asks.

Experimental Procedure for Students:

1) Insert the gel comb into the notches directly above the black band that is seen on the casting tray.
2) Carefully pour the warm agarose fluid into the casting tray until it is halfway up the teeth of the inserted gel comb. Do not move the tasting tray after pouring the agarose! You will notice that the gel becomes cloudy as it solidifies.
3) When the gel has solidified, remove the comb by pulling it straight up. In order to not rip the wells in the gel, have your partner hold down the tray as you pull the comb out.
4) Gently remove the tray from the casting bed. Wipe away any remaining agarose on the bottom of a tray with a paper towel.
5) Place the tray between the pegs in the bottom of the gel box. Make sure that the negative end is located where the comb had been inserted (this will allow for the samples to drift towards the other side of the gel).
6) Now, fill the gel box with 1x TBE until the whole surface of the gel is covered. Try to pour it at either end of the gel box rather than directly over the gel.

Practicing How to Load the Wells:

- Set a small micro-pipette to 10 µl (microliters)
- Draw 10 µl of the 1x loading dye with the pipette while making sure that there are no air bubbles in the tip.
- Position the pipette over the center of the well (an outside well) using two hands. (use only the outside wells for practice; the inside wells are for the actual experiment)
- On a slight angle, pierce the tip through the surface of the buffer and gently release the extracted dye into the well. The dye must be released below the surface but also inside the well (avoid going through the bottom of the well).

Experimental Procedure for Students (continued):

7) When looking at the gel, lanes 1,2,7, and 8 may be used for the purposes of practice. Load the 10 µl of DNA/ loading dye mixture into the assigned well. Label the gel diagram as such:

a) Lane 3: mystery DNA
b) Lane 4: reference DNA 1
c) Lane 5: reference DNA 2
d) Lane 6: reference DNA 3

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8) Once each sample has been loaded, attach the cover onto the electrophoresis box and connect the electrodes correctly. DO NOT move the electrophoresis box once the samples have been loaded.
9) Turn the voltage level knob to the setting of 100 volts and start the gel experiment. Let the gel run for 45 minutes; However, the gel can be run for 30 minutes for reasonable results.
10) Observe the dye move down the gel. The dye's timely movement will indicate that the gel is working properly. It is best that the gel be stopped before the dye runs off the end.
11) Once the gel has successfully run through, unplug the power and remove the electrodes from the electrophoresis box. Now, remove the top and let your instructor/teacher know that your gel is ready to be stained.

Teacher should see the PREPARATION section for staining instructions

EXPLAIN :

Teacher: Before we go into the details of how the gel electrophoresis works, let's first describe the various components of the assay.

NOTE: The assay uses an agarose gel medium rather than some of the other viscous fluids that common assays use.

Teacher Asks: "Can anyone think what is so special about the agarose that we use it in the electrophoresis? Think about what is traveling through it."

Positive student responses to look for:

- The agarose has the perfect gel like consistency for the experiment
- Agarose works well because molecules like DNA can travel through them

Teacher Explains: Those responses are accurate! The agarose gel, when in its gelatin form, contains an intricate system of pores which allow for molecules to pass through (slide 8). The gel electrophoresis allows for DNA strands of various lengths to be sorted accordingly. While longer strands of DNA slow down and stall closer to the well on the gel, the smaller strands are capable of moving more freely through the small pores of the agarose. For this reason, the color dyed bands on the gel can be used to measure the various lengths of DNA strands present in the DNA sample. Another important characteristic of agarose gel, is how it reacts with electrical currents.

Think about why an electrical current was used to run the gel. It is vital to understand the structure of the DNA molecule. DNA is a double helix (twisted ladder) that has lots of phosphates. These phosphates are very negatively charged which cause the whole molecule to

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be negatively charged. When you all inserted the negative probe into the side of the wells and the positive on the opposite end, you were forcing the negative molecules away from the initial point and towards the final point using charge (slide 9).

The wells also are important in the experiment. Each well is made so that lanes are created for each sample to run through. These lanes can then be compared side to side in order to match the "mystery" DNA with one of the reference DNAs.

Even though the gel run is over, you all still need to analyze your data and come to some kind of conclusion about the identities of the mystery DNA samples.

ELABORATE:

When trying to read the electrophoresis gel, there are several key things to look for. The dye is chemically engineered to adhere to the tested molecule (in this case DNA) which helps scientists visually see the similarities and differences between the DNA samples. The way the samples are prepared is important to the experiment. The DNA samples are all put in their individual containers and then treated with a specific digestive enzyme. This enzyme molecule then cuts the DNA samples into parts whenever a particular sequence of nucleotides is detected (slide 10), thus creating the various sized pieces. When the gel run with the samples is complete, one might notice that the dyed bands are very similar in two different lanes. This could mean that the original DNA samples are a match. Since the same enzyme was used for all samples, and

since an organisms DNA is the same throughout their whole body, the enzyme should cut the DNA into identically sized pieces in each matching sample (slide 11).

Teacher: Now it's your turn. Get your dyed gel platelets and look at where the bands are located. Try to copy down the gel on some graph paper by making an x axis (lanes) and y axis (band locations) and plotting the color bands.

Question for students to answer:
Is there a visible match between the "mystery" DNA and one of the reference DNAs? If so, record your thoughts and observations on your lab worksheet as well as answer the questions on the worksheet.

EVALUATE:

Teacher Says: Assays are becoming more prevalent and more useful as new experimental techniques and assay designs are being developed. Two examples of assays have thus far been

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explained that have revolutionized science and the speed at which experimental results can be achieved. The pregnancy test was discussed because of its universal presence and commonness. Next, everyone conducted a gel electrophoresis experiment in order match a “mystery” DNA sample with a reference DNA sample.

Now, it is time to use your knowledge to think a little outside the box. Complete the last portion of your worksheet.

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Name:___________________________________ Date: __________
Name:___________________________________ Date: __________

GEL ELECTROPHORESIS LAB WORKSHEET

In order to successfully complete this lab, each group must follow the directions exactly and not hesitate to ask for help when it is needed. Make sure that you all read each step in the procedure completely before following through with what the step asks. Students are to use the boxes to briefly illustrate the steps of the experiment as they go.

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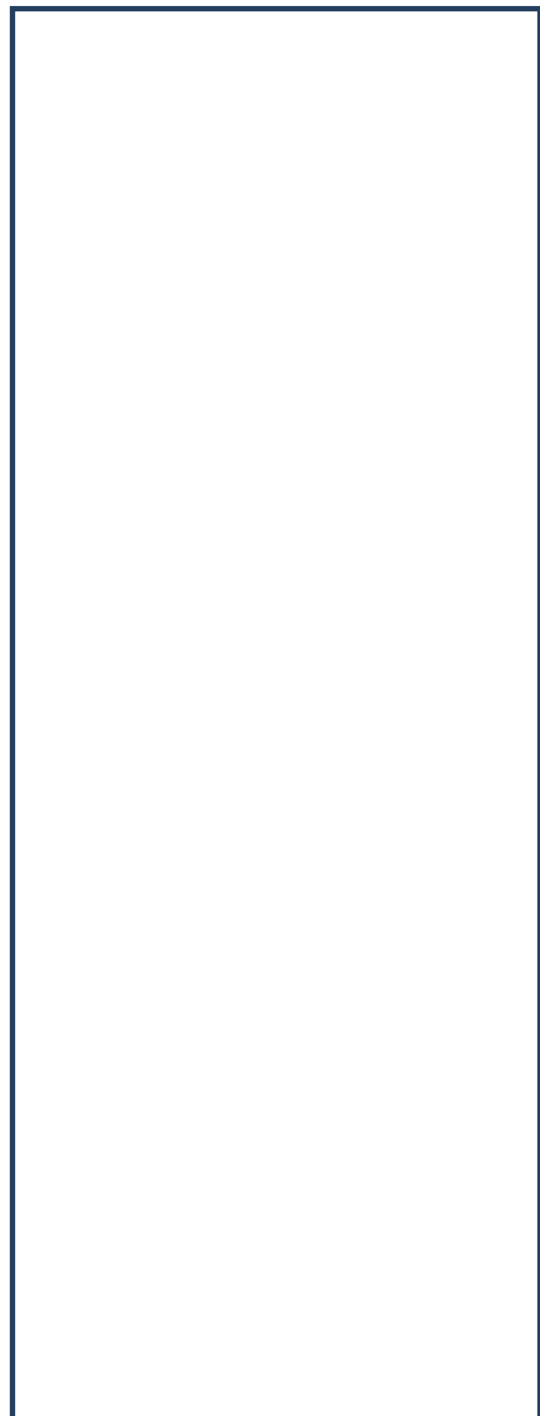

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- Position the pipette over the center of the well (an outside well) using two hands. (use only the outside wells for practice; the inside wells are for the actual experiment)
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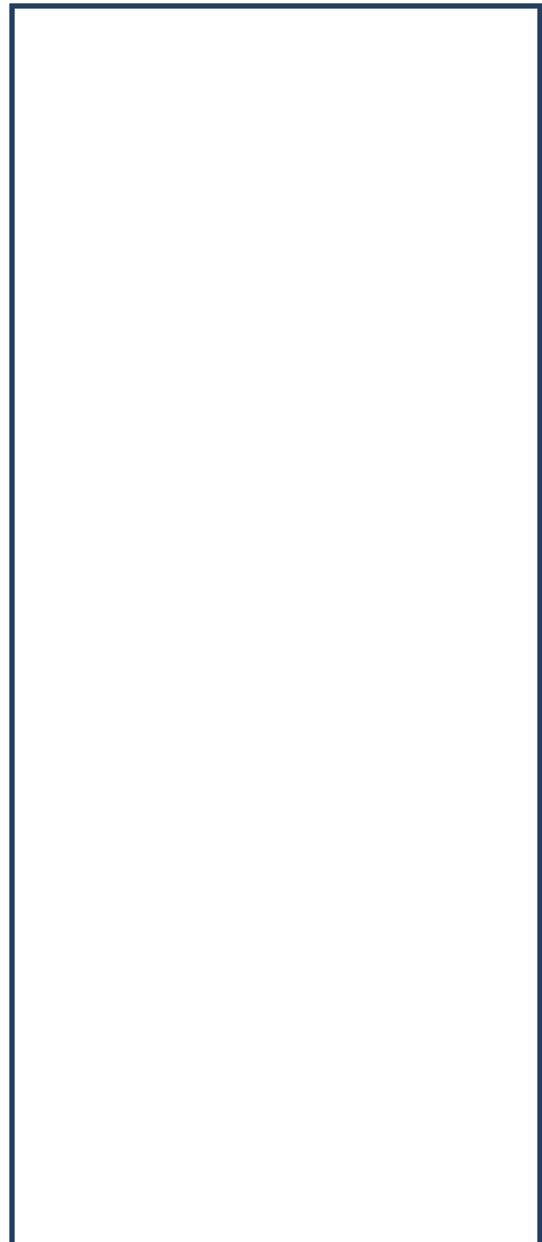

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Part 2

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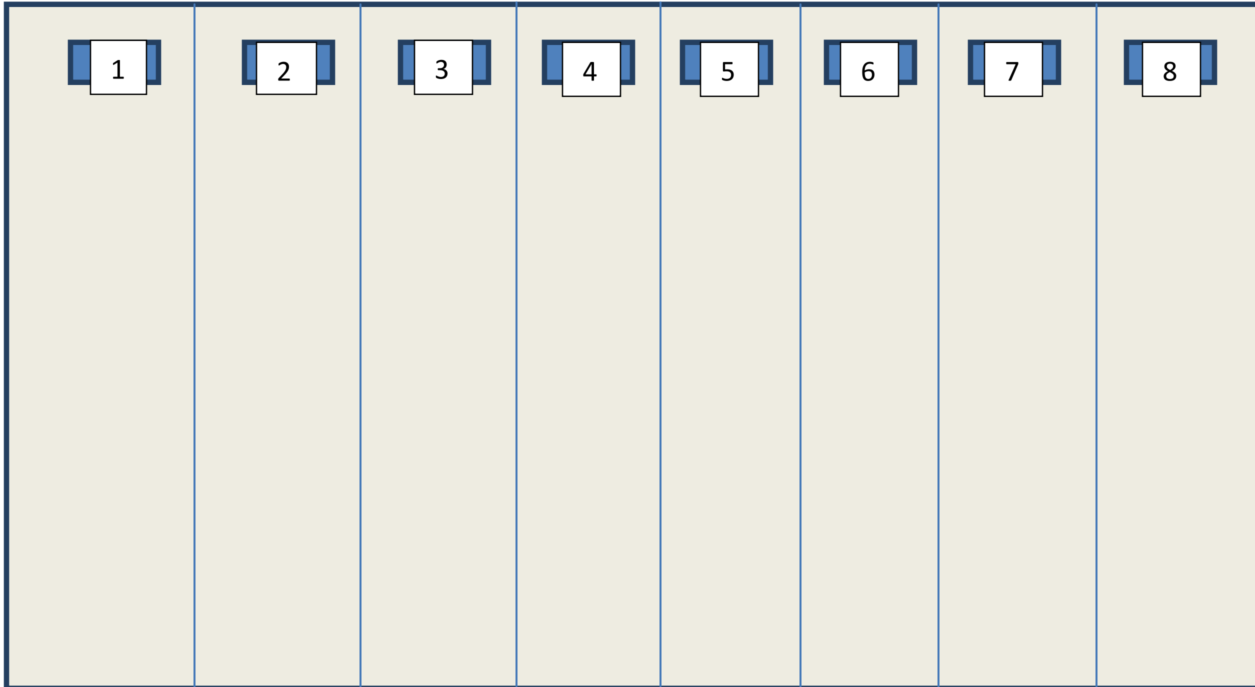
First, each student is to briefly sketch how they gel looks like after the staining has been completed. Make sure to label the lanes by their appropriate number.



On your diagram above, number the bands in each lane in order of decreasing darkness/thickness (1 being the darkest/thickest). Do this for each lane that contains data relevant to the experiment.

By simply looking at the diagram above and your stained agarose gel, which two lanes are the most comparable? What are details and information are you using to compare the different lanes?

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Based on your results, can you confidently say that you found a match with one of your reference DNA's? Why or why not? Is there something that may have occurred during the experiment on your behalf that could have caused this outcome to appear in the gel?

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It is fairly logical that such techniques can be used for crime scene investigation as well as DNA sequencing. You could assume that the mystery DNA was found at a crime scene while the reference DNA's are samples that you have from various suspects or from some sort of DNA data bank for the individuals of a particular location.

Now it's time to think a little outside of the box. The DNA samples were all cut using the same restriction enzyme. Suppose that a complete DNA sample is obtained from all suspects and from the crime scene. Would it be possible to run a proper gel experiment using a different restriction enzyme for each DNA sample? If not, what would be wrong with this type of experiment?

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