

Determining if the Photosystem II Gene in Plants Can be used as a DNA Fingerprint for Crime Analysis

By: Samantha Sinwald and Janelle Silvis

Introduction:

The Photosystem II (PSII) Gene is located in the thylakoid membrane of chloroplasts and is part of the mechanisms involved in harvesting light energy during photosynthesis. Photosynthesis is the process of converting light energy into chemical energy for plants. Photosystem II is the location where water is split into oxygen, protons and electrons, and is crucial for organisms that breathe air. Without the PSII gene these organisms would not live. It is important that the gene is located in the chloroplasts because there is a lot more chloroplast Deoxyribose nucleic acid (DNA) in a cell than genomic DNA

All living things contain unique sequences of DNA, which can be used to differentiate between organisms through genetic fingerprinting. The whole idea of a genetic fingerprint is that an individual can be identified by their DNA. Modern applications of DNA fingerprinting include paternity testing, and matching DNA left at crime scenes (blood, skin cells, hair, etc.) to suspects.

However, plants are not commonly used in crime scene investigations. Spores and specialized parts of plants (such as thick cell walls) can be found on a suspect or victim. This can link them back to the scene of the crime. (Graham, January 28, 2007.)

Microsatellites are repetitive DNA sequences that can also be used to identify plants. Each microsatellite has a unique and specific number of DNA sequence repeats and can be used to identify an unknown plant. Genetic fingerprints are created by these DNA sequence repeats. (Brotten, March 19, 2007.)

In addition, Amplified Fragment Length Polymorphism (AFLP) testing is used for genetically identifying plants. In AFLP testing DNA is amplified by PCR and the PCR products are run on an agarose gel. For example, currently plant DNA is being used to identify poisonous plants because of the increasing number of livestock fatalities. Plant material is extracted within 24 hours of death from livestock, and specific DNA sequences are amplified to create a plant fingerprint. (Moore, March 19, 2007.)

DNA fingerprints can be generated in many ways, but one of the most common ways is through Polymerase Chain Reaction (PCR), and Restriction Fragment Length Polymorphism (RFLP) testing (Riley,

April 6, 2005). These two DNA tests consist of several steps including DNA extraction, PCR (amplification of a specific gene sequence) , restriction digest, and gel electrophoresis. Once DNA is extracted, a gene can be amplified through PCR, produced in mass quantities, and cut with restriction enzymes. When the DNA is then run on a gel the cut patterns are observable and differences can be recorded (Campbell, 2005).

Photosystem II gene can be used to differentiate between plants. If there are differences in the plant's Photosystem II Gene then, when the plant's PSII genes are amplified and cut with specific restriction enzymes they will have different size fragments.

In this experiment DNA was extracted from various plants, and the PSII gene was amplified and cut with various restriction enzymes. A gel was also run to determine if plants' PSII genes have different banding patterns that are distinguishable from each other.

Procedures:

The online tool NCBI was used to find a generic corn PSII Gene sequence. NCBI works by searching a nucleotide database for a gene sequence. Primer sequences were obtained from Bio-Rad labs. Three different gene sequences were found that claimed to have the PSII gene in their given sequence. With the assistance of Dr. Wiseman, it was determined that gene gi>23452827, had the PSII gene in it. With this information the sequences were aligned using Clustal W. Clustal W is an online tool used to align DNA sequences. The sequences were compared to see if there was a matching area, roughly 475 base pairs long, where the PSII gene would be located. After several trials, the primer sequences used in a Bio-Rad Labs GMO kit made for amplifying the PSII gene were obtained. The primers and the gi>23452827 gene were aligned using Clustal W. When no results were found the complements of the primers were also aligned with the gene sequence. However, the PSII gene could not be located, so a new online tool, Primer Show was used to align the primers and the sequence gi>23452827. The online tool Primer Show was used to show where the forward and reverse primers bind. The gene sequence obtained from NCBI was pasted into the top window on Primer Show and the primers were pasted into the bottom window. When that data is submitted then Primer Show matches up the primers and the gene sequence where they are complimentary. The online tool NEB Cutter was then used to find the predicted size fragments for each enzyme used. NEB

Cutter works by pasting the gene sequence from NCBI into the window and selecting custom digest.

Custom digests show which enzymes cut once, twice, and three times.

With the PSII gene sequence found, it was then necessary to figure out which enzymes would be appropriate for an RFLP test. Using NEB cutter, the PSII gene sequence was entered into the system, which then displayed all the single, double, and triple cutting enzymes appropriate for the sequence. Enzymes AluI, RsaI, BccI, Hpy188I, Hpy188III, and AciI were chosen to be used.

Leaf samples of: *Citrus Sinensis* (Washington Navel Orange), *Agropyron cristatum* (Wheat Grass), *Apium graveolens* (Celery), *Zea Mays* (Corn), and *Citrus limonium* (Lemon) were then obtained for DNA extraction. They were stored in individual Ziploc bags with ice in the refrigerator and used the next day. Mortars and pistols were obtained and cleaned thoroughly with bleach and soap. While wearing gloves, each plant was rinsed with distilled water and patted dry. Then 1g of each plant was weighed out using a balance and placed into separate mortars. With a sterile disposable pipette, 5 ml of distilled water was added to each mortar and the plants were ground for 2 minutes each, being careful not to cross contaminate the plants. Another 5ml of distilled water was added. With a new sterile disposable pipette (a new one for each plant), transferred 0.5 ml of the liquid from the ground plant was transferred into individually labeled 1.5 ml screw cap tubes. Each tube was color coded to a corresponding plant. The DNA samples were placed into a water bath at 95 degrees Celsius for 5 minutes. The samples were then centrifuged for five minutes at 6000 rpm. Another method of DNA extraction, called Aqua Pure DNA Extraction, was also used. In doing this protocol the same plants were used. 20mg of each plant was weighed out. 1.5 ml tubes were obtained for each plant and 300ul of Genomic DNA Lysis Solution was pipetted into each. The weighed out plant tissues were then placed into the prepared tubes. In the tubes, each plant was thoroughly homogenized using a microfuge pestle, until there were no chunks left. The lysate was then incubated for 30 minutes at 65 degrees Celsius and the tubes were periodically inverted during the incubation. After 1.5 ul of RNase A Solution (4mg/ml) was added to the cell lysate. The samples were mixed by inverting 25 times and incubated again for 30 minutes at 65 degrees Celsius. 100 ul of Protein Precipitate Solution was added to the cell lysate. Then samples were vortexed for 20 seconds and then incubated on ice for 5 minutes. Samples were centrifuged at 13.2 rpm for 3 minutes. 200ul of the supernatant was then pipetted into new 1.5 ml screw-cap tubes containing 300 ul of 95% isopropanol. The

samples were then inverted gently 50 times and again centrifuged at 13.2 rpm for 1 minute. The supernatant was poured out and drained on paper towels to isolate the pellet. Then 300 ul of 70% ethanol was pipetted into each tube and the tubes were inverted. At 13.2 rpm the tubes were centrifuged for 1 minute and the ethanol was carefully poured off. 300 ul of 95% ethanol was added to each tube, they were all inverted, and centrifuged again at 13.2 rpm for 1 minute. Using a pipette, the ethanol was then carefully removed. The tubes were then left to drain on paper towels and air-dried for 20 minutes. When completely dried, 100 ul of Hydration Solution was added to each tube and they were incubated for 10 minutes at 65 degrees Celsius. Tubes were then vortexed for 5 seconds each at medium speed and samples were stored in the freezer.

Before PCR, the primers and the master mix had to be combined. The primers and master mix need to be at a 2:100 ratio. 20 ul of master mix was needed for each tube thus; 20 times the amount of tubes equals the amount of master mix needed. A proportion was set up to calculate the amount of primers needed. After the master mix and primers was combined and vortexed, 200 ul thin wall PCR tubes were obtained and color coded for each plant. 20 ul of the master mix was pipetted into each tube and 20 ul of extracted plant DNA was pipetted into their appropriate tubes. All tubes were vortexed and centrifuged to make sure that all the liquid was mixed uniformly at the bottom of the tube. Tubes were then placed into the Thermocycler and set to GMO cycle. After they were finished in the Thermocycler they were removed and placed in the freezer.

A restriction digest of the plant DNA samples was then performed with the various enzymes found. Calculations were first done to figure out how much DNA, enzyme, buffer, and water was needed for the restriction digest. The total depends on the amount of buffer. Because the buffer is 10x, if 1.5 ul of buffer is added then the total must be 15 ($10 \times 1.5 = 15$). New 1.5 ml tubes were obtained and color coded for a specific plant. In each tube 2.5 ul of sterile water was pipetted first. The 1.5 ul of 10x buffer was pipetted into each tube. Thirdly, 10 ul of the plant DNA was added to their appropriate tubes. Lastly, 1 ul of enzyme was pipetted in each tube. The tubes were then vortexed and centrifuged, then incubated at 37 degrees Celsius in a water bath for an hour. This process was repeated for all the other enzymes.

Before viewing the results of the restriction digest by electrophoresis a 3% agarose gel was first made. Calculations for how much agarose powder needed were done. Then 1.5 grams of agarose powder

was weighed out with an electric balance and poured into a clean Erlenmeyer flask. Then 50 ml of 1XTAE buffer was added into the flask and mixed. The contents were then nuked in the microwave until it became translucent. The gel was stained by pipetting 22.7 ul of ethidium bromide into the flask and swirled. (When using ready made gels, the gel was soaked in ethidium bromide solution after electrophoresis). The flask was then cooled in a bucket of cold water. A gel tray was obtained and taped tightly and a gel comb was set a penny's width away from the bottom of the gel tray and about a centimeter away from the top. The gel was then poured and left out to solidify.

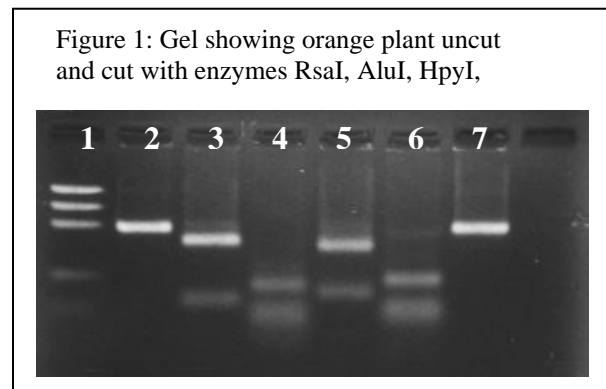
Gel electrophoresis was conducted to view the results of the restriction digest. First, 3ul of 6x Orange G loading dye was added to the DNA samples and mixed thoroughly. The gel box was set up and filled with 1x TAE buffer. After the tape was taken off the gel, the gel was placed in the gel box. The wells of the gel were placed closest to the negative end, because DNA is negatively charged. DNA samples were then loaded along with controls such as Mass Ruler and the positive control from the GMO kit. The gel was then run at 75 voltz for 45 minutes or 100 voltz for 30 minutes. After the gel was run, a picture of the gel was taken with a transilluminator and results were observed and recorded.

Results:

Orange was the first plant DNA that successfully extracted, amplified, and cut. Figure 1 shows that the Photosystem II Gene can be amplified and cut with the enzymes RsaI, AluI, HpyI, and HpyIII.

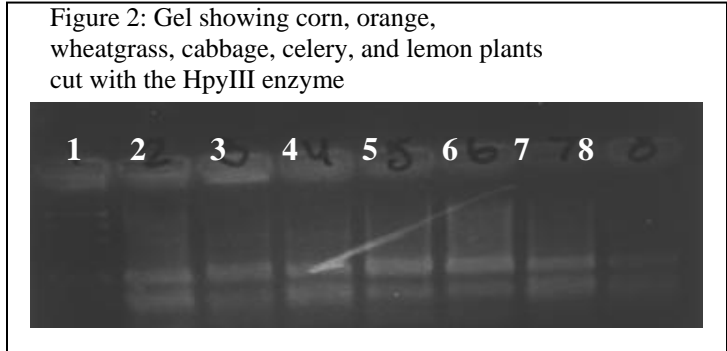
To compare the predicted size fragments to the fragments displayed on figure 1, the sizes of the fragments were determined by using the mass ruler. The standard has known base pair lengths, and by measuring the bands from the wells a standard curve was created. This standard curve can then be used to find the estimated base pair lengths of the other bands. However, the mass ruler standard that was used did not span the distance that the fragments migrated.

In Figure 1, lane 2 is the orange PSII Gene amplified and uncut. This shows that it is possible to amplify the PSII Gene in orange plants. Lanes 3-7 are the orange PS II Gene cut with different enzymes. All enzymes cut differently because the bands are all different



fragment sizes. However, in lane 7 the BccI enzyme did not cut because it is the same as the uncut PSII Gene in lane 2. In addition, some of the predicted bands did not show up because the really small bands ran off the end of the gel.

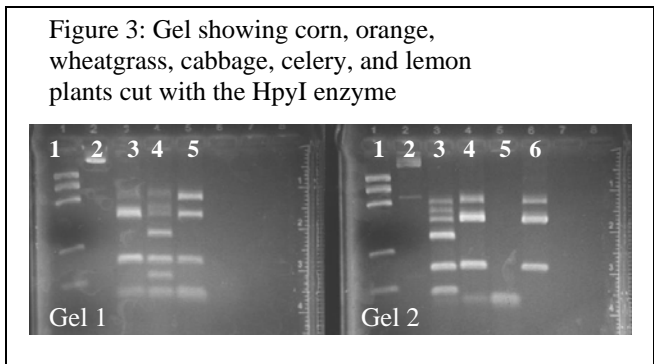
Figure 2 showed that the HpyIII enzyme cuts the PSII gene in locations where various plant sequences are identical. The Mass Ruler can not be seen in Figure 2, but regardless the fragment sizes of the



plants in lanes 3-8 are all the same. The standard used was also the wrong size standard because it didn't span the distance of the cut fragments. The positive control that was used was from the Bio-Rad GMO kit. It was the same generic PSII gene sequence used on NEB Cutter. In addition, the positive control (Figure 2, lane 2) is the same as the plants and therefore shows that the other plants cut as expected.

Figure 3 shows that the plants orange, corn, cabbage, lemon, celery, and wheatgrass cut differently with the HpyI enzyme. The first lane for both gels in Figure 3 is the Mass Ruler which was used to determine the fragment sizes of each plant. The second lane in both gels in Figure 3 is the Lambda HindIII DNA standard which was used to span the distance of the larger size fragments, and determine the

estimated band size. Wheatgrass (lane 5 gel 2) was significantly different from the other plants. It cut really small fragments at less than 100bp. This may be due to the DNA being degraded by random proteases and enzymes. The corn in gel 1 lane 4 cut



differently than the positive control (gel 2 lane 6) which is also a type of corn. This may be because of the fact that they were different types of corn.

Conclusion:

The question explored in this investigation was could differences in the PSII gene be used to create a genetic fingerprint for plants?

The PSII gene sequence used in NEB Cutter was a generic corn PSII gene sequence. The positive control matched that generic corn NCBI gene sequence. Since the genes in figure 3 showed difference when cut, it can be inferred that the sequences are different, even between various species of corn.

The fact that plants can be differentiated by their PSII gene is significant because it can be crucial for crime scene analysis. With the PSII gene technique, only small amounts of plant DNA are needed. In addition, since the gene is located in chloroplasts, there is a lot more DNA available for amplification, which is not the case for genomic DNA. Once a library of plant's different band patterns is created, then unknown plant DNA can be compared to it and the plant can be identified. This DNA can place a suspect at the scene of a crime, or even link where the crime was committed if a body was moved.

In the future we plan to continue to test the same plants as well as different plants and compile a valid reference of plant PSII gene cut patterns. These could then be used to find an unknown in crime scene analysis. We also plan to find other enzymes that cut the plants differently.

Bibliography:

Broten, Krista. "Microsatellites". <<http://www.agwest.sk.ca>>. April 2000. March 19, 2007.

Campbell, Neil. Reece, Jane. Biology: 7th Edition. Pearson Education Inc. 2005.

Moore, Lucy. "Crime scene investigates: The case of the dead cow". <<http://www.eurekaalert.org/>>. April 6, 2006. March 19, 2007.

National Center for Biotechnology Information. <<http://www.ncbi.nlm.nih.gov/>>. March 6, 2007. April 2006.

New England Biolabs. <<http://tools.neb.com/NEBcutter2/index.php>>. May 2006.

Nixon, Peter. "Cyanobacterial and chloroplast molecular biology".

<<http://www.bio.ic.ac.uk/research/pnixon/>>. January 10, 2007.

Primer Show. <http://bioinformatics.org/sms/primer_show.html>. May 2006.

Riley, Donald. "DNA Testing: An Introduction For Non-Scientists An Illustrated Explanation".

<<http://www.scientific.org/tutorials/articles/riley/riley.html>>. April 6, 2005. January 10, 2007.

Graham, Shirley. "Interesting Jogs-Crime Scene Botanicals- Forensic Botany". The Botanical Society of

America. <www.botany.org/PlantTalkingPoints/crime.php> January 28, 2007.

Acknowledgements

Bio-Rad Laboratories. Aqua Pure Kit. GMO Investigator Kit. May 2006-Present day.

Brown, J. Kirk. Discussion about idea, and preparation for writing paper. May 2006-Present day.

New England Biolabs. Donation of enzymes used. May 2006-Present Day.

Wiseman, Bryony. Information on PSII gene and PSII gene primers, and assistance analyzing results. May 2006-Present day.