One Step at a Time:
My Experiences as a 2003 World Food Prize Intern in Peru

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Acknowledgements

I would like to deeply thank the World Food Prize Foundation for allowing me to have had this wonderful and meaningful experience. Secondly, I want to thank Lisa Fleming for all of the hard work that she put into seeing that all of us interns were safe and happy during our stays. I want to extend a deep thank you to Ida Bartolini and her family who were kind enough to let Rachael and me stay in their home. I would also like to thank them for making us both feel welcomed and comfortable while in Peru. I want to thank everyone that I worked with at CIP: Dr. Dapeng Zhang for all of his endless advice, humor, and patience; Jorge Benevides for taking the time and having the patience to teach me all the proper ways and techniques in the lab work that we did together; and I want to deeply thank Luo Hongrong for allowing me to have the privilege of working with her on her project at CIP and for the kindness and care that she treated me with throughout my internship. I cannot thank Luo enough for all that she has taught me! I would also like to thank Rachael Collier for "putting up with me" while we were in Peru together. She was a wonderful source of strength for me, and I cannot thank her enough for that. Finally I would like to thank my twin sister, Suzanne, my parents, and all of my family, friends, and loved ones for their unconditional love and support.

Who I Am

My name is Melissa Ann Fox, and this is my story. I am eighteen years old, and I have lived in Des Moines, Iowa in the same home, all of my life. My parents have been married for 30 years, and I have a twin sister named Suzanne. This year, we both graduated from Saydel High School, a school that is part of a small district that we have attended since kindergarten. As I am typing this, I am realizing all of the redundancy! But it is true! My life so far, has been very constant and simple…

I am going to attend Wartburg College this fall. I am not sure what I am going to be studying, but whatever it is, I want it to be something that I am passionate about. I have many, many things that I am interested in doing. I dream of one day being a veterinarian, a horticulturist, a biotechnologist, a counselor, a nurse, or someone who works with international relations. These are just a few of my passions. The list is long, but there is one thing that connects all of it. I have a strong desire to help others during my life.

People are so much more than what words can describe, though. We each have our own energy, our own passions, and our own experiences that combine to make us each unique. Every now and then, when one of us is lucky enough, we have an experience that has the potential to alter our complete being, and that is what defines us.

My Involvement in the World Food Prize Youth Institute

I first heard about the World Food Prize Youth Institute while I was sitting in advanced chemistry class one day last year. Shawn Thomas was in the classroom typing on the computer. When another student in my class asked our teacher, Mr. Bechtel, what Shawn was doing, Mr. Bechtel responded by telling us about the World Food Prize, a program that Shawn was involved in. Later on, after the 2001 World Food Prize Symposium was held, Mr. Bechtel stood in front of our class and enthusiastically told us all about the World Food Prize. He talked about Norman Borlaug, the founder of the World Food Prize (WFP) and winner of the 1970 Nobel Peace Prize. It was so nice and exhilarating hearing about all of those wonderful things especially about someone from Iowa!

Later on, during the school year, parent-teacher conferences were held. My parents and I ended up talking for quite some time with Mr. Bechtel. I told him of my dream of someday joining the Peace Corps. It was then that Mr. Bechtel told me about the WFP Borlaug~Ruan Summer Internship Program that Shawn was trying to get accepted into. I went home that night with a vision and with the hope that I could somehow become connected to this amazing program.

I was so happy and pleased when I was able to become involved in the WFP Youth Institute, and on October 24th, 25th, and 26th, I attended the 2002 World Food Prize Symposium. My days spent at the WFP Symposium were without a doubt the most

valuable and influential days of my high school career. It is very hard to describe how wonderful it was with words! People from all over the world, from Asia, Africa, India, and Mexico were in Des Moines, Iowa to discuss one of the most empirical issues for the well being of mankind-the alleviation of world hunger. Everyone that was there seemed to possess a certain

type of energy. It was very monumental and inspirational being connected with such a powerful source, and I'll never forget how it felt.

After attending the WFP symposium, I really wanted to apply for a WFP Borlaug~Ruan summer internship. I truly wanted to have a chance to see the world in a different aspect. I wanted to see with my very own eyes that internally, we all wanted and needed the same things regardless of how greatly our lives differed. So, I put my best efforts into the application process.

I was so happy when I found out that I had been accepted for an internship in China. At that time, though, SARS was rapidly spreading in Beijing, the city that I would be staying in. Consequently, I was unable to go to China. However, I was asked if I would like to intern at the International Potato Center in Lima, Peru. After giving it some thought, I knew that I couldn't even consider declining. The opportunity was so great, and I knew that I would learn important life lessons regardless of where I was.

I left for Lima on Thursday, June 12, 2003, and I was very nervous! It was a combination of things. I was leaving behind my family and loved ones for two months! At the same time, I couldn't help but think of my internship as an adventure-one where I could both learn and explore. I would also be able to meet someone new. Because I was originally supposed to go to China, and not Peru, I was going with another World Food Prize Intern, Rachael Collier. My biggest hopes were that we would get along and that I would not miss my family too much! During this entire time, I had so many questions that I couldn't wait to have answered!

Our plane landed at Jorge Chavez International Airport late at night. Walking down the ramp and into the hall of the airport, all I could think about was that I was finally in Peru and that this was going to be different from anything I had ever done in my previously "simple" life. I was also a bit anxious because Rachael and I had to go through customs and pick up our luggage all in a place where the people did not speak our language! Well, we made it through with little trouble, and our driver managed to find us in the large crowd of people. In little time, we were on our way down the road.

My first impression of Lima while we were driving through that night was that the city seemed to be very run-down. The buildings were not as clean as I was used to and the streets had a lot of trash on them. I could almost sense the dreariness of Lima that I had read about. I was trying to keep an open mind and to limit my judgments. After all, I really knew nothing about Peru. I had no doubt, though, that Lima was very different than Des Moines. While we were stopped at an intersection, I was deeply troubled when a little girl came up to the car to ask for money. I could almost see the tired, hopeless look in her eyes. After forty-five minutes of driving, we finally arrived safely at our host family's home.

Peru

When one thinks of Peru, one cannot help but think of the majestic Andes, the beautiful rainforest, and of course, the llamas! But Peru is so much more than that. It is a country that is

blessed with a uniquely diverse population. It is a land where people are friendly and unassuming. It is a place where family is very important to the individual. Sadly, Peru also has many obstacles to its growth as a country. Its unstable government and struggling economy has created numerous problems for the people of Peru.

In the 1980s, Peru was stricken with violence and terrorism. During this period of time, two rebel groups, Sendero Luminoso and Tupac Amaru, reached their highest level of activity. Experts say the groups "arose in response to Peru's entrenched system of race-and-class-based discrimination, which has deeply impoverished most of the country's population, especially citizens of indigenous descent." (Council on Foreign Relations) Most of the members of Sendero Luminoso and Tupac Amaru were lower-class, indigenous people from the Andean highlands and jungle areas of Peru. The main goal of both groups was to remove the existing Peruvian government and replace it with one of their own. Over this reign of terror, hyperinflation was incurred on the economy, and an estimated 30,000 Peruvians were killed.

Alberto Fujimori became president of Peru in 1990, and vowed to put an end to Sendero Luminoso, Tupac Amaru, and Peru's ever-growing drug trade. This change in leadership was not entirely positive. In 1992, Fujimori raised claims that the congress and the judicial system of Peru were blocking his efforts in the fight against terrorism. He soon became dictator-like, diminishing the legislature, legalizing censorship, and arresting his political opponents. (Meneen-Vela) By April of that year, the two top leaders of Tupac Amaru and Sendero Luminoso had been captured along with many of their comrades. (Meneen-Vela) While the reign of terror had ended, governmental corruption had only just begun.

In both the 1995 and the 2000 presidential elections, Fujimori was victorious. In the latter, however, there was hard evidence that election fraud was done by Fujimori. In 2001, after Fujimori had been removed from office, Alejandro Toledo became the president of Peru. (Meleen-Vela)

Even though both terrorism and corruption had been removed from the government, an overwhelming amount of damage had been done. Peru continues to have an unstable economy and an enormous level of poverty. Nearly 50% of the people in Peru are considered below the poverty line, and nearly 9% of the population is unemployed. (CIA) It doesn't help that every time there is an election and a new government is put in place, many of the existing polices and institutions are drastically changed. Peru does not have all of the policies and checks and balances that we are fortunate to have in the United States. Sadly, it seems to be a never-ending cycle. Many of Peru's citizens, who have much to offer because of their education level and status, leave Peru each year. In this way, the continuous cycle of poverty and hopelessness for many of the people continues.

CIP

Both Rachael and I completed our internships at the International Potato Center, commonly known by its Spanish acronym, CIP. Even before I arrived at CIP, I already held deep admiration for those who worked at such an important place, and for those who did such

tremendous things. These feelings of admiration only continued to grow as I began to understand the "bigger picture" and how CIP plays into it.

CIP's work is crucial in feeding our growing world. Projects done at CIP are focused on potatoes, sweet potatoes, and other root and tuber crops. These crops feed the hungry and the poor, the people that tend to be forgotten about in life. CIP focuses not only on the research end of the spectrum, but also on the economic end. This creates a unique balance that is nearly impossible to duplicate. CIP truly does help others help themselves, and that is invaluable.

I had many misconceptions about CIP before my internship. I automatically assumed that because CIP was an international research center and part of the Consultative Group on International Agricultural Research (CGIAR), money would not be a concern when it came to funding projects. Sadly this is not the case. I was able to attend a CIP staff meeting led by the director general of CIP, Hubert Zandstra. Discussion focused mostly on finances. In the year 2002, CIP received $18,393,000 in donations. This amount was 2% less than the donations received in 2001. (CIP) I now realize that money and job security are big concerns that people who work at CIP have.

I was also worried that many of the people that worked at CIP would be hard-core scientists with little to no patience for people with little lab experience, like me. I have never been so wrong in my entire life! My supervisor at CIP was Dr. Dapeng Zhang, a sweet potato breeder and geneticist from China. He felt that it was important for Rachael and me to find projects we found interesting, as well as rewarding. Because of Dr. Zhang's efforts, I was able to explore several areas of CIP and met many of the different people who work there. After my first few days at CIP, my beliefs of the existence of "hard-core impatient scientists" melted away. Throughout my internship I was lucky enough to have been able to work with some very wonderful people.

After a lot of exploring and thinking, I decided to work in the Crop Improvement and Genetic Resources Department. I regularly worked with Luo Hongrong, a professor from China completing her PhD. research at CIP. She taught me many of the crucial procedures in molecular biology including the use of *E.coli* bacterium to multiply plasmid DNA, extraction of plasmid DNA from *E.coli* culture, enzyme digestion reactions, DNA electrophoresis, and the Polymerase Chain Reaction (PCR). Individually, I performed an extraction of plasmid DNA and DNA electrophoresis.

In the latter part of my internship at CIP, I worked with Jorge Benevides, a research assistant at CIP. I assisted him while he was preparing his DNA samples for the Southern Blot Assay. I then observed Mr. Benevides when he labeled 2 different DNA probes and also during the preparation of membranes for hybridization. During the main part of my internship at CIP, I assisted Ms. Hongrong with her project titled "Molecular Characterization of Transgenic Sweet Potato with *dhdps-r1*, Mediated by *Agrobacterium tumefaciens*." All procedures were done with Ms. Hongrong, unless otherwise noted.

"Molecular Characterization of Transgenic Sweet Potato with *dhdps-r1*, Mediated by *Agrobacterium tumefaciens*"

I. Background

The Sweet Potato *(Ipomoea batatas)*

There are 5.2 billion human beings living in developing nations, and 1.2 billion of these people struggle to live on a dollar or less each day. Many of us try to ignore this cold truth. Meanwhile, some of the poorest people of the world struggle to survive on a daily basis. For them, the sweet potato means life.

One of the many sweet potato varieties in CIP's fields.

More than 95% of the world's sweet potato crop is grown in developing nations. The sweet potato plays a crucial role in the livelihoods of the people of these countries. It is the fifth most valuable crop for developing nations. (Woolfe) The sweet potato is a hardy crop with many benefits. First, the sweet potato's pest problems are relatively low compared to other crops. Therefore, a low amount of pesticides can be used. Secondly, the sweet potato can be grown in poor soil with little aid of fertilizers. (CGIAR) Thirdly, sweet potatoes are grown from vine cuttings. Therefore, farmers do not have to buy seed from outside sources or save any of the crop for the following year. (Woolfe) Lastly, the time to harvest can be as little as 3 months so oftentimes farmers in tropical countries can complete 2 harvests in one year. (CGIAR)

Nutritionally, the sweet potato also offers many benefits. The sweet potato is high in carbohydrates and vitamin A. (CGIAR) Also, the sweet potato is ranked first amongst the developing world's 8 most important crops for the amount of energy per hectare per day it can produce. (Woolfe) The sweet potato's green leaves can also be used in human consumption and in animal feed. (CGIAR)

Regardless of its numerous benefits, the sweet potato faces many commercial obstacles. Outside of developing nations, the sweet potato is often thought of as "the poor man's crop." (Woolfe) Because of this reputation and other reasons, the sweet potato has limited commercial success in developed countries. Consequently, the sweet potato remains a hidden treasure. While little research money is given to the sweet potato in comparison to other crops, the Consultative Group on International Agricultural Research (CGIAR) invested 5.7 million dollars in sweet potato research in the year 2001. With their support, scientists at the International Potato Center are making important advances with this priceless crop.

dhdps-r1

At CIP's Crop Improvement and Genetic Resources Department, a great deal of work is being put into creating transgenic sweet potatoes that carry the gene *dhdps-r1*. The success of this project would create two benefits. First and more importantly *dhdps-r1* can be used as a selectable marker, a crucial tool in biotechnology.

Conventionally, *npt II*, a gene that blocks the effects of the antibiotic kanamycin is used as a selectable marker. The desired gene of insertion is "tagged" with this gene. Transformed plants consequently carry both the desired gene of insertion and the *npt II* gene. Transformed plants are then regenerated in a medium containing kanamycin. Under normal circumstances, kanamycin blocks the protein biosynthesis in the chloroplasts of plants; therefore plants are unable to grow. (Hooykaas) The plants that do have the *npt II* gene, however, can successfully grow. In this way, scientists can quickly determine which plants have been successfully transformed. (Hooykaas)

Recently, however, there has been a lot of controversy surrounding this method. Critics argue that the stomach bacteria in humans and animals could develop resistance to kanamycin if the host was fed transformed crops that carry this gene. This belief has yet to be scientifically proven, but it creates an unnecessary poor perception of this type of biotechnical work. It has even been recommended by some governments in the European Union to "phase out" any genetically modified crops that contain antibiotic resistant markers. (European…)

The *dhdps-r1* gene has been shown to be a successful selectable molecular marker in a previous study at CIP done on potatoes. Transformed potato plants with *dhdps-r1* were placed in a growing medium containing AEC, a lysine analogue. It has been hypothesized that the overproduction of lysine in the transformed plants, counteracts against the normally harmful AEC. Now scientists at CIP are studying the effectiveness of *dhdps-r1* as a selectable molecular marker in sweet potato. If dhdps-r1 is proven to be successful as a selectable molecular marker, it would be beneficial to all.

The second benefit of developing transgenic sweet potato plants with the dhdps-r1 gene is that *dhdps-r1* synthesizes an enzyme that is crucial to the biosynthesis of lysine, an essential amino acid. This results in an overproduction of lysine and thus sweet potatoes with a higher quality of protein. Many of the people for whom the sweet potato is a staple food, cannot afford foods high in protein. In Proteins in Human Nutrition, J.C. Abbot writes, "Protein deficits appear to be most probable in the maize-eating countries of Latin America, in Equatorial Africa because of the predominance of starchy foods there…" "In these areas there is a protein/energy imbalance. Energy requirements are being met but the protein intake is low." In many of the areas where protein deficiency is prevalent, the sweet potato is a staple food in the diet. Having a sweet potato with a higher quality of protein could be part of solution to the crucial problem of protein deficiency in today's world. (Woolfe)

The Use of *Agrobacterium tumefaciens* as a Vector

Bacteria have certain characteristics which can be utilized in many parts of biotechnology. (Industry Canada) One of the most important characteristics is that bacteria often contain plasmids, small circular pieces of DNA. Plasmids can be easily passed from one bacterium to another where they become a permanent part of the host bacterium's DNA. Knowing this, scientists are able to manipulate bacteria so they can be used in the transformation of plants. *Agrobacterium tumefaciens*

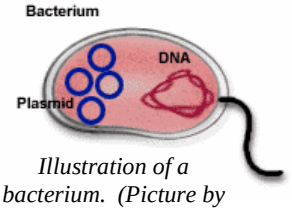


Illustration of a bacterium. (Picture by Canada Stragetis)

is a bacterium that is commonly used in genetic engineering because of its ease of use and accuracy. (Hooykas) *Agrobacterium* has even been referred to as a "natural genetic engineer" because it transfers a segment of tumor-causing DNA to plant cells causing the plant to become infected with crown gall disease. (Hooykas) That segment of DNA comes from Ti (Tumor inducing) plasmids in *Agrobacterium tumefaciens*. The part of the Ti plasmid that is transferred is called the T (transferred) region. Genetic engineers use restriction enzymes to cut the Ti plasmid in specific spots in its T region. Then, the desired gene of insertion, like dhdps-r1, is incubated with the cut plasmid and ligase enzymes. The ligase enzymes work like glue, attaching the gene at the place where the plasmid was cut, and making the plasmid whole once again. (Industry Canada) The genetically altered plasmids from A. tumefaciens are then used to "infect" plant cells. The plasmid DNA becomes incorporated into the original plant DNA, and transformed sweet potato plants can be regenerated and grown from single transformed cells

II. Methods and Procedures

Culturing *E.Coli* for the Multiplication of Plasmids

Background

It is important to check the quality of the plasmids and their construction. Before this is done, however, there must be an adequate amount of plasmids for the necessary tests. Ti plasmids of *Agrobacterium tumefaciens* are large in size, but have low copy numbers. So, *E.coli* bacterium is often used as a copying "vector". Plasmids from *A.tumefaciens* are transferred to *E.coli* by electroporation. During electroporation, an electric current is run through a solution containing *Agrobacterium* plasmids and *E.coli* bacterium. The cell walls of the *E.coli* become temporarily porous, so the plasmids from the *Agrobacterium* are readily taken up by the *E.coli* bacterium. (Hooykaas) *E.coli* bacterium multiplies very quickly if given the right growing conditions. Because the plasmids have become incorporated into the DNA of the *E.coli* bacterium, they are copied as well. Luria-Bertani (LB) medium creates the environment that *E.coli* thrives in.

Culture of colonies of E.coli (Picture by Canada Stragetis)

Procedure

The procedure for the preparation of LB medium for culturing *E.coli* bacterium was as follows: The following substances were added to 1 liter of distilled water. 10.0 grams of Tryptone, 10.0 grams of Sodium chloride (NaCl), 5.0 grams of yeast extract. The solution was then autoclaved for 20 minutes for sterilization. Then, one colony of *E.coli* bacterium, with kanamycin resistance, was placed in tubes containing 5 ml. of the LB medium. 5 tubes were prepared in this manner. The tubes were then incubated at 37°C in a shaking incubator overnight.

Purification of Plasmid DNA

Background

In order to perform the necessary protocols to check the quality of plasmids, they must first be removed from *E.coli* bacterium and purified. Purification of plasmid DNA can be done either with a kit or without a kit. The advantage of using a kit is that there is less opportunity for the occurrence of contamination from an outside source. The following protocol was completed using the Wizard® Plus SV Minipreps DNA Purification System (Promega: 2001).

Procedure (performed individually)

Five samples of the *E.coli* bacterial culture and solution were placed in 1.5 ml. microcentrifuge tubes. Bacterial culture (10ml.) was then harvested by first centrifuging the tubes and then pouring off the supernatant. Cell Resuspension Solution (250µl) was added to each tube, and the cells were resuspended by vortexing. Cell Lysis Solution (250µl) was then added to each tube and the tubes were then incubated for 5 minutes at room temperature. Then, Alkaline Protease Solution (10µl) was added to each tube and the tubes were again incubated for 5 minutes. (The Alkaline Protease Solution is designed to lower the level of protein contaminants in the samples.) After 5 minutes, Promega's Wizard® Plus SV Neutralization Solution (350µl) was added to the samples and the tubes were immediately mixed by inversion. The samples were then centrifuged for 10 minutes at maximum speed. From the above procedure, a cleared lysate was produced.

Before the protocol was continued, one Spin Column was placed into one 2ml. Collection Tube for each sample. The clear lysate was transferred to the Spin Column. The assembly was then centrifuged for 1 minute at maximum speed. The flow through in the Collection Tube was discarded, and the Spin Column was again placed into the Collection Tube. Column Wash Solution (750µl) was added to the Spin Column. Again, the assembly was centrifuged for 1 minute at maximum speed. The flow through was discarded and the wash procedure was repeated using 250µl of the Column Wash Solution. The assembly was centrifuged for 2 minutes at maximum speed and the Spin Column was transferred to a new microcentrifuge tube. 100µl of Nuclease-Free Water was added to the Spin Column and the assembly was centrifuge for 1 minute. The Spin Column was discarded and the 5 purified plasmid DNA samples were stored at -20°C until further use.

Electrophoresis of Plasmid DNA

Background

Electrophoresis of plasmid DNA gives "an indication of the amount and size of DNA and RNA present." (Grinsted and Bennett) This information is used to determine the amount of DNA needed to run further experiments and procedures.

To run DNA electrophoresis, samples of DNA with loading buffer are placed into the wells of an agarose gel containing ethidium bromide (EtBr). The gel is then placed in into an electrophoresis chamber and covered with TBE (Tris-borate-EDTA) buffer. The electrophoresis chamber has 2 cathodes on either end. When an electric current is applied, the DNA fragments migrate towards the positively charged cathode, called the anode. This enables the DNA samples to be separated according to size. When the electrophoresis is completed, the gel is placed in a transilluminator under a UV light. The DNA can then be seen.

Procedure

A 1% agarose gel was prepared by mixing agarose (.30g.), and 10X buffer diluted with distilled water (30ml.) in a flask. The flask was then heated in a microwave until the agarose was completely dissolved. After the solution had cooled, EtBr (5µl) was added. A sample comb was placed into a gel casting tray, and the agarose solution was poured into the tray. Twenty minutes were allowed for the setting of the agarose gel. Then, DNA samples of plasmids containing *dhdps-r1* were placed into the wells using a micropipetor. To get an accurate approximation of how much DNA samples would be adequate for further experimentation, 3 different amounts were placed in the wells: 20µl, 10µl, and
5µl. 5µl of PstI DNA for control was also placed into the first well.

DNA Electrophoresis of PB120 Plasmids (Purified)

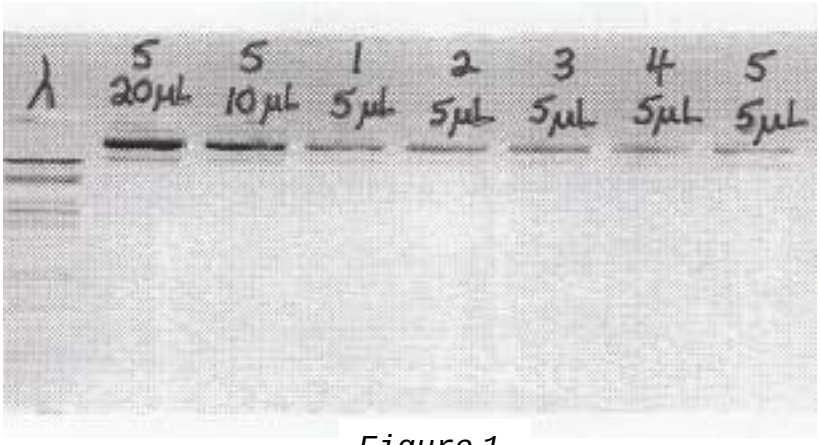


Figure 1

Notice that in *Figure 1*, all of the fragments of DNA can be easily seen, including the bands with only 5µl. of DNA. Therefore, it was concluded that for further experimentation, 5µl.of DNA would be adequate.

Enzyme Digestion of Plasmid DNA

Background

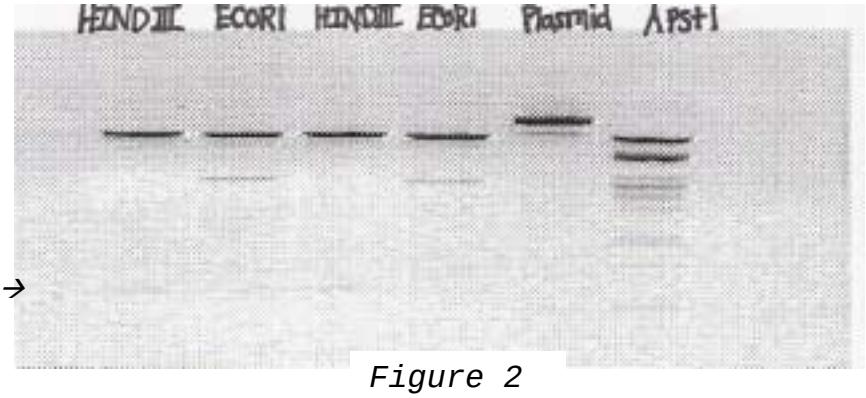
Enzyme digestion of DNA is a very important way through which scientists can check the construct and quality of purified plasmids. (Grinsted and Bennett) Plasmid DNA can be

easily contaminated by outside sources, so it can be very worthwhile to perform an enzyme digestion reaction as a precaution before moving on to other protocols. Scientists have discovered that there are restriction enzymes that always cut in the same spot of specific plasmids. A good hypothesis can therefore be formulated about where the DNA fragments should be in the gel after electrophoresis.

Procedure

Two enzyme digestion reactions using two different enzymes were carried out so that there would be two ways of checking the purity of the plasmid DNA. HindIII and EcoR1 were the enzymes used. Both cut the PB120-dhdps-r1 plasmid in 2 spots. For the reaction, water (16µl), 10X buffer (3µl) specific to the enzyme used, EcoR1 or HindIII enzyme (1µl), and DNA (10µl) were placed in a small 1.5ml. tube and incubated for 2-3 hours at 30 degrees Celsius. Samples 1 and 2 were digested with EcoR1 and samples 3 and 4 were digested with HindIII. A hypothesis was formed about where the DNA fragments of each sample should be located in the gel after electrophoresis.

Electrophoresis of Enzyme Digestion of PB120



1st fragment of DNA →

2nd fragment of DNA (EcoR1) →

2nd fragment of DNA (HindIII) →

Figure 2

Notice in *Figure* 2 that all of the samples of digested plasmids, two fragments can be seen. This was the first sign that the digestion was successful. One must look more carefully for the second sign. Scientists know that EcoR1 cuts the plasmid in 2 spots that are quite far from each other. Consequently, the two fragments are relatively close in size to one another. Now look at figure 1 and notice how close the two fragments of DNA are to each other in the samples digested with EcoR1. This was the second sign that the digestion was successful. Conclusions about the success of the digestion with HindIII can be made in the same way. Scientists know that HindIII also cuts the plasmid in 2 different spots, however, unlike EcoR1, the sites of the cutting are close to each other. Consequently, the two fragments of DNA are very different in size. In the lanes where HindIII was used on the samples, the two fragments of DNA can be seen. They are much farther apart in the gel than the fragments of DNA cut by EcoR1.These were the anticipated results, so it was concluded that the enzyme digestion of the plasmids was successful, and the plasmid DNA had a high level of quality and purity.

The PCR- Polymerase Chain Reaction

Background

The Polymerase Chain Reaction (PCR) is a crucial part to the success of microbiology and genetic engineering. The purpose of this procedure is to amplify a sample of DNA (Industry Canada).

Procedure

The goal of this protocol was to amplify the *dhdps-r1* gene in some samples, and to amplify the npt II gene in the remaining samples. Small tubes were prepared with a small sample of extracted plant DNA containing the dhdps-r1 gene, individual nucleotides, 1 of 2 different primers- a primer for *dhdps-r1* or a primer for *npt II*, water, buffer, and thermos aquaticus polymerase enzyme (Taq enzyme). The tubes were then placed in the PCR machine where they underwent a pre-set heating and cooling process. First, the tubes were heated to a very high temperature near to 95°C. The hydrogen bonds between the 2 strands of the double helix DNA broke causing the strands to separate (Industry Canada). Then the temperature was lowered and the primers, strands of DNA one specifically complementary to *dhdps-r1* and one specifically complimentary to the npt II, bonded to the complementary part of each strand. Taq enzyme then created a complimentary strand for each single strand of DNA using the individual nucleotides in the tube. The cycle was repeated 30 times.

PCR Machines

Electrophoresis of Plant DNA after PCR

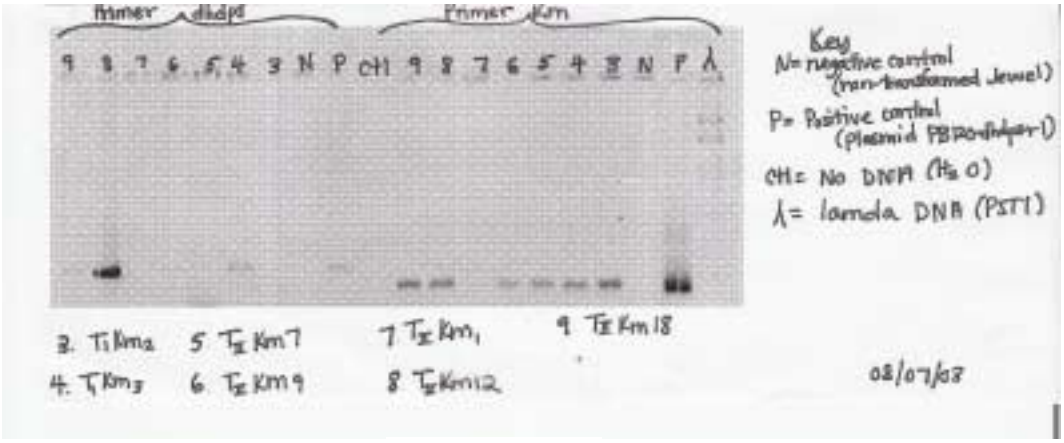


Figure 3

Notice in *Figure 3* that in the samples in which primer km (*npt II*) was used, the fragments of DNA are darker than ones in which primer dhdps (*dhdps-r1*) were used. Therefore, it was concluded that using the km (*npt II*) primer resulted in better gene amplification than using the dhdps (*dhdps-r1*) primer.

III. Observed Procedures

Ms. Hongrong and I had originally planned on performing the Southern Blot Assay on her samples of DNA, with *dhdps-r1*. However, the transformed plants in the greenhouse were not mature enough to gather enough leaves for the extraction of DNA. Mr. Benevides was very kind and allowed me to observe and assist him while he performed the Southern Blot Assay on his transformed sweet potato plants with glutenin.

The Southern Blot

Background

The Southern Blot method is used to determine many characteristics of DNA. One of the ways this method can be utilized is in the determination of the number of copies of a specific gene or DNA sequence in a sample of DNA.

Procedure

First, enzyme digestion reactions were carried out on the DNA samples and electrophoresis was done. The gel was then soaked in 500 ml. of .25 M HCl for 18-20 minutes. This was repeated twice. Then, to denature, or separate, the double stranded DNA, the gel was washed twice with a NaOH solution, each time for 20 minutes. To neutralize the DNA, the gel was soaked in 500 ml. of 1.0 N Tris, 1.5 M NaCl for 30 minutes. A basin was prepared with approximately 350 ml. of 10X SSC, and a glass plate was placed over the sides of the basin. On this plate, 3 large sheets of Whatman 3MM paper were placed so that the ends were soaking in the 10X SSC solution. The gel was then placed face down on top of the Whatman paper. A nylon membrane was then placed on top of the gel. 2 Additional pieces of Whatman 3MM paper were placed on top of the nylon membrane. Finally a large stack of paper towels were placed on top of the entire sit up and weighed down with a bottle. The entire set-up was left overnight in the lab.

Set-up of the Southern Blot. Nylon membrane is in place on top of gel.

How does this process work?

The Whatman paper on the bottom soaks up the 10X SSC solution, and the paper towels on top draw up all of the liquid through the gel. This "pull" enables the DNA to be binded to the nylon membrane, in a mirror image how it was in the gel. A nylon membrane is then placed on top of the gel. The fragments of DNA bind to the membrane in a mirror copy of how they were in the gel. Then, the membrane is incubated with prepared probes (strands of DNA complimentary to the gene of interest) that are labeled with radioactive P32. After incubation, the membrane is then put into contact with a sheet of photographic film that is sensitive to

radioactive emission. Only the sections of the sample where the gene is located, show up on the paper. In this way, conclusions can be made about the number of copies of genes in the DNA samples.

Because only one "blot" reacted with the photographic film, it was concluded more time should have been allowed for the P32 to react to the film.

IV. Discussion and Conclusions

"Molecular Characterization of Transgenic Sweet Potato with *dhdps-r1*, Mediated by *Agrobacterium tumefaciens*" was just a small, but important part of the large study of the dhdps-r1 gene, its effectiveness as a selectable molecular marker in sweet potato. Ms. Hongrong's and my results will be used as a base for further experimentation of the transgenic sweet potato with the *dhdps-r1* gene. Further tests to be done on the transformed sweet potato DNA will include the Rt-PCR and the Southern Blot Assay. Both of the results from these tests will show the level of success of the actual transformation of the sweet potato plants. It is through projects like these, which larger more crucial changes can occur. If the use of the *dhdps-r1* gene as a selectable molecular marker proves to be successful and safe, the window of hope for the millions of people who rely on the sweet potato will be opened just a little farther.

What I Have Learned About Food Security

Although I feel like I have learned a lot about food security and the world, I still have so much to learn! One of the most important lessons that I had about food security was when Rachael and I had the chance to visit with an economist at CIP. During our conversation, I began to realize how crucial it is for administrators, scientists, economists, and governments to communicate and have a working relationship.

Science is a very difficult and trying field to work in. Besides keeping up on all of the changing technologies and discoveries, where to get the funds is always a major concern especially for those working at international research centers like CIP. All of the scientists at CIP worked so hard putting in 10, 11, and sometimes 12 hour days. Even though they do all of this hard work, their pay compensation is very little. This puts an enormous amount of stress on scientists, especially ones with families. Looking outside of Peru for a job looks very promising for young scientists, and many of them do leave the country. CIP needs these scientists, and hopefully someday they will get paid accordingly.

Many times economists and scientists "battle" for funds for their departments. Like many research centers, this is prominent at CIP. During my internship, I realized that for any successful solution, there must be more than one aspect in its development, and it must be looked at from more than one perspective. A genetically modified potato is not always going to be the answer in some cases…maybe an approach that is geared towards community development will be, though. Individuals must not allow themselves to be blinded by their personal beliefs and biases.

I also realized that people work so much better when they have a good motivation behind them. My motivation wavered during some times of my internship. I enjoy working with people so much, and sometimes doing lab work all of the time made me feel distant from the final result of my work and of Luo's work. While in Lima, I really felt like I was sheltered from the "average" life of Peruvians. During Rachael's and my trips, I only got a small glimpse of what poverty in Peru is really like. I guess my only regret that I have from my time in Peru is that I never actually met or talked to the people that CIP's work truly affects-the small farmers of the highlands. Knowing this, though, only makes me more motivated to one day actually come face to face with poverty and hunger and to fight against it.

What I Have Learned About Myself and My Beliefs

I learned how much I take everything for granted here at home in the United States. We have a stable government, a surplus of food, and endless opportunities to improve our lives if we have the desire to do so. Peru and so many other countries do not have half of those things. It struck me so much that the majority of Peruvians are just trying to get by, but here in the United States we always seem to strive for more-a bigger house, nicer car, more money, etc. We seem to always thirst for more even though many of us have plenty. Still, we never seem to be satisfied. I now realize that there is a severe imbalance in the distribution of wealth amongst the citizens of this world..

Our priorities are very different as well. We have celebrities who make over $10 million dollars a film, we spend thousands on plastic surgery for the quest for the perfect body, we spend billions of dollars each on NASA and its programs, yet there are so many people in this world who are hungry, homeless, or who are sick.

Humans are truly far from perfect. Perhaps what get most in our way are our feelings of greed, jealousy, and indifference. Before my internship, I found it simple to ignore a lot of the worldly events that would happen each day. I almost had this, "as long as it isn't happening to me" perception of things. Even to this day, I still struggle with that, and I probably will for the rest of my life. I think that there are a lot of people in this world who share this same perception. What we don't realize, though, is that having indifference and living in ignorance are perhaps two of the most harmful actions we can do.

Imagine all of the good we could do if all of a sudden, we each cared enough to fight the spread of AIDS and the widespread unemployment in parts of the world? What if we all suddenly cared about that drunken poor man on the side of the road in Anytown, USA? Our world would become so much stronger, healthier, and happier for everyone. It is important also that we realize that it shouldn't matter who it is that we help. A human being is a human being whether he or she is from Kenya, India, Mexico, or the United States. That shouldn't even really be an issue. I admire anyone who has the strength and the courage to travel to another country and help those citizens. But at the same time, I admire the social worker who works so hard trying to find a foster home for a brother and sister here in the United States. The beauty of both of those people…of their gifts that they are freely giving to others…it must take incredible strength of heart. I truly believe that we each hold a very special gift. Maybe it's a budding talent in science…or an overabundance of empathy. Maybe it's a wonderful, warm sense of

humor…or an inane ability to solve critical problems. Whatever your gift is, you can use it to help others. And we should never become completely discouraged by the overwhelming amount of troubles in the world. Mother Theresa had wonderful words of wisdom on this subject. "We ourselves feel that what we are doing is just a drop in the ocean. But if that drop was not there, I think the ocean would be less by that missing drop. We don't have to think in numbers. We can only love one person at a time-serve one person at a time." (Chalika and Le Joly)

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