# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Brandon C. Amash

**Project Number**
**S0401**

**Project Title**
## Cloning a Human Gene in Genetically Recombinant Bacterial Colonies

**Abstract**

**Objectives/Goals**
How effective is cloning a human gene in a bacterial strain by transformation as genetic recombination in various bacterial strains?

**Methods/Materials**
1. After sterilizing all materials with the alcohol burner, use the micropipette to transfer 250 µL of calcium chloride into a test tube.
2. Mix the desired plasmid, restriction enzyme, and the human insulin gene and place in an ice bath for 15 minutes
3. Transfer a large (3 mm) colony of the desired bacteria using an inoculating loop into the calcium chloride.
4. Use the micropipette to place 10 µL of the desired plasmid solution in the test tube. Keep the tube on ice for 15 minutes.
5. Heat-shock the cells in the tube by holding the tube in the heated water bath for 90 seconds. It is essential that this is a sharp and distinct shock.
6. Immediately return the cells to the ice for two minutes.
7. Use the micropipette to add 250 µL of pure Luria Broth agar to the tube.
8. Place 100 µL of the solution on the desired agar plate (depending on plasmid solution used). All plates should contain an X-gal solution as well as the antibiotics.
9. Allow plates to set for at least 24 hours. Incubate at 37⁰C.

**Results**
The number of white colonies (with a disrupted lacZ gene) was more than that of the blue colonies. The human gene was successfully uptaken. The transformation efficiencies for each colony of bacteria varied if it was transformed with more than one plasmid. Some bacterial strains such as Bacillus megaterium did not have good transformation efficiencies no matter the plasmid. On the other hand, some had excellent values.

**Conclusions/Discussion**
Depending on the strain of bacteria and its ecological niche, genetics, and morphology, transformation efficiency varies from bacteria. Not very many bacterial strains are naturally competent to uptake naked DNA in the form of a plasmid. Those that do have need for the process in their niche, the genes to produce DNA-uptake proteins, and morphological traits such as a pilus.

**Summary Statement**
How transformation and genetic recombination is used to clone a human insulin gene in various bacterial colonies.

**Help Received**
Parents paid for materials and helped to set up the board

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

Kevin Baik; Jun Noh

**Project Number**

**S0402**

**Project Title**

## Extraction of Bio-Ethanol from Wasted Bio-Wastes through the Process of Fermentation and Distillation

**Abstract**

**Objectives/Goals**

The main objective of the experiment was to observe if the common household wastes, such as apple and banana peelings, were suitable to be used as another source of producing bio-ethanol. The experiment also served as a way to relieve the world's dependency on oil and fossil fuels through the extraction of bio-ethanol from the wasted bio-wastes. Through this experiment, people will discover that energy can be derived from even wastes that are thought to be just useless.

**Methods/Materials**

During the experiment, the peelings of apple and banana were mixed with water and yeast to start the fermentation.(24 hour and 48 hour) From the fermentation process, the bio-ethanol was produced from both the apple and banana. The produced mixture then went through the distillation process, where the ethanol was boiled out at 78.4¡ÆC, to extract the ethanol out of the mixture. After the extraction of the ethanol, the amount of ethanol production was measured. The variables that were altered for the test of the hypothesis were the amount of bio-waste (peelings)and duration of fermentation (24 & 48 hour). The sample size of the banana peelings was 8. The sample size of the apple peelings was 8. Total of 16 samples. 8 trials were observed for both apple and banana peelings. The measurement for amount of bio-waste was in grams and the amount of ethanol was measured in milliliters.

The materials used during the experiment were banana peelings, apple peelings, water, yeast, condenser & still head/receiver/pot (Distillation System), sand bath/stirrer plate, and electric balance.

**Results**

The average mean of bio-ethanol produced from banana was 6.65 mL and the average mean that apple produced was 5.57 mL. Also the mean of ethanol production during the 24 hour fermentation period (both apple and banana) was 5.15 mL, and the mean of ethanol production during the 48 hour was 6.8 mL.

**Conclusions/Discussion**

As a result, the hypothesis of the experiment that banana peelings would produce more ethanol than the apple peelings was supported. The result also showed that both the banana and apple peelings can be used for the production of ethanol. The data supported that the amount of bio-ethanol produced from banana peelings was greater than the amount produced by the apple peelings.

**Summary Statement**

Demonstrating the importance of wasted bio-wastes through the production of bio-ethanol, an alternate fuel source that is more energy-efficient and eco-friendly than the fossil fuel that is used today.

**Help Received**

Used laboratory equipments at University of California Irvine under the supervision of Dr. Yoon Kim.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Aubrea M. Bailis

**Project Number**
**S0403**

**Project Title**
## Genotoxicity of Common Household Substances

**Abstract**

**Objectives/Goals**
The experiment that I have put together is designed to determine the genome toxicity of certain common household chemicals that allegedly cause cancer. I want to know how much truth there is behind the warnings stating that these substances are dangerous.

**Methods/Materials**
To figure this out, I used yeast cells as test subjects because their DNA replication and repair processes are similar to humans#. By using yeast I could conduct my experiment effectively without putting anyone in danger. For my purposes I used an assay for deletion formation to demonstrate the effect of different substances on the yeast. The assay allowed me to see when damage and rearrangement of the yeasts# DNA occurred. I put five filter paper disks on each of 15 selective medium Petri dishes with a set amount of yeast cells. Then I put a varying amount of each substance (with a control of zero) on every disk. By observing the increase or decrease in the growth of recombinant yeast colonies in response to the different substances, I can assess their genotoxicity and perhaps their ability to cause cancer. To reach my conclusion, I calculated the mean number of colonies that arose on each plate, and also the mean number that arose due to exposure to the chemical that gave me the most positive result (hydrogen peroxide).

**Results**
After I completed my procedure and collected al of my data, I concluded that only two of the chemicals were potentially harmful. One was hydrogen peroxide, which produced only a slight result after a lot of exposure. The other was bleach, which was so powerful in its smallest dose that it killed all of the cells.

**Conclusions/Discussion**
The results of my experiment support my hypothesis. Hydrogen peroxide triggered more, very small growths around the highest dose. This leads me to believe that hydrogen peroxide may be carcinogenic, but since the growths were so small, it would take a very long time for an individual to develop cancer from exposure to that product. In that time, an organism is more likely to die from exposure to the toxins than the development of a tumor.

**Summary Statement**
I used the yeast DEL assay to evaluate the potential carcinogenicity of five common household chemicals.

**Help Received**
My father , Dr. Adam Bailis, Phd provided me with the necessary instruction and equipment for performing my procedures. All of my experimentation was done in his lab at the City of Hope.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Laura J. Billiter

**Project Number**
**S0404**

**Project Title**
## Effect of Microwave Radiation on Chlorophyll

**Abstract**

**Objectives/Goals**
The objective of this experiment is to measure the effect of microwave radiation on the amount of chlorophyll a and chlorophyll b in spinach, lettuce, and bok choy extracts.

**Methods/Materials**
Chlorophyll was extracted from fresh spinach, lettuce, and bok choy leaves using the solvent acetone and the resulting solution was microwaved. The amount of chlorophyll a and chlorophyll b per extract was calculated both before and after microwaving using formulas from published journal articles, which required measuring the absorbance of each extract through a spectrophotometer at 662 nm and 645 nm. This procedure was repeated for three trials using 5 mL of chlorophyll extract per cuvette. A total of 10 samples were taken per vegetable, 5 of which were of the nonmicrowaved solution and 5 of which were samples of the same solution that had been microwaved for one minute.

**Results**
In trial 1, the amounts of chlorophyll a and chlorophyll b increased after microwaving the extracts of all three vegetables. In trial 2, the amount of chlorophyll b increased in all the vegetables except lettuce after microwaving the extracts; the amount of chlorophyll a increased in all the vegetables except for spinach. In trial 3, the amount of chlorophyll a increased in all the vegetables after microwaving except for spinach, while the amount of chlorophyll b dropped in all the vegetables. On average, the amount of chlorophyll a and b showed an increase after microwaving instead of the proposed decrease.

**Conclusions/Discussion**
My hypothesis was proved wrong, since neither chlorophyll a nor chlorophyll b showed a consistent decrease in amount after microwaving; the opposite happened to be true, although there were many discrepancies between trials. This can possibly be attributed to errors caused by the high volatility of acetone and perhaps to the insufficient time each extract was microwaved.

**Summary Statement**
Measuring the effect of microwave radiation on the amount of chlorophyll a and chlorophyll b in different vegetable extracts.

**Help Received**
Borrowed equipment and instruments from AP Biology teacher; brother took pictures; parents provided acetone and vegetables.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

Varun Chaturvedi

**Project Number**

**S0405**

**Project Title**

## CD1d Antigen Expression for the Therapeutics of Prostate Cancer

**Abstract**

**Objectives/Goals**

Prostate Cancer is one of the most common type of cancer for men over the age of 50. Thus deciphering the mechanism that promotes this cancer growth in an organism is of great interest to many of these scientists, specifically the interaction between antigen and antibodies in the immune system. The antigen of interest in my experiment is CD1 d Antigen which is recognized by the Natural Killer T-Cells in the immune system. First, we tested whether this CD1 d antigen was present between different cancers (prostate cancer, lung cancer, and liver) through a FACS Machine where the computer detects if CD1 d is expressed through fluorescence. It was through this experiment that we found prostate cancer to express this specific antigen. Our next experiment was to inject these cancer cells into mice with NK T-Cells and one without these cells. To a surprise after 2-3 weeks analysis, we found out that the mice with the NK T-Cells help induce prostate cancer growth, while the mice without these cells suppressed cancer growth. After accomplishing this experiment, it was seen, that the NK T-Cell, which has been proven to be one of the more complex defense mechanism in our bodies, actually helps induce Prostate Cancer growth. The results of this experiment really help show and presents conclusive evidence from cancer growth in mice with the NK T-Cells as opposed to knockout mice for these immune cells. Inclusive of this, results of this experiment can help many other scientists analyze as to why the bodies own defenses help grow cancerous cells and ultimately help in research to fight against prostate cancer.

**Summary Statement**

The main purpose of my project was to examine the role of an anitgen presenting molecule, specifically the CD1 d Antigens, in the growth of prostate cancer.

**Help Received**

Dr. Vipin Kumar helped with the data analysis for the experiment, and helped with the procedures; My father helped review my lab write up

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Jennifer S. Chen

**Project Number**
**S0406**

**Project Title**
# Exploring a Sequencing-based Human Identification Method as a Replacement for Current Fragment Sizing Technology

**Abstract**

**Objectives/Goals**
There is currently an enormous forensic DNA backlog, meaning a large number of DNA samples from criminals have not been analyzed to create DNA profiles and entered into the FBI Combined DNA Index System (CODIS) database. Consequently, not all potential DNA profiles are in the database for comparison with DNA samples collected in the future. These DNA samples are used to provide evidence to determine the guilt or innocence of those accused of serious crimes, making this database critically important. The purpose of my project is to explore an innovative way to reduce the backlog of DNA samples by proposing a more accurate, more efficient, and less costly method of human identification--ultra-high throughput (UHT) DNA sequencing.

**Methods/Materials**
I amplifed the miniSTR D7S820 locus of three individual samples with polymerase chain reaction (PCR) on a thermal cycler. I then sized the PCR products by two methods: 1) agarose gel electrophoresis to represent the current fragment sizing technology and 2) Sanger DNA sequencing, a conventional sequencing method, to simulate UHT DNA sequencing. I also compared the cost and time required to finish processing a DNA backlog of variable size by both methods.

**Results**
Gel electrophoresis yielded blurred bands that were very difficult to size, while the Sanger method produced clear sequences from which size and allele were easy to determine. In addition, comparing fragment sizing with UHT DNA sequencing showed that the latter is both more efficient and less costly when dealing with a large number of DNA samples, such as those of the backlog.

**Conclusions/Discussion**
UHT DNA sequencing holds great promise as a more accurate, more efficient, and less expensive human identification method than what is currently used.

**Summary Statement**
My project explores a human identification method based on ultra-high throughput DNA sequencing as a more accurate, more efficient, and less costly replacement for the current method based on fragment sizing.

**Help Received**
Used lab equipment at the San Jose BioCenter under the supervision of Dr. Chun-Nan Chen.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Kevin N. Chen

**Project Number**
**S0407**

**Project Title**
## Using RNA Interference to Block HIV Entry into Cells

**Abstract**

**Objectives/Goals**
Human immunodeficiency virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS), an epidemic across the world that has no cure. HIV needs two receptors in order to enter a T- cell: CCR5 and CD4. Importantly, some people are born without CCR5 and are resistant to HIV infection. This project investigates the use of a recent genetic approach, RNA interference (RNAi), to reduce CCR5 RNA and thereby prevent HIV from entering target cells. The overall goal is to test several new RNAi reagents and to determine if combinations targeting different regions of CCR5 mRNA are more effective.

**Methods/Materials**
One form of RNAi called Short hairpin RNA (shRNA) was used to target the RNA for the CCR5 receptor. Rather than working directly with HIV or CCR5, which are technically more difficult ( and dangerous), a fluorescent #reporter# was created in order to have an easier and more quantitative method of measuring the amount of CCR5 mRNA. DNA cotransfection was used to introduce shRNAs and the reporter into cells and the effects measured by microscopy and flow cytometry.

**Results**
A CCR5 reporter system was constructed using the red fluorescent gene, mCherry, and used to identify several new shRNAs that target CCR5 mRNA well. Two shRNAs appear to downregulate better in combination than each alone.

**Conclusions/Discussion**
These results show that potent shRNAs can be identified to downregulate the HIV receptor CCR5. Combinations of these shRNAs may be even better at downregulating CCR5. In further studies, the two shRNAs will be combined into a single plasmid to confirm their potency. In the future, combinations of shRNAs targeting CCR5 may be used by other researchers as a stem cell therapy to mimic a natural genetic deficiency of CCR5 and block HIV infection of T-cells.

**Summary Statement**
This study focuses on investigating one potential approach to eliminate the CCR5 receptor.

**Help Received**
Used laboratory equipment at David Geffen School of Medicine at UCLA; supervised and mentored by Dr. Masakazu Kamata.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

**Nicholas K. Davis**

**Project Number**

**S0408**

**Project Title**

## Inhibiting Chemosensory Function via Introduction of Anticonvulsant Ethosuximide for Net Extension of Schmidtea mediterr

**Abstract**

**Objectives/Goals**

Experimentally influencing the chemotaxis of Schmidtea mediterranea, a planarian oft chosen for biomedical research because its genome (which is currently being sequenced) shares certain sequences of DNA also found in humans, and subsequently inducing an increase in planarian lifespan, holds vast implications for substantiating the existence of evolutionarily conserved mechanisms for organismal lifespan control.

**Methods/Materials**

1.Breeding of four different sets of Schmidtea mediterranea and Dugestia tigrina in 500 mL beakers (one in mineral/distilled water environment, others in same setup, characterized by 5%, 10% and 15% net ethosuximide solutions, respectively).

**Results**

The results of this experimentation support my hypothesis that by inhibiting a certain neurological function/sense (as in olfaction), lifespan can be lengthened.

**Conclusions/Discussion**

The fact that inhibiting a planarian#s ability to respond to trophic stimuli after already being deprived from necessary nutrient positively affected the planarian#s lifespan gives insight into possible evolutionary conserved means for lifespan augmentation/control. This is to parallel the phenomenon demonstrated by humans, where the sizeable depreciation or total loss of one sense is compensated with the amplification of another sense. Evolutionarily speaking, it is plausible that an organism as stem-cell-based in composition as a planarian would have the genetic programming to respond to certain drastic threats to survival, such as the loss of an entire function. In this way, it would go well to surmise that the neoblasts of the planarian#s body would respond with the differentiation of its progeny to aid in other biological processes (as the embryonic stems cells of planaria are hypersensitive to DNA damage and automatically induce apoptosis to rid the body of damaged cells, impaired olfactory sense could be tagged as extraneous, with neoblast cells aiding the function of other processes). Such a claim is in part substantiated by the fact that planarian sense of smell actually decreased during prolonged exposure to ethosuximide.

**Summary Statement**

Inhibiting Chemosensory Function for Net Extension of Organismal Lifespan: Unveiling an Evolutionarily Conserved Mechanism for Lifespan Control

**Help Received**

Used laboratory glassware from Clovis West High School; otherwise, experimentation and board executed independently

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

**Samantha M. Guhan**

**Project Number**

**S0409**

**Project Title**

## A Tripeptide Based Study of the Role of Flanking Amino Acids in DOPA Mediated Mussel Adhesion

**Abstract**

**Objectives/Goals**

Mussel adhesion is achieved through secreted proteins such as mfp3 and mfp5, which have a high proportion of the modified amino acid Dopa. Dopa is suspected to be a key player in adhesion due to its ability to bind reversibly to Fe3+ and to be oxidized to quinone. The redox potential of the Dopa-quinone system favors quinone at the pH of sea water; however quinone is absent in mfps indicating that Dopa's oxidation is suppressed when it is a part of mfp proteins. This study employs a tripeptide model system to investigate the hypothesis that amino acids flanking Dopa affect its ability to be oxidized to quinone and to bind Fe3+.

**Methods/Materials**

Published sequences of mfp3 and mfp5 of marine mussels were analyzed to design tripeptides (GYG, KYK, NYN, GYK, GYN, RYN, RYG and GGG where Y is Dopa). Spectrophotometric assays were used to test the peptides at 80µM and 40µM concentration at pH 7 and 8.3 for their capacity to bind Fe3+ and get Dopa oxidized. Experiments were performed to study iron binding, auto oxidation, oxidation in the presence of mushroom tyrosinase, and the oxidizing agent NaIO4. Experiments were also carried out to study the competition between oxidation and iron binding.

**Results**

Results indicate that Dopa alone was best; the presence of flanking amino acids reduced the extent of both Dopa oxidation and iron binding. Auto oxidation was not seen at any pH. In general, oxidation was better at pH 8.3 while iron binding was better at pH 7. Competition studies show that presence of an oxidizing agent reduced iron binding; however oxidation was enhanced in the presence of Fe3+ especially for peptides containing asparagine. The nature of flanking amino acids affects both the extent of conversion of Dopa and its fate. Tripeptides such a GYG and RYG show robust oxidation and iron binding at both pHs. RYG always prefers oxidation and NYN iron binding.

**Conclusions/Discussion**

Flanking amino acids clearly affect DOPA mediated mussel adhesion by controlling the extent to which Dopa gets oxidized and binds to iron. An interesting observation is that Mytilus californianus, a Pacific mussel subject to harsh environment, achieves optimum adhesion by employing robust motifs in mfp3 while using motifs that specialize in iron binding or oxidation in mfp5. Future work could focus on studying whether the relative distribution of mfp3 and mfp5 is a function of iron concentration and oxygen levels.

**Summary Statement**

My project uses a tripeptide model system to demonstrate that flanking amino acids affect DOPA mediated mussel adhesion significantly, both by affecting DOPA's oxidation and its ability to bind to iron.

**Help Received**

Dr. Les Miranda (Amgen) gave peptides; used lab equipment at CSUCI under the supervision of Mike Mahoney and Cathy Hutchinson; Athol Wong communicated with CSUCI; mother helped understand concepts, assisted me in repetitive steps of the assay such as pipetting etc. to optimize lab time.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
John N. Ho

**Project Number**
**S0410**

**Project Title**
## The Effect of pH on Lactase

**Abstract**

**Objectives/Goals**
The objective of this project is to determine the pH level of an environment in which lactase, an enzyme that breaks down lactose to galactose and glucose, is most active.

**Methods/Materials**
In a test tube 3 mL of water, 1 mL of lactose and 1 mL of a specific buffer solution (7.0 pH as the control; 2.0, 4.0, 6.0, 8.0, 10.0 pH as experimentals) were combined to form an assay mixture. Next, 1 mL of a lactase solution was added and ten minutes was allowed to pass. At exactly ten minutes, one droplet of the assay and lactase mixture was tested with a blood glucose meter displaying a number that represented the amount of glucose present in mg/dL. This number is used to gauge lactase activity. It is assumed that all glucose came from lactose converted via lactase. Ten trials were conducted for each control and experimental group.

**Results**
The results from this experiment suggested that the optimum pH level for lactase activity was between 2.0 to 4.0 pH, a moderately acidic environment. Compared to glucose levels for 7.0 pH assay mixtures, glucose levels for 4.0 pH assay mixtures increased by 223.3%. Assay mixtures for 6.0 pH assay mixtures increased by 138.2%. The alkaline experimental groups had extremely low levels of glucose with glucose levels in the 10.0 pH assay mixture decreased by 100% when compared to levels in the control.

**Conclusions/Discussion**
The hypothesis was derived from research conducted in preparation for the project gave that lactase worked the best in a neutral (7 pH) or slightly acidic (6.5 pH) environment. However, the results of this experiment showed that the pH level for optimal latase activity was lower, more moderately acidic environment (4.5 pH). Calculated error bars showed that the results were statistically significant, ruling out major human error regarding measurement of glucose levels. The results suggest that people suffering from lactose intolerance should take lactase supplements in tablet form instead of using droplets applied to liquids containing lactase like milk.

**Summary Statement**
This project explores the activity of lactase based on pH, helping determine the best treatments for lactose intolerant patients.

**Help Received**
Used classroom lab and equipment at CAMS High School under the supervision of Dr. Kathleen O'Neill.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Eric V. Jang

**Project Number**
**S0411**

**Project Title**
## UV Exposure Accelerates Telomere Shortening: An Implication of Premature Aging

**Abstract**

**Objectives/Goals**
This experiment provides a health statistic that demonstrates one of the dangers of UVA in biological systems, particularly in yeast. Telomeres are protein caps that protect chromosomes from natural DNA shortening during mitosis (binds to repeated DNA with lengths varying between organisms), and are responsible for aging on a cellular level. UV-A is a type of ultraviolet light that causes cells to produce DNA-damaging reactive oxygen molecules. The goal of my experiment was to find out whether telomeres themselves shorten faster in the presence of ultraviolet light due to these molecules. The results show the life-shortening effects of UV light on irradiated organisms.

**Methods/Materials**
Yeast cells were divided into groups and allowed to proliferate under specific conditions for three days. Changing DNA patterns through HindIII restriction enzyme digests were recorded. Controlled variables were integrated into the experiment, including colony separation, stable environment, and un-irradiated control groups. The independent variable was the duration of UVA irradiation. Hind III restriction enzymes fragmented non-telomeric DNA but left telomere DNA unaffected. Electrophoresis allowed the separation of the DNA fragments so that the CBI (comparative band intensity) of 200-400bp DNA could be measured using ImageJ software.

**Results**
All test groups experienced semi-linear downward trends in CBI. For example, the B1 group (15 minute exposure) begins with a 171.92% the intensity of the control group average, but dropped down to 139.52% and then 99.66% of CBI. Similar trends reoccur in the other test groups to varying degrees, where surprisingly, the B group experiences a larger total CBI decrease.

**Conclusions/Discussion**
The definite implications of this experiment demonstrate the link between UV light and premature cellular aging caused by telomere shortening. From these results shown in yeast cells, I conclude that sources of excessive ultraviolet radiation also cause telomeres in humans to shorten in the same manner due to identical telomere function in all eukaryotic organisms.

**Summary Statement**
Exposing yeast cells to UV accelerates telomere shortening, implying premature UV aging risk for humans.

**Help Received**
Teacher helped stain toxic gels, borrowed equipment from SCCBEP, Schmahl Sciences, and Lawrence Livermore National Lab.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Ann Kim

**Project Number**
**S0412**

**Project Title**
## The Effects of Glucose on Insulin Receptor Protein Production

**Abstract**

**Objectives/Goals**
The projects objective is to investigate the effects of increased glucose levels on the insulin receptor protein production in tilapia (Oreochromis mossambicus). Fish generally exhibit an impaired ability to up-take glucose, much like humans with type 2 diabetes. It has been shown that plasma insulin levels do increase in fish following a glucose injection. Fish possess many similar physiological characteristics to mammalian metabolism; therefore fish are an ideal model to study vertebrate glucose metabolism. For my project, seven fish were injected with glucose and seven fish were injected with saline (control). Liver tissues were collected four hours post injection to measure the amount of insulin receptor protein. Due to the observation that increased levels of glucose stimulates insulin release, I hypothesize that, in turn, glucose treatment will increase insulin-receptor protein production in tilapia liver cells.

**Methods/Materials**
SDS-PAGE was run, Western blot performed. Transfer proteins onto nitrocellulose membrane. After transfer, expose to primary antibodies (1:200). Leave overnight. Expose to secondary antibodies (1:20,000). Expose to ECL (enhanced chemiluminescence). Take picture

**Results**
Glucose treatment significantly elevated liver insulin receptor-b protein levels. Nevertheless, Glucose treatment significantly elevated plasma glucose.
Insulin receptor-b antibody validation: Tissue homogenates were separated by SDS-PAGE and transferred to nitrocellulose membrane. Blot was probed with a human insulin receptor-b antibody at a 1:200 dilution. To validate the specificity of the antibody; the human insulin-b antibody was pre-absorbed with five-fold by weight excess of the insulin receptor-b blocking protein.

**Conclusions/Discussion**
After four hours, blood glucose levels remain high confirming the problem of glucose up-take.My hypothesis was supported. It can be seen that the insulin receptor protein concentration from glucose is 1.6X higher than the concentration from saline. Since there was an increase in IR proteins from the glucose injection, it should directly correlate with less plasma glucose. Nevertheless, four hours post injection plasma glucose levels remain significantly high. Thus, the amount of insulin receptor proteins present is not the contributing problem to glucose uptake in Tilapia liver cells.

**Summary Statement**
Increasing Insulin Receptor protein production by elevating glucose levels in Tilapia for therapeutic effects to treating type 2 diabetes.

**Help Received**
Used lab equipment and help with experimental design from Dr. Larry Riley, mother helped make project board.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Anna K. Kimball

**Project Number**
**S0413**

**Project Title**
## Antibody Based Analysis of Digestive Enzyme Action on Gluten in Wheat Flour using Enzyme Linked Immunosorbent Assay

**Abstract**

**Objectives/Goals**
The presence of gluten was tested using three different enzyme formulas: Gluten Digest, Gluten Ease, and Ultra-Zyme, to determine which enzyme breaks down gluten the best after 90 minutes in various environments.

**Methods/Materials**
The first analysis used stomach-like conditions (prepared to allow enzymes to break down in approximately their natural pH of 2.5 and 98 degrees F temperature environments) for a period of 90 minutes. Enzyme Linked Immunosorbent Assay test strips (E-Z Gluten) were then used to measure the presence of gluten. A second analysis was performed to test our suspicion that either the sodium bisulfate or the enzyme mixtures were affecting the antibodies in the test strips.

**Results**
Results show that the pH and/or enzymes were affecting the results. Additional tests were performed without the presence of sodium bisulfate and with the destruction the enzymes by boiling samples for 15 minutes prior to analysis.

**Conclusions/Discussion**
Ultra-Zyme breaks down gluten the best. Gluten Ease worked second best. Gluten Digest gave faulty readings, and we were unable to determine actual results. Further experiments should be conducted to account for irregularities in test results.

**Summary Statement**
Antibody Based Analysis of Digestive Enzyme Action on Gluten in Wheat Flour using Enzyme Linked Immunosorbent Assay (ELISA).

**Help Received**
Father helped experimental design; Sister and Mother helped with board; test strips/advice from Laura Allred, Lab Director ELISA tech, Florida; Dr. Margaret Rice Ph.D. Assistant Professor of Biochemistry Cal Poly San Luis Obispo explained antibodies

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

Nitish Lakhanpal

**Project Number**

**S0414**

**Project Title**

## Computational Prediction of Beta Structure from Amino Acid Sequence in a Class of Pathologically Relevant Proteins

**Abstract**

**Objectives/Goals**

Because structure dictates the function of proteins - physiological or pathological - protein structure discovery is of great interest to biological science. Though experimental approaches have yielded good results, these efforts have proven ineffective for beta-rich proteins such as amyloids and autotransport proteins - both implicated in pathologies such as Alzheimer's Disease, meningitis, and pertussis - due to difficulties with crystallization and insolubility. Interest has therefore grown in computationally predicting beta structure from primary amino acid sequence. My objective is to develop an algorithm that improves the prediction accuracy achieved by current algorithms. Based on insights into the nature of protein structure, I suggest an algorithm that is governed by both near and distant interactions among the amino acids comprising the protein.

**Methods/Materials**

2.4 GHz Personal computer, 3 GB RAM. Coded in C++: Take a non-redundant sampling of protein structures in the Protein Data Bank. Extract frequencies of residues and pairs of interacting residues involved in beta structures, yielding tables w(i,j,theta) containing the frequency of pairing between amino acids i and j with orientation theta and v(i,theta) containing the frequency of observing amino acid i with orientation theta - then compute a "solo" and a "duo" propensity for every subsequence of permissible length. Compute a single score by combining the two. Do this for several threshold values. Compute final globally optimum structure assignment.

**Results**

We ran the algorithm on two sets of solved non-redundant proteins: 16 amyloids and 21 autotransporters. Our algorithm outperformed its predecessors in sensitivity to beta strands and in the false positive rate of beta strand discovery, showing approximately a two-and-a-half times improvement.

**Conclusions/Discussion**

Our algorithm improved beta structure prediction substantially by considering close as well as distant interactions in a polypeptide chain. We also explored the relationship between prediction sensitivity and false positives and the threshold level used - enabling the algorithm's use for a spectrum of prediction objectives. Though applied to 2 classes of proteins, our algorithm has broad applicability for predicting beta structure.

**Summary Statement**

We developed an algorithm to predict beta structure in proteins from amino acid sequence based on properties of known beta structures, effectively merging single-amino-acid level analysis with the possibility of long-distance interactions.

**Help Received**

Discussed idea for project with Dr. Bonnie Berger at MIT.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

Katie V. Luong

**Project Number**

**S0416**

**Project Title**

## Banana DNA Extraction

**Abstract**

**Objectives/Goals**

The objective is to determine if the banana#s DNA denatures overtime. In this experiment, the bananas are categorized into three different ripening stages: the under-ripe, ripe, and over-ripe. If the banana is in the under-ripe ripe stage, then it is expected to have the most amount of DNA.

**Methods/Materials**

For this experiment, the methods are spilt into two parts. The first method requires an online #Banana DNA Extraction# protocol to extract the banana#s DNA from its content. This method requires primarily of an antibacterial hand soap, ethanol, pipettes, mortar, pestle, salt, coffee filters, and beakers. As for the second procedure, it involves analyzing the DNA with a centrifuge, vortex, and spectrophotometer. This will give the concentration of the DNA, the DNA/ Protein Absorbance, and its purity. Three trials are done for each stage of the banana.

**Results**

The banana in the under-ripe stage holds the greatest concentration of DNA. In addition, this stage of the banana holds the highest value in DNA/ Protein Absorbance, and its purity when compared to the other stages.

**Conclusions/Discussion**

The results support the project#s hypothesis. This project may indicate that, as time passes, the banana#s DNA denatures overtime. Based on an article by Frank Sherwin, the banana#s DNA resembles fifty percent of the human DNA. This provides information that possibly the human DNA may deteriorate also.

**Summary Statement**

My project is about extracting the DNA from a banana#s content and then analyzing the DNA to see if aging occurs overtime.

**Help Received**

Sister provided access to lab and lab equipment; Parents bought me bananas; Mr. Ho gave great advice about experimental design

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

**Isaac Madan; Haran Sivakumar**

**Project Number**

**S0417**

**Project Title**

## Identifying Two Populations of Neurons in the Developing Cerebellum

**Abstract**

**Objectives/Goals**

In the past, genes for fluorescence have been utilized for tagging viruses. The insertion of these genes allowed for viral strains to be more immediately visible and facilitated additional analysis of the effects of the virus. The purpose of our experiment is to further the use of fluorescent genes in this capacity. We aimed to create separate lentivirus each containing a unique fluorescence and tested to see if the two could be independently distinguished in one specimen.

**Methods/Materials**

Plasmids were injected with their corresponding restriction enzymes. Genes for the chosen fluorescence were inserted into the gaps created by the enzymes. The plasmids were turned into virus from their constructs and were then humanely injected into mouse pups# developing cerebellums. The cerebellums were sectioned, stained, and imaged.

**Results**

Based on the imaging results, it appears that the experiment was successful. In addition to the standard calbindin staining of Purkinje neurons, other neurons seem to have been stained as well. Both the green fluorescent proteins (GFP) and the cyan fluorescent proteins (CFP) appear to have successfully infected cells. Most infected cells appear to exhibit both blue and green staining; however, there are a substantial number of cells with only one visible color, either green or blue.

**Conclusions/Discussion**

The results indicate that it is certainly possible to stain neurons with different fluorescent proteins in the same specimen. While the overwhelming presence of dually infected cells is peculiar, the fact that numerous cells are stained with only one GFP or only CFP seems to suggest that the outcome, while not what was theoretically expected, did successfully demonstrate the potential for multi-colored staining. A multi-colored approach to studying the impact of the cerebellum certainly will open new doors in research in the field of molecular biology. Through the use of multiple fluorescent proteins, researchers can analyze the effects of multiple viruses when targeting different cells or the same cell in a single specimen.

**Summary Statement**

This project sought to demonstrate that cells infected with different virus can be distinguished by using fluorescent proteins.

**Help Received**

Used lab equipment and conducted experiments at Stanford University under the supervision and leadership of Dr. Ashvin Sangoram

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Sanjana Marpadga

**Project Number**
**S0418**

**Project Title**
## Beclin I: A Novel Marker to Evaluate Human Islet Quality

**Abstract**

**Objectives/Goals**
The objective of the current study is to develop novel assays based on autophagic and apoptotic cell death markers to evaluate the function of transplanted islets in Type 1 Diabetic (T1D) mouse models.

**Methods/Materials**
Islet cell death from apoptosis and autophagy was determined by using DNA fragmentation (TUNEL Assay) and Beclin I staining, respectively. Insulin producing beta-cells were detected by staining with insulin specific antibodies. Briefly, human islet sections on glass slides were incubated with primary antibodies followed by appropriate secondary antibodies conjugated to fluorescent dyes. Fluorescence intensity was quantified by using a fluorescence microscope equipped with an iCys laser scanning cytometer (LSC). Islet function was evaluated by monitoring blood glucose levels in streptozotocin induced T1D mice models up to one month post islet transplantation.
Protein levels in cell lysates were determined by immunoblotting with antibodies specific to cell death markers and proteins on blots visualized using chemiluminescence methods. Statistical analysis was performed using Microsoft Excel software.

**Results**
Treatment of human islets with various stress conditions, such as hypoxia, oxidant stress (hydrogen peroxide) and inflammatory cytokines, ƒnincreased levels of proteins involved in both apoptosis (phospho-H2AX, activated caspases) and autophagy (Beclin 1). However, immunostaining of islets showed only an increase in TUNEL and Beclin I positive staining in insulin producing beta-cells, suggesting only the TUNEL assay and Beclin I are useful biomarkers to detect islet cell death. Screening of human islets from different batches displayed varying levels of both Beclin I and TUNEL staining. Furthermore, Beclin 1 and TUNEL positive staining significantly correlated with islet dysfunction, i.e., elevated blood glucose levels in islet transplanted T1D mice.

**Conclusions/Discussion**
These results show that both autophagy and apoptosis play an important role in islet cell damage during isolation. The data with T1D mice clearly demonstrated that islet damage from autophagy or apoptosis during isolation can reduce the ability of beta-cells to produce sufficient insulin to reduce blood glucose levels. Therefore, Beclin 1 can be used effectively in parallel with the apoptosis specific TUNEL assay for the evaluation of islet quality prior to transplantation.

**Summary Statement**
Beclin I was identified as a novel biomarker to evaluate islet quality prior to transplantation into Type 1 Diabetic patients.

**Help Received**
Performed research at Department of Diabetes, Beckman Research Institute of City of Hope, Duarte, CA, under the guidance of Dr. Ivan Todorov.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Shamik Mascharak

**Project Number**
**S0419**

**Project Title**
## Synthesis of Fluorescent Silica Nanoparticles Conjugated with RGD Peptide for Detection of Invasive Human Breast Cancer

**Abstract**

**Objectives/Goals**
The objectives of this project were to synthesize fluorescent silica nanoparticles (FSNPs) with RGD peptide covalently linked on the surface amino groups and to employ these FSNPs as fluorometric probes to detect invasive human breast cancer cells via strong interaction between RGD peptide and integrin proteins on the cell surfaces.

**Methods/Materials**
3-aminopropyltriethoxysilane (APTS), fluorescein-5-isothiocyanate (FITC), tetraethylorthosilicate (TEOS), cyclohexane, n-hexanol, triton X-100, 3-(Trihydroxylsilyl)propylmethylphosphonate (THPMP), DAPI, Perkin-Elmer Fluorescent Spectrometer, Zeiss HAL 100 Fluorescent Microscope, JEOL 1200 EX TEM. Methods: First, a batch of 69 mg of APTS and 5.25 mg FITC was dissolved in 1 mL of absolute ethanol under dry N2 atmosphere to isolate the fluorescent silane reagent. Next, 50 microL of the FITC-ATPS conjugate, 15 microL of THPMP (to prevent aggregation), 100 microL TEOS and 100 microL ammonium hydroxide were reacted in a water/oil microemulsion. After 24 hours, the microemulsion system was destabilized with ethanol, and the FSNPs were collected by centrifugation. The FSNPs were then washed and centrifuged (2000 RPM, 10 min), and checked for quality via TEM and fluorescence microscopy (515 nm). For tumor targeting, the FSNPs were conjugated to RGD peptide with SPDP as the coupling reagent. The fluorescence properties of the peptide-nanoparticle conjugates were checked via fluorescent spectroscopy and TEM imaging. Both RGD-FSNP conjugates and FSNPs were then added to MCF7 (non-invasive breast cancer cells), MDA-MB 435 (transformed human breast cancer cells), and MDA-MB 231 (metastasized breast cancer cells) grown in 8-well trays (2 days, 5% CO2 atmosphere). After 2 hrs, the cells were thoroughly washed, fixed, stained with DAPI, and subjected to fluorescence microscopy.

**Results**
The FSNPs synthesized in this project showed a narrow range of dispersivity (70 nm). They are stable in aqueous buffer over weeks and strongly fluoresce at 515 nm. The FSNP-RGD peptide conjugates selectively got attached to the high concentration of integrins expressed on the surface of the metastasized cancer cells. In the case of the non-invasive cells, the expression of integrin was low and hence such cells showed very few FSNPs on the cell surface.

**Conclusions/Discussion**
The results confirm that FSNP-RGD conjugates are excellent imaging tools for cancer detection.

**Summary Statement**
My project is aimed at synthesizing a non-toxic, fluorometric nanoparticle probe to detect invasive human breast cancer cells.

**Help Received**
Michael Rose (a UCSC grad student) helped in the synthesis and cell experiments; Walter Bray of UCSC provided the cells; Dr. Yang of UCSC helped in obtaining the TEM images.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

**Vijay Menon; Karthik Sreedhara; Abhishek Venkataramana**

**Project Number**

**S0420**

**Project Title**

## Ethanol Sources and Yields: An Analysis of Raw vs. Processed Sources and How Temperature and Enzymes Affect Yield

**Abstract**

**Objectives/Goals**

The purpose of our experimentation was to find the source of ethanol, cellulosic or non-cellulosic, that provided the greatest yield of ethanol content after fermentation. Variables, such as temperature, time, and enzymes were used to find the most productive source. Seen as a potential alternative fuel of the future, the discovery of a viable source of ethanol is a key step in fighting global warming.

**Methods/Materials**

In experiment one, the control experiment, one liter solutions of raw ethanol sources, including corn kernels, husks, sugarcane, and alfalfa, were blended and ground finely. The solutions were heated to kill bacteria, innoculated with yeast, and measured for ethanol content with a hydrometer. Experiment two used the same raw sources which were autoclaved rather than heated and treated with amylase and cellulase enzymes. The solutions were accurately measured for ethanol content using a spectrophotometer to determine absorbance. Experiment three repeated the steps in experiment two, except store-bought processed ethanol sources were used. These included corn starch, brown sugar, and glucose.

**Results**

Experiment one without enzymes yielded negligible ethanol content using the hydrometer as a measurement tool. Experiment two yielded relatively small, but noticeable ethanol production, especially when allowed to ferment at 37C as opposed to room temperature. Experiment three, using processed sources, produced large concentrations of ethanol, with non-cellulosic sources such as glucose and brown sugar producing the greatest amount of ethanol.

**Conclusions/Discussion**

In general, higher temperature and more time led to a greater net yield of ethanol production. The results showed that non-cellulosic sources such as glucose and brown sugar provided the greatest net yield. Store-bought processed sources produced far more ethanol than raw sources which were broken down by hand. Experimentation showed that ethanol production is negligible without the adding of specific enzymes to accommodate the fermentation.

**Summary Statement**

An experiment directed towards discovering the source that provides the greatest net yield of ethanol and an analysis of the factors affecting yield.

**Help Received**

Professor Stephens provided a lab and research guidance.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Danielle P. Monahan

**Project Number**
**S0421**

**Project Title**
# Oligomerization of the P. aeruginosa Aer-z N-terminal Domain May Determine Signaling State

**Abstract**

**Objectives/Goals**
The goal was to determine if the N-terminal domain of the Aer-z affects oligomerization of the receptor when it is oxidized or reduced.

**Methods/Materials**
P. aeroginosa fragments Aer-z PAS 173-289 was examined as a compact monomer in oxidized and reduced states.

**Results**
Aer-z PAS 173-289 elevated as compact monomer in oxidized and reduced states. N-terminal domain Aer-z-289 elevated as a compact monomer when oxidized but not when reduced.

**Conclusions/Discussion**
Aer-z N-terminal domain assumes different conformations in different signaling states and suggests that oligomerization is associated and interacts with a loss of signaling.

**Summary Statement**
The goal was to determine if the N-terminal domain of the Aer-z affects oligomerization of the receptor when its oxidized or reduced.

**Help Received**
Used lab equipment at Loma Linda University under the supervision of Dr. Watts and Dr. Taylor in a summer immersion program.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Megan D. Nicponski

**Project Number**
**S0422**

**Project Title**
## Self-Medicating Plants

**Abstract**

**Objectives/Goals**
The objective of this project was to determine if plants would produce salicylic acid in response to stress.

**Methods/Materials**
Plants were grown for testing and stressed with fungus and physical damage, then frozen using liquid nitrogen prior to being ground into powder. Salicylic acid was extracted with a mixture of ethanol and acetic acid, then sonicated to further break down the cell structure to release the chemicals.

The samples were subjected to a ferric chloride test. If salicylic acid was present, there would be a purple color change. Calibration testing indicated the ferric chloride test method could only detect salicylic acid at levels down to 0.0001 molar concentrations. As a result, to determine if a control or a stressed sample had more salicyclic acid than the other, a know concentration solution of salicylic acid was added to both the control and test samples in equal and known amounts until a color change was observed in one of them. The first one to change color would, therefore, contain more salicylic acid.

**Results**
The testing showed more salicylic acid present in the infected and physically wounded plants than in the control plants in all cases. The test method didn't yield an exact quantity of salicylic acid present in the plant material.

**Conclusions/Discussion**
More advanced methods of measurement are required to determine exact quantities of salicylic acid produced.

The Ferric Chloride test was not sensitive enough to directly detect the low levels of salicylic acid present, but as equal amounts of salicylic acid was added to each sample the test did allow a determination of which sample reacted first. As a result, the first sample to react contained the most salicylic acid.

Since it took a relatively small amount of additional salicylic acid to cause a reaction to the ferric chloride, the amount of acid already present in the stressed samples was higher than expected. More advanced methods of measurement are required to determine exact quantities of salicylic acid present.

The use of the Salicylic acid in plants is not know from this experiment just as we don't really understand how aspirin works in people, but it is definitely present when the plants are stressed. By looking for the presence of salicylic acid we may be able to determine when plants are stressed. This may allow human intervention to assist the plants. This would be very important for food crops resulting in higher yields and possibility a better quality product.

**Summary Statement**
This project was conducted to prove that plants will produce salicylic acid in response to stress.

**Help Received**
Dr.T.Eulgem, Asst.Prof. Plant Cell Biology, UCR, reviewed experimental design; M.Schroeder, Lab Specialist, UCR, main contact at UCR for lab time; Dr. Milner, Chairman, Dept. of Chemistry, Cal Poly Pomona, reviewed experimental design; Father, transportation & guidance; Sister, artwork; L.Rose,

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Marie Nielsen

**Project Number**
**S0423**

**Project Title**
## TSEs: Analyzing Copper 2+ Binding in the Octarepeat Region of the Infectious Prion Protein

**Abstract**

**Objectives/Goals**
By modeling the interactions between Cu2+ and the octarepeat region of the infectious prion protein and then analyzing the effects of temperature on the bonds, new insights can be made into the question of why prions do not denature under normal temperatures.

**Methods/Materials**
Computer Programs: NAMD (NAnoscale Molecular Dynamics); VMD (Visual Molecular Dynamics).
Molecular models and data: prion octarepeat region protein model (pdb); organic component submolecular mass, energy and Van Der Waals force data for all atoms in octarepeat region; atomic mass, energy and Van Der Waals force data for Cu2+.
Model preparation: Using the VMD and prp21, remove water and create two octarepeat region models: one with copper and a second without. Combine the two pdb models with atomic property data to create two psfs (protein structure file). Use NAMD energy program to generate the .xsc file. Run practice tests determining number of steps required for stable results.
Temperature Simulation: Perform steps for both models at many temperatures. Run NAMD simulation for 440 steps to generate .dcd (trajectory file) for octarepeat region. Use RMSD script to extract residue values for both models at each temperature. Visually evaluate simulation of physical movement of molecule.

**Results**
The graphs show the RMSD values at different temperatures for the five residues. Each of the residues is a different amino acid: histidine imidazole, two glycine amides, glycine carbonyl, and trp indole. The values indicate the variability in the distance between the amino acids and the protein strand in Angstroms. A lower distance means the distance is more tightly regulated. Each RMSD simulation generates an animated graphical display of the backbone of the protein. Less flexibility means there is probably some force or bond holding the residue in place.

**Conclusions/Discussion**
This simulation shows that Cu2+ makes the octarepeat region stiffer. Thus providing further evidence that the presence of Cu2+ may be a required aspect fora misfolded prions to be more easily transferred. It provides insight that the Cu2+ changes the octarepeat region of the prion protein in such a way that denaturing it will be more difficult. By understanding why diseased prion proteins are resistant to denaturing, a solution can be found to destroy these proteins once they have been created.

**Summary Statement**
It is difficult to render diseased prion proteins harmless; this experiment suggests that the Cu2+ plays a role in making the infected prion harder to denature, and this may vary with temperature.

**Help Received**
Darrell Steely helped get me started, Dr. Glenn Millhauser gave me the coordinates needed and reviewed my completed project.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Kyle R. Rothschild-Mancinelli

**Project Number**
**S0424**

**Project Title**
# The Effects of UV Radiation on Supercoiled DNA: A Three Year Study

**Abstract**

**Objectives/Goals**
The objective was to determine whether UV radiation was strong enough to nick or break supercoiled DNA in vitro and in vivo. Specifically I tested to see how much damage the DNA backbone would have after exposure to UV radiation in vitro and in vivo, and its correlation to the survival of the exposed cells. This experiment could act as a template for effects of UV radiation on the human skin cell.

**Methods/Materials**
In 2007 I showed that solar radiation could nick and break isolated pUC19 plasmid. In 2008, I exposed pUC19 under a UV hood to test whether the DNA damage occurred at certain sequences. This year, I exposed pUC18 under a UV hood both in live Escherichia coli and as an isolated plasmid in order to begin to understand the effects on a live organism. The E. coli were killed rapidly although a few cells survived to 60 minutes. I also isolated plasmid DNA from exposed cells.

**Results**
Gel electrophoresis revealed that nicking of the DNA increased with exposure time in vivo and in vitro. Also, as the exposure time increased, the survival of the cells decreased.

**Conclusions/Discussion**
As exposure time increased, less DNA was extracted probably due to further breakage of the DNA backbone. I conclude that nicking directly correlates to the number of cells surviving. Any cells surviving at 60 minutes might have been self-shaded or possibly had survival mechanisms.

**Summary Statement**
I tested to see how much UV radiation would inhibit the growth of exposed Escherichia coli cells and damage the DNA backbone in vivo and in vitro.

**Help Received**
Used lab equipment at NASA Ames Research Center under the supervision of Dr. Lynn Rothschild

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
San Singh

**Project Number**
**S0425**

**Project Title**
## Zbtb7 Gene Expression in Various Cancer Cell Lines

**Abstract**

**Objectives/Goals**
The purpose of this investigation is to determine differences in the endogenous expression of the zbtb7 gene in different cancer cell lines.

**Methods/Materials**
The experimental design of this experiment involved comparing various cancer cell lines for expression levels of the zbtb7 gene. First, RNA will be extracted from various cancer cell lines. Next, RNA concentrations will be standardized using UV-Vis spectroscopy. Then qRT-PCR will be used to isolate the zbtb7 gene and amplify it. Finally, gel electrophoresis combined with Kodak Digital Science densitometry software will be used to quantitatively analyze the results.

**Results**
The densitometry results from the gel electrophoresis of the experiment showed that the mean intensity values for the MCF7 breast cancer cells, the A549 lung cancer cells, and the PC3 prostate cancer cells were statistically equivalent to each other.

**Conclusions/Discussion**
These results support the notion that the zbtb7 gene plays an important role in the regulatory processes of the cell cycle and that it is especially important in this role in that it could act as a more universal controller of the oncogenes that lead to the breakdown of the regulatory functions of the cell cycle. It has been suggested that the zbtb7 gene functions by preventing the function of p14ARF, which serves to inhibit mdm2, thus promoting p53, which leads to the activation of p21, inactivating a number of cyclin-CDK complexes which allow cells to pass through the G1/S phase of the cell cycle. Preventing the zbtb7 gene from causing these effects would be an interesting avenue for approaching further research on the universal mechanisms of cancer. Furthermore, this specific avenue of cancer research regarding the control of the cell cycle would also offer the unique benefit of being able to halt the progression of the cancer at any stage by shutting down its ability to rapidly engage in uncontrolled cell division.

**Summary Statement**
This project measured zbtb7 gene expression in various cancer cell lines.

**Help Received**
Used lab equipment at the University of the Pacific under the supervision of Dr. Jesika Faridi and graduate student Ashish Sawhney.

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**
Tyler H. Taylor

**Project Number**
**S0427**

**Project Title**
## DNA Barcoding as a Tool to Identify Commercially Marketed Fish

**Abstract**

**Objectives/Goals**
Overfishing is a major factor in the decline of fish diversity, and productivity of fisheries worldwide. In order to combat overfishing, DNA barcoding could be utilized to identify mislabeled seafood and enforce fishing restrictions. This experiment was intended to test the feasibility of barcoding using a small-scale lab as a species identification tool.

**Methods/Materials**
In this study, DNA was extracted from five generically labeled samples collected in San Diego seafood markets. DNA from each sample was isolated, and Polymerase Chain Reaction (PCR) was performed, in order to amplify the cytochrome c oxidase subunit 1 (CO1) section of mitochondrial DNA for sequencing.

**Results**
Of the five samples, the CO1 fragment was amplified for three, and two yielded an accurate species ID.

**Conclusions/Discussion**
These results demonstrated the possibility of implementing small-scale barcoding operations to expose market substitution and in turn help put an end to overfishing.

**Summary Statement**
This project is intended to test the ability of DNA barcoding using a small-scale lab to identify commercially marketed fish in the context of conservation forensics.

**Help Received**
Used lab equipment at High Tech High under the partial supervision of advisor, Dr. Jay Vavra

# CALIFORNIA STATE SCIENCE FAIR
# 2009 PROJECT SUMMARY

**Name(s)**

**Paul Tran**

**Project Number**

**S0428**

**Project Title**

## Further Investigation of the Inhibitory Effect of HKa on Metastasis of Prostate Cancer Cell Line DU145

**Abstract**

**Objectives/Goals**

Prostate Cancer metastasis caused by the activation of cell surface receptors, such as EGFR, ERK, and AKT1. Cleaved high molecular-weight kininogen (HKa) is an anti-adhesive protein which, in zinc (Zn2+)-dependent manner, induces apoptosis and inhibits angiogenesis in vivo by binding to/preventing activation of cell surface receptors. The purpose of this research was to identify the role HKa and Zn2+ play in inhibiting Prostate Cancer metastasis by preventing phosphorylation of EGFR, ERK, and AKT1.

**Methods/Materials**

(1) Immunofluorescence Microscopy was used to observe cellular proliferation and changes in cellular morphology. In the first study, DU145 cells were treated with a high dose of Zn2+ (13.3µL). In the second study, DU145 cells were treated with HKa (4.5µL) and Zn2+ (2µL). (2) To detect phosphorylation of EGFR, ERK, and AKT1, DU145 cells were harvested from both Zn2+ Treated and HKa+Zn2+ Treated samples. Western Blot was performed using infrared LI-COR Odyssey Machine. (3) After plating DU145 cells in a dose on a 96-well plate, BrdU Immunohistochemistry System (Oncogene) was used to quantify the proliferation of DU145 cells in both Zn2+ Treated and HKa+Zn2+ Treated samples.

**Results**

(1) In the first study, Immunofluorescence Microscopy showed significant proliferation and changes in cellular morphology in Zn2+ Treated samples as the cells grew in clumps and took on a sickle shape. However, in the second study, Immunofluorescence Microscopy showed no proliferation and changes in cellular morphology in the HKa+Zn2+ Treated samples. (2) In the first study, Western Blot showed consistent phosphorylation of EGFR (170 kDa), ERK (42/44 kDa), and AKT1 (56 kDa) in Zn2+ Treated samples. In the second study, Western Blot showed consistent deregulation of EGFR, ERK, and AKT1 in the HKa+Zn2+ Treated samples. (3) In the HKa+Zn2+ Treated samples, Proliferation Assay showed that DU145 cells proliferated in the Control (83 per 100 cells) more so than the HKa+Zn2+ Treated (47 per 100 cells) samples. A p-value of 0.0107 suggested significant difference.

**Conclusions/Discussion**

HKa+Zn2+ have a higher potency of preventing phosphorylation of EGFR, ERK, and AKT1 than Zn2+ alone. This suggests that HKa can potentially inhibit Prostate Cancer metastasis by competitively binding to/preventing the activation of cell surface receptors responsible for cellular proliferation and migration.

**Summary Statement**

HKa, a plasma protein that induces apoptosis and inhibit angiogenesis, is used to inhibit Prostate Cancer metastasis by preventing the activation of cell surface receptors responsible for tumor proliferation, namely EGFR, ERK, and AKT1.

**Help Received**

Utilized facilities and equipment at Sol Sherry Thrombosis Research Center (Temple University School of Medicine, Philadelphia, PA) under supervision of Yuchuan Liu, PhD.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Nicholas J. Tucker

**Project Number**
**S0429**

**Project Title**
# How Does Temperature and Concentration Affect Enzyme Catalyzed Reactions?

**Abstract**

**Objectives/Goals**
The objective of this experiment was to test how the temperature of a substrate and the concentration of a catalyst affect the rate of enzyme-catalyzed reactions. I hypothesized that if the temperature of the substrate increases, then the rate of the reaction will also increase and that if the concentration of the catalyst increases, then the rate of the reaction will also increase.

**Methods/Materials**
In this experiment I used hydrogen peroxide for the substrate and catalase for the catalyst. In addition, beakers, pipettes, filter paper discs, potatoes, water, ice, a graduated cylinder, a cooler, a scale, a thermometer, and a stop watch were used. To begin the experimentation I prepared the catalase from the potatoes. Next, I soaked filter paper discs in the catalase and then placed the disc into a beaker of hydrogen peroxide, because of the chemical reaction that occurred the filter paper disc rose to the top of the beaker. I repeated this step with different temperatures of hydrogen peroxide and different concentrations of catalase, and then compared results. The speed at which the disc rose, acts as an indirect measure of the rate of the reaction.

**Results**
I found in my experimentation that as the temperature of the substrate increased, the rate of the reaction increased, becoming quicker. Also, I found that as the concentration of the catalyst increased, the rate of the reaction increased, becoming quicker.

**Conclusions/Discussion**
Through experimentation, I was able to prove my hypothesis to be true. As the temperature of the substrate increases, the rate of the reaction increases. This occurred because when the substrate is hotter, there is more energy in the reaction and the necessary activation energy to begin the reaction can be obtained more quickly. It was also proven true that as the concentration of the catalyst increases, the rate of the reaction increases. Enzymes are reusable protein binding sites, so at a higher concentration, more enzymes exist in the reaction and more proteins are available for the substrate to bind to.

**Summary Statement**
This experiment tests how certain factors affect the rate of enzyme catalyzed reactions.

**Help Received**
Sister taught me how to use a pipette, father took pictures and helped time the trials.

# CALIFORNIA STATE SCIENCE FAIR
## 2009 PROJECT SUMMARY

**Name(s)**
Y. Symphony Yu

**Project Number**
**S0430**

**Project Title**
## The Rotational Barrier of 6-Methylnicotinamide

**Abstract**

**Objectives/Goals**
The objective of my project was to determine the rotational barrier of the molecule 6-methylnicotinamide in its liquid state. In accordance with the calculated value, the experimental value of the rotational barrier should be 16.012 ± 0.212 kcal/mol.

**Methods/Materials**
Methods in proton nuclear magnetic resonance spectroscopy and computational chemistry were implemented to find the rotational barrier. The 6-methylnicotinamide sample, prepared in a solution of deuterated nitrobenzene, is first analyzed using NMR spectroscopy, with data points collected at incremented temperatures. The raw data collected by the Bruker TOPSPIN program is then transferred to Mathematica 7.0 for nonlinear regression. A composite curve is then obtained and the values of enthalpy and entropy solved for.

**Results**
According to the experiment, the rotational barrier of the molecule 6-methylnicotinamide is 12.6 kcal/mol.

**Conclusions/Discussion**
The results from the experiment yielded a percent error of 27.079% for the rotational barrier. This large percent error shows some unexpected/unknown sources of error. Because of the large discrepancy between the theoretical and experimental values for the rotational barrier, the results of this experiment are inconclusive, and further experimentation will be required.

**Summary Statement**
In my project, I determined the rotational barrier of the molecule 6-methylnicotinamide through the use of proton NMR spectroscopy and the implementation of methods in computational chemistry.

**Help Received**
Used lab equipment at the University of California, Riverside under the supervision of Professor Mueller and graduate students Jin Feng Lai and Ye Tian; Parents helped with putting together the display board.