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Western Oregon University, apalmer09@mail.wou.edu

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Cancer Research:
A Quest for a Cure

By

Alyssa Palmer

An Honors Thesis Submitted in Partial Fulfillment
Of the Requirements for Graduation from the
Western Oregon University Honors Program

Dr. Kristin Latham,
Thesis Advisor

Dr. Gavin Keulks,
Honors Program Director

Western Oregon University

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Introduction

People strive for equality in many aspects of life. They fight to make things fair and balanced for everyone. Cancer is a part of life which does not favor some people over others. It does not care about your background, race, goals, or family. It does not care what mistakes you have made or how you are learning from them.

Cancer is a condition of uncontrolled cell division. It is caused by a series of mutations to the DNA. Most mutations to the DNA are caught and prevented from replicating during the check points of the cell cycle. Cancerous mutations have evaded the checks and are replicated. There are four basic kinds of tumor cells: neoplastic, benign, malignant, and metastatic. The neoplastic cells are the initially modified tumor cells; they are isolated mutated cells. Neoplastic cells can progress to benign tumor cells, which are still localized, but are not isolated from other mutated cells. The malignant tumor cells have progressed to cause harm within the organ. Metastatic tumor cells invade the blood vessels, leading to migration from one organ to another.

In order to understand cancer, the human genome and why the cell cycle checks can be bypassed must be understood. One of the large-scale projects to bring enlightenment is the Human Genome Project. This project is critical for cancer research because the push to

sequence human DNA lead to understanding the genome, but it also directly led to advances in technologies. These technologies are used to help identify and treat cancers, as well as research preventative measures.

Literature Review

Deoxyribonucleic acid (DNA) is the informational storage molecule for nearly all living organisms. It is the coding molecule that instructs for the building of proteins. Each of the DNA molecules is highly condensed most of the time and is wound into packages called chromosomes. The chromosomes partly unwind in order to replicate and then condense again. They are the apparatus of cell government in motion, and “the smallest living structures whose movements and transformations can be seen and followed”[1]. These permanent hereditary structures contain the coding regions of the DNA termed genes[1].

Genes can vary from person to person, as seen in differences in hair and eye color, for example. They are passed from parent to offspring in chromosomes during reproduction. In humans, each child is passed one set of chromosomes from each parent, and the expressed genes are the dominant ones. The organism's genome is the entirety of the DNA sequence, not just the coding regions, found in a single (haploid) set of their chromosomes. Researchers originally estimated

the human genome to contain 50,000 to 100,000 genes, all of which they hoped to map during the Human Genome Project[2].

The Human Genome Project:

The Human Genome Project, also referred to as an "instruction book for human biology,"[3] was founded in 1990. The project brought together individuals from a variety of disciplines and countries. Great Britain, France, Italy, Japan, and the United States, among others, all came together to make the project a success. From the United States, the National Institute of Health (NIH) and Department of Energy (DOE) spearheaded the efforts[4]. While it seems logical that the NIH would have interest in creating a comprehensive map of the human genome, the involvement of the DOE may be more confusing. The DOE was interested in learning more about the effects of the nuclear energy on DNA to better understand the potential effects this form of energy would have on humans[5].

James Watson led the Human Genome Project in a series of milestones. The first five years of the project were to focus on genetic and physical maps of the human genome. Other goals, such as mapping the genomes of other organisms (bacteria, yeast, roundworm, and fruit fly), investigating the ethical consequences of the research, as well as improving technologies to promote better data acquisition were also critical[3].

Thoughtful evaluation led to the "four A's of the Human Genome Project"[6]. The A's are accurate, assembled, affordable, and accessible. The final product must be accurate to at least 99.99%, allowing it to be a reliable reference. The map must be assembled, rather than left in the fragments in which it was mapped. Affordability allowed scientists to conduct the research required to make the project a success. Accessibility to the public within 24 hours helped prevent multiple researchers from wasting time and money reworking a section that was already mapped to within 99.99% accuracy. It also promoted collaborative efforts. In the United States, the information was released every 24 hours on the National Center for Biotechnology Information (NCBI) website[6]. Updates on project progress are still reported at the NCBI website daily.

<u>Instrumentation in the Human Genome Project:</u>

As early as January 2000, 50% of the human genome was mapped to within the 99.99% accuracy, and in 2003 the remainder was completed[5,7]. The Human Genome Project was a success not only due to the collaboration of a variety of individuals, but also because of the instruments and techniques that developed prior to, as well as during, the project. Some include: Yeast artificial chromosome (YAC) cloning, radiation-hybrid (RH) mapping, fluorescence *in situ* hybridization (FISH), and polymerase chain reaction (PCR).

Creating a genetic map involved determining the complete 3 billion base sequence of the human DNA genome. Not only the length of the sequence, but also the microscopic size of it, posed a challenge for researchers. In order to make progress in the mapping, the DNA sequence had to be broken up rather than sequenced in a single run. Breaking the sequence up along known regions of the DNA (such as sequence tagged sites) was the easiest for reassembling later, and could be accomplished using specific enzymes.

After the DNA was broken, one of the methods implemented to aid in the mapping process was yeast artificial chromosome (YAC) cloning. Similar to bacterial artificial chromosome (BAC) cloning, YAC cloning allowed researchers to make copies of the segments of DNA in order to map it piece by piece. YAC cloning quickly became more beneficial than BAC cloning because the YAC clones were larger, and therefore easier for the researchers to analyze. This process allowed for a complete rough mapping of human chromosomes as early as 1992[8].

Radiation-hybrid Mapping helped define the order and orientation of disconnected contigs (overlapping sections of DNA) along the chromosomes. It involved “fragmentation of chromosomes in cultured cells with high doses of x-rays followed by the incorporation of the fragments into stable cell lines[8].” This meant that after the target

chromosomes were broken apart with x-rays, they were recovered using cells that were not broken apart. The chromosomes of interest were transferred into rodent cells to create the radiation-hybrid cells. The hybrid cells were then isolated and examined for the presence, or lack thereof, of the specific DNA markers. The further apart the two markers were on the original chromosome, the more likely the x-rays would break them apart, thereby placing them on two different chromosomal fragments. It was possible from this information to estimate the frequency of breakage (and from that, distance between the markers), and use this to determine the order of the fragments in the original chromosome[9]. This method allowed the DNA puzzle to more easily be put together.

Fluorescence *in situ* hybridization was a method used for orienting the contigs. While RH Mapping helped determine the order and orientation of the contigs on a finer scale, FISH accomplished the same task on a rough scale. It provided the scaffold for further analysis and a basis off which to build. Having a basic image of what the final product will look like can help reduce the overall time and effort spent in assembling the fragments, similar to how the photograph on the back of a jigsaw box can help those piecing the puzzle together work through the piecing process[8].

Polymerase chain reaction was another time-saving technique

implemented. This process allowed for the quick amplification of segments of the DNA strand, which was key because “complex physical maps based on restriction sites are of little value as experimental tools unless they are supported by a collection of clones[8].” Looking merely at a single copy of each DNA region can lead to a plethora of errors, whereas addressing a larger quantity of the same region increases the chances of catching the errors before progressing. These methods, in combination with others, allowed for the complete mapping of the human genome.

The importance of mapping the human genome is understandable, given the title of the project, but the genomes of other organisms can aid by adding the value of comparison. It allowed researchers to follow genes through evolution to find connections between organisms and more accurately determine common ancestors. Although not all regions of DNA are conserved through evolution, otherwise evolution would be unable to occur, many of the conserved region are functional regions. These may code for traits such as gene regulation[7].

Ethics of the Human Genome Project:

Ethical issues were important to address for a project of this magnitude. The research team initially decided to address the potential ethical implications of the results and research them before they

became problematic[4]. They set aside 5% of the annual research budget to this cause[6]. To accomplish the task at hand, they developed a group called the Ethical, Legal, and Social Issues (ELSI) group. This group was comprised of members of the Office of Health and Environmental Research (OHER) through the Department of Energy, as well as the National Center for Human Genome Research (NCHGR) through the National Institute of Health. They based their cause on: "1) recognizing that the human genome is part of humanity's common heritage; 2) adherence to international norms of human rights; 3) respect for the values, traditions, culture and integrity of participants; and 4) the acceptance and preservation of human dignity and freedom"[4]. Based on these four principles, the bioethics program was able to grow to the largest in the world.

Throughout the duration of the project, the current research methods and techniques were evaluated. Although each method used had its benefits, it also had drawbacks or disadvantages. Some methods were time-consuming, while others were not energy efficient. Researchers hoped to find new technologies to replace or improve their practices, as well as reduce the cost to continuing the project. Given this incentive, combined efforts were able to lead to some adaptations.

Yeast artificial chromosome cloning was not able to be used to full capacity at the beginning of the project, for example, and bacterial

artificial chromosome cloning was used instead. This method made small clones which were not as easy to analyze as the larger YAC ones were. As the project progressed, however, YAC cloning became a common method. Radiation-hybrid mapping similarly replaced conventional meiotic mapping because it broke the DNA strand randomly, rather than at a specific location. This allowed for more accurate results[9]. Other methods developed or improved as the project progressed, but more alterations could still be beneficial for the future.

After the completion of the Human Genome Project in 2003, the Department of Energy and National Institute of Health continued their work compiling genomic information and working on applying it. One of the current offshoots of the project is cancer research. DNA replication is critical in cancer research because if the DNA did not replicate, the cancer would not spread but rather would die on its own[10].

DNA Replication

DNA is frequently replicated – cells divide for the organism to grow, as well as to heal injuries. The cell cycle limits how often and how many cells divide, as well as when they do so. Mutations to DNA are often caught in the cell's own proofreading of the cell cycle and prevented from replicating. Some of the mutations are created during the replication process, while others are caused before it takes place. Certain mutations do, however, make it past the cell cycle check to be

copied into new DNA and passed on when the cell divides. Cancerous cells are mutated cells which have made it through the cycle to replicate. When mutated cells are able to divide and spread, they are often harmful. Due to their ability to replicate without being checked, they are able to replicate faster than are non-mutated cells. They are therefore able to become a larger percentage of the cells in a specific area[10].

Once the mutated cells have built up, their chances of displacing and transplanting themselves into another region of the body increases. The individual likelihood of a cell breaking free from its current location and surviving in another area of the body is low, but as the number of mutant cells increases, this small factor is increased drastically[10].

One of the ways DNA becomes mutated DNA that circumvents the checks in the cell cycle is expose to ultraviolet (UV) radiation (such as that found in sunlight). The UV radiation tends to mutate the telomeres of the DNA. Telomeres are critical for stability and integrity in the chromosomes. They are a non-coding region at the ends of the DNA strands, acting much like the caps on shoelaces to prevent unraveling and damage. Since the DNA cannot be replicated to the end of strand, the telomeres are integral in preventing the coding regions of the DNA from being cut off during replication. Human telomeres

have a repeating sequence of 5'TTAGGG/5'CCCTAA. UV radiation has a large impact on the telomeres, which is “thought to be due to the fact that sequences containing guanine triplets are highly sensitive to oxidation”[11]. UV radiation can cause unwanted oxidation to DNA, which the human telomere is unable to consistently and effectively combat. Because the telomere cannot effectively repair the DNA after the oxidation event, radiation can cause mutation. UV radiation is also thought to be “a complete carcinogen,” meaning that it requires “no additional treatments for tumor development”[11]. Given the abundance of UV radiation in many human lives, as well as the lack of ability for the cells to fix the damage it causes, UV contributes to the high frequency of skin cancer developments.

The human genome project will result not only in a better understanding of single gene defects, but also more complex diseases such as cancer. Some researchers argue that information on the human genome can open the door for genomic medicine. This medicine can, in turn, revolutionize the diagnosis and treatment of illnesses. Initial steps involve improved methods for screening and diagnosing the genetic diseases. This will be followed by “potential genetic cures”[4]. Similar to retroviruses, cancerous mutations are constantly changing and vary from person to person, making a single “cure” challenging.

In order to find solutions to genetic problems, researchers from multiple countries are again joining forces. In 2013, organizations from over 40 countries began the process of securely sharing genomic and clinical data. They are developing a standardized method to effectively and responsibly share this information. Since new technologies have been developed since the initial mapping of the human genome, the "ability to inexpensively gather genome sequence information in large numbers of individuals" has increased[12]. This allows for more research into the differences in DNA between people. Further study can help determine in which regions of the DNA mutations correspond with the most adverse effects. The information may also lead to a better understanding of which mutations respond best to which treatments[12].

Although the collaboration and the accessibility of the genomic information when working together are valuable, the ability to do so effectively is still a challenge. As most of this data is gathered and analyzed in isolation, finding a system that individuals from a plethora of organizations agree upon is an obstacle. A lot of work remains to understanding the mutations in human DNA, and this allows for reason for collaboration. If a working system is not developed and put into action before much progress is made, the challenges of working out the system will outweigh the benefits gained from it[12].

Challenges of Cancer

Currently, over 800 targeted cancer drugs are being tested for FDA approval. With the wide variety of cancerous mutations, a large number of cancer-fighting drugs seems logical. Finding the matches between the drugs and the patients is the challenge, and “requires widespread collection and analysis of genomic data in a dynamic and ongoing manner”[12]. With the large numbers of possible DNA mutations that can lead to cancer, a wide test group is also required. One of the benefits of collaboration on this project would be accessibility of a plethora of different mutations, and finding similar ones to compare[12].

Similar to cures for cancer posing a challenge based on the variety of mutations possible, preventing and restoring damaged DNA can be an obstacle. Some of the suggestions for preventing the harmful mutations are broad and cover mutations in general rather than a specific development of the mutations. Others, however, are more specific. Several foods, for example, are considered to be “cancer fighters.” More often than not, these foods are antioxidants. Oxidants are reactive molecules, so a surplus of these in the body can increase the chances of them interacting with DNA molecules and causing mutations. Antioxidants bind to the oxidants and prevent them from reacting with other molecules, reducing this risk.

Other foods can increase the risk of DNA mutations. Many of

these are highly processed, and some of the chemicals they are treated with are reactive. Nitrogenous solutions tend to be used to treat red meat to preserve it, but they can also be harmful for the body. Consuming high quantities of these processed meats are thought to promote leukemia, or blood cancer. Another risk factor in mutating DNA is smoking. This is again due to the increased intake of harmful chemicals that lead to DNA mutations.

Although many methods are implemented to avoid the harmful mutations in DNA which lead to cancer, it is not an exact science. A person can eat healthy and not partake in carcinogens and develop cancer, while someone with an unhealthy lifestyle may never develop it. Addressing this challenge is one of the goals of with more genetic work and an increased understanding of the human genome.

Human Genome Project

Deoxyribonucleic Acid (DNA) is the genetic material for most organisms. It codes for genes, or traits which can be passed on from parent to offspring. Genes are important because they build and maintain organisms. DNA is made up of four bases, adenine, guanine, cytosine, and thymine. These bases alternate to code for genes. Similar to letters being strung together to make words, the order of the bases determines the message they are sending[13].

The Human Genome Project started with the basic concept of

sequencing the DNA base pairs that make up the human genome, identifying the genes in the genome, and comparing the genes between humans and other related animals.

The Human Genome Project (HGP) is an initiative involving many different countries and disciplines. Large-scale initiatives which incorporate a significant portion of collaboration rely on individuals working together effectively and efficiently. When collaborating with many individuals from different countries, finding common standards and agreeing on protocols can be a time-consuming challenge. Finding a balance between the time spent making functioning collaborations and the rewards of working together is important. The balance in this case was found with Great Britain, France, Italy, Japan, and the United States.

Founded in 1990, the HGP soon had a Genome Data Base at John Hopkins University. This location became the central repository for mapping data. By 2000, 50% of the human genome sequence was mapped and available in the public domain. In 2003, the initial project was completed.

Some key accomplishments of the project include completing the project over two years ahead of schedule, using less money than allotted, creating the world’s largest bioethics program, and sequencing the genomes of humans, brewers’ yeast, roundworms, and

fruit flies.

The bioethics program came into play because of concern regarding the potential misuse of genetic information. People were cautious of the possibility of discrimination in obtaining health insurance, discrimination in the workplace, and not crossing the line of which genes were considered okay to alter and not, as well as under what circumstances it was acceptable to alter genes.

The Human Genome Project relates to cancer research by helping develop further understanding into genes. A greater understanding of the genes in DNA helps researchers discover which DNA mutations cause cancers and can help work toward finding preventative and combative measures regarding cancerous mutations.

What is Cancer

Cancer is caused by a series of mutations in an organism's DNA. For the most part, mutations in the DNA lead to apoptosis, or programmed cell death. This is accomplished by the cells marking themselves for death. Cancerous mutations are mutations that are not stopped and marked for death during the cell cycle. Without the checkpoints stopping the cells from replicating, the cancerous cells also procreate at a faster rate than healthy cells do. The more the cancerous cells replicate, the greater the ratio of cancer cells to

healthy cells.

Different types of cancers target different parts of the body. As cancer progresses, the mutated cells can break free of the body organ they are associated with and migrate to other regions in the body. When this occurs, the cancer can affect multiple systems over time. Some examples of cancers include: blood, lung, pancreatic, and skin cancers.

There are many theories about methods for preventing cancers from developing, but no one treatment has been supported at this point. Besides the differences in cancerous mutations, each individual has some natural variation from other individuals. This variation increases the challenge of locating the problem specifically as well as finding a method for addressing it without causing negative side effects.

Once cancer has been diagnosed in a patient, there are several methods which can be taken to prevent the spread of the cancer or to otherwise remove the threat it poses. Some methods have proven more effective in certain situations, and some are harmful to the patient’s healthy cells as well as their cancerous ones. Chemotherapy is an example of a method for treating cancer which is harmful for healthy and mutated cells.

Leukemia:

When a cancerous mutation develops in blood cell-forming tissue, or bone marrow, leukemia is the result. Leukemia is the most common type of childhood cancer which causes mutations in white blood cells. Leukemia cells also do not die when they are old or damaged, as the other blood cells do, but the mutated white blood cells crowd out the healthy blood cells. Without normally functioning white blood cells, the body has difficulties transporting oxygen to the tissues, controlling blood loss, and fighting infections. Over time, the leukemia can spread from the bone marrow to other organs.

In 2013, the estimated new cases of leukemia were 48,610 and the estimated deaths from leukemia were 23,720[14]. Leukemia is a broad term for blood cancer, however, and can be further broken down into categories. The categories of leukemia fall into two broad groups: acute and chronic. Acute leukemia tends to develop faster than chronic leukemia. The acute leukemia cells do not work like normal white blood cells, while the chronic leukemia cells function nearly as well as the normal cells. These differences lead to acute leukemia causing individuals to be very tired, bruise more easily, and get infections more readily, whereas people with chronic leukemia start off feeling normal, and then may progress into experiencing similar symptoms as those who have acute leukemia.

In these two broad groups, there are several more specific types of leukemia. The four most common types are acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphocytic leukemia (CLL). Chemotherapy, targeted therapy, radiation therapy, and stem cell transplant are some examples of how leukemia is treated, depending on the type of leukemia and the age and health of the patient[14,15,16,17].

Lung Cancer:

Lung cancer is one of the cancers with more obvious risk factors associated with it. Tobacco use increases the risk of developing lung cancer, but it is not always the cause of it. Although not using tobacco or quitting using tobacco will decrease one's chances of developing lung cancer, it does not eliminate the risk. Other risk factors include: secondhand smoke, pollutants, and radon.

Increased amounts of people develop lung cancer as they get older, with 71 being the average age of diagnosis. Knowing this statistic can lead younger individuals to the false impression that they are invincible or that the risk is too far in the future for them to worry about in the present.

Being related to someone who did not smoke but developed lung cancer increases the risk of developing lung cancer. Hereditary risk of

cancer is caused because the first step or steps in the cancerous mutation process have already taken place and passed on from parent to offspring.

Lung cancer has two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Small cell lung cancer tends to spread quickly and the cells look similar to oats under a microscope. This variety of lung cancer constitutes less than 20% of cases of lung cancer. To treat SCLC, surgery, radiation therapy, and/or chemotherapy is implemented[18].

Non-small cell lung cancer tends to spread slower than does SMLC, and can be further divided into squamous cell, adenocarcinoma, and large-cell undifferentiated carcinoma. Squamous cell tends to be related to smoking. Adenocarcinoma is the most common type of lung cancer. Large-cell undifferentiated carcinoma tends to proliferate quickly. The treatment options for NSCLC is similar to that of SCLC[18].

<u>Pancreatic Cancer:</u>

The Smithsonian Magazine says that pancreatic cancer is “one of the most lethal cancers, with a five-year survival rate of 6 percent.” Part of why the survival rate is this low is because pancreatic cancer has been challenging to notice until it has progressed beyond what current medicine can combat and has spread to other organs in the

body[19].

Fifteen-year-old Jack Andraka has created a paper sensor for pancreatic cancer in 2011. The sensor has remarkably high accuracy, especially compared to previous methods of detection. Although clinical trials still need to be completed before the product can be marketed, it has a potential to save many lives in the future[20,21].

Andraka's test involves coating filter paper in carbon nanotubes which have been laced with mesithelin antibodies. The patient's blood is then applied to the paper. Using an ohmmeter, the electric potential can be measured. Depending on the results of the ohmmeter, scientists can determine whether or not the patient has pancreatic cancer[20].

Skin Cancer:

Skin cancer is one of the most common cancers which affect both males and females. It is also a cancer for which risk factors are better known, such as ultraviolet (UV) radiation from the sun. Since this risk factor is known, people are encouraged to wear sun screen, which helps to block UV radiation from penetrating the skin and reaching the DNA. Avoiding extended exposure to the UV radiation can help decrease the risk of developing skin cancers[22].

Cancer of the skin can take on the appearance of a mole or

freckle, so being observant and having any newly developed changes to the skin checked can decrease the risk of having any complications if cancer has emerged. If caught early, skin cancer can be more easily removed than many other cancers. With skin being an external organ and quick to heal, the cancerous part can be removed before the cancerous cells migrate to other areas of the body[22].

Since UV radiation increases the risk of developing skin cancer, UV phototherapy has been questioned. UV phototherapy is a technique for treating psoriasis, a cronic immune disease. Regular treatments are key to the treatment, and help slow the growth of the infected cells. Studies have been done on phototherapy to determine if a correlation between UV phototherapy and skin cancer exists23. A literature review by Lee, Koo, and Berger (2005) has examined eleven studies to better understand the possibility of a correlation, but has been unable to find one at that point[22].

Instrumentation

Instrumentation and its advancements were critical to the Human Genome Project being a success, as well as developing anti-cancer methods. The constant changes in technology and techniques allows for progress in many other fields as well. Some of the instruments used in the Human Genome Project are further described in following pages.

Part of what holds scientists back in making advances in research is the nature of DNA research. Frequent and rapid mutation rates in DNA make progress in genome research more challenging. Another factor preventing breakthroughs is the technology. Without an instrument to perform a certain test, having knowledge of a theoretical test will not yield real results. Likewise, the cost of certain instruments and methods can hold research back. These are a couple examples of why instrumentation is crucial to the Human Genome Project, among other projects and research.

Throughout the course of the bulk of the Human Genome Project, advances were made due in part to pressure put on the research community to complete the project. There is continued desire to make advances and to decrease the cost required to obtain the data needed to sequence genomes, indicating investment in a process rather than a result. There is always room for improvement and new ideas to be tested.

Polymerase Chain Reaction

When working on any project, being accurate is critical. One of the ways to promote accuracy is to have multiple trials of the research. Multiple times of working through every procedure and multiple different sets of results allows for easier discovery of any discrepancies and increased ability to analyze potential factors contributing to the

discrepancies. Duplication allows for more trials and thereby more comparisons to see if the result is normal or if there was increased likelihood of something having gone wrong with that trial.

Polymerase Chain Reaction (PCR) is a method for duplicating DNA segments. It allows for exponential duplication in a short space of time. With the rapid duplication, researchers are better able to take small initial concentrations of the DNA and have plenty of material to work with and test[26,27]. This method has been implemented in the Human Genome Project and cancer research in order to yield more DNA fragments to analyze at once.

In general, PCR is a fast and effect method for replicating precise DNA segments. It allows for more flexibility in research because less original DNA is needed than is required for most analyses with the DNA. It applies simple concepts and takes advantage of natural replicating processes. An initial investment into instruments may be costly, but the method itself is cheap and easy to use.

Gel Electrophoresis

Gel Electrophoresis was a method, developed by Arne Tiselius, which helped analyze the segments of broken up DNA. It soon became known as the powerhouse method because it not only yielded results fairly quickly, but accurately, as well as being able to compare multiple

samples simultaneously.

Gel Electrophoresis is based on the migration of DNA fragments through gel. Wells are put in the gel and the stained DNA fragments are loaded into the gel. After all the wells are loaded, a charge is applied across the gel. The negative end is hooked up to the end containing the wells of DNA, while the positive end is hooked up to the opposite end. The DNA, being negatively charged, is attracted to the positive end and repulsed by the negative end. The smaller DNA fragments are able to more quickly and easily move from the negative end to the positive end because they can maneuver through the gel better. The larger, heavier molecules move more slowly through the gel.

Based on the distance each DNA fragment sample moves, relative sizes, charges, or weights of the samples can be determined. This method is able to distinguish between nucleotides of 200-500 bases with only one difference in the bases. Since human DNA is roughly 3 billion bases long, 200-500 bases is a relatively small piece of that sequence, but large in relation to how much can be analyzed at a time using alternative methods.

One of the benefits of this method is its accuracy and speed. The method can take several hours to do, but it does not need constant monitoring. Multiple samples can also be run simultaneously in the

same gel. Depending on the size of the gel and the number of wells it contains, the number of samples processed at one time varies. The gel tends to be inexpensive, but it is also disposable rather than reusable.

One of the disadvantages of Gel Electrophoresis is that although focus during the entire run time is not necessary, constant checking in on the sample is required. If the gel is run for too long, the multiple bands will reach the edge of the gel and it will appear as though they are composed of the same or similar strands of DNA. The method is also sensitive to being jostled, so caution is required. Sometimes the bands in the gel are not crisp, as well as the size being relative. Running a ladder with the sample is key, but the ladder only has certain sized fragments in it. Sample fragment sizes are therefore estimated rather than exactly known.

Variable Number Tandem Repeats

DNA is a large molecule – too large to map while a complete strand. A single cell’s DNA can stretch six feet when it is not condensed into chromosomes. It seems logical, then, to break the DNA into smaller pieces. Once these are sequenced, the sequences can be put back together like a puzzle. Also like a puzzle, you have to be careful about lining up the DNA fragments correctly, rather than forcing pieces to fit together.

There are several ways to break apart DNA. One of them is to break the DNA at specific locations such as Variable Number Tandem Repeats (VNTRs). These are segments of DNA that are repeated a variable number of times. The VNTRs can have the same sequence repeated a different number of times. Some are repeated 2, 3, or 4 times, for example. Breaking the DNA into sections using VNTRs allows researchers to work with the DNA in smaller fragments. Since the VNTRs are known, piecing together the segments is easier than if the DNA was broken up haphazardly. VNTRs were used in the Human Genome Project to sequence the DNA strands in smaller increments and then piece the fragments together to analyze the sequence as a whole.

Sequence Tagged Sites (STS)

When piecing together a puzzle, it can be beneficial to have some reference point. If the only guide you had to piece the puzzle together was how the pieces themselves fit together and there were several pieces that fit the same other piece, it would be nearly impossible to put the puzzle together correctly. Even if you did put it together right, it would be challenging to be sure that you were correct.

Sequence Tagged Sites can help give more perspective to the puzzle so it is less of a chance effort. They are DNA sequences that

range from 200 to 500 base pairs long and have a known sequence. This known sequence can act as a guide when analyzing the DNA fragments and trying to find how they all go back together.

Denaturing High Performance Liquid Chromatography

Chromatography is a method for separating compounds, including fragments of DNA, to analyze them. Mikhail Tswett is recognized for developing the first chromatogram. He used petroleum ether and pigments in green leaves. Depending on the solubility with the petroleum ether, Tswett was able to separate out the pigments from solution at different rates. Martin and Synge further found "an alternative physical basis for chromatography" [24]. They developed partition chromatography, which separates compounds by differing solubility with the two solvents used, rather than only using a single solvent. Since Martin and Synge, chromatography methods have been advanced to include multiple new chromatography methods, such as ion-exchange chromatography and gas-liquid chromatography. Chromatography can be used by itself or with other methods, such as mass spectrometry[24].

One of the challenges with chromatography, depending on the type, is finding a solvent which will dissolve the components of a substance at different rates to effectively separate them. A benefit of the method is that it is able to be coupled with other methods. This

flexibility allows scientists to acquire different data depending on what they are looking for.

Denaturing high-performance liquid chromatography, or DHPLC, is a method for comparing pieces of chromosomes amplified by polymerase chain reaction (PCR). This technique can detect mutations by “differential retention of homo- and heteroduplex DNA”[25]. Homoduplex and heteroduplex DNA are comparisons of the two DNA strands to each other[25].

Mutations involving a single nucleotide insertion, deletion, or substitution can be detected in 2-3 minutes with DHPLC, with a sensitivity of 96% or more. Since single base differences can be detected with this method, it is not only beneficial for locating mutations, but also for differences between multiple strands of DNA. In this manner, denaturing high-performance liquid chromatography has been used to genotype polymorphisms in human, and other animal, genomes. The method is cheap to use and can work with other instruments, such as the mass spectrometer[25]. With mass spectrometry, chromatography is able to effectively separate out compounds and yield information about what a sample is composed of. This technique can be more specific that some other chromatography techniques because the mass spectrometer can indicate the mass of the compounds rather than a less specific relative mass.

Mass Spectrometry

Mass Spectrometry is a method which can determine the composition of samples. It can be used to help determine the bases which compose a fragment of DNA. Knowing the concentrations of each of the bases gives scientists a starting point for determining the sequence of a fragment of DNA. It is a highly effective method for determining the structures of molecules, as well as which elements composed the sample. Qualitative and quantitative analysis of the composition of mixtures can be determined as well.

Depending on the sample properties, the manner in which the sample is processed is different. Volatile substances, or substances which readily vaporize, are simpler to analyze than those that are non-volatile.

One of the benefits of this technique is that relatively little sample is needed in order to obtain accurate results. A setback is that many of the instruments round the measurements of the mass of the molecules and ions, so similar molecules may look the same. Depending on the molecule and the ions it most commonly forms, the ion peaks can help determine which of the possible molecules is being analyzed. The Mass Spectrometer yields graphs showing the initial molecule as well as the ions that are formed when the molecule is bombarded with electrons. Depending on the relative abundances of

the ions, the likely structures will be narrowed down. Some Mass Spectrometers are more accurate than others and can further indicate the ionic masses.

One of the types of Mass Spectrometry is Electron-Impact Mass Spectrometry. For this method, a sample is brought to a high temperature. This produces a molecular vapor. The gas is then bombarded with a beam of electrons with high energy to ionize the sample. Once ionized, the sample is propelled down a time-of-flight tube to the scanner where the time to travel the length of the tube can be analyzed. This method is not very efficient; only about 1 of 1,000,000 molecules is ionized in this manner. The remaining sample stays in the liquid phase and is discarded as waste.

Gas Chromatography-Mass Spectrometry (GC-MS) is another application of mass spectrometry. This method uses samples already in the gaseous phase, so less sample is disposed of as waste. It requires that the sample be volatile enough that the gas phase can be easily reached.

Fluorescent *in situ* Hybridization (FISH)

Fluorescent *in situ* Hybridization (FISH) is a method for roughly mapping DNA. It is useful in helping order the fragments of DNA, or contigs. Properly ordering the fragments helps eliminate potential

discontinuities, which can add ambiguity to data or indicate false results.

To cause the hybridization of the genetic material with the fluorescent marker, the marker is added in solution to the sample DNA. Once the sample is prepared, it can be analyzed using a flow cytometer. A flow cytometer measures the light scatter and fluorescence of the sample. These measurements can then be analyzed using data analysis software.

Besides helping orient the contigs, or fragments of DNA, FISH is a useful method because the cells do not need to be actively dividing for the method to work. Previous methods needed the cells to be currently dividing to yield results, so this method was highly beneficial with that flexibility.

Another benefit of FISH is that you can probe for a desired gene. If you know what gene you are looking for, it makes it simpler to do so. A single strand of the DNA is taken and fluorescent tags are added. They bind to specific bases in the DNA, causing them to fluoresce.

Yeast Artificial Chromosome/Bacterial Artificial Chromosome Cloning

One of the challenges faced with analyzing human DNA is the small size of each fragment. Bacterial Artificial Chromosome (BAC) cloning is a method developed to increase the size of the DNA being

worked with. This is beneficial because the human genome is larger than that of model organisms. Human DNA is inserted in the bacterial DNA to be cloned when the bacteria divides.

A newer method became popular during the Human Genome Project. Yeast Artificial Chromosome (YAC) cloning became a key process for the research because the YAC clones were larger and easier to work with than were the BAC clones.

As early as 1989, segments of the human genome were successfully recovered using YAC cloning. By 1991, a library from human DNA was created. This was a rough picture of the human genome. Similar to the picture on the box of a puzzle, this map was able to guide researchers in piecing together the genome.

To use the YAC clones, they were made of overlapping segments of DNA. Observing where the segments overlapped helped to piece to YAC clones together into a workable map.

Radiation-Hybrid Method

Radiation-Hybrid (RH) Mapping uses X-ray radiation to break apart DNA. The broken DNA is then inserted into non-fragmented cells. Once the non-fragmented cells take up the fragments of DNA, researchers look for DNA markers in the cells. When looking at the DNA, the goal is to analyze how far apart the DNA fragments are. The

distance is measured in map distance, which is related to the frequency of breakage in the fragment rather than to a set distance such as millimeters. This method helps determine the order of the DNA fragments with relation to each other. Understanding the order of the fragments helps to create a map of the DNA sequence as a whole.

BLAST Comparison

A BLAST is a Basic Local Alignment Search Tool. This tool can be used to compare sequences from a genome with sequences in other organisms. BLAST is publically available online at: http://blast.ncbi.nlm.nih.gov/Blast.cgi.

BLAST is an effective method for comparing DNA sequences, but only when going to the website with specific sequence in mind. Comparisons can only be made with a known piece of DNA, rather than being able to search and find a piece of DNA with which to work. Once the DNA sequence is input and the BLAST initiated, other organisms in the database with similar sequences can be displayed. Depending on the size of the sequence, close organism relatives may be more or less challenging to locate.

Applying BLAST to cancer research, knowing a sequence of DNA that is mutated to cause cancer in humans, researchers can potentially find an organism with a similar sequence of DNA and compare how

that organism is able to handle mutations to the segment of DNA.

One benefit of the BLAST technique is it being quick, especially considering how large the database it has to run the segment through. Some sequences may take longer than others and people might be impatient with the amount of time it takes, but given what it is accomplishing, BLAST is timely. BLAST Comparison also gives the opportunity to compare multiple species to the target DNA strand, rather than selecting a single species with which to compare it.

One disadvantage of BLAST, as addressed above is needing to know a specific gene for which to look. Once a gene is known, the benefits of being able to quickly and easily compare that segment to segments in other related organisms is apparent. In order to determine the gene in question, other methods are used.

Capstone Presentation

For my chemistry capstone presentation, I addressed the Human Genome Project and how this relates to cancer research. One of the comments I received about the presentation was that the connection between the Human Genome Project and cancer research was challenging to see. I used this feedback in my blog to make sure I addressed the connection in the beginning, as well as when I started talking more about the specific instrumentation.

Another comment was that my description of the instrumentation was too technical for a general audience. I was advised to add more human aspects to balance with the technical parts, as well as to come up with methods for explaining the instruments in simple terms. In the creation of my blog, one of the aspects I focused on was balancing the technical aspects of the research. I made sure to include diagrams of complex instrumentation which tied the techniques with common concepts. I also added sections about places people could volunteer to work with children with cancer and current cancer research.

Blog

When thinking of the importance of cancer research, one of my first concerns was the lack of general knowledge about the topic. I decided to create a blog to share my research as I progressed in my project. I thought a blog made more sense than a website because blogs allow people to more freely add feedback and interact with the owner and other visitors of the blog than websites tend to do. It also seemed to be a less intimidating forum for information for the public.

Once I decided to create a blog, I needed to find a host, create and account, and figure out the formatting. I found the host that I used by talking to individuals who had experience with blogs. Multiple people told me about Wordpress.com and said that it was a good host. I looked into it and found that it offered many options without charging.

After creating an account and finding a template I liked, I started adding in my information.

In some aspects, my blog is set up similar to a website – with different sections for each different piece of information. To make it more interactive, I began posting every Monday. The posts would either express information or summarize information and the location of it which I added that day elsewhere to the blog – making new pages or adding to old ones.

I sent out an all student and an all faculty/staff E-mail informing people at WOU about my blog and asking people to take a look at it. The day these E-mails went out, the blog had 133 views (Appendix). Most other days have between 0 and 3 views. Part of this low viewership could be because of the specific nature of the content of the blog. It is also a technical blog, so this could deter some potential viewers.

Another method I used for recruiting viewers to the blog was to look at other people's blog. When I found an interesting blog which had similar content, I linked it on my blog. The owner of the blog I linked received an E-mail stated that I had linked their blog on mine. One of the owners of the blogs I linked started following my blog.

I also used social media and conversations to promote my blog to

people I know. Some people were not interested in the blog themselves, but thought of people who might be and asked to pass on the web address to others. In this manner, the blog was passed from person to person.

Besides learning how to set up a blog and promote it, I learned more about my research by maintaining the blog. I realized as I started writing the pages that in order for the information I was sharing to be truly accessible to the public, I needed to be able to share information in layman's terms. After reading each peer reviewed article or each new chapter in a book, I would think through what I had just read and how to explain it to someone without any background in science. It was a challenge at first, and to some extent throughout the project, but I gained a better understanding of the material by being able to explain it in more simple terms.

By creating and maintaining the blog, I was able to share knowledge about cancer research with the public. I was able to reach people beyond just those I knew or who attended my university. I believe communication is critical for advances in cancer research, and helping to open the door for general communication regarding cancer was one goal of creating the blog.

Since it is a public blog, anyone can look at it. The challenge is making the information appealing to people while keeping it in line

with the goal of spreading technical information to the public. Finding a balance with technical aspects and human aspects is important for making the information appealing.

The blog is currently located at: http://humangenomeprojectandcancer.wordpress.com/.

Where to go from here

One of the challenging aspects of cancer research is that the cost is high and the reward is delayed and "low." More money is found in research involving drugs more frequently used by more people than for cancer drugs. This poses a challenge for progress with finding effective means for addressing these diseases. In some ways, the shift of mentality of taking medicine for smaller symptoms might have led to this shift in research; companies producing drugs to treat simpler problems will earn more money because more people will purchase them and they are cheaper to develop.

Another challenge is the daunting process to get drugs approved for human consumption and use to treat diseases. Many of the drugs that show early signs of being successful for treating diseases turn out to not be safe or not be effective when ingested. Working hard and spending lots of money to get results that are not useful can dampen spirits when it is seen as a failure rather than a chance to learn more

and have better awareness moving forward.

Many research companies only have a certain amount of money they are working with, which is less than the amount of money that others annually request from them to do research[28]. Researchers with more “conventional” research ideas tend to be more supported because they are considered safer investments. Although the less conventional ideas tend to have bigger pay-outs when they work as anticipated, they are less likely to conform to the expectations. As a society, deciding where we place out priorities can help with these problems to some extent, but they may remain challenges for years to come.

Conclusions

Cancer research is an important field of study for a number of reasons. Cancer is a wide-spread challenge for people because it comes in many shapes and forms and we do not know how to consistently and effectively combat it when it occurs. People from every walk of life can be affected by cancer, drawing in general interest and concern.

Since cancer is not confined to a single geographic location or to people with a certain background, it is a unifying aspect of life. People have come together from a variety of locations and disciplines to

better understand what causes cancers and how to address them when they arise. Similarly, researchers have come together to sequence genomes.

The Human Genome Project has been a success in part because of how effectively individuals have been able to coordinate research and share results. Research for sequencing the human genome relates to cancer research because it increases understanding what mutations can lead to cancers. Understanding what causes these mutations and how to potentially limit the number of them can lead to advances in cancer research as well.

Another aspect of cancer research benefited by the Human Genome Project is instrumentation. Setting deadlines for sequencing the human genome which incorporated international efforts promoted advancement with technology in order to effectively and efficiently complete the project. This pressure led to the development of new instruments and technologies which were not only beneficial for sequencing genomes, but for other research as well. Several instruments developed or improved as a result of the pressure of the project are used for cancer research.

Research and technology are important for progress with research, but also for bringing people together. Joining together for a common goal allows for more progress to be made, and in a more

timely fashion. In many aspects of life, people with different backgrounds may be hesitant to work together or try to understand each other. Having a common goal of mapping the human genome has helped to unite people and build relationships which can be beneficial for all involved in the future. The Human Genome Project is in this manner a starting point for not only cancer research, but for future collaborative research with endless possibilities.

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Appendix I: Informed consent posted on the blog

The purpose of this study is to help raise awareness on cancer and cancer research. By reading information about cancer, stress or discomfort might occur. In the case that either of these happens, feel free to exit the webpage at any time. Potential benefits from this research include increased awareness about cancer, which can help when confronted with cancer in your life personally or with someone you know. Increased awareness as a society can help lead to more breakthroughs in cancer research, as well as gearing the direction the research takes.

Although you must create an account in order to post a response on the blog, you do not need to use your legal name. The blog is a public blog which anyone can access. If you wish to remain anonymous, please do not use your legal name. You will not be compensated for participating in this research, but you will hopefully learn something new.

If you wish to participate in the project, please start by creating a free account and reading some of the information. Any feedback or comments you have is appreciated. Please note that you can withdraw from the research at any time by leaving the blog.

If you have any questions, please contact Alyssa Palmer at apalmer09@wou.edu.

Appendix II: Blog Screen Shots

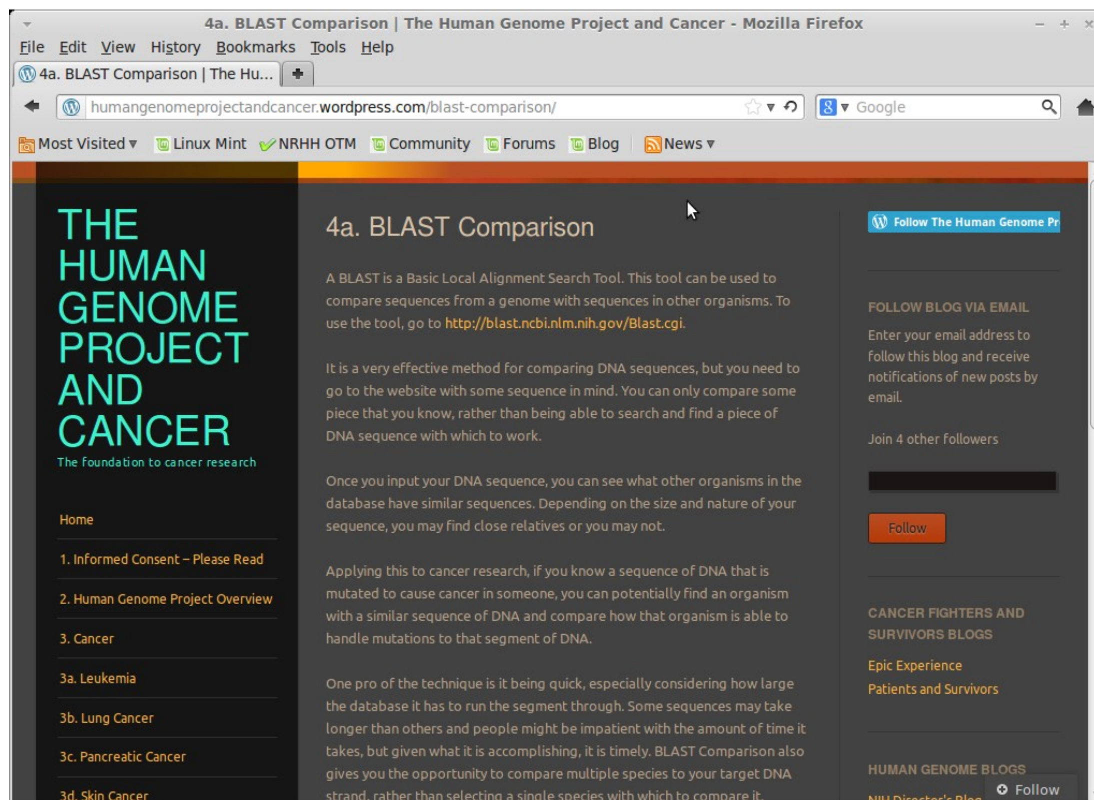

Figure 1: A screen shot of the BLAST Comparison page of the blog. The pages are listed along the left side of the blog. The right side of the page has links to similar blogs to guide readers if they desire other places to look for information.

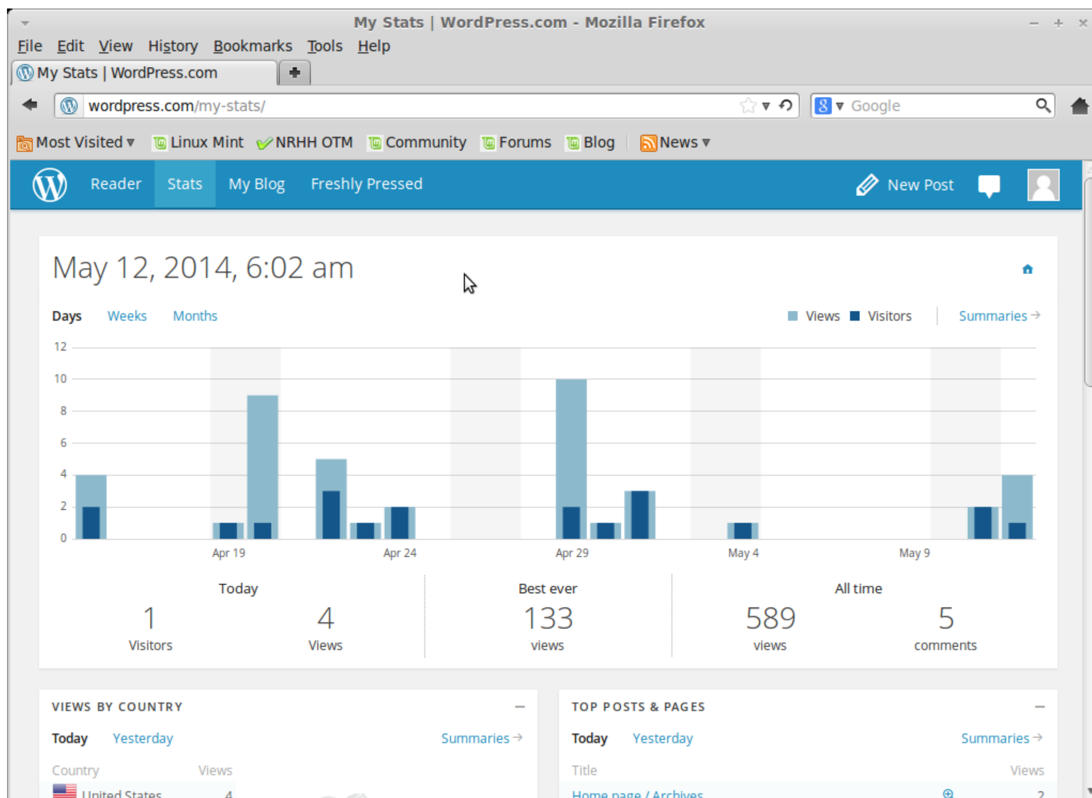

Figure 2: A screenshot of the activity status of the blog as of May 12, 2014. This image indicates the total views, comments, and shows the recent trend for views of various pages of the blog.