Synthetic Biology and the Layman

A Major Qualifying Project

Submitted to the Faculty of

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the
Degree of Bachelor of Science

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PROJECT DESCRIPTION(S)

This Major Qualifying Project (MQP) was completed to satisfy the requirements of both the Biochemistry degree and the Professional Writing degree. The primary effort was to combine an authentic scientific research project conducted in the field of synthetic biology with concurrent analysis of written popular media on the subject. To complete this combined MQP, I conducted the scientific research portion in the Goddard Project Lab, under the advice of Professors Michael Buckholt and Natalie Farny, with the assistance of partner Andrew Baker. Simultaneously, I conducted the literature analysis portion independently under the guidance of Professor Brenton Faber. This exercise in science and communication was a meaningful study with the aim to understand both the technical and humanities disciplines within the context of each other.

This full MQP report is a compilation of all of the work done for both majors. The first chapter contains the entirety of the literature analysis portion of the project in synthetic biology. The second chapter consists of the scientific research portion, performed in the laboratory. Finally, the report concludes with a reflection upon the overall experience, having immersed myself in both the technical and rhetorical aspects of a fascinating and influential new field: synthetic biology.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to the following individuals, without whom I could not have completed my project:

My Biochemistry advisors, Professor Michael Buckholt and Professor Natalie Farny, for their incredible guidance throughout the year. Without their continued patience, encouragement, and understanding, I would not have had an experience as nearly as enriching as mine was. Their teaching in persistence and critical thinking will serve me well beyond my undergraduate career.

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My family, for their love and support throughout my years of education, culminating at WPI with this MQP experience. In particular, I thank my parental units and WPI alumni big brother for attending Project Presentation Day.

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The Biochemistry and Professional Writing departments, for their allowance of my combined MQP. I cannot express how much growth I have obtained from this experience that I could not have otherwise attained.

CHAPTER ONE

Popularized Synthetic Biology: Public Perception & Understanding, 2006-2016

ABSTRACT

Synthetic biology is a continuously emerging field of research and application that comprises engineering principles to further biology. This study explores the public understanding and perceptions of the science from 2006 to 2016, as represented by written popular media within that time, such as newspapers and magazines, both local and national. Though the idea of a synthetic biology originates from before the turn of the century, the field has emerged significantly in the last ten years. Synthetic biology follows similar patterns as other emergent sciences and technologies, as it strives to establish itself as a credible field at the forefront of science and society. However, contrary to other scientific areas such as nanotechnology, synthetic biology seeks to bridge existing areas together rather than offer itself as a unique and separately understood science. With applications ranging from medical care to biofuels, and concerns like bioterrorism and biosafety hazards, this study reveals the popular perceptions and understandings of synthetic biology over the last 10 years, and how this has shaped synthetic biology as a new, hybrid science in society.

INTRODUCTION
Synthetic Biology: An Engineering Revolution in the Biological World

Synthetic biology, more affectionately referred to as synbio, is a broad, interdisciplinary, ever-growing field that invokes engineering principles to devise new biological systems for adapting and creating life. The true definition still has not reached consensus among the scientific community, and its scope as an emerging science expands as related research accomplishes more in the name of synthetic biology.

"Synthetic biology" as a recognizable term originated in 1910 with Stephane Leduc, a French scientist who desired his work to be considered as "synthetic" life forms (Tirard, 2008). Following that time, however, both the term and the idea as a scientific discipline failed to catch on in more established scientific communities. The lack of necessary technology to substantiate and progress the field was a critical factor in its delayed significance. The contemporary understanding of synthetic biology, then, didn't come into prominence until the 1970's, when a notable geneticist by the name of Waclaw Szybalski described the "new era of synthetic biology" as one "where not only existing genes are described and analyzed but also new gene arrangements can be constructed and evaluated" (1978). This concept became more tangible once the century turned, when the first synthetic biological circuit was created in bacteria in the year 2000, with the work published in two separate *Nature* articles (Elowitz, 2000 and Gardner, 2000). The flowchart below conveys some of the major milestones and key players in the advent of synthetic biology following this first success story (Cameron, 2014; Mali, 2013; Purnick, 2009; Specter, 2009; Zimmer, 2006).

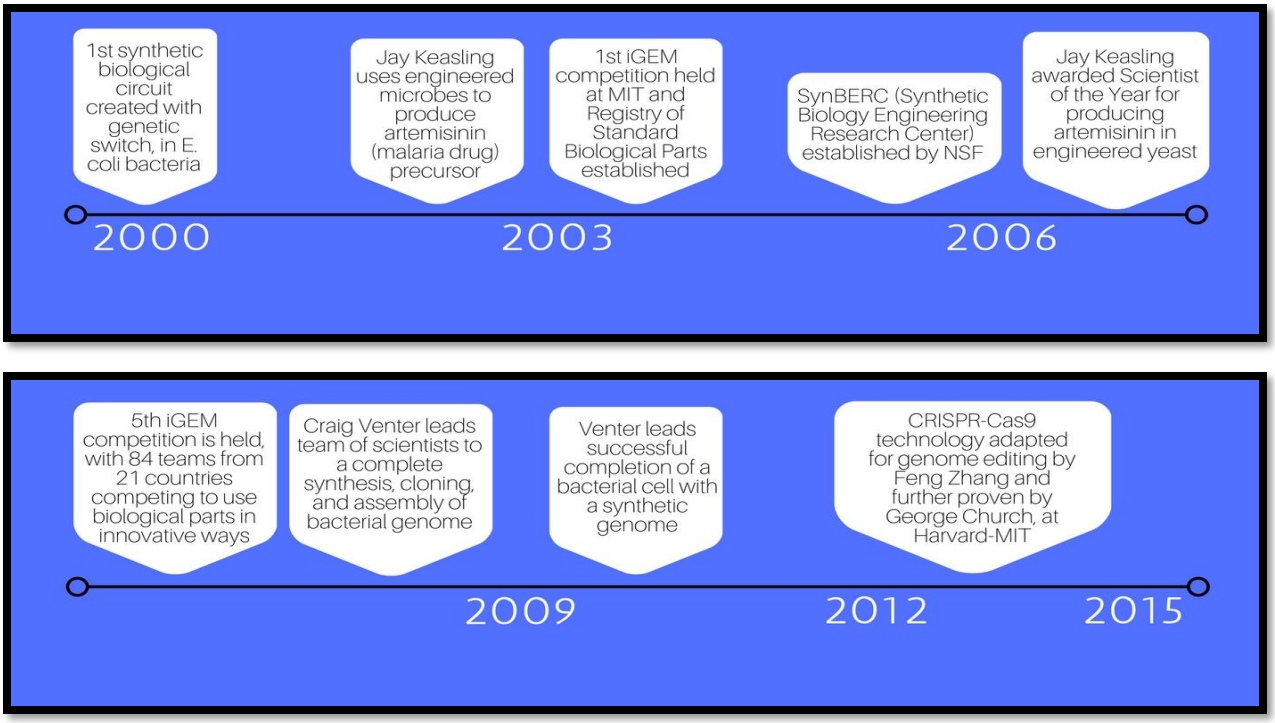

Figure 1: Timeline of Major Synthetic Biology Milestones

In the last few years to now, many of the earliest ideas of synthetic biology are becoming more capable of coming into fruition through advanced genome editing technology such as the CRISPR-Cas9 system, higher rates of industry-produced DNA, and the benefit of time, evidenced by increasing numbers of major publications in prestigious journals such as *Cell, Nature,* and *JBC.*

The Public Appearances of Synthetic Biology

In the last 10 years, as popular media reported on the field of synthetic biology, it associated the science with much of the same applications that the public associates other respected science fields with. Most often expressed as a means to medical advancements, improvements to the environment and agriculture, and a new source for fuel, synbio promises a wide variety of solutions to society's most pressing and timeless needs. The field is dominated by the enduring frontier of genetic engineering and using genetics as a circuitry to life, with standards and parts to build custom systems. Contrary to such a clinical, systematic approach, the field also emphasizes a need for using bacteria as a life-infused research tool. Accordingly, the media also reports synthetic biology as a science raising important questions of policy and supervision, if not expressing an urgent need for increased research regulation.

DATA COLLECTION & ANALYSIS

For this study, to better understand the public understanding of the emerging field of synthetic biology and its potential acceptance within both scientific and nonscientific communities, I went to the written public media as a representation of synthetic biology's public perceptions. I followed a method similar to the one outlined by Faber in his study on the emergence of nanoscience and nanotechnology (2006). To uncover a full spectrum of public perceptions, I used the expansive media database provided by my university, Worcester Polytechnic Institute (WPI). Through this connection, as well as through independent subscriptions, I was able to access newspaper and magazine articles from many sources. In order to represent as well as possible the entirety of public media in North America, I chose two major national sources: the Washington Post and New York Times, as well as eight regional publication sources from across the country: the Boston Globe, San Jose Mercury News, Chicago Tribune, Orlando Sentinel, Houston Chronicle, Seattle Times, Los Angeles Times, and The Day (New London). I determined my time period of the most recent ten years (January 1 of 2006 to December 31 of 2015) as a critical period in synthetic biology's modern popular history, and an appropriate scope for this study. The keyword term remained "synthetic biology", and I included in my search query only those articles that had their full text available online.

The first search with these parameters generated a total of 563 articles. For a shallow understanding of the field and of the material I would be working with, I read the abstracts and/or skimmed through each of the articles. Through this process, I determined many articles to be repeats or not actually relevant to the field of synthetic biology. After manually reviewing each article, and eliminating all repeated or irrelevant articles, I established a final list of 162 articles on synthetic biology that I would then include in the data set for my study. The articles ranged from a few sentences long to sixteen pages. Interestingly, only six of the 162 articles contained the entire phrase "synthetic biology" in their headline titles. Figure 2 below depicts the number of articles about synthetic biology in each year of my chosen ten-year span:

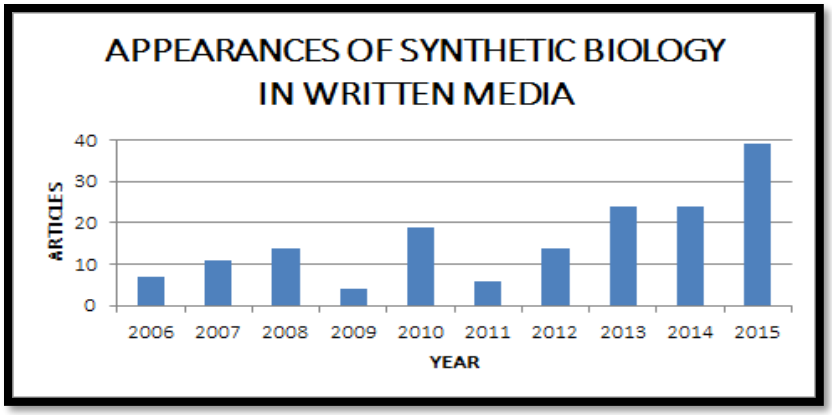

Figure 2: Appearances of Synthetic Biology in Popular Articles from 2006-2016

Understanding the Value of Synthetic Biology

The next point of interest I had in the media's portrayal of synthetic biology over the years was the value of the field: was this emerging scientific discipline regarded as positive in the public eye, negative, or perhaps both, or neutral? Furthermore, did the understanding in written media change significantly as the field grew in prominence in the public sphere? To gain insight into this, I read each article for its overall portrayal of synthetic biology and recorded the articles as positive, negative, neutral, or both (positive and negative).

The Public Associations with Synbio

While going through the process of reading each of the articles in my data set, I recorded the most prominent topics written about as associated to synthetic biology. From this list of 41 topics, I arranged 24 major categories that comprised of all of them. I refer to these 24 major topic categories henceforth as *representations*. After creating this list, I then returned to each of the 162 articles in my data set to read them yet again and track which representations occurred in each article, to gain an idea of what synthetic biology is most associated with over time.

FINDINGS

The Value of Synthetic Biology

The first results generated from this study unveil the portrayal of synbio in written public media as positive, negative, neutral, or both. Figure 3 below shows the percentage of articles about synthetic biology with each indicated value, for each year:

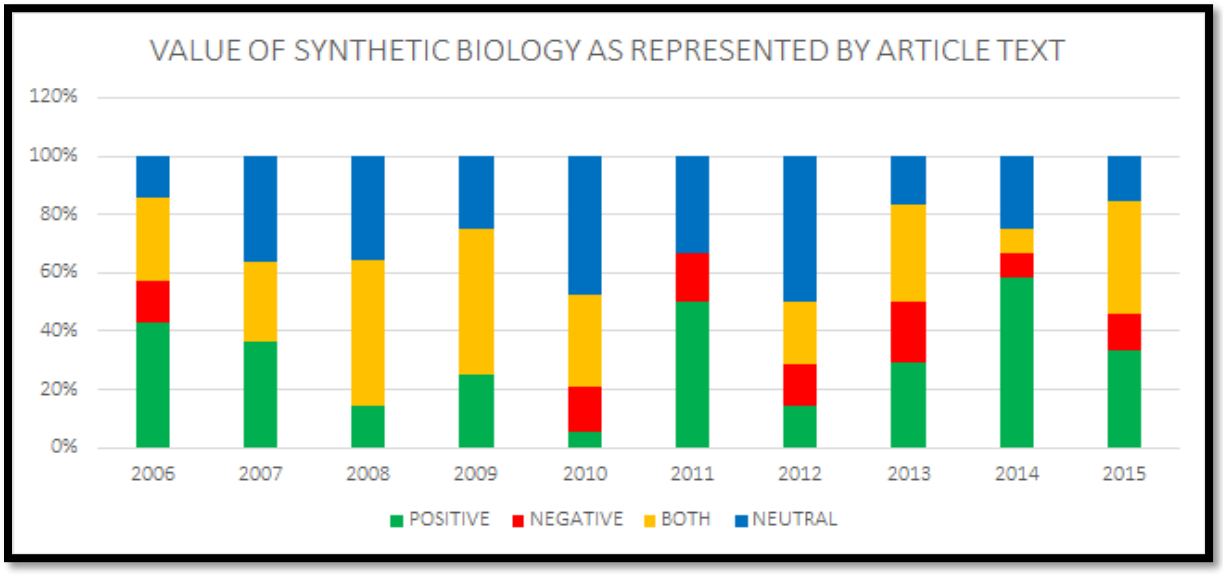

Figure 3: Depiction of Synbio Value – Positive, Negative, Both, and Neutral

For simplification purposes, I then re-generated a graph that no longer includes articles that contain both a positive and negative idea of synthetic biology ("both"), or none at all ("neutral"). Following, Figure 4 shows articles that are more polarized as either overwhelmingly positive or negative:

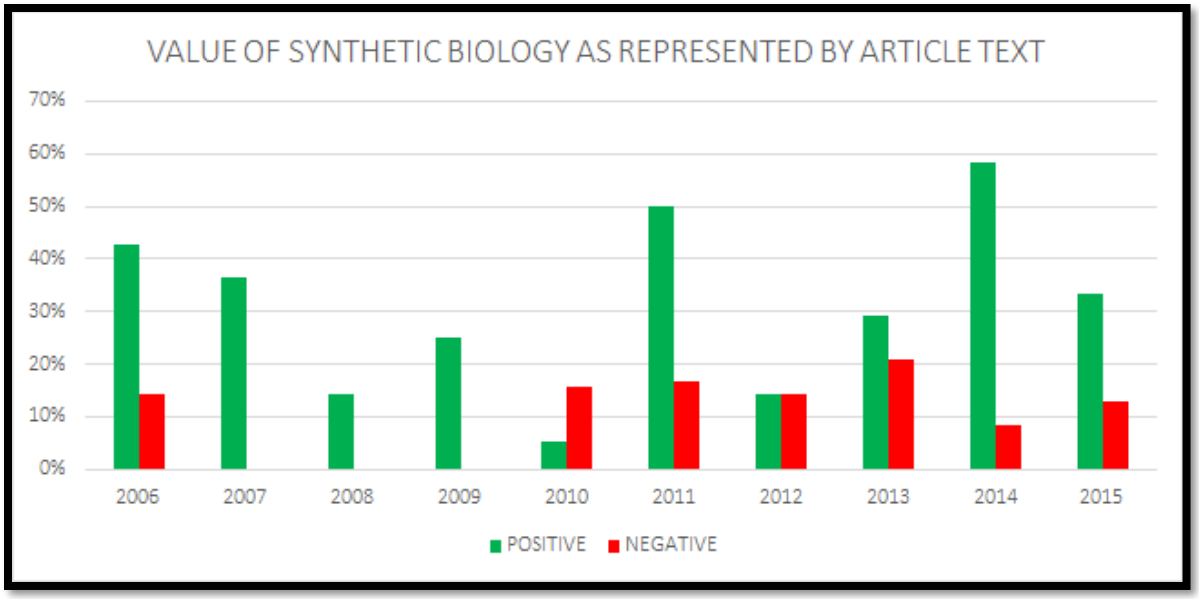

Figure 4: Simplified Depiction of Polarized Synbio Articles – Positive or Negative

There are a few interesting things to note in the portrayal of synthetic biology as positive or negative over time. The period between 2007 and 2009 contained no articles that were overtly negative, and the percentage of negative articles otherwise remains relatively stable between 10% and 20%, with the exception of the year 2013, which sees a spike of negative articles at just over 20%. The three years with the largest percentages of a positive portrayal are 2006, 2011, and 2014, with mainly positive articles consisting over 40% of the data set in those years. Meanwhile, 2010 was the year with the least amount of articles portraying synthetic biology positively, with a meager 5%.

Representation by Association

The list of *representations* of synthetic biology stand for the major topics that the science is associated with, and these categories come directly from the text of the data set. Assuming equal occurrence, each of the 24 representations would in theory comprise of roughly 4% of each year's total articles. However, as this study sought to find, the representations yielded great variability in their rate of occurrence. Regardless of the quantity of articles in each year, every year was dominated by one or more of the representations at 10% or higher of the total representations in that year. These major representations contribute to a common public understanding of synthetic biology in each of the last ten years. Figure 5 depicts which of the representations dominated through the written media of our data set in each year, providing insight into the major "buzzword(s)" of synthetic biology over time:

Figure 5: Table of Top Major Synbio Association by Year from 2006 to 2016

Year	Major Representation(s) with at least 10%
2006	Genetic Engineering, Genetic Circuitry (Standards & Parts), and Industry & Funding/Investment
2007	Biofuels/Energy Applications and Venter- Biography
2008	Bacteria
2009	Synthesizing Artificial Life
2010	Venter- Biography
2011	Bacteria and Competition
2012	Medical Applications
2013	Policy & Regulation (Biosafety)
2014	Medical Applications
2015	Medical Applications and Genetic Engineering

This study desired to gain a more thorough, appreciable view of the understanding of synthetic biology in the public sphere. Therefore, it is crucial that I identified not only the representations with the highest occurrences, but the rate of occurrence for each representation. In this way, I could interpret the understanding and perceptions of synbio from how it is most often associated and least often associated, further altered by time.

The 24 representations previously identified occurred a total of 764 times. On average, each representation appeared in 32 articles, and each article averaged 7 representations. The total number of representations found in each year is directly proportional to the number of articles about synthetic biology in each year. Figure 6 details those 7 representations that had higher than average occurrences. Figure 9 details the 8 representations that had the average amount of occurrences. Finally, Figure 12 details the 9 representations that had less than average occurrences. Figures 7, 10, and 13 each show in a binary fashion the appearance of representations over the 10-year time span. The last charts in each section, Figures 8, 11, and 14 show the trends for each representation in each year, graphed to discover any remarkable occurrences.

Synthetic Biology: High-Occurring Representations

Figure 6: Table of Topic Categories, High Count (>39)

Topic	Total
Medical Applications	68
Genetic Engineering	63
Policy & Regulation (Biosafety)	57
Bacteria	53
Biofuels/Energy Applications	42
Environmental/Agricultural Applications	40
Genetic Circuitry (Standards & Parts)	40

Figure 7: Presence of Topic Categories, High Count (>39), for each year

TOPIC // YEAR	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	ALL
MEDICAL APPLICATIONS	x	x	x	x	x	x	x	x	x	x	x
GENETIC ENGINEERING	x	x	x	x	x	x	x	x	x	x	x
POLICY & REGULATION (BIOSAFETY)	x	x	x	x	x	x	x	x	x	x	x
BACTERIA	x	x	x	x	x	x	x	x	x	x	x
BIOFUELS/ENERGY APPLICATIONS	x	x	x	x	x	x	x	x	x	x	x
ENVIRONMENTAL/ AGRICULTURAL APPLICATIONS	x	x	x	x	x	x	x	x	x	x	x
GENETIC CIRCUITRY (STANDARDS & PARTS)	x	x	x	x	x	x	x	x	x	x	x
ALL	x	x	x	x	x	x	x	x	x	x	x

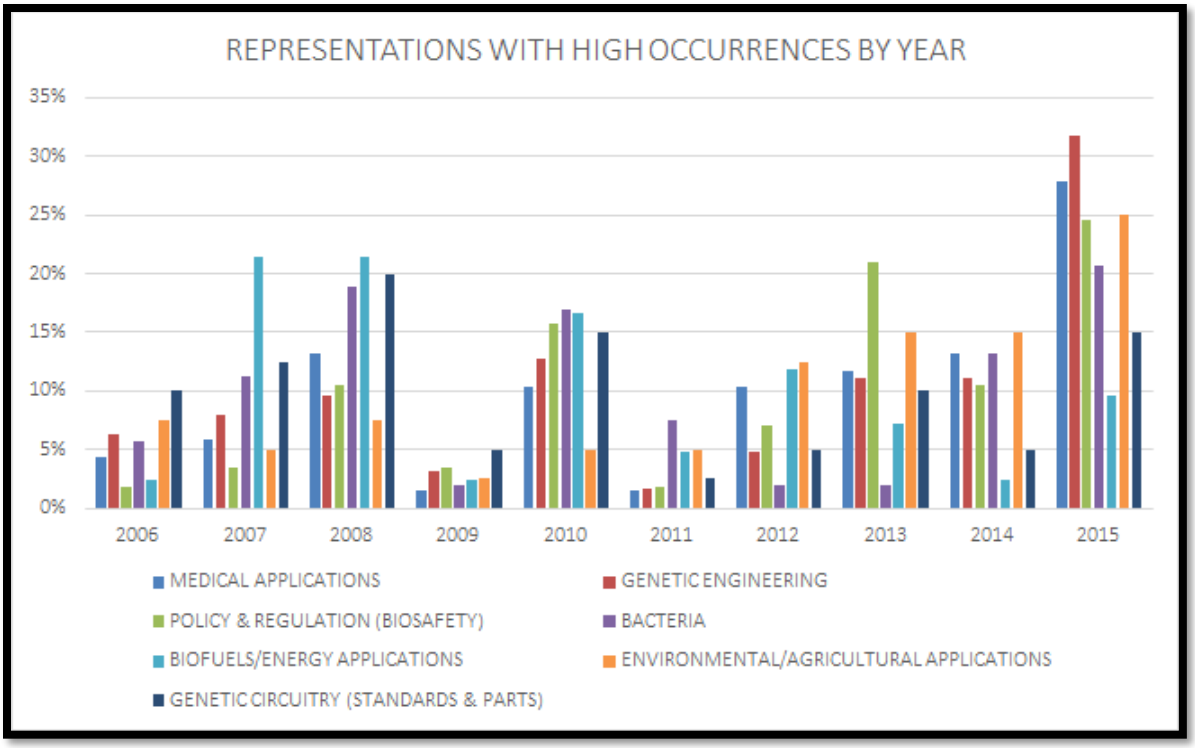

Figure 8: Representations with High Occurrences Graphed by Year

Temporal Findings

Not surprisingly, each of the representations with higher-than-average rates of occurrence appear in each of the ten years from 2006-2015. Medical applications and genetic engineering are by far the representations that are most commonly associated with synthetic biology throughout time, with almost twice as many occurrences as average. Mostly, all of the high-occurring representations follow the overall trend(s) of synbio media. There are two notable exceptions, discussed briefly below.

Biofuels/Energy Applications: This representation (marked in light blue in the graph above) directly contradicts the overall trend of appearance quantity of synthetic biology in written media. While the number of total articles about synbio from 2006-2015 is on a steady rise (with subtle increasing/decreasing trends within that time), the percentage of those articles representing synbio as having to do with biofuels & energy applications is on an overall steady decline over the course of those ten years.

Genetic Circuitry (Standards & Parts): This representation (marked in very dark blue in the graph above) follows the three separate trend intervals as the overall article data (an increase from 2006-2008, brief increase from 2009-2010, and the largest increase from 2011-2015). It differs, however, in that the percentage of genetic circuitry representations

continuously decreases over 10 years as the number of total articles about synbio increases- similar to the biofuels/energy applications representation overall trend.

Synthetic Biology: Average-Occurring Representations

Figure 9: Table of Topic Categories, Average Count (25-39)

Topic	Total
Industry & Funding/Investment	38
Venter- Biography	37
GMO & Foods	36
Morality (Bioethics)	35
Synthesizing Artificial Life	32
Competition	28
Globalism	28
Bioterrorism	27

Figure 10: Presence of Topic Categories, Average Count (25-39), for each year

TOPIC // YEAR	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	ALL
INDUSTRY & FUNDING/ INVESTMENT	x	x	x	x	x	x	x	x	x	x	x
VENTER- BIOGRAPHY	x	x	x		x	x	x	x	x	x	
GMO & FOODS	x		x		x	x	x	x	x	x	
MORALITY (BIOETHICS)		x	x	x	x	x	x	x	x	x	
SYNTHESIZING ARTIFICIAL LIFE		x	x	x	x	x	x	x	x		
COMPETITION	x	x	x	x	x	x	x	x	x	x	x
GLOBALISM	x	x	x		x	x	x	x	x	x	
BIOTERRORISM	x	x	x	x	x	x		x	x	x	
ALL			x		x	x		x	x		

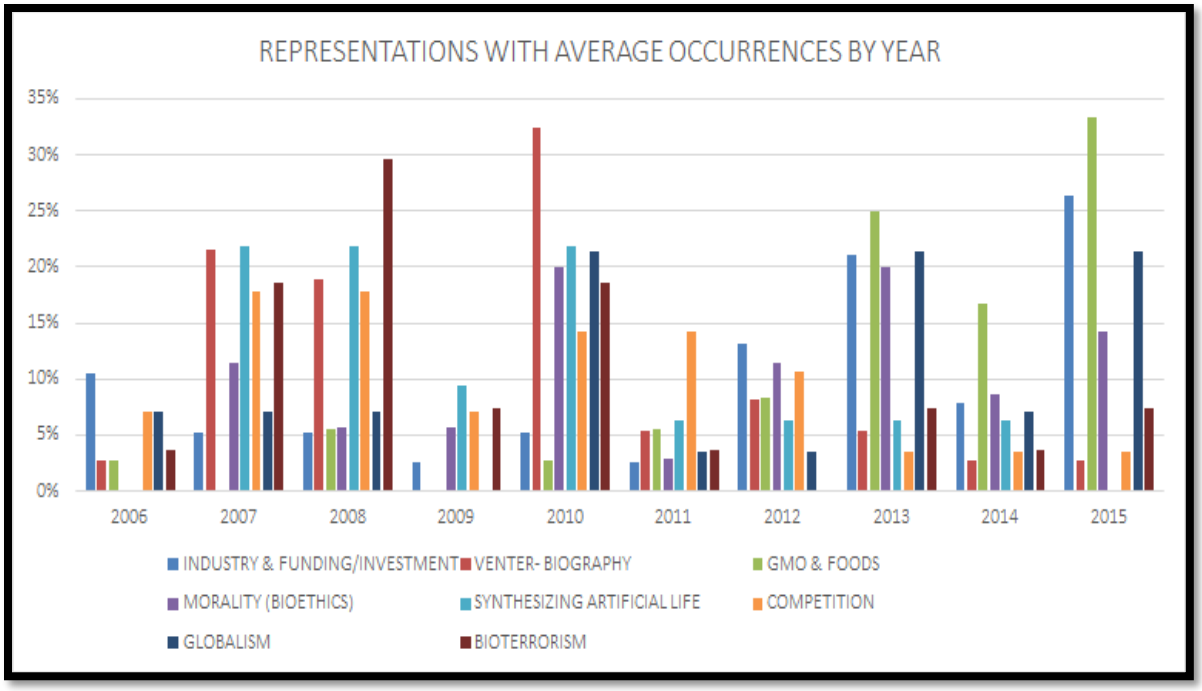

Figure 11: Representations with Average Occurrences Graphed by Year

Temporal Findings

The years 2008, 2010, 2011, 2013, and 2014 each contain at least one occurrence of every one of the average-occurring representations. However, the only two average-occurring representations that occur in at least one article every year are Industry & Funding/Investment and Competition. Only about half of the average-occurring representations follow the same trend(s) as the total quantity of synbio articles does.

Industry & Funding/Investment: This representation (marked in medium blue in the graph above) somewhat contradicts the overall trend of appearance quantity of synthetic biology in written media. The percentage of this representation actually decreases from 2006 to 2009 while most representations increase, only to then follow the trend by increasing from 2011 to 2015.

Venter- Biography: This representation (marked in red in the graph above) is noticeable for the significant spike in its percentage in the year 2010, when all other representations are significantly reduced compared to the other years. Furthermore, there is only a small, rather stable percentage of these representations from from 2011 to 2015, while the total number of representations/articles about synbio increases drastically.

Synthesizing Artificial Life: This representation (marked in light blue in the graph above) creates its own intriguing pattern. With the exception of none in 2006 and a reduced percentage in 2009, this representation contributes a high percentage from 2007-2010, where it then drops to provide only a small, stable percentage of representations from 2011-2014 (and none in 2015).

Competition: This representation (marked in yellow-orange in the graph above) follows a trend opposite to the overall article trend; barring 2006, it decreases over the 10 years while all others generally increase.

Bioterrorism: This representation (marked in brown in the graph above) is dominated by a majority percentage of its representations being in 2006 to 2008, as well as 2010. Yet, these years are the ones characterized by the lowest overall articles on synbio.

Synthetic Biology: Low-Occurring Representations

Figure 12: Table of Topic Categories, Low Count (<25)

Topic	Total
Church- Biography	24
High-Throughput Methods	20
Yeast	20
Science Fiction/Fantasy	17
Revolutionary/ "Cutting-edge"	16
Other (miscellaneous)	14
Software/Computer Applications	12
Biomaterials Applications	9
Keasling- Biography	8

Figure 13: Presence of Topic Categories, Low Count (<25), for each year

TOPIC // YEAR	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	ALL
CHURCH- BIOGRAPHY		x	x	x	x	x	x	x	x	x	
HIGH-THROUGHPUT METHODS	x	x	x		x	x	x	x	x	x	
YEAST	x	x	x		x			x	x	x	
SCIENCE FICTION/ FANTASY		x	x	x	x	x	x	x	x	x	
REVOLUTIONARY/ "CUTTING-EDGE"	x		x		x	x		x		x	
OTHER								x	x	x	
SOFTWARE/ COMPUTER APPLICATIONS	x						x	x	x	x	
BIOMATERIALS APPLICATIONS	x	x	x		x			x	x	x	
KEASLING- BIOGRAPHY	x	x	x			x		x			
ALL								x			

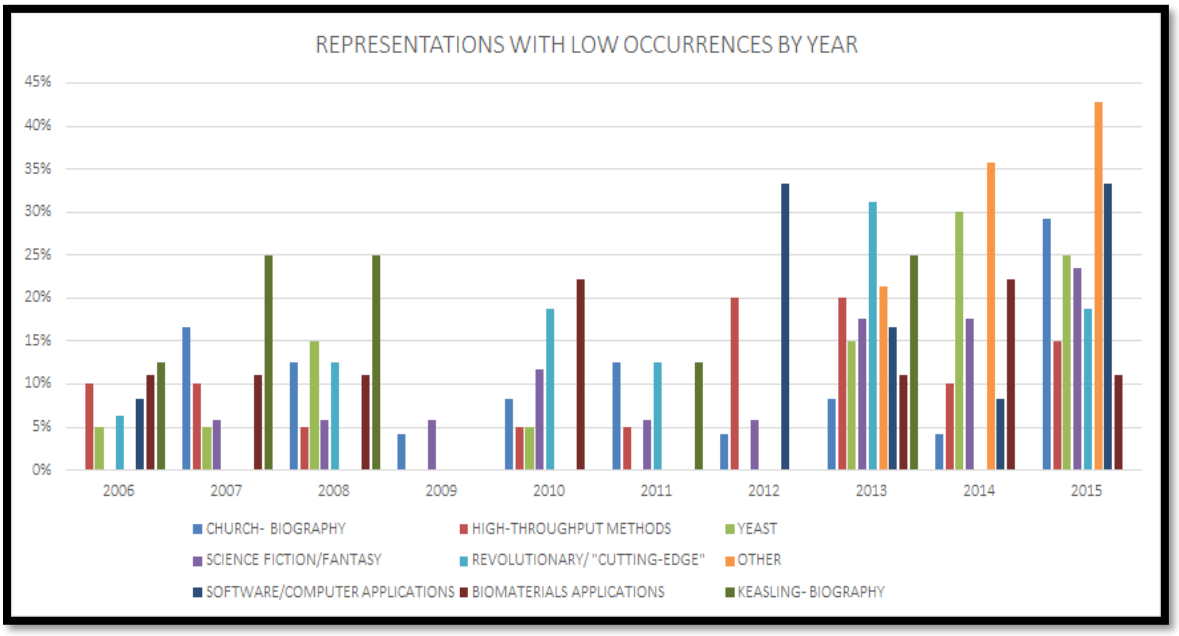

Figure 14: Representations with Low Occurrences Graphed by Year

Temporal Findings

Only the year 2013 contains each of the low-occurring representations. None of the low-occurring representations persist throughout all of the years. In their limited appearances, these less enduring topics associated with synthetic biology follow similar patterns as the overall trend(s) of synthetic biology articles. Their adherence to the prescribed trend(s) is discussed at large in the following section (Discussion).

DISCUSSION
The Dynamic Face of #Synbio

There is little doubt that synthetic biology is in an exciting era of technological advancement, benefitting from a snowball effect of enthusiasm and support from a variety of stakeholders: students, teachers, researchers, the government, and industry. It is important to recognize, however, that in very recent years, there is a stable percentage of negative articles. Rather than overwhelmingly positive coverage as more information about synthetic biology and its applications are published, the public remains hesitant. It is only logical for the public to be skeptical when synbio first appears as a new science in media in 2006, and again logical that when there are not many articles about synbio in general, the concerns about the science are also diluted. The turning point that reveals the continued hesitancy is in 2010 when there is a large spike in total synbio-related articles, accompanied by a proportionally large spike in negative article portrayals. Even though the percentage of negative articles remains the same in 2011 as it was in 2010, the percentage of positive articles greatly increases, (in fact, taking the place of neutral articles). This seems to serve as a reinforcement of the durability of synthetic biology as an emerging field, despite all concerns. While this study is obviously limited by the current time, it would be incredibly beneficial and revealing to conduct a more longitudinal study to observe these value trends continued from 2016 onwards, as the field undergoes more growth and coverage.

The ten-year span between 2006 and 2016 encompasses much of, but not all, of synthetic biology's earliest years. This particular timeframe, though, is fitting as it was in 2006 that artemisinin (an antimalarial) became known as the first "marketable product" attributable to synthetic biology, and SynBERC (Synthetic Biology Engineering Research Center) was created: two major recognitions for a still-emerging field needing a foothold in society's mind. Only the year 2013 contains every one of the 24 representations. This is despite the fact that 2013 is not the year with the highest amount of total articles or representations. This may mark 2013 as the year in which synthetic biology branches itself out, having enough foundational support and recognition to begin developing in new years. It will be fascinating to see if the 2013 invention of CRISPR-Cas9 gene editing technology will coincide with this allowance of synthetic biology to apply itself to even more of society's needs and researcher's curiosities.

Some of the most surprising data arising from this study actually arises from several of the representations that occur less than average (low occurrences). Faber used nanoscience as his example emergent scientific discipline in a comparative media study, and in his results, "Science Fiction" was a high-occurring representation, whereas it is one of the lowest in this study. However, synthetic biology does appear to follow the trend in nanoscience to associate itself with science fiction/fantasy in the latter years of

its establishment- specifically, mostly from 2013-2015 (Faber, 2006). It appears that only once synthetic biology was presented in media with "known" science for the first half of its years did it venture into the more "unknown", where more Other representations are seen in addition to Science Fiction/Fantasy, as well as Software/Computer Applications (not typically a topic combined with biology).

Synthetic biology as newly formed scientific field is, therefore, following some of the same patterns and aligning with some of the same categories as other emerging scientific disciplines, in a general way. Much of the written media presents synbio in association with broad categories and what the science could mean for the advancement of public society, rather than explaining the hard science behind it. The claim to "cure cancer" and "invent a new sustainable biofuel" is not unprecedented, and furthermore unspecific. In particular, Medical Applications was also one of the highest-occurring representations of nanoscience as it struggled to emerge itself. This familiar scientific aim time and time again yields to both the public's idea of scientific research as well as the much-needed conveyance of the scientific world to gain support and funding from the public- who would refute a science discipline that holds the key to unlocking disease, obesity, global warming, clean water, and food shortages? Alas, even with this common theme to emerging science, much of the same criticisms appear throughout the media as well. As a new, not-yet-understood science presents itself to the layman, making lavish claims and expressing itself as the magic to life, there is also the stream of concern and discussion of regulation. Another noticeable similarity in the process of the emerging science is the prominence of a scientific figure- while Drexler led the nanotech world, Craig Venter plays a gargantuan role not only in the research of synthetic biology but in the public representation of it.

But, synthetic biology deviates from the pattern(s) of emerging science in at least one major way. Other fields such as nanoscience have attempted to substantiate themselves by proving that they are radically different, and wanting to establish their own programs/departments/labs dedicated to purely this new field of science, arguing that it will replace previous notions of physics or biology, and needing its own place in the scientific community. Synbio, on the other hand, is a true bridge between current understandings of engineering and biology, and instead of pressing for separate recognition, throughout the articles of this data set, it consistently announces itself as such. It capitalizes on the known area of genetic engineering and relies on this credibility to establish its own further aims and progresses. Instead of fearing the association with other existing sciences and technologies, it is grateful to the work that these disciplines have given for its own creation. Ultimately, perhaps this will prove to succeed in ways other disciplines haven't, not only in the lab but in the public eye.

CONCLUSION
Synthetic Biology as a Hybrid

The nexus of synthetic biology in biology and engineering - life and machines - is a fascinating blend of two long-well-known sciences. The interplay between these two is the force that stabilizes and propels the emerging area of synbio, and the interface between lifeless and life-prone areas is perhaps what lends to synbio's substantial current success. Only time will tell how the public continues to perceive and value the emergent discipline. For now, scientists will continue to tinker with DNA, repress genes, transplant bacterium genomes, reinvent species, alter foods- many of the old scientific ideas rebranded in a heightened way, under the new guise of synthetic biology.

CHAPTER TWO

Single-Base Editing of Cellular mRNAby CRISPR/Cas9

**Completed in partnership with Andrew Baker (BBT/CBC)*

ABSTRACT

Genetic engineering is currently dominated by CRISPR/Cas9 technology, promising precise multipurpose genome editing. The 2016 WPI iGEM team investigated the potential of adapting this technology to direct single-base editing of mRNA by linking deactivated Cas9 to the C-to-U RNA editor enzyme APOBEC1. We pursued the re-cloning and characterization of the dCas9/APOBEC fusion to identify and eliminate problems related to expression and toxicity. Ultimately, this adapted CRISPR/Cas9 system could provide an advantageous method of editing, particularly for therapeutics.

INTRODUCTION
A Revolution in Synthetic Biology: The CRISPR Way

The natural CRISPR/Cas9 system employs a bacterial adaptive immune response in which clustered regularly-interspaced short palindromic repeats (CRISPRs) are recognized by the nuclease Cas9 and cleaved for the purpose of editing the gene in an effort to ensure organism survival. This system has been specially adapted for the use of targeting virtually any position in the genome by guiding the Cas9 to recognize specific sites of double-stranded DNA (dsDNA), usually via engineered single-guideRNAs (sgRNAs) (Ran et al. 2013). A simple diagram of the mechanism of CRISPR/Cas9 technology is below:

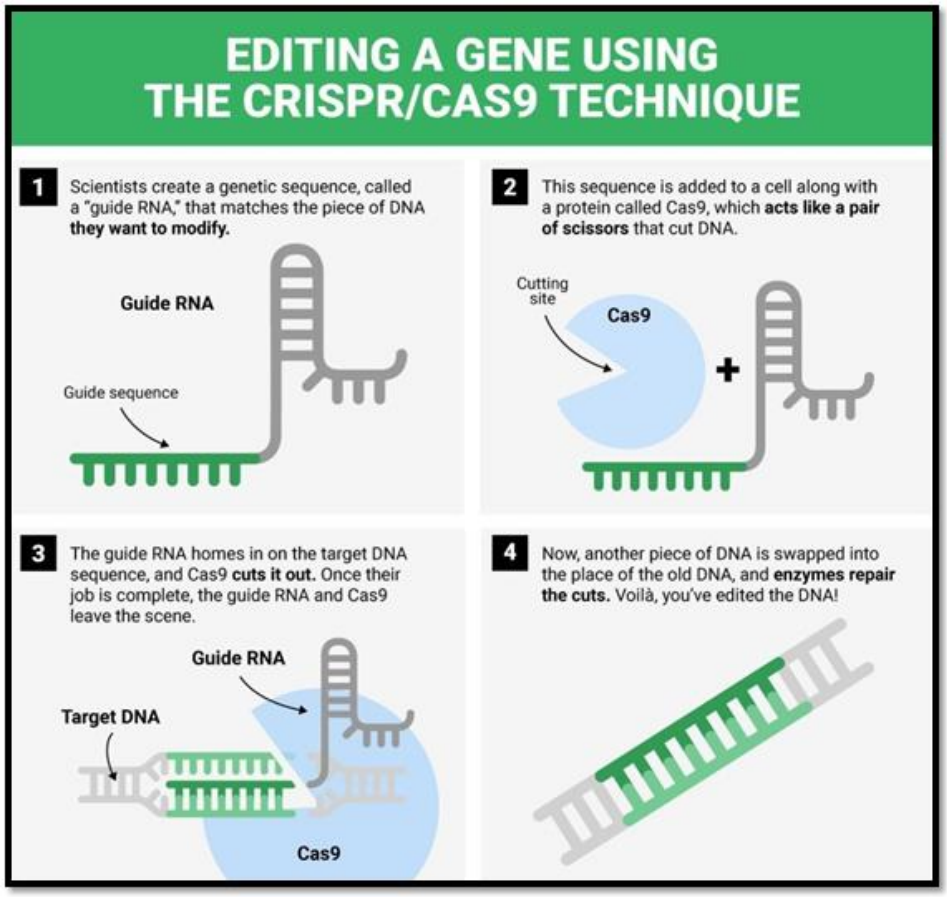

Figure 15: A Diagram of CRISPR/Cas9 Editing Technology (Lewis 2015)

Because of its natural derivation and high efficiency, the CRISPR/Cas9 system has quickly developed into a highly-regarded gene editing technology, through both recombination-based and single-base editing. In recombination-based editing, Cas9 cuts the dsDNA at a target locus, where the DNA is edited in the desired way (i.e. gene edits,

knockouts), and the cell undergoes either non-homologous end joining (NHEJ) or homology-directed repair (HDR), although typically NHEJ (Ran et al. 2013). In single-base editing, the CRISPR/Cas9 system foregoes double-stranded DNA cleavage but retains editing power through an attached DNA-editing deaminase enzyme called APOBEC, which targets specific bases to be changed to a different nucleotide, such as a C→U substitution (Komor et al. 2016).

While CRISPR technology has become a rather valuable tool in modern molecular biology, the current system is not without its issues, both technically and ethically. Biologically, Cas9's ability to cut so efficiently at specific sequences on the genomic level is what makes it such a useful tool; however, sequences similar to the target sequence are often also cut, resulting in unpredictable and irreversible off-target effects (Harrison et al. 2014). After the initial cut by Cas9, cells routinely use the NHEJ repair pathway to repair the genomic DNA. This repair mechanism is often inaccurate and leads to frequent insertions, deletions, and changes of nucleotides. These indel and single-point mutations are unpredictable and irreversible, with the potential to yield drastic, undesirable results (Harrison et al. 2014). Lastly, the extent of complex post-transcriptional modifications and splicing variation allows for a myriad of uncontrollable outcomes, due to the nature of editing primary genetic material. Our project was motivated partially due to these technical concerns, and partially due to the share of ethical and safety concerns that naturally come from genetic engineering.

In response to these concerns, one adaptation to the current CRISPR/Cas9 gene-editing system has been to target RNA instead of DNA. It has been shown that CRISPR/Cas9 can in fact be programmed to recognize single-stranded RNA (ssRNA) while avoiding the corresponding DNA sequences, and further cleave the targeted RNA in this manner (O'Connell et al. 2014). Furthermore, the CRISPR/Cas9 system has been demonstrated to be useful for live RNA tracking in vivo (Nelles et al. 2016). Finally, very recent progress by the Huang group has addressed the need to develop an mRNA-based CRISPR tool; their work validated the possibility of efficient targeting of cellular mRNA by an enzymatically dead Cas9, without affecting its corresponding DNA segments (2016).

Therefore, understanding the technical and ethical issues presented by the common system of DNA-targeting CRISPR/Cas9, having studied the precedent of RNA targeting with CRISPR/Cas9, and inspired by the foundational work of the 2016 Worcester Polytechnic Institute (WPI) iGEM team in this field, we developed an adapted CRISPR/Cas9 system that also targets mRNA (WPI_Worcester, 2016). Another significant change from the current technology is that it utilizes the DNA/RNA editing enzyme APOBEC to cause specific single-base edits.

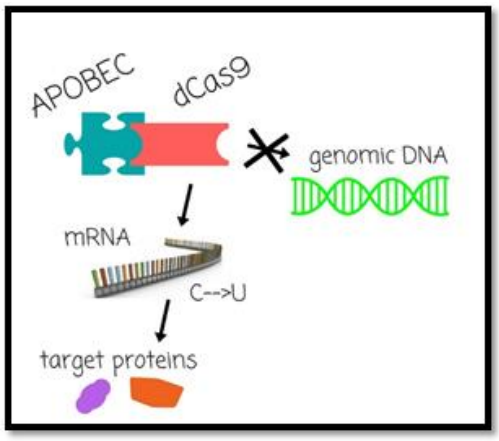

Figure 16: A Diagram of Targeted mRNA Editing by dCas9/APOBEC

In this way, we can make very precise adjustments to the mRNA of a desired organism, regulating its related protein levels, without editing a single nucleotide of DNA. Additionally, the application of this tool would be a temporary therapy; in the case of any unforeseen error or mutation, the same regulation could be easily reversed by stopping the editing of mRNA. We propose that this mRNA/CRISPR/Cas9-APOBEC system can be used for the same research and therapeutic purposes as the original CRISPR gene-editing technology, but with significantly reduced scientific and ethical risk, as well as increased accuracy.

Our project continued the foundational work done by the 2016 WPI iGEM team in its efforts to develop this promising adapted CRISPR/Cas9 therapy. Herein, we describe our attempt to re-clone the dCas9/APOBEC construct. We also describe our conclusion of the project with transfection trials of several constructs into mammalian cells.

MATERIALS & METHODS

Preparation of pcDNA3.1+

We transformed 2 uL pcDNA3.1+ template vector DNA (provided by the WPI 2016 iGEM team) into 50 uL chilled, thawed competent DH5α *e. coli* cells. They were then incubated on ice for 30 minutes, heat shocked at 42°C for 1 minute, and incubated back on ice for 5 minutes. 200 uL of SOC media was added to each 52 uL cell/DNA mix for a brief outgrowth period. The SOC media contained the following ingredients and then was sterilized and filtered: 0.5% yeast extract, 2% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, and 20 mM Glucose. The cells in the media were put into a shaking incubator at 37°C for 2 hours, then plated (in various quantities) on LB plates (made with 1 mL Ampicillin at a concentration of 100 mg/mL). These sat overnight in a 37°C incubator. Single colonies were picked for overnight cultures in 3 mL LB (w/Amp) each. These were mini-prepped using the Macherey-Nagel NucleoSpi Plasmid Mini Kit (Bethleme, PA) per the manufacturer's instructions. The mini-prep products were run through a 1% agarose gel at room-temperature and 100 Volts, visualized by SYBR (from Qiagen), then cut out and gel-purified by Macherey-Nagel Gel and PCR Clean-Up Kit per the manufacturer's instructions (Registry of Standard, n.d.). Verification gel(s) can be found in Figure 1 of Appendix B.

Amplification of dCas9 and APOBEC

dCas9: We amplified dCas9 DNA template (provided by the WPI 2016 iGEM team) via PCR reactions with Q5 master mix (New England BioLabs, Beverly, MA), using the following protocol:

1x	[95 for 02:00]
30x	[95 for 00:30]
	[59 for 00:45]
	[72 for 04:30]
1x	[72 for 10:00]
Hold at 10 degrees for infinity	

The PCR product was run in a 1% agarose gel, visualized by SYBR (from Cambrex in Rockland, Maine), cut out and gel-purified by Qiagen purification kit. Verification gel(s) can be found in Appendix B.

The temperature for annealing in the dCas9 amplification protocol was established by performing a gradient-temperature PCR reaction. We ran 12 identical reactions, each of 15 uL, spanning from 45 to 62 degrees Celsius. The brightest bands when visualized in an agarose gel by SYBR allowed us to calculate the optimal annealing temperature to be 59 degrees. For the gel results and calculation, please refer to Figure 2 of Appendix B.

APOBEC: We amplified APOBEC with 1Xten linker, 2Xten linker, and 3Xten linker (provided by the WPI 2016 iGEM team) via PCR reactions with Q5 master mix, using the following protocol:

1x [95 for 02:00]
30x [95 for 00:30]
[55 for 00:45]
[72 for 01:00]
1x [72 for 10:00]
Hold at 10 degrees for infinity

The PCR products were treated as described above.

Cloning of dCas9 into pcDNA

We used Gibson cloning method to attempt to clone dCas9 into our pcDNA3.1+ vector. We mixed 100 ng of gel-purified pcDNA3.1+ vector DNA with 200 ng of gel-purified dCas9 insert DNA, along 10 uL of NEBuilder HiFi DNA Assembly Master Mix and the remaining amount needed of deionized water to create a 20 uL total reaction. The reactions were incubated in a PCR machine for 30 minutes at 50°C. The reaction product was then transformed by mixing 2 uL of the product into 50 uL of thawed competent DH5α *e. coli* cells (obtained by Professor Farny's competent cell stock in the -80 degrees Celsius freezer), incubated on ice for 30 minutes, heat shocked at 42°C for 30 seconds, and put back on ice for 2 minutes. 950 uL of room temperature SOC media was added, the mix was then incubated at 37°C with vigorous shaking for 1 hour, then plated on prewarmed LB (w/Amp) plates, and incubated overnight at 37°C (Gibson, 2009 and New England BioLabs, n.d.). We further attempted to repeat this with un-gel-purified dCas9 insert DNA, all the rest remaining the same. Our control for these reactions was to plate the plasmid without insert DNA, and cells without any transformation plasmid or insert DNA (Registry of Standard, n.d.).

Transfection of dCas9/APOBEC Constructs

For transfection, we followed the protocol found with TransFectin (from Bio-Rad), a highly efficient, lipid-based transfection reagent. We plated 600,000 cells into the wells of a 6-well plate and allowed 24 hours for adherence. We then added 2 μg of DNA with 10 μL TransFectin and counted this as time = 0 hours for imaging by microscopy. We performed two transfections using this basic process.

The first transfection was conducted with H1299 cells, which is a human non-small cell lung carcinoma cell line, and was done as described above. The DNA inserted into the cells were the following constructs: APOBEC 1X, APOBEC 2X, APOBEC 3X, BE2, dCas9, and pRETRO. The control was cells with no DNA. The cells were incubated at a stable 37 degrees Celsius. After 48 hours, the cells were imaged (please see Results section).

The second transfection was conducted with MCF7 cells, a breast cancer cell line, and was done as described above, with the following exception: the cells were incubated for 4 hours before changing media, completely removing the transfection reagent. The media used was standard DMEM, 10% FBS and 1% Pen-Strep (Gibco). The following three variations were the conditions used: transfection reagent and DNA, DNA only, and transfection reagent only. The DNA added for this transfection was the BE2 construct (Addgene Plasmid #73020). The control well contained only cells. All cells were imaged at 24 hours (please see Results section).

RESULTS

The first part of our project consisted of the re-cloning of dCas9 into the pcDNA3.1+ vector, to be followed by APOBEC with the 1X, 2X, and 3X size linkers. We first amplified our dCas9 DNA through multiple PCR reactions, but rarely were able to produce enough DNA to begin molecular cloning. Because of this issue, we optimized the PCR protocol by performing a gradient-temperature PCR reaction in which the annealing temperature of the reaction was increased incrementally. Below, Figure 17 illustrates the results collected.

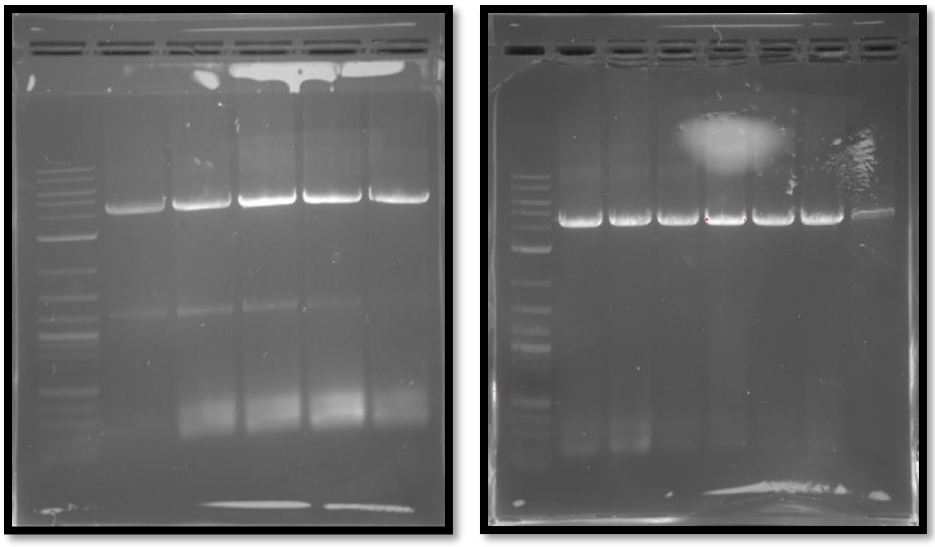

Figure 17: Gradient-Temperature PCR Gel Results

The lanes in the two gels sequentially represent reactions 1-12 of the temperature gradient, ranging from 45 to 62 degrees. The results shown in Figure 1 suggest an optimal annealing temperature between 57.78 (reaction 9) and 59.2 (reaction 10) degrees. Based on these results, the annealing temperature used in future PCRs was set to 59 degrees (full calculation can be found in Figure 2 of Appendix B). Using this modified PCR protocol, we continued amplifying the dCas9 DNA and began attempting to clone the DNA into pcDNA 3.1+ via Gibson assembly.

Inserting the dCas9 DNA into the pcDNA 3.1+ vector proved difficult for unknown reasons. We believe that because of dCas9's size (approximately 4.2 kb), the DNA was simply too big to successfully integrate into the pcDNA 3.1+ plasmid in one step. We suggest that in the future, the dCas9 gene could be split into two or three parts, amplified separately via PCR and inserted individually into the plasmid. By splitting the gene into

parts, the size of the insert is dramatically reduced - possible making the cloning more successful.

Because we had little time left in our MQP to complete the cloning process and transfect with our own constructs, we had the fortune to use iGEM's previously made/purchased constructs for investigating transfection/expression of dCas9 and APOBEC in mammalian cells. We began by first taking each individual component of the pRETRO iGEM-constructs, as well as the AddGene plasmid BE2, and transfecting H1299 mammalian cells. Figure 18 below clarifies each of the DNA constructs we added to our transfection(s):

Figure 18: Table of DNA Constructs Used in Transfection(s)

DNA Construct	**Plasmid Backbone**	**Contents**	**Source**
"pRETRO"	pRETRO	none	AddGene
"dCas9"	pRETRO	dCas9	iGEM
"APOBEC 1X"	pRETRO	APOBEC 1X	iGEM
"APOBEC 2X"	pRETRO	APOBEC 2X	iGEM
"APOBEC 3X"	pRETRO	APOBEC 3X	iGEM
"BE2"	pCMV	dCas9/APOBEC	AddGene

Following, Figure 19 shows the images taken from the first transfection, which was done with H1299 cells:

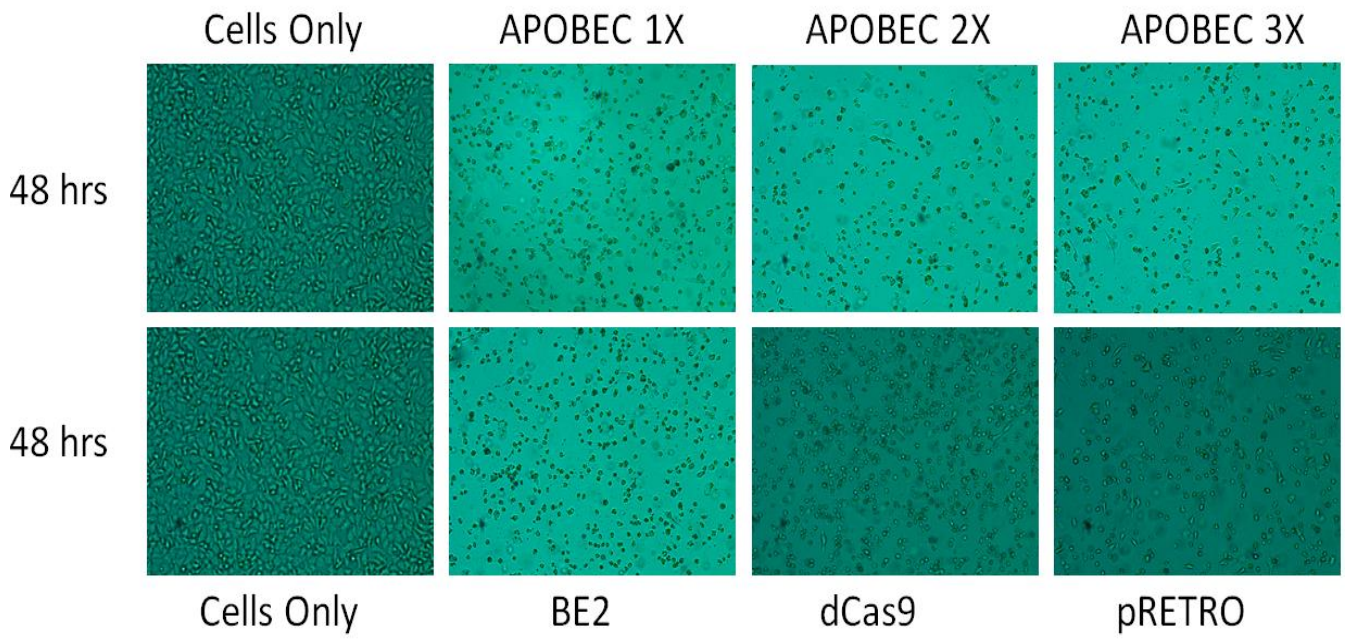

Figure 19: Cells 48 Hours Post-Transfection with Various Constructs Compared to Control

From this transfection, we realized the incidence of massive cell death in every well except for the one that contained only cells (no transfection reagent and no DNA) - specifically, after 48 hours, the cells with DNA added became suspended in the media, over 80% dead as compared to the anchored, 90% alive "cells only" well. This was an unexpected result, especially because a simple vector such as pRETRO should not theoretically cause cell death. Therefore, we wanted to determine which of the two added transfection ingredients (reagent or DNA) was the one responsible for killing the cells. To do this, we conducted a second experiment including proper controls to make a more thorough and substantiated conclusion. This second transfection involved transfecting MCF7 mammalian cells with only BE2. We decided to switch to the new cell type, MCF7, to determine if the cell type played any role in the results, and to prevent cell death if caused easily to the previous cell types tried (293T's and H1299's).

Following, Figure 20 shows the images taken from the second transfection, which was done with MCF7 cells:

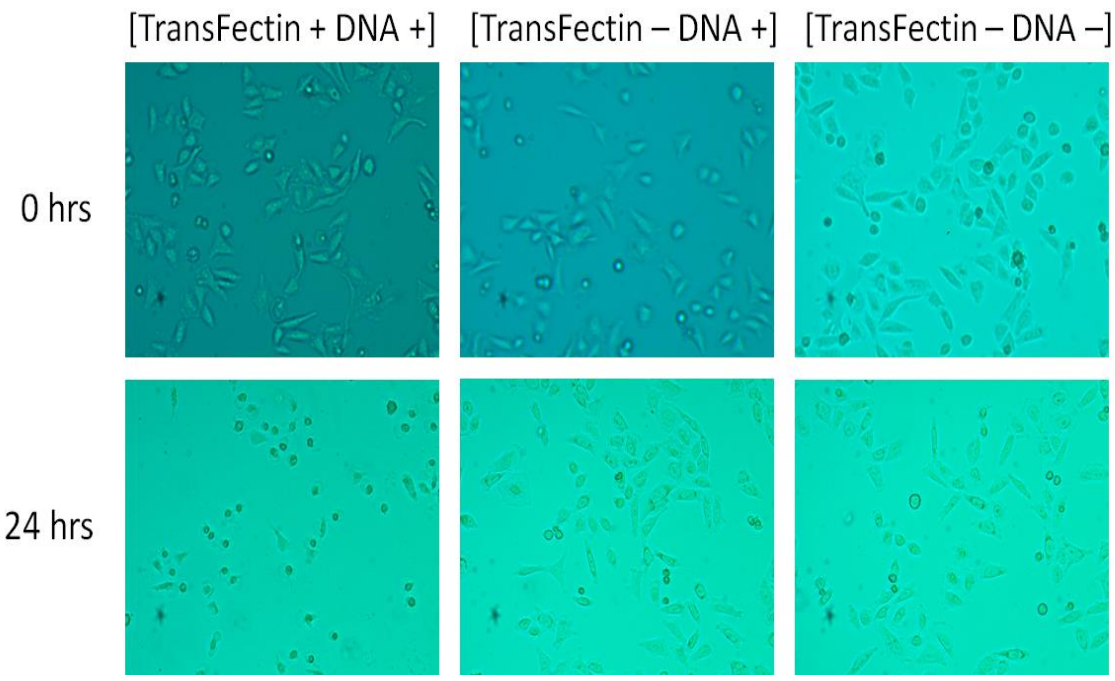

Figure 20: Cells 0 and 24 Hours Post-Transfection, Testing for Toxicity Cause

The images collected above indicate that the transfection reagent used (TransFectin) is responsible for the quantified cell death observed in both Figures 19 and 20. This result could be due to a variety of reasons including: the age of the TransFectin (4+ years), or the plasmid itself. While the second column in Figure 20 indicates that the DNA is not killing the cells, the DNA is not able to enter the cells because of the absence of TransFectin, thus it would be useful to recapitulate these results with a Transfectin-only culture. This is important to note because once TransFectin is added, the DNA forms complexes with the TransFectin and enters the cells. BE2 contains pRETRO as its plasmid backbone - a DNA sequence that originates from viruses. We suggest that cell death may be occurring in Figures 19 and 20 because of the use of pRETRO. As the DNA enters the cell, it may be integrating into the host genome and inducing apoptosis through either integrating in a non-optimal location (disrupting essential genes), or leading to interferon production in the cell which then leads to cellular apoptosis. We also recommend attempting the same transfections as above in media that does not contain antibiotic. While bacterial antibiotic, like Penn Strep, should not usually cause mammalian cell death, the increase in cell permeability via lipid-based transfection may be contributing to the observed cell death. Similarly, it is recommended that alternative transfection protocols be attempted. All of our recommendations are discussed in the following section.

DISCUSSION

The foundational work completed by the iGEM team led to an exciting prospect for our MQP. It is indisputable that CRISPR technology offers boundless potential for applications ranging from medical to environmental. However, its technical concerns simultaneously present ethical concerns for the scientific community and the society at large. Firstly, accidental release of any genetically modified organism could lead to severe ecological disruptions. Any experimental organisms released into an ecosystem could quickly proliferate, spreading possibly hazardous DNA sequences throughout the ecosystem (Rodriguez 2016). A second, more economical concern, is the recent practice of patenting DNA sequences. CRISPR technology is frequently used to create and insert laboratory-produced DNA sequences that are then often used in applications for patents. This creates the ethical concern of whether it is morally right to patent genes or other DNA sequences (Rodriguez 2016). A final and most severe ethical concern associated with CRISPR is the practice of editing human genomes. Already, UNESCO has issued the Universal Declaration on the Human Genome and Human Rights which recommends a moratorium on human germline genome editing. Despite similar recommendations to avoid using tools like CRISPR on the human genome, some have argued the potential to treat a wide range of diseases, such as cystic fibrosis or sickle cell disease, through the tool (Rodriguez 2016). It is these concerns that have placed CRISPR into a negative light in terms of public opinion and have founded the need for a safer, less controversial alternative to CRISPR.

While we encountered obstacles in the lab that prevented us from progressing further in the development of this adapted CRISPR/Cas9 technology, we enjoyed the experience immensely and learned boundless skills in troubleshooting and critical thinking. We stress the importance of further developing this adapted CRISPR technology and highly encourage a future MQP team to pick up where we left off. To this end, we have compiled a list of major routes that we suggest such a team to proceed on. We look forward to the future development of this project.

Our first major suggestion is the continued cloning of dCas9/APOBEC into the pcDNA3.1+ vector. Future persistence in these cloning aims should include APOBEC inserts of various size linkers into pcDNA3.1+, as well as into other standard and atypical vectors. Given the trouble we faced with cloning in dCas9, we propose splitting the dCas9 DNA into multiple parts and cloning them into the vector in pieces. Reducing the substantial size of the insert when cloning the DNA may facilitate uptake of the gene into a plasmid.

Our second major suggestion is determining successful alternate transfection protocols to reduce toxicity to the cells. We suggest attempting the same transfection as done above,

but in media that does not contain any antibiotic (our attempt at this yielded large contamination, making the cells difficult to image- we suggest trying several concentrations/quantities of antibiotic). We also suggest transfecting the cells using plasmids that do not originate from viruses in order to reduce possible interferon production post-transfection. Finally, we also suggest finding alternate methods of transfecting the DNA into the cells, in order to accurately determine the effect of the dCas9 construct(s), such as a calcium phosphate method.

Once the dCas9/APOBEC construct(s) have successfully been cloned into an appropriate plasmid vector and the construct(s) have been transfected into mammalian cells, the next major step in the project can be continued. Firstly, one would need to verify the expression of the construct in vivo. Following that is to proceed with introducing guide RNA to target the CRISPR/Cas9 system for editing. Finally, conduction of RNA collection and sequencing, as well as protein analysis, would both be done to test for successful mRNA editing.

CHAPTER THREE

A Reflection on the Socio-Technical Paradigm

A Personal Reflection on the Socio-technical MQP Experience

"This doesn't belong here."

These are the words I continue to remember the most from Project Presentation Day on April 20, 2017. I was presenting my MQP at the CBC/BBT poster session in the Campus Center Odeum. This included a poster with my partner representing our work in the laboratory in the area of synthetic biology, on developing an adapted CRISPR/Cas9 technology for genetic engineering, accompanied by a poster representing my individual work on popularized synthetic biology in media. I had set the posters up, side-by-side, as I had been designated to do, and was excited to share my experience with the many students, faculty, and family that would be attending the events of the day.

The students of the CBC/BBT departments were instructed to prop up our posters and then attend a lecture in the room next door before the poster session would begin. My peers did this, and I followed suit. However, before the speaker officially began, I realized I had forgotten something back at my posters, so I walked quickly over to where my backpack was lying near them. There stood a professor having a conversation with another faculty member, and that's when I heard the words:

"This doesn't belong here".

They had not realized I was there, or at least that it was ***my*** posters they were talking about. It was obvious that the CBC/BBT professor was referring to my Professional Writing poster on synthetic biology, as she peered at it, scrutinizing. The tone of the words, said so casually, hit me in a profound way. At first, I couldn't help but be hurt. "My work doesn't belong here?", I thought to myself questioningly in a broken-hearted way. I didn't have much time to ponder it further, as I hurried back to claim my seat at the morning lecture. But, as the speaker continued on to the better half of an hour, my mind began to drift away from the antibiotic resistance content she was explaining so intelligently, and back to the claim I had heard just a while ago in the adjacent room.

"This doesn't belong here".

On one hand, I took it personally. I fervently believe I deserved to present my combined MQP work at the CBC/BBT session. Even if one of my two posters was Professional Writing-focused, it was relevant to my work in the lab, relevant to the scientific community at WPI. I had not replaced my work in the lab with a silly composition on the subject- I was not trying pompously to earn the same credit with a lesser project. Rather, the PW poster was an addendum to my work in the lab, a sister study. Of course, I am biased to the importance of my own research. But, as I mulled over those same words, ringing over and over again in my head, I also realized the inherent bias as demonstrated

by the CBC/BBT professor's casual phrase. In one striking moment, I couldn't help but suddenly think how ***perfect*** those words really were in embodying the entirety of my project. After all, it's true- as science and humanities currently stand, my project ***didn't*** belong there. Given their way, the scientific community would not want "my humanities" taking up "their space". Here, I don't talk pointedly anymore about just the one CBC/BBT professor and the departments at WPI, but rather the general academic and practical disciplines of the "sciences" and the "humanities". In that moment, I no longer took the words personally, but genuinely smiled at the magic in the meaning they held, relishing them as the exact, most perfect embodiment of a reason for my project in the first place. This revolutionary moment in my mind fueled my fire to share my entire MQP experience throughout the day that followed.

When I began my combined MQP, I really had no idea what I had signed up for. I set my synthetic biology project up with my CBC/BBT advisors first because it was easier to do so and it required more advanced planning. I confirmed with them the capability to combine lab work with an extra writing aspect guided by an additional advisor. Then I found my PW advisor and we tossed ideas at each other through scattered emails while I was on IQP and over the summer, in regards to what avenues I could explore at the interface between research in a scientific discipline like synthetic biology and scientific communication. At the start of senior year, I was excited to dive into the MQP headfirst. Having prior experience with CRISPR in the lab, I felt fortunate to be given the opportunity to continue in this topic as one of the synthetic biology teams in the project lab. Narrowing down my focus for the writing side was not as easy of a task – there are too many fascinating avenues to explore in scientific communication and rhetoric. Once I read a prior study conducted by my advisor on popularized nanotechnology, however, I felt passionate about doing a similar project for the emerging field of synthetic biology.

As a primarily Biochemistry major knee-deep in scientific material, my perspective on scientific articles in popular media is at a stark contrast to how the audience outside of the scientific community views them. My interest lies in the understanding of synbio by the everyday readership of popular general magazines, like the New York Times and Boston Post. How do journalists portray the science, and in return, how do the readers perceive it? How in sync are these portrayals with the hard science being done in the lab, with the aims and achievements that scientists are actually striving for and accomplishing? Which community, the sciences or the humanities, is given the responsibility of determining this disconnect, if any, and how it should be remedied? Regardless of the accuracy of media coverage, scientists must address the public in their concerns and misunderstandings. Yet, as long as the science is propelled with promise to further the good of humankind, its investment is secured, and the two communities remain largely separated and in conflict with each other.

It seems that these very separate circles of intelligent communities worry about the harm of each other before it happens, or point blame after harm occurs, but cannot seem to find

an adequate way of addressing harm ***in*** the moment. When humanities professionals, such as sociologists and philosophers, raise questions of concern in ethics and safety, for example, the morality of "playing God to synthesize artificial life", the biohazard of releasing genetically modified organisms, or the fear of terrorist attacks by designer viruses, science is not halted- and nor should it be, as science plays a critical role in everyday society. Instead, politicians and other social agents react in one of two ways: (1) desiring to put regulations in place to *prevent overstepping the bounds* of "proper science" or (2) *chastising* a scientific act as having *already stepped over those bounds*. This description of ineffective pre-interruption or post-interruption is clinically explored in Paul Rabinow's experience with SynBERC, outlined in his book "Designing Human Practices: An Experiment with Synthetic Biology". Consistently, policies and regulations on scientific practice, as set primarily by humanities communities, are deemed outdated, extreme, or invalid for another reason. Similarly, scientific advances are often accepted with immense hesitation or outright refuted in humanities communities. In other words, there have either been little to no attempts at using the two disciplines together other than imposing one over the other, no attempts at bridging the two communities together to establish common goals and laws that can be agreed upon and followed by both- either this or all large-scale attempts thus far have proven unsuccessful.

My limited socio-technical experience, working in both areas within the context of synthetic biology- conducting biology research and studying its social implications, has taught me very little and a great deal about this problem and its potential solutions at the same time. I have learned that there is no easy or immediate answer, at least not in present society, to the disconnect between the disciplines. Simply, the humanities does not understand science, just as science does not understand humanities, and the lack of understanding lends itself all too easily to impatience and under-appreciation. When the professor spoke the words that my PW poster did not belong in the CBC/BBT sphere of work, she said this not out of malice but out of an ingrained ideal of separation between the two departments. It is this barrier that prevents successful complementarity and integration between them that would prevent future tension.

To the professor I overheard by chance, my PW poster was regarded as an irrelevant piece of work without taking the time to stop and look for what it really was. Moreover, it was disregarded as "just a project in the humanities", without acknowledging its very tangible relation to synthetic biology and what value it had in telling scientists how the emerging discipline has established itself in the mind of everyday readers, the ones who would be contributing to the funding and societal acceptance of the very same science researchers are trying to promote in society. My goal in completing this MQP was to do what little I could to broaden the minds of individuals in both academic communities to the importance of each other. I could not have learned what I did had I only kept my head down in the laboratory performing genetic engineering, and I could not have learned what I did had I only read popularized articles on synthetic biology. I wholeheartedly admit that because of my work across disciplines, I may have lost short-term efficiency

in the progress of either project, but it is truly fact that I am better served for the future by having done them together in one cohesive MQP experience.

Through this MQP, I have embarked upon a personal journey in the socio-technical paradigm. As I venture on to a career combining both, learning and living the potential of a sociotechnical perspective, I know that regardless of what framework I'm presenting in, whether this be one of the humanities or of science, and regardless of the allowance or acceptance of any poster, I'll always need to remember the importance of this broader perspective. As an aspiring scientist and communicator, I hope my MQP shows the incredible power of an exercise in both. We are all "layman" to what is outside of our fields, but it is our decision to remain captive by our bias or to find the value in what lies outside. It was my decision to pursue both a technical and a rhetorical degree, and because of this, truly combining my expertise in the two in this final MQP, that I feel prepared to look beyond the barriers of one discipline, and to always strive for the successful integration of both social and technical for the betterment of society as a whole. It is because of this decision, that even as a layman, my work will always "belong".

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APPENDIX A.
Supplemental to CHAPTER ONE

Figure 1: Table of Articles by Source and Year

PUBLICATION/YEAR	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	TOTAL
(1) Boston Globe	4	0	4	2	3	2	4	7	1	3	30
(2) Chicago Tribune	0	0	0	0	0	0	0	0	2	1	3
(3) Houston Chronicle	0	1	0	0	0	0	1	2	3	2	9
(4) Los Angeles Times	1	0	0	0	0	0	0	1	5	8	15
(5) New York Times	1	3	2	0	11	3	4	6	5	10	45
(6) Orlando Sentinel	0	0	0	0	0	0	0	0	2	2	4
(7) San Jose Mercury News	0	2	3	0	0	0	0	2	0	1	8
(8) Seattle Times	0	2	2	1	2	1	0	3	0	1	12
(9) The Day	0	1	2	0	0	0	1	0	1	0	5
(10) Washington Post	1	2	1	1	3	0	4	3	5	11	31
TOTAL	7	11	14	4	19	6	14	24	24	39	***162***
National Total	2	5	3	1	14	3	8	9	10	21	76
Local Total	5	6	11	3	5	3	6	15	14	18	86

Figure 2: Graph of Media Source, National vs. Regional, by Year

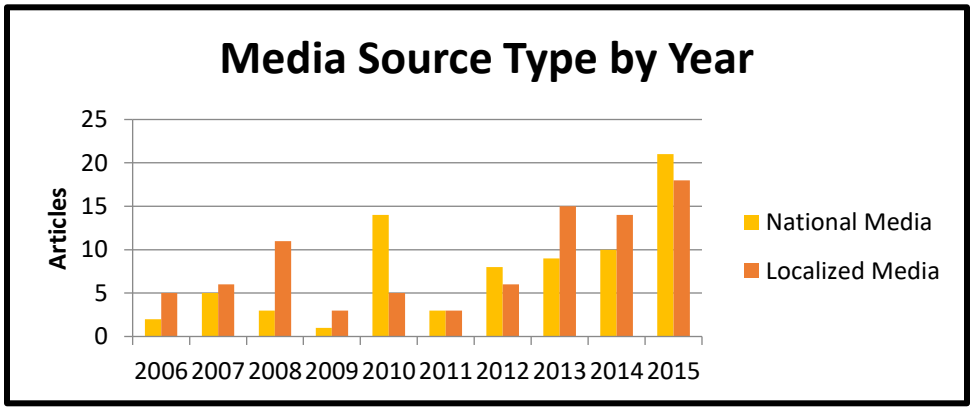


Figure 3: Value Tracking by Article

ARTICLE CODE	VALUE CLASS	ARTICLE CODE	VALUE CLASS	ARTICLE CODE	VALUE CLASS	ARTICLE CODE	VALUE CLASS
1_2006_1	Positive	**3_2015_1**	Negative	**_4**	Both	**_2**	Neutral
_2	Positive	**_2**	Positive	**5_2013_1**	Positive	**8_2011_1**	Positive
_3	Both	**4_2006_1**	Positive	**_2**	Positive	**8_2013_1**	Negative
_4	Neutral	**4_2013_1**	Both	**_3**	Negative	**_2**	Negative
1_2008_1	Neutral	**4_2014_1**	Negative	**_4**	Both	**_3**	Both
_2	Both	**_2**	Neutral	**_5**	Both	**8_2015_1**	Positive
_3	Both	**_3**	Positive	**_6**	Both	**9_2007_1**	Neutral
_4	Positive	**_4**	Neutral	**5_2014_1**	Neutral	**9_2008_1**	Neutral
1_2009_1	Positive	**_5**	Neutral	**_2**	Positive	**_2**	Both
_2	Neutral	**4_2015_1**	Both	**_3**	Both	**9_2012_1**	Negative
1_2010_1	Neutral	**_2**	Positive	**_4**	Positive	**9_2014_1**	Positive
_2	Both	**_3**	Positive	**_5**	Positive	**10_2006_1**	Negative
_3	Negative	**_4**	Neutral	**5_2015_1**	Neutral	**10_2007_1**	Neutral
1_2011_1	Positive	**_5**	Positive	**_2**	Both	**_2**	Both
_2	Positive	**_6**	Both	**_3**	Both	**10_2008_1**	Neutral
1_2012_1	Positive	**_7**	Both	**_4**	Both	**10_2009_1**	Both
_2	Neutral	**_8**	Both	**_5**	Neutral	**10_2010_1**	Both
_3	Neutral	**5_2006_1**	Both	**_6**	Neutral	**_2**	Neutral
_4	Neutral	**5_2007_1**	Positive	**_7**	Negative	**_3**	Negative
1_2013_1	Both	**_2**	Positive	**_8**	Both	**10_2012_1**	Negative
_2	Positive	**_3**	Both	**_9**	Positive	**_2**	Neutral
_3	Neutral	**5_2008_1**	Both	**_10**	Both	**_3**	Neutral
_4	Both	**_2**	Neutral	**6_2014_1**	Neutral	**_4**	Positive
_5	Positive	**5_2010_1**	Positive	**_2**	Positive	**10_2013_1**	Positive
_6	Positive	**_2**	Neutral	**6_2015_1**	Positive	**_2**	Negative
_7	Neutral	**_3**	Neutral	**_2**	Both	**_3**	Both
1_2014_1	Positive	**_4**	Negative	**7_2007_1**	Positive	**10_2014_1**	Positive
1_2015_1	Both	**_5**	Neutral	**_2**	Positive	**_2**	Positive
_2	Positive	**_6**	Both	**7_2008_1**	Both	**_3**	Both
_3	Positive	**_7**	Neutral	**_2**	Positive	**_4**	Positive
2_2014_1	Neutral	**_8**	Neutral	**_3**	Both	**_5**	Positive
_2	Positive	**_9**	Both	**7_2013_1**	Positive	**10_2015_1**	Neutral
2_2015_1	Positive	**_10**	Neutral	**_2**	Negative	**_2**	Negative
3_2007_1	Neutral	**_11**	Both	**7_2015_1**	Positive	**_3**	Positive
3_2012_1	Neutral	**5_2011_1**	Neutral	**8_2007_1**	Neutral	**_4**	Both
3_2013_1	Neutral	**_2**	Neutral	**_2**	Both	**_5**	Neutral
_2	Neutral	**_3**	Negative	**8_2008_1**	Neutral	**_6**	Negative
3_2014_1	Negative	**5_2012_1**	Both	**_2**	Both	**_7**	Positive
_2	Positive	**_2**	Both	**8_2009_1**	Both	**_8**	Both
_3	Positive	**_3**	Neutral	**8_2010_1**	Both	**_9**	Both
						_10	Both
						_11	Negative

Figure 4: Graph of Synthetic Biology Value by Year

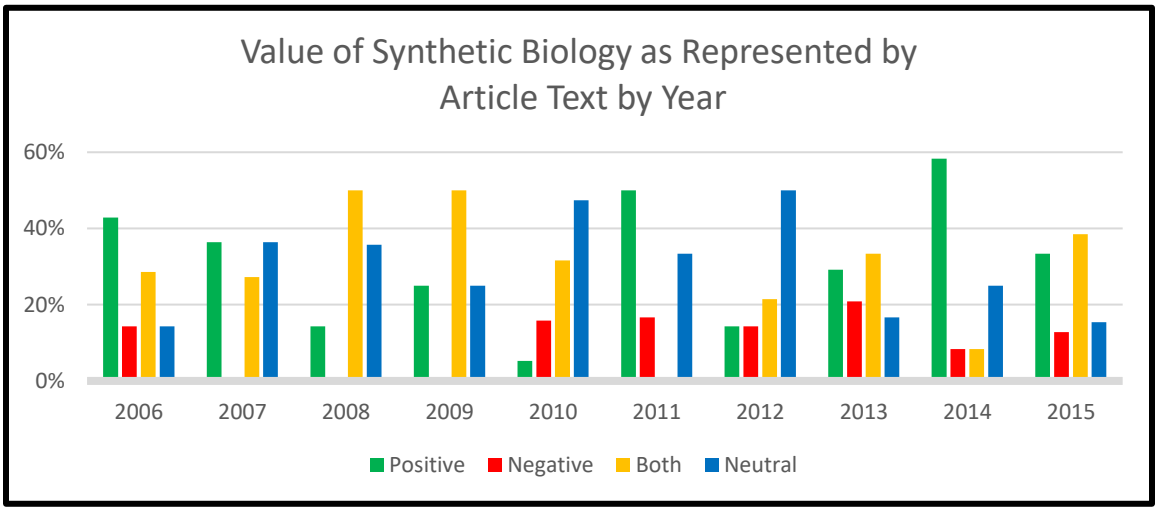


Figure 5: Full List of Representation Categories

Numerical Tracking Designation	Representation Topic	Articles with Representation Occurrence
1	Bacteria	53
2	Biofuels/ Energy Applications	42
3	Biomaterials Applications	9
4	Bioterrorism	27
5	Church- Biography	24
6	Competition	28
7	Environment/ Agricultural Applications	40
8	GMO & Foods	36
9	Genetic Circuitry (Standard & Parts)	40
10	Genetic Engineering	63
11	Globalism	28
12	High-Throughput Methods	20
13	Industry & Funding/ Investment	38
14	Keasling- Biography	8
15	Medical Applications	68
16	Morality (Bioethics)	35
17	Policy & Regulation (Biosafety)	57
18	Revolutionary/ "Cutting-Edge"	16
19	Science Fiction/ Fantasy	17
20	Software/ Computer Applications	12
21	Synthesizing Artificial Life	32
22	Venter- Biography	37
23	Yeast	20
24	Other	14

Figure 6: Representation Tracking by Article, for Representations 1-12

		Representation Tracking											
	Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Article Tracking	1_2006_1	1	1				1						1
	_2							1			1		
	_3	1					1	1	1	1	1	1	
	_4									1			1
	1_2008_1	1	1							1			
	_2	1	1		1			1		1	1		1
	_3	1	1		1		1		1	1			
	_4							1		1	1		
	1_2009_1					1							
	_2												
	1_2010_1						1			1			
	_2	1			1								
	_3				1							1	
	1_2011_1	1					1	1					
	_2	1	1			1	1	1	1	1			1
	1_2012_1		1				1	1			1		1
	_2												
	_3		1				1	1					1
	_4												
	1_2013_1				1								
	_2					1							
	_3												
	_4					1				1	1	1	
	_5												1
	_6												1
	_7												
	1_2014_1	1									1		
	1_2015_1	1	1			1		1	1				
	_2							1					1
	_3												
	2_2014_1												
	_2	1											
	2_2015_1								1		1		
	3_2007_1	1				1	1				1		
	3_2012_1									1			
	3_2013_1												
	_2		1										
	3_2014_1								1		1		
	_2										1		
	_3		1					1		1			1
	3_2015_1										1		
	_2												
	4_2006_1			1							1		
	4_2013_1				1						1	1	
	4_2014_1								1		1		
	_2												
	_3	1											
	_4												
	_5									1			
	4_2015_1	1				1		1			1		
	_2							1			1		
	_3		1					1	1				
	_4												
	_5	1											

		Representation Tracking											
	Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Article Tracking	_6	1								1			
	_7											1	
	_8										1		
	5_2006_1	1						1		1	1		
	5_2007_1		1								1		
	_2	1	1			1	1			1		1	
	_3		1		1					1	1	1	
	5_2008_1	1			1	1	1				1		
	_2									1	1		
	5_2010_1	1	1	1	1		1			1	1	1	
	_2												
	_3	1	1			1	1				1		
	_4	1										1	
	_5									1			
	_6	1											
	_7	1										1	
	_8					1				1			1
	_9		1								1	1	
	_10		1				1				1		
	_11		1							1	1		
	5_2011_1	1				1	1						
	_2					1							
	_3				1								
	5_2012_1		1						1				1
	_2		1				1	1	1				
	_3	1											
	_4								1				
	5_2013_1												
	_2	1									1		
	_3								1				
	_4								1		1		
	_5											1	
	_6								1			1	
	5_2014_1					1		1					
	_2	1							1				
	_3	1											
	_4								1				
	_5							1	1		1		
	5_2015_1	1				1			1		1		
	_2										1		
	_3								1		1		
	_4							1	1		1		
	_5									1			
	_6										1		
	_7								1		1		
	_8								1		1		
	_9					1							1
	_10					1		1			1		
	6_2014_1												
	_2	1											
	6_2015_1	1											
	_2	1								1			
	7_2007_1		1					1					
	_2		1										

		Representation Tracking											
	Code	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Article Tracking	7_2008_1	1	1		1					1		1	
	_2		1									1	
	_3	1	1	1	1			1		1	1		
	7_2013_1							1		1			
	_2							1	1				
	7_2015_1	1								1	1		
	8_2007_1	1	1		1	1	1				1		
	_2	1	1		1					1			1
	8_2008_1	1	1		1	1	1						
	_2	1	1		1		1		1	1			
	8_2009_1	1			1		1			1	1		
	8_2010_1	1								1	1		
	_2										1	1	
	8_2011_1	1	1				1		1		1	1	
	8_2013_1								1		1		
	_2							1	1		1		
	_3		1	1				1	1	1			
	8_2015_1												1
	9_2007_1	1			1					1			
	9_2008_1				1								
	_2	1	1								1		
	9_2012_1												
	9_2014_1			1			1	1			1		
	10_2006_1				1					1		1	
	10_2007_1	1	1			1	1						
	_2		1	1	1		1	1		1	1		1
	10_2008_1	1				1	1						
	10_2009_1		1		1		1	1		1	1		
	10_2010_1	1	1		1			1	1		1		
	_2												
	_3	1	1	1	1			1					
	10_2012_1		1					1			1		
	_2					1							1
	_3							1			1	1	
	_4									1			
	10_2013_1						1						
	_2							1	1			1	1
	_3		1					1	1	1	1	1	1
	10_2014_1												
	_2							1	1		1	1	1
	_3				1								
	_4											1	
	_5	1		1				1					
	10_2015_1											1	
	_2	1							1				
	_3	1							1	1	1		
	_4										1		
	_5												
	_6				1						1		
	_7	1		1			1	1		1	1	1	
	_8		1		1			1					
	_9					1					1	1	
	_10								1			1	
	_11		1			1		1	1		1	1	

Figure 7: Representation Tracking by Article, for Representations 13-24

		Representation Tracking												
	Code	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**ALL**
Article Tracking	1_2006_1	1		1										6
	_2	1												3
	_3						1		1			1		10
	_4	1												3
	1_2008_1			1						1	1			6
	_2		1	1		1				1	1	1		13
	_3			1		1								8
	_4	1		1										5
	1_2009_1									1				2
	_2	1												1
	1_2010_1			1										3
	_2					1	1			1	1			6
	_3					1					1			4
	1_2011_1	1												4
	_2						1			1				10
	1_2012_1	1		1										7
	_2				1				1	1				3
	_3	1												5
	_4	1												1
	1_2013_1				1	1								3
	_2			1			1	1						4
	_3	1												1
	_4			1	1		1							7
	_5			1							1			3
	_6			1										2
	_7	1												1
	1_2014_1													2
	1_2015_1					1								6
	_2	1		1										4
	_3	1											1	2
	2_2014_1											1		1
	_2	1		1										3
	2_2015_1													2
	3_2007_1									1	1			6
	3_2012_1			1						1				3
	3_2013_1								1					1
	_2													1
	3_2014_1					1								3
	_2												1	2
	_3			1										5
	3_2015_1			1	1	1		1						5
	_2								1					1
	4_2006_1			1										3
	4_2013_1				1	1	1	1		1				8
	4_2014_1					1								3
	_2											1		1
	_3	1		1										3
	_4							1					1	2
	_5												1	2
	4_2015_1													4
	_2													2
	_3													3
	_4	1											1	2
	_5			1										2

		Representation Tracking												
	Code	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**ALL**
Article Tracking	_6			1	1									4
	_7												1	2
	_8			1								1		3
	5_2006_1	1	1	1							1			8
	5_2007_1	1	1	1										5
	_2									1	1			8
	_3										1			6
	5_2008_1				1	1				1	1			9
	_2						1			1	1			5
	5_2010_1			1								1		10
	_2	1		1										2
	_3			1	1					1	1			9
	_4					1					1			4
	_5									1				2
	_6				1					1	1			4
	_7									1	1			4
	_8													3
	_9					1					1			5
	_10	1				1	1	1			1			8
	_11			1	1	1								6
	5_2011_1										1			4
	_2				1		1	1		1	1			6
	_3		1			1								3
	5_2012_1	1		1		1								6
	_2	1		1	1						1			8
	_3			1					1		1			4
	_4					1								2
	5_2013_1								1					1
	_2				1	1								4
	_3					1								2
	_4	1				1								4
	_5				1		1	1						4
	_6	1	1			1						1		6
	5_2014_1				1	1		1						5
	_2			1	1	1								5
	_3			1	1	1				1				5
	_4											1		2
	_5					1					1	1	1	7
	5_2015_1			1		1								6
	_2			1		1						1		4
	_3					1								3
	_4					1								4
	_5	1												2
	_6			1								1		3
	_7					1								3
	_8					1								3
	_9								1					3
	_10			1										4
	6_2014_1											1		1
	_2	1		1										3
	6_2015_1			1										2
	_2			1										3
	7_2007_1			1										3
	_2	1												2

		Representation Tracking												
	Code	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**ALL**
Article Tracking	7_2008_1			1	1	1				1	1	1		11
	_2	1		1										4
	_3		1	1		1	1					1		12
	7_2013_1		1	1										4
	_2	1				1								4
	7_2015_1			1										4
	8_2007_1				1					1	1			9
	_2		1	1	1	1		1		1	1	1		13
	8_2008_1									1	1			7
	_2			1		1								8
	8_2009_1				1	1				1				8
	8_2010_1				1	1				1	1			7
	_2						1	1						4
	8_2011_1			1										7
	8_2013_1					1								3
	_2	1				1								5
	_3			1	1	1						1	1	10
	8_2015_1								1					2
	9_2007_1				1					1	1			6
	9_2008_1							1						2
	_2			1										4
	9_2012_1				1	1								2
	9_2014_1													4
	10_2006_1					1								4
	10_2007_1									1	1			6
	_2			1	1	1				1	1			13
	10_2008_1									1	1			5
	10_2009_1			1	1	1		1		1				11
	10_2010_1			1	1	1				1	1			11
	_2				1						1			2
	_3			1	1	1					1			9
	10_2012_1			1	1	1					1			7
	_2								1					3
	_3			1										4
	_4							1	1					3
	10_2013_1	1		1			1				1			5
	_2					1							1	6
	_3	1		1	1	1				1		1	1	14
	10_2014_1			1				1	1	1				4
	_2											1		6
	_3			1										2
	_4			1										2
	_5												1	4
	10_2015_1												1	2
	_2			1		1		1						5
	_3	1				1						1	1	8
	_4			1								1		3
	_5	1		1										2
	_6				1	1	1							5
	_7	1		1			1		1					11
	_8	1		1									1	6
	_9	1		1	1	1	1	1						9
	_10	1				1		1						5
	_11			1	1	1					1			10

Figure 8: Occurrence Totals for Each Representation by Year

REPRESENTATION	1	2	3	4	5	6	7	8	9	10	11	12
ALL 2006	3	1	1	1	0	2	3	1	4	4	2	2
ALL 2007	6	9	1	5	4	5	2	0	5	5	2	2
ALL 2008	10	9	1	8	3	5	3	2	8	6	2	1
ALL 2009	1	1	0	2	1	2	1	0	2	2	0	0
ALL 2010	9	7	2	5	2	4	2	1	6	8	6	1
ALL 2011	4	2	0	1	3	4	2	2	1	1	1	1
ALL 2012	1	5	0	0	1	3	5	3	2	3	1	4
ALL 2013	1	3	1	2	2	1	6	9	4	7	6	4
ALL 2014	7	1	2	1	1	1	6	6	2	7	2	2
ALL 2015	11	4	1	2	7	1	10	12	6	20	6	3
TOTAL	53	42	9	27	24	28	40	36	40	63	28	20

REPRESENTATION	13	14	15	16	17	18	19	20	21	22	23	24	ALL
ALL 2006	4	1	3	0	1	1	0	1	0	1	1	0	37
ALL 2007	2	2	4	4	2	0	1	0	7	8	1	0	77
ALL 2008	2	2	9	2	6	2	1	0	7	7	3	0	99
ALL 2009	1	0	1	2	2	0	1	0	3	0	0	0	22
ALL 2010	2	0	7	7	9	3	2	0	7	12	1	0	103
ALL 2011	1	1	1	1	1	2	1	0	2	2	0	0	34
ALL 2012	5	0	7	4	4	0	1	4	2	3	0	0	58
ALL 2013	8	2	8	7	12	5	3	2	2	2	3	3	103
ALL 2014	3	0	9	3	6	0	3	1	2	1	6	5	77
ALL 2015	10	0	19	5	14	3	4	4	0	1	5	6	154
TOTAL	38	8	68	35	57	16	17	12	32	37	20	14	764

Figure 9: List of Articles Included in Final Data Set Database by Source & Title

1_2006_1_Build to order is the plan at Codon
1_2006_2_Target Mosquitoes; To fend off the deadly disease-carriers, scientists worldwide are hot on the trail of new scents that can repel them
1_2006_3_Genetic 'jamboree' draws innovators; Science students the world over share research
1_2006_4_DNA builder closes 20m in financing
1_2008_1_Making cells like computers
1_2008_2_A quest to create life out of synthetics. New science spurs high hopes, worry
1_2008_3_Accessible science. Hackers aim to make biology household practice
1_2008_4_In largest gift, Harvard gets 125m for biological institute
1_2009_1_Harvard fuels quest to create life from scratch. Machine mimics ribosome activity, professor asserts
1_2009_2_Two biotechs moving operations to Boston
1_2010_1_Ginkgo BioWorks sells kit to connect pieces of DNA
1_2010_2_Partially synthetic cell created. Scientists say its genetic heritage began in computer
1_2010_3_Lab regulations haven't kept pace with biotech, critics say. A call for tougher US standards to protect workers
1_2011_1_Start-up plans to seek out, kill bacteria
1_2011_2_Aiming to build a better bacteria. Researchers at Harvard work to edit the genome, with eye toward industrial uses
1_2012_1_Researchers making microbes that can do the dirty work. Innovation Economy
1_2012_2_Introducing the human computer. Could a circut made of people be the building block of a new field
1_2012_3_Gen9 hopes to be the Intel of custom DNA. INNOVATION ECONOMY
1_2012_4_Innovation Center must make an effort to energize Seaport. Innovation Economy

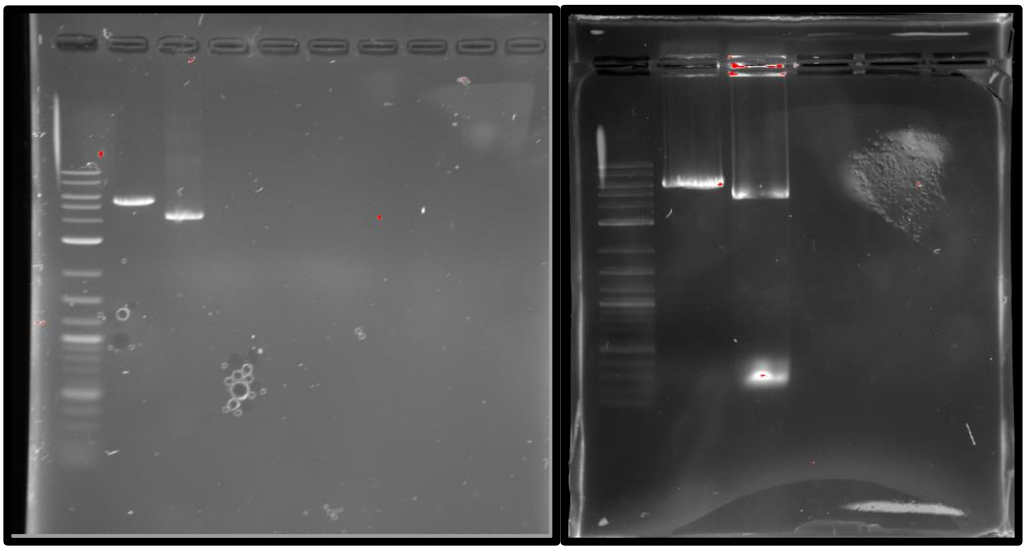
- 1_2013_1_Expert group aims to keep biology safe for amateurs
- 1_2013_2_Genetics. the final frontier. katharine whittemore
- 1_2013_3_Gen9 gets 21m boost from Agilent
- 1_2013_4_MIT summit to weigh how to hack cells
- 1_2013_5_Making vaccine IN A HURRY. Injections for '09 flu arrived too late to matter, but work at Novartis may change the game in the future
- 1_2013_6_Flu vaccines. An end to pandemics
- 1_2013_7_BU plans life sciences facility on Charles River campus
- 1_2014_1_but great mentors can help
- 1_2015_1_Harvard, Yale teams leash life forms developed in lab. Technique keeps altered bacteria from escaping
- 1_2015_2_Manufacturing's cutting edge -- custom organisms
- 1_2015_3_Dollars and scents. Gingko Bioworks gets 45m. Biology startup custom designs bacteria to enhance smells, tastes
  - 2_2014_1_Scientists create designer chromosome in brewer's yeast
  - 2_2014_2_Bioengineer is working to make E. coli fight obesity, depression
  - 2_2015_1_DNA engineering could lead to new food flavors and ingredients
    - 3_2007_1_Genome swap in bacterium creating buzz
    - 3_2012_1_Researchers replicate artificial genetic data
    - 3_2013_1_Study. Digital information can be stored in DNA
    - 3_2013_2_Algae is proving slippery in quest to make fuel
    - 3_2014_1_The FDA must stand firm
    - 3_2014_2_Fish found common genetic ground to develop electric organs
    - 3_2014_3_Rice, Kansas researchers build more sophisticated synthetic gene circuits
    - 3_2015_1_Florida is abuzz over plan to introduce mutant mosquitoes to fight disease
    - 3_2015_2_Storage on DNA can keep data safe for centuries
      - 4_2006_1_Of spiders and stitches and more
      - 4_2013_1_'Creation' explains how science reinvents life
      - 4_2014_1_FDA must stand firm in the 'natural' food fight
      - 4_2014_2_Scientists create 'designer chromosome' in brewer's yeast
      - 4_2014_3_Bioengineer is workig to make E. coli fight obesity, depression
      - 4_2014_4_Moment of Friday. 'Biomass Man' creator Megan Daalder sees into 'Black Mirror'
      - 4_2014_5_Sci-fi meets wi-fi in a mind over gene experiment
      - 4_2015_1_Creating a 'genetic firewall' for GMOs
      - 4_2015_2_Can scientists engineer drought-tolerant plants
      - 4_2015_3 Surviving the next population boom with resistant crops and biofuels
      - 4_2015_4_What I Learned. L.A.'s role as a major manufacturing hub
      - 4_2015_5_Talented bacteria detect cancer, diabetes
      - 4_2015_6_Designer microbiome. MIT biologists program common gut bacteria
      - 4_2015_7_UC Irvine to exhibit artworks created via biological engineering
      - 4_2015_8_Team makes Vicodin component in yeast, says 'home brew' opiates still not feasible
        - 5_2006_1_Custom-Made Microbes, at Your Service
        - 5_2007_1_First, cure Malaria. Next, global warming.
        - 5_2007_2_Pursuing Synthetic Life, Scientists Transplant Genome of Bacteria
        - 5_2007_3_Genetic engineers who don't just tinker
        - 5_2008_1_Researchers announce a step toward synthetic life
        - 5_2008_2_Pursuing synthetic life, dazzled by reality
        - 5_2010_1_Do-it-Yourself Genetic Engineering
        - 5_2010_2_Betting on a breast cancer cure
        - 5_2010_3_Synthetic bacterial genome takes over a cell, researchers report
        - 5_2010_4_Perils in the Biotech Frontier
        - 5_2010_5_Baby steps to new life-forms
        - 5_2010_6_One Cell Forward
        - 5_2010_7_Peering over the fortress that is the mighty cell
        - 5_2010_8_On a mission to sequence the genomes of 100,000 people
        - 5_2010_9_Not just pond scum. Bioengineering a superalgae to make a green fuel
        - 5_2010_10_His Corporate Strategy. The Scientific Method
        - 5_2010_11_Presidential Bioethics Panel gives a Green Light to Research in Synthetic Biology
        - 5_2011_1_Genetic Code of E. coli is hijacked by Biologists
        - 5_2011_2_'It's Alive! It's Alive!' maybe right here on Earth
        - 5_2011_3_Lab Fight Raises U.S. Security Issues

- 5_2012_1_White House announces intention to encourage biological manufacturing methods
- 5_2012_2_God of Small Things
- 5_2012_3_In a First, an Entire Organism (all 525 genes) is Simulated with Software
- 5_2012_4_Engineered fish moves step closer to approval
- 5_2013_1_Double Helix serves Double Duty. A new technique improves the potential of storing vast amounts of data on DNA
- 5_2013_2_Come Slither Look
- 5_2013_3_Grocers won't sell altered fish, groups say
- 5_2013_4_A dream of trees aglow at night. Plan to make bioiluminescent plants is assailed as gene-tinkering
- 5_2013_5_Where science is going
- 5_2013_6_Genetic engineering for the spice rack
- 5_2014_1_The New Origin of the Species
- 5_2014_2_Scientists create cells with artificial genetic code; Technology could bring new vaccines, antibiotics and industrial products
- 5_2014_3_Scientists add letters to DNA's Alphabet, Raising hope and fear
- 5_2014_4_Strange brews. The genes of craft beer; It is still not understood how brewing yeast can create such varying tastes
- 5_2014_5_Changing the Science of Soap
- 5_2015_1_Scientists work to contain modified organisms to labs
- 5_2015_2_Makings of a New Heroin
- 5_2015_3_Obama Administration Orders Review of Rules for Genetically Modified Crops
- 5_2015_4_Replacing pesticides with genetics; To fight farm pests, scientists infuse DNA into moths to kill larvae
- 5_2015_5_Pentagon reaches out to tech field; Forum aims to bridge divide between Silicon Valley and government
- 5_2015_6_Newly Risen From Yeast. THC
- 5_2015_7_The Risks of Assisting Evolution
- 5_2015_8_Genetic engineers land bigger fish for U.S. plates
- 5_2015_9_Synthetic DNA is seen as way to store data for centuries
- 5_2015_10_Scientists build the imperfect pest; Gene drives can control an insect population, but will it work in the wild
  - 6_2014_1_Scientists create designer chromosome in brewer's yeast
  - 6_2014_2_Bioengineer is working to make E. coli fight obesity, depression
  - 6_2015_1_Talented bacteria detect cancer, diabetes
  - 6_2015_2_Designer microbiome. MIT biologists program common gut bacteria
    - 7_2007_1_VC John Doerr presses Silicon Valley to fight climate change. VC tells valley leaders there's much more to do
    - 7_2007_2_Innovative Winners
    - 7_2008_1_Creating life from scratch moves one step closer. COULD BE USED FOR GOOD (BIOFUELDS) OR EVIL (BIOWEAPONS), EXPERTS SAY
    - 7_2008_2_Emeryville's Amyris announces deal to make renewable diesel fuel in Brazil. COMPANY BEST KNOWN FOR MALARIA DRUG
    - 7_2008_3_Conference explores hot new field of synthetic biology
    - 7_2013_1_Researchers make computer that fits inside a living cell
    - 7_2013_2_Project about much more than glowing plant
    - 7_2015_1_Synthetic biology. Engineered cells detect diabetes and cancer
      - 8_2007_1_DNA transplant gives cells new identity
      - 8_2007_2_As DNA research advances, science plays God ever more
      - 8_2008_1_Synthetic life forms on horizon
      - 8_2008_2_Hobbyists are trying genetic engineering at home
      - 8_2009_1_Competitors strive to create new life-forms
      - 8_2010_1_A step to artificial life. Manmade DNA powers cell
      - 8_2010_2_Biology inspires future technology
      - 8_2011_1_UW team wins genetic-engineering contest
      - 8_2013_1_Markets pledge not to sell genetically-modified salmon
      - 8_2013_2_Glowing plants spark environmental debate
      - 8_2013_3_Biotech builds cells like you build an app
      - 8_2015_1_UW, Microsoft studies new place to keep digital info. DNA
        - 9_2007_1_Scientists Successfully Move Microbe's Entire DNA To Change Its Species
        - 9_2008_1_Woolly mammoth in our future
        - 9_2008_2_Amateurs Are Trying Genetic Engineering At Home
        - 9_2012_1_Whistleblower now reluctant biotech safety spokeswoman
        - 9_2014_1_NASA team builds biodegradable drone using fungi, wasp spit, cellulose

10_2006_1_Custom-built pathogens raise bioterror fears
10_2007_1_Scientists report DNA transplant; Organisms adopt donor traits
10_2007_2_Synthetic DNA on the brink of yielding new life forms
10_2008_1_Md. scientists build bacterial chromosome
10_2009_1_New works of science nonfiction; U-Va. students are using 'BioBricks' to try to build an original life form
10_2010_1_Creating a cell from scratch; 'Important advance' Scientists transplant genome into bacterium
10_2010_2_Chair of new bioethics commission is a firm believer in deliberative democracy
10_2010_3_Concerns in 'synthetic biology'
10_2012_1_Coalition urges tighter controls on 'extreme genetic engineering'
10_2012_2_Researchers write a book in DNA
10_2012_3_Fighting the Asian Tiger Mosquito
10_2012_4_An app lets users study Einstein's brain; magazine explores longevity research
10_2013_1_A prize for life
10_2013_2_Glowing plants illuminate regulatory debate
10_2013_3_Creating life forms. There's an app for that
10_2014_1_How technology will drive the promising future of medicine
10_2014_2_Start-up's lab experiment aims to take the cow out of milk
10_2014_3_Silicon Valley must join Ebola battle to avert pandemic disaster
10_2014_4_Innovations. The Indian start-up that could disrupt health care with its powerful, affordable diagnostic tool
10_2014_5_NASA team builds biodegradable drone using fungi, wasp spit, cellulose
10_2015_1_Can we bring back the woolly mammoth
10_2015_2_Florida is abuzz over plan to introduce 'mutant mosquitoes' to fight disease
10_2015_3_DNA engineering could lead to new food flavors and ingredients
10_2015_4_Home-brewed heroin could be a thing, thanks to genetically engineered yeast
10_2015_5_The start-up philanthropist
10_2015_6_There's an urgent need for a moratorium on gene editing
10_2015_7_World's youngest synthetic biologists show that the future of innovation is in the genes
10_2015_8_Trends in synthetic biology include military, space issues
10_2015_9_Investors see gene-editing as a hot technology
10_2015_10_China looks to solve future food shortage with test tube beef
10_2015_11_Genetically engineering food is one thing; tinkering with humans is another

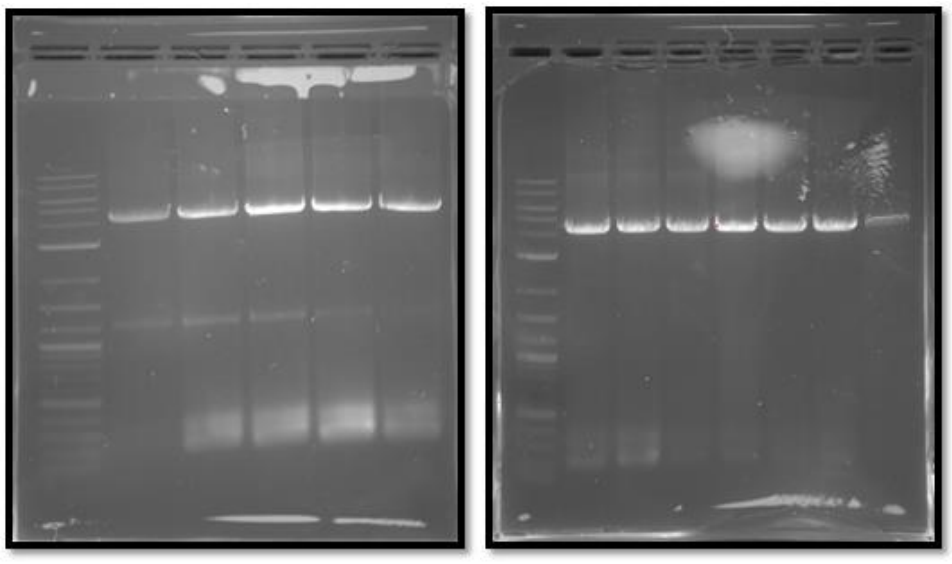
APPENDIX B.
Supplemental to CHAPTER TWO

Figure 1: Verification gels of pcDNA3.1+ and dCas9



Lane 1 in both gels contains pcDNA3.1+
Lane 2 in both gels contains amplified dCas9

Figure 2: Visualization Gels for Gradient-temperature Reactions in dCas9 PCR Protocol Optimization



The temperature range over the 12 reactions was 45 to 62, spanning 17 degrees, each reaction representing a difference of 12/17=1.42 degrees.

Reactions 8-11 are the most effective,
evidenced by the brightest, cleanest, thickest bands.

Therefore, Reaction 9 is 45+(9*1.42)=57.78 degrees.
Reaction 10 is 45+(10*1.42)=59.2 degrees.

Averaging the temperatures of reactions 9 and 10,
we can conclude that the optimal temperature for the annealing step in our PCR protocol for dCas9 should be 58.49 or 58.5 degrees.

The temperature we were using before performing this optimization was 55 degrees.

Figure 3: Verification gel of dCas9 Amplification

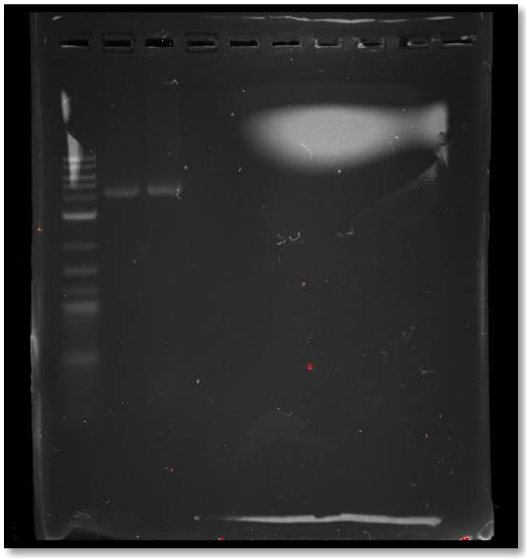


Figure 4: Vector Map of pcDNA3.1+ Plasmid (Addgene)

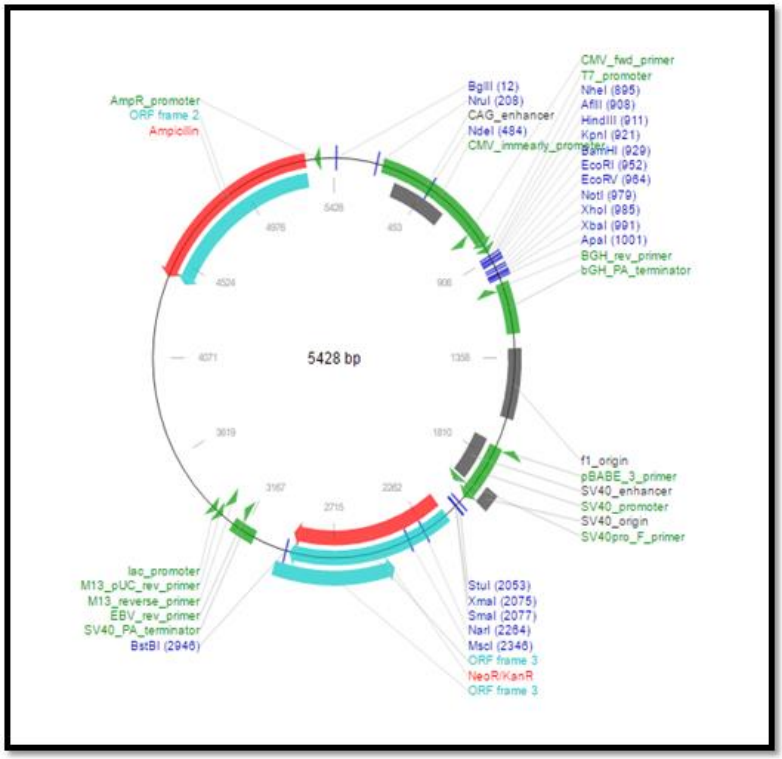


Figure 5: Vector Map of BE2 Plasmid Construct (Addgene)

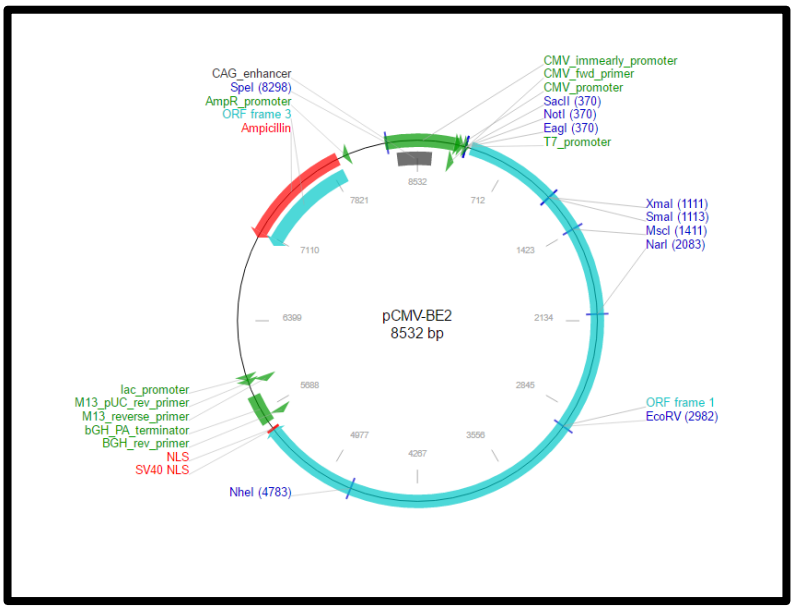


Figure 6: Vector Map of pRETRO Plasmid (Addgene)