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Should We? Possibilities, Perils, and Unintended Consequences of Genetic Engineering

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Introductions

Manipulating Biology

Mankind has been manipulating the biology of living organisms for over 15,000 years when nomadic tribes began to use wild wolves to guard their encampments. They were able to do so because some of the wolves were less ferocious and able to be "trained" to perform tasks necessary for the tribe's survival: thus, began the domestication of dogs. The domestication of wild plants began later, around 11,000 years ago, when hunter-gatherers noticed that certain species of wild grains had characteristics that made them easier to gather and plant. One of these wild organisms was wheat which has two naturally occurring species: the dehiscent and the indehiscent variety. Dehiscent wheat has ears that shatter upon maturity and the spikelets (containing the seeds) scatter onto the ground; the indehiscent species has spikelets that remain attached to the mature ears: thus, the spikelets (and seeds) can be easily gathered and stored.[1] As a result, early farmers would (whenever possible) choose the indehiscent wheat over its dehiscent counterpart. Their preference for one particular species was a form of artificial selection as it gave one variety of wheat an evolutionary advantage over all others. Over time indehiscent wheat became dominant as it was able to more successfully pass its genetic structure onto succeeding generations. In this manner humanity "domesticated" the wild grain as our actions ultimately determined the genetic structure of the wheat we use today. Domestication can be defined "as the genetically determined physical and physiological changes a plant undergoes in response to human behavior".[2] In a similar fashion, Neolithic farmers discovered advantageous traits in 8 species of wild plants (emmer and einkorn wheat, barley, lentils, pea, chickpea, bitter vetch, and flax) that they successfully domesticated. The suite of organisms would become the "founder crops" of the early farms that populated the Fertile Crescent .[3] Along with this domestication of wild plants, early farmers also began to select animals from wild herds (cattle, sheep, goats, pigs, and horses) that became valuable sources of food, energy, and clothing on early farms. As in the case of plants, they selected organisms with traits that would facilitate their domestication. Animals were selected based on "pre-adaptive" characteristics such as high meat yield, non-aggressiveness, tamable, and feeding habits, that eased their transition into the farm's economy.[4] Once again our preference for particular species determined the genetic structure of the many farm animals we have today. As the farms and their surrounding settlements grew in complexity, humans continued domesticating an increasing number of wild organisms and animals that provided resources for the

burgeoning populations. Their increasing ability to grow vast quantities of food, harness the power of animals, and invent an array of technologies laid the foundations of the economic, cultural, and sociocultural institutions that define the modern world.[5]

> ...viewing history in the light of evolution we see that it was intelligently designed changes to the genetic composition of natural biota that made the real [difference]. In some sense, Neolithic farmers were the first geneticists and domestic agriculture was the lever with which they moved the world.[6]

Perils, and Unintended Consequences

Manipulating the biology of living organisms helped mankind establish the economic and sociopolitical institutions that have made us the dominant species on our planet. Our actions however have had a profound impact on the earth's ecological balance. While we (many but not all members of the human population) have reaped countless benefits from our ability to manipulate nature, mankind has endangered (and driven to extinction) countless living organisms, eradicated over 50% of the planet's forests and has imperiled the sustainability of most of our planet's ecosystems.[7] It is disheartening to consider that the deleterious effects of our actions will continue for thousands of years. They can be considered as an ecological inheritance bequeathed to future generations. We must also recognize that our manipulation of biological systems has created a host of unintended social, political, and economic consequences that have negatively impacted the lives of the more vulnerable members of our earth family. The combination of these environmental, social and cultural effects of our technologies pose great risks to the survival of our species.

Since the dawn of agriculture, three-quarters of the terrestrial biosphere has been converted from wilderness. Somewhere between a quarter and a third of the planet's biological productivity...is now appropriated for human use. If the same cultural practices that have reworked the planet so dramatically over the past ten thousand years are inherited by future generations, we may push many species to extinction and threaten our own well-being [8]

Unit Rationale

Modern Technology (in all of its varied applications) is considered by many to be the driving force of the continuing evolution of our species: there are few aspects of our lives that are not impacted by some sort of technological innovation. This is especially true of biological technologies, as us increasing abilities to manipulate genomes has given mankind unprecedented control of the biochemistry of many organisms on our planet. And although our technological prowess can provide a wide range of benefits, they have the potential to cause great harm to other living organisms, to our planet's ecosystem, and to many less privileged members of the human family.

I feel that it is critically important that we remain aware of this fact because it is our responsibility (as stewards of our environment) to use our technology in ways that do not endanger other organisms, exacerbate structural inequalities, or threaten the sustainability of our ecosphere.

Understanding the risks associated with biotechnological technologies is even more crucial at this moment in history because new bioengineering processes (such as CRISPR-Cas 9) have made gene editing an inexpensive, highly efficient, and versatile way to edit genetic structures. Although the process is still in the early stages of development, it may soon become a widely used means of manipulating the genome of a vast number of organisms (including the human germ line). The impacts of these new technologies have, however, yet to be adequately evaluated. As a science educator I feel that is my duty to inform my students of the benefits, perils and unintended consequences of emerging biotechnologies, as they will very likely affect their lives in the not too distant future. It is important that our students understand their responsibility as stewards of our planet, to become informed citizens who will add their voice to the public discourse on these issues.

Demographics

I teach in the Philadelphia High School for Girl's which is located in North Philadelphia. Our school population is composed of African-American (66%), Asian (16%), Hispanic (10%), Caucasian students (4%), and approximately (6%) mixture of other ethnic groups. Girl's High is a special admission school which means that in order to be accepted students must have scored proficient or advanced on mathematics and science standardized tests. I mention this because although most of my students are able to understand the science content, few are able to fully connect their science learning to their everyday lives. As a result, most are unable to engage in substantive discussions on the role of science in society or how they will respond to the various ways in which science and technological innovation will impact their future. This unit will address these issues by providing my students a forum in which to explore the benefits, risks, and consequences of our developing biotechnologies.

Content Objective

Students in this unit will be asked to evaluate the human, environmental, and ethical consequences of modern biotechnologies such as CRISPR and gene drives. In order to do so they will need to have an in depth understanding of how genetic information is encoded in the DNA molecule, how genetic codes are used to regulate biochemical processes, and how changes in genetic structures (double stranded breaks, insertions, and deletions) affect those processes.

Although scientists have known how to edit DNA for many years, the emerging CRISPR technology has given genetic engineers the ability to efficiently alter the genome of many organisms. To fully evaluate the impact of this technology, students will first explain the function of the varying structures in the CRISPR array, how the system defends bacteria against organisms invading the cell, and then analyze how this system differs from the human immune system response.

The ease with which this technology is able to alter genetic structures has given mankind the ability to engineer solutions to a wide array of problems in human society. One area of intense interest is the possibility of combining CRISPR and gene drives to alter or eradicate species of organisms that transmit diseases. In

order to evaluate the utility of these solutions students will need to understand how CRISPR is used to edit genomes, how it is used to engineer gene drives and how the combination of the two technologies are able to alter traditional patterns of Mendelian inheritance. While many in the public sphere consider these proposed solutions a great benefit, there are a host of ethical and environmental risks posed by our use of these technologies.

Teaching Strategies

(Aligned to the Content Objectives)

To analyze the structure and processes of our genetic codes, students will engage in three self-guided inquiries (POGILS) that will explore the structure, function and biochemical processes of the genetic information stored in our DNA. This will address the common core standard **CCSS.ELA-Literacy.RST.11-12.9** that ask student to synthesize information from a range of sources into a coherent understanding of a process, phenomenon, or concept.

To compare and evaluate the two immune systems students will work cooperatively on a series of Guided Discovery problems that they will use to build models of the components of the CRISPR-Cas 9 system. Students will use the models to create a presentation that explains how the two systems differ. This strategy will address the standard **HS-ETS1-4 Engineering Design** that asks students to create a simulation that models the impact of proposed solutions to complex real-world problem.

To compare the two types of inheritance patterns students will engage in a Directed Modeling Inquiry in which they will use Punnett squares to predict patterns of inheritance. Once complete each student will write a narrative summary that explains how gene drives alter these traditional patterns. This strategy will address the **HS-ESS3-4 Earth and Human Activity** standard that asks students to evaluate or refine a technological solution that reduces the impacts of human activities on natural systems.

In order to evaluate the ethical dimensions of biotechnology, students will conduct an independent research on the application of CRISPR and gene drive technologies. Research information will be used to write a critical evaluation that analyzes the benefits, risks, and unintended consequences of biogenetic engineering. This strategy will address the common core standard **HS-ETS1-1 Engineering Design that** asks students to analyze a major global challenge and specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.

Background Content

Establishing A Genetic Model

Though humans had used the natural diversity of living organisms to their advantage, they knew little of the structures or biological processes responsible for the genetic variation within species. In 1866 Gregor Mendel proposed three laws of inheritance to explain the transmission of characteristics between successive

generations of pea plants. He proposed that the interaction between alternate forms of each trait produced the variations observed in offspring of given organisms. The work of other scientists during the early years of the 20th century refined Mendel's work and introduced modern terms such as genotype / phenotype, genes, alleles, and chromosomes into the developing field of genetics. Thomas Hunt Morgan worked with varying species of Drosophila (fruit flies) and proposed that genes resided on specific locations on the chromosomes located within the nucleus of the cell. Although Hunt's work established a physical relationship between genes and chromosomes scientists did not yet fully understand the biochemistry of genetic structures, how genetic information was stored, or how genes were able to influence biochemical processes at the cellular level.

Understanding Genetic Structure

The discovery of the structure and composition of the DNA molecule (by Franklin, Crick, and Watson in 1952-1953) provided our first understanding of how genetic information is stored at the molecular level. Francis Crick expanded this understanding with his model of the manner in which molecules of mRNA and tRNA work (along with other structures in the cell) to translate genetic information into molecules (proteins, enzymes, hormones) that regulate bodily functions. From the earliest understandings of the molecular basis of genetic structures, scientists wondered if they could influence the biology of living organisms by manipulating the genetic codes stored within our DNA.

Genetic Modification

The discovery that yeasts and bacteria had endogenous mechanisms that allowed them to repair double stranded breaks in their DNA, provided the first clues that site specific targeting and modification of the genome was possible.[9] Soon after that, genetic engineers began experimenting with an array of endonucleases and restriction enzymes that could recognize specific base pair sequences on an organism's DNA. Once a specific site was located these enzymes could be used to cleave the DNA and introduce chromosomal modifications to an organism's genetic structure. The resulting recombined organisms would be a transgenic species as it contained DNA from two (or more) differing sources. Recombinant DNA technology became a reality in 1972 when researchers used the restriction enzyme Eco-RI to insert genes encoding proteins into bacteria. The bacteria would become the first transgenic organism created by genetic engineers. In 1979 Genentech corporation used the technology to engineer a transgenic bacterium that produced the growth hormone somatostatin.[10] Using the same technology companies soon learned to genetically modify plants to make them resistant to pathogens, since then the world has seen countless applications of the technology.

In the ensuing years, genetic engineers developed various technologies (such as Zinc Fingers, TALENS, and PCR) **to** increase the range of biogenetic engineering,[11] their work was however, tedious, expensive, and time consuming: that was until CRISPR- Cas-9 revolutionized the industry [12] .

CRISPR Cas- 9 Technology

In 1987 researchers exploring the *iap* gene in the bacteria *Escherichia coli* reported the discovery of a series of 29 segments of RNA separated by short repeats.[13] Analysis of the function of the array showed that it was a system created by the bacteria as part of an adaptive defense mechanism. Most bacteria seem to live in ever-changing environments where bacteria-specific genetic elements, (such as plasmids and phages), attempt to enter the cell and harness the cellular metabolism and molecular machinery to replicate themselves. As such, bacteria benefit from having a flexible and readily adaptive immune response to withstand such invaders.

Rather than having a system of specific immune cells that are trained to recognize and degrade invading pathogens (like many eukaryotes), single-celled bacteria that survive an attack can then incorporate copies of a portion of the invader's genome into what has become termed a CRISPR array. The name CRISPR-Cas-9 refers to the combined system of non-coding RNA sequences (spacers) and associated Cas proteins used to identify and cleave the genome of invading pathogens.

The CRISPR Cas-9 Array

The CRISPR array is a region on the genome of a bacteria (or archaebacteria) containing a **C**luster of **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats [14] in association with a series of proteins known as the **C**RISPR **A**ssociated **P**roteins or simply **Cas-9.** The array is composed of short segments called spacers that contain the memorialized segments of the genome of invading organism separated by short segments (approximately 28-37 base pairs) of DNA.

The array is a kind of reference library that is used to alert the organism upon reinvasion by a phage or plasmid. CRISPR is an adaptive system as it can continually respond to its environment (by adding or removing spacers) during the bacterium's lifetime. Many scientists consider CRISPR to be an example of Lamarckian (rather than Darwinian) evolution as it is a heritable adaptation developed during the lifetime of the organism rather than one that emerged in a population in response to selective environmental pressures.[15]

The System

The CRISPR system is composed of three phases: Spacer Acquisition, crRNA Processing, and Interference. It is important to note that there are three known systems (CRISPR- types I, II and III). The basic components of the systems are similar; however, there are variations in the number, function, and orientation of the Cas proteins.

Spacer Acquisition

When a genetic element (usually a phage or plasmid) invades a bacterium (or archaeal cell), two Cas proteins (Cas-1 and Cas-2) cleave the invading genome (either DNA or RNA depending on the invader) and sequester a short segment (a protospacer of approximately 32-38 base pairs [b.p.]) into the array along with a repeat. Every time an invasion occurs, the Cas proteins cleave the foreign DNA and integrate a section of its genome (along with a repeat) into the array.[16] It is important to note that the Cas proteins do not cut at random locations, rather they select sequences that are adjacent to specific sites on the invading genome. These sites are known as PAM (Protospacer Adjacent Motif) are not included in the spacers stored in the CRISPR array. When reinfection occurs, the bacteria will use the PAM sequences to tell the difference between the DNA of an invader and the corresponding protospacer of its CRISPR array: in this way the bacteria will attack the invading entity and not itself.

crRNA Processing

To create the defense mechanism the bacteria transcribes the CRISPR array (protospacers and repeats) into a strand of mRNA (the crRNA). In the type-I CRISPR system this crRNA strand is cut into sections each of which contains a segment of an invader's genome and a repeat. In this system the repeat is in the form of a loop. Each of these crRNA molecules is then merged with a Cas protein forming a complex that will remain in the cell. The various complexes (each with a different segment of foreign DNA) function much like a group of

sentinels that will be activated if reinfection by one of the “known” invaders occurs.

In the type-II CRISPR system a second sequence of RNA (the trans activating, or tracRNA) is bound to each repeat. Each protospacer in this system is therefore bound to two segments of RNA (crRNA: tracRNA) which are called the guide RNAs. After the array is transcribed, Cas proteins (Cas-9 and RNase-III), cut the (crRNA: tracRNA) segments into smaller sections, each containing the two segments of RNA repeats and a spacer. Once again, the segments are each merged with Cas proteins forming the complexes that will defend against reinfection (see Figure 1).

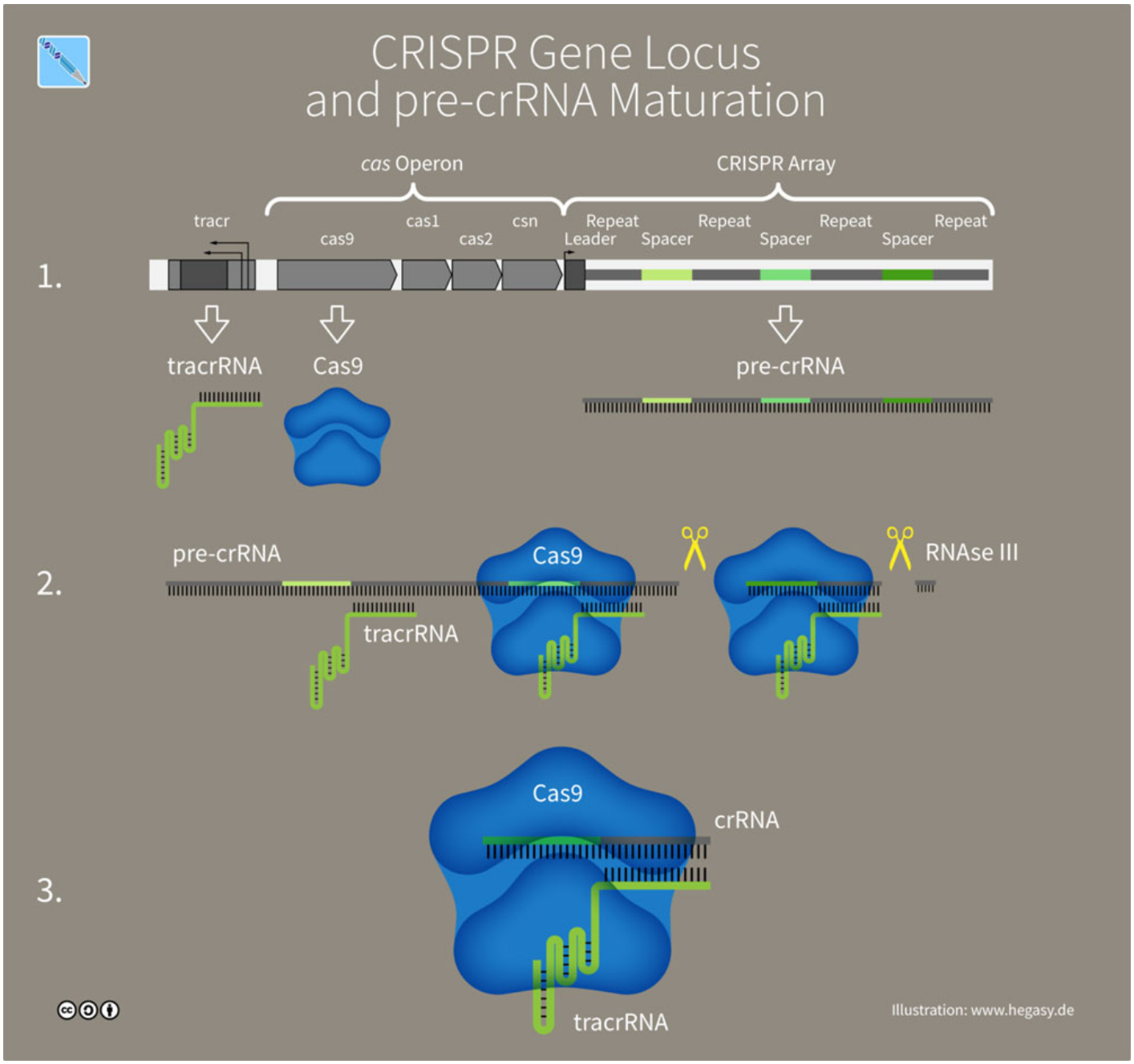

Figure 1:CRISPR-Cas-9 Array

Interference

The Cas-protein complexes formed during the crRNA processing are activated whenever a known invader reenters the cell. When this happens, the Cas complexes will recognize the DNA sequence and the corresponding PAM site on the genome; which will identify the genome as foreign genetic material. The protospacer sequence will then bond (through complementary base pairing) with the foreign DNA. Once this occurs one of a series of CASCADE (**C**RISPR-**A**ssociated **C**omplex for **A**ntiviral **D**efense) proteins will deactivate the invading genome. In type-I systems the CASCADE protein is a Cas-3 protein that cleaves the DNA into small inactive pieces. In type - II systems, the CASCADE protein is the Cas-9 protein which uses various enzymes to make a double strand break in the DNA.

Editing and Repair

Once a double strand break (DSB) occurs, the cell will attempt to repair the break using one of two mechanisms: Non-Homologous End Joining (NHEJ) or Homology Directed Joining (HDJ).

In the former mechanism the cell will join the ends of the DNA together using a ligase enzyme. This method is error prone as the process can create insertions or deletions of the gene sequences leading to a frame shift that will lead to a variety of negative biochemical consequences.[17] The second method of correcting a double stranded break is to use homology directed repair. Cells have traditionally used this repair pathway (during mitosis) when a sister chromatid is nearby, and the homologous strand can be used as a template to repair a break.

Frame Shifts in DNA

Our DNA contains the genetic code that directs the synthesis of the countless chemical compounds that control the body's biological processes. Each of our body's approximately 20,000 [18] genes is composed of a long sequence of precisely ordered nucleotides. The sequence of nucleotides is the code for amino acids which (when linked together) form the vast array of biochemicals that control every aspect of our existence. The process of transcription, to translation and synthesis of proteins is often referred to as the "central dogma of molecular biology".[19] The process begins when a molecule of mRNA makes a complimentary copy of a given sequence of nucleotides. The strand of mRNA moves into the cytoplasm where it interacts with ribosomes which read the sequence of nucleotides (three nucleotides at a time). Each set of three nucleotides (known as codons) correspond to a given amino acid (according to the underlying genetic code). It is critical that the sequence of nucleotides be read in the exact order specified by the DNA code. Changes to the code as a result of single point mutations, or insertions / deletions (indels) create frame shifts which alter the sequence of base pairs and the resulting chain of amino acids.

Applications of CRISPR Cas-9

The ability of the CRISPR system to achieve targeted editing of a precise location in a genome provides a highly efficient, relatively inexpensive, and accurate method of altering an organism's genes. While the constraints of existing genetic technologies limited the range and efficacy of genetic engineering, the advent of CRISPR technology has given researchers in various fields a reliable tool to solve long standing problems in agriculture, medical research, disease prevention, and ecosystem restoration. Researchers are now able to direct the Cas proteins to cleave a specific region of an organism's genome. They can also provide the cell with the genetic information it will use to repair its DNA. In this manner new genetic material can be introduced into the cell's genome.[20]

Benefits of CRISPR Cas-9 Technology

In agriculture, CRISPR "has become a simple, most user friendly and efficient, precise genome editing tool for development of genetically edited crops".[21] The system can be used to modify plant genomes so that they become more resistant to viruses, bacterial pathogens, and pests. This should make farming less toxic as it would reduce or eliminate the need for pesticide use.

In addition to conferring resistance, researchers have used the technology to engineer mutations that increase size, nutritional value, ease of cultivation, and other desirable traits which would increase crop yields throughout the world.[22] The technology also provides novel approaches to address plant, animal and human diseases that have proven difficult to treat or cure. Recently scientists proposed a method of excising the HIV virus from cells as shown in a mouse model.[23]

It is also proposed as a method for editing genes responsible for genetic diseases such as sickle cell anemia, forms of muscular dystrophy, beta thalassemia,[24] as well as using it to develop genetic therapies in cancer research.[25]

Gene Drive Technology

Although genetic engineers have developed the ability to alter the genomes of organisms that transmit diseases, they have never been able to efficiently insert a mutant gene into an entire population to address a disease problem. If they inserted a mutant gene into an insect (or rodent), the gene (according to the laws of Mendelian inheritance) would have at most a 50% chance of being expressed.[26] Over time the mutation would fade into the larger gene pool and perhaps disappear. This is not the case for a gene drive, which could be used to forcibly introduce a mutation into a target population.

A gene drive is composed of a gene that has been engineered to achieve a given result (e.g., decreased reproduction, reduced ability to transmit a pathogen, or limited life span), along with a mechanism (for example a CRISPR complex) that will drive the gene into the genome of all members of a given population. Gene drives can be defined as systems of biased inheritance in which the ability of a genetic element to pass from a parent to its offspring through sexual reproduction is enhanced.[27]

When organism's mate, the offspring receive one chromosome from each parent. However, when one parent carries a gene drive mutation, one of the offspring's two chromosomes will have the gene drive, the other chromosome (from the wild type) will not. When the chromosomes interact, the CRISPR complex will cause a double stranded break in the (wild type) chromosome. When the cell initiates its repair, it will use the mutated gene as a template: thus, creating two mutated chromosomes. This process will continue every time mating occurs thus increasing the number of organisms carrying two mutated genes. In subsequent generations, the percentage of organisms carrying the mutated gene will increase, and (depending on the rate at which the organism reproduces) all members of the species will eventually carry the mutant gene.

It is compelling to consider the possibilities that the gene drives offer our species given our ability to create precise mutations in the genomes of any living organism. The applications for this technology are wide ranging as it can be used in theory, to control organisms that transmit diseases to human populations, control invasive species that disrupt ecosystems, and eliminate pests that impact agricultural production.[28]

Risks and Unintended Consequences

Gene drives hold great promise in our efforts to improve the quality of life for our species. However, it is incumbent on us, to realize that these technologies can pose grave risks to the Earth and all the species that live on it. Before these engineered organisms are released substantial research should inform a body of public policy that will safeguard our ecosystems and other living organisms. Gene drives are most impressive in their (projected) ability to eradicate organisms that transmit diseases that cause millions of deaths each year. However, there is no guarantee that a mutated gene will remain in the target organism and will not migrate into another species (horizontal gene transfer),[29] or that the gene will not mutate in response to selective pressure, or that it will occasion the rise of other more virulent pathogens.[30] The ongoing loss of lives due to mosquito borne illnesses will likely motivate some in our society to vigorously advocate for the deployment of a gene drive somewhere on the planet, despite possible risks. While the use of the technology is a likely inevitability it is critical that we realize that

Continuous evaluation and assessment of the social, environmental, legal, and ethical considerations of gene drives will be needed to develop this technology responsibly and adapt research and governance to the field's complex and emerging challenges.[30]

The CRISPR-Cas-9 system poses different but an equally dangerous set of risks as it can also occasion unintended mutations. While researchers can use the system to target a specific site on a genome, there is the possibility that the complex will act mistakenly on other non-target sites in the genome and occasion off target double stranded breaks.[32] The resulting mutations can endanger the organism, or (if the target site is seriously affected) cause its death. CRISPR poses another set of ethical problems as it can be used to modify somatic and germline cells.

Ethical Concerns

Editing germ line cells is a highly controversial application of CRISPR technology as it has the potential to alter human patterns of heredity. While the use of this technology may provide alternatives to parents with no other option through which they can save a child,[33] the moral and ethical ramifications of embryonic engineering are quite troubling. Moreover, such technologies (when they become widely available) will cost more than most can afford. What will happen when poorer citizens need such technology? What will happen when only the richer nations have such access?

Socio-Political Implications

It is important to note that new gene editing technologies (such as CRISPR) differ from traditional methods of modifying an organism's genetic structure. Those techniques rely on the replacement of an organism's genes with those from another: techniques such as CRISPR alter the organisms own genotype and are therefore not considered a "trans-genetic modification".[34] This is an important distinction as the United States federal government has recently listed regulations that may exempt new gene editing technologies from existing regulations on GMO's.[35] While these regulations hold for American interests, such is not the case internationally where a debate as to classification of new genetically edited organisms is ongoing.[36] This is a serious consideration given the need for policies that will safeguard all nations.

Although the potential benefits of new gene editing technologies are very promising, there are a wide range of potential risks that have yet to be fully articulated and explored. Gene drives are at the forefront of research

efforts given the toll that mosquito borne diseases have on mankind. Recent outbreaks of Zika and Dengue fever, along with the historic death toll of malaria outbreaks, have increased interest in gene drives as a way to eradicate these mosquito-borne diseases. While we have already released genetically altered organisms into our ecosystems, the intentional eradication of an entire species raises profound environmental, ecological, and ethical considerations.[37] All experts agree that an extensive amount of discussion within national and international forums must take place before such technologies are deployed;[38] however, the discourse on the safety and security of these technologies needs to include a diversity of voices (from developed and developing nations). This is critically important in the case of the proposed deployment of gene drives as they will likely be used in underdeveloped nations that are traditionally overlooked when the interests of developed nations are at stake.[39]

Activities

DNA and Protein Synthesis Guided Inquiry

NOTE: It is assumed that students have a cursory knowledge of the structure of DNA; (the nucleotides, rules of base pairing, various functions of RNA) as well as an understanding of ribosomes and their role in protein synthesis. Although the activities will review these concepts, they will not reteach all aspects of these concepts.

Protein Synthesis POGIL

Objective: To Analyze the biochemical processes that translate information stored in the DNA into sequences of amino acids.

Standard:CCSS.ELA-Literacy.RST.11-12.9

Instruction: Genetic information stored in the DNA contains all of the necessary information for the synthesis of biochemicals that direct all aspects of an organism's survival. An organism's genetic information (its genes) is stored as a sequence of nucleotides (Guanine (G), Cystine (C), Thymine (T), and Adenine (A)), that are paired with their complements in the DNA double helix. Given sections of the DNA (genes) code for the specific proteins that direct and control cellular functions. This activity will guide students through an inquiry that explores protein synthesis.

Task One: Once the DNA helix is separated: use the rules of base pairing to create an mRNA copy of the sequence: your strand should be at least twenty-one base pairs (b.p.) long.

Task Two: Once completed use a strand of mRNA and the genetic code guide to translate sets of three b.p.'s into their corresponding amino acid.

Task Three: Assemble the amino acid sequences into a chain.

Additional Information: DNA sequences must be translated with fidelity in order to ensure that amino acids are sequenced correctly. Insertions or deletions of the DNA code alter the amino acid sequence which can lead to frame shifts mutations. These mutations can disrupt the proper translation of the cell's genetic information.

Task Four: Using your mRNA strand remove one to three base pairs. Use this altered strand to carry out your protein synthesis. How does this new sequence compare with the original?

Could this new sequence code for the same protein? How would this affect the cell's processes?

CRISPR – Cas 9 Guided Inquiry

Objective: To Analyze the CRISPR – Cas 9 Immune Response

Standard:HS-ETS1-4 Engineering Design

Instruction: Bacterial cells use the CRISPR array and associated Cas proteins as an immune response. Upon reinvasion the cells use the segments stored in the array to guide the Cas proteins that cleave the invading genome. This is a collaborative activity in which groups of students (at least four per group) build a CRISPR array.

Task One: Each group of students will receive a DNA sequence of an "invading genome": (NOTE: there should be at least 6 sequences for this activity). Each group will select a protospacer sequence (approximately 15 -20 b.p.) that they will be incorporated into the CRISPR array (students also should note the PAM sequence on the DNA sequence). Each group's sequence will be used to build the CRISPR array.

Task Two: Each student group will translate their DNA sequence into an RNA copy (the crRNA) which serves as the guide RNA. Each group attaches their crRNA to a cutout representing the CAS protein. The complex will serve as a facsimile of the CAS protein complex.

Additional Information: The series of associated CAS protein complexes are used to defend against reinvasion. When this occurs, the CAS complexes use the guide sequences to identify the invading genome which is cleaved by Cas proteins.

Task Three: Groups will interchange their CAS protein complexes. Teacher will show each of the original DNA sequences; student groups will determine which CAS protein corresponds to the genome and thence simulate cleavage by the CAS proteins. (Note: Students will need to identify the appropriate PAM sequence of the genome before "cleaving" the DNA.)

Practical Applications of CRISPR Technology: Independent Research

Objective: To evaluate the practical applications of CRISPR Technology

Standard:HS-ETS1-1 Engineering Design

Instruction: While bacteria use CRISPR as an immune response, the system can be used to modify an organism's genetic structure. Once the CAS complex cleaves an organism's DNA the cell will use either Non-Homologous End Joining (NHEJ) or Homology Directed Joining (HDJ) to repair the break. Each of these processes can be used by researchers to alter (or remove gene sequences) or to insert genetic information.

In this activity, students will engage in a web quest to research the applications of CRISPR in a variety of fields. The teacher will list the various fields in which the technology is currently being used. Each student group will select one application and prepare a brief explanation of how CRISPR is being used, along with the proposed benefits and possible consequences its use poses to society.

Gene Drive Technology Directed Modeling Activity

Objective: To analyze how gene drives alter traditional patterns of Mendelian inheritance

Standard: HS-ESS3-4 Earth and Human Activity

Note: Students should have a cursory knowledge of Punnett squares and a basic understanding of Mendelian laws of inheritance.

Instruction: In traditional patterns of inheritance in sexually-reproducing organisms, each offspring receives a gene from each parent. This is true for each successive generation; thus, each gene has at most a 50% chance of being passed on. This activity begins with a brief analysis of three generations to verify this pattern. Students should note that inherited genes remain the same across the generations.

Task One: Each group of students will be given two sets of alleles (father and mother) which they will cross (for this activity the alleles should be heterozygous). Students should complete Punnett squares for at least four generations.

Additional Information: When a gene drive is employed one gene has the capacity to overwrite the genetic information of an inherited gene (designated as the wild type for this activity). In this manner the frequency of the designed gene will steadily increase with each successive generation.

Task Two: Students will repeat task one; however, one of the genes that are passed on will be a gene drive gene. Students should complete task one and notice the gradual increase in the frequency of the gene drive gene: eventually every offspring will carry the gene drive gene.

Additional Information: There are many proposed uses for gene drive technologies. Students and teacher will explore these proposed uses of the technology and add them to the list generated during the previous research activity.

Ethical Dimensions of Genetic Engineering: Guided Research

Objective: To evaluate the ethical, societal, and environmental consequences of bioengineering technology

Standard: HS-ETS1-1 Engineering Design

Instruction: Students will use their research on the benefits and consequence of genetic engineering to create a position paper. The class will first engage in a structured discussion to elicit as many perspectives as possible. Each group will take notes and then use their collected information to answer the guiding question: “Should We?”.

Appendix: Standards Narrative

CCSS.ELA-Literacy.RST.11-12.9 is a common core standard that asks student to synthesize information form a range of sources to create a coherent understanding of a phenomena or concept. The standard is relevant to the first activity as students will be required to bring together their prior knowledge of biological

systems, the genetic code and protein synthesis to fully analyze how insertions and deletions can alter genetic codes. This will be important in later activities that explore how CRISPR technology can be used to modify genetic structures.

HS-ETS1-4 Engineering Design is a Next Generation science standard that asks students to create a model of a proposed solution to a real-world problem. Being able to explain how the CRISPR system functions is important because the technology has the possibility of providing solutions to a wide range of problems in our society.

Although there are many proposed benefits to our use of CRISPR technology, there are many risks and unforeseen consequences that researchers have yet to fully articulate. Standard **HS-ESS3-4 Earth and Human Activity** is relevant to this unit as it asks students to evaluate or refine a technological solution that reduces impacts of human activities on natural systems.

Given that the debate over the risks and benefits of genetic engineering has yet to be resolved, students will need to examine a range of perspectives in order to form their opinions on the use these technologies. Standard **HS-ETS1-1 Engineering Design** is included in this unit as it asks student to analyze the criterial and constraints of proposed solutions to major global challenges in light of societal needs and wants.

Bibliography

Balter, Michael. “Seeking Agriculture’s Ancient Roots.” *Source: Science, New Series* 316, no. 5833 (2007): 1830–35. http://www.jstor.org/stable/20036560.

Bhaya, Devaki, Michelle Davison, and Rodolphe Barrangou. “CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation.” *Annual Review of Genetics*, 2011. https://doi.org/10.1146/annurev-genet-110410-132430.

Chen, Longzheng, Wei Li, Lorenzo Katin-Grazzini, Jing Ding, Xianbin Gu, Yanjun Li, Tingting Gu, et al. “A Method for the Production and Expedient Screening of CRISPR/Cas9-Mediated Non-Transgenic Mutant Plants.” *Horticulture Research* 5, no. 1 (December 2, 2018): 13. https://doi.org/10.1038/s41438-018-0023-4.

Doudna, Jennifer A, and Emmanuelle Charpentier. “Genome Editing. The New Frontier of Genome Engineering with CRISPR-Cas9.” *Science (New York, N.Y.)* 346, no. 6213 (2014): 1258096-1-1258096–99. https://doi.org/10.1126/science.1258096.

Driscoll, Carlos A, David W Macdonald, and S. J. O’Brien. “From Wild Animals to Domestic Pets, an Evolutionary View of Domestication.” *Proceedings of the National Academy of Sciences* 106, no. Supplement_1 (2009): 9971–78. https://doi.org/10.1073/pnas.0901586106.

Genetech. “First Successful Bacterial Production of Human Hormone Announced,” 1979. https://www.gene.com/media/press-releases/4161/1979-07-11/first-successful-bacterial-production-ofG.

Guernet, Alexis, and Luca Grumolato. “CRISPR/Cas9 Editing of the Genome for Cancer Modeling,” 2017. https://doi.org/10.1016/j.ymeth.2017.03.007.

Hsu, Patrick D, Eric S Lander, and Feng Zhang. “Development and Applications of CRISPR-Cas9 for Genome Engineering.” *Cell* 157,

no. 6 (2014): 1262–78. https://doi.org/10.1016/j.cell.2014.05.010.

Ishino, Yoshizumi, Hideo Shinagawa, Kozo Makino, Mitsuko Amemura, and Atsuo Nakata. “Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product.” *JOURNAL OF BACTERIOLOGY* 169, no. 12 (1987): 5429–33. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC213968/pdf/jbacter00202-0107.pdf.

K.Oye, K.Estevez, A.Flamina. “Regulating Gene Drives.” *Science* 345, no. 6197 (2014). http://science.sciencemag.org/content/sci/345/6197/626.full.pdf.

Kareiva, Peter, Sean Watts, Robert Mcdonald, and Tim Boucher. “Domesticated Nature: Shaping Landscapes and Ecosystems for Human Welfare.” *Source: Science, New Series* 316, no. 5833 (2007): 1866–69. http://www.jstor.org/stable/20036578.

Khatodia, Surender, Kirti Bhatotia, and Narendra Tuteja. “Development of CRISPR/Cas9 Mediated Virus Resistance in Agriculturally Important Crops.” *Bioengineered* 8, no. 3 (2017): 274–79. https://doi.org/10.1080/21655979.2017.1297347.

Ledford, Heidi. “CRISPR, THE DISRUPTOR.” *Nature* 522, no. 4 (2015): 20–15. https://www.nature.com/polopoly_fs/1.17673!/menu/main/topColumns/topLeftColumn/pdf/522020A.pdf.

Lya, I L. “Expanded Human Gene Tally Reignites Debate.” Accessed August 17, 2018. http://doi.org/cq5s;

Ma, Hong, Nuria Marti-Gutierrez, Wook Park, Jun Wu, Yeonmi Lee, Keiichiro Suzuki, Amy Koski, et al. “Correction of a Pathogenic Gene Mutation in Human Embryos.” *Nature Publishing Group* 548 (2017). https://doi.org/10.1038/nature23305.

Martinez-Lage, Marta. “CRISPR/Cas9 Technology: Applications and Human Disease Modeling.” *Progress in Molecular Biology and Translational Science* 152 (January 1, 2017): 23–48. https://doi.org/10.1016/BS.PMBTS.2017.09.002.

National Academy of Science. “Gene Drives on the Horizon Board on Life Sciences DIVISION ON E ARTH AND LIFE STUDIES,” 2016. http://nas-sites.org/gene-drives/files/2015/08/Gene-Drives-Brief06.pdf.

Noble, Charleston, Ben Adlam, George M Church, Kevin M Esvelt, and Martin A Nowak. “Current CRISPR Gene Drive Systems Are Likely to Be Highly Invasive in Wild Populations.” Accessed July 13, 2018. https://doi.org/10.1101/219022.

Pukkila, Patricia J. “Molecular Biology: The Central Dogma,” 2001. www.els.net.

Regalado, Anthony. “These Are Not Your Father’s GMOs - MIT Technology Review.” MIT Technology Reviews , 2017. https://www.technologyreview.com/s/609230/these-are-not-your-fathers-gmos/.

Rodriguez, E. “Ethical Issues in Genome Editing Using Crispr/Cas9 System.” *Journal of Clinical Research & Bioethics* 07, no. 02 (2016). https://doi.org/10.4172/2155-9627.1000266.

Shin, Ha Youn, Chaochen Wang, Hye Kyung Lee, Kyung Hyun Yoo, Xianke Zeng, Tyler Kuhns, Chul Min Yang, Teresa Mohr, Chengyu Liu, and Lothar Hennighausen. “CRISPR/Cas9 Targeting Events Cause Complex Deletions and Insertions at 17 Sites in the Mouse Genome.” *Nature Communications* 8 (May 31, 2017): 15464. https://doi.org/10.1038/ncomms15464.

Shui, Bing, Liz Hernandez Matias, Yi Guo, and Ying Peng. “The Rise of CRISPR/Cas for Genome Editing in Stem Cells.” Accessed July 18, 2018. https://doi.org/10.1155/2016/8140168.

Soyk, Sebastian, Zachary H. Lemmon, Matan Oved, Josef Fisher, Katie L. Liberatore, Soon Ju Park, Anna Goren, et al. “Bypassing Negative Epistasis on Yield in Tomato Imposed by a Domestication Gene.” *Cell* 169, no. 6 (June 1, 2017): 1142–1155.e12. https://doi.org/10.1016/J.CELL.2017.04.032.

Tanno, Ken-Ichi, George Willcox, Ken-Ichi Tanno1, and George Willcox2. “How Fast Was Wild Wheat Domesticated? How Fast Was Wild Wheat Domesticated? On the Position on the Ear and Environmental.” *Source: Science, New Series* 311, no. 5769 (2006). http://www.jstor.org/stable/3845609.

Thurtle-Schmidt, Deborah M., and Te-Wen Lo. “Molecular Biology at the Cutting Edge: A Review on CRISPR/CAS9 Gene Editing for Undergraduates.” *Biochemistry and Molecular Biology Education* 46, no. 2 (March 1, 2018): 195–205. https://doi.org/10.1002/bmb.21108.

USDA. “Secretary Perdue Issues USDA Statement on Plant Breeding Innovation | USDA,” 2018. https://www.usda.gov/media/press-releases/2018/03/28/secretary-perdue-issues-usda-statement-plant-breeding-innovation.

Weiss, Ehud, and Daniel Zohary. “The Neolithic Southwest Asian Founder Crops.” *Current Anthropology* 52, no. S4 (2011): S237–54. https://doi.org/10.1086/658367.

Yin, Chaoran, Ting Zhang, Xiying Qu, Yonggang Zhang, Raj Putatunda, Xiao Xiao, Fang Li, et al. “In Vivo Excision of HIV-1 Provirus by SaCas9 and Multiplex Single-Guide RNAs in Animal Models.” *Molecular Therapy* 25 (2017): 1168–86. https://doi.org/10.1016/j.ymthe.2017.03.012.

Zimmer, Carl. *She Has Her Mother’s Laugh: The Powers, Perversions, and Potential of Heredity*. New York: Dutton, 2018.

Endnotes

1. Tanno et al., “How Fast Was Wild Wheat Domesticated? How Fast Was Wild Wheat Domesticated? On the Position on the Ear and Environmental.”
2. (Balter 2007 p. 1830)
3. Weiss and Zohary, “The Neolithic Southwest Asian Founder Crops.”
4. Driscoll, Macdonald, and O’Brien, “From Wild Animals to Domestic Pets, an Evolutionary View of Domestication.”
5. (Driscoll, Macdonald, and O’Brien 2009)
6. (Driscoll, Macdonald, and O’Brien 2009, p 9971)
7. Kareiva et al., “Domesticated Nature: Shaping Landscapes and Ecosystems for Human Welfare.”
8. Zimmer, *She Has Her Mother’s Laugh: The Powers, Perversions, and Potential of Heredity*.
9. Doudna and Charpentier, “Genome Editing. The New Frontier of Genome Engineering with CRISPR-Cas9.”
10. Genetech, “First Successful Bacterial Production of Human Hormone Announced.”
11. Shui et al., “The Rise of CRISPR/Cas for Genome Editing in Stem Cells.”
12. Ledford, “CRISPR, THE DISRUPTOR.”
13. Ishino et al., “Nucleotide Sequence of the Iap Gene, Responsible for Alkaline Phosphatase Isozyme Conversion in Escherichia Coli, and Identification of the Gene Product.”
14. Palindromes are sequences of base pairs that read the same in both directions: for example, the sequence GGTTGG: or CCAACC are palindromes.
15. Bhaya, Davison, and Barrangou, “CRISPR-Cas Systems in Bacteria and Archaea: Versatile Small RNAs for Adaptive Defense and Regulation.”
16. Thurtle-Schmidt and Lo, “Molecular Biology at the Cutting Edge: A Review on CRISPR/CAS9 Gene Editing for Undergraduates.”
17. Martinez-Lage, “CRISPR/Cas9 Technology: Applications and Human Disease Modeling.”
18. Lya, “Expanded Human Gene Tally Reignites Debate.”
19. Pukkila, “Molecular Biology: The Central Dogma.”

20. Hsu, Lander, and Zhang, “Development and Applications of CRISPR-Cas9 for Genome Engineering.”
21. (Khatodia, Bhatotia, and Tuteja 2017 p.274)
22. Soyk et al., “Bypassing Negative Epistasis on Yield in Tomato Imposed by a Domestication Gene.”
23. Yin et al., “In Vivo Excision of HIV-1 Provirus by SaCas9 and Multiplex Single-Guide RNAs in Animal Models.”
24. Ledford, “CRISPR, THE DISRUPTOR.”
25. Guernet and Grumolato, “CRISPR/Cas9 Editing of the Genome for Cancer Modeling.”
26. (National Academy of Science 2016)
27. (National Academy of Science 2016 p.18)
28. K.Oye, K.Estevez, “Regulating Gene Drives.”
29. Noble et al., “Current CRISPR Gene Drive Systems Are Likely to Be Highly Invasive in Wild Populations.”
30. Ledford, “CRISPR, THE DISRUPTOR.”
31. (National Academy of Science 2016 p.24)
32. Shin et al., CRISPR/Cas9 Targeting Events Cause Complex Deletions and Insertions at 17 Sites in the Mouse Genome.”
33. Ma et al., “Correction of a Pathogenic Gene Mutation in Human Embryos.”
34. Chen et al., “A Method for the Production and Expedient Screening of CRISPR/Cas9-Mediated Non-Transgenic Mutant Plants.”
35. USDA, “Secretary Perdue Issues USDA Statement on Plant Breeding Innovation | USDA.”
36. Regalado, “These Are Not Your Father’s GMOs - MIT Technology Review.”
37. Rodriguez, “Ethical Issues in Genome Editing Using Crispr/Cas9 System.”
38. K.Oye, K.Estevez, “Regulating Gene Drives.”
39. National Academy of Science, “Gene Drives on the Horizon Board on Life Sciences DIVISION ON EARTH AND LIFE STUDIES.”

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