6-8-2015

# A TALE of Two Nucleases: Using TALENs to Edit the Genome of C. elegans

Clare Bartlett
*Santa Clara Univeristy*

Kriszten Kocmond
*Santa Clara Univeristy*

Erin Root
*Santa Clara Univeristy*

**SANTA CLARA UNIVERSITY**

Department of Bioengineering

I HEREBY RECOMMEND THAT THE THESIS PREPARED
UNDER MY SUPERVISION BY

Clare Bartlett, Kriszten Kocmond, Erin Root

ENTITLED

# A TALE OF TWO NUCLEASES: USING TALENs TO EDIT THE GENOME OF *C. ELEGANS*

BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

**BACHELOR OF SCIENCE**
IN
**BIOENGINEERING**

6/1/15

Thesis Advisor date

6/1/15

Department Chair date

# A TALE OF TWO NUCLEASES: USING TALENs TO EDIT THE GENOME OF *C. ELEGANS*

By

Clare Bartlett, Kriszten Kocmond, Erin Root

**SENIOR DESIGN PROJECT REPORT**

Submitted to
The Department of Bioengineering

Of

SANTA CLARA UNIVERSITY

in Partial Fulfillment of the Requirements
for the degree of
Bachelor of Science in Bioengineering

Santa Clara, California

Spring 2015

## Abstract

Genetic engineering is an emerging technology that offers the potential to prevent, treat, or cure genetic diseases. The technology can permanently alter the genome, providing an alternative therapy to drugs and surgery. Specifically, gene therapy is a promising treatment option for many incurable genetic diseases, such as cystic fibrosis and muscular cell dystrophy. Our project gives rise to a better understanding of TALENs and its uses in the genetic engineering field.

TALENs, transcription activator-like effector nucleases, are a genetic engineering technology that can be used for targeted gene modification. They are engineered proteins that can bind to specific sequences of DNA and induce a double-stranded break. The DNA sequence that the TALENs bind to is determined by the user; therefore the TALENs can be engineered to target specific DNA sequences that cause genetic diseases. We used TALENs within the model organism, nematode *C. elegans*, to explore their potential for use in gene therapy. By utilizing TALENs to introduce a *lin-31* mutant into the genome of *C. elegans* we aim to advance the understanding of TALENs as a genetic engineering tool and contribute to the research on the docking site of LIN-31 in the Ras/MAPK signaling pathway. Our group was successful in creating the DNA that encodes for these TALENs proteins, providing a foundation for future student researchers to continue on the project.

## Acknowledgments

We would like to extend our thanks to Dr. Leilani Miller and Amanda Dewey for their support and assistance with our Senior Design Project this year. They are both incredibly patient, knowledgeable and passionate scientists from whom we have learned so much. We would also like to thank Santa Clara University's Undergraduate School of Engineering for their generous donation to our project.

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## List of Terms and Abbreviations

**Nuclease:** an enzyme that can induce a double stranded break (DSB) in the DNA

**NHEJ**: **N**on-**H**omologous **E**nd **J**oining

**HR**: **H**omologous **R**ecombination

**TALENs- T**ranscription **A**ctivator-**L**ike **E**ffector **N**ucleases

**ZFNs- Z**inc **F**inger **N**ucleases

**CRISPR- C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

**RVDs- R**epeat **V**ariable **R**esidue

**PCR- P**olymerase **C**hain **R**eaction

**NEB- N**ew **E**ngland **B**iolabs

**CEL-1:** An enzyme from celery that cuts mismatched DNA

## Introduction and Significance

### Background/Motivation

Genetic engineering is an emerging technology that offers the potential to permanently alter the genome of organisms. Gene therapy uses genetic engineering technologies to treat many incurable genetic diseases, such as cystic fibrosis and muscular cell dystrophy. It is an exciting field because the technology can permanently alter the genome, providing an alternative therapy to drugs and surgery.

There are many ways by which genetic engineering can be achieved. One of the most common techniques is to use a nuclease to induce a double-stranded break in the genome. New genetic information can be inserted at this break site (an insertion mutation) or genetic information can be deleted from the break site (a deletion mutation). After the genome is mutated, it is repaired either by non-homologous end joining (NHEJ) or by homologous recombination (HR). Non-homologous end joining allows the DNA to crash back together, while homologous recombination requires the use of template DNA (which contains the same sequence as the break site) to repair the broken ends. The variety in genetic engineering technology arises from the different types of mutations they induce after the double-stranded break in the genome and how this mutation is repaired.

TALENs, transcription activator-like effector nucleases, are a genetic engineering technology that can be used for targeted gene modification. They are engineered proteins which can bind to specific sequences of DNA and induce a double-stranded break. This break is then repaired by either NHEJ or HR using template DNA. The user determines the DNA sequence that the TALENs binds to; therefore the TALENs can be engineered to target specific DNA

sequences that cause genetic diseases, and therein lies its great potential as a therapeutic tool (Cermak). For our senior design project we used TALENs within the model organism, nematode *C. elegans*, to explore their potential for use in gene therapy. As a result of our research, we concluded that TALENS enables much more precise targeting of DNA sequences, and is therefore worthy of continued testing and development. At the end of our report we will indicate the next steps that might be taken with this promising technology.

**Critique of Competing Technologies**

The established methods for gene therapy include retroviruses, zinc finger nucleases, and CRISPR. However, each of these technologies has significant drawbacks. As seen in Table 1, TALENs offers an exciting approach to gene therapy because it addresses the issues seen with the established methods for gene therapy.

**Table 1.** A comparison of TALENs to the current approaches to gene therapy

| Technology | What it Does | Drawbacks | How TALENs Addresses these problems |
|---|---|---|---|
| • Retroviruses | • The virus can insert its DNA into the host's genome<br>• The virus's DNA permanently becomes part of the host's genome and is expressed using the host's DNA transcription and translation machinery | • Retroviruses currently cannot be engineered to target specific DNA sequences<br>• This can result in undesired gene modification: the retrovirus could insert at a location in the genome that turns on an oncogene, causing cancer<br>• Takes weeks or months to engineer | • TALENs can be engineered to target specific DNA sequences<br>• TALENs gives the genetic engineer more control over the gene modification; they will not randomly modify the genome<br>• Takes 5 days to engineer |
| • Zinc Finger Nucleases (ZFNs) | • Engineered proteins that can target DNA sequences and induce a double stranded break<br>• This break is then repaired by injecting DNA that acts as a template for HR | • Difficult to engineer<br>• Have a significant failure rate<br>• Takes weeks or months to engineer | • The creators of this technology have developed a kit which provides a step by step protocol to engineer the TALENs<br>• Data has been published showing the success of TALENs<br>• Takes 5 days to engineer |

| Technology | What it Does | Drawbacks | How TALENs Addresses these problems |
|---|---|---|---|
| • CRISPR | • Engineered proteins composed of a DNA binding domain and a nuclease<br>• Break is then repaired by injecting foreign DNA | • Recently developed technology<br>• High rate of off-target effects | • TALENs is a more established approach to gene therapy and has been studied in *C. elegans*<br>• Lower incidence of off-target effects |

A retrovirus is an RNA virus that stores genetic information in the form of mRNA. The mRNA is then converted into DNA with the use of the enzyme reverse transcriptase and can then be incorporated into the host cell's genome (Anson). Scientists have used retroviruses as vehicles for delivering corrected gene segments (Anson). This has shown that retroviruses have high efficiency when spreading their genetic information. However, a major problem with using retroviruses to correct genetic mutations is that they cannot be engineered to target specific DNA sequences and therefore could cause undesired gene modification. The retrovirus could insert DNA in a location within the genome that turns on an oncogene, causing cancer. Because of this drawback, new approaches to gene therapy were developed.

Zinc finger nucleases (ZFNs) attempt to address the problems seen with retroviruses. ZFNs can be engineered to target specific sequences within the genome, as illustrated in Figure 1 below:

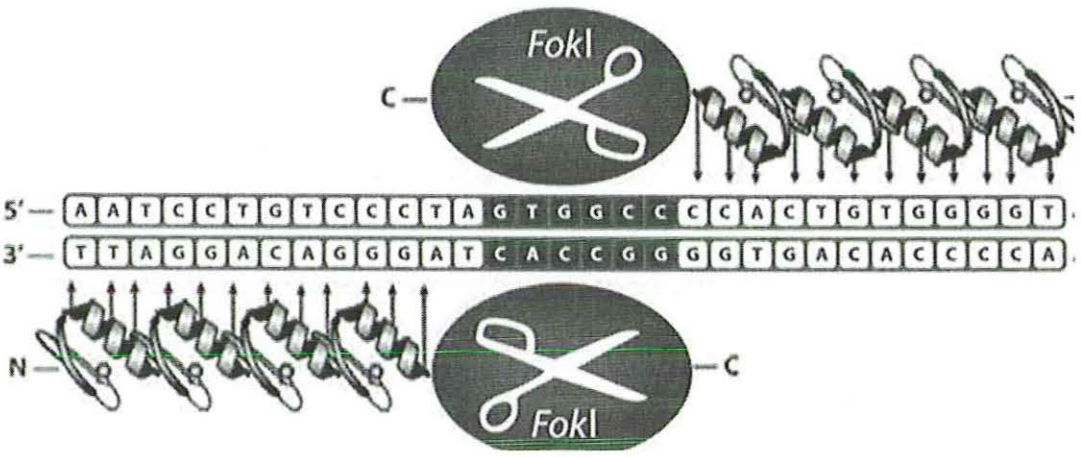


Figure 1: Structure of a Zinc Finger Nuclease ("What is").

As shown in Figure 1, after the ZFNs bind to the DNA, they are able to induce a double-stranded break. The genome is then modified either through homologous recombination with an inserted plasmid or non-homologous end joining. ZFNs are composed of $C_2H_2$ zinc-finger motifs, which are the DNA binding domain and a Fok1 nuclease, an enzyme that can cut DNA. Together these components create a targeted approach to genetic engineering and have been used successfully to disrupt genes in the model organism *C. elegans* (Wood). However, ZFNs have not become a widely used approach to genetic engineering because they are difficult to engineer and expensive to create.

Recently developed approaches to gene therapy include CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and TALENs (Transcription Activator-Like Effector Nucleases). Both of these technologies address the problems seen with retroviruses and ZFNs. TALENs and CRISPR are similar to the zinc finger nucleases; they are engineered proteins composed of a DNA binding domain and a nuclease. The DNA binding domain in the CRISPR system is guide RNA derived from bacteria and is fused to the endonuclease Cas9 (Chen). This complex can then bind to and cut DNA. However, CRISPR is a recently developed technology

and preliminary research has shown that it has more off-target effects, or binding in undesired locations. This is highlighted by a recently published study in which CRISPR was used to modify the genome of non-viable human embryos. Eighty-six embryos were used in this experiment, and only four contained the correct mutation (Cyranoski). Further data analysis indicated that many of the embryos used in the study contained off-target mutations, meaning that CRISPR had modified the embryo's genome in an undesired location.

We have chosen to use the TALENs approach to genetic engineering because they can be engineered to target specific DNA, have few limitations regarding the sequences they target, are easy to engineer, and have less off-target effects than CRISPR.

**Review of TALENs Technology**

TALENs are engineered proteins comprised of transcription activator-like (TAL) effectors, the DNA binding domain of the protein, and a Fok1 nuclease (Cermak). The TAL effectors are DNA binding proteins produced by bacteria in the *Xanthomonas* genus (Cermak). Because of this DNA binding capability, different TAL-effectors can be combined to create the DNA binding domain of the TALENs protein. The binding domain is then fused to a Fok1 nuclease, creating a protein that can target and induce a double stranded break at a specific location. TALENs have been used to successfully alter the genomes of *C. elegans*, zebrafish, fruit flies, frogs, rats, and pigs (Joung). Most of these studies have used TALENs to induce knockout mutations, or mutations facilitated by non-homologous end joining, in the organism's genome (Joung). Specifically, our project will use TALENs to create a double stranded break, which will then be repaired through homologous recombination with a repair plasmid. Such

homologous recombination is demonstrated in the image below through the process of "gene correction" or "gene addition".

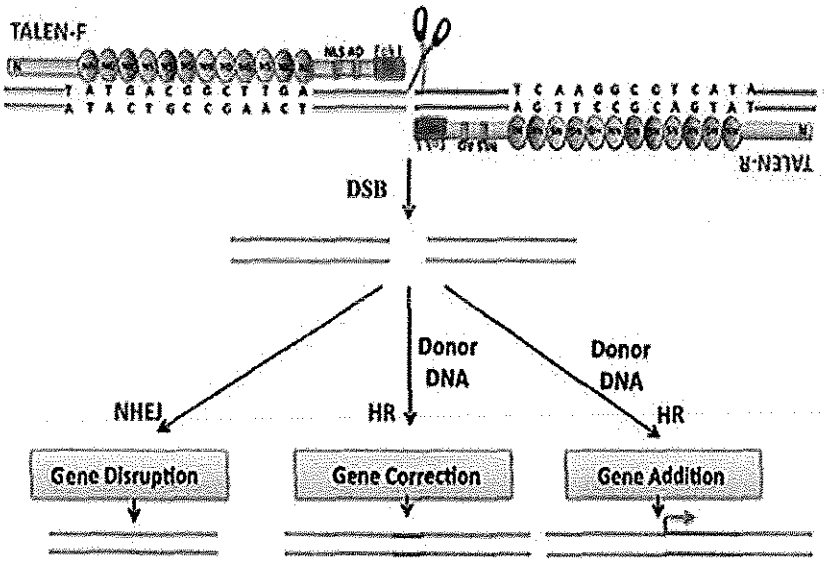


Figure 2. Process of gene editing with a TALENs protein ("Custom").

As indicated in the graphic, the first step in using the TALENs technology is to identify the DNA sequence that will be altered. This sequence is then put into the TALENs software, a free online program developed by the creators of TALENs. The user also inputs the location in the genome where the TALENs should cut. The program then generates the DNA sequence for constructing TALENs that will target the desired location in the genome. Once the template has been generated, the appropriate subunits from the TALENs plasmid kit are used to create the TALENs DNA. This DNA is then transcribed into mRNA using an *in vitro* transcription kit. After the mRNA is created, it is microinjected into the *C. elegans*, along with the repair plasmid. The *C. elegans*' ribosomes then translate the TALENs mRNA into TALENs protein; this protein can then target the desired DNA sequence. Targeting occurs because the TALENs DNA binding domain contains only four types of repeating subunits, each corresponding to a specific nucleotide. This allows the user to customize the order of these subunits, creating two TALENs

proteins that will bind to complementary strands of the desired DNA. Once the TALENs proteins bind, the FokI nuclease that is attached to the TALENs will induce a double stranded break in the DNA.

In addition to making the TALENs DNA and mRNA, we will also construct a separate plasmid containing the desired mutant sequence that will also be microinjected with the mRNA. DNA repair machinery utilizes this plasmid as a template for homologous recombination. This results in an altered gene at its endogenous site within the genome.

**Statement of Project Goal**

Our project focuses on engineering TALENs to alter the gene *lin-31*, an important gene in cell fate determination in *C. elegans*. Our goal is to create a modified version of the *lin-31* gene, which is then translated into a mutated LIN-31 protein. We hypothesized that this mutation will alter a putative Map Kinase docking site at the end of a Ras/Map Kinase signaling pathway.

The success of this project will be analyzed by screening the microinjected *C. elegans* for mutations. Both the constructed TALENs and the repair plasmid containing our altered sequence will be microinjected into *C. elegans.* If the TALENs were successful, the nematode's progeny would contain the mutated version of our selected sequence. This mutation was hypothesized to create a visible mutant phenotype in a specific sensitized genetic background.

Beyond scoring the phenotypes of the progeny of our TALENs *C. elegans,* a cel-1 assay will also be used to analyze the project. Pools of worms will be lysed in order to extract their DNA. This DNA will then be amplified using PCR or polymerase chain reaction. Cel-1, an enzyme that cuts mismatched DNA, will be extracted from celery, and when incubated with the PCR products from pools of worms, should reveal if the mutation is present. This DNA will also

be sequenced to determine if the TALENs were successful in mutating the *C. elegans* genome at the desired location.

**Back-up Plan**

Due to the nature of scientific research, our design team ran into many issues that required troubleshooting and redoing experiments. Therefore, we were unable to complete the project in its entirety. However, we were able to create the TALENs DNA and have left a detailed lab notebook for future students to use as a guide to further explore the potential of TALENs as a genetic engineering tool. Our plan is to present our completed work and explore possible explanations as to what went wrong.

**Significance**

Not only does this project give rise to a better understanding of cell fate determination in *C. elegans*, but it also has the potential to increase our understanding of how transcription factors work at the end of other Ras/Map Kinase signaling pathways. Mutations in these pathways are found on approximately 70% of all human cancers (Sundaram). Therefore, successful completion of our project will give rise to a better understanding of the details of the working of this signaling pathway.

With our project we will be able to gain a deeper understanding of the genetic engineering process. Because TALENs is a relatively new technology, our research has the potential to advance the general understanding of TALENs and how they can be used in genetic engineering, especially as a research tool. These advancements will bring TALENs-based genetic therapy closer to the ultimate goal of using this technology to treat human genetic diseases.

**Team and Management**

*Senior Design Team:*

Clare Bartlett - Biomolecular Track Bioengineering

Kriszten Kocmond - Device Track Bioengineering

Erin Root - Pre-med Track Bioengineering

*Advisors:*

Dr. Leilani Miller - Biology Professor, Director of Office of Fellowships, Director of University Honors Program, Director of LEAD Scholars Program

Amanda Dewey - Graduate of Santa Clara University, Miller Lab Manager

**Budget**

| Item | Name | Vendor | Catalog # | cost |
|---|---|---|---|---|
| CEL-1 Purification | SpectraPor Dialysis Tubing | Spectrum | 132119T | $130 |
| CEL-1 Purification | Ammonium Sulfate | Fisher | A702-500 | $79 |
| mRNA Synthesis | RNAase Away Decontaminant | Fisher | 21-402-178 | $50 |
| Enzymes and<br>Competent Cells | BsaI, BsmBI, Taq DNA<br>Polymerase,<br>5-alpha Competent *E. coli* | New England<br>Biolabs | R0535S<br>R0580S<br>M0273X<br>C2987H | $63<br>$68<br>$368<br>$443 |
| **TOTAL** | | | | **$1,211** |

**Timeline**

*September 2014-December 2014:*

- Preliminary research
- Lab safety training
- Familiarization with lab techniques
- Identification of the sequence within the *C. elegans* LIN-31 gene that our TALENs will target
- Create repair plasmid
- Begin working on creating TALENs DNA

*January 2015-March 2015:*

- Continue work on TALENs DNA
- Begin research on Thesis
- Create PowerPoint for Presentation for Senior Design Conference and West Coast Biological Sciences Undergraduate Research Conference

*April 2015- May 14th 2015:*

- Prepare TALENs DNA for transcription into mRNA
- Seminar Presentation at West Coast Biological Sciences Undergraduate Research Conference
- Presentation at Senior Design Conference
- West Coast Biological Sciences Undergraduate Research Conference
- Prepare project to be taken over by future student researchers

## 1.0 Design and Construct

### Ras/MAPK Signaling Pathway

One of the major goals of our project was to study a pathway known as the Ras/MAPK signaling pathway. This pathway is conserved among many organisms including humans. Ras/MAPK is involved in cell fate specification or the process by which cells determine their specific cell type. This is because activation of the pathway causes a phosphorylation cascade resulting in phosphorylated MAPK entering the nucleus and phosphorylating transcription factors. Phosphorylation is a means of communication because it alters structural conformation through the addition of a charged phosphate group. Transcription factors, or proteins that turn

genes on or off, play an important role in cell development, including cell fate specification, depending on when they are activated or deactivated.

Specifically, we studied the effect of Ras/MAPK on the LIN-31 transcription factor. The Miller lab has discovered a putative docking site on LIN-31 that tells MAP kinase where it should bind and therefore indicates that LIN-31 should be phosphorylated. Because *C. elegans* have been studied so extensively, we know they contain an anchor cell that utilizes the Ras/MAPK signaling pathway to communicate with neighboring cells and affect their development. If MAP kinase recognizes the docking site on LIN-31, it phosphorylates LIN-31 and the cells receive the proper signal to develop normally. However, we wanted to confirm that the putative docking site is indeed a significant aspect of cell fate specification because of its role in the Ras/MAPK signaling pathway. Therefore, if we can create a mutation in the *lin-31* gene at the location that codes for the docking site, we believe MAP kinase will be unable to recognize where it should bind and therefore not properly phosphorylate LIN-31.

Within *C. elegans*, the anchor cell directs cells whether or not to become vulva cells. If all of the processes occur properly, one vulva develops. If the docking site and therefore proper phosphorylation of LIN-31 is disrupted, the cells do not receive the signal to develop into a vulva at the transcriptional activation level, causing a vulvaless animal. This is a convenient phenotype used to confirm our success because as hermaphrodites, vulvaless worms will internally fertilize oocytes causing an easily visible "bag of worms."

## 1.1 Repair Plasmid

*Introduction*

In order to induce a specific mutation into the genome of *C. elegans* we first had to create a repair plasmid with the desired mutation in it. This repair plasmid served as a template for homologous recombination for the DNA strands and was incorporated into the genome of *C. elegans*. In our project we aimed to mutate the putative docking site on the LIN-31 protein. The potential docking site in the wild-type *C. elegans* contains arginine and lysine amino acids. Our repair plasmid contains the DNA that codes for two alanine amino acids. By incorporating the repair plasmid into the DNA we will disrupt this potential docking site and determine the effect on cell fate development.

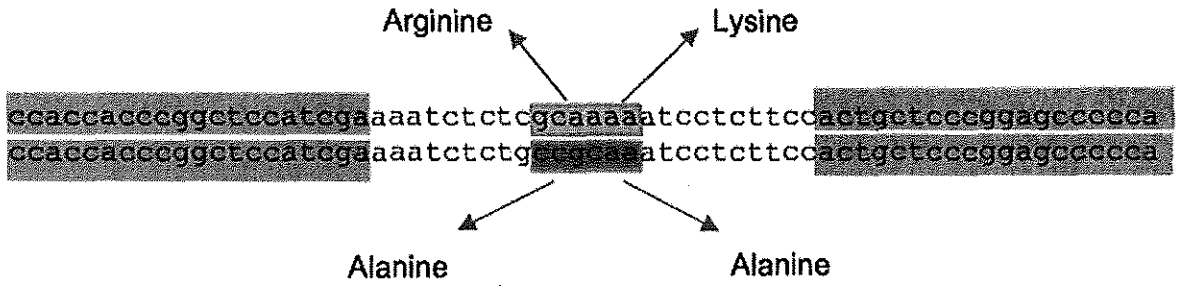


Figure 3. The original wild-type DNA and the DNA for the repair plasmid. The top strand of DNA shown is the DNA from wild-type C. elegans with the DNA for the docking site in orange. The bottom strand is from the repair plasmid DNA and contains the desired mutation. The DNA highlighted in purple is where our TALENs proteins bind.

*Details and Key Constraints*

Dr. Leilani Miller, Associate Professor in Biology at Santa Clara University, and her lab identified the site shown in Figure 3 as a potential docking site through analysis of consensus sequences. This segment of DNA was identified to be a potential docking site through consensus sequence analysis. This involves looking at DNA sequences of similar docking sites and identifying the similarities in the sequences. More specifically, other studies looked at conserved

sequences within MAP kinase targets until they determined sequences that act as docking sites by knocking them out and performing kinase assays. The Miller Lab received the information on what the docking site amino acid consensus sequences were, and then scanned *lin-31* until that site was found. The DNA (pLM 545) for *lin*-31 with these docking site mutations was already created by Dr. Miller's lab. In order to create the desired repair plasmid we performed a TOPO cloning reaction, which is seen in Figure 4.

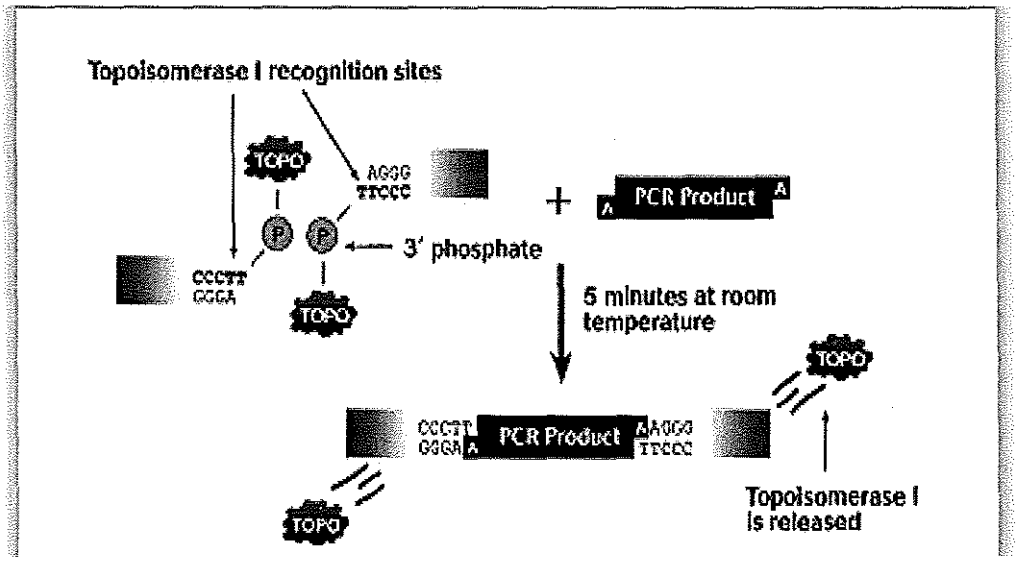


Figure 4. TOPO cloning reaction. The Topoisomerases create a break at their recognition site. The PCR product then incorporates into the plasmid at this break (New).

TOPO cloning reactions use topoisomerases to uncoil and cut the plasmid DNA. This allows the new DNA to insert itself into the plasmid before the plasmid rejoins. In order to do this we amplified a short segment of DNA containing the mutations via a PCR reaction. By amplifying the DNA, we ensured that it would be in high concentrations and have A overhangs, making it ready to integrate when the topoisomerases cut the plasmid DNA.

*Detailed Design Description*

To amplify our DNA we used the PCR protocol from NEB entitled PCR Protocol for Phusion® High-Fidelity DNA Polymerase (M0530). PCR reactions use the ability of DNA to create a new strand from a complementary strand of DNA to create thousands of new copies of the same DNA. In order to do this, primers (short, complimentary sequences at the start and end of the desired fragment), a buffer, and nucleotides are added to the template DNA and put into a thermocycler. While in the thermocycler, the contents are heated to denature the DNA and then cooled and heated to promote replication in cycles, to amplify the DNA fragment.

The TOPO cloning reaction uses blue/white selection to make screening for insertion easier. The Topoisomerases insert DNA within the LacZ gene on the plasmid. When the LacZ gene is disrupted, then no ß-galactosidase enzyme is produced. This allows for an easy screening method because when grown on X-gal and IPTG plates the colonies with an intact LacZ and no insert will be blue. The colonies in which the LacZ was disrupted and the PCR product was inserted will be white.

*Detailed Supporting Analysis*

The TOPO cloning reaction is an established practice in the field of molecular biology and has been cited in over 20,000 papers (TOPO).

*Expected Results*

As stated earlier it is easy to screen for the success of the TOPO cloning reaction because it disrupts the LacZ gene. This process is called blue white selection. From this we expect to have white colonies, which indicate that the DNA was integrated into the plasmid. After this screening we will run the DNA from the white colonies on gel electrophoresis, a technique used

to determine the size of DNA. We expect to see a single band at around 1500 base pairs. When this is confirmed we will send the DNA out for sequencing to confirm that the mutation was inserted into the plasmid.

*Back-up Plan*

We will still use TALENs and disrupt the docking site via non-homologous end-joining. When the two ends of DNA crash back together in NHEJ, base pairs are lost and the gene is disrupted.

*Materials and Methods*

For our reactions we followed two widely used protocols to determine the materials and methods. We followed the PCR Protocol for Phusion® High-Fidelity DNA Polymerase (M0530) protocol for the PCR reaction. We then used Invitrogen's guide for the TOPO® TA Cloning® Kit for Sequencing.

*Results*

After performing the PCR and TOPO cloning reactions we observed approximately 50% white colonies. The white color implied that the LacZ gene had been disrupted and the desired DNA had been inserted into the repair plasmid. On the electrophoresis gel the DNA had a single band at the expected size. This gel is shown in Figure 5. We then confirmed that we had the correct DNA in the plasmid by sending the plasmid out for sequencing. The sequencing showed that we had a plasmid with the correct mutation in it.

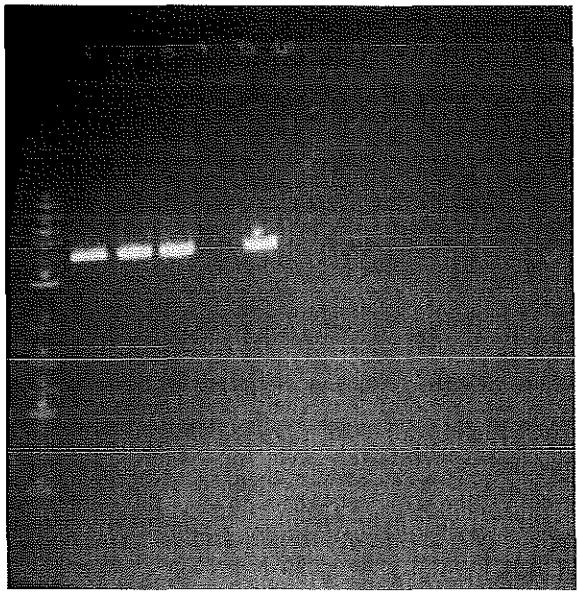

Figure 5: Electrophoresis Gel of repair plasmid. A single band around 1500bp was observed as expected.

*Discussion*

These results confirm that we created a repair plasmid containing the desired mutation we hope to induce into the *C. elegans*. After the TALENs and repair plasmid have been microinjected into the *C. elegans*, it can be determined if the specific mutation was induced in the *C. elegans* progeny by comparing the sequencing results to the mutant sequence in the repair plasmid. This is an important step in the genetic engineering process, because it demonstrates TALENs ability to do gene replacement.

## 1.2 TALENs DNA and mRNA

*Introduction*

TALEs are proteins discovered from the plant pathogen *Xanthomomas.* These TALE proteins are able to recognize and bind to specific sequences of DNA (Joung).

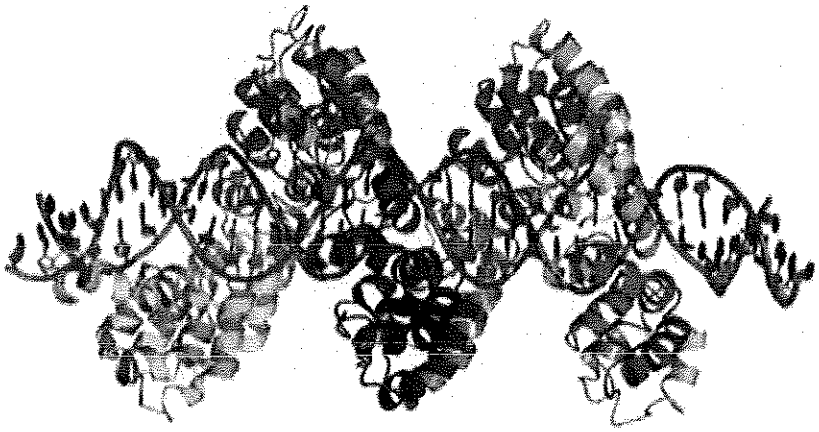

Figure 6: TALE Binding protein ("TALEN").

Specifically, TALE proteins contain a DNA-binding structure made up of repeating loops of 33-35 amino acids that allow the protein to bind to a specific DNA sequence. Each loop varies with only two amino acids (Joung). This variety in amino acid loops determines which DNA base pair the TALE will bind to. This is seen in Figure 7.

Figure 7: TALE binding loop bound to a specific DNA nucleotide ("TALENs for").

The users determine the order of the individual TALE loops (RVDs), and the order determines where the TALENs protein will bind. Each RVD is specific to a single base pair of DNA. RVD NH binds to G, NI binds to A, HD binds to C, and NG binds to T (Joung). Therefore, the order in which the RVDs are linked together determines where the TALENs will

bind, allowing for targeted genome editing. It is necessary to design two TALENs proteins, one that can bind to the top strand of DNA and one that can bind to the bottom strand of DNA. The FokI nucleases of each of these TALENs then work together to create a double-stranded break in the DNA sequence; this is seen in Figure 8.

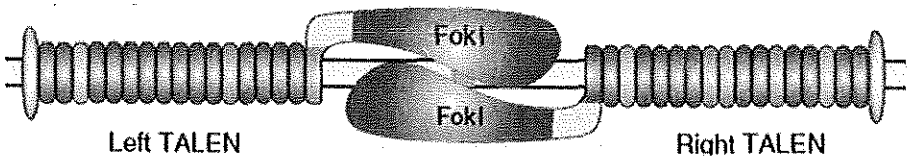


Figure 8: TALENs proteins (Sanjana).

Figure 8 shows the two domains that make up the TALENs proteins, the DNA binding domain and the nuclease. This DNA binding domain of the TALENs protein is fused to a FokI nuclease. The TALE part of the protein recognizes and binds to the DNA sequence of the user's choosing, and the nuclease forms a dimer to create a double stranded break. Once this break is created, the cell is prompted to repair itself by homologous recombination with the template provided by the repair plasmid.

*Details and Key Constraints*

In order to create the double stranded break at the specific location, two TALENs proteins must be made. One TALENs binds to the top strand of DNA and the other binds to the bottom strand of DNA. The nucleases of these two proteins then dimerize and induce a double stranded break at the specific site. In order to create the two TALENs proteins, first the DNA

that encodes for these subunits must be put in the correct order. This is done *in vitro*. After the TALENs DNA is made, it must be transcribed into mRNA. This mRNA, along with the repair plasmid, can then be microinjected into the *C. elegans*. The ribosomes of the *C. elegans* will then translate the TALENs mRNA, creating TALENs proteins *in vivo*. These proteins can then target the specific DNA sequence and induce a double-stranded break, which can then be repaired by the repair plasmid.

*Detailed Design Description and Analysis*

Design of the TALENs DNA is done by choosing the specific DNA sequence where you would like to induce a double-stranded break, then customizing the TALENs binding domain to target the chosen DNA sequence.

All components necessary for making TALENs are supplied in the Golden Gate TAL Effector TALENS Kit 2.0, which contains a library of plasmids. Each of these plasmids containing an RVD sandwiched between two type IIS restriction enzyme recognition sites.

Type IIS restriction enzymes cut DNA at short distances from the recognition site, creating a break with a sticky end- single strand overhang as shown in Figure 9.

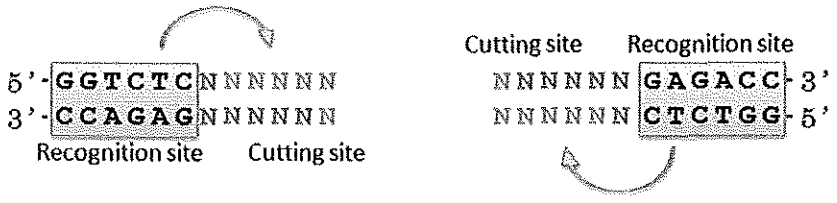


Figure 9: Type IIS Restriction Enzymes ("Illustrations").

These sticky ends allow the subunits to be assembled in the correct order: the overhang for subunit 1 of the protein will only link together with the overhang of subunit 2. Therefore,

when all the subunits are digested together with a type IIS restriction enzyme and allowed to undergo a digestion and ligation reaction, all the different subunits are ligated together in the desired order. This allows for the design of a TALENs protein that will target the sequence of the user's choosing.

After all of the different RVDs are linked up in the digestion and ligation reaction, the TALENs DNA plasmid has been created. This DNA is then transformed into *E. coli*. Transformation is a molecular biology technique that allows for the insertion of foreign plasmids into the bacteria. The bacteria then amplify the plasmid to create large quantities of it.

Colony PCR allows for the analysis of DNA sequences, and allows us to determine the success of our TALENs creation. In the colony PCR reaction the DNA is amplified and can then be analyzed by gel electrophoresis and DNA sequencing. Gel electrophoresis is a technique that allows for the determination of the size of a segment of DNA, and DNA sequencing allows for the determination of the exact base pair sequence of a segment of DNA.

*Results*

It was expected that our gel electrophoresis results would show a band which was approximately 1200 base pairs in length because our group knew the size of all the individual components which made up the TALENs DNA. Our group was able to obtain our expected results, as seen in Figure 10.

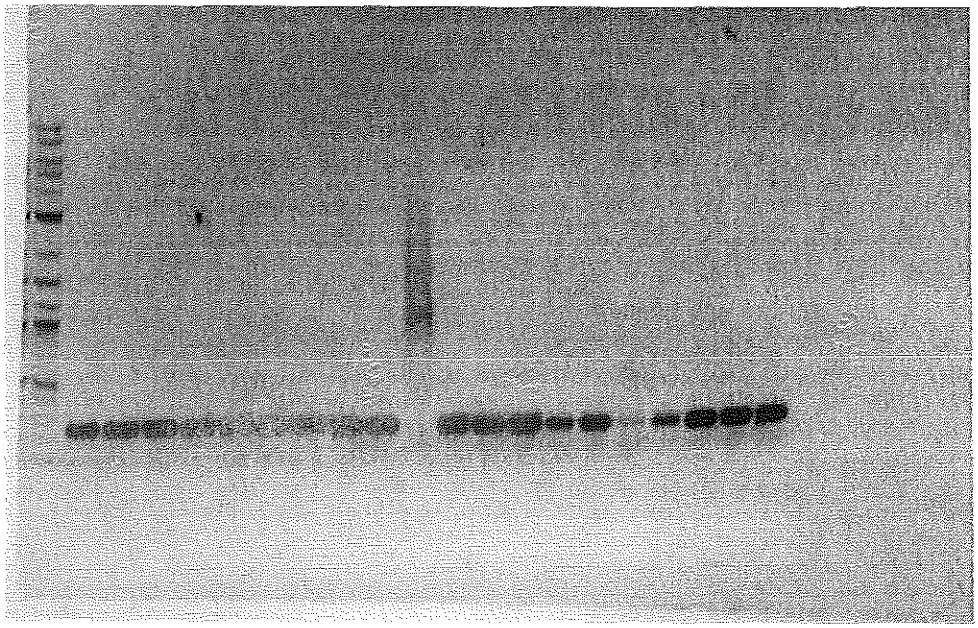

Figure 10. Gel Electrophoresis results of TALENs DNA.

After determining that our TALENs DNA was the right size, our next step was to determine the RVDs had linked in the correct order. This was done by sending the TALENs DNA out for sequencing. Because our group knew the DNA sequence of each of the individual RVDs, we were able to determine what the DNA sequence of the TALENs DNA obtained from colony PCR should be. We compared the expected DNA sequence and the DNA sequence obtained from the sequencing company and found that our results matched the expected DNA sequence. This indicated we had been successful in creating TALENs DNA whose RVD components were linked up in the order we desired.

Our group has successfully created the TALENs DNA and is currently finishing the final steps of preparing the TALENs DNA for microinjection. When creating the plasmids that contain the TALENs DNA, only 10 subunits can be linked together in one reaction. The DNA we are creating contains 19 subunits. Therefore, creating the full length TALENs DNA plasmid

is a two step process. The first step involves combining subunits 1-10 in one reaction and subunits 11-19 in another. This creates the two halves of the TALENs DNA. The second step combines these two halves together, creating a DNA plasmid containing the full length TALENs DNA. Our group was able to successfully create the four halves of TALENs DNA necessary for our project.

*Back-up Plan*

Our group's back-up plan was to repeat the experiment, troubleshooting various components of the reactions, until we were able to successfully create the TALENs DNA. This would allow us to optimize the process of creating the TALENs DNA until we could successfully create it. Additionally, we have maintained a detailed notebook for future student researchers to follow, which allows for the continuation of the project.

**1.3 Microinjection**

*Introduction*

Once we have successfully transcribed our TALENs DNA into mRNA with the *in vitro* transcription kit, we will be microinjecting the mRNA along with our repair plasmid into the gonad of the *C. elegans*. Microinjection is defined as "the use of a glass micropipette to inject a liquid substance at a microscopic or borderline macroscopic level" (Evans). This can be seen in Figure 11.

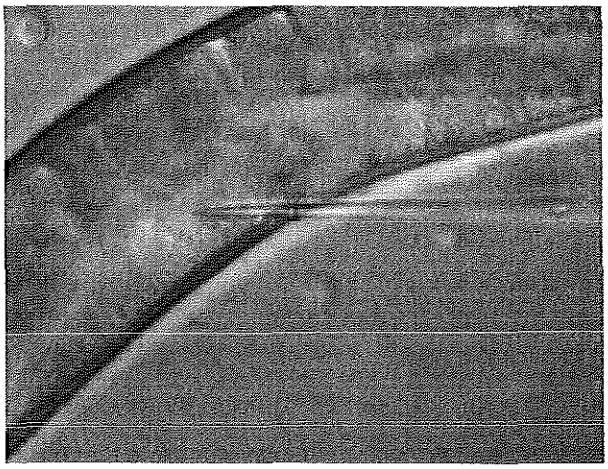

Figure 11: Microinjection into *C. elegans.*

*Details and Key Constraints*

The *C. elegans* are one millimeter in length and therefore the injection process must be precise. A high resolution-inverting microscope with a microinjecting device to hold the micropipette in place is used.

*Detailed Design Description and Analysis*

Once inside the gonad of the *C. elegans,* the developing oocytes will take up the DNA and mRNA, and their ribosomes should then translate the TALENs mRNA into TALENs protein. The TALENs protein can then target and bind to the desired sequence on the *lin-31* gene and create a double stranded break at the potential docking site. The ends of our repair plasmid should be complementary to the break in the genome and hopefully integrate into the *C. elegans* DNA through homologous recombination.

If the repair plasmid is able to successfully integrate into the genome at the created double stranded break, the *C. elegans'* progeny should have the mutated version of the *lin-31* gene. The docking site on LIN-31 will be altered, making MAP kinase unable to recognize it as a

phosphorylation target. The surrounding cells will then not receive the correct signals and a vulva will not develop. Because *C. elegans* are hermaphrodites and contain both sex organs, the progeny will develop and be unable to escape through the vulva. We will then observe a "bag of worms," or an adult worm with live progeny inside, this is shown in Figure 12.

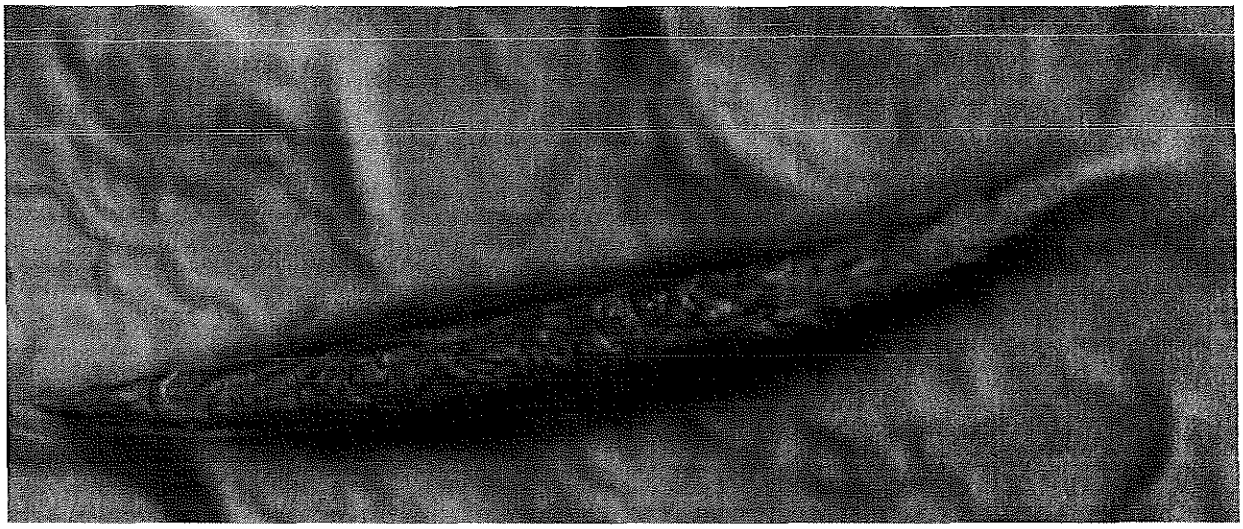

Figure 12: Bag of worms phenotype.

*Results*

Unfortunately, we were unable to reach this step in our experiment before June. However, once we are able to successfully create TALENs mRNA from our TALENs DNA, we believe this process will be straightforward and easily accomplished. We should be able to obtain results fairly quickly because of the short maturation cycle of the *C. elegans.*

### 1.4 Screening for Results

*Introduction*

If the activity of LIN-31 is disrupted, unspecified cells in *C. elegans* do not receive the signal to develop into vulva cells and the organism will display the vulvaless phenotype. This is

a convenient phenotype to use to confirm our success because it is easily visible. As seen in Figure 13, it is easy to distinguish between vulvaless and wild type *C. elegans*.

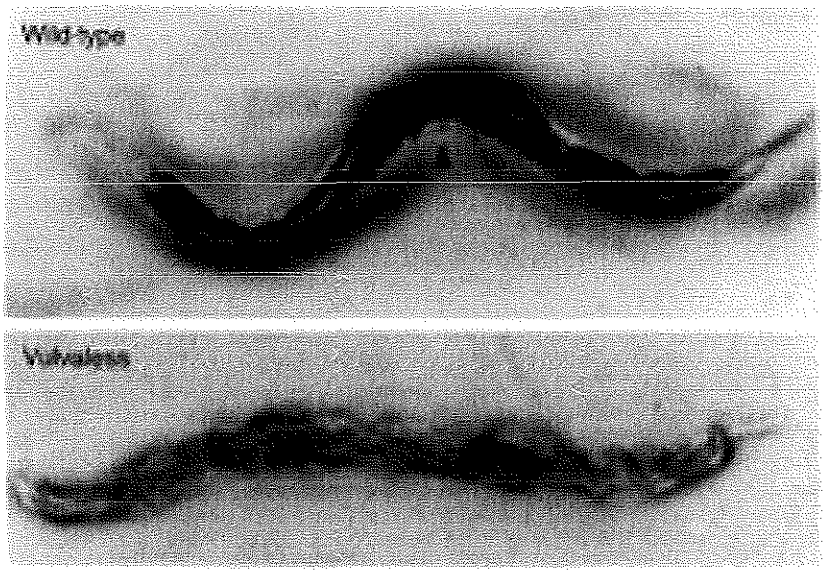


Figure 13: Comparison of *C. elegans* phenotypes (Rawat).

It is expected that the *C. elegans* that were not mutated by TALENs will display the wildtype phenotype, while the mutated *C. elegans* will display the vulvaless phenotypes. These phenotypes will be determined by visualizing the organisms under a dissecting microscope.

A successful mutation will then be confirmed by analyzing the DNA of the vulvaless *C. elegans*. This will be done by sending out their *lin-31* DNA for sequencing. The DNA sequence of a successful mutation will contain the repair plasmid sequence inserted at the cut site on *lin-31*; this is shown in Figure 14.

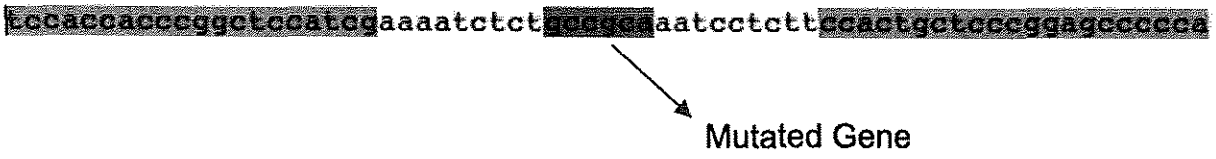


Figure 14: DNA sequence containing the desired mutation of *lin-31*.

*Details and Key Constraints*

Each animal is injected and allowed to grow for several days on Nematode Growth Media plates at 20-25 degrees. After that time the injected *C. elegans* should have produced offspring that are now old enough to be screened for phenotypes under a dissecting microscope. The *C. elegans* with the correct phenotype are picked and lysed for their DNA. This DNA is then amplified with PCR and sent out for sequencing.

*Detailed Design Description*

By mutating the *lin-31* gene in *C. elegans* we are affecting the vulval cell development in the organisms. *C. elegans* are hermaphrodites and therefore able to fertilize internally. When this occurs in a vulvaless *C. elegan* the progeny are unable to escape and therefore begin consuming the original organism. This results in the phenotype commonly referred to as a bag of worms. This can be seen above in Figures 12 and 13.

*Detailed Supporting Analysis*

*C. elegans* have been studied extensively and shown to have many different mutant phenotypes. Due to their self-fertilization capabilities they allow us to study cell fate determination by their ability or inability to develop vulvas and the visible phenotype occurs as a

result. The vulvaless phenotype has been induced through other methods of mutating the genome of *C. elegans* such as exposure to radiation.

*Expected Results*

For our results we would expect to see *C. elegans* with the vulvaless phenotype. When the DNA from these *C. elegans* are sent out for sequencing we would expect them to contain the *lin-31* sequence shown in Figure 14 that codes for the alanine amino acids in the place of the arginine and lysine residues in the potential docking site.

*Back-up Plan*

If the progeny do not display the expected phenotype, we will run a Cel-1 assay. The Cel-1 assay allows us to screen many *C. elegans*, with any mutation, at one time. Cel-1 is an enzyme isolated from celery that cuts mismatched DNA. We would expect that the pools of C. elegans that contain individual *C. elegans* with mutations would show mismatches when lysed and the *lin-31* gene is amplified through PCR. The PCR product from the pools of worms is then incubated with Cel-1, run on an agarose gel, and the fragment is cut. We would see three bands, indicating that at least one of the worms in the original pool contains a mutation. The three bands are only present when the mutated DNA creates a mismatch with the wild type DNA. From there, individual worms can be mixed with wild type worms, lysed, amplified with PCR, and incubated with Cel-1 to determine which individual worms contain mutations. Then, these mutant PCR products can be sent for sequencing.

*Materials and Methods*

For this step we will require a dissecting microscope and the microinjected *C. elegans* and their progeny. In order to isolate the DNA for sequencing from the *C. elegans* the worms are placed in a buffer containing Proteinase K, and heated in the thermocycler. This lyses the worms, releasing their DNA, which is then used in a PCR reaction to amplify the docking-site coding fragment of the *lin-31* gene.

*Results*

Until the repair plasmid and TALENs are injected we cannot complete this step. However this step is straightforward and will take little time to complete.

*Discussion*

If this step is successful it will not only confirm that TALENs is an effective technology for genome editing, but also support the research done to prove that the sequence targeted in this experiment codes for a docking site on LIN-31. This has the potential to contribute to the research on TALENs as well as research on LIN-31 and the Ras/MAP kinase pathway.

## Summary and Conclusion

TALENs, transcription activator-like effector nucleases, are a genetic engineering technology that can be used for targeted gene modification. For our senior design project we used TALENs to explore their potential for use in gene therapy. Gene therapy is an exciting field as it provides the potential to cure many genetic diseases. Gene therapy offers the potential for a cure to these diseases because it can be used to permanently repair the genome. Specifically, our project with TALENs will provide a service to society as it will give rise to a better understanding of the technology and its uses as a genetic engineering research tool.

Over the course of our senior year, we have been able to successfully construct TALENs DNA that codes for a protein that will bind specifically and cut the genome at our desired location. We have validated the ability to create customizable DNA binding domains designed by the user. The materials and methods involved in creating TALENs are fairly common and straightforward. A majority of laboratories have all of the necessary facilities and knowledge to utilize this technology. Such accessibility is testament to the relevance and future of the TALENs technology. We have high hopes that our work will lead to further exploration of TALENs at Santa Clara University and that researchers will continue to improve TALENs for its use in gene therapy.

## Engineering Standards and Realistic Constraints

### Health & Safety

*Current Concerns*

When evaluating the health and safety concerns associated with the TALENs technology it is important to consider them in the context of our project as it stands currently and in the context of future applications in gene therapy. Currently our own safety is the greatest concern. While working with any toxic chemicals or dangerous microorganisms it is important that we are always using good lab technique to protect ourselves and those in the lab around us. Hazards in our lab include ethidium bromide (a known carcinogen), *E. coli* bacteria, flammable chemicals, and UV light. It is important that we always use the appropriate precautions such as wearing gloves and disposing of all materials properly.

*Future Concerns*

When considering the safety and risk involved with future treatments for patients using TALENs there are many. It is important that we currently document all observations so that when the treatment is being tested in humans, we can provide them with as much information as possible. Informed consent requires that the patients and doctors know the risks involved with the treatment. In order to fully explain the risks, there has to be good documentation throughout the entire development, especially in regards to any adverse or unintended effects. While all clinical trials involve risk, it is important that all possible precautions are taken to minimize these risks before they are tested in humans.

The need for precautions in using genetic engineering technologies is highlighted by a recent publication about targeted genome editing; specifically, CRISPR (a genetic engineering technology similar to TALENs) was used to edit the genome of non-viable human embryos (Liang). This research team attempted to edit the gene that causes β-thalassaemia, a genetic mutation involved in blood disorders (Cyranoski). Eight-six embryos were used in this experiment, and only four contained the correct mutation (Cyranoski).

Further data analysis indicated that many of the embryos used in the study contained off-target mutations, meaning that CRISPR had modified the embryo's genome in an undesired location. This is a major health and safety threat because these mutations could be harmful. This study highlights the health and safety concerns surrounding these genetic engineering technologies because they do have the potential to mutate the genome in undesired locations. Therefore, further research needs to be done to ensure that these technologies only mutate the

genome at a specific, targeted location, minimizing the health and safety risks associated with the technology.

## Social Considerations

### *Introduction*

The ability to specifically target a desired location of the genome is an incredibly powerful tool with the potential to be developed for numerous applications. The application of gene therapy is an honorable one that aims to provide relief to victims of genetic disorders. Proponents argue that if we have the ability to prevent excruciating pain then we should be obligated to do so, while opponents believe gene therapy is a slippery slope to genetic enhancement. Whatever the case, many other considerations need to be taken into account that will ultimately affect social perceptions of gene therapy. For example, if gene therapy becomes a viable option in the future, should women be required to receive genetic screening? Is it wrong for a family to bring a child into the world if they know his or her life will be filled with suffering? This brings up the question of when exactly such testing should be performed. Also, if people or companies have the ability to alter a genome, are they creating new patentable life forms? The Supreme Court decision, *Diamond vs. Chakrabarty*, granted permission for just that (Ramsdale). To many it seems wrong that anyone can own the rights to life.

### *Future Directions*

Society ultimately needs to decide whether or not to pursue technology used for gene therapy and how it will be done. Many members of the scientific community believe the FDA and RAC are too restricting and discourage progress and advancements. Others are fearful that we are pursuing dangerous technologies too hastily. The public will need to decide for itself and

make crucial decisions with little guidance or technical understanding. It is therefore important that we remain cautiously optimistic of technology that has the potential to revolutionize science and the way we approach treating genetic diseases. With so many variable outcomes, it is not unwise to consider what exactly such a powerful tool can bring about.

## Sustainability

### *Introduction*

The science of sustainability involves "examining the interactions between human, environmental, and engineered systems to understand and contribute to solutions for complex challenges that threaten the future of humanity" (Clark). Our project aimed to impact this field by exploring a technology that has the potential to provide permanent solutions to debilitating genetic disorders. Because the TALENs technology has a DNA binding domain attached to a DNA cleavage domain, the user is able to create a protein that can specifically target and create a double stranded break at any desired location. The ability to target an exact location allows for efficient and repeatable results.

### *Sustainability and TALENs*

Since the TALEs used in the TALENs technology are designed after those found in the bacteria *Xanthomonas*, the TALENs technology is an efficient and accurate resource. The TALEs recognize DNA sequences based on RVDs, or two amino acids found within the longer repeat sequence from which they are coded. The genetic constructs that encode for the TALEs are produced by gene synthesis or modular assembly. These techniques are considered economical and are offered by many companies worldwide. It is therefore relatively easy to both create and obtain a library of TALEs. The organization Addgene, provides a plasmid kit

containing a library of TALEs for the creation of customizable TALENs proteins. If the technology were to be further developed and validated, the creation of personalized TALENs would be a sustainable and desirable option.

## Manufacturability

Our project involved exploring the potential of TALENs for as a research tool. In order to ensure the integrity of our research and design project, the whole group was trained in good laboratory practices. This included keeping a detailed lab notebook and recording all data and results. Each member of the group analyzed data separately and then compared our findings. This helped ensure our data's accuracy, allowing us to ensure the integrity of our research.

Keeping a detailed lab notebook will make the transition to manufacturing TALENs easier. If our project proves successful, eventually many different TALENs could be manufactured for gene therapy. This process will require a scale-up of our reactions to industrial levels. However, we have figured out the necessary concentrations for all our reactions, making this scale-up process easier and more feasible.

## Ethical Considerations

### *Introduction*

The presidential commission for the study of bioethical issues developed a framework for addressing developing technologies such as gene therapy. Their main focus is on defining and applying the concept of responsible stewardship. Responsible stewardship is the responsibility of us all to act in a way that demonstrates concern for those that cannot represent themselves, such as children, the environment, or the future. This means ensuring we are fully

aware of the risks and consequences of our actions on others. The commission's attitude towards addressing new technologies coincides with the Markkula Center for Applied Ethics' Framework for Thinking Ethically.

The Center's approach begins with recognizing an ethical issue. The technology of gene therapy is a rapidly growing field that has the potential to prevent, treat, or cure genetic diseases. While this capability would allow for the treatment of horrible diseases such as cystic fibrosis or sickle cell anemia, if analyzed ethically, it may seem too good to be true. Gene therapy could provide a solution for those suffering from debilitating illnesses, but it could also allow people to alter undesirable traits or create designer babies.

This idea raises many ethical questions. Should people be able to use genetic engineering to fix any trait or characteristic they are unsatisfied with? Where is the line drawn between someone that wants to use gene therapy to treat muscular cell dystrophy and someone that wishes they were genetically more capable of building muscle? The amazing capability of gene therapy to do good could quickly create unforeseen consequences and change the world in which we live. Parents would have the ability to alter their children, threatening their autonomy. According to Kantian ethics, because humans are autonomous agents and capable of making rational decisions, we all deserve dignity and respect. Autonomy gives everyone the right to make fully informed decisions about what happens to them and whether or not they want to participate in medical research. Altering a child's genetic information strips them of this choice. It is important that we recognize this and consider all possible outcomes and ethical responses. Although the gene therapy technology is nowhere near this advanced, by looking ahead we will be prepared if human experimentation becomes a possibility.

Another question raised by the TALENs technology is: whether or not the ability to alter an organism's genome is playing God. If the technology continues to develop, it is likely to be used to treat human diseases. One argument states that human DNA should be off-limits to any tampering. We should not interfere with natural selection or challenge our relationship with nature. If we allow pride or arrogance to cloud our judgment, we will lose reverence for life. An opposing argument believes that if we have the capability to cure horrible diseases and ease potentially excruciating pain we have the responsibility to do so. The solution is in finding a balance between these two viewpoints. Using genetic engineering to alter a person's basic traits would be an abuse of the technology. The technology should be respected and used only to provide relief to those suffering from terrible illnesses. Acknowledging the sacredness and privilege of life will ensure that such a powerful tool will not be abused.

*Evaluation of Alternative Actions*

However, before we can realistically predict the development of gene therapy technologies, we need to ensure we have a deep understanding of the technology and its potential. Judgment cannot be made upon something that is not fully understood. Before committing to using TALENs as the chosen form of genetic engineering, a full analysis of competing technologies should be done in order to either discover a more ethical method or confirm that TALENs is the best option. Some of the competing technologies include retroviruses, zinc finger nucleases, and clustered regularly interspaced short palindromic repeats (CRISPR). TALENs outcompetes each of these technologies because it has less off target effects and is therefore the safest option. Off target effects refers to the genetic engineering tool (such as CRISPR) integrating at the wrong place in the genome. The consequences of this could be catastrophic because the technology could alter the genome in an uncontrolled manner, causing

unknown effects on the genome. This occurred in a clinical trial that aimed to use retroviruses to treat severe combined immunodeficiency. In this trial, eleven children were given retroviral treatment, and nine recovered. However, of these nine children, four developed leukemia. It was discovered that the retroviruses had randomly integrated in the genome and turned on an oncogene, giving the kids cancer (Check). This highlights the need for the development of new approaches to gene therapy, specifically approaches which are safe and effective.

From a utilitarian standpoint, TALENs would be the obvious choice since it can accomplish what the other technologies do more effectively and in a less harmful manner. It provides the most hope for furthering the field of genetic engineering because it is targeted, efficient, and safe.

*Choice of C. elegans*

Our project aimed to demonstrate the safety and efficacy of TALENs by using the technology in a model organism, nematode *C. elegans*. This allowed for the study of the safety of TALENs without experimentation in humans. Additionally, it will give the scientific community a better understanding of the technology, which will aid in determining regulation as to what TALENs can and cannot be used for.

Even though the use of a model organism prevents experimentation in humans, there are ethical concerns that must be taken into consideration when conducting research on living organisms. *C. elegans* are considered a model organism in the scientific community because they are one of the simplest systems to possess a nervous system, they can be frozen and thawed without damaging the organism, and they possess a quick reproduction rate (Comstock) . Therefore these nematodes are well studied and have been used in a variety of scientific

experiments. However, there are some questions which must be addressed when conducting research on live organisms. These include: Will the experiment create an unnatural organism?, Who takes responsibility for the organisms used?, and How will the organisms be treated during the experimentation?. Our group carefully considered the implications of each of these questions before moving forward with our project.

We determined that yes, if successful, our project will create an unnatural organism. We used TALENs to mutate the *lin-31* gene of *C. elegans* in order to learn more about cell fate specification in a RAS/MAPK signaling pathway. Therefore our project aimed to create *C. elegans* with a mutated signaling pathway. We determined that the benefits gained from learning more about this signaling pathway and demonstrating the safety and efficacy of TALENs outweighed the negative consequences of creating a mutated organism.

Our group also addressed concerns of who would take responsibility for the organisms used in our experiment and how they would be treated. *C. elegans* are organisms that respond to stimuli and thus could be subject to inhumane laboratory practices. We addressed these concerns by choosing an advisor who is well experienced in working with *C. elegans*. Our advisor has many years of experience in working with *C. elegans* and therefore was able to train us in proper handling of the organisms. Our group was trained to use *C. elegans* in a manner that minimizes the pain the organisms could experience and ensured that the organisms are not subjected to inhumane laboratory practices. Both our senior design group and our advisor ultimately took responsibility for the organisms used in the experiment and therefore committed ourselves to conducting our experiment in accordance to good lab techniques.

*C. elegans* do have a nervous system and therefore could respond to pain caused by improper handling. However their nervous system is not highly developed and the level of pain would be minimal. Additionally, our project itself does not cause pain to the *C. elegans* as the TALENs mRNA is microinjected into the organisms and then the *C. elegans* ribosomes are used to produce the TALENs protein *in vivo*. Microinjection is a process that uses a very thin needle to inject mRNA into the *C.elegans*, the needle is so thin that it can barely be felt by the organisms. After microinjection, our experiment simply involved microscopically monitoring the development of the *C. elegans*. This meant the *C. elegans* would experience minimal handling by our group. This allowed our group to ensure that the organisms were humanely treated and allowed our group to take responsibility for the organisms that we genetically altered.

*Future Implications*

Once the technology has been thoroughly assessed and all potential outcomes considered, a choice needs to be made about whether or not to continue developing the technology. The presidential commission for the study of bioethical issues created a method for approaching this decision making process called prudent vigilance. Prudent vigilance is the need to continue assessing the benefits and risks of a technology, both before, during, and after its development. As TALENs is explored and implemented, experimental results will provide further information about the efficacy and safety of the technology. Based off of this data, we can reevaluate the direction and goals of the technology and our project. In this manner, scientific advancement will not be hindered but it will also not progress unchecked. Continuing to evaluate the ethical issues of a project as it develops ensures it will not take an undesirable direction and that the community stays informed and involved.

*Team and Organizational Ethics*

We upheld distributive justice by equally distributing the work between the members. One way we ensured that the work was distributed evenly was by everyone scheduling the same number of hours in the lab. We also tried to ensure that there were at least a few hours in which we were all present in the lab together, so that everyone could have a voice in the decisions. In this way we used technology to assist us in communication between our members. We each prepared our own lab notebooks as well as created a Google doc with the main experiments for the day so that each member knew what had been done, and what still needed to be done. For resolving any issues and working to uphold the common good of the group we established that if any conflict between members arose we would have a conversation and resolve the issue ourselves. If it could not be resolved between two members we would bring the other member of the group in to help address the problem. As a last resort we would speak with the advisor if an agreement could not be made between group members.

In our project it was important that we analyzed the contributions that everyone made to the project, including our advisor and lab technician. Our team had the privilege of working with Dr. Miller, our advisor, and her employees to contribute to their research. When preparing our report we ensured that we gave credit to the other members of the lab for their contributions to the project, taking into account the justice and fairness of our project. All the reports were written and prepared by our team members. In addition we wanted to give credit to the previous researchers who provided the framework for us to do our work. Many of these researchers are included in our works cited, however two groups were especially important in our research. The first group was Carson Harms and Serena Lertkantitham who worked with TALENs in the Miller

lab the previous year and laid the framework for our project. The second group was the Voytas lab group. The Voytas lab pioneered the TALENs technology and produced the kit we used.

As demonstrated by our continuation of the project from the previous year it was important that we maintained detailed documentation of our work. One of the virtues possessed by good scientists and engineers is the ability and dedication to habitually document their work. Our team aimed to be thorough in describing our work, so that future scientists and engineers could use our work. In our project the goal was to prove the efficacy and safety of TALENs, and this could only be achieved with thorough, detailed reports. In addition all results and data were accurate and true. No data was falsified or exaggerated to support our hypothesis.

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# Appendix

Table 2. Modified Gannt Chart.

| Task description | Start date | Finish date | Progress | Resources |
|---|---|---|---|---|
| | | | | |
| Complete Lab Safety | 9/30/14 | 10/2/14 | completed | |
| School of Engineering Grant Proposal | 10/2/14 | 10/10/14 | completed | |
| Identify target Sequence and RVD | 10/20/14 | 10/20/14 | completed | TAL targeter |
| Make Repair plasmid | 10/27/14 | 10/31/14 | completed | pLM 545 |
| Golden Gate reaction 1-1 | 10/27/14 | | completed | RVD colonies |
| Golden Gate reaction 1-2 | 10/27/14 | 11/18/14 | completed | RVD colonies |
| Golden Gate reaction 2-1 | 10/27/14 | | | RVD colonies |
| Golden Gate reaction 2-2 | 10/27/14 | 11/7/14 | completed | RVD colonies |
| Check 1-1 by colony PCR | 10/31/14 | 1/23/14 | completed | Taq supplies |
| Check 1-2 by colony PCR | 10/31/14 | | | Taq supplies |
| Check 2-1 by colony PCR | 10/31/14 | | | Taq supplies |
| Check 2-2 by colony PCR | 10/31/14 | 11/7/14 | completed | Taq supplies |
| Check 1-1 by sequencing | 1/26/14 | | | primers |
| Check 1-2 by sequencing | | | | primers |
| Check 2-1 by sequencing | 1/26/14 | | | primers |
| Check 2-2 by sequencing | 1/14/15 | 1/15/15 | completed | primers |
| 2nd Golden Gate for plasmid 1 | | | | golden gate primers and colonies |
| 2nd Golden Gate for plasmid 2 | | | | golden gate primers and colonies |
| Check plasmid 1 | | | | |
| Check plasmid 2 | | | | |
| Make TALENs mRNA for 1 | | | | |
| Make TALENs mRNA for 2 | | | | |
| Inject mRNA | | | | |
| Pick correct phenotypes | | | | |
| Preform Cel-1 assay | | | | |
| Sequence correct C. elegans | | | | |
| Write Thesis Intro | | | completed | |
| Write Thesis Body | | | completed | |
| Complete Thesis | | | completed | |
| First Draft of Presentation | | | completed | |
| Final Presentation Complete | | | completed | |