

# Functional Analysis of FD36 in *A. thaliana*

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2017 Borlaug-Ruan International Internship  
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## Table of Contents

Acknowledgements	3
My Story	4
Host Institution and Colleagues	5
Abstract	6
Introduction	7
MADS-box	7
Justification of Research of FD36	7
Explanation of Approach	7
Objectives	8
Research Methodology	9
Experimental Overview of CRISPR/Cas System	9
Part 1: DNA Extraction and Cloning of WT	11
Part 2: RNA Extraction and Inquiry into Expression	11
Part 3: CRISPR/Cas9-Mediated Mutation of FD36	12
Research Analysis and Contributions to Food Security	15
Results	15
Next Steps	16
Contributions to Food Security	16
Reflection	17
Photos	20
References	21

## **Acknowledgements**

First, I have to thank God for guiding my life in a way that brought me to this internship and this research. Never once this summer was it easy... to understand the experiment I was chosen to do, to adapt to what life was like in a city of 23 million when I had grown up near a town of only 457, and to become comfortable living in a place where English and English customs were scarce. However, I know that this experience will be never be something I forget and, even though it was difficult, it was also positively life changing.

This experience would only be possible through the hard work and dedication of the members of the World Food Prize Foundation, and I have them to thank for making this opportunity available for those who want to invest their lives in the betterment of others. I must thank especially Ambassador Quinn, a man whom I admire for not only his faith in the world but for his deep understanding for what it takes to change it. As an aspiring biochemist with interests in medicine, not agriculture, I am eternally grateful for the support the foundation showed me by allowing me to learn from this internship. What I have gained here has transcended plant molecular biology and given me inspiration for how I may apply my passion for medicine and health to those who are food insecure and otherwise disadvantaged. The World Food Prize has, indeed, shown me what I want to do with my life, and for that there are not enough words of thanks.

I must also thank those who were integral to my academic experience at Peking University: Dr. Li-jia Qu, Dr. Hongya Gu, and Ms. Li Zhang, who were my ever-patient mentors during my stay. Their passion, knowledge, and steadfast belief in their research was inspiring.

Last, but certainly not least, I thank the students at Peking University who dedicated their valuable time toward making my stay in China as memorable as possible: Dongshu, Yihao, Xue Fei Wang, Jiaying Huang and more. No matter the question or the problem, they were always willing to assist. My experience here would not have been the same without their support and kindness.

## My Story

### A Brief Overview of the Journey Which Brought Me Here

“Today is the day. You’ve done your research. Now, you have to fit all you’ve learned into a presentation less than ten minutes long. That’s nerve wracking, but remember that you are representing more than yourself. You are representing your people and their struggles, because they can’t speak for themselves. Be passionate. Be honest. You are the voice of hunger now.” Those words by Jacob Hunter have remained with me vividly since I first heard them as an eager sophomore at the 2015 Iowa Youth Institute. I had done my research. I was full of statistics, theories, and ideas for Kenya’s fragile, inequitable education system. I had an intense interest in what I had studied but, after hearing Mr. Hunter’s words, I had my first taste of inspiration. That moment marked a shift in perspective altering my entire frame of mind going into the Iowa Youth Institute, the Global Youth Institute, and even later as I contemplated my college major and my career. What Mr. Hunter had made me realize was not that I was capable of making a difference but that it was my responsibility, and that it was the course I wanted to dedicate my life to.

So often becoming aware of the flaws in the world— hunger, poverty, inequality, disrespect of human rights, violence, and many other examples— results in feelings of helplessness and worse, apathy. It should ignite a will within for change. Fortunately for me, it did that. I entered the IYI in 2015 as an average high schooler from a very rural part of Iowa. At that time, the biggest dreams I had consisted of wanting to attend Saint Cloud State University for journalism. The idea of educating people and telling people’s stories greatly appealed to me. However, although that part of me hasn’t changed, by the time I had advanced to GYI I had completely evolved. Not only did my my expectations for myself become higher, but I also realized what I was really passionate was science. I also realized the potential for me to apply my talents and passions toward the inspiration for change that had been kindled at IYI. By the time the 2016 IYI had begun, I had shifted my focus from education to biology and chemistry. I delved into the topic of malnutrition in India like it was my doctoral dissertation, readings dozens of articles, researching every aspect of the chemistry of micronutrient deficiency. Why is this issue present in India. Who is most affected? How is the problem currently being handled? What methods or materials are readily available to enhance what is being done or change it? Most importantly, I wanted to know what opportunities could provide a sustainable solution. That research solidified my growing love of science and helped lead me to the Borlaug-Ruan Internship.

As a high schooler in rural Iowa with minimal lab experience, the chance at being able to become involved in a lab before college is slim to none. Luckily, I was able to partake in shadowing and working in my local hospital lab for some weeks over the summer of my junior year. By my senior year, though, I was ready for something more. I wanted to gain the experience that would prepare me for pursuing science and put me in a position to immediately begin doing research at a top-tier university. The Borlaug-Ruan Internship provided me that

opportunity as well as an incredible chance to explore the culture of a new country and witness firsthand the issues which I learned I wanted to spend the rest of my life fighting.

As you will read in my reflection, this internship did not disappoint.

### Host Institution and Colleagues

Peking University is, in short, a wonderfully intellectual place where collaboration and new ideas abound. The lab shared by Drs. Gu and Qu is the foremost lab involved in *Arabidopsis thaliana* genetic research in China. It is also securely known throughout the world as a successful and rigorous lab setting which produces thoughtful and impactful research in plant biology. I am blessed to have been allowed to work with such gifted people, and I cannot provide high enough praise for them.

The experiment I worked on in Peking University is under the supervision of Professor Hongya Gu., a former Ph.D graduate of Washington University in Saint Louis. She has dedicated her research to studying “systemic and evolutionary botany and plant molecular biology” as well as genetic diversity and the evolution of certain gene families. The original investigation into FD36 was inspired by her research, which, when analyzing the evolution of genes acquiring novel traits throughout evolutionary progression, resulted in finding genes related to FD36.

Her colleague and co-director of the lab is Professor Li-jia Qu, a professor of biotechnology, who completed his bachelor’s, master’s, and doctoral degrees at Peking University, graduating in 1995. From 1999 to 2000, he served as a Senior Visiting Scholar within the MCDB Department at Yale University and in 2001 became a full-time professor at Peking University. Since 2004, he has held editorial positions in many prestigious journals of botany and genetics, including *Science China*. Since his time as a PI in the biotechnology lab, he has pushed for research of a molecular examination of the reproductive process of *A. thaliana*, including pollen tube guidance by peptide signaling, receptor-like kinases, and E-3 ligases. He has additionally commandeered the research of and analyzation of *Arabidopsis* transcription factors, rice breeding technologies, and the methylation of phytohormones.

My mentor who guided me from the first day with my project was Ms. Li Zhang. She received her master’s degree from Peking University in 2000. Her current research focus involves testing the effectiveness of various CRISPR techniques using different vectors and different strains of bacteria. She has been the researcher responsible for the project involving the mutations of the FD36-related genes under Dr. Gu, and so I was taken under her wing immediately after I arrived here to begin work on mutating FD36.

## Abstract

In this experiment, functional analysis of the gene FD36 was conducted in *Arabidopsis thaliana* in order to determine the gene's role in gametophyte development, flower development, and fertilization. Previous RNA profiling by Dr. Huang and Dr. Yang revealed that FD36 is strongly expressed in the petal and ovule of the flower. However, little else known about FD36, making it an intriguing source of research among biologists. Here, CRISPR/Cas9 was used in an effort to knockout FD36, which was defined as a mutation in the gene resulting in disrupted protein production. The genome from a wild-type (WT) *A. thaliana* plant was extracted and cloned for further experimentation, and RNA expression profiling was completed. Expression levels shown by RT PCR support Dr. Huang and Dr. Yang's results. In the key point of the experiment, CRISPR/Cas9 was used to attempt to mutate FD36 and the mutated gene was transformed into a WT plant through an *Agrobacterium* flower dip. By creating a hereditary knockout mutation, whose success will be verified by sequencing the genome of the progeny produced by the seeds of the flower-dipped plants, scientists will be able to move on to answering the metathesis: are FD36 and its close relatives integral in flower development?

A phenotypic change is not expected through the single mutation of FD36, however by effectively knocking out other redundant genes it is reasonable to suggest the ability to functionally analyze the gene. If FD36 is involved in flower development and fertilization, then it holds the potential to be influential in food security through direct correlation to successful fertilization. Although existing research has not provided a homolog for FD36 outside of *A. thaliana*, it is possible and even likely that as more information becomes available a homolog will be found, potentially even in agriculturally significant crops. With the existence of a homologous gene, the research done here will provide crucial knowledge guiding scientists as they begin gene editing with the purpose of agricultural application.

# Introduction

## MADS-box

FD36 belongs to the MADS-box gene family, therefore it is necessary to discuss the significance of this categorization. The MADS-box gene family is identifiable by the presence of a conserved motif and affectionately named for the first proteins found associated with the motif: MCM1, AG, DEFA, SRF. The motif itself is a 56-amino-acid region found within the DNA-binding domains of the proteins the genes code for. The relevance of MADS-box lies both in its membership size and diversity. The motif and therefore the gene family are found in mammals controlling muscle gene regulation and yeast controlling cell type determination and mating but, most importantly, in *Arabidopsis thaliana* and in other plants MADS-box plays an integral role in the transcription factors involved in flower organ development (Shore & Sharrocks). Transcription factors are conserved proteins (functionally independent from the protein they may be bound to) which bind to DNA to regulate the expression of the gene. This process is known as transcriptional regulation as it involves the suppression or enhancement of transcription, often through manipulating the ability of RNA polymerase to bind to DNA. Therefore, the role of MADS-box in flower organ development is quite vast. These genes have been shown to control everything from development timing to full organ development. Consequently, this makes MADS-box genes, of which FD36 is one, absolutely necessary for proper fertilization to occur.

## Justification of Research of FD36

This lab is interested in the function of FD36 based on the experimental data of Dr. Yang's thesis "Origination and Neofunctionalization of New Genes of MADS-box Family in *Arabidopsis thaliana*," which indicated that the gene was highly expressed in the ovule of the flower. Referenced in that paper was information by Dr. Huang, who states that FD36 is also highly expressed in the petal. The gene's presence in the reproductive organs of the plant indicates that it plays a role in flower development and fertilization. This is relevant when looking into the possibility of breaking species isolation. *Arabidopsis*, along with many other plants, is self-pollinating, meaning that it has a complex mechanism put into place to prevent cross fertilization from other species. If it could be determined how to dismantle or otherwise overcome this barrier, hybridization could be completed with ease. This opens doors to new genomic editing possibilities as well as significantly simpler ways to edit the genome of the plant.

## Explanation of Approach

The most standard method of deducing the function of a gene is most simply done through the observance of the phenotypic consequences of mutations. Mutations completed in the lab using various mutagens drastically change the way DNA is read by altering the amino acids being originally coded for either by indels (insertions and deletions) or by covalent bonding and other disruptive methods of interrupting how the sequence is read by RNA. This causes a change in the

protein produced by the gene, usually resulting in the deficiency in a certain protein that, in turn, alters a metabolic process, rendering the gene unable to perform its purpose (nonfunctional). If all other redundant genes are targeted and mutate successfully, then this process will produce a phenotype. In the study of FD36 and its redundant genes, CRISPR/Cas has been selected as the technology which will be used to complete this task.

This lab is approaching the task of genome editing using the CRISPR/Cas system for multiple reasons. First and foremost is the simplicity of the process. CRISPR is unique from other genome editing methods due to the ease and efficiency by which a genomic target may be chosen and changed. Unlike previous methods, such as zinc finger nucleases or transcription-activator-like effector nuclease which require DNA-binding nucleases to be created and customized every time a new target is chosen, CRISPR, on the other hand, allows for the genomic target to be changed by changing the targeting sequence within the guide RNA (see Experimental Overview of CRISPR/Cas System). This can be completed easily. The ability to quickly create a guide RNA with a new target sequence makes CRISPR a popular genome technology. Additionally, CRISPR is currently the most accurate genome editing process available. That is to say it has a very high success rate of creating mutations in the target sequence if the guide RNA is designed correctly. Lastly, CRISPR is the only genome editing process available that allows scientists to make mutations in multiple genes. As FD36 has other homologous members within *Arabidopsis*, a crucial part of the experimental process being used the future attempt to create double and triple mutants with FD36 and its redundant genes. This can only be done with CRISPR.

### Objectives

The objective of this experiment is to knockout FD36. In this case, knockout is defined by sequencing results showing a mutation in the gene. It is not defined by the absence of RNA expression. This is because a potential mutation may result in altered protein production which would still accomplish changing the original function of the gene. Therefore, the result would still be a successful knockout. In the case of the single mutant FD36, a phenotype is not expected as the other redundant genes have yet to be targeted. However, it is the aspiration of this lab to continue toward the creation of a plant with FD36 and its redundant genes successfully mutated in it which will ultimately reveal the function of FD36 itself.



## Research Methodology

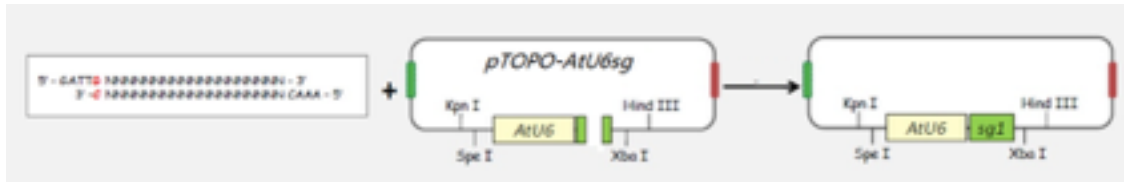
### Experimental Overview of CRISPR/Cas System

When the laboratory protocol of CRISPR/Cas was discovered by Jennifer Doudna and Emmanuel Charpentier in 2012, it bespoke of genome editing capability only dreamed of previously. CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats, is the name given to an arcane bacterial self-defense system created to render a bacteriophage obsolete by selectively cleaving very specific loci in the virus's genome. In CRISPR/Cas9, specifically used in this project, the bacterium is "tricked" so it will recognize and cut programmed target sequences using the endonuclease Cas9, providing scientists with a multitude of opportunities for genomic editing. For instance, harmful mutations could be made by cutting both strands of DNA and allowing them to repair by non-homologous end joining (NHEJ) or homologous recombination (HR) pathways where the repairs usually lead to the addition of extra bases, and thus different amino acids than the original sequence. This changes gene translation and often renders the gene unable to complete its original function. Additionally, scientists may insert a modified sequence in where the original cut(s) were made, leading to the most exciting capability of CRISPR: gene therapy. For the purposes of this experiment, CRISPR was used in an attempt to knockout a gene named FD36. The following paragraphs will provide an overview of how CRISPR was used in this endeavor.

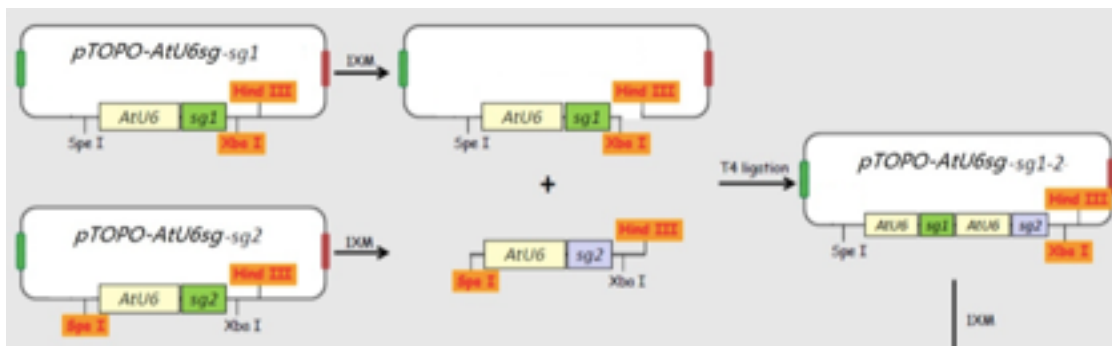
The first step of CRISPR-Cas9 system begins with the selection and creation of a ~20 bp protospacer element, also known as a spacer, within the gene of interest. The creation of a spacer is extremely easy and one of the foremost reasons why CRISPR is such a popular genome editing technology. This spacer is one part of a three part gRNA complex comprised of crRNA (containing the spacer and the PAM), tracrRNA, and a linker loop). As a whole, gRNA works by providing a target sequence, the spacer, which when read signals for the endonuclease Cas9 to cut. The consequential repair following the cut will ideally render the gene "knocked out" or nonfunctional in its original purpose. When deciding on a spacer, certain criteria must be met. The spacer may be any ~20 nucleotide sequences provided that is it 1) unique to the rest of the genome and, 2) upstream (5') of a protospacer adjacent motif (PAM) which in the use of *Streptococcus pyogenes* (*SP Cas9*) is NGG "CRISPR/Cas9 Guide". So, the final target sequences will appear as 5'— ~20 bp + NGG—3'. Understanding the nature of DNA allows one to also give a reverse reading option appearing as 5' — CCN + 20 bp —3', which will also work.

As was stated previously, when the target sequence is recognized, Cas9 is signaled to cut. However, there are many steps between choosing the spacer and the final cut. After creating the spacer, it must be inserted into the entry vector (pTOPO for this experiment), which is foreign DNA which carries the sequence into *E. coli*. Within the vector pTOPO is a promoter (AtU6 for this experiment), which is the chemical initiating gene transcription, and a selection gene (in this case an antibiotic resistant gene, kanamycin). Therefore, each growth plate used to grow clones will be laced with kanamycin to eliminate all other bacteria which may grow in the plate. To

insert the spacer into the plasmid vector, the plasmid is cleaved using the restriction enzyme BbsI. The spacer is then ligated into the vector, resulting in the initial construct. Each spacer chosen will initially receive its own vector.



In the case of this CRISPR experiment, two spacers will be used. The next step is to combine the spacers into one vector. This is done by cleaving certain restriction sites present in the vectors to cut the second spacer out entirely while only opening a small gap for insertion into the first vector. In the second vector, SpeI and HindIII are used to cut the second vector out entirely. In the first vector, XbaI and HindIII are used to cut an opening to insert the second spacer. The second spacer is ligated into the first vector, resulting in one plasmid vector containing instructions for two target sequence cuts.



In the final steps before transformation to *Agrobacterium*, an LR reaction is performed through gateway-compatible CRISPR. The LR reaction allows for a DNA fragment to be transferred from vector to vector, in this case, from pTOPO to p167, which includes the Cas9 endonuclease, a spectinomycin-resistant selection gene, and a promoter for Cas9 with its selection based on the time in the development process in which the cut is to be made. As the last vector used in the experiment, p167 is named the “destination vector.” The combination of the spacers, AtU6 promoter, Cas9, Cas9’s promoter, and the antibiotic resistant gene within the vector is referred to as T-DNA. It is the T-DNA which is inserted into the *Agrobacterium* is labeled as the “target.”



After the target is transformed into the *Agrobacterium* and transfected into to a WT plant, the target will work within the genome of the plant to actively search and alter the gene by cleaving at the spacer sequence, creating a double strand break. In the case of this experiment, a simple target cleavage will be completed to “knock out” the gene. During the repair, usually completed by Non-Homologous End Joining (an uneven cut that is very efficient but often causes InDels) or Homology Direct Repair (an even cut that results in inefficient repair) mutation-rich repair processes occur. Ideally, the mutations created in these processes it will be so extreme as to render original message translation impossible and the gene therefore non-functional. Assuming that all other redundant genes have also been knocked out, the result should yield a plant with a noticeable phenotypic anomaly that can be attributed to the mutation. The ultimate goal of the experiment is to create a hereditary mutation that may be used for further experimentation.

### Part 1: DNA Extraction and Cloning of WT

For the use of further experimentation, such as GUS staining or GFP, four wild type genome samples were collected from wild type plants grown in this lab to isolate and collect FD36. Each sample contained half of a leaf. DNA was extracted through alcohol precipitation.

After the genome was extracted, standard PCR was performed with primers FD36f and FD36r. A agarose gel electrophoresis was used to determine a successful extraction and pure sample and instead revealed a primer issue. PCR was repeated using an annealing temperature test and another gel was run using the results to determine the proper annealing temperature. After identifying appropriate annealing temperatures, the annealing temperature of PCR was set to 49 C and the gel was repeated, providing a positive result. Gel DNA extraction was completed using the protocol and materials in the EasyPure Quick Gel Extraction Kit by TransGen. A NanoDrop machine was used to test the quantity of DNA extracted, and the concentration revealed that it was possible to continue to ligate the PCR product into the pTOPO vector and begin cloning.

After the DNA fragment was cloned, it was then transformed using *E. coli* DH5 $\alpha$  competent cells. Then, the pTOPO vector was extracted using CWBioTech’s PurePlasmid Mini Kit protocol and materials, and the results were sent in for sequencing to ascertain whether the gene was successfully isolated from the genome and amplified.

### Part 2: RNA Extraction and Inquiry into Expression

RNA extraction procedure and materials were taken from the Plant Total RNA Purification Kit made by GeneMark following protocol from Lysis Solution A. Samples were collected from the stem, flower, and leaf of WT plants to determine the relative expression levels of FD36 and to add to the findings of Dr. Yang and Dr. Huang. NanoDrop determined the quantity of RNA extracted to be the following:

Sample	Concentration ng/uL	A260/A280	A260/A230
Flower 1	662.4	2.14	2.02
Leaf 1	45.7	2.05	0.65
Stem 1	186.7	2.15	1.78
Flower 2	1202.4	2.20	2.34
Leaf 2	190	2.16	1.83
Stem 2	308.3	2.22	2.07

Reverse transcription was followed by placing the samples in a thermo cycler with preparation of RT PCR mix being completed the next day. The RNA samples were not diluted before RT PCR was completed.

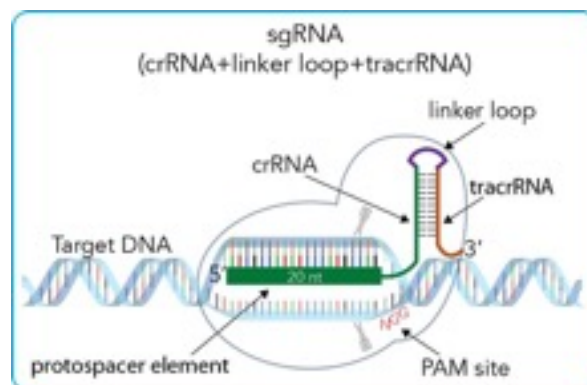
### Part 3: CRISPR/Cas9-Mediated Mutation of FD36

Using the nucleotide sequence of FD36, spacers were picked manually by identifying the given pattern of the PAM (NGG). After multiple options had been selected and recorded, the website NCBI was opened to Basic Local Alignment Search Tool (BLAST) where nucleotide collection was selected for the *Arabidopsis thaliana*. Two spacers were chosen to increase the efficiency of the knock-out.

The spacers identified as the 20 base pairs preceding NGG and following CCN (notice that CCN is a reflection of NGG), and for the purposes of composing a spacer the following linkage bases are added. Additionally, reverse complements were created manually as well.

**CRISPR Spacer**  
 sp1-F: gattgACATTATCTAAGCGTCGAAA  
 sp1-R: aaacTTTCGACGCTTAGATAATGtc  
  
 sp2-F: gattgAACTTCAGCACCACAGAGAA  
 sp2-R: aaacTTCTCTGTGGTGCTGAAGTtc

The spacers for the target sequence within the sgRNA. As the project is working with double strand DNA, F indicates the forward reading sequence while R indicates the reverse reading sequence. Capital letters indicate the nucleotide sequence within the gene. Lower case letters indicate the addition of linkage bases (the linker loop).



Model of the sgRNA now created: the spacer and PAM are part of the crRNA segment and the linker loops are the added base pairs in lower case indicated in the image to the left. The tracrRNA leads as the segment “reading” the strand. (Image by Prediger.)

After the two spacers were ordered and obtained, they were separately annealed to create a spacer one (Sp 1) and spacer two (Sp 2) and then each ligated to a pTOPO vector containing the promoter AtU6. This creates an initial construct. The constructs were then cloned and transformed using competent *E. coli* DH5 $\alpha$  cells. The selection site of pTOPO contained a kanamycin-resistant gene, therefore kanamycin plates were used for culture. Next, fifteen clone samples were selected from the plate of transformed cells and PCR was completed. The PCR results were run in an agarose gel to ascertain whether the vectors containing the promoter and spacer were successfully transformed into the *E. coli* cells. One successful clone from each was selected and cultured.

Vector extraction for both vectors was completed using the protocol and materials given in the CWBiotech PurePlasmid Minikit. This was followed by an agarose gel to test the quality of the DNA, which provided a positive result. Endonuclease digestion followed.

During endonuclease digestion, in the vector containing the first spacer, the Xba I and Hind III restriction enzymes were targeted to open a space in the vector for the insertion of the second spacer. Therefore, 10  $\mu$ l of buffer 1xM as well as 1.0  $\mu$ l of each of the enzymes and 88  $\mu$ l of the plasmid were added to a tube and transferred to an incubator to sit at 37 C overnight. For the vector containing the second spacer, the Hind III and Spe I restriction enzymes were targeted to cut out the second spacer (see Experimental Overview of CRISPR/Cas System fig. 2).

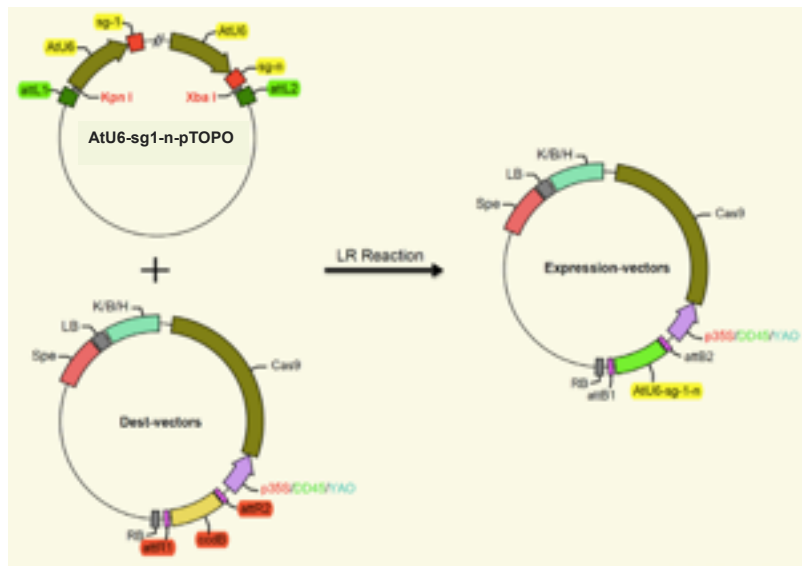
The digested products were purified through an agarose gel and the products of the gels were cut away under UV light. Gel DNA extraction was completed using EasyPure Quick Gel Extraction Kit by TransGen and a NanoDrop machine was used to collect the following information:

DNA Fragment	bp	$\mu$ l	ng	concentration
Sp1	3200	2.7	500	168 ng/ $\mu$ l
Sp2	300	0.5	50	93 ng/ $\mu$ l

The DNA fragments were used in the ligation process to complete the final construct of two spacers within one vector. The new vector was then transformed into *E. coli* DH5 $\alpha$  cells. Fifteen samples were collected and

cultured and PCR was completed with those samples. The following gel revealed a successful transformation process and a successful clone was selected for vector extraction using the PurePlasmid Mini Kit protocol and materials. An agarose gel electrophoresis provided a basis for examining the quality of each construct based on the different concentrations of DNA.

Using the gateway-compatible CRISPR process, an LR reaction was completed using 1.0  $\mu$ l of DNA to transfer the spacers and promoters from pTOPO to p167, containing Cas9, Cas9 DD45 promoter, and a gene for spectinomycin (Spe) resistance. This creates the expression vector.



The expression vector was transformed into *E. coli* DH5 $\alpha$  cells. Subsequent PCR was completed with a primer change to M13R. After the following gel revealed successful transformation, vector extraction was completed using the CWBiotech PurePlasmid Mini Kit materials and protocol, and another agarose gel electrophoresis was run to test the quality of the DNA fragment.

Transformation to *Agrobacterium* strain GV3101 was completed successfully using electroporation (Weigel and Glazebrook, pg. 123). Thirteen samples were collected and PCR was completed. The subsequent gel displayed a successful transformation, and a suitable clone was selected and incubated at 28 C overnight. The culture was obtained from the incubator and transfection buffer was added. The resulting mixture was cultured again with the antibiotics spectinomycin, rifampicin, and gentamicin then returned to 28 C for 12-16 hours. The mixture was then divided equally between two sterilized bottles and centrifuged for fifteen minutes at 3,000 rpm. The supernatant was emptied and 40 mL of transfection buffer was added to each bottle and suspended manually.

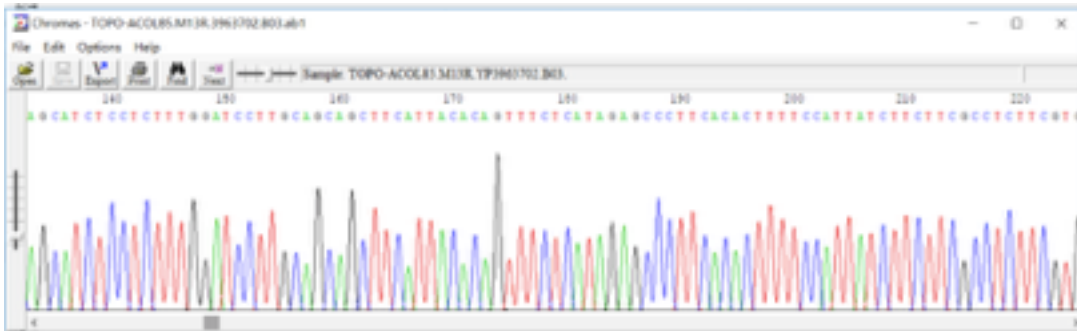
Plant preparation for transfection began by removing all blooming flowers and siliques from the WT *Arabidopsis* while leaving the buds. Lastly, the prepared WT plants were flower dipped (Weigel and Glazebrook, pg. 129). After the flower dip, the transformed plants were stored in darkness for 24 hours. After 24 hours, the plants were moved to the greenhouse. The seeds will be collected at the end of August then planted. The plants produced by the seeds will be monitored for phenotypic abnormalities throughout the growing process. Once they reach maturity, their genomes will be sequenced scanning for evidence of a successful mutation in FD36.

# Research Analysis and Contributions to Food Security

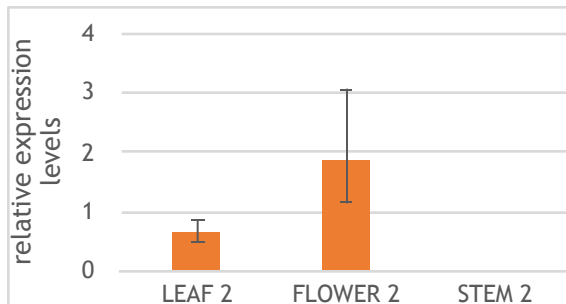
## Results

Until the seeds from the transfected plants have been collected, grown, and their DNA sequenced, it is impossible to know if a successful mutation took place.

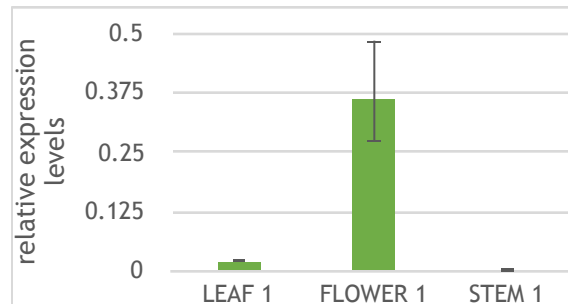
DNA that was extracted and cloned from the WT plant was sequenced, and results verified success.



Additionally, RNA extraction and expression level analysis provided important information that supported Dr. Huang's, which showed that relative gene expression was highest in the petal, and Dr. Yang's, which showed that the relative gene expression was highest in the ovule. The relative expression levels collected from this research indicate strongly that the gene is highly expressed in the flower while low expression occurs in the leaf and stem.



a.



b.

These figures a (left) and b (right) clearly display a consistent result of higher expression in the flower relative to the leaf and stem. This supports the belief that the FD36 is involved in flower development and fertilization.

The result of using CRISPR/Cas9 to knockout FD36 was negative. This was the third unsuccessful attempt made by this lab to produce a mutated FD36 heterozygous or homozygous progeny of *A. thaliana*. Although the lab is unsure of the reason for this result, there are a few ideas circulating which have merit, including using an incorrect spacer or FD36-mediated fatality in the plant upon mutation. One important thing to note, though, is that a third unsuccessful attempt may not indicate scientific error of the standard process, but may instead push a new idea that the standard CRISPR process of mutation may have to be changed in order to be successful for this gene. Much has yet to be seen, and this adds yet another incentive to further research FD36.

### Next Steps

The result of the attempted gene knockout was negative. No mutant was obtained. This was the third unsuccessful attempt made in the lab to create a mutant of FD36, therefore it is reasonable to discuss that a mutation of FD36 may not be attainable.

There are a few possibilities:

1. FD36 is so vital to plant fertilization and successful production that manipulation of it is fatal to the progeny.
2. A highly successful spacer combination has not been achieved yet.
3. For reasons yet unknown to us, this gene can not be manipulated by CRISPR.

As of now, the lab is undecided as to how to proceed, and it is likely that further experimentation of this gene will be done to determine whether it results in fatality or if the spacer sequences used are for some reason to blame.

### Contributions to Food Security

Knowing for certain the role FD36 plays in fertilization and flower development in *Arabidopsis thaliana* holds the potential to provide functional knowledge to biologists interested research pertaining to fertilization, flower development, and yield factors. As *A. thaliana* is the model plant for genetics research, the significance this project should not be overlooked. The purpose of *A. thaliana* research is to provide a foundation for other scientists interested in more field-based plant science to build upon. If homologs are found in commercial agriculture crops or staple foods, the results of this experiment will be important data.



## Reflection

Before formally beginning my reflection of China, I would like to provide an explanation as to why I recorded an account in the manner I did. As a Borlaug-Ruan intern, I feel the need to be transparent and objective in what I was able to experience in China, both the good and the bad. As a recently developed country, China is still striving to deal with its own growth, and the consequences of that are extremely eye opening. Thus, my experience in China brought real context to things I had before only heard of— statistics on pollution, overpopulation, and food insecurity. It is in no way my intent to degrade China or Chinese culture; however, I feel it is my responsibility as a Borlaug-Ruan intern to shed light on the issues this program is working to address. Therefore, the main focus of my reflection will be the juxtaposition of modernism versus rural poverty, which as a rural Iowan struck me most potently. As I say later, “I would have to say that I am glad not to be ignorant. If no one knows, then nothing will be done. If someone knows and feels the passion for change, then there is a real opportunity for a difference to be made.” Now, I give you my personal takeaways from China.

I entered this internship fairly ignorantly. I really didn’t understand what kind of situation I would be encountering in China; therefore, I was unprepared for the contrast of extreme poverty with extreme wealth and modernization I became accustomed to seeing in and around Beijing. A beautiful city which has over the past decade evolved to be a picture of diplomatic strength and prosperity, Beijing is an urban marvel and a powerful symbol to the world. However, in the race against the West, many have been left behind— field workers, factory workers, the uneducated, the unfortunate, not unlike in America. It is because of the contrast between the center of Beijing and the outskirts that I realized reading and being lectured about food insecurity is much different than going out and seeing it face to face every single day. This internship removed any ignorance I may have had about how blessed I was to be born into a middle class family in the United States. And, of course, from that my views on food insecurity were completely transformed.

Outside of Beijing, in the villages of industrial and agricultural workers, those not lucky enough to contain an urban working card, winding alleys of cement boxes pushed tightly together were the definition of homes and neighborhoods. Piles of cement rubble and trash made little walls in the street like the defensive lines. There were no parks, no yards, no windows, and only a few trees. On the main street through the village young men sat outside smoking and old women sat on the ground with small piles of fruit on a piece of newspaper in front of them. Moving out of the village, shirtless, shoeless old men sat in lawn chairs on the side of the road with their bones protruding from their elbows, knees, and cheeks. Women slept in the shade of trees on cardboard mats or just in the dirt. It was an extremely saddening experience, and it was shocking how these people could live such a different life than me. I

I understood that the source of food insecurity and poverty in China is largely a consequence of poor governance. As China has become engaged in the world economy, much like as in America,

its focus has shifted away from its citizens. The result of that is only major coastal cities are benefiting from this increase in trade, leaving western China and rural areas in the dust of the country's recent, uncontrolled growth. The situation occurring in China is not at all similar to undeveloped countries in which proper farming methods and resources are out of reach due to money or some kind of regional challenge. This was simply neglect, and subjectively it posed the strong question for me to think of what could be done to help these people who I felt were so wronged. On the other hand, objectively, I had to ask was this a fair assessment of China? Without a doubt there are issues that are simply insurmountable, and it is not necessarily my place to dictate what can and must be changed especially in a country with so many people where change takes time. However, I feel that it is beyond reproach to say that China needs to begin to value all of its people, from the west and the east, rural to urban, and begin a developmental process that benefits all, not some. This is a cry heard in many countries around the world, including America: equality, quality jobs, human rights of access to health care, food, and shelter. This is not new, nor is it something which should be taken offensively by any Chinese which read this for it is a global issue, not an isolated one. I feel it is my duty as someone involved in the World Food Prize to identify issues which are of pertinence to the organization and to provide an honest and personal reflection of my experience in China which, I add, truly was an exceptional one.

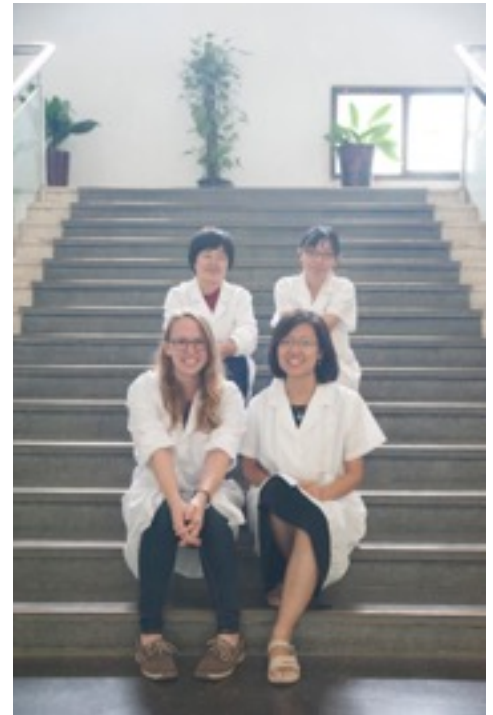
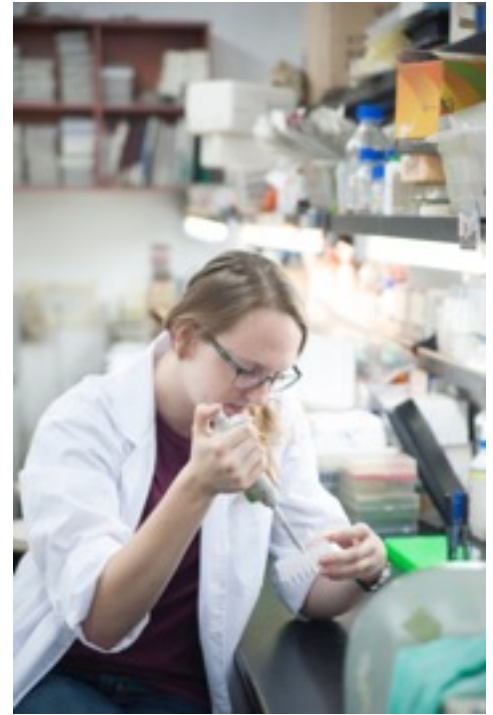
What I have been able to take part in in China is an unbelievable blessing. The beauty I have been able to experience in the parks and historic sites of Beijing is unparalleled by anything I have ever seen in America. The landscape of China is luscious and exotic, and the traditional architecture that has been preserved is simply a form of incredible dynamic art that American architecture can't compare to. I could not imagine anything more different from Iowa than the forests and mountains surrounding Beijing and the vibrant colors that can be seen in the Forbidden City, the Summer Palace, the Beijing Confucius Temple, and throughout the rest of the parks, gardens, and temples of the dynasties. Through the incredible displays of advancement present in that art, science, and architecture, it is with confidence I can say that China is undoubtedly a place of history whose intellectual feats are indicative of an impressive pursuit of knowledge that lasts in the culture's value of education today. With this, it has been an enriching experience to learn about China's evolution from aesthetic historical grandeur to modernized economic power.

Leaving China I have been reaffirmed in my passion for the injustice of poverty. Driving me forward in my life will always be the faces I saw in the villages on the outskirts of Beijing, because at my core I feel that the existence of that is immoral and an unforgivable negligence of mankind to its fellow human. I have firmly decided that I wish to pursue the study of disease and medicine in college, and my project at Peking University proved to be an intellectually challenging experience that will play an integral role as I continue my future in biology and public health. The fact I came to realize is that ignorance simply isn't acceptable when faced with poverty. Neither is apathy. It is a burden to know what exists in the world from a first hand

experience, but I would have to say that I am glad not to be ignorant. If no one knows then nothing will be done. If someone knows and feels the passion for change, then there is a real opportunity for a difference to be made.

During my time in China I have been given a glimpse of what it's like to live in a country with thousands of years of tradition and patriotic loyalty engrained in the very fiber of everyday existence. For the people of China, no other way of life is known besides the utmost loyalty to government and its process. And, through my experience speaking to other foreigners here— political analysts, missionaries, and consistent visitors— I have been able to learn how China is setting itself up to be the world's more influential power in the next decade. However, this growth in power does not exactly reflect through a growing standard of living for all of China. Much of western China faces an extreme disadvantage through poor infrastructure and development, and the high population density of coastal cities puts pressure on the resources available. The modernized economic power that China has become contains many faces of food insecurity and the real benefit I gained from China was one of new and real understanding of what food insecurity looked like in one of the most developed nations in the world. It is because of that China will always be a bittersweet memory. It will also be a source of motivation in my future endeavors.

## Photos



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