GENOTYPIC RESULT OF THE INSULIN-LIKE

RECEPTOR GENE KNOCKOUT IN

DAPHNIA PULEX EB1

by

ANISA KAMMAZ

Presented to the Faculty of the Honors College of

The University of Texas at Arlington in Partial Fulfillment

of the Requirements

for the Degree of

HONORS BACHELOR OF SCIENCE IN MICROBIOLOGY

THE UNIVERSITY OF TEXAS AT ARLINGTON

Dec 2023

ACKNOWLEDGMENTS

I would like to formally express my gratitude for my supervisor, Dr. Sen Xu, Associate Professor of Biology at The University of Texas at Arlington, for his guidance and support throughout this project. His scholarly advice and recommendations have helped me complete my Honors Capstone Project.

This endeavor would not have been possible without my mentor Thinh Pham. Thank you for your endless moral support and expertise. I am also grateful for Dr. Swatantra Neupane, Dr. Trung Huynh, and Dr. Hongjun Wang for their assistance regarding CRISPR-Cas9 and protein analysis.

Lastly, I would like to recognize my family and friends for their support throughout my undergraduate career. They have always had faith in me, and I'm happy to finally show them what I have been working on all this time.

April 21, 2023

ABSTRACT

GENOTYPIC RESULT OF THE INSULIN-LIKE RECEPTOR GENE KNOCKOUT IN DAPHNIA PULEX EB1

Anisa Kammaz, B.S. Microbiology
The University of Texas at Arlington, 2023

Faculty Mentor: Sen Xu

CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 has been used in multiple gene modification studies in various organisms. *Daphnia*, water fleas, are used in ecological studies due to their ability to sense and respond to environmental factors. Their quick life cycles make them prime candidates for gene modification and evolutionary studies. In our experiment, *Daphnia pulex* EB1 was used in the knockout of the *Insulin-like Receptor* gene via CRISPR-Cas9 due to its effect on dwarfism in humans and developmental disorders in mice. Polymerase Chain Reaction (PCR) sequencing was done to compare the EB1 gene of interest to the PA42 reference genome, generating a single guide RNA (sgRNA). There were occasional single nucleotide polymorphisms (SNPs); to overcome this, the sgRNA was designed to match the EB1 sequence. In-vitro testing and gel electrophoresis was performed on the digested sample to confirm the knockout was

successful. In-vivo studies with PCR will need to be performed in the future to further the advancement of CRISPR-Cas9 studies.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Water fleas: Daphnia spp.

Daphnia are planktonic crustaceans found in still bodies of freshwater, such as lakes and ponds. The animal can switch between sexual and asexual reproduction (parthenogenesis) based on environmental stimuli. While some clones are exclusively asexual, known as obligate parthenogenesis, cyclical parthenogenesis is also observed (Decaestecker et al., 2009). In drought seasons, *Daphnia* can switch to sexual reproduction to produce ephippia, protecting the eggs until conditions are favorable again. In regular environmental conditions where there are ample resources and space, *Daphnia* are asexually reproductive (Decaestecker et al., 2009). Haploid eggs are generated and deposited into the female brood chamber where they will develop into larvae. After three days, they are released into the water and become independent of the parent (Ebert, 2005).

1.2 Significance of Research

Insulin-like Receptor gene (*InR*) knockout has never been done in *Daphnia* before. The research has primarily been conducted in mice (Laron, 2001) and other model organisms (Brogiolo et al., 2001). This experiment will be the first of its kind and will contribute to the knowledge of *Daphnia* behavior without the gene. Atypical quadruple duplication of the *InR* gene in *D. pulex* has recently been discovered, and further research aims to discover the synthetic lethality of the gene. The genotype is expected to change, and experiments of plasticity can be performed in the future based on this research. *InR* was shown to be downregulated during sexual reproduction in

Daphnia, and this experiment will help contribute to the knowledge of the role of the gene in cyclical parthenogenesis clones. Measuring expression levels in in-vivo knockouts of cyclical parthenogenesis *D. pulex* EB1will help determine its role in reproduction.

In humans, the homologous gene would be the *insulin-like growth factor* gene (I*gf-1*) that encodes the protein IGF-1, an important growth hormone that promotes normal cell and tissue development (Laron, 2001). Both IGF-1 and growth hormone (GF) are responsible for promoting normal growth of bones and tissues. In humans with IGF-1 deficiency, their organ growth, muscular system, and skeletal maturation are highly impacted (Laron, 2001). If left untreated, deficiency can lead to dwarfism (Laron, 2001).

Research regarding CRISPR-Cas9 focuses on the improvement of human health by genome editing (Abdelnour et al., 2021). Focusing primarily on inherited diseases that cannot be mitigated by traditional medicine, CRISPR-Cas9 gene editing therapy could become a viable solution. The enzyme could have many agricultural, biological, and biomedical uses in the near future (Abdelnour et al., 2021).

CHAPTER 2

LITERATURE REVIEW

2.1 CRISPR-Cas9

CRISPR-Cas9 is a targeted nuclease derived from *Streptococcus pyogenes* that can cleave genomic sequences with high specificity (Ran et al., 2013). The enzyme requires a sgRNA in order to efficiently cleave the sequence at the desired location (Ran et al., 2013). The protospacer adjacent motif (PAM) is directly adjacent to the sgRNA and is required for the Cas nuclease to cleave the sequence (Ran et al., 2013).

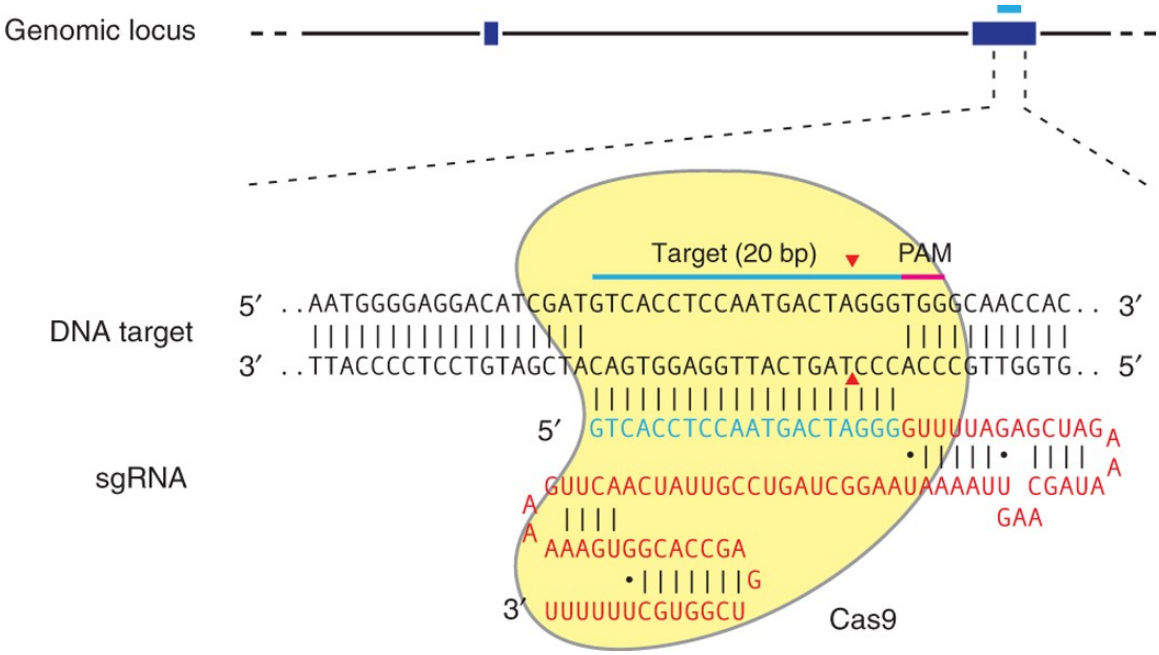

Figure 2.1: Schematic of the RNA-guided Cas9 Nuclease (Ran et al., 2013).

CRISPR-Cas9-mediated homology-directed repair (HDR) is a natural nucleic acid repair system used in many organisms, including humans (Abdelnour et al., 2021).

Nonetheless, it can be ineffective and cleave off-target sites leading to double-strand breaks (DSBs), which is not conducive to the clinical use of the enzyme (Abdelnour et al., 2021). A newly developed approach using Cas9 fusion proteins called base editors (BE) are able to decrease insertions and deletion errors (Abdelnour et al., 2021), while allowing access to previously inaccessible genetic loci (Antoniou et al., 2021). There are two main classes of BEs: cytidine base editors (CBEs) and adenine base editors (ABEs).

2.2 Insulin-like Growth Factor Gene

IGF-1 is an important growth hormone promoting normal human cell development (Laron, 2001). It is a small peptide (70 amino acids) and is structurally similar to insulin. In mice knockouts, a similar conclusion was reached, which has shown that knocking out the IGF-1 gene or IGF-1 receptor (IGF-1R) gene causes retarded growth by 40-45% (Accili et al., 1999). Furthermore, the result was impaired growth and skeletal development (Sjögren et al., 2000). On the other hand, if the mice did not have IGF-1R at birth, the result was death from respiratory failure (Sjögren et al., 2000). The mice were not able to properly develop their diaphragm and intercostal muscles. Bone development was also severely affected in mice without IGF-1Rs.

In *Drosophila,* the insulin receptor homolog (DInr) is able to regulate organ size (Brogiolo et al., 2001). Varying the activity of DInr during developmental stages changed the cell size and cell number autonomously (Brogiolo et al., 2001). *Drosophila* has seven insulin-like peptide coding genes, and overexpression of any gene encoding an insulin-like peptide increased body size (Brogiolo et al., 2001). Body size is normally regulated by temperature and food availability; however, hormones and growth factors also play a role in mammalian species (Brogiolo et al., 2001). *Drosophila melanogaster* has also been used

as a model organism in type 2 Diabetes Mellitus studies. Impaired insulin signaling in *D. melanogaster* triggers an abnormal metabolic state. The homozygous mutant for genes in the insulin pathway is born diabetic (Álvarez-Rendón et al., 2018). The most commonly observed phenotypes are a decrease in fertility, decreased body size, changes in longevity, (decrease in normal conditions, often an increase in longevity when there is caloric restriction), defects in fat body morphology, in heart, retina, and brain physiology, increased levels of triacyl glycerides, and higher amounts of circulating sugars in the hemolymph (Álvarez-Rendón et al., 2018).

Studies involving *Caenorhabditis elegans* have also shown the importance of the insulin/IGF-1 signaling pathway (IIS). Insulin-like peptides activate the DAF-2/insulin/IGF-1 receptor tyrosine kinase triggering a PI3/AKT/SGK kinase cascade that phosphorylates the DAF-16/FOXO transcription factor (Antebi, 2007). DAF-16/FOXO is able to enter the nucleus and turns on survival genes. Changes in expression impact overall stress resistance and longevity (Antebi, 2007).

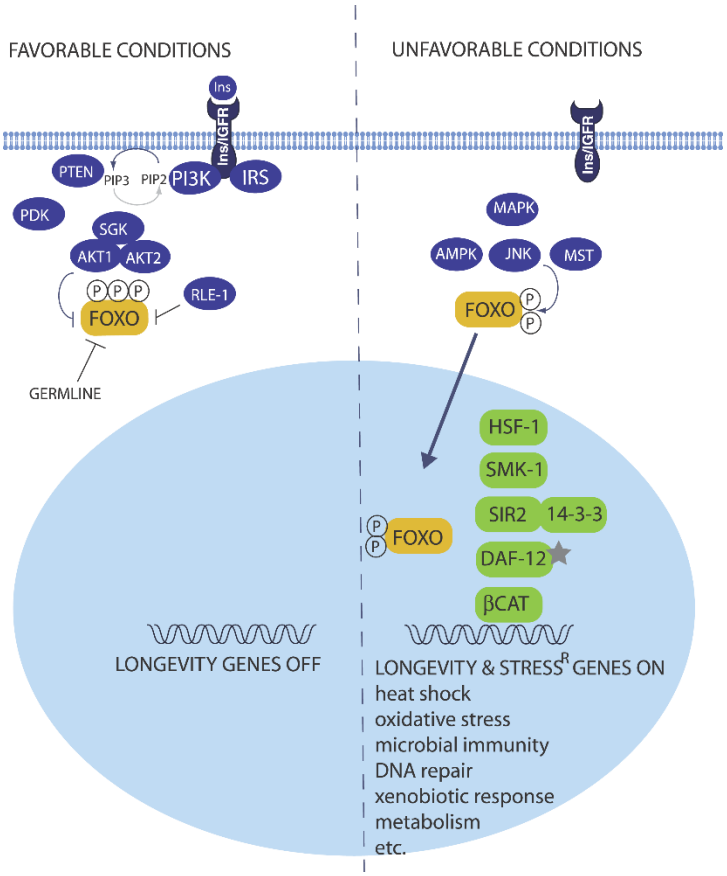

Figure 2.2: Insulin/IGF-1 Signal Transduction Pathway in *C. elegans* (Antebi, 2007).

2.3 Daphnia Insulin Signaling Pathway

Research in Daphnia's insulin signaling pathway (ISP) is scarce since most research has been performed in model organisms. One study found that *D. pulex* duplicates its insulin receptor genes (*InR*), which could have an effect on plasticity (Boucher et al., 2010). The study showed four different *InRs* most likely resulted from duplication events (Boucher et al., 2010). The *InRs* had different features, but they concluded the *D. pulex* ISP is largely similar to the mammalian ISP. A graduate student in the Xu Laboratory confirmed that the gene was differentially expressed, more specifically, downregulated, during sexual reproduction stages in *Daphnia* (Huynh and Xu, 2022).

CHAPTER 3

METHODOLOGY

3.1 Animal Care

To maintain healthy animals, the *Daphnia* are kept in incubators set to 18°C with a photoperiod of 18 hours light and 6 hours dark. They are fed algae every other day, and the beakers are cleaned every week. *Daphnia* can become stressed in crowded and dirty conditions and switch to sexual reproduction, which is not conducive to the experiment. During the cleaning, babies and dead algae are removed from the beaker. Artificial lake water is added to maintain a water level of 25 mL. Asexual (clonal) embryos are needed, so it is crucial to maintain healthy *Daphnia* culture.

3.2 Target Design

First, the EB1 (an isolate of *D. pulex*) sequence must be obtained to determine the protospacer adjacent motif (PAM) in the gene of interest. The PAM sequence is the recognition site for the cleavage. The purpose will be to design a single guide RNA (sgRNA) that will guide the Cas9 protein to the target. The sgRNA combined with trans-activating crisprRNA (tracrRNA) to form the ribonucleoprotein (RNP).

3.2.1 Obtaining the EB1 sequence

Using polymerase chain reaction (PCR), the EB1 sequence was obtained. Five primers were designed using Primer3 to find a functional protein domain within *InR* to

disrupt. Since the EB1 sequence remained unknown at the time, the PA42 isolate reference genome was used to design primers for PCR.

In every tube, the reagents were added following the table below (Table 3.1). After adding all the components of the reactions, all samples were then put into a thermocycler to run with a program of 3 minutes at 94°C, 30 cycles of 45 seconds at 94°C, 45 seconds at 50°C, 45 seconds at 72°C, and 4 minutes at 72°C for final elongation then consecutively paused at 4oC for preserving purposes.

Table 3.1: Standard PCR Protocol

Reagents	Volume (µL)
Ultra-Pure H_2O	19.515
10x Taq Buffer	2.5
$MgCl_2$	0.36
dNTP	0.50
Forward Primer	0.50
Reverse Primer	0.50
Template DNA	1.0
Taq Polymerase	0.125
Total	25.0

This process was repeated for all five of the primers.

Table 3.2: PCR Primers for EB1 Sequence

Forward Primer	Reverse Primer
1. TCCTTTCCCATCTTGTCGCT	1. TTATCCGGCGATGTCTCCTC
2. ggcgcgttctagtttcgaat	2. GTGAAATGTCCACGTCCGAG
3. CAACGCCATAGAATTCCCCG	3. TGGCACATTTCACTTCAGGC
4. TCCCATCTTGTCGCTCAGTT	4. GGCGATGTCTCCTCCAAAAC
5. CTCGGACGTGGACATTTCAC	5. CGGGGAATTCTATGGCGTTG

Note that exons are denoted with capital letters while introns are denoted with lowercase letters.

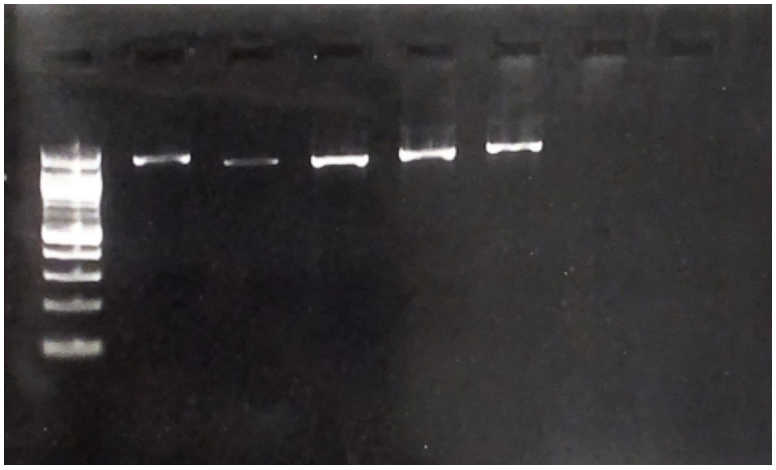

Figure 3.1: Gel Electrophoresis of PCR Product.

After running gel electrophoresis to confirm successful amplification, the PCR product was used as the template for the BigDye Termination Reaction from ThermoFisher. The reagents as shown in Table 3.3.

Table 3.3: BigDye Termination Reaction Protocol

Reagents	Volume (μL)
H2O	4.5
MgCl2 (5mM)	3.0
Primer	0.5
BigDye	0.5
Template from PCR	1.5
Total	10.0

After adding all the components of the reactions, all samples were then put into a thermocycler to run with a program of 2 minutes at 96°C, 30 cycles of 30 seconds at 96°C, 30 seconds at 55°C, 3 minutes and 45 seconds at 65°C, and then consecutively paused at 4°C for preserving purposes.

The final products were then condensed and cleaned up using homemade magnetic beads and eluted using HiDi. After the DNA got eluted using HiDi, it was denatured at 95°C for five minutes and quenched on ice to open the double-strand structure, which was required for Sanger Sequencing performed by 3130xl Genetic Analyzer.

3.2.2 sgRNA design

Out of the five primers that were designed, Primers 1 and 4 had readable sequences and were used to design the sgRNA. While Primer 3 generated a readable sequence, it was further downstream in the sequence. To complete the project in a timely manner, further upstream sequences were used. PCR sequence from primers 2 and 5 had a large percentage of unidentified nucleotides, as shown in Appendix A. Although these primers had readable sequences, the IDT Custom Alt-R™ CRISPR-Cas9 guide RNA tool was not able to use it due to the occasional Ns in the sequence. Therefore, the PA42 sequence was used to design the sgRNA initially. Once the sgRNA was found, it was compared to the sequence of the EB1 isolate to ensure there were no single nucleotide polymorphisms (SNPs). There was one sgRNA that had matched completely with the EB1 isolate located in the fibronectin type III domain. The protein is responsible for cell adhesion, migration, growth, and differentiation. The sgRNA sequence was confirmed to be a part of the *InR* region through NBCI BLASTn.

Table 3.4: sgRNA Information

	Sequence	PAM	On-Target Score	SNPs
sgRNA 1.1	GACATTTCACCTTGGTCCAA	TGG	99%	2
sgRNA 1.4	CGTCACTATTGTTAATAAGT	CGG	71%	0

3.2.3 Knockout Design

To ensure the knockout is successful, primers were designed using Primer 3 to amplify a sequence including sgRNA 1.1 and sgRNA 1.4. The forward primer has an M13 tail attachment for future neonate genotyping (Boutin-Ganache et al., 2001). The amplicon is 364 base pairs long. After successfully designing an sgRNA for the EB1 sequence, the ribonucleoprotein was constructed.

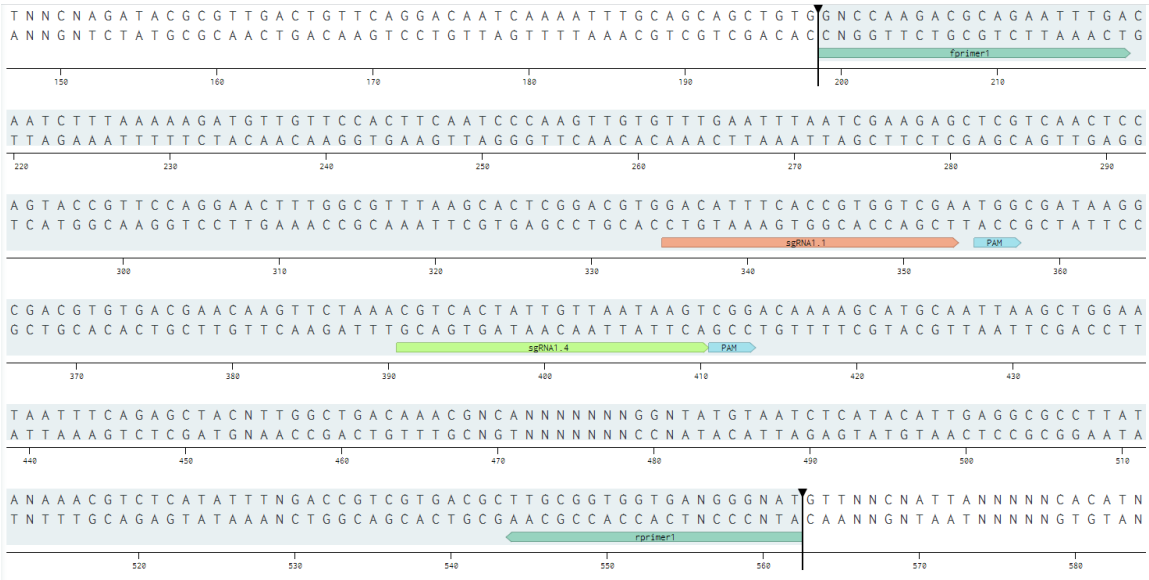

Figure 3.2: Region of Interest in FN_3 Domain Located on Chromosome 11.

Table 3.5: Target Gene Knockout Primers

Forward Primer with M13	CACGACGTTGTAAAACGACGCCCAAGACGCAGAATTTGA
Reverse Primer	atacCCATCACCACCGCAA

PCR and gel electrophoresis were performed to ensure the primers were functional. The gel electrophoresis was run with a 1000 kb ladder for comparison.

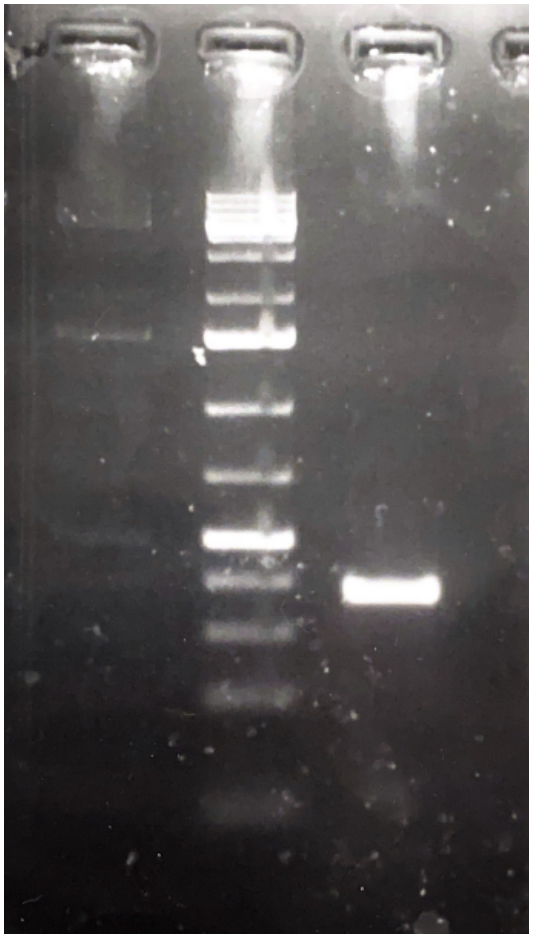

Figure 3.3: Gel Electrophoresis of Region of Interest.

RNP Preparation Protocol

1. Food Dye and TE buffer solvent

    a. For the Food Dye solution, mix 500 uL Regular/Commercial Food Dye (from Walmart or any big box store) with 500 uL of ddH20. After homogenized, use a syringe to filter the solution through a 0.2 um Cellulose Acetate filter before moving onto the next step.
    b. In an Eppendorf tube, mix 10 uL of 1M Tris and 1 uL of 100 mM EDTA.
    c. Finally, add 989 uL of Food dye solution and vortex the mixture.

2. Prepare sgRNA

    a. Add 50 uL of Nuclease-free duplex buffer to the dry crRNA (10 nmol). The crRNA concentration now will be 200 uM or 125 ng/uL
    b. Add 100 uL of Nuclease-free duplex buffer to the dry tracrRNA (20 nmol). The tracrRNA concentration now will be 200 uM.
    c. After dissolve the crRNA and tracrRNA, in a 200 uL PCR tube, add 10 uL of tracrRNA and 10uL of crRNA. **Note:** for testing, 5 uL of each RNA can be used instead of 10 uL.
    d. Incubate the mixture at 95^{o}C for 5 minutes, then cool down to room temperature.

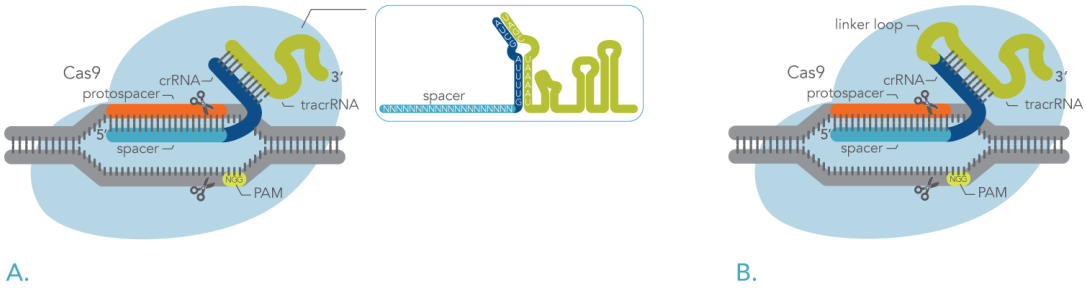

Figure 3.4: CRISPR-Cas9 Mechanism (IDT, 2023).

3. Assemble the RNP

    a. Mix and incubate for 15 minutes at room temperature. This was used for in vitro/ activity testing.

Table 3.6: RNP Complex Assembly

Reagents	1x (25 µL)	0.2x (5 µL)
sgRNA (200 µM or 125 ng/µL)	1.85	0.37
Cas9 (600 ng/µL)	3	0.6
TE buffer with Dye	20.15	4.03

4. In-vitro/ Activity Testing

    a. Mix thoroughly and incubate at 37°C for 60 minutes

    b. Check digested and undigested sample on 1.5% agarose gel

Table 3.7: In-vitro RNP Assembly

Reagents	1x (10 µL)
10x Nuclease Reaction Buffer	1
Prepared RNP complex	1
DNA substrate	X µL (150 ng)
ddH20	10-1-1-x

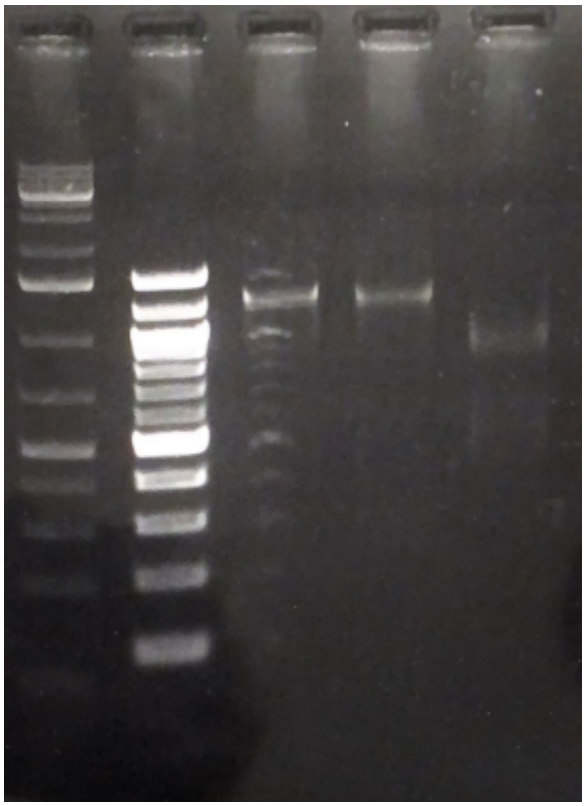

Figure 3.5: Gel Electrophoresis of Digested Sample.

3.3 In-vivo Testing

Once healthy asexual animals are attained, the microinjection process can begin. The RNP system is loaded into the microinjection beveled needle and loaded onto the Eppendorf FemtoJet 4i and InjectMan4 machines. The microscope used is the Nikon

SMZ800N. The animals are separated onto petri dishes for observation. Animals are closely monitored in order to harvest the embryos as soon as they move into the brood chamber. When *Daphnia* molt and discard their old carapace, they are expected to drop embryos approximately 13 minutes later (Hiruta et al., 2010). Once the embryos are approximately 60% loaded in the brood chamber, they are placed in an ice bath for six minutes to slow their movement to ease dissection. To dissect the animal, the animal is pinned down by its eye and the carapace is lifted apart with tweezers. The embryos will flow into the solution and the animal is discarded. The embryos are lined up against the wall of the petri dish to aid injection. After injecting the embryos, they are moved to wells containing 100 µL 60mM sucrose solution and placed in an incubator at 25°C. The embryos are observed every 12 hours for signs of development and hatching.

Table 3.8: Injected Embryo Data

Injection Date	Embryos Injected	Embryos Intact	Hatched
03/03/23	23	10	0
03/10/23	64	26	0
03/16/23	15	10	3
03/24/23	10	2	0
03/27/23	25	21	0
03/31/23	12	10	0
Total	149	79	3

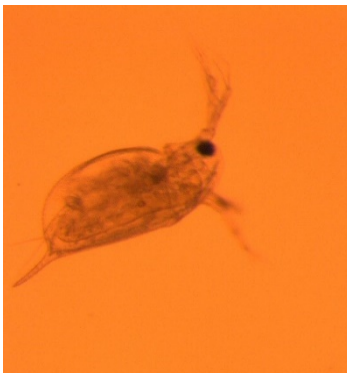

Figure 3.6: Post injection Hatched *D.pulex* EB1.

CHAPTER 4

DISCUSSION

4.1 PCR Primer Design

To design primers to sequence the EB1 clone, the reference genome PA42 was used. Primer3 web version was used to design five primers to sequence the EB1 genome. As shown in Appendix A, primers 2 and 5 were unsuccessful in generating a readable sequence. The large percentage of unidentified nucleotides in the primer 2 sequence prohibited the use of the sequence in sgRNA design. Primer 5 yielded a completely unreadable sequence and was unusable. While the PCR gel electrophoresis was successful, the Sanger Sequencing was less so. PCR reactions can use a mismatching primer site; however, sequencing needs a complete match. Since the primers were designed using the PA42 genome, there were most likely SNPs present.

4.2 Knockout Design

After the sequence was derived using primers 1, 3, and 4, the sgRNA was designed using IDT. After finding two sgRNA with directly adjacent PAMs that were in the region of interest (FN_3 domain), primers were designed using Primer3 to generate an amplicon between 200-400 base pairs. The final amplicon measured 364 base pairs. PCR and gel electrophoresis were performed with the newly designed primers to ensure functionality. On the gel electrophoresis, from the bottom up, each ladder increases by

100 base pairs until 1,000 base pairs. Based off this measurement, the amplicon was confirmed to be between 300-400 base pairs (see Figure 3.2), concluding the primer success.

4.3 In-vitro Testing

Once the PCR product was obtained, the in-vitro testing with the RNP assembly was performed. The sgRNA was designed in lab and ordered from IDT, and the tracrRNA was directly manufactured and shipped from IDT. Combining these two creates crRNA, which is a necessary ingredient in performing the CRISPR-Cas9 cleavage. Referring to Figure 3.3, the digested sample showing two bands in the fifth well containing sgRNA1.4 was successful. The sgRNA1.1 in the fourth well was not successful due to the SNPs when comparing the PA42 genome to the EB1 genome. It was an oversight made by human error. In the third well PCR product without the RNP was placed as a control for the system. It appears similar to the sgRNA1.1 because neither sample had DNA cleavage, therefore the DNA appears as one band. The two remaining wells are ladders: 1 kilobase (first well) and 100 base pair (second well).

4.4 In-vivo Testing

After confirming the success of sgRNA1.4 in-vitro, microinjection of *Daphnia* embryos was performed throughout the month of March. Out of the six injection days, 149 total embryos were injected; yet only three hatched (refer to Table 3.7). The three that hatched died in three days. They were weak compared to other hatched babies of the same age and died three days later. Knocking out the *InR* gene proved to be difficult as it is not a cosmetic gene, like *scarlet*. Previously in the Xu Laboratory, graduate students and post-doctoral fellows successfully and repeatedly knocked out the *scarlet* gene in *D.*

pulex and *Daphnia magna.* The *InR* gene is important for normal development -- knocking it out and expecting the embryos to hatch out was optimistic. Moreover, time was of the essence since the project was to be completed within the semester. The three babies that hatched did not survive long enough to create cell lines for PCR and genotyping.

CHAPTER 5

CONCLUSION

In the future, further in-vivo experiments will need to be conducted to determine whether the *InR* gene is a lethal gene required for *D. pulex* EB1 development. The atypical duplication of the *InR* gene in *D. pulex* introduced the idea of synthetic lethality for the gene; however, our current research is not extensive enough to support or reject that hypothesis. Successful lineage needs to be established in order to further test the expression of the *InR* gene in cyclical parthenogenesis *D. pulex* EB1.

APPENDIX A

PCR PRODUCT ANALYSIS

Primer	Number of Unidentified Nucleotides	Total Base Pairs in Sequence	Unidentified Nucleotide Percentage
1	401	1177	34.07
2	747	1148	65.07
3	380	1183	32.12
4	453	1156	39.19
5	N/A	N/A	N/A

The number of unidentified nucleotides from the sequences, respective to their primer pairs.

REFERENCES

Abdelnour SA, Xie L, Hassanin AA, Zuo E, Lu Y. 2021. The Potential of CRISPR/Cas9 Gene Editing as a Treatment Strategy for Inherited Diseases. *Frontiers in Cell and Developmental Biology.* 9. doi:https://doi.org/10.3389/fcell.2021.699597.

Accili D, Nakae J, Kim JJ, Park B-C., Rother KI. 1999. Targeted Gene Mutations Define the Roles of Insulin and IGF-I Receptors in Mouse Embryonic Development. *Journal of Pediatric Endocrinology and Metabolism*. 12(4). doi:https://doi.org/10.1515/jpem.1999.12.4.475.

Álvarez-Rendón JP, Salceda R, Riesgo-Escovar JR. 2018. Drosophila melanogasteras a Model for Diabetes Type 2 Progression. *BioMed Research International*. 2018:1–16. doi:https://doi.org/10.1155/2018/1417528.

Antebi A. 2007. Genetics of Aging in Caenorhabditis elegans. PLoS Genetics. 3(9):e129. doi:https://doi.org/10.1371/journal.pgen.0030129.

Antoniou P, Miccio A, Brusson M. 2021. Base and Prime Editing Technologies for Blood Disorders. *Frontiers in Genome Editing*. 3. doi:https://doi.org/10.3389/fgeed.2021.618406.

Boucher P, Ditlecadet D, Dubé C, Dufresne F. 2010. Unusual duplication of the insulin-like receptor in the crustacean Daphnia pulex. *BMC Evolutionary Biology*. 10(1):305. doi:https://doi.org/10.1186/1471-2148-10-305.

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF. 2001. M13-Tailed Primers Improve the Readability and Usability of Microsatellite Analyses Performed with Two Different Allele- Sizing Methods. *BioTechniques.* 31(1):25–28. doi:https://doi.org/10.2144/01311bm02.

Brogiolo W, Stocker H, Ikeya T, Rintelen F, Fernandez R, Hafen E. 2001. An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. *Current Biology*. 11(4):213–221. doi:https://doi.org/10.1016/s0960-9822(01)00068-9.

Decaestecker E, De Meester L, Mergeay J. 2009. Cyclical Parthenogenesis in Daphnia: Sexual Versus Asexual Reproduction. *Lost Sex*. 295–316. doi:https://doi.org/10.1007/978-90-481-2770-2_15.

Ebert D, 2005. Ecology, Epidemiology, and Evolution of Parasitism in Daphnia [Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books

Hiruta C, Nishida C, Tochinai S. 2010. Abortive meiosis in the oogenesis of parthenogenetic Daphnia pulex. *Chromosome Research*. 18(7):833–840. doi:https://doi.org/10.1007/s10577-010-9159-2.

Huynh T, Xu S. 2022. Transcriptomic signal of reproductive modes and early speciation in daphnia pulex-pulicaria system. [accessed 2023 Apr 10]. https://rc.library.uta.edu/uta-ir/bitstream/handle/10106/31037/HUYNH-DISSERTATION-2022.pdf?sequence=1&isAllowed=y.

IDT. 2023. CRISPR-Cas9 genome editing system. Integrated DNA Technologies. [accessed 2023 Apr 5]. https://www.idtdna.com/pages/technology/crispr/crispr-genome-editing/Alt-R-systems/cas9.

Laron Z. 2001. Insulin-like growth factor 1 (IGF-1): a growth hormone. *Molecular Pathology.* 54(5):311–316. doi:https://doi.org/10.1136/mp.54.5.311. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1187088/.

Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. 2013. Genome engineering using the CRISPR-Cas9 system. *Nature Protocols.* 8(11):2281–2308. doi:https://doi.org/10.1038/nprot.2013.143. https://www.nature.com/articles/nprot.2013.143.

Sjögren K, Bohlooly YM, Olsson B, Coschigano K, Törnell J, Mohan S, Isaksson OGP, Baumann G, Kopchick J, Ohlsson C. 2000. Disproportional Skeletal Growth and Markedly Decreased Bone Mineral Content in Growth Hormone Receptor −/− Mice. *Biochemical and Biophysical Research Communications.* 267(2):603–608. doi:https://doi.org/10.1006/bbrc.1999.1986.

BIOGRAPHICAL INFORMATION

Anisa Kammaz will be completing her Honors Bachelor of Science in Microbiology from The University of Texas at Arlington in December of 2023. She plans to pursue the medical science program at The University of Texas Southwestern. She has been a part of the Xu *Daphnia* Laboratory since February of 2022. During her time there she assisted with a CRISPR-Cas9 microinjection project involving the knockout of the *scarlet* gene. She served as President of the Science Constituency Council for the 2022-2023 academic year and is a member of the Honors College and Vietnamese Student Association.