HIGH SCHOOL EDITION
Journal of Student Research
Volume 10 Issue 4 (2021)

Designing a Novel CRISPR/Cas9 Endogenous Tag of the Amph-1 Gene for Treatment Research Purposes

Angelina Yang[1] and Ryan Doonan[#]

[1]Plano West Senior High School, Plano, TX, USA
[#]Advisor

ABSTRACT

Amphiphysin 1, or amph-1, is a *c. elegans* ortholog of the genes amphiphysin (AMPH) and bridging integrator 1 (BIN 1) present in humans. Amph-1 is located primarily in the endomembrane system of the cell. Its protein count is highly affected by diseases under dementia and carcinoma. To gain further understanding of amph-1 protein activity in response to such diseases, this research centers around the creation of a CRISPR/Cas9 design plan to inject modified plasmids into *c. elegans* eggs and produce specimens available for endogenous activity research. An N20 sequence and 5' and 3' homology arms were located near the N-terminus, where a bioluminescent protein tag would be inserted. A plasmid dedicated to synthesizing the Cas9 protein and a second plasmid carrying the genetic code for bioluminescent protein are then modified using amph-1 genetic code as a base template. Following silent mutation edits to the N20 sequence, this plasmid design has potential to be developed in labs for depper research into the amph-1 gene behavior.

Introduction

Amphiphysin 1, or amph-1, is a *c, elegans* ortholog of the genes amphiphysin (AMPH) and bridging integrator 1 (BIN 1) present in humans. Subcellular locations of amph-1 include the recycling endosome, nuclear envelope, plasma membrane, and the cytoplasm's perinuclear region. Amph-1 plays a large role in the development of carcinoma and dementia-type diseases in humans. Examples include breast carcinoma, prostate adenocarcinoma, centronuclear myopathy 2, and Alzheimer's disease. As such diseases of affluence are diagnosed at an increasing rate (prevalence of Alzheimer's in low/middle income families expected to rise from 60% to 70% by 2050), it is becoming increasingly vital for scientists to gain a higher understanding of the amph-1 gene's local activity and response to carcinoma and dementia.

Process

For further research of amph-1, it was appropriately concluded that designing a CRISPR/Cas9 plan that endogenously tags the gene amph-1 in *c. elegans* with a bioluminescent protein, without any predictable lethal effects in the animal model, is best suited for deeper research into amph-1's activity at the nuclear level. Using the *c. elegans* as an animal model to implant the CRISPR design would evade impractical and ethical issues of planting this design into humans. It was decided that this would be an N-terminus tag, centralizing plasmid design within ~700 base pairs of the N-terminus region. While creating the CRISPR/Cas9 design plan, there needed to be an identified N20 sequence guide RNA from amph-1's genetic sequence. It is vital in order to guide Cas9 protein's controlled activity in cutting strands of DNA. In addition, 5' and 3' homology arms derived from amph-1 sequence are important to integrate into the plasmid design and prevent the plasmid from being rejected by the *c. elegan* without the homology arms. Moreover, silent mutations were made to the N20 sequence so that its function and amino acid sequence stay original while not

identified for cutting by Cas9. In total, there will be two plasmids to be injected into the *c. elegans*: one plasmid responsible for synthesizing the Cas9 protein, and a second plasmid storing the bioluminescent marker genetic sequence that will be copied into *c. elegan* DNA through homology directed repair. These two plasmids will then be injected into the gonad region of *c. elegans*, where they will integrate the genetic sequence for bioluminescent markers into the eggs' chromosomes and produce offspring that have trackable amph-1 activity.

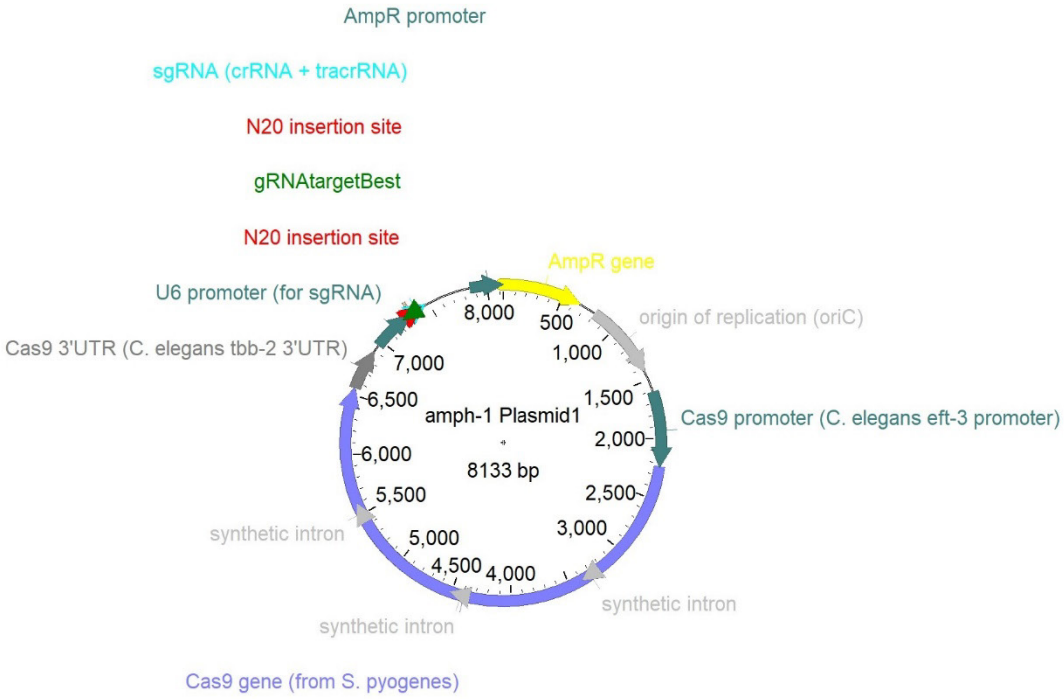

Figure 1. A schematic diagram of amph-1 Plasmid 1's DNA sequence for Cas9 synthesis; gRNA general location colored-coded with forest green.

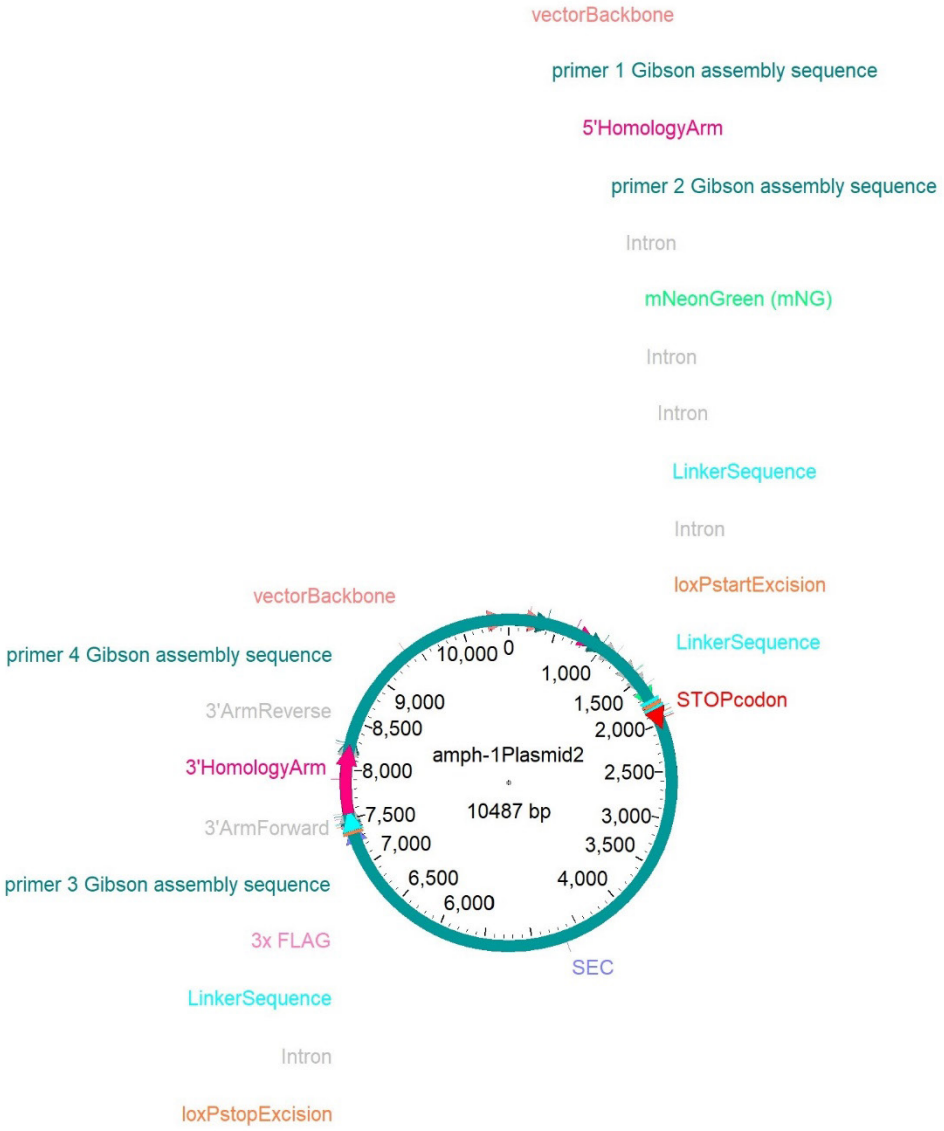

Figure 2. A schematic diagram of amph-1 Plasmid 2's DNA sequence as template to be integrated into amph-1 and synthesized as bioluminescent protein tags; homology arm general locations color-coded with hot pink.

Conclusion

Through completing this CRISPR design of amph-1, scientists will be able to quickly begin endogenously tagging the amph-1 gene locus in *c. elegans* and develop a further understanding of the gene aside from name and general function in humans. As amph-1 concentration is tied to developments of Alzheimer's breast carcinoma, and many more in the unforeseeable future, designing a CRISPR plan to tag the gene for research purposes is a step forward in developing possible treatment for dementia patients.

Acknowledgements

I would like to thank Professor Ryan Doonan (doonan@utexas.edu) at UT Austin's High School Summer Research Academy for providing me the opportunity and assistance to explore the possibilities that lie in CRISPR designs. Through his mentorship, I was opened to the boundless possibilities biotechnology has to offer for the betterment of human life.

References

Alzheimer's Disease International. (2017). ADI - Dementia Statistics. Alzheimer's Disease International (ADI). https://www.alzint.org/about/dementia-facts-figures/dementia-statistics/.

UniProt ConsortiumEuropean Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics. (2021, June 2). Amphiphysin homolog. UniProt ConsortiumEuropean Bioinformatics InstituteProtein Information ResourceSIB Swiss Institute of Bioinformatics. https://www.uniprot.org/uniprot/Q21004.

WormBase. (2018). Amph-1 (gene) - wormbase : Nematode information resource. WormBase. https://wormbase.org/species/c_elegans/gene/WBGene00010272?redirect=1#0-9f1e6bd-10.