PCR Analysis of Transgenic Zebrafish: Zoe Coutu and Abigail Shafer under the direction of Dr. Bailey

Abstract:

Our goal for this project is to verify that the neurod4 gene was indeed passed down to future generations of zebrafish from the original transgenic fish. In order to correctly genotype the zebrafish used for regenerative research in the Bailey lab, DNA extraction and isolation, along with PCR analysis and gel electrophoresis are used. We are specifically amplifying the neurod4 gene responsible for regulation of neuronal development and differentiation. Using various polymerases purchased through the Geneseo TRAC Grant and multiple methods of experimentation, we have been attempting to amplify the specific region of the zebrafish genome that contains this gene which would allow us to genotype the fish correctly. These correctly genotyped fish could then be used for further research in regenerative and developmental biology by students in the Bailey lab.

How do we get the DNA?

DNA isolation via the Zymo Isolation kit. Zebrafish have regenerative abilities, meaning they can grow back parts of their bodies that have been damaged; this is part of the reason why we are studying them in the first place. While under anesthesia, a portion of the fish's tail is removed and that is what is used for DNA isolation. Within a week or two, the tail is fully healed and grown back. The tail clip is then run through a procedure that breaks down the tissue, and degrades proteins, leaving the DNA. Products of this procedure are tested for concentration and contamination before they are run through the PCR process.

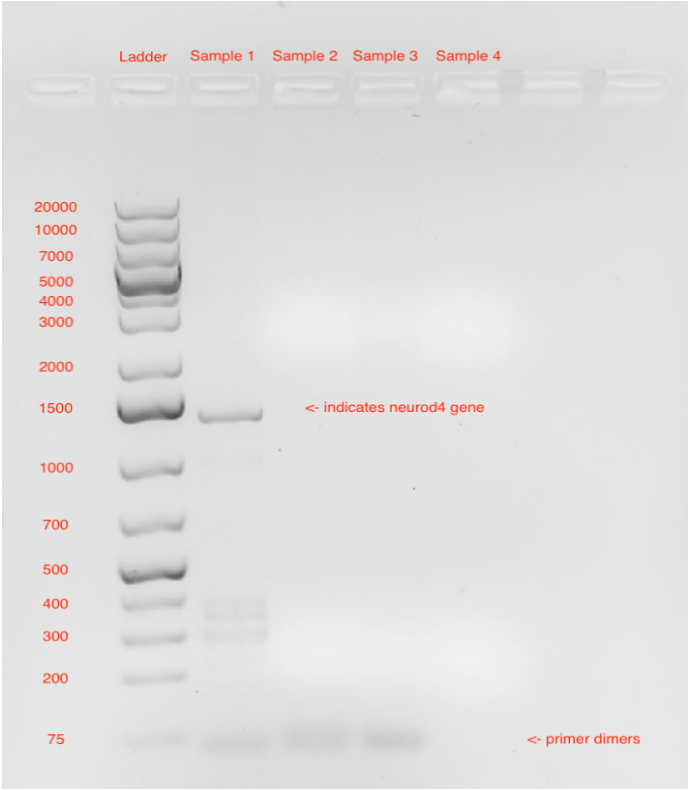
What is Neurod4 and why are we amplifying it?

It is a gene expressed mostly in progenitor cells and plays a part in the development of the cerebral cortex of the brain and in regions of the central nervous system. In addition to neural regulation, neurod4 also acts to regulate gene expression and has been seen to reprogram differentiated non-neuronal cells to neurons. When phosphorylated, it limits the ability to drive neuronal differentiation during neurogenesis: the process of the growth and development of nervous tissue (Hernandez-Miranda,Tarabykin, and Tutukova, 2021). Some of the zebrafish express the neurod4 gene while others do not; our aim is to figure out which ones are which by specifically amplifying the neurod4 region found in the fish using long-range PCR analysis. This will allow us to ensure that the fish are correctly genotyped which can be used to further future research on regeneration and development in the Bailey lab.

What is PCR?

Polymerase Chain Reaction (PCR) is a way to replicate DNA by mimicking the steps of DNA replication in a cell. Rather than using a helicase enzyme, PCR uses extreme temperatures (94C) to separate the strands of DNA. Then primers chosen for the region of interest bind to the DNA (annealing step at around 55C) which gives the polymerase something to work off of to add nucleotides (extension at 72C) and replicate the DNA segment. As long as both forward and reverse primers are added and enough extension time and nucleotides are given, PCR will copy the DNA segment each cycle. Different DNA polymerases can be used in PCR protocols depending on the length of the target region to be amplified, and the exact temperature and length of cycling. A typical PCR will run for about 40 cycles. Thus far PCR has failed to amplify the neurod4 gene in these fish, so we will attempt to find a procedure that works by varying the enzyme, primers, and buffer solution used as well as the lengths and temperatures of each step of the PCR cycle. This is less of a science than an art, we will essentially just be trying every combination of variables until something works

After PCR we will run agarose gel electrophoresis on the PCR products to determine if the neurod4 gene was amplified. Agarose gel electrophoresis separates nucleic acids by size and charge, so if the PCR works we'd expect to see a concentrated band representing the neurod4 region (sized by comparison to a ladder containing nucleic acids of known lengths).



Results and Next Steps:

From our PCR using the SuperFi-1 Polymerase we were able to get a band the right size to be the neurod4 gene. From the successful genomic PCR using the Platinum SuperFi Polymerase on sample one, we will extract the amplified DNA and send it to be sequenced commercially to verify that the PCR product is actually the neurod4 gene. It will likely take several weeks to get the results of the sequencing. In the meantime we will continue to experiment with the PCR procedure: testing the other polymerases to see if they could potentially work better to amplify the neurod4 region.

References

U.S. National Library of Medicine. (2023, January 8). *Neurod4 neuronal differentiation 4 [Danio rerio (zebrafish)] - gene - NCBI. National Center for Biotechnology Information. Retrieved February 23, 2023, from https://www.ncbi.nlm.nih.gov/gene/266958*

Hernandez-Miranda, L., R., Tarabykin, V., and Tutukova, S. (2021). The Role of Neurod Genes in Brain Development, Function, and Disease. *Frontiers in Molecular Neuroscience, 14.* doi: 10.3389/fnmol.2021.662774