Fluorescence In Situ Hybridization in Zebrafish with the Neurod4 Gene

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Abstract

Neurod4 is a protein coding gene, also known as neurogenic differentiation 4. This gene prevails within the nervous system, specifically expressed in the brain. Neurod4 helps control any extreme, rapid growth of the photoreceptors in zebrafish, *Danio rerio*, retina. Zebrafish have a similar, yet not identical gene to humans, thus, research done with zebrafish can correlate to how neurod4 impacts human retinal neuronal development. Zebrafish used in this study are transgenic organisms, containing the Tg (neurod4: GFP). Transgenes are sections of genetic material used for genome modification of specific organisms. Zebrafish were genetically manipulated to express neurod4. It is assumed that Tg will share similar activity as neurod4 gene, the transgene displays the same transcript expression as the endogenous gene. For Tg to be expressed, it is necessary to polymerase the RNA strand. If this were to work in zebrafish, we could then apply this understanding to human neuronal development studies.

Objective/Goal

The goal of this study is to find the localization and distribution rate of the neurod4 mRNA , as well as if the Tg in the zebrafish shares the same activity and function as the endogenous gene. Localizing where neurod4 is being expressed, will accept or reject our hypothesis, and the results will determine the significance that it holds in zebrafish and humans.

Materials and Methods

MiniPrep. We first conducted a series of Minipreps to collect DNA samples from bacteria. The bacteria possessed our gene of interest. We grew bacterial colonies in teriffic broth and ampicillin solutions then proceeded with the miniprep protocol.
DNA Digest. Then, we chose an enzyme to digest the DNA, resulting in a linearized plasmid. We chose to use NcoI restriction enzyme because it cuts once outside of important promotor regions.
Flourescent Dye-Labeled RNA Probe. The required materials utilized were provided in the Invitrogen FISH Tag ™ RNA Kit. The important protocols followed included Synthesis of Amine-Labeled RNA and Labeling the Amine-Modified RNA with Fluorescent Dye.

Results

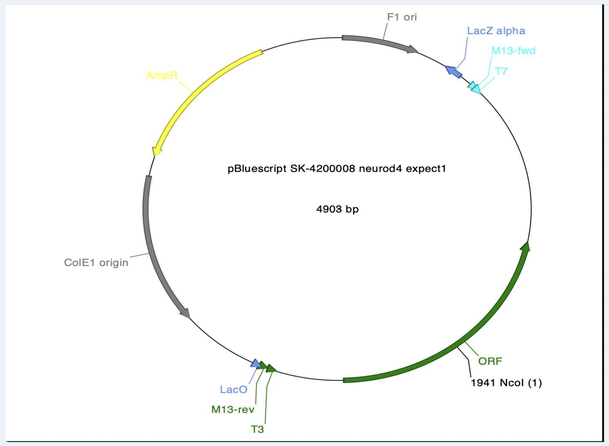

Figure 1. . A vector map indicating the NcoI enzyme cut site.

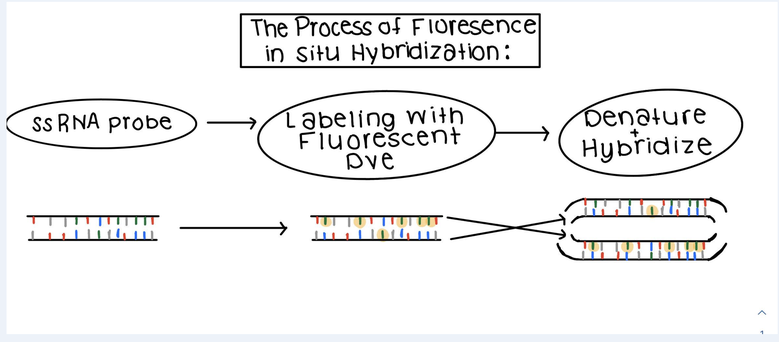

Figure 2. The Process of Florescence Hybridization, a step-by-step map.

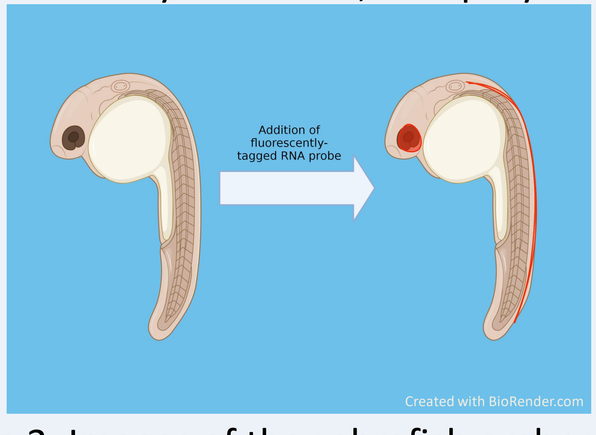

Figure 3. Images of the zebrafish embryos, hope of florescence being expressed by neurod4:GFP in the eyes and spinal cord. No expression shown in this run of the experiment.

Discussion

The Neurod4 gene is expressed primarily in the retina and spinal cord of zebrafish. Neurod4 converts endogenous neural stem cells to neurons with synaptic formation after spinal cord injury. Thus, this gene has a large stimulating function in neuron regeneration. We are attempting to understand neurod4's role in regeneration and development through in situ hybridization, visualizing the gene in embryos at different stages. The fluorescence of in situ hybridization allows us to specify one gene out of several as it will glow under red microscopic light.
After a couple of days, to quantify our sample, we used a spectrometer to get an IR from the digested DNA samples. All initial DNA sample concentrations were less than 60 ng/ml, which did not provide enough digested DNA to continue to the RNA probe-making protocol. Once successful in digesting enough DNA, we will be able to make an RNA probe capable of binding to embryonic tissues. Thus, it will provide confirmation that the neurod4 gene is functioning and producing mRNA in nervous tissues such as the retina and spinal cord. Once confirmed, we would need to perform more trials and test with antibodies for proper analysis. With a continuation in this experiment, we may be able to not only understand the neurod4 gene in zebrafish, but multiple genes of the same family within other organisms.

References

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