Embryo Microinjection of CRISPR-Cas9 into a Caenorhabditis elegans model using the C9ORF72 ortholog: Alfa-1

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative and ultimately fatal disorder. ALS can be described as the degradation of motor neurons and will end the life of patients typically within 3-5 years of being diagnosed. The most common cause of death among victims of ALS is respiratory failure. Respiratory failure is the result of the progression of ALS to the lungs causing the nervous system (motor neurons) to become unable to operate the patient's lungs. There are two different categories of ALS: familial and sporadic. Familial ALS (FALS) is the effect of a genetic disposition. In contrast, Sporadic ALS (SALS) is the effect of an unpredictable modification to the genes involved in the product of ALS. Approximately 25-40% of FALS cases and 7% of SALS cases are caused by an alteration in the C9ORF72 (Chromosome 9 Open Reading Frame 72) gene. In a human without ALS, the C9ORF72 gene is composed of a 6 nucleotide DNA sequence, four guanines followed by two cytosines (GGGGCC). However, in patients with ALS, this sequence is repeated numerous times (known as a hexanucleotide repeat expansion (HRE)). It is still unclear how many times the HRE must be repeated to produce ALS, but it is believed that when the sequence is repeated more than 30 times, the organism will develop this disorder. Researchers are also uncertain if the HRE reduces the function of the transcribed protein or if it creates an abnormal protein from the mutated C9ORF72 gene, but it has been confirmed that the HRE can lead to ubiquitin-protein aggregates. Ubiquitin-protein aggregates are misfolded proteins that are prone to clustering into a dysfunctional form of the desired protein. Unlike other neurodegenerative diseases (e.g. Huntington's, Alzheimer's, and Parkinson's) that are also caused by ubiquitin-protein aggregates, the proteins transcribed from the modified C9ORF72 gene cause premature cell death in motor neurons.

In this study, the ortholog of the C9ORF72 gene in Caenorhabditis elegans, *alfa-1*, will be genetically modified using the microinjection technique to administer the CRISPR-Cas9. Microinjection is a fairly new and uncommon method of CRISPR-Cas9 delivery in C. elegans. In the past, microinjection has been used when executing RNAi with C. elegans, but not CRISPR-Cas9. To create the needles for microinjection, Pasteur Pipettes will be under external conditions causing the needle to become soft and able to be pulled to create a needle with a point of ≥ 1 µm in diameter. The Cas-9 injection mix will be injected into the ovary of a hermaphrodite C. elegans. Once the embryos of the injected hermaphrodites hatch, their DNA will then be isolated and run in 2% gel electrophoresis to determine whether microinjection with CRISPR-Cas9 is an efficient method of targeting and modifying the *alfa-1* gene of C. elegans.