Determining if a Novel CRISPR Enzyme Can Efficiently Induce Deletions of Hexanucleotide G4C2 Repeat Mutations in the ALS Gene C9ORF 72 *In Vitro*

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CRISPR technology utilizes the enzyme Cas 9 from various bacteria to act an efficient endonuclease in inducing deletions and NHEJ of DNA. However, AAVs can only package ~ 4.7 kb of DNA, and the DNA required to produce Cas9 is greater than 4.7 kb. Cas 12f, a smaller Cas enzyme, can fit in AAV vectors. In this study, we sought to determine if Cas12f can efficiently induce deletions of G4C2 Repeat Mutations in the ALS Gene C9ORF 72 *in vitro.* Plasmids containing the DNA for Cas12f production were digested by restriction enzyme and then purified, and gRNA polynucleotide sequences were ligated to the leftover backbone of the plasmid. Bacterial cells were then transformed utilizing the new plasmid, cultured and then the plasmid DNA was harvested utilizing Qiagen Mini-Prep and Large-Prep kits. HEK293T cells were then transfected with various gRNA and Cas 12f pairings. gDNA (genomic DNA) was then harvested from the cells and amplified utilizing PCR. Gels were run to observe any editing from the wild type. Simultaneously, another plate of the same HEK293T cells were being selected with Puromycin, and the selected HEK293T cells underwent the same procedures as above. The significance of this study is that if Cas12f is efficient in producing deletions, that gene therapy clinical trials utilizing AAVs could be more efficient. The study is still ongoing, so future experiments include comparing Cas9 and Cas12f efficiencies, mRNA deliveries of Cas12f, and/or different methods of delivering Cas12f DNA such as using lipid nanoparticles. Supported by R25ES020721 and the Rutgers Office for Research and Economic Development.