**Name(s)**
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**Project Number**
S0506

**Project Title**
# Augmenting Expression of Gene Editing CRISPR-SaCas9 through Codon Optimization to Boost in vivo Therapeutic Application

**Abstract**

**Objectives/Goals**
CRISPR-Cas9 shows great promise in genetic medicine, but the current focus on treatment by ex vivo delivery is limited to a few diseases. In vivo treatment can substantially extend the reach of the technology but is limited by a challenging delivery process. Our project aims to compensate for the low delivery efficiency by utilizing codon optimization to increase the expression levels of the Cas9 gene. A success has great potential to increase the viability of in vivo CRISPR-Cas9 treatments.

**Methods/Materials**
HepG2 liver cells were purchased from Sigma. A commonly used and small SaCas9 sequence was selected as a reference. A codon-optimized novel sequence was then engineered and synthesized through Genscript and cloned in the pcDNA3.1 vector. Plasmids were transfected into HepG2 cells, and protein expression was confirmed by viewing GFP under a fluorescent microscope. Samples were then run through ELISA assays to quantify and compare the SaCas9 expression levels.

**Results**
HepG2 liver cells were successfully cultured in a sterile environment. SaCas9 codon optimization using Genscript's OptimumGeneTM algorithm improved the Codon Adaptation Index from 0.81 to 0.96 and the Optimal Codon Frequency from 53% to 82%. HepG2 transfection and SaCas9 protein expression succeeded with high efficiency as visualized by GFP-tagged saCas9 under a fluorescent microscope. ELISA analysis of two sample groups demonstrated that the codon optimized gene expressed the saCas9 protein 3.5x the rate of the commercial sequence.

**Conclusions/Discussion**
Addgene's public plasmid database was efficiently utilized to identify saCas9 sequences from literature. Genscript's synthetic biology services were successfully used as a method of codon optimization and gene synthesis. Liver cell culture, plasmid transfection, and protein visualization and quantification have been established at Nueva. The novel SaCas9 sequence engineered demonstrated a 3.5-fold increase in expression and has the potential to greater enable in vivo CRISPR-Cas9 treatments.

**Summary Statement**
The project successfully increased Cas9 protein expression by 3.5x in human liver cells through codon optimization, which can facilitate the success of in vivo CRISPR-Cas9 gene editing treatments for a much broader range of genetic diseases

**Help Received**
Our teacher, Luke De, acted as our mentor and supervisor for this project, providing general guidance, and the Nueva School provided funding.