Assessing the Impacts of CRISPR Editing on Protein Expression; Utilizing dCas9 as a Potential Roadblock to DNA Transcription

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The field of genetic engineering is rapidly developing with exciting new discoveries being made every day. However, existing gene editing technologies do not have perfect accuracy, and create permanent edits to the genome. Off target effects, or DNA editing at an unintended locus, can have severe consequences including the introduction of mutations into the genome. Our technology of interest is dCas9 - a programmable protein that can bind to specific regions of the genome using a small guide RNA. dCas9 is a valuable tool for targeting specific genes, and we hypothesize that by binding to these genes dCas9 will inhibit DNA polymerase from copying the gene and thus reduce the overall level of protein expression. We targeted dCas9 to the promoter and start codon of a cyan fluorescent protein. dCas9 induced ~38% repression when targeting the open reading frame as compared to Cas9 with ~57% repression. We believe that dCas9 is a protein worth further study as it does not permanently edit DNA, and this substantially reduces the chance of DNA mutation. We are currently expanding our study to endogenous genes including ROSA26 and CCR5. We envision that dCas9 could eventually be administered in the form of a therapy that enables patients to control levels of protein expression within their own bodies.

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