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Class of 2019

Genetics & Biotechnology, Forensic Science

Evaluating the Efficiency of Transgene Knock-In by CRISPR-Cas9 Mediated Nonhomologous End Joining

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CRISPR/Cas9 genome engineering has received a significant amount of attention in recent years for its ability to cut DNA at a specific site. This technology has the potential for genetic research, biotechnology, as well as in medicine for treatment of many genetic diseases such as cancer. However, current methodologies for DNA integration into a specific target region typically do not have high enough efficiency for use in human models. The high levels of off-target effects present a problem for the system's use *in vivo* as well. The development of methodologies which allow for efficient CRISPR mediated knock-in transgenesis with zero or very low off targeting effects is essential in optimizing this technology. Zebrafish are a powerful model for studying CRISPR-based technologies, since the embryonic development of the organism can be closely monitored, in a relatively short span of time. To visualize a phenotypic change as a result of CRISPR mediated transgenesis, the gene SLC45A2 was chosen to be eliminated for this study leading to a "knock-out" site. Major alteration and mutation of this locus will cause albinism. Insertion of a reporter gene such as green fluorescent protein (GFP) under a strong ubiquitous promoter into this site, would indicate a successful knock in. The goals of this research were to knock in a large reporter gene by Non-homologous end joining (NHEJ) utilizing a two sgRNA system previously reported and to compare to a novel strategy utilizing a single sgRNA targeting both the SLC45A2 and the plasmid carrying the reporter gene. By reducing the number of variables involved in integration of the donor, we hope to improve the efficiency of integration and reduce the potential off targeting effects in an effort to optimize this system for future research.

Our findings suggest that CRISPR Cas9 mediated NHEJ can be utilized to integrated large reporter genes with high efficiency. Some researchers have reported success with integration of donor plasmids up to 5 kilobase pairs(kb). This research has demonstrated that integration is possible with much larger fragments, upwards of 9kb. Future experiments have been designed to analyze the extent of off targeting effects and to determine if our novel method of integration shows a reduction in off targeting and an increase in efficiency of integration. PCR amplification of SLC45A2 cut site will be utilized to confirm integration of GFP. Additionally, qPCR will be used to compare the single and double sgRNA methods; to determine which shows greater efficiency of integration.