Enhancer Biology

Enhancers are cis-regulatory elements (CREs) that enable precise spatiotemporal patterns of gene expression during development and are notable for being able to function at large distances from their target genes. This research looked at a transcriptional enhancer of the ZFPM2 gene (zinc finger protein, FOG family member 2), which modulates the activity of GATA family proteins, which are important regulators of blood and heart tissue cell formation.

Methods

Find all potential genomic sites of the form 5'-N<sub>18</sub>NGG-3' near intended target site using ZiFit.

Look for off-target binding sites using the ZiFit Target Predictor.

Incorporate 16-17 basepairs of the target site into two 58mer oligonucleotides (forward and reverse).

Use Gibson assembly to incorporate the 100 bp DNA fragment into the gRNA cloning vector.

Transfer Top10 Z competent cells using 3-6 μl of the Gibson Assembly reaction, shake for one hour, then spread on Kanamycin plate.

Run 5 μl on 2% agarose gel to confirm assembly, and clean up the rest using a Qiagen PCR Cleanup Kit.

Use 9700 PCR machine to anneal the two oligos and extend them to make 100 bp double stranded DNA fragment using Phusion polymerase (NEB).

Send 10 μl of one correct done per gRNA to Operon for sequencing and sequence analysis.

Results

Confirmation of Gibson-assembled vector (band around 540 bp instead of 360 bp).

Sequence trace file showing the inserted gRNA from Operon.

Introduction and Background

CRISPR (clustered regularly interspaced palindromic repeats) contain short repetitions of basepairs in DNA. CRISPRs are often associated with cas genes, which code for proteins related to CRISPRs. CRISPR-Cas systems are prokaryotic immune systems, recognizing and cutting out exogenous genetic elements. CRISPR-Cas systems are capable of being used to create targeted modifications in the genomes of many eukaryotes. CRISPR-Cas systems rely on short guide RNAs (gRNAs) in association with Cas proteins to direct degradation of complementary sequences. These engineered systems greatly increase the ease and efficiency of genome editing. The purpose of this research was to use CRISPR-Cas genome editing in order to characterize cis-regulatory elements (CREs) in the non-coding genome. Specifically, this research looked at how to characterize distal enhancers through loss of function analysis via qPCR.

Future Directions

This research was successful in designing and creating six gRNAs and assembling them into vectors. Future directions include successfully nucleofecting K562 cells with three plasmids (gRNA vector, Cas gene, and GFP gene). After successful nucleofection, qPCR with primers designed to the “left” of the enhancer, in the “middle” of the enhancer, and to the “right” of the enhancer will confirm deletion of the enhancer when there is little amplification of the enhancer targeted by the “middle” primer. Transcriptional analysis “loss of function” testing would then be conducted.

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