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Comparative Analysis of Single vs. Multiple gRNA on NGN3 Expression Using a Controllable dCas9-VP192 Activator (CRISPRa)

Authors : Nicholas Abdilmasih, Habib Rezanejad

Abstract : This study investigates the gene expression induction efficiency of single versus multiple guide RNAs (gRNAs) targeting the NGN3 gene using the CRISPR activation system in HEK293 cells. Our study aimed to contribute to optimizing the use of gRNAs in gene therapy applications, particularly in treating diseases like diabetes, where precise gene regulation is essential. The experimental design involves culturing HEK293 cells, and once they reach approximately 70-80% confluence, cells were transfected with specific gRNAs targeting the NGN3 gene promoter. Specific gRNAs targeting the NGN3 promoter that was previously designed, incorporated into plasmid clone cassettes and introduced into HEK293 cells through co-transfection using pCAG-DDdCas9-VP192-EGFP transactivator. Post-transfection, cell viability, and fluorescence were monitored to assess transfection efficiency. RNA was extracted, converted to cDNA, and analyzed via qPCR to measure NGN3 expression levels. Results indicated that specific combinations of fewer gRNAs led to higher NGN3 activation compared to multiple gRNAs, challenging the assumption that more gRNAs result in synergistic gene activation. These findings suggest that optimized gRNA combinations can enhance gene therapy efficiency, potentially leading to more effective treatments for conditions like diabetes.

Keywords : CRISPR activation, Diabetes mellitus, gene therapy, guide RNA, Neurogenin3
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