Directed Evolution of Genetic Regulatory Elements using CRISPR/Cas Genome Engineering

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Overview
Gene regulation in humans is driven by a complex network of genetic regulatory elements that are responsible for turning genes on and off in a cell type specific manner using both spatial and temporal control. These regulatory elements are often conserved across species but many are unique to humans. To uncover the evolutionary ability of human specific enhancers to arise and examine the ability of cell type specific enhancers to switch specificity, we propose a system to create thousands of new DNA mutations using CRISPR/Cas mediated genome engineering.

Results: Cas9 Optimization triples HDR rate

Figure 1A. gRNA Efficiency

Figure 1B. HDR Efficiency Across Conditions

Figure 1C. Integration Efficiency by Distance

Relevance
Distal enhancers are essential in development and cell-type specific gene expression patterns. Given their complex nature, enhancer variants have the potential to lead to a broad range of phenotypic variation and common disease risk. Regulatory elements often have cumulative or redundant function and, consequently, mutations in many of these elements may have low fitness burdens allowing them to reach higher than expected frequencies. This suggests that mutations in enhancer and other regulatory regions are excellent candidates for factors contributing to common, polygenic disease risk. This research will add to the body of knowledge used to characterize human enhancer elements and the evolution of novel variants allowing more accurate predictions of regulatory sites, function, and consequences of mutation.

Future Directions
Donor DNA libraries will efficiently introduce multiple mutations in 5 loci within two commonly used human cell lines. Transfected cells will then undergo Dnase hypersensitivity and CHIP-seq assays to identify biochemical signatures unique to enhancers, and DNA sequencing to determine the number and type of mutations that create de novo enhancer elements or switch cell type specificity.

Method for Directed Evolution
Selection of Non-Enhancer Candidate Regions and CRISPR/Cas guide RNA design

Optimization of Conditions for Homology Directed Repair

Machine Learning based Design of Variant Libraries

AGTCGTTCACGCTGTGTAAT
GGGCCGTAAGCCGCTCATT
AGTCGTTCACGCTGTGTAATATAGCGCTCATT
AGTCGTTCACGCTGTGTAATTAGCGCTCATT
AGTCGTTCAGCGCTGTGTAATAGCGCTCATT

CRISPR/Cas9 Mediated Genome Engineering of Candidate Enhancer Cell Library

Biochemical Screen for Enhancer Function (Dnase Hypersensitivity and Chip-seq) and Quantitative Sequence Analysis

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