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Research Horizons Day
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CRISPR-Cas Mediated Genome Engineering of Genetic Regulatory Elements

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Overview

Gene regulation in humans is driven by a complex series of "switches", or genetic regulatory elements, that are responsible for turning genes on and off.

A fraction of these regulatory elements, including enhancers, are unique to humans, or have switched cell type specificity during evolution.

To uncover the evolution of human enhancers, we developed a system to create thousands of new DNA mutations using CRISPR/Cas mediated genome engineering.

Hypothesis: We can model the path of DNA mutations that create unique or repurposed enhancers to better understand the evolution of gene regulation in mammals.

Figure 1. Classes of Mammalian Enhancers

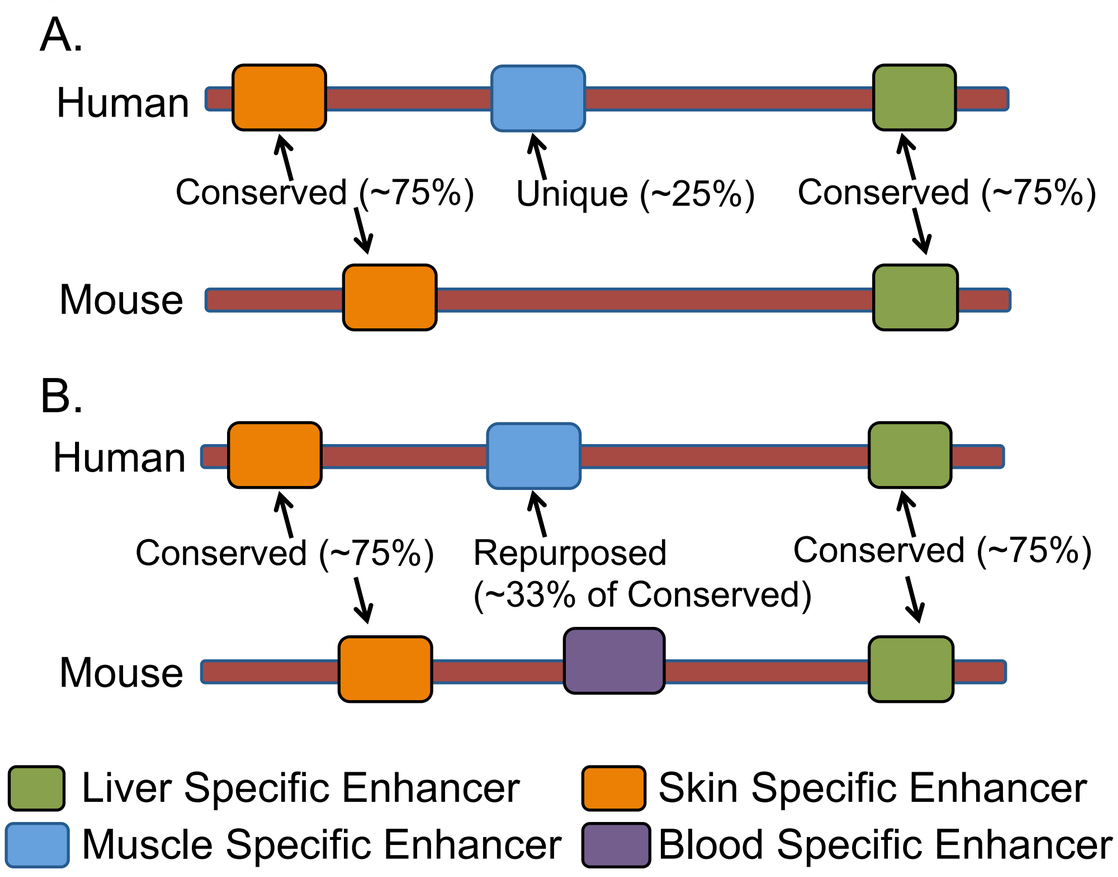


Figure 1. (A) Genome wide analysis has shown most enhancers are conserved across mammalian species (~75%) while a a minor fraction are unique to humans. (B) Of the conserved enhancers, many appear to have been repurposed during evolution for regulation in an alternate cell type. (Denas et al. 2014)

Figure 2. Method for Directed Evolution

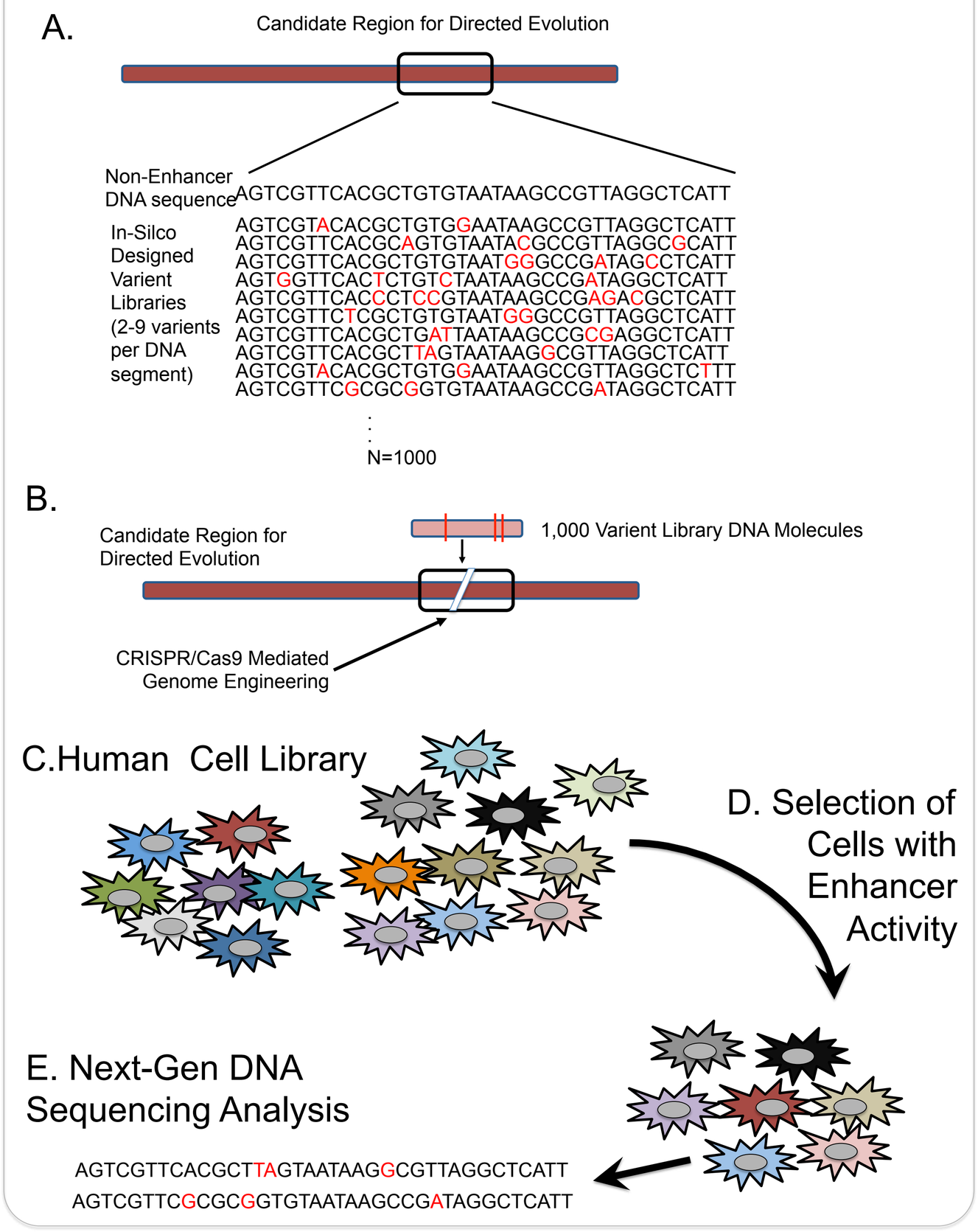
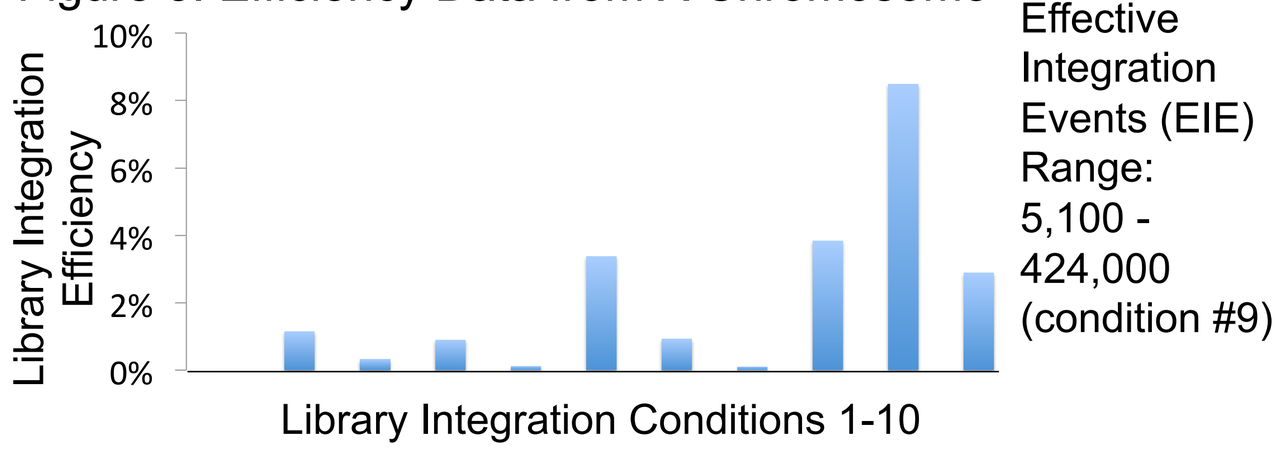


Figure 3. Efficiency Data from X Chromosome



Key Findings

Crispr/Cas9 Mediated Genome Engineering can be used to incorporate up to 400,000 DNA variants (400X Coverage) into K562 cells. These variants can be isolated by PCR and quantitatively measured by Next-Gen DNA Sequencing.

Impact

A robust method to test a significant number of DNA variants to model the mechanisms of mammalian enhancer evolution.

Future Directions

The next set of experiments will be to test biochemical aspects of regulatory elements and determine the number and type of mutations that create new regulatory elements.

Acknowledgements

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