Randomizing Gene Regulatory Regions Using CRISPR/Cas9 Prime Editing And The Cre Lox System

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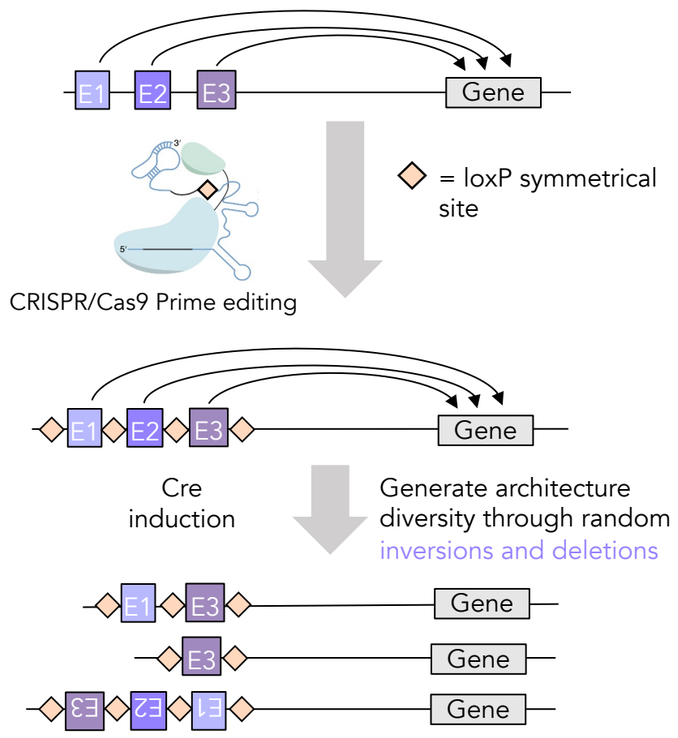
Introduction

Gene expression is commonly regulated by multiple regulatory elements. We combine Prime Editing (PE) and the Cre-Lox system to rearrange endogenous enhancer clusters.



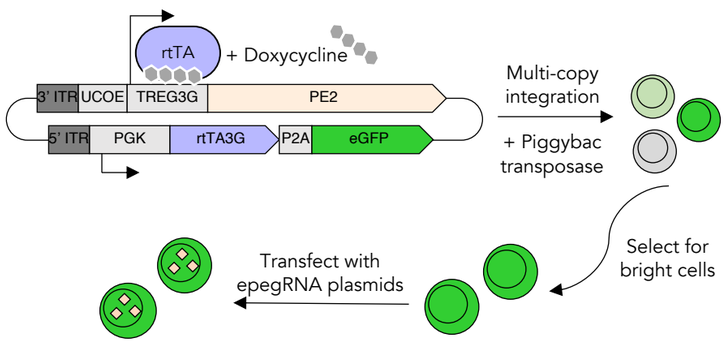
How do individual enhancers interact and how does their spacing and relative orientations drive gene expression ?

Project Outline

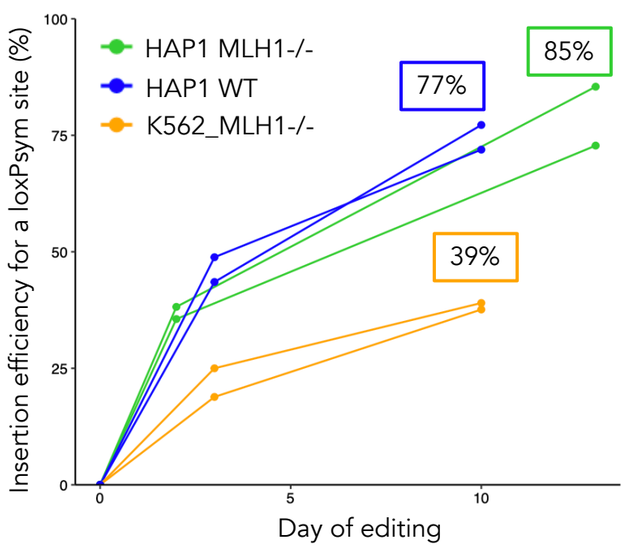
1. Generate efficient prime editing cell lines
2. Pick interesting enhancer clusters
3. Insert loxPsym sites between enhancers
4. Trigger stochastic rearrangements and long reads sequencing

1. Prime Editing Cell Lines

Generation of a prime editing cell line



Prime editing efficiency of cell lines



Efficiency of inserting a **loxPsym** site into the **HEK3 locus** using prime editing

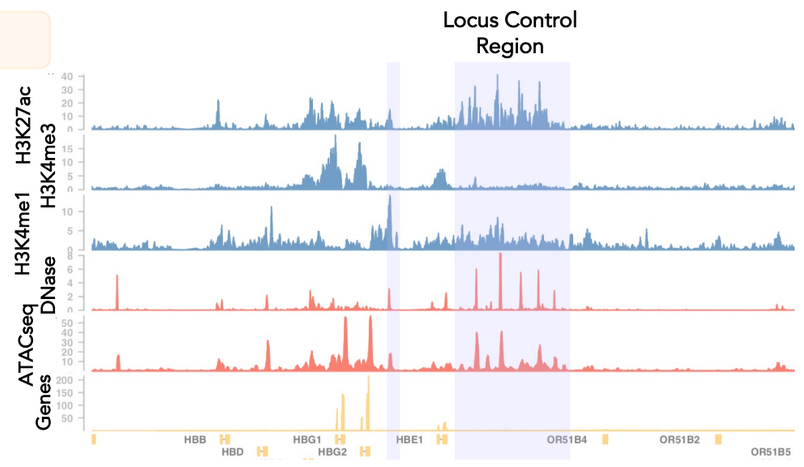
2. Enhancer Cluster Target Discovery

1 Beta-globin Locus in K562 cells

Chromatin datasets in K562

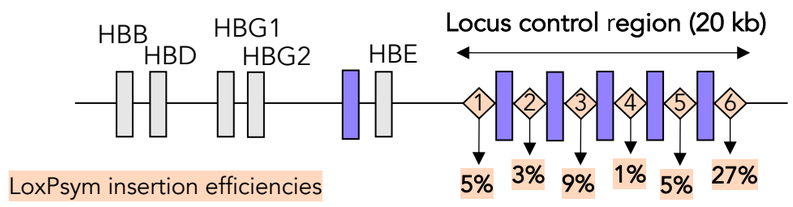
Beta-Globin Locus Control Region

- Cluster of 5 enhancers
- Beta-globin locus

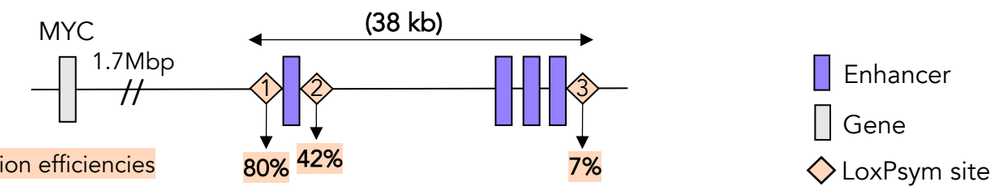


3. Insertion Of LoxPsym Sites Between Enhancers

1 Beta-globin Locus in K562 cells



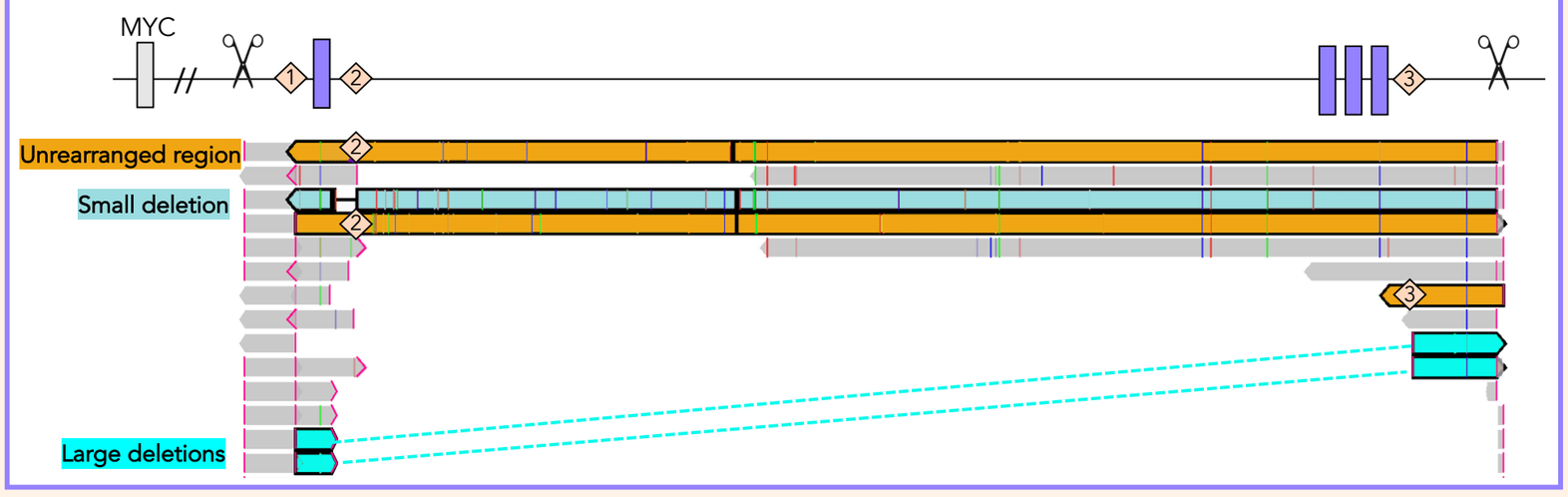
2 MYC Locus in HAP1 MLH1-/- cells



4. Rearrangements And Long Reads Sequencing (>15kb)

2 MYC Locus in HAP1 MLH1-/- cells

- Induction with Cre recombinase in MYC enhancer cluster
- Sequencing of the new enhancer architecture with Oxford Nanopore Cas9-enrichment technology



Conclusions

- Prime editing can be used to efficiently insert loxPsym sites in multiple cell lines
- Cre recombinase can generate diverse enhancer architectures
- Cas9-enrichment with Oxford Nanopore sequencing can capture enhancer architectures
- This strategy could be reproduced for other enhancer clusters and in different cell lines

Anzalone AV, Randolph PB, Davis JR, et al. (2019) *Nature.* 576(7785):149-157. **Nelson JW, Randolph PB, Shen SP, et al.** (2022) *Nat Biotechnol.* 40(3):402-410. **Chen PJ, Hussmann JA, Yan J, et al.** (2021) *Cell.* 184(22):5635-5652.e29. **Gilpatrick T, Lee I, Graham JE, et al.** (2020) *Nat Biotechnol.* 38(4):433-438.

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