Understanding Rules for Engineering Large Genomically Integrated Mammalian Gene Circuits

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Introduction

- Synthetic biology aims to engineer human cells capable of conducting therapeutic functions
- DNA-level manipulations are used to achieve a desired cellular phenotype where a variety of stimuli can trigger a diverse array of responses
- These manipulations are limited by a lack of understanding of parts and their relationships to one another in expression units and arrays of expression units

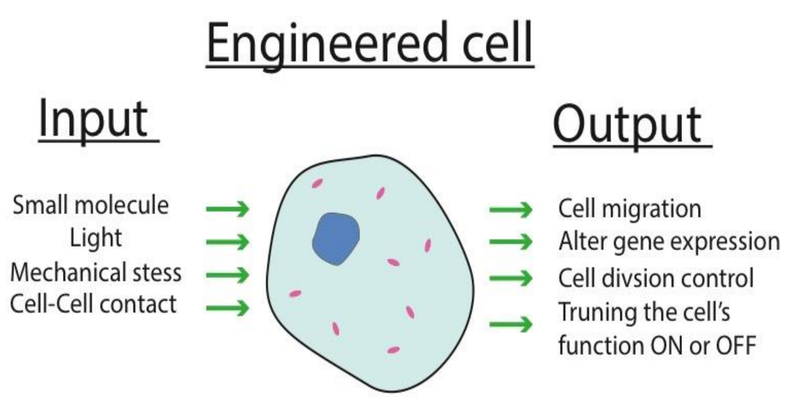


Figure 1: Inputs and outputs of an engineered cell

Approach and objectives

- TypeII restriction enzyme-based cloning allows rapid generation of a modular library of individual expression units containing unique combinations of parts: chromatin insulator, promoter, open reading frame, RNA stabilization domain, and terminator
- With this framework we seek to determine rules for performance and compatibility of these parts.
- We hypothesize that chromatin insulator-promoter pairs will exhibit differential expression.
- We focused on testing combinations of chromatin insulators and promoters driving expression of mCherry. Output fluorescence was quantified via flow cytometry.

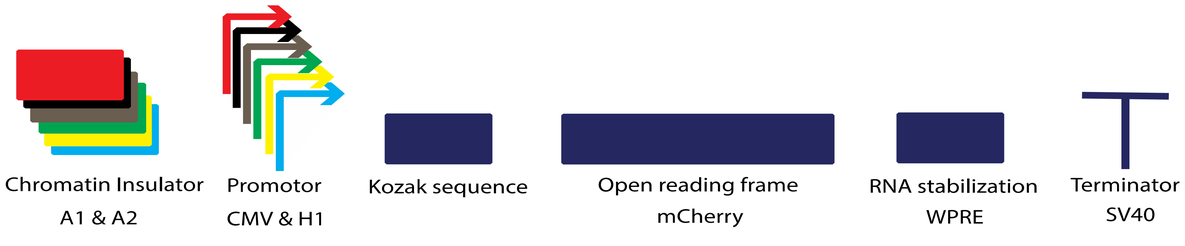
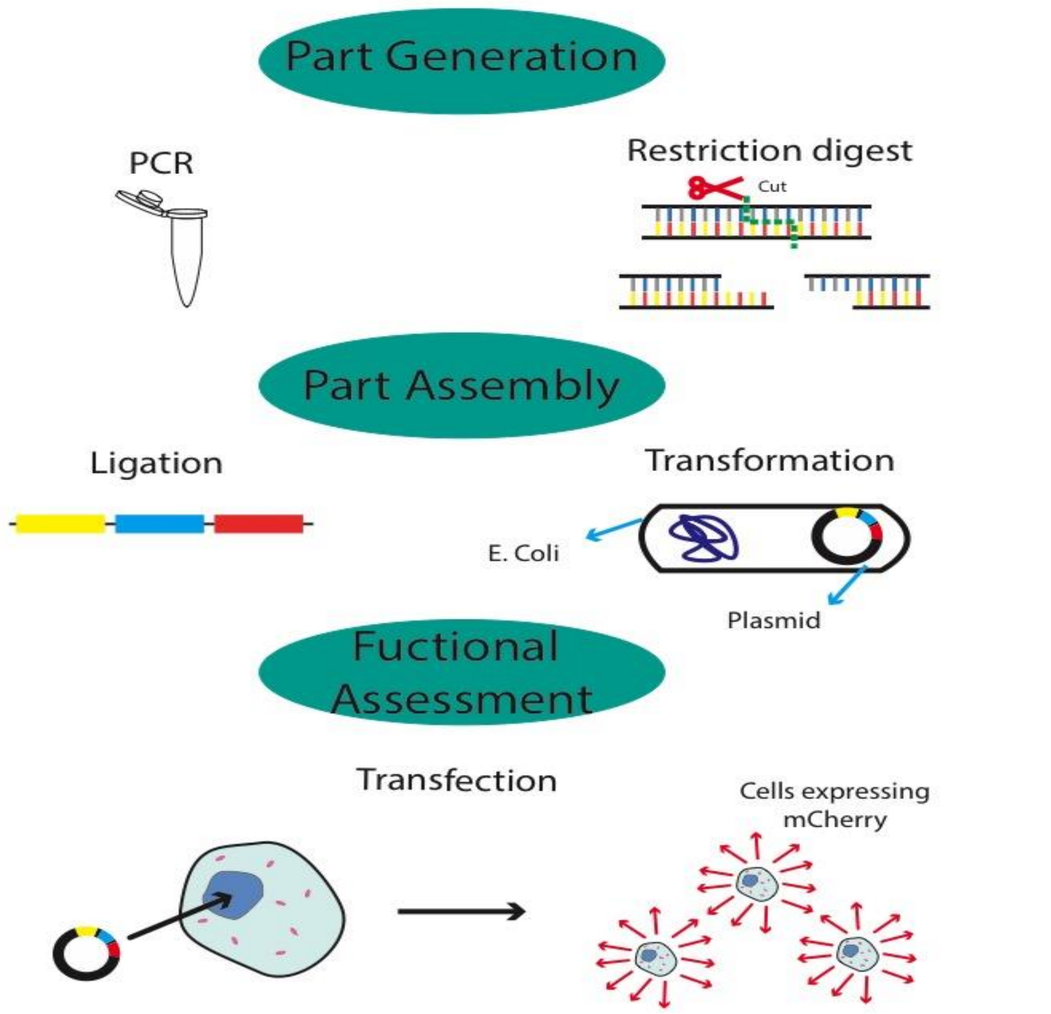


Figure 2: Modular expression unit to understand relationships between genetic parts

Methods



Results

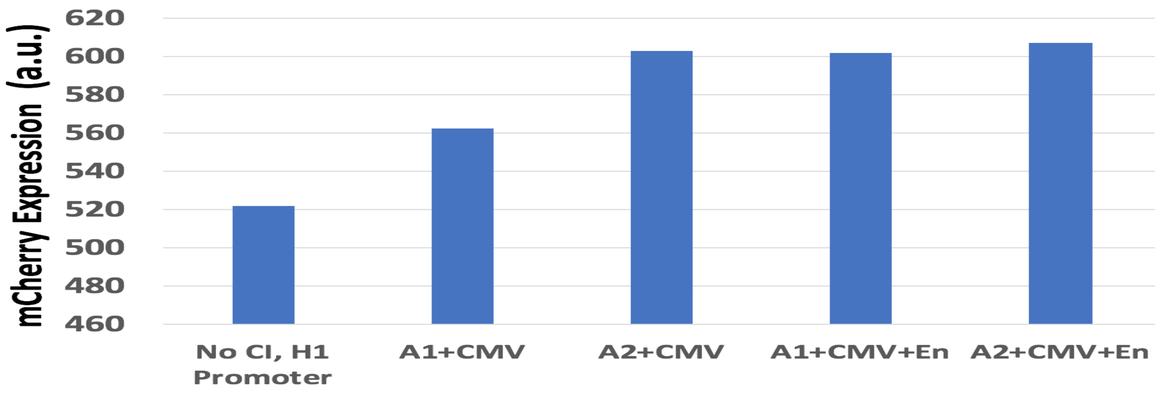


Figure 3: Magnitude of mCherry expression is dependent upon the promoter and chromatin insulator pair that drives it.

Conclusion

Understanding these context rules will help us reach the ultimate goal of predictively tunable multi-gene circuitry that can support engineered therapeutic function