A Platform for Designing Neuron, Astrocyte and Hepatocyte Specific Promoters for Gene Therapy

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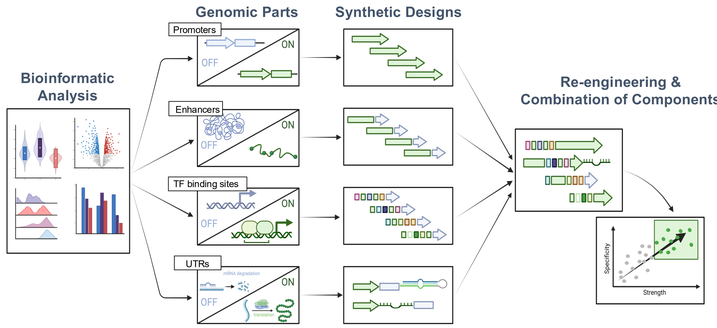
Promoter Discovery Platform



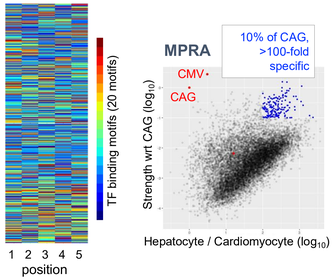
Overview of Promoter Discovery Platform. Extensive bioinformatics analysis in ON and OFF target cell types or states yields sets of genomic parts (promoters, enhancers, transcription factor (TF) binding sites and UTRs) used to create initial synthetic promoter designs. These are based on derivatized natural promoter, enhancer and UTR sequences used in arrayed screens, as well as combinatorial libraries of TF binding sites and/or other elements used to identify candidates in a Massively Parallel Reporter Assay (MPRA). High performing sequences are then combined in an iterative manner to derive final synthetic designs with the desired strength and specificity metrics.

Hepatocyte Specific Promoters

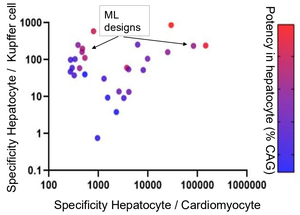
ON in Hepatocytes
OFF in Cardiomyocytes and Kupffer cells

Promoter profile

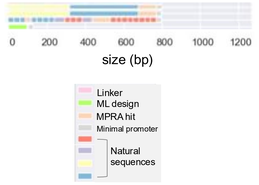
TF binding motifs library (ca. 16,000 elements)



Final promoter metrics



Final promoters structural features



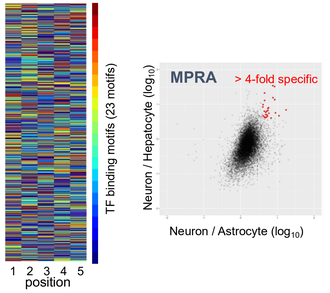
Design of hepatocyte specific promoters. The same general strategy used in the design of neuron and astrocyte specific promoters (right panel) was also applied for this promoter profile. Some of the final designs were also informed by regression machine learning (ML) models utilizing data from the analysis of the MPRA library by mapping DNA sequence to promoter metrics (ML design, green on the structural features panel).

Motivation

Gene therapies that rely on the targeted expression of transgene(s) in specific cell type, organ or system, have become more prevalent and important in the treatment of various diseases. However, limiting the expression of therapeutic transgenes to the intended ON-target cell types remains a challenge. Risks associated with transgene(s) expression in OFF-target cell types include immunogenicity and/or inflammatory responses. In this work, we developed highly specific, potent and compact synthetic promoters for three clinically relevant cell types - neurons, astrocytes, and hepatocytes.

Neuron Specific Promoters

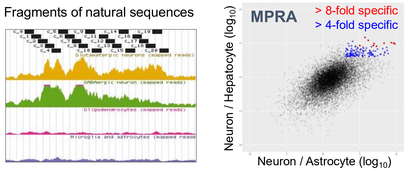
TF binding motifs library (ca. 16,000 elements)



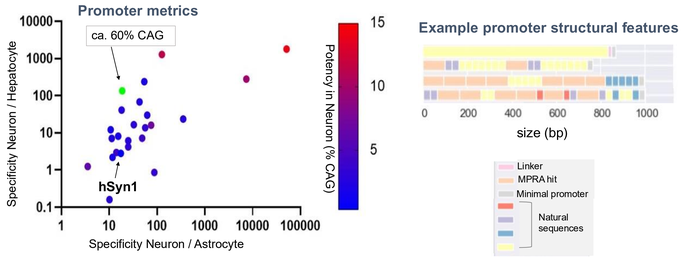
Promoter profile

ON in Neurons
OFF in Astrocytes and Hepatocytes

Enhancer fragments library (ca. 15,000 elements)

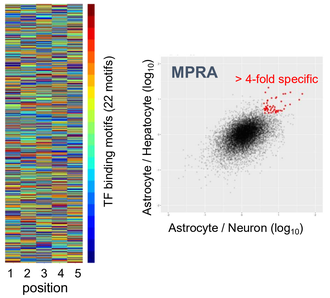


Final promoters



Astrocyte Specific Promoters

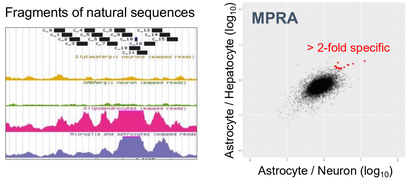
TF binding motifs library (ca. 16,000 elements)



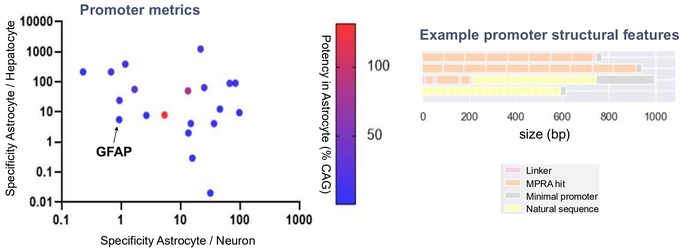
Promoter profile

ON in Astrocytes
OFF in Neurons and Hepatocytes

Enhancer fragments library (ca. 16,000 elements)



Final promoters



Design of neuronal (top) and astrocyte (bottom) specific promoters. For each promoter OMICs data was analyzed to identify natural DNA sequences (promoters and enhancers) displaying the desired expression patterns. These were then derivatized and tested individually to define parts to be used in downstream synthetic promoter designs. Additionally, for each promoter profile, two MPRA libraries were also constructed: (1) TF binding motifs arrays (left), and (2) overlapping segments of genomic regions exhibiting the desirable chromatin accessibility between ON and OFF target cell types. Initial sets of hits were identified from both libraries were identified using Massively Parallel Reporter Assay (MPRA)(scatter plot insets) and used for downstream designs. Synthetic promoters were constructed iteratively by combining parts until the desired target metrics were achieved (strength and specificity). In both cases a number of the final designs (right) meet or exceed the target metrics. For all profiles, relevant cell models were used as stand-ins for ON and OFF target cell types during the design and screening process.

Conclusion

Our multi-pronged promoter design platform is capable of reliably designing potent, compact and highly specific promoters relevant in the Gene and Cell therapy space. The platform relies on combining bioinformatics analysis, machine learning (ML) and GeneFab's expertise in engineering diverse and challenging cell types. Here we present three examples of utilizing it to design such promoters for neural tissues (neurons and astrocytes), and hepatocytes.