A Novel Genomics-Based Platform for the Creation of Environmental-Responsive Gene Promoters

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ABSTRACT

We have developed a gene promoter design and construction platform that integrates data derived from large-scale functional genomics datasets and employs machine learning algorithms in data analysis with a view to identify novel regulatory elements.

Elements are ranked according to many distinct criteria and subsequently used as component parts in synthetic promoter construction, using engineering biology principles.

Here we introduce the platform and present results detailing how we have employed it to create cell type selective promoters in muscle, liver and retina target cell populations.

In the liver, the activity of our synthetic promoter candidates is significantly higher than currently used industry standard liver-selective promoters.

In muscle cells, we show that promoters are active in mature differentiated myotubes and we are further able to show a large dynamic range of activity.

RPE-selective promoter candidates are several-fold higher in vitro than is achievable with endogenous RPE-specific promoters such as RPE65, CRALBP and Bestrophin.

We have also used the platform to generate novel inducible promoters responsive to different chemical and biological stimuli, and whose activity is stimulated solely with the addition of an inducer, without the requirement for the co-expression of a trans-activator.

GENE & CELL THERAPY

1. Liver Promoters

2. Muscle Promoters

3. RPE Promoters

4. Ubiquitous Promoters

5. Inducible Promoters
   a. Single Element Induction
   b. Multiple Element Induction – Logic Gate

CONCLUSIONS

We present data showing promoters active in different cell types with large dynamic ranges of activity

Promoters range in size from 150bp upwards; can include UTRs and introns

Promoters mediate highly selective gene regulation in the target cell type

Different types of elements can be combined to create logic gates based on transcriptional regulation.

In summary, we have developed a novel genomics-based platform that enables the rational design, synthesis and testing of mammalian gene regulatory elements, and whose output can help support the construction of the next generation of cell and gene therapeutics.

REFERENCES