Massively Parallel and Systematic Engineering Platform for Highly Compact, Cell-type Specific, and Potent Smart Sensor Promoters for Precision Retinal Gene Therapies

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Disclosures

Magdalena Cichewicz is a paid employee of Senti Biosciences, Inc.
Gene therapies are now a proven therapeutic modality for ocular diseases, including Leber congenital amaurosis type 2.

However, ectopic expression of transgenes, e.g. photoreceptor-specific proteins in RPE, creates the potential for toxicities/off-target effects in Ocular-directed gene therapy.


Axons of Retinal Ganglion Cells

<table>
<thead>
<tr>
<th>Cell type of interest:</th>
<th>ON-target cell line:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photoreceptors</td>
<td>Y79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OFF-target cells:</th>
<th>OFF-target cell line:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinal pigment epithelium</td>
<td>ARPE-19</td>
</tr>
</tbody>
</table>

https://www.labiotech.eu/in-depth/gene-therapy-blindness-cure/
Smart Sensor Promoters are Designed to Address Key Challenges in Gene Therapy

### AAV Gene Therapy with Cell Type-Specific Smart Sensor

**Spark**

Collaboration for gene therapies

- **AAV Capsid**
- **Senti Synthetic Promoter**
- **Therapeutic Payload**

### Synthetic Promoter Performance Profile:

- **High payload expression in ON Target cell type**
- **Low payload expression in OFF Target cell type(s)**
- **Compact promoter size to accommodate therapeutic payload transgene within ~4.5 kb AAV vector**

### Gene Therapy Challenges

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Off-target cell toxicity</td>
<td><strong>Smart Sensor</strong> Enhance target cell specificity and limit off-target cell toxicity</td>
</tr>
<tr>
<td>Sub-optimal therapeutic performance</td>
<td><strong>Smart Sensor</strong> Improve expression and increase potency</td>
</tr>
</tbody>
</table>

### Senti’s Gene Circuit Solutions

**Smart Sensor promoters enable next-generation gene therapy by:**

- Enhancing specificity to target cell(s) (and thus limiting off-target cell toxicities) and
- Increasing strength, potentially enabling more efficacious therapies
Two Parallel Workflows Lead to Discovery of Ocular-Specific Promoters

**Discovery of endogenous regulatory elements**

**Data mining**
- Chromatin: ATAC-Seq
- Transcription: scRNA-Seq
- Literature search
- Motif enrichment analysis

**Initial components**
- 24 enhancers
- 91 promoters
- 16 transcription factor binding sites

**Generation 1**
- Clonal screening
- High-throughput screening

**Generation 2**
- 14 mutation screening batches
- 51 MPRA hits evaluated clonally

**Generation 3**
- > 100 chimeric sequences (combination of natural and synthetic cores)

**Design of synthetic regulatory elements**
Batched Screening Allows Efficient Quantitative Analysis of Numerous Candidate Sequences

Establishment of a quantitative functional assay:

- Robust transfection of ON and OFF target surrogate cell types
- High throughput single cell, flow cytometry assay
- Quantitative analysis pipeline
High-Scale Clonal Evaluation Allows Efficient Quantitative Analysis of Numerous Candidate Sequences

**STRENGTH** = AREA UNDER THE CURVE

**SPECIFICITY** = STRENGTH Y79 / STRENGTH ARPE-19
Evaluation of Native Sequences Leads to Discovery of Potent Endogenous Core Regulatory Elements

14 sequences (>1% CAG) were selected for bioinformatically guided mutational analysis.
Evaluation of Native Sequences Leads to Discovery of Potent Endogenous Core Regulatory Elements
Massive Parallel Reporter Assay (MPRA) Enables Pooled Screening of >10K Synthetic Promoters

TF Binding Array \(\rightarrow\) minP \(\rightarrow\) NGS Barcode \(\rightarrow\) Reporter Gene

>10,000 arrays of binding sites

co-transfect: 13M ON target cells (Y79)
18M OFF target cells (ARPE-19)

Count plasmid barcodes

- 189
- 76
- 227
- 43

Count mRNA barcodes

- AAAA
- AAAAA
- AAAAA
- AAAAA

Calculate DNA:RNA barcode ratios

<table>
<thead>
<tr>
<th>DNA Barcode</th>
<th>mRNA Barcode</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>189</td>
<td>AAAA</td>
<td>477</td>
</tr>
<tr>
<td>76</td>
<td>AAAA</td>
<td>689</td>
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<tr>
<td>227</td>
<td>AAAA</td>
<td>818</td>
</tr>
<tr>
<td>43</td>
<td>AAA</td>
<td>57</td>
</tr>
</tbody>
</table>
MPRA Screening Yields Compact Transcription Factor Binding Site Arrays with High Strength and Cell Type-specificity

**QUANTIFICATION OF TRANSFECTED LIBRARY**

- **ON-target cells**
  - Replicate 2
  - Replicate 1

- **OFF-target cell**
  - Replicate 2
  - Replicate 1

**Correlation between biological replicates**

**PROMOTER SELECTION**

**ANALYSIS OF TOP PERFORMING SYNTHETIC PROMOTERS**

- Transcription factor binding site arrays
- Position in TFBS array

**SPECIFICITY 1**

\[ \text{SPECIFICITY 1} = \frac{\text{RNA:DNA ON target}}{\text{RNA:DNA OFF target 1}} \]

**SPECIFICITY 2**

\[ \text{SPECIFICITY 2} = \frac{\text{RNA:DNA ON target}}{\text{RNA:DNA OFF target 2}} \]

Note: OFF-target 2 is an undisclosed second OFF-target cell line tested
3rd Generation Promoter Design: Natural and Synthetic Core Sequences Were Integrated to Generate Diverse, Potent, and Specific Synthetic Promoters

**Generation 1**
- Clonal screening
- High-throughput screening

**Generation 2**
- 14 mutation screening batches
- 51 MPRA hits evaluated clonally

**Generation 3**
- > 100 chimeric sequences (combination of natural and synthetic cores)

**EXEMPLARY PARTS**
- Minimal Promoter
- Linker
- Enhancer
- MPRA hit TF array

**EXEMPLARY COMPOSITES**

Promoter Length (bp)
3rd Generation Promoter Design: Natural and Synthetic Core Sequences Were Integrated to Generate Diverse, Potent, and Specific Synthetic Promoters

Fold Specificity (Y79:ARPE19) vs. Potency (fraction of CAG)

- 1st generation
- 2nd generation
- 3rd generation

CAG-GFP
Conclusions

- Development of Smart Sensors that achieve **100 to 10,000-fold specificity** for the photoreceptor surrogate line Y79 over ARPE-19 (RPE)

- Our photoreceptor-specific synthetic promoters achieve **expression levels equivalent to the strong constitutive CAG promoter** currently in clinical use gene therapies

- All synthetic promoters are <= 500 bp in length, there are examples as short as 120 bp

- This application of **massively parallel and systematic workflow** for designing highly compact, specific, and potent synthetic Smart Sensor promoters can be applied across various cell types and diseases of interest
Thank you!

Acknowledgements

Senti Team:

Joseph Draut, Thant Zaw, Myles MacEachern, Assen Roguev, Rocky Chueng, Michelle Hung, Frances Liu, Rebecca Cottman, Nicholas Frankel, Tony Hua, Gary K. Lee, Curt Herberts, Philip Lee, Timothy Lu, Russell Gordley

Spark Team: