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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[3]former employee of Spark Therapeutics, Inc., Philadelphia, PA
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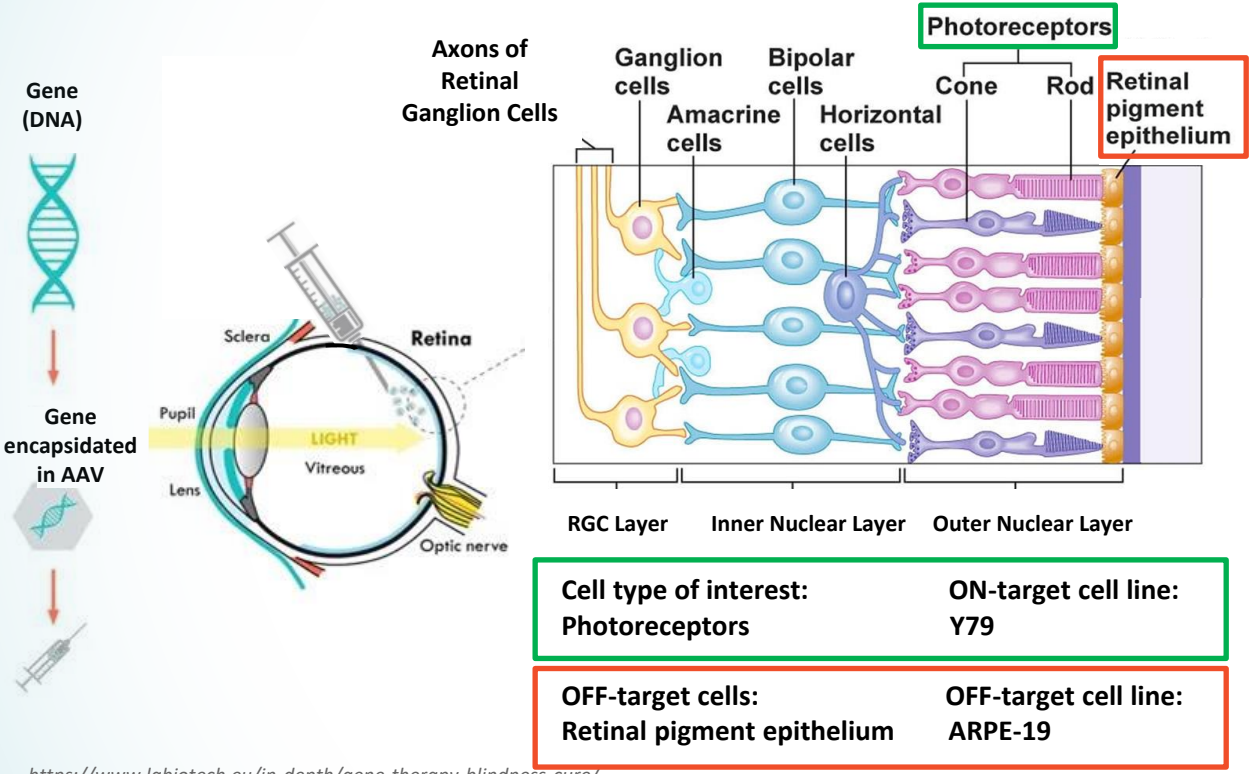
May 20, 2023

Disclosures

Magdalena Cichewicz is a paid employee of Senti Biosciences, Inc.

Challenges of Ocular-directed Gene Therapies



Cell type of interest:	ON-target cell line:
Photoreceptors	Y79

OFF-target cells:	OFF-target cell line:
Retinal pigment epithelium	ARPE-19

https://www.labiotech.eu/in-depth/gene-therapy-blindness-cure/
https://www.toppr.com/ask/question/the-order-of-the-three-layers-of-cells-in-the-retina-of-human-eye-from/

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

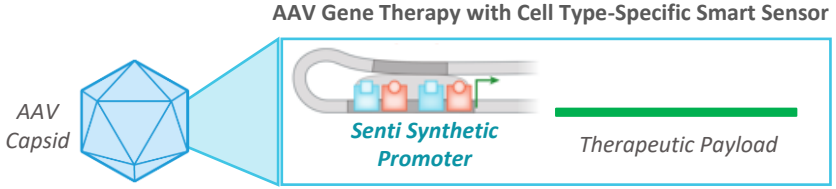
Xiong, W.; Wu, D.M.; Xue, Y.; Wang, S.K.; Chung, M.J.; Ji, X.; Rana, P.; Zhao, S.R.; Mai, S.; Cepko, C.L. AAV cis-regulatory sequences are correlated with ocular toxicity. Proc. Natl. Acad. Sci. USA 2019, 116, 5785–5794.

Smart Sensor Promoters are Designed to Address Key Challenges in Gene Therapy

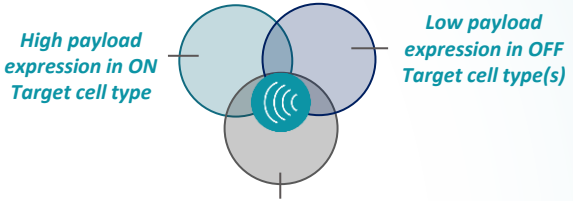
Spark

Collaboration for gene therapies

AAV Gene Therapy with Cell Type-Specific Smart Sensor



Synthetic Promoter Performance Profile:



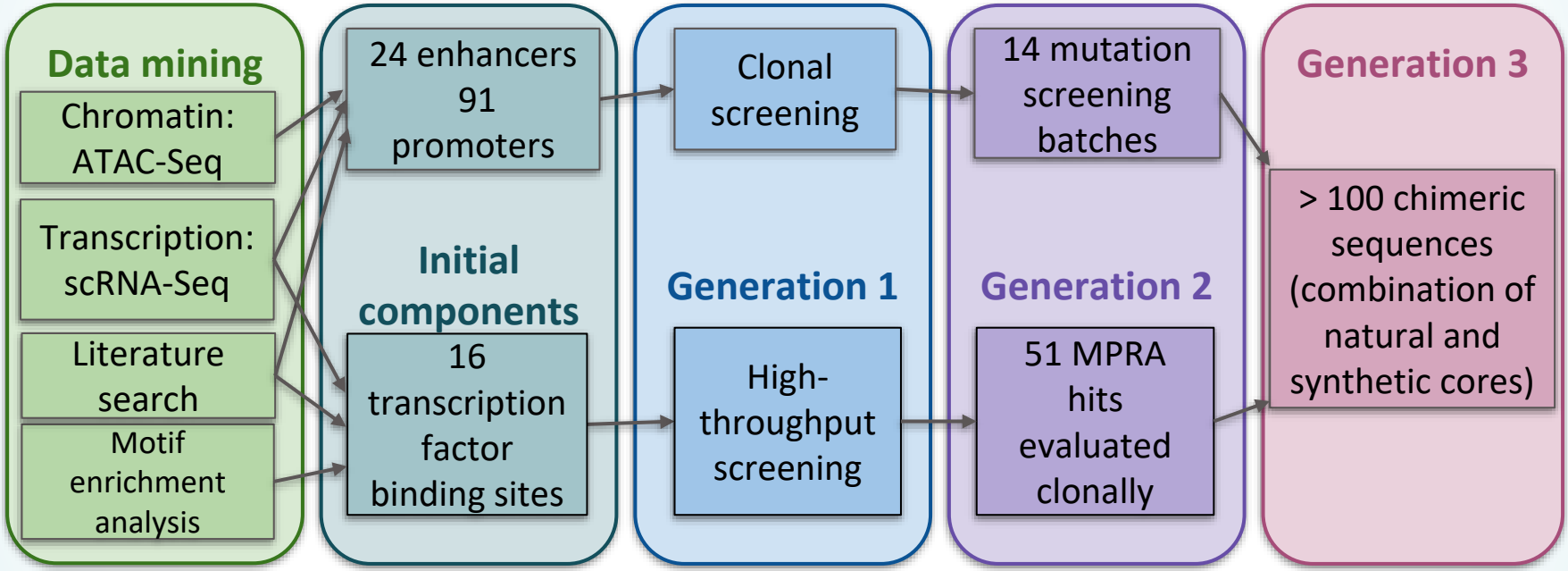
Gene Therapy Challenges	Senti's Gene Circuit Solutions	
Off-target cell toxicity	Smart Sensor	Enhance target cell specificity and limit off-target cell toxicity
Sub-optimal therapeutic performance	Smart Sensor	Improve expression and increase potency

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

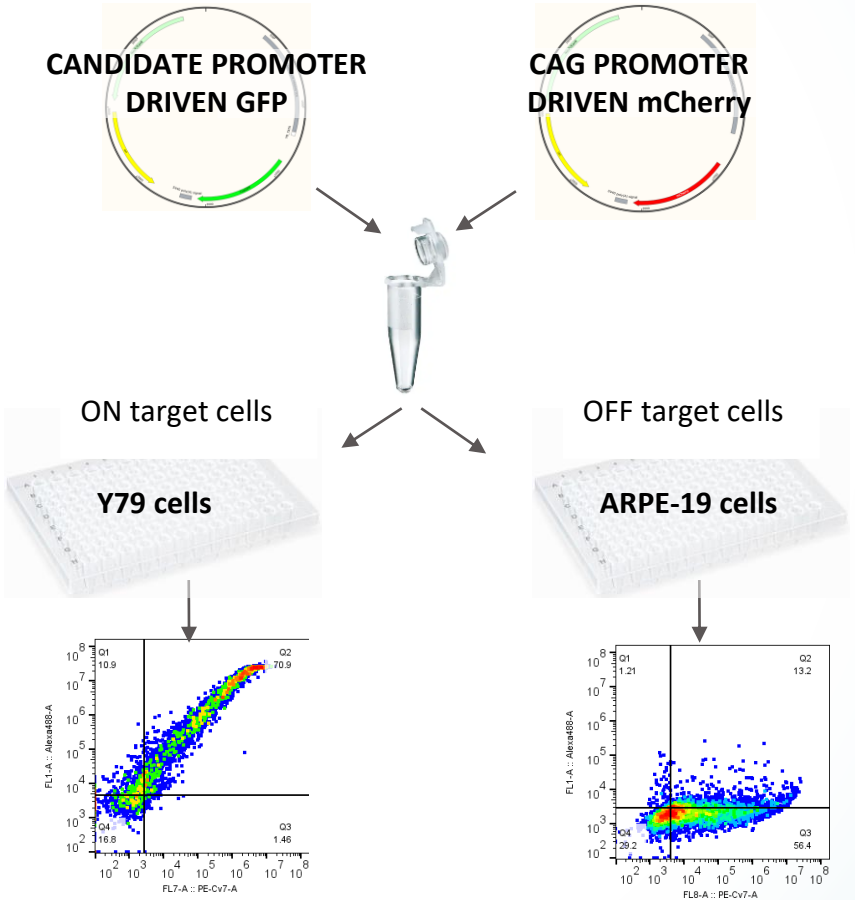


Design of synthetic regulatory elements

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



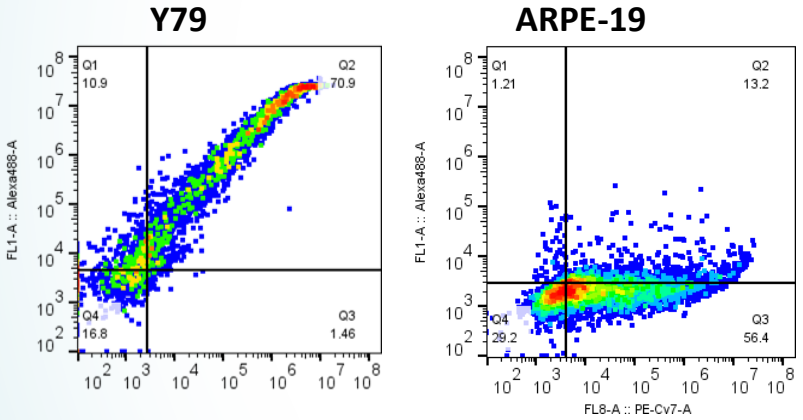
High-Scale Clonal Evaluation Allows Efficient Quantitative Analysis of Numerous Candidate Sequences

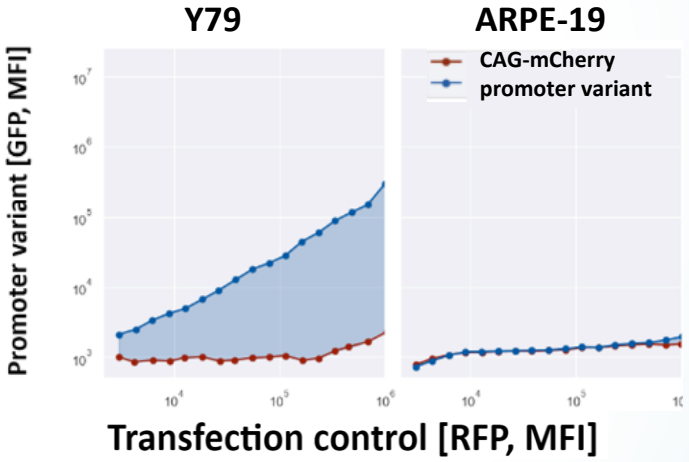
Manual gating in FlowJo

Gate cells → Gate live cells → Gate singlets → Gate transfected cells

Automated

Bin transfected cells → Extract dose response → Average curves → Generate plots & statistics





STRENGTH = AREA UNDER THE CURVE

SPECIFICITY = STRENGTH Y79 / STRENGTH ARPE-19

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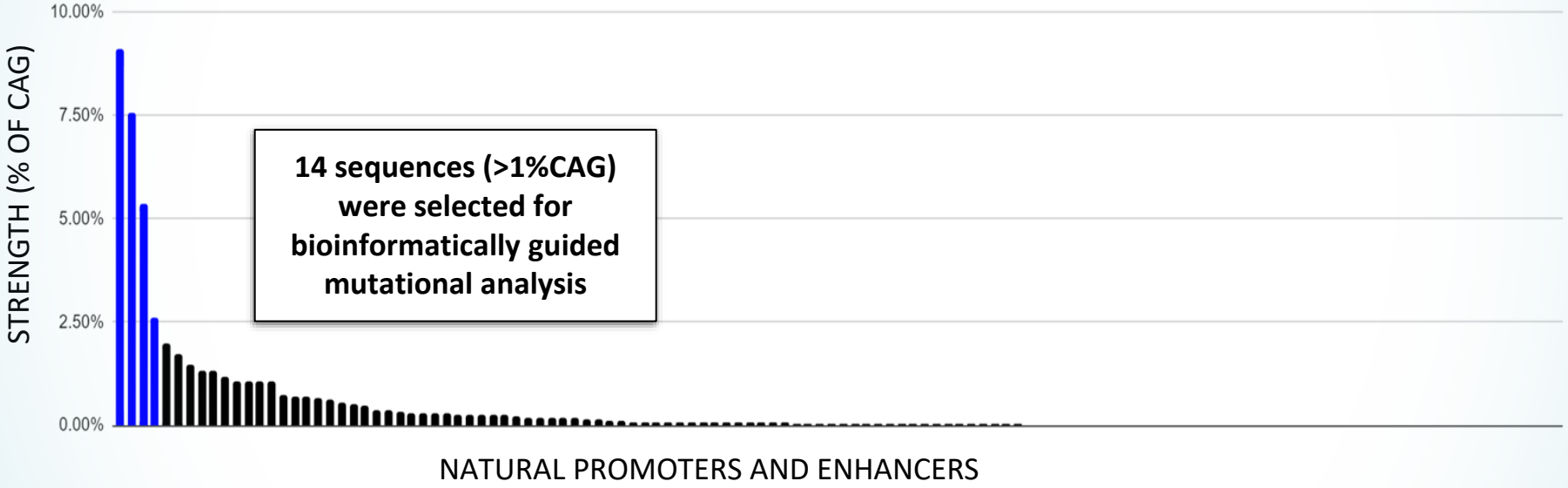
Evaluation of Native Sequences Leads to Discovery of Potent Endogenous Core Regulatory Elements



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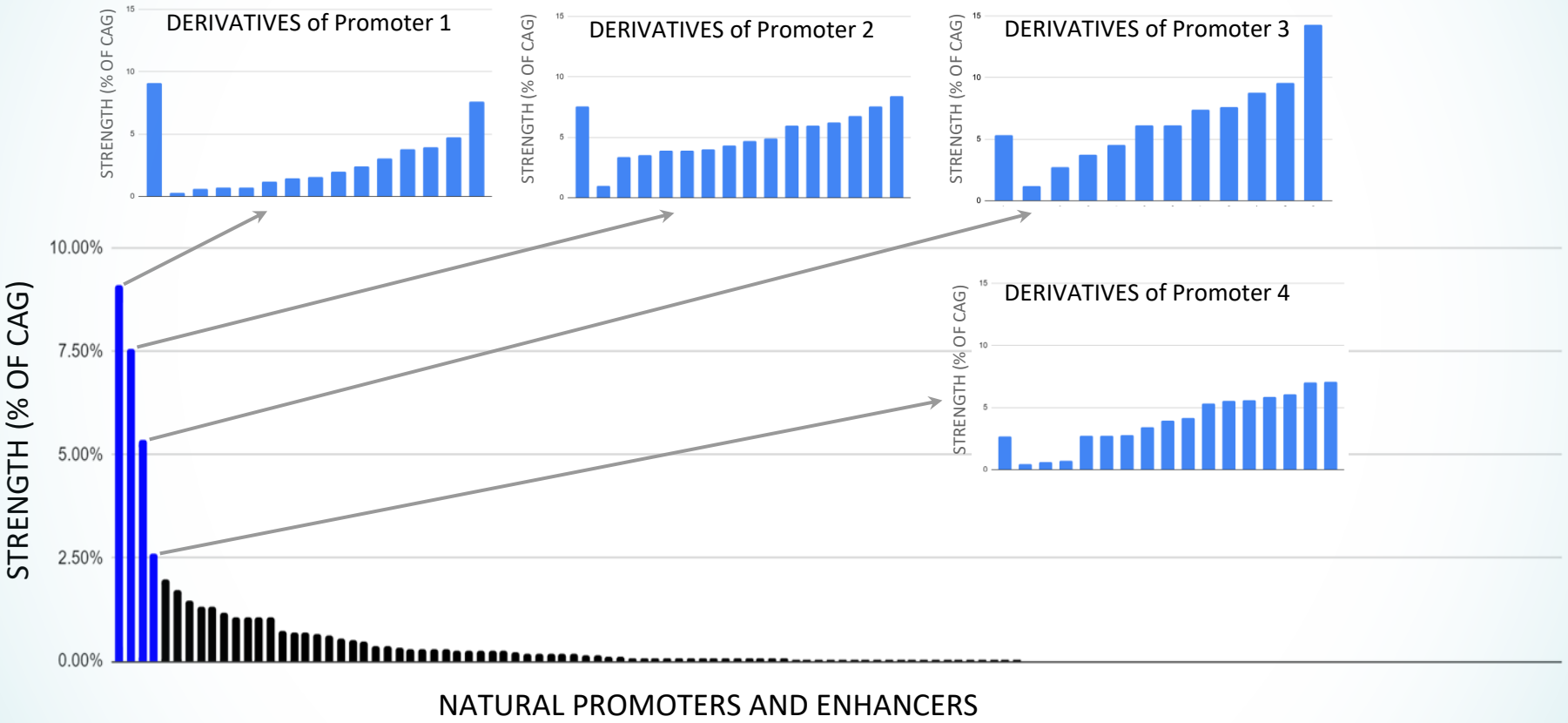
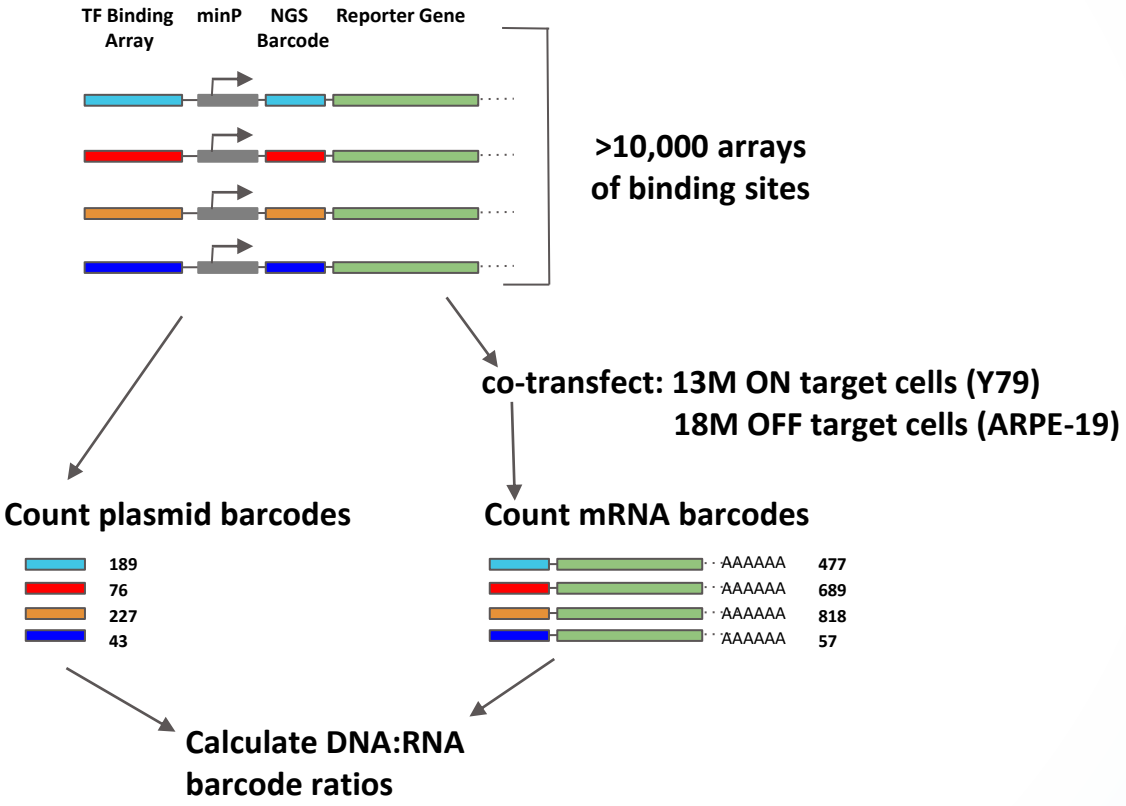
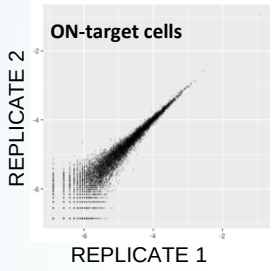


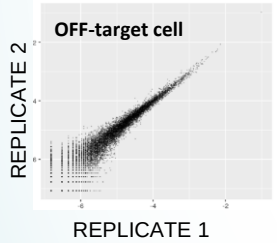
Massive Parallel Reporter Assay (MPRA) Enables Pooled Screening of >10K Synthetic Promoters



MPRA Screening Yields Compact Transcription Factor Binding Site Arrays with High Strength and Cell Type-specificity

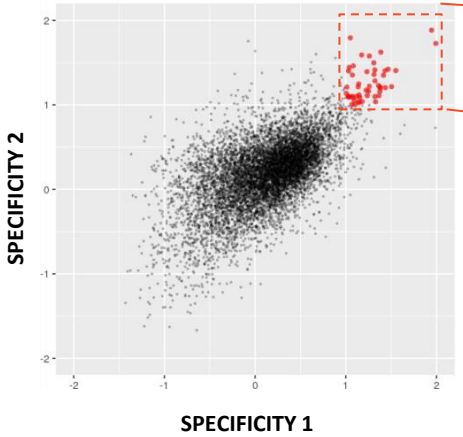
QUANTIFICATION OF TRANSFECTED LIBRARY





Correlation between biological replicates

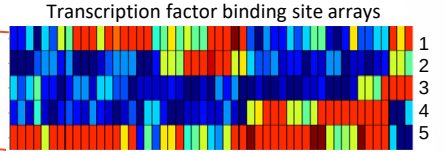
PROMOTER SELECTION

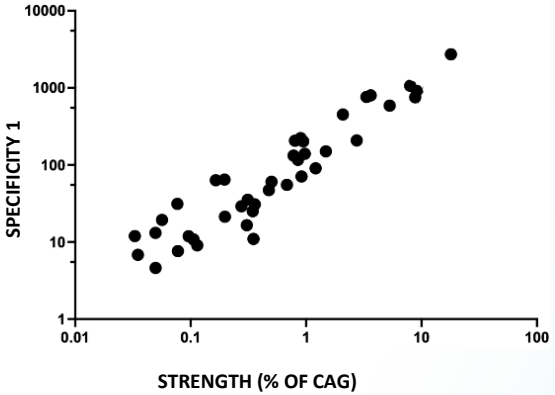


$$\text{SPECIFICITY 1} = \frac{\text{RNA:DNA ON target}}{\text{RNA:DNA OFF target 1}}$$

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

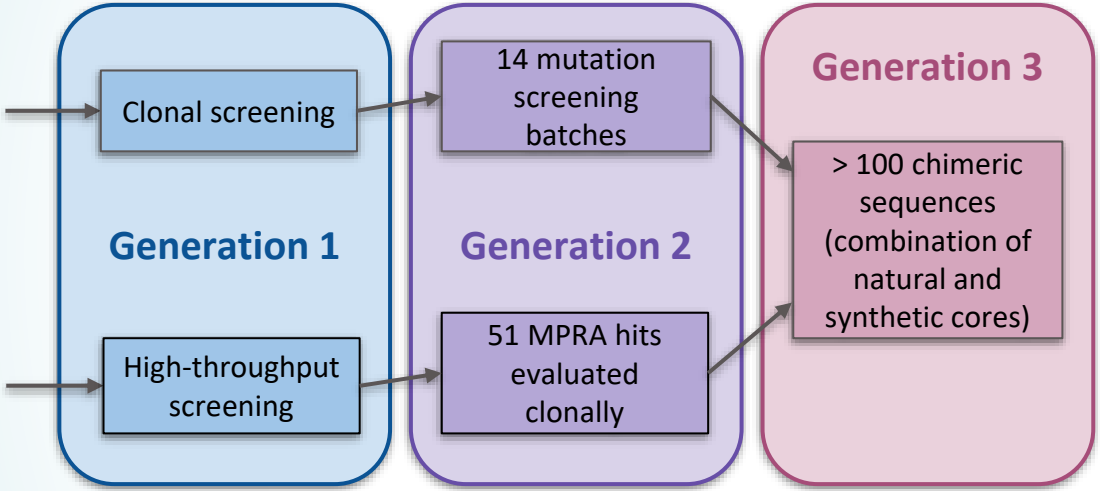
ANALYSIS OF TOP PERFORMING SYNTHETIC PROMOTERS

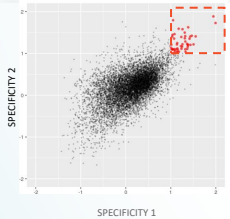


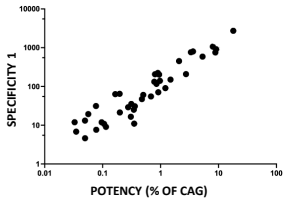


Note: OFF-target 2 is an undisclosed second OFF-target cell line tested

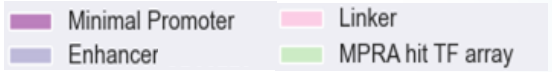
3rd Generation Promoter Design: Natural and Synthetic Core Sequences Were Integrated to Generate Diverse, Potent, and Specific Synthetic Promoters



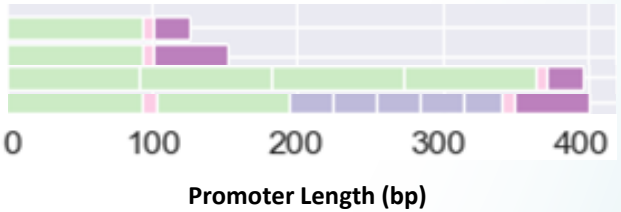




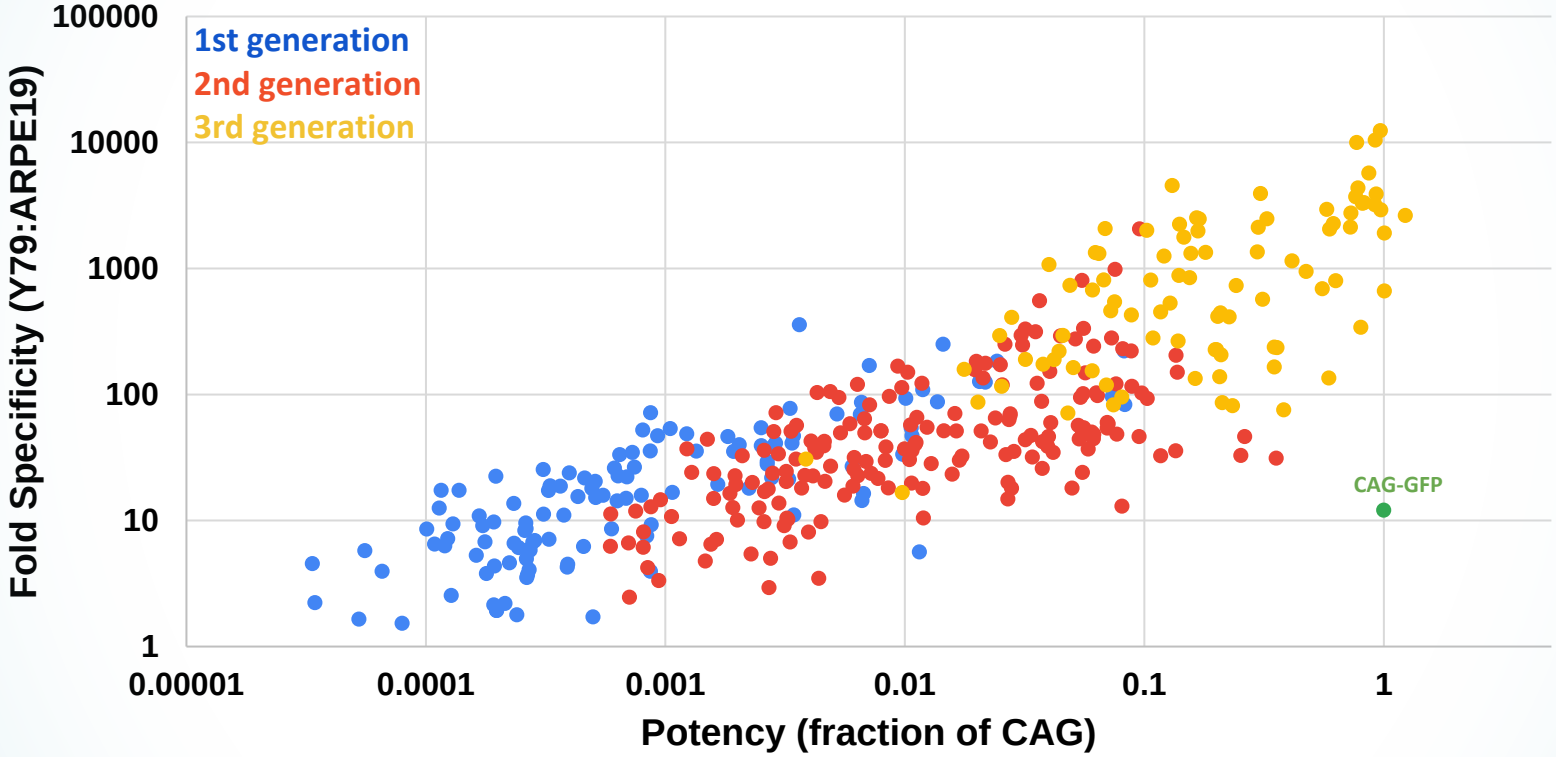
EXEMPLARY PARTS



EXEMPLARY COMPOSITES



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



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

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Thank you!

Acknowledgements

Senti Team:

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