Poster #0129

Identification of highly potent and tissue-specific promoters with massively parallel screening

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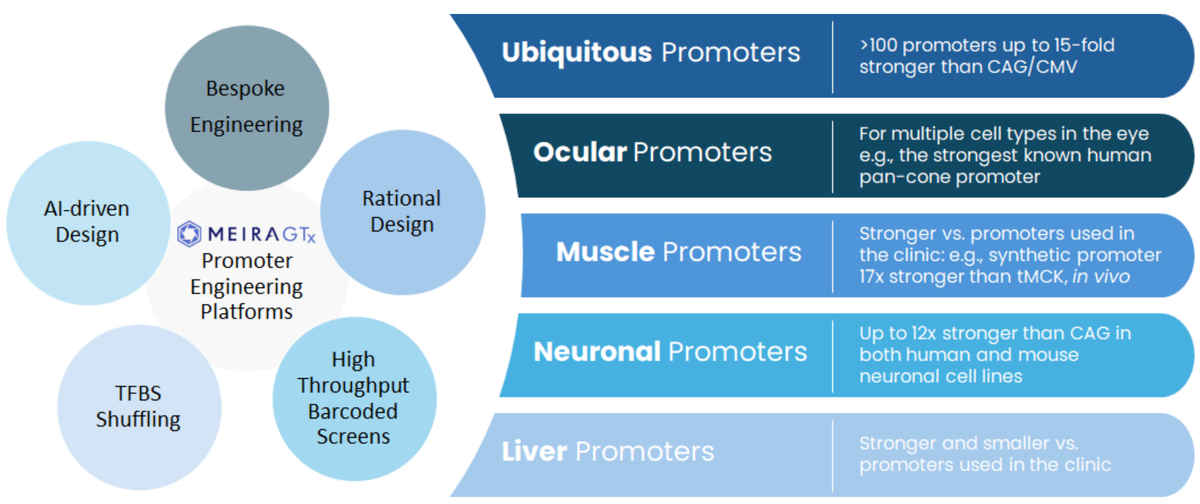
Optimizing *Cis*-Regulatory Elements to Develop Stronger and Safer Gene Therapies

Benefits of Promoter Engineering for Gene Therapies

- Precise control of therapeutic gene expression[1]
- Cell-specific expression independent of capsid[2]
- Increased potency may decrease immune responses and safety risks[3]
- Promoter kinetics impact the durability of gene therapy
- Reducing size without sacrificing strength or specificity allows efficient packaging or larger cargo[4]

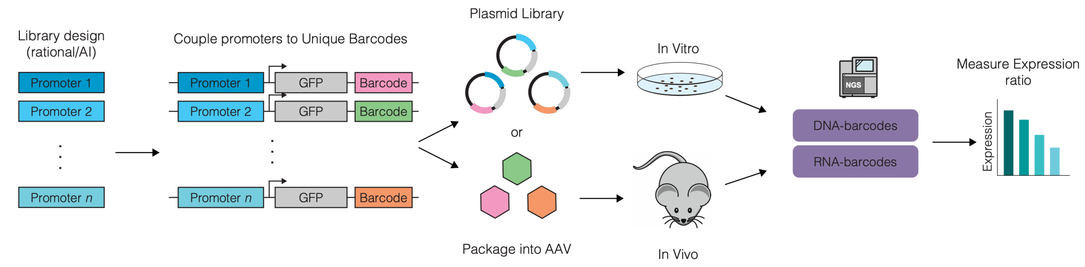


We have built libraries of synthetic promoters and *cis*-regulatory elements available for viral vector optimization. These libraries have a broad range of size, cellular specificity, and potency which can be used in a diverse range of therapeutic applications. Here, we identify potent and small promoters by screening libraries of >200,000 sequences in various tissues.

Identification of Potent and Specific Mini Promoters using MeiraGTx Promoter Design Platform

MPRA screens identify small, but potent promoters in various tissues. (A) Bespoke libraries of >200,000 sequences can be screened using our MPRA platform. **(B)** Representative analysis of the NGS-based expression of a synthetic promoter library, here showing screening results from N2A cells, a mouse neuroblastoma line. Higher ratios indicate stronger promoter activity. **(C)** Specificity and expression level of high-performing candidates selected from screens in various tissues.

A.



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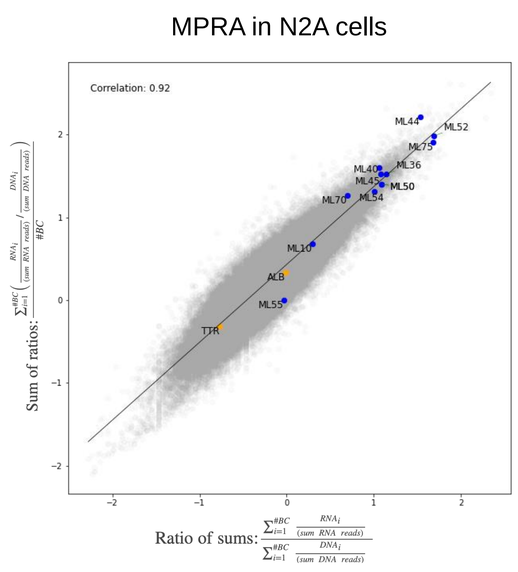

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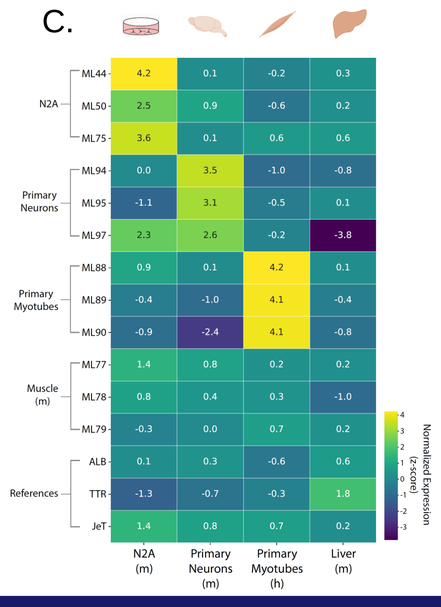
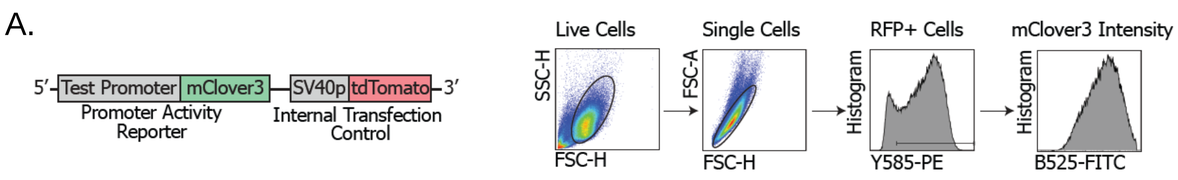


Mini Promoters are up to 12x Stronger than CAG

Mini promoters identified by screening in N2A cells are stronger than CAG in vitro. (A) The activity of promising mini-promoter candidates was validated using a flow cytometry-based *in vitro* assay. Promoter candidates were cloned upstream of mClover3 in a dual-reporter plasmid, which also contains a tdTomato (RFP) expression cassette that is used as an internal transfection control. Promoter activity is quantified as the ratio of the median fluorescence intensity of mClover3 and tdTomato in single, live tdTomato+ cells. **(B)** Relative expression of promoters in mouse N2A and human HuH7 cells using the dual reporter assay. **(C)** Strong correlation of NGS expression (barcodes) and independent assay expression (protein fluorescence) shows high confidence in the predictive power of high-throughput screening and bioinformatic hit selection.

A.



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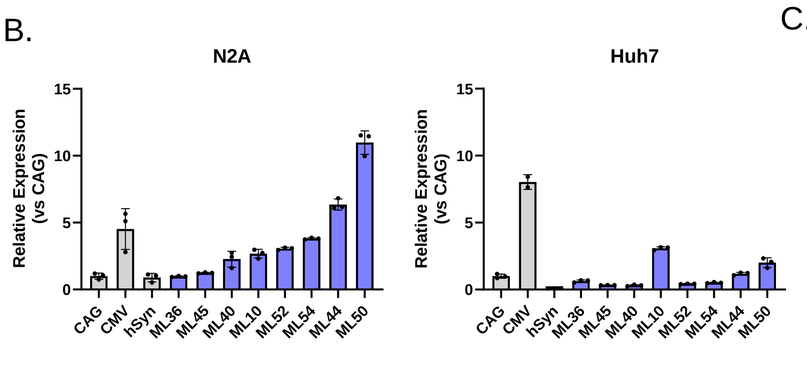

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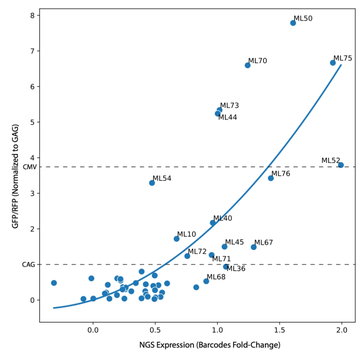
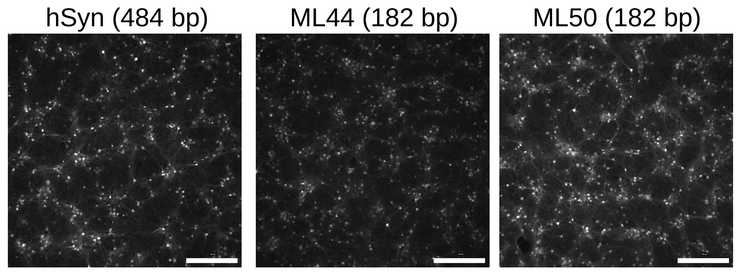


Mini Promoters Drive High Expression in the CNS

Expression and specificity of MeiraGTx CNS promoter candidates. (A) On day 4 *in vitro*, primary mouse cortical neurons were transduced at 50,000 multiplicity of infection with AAV9-miRFP713 driven by candidate promoters. After 7 days, neurons were imaged to assess miRFP713 expression. Scale bar: 300 µm. **(B)** Image quantification at 7 days post infection shows that ML44 and ML50 express equal to or stronger than JeT and hSyn despite their small size. **(C)** Promoter activity was assessed *in vivo* by bioimaging the mIRFP713 reporter gene 4 weeks after systemic or direct injection. **(D)** Quantification of promoter expression in the mouse brain (left), liver (middle) and muscle (right).

A.



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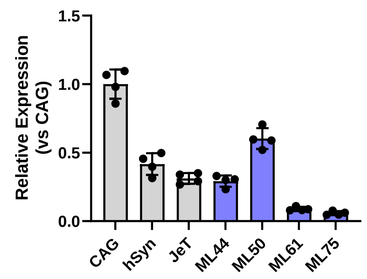

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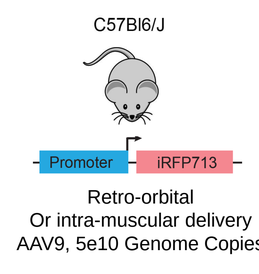

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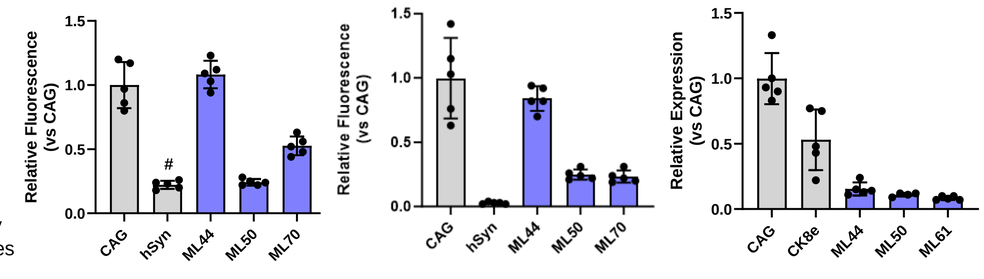
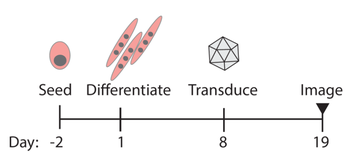


MeiraGTx Promoters are Stronger than Ck8e in Human Myotubes

Expression of MeiraGTx muscle promoter candidates in human iPSC-derived myotubes. (A) Experimental paradigm to test promoter expression in human induced pluripotent stem cell (hiPSC) derived myotubes. hiPSC-derived myoblasts are differentiated for 8 days. Cultures are then transduced with 200,000 multiplicity of infection with AAV9-mIRFP713 driven by candidate promoters. Cells are imaged and quantified 11 days after infection. **(B**) Representative images of transduced myotubes showing higher expression driven by MeiraGTx promoters compared to Ck8e. **(C)** Mini promoters identified from the mouse muscle barcode screen are equal to or stronger than Ck8e while 60% smaller.

A.



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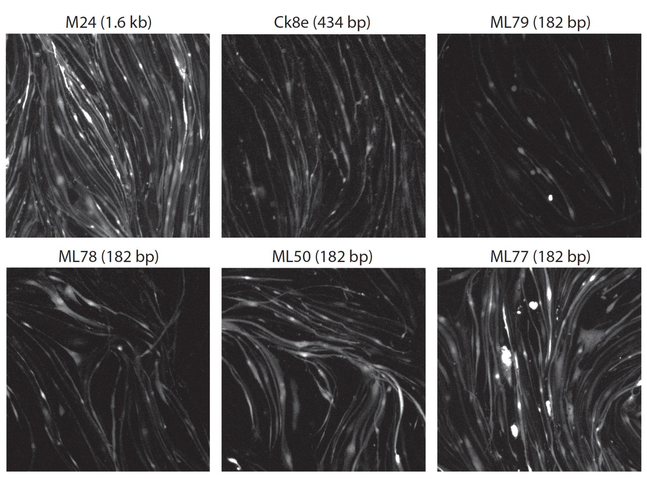

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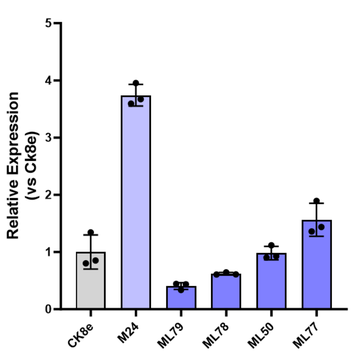

Conclusions

- MeiraGTx has a robust and predictive platform to screen hundreds of thousands of sequences in complex models *in vitro* and *in vivo,* independent of species.
- Here we show high-performing 182 bp promoters selected by our platform in brain, muscle and liver models.
- Independent validation at the protein expression level confirms the predictive power of our promoter engineering platform.
- ML44 and ML50 show strong expression in neuronal models despite small size.
- Muscle candidate ML77, is 56% stronger than Ck8e in hiPSC-derived myotubes and is ~60% smaller (182 bp vs 443 bp).

- <u>MeiraGTx synthetic mini promoters exhibit greatly improved potency for driving therapeutic gene expression in the CNS and muscle.</u>

- Future work:
  - Quantify expression and cellular specificity in the mouse CNS *in vivo*
  - Evaluate potency of neuronal and muscle candidates in human iPSC-derived models and *in vivo*
  - Examine promoter kinetics using long-term *in vivo* bioimaging

References

1. Wang et al. Adeno-associated virus vector as a platform for gene therapy delivery. *Nat Rev Drug Discov*. 2019 May;18(5):358-378.
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3. Cordier et al. Muscle-specific promoters may be necessary for adeno-associated virus-mediated gene transfer in the treatment of muscular dystrophies. *Hum Gene Ther*. 2001 Jan 20;12(2):205-15.
4. Wu et al. Effect of genome size on AAV vector packaging. *Mol Ther*. 2010 Jan; 18(1): 80–86.