A computational platform for next-generation synthetic promoter design and engineering

Compact synthetic promoters tailored for expression in liver, muscle, and NK cells, with >10× dynamic range and minimal off-target activity

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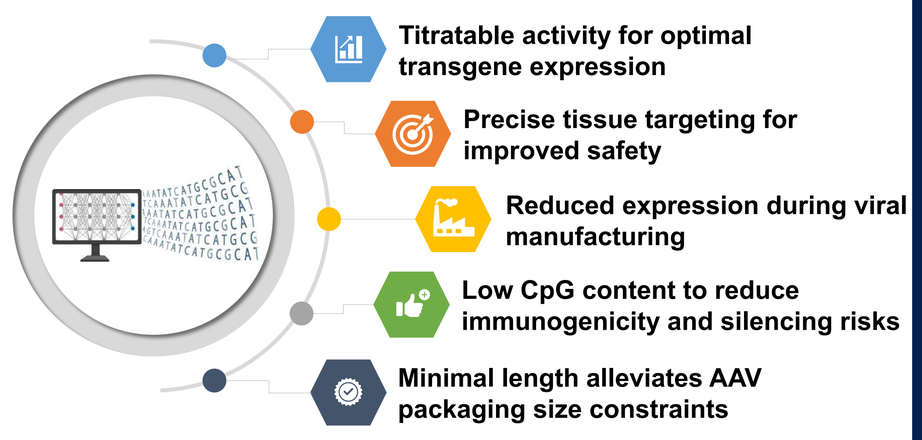
A proprietary platform for the design of synthetic promoters

Promoter choice is critical to the success of cell and gene therapies, determining the timing, location, and level of therapeutic gene expression. Conventional viral and endogenous promoters are often suboptimal for many intended applications, exhibiting undesirable off-target activity, inefficient gene expression, and immunogenicity.

Instead, our proprietary platform quantitatively surveys the transcriptional landscapes of all human tissues to identify transcriptionally active regulatory elements, which are then assembled *in silico* into novel synthetic promoters with user-defined characteristics, such as tissue-specificity, expression level, promoter length, and CpG content.

Here we exemplify the capability of our platform to design libraries of patentable synthetic promoters that drive targeted expression in liver, muscle, and NK cells, offering an additional layer of control to enhance the safety and efficacy of next-generation cell and gene therapies beyond delivery vehicle selection alone.

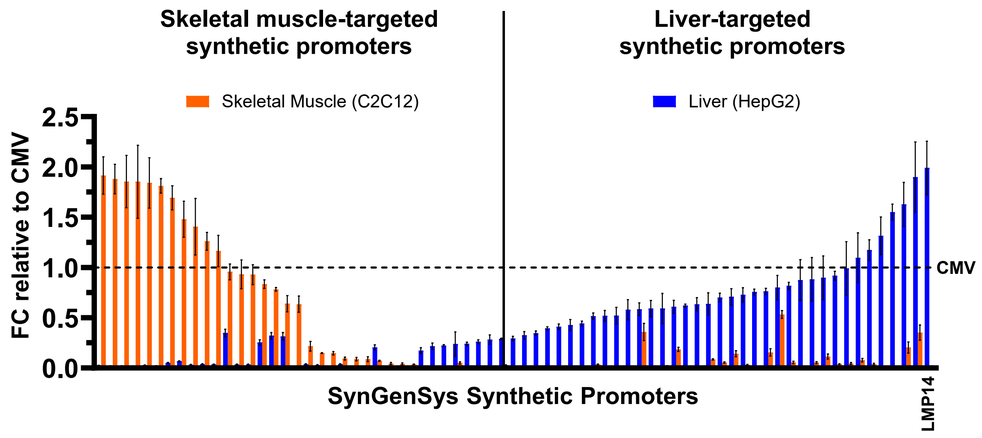


Promoter-driven specificity for gene therapy

SynGenSys demonstrates liver- and skeletal muscle- targeted expression with libraries of novel off-the-shelf synthetic promoters characterised by:

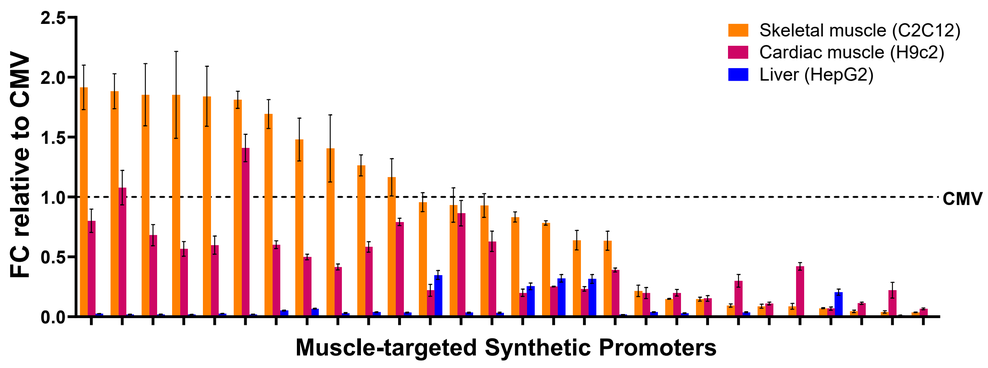
- ✓ Over 10-fold range of expression in the intended target cell line(s) with minimal activity in an off-target cell line (A, B)
- ✓ Reduced expression in HEK293 producer cells for improved AAV manufacturing (C)
- ✓ Reproducibility of expression between HepG2 cell line and primary human hepatocytes (D)
- ✓ Targeted expression to the liver demonstrated *in vivo* (E)
- ✓ Small size (~200-650 bp) and low CpG content

A



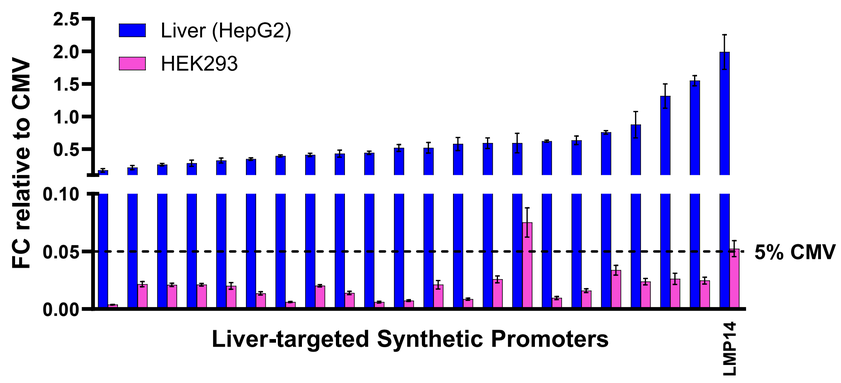
SynGenSys liver and skeletal muscle targeted synthetic promoters transiently transfected in the liver cell line, HepG2 and the differentiated skeletal muscle cell line, C2C12. Bars show the mean luciferase intensities relative to a CMV promoter control, +/- SEM, N=3.

B



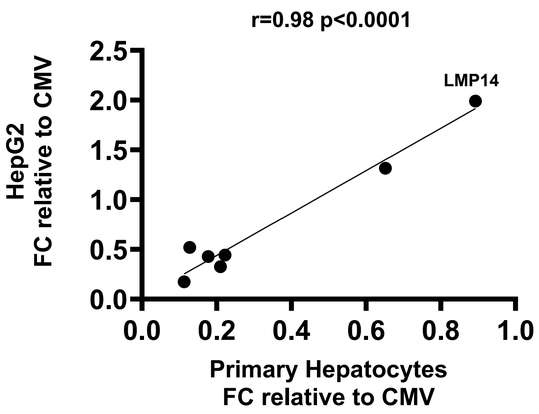
SynGenSys skeletal muscle targeted synthetic promoters transiently transfected in the differentiated skeletal muscle cell line, C2C12, differentiated cardiac muscle cell line, H9c2, and the liver cell line, HepG2. Bars show the mean luciferase intensities relative to a CMV promoter control, +/- SEM, N=3.

C



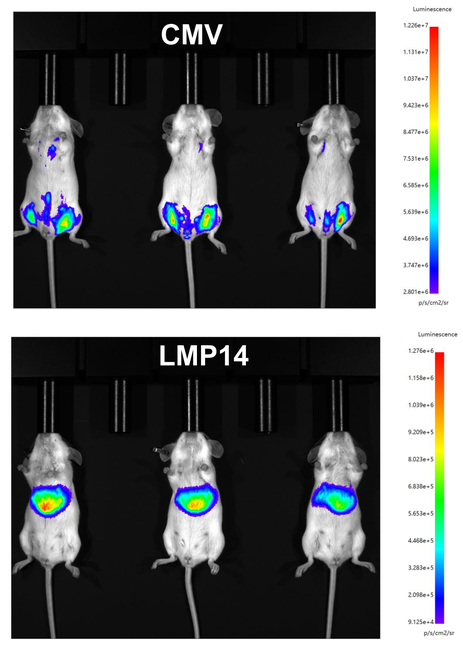
SynGenSys liver-targeted synthetic promoters transiently transfected in the liver cell line HepG2 and a HEK293 cell line. Bars show the mean luciferase intensities relative to a CMV promoter control, +/- SEM, N=3. Dotted line at 5% activity relative to the CMV promoter.

D



***(D)** Correlation between the mean promoter activity in HepG2 cells and primary hepatocytes for a selection of liver-targeted SynGenSys promoters. N=3 independent transfections, and primary hepatocytes from 3 different donors.*
***(E)** Luciferase reporter plasmids containing either the CMV promoter or our liver-targeted LMP14 synthetic promoter were packaged into AAV9 capsids and systemically delivered to mice via tail vein injection. Three weeks post-injection full-body bioluminescence images were taken to assess transgene expression.*

E

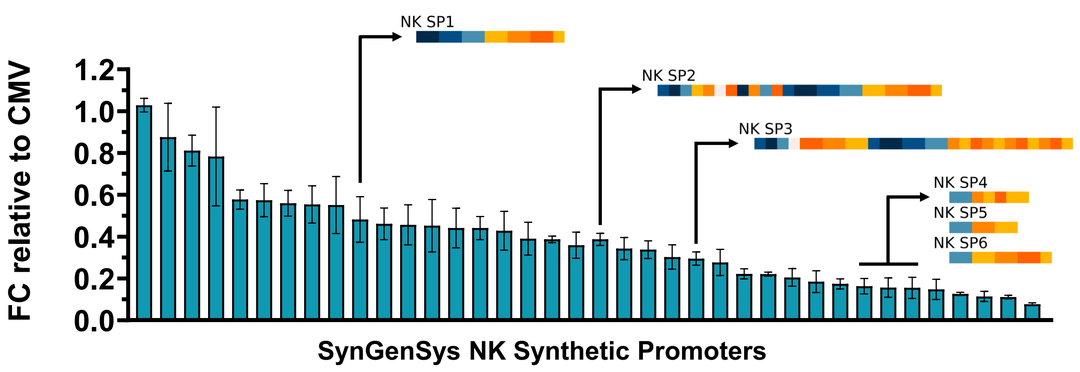


Synthetic promoters enable NK cell-restricted expression

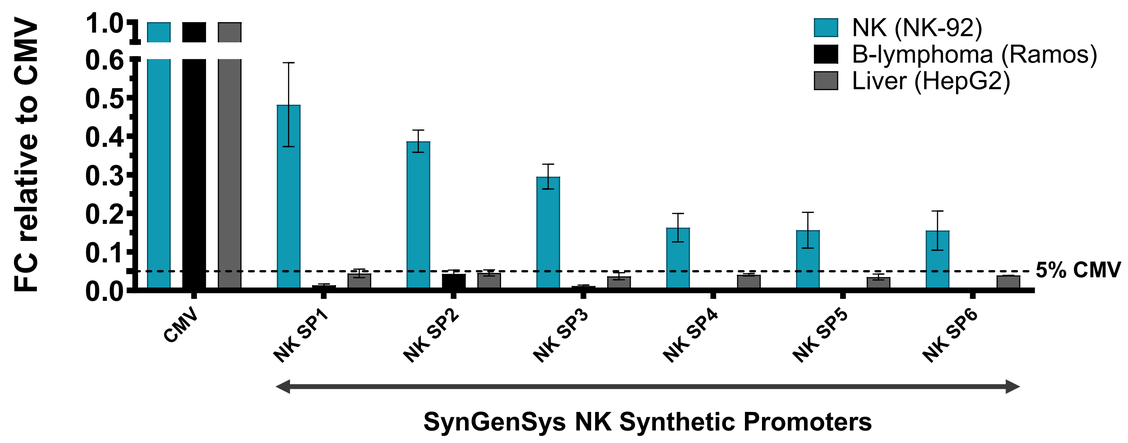
SynGenSys has developed a library of novel, off-the-shelf synthetic promoters designed to enable targeted, tuneable gene expression in NK cells exhibiting:

- ✓ A broad range of activity in target NK-92 cells (A)
- ✓ Minimal off-target expression in Ramos B-cell lymphoma and HepG2 hepatocyte cell lines (B)
- ✓ Compact size (~200-600 bp)
- ✓ Lower CpG content than CMV

A



B



***(A)** SynGenSys NK synthetic promoters transiently transfected in NK-92 cells. Bars show the mean GFP intensities relative to the CMV promoter, +/- SEM, N=3.*
***(B)** Selected SynGenSys NK-targeted synthetic promoters transiently transfected in NK-92, Ramos, and HepG2 cells. Bars show the mean GFP intensities relative to the CMV promoter, +/- SEM, N=3. Dotted line at 5% activity relative to the CMV promoter.*