Synthetic promoters for safe and controlled expression in cell and gene therapy

syngensys

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made in Sheffield

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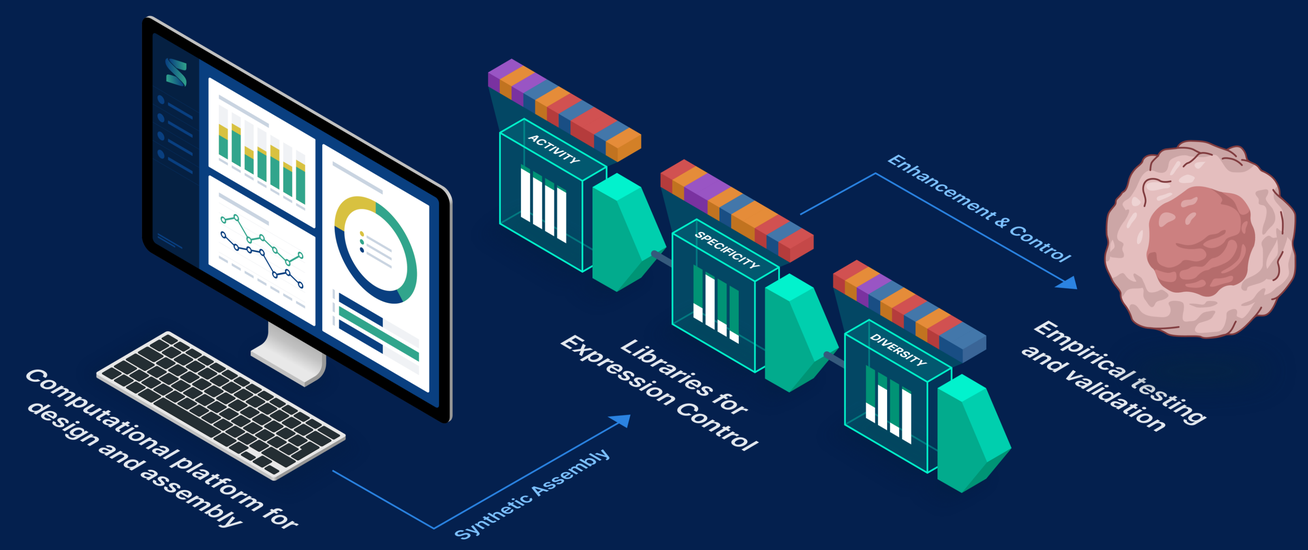
A proprietary platform for the design of synthetic promoters

Promoter choice is crucial to the success of cell and gene therapies. Conventionally utilised viral and native promoters are suboptimal for many intended applications. Common limitations include undesirable off-target activity, inefficient gene expression and immune responses.

Our proprietary platform enables the creation of novel synthetic promoters with user-defined requirements such as cell-type specificity, high activity, and low immunogenicity (low CpG content).

We exemplify here the capability of our platform for the design of novel patentable NK-targeted and liver-targeted synthetic promoters showing broad range of activity in the target cells and negligible activity in off-target cells.

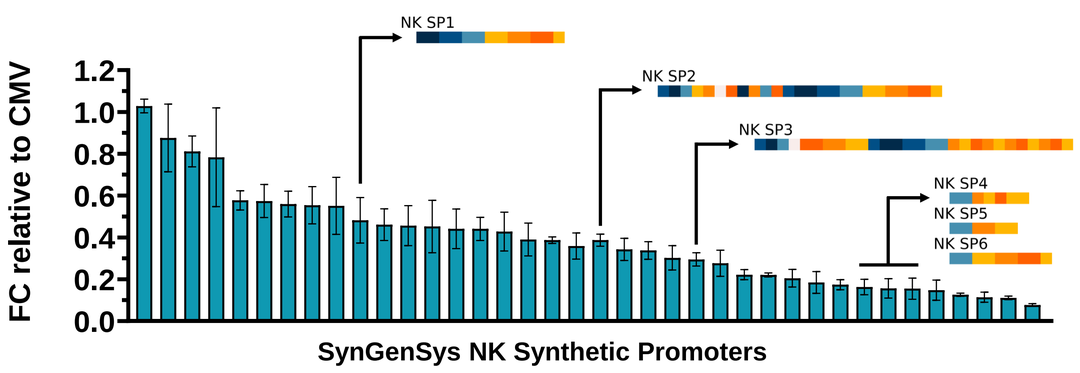


Highly targeted NK promoters

SynGenSys has developed a library of novel off-the-shelf NK synthetic promoters that exhibit:

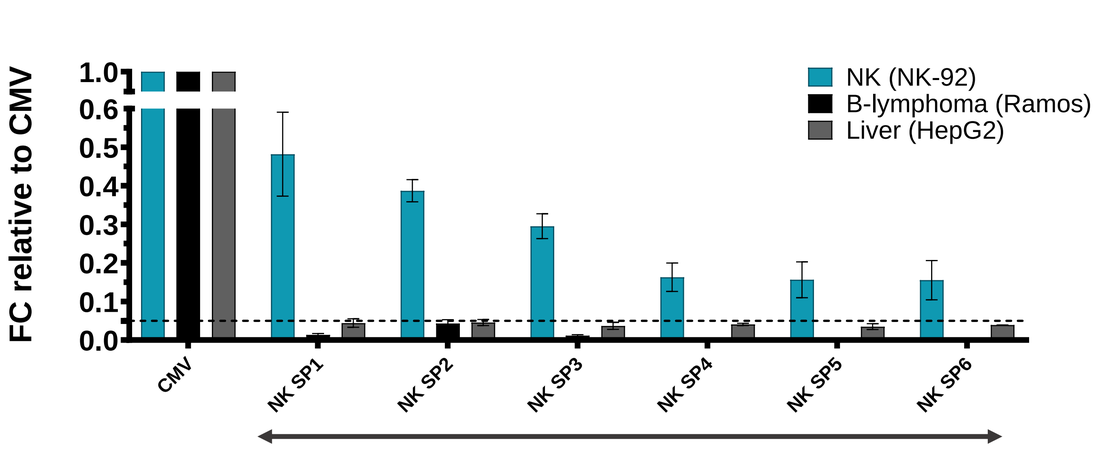
- ✓ A broad range of activity in target NK-92 cells (A)
- ✓ Pre-designed minimal off-target expression in Ramos B-cell lymphoma and HepG2 hepatocyte cells (B)
- ✓ Minimal size (C)
- ✓ Lower CpG content than CMV (C)

A



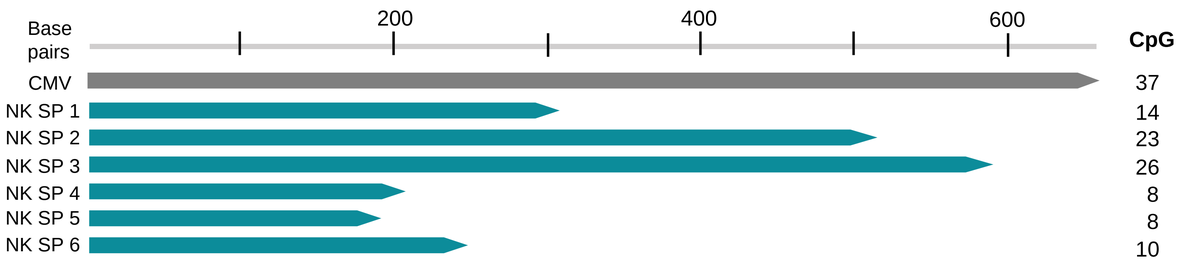
SynGenSys NK synthetic promoters transiently transfected in NK-92 cells are compared to the CMV promoter. Bars show the mean GFP intensities from at least n=3 independent transfections, +/- SEM.

B



SynGenSys NK-targeted synthetic promoters transiently transfected in NK-92 cells, Ramos and HepG2 cells are compared to the CMV promoter. Bars show the mean GFP intensities from at least n=3 independent transfections, +/- SEM. Dotted line at 5% activity relative to CMV promoter.

C



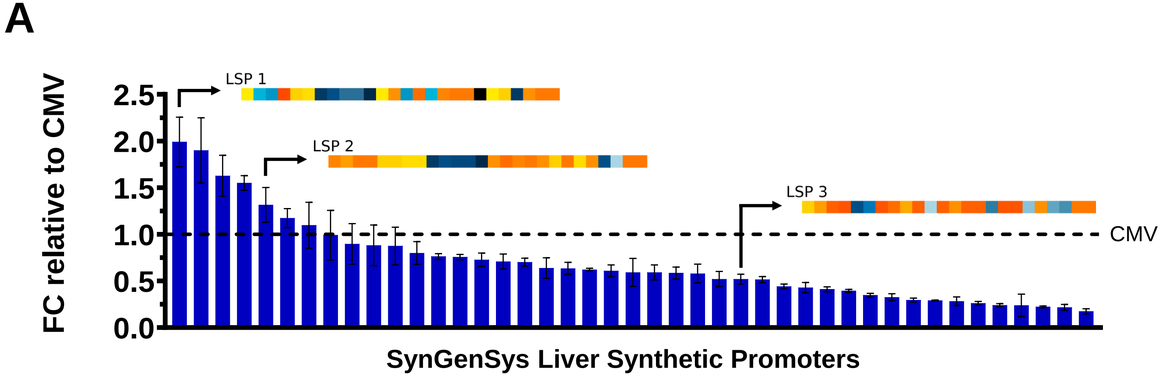
Sizes and CpG content of selected SynGenSys NK-targeted synthetic promoters.

Titratable expression with Liver promoters

SynGenSys has created a library of patentable off-the-shelf liver synthetic promoters characterised by:

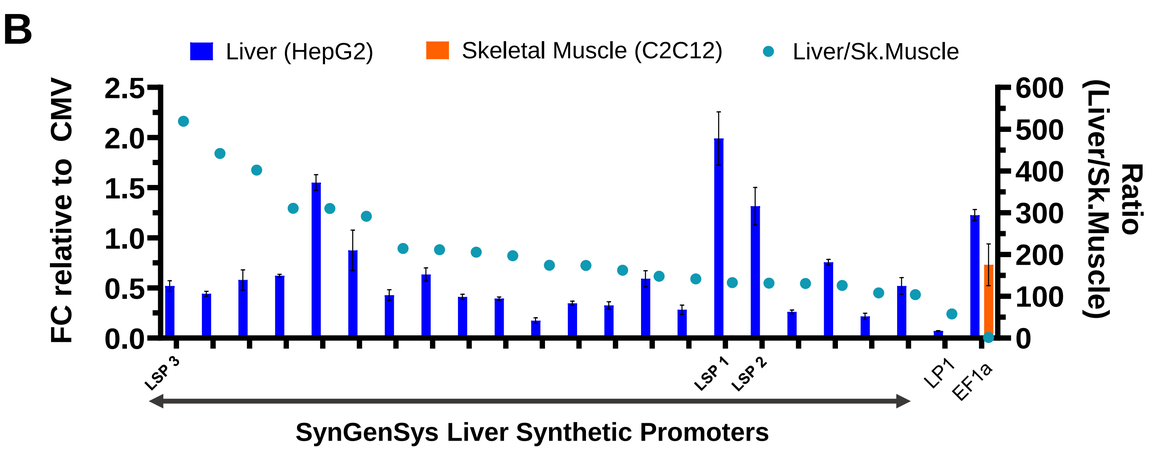
- ✓ Over 10-fold range of expression in the liver cell line HepG2 (A)
- ✓ Negligible activity in differentiated C2C12 skeletal muscle cells (B)
- ✓ Lower CpG and CpG island content than industry standard liver benchmark LP1
- ✓ Smaller size than industry standard liver benchmark LP1 (325-667 bp)
- ✓ Reproducibility of expression between HepG2 cell line and primary human hepatocytes (C)

A



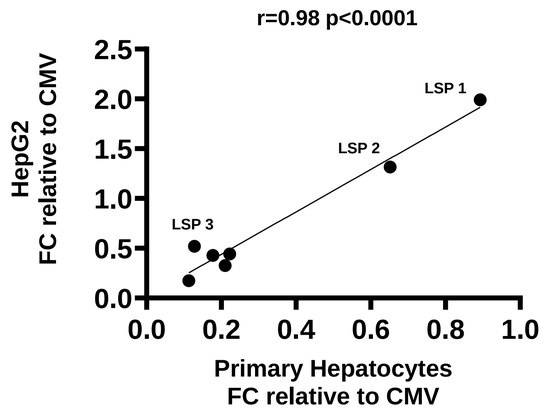
SynGenSys liver synthetic promoters transiently transfected in the liver cell line HepG2 are compared to the CMV promoter. Bars show the mean luciferase intensities from n=3 independent transfections, +/- SEM.

B



SynGenSys liver-targeted synthetic promoters transiently transfected in the liver cell line HepG2 and the skeletal muscle cell line C2C12. Activity from industry benchmark LP1 and ubiquitous EF1a promoter is also reported. Bars show the mean luciferase intensities from n=3 independent transfections, +/- SEM. Dots indicate the ratio of the expression fold changes of HepG2/C2C12.

C



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.