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A novel genomics-based platform for the creation of synthetic promoters for cell and gene therapy

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The wealth of genomic data generated by high-throughput sequencing methods (HTS) has made it possible to mine the genome for gene regulatory elements that can then be used in the design of synthetic promoters. These synthetic promoters can be tuned to deliver exquisite expression control of the GOI within the context of the gene therapy vector, thereby increasing the safety profile of the therapeutic product.

By harnessing the power of HTS methods, Synpromics has developed a promoter design platform that integrates data from large-scale functional genomics datasets with machine learning algorithms to identify regulatory elements. These regulatory elements are then ranked using multiple distinct criteria and subsequently used as component parts in synthetic promoter construction, which are designed using engineering biology principles.

Using this platform, Synpromics has engineered liver and muscle selective promoters, and generated novel inducible promoters which are responsive to either chemical or biological stimuli. Activity from these inducible promoters is triggered solely by the addition of an inducer, without the requirement for the co-expression of a trans-activator. This simple mechanism has many advantages over currently used 2 component systems, e.g. decreased size and ease of use. The liver and muscle selective promoters showed a broad range of activities with high specificity, and outperform frequently used non-selective viral promoters.

In addition to promoter design, Synpromics' platform can potentially be applied to the identification of post-transcriptional regulatory elements to combine multiple layers of regulation (transcription, splicing, translation, etc.) to create expression cassettes optimised for different therapeutic applications. In summary, Synpromics has developed a novel genomics-based platform that enables the rational design, synthesis and testing of mammalian regulatory elements, and whose output can help support the construction of the next generation of cell and gene therapeutics.

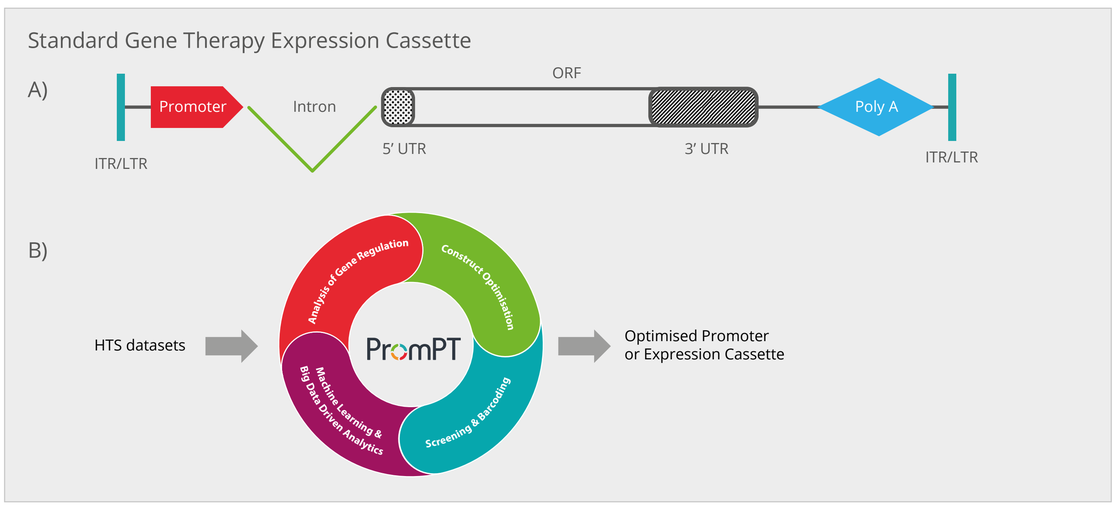

Fig 1. PromPT platform integrates bioinformatics tools and screening technologies to enable the rational design of next generation cell and gene therapy tools. **A)** Representation of a standard gene therapy expression cassette. PromPT has been successfully applied to the design of synthetic promoters and can be also applied to the identification of candidate post-transcriptional regulatory elements to optimise the expression of the GOI for gene and cell therapy applications. **B)** PromPT applies engineering principles to integrate "*in silico*" and wet lab tools. A standard PromPT project starts with the collection and analysis of relevant genomic datasets for the identification of regulatory regions of interest. Candidate regulatory regions are synthesised and functionally assayed *in vitro* and/or *in vivo*. The output of these functional tests feeds back into the first step of the cycle. Multiple iterations of the cycle might be needed until promoter or expression cassette matches the customer specifications.

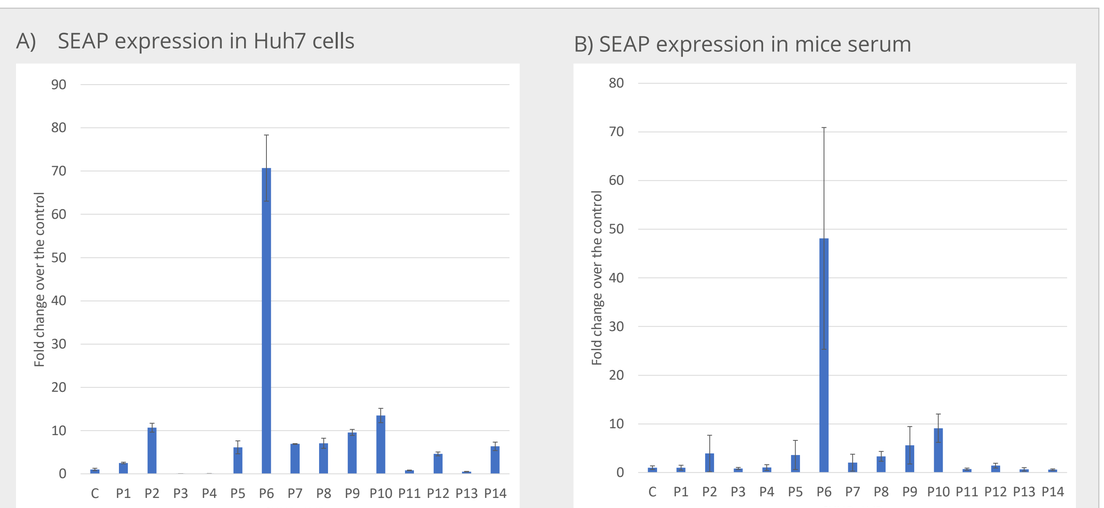

Fig 2. Validation of PromPT platform for the design of synthetic liver specific promoters. Synpromics' proprietary bioinformatic analysis was used to identify liver specific CREs and to design new synthetic liver specific promoters. **A)** Synthetic promoter activity assayed in a hepato cellular carcinoma cell line. Huh7 cells were infected with AAV reporter vectors using SEAP as reporter gene. Media was collected 72 hours after infection and SEAP activity was assayed using a luminescence assay. **B)** *In vivo* assay of synthetic promoter activity in wildtype mice (C57BL/6JRj) injected with AAV/SEAP reporter vectors. AAV viral preparations were injected intravenously, 6 weeks after injection mice were bled and SEAP activity measured to quantify synthetic promoter activity.

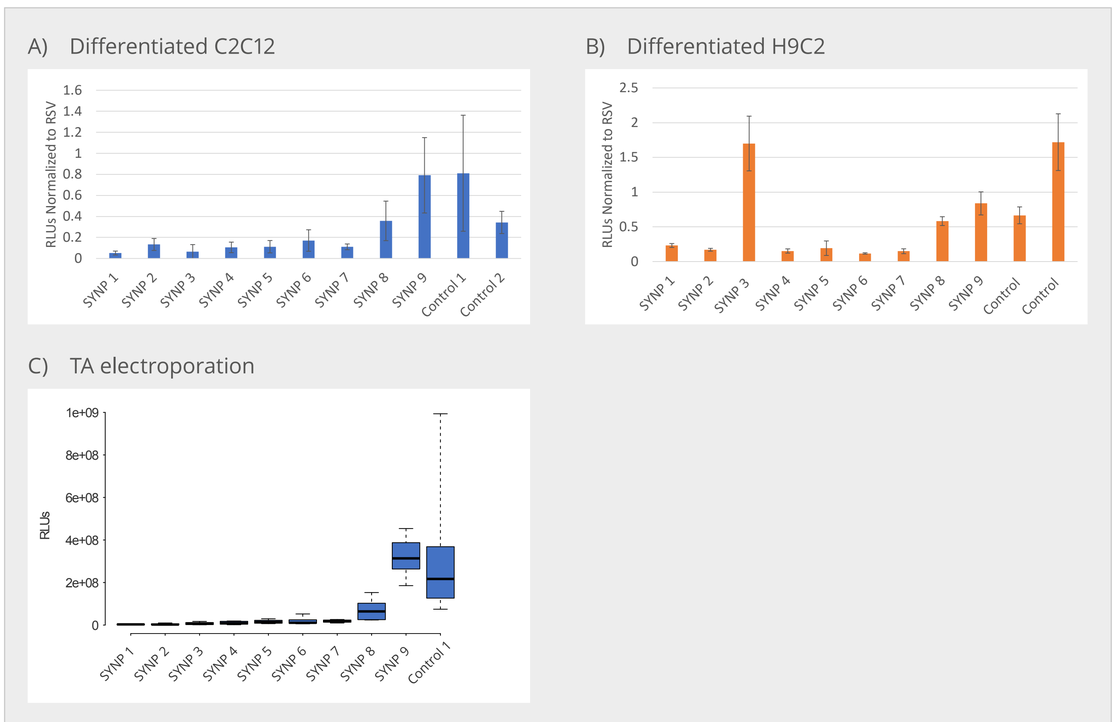

Fig 3. Optimization of PromPT platform for the design of synthetic muscle specific promoters. **A)** Luciferase activity of muscle specific promoters in C2C12 mouse myoblast cell line. Cells were transfected and 24 hours later induced to differentiate. Promoter activity was assayed 5.5 days after differentiation was triggered. **B)** Luciferase activity of muscle specific promoters in H9C2 rat cardiomyobast cell line. Cells were transfected and 24 hours later induced to differentiate. Promoter activity was assayed 7 days after differentiation was triggered. SYNP 3 promoter is a control designed to be active in heart but not skeletal muscle. **C)** *In vivo* validation of muscle synthetic promoter activity by electroporation of luciferase reporter plasmids into TA muscle of BALB/c wildtype mice. Promoter activity was assayed 7 days after electroporation.

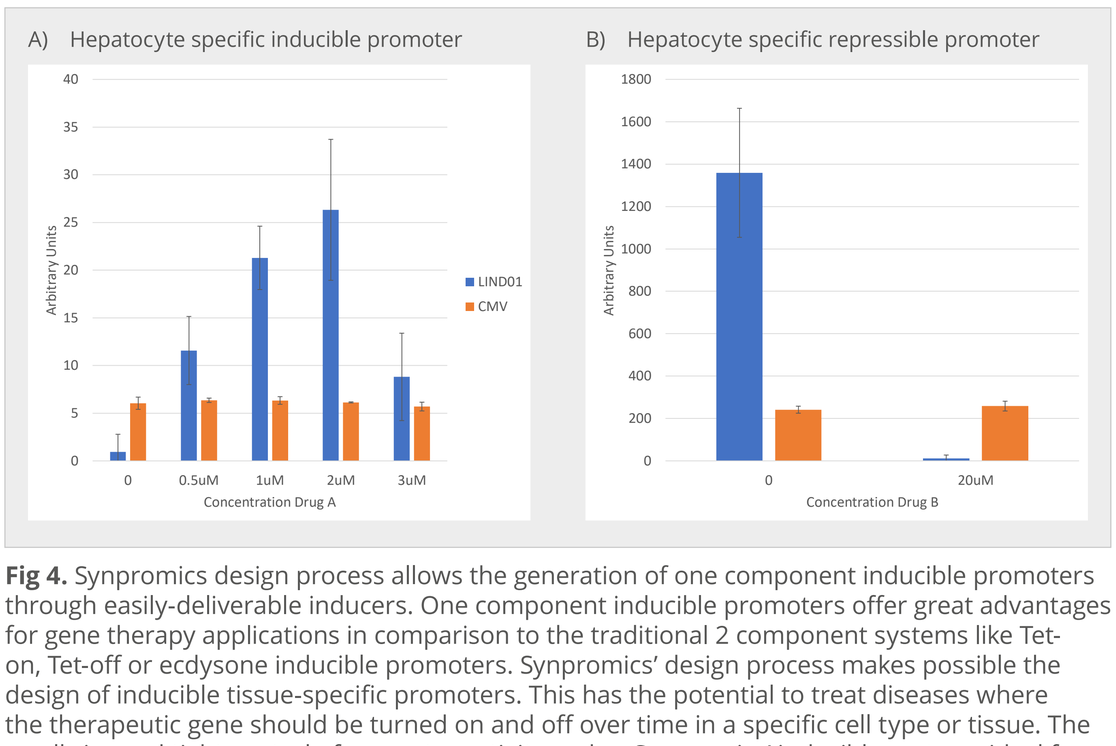

Fig 4. Synpromics design process allows the generation of one component inducible promoters through easily-deliverable inducers. One component inducible promoters offer great advantages for gene therapy applications in comparison to the traditional 2 component systems like Tet-on, Tet-off or ecdysone inducible promoters. Synpromics' design process makes possible the design of inducible tissue-specific promoters. This has the potential to treat diseases where the therapeutic gene should be turned on and off over time in a specific cell type or tissue. The small size and tight control of promoter activity makes Synpromics' inducible systems ideal for gene therapy applications. **A)** Example of a synthetic inducible liver specific promoter showing increased activity after addition of the inducer in human primary hepatocytes. Inducer was added 3 hours after transfection and EPO expression was measured, by ELISA, 24hrs later. In addition to tight control, the promoter shows dose dependent expression of the EPO gene used in the assay as transcriptional reporter. **B)** Example of a hepatocyte specific repressible promoter. Luciferase reporter constructs were transfected in human primary hepatocytes. Inducer was added 3 hours after transfection. Promoter activity was assayed 24 hours after induction was triggered.