Designer Promoter: A Novel Strategy for CIS Engineering

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In present era, in the field of genetic engineering and synthetic biology, new advancement everyday open new possibilities. Among these advancement, one most promising concept of a Designer Promoter has emerged as a novel strategy, particularly for cis-regulatory engineering. This innovative approach offers a control gene expression with unprecedented precision, enabling researchers to explore and manipulate genetic construct with greater control.

Designer Promoter

A Designer Promoter is a synthetically engineered DNA sequence that govern gene expression in a highly precise and anticipated manner. Unlike natural promoters, which are limited by their evolutionary constraints, Designer Promoters are custom-built to accomplish desired levels of gene activation or repression. This customization is particularly valuable in cis-regulatory engineering, where precise control over the spatial and temporal expression of genes is crucial.

The Role of Designer Promoters in CIS Engineering

Cis engineering in molecular biology is a technique that involves altering cis-regulatory regions (promoter, operator and effector like regulatory sequences) of genes to introduce genetic variation. This can be used to improve the characteristics of crops. Designer Promoters provide a transformative tool in this domain by:

- **Enhancing Precision**: By tailoring promoter sequences to specific transcription factors, Designer Promoters ensure highly selective gene activation. This is particularly useful for creating tightly regulated gene circuits in synthetic biology.
- **Optimizing Expression Levels**: Fine-tuning gene expression is essential for balancing metabolic pathways or achieving therapeutic outcomes. Designer Promoters allow researchers to modulate expression levels with exceptional accuracy.
- **Expanding Functional Diversity**: Natural promoters often have limited compatibility with different host systems or pathways. Designer Promoters can be engineered to function effectively across diverse organisms and conditions, broadening their utility.
- **Mitigating Off-Target Effects**: Customizing promoter sequences reduces the likelihood of unintended interactions with non-target genes, enhancing the specificity of genetic interventions. (Bhadouriya *et al*.,2024)

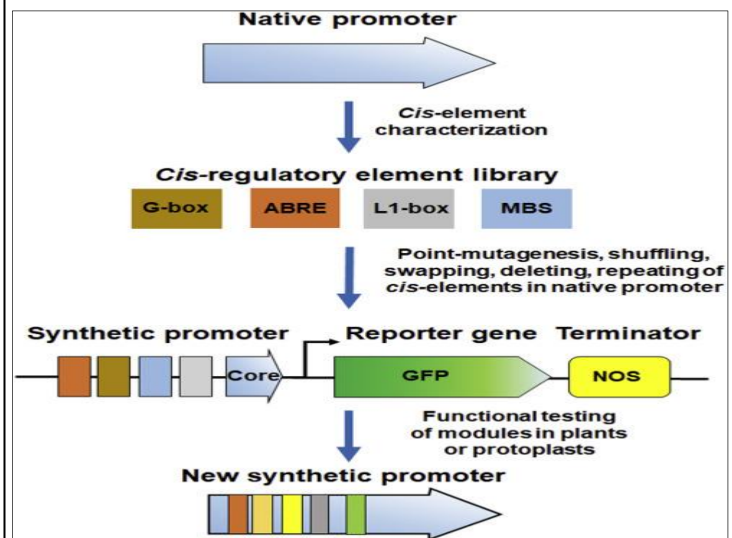

Fig. 1. Traditional approach for generating new synthetic promoter. ABRE, ABA-responsive element; MBS, MYB transcription factor binding site; Core, core promoter; NOS: nopaline synthase gene terminator. (Yasmeen *et al*.,2023)

Traditional approach for generating new synthetic promoters

The creation of Designer Promoters involves several cutting-edge techniques:

- Synthetic promoters were initially constructed by manipulating the position, spacing, and number of key *cis* elements by incorporating them individually or in combination into existing native/core promoters (Kumar *et al*.,2012 and Deb *et al*.,2018)
- Traditionally, the *cis* elements in native promoters were point mutated, shuffled, repeated, or replaced with novel motifs to generate synthetic promoters with desirable functions

- The mutated promoters are functionally tested in plants or protoplasts to guide subsequent generation of artificial promoters (Joreset *al*., 2021).

Table 1. Synthetic promoters generated in plants. (Yasmeen *et al*., 2023)

Promoter name	*cis*-element resource	Method used	Function	Tested plant
FUASCsV8CP	cassava vein mosaic virus	artificially synthesized	enhanced antifungal	tobacco
pOp4; pOp6	*Lac* operator *CaMV35S* mini promoter	manually synthesized	steroid, dexamethasone	*Arabidopsis*
Pmec	spacer element, TAGC repeat	manually synthesized	salt, ABA inducible	tobacco
GAL1	Yeast	manually synthesized	IPTG inducible	yeast
BiGSSP2; SP7	*Act1*, *rbcs1* promoter from rice	promoter synthesized	Bi-directional expression in	rice
At2S3	minimal promoter	artificially synthesized	phosphate responsive	*Arabidopsis*
SynP14-SynP19	CREs from soybean, *CaMV35S*	synthetic assemblies	root specific, drought	soybean hairy roots, *Arabidopsis*
CaMV35S with riboswitch	CaMV35S	module *aTheoAz*	theophylline responsive	*Arabidopsis*
TCSn	bacterial two component signaling	artificially synthesized	cytokinin responsive	rice protoplasts
SD18-1; SD9-2; SS16	CRES from poplar	SD and SS subdomains	osmotic stress inducible	poplar, tobacco, *Arabidopsis*
STAPs	*35S* promoter, effector-binding elements	small synthetic TALE-activated	dTALE protein inducible	rice
ZmDRO1	*cis* elements from maize	manually synthesized	ABA/drought inducible	maize
pATFs	gRNA and 35S minimal promoter	synthetic assemblies	inducible by gRNAs	tobacco, *Arabidopsis*
SlDFR	*CaMV35S* promoters	manual combination	promoted by fungal mPAF	tobacco

Applications of Designer Promoters

The utility of Designer Promoters extends across various fields of research and industry:

- **Synthetic Biology**: Designer Promoters are instrumental in constructing synthetic gene networks, enabling the creation of biological systems with novel functionalities.
- **Biopharmaceuticals**: In therapeutic development, Designer Promoters can be used to drive the expression of therapeutic proteins or regulate cellular behavior in gene therapies.
- **Metabolic Engineering**: Optimizing metabolic pathways for the production of biofuels, chemicals, or pharmaceuticals often requires

precise gene expression, which Designer Promoters can achieve.

Agricultural Biotechnology: Enhancing crop traits, such as stress resistance or yield, can benefit from the application of Designer Promoters to regulate plant gene expression in response to environmental cues

Challenges and Future Directions

Despite their potential, Designer Promoters face challenges that require further research:

- **Context Dependency**: Promoter activity can vary depending on the genomic and cellular context, necessitating careful optimization for each application.
- **Ethical Considerations**: As with all genetic engineering tools, the use of Designer Promoters raises ethical questions regarding their applications and long-term impacts.

Looking ahead, advancements in computational biology, machine learning, and high-throughput experimentation will continue to refine the design and functionality of Designer Promoters. As a result, their role in cis-regulatory engineering and beyond will become even more pivotal.

Conclusion

The development of Designer Promoters marks a significant leap forward in the field of cis-regulatory engineering. By enabling precise control over gene expression, they unlock new possibilities for research, innovation, and application in synthetic biology, medicine, agriculture, and more. As we continue to explore their potential, Designer Promoters are poised to become indispensable tools in the quest to engineer life at the molecular level.

References

Bhadouriya, S. L., Karamchandani, A. N., Nayak, N., Mehrotra, S., andMehrotra, R.(2024). Artificially designed synthetic promoter for a high level of salt induction using a cis-engineering approach. *Scientific Reports,* **14(1)**, 13657.

Deb, D., Shrestha, A., Maiti, I.B., and Dey, N. (2018). Recombinant promoter (MUASCsV8CP) driven rotaviral killer protein 4 (KP4) imparts resistance against fungal pathogens in transgenic tobacco. *Front. Plant Sci*. **9:**278.

Jores, T., Tonnies, J., Wrightsman, T., Buckler, E.S., Cuperus, J.T.,Fields, S., and Queitsch, C. (2021). Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. *Nat.Plants*.**7**:842–855.

Kumar, D., Patro, S., Ghosh, J., Das, A., Maiti, I.B., and Dey, N. (2012). Development of a salicylic acid inducible minimal sub-genomic transcript promoter from Figwort mosaic virus with enhanced roundleaf-activity using TGACG motif rearrangement. *Gene* **503**:36–47.

Yasmeen, E., Wang, J., Riaz, M., Zhang, L., and Zuo, K. (2023). Designing artificial synthetic promoters for accurate, smart, and versatile gene expression in plants. *Plant Communications*, **4**, 100558.

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