Design, construction, and function of a genetic circuit that regulates gene transcription in response to a pair of mutually exclusive induction events

Melika Sadeghi | Supervised by: Dr. James Davey | 39th Annual Cameron Conference 2025

Introduction

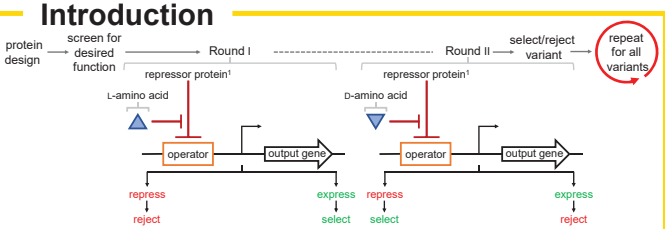


Figure 1. Engineering proteins that recognize or produce metabolites with enantiomeric specificity requires that protein variant functions be screened using multiple rounds of the protein engineering workflow. For example, engineering a repressor that selectively recognizes an L-amino acid requires screening the same candidate protein to ensure no interaction with the D-amino acid enantiomer. This renders the conventional protein engineering workflow intractable and prone to failure.

Problem: There is no protein engineering platform that mediates simultaneous selection of multiple protein functions in a single experiment: Genetic circuits can serve as a protein engineering platform that condenses multiple protein functions into a single gene expression event.

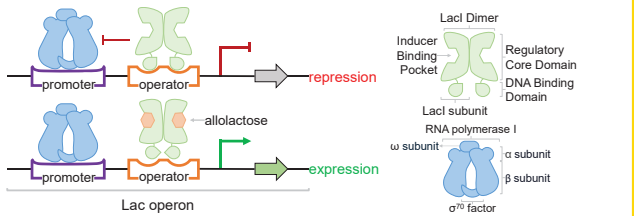
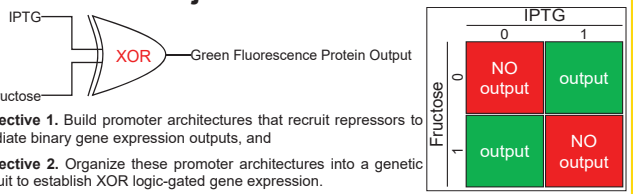


Figure 2. Genetic circuits are a system of transcription factors that function in concertation with genetic elements to control gene transcription: The **Lac operon** is an example of a natural genetic circuit.[3]

Research Objectives



Objective 1. Build promoter architectures that recruit repressors to mediate binary gene expression outputs, and

Objective 2. Organize these promoter architectures into a genetic circuit to establish XOR logic-gated gene expression.

Hypothesis

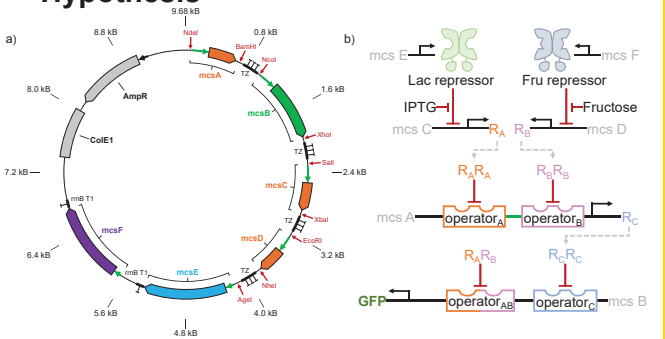


Figure 3. The plasmid architecture (a) and genetic circuit design (b) will enable XOR logic-gated control of mNeon protein (GFP) expression, producing binary output responses to IPTG and fructose. This could serve as a platform for selecting designed variants when engineering systems of protein functions and sequences.

Design: Promoter Architecture

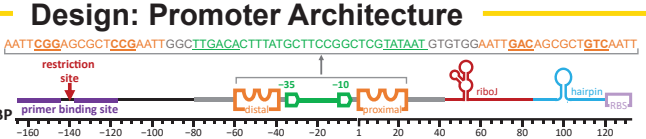


Figure 4. Promoter design incorporates genetic elements that mediate control of gene transcription: **Promoter sequences**, including **-35** and **-10 boxes**, recruit RNA polymerase to initiate gene transcription. **Operator sequences**, placed distal and proximal relative to the promoter[5], recruit repressor proteins to repress gene transcription. The genetic insulator **riboJ** ensures standardized transcriptional output.[1] The **ribosome binding site** (RBS) recruits ribosome to the mRNA transcript to initiate protein translation. The **hairpin** structure enhances recruitment of the ribosome to the RBS.[2]

Design: Repressor Architecture

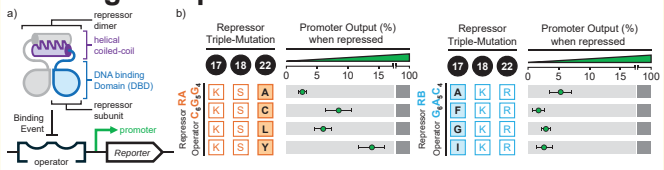


Figure 5. R_A, R_B, R_C are repressor dimers that share the same architecture (a) except for residues in their DNA binding domain at positions 17, 18, and 22. Four triple-mutations were introduced in R_A's and R_B's DBD to recognize distal and proximal operators at multiple cloning site A (b). The triple-mutations were introduced to provide binary gene expression outputs that maximize transcript count from gene expression events and minimize background expression during repression.[4]

Methodology: Synthesis Workflow

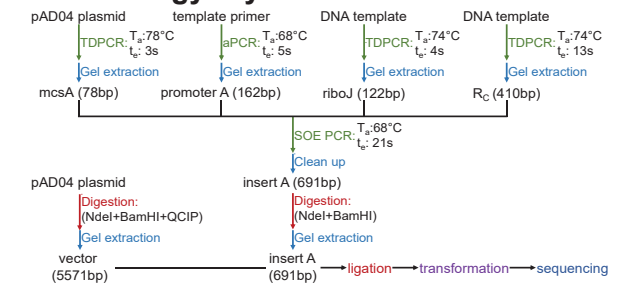


Figure 6. Synthesis workflow of multiple cloning site (mcs) A. Promoter cassette elements were synthesized by touchdown (TD) PCR, assembly PCR (aPCR), and overlap extension (SOE) PCR. A similar workflow was used to synthesize insert B, insert C, and insert D.

Methodology: Synthesis Protocols

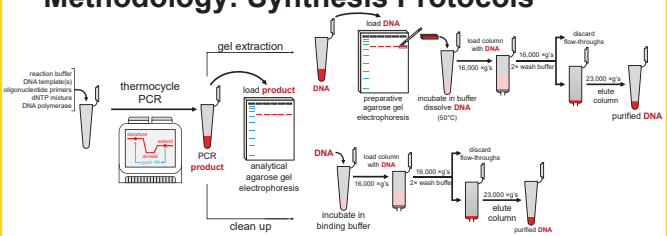


Figure 7. PCR, gel extraction, and DNA cleanup protocol. A PCR product can be purified by gel extraction or clean up.

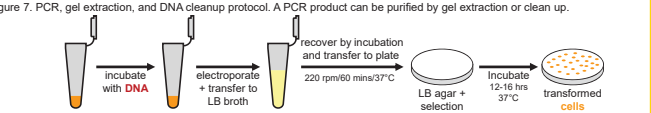


Figure 8. Transforming electrocompetent *E. coli* cells with plasmids subjected to ligation with the synthesized promoter cassettes.

Result: Circuit Components Synthesis

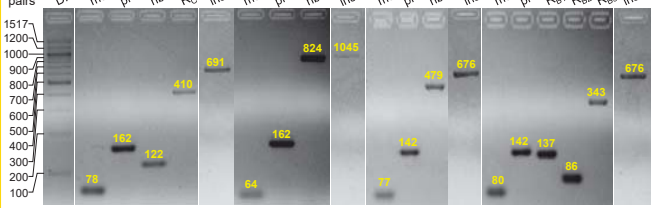


Figure 9. 2% (w/v) analytical agarose gel electrophoresis of the synthesized multiple cloning site (mcs) A, mcs B, mcsC, and mcs D components including promoter (pro), riboJ, and repressor (R) sequence.

Result: Transformations

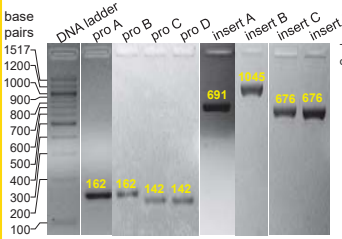


Figure 10. 2%(w/v) analytical agarose gel electrophoresis: successful transformation of *E. coli* cells with pAD04 plasmids containing the synthesized promoter (pro) and insert sequences.

Table 1. Sanger sequencing results following the transformation of *E. coli* cells with the synthesized pAD04 plasmids.

multicloning site	promoter component	gene component
mcsA	sequence verified	sequence verified
mcsB	sequence verified	sequence verified
mcsC	sequence verified	sequence verified
mcsD	assembly error	sequence verified
mcsE	sequence verified	sequence verified
mcsF	sequence verified	sequence verified

Next Steps and Discussion

Figure 11. Positive control: Constitutive expression of GFP by introducing triple mutations in the DNA binding domains of the repressors, which are no longer able to bind to the operator. Negative control: A mutation in mNeon protein that disrupts the formation of a mature chromophore, rendering the protein incapable of fluorescence.

The next steps include:

1. Fixing assembly errors in mcsD promoter.
2. Characterization and quantification of XOR-logic gated control of GFP protein.

Conclusions

Genetic circuits with modular components designed for logic-gated control of gene expression can serve as a valuable platform for simultaneous selection of a desired function. e.g. the biocatalysis of enantiospecific chemical commodities like D- vs. L- amino acids acids.

Figure 12. Diagram of XOR logic-gated production of antibiotics with enantiospecific chemical commodities, D- and L- amino acids.

References

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Davey Lab (left to right): Olya Akay, Jaime Barrett, Ally Roberts, Jordan Cucksey, Melika Sadeghi, Amina Menhour, James Davey