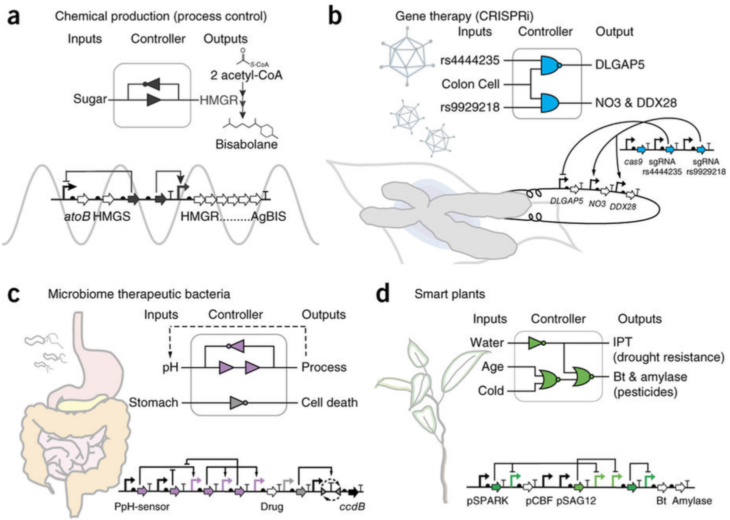
Engineering Genetic Circuits

Chris J. Myers

Lecture 11: Principles of Genetic Circuit Design

Potential Uses of Synthetic Genetic Circuits



(Courtesy of Brophy/Voight, Nature Methods, 2014)

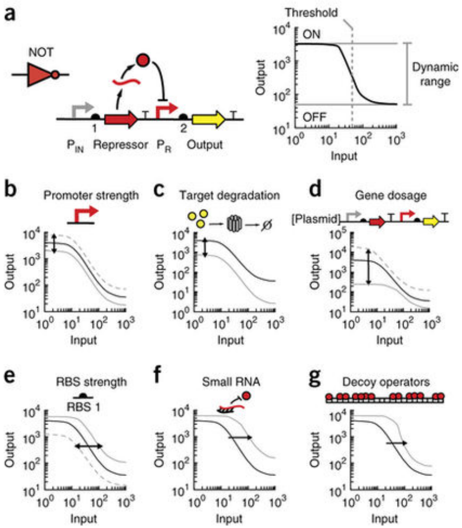
Challenges to Genetic Circuit Design

- Require precise balancing of regulators to generate a correct response.
- Can be difficult to screen for correct performance.
- Few tools available to measure circuit performance other than flurorescent reporters, which are limited for measuring dynamics.
- Sensitive to environment, growth conditions, and genetic context.
- Assembly of large genetic circuits is difficult and often has errors.

Overview

- Methods of modifying circuit behavior.
- Common failure modes from connecting circuits.
- Circuit performance within the context of a living cell.
- Alternative regulatory mechanisms for genetic logic gates.

Methods of Modifying Circuit Behavior



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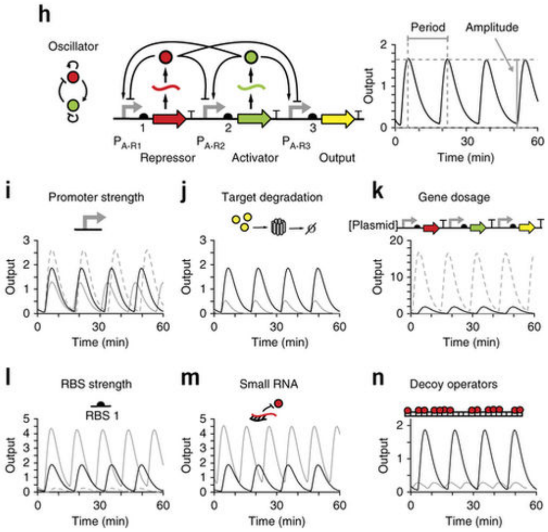
Methods of Modifying Circuit Behavior (cont)



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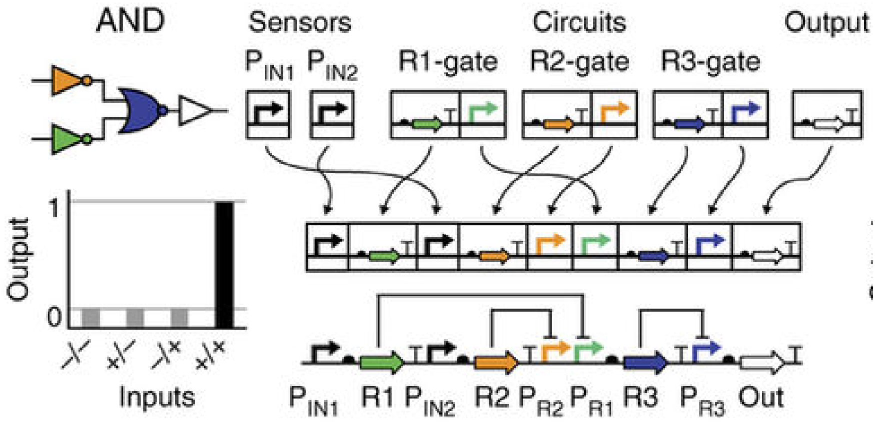

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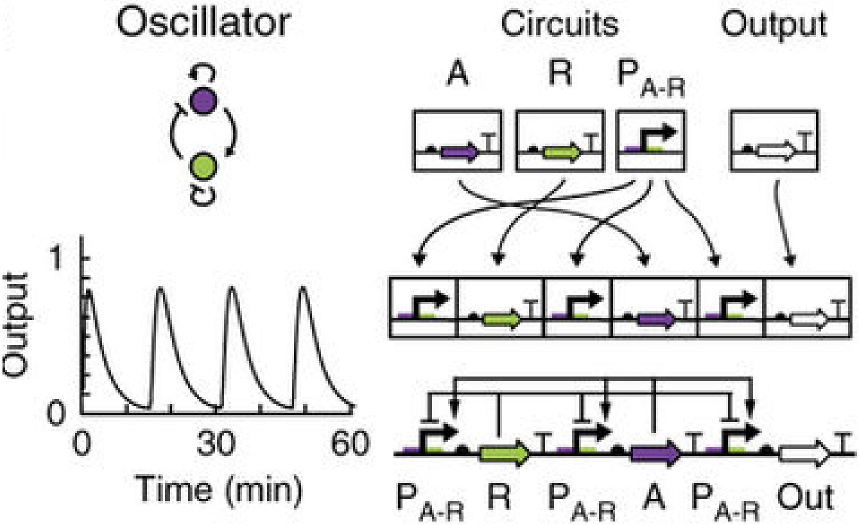
Connecting Circuits: Oscillator Example



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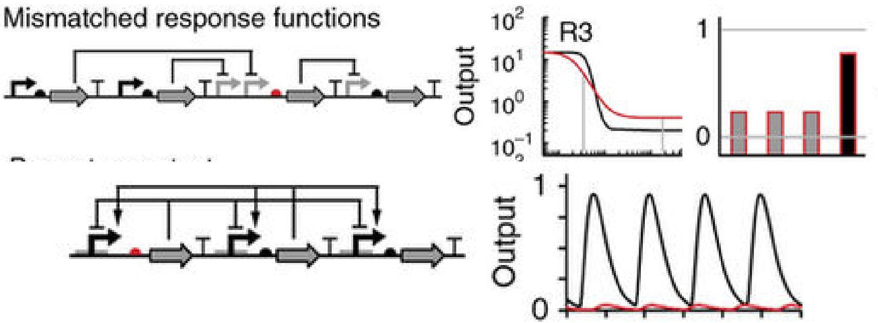
Failure Modes: Mismatched Response Functions



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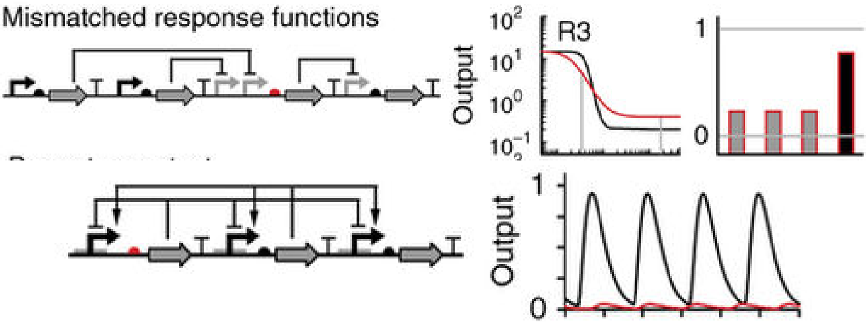
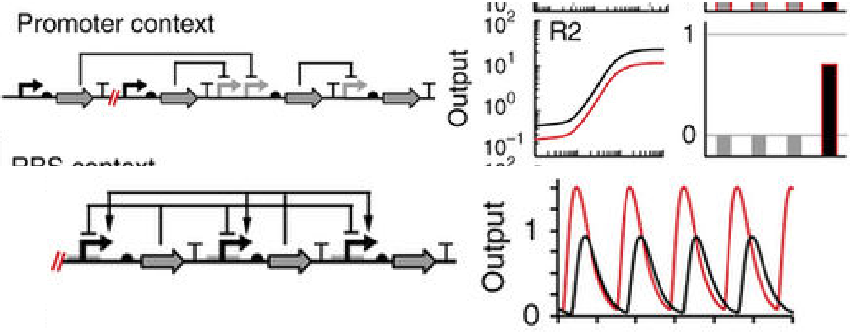
Failure Modes: Mismatched Response Functions



(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:
Select RBSs and promoters to achieve the required expression levels.



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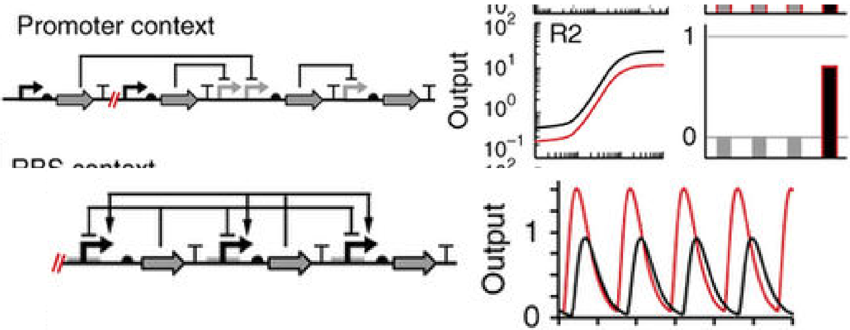
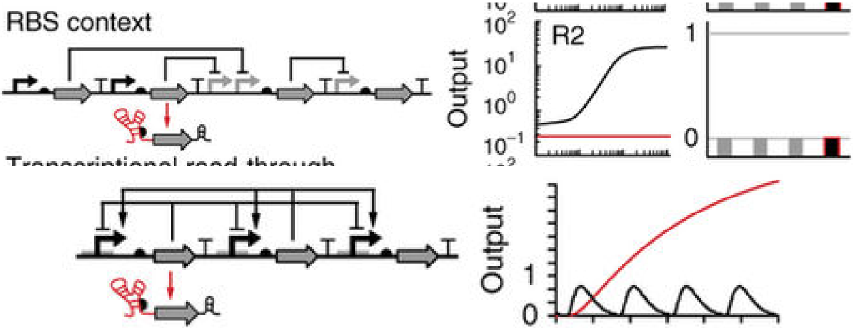
Failure Modes: Promoter Context



(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:
Insulator sequences standardize the DNA sequences flanking promoters.

Failure Modes: RBS Context



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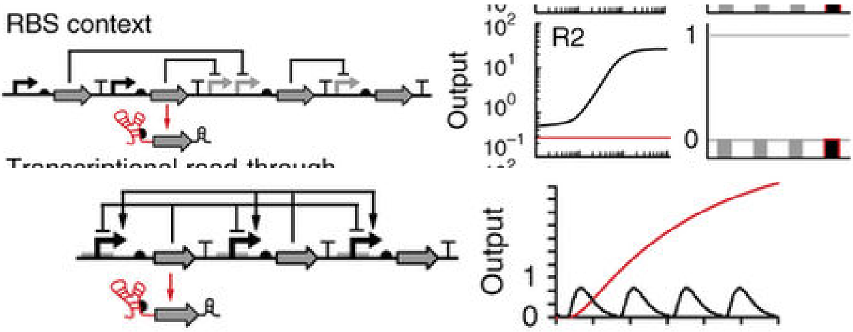
Failure Modes: RBS Context

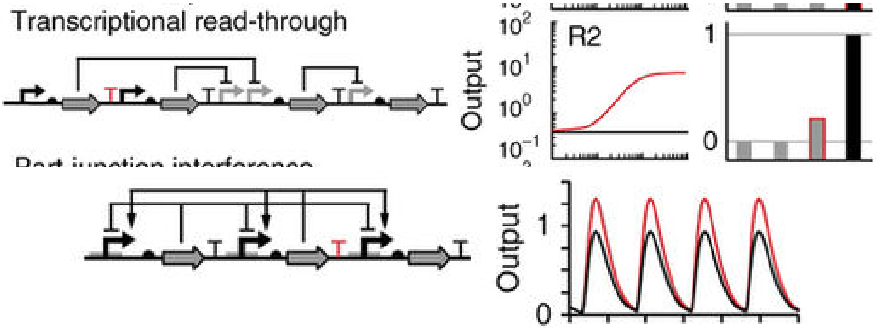


(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:

5' UTR can be cleaved with ribozymes to standardize RBS accessibility.

Failure Modes: Transcriptional Read Through



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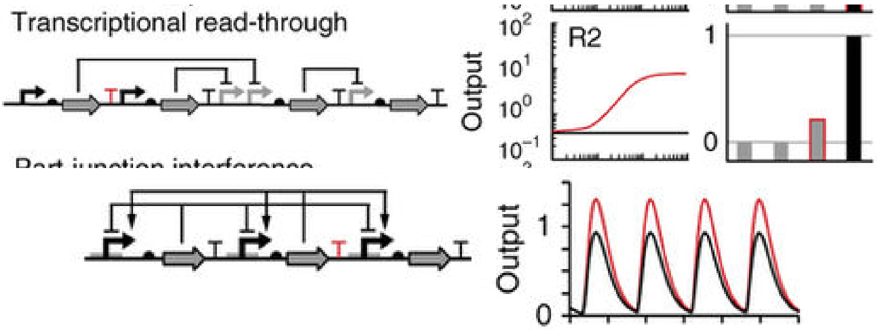
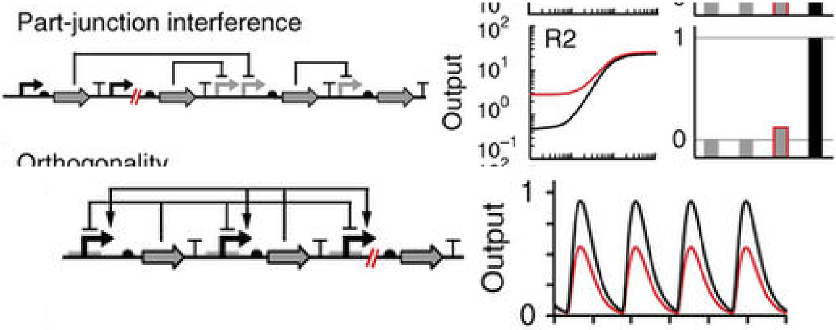
Failure Modes: Transcriptional Read Through



(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:
Use strong, tandem terminators.

Failure Modes: Part Junction Interference



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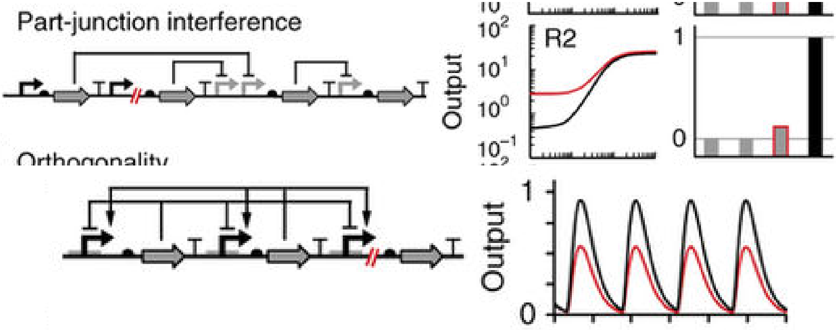
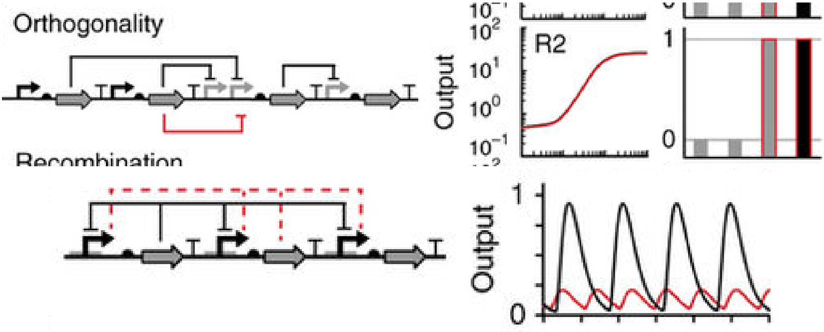


(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:
Scan for unintended functional sequences.

Failure Modes: Orthogonality



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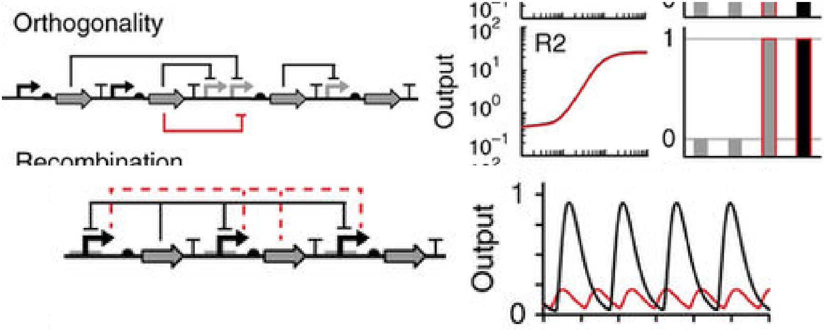
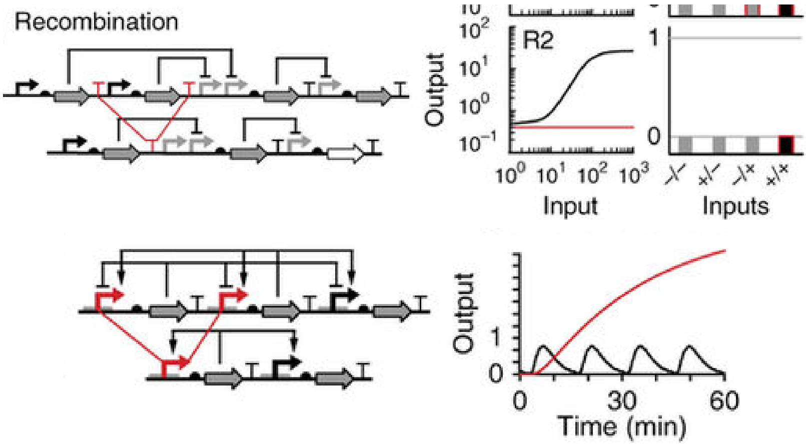
Failure Modes: Orthogonality



(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:
Screen parts to test every combination of promoter and regulatory element.

Failure Modes: Recombination



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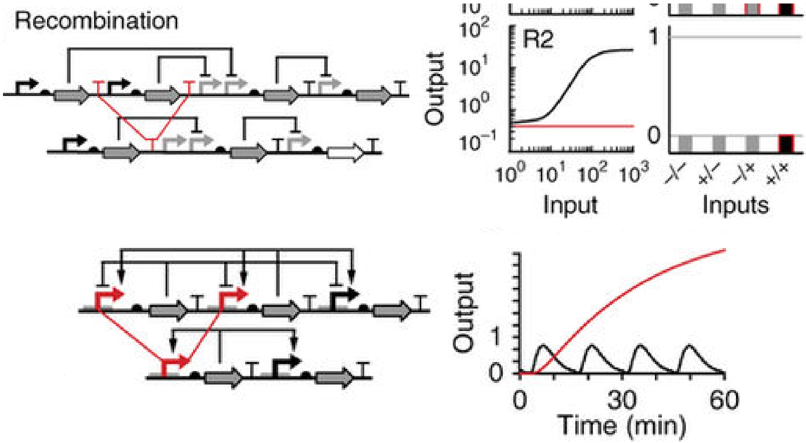
Failure Modes: Recombination

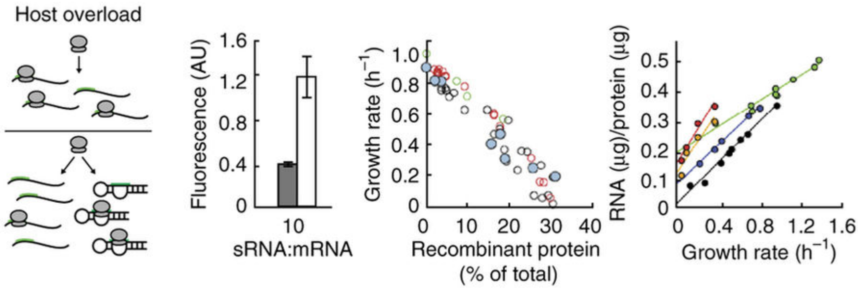


(Courtesy of Brophy/Voight, Nature Methods, 2014)

SOLUTION:

Use large libraries of parts with enough sequence diversity.

Host Context Issues: Host Overload



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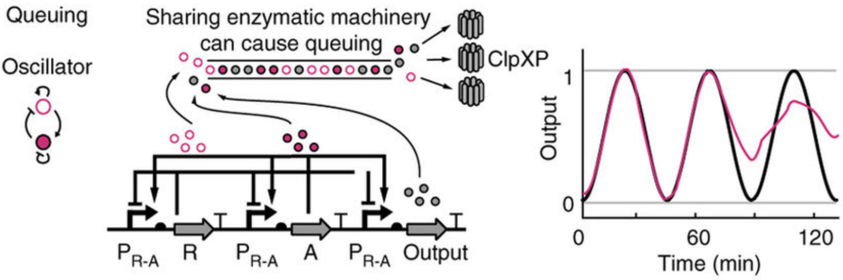
Host Context Issues: Queuing



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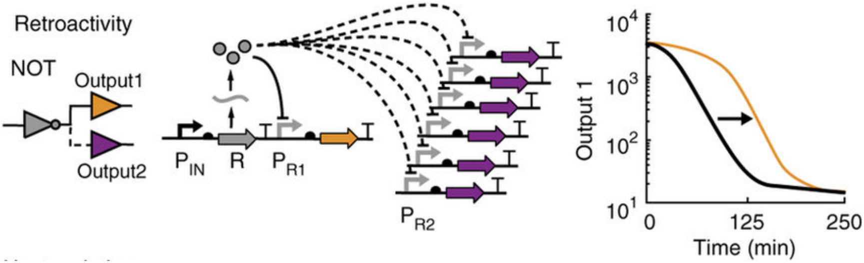
Context Issues: Retroactivity



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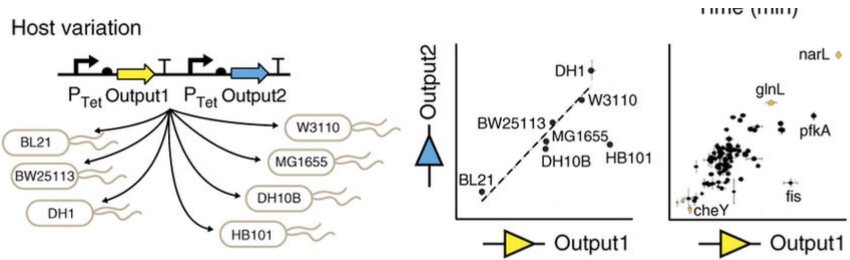
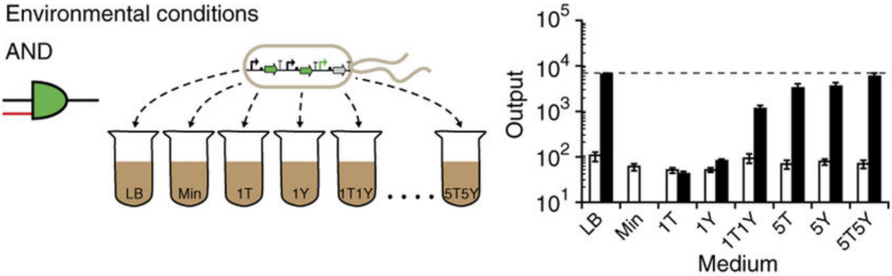


(Courtesy of Brophy/Voight, Nature Methods, 2014)

Context Issues: Environmental Conditions



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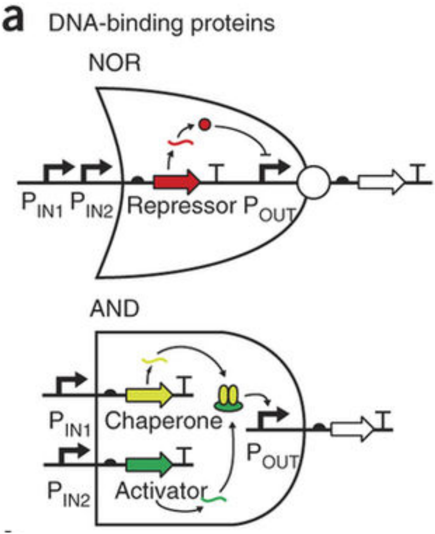
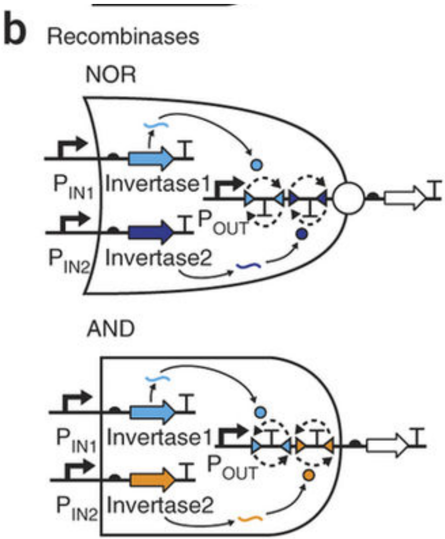
DNA Binding Proteins



(Courtesy of Brophy/Voight, Nature Methods, 2014)

Challenges with DNA Binding Proteins

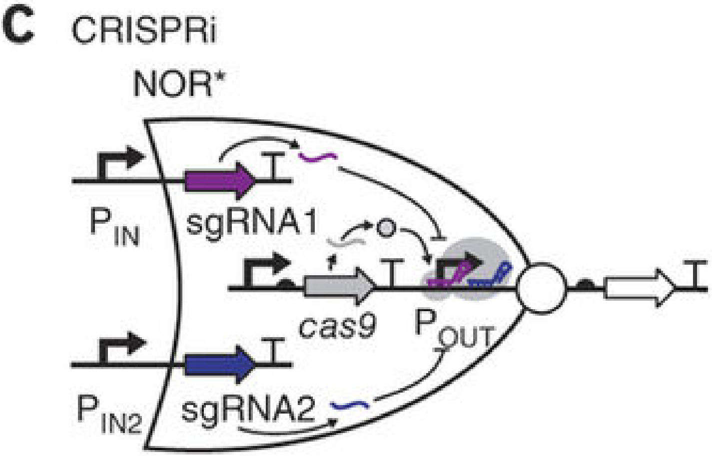
- Expanding protein libraries is difficult, since they must be orthogonal.
  - zinc-finger proteins
  - transcription activator-like (TAL) effectors
  - TetR and LacI homologs.
- Even when individual transcription factors are nontoxic, multiple regulators may be toxic.
- Circuits are dependent on growth rate, since dilution rate affects steady-state concentration of regulators.
- Response functions are often suboptimal and difficult to control (high OFF states and low dynamic ranges).



(Courtesy of Brophy/Voight, Nature Methods, 2014)

Recombinases: Advantages/Disadvantages

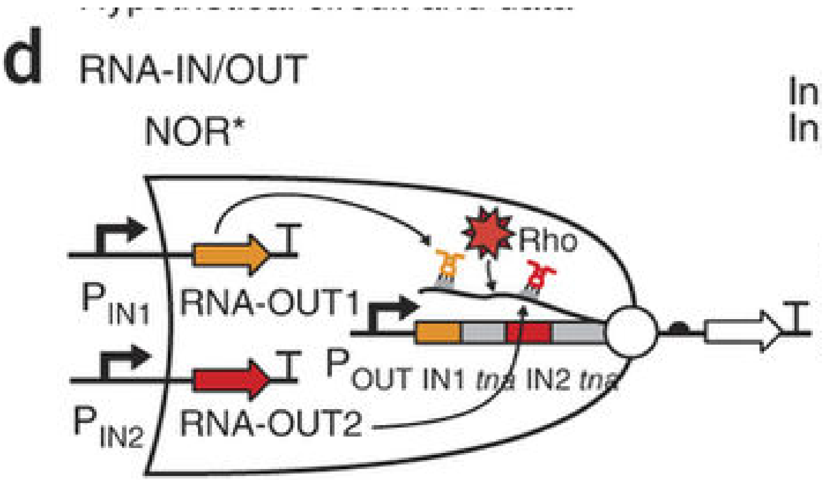
- Tyrosine recombinases (Cre, Flp, FimBE) - require host-specific factors, can be reversible and irreversible.
- Serine integrases - catalyze unidirectional reactions to invert DNA without host factors and often have excisonases to return to original orientation.
- Advantages:
  - Ideal for memory storage since they flip DNA permanently.
  - All two-input logic gates have been built using serine integrases.
- Disadvantages:
  - Reactions can be slow and generate mixed populations.
  - Reversing the state change requires extra circuitry.



(Courtesy of Brophy/Voight, Nature Methods, 2014)

CRISPRi: Advantages/Disadvantages

- *Clustered, regularly interspaced, short palindromic repeat* (CRISPR) function as a bacterial immune system that targets specific DNA sequence motifs for degradation.
- Use a Cas (CRISPR-associated) nuclease and guide RNA to introduce double-strand breaks to specific DNA sequences.
- CRISPR *interference* (CRISPRi) uses dCas9/Cas9_{N-}, which does not have a nuclease, as a transcription factor to repress gene expression by producing a DNA bubble.
- Advantages:
  - Can create orthogonal set of guide sequences to target different promoters.
  - Operate at speeds similar to protein-based circuits.
- Disadvantages:
  - Predicting guide RNA orthogonality is complicated.
  - Toxicity can result if Cas9 binds to and interferes with the host genome.
  - Retroactivity can be an issue since Cas9 is a shared resource.

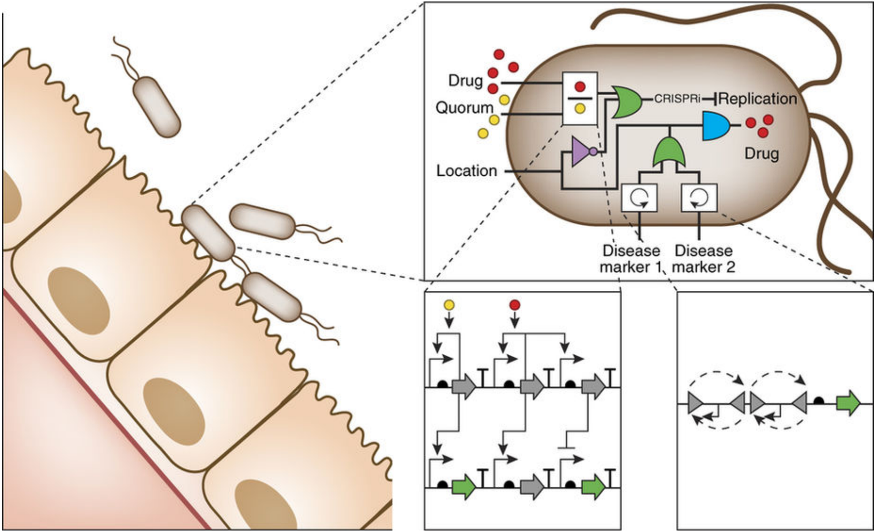


(Courtesy of Brophy/Voight, Nature Methods, 2014)

RNA IN/OUT: Advantages/Disadvantages

- Natural system represses translation with a short non-coding RNA.
- Synthetic system adapted to repress transcription using the transcriptional adapter, *tna*, composed of RBS and CDS for TnaC.
- When TnaC translation is blocked, Rho binds knocking off the RNAP.
- Advantages:
  - Could be used to generate a large set of orthogonal regulators.
  - Have been successfully used for 2/3/4-input NOR gates.
- Disadvantages:
  - Each transcriptional regulator requires the same *tna* regulatory element ($\sim$290 bp), which could lead to homologous recombination.

Conceptual Circuit for a Therapeutic Bacterium



(Courtesy of Brophy/Voight, Nature Methods, 2014)

The Road Ahead

- Standards:
  - Experimental synthetic biologists should be encouraged by journals and funding agencies to share their data using standards and repositories.
  - Computational synthetic biologists need to develop software tools that can easily create models and capture design information using standards.
- Abstraction:
  - Experimental synthetic biologists must determine design details that are critical to achieving a desired phenotypic behavior.
  - Computational synthetic biologists must fall out of love with their models, accept abstraction, and create tools supporting multiple abstractions levels.
- Decoupling:
  - Experimental synthetic biologists need to create libraries of components that are more orthogonal and well characterized.
  - Computational synthetic biologists need to develop design flows that allow the biologically naive to build genetic circuits that actually work.

J. R. R. Tolkien

Faithless is he that says farewell when the road darkens.

Sources

- Brophy/Voight, Nature Methods, 2014.
- Myers, IEEE Transactions on Multi-Scale Computing Systems, 2015.