Recombinase-Based Circuits for Environmental Detection, Diagnostics, and Logging

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Outline
I. Event detection and introduction to recombinases
II. Event diagnostics using population-level, stochastic response
III. New directions: event logging, field-programmability
IV. Summary and next steps
Environmental Detection, Diagnostics, and Logging

**Approach**
- Component technologies: signal detection, memory, species comparison, logic functions
- Event detectors: $A > B$, $A$ followed by $B$, $A > \text{thresh}$
- Interconnection framework: modular techniques for interconnecting components & detectors

**Applications**: environmental monitoring, diagnostics for health, circuit debugging, …
Integrases and Excisionases

(Serine) Integrases
• Mechanism by which phage insert DNA into the chromosome of a bacterial host
• $attP$ = phage recognition site
• $attB$ = bacterial recognition site
• Integrase action: insert phage DNA into bacterial chromosome, leaving changing recognition sites

Excisionases
• Reverse reaction requires second phage-coded protein, excisionase

Other recombinases
• Cre recombinase - tyrosine recombinase
• Cre-Lox & Flp-FRT recombinases - insertion, excision, inversion, translocation
• CRISPR/Cas9 - guide RNA-directed excision, insertion

Repurposing Recombinases for Synthetic Biology

Basic trick: put attB/attP on same piece of DNA
- Integrase activity causes DNA between sites to flip
- Excisionase (+ integrases) causes reverse flip

Effects depend on orientation of attachment sides
- Attachment sites pointing toward each other: flip
  \[
  \text{attB-attP} \quad \text{attL-attR} \\
  \begin{array}{ccc}
  a & b & c \\
  \rightarrow \\
  a & \overline{q} & c
  \end{array}
  \]
- Attachment sites in same direction: excise
  \[
  \begin{array}{ccc}
  a & b & c \\
  \rightarrow \\
  a & c
  \end{array}
  \]


Recombinase-Based Circuit Examples


Event Ordering Detection (A then B)

DNA layout

Integrases:
- TP-901
- Bxb1

Reporter
Terminator
Promoter
Reporter

A and B simultaneously

A at t = 0 hr
B at t = 1 hr

Number of cells that switch depends on interval between the two inputs

Steady state response

"B"

A only
B only
No inducer
A then B
B then A

Markov process model

Hsiao, Hori and M., MSB, 2016
Additional Event “Diagnostics”

Q: can we keep track of other things in addition to \( \Delta T \)?

- Have two measurements: \#red, \#green (versus total concentration)
- Idea: GFP population depends on the duration of pulse B => can also measure PWb

Use calibration phase (or models -:) to create lookup table and determine properties of inputs
Event Logging Circuit

Objectives

- Implement a genome ‘recording site’ with a chronological order of inserted DNA fragments
- Utilize plasmids as the source of recording material, and use integrases as the means for DNA insertion

Status

- Built event logger design consisting of four modules: event plasmid (ID sequence), input selector (not shown), controller, logging site
- One event detector circuit tested and working
- Event plasmid selector using Cas9 gRNA to block integrases working in TX-TL

Event logging circuit using DNA integrases

Event Plasmid  Integrate Controller  Genome site

Spin down & resuspend in fresh media

Proof-of-concept experimental validation

A. Shur (unpublished)
Field Programmable Circuits

Idea: use dCas9 to block integrases
- Use gRNA to guide dCas9 to recognition site => no integration
- Can create different circuits by controlling insertion of elements

Similar to field programmable gate arrays (FPGA) technology in circuits
- Use expression of different integrases to interconnect circuits

Preliminary experiments: it works!
- Cell-free assays show repression of integrates activity

Recombinase-Based Circuits for Environmental Detection, Diagnostics, and Logging

Recombinase-based circuits compliment capabilities of genetic networks

- Ability to reconfigure DNA in cells can be used for logic and memory (detection logic)
- Stochastic response across populations of cells provides diagnostic capability
- Use DNA as a “recording tape” (logging) [see also recent paper by Shipman et al.]

Other uses to be explored

- Integrases as a means of “programming” circuits (FPGA-style)
- Use integrase/excisionase pairing as feedback mechanism (see Folliard poster)

Some open problems

- Compilers: specs → (recombinase) circuits
- Leaks are still a problem (leaky integrate expression => noisy flipping)
- Better exploiting stochastic dynamics
Some Challenges and Research Directions (BFS)

Better understanding of uncertainty
- How do we capture observed behavior using structured models for (dynamic) uncertainty

Stochastic specifications and design tools
- How do we describe stochastic behavior in a systematic and useful way?
- How do we design stochastic behavior?
- What are the right design “knobs”?

Higher level design abstractions
- What are the right “device-level” design abstractions (and corresponding diagrams)?

Redundant design strategies
- Start implementing non-minimal designs
- Analogy: stochastic memory storage

Scaling up: components → devices → systems
- How can we use in vitro “breadboarding” to design and implement complex systems