Engineering Genetic Circuits

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Lecture 3: Genetic Devices

What are Devices?

- Aggregations of parts.
- Modular in nature (i.e., can be assembled to form larger devices).
- Encapsulate behavior (ideally).
- In other words, parts are assembled to produce new devices that perform a useful defined function.

Some Basic Rules for Composing Devices from Parts

- Coding sequences should have a RBS preceding them.
- Promoters should be upstream (in front of) the RBS and the coding sequence.
- Use transcription terminators to stop transcription.
- Use three stop codon in three frames to stop translation.
- Coding sequences in a operon need their own RBS but can share a promoter.
- Promoters can be combined by putting them next to each other.

Types of Devices

- The iGEM registry defines the following devices:
  - Protein generators
  - Reporters
  - Receivers and senders
  - Measurement devices
  - Inverters
- Logic gates are also desirable for engineering control and computation.

Protein Generators

- Essentially protein coding sequences with promoters and RBS to enable transcription and translation.
- Many also include transcription terminators.

Protein Generators

Available Protein Generators PRCT *Show 28 more parts* *Edit*

		Name	Protein	Description	RBS	Tag -?-	Performance O_H	Length
1☆		BBa_J45270	ATF1	Stationary-phase-dependent banana odor generator	BBa_B0032	None		1802

Edit Table specification

Available Protein Generators RCT *Show 46 more parts* *Edit*

		Name	Protein	Description	RBS	Tag -?-	Performance O_H	Length
1☆	W	BBa_I0462	luxR	luxR Protein Generator	BBa_B0034	None		936
1☆	W	BBa_J45199	ATF1	Banana odor enzyme (ATF1) generator	BBa_B0030	None		1739
A	W	BBa_J45219	ATF1	Banana odor enzyme (ATF1) generator	BBa_B0032	None		1737
A	W	BBa_J45299	PchA & PchB	PchA & PchB enzyme generator		None		1894
1☆	W	BBa_J45319	PchA & PchB	PchA & PchB enzyme generator		None		1892

- A type of protein generator where the amount of protein indicates level of expression.
    - Visual output
    - Enzyme activity
    - Other quantifiable biochemical or phenotypic trait
- Measures the combination of transcription and translation.
- Reporter genes include:
    - Those encoding fluorescent proteins such as GFP, RFP, etc.
    - lacZ encoding beta-galactosidase
    - Luciferase genes

Reporters

Available reporters PRET *Show 67 more parts* *Edit*

		Name	Description	Tag -?-	Excitation	Output	Length
1☆	W	BBa_I13521	Ptet mRFP	None		RFP	923
1☆	W	BBa_I13522	pTet GFP	None		GFP	937
1☆	W	BBa_I13600	Tet with CFP reporter (without LVA tag)	None		CFP	940
1☆	W	BBa_I13602	Tet operator with CFP reporter (with LVA tag) [R/Tc+]	LVA		cyan	979
1☆	W	BBa_I763007	promoter lambda (cI regulated) with RFP reporter				918

Receivers and Senders

- Exploit cell signalling systems to coordinate behavior of populations.
- Based on bacterial signalling method called *quorum sensing*.
- Small molecules produced by *sender* and sensed by *receiver* device.
  - *Acy-homoserine lactones* (AHLs) used in *Gram negative*, such as *E. coli*.
  - Small peptides are used by *Gram positive* organisms, such as *B. subtilis*.

Cell-Cell Signalling

Cell-cell signalling devices allow communication between an individual cell and its neighbors in culture or on a plate. This capability allows synchronized behavior across a cell population or the communication of information between cells hosting different systems. A cell can send a signal and it can receive an averaged signal from all its neighbors carrying the same signalling device. The two fundamental devices to perform cell-cell signalling are therefore a Sender device and a Receiver device. The current families of sender and receiver devices are all based on the Lux system of V. Fischeri or its analogs in other organisms (see references). These two families of devices are defined below.

Available signal senders *Show 4 more parts* *Edit*

		Name	Description	Family	Signalling Molecule	Control	Proteins	Molecules Cell Sec	Delay
1☆	W	BBa_F1610	$3OC_6HSL$ Sender Device		$3OC_6HSL$		LuxI		
A		BBa_F1780	AI-1 Sender Device		AI-1		LasI		
A		BBa_K574004	3OC12HSL regulated by TetR		3OC12HSL				
A		BBa_K574005	3OC12HSL and YFP regulated by pBad		3OC12HSL				

Edit Table specification

Available signal receivers *Show 6 more parts* *Edit*

		Name	Description	Family	Signalling Molecule	Control	Proteins	Switch Point	Delay
1☆	W	BBa_F2620	$3OC_6HSL$ -> PoPS Receiver		$3OC_6HSL$	R0040	LuxR, TetR	2nM	Seconds
1☆	W	BBa_F2621	$3OC_6HSL$ Receiver Device		$3OC_6HSL$	R0063	LuxR	2nM	Seconds
1☆	W	BBa_F2622	$3OC_6HSL$ Receiver Device		$3OC_6HSL$	R0011	LuxR, LacI		

- Essentially reporter constructs and standard measurement protocols.
- Measure the strength of:
  - Promoters (i.e., transcription)
  - Ribosome binding sites (i.e., translation)
- Require validation of copy number and cell metabolic status
- Promoter often measured as *polymerases per second* (PoPS).
- Ribosomal binding site measured as *Ribosomes per second* (RiPS).

Measurement Devices

Information about measurement systems

Table of promoter output high measurement system parts

Available Measurement Devices *Show 892 more parts* *Edit*

		Name	Type	Description	Output	Length
A	W	BBa_I13513	Measurement	Screening Plasmid 2		2885
A	W	BBa_I13515	Measurement	Screening Plasmid 4		2825
A	W	BBa_I732902	Measurement	R0010 I732020		597
A	W	BBa_I732903	Measurement	R0011 I732020		452
A	W	BBa_I732913	Measurement	[aTC] -> RFP		1757
A	W	BBa_I732916	Measurement	P_NOR_U037O11D002O22 + RFP		954
A	W	BBa_J107011	Measurement	PlacIQ measurement system		921
A	W	BBa_J107028	Measurement	J23100 measurement system (with RFP)		904

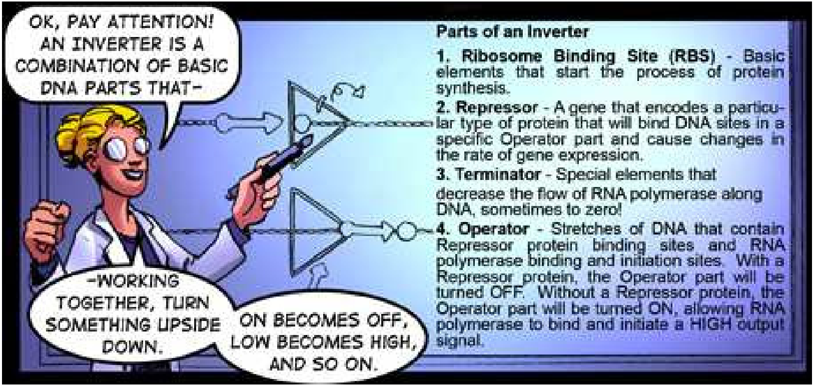
Logic Gates

- Term logic gates derived from electronic circuits.
- Devices constructed to produce a single output from multiple inputs.
- Logic gates assembled to produce complex electrical circuits.
- Operations described by Boolean algebra using binary states.
  - 1 for on, 0 for off.
- Allows biological systems to be programmed.
- Logic gates constructed using biological systems.
  - Compute inside cells.
  - Use genetic circuits, biochemical networks, and nucleic acids.

Biological Logic Gates

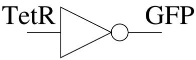
- Biology adds to the complexity of design.
- Gates may be noisy in practice.
    - Reduces true digital behavior.
    - Fluctuations in expression levels.
    - Unwanted interactions.
    - Can engineer gates to amplify or reduce noise.
    - Autorepression can reduce noise.

Genetic Inverter

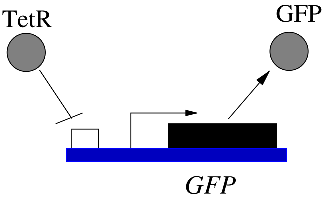


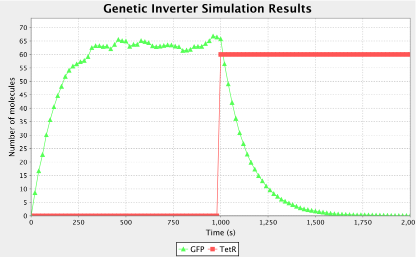
(From “Adventures in Synthetic Biology” - Endy et al.)

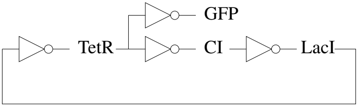
Genetic Inverter

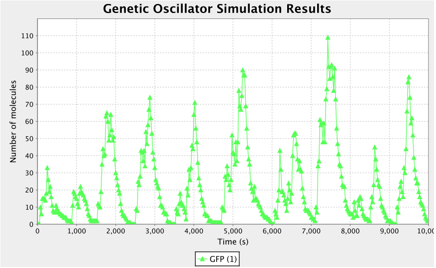


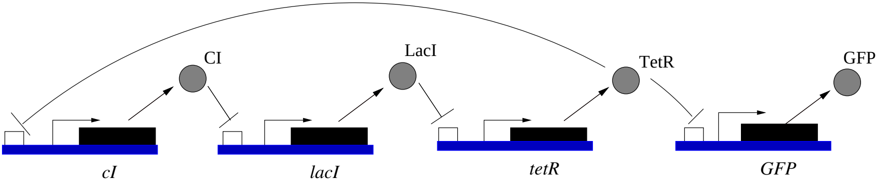
TetR	GFP
0	1
1	0






GFP
TetR
CI
LacI

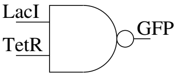

Genetic Oscillator Simulation Results
Number of molecules
Time (s)
GFP (1)


CI
LacI
TetR
GFP
cI
lacI
tetR
GFP

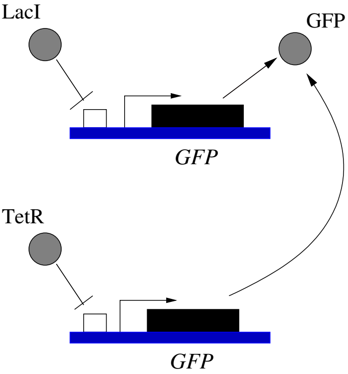
Genetic Oscillator

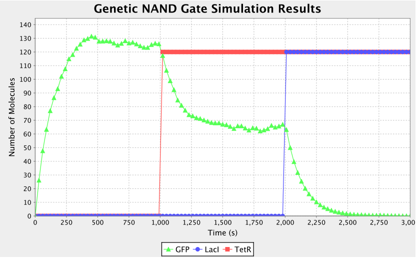
- Oscillations used as central clocks to synchronize behavior.
- *Circadian rhythms* manifest as periodic variations of concentrations of particular proteins in the cell.
- Though precise mechanism is unknown can generate a network that has a similar behavior.
- Note that not all parameter choices lead to oscillations.
- High protein synthesis and degradation rates, large cooperative binding effects, and efficient repression are all necessary.
- As a result, strong and tightly repressible promoters are selected, and proteins are modified to make easy targets for proteases.

Genetic NAND

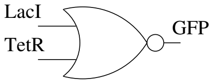


LacI	TetR	GFP
0	0	1
0	1	1
1	0	1
1	1	0

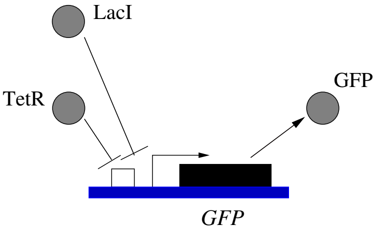


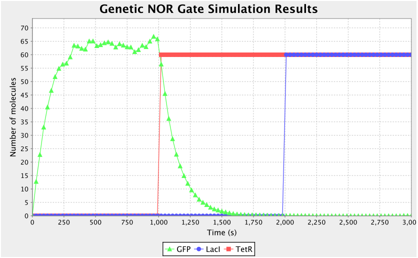


Genetic NOR Gate

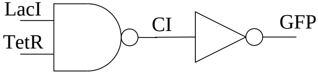


LacI	TetR	GFP
0	0	1
0	1	0
1	0	0
1	1	0

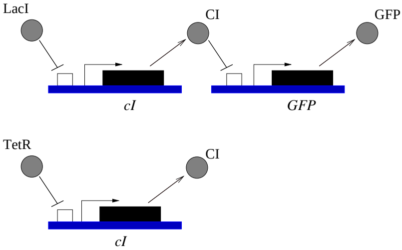


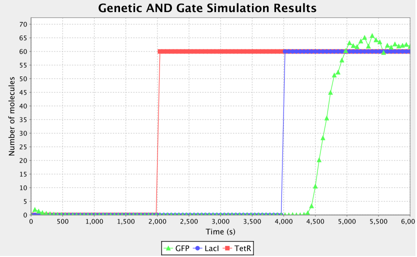


Genetic AND Gate

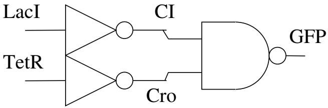


LacI	TetR	GFP
0	0	0
0	1	0
1	0	0
1	1	1

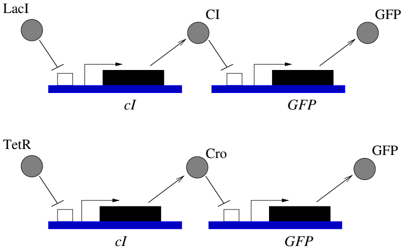


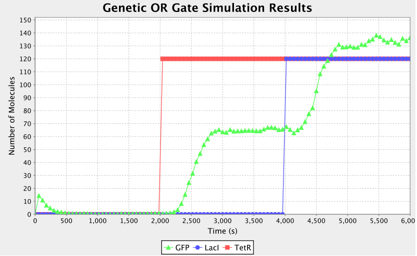


Genetic OR Gate

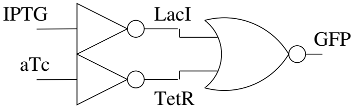


LacI	TetR	GFP
0	0	0
0	1	1
1	0	1
1	1	1

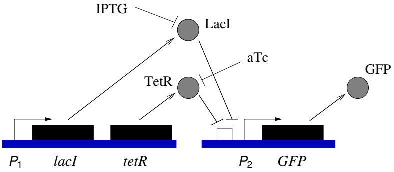


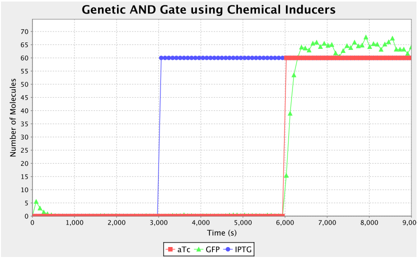


Genetic AND Gate using Chemical Inducers

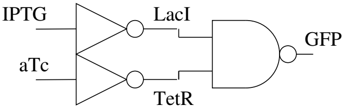


IPTG	aTC	GFP
0	0	0
0	1	0
1	0	0
1	1	1

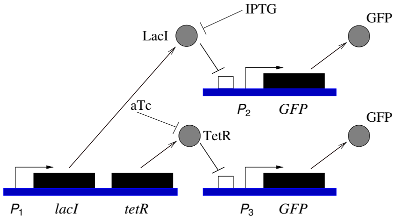


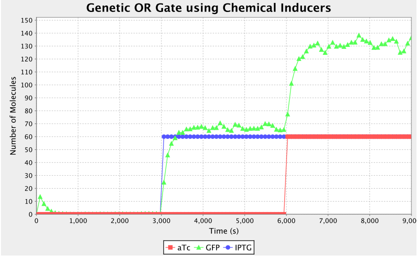


Genetic OR Gate using Chemical Inducers

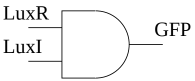


IPTG	aTC	GFP
0	0	0
0	1	1
1	0	1
1	1	1

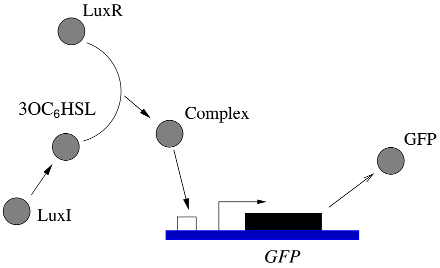


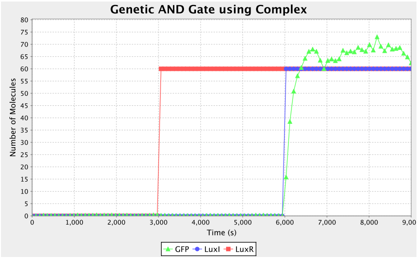


Genetic AND Gate using One Gene



LuxR	LuxI	GFP
0	0	0
0	1	0
1	0	0
1	1	1

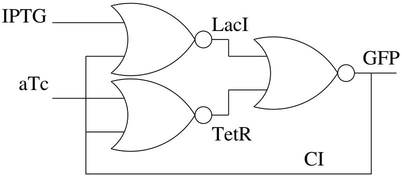




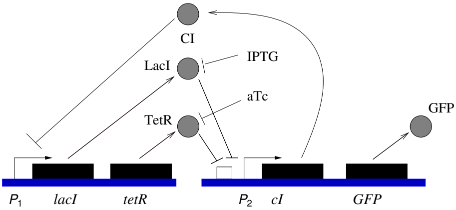
Sequential Logic Circuits

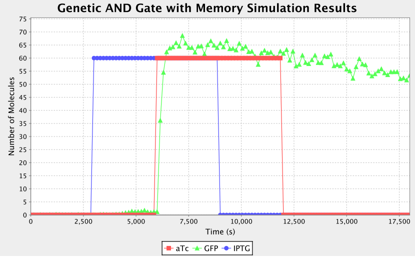
- The output of sequential circuits depend not only on the current input, but also on the recent history of inputs.
- This history is recorded in the state of the circuit.
- State is maintained through the use of *feedback*.
- Feedback loops are important for stability in control systems.
- In *autoregulation*, protein modifies own rate of production.
- Feedback can be either positive or negative.
- Genes regulated by negative feedback should be more stable than those unregulated or regulated by positive feedback.

Genetic AND Gate with Memory

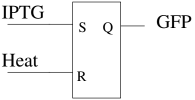


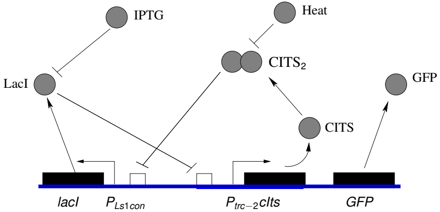
CI	IPTG	aTC	CI	GFP
0	0	0	0	0
0	0	1	0	0
0	1	0	0	0
0	1	1	1	1
1	0	0	1	1
1	0	1	1	1
1	1	0	1	1
1	1	1	1	1



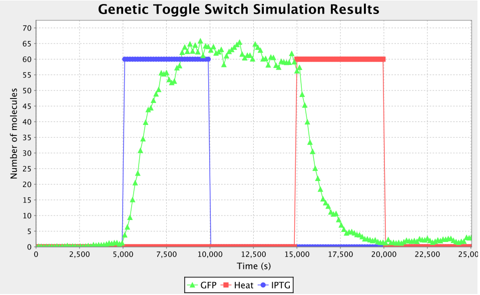


Genetic Toggle Switch (Gardner et al. 2000)



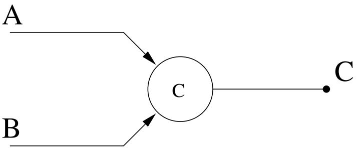


IPTG	Heat	GFP
0	0	GFP
0	1	0
1	0	1
1	1	?



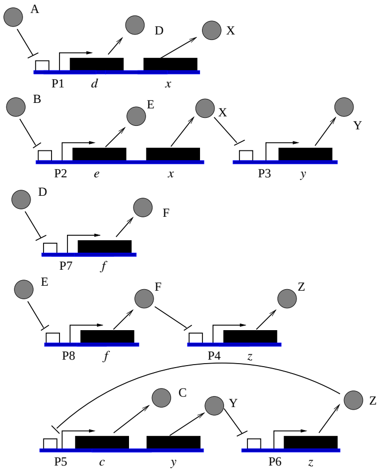
Genetic Muller C-Element

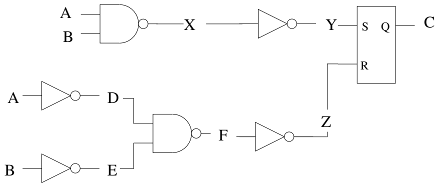
- A Muller C-element is a state holding gate common in many asynchronous design methods that is used to synchronize multiple independent processes.
- A genetic Muller C-element would allow for the design of any asynchronous FSM.

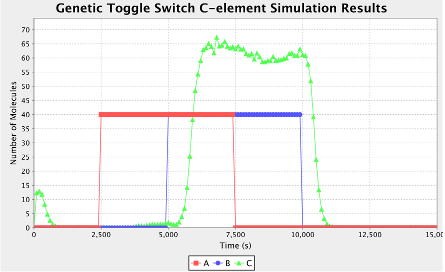


A	B	C
0	0	0
0	1	C
1	0	C
1	1	1

Genetic Toggle Switch Muller C-Element



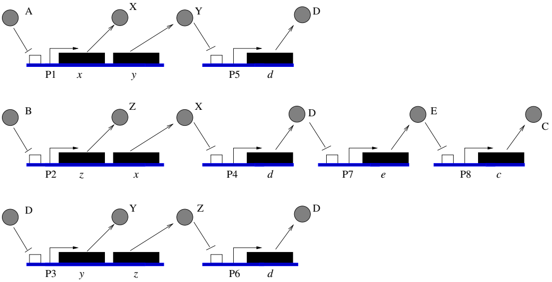


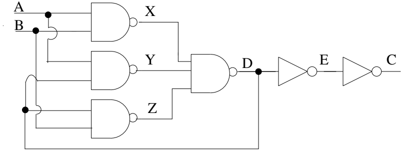


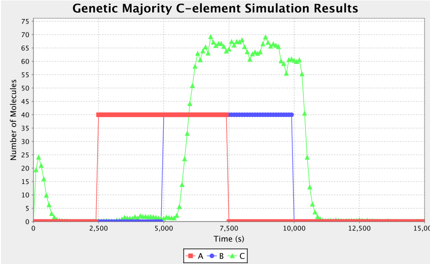
Nguyen et al., 13th Symposium on Async. Ckts. & Sys., 2007 (**best paper**)

Nguyen et al., Journal of Theoretical Biology, 2010.

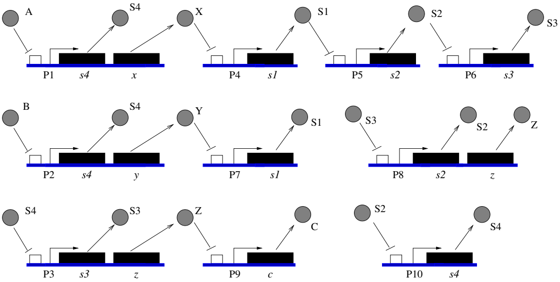
Genetic Majority Gate Muller C-Element

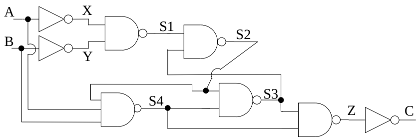


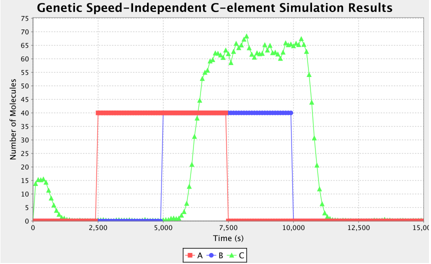




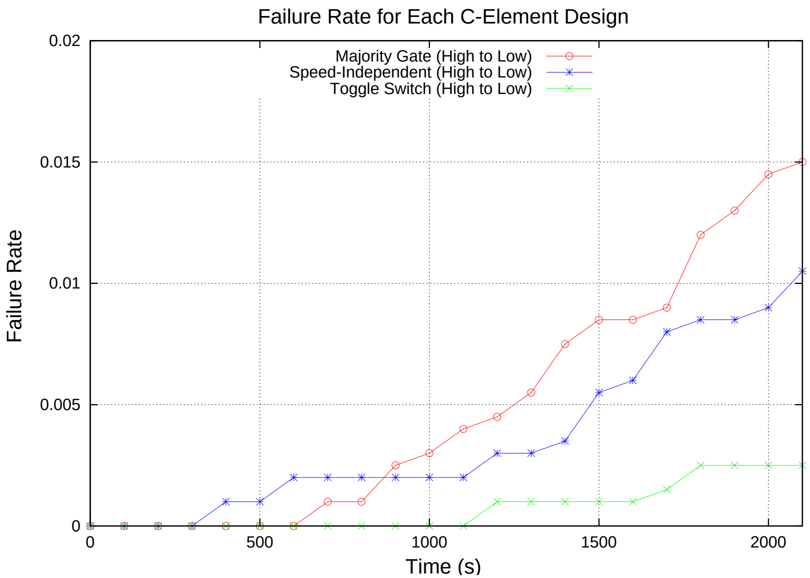
Genetic Speed-Independent Muller C-Element



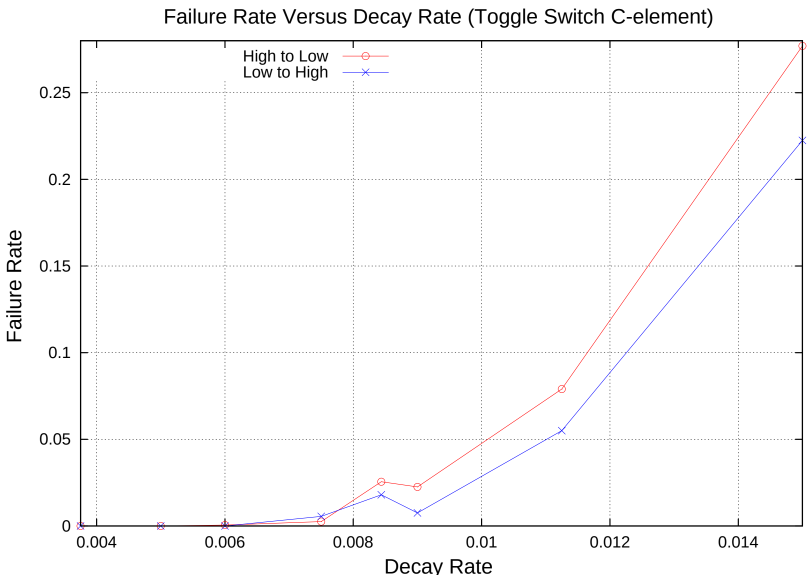




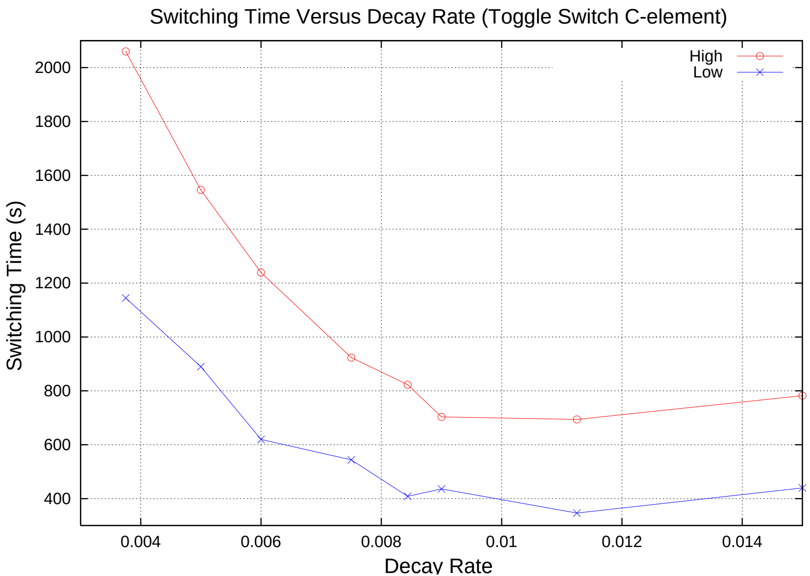
Failure Rate for Each C-Element Design



Failure Rate Versus Decay Rate



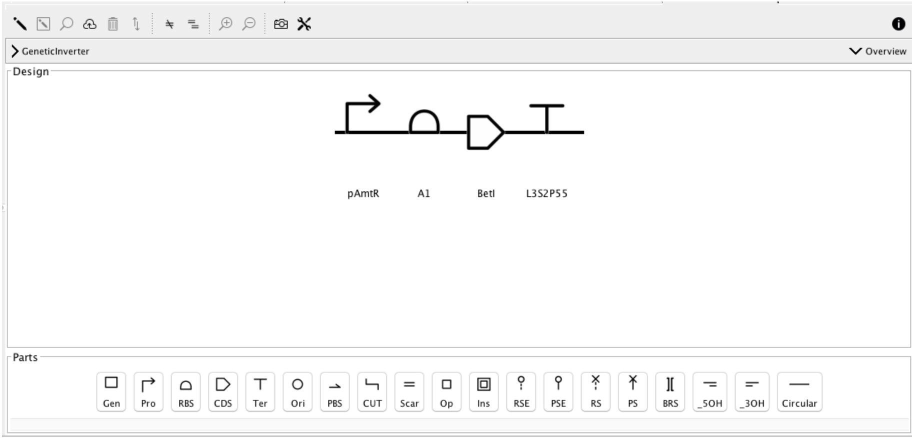
Switching Time Versus Decay Rate



Genetic Circuit Design Challenges

- Genetic circuits have no signal isolation.
- Circuit products may interfere with each other and the host cell.
- Gates in a genetic circuit library usually can only be used once.
- Behavior of circuits are non-deterministic in nature.
- No global clock, so timing is difficult to characterize.
- QUESTION: Can asynchronous synthesis tools be adapted to requirements for a genetic circuit technology?

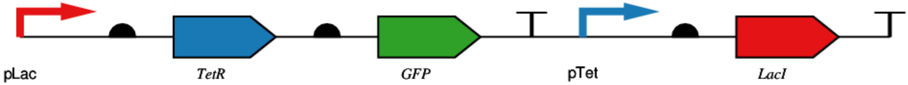
SBOLDesigner



Zhang et al., ACS Synthetic Biology (2017)

http://www.async.ece.utah.edu/SBOLDesigner

Genetic Toggle Switch (Gardner et al. 2000)



Assignment #2

1. Using SBOLDesigner, construct the genetic toggle switch device.
    1. Construct the LacI inverter device.
    2. Construct the TetR inverter device.
    3. Construct the toggle switch using these two devices on opposite strands.
    4. Upload your completed design to your private repository at `https://synbiohub.utah.edu` and provide a share link.
2. Using SBOLDesigner, construct the genetic device in your paper.
    1. Be sure to use hierarchy to construct a modular design.
    2. Upload your completed design to your private repository at `https://synbiohub.utah.edu` and provide a share link.