Engineered Communications for Microbial Robotics

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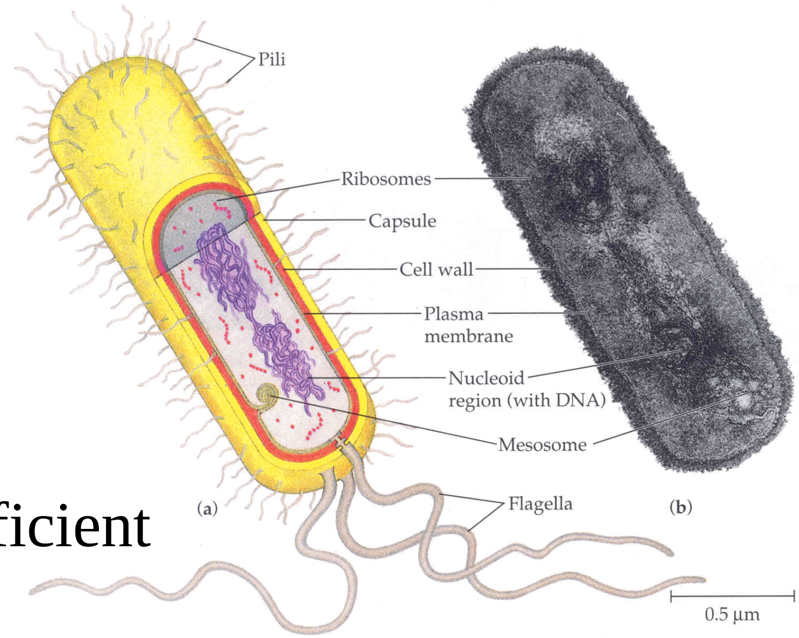
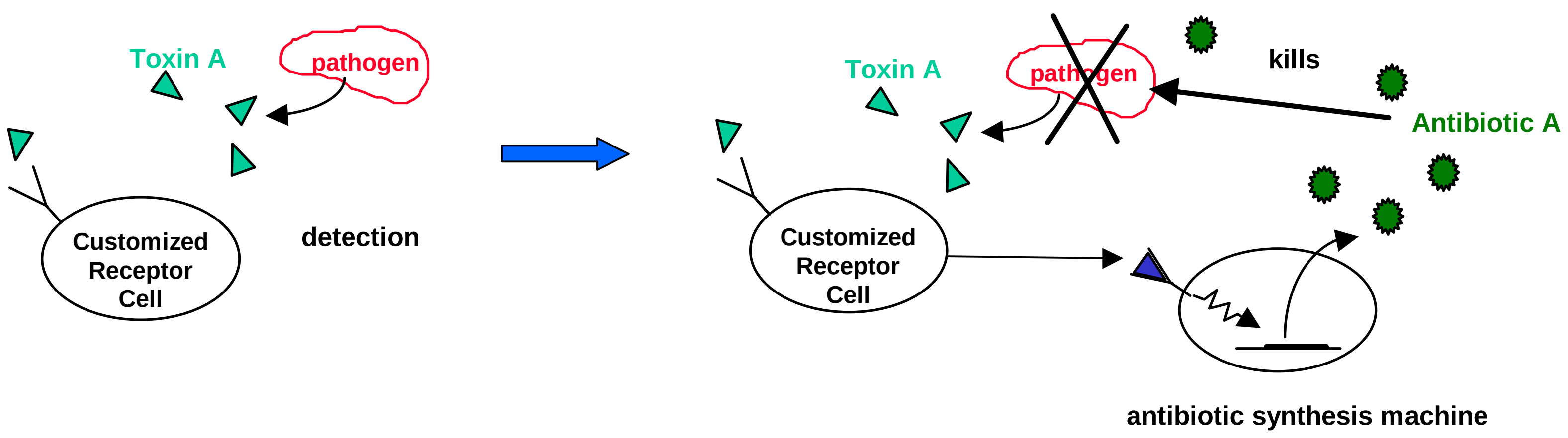
Microbial Robotics



- Goal:
  Design and implement cellular computers / robots using engineering principles
- Special features of cells:
  - small, self-replicating, energy-efficient
- Why?
  - Biomedical applications
  - Environmental applications (sensors & effectors)
  - Embedded systems
  - Interface to chemical world
  - Molecular scale engineering

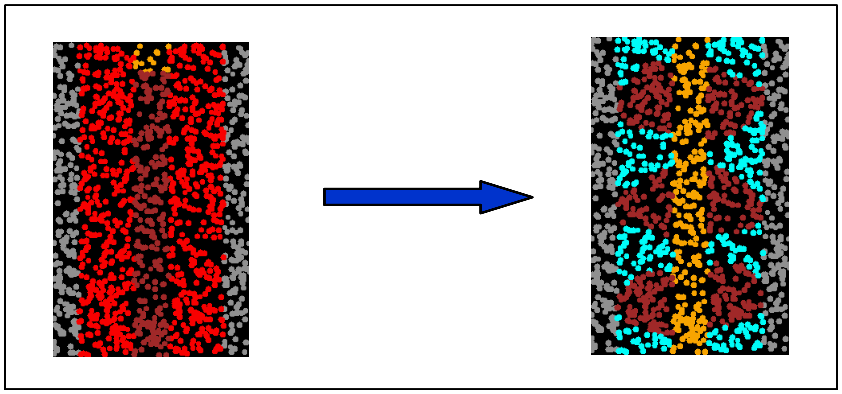
Engineered Behavior

- Potential to engineer behavior into bacterial cells:
  - phototropic or magnetotropic response
  - control of flagellar motors
  - chemical sensing and engineered enzymatic release
  - selective protein expression
  - molecular scale fabrication
  - selective binding to membrane sites
  - collective behavior
    - autoinducers
    - slime molds
    - pattern formation
- Example: timed drug-delivery in response to toxins



Communications

- Cellular robotics requires
  - Intracellular control circuits
  - Intercellular signaling
- First, characterize communication components
- Engineer coordinated behavior using diffusion-based communications



Example of pattern generation in an amorphous substrate, using only diffusion-based signaling

➢ Demonstrate engineered communications using the lux Operon from *Vibrio fischeri*

Outline

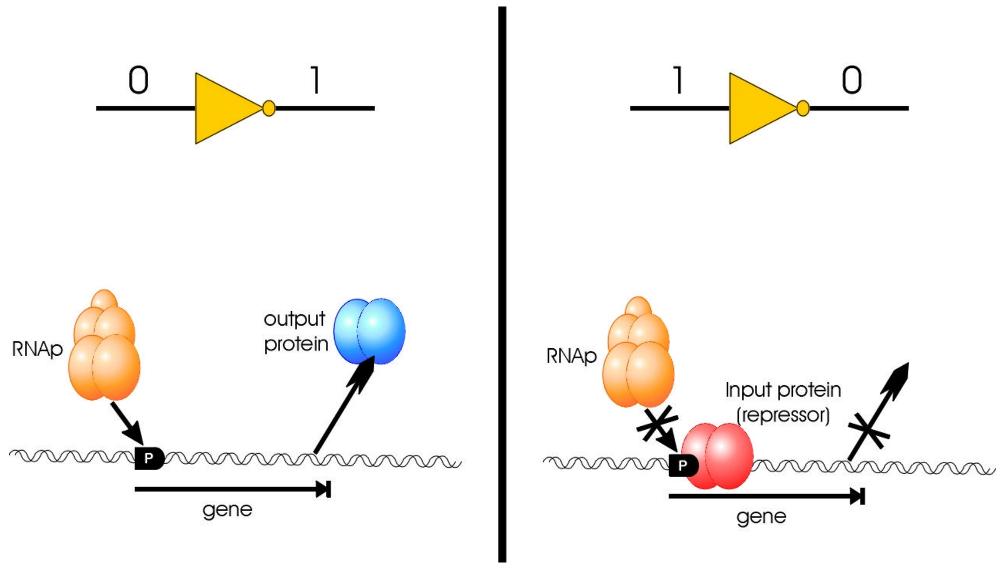
- Previous Work
- Implementing computation & communications
  - Intracellular regulation of transcription
  - Intercellular regulation of protein activity
- Quorum sensing
- Experimental Results
- Conclusions

Previous Work

- Cellular gate technology
  [Knight & Sussman, ’98]
- Simulation & characterization of gates and circuits
  [Weiss, Homsy, Knight, ’98, ’99]
- Toggle Switch implementation
  [Gardner & Collins, ’00]
- Ring Oscillator implementation
  [Elowitz & Leibler, ’00]

Intracellular Circuits: The Inverter

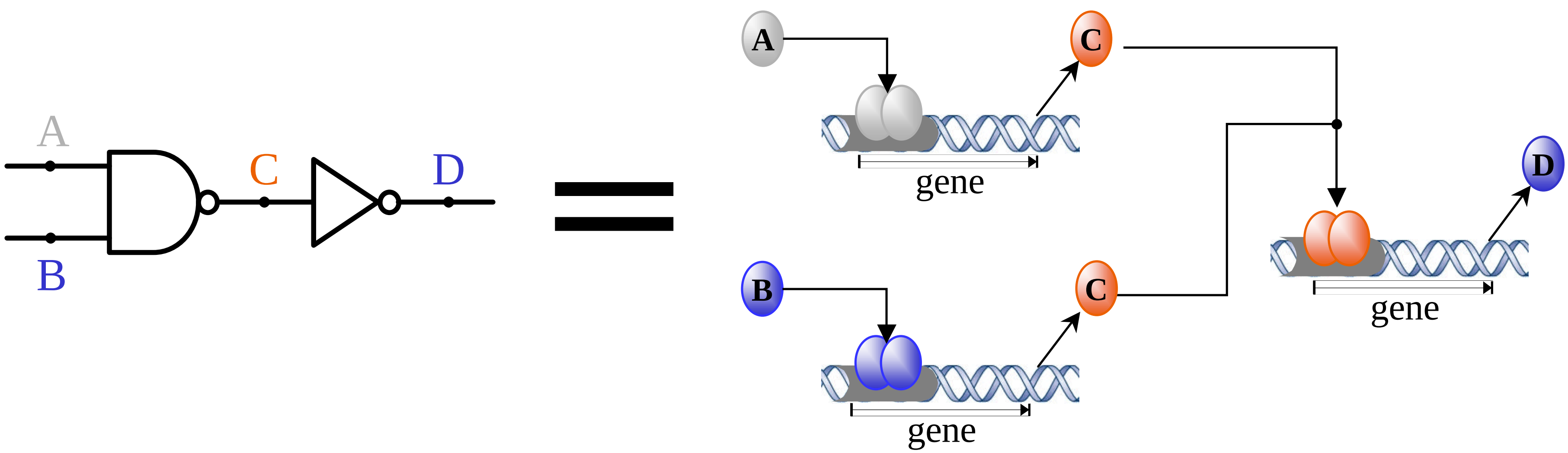
- *In-vivo* digital circuits:
  - signal = concentration of a specific protein
  - computation = regulated protein synthesis + decay
- The basic computational element is an **inverter**



➢Allows building any (complex) digital circuit in individual cells

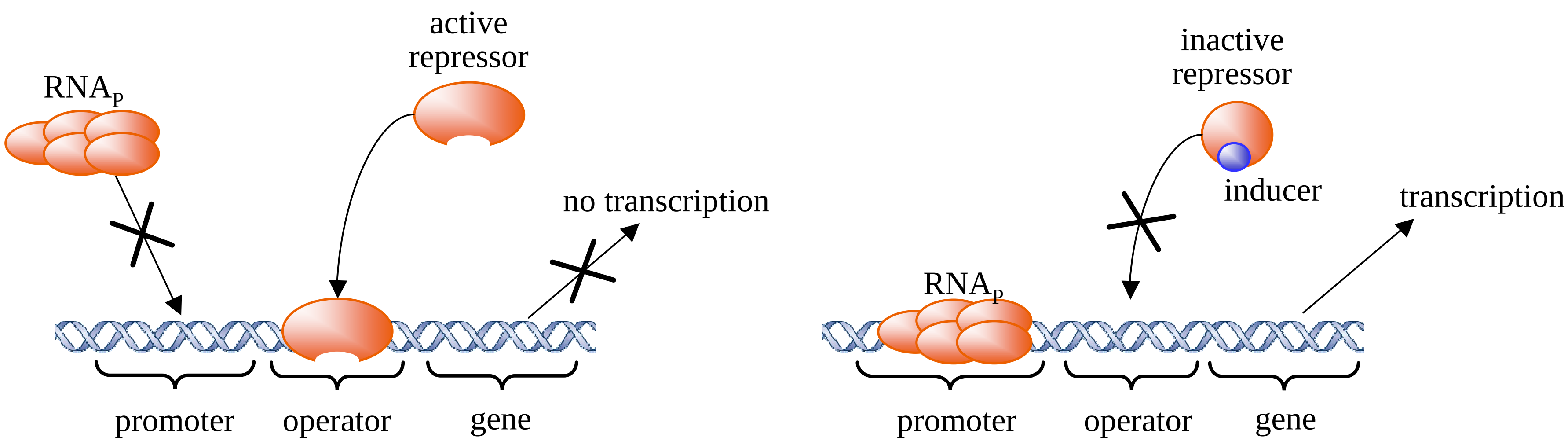
Digital Logic Circuits

- With these inverters, any (finite) digital circuit can be built

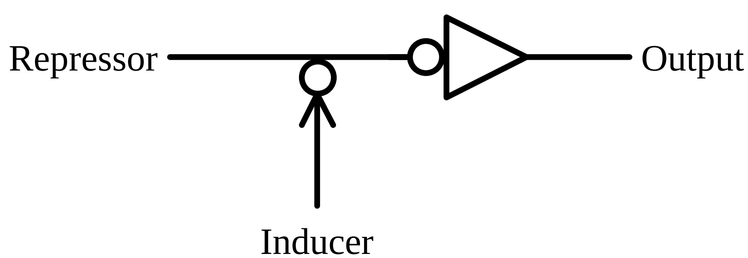


- proteins are the wires, genes are the gates
- NAND gate = “wire-OR” of two genes
- NAND gate is a universal logic element

Repressors & Small Molecules

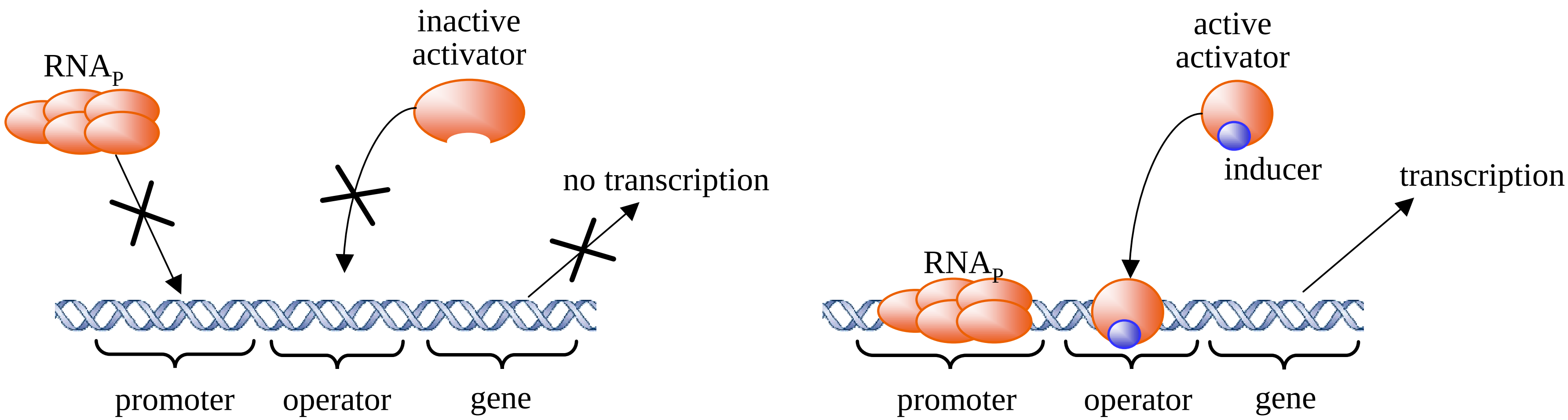


- Inducers can inactivate repressors:
  - IPTG (Isopropylthio-ß-galactoside) → Lac repressor
  - aTc (Anhydrotetracycline) → Tet repressor
- Use as a logical gate:

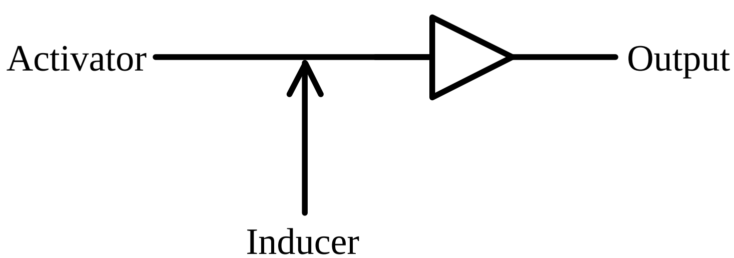


Repressor	Inducer	Output
0	0	1
0	1	1
1	0	0
1	1	1

Activators & Small Molecules



- Inducers can also activate activators:
  - VAI (3-N-oxohexanoyl-L-Homoserine lacton) → luxR
- Use as a logical (AND) gate:



Activator	Inducer	Output
0	0	0
0	1	0
1	0	0
1	1	1

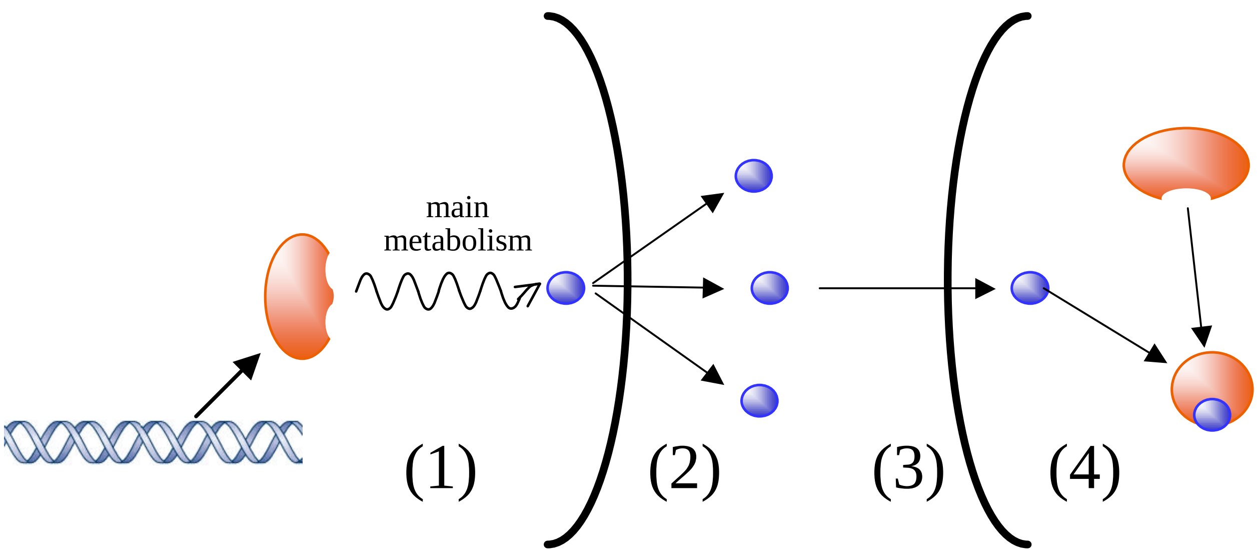
Summary of Effectors

	Protein : Effector	Effector present		Effector not present	
		binds DNA	transcription	binds DNA	transcription
inducers	TetR : aTc	+	-	-	+
	LuxR : VAI	-	-	+	+
co-repressors	TrpR : tryptophane	+	+	-	-
	? : ?	-	+	+	-

- Inducers and Co-repressors are termed effectors
- Reasons to use effectors:
  - faster intracellular interactions
  - intercellular communications

Intercellular Communications

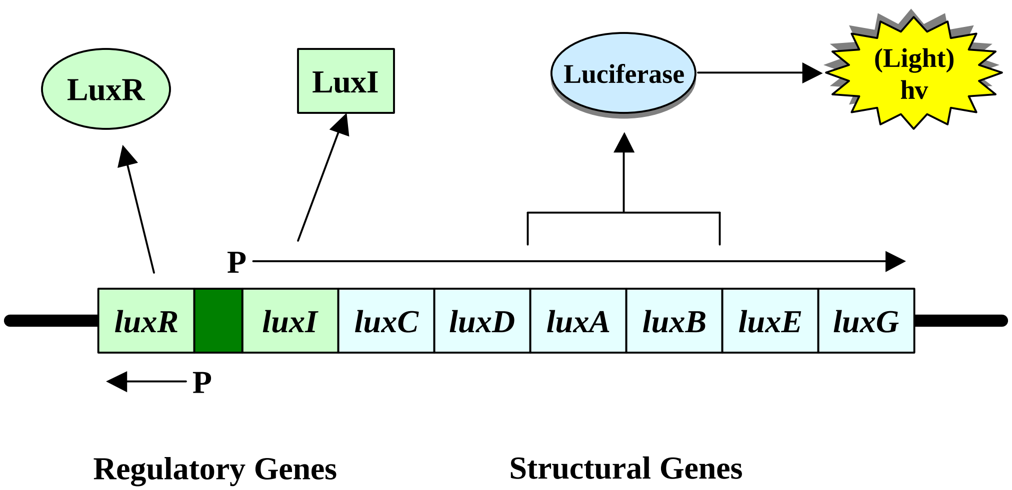
- Certain inducers useful for communications:
  1. A cell produces inducer
  2. Inducer diffuses outside the cell
  3. Inducer enters another cell
  4. Inducer interacts with repressor/activator → change signal



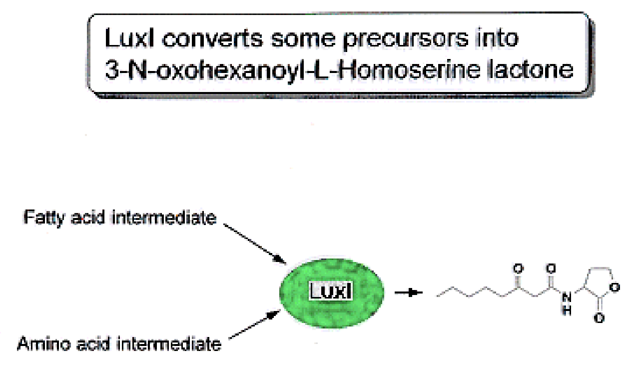
Quorum Sensing

- Cell density dependent gene expression

Example: *Vibrio fischeri* [density dependent bioluminscence]



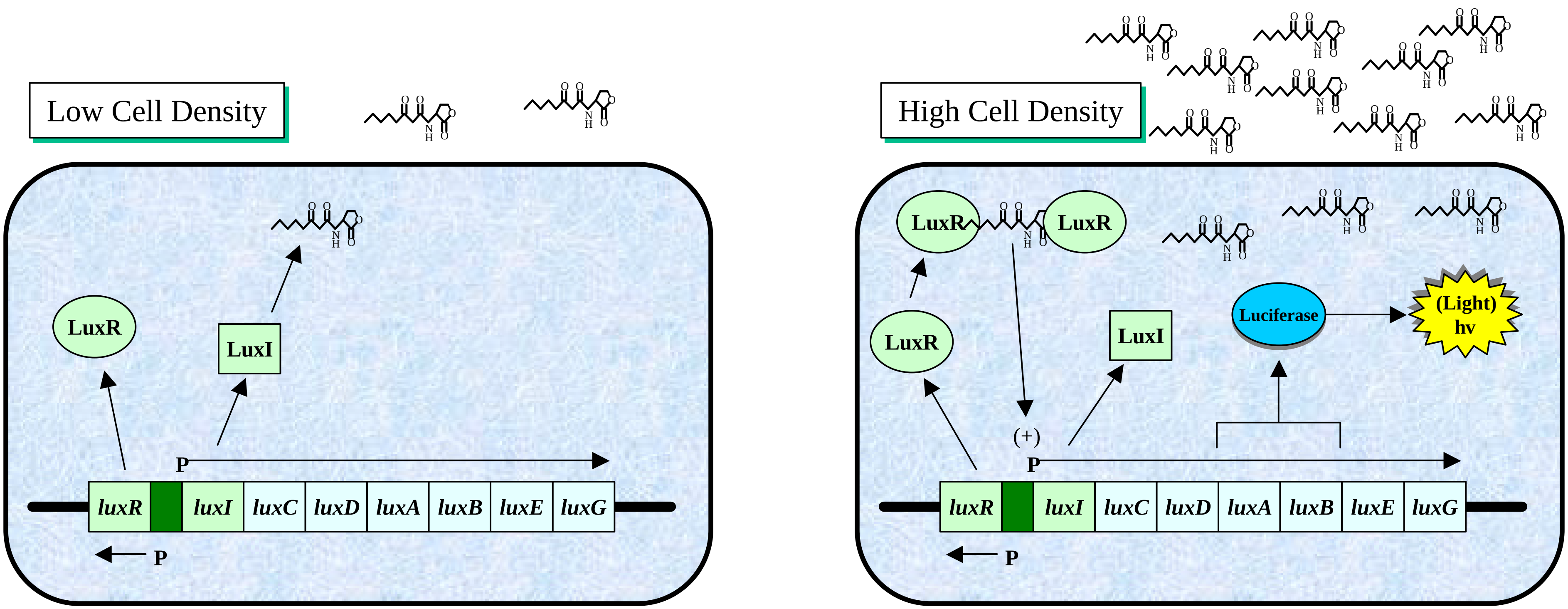
The lux Operon



LuxI metabolism

→ *autoinducer* (VAI)

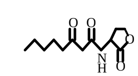
Density Dependent Bioluminescence



free living, 10 cells/liter
<0.8 photons/second/cell

symbiotic, 10^{10} cells/liter
800 photons/second/cell

- A positive feedback circuit

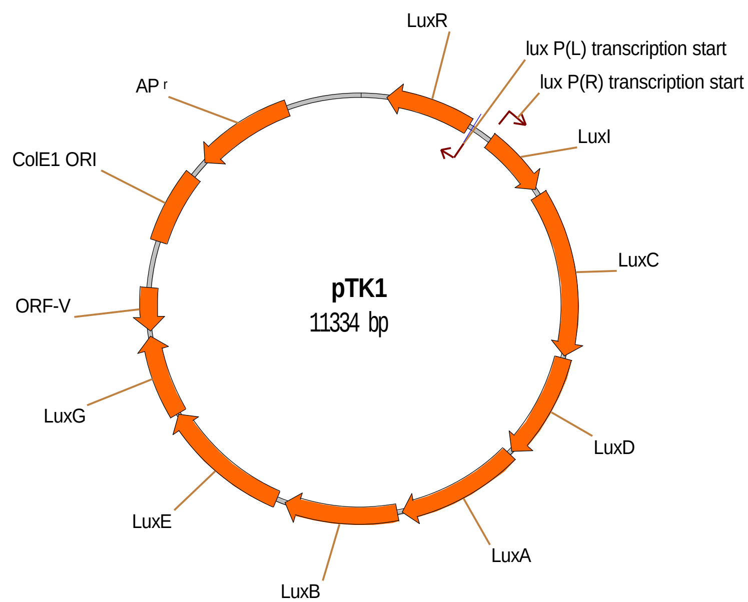


Similar Signalling Systems

N-acyl-L-Homoserine Lactone Autoinducers in Bacteria

Species	**Relation to Host**	**Regulate Production of**	**I Gene**	**R Gene**
Vibrio fischeri	marine symbiont	Bioluminescence	*luxI*	*luxR*
Vibrio harveyi	marine symbiont	Bioluminescence	*luxL,M*	*luxN,P,Q*
Pseudomonas aeruginosa	Human pathogen	Virulence factors	*lasI*	*lasR*
		Rhamnolipids	*rhlI*	*rhlR*
Yersinia enterocolitica	Human pathogen	?	*yenI*	*yenR*
Chromobacterium violaceum	Human pathogen	Violaceum production Hemolysin Exoprotease	cviI	cviR
Enterobacter agglomerans	Human pathogen	?	*eagI*	?
Agrobacterium tumefaciens	Plant pathogen	Ti plasmid conjugation	*traI*	*traR*
Erwinia caratovora	Plant pathogen	Virulence factors Carbapenem production	*expI*	*expR*
Erwinia stewartii	Plant pathogen	Extracellular Capsule	*esaI*	*esaR*
Rhizobium leguminosarum	Plant symbiont	Rhizome interactions	*rhiI*	*rhiR*
Pseudomonas aureofaciens	Plant beneficial	Phenazine production	*phzI*	*phzR*

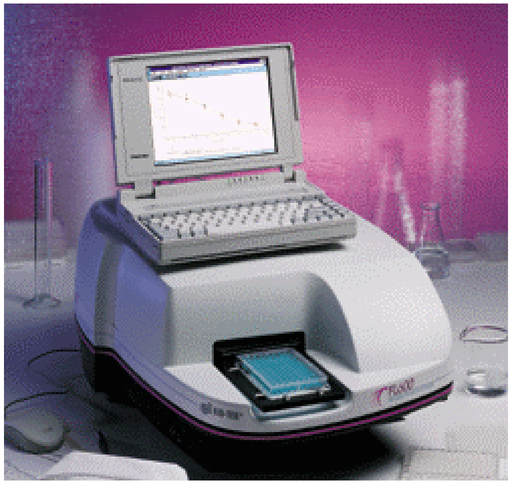
Cloning the lux Operon into E. coli



- First, we shotgun cloned the lux Operon from *Vibrio fischeri* to form plasmid pTK1
- Sequenced the operon [Genbank entry AF170104] (thanks to Nick Papadakis)
- Expressed in E. coli DH5a → showed bioluminescence

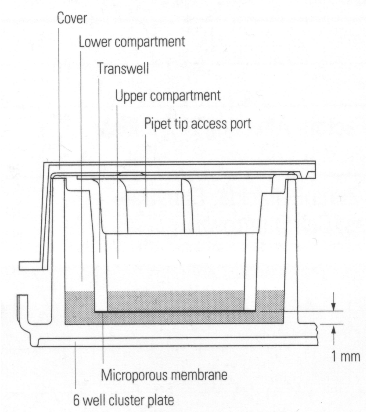
Experimental Setup

- BIO-TEK FL600
  Microplate Fluorescence Reader



insert

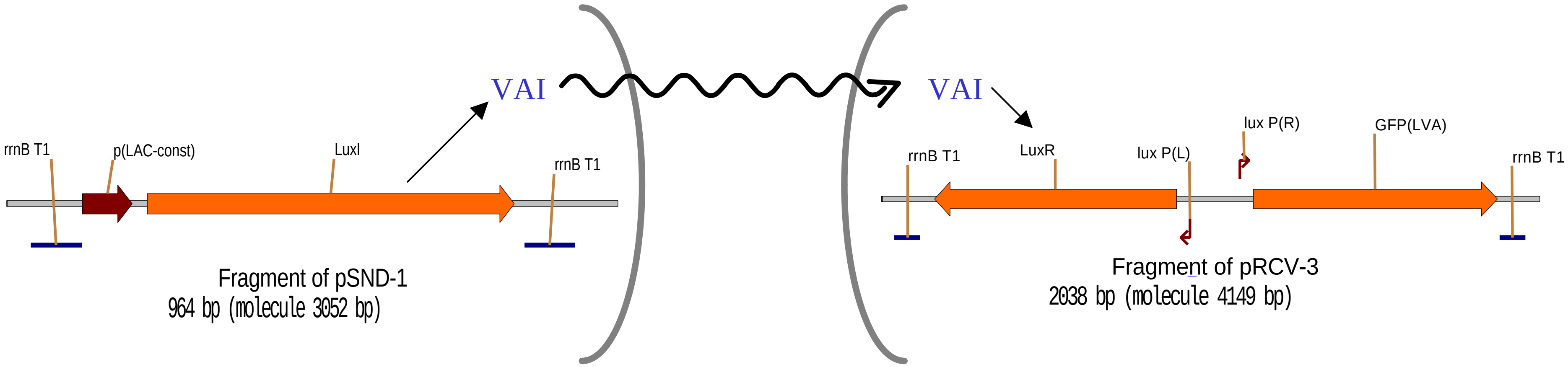
- Costar Transwell microplates and cell culture inserts with permeable membrane (0.1µm pores)
- Cells separated by function:
  - Sender cells in the bottom well
  - Receiver cells in the top well



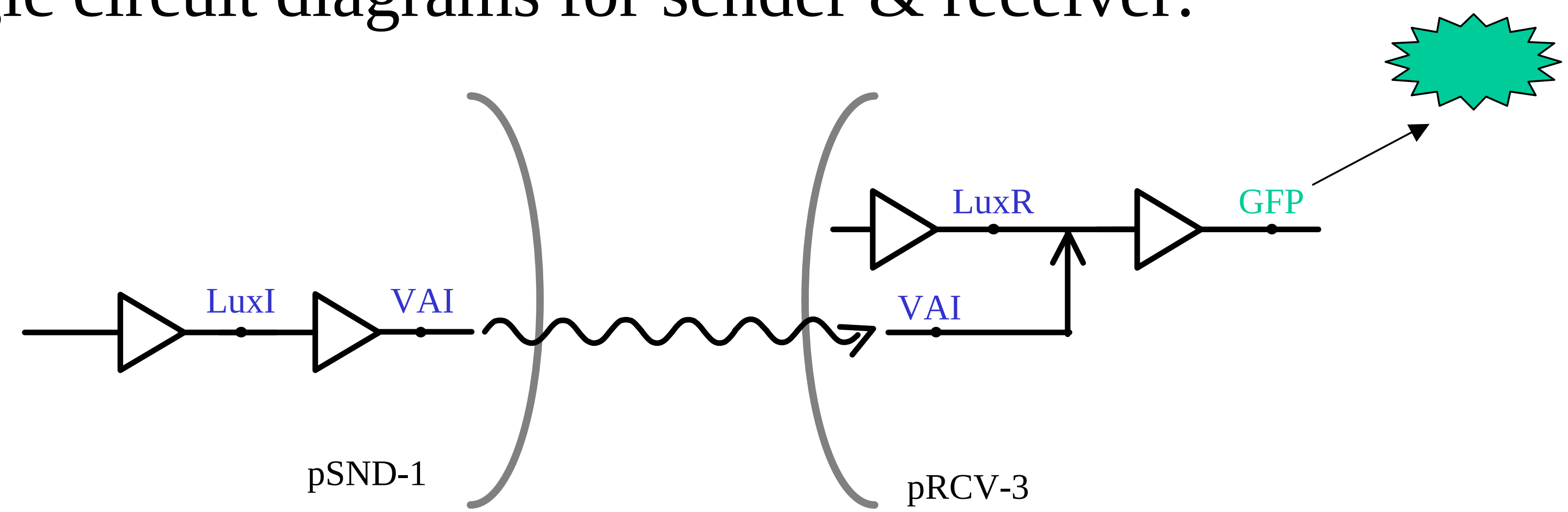
- Top excitation and emission fluorescence readings

Experiment I: Constant Signaling

- Genetic networks for sender & receiver:

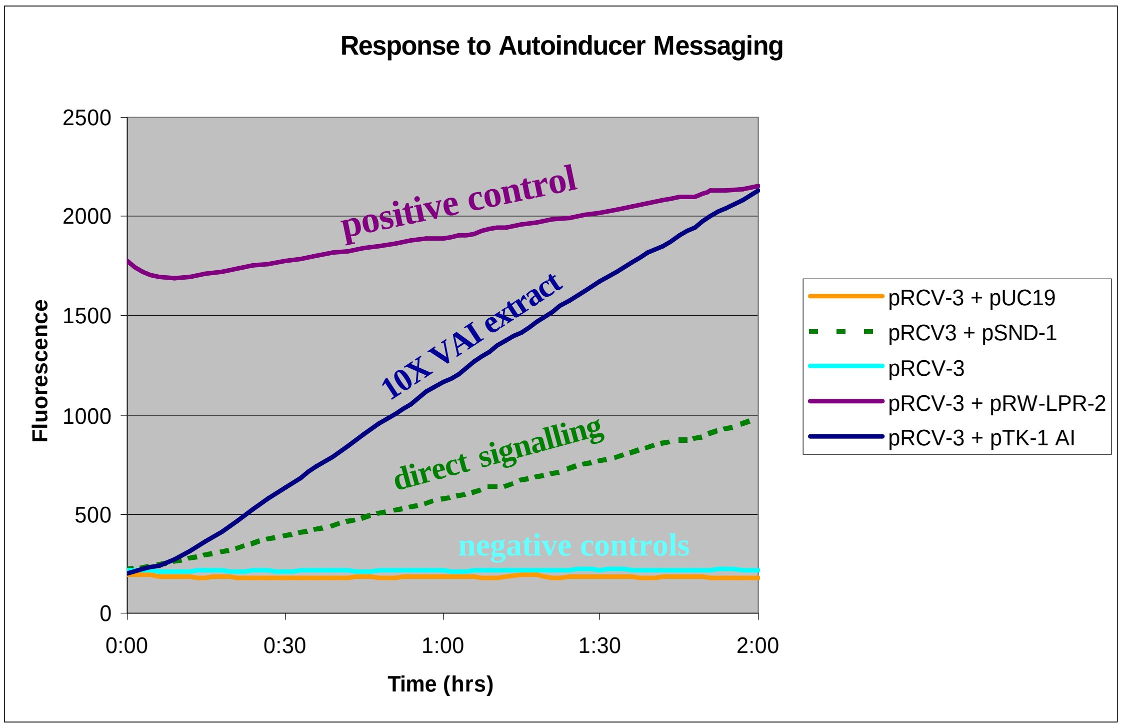


- Logic circuit diagrams for sender & receiver:



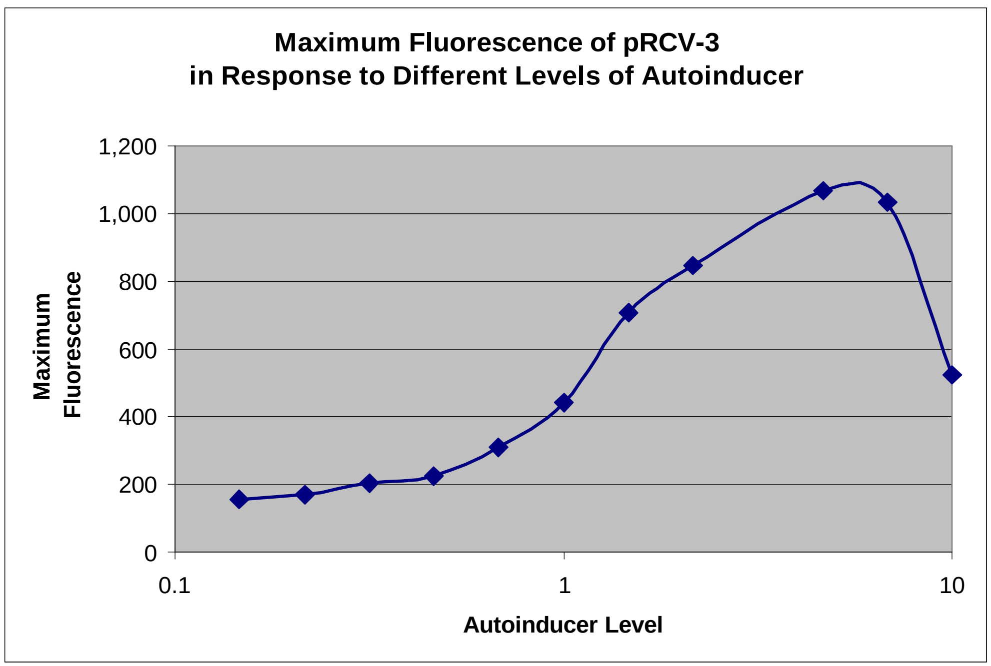
Experiment I: Constant Signalling

- Figure shows fluorescence response of receiver (pRCV-3)
  - Several cultures grown seperately overnight @37°C
  - Cultures mixed in 5 different ways and incubated in FL600 @37°C
  - Fluorescence readings taken every 5 minutes for 2 hours



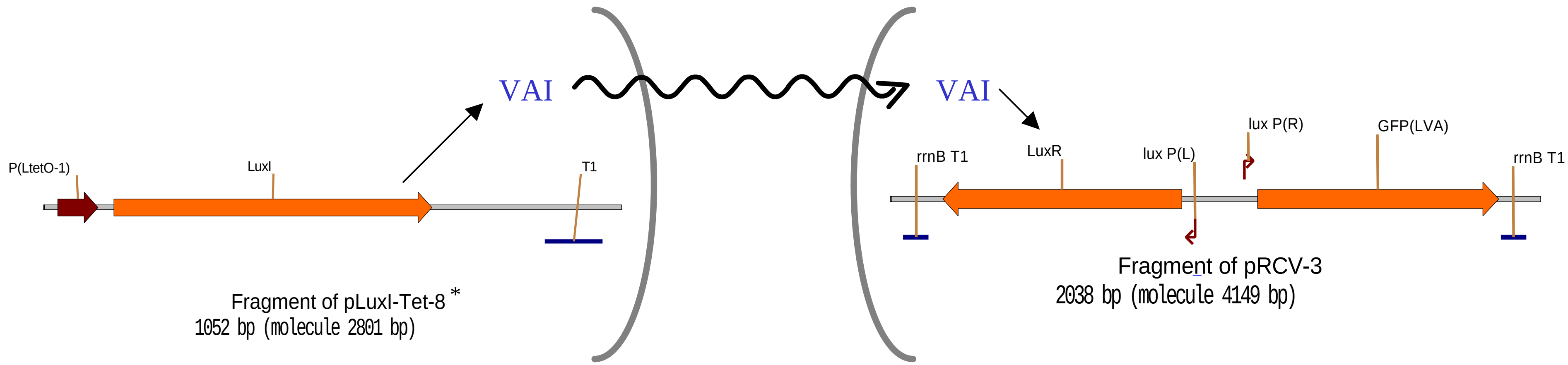
Experiment II: Characterizing the Receiver

- Figure shows response of receiver to different levels of VAI
  - VAI extracted from pTK1 culture
  - Receiver cells (pRCV-3) grown @37°C to late log phase
  - Receiver cells incubated in FL600 for 6 hours @37°C with VAI
  - Data shows max fluorescence for each different VAI level



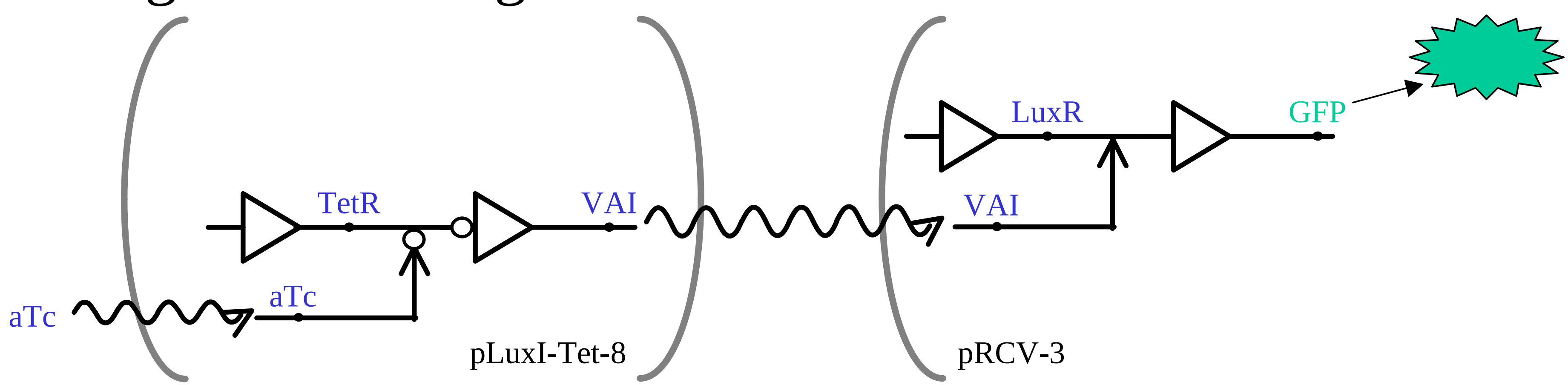
Experiment III: Controlled Sender

- Genetic networks for controlled sender & receiver:



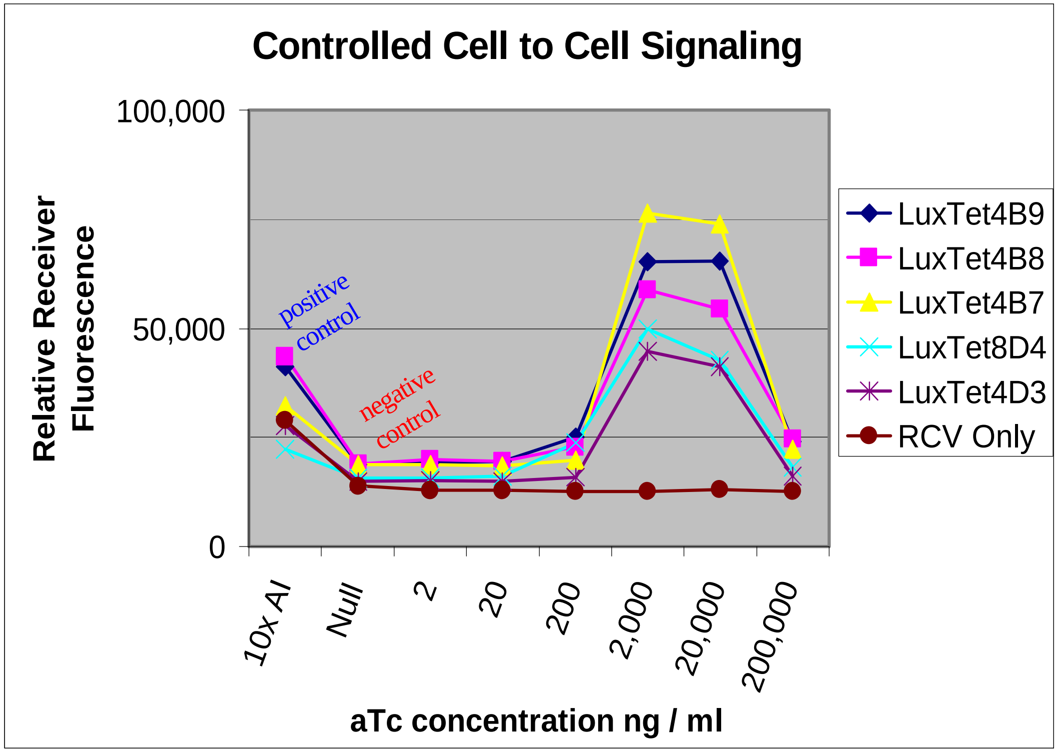
* E. coli strain expresses TetR (not shown)

- Logic circuit diagrams for controlled sender & receiver:



Experiment III: Controlling Sender

- Figure shows ability to induce stronger signals with aTc
  - Non-induced sender (pLux8-Tet-8) & receiver cells grown seperately @37°C to late log phase
  - Cells were combined in FL600, and sender cells were induced with aTc
  - Data shows max fluorescence after 4 hours @37 °C for 5 separate cultures plus control [positive cultures have same DNA → variance due to OD]



Conclusions & Future Work

- This work:
  - Isolated an important intercellular communications mechanism
  - Analyzed its components
  - Engineered its interfaces with standard genetic control and reporter mechanisms
- Future:
  - Additional analysis of lux characteristics
  - Examine and incorporate additional, non-cross reacting, communications systems
  - Integrate communications with more sophisticated in-vivo circuits
  - Engineer coordinated behavior (e.g. to form patterns)