

Addressable Conjugation in Bacterial Networks



The University of California, Berkeley

High School

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Postdocs

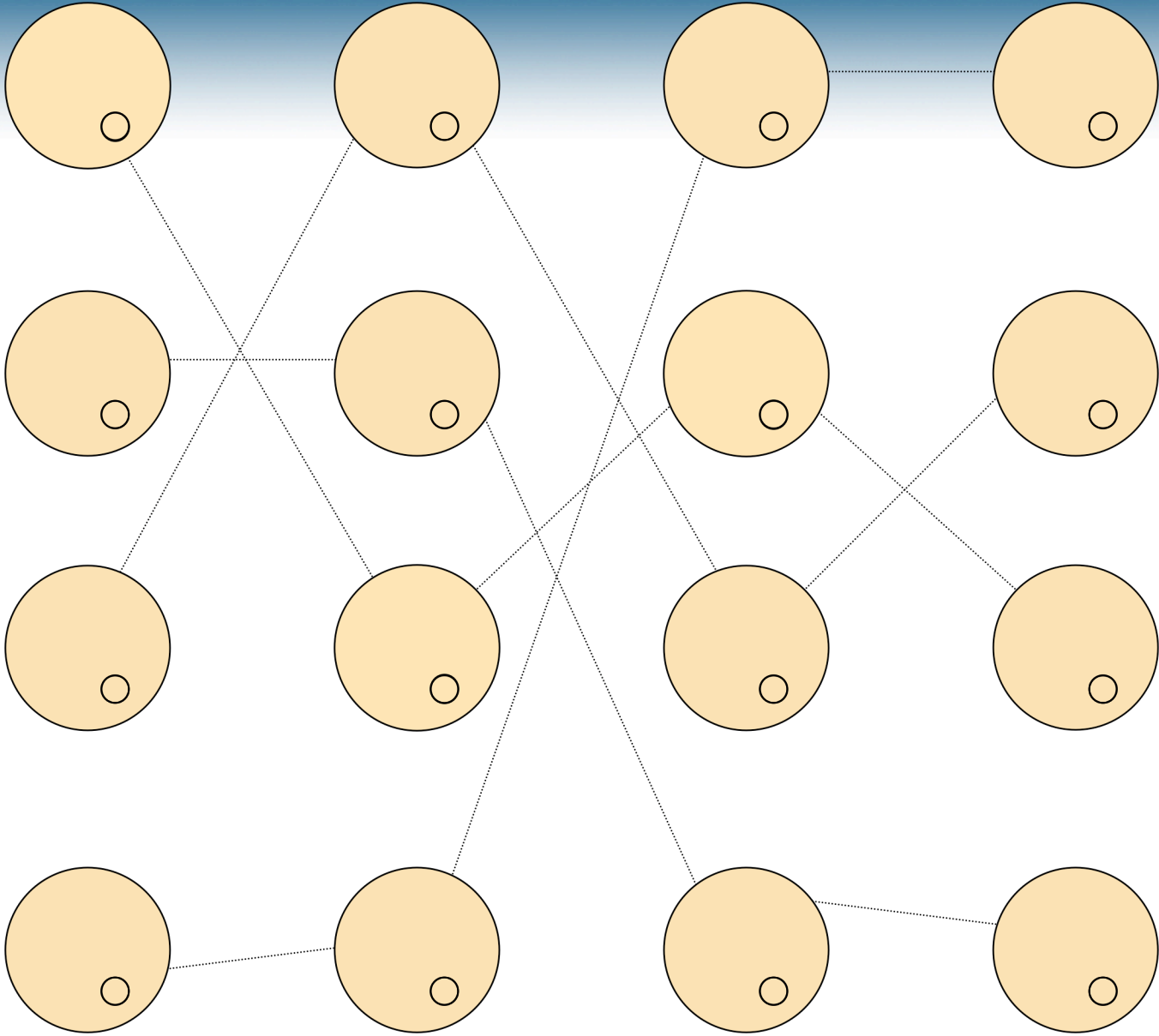
John E. Dueber
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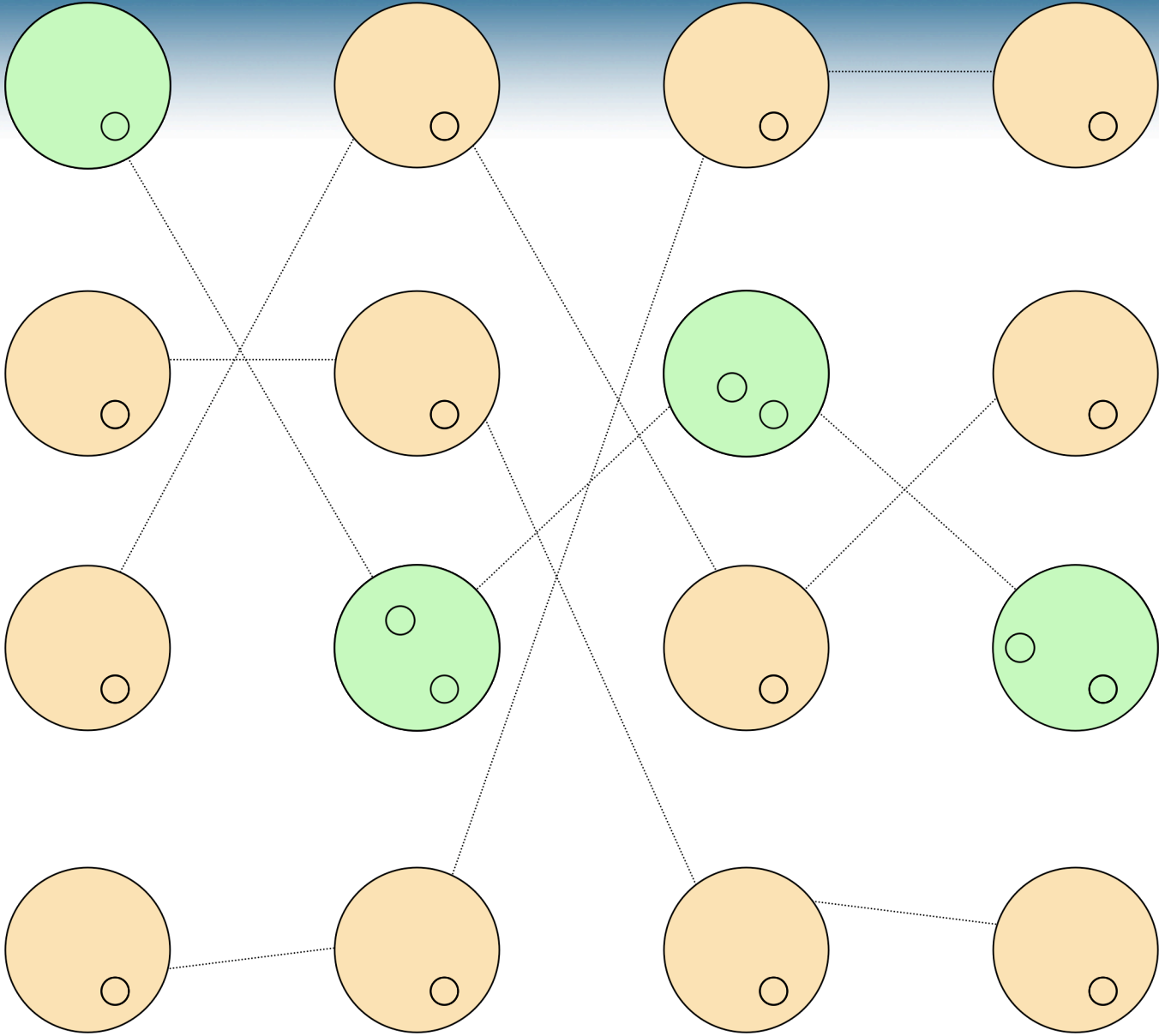
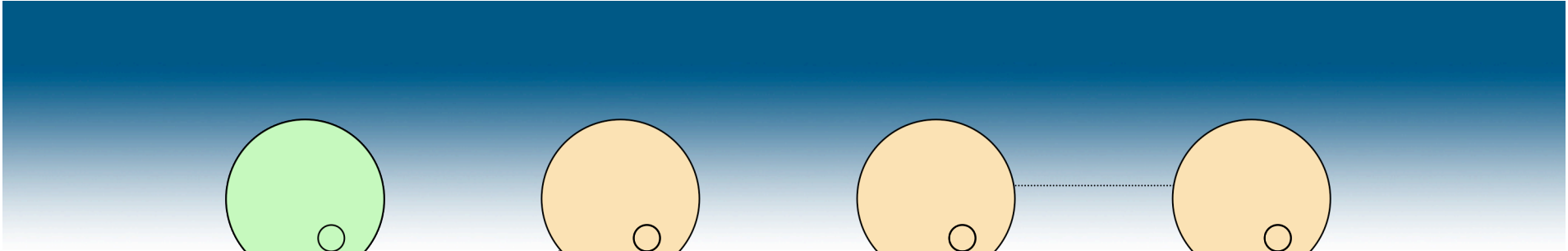
Undergrads

Bryan Hernandez	Freshman
Jennifer Lu	Freshman
Samantha Liang	Freshman
Will Bosworth	Sophomore
Daniel Kluesing	Junior

Faculty Advisors

Adam P. Arkin
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Every Cell is assigned a Unique RNA Address

Cell 1	UGAUCUAAGUGGAGGACGAA
Cell 2	GUUCAAGACAAUCACUCACG
Cell 3	UCAAUUAUAAAACUACAAACU
Cell 4	GAUGAUAGAGGUUUCUUUUA

Implementation

Need: To transfer DNA messages from one bacterial cell to another

Means: Bacterial Conjugation

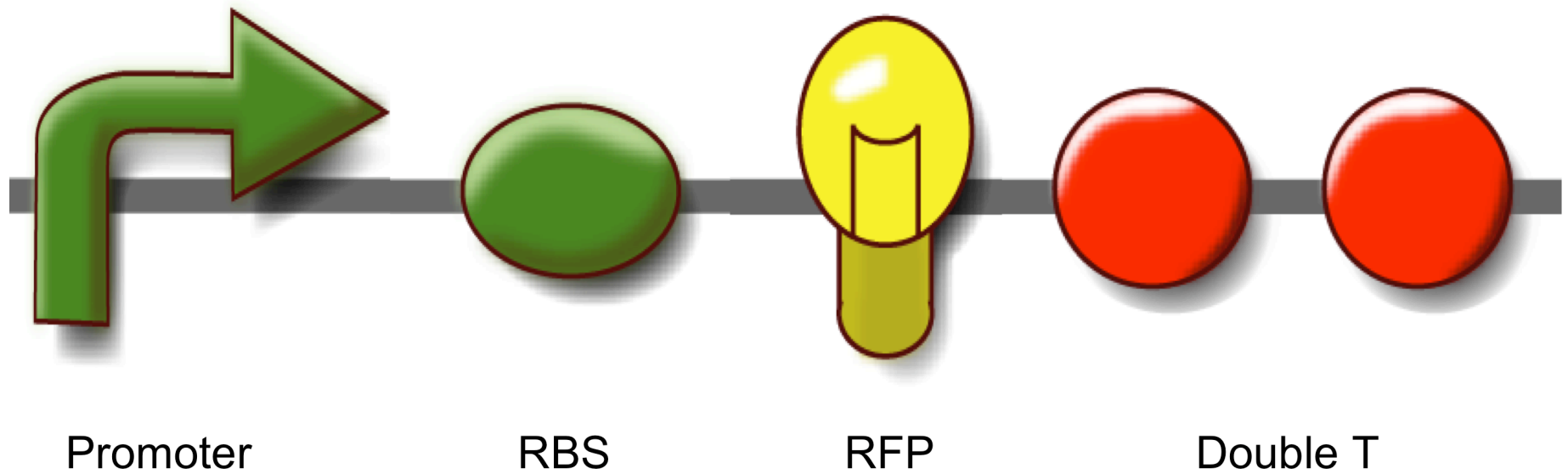
Need: To specifically control who can read the DNA message

Means: Riboregulation

Our specific goals:

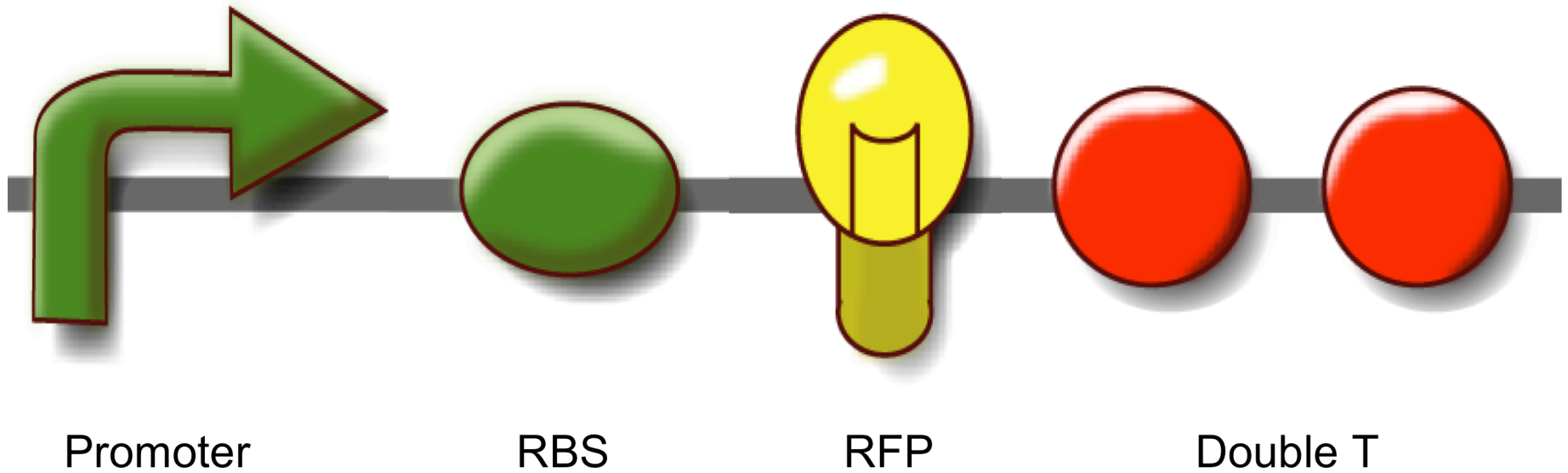
- Construct high-performance riboregulators**
- Harness bacterial conjugation**
- Transmit a coded message**
- Construct a bacterial learning network**

The Riboregulator: DNA

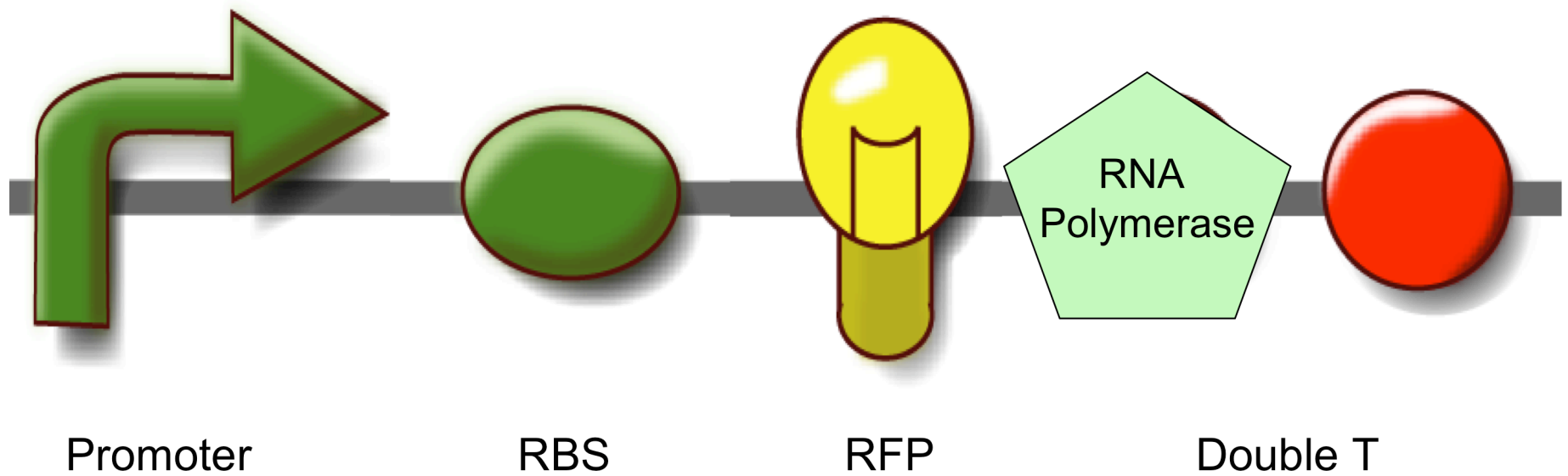


RNA
Polymerase

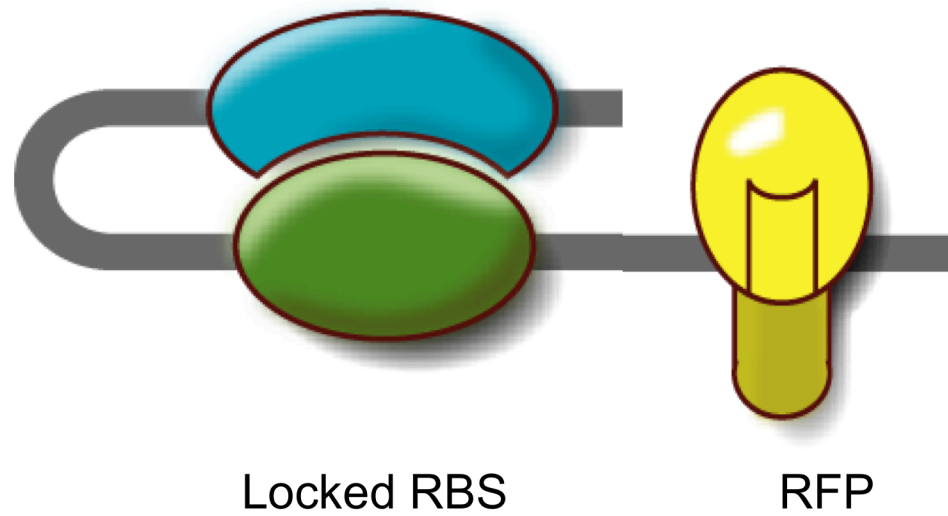
The Riboregulator: DNA



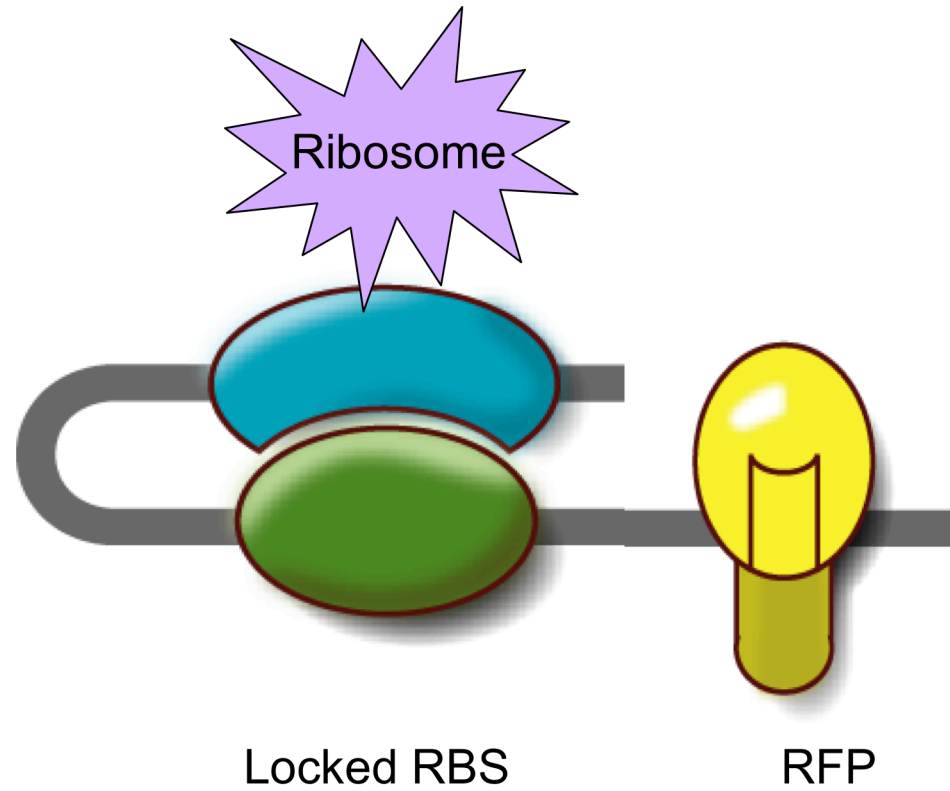
The Riboregulator: DNA



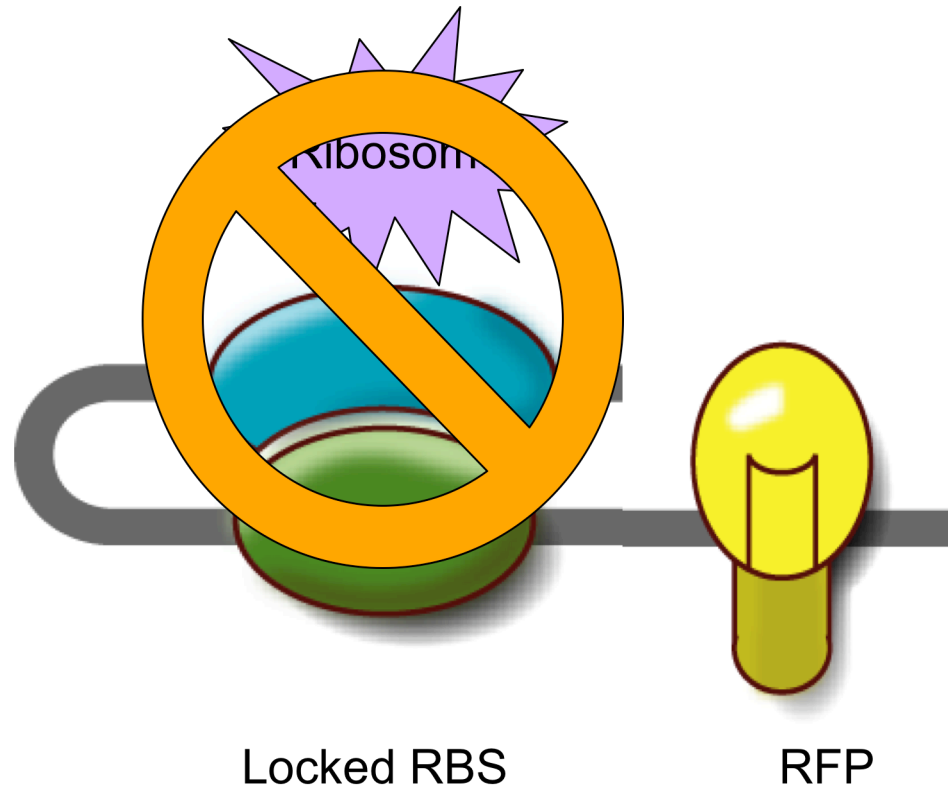
Locked Riboregulator: RNA



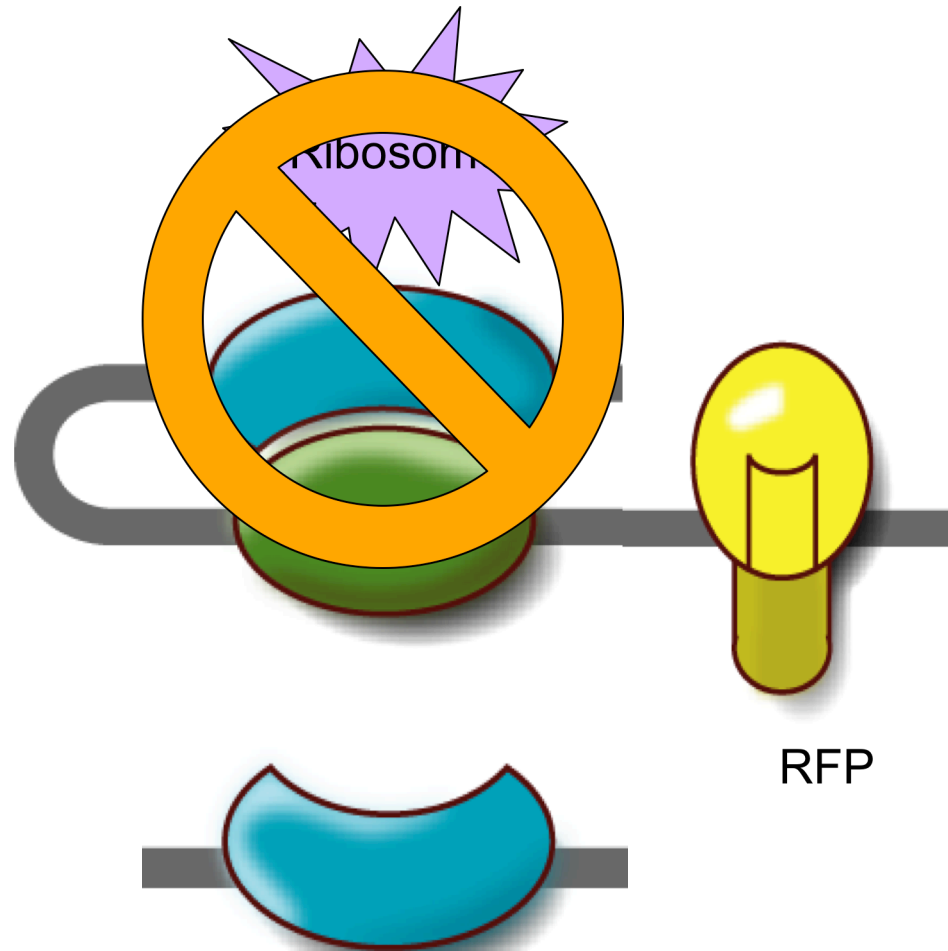
Locked Riboregulator: RNA



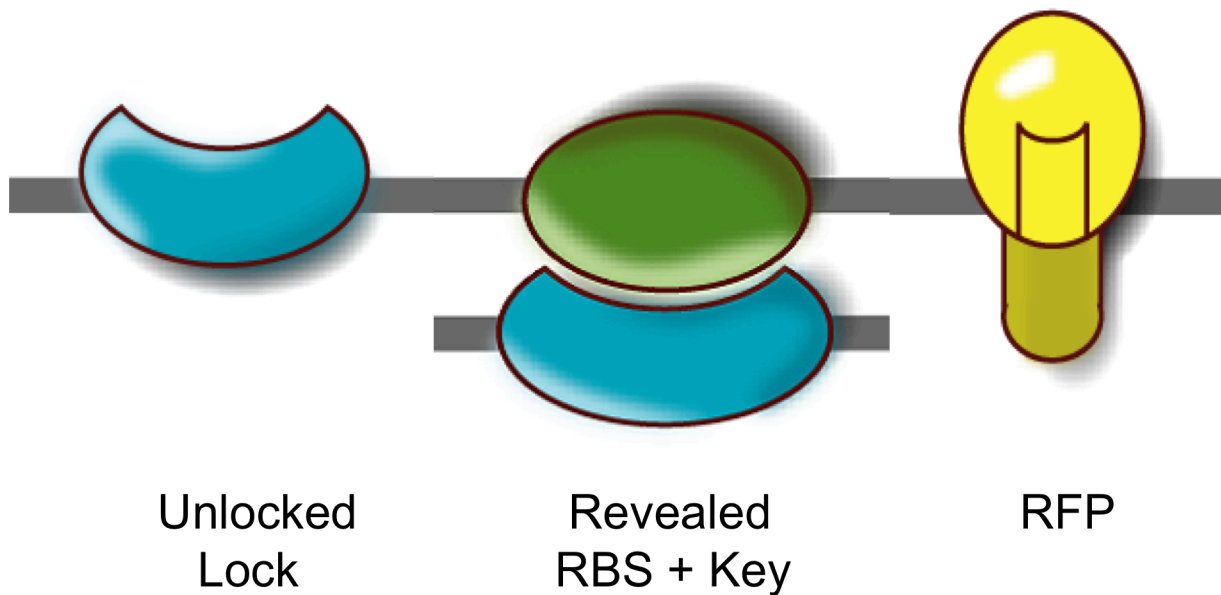
Locked Riboregulator: RNA



Locked Riboregulator: RNA

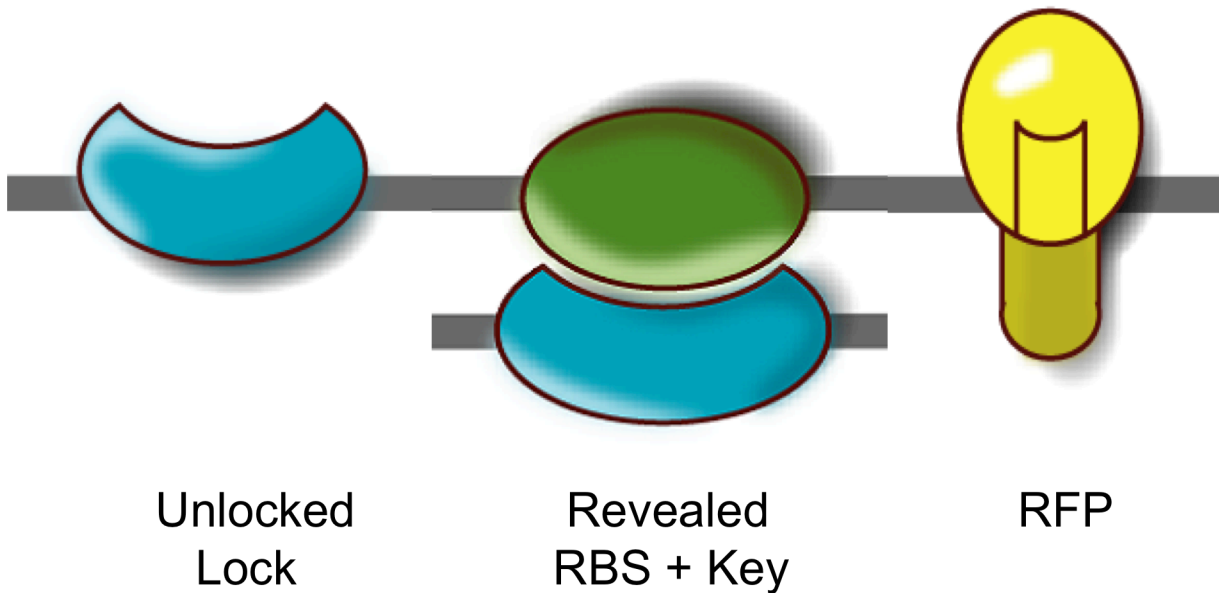


Unlocked Riboregulator

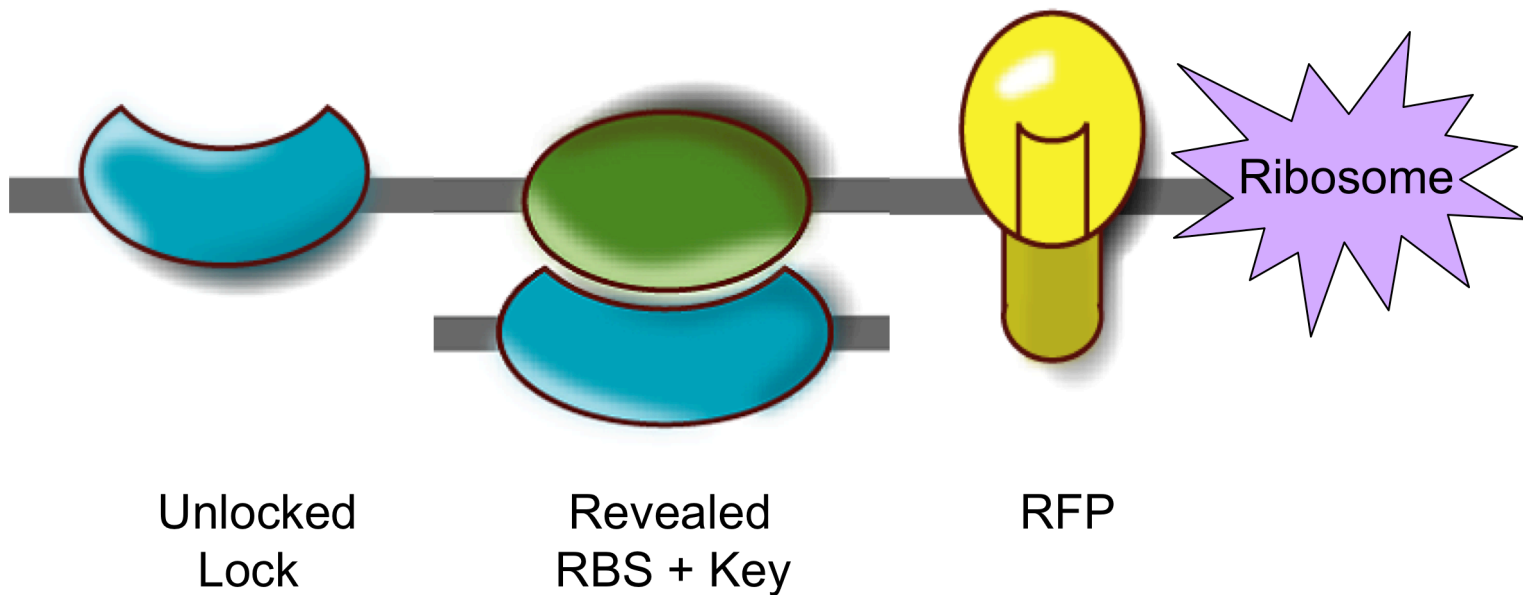


Ribosome

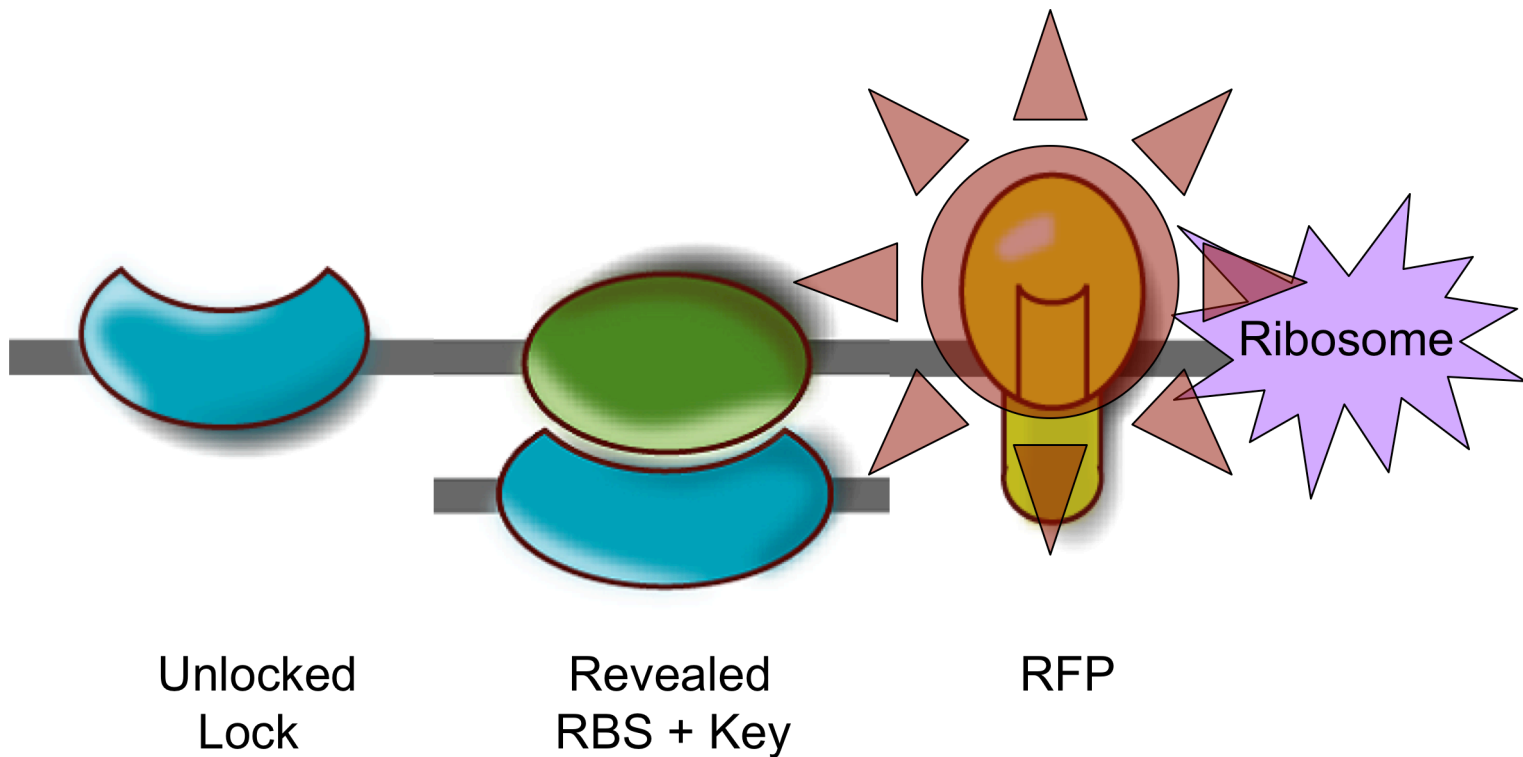
Unlocked Riboregulator



Unlocked Riboregulator



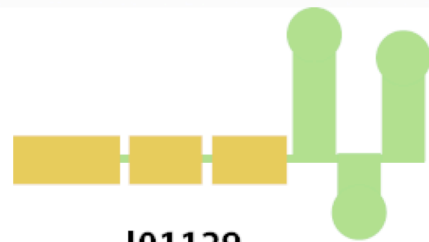
Unlocked Riboregulator



The Locks

		Alone ¹	Weak Key	Strong Key
	J01122	0.4% ²	1.2%	8.4%
	J23071	6.4%	4.5%	19.1%
	J23048	0.5%	0.9%	3.8%
	J23049	1.0%	1.3%	6.0%
	J23077	0.3%	ND	14.7%

Mismatches are not necessary for Unlocking

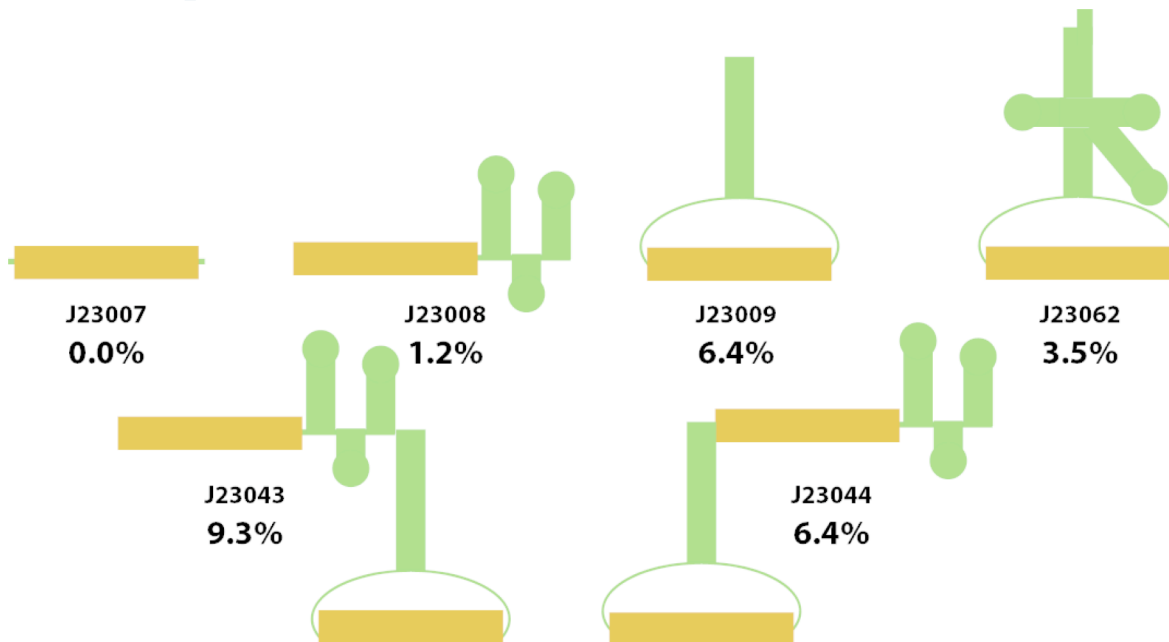


J01129
1.2%*

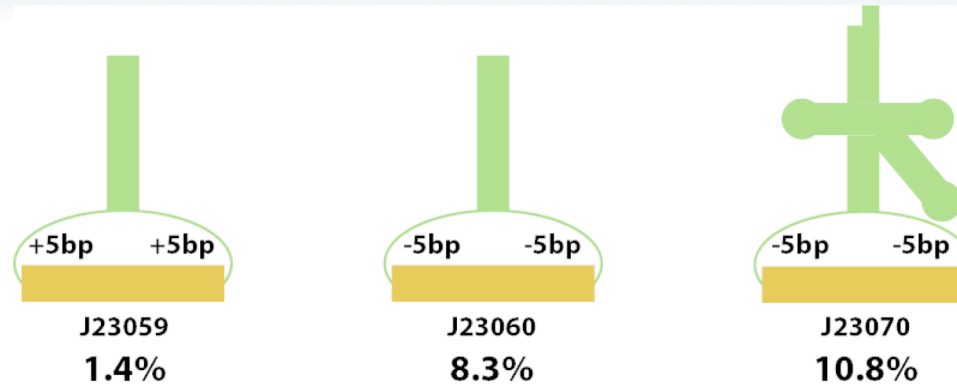


J23008
1.2%

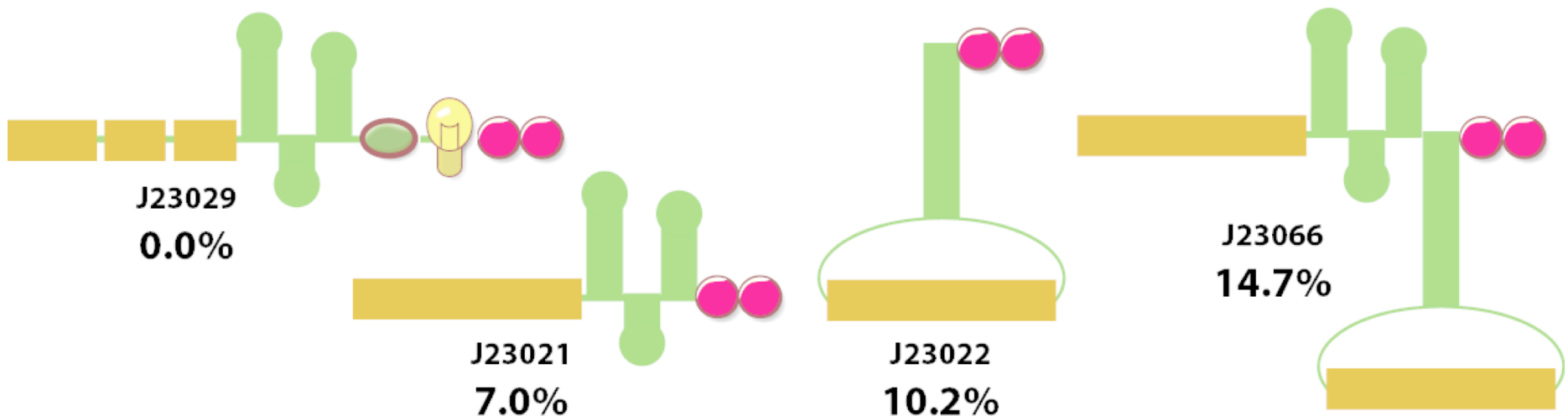
Secondary structure is critical for activity



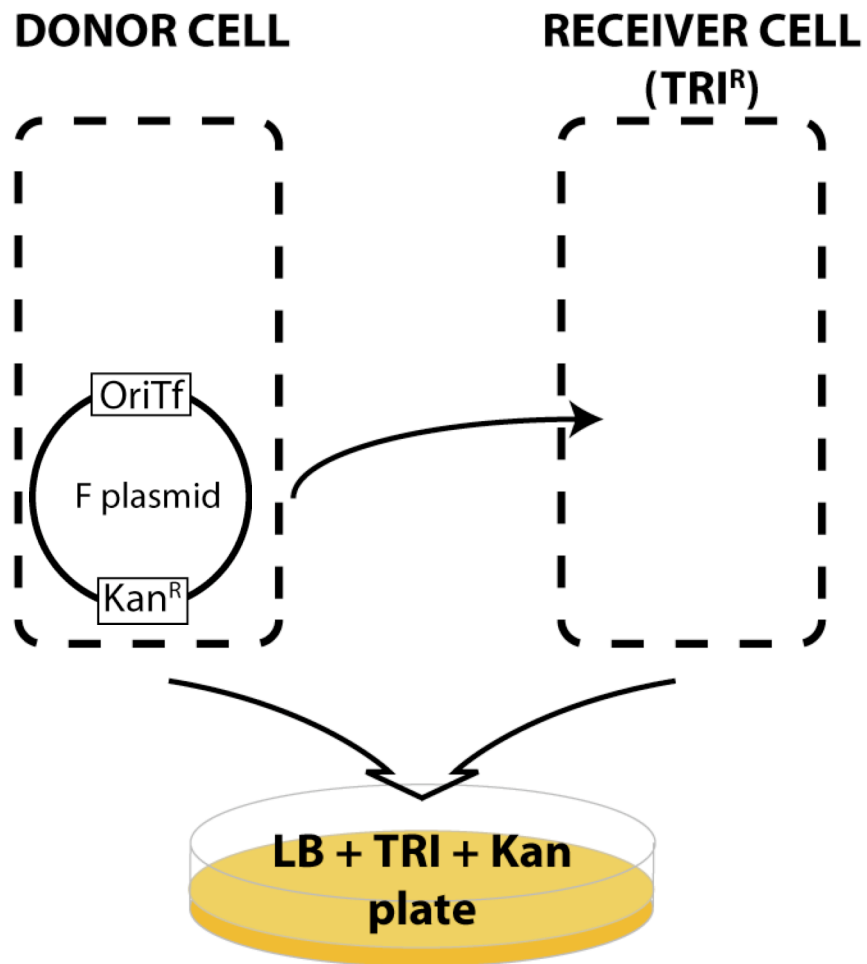
Shorter loops unlock better



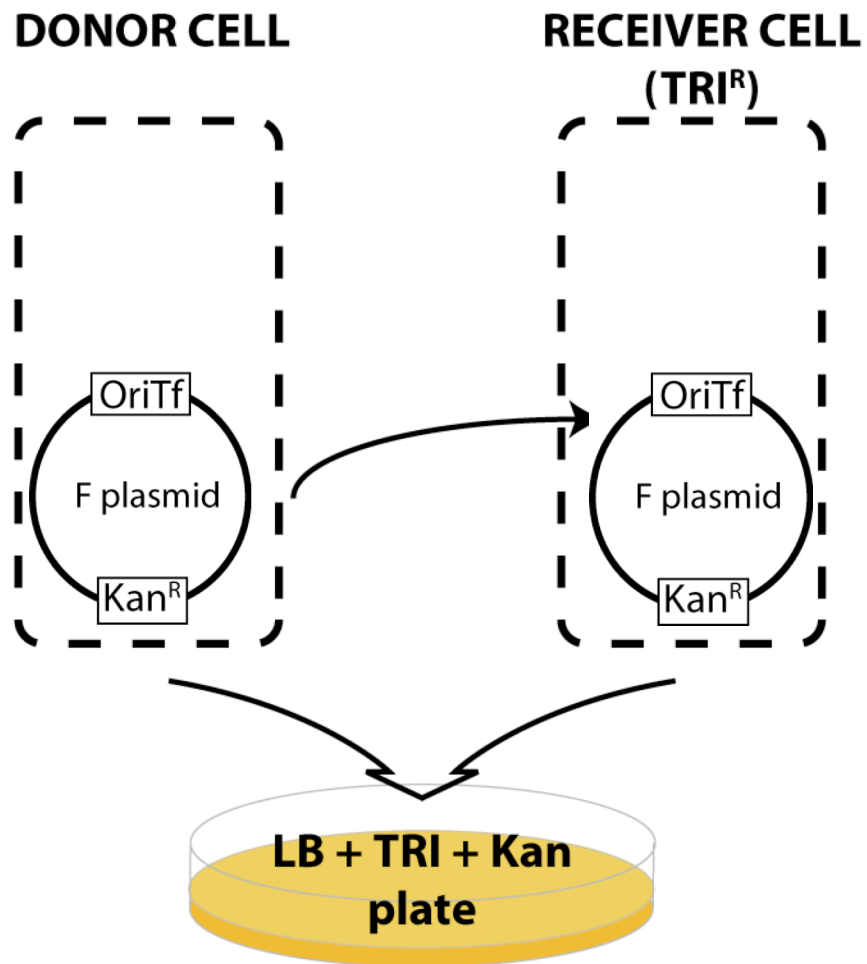
Transcriptional terminators but not open reading frames improve activity



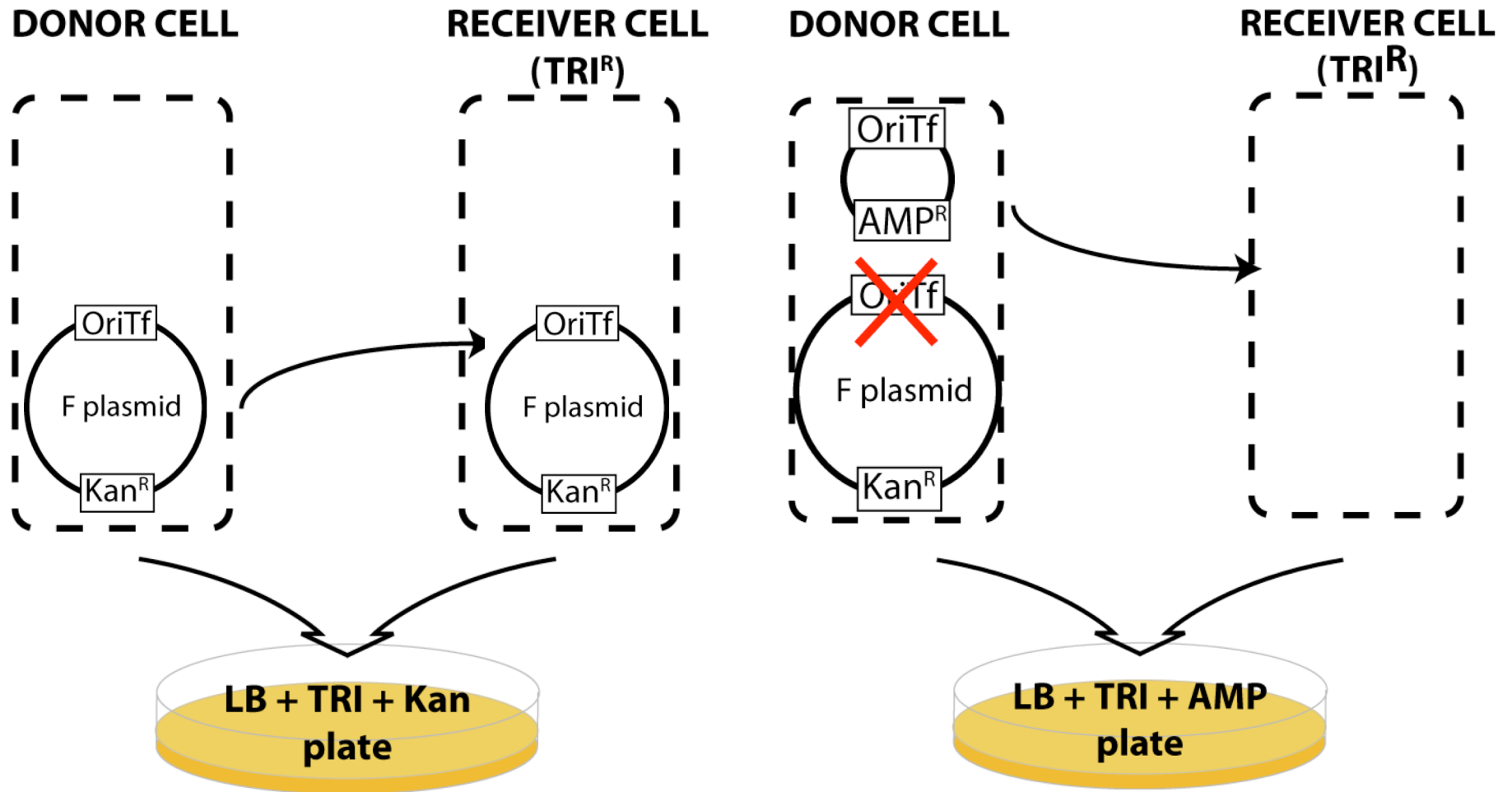
Bacterial Conjugation



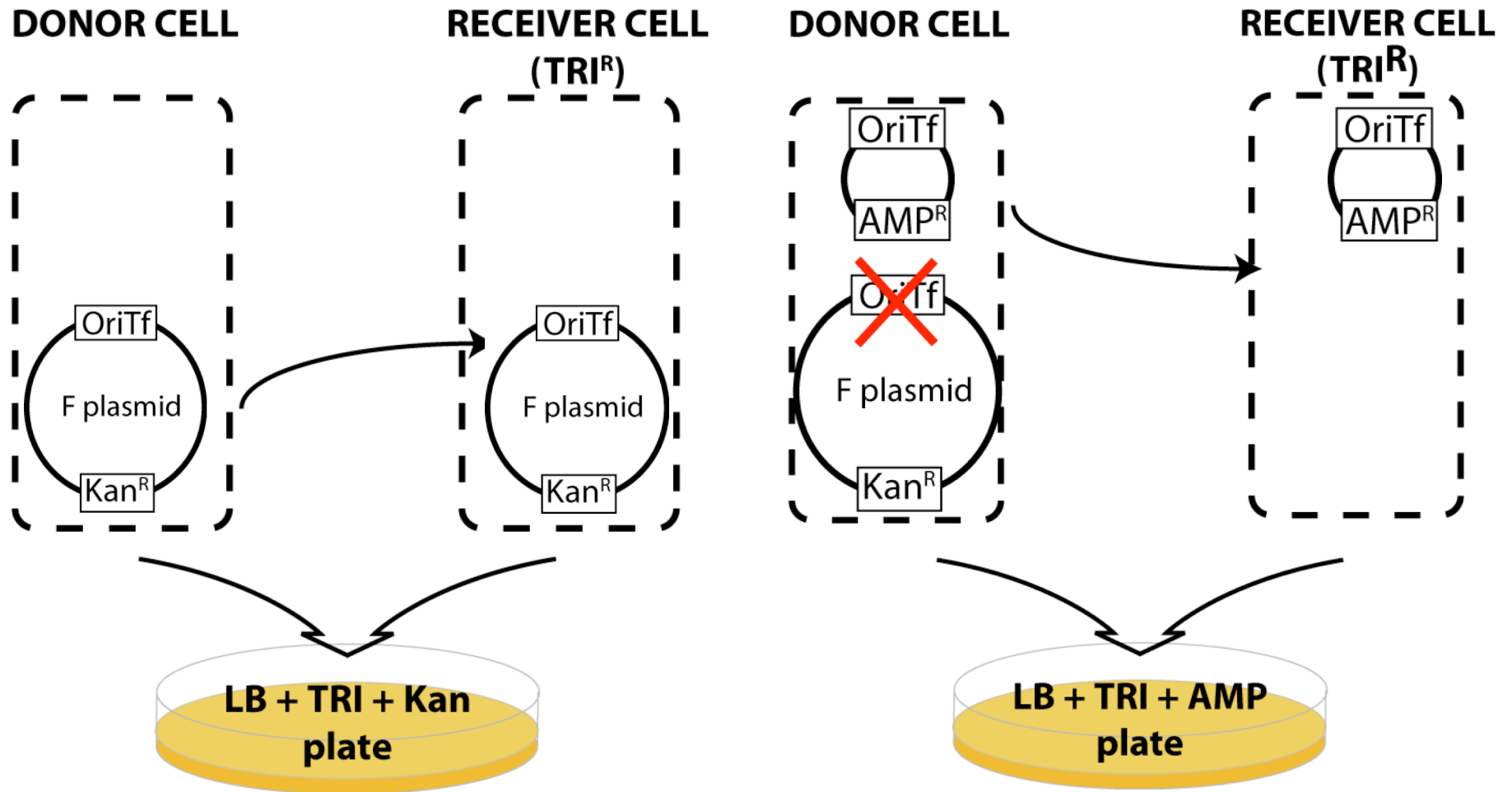
Bacterial Conjugation



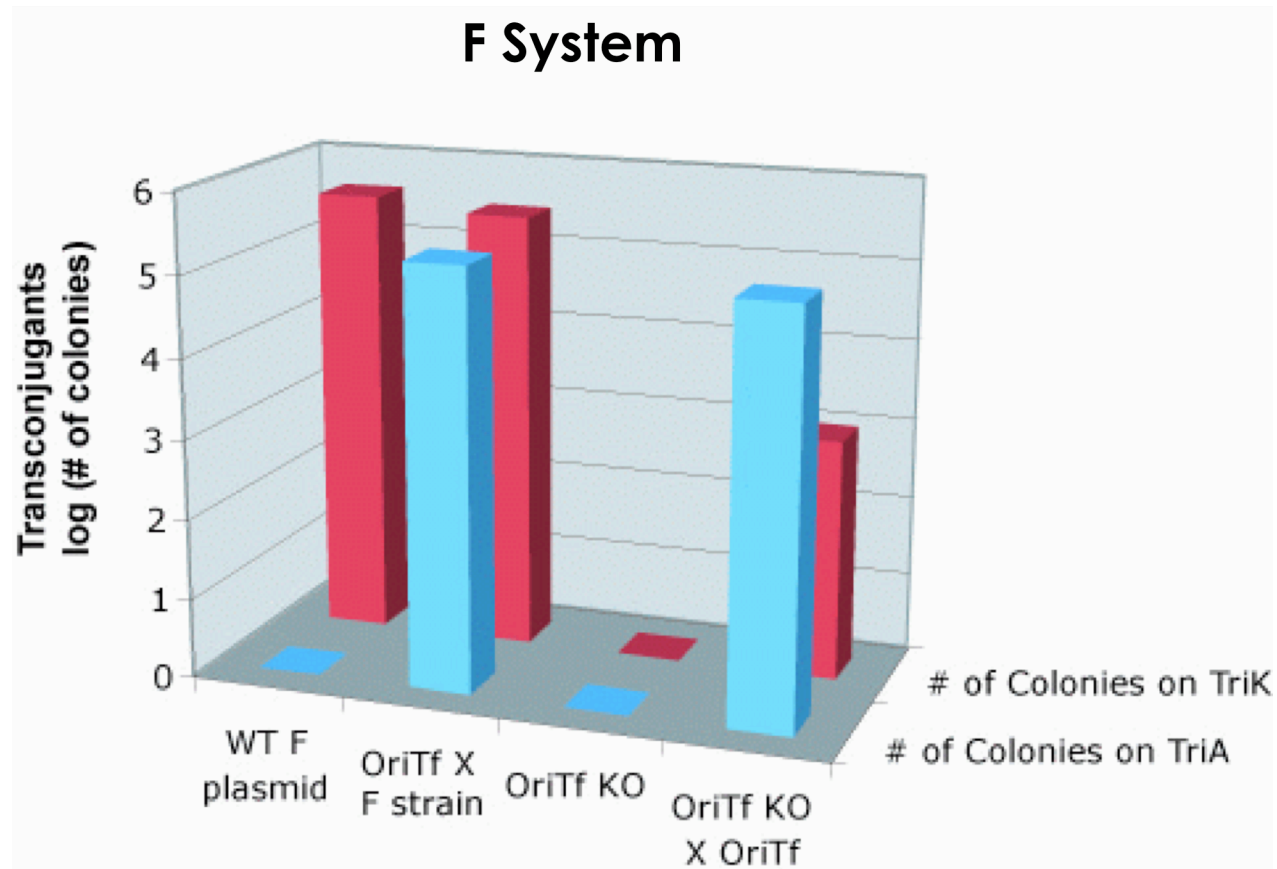
Bacterial Conjugation



Bacterial Conjugation

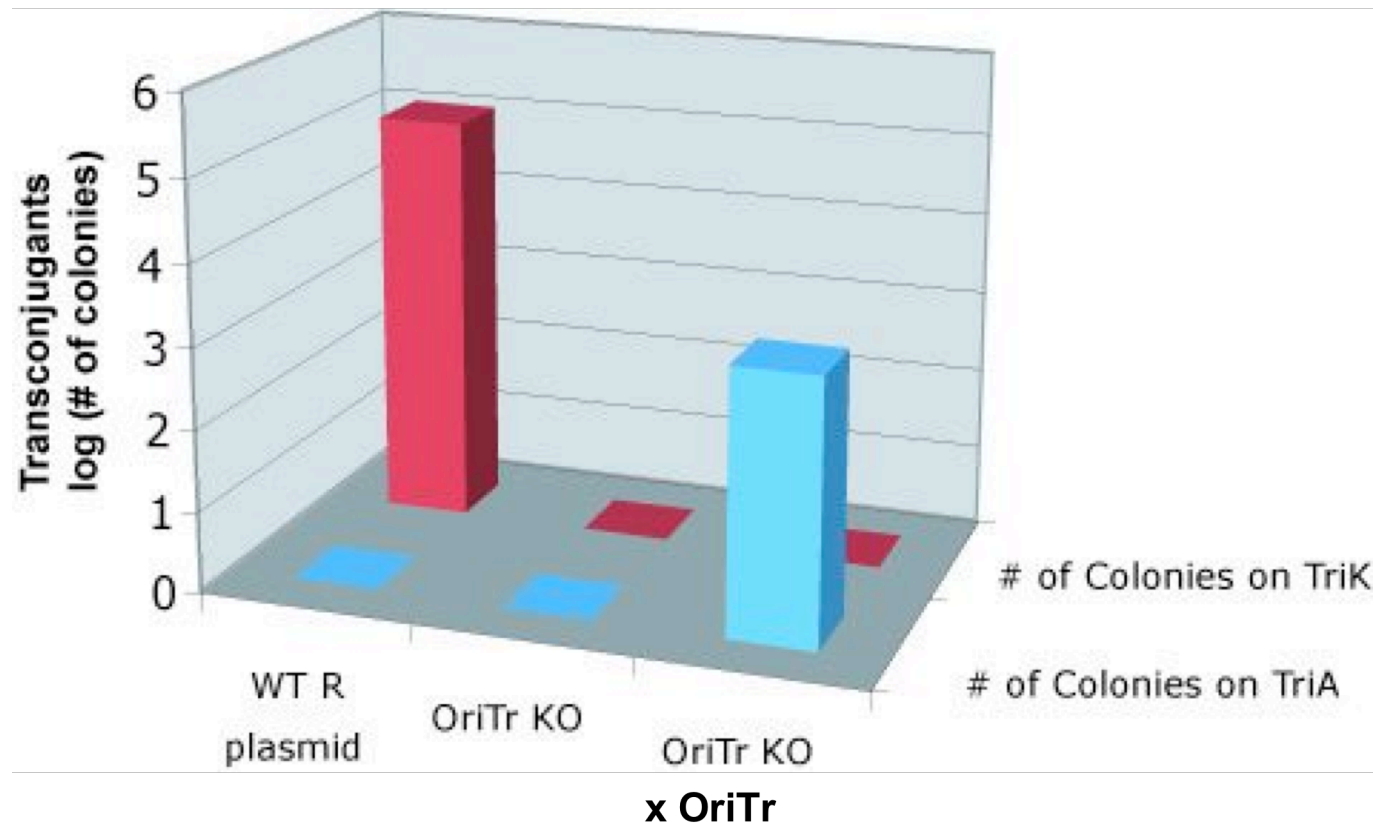


Transfer of Message Plasmids



Transfer of Message Plasmids

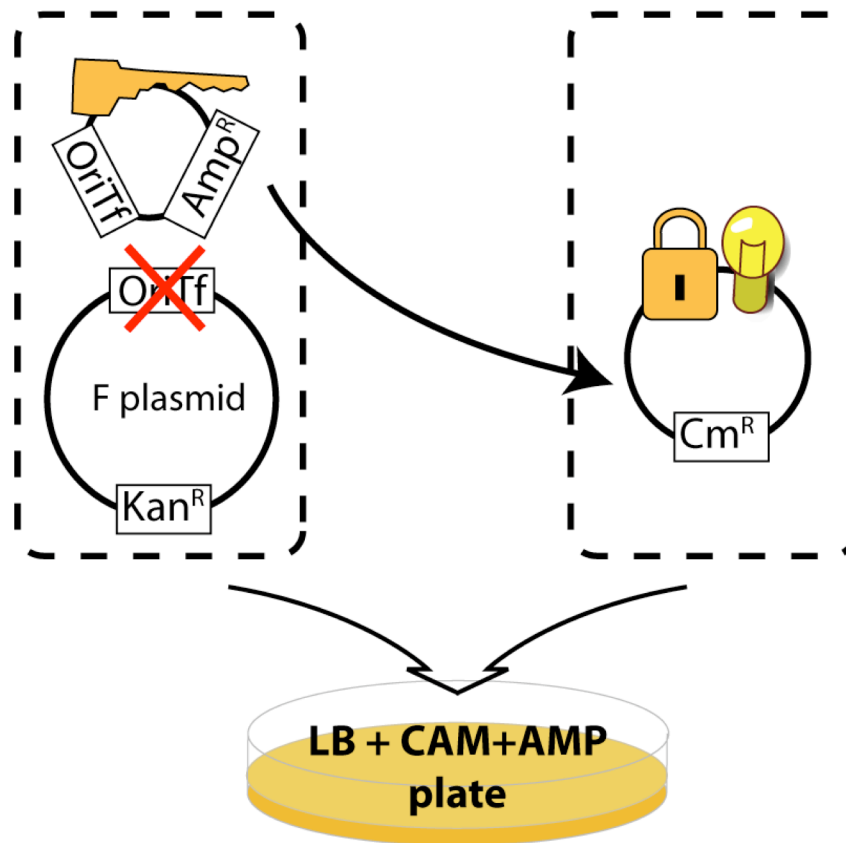
R System



Cell Sends a Coded Message to Recipient Cell

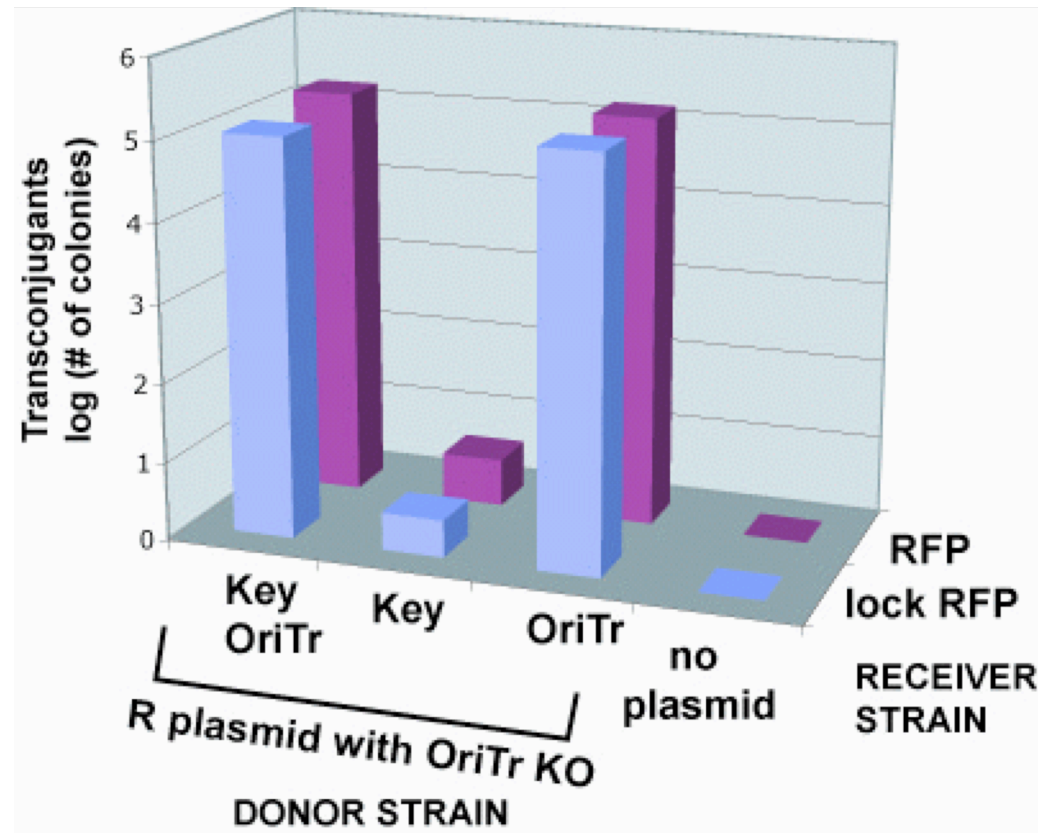
DONOR CELL

RECEIVER CELL



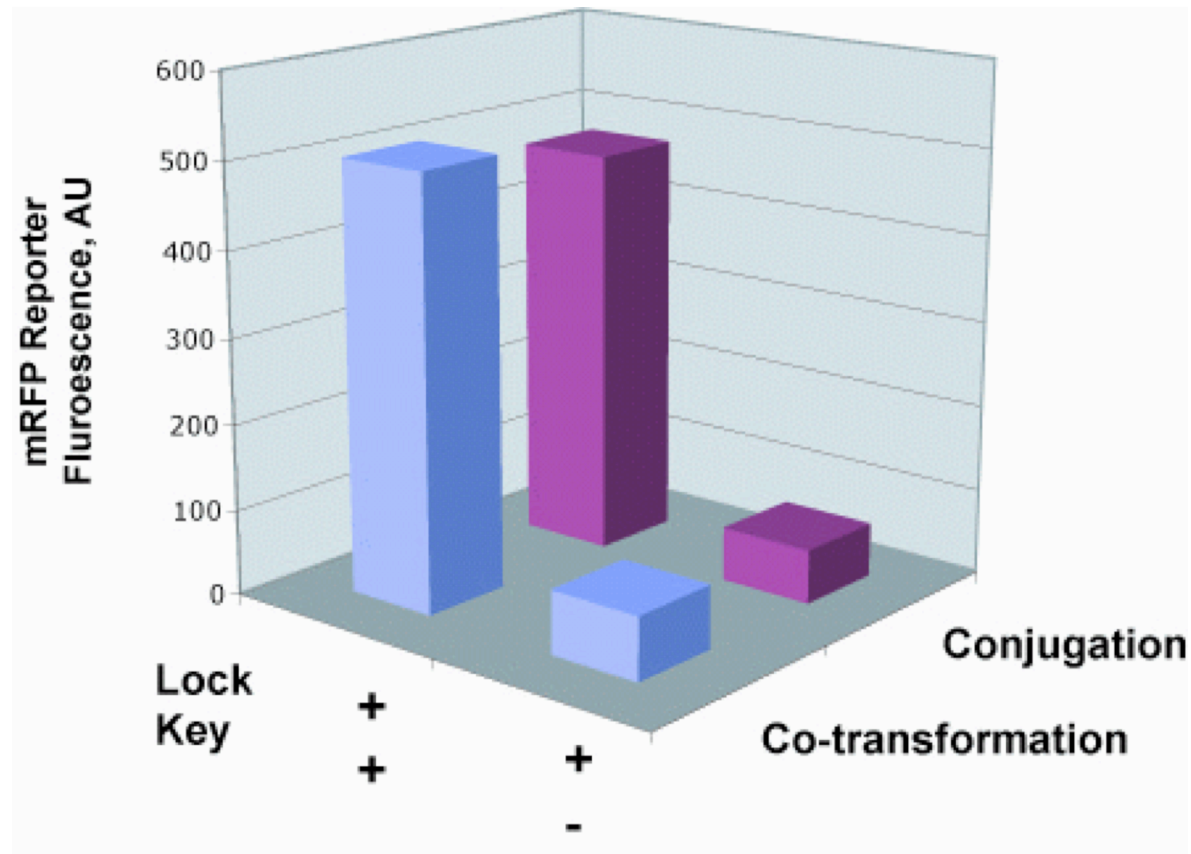
Conjugation and Riboregulators

Riboregulators Don't Effect Conjugation Efficiency

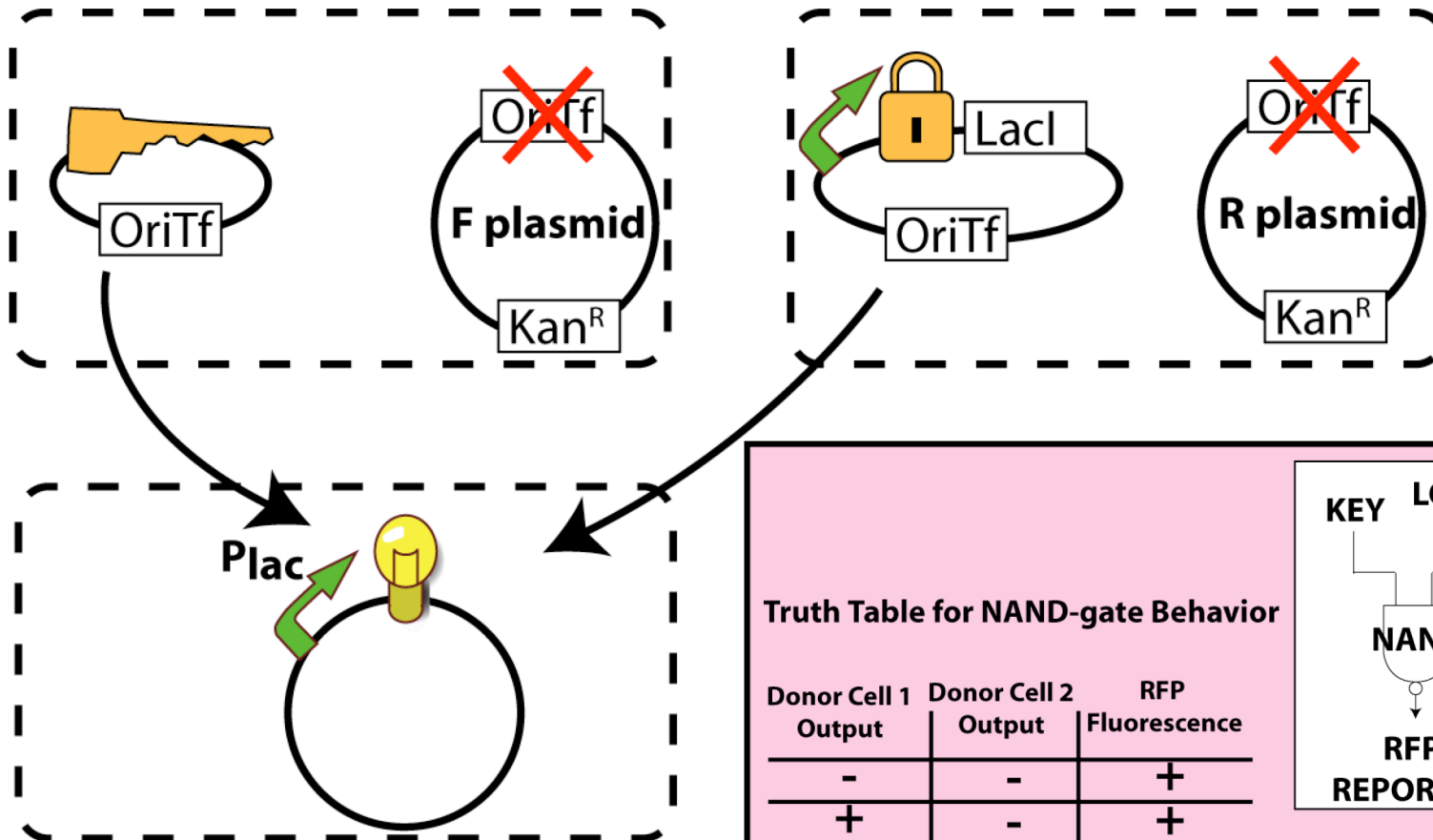


Conjugation and Riboregulators

Conjugation Does Not Impede
Riboregulator Function



NAND Logic Gates



Truth Table for NAND-gate Behavior

Donor Cell 1 Output	Donor Cell 2 Output	RFP Fluorescence
-	-	+
+	-	+
-	+	+
+	+	-

KEY LOCKED
LacI

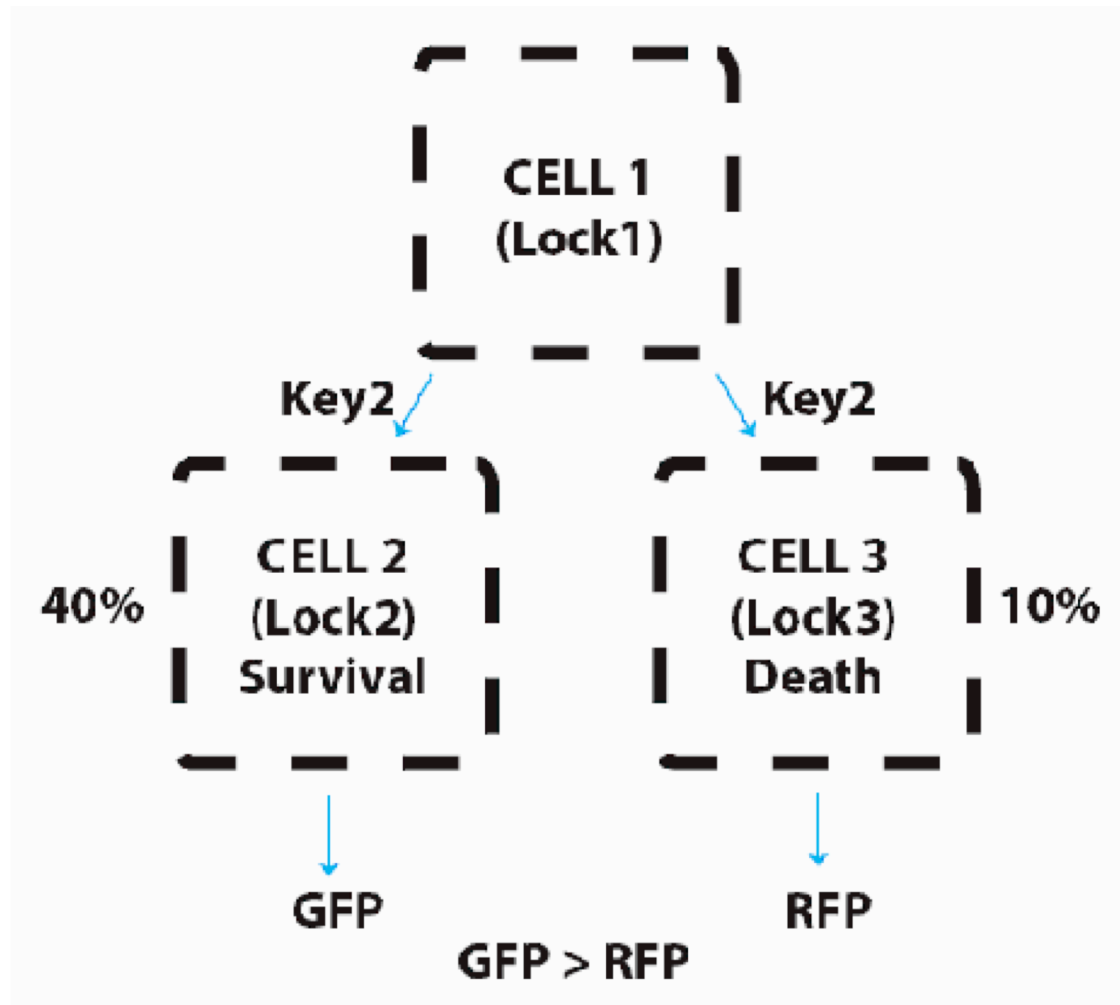
NAND

RFP REPORTER

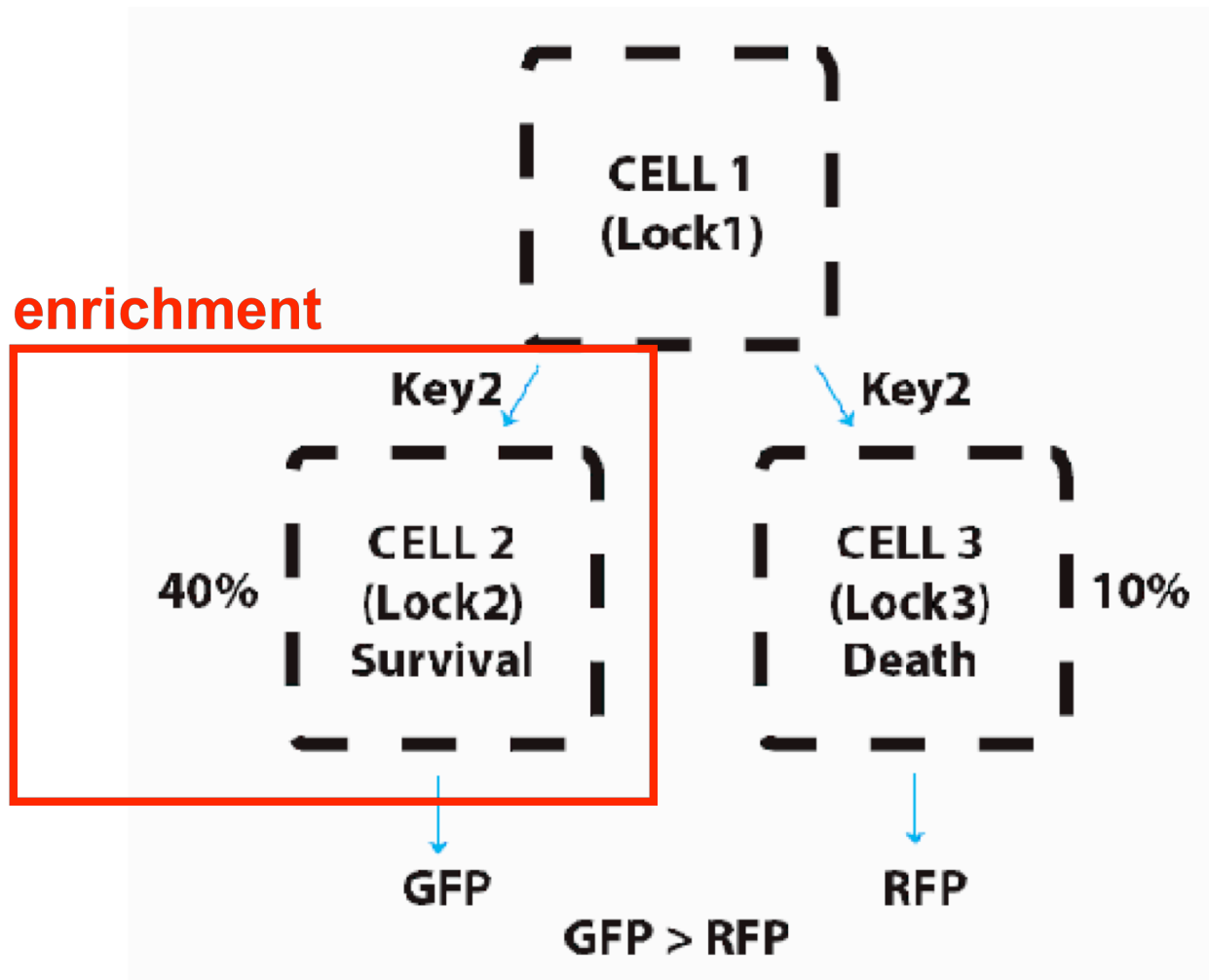
Building Computational Networks

- **Graded Response**
- **Inhibitory Signals**
- **Stimulatory Signals**
- **Parallel Signals**

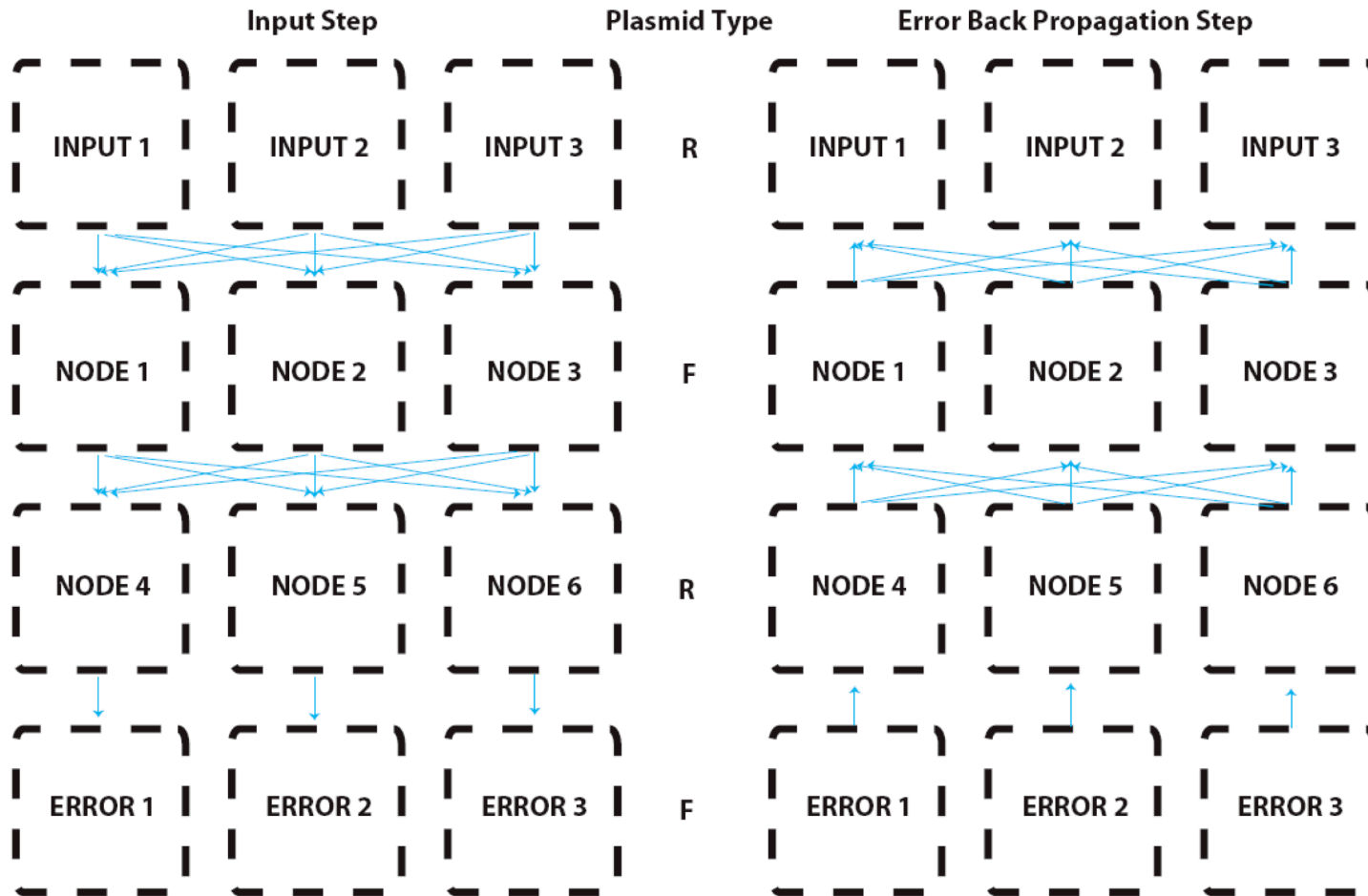
Concentration in Culture gives Graded Responses



Concentration in Culture gives Graded Responses



Trainable Bacterial Networks



Acknowledgements

Arkin and Keasling Labs

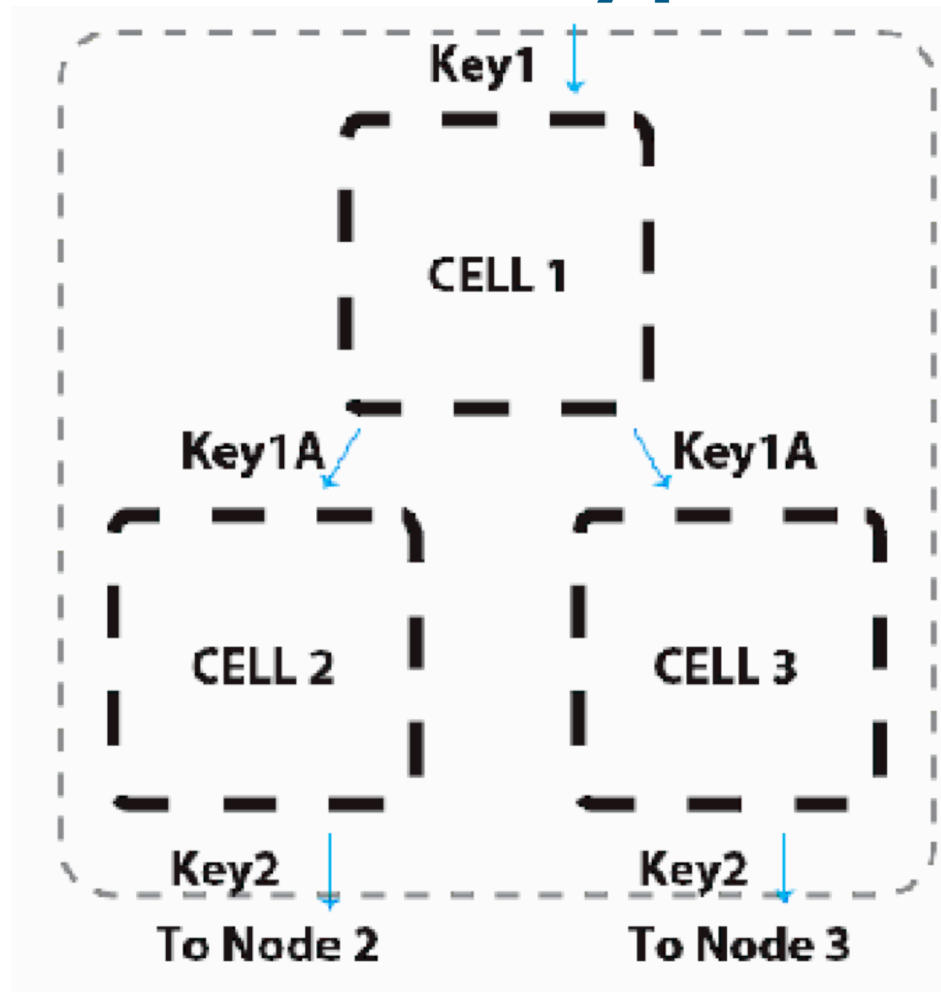
Funding from Microsoft

LBL EHS

Jonathon Goler, Melissa Li



Nodes are Built from Multiple Cell Types



Addressable Networks

