Addressable Conjugation in Bacterial Networks

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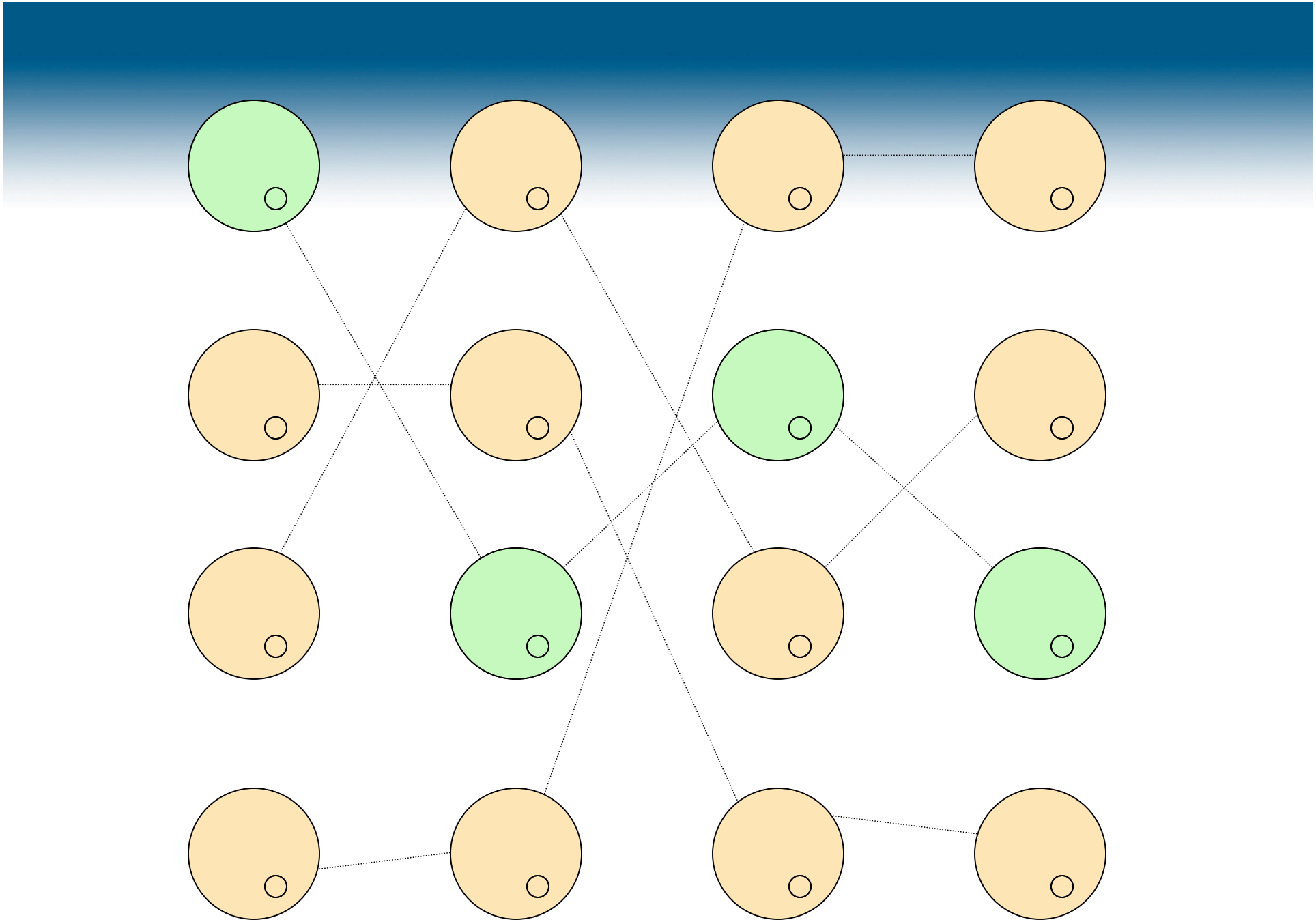


Every Cell is assigned a Unique RNA Address

Cell 1	UGAUCUAAGUGGAGGACGAA
Cell 2	GUUCAAGACAAUCACUCACG
Cell 3	UCAAUAUAAAACUACAAACU
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

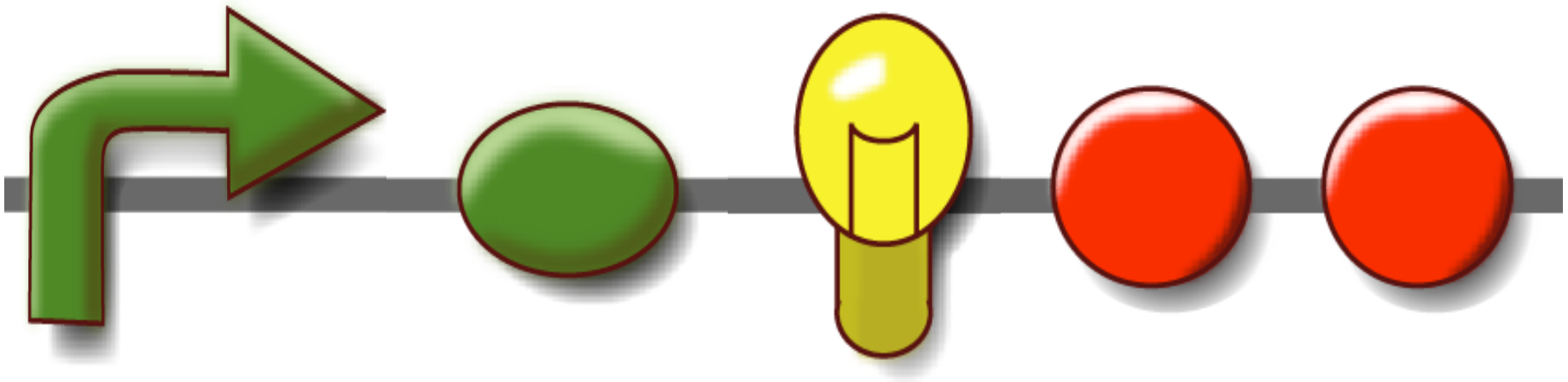
Means: Riboregulation

Our specific goals:

- [x] Construct high-performance riboregulators
- [x] Harness bacterial conjugation
- [x] Transmit a coded message
- [ ] Construct a bacterial learning network

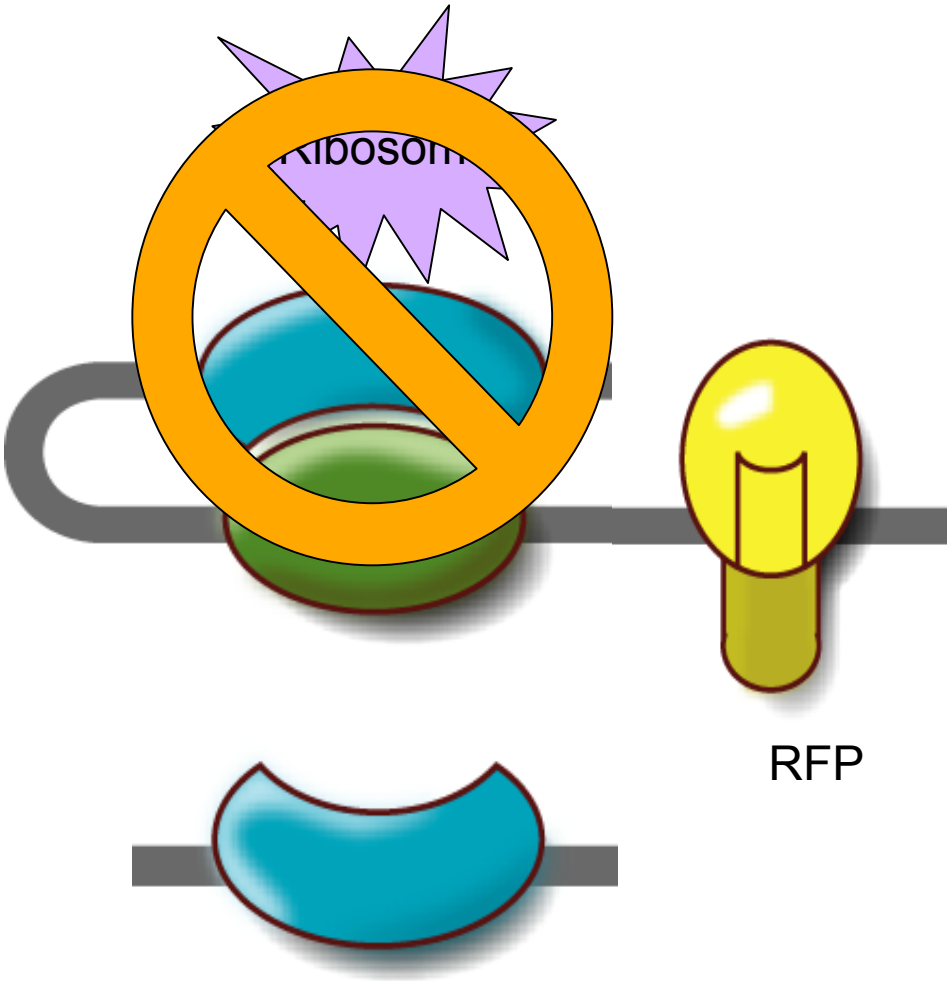
RNA Polymerase

The Riboregulator: DNA



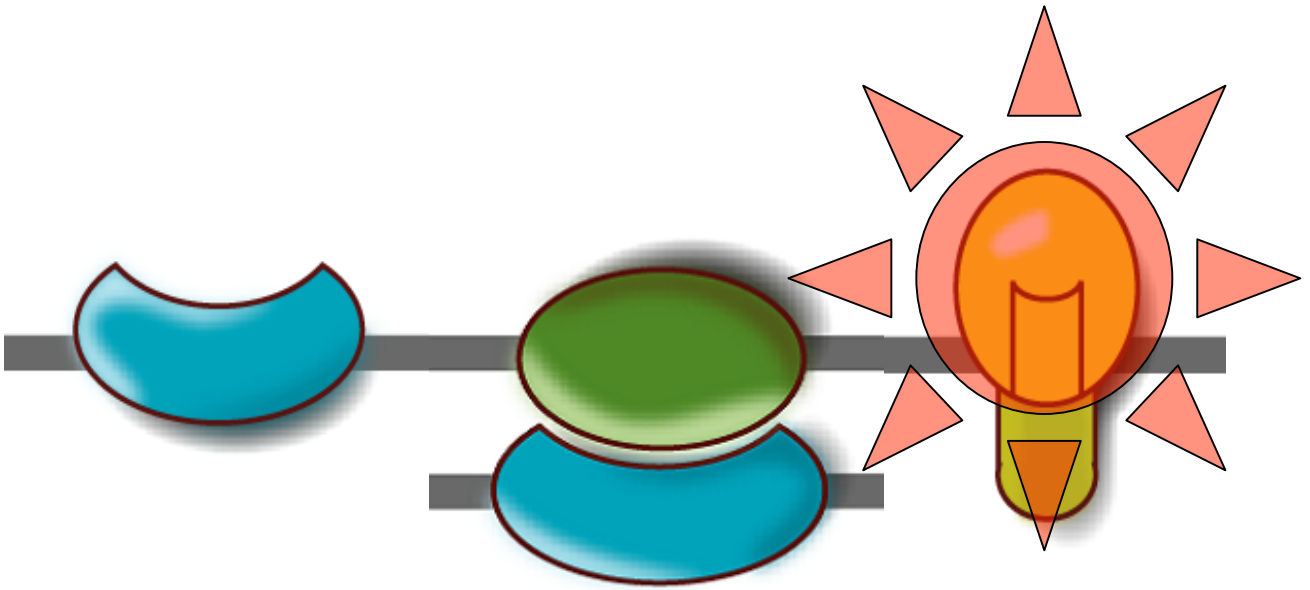
Promoter RBS RFP Double T

Locked Riboregulator: RNA



Ribosome

Unlocked Riboregulator



Unlocked Lock

Revealed RBS + Key

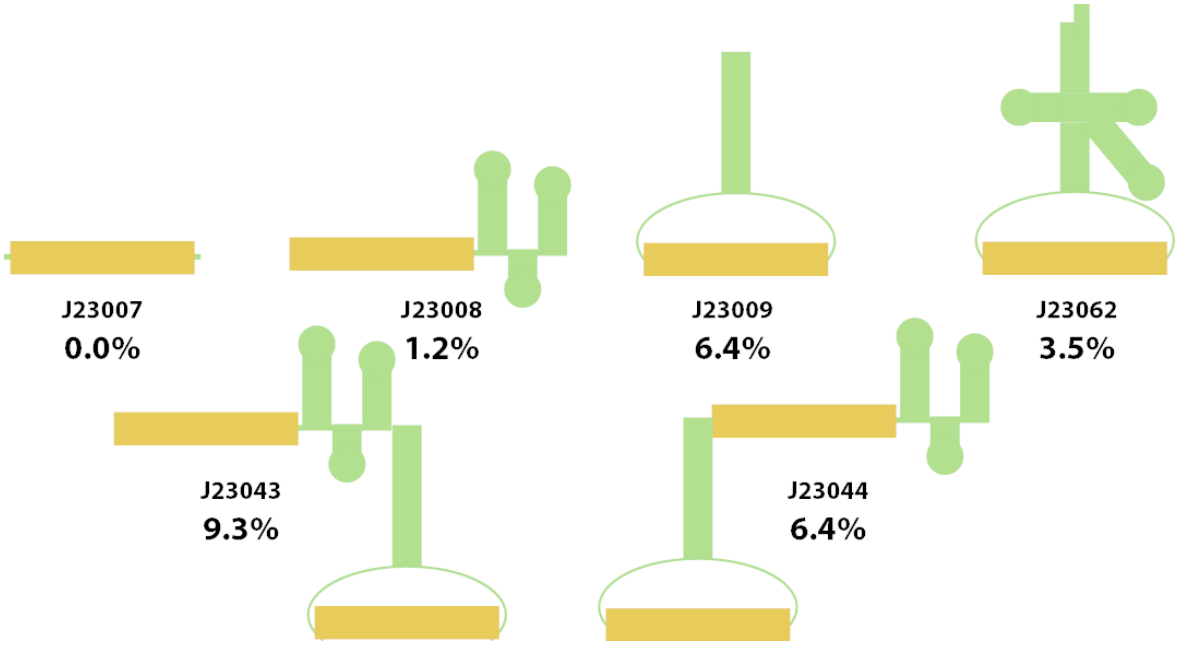
RFP

The Locks

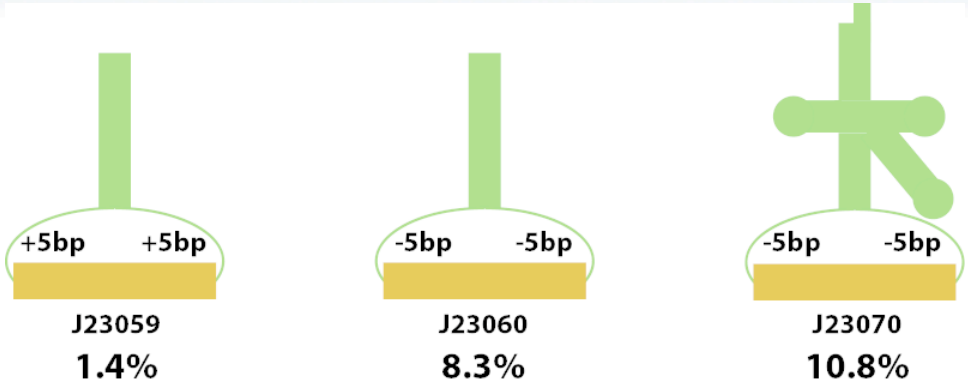
	Alone[1]	Weak Key	Strong Key
J01122	0.4%[2]	1.2%	8.4%
J23071	6.4%	4.5%	19.1%
J23048	0.5%	0.9%	3.8%
J23049	1.0%	1.3%	6.0%
J23077	0.3%	ND	14.7%

Mismatches are not necessary for Unlocking

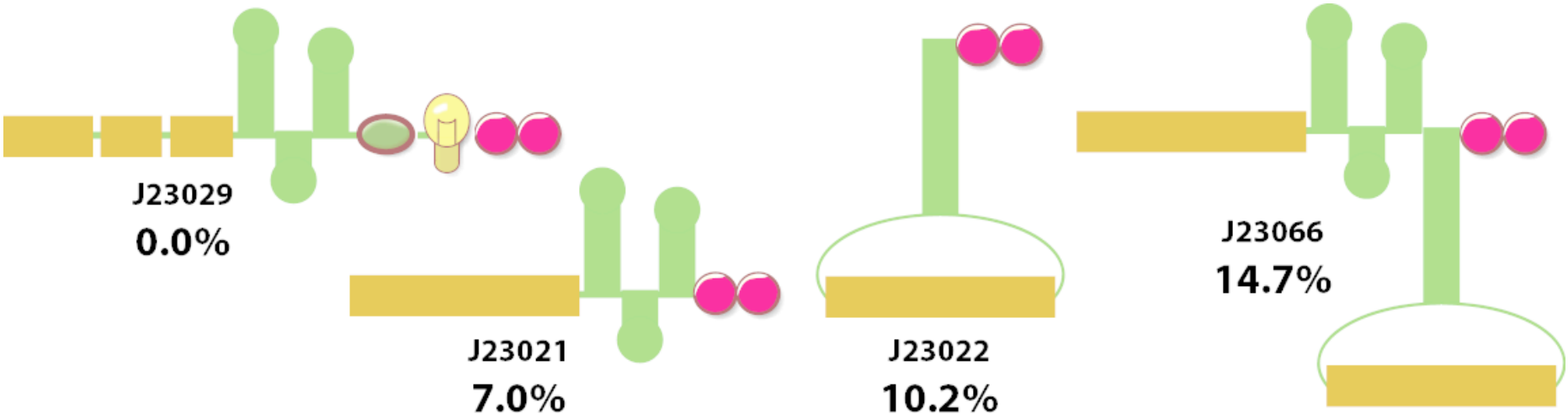
Secondary structure is critical for activity



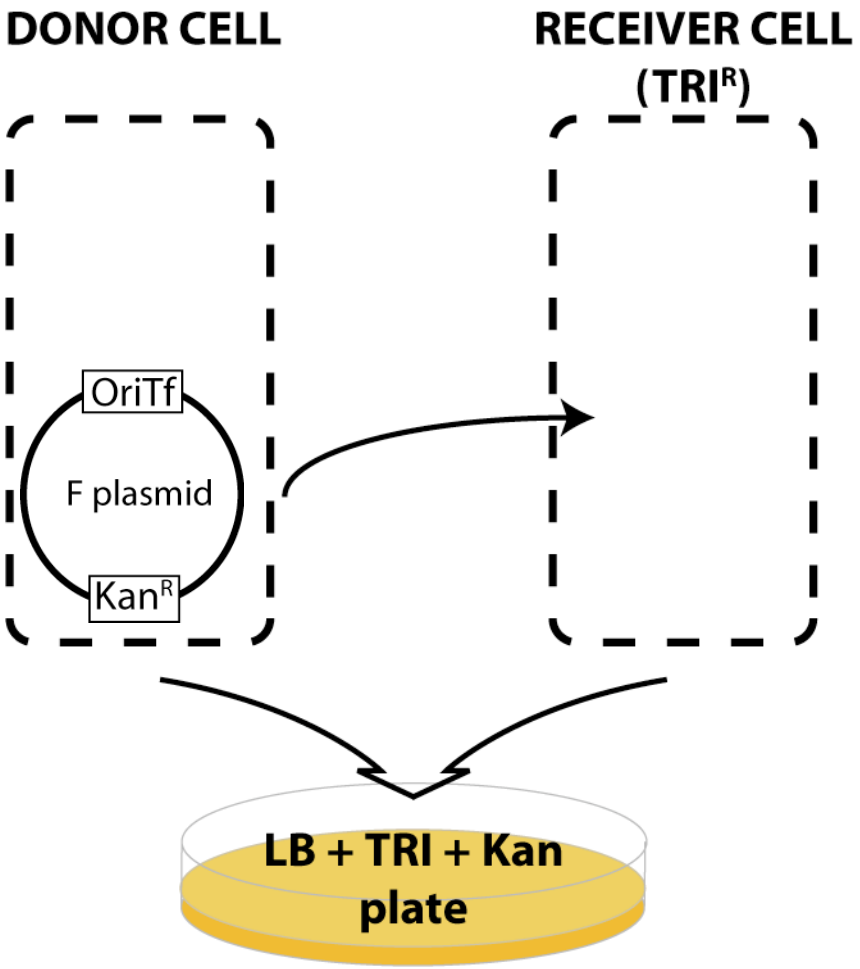
Shorter loops unlock better



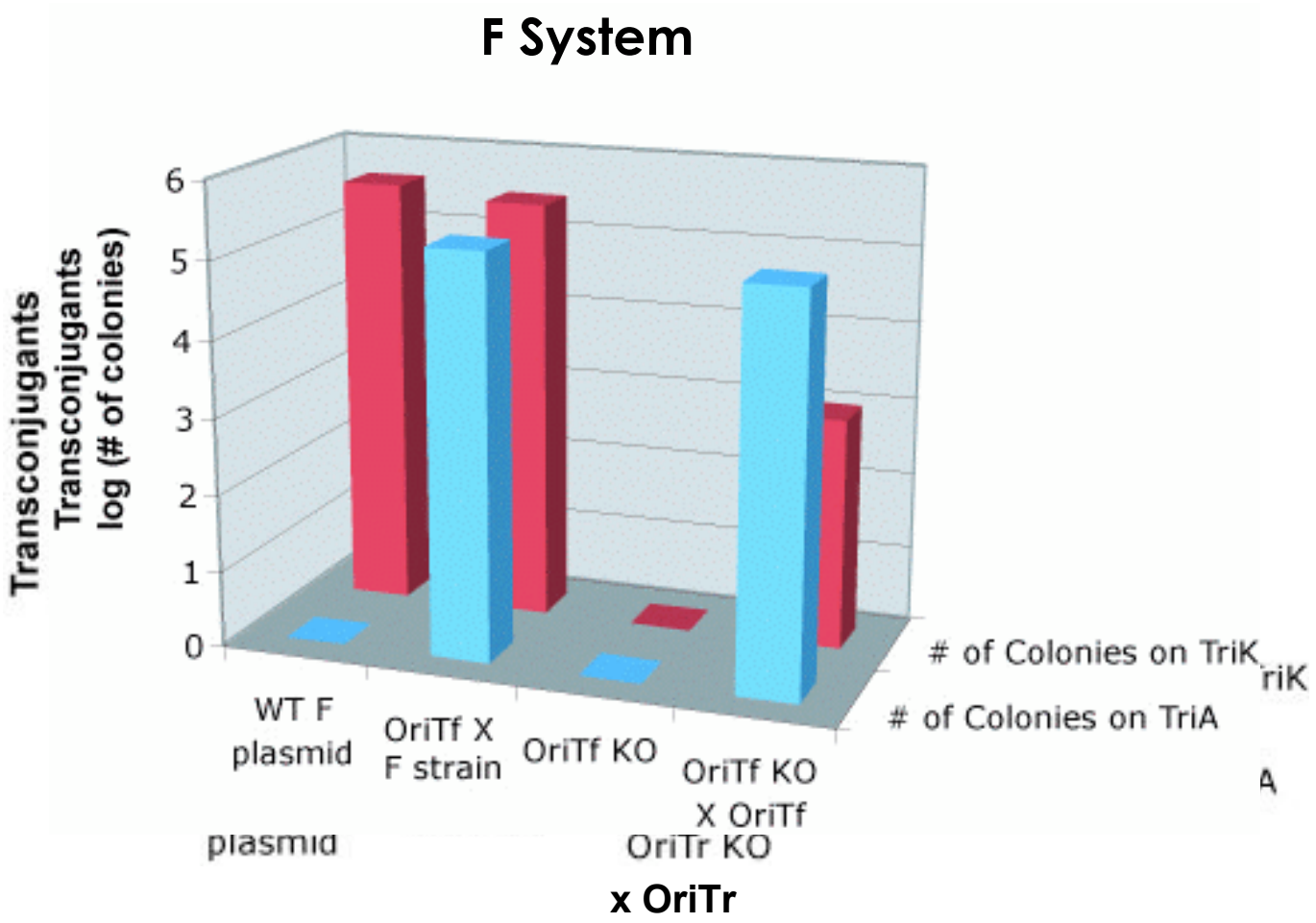
Transcriptional terminators but not open reading frames improve activity



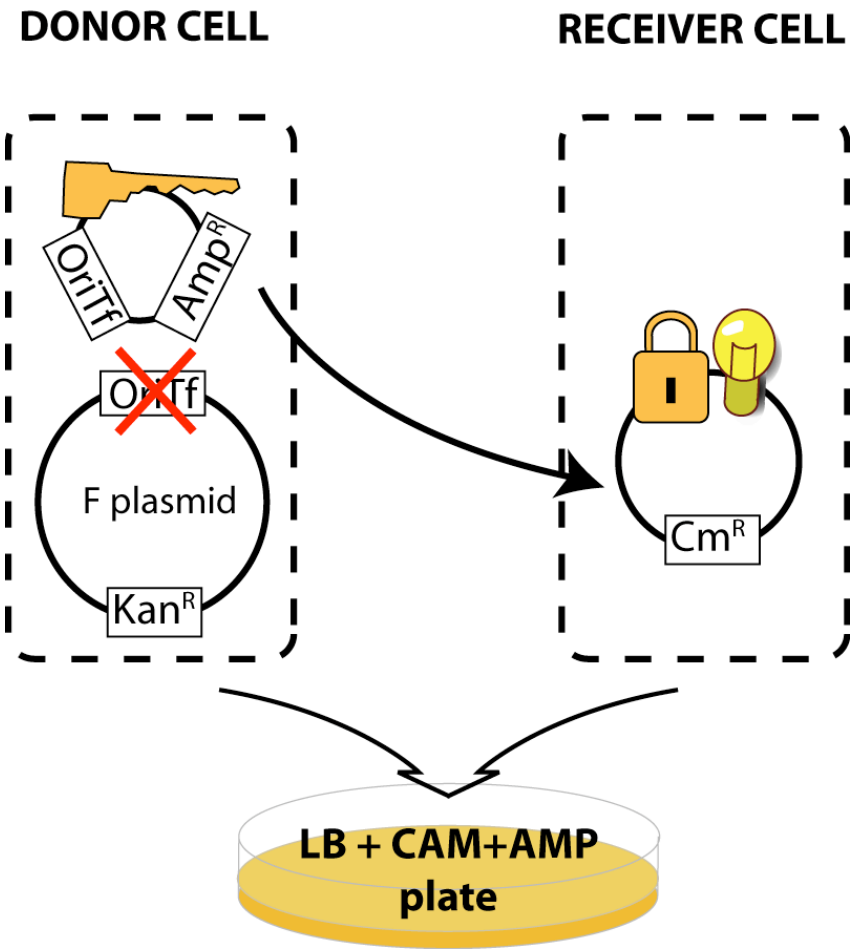
Bacterial Conjugation



Transfer of Message Plasmids



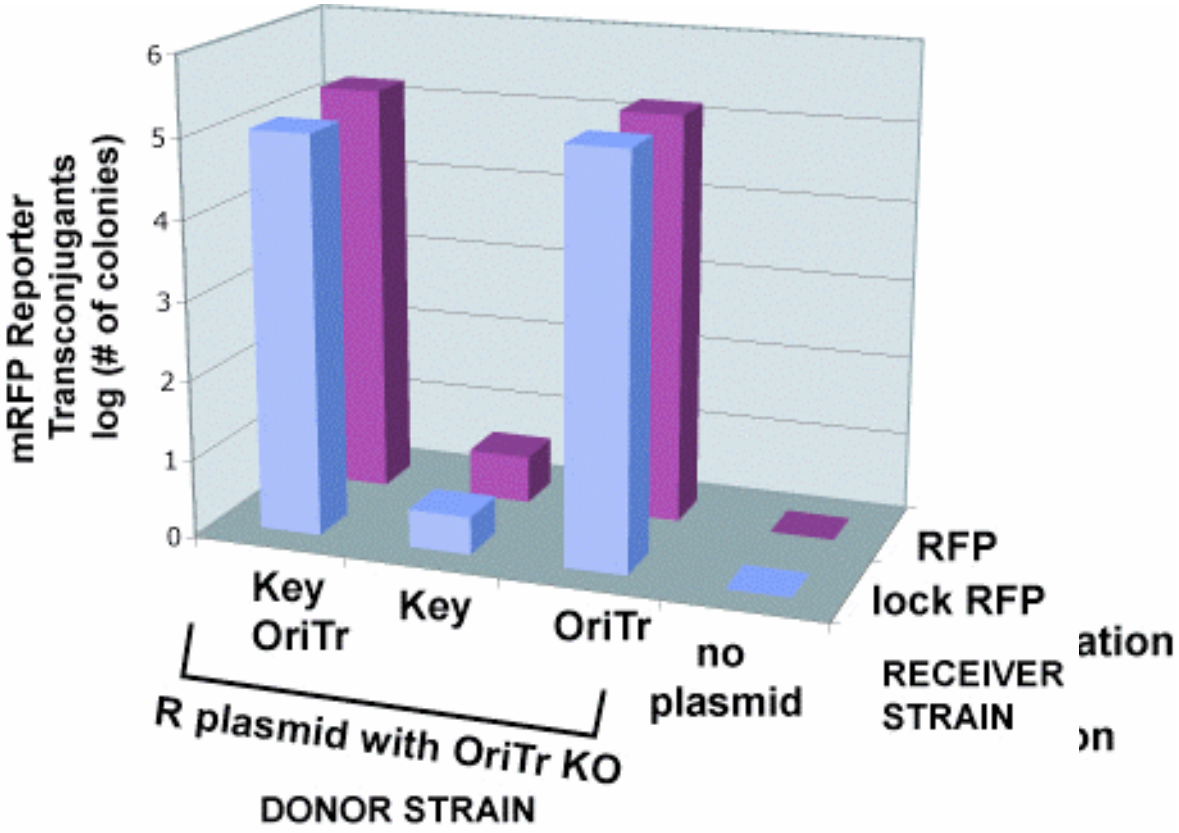
Cell Sends a Coded Message to Recipient Cell



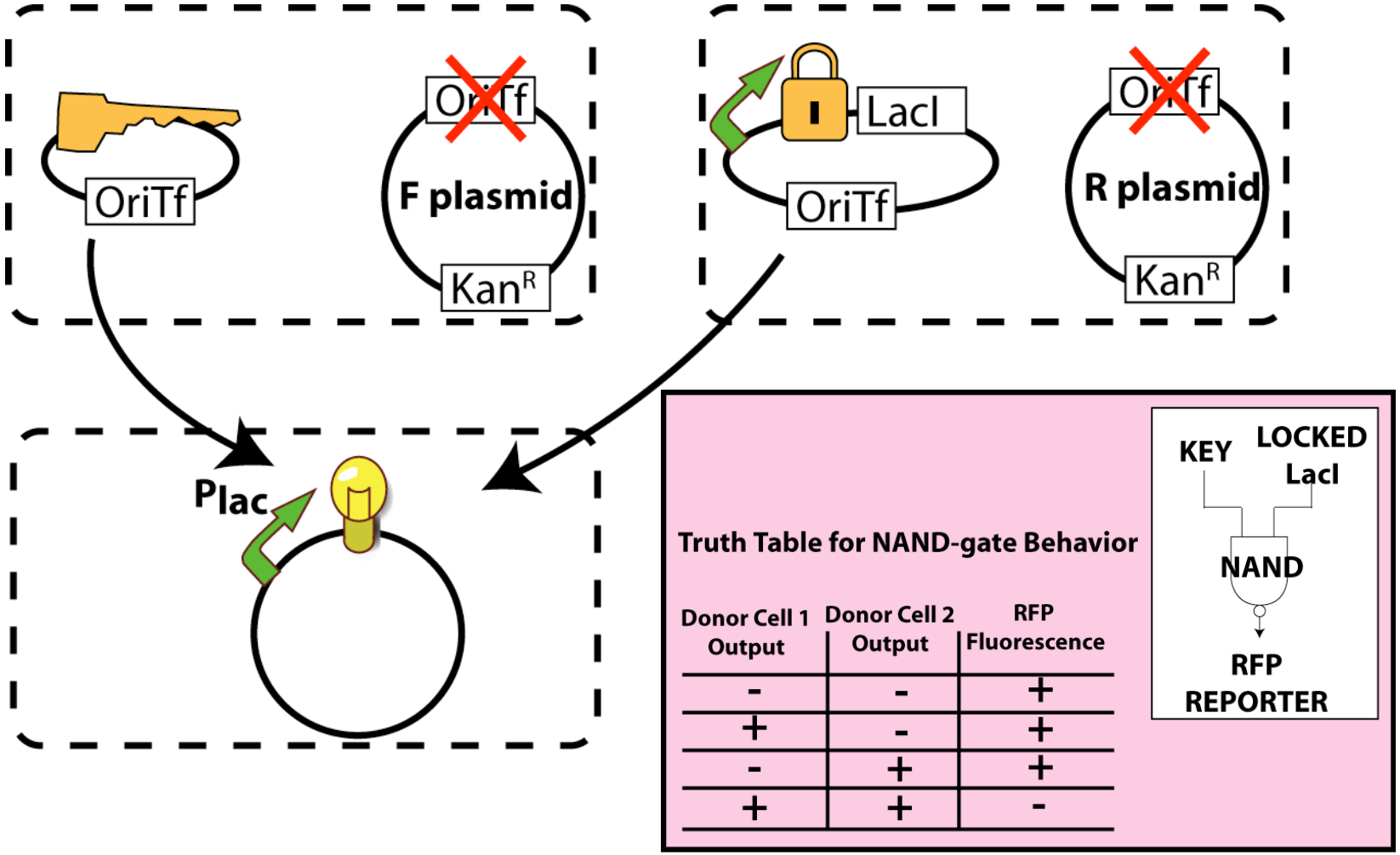
Conjugation and Riboregulators

Riboregulators Don't Effect Conjugation Efficiency

Conjugation Does Not Impede Riboregulator Function



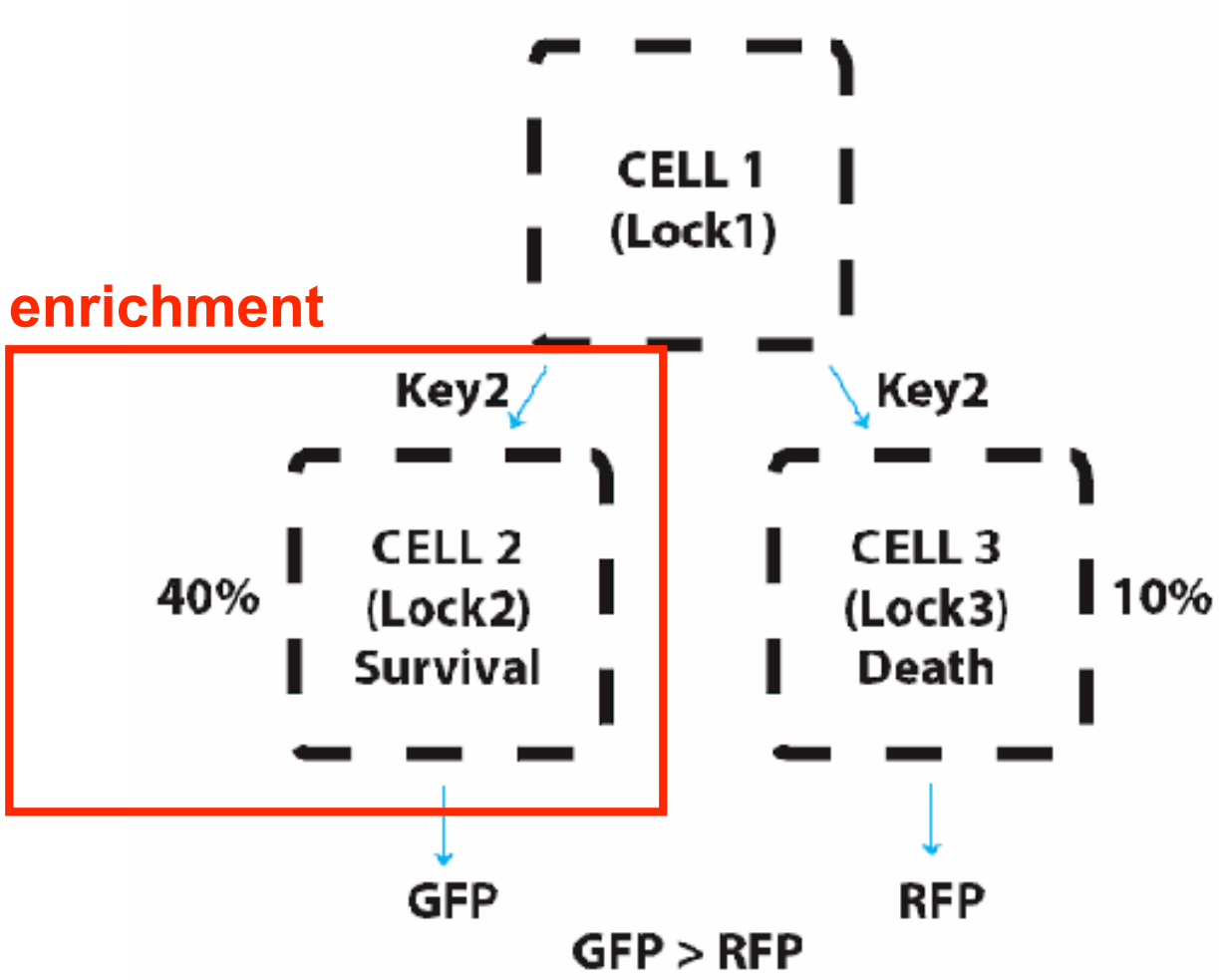
NAND Logic Gates



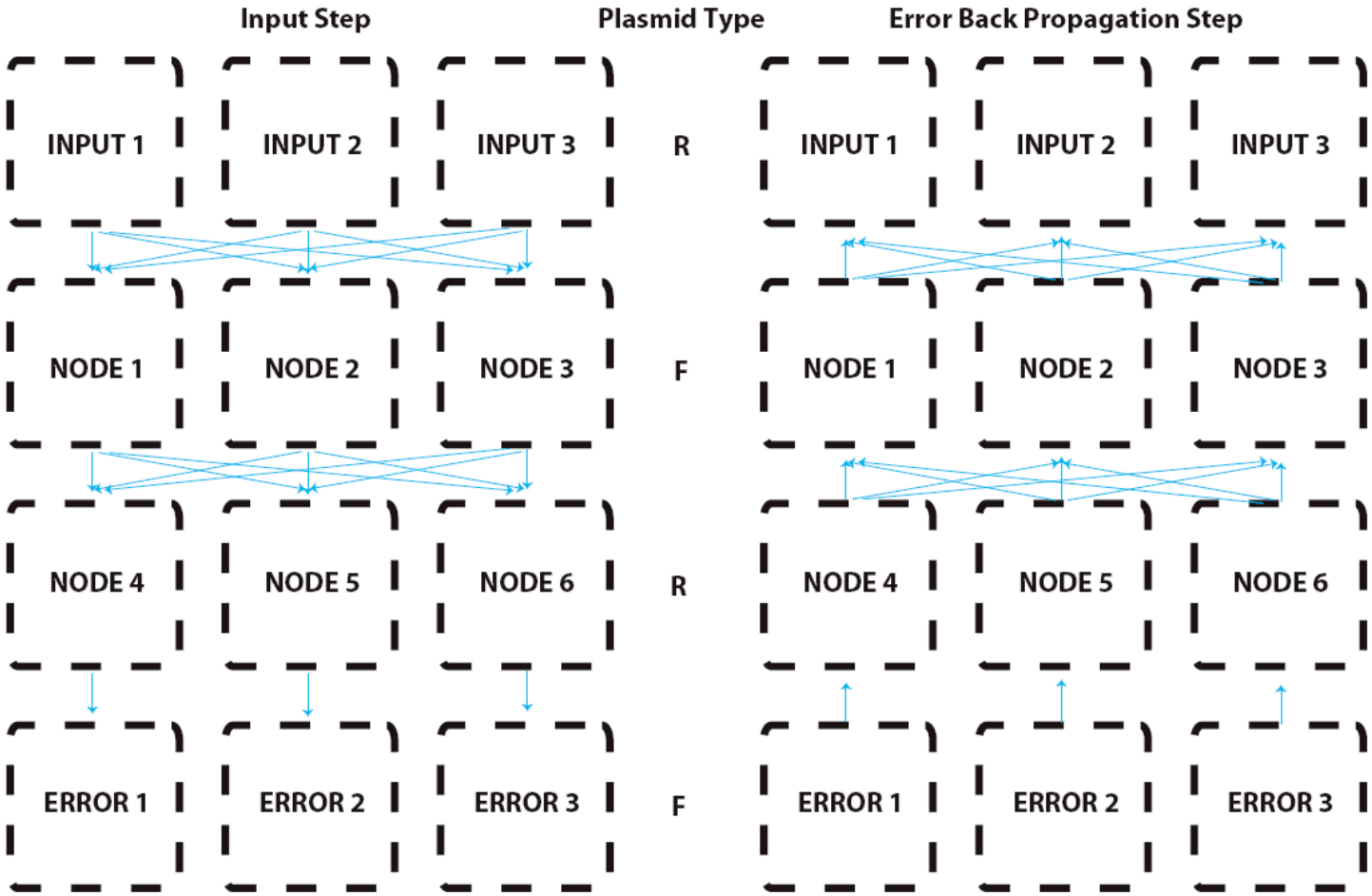
Building Computational Networks

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

Concentration in Culture gives Graded Responses



Trainable Bacterial Networks



Acknowledgements

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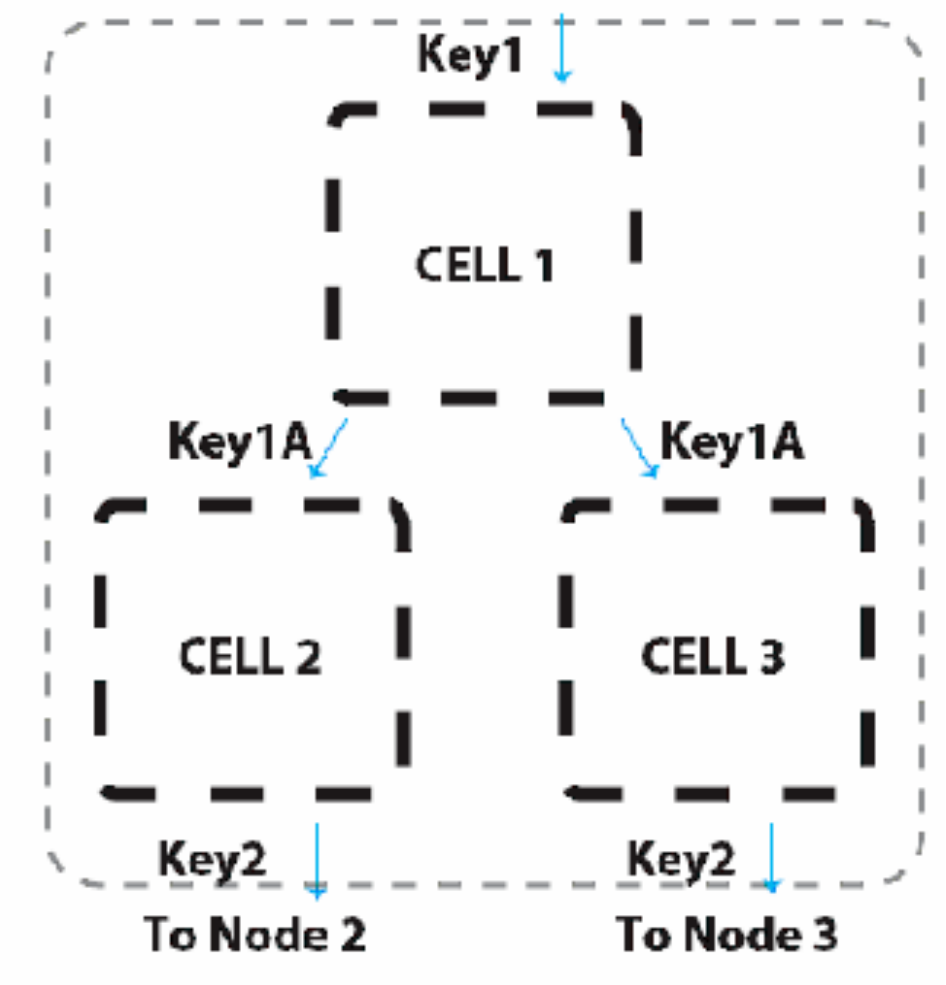
Nodes are Built from Multiple Cell Types



Addressable Networks