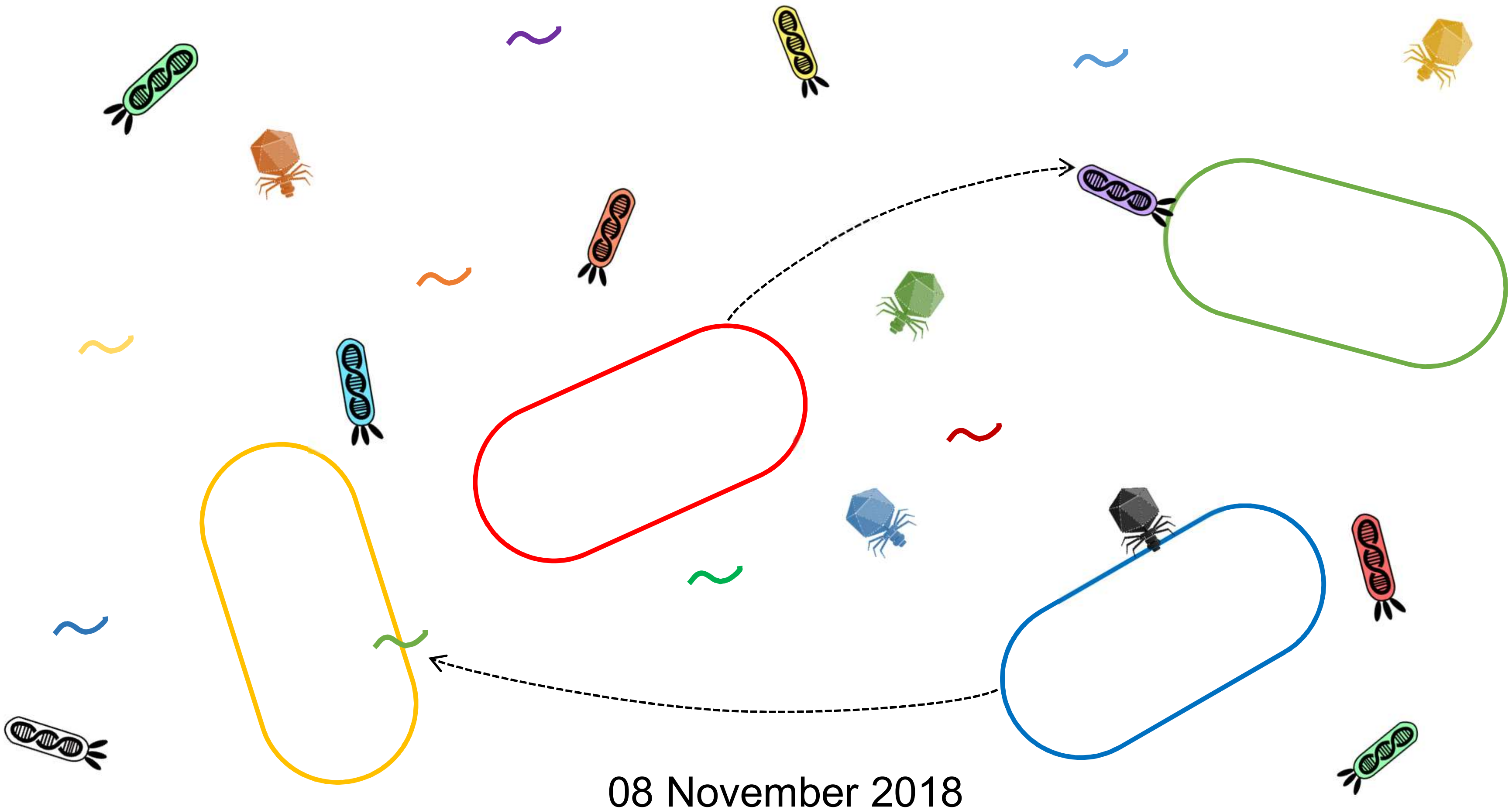
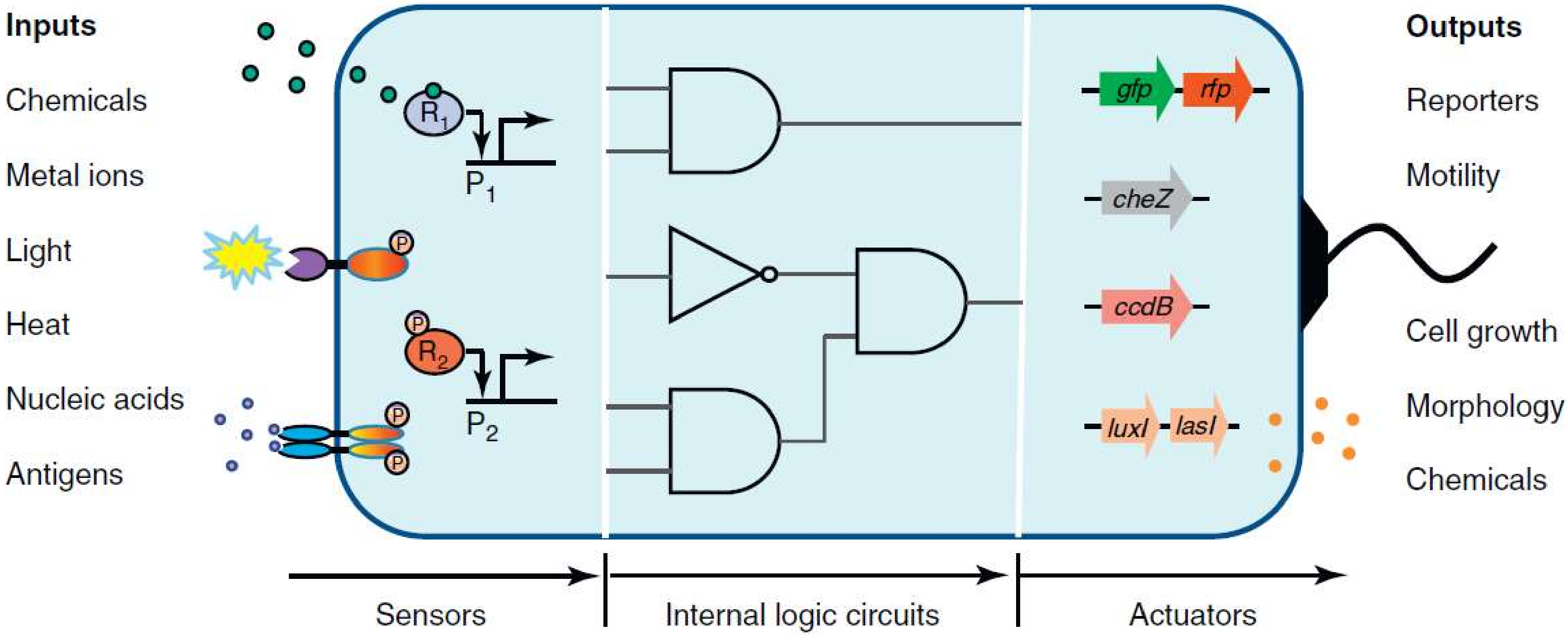
Exploiting bacteriophage-derived signals as external wires for biological computing



08 November 2018

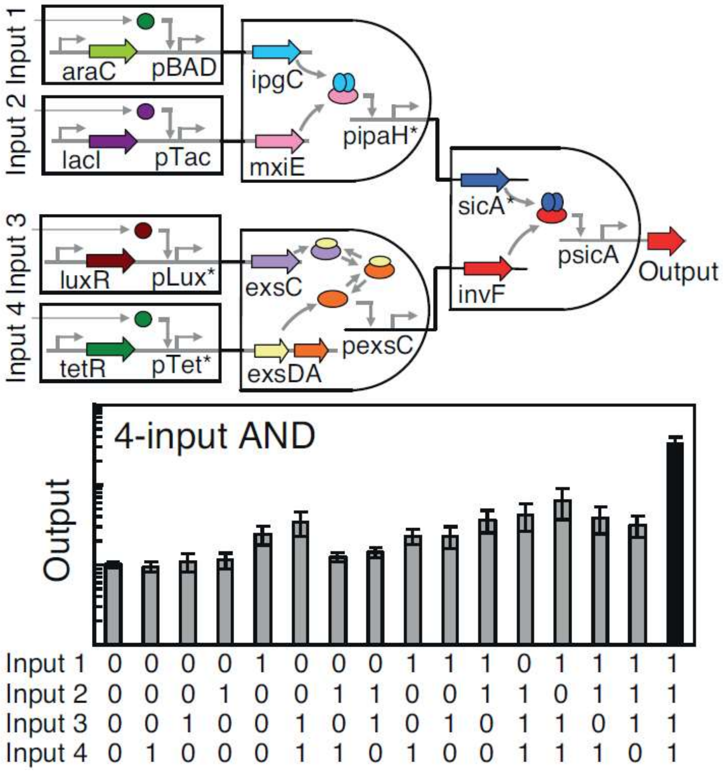
Manish Kushwaha
MICALIS Institute, INRA, Jouy-en-Josas

Typical Organization of a Synthetic Genetic Network

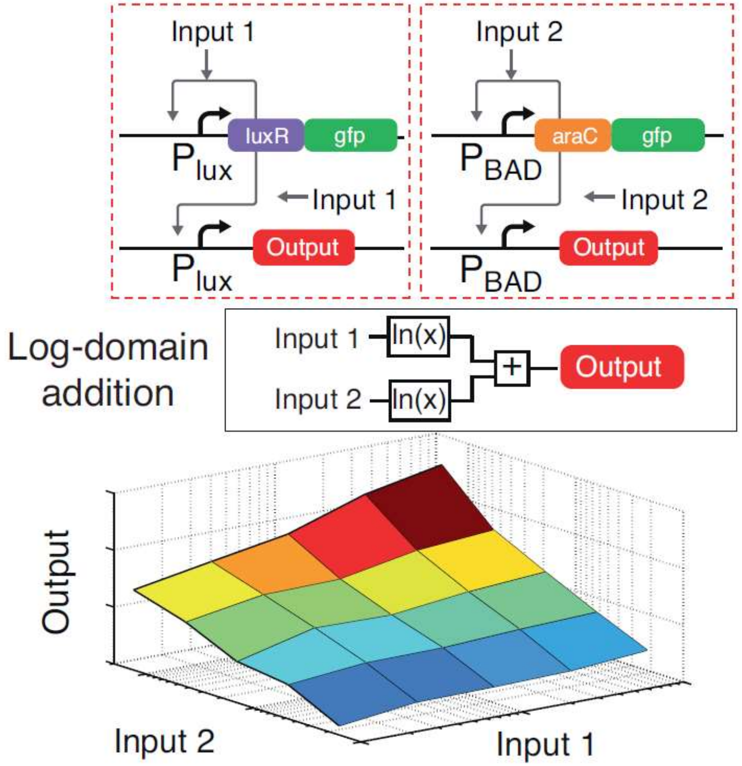


(Wang & Buck, 2012. Trends in Microbiology)

Biological computation in Synthetic Biology



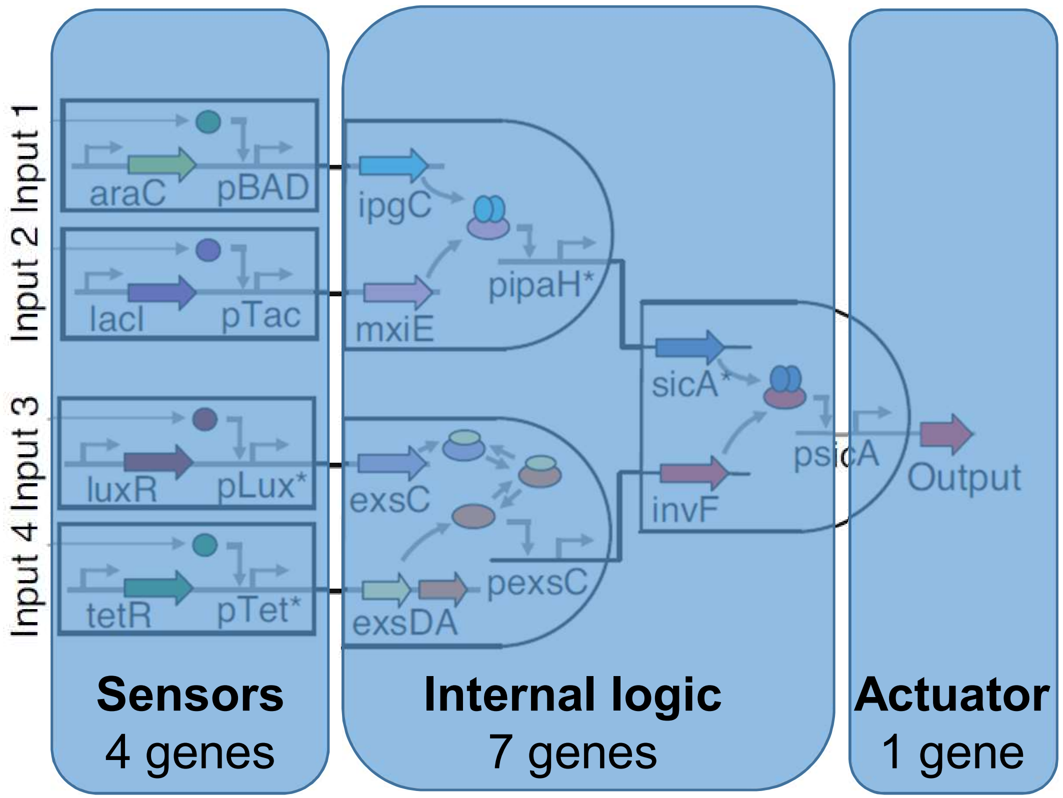
Digital computation
(Moon *et al.*, 2012. Nature.)

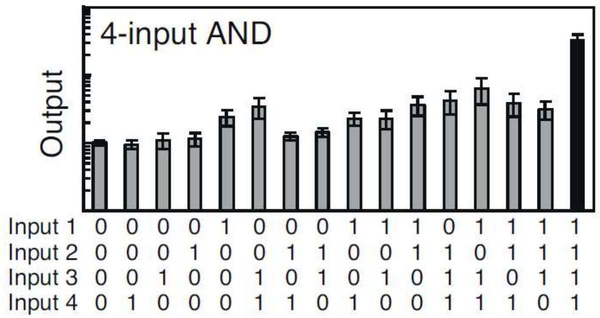


Analog computation
(Daniel *et al.*, 2013. Nature.)

- Synthetic genetic circuits can process digital and analog information
- Their construction requires the careful wiring of different genetic components

Connecting wires: Expenses add up

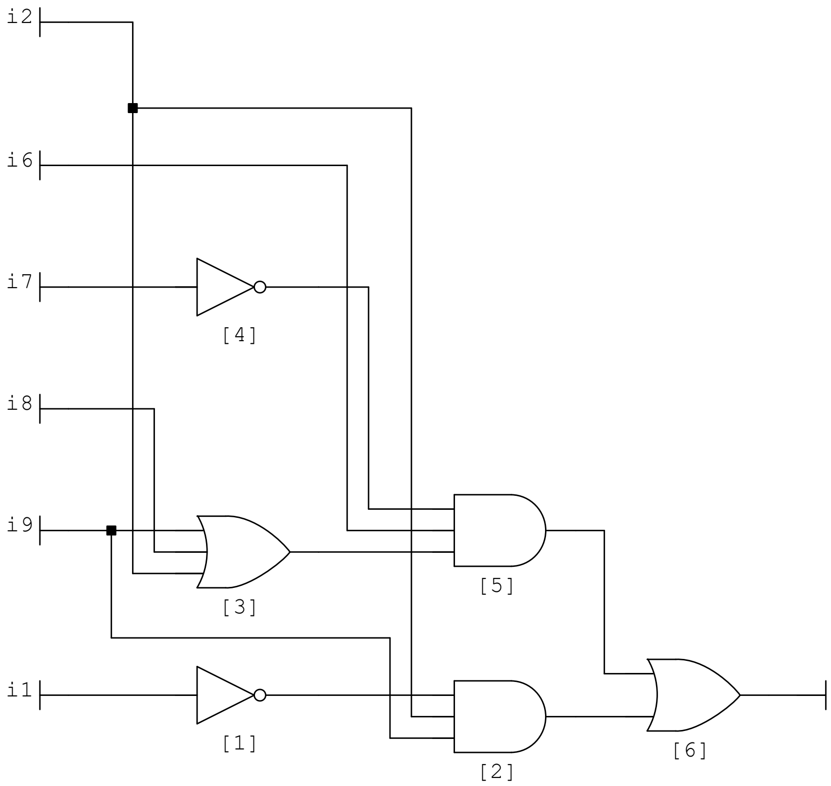




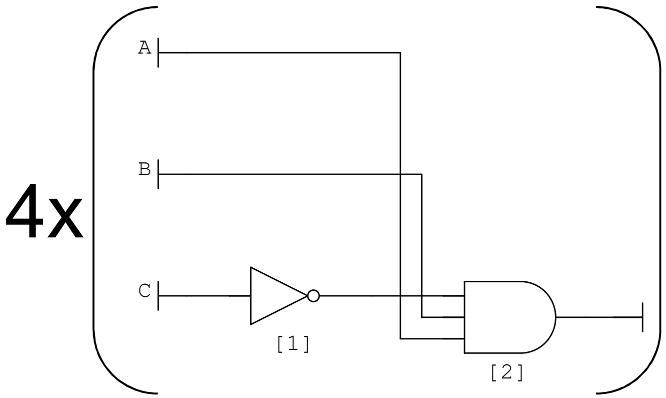
Digital computation
(Moon *et al.*, 2012. Nature.)

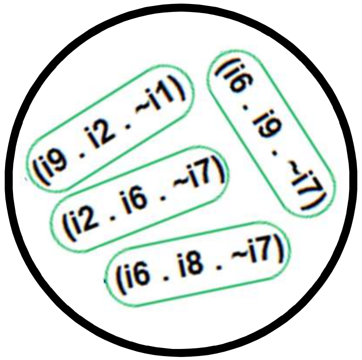
- Adding multiple “wires” is expensive for the cell
- Multiple internal circuit layers often dissipate the signal

Single versus multiple cells for Biological computing



~i7 . i6 . (i9 + i8 + i2) + (~i1 . i2 . i9)

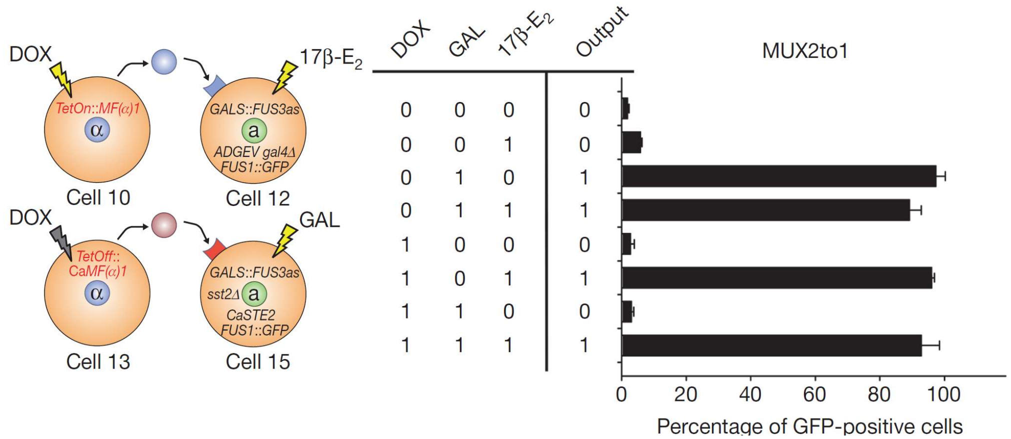




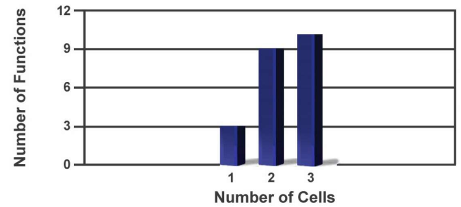
- “Distributed computing” could allow decomposition of a complex problem into multiple smaller parts that can be solved by different computers

(Kushwaha, unpublished)

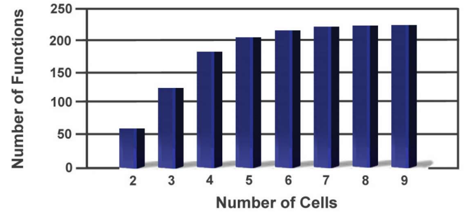
Distributed Computing



2-input

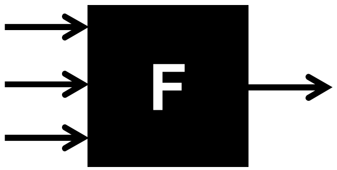


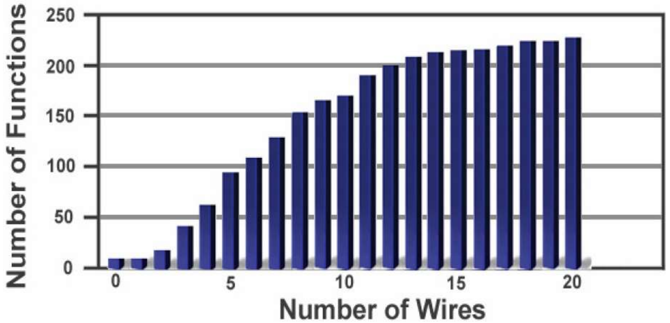
3-input



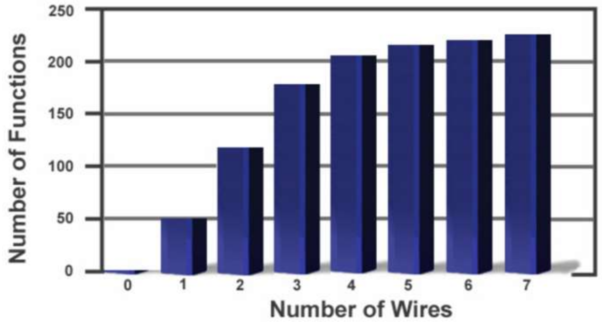
(Regot *et al.*, Nature 2011)

3-input NOR only logic





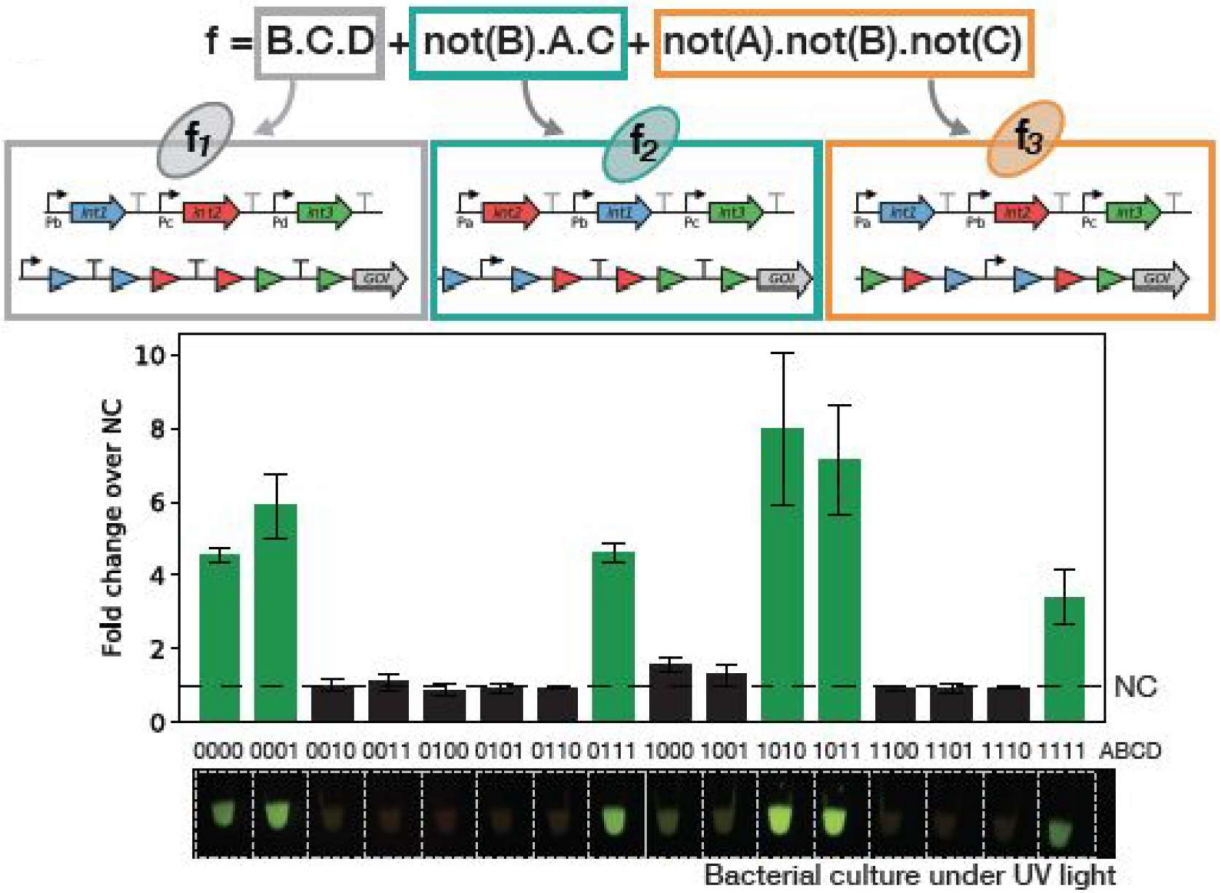
Single Cell



Distributed

- As circuit complexity increases, the number of genetic parts and their connecting “wires” increases rapidly
- Distributed computing significantly **reduces wiring requirements** and enables **re-use of sub-circuits**

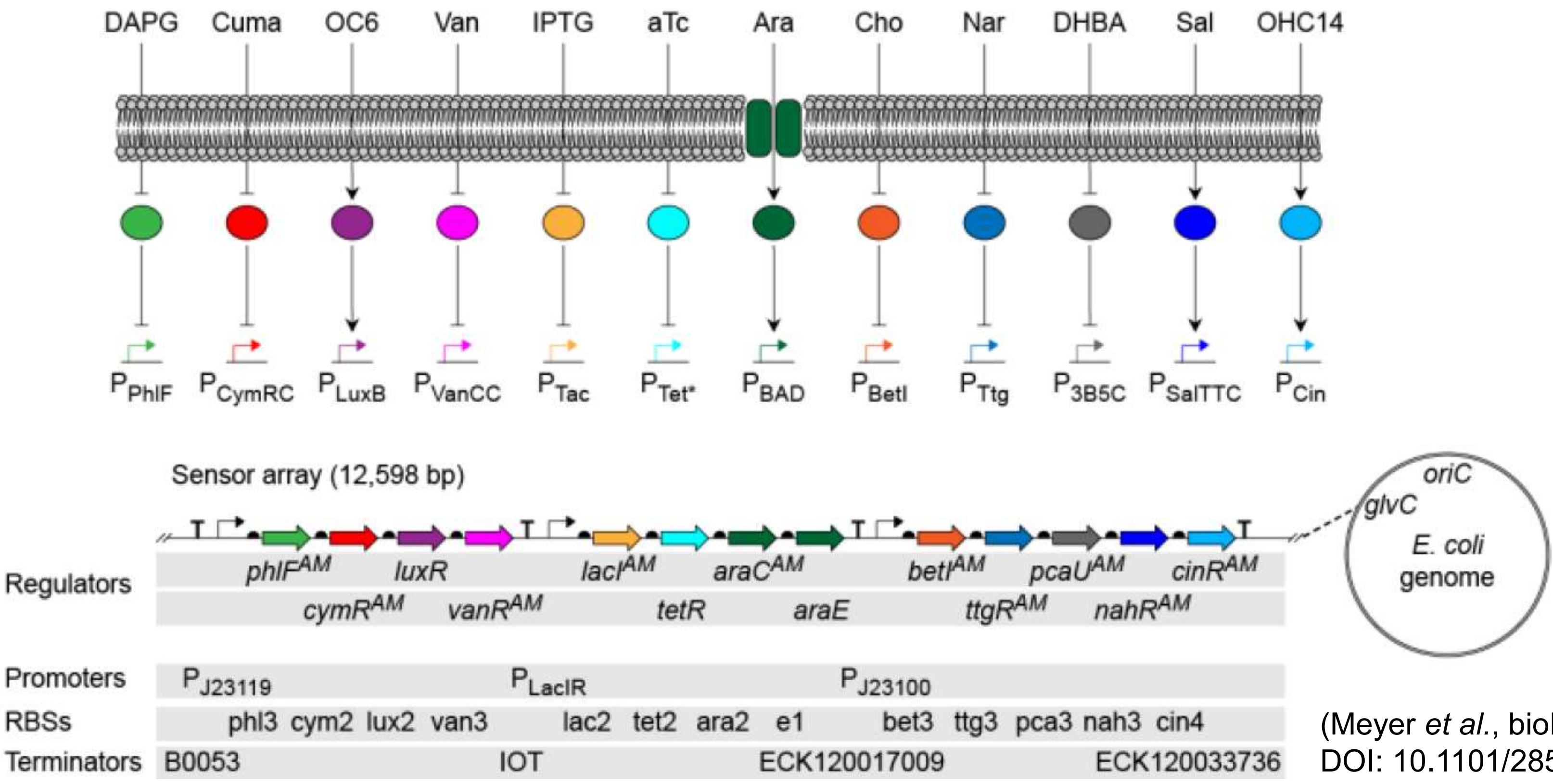
More Multicellular Computing



(Guiziou *et al.*, bioRxiv 2018)
DOI: 10.1101/390823

- Combining recombinase “state machine” logic with multicellular computing

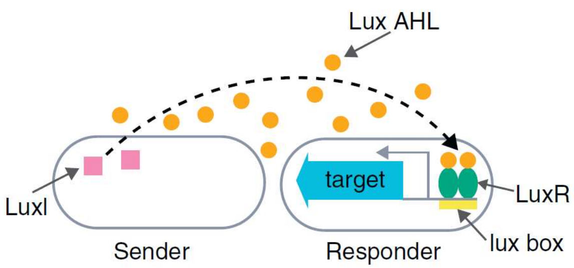
More Internal Wires



(Meyer *et al.*, bioRxiv 2018)
DOI: 10.1101/285866

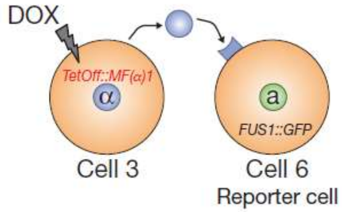
- Recent work has built strains with 12 different “sensor” modules

Limited external wires



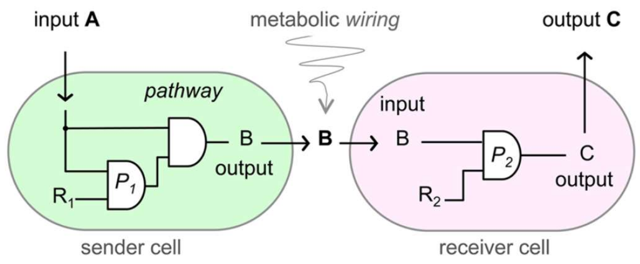
Quorum sensing molecules

(Macia *et al.*, Trends in Biotech. 2012)



Hormone molecules

(Regot *et al.*, Nature 2011)



Metabolites

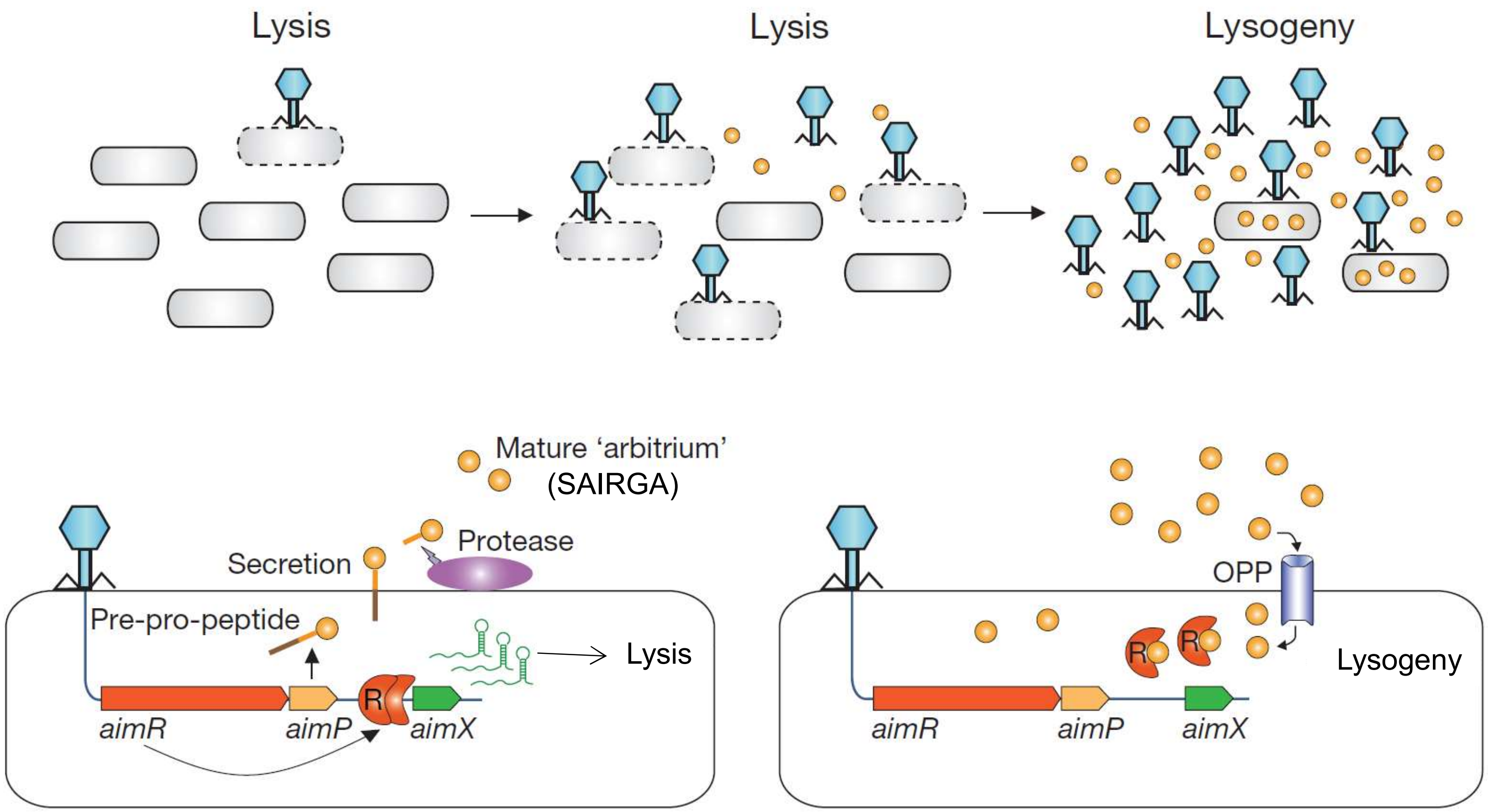
(Silva-Rocha *et al.*, ACS SynBio. 2013)

- A limited set of orthogonal external wires exist for cell-to-cell communication

Part-1:
Bacteriophage-derived signals as External Wires

Small Signaling Peptides

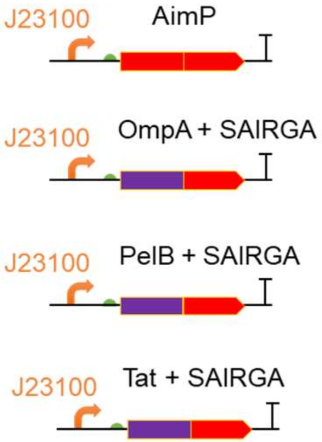
Phage Quorum sensing system



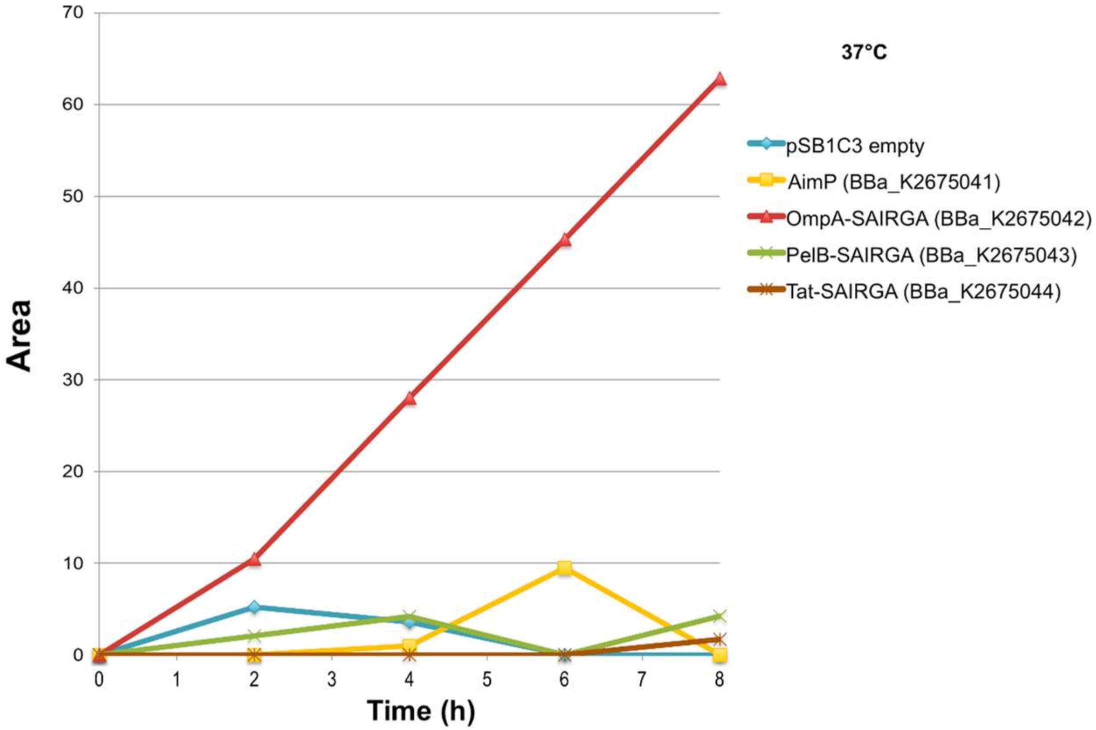
(Erez *et al.*, Nature 2017)

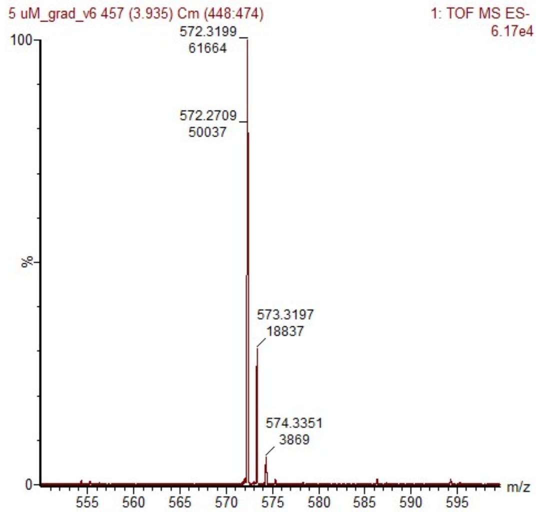
- Quorum sensing system of *Bacillus* phage phiT3 uses a hexapeptide as signaling molecule

Re-engineering peptide secretion



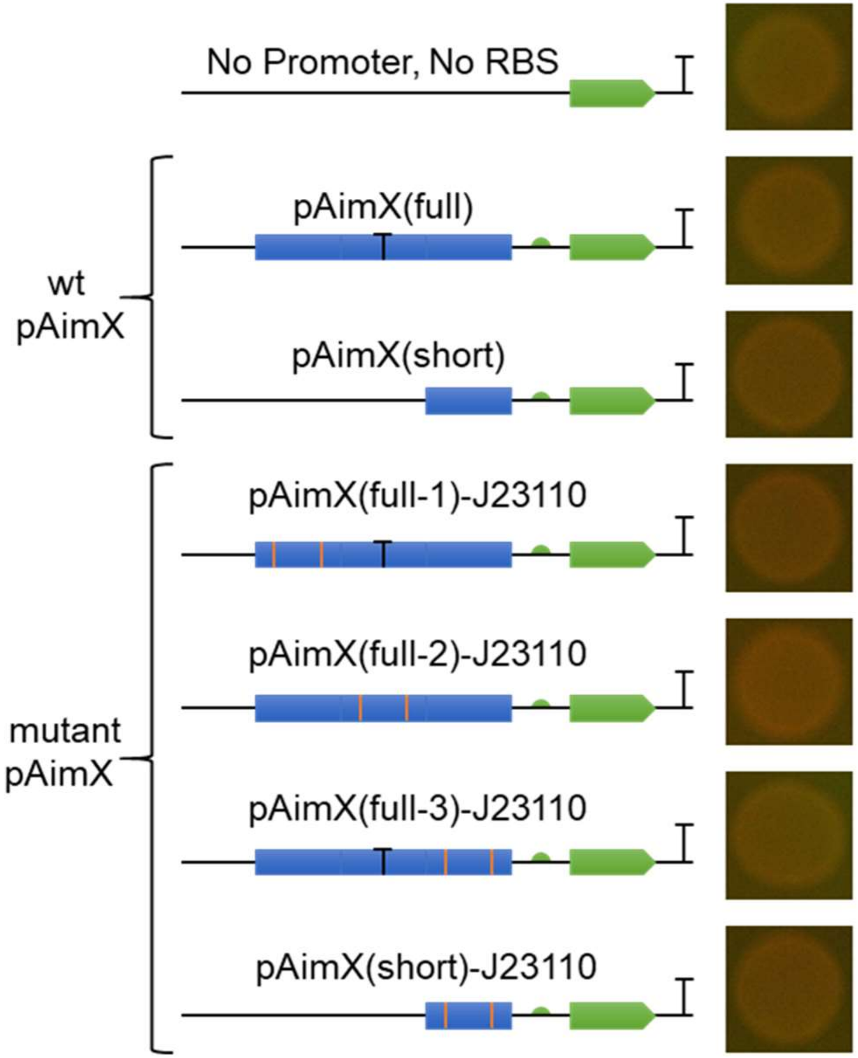
- While one system was characterized, genomic searches revealed at least 17 different peptide signals and their receptors
- This repertoire can be expanded by directed evolution of the peptide-receptor pair
- We decided to adapt this system for peptide secretion in *E. coli*





(iGEM Evry Paris-Saclay 2018. https://2018.igem.org/Team:Evry_Paris-Saclay)

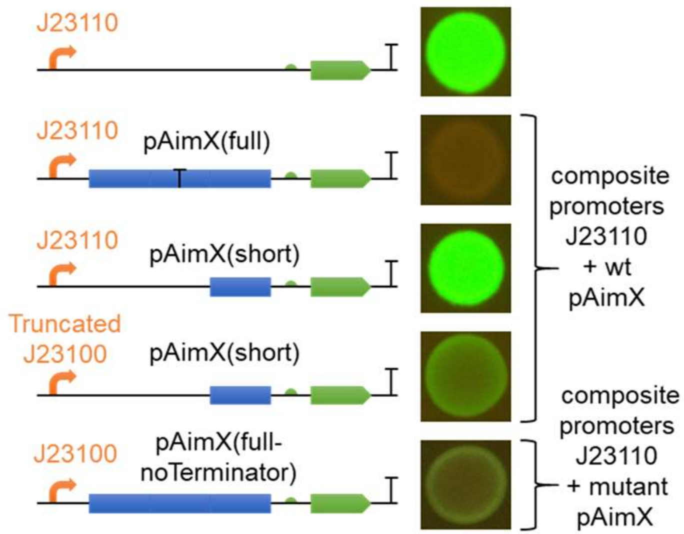
Re-engineering promoters

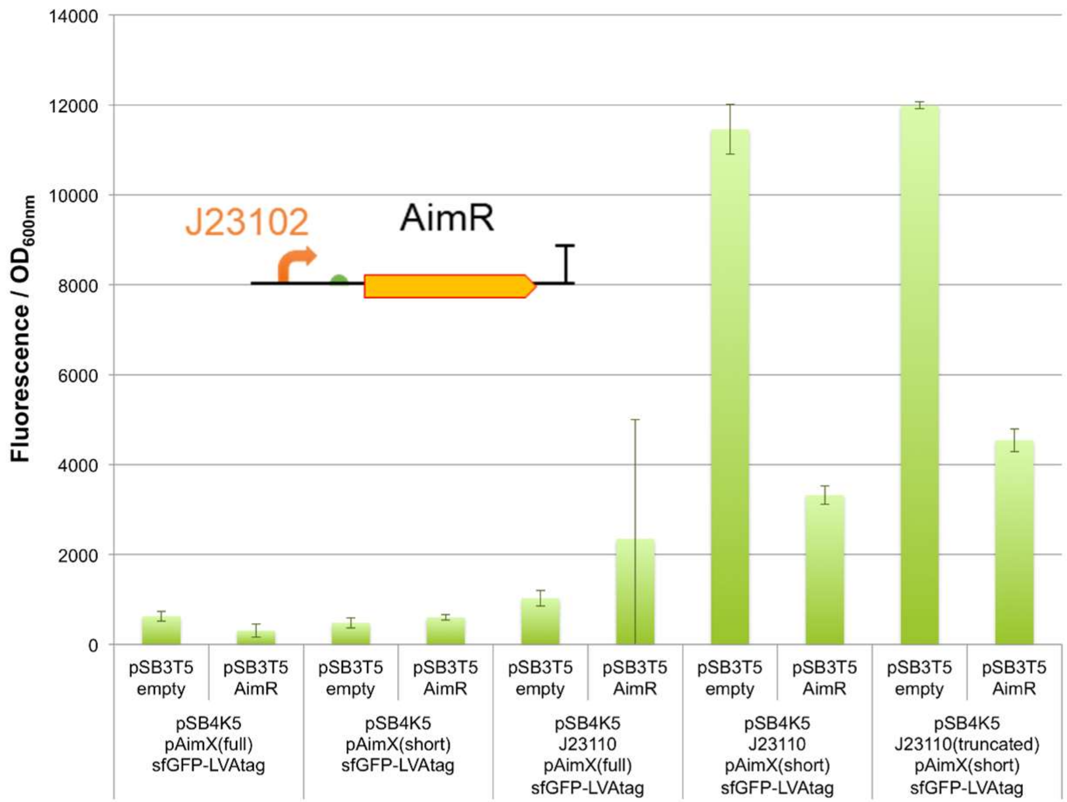


- *Bacillus* promoters were not functional in *E. coli*, with or without the AimR activator

(iGEM Evry Paris-Saclay 2018. https://2018.igem.org/Team:Evry_Paris-Saclay)

Re-engineering promoters





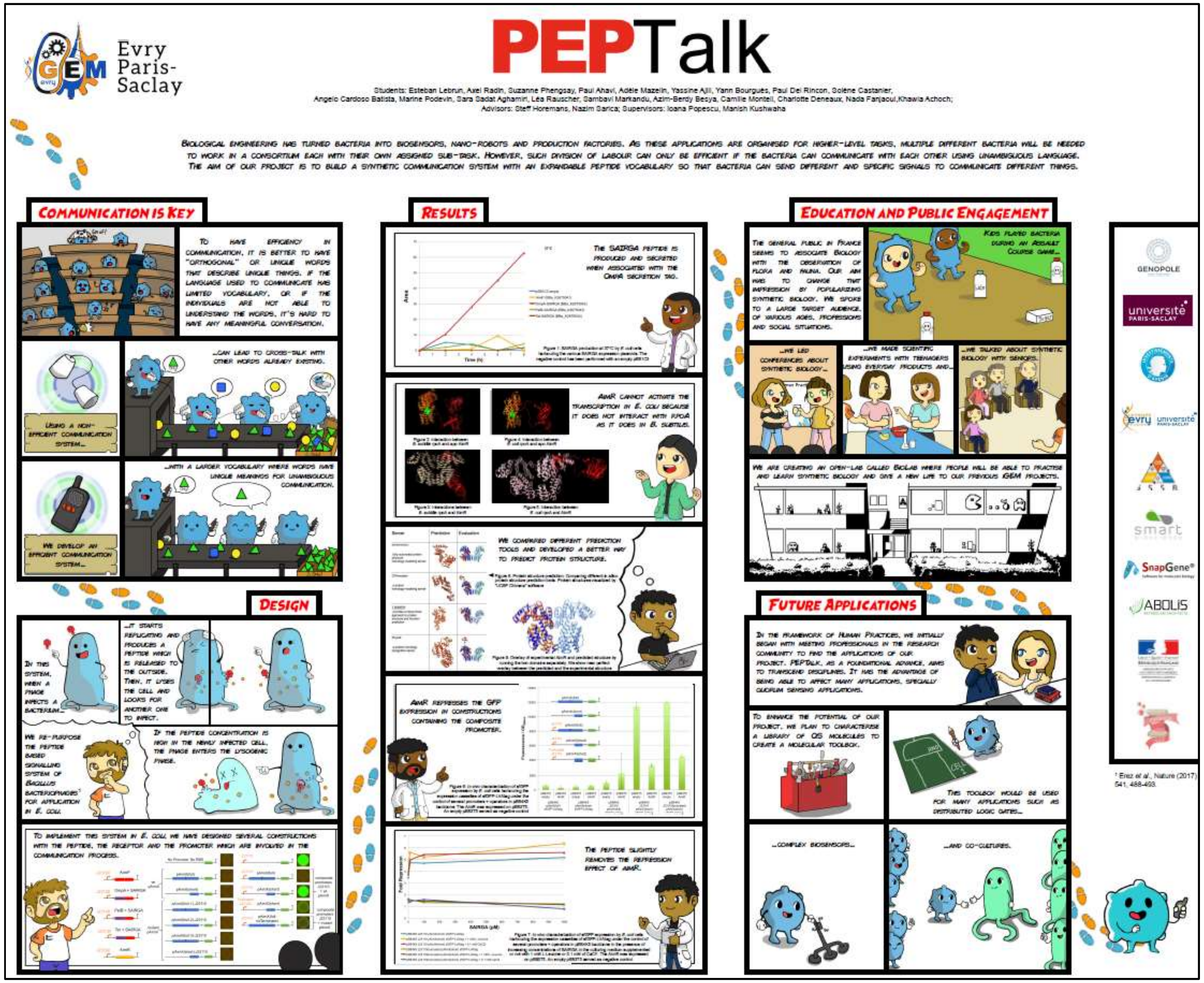
- So, activatable promoters were re-engineered as repressible promoters in *E. coli*

(iGEM Evry Paris-Saclay 2018. https://2018.igem.org/Team:Evry_Paris-Saclay)

Presented at the Giant Jamboree

Evry Paris-Saclay iGEM

Boston
October 24-28th 2018



(iGEM Evry Paris-Saclay 2018. https://2018.igem.org/Team:Evry_Paris-Saclay)

Next Steps

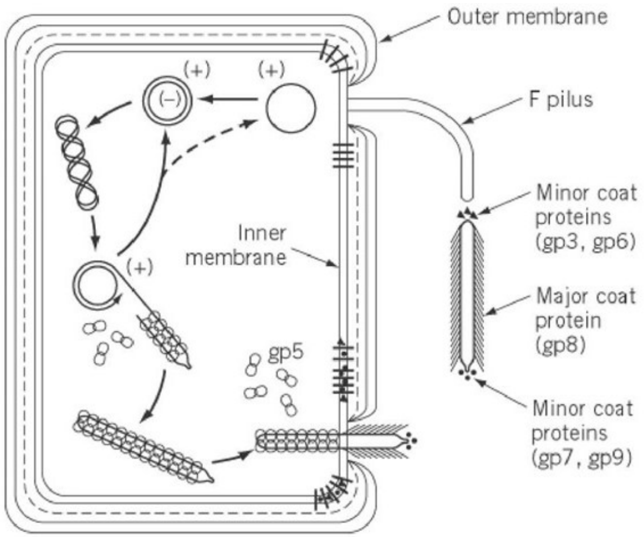
1) Test the effect of peptide on the repressible promoters
2) Test the other 16 peptide-receptor pairs similarly
3) Perform directed evolution of the peptide-receptor pair to expand repertoire

(iGEM Evry Paris-Saclay 2018.
https://2018.igem.org/Team:Evry_Paris-Saclay)

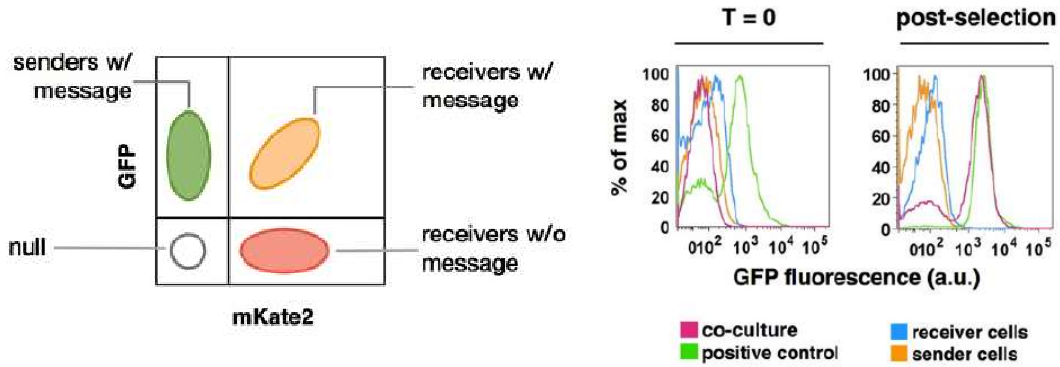
Part-2:
Bacteriophage-derived signals as External Wires

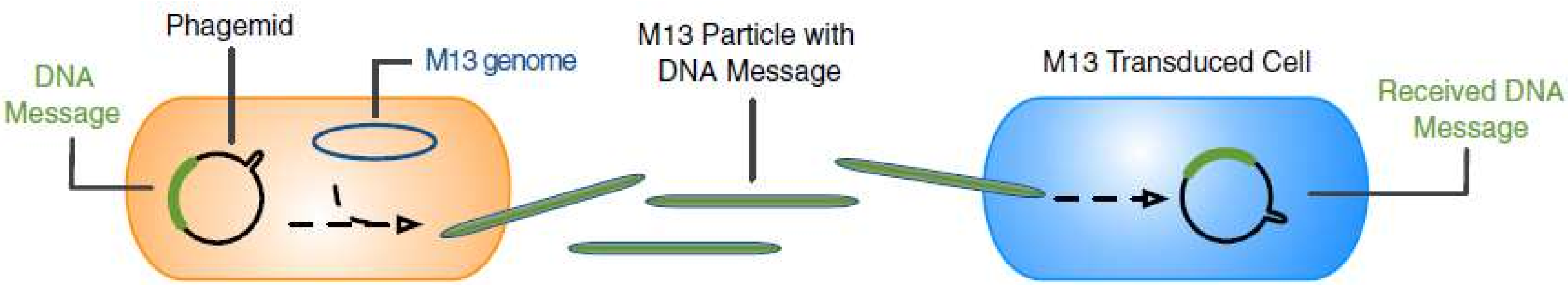
Packaged DNA

Engineering Filamentous bacteriophages for messaging



- Filamentous phages are non-lytic phages that reproduce in bacterial cells





(Ortiz & Endy. J. Biol. Engg. 2012)

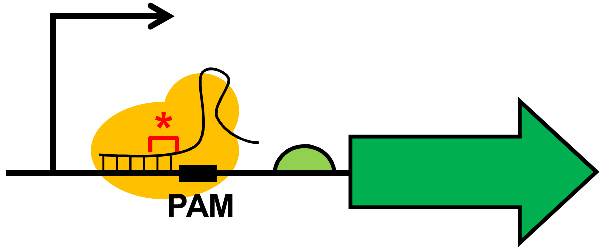
- They can be engineered to send “phagemid” messages between cells
- The Sender cells have the secretion machinery, while the Receiver cells have the surface receptors

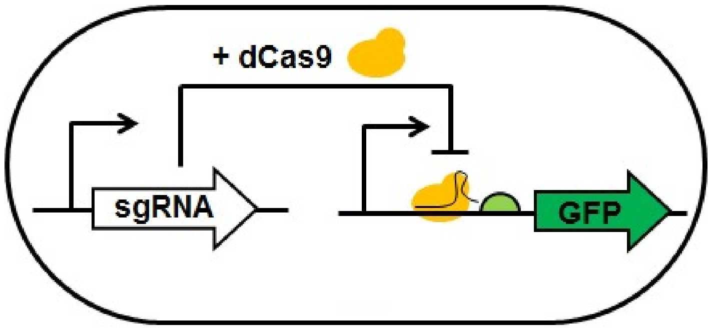
Orthogonal RNA signals encoded in phagemid particles

- dCas9 protein is an RNA-programmable repressor
- Computational design was used to generate a panel of orthogonal gRNAs
- Orthogonality was experimentally tested

sgRNA

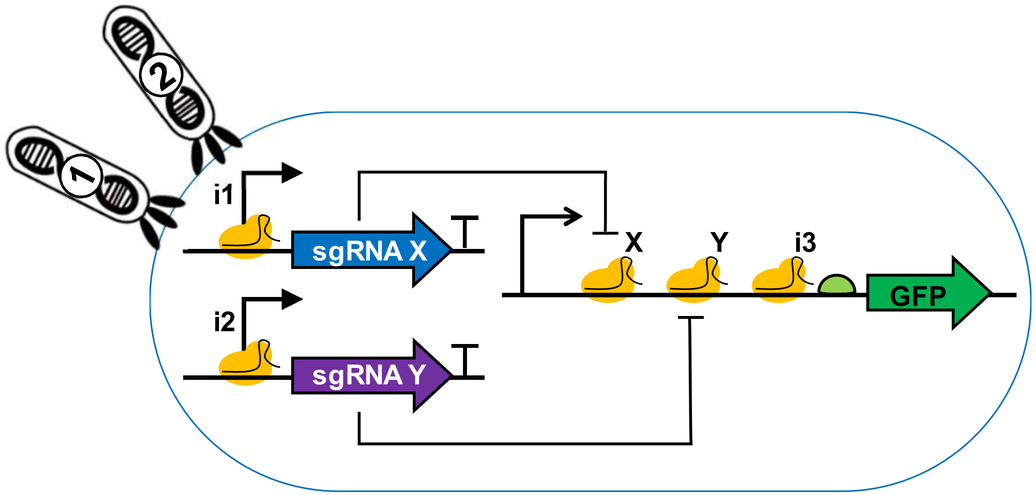
GFP promoter	1	2	3	4	5	6	7	8	9	10	11	12
1	0.03	0.61	0.87	0.60	0.68	0.73	0.52	0.71	0.65	0.78	0.62	1.00
2	0.63	0.16	0.73	0.64	0.63	0.72	0.80	0.78	0.70	0.63	0.52	0.56
3	0.80	0.61	0.09	1.00	0.66	0.87	0.99	0.80	0.78	0.87	0.94	0.94
4	0.72	0.69	0.74	0.09	0.75	1.00	0.78	0.91	0.89	0.99	0.60	0.76
5	0.95	0.82	1.00	0.67	0.01	0.35	0.61	0.59	0.82	0.57	0.81	0.83
6	0.74	0.63	0.88	0.54	0.72	0.08	0.84	0.63	0.45	0.85	0.76	0.57
7	0.85	0.55	0.91	0.46	0.81	0.91	0.07	0.64	0.55	1.00	0.67	0.77
8	0.81	0.58	0.71	0.74	0.77	0.77	0.74	0.05	0.63	0.62	0.75	0.59
9	1.00	1.00	0.99	0.29	1.00	0.72	0.73	0.77	0.02	0.57	0.60	0.81
10	0.91	0.63	0.85	0.92	0.75	0.82	1.00	1.00	1.00	0.09	1.00	0.83
11	0.80	0.58	0.74	0.37	0.98	0.60	0.92	0.72	0.63	0.59	0.15	0.80
12	0.80	0.84	0.96	0.64	0.89	0.81	0.92	0.69	0.76	0.95	0.80	0.08



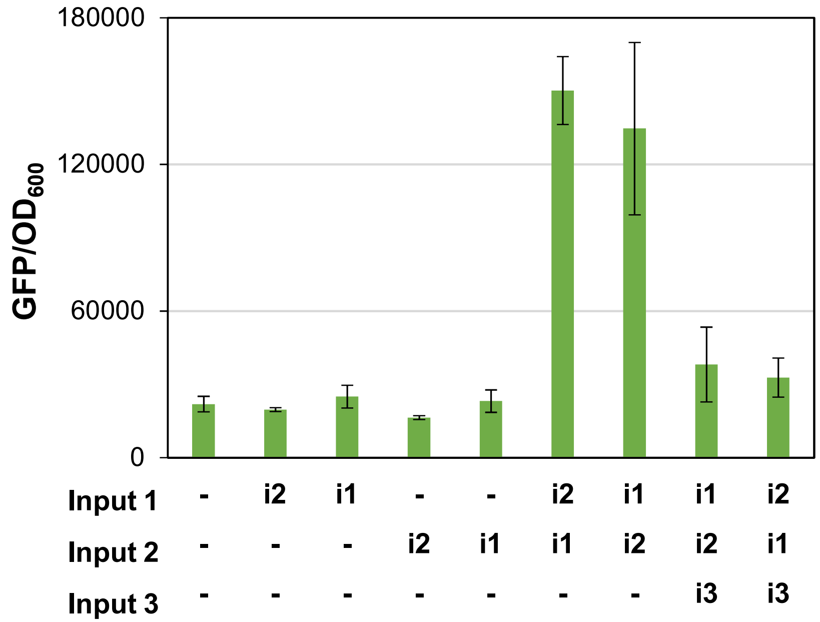


(Kushwaha, unpublished)

Logic gates based on phage-delivered signals



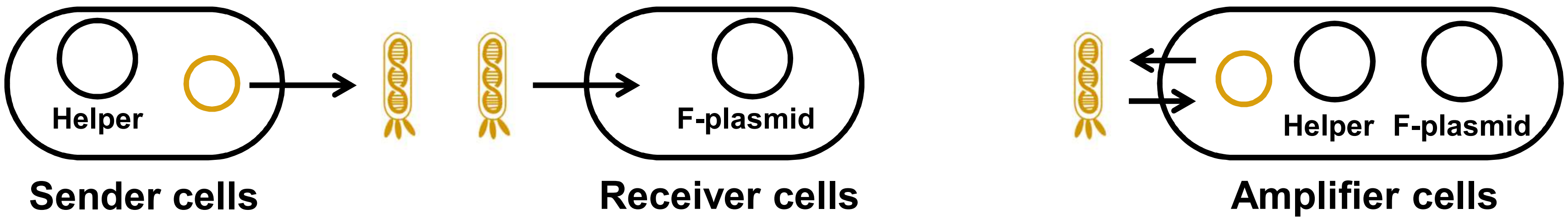
(i1 . i2 . ~i3)
= ~(~i1 + ~i2 + i3)
= ~(X + Y + i3)

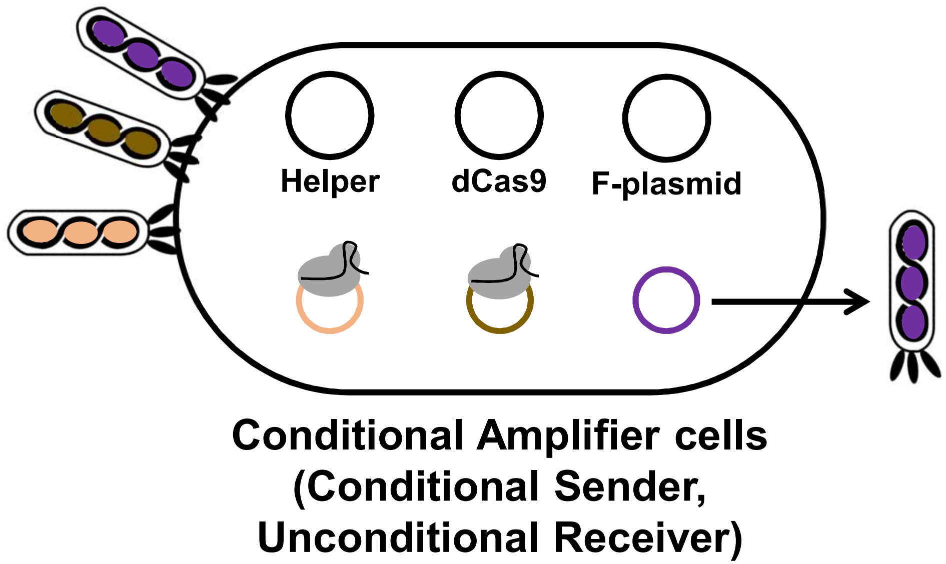


- de Morgan´s rule allows re-coding of AND-AND-NOT logic
- Two internal gRNAs (X and Y) were used as inverters (NOT gates)
- The AND-AND-NOT gate was implemented using a gRNA-only strategy

(Kushwaha, unpublished)

Conditional amplification of phagemid signals

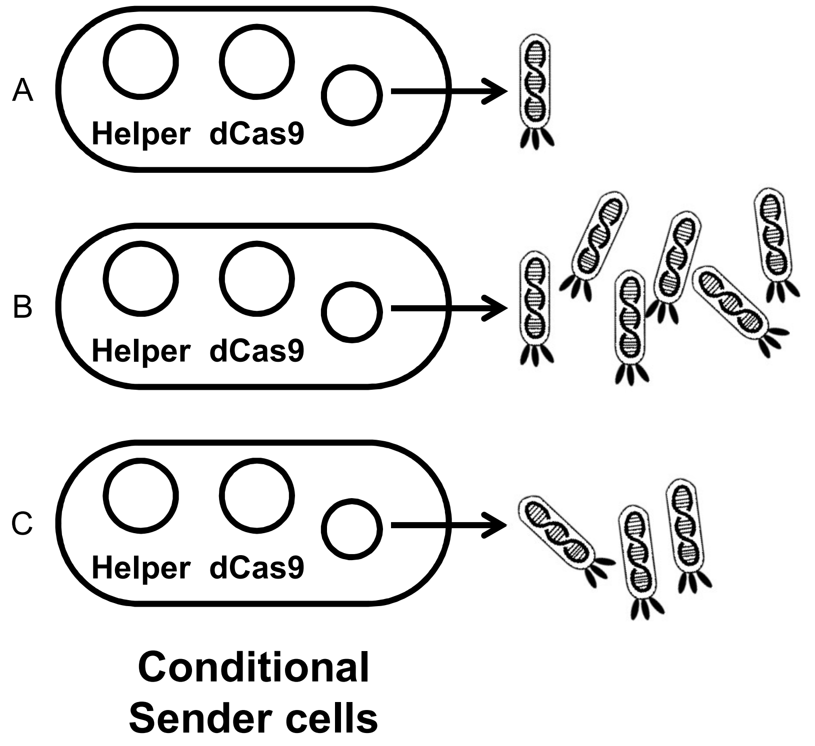


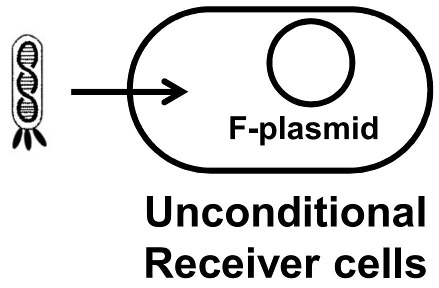


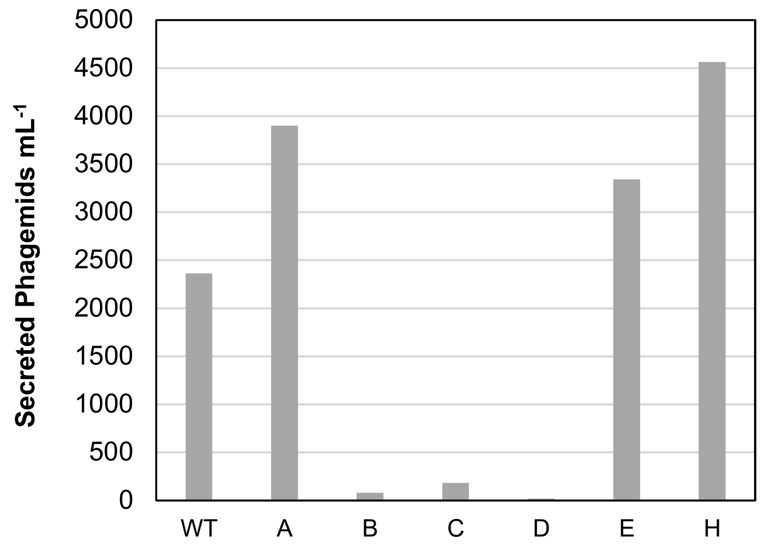
- A conditional amplification system is designed to prevent packaging of some phagemids, while allowing packaging for others

(Kushwaha, unpublished)

Conditional amplification of phagemid signals







- Conditional Senders can select which phagemids to package for secretion

(Kushwaha, unpublished)

Next Steps

1) Phagemids B, C and D will be tested for Conditional amplification

2) Cell-to-cell signaling cascades will be tested for multiple layers

3) The cascades will be coupled with metabolic pathways to optimize production

Acknowledgments

Alfonso Jaramillo
Warwick Integrative Synthetic Biology Center (WISB), UK

Jean-Loup Faulon
MICALIS Institute, INRA, Jouy-en-Josas, France

Vijai Singh
Université Paris-Saclay, Genopole, France

Ioana Popescu
Université Paris-Saclay, Genopole, France

Amir Pandi

Thomas Duigou
Olivier Borkowski
Angelo Batista
Paul Soudier
Mathilde Koch
Melchior Du-Lac
Alexandra Zaharia