Iterative plug-and-play methodology for constructing and modifying synthetic gene networks

Kevin D Litcofsky, Raffi B Afeyan, Russell J Krom, Ahmad S Khalil & James J Collins

Presented by Divya Chhabra

May 1, 2013

20.385

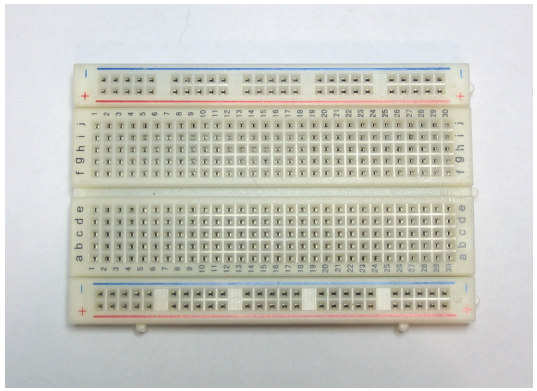
Nat Methods(2012) 9(11):1077-80. doi: 10.1038/nmeth.2205

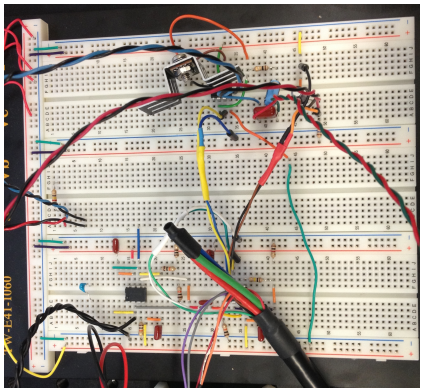
The Need

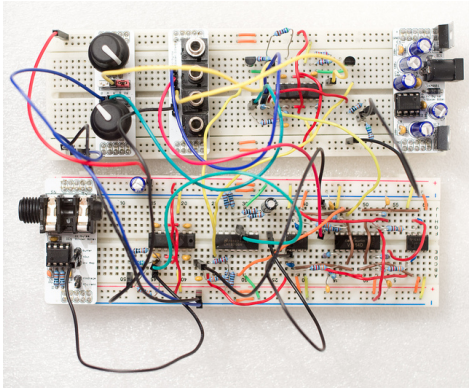
- Current approach is unwieldy
  - *ad hoc* construction and modification
- Increasing scale and complexity in synthetic gene networks
- Desire to modify network after observing network performance

The Solution

Iterable plug-and-play approach to modifying gene networks

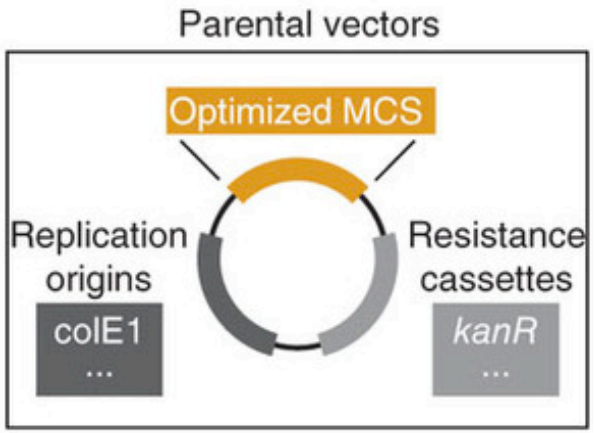






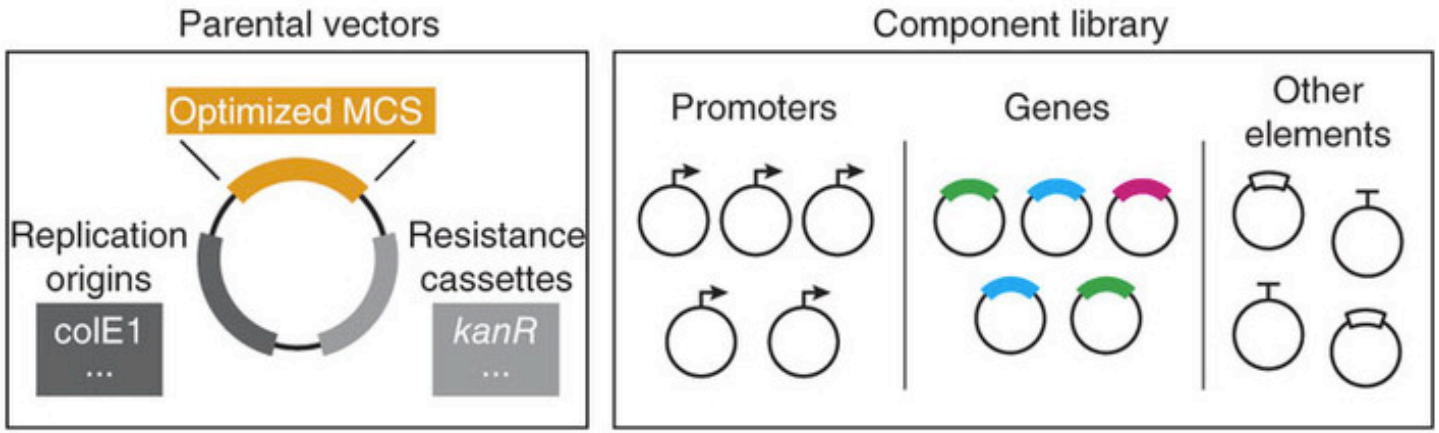
Scalable, Rapidly Changeable

Overview of Plug-and-Play Methodology



Type IIP restriction enzymes with restriction sites in MCS

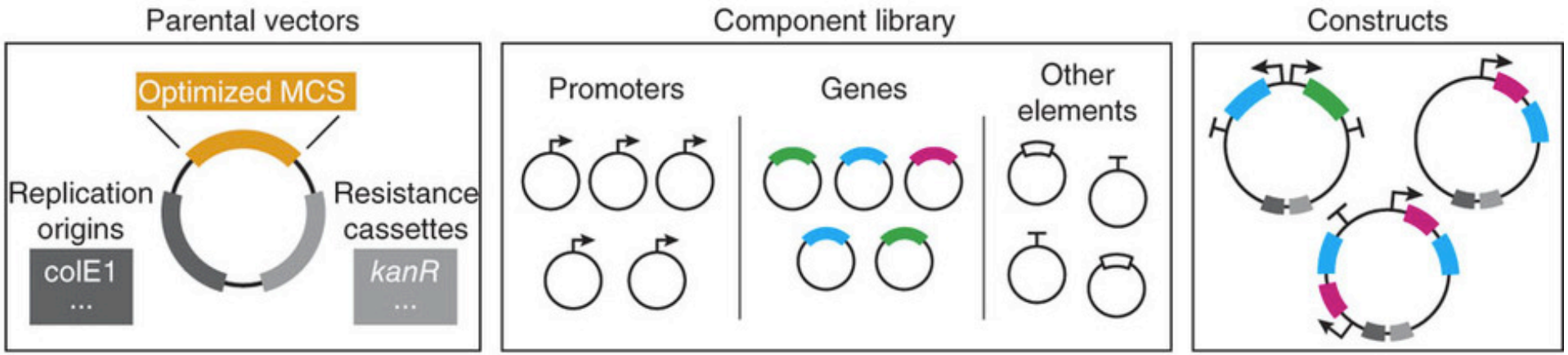
Overview of Plug-and-Play Methodology



Type IIP restriction enzymes with restriction sites in MCS

26 genetic components without restriction sites internally located

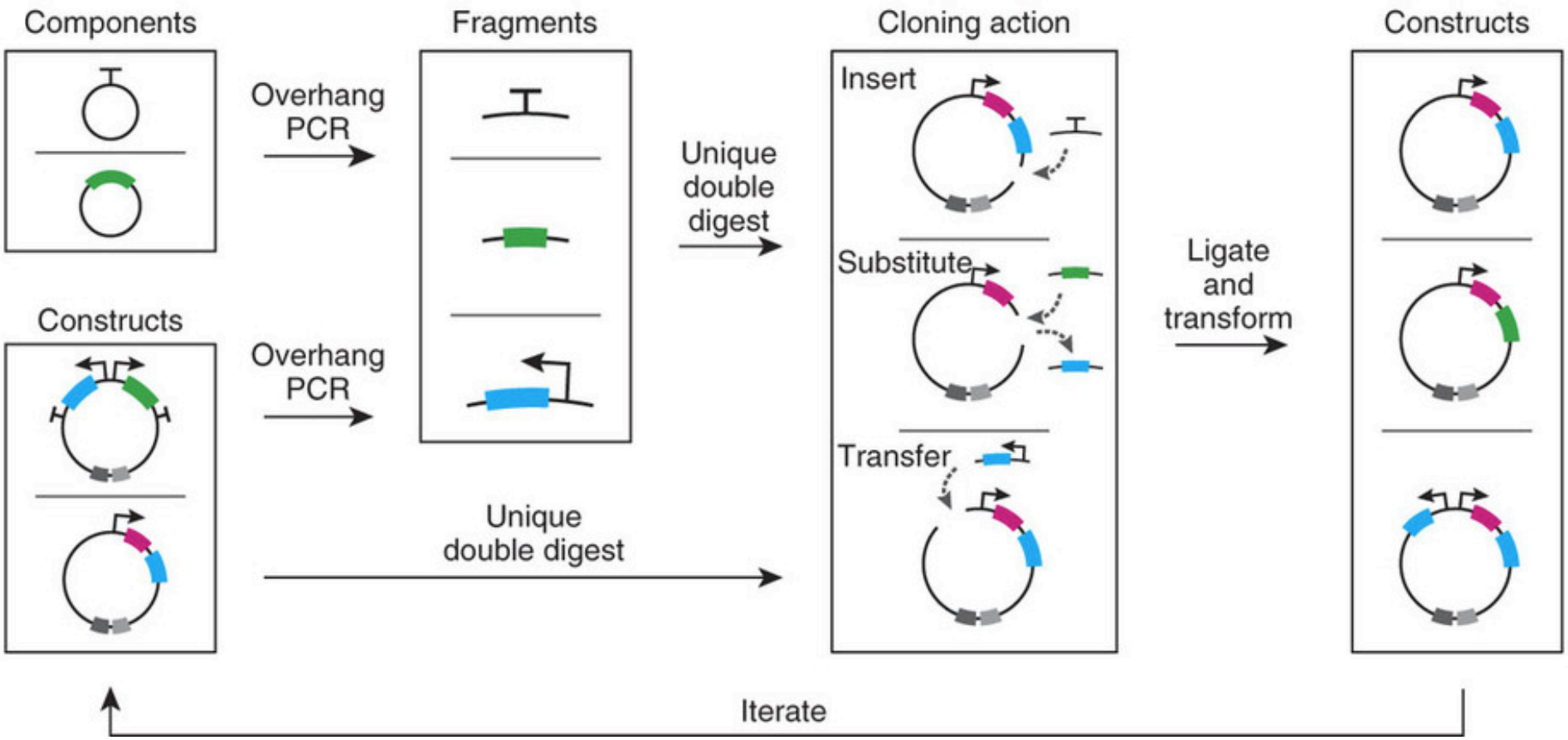
Overview of Plug-and-Play Methodology



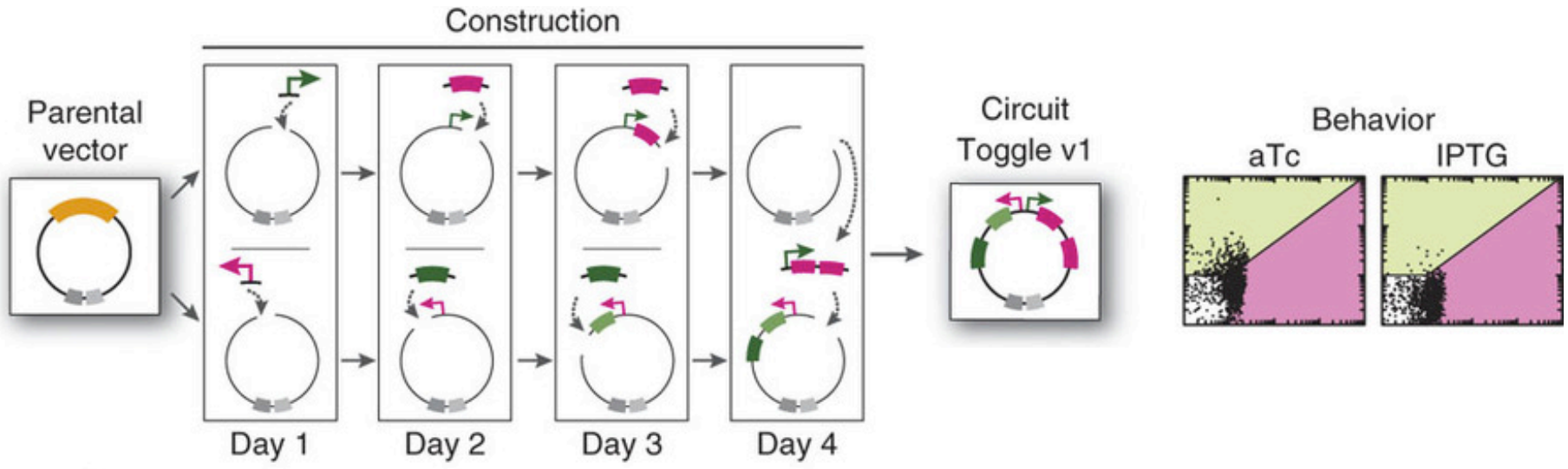
Type IIP restriction enzymes with restriction sites in MCS

26 genetic components without restriction sites internally located

Construction of Synthetic Gene Networks



Construction of bistable genetic toggle switch



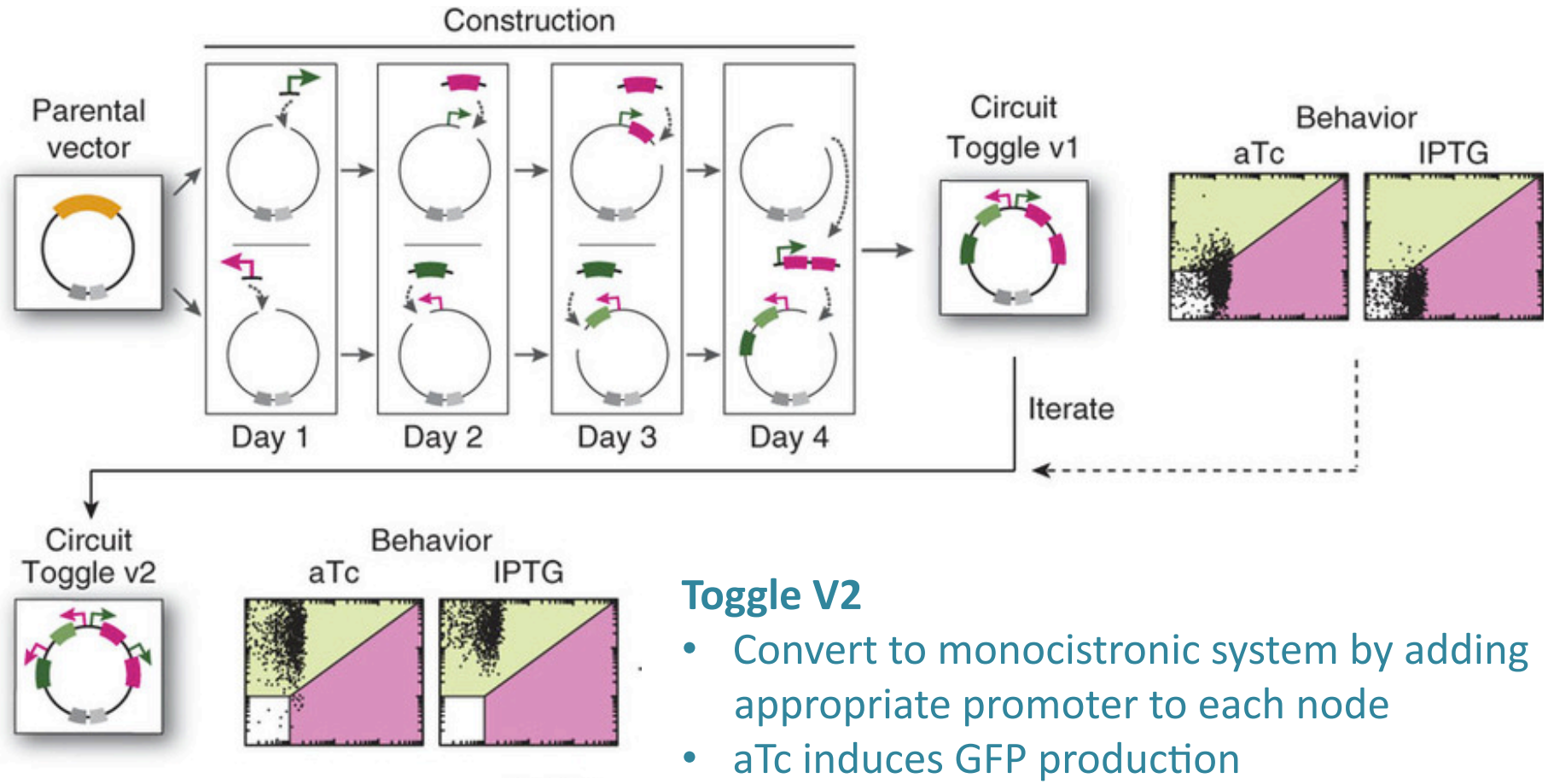
Goal

- aTc→ lacI and GFP
- IPTG→ tetR and mCherry

Toggle V1

- Bicistronic system
- Toggle v1 did not activate in response to either inducer

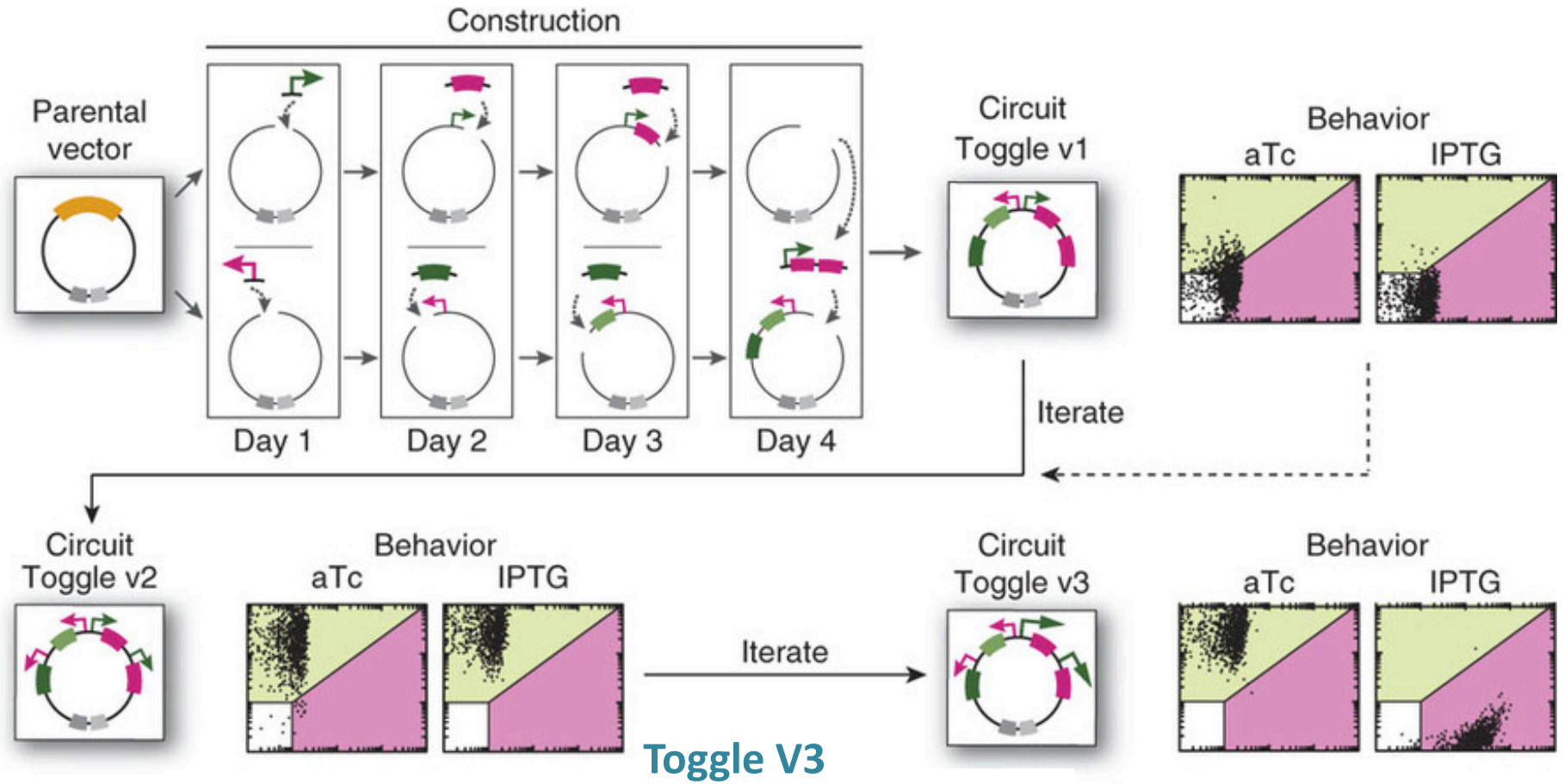
Construction of bistable genetic toggle switch



Toggle V2

- Convert to monocistronic system by adding appropriate promoter to each node
- aTc induces GFP production
- IPTG fails to induce mCherry production

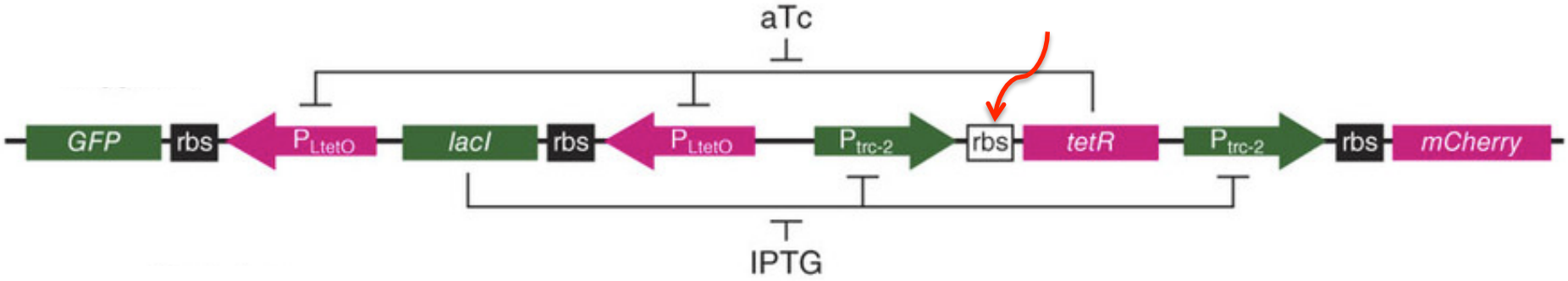
Construction of bistable genetic toggle switch



Toggle V3

- Use different promoters
- Unable to maintain GFP state in absence of aTc
- Bias towards high mCherry state

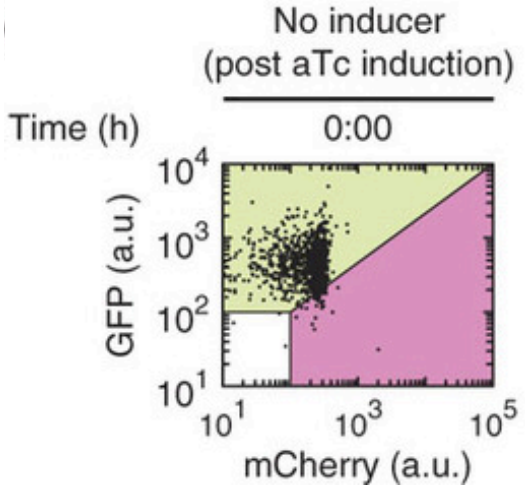
Toggle V4: Final Genetic Toggle Switch



Toggle V4

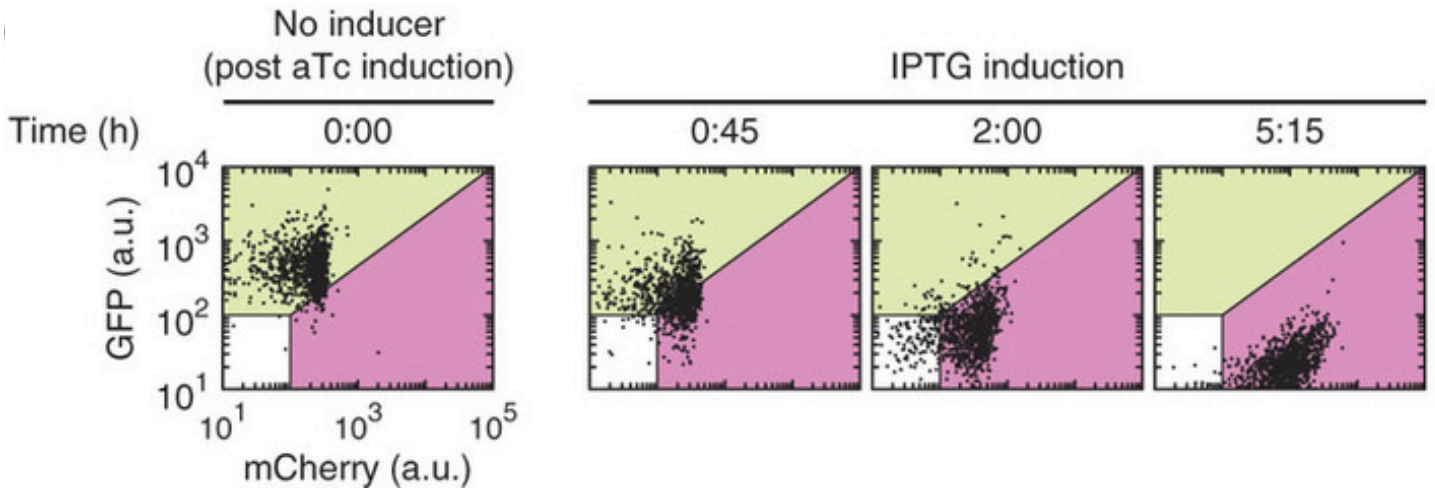
- Balance expression by random mutagenesis of Shine-Dalgarno sequence in rbs that controls *tetR* translation
- Addition of aTc results in high expression of *lacI* and *GFP*
- Addition of IPTG in high *tet*R and *mCherry*

Switching Between Switches



GFP state post aTc induction

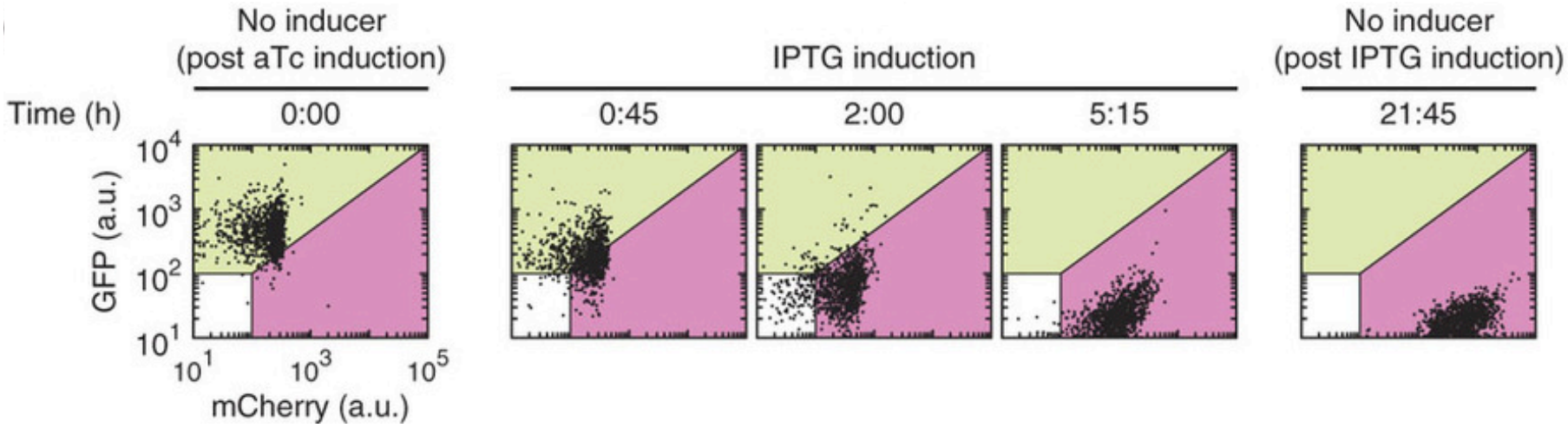
Switching Between Switches



GFP state post aTc induction

Addition of IPTG results in switching to mCherry state

Switching Between Switches

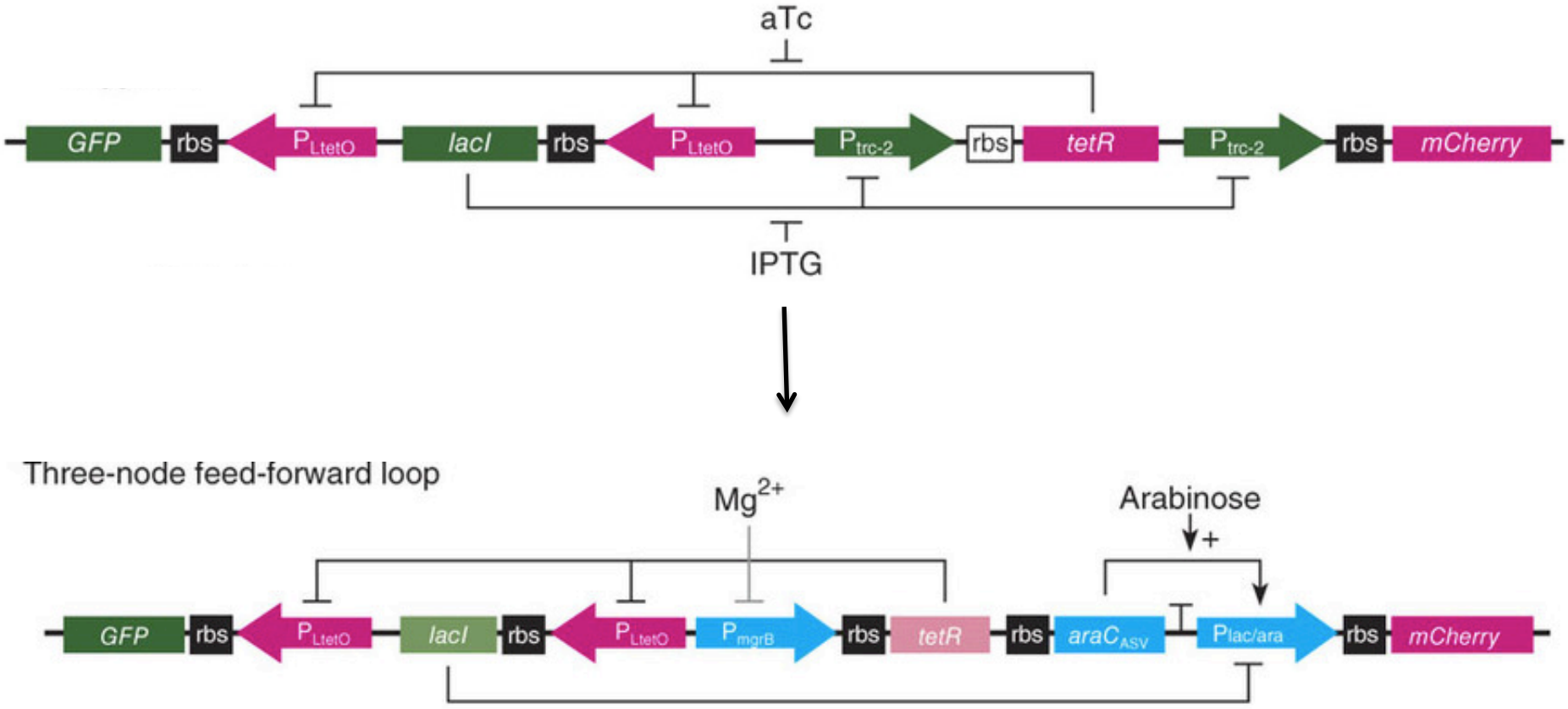


GFP state post aTc induction

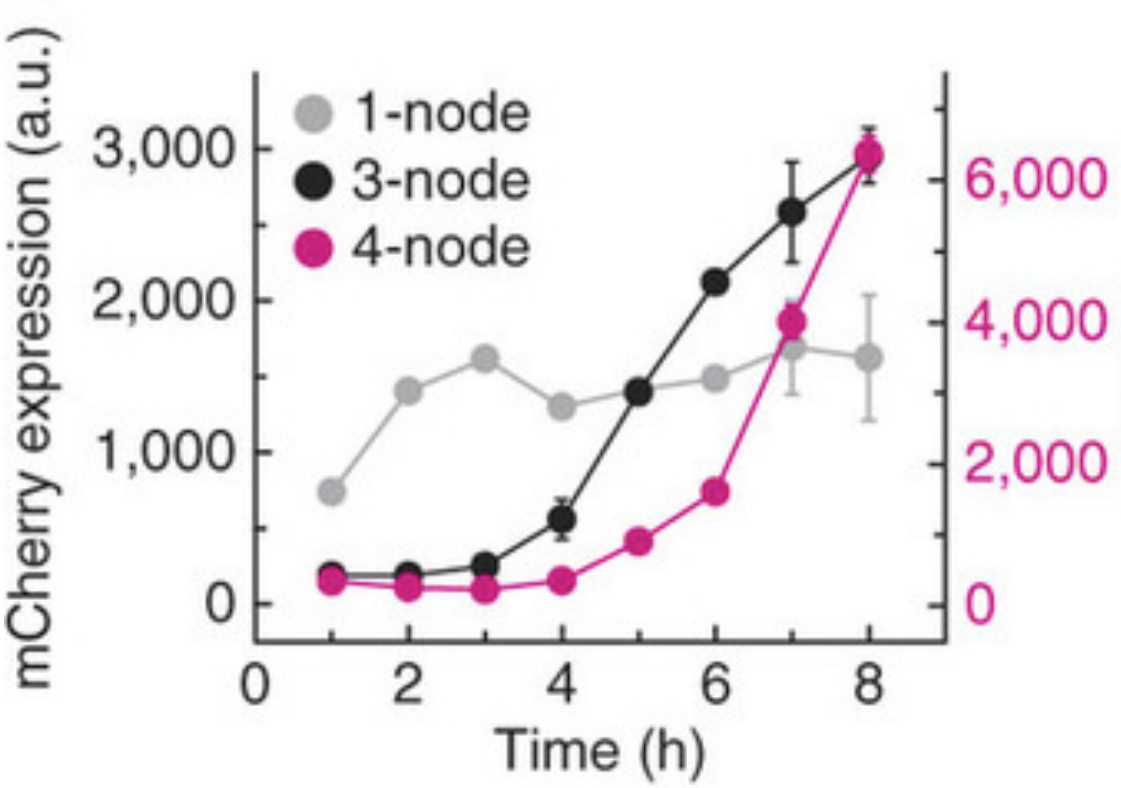
Addition of IPTG results in switching to mCherry state

Maintenance of mCherry state post IPTG removal

Using Toggle V4 as a Starting Point for Creation of Novel Gene Networks



Time Delay in Three and Four Node Feed Forward Loops



Assumptions

- Readout can always be easily detectable
- Speed of construction (as opposed to computational modeling) is desired
- Only way to speed construction is using plug-and-play
- Worthy investment to create a complex library
- Others can also build as rapidly as they did

Limitations

- Number of available, well-characterized genetic components
- Need to exclude restriction site sequences from genetic components
- Ability to translate to other chassis
- Ease of integration into DNA
- Currently only at plasmid-level
- Limited number of inducer molecules

Significance

- Proof of concept of using rapidly interchangeable parts in biology
- Methodology for systematically "troubleshooting" synthetic gene clusters
- Creation of a greater number of more complex building blocks
- Creation of time-delayed responses

Discussion Questions

- What are the implications of this approach?
- Is there a limit to the complexity that these gene networks can reach before becoming too complicated?
- What are the advantages of speeding up construction as opposed to modeling?
- Is there a need for this approach?