Reset - Workshop on microbial growth control and biotechnological applications 2017

Measuring and controlling the burden of synthetic gene expression in *E. coli*

Francesca Ceroni
Junior Research Fellow
Dept. of Chemical Engineering
Imperial College London

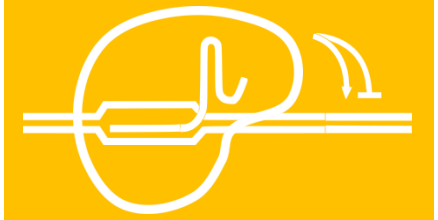
Cellular Burden

Understand it

Cellular Burden

Understand it

burden-based molecular feedback



Cellular Burden

Understand it

burden-based molecular feedback

to Overcome it

Cellular Burden

Understand it

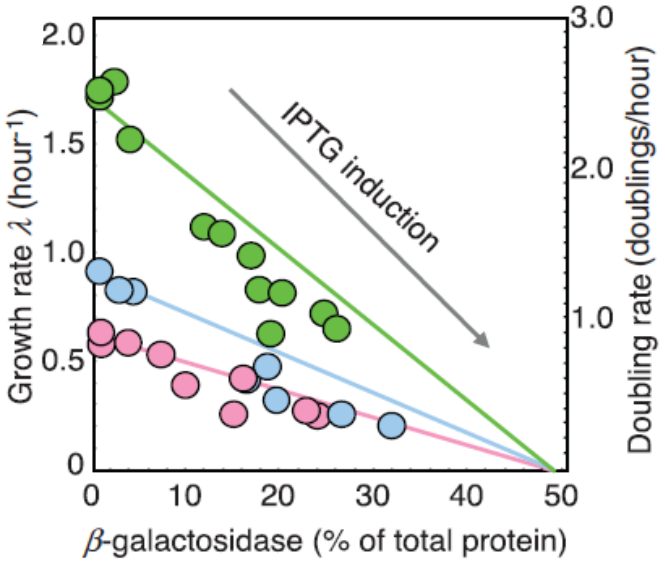
The “Burden problem”

Heterologous gene expression imposes a load to host cells leading to unanticipated effects

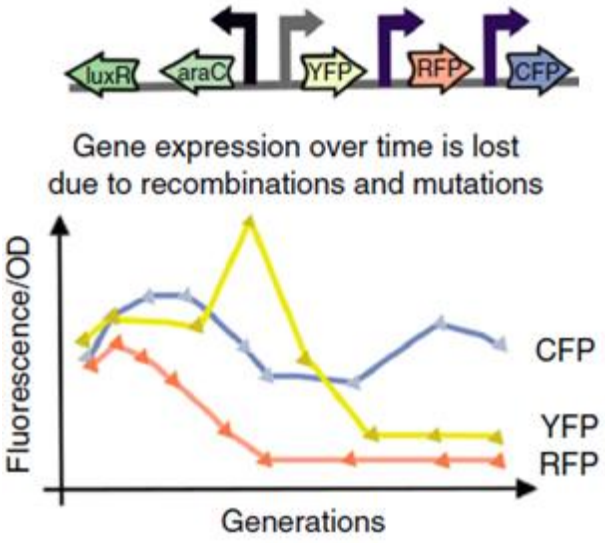
The “Burden problem”

Limitation in the predictability of the behavior of synthetic devices

Decreased growth



Escape mutants

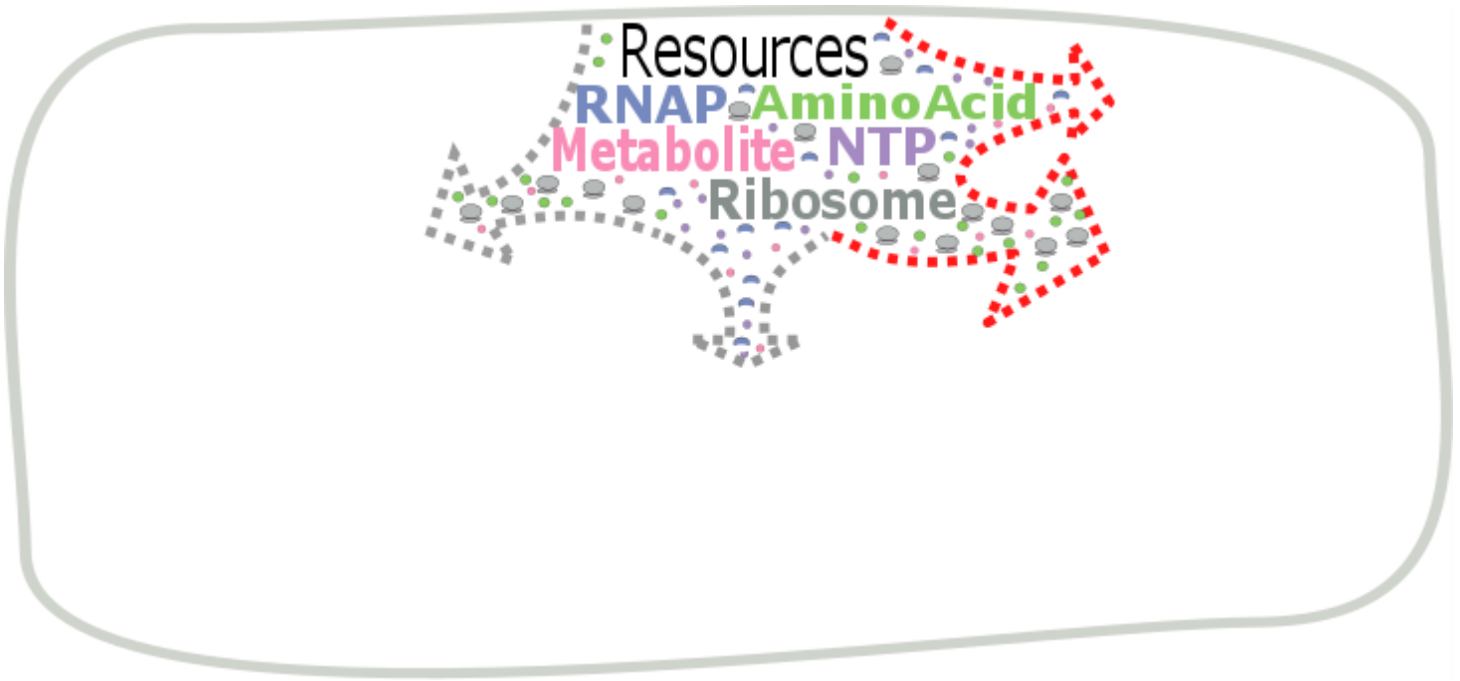


Scott *et al.* ***Science*** *2010*

Adapted from *Sleight et al.* ***ACS Synth Biology*** *2013*

Burden is caused by

competition for shared intracellular resources in the cell



Borkowski O, ***Ceroni F,*** *Stan GB, Ellis T.* ***Curr Opinion in Microbiology***

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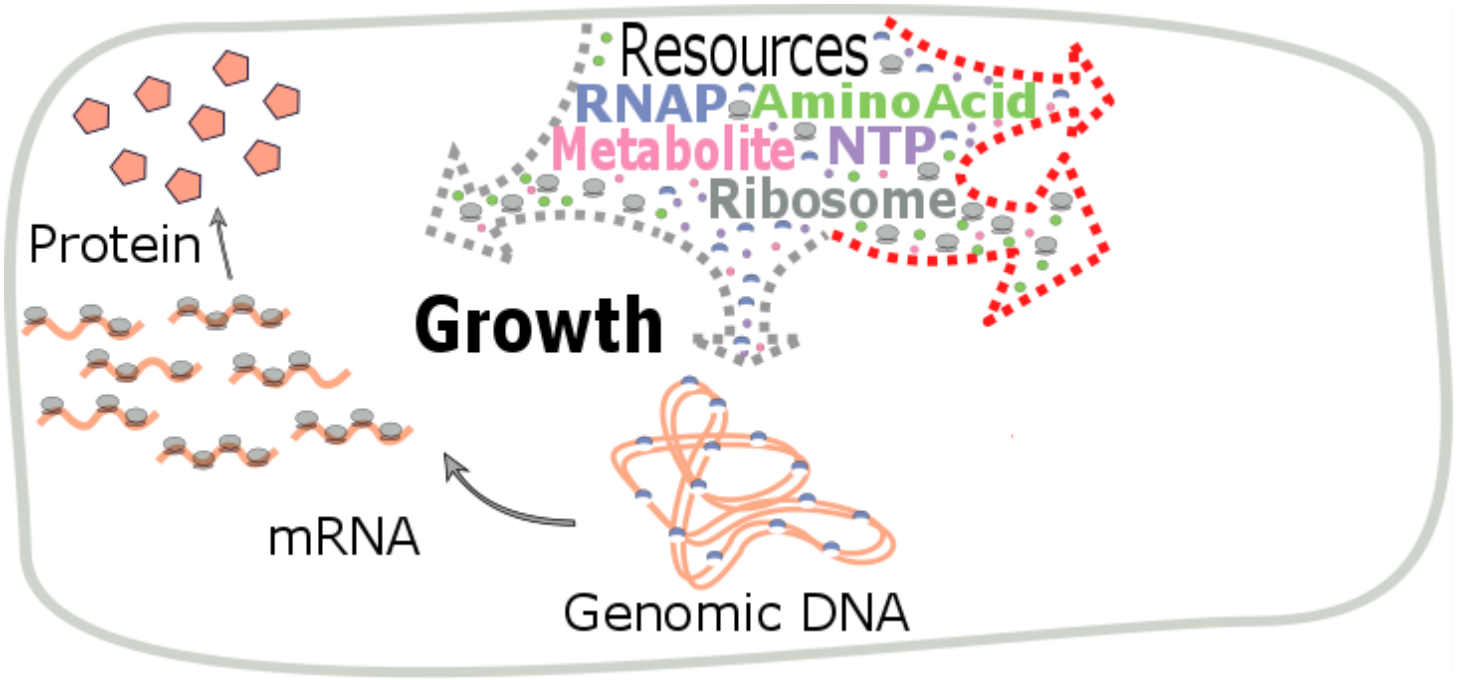

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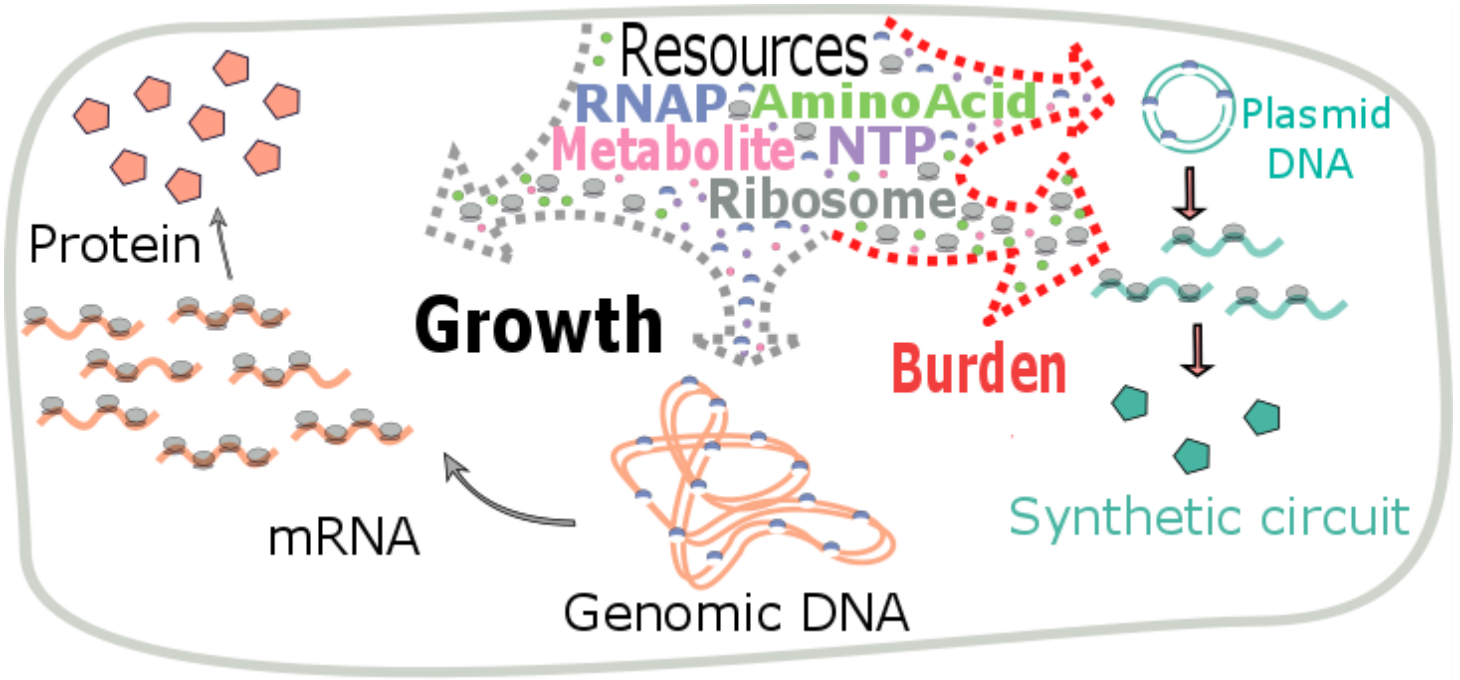

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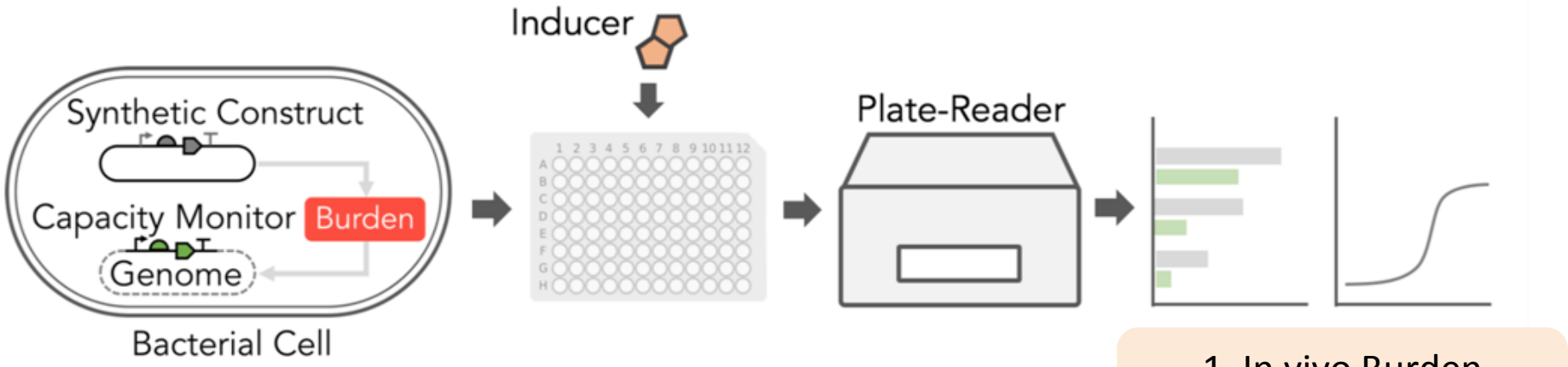
Understanding the impact of synthetic constructs on the host cell



1. In vivo Burden Analysis

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

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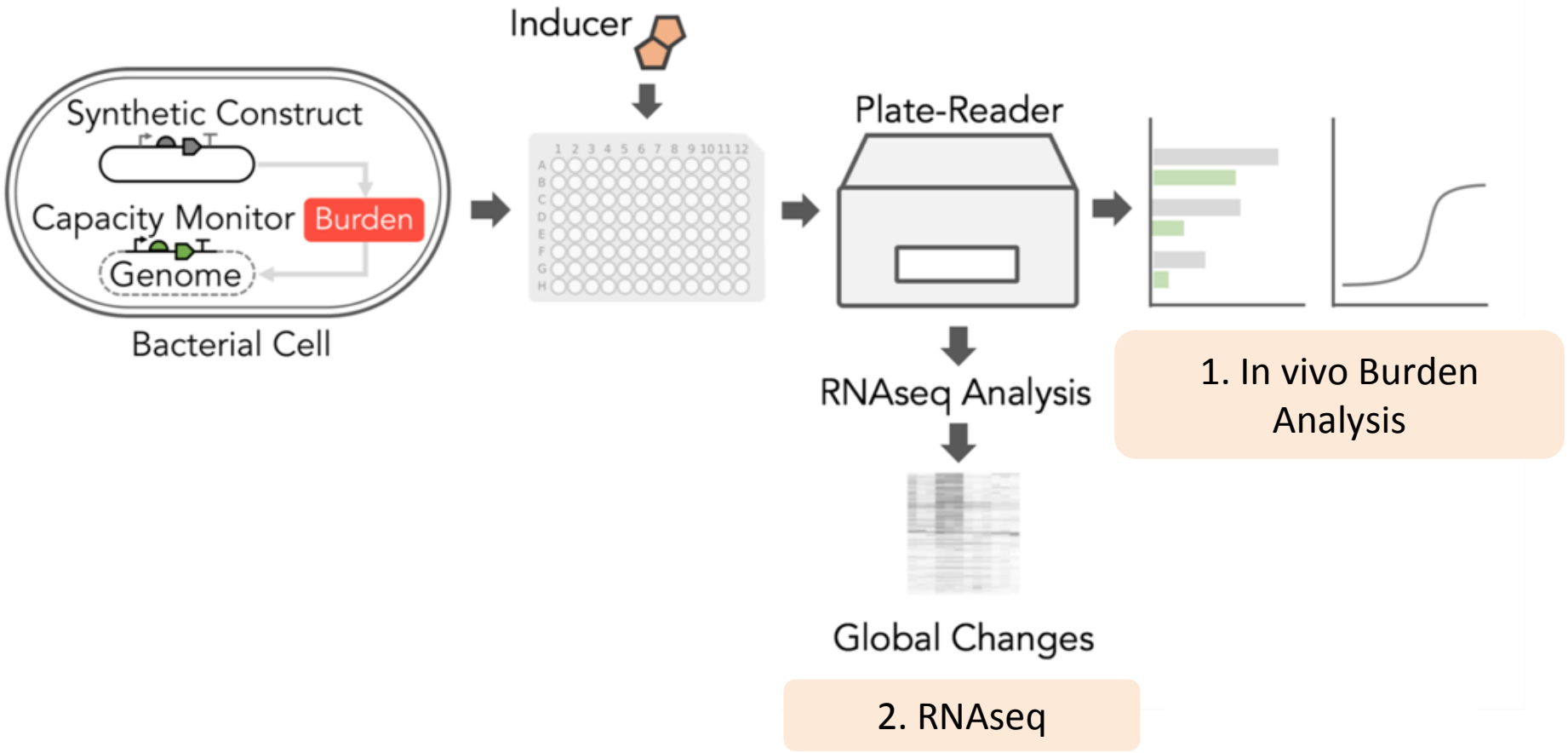

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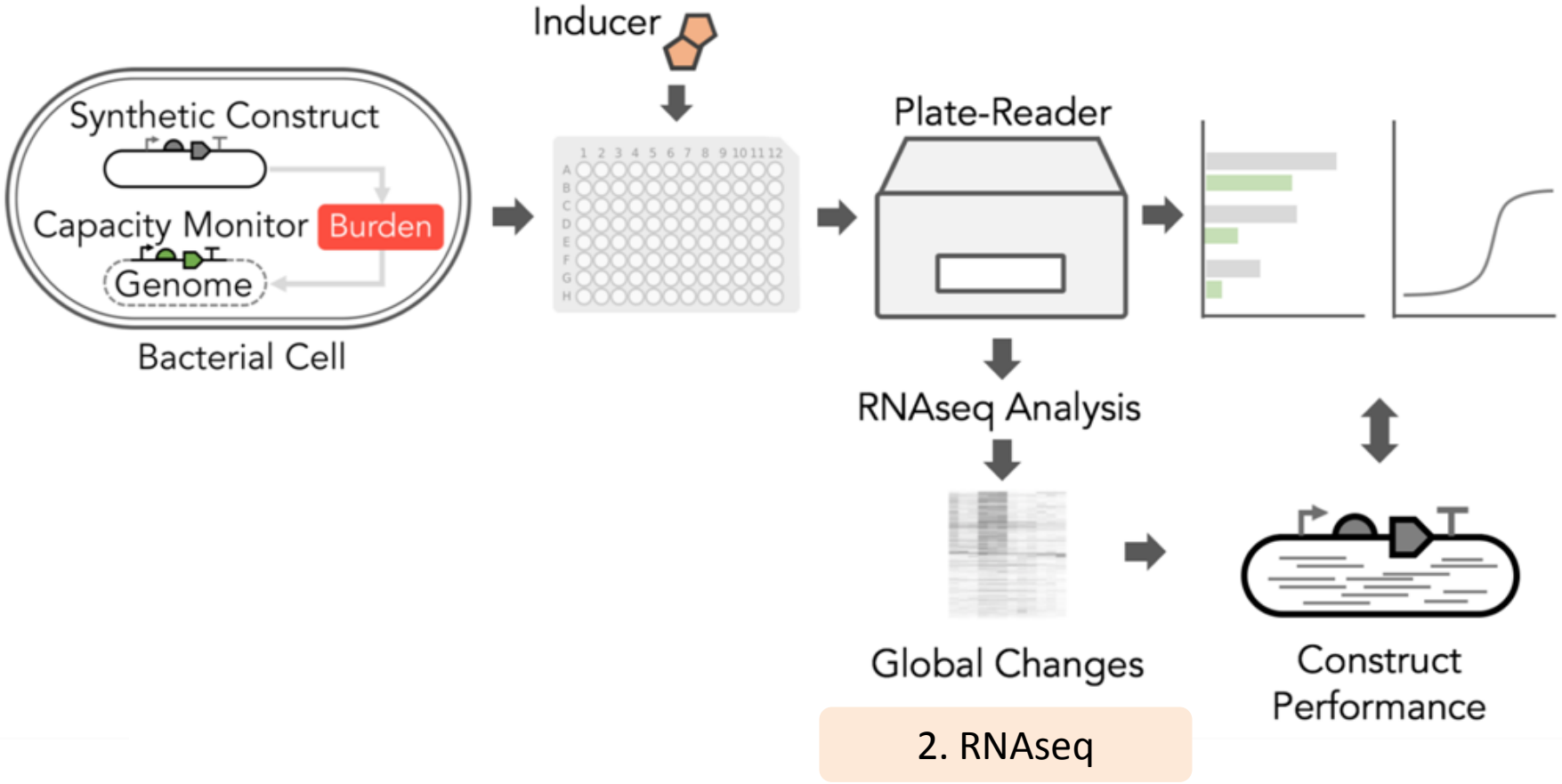

2. RNAseq

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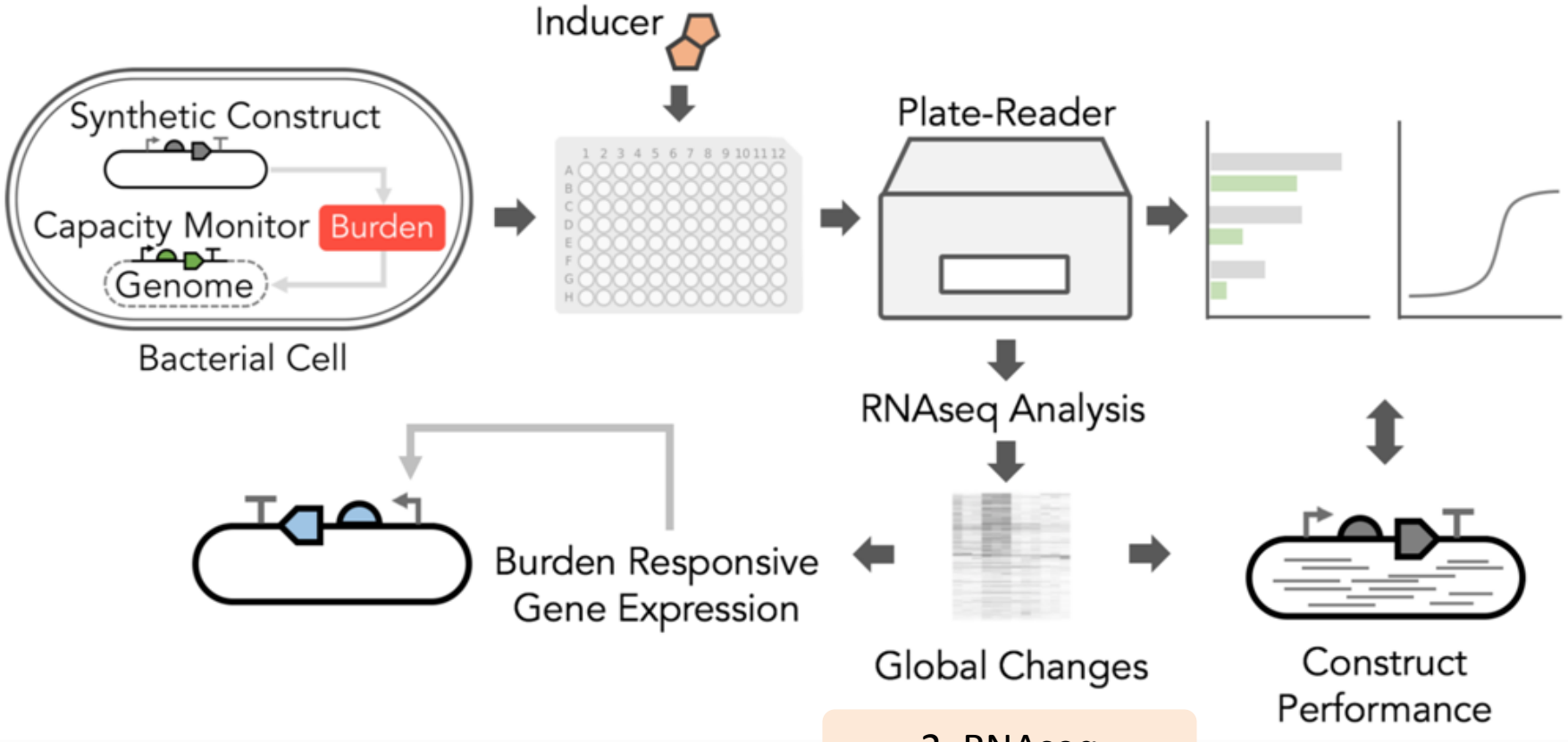

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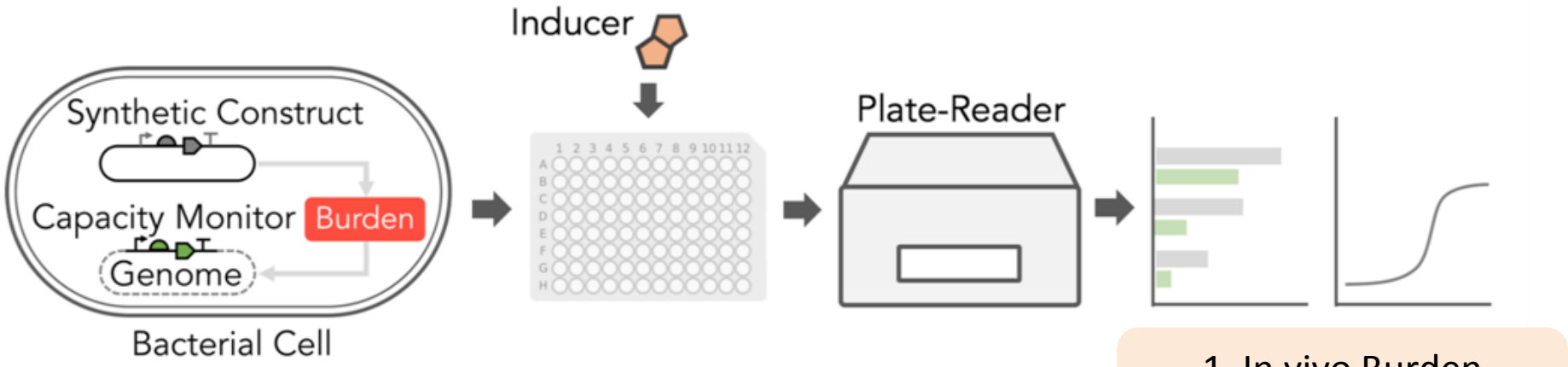

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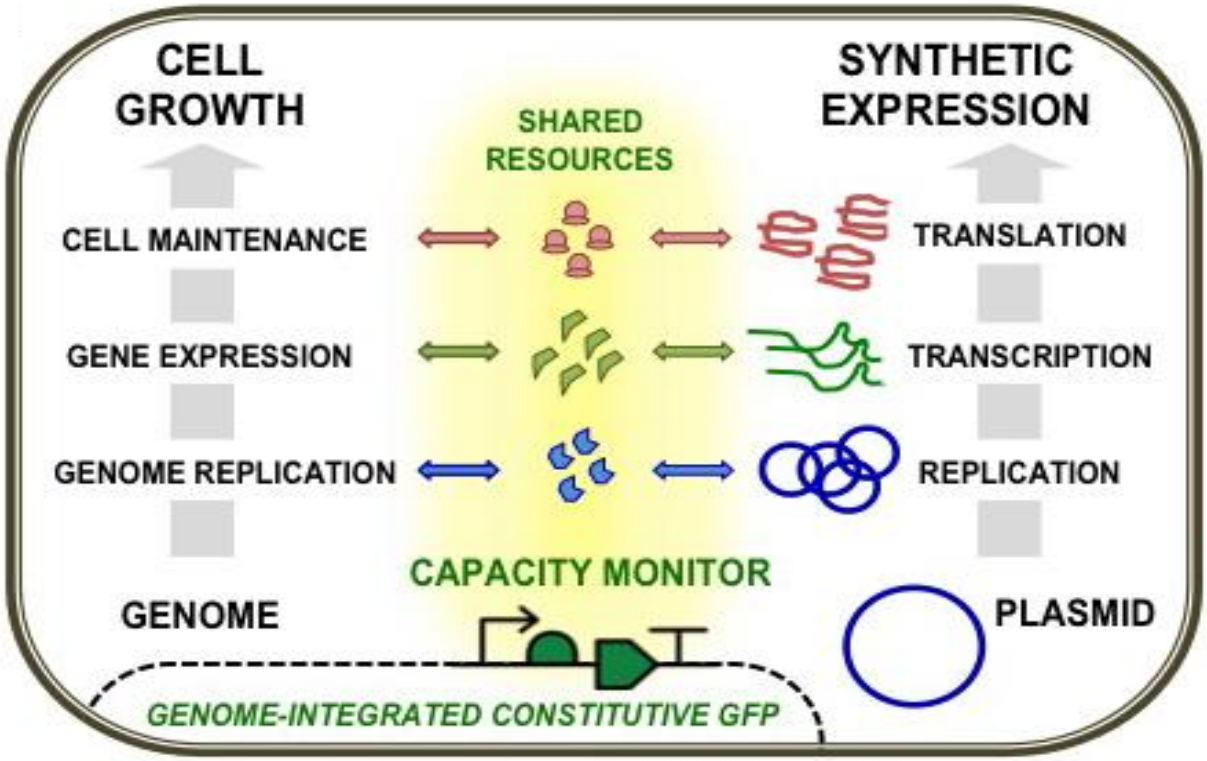


DH10B::GFP

GFP genome integration

Stringent response OFF [RelA inactivation]

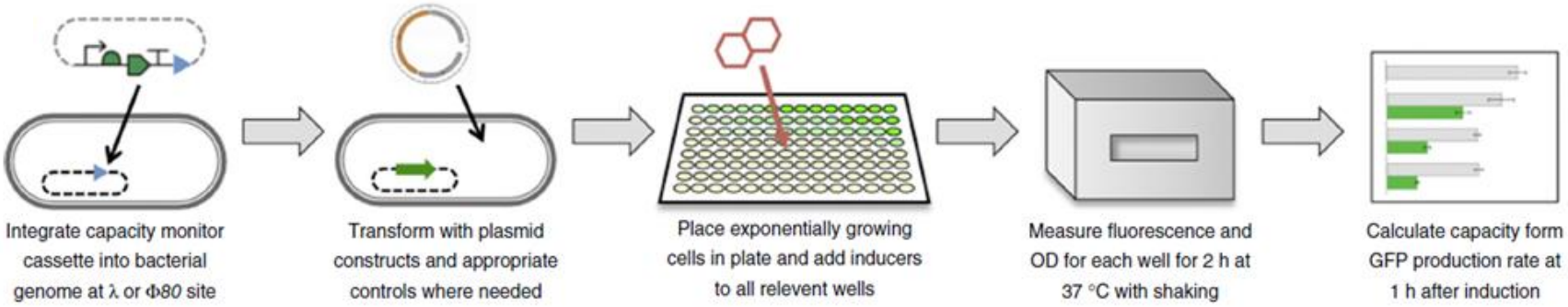
MG1655::GFP

GFP genome integration

Wild Type

***Ceroni F**, Algar R, Stan GB, Ellis T. **Nature Methods** 2015*

Plate- based Assay



$$GFP\ \ Capacity|_{t=t_2} = \frac{\left[\frac{total\ \ GFP_{t_3} - total\ \ GFP_{t_1}}{t_3 - t_1}\right]}{OD_{t_2}}$$

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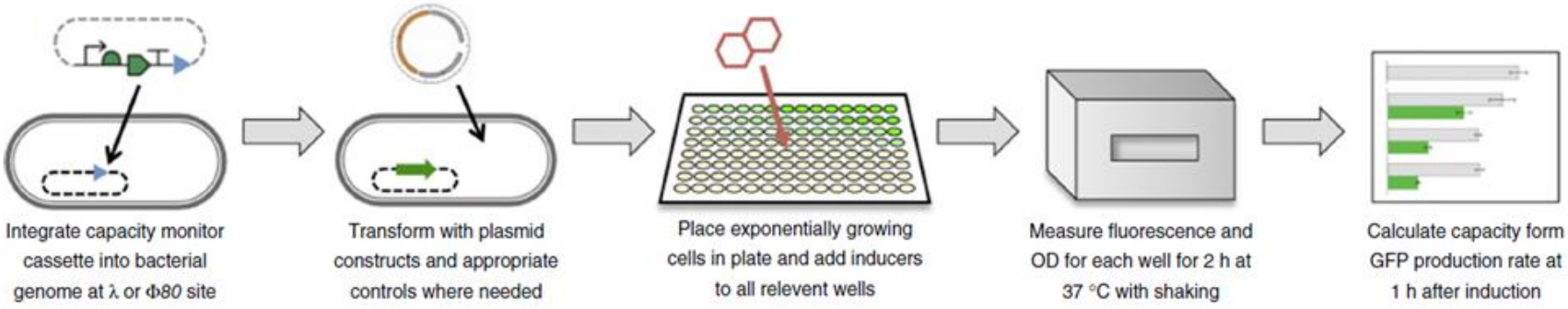

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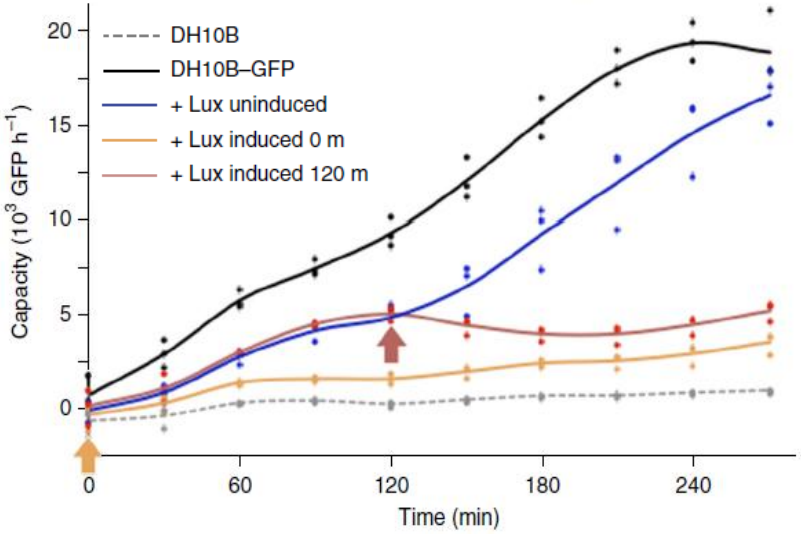
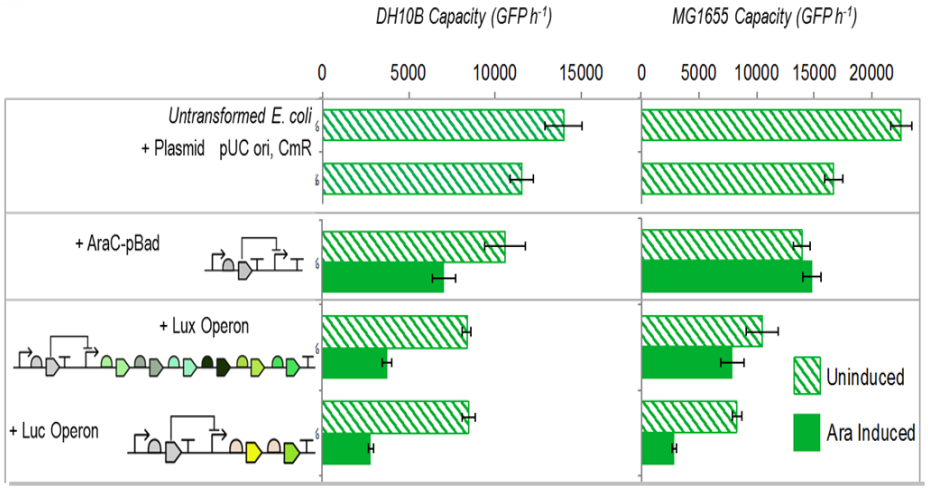
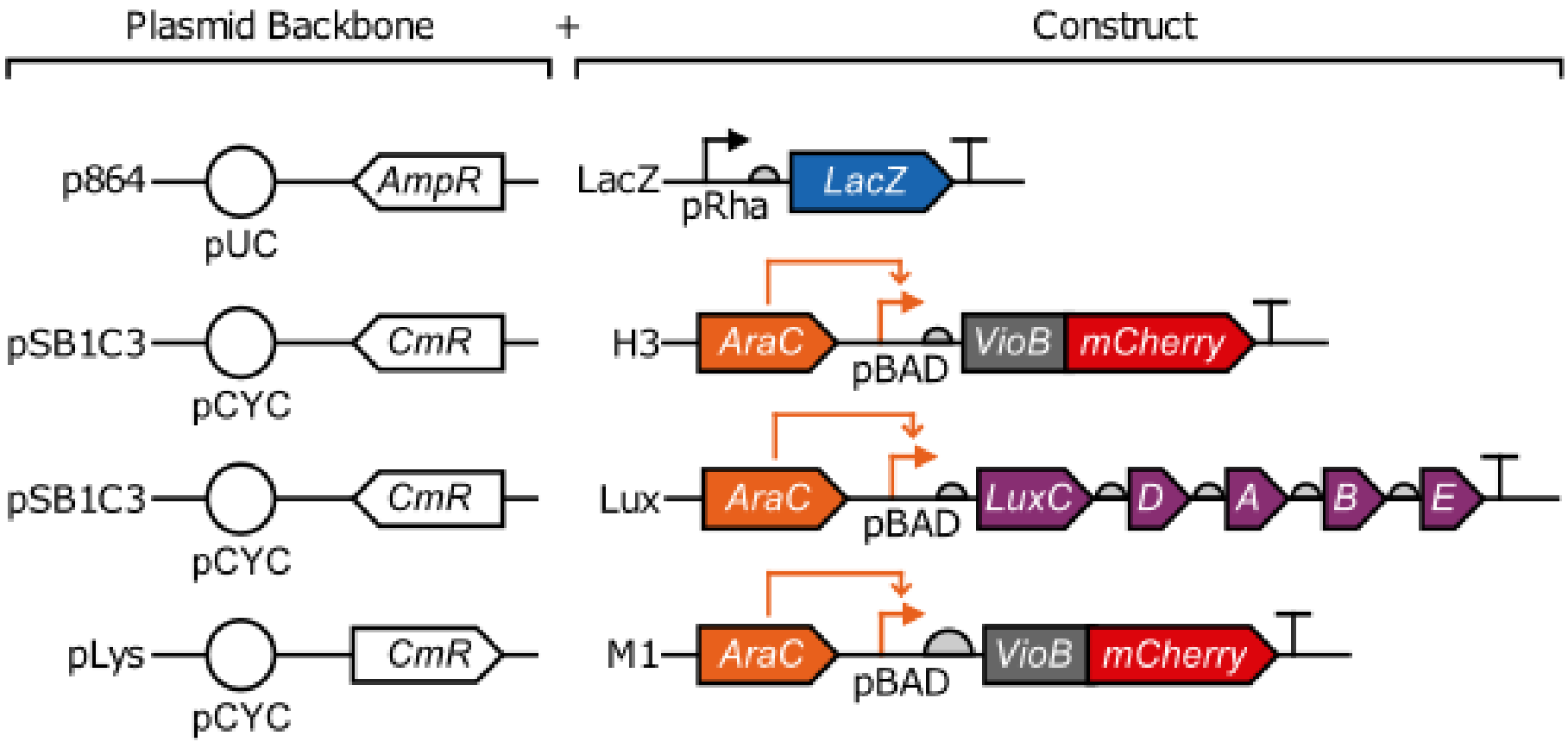




***Ceroni F**, Algar R, Stan GB, Ellis T. **Nature Methods** 2015*

A collection of diverse synthetic constructs



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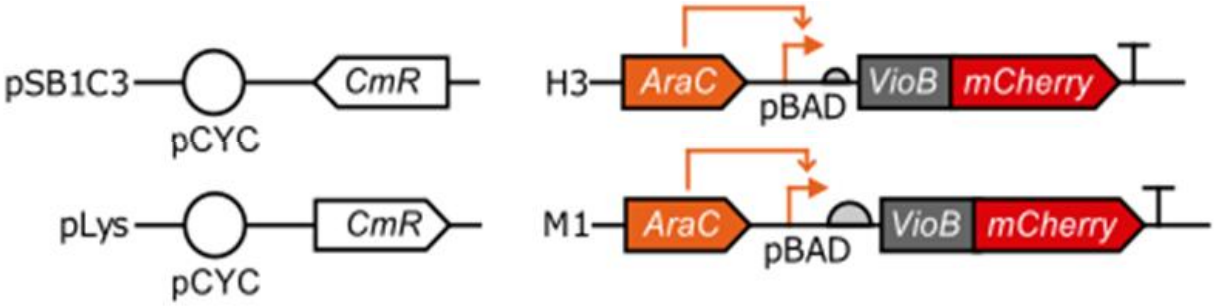

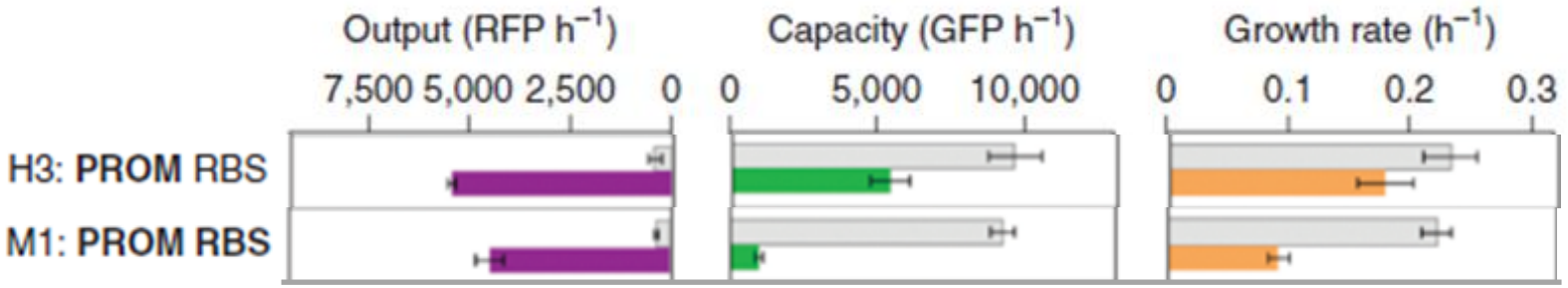

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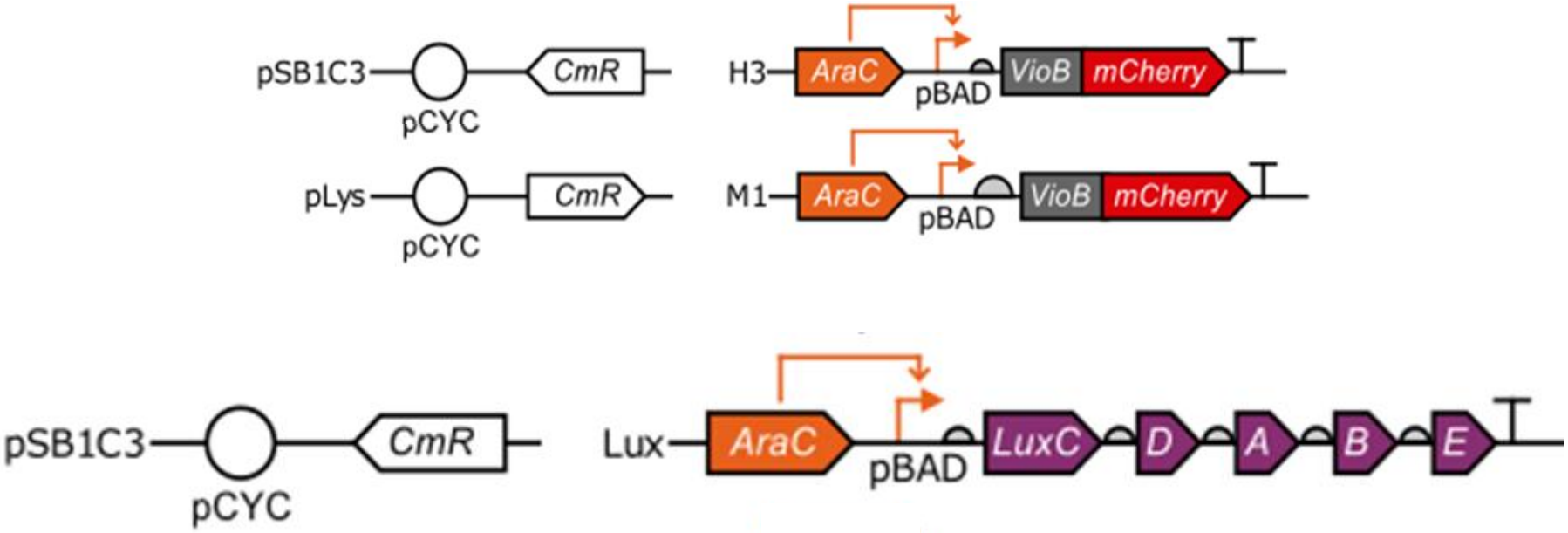

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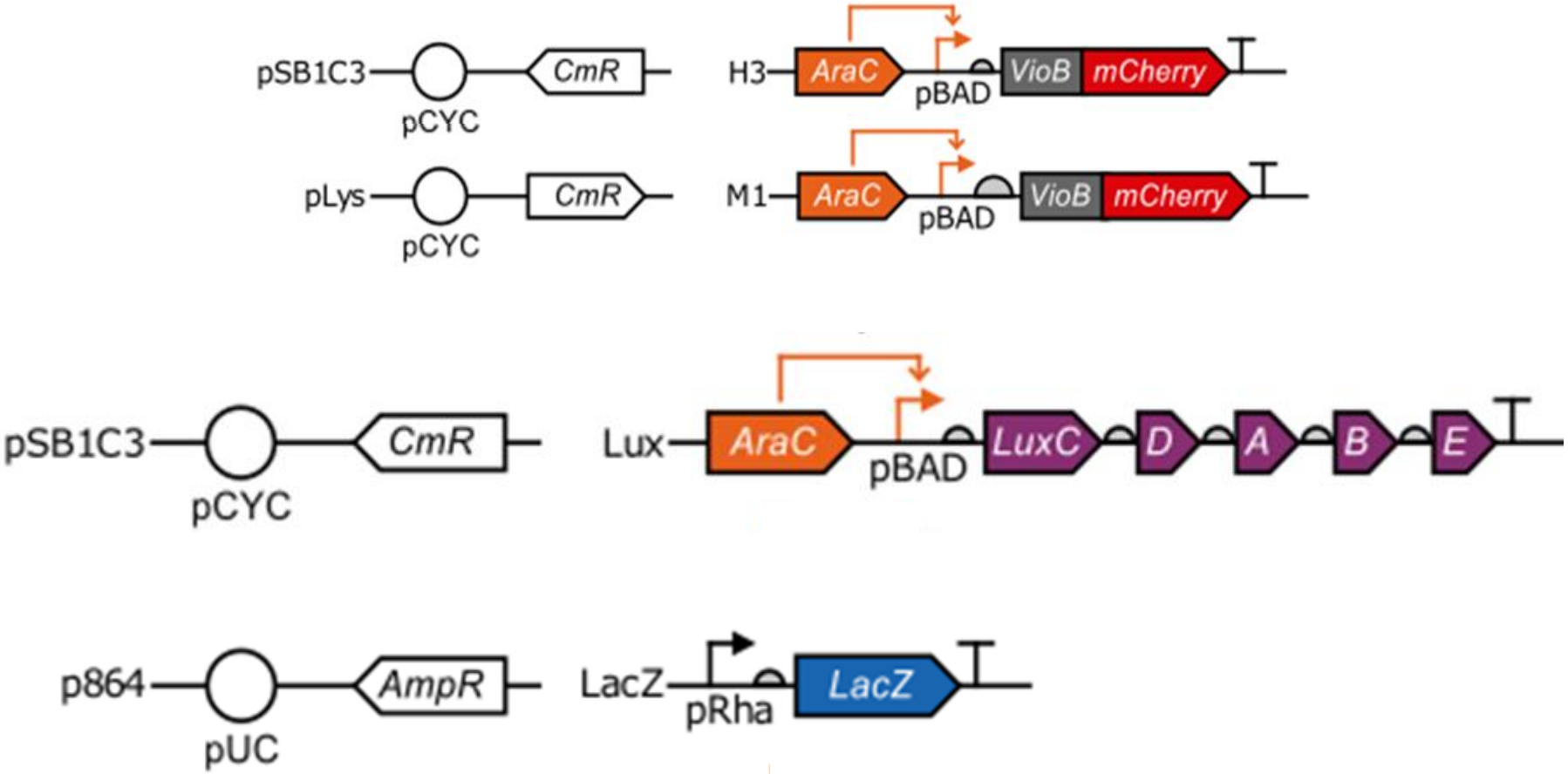

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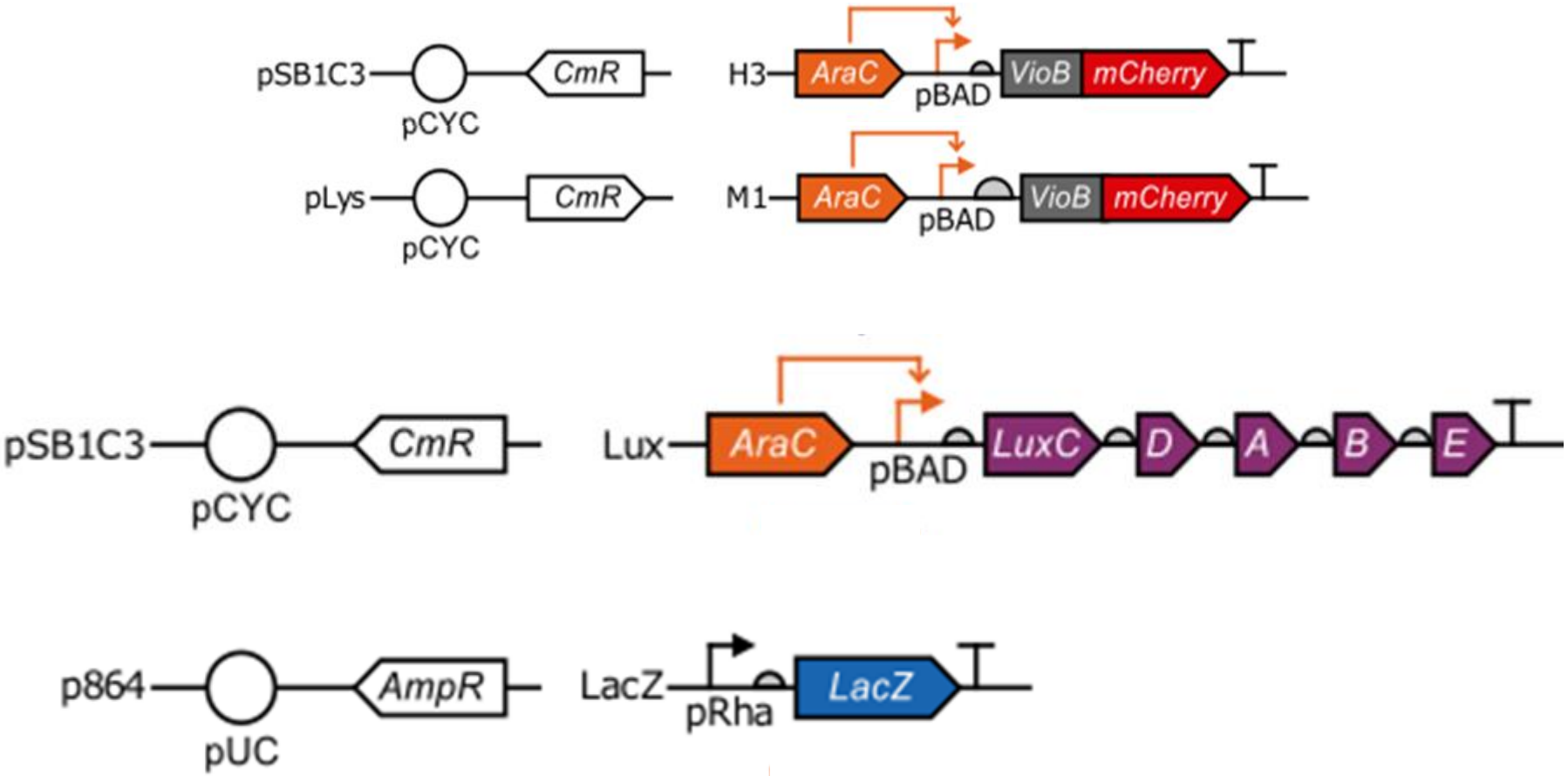
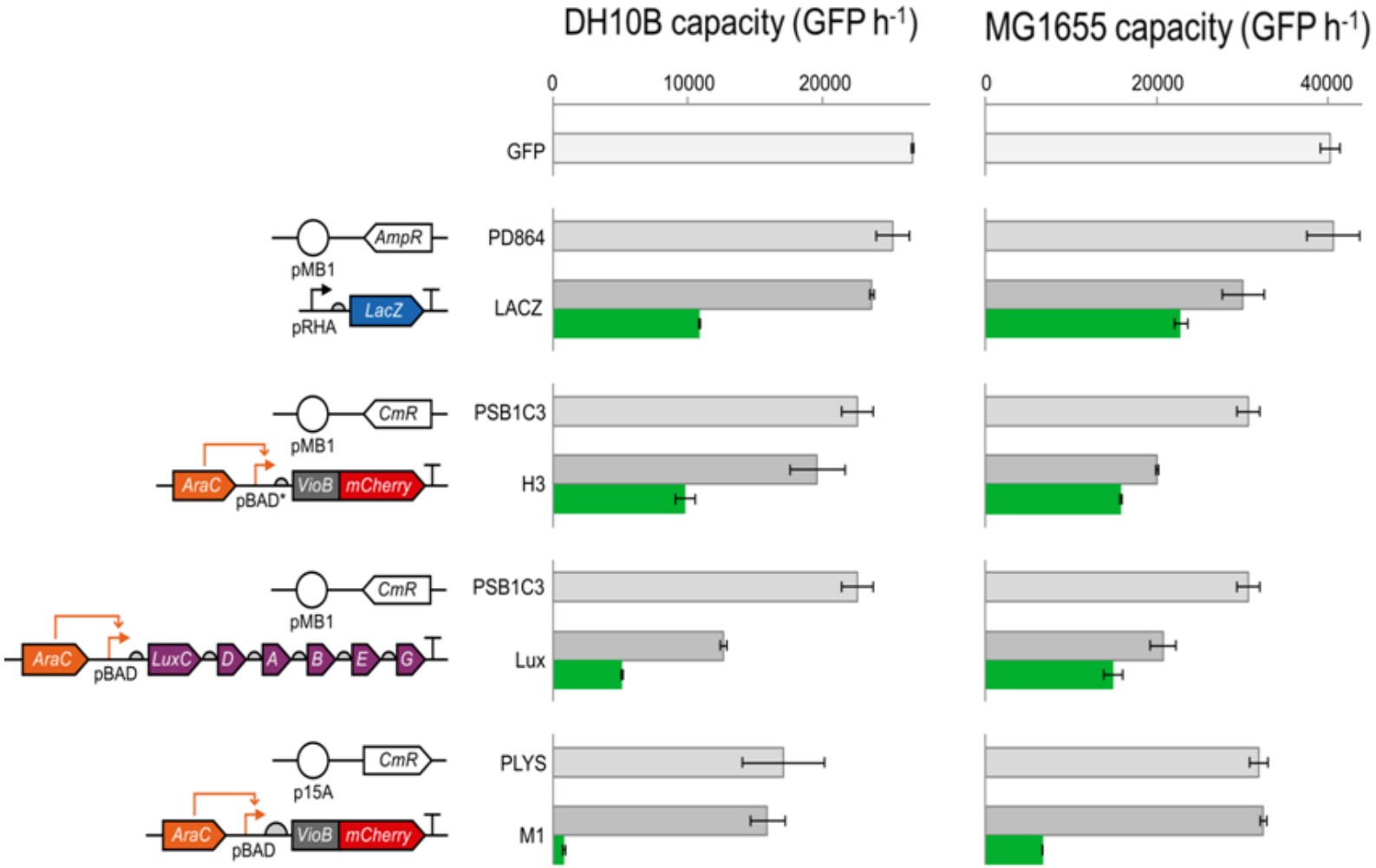


Inducible gene expression

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

In vivo Burden analysis

Cellular capacity 1h post induction of gene expression



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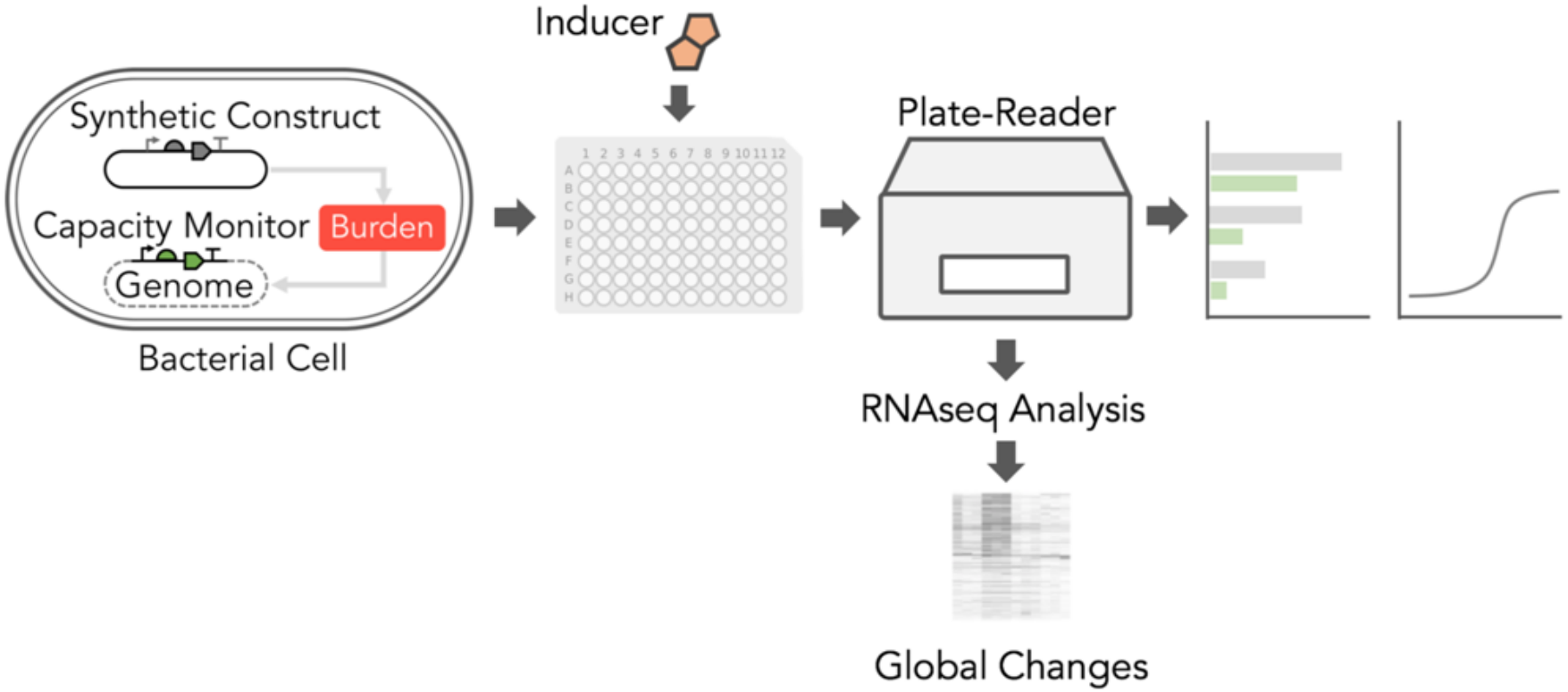
Understanding the impact of synthetic constructs on the host cell



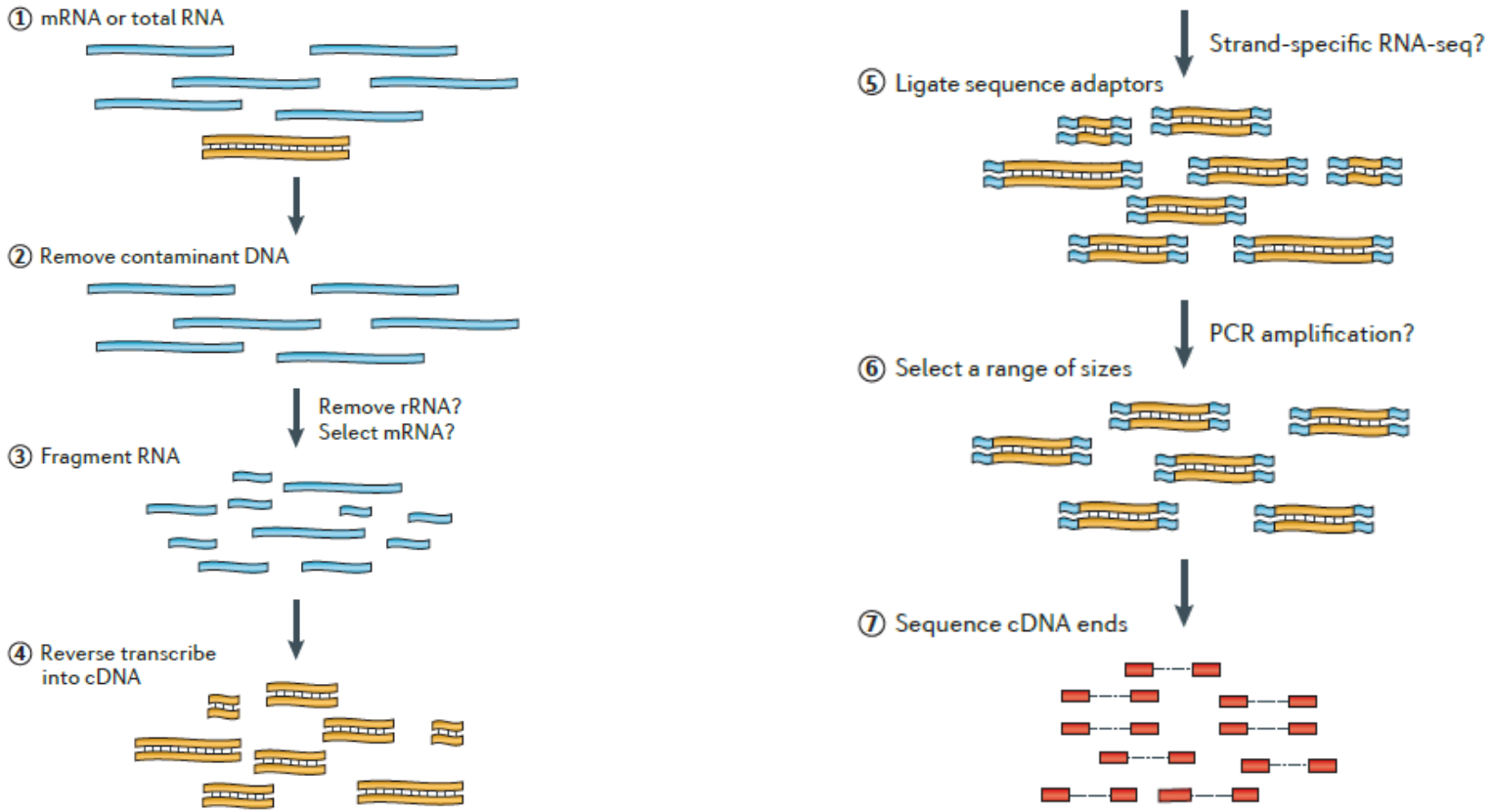
2. RNAseq

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

RNAseq analysis

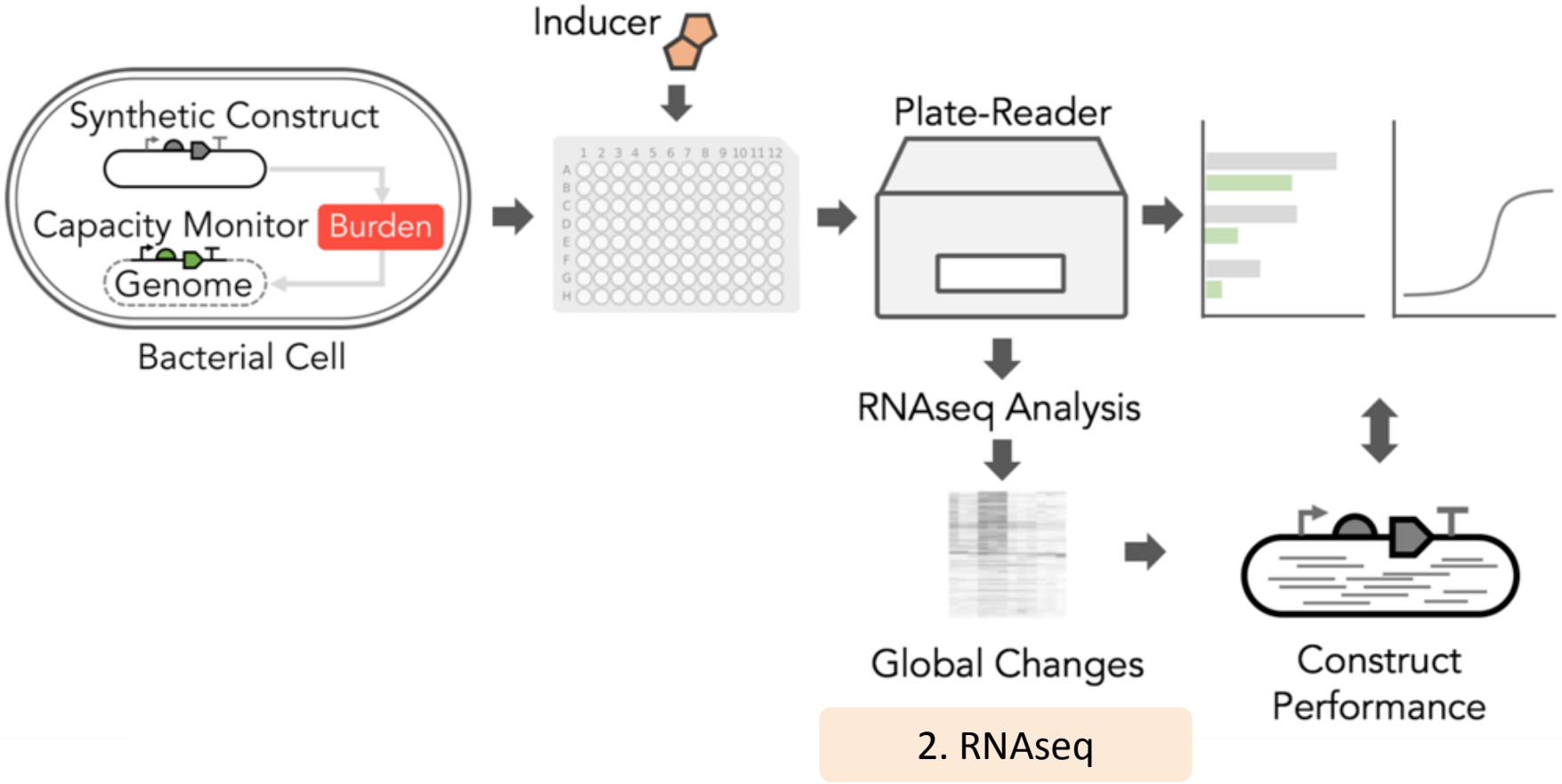
7 constructs X 2 strains X 3 replicates X 2 time points plus empty DH10B

= Total of 90 samples



Jeffrey A et al. ***Nature Reviews Genetics*** 2011

Understanding the impact of synthetic constructs on the host cell



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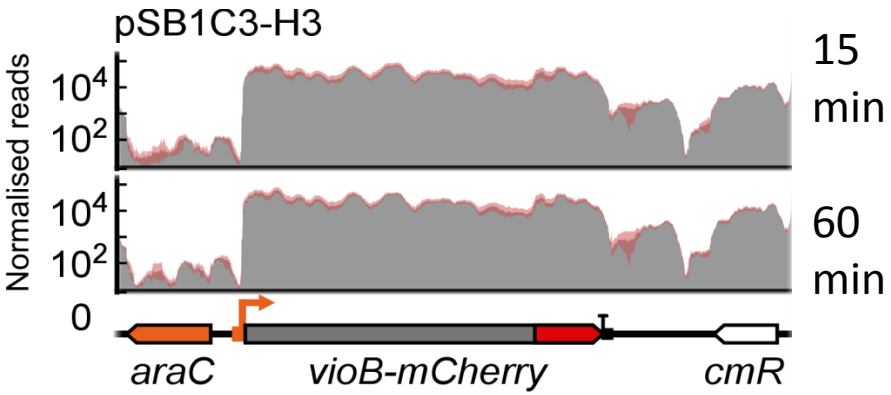
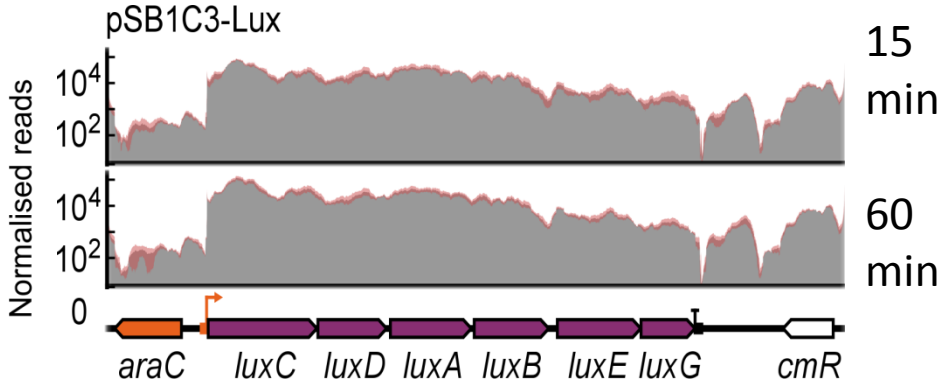
Construct performance

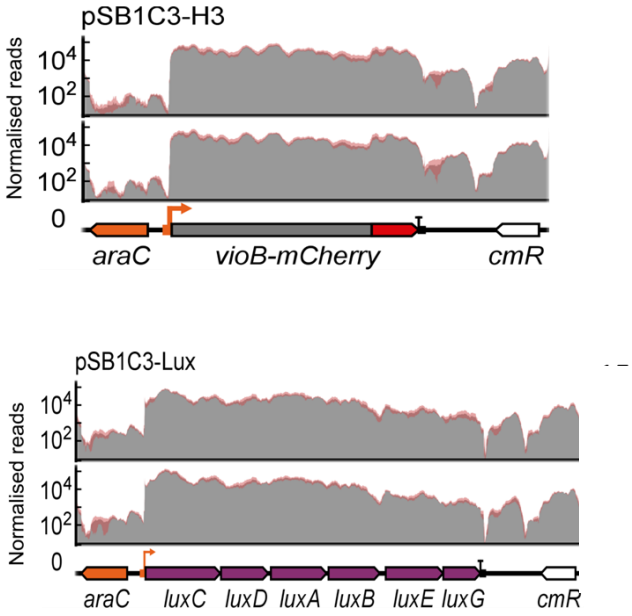


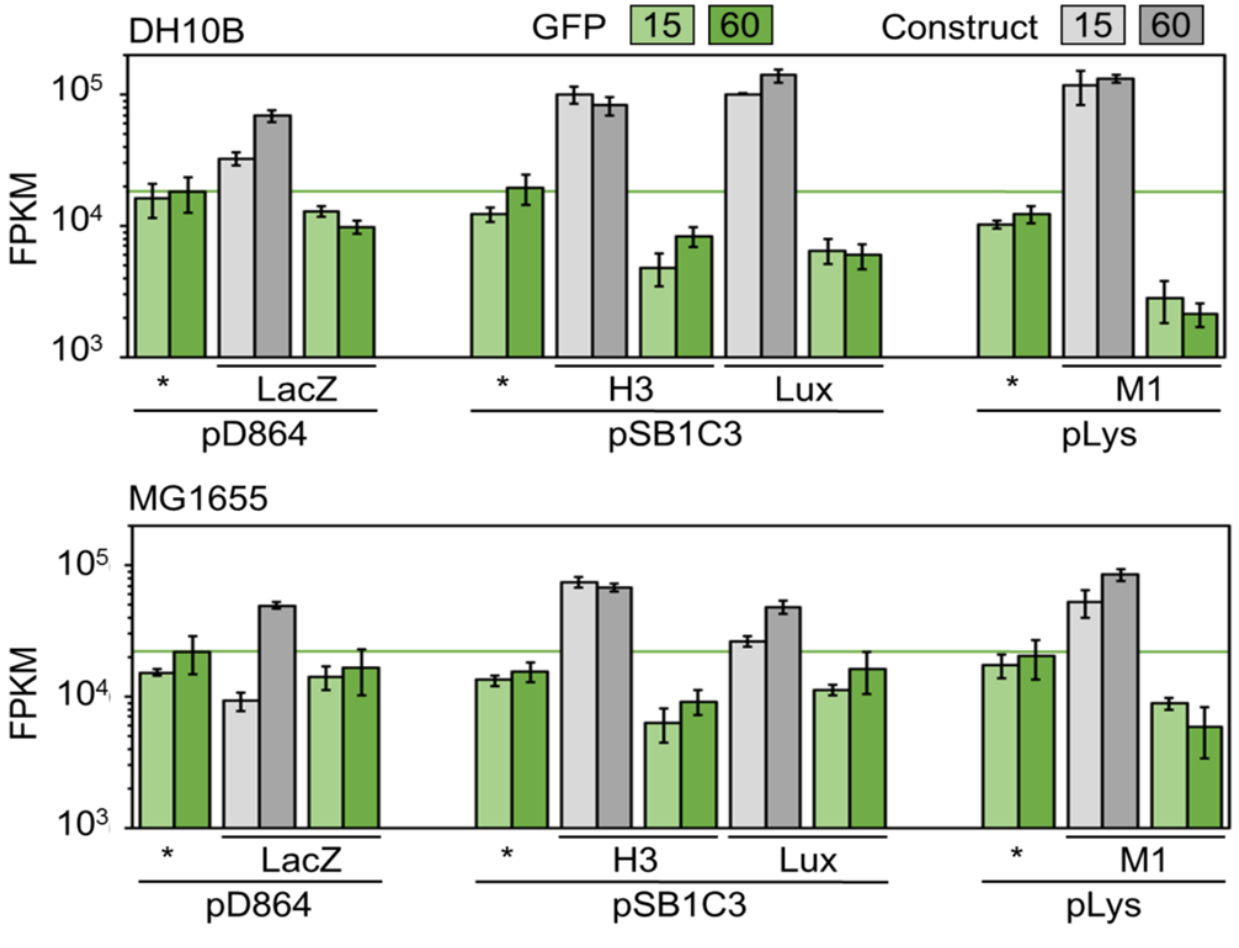


Transcriptional Profiles

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

Construct performance





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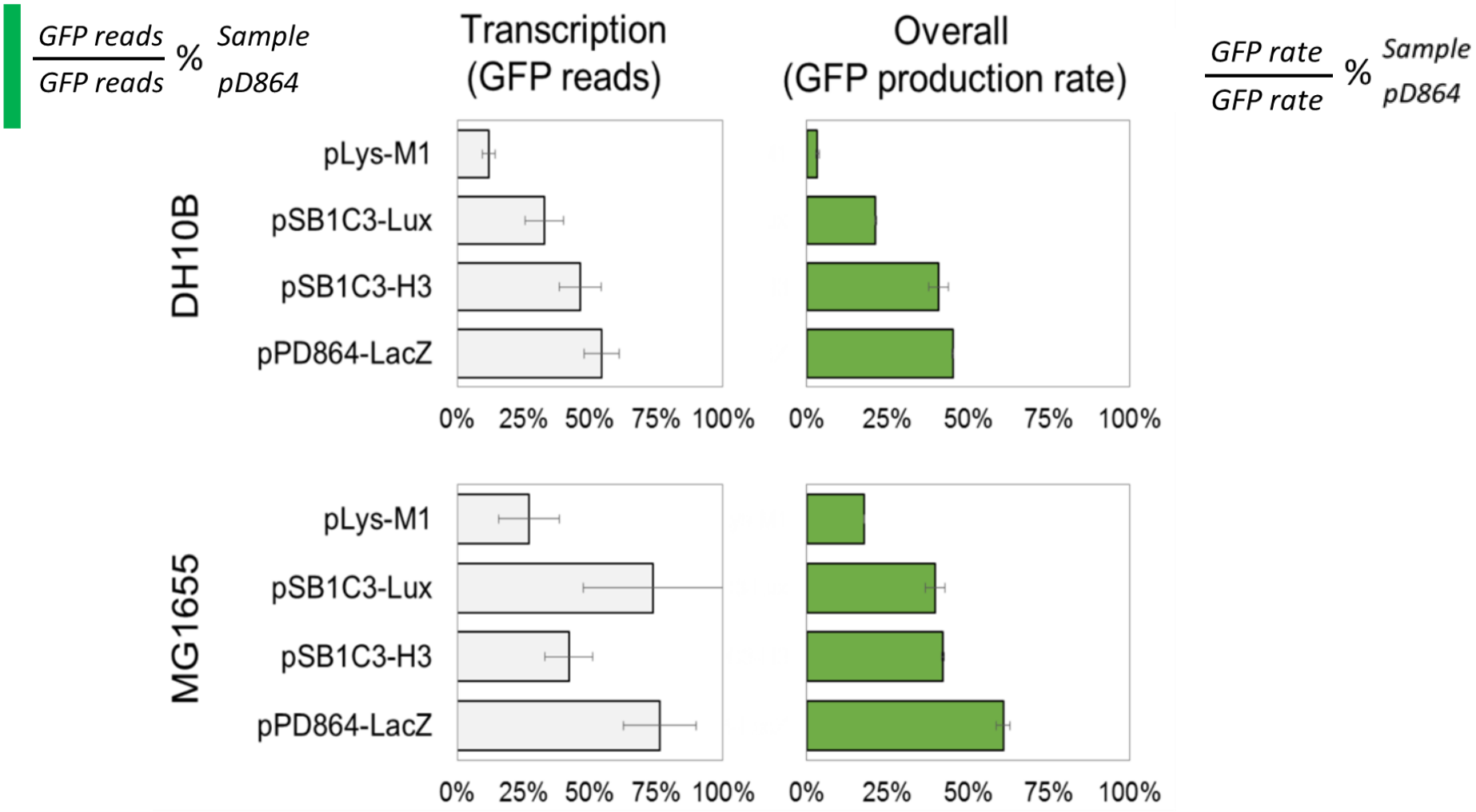

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

Global transcriptional changes

in DH10B 1h post induction

Ceroni F *et al, Stan GB, Ellis T.* ***Nature Methods*** *under revision*

Global transcriptional changes

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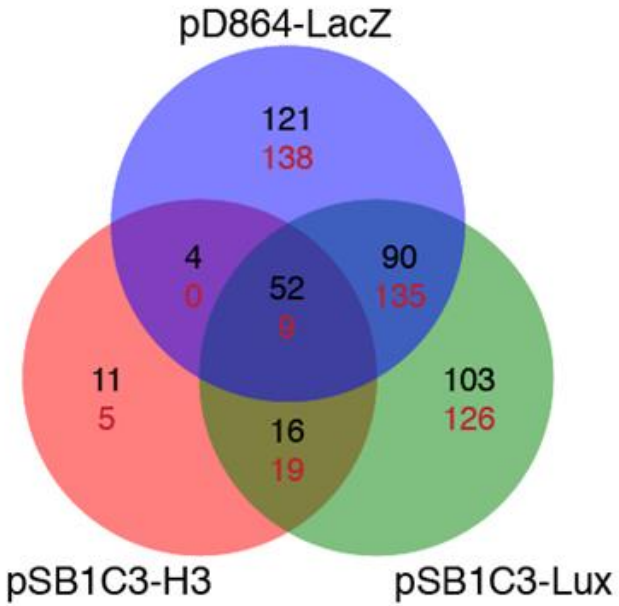

shared		pSB1C3-Lux		pSB1C3-H3		pD864-LacZ	
ibpB	$\sigma^{32/54}$	**ibpB**	$\sigma^{32/54}$	**tktA**	N/A	gadC	$\sigma^{24/70/S}$
ibpA	σ^{32}	**ibpA**	σ^{32}	**malE**	σ^{70}	ibpB	$\sigma^{32/54}$
ycjX	σ^{32}	ypdH	N/A	ptsA	N/A	gadA	$\sigma^{70/5}$
htpG	σ^{32}	ypdG	N/A	metE	N/A	gadB	$\sigma^{70/5}$
dnaK	σ^{32}	**ypdD**	σ^{28}	**ydeN**	σ^{70}	ibpA	σ^{32}
mutM	σ^{32}	**ypdF**	N/A	**malM**	σ^{70}	hdeB	$\sigma^{70/S}$
dnaJ	σ^{32}	**malE**	σ^{70}	**lamB**	σ^{70}	hdeD	σ^{70}
hslO	σ^{32}	ypdE	N/A	**clpB**	$\sigma^{32/70}$	hdeA	$\sigma^{70/S}$
hslR	σ^{32}	cysC	σ^{32}	malF	σ^{70}	slp	σ^{70}
clpB	$\sigma^{32/70}$	**lamB**	σ^{70}	**htpG**	σ^{32}	gadE	$\sigma^{70/5}$

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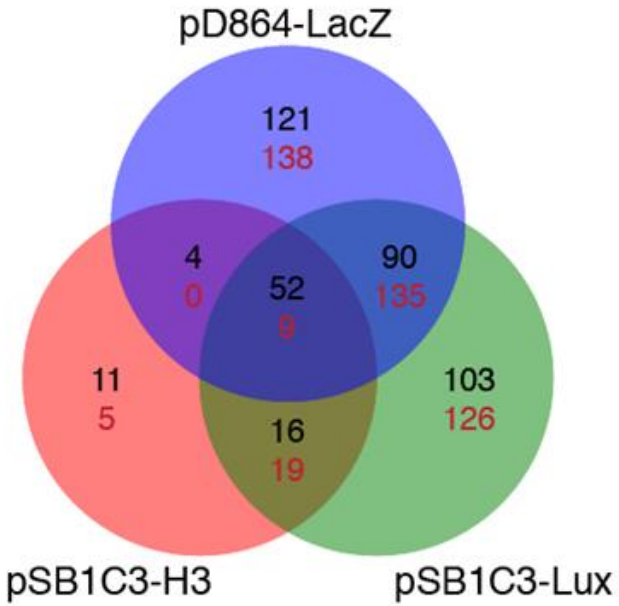
in DH10B 1h post induction



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htpG	σ^{32}	ypdG	N/A	metE	N/A	gadB	$\sigma^{70/5}$
dnaK	σ^{32}	**ypdD**	σ^{28}	**ydeN**	σ^{70}	ibpA	σ^{32}
mutM	σ^{32}	**ypdF**	N/A	**malM**	σ^{70}	hdeB	$\sigma^{70/S}$
dnaJ	σ^{32}	**malE**	σ^{70}	**lamB**	σ^{70}	hdeD	σ^{70}
hslO	σ^{32}	ypdE	N/A	**clpB**	$\sigma^{32/70}$	hdeA	$\sigma^{70/S}$
hslR	σ^{32}	cysC	σ^{32}	malF	σ^{70}	slp	σ^{70}
clpB	$\sigma^{32/70}$	**lamB**	σ^{70}	**htpG**	σ^{32}	gadE	$\sigma^{70/5}$

Early responsive promoters

ibpAB *htpG*
groSL *dnaKJ*

σ32

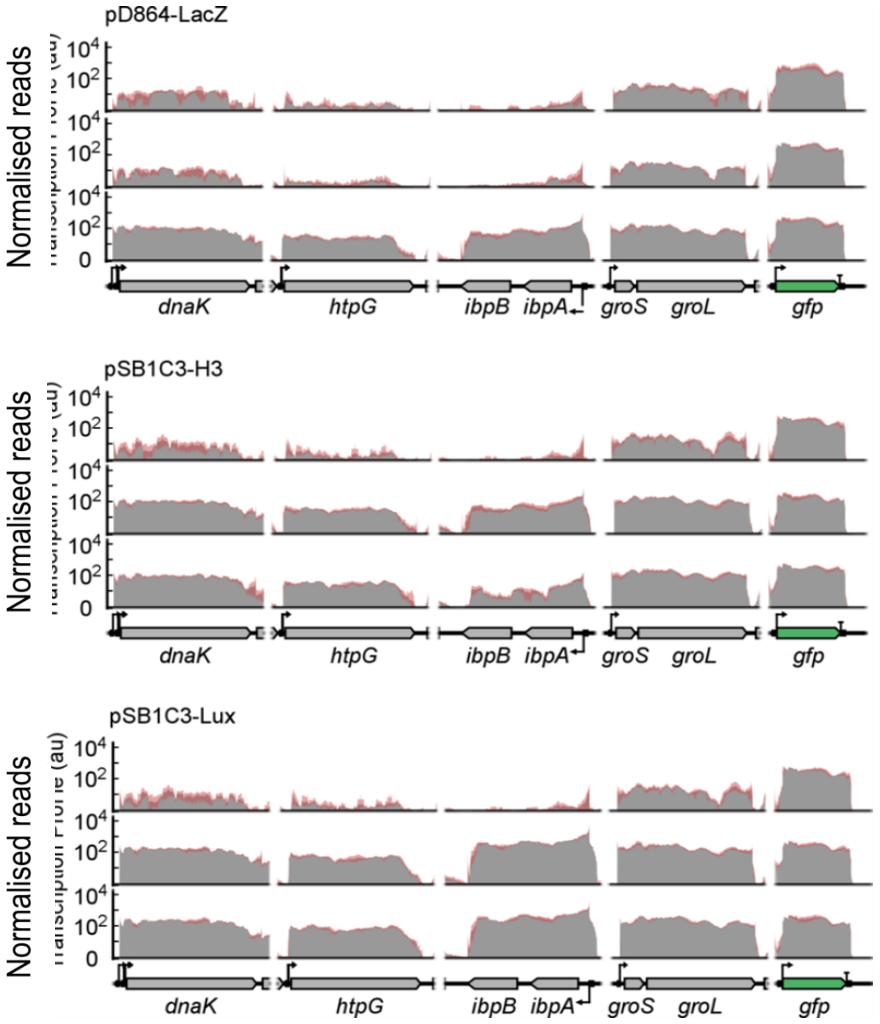
Ceroni F *et al, Stan GB, Ellis T.* ***Nature Methods*** *under revision*

Early responsive promoters

Behavior on the Genome

Early responsive promoters

ibpAB *htpG*
groSL *dnaKJ*



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Early responsive promoters

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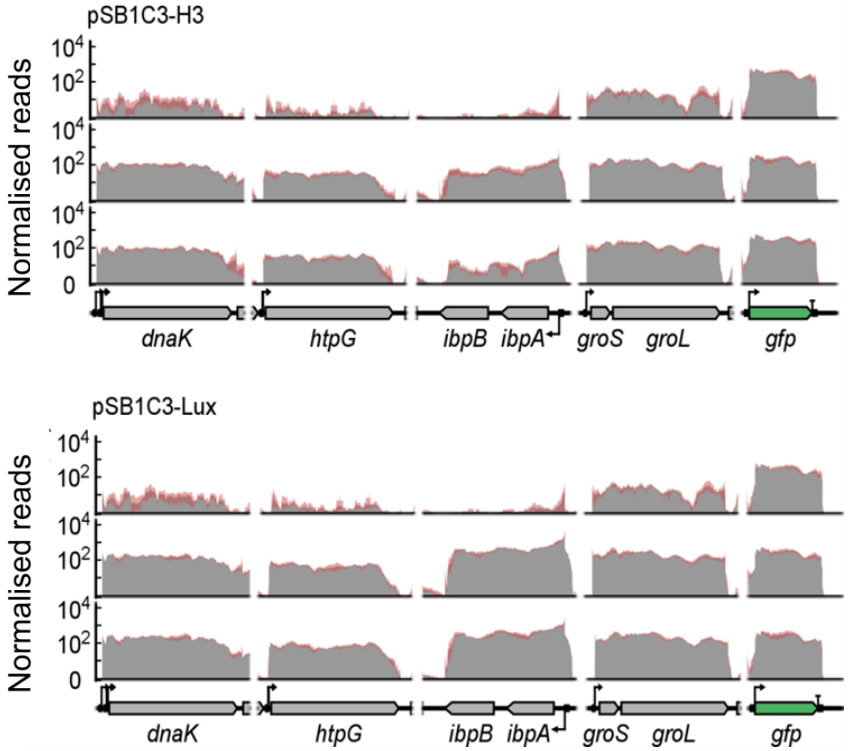
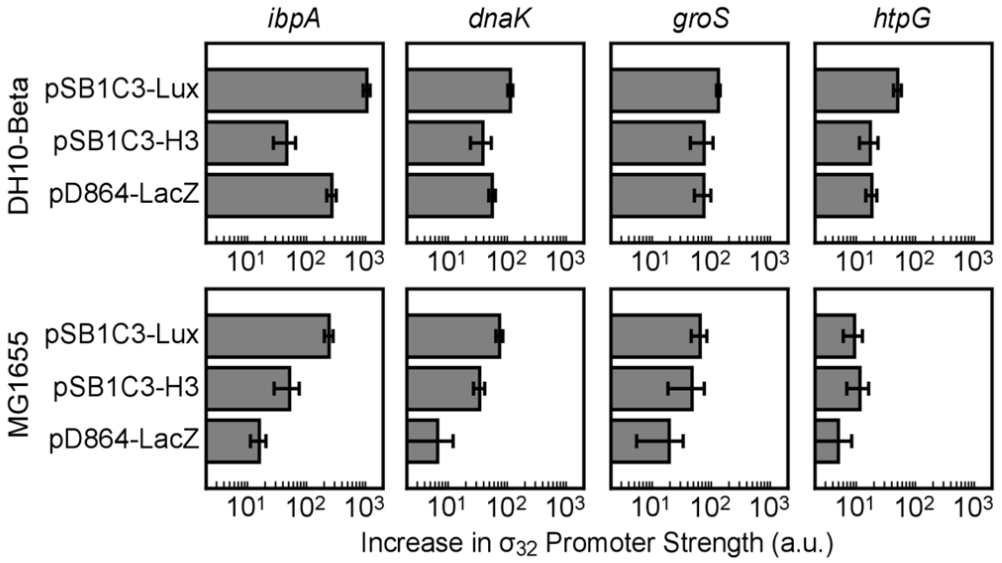
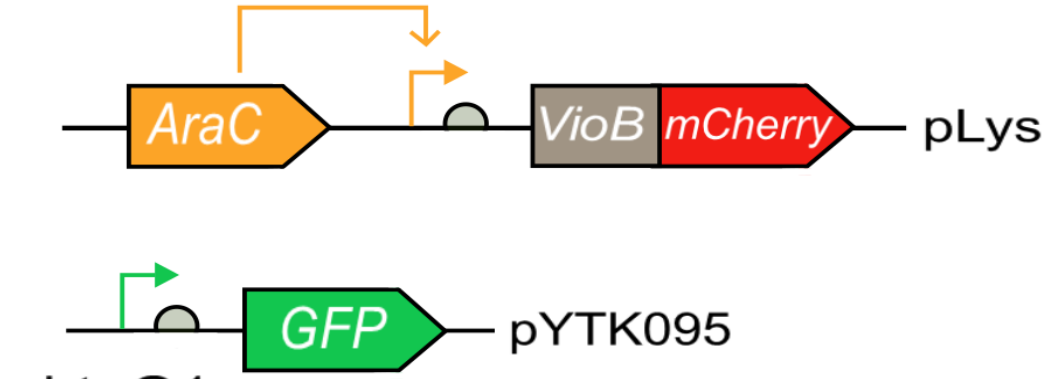




***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

Early responsive promoters

Behavior on a plasmid



phtpG1

phtpG2

pgroSL

pibpAB

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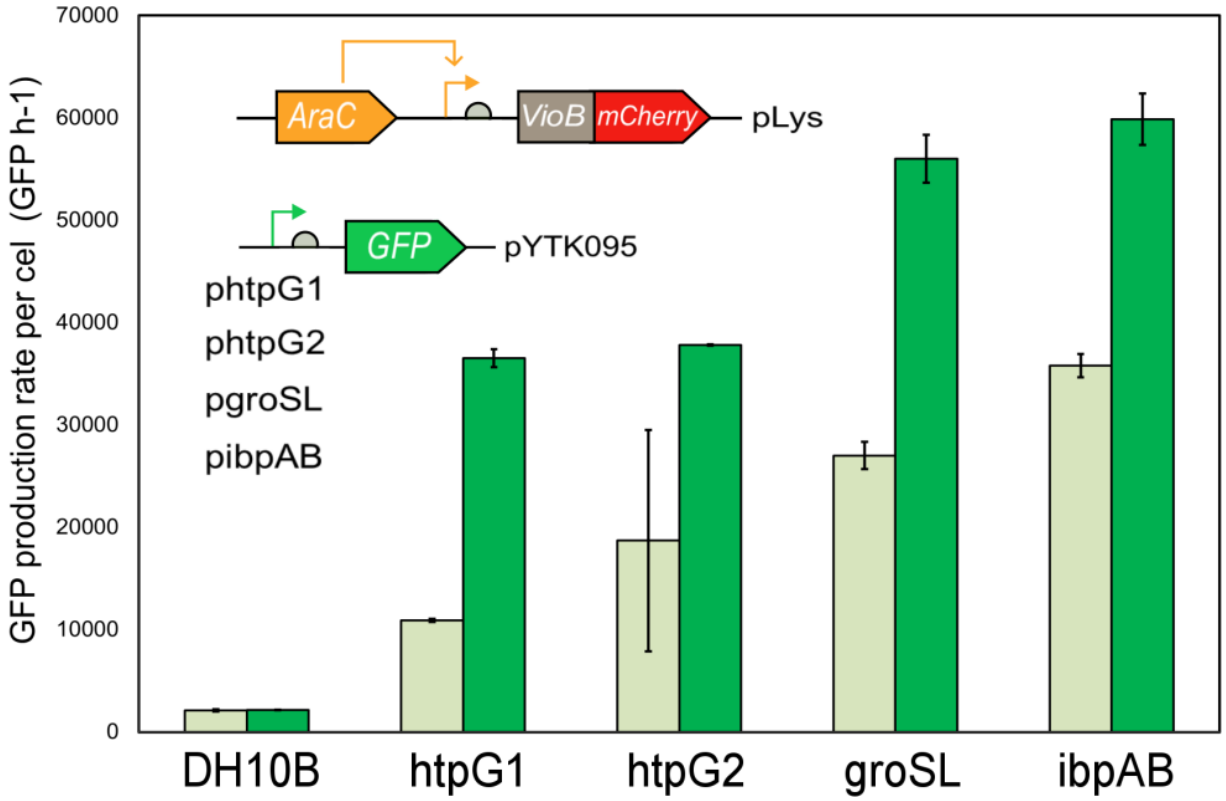
Early responsive promoters

Behavior on a plasmid



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Cellular Burden

Understand it

burden-based molecular feedback

to Overcome it

A biomolecular feedback system

1. Burden- responsive

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A biomolecular feedback system

1. Burden- responsive

2. Fast

***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

A biomolecular feedback system

1. Burden- responsive

2. Fast

3. Modular

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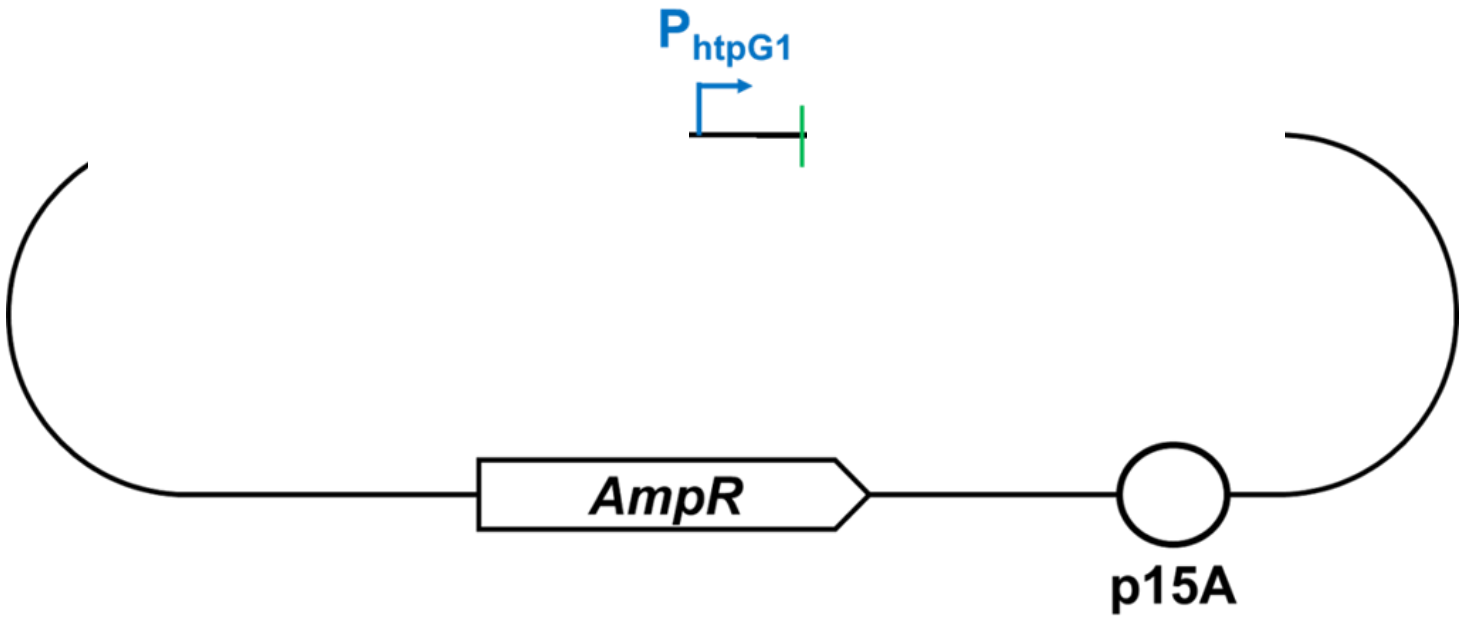
A biomolecular feedback system



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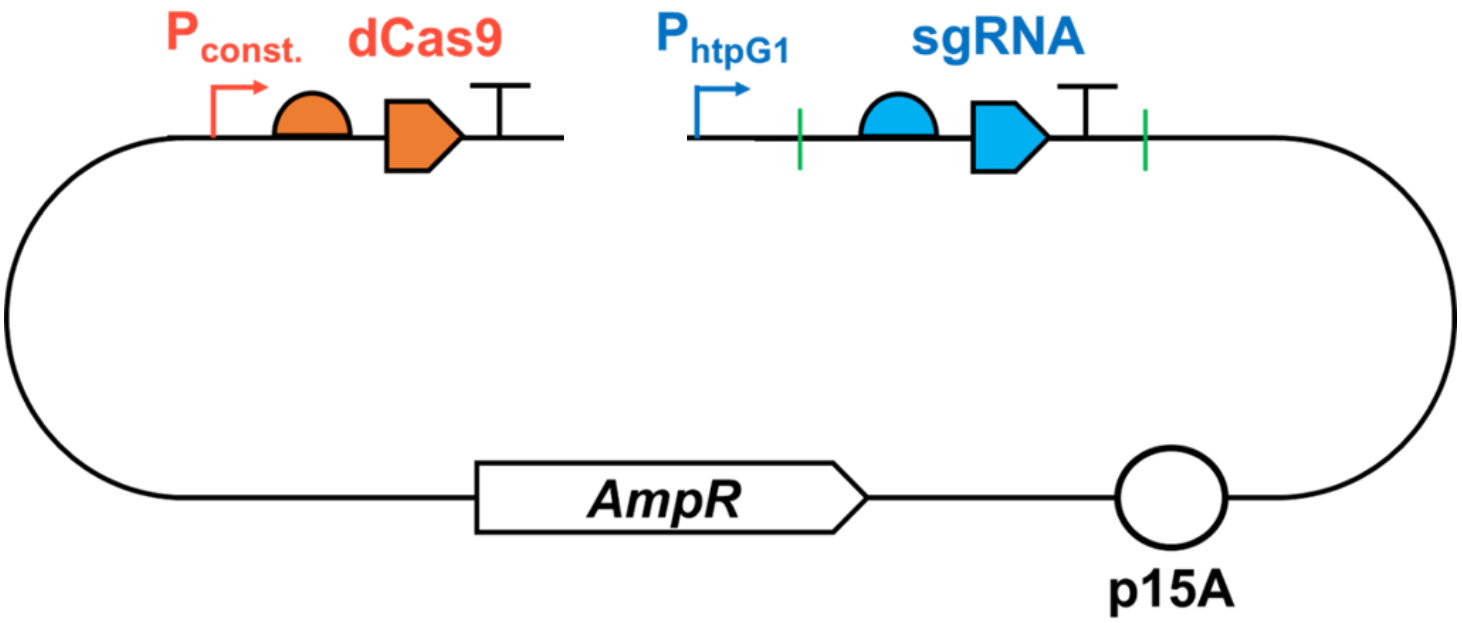
A biomolecular feedback system

dCas9-gRNA based regulation



***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

A biomolecular feedback system

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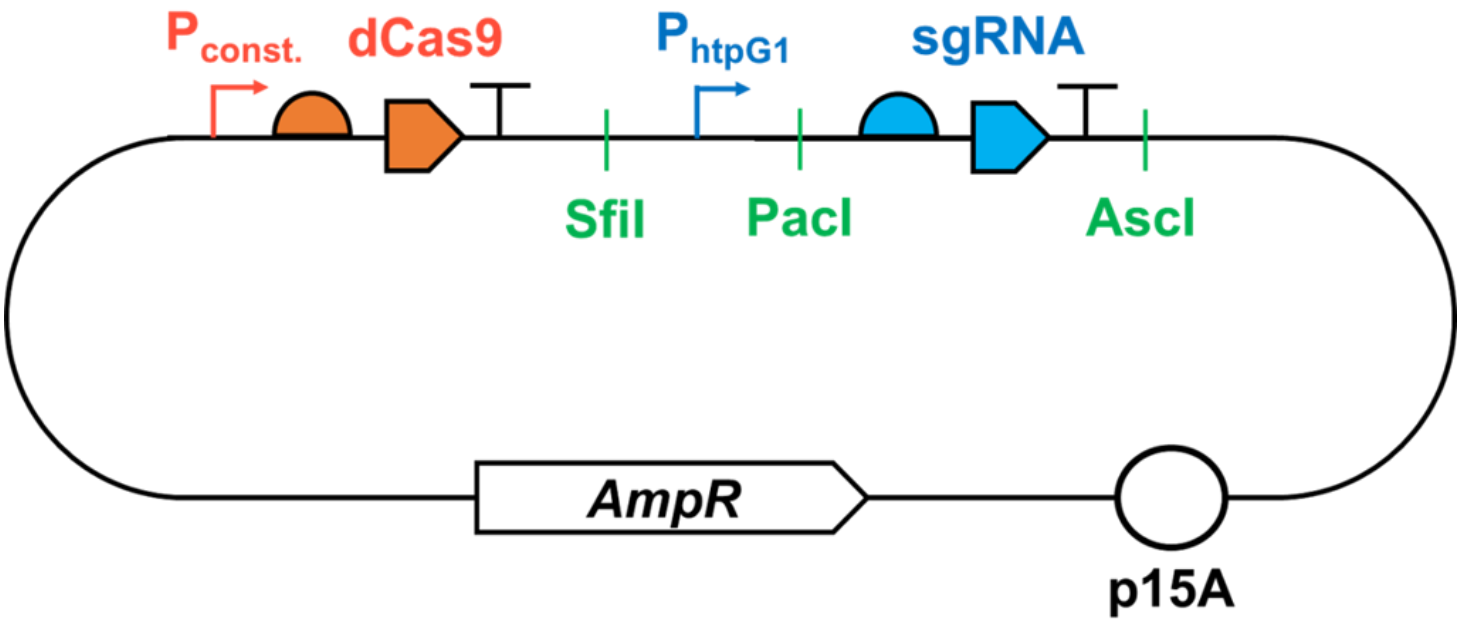

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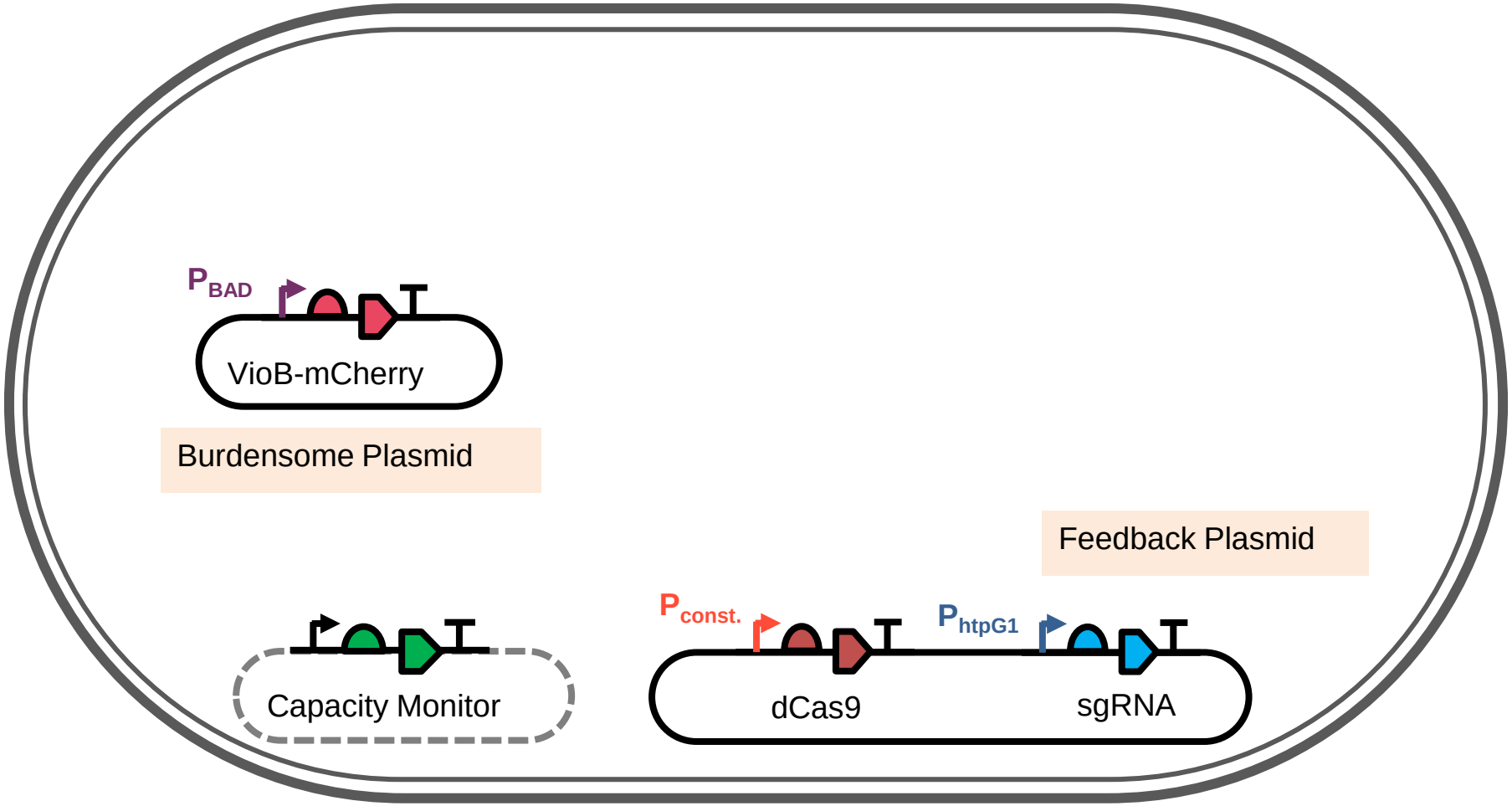
A biomolecular feedback system



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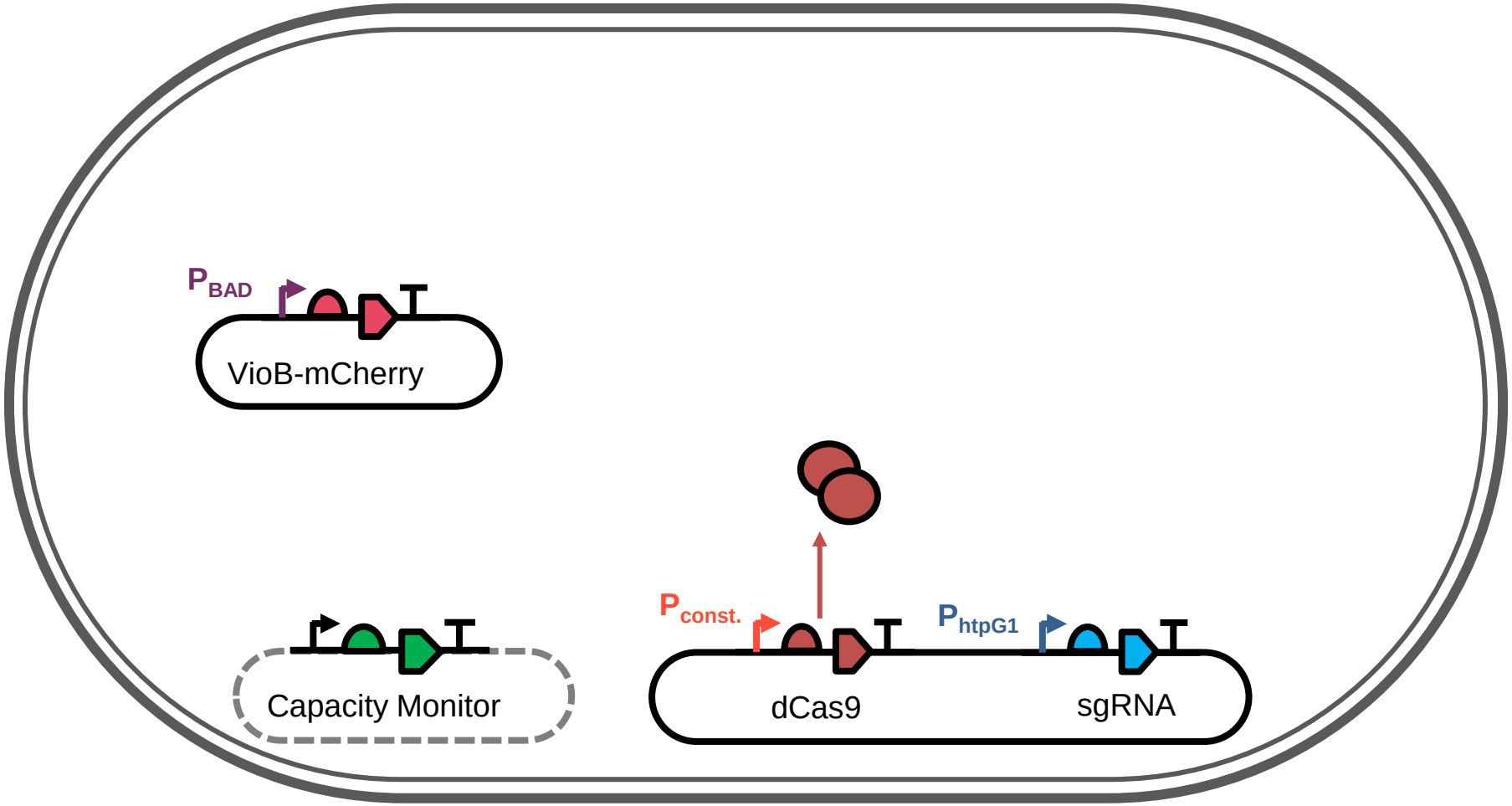
A biomolecular feedback system



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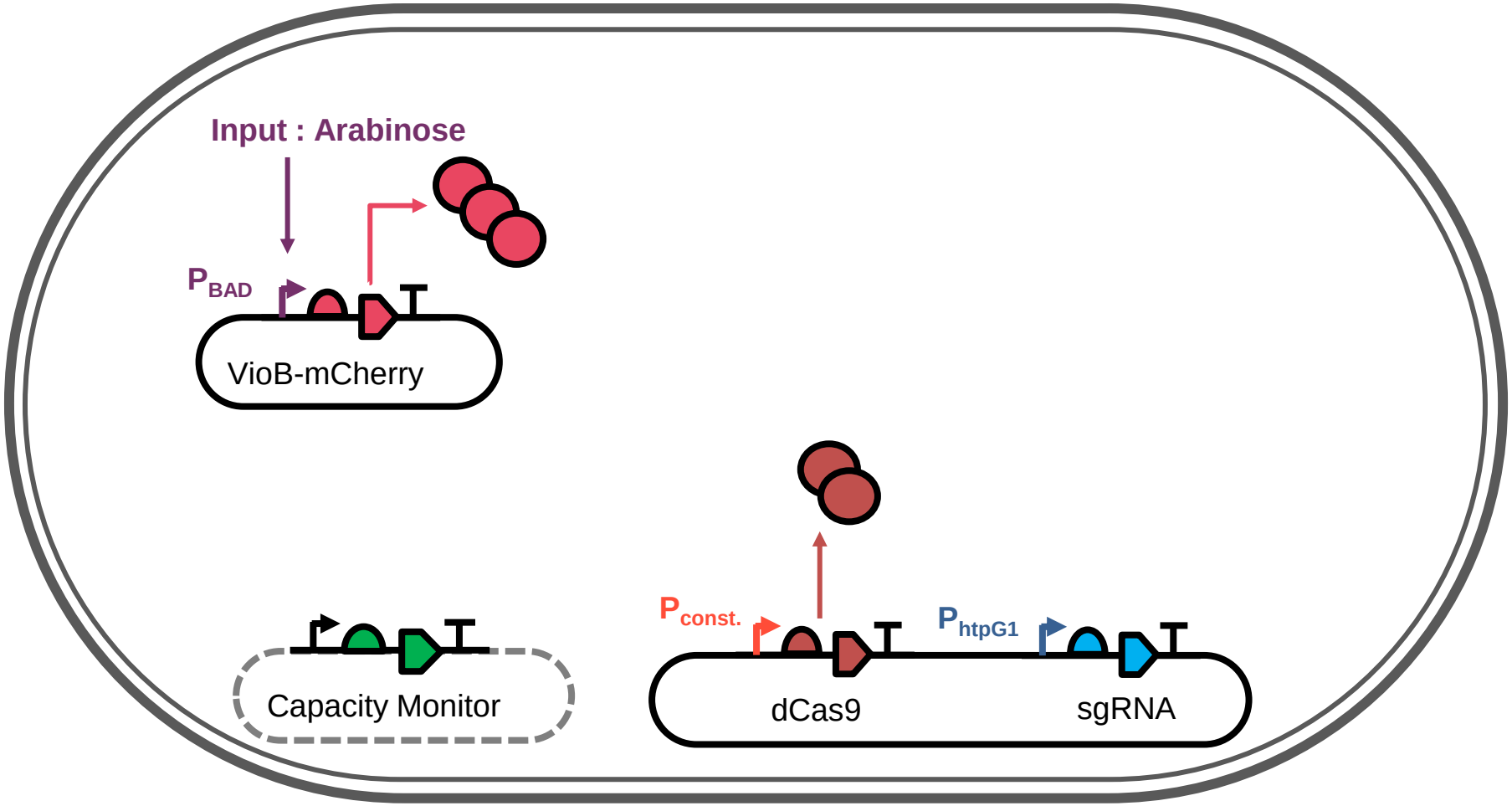
A biomolecular feedback system



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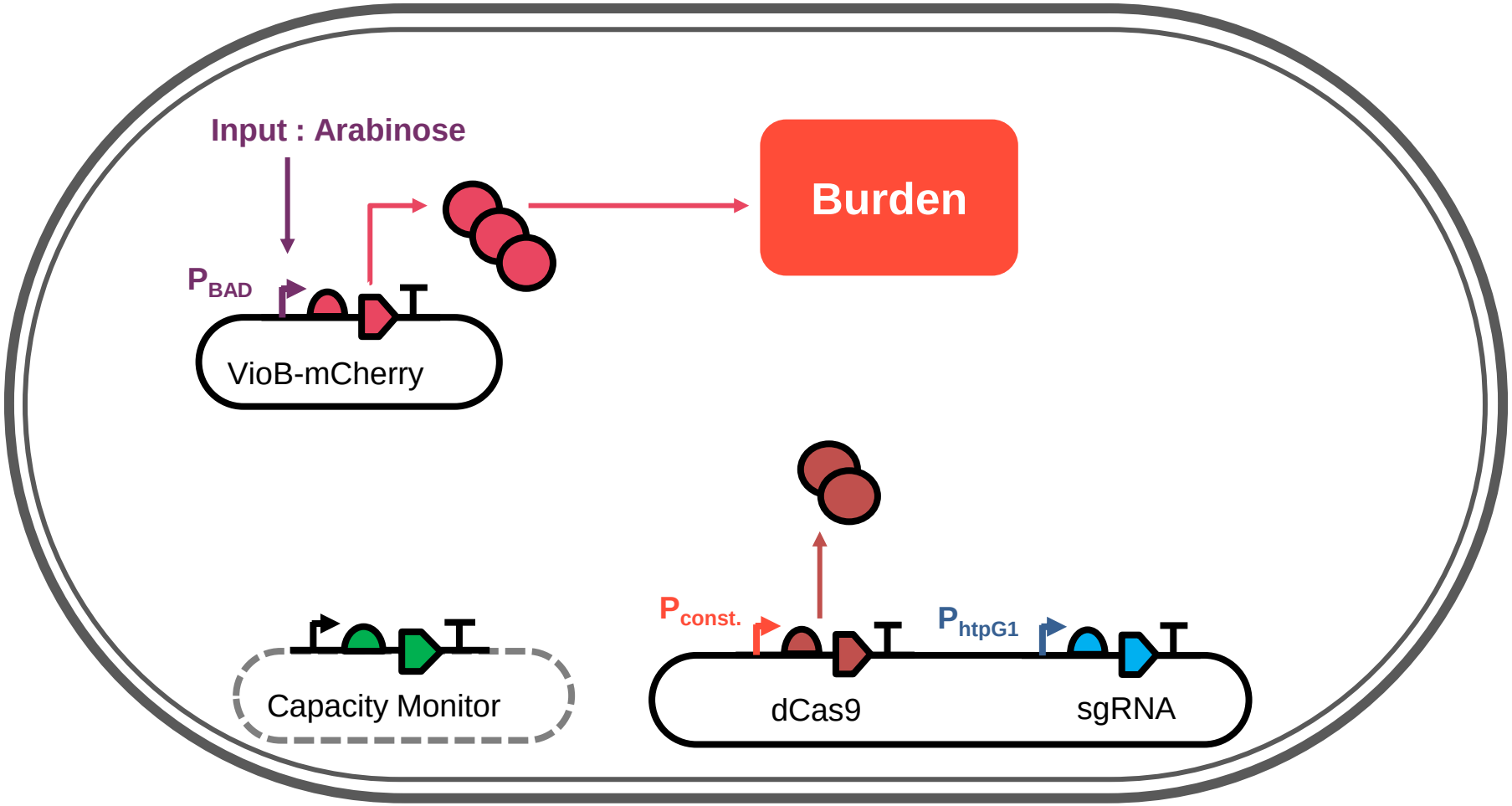

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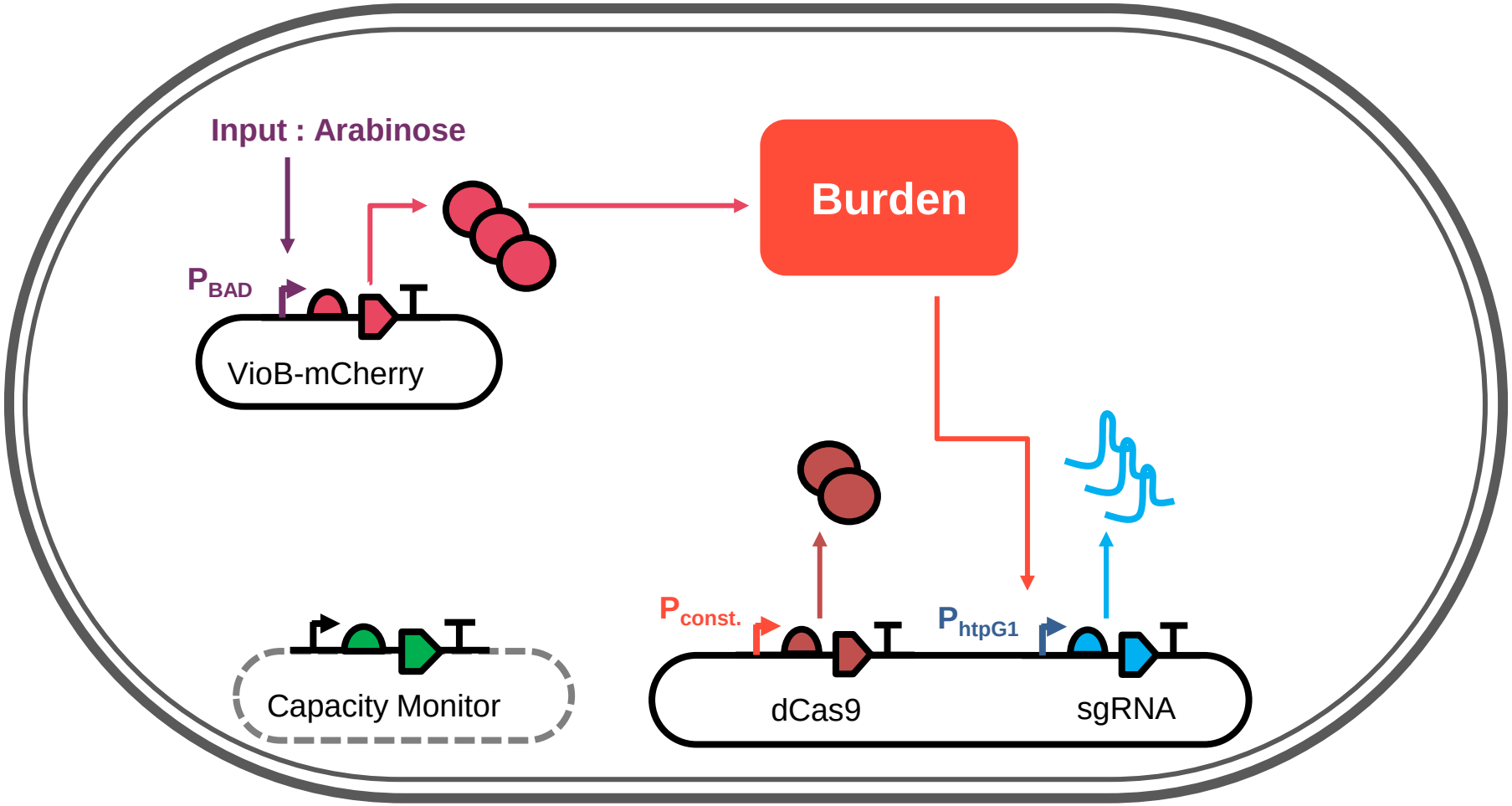
A biomolecular feedback system



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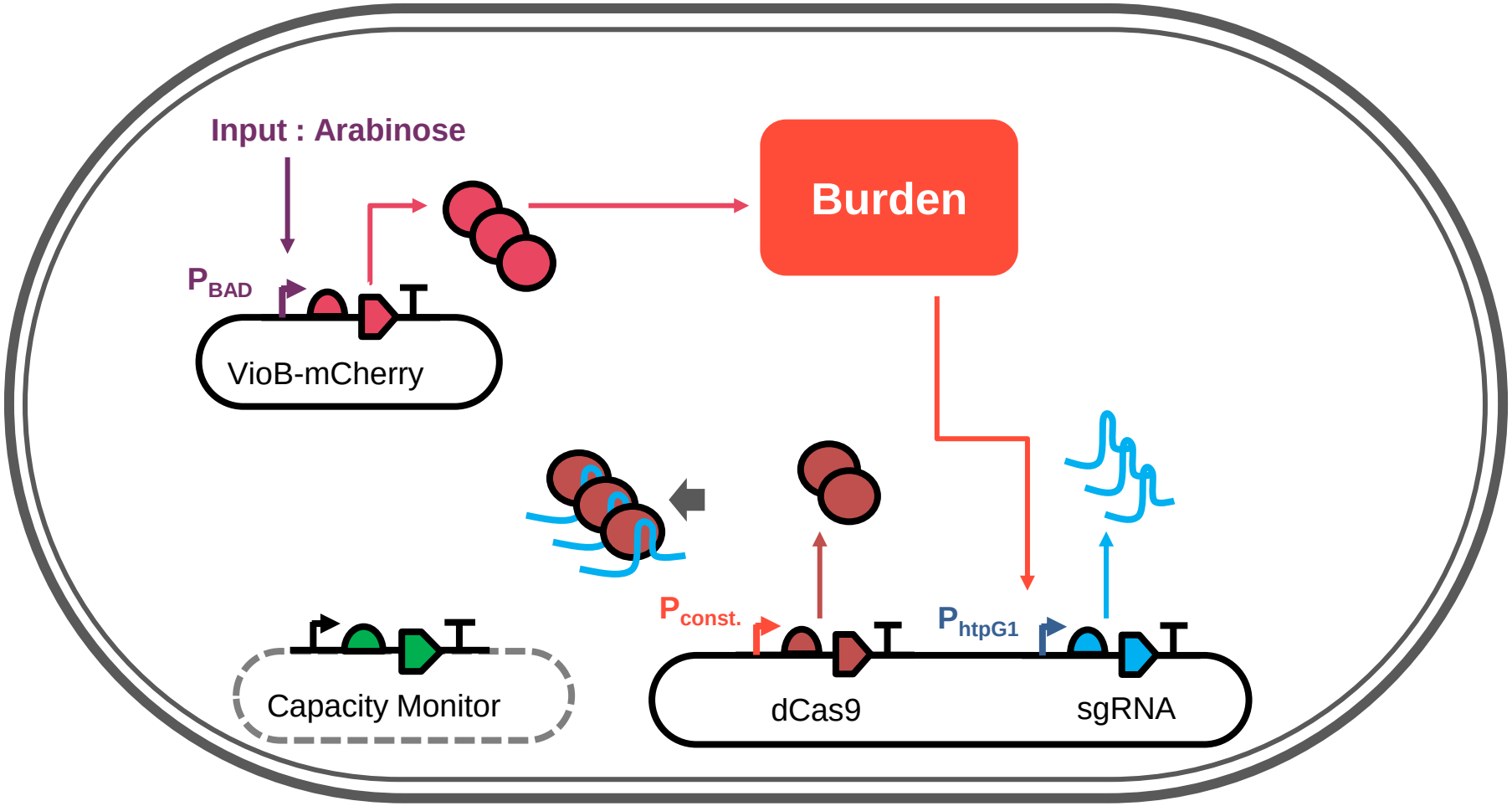

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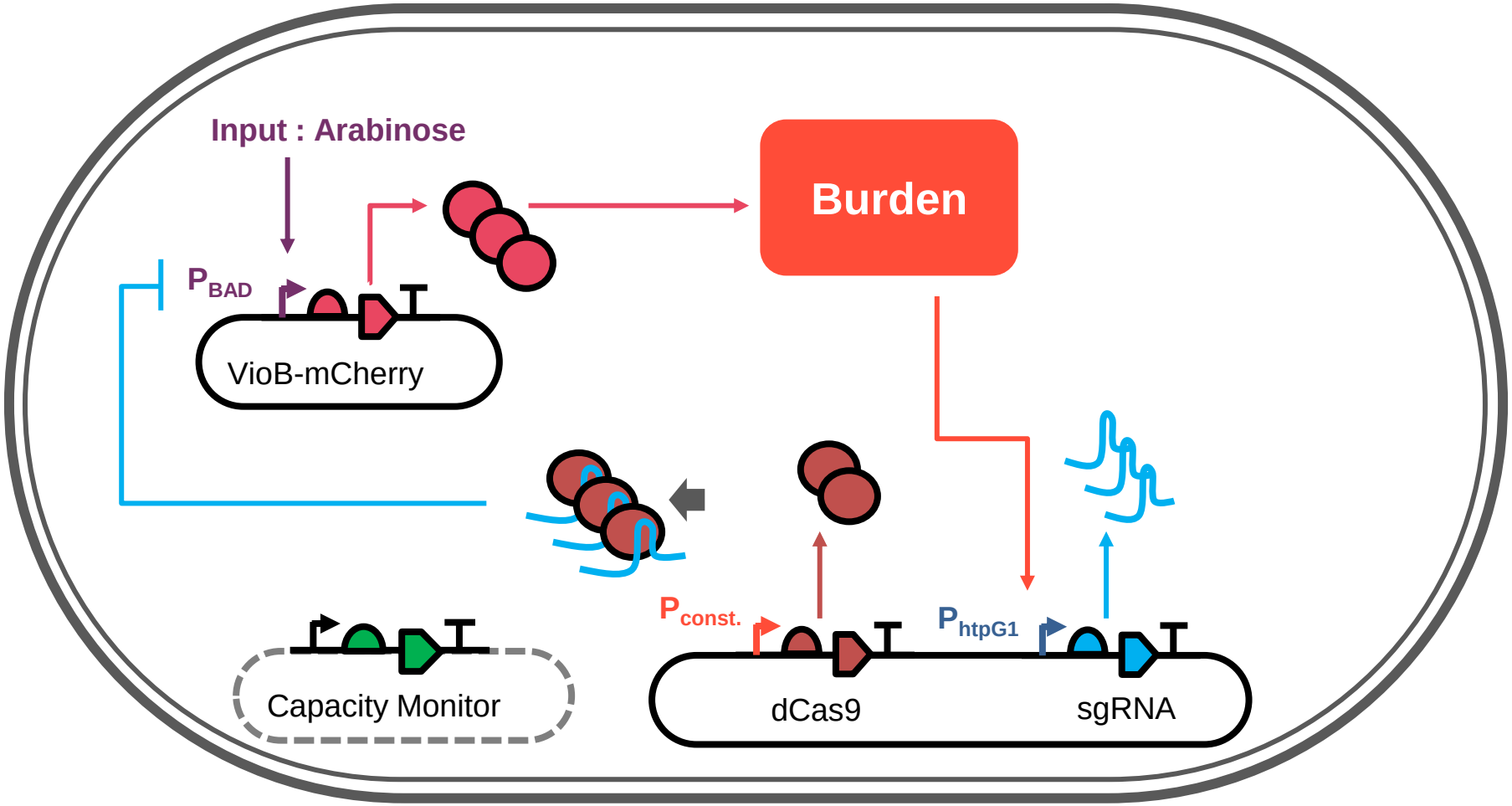

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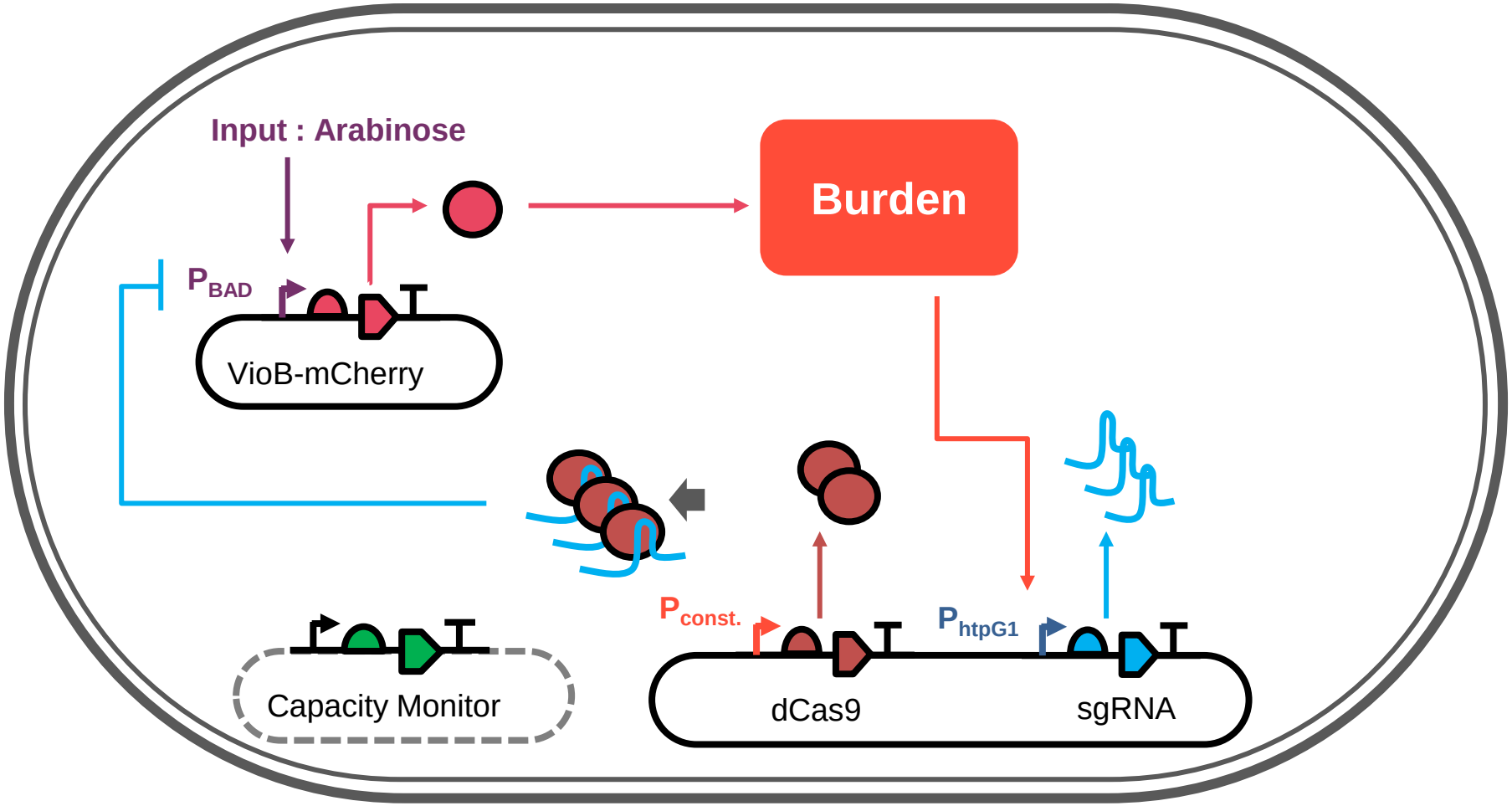

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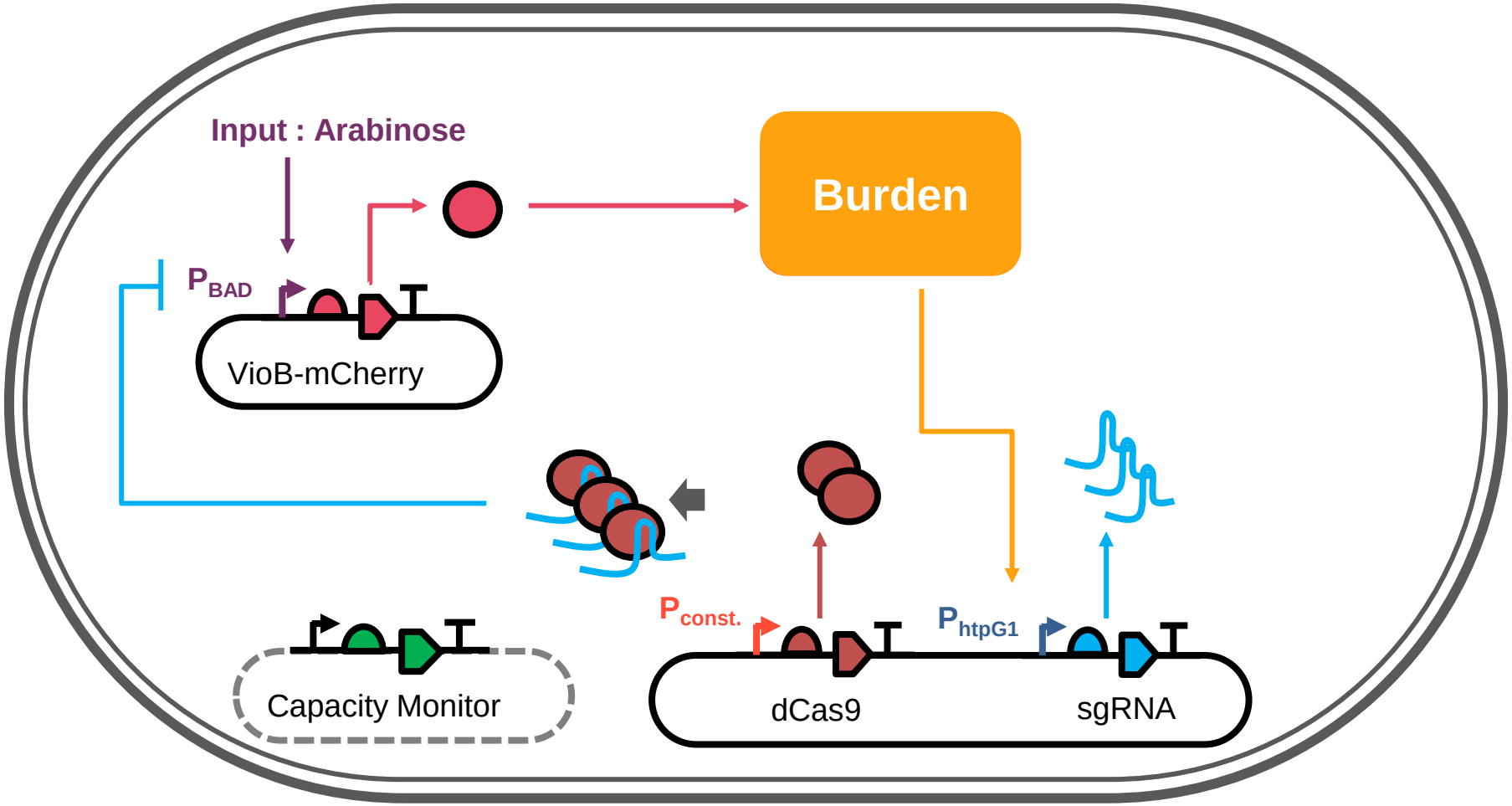

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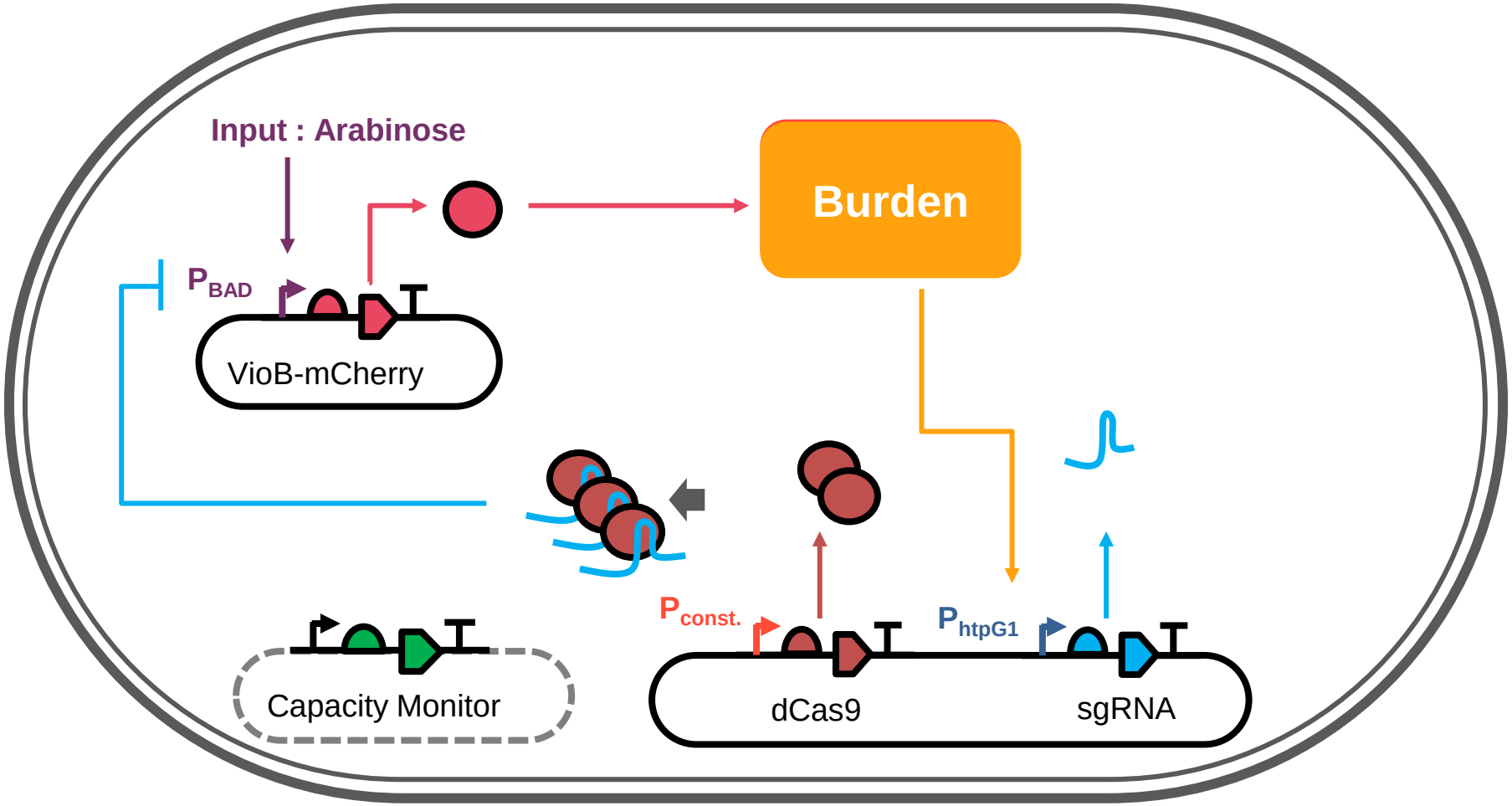

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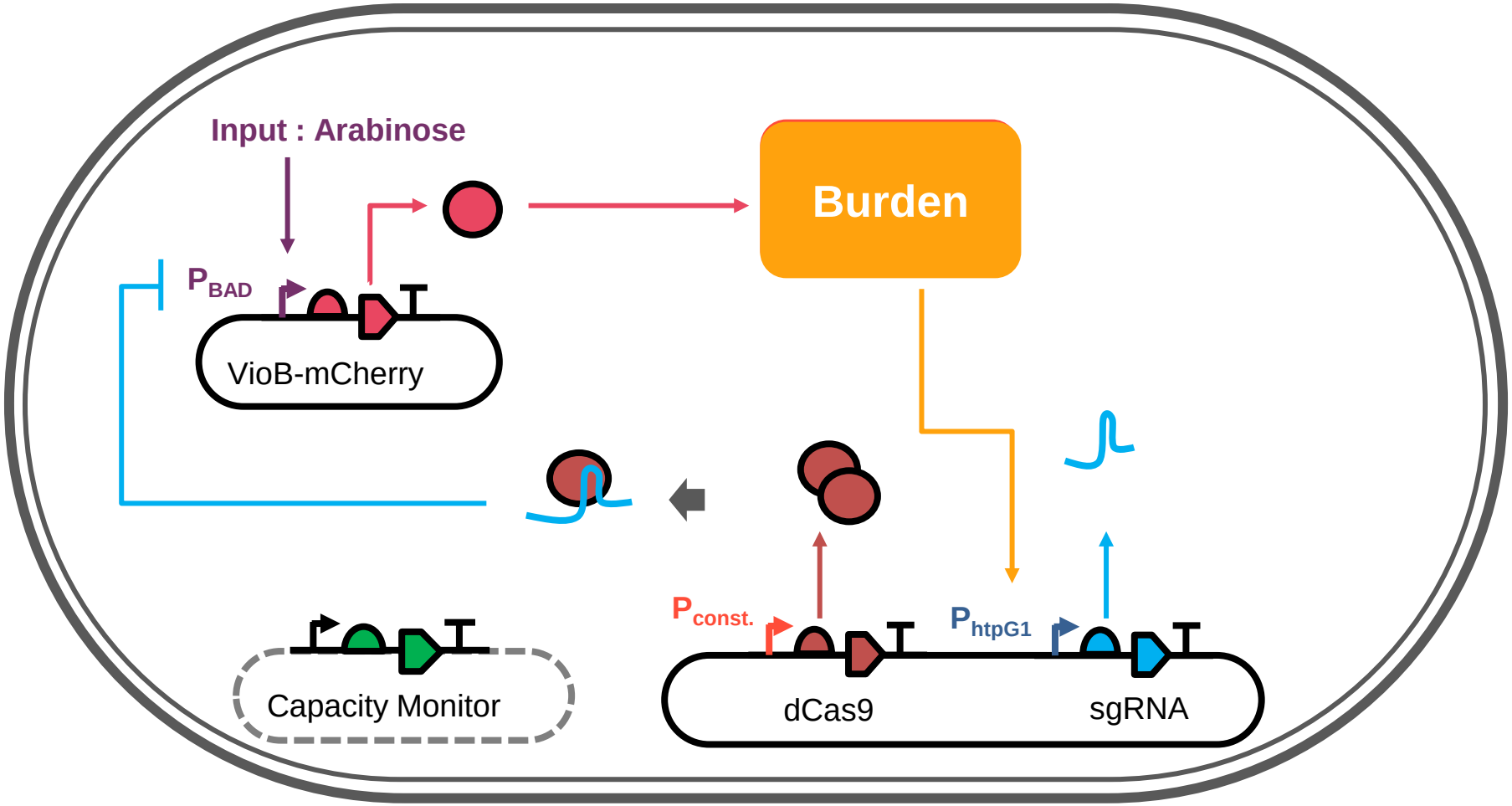

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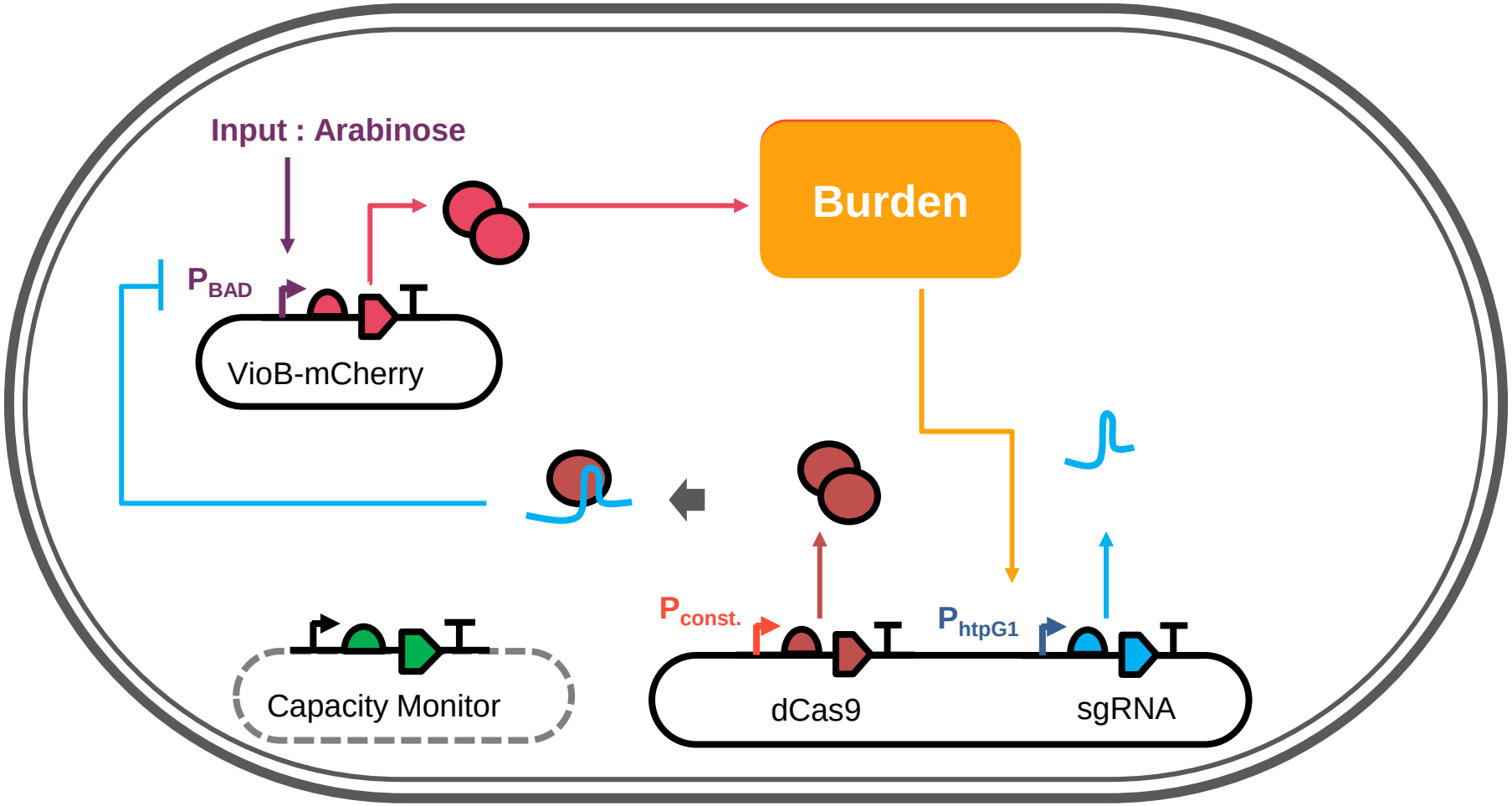

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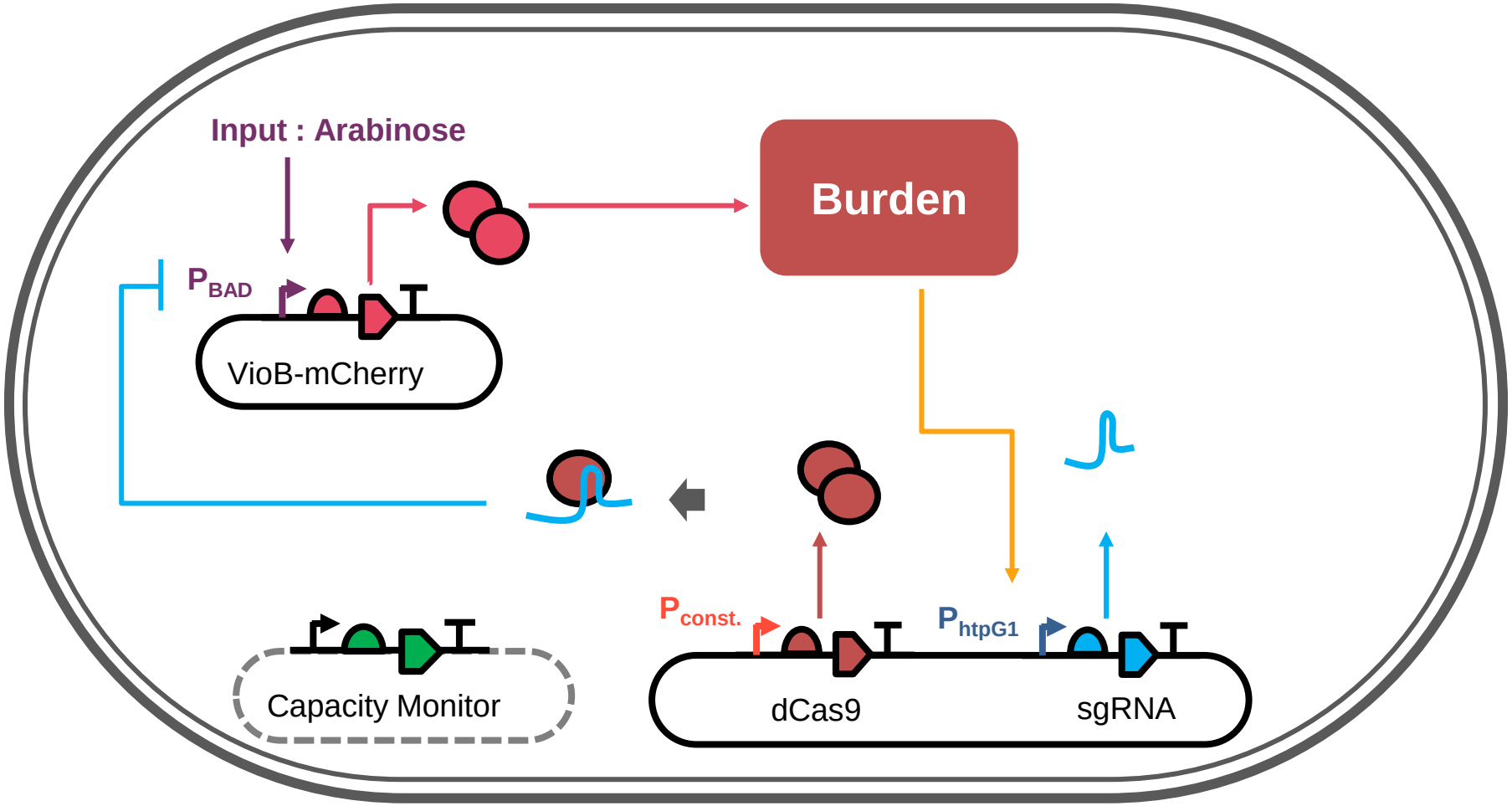

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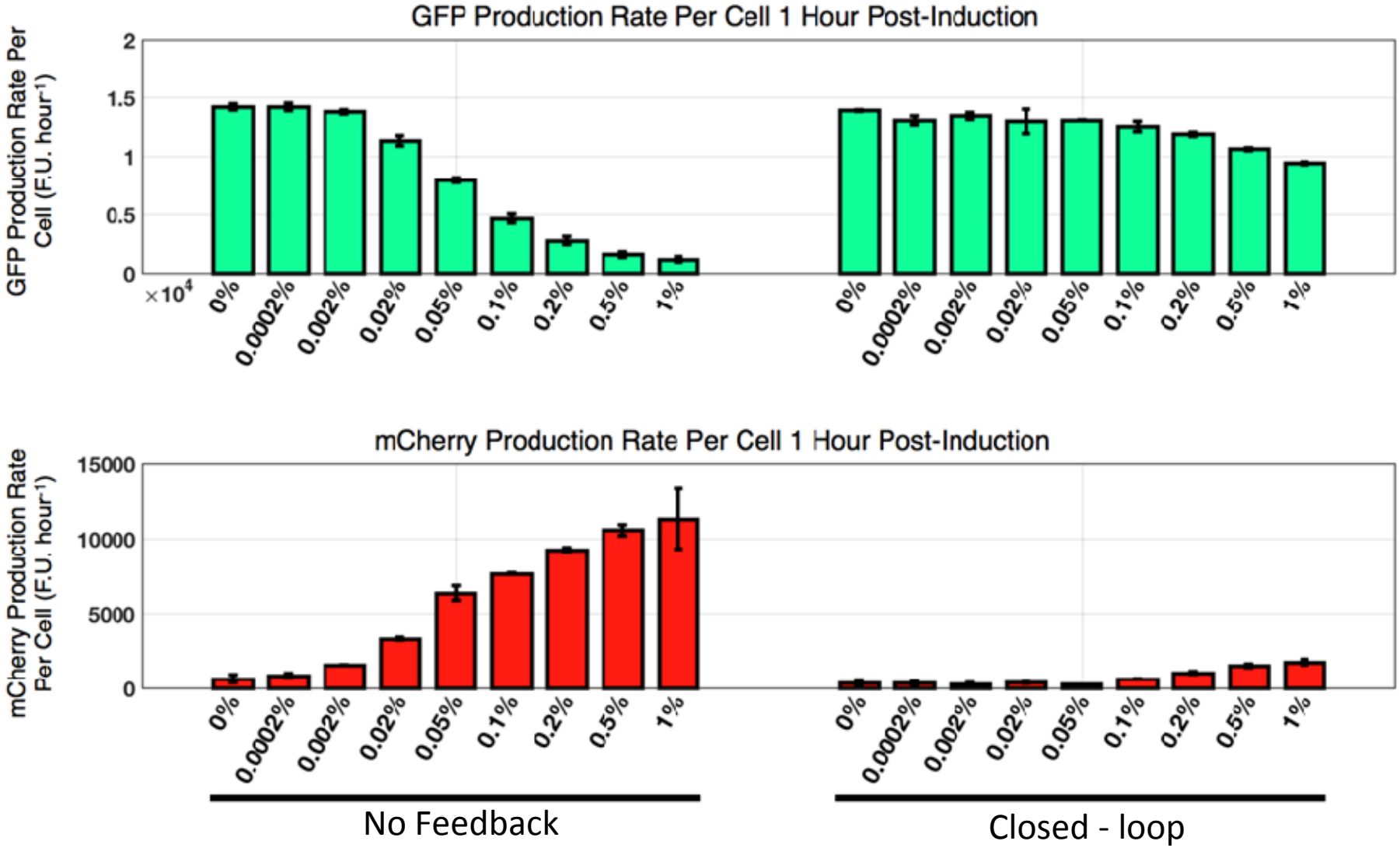
The feedback controls output expression leading to capacity rescue



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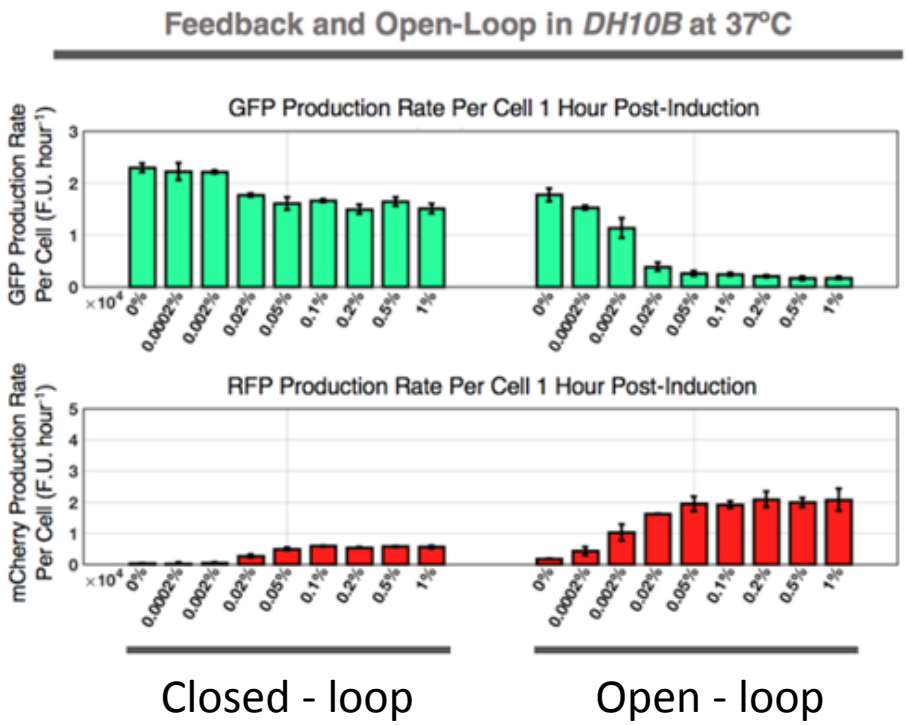
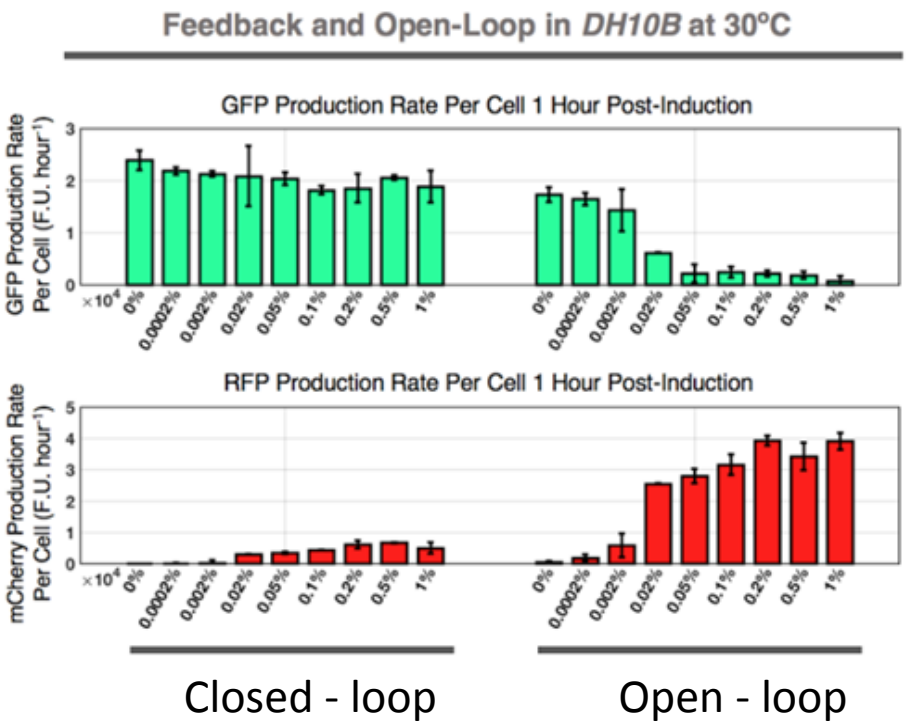
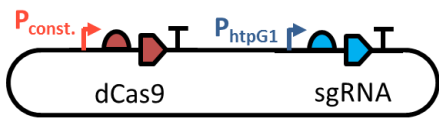
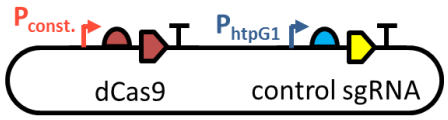
Robustness
The feedback control at different temperatures





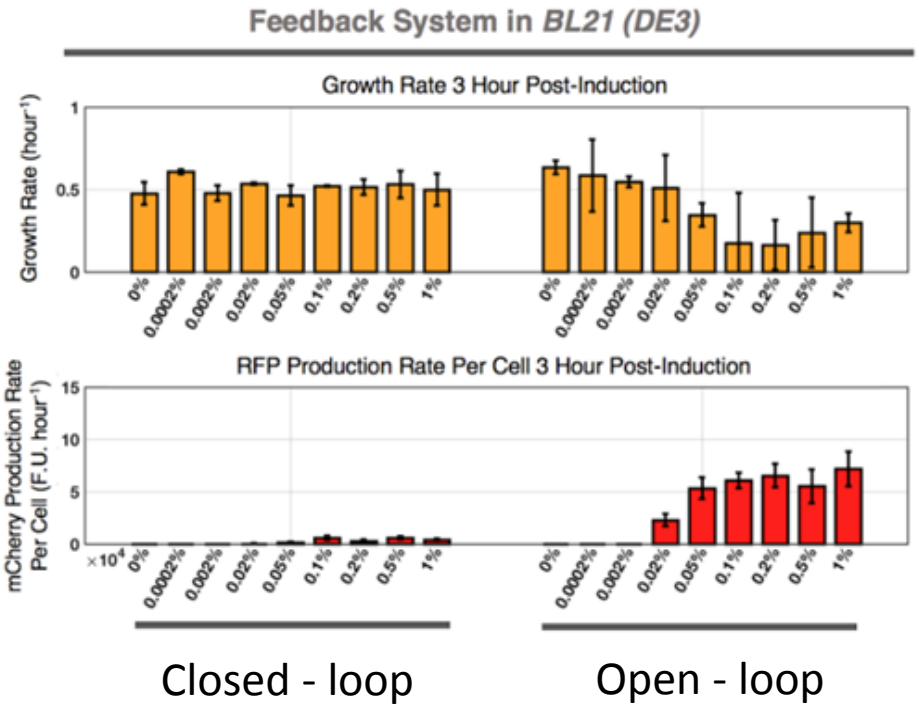




***Ceroni F** et al, Stan GB, Ellis T. **Nature Methods** under revision*

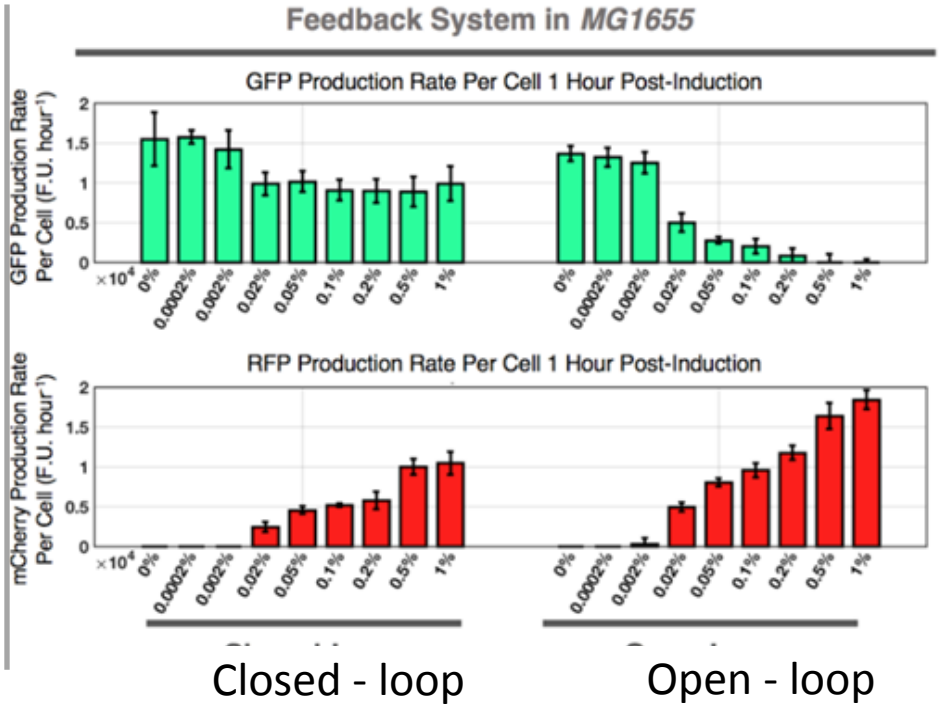
Robustness

The feedback control in different genetic backgrounds



Closed - loop

Open - loop



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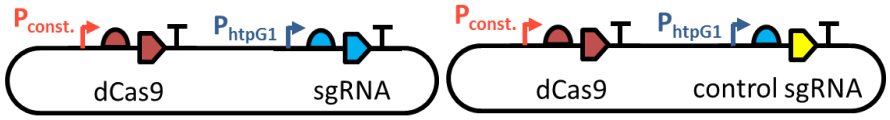

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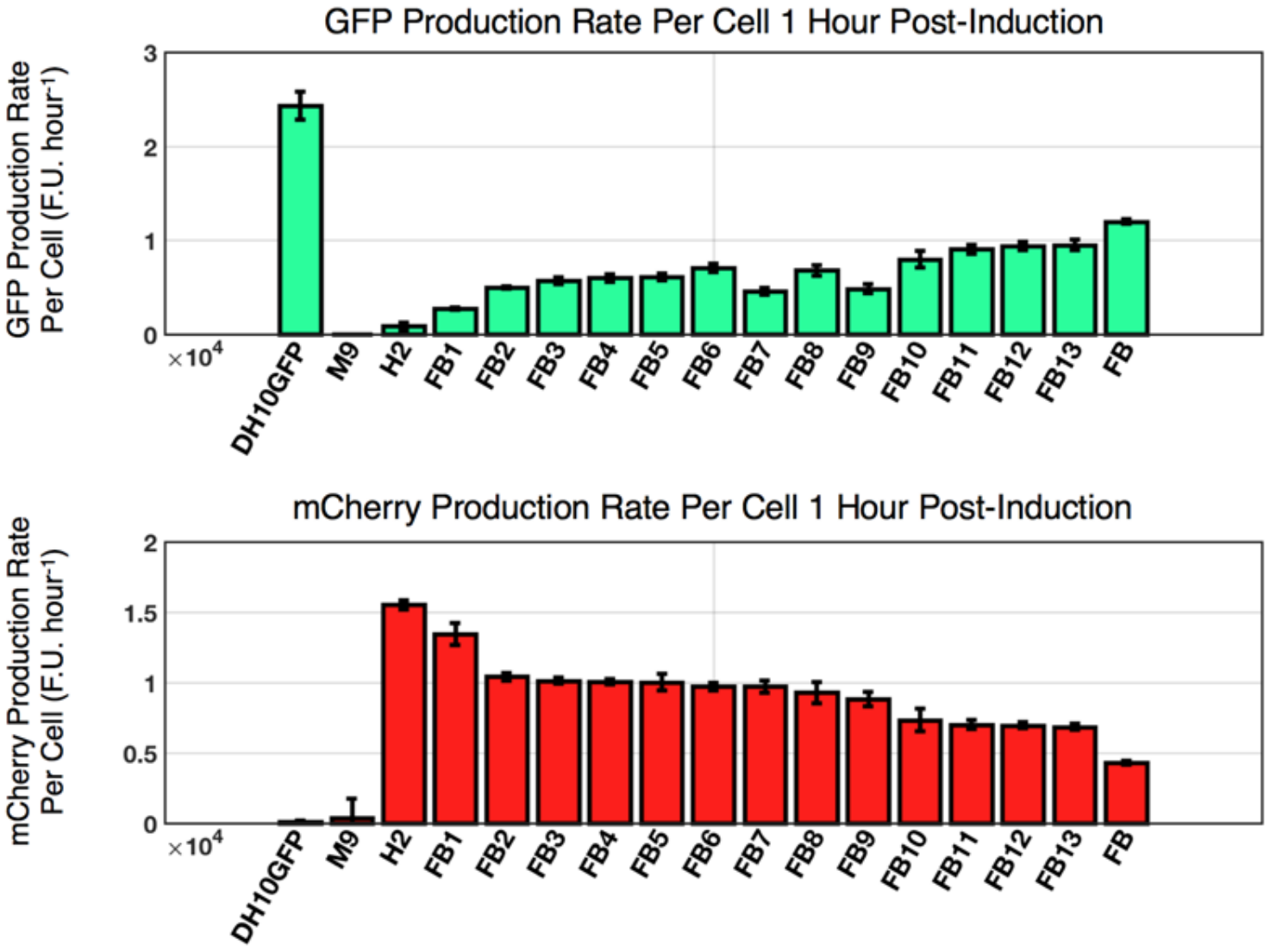
Tunability

A library of gRNAs to get the desired output



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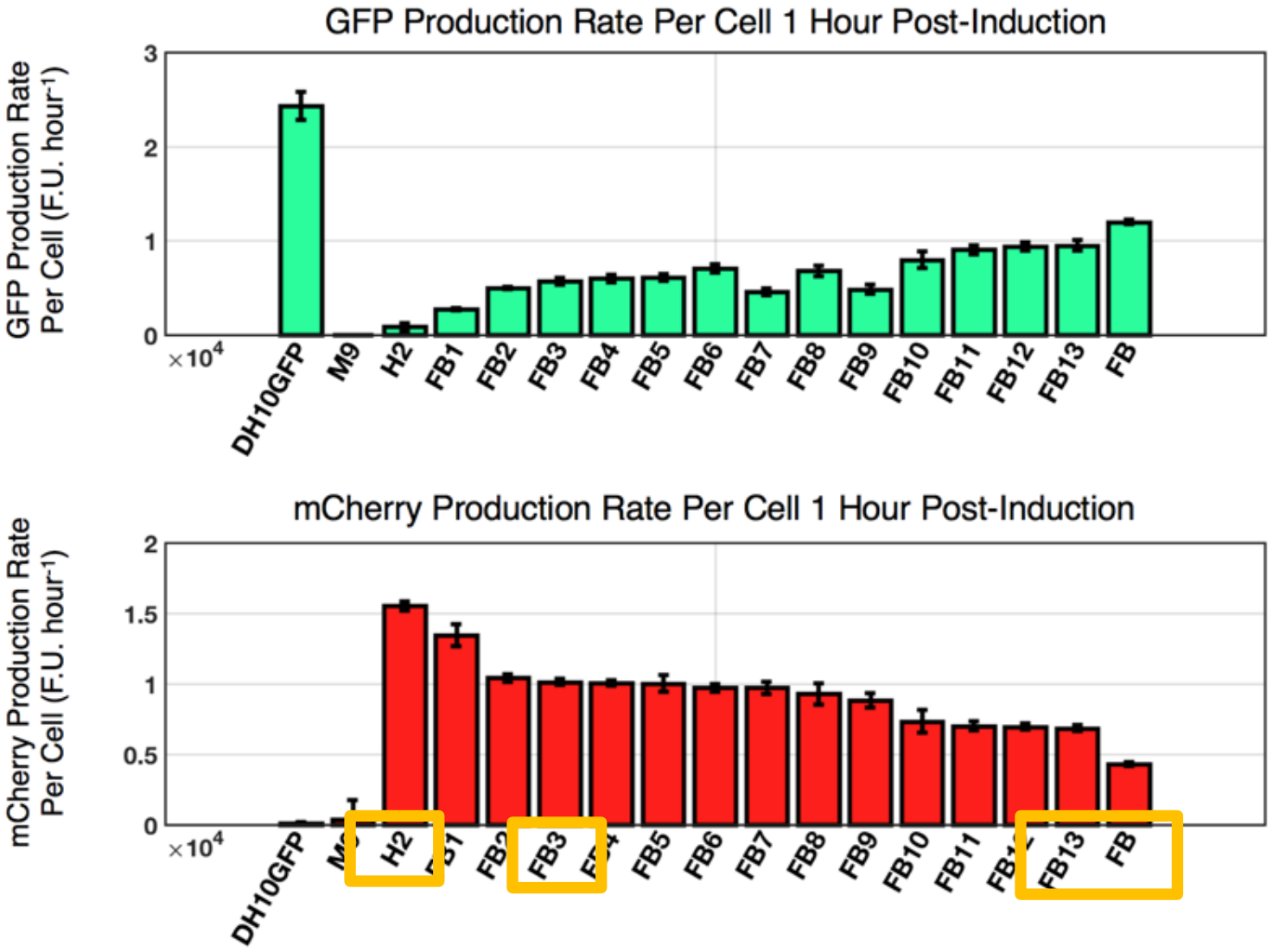
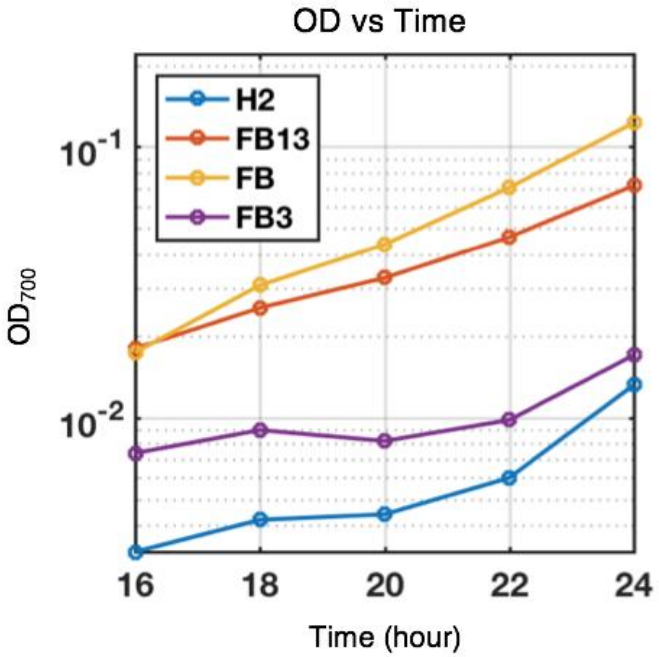
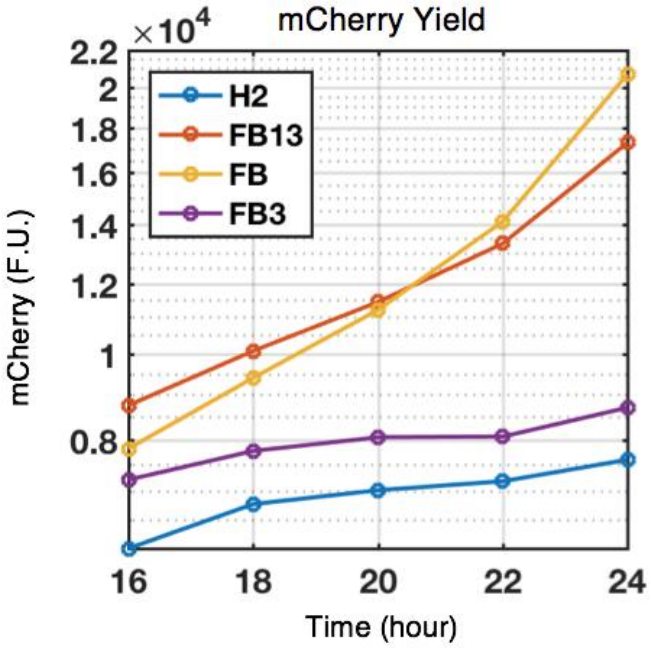


Performance of the feedback over 24 hours





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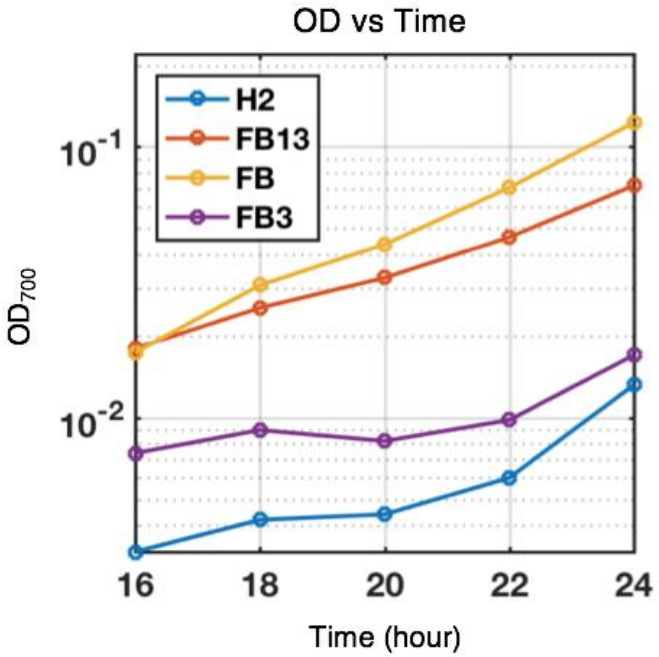
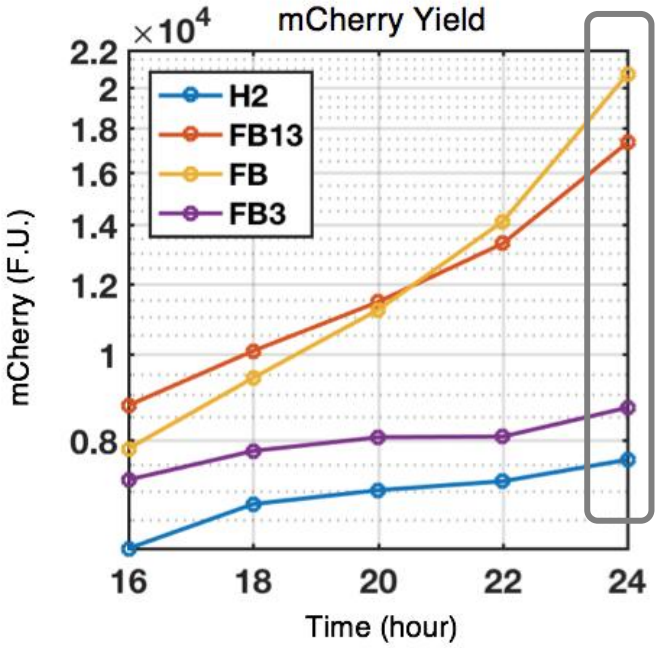
Performance of the feedback over 24 hours





Total yield is higher when the feedback is in place

Ceroni F *et al, Stan GB, Ellis T.* ***Nature Methods*** *under revision*

Conclusions

- Look at the impact of synthetic constructs in vivo and with RNAseq;

- Identify global transcriptional changes occurring upon burden induction;

- Develop a burden- based molecular feedback that robustly controls gene expression

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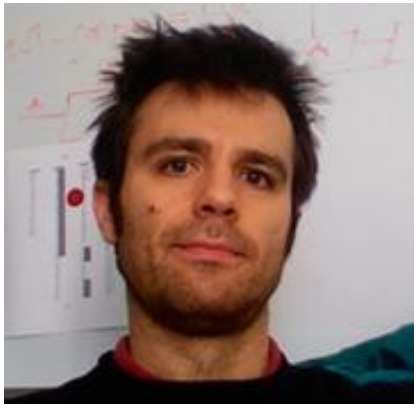

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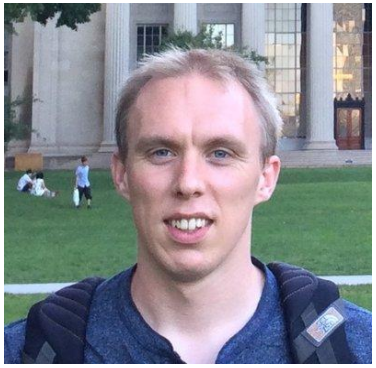

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Ali Awan

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Chemical Engineering Department

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Junior Research Fellowship Scheme

Questions