

# SCIE5508

## Synthetic Biology – Solving global challenges

Workshop 1 – Designing orthogonal circuits based on  
extracytoplasmic function (ECF)  $\sigma$  factors



THE UNIVERSITY OF  
**WESTERN**  
AUSTRALIA

**Dr. Georg Fritz**  
Senior Lecturer  
School of Molecular Sciences



# Roadmap for today



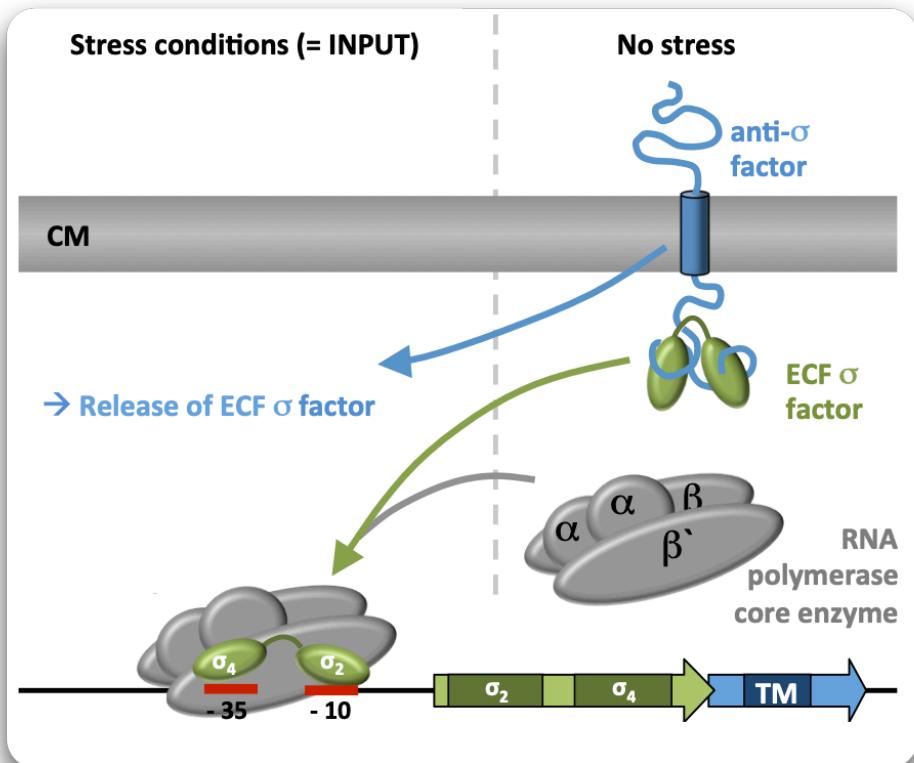
- 1. Recap of ECF-based synthetic circuits**
- 2. Recap of MoClo system**
- 3. Introduction to Geneious: Build your first GFP expression cassette**
- 4. Hands-on: *in silico* cloning of your own ECF circuit**



# Circumventing regulatory cross-talk through orthogonal systems

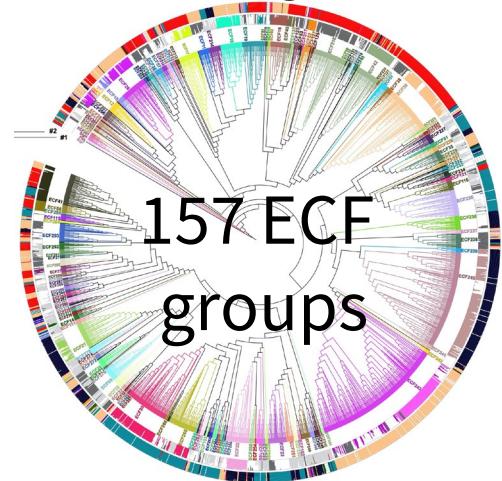


Extracytoplasmic function (ECF)  
 $\sigma$  factors control orthogonal regulons  
 under stress conditions

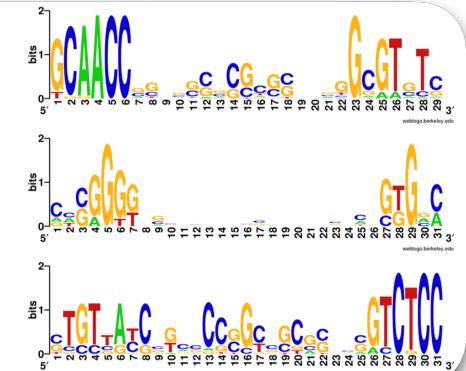


Mascher, Curr. Opin. Microbiol. (2013)

Diverse ECF groups feature different promoter preference

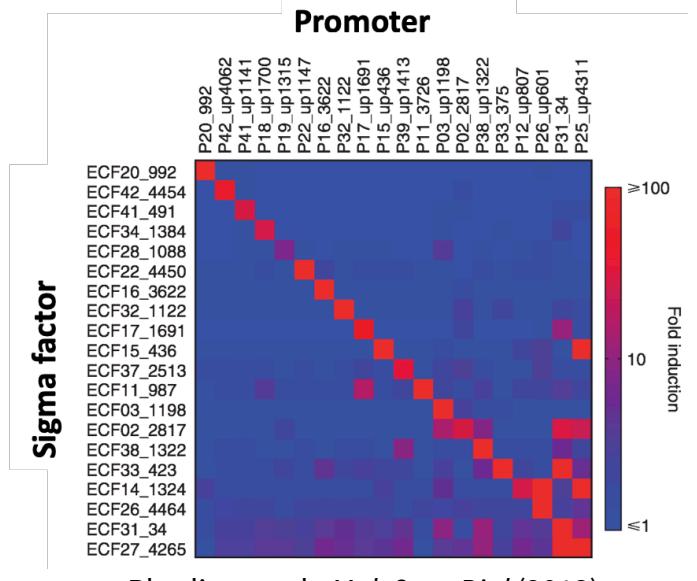


Casas-Pastor et al, bioRxiv (2019)  
<https://doi.org/10.1101/2019.12.11.873521>



Huang, Pinto, Fritz & Mascher, J. Bacteriol. (2015)

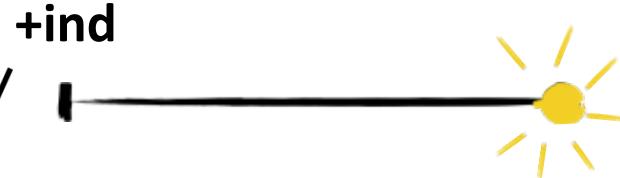
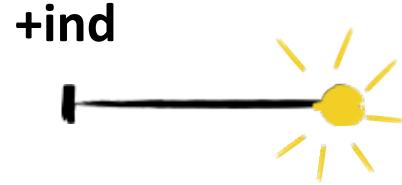
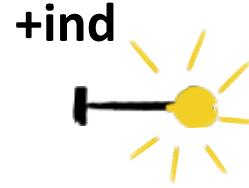
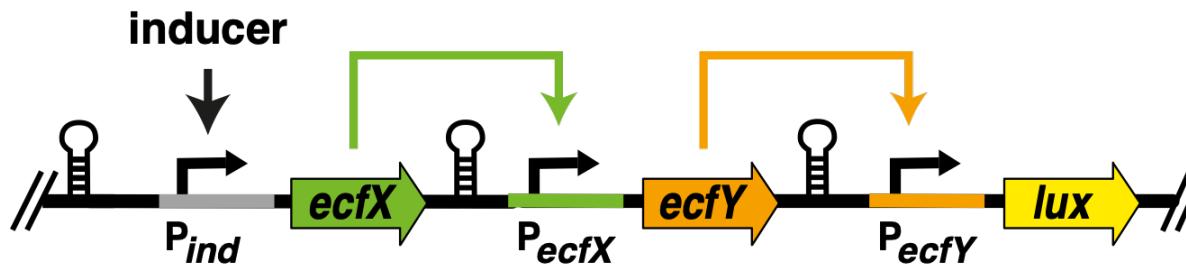
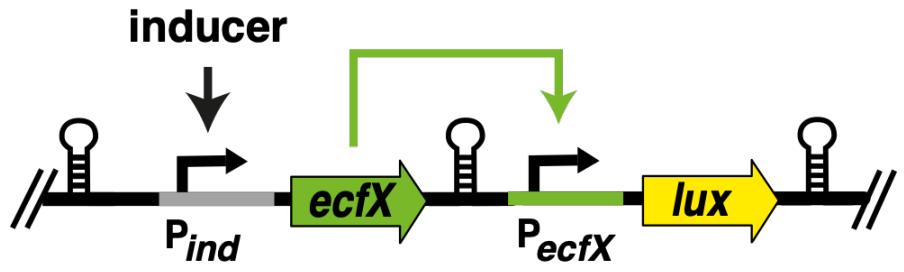
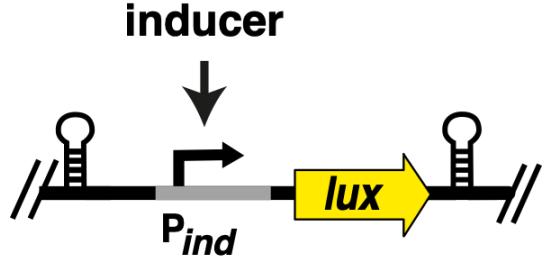
**Heterologous expression of ECFs from diverse species in *E. coli* leads to recognition of cognate promoters**



Rhodius, et al., Mol. Syst. Biol (2013)



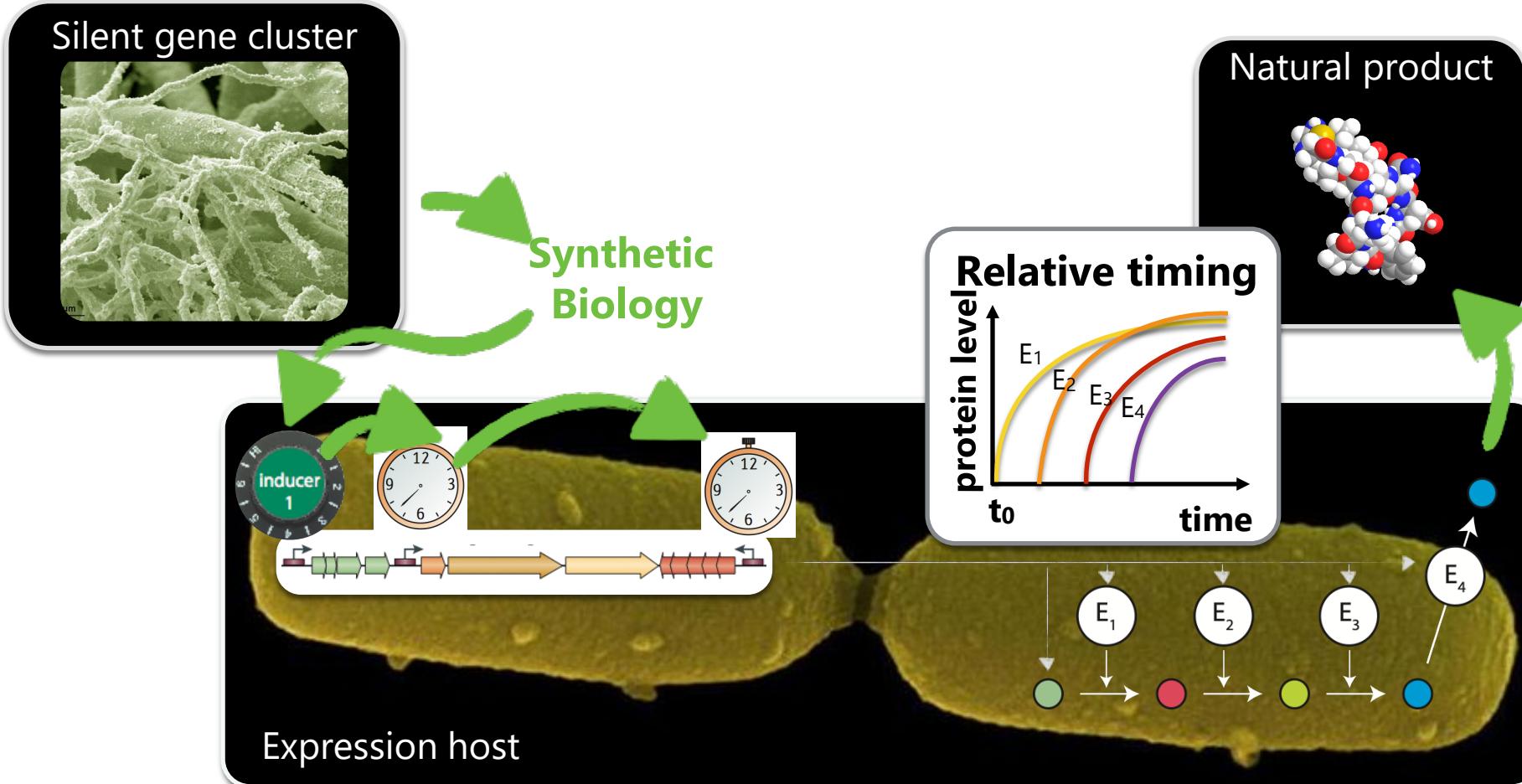
# Pinto et al provide proof of concept: Building autonomous timer circuits

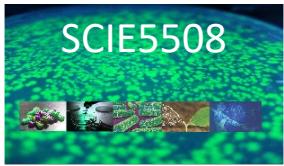


time delay



# Application of autonomous timer circuits

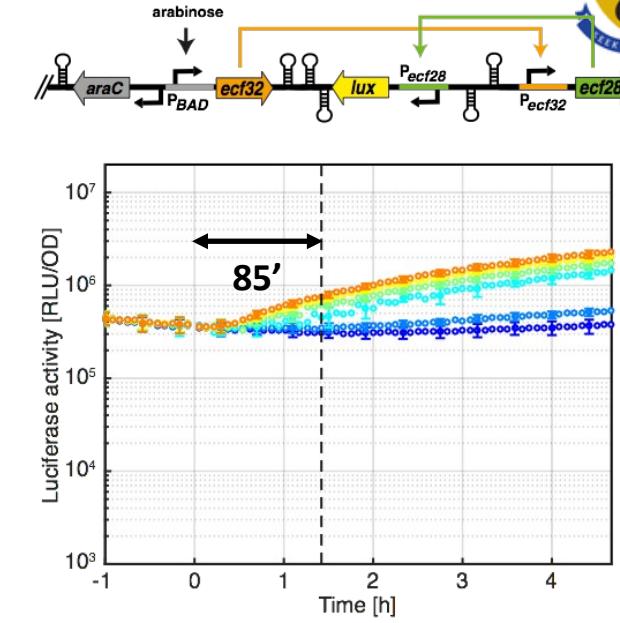
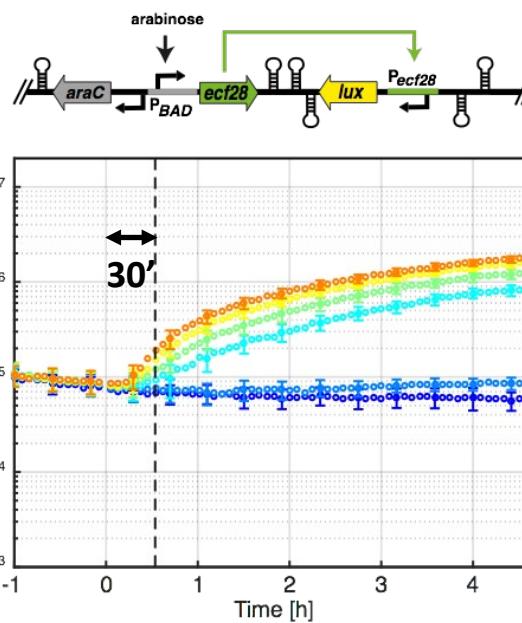
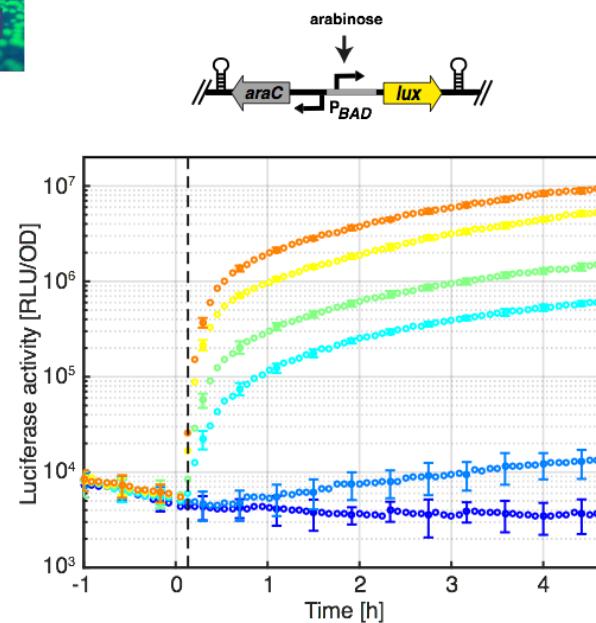




# Autonomous timers in *E. coli*



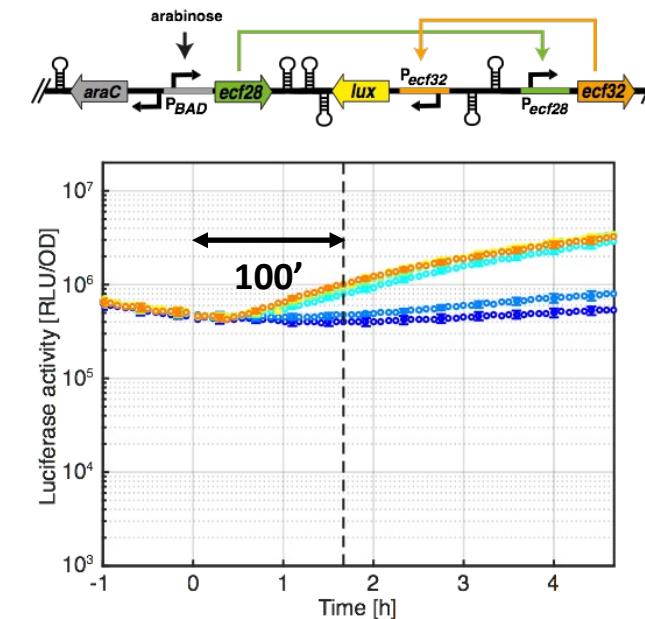
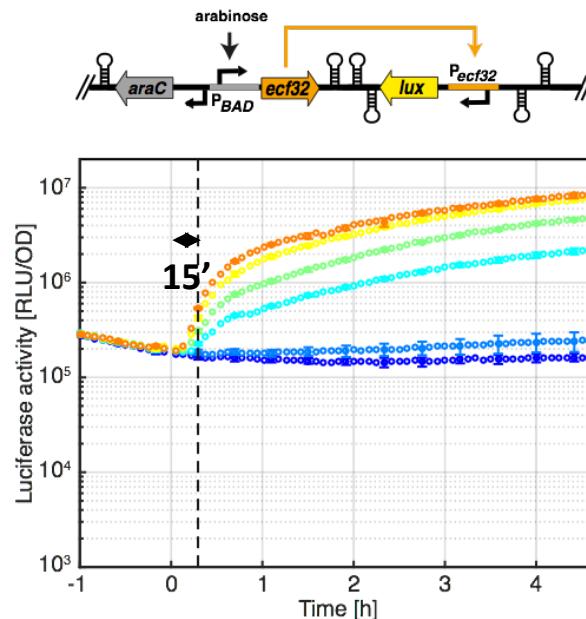
THE UNIVERSITY OF  
WESTERN  
AUSTRALIA



arabinose [%]

- 0
- $1 \times 10^{-6}$
- $1 \times 10^{-5}$
- $2 \times 10^{-5}$
- $5 \times 10^{-5}$
- $1 \times 10^{-4}$

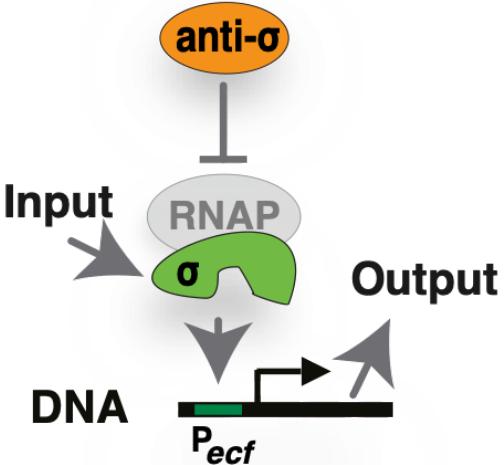
Pinto, Vecchione *et al.*,  
NAR, 2018



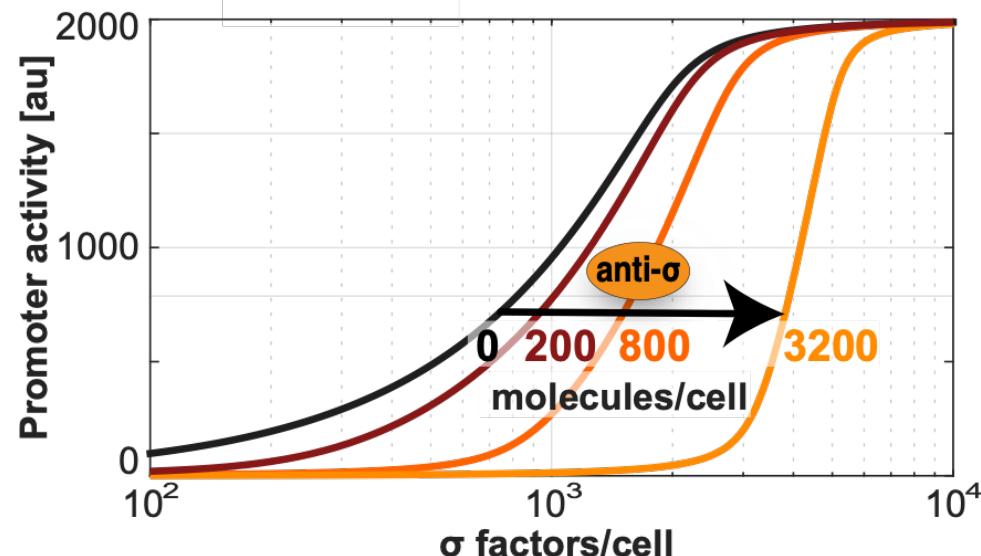


SCIE5508

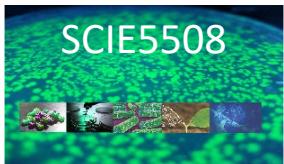
# Digitalising switching behaviour via anti- $\sigma$ factors



## Mathematical model

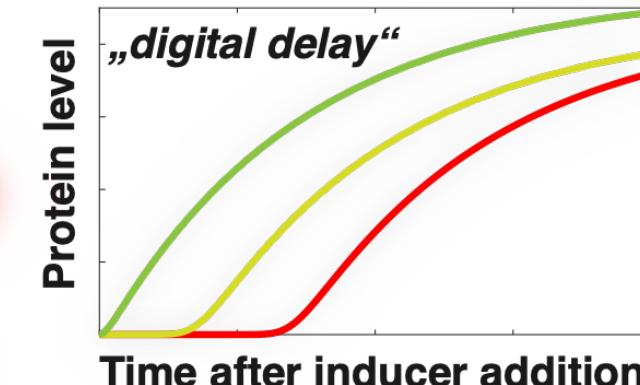
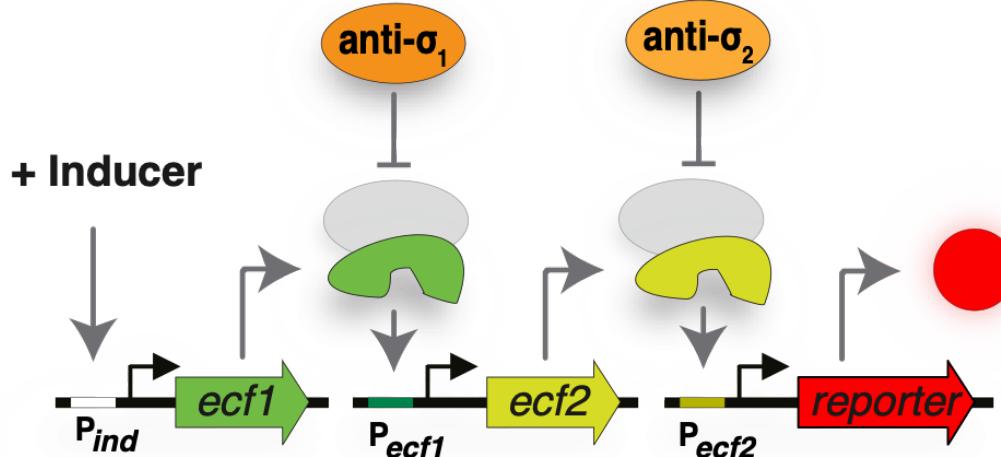
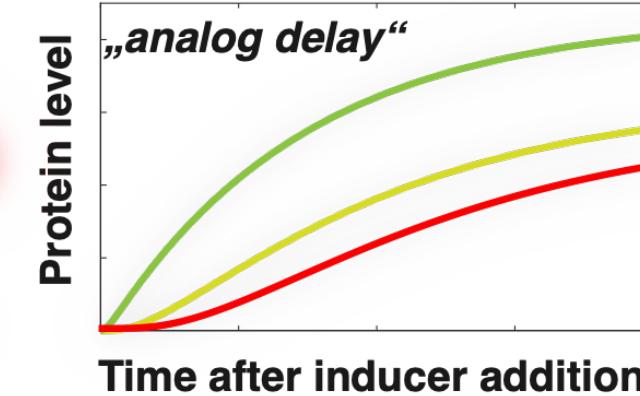
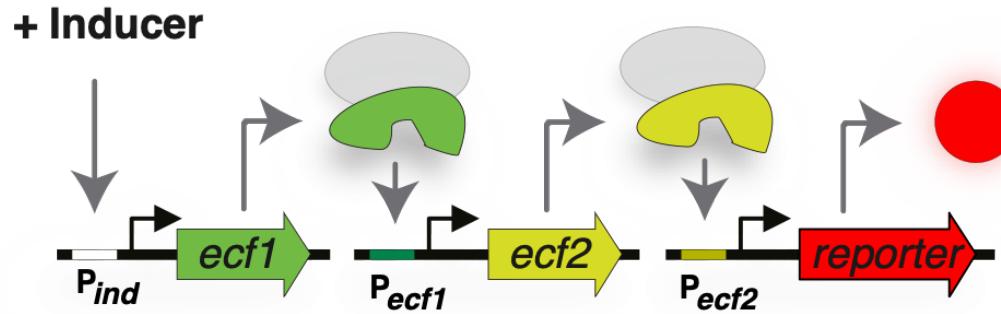


Tunable non-linearity!



SCIE5508

# Digitalising switching behaviour via anti- $\sigma$ factors





SCIE5508

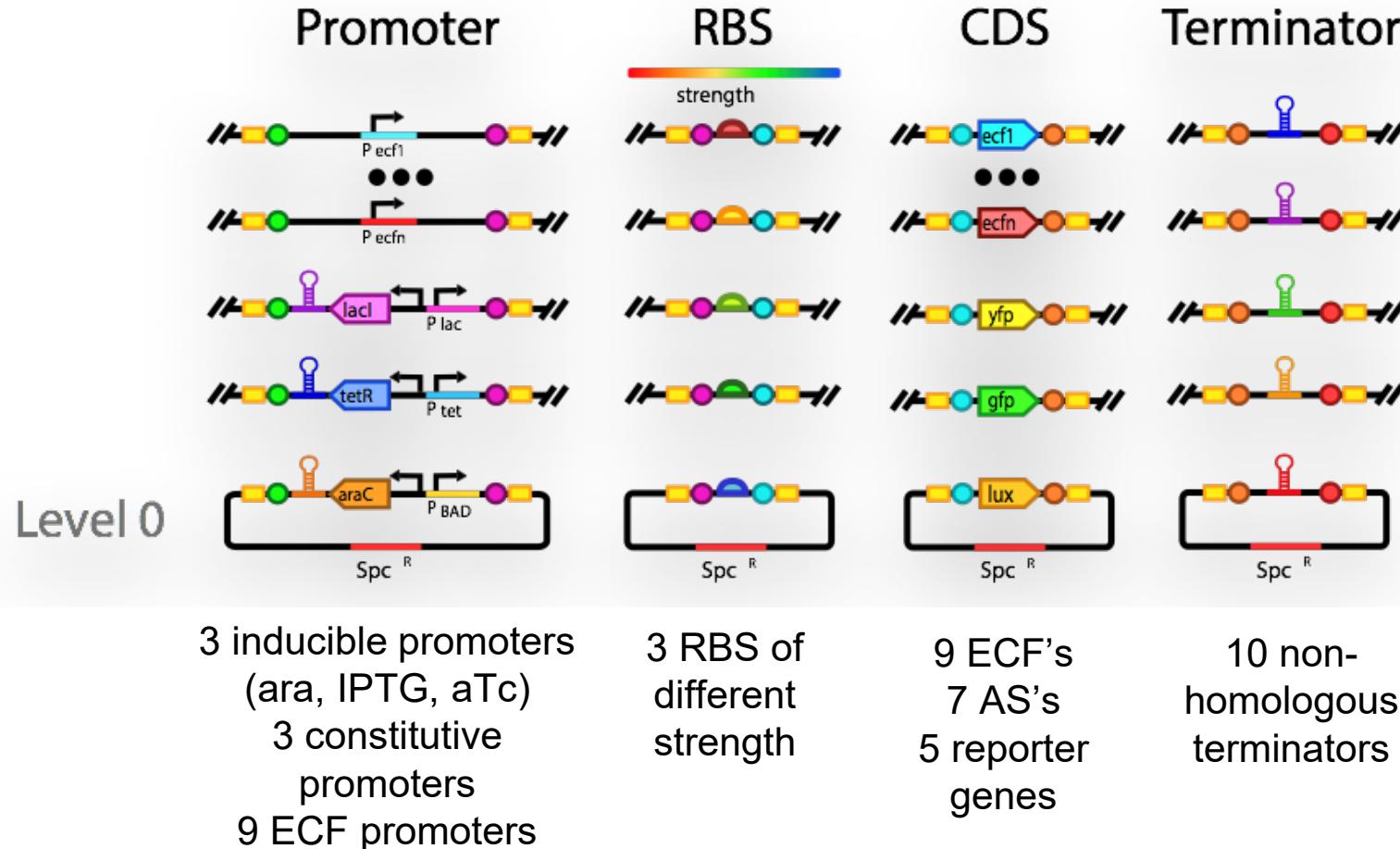
# Goal of this workshop



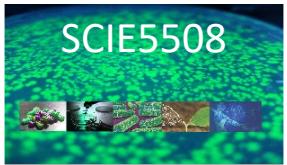


SCIE5508

# Library of parts

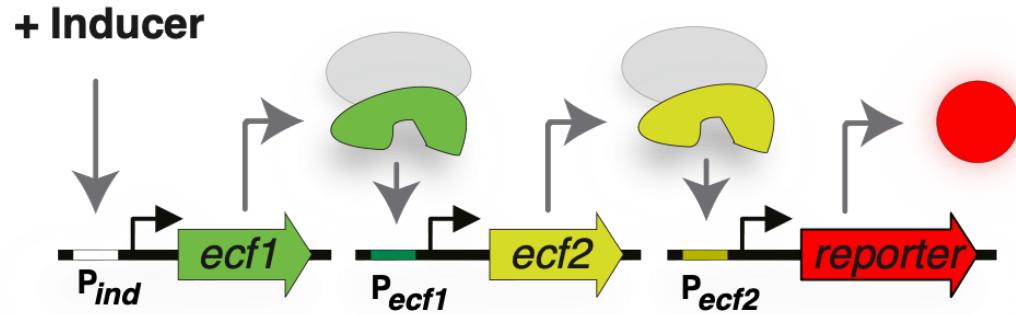


Available on LMS: SCIE5508 workshop1.geneious



# How to design a genetic circuit?

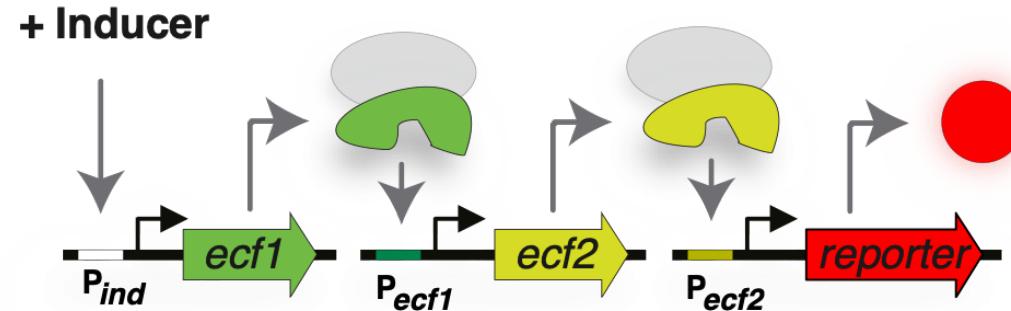
**1. Concept**  
**(anticipate desired function in a wiring diagram)**



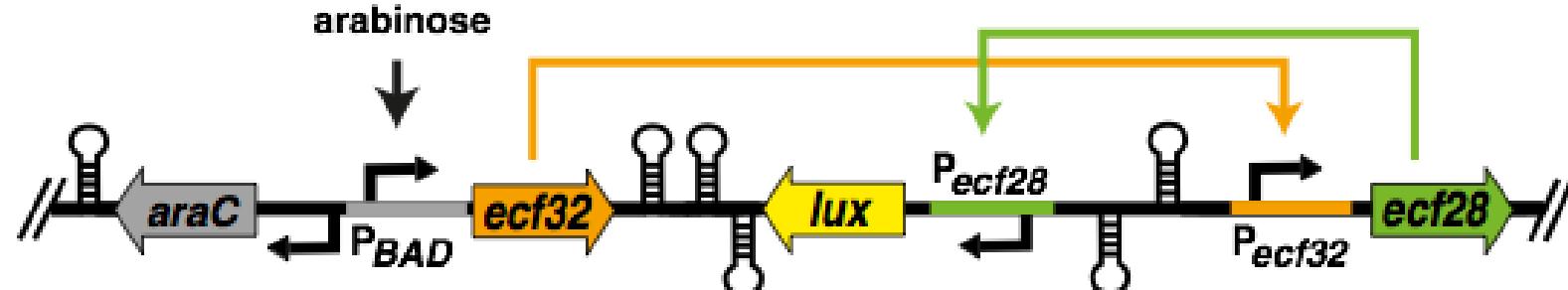
Detail

# How to design a genetic circuit?

**1. Concept**  
 (anticipate desired function in a wiring diagram)



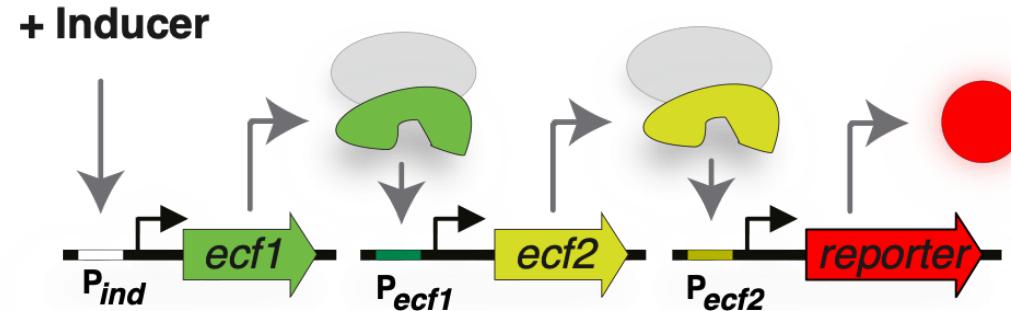
**2. Genetic design**  
 (envise the parts used, orientation of genes, which plasmids to use, ...)



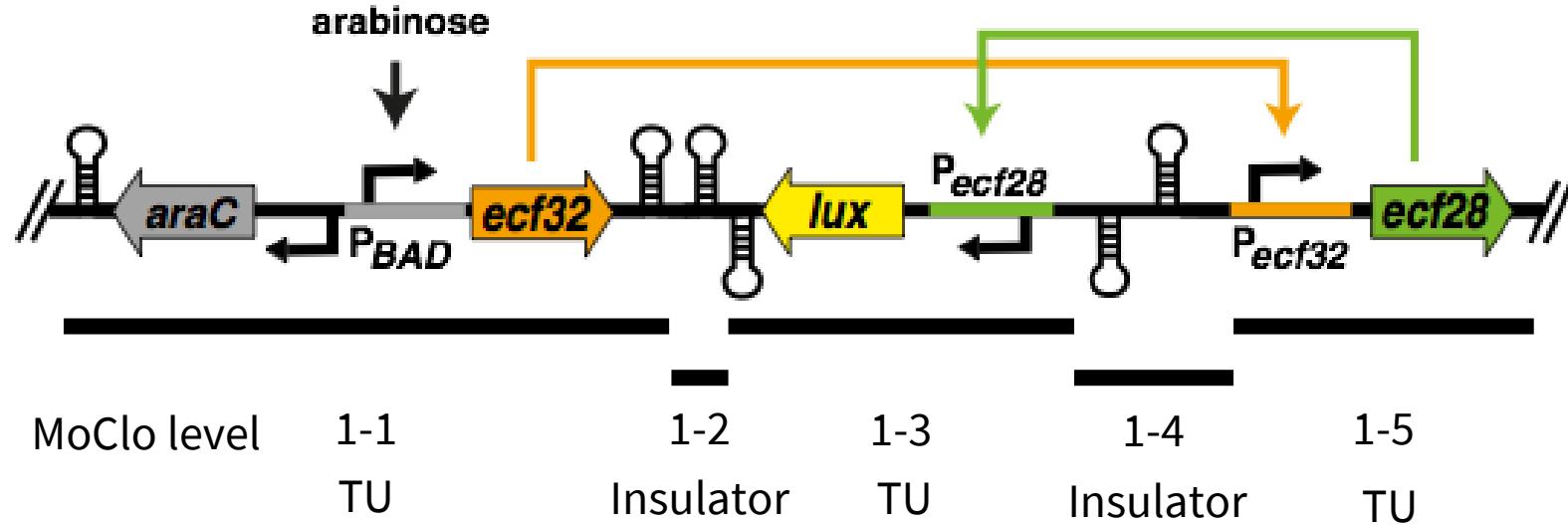
Detail

# How to design a genetic circuit?

**1. Concept**  
 (anticipate desired function in a wiring diagram)



**2. Genetic design**  
 (envise the parts used, orientation of genes, which plasmids to use, ...)



**3. *In silico* Golden Gate assembly in Geneious bioinformatics software**

Detail

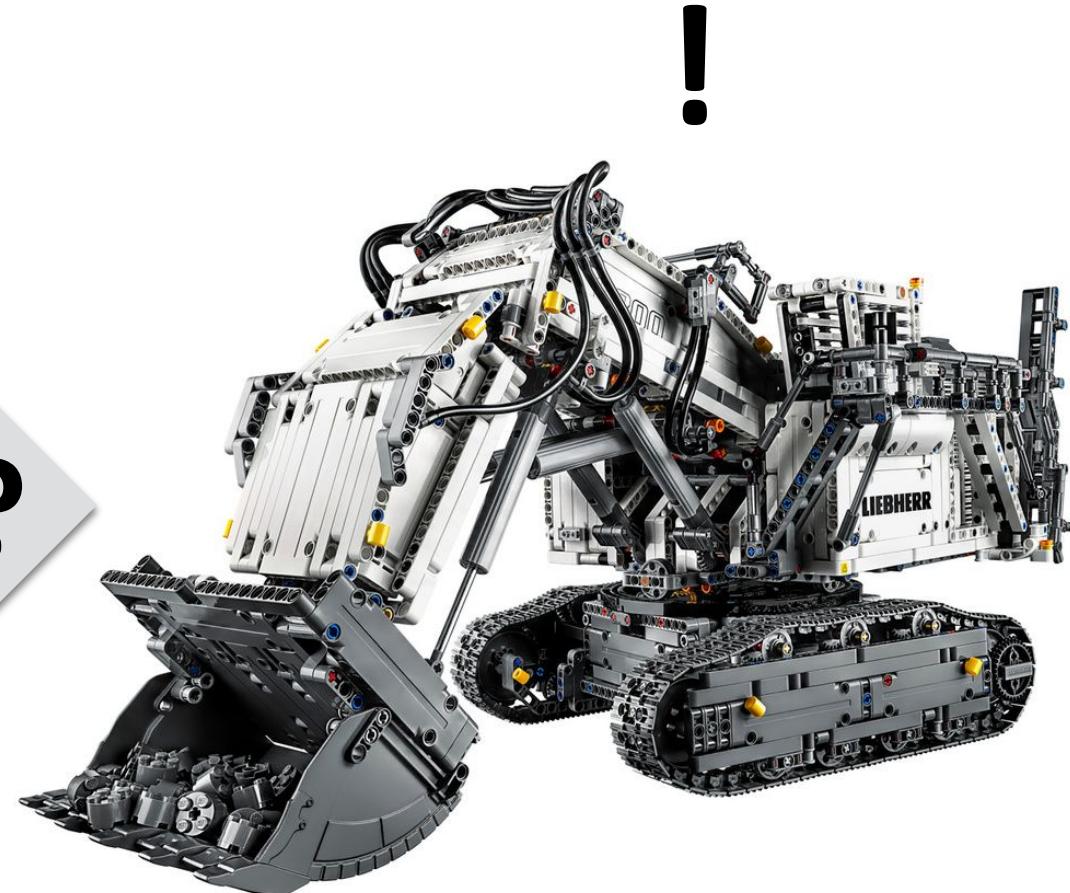
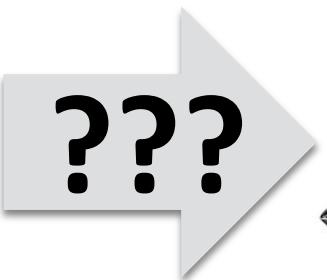
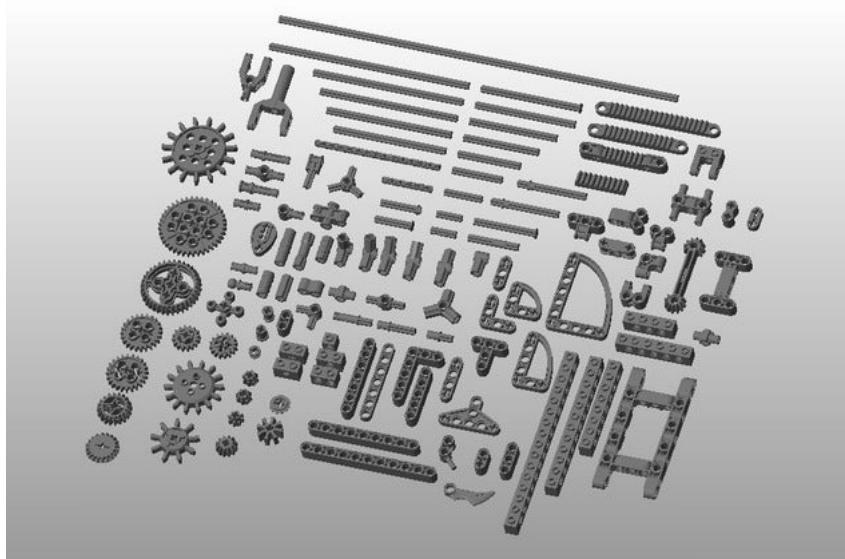


SCIE5508



THE UNIVERSITY OF  
WESTERN  
AUSTRALIA

# How to build it?

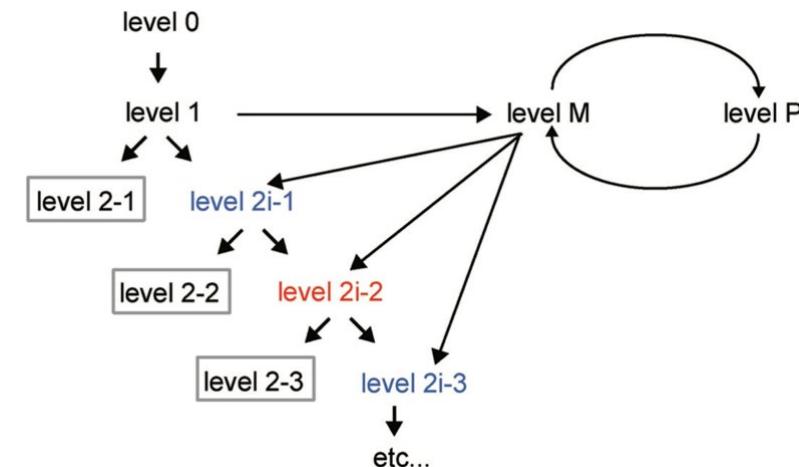
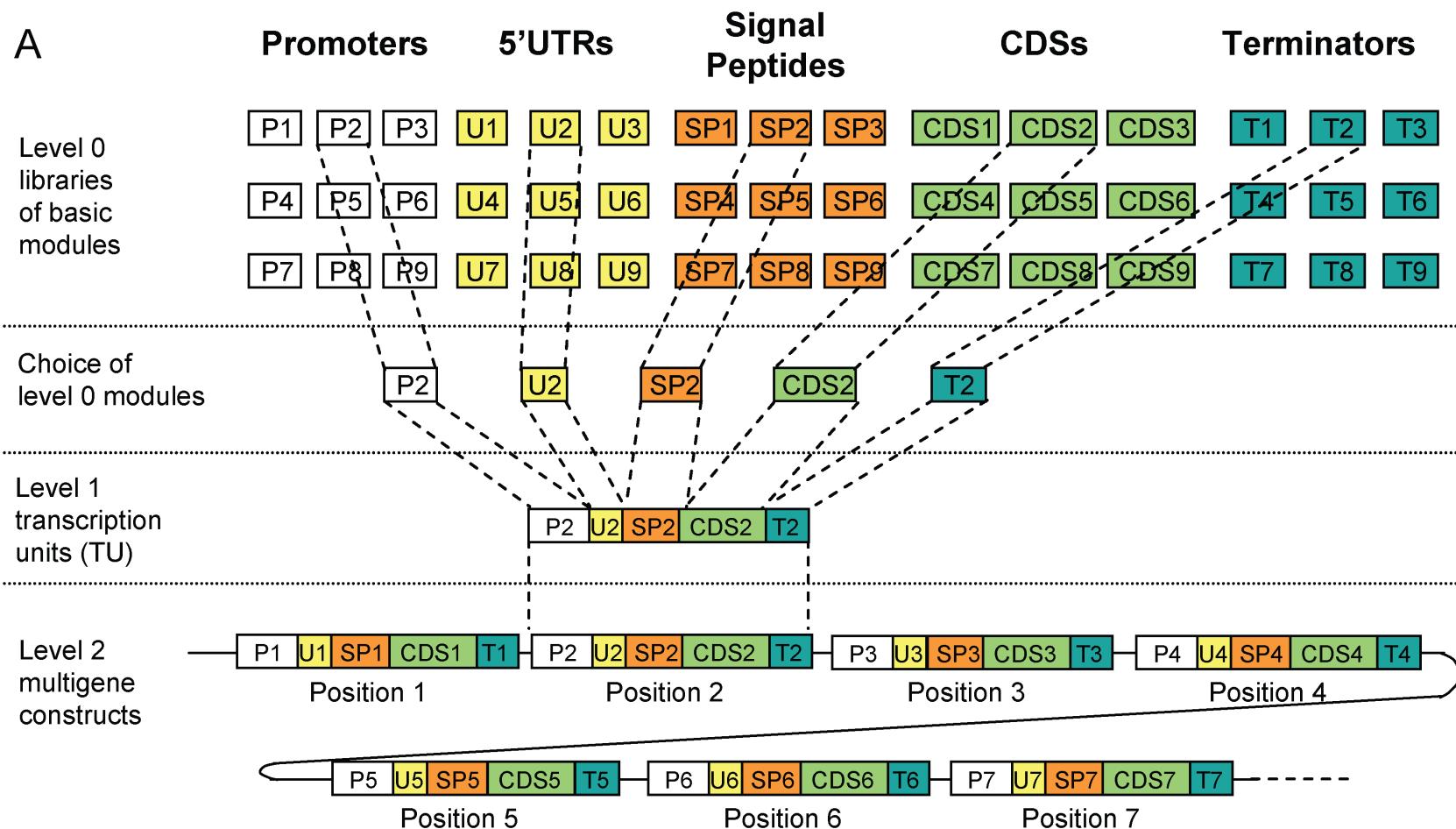




# Recap of the modular cloning (MoClo) standard



A

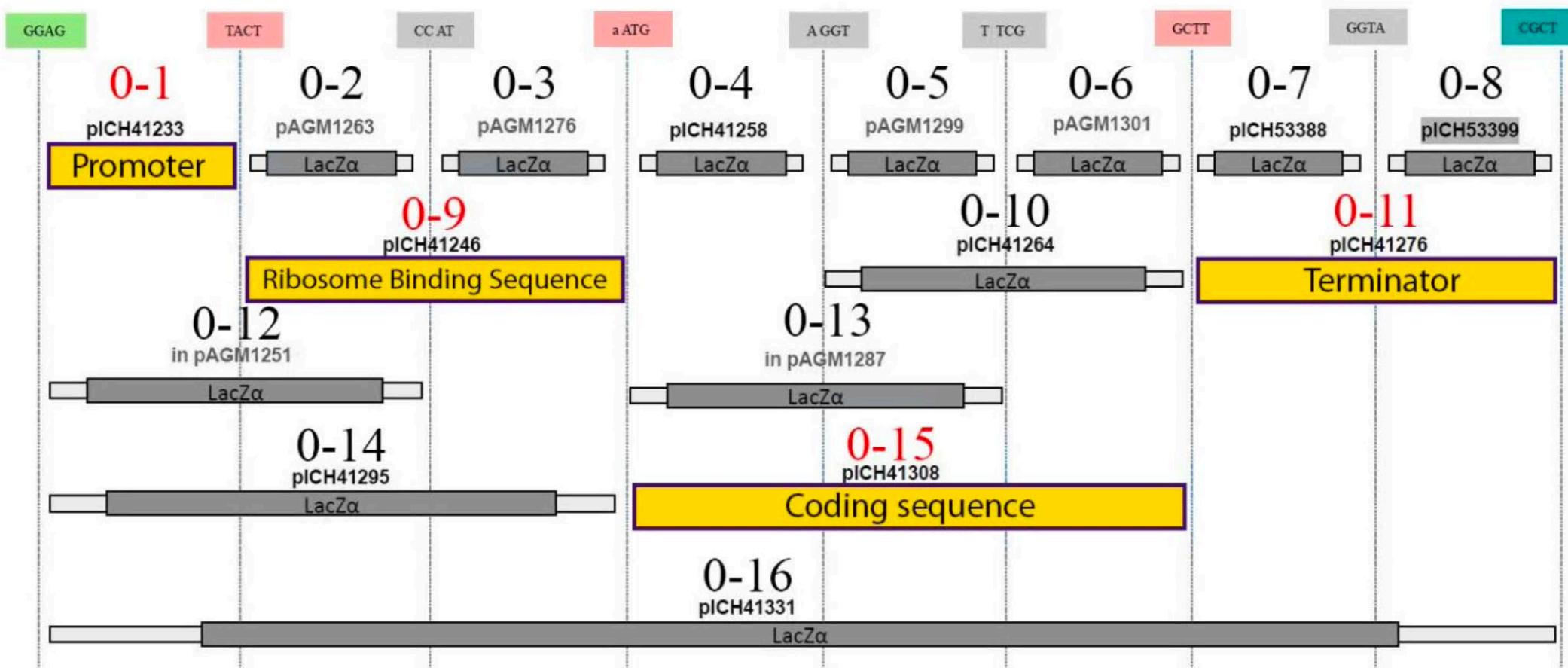


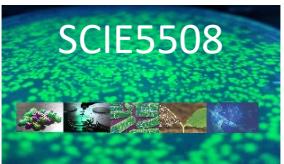
Stefan Werner, Carola Engler, Ernst Weber,  
Ramona Gruetzner & Sylvestre Marillonnet  
(2012) Fast track assembly of multigene  
constructs using Golden Gate cloning and the  
MoClo system, *Bioengineered*, 3:1, 38-43, DOI:  
10.4161/bbug.3.1.18223



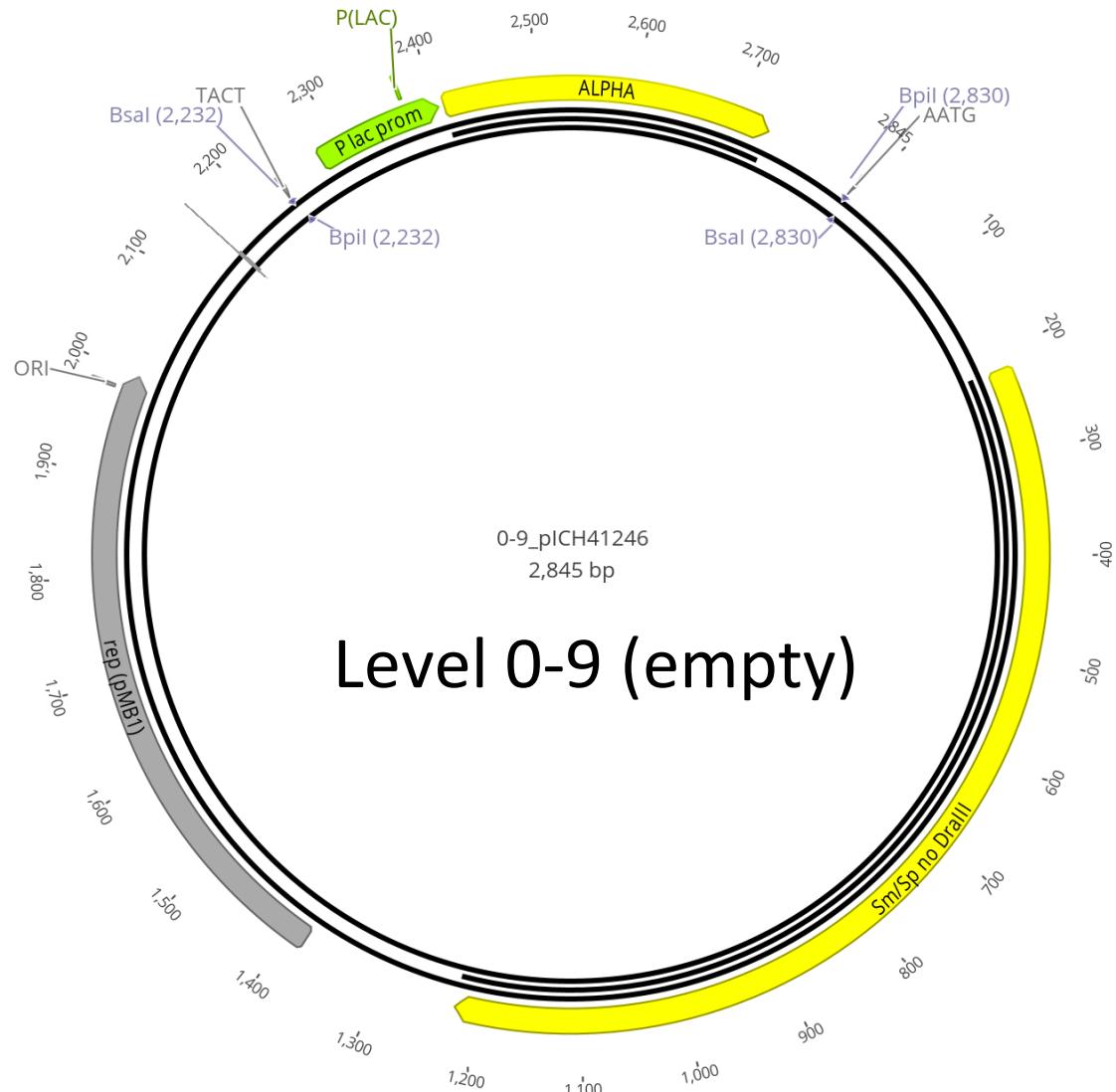
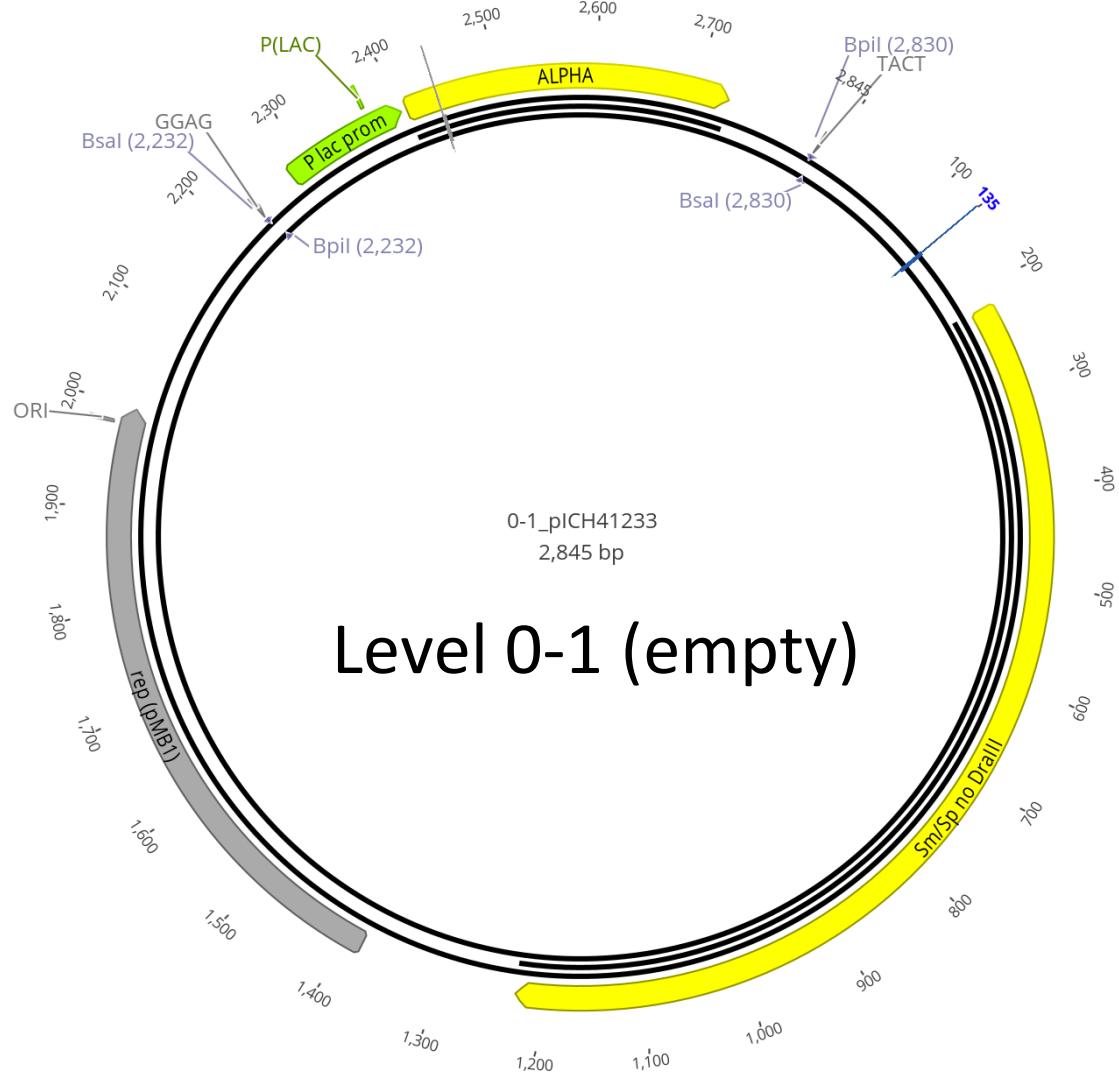
# Our part library is stored in vectors containing these fusion sites

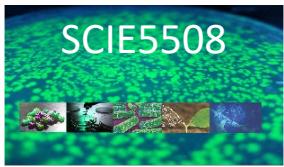
## Level 0 cloning vectors



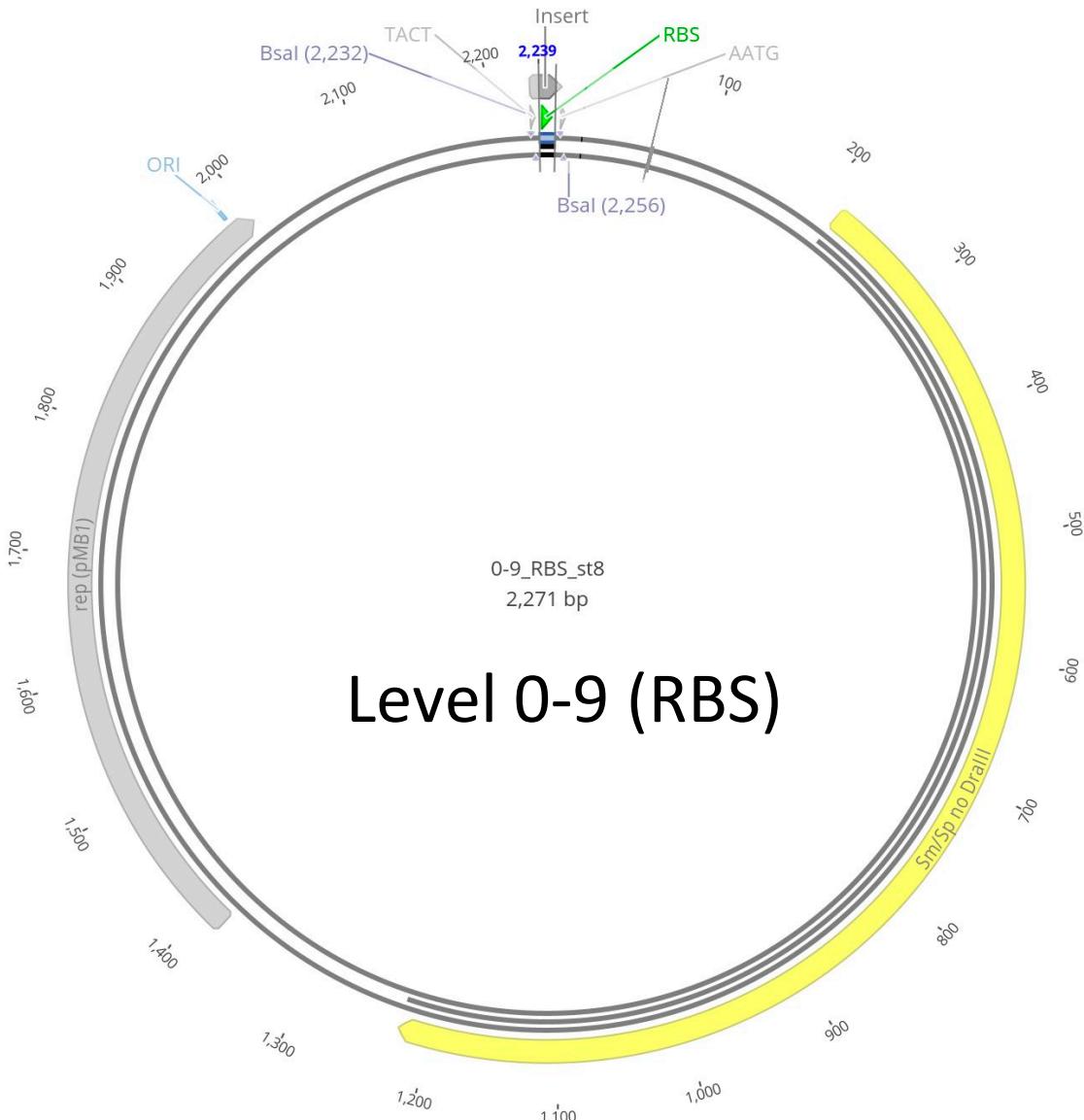
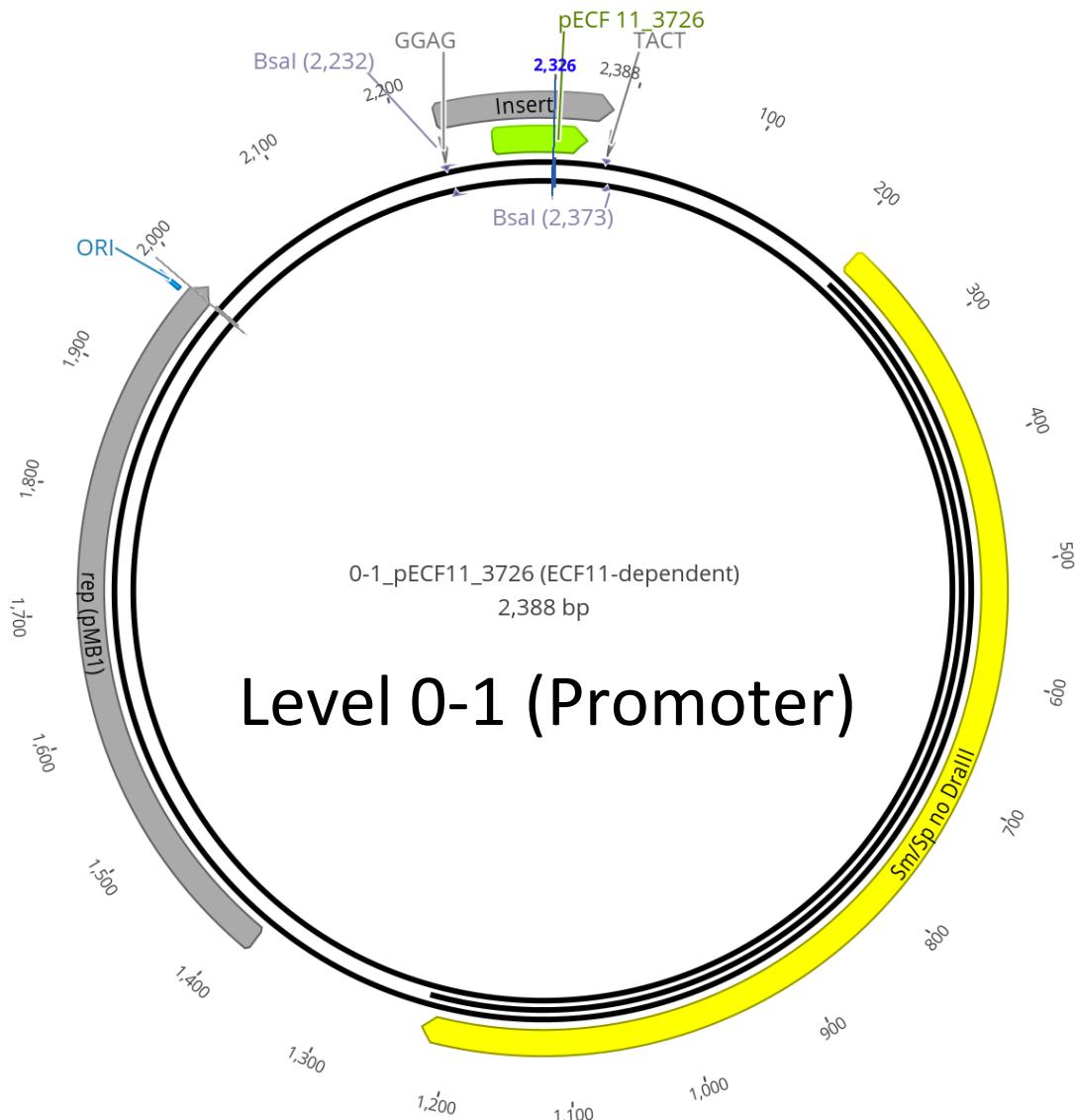


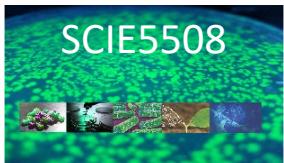
# Examples of level 0 entry vectors





# Examples of “loaded” level 0 vectors





SCIE5508

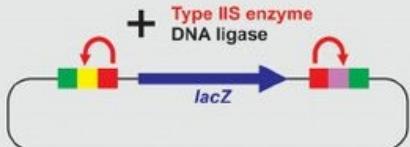
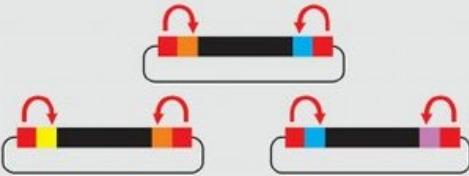
# Building level 1 transcription units (TUs)

Level 0 modules

Level 1  
destination  
vectorLevel 1  
transcription unitRe-ligated vector  
= blue colony

Level 1 part release

## Type-IIS-based assembly

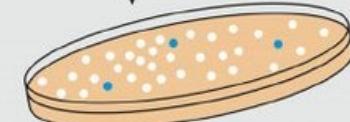


One pot reaction:  
Type IIS enzyme  
DNA ligase

Bsal

Bsal

Transformation  
&  
Selection marker B



Type IIS enzyme

Bpil

Bpil

Bpil

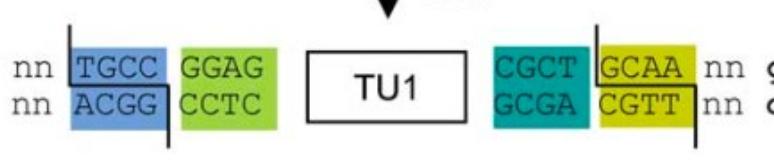
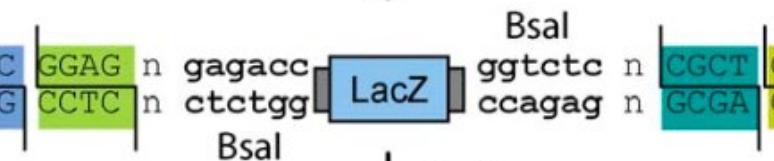
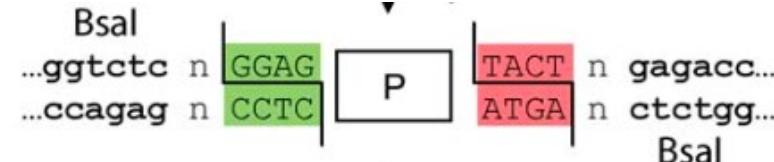
Bsal

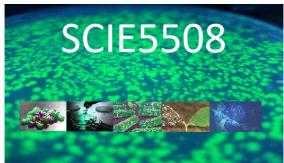
Bpil

Bsal

TU1

Bpil





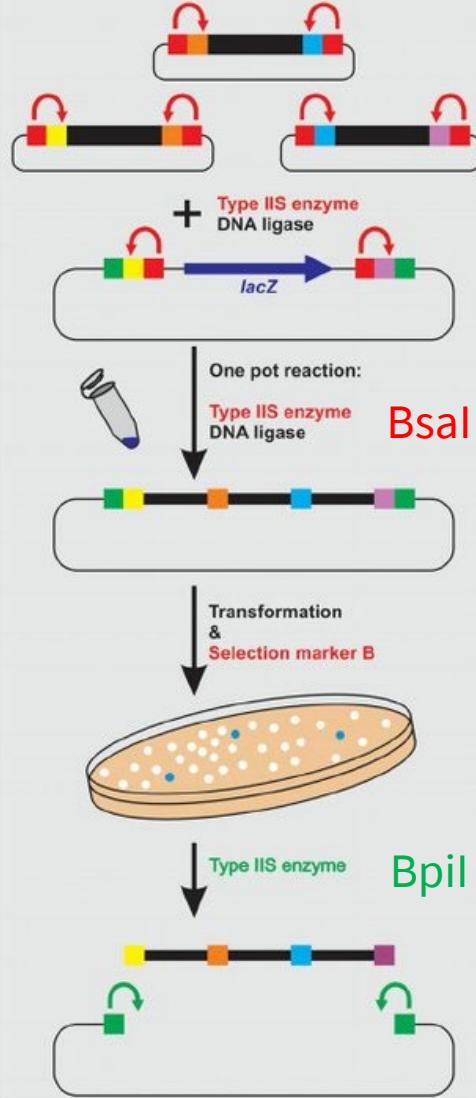
SCIE5508

Level 0 modules

Level 1  
destination  
vectorLevel 1  
transcription unitRe-ligated vector  
= blue colony

Level 1 part release

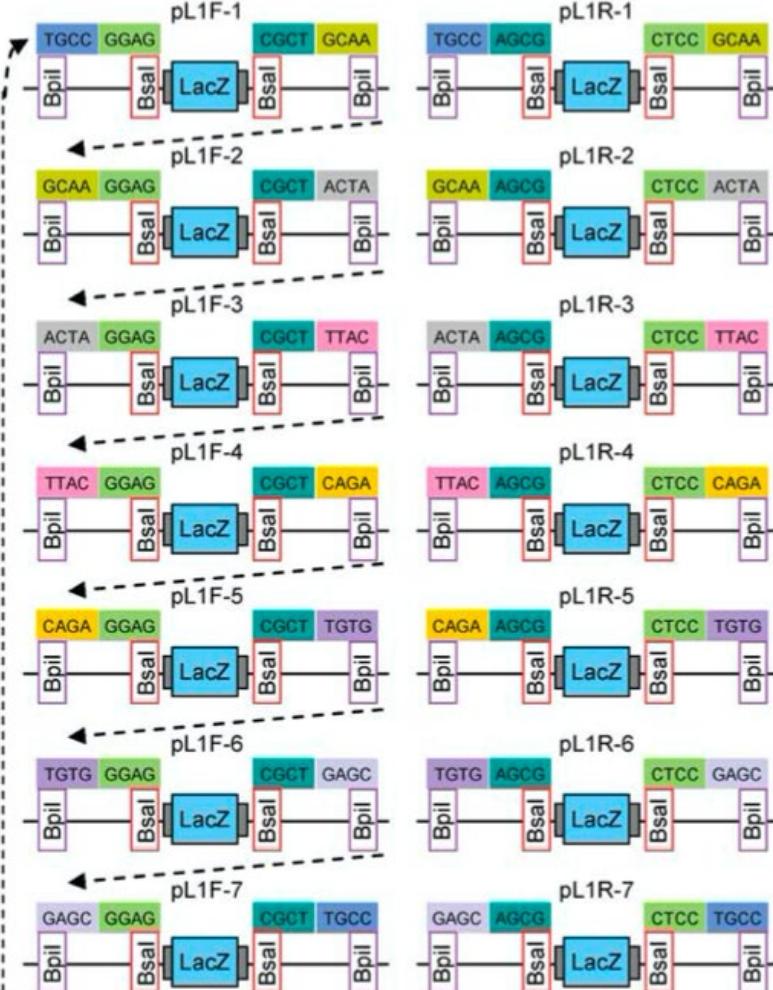
### Type-IIS-based assembly



## Building level 1 transcription units (TUs)

THE UNIVERSITY OF  
WESTERN  
AUSTRALIA

### Level 1 destination vectors ( $\text{Ap}^R$ )



Positions 1-7

Positions 1-7  
(reverse orient.)



# Hands on!



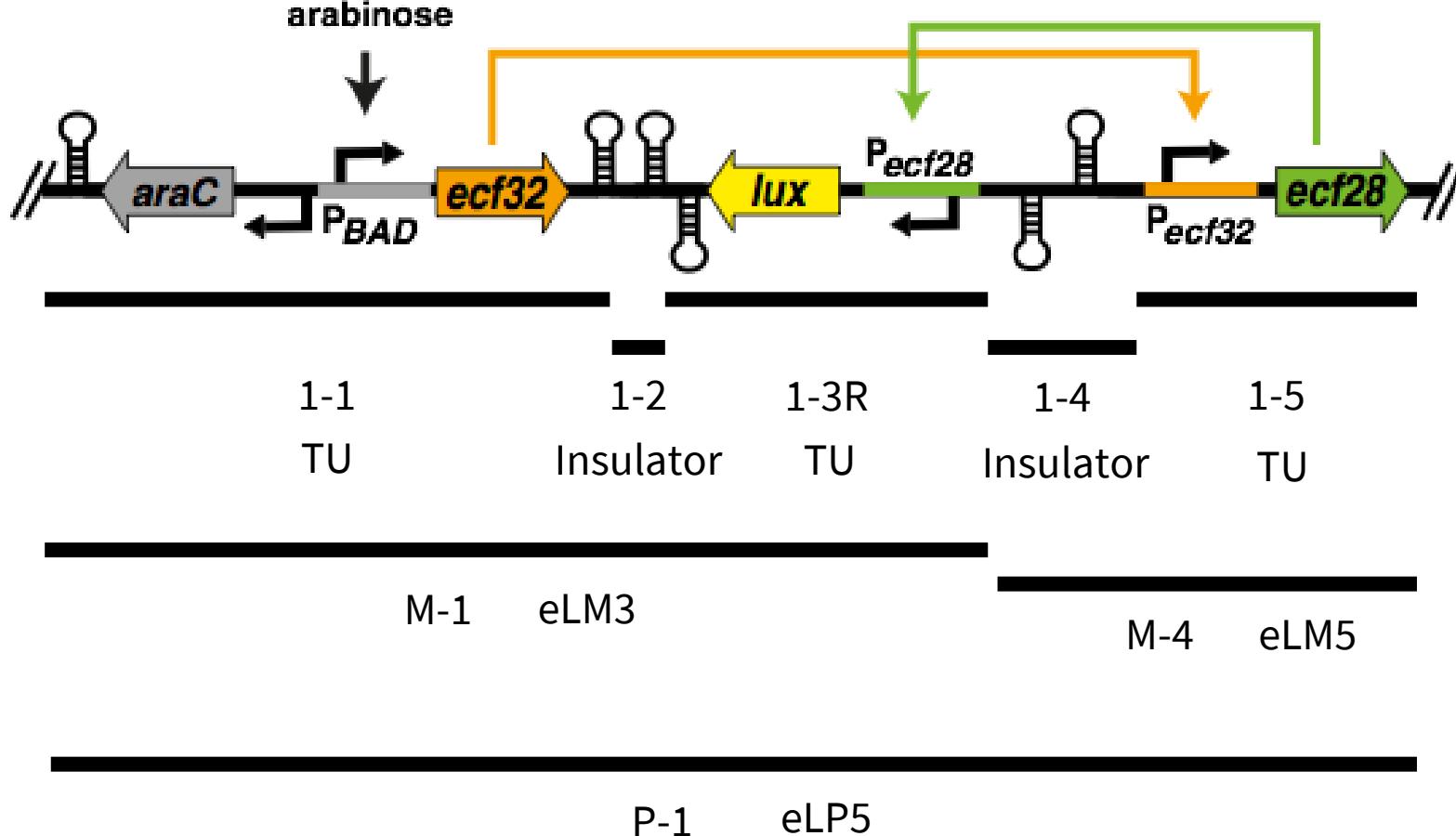
**Find your  
team mates!**

1. Start software
2. Make sure MoClo library is imported (drag & drop MoClo collection.geneious to your Sources folder)
3. Check out part library
4. Visualize restriction sites, add annotations, etc.
5. Start test assembly: Build a GFP/RFP dual expression cassette

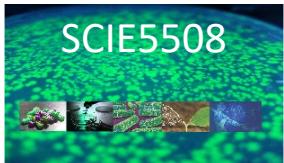


# Now: build a more complex genetic design in MoClo

MoClo level

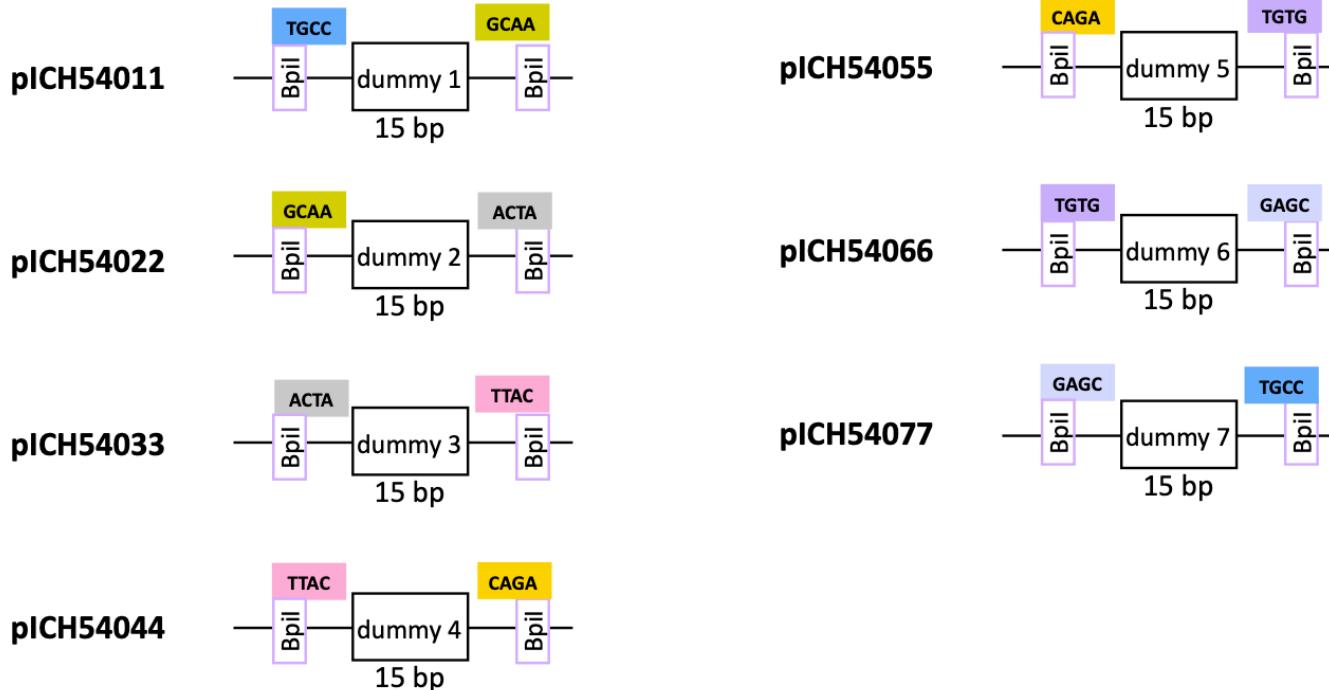


Design:  
Convergent &  
divergent TUs  
separated by  
insulators (100's  
of bp random DNA  
+ terminators)



# Dummies to close "gaps" in the design

## 15 bp dummies for level 1 parts



## 15 bp + 300 bp dummies for level 0 parts

## Building level M circuits

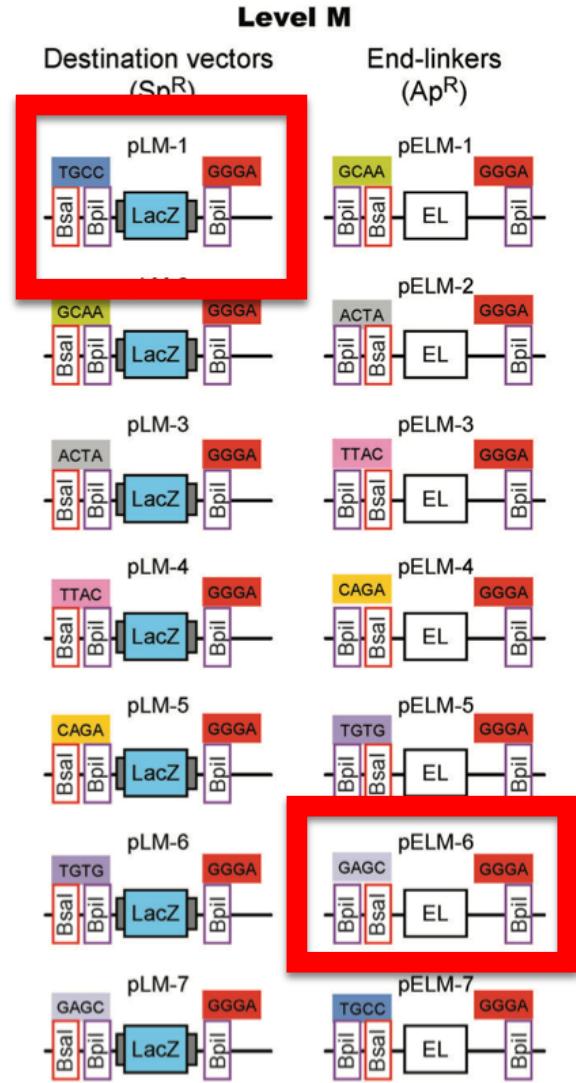
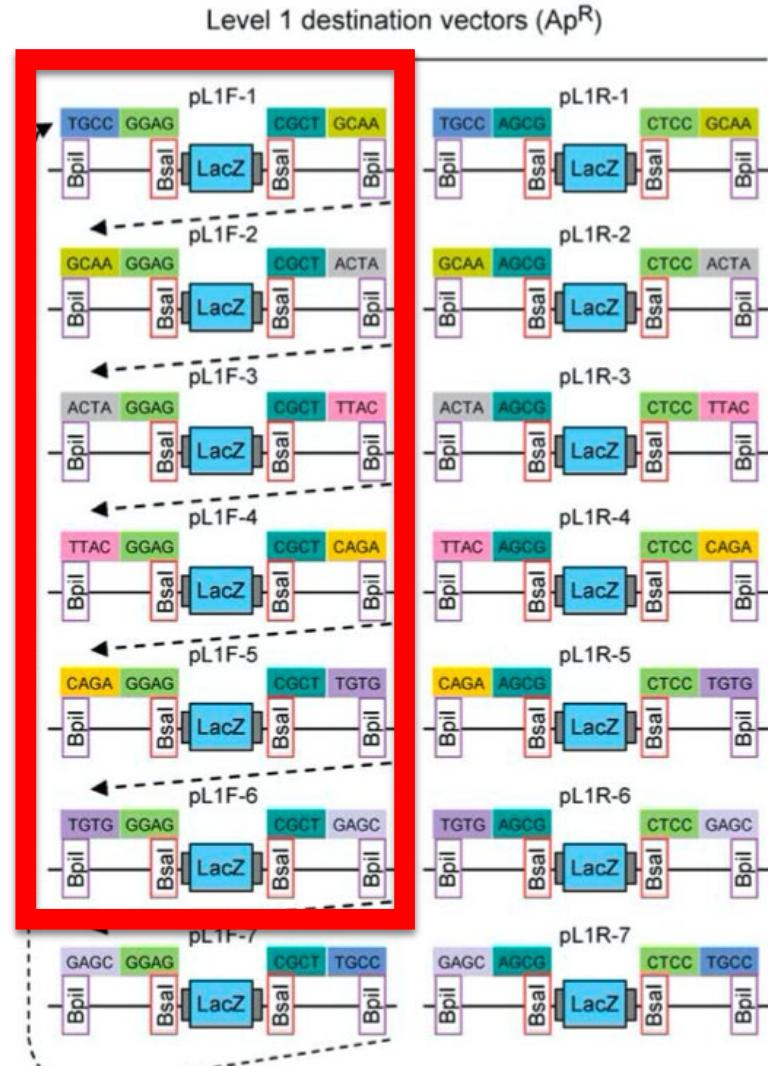
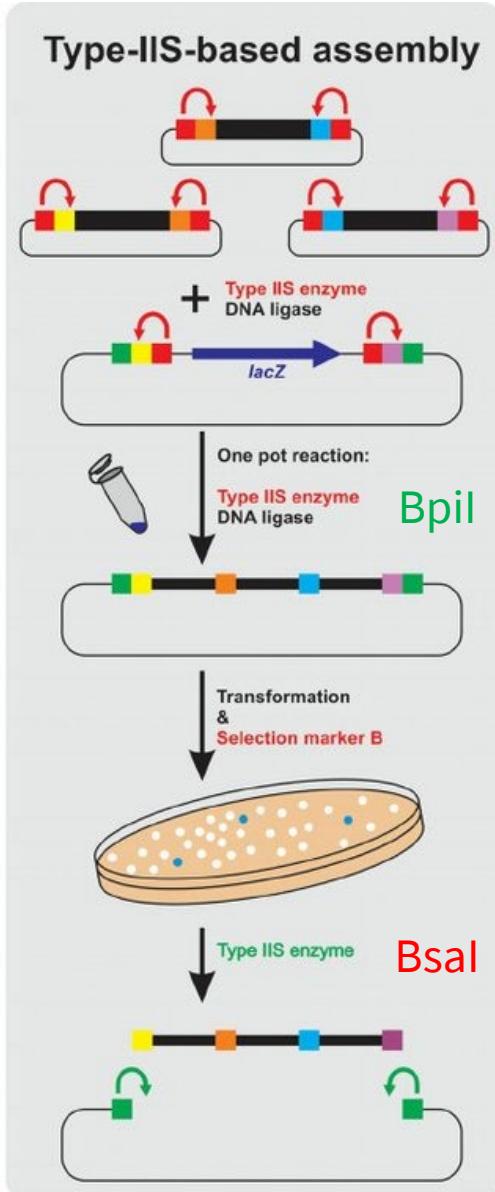
# Level 1 transcription units

## Level 2 destination vector

## Level 2 circuit

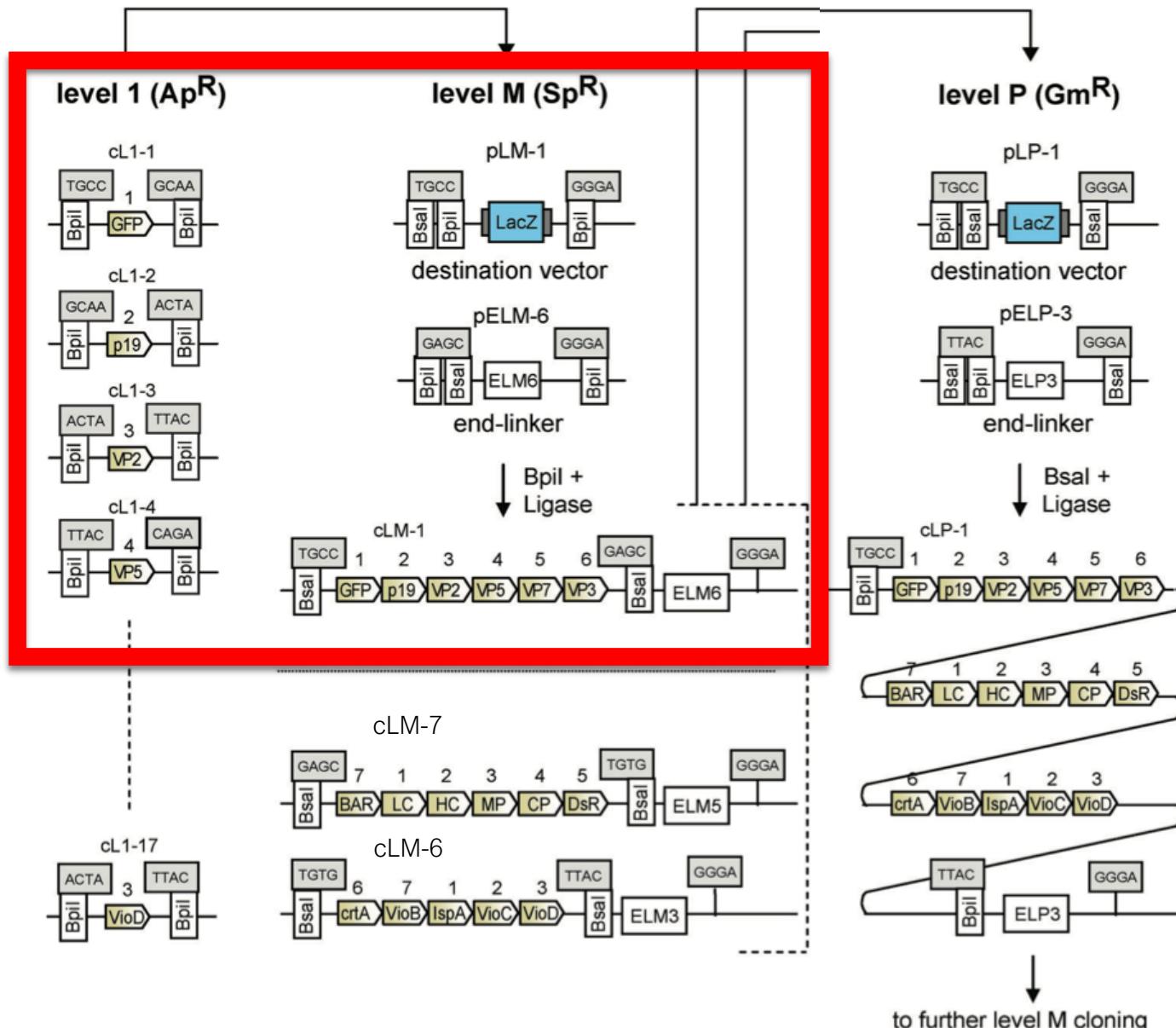
Re-ligated vector  
= blue colony

## Level 2 part release





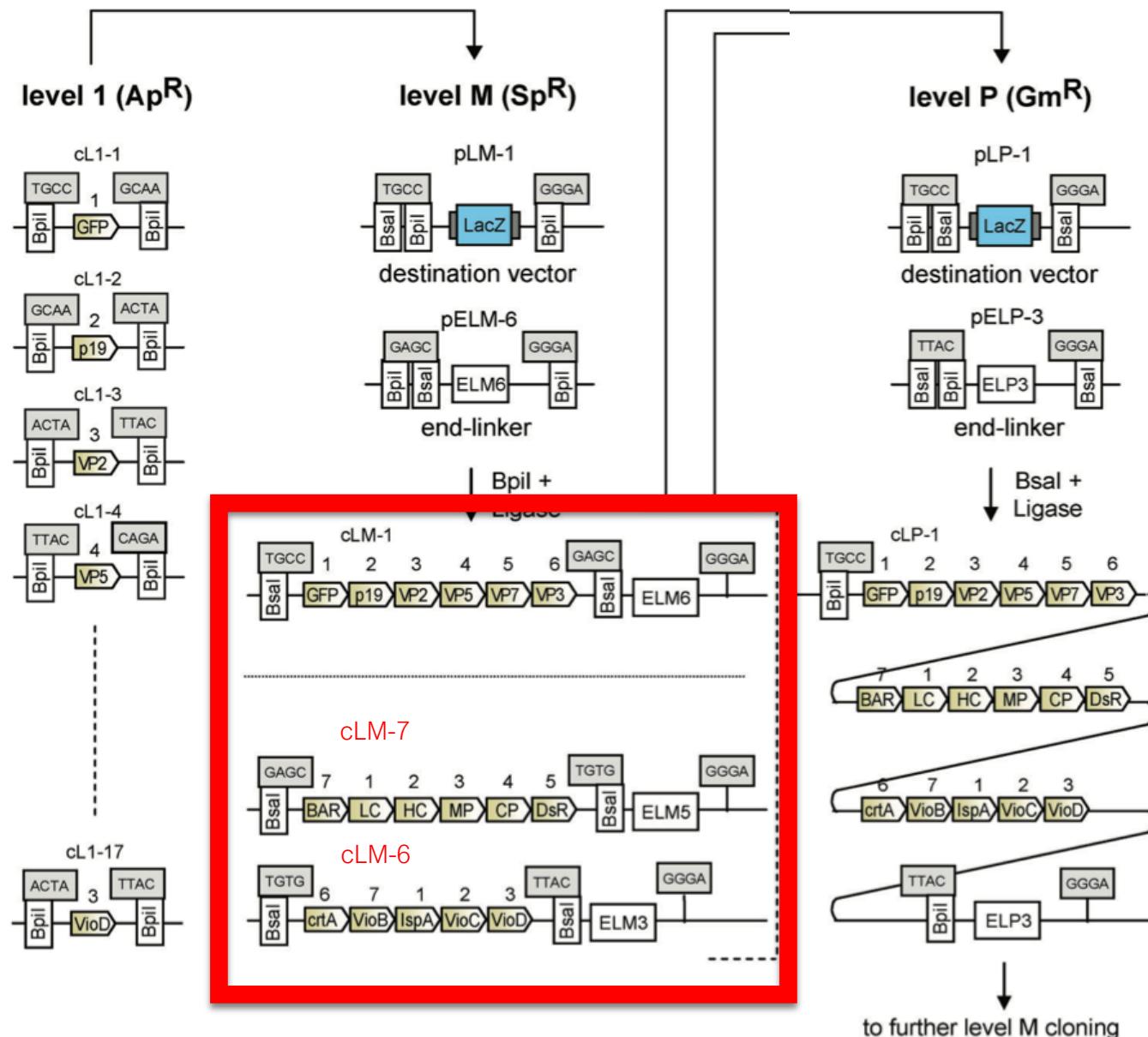
# From level 1 TUs to level P circuits



Stefan Werner, Carola Engler, Ernst Weber,  
Ramona Gruetzner & Sylvestre Marillonnet  
(2012) Fast track assembly of multigene  
constructs using Golden Gate cloning and the  
MoClo system, *Bioengineered*, 3:1, 38-43, DOI:  
10.4161/bbug.3.1.18223



# From level 1 TUs to level P circuits



Stefan Werner, Carola Engler, Ernst Weber,  
Ramona Gruetzner & Sylvestre Marillonnet  
(2012) Fast track assembly of multigene  
constructs using Golden Gate cloning and the  
MoClo system, Bioengineered, 3:1, 38-43, DOI:  
[10.4161/bbug.3.1.18223](https://doi.org/10.4161/bbug.3.1.18223)



SCIE5508

14 constructs

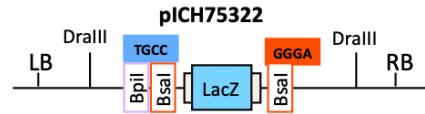
## Level P vectors and end-linkers

Level P cloning vectors, Kan<sup>R</sup>

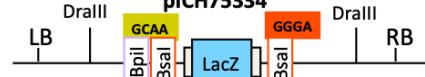
ColE1 and pVS1 ori

backbone derived from pPZP200  
(pVS1 ori) and pUC19 (ColE1 ori)  
replicate in E.coli and agrobacterium

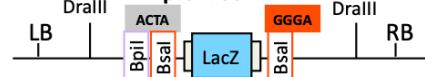
P-1



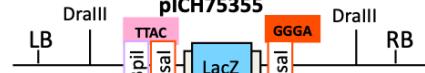
P-2



P-3



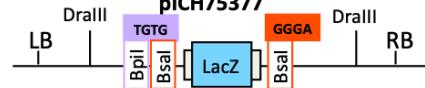
P-4



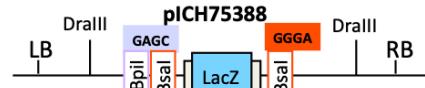
P-5



P-6



P-7



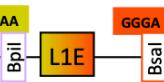
the DralII fragment  
can be subcloned in  
new vector backbones

End linkers, Amp<sup>R</sup>

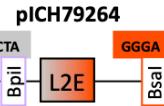
ColE1 ori

pUC19-derived  
backbone

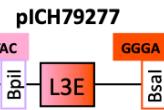
pICH79255



P-eL1



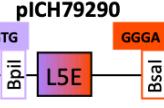
P-eL2



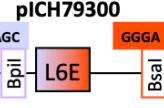
P-eL3



P-eL4



P-eL5



P-eL6



P-eL7



# Questions?





# Summary



- 1. Deep dive into ECF-based synthetic circuit design**
- 2. From schematic circuit design to implementation using the MoClo system**
- 3. Hands-on *in silico* cloning of your circuit using Geneious**

Thanks for your participation!



THE UNIVERSITY OF  
**WESTERN**  
**AUSTRALIA**