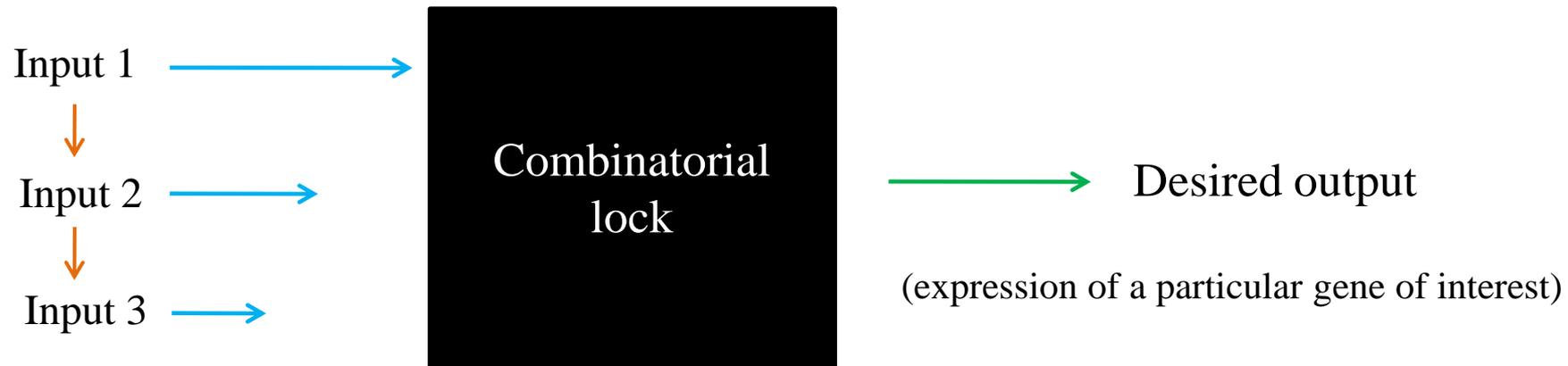

PLASMID

Plasmid **L**ocking **A**ssembly for
Sustaining **M**ultiple **I**nsert **D**NA

IIT MADRAS – IGEM 2009

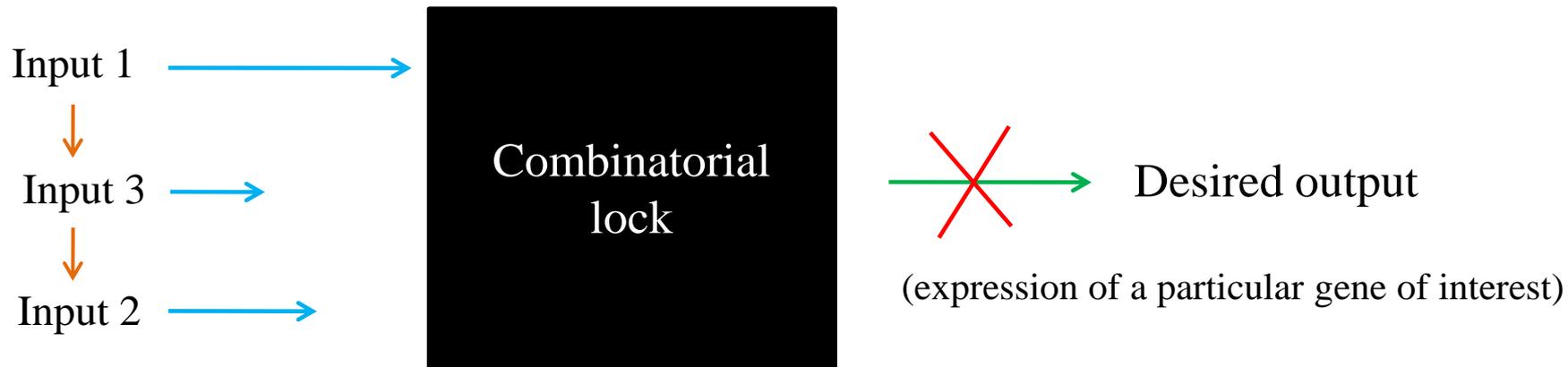
Motivation

Combinatorial lock



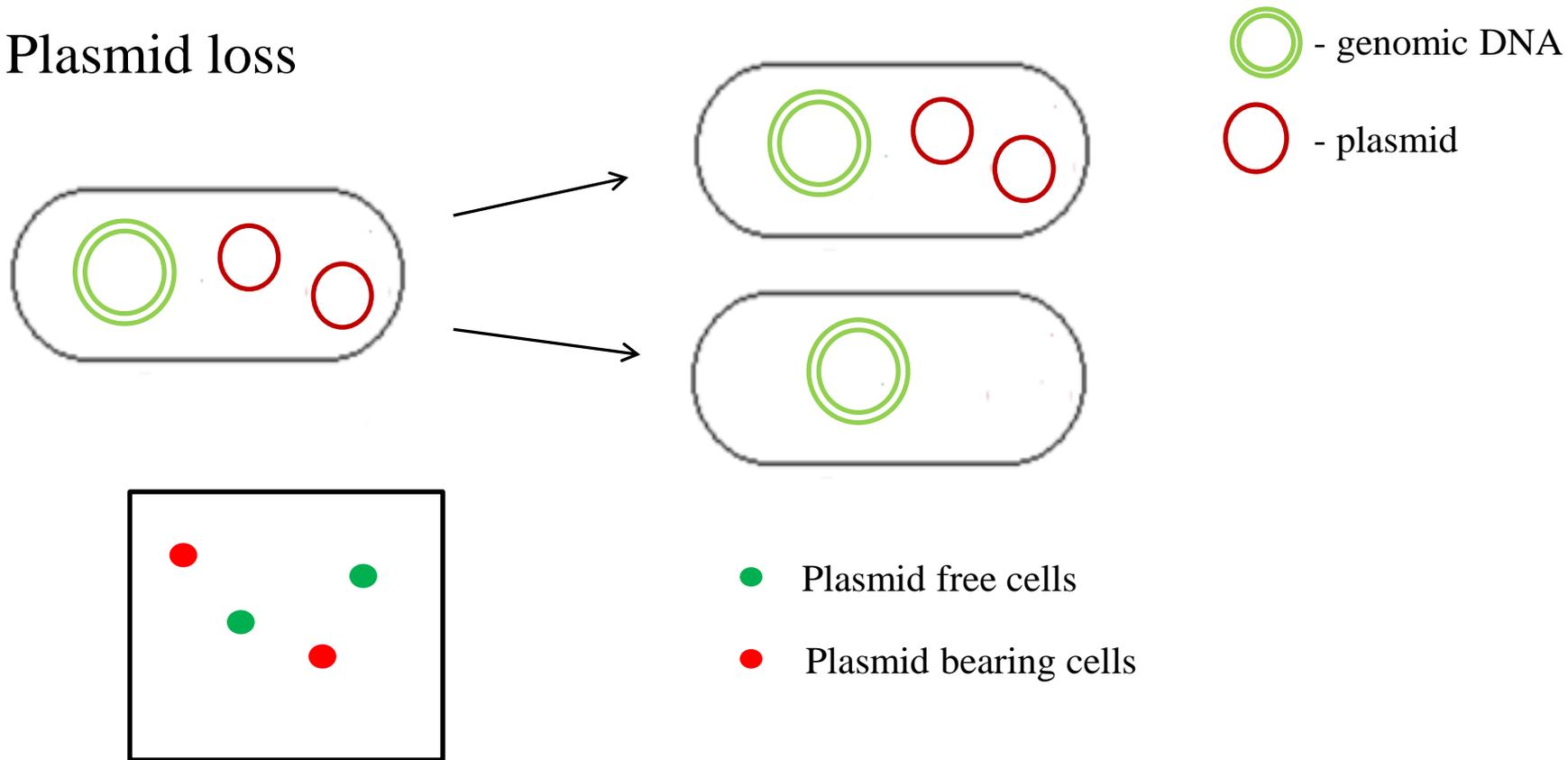
Motivation

Combinatorial lock



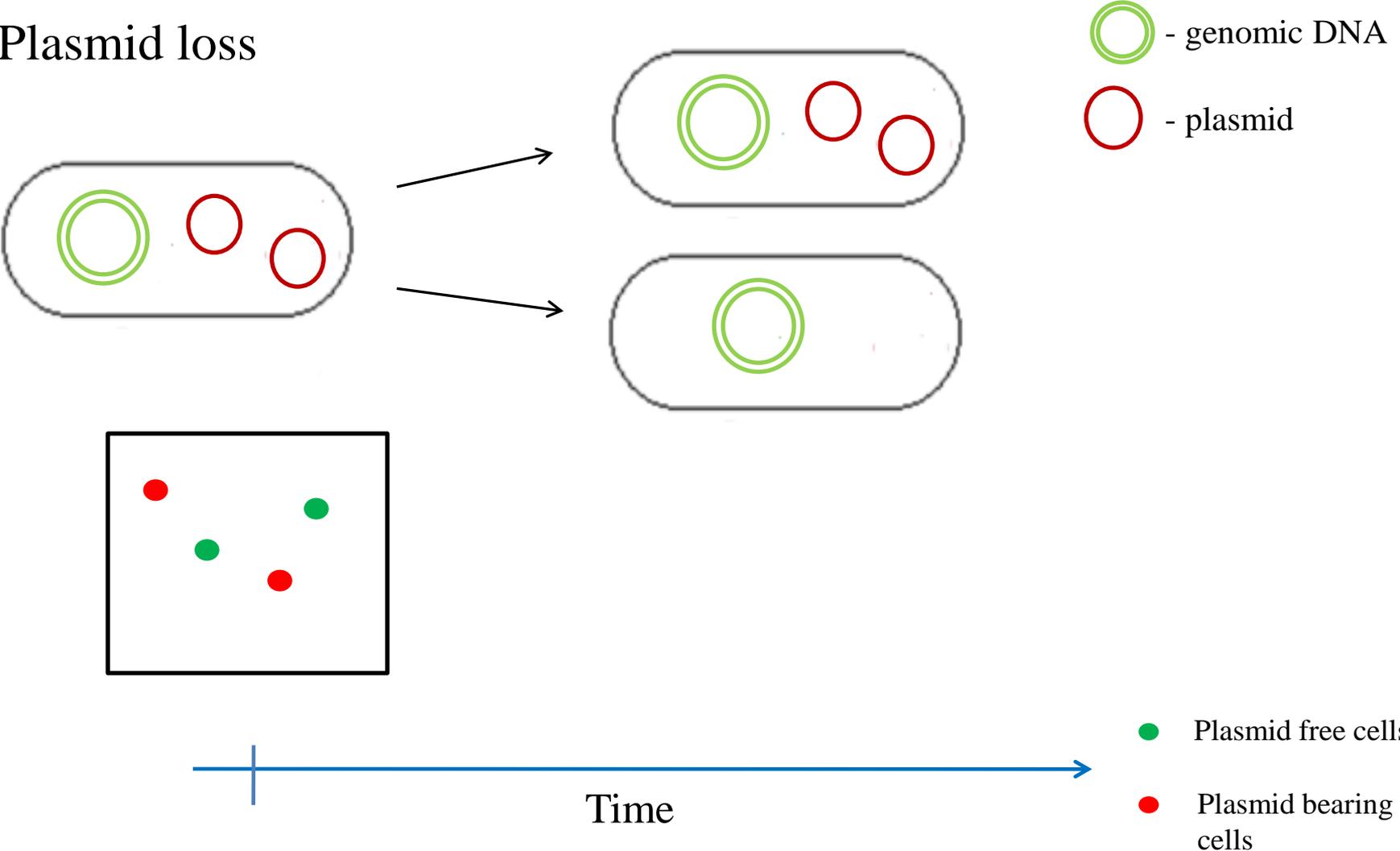
Background

- Plasmid loss



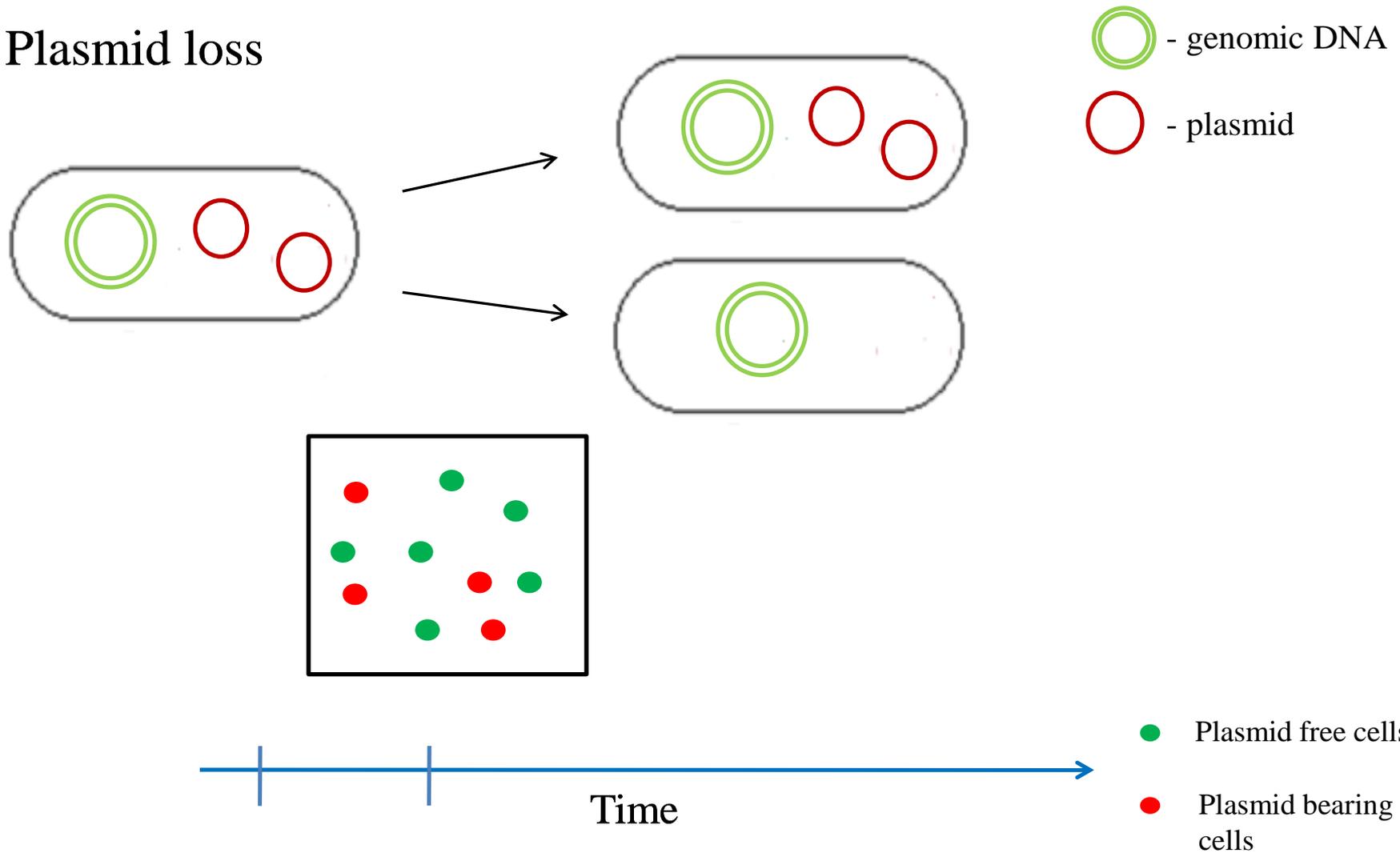
Background

- Plasmid loss



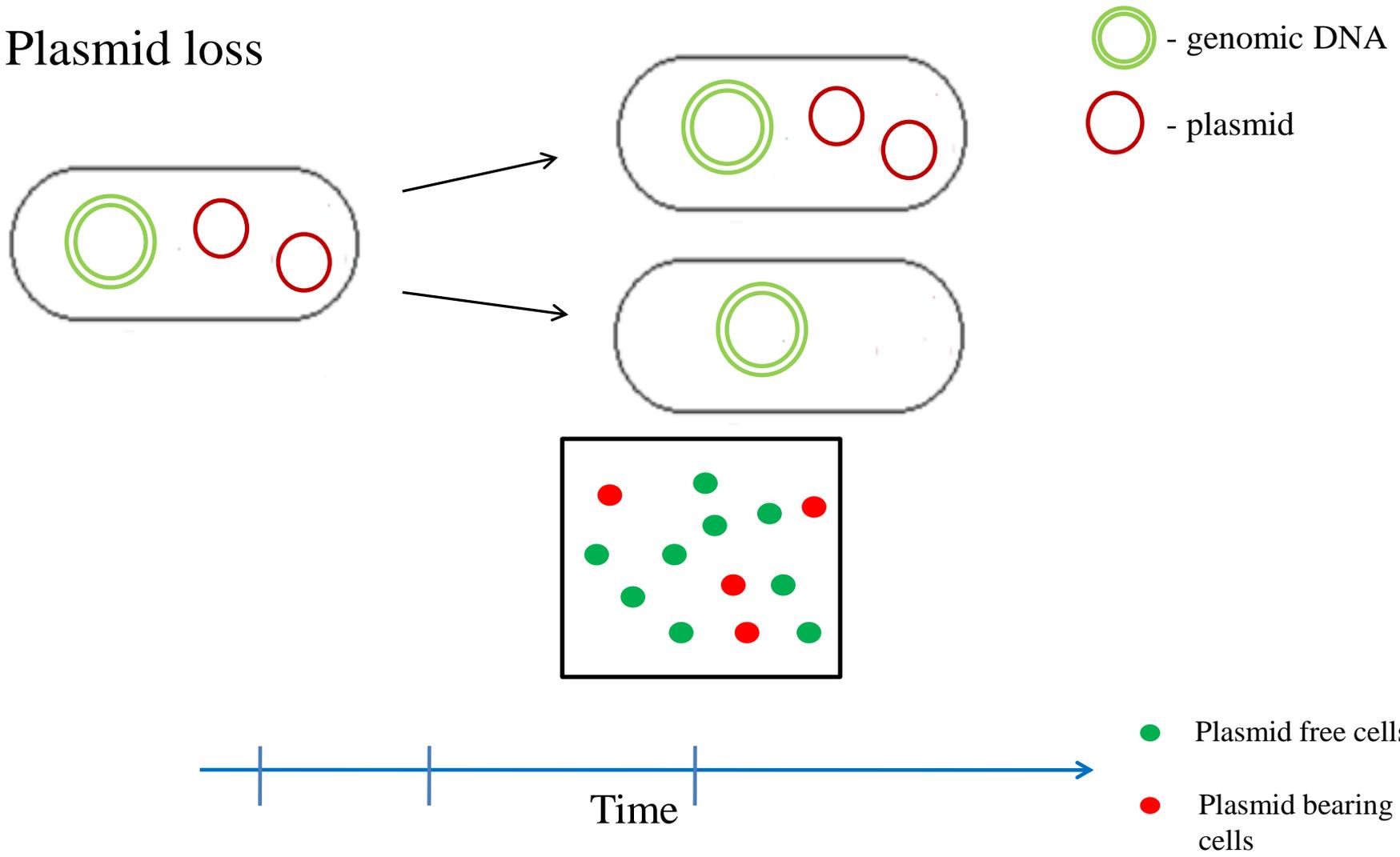
Background

- Plasmid loss



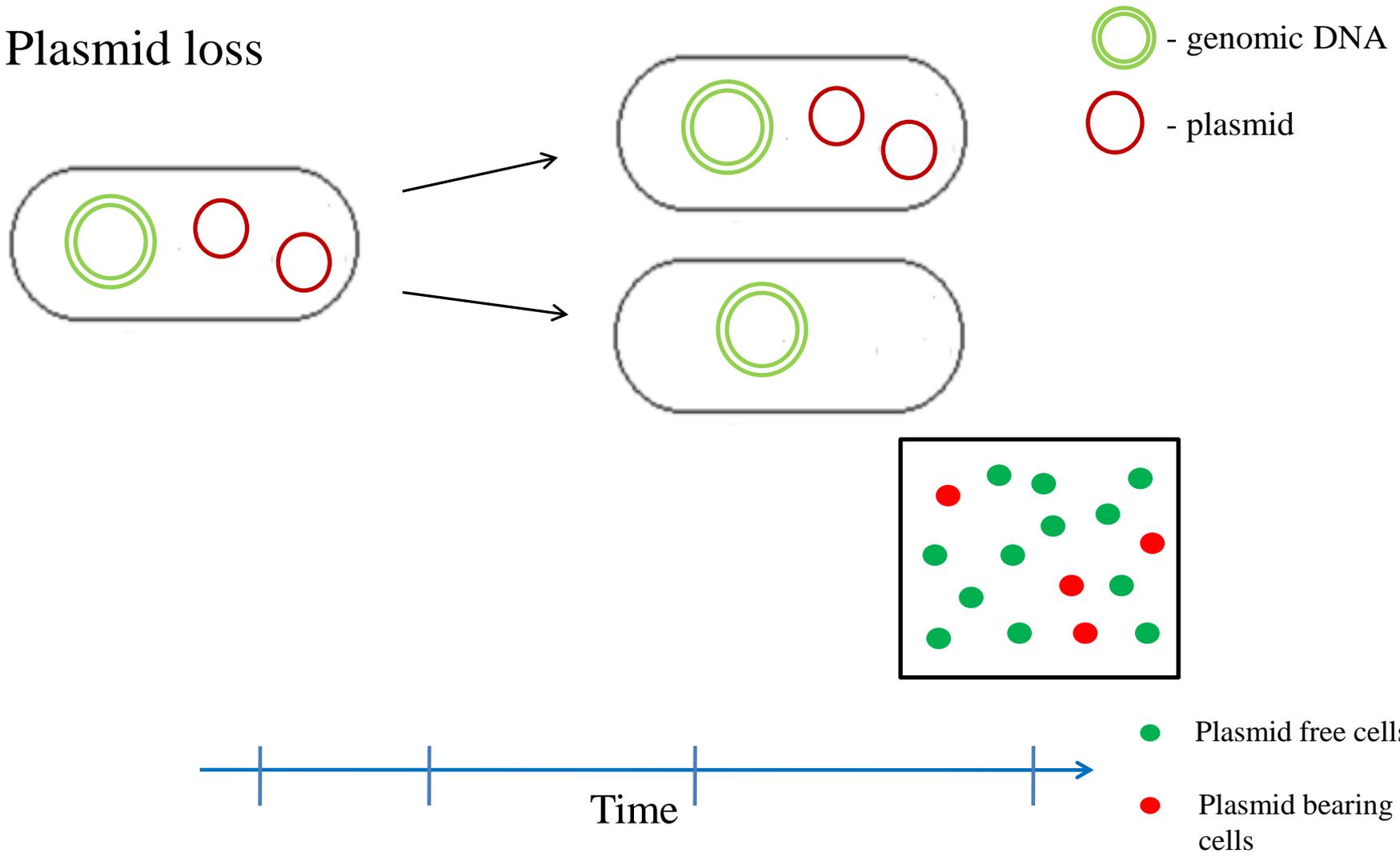
Background

- Plasmid loss



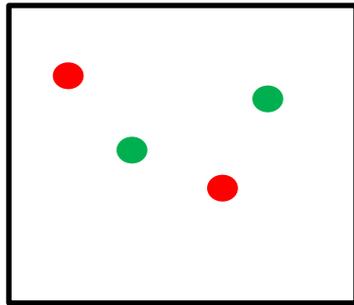
Background

- Plasmid loss



Background

- Selection pressure ensures the cell maintains the plasmid



- Plasmid free cells
- Plasmid bearing cells

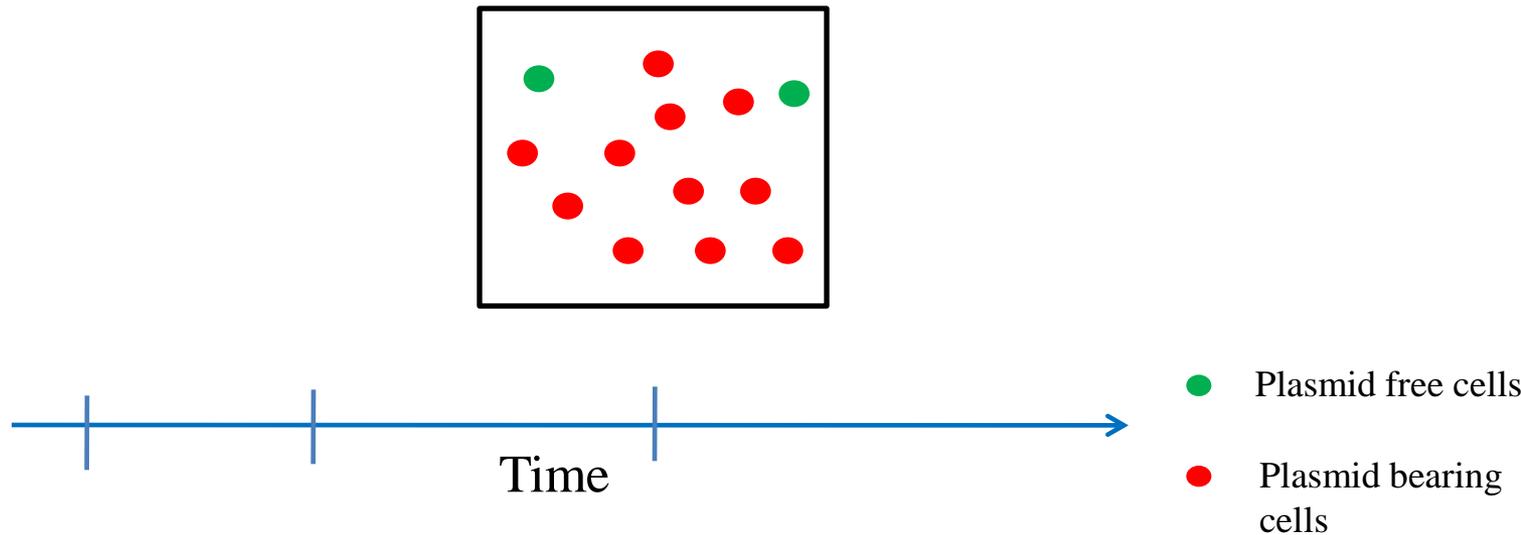
Background

- Selection pressure ensures the cell maintains the plasmid



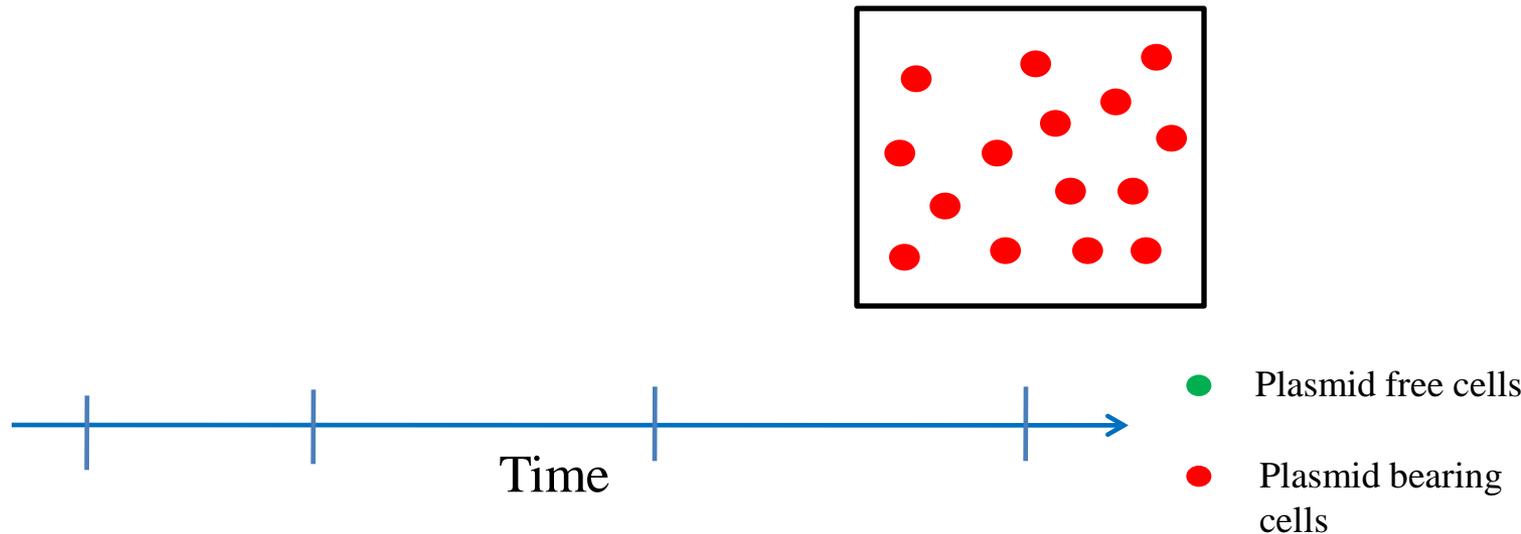
Background

- Selection pressure ensures the cell maintains the plasmid



Background

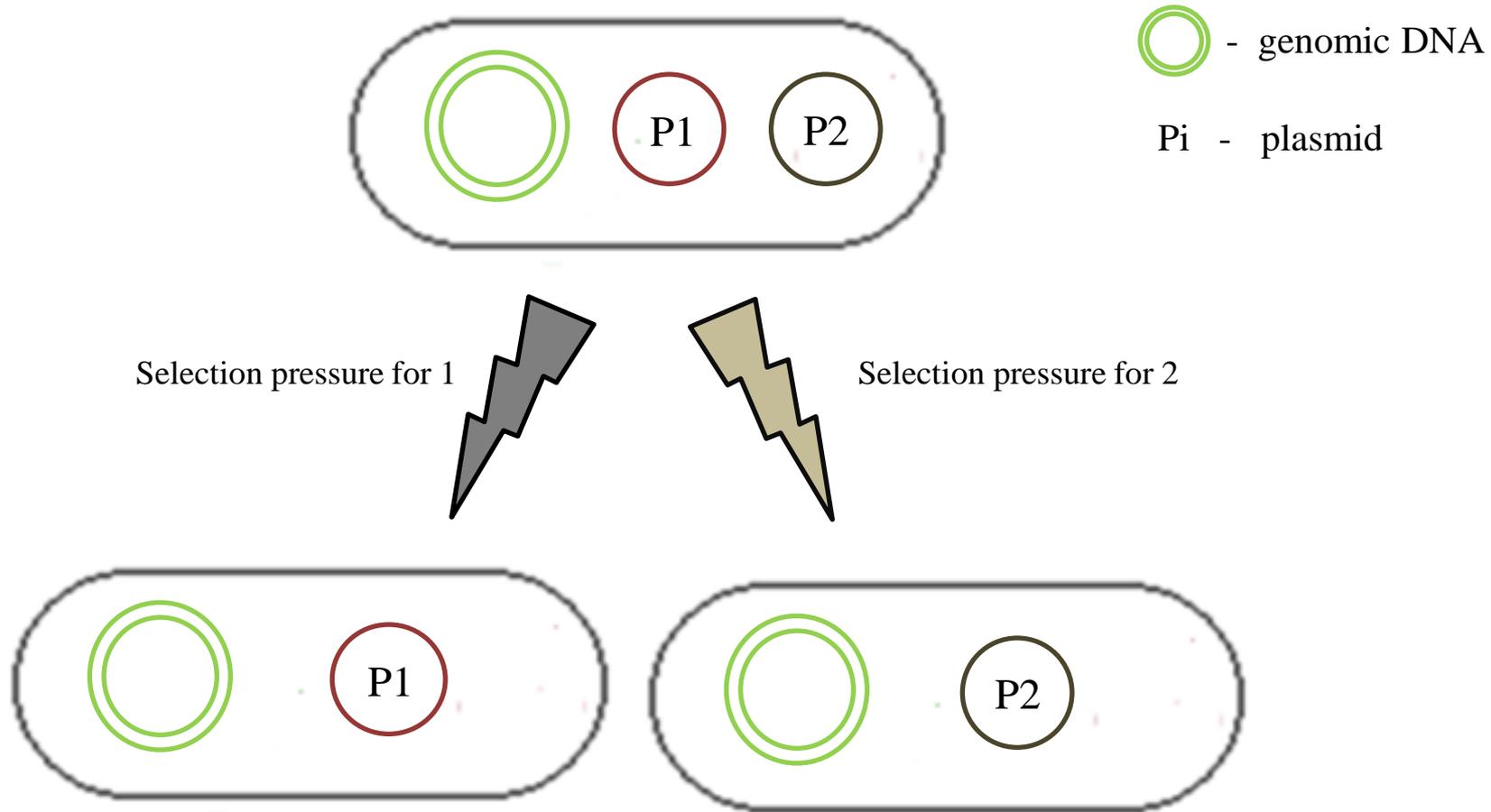
- Selection pressure ensures the cell maintains the plasmid



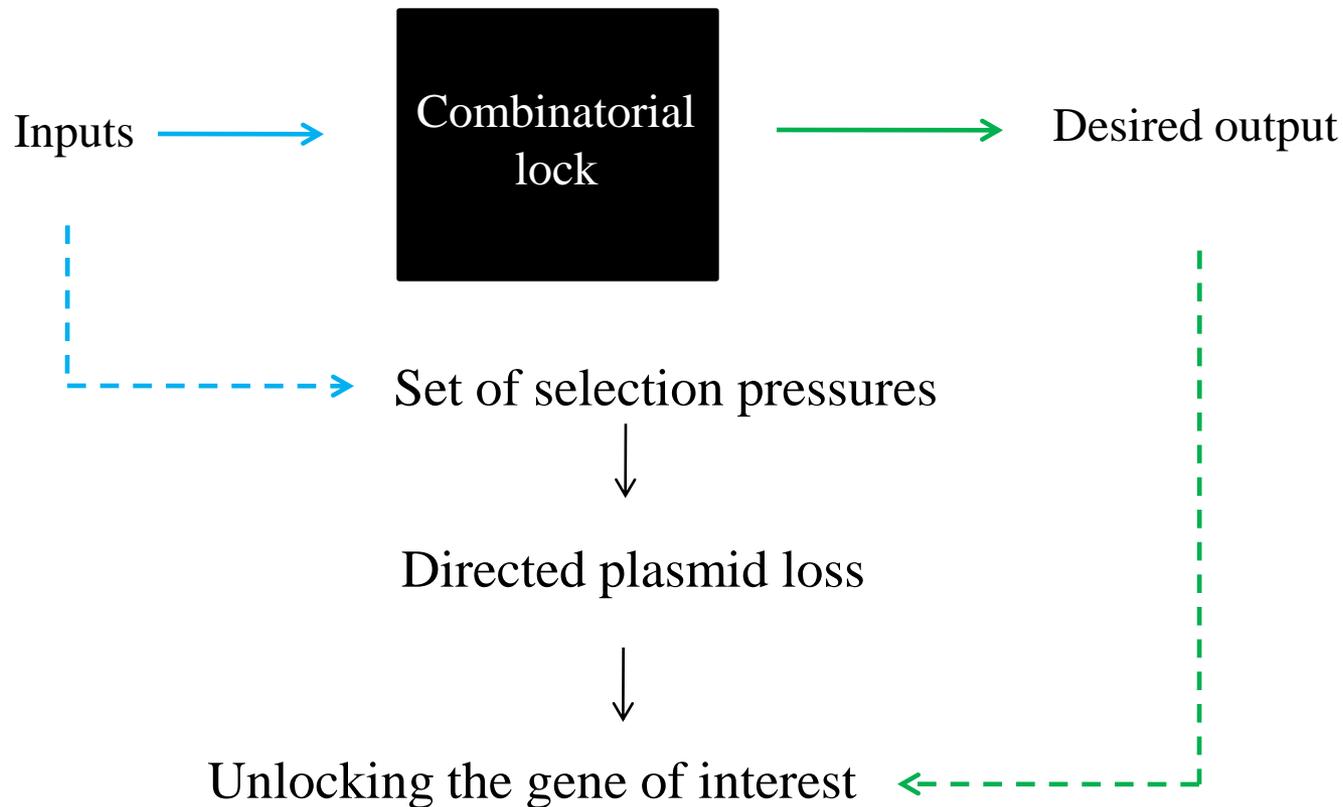
In this case – an antibiotic in the growth medium

System Design

- Directed plasmid loss

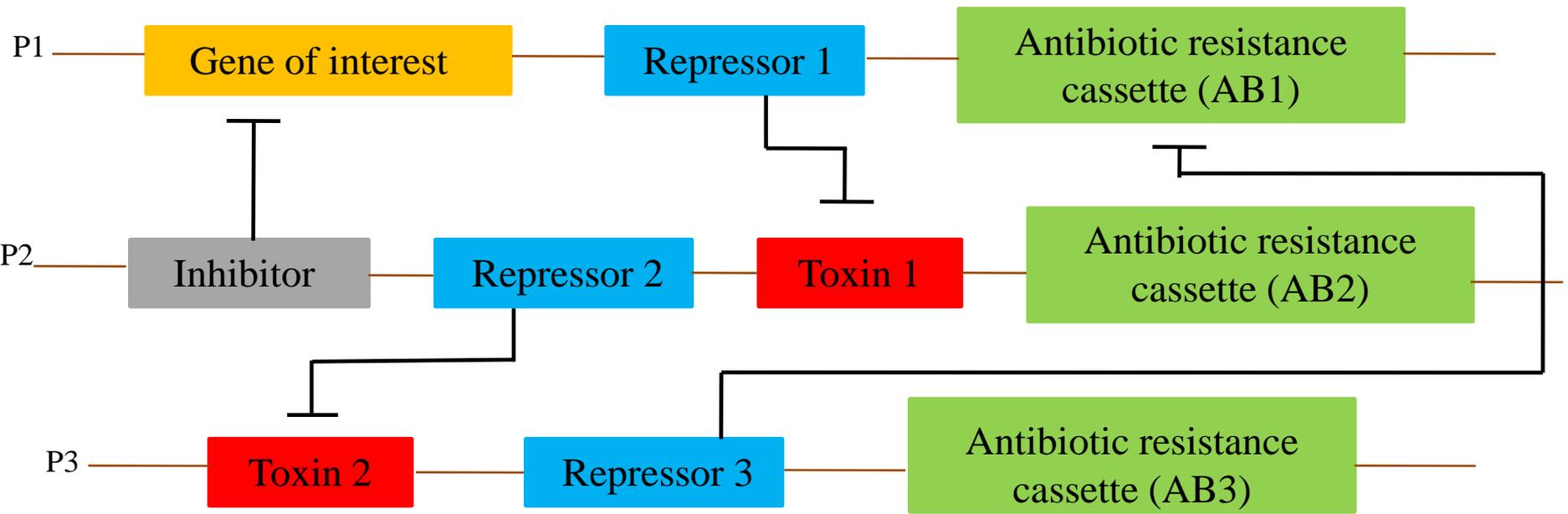


System Design



Constructs

- 3-plasmid system

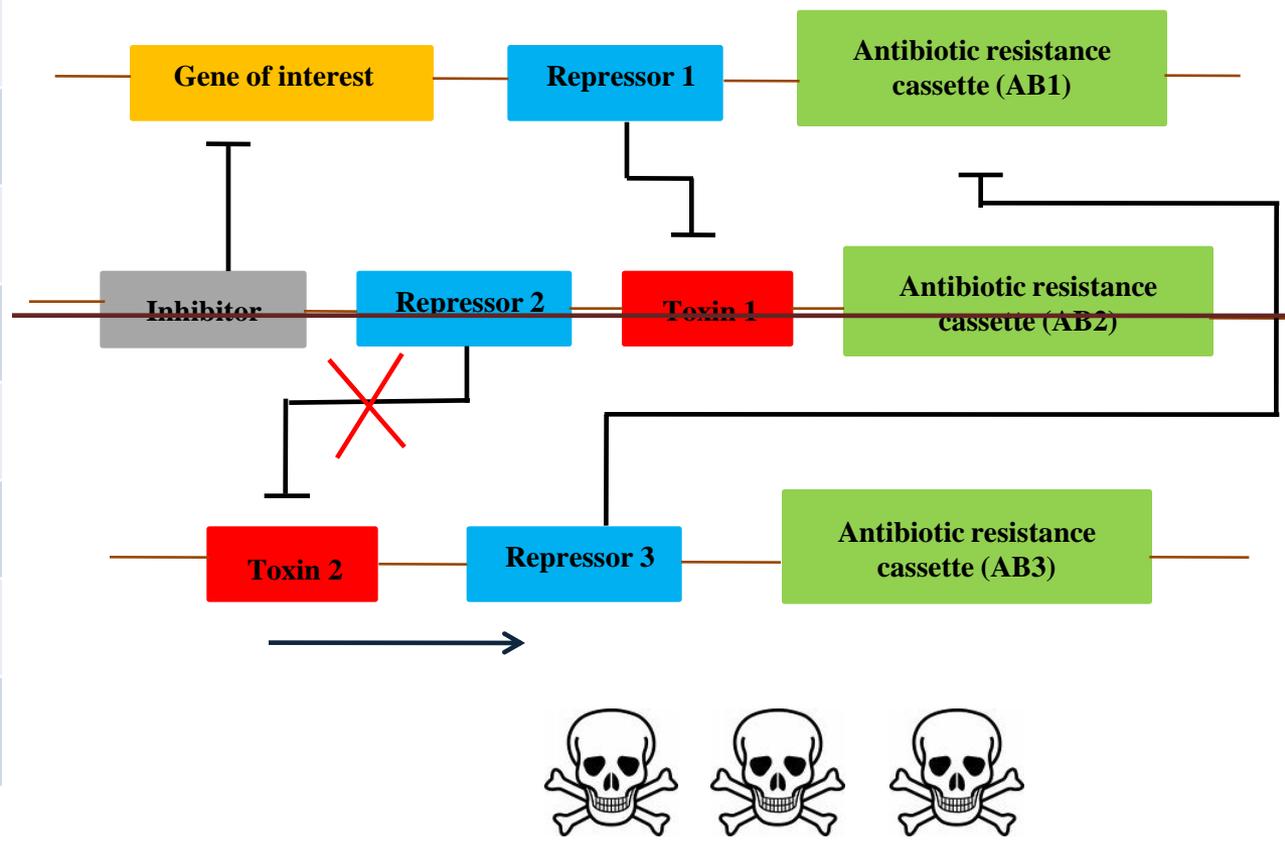


3-plasmid system

STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4	●	●	○
5	○	●	●
6	●	○	●
7	●	●	●

Ab3 in growth medium

- If plasmid 2 is lost

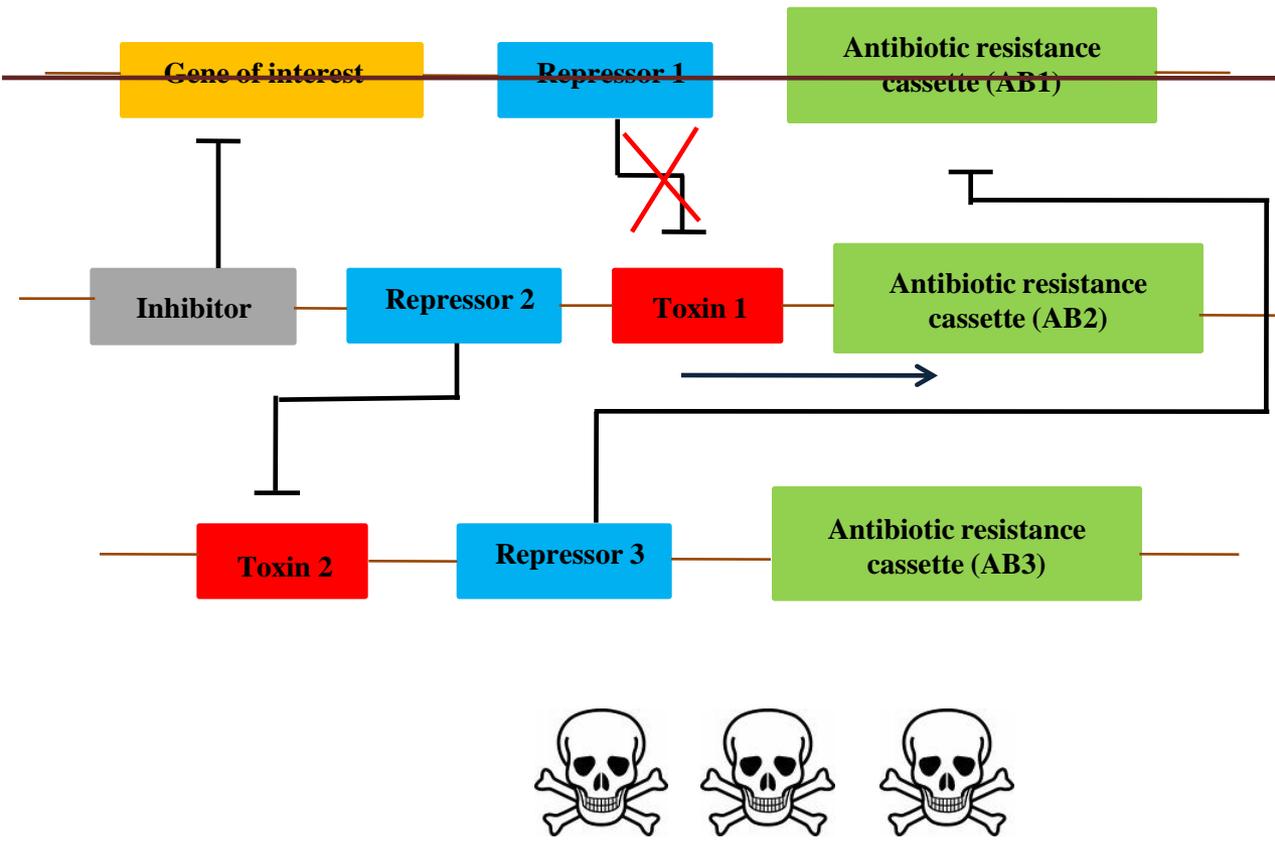


3-plasmid system

STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4	●	●	○
5	○	●	●
6	●	○	●
7 ✓	●	●	●

Ab3 in growth medium

- If plasmid 1 is lost

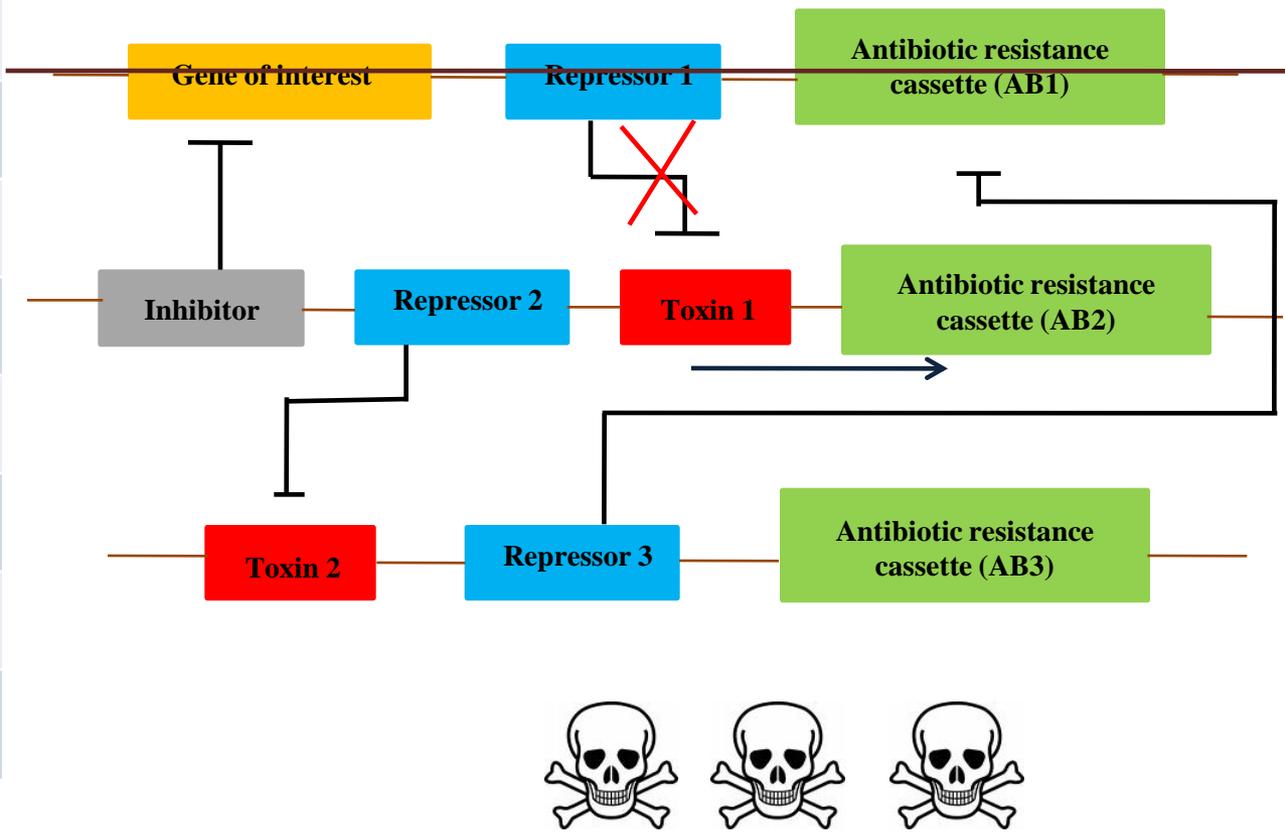


3-plasmid system

STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4	●	●	○
5	○	●	●
6	●	○	●
7	●	●	●

Ab2 in growth medium

- If plasmid 1 is lost

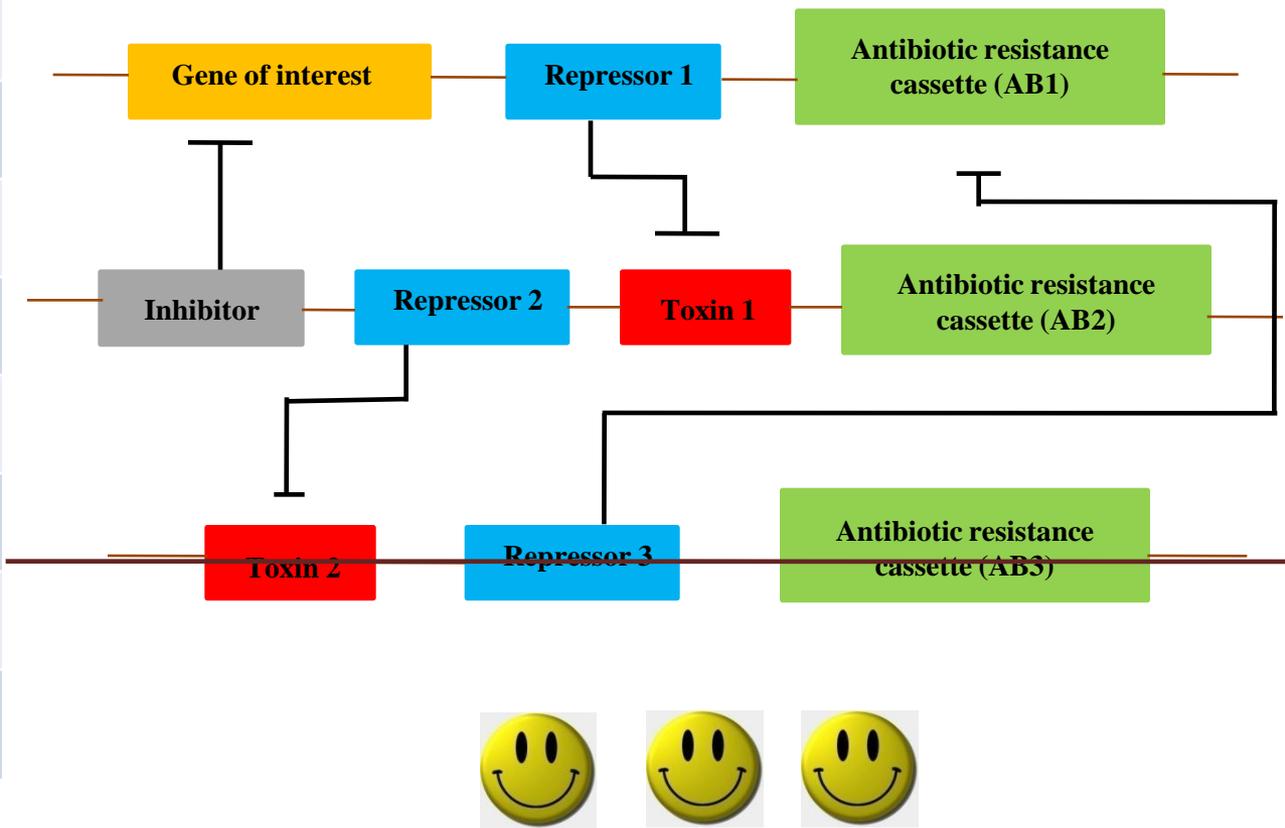


3-plasmid system

STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4 ✓	●	●	○
5	○	●	●
6	●	○	●
7	●	●	●

Ab2 in growth medium

- If plasmid 3 is lost

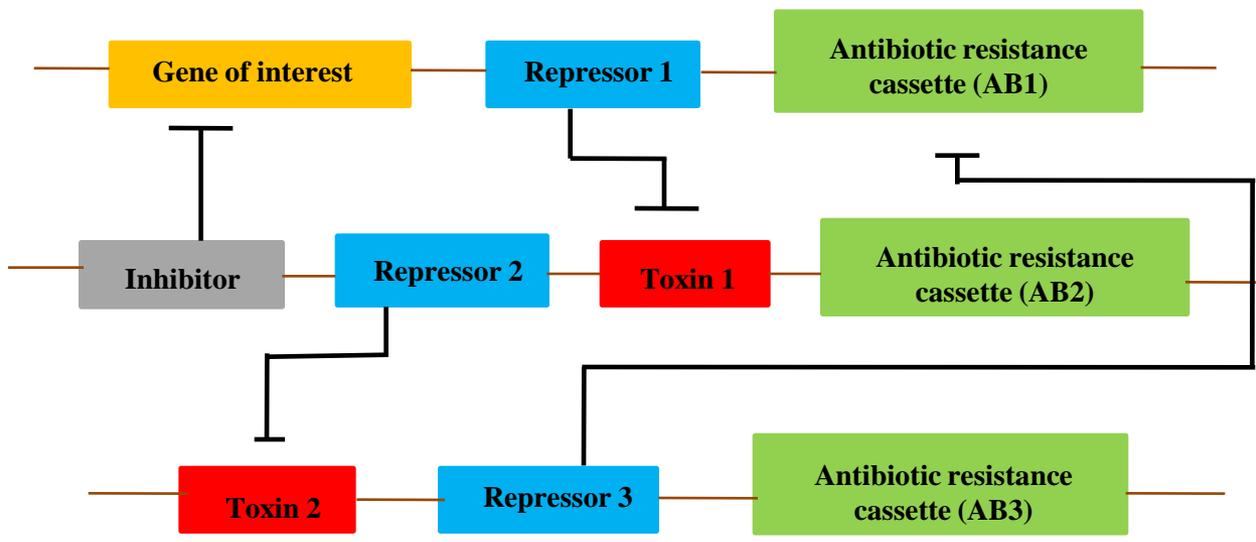


3-plasmid system

STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4 ✓	●	●	○
5	○	●	●
6	●	○	●
7 ✓	●	●	●

Ab2 in growth medium

- State 7



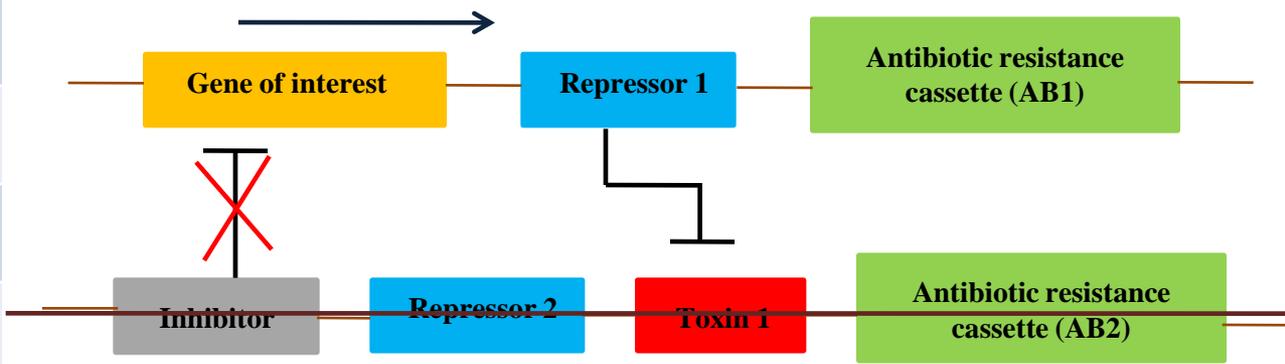
However, no selection pressure for plasmid 3.

3-plasmid system

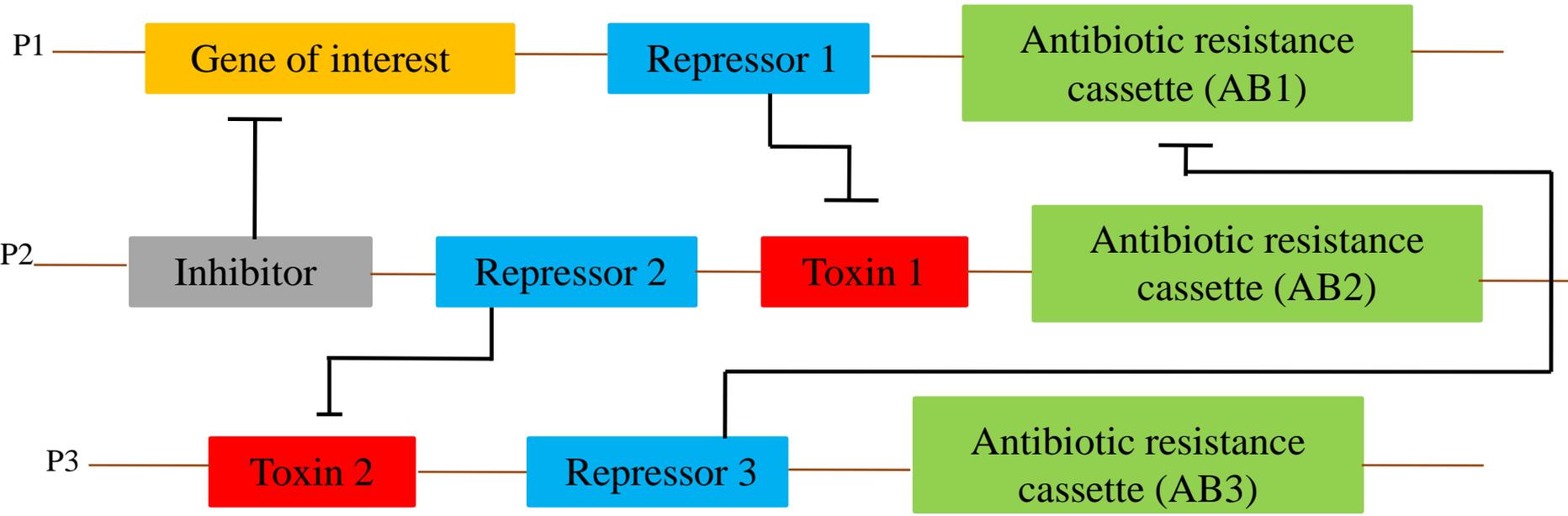
STATE	P1	P2	P3
0	○	○	○
1	●	○	○
2	○	●	○
3	○	○	●
4 ✓	●	●	○
5	○	●	●
6	●	○	●
7	●	●	●

Now, with P1 and P2 intact, changing to **Ab1**

- If plasmid 2 is lost

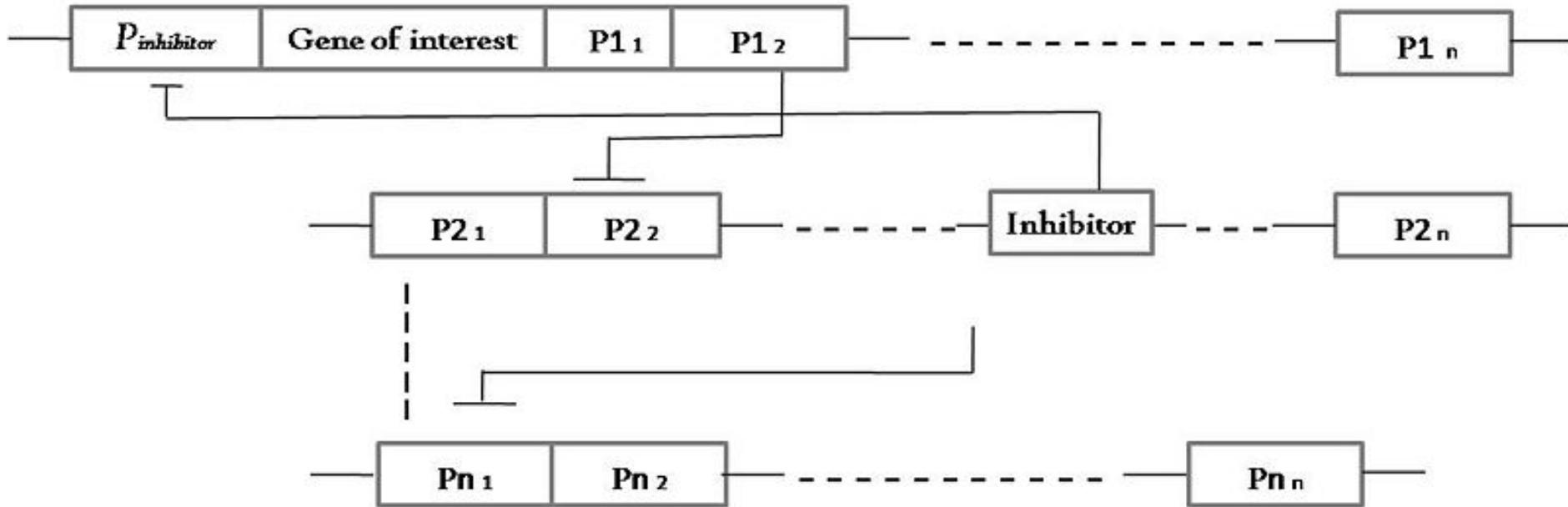


3-plasmid system



AB3 → AB2 → AB1 → Gene is expressed

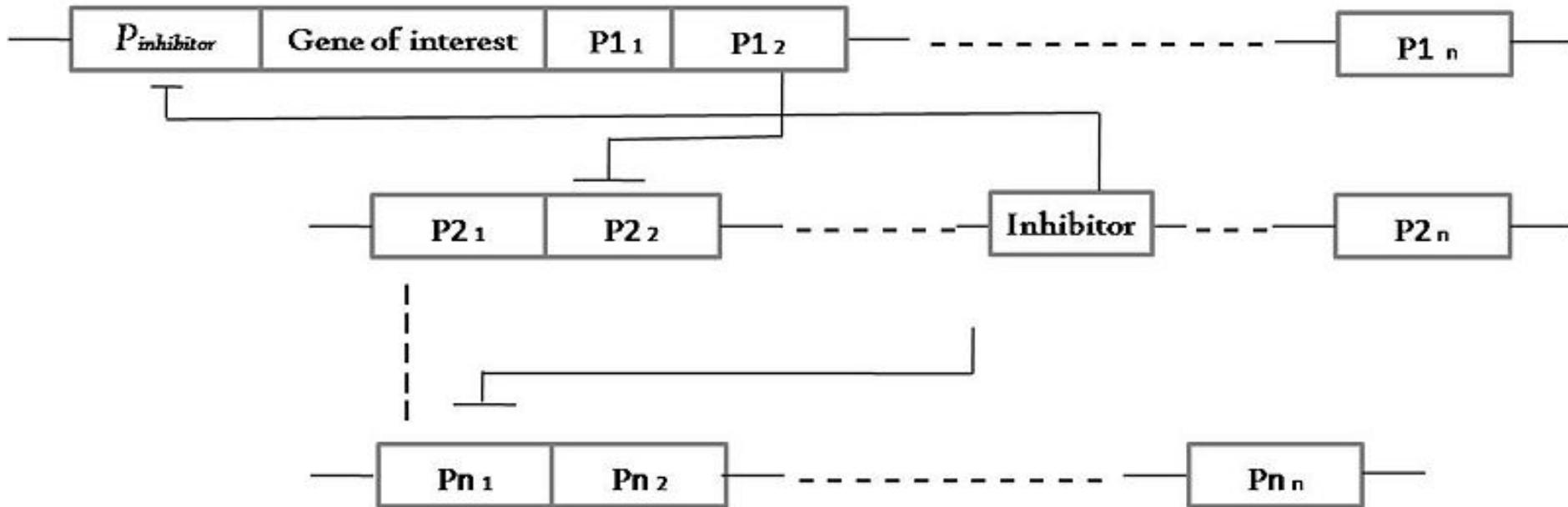
N-plasmid system



Code length: N

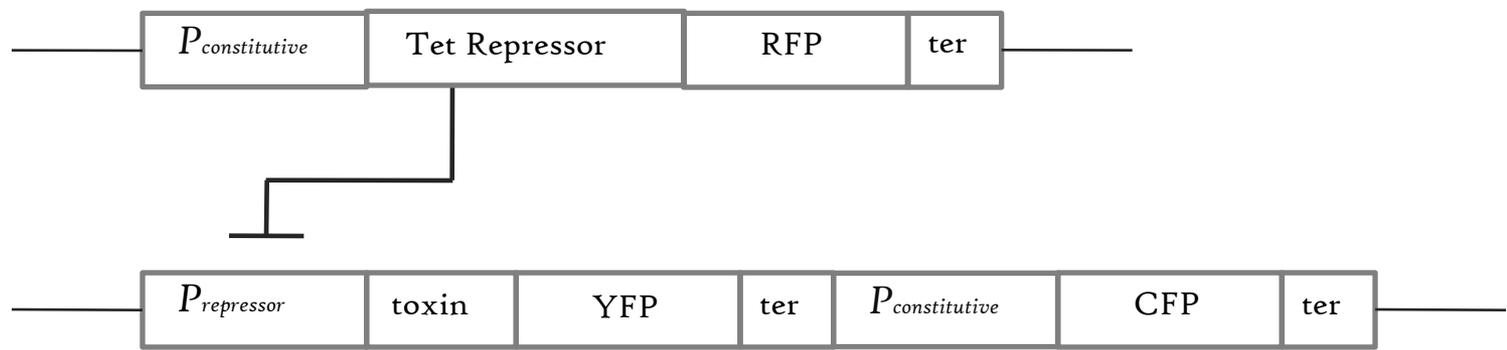
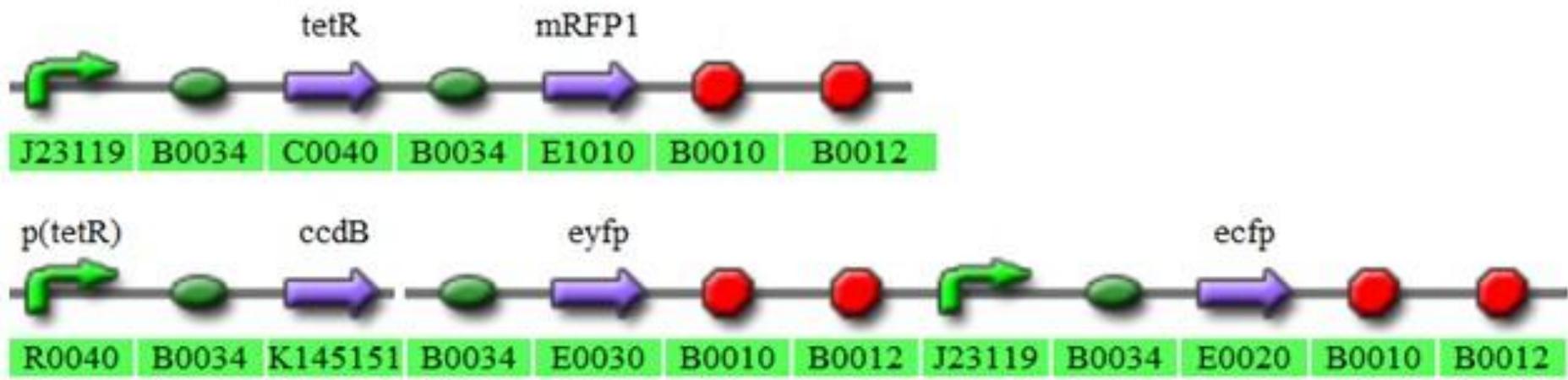
Code: $AB_n \rightarrow AB_{n-1} \rightarrow \dots \rightarrow AB_2 \rightarrow AB_1$.

Sustaining multiple insert DNA



A **single selection pressure** ensures that all the plasmids are intact in the system.

Biobricks construction



Proof-of-concept

Parts submitted

- BBa_K272001

Constitutive Expression Cassette for RFP

Emission 607 nm

Excitation 584 nm

Protein mRFP1

Tag None

- BBa_K272002

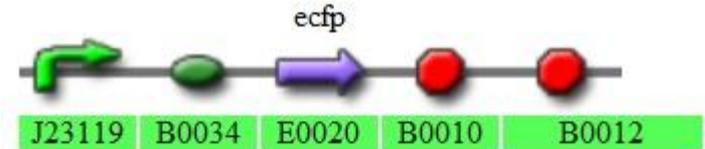
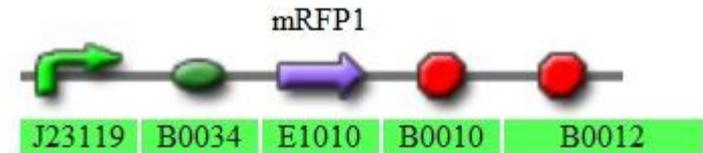
Constitutive Expression Cassette for CFP

Emission 476 nm

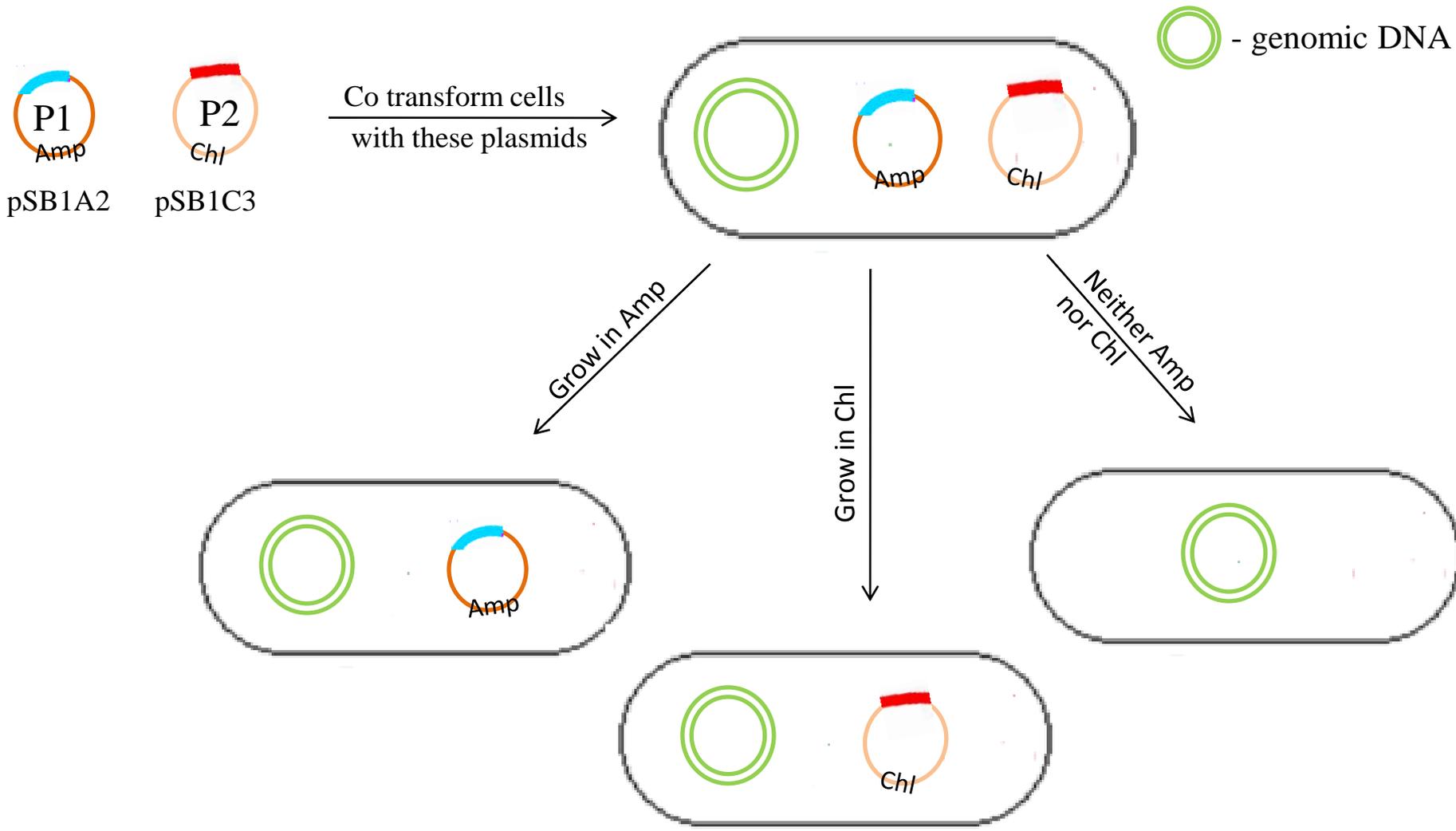
Excitation 439 nm

Protein ECFP

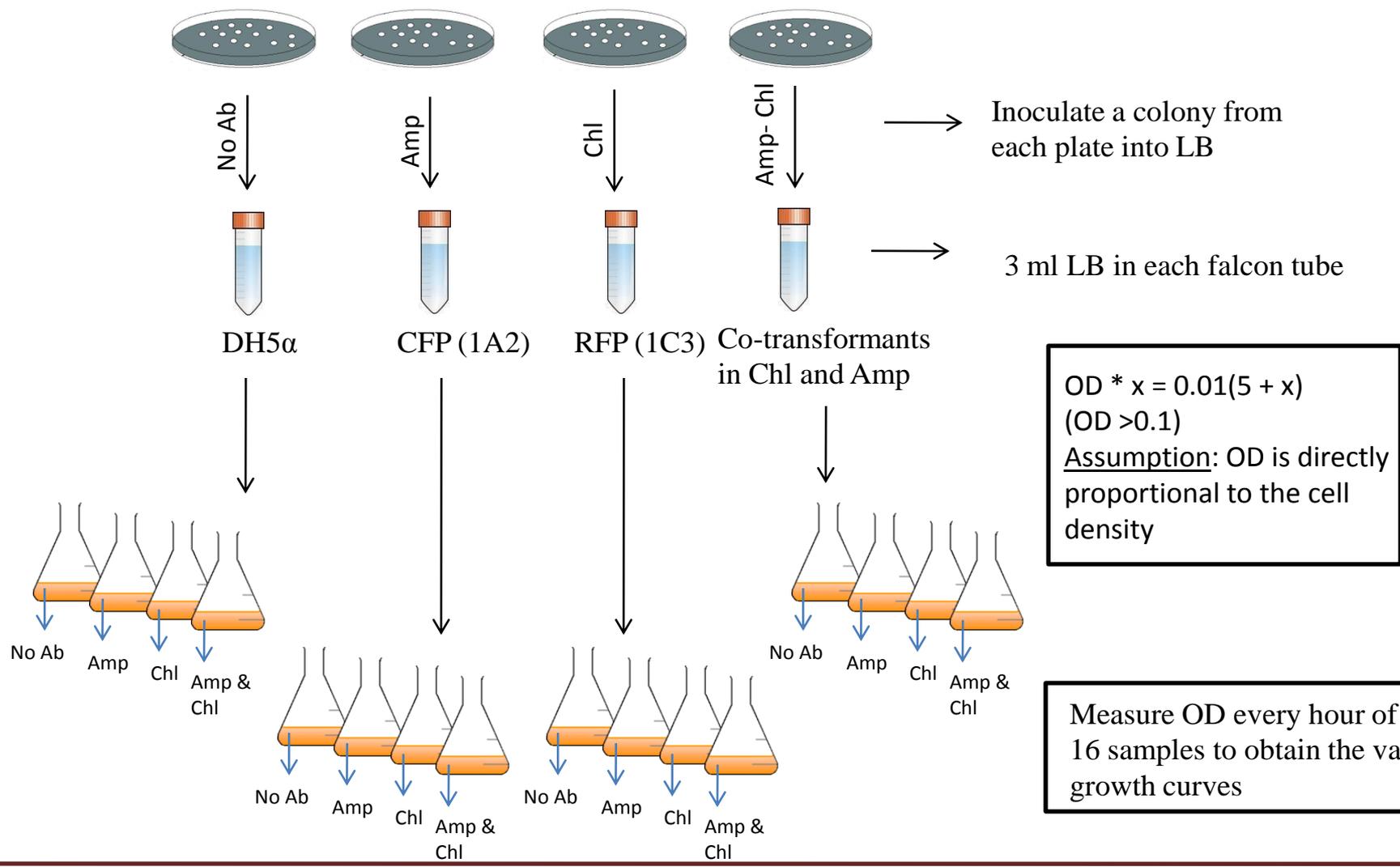
Tag None



Proof-of-concept

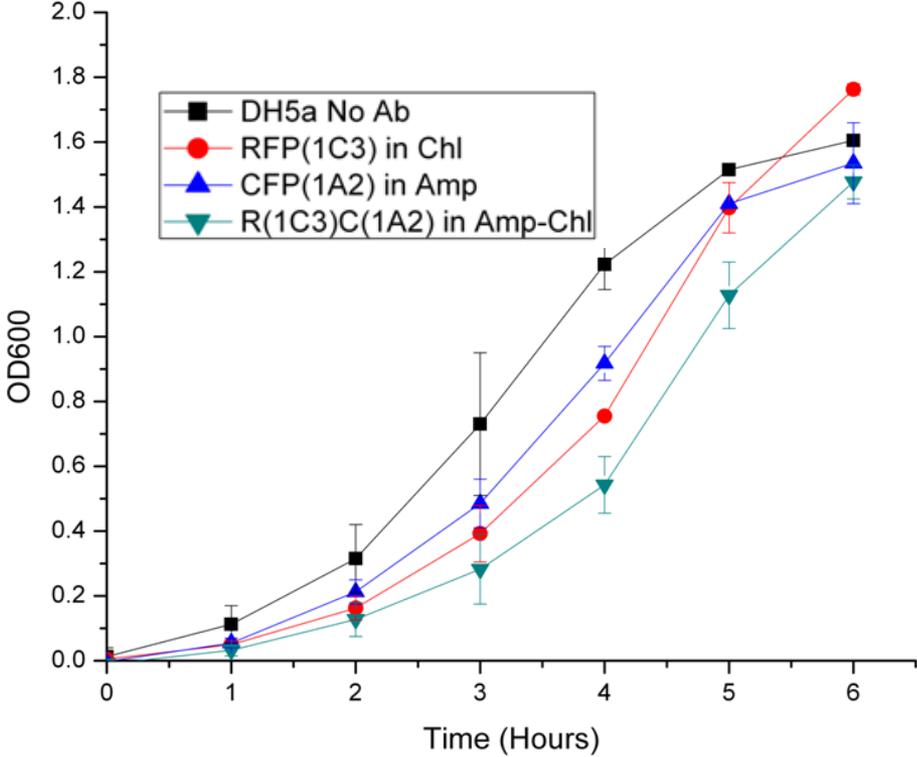
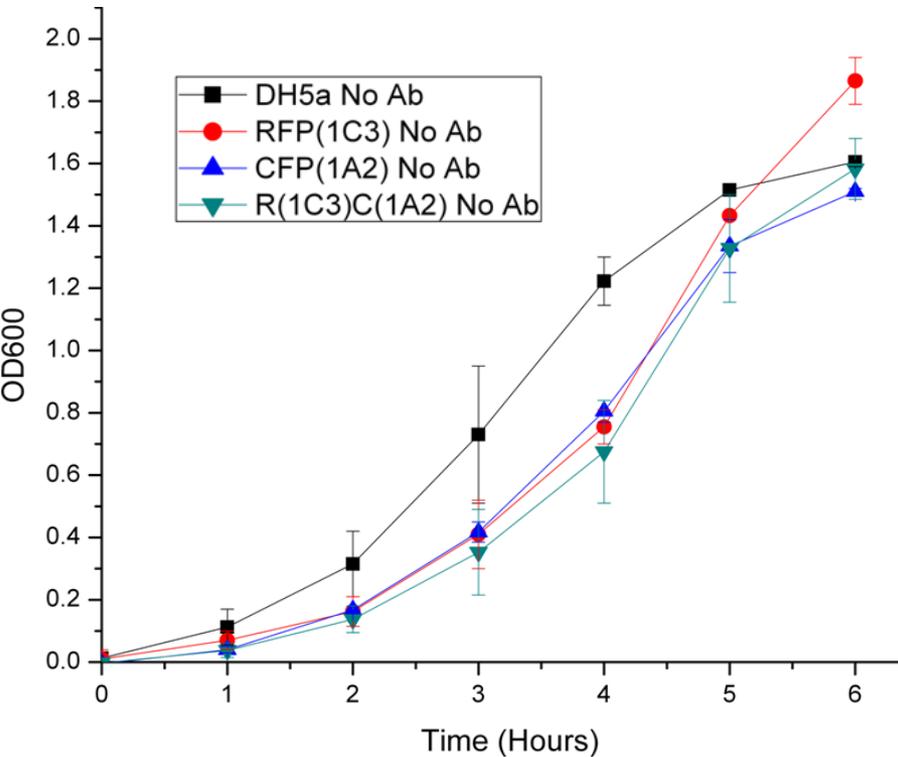


Design of Experiments

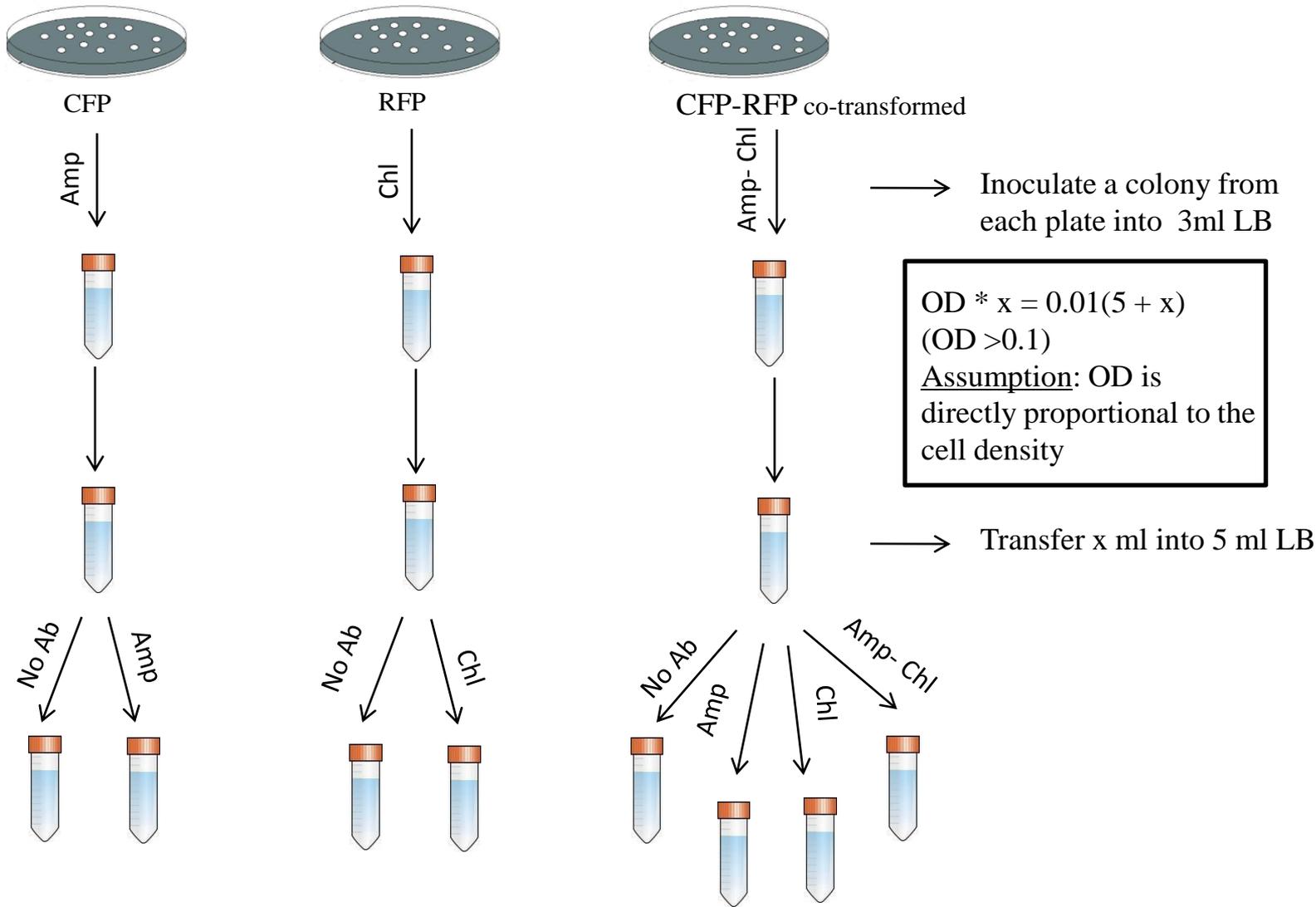


Growth curves

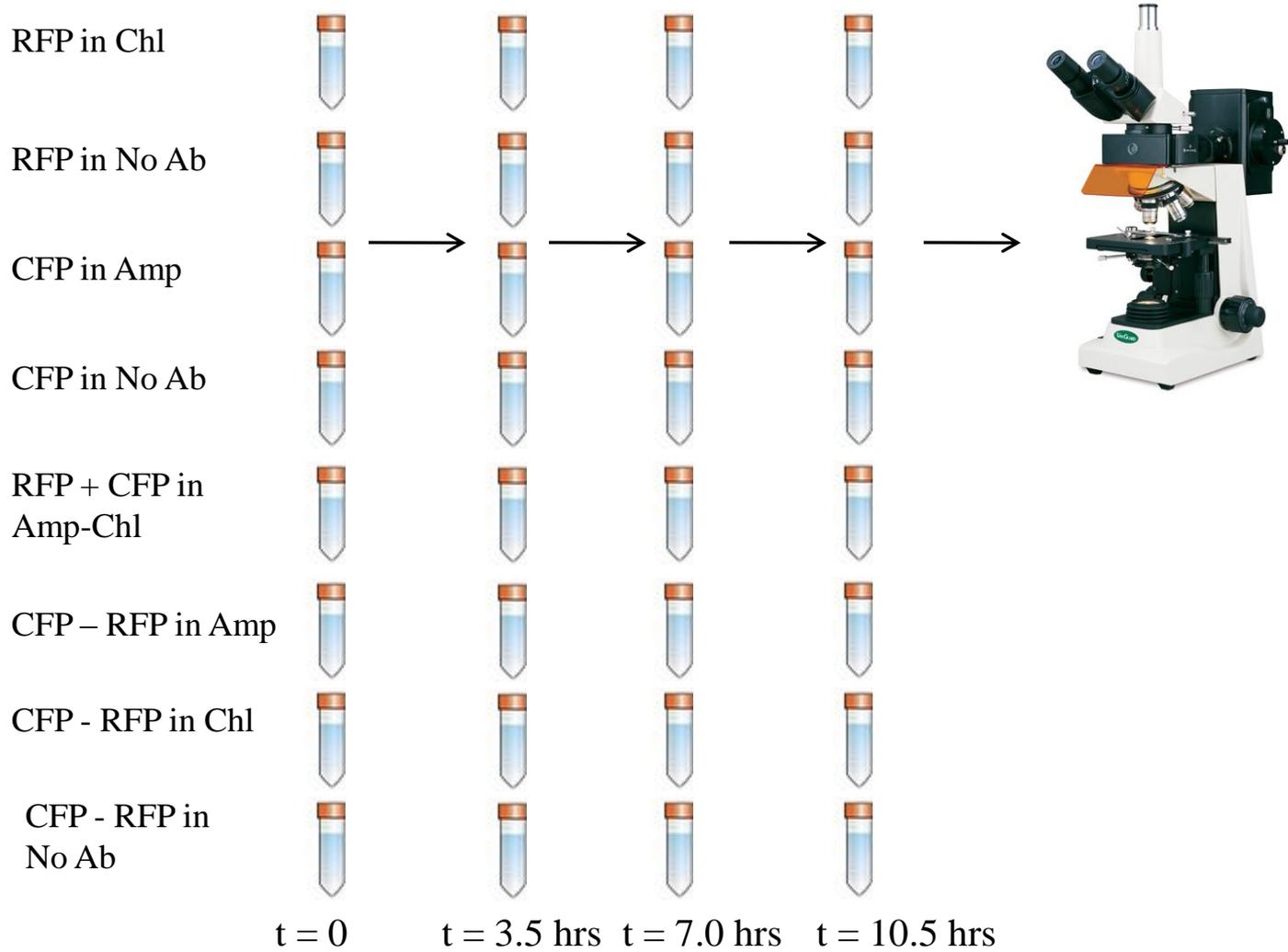
With no antibiotic(s) and corresponding antibiotic(s) in the growth medium



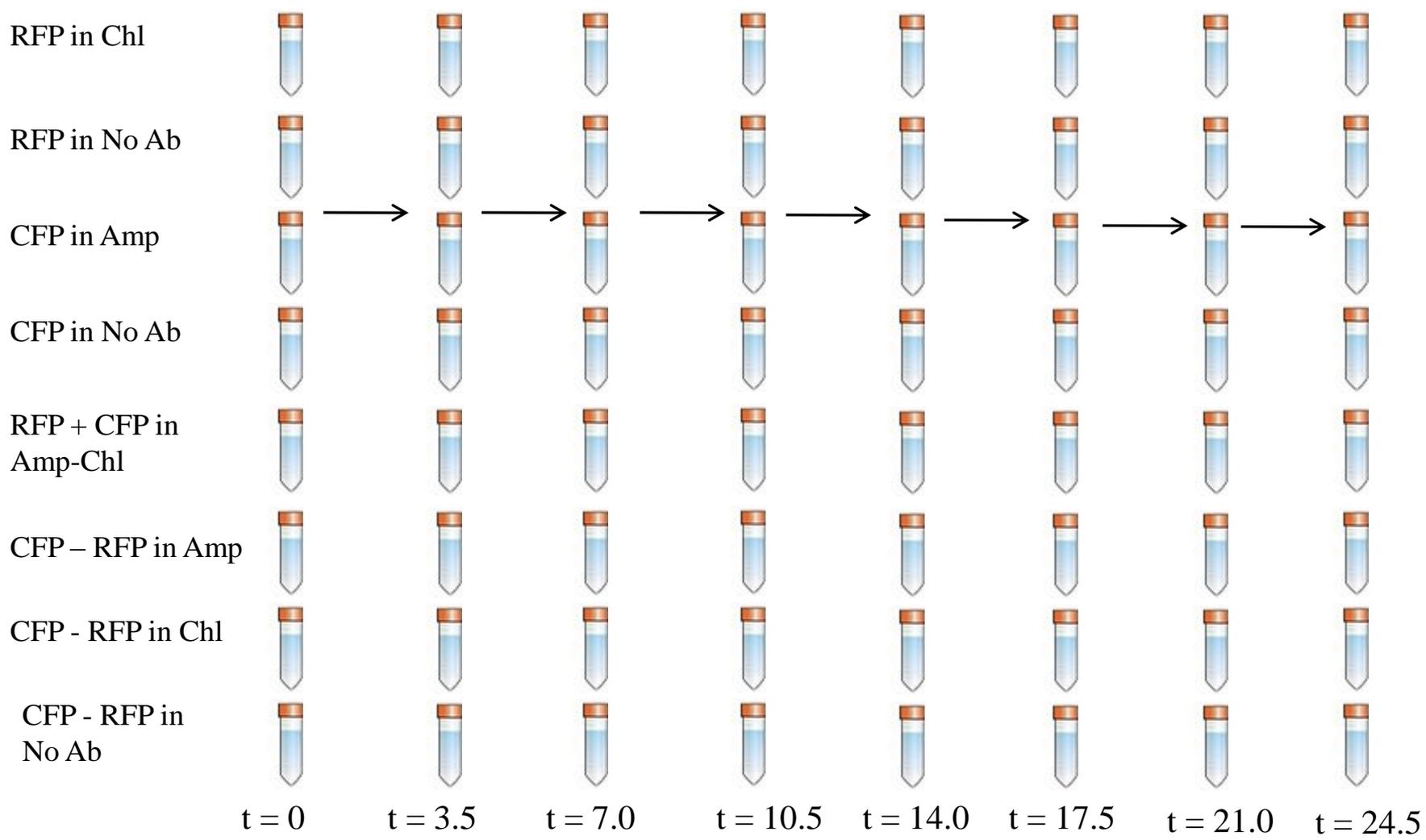
Design of Experiments



Design of Experiments

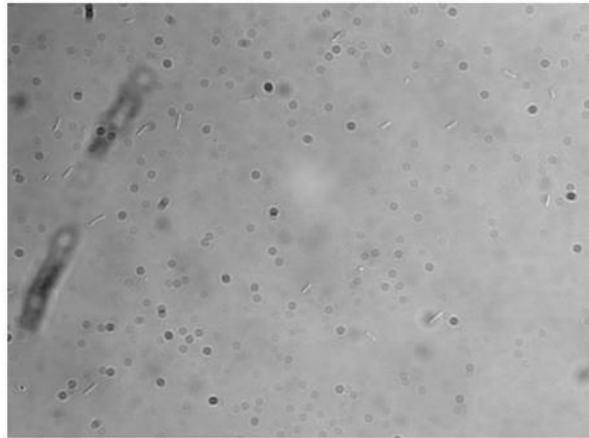


Design of Experiments

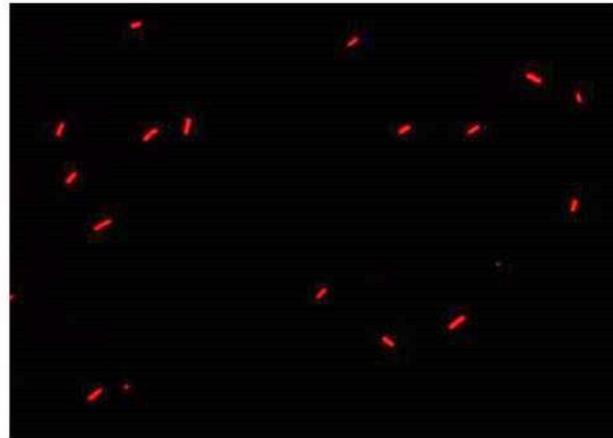


Images

- RFP (in pSB1C3)



DIC



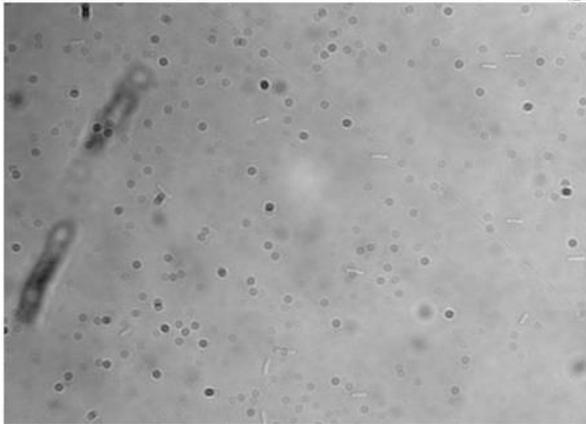
Red filter



Green filter

Images

- CFP (in pSB1A2)



DIC



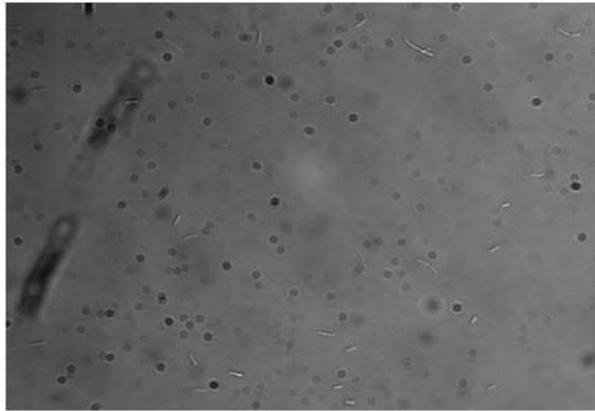
Red filter



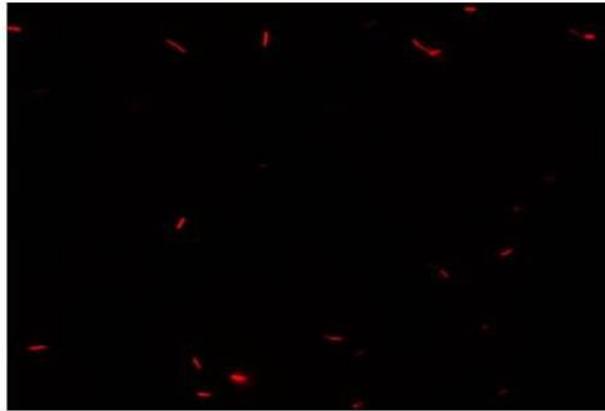
Green filter

Images

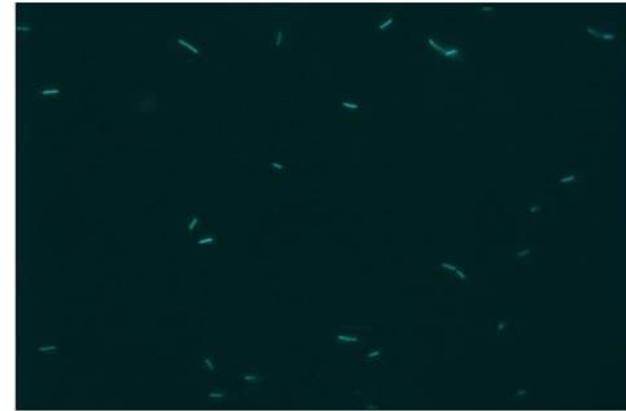
- Cells co-transformed with CFP (pSB1A2) and RFP (pSB1C3)



DIC



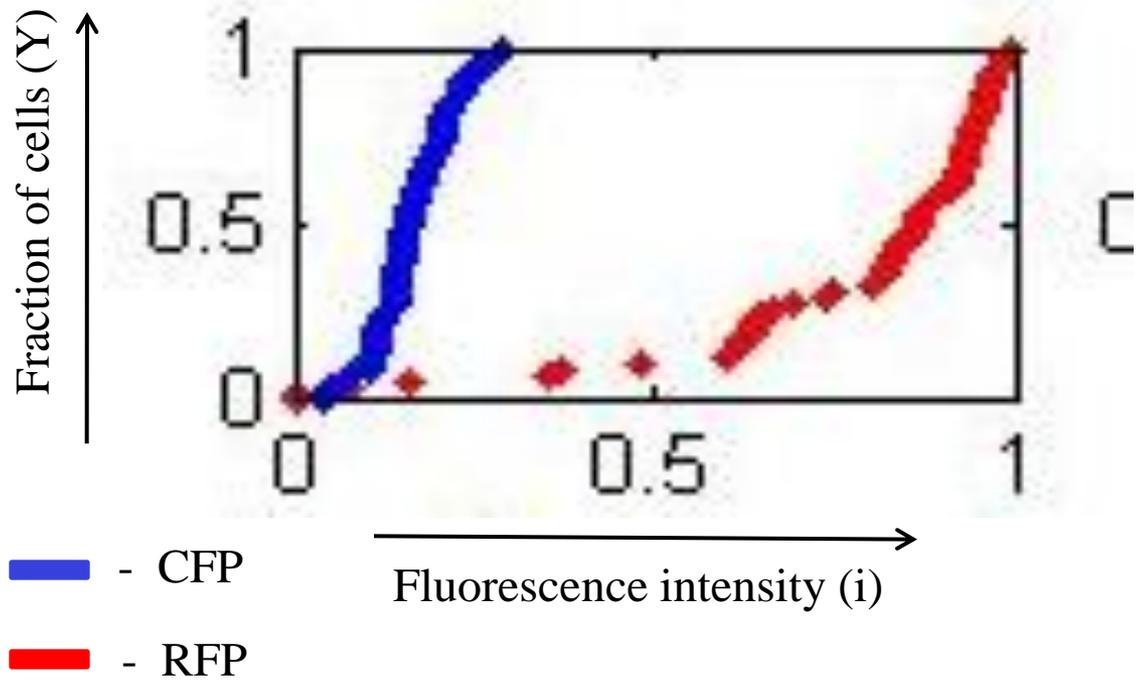
Red filter



Green filter

Interpreting images

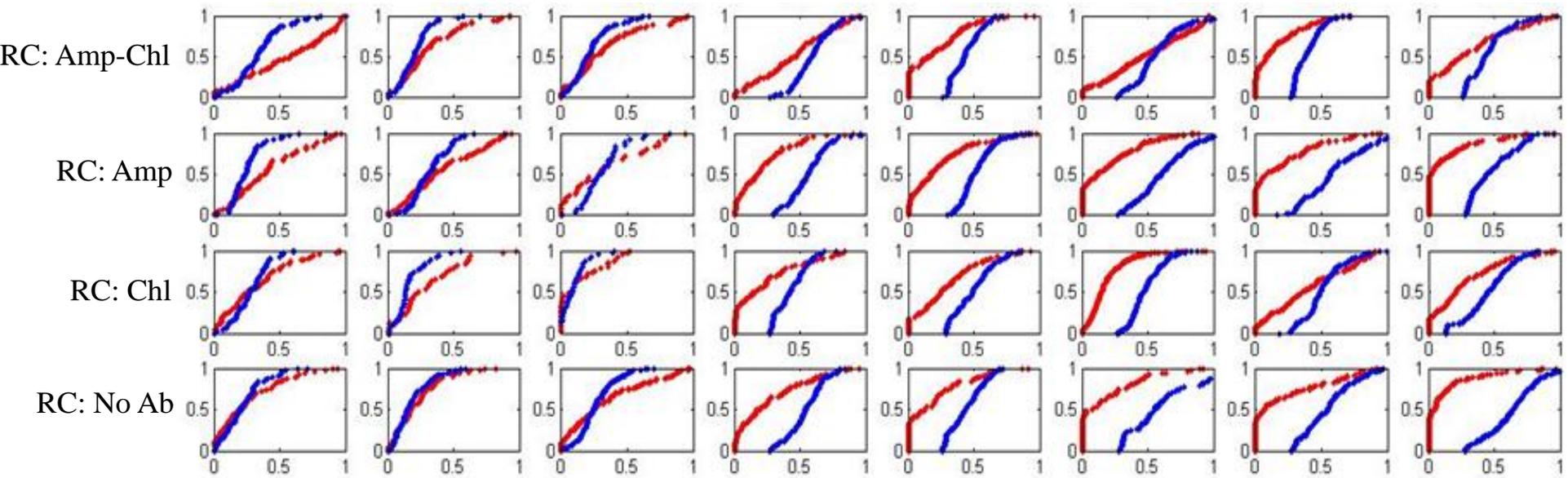
Cumulative Distribution Curve



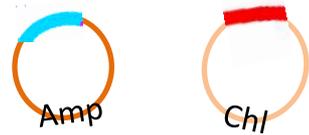
$$y = f(i)$$
$$Y = \int_0^i y \, dy$$

Results

Cumulative distribution curves for co-transformed cells

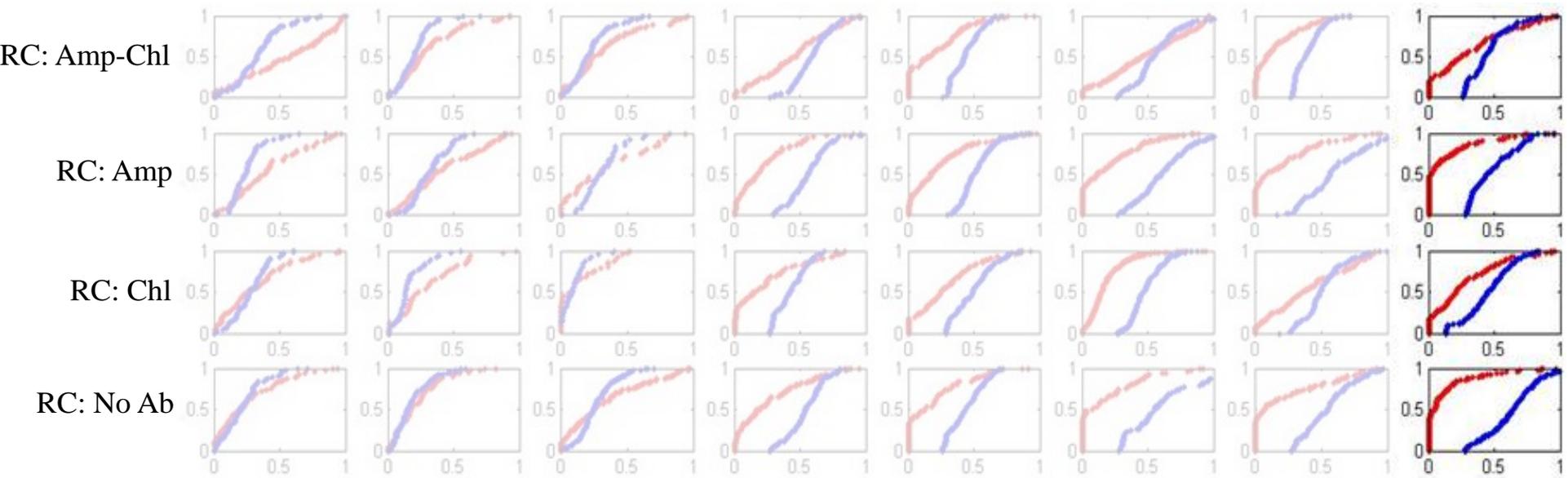


Time points →

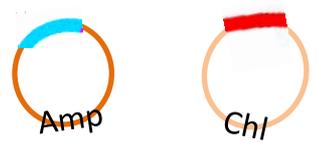


Results

Cumulative distribution curves for co-transformed cells



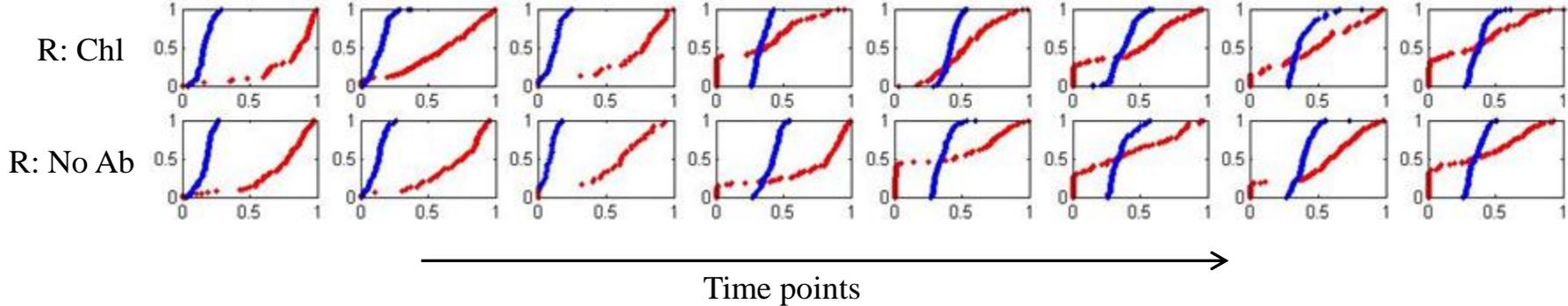
Time points →



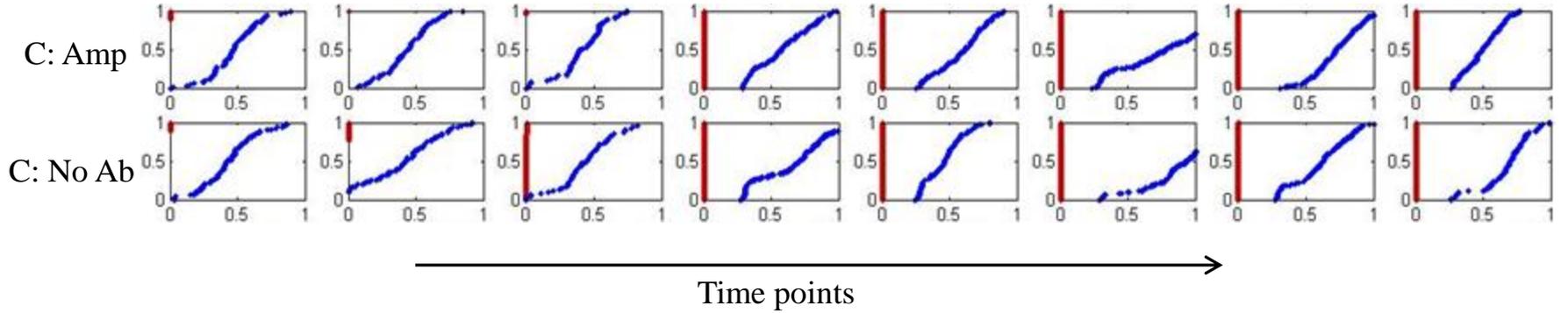
Results



Cumulative distribution curves for RFP transformed cells

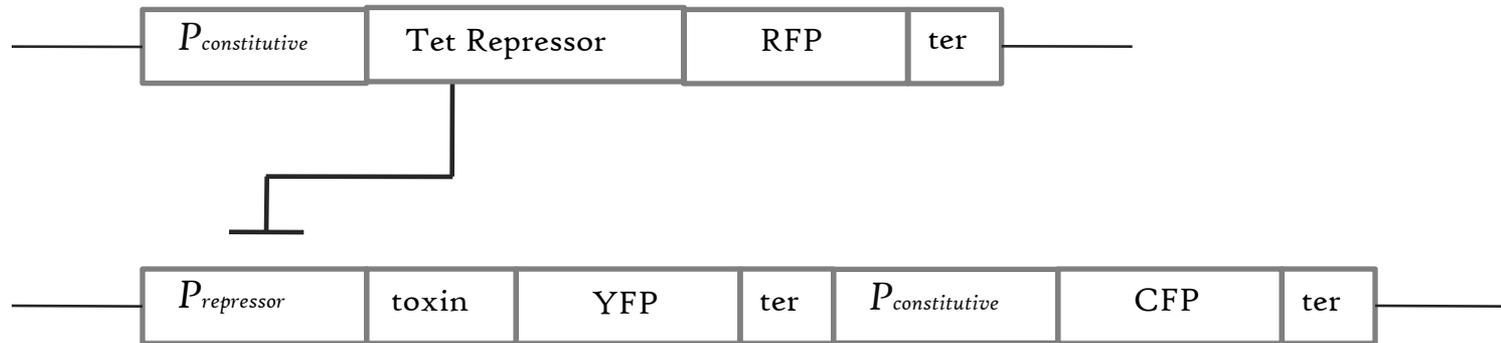


Cumulative distribution curves for CFP transformed cells



Conclusions and Implications

- Directed plasmid loss works!



- Locking Assembly
- Sustaining multiple inserts with a single selection pressure

Acknowledgements

- The team

Abdul
Ajit
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Swathi



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Dr. Madhulika Dixit

Dr. Mukund Thattai



ARCH Pharmalabs



Industrial Consultancy &
Sponsored Research -
IIT Madras



Council of Scientific &
Industrial Research



Biocon

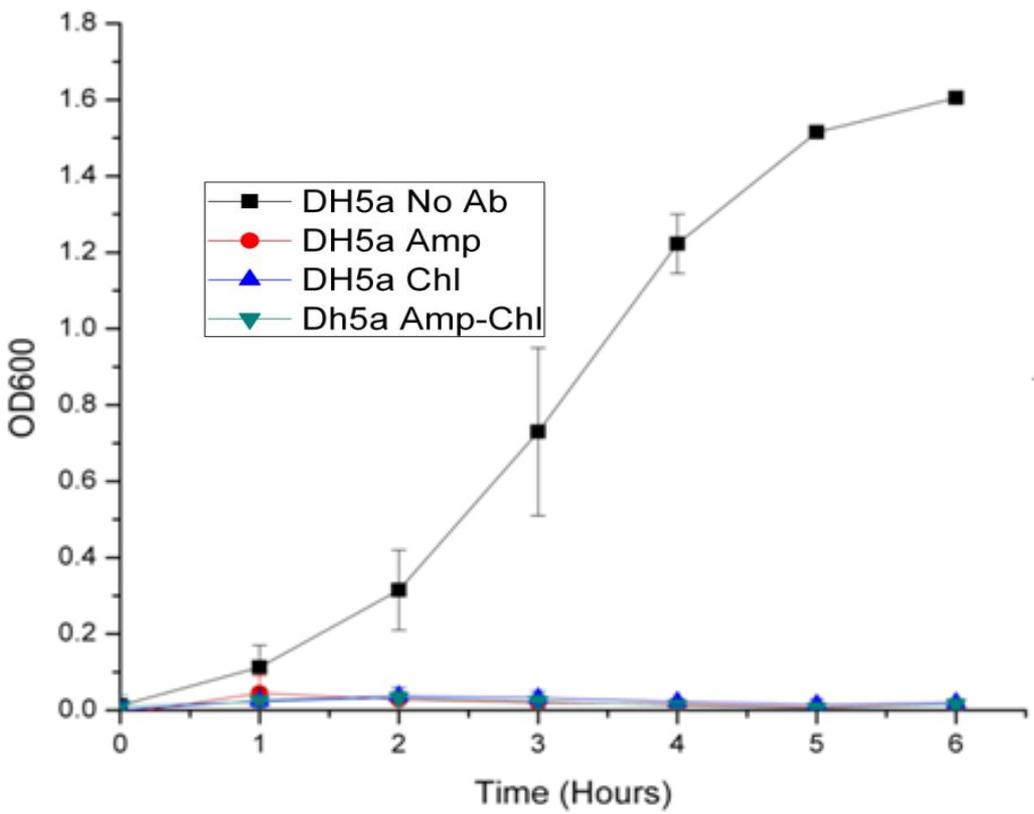
Growth curves

DH5 α

CFP (1A2)

RFP (1C3)

Co-transformants
in Chl and Amp



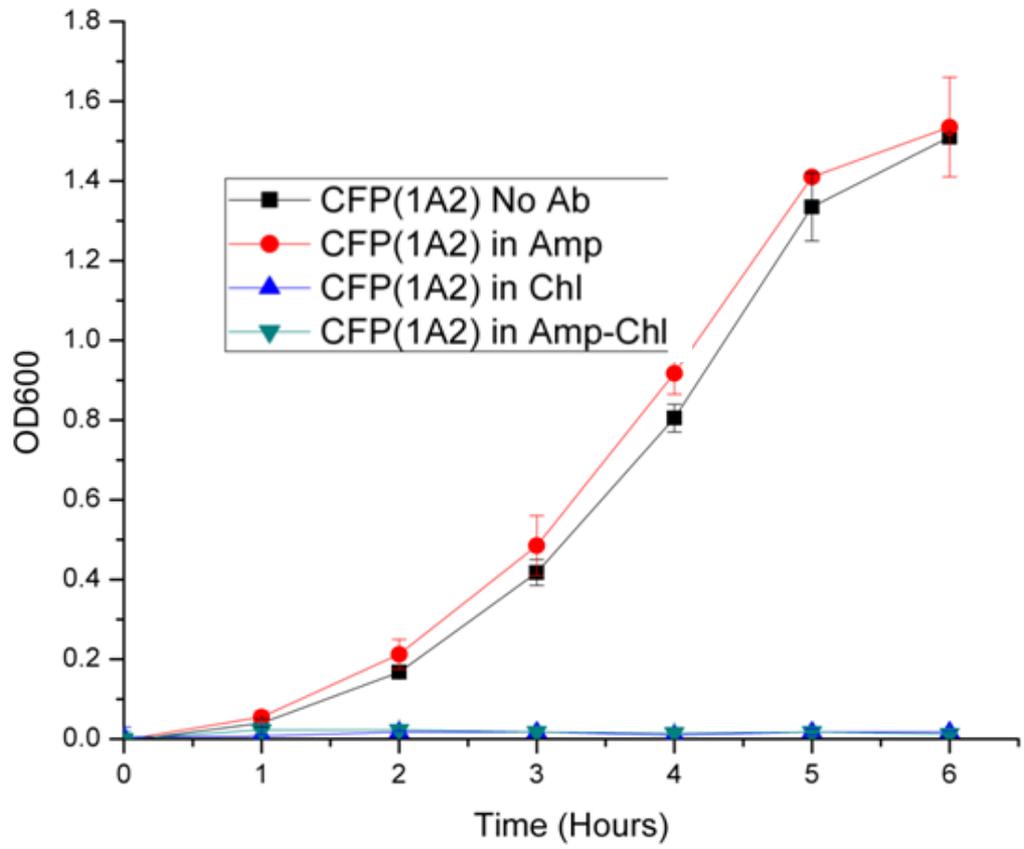
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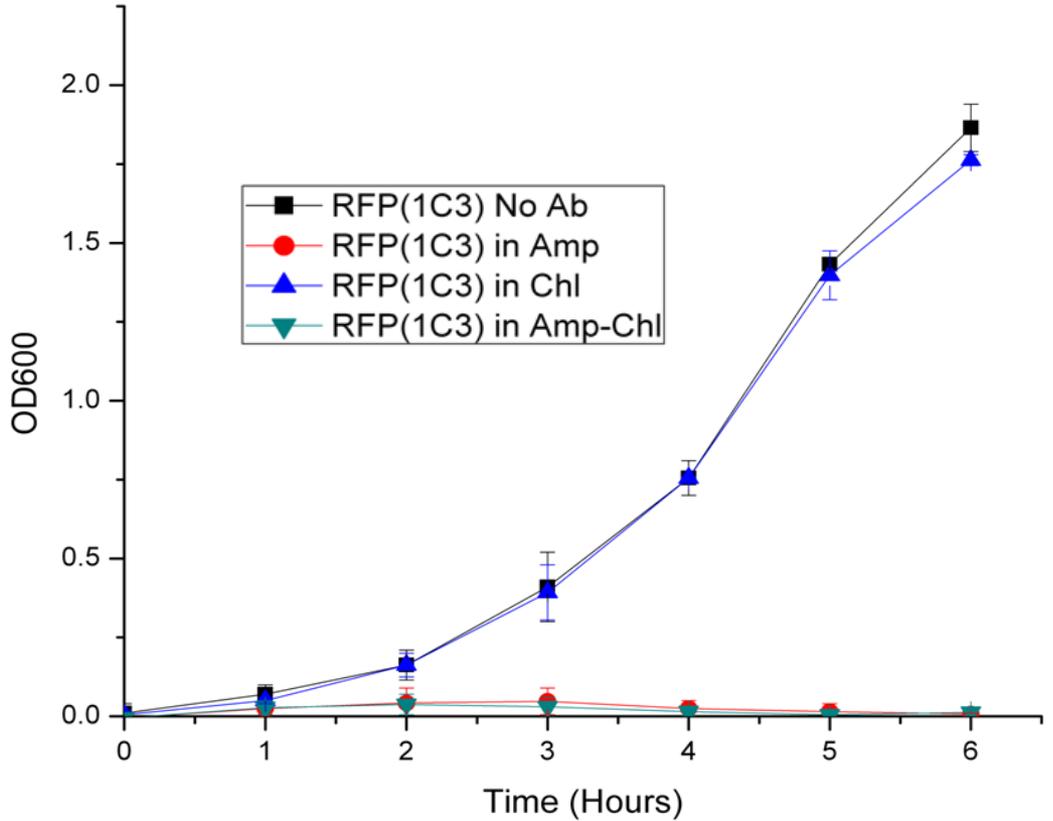
Growth curves

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Growth curves

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