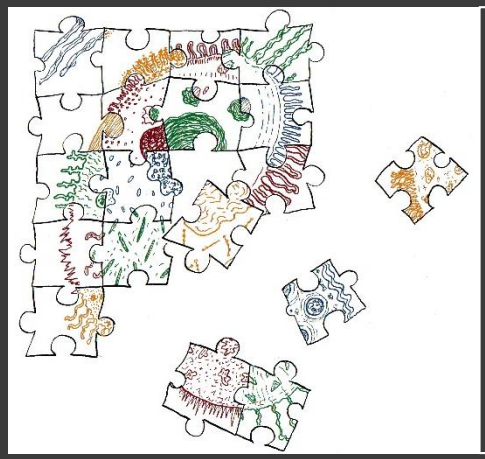
InteGreator :
How to improve biological pathways?



ULB
Brussels
team

Who we are:

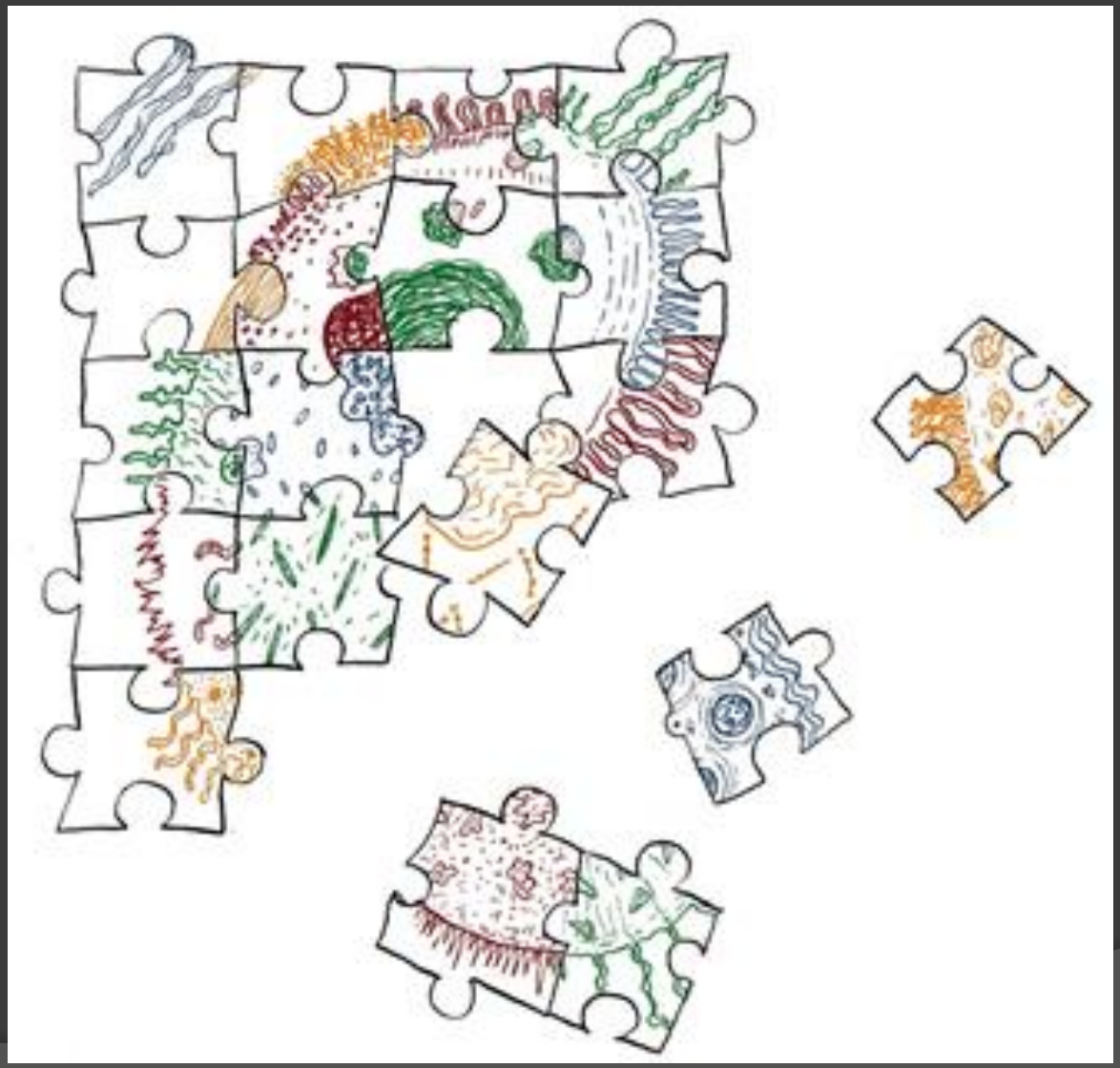
Wet lab crew

Institute for Molecular Biology and Medicine (IBMM)

Modeling crew

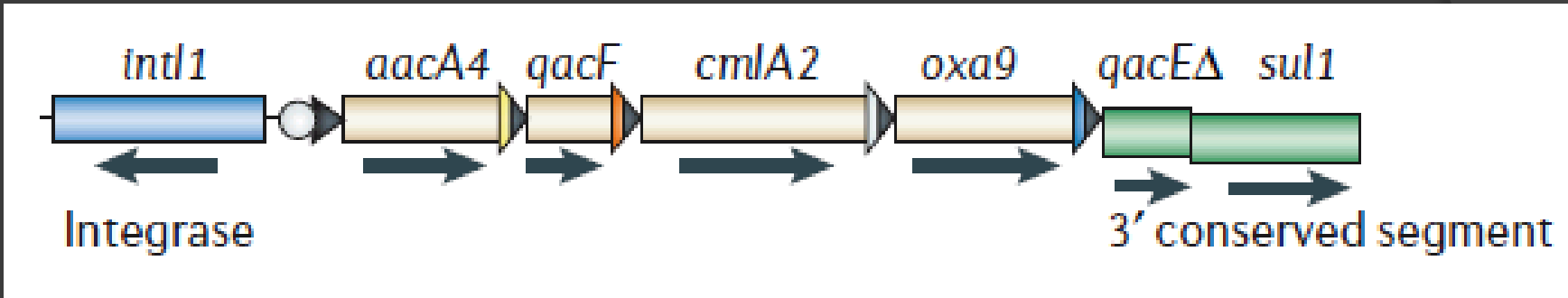
ULB main campus - Brussels

What is inteGreator?



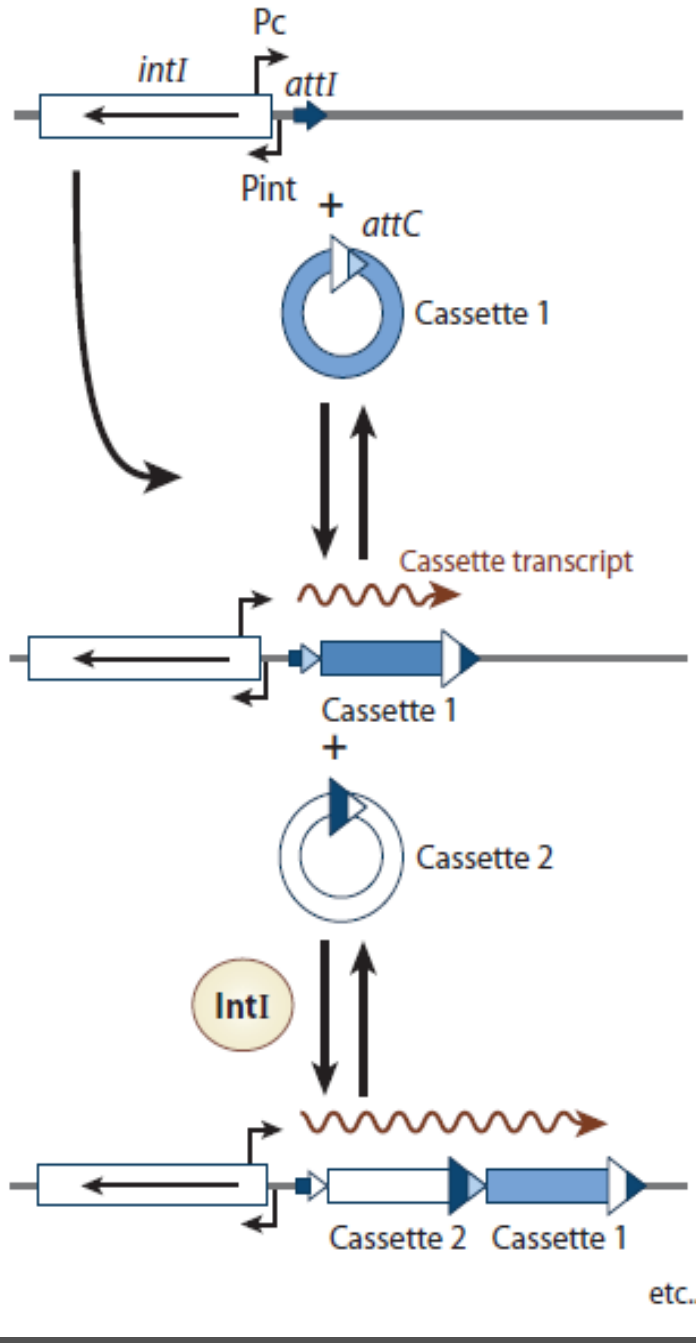
Using the integron system to optimize pathways

Introduction: the integron



Mazel D. 2006

- Dynamic genetic platform
- Contains gene cassettes that can be excised, integrated and their order rearranged



- IntI: integrase
- attC: excision sites
- attI: primary recombination site
- Pc promoter

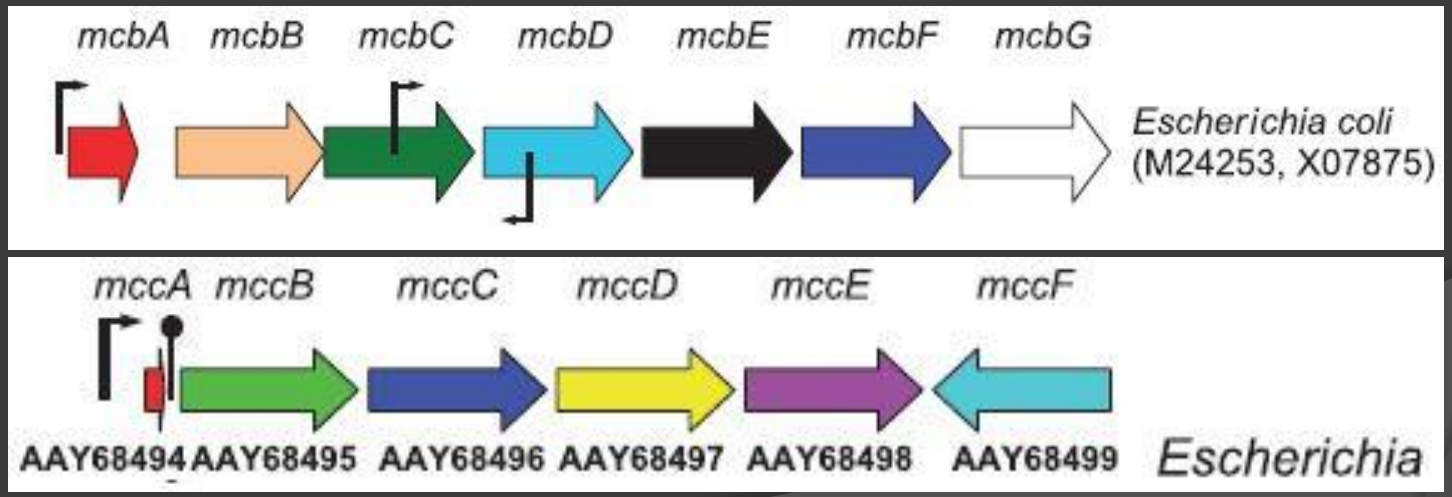
Cambray et al. 2010

Goal

- Use the integron as a natural genetic optimization tool to produce proteins in bacteria

Proof of concept : microcins

- Natural antibacterial peptides produced by *Enterobacteria*
- Low molecular weight
- Extensive post-translational modifications
- Target essential physiological processes

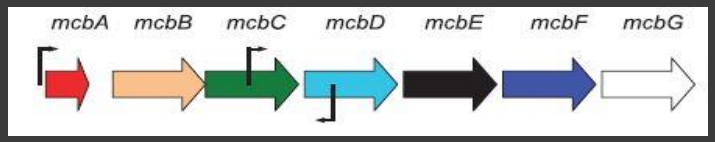


Severinov et al. 2007

Results

Construction of the biobricks

- Biobricks → integron



- Typical structure

RFC10 sequences: insertion of microcin gene into biobricks and in the “integron” plasmid.

RBS: ribosome binding site

attC site: gene rearrangement in the integron

Results

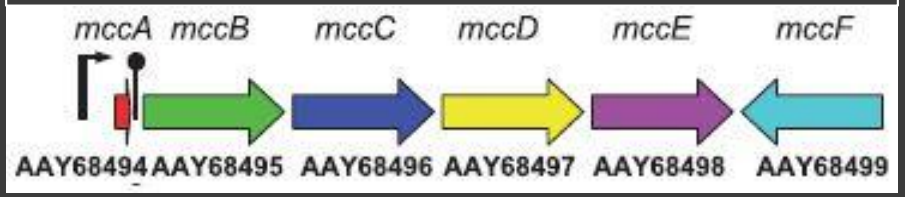
7 biobricks

Microcin C7

BBa_K908020 : pSB1K3-mccC

BBa_K908019 : pSB1K3-mccE

BBa_K908021 : pSB1K3-mccF



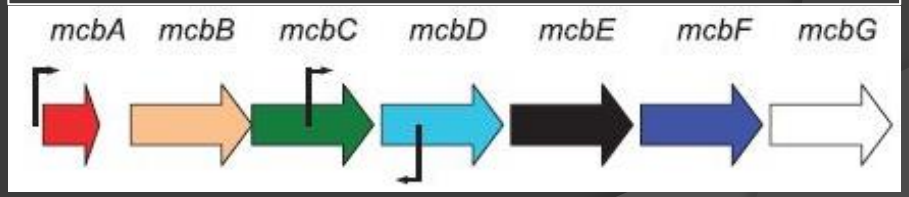
Microcin B17:

BBa_K908015 : pSB1C3-mccA

BBa_K908016 : pSB1K3-mccB

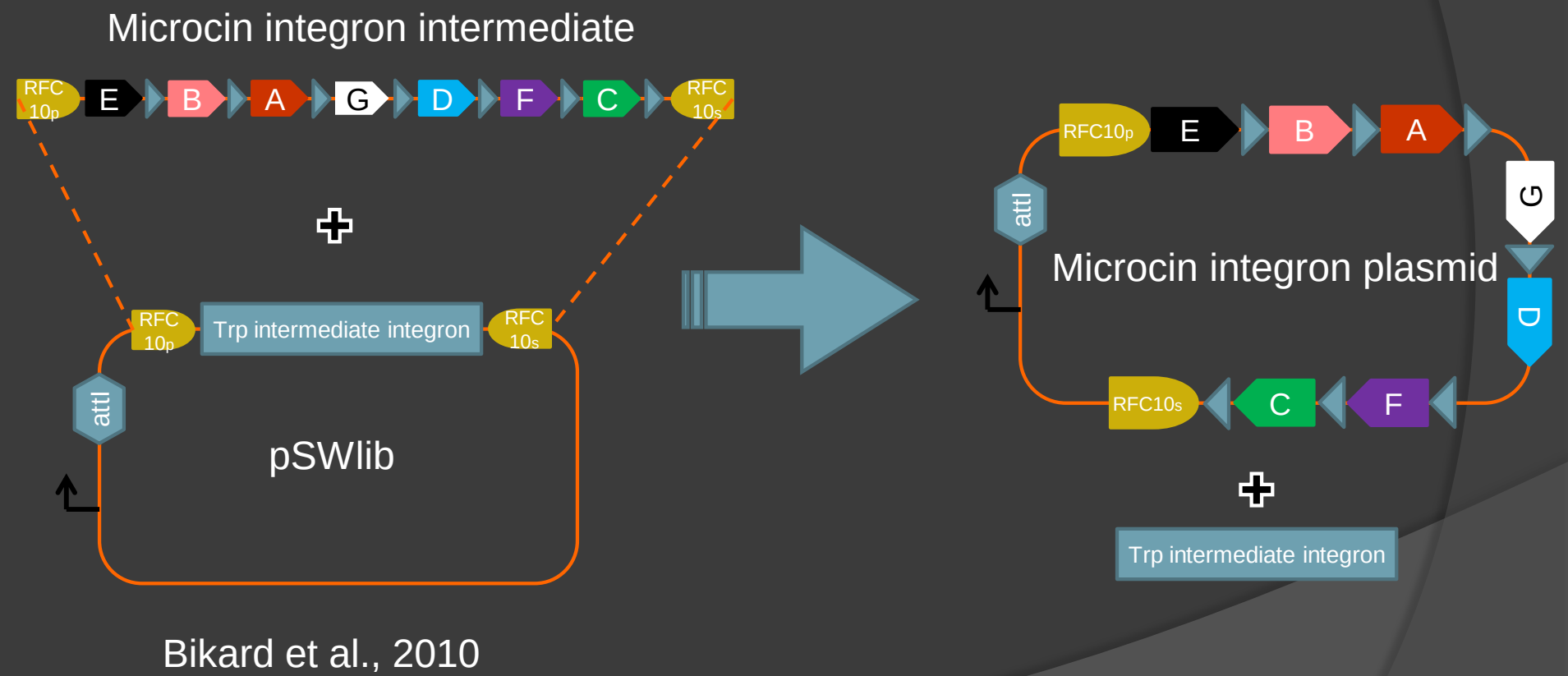
BBa_K908017 : pSB1K3-mccC

BBa_K908018 : pSB1C3-mccF



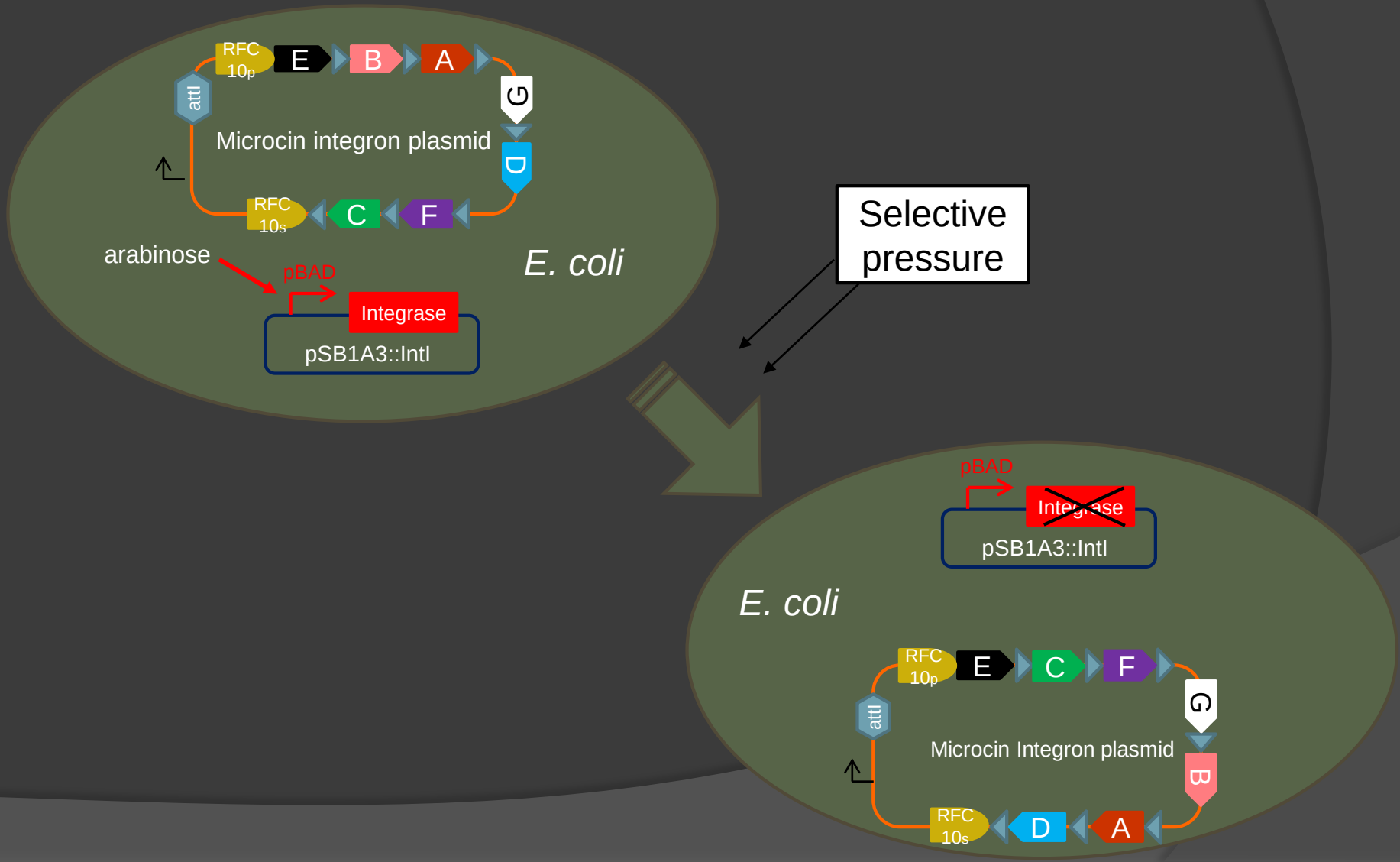
Construction of the Microcin integron

Transfer the integron in pSWIib



Bikard et al., 2010

How InteGreator could optimize microcin production



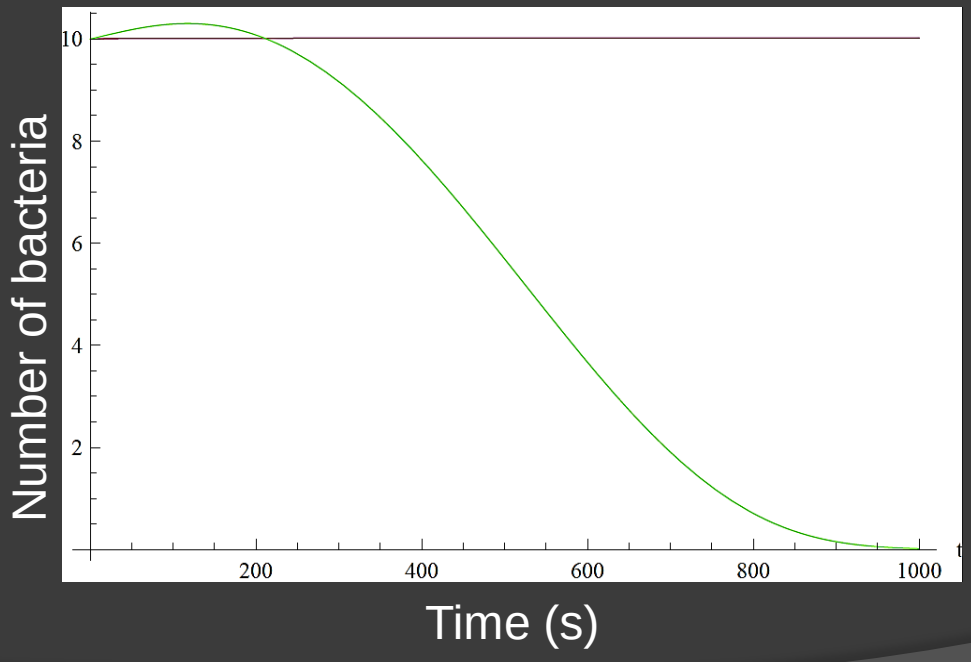
Modeling

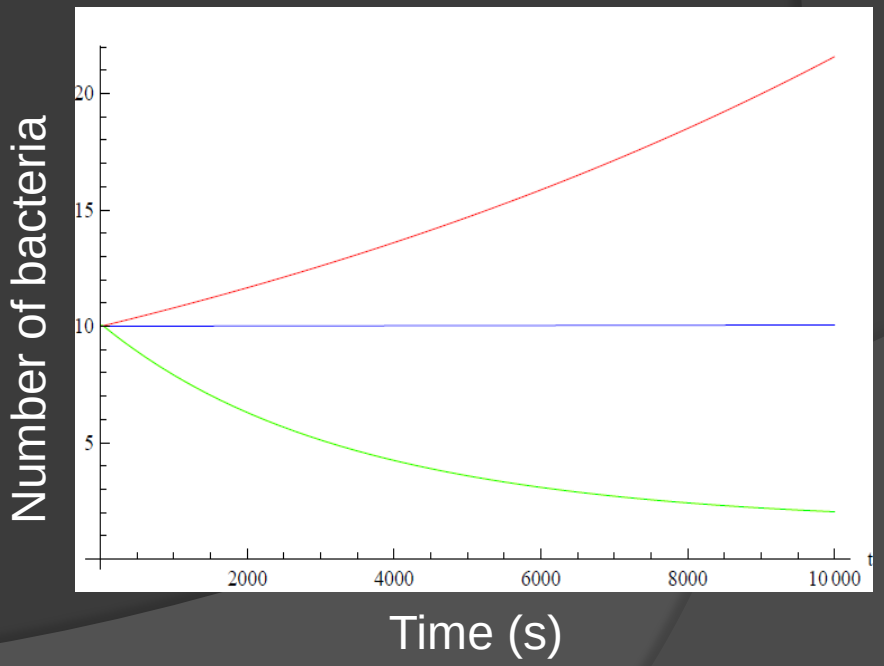
- First approach: put into competition bacteria with all possible gene orders.

$$
\begin{cases}
\dot{N}_{Bi} = k\dfrac{D_{Bi}/N_{Bi}}{1 + D_{Bi}/N_{Bi}} N_{Bi} \\
\dot{N}_{Ci} = kN_{Ci} - \alpha A_{Ci} \\
\dot{A}_{Ci} = \alpha^B N_{Ci} \dfrac{\gamma^B_{Ci} M^B_{Ci}/N_{Ci}}{1 + \gamma^B_{Ci} M^B_{Ci}/N_{Ci}} - \delta A_{Ci} \\
\dot{D}_{Bi} = \alpha^C N_{Bi} \dfrac{1}{1 + \gamma^C_{Bi} M^C_{Bi}/N_{Bi}} - \delta' D_{Bi} \\
\dot{M}^Y_{Xi} = \rho^Y N_{Xi} \dfrac{M^Y}{N} - (\delta^Y + \pi^Y_{Xi}) M^Y_{Xi} \qquad (X \neq Y) \\
\dot{M}^X = \sum_i \pi^X_{Yi} M^X_{Yi} - \rho^X M^X + \sum_i \tau_{Xi} N_{Xi} \qquad (X \neq Y)
\end{cases}
$$

Modeling

- Problem: no natural selection based on production rate can occur in this system!
- Only immunity-based selection can occur…





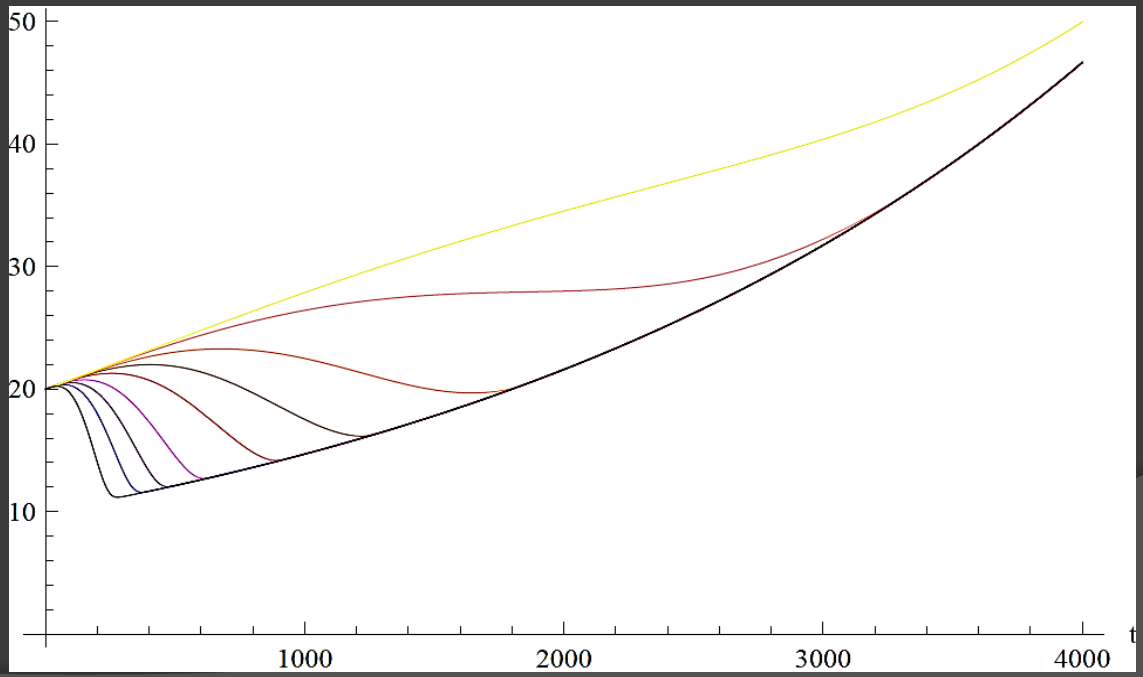
Modeling

- Second approach: put together
  - producing bacteria with common gene order
  - non-producing, slightly immune bacteria.

$$
\begin{cases}
\dot{N}_{Bi} = kN_{Bi} \\
\dot{N}_n = kN_n - \alpha A_n \\
\dot{A}_n = \alpha^B \dfrac{\gamma_n M_n^B / N_n}{1 + \gamma_n M_n^B / N_n} N_n - \delta A_n \\
\dot{M}_n^B = \rho^B N_n \dfrac{M^B}{N} - \pi^B M_n^B \\
\dot{M}^B = \pi^B M_n^B - \rho^B N_n \dfrac{M^B}{N} + \tau_{Bi} N_{Bi}
\end{cases}
$$

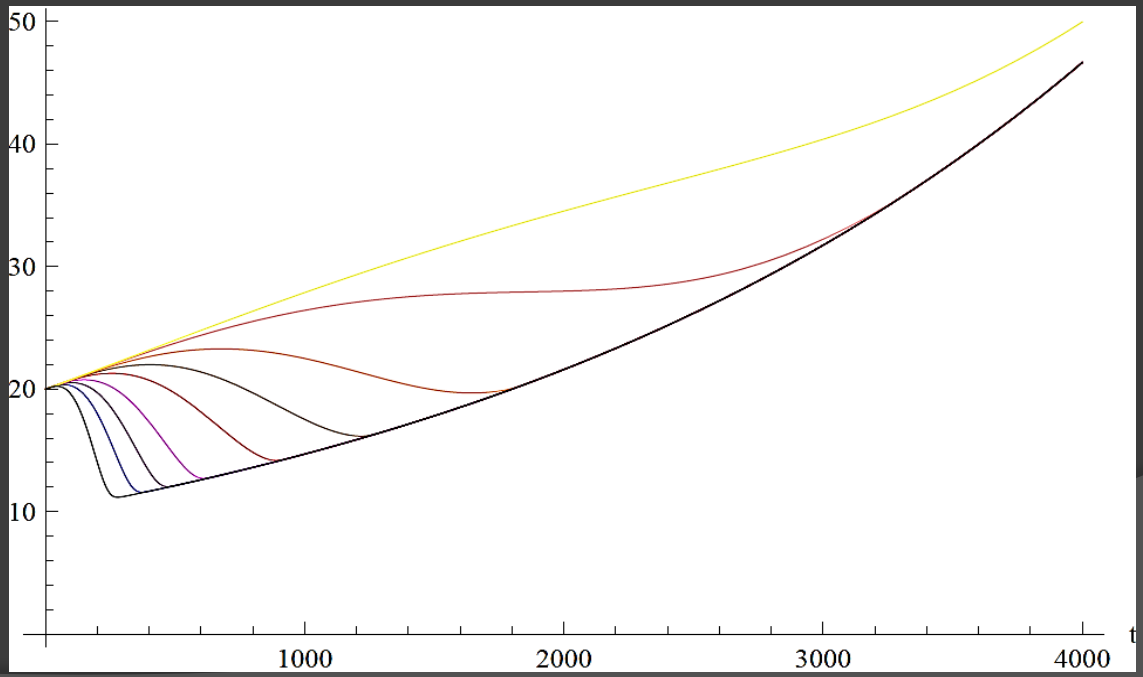
Modeling

- Non-producing bacteria will disappear.
- BUT: shift in the total population growth allows to deduce production rate!



Modeling

- Comparing such experiments with different groups of bacteria allows to find the most productive ones.



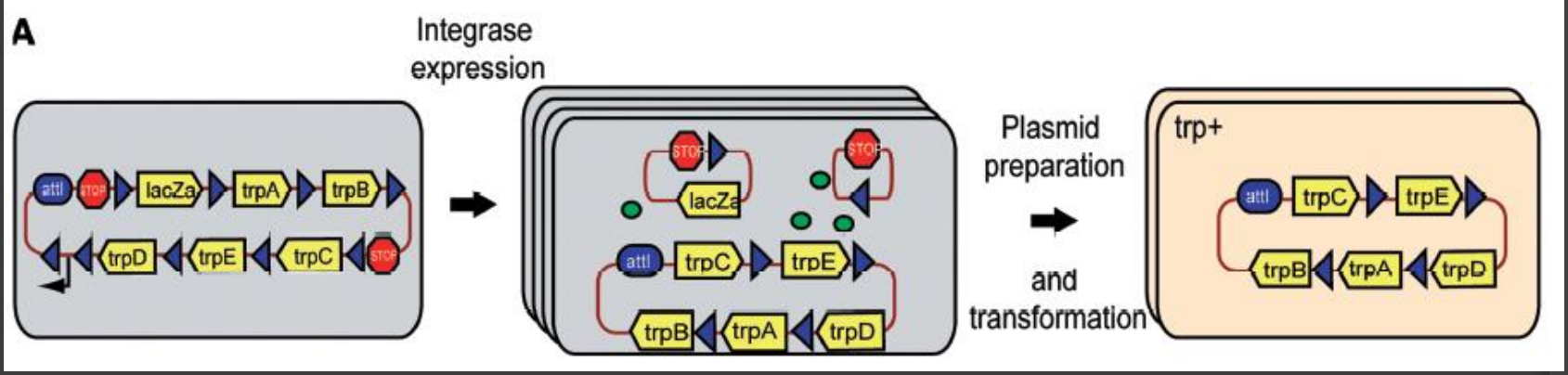
Biosafety

- Non pathogenic *E. coli* strains
- Integron plasmid can only replicate in specific *E. coli* strain
- All biobricks and plasmids were designed in the final goal to be used in bioreactor

Perspectives - Conclusion

Could we do better than evolution?

- Yes! It has been done before…



Bikard et al., 2010

- The modeling team has found an experimental setup to select better microcin producing bacteria.

Perspectives - Conclusions

Improvement of production in bio-industry

- The integron could be used to optimize production of antibiotics, drugs, glue (ULB Brussels team 2009: Glucoli), …

Acknowledgements

Our team leaders:
Laurence Van Melderen, Gilles Vanwalleghem and Dimitri Gilis

OUR TEAM