

Systems assembly

12 July

- ZR Miniprep Kit from all the genes
 - AppA
 - PpsR
 - PrrA
 - PrrB
 - PrrA Promoter
 - PpsR Promoter
- Transformation of E. coli TOP10

13 July

- Colony PCR
 - Taq Buffer 5 μ L
 - MgCl₂ 5 μ L
 - dnTP's 1 μ L
 - Forward 1 μ L
 - Reverse 1 μ L
 - Taq polymerase 0.5 μ L
 - H₂O 36.5 μ L
 - TOTAL 50 μ L

NEGATIVE

14 July

- Culture from 4 colonies per gene. 6hrs
- ZR Miniprep Kit
- Agarose gel electrophoresis 1%
- PCR from plasmid
 - Taq Buffer 2 μ L

○ MgCl ₂	2μL
○ dnTP's	0.5μL
○ Forward	1μL
○ Reverse	1μL
○ Taq polymerase	1μL
○ H ₂ O	11.5μL
▪ TOTAL	20μL

POSITIVE

- Band purification
- Plasmid digestion only for genes (1.5h)
 - Xba1 and Pst1
- Plasmid digestion only for promoters (1.5h)
 - EcoRI and SpeI
 - Agarose gel electrophoresis 1%
 - Genes (+)
 - Promoters (-)
- Ligations
 - RBS+GENES
 - 20μL Reaction (see protocols)
- AGAIN!!!! Plasmid digestion, only for promoters (1.5h)
 - EcoRI and SpeI
 - Promoters (-)
 - Agarose gel electrophoresis 4%
 - This was 4 more times!!!!!!!!!!!!!!!

15 July

- Transformation by heat-shock

16 July

- Ligation of promoters in psB1A3
- Cultures of RBS+GENES (6hr)
 - ZR Miniprep kit

17 July

- Digestion of RBS+GENS (1.5h, see protocols)
 - RBS+PrrA S+P
 - RBS+PrrB X+P
 - RBS+PrrC E+S
 - DT E+X
 - RBS+AppA X+P
 - Prom J23104 S+P
 - RBS+PpsR E+S
- Agarose gel electrophoresis 1%
 - POSITIVE (+)
- Band Purification
- Ligations A
 - **1A** RBS+PrrA +RBS+PrrB
 - **2A** RBS+PrrC+DT
 - **3A** Prom J23104+RBS+AppA
 - **5A** RBS+PpsR+ DT
- Overnight cultures of promoters+psB1A3

18 July

- Transformation of ligations A
- ZR Miniprep Kit
- Digestion

- POSITIVE
- Band purification
- Ligation D
 - PrrA Promoter+GFP+DT
 - PpsR Promoter+GFP+DT

19 July

- Transformation of ligations D
- Cultures of ligations A (6h)
 - ZR Miniprep Kit
 - Digestion (1.5h)
 - **1A** S+P
 - **2A** X+P
 - **3A** S+P
 - **5A** X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE (+)
 - Band purification
- Ligations C
 - **1C** RBS+PrrA +RBS+PrrB +RBS+PrrC+DT
 - **2C** Prom J23104+RBS+AppA+RBS+PpsR+ DT

20 July

- Overnight cultures of ligations D (6h) (small colonies)
- Transformation of ligations C

21 July

- Cultures of Ligation C
 - Miniprep Kit
 - Digestion E and E+P
 - Agarose gel electrophoresis 1%

- POSITIVE (+)
- ZR Miniprep Kit of ligations D
 - Digestion E and X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE (+)
 - Band purification!
 - NEGATIVE
 - Overnight culture

22 July

- ZR Miniprep Kit ligations D
 - Digestion E and E+P
 - Ppsr promoter+GFP+DT X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE (+)
 - High concentration! 😊
 - Band purification
 - POSITIVE +
- Miniprep Kit ligation C
 - Digestion
 - PromJ23104 S+P
 - RBS+PrrA +RBS+PrrB +RBS+PrrC+DT X+P
 - Prom J23104+RBS+AppA+RBS+PpsR+ DT S+P
 - Agarose gel electrophoresis 1%
 - High concentration! 😊
 - Band purification
 - POSITIVE +
 - Ligations E
 - **1E** PromJ23104 +RBS+PrrA +RBS+PrrB +RBS+PrrC+DT
 - **2E** Prom J23104+RBS+AppA+RBS+PpsR+ DT+ Ppsr promoter+GFP+DT

23 July

- Transformation of ligations E
- WE are also worked in the assembly of the butanol pathway + regulatory systems

24 July

- **1E NOTHING!!!!!!!!!!!!**
 - Again ligation!!
 - Miniprep Kit ligation C
 - Digestion
 - PromJ23104 S+P
 - RBS+PrrA +RBS+PrrB +RBS+PrrC+DT X+P
 - Agarose gel electrophoresis 1%
 - High concentration! 😊
 - Band purification
 - POSITIVE +
- **2E; 3 COLONIES!!... ; Prom J23104+RBS+AppA+RBS+PpsR+ DT+ Ppsr promoter+GFP+DT**
 - 6h culture!
 - ZR Miniprep Kit
 - Digestion (1.5h) S+P and X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE!!!!!!!!!!!!!!!!!!!!!!!!!!!!
 - Band purification
 - FIRST SYSTEM READY

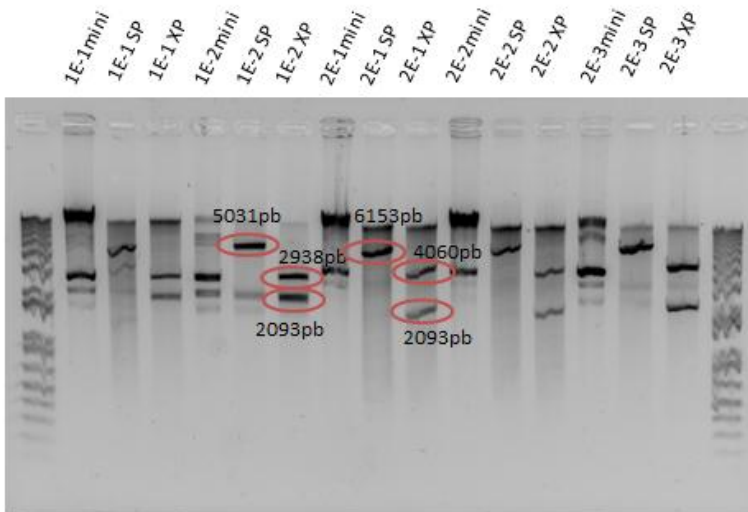
25 July

- Transformation of ligation 1E

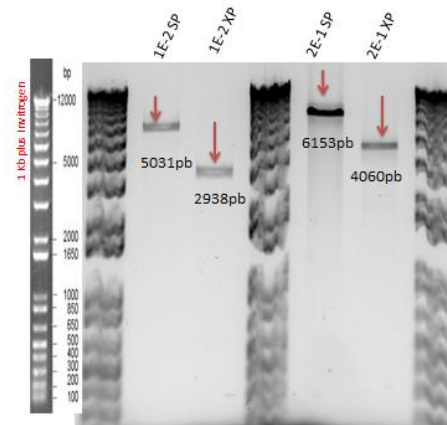
26 July

- **1E; 2 COLONIES!!! ; PromJ23104 +RBS+PrrA +RBS+PrrB +RBS+PrrC+DT**
 - 6h culture!

- ZR Miniprep Kit
- Digestion (1.5h)
 - S+P and X+P
- Agarose gel electrophoresis 1%
 - POSITIVE!!!!!!!!!!!!!!!!!!!!!!
 - Band Purification
 - JUST ONE MORE LIGATION
 - Overnight digestion



Gel . Digestión de ligaciones E



Gel. Band Purification ligations E

27 July

- ZR Miniprep Kit ligations 1D
 - Digestion X+P
 - PrrA promoter+GFP+DT X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE (+)
 - High concentration! 😊
 - Band purification
 - POSITIVE

- **1E PromJ23104 +RBS+PrrA +RBS+PrrB +RBS+PrrC+DT**
 - ZR Miniprep Kit
 - Digestion (1.5h)
 - S+P and X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE!!!!!!!!!!!!!!!!!!!!!!
 - Band Purification
 - POSITIVE
- Ligation 2F:
 - PromJ23104 +RBS+PrrA +RBS+PrrB +RBS+PrrC+DT+ PrrA promoter+GFP+DT

28 July

- Transformation ligation 2F

29 July

- **NOTHING, AGAIN!**
- ZR Miniprep Kit ligations 1D
 - Digestion X+P
 - PrrA promoter+GFP+DT X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE (+)
 - High concentration! 😊
 - Band purification
 - POSITIVE
- **1E PromJ23104 +RBS+PrrA +RBS+PrrB +RBS+PrrC+DT**
 - ZR Miniprep Kit
 - Digestion (1.5h)
 - S+P and X+P
 - Agarose gel electrophoresis 1%
 - POSITIVE!!!!!!!!!!!!!!!!!!!!!!
 - Band Purification

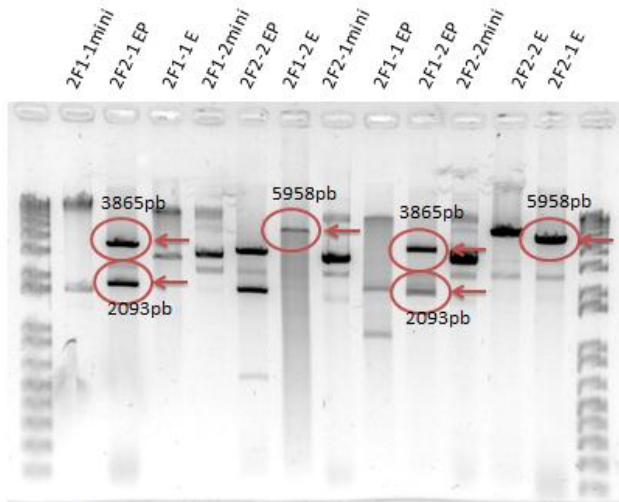
- POSITIVE
- Ligation 2F

30 July

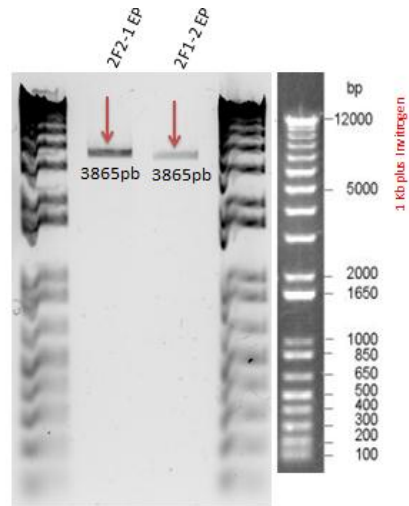
- Transformation

1 August

- **2F, 4 COLONIES**
 - 6h cultures
 - ZR Miniprep Kit
 - Digestion (1.5h)
 - E and E+P
 - Agarose gel electrophoresis 1%
 - POSITIVE!!!!!!!!!!!!!!!!!!!!!!
 - Band Purification
 - POSITIVE
 - **SECOND SYSTEM READY**



Gel . Digestion Ligation 2F



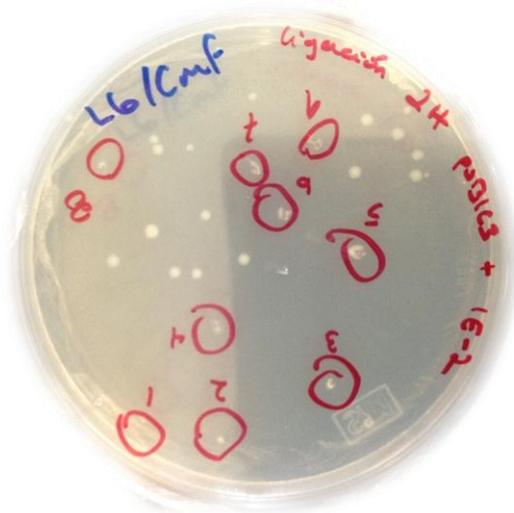
Gel . Band purification Ligation 2F

2 August

- **Party! all the systems are ready...**

Biobricks Final assembly to psB1C3

- From 3 of August to 15 of August, we did the following things.
 - Overnight and 6h cultures
 - ZR Miniprep Kit
 - Digestion (1.5h)
 - For ligate
 - For confirmation
 - Agarose gel electrophoresis 1%
 - Band purification
 - Ligation
 - Transformation; and again!
 - **TOTAL** **11 parts in psB1C3**



Big colonies 😊