ComRS approach

MERCREDI 12/07/2017

Made with benchling

Several strains obtained from M-C (DH5α)
> pCMMCD 102 no promoter control
> pCMMCD 105 promoter p1655
> pCMMCD 106 promoter Pshp200649
Reporter : glucuronidase (GusA)

Plated out on algar LB + erytromicin (250 µg/mL) and incubated overnight at 37°C.

JEUDI 13/07/2017

Preculture of all three strains + Gus- strain (KW1, from Marie-Clémence Duchêne).

VENDREDI 14/07/2017

Glycerol stocks of Gus-, 102, 105 and 106 (10% glycerol). Stored at -80°C.
DNA extraction of 102, 105 and 106 (High Pure Plasmid Isolation Kit, Roche).

Table1

	A	B	C	D
1		102	105	106
2	Mesure 1	87.9	54.2	77.3
3	260/280	1.81	1.87	1.51
4	Mesure 2	86.9	51.9	15.5
5	260/280	1.78	1.71	1.52

Low concentration for 106: redo.

TOP10 and Gus- electrocompetent cells
Follow protocol.

LUNDI 17/07/2017

Preculture of 106 (ErR) and Gus- in liquid LB.

MARDI 18/07/2017

DNA extraction of 106 (Roche kit) and purification with Monarch Nucleic Acid Purification kit: 40 ng/µL.

Centrifugated Gus- preculture at 5000 RPM for 3 minutes.
Rinced 3x1mL H2O and centrifugated 6000 RPM.
Electroporation with 102, 105 and 106 plasmid. Cells recovered at 37°C in liquid LB for 30 minutes, then plated out on agar LB + Ery.

MERCREDI 19/07/2017

4 precultures of the 3 plasmids in Gus- cells.

JEUDI 20/07/2017

102: preculture 1 and 2 grew
105: preculture 2 and 4 grew
106: preculture 1 and 2 grew

Test for the ComS induction in Gram- bacteria: follow protocol. Incubation with 8 µM of ComS (of *S. thermophilius*, sequence: LPYFAGCL).

Table2

	A	B	C	D	E
1			Abs 405 nm	5x diluted	100x diluted
2	102	1	0.320		0.067
3		2	0.254		0.065
4		3	0.285		0.072
5		4	0.283		0.065
6	105	1	4.058	1.919	0.170
7		2	4.177	3.83	0.315
8	106	1	0.268		0.064
9		2	0.304		0.066

Induction works for 105 but not 106.

LUNDI 24/07/2017

Redid test from 20/07.
Induction with 8 µM of ComS.

Table3

	A	B	C
1		Abs after induction (405 nm)	Abs 405 nm (10x diluted)
2	102	0.242	0.010
3		0.240	0.011
4	105	0.140	0.316
5		0.179	0.099
6	106	0.156	0.009
7		0.120	0.012

DNA extraction for 102, 105 and 106 plasmids.
Concentration (NanoDrop) :

Table4

	A	B	C
1		Conc (ng/µL)	260/280
2	102	280.0	1.91
3		114.8	1.95
4	105	381.3	1.85
5		408.7	1.90
6	106	461.8	1.87
7		642.3	1.88

MERCREDI 26/07/2017

Preculture of 102, 105 and 106.

JEUDI 27/07/2017

Redid test from 24/07 with 8 µM ComS.

Table5

	A	B	C	D	E	F	G
1		Abs 405 nm (with ComS)	5x diluted	10x diluted	Abs 405 nm (without ComS)	5x diluted	10x diluted
2	102	0.078			0.096		
3		0.061			0.092		
4	105	4.052	2.989	1.514	3.895	1.007	0.495
5		0.743	0.148		3.461	0.628	
6	106	0.077			0.082		
7		0.079			0.086		

Test with X-gluc on agar plates.

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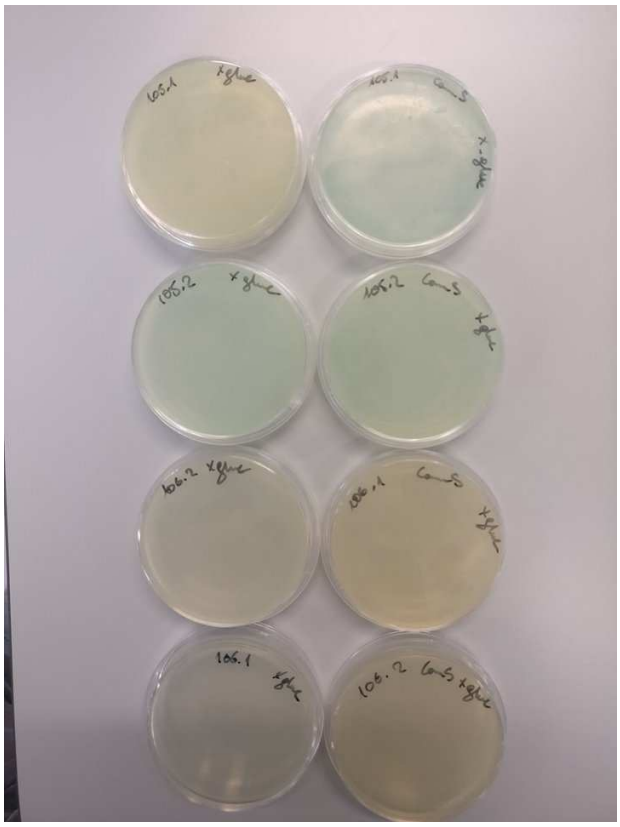

MERCREDI 02/08/2017

Preculture 105 and 106.

JEUDI 03/08/2017

Test induction ComS with different concentrations of erytromicin.
0, 50, 100, 200 µg/mL
Best growth with ~100 µg/mL

Optimisation of ComS concentration
105 and 106 plated out on agar LB + Ery + ComS + X-gluc.
Concentrations: 2, 4, 8, 16 and 32 µM of ComS.

(105 and 106 label inverted)

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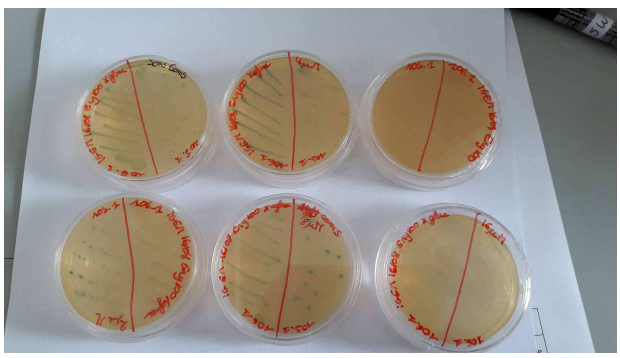

MERCREDI 23/08/2017

Test glucuronidase activity

Protocol Gus.

Control: incubation without ComS.

Different incubation time: 0-120 minutes

Table8

	A	B	C	D	E	F	G	H	I
1		Control	0	20	40	60	80	100	12
2		1	2	3	4	5	6	7	
3	A	0,0688	0,1089	0,99370003	0,60530001	2,80489993	0,40970001	1,05840003	0,5989999
4	B	0,0708	0,1109	0,87279999	0,63330001	2,80539989	0,36770001	1,0165	0,5439000
5	C	0,0683	0,1246	0,88150001	0,84060001	3,38910007	0,3766	0,98250002	0,5787000
6	D	0,1079	0,1125	0,85650003	0,97979999	2,77859998	0,38679999	0,98269999	0,584
7	E	0,069	0,114	0,83530003	0,67949998	2,48200011	0,37920001	0,98089999	0,592
8	F	0,0908	0,1131	0,99900001	0,95459998	2,68959999	0,375	0,99430001	0,5881999
9	G	0,0759	0,1201	0,95880002	0,80680001	2,79780006	0,36759999	1,05079997	0,867
10	H	0,0723	0,117	1,04159999	0,69029999	2,81329989	0,43709999	1,82179999	0,5630000
11	Moyenne	0,077975	0,1151375	0,92990001	0,773775	2,22008749	0,3874625	1,1109875	0,61462

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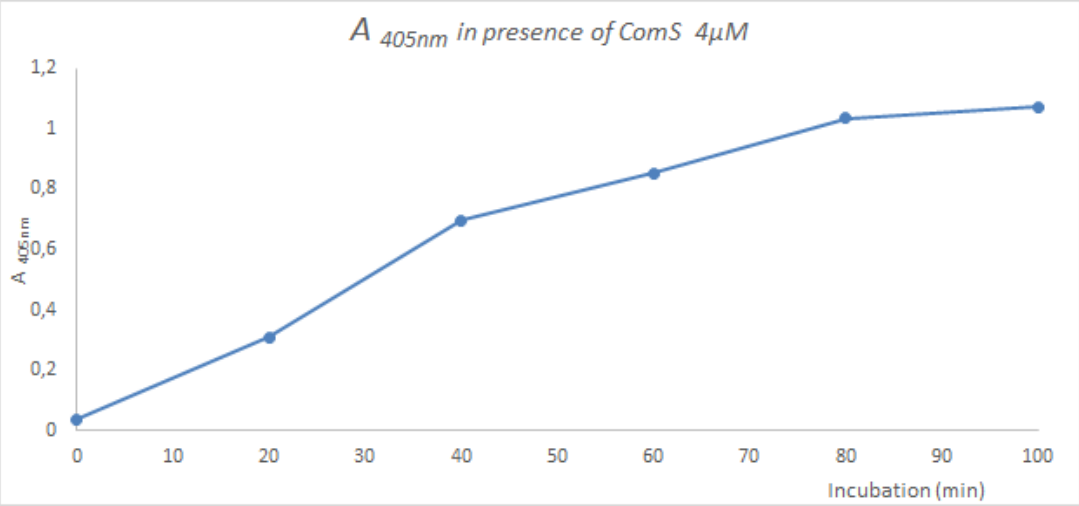

Influence of ComS concentration

ComS: 2-8 µM, 1h incubation

Table9

	A	B	C	D
1	2	4	6	8
2	12	11	10	9
3	1,55470002	2,67090011	1,60070002	1,74020004
4	1,4691	2,25760007	1,23310006	1,27620006
5	1,67260003	0,58899999	1,28910005	1,27520001
6	1,54499996	2,28500009	1,31130004	1,35259998
7	1,66989994	2,3822999	1,32309997	1,35650003
8	1,16770005	2,35450006	1,26380002	1,41929996
9	1,50450003	2,60719991	1,28470004	1,57360005
10	0,1133	1,05130005	1,52690005	1,72819996
11	1,3371	2,02472502	1,35408753	1,46522501
12	1,259125	1,94675002	1,27611253	1,38725001

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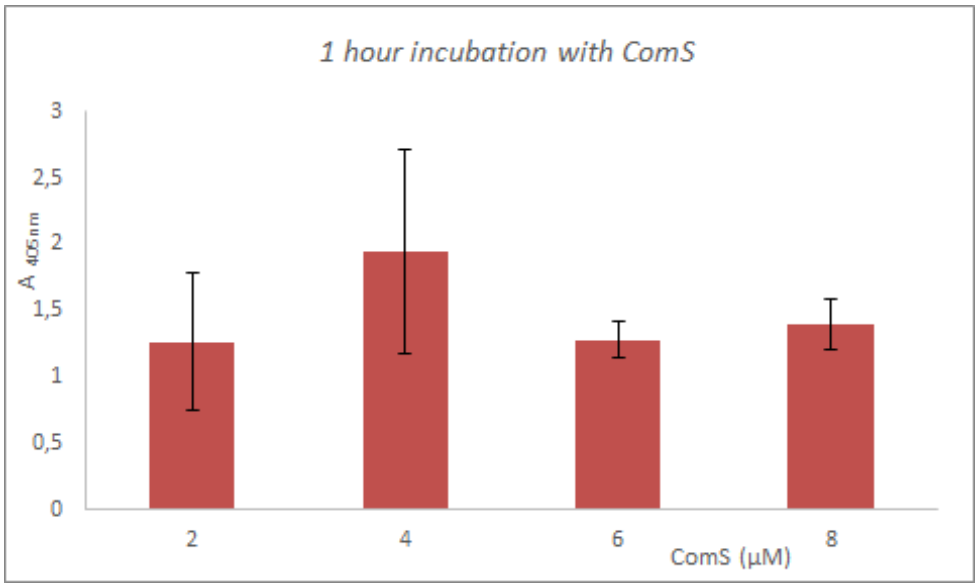

> 4 µM works best

Precultures of 105 and 106 in liquid LB + Ery.

JEUDI 24/08/2017

Mesure kinetic of ComS induction with 105. Follow protocol, incubation with 2 µM ComS.

Table6

	A	B
1	Time	Abs 405 nm
2	30 minutes	0.380
3	1h	0.734
4	1h30	0.809
5	2h	1.045

JEUDI 31/08/2017

Insertion of RFP in 105 plasmid with Gibson assembly

> 105 (backbone) 329 ng/µL

> RFP (PCR product) 123 ng/µL

>2 PCR (50 µL):

REV primer (10 µM)	2.5 µL		
FWD primer (10 µM)	2.5 µL		
5x buffer HF	10 µL		
dNTPs (20 mM)	1 µL		
DNA (30 ng/µL)		1 µL (RFP)	0.5 µL (105)
Phusion Polymerase	0.5 µL		
H2O		31.5 µL (RFP)	32 µL (105)

Program: 98°C 30 sec

98°C	10 sec	25 cycles
TM	15 sec	
72°C	t anneling	
72°C	7 minutes 30	
4°C	∞	

RFP: TM = 72°C t anneling = 1 minute (721 bp)

105: TM = 65°C t anneling = 4 minutes (4382 bp)

>Digestion with DpnI (20 µL)

DpnI	2 µL
CutSmart buffer	2 µL
PCR product	16 µL

Incubated 30 minutes at 37°C. Heat-kill at 80°C for 20 minutes.

>Purification (Monarch kit)

Table7

	A	B	C
1		Conc (ng/µL)	260/280
2	105	16.6	1.49
3		13.6	1.50
4	RFP	88.1	1.78
5		85.0	1.90

VENDREDI 01/09/2017

ComS photocaged peptide synthesis
Vincent Stroobant (de Duve institute)
>Couldn't synthetise the peptide: cage is on the carboxy-group of the tyrosine and inhibit the rest of the synthesis.
Use another ComS with tyrosine at the end

Gibson Assembly

Gibson mastermix 2X	10 µL
105	5 µL
RFP	2.5 µL
H2O	2.5 µL

DNA = 0.485 pmol
Incubated at 50°C for 1h. Stored at -20°C.

LUNDI 04/09/2017

Plasmid transformation into TOP10 electrocompetent cells. Recovered in liquid LB for 1h then plated out on agar LB + Ery.
Incubated overnight at 37°C. Negative control: irrelevant plasmid (TetR) transformed into TOP10.

MARDI 05/09/2017

Uniform cell layer: redid transformation, plated out only 10 µL.

Mesuring reaction time and optimized ComS concentration.
Follow protocol on induction.
Controls: Gus-, 105 without ComS, 105 without ComS and without ONPG.

>ComS concentration: 2, 4, 6 and 8 µM
>Reaction time: induce with 2 µM ComS every 10 minutes (from 0 to 60).

Fill a 96-well plate with 4 µL of ONPG (12.5 mM stock) + 100 µL of supernatant + 150 µL of "stop tampon" after 3 minutes.

VENDREDI 08/09/2017

>Screening Gibson product + Ery (agar LB) + ComR/RFP
>32 colonies used for new screening

Gibson product + ComS and x-gluc
All colonies grew. All have a light blue center.
Except: 12, 13, 23, 25, 26 (blue) and 28, 29 (very blue).
-> No integration of RFP in the place of GusA

ComS + Ery

All colonies grew. No color change.