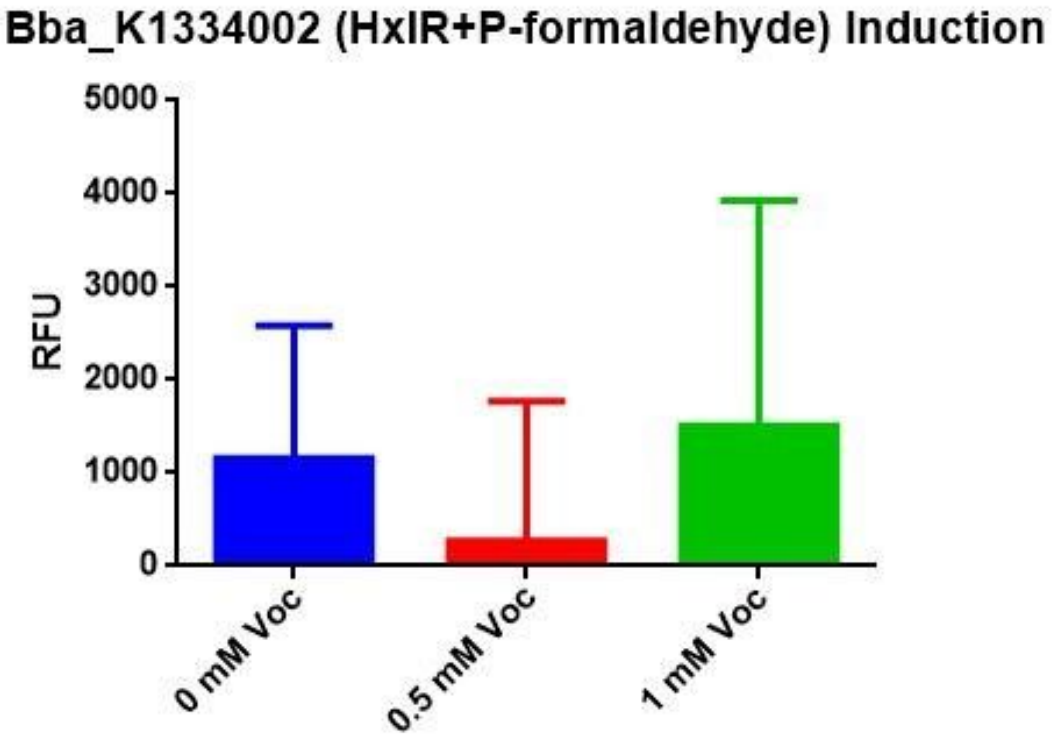
Abbreviations and notes:
Col: Colony
Ver: Verified
1:1000 atc corresponds to 250 ng/µl
2log is used as a ladder for all gel electrophoresis experiments.

04.06.2017



PRO1 UN	PRO2 UN	PRO 3 UN	I1 UN	I2 UN	I3 UN
PRO1 0.5	PRO2 0.5	PRO3 0.5	I1 0.5	I2 0.5	I3 0.5
PRO1 1	PRO2 1	PRO3 1	I1 1	I2 1	I3 1

The overnight measurement indicates that the error bars are almost equal or more than the fold change of the each induction. This inconsistency can also be seen from the uninduced and 0.5 mM bars. Uninduced bar is almost 5 times more than the induced one. In conclusion, induction of the part Bba_K1334002 with formaldehyde is inconsistent which prompts us to improve this part.

24.06.17

- For pza-HxlR-sfGFP and psb1c3-HxlR PCR, Gibson ve transformation are performed.
-Cultures are prepared.

25.06.17

-psb1c3-hxlR plate contains no colony.
-pza-hxlR-sfgfp plate (plate 1) has 5 colonies.

28.06.17

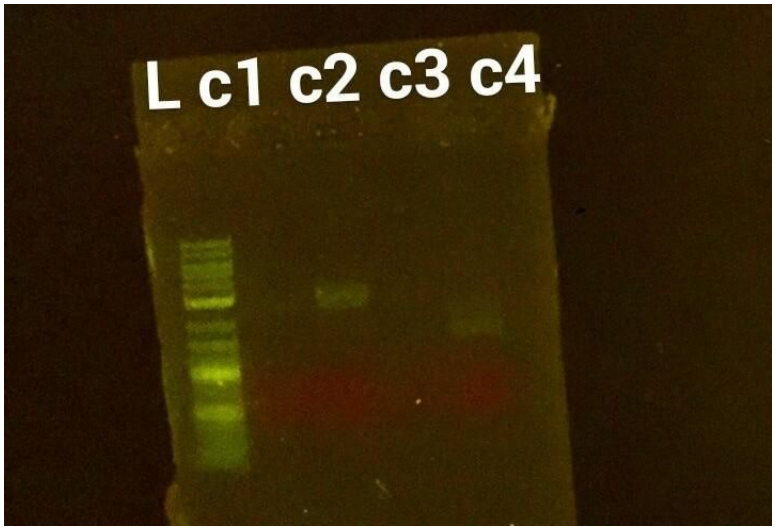
-Cultures are prepared from colony 1, 2, 3 and 4 of plate 1 (pza-hxlR-sfgfp).

29.06.07

-To the colony 1, 2, 3 and 4 miniprep is performed (pza-hxlR-sfgfp, plate 1).
Col 1- 236.5 ng/µl col 2- 57.0 ng/µlcol 3- 226.6 ng/µl col 4- 166.5 ng/µl

-The plasmids isolated from miniprep experiment, digestions are prepared with SalI ve BglII.

Figure 1 : Pza-hxlR-sfgfp, colony(1-4), plate 1, digest SalI + BglII :



30.06.17

-Miniprep samples from colony 1 and 2 are sent for sequencing (pza-hxlR-sfgfp plate 1).

03.07.17

-Cultures are prepared again from colony 1 and 2 from plate 1.

04.07.17

-Induction is performed for colony 1 and 2. (unind / atc / atc+voc / voc)
1:50 dilution for fresh LB
1:1000 dilution for atc (2-2.5 hours of incubation)

05.07.17

-GFP signals are measured. Unind / atc / voc / atc+voc / pro (c1-5 col1, d1-5 col2)

06.07.17

-Induction is performed for colony 1.
1:1000 atc - 0 / 0.05 / 0.1 / 0.2 / 1 mM formaldehyde

10.07.17

Figure 2: Induction results of pza-hxlR-colony 2. M5 order: LB unind atc 0.05 0.1 0.2 1 (in mM), Induction results of pza-hxlR-colony 2. X axis indicates the GFP measurement after voc addition: 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

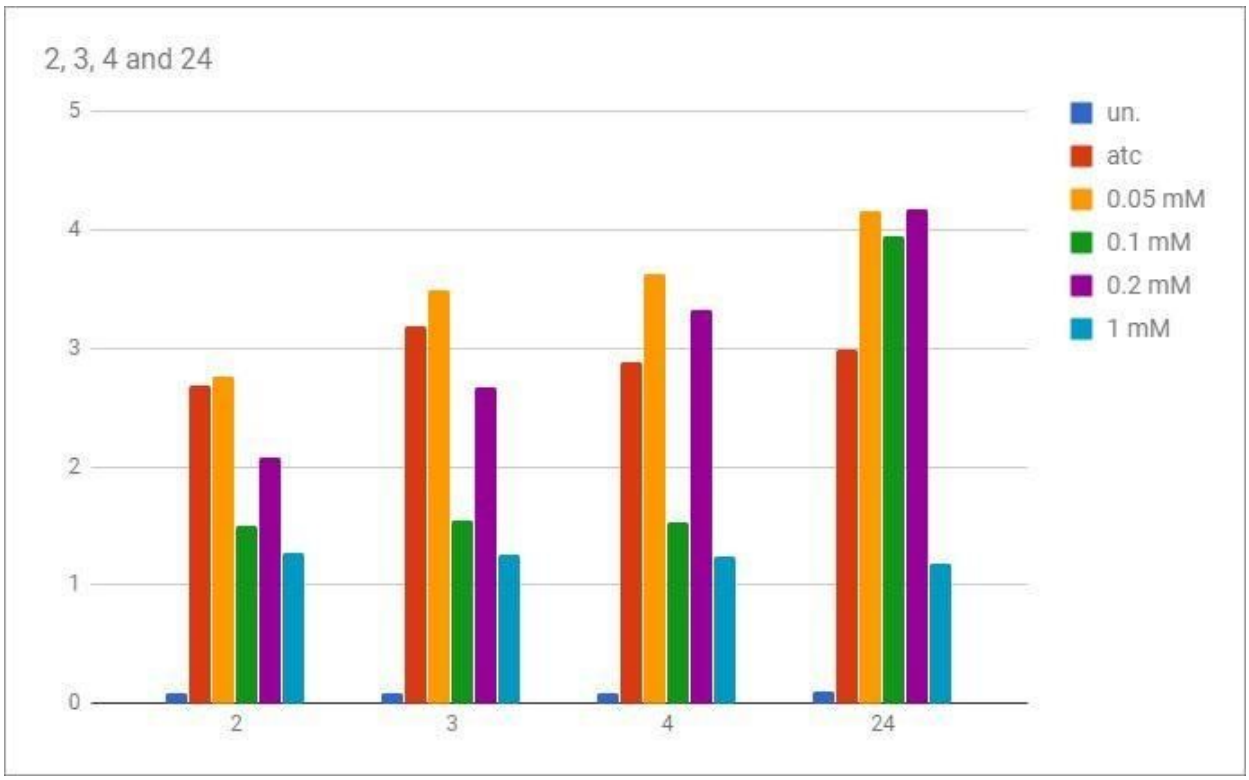


Table 1: Normalized GFP signal data from M5 reader for colony 2 induction

	un.	atc	0.05 mM	0.1 mM	0.2 mM	1 mM
2 h	0.08	2.686	2.764	1.4983	2.0749	1.2638
3 h	0.0815	3.181	3.4851	1.5375	2.6687	1.2503
4 h	0.0886	2.8799	3.6198	1.5309	3.326	1.2403
24 h	0.1048	2.9891	4.1495	3.9383	4.1727	1.184

-Pza-hxlR-sfgfp colony 2 (plate 1) is prepared for cell culture for induction.

11.07.17

-Pza-hxlR-sfgfp-col2 2nd induction: unind. - Atc- 0.05 mM - 0.1 mM - 0.4 mM - 1 mM and pro

Dilution:
For colony 2, 30 ml fresh LB + 600 µL o.n col2 + 30 µL cmr
For pro 8 ml fresh LB + 160 µL + 8 µL spectinomycin

After 2 hours and 15 minutes incubation 0.38 OD level is achieved.
After 3 hours 25 minutes atc is added.

3 hours later formaldehyde is added. Each sample contains 4 mL of culture.
13 M formaldehyde is diluted to 20 mM. (1ml formaldehyde + 650 µL ddH_2O)

For 0.05 mM 10 µL diluted formaldehyde is used.
For 0.1 mM 20 µL diluted formaldehyde is used.
For 0.4 mM 80 µL diluted formaldehyde is used.
For 1 mM 200 µL diluted formaldehyde is used.

12.07.17

Figure 3: Induction results of Col2-pza-hxlR-sfgfp plate 1. GFP measurement order: LB, pro, unind, atc, 0.05, 0.1, 0.4, 1 (mM). X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

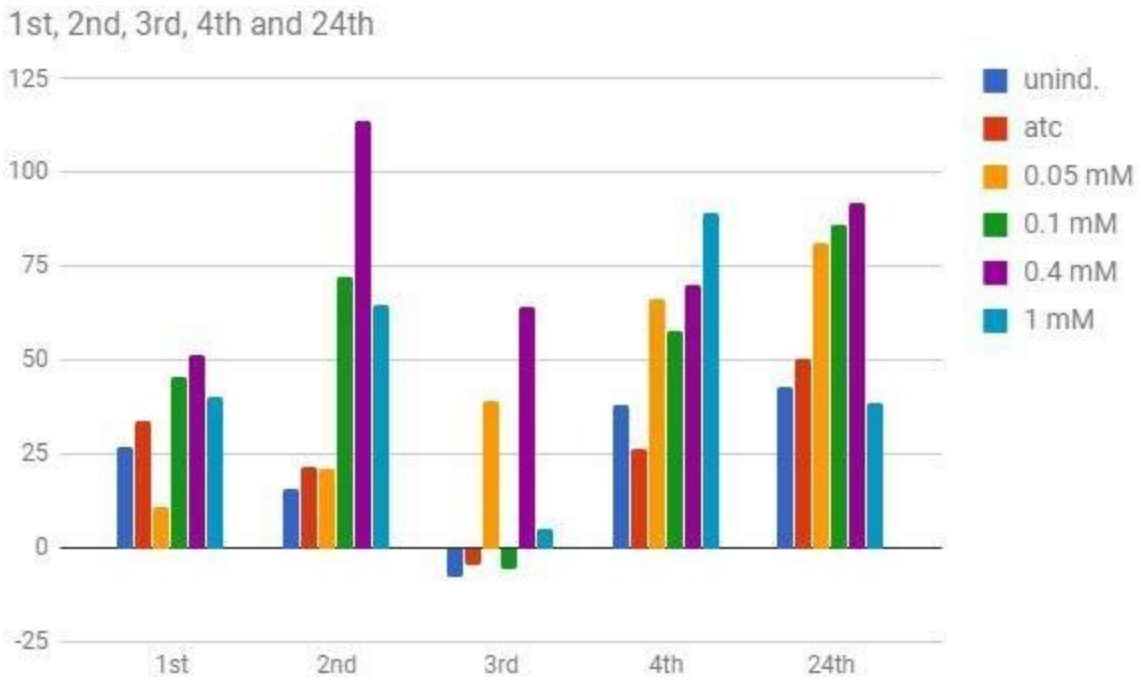
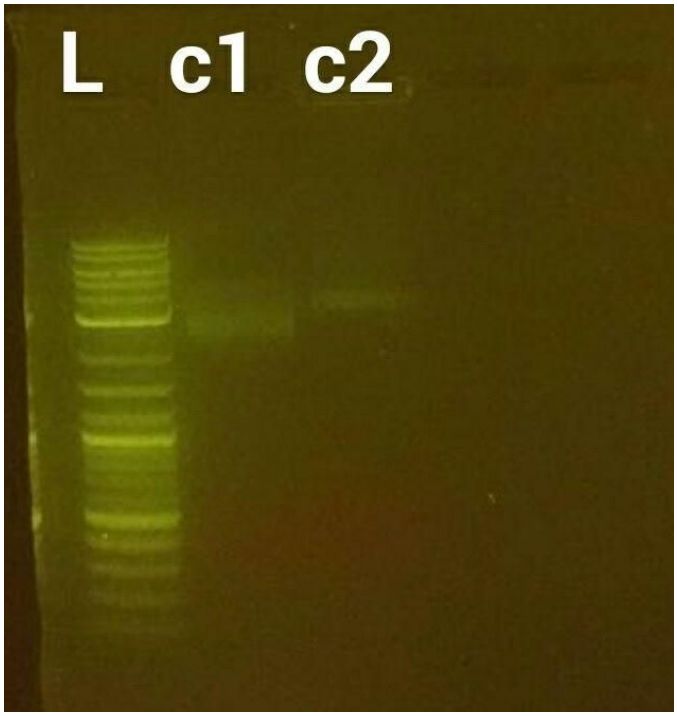


Table 2: Normalized GFP signal data from M5 reader for colony 2 induction

	unind.	atc	0.05 mM	0.1 mM	0.4 mM	1 mM
1st	26.9191	33.601	10.7735	45.3964	51.3835	40.2459
2nd	15.6303	21.6393	21.2892	72.3037	113.9423	64.7239
3rd	-7.813	-4.7165	39.3812	-5.7551	64.3198	4.9212
4th	37.9545	26.1423	66.3795	57.971	69.8467	89.229
24th	42.8865	50.1338	81.3497	86.1527	91.7562	38.5177

Figure 4: XbaI digest for colony 1 and 2 (miniprep products on 29.06.17). On 29.06.17, miniprep products of colony 2 has been verified.



13.07.17

-Miniprep is performed to colony 2. Concentration is 25.7 ng/µL

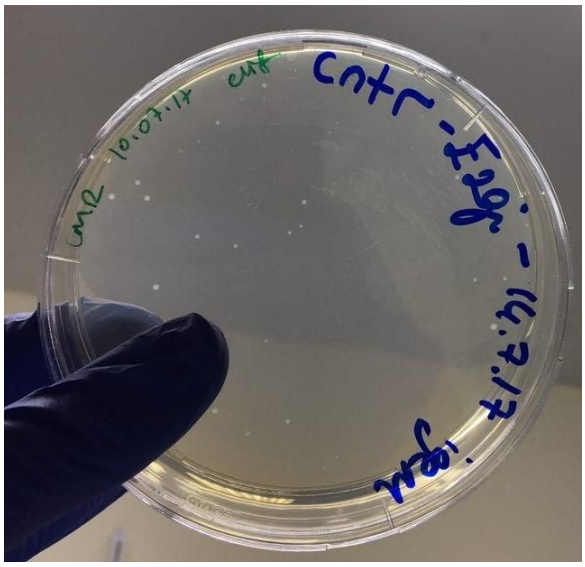
14.07.17

- Transformation is performed to miniprep products from 29.06.17 of colony 2.
Since there was little left, it has been vortexed with 10 µL ddH_2O and used after this.
Plates were put to the incubator at 17:00.

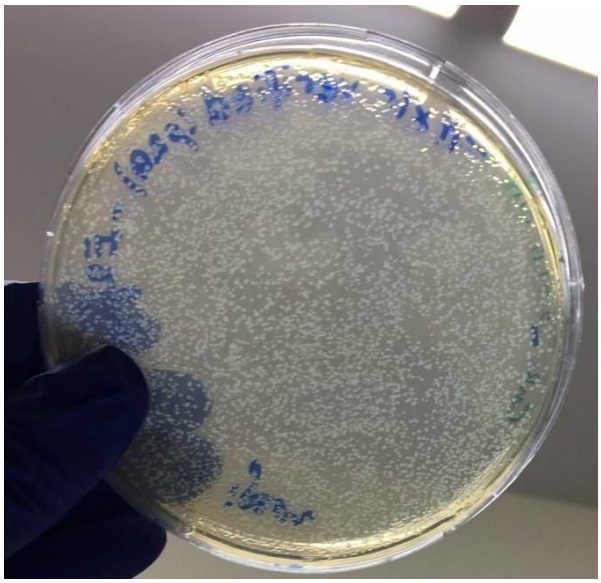
Plate name: hxlR verified

15.07.17

Figure 5: Control plate for 14.07.17 transformation



Fgure 6: Pza-hxlR-sfgfp (verified)



16.07.17

-HxlR ver. col1, ver. col2, ver. col3 and pro are prepared for cell culture. (at 16.30) each 3 mL

17.07.17

-HxlR verified col1-2-3, 2 for each, glycerol has been taken from stock. (200 µL o.n + 200 µL %50 glycerol) One of them is sterile and covered with parafilm. Other is for cell culturing.
-Induction is performed to HxlR ver. Col1, ver. col2 and ver. col3.

For verified col1-2-3 fresh LB:

30 ml LB + 30 µL cmr + 600 µL o.n
Pro:
8 ml LB + 8 µL spek + 160 µL o.n

-For verified col1, col2, col3 and pro amounts of atc:
4 ml - Unind 0
4 ml - 1:0 0
4 ml - 1:1,000 --- 4 µL
4 ml - 1:2,500 --- 1.6 µL
4 ml - 1:5,000 --- 0.8 µL
4 ml - 1:10,000 -- 0.4 µL
4 ml - 1:25,000 -- 0.16 µL
4 ml - pro 0

- Formaldehyde amounts for verified col1, col2, col3 and pro: (0.4 mM each induced one) Dilution: (13 M)(2 µL) = (0.02 M)(1300 µL)
(20 mM)(40 µL) = (0.4 mM)(2000 µL)

4 ml - Unind 0
2 ml - 1:0 -------- 40 µL
2 ml - 1:1,000 --- 40 µL
2 ml - 1:2,500 --- 40 µL
2 ml - 1:5,000 --- 40 µL
2 ml - 1:10,000 -- 40 µL
2 ml - 1:25,000 -- 40 µL

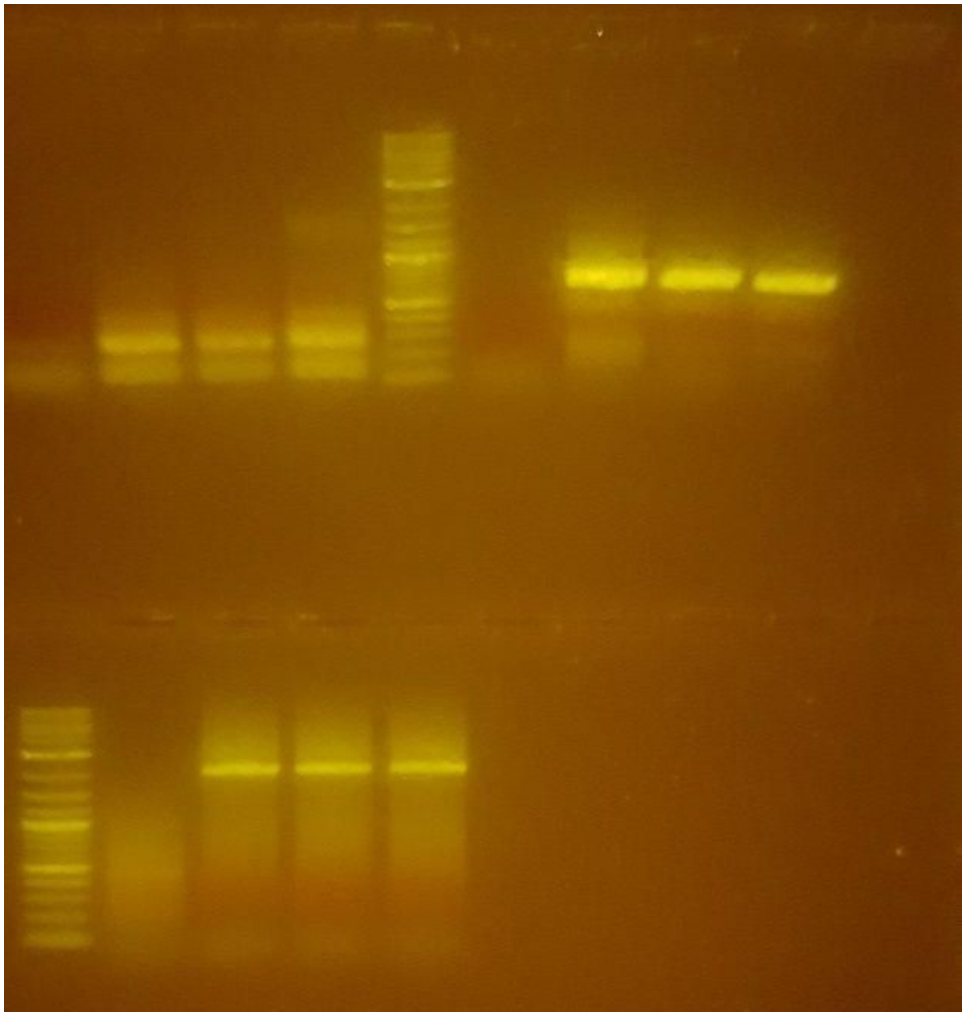
2 ml - 1:0 0
2 ml - 1:1,000 --- 0
2 ml - 1:2,500 --- 0
2 ml - 1:5,000 --- 0
2 ml - 1:10,000 -- 0
2 ml - 1:25,000 -- 0
4 ml - pro 0

18.7.17

In order to put HxlR into psB1C3; AlkR, hxlR and psB1C3 PCR was conducted.

Alkr-> Tm: 64 Hxlr-> ™: 64 psb1c3-> Tm:71 Q5

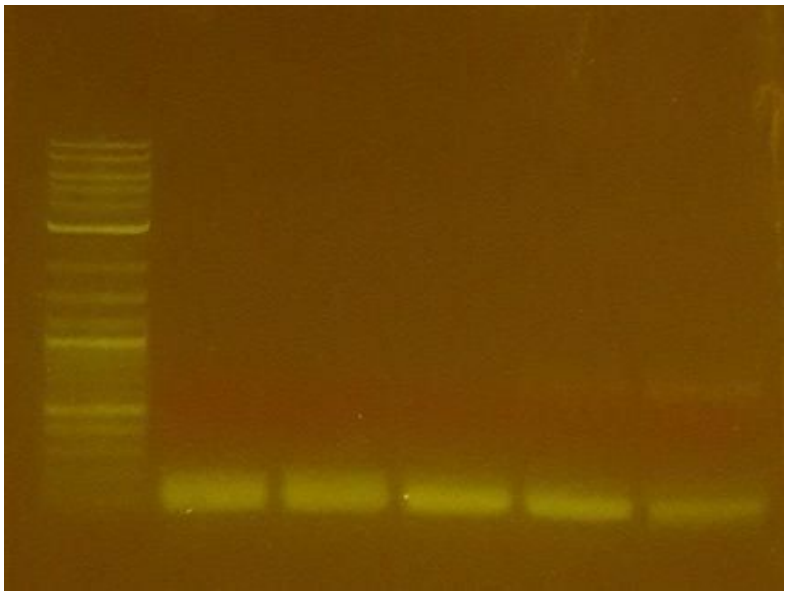
Figure 7: psB1c3 PCR results. Left:AlkR right:Hxlr bottom: pSB1C3 expected band lenghts respectively: ~1300 bp,700bp, 2000bp



HxlR and psB1C3 were combined by Gibson assay. (3:1, 50 ng:49.42 ng)
19.07.17

For HxlR pSB1C3, 4 colony was selected and colony PCR was performed

Figure 8: Colony PCR results for HxlR psb1c3. Gel: L C 1 2 3 4 3rd and 4th colonies were seen to be have the expected band lenghts. (682bp)

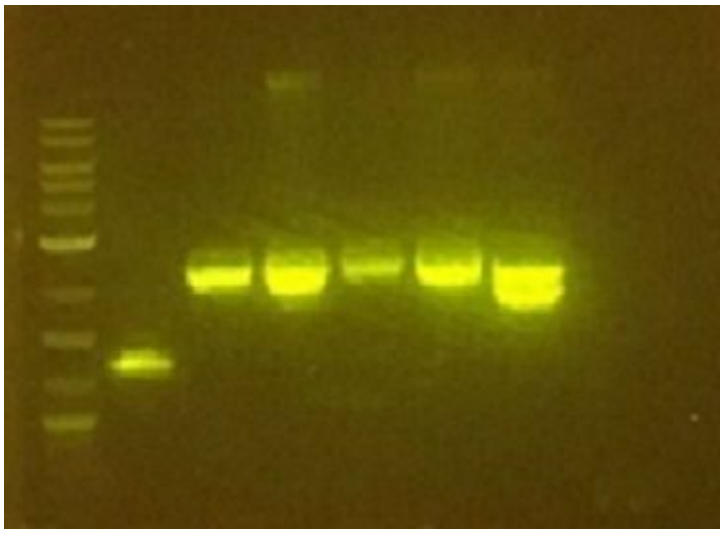


4 HxlR pSB1C3 colony cell culture were prepared.

20.7.17

4 HxlR psB1C3 miniprep was conducted.

Figure 9: 4 HxlR pSB1C3 and mimR pSB1C3 and AlkR's PCR product was cut with XbaI and SpeI enzymes. Gel: Alkr PCR, HxlR pSB1C3 1-2-3-4, mimR pSB1C3 was all cut by XbaI and SpeI.
1282 bp/2062-496-144 bp/2062 bp-1790 bp

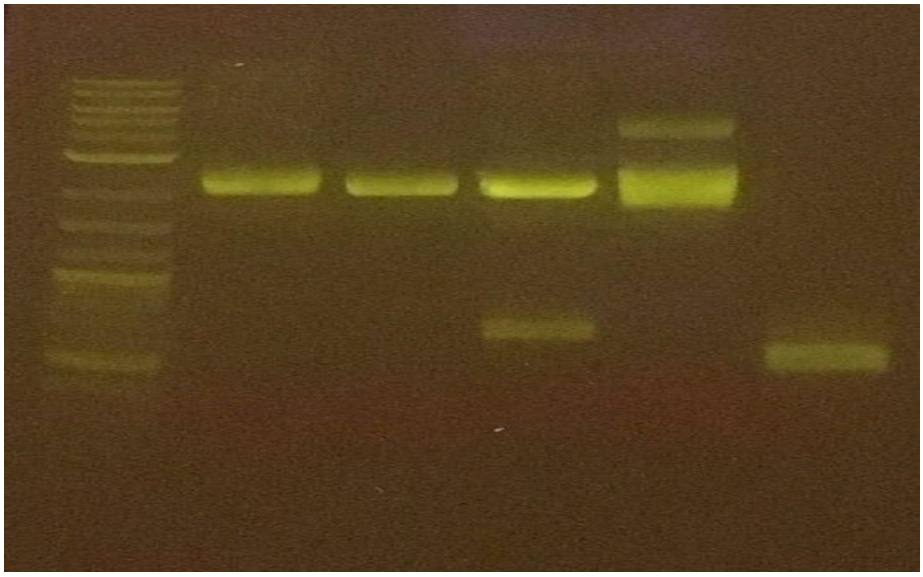


-HxlR pSB1C3 was not verified by the cut with XbaI SpeI enzymes. Cell culture was prepared with newly selected 3 colonies from MimR pSB1C3.

21.07.17

HxlR pSB1C3 5-6-7 miniprep was conducted.

Figure 10: The HxlR's PCR and mimR pSB1C3 and Hxlr pSB1C3 5-6-7 which we already have was digested with XbaI and PstI enzymes. Gel: HxlR psB1C3 5-6-7 , MimR pSB1C3 and HxlR PCR was all digested with XbaI and PstI enzymes.



2044-496-162 bp/ 2044-1808 bp/ 658 bp expected bands were not seen. The very end band was seen correct but when we looked zoomed 2 bands were seen.
pSB1C3 mimr 5th colony was verified with the sequencing.

24.7.2017

Figure 11: Alkr psb1c3 PCR Control-1-2-3 Hxlr psb1c3 PCR Control- 1-2-3 to be cloned into the pSB1C3.

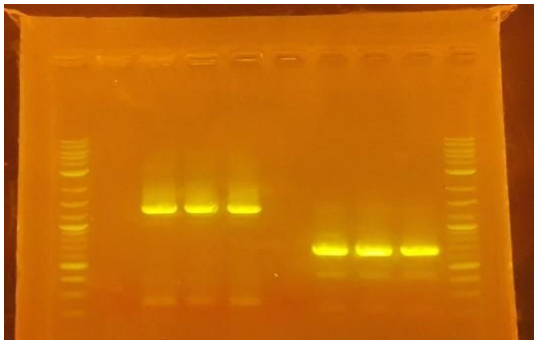


Figure 12: HxlR PCR and AlkR's PCR was digested with XbaI and pstI. But we learned that xbaI also cuts HxlR from the very middle.

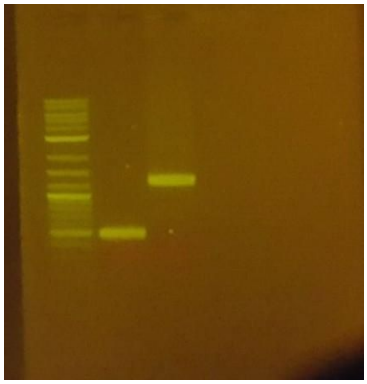


Figure 13: Ver. col1 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

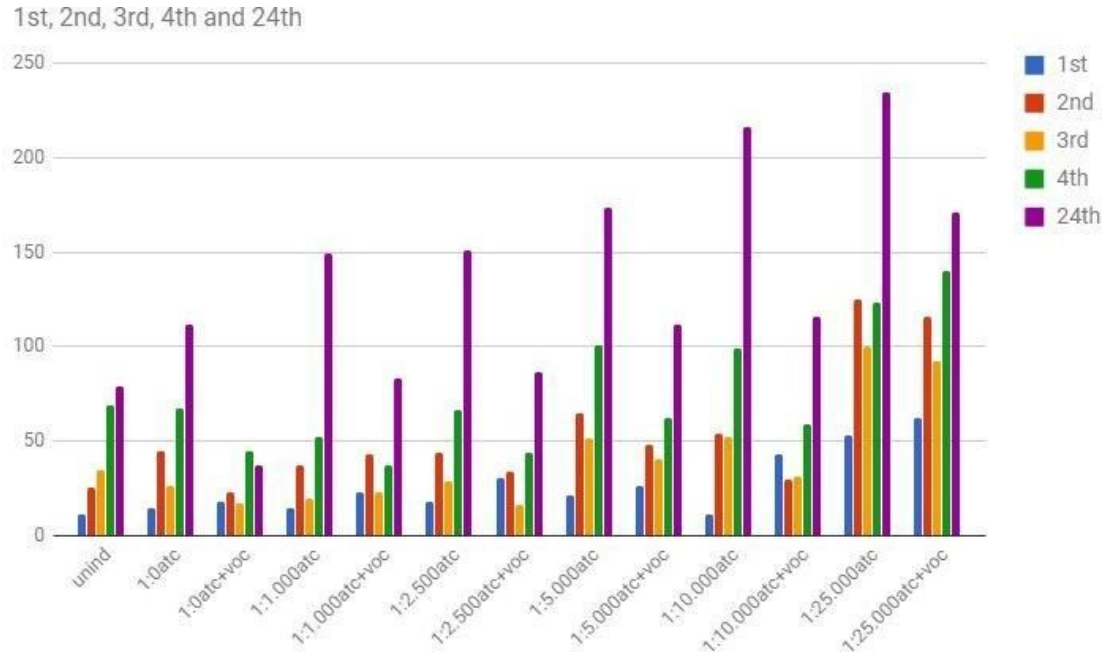


Figure 14: Ver. col2 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

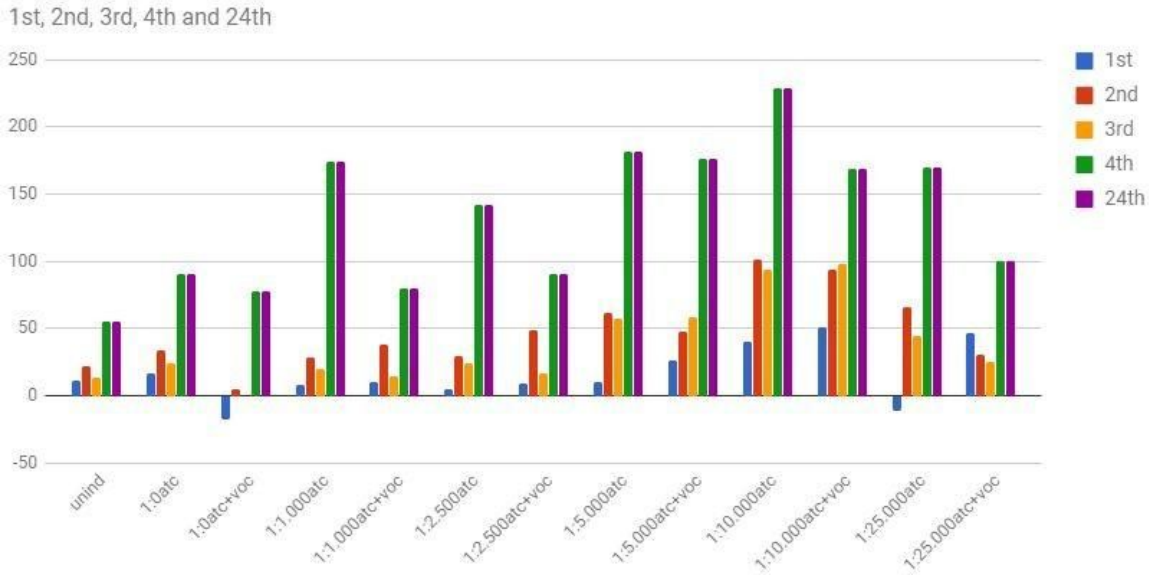
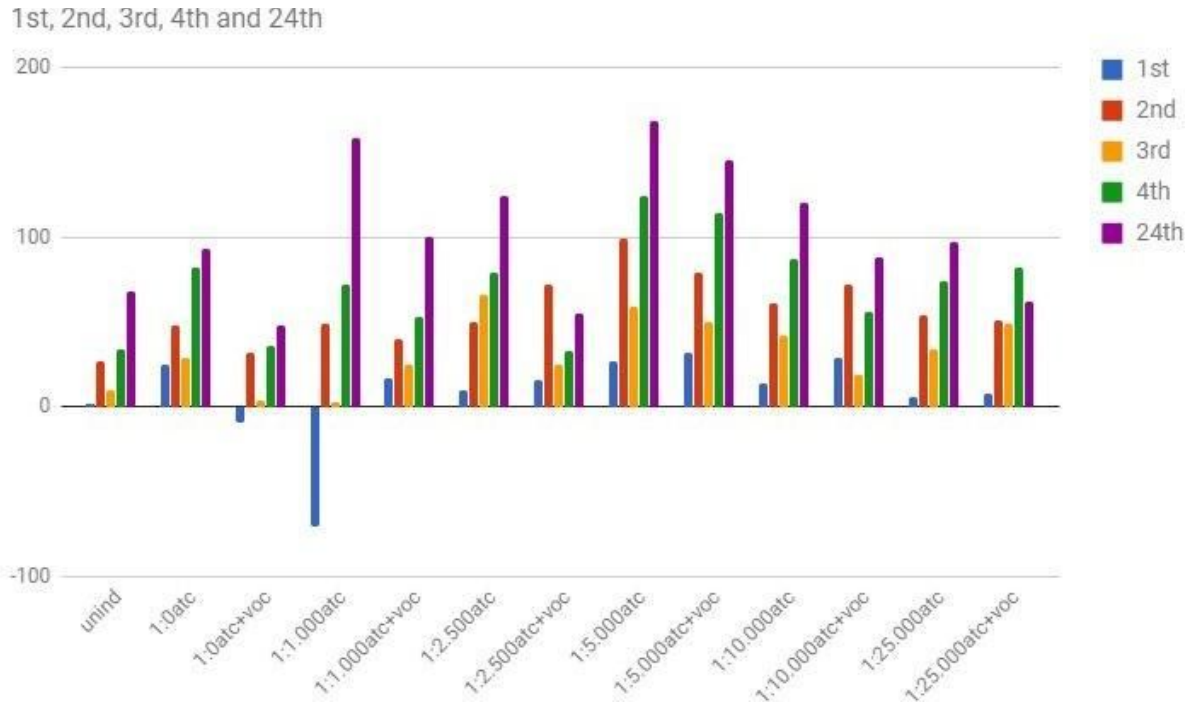


Figure 15: Ver. col3 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.



26.7.17

Miniprep was conducted to pSB1C3 HxlR colonies 7-8-9 and digested with EcoRI and PstI.
Then its ligation and transformation is completed.

18.8.17

miniprep was conducted to pza HxlR colonies 1-2-3-4.
These samples were sent to sequencing.

21.8.17

Histag HxlR digest was conducted with XhoI.

12.10.17

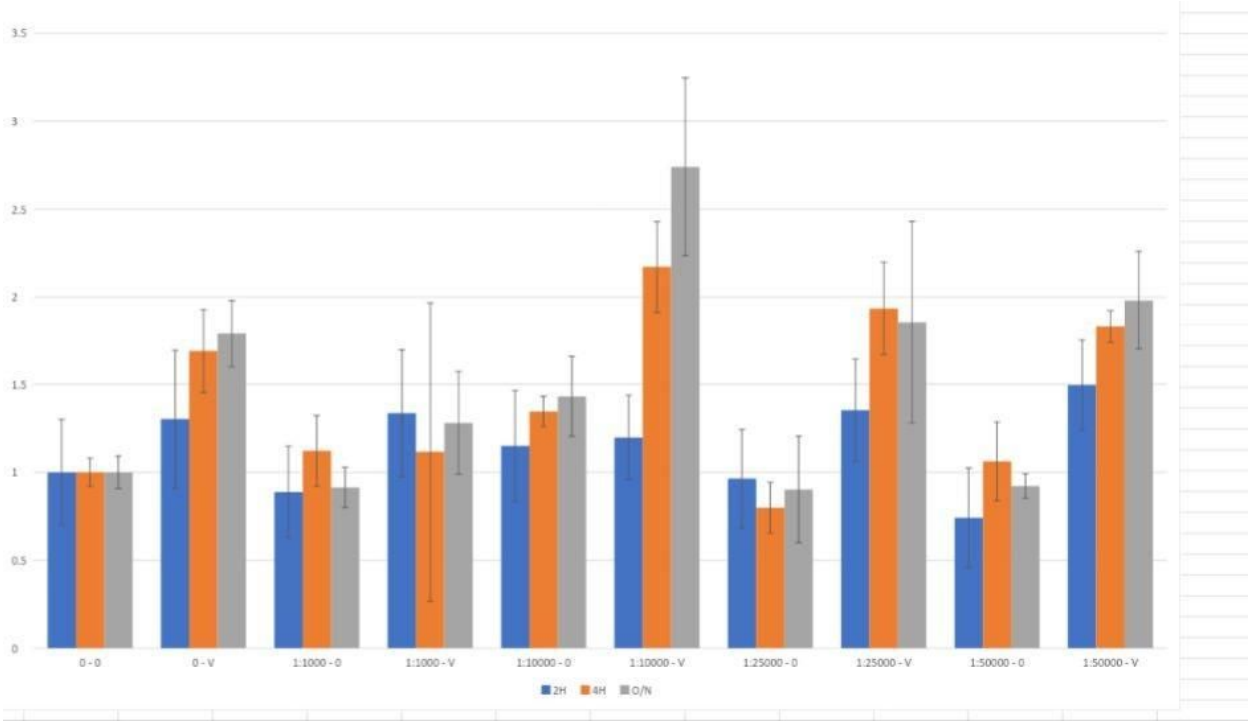
-New induction trials for HxlR verified colony 2:

Table 3 : Experimental design for induction, with 3 replicas and formaldehyde (voc) concentration 1 mM

Hxlr X3	No atc	No atc + voc	1:1000 atc	1:1000 atc + voc	1:10,000 atc	1:10,000 atc + voc	1:25,000 atc	1:25,000 atc + voc	1:50,000 atc	1:50,000 atc + voc
Pro X3	No atc	No atc + voc	1:1000 atc	1:1000 atc + voc	1:10,000 atc	1:10,000 atc + voc	1:25,000 atc	1:25,000 atc + voc	1:50,000 atc	1:50,000 atc + voc

Figure 16: GFP measurement results.X axis indicates samples with different formaldehyde concentrations shown in table 3 and incubation periods: 1i 4 and 16 hours. Y axis indicates the normalized GFP reading.

Note: GFP signal is measured in PBS rather than LB.



14.10.17

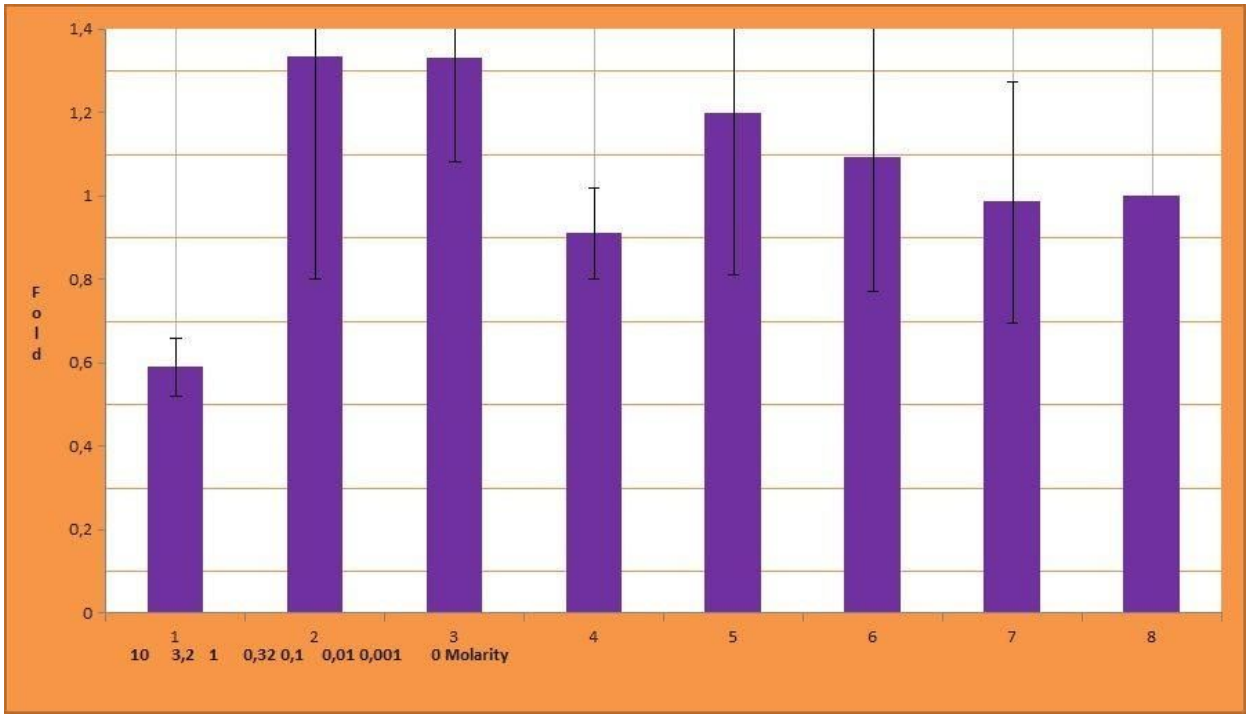
-New induction trials for HxlR verified colony 2:

Table 4 : Experimental desing for induction of HxlR verified col 2 . Gradient formaldehyde (voc) with 3 replicas, 1:10,000 atc

Hxlr X3	atc+ 10 mM voc	atc+ 3.2 mM voc	atc+ 1 mM voc	atc+ 0.32 mM voc	atc+ 0.1 mM voc	atc+ 0.01 mM voc	atc+ 0.001 mM voc	atc+ 0 mM voc
Pro X3	atc+ 10 mM voc	atc+ 3.2 mM voc	atc+ 1 mM voc	atc+ 0.32 mM voc	atc+ 0.1 mM voc	atc+ 0.01 mM voc	atc+ 0.001 mM voc	atc+ 0 mM voc

Figure 17: GFP measurement results after 16 incubation..X axis indicates samples with different formaldehyde concentrations shown in table 4. Y axis indicates the normalized GFP reading.

Note: GFP signal is measured in PBS rather than LB.



15.10.17