**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.

**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**

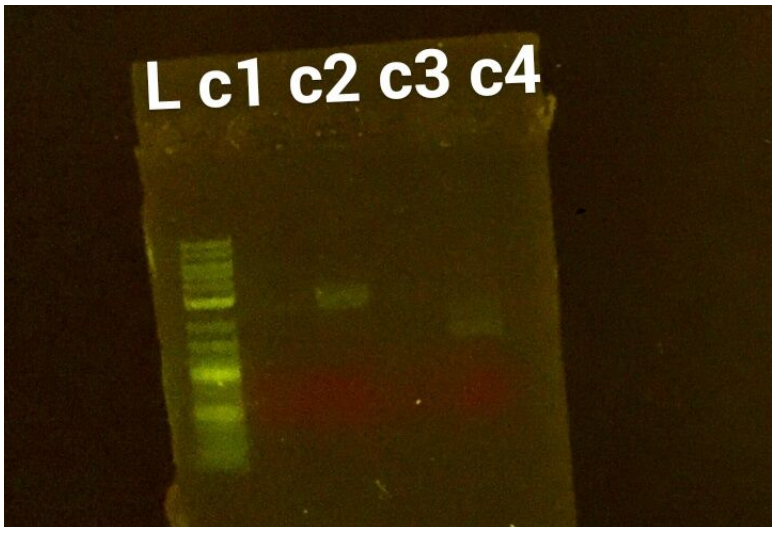
-Cultures are prepared from colony 1, 2, 3 and 4 of plate 1 (pza-hxlR-sfgfp).

**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.

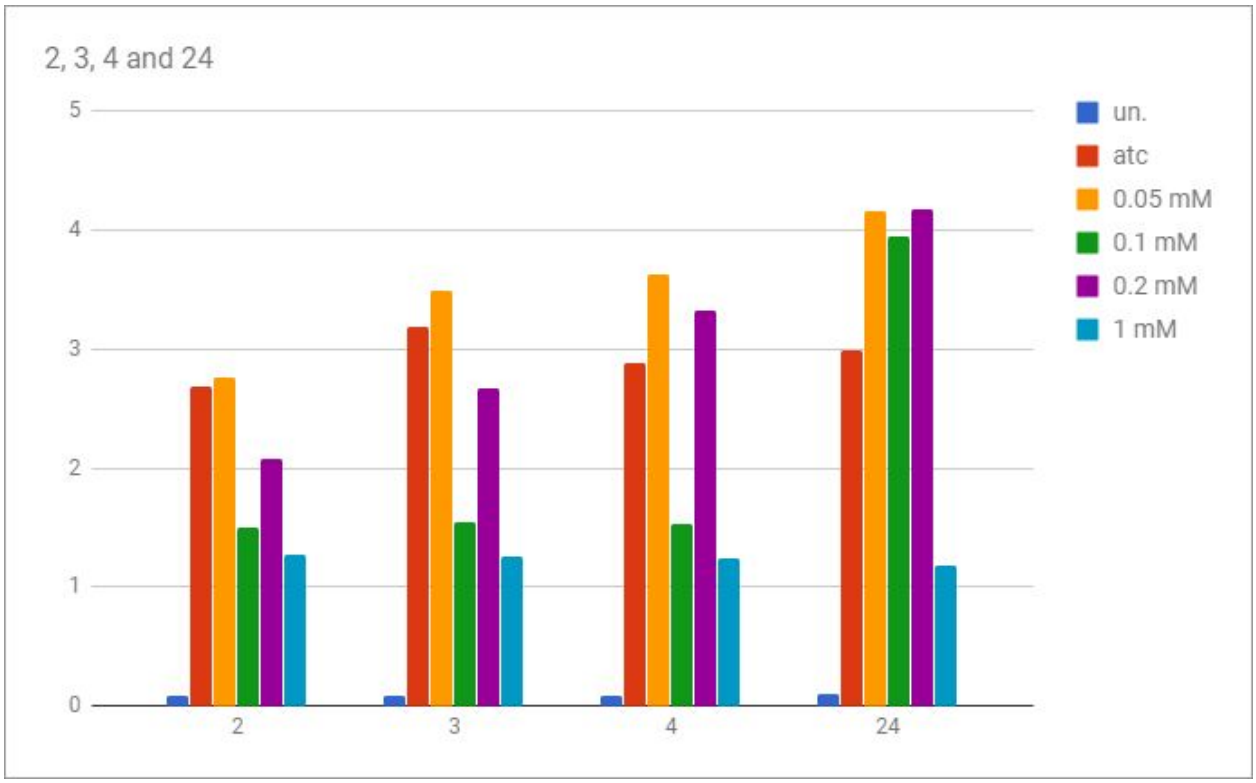



Table 1: Normalized GFP signal data from M5 reader for colony 2 induction

| | 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.

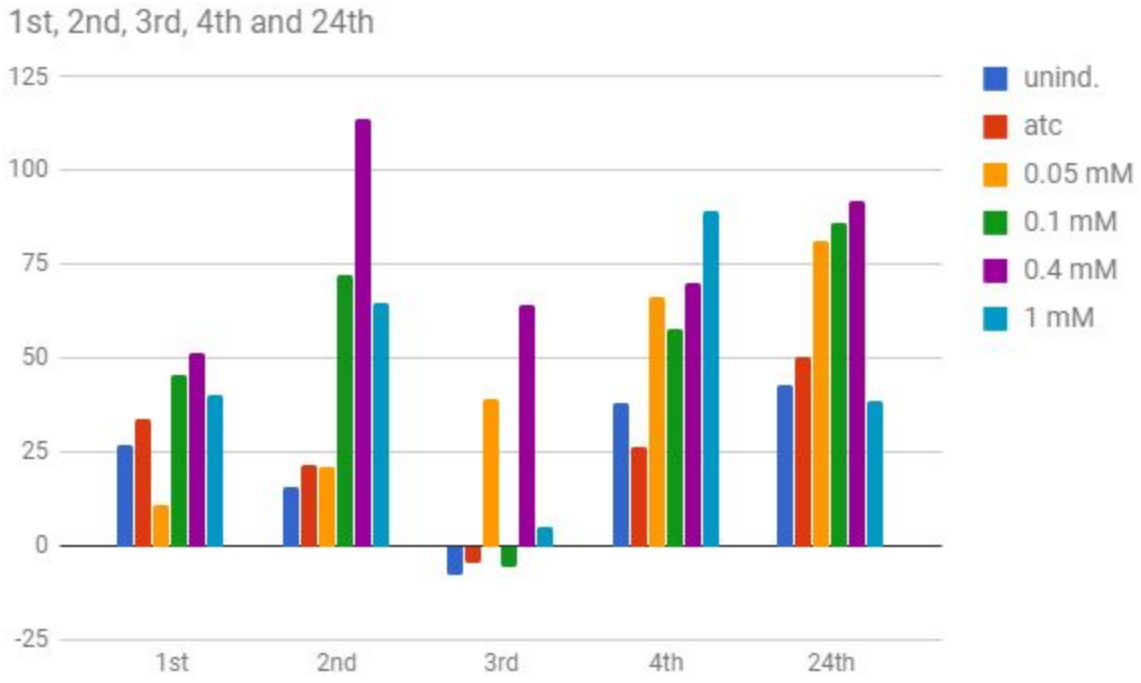
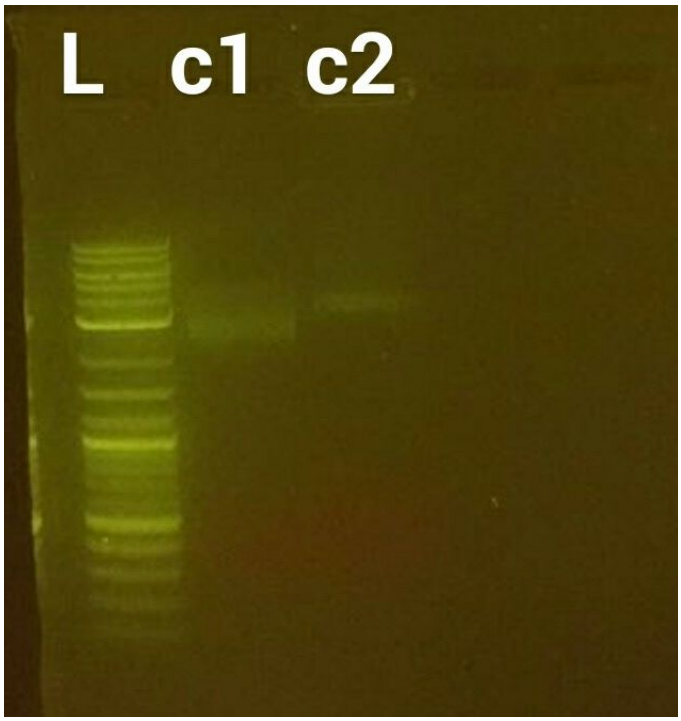


Table 2: Normalized GFP signal data from M5 reader for colony 2 induction

| | 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

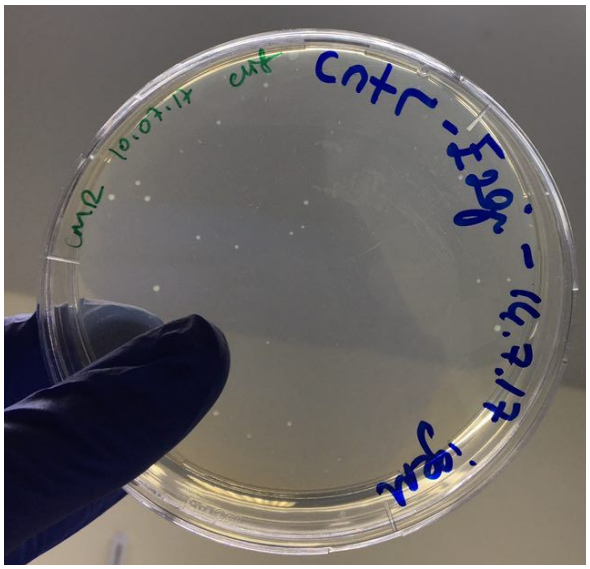
**14.07.17**

- 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 dd$H_2O$ and used after this.
Plates were put to the incubator at 17:00.

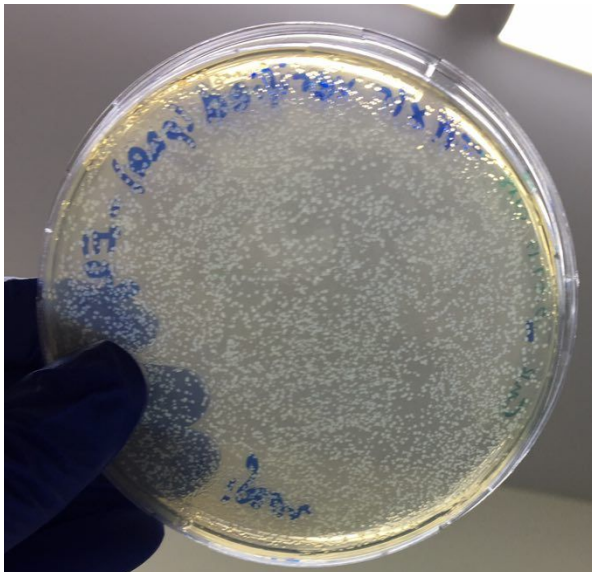
Plate name: hxlR verified

**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

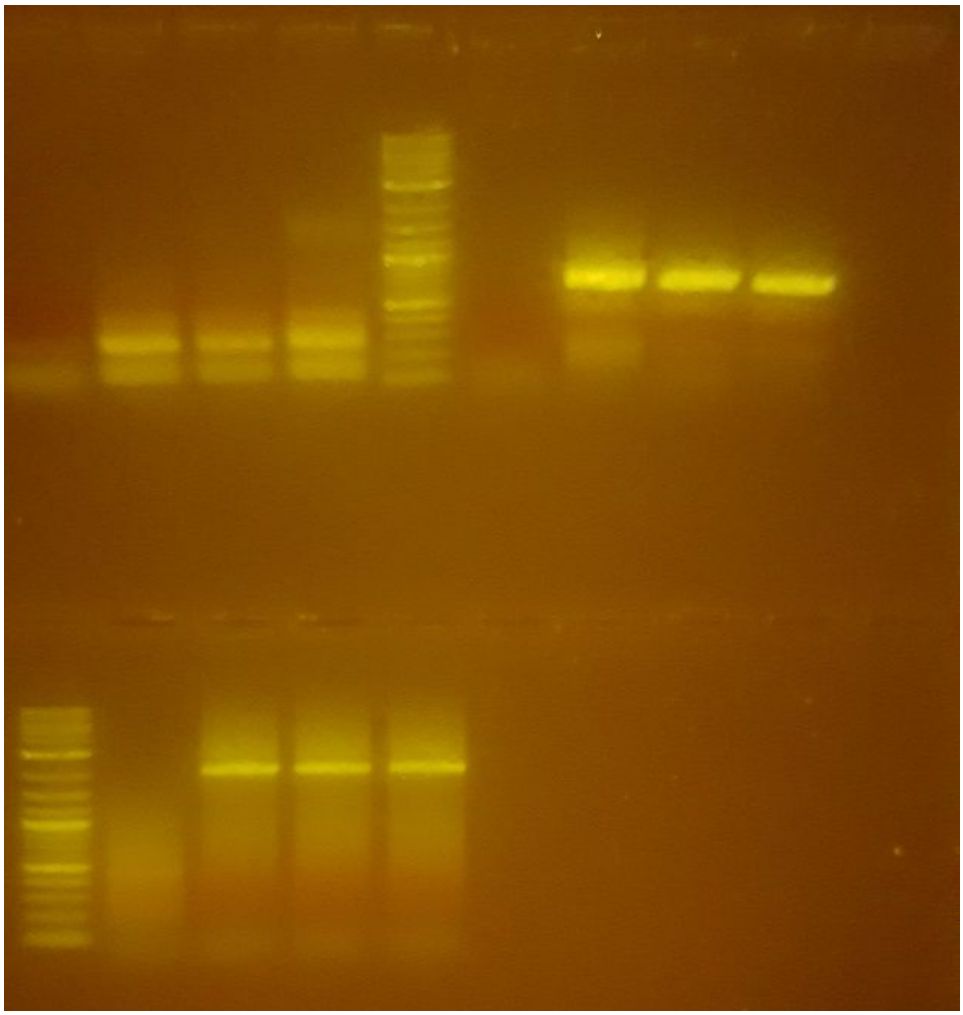
2 ml - 1:0 -------- 0
2 ml - 1:1,000 --- 0
2 ml - 1:2,500 --- 0
2 ml - 1:5,000 --- 0
2 ml - 1:10,000 -- 0
2 ml - 1:25,000 -- 0
4 ml - pro ---------- 0

**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

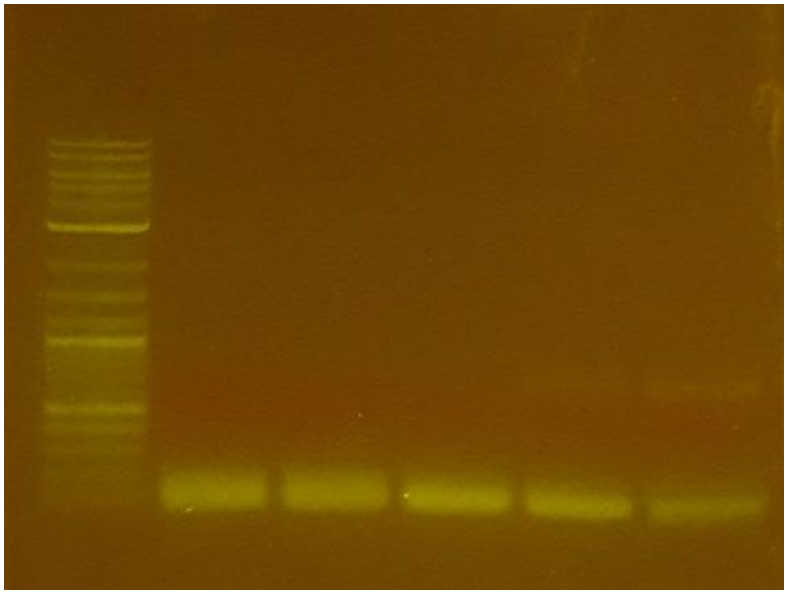
Figure 7: psB1c3 PCR results. Left:AlkR right:Hxlr bottom: pSB1C3 expected band lenghts respectively: ~1300 bp,700bp, 2000bp



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)



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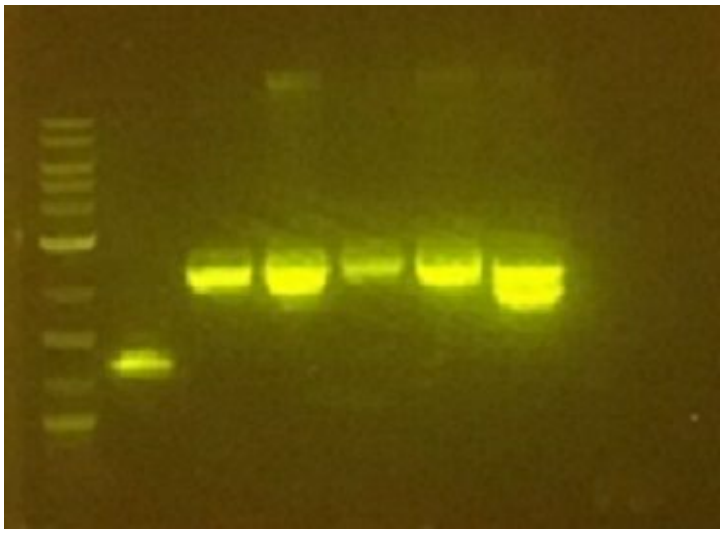
4 HxlR pSB1C3 colony cell culture were prepared.

**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

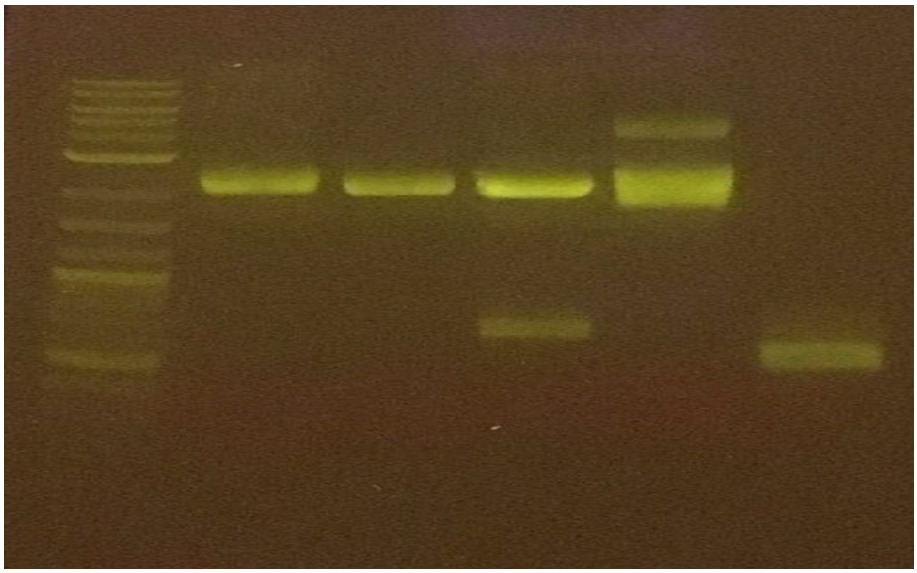


-HxlR pSB1C3 was not verified by the cut with XbaI SpeI enzymes. Cell culture was prepared with newly selected 3 colonies from MimR pSB1C3.

**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.

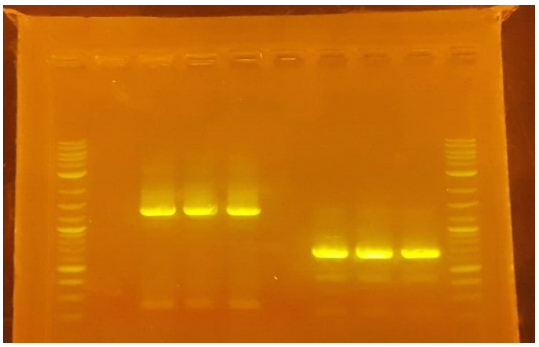


Figure 12: HxlR PCR and AlkR's PCR was digested with XbaI and pstI. But we learned that xbaI also cuts HxlR from the very middle.

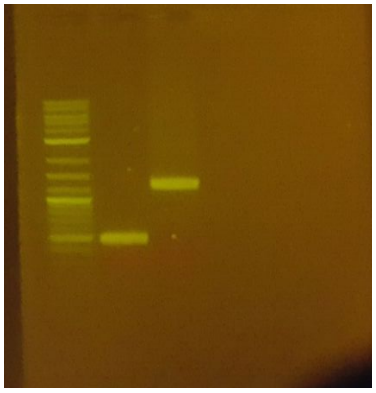


Figure 13: Ver. col1 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

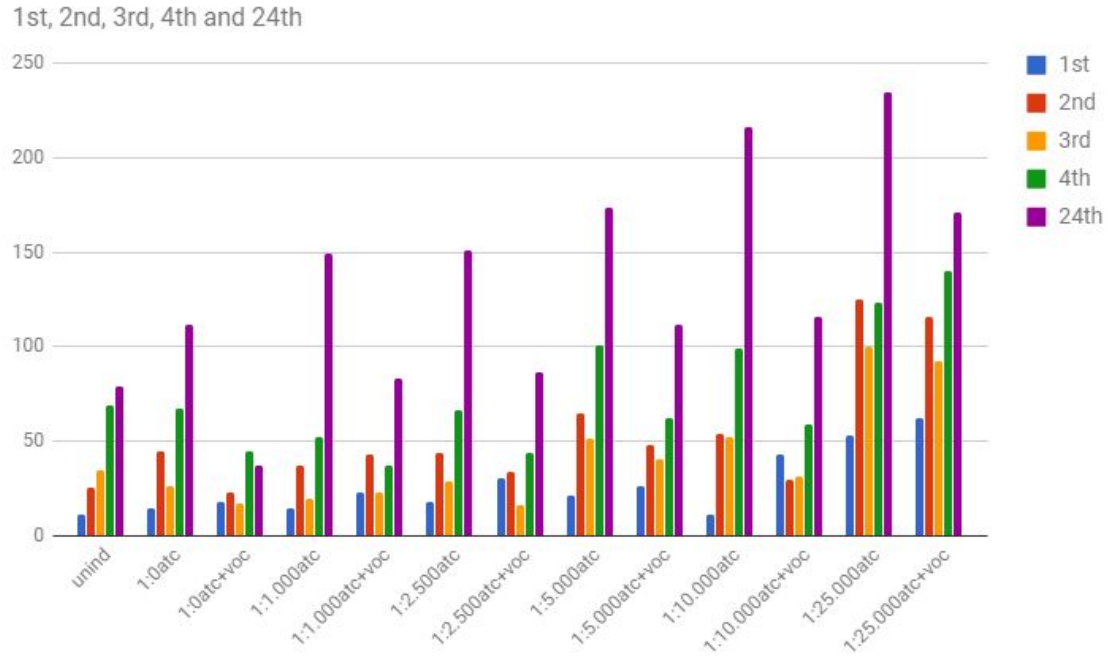



Figure 14: Ver. col2 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.

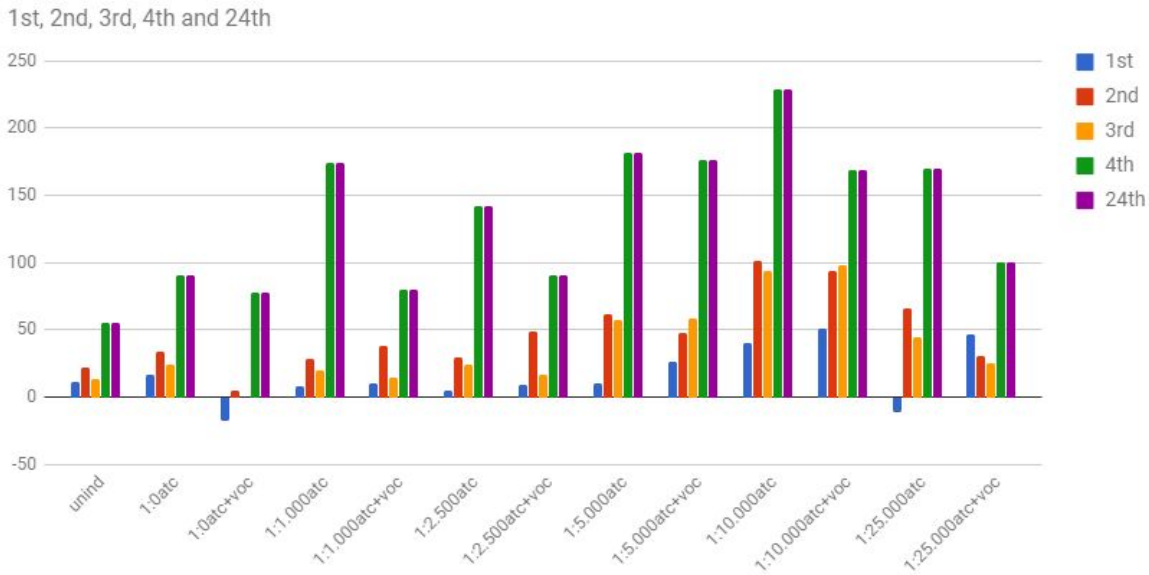
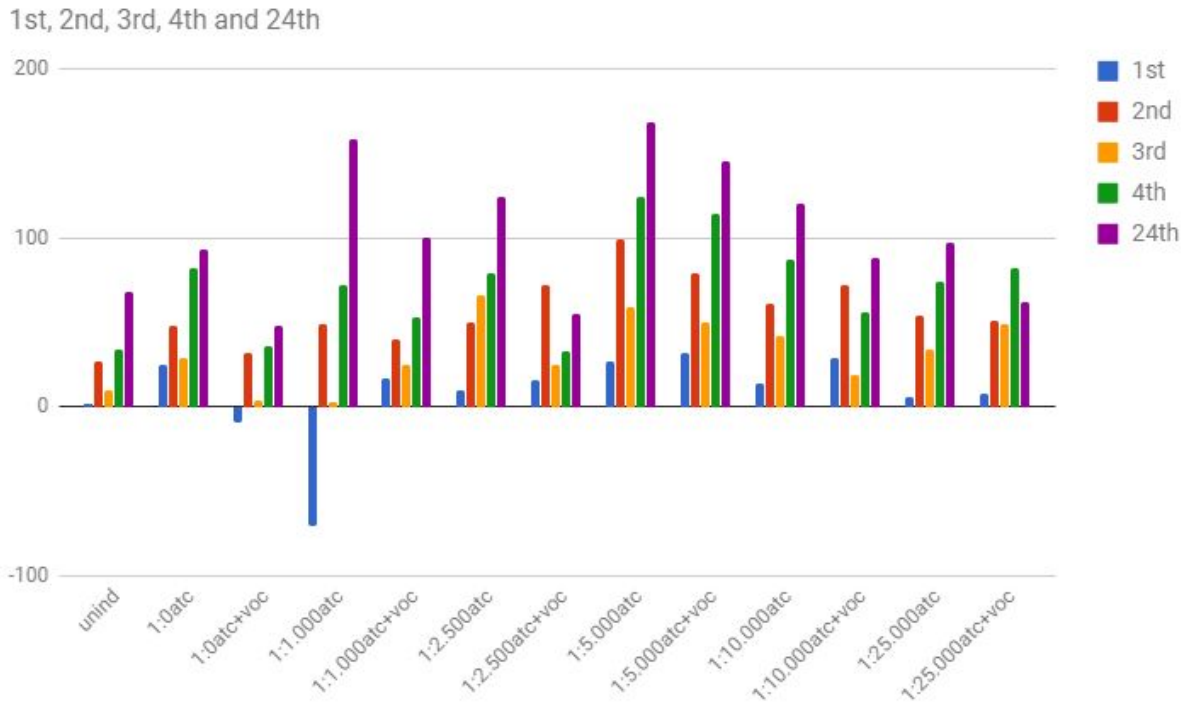


Figure 15: Ver. col3 GFP measurement results. X axis indicates the GFP measurement after voc addition: 1, 2, 3, 4 and 24 hours. Y axis indicates the normalized GFP reading.




**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.

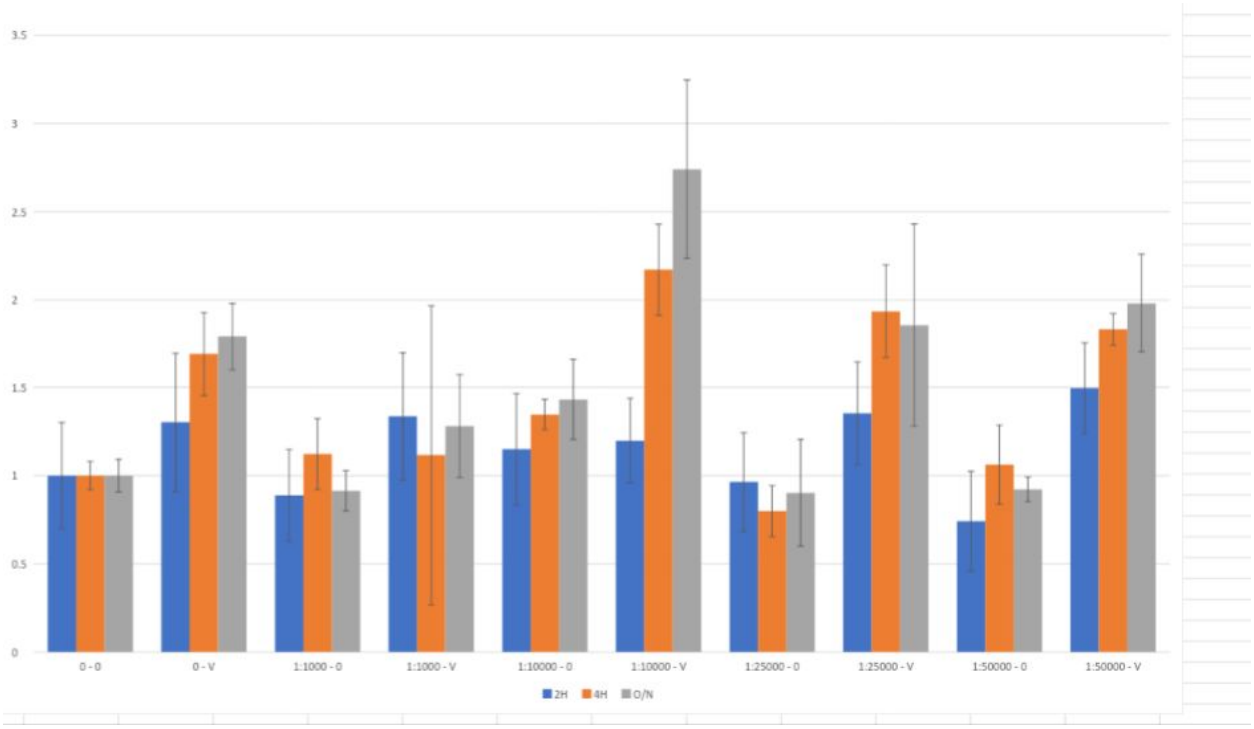
**12.10.17**

-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.

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