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Experiment T32
Integrases on addgene plasmids with our Gates
siG0073: ptet-bxb1, pBAD-tp901, tp901-BUFFER
siG0077: plac-phiC31, bxb1-BUFFER
siG0078: plac-phiC31, tp901-BUFFER

Experiment T01

Dose-Response Kinetics
siG0024: LuxR with sfGFP under plux Promoter and standard RBS
2014-08-06

Goal of the experiment:

- Determine dose-response curve for the lux AHL (3OC6-HSL)
- Collect kinetic data to plan next experiments
- Optimize gain for future experiments
- Choose concentration range for future experiments
- Establish assay

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 36 wells with siG0001 (“empty” plasmid reference)
        - 36 wells with siG0024
        - 3 wells LB as blank
    - Induction after 2 hours in triplicates with 12 dilutions of 3OC6-HSL:
        - 0, 10^{-15}, 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

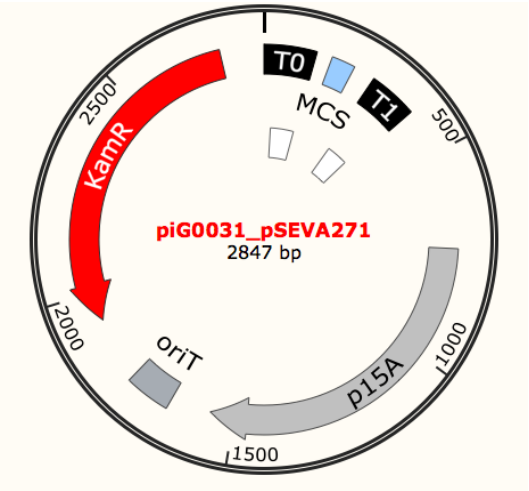
- Tecan infinite M200 PRO

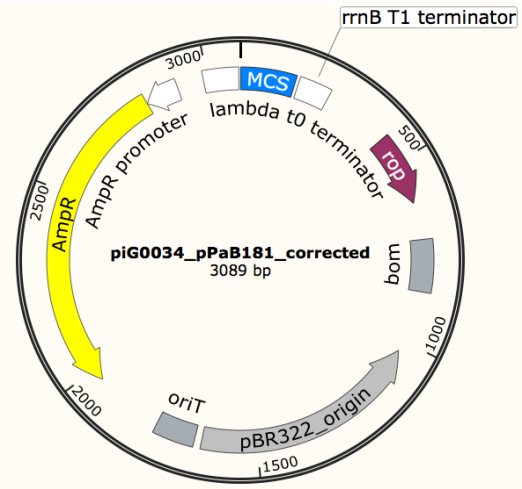
Raw Data:

- Lab/Microtiterplate/crosstalk/20140806_s24_lux-AHL_gradient.xlsx

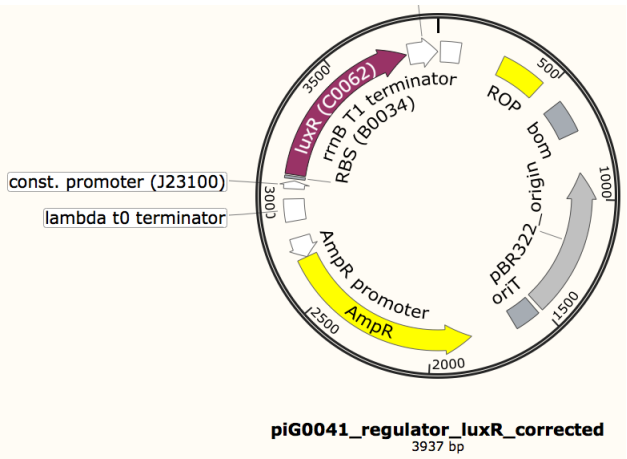
Plasmids in play:

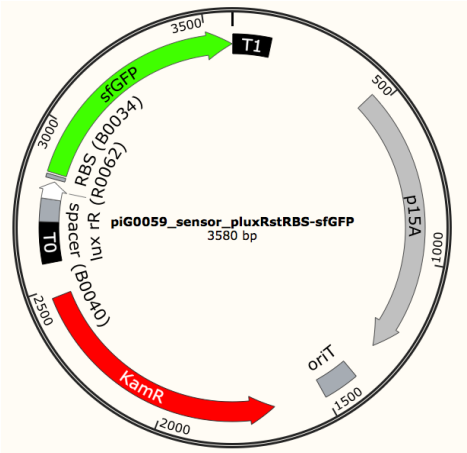
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





Graphs of Data:

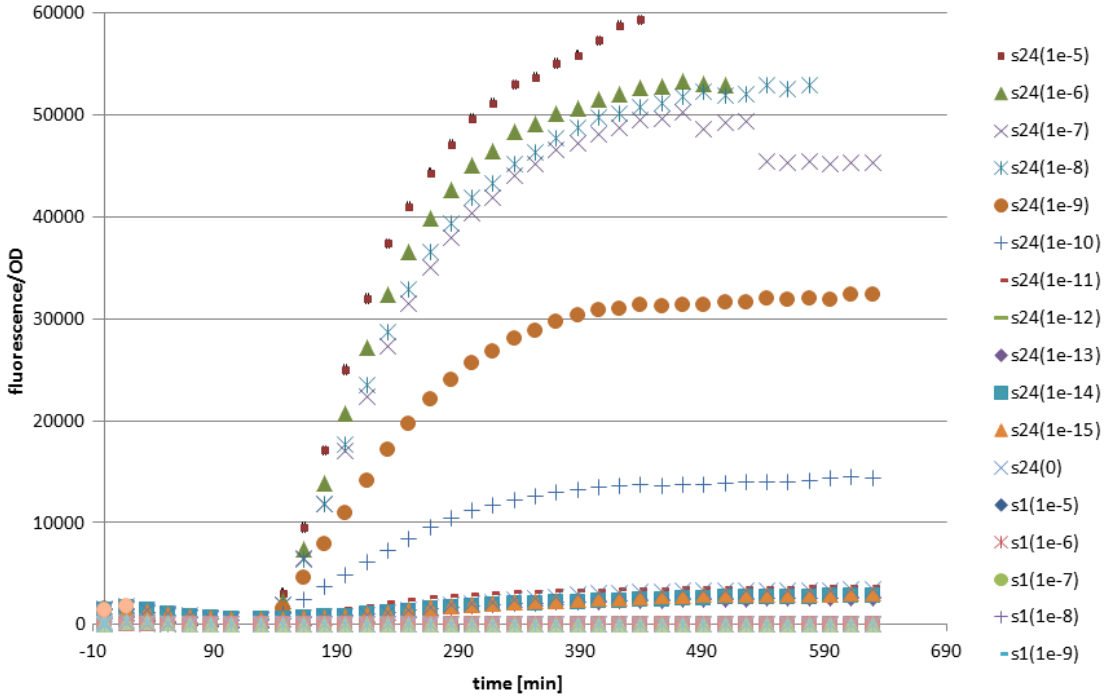
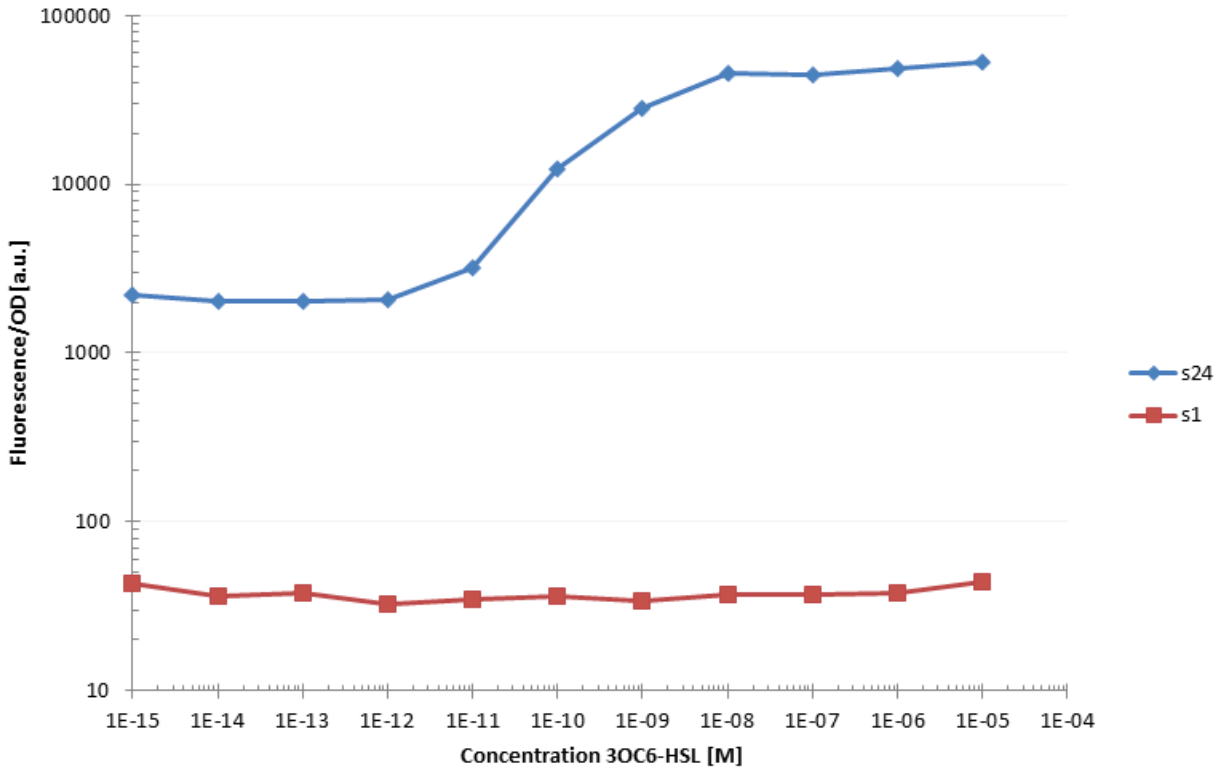


Fig. 1 siG0001 and siG0024 responding to different lux AHL concentrations over time



y

Fig. 2 siG0001 and siG0024 dose-response curve 200 min after induction

Interpretation of Data:

- Gain of 70 was too high (highly induced wells exceeds measurement limits), use gain of 60 for all following experiments that are compared to each other
- dynamic range for 3OC6-HSL **10^{-12} - 10^{-8} M**
- AHL range can be reduced to 10^{-13} to 10^{-5} M (additionally 0 M, no AHL)
- siG0001 works as negative control or background reference, it's not responding to AHL and showing low fluorescence
- 500 minutes are enough for one run

Experiment T02

Dose-Response Kinetics and Crosstalk
siG0024: LuxR with sfGFP under plux Promoter and standard RBS
2014-08-07

Goal of the experiment:

- Analyse crosstalk in siG0024 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0024
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-21}, 10^{-19}, 10^{-17}, 10^{-15}, 10^{-13}, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} M
        - ! dilutions not as planned between 10^{-13} to 10^{-5} M

Machines used:

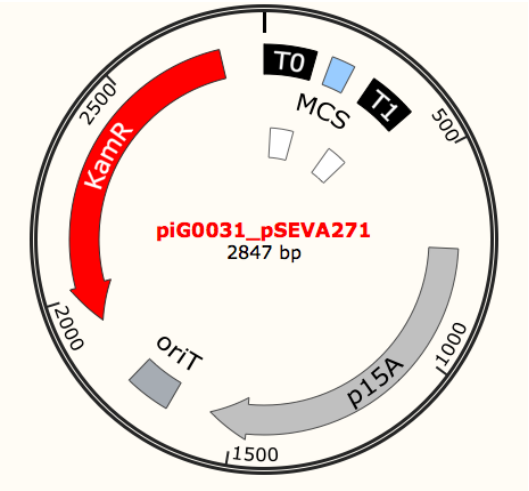
- Tecan infinite M200 PRO

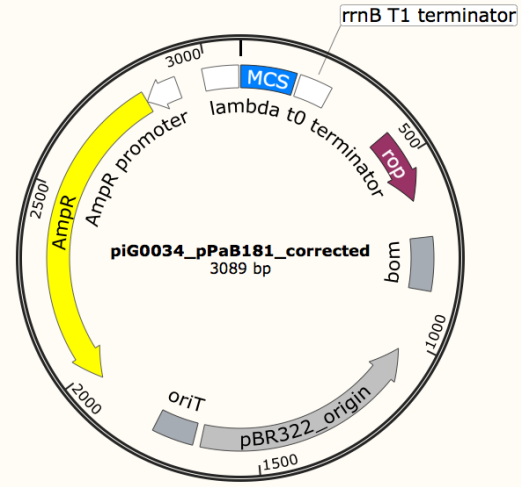
Raw Data:

- Lab/Microtiterplate/crosstalk/20140807_s24_crosstalk.xlsx

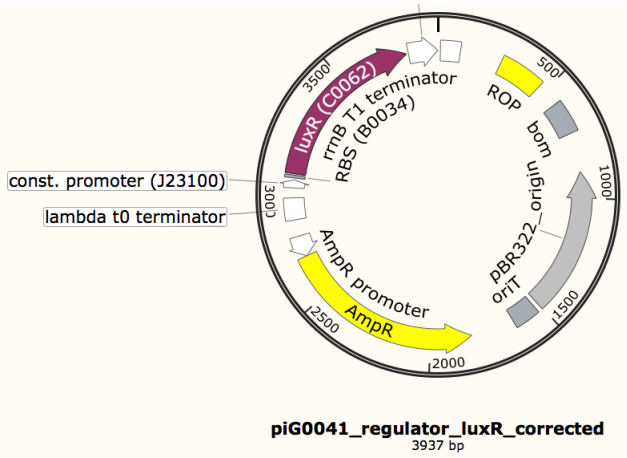
Plasmids in play:

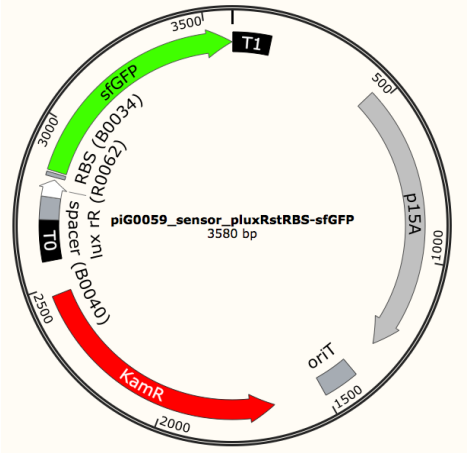
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





Graphs of Data:

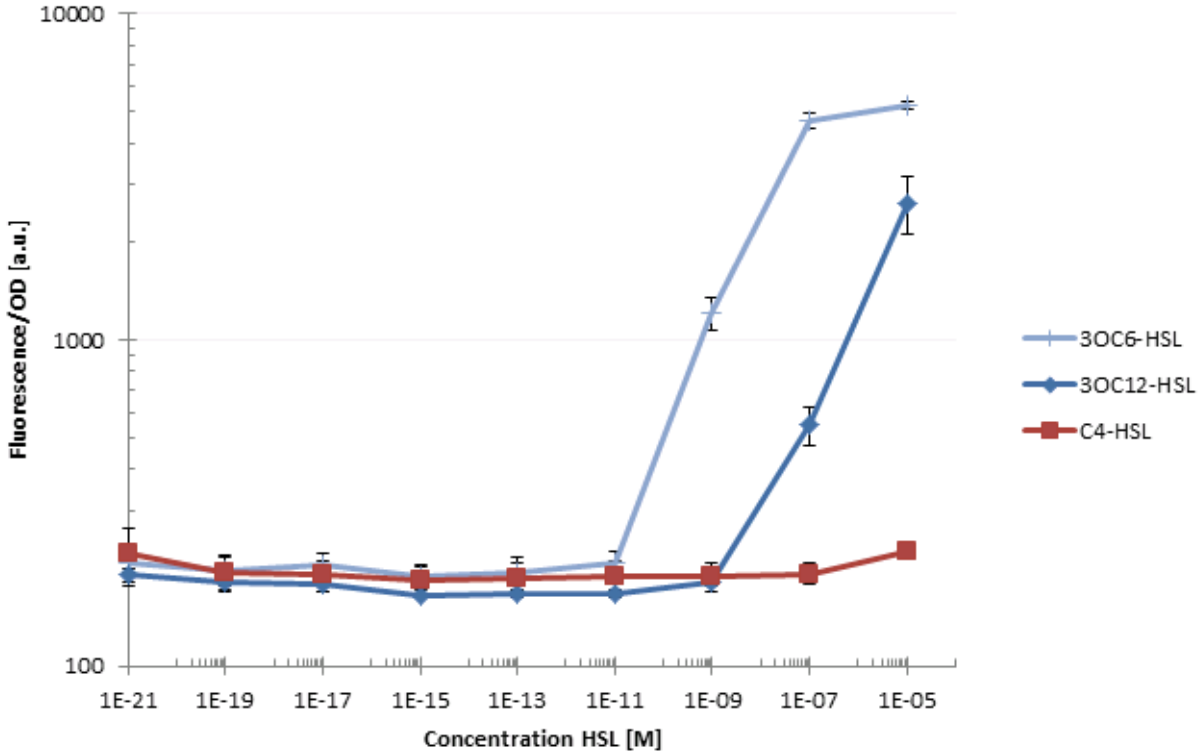


Fig. 1 siG0024 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- Gain of 50 was too low, use gain of 60 for all following experiments that are compared to each other
- dynamic range for 3OC6-HSL **10^{-11} - 10^{-7} M**
- dynamic range for 3OC12-HSL **10^{-9} - min. 10^{-5} M**

Experiment T03

Dose-Response Kinetics and Crosstalk
siG0024: LuxR with sfGFP under plux Promoter and standard RBS fine tuning
2014-08-08

Goal of the experiment:

- Repeat experiment T02 with a gain of 60
- Analyse crosstalk in siG0024 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0024
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-21}, 10^{-19}, 10^{-17}, 10^{-15}, 10^{-13}, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} M
        - ! dilutions not as planned between 10^{-13} to 10^{-5} M

Machines used:

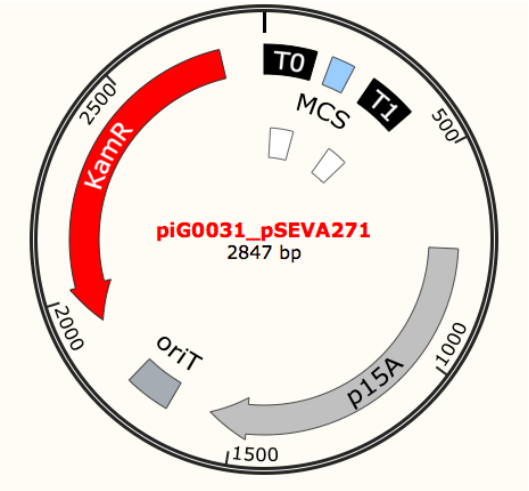
- Tecan infinite M200 PRO

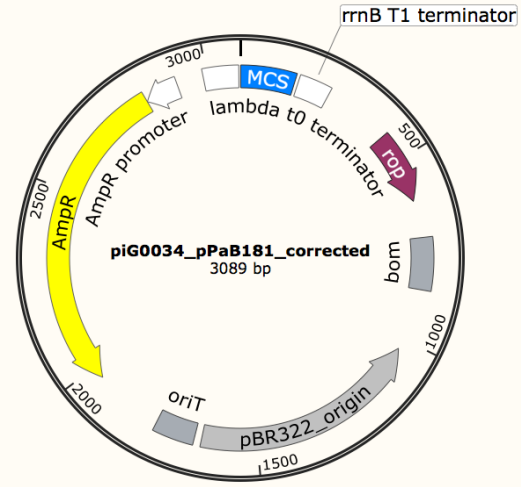
Raw Data:

- Lab/Microtiterplate/crosstalk/20140808_s24_crosstalk.xlsx

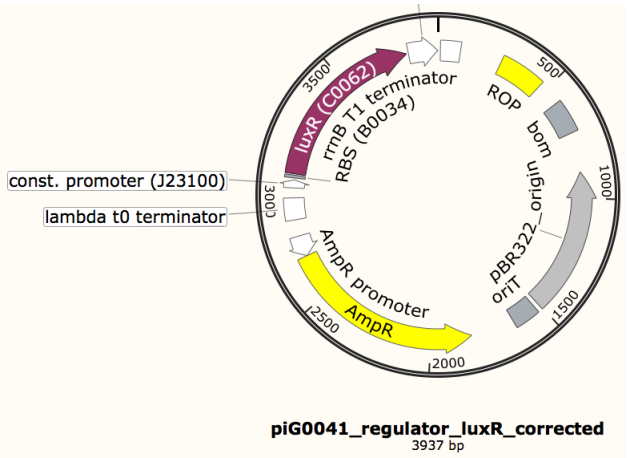
Plasmids in play:

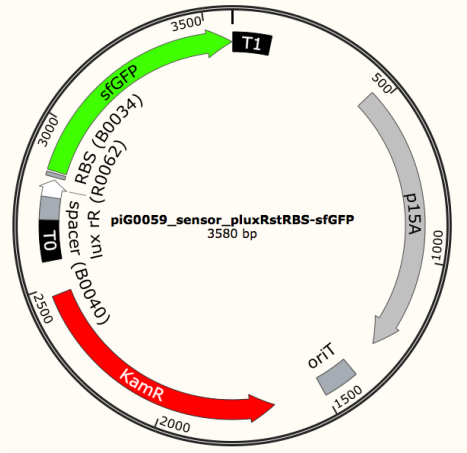
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





Graphs of Data:

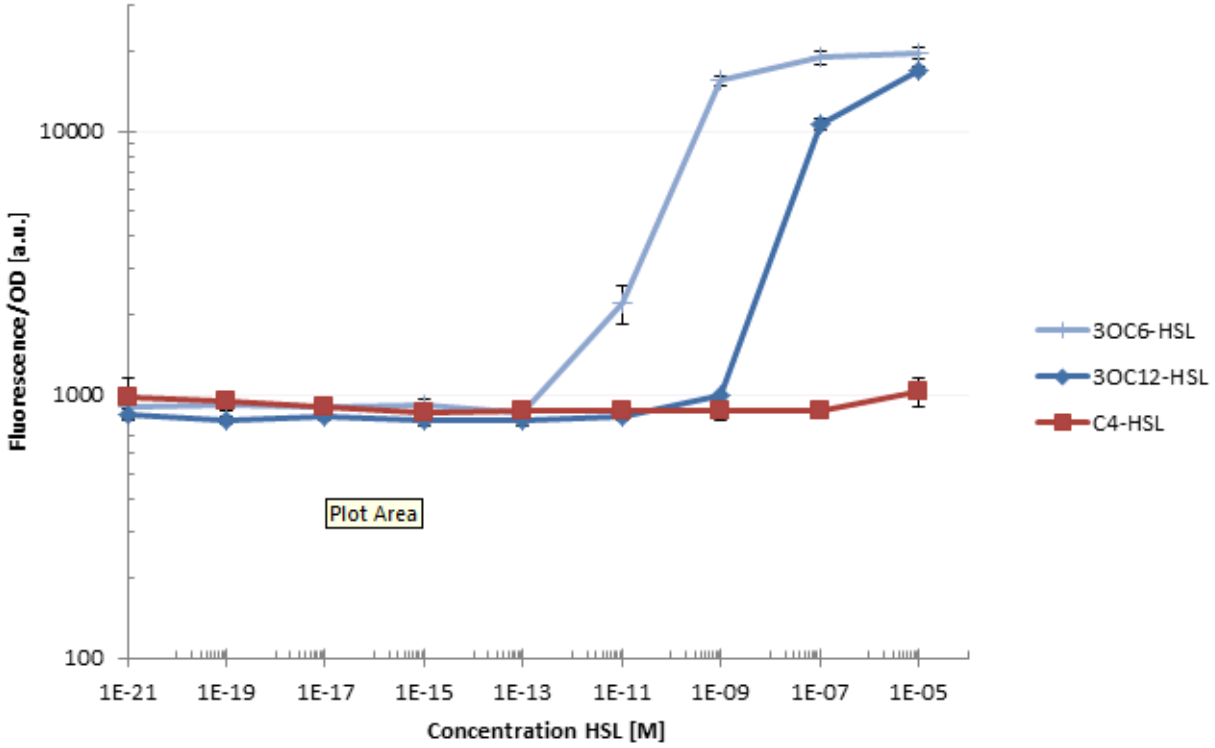


Fig. 1 siG0024 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- LuxR with plux
    - shows almost no response to the rhl AHL (C4-HSL)
    - is more sensitive to the lux AHL (3OC6-HSL) than to the las AHL (3OC12-HSL)
- dynamic range for 3OC6-HSL **10^{-13} - 10^{-9} M**
- dynamic range for 3OC12-HSL **10^{-9} - 10^{-5} M**

Experiment T04

Dose-Response Kinetics and Crosstalk
siG0042: low LuxR with sfGFP under plux Promoter and standard RBS
2014-08-08

Goal of the experiment:

- Investigate the influence of the LuxR concentration by using a weak constitutive promoter (J23100) for luxR
- Analyse crosstalk in siG0042 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0042
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-21}, 10^{-19}, 10^{-17}, 10^{-15}, 10^{-13}, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} M
        - ! dilutions not as planned between 10^{-13} to 10^{-5} M

Machines used:

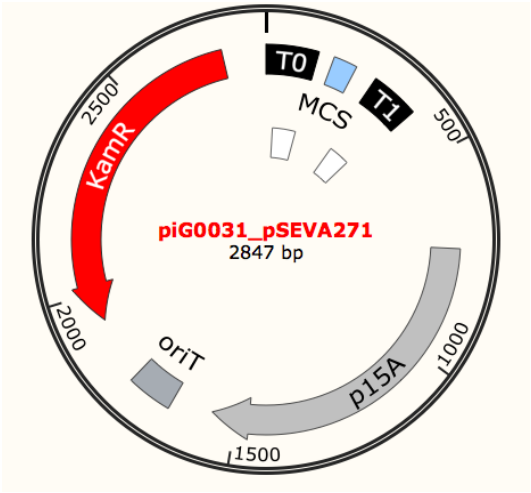
- Tecan infinite M200 PRO

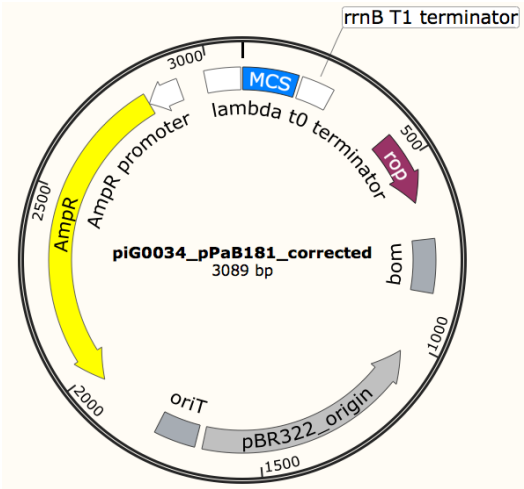
Raw Data:

- Lab/Microtiterplate/crosstalk/20140808_s42_crosstalk.xlsx

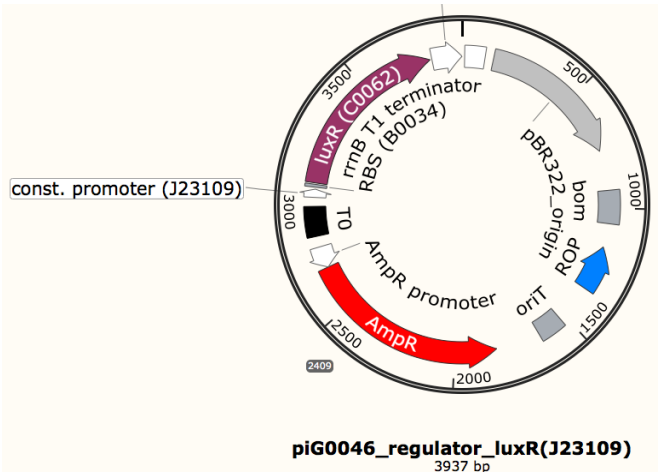
Plasmids in play:

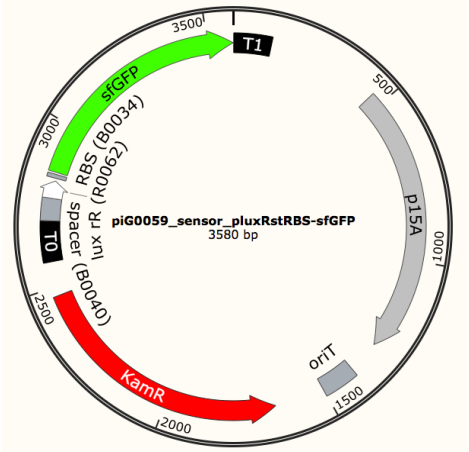
- siG0001: piG0031, piG0034





- siG0042: piG0046, piG0059





Graphs of Data:

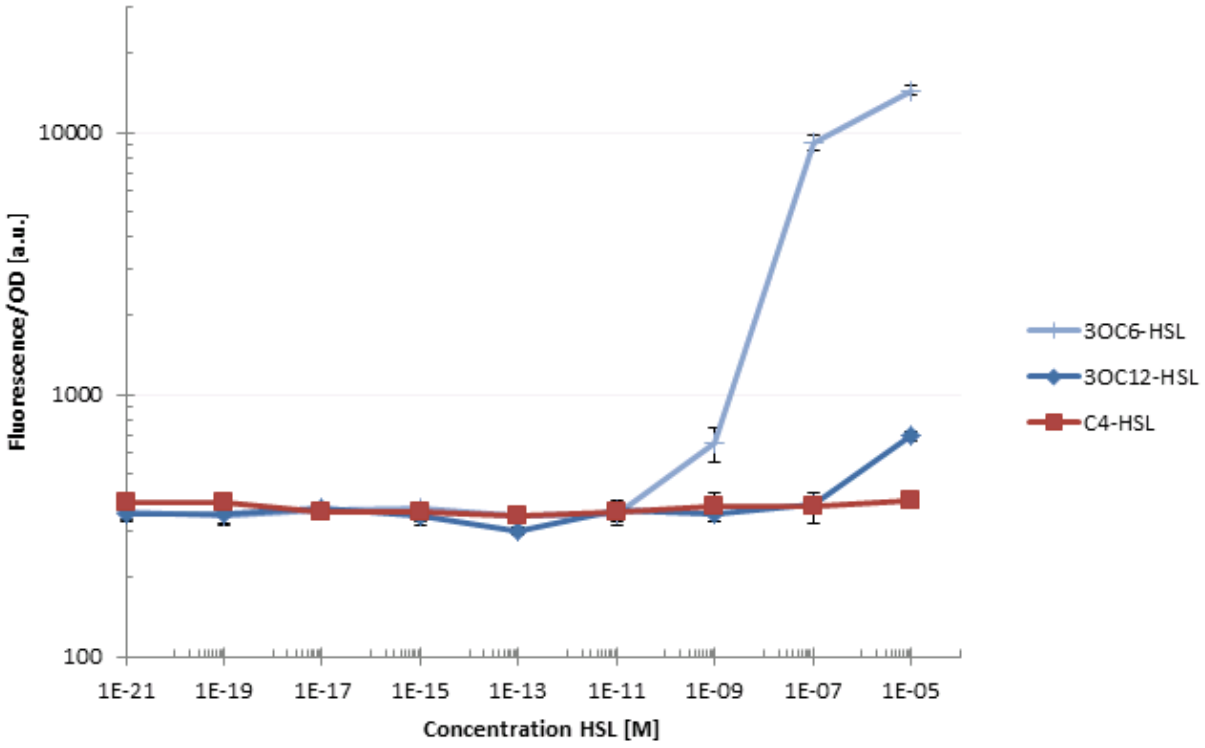


Fig. 1 siG0042 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- having a low concentration of LuxR with plux
    - shows lower leakiness: Fluorescence/OD goes down from ~1000 to ~400 [a.u.]
    - leads to less sensitivity: ~10^{-11} to ~10^{-9} M 3OC6-HSL (first value over basal level)
- dynamic range for 3OC6-HSL **10^{-11} - 10^{-5} M**

Experiment T05

Dose-Response Kinetics and Crosstalk
siG0014: LasR with sfGFP under plas Promoter and standard RBS
2014-08-09

Goal of the experiment:

- Analyse crosstalk in siG0014 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0014
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-21}, 10^{-19}, 10^{-17}, 10^{-15}, 10^{-13}, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} M
        - ! dilutions not as planned between 10^{-13} to 10^{-5} M

Machines used:

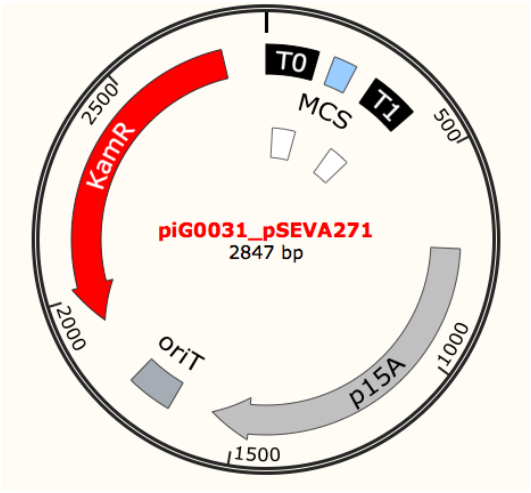
- Tecan infinite M200 PRO

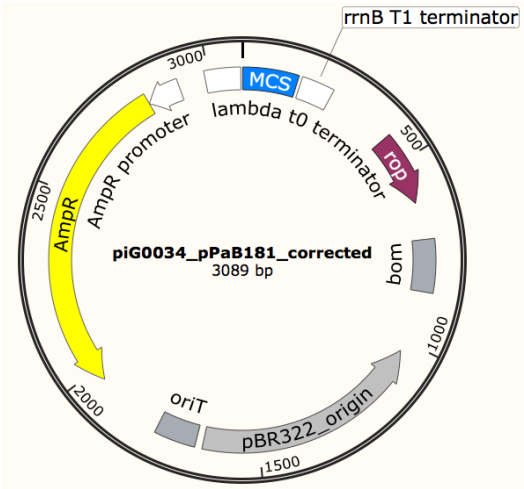
Raw Data:

- Lab/Microtiterplate/crosstalk/20140809_s14_crosstalk.xlsx

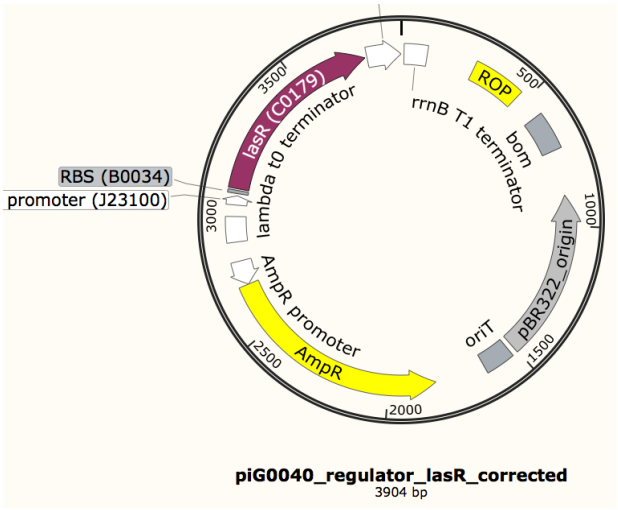
Plasmids in play:

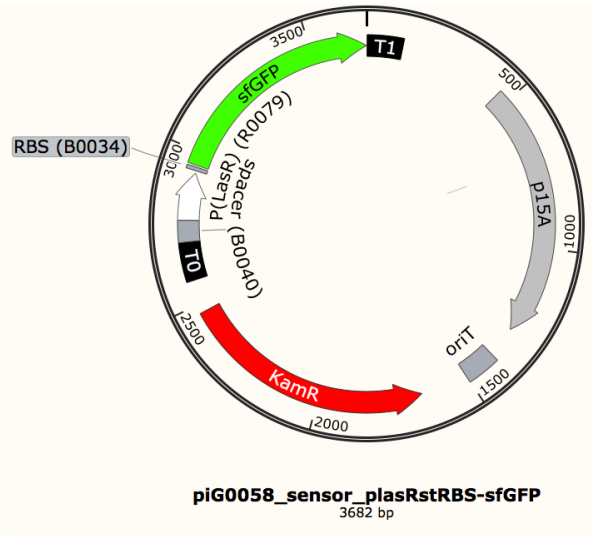
- siG0001: piG0031, piG0034





- siG0014: piG0040, piG0058





Graphs of Data:

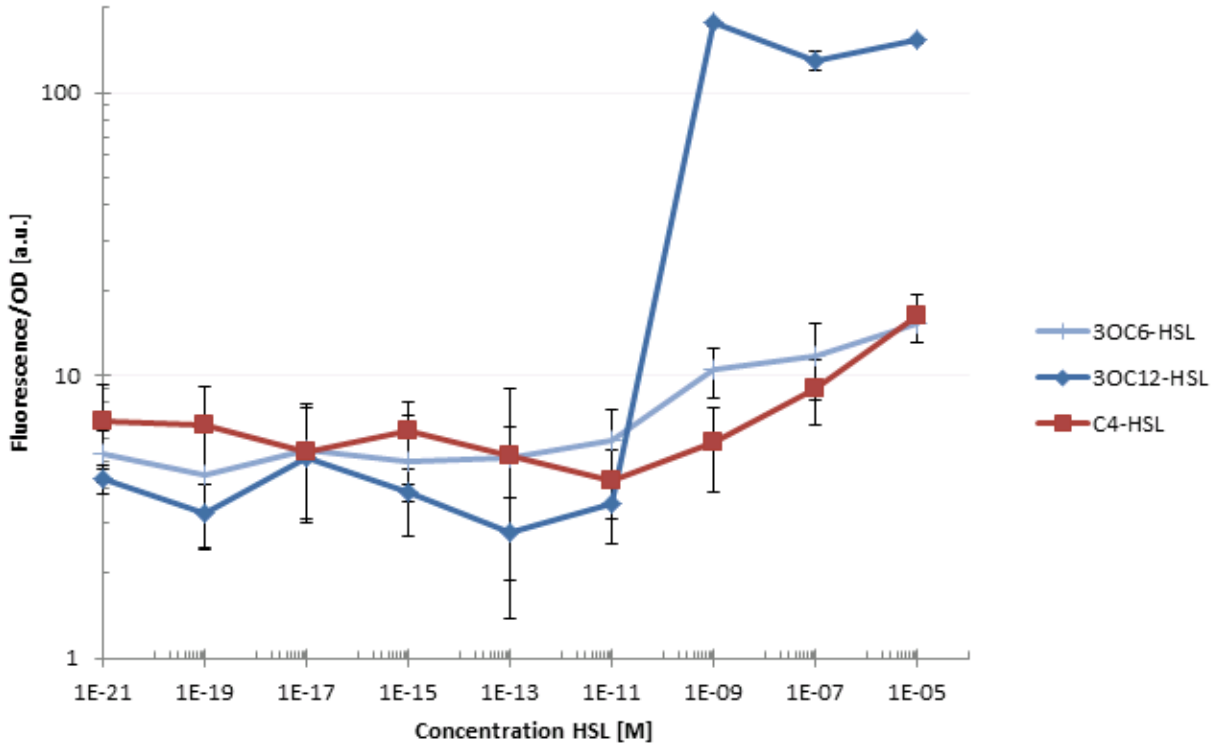


Fig. 1 siG0014 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- plas regulated by LasR is most sensitive to 3OC12-HSL
- very low leakiness could be observed:
  basal level is 100 times lower than in siG0024 (luxR, plux)
- dynamic range for 3OC12-HSL **10^{-11} - 10^{-9} M**

Experiment T06

Dose-Response Kinetics and Crosstalk
siG0048: low LuxR with sfGFP under plux Promoter and Riboregulator 12y
2014-08-09

Goal of the experiment:

- Determine whether the riboregulator decreases leakiness
- Analyse crosstalk in siG0048 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0048
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-21}, 10^{-19}, 10^{-17}, 10^{-15}, 10^{-13}, 10^{-11}, 10^{-9}, 10^{-7}, 10^{-5} M
        - ! dilutions not as planned between 10^{-13} to 10^{-5} M

Machines used:

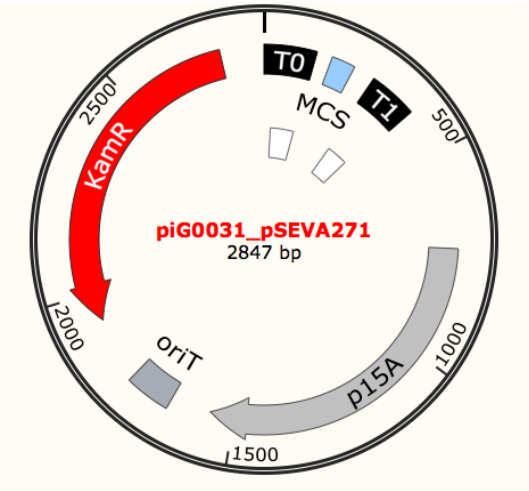
- Tecan infinite M200 PRO

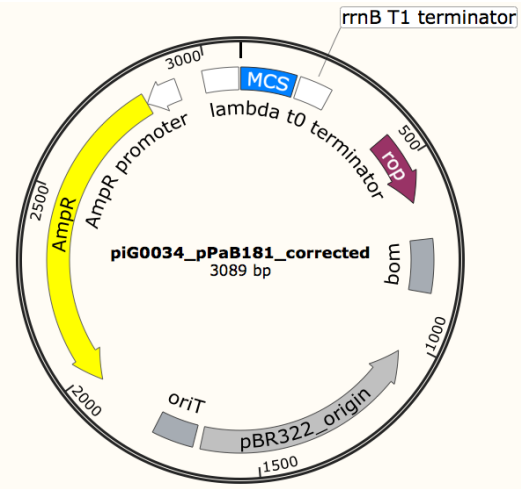
Raw Data:

- Lab/Microtiterplate/crosstalk/20140809_s48_crosstalk.xlsx

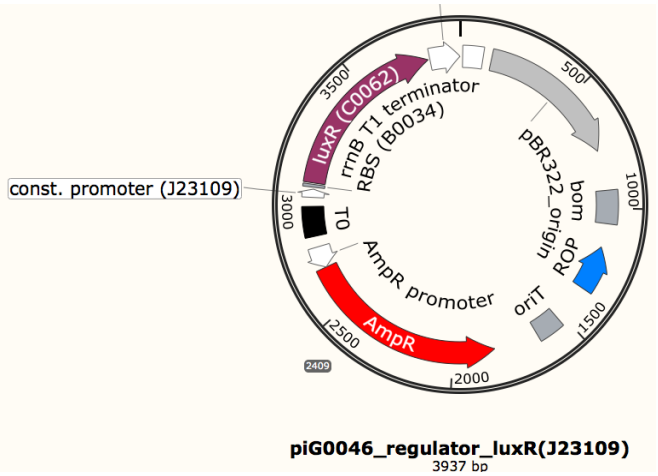
Plasmids in play:

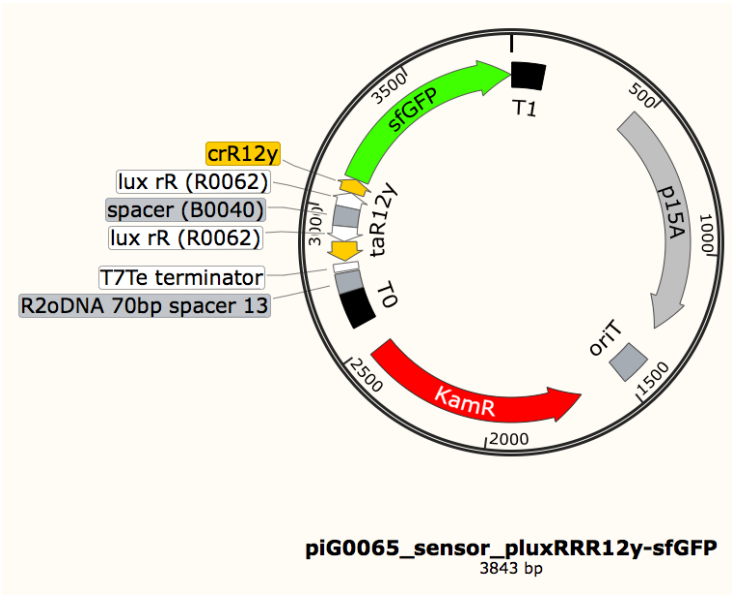
- siG0001: piG0031, piG0034





- siG0048: piG0046, piG0065





Graphs of Data:

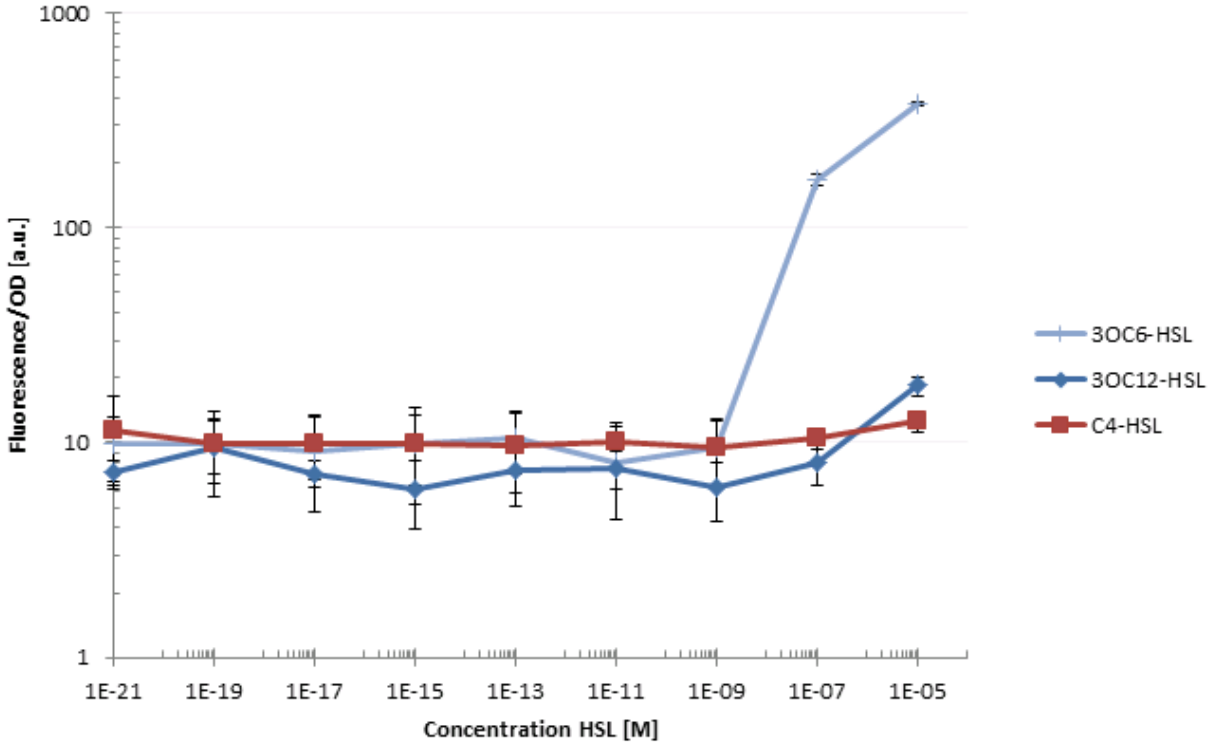


Fig. 1 siG0048 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- 30 times reduced leakiness compared to the plux without riboregulator siG0042 (Experiment T04)
- reduced dynamic range compared to siG0042
- dynamic range for 3OC6-HSL **10^{-9} - 10^{-5} M**

Experiment T07

Dose-Response Kinetics and Crosstalk
siG0051: medium LuxR with sfGFP under plux Promoter and standard RBS
2014-08-10

Goal of the experiment:

- Find effects of promoter strength (J23111) controlling LuxR production
- Compare to siG0042 (T03) and siG0024 (T02)
- Analyse crosstalk in siG0051 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0051
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

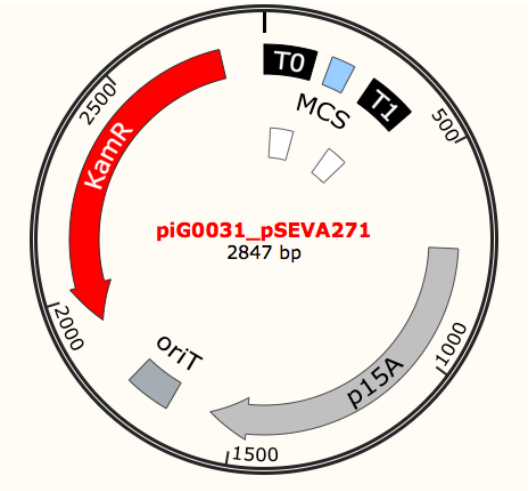
- Tecan infinite M200 PRO

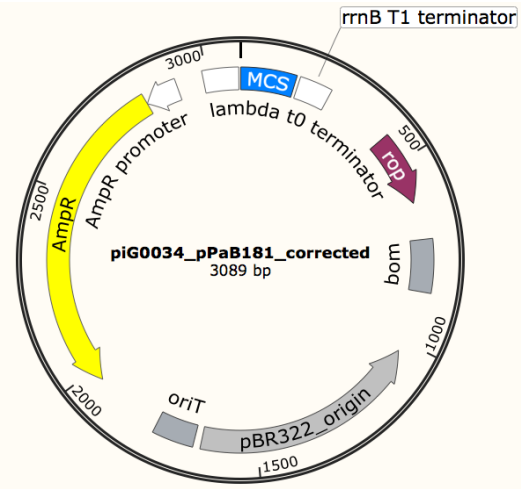
Raw Data:

- Lab/Microtiterplate/crosstalk/20140810_s51_crosstalk.xlsx

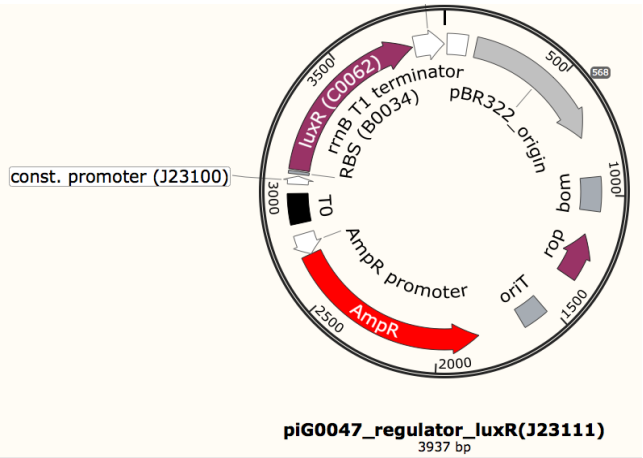
Plasmids in play:

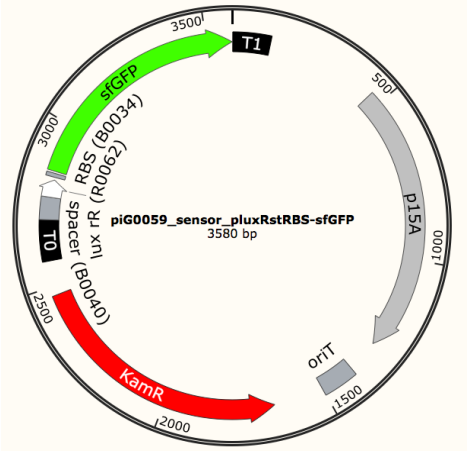
- siG0001: piG0031, piG0034





- siG0051: piG0047, piG0059





Graphs of Data:

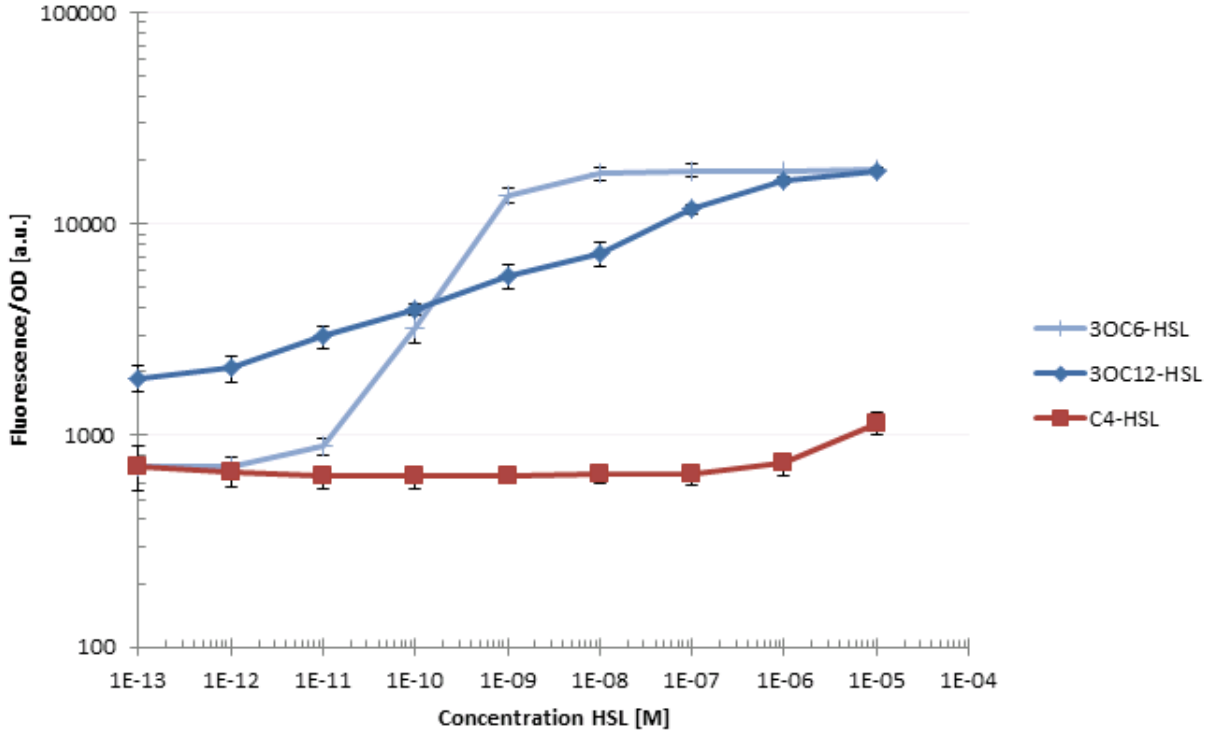


Fig. 1 siG0051 dose-response curve 200 min after induction for three AHL molecules

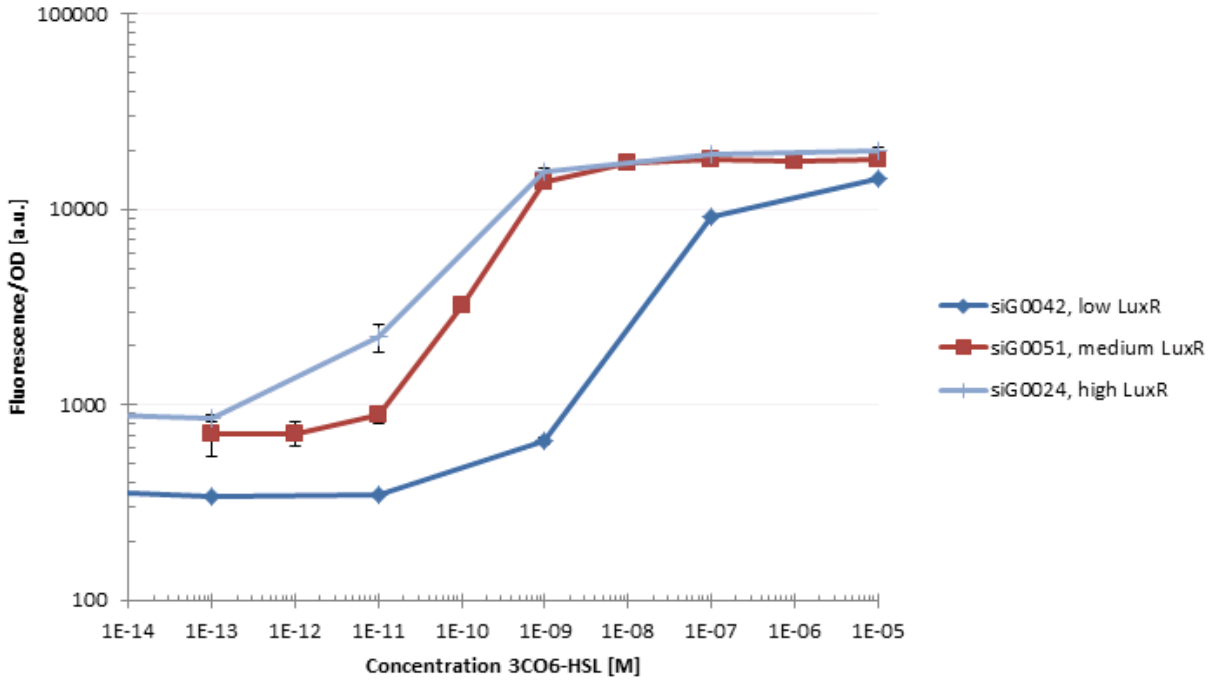


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for the three variants with different promoter strengths for LuxR production

Interpretation of Data:

- unexpected shallow response to 3OC12-HSL (Fig. 1)
- could be a pipetting error, if data is further used, the experiment needs to be repeated!
- influence of LuxR amount on sensitivity to 3OC6-HSL
- dynamic range for 3OC6-HSL **10^{-12} - 10^{-8} M**

Experiment T08

Dose-Response Kinetics and Crosstalk
siG0030: LuxR with sfGFP under plux Promoter and Riboregulator 12y
2014-08-11

Goal of the experiment:

- Does the riboregulator decrease leakiness?
- Compare to siG0024 (T02)
- Analyse crosstalk in siG0030 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0030
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

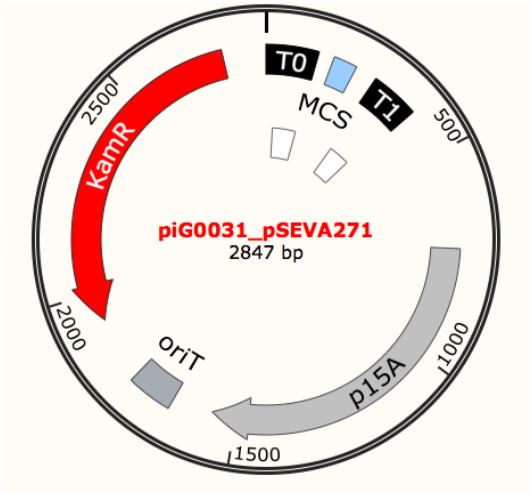
- Tecan infinite M200 PRO

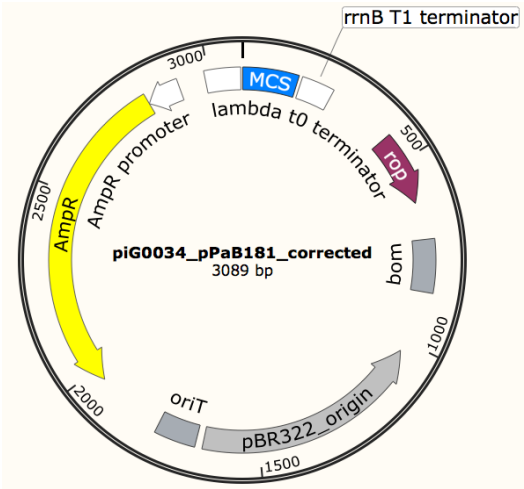
Raw Data:

- Lab/Microtiterplate/crosstalk/20140811_s30_crosstalk.xlsx

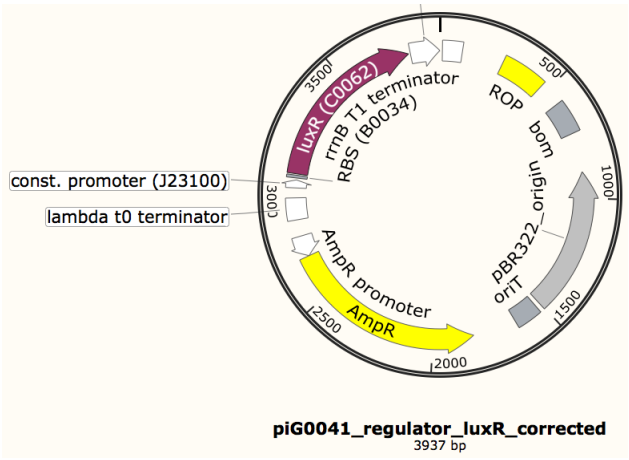
Plasmids in play:

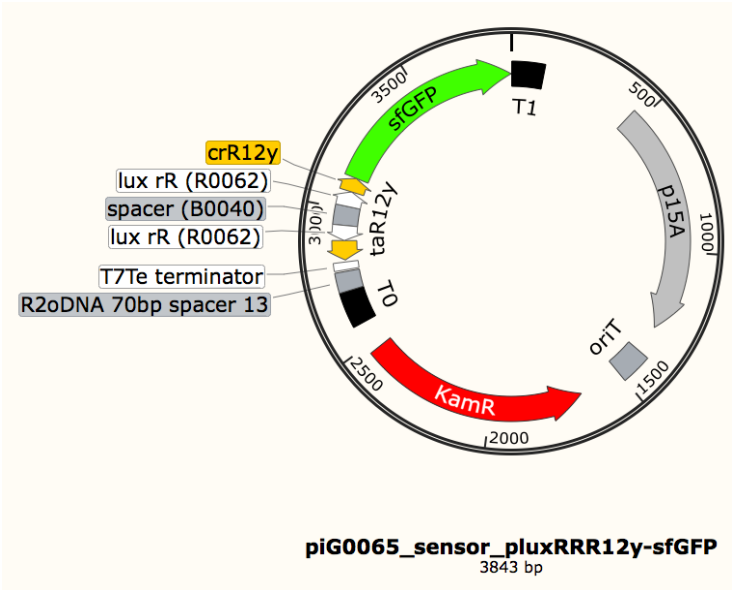
- siG0001: piG0031, piG0034





- siG0030: piG0041, piG0065





Graphs of Data:

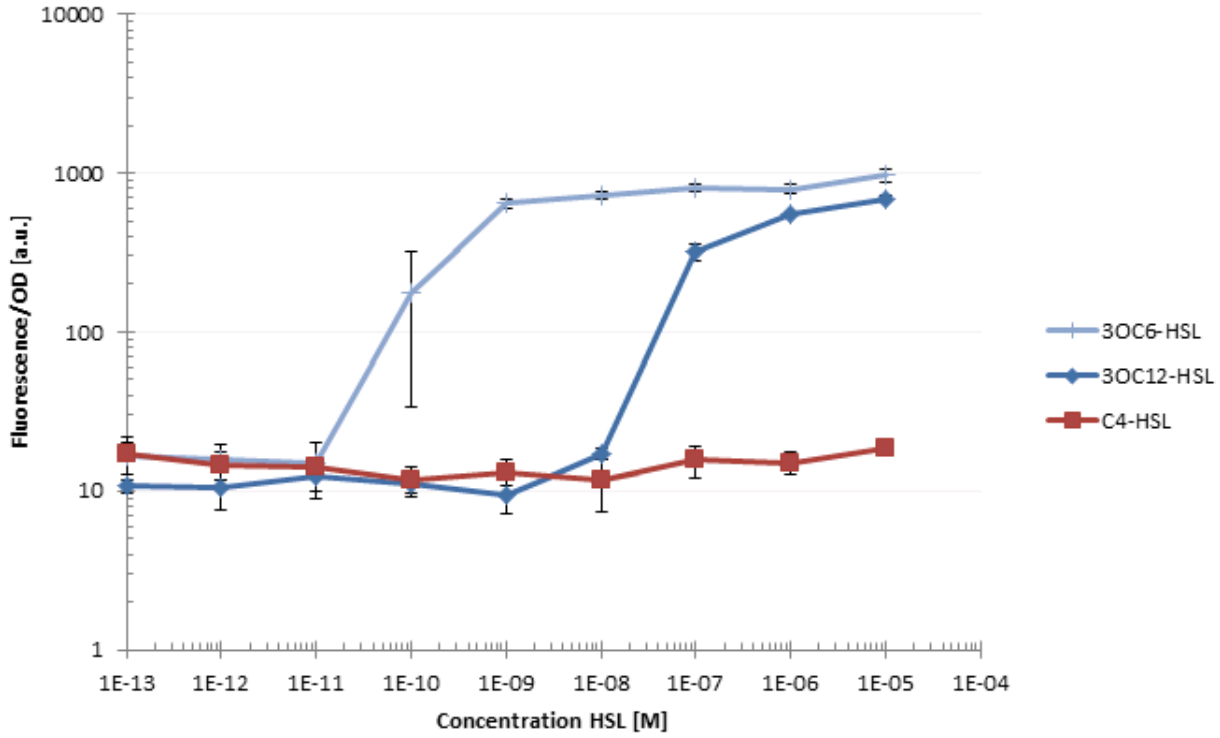


Fig. 1 siG0030 dose-response curve 200 min after induction for three AHL molecules

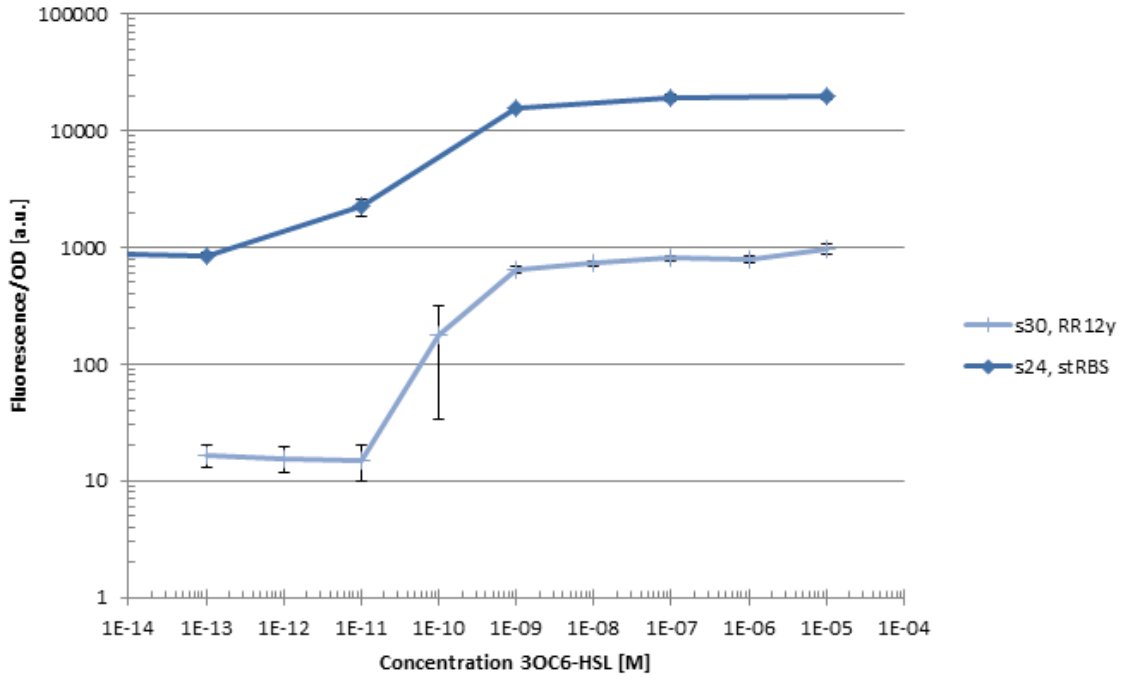


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0024 (without RR12y) and siG0030 (with RR12y)

Interpretation of Data:

- leakiness ~100 times decreased with RR12y (see Fig. 2)
- ON/OFF ratio more than 2 times increased with RR12y (Fig. 2)
- dynamic range for 3OC6-HSL **10^{-11} - 10^{-9} M**
- dynamic range for 3OC12-HSL **10^{-9} - 10^{-6} M**

Experiment T09

Dose-Response Kinetics and Crosstalk with Producer Supernatants siG0030: LuxR with sfGFP under plux Promoter and Riboregulator 12y 2014-08-11

Goal of the experiment:

- Do the strains containing the producer plasmids with genes for LasI, LuxI or RhlI activate the receiver strain siG0030?
- Analyse crosstalk
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0030
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of sterile filtered overnight supernatant of constitutive AHL producers piG0049(LasI), piG0050(LuxI), piG0051(RhlI):
        - 0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} final supernatant (v/v)

Machines used:

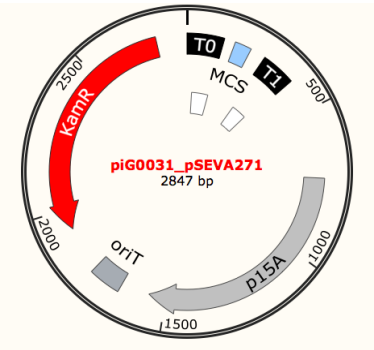
- Tecan infinite M200 PRO

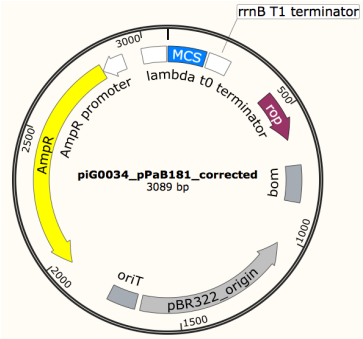
Raw Data:

- Lab/Microtiterplate/crosstalk/20140811_s30_crosstalk_supernatant(too low producer OD).xlsx

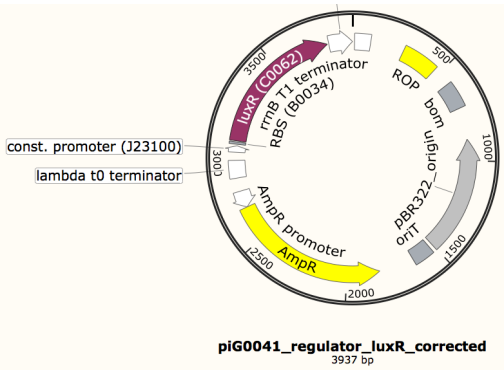
Plasmids in play:

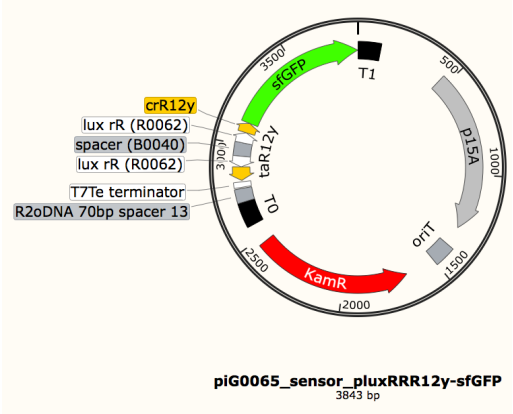
- siG0001: piG0031, piG0034



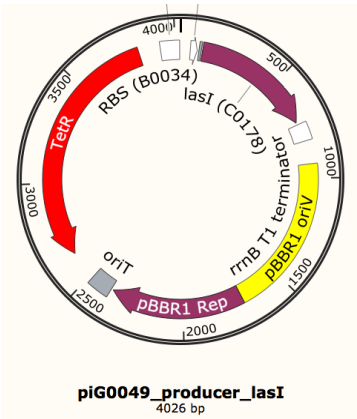


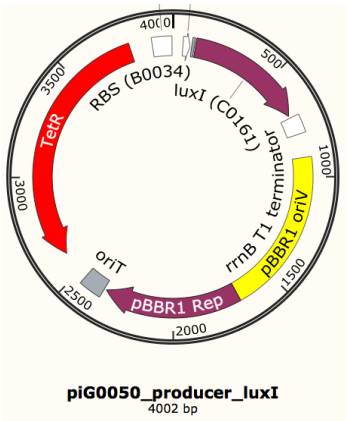
- siG0030: piG0041, piG0065

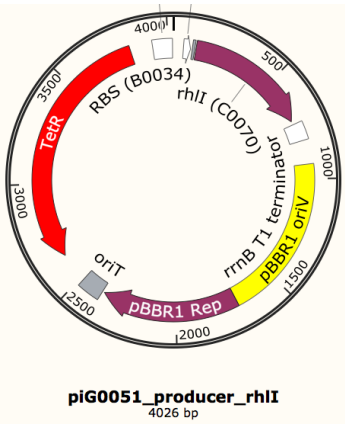




piG0049 (LasI producer) piG0050 (LuxI producer) piG0051 (RhlI producer)







Graphs of Data:

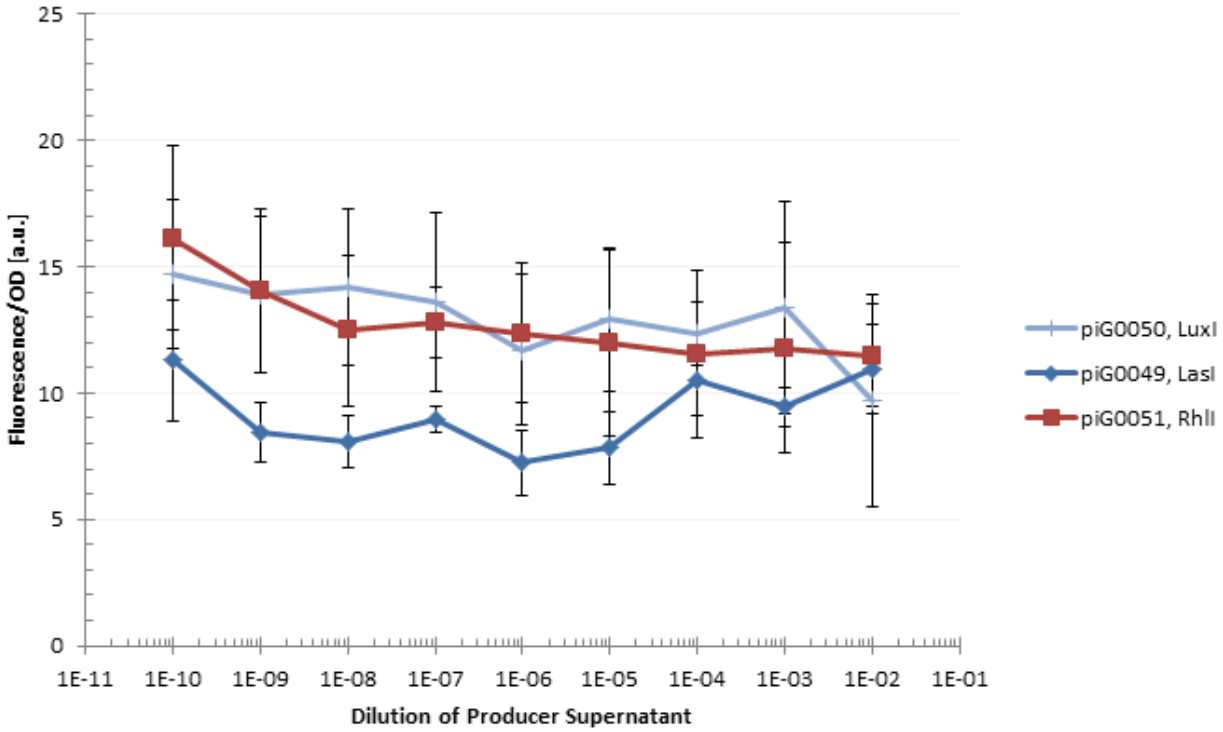


Fig. 1 siG0030 dose-response curve 200 min after induction with the three different producer supernatants

Interpretation of Data:

- concentration in producer supernatant seems to be too low for this experiment
- after overnight cultivation AHL might be degraded
- or overnight culture density was too low (OD600 ~< 1), repeat higher inoculated and measure OD600 before supernatant harvesting

Experiment T10

Dose-Response Kinetics and Crosstalk
siG0014: LasR with sfGFP under plas Promoter and standard RBS - higher resolution
2014-08-12

Goal of the experiment:

- Repeat experiment T05 with higher resolution of concentration steps
- Compare to siG0014 (T05) and siG0030 (T08)
- Is siG0030 crosstalk with 3OC12-HSL close to the siG0014 response?
- Analyse crosstalk in siG0014 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0014
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

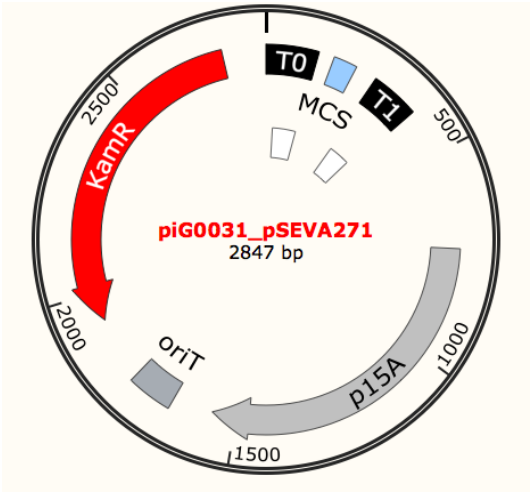
- Tecan infinite M200 PRO

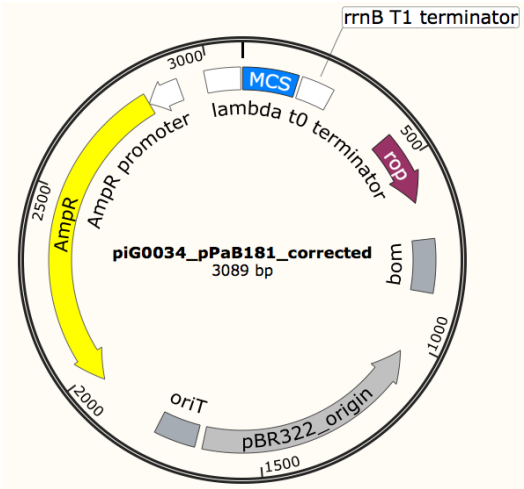
Raw Data:

- Lab/Microtiterplate/crosstalk/20140812_s14_crosstalk.xlsx

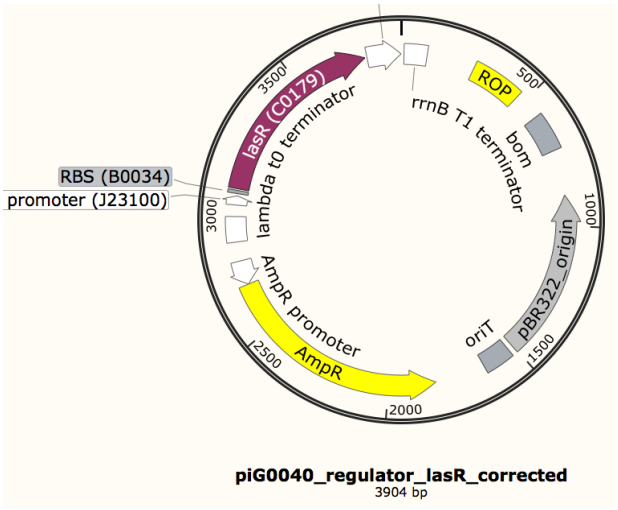
Plasmids in play:

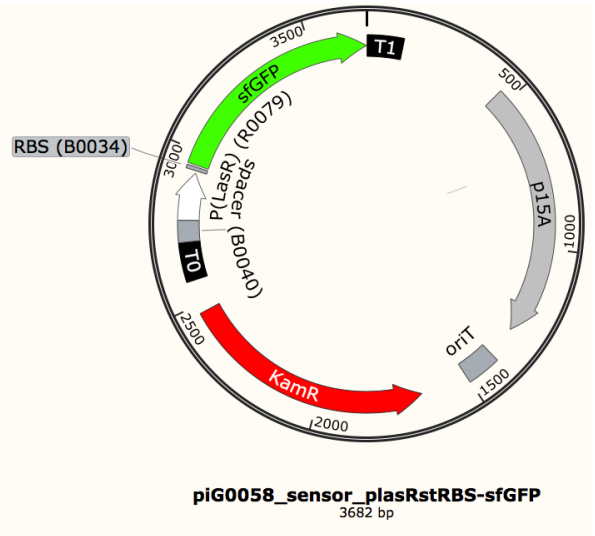
- siG0001: piG0031, piG0034





- siG0014: piG0040, piG0058





Graphs of Data:

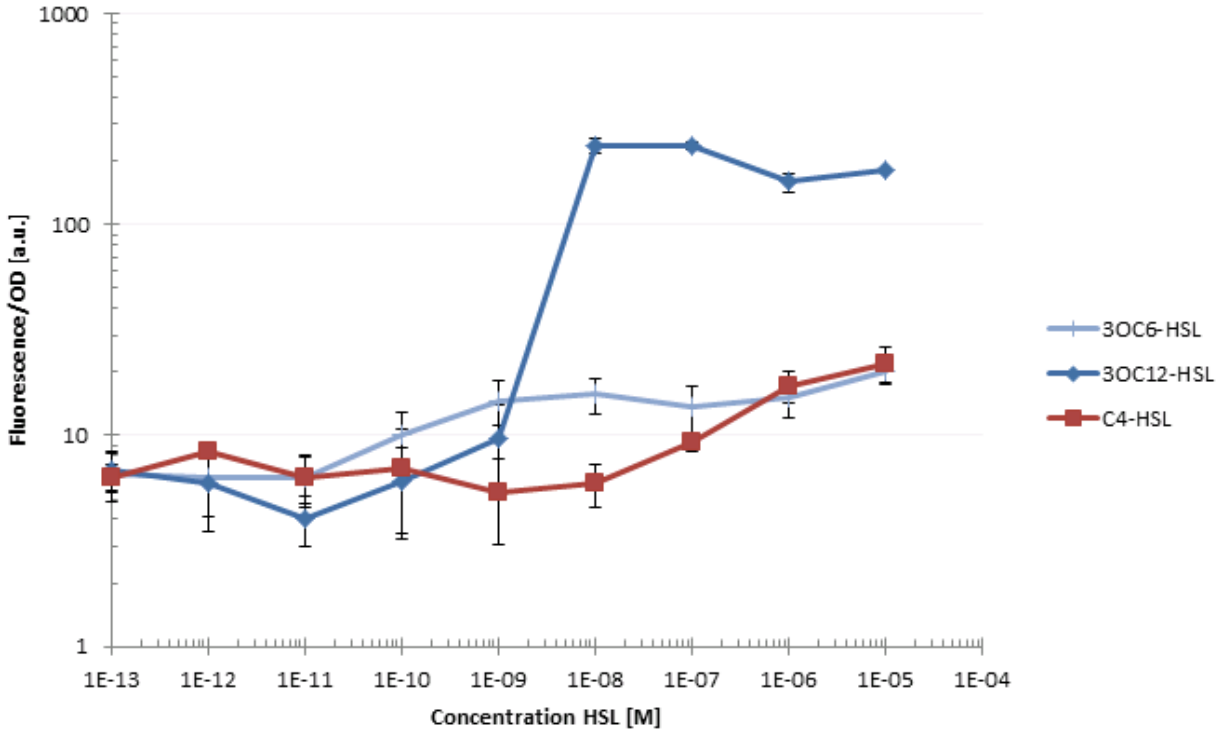


Fig. 1 siG0014 dose-response curve 200 min after induction for three AHL molecules

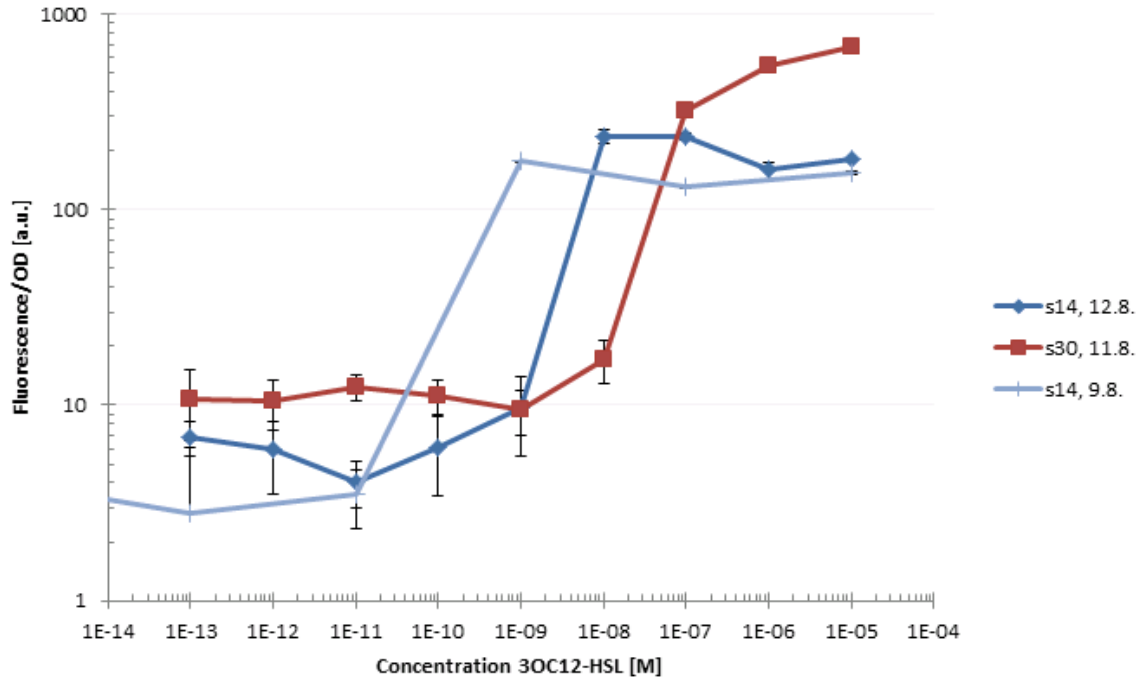


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for siG0014 (las receiver) and siG0030 (lux receiver with RR12y)

Interpretation of Data:

- high variation between the two measurement is likely due to serial dilution errors, 1:100 dilutions compared to 1:10 dilutions (see Fig. 2)
- 3OC12-HSL ON switching point is with 10^{-8} M close to the one from the lux sensor siG0030 at 10^{-7} M (Fig. 2)
- dynamic range for 3OC12-HSL **10^{-11} - 10^{-8} M**
- very weak response to the other two AHLs (see Fig. 1)

Experiment T11

Dose-Response Kinetics and Crosstalk with Producer Supernatants siG0030: LuxR with sfGFP under plux Promoter and Riboregulator 12y - repetition of T09

2014-08-12

Goal of the experiment:

- Repeat experiment T09 since previously the OD600 of the overnight cultures for supernatant harvesting was low (< 1.0), this time the OD600 was ~1.7 for all three
- Analyse crosstalk
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0030
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of sterile filtered overnight supernatant of constitutive AHL producers piG0049(LasI), piG0050(LuxI), piG0051(RhlI):
        - 0, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2} final supernatant (v/v)

Machines used:

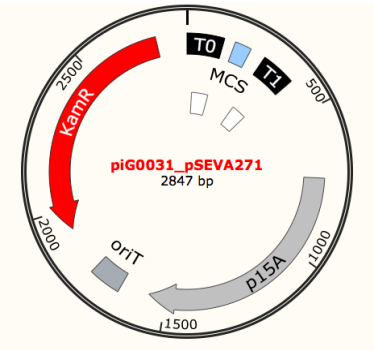
- Tecan infinite M200 PRO

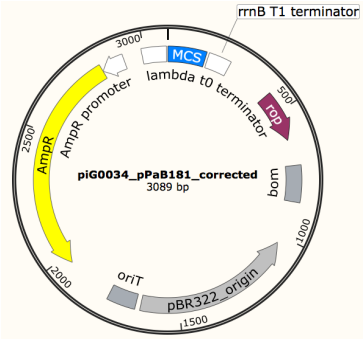
Raw Data:

- Lab/Microtiterplate/crosstalk/20140812_s30_crosstalk_supernatant.xlsx

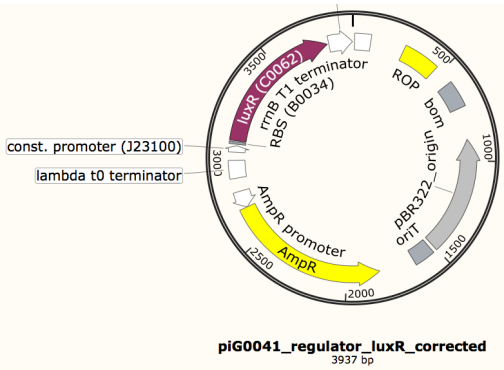
Plasmids in play:

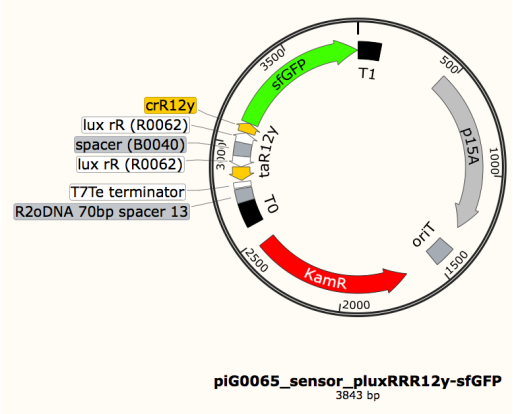
- siG0001: piG0031, piG0034



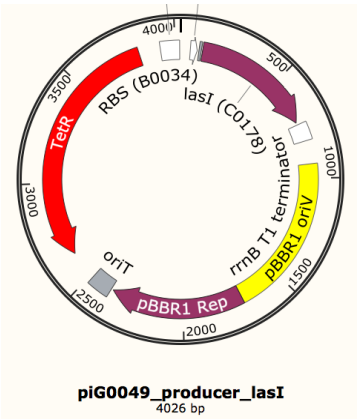


- siG0030: piG0041, piG0065

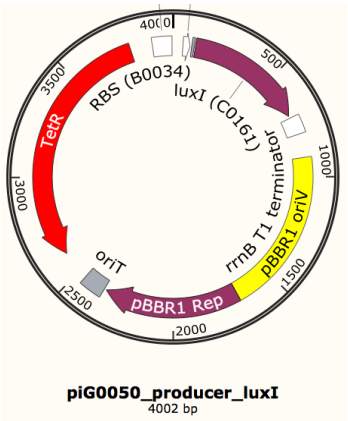




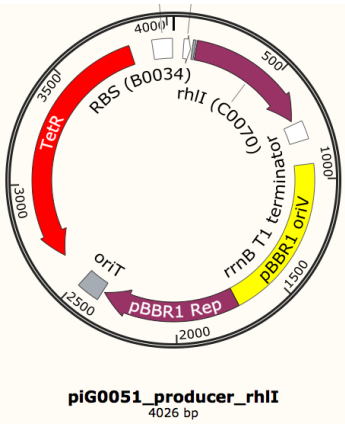
piG0049 (LasI producer)



piG0050 (LuxI producer)



piG0051 (RhlI producer)



Graphs of Data:

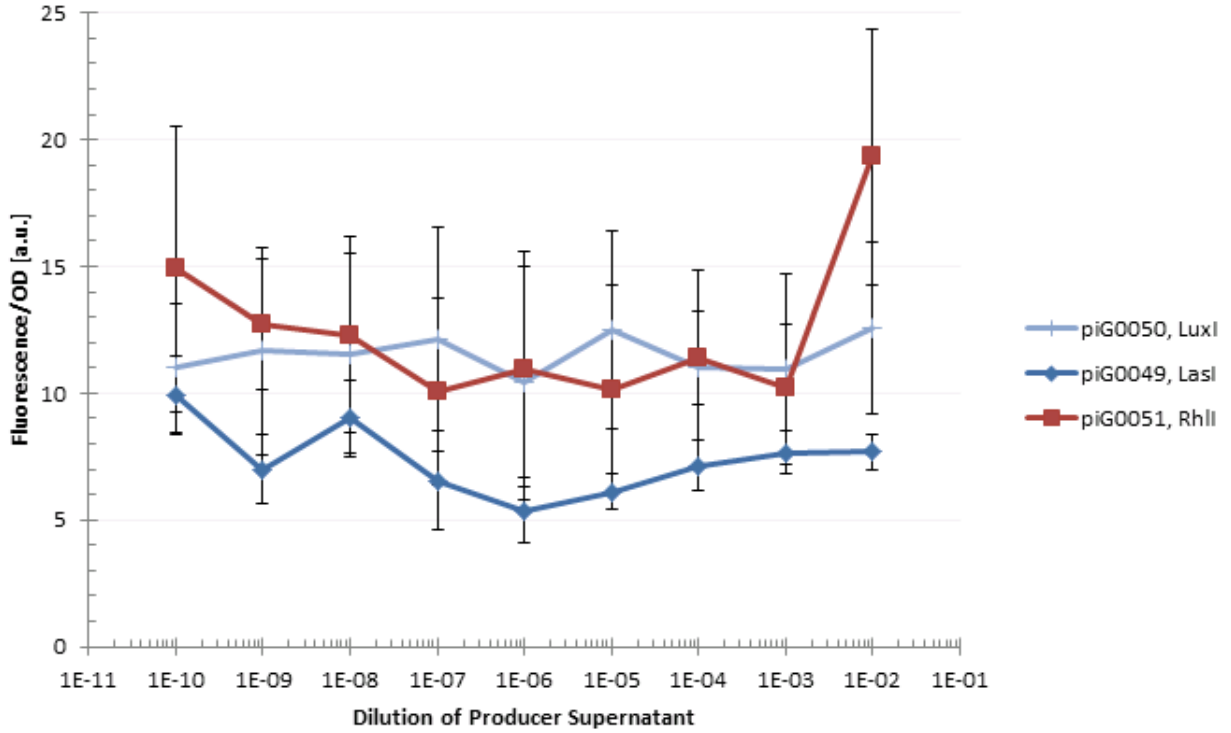


Fig. 1 siG0030 dose-response curve 200 min after induction with the three different producer supernatants

Interpretation of Data:

- concentration in producer supernatant seems still to be too low for this experiment
- after overnight cultivation AHL might be degraded

Experiment T12

Dose-Response Kinetics and Crosstalk
siG0027: LuxR with sfGFP under plux Promoter and cis-repressed RBS 12y 6+2014-08-13

Goal of the experiment:

- Does the cis-repressed RBS without trans-activating RNA show a weaker expression than the full riboregulator system?
- Compare to siG0024 (T03) and siG0030 (T08)
- Analyse crosstalk in siG0027 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0027
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

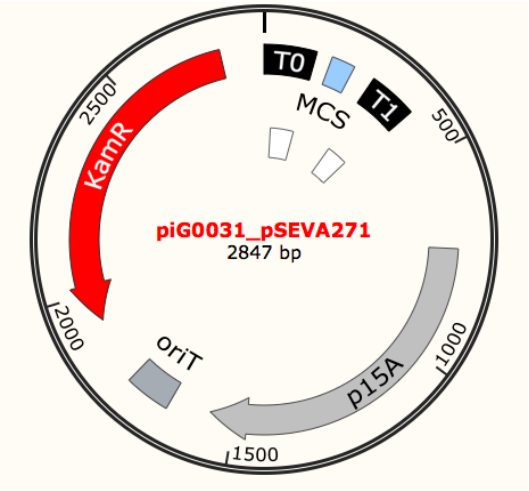
- Tecan infinite M200 PRO

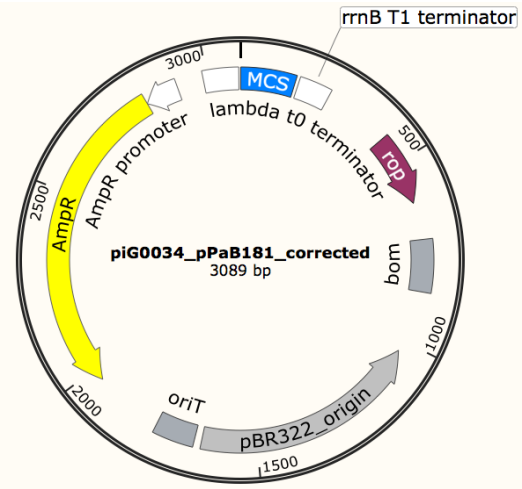
Raw Data:

- Lab/Microtiterplate/crosstalk/20140813_s27_crosstalk.xlsx

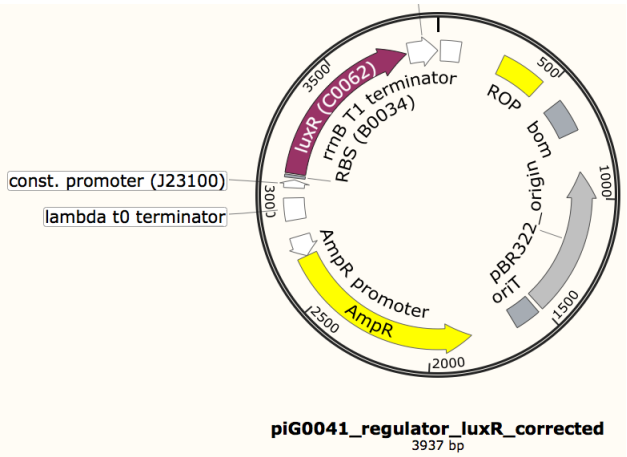
Plasmids in play:

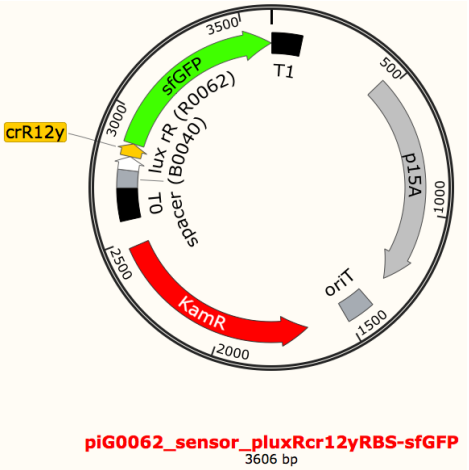
- siG0001: piG0031, piG0034





- siG0027: piG0041, piG0062





Graphs of Data:

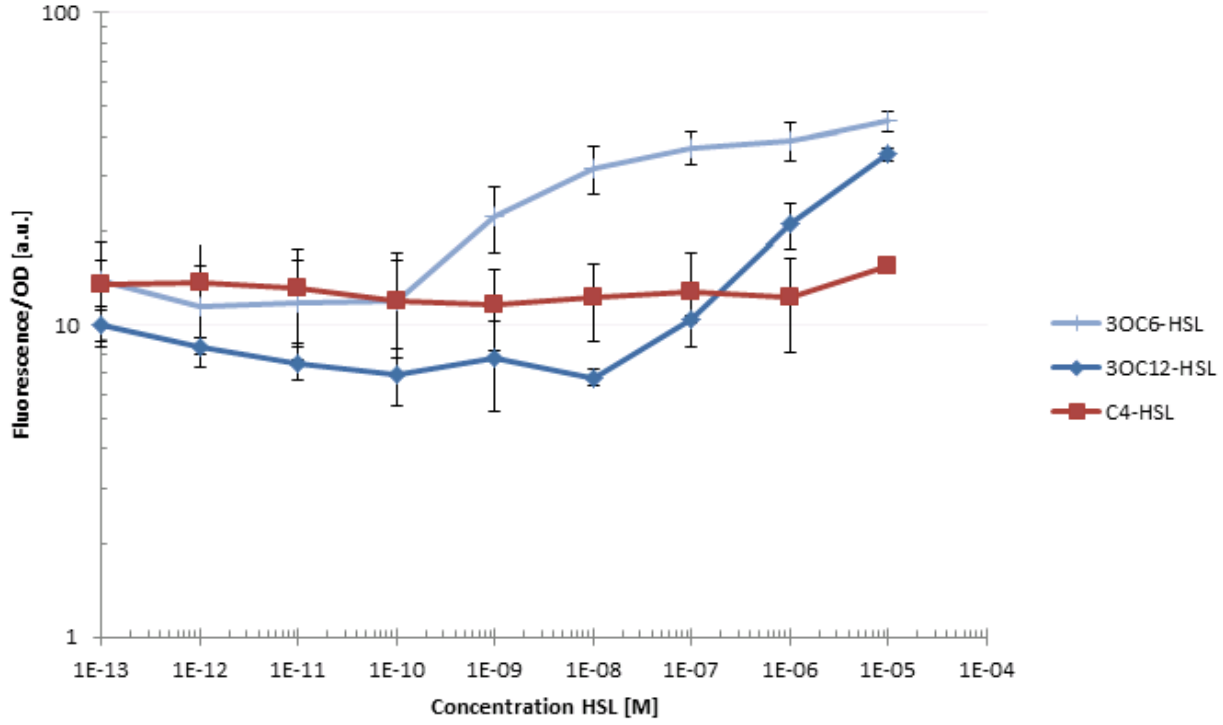


Fig. 1 siG0027 dose-response curve 200 min after induction for three AHL molecules

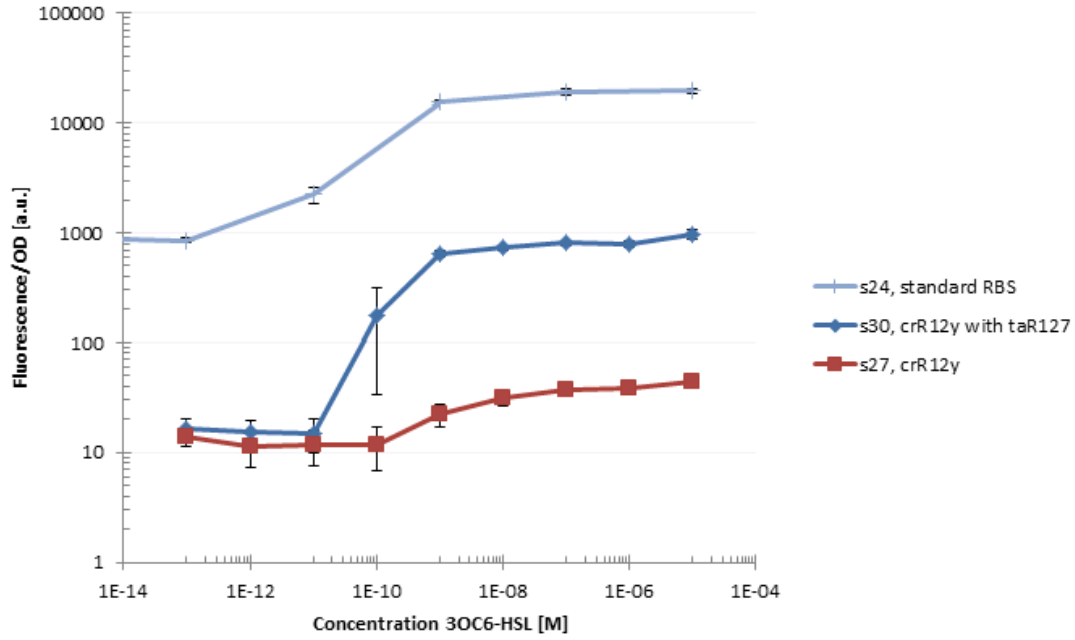


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0024, siG0030, and siG0027

Interpretation of Data:

- without trans-activating RNA the cis-repressed systems shows a ~20 times lower ON level while having the same OFF level (see Fig. 2)
- very weak response to the other two AHLs (see Fig. 1)

Experiment T13

Dose-Response Kinetics and Crosstalk
siG0006: without LuxR, but with sfGFP under plux Promoter and standard RBS
2014-08-14

Goal of the experiment:

- How high is the leakiness without LuxR?
- Determine dose-response curves
- Record dynamic behavior
- Compare to siG0024, siG0042, siG0051

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0006
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

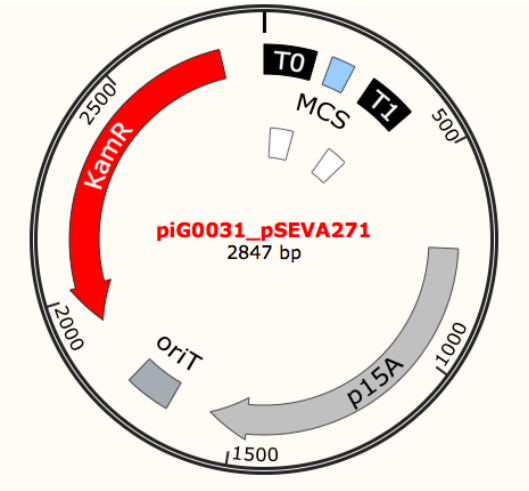
- Tecan infinite M200 PRO

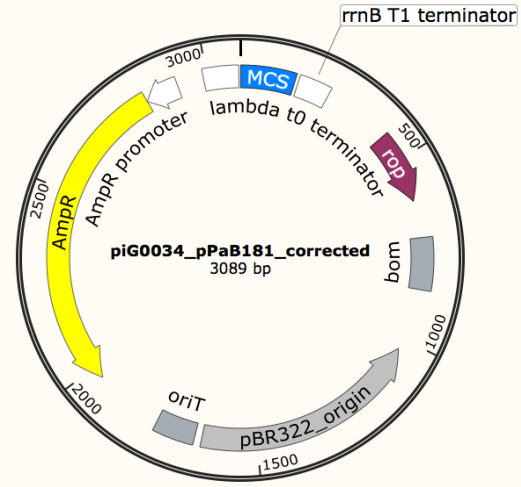
Raw Data:

- Lab/Microtiterplate/crosstalk/20140814_s6_crosstalk.xlsx

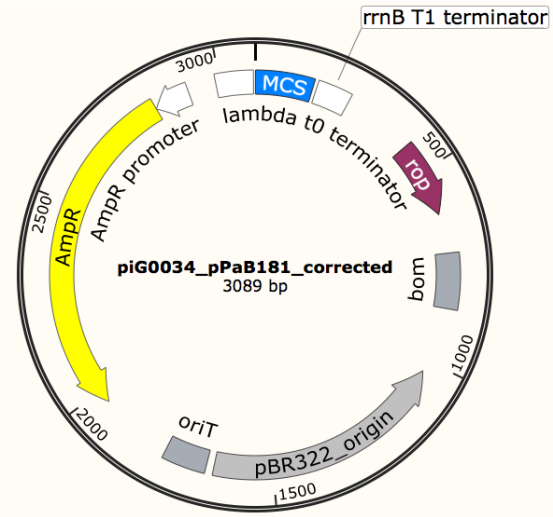
Plasmids in play:

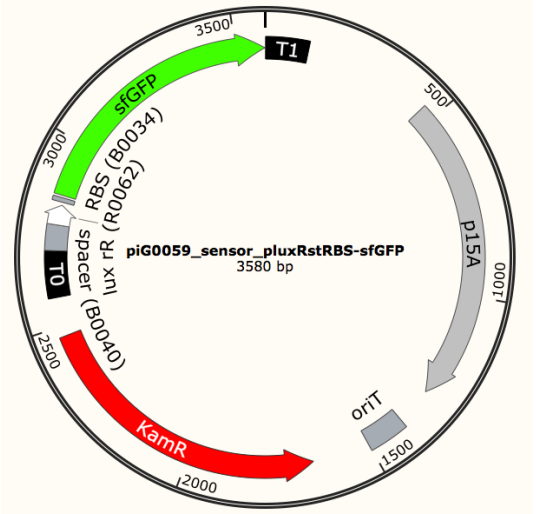
- siG0001: piG0031, piG0034





- siG0006: piG0041, piG0059





Graphs of Data:

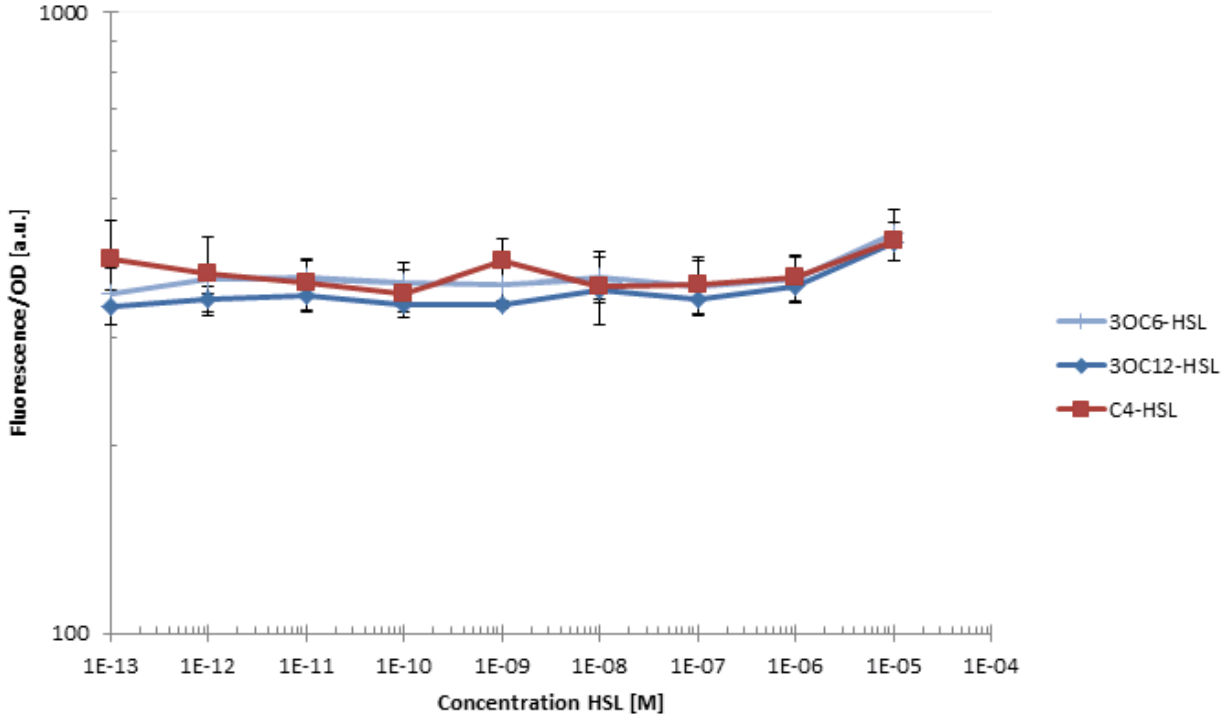


Fig. 1 siG0006 dose-response curve 200 min after induction for three AHL molecules

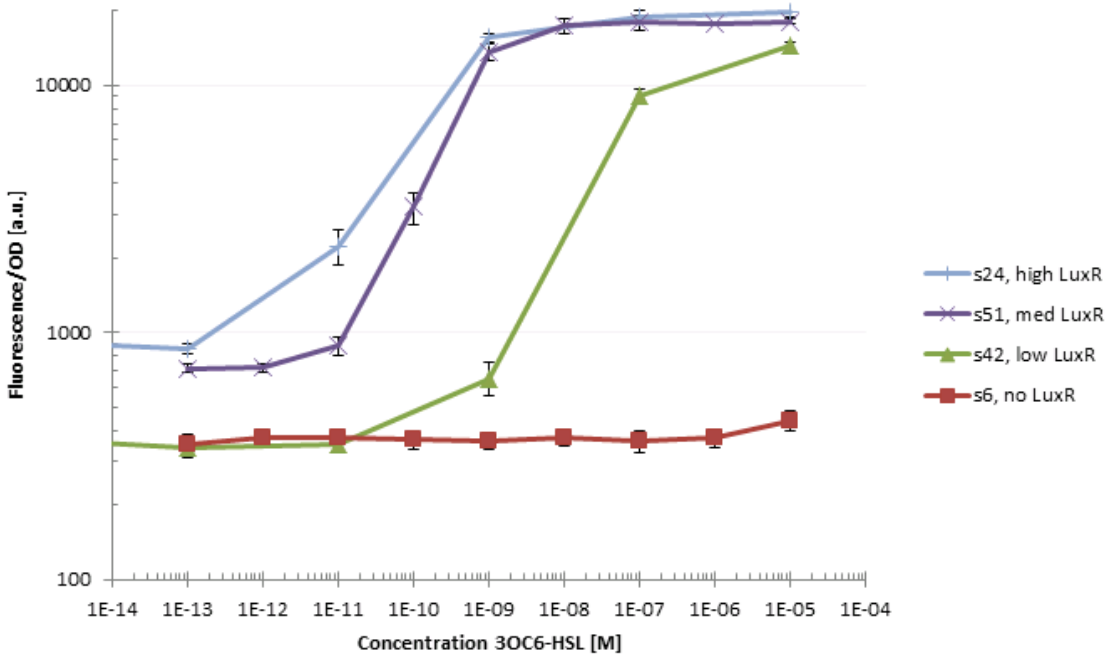


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0006, siG0024, siG0030, siG0042, and siG0051

Interpretation of Data:

- with less LuxR there is less leakiness (see Fig. 2)
- the leakiness limit without LuxR is still comparably high (see experiment T11 fig. 2)

Experiment T14

Dose-Response Kinetics and Crosstalk
siG0048: low LuxR, sfGFP under plux Promoter and riboregulator 12y - repetition of T06
2014-08-14

Goal of the experiment:

- Same experiment as T07, but with riboregulator
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0048
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

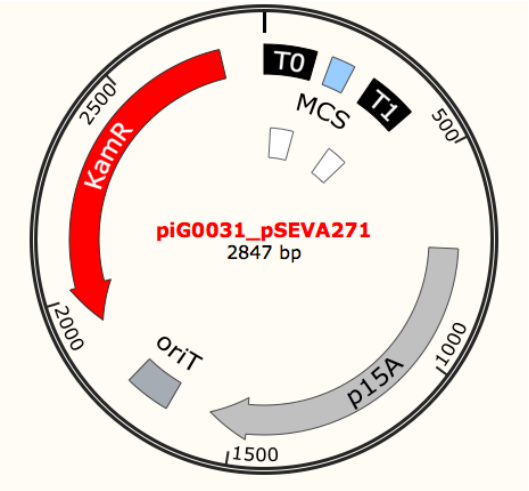
- Tecan infinite M200 PRO

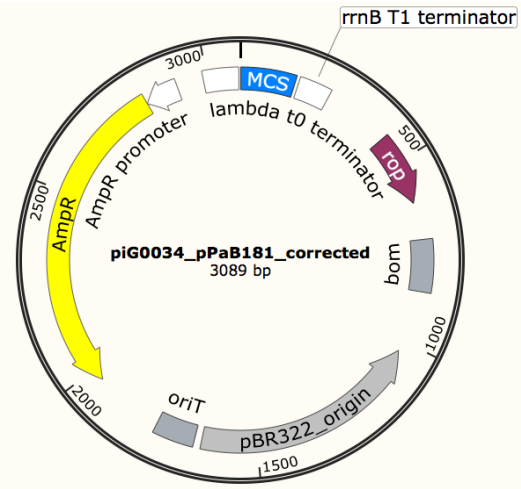
Raw Data:

- Lab/Microtiterplate/crosstalk/20140814_s48_crosstalk.xlsx

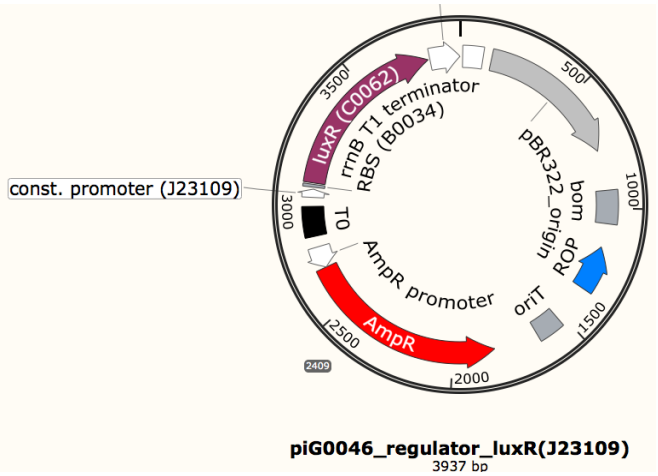
Plasmids in play:

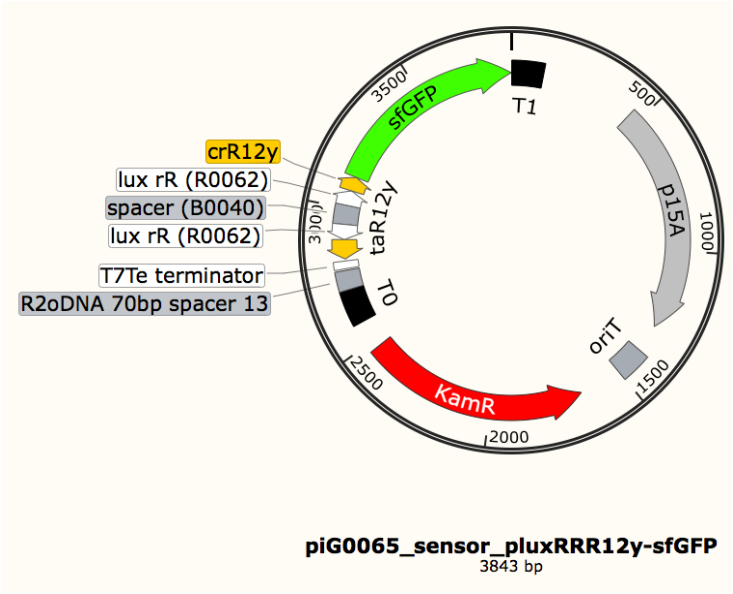
- siG0001: piG0031, piG0034





- siG0048: piG0046, piG0065





Graphs of Data:

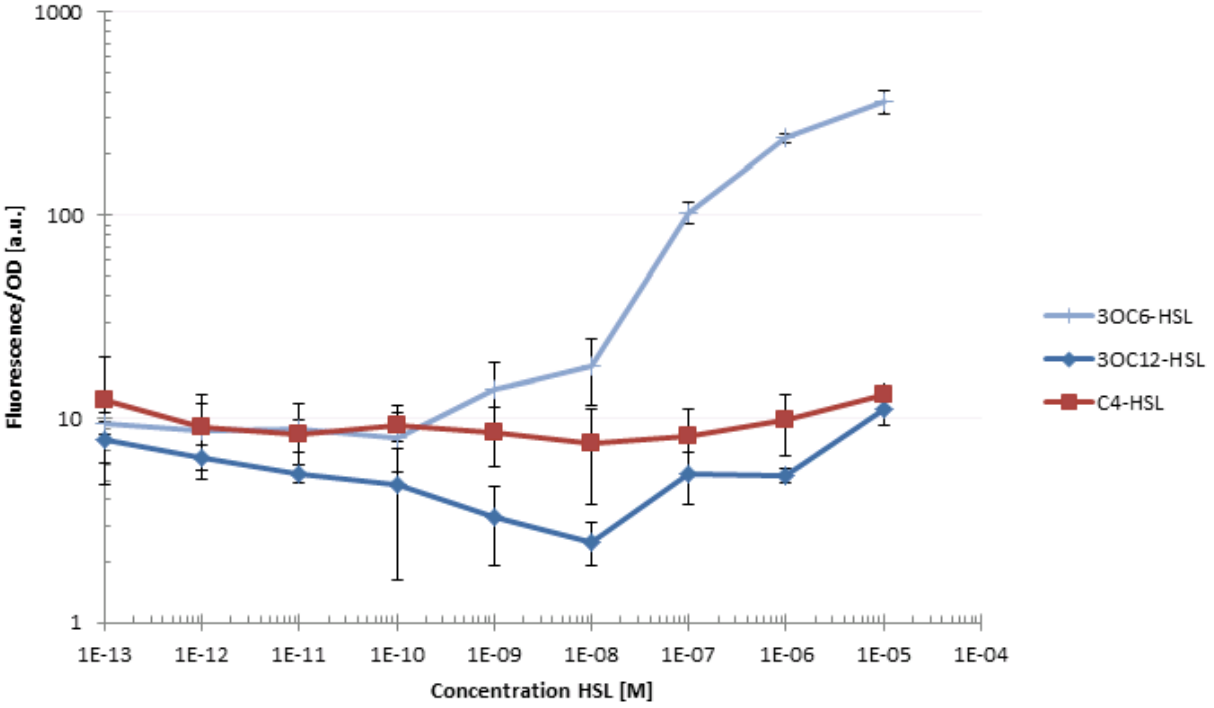


Fig. 1 siG0048 dose-response curve 200 min after induction for three AHL molecules

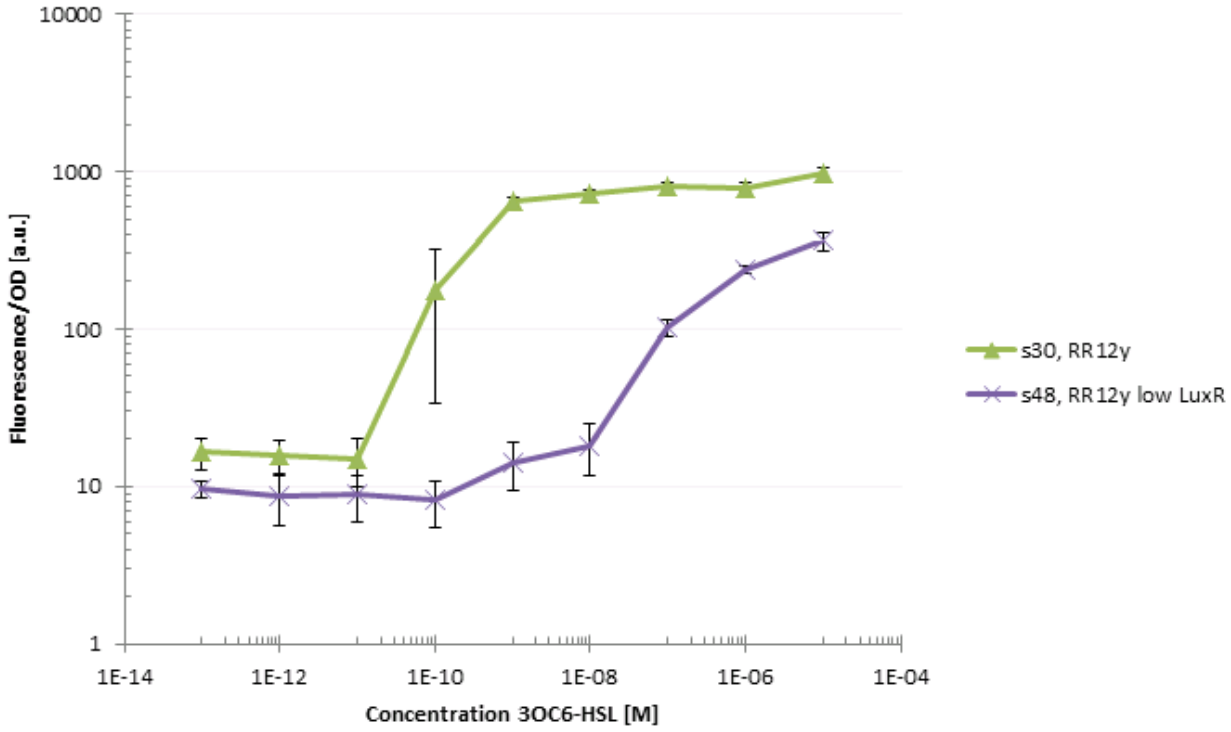


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0048 and siG0030

Interpretation of Data:

- with less LuxR there is a small amount less leakiness (see Fig. 2)
- but the sensitivity decreases, full ON from 10^{-9} M to 10^{-5} M (siG0030 compared to siG0048)
- increased dynamic range
- decreased maximum ON level

Experiment T15

Dose-Response Kinetics and Crosstalk
siG0040: RhlR, sfGFP under prhl Promoter and Riboregulator 12
2014-08-17

Goal of the experiment:

- Analyse crosstalk in siG0040 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0040
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

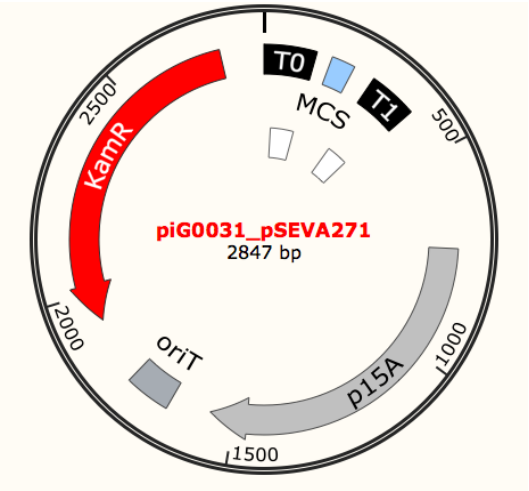
- Tecan infinite M200 PRO

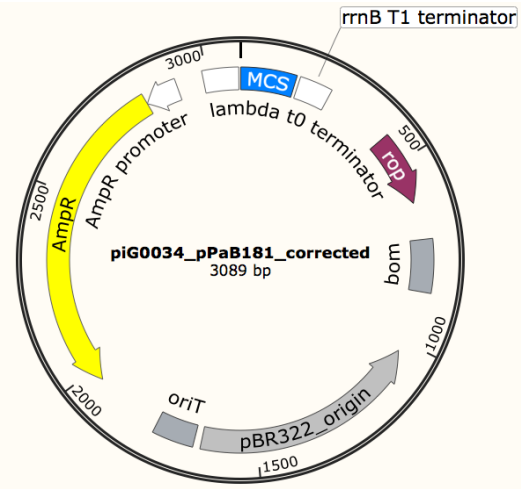
Raw Data:

- Lab/Microtiterplate/crosstalk/20140817_s40_crosstalk.xlsx

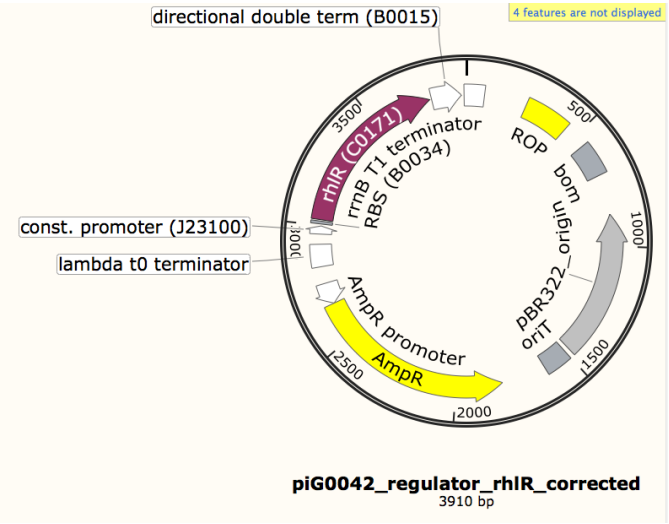
Plasmids in play:

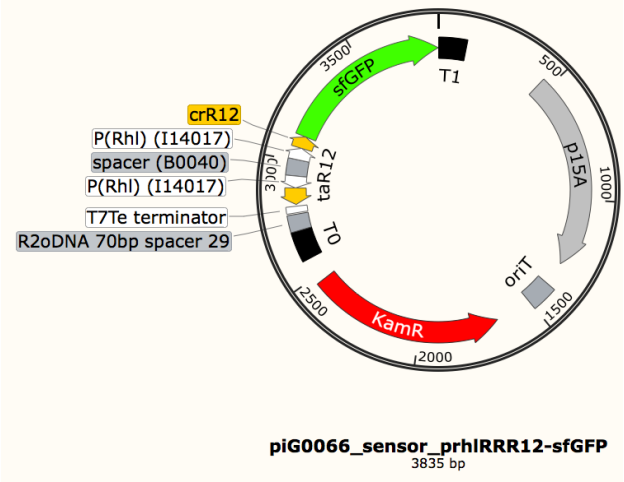
- siG0001: piG0031, piG0034





- siG0040: piG0042, piG0066





Graphs of Data:

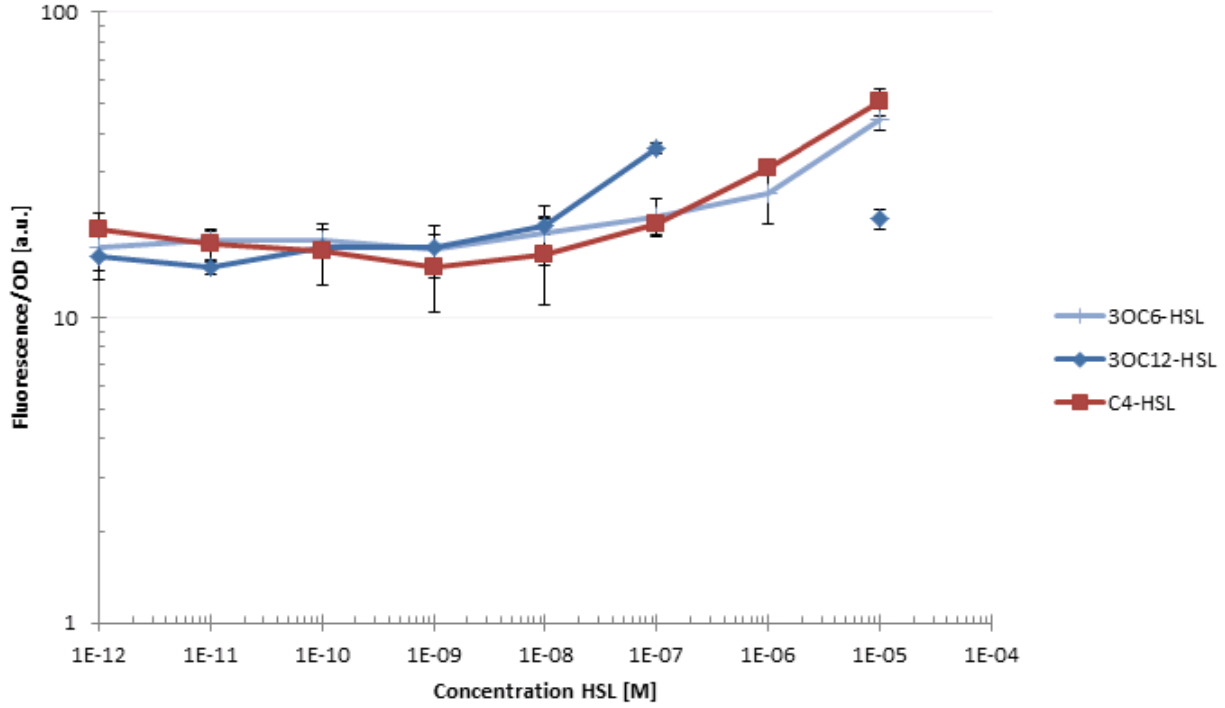


Fig. 1 siG0040 dose-response curve 200 min after induction for three AHL molecules, reference fluorescence of s1 was not subtracted due to unusual high fluorescence of s1

Interpretation of Data:

- very weak response overall
- no clearly specific response for one of the AHLs visible

Experiment T16

Dose-Response Kinetics and Crosstalk
siG0021: LasR, sfGFP under plux Promoter and riboregulator 12y
2014-08-18

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LasR also activate the plux promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0021
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

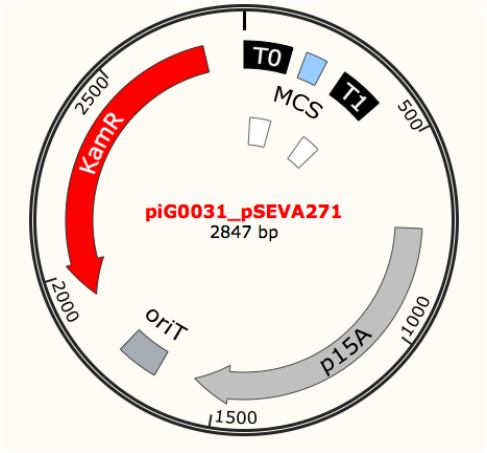
- Tecan infinite M200 PRO

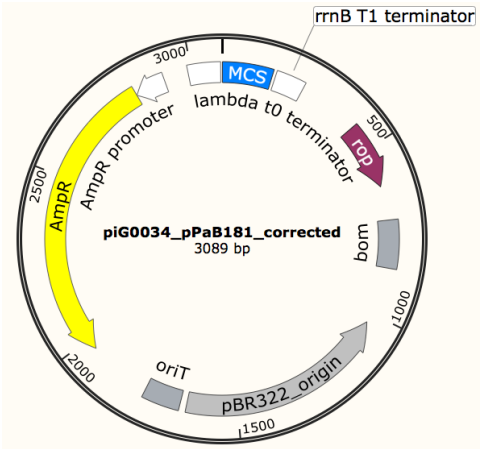
Raw Data:

- Lab/Microtiterplate/crosstalk/20140818_s21_crosstalk.xlsx

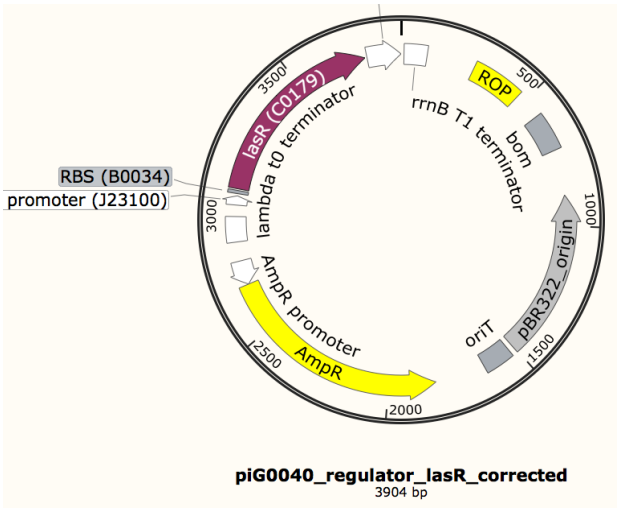
Plasmids in play:

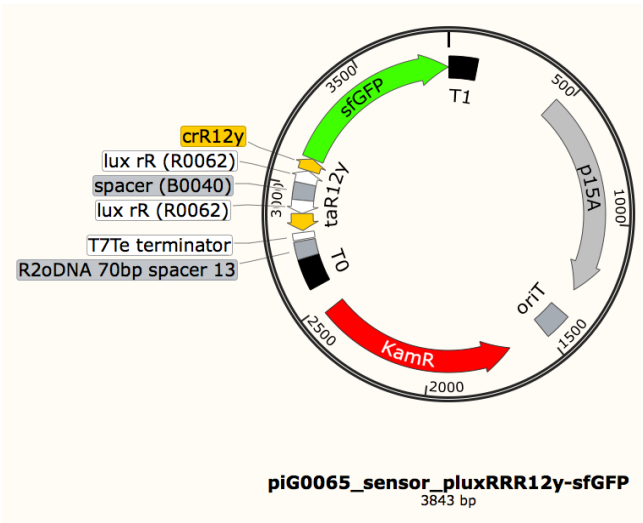
- siG0001: piG0031, piG0034





- siG0021: piG0040, piG0065





Graphs of Data:

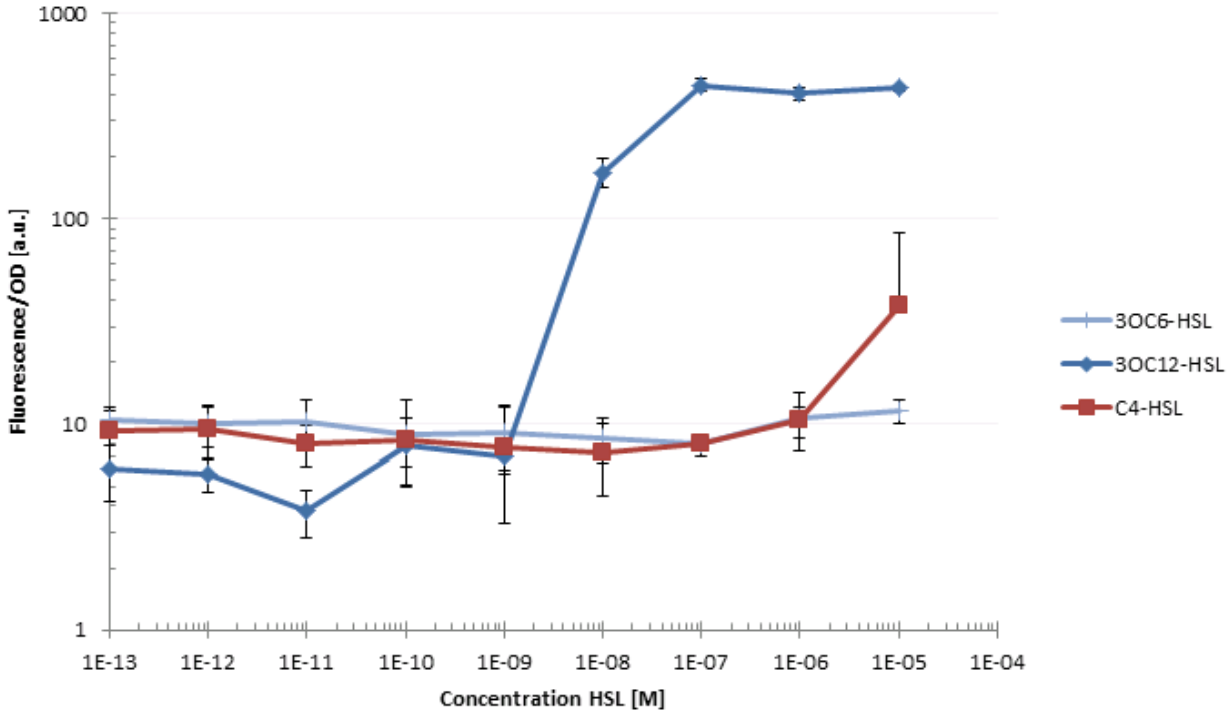


Fig. 1 siG0021 dose-response curve 200 min after induction for three AHL molecules

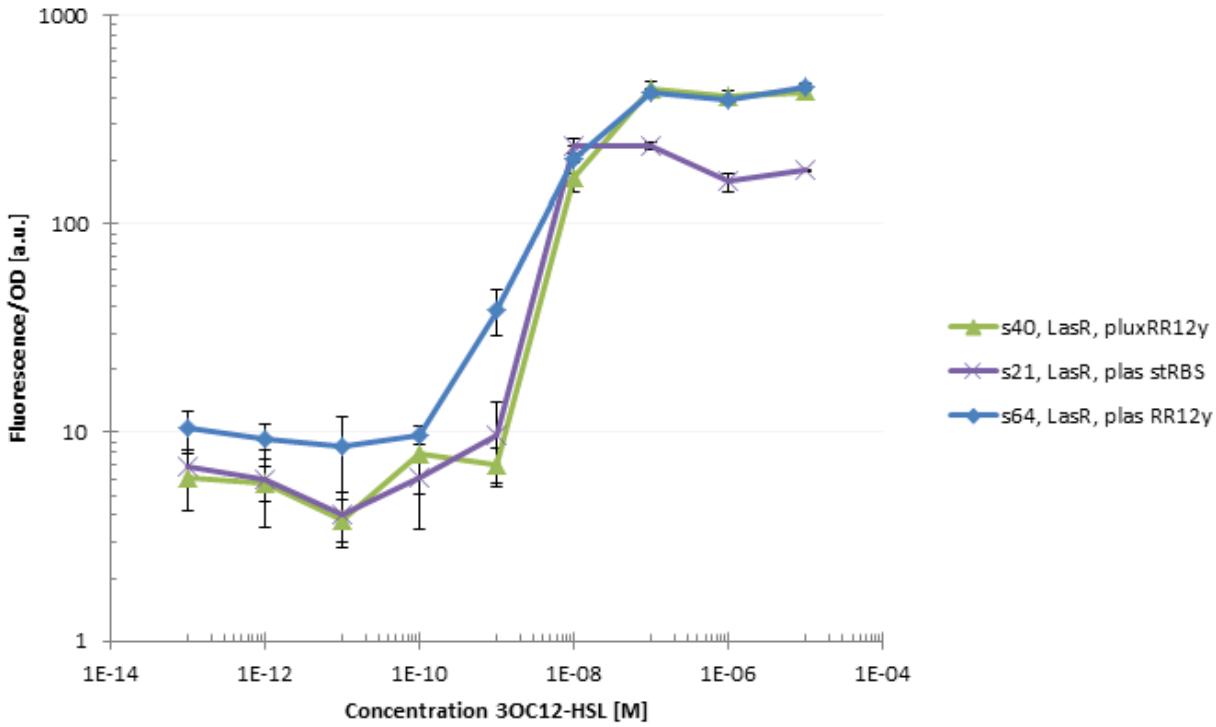


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for siG0040, siG0021 and siG0064

Interpretation of Data:

- with a plux promoter and riboregulator 12y the sensitivity and response curve is very similar to the plas promoter with standard RBS
- LasR can activate both promoters

Experiment T17

Dose-Response Kinetics and Crosstalk
siG0023: LuxR, sfGFP under plas Promoter and standard RBS
2014-08-19

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LuxR also activate the plas promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0023
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

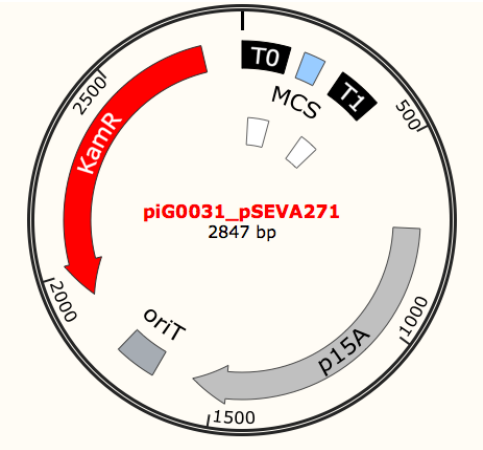
- Tecan infinite M200 PRO

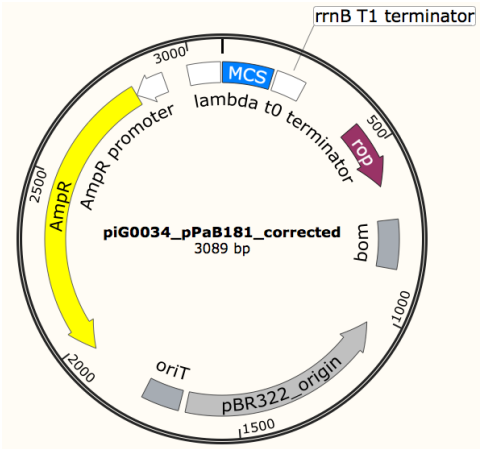
Raw Data:

- Lab/Microtiterplate/crosstalk/20140819_s23_crosstalk.xlsx

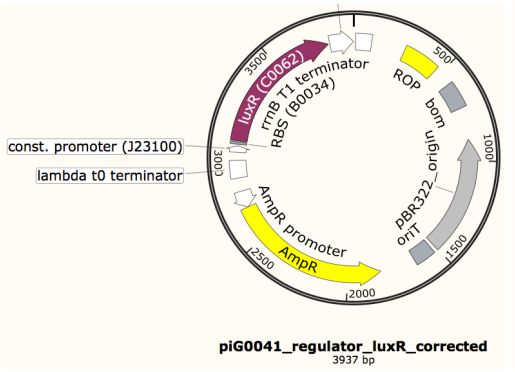
Plasmids in play:

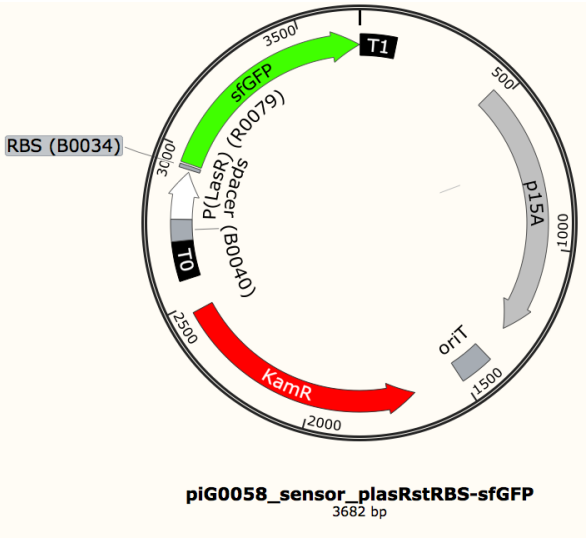
- siG0001: piG0031, piG0034





- siG0023: piG0041, piG0058





Graphs of Data:

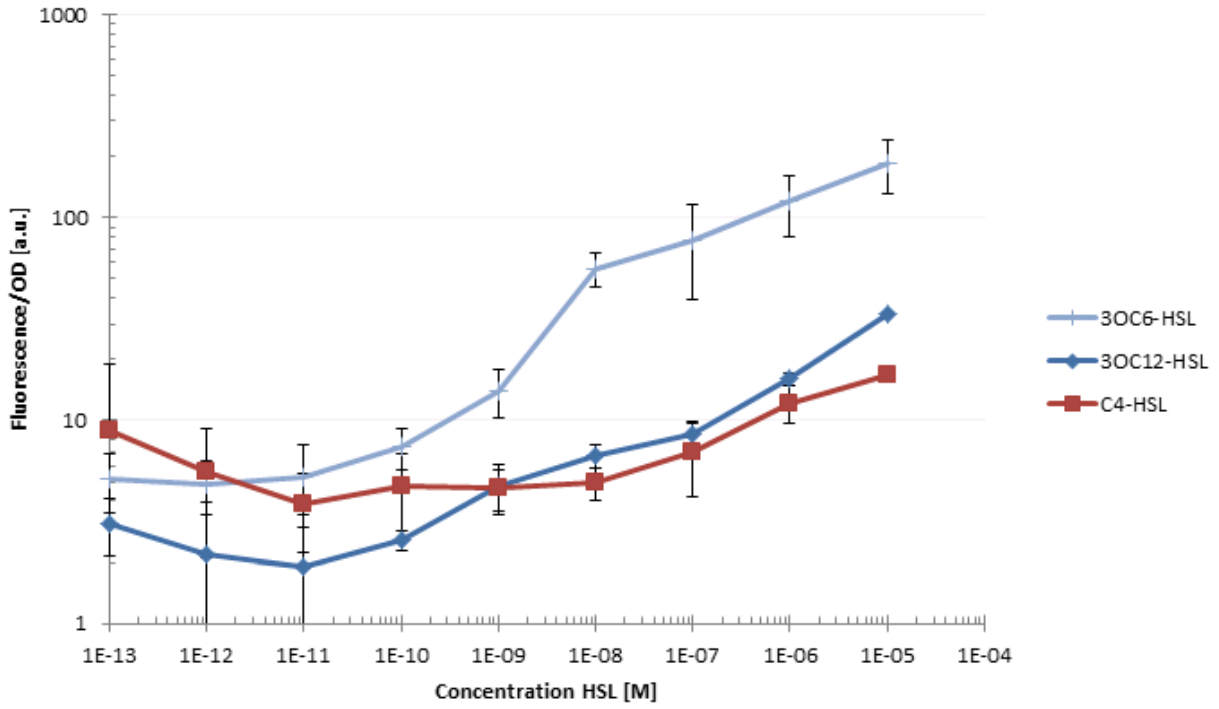


Fig. 1 siG0023 dose-response curve 200 min after induction for three AHL molecules

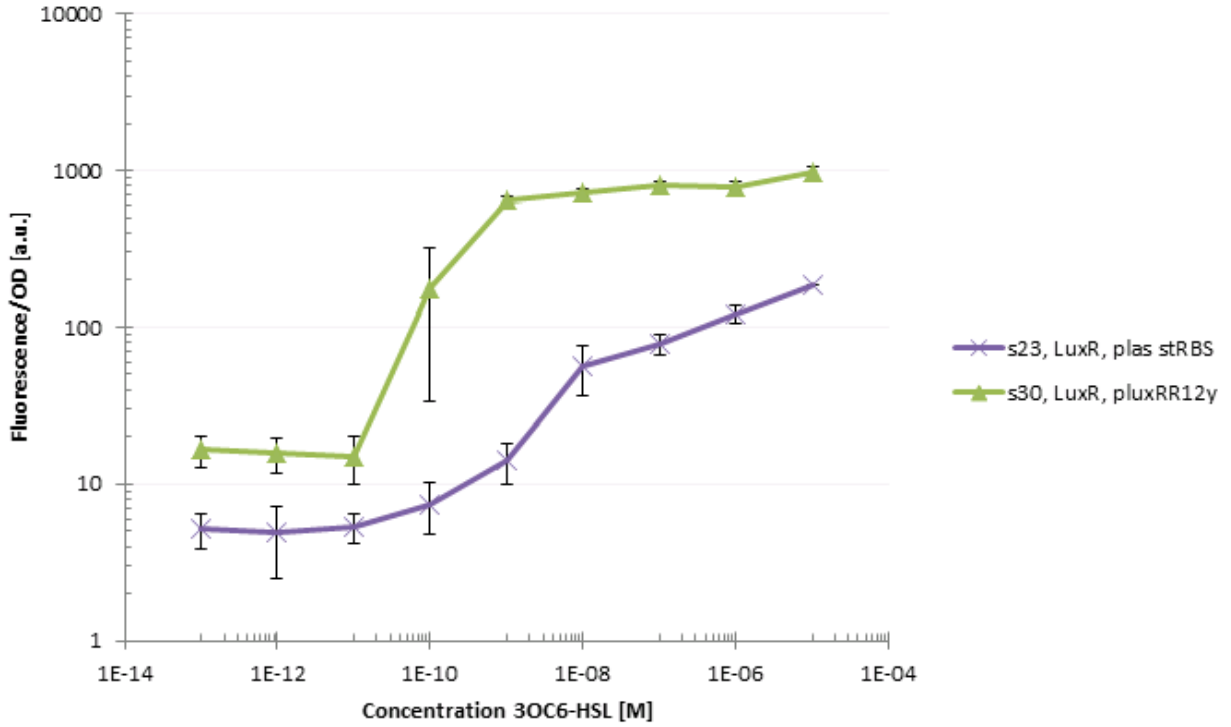


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0023 and siG0030

Interpretation of Data:

- LuxR can activate both promoters, plas and plux
- but with plas the response is shallower (Fig. 2)

Experiment T18

Dose-Response Kinetics and Crosstalk
siG0057: medium LuxR with sfGFP under plux Promoter and Riboregulator 12y
2014-08-19

Goal of the experiment:

- Compare to experiment T07
- Find effects of promoter strength (J23111) controlling LuxR production
- Compare to siG0048 (T06) and siG0030 (T08)
- Analyse crosstalk in siG0057 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0057
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

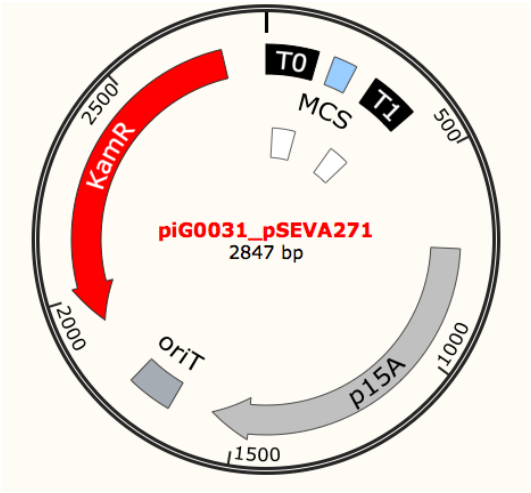
- Tecan infinite M200 PRO

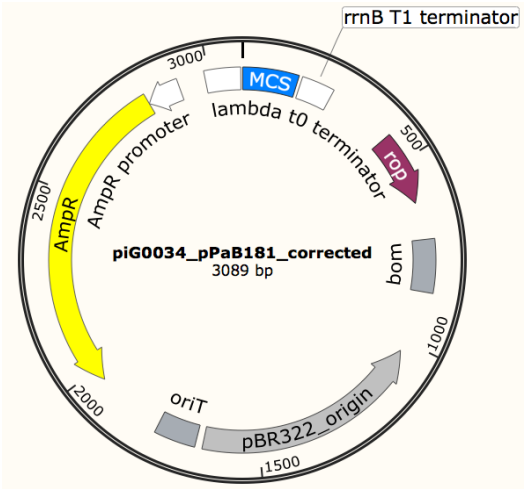
Raw Data:

- Lab/Microtiterplate/crosstalk/20140819_s57_crosstalk.xlsx

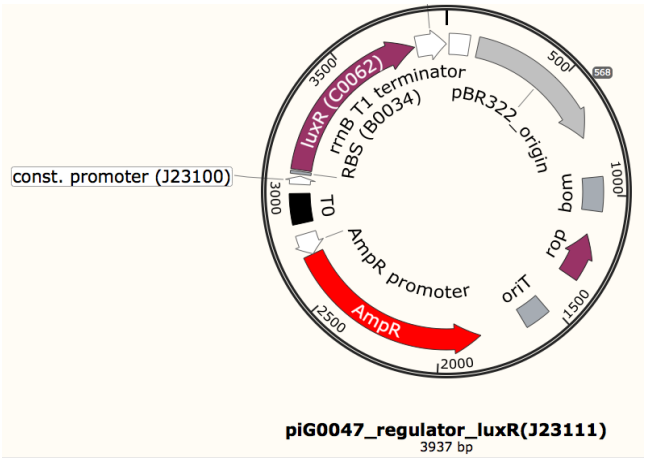
Plasmids in play:

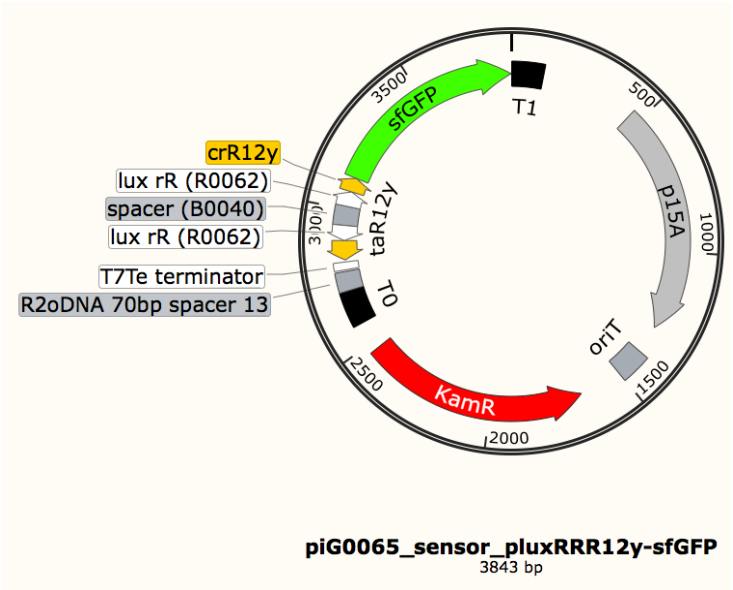
- siG0001: piG0031, piG0034





- siG0057: piG0047, piG0065





Graphs of Data:

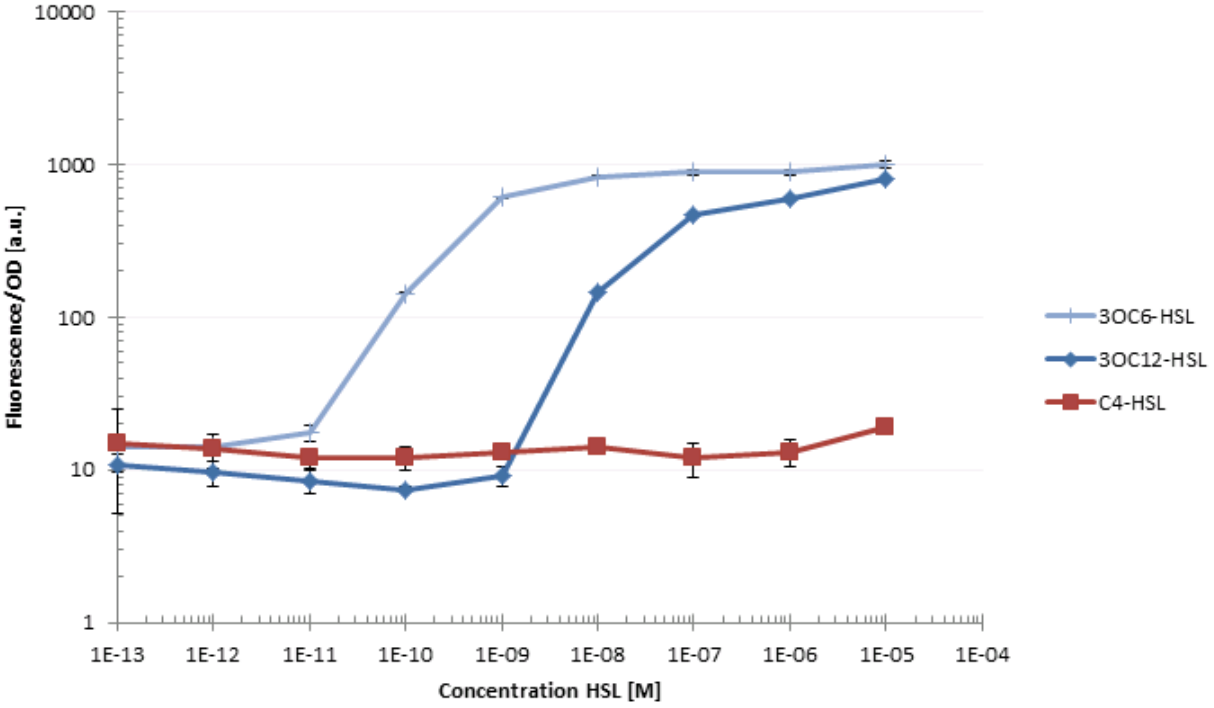


Fig. 1 siG0057 dose-response curve 200 min after induction for three AHL molecules

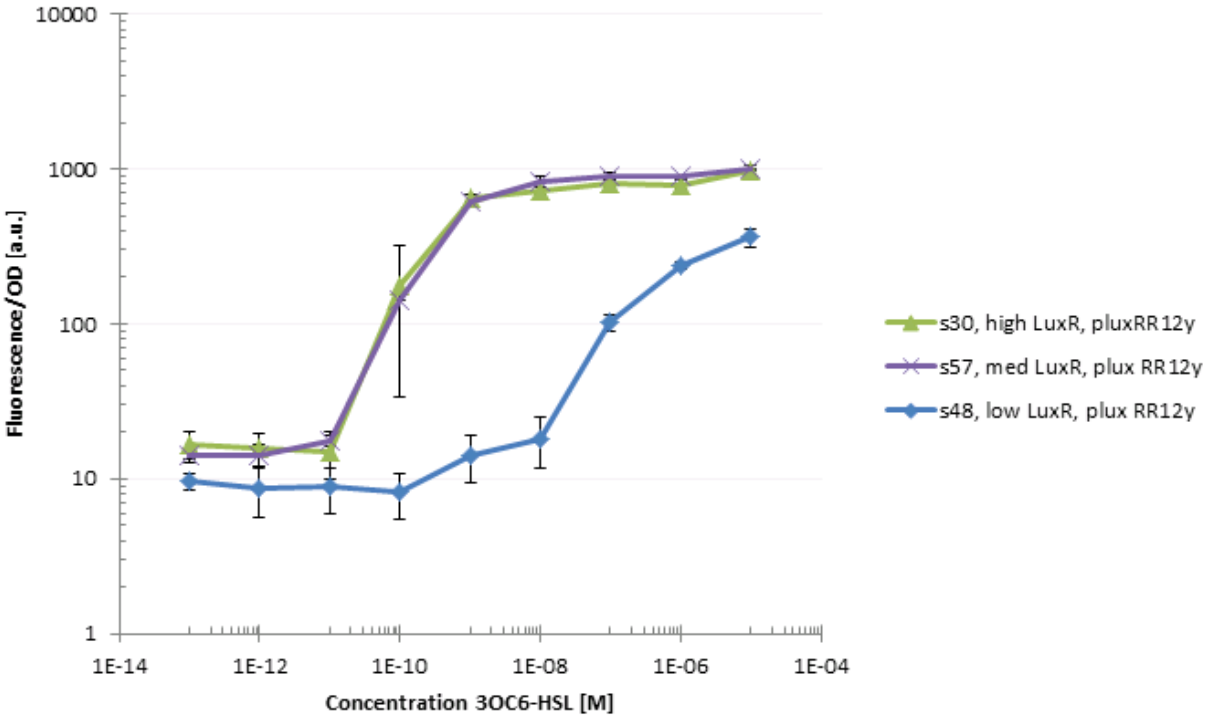


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for the three variants with different promoter strengths for LuxR production

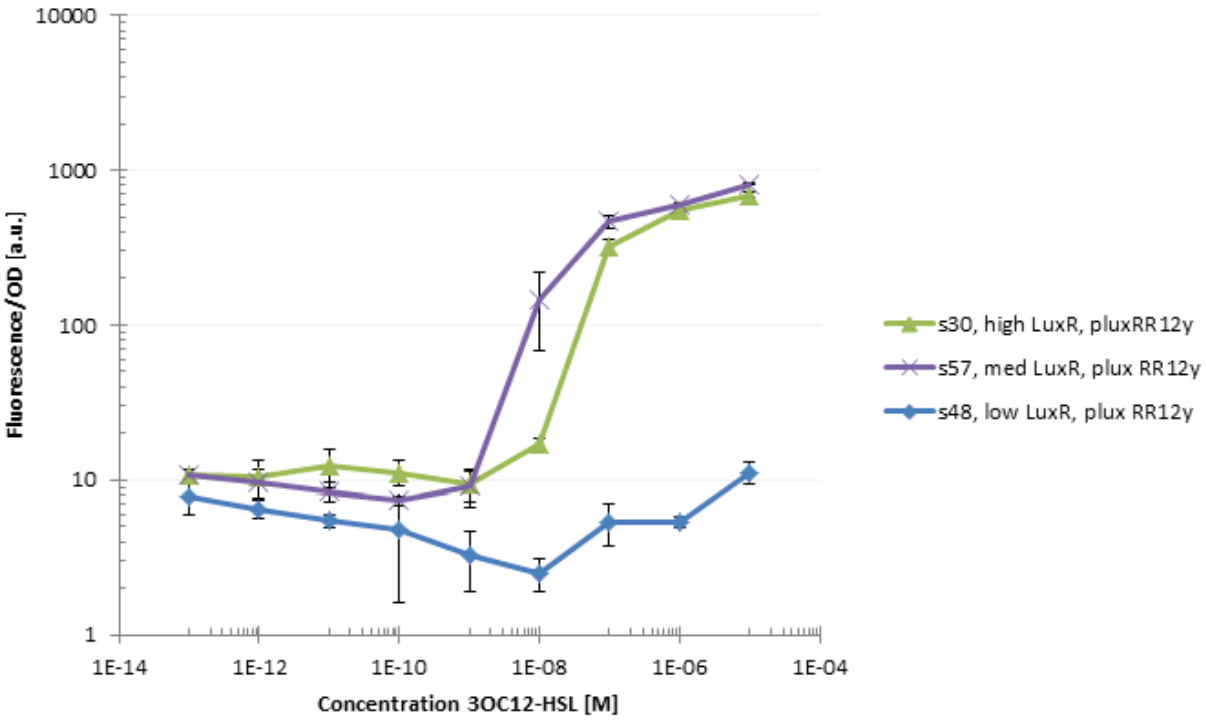


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for the three variants with different promoter strengths for LuxR production

Interpretation of Data:

- influence of LuxR amount on sensitivity to 3OC6-HSL
- with riboregulator the difference in 3OC6-HSL is marginal comparing high and medium LuxR, this is different for the standard RBS see experiment T07
- dynamic range for 3OC6-HSL **10^{-11} - 10^{-8} M**
- dynamic range for 3OC12-HSL **10^{-9} - 10^{-5} M**

Experiment T19

Dose-Response Kinetics and Crosstalk
siG0032: RhlR, sfGFP under plas Promoter and standard RBS
2014-08-20

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does RhlR also activate the plas promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0032
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

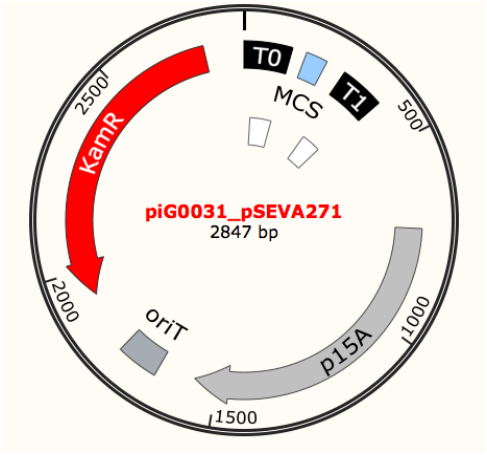
- Tecan infinite M200 PRO

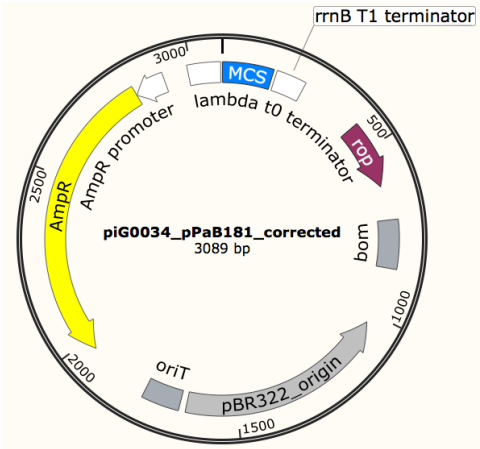
Raw Data:

- Lab/Microtiterplate/crosstalk/20140820_s32_crosstalk.xlsx

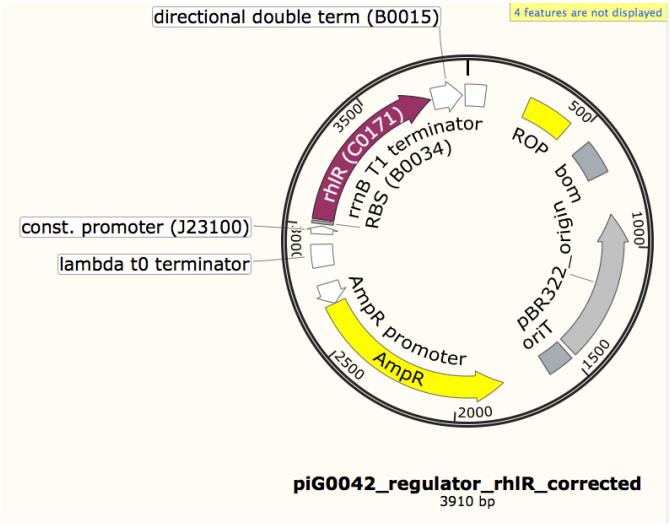
Plasmids in play:

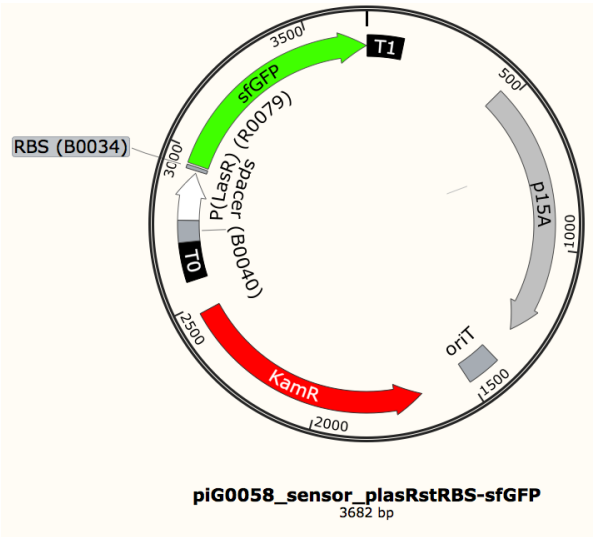
- siG0001: piG0031, piG0034





- siG0032: piG0042, piG0058





Graphs of Data:

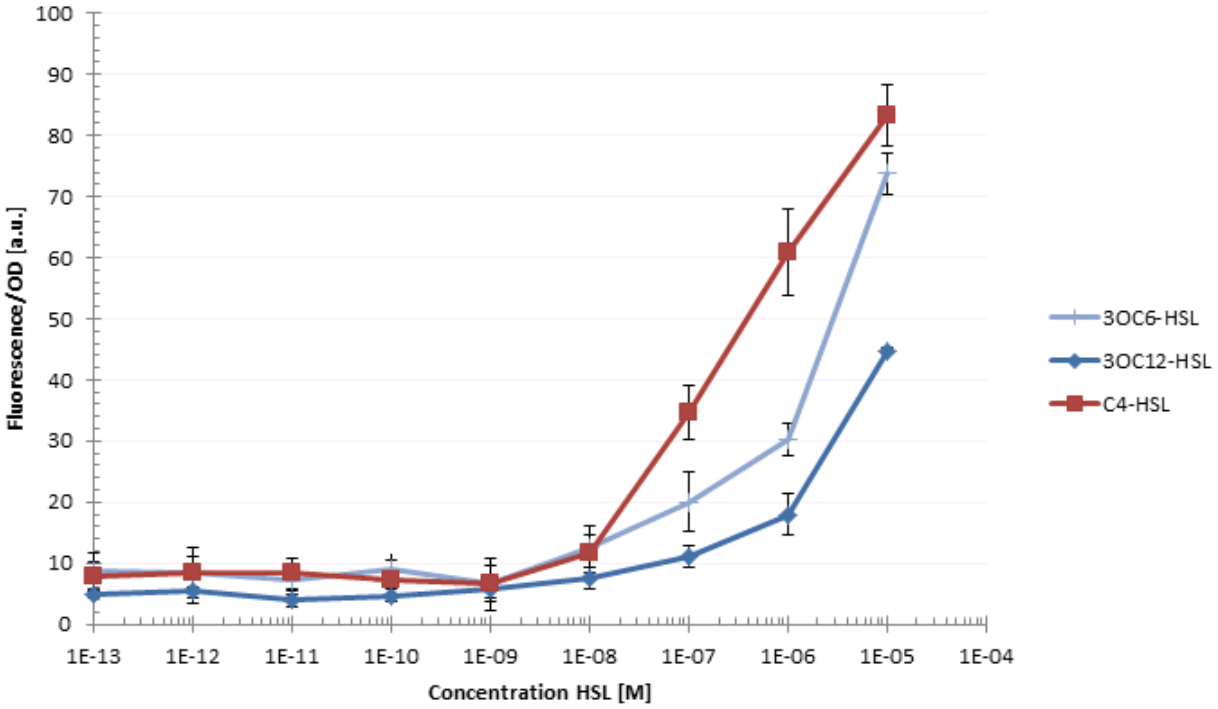


Fig. 1 siG0032 dose-response curve 200 min after induction for three AHL molecules (linear fluorescence scale!)

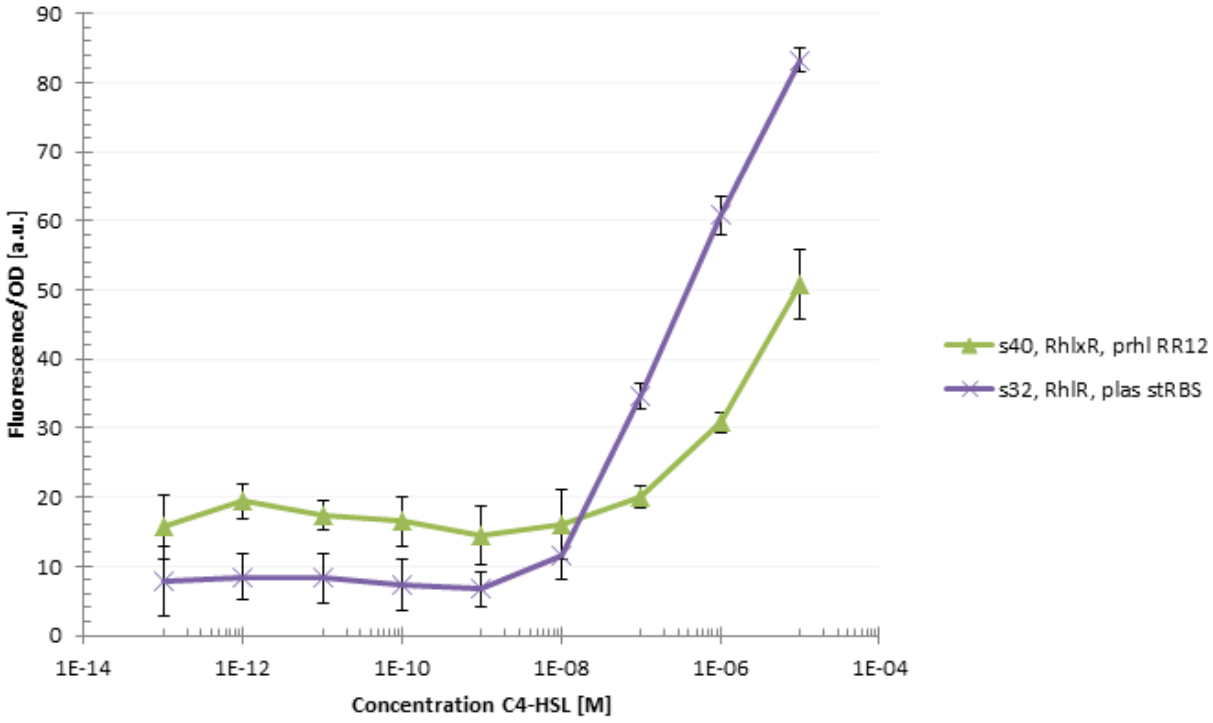


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0040 and siG0032

Interpretation of Data:

- RhlR can activate both promoters, prhl and plas (Fig. 2)
- the activation of plas by RhlR is similar for all three AHLs (Fig. 1)

Experiment T20

Dose-Response Kinetics and Crosstalk
siG0022: LasR, sfGFP under prhl Promoter and Riboregulator RR12
2014-08-21

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LasR also activate the prhl promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0022
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

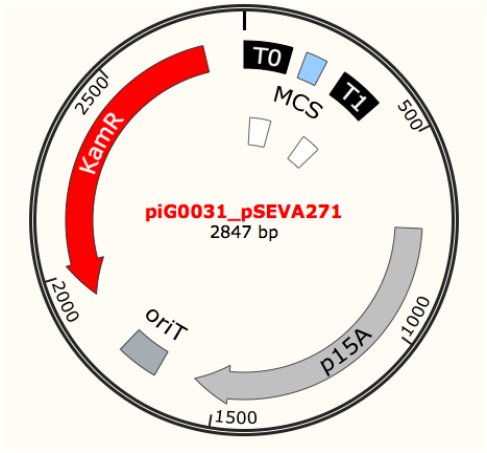
- Tecan infinite M200 PRO

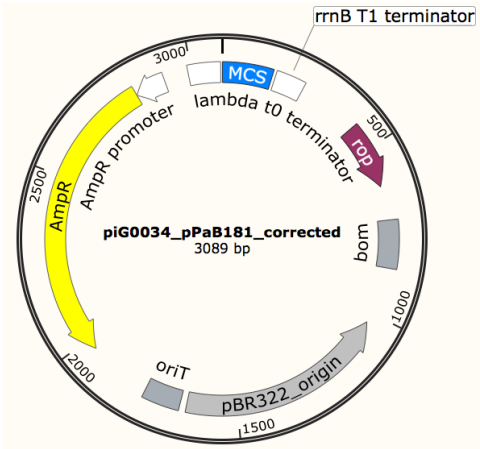
Raw Data:

- Lab/Microtiterplate/crosstalk/20140821_s22_crosstalk.xlsx

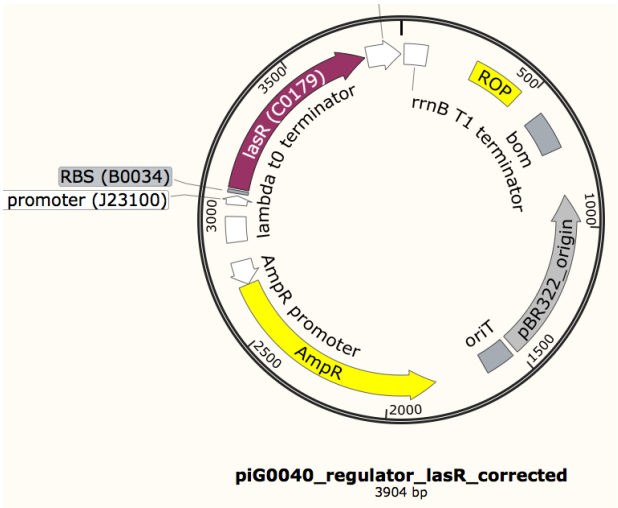
Plasmids in play:

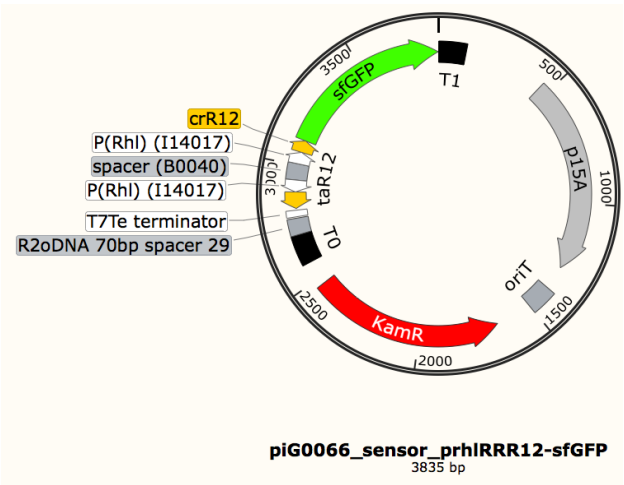
- siG0001: piG0031, piG0034





- siG0022: piG0040, piG0066





Graphs of Data:

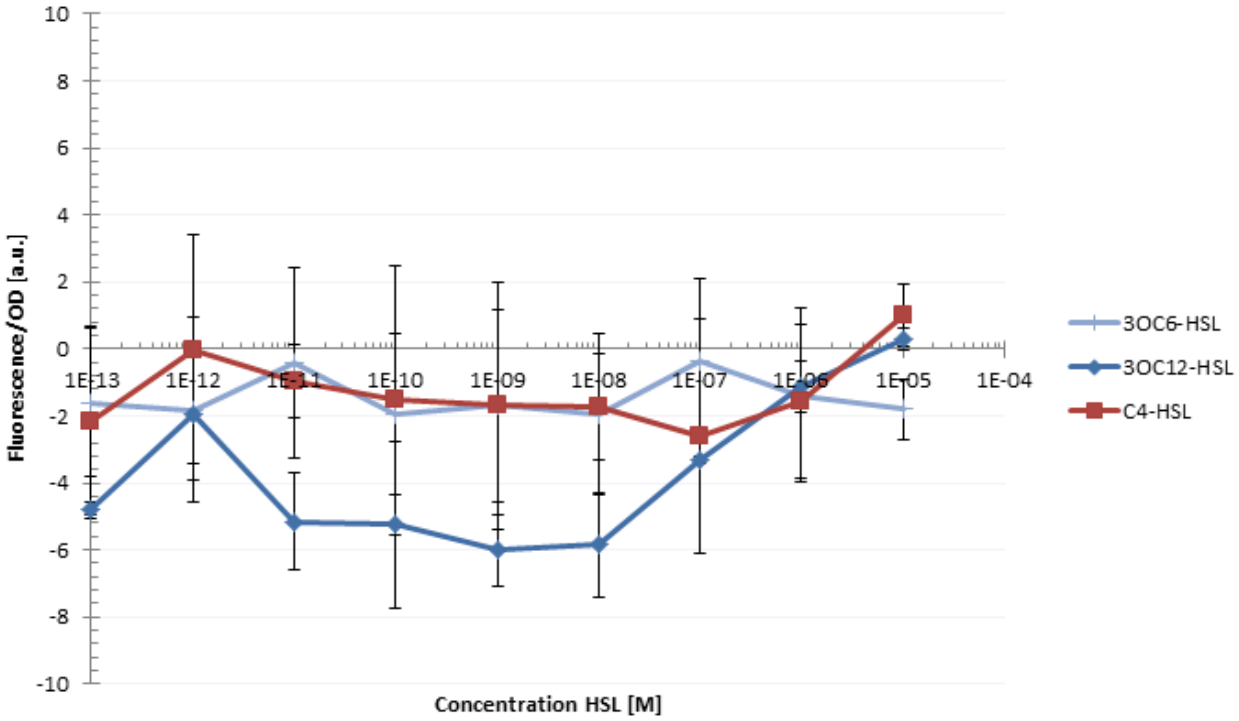


Fig. 1 siG0022 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- LasR **cannot** activate prhl and plas when RR12 is used (Fig. 1)
- the fluorescence does not exceed background noise

Experiment T21

Dose-Response Kinetics and Crosstalk
siG0065: optimized RBS RhlR, sfGFP under prhl Promoter and Riboregulator 12
2014-09-01

Goal of the experiment:

- Compare to experiment T15 (siG0040)
- Find effects of RBS (higher TIR) controlling RhlR production
- Analyse crosstalk in siG0065 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0065
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

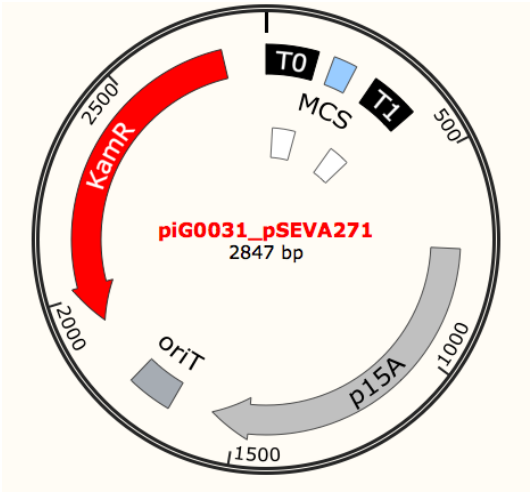
- Tecan infinite M200 PRO

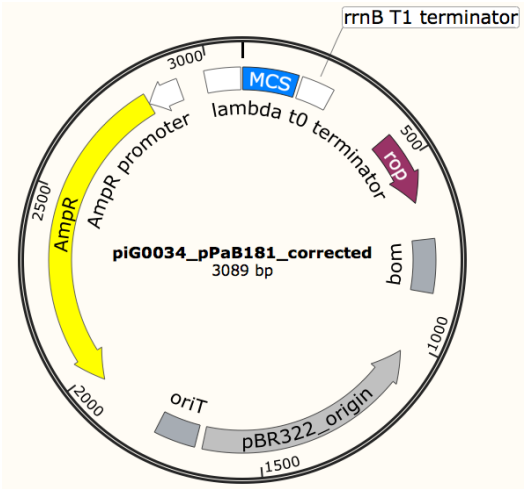
Raw Data:

- Lab/Microtiterplate/crosstalk/20140901_s65_crosstalk.xlsx

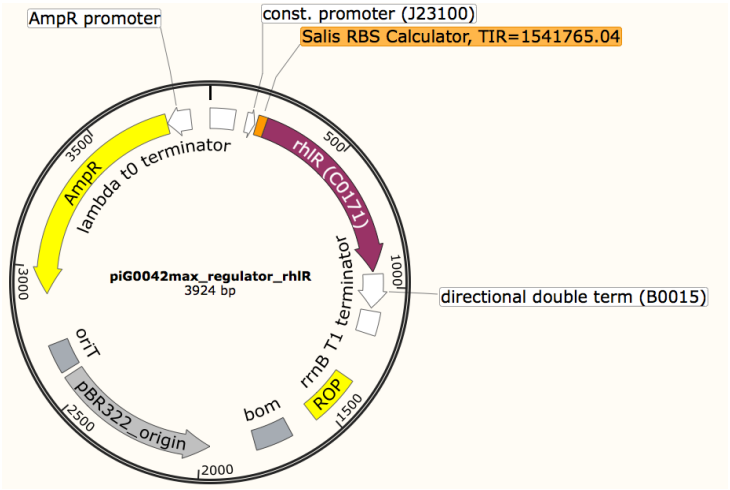
Plasmids in play:

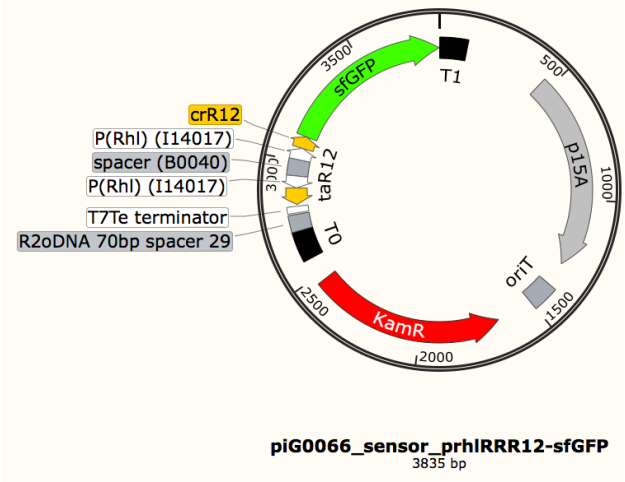
- siG0001: piG0031, piG0034





- siG0065: piG0042max, piG0066





Graphs of Data:

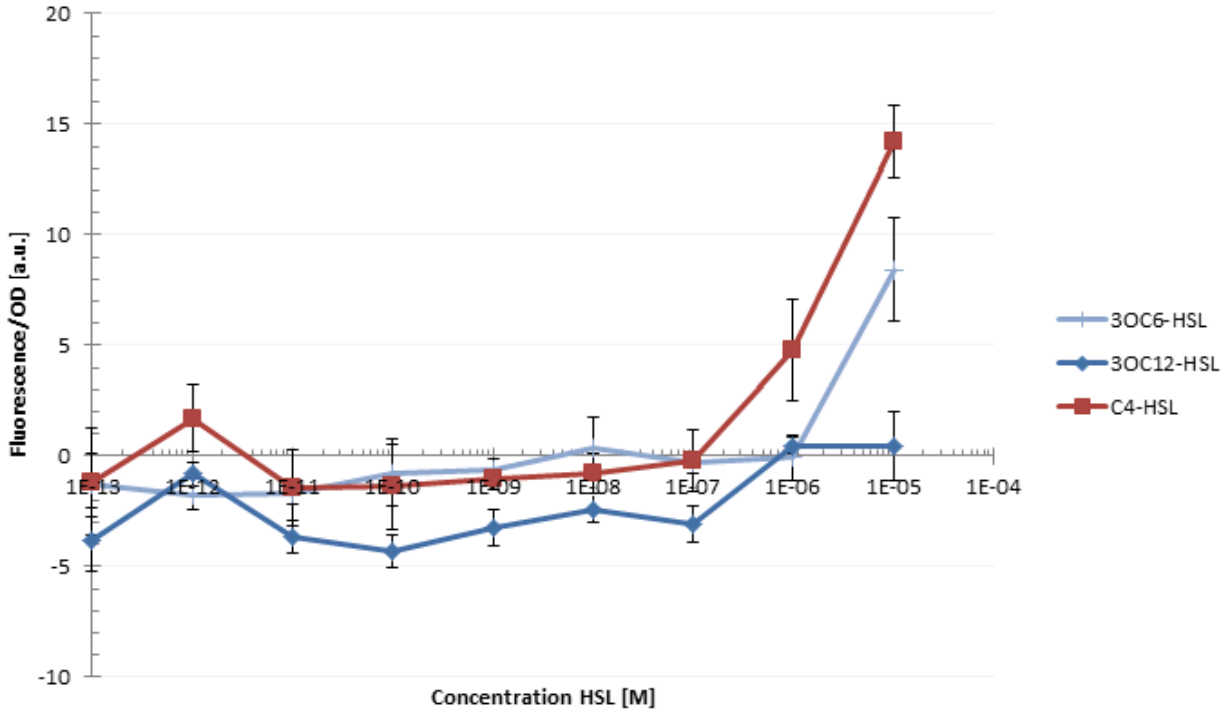


Fig. 1 siG0065 dose-response curve 200 min after induction for three AHL molecules

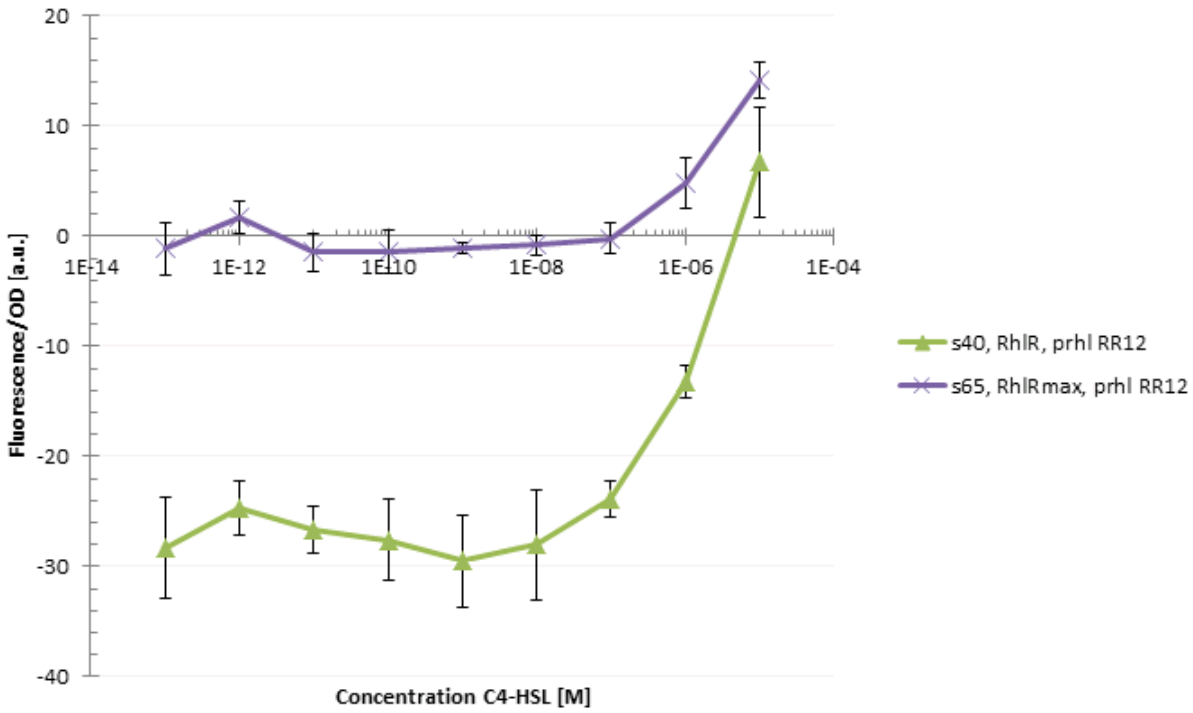


Fig. 2 dose-response curve 200 min after induction for C4-HSL for the two variants with different RBS strengths for RhlR production

Interpretation of Data:

- slight influence of RhlR amount? Or was the s40 not correct (highly negative values)
- no dramatic difference, but tendency

Experiment T22

Dose-Response Kinetics and Crosstalk
siG0064: LasR, sfGFP under plas Promoter and Riboregulator 12y
2014-09-04

Goal of the experiment:

- Does the riboregulator further decrease leakiness?
- Compare to siG0014 (T10)
- Analyse crosstalk in siG0064 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0064
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

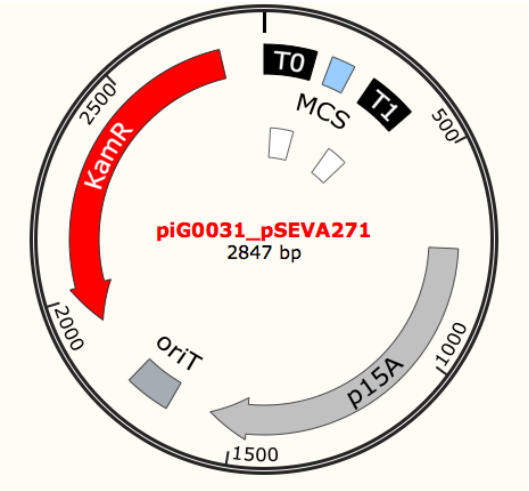
- Tecan infinite M200 PRO

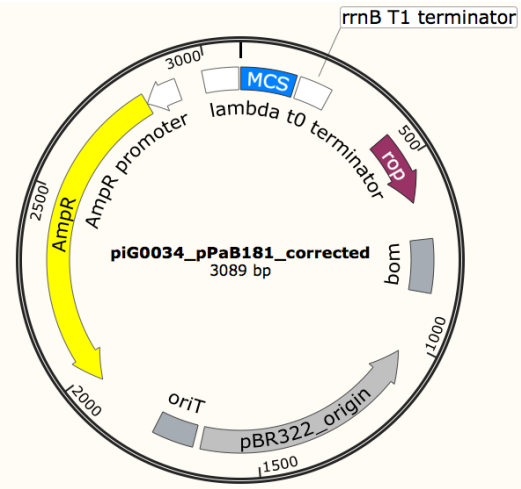
Raw Data:

- Lab/Microtiterplate/crosstalk/20140904_s64_crosstalk.xlsx

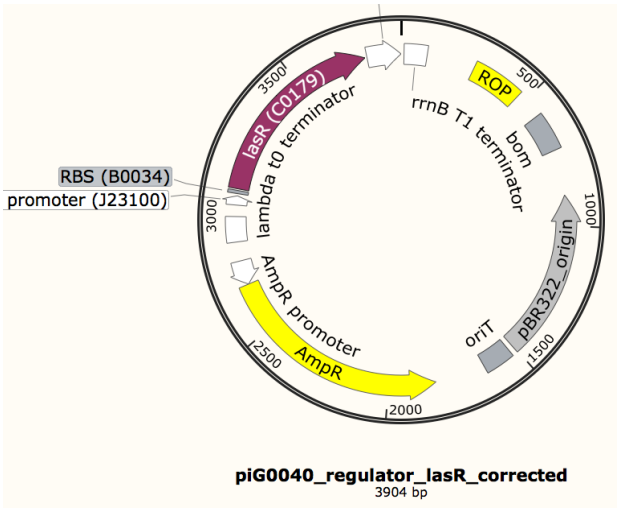
Plasmids in play:

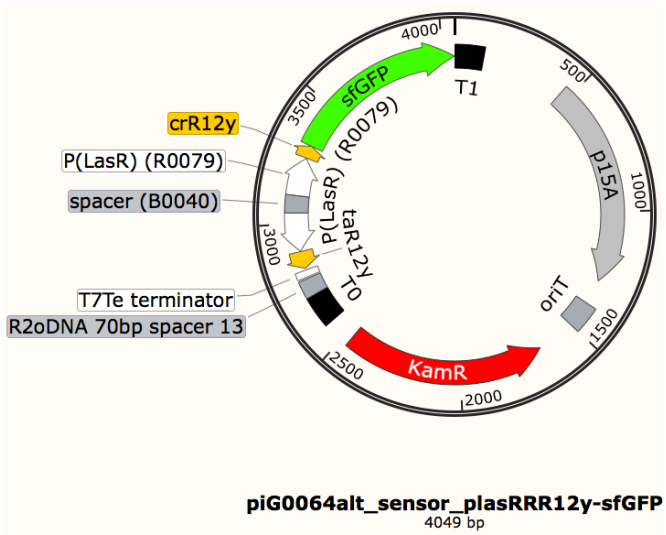
- siG0001: piG0031, piG0034





- siG0064: piG0040, piG0064alt





Graphs of Data:

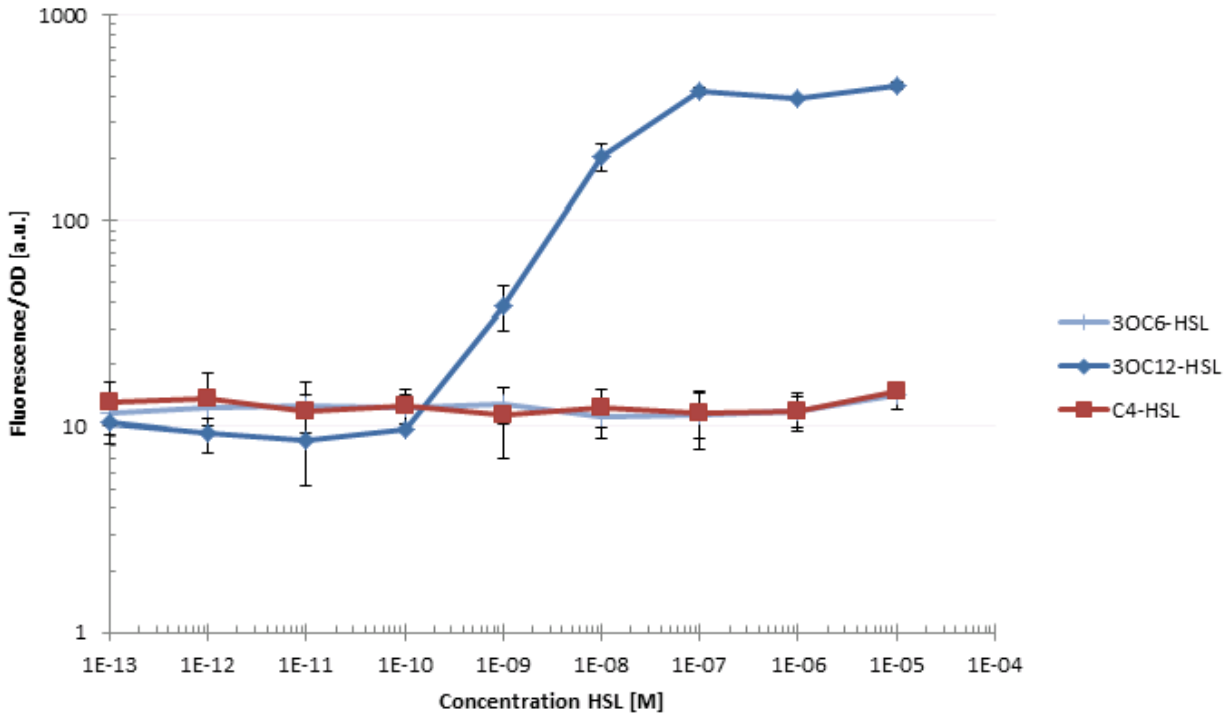


Fig. 1 siG0064 dose-response curve 200 min after induction for three AHL molecules

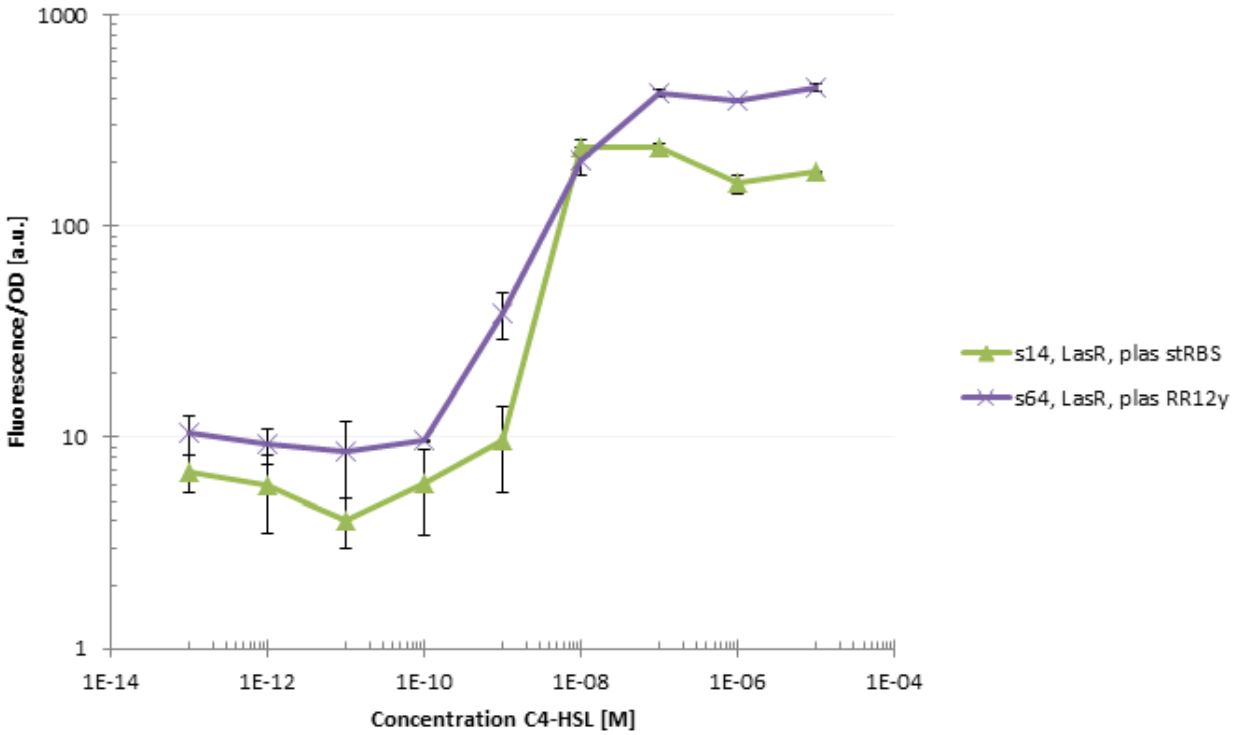


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0014 (without RR12y) and siG0064 (with RR12y)

Interpretation of Data:

- leakiness did not decrease, but ON level almost doubled (see Fig. 2)
- ON/OFF ratio stays almost the same with RR12y (Fig. 2)

Experiment T23

Dose-Response Kinetics and Crosstalk
siG0065: optimized RBS RhlR, sfGFP under prhl Promoter and Riboregulator 12 - repetition of T21
2014-09-01

Goal of the experiment:

- Repeated T21 for unknown reason
- Use result to compare day-to-day variation

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0065
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 10^{-14}, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

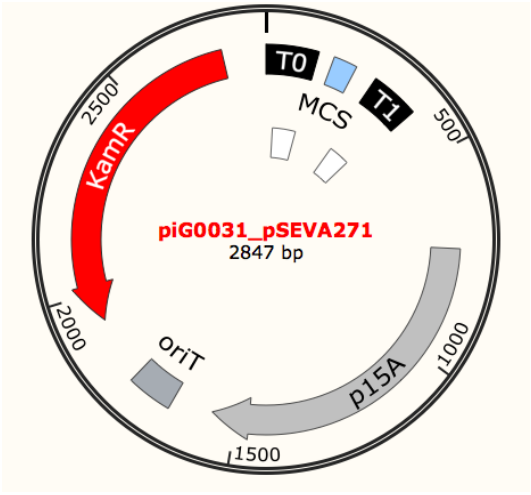
- Tecan infinite M200 PRO

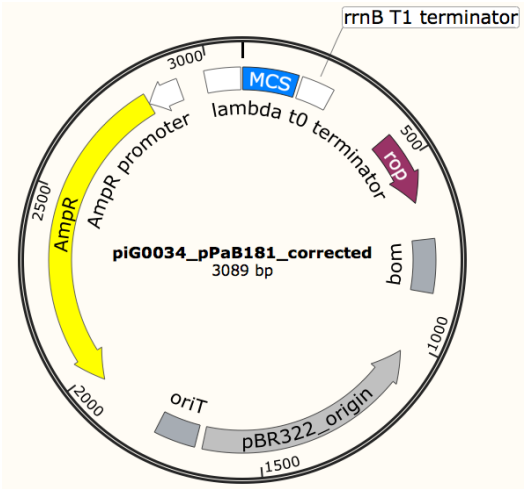
Raw Data:

- Lab/Microtiterplate/crosstalk/20140906_s65_crosstalk.xlsx

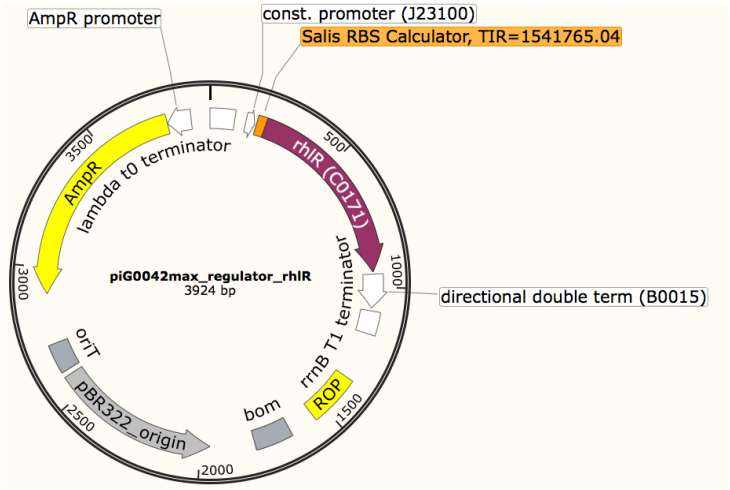
Plasmids in play:

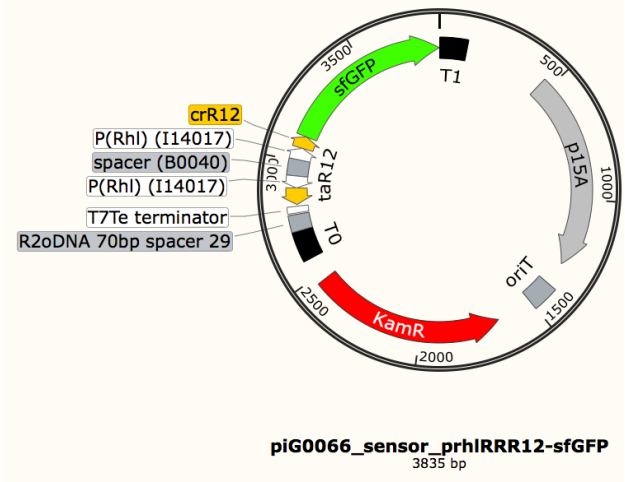
- siG0001: piG0031, piG0034





- siG0065: piG0042max, piG0066





Graphs of Data:

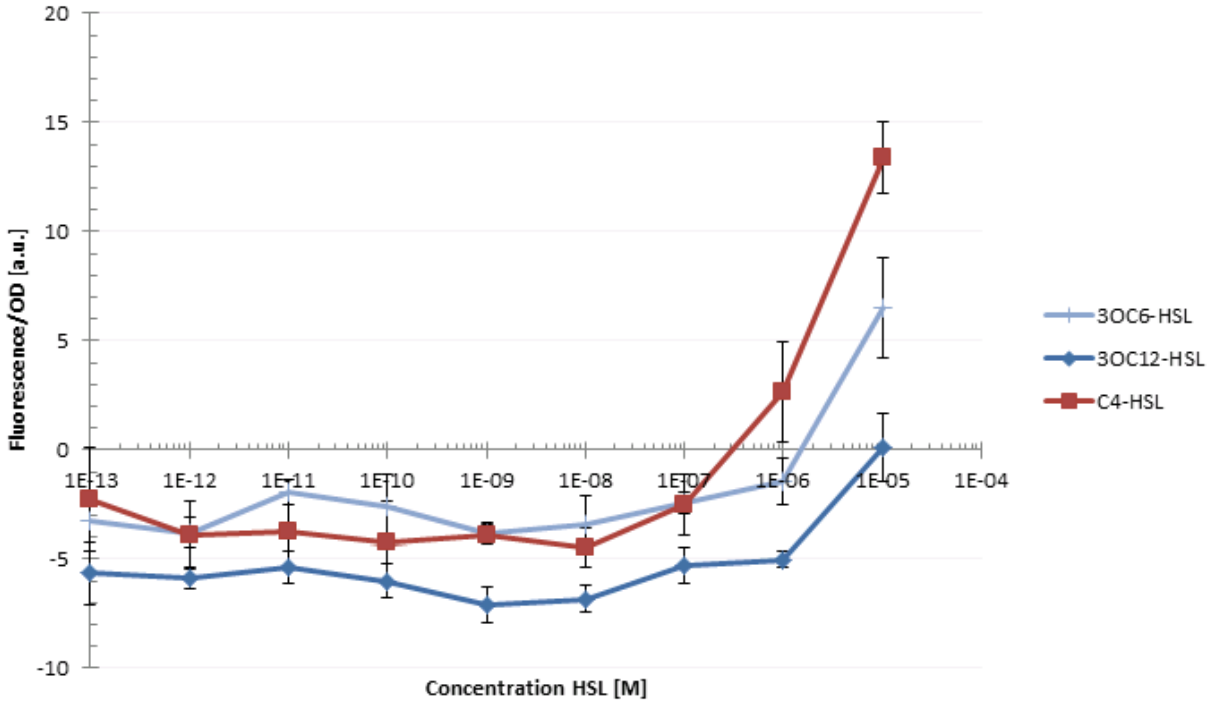


Fig. 1 siG0065 dose-response curve 200 min after induction for three AHL molecules

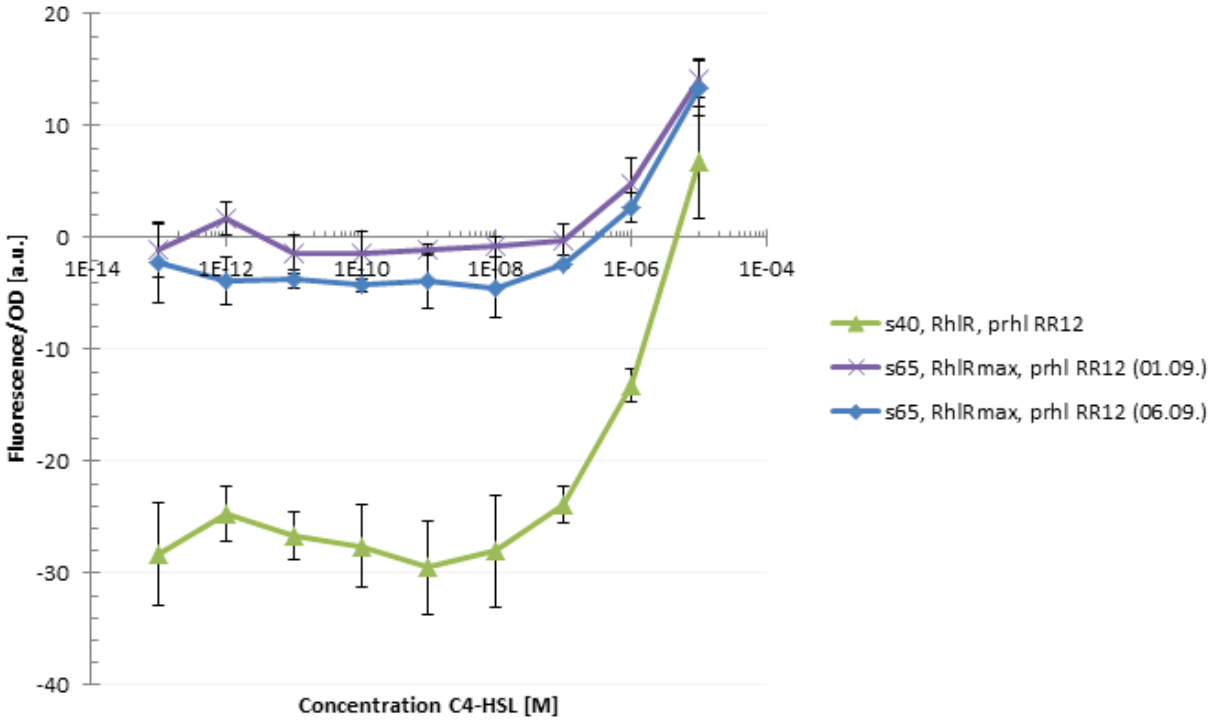


Fig. 2 dose-response curve 200 min after induction for C4-HSL for the two variants with different RBS strengths for RhlR production and comparison to repetition

Interpretation of Data:

- previous results could be confirmed (Fig. 2)

Experiment T24

Dose-Response Kinetics and Crosstalk
siG0067: RhlR optimized RBS, sfGFP under prhl Promoter and standard RBS
2014-09-07

Goal of the experiment:

- Does the riboregulator further decrease leakiness?
- Compare to siG0065 (T23)
- Analyse crosstalk in siG0067 between three AHLs (3OC6-HSL, 3OC12-HSL, C4-HSL)
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0067
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

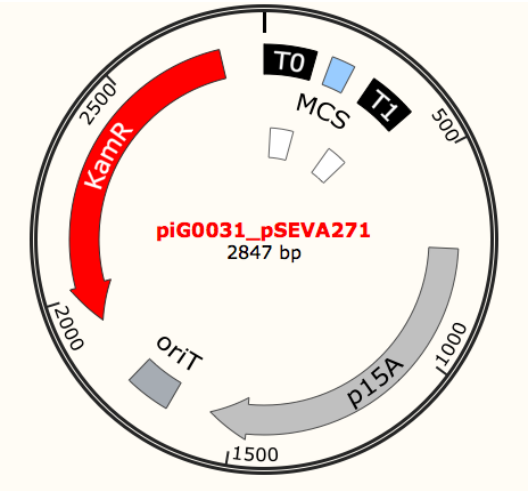
- Tecan infinite M200 PRO

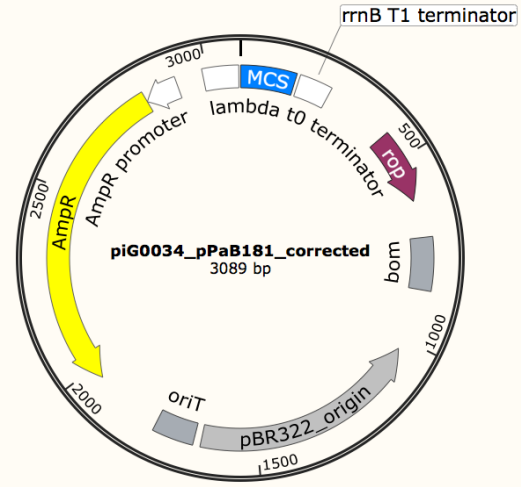
Raw Data:

- Lab/Microtiterplate/crosstalk/20140907_s67_crosstalk.xlsx

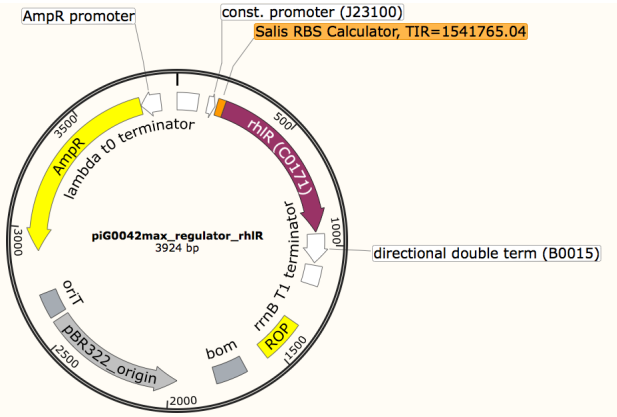
Plasmids in play:

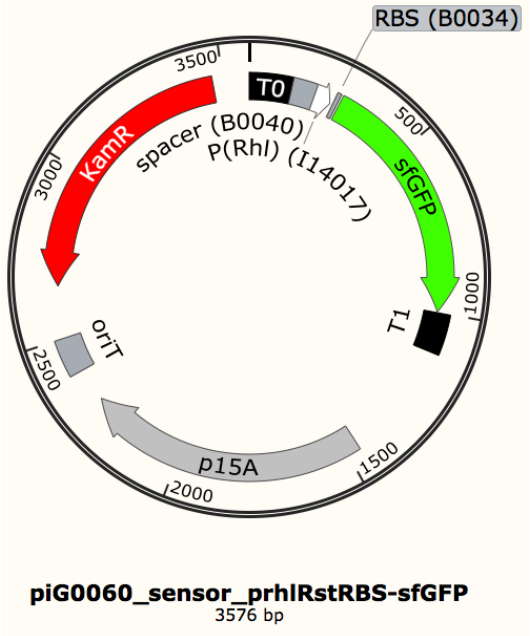
- siG0001: piG0031, piG0034





- siG0067: piG0042max, piG0060





Graphs of Data:

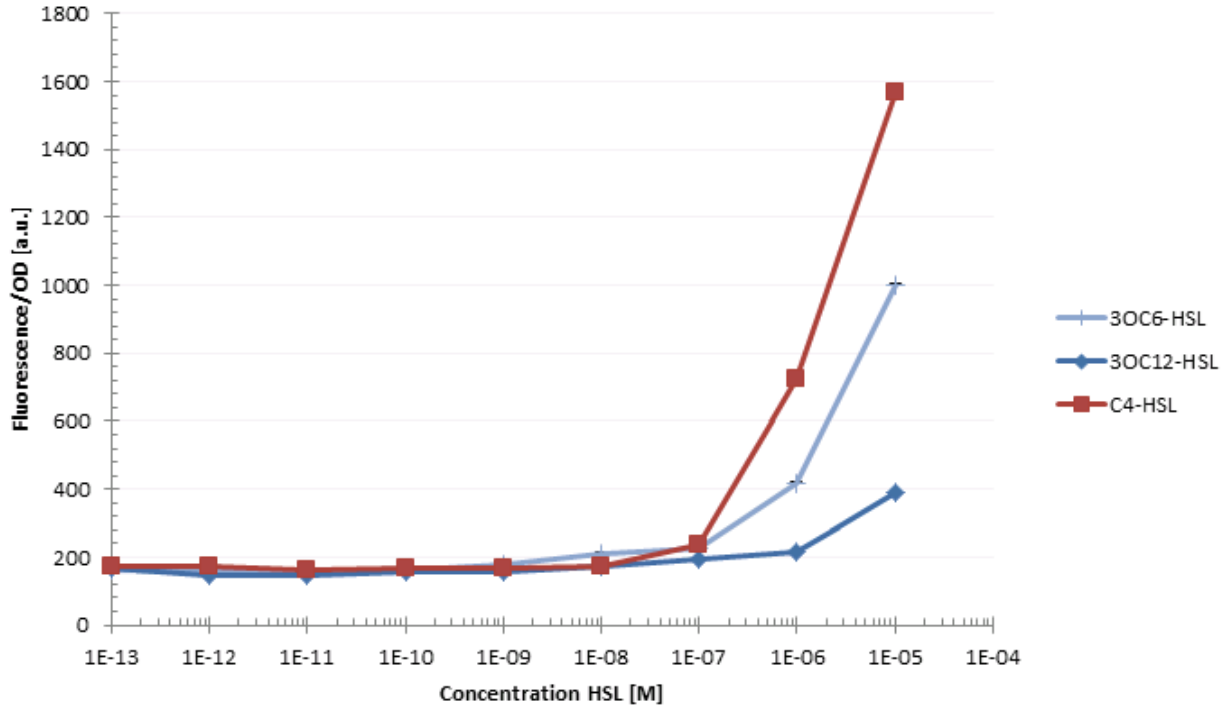


Fig. 1 siG0067 dose-response curve 200 min after induction for three AHL molecules

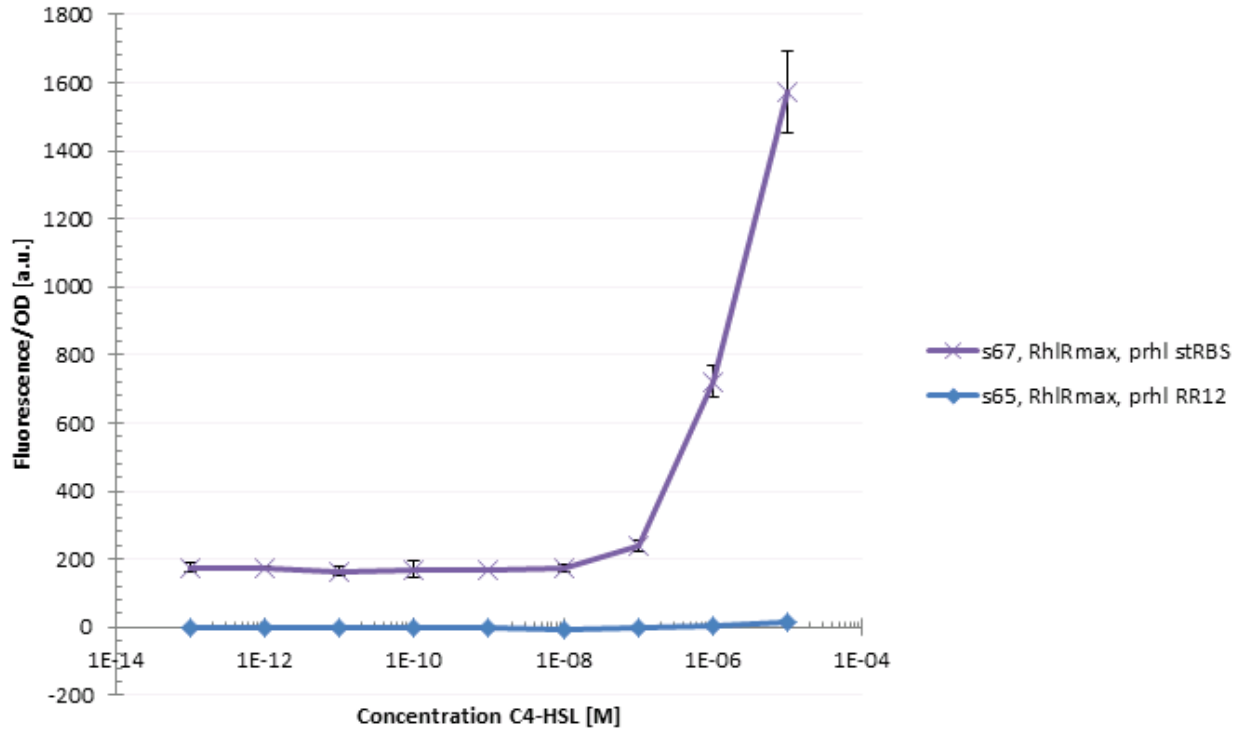


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0067 (without RR12) and siG0065 (with RR12)

Interpretation of Data:

- leakiness highly decreased with RR12 (see Fig. 2)
- However, ON response is ~100 times decreased as well (Fig. 2)
- Full ON (saturation) couldn't be observed in this concentration range

Experiment T25

Dose-Response Kinetics and Crosstalk
siG0066: RhlR optimized RBS, sfGFP under plas Promoter and Riboregulator 12y
2014-09-08

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does RhlR also activate the plas promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0066
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

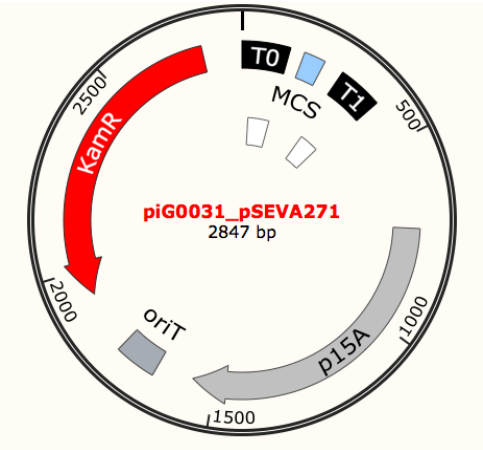
- Tecan infinite M200 PRO

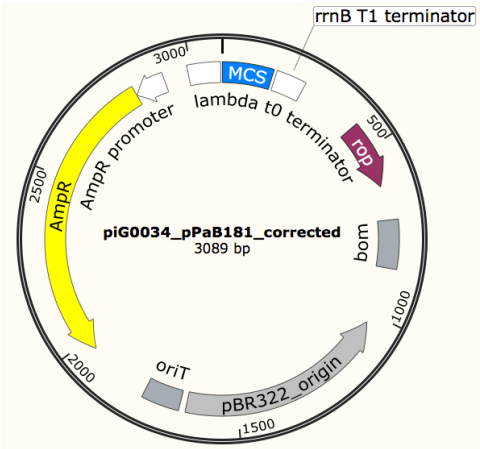
Raw Data:

- Lab/Microtiterplate/crosstalk/20140908_s66_crosstalk.xlsx

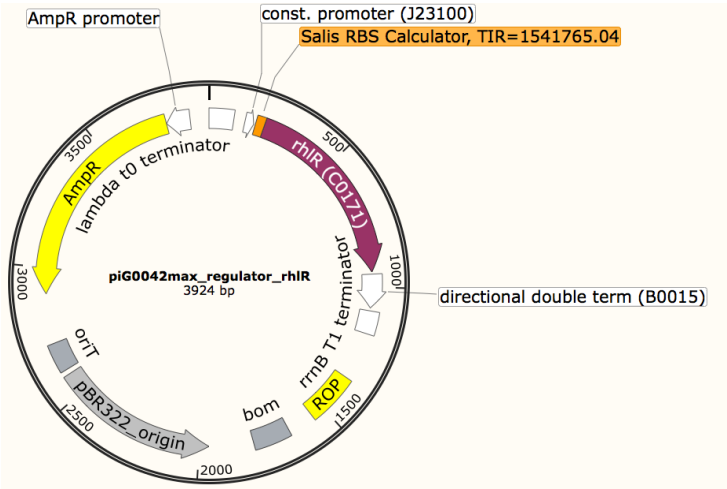
Plasmids in play:

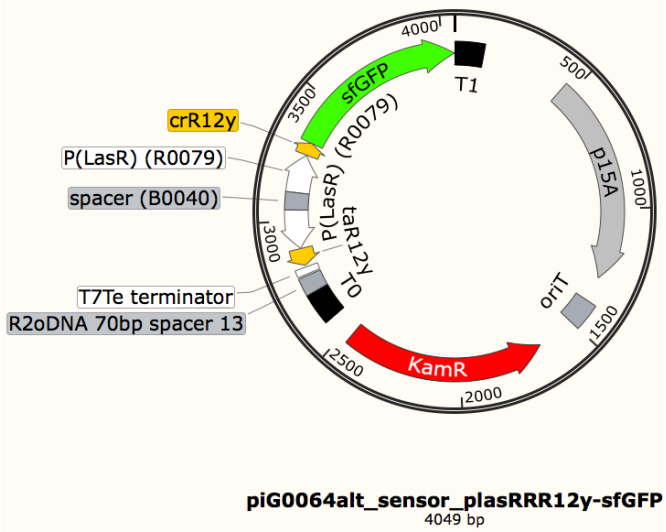
- siG0001: piG0031, piG0034





- siG0066: piG0042max, piG0064alt





Graphs of Data:

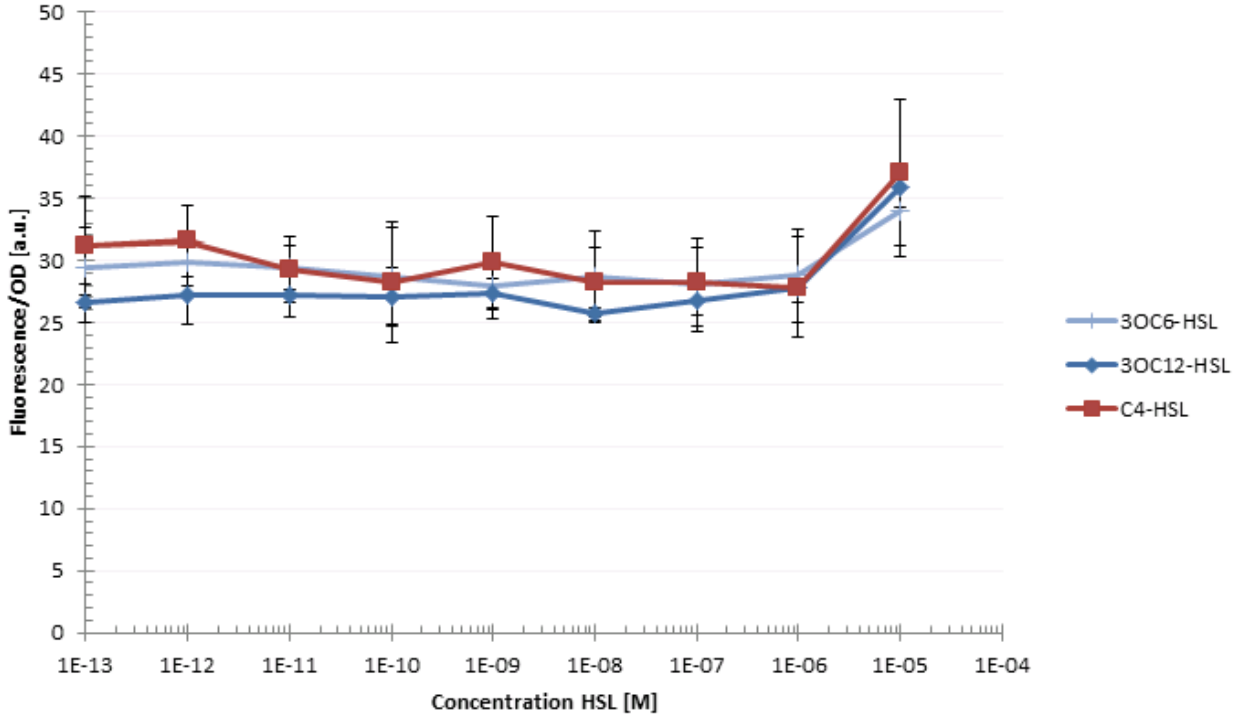


Fig. 1 siG0066 dose-response curve 200 min after induction for three AHL molecules

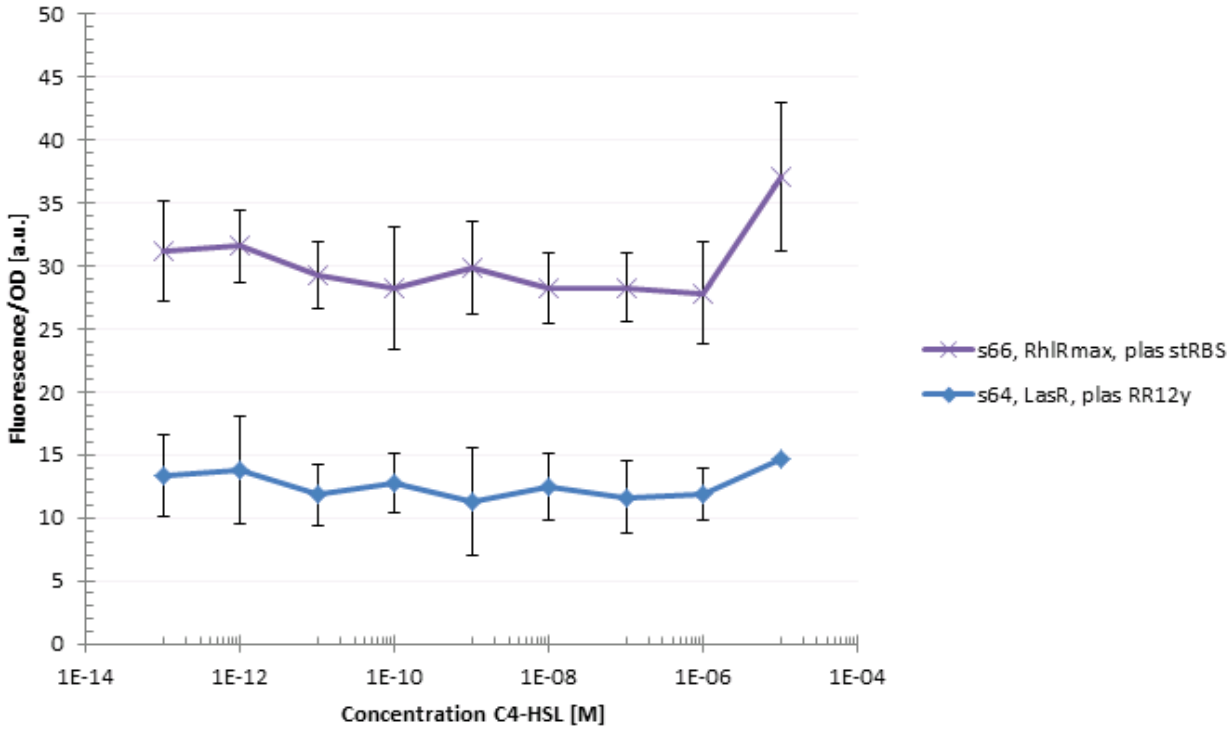


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0064 (with LasR) and siG0067 (with RhlR)

Interpretation of Data:

- RhlR does not clearly activate plas RR12y (Fig. 1)
- However, the leakiness is increased in comparison to the strain with LasR (Fig. 2)

Experiment T26

Dose-Response Kinetics and Crosstalk with Producer Supernatants siG0030: LuxR with sfGFP under plux Promoter and Riboregulator 12y - same as T9/T11 but new concentrations of supernatant

2014-09-11

Goal of the experiment:

- Investigate how much AHL is produced in piG0050max, 3OC6-HSL producer with optimized RBS
- Analyse crosstalk
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0030
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of sterile filtered overnight supernatant of constitutive AHL producers piG0049max(LasI), piG0050max(LuxI), piG0051max(RhlI):
        - 0, 1, 5, 10, 25, 40, 55, 70, 85, 100% final supernatant (v/v)

Machines used:

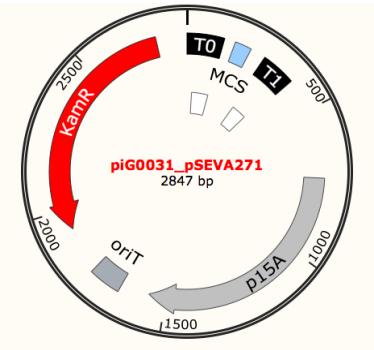
- Tecan infinite M200 PRO

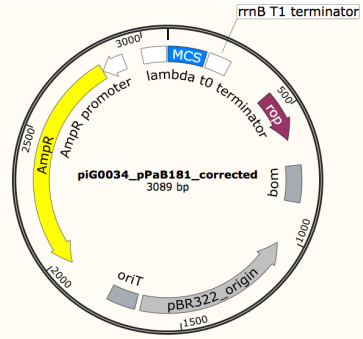
Raw Data:

- Lab/Microtiterplate/crosstalk/20140911_s30_crosstalk_supernatants_narrow_Lux_range.xlsx

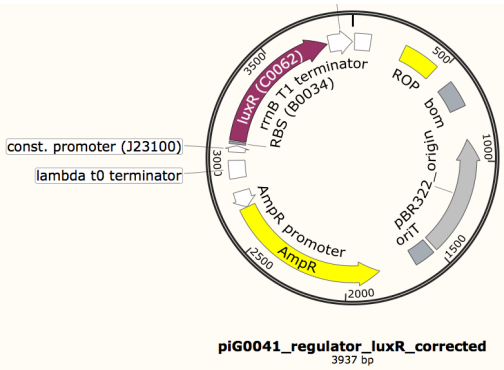
Plasmids in play:

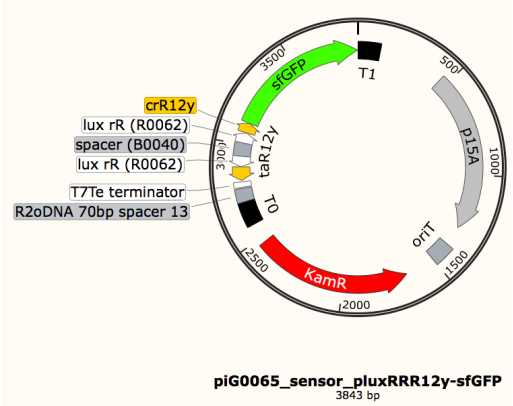
- siG0001: piG0031, piG0034



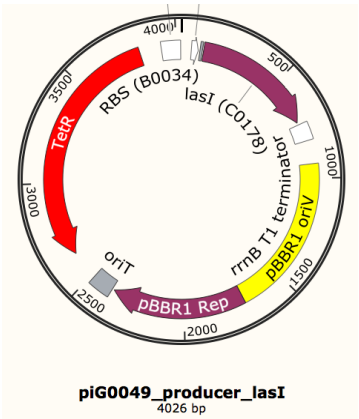


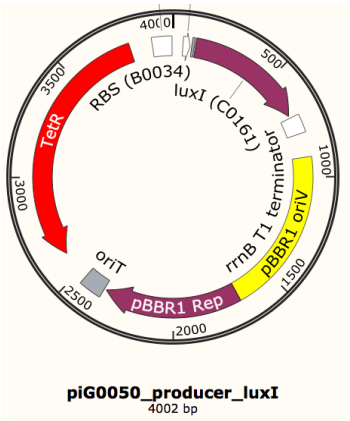
- siG0030: piG0041, piG0065

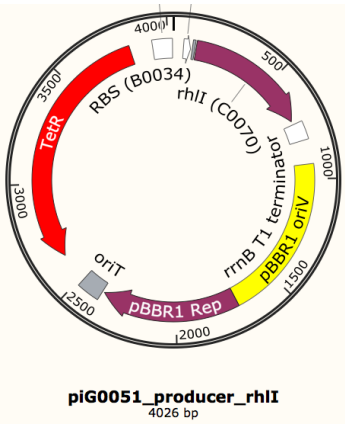




piG0049max (LasI producer)piG0050max (LuxI producer)piG0051max (RhlI producer)







Graphs of Data:

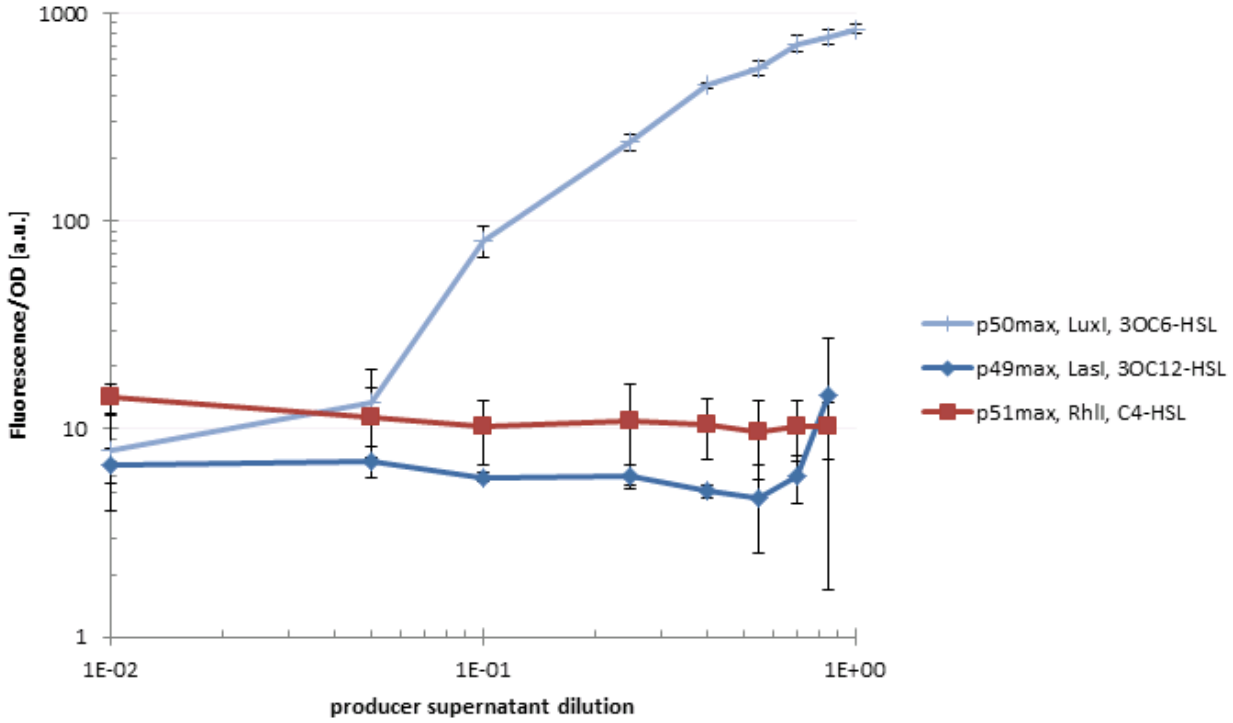


Fig. 1 siG0030 dose-response curve 350 min after inoculation in LB/supernatant mix with the three different producer supernatants

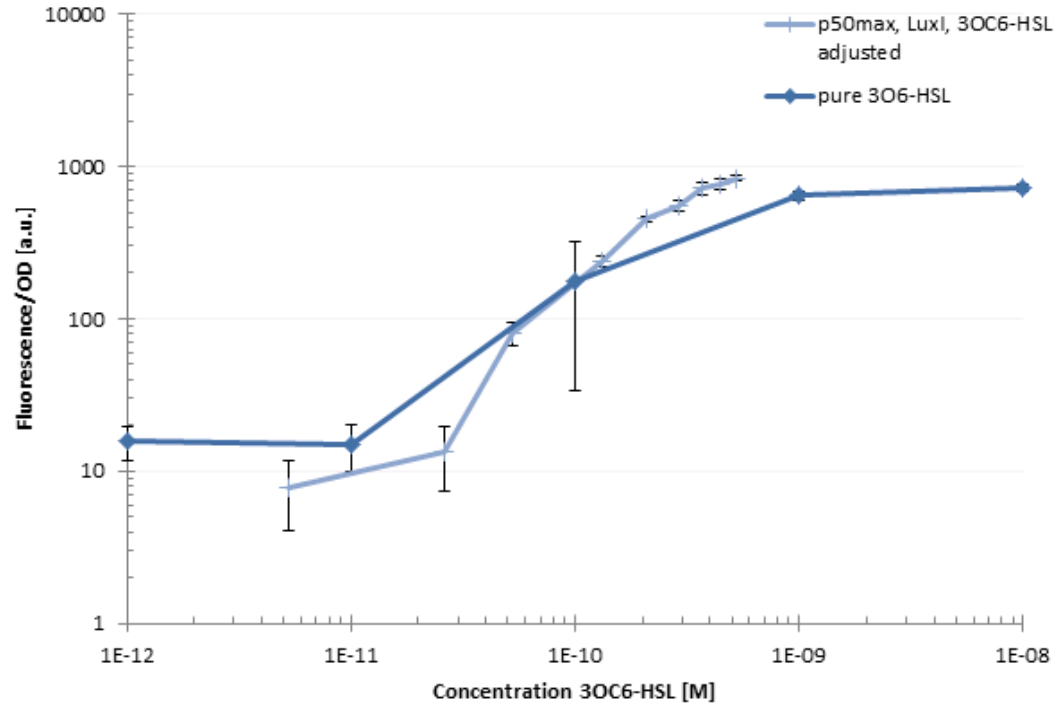


Fig. 2 siG0030 dose-response curve of supernatant fit into the dose response curve of pure 3OC6-HSL

Interpretation of Data:

- the LuxI supernatant can induce siG0030 (Fig. 1)
- a rough estimate with help of previous measurement with purified 3OC6-HSL would give 1 nM 3OC6-HSL as the supernatant's AHL concentration

Experiment T27

Dose-Response Kinetics and Crosstalk
siG0015: LasR, sfGFP under plux Promoter and standard RBS
2014-09-18

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LasR also activate the plux promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0015
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

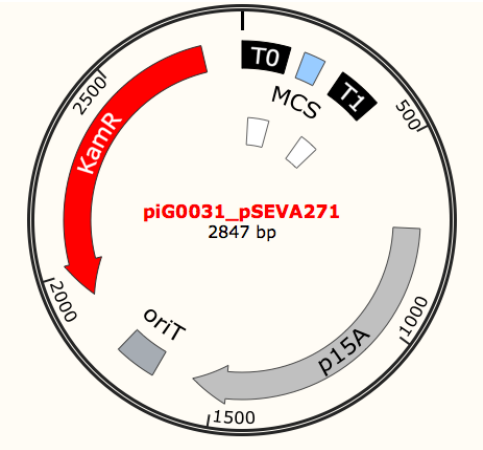
- Tecan infinite M200 PRO

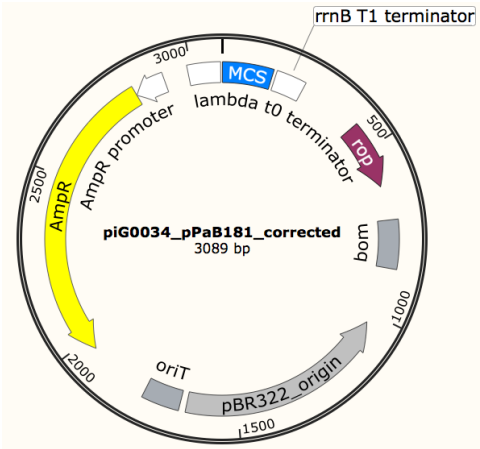
Raw Data:

- Lab/Microtiterplate/crosstalk/20140918_s15_crosstalk.xlsx

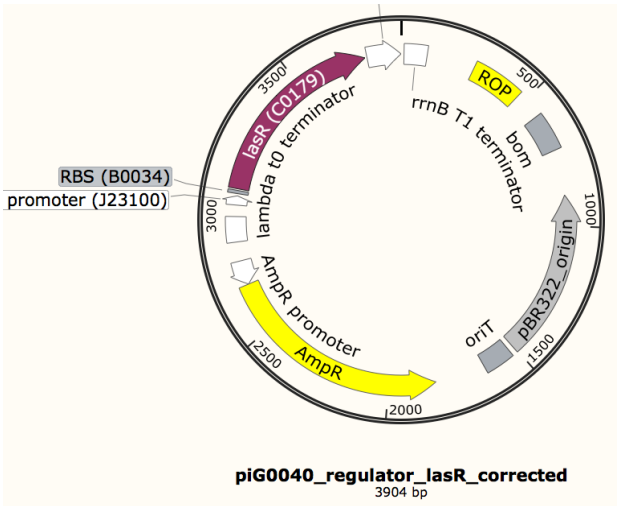
Plasmids in play:

- siG0001: piG0031, piG0034





- siG0015: piG0040, piG0059



Graphs of Data:

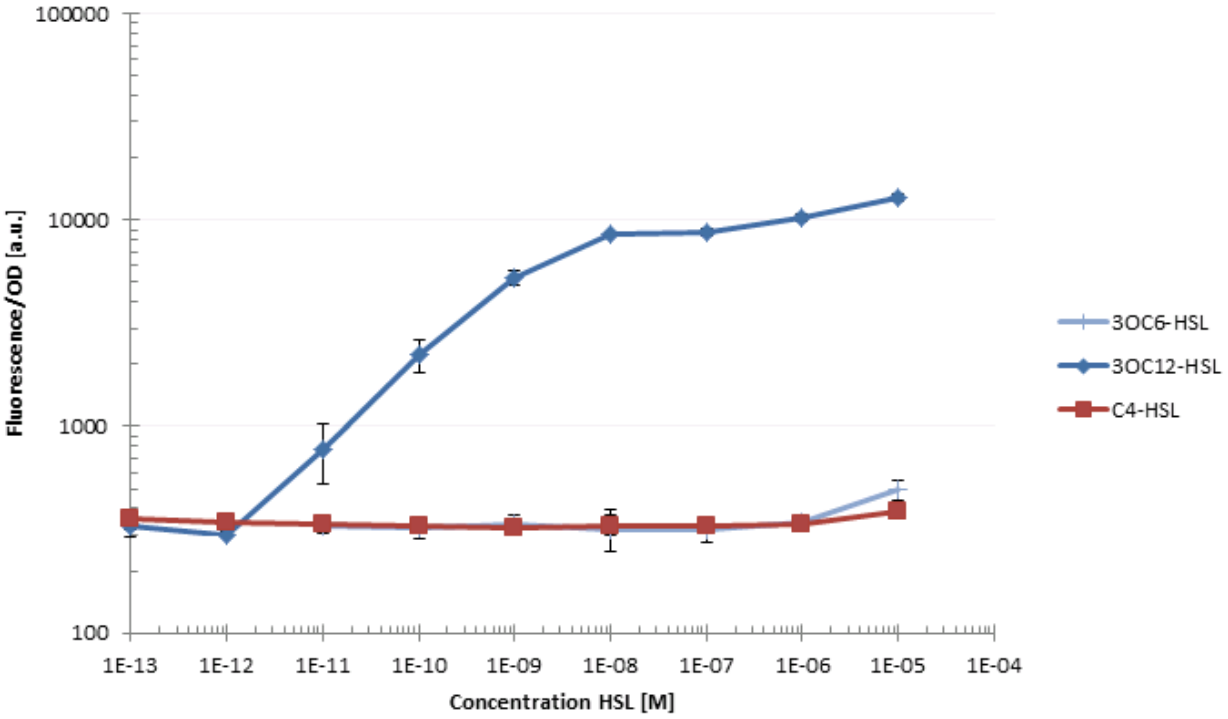


Fig. 1 siG0015 dose-response curve 200 min after induction for three AHL molecules

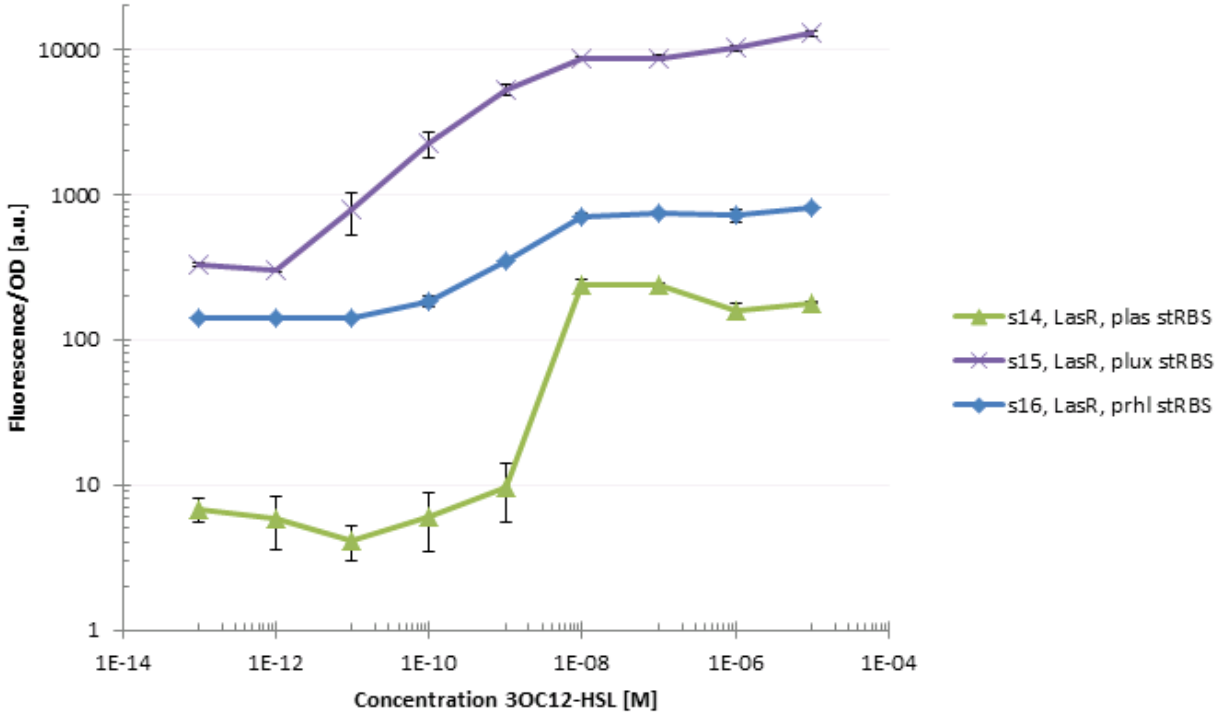


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0015, siG0014, siG0016

Interpretation of Data:

- LasR activates all three promoters (Fig. 2)

Experiment T28

Dose-Response Kinetics and Crosstalk
siG0081: RhlR optimized RBS, sfGFP under plas Promoter and standard RBS
2014-09-19

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does RhlR also activate the plas promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0081
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

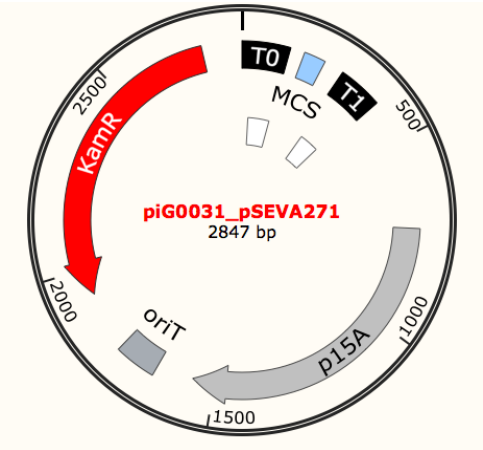
- Tecan infinite M200 PRO

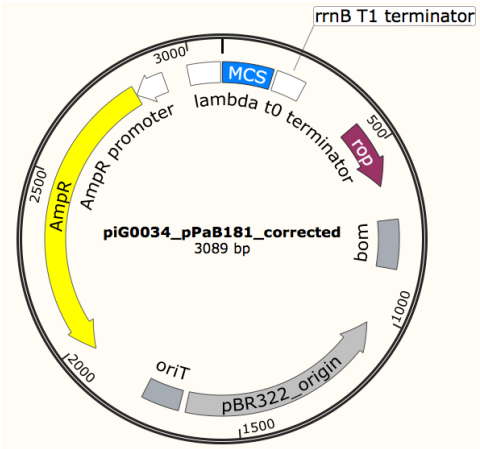
Raw Data:

- Lab/Microtiterplate/crosstalk/20140919_s81_crosstalk.xlsx

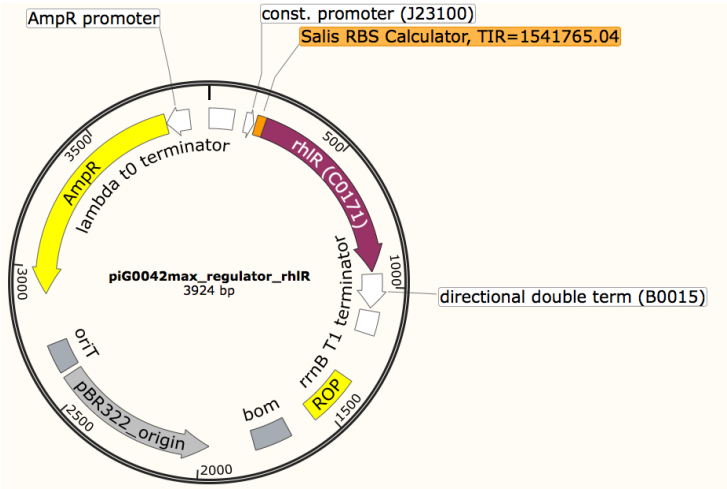
Plasmids in play:

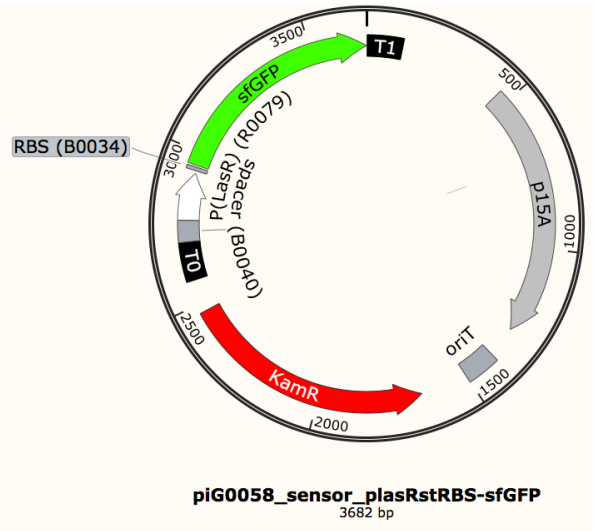
- siG0001: piG0031, piG0034





- siG0081: piG0042max, piG0058





Graphs of Data:

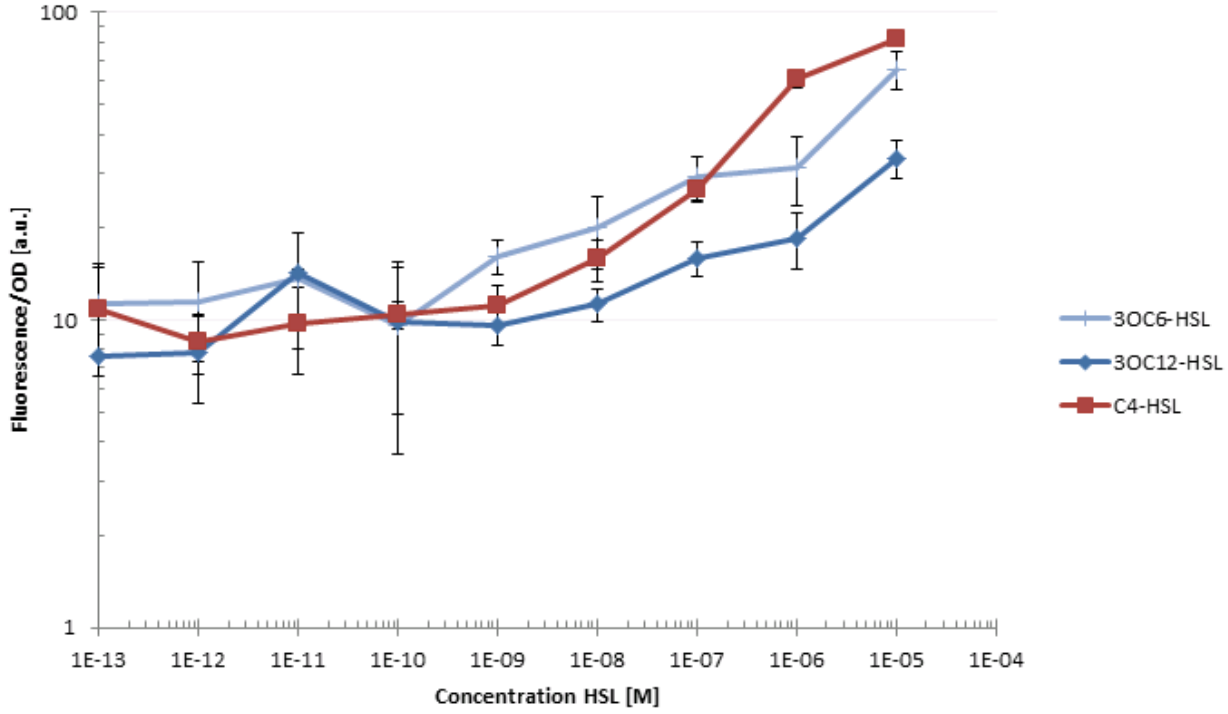


Fig. 1 siG0081 dose-response curve 200 min after induction for three AHL molecules

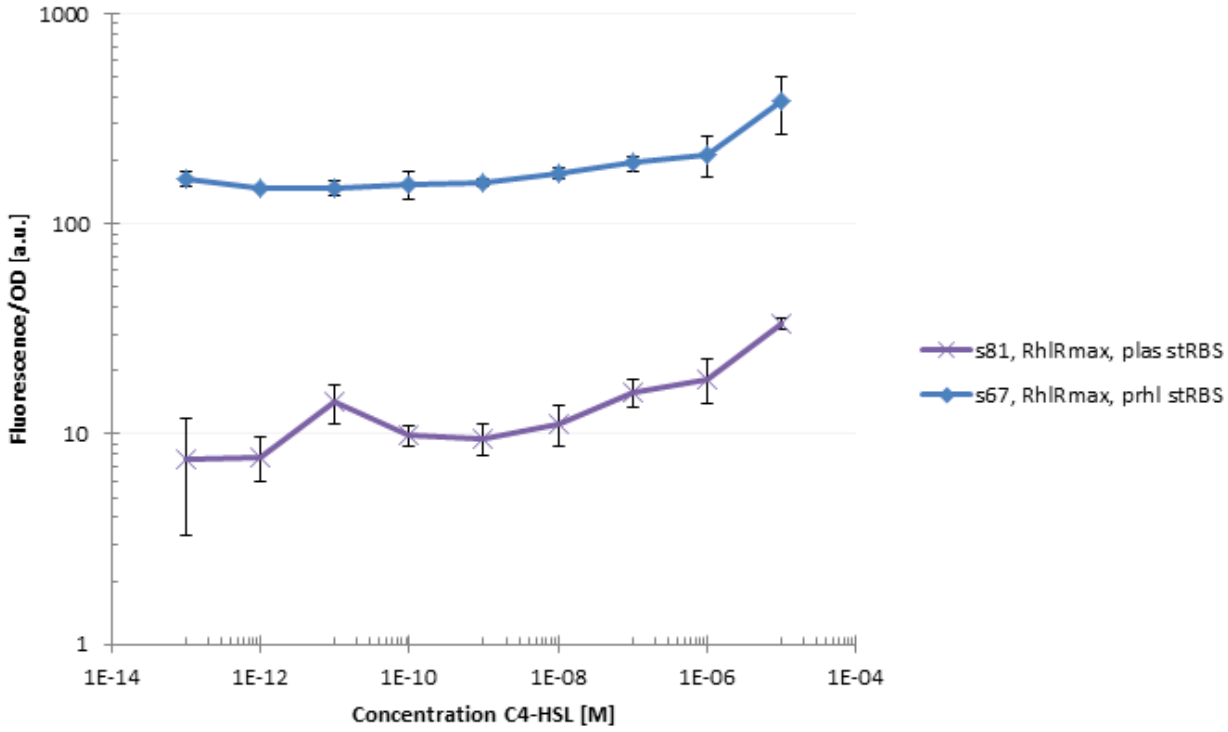


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0081 and siG0067

Interpretation of Data:

- RhlR shows almost no specificity with plas, but is only weakly activating (Fig. 1)

Experiment T29

Dose-Response Kinetics and Crosstalk
siG0016: LasR, sfGFP under prhl Promoter and standard RBS
2014-09-19

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LasR also activate the prhl promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0016
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

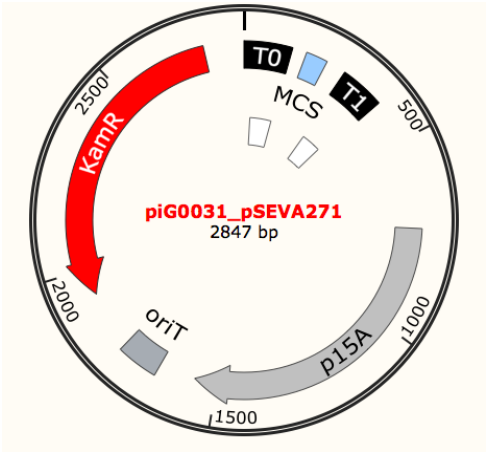
- Tecan infinite M200 PRO

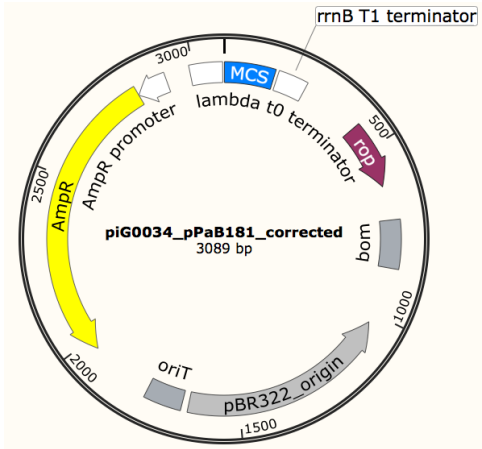
Raw Data:

- Lab/Microtiterplate/crosstalk/20140919_s16_crosstalk.xlsx

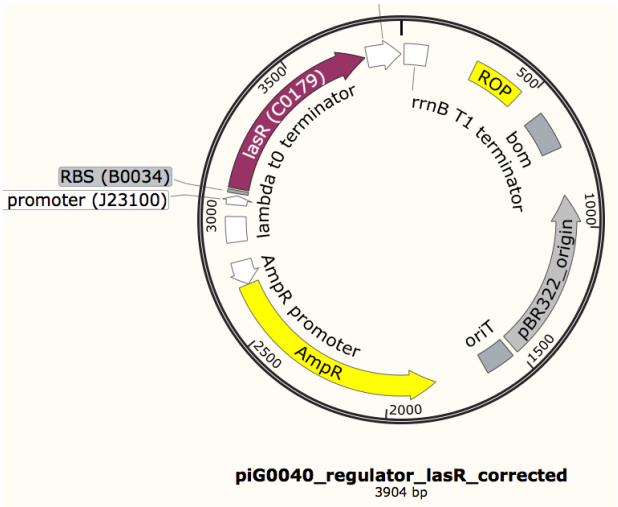
Plasmids in play:

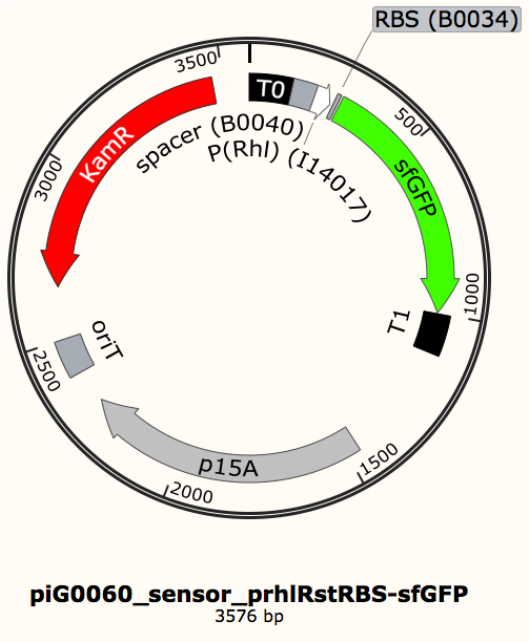
- siG0001: piG0031, piG0034





- siG0016: piG0040, piG0060





Graphs of Data:

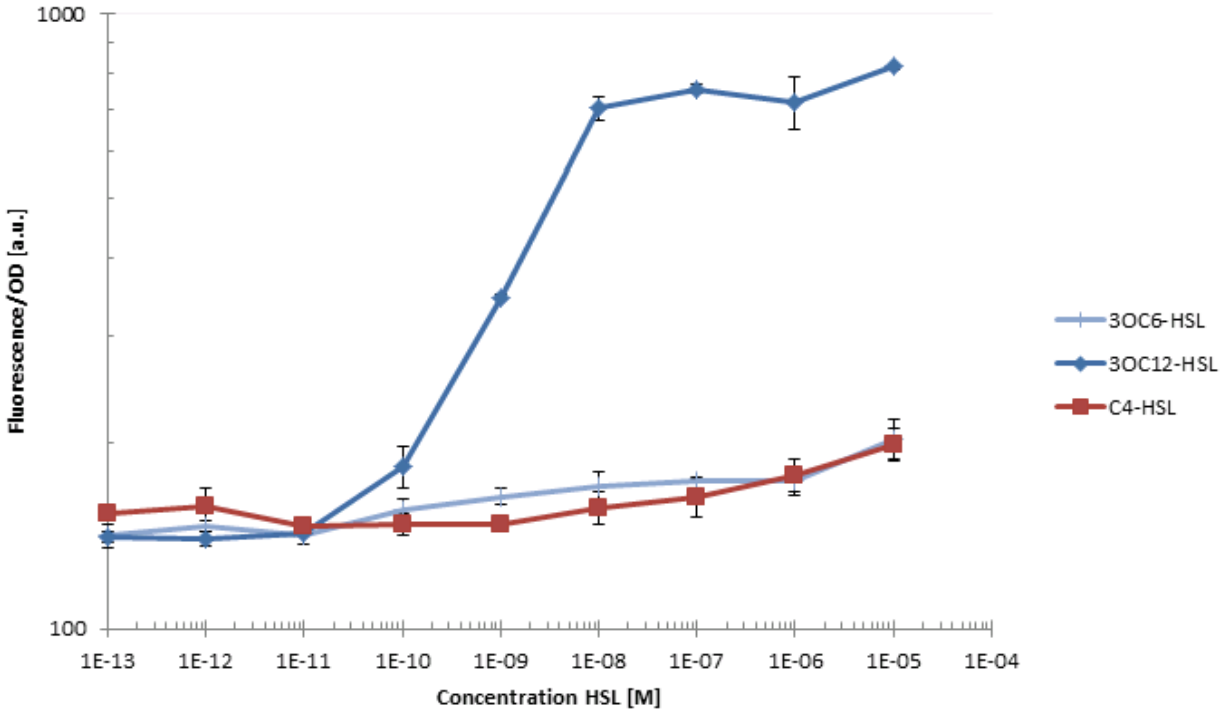


Fig. 1 siG0016 dose-response curve 200 min after induction for three AHL molecules

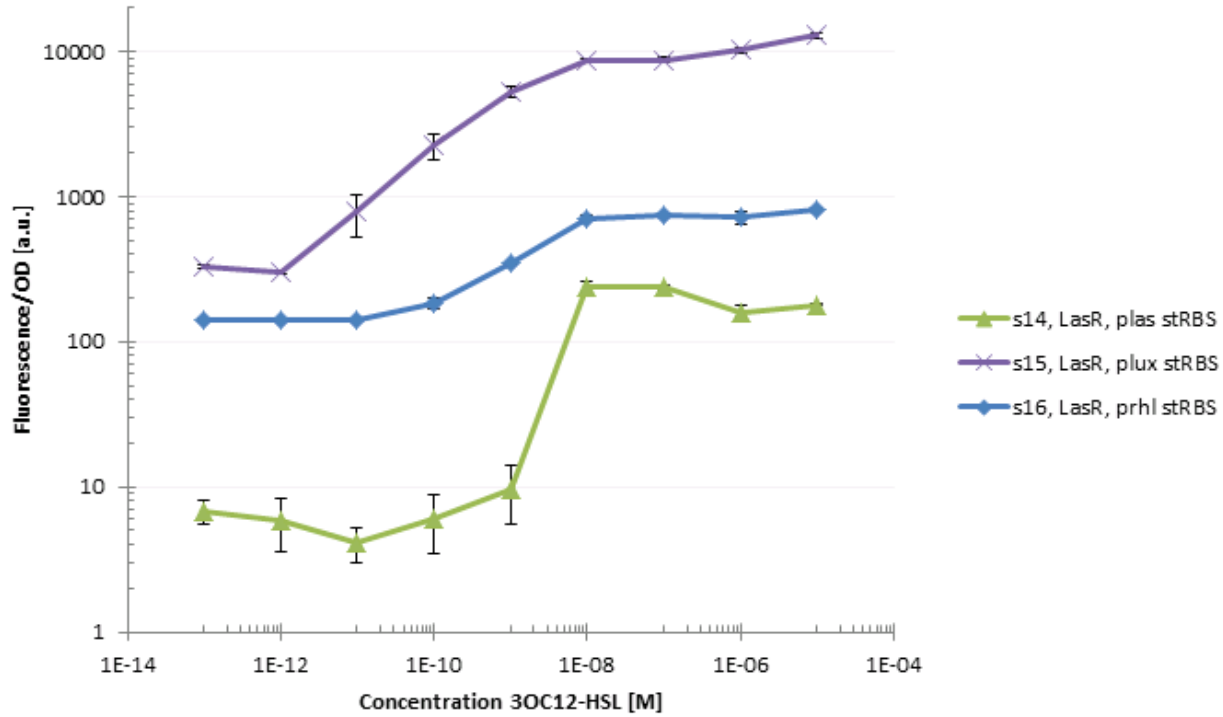


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0015, siG0014, siG0016

Interpretation of Data:

- LasR activates prhl reacting specifically to 3Oc12-HSL (Fig. 1)
- LasR activates all three promoters in a similar range, full ON at 10^{-8} M 3OC12-HSL (Fig. 2)

Experiment T30

Dose-Response Kinetics and Crosstalk
siG0025: LuxR, sfGFP under prhl Promoter and standard RBS
2014-09-21

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does LuxR also activate the prhl promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0025
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after 2 hours in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

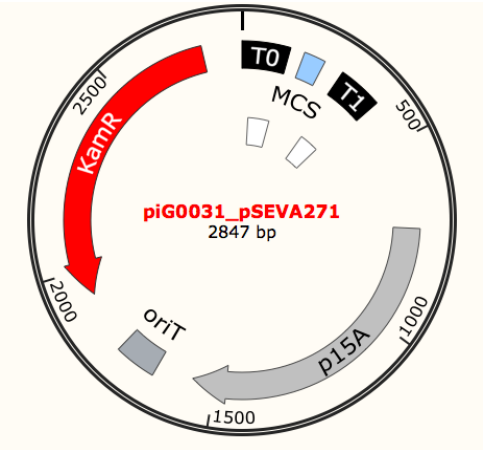
- Tecan infinite M200 PRO

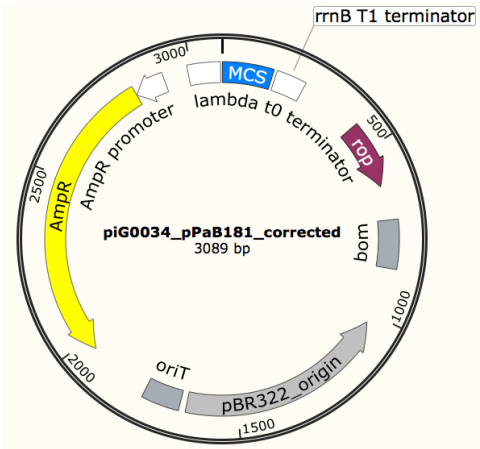
Raw Data:

- Lab/Microtiterplate/crosstalk/20140921_s25_crosstalk.xlsx

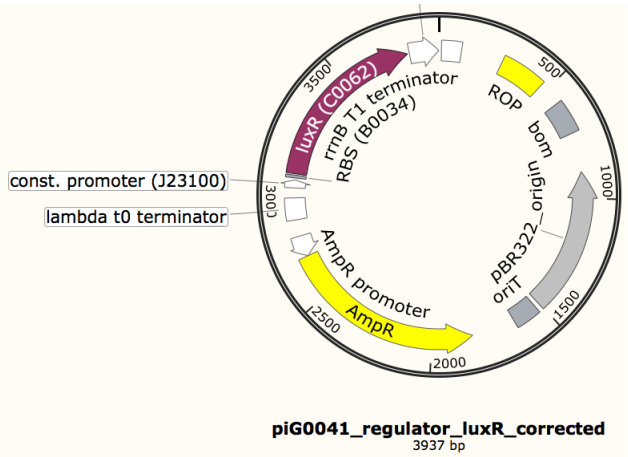
Plasmids in play:

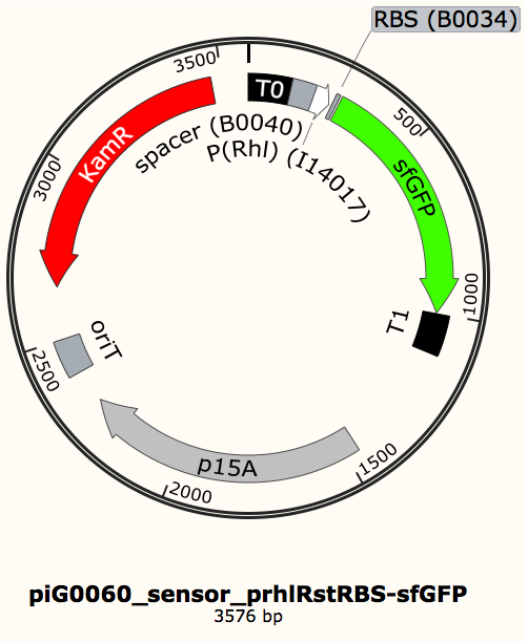
- siG0001: piG0031, piG0034





- siG0025: piG0041, piG0060





Graphs of Data:

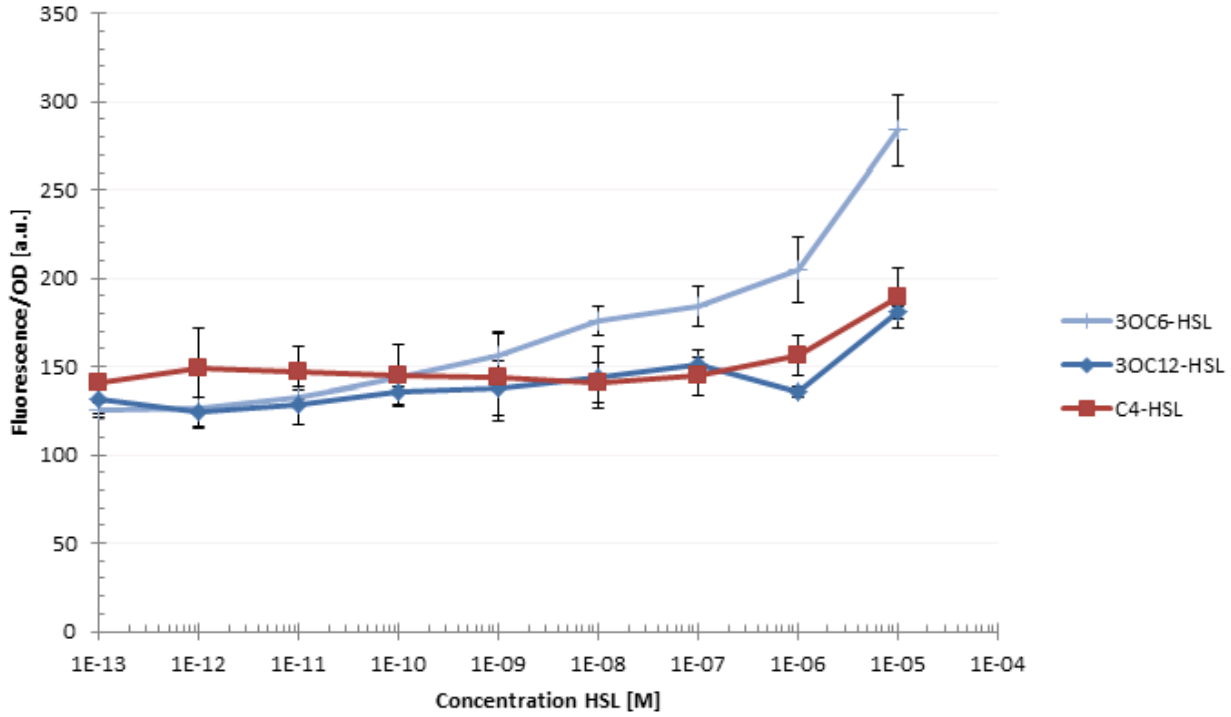


Fig. 1 siG0025 dose-response curve 200 min after induction for three AHL molecules

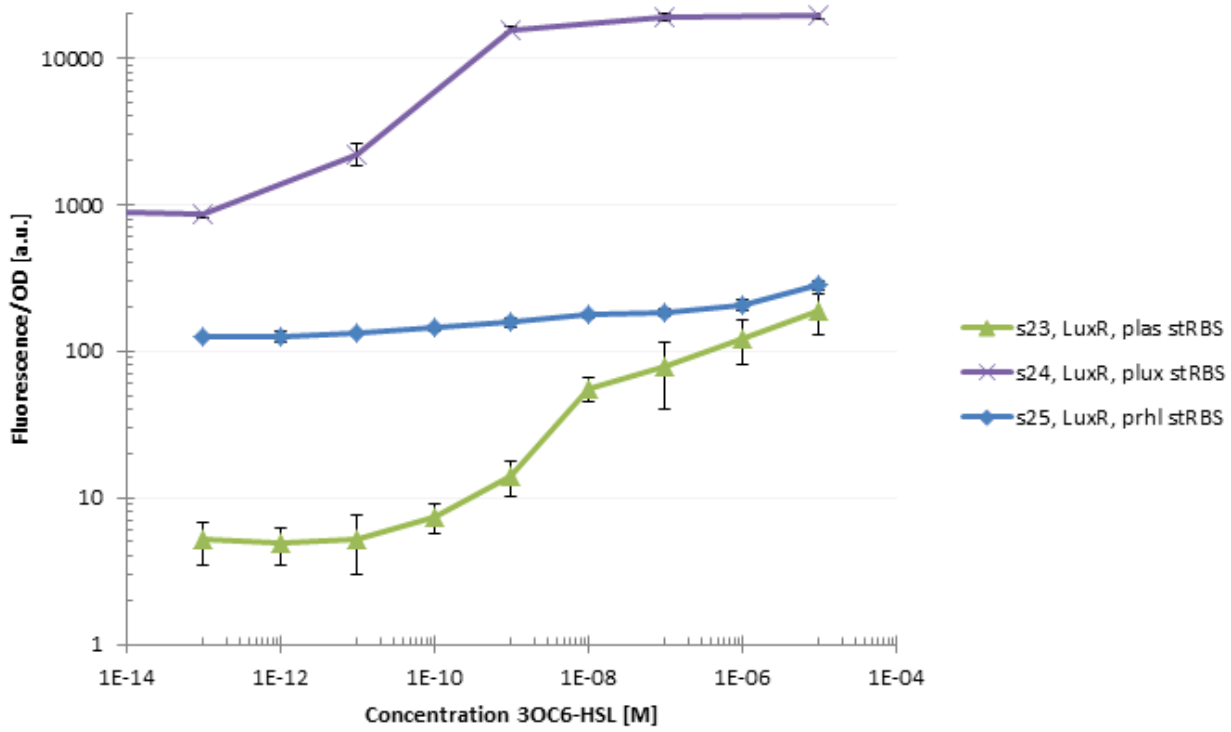


Fig. 2 dose-response curve 200 min after induction with 3OC6-HSL for siG0024, siG0025, siG0026

Interpretation of Data:

- LuxR activates prhl only weakly (Fig. 1)
- LuxR activates the other two promoters in a similar range 3OC6-HSL, but stronger, i.e. at least one order of magnitude (Fig. 2)

Experiment T31

Integrases on addgene plasmids with our Gates
siG0073: ptet-bxb1, pBAD-tp901, tp901-BUFFER
siG0075: ptet-bxb1, pBAD-tp901, bxb1-BUFFER
siG0076: ptet-bxb1, pBAD-tp901, bxb1/phiC31-XOR
2014-09-21

Goal of the experiment:

- Determine whether gate switching can be observed
- Is there integrase crosstalk?
- Can ON be distinguished from OFF?

Experimental SetUp:

- **gain 104**
- 96-well plate with overnight cultures diluted 1:1 with PBS
    - Overnight culture inoculated with the three strains in 6 different dilutions of arabinose in LB:
        - 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} % w/v

Machines used:

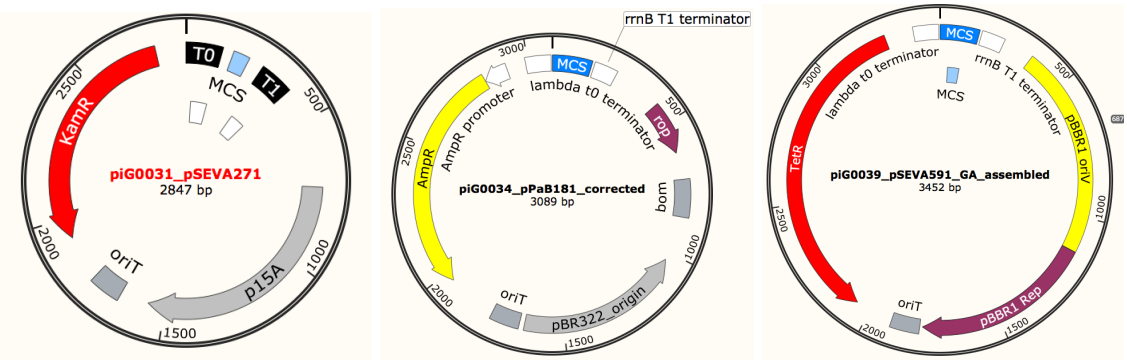
- Tecan infinite M200 PRO

Raw Data:

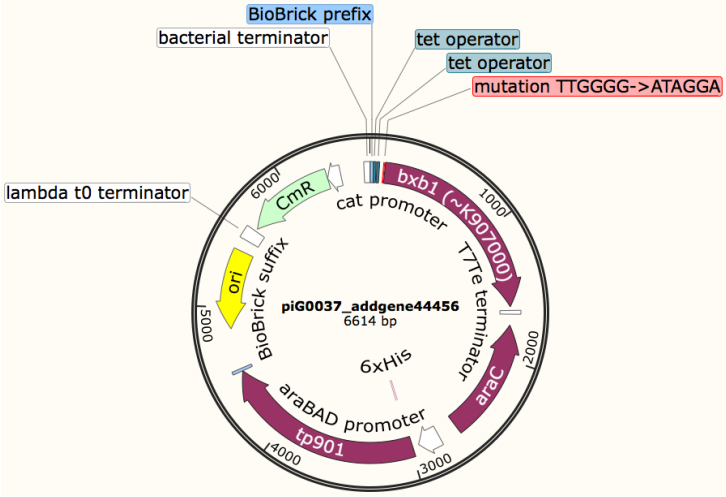
- Lab/Microtiterplate/integrase/20140921_integrase_test_s73,75,76,63_with_ara.xlsx

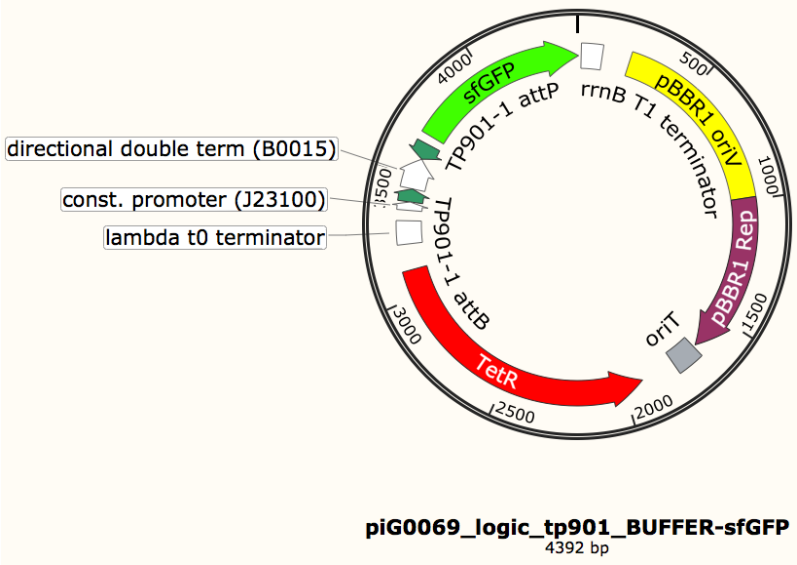
Plasmids in play:

- siG0063: piG0031, piG0034, piG0039

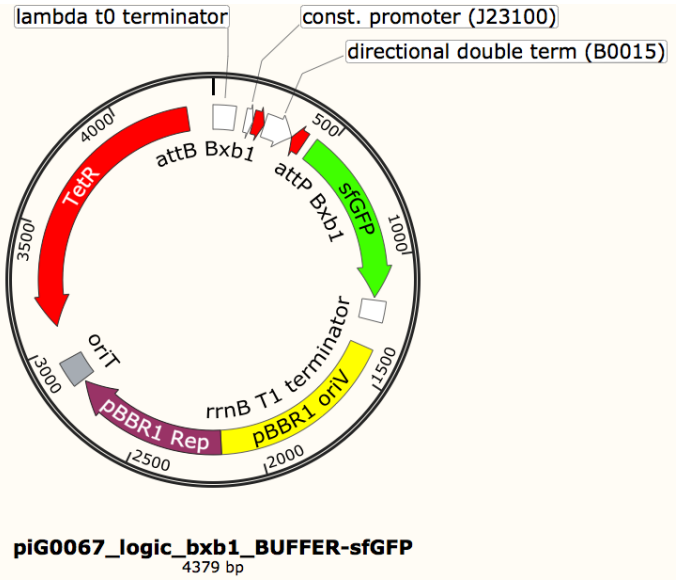


- siG0073: piG0037, piG0069

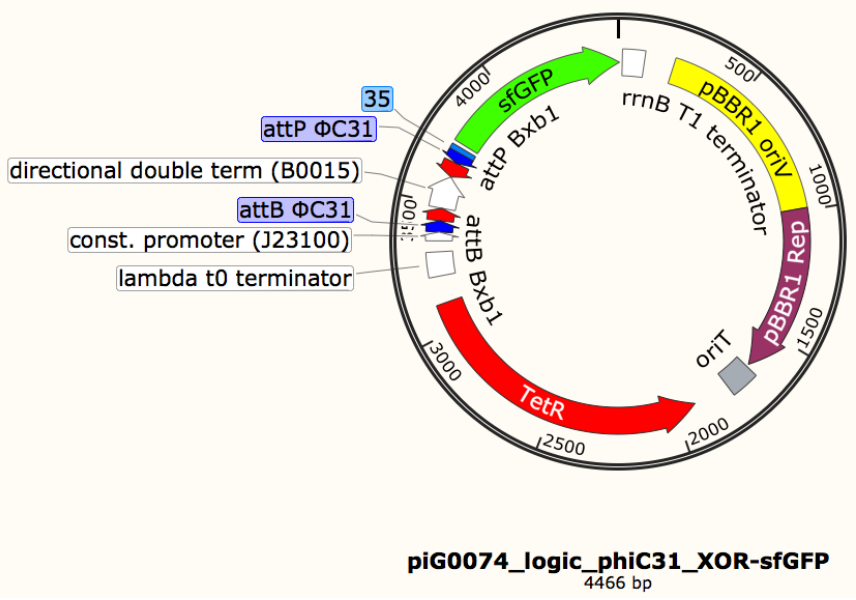




- siG0075: piG0037, piG0067



- siG0076: piG0037, piG0074



Graphs of Data:

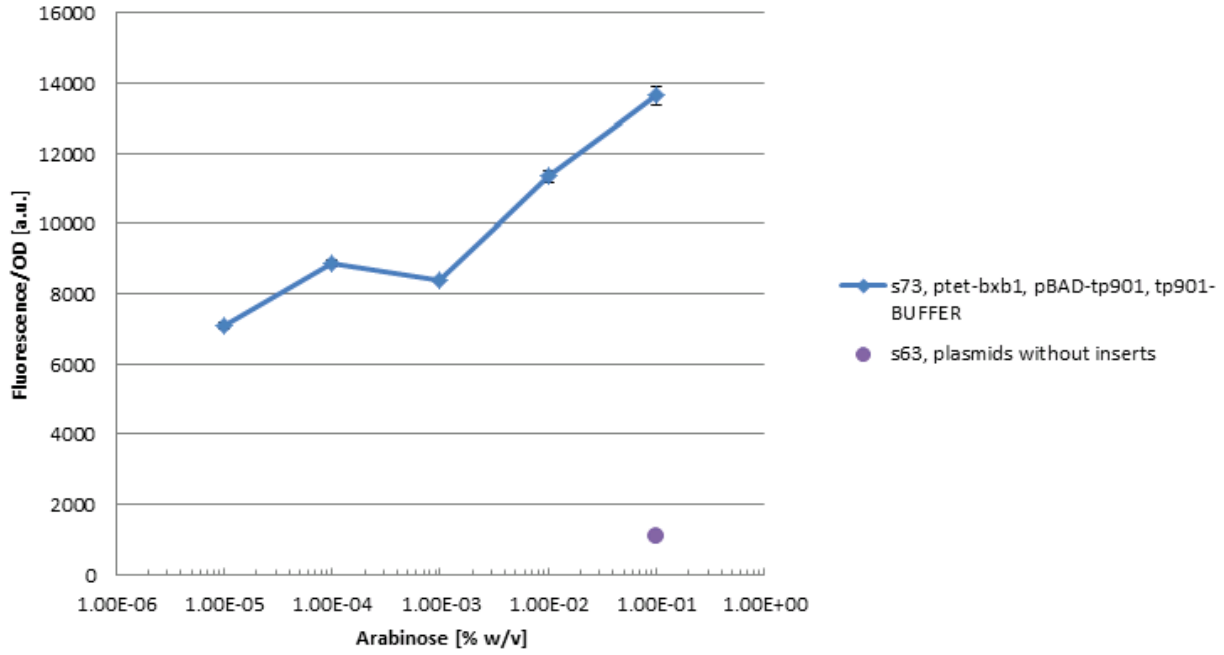


Fig. 1 siG0073 dose-response curve of overnight culture to different arabinose concentrations, siG0063 as GFP-free reference

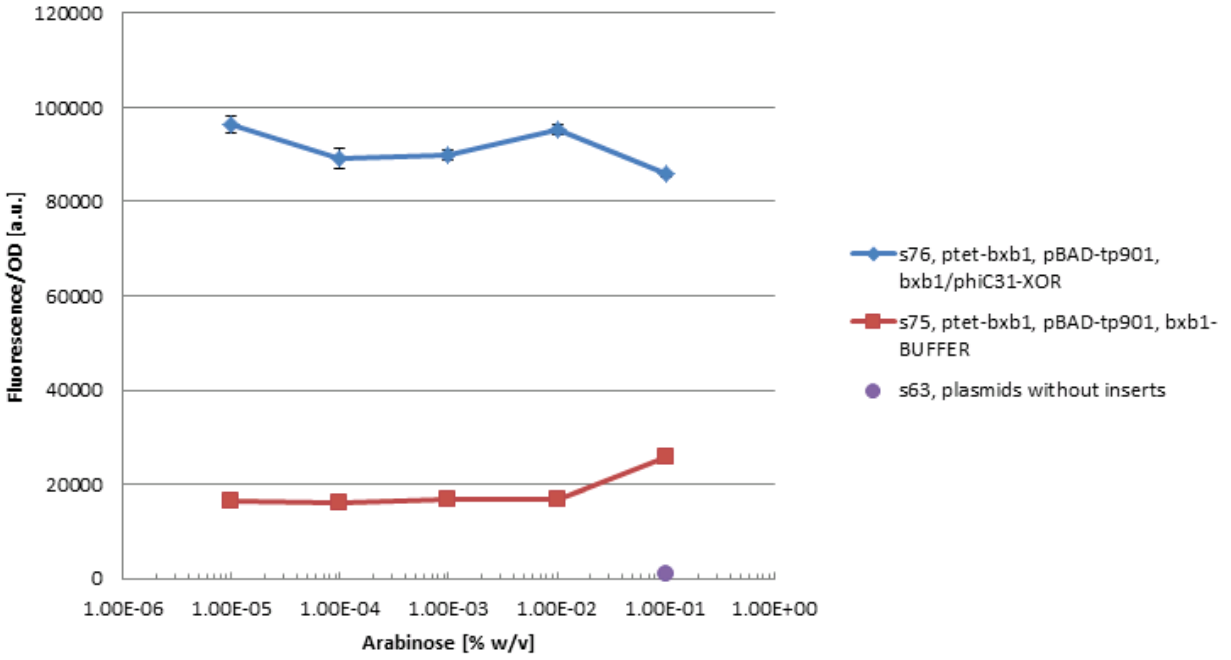


Fig. 2 siG0075 and siG0076 dose-response curve of overnight culture

Interpretation of Data:

- activation of the tp901 BUFFER gate could be observed (Fig. 1), however it is a weak signal and only ON/OFF ration of ~2
- the bxb1 BUFFER and the bxb1/phiC31 XOR gates are supposed to be ON due to constitutive production of Bxb1, however due to the lack of a negative control this is hard to determine

Experiment T32

Integrases on addgene plasmids with our Gates
siG0073: ptet-bxb1, pBAD-tp901, tp901-BUFFER
siG0077: plac-phiC31, bxb1-BUFFER
siG0078: plac-phiC31, tp901-BUFFER
siG0079: plac-phiC31, bxb1/phiC31-XOR
2014-09-22

Goal of the experiment:

- Determine whether gate switching can be observed
- Is there integrase crosstalk?
- Can ON be distinguished from OFF?

Experimental SetUp:

- **gain 141**
- 96-well plate with overnight cultures diluted 1:1 with PBS
    - Overnight culture inoculated with the three strains in 6 different dilutions of arabinose (for siG0073) and IPTG (for the other three) in LB:
        - 0, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} % w/v arabinose
        - 0, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M IPTG

Machines used:

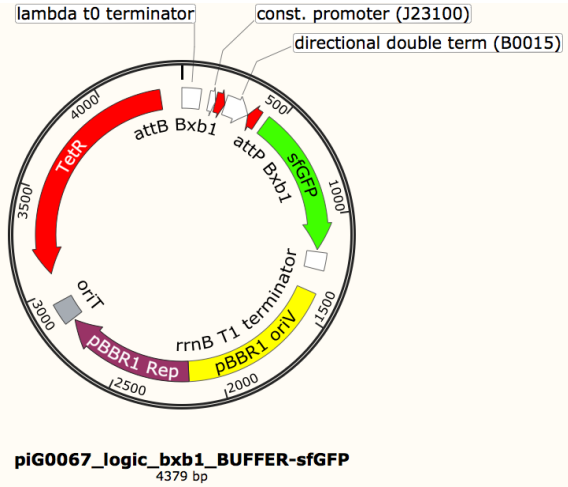
- Tecan infinite M200 PRO

Raw Data:

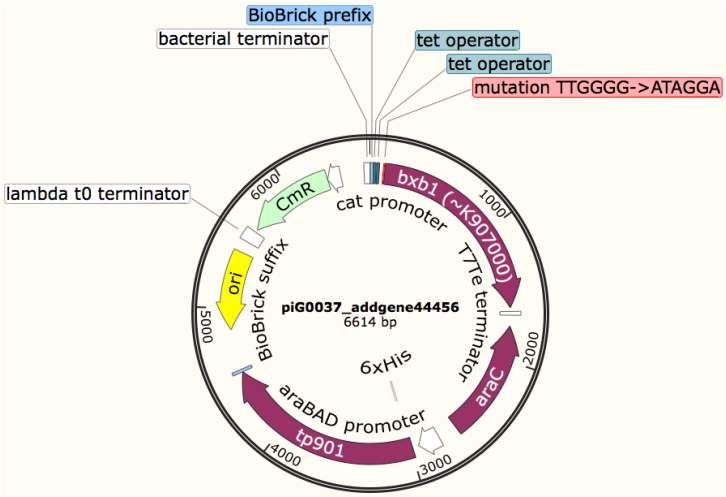
- Lab/Microtiterplate/integrase/20140922_s77,78,79_IPTG_73_ara.xlsx

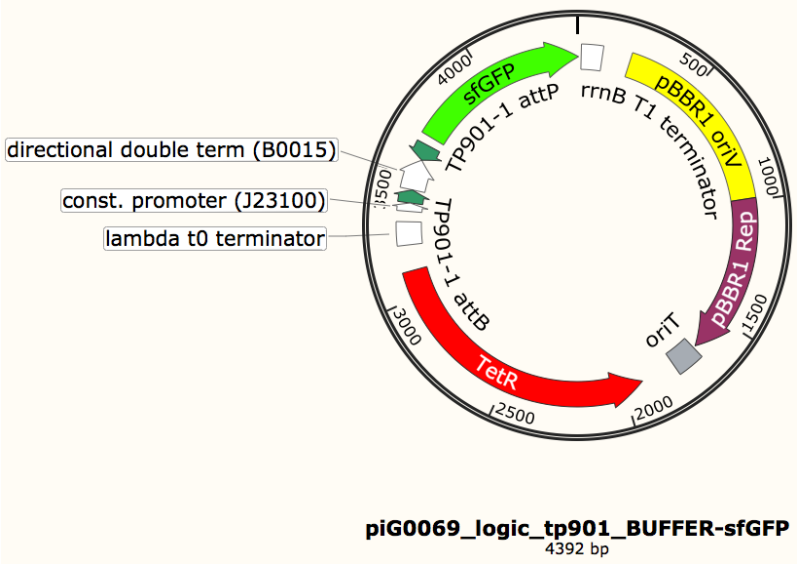
Plasmids in play:

- piG0067

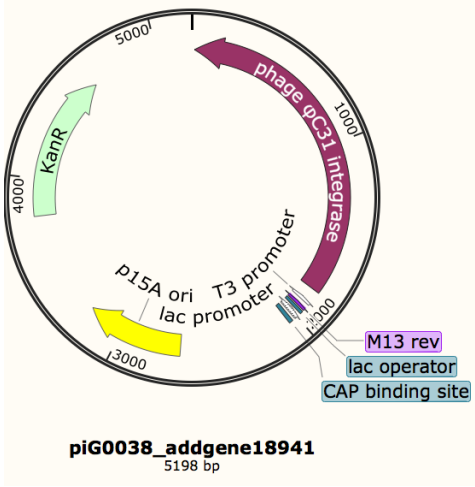


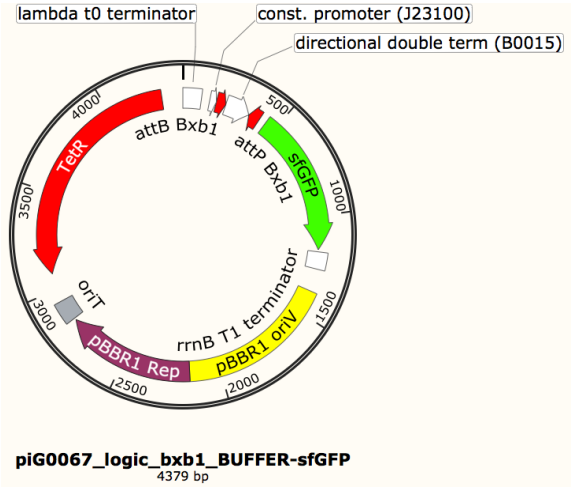
- siG0073: piG0037, piG0069



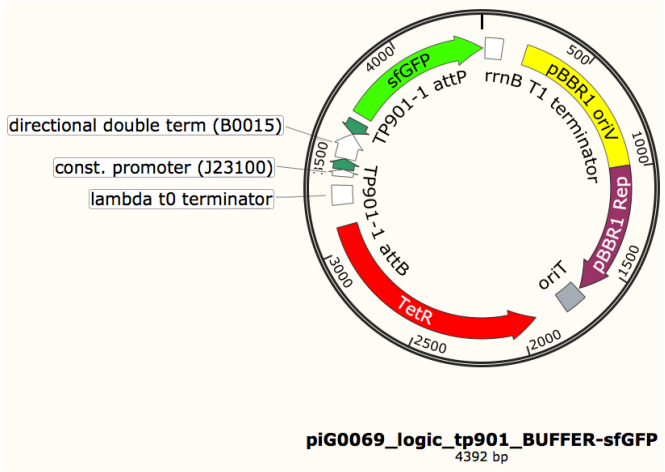


- siG0077: piG0038, piG0067

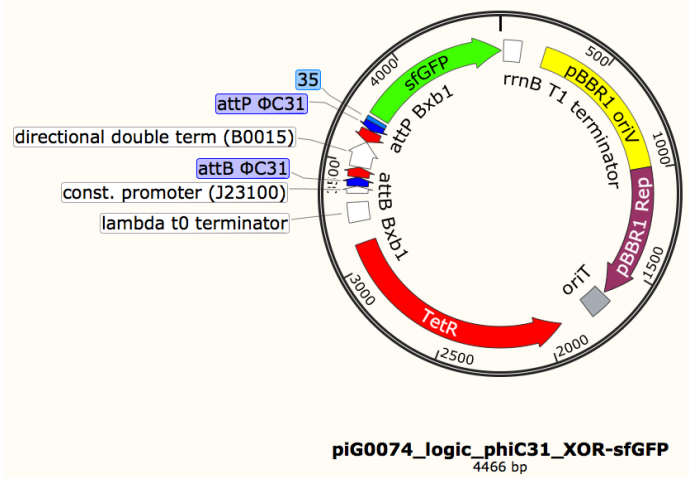




- siG0078: piG0038, piG0069



- siG0079: piG0038, piG0074



Graphs of Data:

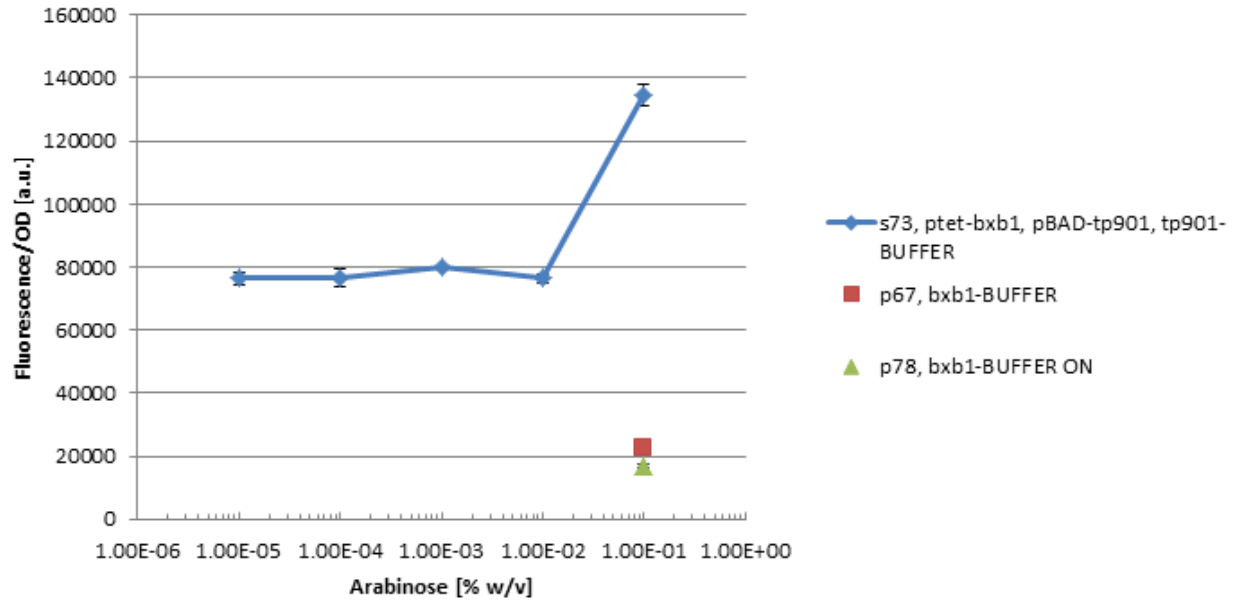


Fig. 1 siG0073 dose-response curve of overnight culture to different arabinose concentrations, compare to previous experiment T31

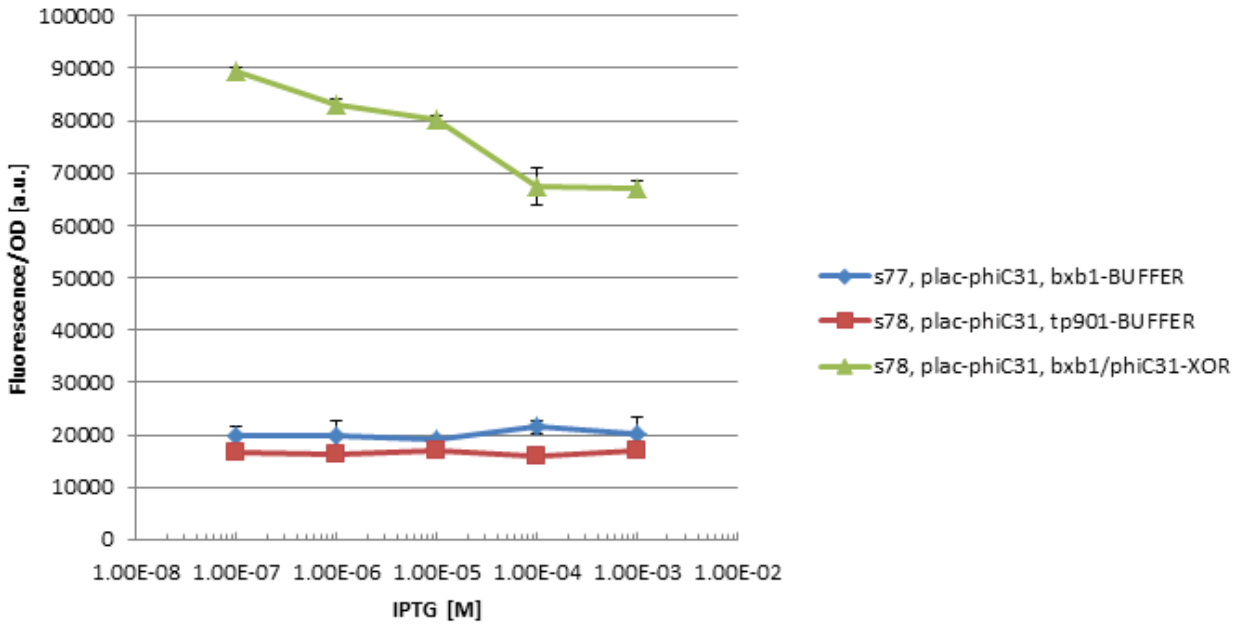


Fig. 2 siG0077, siG0078, and siG0079 dose-response curve of overnight culture

Interpretation of Data:

- the tp901 gate could again be activated with Tp901 (Fig. 1), however the shape of the response curve is different to the previous experiment T31. The curved cannot be directly compared though, since the gain was different.
- the activation of PhiC31 seems to be inversely related to the IPTG concentration, wait for FACS data to draw conclusions

Experiment T33

Dose-Response Kinetics and Crosstalk
siG0080: RhlR optimized RBS, sfGFP under plux Promoter and standard RBS
2014-09-24

Goal of the experiment:

- Investigate crosstalk on the regulator level
- Does RhlR also activate the plux promoter?
- Determine dose-response curves
- Record dynamic behavior

Experimental SetUp:

- 96-well plate with LB containing kanamycin (50 µg/mL) and ampicillin (200 µg/mL)
    - Inoculation of 200 µL medium with 5 µL overnight culture (OD_{600} ~1.5)
        - 90 wells with siG0080
        - 3 wells with siG0001
        - 3 wells with LB blank
    - Induction after ~3 hours (longer than usual, due to prolonged lag phase) in triplicates with 10 dilutions of 3OC6-HSL/3OC12-HSL/C4-HSL:
        - 0, 10^{-13}, 10^{-12}, 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M

Machines used:

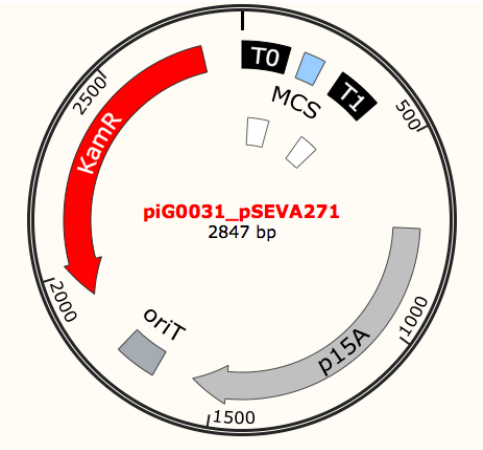
- Tecan infinite M200 PRO

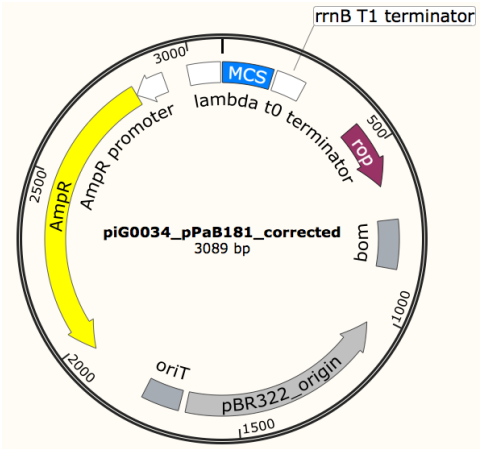
Raw Data:

- Lab/Microtiterplate/crosstalk/20140924_s80_crosstalk.xlsx

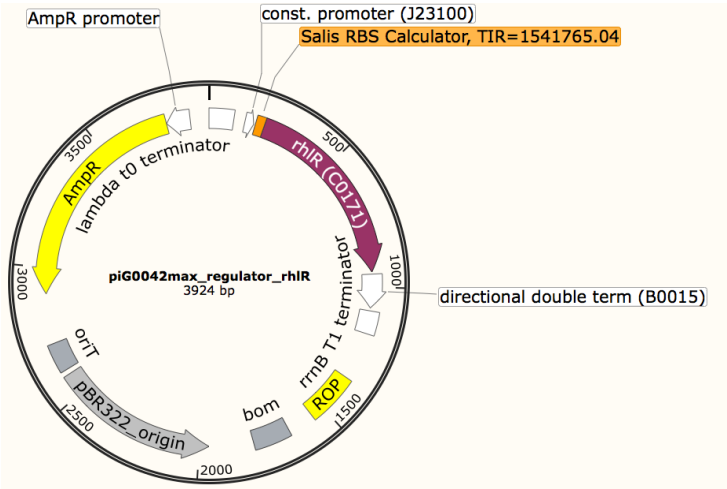
Plasmids in play:

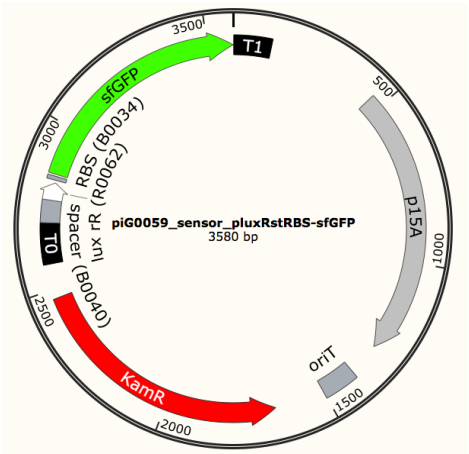
- siG0001: piG0031, piG0034





- siG0080: piG0042max, piG0059





Graphs of Data:

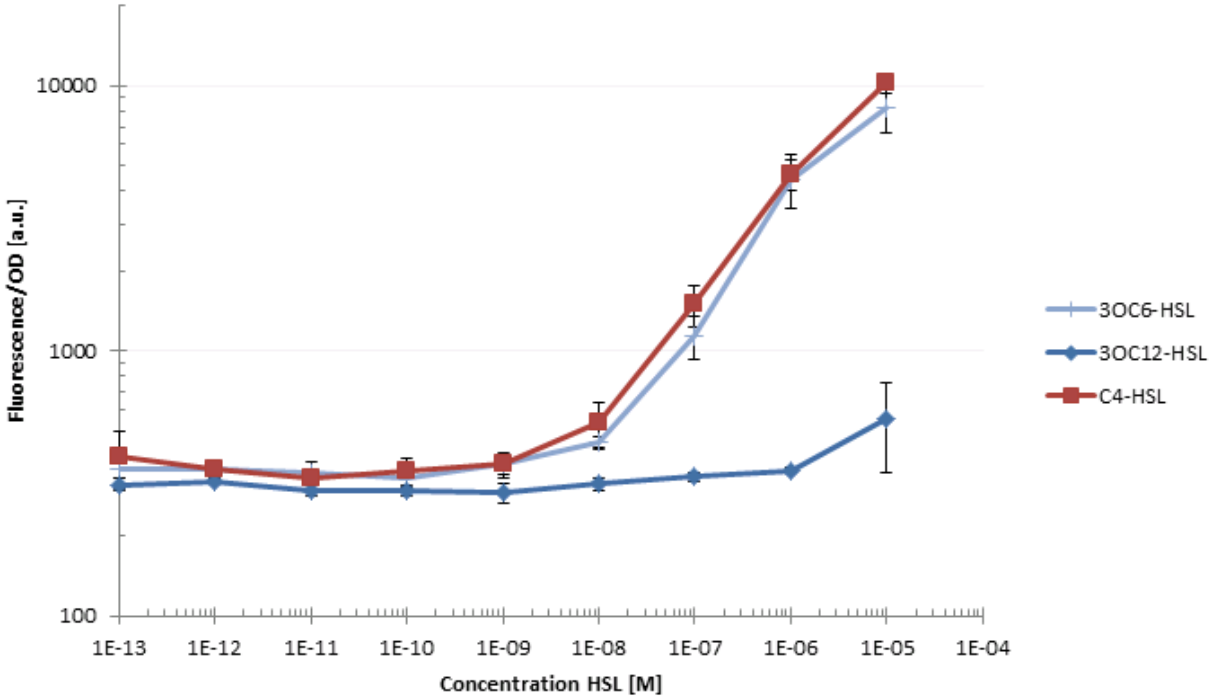


Fig. 1 siG0080 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

- RhlR activates plux strongly, ~30 times ON/OFF, with C4-HSL and 3OC6-HSL (Fig. 1)