Experiments Overview

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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:

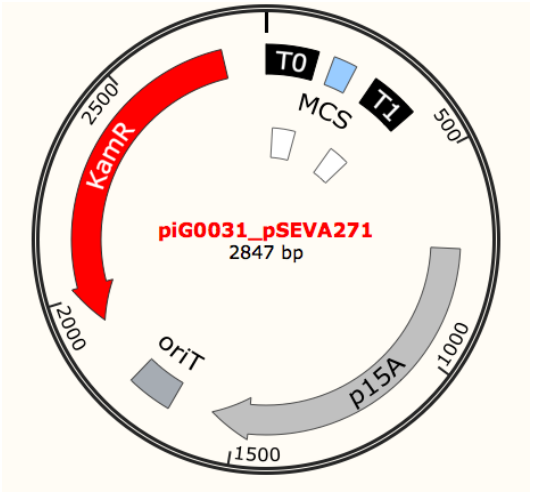
- Tecan infinite M200 PRO

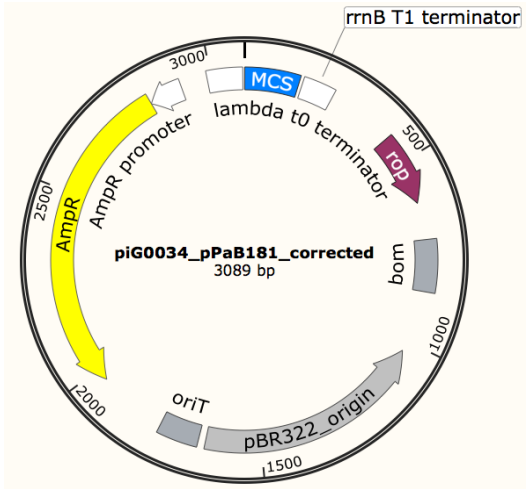
Raw Data:

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

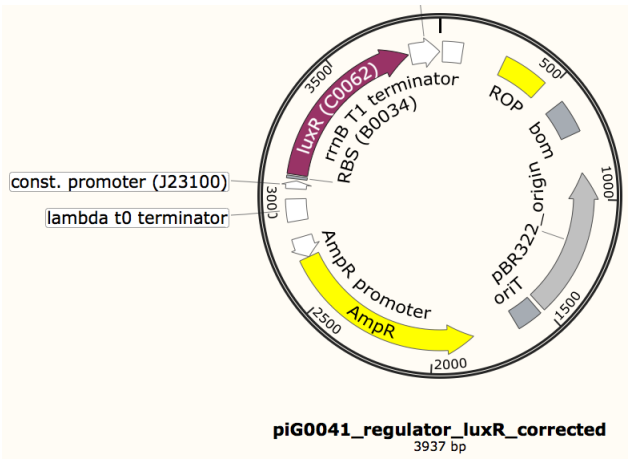
Plasmids in play:

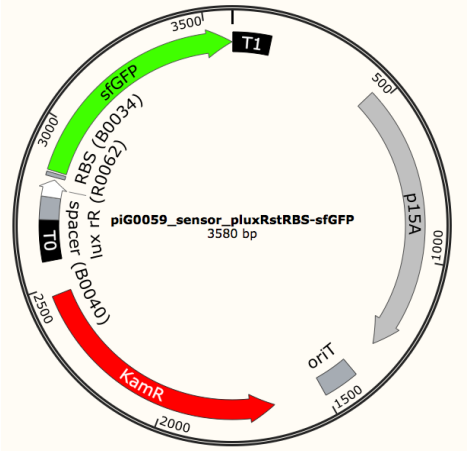
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





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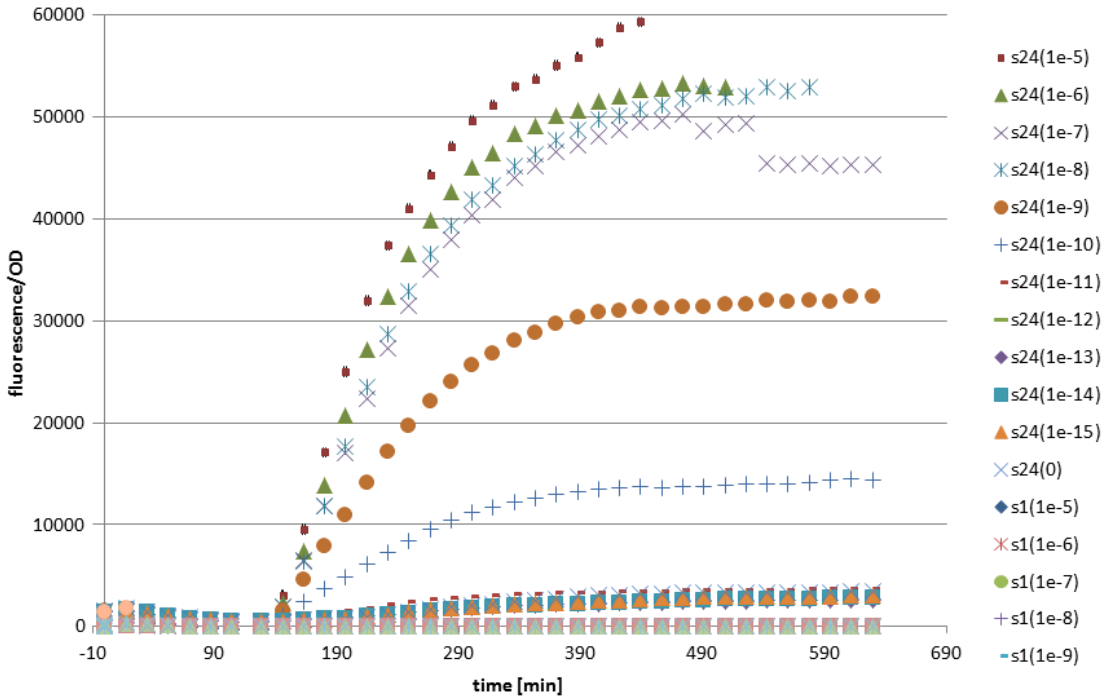

Fig. 1 siG0001 and siG0024 responding to different lux AHL concentrations over time

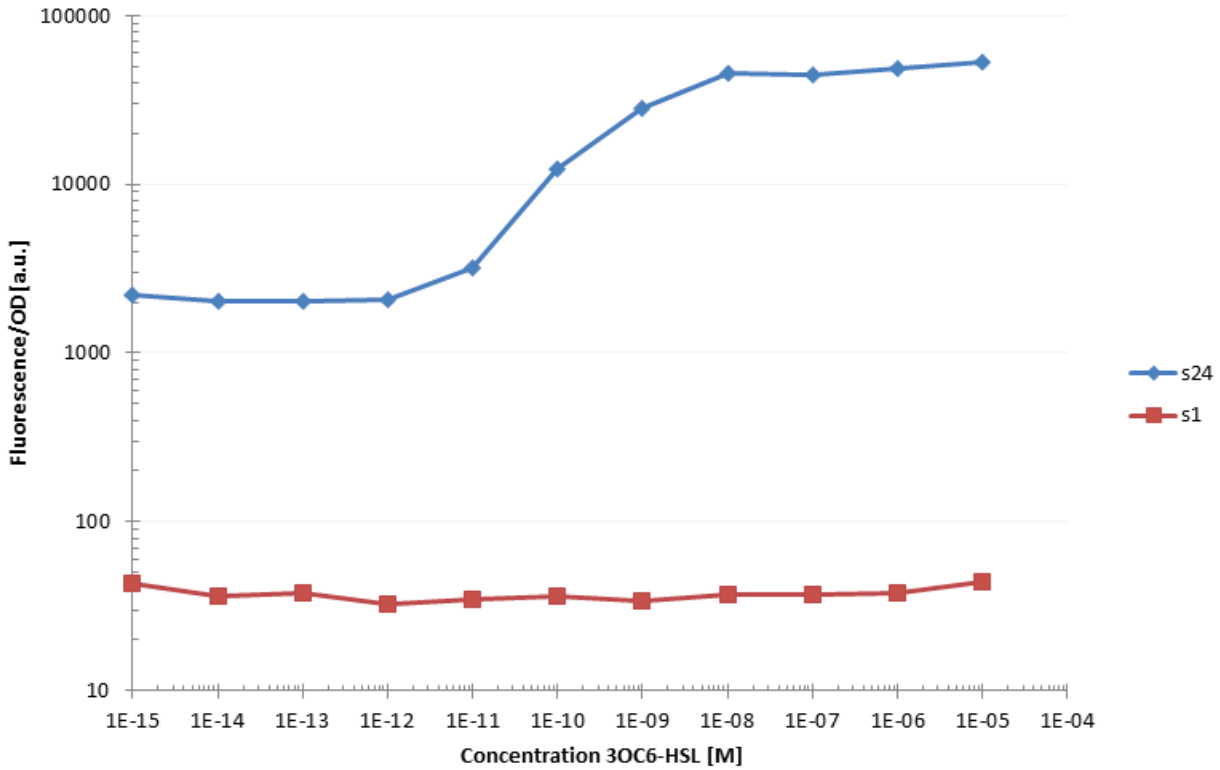

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Fig. 2 siG0001 and siG0024 dose-response curve 200 min after induction

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:

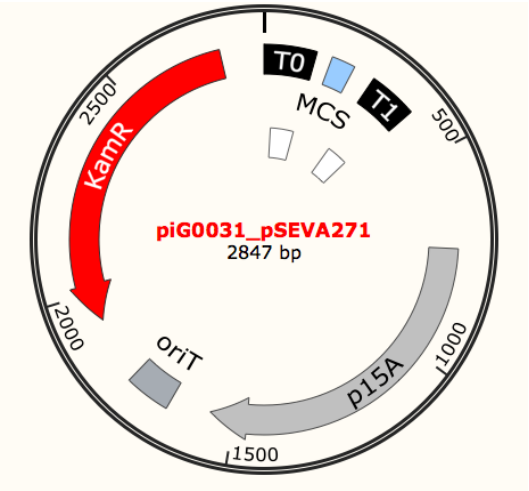
- Tecan infinite M200 PRO

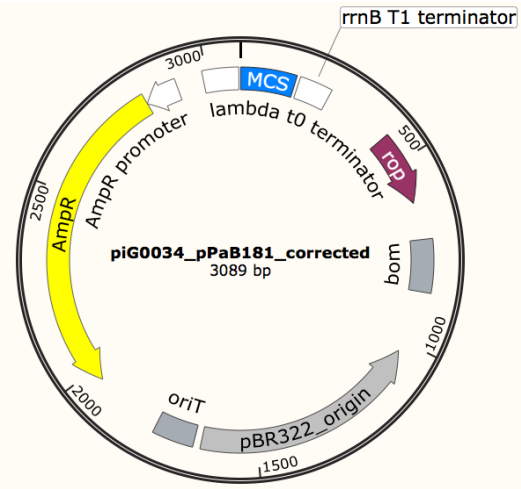
Raw Data:

- Lab/Microtiterplate/crosstalk/20140807_s24_crosstalk.xlsx

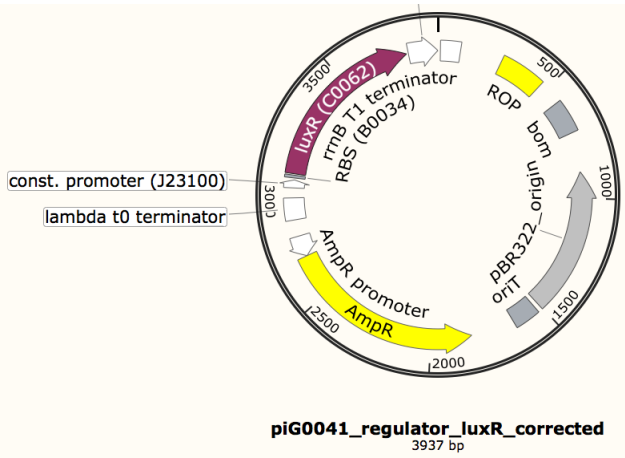
Plasmids in play:

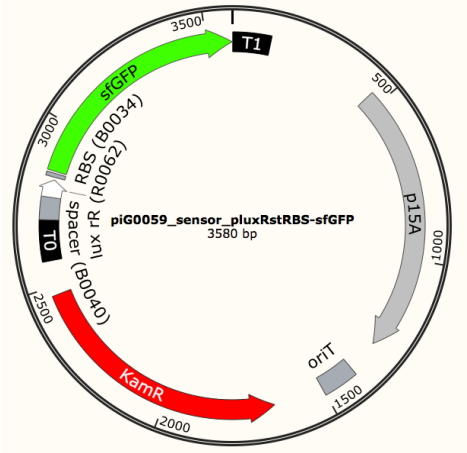
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





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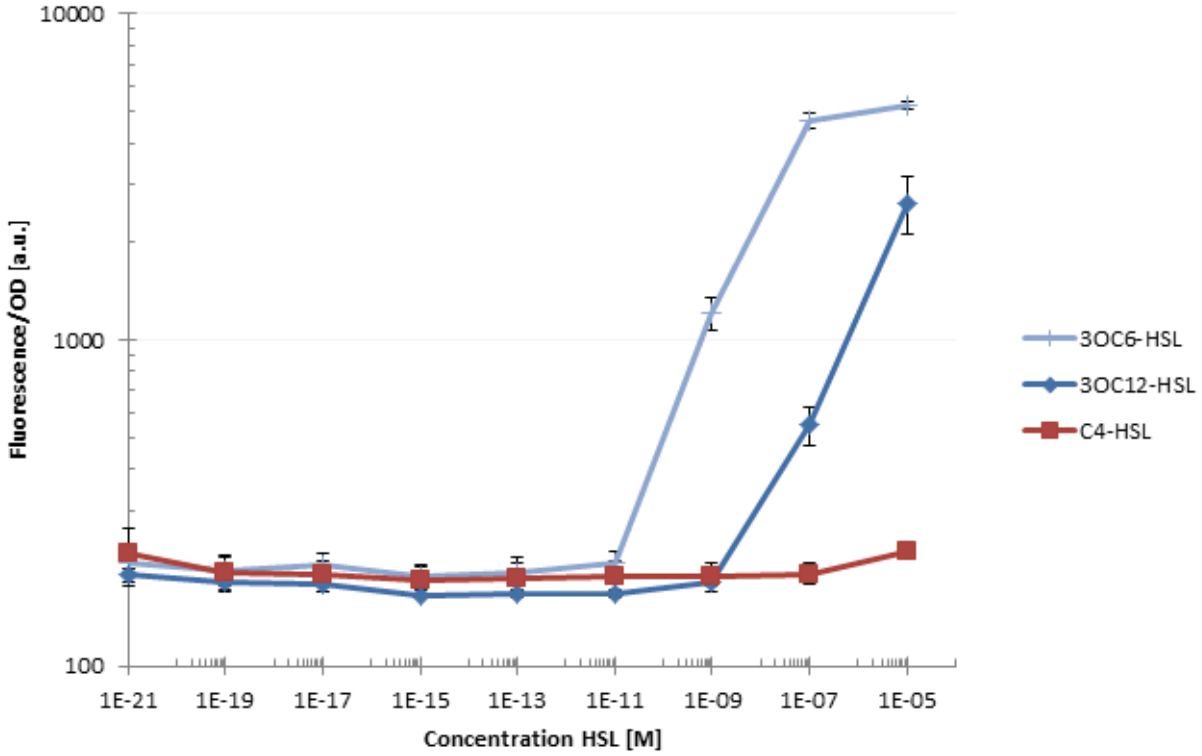


Fig. 1 siG0024 dose-response curve 200 min after induction for three AHL molecules

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:

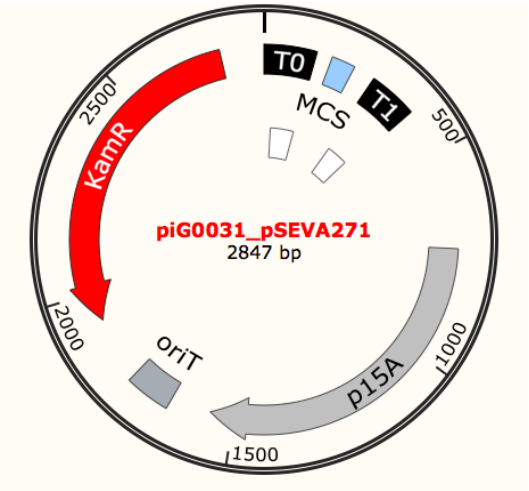
- Tecan infinite M200 PRO

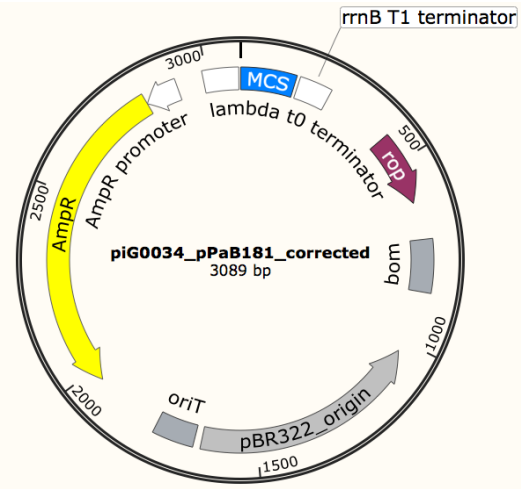
Raw Data:

- Lab/Microtiterplate/crosstalk/20140808_s24_crosstalk.xlsx

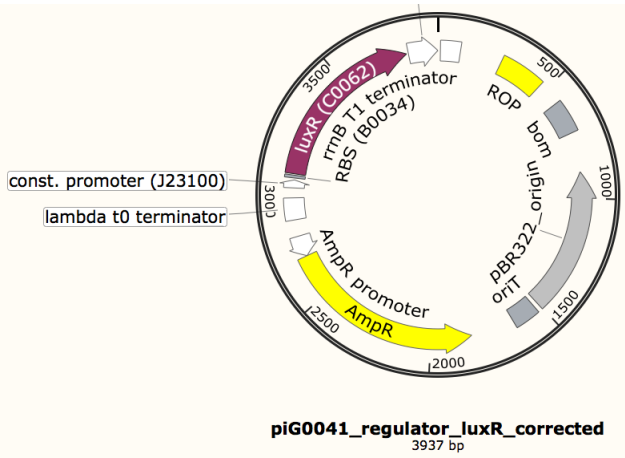
Plasmids in play:

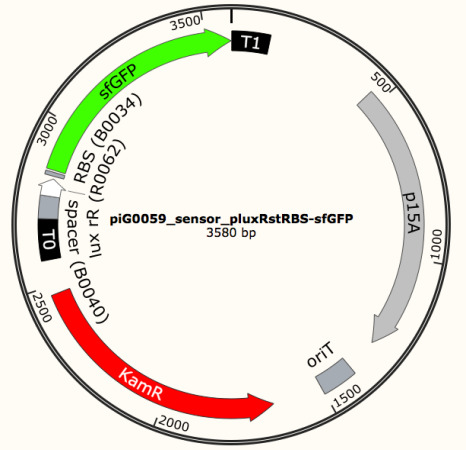
- siG0001: piG0031, piG0034





- siG0024: piG0041, piG0059





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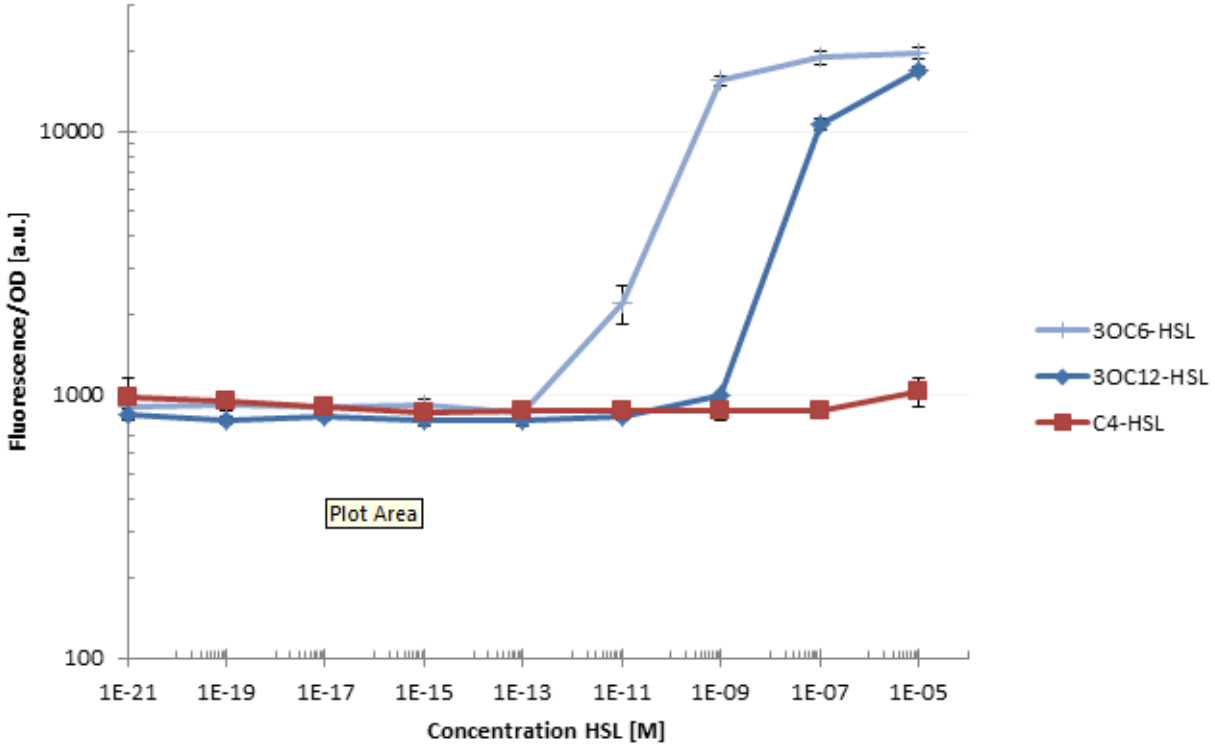


Fig. 1 siG0024 dose-response curve 200 min after induction for three AHL molecules

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:

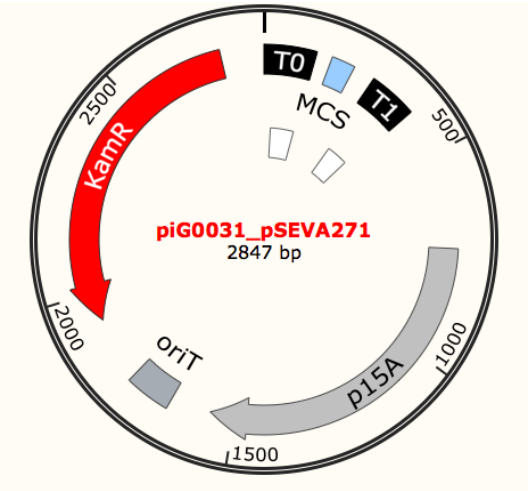
- Tecan infinite M200 PRO

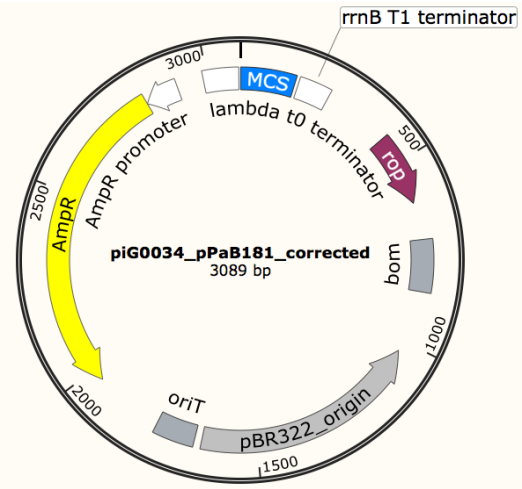
Raw Data:

- Lab/Microtiterplate/crosstalk/20140808_s42_crosstalk.xlsx

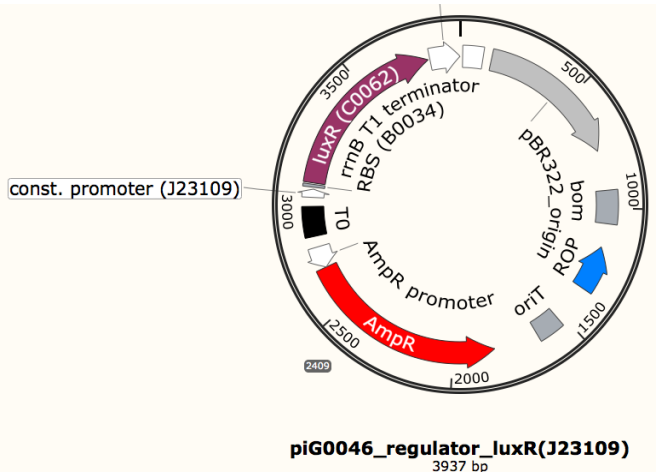
Plasmids in play:

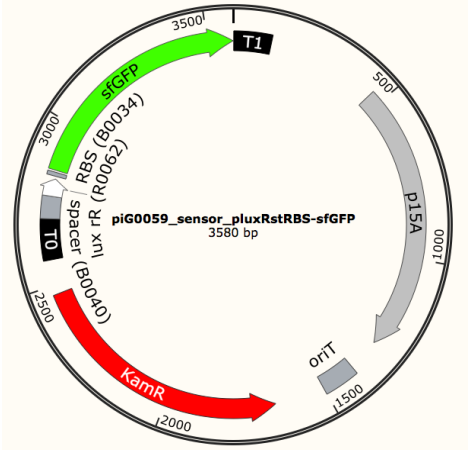
- siG0001: piG0031, piG0034





- siG0042: piG0046, piG0059





Graphs of Data:

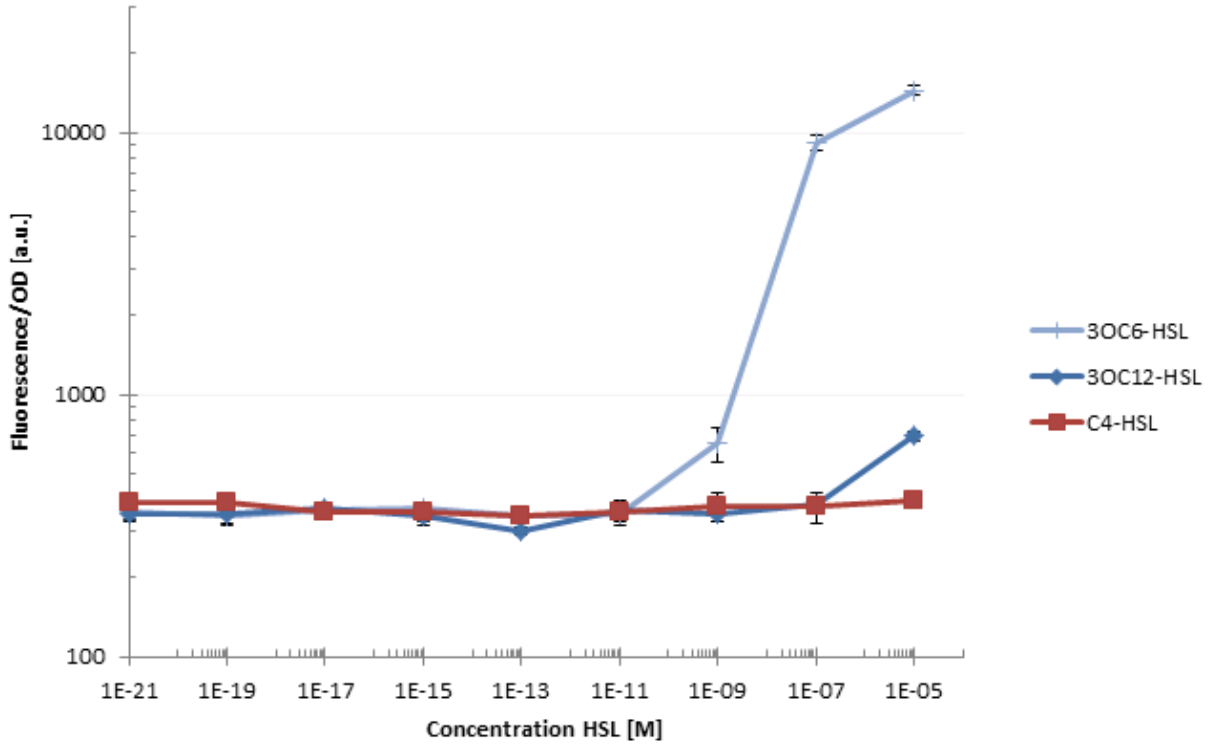


Fig. 1 siG0042 dose-response curve 200 min after induction for three AHL molecules

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:

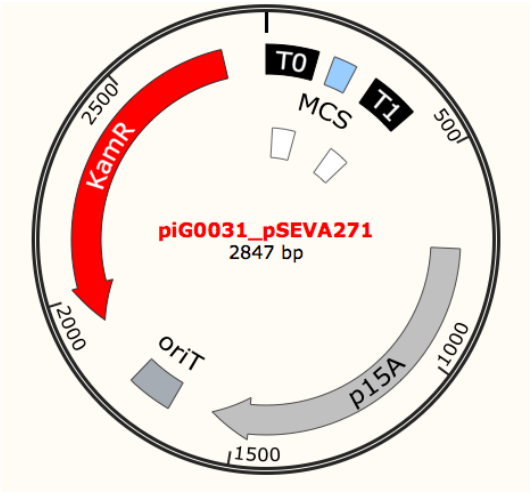
- Tecan infinite M200 PRO

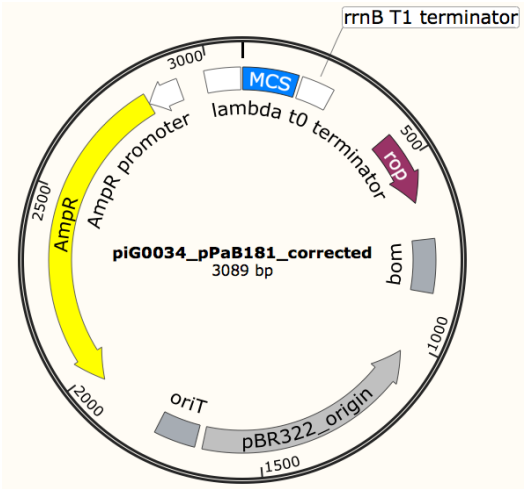
Raw Data:

- Lab/Microtiterplate/crosstalk/20140809_s14_crosstalk.xlsx

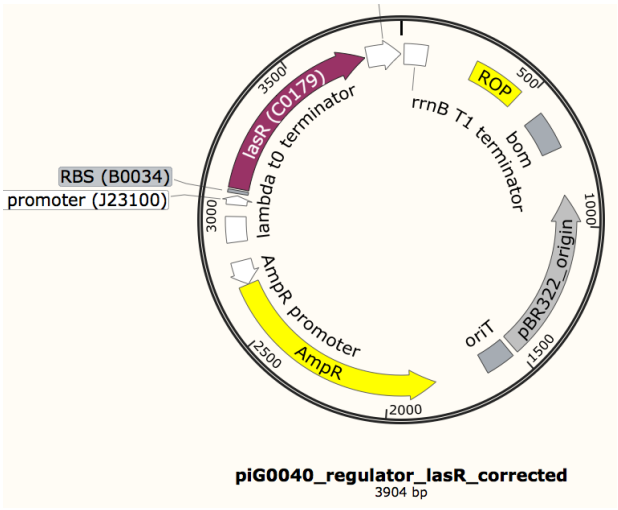
Plasmids in play:

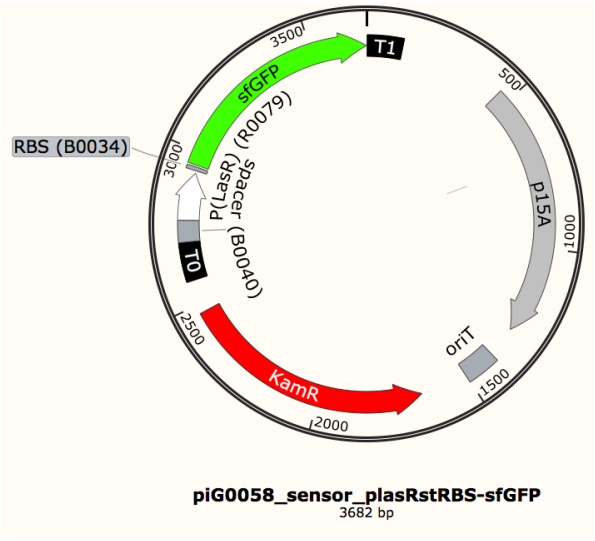
- siG0001: piG0031, piG0034





- siG0014: piG0040, piG0058





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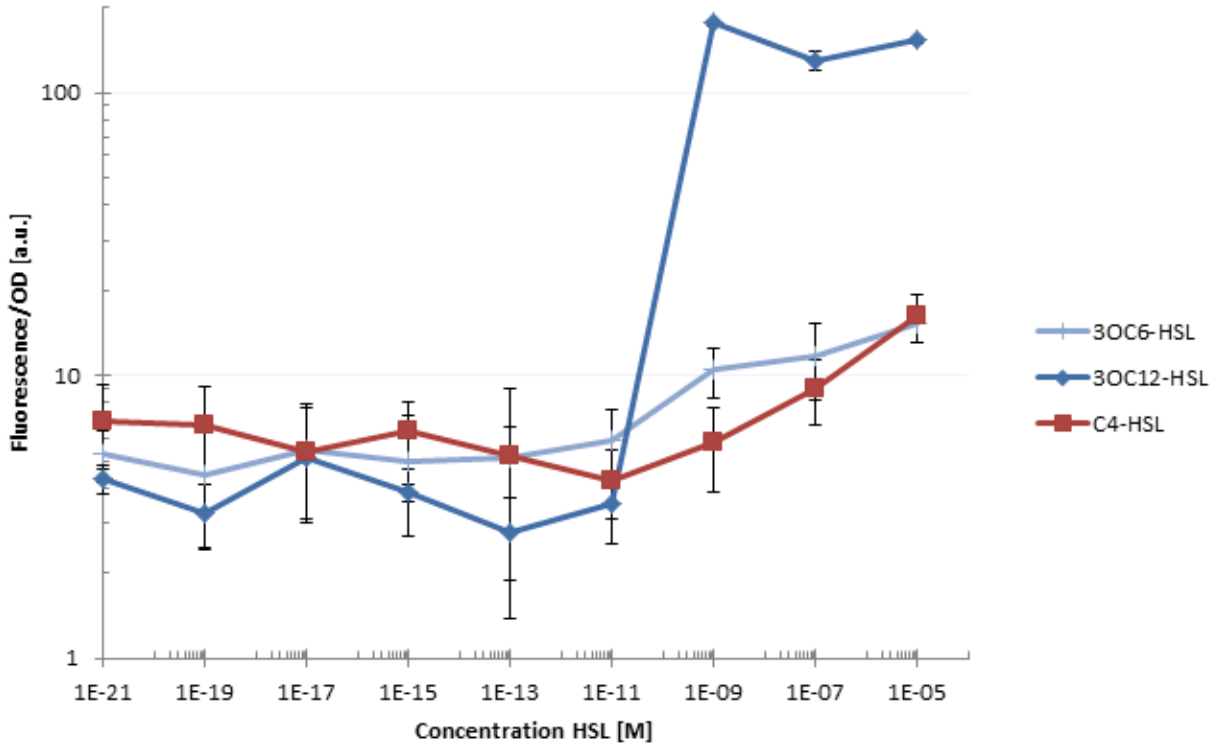


Fig. 1 siG0014 dose-response curve 200 min after induction for three AHL molecules

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:

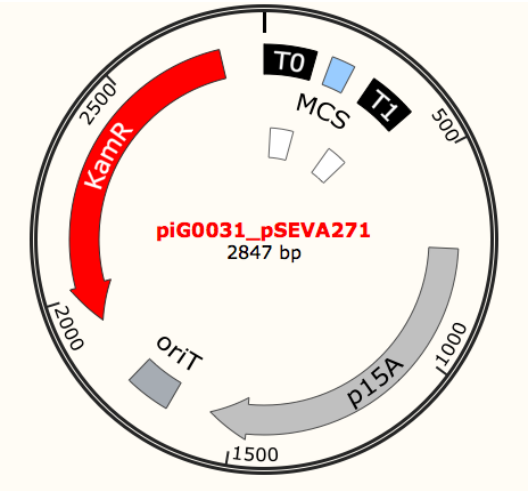
- Tecan infinite M200 PRO

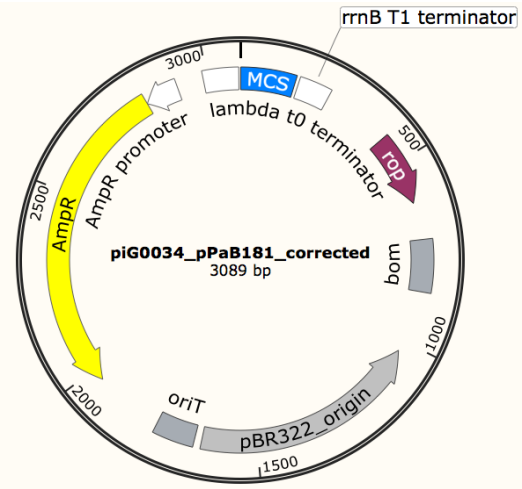
Raw Data:

- Lab/Microtiterplate/crosstalk/20140809_s48_crosstalk.xlsx

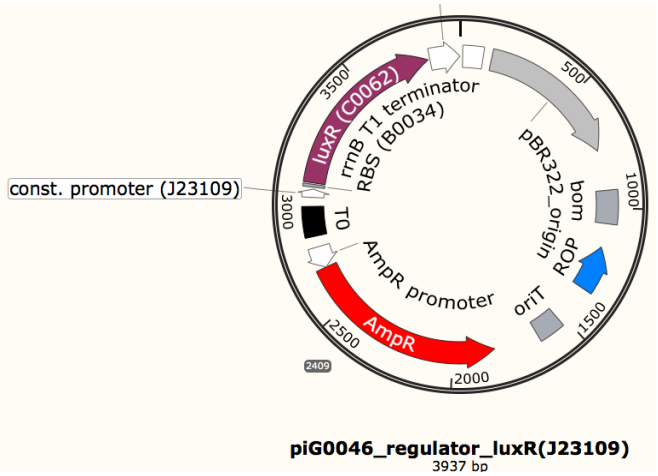
Plasmids in play:

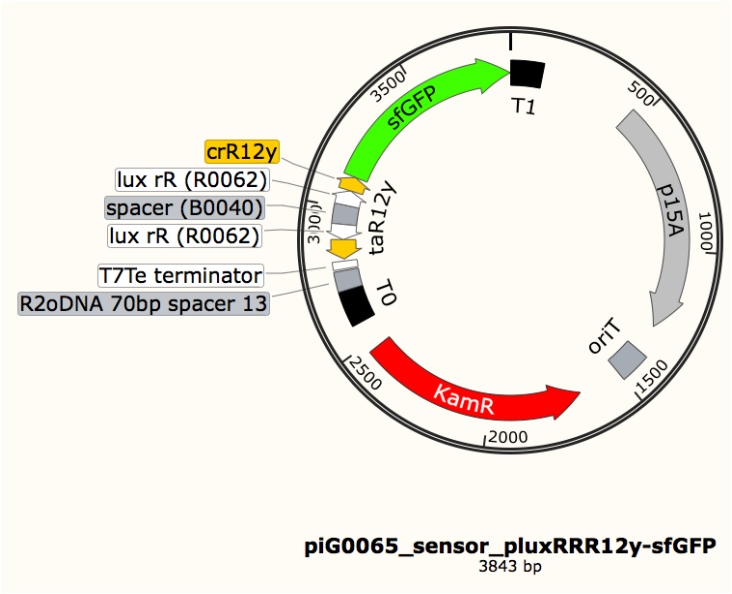
- siG0001: piG0031, piG0034





- siG0048: piG0046, piG0065





Graphs of Data:

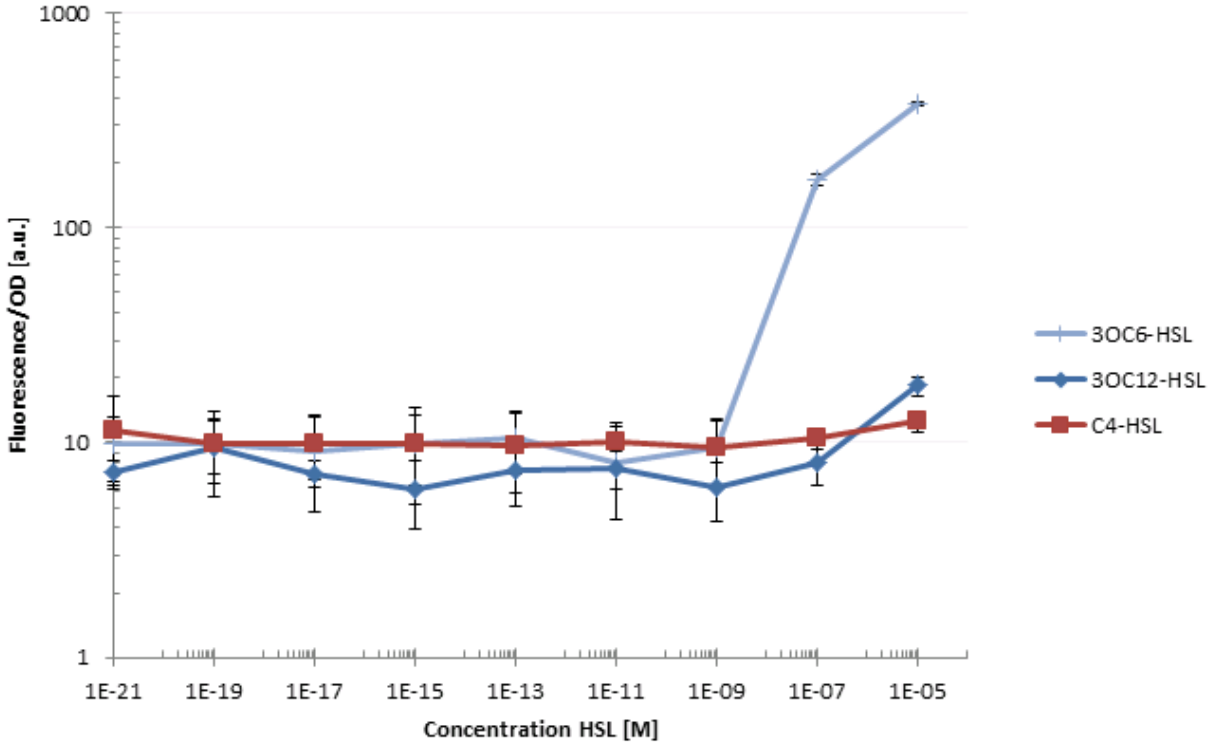


Fig. 1 siG0048 dose-response curve 200 min after induction for three AHL molecules

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:

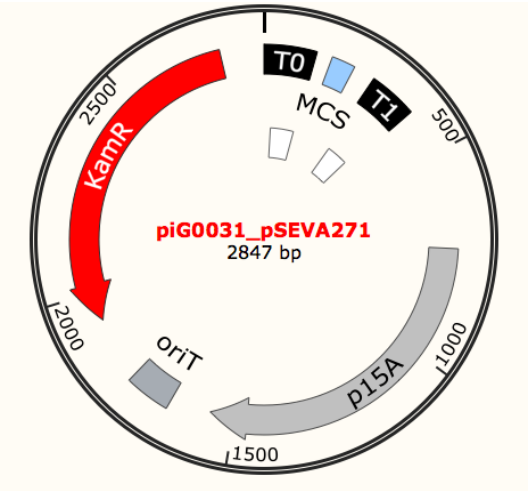
- Tecan infinite M200 PRO

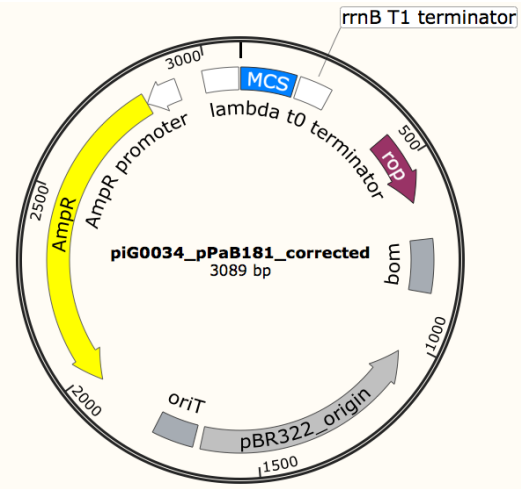
Raw Data:

- Lab/Microtiterplate/crosstalk/20140810_s51_crosstalk.xlsx

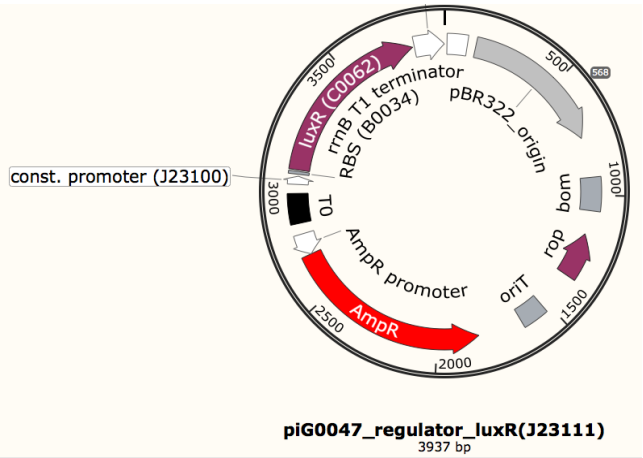
Plasmids in play:

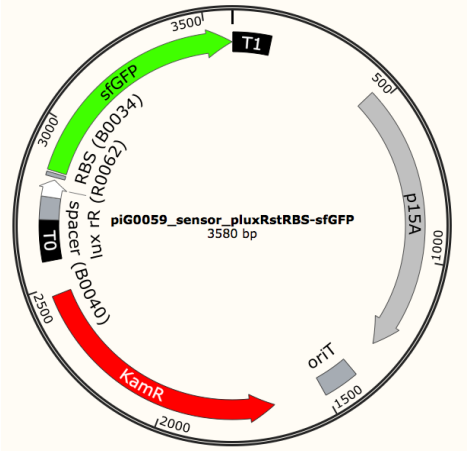
- siG0001: piG0031, piG0034





- siG0051: piG0047, piG0059





Graphs of Data:

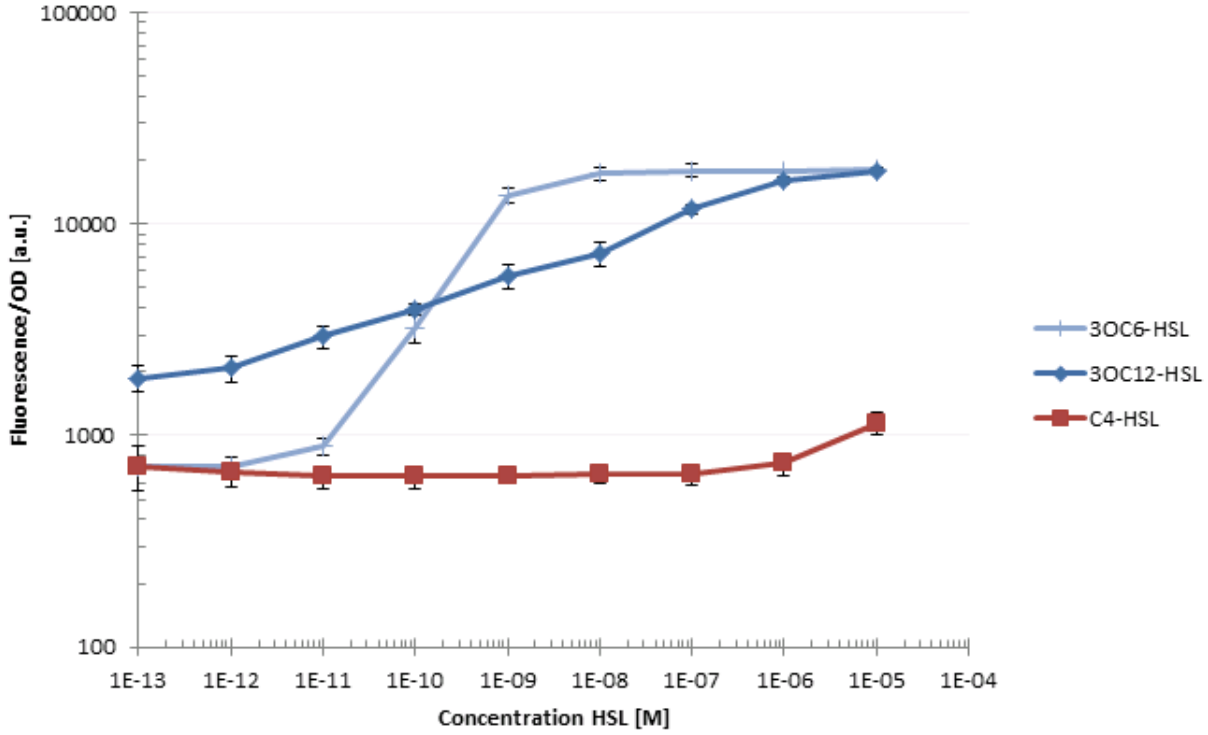


Fig. 1 siG0051 dose-response curve 200 min after induction for three AHL molecules

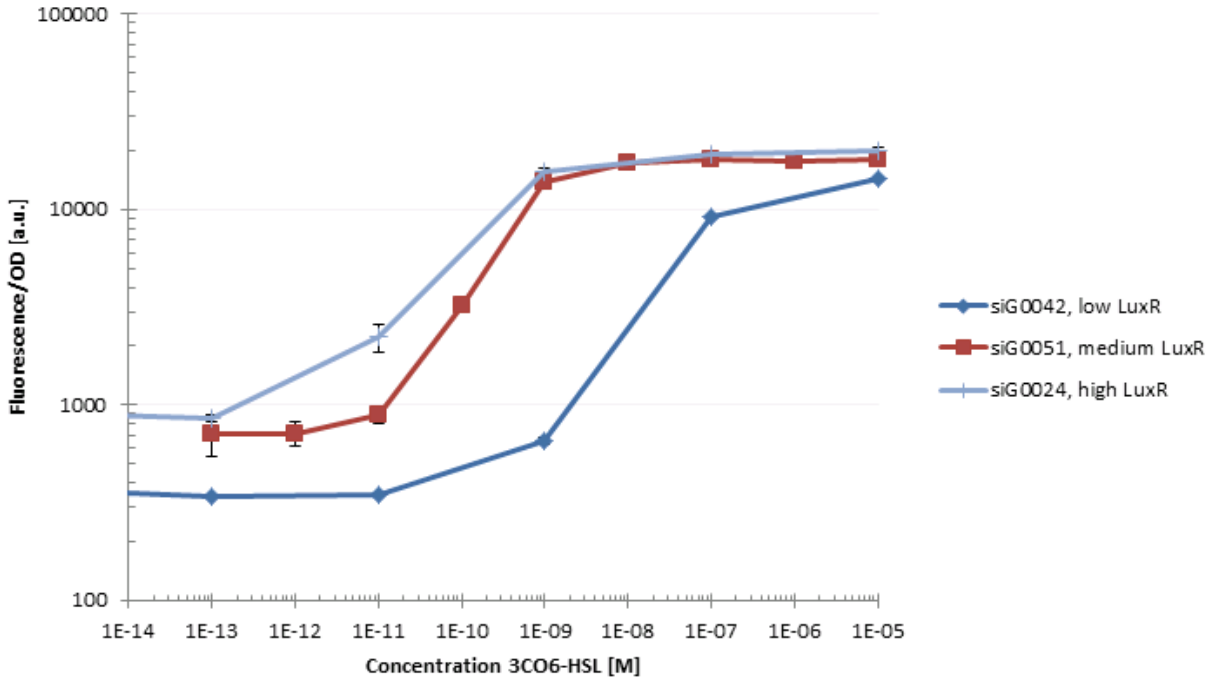


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for the three variants with different promoter strengths for LuxR production

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:

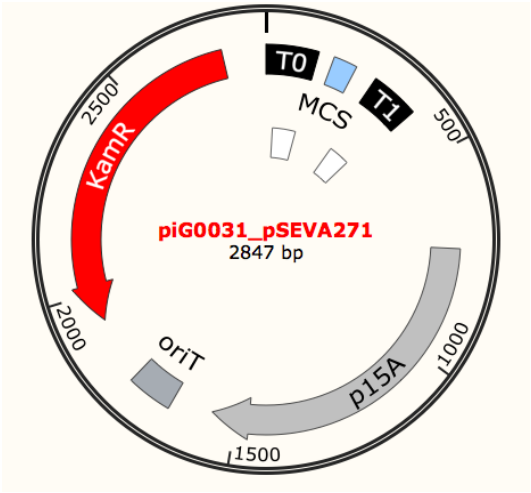
- Tecan infinite M200 PRO

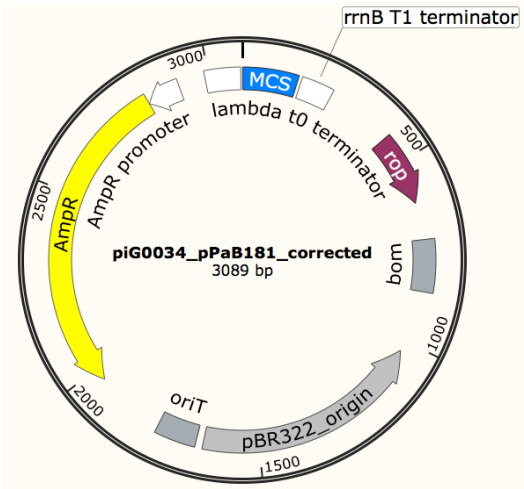
Raw Data:

- Lab/Microtiterplate/crosstalk/20140811_s30_crosstalk.xlsx

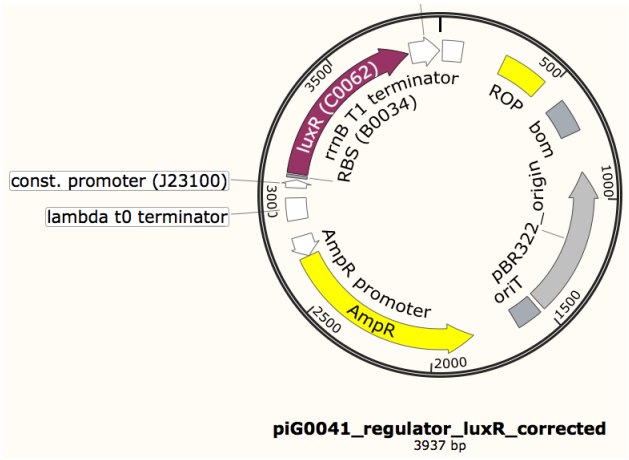
Plasmids in play:

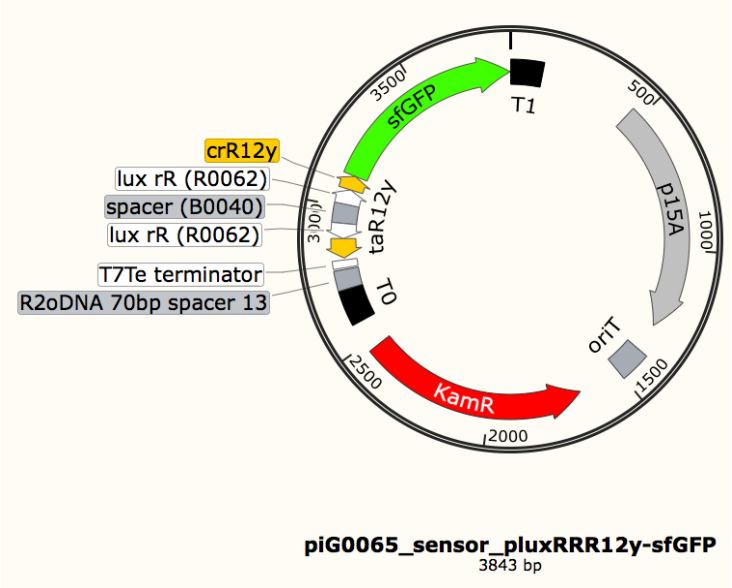
- siG0001: piG0031, piG0034





- siG0030: piG0041, piG0065





Graphs of Data:

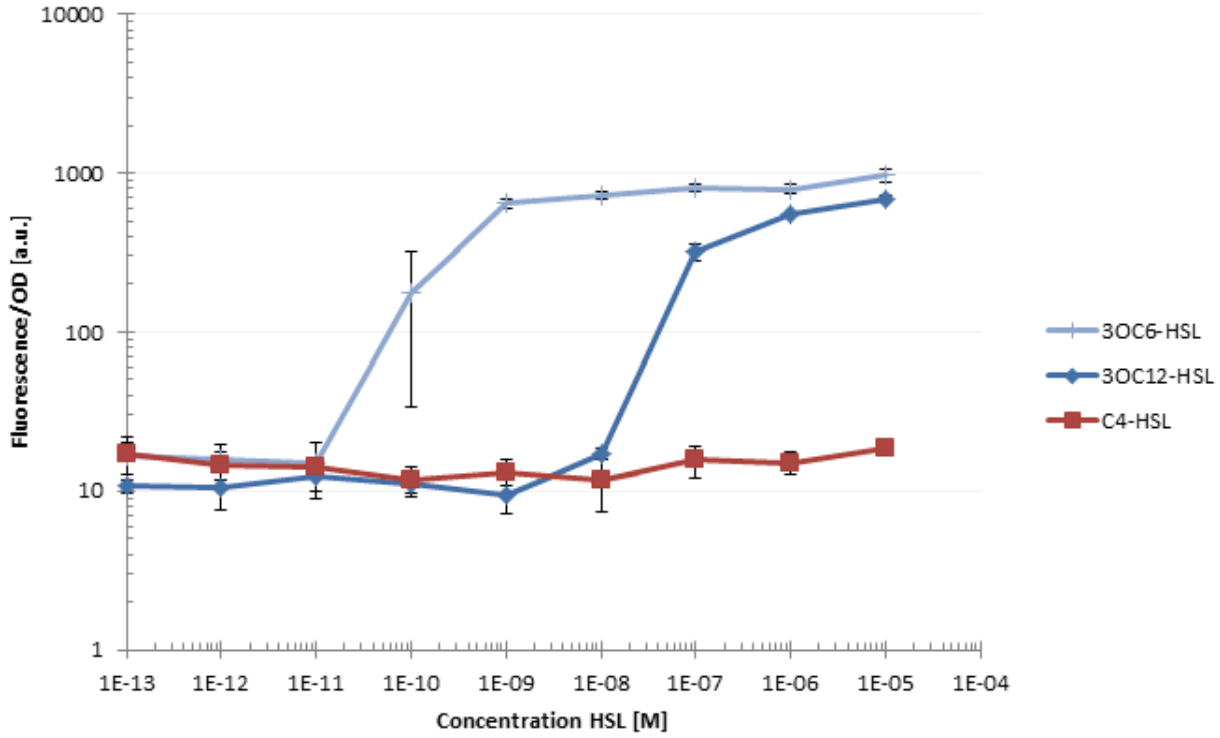


Fig. 1 siG0030 dose-response curve 200 min after induction for three AHL molecules

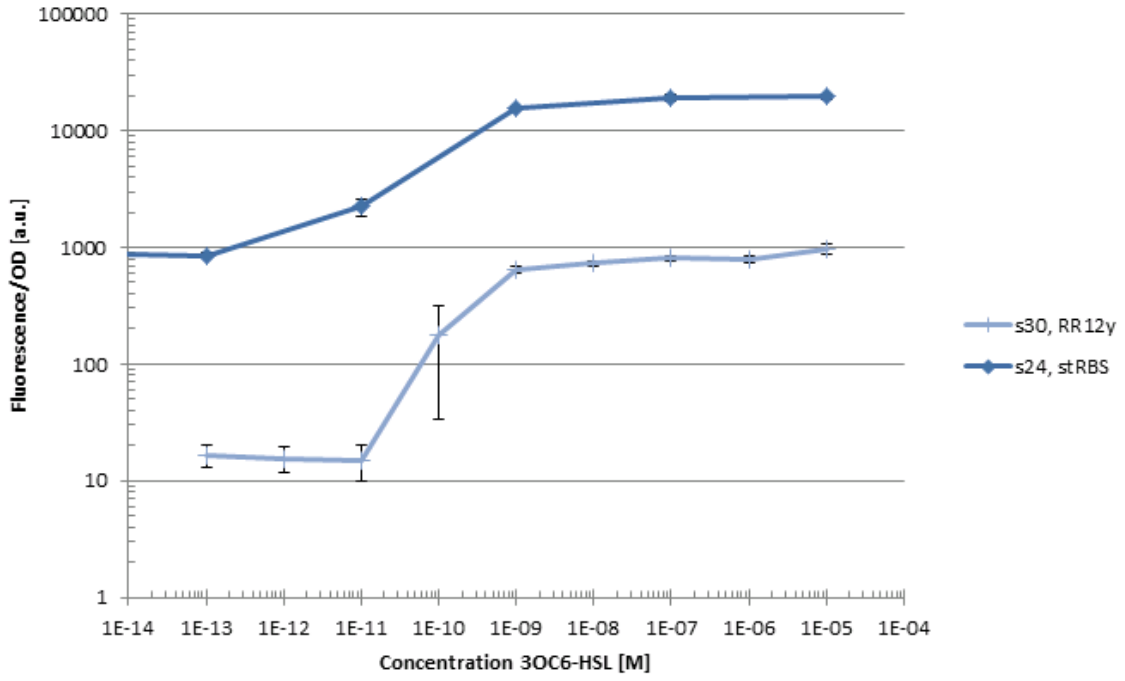


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0024 (without RR12y) and siG0030 (with RR12y)

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:

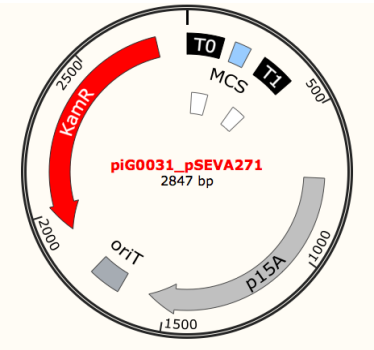
- Tecan infinite M200 PRO

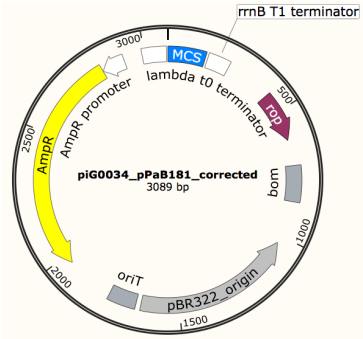
Raw Data:

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

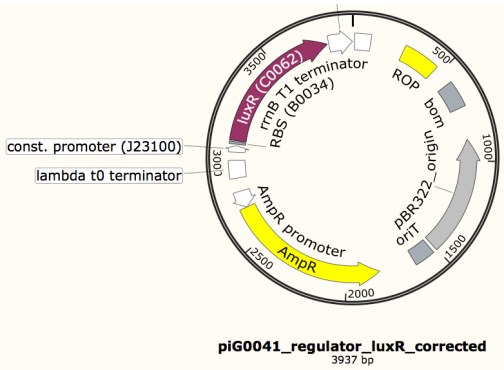
Plasmids in play:

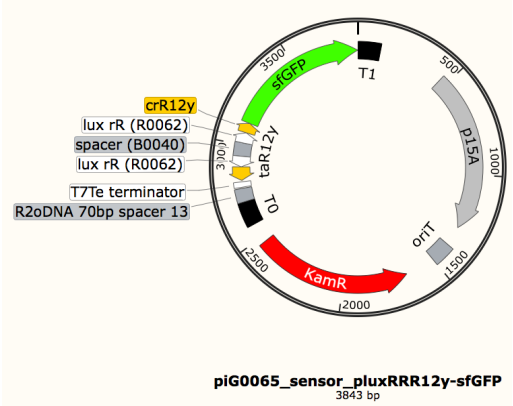
- siG0001: piG0031, piG0034



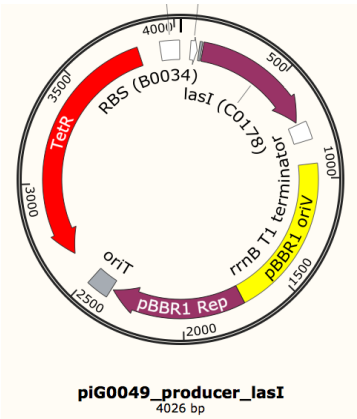


- siG0030: piG0041, piG0065

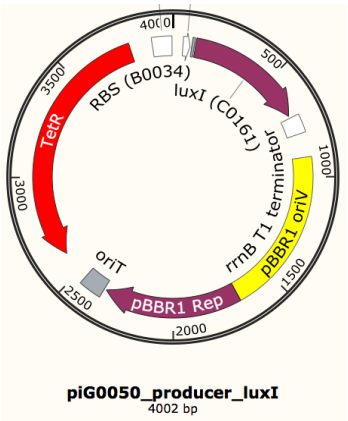




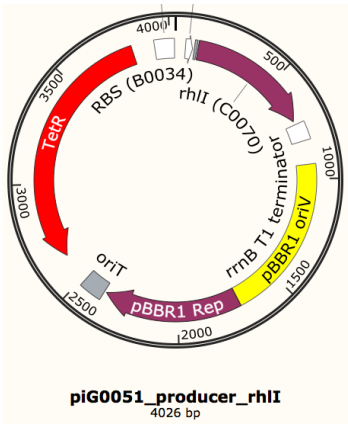
piG0049 (LasI producer)



piG0050 (LuxI producer)



piG0051 (RhlI producer)



Graphs of Data:

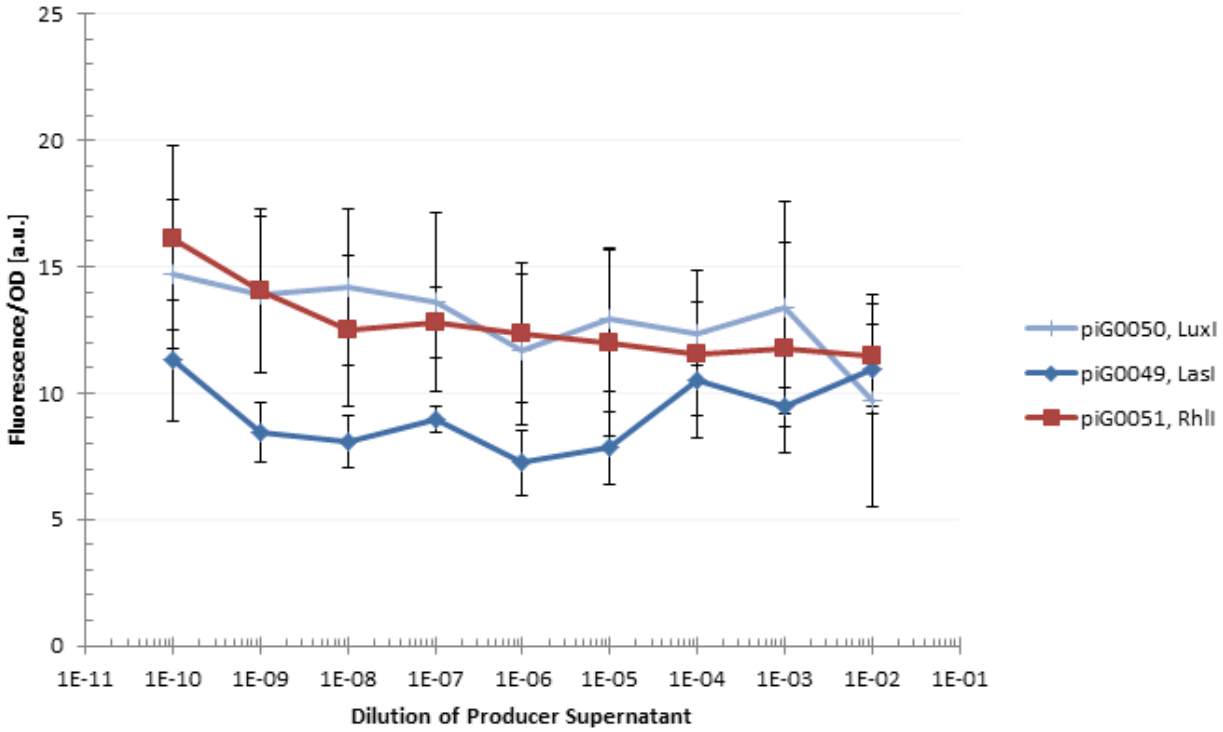


Fig. 1 siG0030 dose-response curve 200 min after induction with the three different producer supernatants

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:

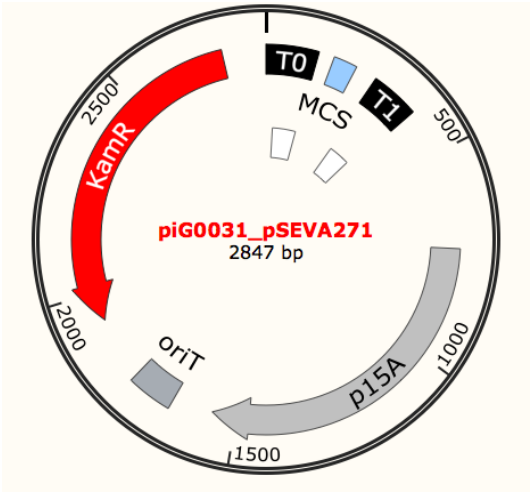
- Tecan infinite M200 PRO

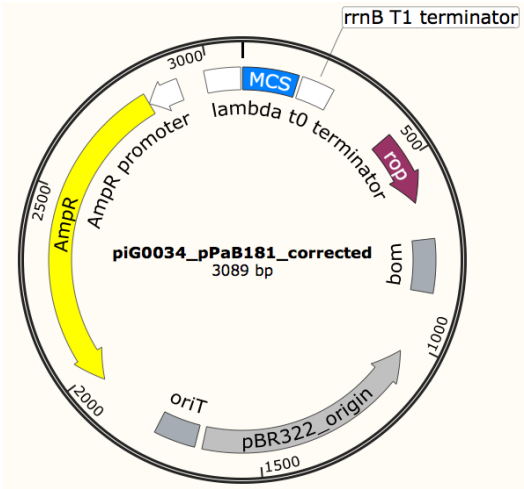
Raw Data:

- Lab/Microtiterplate/crosstalk/20140812_s14_crosstalk.xlsx

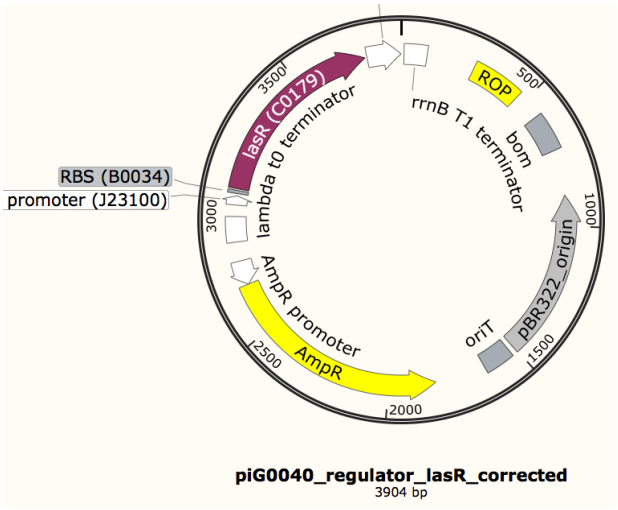
Plasmids in play:

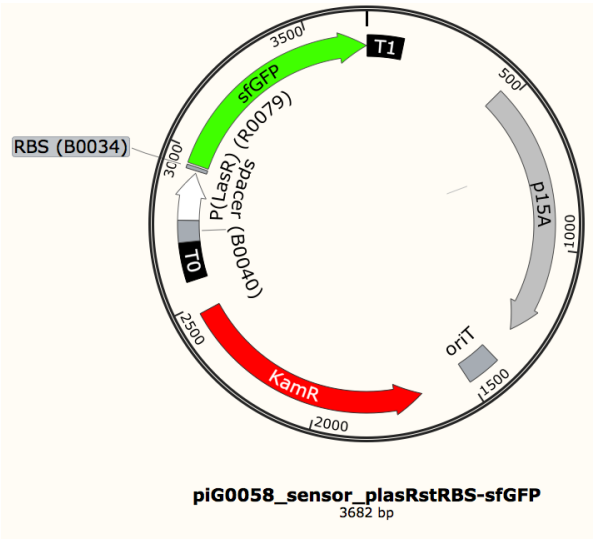
- siG0001: piG0031, piG0034





- siG0014: piG0040, piG0058





Graphs of Data:

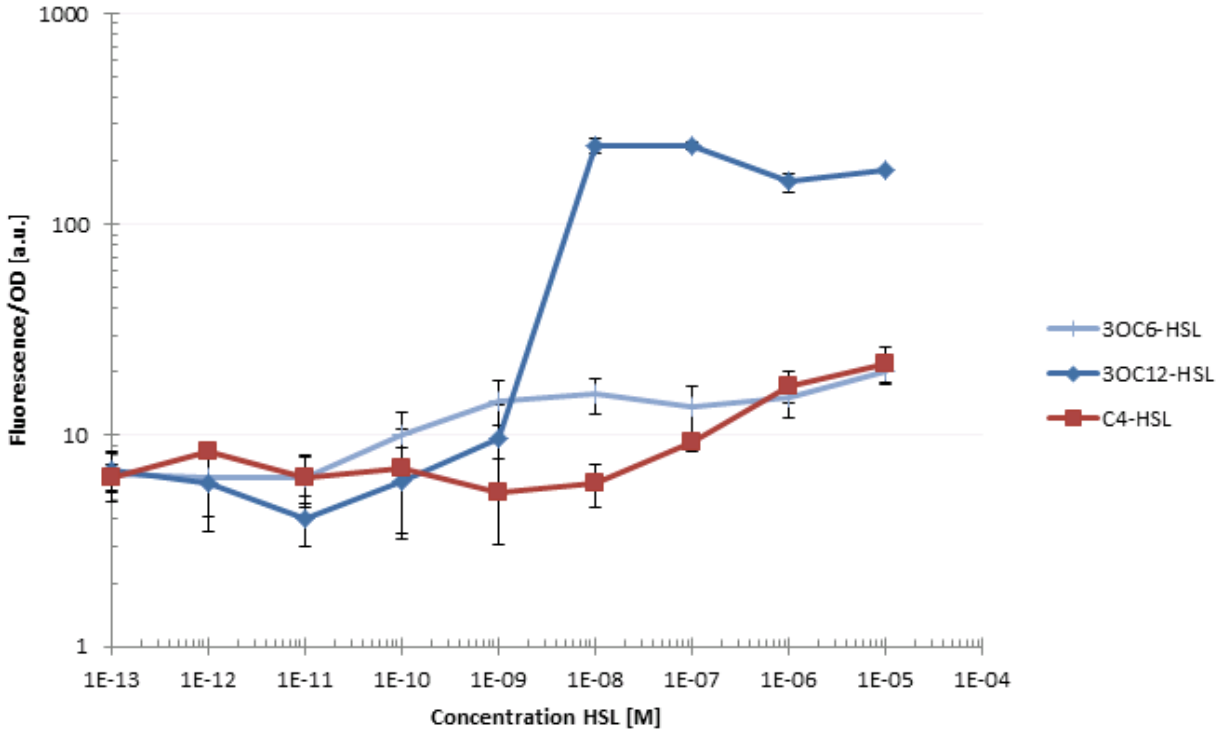


Fig. 1 siG0014 dose-response curve 200 min after induction for three AHL molecules

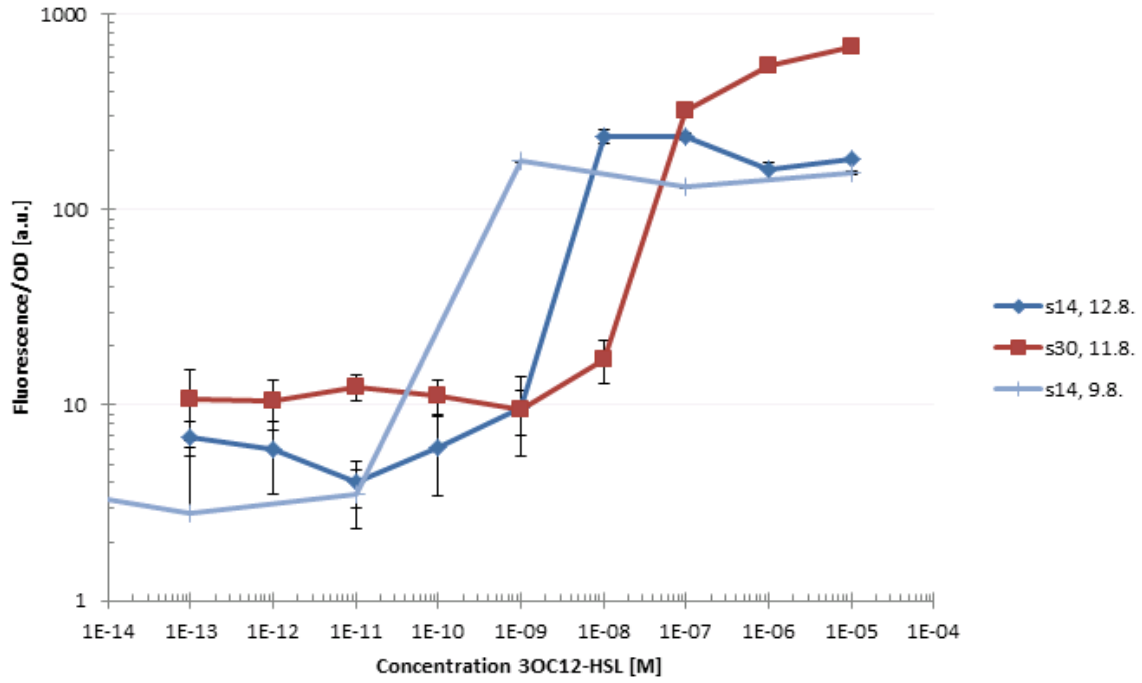


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for siG0014 (las receiver) and siG0030 (lux receiver with RR12y)

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:

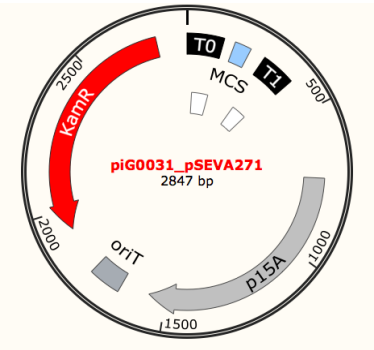
- Tecan infinite M200 PRO

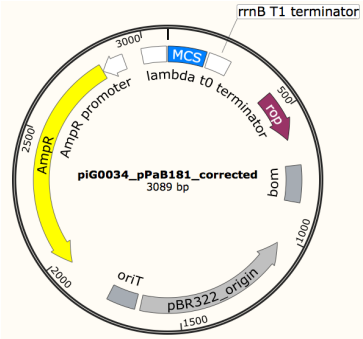
Raw Data:

- Lab/Microtiterplate/crosstalk/20140812_s30_crosstalk_supernatant.xlsx

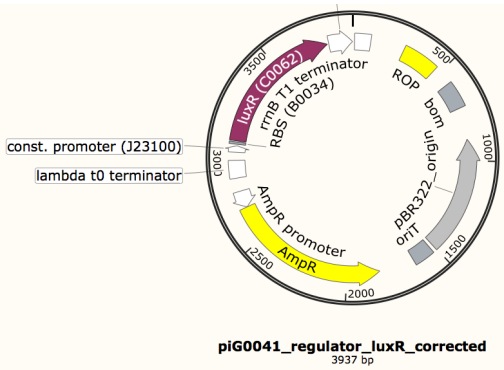
Plasmids in play:

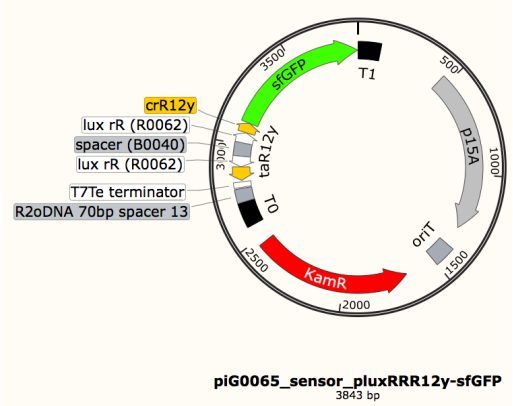
- siG0001: piG0031, piG0034



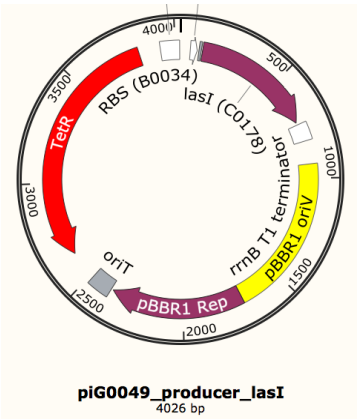


- siG0030: piG0041, piG0065

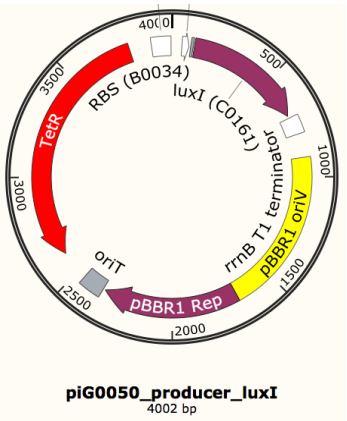




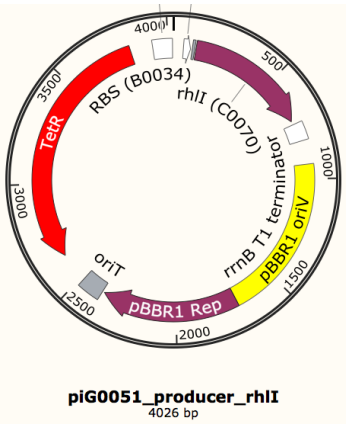
piG0049 (LasI producer)



piG0050 (LuxI producer)



piG0051 (RhlI producer)



Graphs of Data:

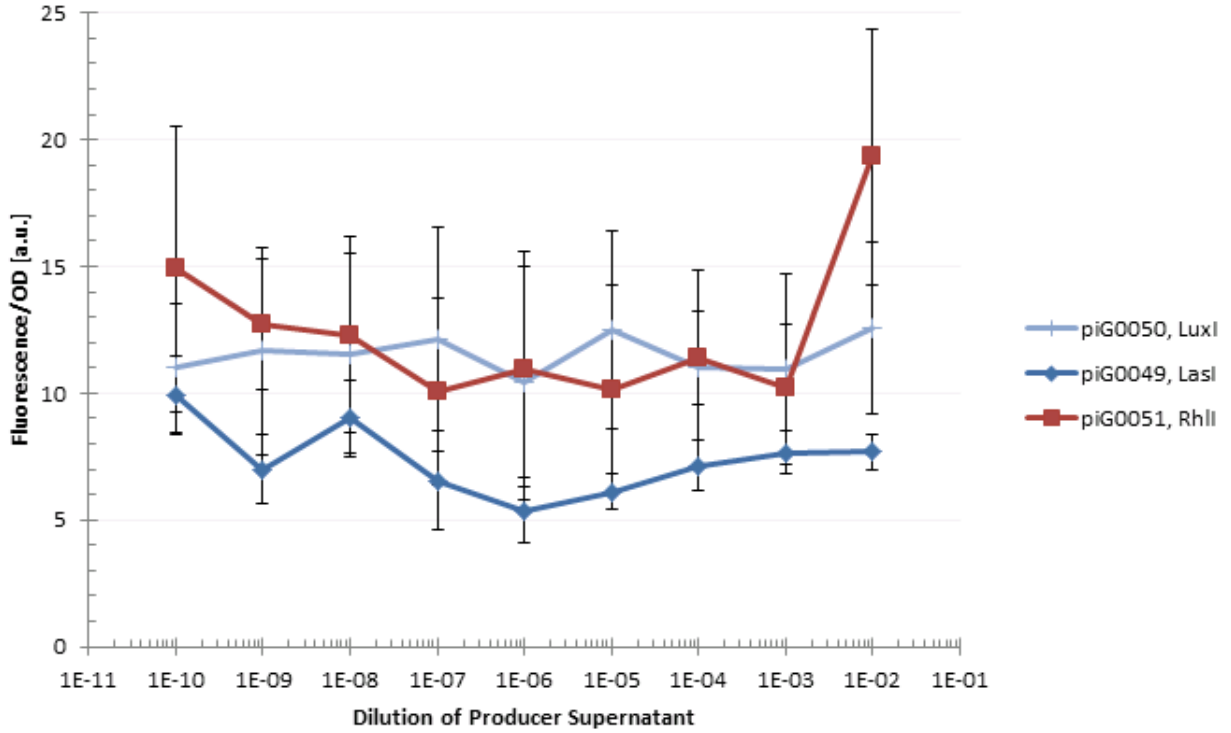


Fig. 1 siG0030 dose-response curve 200 min after induction with the three different producer supernatants

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:

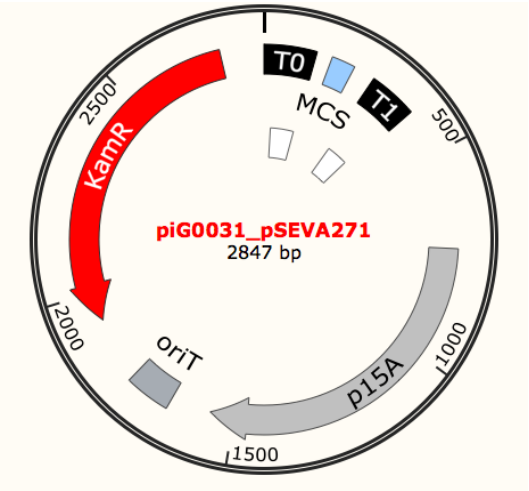
- Tecan infinite M200 PRO

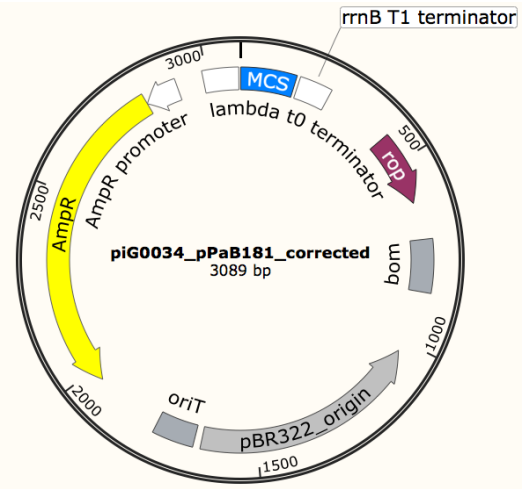
Raw Data:

- Lab/Microtiterplate/crosstalk/20140813_s27_crosstalk.xlsx

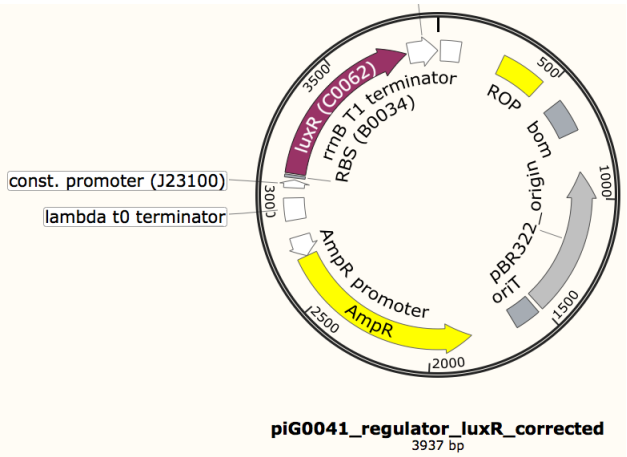
Plasmids in play:

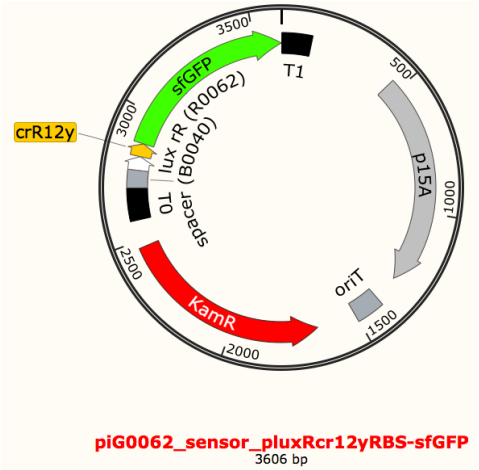
- siG0001: piG0031, piG0034





- siG0027: piG0041, piG0062





Graphs of Data:

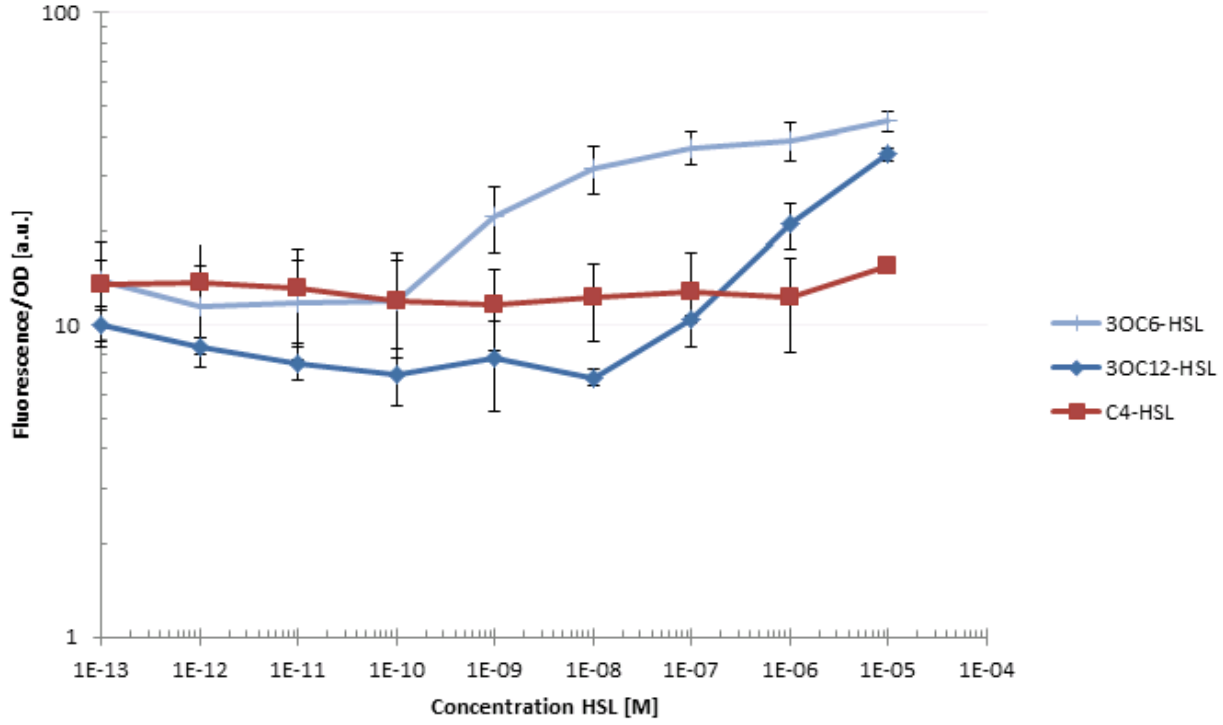


Fig. 1 siG0027 dose-response curve 200 min after induction for three AHL molecules

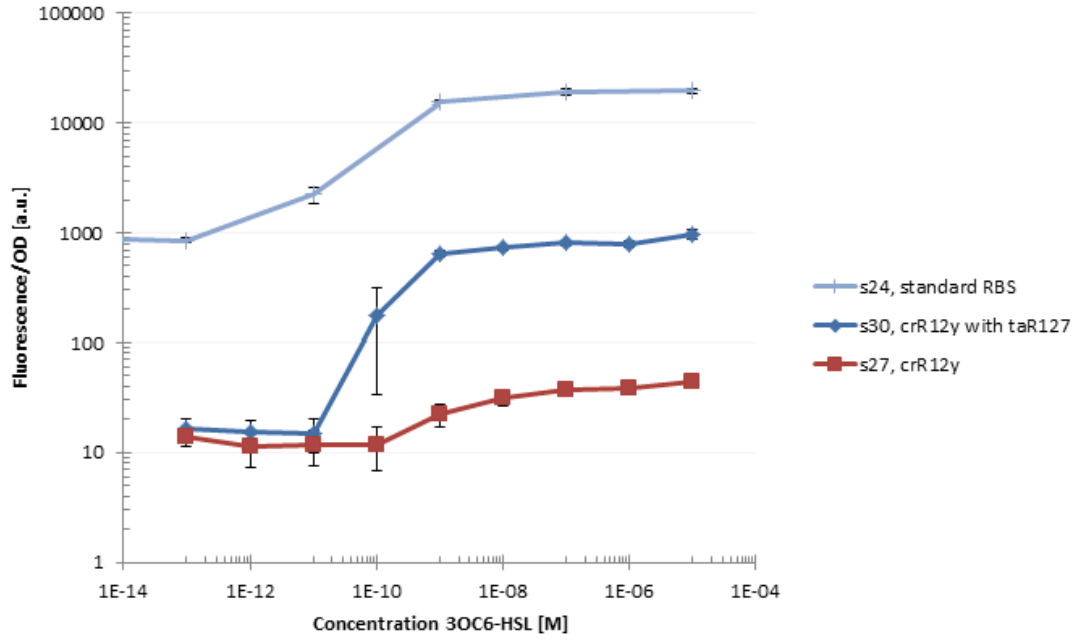


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0024, siG0030, and siG0027

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:

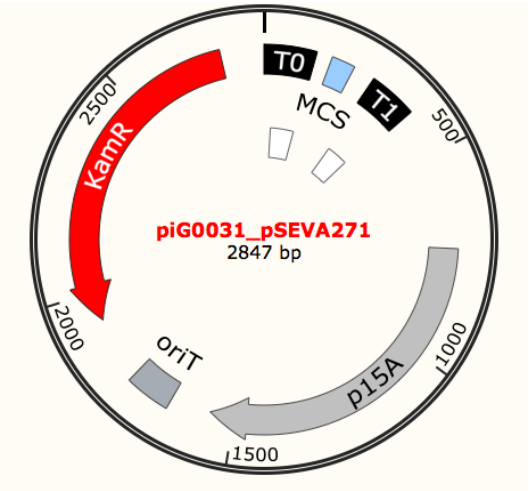
- Tecan infinite M200 PRO

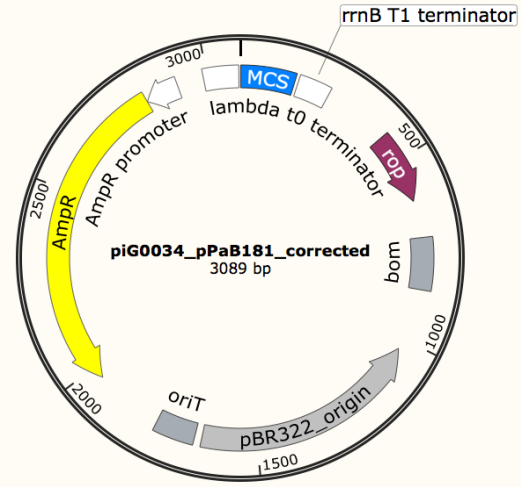
Raw Data:

- Lab/Microtiterplate/crosstalk/20140814_s6_crosstalk.xlsx

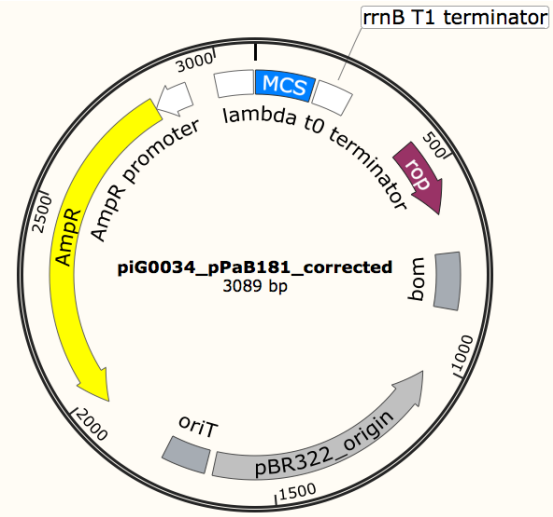
Plasmids in play:

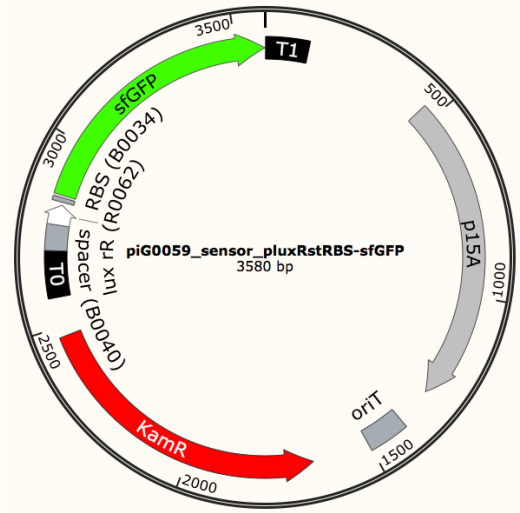
- siG0001: piG0031, piG0034





- siG0006: piG0041, piG0059





Graphs of Data:

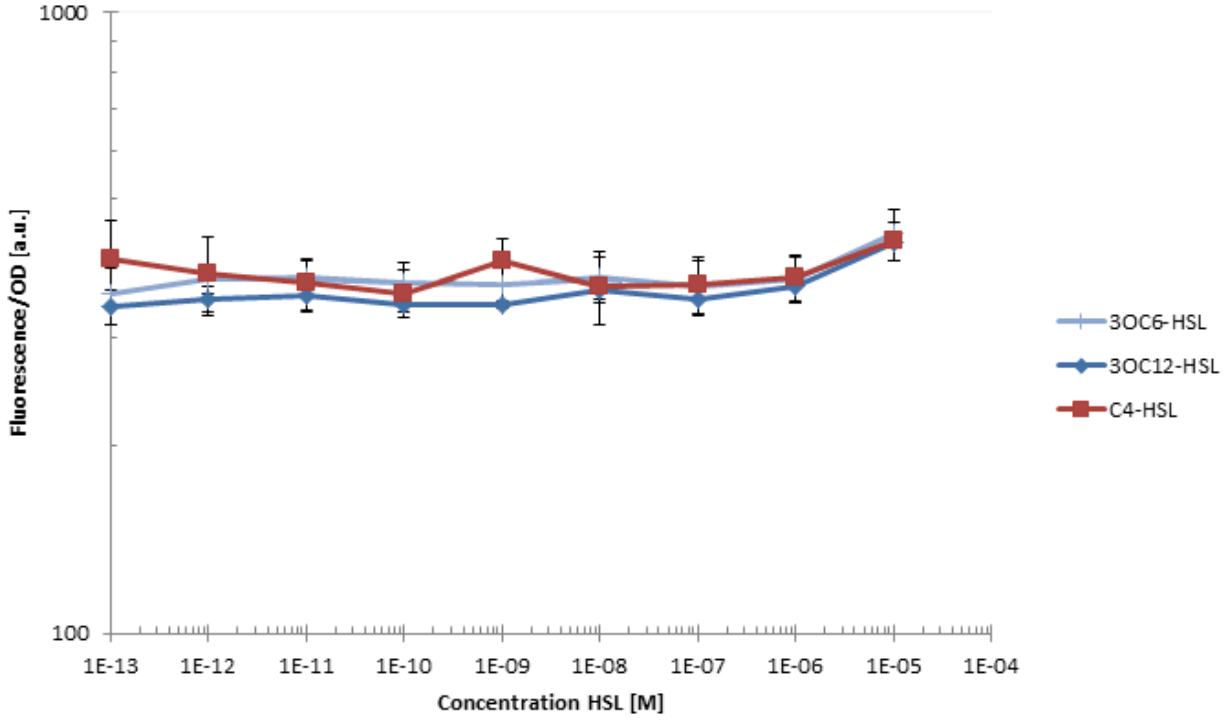


Fig. 1 siG0006 dose-response curve 200 min after induction for three AHL molecules

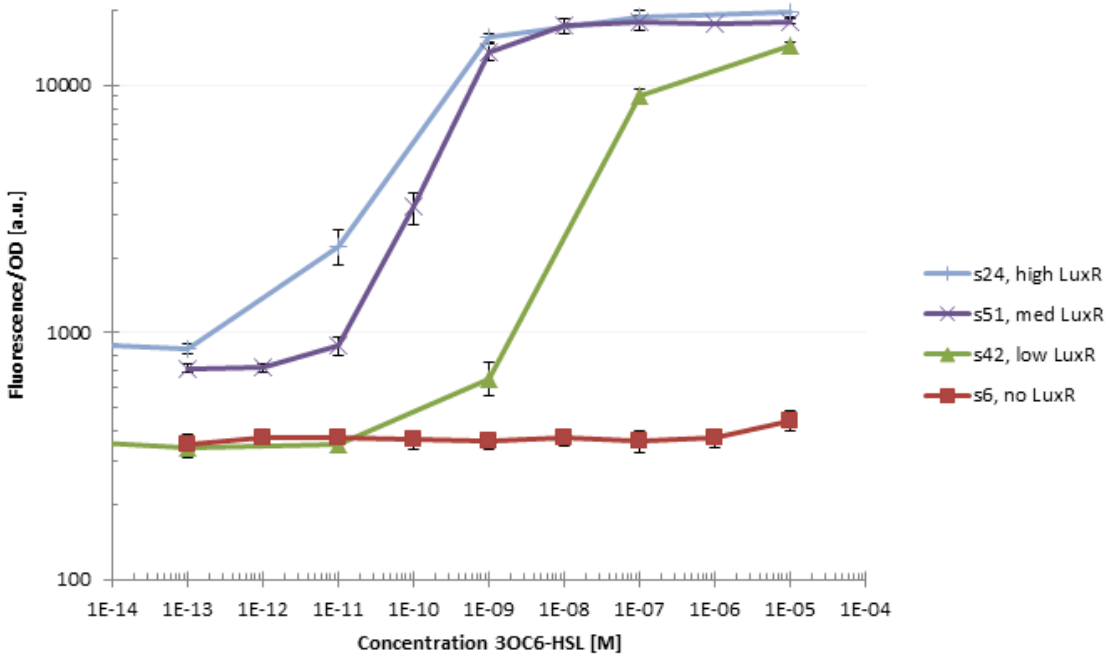


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0006, siG0024, siG0030, siG0042, and siG0051

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:

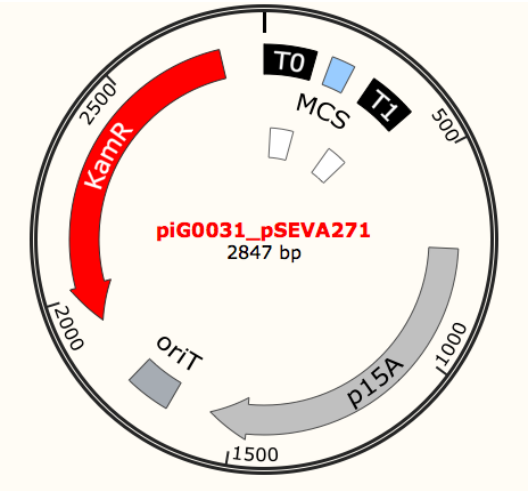
- Tecan infinite M200 PRO

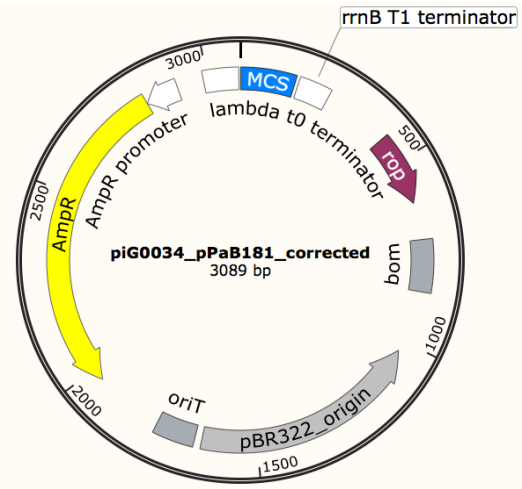
Raw Data:

- Lab/Microtiterplate/crosstalk/20140814_s48_crosstalk.xlsx

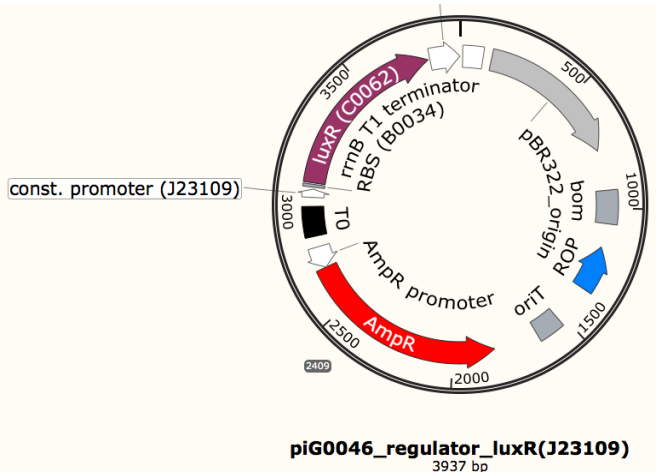
Plasmids in play:

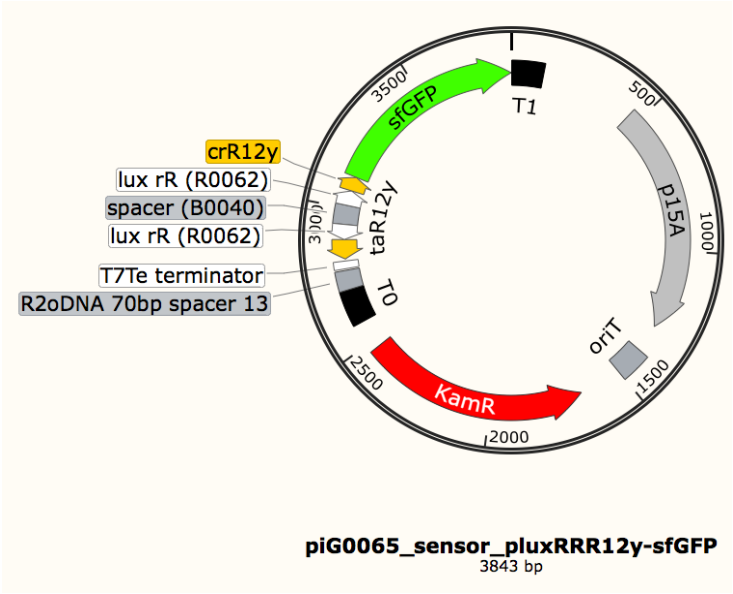
- siG0001: piG0031, piG0034





- siG0048: piG0046, piG0065





Graphs of Data:

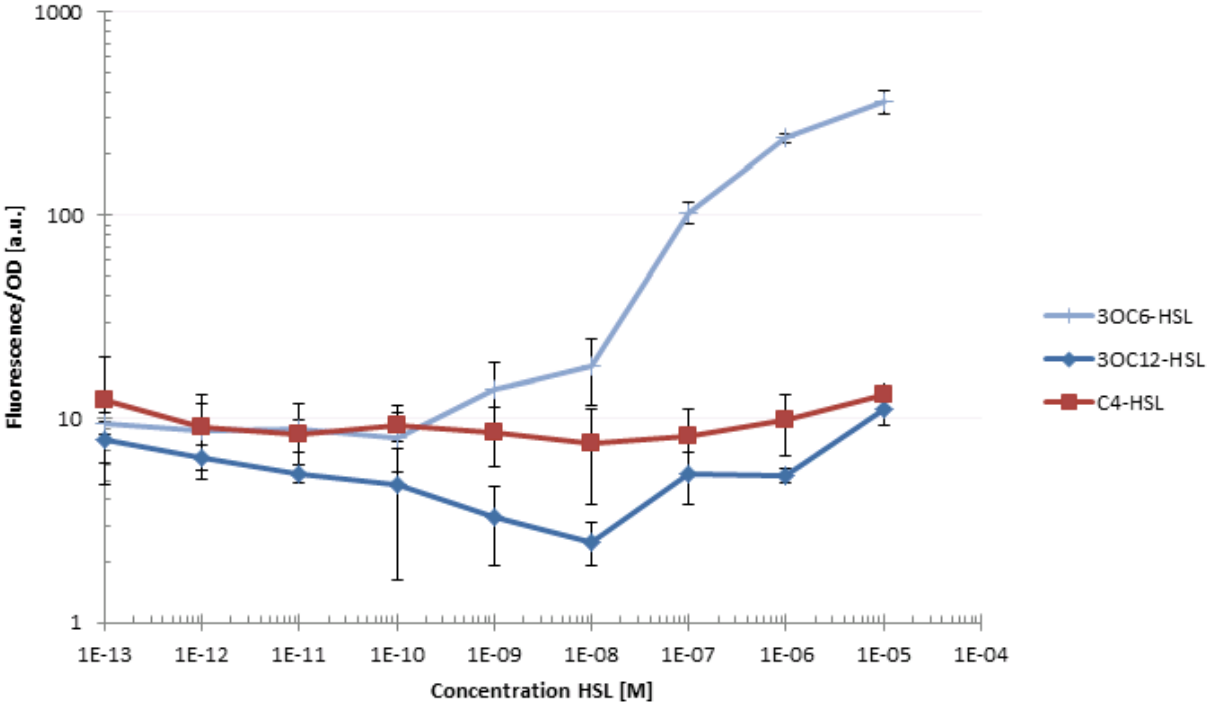


Fig. 1 siG0048 dose-response curve 200 min after induction for three AHL molecules

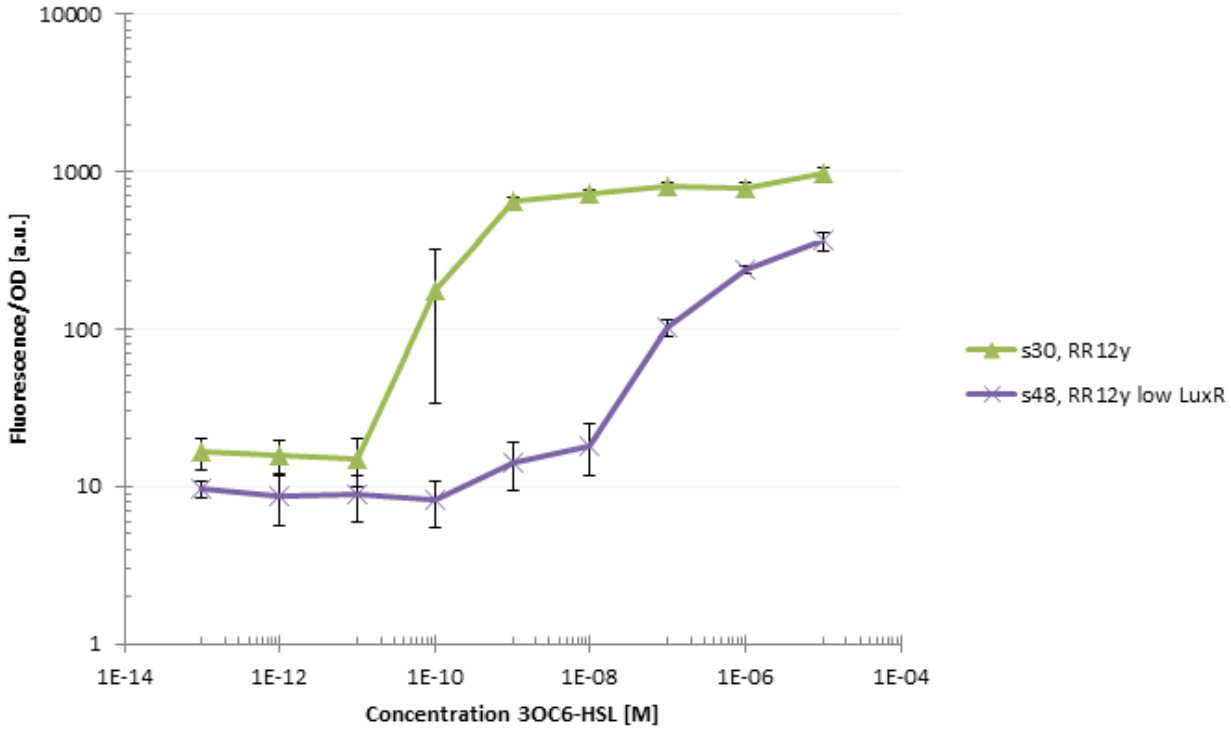


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0048 and siG0030

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:

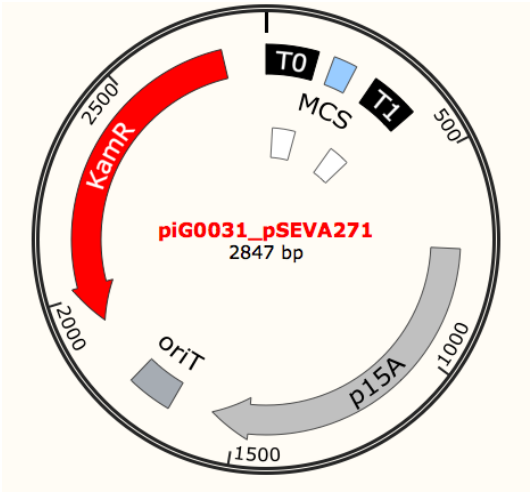
- Tecan infinite M200 PRO

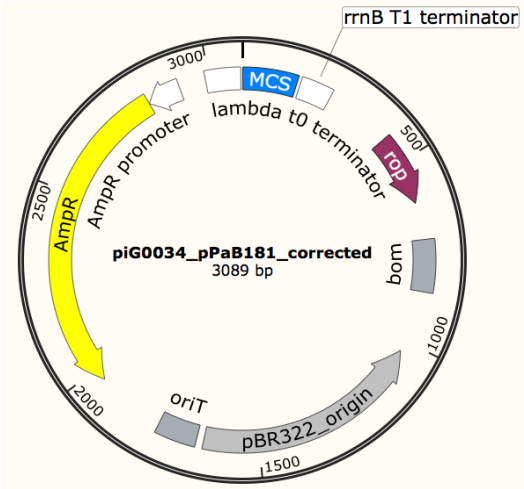
Raw Data:

- Lab/Microtiterplate/crosstalk/20140817_s40_crosstalk.xlsx

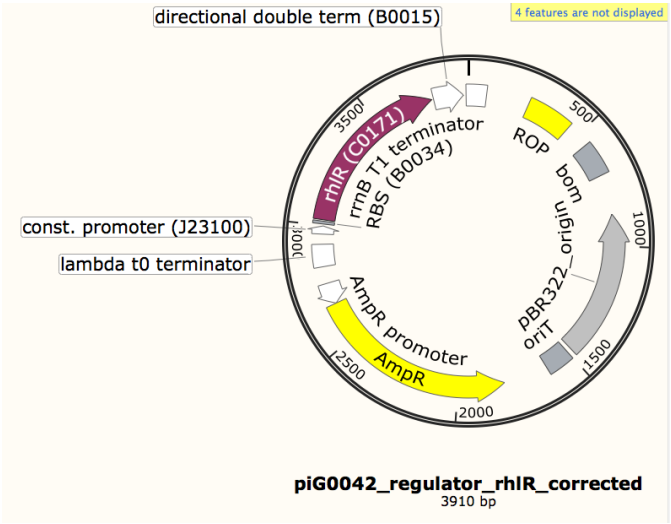
Plasmids in play:

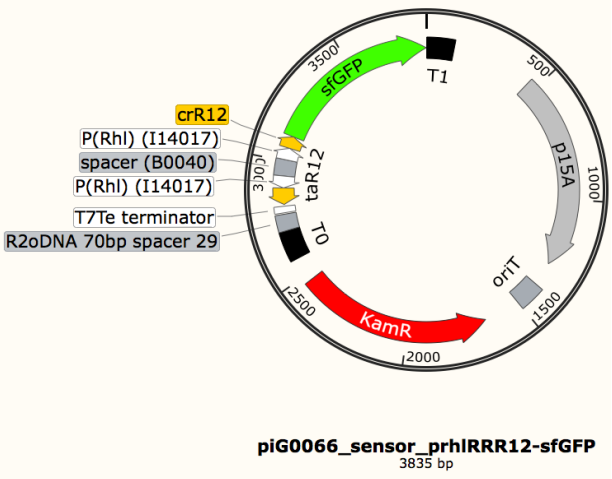
- siG0001: piG0031, piG0034





- siG0040: piG0042, piG0066





Graphs of Data:

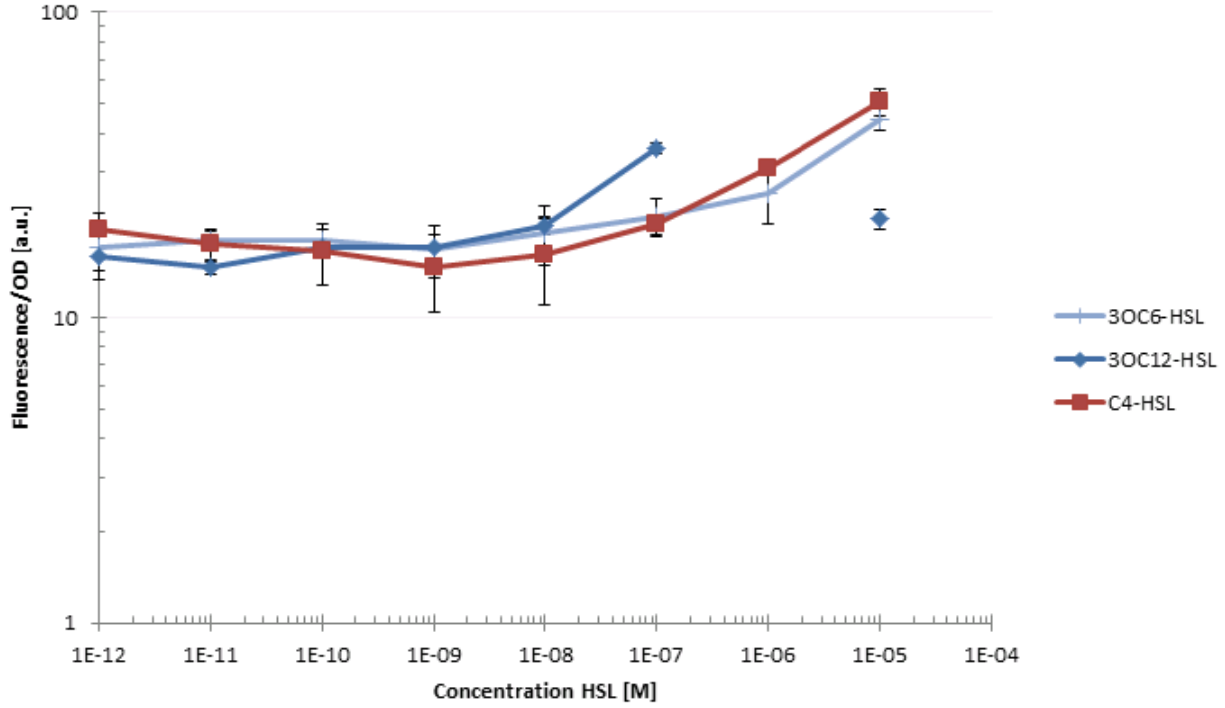


Fig. 1 siG0040 dose-response curve 200 min after induction for three AHL molecules, reference fluorescence of s1 was not subtracted due to unusual high fluorescence of s1

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:

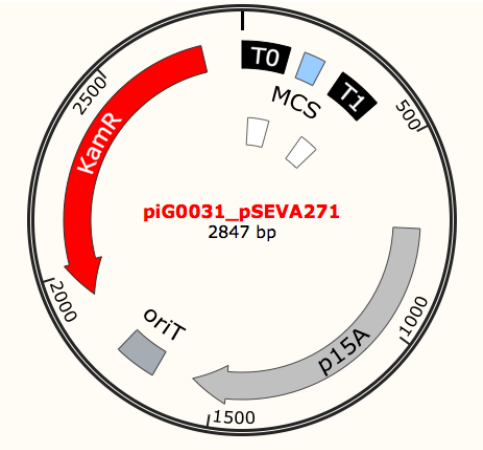
- Tecan infinite M200 PRO

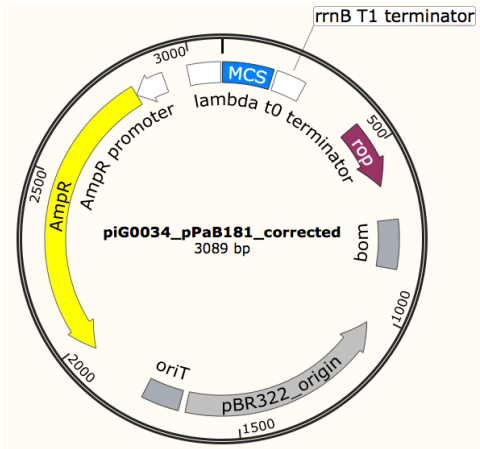
Raw Data:

- Lab/Microtiterplate/crosstalk/20140818_s21_crosstalk.xlsx

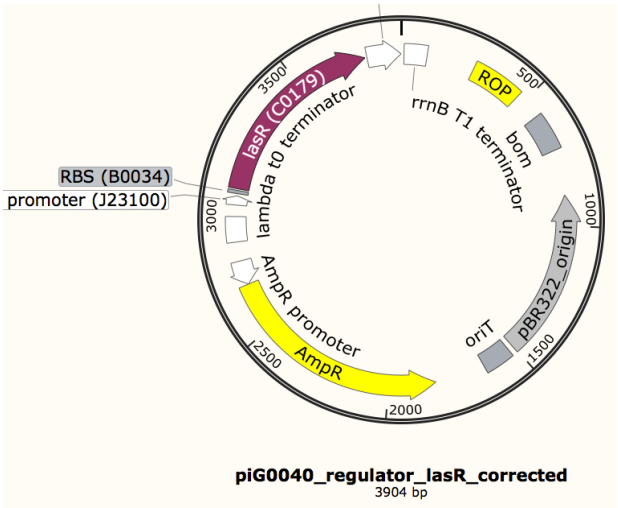
Plasmids in play:

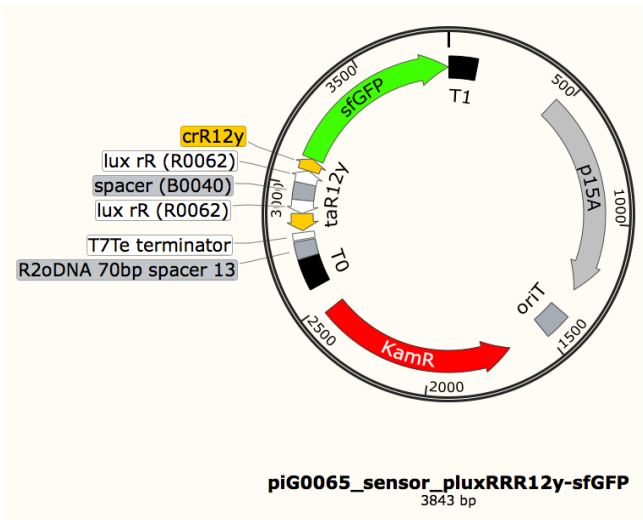
- siG0001: piG0031, piG0034





- siG0021: piG0040, piG0065





Graphs of Data:

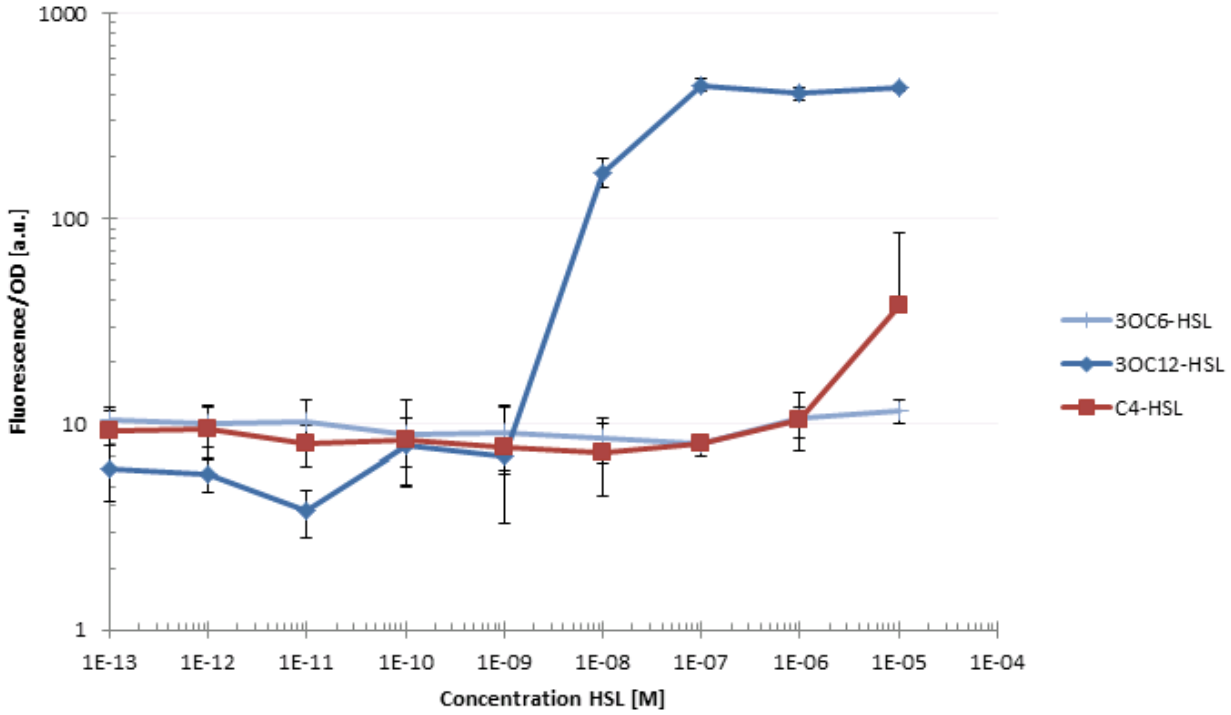


Fig. 1 siG0021 dose-response curve 200 min after induction for three AHL molecules

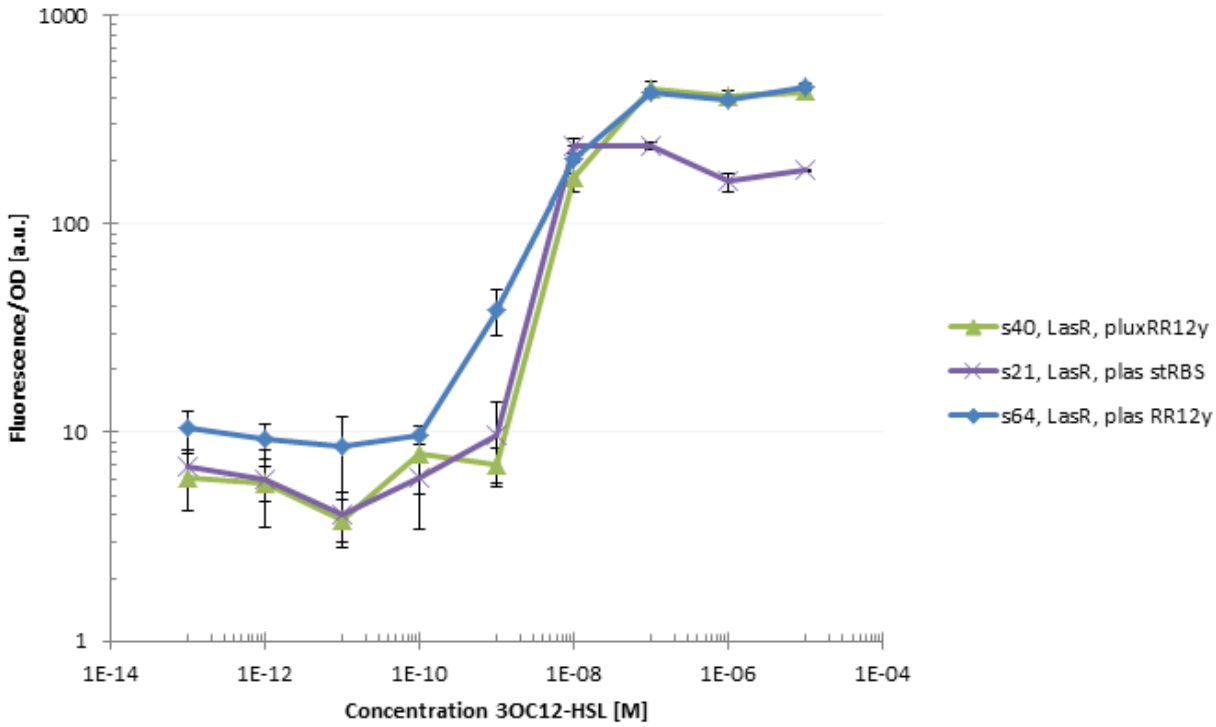


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for siG0040, siG0021 and siG0064

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:

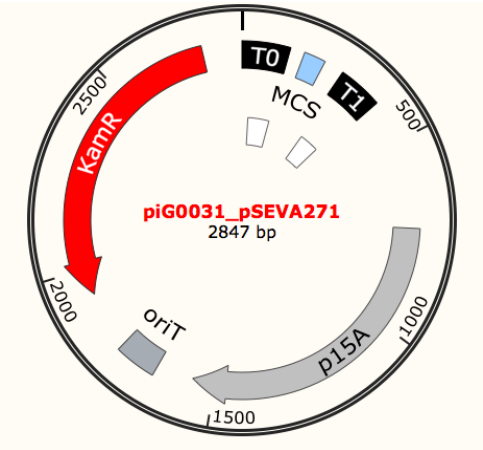
- Tecan infinite M200 PRO

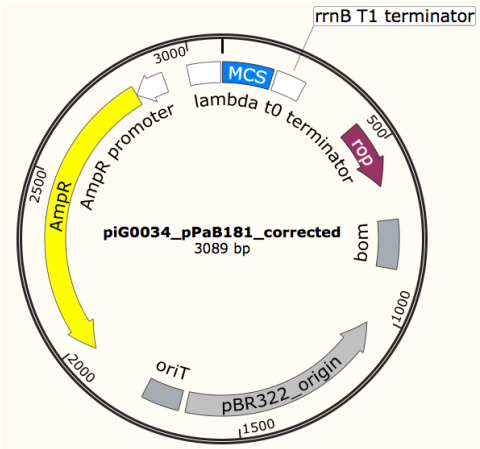
Raw Data:

- Lab/Microtiterplate/crosstalk/20140819_s23_crosstalk.xlsx

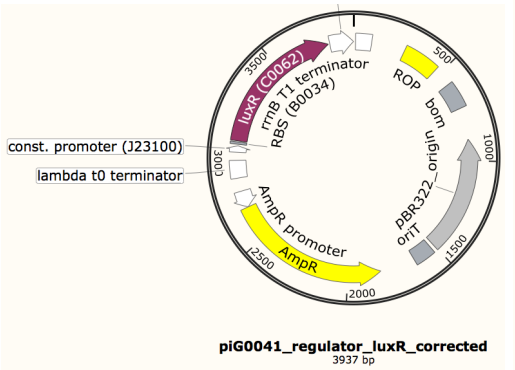
Plasmids in play:

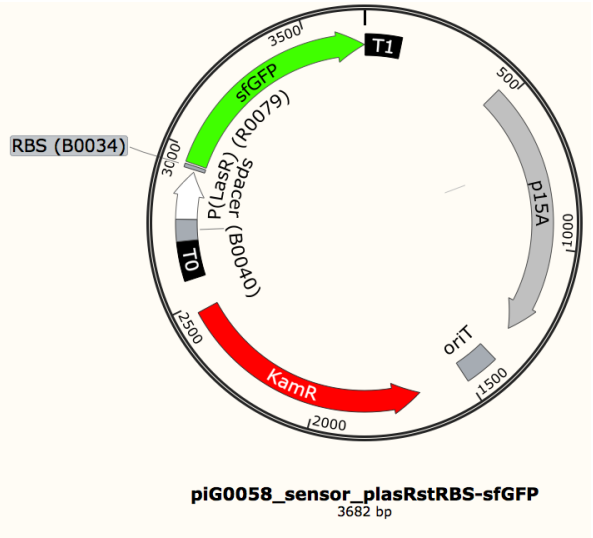
- siG0001: piG0031, piG0034





- siG0023: piG0041, piG0058





Graphs of Data:

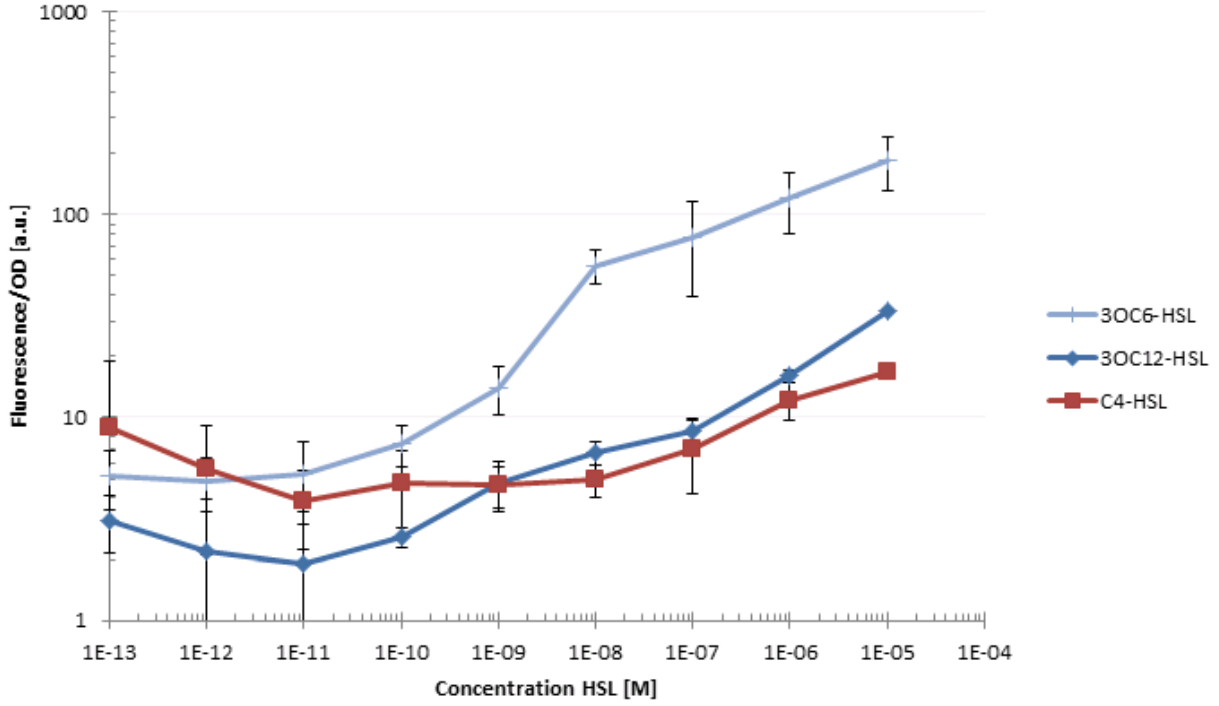


Fig. 1 siG0023 dose-response curve 200 min after induction for three AHL molecules

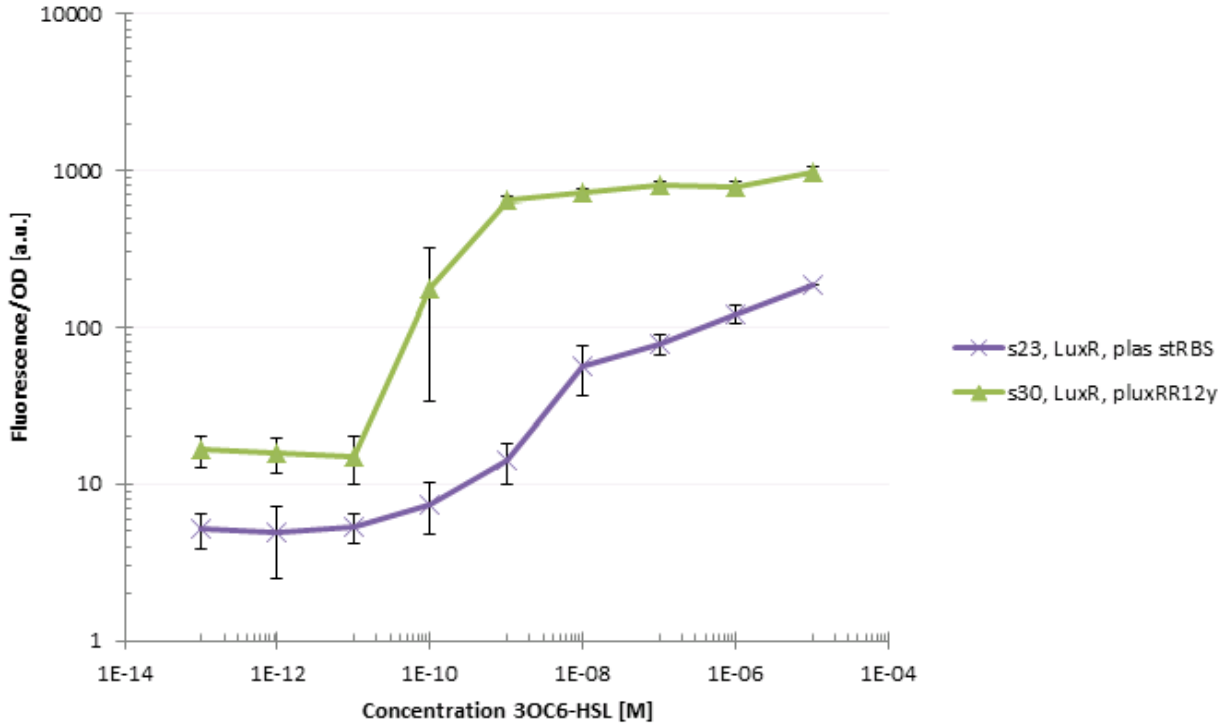


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0023 and siG0030

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:

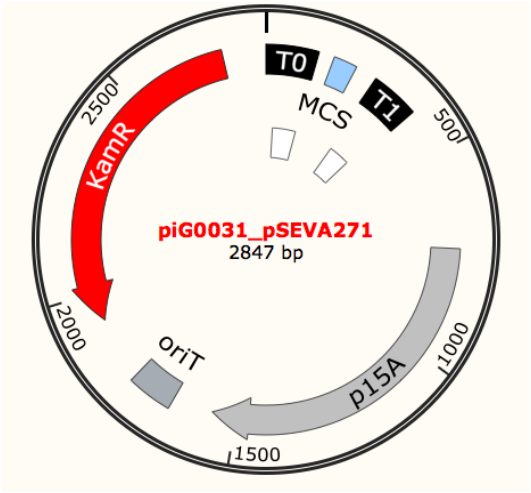
- Tecan infinite M200 PRO

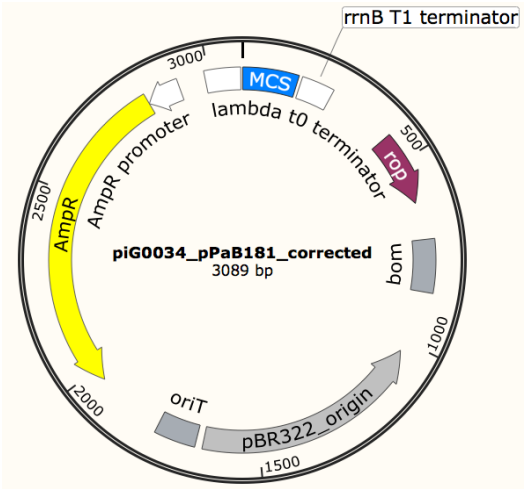
Raw Data:

- Lab/Microtiterplate/crosstalk/20140819_s57_crosstalk.xlsx

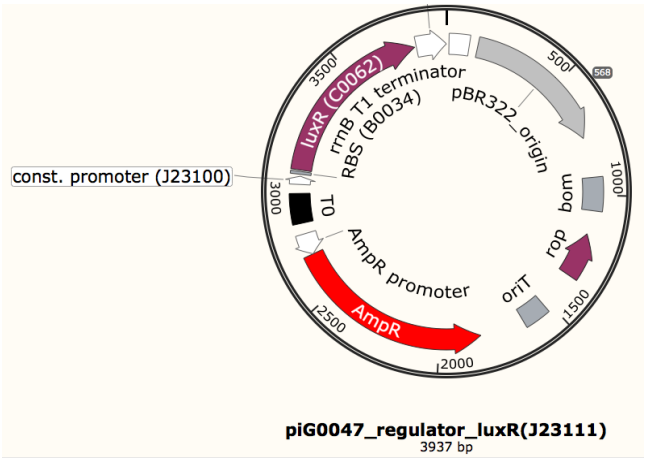
Plasmids in play:

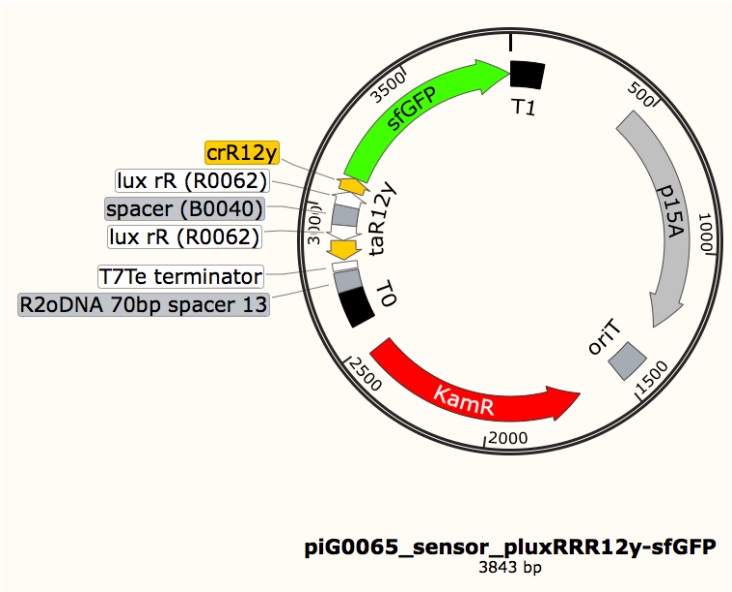
- siG0001: piG0031, piG0034





- siG0057: piG0047, piG0065





Graphs of Data:

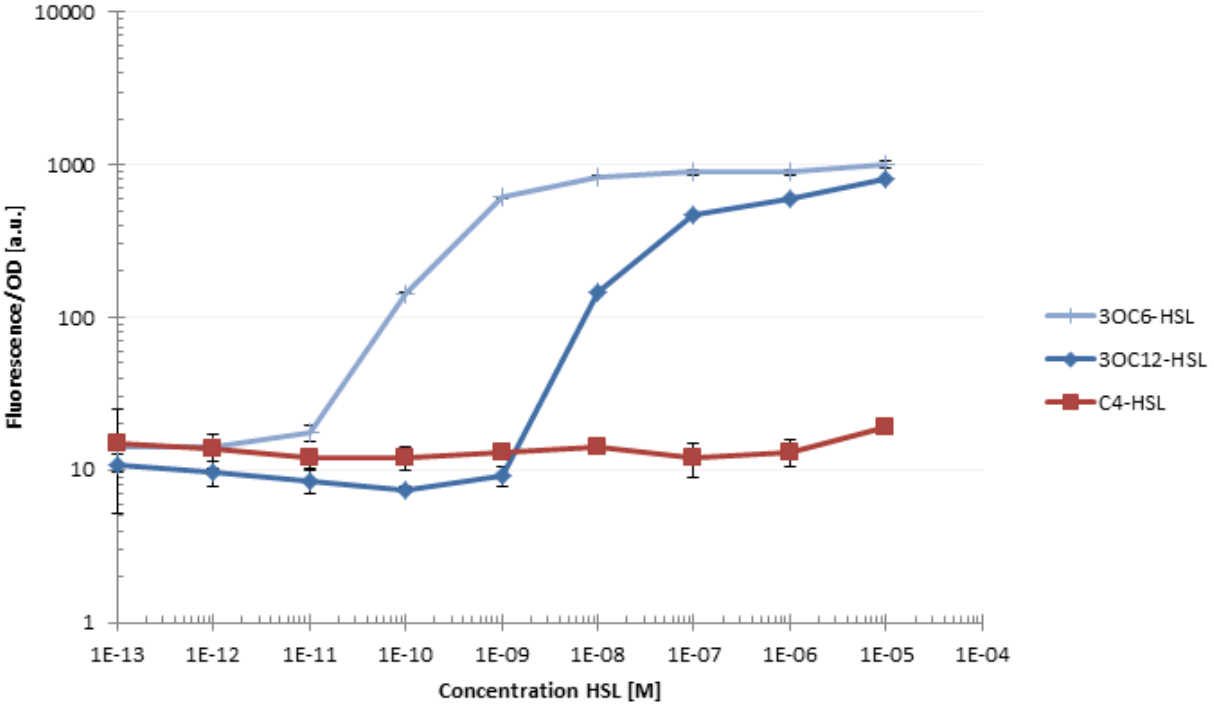


Fig. 1 siG0057 dose-response curve 200 min after induction for three AHL molecules

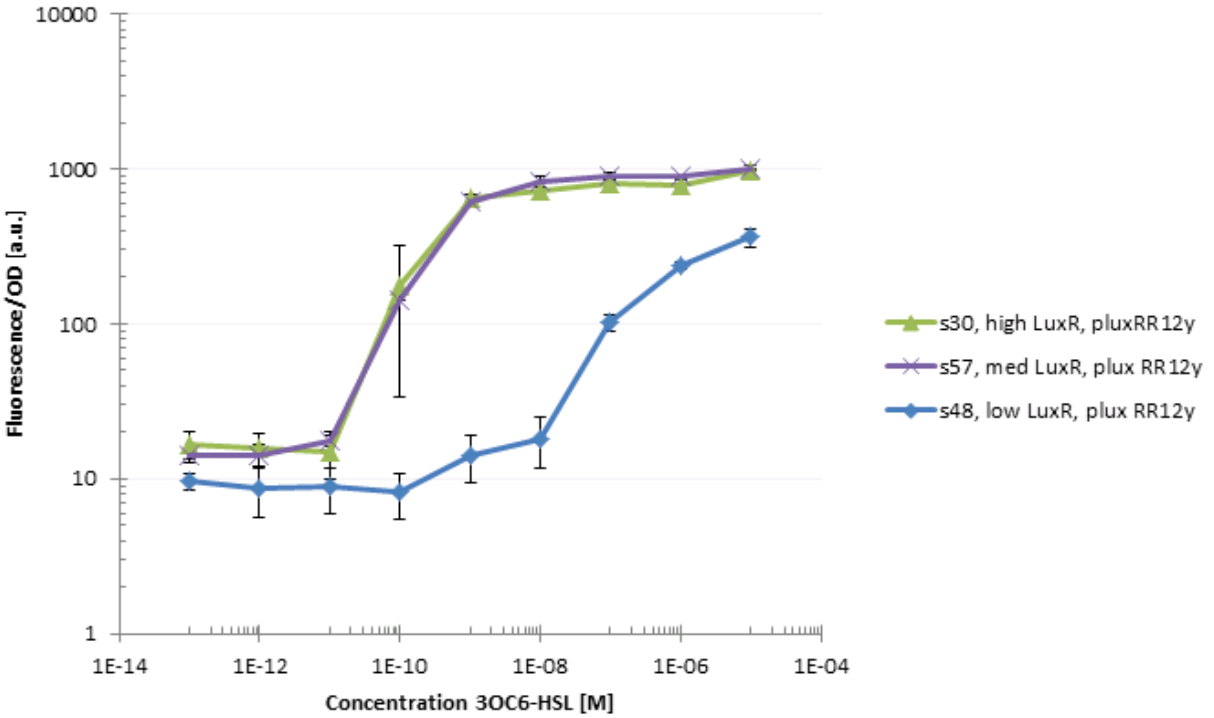


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for the three variants with different promoter strengths for LuxR production

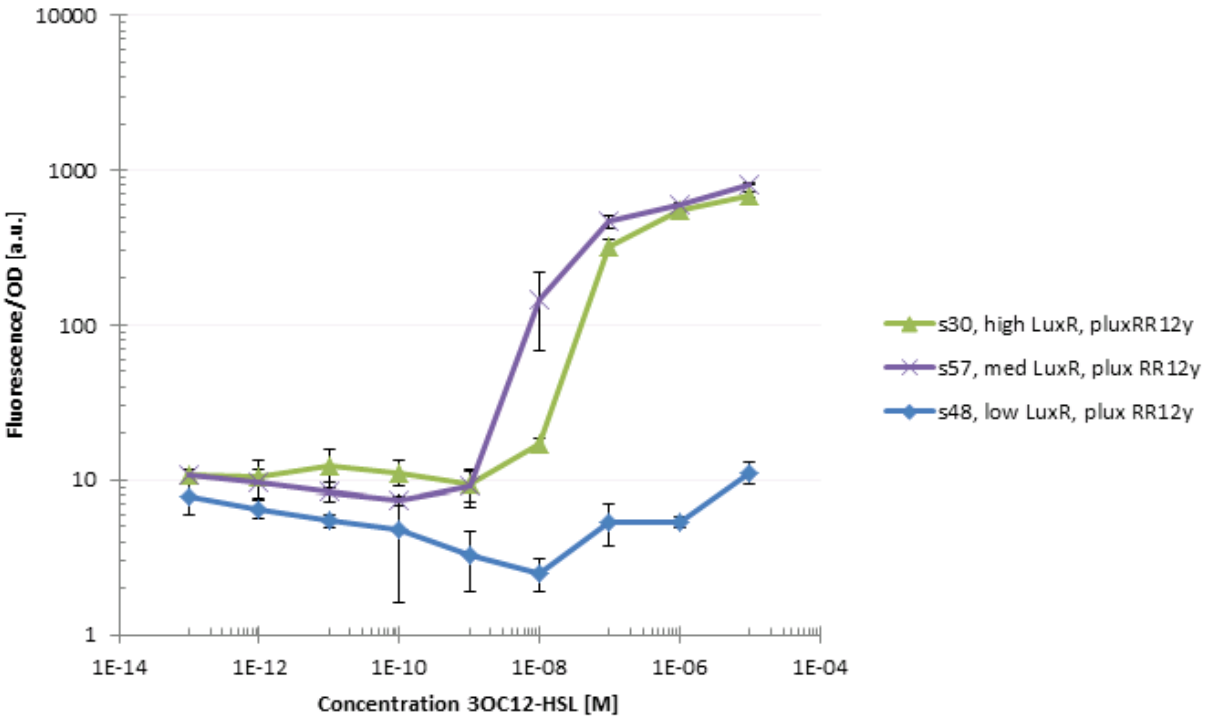


Fig. 2 dose-response curve 200 min after induction for 3OC12-HSL for the three variants with different promoter strengths for LuxR production

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:

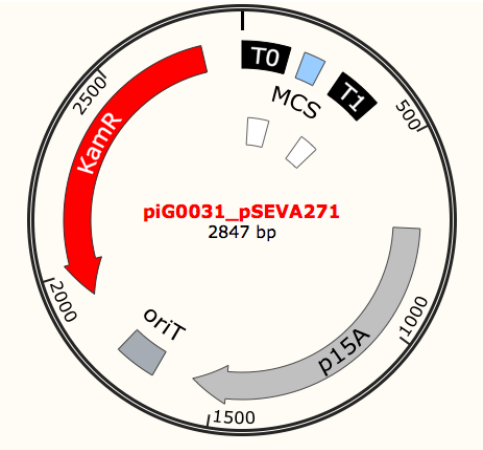
- Tecan infinite M200 PRO

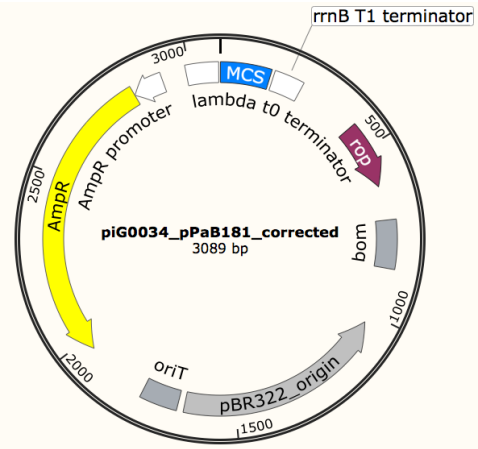
Raw Data:

- Lab/Microtiterplate/crosstalk/20140820_s32_crosstalk.xlsx

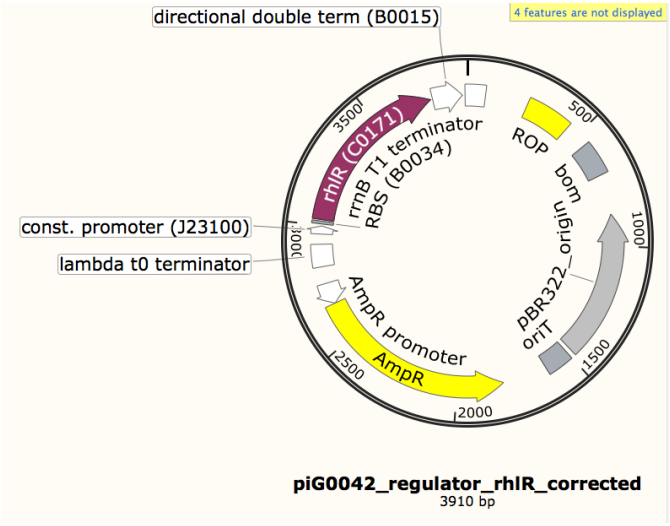
Plasmids in play:

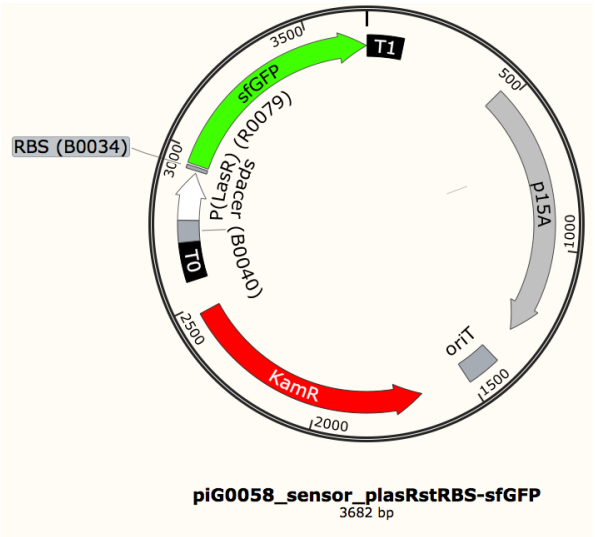
- siG0001: piG0031, piG0034





- siG0032: piG0042, piG0058





Graphs of Data:

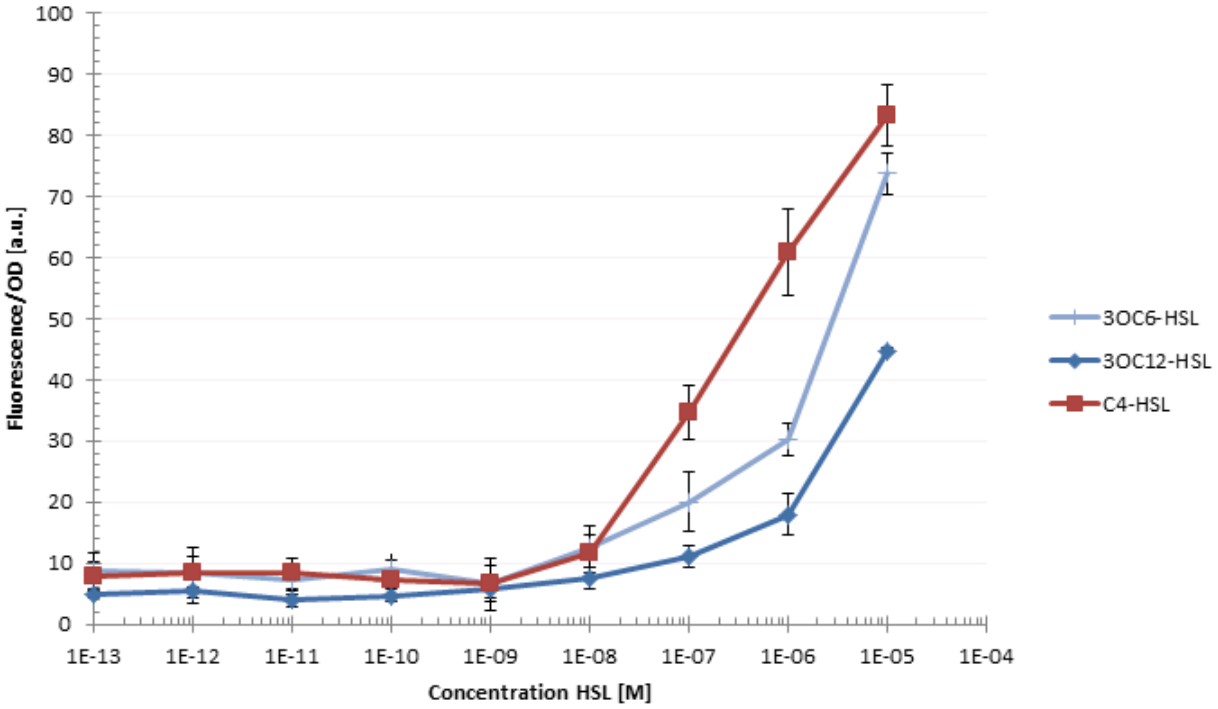


Fig. 1 siG0032 dose-response curve 200 min after induction for three AHL molecules (linear fluorescence scale!)

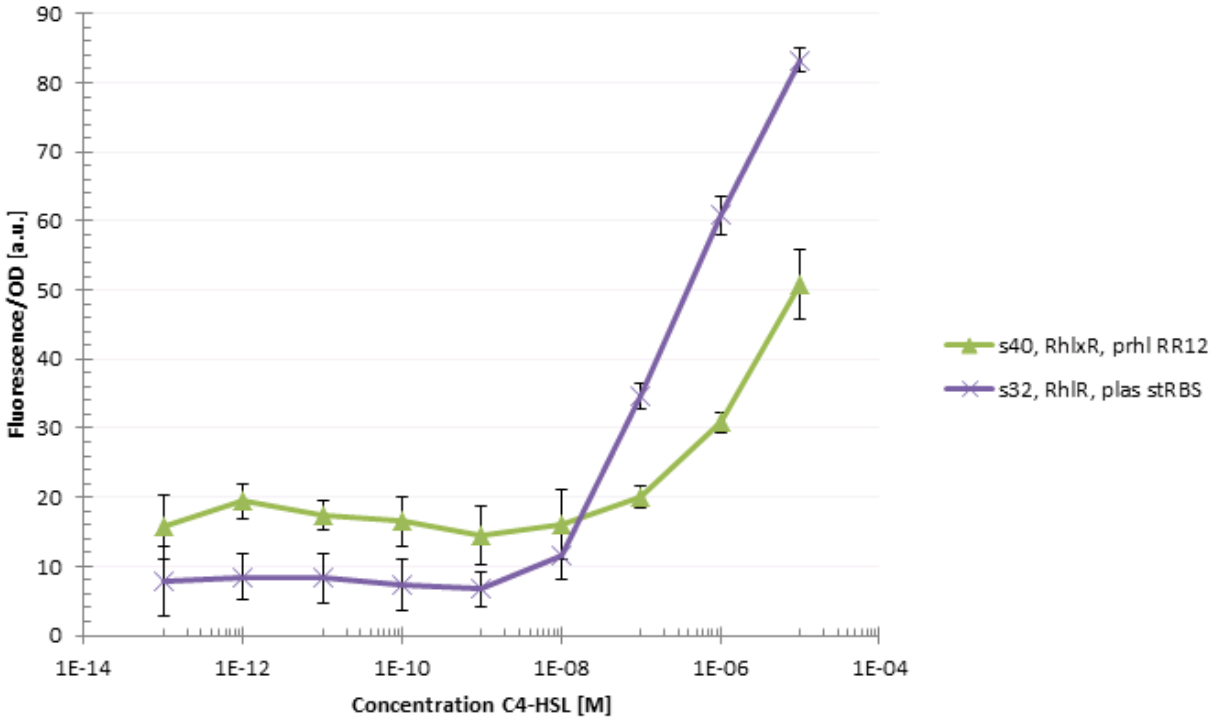


Fig. 2 dose-response curve 200 min after induction for 3OC6-HSL for siG0040 and siG0032

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:

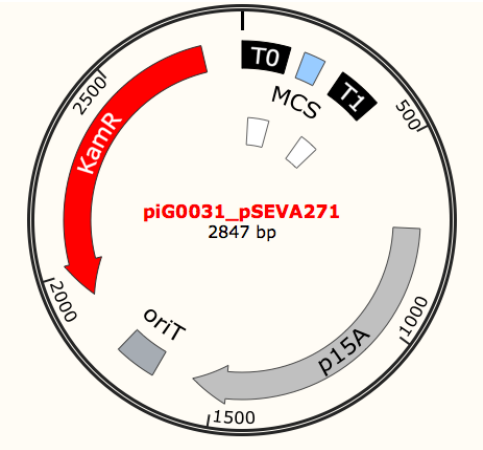
- Tecan infinite M200 PRO

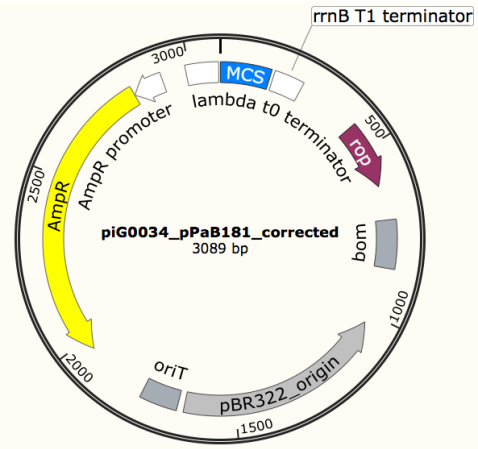
Raw Data:

- Lab/Microtiterplate/crosstalk/20140821_s22_crosstalk.xlsx

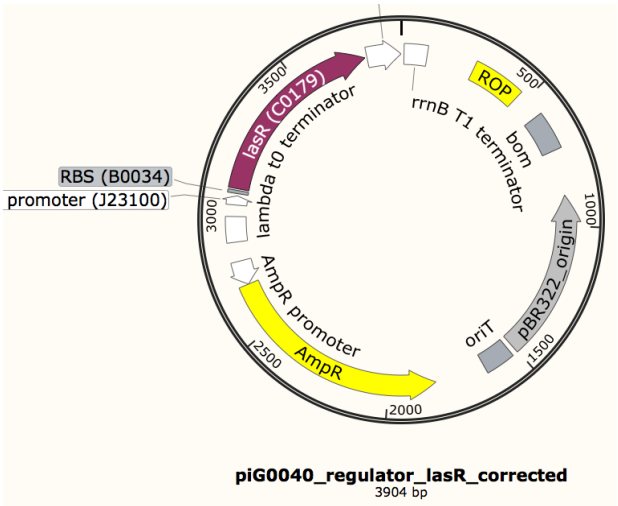
Plasmids in play:

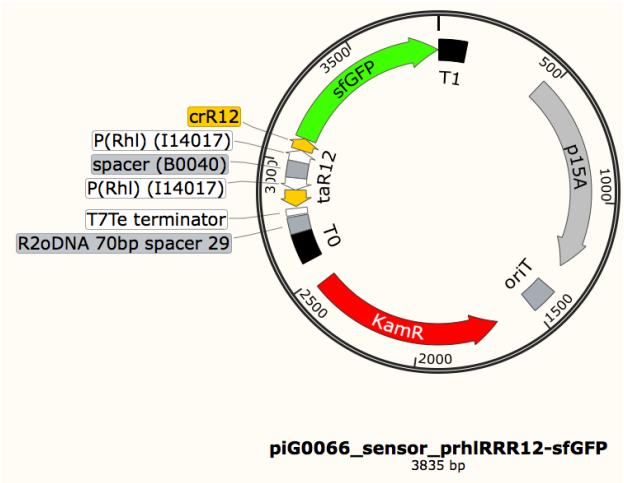
- siG0001: piG0031, piG0034





- siG0022: piG0040, piG0066





Graphs of Data:

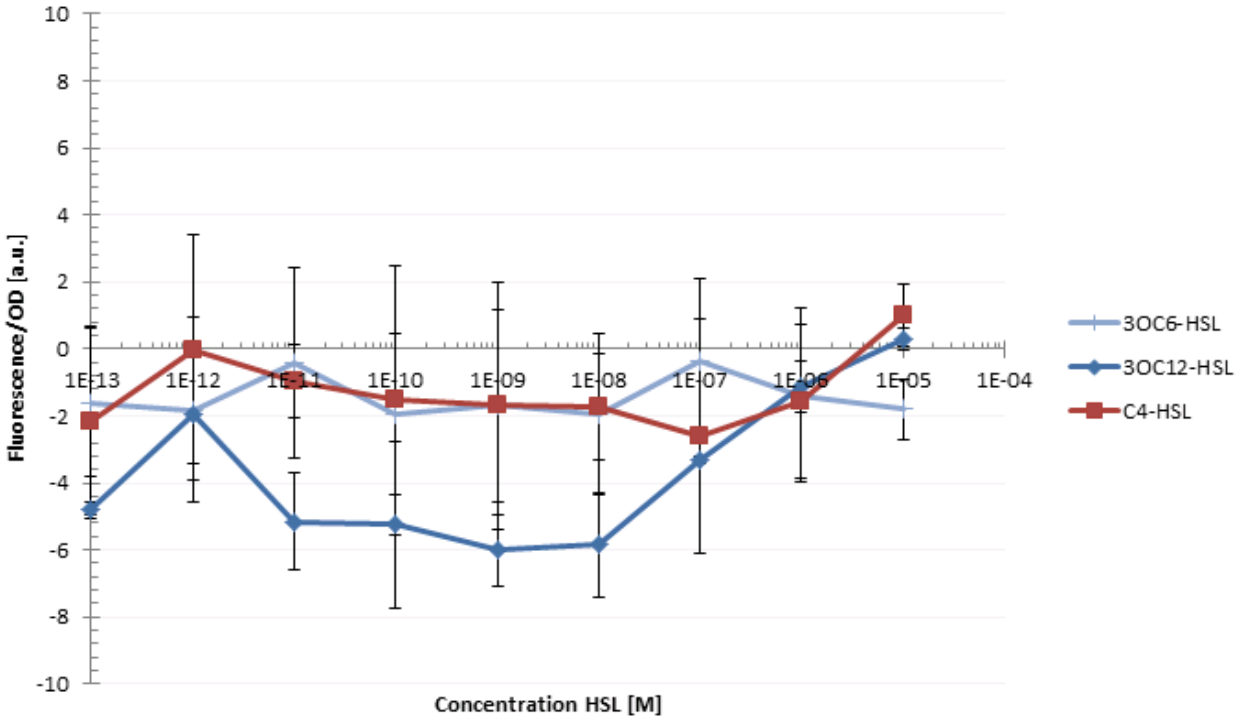


Fig. 1 siG0022 dose-response curve 200 min after induction for three AHL molecules

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:

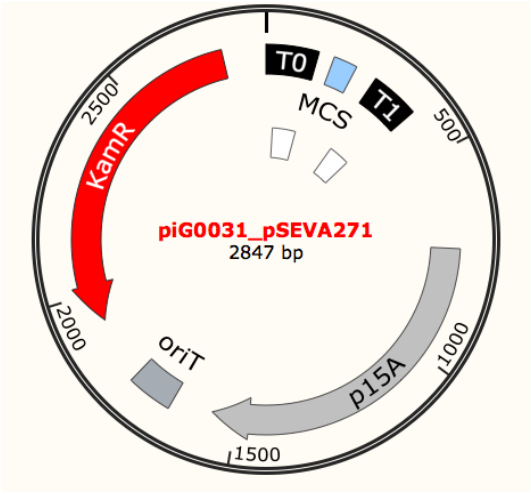
- Tecan infinite M200 PRO

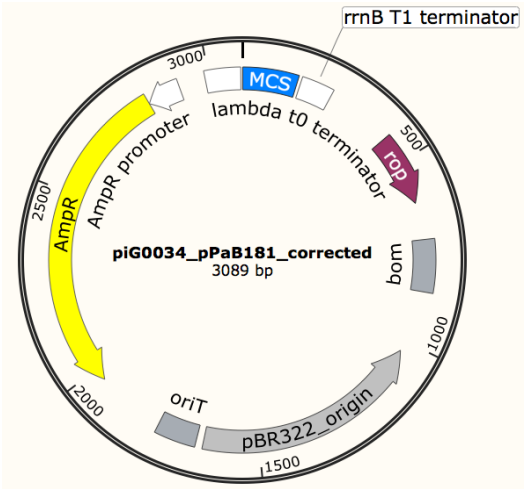
Raw Data:

- Lab/Microtiterplate/crosstalk/20140901_s65_crosstalk.xlsx

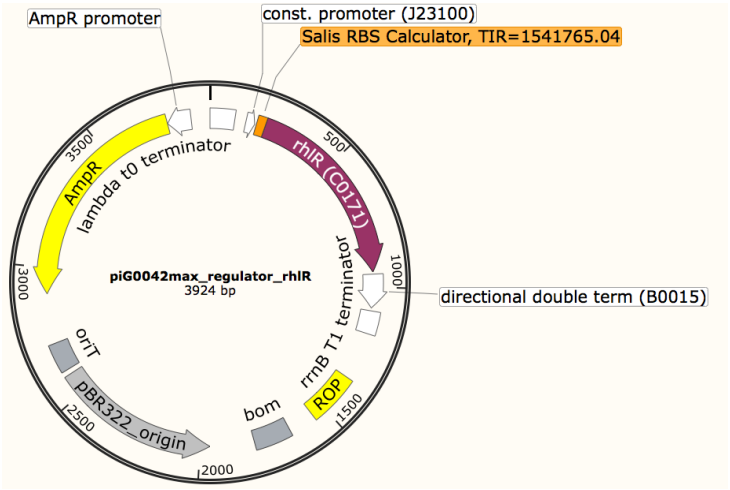
Plasmids in play:

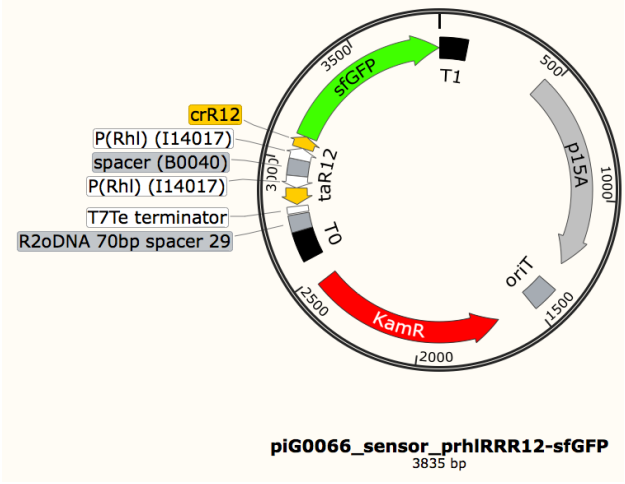
- siG0001: piG0031, piG0034





- siG0065: piG0042max, piG0066





Graphs of Data:

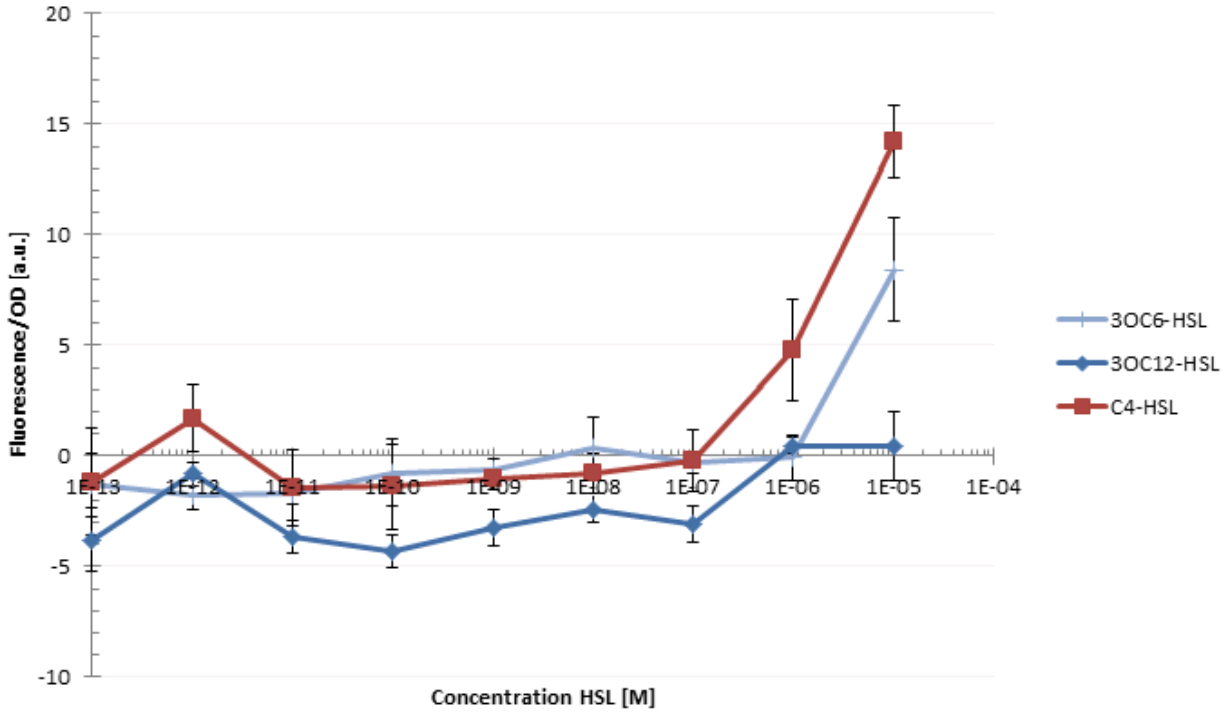


Fig. 1 siG0065 dose-response curve 200 min after induction for three AHL molecules

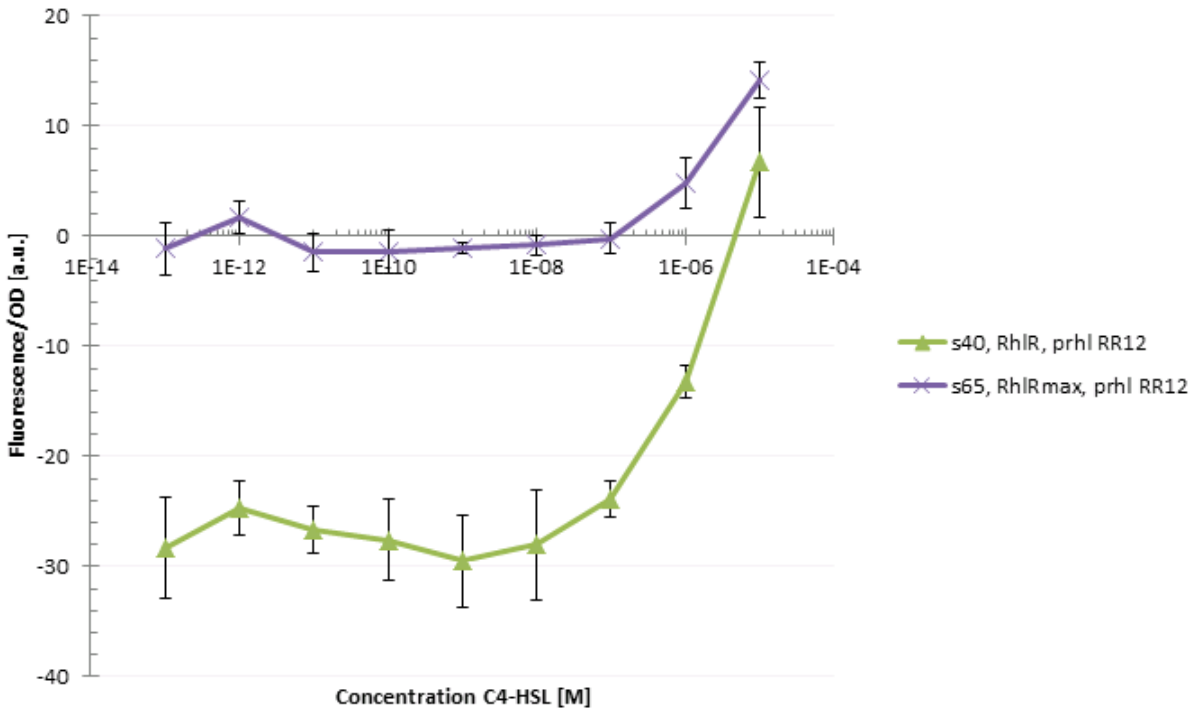


Fig. 2 dose-response curve 200 min after induction for C4-HSL for the two variants with different RBS strengths for RhlR production

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:

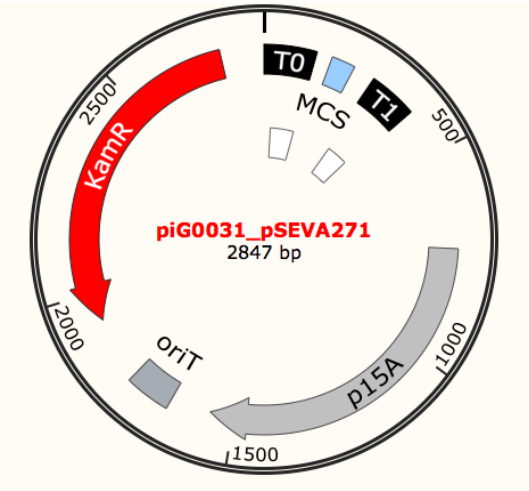
- Tecan infinite M200 PRO

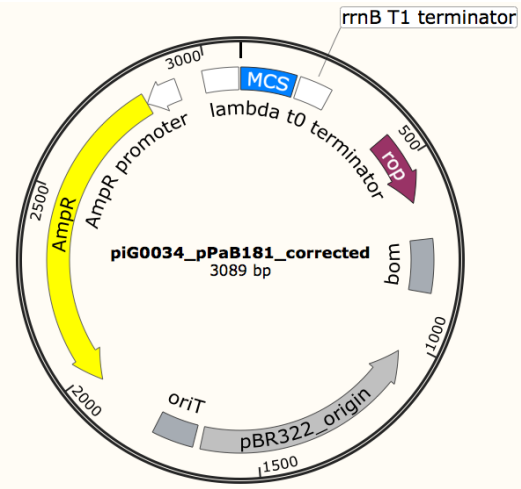
Raw Data:

- Lab/Microtiterplate/crosstalk/20140904_s64_crosstalk.xlsx

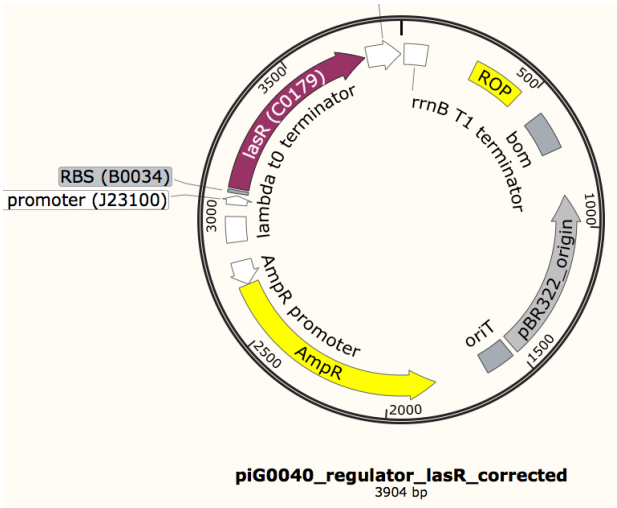
Plasmids in play:

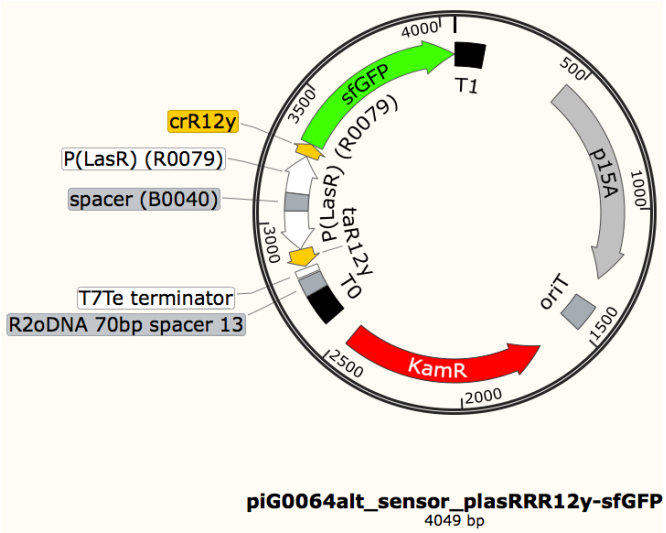
- siG0001: piG0031, piG0034





- siG0064: piG0040, piG0064alt





Graphs of Data:

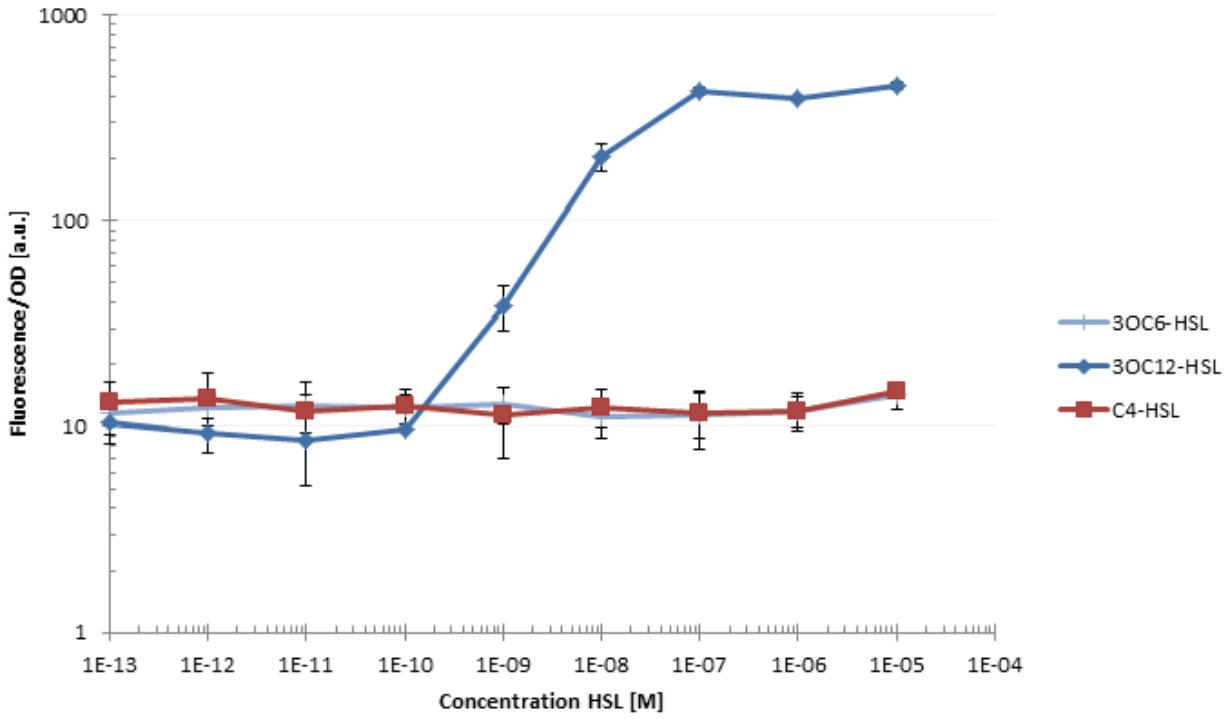


Fig. 1 siG0064 dose-response curve 200 min after induction for three AHL molecules

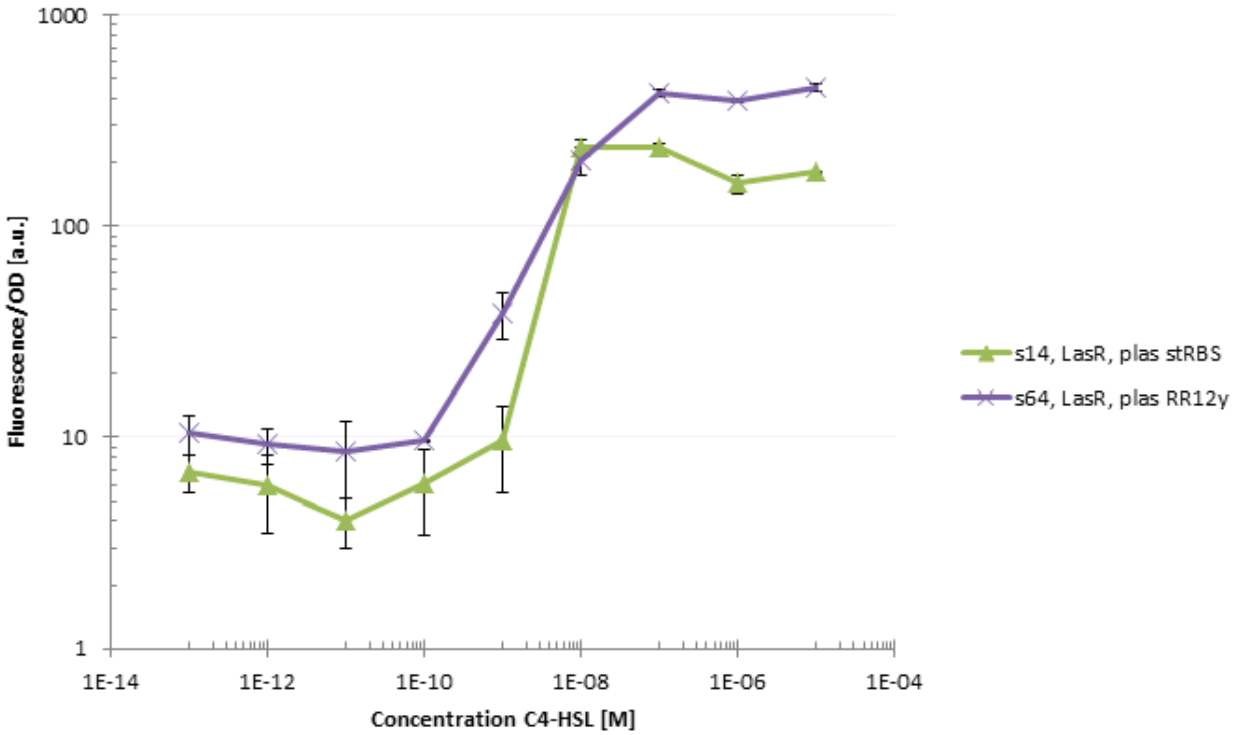


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0014 (without RR12y) and siG0064 (with RR12y)

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:

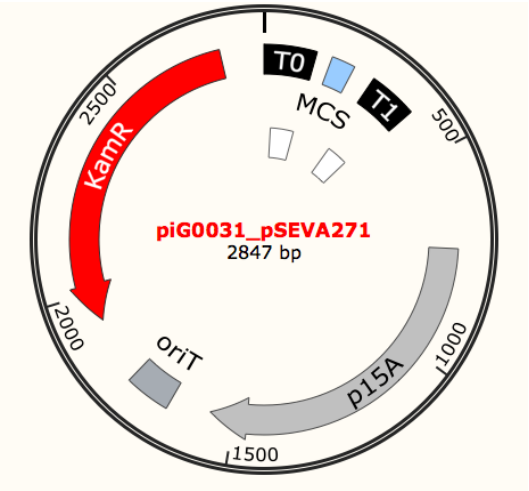
- Tecan infinite M200 PRO

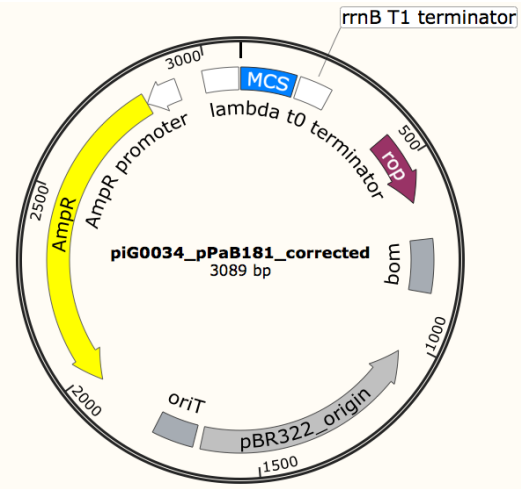
Raw Data:

- Lab/Microtiterplate/crosstalk/20140906_s65_crosstalk.xlsx

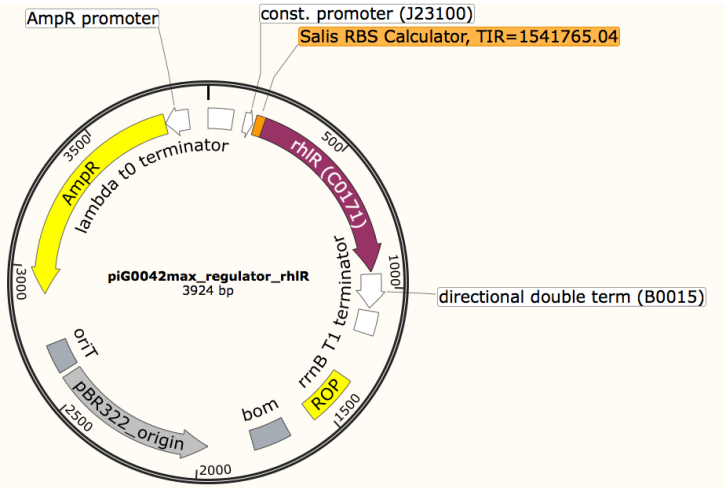
Plasmids in play:

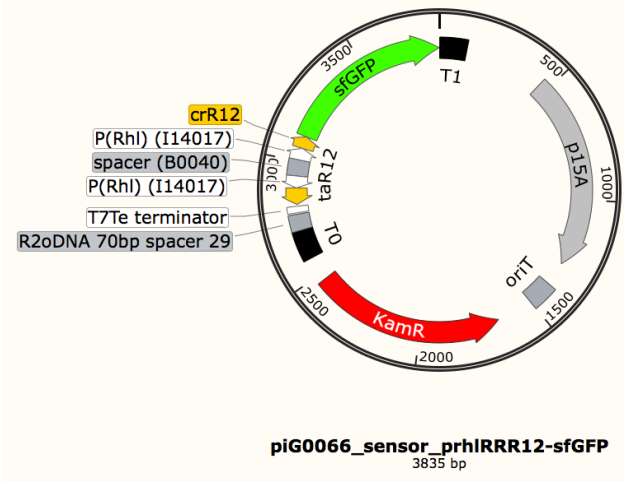
- siG0001: piG0031, piG0034





- siG0065: piG0042max, piG0066





Graphs of Data:

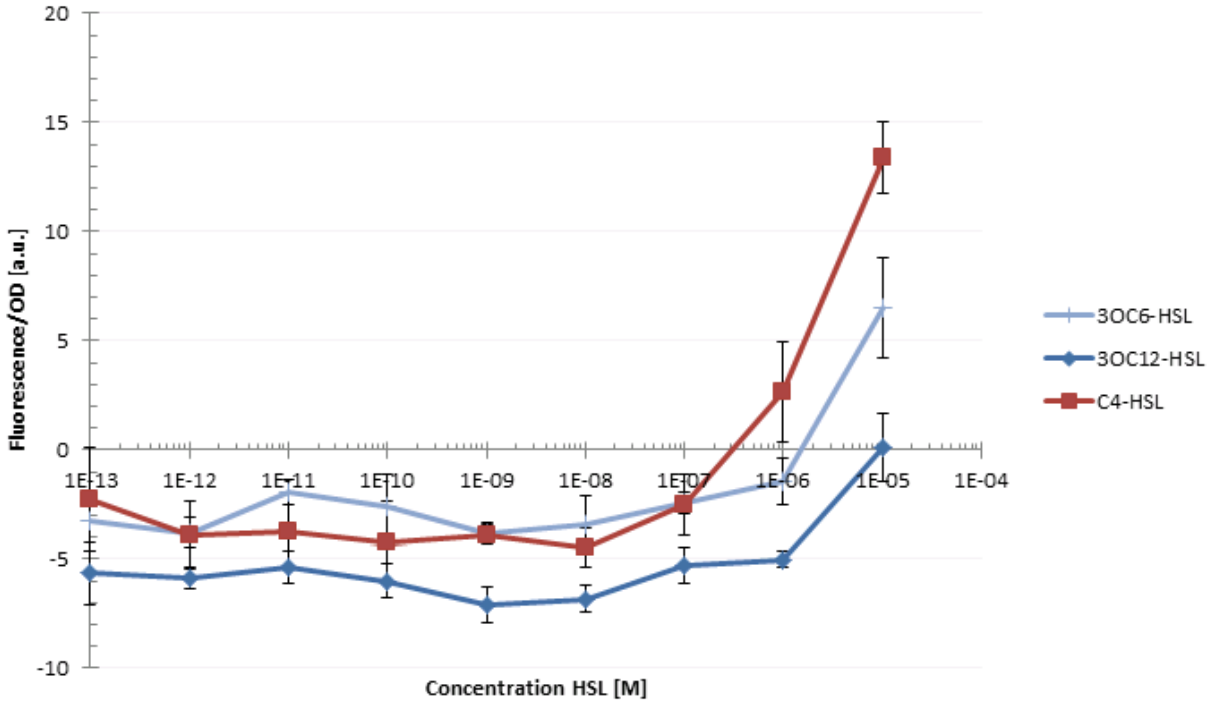


Fig. 1 siG0065 dose-response curve 200 min after induction for three AHL molecules

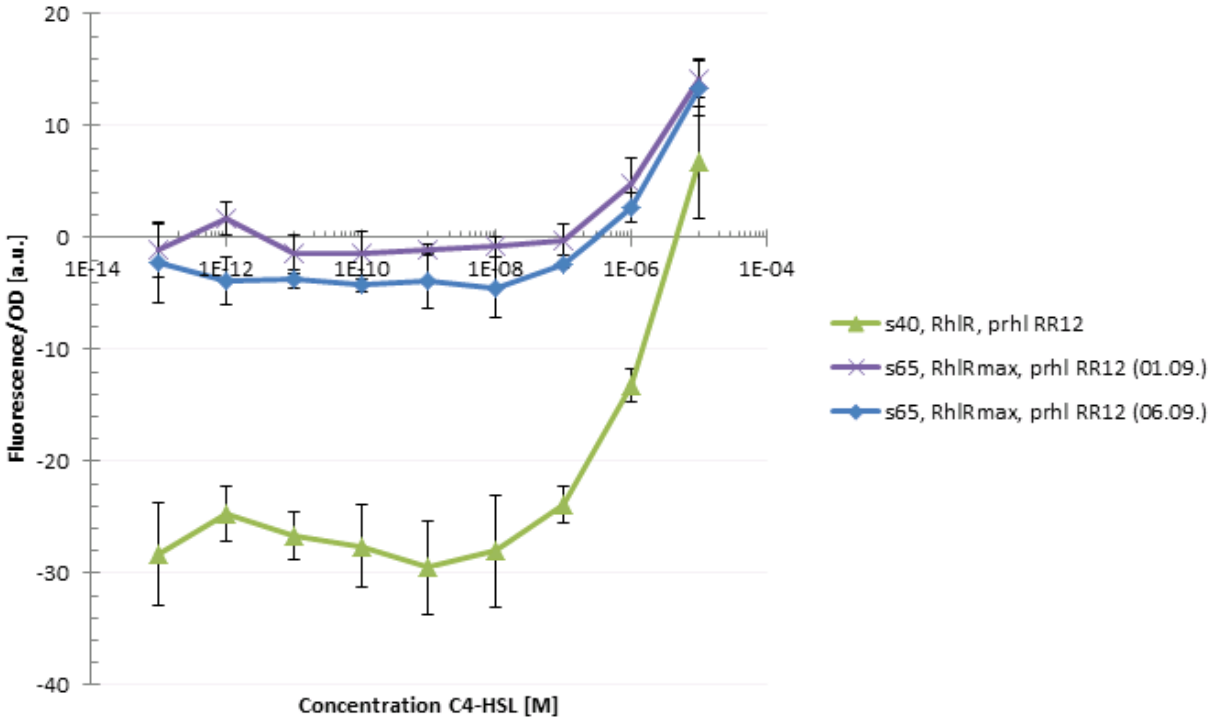


Fig. 2 dose-response curve 200 min after induction for C4-HSL for the two variants with different RBS strengths for RhlR production and comparison to repetition

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:

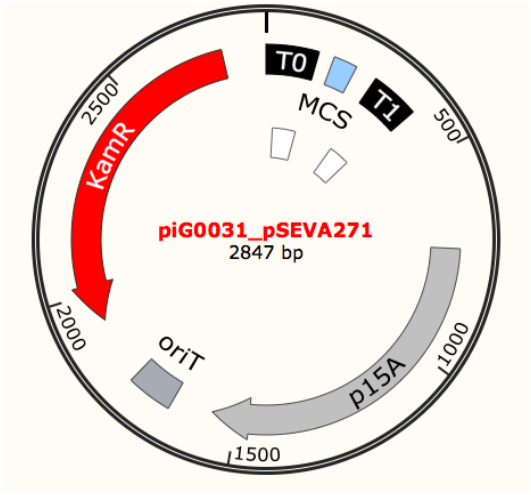
- Tecan infinite M200 PRO

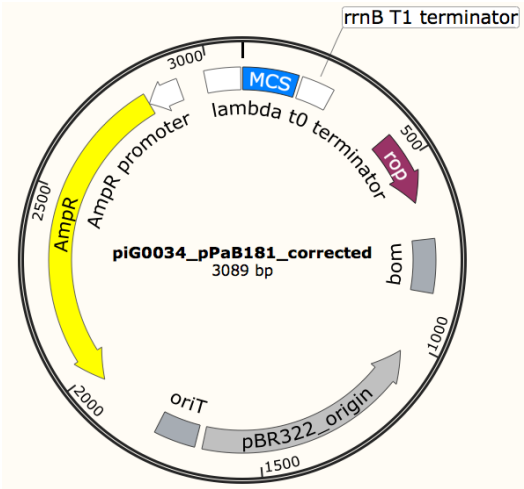
Raw Data:

- Lab/Microtiterplate/crosstalk/20140907_s67_crosstalk.xlsx

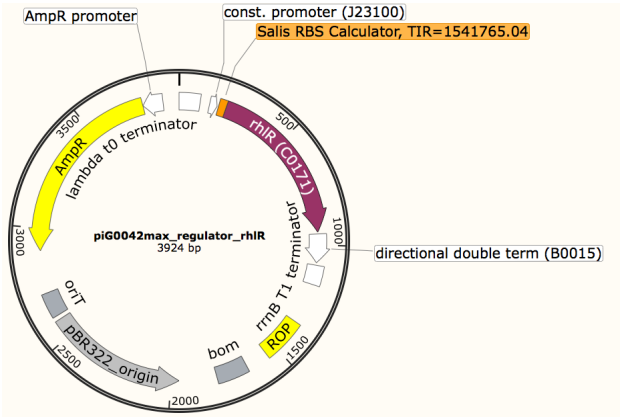
Plasmids in play:

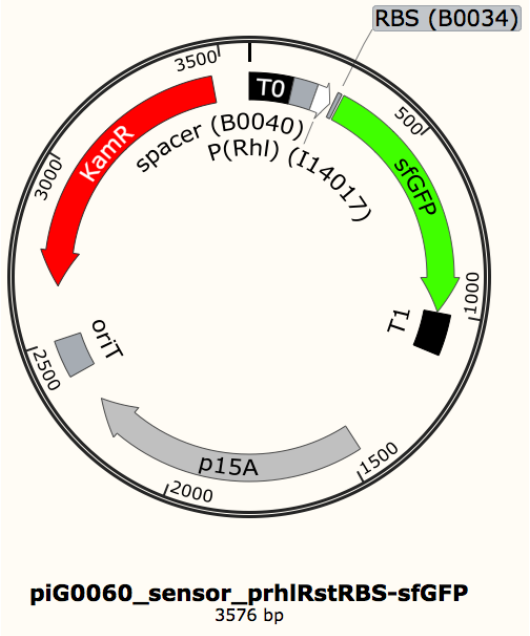
- siG0001: piG0031, piG0034





- siG0067: piG0042max, piG0060





Graphs of Data:

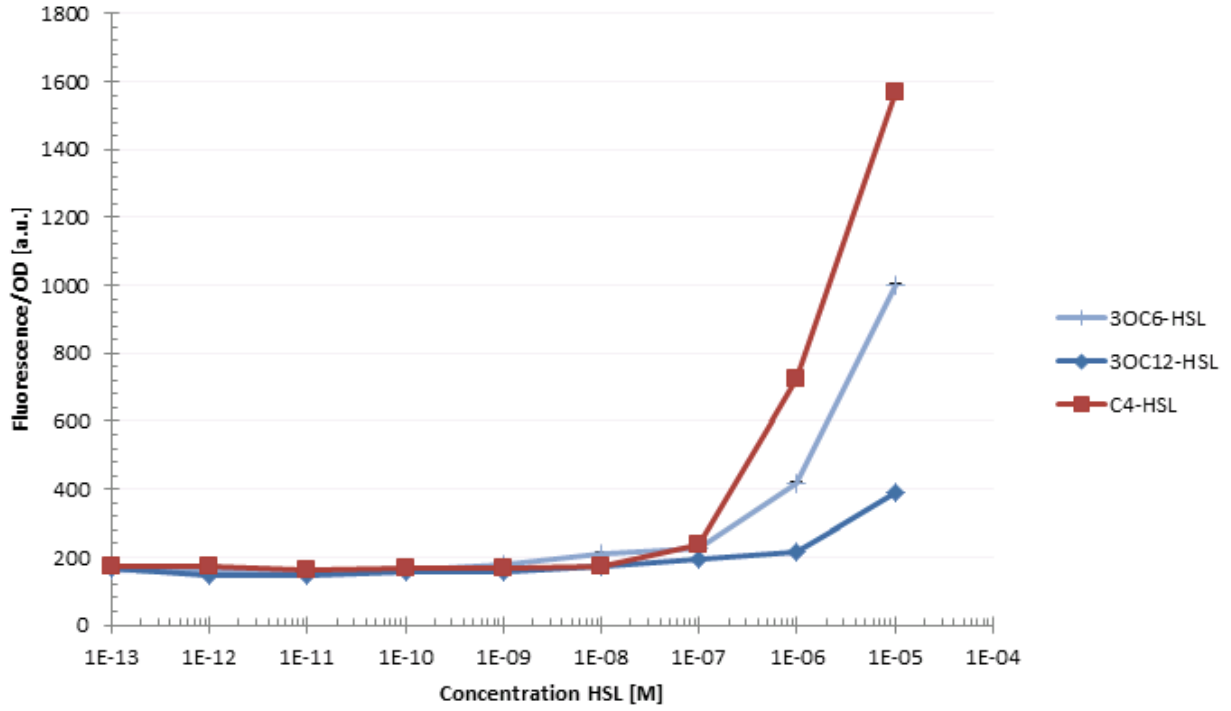


Fig. 1 siG0067 dose-response curve 200 min after induction for three AHL molecules

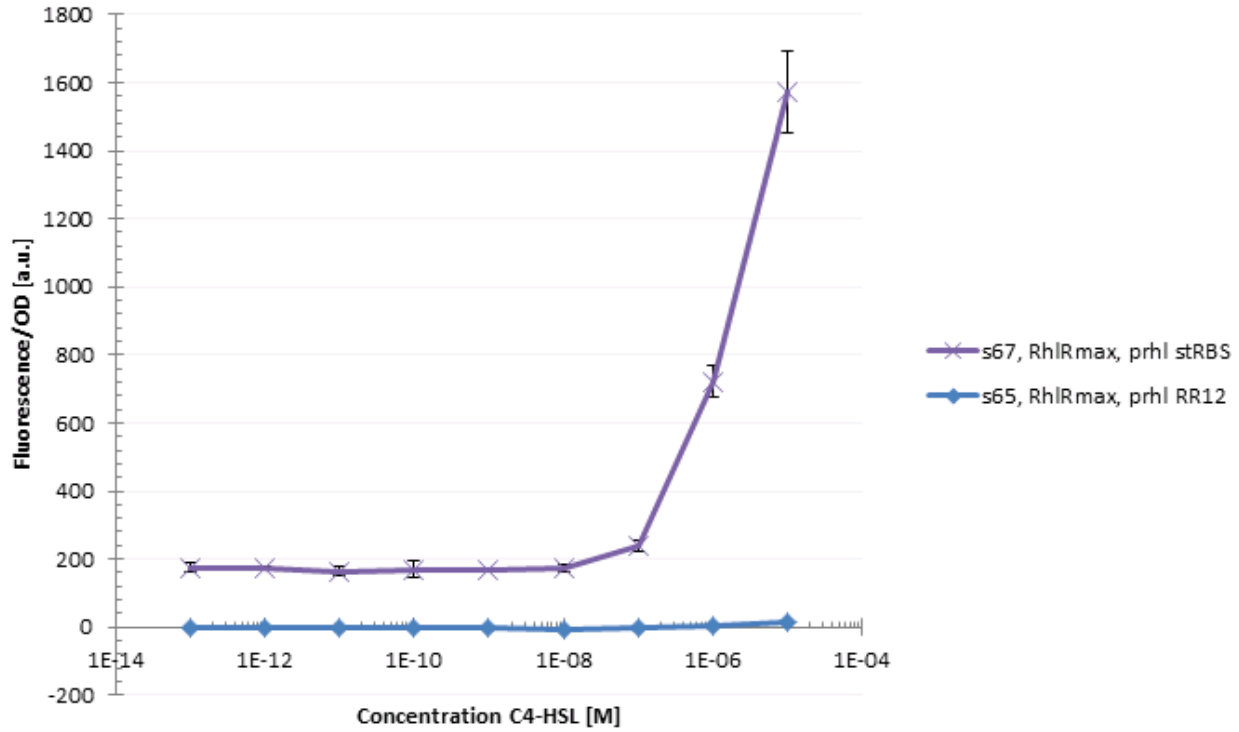


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0067 (without RR12) and siG0065 (with RR12)

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:

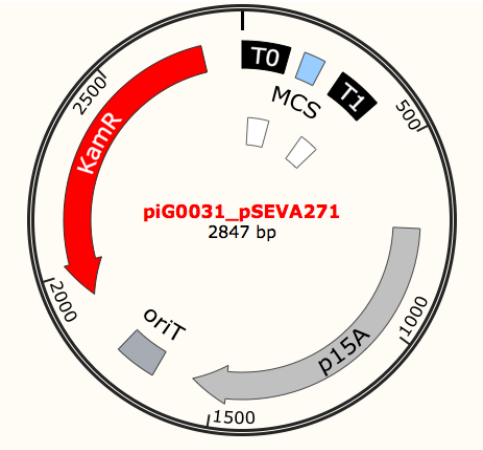
- Tecan infinite M200 PRO

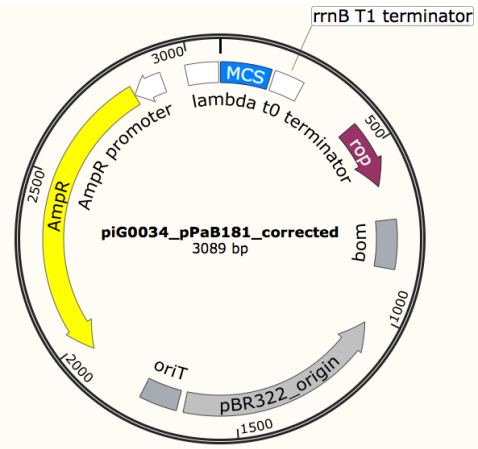
Raw Data:

- Lab/Microtiterplate/crosstalk/20140908_s66_crosstalk.xlsx

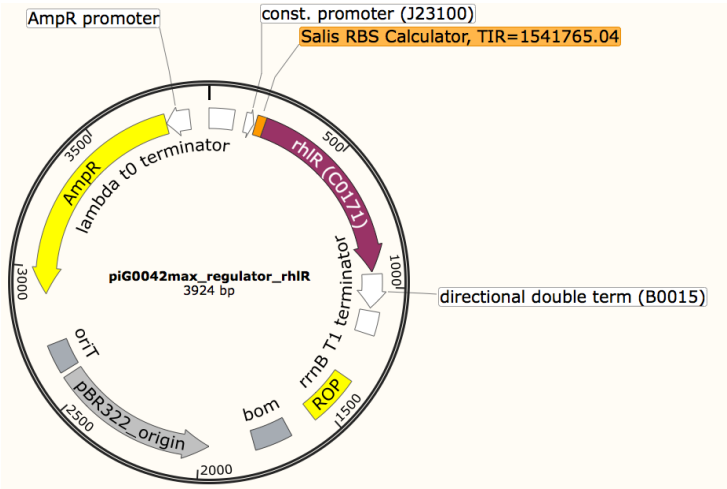
Plasmids in play:

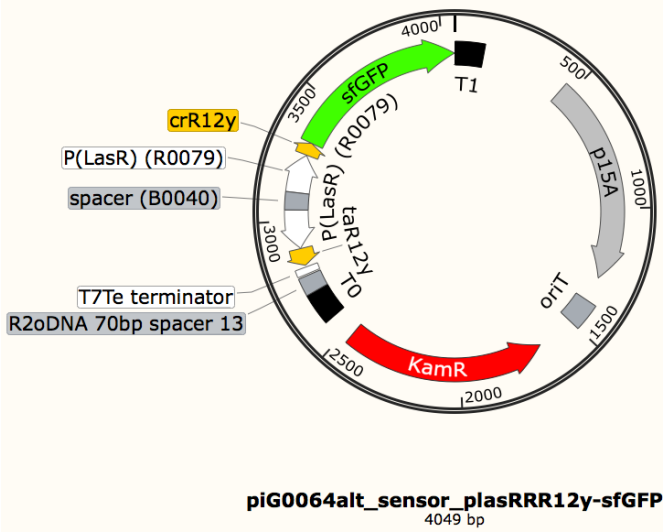
- siG0001: piG0031, piG0034





- siG0066: piG0042max, piG0064alt





Graphs of Data:

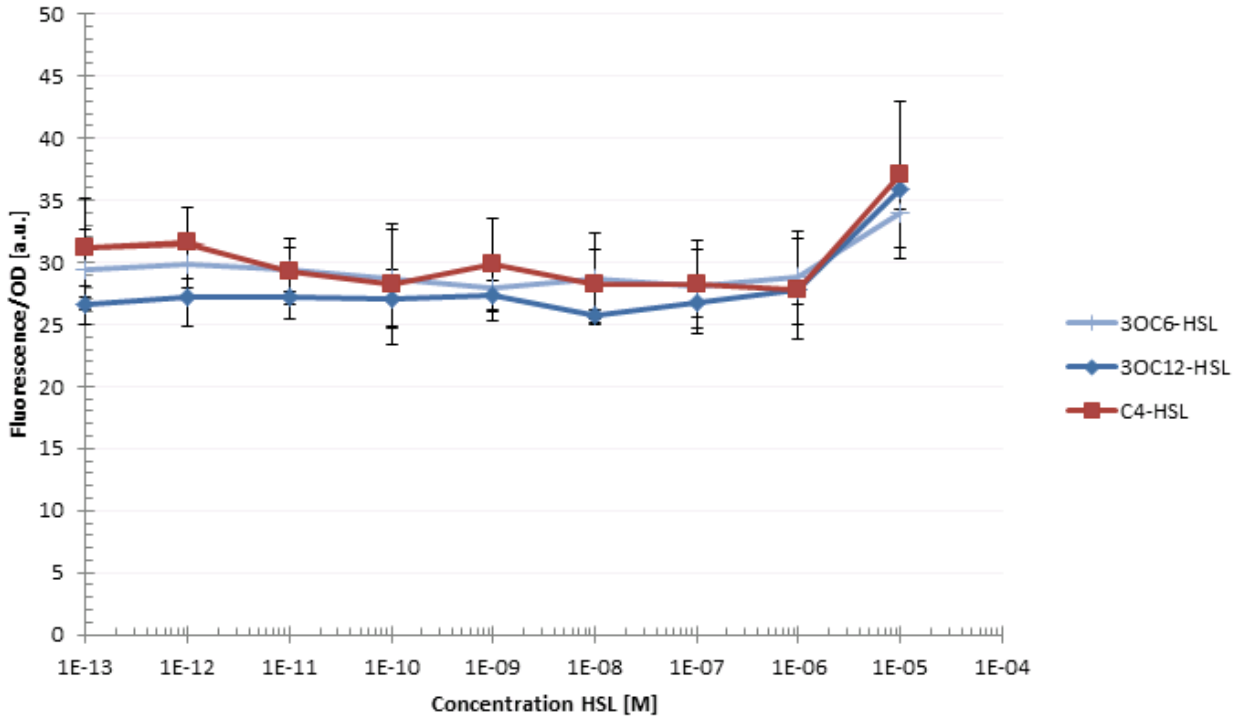


Fig. 1 siG0066 dose-response curve 200 min after induction for three AHL molecules

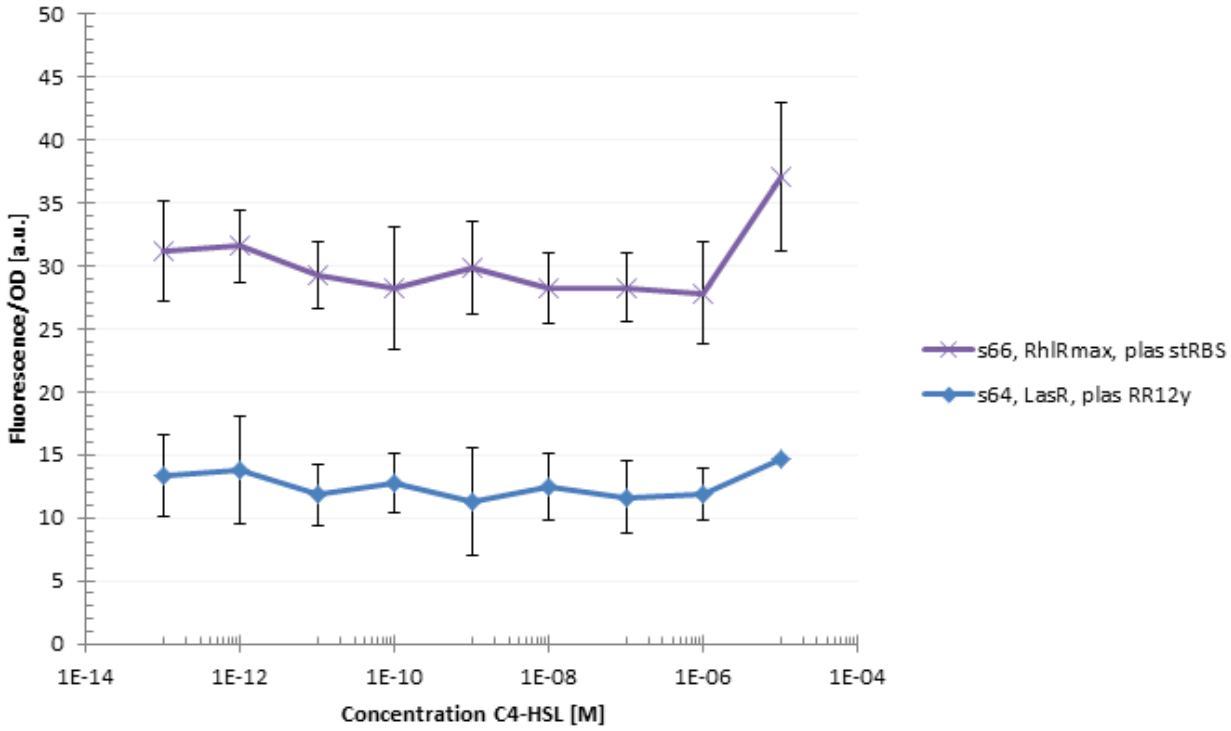


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0064 (with LasR) and siG0067 (with RhlR)

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:

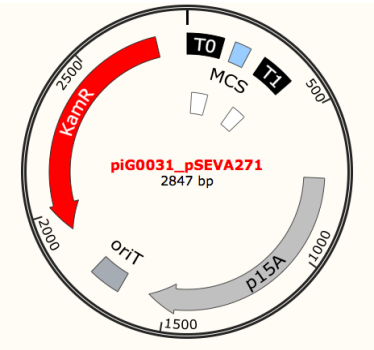
- Tecan infinite M200 PRO

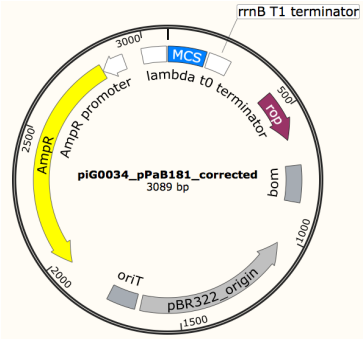
Raw Data:

- Lab/Microtiterplate/crosstalk/20140911_s30_crosstalk_supernatants_narrow_Lux_range.xlsx

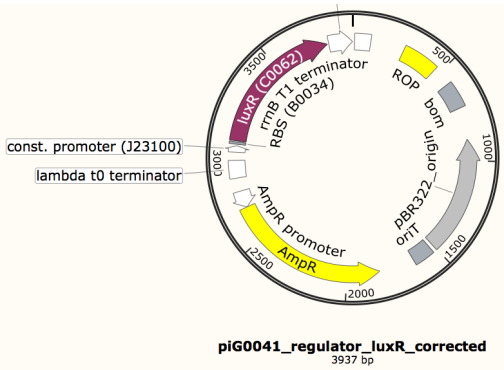
Plasmids in play:

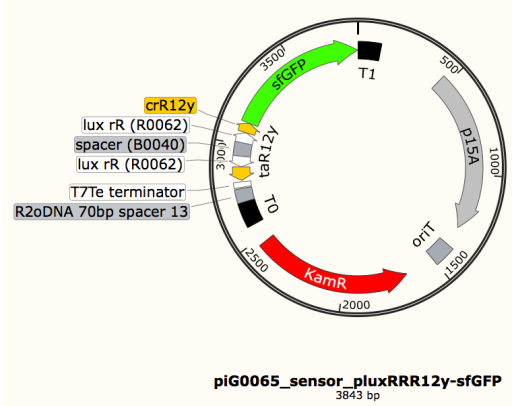
- siG0001: piG0031, piG0034



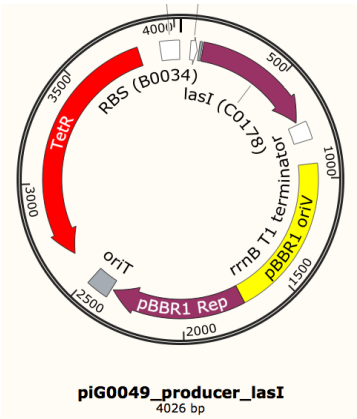


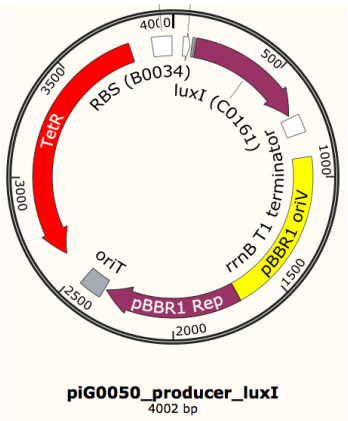
- siG0030: piG0041, piG0065

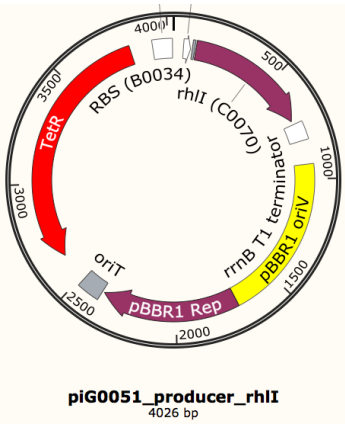




piG0049max (LasI producer)piG0050max (LuxI producer)piG0051max (RhlI producer)







Graphs of Data:

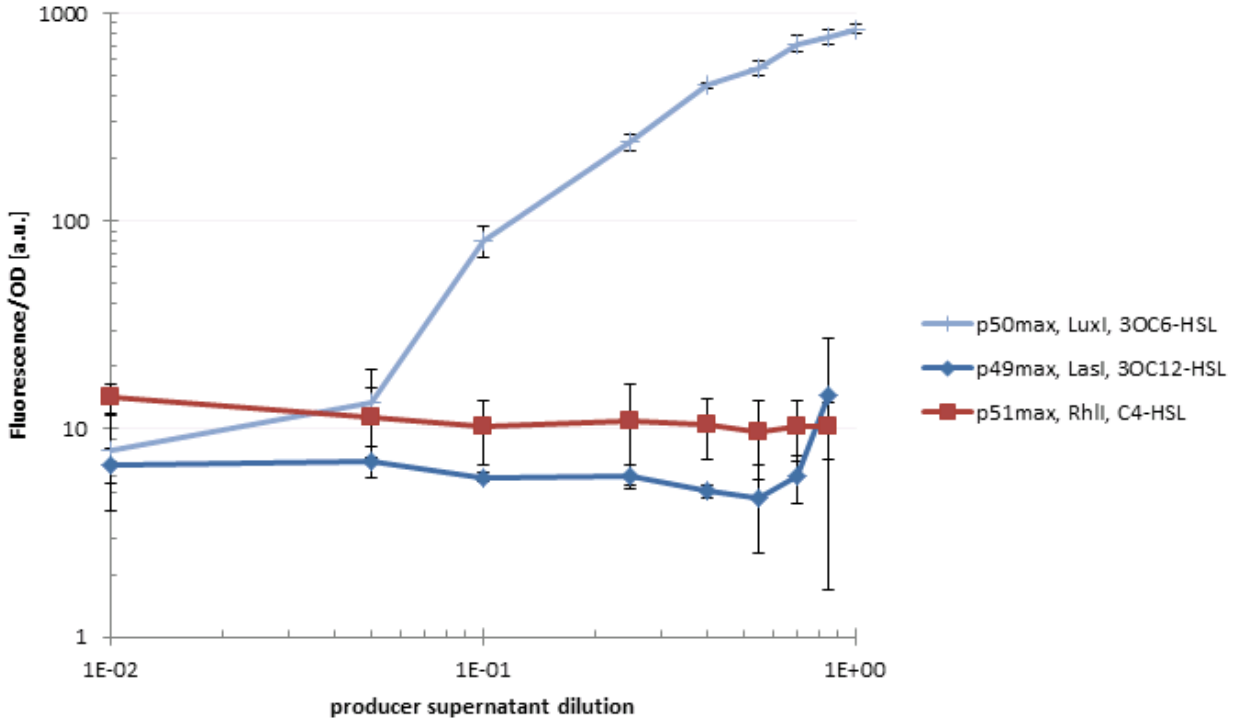


Fig. 1 siG0030 dose-response curve 350 min after inoculation in LB/supernatant mix with the three different producer supernatants

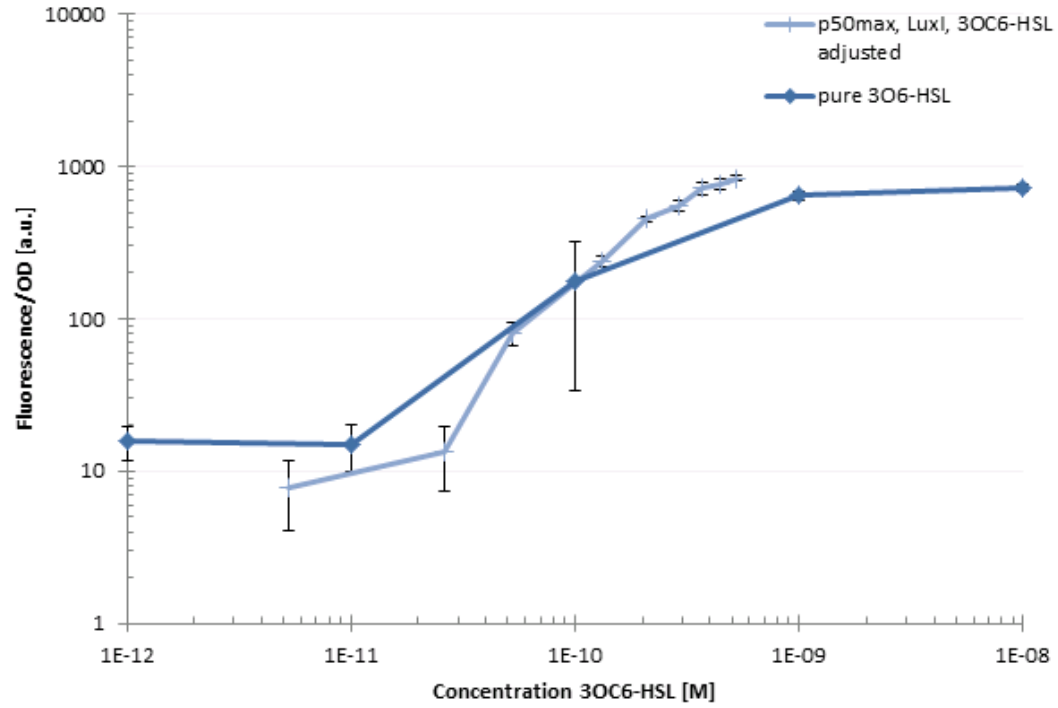


Fig. 2 siG0030 dose-response curve of supernatant fit into the dose response curve of pure 3OC6-HSL

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:

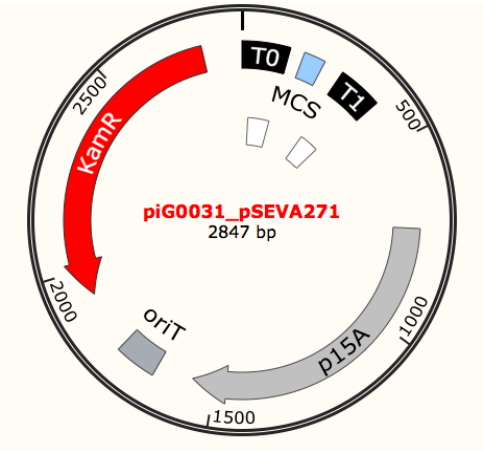
- Tecan infinite M200 PRO

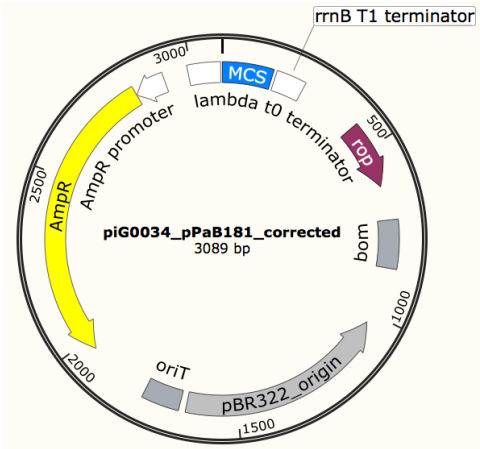
Raw Data:

- Lab/Microtiterplate/crosstalk/20140918_s15_crosstalk.xlsx

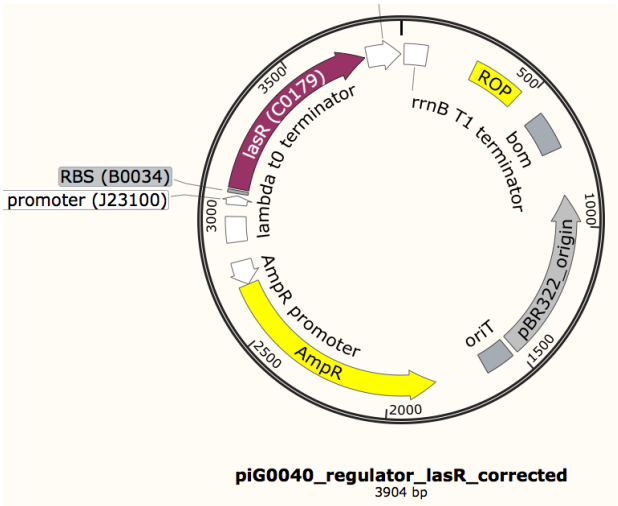
Plasmids in play:

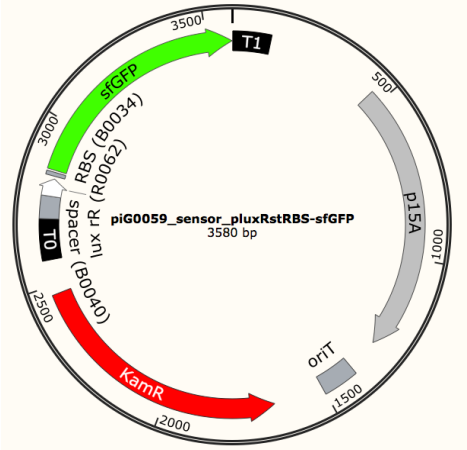
- siG0001: piG0031, piG0034





- siG0015: piG0040, piG0059





Graphs of Data:

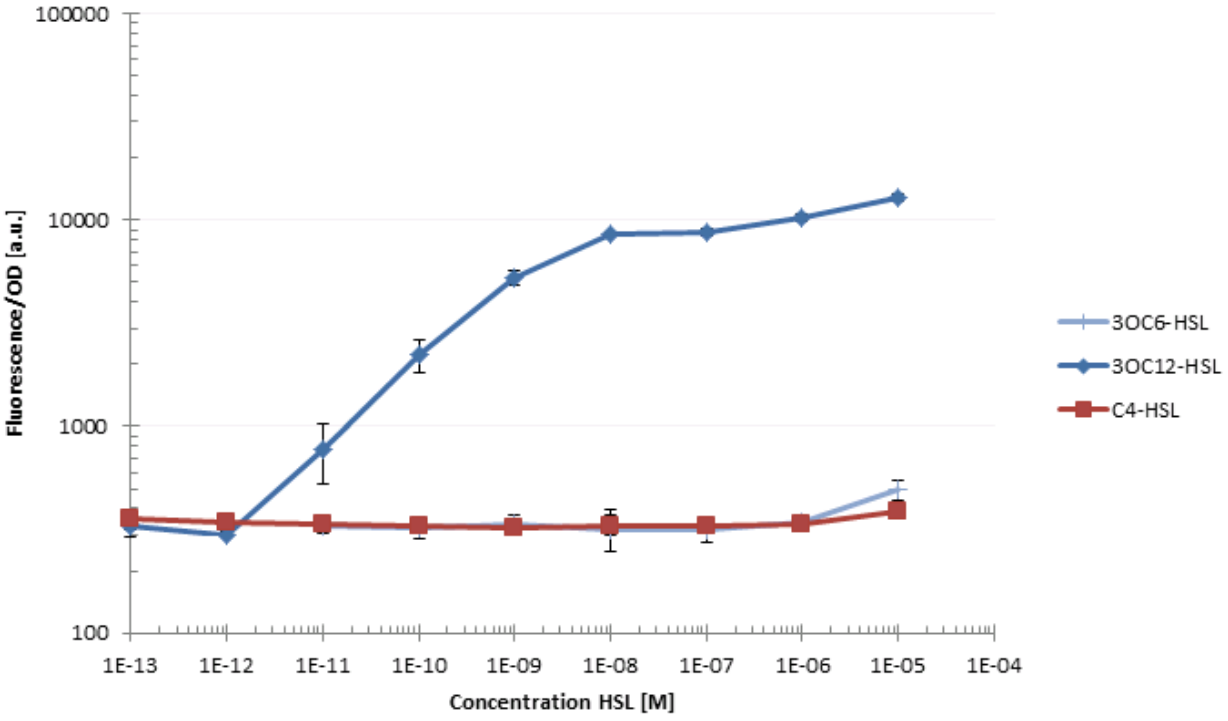


Fig. 1 siG0015 dose-response curve 200 min after induction for three AHL molecules

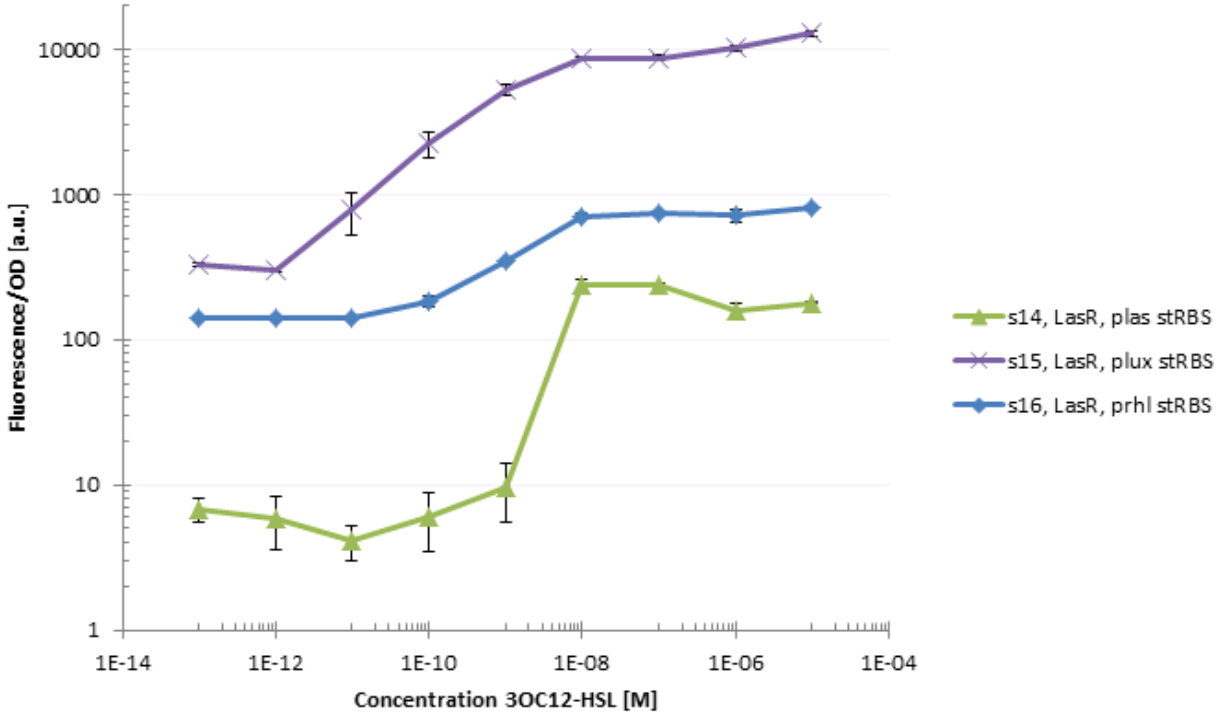


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0015, siG0014, siG0016

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:

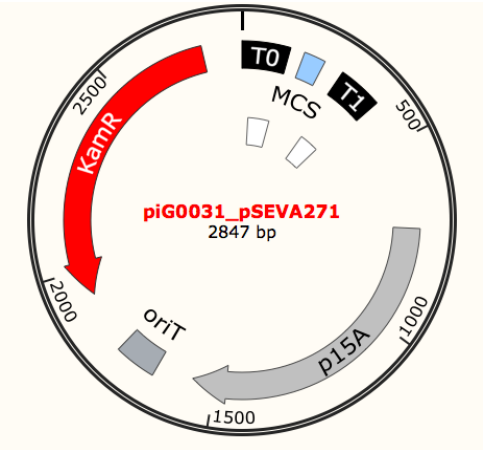
- Tecan infinite M200 PRO

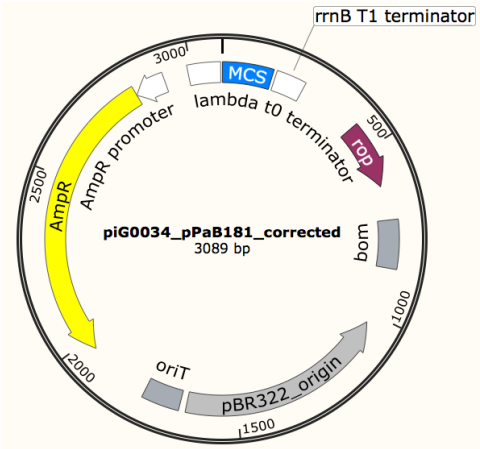
Raw Data:

- Lab/Microtiterplate/crosstalk/20140919_s81_crosstalk.xlsx

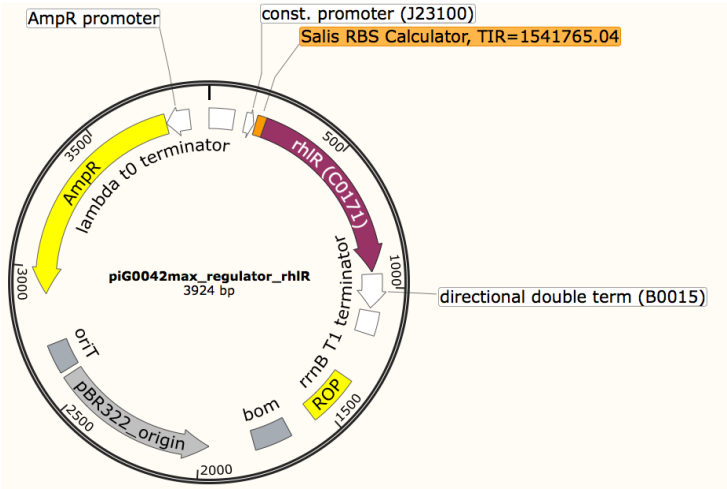
Plasmids in play:

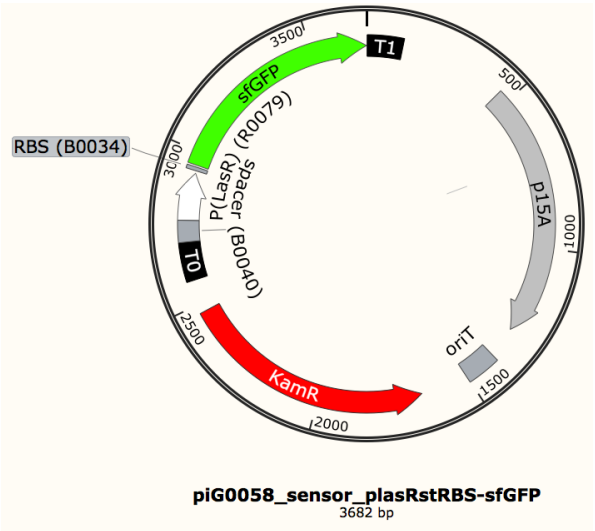
- siG0001: piG0031, piG0034





- siG0081: piG0042max, piG0058





Graphs of Data:

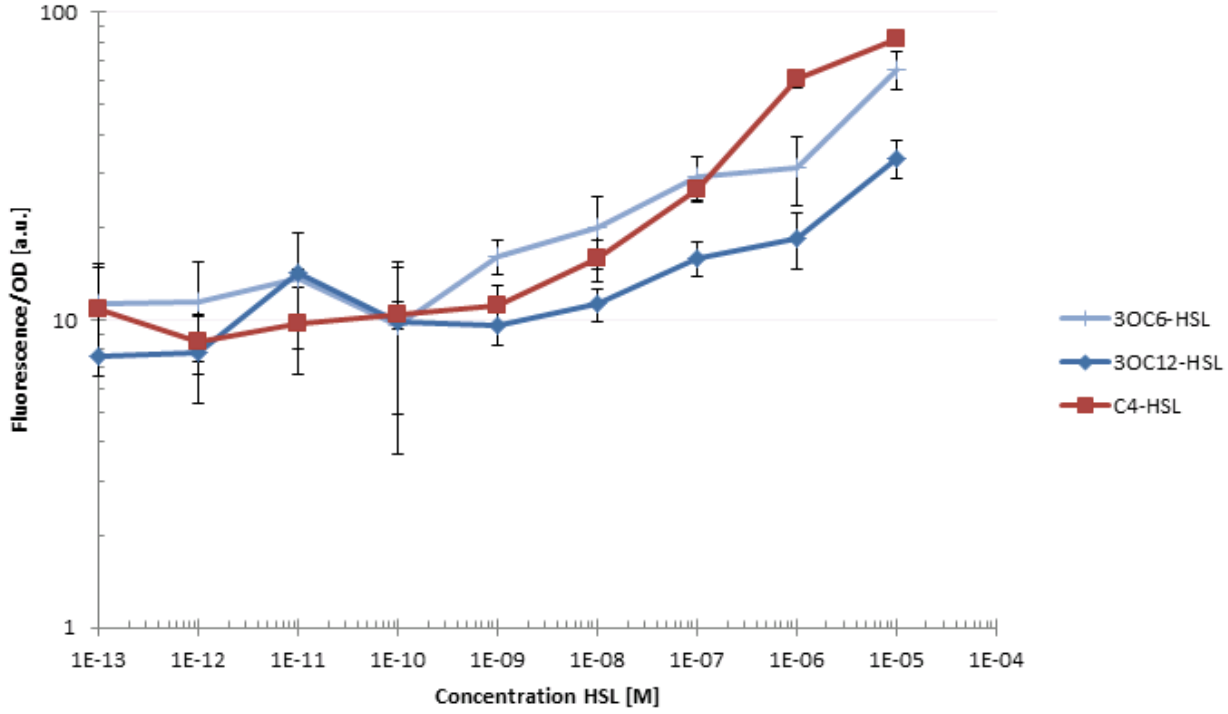


Fig. 1 siG0081 dose-response curve 200 min after induction for three AHL molecules

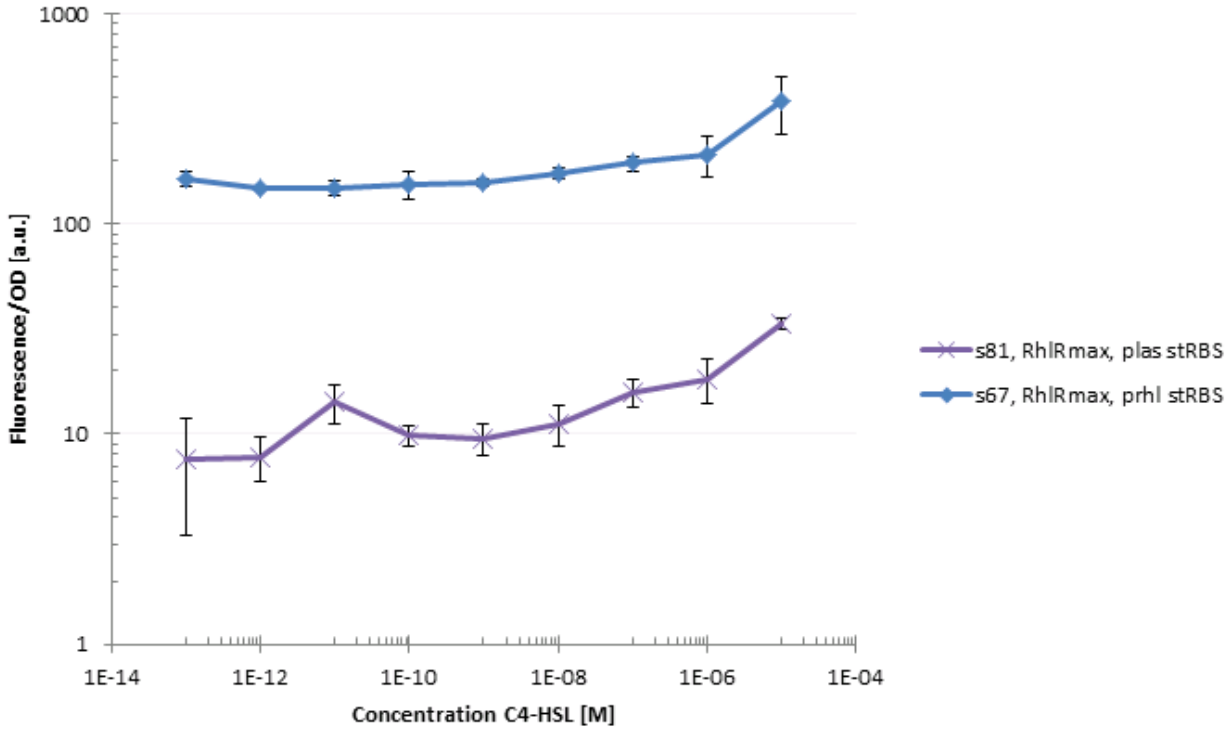


Fig. 2 dose-response curve 200 min after induction with C4-HSL for siG0081 and siG0067

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:

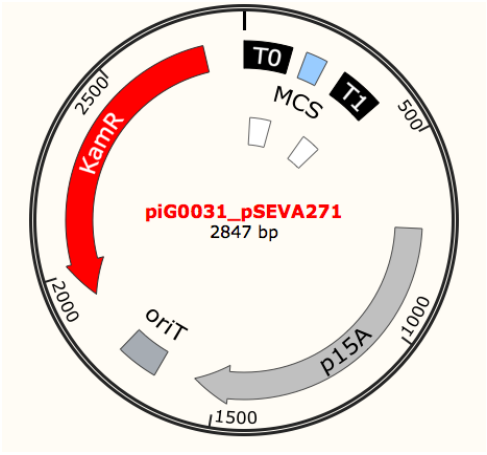
- Tecan infinite M200 PRO

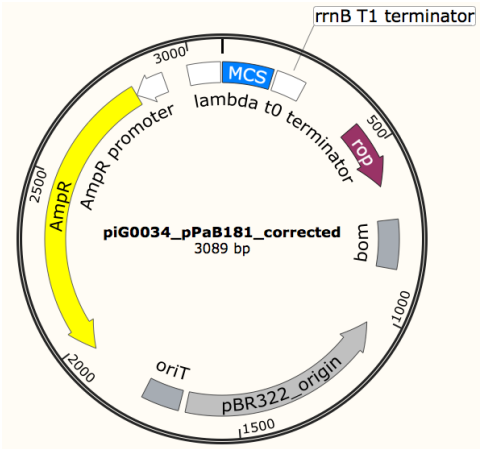
Raw Data:

- Lab/Microtiterplate/crosstalk/20140919_s16_crosstalk.xlsx

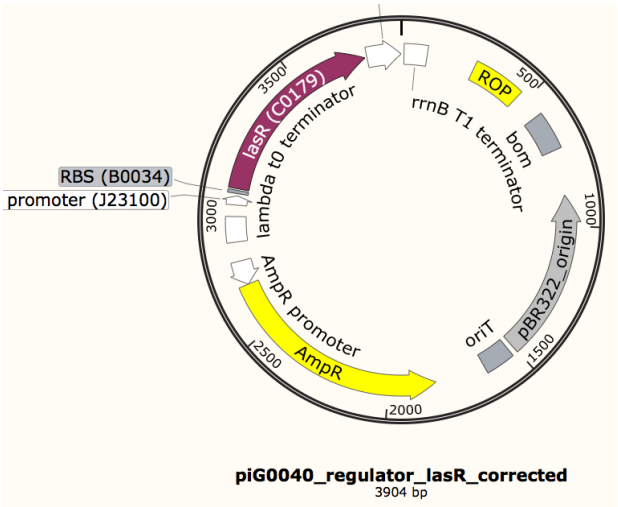
Plasmids in play:

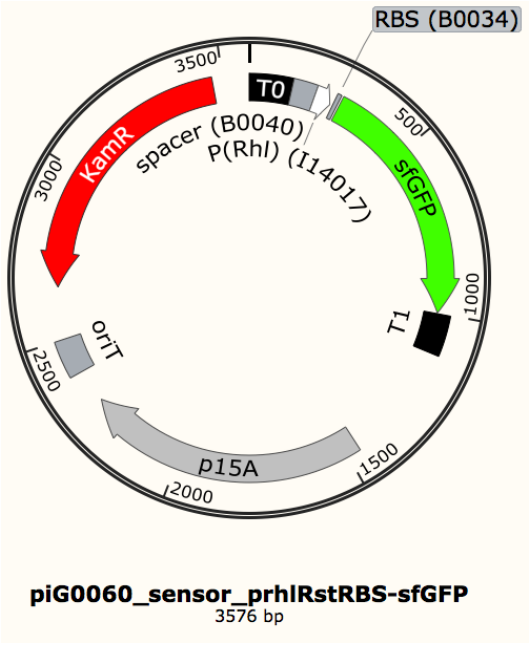
- siG0001: piG0031, piG0034





- siG0016: piG0040, piG0060





Graphs of Data:

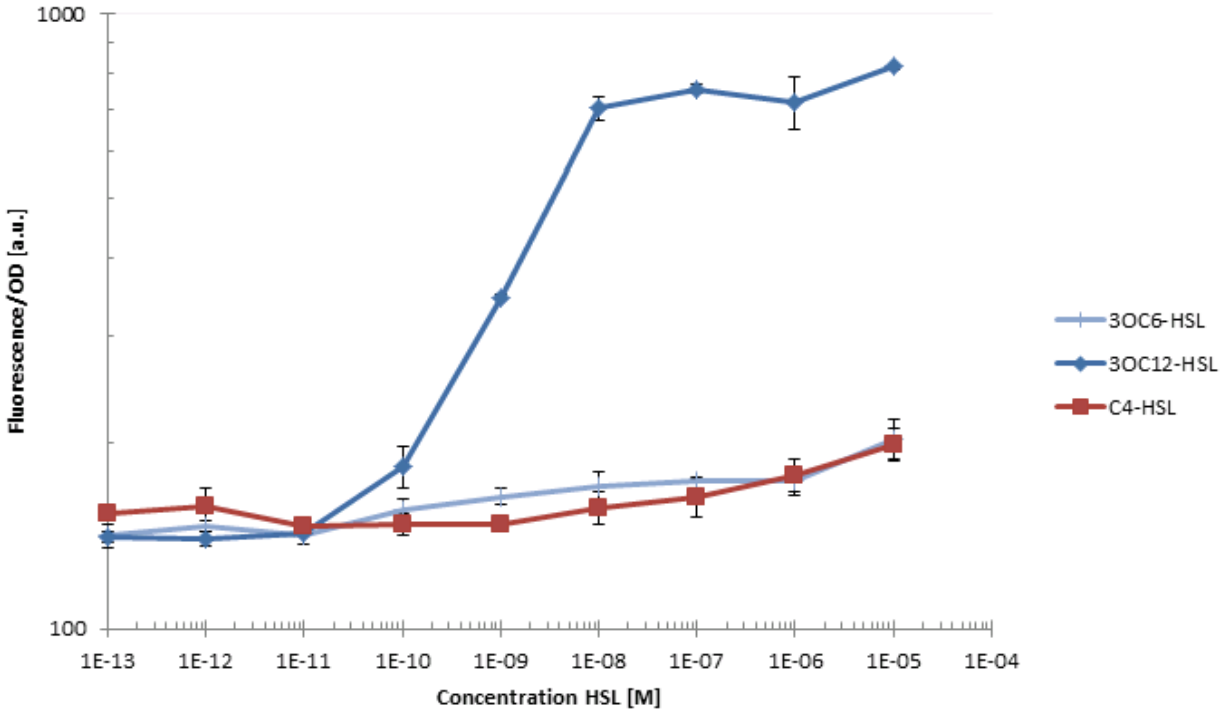


Fig. 1 siG0016 dose-response curve 200 min after induction for three AHL molecules

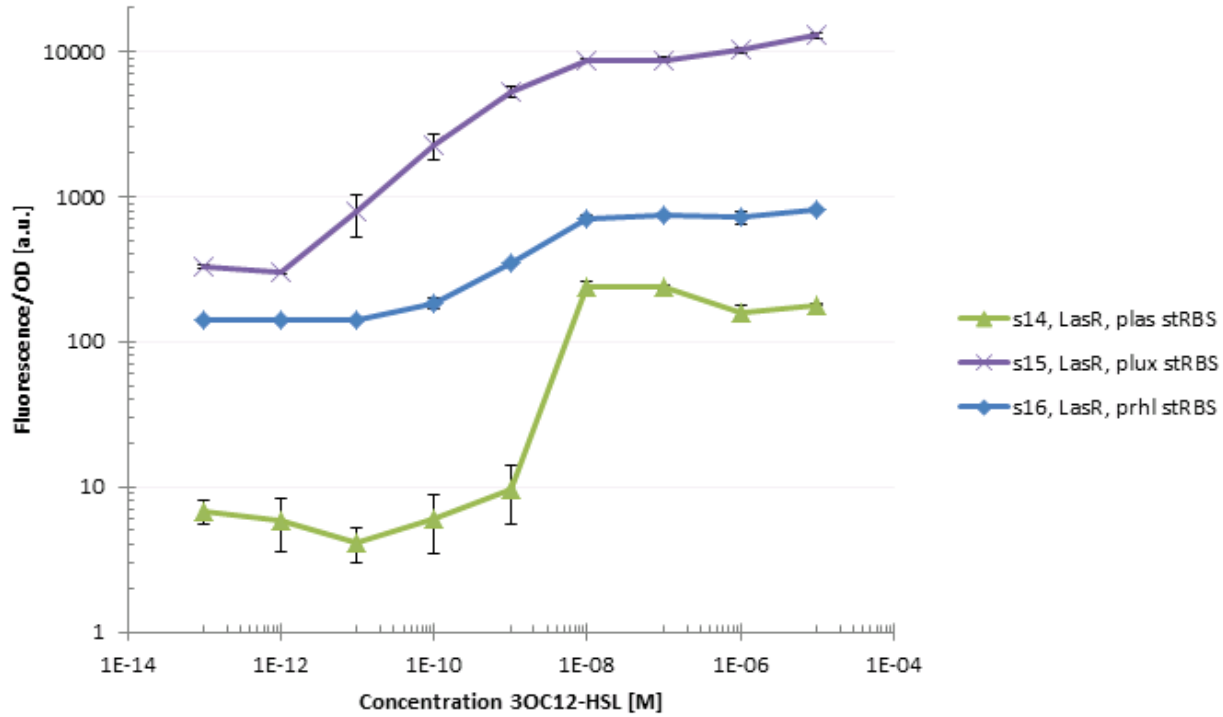


Fig. 2 dose-response curve 200 min after induction with 3OC12-HSL for siG0015, siG0014, siG0016

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:

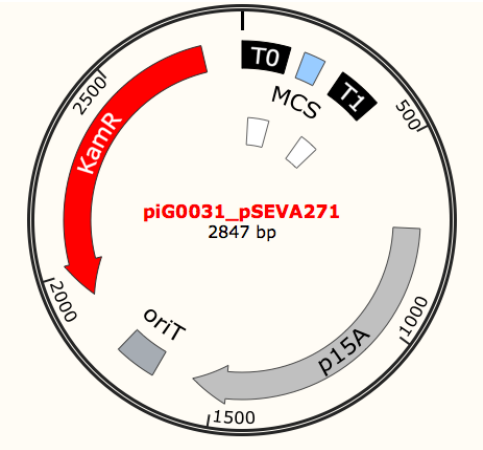
- Tecan infinite M200 PRO

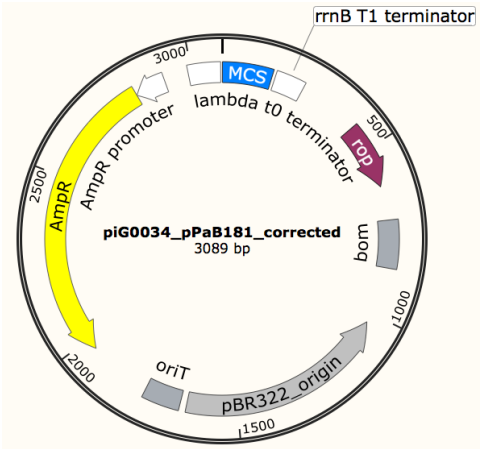
Raw Data:

- Lab/Microtiterplate/crosstalk/20140921_s25_crosstalk.xlsx

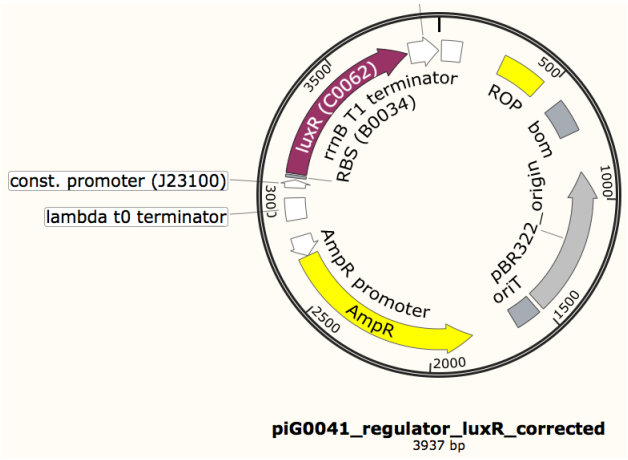
Plasmids in play:

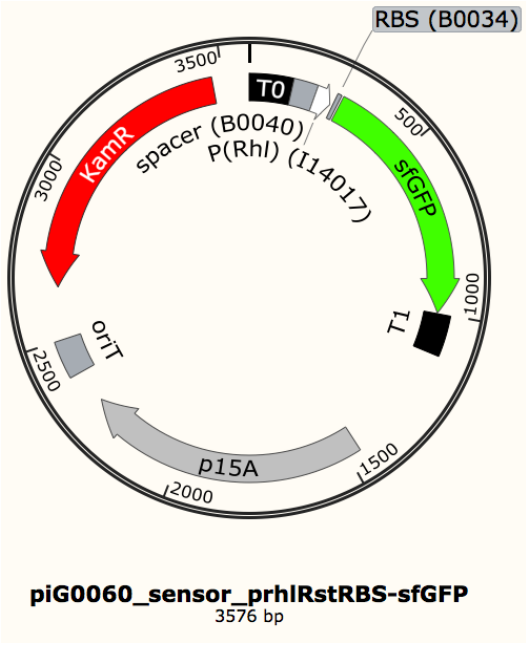
- siG0001: piG0031, piG0034





- siG0025: piG0041, piG0060





Graphs of Data:

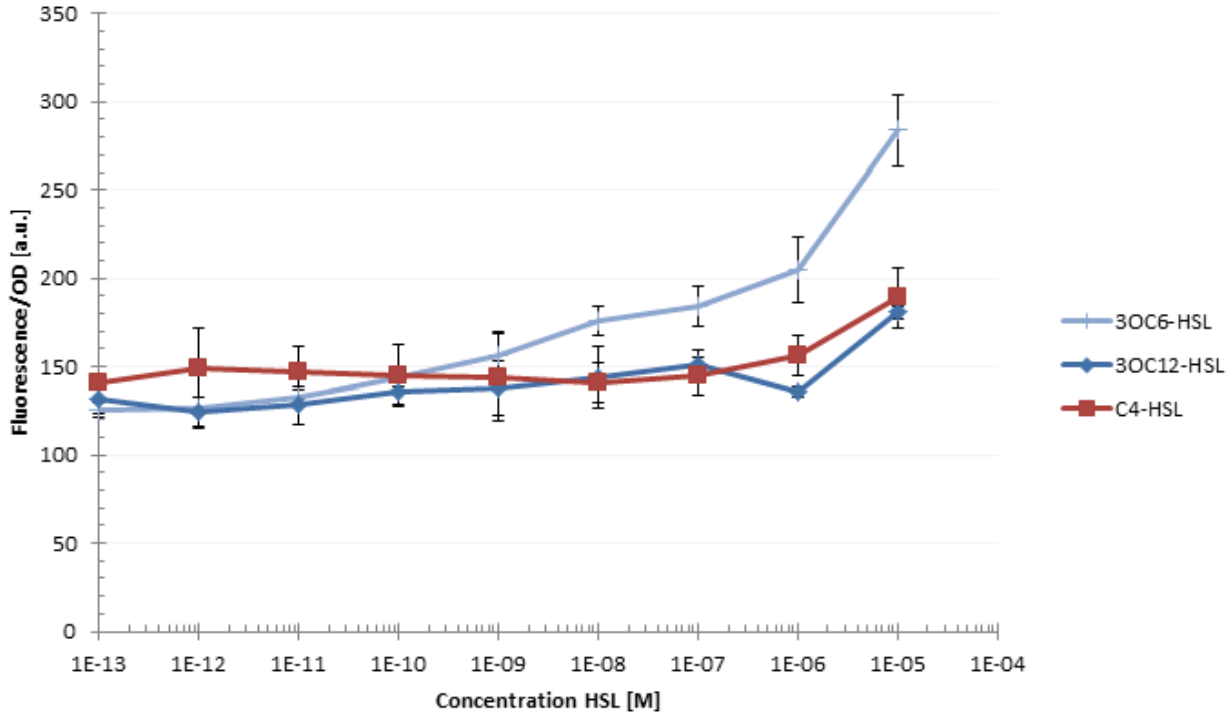


Fig. 1 siG0025 dose-response curve 200 min after induction for three AHL molecules

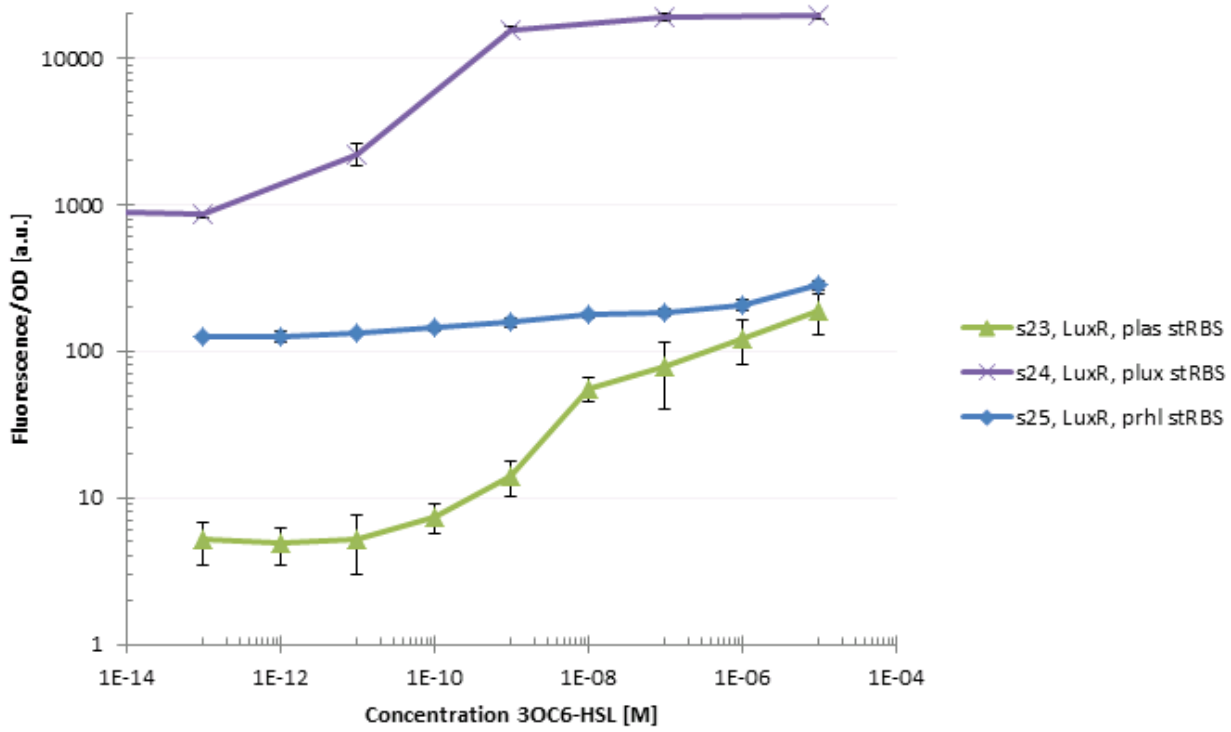


Fig. 2 dose-response curve 200 min after induction with 3OC6-HSL for siG0024, siG0025, siG0026

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

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:

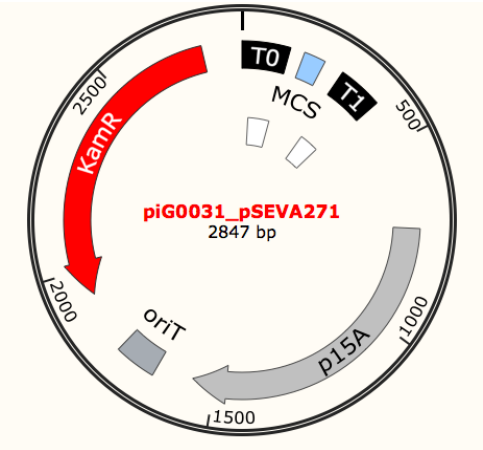
- Tecan infinite M200 PRO

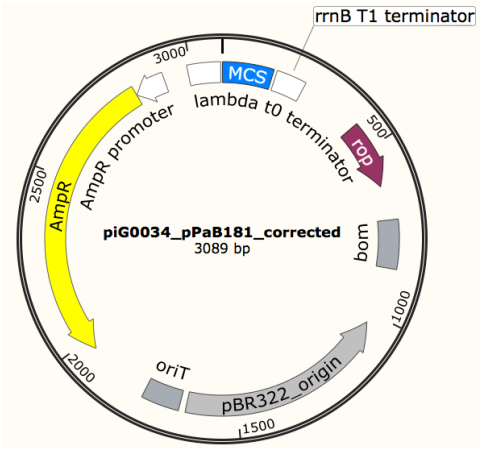
Raw Data:

- Lab/Microtiterplate/crosstalk/20140924_s80_crosstalk.xlsx

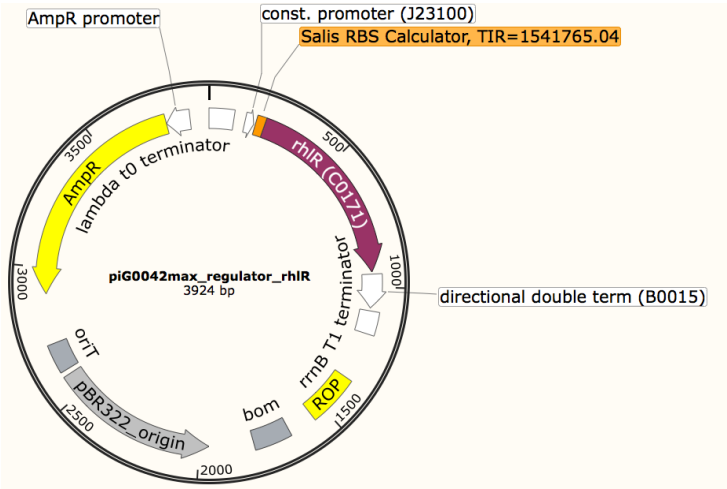
Plasmids in play:

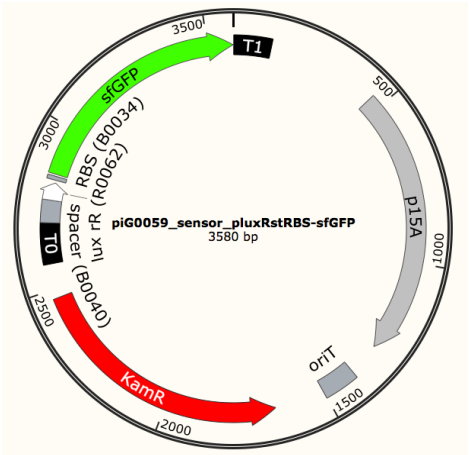
- siG0001: piG0031, piG0034





- siG0080: piG0042max, piG0059





Graphs of Data:

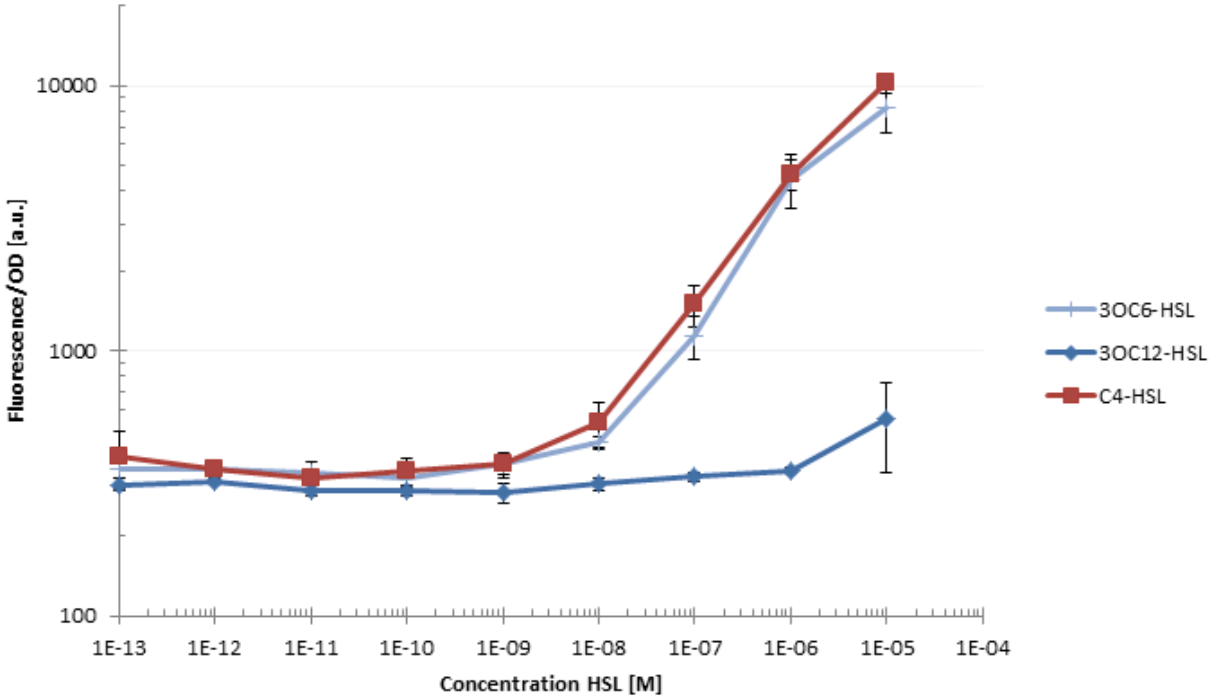


Fig. 1 siG0080 dose-response curve 200 min after induction for three AHL molecules

Interpretation of Data:

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

Experiment T32

Dose-Response Kinetics and Crosstalk
siG0098: LuxR, sfGFP under plux Promoter and Riboregulator 12y
2014-10-13

Goal of the experiment:

- Investigate crosstalk on the regulator level when EcoRI and XbaI sites are removed
- Does the removal of these site destroy the riboregulating function?
- 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 siG0098
        - 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:

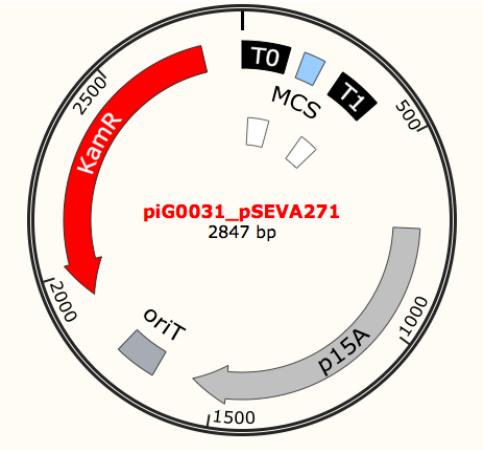
- Tecan infinite M200 PRO

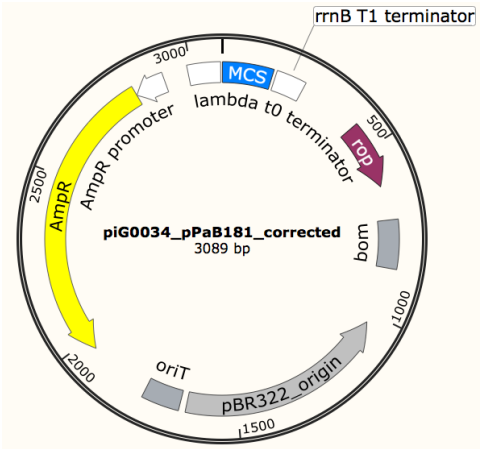
Raw Data:

- Lab/Microtiterplate/crosstalk/20141013_s98_crosstalk.xlsx

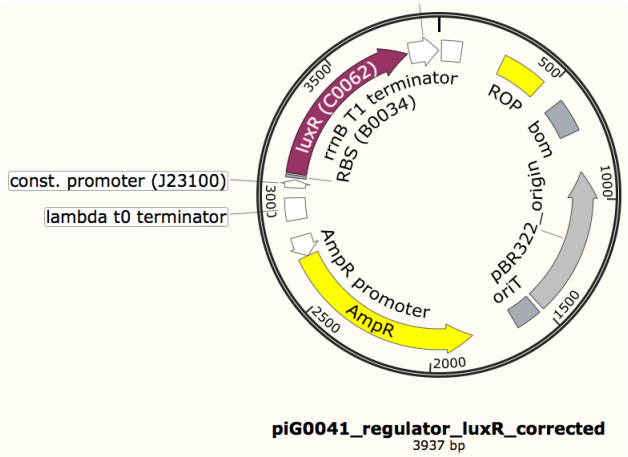
Plasmids in play:

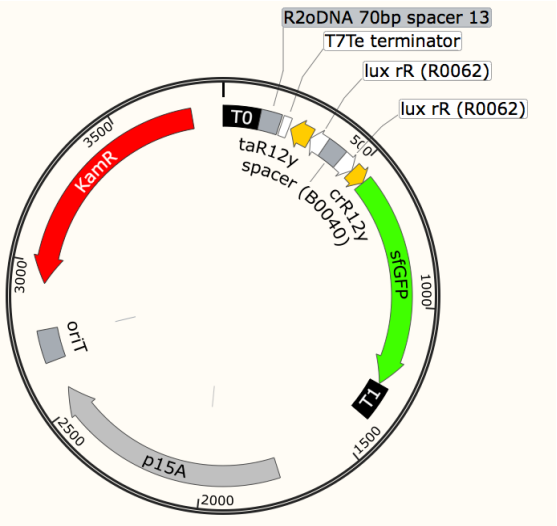
- siG0001: piG0031, piG0034





- siG0098: piG0041, piG0109





Graphs of Data:

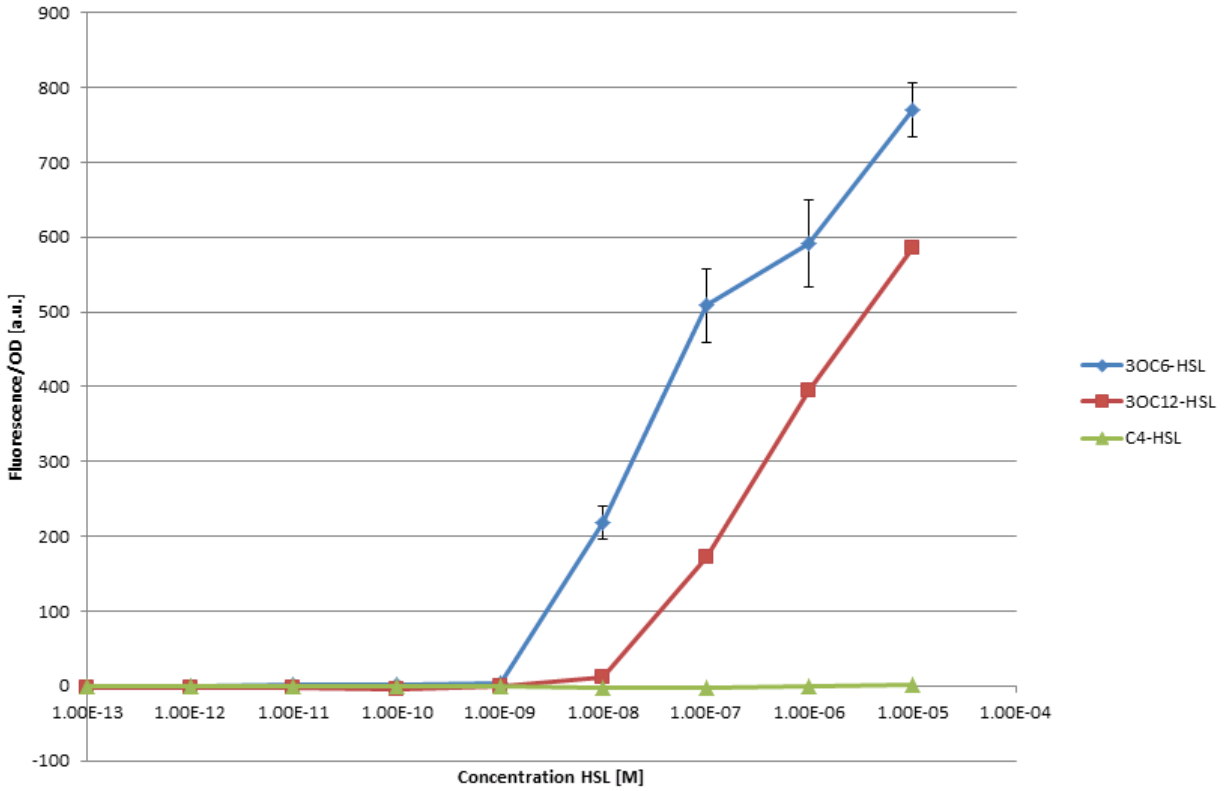


Fig. 1 siG0098 dose-response curve 200 min after induction for three AHL molecules

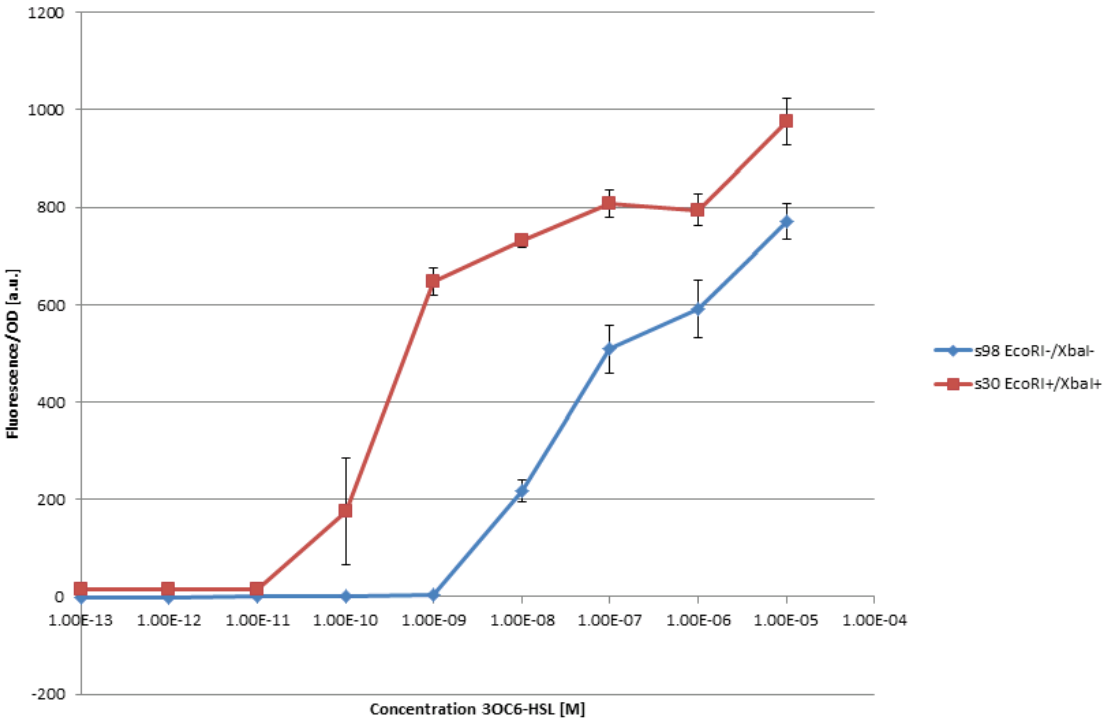


Fig. 2 siG0098 and siG0030 compared dose-response curve to 3OC6-HSL

Interpretation of Data:

- The riboregulator is still reducing the leakiness, but the sensitivity is lowered (Fig. 2)

Experiment T33

Dose-Response Kinetics and Crosstalk
siG0099: RhlR with optimized RBS, sfGFP under prhl Promoter and Riboregulator 12
2014-10-13

Goal of the experiment:

- Investigate crosstalk on the regulator level when EcoRI and XbaI sites are removed
- Does the removal of these site destroy the riboregulating function?
- Determine dose-response curves
- Record dynamic behavior
- Try higher concentration, since the rhl system was so far less responsive than the others

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 siG0099
        - 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^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M

Machines used:

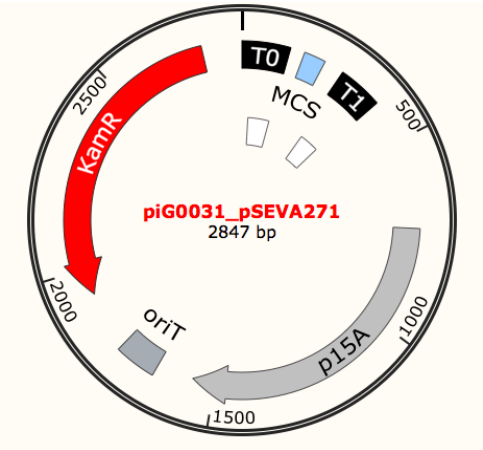
- Tecan infinite M200 PRO

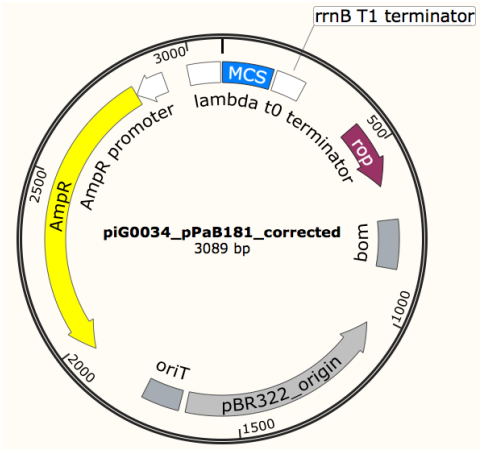
Raw Data:

- Lab/Microtiterplate/crosstalk/20141013_s99_crosstalk.xlsx

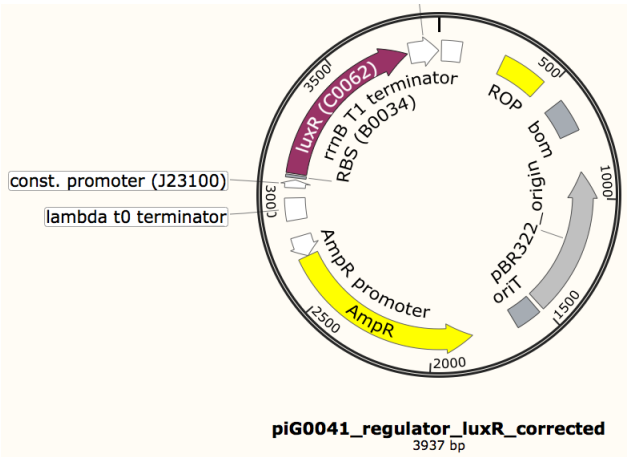
Plasmids in play:

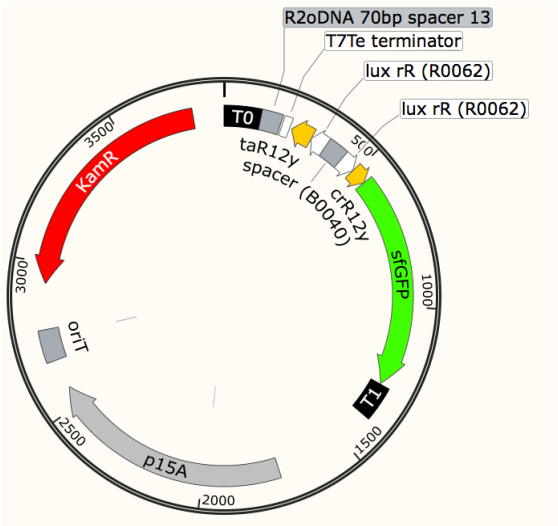
- siG0001: piG0031, piG0034





- siG0098: piG0042max, piG0110





Graphs of Data:

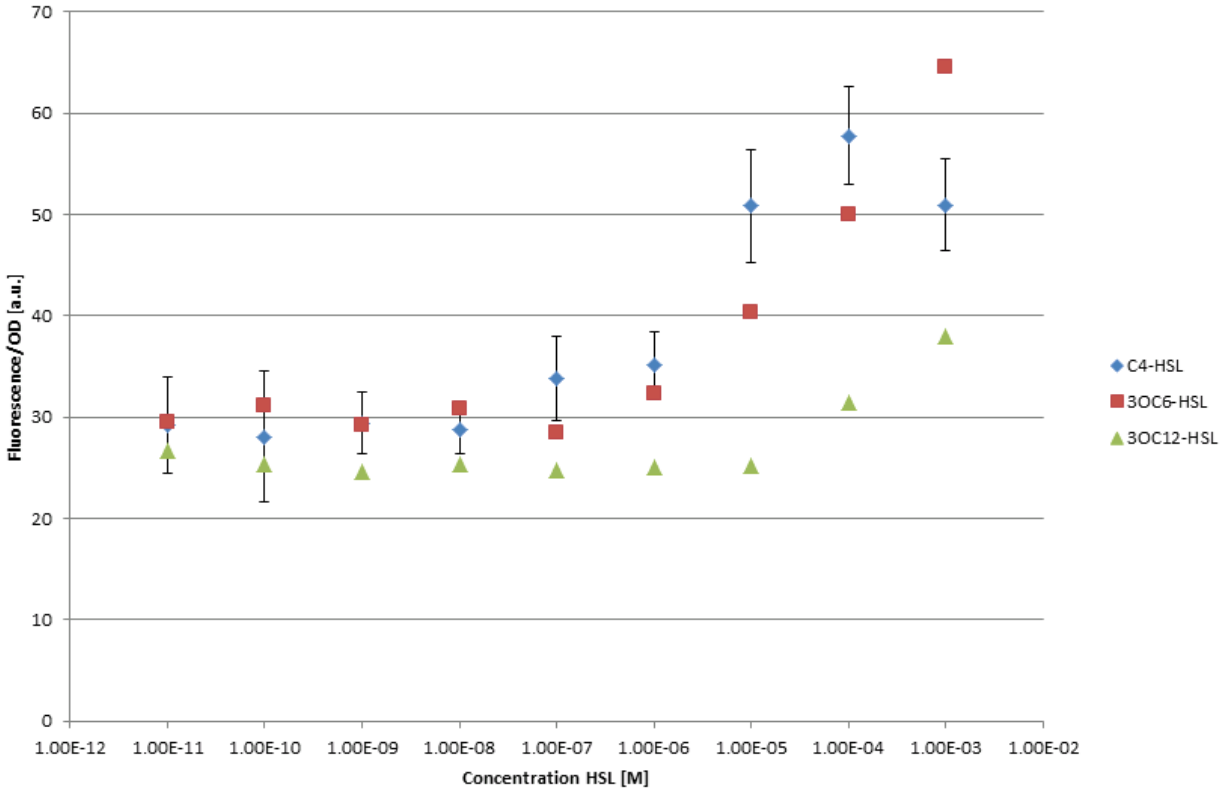


Fig. 1 siG0099 dose-response curve 200 min after induction for three AHL molecules

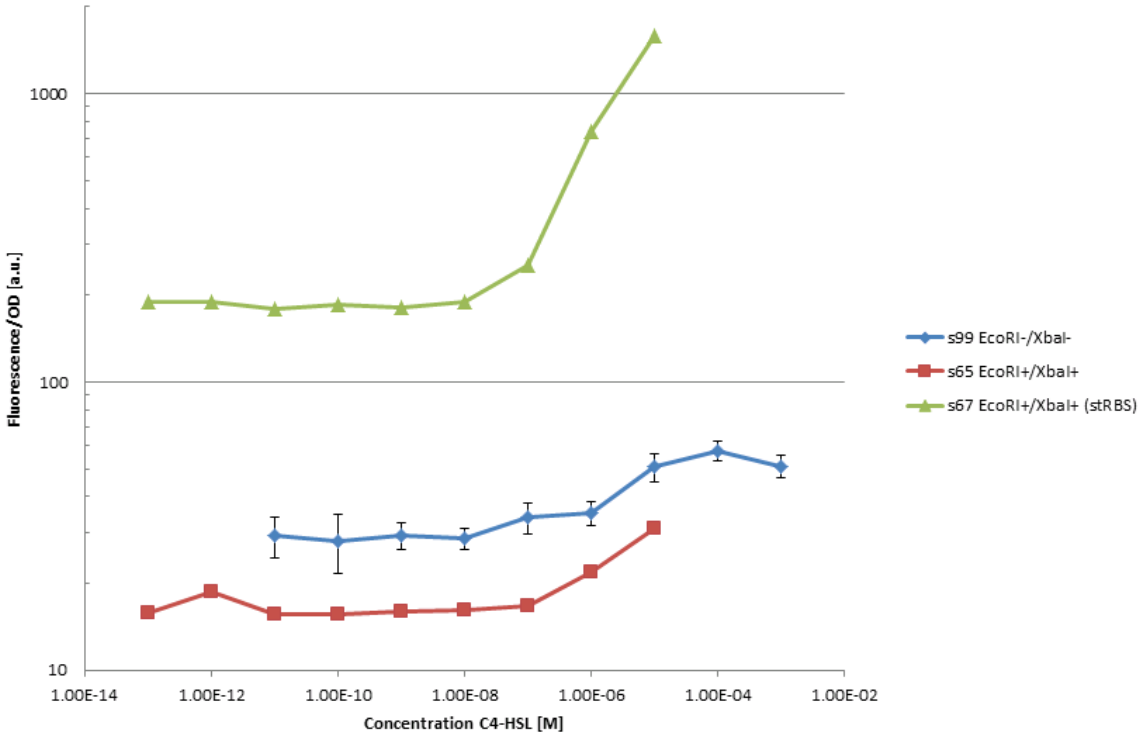


Fig. 2 siG0099, siG0065, and siG0067 compared dose-response curve to C4-HSL

Interpretation of Data:

- The riboregulator is still reducing the leakiness, but to a lesser extend (Fig. 2)
- With the higher concentration a plateau might be reached for C4-HSL, but the other inducers might still need higher concentrations to reach this plateau (Fig. 1)