

## Supplementary data 2

### MoBioS: Modular platform technology for high-throughput construction and characterization of tunable transcriptional biological sensors

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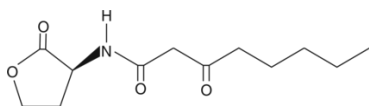
<sup>1</sup> = Equal contribution

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#### Overview

ID-sheets of the biosensors constructed in this research. They contain information on the ligand, response curve, meta-data about the strain and experiment and the DNA-sequences of the transcription factor and promoter part.

## LasR:3OC12-HSL



Ligand: 3-oxo-dodecanoyl-L-homoserine lactone

Source: Sigma-Aldrich O9139

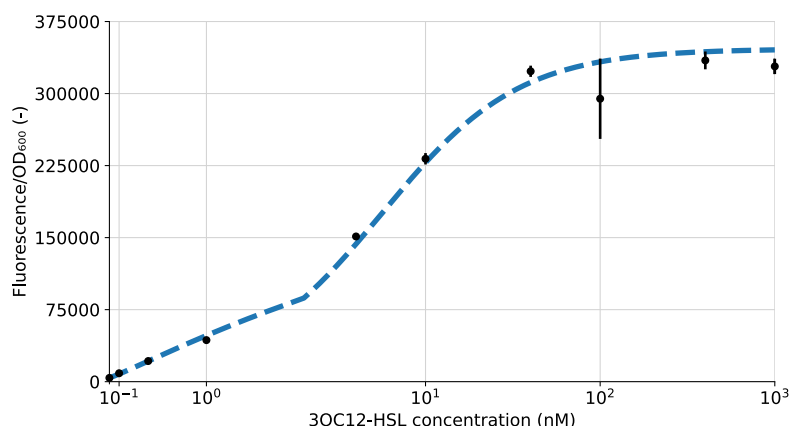
CAS ID: 168982-69-2

Stock: 1 mM

Solvent: DMSO

Storage: -80 °C (months)

### Response curve: SynLasR



#### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}} \right)_{\text{cor}} = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

#### Parameters Hill function

$$a \text{ (a.u.)} = 4.09 \cdot 10^3 \pm 0.11 \cdot 10^3$$

$$k \text{ (a.u.)} = 3.42 \cdot 10^5 \pm 0.05 \cdot 10^5$$

$$M \text{ (a.u.)} = 3.47 \cdot 10^5 \pm 0.05 \cdot 10^5$$

$$n = 1.11 \pm 0.02$$

$$K \text{ (nM)} = 5.63 \pm 0.23$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, nM)

C = the ligand concentration in the growth medium (nM)

### Metadata

Host *Escherichia coli* TOP10 (F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *araD139*  $\Delta$ (*araleu*)7697 *galU* *galk* *rpsL* (*StrR*) *endA1* *nupG*)

Origin *Pseudomonas aeruginosa* PAO1

Parts LasR: Gene ID: 881789 codon optimized for *Escherichia coli*

$P_{\text{LasI}}$ : Gene ID: 881777, promoter upstream of *lasI*

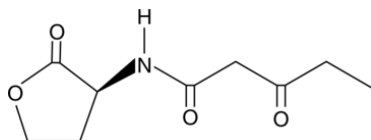
Architecture Synthetic

Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
Fluorescence after 24h of growth

Notes /

# LasR:30C12-HSL

<p><i>lasR</i></p>	<p>GATGATGGCGAGCATT<u>CACCTGCGGTA</u><u>AGCTATTACAGGGTAATCAGACCCAGATTA</u>ACT</p> <p><small>PaqCI recognition sequence      PaqCI restriction site</small></p> <p>GCCATAATTGCTGCAACACGACGGCTGGTAACACCAAATTTGCGACGAATATTACCC</p> <p>ATATGAAAGTTCACGTTGGCTTCGCTACAATTACAAATAACGCTAATTTCCAGCTG</p> <p>GTTTTACCAATTGCACACCACTGCAGAACTTCTTTTTACGGCTGGTCAGAACCACC</p> <p>GGTTTGCTAACCGGATGTTCAAATGCCAGACCGGCACCGCTCTGCAGTGCATAATCT</p> <p>TTCAGCATCCACAGTGTCTGGCAGCACGCTTCCATAAAACGATTTGCTTCGGCACGA</p> <p>TTTTCTGCTTCAACGCTCAGGCTCAGTGCACCCAGTTCACCACGTGCGCCATGCAGC</p> <p><small><i>lasR</i></small></p> <p>GGCATGGTCAGACCATAAACCAGACCTGCTGCGCTTGCTTCTTCAAAAAATTCTGTGC</p> <p>TGTTTACGGGTCTGATAAATGCTCGGTTCCCAAAAAATCGGCAGAACGCTCTGGGTA</p> <p>CAATGGCTAACGGTCGGATCAACACGTGCATAACCTGCACGATCATAATGTTACGC</p> <p>CATGCTGCCGGATAATTACCCACGATAAAGGCATTTTCATAATCCTGGCTATCTTTC</p> <p>GGCAGCAGACCAAACAGAATTTTGCTAAAACCCAGATCGCTTGCCATTTTCTGCAGG</p> <p>ATTGCTGACCATTCCAGTTTACCGCTGCTACGTTCCAGTTCCAGAAAACCATCAACC</p> <p>←</p> <p><b>AGTGCCAT</b>AGAGACCTCCTTGGACCTGGTACGTCGCTAGCGGGTAGTGT<b>GACGTGGCGCA</b></p> <p><small>BsaI restriction site      BsaI recognition sequence      GoldenRBS      PaqCI restriction site</small></p> <p><u>GGTGATGGACTTCATGCTGAC</u></p> <p><small>PaqCI recognition sequence</small></p>
<p>PlasI</p>	<p>GATGATGGCGAGCATT<u>CACCTGCGGTA</u><u>CTTATTCGAGCCTAGCAAGGGTCCGGGTTCAC</u></p> <p><small>PaqCI recognition sequence      PaqCI restriction site</small></p> <p>CGAAATCTATCTCATTGCTAGTTATAAAATTATGAAATTTGCATAAATTCTTCATCT</p> <p><small>P<sub>LasI</sub></small></p> <p>AGAGATTAAAGAG<b>GAGAAATACTAG</b>ATGGTGGCGCAGGTGATGGACTTCATGCTGAC</p> <p><small>Bba_B0030      PaqCI restriction site      PaqCI recognition sequence</small></p>

**EsaR:3OC6-HSL**

Ligand: 3-oxo-hexanoyl-L-homoserine lactone

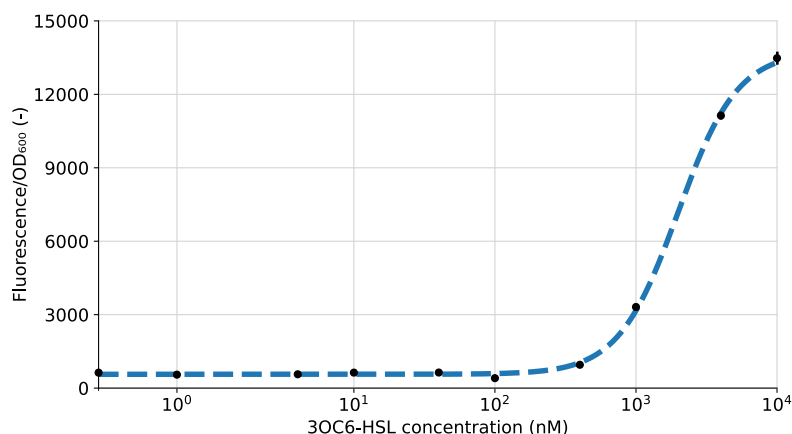
Source: Sigma-Aldrich K3007

CAS ID: 143537-62-6

Stock: 1 mM

Solvent: DMSO

Storage: -80 °C (2 months)

**Response curve: SynEsaR****Hill function**

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}} \right)_{\text{cor}} = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

**Parameters Hill function**

$$a \text{ (a.u.)} = 5.64 \cdot 10^2 \pm 0.23 \cdot 10^2$$

$$k \text{ (a.u.)} = 1.32 \cdot 10^4 \pm 0.04 \cdot 10^4$$

$$M \text{ (a.u.)} = 1.38 \cdot 10^4 \pm 0.04 \cdot 10^4$$

$$n = 2.05 \pm 0.10$$

$$K \text{ (nM)} = 1.98 \cdot 10^3 \pm 0.11 \cdot 10^3$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, nM)

C = the ligand concentration in the growth medium (nM)

**Metadata**

Host *Escherichia coli* TOP10 (F- mcrA  $\Delta$ (mrr-hsdRMS-mcrBC)  $\Phi$ 80lacZ $\Delta$ M15  $\Delta$ lacX74 recA1 araD139  $\Delta$ (araleu)7697 galU galk rpsL (StrR) endA1 nupG)

Origin *Pantoea stewartii*

Parts EsaR: GenBank L32184.1 partly codon-optimized for *Escherichia coli*

P<sub>esaR</sub>: GenBank L32184.1

Architecture Synthetic

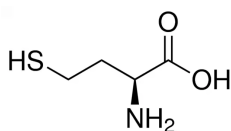
Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
PerkinElmer Ensignht, exc. 588nm, emm. 633nm at 30°C  
Fluorescence after 24h of growth

Notes EsaR works as a repressor of P<sub>esaR</sub> in the absence of the ligand. The promoter region is bidirectional and EsaR activates P<sub>esaS</sub> in the absence of the ligand.

## EsaR:30C6-HSL

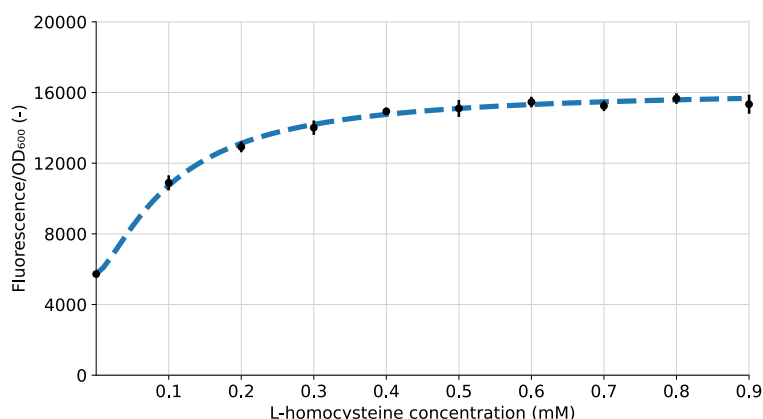
<p><i>esaR</i></p>	<p>GATGATGGCGAGCATT<b>CACCTGCGGTA</b><b>GCTA</b><b>CTACCTTGCAGCTGATGCTGCCGGTCTG</b></p> <p><small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p><b>ATAAGATCCAGTTCTACACCCAGTCTGATAGCCTGTCGGGCGTTACTGACGCCCAGT</b></p> <p><b>TTCACGACCACATTCTTGATGTGAAACTTCACGGTACTCACAGAAATGCCCCGTAATA</b></p> <p><b>GCGGCAATCTCAGCATAGGTTTTGCCATACTCGCCCAGTACAACACCTCATTTTCA</b></p> <p><b>CGCGAGGAAAATATCGTTTTGTCCGCGCTCTGATTTAACGCCGGGGCTCGCTCGCCT</b></p> <p><b>TCGGTACCGGCCAGGCGGTACATCTGCTCGTTAAATCAATCAGCAGCATCTGCATC</b></p> <p><b>GTGCCCTGTTCCGCAGCAAGGCGTTGCTCCAGCGCAGTCTGATCGTTGCCTTTAATG</b></p> <p><small>esaR</small></p> <p><b>ATCACGGACAACAGAGCAAGGTTGTTTCATGTGGTCATGCAGGACATAGGTAAAGCC</b></p> <p><b>GTTAACGATGTTGTATTGCTTGGATAAAGAGAAAATTTTGGTGAACCGCAGGTCGG</b></p> <p><b>ACATCAGCGTAATATTCTCATCCCAGGCAAACGGCGAGGTGCGTTTAAAGGCCGTG</b></p> <p><b>AGAATAACCGGATCGGTCAGCTGAAAGTTGTTAGCGCGGTATAACCTAATCCATTC</b></p> <p><b>GTCAGGATAACTGGAAATAATCAGAACATTTGAAGGATTTTTTTTGTCTACAACAGT</b></p> <p><b>GTAAGCGTAATCCGGACTACCCAGCGGAGATAACTTTCTCTGTATGTAAGTCTGAAG</b></p> <p><b>CGTATCCGTAATGGTCTGGTTTTCCAGGAAGAACGAGA</b><b>ACAT</b><b>AGAGACCTCCTTGGAC</b></p> <p><small>BsaI restriction site</small> <small>BsaI recognition sequence</small></p> <p><b>CTGGTACGTCGCTAGCGGGTAGTGT</b><b>GACG</b><b>TGGCGCAGGTGATGGACTTCATGCTGAC</b></p> <p><small>GoldenRBS</small> <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>
<p><math>P_{esaR}</math></p>	<p>GATGATGGCGAGCATT<b>CACCTGCGGTA</b><b>CTTAGGTCTCG</b><b>ACAT</b><b>CTAGTACTTTCTGTGTGACT</b></p> <p><small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small> <small>Bba_B0032</small></p> <p><b>CTAGTATCCGCTAAACAACCTGAAGCCATTGTAACTCTGAATGATTCATTGTAAGTTA</b></p> <p><small>P<sub>esaR</sub></small></p> <p><b>CTCTTAAGTATCATCTTGCCTGTACTATAGTGCAGGTAAAGTCCACGTAAAGTAAAA</b></p> <p><b>GAAGCAGCTCTAGAGATTAAAGAGGAGAAATACTAG</b><b>ATGGTGGCGCAGGTGATGGACTTCA</b></p> <p><small>Bba_B0030</small> <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p> <p>TGCTGAC</p>

## MetR:L-homocysteine



Ligand: L-homocysteine  
 Source: Sigma- Aldrich 69453  
 CAS ID: 6027-13-0  
 Stock: 1 g/L  
 Solvent: H<sub>2</sub>O  
 Storage: -20 °C (2 months)

### Response curve: SynMetR



### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}_{\text{cor}}} \right) = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

### Parameters Hill function

$$\begin{aligned} a \text{ (a.u.)} &= 5.73 \cdot 10^3 \pm 0.23 \cdot 10^3 \\ k \text{ (a.u.)} &= 1.04 \cdot 10^4 \pm 0.06 \cdot 10^4 \\ M \text{ (a.u.)} &= 1.62 \cdot 10^4 \pm 0.06 \cdot 10^4 \\ n &= 1.40 \pm 0.31 \\ K \text{ (mM)} &= 1.06 \cdot 10^{-1} \pm 0.11 \cdot 10^{-1} \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

### Metadata

Host *Escherichia coli* TOP10 (F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *araD139*  $\Delta$ (*araleu*)7697 *galU* *galk* *rpsL* (*StrR*) *endA1* *nupG*)

Origin *Salmonella enterica* LT2

Parts MetR: GenBank AE006468.2

P<sub>metER</sub>: Intergenic region between *metR* and *metE*;  
 GenBank AE006468.2

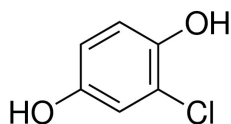
Architecture Synthetic

Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
 PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
 Fluorescence after 24h of growth

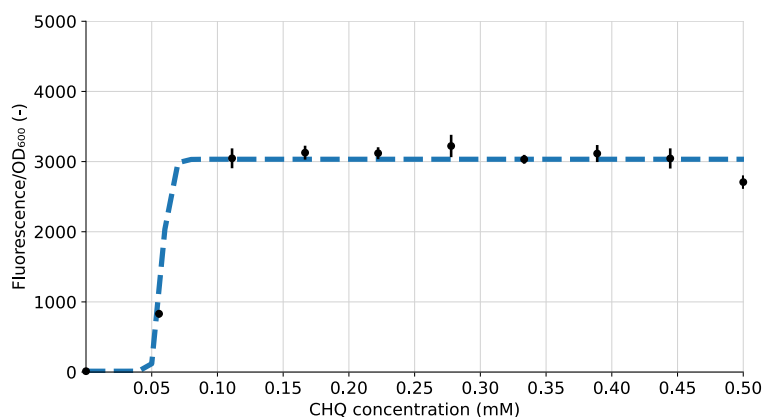
Notes /

## MetR:L-homocysteine

<i>metR</i>	<p>GATGATGGCGAGCATTACCTGCGGTAGCTATTTAAATGTTTTTCACATTTTTGAAAAAGTG  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p>CGGTTTCTGGGTATCACGGATAAATTTACAGGTGGGTACAAACATGCAGACGAATGGC</p> <p>ATAAATGAAGTTCTTGATGATCAGTTCTTTCTGATCACCATCACGAATGGCTGCATAC</p> <p>AGACGTTTCCAAATACCATCACCTAACTTTTTGGTCACCACCAGACCTTGTTTTTCAAA</p> <p>GGTATCAACAACCAATGCGGCAGTGCGGTAATACCCATTTTTGCTGCAACCATCTGA</p> <p>ATCAGCAGCAGCGTGTTATTCACGTTTTTAAAGATCGGAATAATACCTGCAGGCTGCA</p> <p>GAAAGAATTTCCAGATGTCCAGTTTATCACGTTTCACCGGATAAATCATCAGGATTTCT</p> <p>GCTGATCAGATCTTCCGGCAGAATATTATGCTTTTTCTGTGCCAGCGGATGATTCGGG</p> <p>CTCAGAACCAGACGAACTTCAAAATCAAAGATCGGGAAATAAAACAGGTTGCTACGA</p> <p>GGCAGAACTTCGCTGGTCAGAACAAATATCCAGTTTACCCTGCTGCAGGCTCGGCTGC  <small>metR</small></p> <p>GGACTAAAAATCATATCGCTATAAAAGTCGATTTCACACGAGGCCACTTTTTCTGAA</p> <p>AAATCTTCAGAGCCGGTGTGAGCCACTGAATACAGCTATGACATTCAATGGCCAGTTT</p> <p>GATAATCATCTGGTGGGTCTTTTTGCAATTCTGGATGGCTTTATGAATTTTCGGCAGG</p> <p>ATTTCTTTTGACAGCTGCAGCAGAATTTACCCTGTGCGGTAAATTTGATCGGGTTGC</p> <p>TTTTACGAATAAACAGTTTGAAGCCCAGCTTCTTTTCCAGTTCATTAAACTGATGGCT</p> <p>AATTGCGCTCTGGGTCTGATGCAGCTGAATTGCTGCTGCGCTCAGGCTACCGTTATTT</p> <p>TTCAGTGCCTGCAGGGTACGCAGATGTTTAATCTCGATCATAGAGACCTCCTTGGACCT  <small>BsaI restriction site</small> <small>BsaI recognition sequence</small> <small>GoldenRBS</small></p> <p>GGTACGTCGCTAGCGGGTAGTGTGACGTGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>
$P_{metER}$	<p>GATGATGGCGAGCATTACCTGCGGTACTTACGGTCTCTTCATGAGATTCTTCATATCGAG  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p>AATTA ACTATTTGCGCTTGCGAATTTTACTGGATATGAGAATTATAAGATATCTGATT</p> <p>AAATAAATCAATATATAAAATTTTATTAATTGATAATTTTATTATGAATGATAAGGAA  <small>P<sub>metER</sub></small></p> <p>AAAGATGGTGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>

**LinR:CHQ**

Ligand: Chlorohydroquinone  
 Source: Sigma- Aldrich 224081  
 CAS ID: 615-67-8  
 Stock: 10 g/L  
 Solvent: H<sub>2</sub>O  
 Storage: 2-8 °C (2 months)

**Response curve: SynLinR****Hill function**

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}_{\text{cor}}} \right) = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

**Parameters Hill function**

$$\begin{aligned} a \text{ (a.u.)} &= 1.22 \cdot 10^1 \pm 1.13 \cdot 10^1 \\ k \text{ (a.u.)} &= 3.02 \cdot 10^3 \pm 0.04 \cdot 10^3 \\ M \text{ (a.u.)} &= 3.03 \cdot 10^3 \pm 0.04 \cdot 10^3 \\ n &= 2.20 \cdot 10^1 \pm 1.07 \cdot 10^4 \\ K \text{ (mM)} &= 5.81 \cdot 10^{-2} \pm 1.24 \cdot 10^1 \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

**Metadata**

Host *Escherichia coli* TOP10 (F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(araleu)7697 galU galk rpsL (StrR) endA1 nupG)

Origin *Sphingobium japonicum* UT26S

Parts LinR: GenBank AP010805.1

P<sub>linER</sub>: Intergenic region between *linR* and *linE*;  
 GenBank AP010805.1

Architecture Synthetic

Experiment 150 μL EZ Rich medium, 96-well black microtiter plate  
 PerkinElmer Ensign, exc. 588nm, emm. 633nm at 30°C  
 Fluorescence after 24h of growth

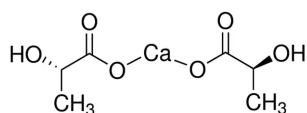
Notes The Hill function could not be fitted properly to the data. This has resulted in high errors for the n and K parameters.



# LinR:CHQ

<p><i>linR</i></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>GCTAT</b>CACATCCGCGCGGACAGCACCTTGTGCA  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p>GGAGCTGTCGCAACCAGCGGTGAGGCGGGTCGTCTTGTTCTCGCATGCCAATATT</p> <p>GCCTGACCTCAAATGCTTCCATAAGAATTGGCGGCCGCACGGCCCCGAGTCCGTAGA</p> <p>CCTCGGCGAGCCGACCAATCACGCGAGCCGAGCGGTAAGAATAAGGTCCGACTCGA</p> <p>AGCAAGCTGCCAACGCCACGAGAAAGCTGCTCGCGACGATCCTGATCCGATCGGGAT</p> <p>GGATCTTGTGAGGAGAGCGCGTTTCGACGGCACGATGGGCATGGGCCATACCCTTGG</p> <p>TGCTGACCACGATATGATCTGCGGCCATGAAGTCATCTGTCTCACCGGACTTGATGAA</p> <p>CGGATGACCTTCCTTTCCGAAGCACAGATATTCCTCCTGGTAGAGCCGTTGCGTCTTG</p> <p>ATGCCGGCAACAAGGCTCGGATAGGCGCCGACGGCGATATCGACATGCCCTGTCTCG</p> <p>AGCGCGCTCACCATTTCATCGCCGCTCAGCGTGAGTGCGCGGTAGCTGGTGTGTGGT</p> <p>GCCTCGAACC GCGCCGCGAATGCAACGCCGTCAAACGACAAGATGGGCGATGTCC</p> <p>GATCCGGCAATGATGAATTCGCGCTTAAGACGGTCAGGTGAGAATGTCTGCCCCGCT</p> <p>TCAGCCAGGTGCGGACGCGAGTTCCAGCAGTTGCTGAACAACCCCGGCGATCGCCTCG</p> <p>GCACGTGGTGTGCGCTCCATGCCGTGCGCGCGCCGGACGAGAAGCGGGTCGCCCAAT</p> <p>GCTTTGCGCAGGCGCGCCAGCCCATGGCTGGCGGTGGGCTGCGGTAGGTCGAGTTCT</p> <p>CGTGACGCGGCGCTGACGCTGTGCCGCTTTAACAGCGCATCCAAAAGCACCAAGTGC</p> <p>CGAAAATCCAGGTCATCTATAT<b>TT</b>CATAGAGACCTCCTTGGACCTGGTACGTCGCTAGCGG  <small>BsaI restriction site</small> <small>BsaI recognition sequence</small> <small>GoldenRBS</small></p> <p>GTAGTGT<b>GACG</b>TGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>
<p><math>P_{linER}</math></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>CTT</b>ACGGTCTCT<b>TT</b>CATGCAGCGGCGCTGACGCTGT  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p>GCCGCTTTAACAGCGCATCCAAAAGCACCAAGTGCCGAAAATCCAGGTCATCTATATT</p> <p>CACAATCTGAATTGTATCTATGAAGGTCGCCAATTGTCAAATACTCCCTGATCCCTC</p> <p>AAACAGGCTGCAGCAGCGATCGTTGTGCAGGTTTGAAGAGACGATCCGCAATCGCAG</p> <p>AAAATAGTGAGAGCGATCTCTAGAGATTAAAGAGGAGAAATACTAG<b>ATGGTGGCGC</b>  <small>PaqCI restriction site</small></p> <p>AGGTGATGGACTTCATGCTGAC  <small>PaqCI recognition sequence</small></p>

## LldR:Ca-lactate



Ligand: Ca-lactate·5H<sub>2</sub>O

Source: VWR 5551

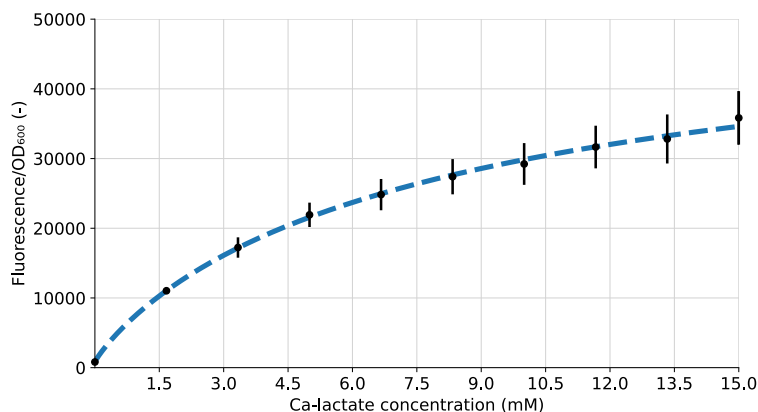
CAS ID: 5743-47-5

Stock: 25.51 g/L

Solvent: H<sub>2</sub>O

Storage: 2-8 °C (8 months)

### Response curve: SynLldR



### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}_{\text{cor}}} \right) = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

### Parameters Hill function

$$a \text{ (a.u.)} = 8.13 \cdot 10^2 \pm 0.70 \cdot 10^2$$

$$k \text{ (a.u.)} = 5.41 \cdot 10^4 \pm 2.38 \cdot 10^4$$

$$M \text{ (a.u.)} = 5.49 \cdot 10^4 \pm 2.38 \cdot 10^4$$

$$n = 8.95 \cdot 10^{-1} \pm 2.30 \cdot 10^{-1}$$

$$K \text{ (mM)} = 8.49 \pm 8.52$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

### Metadata

Host *Escherichia coli* TOP10 (F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(araleu)7697 galU galk rpsL (StrR) endA1 nupG)

Origin *Escherichia coli*

Parts LldR:GenBank EII2917618.1

P<sub>LldPRD</sub>: BBa\_K1847008-RBS0034

Architecture Synthetic

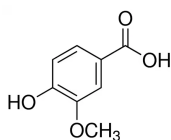
Experiment 150 μL EZ Rich medium, 96-well black microtiter plate  
PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
Fluorescence after 24h of growth

Notes /

# LldR:Ca-lactate

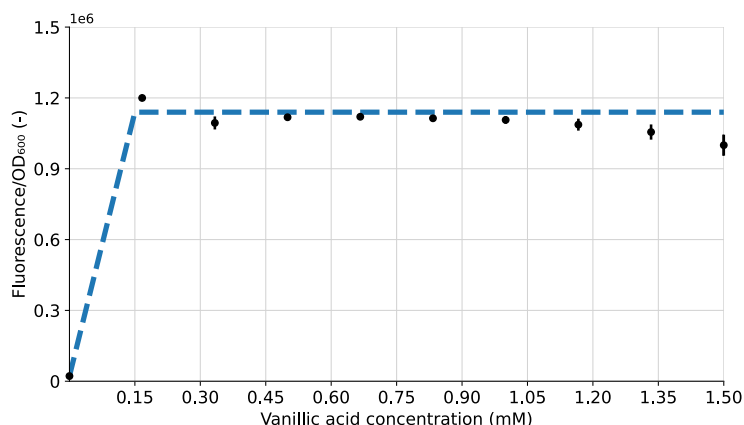
<p><i>lldR</i></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>GCTATCATGCGTTTTTCTCCCTCGAATGCTCATT</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p><b>ATGCTCACCGGGCAGGCGGGTAATCCGTGCGTGGCGAGCCTGATCTTCATCGAATCG</b></p> <p><b>TTTCATGGTGGTGTGAACAAACTAAGGTGCGCCATCATTGCTTTACGCGCCCCGTCA</b></p> <p><b>GCATCACCGGCAAAAATGGCGTCAATGACAGCCTGATGTTGTTGGTCAGTTGTGAA</b></p> <p><b>AAAACCGGTGGCACCAGATACATCCGCTGACGGCTATGCTTCACTGAGGATTGCAGG</b></p> <p><b>ACATCGAAGAAACCGCGCATGGTTTGCAGCAGCACGATGTTATGTGAGGCTTCGGCA</b></p> <p><b>ATCGCCAGATGAAAACGAACGTCCGCTTGTGAGGCGATATCCGGGTCTTCACTTAGC</b></p> <p><small><i>lldR</i></small></p> <p><b>GTTGCTTCAAAGCAAAGCTGAATCTTTTCTTTGTCGCCAGGTGTGGCGCGCATTGCCG</b></p> <p><b>CATGCCATGCGGTGCTGGCTTCAATGGCGTAGCGGGCTTCAGAATATCGAAACTGT</b></p> <p><b>AATCCGGATCATCGGCCATCAGTGTTTTTAGCGGCTGGACGATGTTTTGCTCCGACCA</b></p> <p><b>TGTGTCATGACGCCAGCGAATAAACGTCCCGCCGCGCGTCTGACTGAGCAGCACGCC</b></p> <p><b>TTCACTCACCAGTTTTGCCAGCGCCTCGCGCAGTGAATTACGTGATACGCCGAGTTGC</b></p> <p><b>ATCGCCAGTTGGCGCTCAGCGGGCAACTTCATGCCCGCTTCAGGTTTTTTTCATCAA</b></p> <p><b>TCAGCGCCCGCACACGATCGGCAACCTCGTCTGACAGGCGTCTGGGTAAAACAATCA</b>  <small>BsaI restriction site</small></p> <p><b>TAGAGACCTCCTTGGACCTGGTACGTCGCTAGCGGGTAGTGTGACGTGGCGCAGGTGATGG</b>  <small>BsaI recognition sequence</small> <small>GoldenRBS</small> <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p> <p>ACTTCATGCTGAC</p>
<p><math>P_{lldPRD}</math></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>CTTACGGTCTCTTCATAATTGGCCCTACCAATTCT</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p><b>TCGCTTATCTGACCTCTGGTTCACAATTTCCCAATTAAACTCACATCAATGTTGACAG</b></p> <p><small><math>P_{lldPRD}</math></small></p> <p><b>CTAGCTCAGTCCTAGGGATTGTGCTAGCTCATTATCCCTACACAACACAATTGGCAGT</b></p> <p><b>GCCACTTTCTAGAGAAAGAGGAGAAATACTAGATGGTGGCGCAGGTGATGGACTTCATGCTG</b>  <small>Bba_B0034</small> <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p> <p>AC</p>

## VanR:Vanillic acid



Ligand: Vanillic acid  
 Source: Sigma- Aldrich 94770  
 CAS ID: 121-34-6  
 Stock: 3.75 g/L  
 Solvent: EtOH  
 Storage: -20 °C (8 months)

### Response curve: SynVanR



### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}_{\text{cor}}} \right) = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

### Parameters Hill function

$$\begin{aligned} a \text{ (a.u.)} &= 2.19 \times 10^4 \\ k \text{ (a.u.)} &= 2.24 \times 10^6 \\ M \text{ (a.u.)} &= 2.26 \times 10^6 \\ n &= 5.21 \times 10^{-9} \\ K \text{ (mM)} &= 4.65 \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

### Metadata

Host *Escherichia coli* TOP10 (F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *araD139*  $\Delta$ (*araleu*)7697 *galU* *galk* *rpsL* (*StrR*) *endA1* *nupG*)

Origin *Caulobacter crescentus*

Parts VanR<sub>am</sub>: Evolved, GenBank MH101730.1

P<sub>vanCC</sub>: Evolved, GenBank MH101730.1

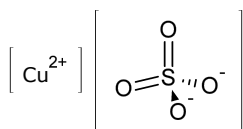
Architecture Synthetic

Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
 PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
 Fluorescence after 20h of growth

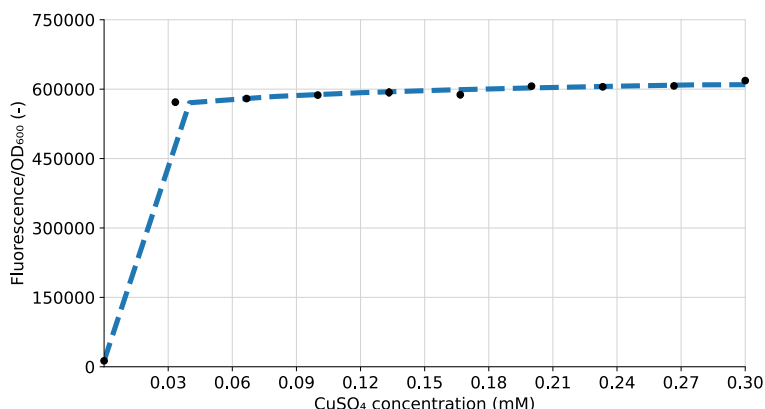
Notes The Hill function could not be fitted properly to the data. The Hill function parameter errors could not be calculated.

## VanR:Vanillic acid

<p><i>vanR</i></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>GCTATCAATCTGCACGAATTGACCATGCTGCAC</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p><b>CCAGCGGTGCGCCTGCGCTTGCTGCTGCTTCAAAAACCTTTGCATTACGAATTGCTGC</b></p> <p><b>CAGTGCATGATCACGCATAATACGTTCTGCACCTTCGGCATCACCACAGCTAACTGCA</b></p> <p><b>TCCAGAACTGCCTGATGCTGACGATGTGCTGCCAGCAGATGTTTCATATTCGGCAGAC</b></p> <p><b>AGGTCCATCAGATCCAGGGCCAGTGCACCGGCTGCTGCAAACGGTTCAAACCATTA</b></p> <p><b>CGTGCCAGTGCGCTTTCAACTGCACCATTACCTGCTGCGCTAACCAGGGTATCATGAA</b></p> <p><b>ATGCCTGATTATATGCGGCATAACGATCCAGATCTTCACCATTAGGGCGACCGGCTGC</b></p> <p><b>AAACAGTGCTTCACCTTCTGCAAT</b><small><i>vanR</i></small><b>CAGTACAACAAAACGTGCATGGGTTTCTGCGGTC</b></p> <p><b>ATACCACGTTCTGCCAGACGACGTGCTGCAAAACCTTCAGAACACCACGAACTTCAA</b></p> <p><b>TTGCATCACGAATCTGATCGCTGCTAACACCACGGGCTGCATAACCACGTGCACCCAG</b></p> <p><b>ACGAACAACCAGACCTTCTTGTTCCAGTGAACGCAGTGCGATACGAACCGGCATACG</b></p> <p><b>GCTAACACCCAGTGCTGCTGCGGTCGGAATTTCTGCAATACGTTACCACTTTTGATT</b></p> <p><b>TCACCGCTTGCAATCATTTTACGCAGTGCCATCATAACACGCTGACCCGGTTTAATAC</b></p> <p><b>GAGGCATGTCCAT</b><small>BsaI restriction site</small><b>AGAGACCTCCTTGGACCTGGTACGTCGCTAGCGGGTAGTGT</b><small>BsaI recognition sequence</small><b>GACGTGG</b><small>GoldenRBS</small><small>PaqCI restriction site</small></p> <p><b>CGCAGGTGATGGACTTCATGCTGAC</b>  <small>PaqCI recognition sequence</small></p>
<p><math>P_{\text{vanCC}}</math></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>CTTACGGTCTCTCCATATTGGATCCAATTGACAGC</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p><b>TAGCTCAGTCCTAGGTACCATTGGATCCAATAGCTGTCACCGGATGTGCTTCCGGTC</b></p> <p><b>TGATGAGTCCGTGAGGACGAAACAGCCTCTACAAATAATTTGTTTAA</b><b>TACTAGAGAAA</b></p> <p><b>GAGGGGAAATACTAG</b><small>synthetic RBS</small><b>ATGGTGGCGCAGGTGATGGACTTCATGCTGAC</b>  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p> <p><small><math>P_{\text{vanCC}}</math></small></p>

**CueR:CuSO<sub>4</sub>**

Ligand: CuSO<sub>4</sub>·5H<sub>2</sub>O  
 Source: Sigma-Aldrich 209198  
 CAS ID: 7758-98-7  
 Stock: 1.2 g/L  
 Solvent: H<sub>2</sub>O  
 Storage: -20 °C (8 months)

**Response curve: SynCueR****Hill function**

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}_{\text{cor}}} \right) = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

**Parameters Hill function**

$$\begin{aligned} a \text{ (a.u.)} &= 1.29 \cdot 10^4 \\ k \text{ (a.u.)} &= 1.68 \cdot 10^8 \\ M \text{ (a.u.)} &= 1.68 \cdot 10^8 \\ n &= 3.48 \cdot 10^{-2} \\ K \text{ (mM)} &= 4.72 \cdot 10^{69} \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)  
 a = the basal normalized fluorescent signal (leaky expression, a.u.)  
 k = the maximum normalized fluorescent signal relative to a (a.u.)  
 M = a + k = the maximum normalized fluorescent signal (a.u.)  
 n = the Hill coefficient (cooperativity, sigmoid character)  
 K = the Hill constant (TF-ligand affinity, mM)  
 C = the ligand concentration in the growth medium (mM)

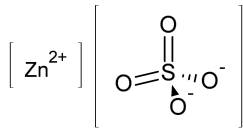
**Metadata**

Host	<i>Escherichia coli</i> TOP10 (F- <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ <i>lacX74</i> <i>recA1</i> <i>araD139</i> $\Delta$ ( <i>araleu</i> )7697 <i>galU</i> <i>galk</i> <i>rpsL</i> ( <i>StrR</i> ) <i>endA1</i> <i>nupG</i> )
Origin	<i>Escherichia coli</i> K12
Parts	CueR: GenBank EGQ7458477.1 P <sub>copA</sub> : GenBank CP097884.1
Architecture	Synthetic
Experiment	150 $\mu$ L EZ Rich medium, 96-well black microtiter plate PerkinElmer Ensign, exc. 588nm, emm. 633nm at 30°C Fluorescence after 24h of growth
Notes	The Hill function could not be fitted properly to the data. The Hill function parameter errors could not be calculated.

# CeuR:CuSO<sub>4</sub>

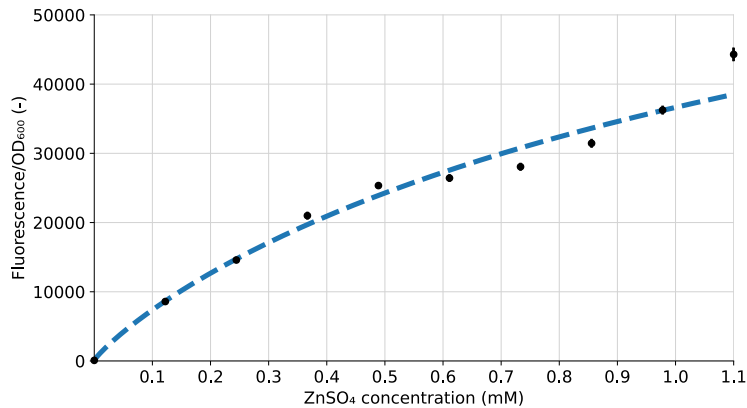
<i>ceuR</i>	<p>GATGATGGCGAGCATTACCTGCGGTAGCTATTACCCTGCGCGATGATGACAACAACCCG</p> <p>PaqCI recognition sequence PaqCI restriction site</p> <p>ACAGGTTTTCAATAATCGGACAGTCGGCACTATCGTCGCCCCGGACACGCGTTAGCCA</p> <p>GCGCCAGCAGTTGGTCCCGCATGCTCTGTAATTCTTCGATATGGCGCTCAATTTCCGC</p> <p>AACTTTTTCAAGGGTACGGCGTTTTACATCAGCAGAGTGACGTTGCGGATCGTTGAA</p> <p>cueR</p> <p>CAAATTGACCAGTTCACCGCTCTCCTCAAGGTTGAACCCTACTTGACGGGCCTGGCGC</p> <p>AGTAACGTCAACTCATTGAGGTGCTGTTGCGTATAGGTGCGATAACCATTCTCTGAGC</p> <p>GCATCGGCGGAGTAACCAAACCTTTTTCTTCATAAAAGCGGATTGCTTTGCTGGTCAG</p> <p>ACCTGTGATCTTCGCCACATCGGAGATGTTTCATAGAGACCTCCTGGACCTGGTACGTCG</p> <p>BsaI restriction site BsaI recognition sequence GoldenRBS</p> <p>CTAGCGGGTAGTGTGACGTGGCGCAGGTGATGGACTTCATGCTGAC</p> <p>PaqCI restriction site PaqCI recognition sequence</p>
P <sub>copA</sub>	<p>GATGATGGCGAGCATTACCTGCGGTACTTACGGTCTCTTCATGGACTTTTACCCGCCTGGT</p> <p>PaqCI recognition sequence PaqCI restriction site BsaI recognition sequence BsaI restriction site</p> <p>TTATTAATTTCTTGACCTTCCCCTTGCTGGAAGGTTTAACTTTATCACAGCCAGTCAA</p> <p>P<sub>copA</sub></p> <p>AACTGTCTTAAAGGAGTGTTTTAGCTGTCACCGGATGTGCTTTCCGGTCTGATGAGTC</p> <p>CGTGAGGACGAAACAGCCTCTACAAATAATTTGTTTAACTAGAGAAAGAGGAGAAAT</p> <p>synthetic RBS</p> <p>ACTAGATGGTGGCGCAGGTGATGGACTTCATGCTGAC</p> <p>PaqCI restriction site PaqCI recognition sequence</p>

# ZntR:ZnSO<sub>4</sub>



Ligand: ZnSO<sub>4</sub>·7H<sub>2</sub>O  
Source: VWR 1884  
CAS ID: 7446-20-0  
Stock: 2.2 g/L  
Solvent: H<sub>2</sub>O  
Storage: -20 °C (8 months)

## Response curve: SynZntR



### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}} \right)_{\text{cor}} = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

### Parameters Hill function

$$\begin{aligned} a \text{ (a.u.)} &= 8.81 \cdot 10^1 \pm 5.24 \cdot 10^1 \\ k \text{ (a.u.)} &= 9.30 \cdot 10^4 \pm 3.34 \cdot 10^4 \\ M \text{ (a.u.)} &= 9.31 \cdot 10^4 \pm 3.34 \cdot 10^4 \\ n &= 8.82 \cdot 10^{-1} \pm 0.89 \cdot 10^{-1} \\ K \text{ (mM)} &= 1.64 \pm 1.13 \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)  
a = the basal normalized fluorescent signal (leaky expression, a.u.)  
k = the maximum normalized fluorescent signal relative to a (a.u.)  
M = a + k = the maximum normalized fluorescent signal (a.u.)  
n = the Hill coefficient (cooperativity, sigmoid character)  
K = the Hill constant (TF-ligand affinity, mM)  
C = the ligand concentration in the growth medium (mM)

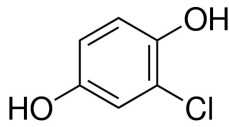
## Metadata

Host	<i>Escherichia coli</i> TOP10 (F- <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ <i>lacX74</i> <i>recA1</i> <i>araD139</i> $\Delta$ ( <i>araleu</i> )7697 <i>galU</i> <i>galk</i> <i>rpsL</i> ( <i>StrR</i> ) <i>endA1</i> <i>nupG</i> )
Origin	<i>Escherichia coli</i> K12
Parts	ZntR: GenBank OSL45746.1 P <sub>ZntAR</sub> : GenBank AP027461.1
Architecture	Synthetic
Experiment	150 $\mu$ L EZ Rich medium, 96-well black microtiter plate PerkinElmer Ensignt, exc. 588nm, emm. 633nm at 30°C Fluorescence after 24h of growth
Notes	/

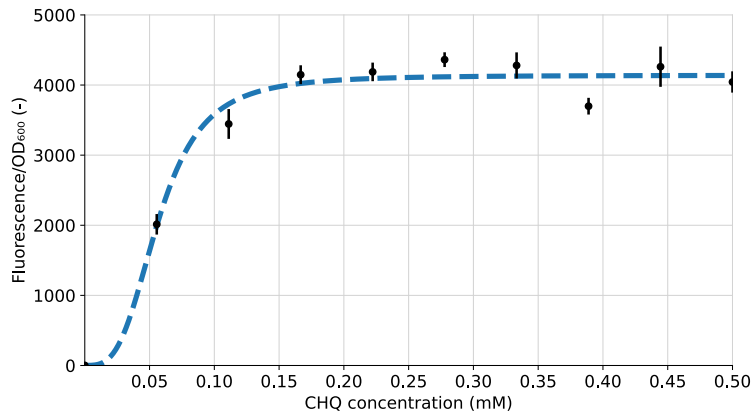


# ZntR:ZnSO4

<p><i>zntR</i></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>GCTATCAACAACCACTCTTAACGCCACTCGCCC</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p><b>CTTGTTCAAGAGCTTCAAGAATCGAACAATAAACTGCTATGAGCAGTCCCACAAC</b></p> <p><b>AGGCATCGTTAAGGCGTTGCAAGGAACGCTGCATACTCTGCAACTCGGCTATCCGTG</b></p> <p><b>CTTCGACTTCCTGCAATCTTTCCTGCACAATGCCTTTTGACTCCTGACAGGTATGGTGT</b></p> <p><b>TCAGGATCGATGCGGATCGACAGCAACTCGCGGATCGACTCCAGACTGAAACCTAGT</b></p> <p><small>zntR</small></p> <p><b>TGTCTGGCATGGCGGATAAATTTCAATCGCTGGAGATCGCTTTCGGTATATAGGCGA</b></p> <p><b>AACCCACCTTCAGTACGCACTTCATGCTCCATCATCTGCTGTTTTTCGTAATAACGAAT</b></p> <p><b>CGTGTCGGGTGTTACTTCCGCCATTTTTGCCAGCTCACCAATGCGATACATAGAGACCT</b>  <small>BsaI restriction site</small> <small>BsaI recognition sequence</small></p> <p><b>CCTTGGACCTGGTACGTCGCTAGCGGGTAGTGTGACGTGGCGCAGGTGATGGACTTCATGCT</b>  <small>GoldenRBS</small> <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p> <p>GAC</p>
<p><math>P_{zntAR}</math></p>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>CTTACGGTCTCTACATTAAAACTTGACTCTGGAGT</b>  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p><b>CGACTCCAGAGTGTATCCTTCGGTTAATGAGAAAAAACTTAACCGGAGGATGCCATGG</b>  <small>P<sub>zntAR</sub></small> <small>PaqCI restriction site</small></p> <p><b>TGGCGCAGGTGATGGACTTCATGCTGAC</b>  <small>PaqCI recognition sequence</small></p>

**LinR:CHQ**

Ligand: Chlorohydroquinone  
 Source: Sigma- Aldrich 224081  
 CAS ID: 615-67-8  
 Stock: 10 g/L  
 Solvent: H<sub>2</sub>O  
 Storage: 2-8 °C (2 months)

**Response curve: NatLinR****Hill function**

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}} \right)_{\text{cor}} = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

**Parameters Hill function**

$$a \text{ (a.u.)} = 1.40 \pm 1.84$$

$$k \text{ (a.u.)} = 4.14 \cdot 10^3 \pm 0.07 \cdot 10^3$$

$$M \text{ (a.u.)} = 4.14 \cdot 10^3 \pm 0.07 \cdot 10^3$$

$$n = 3.29 \pm 0.80$$

$$K \text{ (mM)} = 5.68 \cdot 10^{-2} \pm 0.25 \cdot 10^{-2}$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

**Metadata**

Host *Escherichia coli* TOP10 (F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *araD139*  $\Delta$ (*araleu*)7697 *galU* *galk* *rpsL* (*StrR*) *endA1* *nupG*)

Origin *Sphingobium japonicum* UT26S

Parts LinR: GenBank AP010805.1

P<sub>linER</sub>: Intergenic region between *linR* and *linE*;  
 GenBank AP010805.1

Architecture Natural

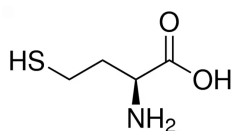
Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
 PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
 Fluorescence after 24h of growth

Notes /

## LinR:CHQ

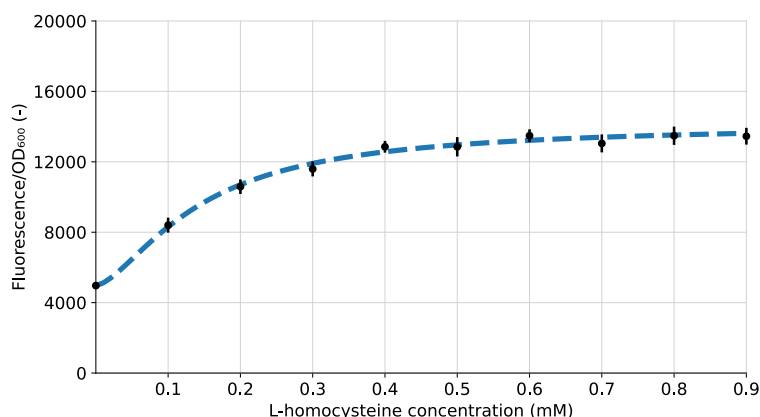
<i>linR</i>	<p>GATGATGGCGAGCATTACCTGCGGTA<b>GCTAT</b>CACATCCGCGGGACAGCACCTTGTGCA  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p>GGAGCTGTCGCAACCAGCGGTGAGGCGGGTCGTCTTGTTCTCGCATGCCAATATT</p> <p>GCCTGACCTCAAATGCTTCCATAAGAATTGGCGGCCGCACGGCCCGAGTCCGTAGA</p> <p>CCTCGGCGAGCCGACCAATCACGCGAGCCGGAGCGGTAAGAATAAGGTCCGACTCGA</p> <p>AGCAAGCTGCCAACGCCACGAGAAAGCTGCTCGCGACGATCCTGATCCGATCGGGAT</p> <p>GGATCTTGTGAGGAGAGCGCGTTTCGACGGCACGATGGGCATGGGCCATACCCTTGG</p> <p>TGCTGACCACGATATGATCTGCGGCCATGAAGTCATCTGTCTCACCGGACTTGATGAA</p> <p>CGGATGACCTTCCTTTCCGAAGCACAGATATTCTCCTGGTAGAGCCGTTGCGTCTTG</p> <p>ATGCCGGCAACAAGGCTCGGATAGGCGCCGACGGCGATATCGACATGCCCTGTCTCG</p> <p>AGCGCGCTCACCATTTCATCGCCGCTCAGCGTGAGTGCGCGGTAGCTGGTGTGTGGT</p> <p>GCCTCGAACC GCGCCGCGAATGCAACGCCGTCAAACGACAAGATGGGCGATGTCC</p> <p>GATCCGGCAATGATGAATTGCGCTTAAGACGGTCAGGTGAGAATGTCTGCCCGCCT</p> <p>TCAGCCAGGTGCGGACGCGAGTTCCAGCAGTTGCTGAACAACCCCGGCGATCGCCTCG</p> <p>GCACGTGGTGTGCGCTCCATGCCGTGCGCGCGCCGGACGAGAAGCGGGTCGCCCAAT</p> <p>GCTTTGCGCAGGCGCGCCAGCCCATGGCTGGCGGTGGGCTGCGGTAGGTCGAGTTCT</p> <p>CGTGACGCGGCGCTGACGCTGTGCCGCTTTAACAGCGCATCCAAAAGCACCAAGTGC</p> <p>CGAAAATCCAGGTCATCTATAT<b>TT</b>CATAGAGACCTCCTTGGACCTGGTACGTCGCTAGCGG  <small>BsaI restriction site</small> <small>BsaI recognition sequence</small> <small>GoldenRBS</small></p> <p>GTAGTGT<b>GACG</b>TGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>
$P_{linER}$	<p>GATGATGGCGAGCATTACCTGCGGTA<b>CTT</b>ACGGTCTCT<b>TT</b>CATGCAGCGGCGCTGACGCTGT  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p>GCCGCTTTAACAGCGCATCCAAAAGCACCAAGTGCCGAAAATCCAGGTCATCTATATT</p> <p>CACAATCTGAATTGTATCTATGAAGGTCGCCAATTGTCAAATACTCCCTGATCCCTC</p> <p>AAACAGGCTGCAGCAGCGATCGTTGTGCAGGTTTGAAGAGACGATCCGCAATCGCAG</p> <p>AAAATAGTGAGAGCGATCTCTAGAGATTAAAGAGGAGAAATACTAG<b>ATGGTGGCGC</b>  <small>PaqCI restriction site</small></p> <p>AGGTGATGGACTTCATGCTGAC  <small>PaqCI recognition sequence</small></p>

## MetR:L-homocysteine



Ligand: L-homocysteine  
 Source: Sigma- Aldrich 69453  
 CAS ID: 6027-13-0  
 Stock: 1 g/L  
 Solvent: H<sub>2</sub>O  
 Storage: -20 °C (2 months)

### Response curve: NatMetR



### Hill function

$$\left( \frac{\overline{\text{Fluo}}}{\text{OD}_{600}} \right)_{\text{cor}} = f(C) = a + k \left( \frac{C^n}{C^n + K^n} \right)$$

### Parameters Hill function

$$\begin{aligned} a \text{ (a.u.)} &= 4.97 \cdot 10^3 \pm 0.13 \cdot 10^3 \\ k \text{ (a.u.)} &= 9.15 \cdot 10^3 \pm 0.64 \cdot 10^3 \\ M \text{ (a.u.)} &= 1.41 \cdot 10^4 \pm 0.07 \cdot 10^4 \\ n &= 1.55 \pm 0.35 \\ K \text{ (mM)} &= 1.43 \cdot 10^{-1} \pm 0.18 \cdot 10^{-1} \end{aligned}$$

Error bars depict standard errors over four biological replicates (n=4)

a = the basal normalized fluorescent signal (leaky expression, a.u.)

k = the maximum normalized fluorescent signal relative to a (a.u.)

M = a + k = the maximum normalized fluorescent signal (a.u.)

n = the Hill coefficient (cooperativity, sigmoid character)

K = the Hill constant (TF-ligand affinity, mM)

C = the ligand concentration in the growth medium (mM)

### Metadata

Host *Escherichia coli* TOP10 (F- *mcrA*  $\Delta$ (*mrr-hsdRMS-mcrBC*)  $\Phi$ 80*lacZ* $\Delta$ M15  $\Delta$ *lacX74* *recA1* *araD139*  $\Delta$ (*araleu*)7697 *galU* *galk* *rpsL* (*StrR*) *endA1* *nupG*)

Origin *Salmonella enterica* LT2

Parts MetR: GenBank AE006468.2

P<sub>metER</sub>: Intergenic region between *metR* and *metE*;  
 GenBank AE006468.2

Architecture Natural

Experiment 150  $\mu$ L EZ Rich medium, 96-well black microtiter plate  
 PerkinElmer Enight, exc. 588nm, emm. 633nm at 30°C  
 Fluorescence after 24h of growth

Notes /

## MetR:L-homocysteine

<i>metR</i>	<p>GATGATGGCGAGCATTACCTGCGGTAGCTATTAAATGTTTTTACATTTTTGAAAAAGTG  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small></p> <p>CGGTTTCTGGGTATCACGGATAAATTTACAGGTGGGTACAAACATGCAGACGAATGGC</p> <p>ATAAATGAAGTTCTTGATGATCAGTTCTTTCTGATCACCATCACGAATGGCTGCATAC</p> <p>AGACGTTTCCAAATACCATCACCTAACTTTTTGGTCACCACCAGACCTTGTTTTTCAAA</p> <p>GGTATCAACAACCAATGCGGCAGTGCGGTAATACCCATTTTTGCTGCAACCATCTGA</p> <p>ATCAGCAGCAGCGTGTTATTCACGTTTTTAAAGATCGGAATAATACCTGCAGGCTGCA</p> <p>GAAAGAATTTCCAGATGTCCAGTTTATCACGTTTCACCGGATAAATCATCAGGATTTT</p> <p>GCTGATCAGATCTTCCGGCAGAATATTATGCTTTTTCTGTGCCAGCGGATGATTCGGG</p> <p>CTCAGAACCAGACGAACTTCAAAATCAAAGATCGGGAAATAAAACAGGTTGCTACGA</p> <p>GGCAGAACTTCGCTGGTCAGAACAAATATCCAGTTTACCCTGCTGCAGGCTCGGCTGC  <small>metR</small></p> <p>GGACTAAAAATCATATCGCTATAAAAGTCGATTTCCACACGAGGCCACTTTTTCTGAA</p> <p>AAATCTTCAGAGCCGGTGTGAGCCACTGAATACAGCTATGACATTCAATGGCCAGTTT</p> <p>GATAATCATCTGGTGGGTCTTTTTGCAATTCTGGATGGCTTTATGAATTTTCGGCAGG</p> <p>ATTTCTTTTGACAGCTGCAGCAGAATTTACCCTGTGCGGTAAATTTGATCGGGTTGC</p> <p>TTTTACGAATAAACAGTTTGAAGCCCAGCTTCTTTTCCAGTTCATTAAACTGATGGCT</p> <p>AATTGCGCTCTGGGTCTGATGCAGCTGAATTGCTGCTGCGCTCAGGCTACCGTTATTT</p> <p>TTCAGTGCCTGCAGGGTACGCAGATGTTTAATCTCGATCATAGAGACCTCCTTGGACCT  <small>BsaI restriction site</small> <small>BsaI recognition sequence</small> <small>GoldenRBS</small></p> <p>GGTACGTCGCTAGCGGGTAGTGTGACGTGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>
$P_{metER}$	<p>GATGATGGCGAGCATTACCTGCGGTACTTACGGTCTCTTCATGAGATTCCTTCATATCGAG  <small>PaqCI recognition sequence</small> <small>PaqCI restriction site</small> <small>BsaI recognition sequence</small> <small>BsaI restriction site</small></p> <p>AATTA ACTATTTGCGCTTGCGAATTTTACTGGATATGAGAATTATAAGATATCTGATTT</p> <p>AAATAAATCAATATATAAAATTTTATTAATTGATAATTTTATTATGAATGATAAGGAA  <small>P<sub>metER</sub></small></p> <p>AAAGATGGTGGCGCAGGTGATGGACTTCATGCTGAC  <small>PaqCI restriction site</small> <small>PaqCI recognition sequence</small></p>