1 Detailed description of the 'modelbricks' used.

1.1 E.coli chassis

This is the cellular environment of the device. Next to the features which are characteristic for the e.coli strain used, the chassis contains so called transcription factor and signalling pools. The pool symbolizes the freedom of the species to move around within the cell and therefore its ability to interact with any of the parts inside the cell, at any time. Transcription factors (TF's) are species which influence an operator by either activating or repressing transcription. Signalling molecules are any species that interact with the extracellular world. The chassis should contain one pool for every TF and signalling species. A species resides in its pool whenever it is not interacting with any of the parts, decay and particular transformation processes (e.g. protein maturation, polymerization) take place exclusively inside the pool.

In this modelling scheme it is considered sufficient to characterize a particular strain by its polymerase and ribosome contents and its volume. The polymerases and ribosomes each have their own pool, which are assumed to be present in the chassis by default.

e.coli specifcations	
Volume (litre)	7.0e-16
Number of species' pools	6

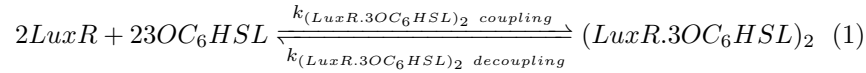| e.coli species		
name	initial value	description
RNAp	2.1e-8M	Free polymerases
rRNA	2.1e-8M	Free ribosomes

1.2 Pools

Next to the standard ribosome and polymerase pools the chassis contains two transcription factor pools, for $LuxR$ and cI, and two signalling pools, for $3OC_6HSL$ and GFP. Below is a list of all of the species and parameters used in these pools, this property of the species is emphasized by means of the adjective 'floating'.

List of 'floating' species	
name	description
$LuxR$	LuxR protein
$3OC_6HSL$	Homoserine lactone signalling molecule
$(LuxR.3OC_6HSL)_2$	LuxR, HSL complex
$cI_{monomer}$	Undimerized cI protein
cI	Free cI
$GFP_{immature}$	Unfolded green fluorescent protein
GFP	Folded, green fluorescent protein

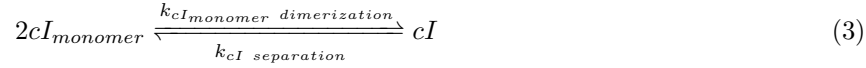| List of 'floating' parameters			
name	value	units	description
$k_{decay\ LuxR}$	1.16e-3	s^{-1}	decay rate for $LuxR$
$k_{decay\ 3OC_6HSL}$	1.16e-3	s^{-1}	decay rate for $3OC_6HSL$
$k_{decay\ cI_{monomer}}$	1.16e-3	s^{-1}	decay rate for $cI_{monomer}$
$k_{decay\ cI}$	1.16e-3	s^{-1}	decay rate for cI
$k_{decay\ GFP_{immature}}$	1.16e-3	s^{-1}	decay rate for $GFP_{immature}$
$k_{decay\ GFP}$	1.16e-3	s^{-1}	decay rate for GFP
$k_{(LuxR.3OC_6HSL)_2\ coupling}$	1e8	$M^{-3}s^{-1}$	association rate for $(LuxR.3OC_6HSL)_2$
$k_{(LuxR.3OC_6HSL)_2\ decoupling}$	1e-3	s^{-1}	dissociation rate for $(LuxR.3OC_6HSL)_2$
$k_{cI_{monomer}\ dimerization}$	1e9	$M^{-1}s^{-1}$	$cI_{monomer}$ dimerization rate
$k_{cI\ separation}$	1e1	s^{-1}	cI separation rate
$k_{cI\ separation}$	1e4	s^{-1}	$GFP_{immature}$ maturation rate

1.2.1 The Lux pool

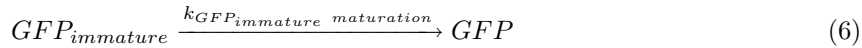This pool governs the decay of $LuxR$ and the formation of the TF $(LuxR.3OC_6HSL)_2$.

$$2LuxR + 23OC_6HSL \underset{k_{(LuxR.3OC_6HSL)_2\ decoupling}}{\overset{k_{(LuxR.3OC_6HSL)_2\ coupling}}{\rightleftharpoons}} (LuxR.3OC_6HSL)_2 \quad (1)$$

$$LuxR \xrightarrow{k_{decay\ LuxR}} \quad (2)$$

1.2.2 The cI pool

This pool governs the formation of the TF cI and its decay as well as the decay of $cI_{monomer}$.

$$2cI_{monomer} \underset{k_{cI\ separation}}{\overset{k_{cI_{monomer}\ dimerization}}{\rightleftharpoons}} cI \quad (3)$$

$$cI_{monomer} \xrightarrow{k_{decay\ 3OC_6HSL}} \quad (4)$$

$$cI \xrightarrow{k_{decay\ cI_{monomer}}} \quad (5)$$

1.2.3 The GFP pool

Green fluorescent protein (GFP) is a common reporter protein, since its presence is considered to affect only the extracellular environment (via photon absorption and emission) it is termed a signalling species. This pool governs keeps track of its presence and the maturation process of the protein.

$$GFP_{immature} \xrightarrow{k_{GFP_{immature}\ maturation}} GFP \quad (6)$$

$$GFP_{immature} \xrightarrow{k_{decay\ cI}} \quad (7)$$

$$GFP \xrightarrow{k_{decay\ GFP_{immature}}} \tag{8}$$

1.2.4 The HSL pool

The signalling molecule $3OC_6HSL$ is small enough to diffuse in and out of the cell throught the membrane, therefore it can be exchanged between cells. Additionally it forms a connection between the intracellular environment and the external medium which allows, in principle, for manual addition and detection of the species.

$$3OC_6HSL \xrightarrow{k_{decay\ GFP}} \tag{9}$$

1.2.5 The polymerase and ribosome pools

These pools have a special role in the model. First of all there is no decay, the species amount is considered to be constant over time since it is a property of the chassis. This does not mean that the content of the pools do not vary over time. Species are taken out of the pools, are used and are subsequently returned to the pools by the transcription units. The connections of these pools with every transcription unit obey the following rules: The polymerase pool is connected to every promoter and to every terminator. The ribosome pool is connected to every ribosomal binding site (RBS) and to every coding region (CR).

1.3 Part R0065

1.3.1 Species

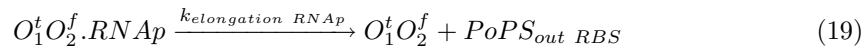$cI/LuxR$ hybrid, two-operator promoter, it is connected to the Lux pool, the cI pool and to part B0034.

List of R0065 species	
name	description
$O_1^f O_2^f$	Unoccupied hybrid promoter
$O_1^t O_2^f$	Hybrid promoter occupied by $(LuxR.3OC_6HSL)_2$
$O_1^f O_2^t$	Hybrid promoter occupied by cI
$O_1^t O_2^t$	Hybrid promoter occupied by $(LuxR.3OC_6HSL)_2$ and cI
$O_1^t O_2^f.RNAp$	$RNAp$ coupled to $O_1^t O_2^f$
$PoPS_{out\ RBS}$	Polymerases per second flow to RBS

1.3.2 Parameters

	List of R0065 parameters		
name	value	units	description
$k_{assoc\ (LuxR.3OC_6HSL)_2}$	3.3e4	$M^{-1}s^{-1}$	association rate for $(LuxR.3OC_6HSL)_2$
$k_{dissoc\ (LuxR.3OC_6HSL)_2}$	1.7e-1	s^{-1}	dissociation rate for $(LuxR.3OC_6HSL)_2$
$k_{assoc\ cI}$	3.3e-6	$M^{-1}s^{-1}$	association rate for cI
$k_{dissoc\ cI}$	1.7e-3	s^{-1}	dissociation rate for cI
$k_{decay\ (LuxR.3OC_6HSL)_2}$	1.6e-3	s^{-1}	decay rate for $(LuxR.3OC_6HSL)_2$
$k_{decay\ cI}$	1.6e-3	s^{-1}	decay rate for cI
$k_{assoc\ RNAp}$	1e5	$M^{-1}s^{-1}$	association rate for $RNAp$
$k_{dissoc\ RNAp}$	1e-2	s^{-1}	dissociation rate for $RNAp$
$k_{elongation\ RNAp}$	5e-1	s^{-1}	$RNAp$ elongation rate

1.3.3 Reactions

$$O_1^f O_2^f + (LuxR.3OC_6HSL)_2 \underset{k_{dissoc\ (LuxR.3OC_6HSL)_2}}{\overset{k_{assoc\ (LuxR.3OC_6HSL)_2}}{\rightleftharpoons}} O_1^t O_2^f \tag{10}$$

$$O_1^f O_2^f + cI \underset{k_{dissoc\ cI}}{\overset{k_{assoc\ cI}}{\rightleftharpoons}} O_1^f O_2^t \tag{11}$$

$$O_1^t O_2^f + cI \underset{k_{dissoc\ cI}}{\overset{k_{assoc\ cI}}{\rightleftharpoons}} O_1^t O_2^t \tag{12}$$

$$O_1^f O_2^t + (LuxR.3OC_6HSL)_2 \underset{k_{dissoc\ (LuxR.3OC_6HSL)_2}}{\overset{k_{assoc\ (LuxR.3OC_6HSL)_2}}{\rightleftharpoons}} O_1^t O_2^t \tag{13}$$

$$O_1^t O_2^t \xrightarrow{k_{decay\ (LuxR.3OC_6HSL)_2}} O_1^f O_2^t \tag{14}$$

$$O_1^t O_2^t \xrightarrow{k_{decay\ cI}} O_1^t O_2^f \tag{15}$$

$$O_1^f O_2^t \xrightarrow{k_{decay\ cI}} O_1^f O_2^f \tag{16}$$

$$O_1^t O_2^f \xrightarrow{k_{decay\ (LuxR.3OC_6HSL)_2}} O_1^f O_2^f \tag{17}$$

$$O_1^t O_2^f + RNAp \underset{k_{dissoc\ RNAp}}{\overset{k_{assoc\ RNAp}}{\rightleftharpoons}} O_1^t O_2^f.RNAp \tag{18}$$

$$O_1^t O_2^f.RNAp \xrightarrow{k_{elongation\ RNAp}} O_1^t O_2^f + PoPS_{out\ RBS} \tag{19}$$

1.4 $\mathbf{Pr}_{Lux\ mut}$

cI, one-operator promoter, it is connected to the Lux pool and to part B0031.

1.4.1 Species

<div align="center">

List of $\mathrm{Pr}_{Lux\ mut}$ species
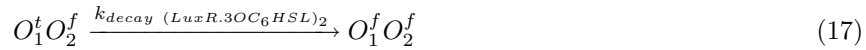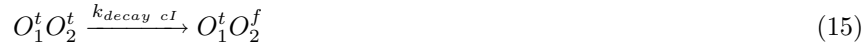
name	description
O^f	Unoccupied Lux promoter
O^t	Lux promoter occupied by $(LuxR.3OC_6HSL)_2$
$O^t.RNAp$	$RNAp$ coupled to O^t
$PoPS_{out_{RBS}}$	Polymerases per second flow to RBS

</div>

1.4.2 Parameters

<div align="center">

List of $\mathrm{Promoter}_{Lux\ mut}$ parameters

name	value	units	description
$k_{assoc\ (LuxR.3OC_6HSL)_2}$	1e4	$M^{-1}s^{-1}$	association rate for $(LuxR.3OC_6HSL)_2$
$k_{dissoc\ (LuxR.3OC_6HSL)_2}$	1	s^{-1}	dissociation rate for $(LuxR.3OC_6HSL)_2$
$k_{decay\ (LuxR.3OC_6HSL)_2}$	1.6e-3	s^{-1}	decay rate for $(LuxR.3OC_6HSL)_2$
$k_{assoc\ RNAp}$	1e5	$M^{-1}s^{-1}$	association rate for $RNAp$
$k_{dissoc\ RNAp}$	1e-2	s^{-1}	dissociation rate for $RNAp$
$k_{elongation\ RNAp}$	5e-1	s^{-1}	elongation rate

</div>

1.4.3 Reactions

$$O^f + (LuxR.3OC_6HSL)_2 \underset{k_{dissoc\ (LuxR.3OC_6HSL)_2}}{\overset{k_{assoc\ (LuxR.3OC_6HSL)_2}}{\rightleftharpoons}} O^t \tag{20}$$

$$O^t \xrightarrow{k_{decay\ (LuxR.3OC_6HSL)_2}} O^f \tag{21}$$

$$O^t + RNAp \underset{k_{dissoc\ RNAp}}{\overset{k_{assoc\ RNAp}}{\rightleftharpoons}} O^t.RNAp \tag{22}$$

$$O_1^t O_2^f.RNAp \xrightarrow{k_{elongation\ RNAp}} O^t + PoPS_{out_{RBS}} \tag{23}$$
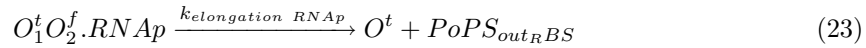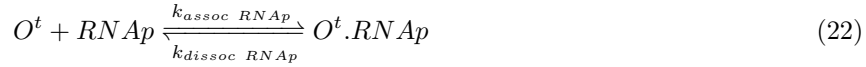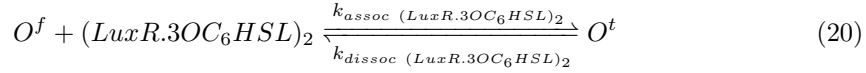
1.5 R0040

This is the TetR promoter, here it is used as a zero-operator promoter which is constitutively on, it is connected to part B0034.
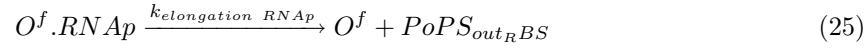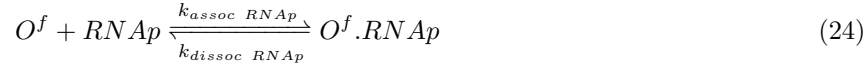
1.5.1 Species

<table>
<tr><td colspan="2" align="center">List of $\mathrm{Pr}_{Lux\ mut}$ species</td></tr>
<tr><td>name</td><td>description</td></tr>
<tr><td>O^f</td><td>Unoccupied Tet promoter</td></tr>
<tr><td>$O^f.RNAp$</td><td>$RNAp$ coupled to O^f</td></tr>
<tr><td>$PoPS_{out_{RBS}}$</td><td>Polymerases per second flow to RBS</td></tr>
</table>

1.5.2 Parameters

<table>
<tr><td colspan="4" align="center">List of $\mathrm{Promoter}_{Lux\ mut}$ parameters</td></tr>
<tr><td>name</td><td>value</td><td>units</td><td>description</td></tr>
<tr><td>$k_{assoc\ RNAp}$</td><td>1e7</td><td>$M^{-1}s^{-1}$</td><td>association rate for $RNAp$</td></tr>
<tr><td>$k_{dissoc\ RNAp}$</td><td>1e-2</td><td>s^{-1}</td><td>dissociation rate for $RNAp$</td></tr>
<tr><td>$k_{elongation\ RNAp}$</td><td>5e-1</td><td>s^{-1}</td><td>elongation rate</td></tr>
</table>

1.5.3 Reactions

$$O^f + RNAp \underset{k_{dissoc\ RNAp}}{\overset{k_{assoc\ RNAp}}{\rightleftharpoons}} O^f.RNAp \tag{24}$$

$$O^f.RNAp \xrightarrow{k_{elongation\ RNAp}} O^f + PoPS_{out_{RBS}} \tag{25}$$

1.6 B0031

RBS with efficiency 0.07 it is connected to $\mathrm{Pr}_{Lux\ mut}$ and to part C0051.

1.6.1 Species

<table>
<tr><td colspan="2" align="center">List of B0031 species</td></tr>
<tr><td>name</td><td>description</td></tr>
<tr><td>$PoPS_{in\ Pr}$</td><td>Polymerases per second flow from promoter</td></tr>
<tr><td>$B.RNAp$</td><td>mRNA binding site B occupied by $RNAp$</td></tr>
<tr><td>$B(b)$</td><td>Unoccupied mRNA binding sites B and b</td></tr>
<tr><td>$b.rRNA$</td><td>mRNA binding site b occupied by $rRNA$</td></tr>
<tr><td>$PoPS_{out\ CR}$</td><td>Polymerases per second flow to coding region</td></tr>
<tr><td>$RiPS_{out\ CR}$</td><td>Ribosomes per second flow to coding region</td></tr>
</table>

1.6.2 Parameters

<table>
<tr><td colspan="4" align="center">List of B0031 parameters</td></tr>
<tr><td>name</td><td>value</td><td>units</td><td>description</td></tr>
<tr><td>$k_{elongation\ RBS}$</td><td>2</td><td>s^{-1}</td><td>elongation rate</td></tr>
<tr><td>$k_{assoc\ rRNA}$</td><td>7e4</td><td>$M^{-1}s^{-1}$</td><td>association rate for $rRNA$</td></tr>
<tr><td>$k_{dissoc\ rRNA}$</td><td>1e-2</td><td>s^{-1}</td><td>dissociation rate for $rRNA$</td></tr>
<tr><td>$k_{clearing}$</td><td>2e-2</td><td>s^{-1}</td><td>clearance rate</td></tr>
<tr><td>$k_{decay\ mRNA}$</td><td>5.8e-3</td><td>s^{-1}</td><td>decay rate for mRNA</td></tr>
</table>

1.6.3 Reactions

$$PoPS_{in\ Pr} \xrightarrow{k_{elongation\ RBS}} B(b) + PoPS_{out\ CR} \tag{26}$$

$$B(b) + rRNA \underset{k_{dissoc\ rRNA}}{\overset{k_{assoc\ rRNA}}{\rightleftharpoons}} b.rRNA \tag{27}$$

$$b.rRNA \xrightarrow{k_{clearing}} B(b) + RiPS_{out\ CR} \tag{28}$$

$$B(b) \xrightarrow{k_{decay\ mRNA}} \tag{29}$$

$$b.rRNA \xrightarrow{k_{decay\ mRNA}} rRNA \tag{30}$$
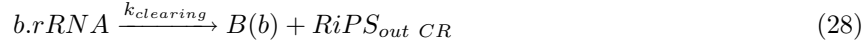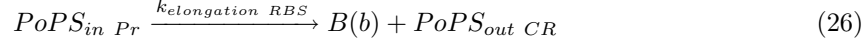
1.7 B0034

Elowitz repressilator with efficiency 1, it is used twice; it is connected to parts R0065 and E0040 and to parts R0040 and C0062.
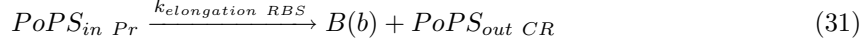
1.7.1 Species

List of B0034 species	
name	description
$PoPS_{in\ Pr}$	Polymerases per second flow from promoter
$B.RNAp$	mRNA binding site B occupied by $RNAp$
$B(b)$	Unoccupied mRNA binding sites B and b
$b.rRNA$	mRNA binding site b occupied by $rRNA$
$PoPS_{out\ CR}$	Polymerases per second flow to coding region
$RiPS_{out\ CR}$	Ribosomes per second flow to coding region

1.7.2 Parameters

List of B0034 parameters			
name	value	units	description
$k_{elongation\ RBS}$	2	s^{-1}	elongation rate
$k_{assoc\ rRNA}$	1e6	$M^{-1}s^{-1}$	association rate for $rRNA$
$k_{dissoc\ rRNA}$	1e-2	s^{-1}	dissociation rate for $rRNA$
$k_{clearing}$	2e-2	s^{-1}	clearance rate
$k_{decay\ mRNA}$	5.8e-3	s^{-1}	decay rate for mRNA

1.7.3 Reactions

$$PoPS_{in\ Pr} \xrightarrow{k_{elongation\ RBS}} B(b) + PoPS_{out\ CR} \tag{31}$$

$$B(b) + rRNA \underset{k_{dissoc\ rRNA}}{\overset{k_{assoc\ rRNA}}{\rightleftarrows}} b.rRNA \tag{32}$$

$$b.rRNA \xrightarrow{k_{clearing}} B(b) + RiPS_{out\ CR} \tag{33}$$

$$B(b) \xrightarrow{k_{decay\ mRNA}} \tag{34}$$

$$b.rRNA \xrightarrow{k_{decay\ mRNA}} rRNA \tag{35}$$

1.8 E0040

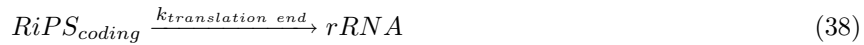GFP coding region, it is connected to parts B0034 and B0015 and to the GFP pool.

1.8.1 Species

List of E0040 species	
name	description
$PoPS_{in\ RBS}$	Polymerases per second flow from RBS
$RiPS_{in\ RBS}$	Ribosomes per second flow from RBS
$RiPS_{coding}$	Ribosomes per second flow during translation
$PoPS_{out\ Term}$	Polymerases per second flow to terminator

1.8.2 Parameters

List of E0040 parameters			
name	value	units	description
$k_{elongation\ RNAp}$	5.6e-2	s^{-1}	elongation rate
$k_{translation\ start}$	4.9e-2	s^{-1}	rate of translation initiation
$k_{translation\ end}$	5e-2	s^{-1}	rate of $rRNA$ decoupling

1.8.3 Reactions

$$PoPS_{in\ RBS} \xrightarrow{k_{elongation\ RNAp}} PoPS_{out\ Term} \tag{36}$$

$$RiPS_{in\ RBS} \xrightarrow{k_{translation\ start}} RiPS_{coding} + GFP_{immature} \tag{37}$$

$$RiPS_{coding} \xrightarrow{k_{translation\ end}} rRNA \tag{38}$$

1.9 C0051

cI coding region, it is connected to parts B0031 and B0015 and to the cI pool.

1.9.1 Species

List of C0051 species	
name	description
$PoPS_{in\ RBS}$	Polymerases per second flow from RBS
$RiPS_{in\ RBS}$	Ribosomes per second flow from RBS
$RiPS_{coding}$	Ribosomes per second flow during translation
$PoPS_{out\ Term}$	Polymerases per second flow to terminator

1.9.2 Parameters

List of C0051 parameters			
name	value	units	description
$k_{elongation\ RNAp}$	5.6e-2	s^{-1}	elongation rate
$k_{translation\ start}$	4.7e-2	s^{-1}	rate of translation initiation
$k_{translation\ end}$	5e-2	s^{-1}	rate of $rRNA$ decoupling

1.9.3 Reactions

$$PoPS_{in\ RBS} \xrightarrow{k_{elongation\ RNAp}} PoPS_{out\ Term} \tag{39}$$

$$RiPS_{in\ RBS} \xrightarrow{k_{translation\ start}} RiPS_{coding} + cI_{monomer} \tag{40}$$

$$RiPS_{coding} \xrightarrow{k_{translation\ end}} rRNA \tag{41}$$

1.10 C0062

LuxR coding region, it is connected to parts B0034 and B0015 and to the Lux pool.

1.10.1 Species

List of C0062 species	
name	description
$PoPS_{in\ RBS}$	Polymerases per second flow from RBS
$RiPS_{in\ RBS}$	Ribosomes per second flow from RBS
$RiPS_{coding}$	Ribosomes per second flow during translation
$PoPS_{out\ Term}$	Polymerases per second flow to terminator

1.10.2 Parameters

<div align="center">

List of C0062 parameters

name	value	units	description
$k_{elongation\ RNAp}$	5.6e-2	s^{-1}	elongation rate
$k_{translation\ start}$	4.6e-2	s^{-1}	rate of translation initiation
$k_{translation\ end}$	5e-2	s^{-1}	rate of $rRNA$ decoupling

</div>

1.10.3 Reactions

$$PoPS_{in\ RBS} \xrightarrow{k_{elongation\ RNAp}} PoPS_{out\ Term} \tag{42}$$

$$RiPS_{in\ RBS} \xrightarrow{k_{translation\ start}} RiPS_{coding} + LuxR \tag{43}$$

$$RiPS_{coding} \xrightarrow{k_{translation\ end}} rRNA \tag{44}$$

1.11 B0015

Terminator, used three times, it is connected to parts E0040, C0051 and C0062.

1.11.1 Species

table of part species

<div align="center">

List of B0015 species

name	description
$PoPS_{in\ CR}$	Polymerases per second flow from coding region

</div>

1.11.2 Parameters

<div align="center">

List of B0015 parameters

name	value	units	description
$k_{terminate}$	3.125e1	s^{-1}	rate of trancription termination

</div>

1.11.3 Reactions

$$PoPS_{in\ CR} \xrightarrow{k_{terminate}} RNAp \tag{45}$$

2 Additional information

All parts above are available as .xml and .sbproj (SimBiology) files. Additionally there is a Matlab script file which can generate genetic systems by merging these parts. They can be requested at the author, and will later be added to the team Wiki at the iGEM website: http://2008.igem.org/Team:Groningen (October 1, 2008).