iGEM 08
Team ESBS Strasbourg

Cell division counter

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ESBS Strasbourg
Cell division counter

- Binary system
- Extendable
  ⇒ Cell cycle dependent toggle switch
- Chassis: Yeast
Applications

- Detection of abnormal growth (cancer)
- Coupling to cell cycle specific factors
- Age determination in cell culture
- ...

iGEM 08
Team E58S Strasbourg
The Project

In Detail ...
external signal

counting device

PoPS

Generation No.
external signal

inducer

0 – module

1 – module

PoPS

Generation No.
inducer → sA0 → R0 → cA0 → 1 – module
inducer → sA0 → R0 → cA0

external signal

internal signal

APC

1 – module

PoPS

Generation No.
external signal

sA0 → R0 → cA0

internal signal
APC

1 – module

PoPS

0 1 2 3 4 5 6

Generation No.
external signal

internal signal

APC

sA0 — R0 — cA0

sA1 — R1 — cA1
Modeling

Characterization of parts ...
Modeling: questions to be solved

I. Protein half life time
dilution sufficient? PEST?

II. APC degradation motif
time frame of activity?

III. Promoter construction
sA/cA/R expression strength?
N° of operator repetitions?
same operator for A and R?
Modeling: promoter construction

One-operator system:

\[
\frac{dX}{dt} = s + \frac{\beta \cdot \Lambda^n}{K^n_A \left(1 + \frac{R^n}{K^n_R}\right)} + \Lambda^n
\]

Two-operator system:

\[
\frac{dX}{dt} = s_R + \left(\frac{s_A + \frac{\beta \cdot \Lambda^n}{K^n_A + \Lambda^n}}{K^{-n}_R + R^{-n}}\right) \cdot R^{-n}
\]
Modeling: cell cycle dependent toggle switch

binary expression pattern

0 – specific expression   1 – specific expression

TF concentrations

Repressor0  Repressor1  cActivator0  cActivator1
Outcome

What we reached to do ...
Modular promoter

- 3-7 operator copies
- Mutated Cyc1 promoter
external signal

internal signal

APC

sA0 → R0 → cA0

sA1 → R1 → cA1

Generation No.

PoPS
Parts construction
New fusion vector

Phillips and Silver (2006)
New fusion vector

BioBrick I
TCTAGA
AGATCT

in frame

BioBrick II
CTAGA
T
ACTAGT
TGATCA

BioBrick I
ACTAGA
TGATCT

BioBrick II
- VP16 activator domain for eucaryotic polymerase III from Herpes Simplex Virus
- repression domain of S. cerevisiae transcription factor TUP1
- DNA binding domains with specific bait sequences (tetR, cl, lacI, lexA, Gal4)
- degradation motifs of hsl1 & cln2
Measurement

- Optimal arrangement of domains
- Strength of the promoters
- Functioning of the tags
Optimal arrangement of domains
# Parts submitted

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