Does God play dice with the cell?

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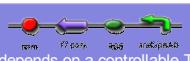
Overview

Our team, enlightened by the idea of gambling machines, aims to construct a synthetic device - the *Randomizer* - which could generate randomness. The core part of the device relies on the random but exclusive binding of *T7 polymerase* to a pair of *overlapping T7 promoters* to generate a logic signal of 0 or 1 - in this case, either green or red fluorescence - which is then stabilized by reciprocal repression of the two T7 promoters.

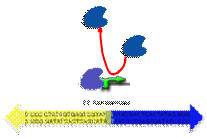
Design

Randomizer

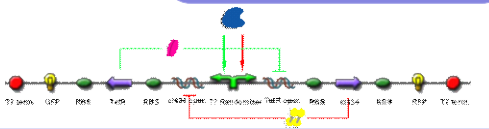
"Kicking Start"



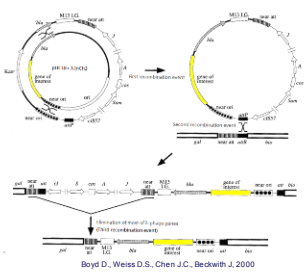
Success of the randomizer depends on a controllable T7 polymerase production by inducible pBAD/araC promoter. Low copy number of T7pol reduces T7 promoter firing frequency, which can benefit our design.



The origin of randomness arises from T7 polymerase's random but exclusive binding to an overlapping T7 promoter pair. The identical promoter sequence could potentially cause random binding of polymerase. This design can also reduce extrinsic and intrinsic noise to minimum.



Optimally the first binding and transcription event could effectively generate repressors which silences the opposite promoter thus stabilizing randomizing outcome.



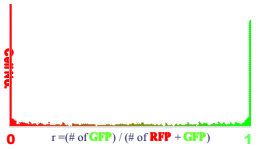
Boyd D., Weiss D.S., Chen J.C., Beckwith J, 2000

A randomizer bacterium requires stringently ***one*** copy of the randomizer gene inside its genome.

The ***λ InCh*** system can stably integrate a single copy gene into *E. coli* chromosome through three homologous recombination events. (left figure) Successfully integrated *E. coli* can be selected by heat shock at 42°C.

Simulation

Sample run of the simulation program



For the total pool of cells,

➢Define

$$\text{SuccessRate} = \frac{\#\text{ of } \textit{distinct} \text{ cells}}{\text{total \# of cells}}$$

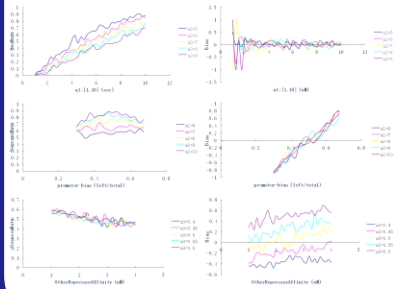
- define a cell as ***distinct*** if

(i) (# of GFP+RFP) > threshold

(ii) r<0.1 or r>0.9, $r = \frac{\#\text{ of GFP}}{\#\text{ of GFP+RFP}}$

➢ Define

$$\text{Bias} = \frac{\#\text{ of } \textit{distinct} \text{ (green - red) cells}}{\#\text{ of } \textit{distinct} \text{ cells}}$$



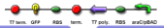
Success Rate *Bias*

← Single Variable Plots

- plus series: semi-double-variable
- Polymerase binding time intervals, represser action delay, promoter bias, repressor asymmetry

Promoter & parts tests

T7 polymerase production test



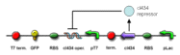
GFP test with intact T7 promoter

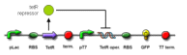
Truncated promoter test

Left/right induction test

Adjacent bidirectional promoters test

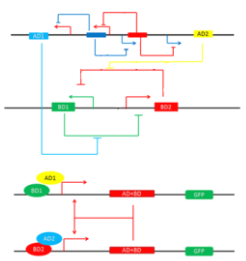
Reciprocal inhibition test





Design extension

Memorizer + Reporter



The output of the Randomizer could potentially be read by a *Memorizer* and *Reporter*, which compares two successive logic signals and reports a jackpot hit. Memorization could be achieved by *E. coli* two hybrid toggle switch. While AD-BD act as antagonists which couldn't stably co-exist. Thus non-interacting AD-BD could serve as memory molecules.
Reporter could work by transient AD-BD activation followed by self activation.

References

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Acknowledgements

This project is supported by the Hong Kong University of Science & Technology, and Hysan Foundation Inc.
Thanks should go to Dr. Dana Boyd for his gift of *lambda InCh* system, Zhenggang Wang for his help on simulation, Peking University iGEM Team (2007) for their sharing of part and iHKU for their provision of *pBAD/araC* part.