A dual selection module for directed evolution of genetic circuits

Yohei Yokobayashi and Frances Arnold
Journal of Natural Computing, 2005

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20.385

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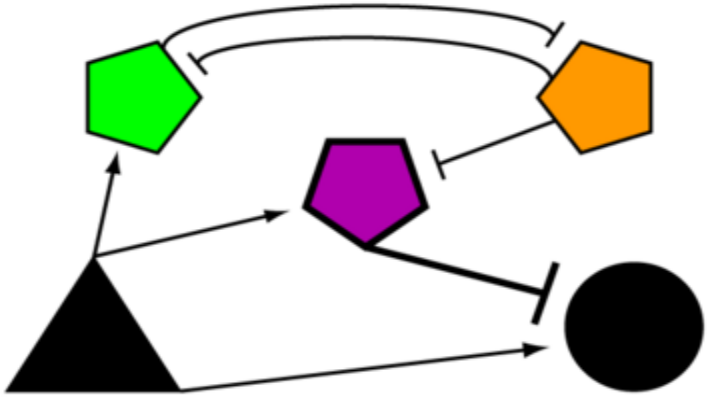
Implementing Genetic Circuits

- Want to program cells



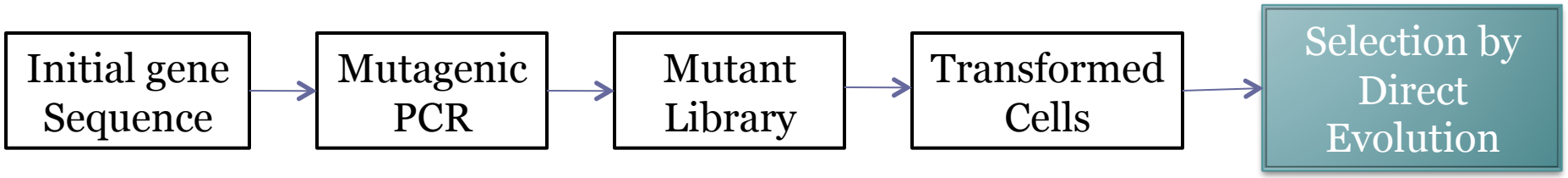
- Problem :
  - inserted genetic circuits do not work as expected
  - need to optimize circuit parameters to obtain desired output characteristics

Optimization Approaches

- Rational Debugging
  - Use what we know about cell biology to fix the problem
  - Use computational methods to predict outcomes
  - Mutagenize RBS, Promoter strengths
- Directed Evolution
  - Circumvent unknowns such as RNA/protein stability, molecular affinities, and effect of host genotype
  - Screen for cells that display the desired output

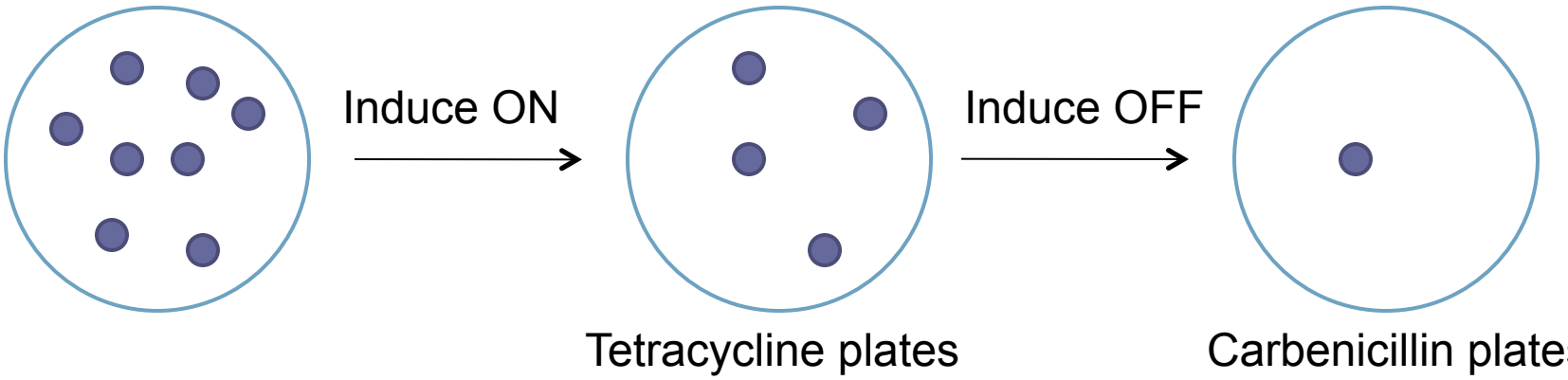
Directed Evolution

- Established as a powerful tool for protein evolution
- First applied to genetic circuits by Yokobayashi, Weiss, and Arnold (PNAS 2002)
- Main selection strategies:
  - Selectable markers (e.g. antibiotic resistance)
  - Observable outputs (e.g. fluorescent protein)



Dual Selection Module

- Develop generic selection module to screen for any circuit with an ON/OFF gene expression output
- Link ON/OFF outputs to different antibiotic resistance
  - ON – tetracycline resistant
  - OFF – carbenicillin resistant



Selection Module Design

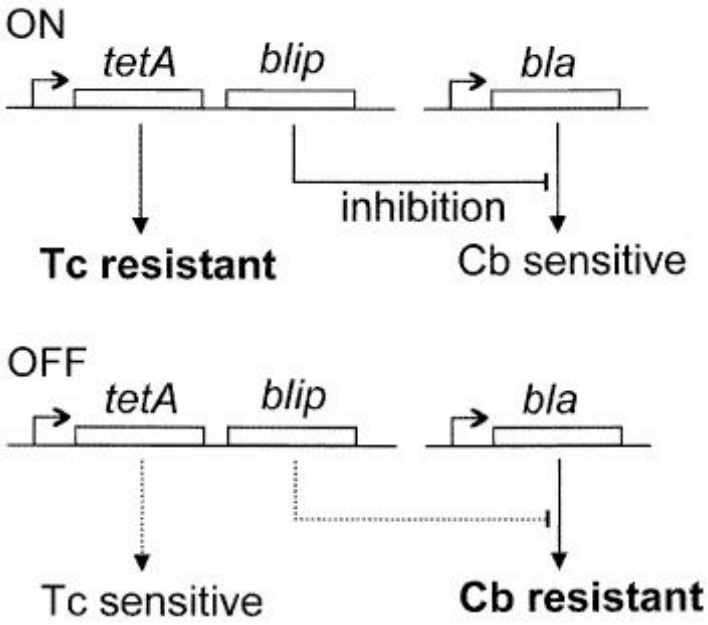
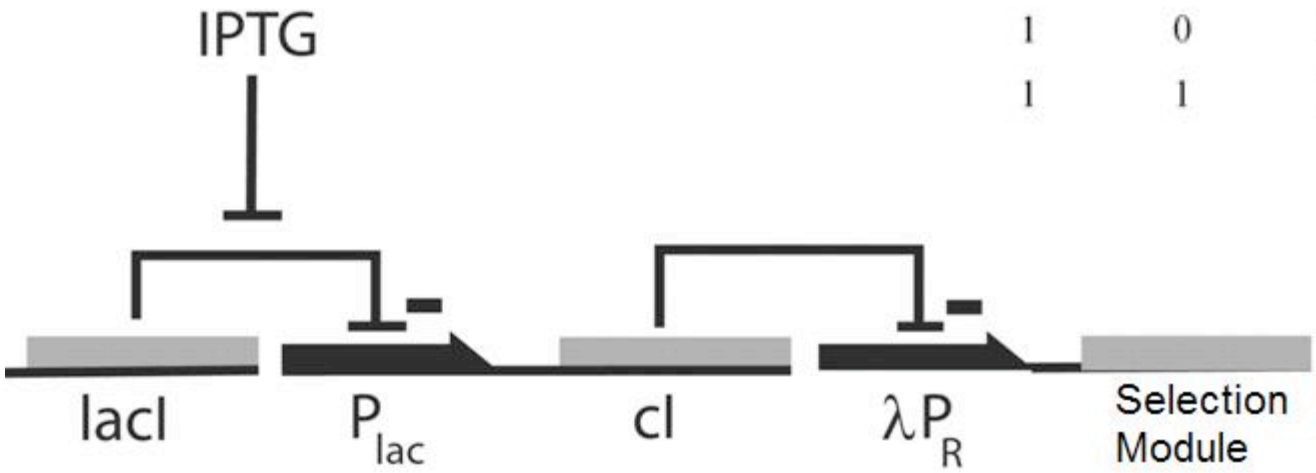


Figure 1. Schematic of the selection module. Two genes *bli* and *tetA* are placed under the control of the output promoter of the circuit of interest, while *bla* is expressed constitutively from its own promoter. Depending on the expression state of the output promoter, the host cell becomes resistant to tetracycline (Tc, ON state) or carbenicillin (Cb, OFF state). The dotted lines indicate the absence of expression in the OFF state.

Inverter Circuit

- Test module with functional genetic inverter
  - IPTG input
  - Output connects to selection module

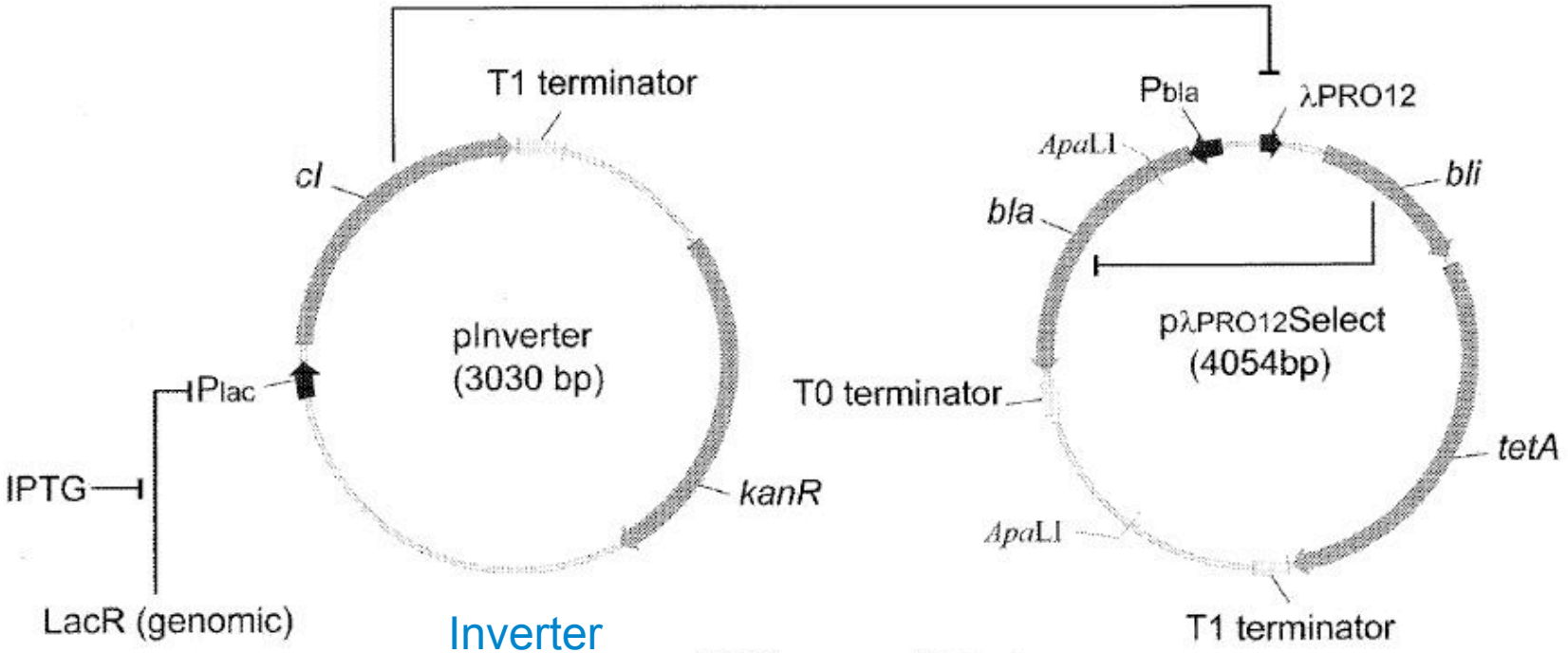
lacI	IPTG	cI	Output
1	0	0	1
1	1	1	0



(genomic, constitutive)

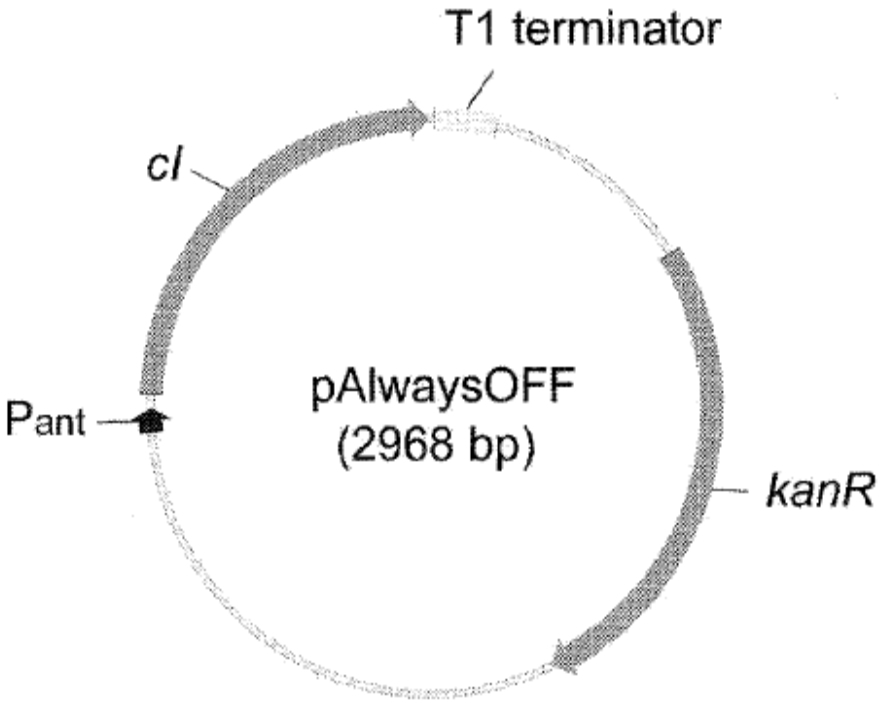
Inverter Circuit + Selection Module

- Two plasmid system

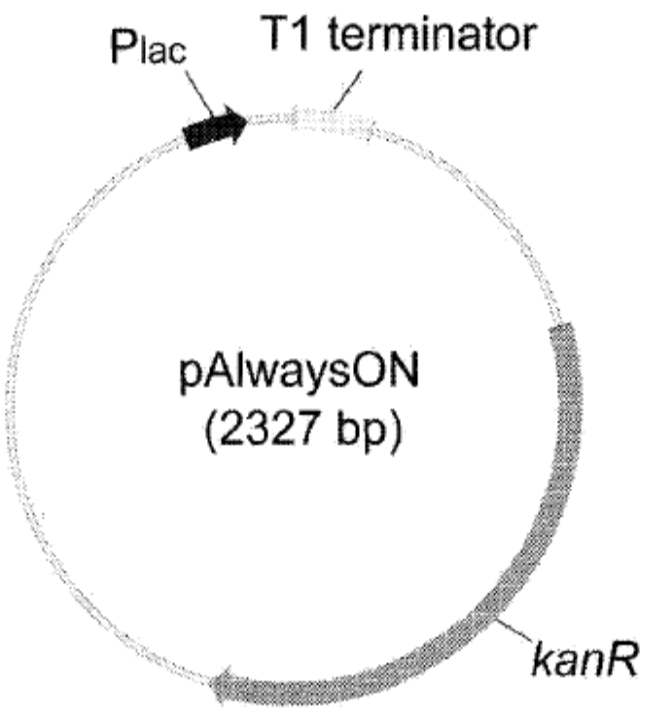


IPTG	Output
OFF	ON
ON	OFF

Control Plasmids

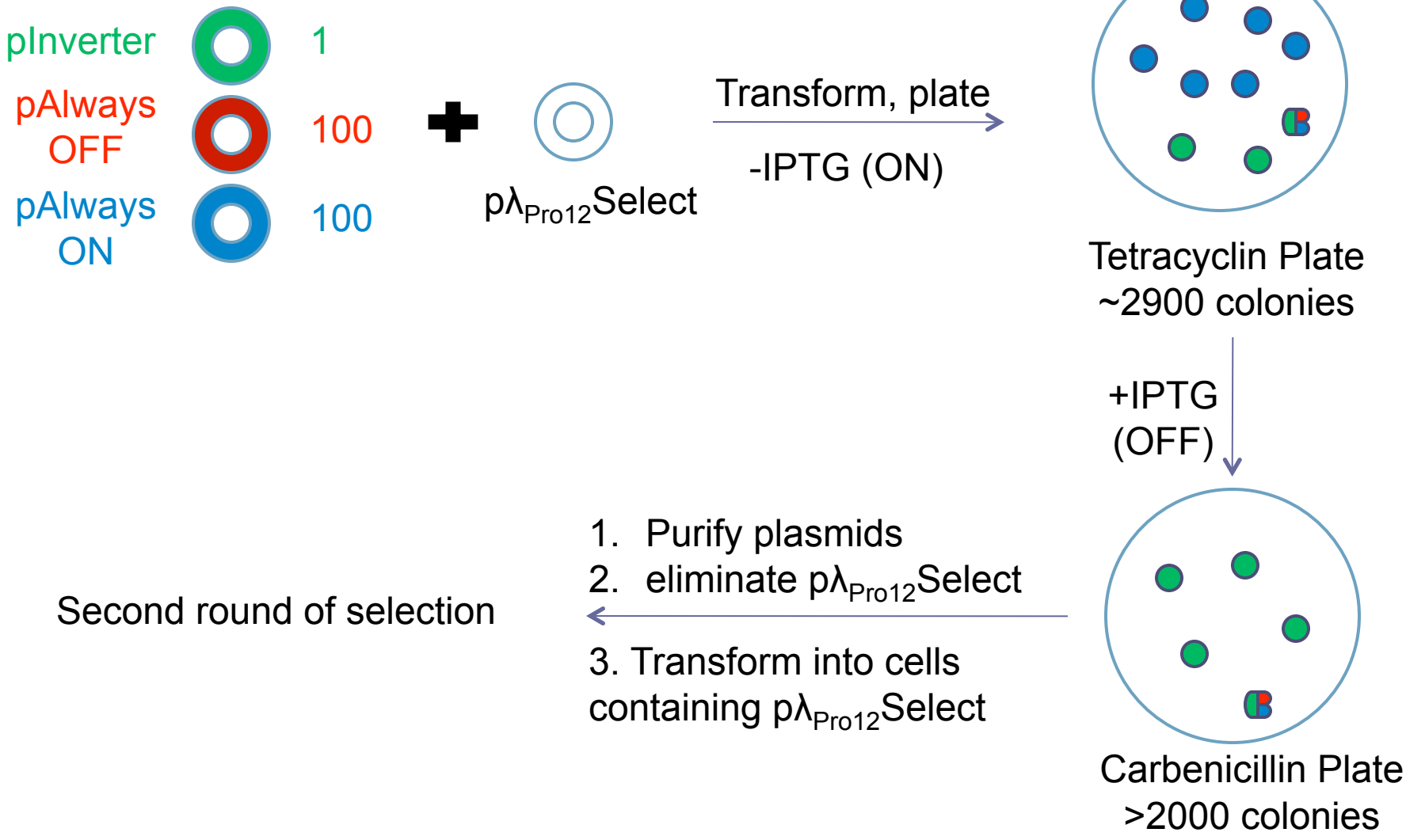


- Constitutive cI expression
- Output = OFF



- No cI expression
- Output = ON

Selection Scheme



Results

- Randomly test colonies from carbenicillin/IPTG plates of 1st and 2nd rounds of selection
  - 1st round - 37/48 (77%) contained pInverter
  - 2nd round – 48/48 (100%) contained pInverter

- Up to 0.1% of transformed cells were false positives
  - Recovered larger plasmids → indicate recombination
  - Suspected mutations

Implications

- Selection module allows isolation of rare circuits from large libraries
- Useful for initial stages of constructing complex circuits to determine functional candidates
- Possible to adjust antibiotic concentrations and marker threshold levels to select for specific circuit performance criteria
- Can be used with variety of host genotypes

Limitations

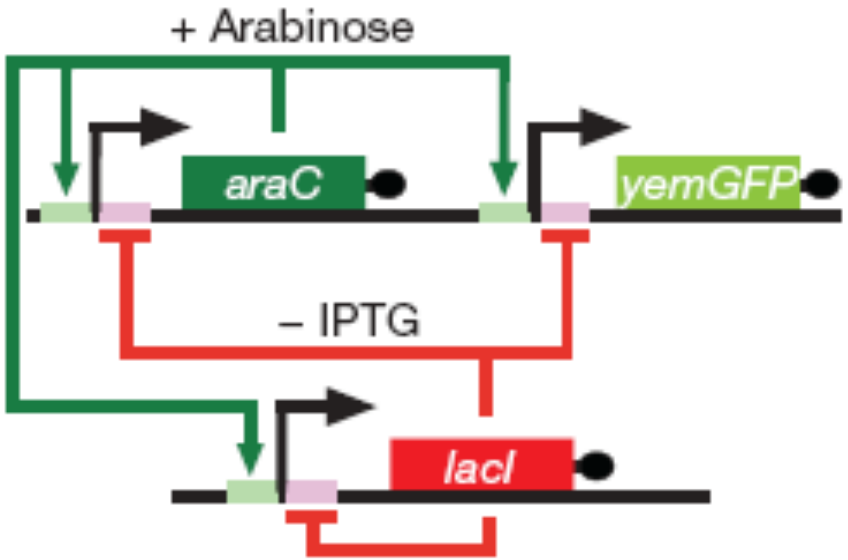
- Strategy is only valid for circuits with a clear ON/ OFF output
- Accuracy – larger library, more false positives
- Authors did not validate many of their claims
  - Small library
  - Tested on only one pre-validated construct – did not actually evolve a circuit
  - System tested in only one host organism
  - Did not demonstrate ability to generate different thresholds

Subsequent Work

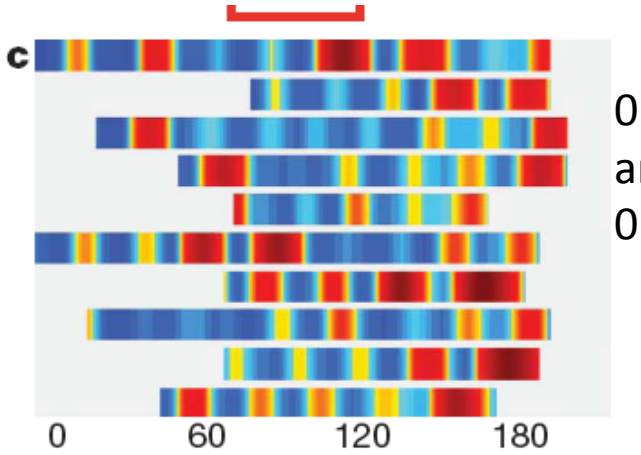
- Muranaka N, Sharma V, Nomura Y, Yokobayashi Y. An efficient platform for genetic selection and screening of gene switches in Escherichia coli. *Nucleic Acids Res* 2009
- Sharma V, Nomura Y, Yokobayashi Y. Engineering complex gene regulation by dual genetic selection. *J Am Chem Soc* 2008
- Nomura Y, Yokobayashi Y. Dual selection of a genetic switch by a single selection marker. *BioSystems* 2007

A fast, robust and tunable synthetic gene oscillator

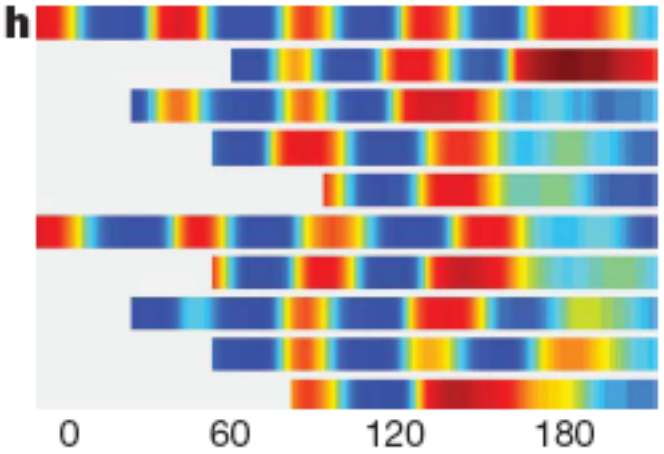
Jesse Stricker[1]*, Scott Cookson[1]*, Matthew R. Bennett[1,2]*, William H. Mather[1], Lev S. Tsimring[2] & Jeff Hasty[1,2]



Measurements for single cell oscillations



0.7% arabinose and 0 IPTG



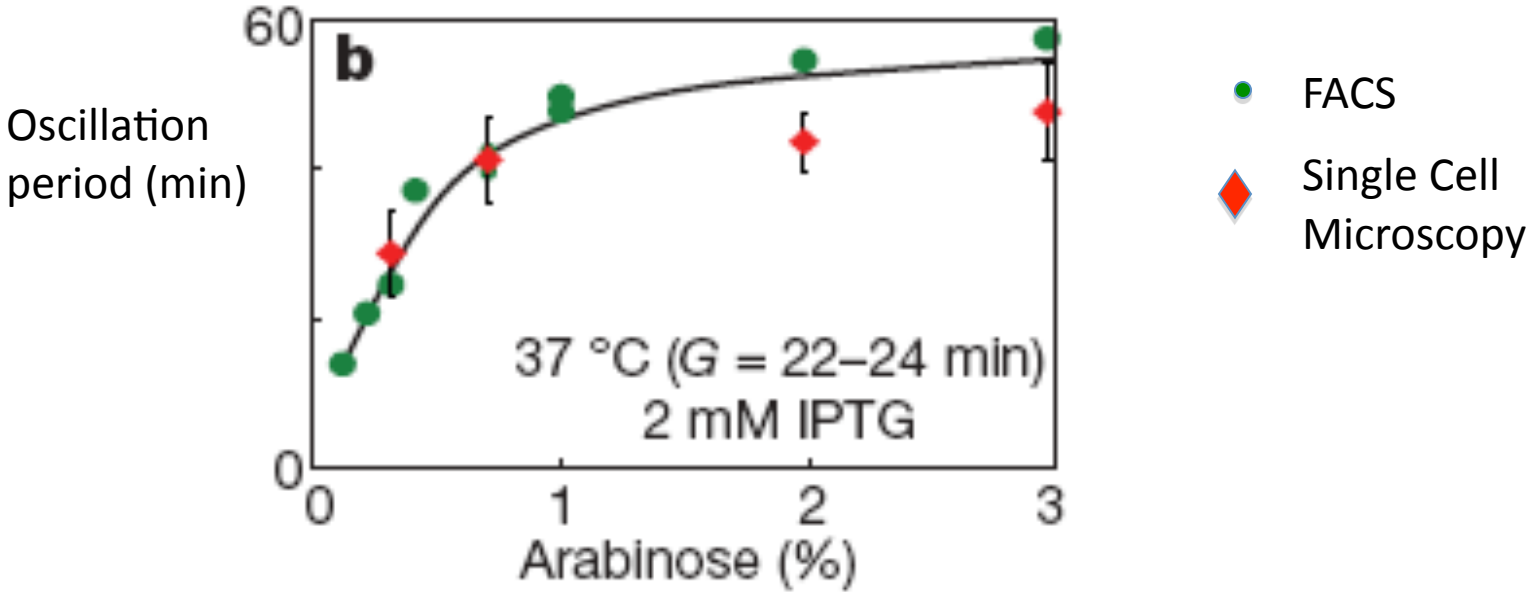
0.7% arabinose and 5mM IPTG

A fast, robust and tunable synthetic gene oscillator

Jesse Stricker[1]*, Scott Cookson[1]*, Matthew R. Bennett[1,2]*, William H. Mather[1], Lev S. Tsimring[2] & Jeff Hasty[1,2]

"tunable"

At a fixed value of 2mM IPTG and at 37 uC, the oscillatory period can be tuned from 13 min to 58 min by varying the arabinose level from 0.1% to 3.0% (Fig. 2b).



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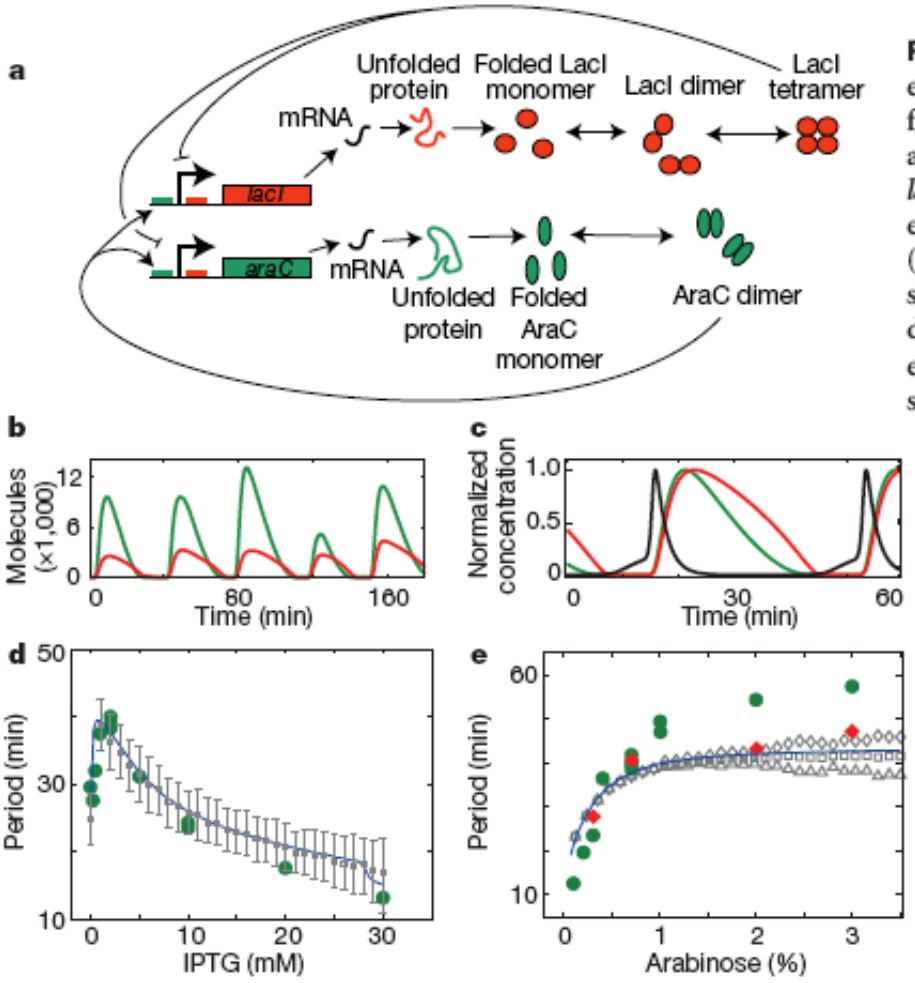

Figure 4 | Modelling the genetic oscillator. **a**, Intermediate processes are explicitly modelled in the refined oscillator model. **b**, **c**, Simulation results from Gillespie simulations (**b**) or deterministic modelling (**c**) at 0.7% arabinose and 2 mM IPTG. AraC dimers (green), LacI tetramers (red) and *lacI* mRNA (black) are shown. **d**, **e**, Comparison of modelling and experiment for oscillation period at 0.7% arabinose (**d**) or 2 mM IPTG (**e**). Values from deterministic modelling (blue curve), stochastic simulations (grey symbols, Supplementary Fig. 18), and microscopy (red diamonds) or flow cytometry (green circles) are shown. Lower and upper error bars in **d** represent the 16th and 84th percentiles, respectively, of the stochastic data, corresponding to ±1 s.d. for a normal distribution.

"In the context of synthetic biology, our findings indicate that caution must be exercised when making simplifying assumptions in the design of engineered gene circuits. We found that a full model of the system that takes into account intermediate steps such as multimerization, translation, protein folding and DNA looping is essential."