Sinteznobiološki sistemi

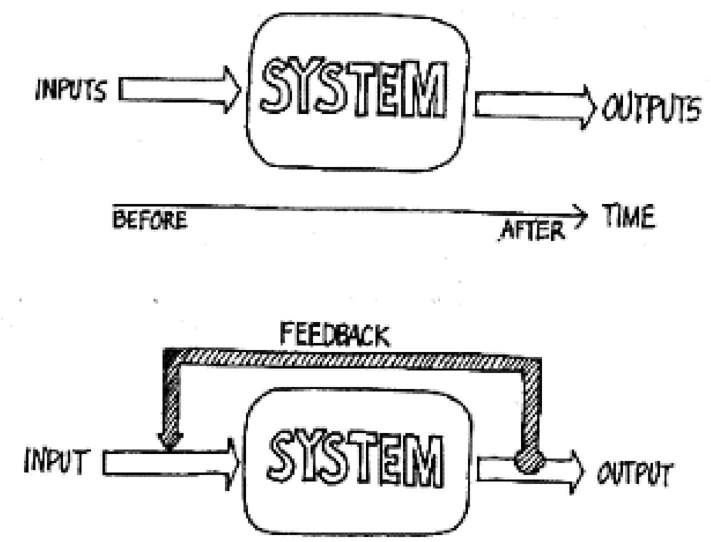
Značilni primeri

Povratne zanke

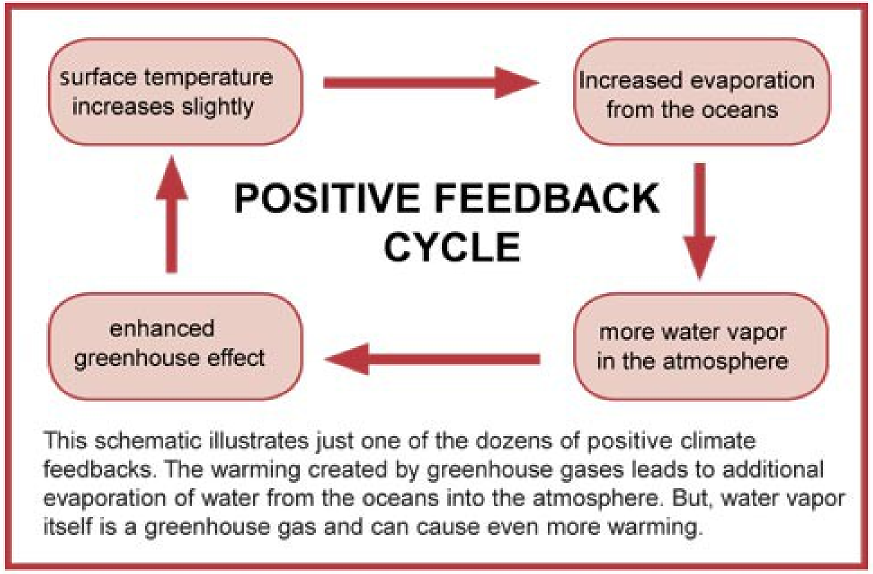
Feedback loops

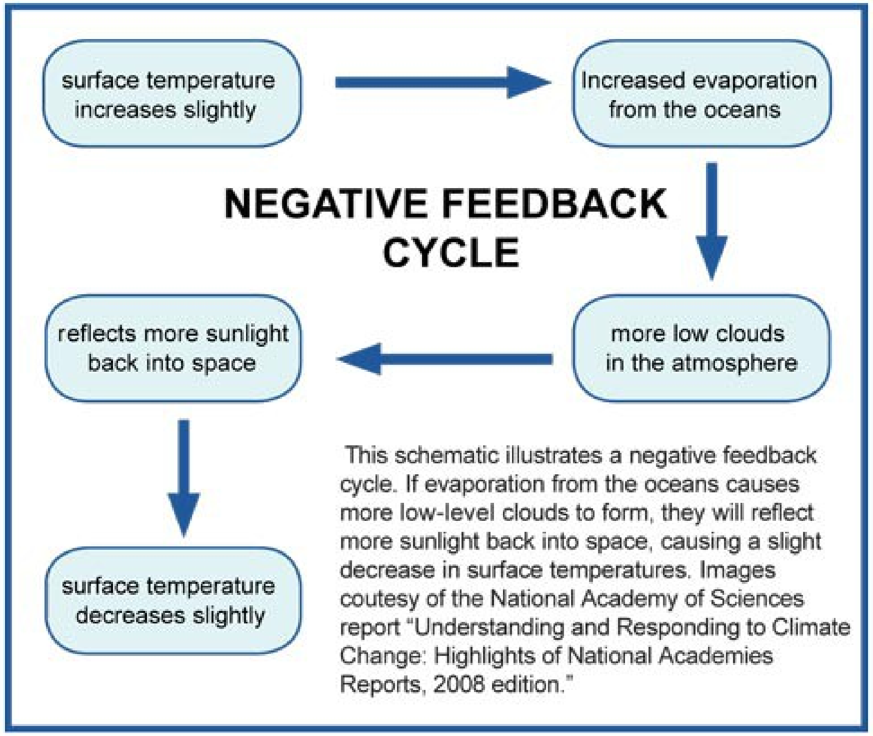
Povratne zanke v Registru niso uvrščene med sisteme. Gre za princip uravnavanja mehanizmov, pri katerem izhod iz naprave vpliva na nadaljnje obnašanje iste naprave. Obstajajo pozitivne in negativne povratne zanke, pri čemer izhodni signal vpliva tako, da poveča oz. zmanjša izhodni signal za v bodoče.

Uvedba povratnih zank poveča robustnost in zmanjšuje šum in biološko variabilnost celičnega sistema.

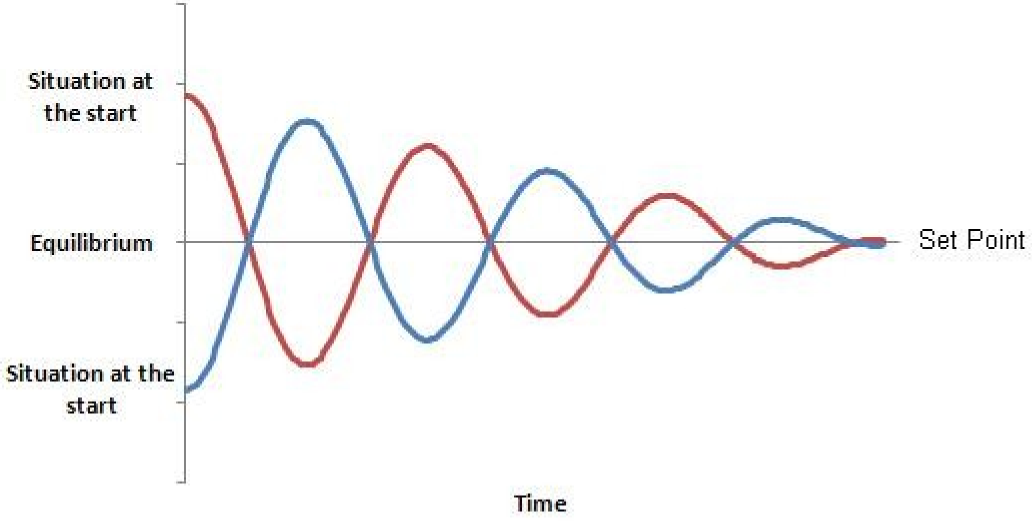


http://pespmc1.vub.ac.be/FEEDBACK.html

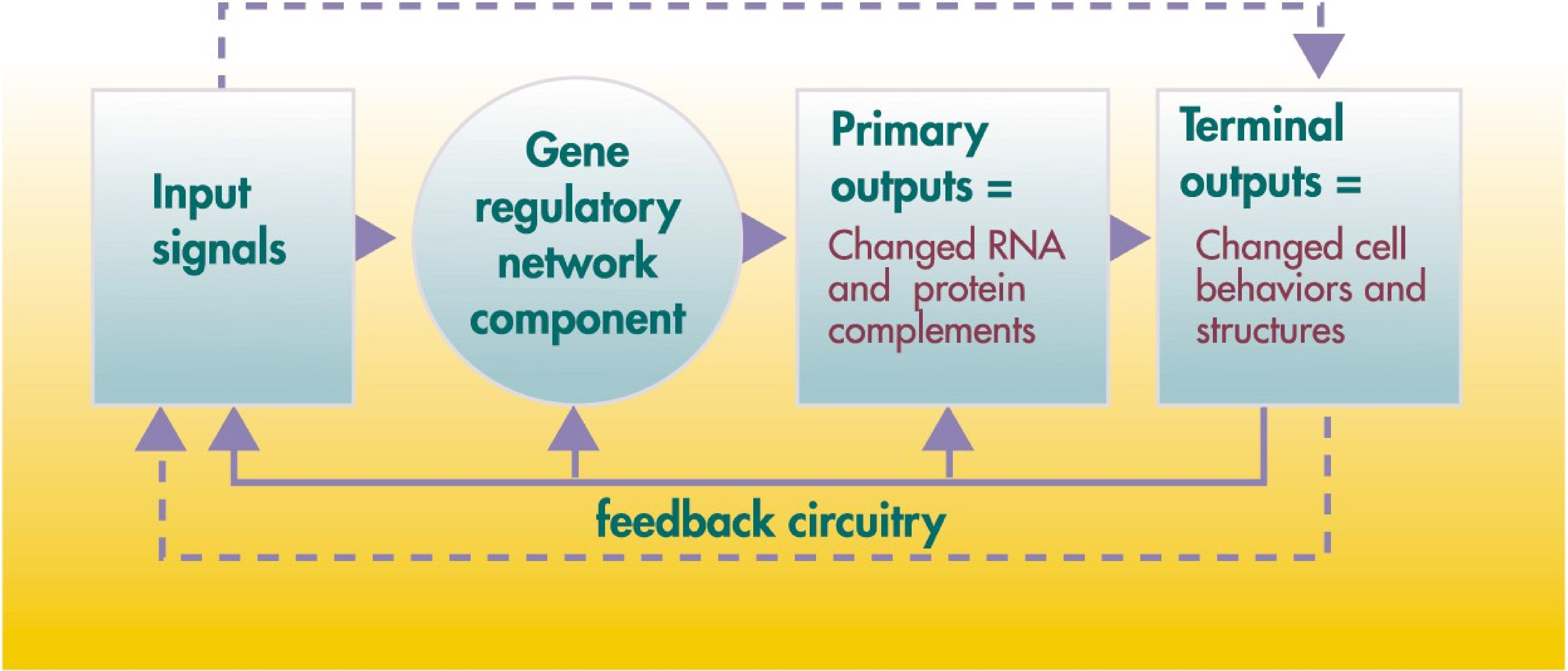




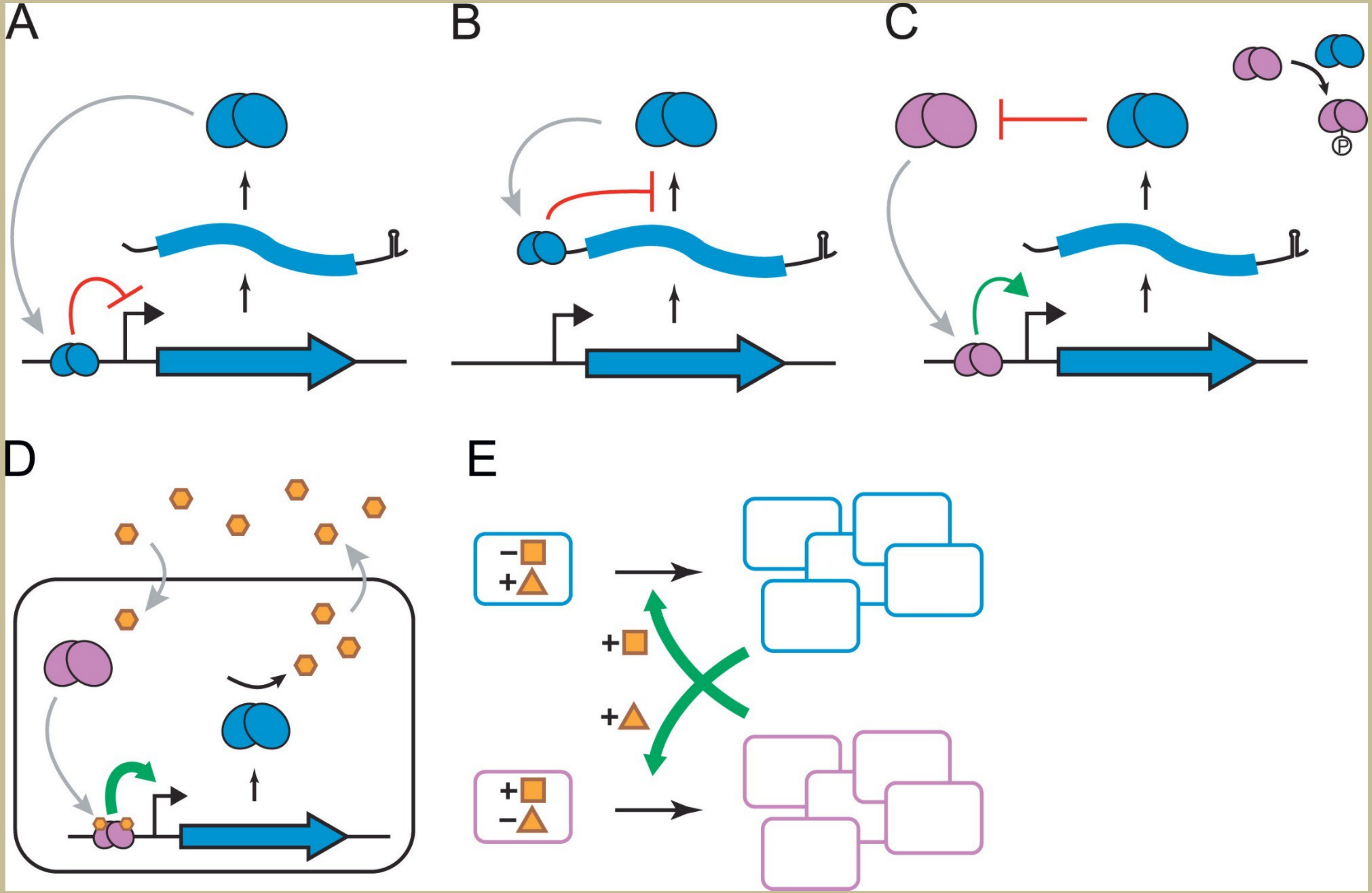
http://www.southwestclimatechange.org/figures/feedback_cycles



https://controls.engin.umich.edu/wiki/index.php/Feedback_control



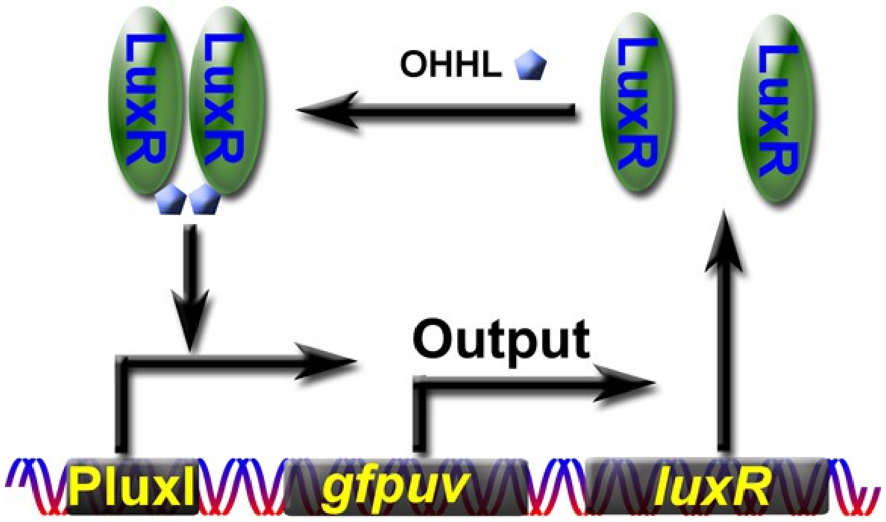
http://en.wikipedia.org/wiki/Gene_regulatory_network



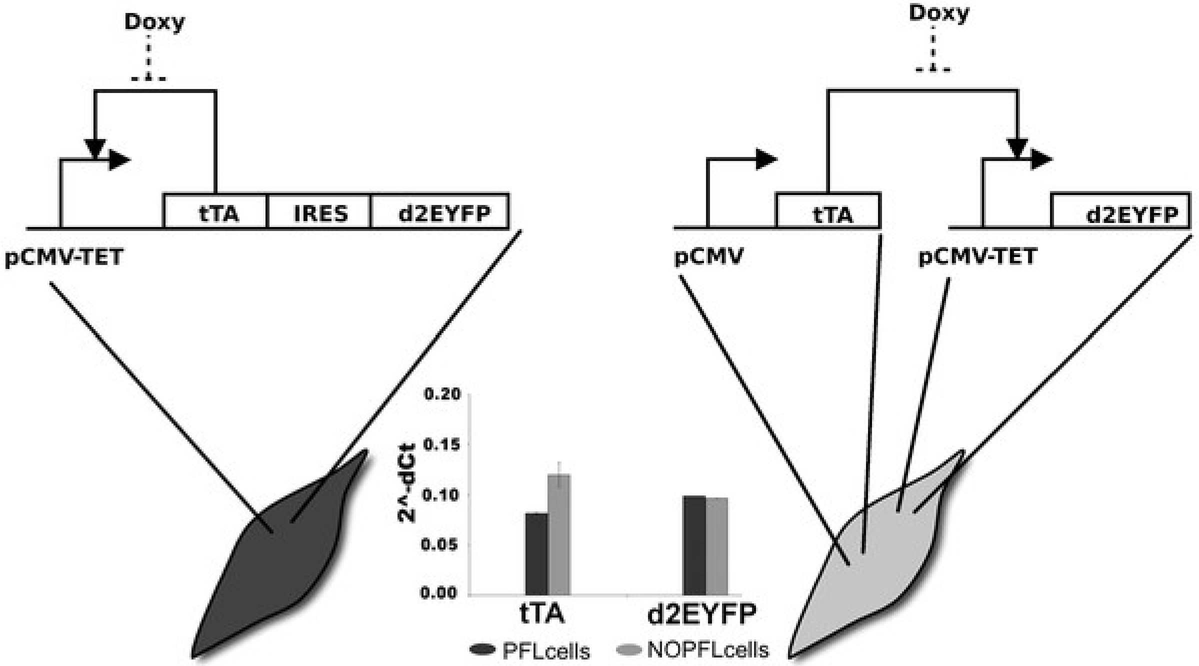
Chemical Engineering Science 103,
15 November 2013, pp. 79–90

Povratne zanke pri mikroorganizmih. Povratne zanke lahko uvedemo na različnih stopnjah izražanja genov v celici (A)–(C) in preko interakcij med celicami (D in E).
Rdeče: negativna regulacija, zeleno: pozitivna regulacija. (A) Transkripcijska regulacija pri avtorepresiji. (B) Posttranskripcijska regulacija na primeru RNA-vezavnega proteina, ki zavira lastno translacijo. (C) Posttranslacijska regulacija na primeru fosforilacije transkripcijskega aktivatorja, ki zavira vezavo na DNA. (D in E) Medcelične interakcije na primeru sinteze in izločanja molekule, ki aktivira izražanje sintetaze te iste molekule (D), in primer medsebojne interakcije med dvema sevoma, kjer en sev proizvaja esencialni metabolit, ki ga drugi sev ne proizvaja (E).

Pozitivna povratna zanka

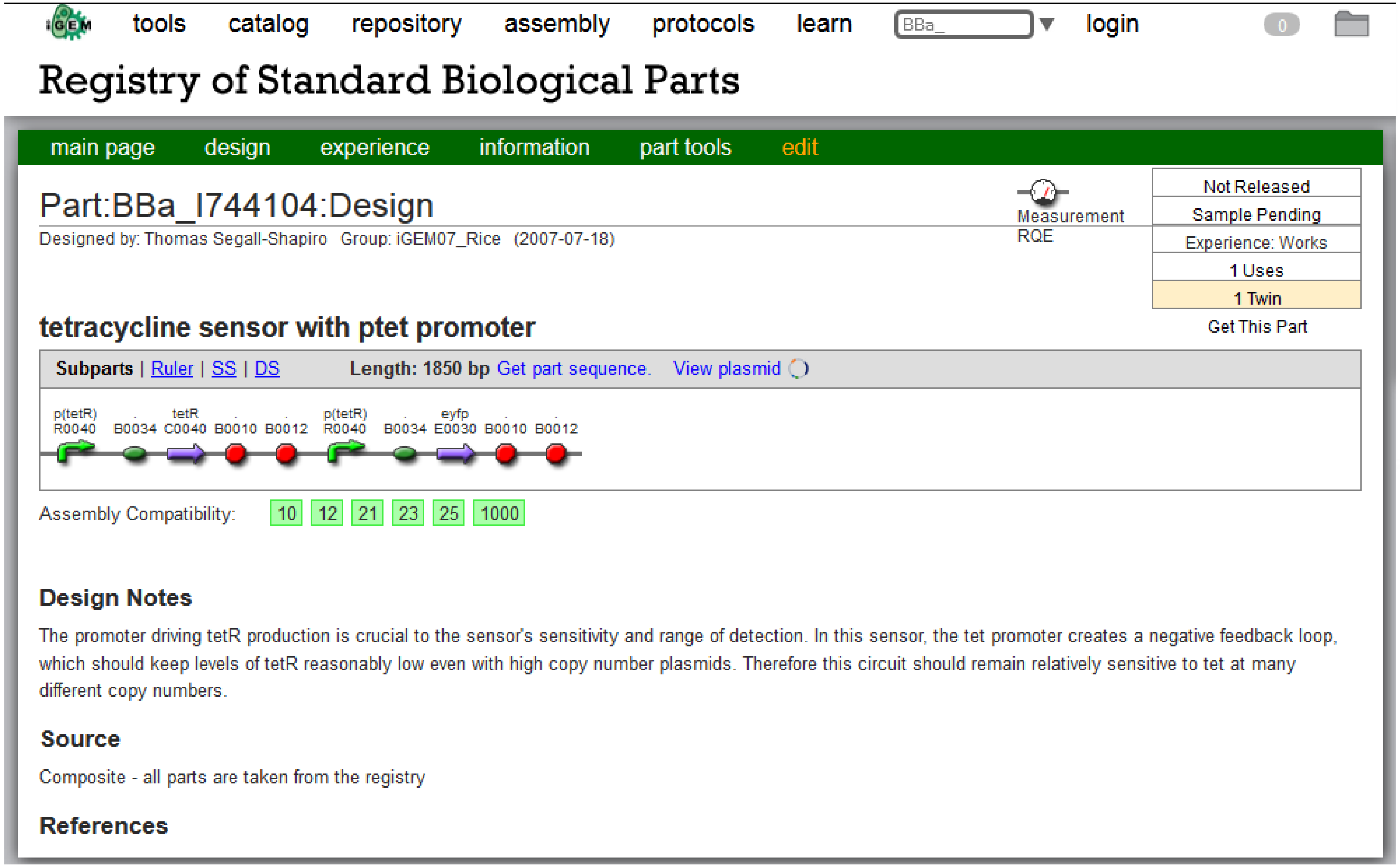


http://staff.ustc.edu.cn/~lianhong/research.html



PLoS Comput Biol 7(6): e1002074 (2011)

Negativna povratna zanka


tools catalog repository assembly protocols learn BBa_ login 0

Registry of Standard Biological Parts

main page design experience information part tools edit

Part:BBa_I744104:Design

Designed by: Thomas Segall-Shapiro Group: iGEM07_Rice (2007-07-18)

Measurement RQE

Not Released
Sample Pending
Experience: Works
1 Uses
1 Twin

Get This Part

tetracycline sensor with ptet promoter

Subparts | Ruler | SS | DS **Length: 1850 bp** Get part sequence. View plasmid

Assembly Compatibility: 10 12 21 23 25 1000

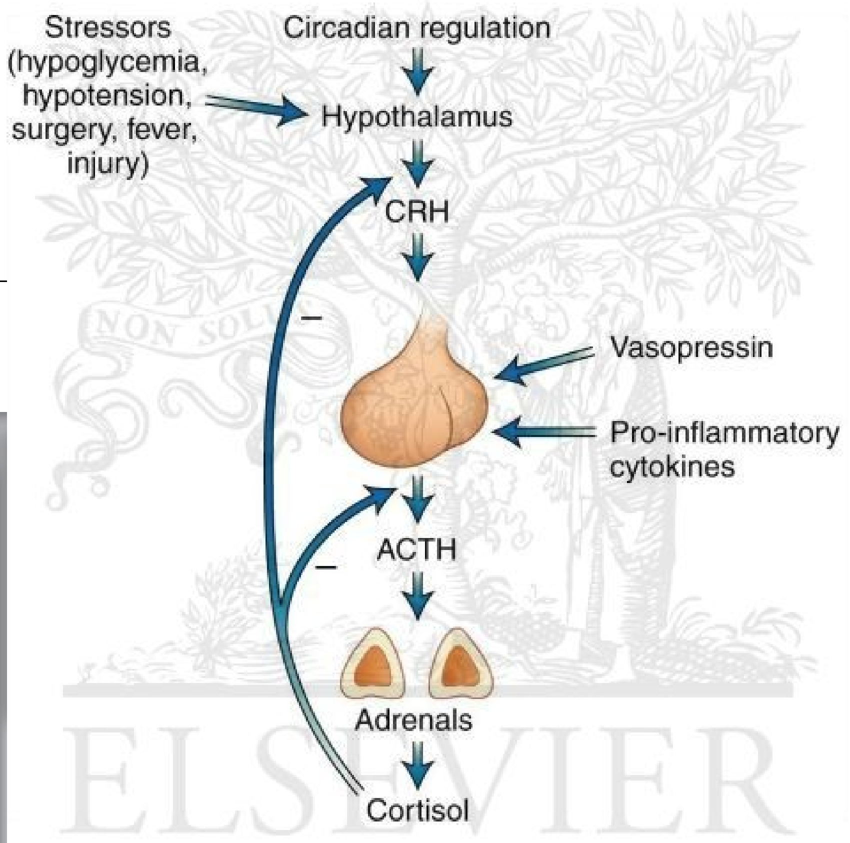
Design Notes

The promoter driving tetR production is crucial to the sensor's sensitivity and range of detection. In this sensor, the tet promoter creates a negative feedback loop, which should keep levels of tetR reasonably low even with high copy number plasmids. Therefore this circuit should remain relatively sensitive to tet at many different copy numbers.

Source

Composite - all parts are taken from the registry

References

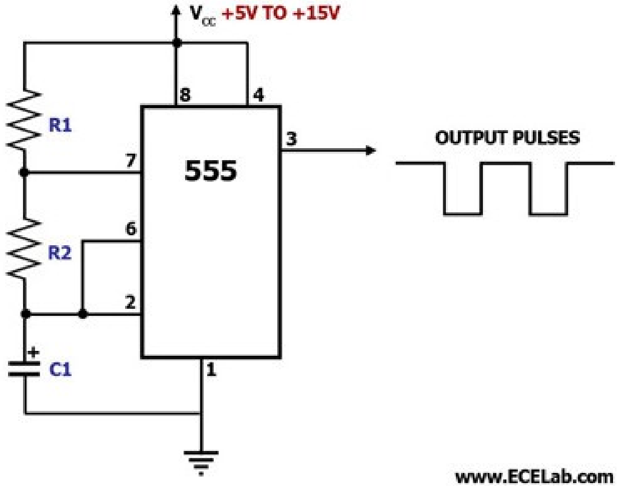


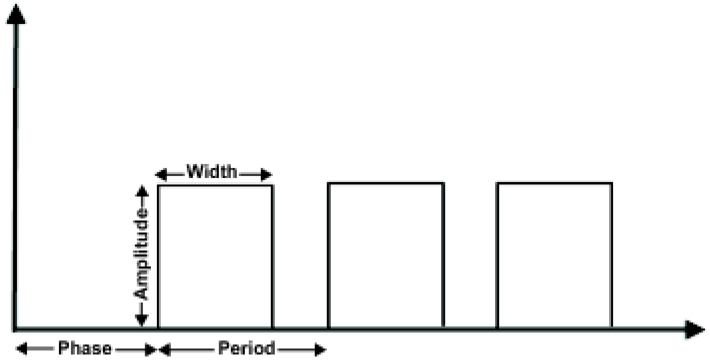
http://www.elsevierimages.com/image/28381.htm

Generatorji impulzov

Pulse generators

V elektroniki je generator impulzov vezje oz. aparatura, ki generira pravokotne impulze napetosti. Obstajajo tudi generatorji svetlobnih impulzov, če so frekvence impulzov visoke, govorimo o pulzerjih mikrovalov. Večkanalni generatorji impulzov imajo več vstopnih signalov. Izhodni signal ima svojo amplitudo, širino, periodo (takt) in fazni zamik.

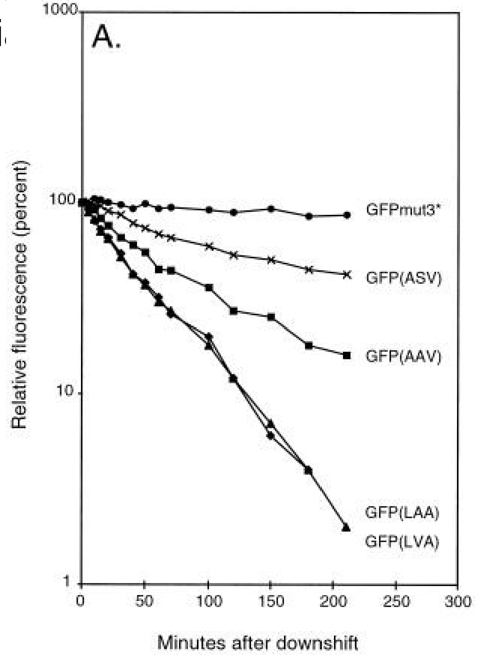




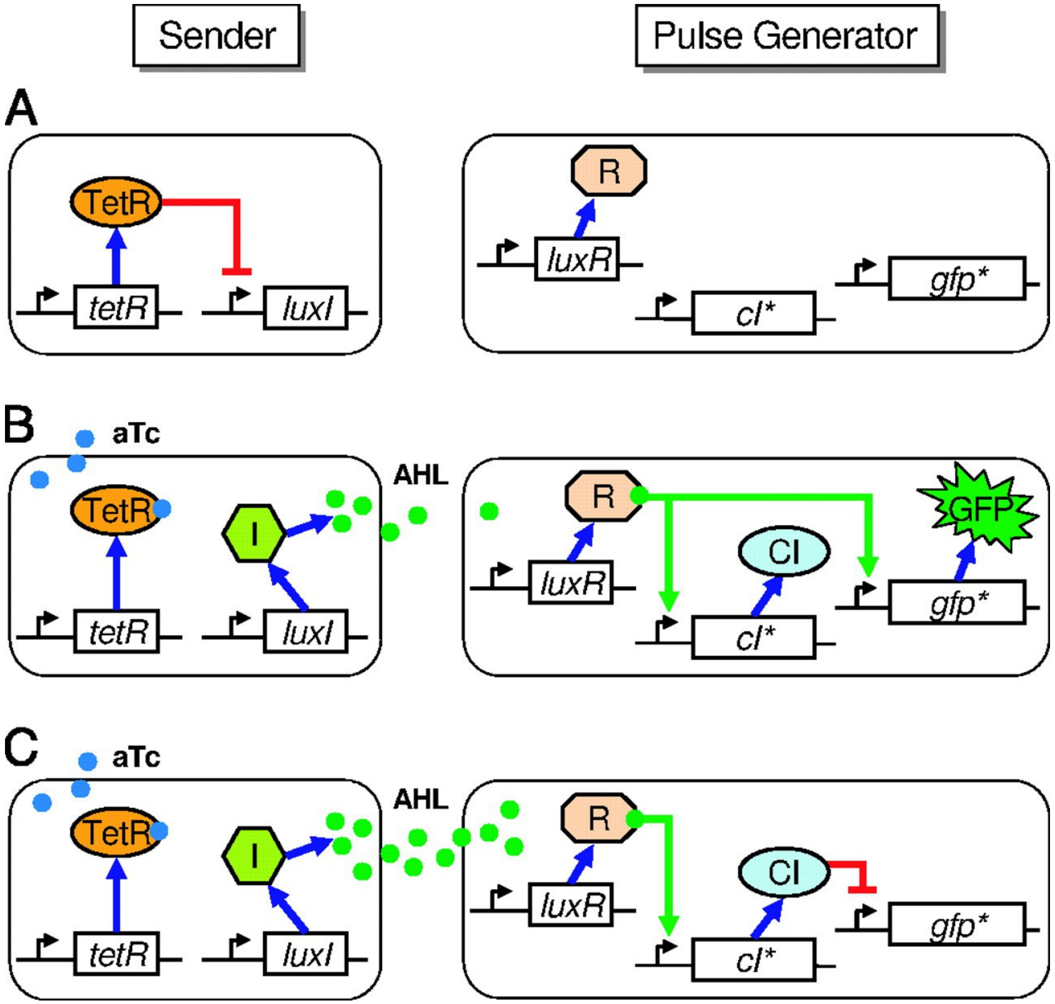
Generatorji impulzov v sintezni biologiji

V SB je generator impulzov (genetsko) vezje, ki generira izhodni signal v obliki reporterja, po začetnem izražanju pa signal upade. Pogosto gre za povezavo dveh celic, pri čemer prva sintetizira in izloča signalno molekulo (vstopni signal), druga pa deluje kot sprejemnik signala, ki kot odziv generira impulz.

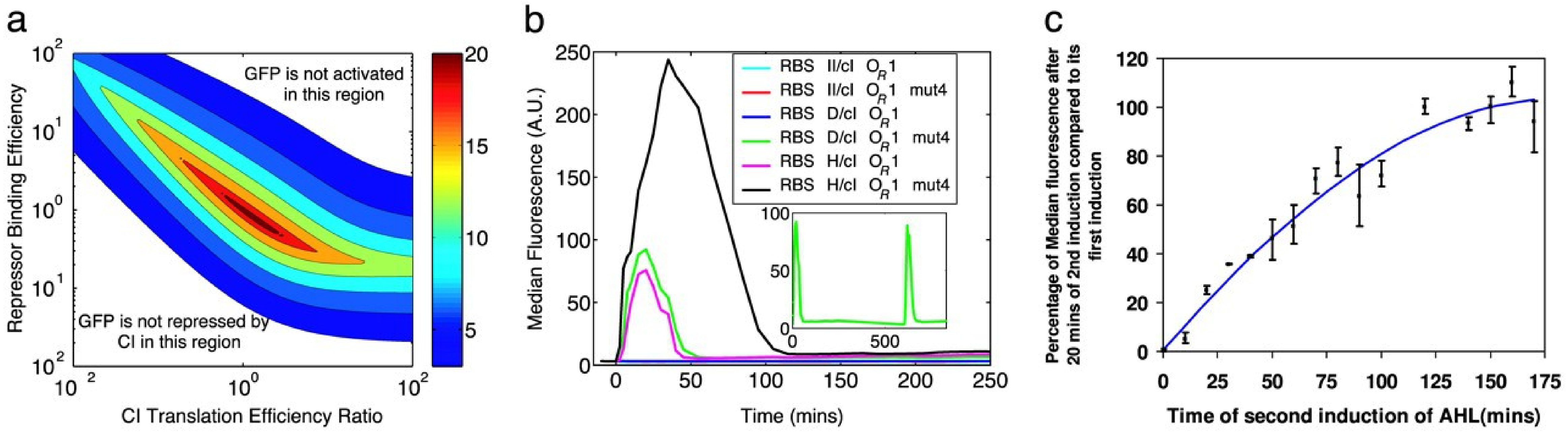
V prvem takem opisanem primeru je kot signalna molekula deloval acil-homoserinlakton, izhodni signal pa je bila mutirana oblika proteina GFP (varianta LVA: na C-koncu je podaljšek RPAANDENYALVA). Zaradi tega podaljška je protein bolj občutljiv na delovanje celičnih proteaz, s tem pa se skrajša razpolovni čas fluorofora in impulz zato upade. Uporabili so tudi vari… represor.



Appl Environ Microbiol.64(6): 2240–2246 (1998)



PNAS 101(17), 6355-6360 (2004)



PNAS 101(17), 6355-6360 (2004)

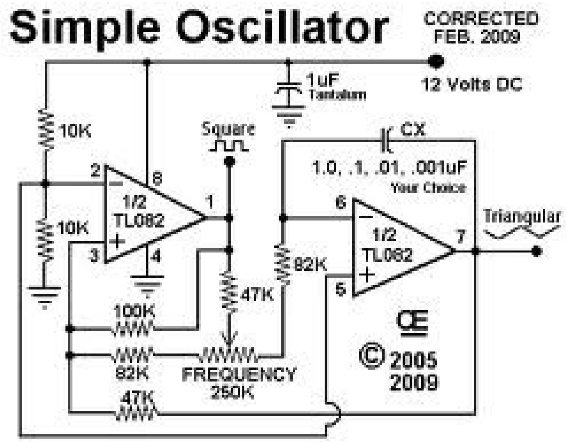
Oscilatorji

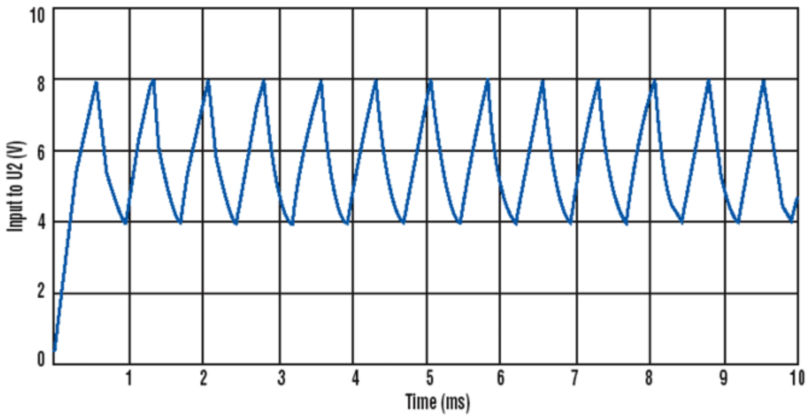
Oscillators

Oscilatorji oddajajo definiran signal, ki se s časom ponavlja in ima običajno obliko valovanja. Pri načrtovanju vezij si želimo, da bi delovali stabilno. Primer oscilatorja v elektroniki je pretvornik enosmernega v izmenični tok, med oscilatorje pa štejemo tudi avdiooscilatorje (izhodni signali v slišnem območju 16 Hz – 20 kHz), radiofrekvenčne oscilatorje (100 kHz – 100 GHz).

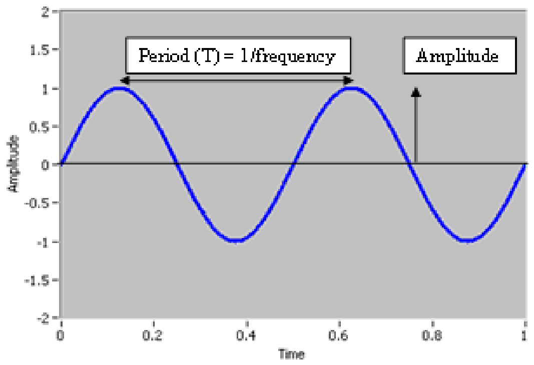
Oscilacijo definirata perioda (frekvenca) in amplituda. Linearni oz. harmonski oscilatorji imajo sinusoidni izhodni signal. Razen teh poznamo tudi nelinearne oz. relaksacijske oscilatorje, ki generirajo izhodni signal trikotne ali pravokotne oblike.

Obstajata dva tipa linearnih oscilatorjev: oscilator s povratno zvezo (feedback oscillator) in oscilator z negativno upornostjo (negative resistance oscillator).



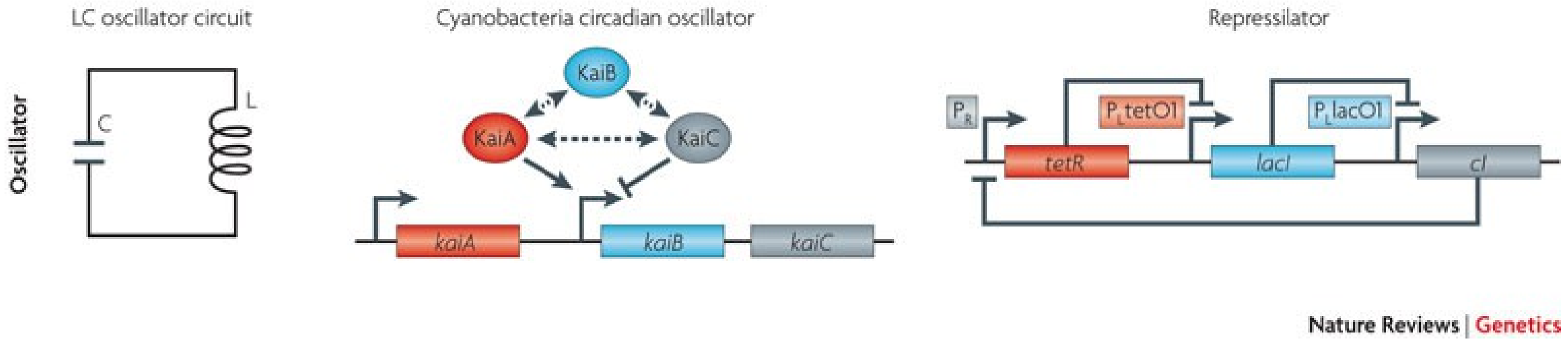


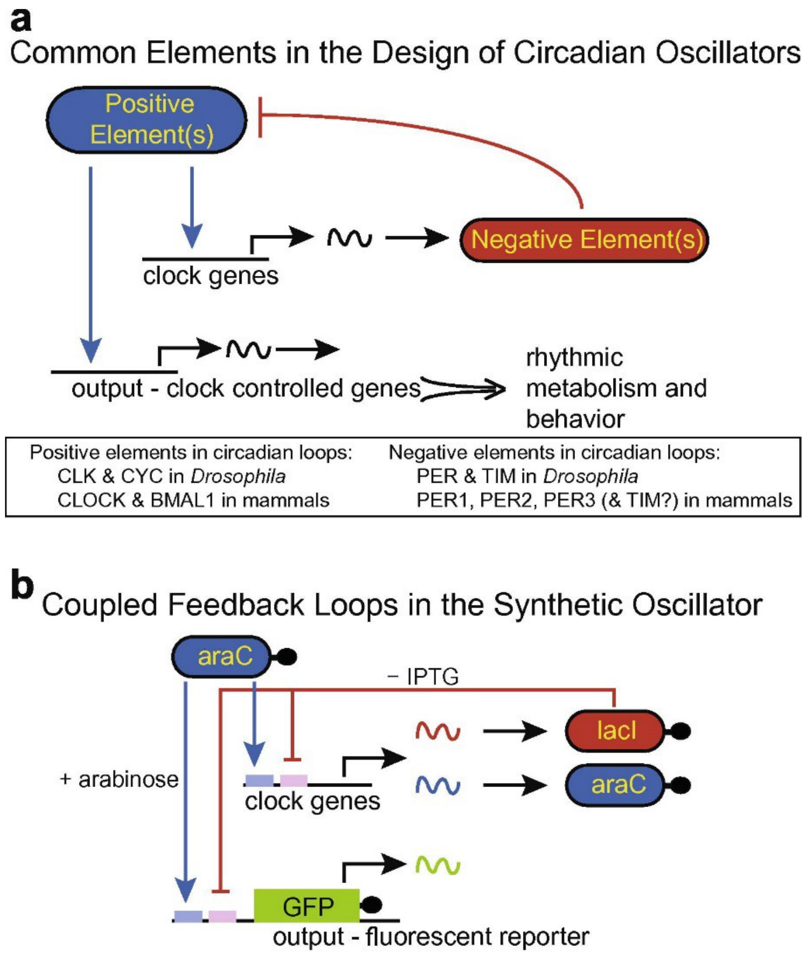
2. This waveform shows the triangle wave appearing at U2's noninverting input.



Oscilatorji

Najpogostejše oscilacije v naravi so cirkadiani ritmi, ki jih uravnavajo endokrini sistem v povezavi z živčnim in na osnovi ciklično regulirane transkripcije določenih genov.





FEBS Lett. 583(24), 3931–3937 (2009)

Represilator

Najbolj znan oscilator v sintezni biologiji je Elowitzev ‚represilator‘, ki ga sestavljajo trije medsebojno povezani represorski sistemi.

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler

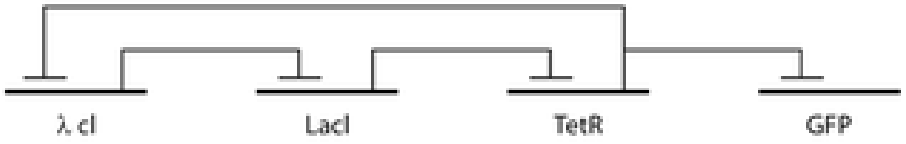
Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA

Networks of interacting biomolecules carry out many essential functions in living cells[1], but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems[2]. Here we present a complementary approach to this problem: the design and construction of a synthetic network to implement a particular function. We used three transcriptional repressor systems that are not part of any natural biological clock[3–5] to build an oscillating network, termed

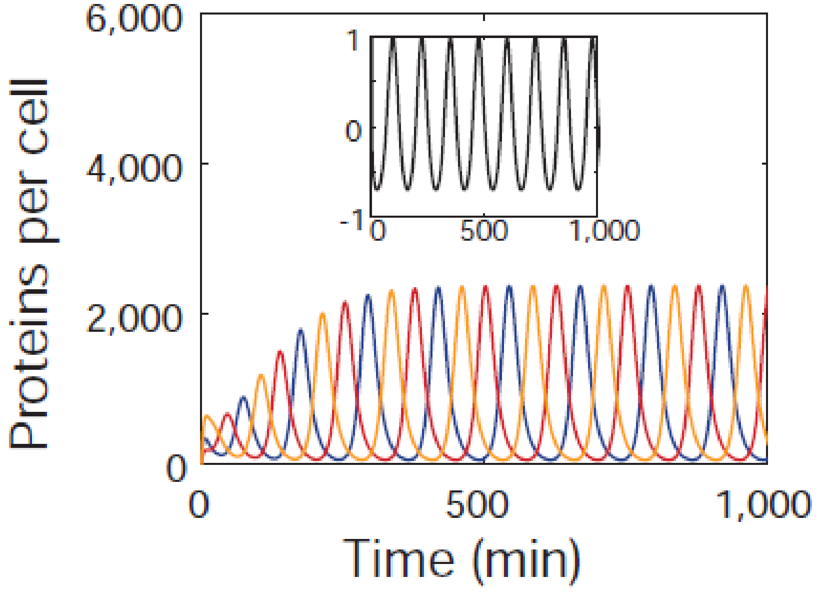
(ref. 8) and diminish the half-life of green fluorescent protein (GFP) to about 30–40 min (ref. 11).

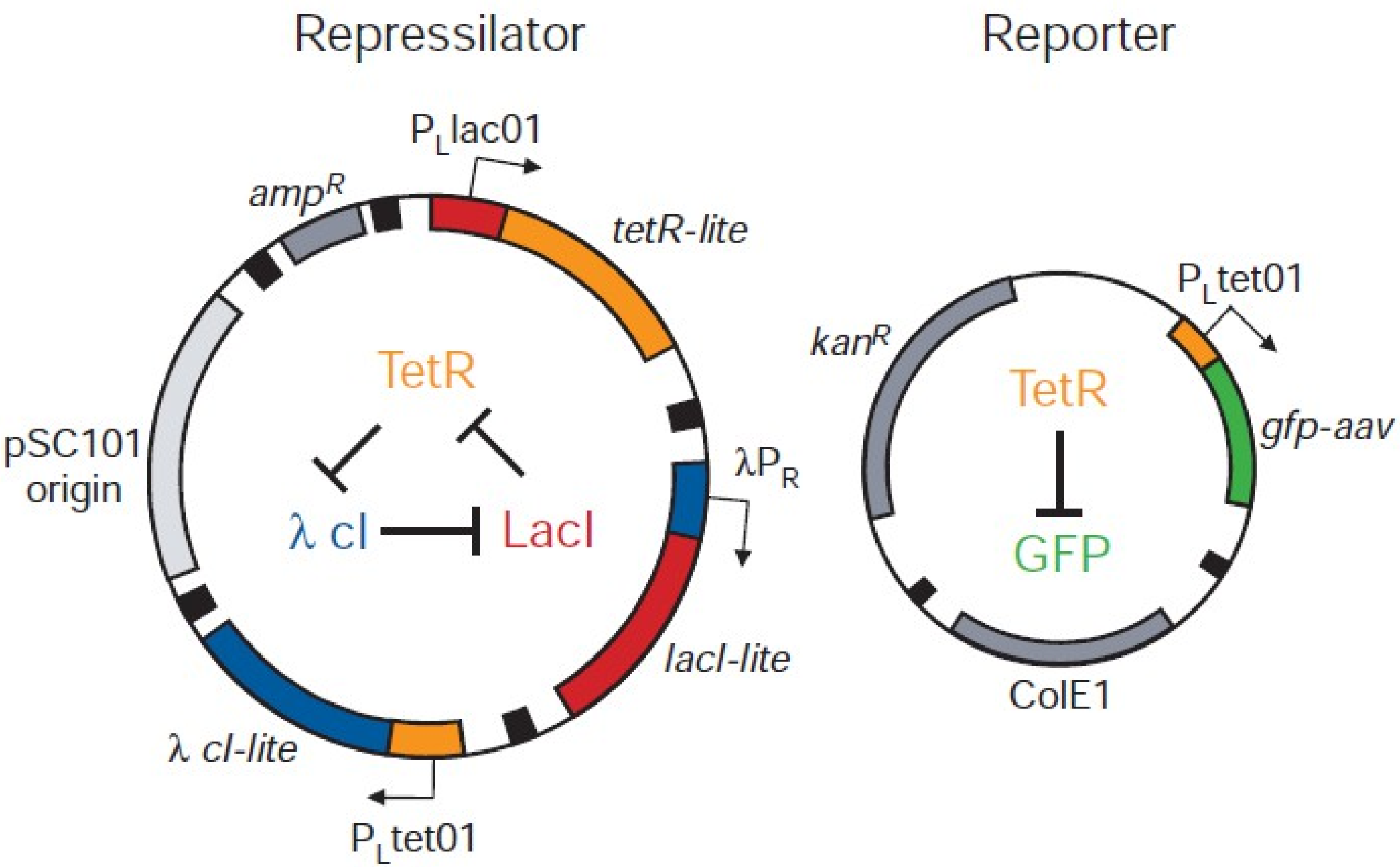
With these considerations in mind, we used standard molecular biology techniques to construct a low-copy plasmid encoding the repressilator and a compatible, higher-copy reporter plasmid containing the tet-repressible promoter $P_L$tetO1 (ref. 6) fused to an intermediate stability variant of *gfp*[11] (Fig. 1a). Because the inducer IPTG interferes with repression by LacI, we expected that a transient pulse of IPTG might be capable of synchronizing a population of repressilator-containing cells. A culture of *E. coli* MC4100 containing the two plasmids and grown in media containing IPTG displayed what appeared to be a single damped oscillation of GFP fluorescence per cell after transfer to media lacking IPTG (results not shown). Because individual cells have no apparent means of maintaining synchronization, we studied the repressilator by isolating single cells under the microscope and monitoring their fluorescence intensity as they grew into small two-dimensional microcolonies consisting of hundreds of progeny cells. In these experiments, total observation time was limited by the colony entering a stationary phase after about 10 hours of growth at

NATURE | VOL 403 | 20 JANUARY 2000 | www.nature.com 335

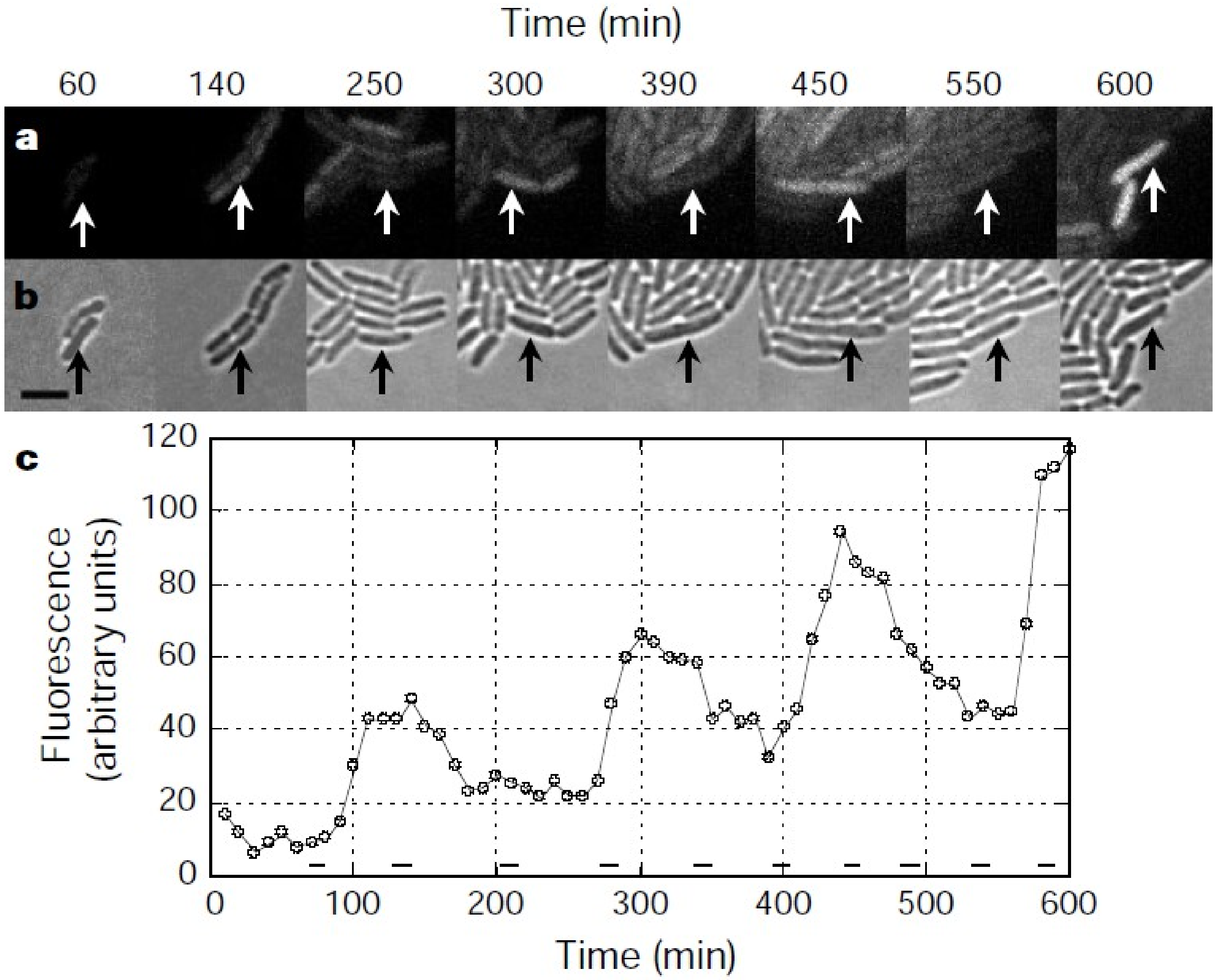


Represilatorsko vezje



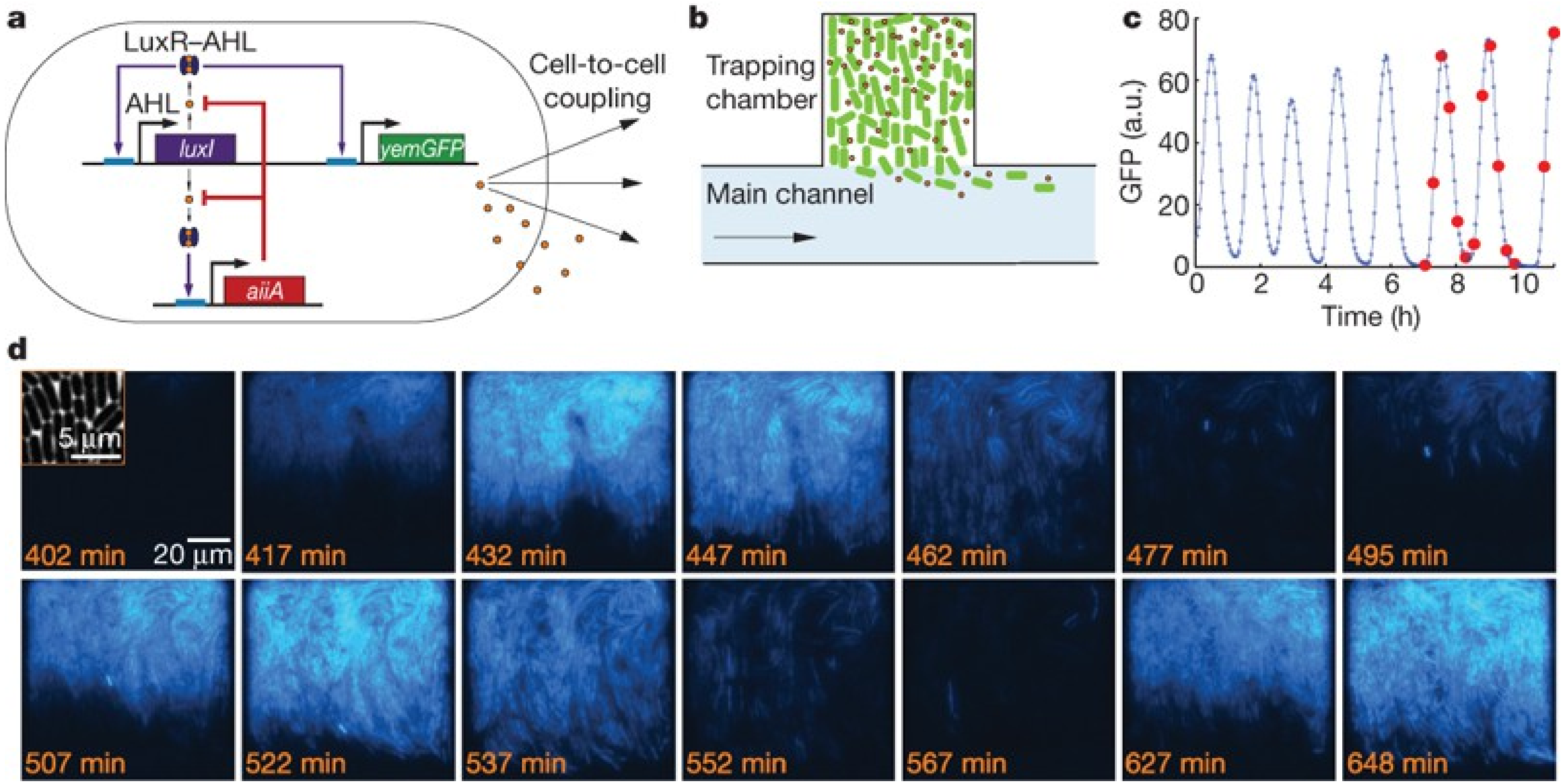


Uporabili so variante represorjev s krajšo razpolovno dobo (4 min. namesto ~60 min.), ki je bila podobna razpolovni dobi mRNA (2 min.). Prav tako so uporabili kot reporter GFP z oznako za razgradnjo.



Izmerjena perioda ~150 min. (~3 generacijski časi), vendar hčerinske celice izgubljajo sinhronizacijo po nekaj več kot 1 generacijskem času, kar predstavlja šum v vezju. Glede na to, da je bila frekvenca ves čas enaka, sistem deluje kot biološka ura.

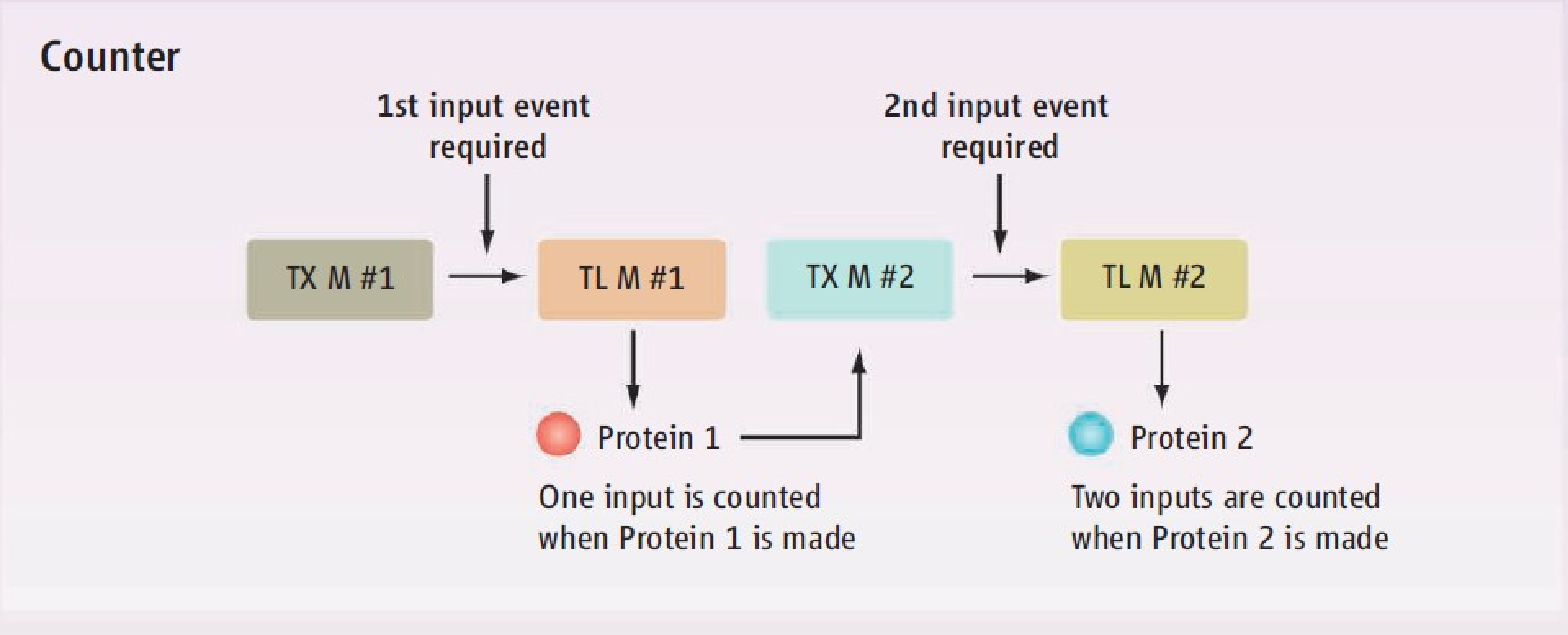
Sinteznobiološke ure



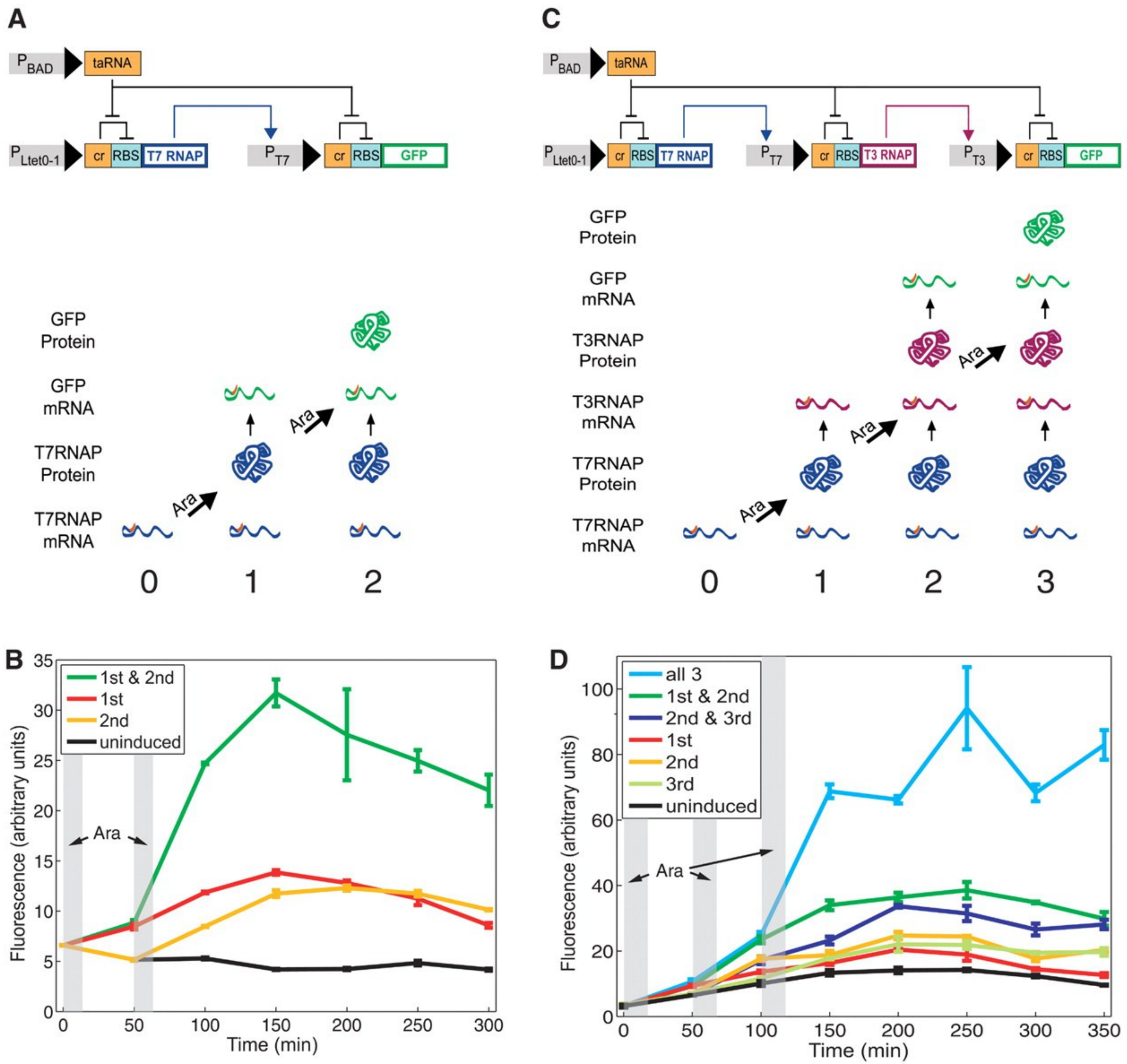
Nature **463**, 326-330 (2010)

Sinteznobiološki števci

Števci so sistemi, ki zapisujejo pojave dogodkov in morajo torej hkrati zagotavljati spominski zapis o dogodkih, ki so se zgodili v preteklosti. To je mogoče s povezovanjem generatorjev proteinov, pri čemer je sinteza drugega možna šele, če je predhodno prišlo do sinteze prvega itd. Nizanje medsebojno odvisnih generatorjev proteinov vodi do razvoja zelo kompleksnih vezij.



Science, 29 May 2009: 1156-1157.

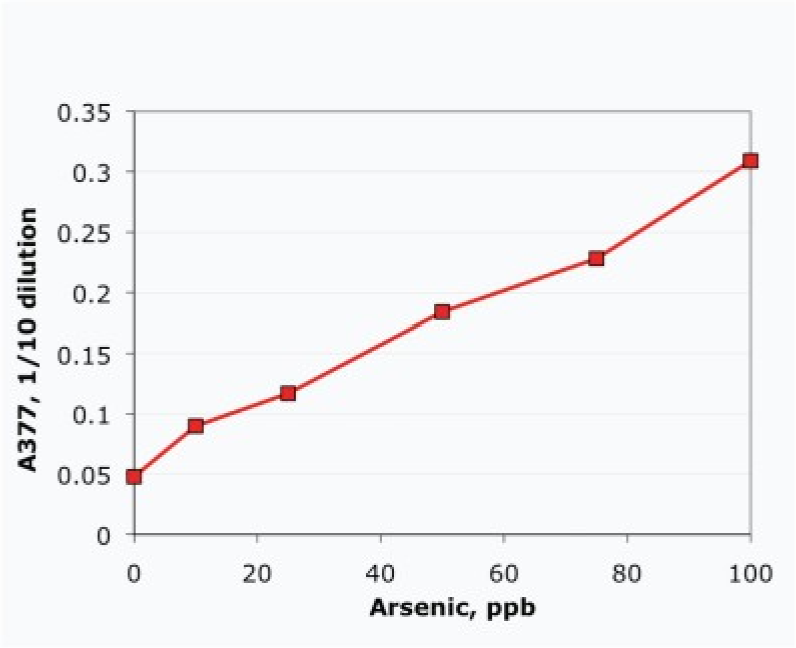


Spomin temelji na povezanih napravah, pri katerih je ključna vloga RNA-regulatorjev (riboregulators). Razvili so sistem z dvema in s tremi stanji.

Science 324(5931), 1199-1202 (2009)

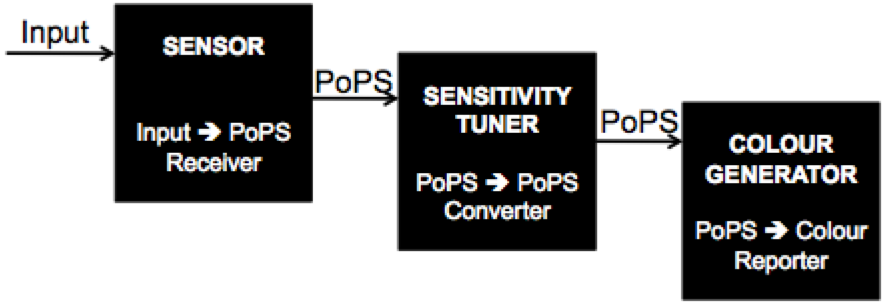
Okoljski senzorji in reporterji

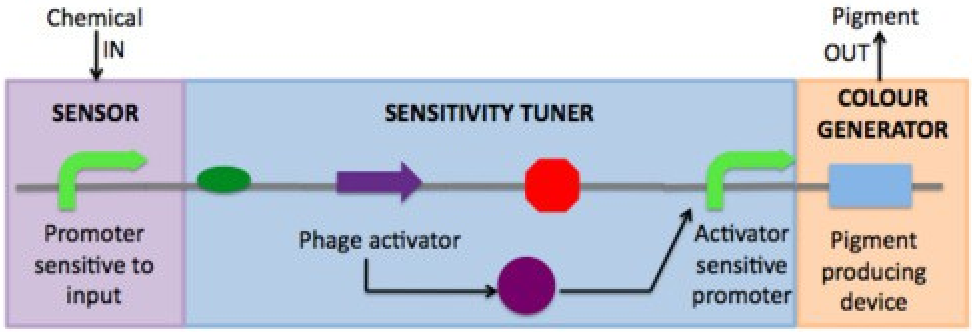
Smiselna povezava dveh naprav, oddajnika in sprejemnika, pri katerih se oddajnik odziva na okoljski signal, sprejemnik pa je hkrati generator reporterskega proteina (merljiv izhodni signal). Primer na seminarju: senzor za vanilin. Bolj uporabni so npr. senzorji za nevarne snovi v okolju, pri čemer v gojišče s senzorskim sistemom dodamo vodno raztopino okoljskega vzorca.



Detection of arsenic by B. subtilis 168/pTG262-arsR-xylE: absorbance at 377 nm vs. arsenic concentration (ppb arsenic as sodium arsenate).

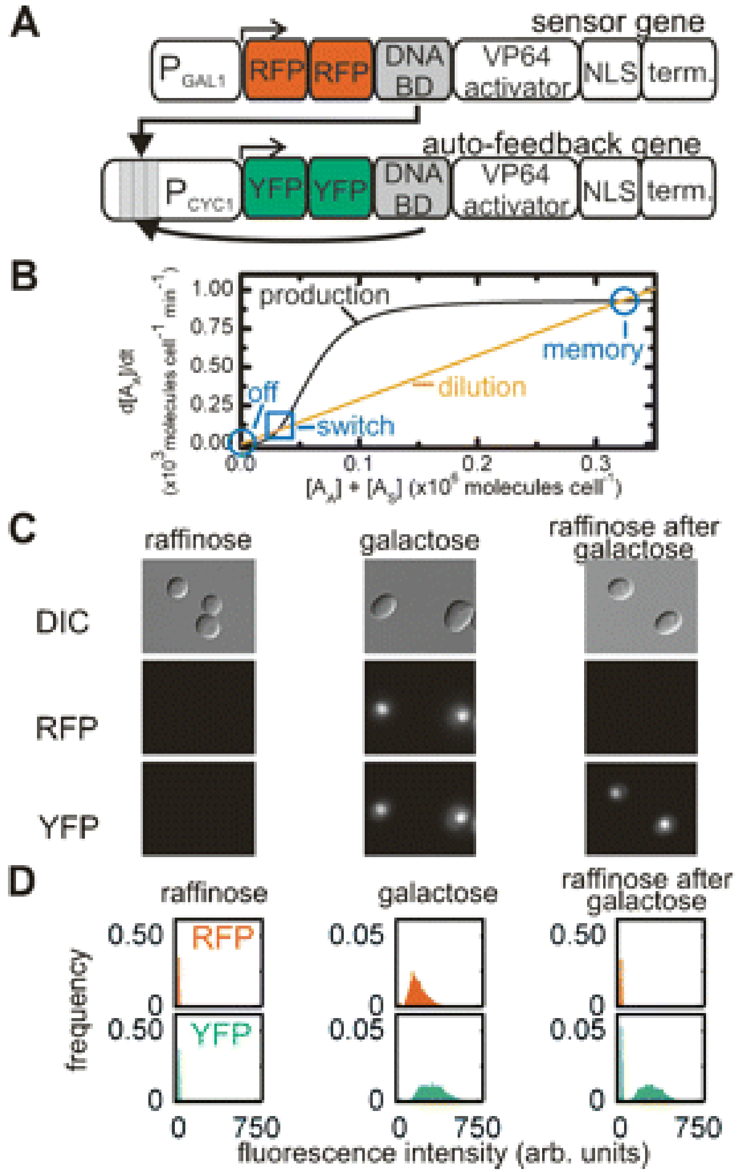
The Science and Applications of Synthetic and Systems Biology: Workshop Summary. Institute of Medicine (US) Forum on Microbial Threats. Washington (DC): National Academies Press (US); 2011.





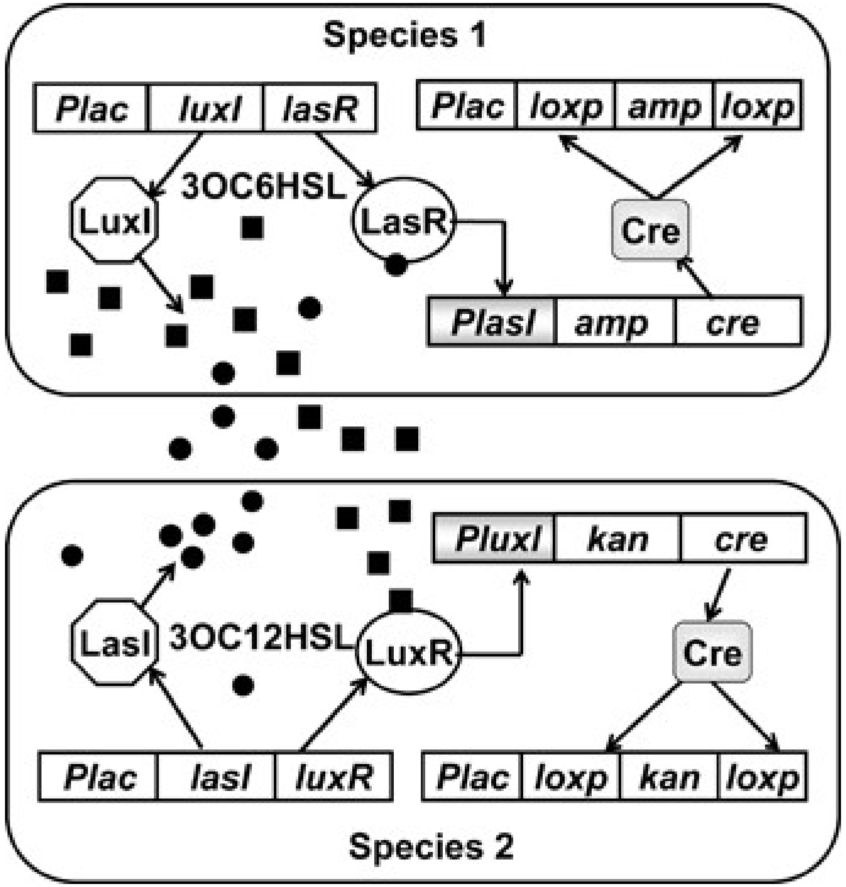
http://2009.igem.org/Team:Cambridge

Sinteznobiološki spomin



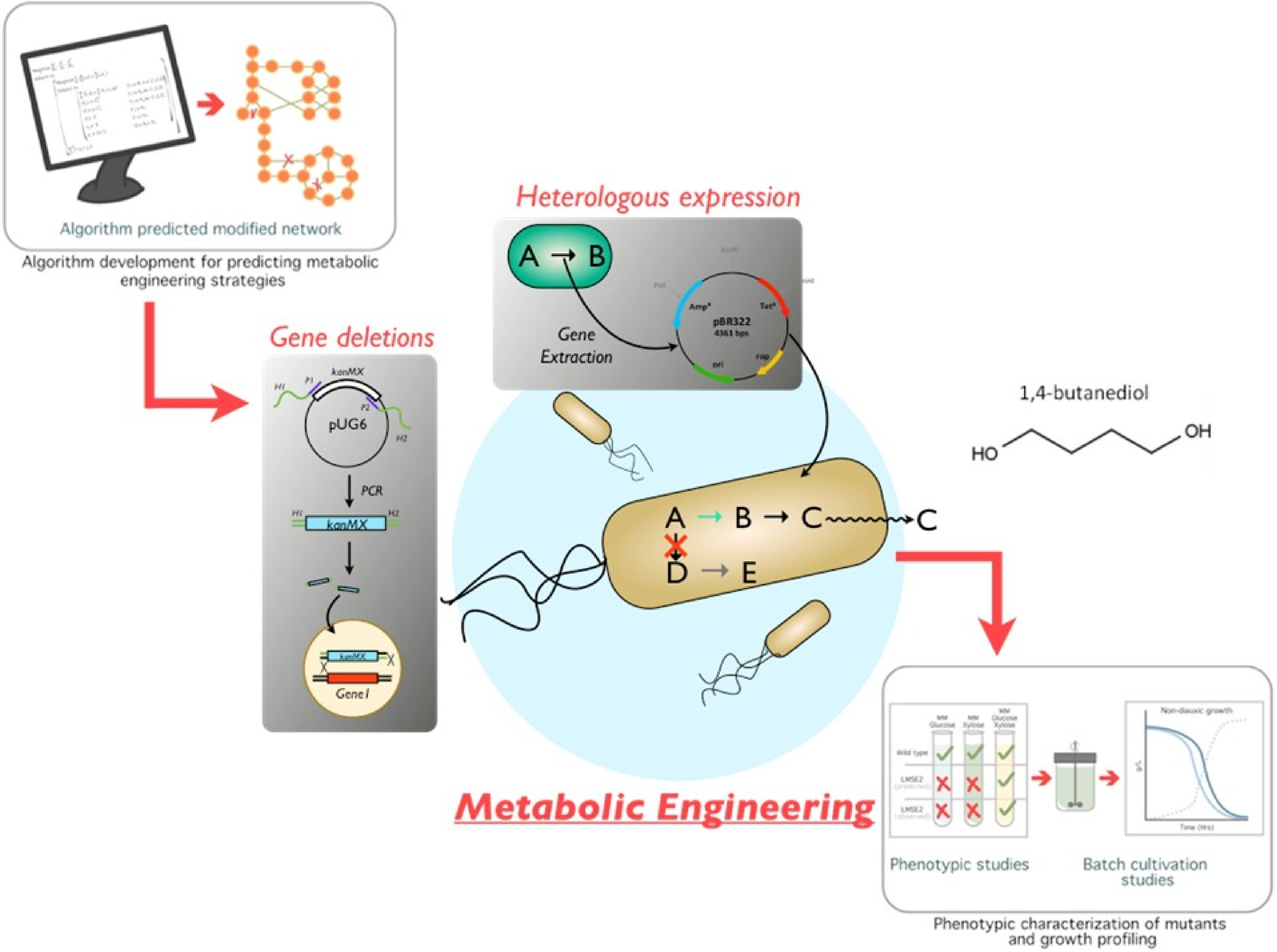
Genes & Dev. 2007. 21: 2271-2276

Sinteznobiološki ekosistem

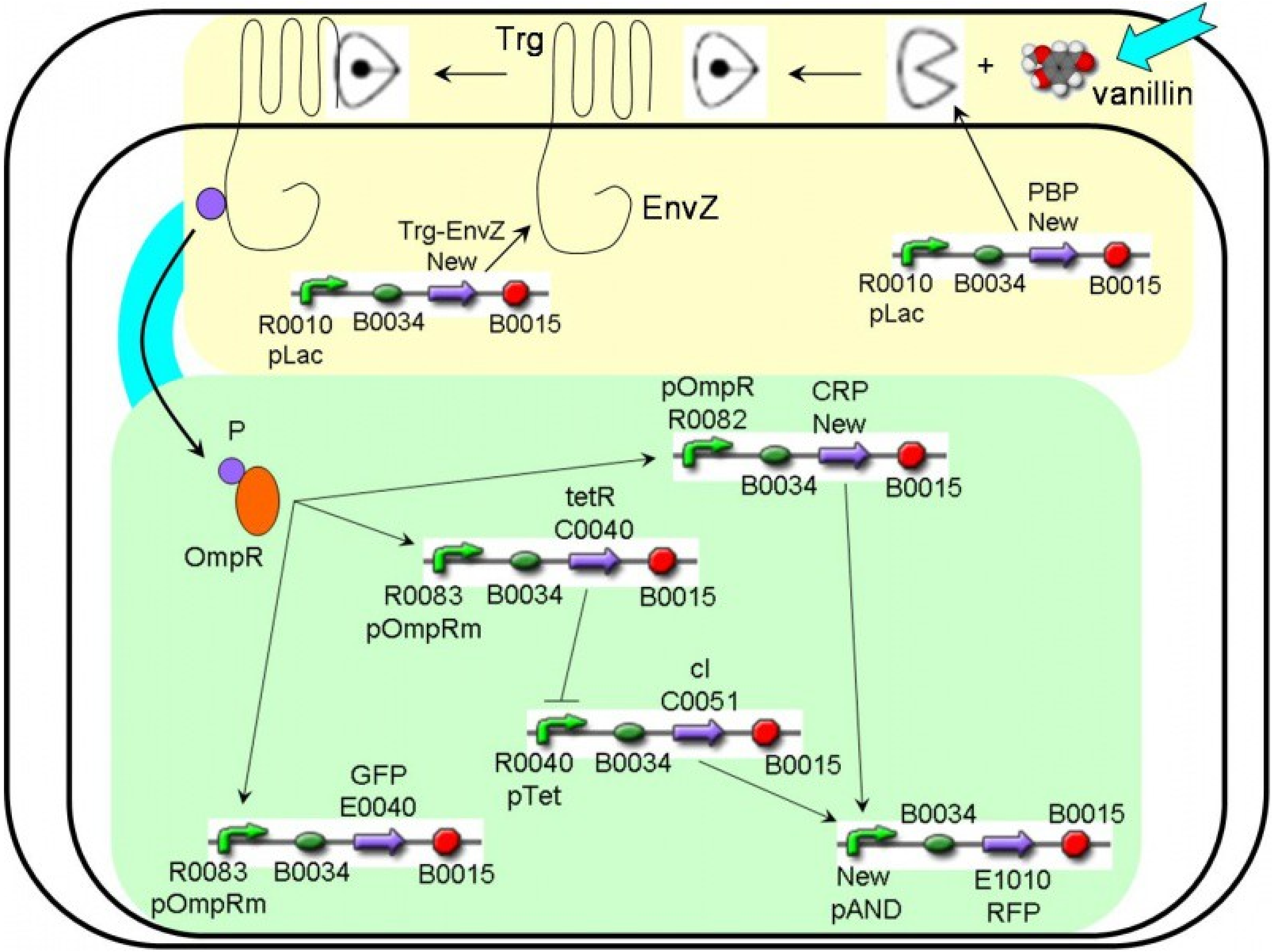


http://staff.ustc.edu.cn/~lianhong/research.html

Metabolno inženirstvo



http://www.labs.chem-eng.utoronto.ca/mahadevan/files/2010/07/MetEng.jpg



http://www.igem.upv.es/igem06/index.php?title=Main_Page