DIY Synthetic Biology @ Maker Revolution

26 Apr 2009

http://digg.com/d1nuSY
and pour into overcrowded cities. There are now ten megacities in the world with populations twice as large as New York City. Soon there will be more. Mexico City is one of them. The increase of human population is one of the causes of the migration. The other cause is the poverty and lack of jobs in the villages. Both the population explosion and the poverty must be reversed if we are to have a decent future. Many experts on population say that if we can mitigate the poverty, the population will stabilize itself, as it has done in Europe and Japan.

I am not an expert on population, so I will say no more about that. I am saying that the poverty can be reduced by a combination of solar energy, genetic engineering, and the internet. And perhaps when the poverty stops increasing, the population will stop exploding.

I have seen with my own eyes what happens to a village when you help it. This happened in a godforsaken Mexican village. How can a godforsaken Mexican village become a source of wealth? Three facts can make it possible. First, solar energy is distributed equitably over the earth. Second, genetic engineering can make solar energy usable everywhere for the local creation of wealth. Third, the internet can provide people in every village with the information and skills they need to develop their talents. The sun, the genome, and the internet can work together to bring wealth to the villages of Mexico, just as the older technology of electricity and automobiles brought wealth to the villages of England. Each of the three new technologies has essential gifts to offer.
Towards a Biotech Society

The Pace and Proliferation of Biological Technologies

ROBERT CARLSON

The advent of the home molecular biology laboratory is not far off. While there is no Star Trek “Tricorder” in sight, the physical infrastructure of molecular biology is becoming more sophisticated and less expensive every day. Automated commercial instrumentation handles an increasing fraction of laboratory tasks that were once the sole province of doctoral level researchers, reducing labor costs and increasing productivity. This technology is gradually moving into the broader marketplace as laboratories upgrade to new equipment. Older, still very powerful instruments are finding their way into wide distribution, as any cursory tour of eBay will reveal.1 These factors are contributing to a proliferation that will soon put highly capable tools in the hands of both professionals and amateurs worldwide. There are obvious short term risks from increased access to DNA synthesis and sequencing technologies, and the general improvement of technologies used in measuring and manipulating molecules will soon enable a broad and distributed enhancement in the ability to alter biological systems. The resulting potential for mischief or mistake causes understandable concern—there are already public calls by scientists and politicians alike to restrict access to certain technologies, to regulate the direction of biological research, and to censor publication of some new techniques and data. It is questionable, however, whether such efforts will increase security or benefit the public good. Proscription of information and artifacts generally leads directly to a black market that is difficult to monitor and therefore difficult to police. A superior alternate is the deliberate creation of an open and expansive research community, which may be better able to respond to crises and better able to keep track of research whether in the university or in the garage.

FACTORS DRIVING THE BIOTECH REVOLUTION

The development of powerful laboratory tools is enabling ever more sophisticated measurement of biology at the molecular level. Beyond its own experimental utility, every new measurement technique creates a new mode of interaction with biological systems. Moreover, new measurement techniques can swiftly become means to manipulate biological systems. Estimating the pace of improvement of representative technologies is one way to illustrate the rate at which our ability to interact with and manipulate biological systems is changing.

For example, chemically synthesized DNA fragments, or oligonucleotides, can be used in DNA computation, in the fabrication of gene expression arrays ("gene chips"), and to make larger constructs for genetic manipulation. Mail-order oligonucleotides were with much fanfare recently used to build a functional poliovirus genome from constituent molecules for the first time.2 The rate at which DNA synthesis capacity is changing is thus a measure of the improvement in our ability to manipulate biological systems and biological information. Similarly, improvements in DNA sequencing capabilities are a measure of our ability to read biological information; in particular the ability to proofread the results of DNA synthesis. Here I refer to such technology, whether instrument or molecule, as “biological technology.”

THE PACE OF TECHNOLOGICAL CHANGE THROUGH THE PRISM OF MOORE’S LAW

Figure 1 contains estimates of potential daily productivity of DNA synthesis and sequencing based on commercially available instruments, including the time necessary to prepare samples. There have been only a few generations of instruments—there is thus a limited amount of data for examination. These estimates are not intended to absolutely quantify a rate of change, but rather to capture the essence of the trends. Several tech-

Robert Carlson, PhD, is a Research Scientist in the Department of Electrical Engineering at the University of Washington and an Adjunct Research Fellow at the Molecular Sciences Institute in Berkeley, California.

1See http:// listings.ebay.com/pool1/listings/list/all/category 1181 I/index.html.
NOTICE PERTINENT TO THE APRIL 2002 REVISIONS OF THE NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT DNA MOLECULES

ROLES AND RESPONSIBILITIES OF THE PRINCIPAL INVESTIGATOR (PI)

Under the amendment to Section IV-B-7, which was published in the Federal Register on November 19, 2001 (66 FR 57970, specifically: 57975) and became effective on December 19, 2001, a PI may delegate the reporting responsibilities set forth in Appendix M-I-C, Reporting Requirements, to another party, with written notification of the delegation to OBA. A letter from each PI indicating to whom they have delegated the reporting requirements set forth in Appendix M-I-C must be on file with OBA. This delegation of reporting responsibility may, if appropriate, be extended to include the material submitted under Appendix M-I-A, Requirements for Protocol Submission. To that end, a letter from the PI should be submitted to OBA, either directly by the investigator or as part of the material submitted under Appendix M-I-A.

Summary of Amendments [Major Actions]

Page 10
Section I-E: Additions to General Definitions. New sections -- I-E-8, I-E-9, I-E-10
Page 36
Appendix B-1 [Lines 5-8]: New – General definition of an E. coli strain as a RG1 agent
Page 97-98
Appendix M-1-C-3: Annual Reports. New – (Harmonized submission requirements)
Page 98
Appendix M-1-C-4: Safety Reporting. New appendix – (Harmonized reporting requirements)
Page 99
Appendix M-1-C-5: Confidentiality. New appendix
Page 99
Appendix M-1-D: Safety Assessment. New appendix
Page 106
Appendix M-IV: Privacy; deleted “…and Confidentiality” from heading; clarification of protection measures.

Standardization & abstraction; SB tools; Part Collections

DIY and off-the-shelf equipment and services

framework that minimizes safety risks and repercussions for society and garage biohackers

$3000 Lab-in-a-box: Affordable tools, equipment & supplies

DIYbio Safety Guide
international Genetically Engineered Machine Competition 2002 - 2008
Teams Registered for iGEM 2008

These 85 teams are registered for iGEM 2008

<table>
<thead>
<tr>
<th>Team Name</th>
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<td>Example</td>
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Applications for these 4 teams are pending approval by iGEM Headquarters
# Teams Registered for iGEM 2009

These 107 teams are registered for iGEM 2009:

<table>
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<tr>
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iGEM

an existence proof for small team-based biotech innovation

• Resveratrol Beer
• Bacterial Photography
• Oderant synthesis (banana!)
• Arsenic & lead biosensors
• H. pylori vaccine

Opportunity for DIYbio teams to compete in 2010
HMM... ARE YOU SURE YOU UNDERSTAND ENOUGH ABOUT WHAT YOU WANT TO DO?
YOU DON'T WANT TO MAKE THINGS WORSE.

LET'S GRAB ONE!

WE'LL ONLY FIND OUT BY TRYING!

HI THERE, BUDDY.
Case Study: pUC19

One of the most popular genetic engineering tools
It’s a cloning vector - built to grab and carry DNA
Where did it come from?
Finding resistance genes

sensitive vs. resistant

live sensitive cells + resistant DNA = rare survivors
Mini-genomes as tools

How do I recover the DNA?

Put it on a plasmid - a small, self-replicating genetic device.

Modularity is your friend.
Synthetic resistance vector

- **ori**: Origin of replication, from ColE1 plasmid
- **ampR**: Gene for digesting penicillin-like drugs
Lactose operon

If lactose and no glucose...then eat lactose
Test kit for simplified *lac*

LacI binding site

Half a lactase

$P_{Lac}$
Hack it for cloning
(add restriction sites)
How can I reuse this?

We need abstractions.
And documentation.
Otherwise, we’re stuck doing all that work again.
Phage $\lambda$: Inside the black box

A complicated regulatory pathway, which has kept scientists busy for half a century at least
Figure 3. Bio-SPICE simulation of “carry” detection.
Blue line: CI activator. Purple line: Cro repressor. Black line: carry signal. Simulation was performed on a representation of the carry detector, using 15 mass action equations.
Actually not vaporware
Benchmarking: what is possible?

- One geek, one closet, one month’s rent (and roughly two months’ time)

Figure 2a. Carry detection system: theory plus prototype.
ADVENTURES IN SYNTHETIC BIOLOGY

WANNA BET?

ALL I NEED TO DO IS MAKE THEM FORM A CLOSED FILM.

DNA'S READY TO GO.

HELP ME CATCH HIM!

MEEP!

HE'S CHANGING!

GET THEM FROM THE CATLOGS.

ONE BALLOON-O-GENESIS—

AND ONE GAS-O-MATIC MODULE.

COOL, HERE THEY ARE!

NOW WHAT?

LOAD THE DNA INTO OUR LITTLE FRIEND HERE.

LOAD THE DNA INTO OUR LITTLE FRIEND HERE.

WHAT DO YOU THINK, BUDDY?
SB 101

- Abstraction
- Standards
- Synthesis

R0080

gcgtaca
caagcgagaa
atfattg
caacattttcagta
ttttatccataagattctactcgagaacctccagcttttt
aaccctctactgcttttt
“A good device standard defines sufficient information about discrete parts to allow the design of predictable complex composite systems. It also provides guidelines for the minimal characterization and manufacturing tolerances of new elements.”

- Arkin, Setting the Standard.
Figure 1  A possible hierarchy for synthetic biology is inspired by computer engineering.
Inputs and outputs to the device are defined and the component parts are no longer explicitly considered (this work).

Engineers reimplement the receiver using BioBrick standard biological parts, thereby enabling ready reuse of the device (this work).

Engineers construct a proof-of-principle device using a subset of the natural quorum-sensing regulatory elements.

The mechanisms and genetic sequences necessary for bacterial quorum-sensing are shared via peer-reviewed publications. Such publications are currently the major channel of communication between biologists and device engineers.

Biologists elucidate the minimal set of genetic elements encoding quorum-sensing regulated bioluminescence (the lux genes of V. fischeri).

Scientists identify a bioluminescent bacteria (V. fischeri) that colonizes the light organ of a squid (Euprymna scolopes). Bioluminescence is regulated via quorum-sensing (cell-cell communication) between individual V. fischeri bacteria.

Device engineers

Biologists
The receiver is used in systems in which the device characteristics fulfill the system specification (http://partsregistry.org/).

The behavior of the receiver is characterized to produce a device datasheet. The datasheet forms the interface between device and system engineers, eliminating the need for extensive interaction between the two groups (this work).

Inputs and outputs to the device are defined and the component parts are no longer explicitly considered (this work).

Engineers reimplement the receiver using BioBrick standard biological parts, thereby enabling ready reuse of the device (this work).

Engineers construct a proof-of-principle device using a subset of the natural quorum-sensing regulatory elements14,15,25–29.
The Problem

We Expect This

We Build This

transistor, v1
The Problem

We Build This

We Expect This

**transistor, v1**
BBa_F2620
3OC6-HSL → PoPS Receiver

Mechanism & Function
A transcription factor (LuxR) that is active in the presence of a cell-cell signaling molecule (3OC6-HSL) is controlled by a regulated operator (P_{LuxR}). Device input is 3OC6-HSL. Device output is PoPS from a LuxR-regulated operator. If used in a cell containing TerR then a second input such as ac can be used to produce a Boolean AND function.

Static Performance

\[ P_{\text{out}} = \frac{P_{\text{max}} [3\text{OC}_6\text{HSL}]}{K + [3\text{OC}_6\text{HSL}]} \]

\[ P_{\text{max}} = 6.6 \text{ PoPS cell}^{-1} \]

\[ K = 1.5 \times 10^{-9} \text{ M 3OC}_6\text{HSL} \]

\[ n = 1.6 \]

Dynamic Performance

BBA_F2620 Response Time: <1 min
BBA_T9002 Response Time: >61 min
Inputs: 0 M (Low), 1E-07 M (High) 3OC6-HSL

Input Compatibility

Part Compatibility (qualitative)
Chassis: MC4100, MG1655, and DH5α
Plasmids: pSB3K3 and pSB1A2
Devices: E0240, E0430 and E0434

Transcriptional Output Demand (low/high input)
Nucleotides: 0 / 6xNt nucleotides cell\(^{-1}\) s\(^{-1}\)
Polymerases: 0 / 1.5E-1xNt RNAP cell\(^{-1}\)
(\(Nt = \) downstream transcript length)

Reliability

Genetic: >92>56 culture doublings
Performance: >92>56 culture doublings
(low/high input during propagation)

Conditions (abridged)
Output: PoPS measured via BBA_E0240
Culture: Supplemented M9, 37°C
Plasmid: pSB3K3
Chassis: MG1655
*Equipment: PE Victor3 multi-well fluorimeter
**Equipment: BD FACScan cytometer
BBa_J04450
Red Fluorescent Protein Generator

Mechanism & Function
The expression of the gene for mRFP1 is controlled by a regulated operator (PLac). If used in a cell containing lac repressor (LacI) then an input such as IPTG can be used to modulate the expression of mRFP1.

Usage
Cells expressing mRFP1 are visibly red under white light and for optimal fluorescence detection should be excited at ~564nm and detected with a 600nm long pass filter.

Origin
mRFP1 is an engineered variant of dsRED fluorescent protein originally isolated in Discosoma sp. (Mushroom Coral)
BBa_J45200
Banana Odor Generator

Mechanism & Function
The banana odor generator (BBa_J45200) catalyzes the conversion of the precursor isoamyl alcohol to the odor isoamyl acetate that has a banana smell. The biosynthetic device is composed of two transcriptional devices: a constitutive transcription source (BBa_R0040) and an odor enzyme generator (BBa_J45199). Odor enzyme generators produce as output an enzyme that catalyzes production of an odor from a chemical precursor.

Activity

Isoamyl acetate concentration increases with cell density

Usage

Cells expressing ATF1 should be grown in the presence of isoamyl alcohol (5mM).

Chromatography

High levels of isoamyl acetate are produced when the precursor isoamyl alcohol is added to the culture medium (A), unless the cellular chassis (E. coli strain TOP10) does not contain J45200 (B). The retention time of the isoamyl acetate peak from J45200 is identical to that of the pure isoamyl acetate standard (C). Most E. coli strains produce indole. Octyl acetate was used as an internal standard for all samples containing isoamyl acetate.
BBa_G10001
Visible Light Generator

Mechanism & Function
The expression of the Lux operon is controlled by a regulated operator (P_Lac). If used in a cell containing lac repressor (LacI) then an input such as IPTG can be used to modulate the expression of the Lux operon. When expressed this operon produces the necessary enzymes to generate the fatty aldehyde substrates as well as the luciferase enzyme that converts luciferin to visible light.

Bacterial luciferin is a reduced riboflavin phosphate (FMNH₂, above) which is oxidized in association with a long-chain aldehyde, oxygen, and a luciferase to produce visible light.

The fatty acid reductase enzyme complex is needed to recycle the fatty aldehyde substrate in the reaction and luciferase is required to catalyze the reaction. No other exogenous enzymes are necessary since FMNH₂ is provided by the native electron transport chain in E. coli.

Usage
Cells expressing the lux operon are visible in low light in liquid culture or as colonies.

Origin
The Lux operon was isolated from Vibrio fischeri a bacteria found predominantly in symbiosis with marine animals such as the bobtail squid (above).
BioBricks

“I was surprised to find that molecular biologists were spending something like 50% of their time at the bench just on manipulating DNA to build particular constructs” – Tom Knight
BioBrick Standard Assembly (BBa)
A Catalog
- 6491 parts defined
- 1880 physically available
-July 2008
Parts Registry kits
RANDOM DATA
By Robert Reiling

Computer clubs continue to form around the country. E. Brooner would like to have material to help him get started with the "Flathead Computer Society" in the Kalispell area. His address is P.O. Box 236, Lakeside, Montana 59922.

Did you see the SOL terminal demonstrated by Bob Marsh at the Sept. 1st meeting? An excellent design that will interest hobbyists and commercial users alike. It's available from Processor Technology, 6209 Hollis St., Emeryville, CA 94608. Write them for prices and specifications.

The OSI Systems Journal has been sent to all OSI customers (free—at least for the time being). It's a bimonthly magazine with plans to go monthly in the future. There are 28 pages in the first issue (August 1976, Vol. 1, No. 1) with a hardware feature covering the OSI 440 Video Graphics System and software, features concerning Tiny BASIC for the 6500 and a Graphics Editor for the 6502. It also includes OSI product and software catalog data. The BASIC is, of course, the SK Tiny BASIC developed by Tom Pittman. Many of you have met Tom at the Homebrew computer Club meetings. The OSI Systems Journal is a good way to learn more about the OSI computer hardware and software along with helpful user information. The contact address is The OSI Systems Journal, P.O. Box 134, Hiram, Ohio 44234.

KIM-1 users now have a newsletter. Eric Behnke is producing the newsletter every 3-5 weeks, MOS Technology, Inc. helped get it started by sending copies to all known KIM owners. The user group, however, is independent of MOS Technology, Inc. The newsletter is devoted to KIM-1 support. Subscriptions are $5.00 for the next six issues. Contact "KIM-1 User Notes," c/o Eric Behnke, Apt. 307, 456 Broadview Ed., Parma, Ohio 44134.

The BAMUG club has a new contact address. It is BAMUG, c/o Timothy O'Hare, 1211 Santa Clara Ave., Alameda, CA 94501. Write Timothy for club information. I suggest you include a stamped, self-addressed envelope.

Beware of board snatchers! Glenn Ewing reports 11 boards were taken out of his IMSAI computer. The boards are: MPU, 4 RAM-4's, SID-2, P16-4, PIC-8, PROM-4, IFM and PIB. Glenn suspects you consider providing good security for your computer and associated equipment. In his case the computer was in a locked office which was burglarized. In the event you have information on the above boards, write Lt. Glenn Ewing, Code 0261, Naval Post Graduate School, Monterey, CA 93940.

For family and friends of people who always wanted to know about computers, but didn't want to ask them, four easy-going classes are available starting Oct. 18th on Tuesdays from 7 to 9 p.m. You can learn how computers work and what they can and can't do. You will also have some of the jargon deciphered, see what you can do with a computer, play some games and learn to program. The cost is $25. Contact the Old Macintosh Center, 1919 Menalto Ave., Menlo Park, CA 94025, phone (415) 325-4444.

A call for papers in personal computing has been issued by the 1977 National Computer Conference. The conference is scheduled for June 13-16, 1977. I have a few copies of the guidelines if you would like to submit a paper.

The First West Coast Computer Faire will be held April 16 and 17, 1977 at the San Francisco Civic Auditorium. This fair is shaping up rapidly. If you would like to lead a conference or participate in a conference section, please contact me. More information about the Faire is in the accompanying article.

THE FIRST WEST COAST COMPUTER FAIRE
A Call For Papers And Participation

The San Francisco Bay Area is finally going to have a major conference and exhibition exclusively concerned with personal and home computing—the First West Coast Computer Faire. And, it promises to be a massive one! It will take place in the largest convention facility in Northern California: The Civic Auditorium in San Francisco. It will be a two-and-a-half day affair, starting on Friday evening and running through Sunday, evening, April 15-17.

It is being sponsored by a number of local and regional hobbyist clubs, educational organizations and professional groups. These include:

- The two largest amateur computer organizations in the United States—the Homebrew Computer Club and the Southern California Computer Society
- Both of the Bay Area chapters of the Association Of Computing Machinery—the San Francisco Chapter and the Golden Gate Chapter
- Stanford University's Electrical Engineering Department
Local Groups

There are DIYbioers all over the globe! See if there is a meetup near you on the map below. If there is not, add your location and your contact information to the map, so others can get in touch with you - just don’t forget to update it once you start a regular meetup!

View a larger map, or add yourself or your group to the map. You’ll need to sign into your Google account in order to add a new point. It’s a little unclear, so here’s a screenshot of adding a new point.

DIYbio is an organization that aims to help make biology a worthwhile pursuit for citizen scientists, amateur biologists, and DIY biological engineers who value openness and safety. This will require mechanisms for amateurs to increase their knowledge and skills, access to a community of experts, the development of a code of ethics, responsible oversight, and leadership on issues that are unique to doing biology outside of traditional professional settings.

recent comments

- **Ana (Quo):** Hola Fernando, Soy una redactora de la revista Quo y estoy ...
- **Nick See Weinberg:** Would someone please add CodeCon to the DIYbio G-Cal? Thanks...
- **Charles Stone:** Hey everyone!
diybio is naturalism

macroscopic to microscopic
diybio is engineering

- graft a hybrid cranberry-apple tree
  or
- add resveratrol production to yeast
  (healthier beer!)
diybio is more

- hardware
- informatics
- art

Alba, the fluorescent bunny (Eduardo Kac, 2000)

working on the SmartLab table, Dec 08
SmartLab Project
multitouch augmented reality lab bench for recording and teaching molecular biology
self-genotyping
Is Kay a carrier of hemochromotosis on her 6th chromosome?

1. Allele-specific PCR at home
2. Mail sample for sequencing
bioweathermaps

flashmob + science = distributing tracking of bacterial populations across cities
Gel Box 2.0
for sorting DNA by size

the best commercial boxes cost > $1200.
build an open source alternative for ~$100
Gel Box 2.0 v0.1 circa feb 09 ($150)
GloGurt & Melaminometer

lactobacillus “hello world” + biosensing melamine
$3000 lab
at a boston-based coworking space

testing diy hardware
testing diy protocols
(DNA extraction, transformation, culturing, gels, PCR
demonstrating it works
Acinetobacter Baylyi
ADP1

- gram-negative
- genome sequenced
- naturally competent!
Acinetobacter Baylyi ADP1

- gram-negative
- genome sequenced
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Acinetobacter Baylyi ADP1

- gram-negative
- genome sequenced
- naturally competent!

...for genetic analysis and genome engineering

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Acinetobacter Baylyi ADP1 is a naturally transmissible gram-negative bacterium with simple cultivation requirements, a prototrophic metabolism and a compact genome of 3.7 Mb which has recently been sequenced. Wild-type ADP1 can be genetically manipulated by the direct addition of linear DNA constructs to the organism for the automation of complex strain construction. Here, we demonstrate the flexibility and versatility of ADP1 as a genetic model through the construction of a broad variety of mutants. These include marked and unmarked insertions and deletions were achieved by the same process of splicing PCR, direct transformation of growing cultures and plating on selective media. The simplicity of these methods make ADP1 accessible to laboratories with minimal microbial genetics expertise and very little equipment. They are also compatible with complete automation of genetic analysis and engineering protocols.

INTRODUCTION

Manipulative genetics is a primary method in both descriptive and constructive molecular biological investigations. Whole-genome analysis of gene deletions and complementary replacements. The replacement of wild-type genes with alternate alleles is also used to identify the function of specific features of genes and proteins. Biochemical analysis of naturally expressed proteins requires the addition of sequences coding for binding tags to chromosomal genes. The development of new biochemical pathways for biomedical and biotechnological industries requires insertion and deletion of many genes in the same strain, and often alteration of those genes in the process. These uses of manipulative genetics are essential to the current progress of biological research, and often determine the cost and efficiency of the experimental process.

Many fields of biology have either chosen or happened to mycologists, and offers the same to mycologists, and offers the same. Biochemistry and molecular biology of plant pathogens has been the primary genetic model since the first use of transgenic plants in the laboratory. Agrobacterium/Arabidopsis transformation capabilities. This must be overcome by the addition of recombination functions from other organisms and the simultaneous deletion or inhibition of native nuclease activities that prevent recombination through direct destruction of the genetic material. This is achieved by the use of mineral oil, which is relatively resistant to genetic manipulation. The archetypal model organism for all genetics, Escherichia coli, is relatively resistant to genetic manipulation. Functional studies on bacterial metabolism often offer the best experimental information. The Gram-negative bacteria, for example, offer a reasonable approach to study Saccharomyces cerevisiae metabolism. However, a robust system is required. E.coli, by virtue of its abilities to functional studies on bacterial metabolism, offers a robust system.

...
Fig. 2. Schematic representation of different models proposed to explain the mechanism and/or energisation of DNA uptake. (A) The hydrolysis of one strand provides the energy to drive the uptake of the complementary strand. (B) DNA is taken up electroneutrally in symport with protons and is thus driven by the transmembrane pH gradient. Hypothetically, DNA may be extruded electrogenically using the existing transmembrane electrical potential. (C) A pHb cylinder filled with a polyphosphate core [poly(P)-core] and stabilised by Ca$^{2+}$ may constitute a DNA transporter. The DNA is bound to the polyphosphate core, which is subsequently degraded allowing the DNA to be internalised. (D) Analogous to glucose-6-phosphate (G-6-P) transport by the E. coli UhpT transporter, negatively charged ds DNA is transported towards the cytoplasm, encounters an endonuclease and is hydrolysed, resulting in monovalent negatively charged ss DNA and nt. The monovalent charged ss DNA and nt liberate a proton into the more alkaline cytoplasm and the now divalent negatively charged nt are expelled. This results in an anion exchange reaction dependent on proton cycling. (E) ds DNA is hydrolysed by an endonuclease, the nt are liberated, and the ss DNA is internalised via an ATP-dependent uptake system.
expression of the tkt gene, together with supplemen-
tary thymidine, is essential in the absence of thyA activity as
Acinetobacter sp. ADP1 does not contain an endogenous thyA allele.

DISCUSSION

Acinetobacter ADP1 provides a remarkably simple, inexpensive and robust model system for genetic manipulation. Most of the experiments were done using an array of backbone plasmids that were functional in ADP1. These markers were used to build positive/negative selection cassettes, which were in turn used to efficiently construct a wide variety of mutations including gene disruptions and deletions, expressed chromo-
somal insertions, targeted chromosomal genes and various com-
binations of these types of mutations. Moving mutations from one strain to another was as straightforward as amplifying the donor strain and inoculating a growing culture of the recipient strain with the PCR product, or even simply transforming with purified genomic DNA from another strain. All constructions shown here utilized approximately 1 kb flanking regions to specifically integrate constructs into the ADP1 genome. Attempts to use shorter flanks were generally unsuccessful. Splitting PCRs with shorter flanks resulted in high yields of products, but no trans-
formants were recovered in selection. This limitation may be
due to the minimal volume of our transformations.

The techniques used in this paper were reiterative and highly similar. All manipulations aside from the initial cassette constructions were performed using splicing PCR and selective plating only. Primers were chosen using very simple rules based on melting temperature, GC content, potential inter-
primer misextension and position with regard to the affected ORF. All PCRs were performed in identical conditions. Furthermore, all direct manipulations of ADP1 cells were performed in minimal volumes similar to those found in stand-
ard 96-well plate formats. The high rate of success under these conditions suggests that this system could be readily adapted to an automated platform, allowing for all steps to be automated robotically. Similarly, the simplicity of the ADP1 genetic engineering protocols developed here should allow this system to be adopted by both training institutions and laboratories that have a need for an inexpensive and user-
friendly method for generating genetically manipulated strains. The Acinetobacter constructions described here required only a PCR machine, incubators and access to old-
gene synthesis. It is notable that the majority of all constructions, including the cassette constructions, antibiotic tests, resistance allele tests and associated design efforts, were achieved by one researcher (D. Metzgar), with very little previous genetic manipulation experience in the course of one year. Attempts by other researchers to use the same system were generally equally successful, but it was noted that suc-
cess was dependent on careful and consistent choice of primer sequences (see Materials and Methods).

Together, the paired traits of natural competence and recombination allow for rapid production of genetically engi-
nereed strains. Replacement of existing genetic models with Acinetobacter ADP1 should be straightforward, as ADP1 pre-
sented no particular challenges with respect to culturing con-
ditions, bioinformatic prediction of metabolic pathways, or

mutational stability in culture, even in conditions that were optimized for E.coli rather than Acinetobacter. In the short
time during which this model has been under development in our laboratory, it has allowed us to test a number of biological questions (27) in a much more efficient manner than would have been possible with previously utilized model organisms.

ACKNOWLEDGEMENTS

We thank L. Nicholas Ornston for critical reading of this work, and Integrated Genomics for access to ERGO. This work was supported by National Institute of Health Grant GM23562, National Science Foundation Grant MCB-0128901 and a fel-
lowship from the National Foundation for Cancer Research.

REFERENCES

• 2.1 - j04550 - 2.2: fail (RFP)
• 2.1 - p1003 - 2.2: worked (Kan resistance)

not all E. coli promoters compatible?
Part:BBa_J04450

Designed by Tamar Ocole  Group: iGEM_Davidson

RFP Coding Device switched on by IPTG

LacI
R0010  B0034  E1010  B0015

Constitutive RFP device. The colonies are clearly red in color under natural light after about 18 hours. Smaller colonies are visibly red under UV. The RFP part does not contain a degradation tag and the RBS is strong.

Pictures

BBa_J04450 visualized under non-UV lightbox
BBa_J04450 visualized under 254nm wavelength UV lightbox
BBa_J04450 Colonies
kanamycin resistance cassette
Kanamycin resistance cassette including promoter and coding sequence. It lacks a terminator.

Usage and Biology
- Used for modular BioBrick vector construction using BioBrick base vector BBa_I51020.

References
Engineering BioBrick vectors from BioBrick parts
Journal of Biological Engineering, 2008 Apr 14:25
Reshma Shetty, Drew Endy, Tom Knight
URL
GenBank EU496093

Sequence and Features

Format: Subparts | Ruler | SS | DS
Search: | Length: 987 bp | Context: Part only | Got selected sequence

kanamycin resistance
• 2.1 - j04550 - 2.2: fail (RFP)
• 2.1 - p1003 - 2.2: worked (Kan resistance)

Genes work; Promoters didn’t?

NEXT

• RFP ADP1!

• ID promoters that work in ADP1

• minimize 2.1 & 2.2
  • 1000bp -> 100bp

• or find ways of using plasmids
  • don’t integrate into genome
  • circular, not linear
  • easier to isolate (miniprep)
Safety

"Thou shalt not design, nor build, nor isolate, nor modify, nor grow, nor release any self replicating organism, with the intent of causing harm?"

-Roger Brent

DIYbio creed:

Safe as an undergrad lab or better: safe enough to eat
Need safety norms before we can expect broad-scale innovation must preempt stupidity

social hack: what is the 1-5 year strategy for DIYbio as a movement to be successful?

safety working group: safety@diybio.org
get involved?

Periodic meetups in San Francisco, Boston, NYC, Seattle and Chicago - email diybio@googlegroups.com

visit diybio.org for more info
5-min DNA extraction in a shot glass

just add:
saliva + soap + salt + 160 proof rum
DIYbio in space

- $1000 DIY cubesat
- launching in 6 mo
- altoids-size DIYbio payload
- 100-200g
- 5v, 100 mA
- -80c to 100c
- DTMF downlink

Software interchange formats for libraries and modeling.
The PoBoL project aims to define an RDF-based data exchange standard for standard biological parts, BioBricks. The goals are to:

- Capture the minimal information needed to describe a BioBrick.
- Allow the connection of additional data to BioBricks.
- Remain open for extension and interlinking.

“PoBoL” stands for Provisional BioBrick Language. “Pobol” is also Welsh for “people”, which reflects our desire for a community-driven format. Find out more at our:

- OpenWetWare Wiki page
- Google Group for discussion
- Google Code project for specifications and code
BioBrickFamily
- Name
- Description
- Any parent families

BioBrickFormat
- Prefix
- Suffix
- Self scar
- Description

BioBrick
- Format
- Part sequence
- Family
- Description
- Subparts (if it is composite)
- Is it circular? (for vectors)

DNA
- Vector
- Insert

Sample
- DNA
- Label
:BBa_P1010
  rdf:type bbf:BiobrickBasic ;
  bbf:author usr:Leon_Chan ;
  bbf:date "2008-05-31"^^xsd:date ;
  bbf:format bbx:BBa ;
  bbf:longDescription "negative selection marker for construction plasmids. Only certain E.coli strains (DB3.1) can survive the expression of this marker."^^xsd:string ;
  bbf:partSequence "actggctgttgtata.......atccacgcgt"^^xsd:string ;
  bbf:shortDescription "cccdB death cassette"^^xsd:string .
Athena
Basic Features

- Construct a module from the selected items
- Insert genes, promoters/operator, terminators, rbs, molecular species, etc..
- Make reactions with reactants being converted to products
- Add a modifier or a reaction or a transcription factor to a promoter
- Use this to connect two modules by indicating where they overlapping
- Simulate a model or visualize rate equations
- Get a list of properties for the selected item(s)
- Interface to the R language
- View sequence of selected item(s)
- Automatic transcription rate derivation
- Get a list of properties for the selected item(s)
Looking at the complete model

The Module Viewer shows the rates of each reaction, concentration of all molecules, PoPS across each part, and parameters of the module.

The module viewer updates when you select different modules. If you select nothing, everything will be shown.
Property Viewers

As you select different items on the screen, the viewer will be updated. For example, when a part is selected, the Part Viewer replaces the Module Viewer.

Selected item influences the properties shown on the right.
Connecting Parts (DNA stands)

Connecting two parts together:

Option 1: bring one part close enough to the other, so that they touch

Option 2: Click and drag the red circle so that the red line meets the other part
Making a Module

Select all the items you want to construct the module with, and click “Make Module”. Other options:

Option 1) Ctrl+M

Option 2) Right click on selected items, and select “Make Module” from Module Tool menu
Simulating a Module

Only the selected Module is simulated so that you can analyze different modules independently.

Opens the Simulation window.

Use the Rate Functions tab to see how different variables affect the different rates.
Connecting two or more modules

S4 and Node0 are now the same molecule, but the separate modules have not been altered.

Link two modules together using this button.

Observe that there is no Node0 in the module viewer.
BioJADE
How to use this site:

GenoCAD™ is an experimental tool allowing you to build and verify complex genetic constructs derived from a library of standard genetic parts.

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contact information

http://genocad.org