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Illustrator: Rebecca Demarest

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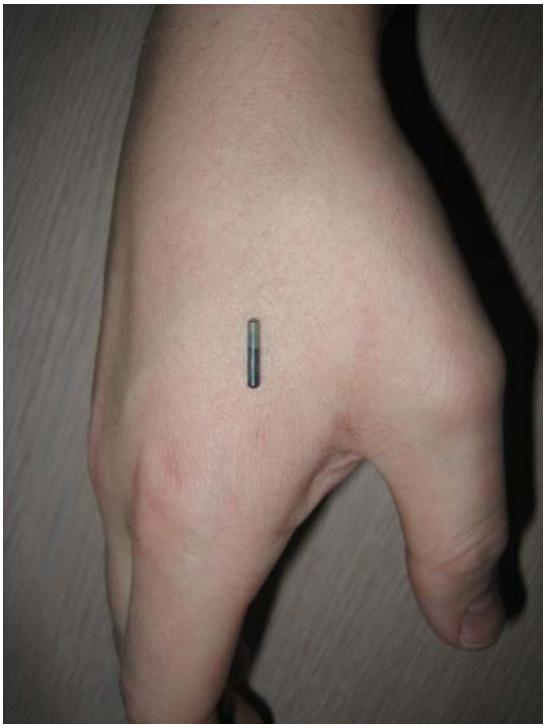
Implanting Evolution

Technology for Human Enhancement

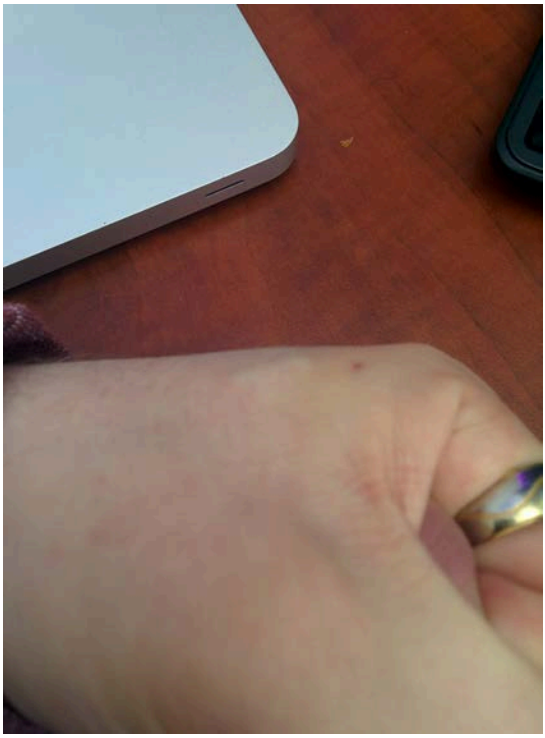
Mitch "Rez" Muenster

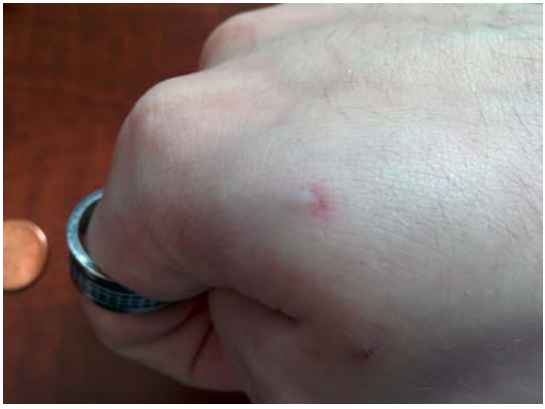
"So what is that in your hand?" I get asked at a local meeting for hackers, programmers, and other likeminded geniuses. "My phone?" I ask the observer. "No, your hand, that weird lump by your thumb," he replies. "Oh, that's my implant. It's an NFC tag." I get a confused look back. You know those white tags you see or the new tap-to-pay cards, they all use NFC. I currently use it to unlock my phone, but I'm working on getting it to do more. "Why would you put tech in yourself?"

I get questions like this rather frequently. The concept of humans implanted with technology is not a new concept, but it is really found only in sci-fi. There are those who currently implant themselves with technology; they are called grinders and are part of a subculture of biohacking. Grinders believe that the next step in human evolution will come from technology; however, we are not willing to wait for the slow, drawn-out process of the corporate machine to invent this implantable tech. Even if a medical or giant tech corporation comes out with implantable tech, it will most likely be designed to turn a profit rather than with the idea of human advancement in mind. It is likely to be limited rather than achieving its full potential and will probably not be shared with the world to allow for enhancements and code improvements.



These reasons and many others set grinders and their implantable tech apart from the medical corporations that put pacemakers, artificial bones, and splints into people's bodies. While insulin pumps and pacemakers are implanted tech, these are cases where the implanted technology means life or death, rather than enhancing the body or providing convenience. In the case of my NFC (and RFID) implant, it can be used in many ways; the more common uses would be tasks like unlocking and starting your car or motorcycle, unlocking your phone, turning off the lights in a room, booting up your computer, and being the decryption key for an external hard drive or secure messages. You can even use it to carry your access to bitcoins with you as you would carry a credit card.





There is another distinct advantage to using RFID and NFC implants versus some of the other technology used in the market today. RFID and NFC do not need to store power in a battery, and therefore it is much safer to implant because there is no risk of battery acid getting into the body and bloodstream. The implant can be made so that it is not discoverable when walking through a metal detector, and

since it is a passive tech, there is no inherent security danger to having an implant on you while you are on a plane or in an area where security is a top priority. Also, unlike your cellphone, which gives away your location via cell towers and GPS, NFC and RFID have a readable range of 5 to 10 mm. So unless you are standing right next to an NFC or RFD implant, you can't obtain its location.

All the technical talk aside, having an implant has greatly changed the way I go about my day. Its most common noticeable use is with my mobile phone and an app called TapUnlock. I use it to unlock my phone via NFC. You scan the tag you want to use, and it stores the UID. That tag is now registered to unlock your phone. There is no longer any need for me to use a PIN, pattern, or swipe to unlock. At the same time, I can change out what is actually coded on the tag. For instance, when visiting user groups or conventions, I have my tag programmed to be a vCard so that I may share my contact info with those I meet and carry around fewer business cards. While at home, I have the tag set to turn the lights on and off in my room via Philips Hue connected bulbs. My RFID implant is currently being used to unlock the doors at work, unlock my computer, and there are future plans for it to unlock and lock my car door (in theory, one could also start and turn off the car, though to do so is a bit more complex than locking a car door). There are also various other situations for which one can program the NFC or RFID tags—the options are limited only by the developer.

There are many other implant and grinder projects in development by grinders and DIY experts around the world. Experts include Kevin Warwick, who has nerve implants allowing him to remotely control a robotic hand; and Neil Harbisson, a color-blind artist who developed an implant that allows him to perceive color via sounds and vibrations. But while these modifications and the tech being developed by those at grind houses have untold scientific and medical advancement potential, grinders are faced with overcoming the notions in the scientific and medical communities that the way nature built us is the way we are intended to be and that the desire to improve is not being appreciative of what we already have. Beyond the obstacles created by personal opinions, more complex tech is also limited by the ability to supply power. Implants requiring a constant power source (such as Bluetooth low energy [BLE] or wireless) mean you have to store energy in the body. While the medical community has come up with ways to do so, safety is not guaranteed and for the DIY enthusiast, very cost prohibitive. The potential for conventional power storage methods to rupture or corrode due to the body's natural acidity causes safety issues, outweighing the benefits of the implant. Still, there are some ideas and concepts floating around, such as a publication from the Korean Instutute by

Sun Jin Kim, Ju Hyung Wea, and Byung Jin Cho, "A wearable thermoelectric generator fabricated on a glass fabric" (doi: 10.1039/C4EE00242C).

The concept of bringing man and machine together is not just science fiction. It is now, and it is happening all around us.

Mitch "Rez" Muenster is a mobile developer and grinder out of Madison, WI, who has a passion for bringing mobile tech and the human body together as well as developing out-of-the-box ideas to grow how programmers and the mobile industry look at the mobile platform. You can follow or contact him via @MobileRez on Twitter or attend one of his many speaking events.

Open Source Biotech Consumables

John Schloendorn

Introduction

Let's face it, biotech startups are expensive. And they're not just expensive, they're also hard. Not just scientifically hard, but also unnecessarily, bureaucratically hard. Even if you're somehow independently wealthy and can afford to plunk down $100k to get your own lab started (and you don't mind getting looked at funny by your friends, "What? You can't do biotech on $100k!"), it still takes a long time to find a seller of biological materials that's willing to do business with an individual or newly formed startup company operating out of a basement. Both of these problems, cost and inaccessibility, are preventing the biotech revolution from taking off in the way the computer tech revolution took off when people started doing things in garages. We need to fix this.

First, I want to point out that there has been great progress on the lab equipment front. Access to a basic molecular lab is now obtainable and affordable both through hardware makers (e.g., OpenPCR) and through community equipment pools (Biocurious, Berkeley Biolabs). The most comprehensive list of these resources is probably Quitterie Largeteau's biotech/hackerspace list. Getting access to higher-end cell culture and regenerative medicine gear still requires more creativity. But I can see the momentum now that will take us there with time.

In this article, I will highlight another front where there has been much less progress until now—the need for access to inexpensive biological consumable reagents, outside the confines of academia and industry and absent of limited-use restrictions.

Price Versus Cost

A poorly kept secret that we biotech overlords have is that biotechnology reagents don't have a cost. They have a price. Big difference.

Frankly speaking, after all these years in the industry, I still have no idea how people can get away with charging several thousand dollars for a milligram of recombinant protein. That's an amount that you can see with the naked eye, if your eyesight is really good, but even then, you can see it only just barely. If you had to make a recombinant protein in your undergraduate biology class, then you know that the cost of doing this is essentially the cost of highly refined sugar water (= culture media) plus the cost of highly refined salt water (= chromatography buffers). Using sophisticated 1970s hardware like a shaking incubator and an Akta purification pump, any undergaduate biotech student can make many milligrams of protein in a single day, for each liter of culture medium and chromatography buffers consumed. The sales price of these things is tens of thousands of times their production cost, including highly skilled labor. If this doesn't make sense to you, then that's because it doesn't make sense period. I would simply refuse to believe the facts of nature described in this paragraph if I hadn't made numerous recombinant proteins with my own hands at virtually zero cost and if I hadn't spent some $500,000 of other people's money purchasing them. There is no reason these things should be happening at the same time on the same planet. But they do happen. On our planet.

Inaccessibility

People in the field have a lot of different views on what's causing the price of biotech to be so much higher than its cost. And they're all correct. High knowledge barriers—yes. Up-front capital investments required—absolutely. An enormous government funding program bidding up prices—totally. And many more. But one of them is most interesting. One is the master barrier. One barrier is protecting all the others from falling to the onslaught of human ingenuity. It's what I like to summarize as inaccessibility.

Large companies and governments have the ability to keep biotechnology profitably inaccessible by exploiting a fundamental principle that has become known as the "Central Dogma of Molecular Biology." The Central Dogma governs which biological resources can by nature be reproduced, and which cannot. Most functional biologics undergo something like the life cycle in Figure 2-1.

Figure 2-1. The Central Dogma of molecular biology

DNA is the most upstream source of biological information. It harbors the genes, which are the "blueprints" for making functional biological entities. Uniquely, DNA has the gift of self-renewal, as it gets replicated during cell division. The nonfunctional "blueprints" written in the DNA get translated into protein (through an RNA intermediate, which we will neglect for the purpose of this article). Proteins are the functional agents that make biology go, and they are also functional tools that scientists need to do biological research. In a way, this is analogous to "compiling" a software program. The important difference to software, however, is that once "compiled," proteins are a dead end. If you have only a protein, but you're denied access to the DNA coding for it, then there is no known way to make more of it, and there is no known way to turn it back into DNA.

What the Central Dogma allows is the sale of functional proteins that scientists can never make more of. By physical law, they're required to come back for more, guaranteeing a steady stream of profits whenever someone wants to test a new idea. For good measure, a material transfer agreement is slapped onto these purchases, making sure that the scientist can never enter into commercial competition, even if she should ever get a hold of the DNA. Meanwhile the government keeps printing money and funnels it to the scientists by various routes to ensure they can afford the ongoing extortion. It's an MBA's paradise. A government-approved establishment scientist wouldn't mind enough to cause real trouble either. But it's killing the ability of self-funded individuals and startups to access biological resources that would be free, if they weren't restricted.

But is this really happening? A casual observer of the field might be confused for a minute: at first glance, the Internet appears to be full of nonprofits purporting to "share biological resources" with names like ATCC or Addgene. They have DNA for sale, or cells containing DNA, and it's not for a terrible amount of money. That's true, until you try to order something from them. If you don't have a university affilitation, you're not even going to get an email back from these folks. And those readers who do have a university affiliation will remember getting inundated with 20 pages of material transfer agreements that have to be signed in blue ink and mailed around physically, because they're just too important to leave to email. This is where you have to promise to never share the DNA with anyone and do everything

in your power to prevent it from being used commercially. It is ironic, although perhaps unsurprising, that these nonprofit "resource sharing" entities have made themselves into the stalwart guardians of the closed-source reagent vending industry (see Figure 2-2).

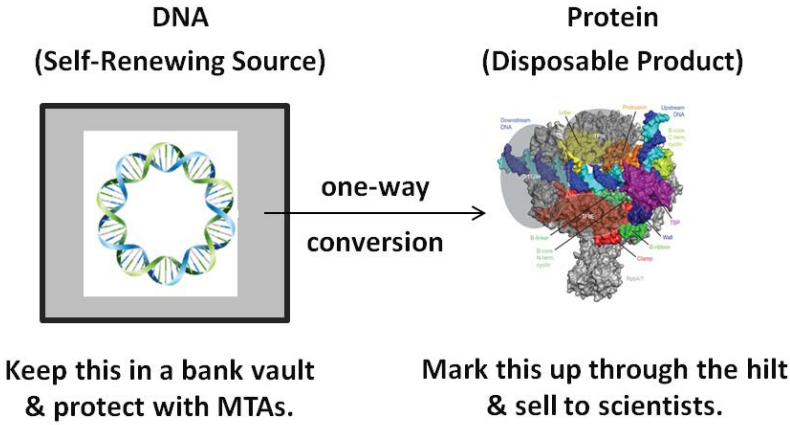


Figure 2-2. Capitalizing on the Central Dogma

The proprietary biologics vending industry protects its astronomical markups by guarding its self-renewing source of DNA by physical possession and through restrictive contracts with those willing to sign them.

The next wave of life-saving, economy-saving, and planet-saving biotechnologies is going to come from small teams of startup entrepreneurs with no access to any variety of "other people's money," as it always has. These fine folks need to be given unbureaucratic access to the basic tools to do their work.

Pricking the Bubble

The protections of the closed-source biologics vending industry are actually thin as paper and brittle as glass. For most of this stuff, they have no patents, no copyright, no government regulations, hardly a lobby to speak of, and no monopolies of any kind. They manage to lock biotechnology away from new entrants and to keep the cost of doing science in the stratosphere for establishment professionals, solely through the physical possession of the source DNA and by imposing contractual restrictions on those willing to sign them.

What if there was an alternative? What if there was a source of DNA, tested and certified for the production of high-value biological reagents, that does not

impose any restrictions on how the DNA gets used? If this alternative was available, the existing closed-source system would become obsolete overnight. Different reagent production companies could snap up these DNA constructs and start competing on efficiency of gene expression and reagent production, rather than on efficiency in keeping secrets. The prices of biological reagents would collapse, and the quality would improve, as these characteristics take the place of corporate secrets as the main criteria for competitive success. The power of free-market capitalism (meaning the nonsecretive, noncrony kind) would finally be unleashed to tear down the barriers to biotechnology-based scientific wealth, as it has done with so many barriers before it (Figure 2-3).

Figure 2-3. Pricking the bubble

Today I am delighted to announce that the pricking process has begun. I have synthesized, manufactured, tested, and fully validated a collection of open source plasmids coding for some of the very basic building blocks of biotechnology. I do charge an initial purchase price to pay for storage, ongoing quality control, and the provision of a reliable source of these molecules. But there is no proprietary barrier of any type on their use. You may grow them on your own, modify them, give them to others, sell them, sell products derived from them, and do whatever you (legally) want to do with them. I offer the following constructs today, and as of this writing am still the only one in the world offering for unlimited use:

- Expression plasmids for Taq polymerase, Pfu polymerase, and DNA ligase
- Plasmids for producing DNA ladders (size standards) for 100 bp, 1 kb, and mass standards
- Mammalian resistance plasmids (neo, hygro, puro, and blasti)

And this is only the beginning. I am first creating a basic suite of molecular tools. These tools are already supporting the creation of a second tier of tools with direct applications in regenerative medicine and industrial biotechnology. As more patents in these fields expire, open source stands ready to make all these wonderful tools available and accessible to anyone willing to use them.

I create the open source plasmids with with my private corporation Gene And Cell Technologies, of which I am the majority shareholder. There is no contract assigning the open source plasmids to the company. I have no corporate investors (although I do gratefully acknowledge a number of true "angel" investors, who all support my charitable open source mission; none of them required any restrictive paperwork). I do not know of any patents covering these plasmids or any of their elements, and do not believe them to be restricted by any form of intellectual property. My material transfer agreement (MTA) consists of a single line: "These plasmids may be used for any legal purpose."

The first open source DNA construct was pOpenTaq, containing the DNA for Taq polymerase protein. Taq is the workhorse driving polymerase chain reaction (PCR), the "DNA copy machine," which is an essential component of virtually every molecular laboratory. pOpenTaq is already enjoying great popularity throughout the world, as can be seen in Figure 2-4.

pOpenTaq is my open source Taq polymerase production plasmid, available for unrestricted use. It appears that people around the world are now using my first open source DNA construct and are talking about it in all kinds of languages. I have no idea what they're saying, and I didn't get paid for much of this, because they gave it to each other. But it cost me little to make it, and it makes me very happy to see that my first open source gift to the world is being appreciated and used.

I must acknowledge that not all of Gene And Cell Technologies is open source. The main purpose of the company is to support my own personal regenerative medicine research through supply chain integration. If and when I can afford to share one of the tools I create, then I will continue to offer it to the public on open source terms that combine doing good with doing well.

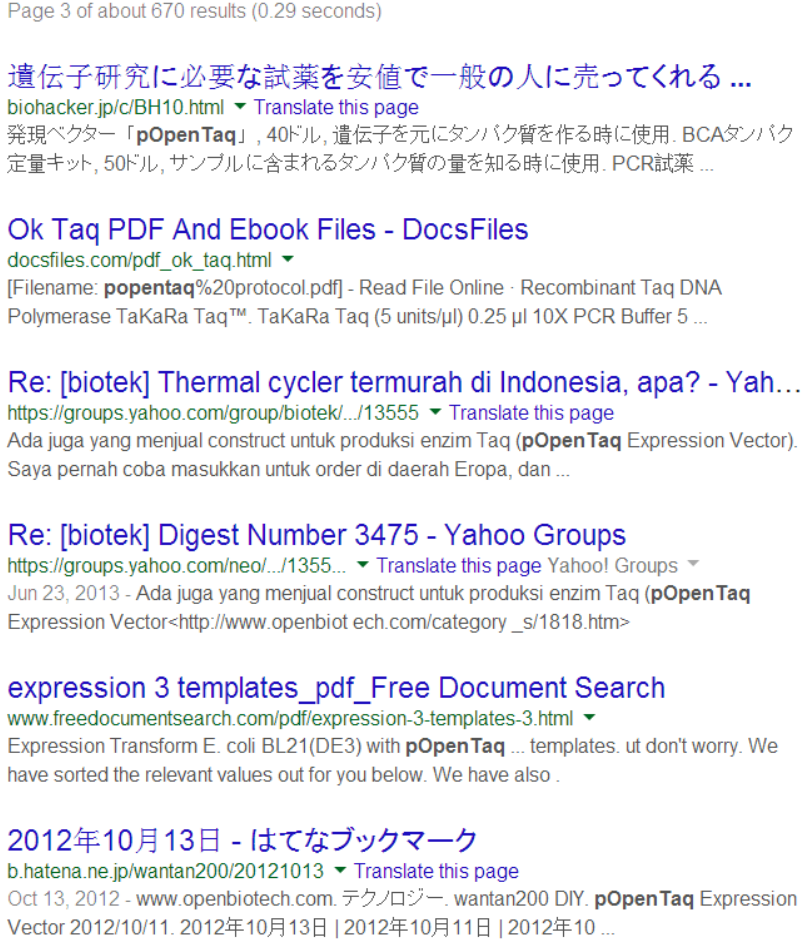


Figure 2-4. Search results for "pOpenTaq"

I would like to thank Dr. Justin Rebo for his valuable insights into the mechanics of the biotechnology industry and for working with me during the earliest stages of my adventures in open source biotechnology.

John Schloendorn is the founder and CEO of Gene And Cell Technologies, Inc., an integrated regenerative medicine startup. Prior to founding Gene And Cell Technologies, John was the CEO of ImmunePath, which he developed from a clean bench in his living room to a venture-backed company with positive preclinical results. ImmunePath was ultimately unable to raise enough money for a clinical trial and closed its doors in 2012. John also served as the director of SENS Foundation's intramural Research Center from 2006 to 2009, where he oversaw the development of enzyme therapies for age-related storage diseases and a variety of other projects through the preclinical stages. A native of Germany, John holds a green card under the National Interest Waiver, a program designed to retain technology leaders considered of national interest to the United States. At the young age of 34, he is the author of several pubmed-indexed publications and inventor on several active patent applications.

Leukippos: A Synthetic Biology Lab in the Cloud

Pablo Cárdenas, Maaruthy Yelleswarapu, Sayane Shome, Jitendra Kumar Gupta, Eugenio Maria Battaglia, Pedro Fernandes, Alioune Ngom, and Gerd Moe-Behrens

Abstract

As we move deeper into the digital age, the social praxis of science undergoes fundamental changes, driven by new tools provided by information and communication technologies. Specifically, social networks and computing resources such as online cloud-based infrastructures and applications provide the necessary context for unprecedented innovations in modern science. These tools are leading to a planetary-scale connectivity among researchers and enable the organization of in silico research activities entirely through the cloud.

Research collaboration and management via the cloud will result in a drastic expansion of our problem-solving capacity, since groups of people with different backgrounds and expertise that openly gather around common interests are more likely to succeed at solving complex problems. Another advantage is that collaboration between individuals becomes possible regardless of their geographic location and background.

Here we present a novel, open-web application called Leukippos, which aims to apply these information and communication technologies to in silico synthetic biology projects. We describe both the underlying technology and organizational structure necessary for the platform's operation. The synthetic biology software search engine, SynBioAppSelector, and the game, SynBrick, are examples of projects being developed on this platform.

Cloud-Based Collaboration Can Potentially Accelerate and Transform the Scientific Discovery Process

Social networks and the ability to organize collaborative work via the cloud will become important factors in driving innovations in modern science and technology.

This kind of social networking provides a potential frame for global connectivity among researchers. This dramatically expands our combined brain power because large groups of different people are more likely to find solutions to complex problems. Moreover, collaborations become independent of the physical location of the collaborators or the development level of the member countries. This independency of the physical location reduces the transaction costs nearly to zero.

Furthermore, such combined brainpower can help deal with another major challenge for contemporary science, the so-called Big Data problem. In recent years, scientific research has increasingly produced vast amounts of data from high-throughput or large-scale experiments. We can also observe an exponential increase in the number and/or size of data sets, particularly in biology and bioinformatics research. For example, the 1000 Genomes Project has produced 200 TB of publicly available data sets since its inception. The output in scientific literature has become so vast and complex that it has become difficult for a single person to read, assimilate, and process it. Social networks can help. For example, the use of Twitter analytics can point someone toward relevant recent publications.

This new organizational model of collaborative research is still in its infancy, and recently, different approaches to distributed problem solving have been gathering attention: examples include crowdsourcing, crowdfunding, and topic-specific science forums. Successful examples of such an effort include games like FoldIt and EteRNA, which gamify computational predictions of protein and RNA structures, respectively.

There are, however, a series of barriers that prevent online communities of scientists from adopting cloud-based collaboration. An inherent conservatism in established science praxis discourages many scientists to share data publicly. A large number of publications in high-impact journals are still essential for scientists to build a career. Going public with data and knowledge would mean giving their competitors an undue advantage. Thus, the ideas of open source are convincing in theory, but are often not put in praxis due to the traditional business model of academic science (see *http://bit.ly/1iqYzl8*).

Another major limitation in adopting distributed problem-solving approaches is the lack of a fully functional, complete, and self-contained infrastructure for

collaboration. That is, an infrastructure that would allow access to data of any type, from anywhere, and by any collaborator. This would also allow processing and analysis of such data and results by anyone, from anywhere.

In Silico Synthetic Biology as an Example Area for Cloud Collaboration

Synthetic biology (SynBio) is a recently established, novel discipline that aims to design and engineer biological elements, circuits, devices, and systems not found in nature and redesign existing natural biological systems for useful purposes.

SynBio is especially well suited for cloud-based collaboration due to a specific social culture in the field and specific scientific needs. The specific culture of SynBio is characterized by a dominance of young, innovative people. Every year the International Genetically Engineered Machine competition engages students from around the world in synthetic biology projects. Moreover, there is a strong DIY/biohacker community interested in SynBio. These people are early adopters and are open to novel open source ideas and used to social networking via the cloud in their scientific work. Additionally, SynBio can be viewed as a practical application of systems biology because it deals with complex systems and large amount of data. As previously discussed, these kinds of problems are especially suited for cloud collaboration. Moreover, SynBio relies heavily on bioinformatics. Standardization of biological parts and subsequent usage of hierarchical abstraction to assemble complex systems are used extensively in SynBio designs. This makes SynBio especially suitable for digitization and computer-aided design software. Hence, in silico work is a crucial part of the daily work of the SynBiologist. Access to a laptop or a mobile device makes it possible to do essential work in this field. This technology makes it possible to organize an in silico SynBio lab in the cloud.

Components of the Leukippos Platform

The Leukippos Institute was founded to harness the power of the crowd and social media in order to collaboratively carry out bioinformatics work for solving nontrivial problems in synthetic biology. Thus, the aim of the Leukippos Institute is to build an in silico synthetic biology lab in the cloud. The output of our work will be different synthetic biology–related web applications.

This proposal embraces a native digital environment: the Internet. Since all the activity occurs on the Web, it is a perfect venue for in silico work and for professionals in different fields and sections of academia and different parts of the world to connect and collaborate. By adopting an open science model and harnessing the

networking capabilities of the Web, Leukippos can harness the contributions, labor, and computing power of online volunteers. In this manner, the Leukippos Institute provides a way to take advantage of the computerized character of synthetic biology to produce an open network for collaborative work in the field.

The Leukippos Institute is based on the crowdsourcing concept, in which collaborators contribute their own expertise from their own areas of interest or research, and then get attributed for their contribution. Originally, some in silico SynBio projects were initiated by a small group of participants on Facebook. The Facebook group has since grown to 472 members. Facebook, in essence, is an integral part of this project in which we discuss ideas, methods, and solutions to problems.

In order to go beyond Facebook, we are now in the process of developing a platform that will serve as a synthetic biology lab in the cloud (see Figure 3-1).

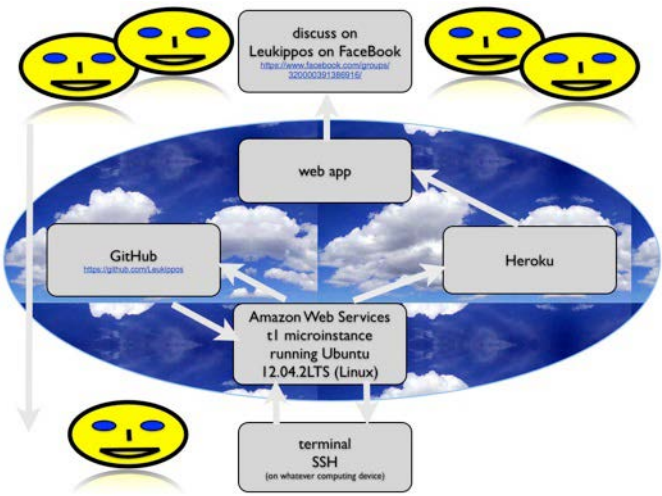


Figure 3-1. The workflow of the coding platform of the Leukippos Institute. The various components of our platform are the following: (1) First, we use an SSH (Secure Shell) terminal on any computing device. (2) This is used to manage a T1 microinstance running Ubuntu 12.04.2LTS (Linux) on Amazon Web Services. (We are in addition using a server from the University of Windsor as an alternative to Amazon Web Services). (3) GitHub, which will be used as a repository, is where we store the versions of our project and can get easy access to the code under development. (4) We will use Heroku to host our web app. (5) Thus anybody participating in a specific project can work on his or her own version or part of the web app under development. (6) Facebook will be used to discuss the different versions of the web app and to agree on an official merged version.

Idea Testing: Projects and Testbeds

The Leukippos Institute has two ongoing projects that serve as testbeds for the crowdsourcing, collaborative methodology. The first is SynBio App Selector, an interactive repository of synthetic biology–related software (see Figure 3-2).

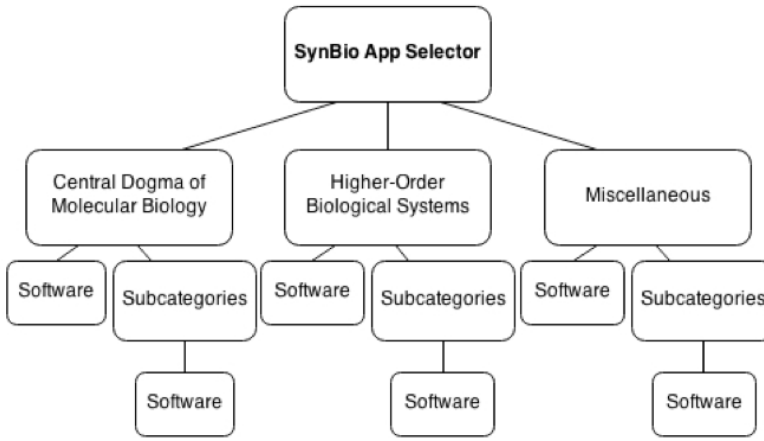


Figure 3-2. The SynBio App Selector (from http://www.iwbdaconf.org/2013/proceedings/) is a hierarchical structured web application that provides the user with an easy and intuitive way to find synthetic biology–related software. The user interface of the app consists of three different menus. These menus are rendered in the form of icons on a 3D sphere, and the user navigates them by dragging and zooming the sphere. The first of these menus displays a schematic representation of the Central Dogma of molecular biology and leads to software that works with the different molecules and processes involved. This ranges from DNA plasmid and RNA primer design to protein analysis. The other menus represent higher-order biological systems and other useful tools. A prototype of this app can be found at http://bit.ly/1qhoiQt.

Synthetic biology is deeply embedded in modern Big Data science, and computational tools play a vital role. However, given the abundance and diversity of software available, it is often hard to find the right tool for the job. SynBio App Selector aims to solve this problem through an online quick reference guide app using HTML5, JavaScript, and other web technologies. The app categorizes synthetic biology software into different classes and subclasses, such as "lab tools," "simulations," or "primer design." Users can navigate these hierarchies by means of a 3D interactive display. The app stores information on each software tool's description, development status, and licensing, as well as other pertinent info. In all,

the app indexes over 180 different software tools. Figure 3-3 shows an early version of the app, which is still under development.

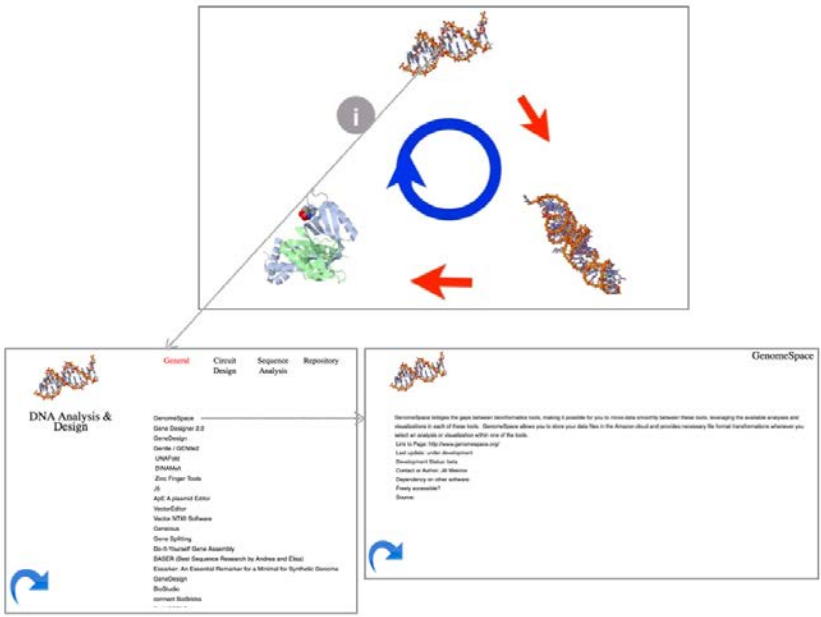


Figure 3-3. The SynBio App Selector is an intuitive-to-use, all-in-one collection of software applications, tutorials, and resources related to synthetic biology. Navigate the menus by dragging and scrolling up and down, and click on the icons to view a list of software belonging to that category.

The second project under development at Leukippos is SynBrick (Figure 3-4), a crowdsourcing game in which players work together to solve engineering challenges using synthetic biology: designing biological systems to produce biofuels or medicines, diagnose diseases, or clean hazardous waste, to mention a few possibilities. SynBrick takes advantage of the concept of BioBricks, standardized genetic components that can be mixed and matched to build different biological systems, and is built on a similar modular scheme.

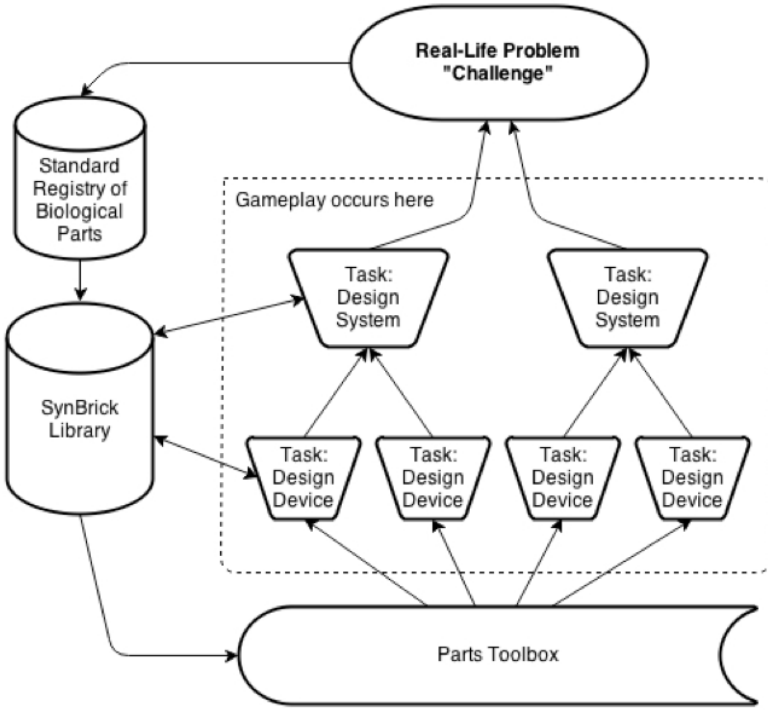


Figure 3-4. SynBrick (from http://www.iwbdaconf.org/2013/proceedings/) is a game played in teams where the aim is to solve complex synthetic biology problems. This figure shows SynBrick's structure and problem-solving design strategy. Arrows denote flow of information. Standardized biological parts are the building blocks of the game.

Complex problems like the ones outlined are decomposed into simpler tasks. For example, if we are building a system that turns water red when a pollutant is detected, we can break it down into two separate devices, one that detects the pollutant and another that produces red pigment. These simpler tasks are then solved by players (i.e., collaborators or any willing participants) who devise in silico biological parts (biogates, biocircuits, biosystems) called BioBricks; these BioBricks are further combined in an appropriate manner and in such a way that the simple tasks are solved. In SynBrick, players will be challenged to solve specific and simple tasks using a virtual BioBrick toolbox. The game evaluates the best solutions by simulating the genetic circuits built by players based on the characterization information available for each BioBrick in the Standard Registry of Biological Parts. The first version of SynBrick is still under development. However, you can read more on

this project or SynBio App Selector in the Proceedings of the International Workshop on Bio-Design Automation 2013 (see page 64).

The authors thank Kevin Chen (McGill University) for critical reading of the paper and his valuable comments.

Correspondence to Dr. Gerd Moe-Behrens: *leukipposinstitute@googlemail.com*.

Pablo Cárdenas is an undergrad student at Universidad de Los Andes in Bogotá, Colombia.

Maaruthy Yelleswarapu is a master's student at ETH Zurich and has been collaborating at the Leukippos Institute since 2013.

Sayane Shome completed her undergraduate studies in bioinformatics from Vellore Institute of Technology in Vellore, India, in May 2013. She works as a project trainee at the Sub-DIC Bioinformatics Centre at NIT Raipur, India. She also works as an external student researcher and virtual classroom trainer for bioinformatics modules at King Abdulaziz University, Rabigh, Saudi Arabia. She served as the president of RSG-India, a student body affiliated to the International Society of Computational Biology (ISCB) Student Council, from 2012 to 2014.

Jitendra Kumar Gupta works as a programmer and research assistant at Shodhaka Life Sciences Private Limited, incubated within the Institute of Bioinformatics and Applied Biotechnology. He has a Masters of Science in Bioinformatics from Mangalore University. For a short period of time he worked in the Manipal Institute of Technology as a research scholar. He joined the Open Source Drug Discovery Project (an initiative by CSIR) at the Indian Institute of Science.

Eugenio Maria Battaglia is an undergraduate student in molecular biotechnology at the University of Turin with a specialization in integrative neuroscience. Currently he's developing a Bio Commons Licence in an European framework named Synenergene. He's developing the first Italian biohacker space, named BioPeers. Eugenio has been active in a series of groups about open science and scholarly communication: he is a member of Force11, a community manager in HackYourPhD, and a board member of the Open Knowledge Foundation – Open Science Italy.

Pedro Fernandes is a bioinformatics training coordinator at Instituto Gulbenkian de Ciência, in Oeiras, Portugal. He also organizes brainstorming events on challenging themes such as "Distance and eLlearning Technologies," "Systems Biology and P4

Medicine" and "Pathway Analysis in Proteomics." He is an advisor to Figshare, an ambassador to iAnn, and plays team-leading roles in organizations like EMBnet and GOBLET.

Alioune Ngom received his BSc degree in mathematics and computer science from the Universite du Quebec a Trois-Rivieres in 1990 and his MSc and PhD degrees in computer science from the University of Ottawa in 1994. He is currently a professor at the University of Windsor, Ontario, Canada. Prior to joining the University of Windsor, he was an assistant professor at the Department of Mathematics and Computer Science at Lakehead University, Thunder Bay, Ontario, Canada, from 1998 to 2000. During his short stay at Lakehead University in 1999, he cofounded Genesis Genomics Inc. (now, Mitomics Inc.), a biotechnology company specializing in the analysis of the mitochondrial genome and the identification and design of mtDNA biomarkers for the early detection of cancer. He is member of the IEEE-BBTC and IAPR-Bioinf and coleads the Pattern Recognition in Bioinformatics group at the University of Windsor.

Gerd Moe-Behrens has a BSc and MSc from the University of Oslo and a PhD from the Faculty of Medicine, University of Oslo, Norway. He founded the Leukippos Institute for Synthetic Biology, a research institute solely in the cloud. Moreover, he is founder and CEO of CytoComp, a young startup focusing on biological computing.

Why the Synthetic Biology Movement Needs Product Design

Sim Castle

The global synthetic biology industry is expected to be worth over $16 billion in 2018—growing at a rate of 41% a year. But without the application of product design, could the world's fastest growing industry be doomed to remain the muse of scientific research and hobbyist hacking?

It is often said that the electronics industry of the 1970s was in a similar stage to synthetic biology today—a mix of state-of-the-art scientific research and geeky amateurs experimenting.

Steve Jobs and Steve Wozniak were two such amateurs of the electronics movement. It wasn't the technical aspects of their products that led to Apple's rise and its pivotal role in creating the tech revolution we see today: it was their recognition that they had to create products that had the user in mind and be technically innovative. The simplified graphical interface of the Apple II (in comparison to other products, many of which didn't even have a screen) and the creation of the first effective mouse (designed in conjunction with product-design firm IDEO) are examples of how great design made these new technologies accessible to the everyday user.

If Steve Jobs had never envisioned a world of casual computer use, where would the tech industry be now—a few large computers restricted to universities? Would we have progressed to anywhere near the level of technology we have today? I would argue almost certainly not. To really fulfill a technology to its potential, it must be adapted to a human user; this is what Steve Jobs did with the Apple II and what Henry Ford did with the Model T. It is not the discovery or even the technology that starts a revolution, it's the product that brings the technology to the masses.

With such rapid scientific progress being made in the new field of synthetic biology, it can be easy to think that design has no place within it yet and that to be thinking about commercial products is premature at best.

After all, it is true that every advancement in technology must progress through distinct stages of development before its full potential is reached. Firstly, the fundamental scientific discoveries need to be made, allowing greater understanding of principles that can eventually be used to create parts with simple functions. These parts then become the building blocks of engineered systems—both physical and informational—which can be used to complete ever increasingly complex tasks. Once you have a engineered machine or system, this can then be used to design a product. This is something that not only simply performs a function, but performs it well, in a way that suits the user. This stage provides revenue streams through commercialization of a technology that can support the lower levels of research and engineering, thereby accelerating progress.

Until recently, synthetic biology has been strictly in the research phase. However, the efforts of iGEM and BioBricks have created the beginnings of the standardized parts required for engineered systems to be created. While for now these systems remain relatively simple, we are already seeing systems and machines with real-life applications. For instance, the Beijing Insitute of Technology has created a reusable device that can detect levels of antibiotic in milk that is expected to be available in stores as early as next year.

Some designers are already utilizing biology for their own purposes. Suzanne Lee of Biocouture is a fashion designer who works with biomaterials that have consumer applications in clothing. The focus of her work is not just to create a novel material from biotechnology, but to shape exciting, viable products from it. Most of these designers are currently working with *low-tech* biodesigns: that is, designs that exploit and include existing natural organisms rather than the products of synthetic biology. Since natural and synthetic biology share the same architecture, so too they share the same broad design considerations.

In fact, synthetic biology would allow for designs that are even more efficient, reliable, and useful than purely natural applications, as synthetic biology can be better designed and optimized toward the focus of the product. One such example is the collaboration of the Cornell iGEM team with Evocative Design, a manufacturer of a mycillium-based alternative to Styrofoam, used in applications ranging from surfboards to packaging. Evocative Design used an existing organism (mycillium-producing fungus) to produce a useful material that is now being proved via synthetic biology. The Cornell iGEM team is working to improve its

disease resistance. As synthetic biology becomes both cheaper and more advanced, product design will increasingly shift toward more synthetic biology, allowing an ever greater range of applications.

There are several questions about synthetic biology's future that can only be answered by design. How will we as humans interface with new biological products? How will we create social acceptance for a misunderstood and often feared technology? How will we create scalable, cost-effective manufacturing processes for biological products? And, perhaps most excitingly, what can be achieved by combining synthetic biology with existing (and future) technologies? These are all questions that good design seeks to answer—in addition to bringing to light further questions of what will be possible, just like design questions in the tech industry have spurred on incredible progress and innovation.

In the future we can expect to see engineered biological systems performing as wide a range of functions as today's electronics industry—not only producing novel new materials and medicines, but spurring innovation in consumer products, architecture, and even fashion. With a little vision, through collaboration between scientists, DIY biologists, and designers, and as the synthetic biologist's toolkit continues to expand, this future can be designed now. It is therefore vital that we begin to develop the design language necessary in order for synthetic biology to fulfill its true potential to change the world.

Sim Castle is an industrial design engineering graduate student at TU Delft who is exploring the applications of biology in future products. Find him on Twitter: @simcastle.

Chemical Safety in DIYbio

Courtney Webster

If chemical safety was modeled after economic policy, many biology labs would follow a laissez-faire approach. Don't get me wrong—I don't blame the biologists. I blame the "kit"-ification of most of their experiments. Calling something "Wash Buffer B" abstracts away from proper chemical names. If you have to dig through fine print to find the ingredients, you have no way of knowing how hazardous it might (or might not) be.

Many DIYbio labs don't have to deal with chemical reagents, so biological safety[1] is the primary concern. But if you need more than ethanol and bleach to run a lab, you should know some basic chemical safety skills.

PPE: Personal Protective Equipment

The first step is (obviously) to protect yourself. If you're handling a chemical, you should at least wear safety glasses and gloves. By the way, not all gloves are created equal. For aqueous (water-based) solutions, latex gloves work just fine. If you're handling a powdery chemical or organic solvent, you'll want nitrile gloves (which are more chemically resistant).[2]

1. Biocoder Winter 2014, "Big Thing #1: Keep it Legal" by Raymond McCauley
2. OSHA: Personal Protective Equipment

Glove Choice

Latex gloves
: Provide the best protection from aqueous (water-based) solutions (such as media)

Nitrile gloves
: Provide the best protection from organic solutions (such as DMSO)

Pro Tip
A chemical's material safety data sheet (MSDS) specifies the best type and thickness of glove to use.

Rookie Tip
If the glove rips when you're trying to put it on, it's too thin.

Preferably, you'll be wearing a lab coat as well. My lab coats testify with numerous spots and stains (and I'm a pretty careful chemist). If you're working with particularly volatile, toxic, or smelly materials, using a chemical fume hood is a good idea.

Hazard Pictograms: The Primary Hazard of a Chemical

Let's move on to the important stuff. If you have the name of a chemical (the *real* name), how can you tell if it is hazardous? The quickest indication will be a little picture on the bottle (a hazard pictogram).

TOXICITY HAZARDS

Next are fairly intuitive pictograms from OSHA's website.[3] We'll start with the health and contact hazard pictograms. Chemicals with minor[4] toxicities (irritants) will be labeled with the "warning" pictogram (sometimes represented with a large "X" instead of an exclamation point; see Figure 5-1). A corrosive pictogram is

3. OSHA pictograms
4. Don't take my word "minor" literally—read an MSDS to get an idea of the toxicity. Oh, you don't know how to read an MSDS? Well it's your lucky day—read on.

common for acids and bases, meaning you'll get a chemical burn (*a la* Fight Club) if you spill it on your skin.

Figure 5-1. Toxicity pictograms I: avoid contact (irritant, corrosive)

When you move into more serious territory, the pictograms distinguish between an acute (immediate) effect, indicated with a skull and crossbones, and a chronic effect, clearly represented by the person with the alien-like hole in his chest (see Figure 5-2). Chemicals with chronic toxicities are carcinogens, mutagens, sensitizers—generally nasty stuff. You might also see the "dangerous for the environment" pictogram, indicating the disposal of this chemical (and any waste containing this chemical) needs to be carefully managed. If it's dangerous for the fishies, it's dangerous for you.

Figure 5-2. Toxicity pictograms II: handle with extreme care (acute toxicity, chronic toxicity, dangerous for the environment)

PHYSICAL HAZARDS

Moving on to physical hazards, you'll often encounter the flammable pictogram (see Figure 5-3). Keep this away from your Bunsen burners and flame sterilization equipment. If you observe the explosive or oxidizing pictogram, you'll want to get some trained advice on handling this material. It's generally advised to keep any

waste you generate containing oxidizers in its own waste container, as these can react with other common lab chemicals.

Figure 5-3. Physical hazards (flammable, explosive, oxidizing)

A pictogram will give you a quick indicator of the presence of a hazard, but it doesn't provide hard guidelines on the scale of that hazard. If you like numbers (like me), you can take a look at the NFPA (National Fire Protection Association) rating.

NFPA: National Fire Protection Association (an Aggregate Safety Rating)

The NFPA gives you an overall rating of the chemical in each hazard category (health, fire, reactivity, and other specific hazards). The higher the number, the worse the hazard (see Figure 5-4).

You can (and should) make an NFPA label for your entire laboratory. It's easy—for each category, find the chemical in your lab with the highest number in that category (health, fire, etc.) and put that number on your lab NFPA.

TIP

Pro Tip

If you have a DIYLab, you should make an NFPA sticker to indicate the overall hazard level for your entire laboratory. It should be posted on an exterior window in case of emergency.

If any of your chemicals contains a specific hazard (especially water-reactive or oxidizer), add those, too. This helps first responders prepare if there is an emergency in your lab.

Now we can identify the presence of a hazard with a pictogram and even get a feel for the scope of that hazard with an NFPA, but to get the full scoop on how to handle a chemical, you'll need to look at its MSDS.

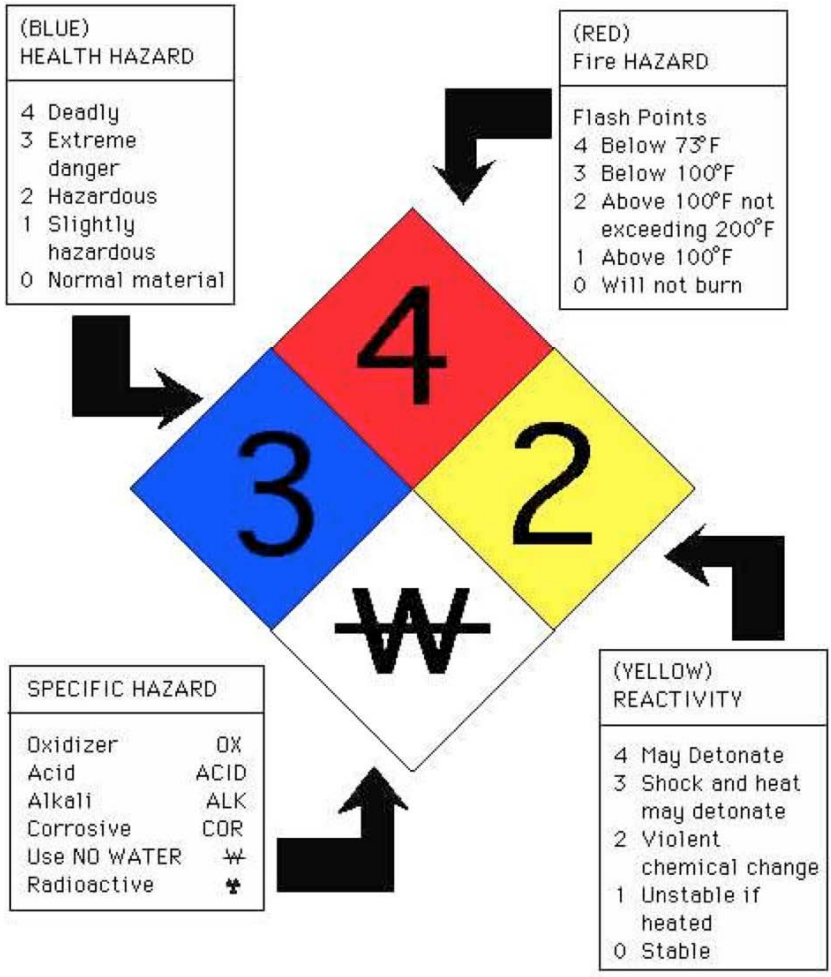


Figure 5-4. NFPA, deconstructed

MSDS: Material Safety Data Sheet (Soon to Be Called SDS)

Any chemical you order should come with an MSDS. You can find them online (free of charge) as well. Rumor has it the name is changing from MSDS to simply SDS, so keep that in mind. If you search for this document using a chemical name (e.g., caffeine MSDS), it might be difficult to find the exact product you have—in

that case, search the manufacturer's website using the product number, or search using the CAS number of the chemical instead.

Pro Tip
You should keep a copy of the MSDS for every chemical you store in your laboratory.

Pro Tip
Read an MSDS before working with a new or unfamiliar chemical.

CAS Number

A unique numerical identifier assigned to every chemical described in the open scientific literature.

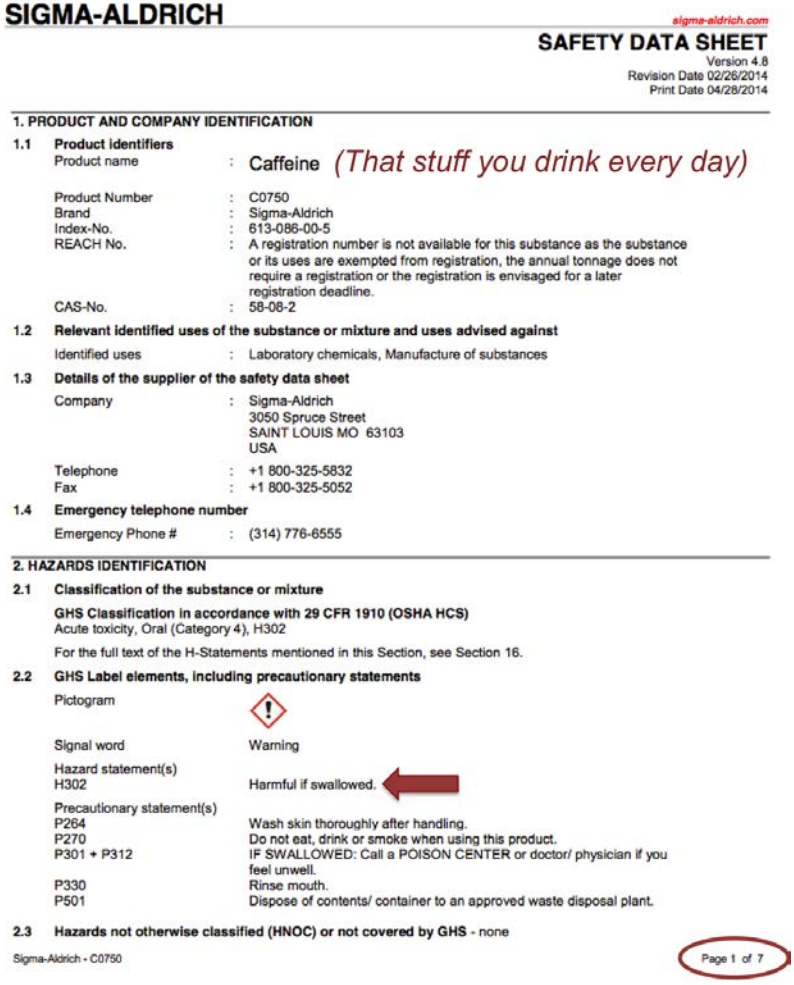
An MSDS will provide exhaustive information about the chemical at hand, but it won't make it easy to glean the most important information (see Figure 5-5). On the plus side, it's great for scary bedtime stories.

I'm not sure what's worse—"POISON CENTER" in all caps, or the fact that this MSDS is seven pages long. The point here is that everything is a chemical and everything has a dose-dependent toxicity. Though it's important to have a healthy fear of chemicals, you don't need an all-encompassing, enclose-yourself-in-a-bubble kind of fear. So how do you take this long and technical document and whittle it down?

If you see toxicity pictograms, look for the words "fatal" or "very toxic" to indicate serious danger. (Note that caffeine simply said "harmful if swallowed," not "toxic" or "very toxic.") The "Toxicological Information" section will list an LD_{50} (acute toxicity) and any chronic hazards the chemical might have.

LD_{50}: Lethal Dose at 50%

The dose (in milligram of chemical per kilogram of body weight) at which 50% of rats died within a specified time period (typically 1–14 days).



SIGMA-ALDRICH

sigma-aldrich.com

SAFETY DATA SHEET

Version 4.8
Revision Date 02/26/2014
Print Date 04/28/2014

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Caffeine *(That stuff you drink every day)*

Product Number : C0750
Brand : Sigma-Aldrich
Index-No. : 613-086-00-5
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 58-08-2

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Acute toxicity, Oral (Category 4), H302

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Warning

Hazard statement(s)
H302 Harmful if swallowed.

Precautionary statement(s)
P264 Wash skin thoroughly after handling.
P270 Do not eat, drink or smoke when using this product.
P301 + P312 IF SWALLOWED: Call a POISON CENTER or doctor/ physician if you feel unwell.
P330 Rinse mouth.
P501 Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

Sigma-Aldrich - C0750

Page 1 of 7

Figure 5-5. The MSDS for caffeine (found on Sigma-Aldrich)

An LD_{50} provides a quantitative toxicity value. In general, the lower the initial number (the milligrams required to cause toxicity per kilogram of body weight), the more toxic the chemical. If you see materials with single- or double-digit LD_{50}s,

you should wear protective equipment and handle and dispose of these chemicals with extreme care.[5]

After checking acute toxicity, you want to look for chronic toxicity or other sensitivities. The following words should trigger some alarm bells: carcinogen, mutagen, teratogen, or sensitizer. In those cases, you'll want to suit up well (gloves, goggles, lab coat) and use good lab technique to avoid any contact with the chemical (if you're sure you have to use it at all).

Pro Tip

Keep a copy of the MSDS for every chemical in your lab (either in paper form or on a small USB drive). If you get exposed to a particular chemical, bring the MSDS with you when you seek medical treatment.

If you see flammability warnings, check out the "Physical and Chemical Properties" and "Stability and Reactivity" sections to determine boiling points and flash points. Be extremely careful if you see words like "flammable solid," "ignites in contact with water," or "handle under inert gas." Have a trained chemist take a look, and see if you can avoid using this chemical altogether.

Ta Ta for Now

That's it for basic chemical safety! Keep an eye out for upcoming safety topics (like chemical storage and waste disposal), and stay safe out there.

5. For example, caffeine has an LD_{50} of 367.7 mg/kg with oral ingestion. While human LD_{50}s aren't provided (for obvious ethical reasons), you can generally assume that you and a rat will have a similar toxic reaction to a chemical. You simply scale the LD_{50} by weight to determine human toxicity. The average human weighs 154 lbs (or 70 kg). That means 50% of humans that ingest 25,739 mg of caffeine (25.7 grams, or 172 Starbucks lattes) might die. In comparison, the LD_{50} of sodium cyanide (a very toxic chemical) is 4.8 mg/kg for oral ingestion. That means a human would reach a possibly fatal toxicity level with only 336 mg (an amount that's about a third of the size of an M&M).

Resources

1. The Occupational Health and Safety website is a gold standard for information on PPE, pictograms, and other lab safety tips.
2. Sigma-Aldrich is my go-to website for MSDSs.

Courtney Webster is a reformed chemist in the D.C. metro area. She spent a few years after grad school programming robots to do chemistry and is now managing web and mobile applications for clinical research trials. She likes to work at the interface of science and software and write for scientists and engineers. You can follow her on Twitter @automorphyc and find her blog at http://automorphyc.com/.

A High-Voltage Power Supply for Systems Biology

Jonathan Cline

Introduction

I am sharing the design, along with the project files for home-lab manufacturing, of a low-current, high-voltage power supply that may be used for various experiments in Systems and Synthetic Biology. The schematic, board layout, and a suggested enclosure is provided. This circuit outputs up to +1,866 VDC at under 1 mA or can be tapped at various points to yield +622 VDC or +933 VDC. All components are easily obtained through common, hobbyist-friendly distributors.

The cost of this supply if built using high-quality components is easily under $100. The building blocks of the design are also reusable in other projects to further reduce total cost. This is in contrast to equipment used in typical journal research papers, which recommend scientific supplies costing more than $5,000.

This power supply may be useful for either DIY biology or institutional research experiments, such as:

- Digital microfluidics using electrowetting-on-dielectric[1]
- Electroporation

1. R. B. Fair. "Digital microfluidics: is a true lab-on-a-chip possible?" *Microfluidics and Nanofluidics*. June 2007, 3:3 (245–281), http://bit.ly/1qgTGSg. doi: 10.1007/s10404-007-0161-8.

- Electrokinetic experiments, such as dielectrophoresis[2]
- And, lastly of course, generating large sparks that blast with a PAHHH-POP!

An important aspect of this design is the built-in current limiting of the components. Never connect the printed circuit board directly to a wall outlet.

Warning Regarding High Voltages

This circuit creates high voltages with enough circuit current to mandate a serious warning. Any contact with high voltages may cause serious physical harm. Build this project at your own risk. Please read and reread the paragraphs on potential misuse of this circuit contained in this article. Make note that the circuit charge remains even after the circuit is unplugged from the input voltage, even after a significant time; always discharge the final output after disconnecting the input voltage. Under no circumstances should the circuit board be connected to a wall outlet (which are typically rated at 20 A or 30 A), or to any power source which does not inherently perform current limiting. In practical use, avoid using two hands near the circuit and near the outputs, to avoid an accident where the shortest path for current could pass through the arms to the user's chest and heart. All electrical components should be kept away from or shielded from the liquids in the wet lab during operation.

This article follows an engineering format by listing specific usage requirements for the project, followed by discussion of the possible design options that fit the requirements, and a theory of operation for the winning design. Projects are best designed by doing a bit of homework first.

This supply is intended to be used with a simple, computer-controlled, high-voltage switching circuit, to be published as a separate paper. Comments and suggestions regarding the construction and use of this project are welcomed on the DIYbio mailing list. The figures in this article can be used to fabricate the circuit

2. Peter R. C. Gascoyne and Jody Vykoukal."Particle separation by dielectrophoresis." *ELECTROPHORESIS*. July 2002, 23:13 (1973–1983), doi: 10.1002/1522-2683(200207)23:13<1973::AID-ELPS1973>3.0.CO;2-1.

board using circuit board etching techniques; see the instructions in "Using These Figures to Etch a Printed Circuit Board" on page 49.

> **NOTE** This power-supply circuit is not intended for and does not supply sufficient output current for a typical tray-style gel electrophoresis setup. The power-supply output is purposely current constrained. For running gels, a different circuit board is needed that reuses some components of this project. For example, a typical agarose gel electrophoresis requires >100 mA; this current varies depending on the gel's cross-sectional area.

Requirements

Scientific power supplies are well regulated and yield very stable output, especially if regularly calibrated. However, a survey of published results has not revealed experimental differences when ripple or electrical noise is either purposely or accidentally applied (this is an area where more research is needed). We assume, therefore, that if ripple and electrical noise is of little consequence, it is possible to design and use a simpler, less expensive high-voltage power supply. Many biological operations seem to activate from a wide range of electromagnetic field strengths, usually dependent on the particular organism or strain being studied.

The design should be easy to reproduce by others, to allow labs or individuals to build their own homebrew supplies. The desired circuit must be simple enough to allow multiple high-voltage output levels, in case voltages need to be much higher or much lower than the reference design. Where possible, the design should use off-the-shelf components that are reusable in other projects.

The laws of physics and today's scale of technology dictate the costs and trade-offs of today's power-supply designs, and these trade-offs factor into the requirements. Simultaneously producing both high voltage and high current is more difficult, expensive, and complex. Producing high voltage and low current, or vice versa, is simpler, lower cost, and fits the need for a range of scientific experiments.

Any power-supply design must allow the circuit to limit the current at the final output. It is important to limit the current available to the user to prevent mishaps in case of accidental shorts. The low current output in this design may be treated as a benefit.

Design and Theory of Operation

High output voltages, in excess of +400 VDC, are more easily obtained if starting with a high input voltage. There are several design choices:

- Starting from a typical AC outlet, a step-up transformer could be directly used, with the AC rectified to DC just prior to the final output. These transformers have marginal cost, and the output would be fixed.
- Starting from a typical AC outlet, a common low voltage AC-to-AC power adapter could be used, such as a 120 VAC to 24 VAC wall adapter, and this low AC voltage run through a secondary step-up transformer with high ratio (such as 1:200) and finally rectified to yield DC. These transformers can be found in some appliances, such as televisions, as flyback transformers. These transformers may be large, heavy, and either expensive or require a combination of reverse engineering and scavenging to minimize cost. Sharing a design that requires scavenging typically means the design is not reproducable by others.
- A high-voltage AC outlet source could be directly used, with current limiting. This requires some safety components and is typically not recommended for a homebrew build due to the possibility of manufacturing mistakes.
- A high-voltage AC outlet source could be converted to DC with a typical benchtop power supply, then converted to a high DC voltage with a DC-to-DC converter. This requires a benchtop power supply at some expense, and an expensive circuit. An example of this type of design can be seen in the popular homebuilt nixie-tube high voltage switching power supplies, which output a maximum of approximately 200 VDC at very low maximum current (50 mA).
- A high-voltage AC outlet source could be converted to a low-voltage, current-limited DC with a common wall wart, such as a 120 VAC to 12 VDC wall adapter, then the DC converted to AC with a commonly found DC-to-AC inverter, for example, an automobile 12 VDC to 120 VAC inverter, followed by a smaller voltage step-up circuit to further boost the voltage. These automobile inverters are mass-produced, so they are inexpensive, easy to find, and relatively small.

Based on the prior requirements, the winning design is the latter, use of a common AC-to-DC wall wart, followed by a 12 VDC automobile inverter, with a twist: by using a European automobile inverter, it is easy to obtain a safe, current-limited 240 VAC as power input to a custom circuit. Sourcing this inverter also provides a ready, current-limited supply for 240 VAC directly. The 240 VAC is then

connected to a custom circuit to further boost and rectify the voltage. Note that a typical low-cost 12 VDC automobile inverter does not output a pure sine wave, thus the AC output will have additional noise characteristics.

The design of the custom circuit is straightforward (following the "Villard cascade voltage multiplier" circuit; see "Brief Overview of Cascade Voltage Multipliers" on page 47) and uses the principle of switched capacitors. Each capacitor will only have Vpk voltage across it (the half wave of the AC input) as the diodes are forward biased. The charge pump creates a doubling effect after the first stage of 2 × Vpk and a tripling after the second stage, to 3 × Vpk, up to 6 × Vpk in this circuit. Since the capacitors are "small," the charge capacity and hence the output current is not high. Also, since there are losses in the components, the efficiency will decrease for each stage added, so practically speaking, after a multiplier of 6, a large drop-off is expected—this depends on the components used. The losses are expended in heat and vibration. The resulting HVOUT voltage potential is measured between the last stage and the AC-VIN ground. Larger capacitors will allow for larger current capacity, at the trade-off of circuit cost.

Brief Overview of Cascade Voltage Multipliers

Villard cascade voltage multipliers use simple interconnections of diodes and capacitors to boost the voltage of an alternating-current (AC) input. When built with multiple stages, large direct-current (DC) output voltages are created. Keep in mind the common saying from physics, "You can't get something for nothing," meaning, a real circuit's output power is always less than its input power ($V_{in} \times I_{in} = P_{in} > P_{out}$). If voltage is doubled, the current will be theoretically halved; in practice, due to losses, it is more than halved. An added benefit of the cascade voltage multiplier circuit is the ability to directly use the voltage at the output of each stage, if intermediate voltages are desired. Read more about voltage doublers and voltage multipliers at Wikipedia.

Cost and Components

Table 6-1. Bill of materials

12 VDC to 240 VAC European automobile inverter	$15
120 VAC to 12 VDC wall wart with 12 VDC automobile plug output	$9
Power box for custom circuit	$3
Custom circuit components (see schematic)	$10
Double-sided copper clad board	$4
Homebrew etching materials	$8
Three-prong AC computer power socket	$1.50
Computer power cable	$1
European AC plug to USA plug adapter	$1

Building the Project

The schematic is pictured in Figure 6-1. The schematic includes sourcing information and part numbers for all of the electronic components needed.

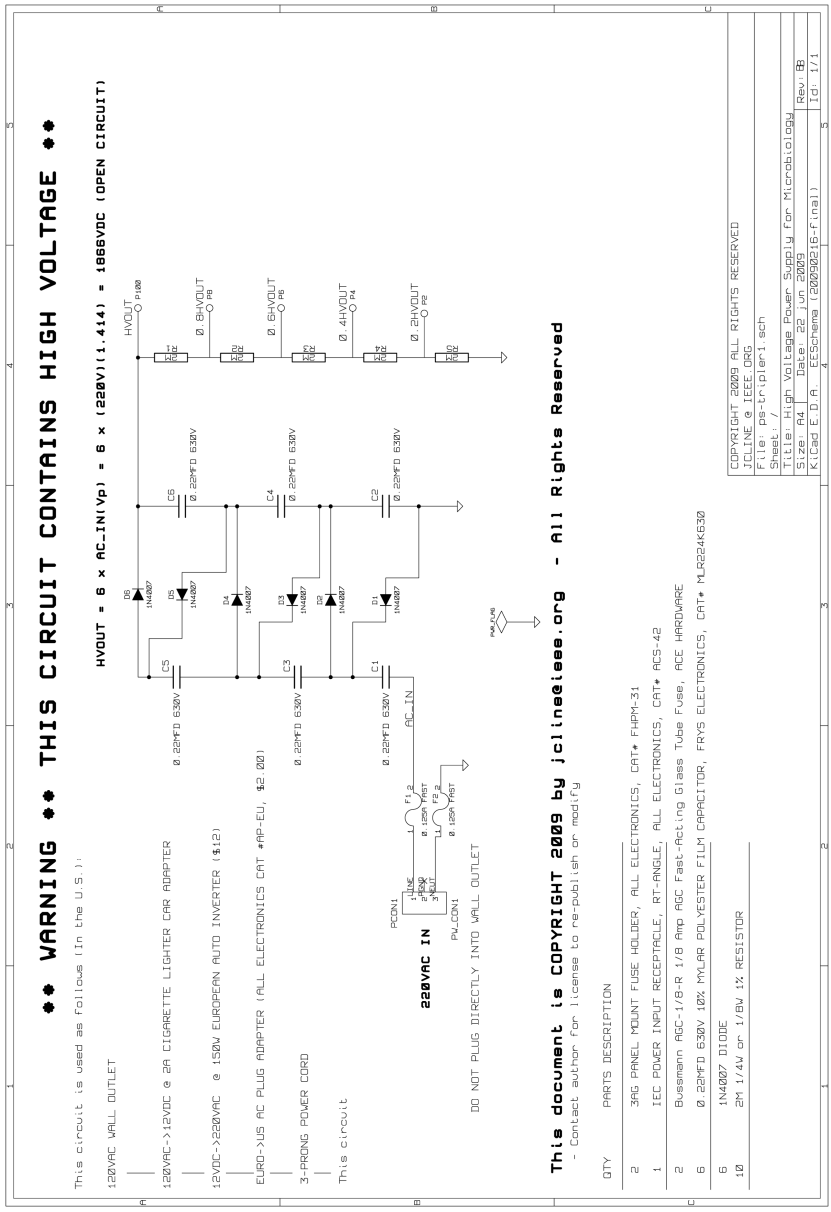
The board may be etched using the positives or negatives of Figure 6-2 and Figure 6-3. Figure 6-4 is used for the final board marking. Either toner transfer or direct inkjet printing may be used on a copper PCB to prepare the board for etching. If using toner transfer, a tabletop laminator is recommended. For etching, a solution of hydrogen peroxide and hydrochloric (muriatic) acid is a good choice, as this solution is reusable and more environmentally safe than ferric chloride. Muriatic acid for use in the etching solution is available from a home improvement store or a pool supply center. Both solutions will etch faster if slightly heated above room temperature. The spent H_2O_2 solution is easily disposable, unlike ferric chloride, although refreshing the solution by bubbling or stirring for reuse is preferred to disposal. Dispose of the H_2O_2 solution, after 20x dilution, in the toilet (if this statement causes any doubt, contact the local city water treatment and/or waste management office). Spent ferric chloride solution, which is not reusable, must be handled as toxic material and disposed of only at a certified hazardous waste disposal site.

If etching equipment is unavailable, the circuit board may also be assembled using a perforated board using point-to-point wiring. Use AWG 12 wire or heavier for construction.

Using These Figures to Etch a Printed Circuit Board

The figures are presented at their actual size in order to be used with the toner transfer method of printed-circuit-board (PCB) etching on a double-sided, copper-clad board. A variety of guides are available for PCB etching in a home lab. Other methods, or modifications of this method, are quite commonly used, such as printing the artwork on overhead transparencies instead of paper, using a modified ink-jet printer with special ink to print artwork directly on the board, or UV light (or sunlight) exposure of a commercially-prepared photoresist copper board. The toner transfer method using paper is suggested as a starting point, as it is widely practiced and, if completed carefully, is suitable for the copper trace widths used on this board. A simple overview of the process is detailed as follows. Print out the figures from this article with a laser printer, on standard printer paper. Prepare the double-sided copper-clad board by wiping with isopropyl alcohol then roughen the copper by scouring the surface with a green 3M Scotch-Brite pad. Clean the surface again with isopropyl alcohol and subsequently handle the board by the edges only; avoid touching the copper. Tape the figures in place, toner-side toward the copper, to both the front and back of the board, using the alignment marks to match the two sides. Transfer the toner to the copper by ironing with a typical household iron set on the cotton setting. The iron will heat the toner to bond it to the copper. Steady ironing pressure, constant motion of the iron, and a minute of heat should be sufficient on each side of the board. For etching boards regularly, a table-top laminator is quicker and more consistent than an iron, and a worthy investment. Allow the board to cool. Soak the board in hot water; the water will saturate the paper. Carefully peel away the paper, leaving the toner affixed to the copper board. Protective gloves (Latex or nitrile) and safety glasses are recommended for the steps that follow. Place the board in the warm etching solution and agitate the bath or stir the solution gently. Monitor the progress of the etching process periodically. Remove the board as soon as all exposed copper is dissolved. Removing the board from etchant early is preferable to etching too long, which may result in pitting of the copper. Rinse the board in water and prepare for drilling

each through-hole. Use a carbide drill bit in either a hand-drill or drill press with light pressure, or use a Dremel tool with an engraving bit, to bore a hole at each component location indicated with a center dot. Double check that all holes have been drilled. Scrub away the toner with a Scotch-Brite pad and clean the board again with isopropyl alcohol. Align the silkscreen figure to the component side of the board and transfer the toner to the board with an iron, as in the previous steps. Soak the board in hot water and carefully peel away the paper from the silkscreen figure as the final step. The toner of the silkscreen remains on the board permanently, for ease of assembly and later reference. The circuit board is now ready for component placement and soldering.



QTY	PARTS DESCRIPTION
2	3AG PANEL MOUNT FUSE HOLDER, ALL ELECTRONICS, CAT# FHPM-31
1	IEC POWER INPUT RECEPTACLE, RT-ANGLE, ALL ELECTRONICS, CAT# ACS-42
2	Bussmann AGC-1/8-R 1/8 Amp AGC Fast-Acting Glass Tube Fuse, ACE HARDWARE
6	Ø.22MFD 630V 10% MYLAR POLYESTER FILM CAPACITOR, FRYS ELECTRONICS, CAT# MLR224K630
6	1N4007 DIODE
10	2M 1/4W or 1/8W 1% RESISTOR

Figure 6-1. Circuit board schematic and part numbers

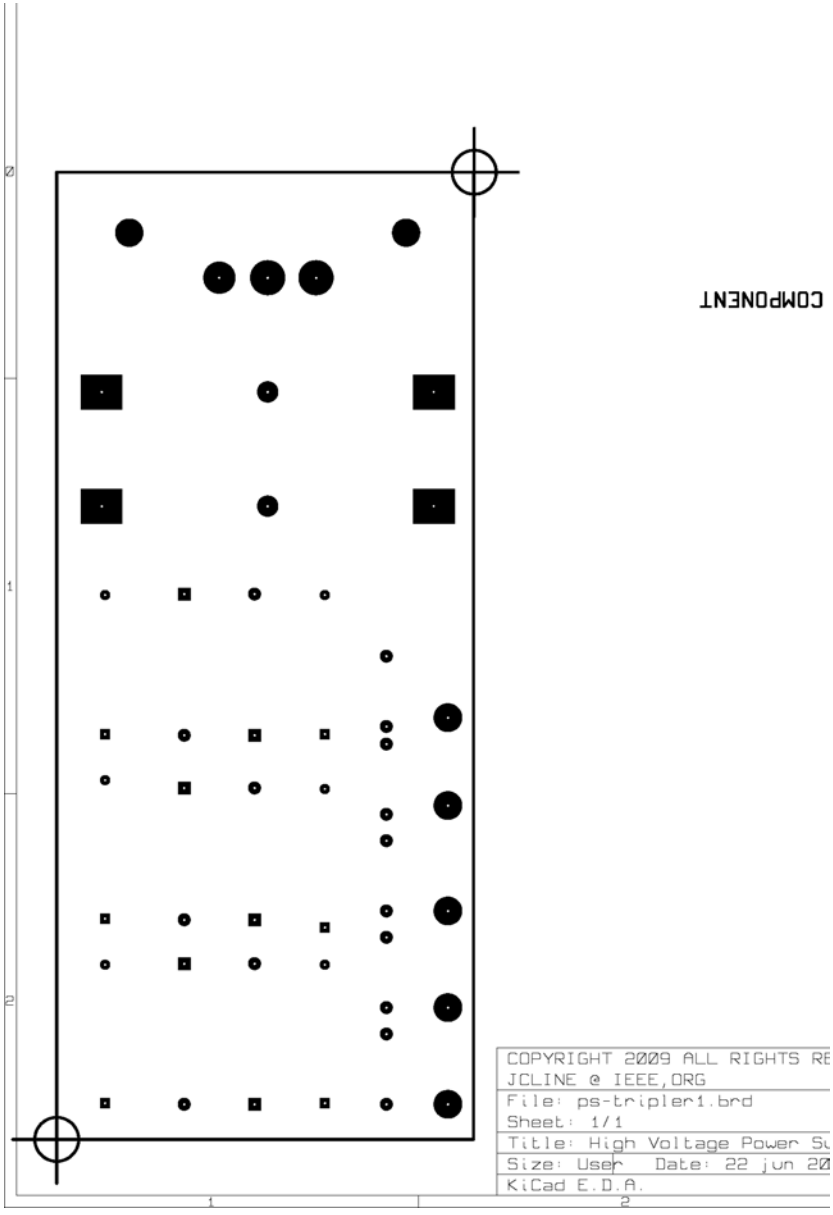


Figure 6-2. Circuit board component-side pattern

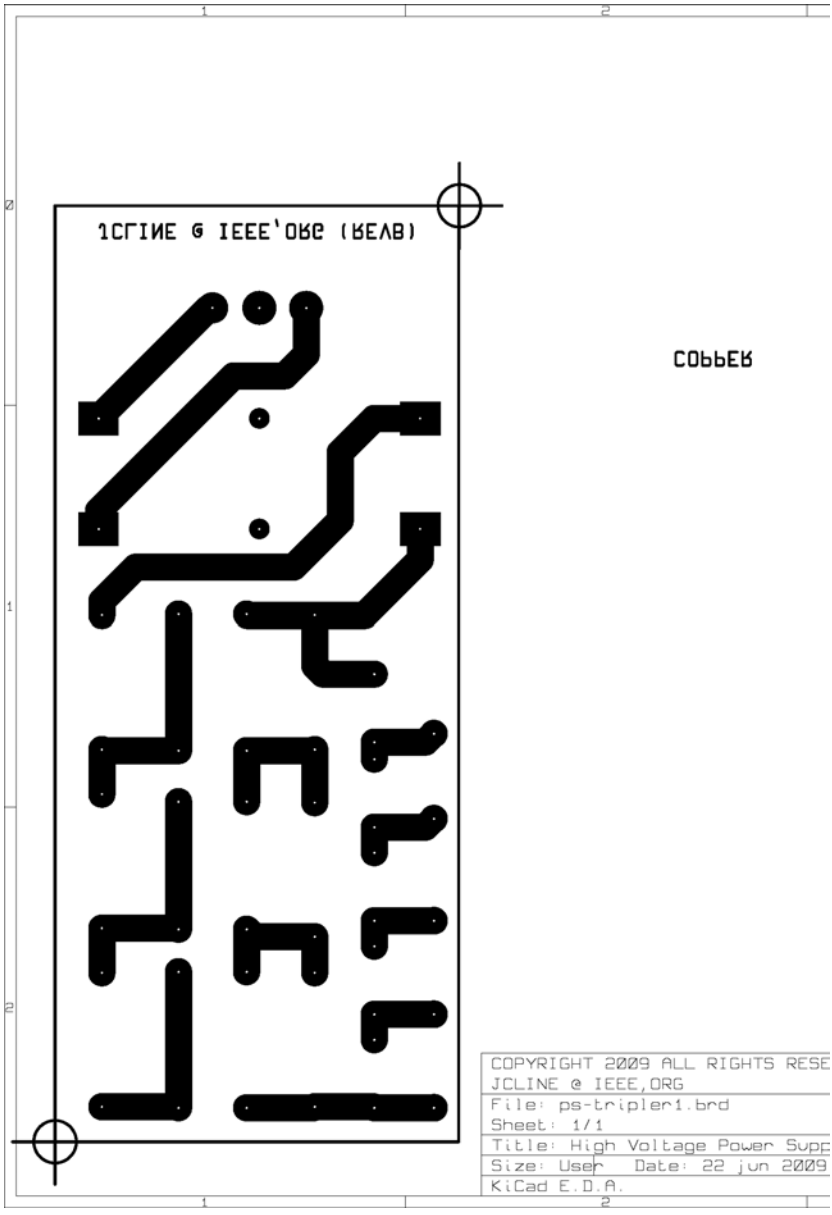


Figure 6-3. Circuit board copper-side pattern

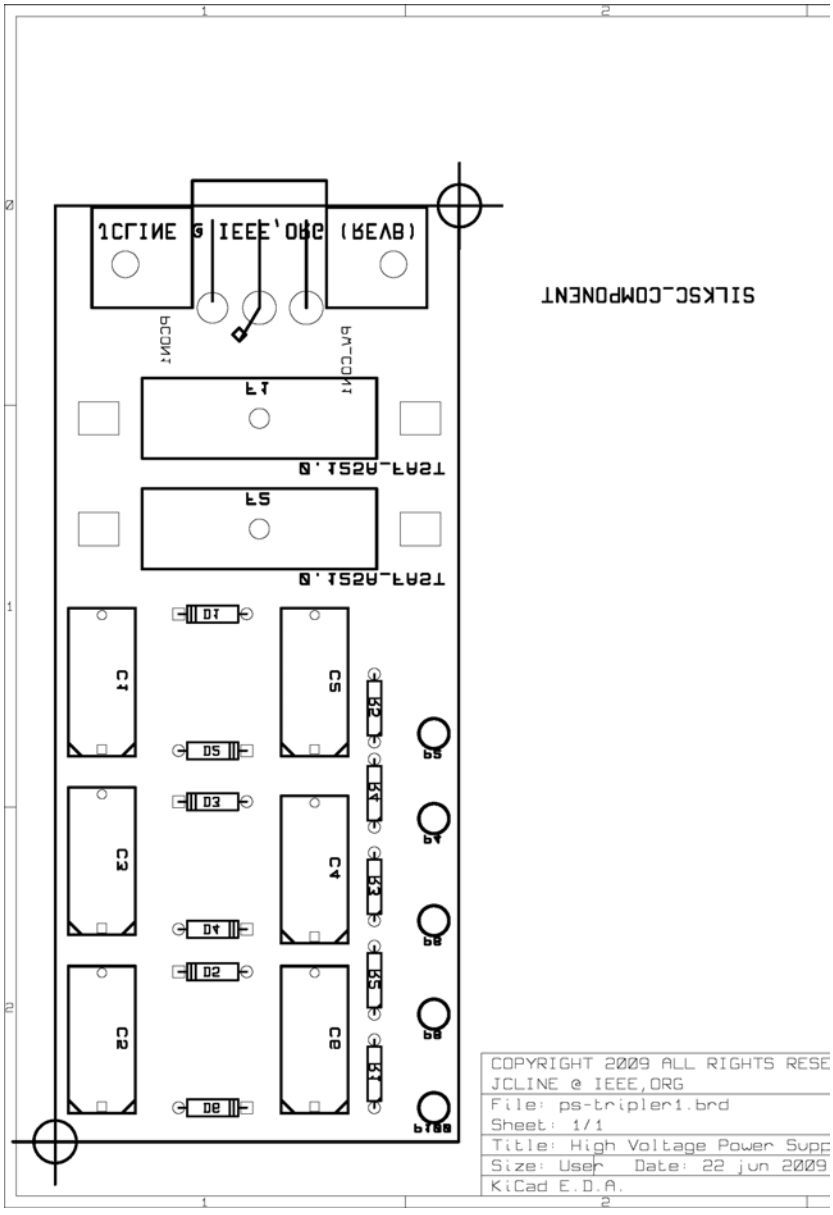


Figure 6-4. Circuit board silkscreen pattern

Assemble the circuit board using standard prototyping techniques, as discussed above. Verify continuity between major components of the circuit; this verification is an important step. Insert two fuses into the fuse holders. Fit the board into a power box with cover. A snapshot of a roughly completed assembly is shown in Figure 6-5.

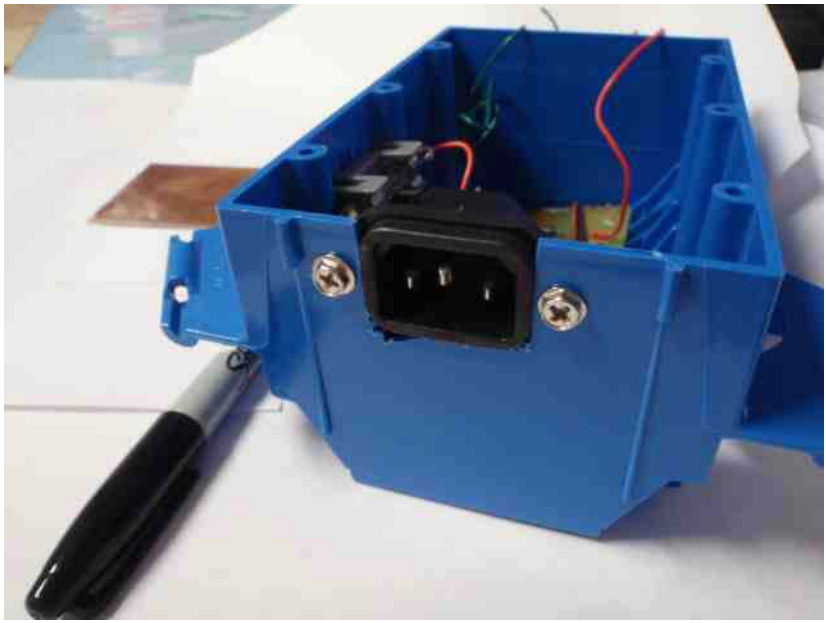


Figure 6-5. Rough cut of completed assembly

Connect power leads as desired from the output points of the circuit to the experimental setup. Power leads should be kept as short as possible. If the circuit is used frequently, consider using stranded high voltage transmission wire (an ignition cable or spark plug wire) on the output connection.

Usage

Prior to using the supply for the first time, or after a period of disuse, perform a continuity check on the major components. The outputs must register as an open circuit.

Power the circuit and use a voltmeter to measure the output voltage at each output tap. Disconnect and discharge the supply, then connect the desired output voltage tap to the experiment. Power the circuit and again measure the voltage at

the output voltage tap while connected to the experiment with a set of control or calibration reagents. Under load, the output voltage may drop. A significant voltage drop indicates that the supply is not providing enough current to the experiment, and a change to the experimental setup may be needed.

When powered and fully charged, the printed circuit generates a nice BZZZZ sound. This sound is normal and expected. The sound is created by the circuit board components internally discharging as the rated voltage potentials are met.

After the supply is no longer needed, disconnect the input power and discharge the outputs through a 1/4 W, 1 Megaohm resistor or larger. The supply should be allowed to discharge for a minute prior to disconnecting the outputs.

It is handy to perform output voltage switching operations through an automated, software-controlled circuit.

Conclusion

This high-voltage DC power supply design is deemed appropriate for various systems biology experiments where some voltage and current instability can be exchanged for simplicity and low cost. Of course, no claims can be or are made for either results or safety in practice.

Jonathan Cline provides his genome sequence data for immediate download as a participant in the Personal Genome Project, and invites readers to hack his genome, available at http://88proof.com/about. He holds a degree in electrical engineering, and specializes in embedded systems hardware and software, network protocols, and cryptography. Cline has proposed and demonstrated several biohacking projects. He can be reached via email at jcline@ieee.org. Digital copies of the PCB artwork are also available via email.

Beer Bottle Minipreps

Joe Rokicki

Introduction

Minipreps are the bread and butter of the genetic engineer. After constructing or procuring a DNA sequence of interest, one of the first next steps is often to insert that DNA into a larger circular piece of DNA called a plasmid. We do this because plasmids have the wonderful property of being autonomously amplified inside of bacteria. If you can get your DNA of interest into a plasmid and you can get that plasmid into a bacterium, all of the fancy, error-correcting, DNA copying machinery of the bacterium will go to work for you, automatically copying your DNA. You can sit back and relax and grow up a bacterial broth that will be rich in your DNA sequence. The only thing you have to worry about is breaking the cells open and purifying your amplified DNA back out, away from all the other genomic DNA, RNA, proteins, and miscellaneous cellular debris. This is where the miniprep comes in.

A miniprep is a column-based plasmid purification protocol where crude cell lysate is passed through a resin that specifically binds plasmid DNA and lets everything else pass through. The plasmid DNA is later released from the column in a highly pure and concentrated form. The miniprep's speed, ease of use, and purity have made it the de facto standard over other plasmid purification protocols such as phenol chloroform extractions and cesium chloride gradients. Yet, despite being one of the most universally performed protocols in molecular biology, the miniprep is also often one of the least understood, even among scientists, for the simple reason that the vast majority of labs take advantage of commercially prepared kits that require zero understanding of what you are actually doing. A little investigation quickly reveals that the active ingredient of the mystery column resin is actually silica, the most abundant mineral on earth. Further, the reaction that causes silica to specifically and reversibly bind to DNA turns out to require nothing more than salt. While the identity of the salt affects the efficiency of the binding (I will go into

more detail about this in the next section), at the end of the day, this isn't an exotic reaction requiring rare chemicals or dangerous reactions. It is simply sticking DNA to sand with salt.

Richard Feynman once famously said eight words that, decades later, scientists at the Venter Institute went on to immortalize (mortalize?) by encoding into the genomic sequence of the first synthetic organism: "What I cannot create, I do not understand." In this article, I will explain the basic principles that allow the miniprep process to isolate plasmids and show you how to create (and therefore understand!) your own DIY miniprep spin columns from commonly available materials.

Background

Commercial minipreps work on two principles. The first is alkaline lysis. In a highly basic solution with a little detergent, bacterial cells break open and their contents denature. DNA's iconic double-stranded helix unwinds into separate single strands, and proteins unfold from the characteristic shapes they need to function. If the pH is just right, the plasmid DNA will behave differently from all the genomic DNA. Because it is in a highly twisted "supercoiled" state, it is able to better resist the DNA unwinding effects of a basic solution. As a result, when the alkaline lysate is later neutralized with acid, the denatured proteins and genomic DNA will all coagulate into an insoluble mass and precipitate with the detergent; but the plasmid DNA, which was never denatured in the first place, will stay separate and in solution.

The second principle minipreps leverage is silica adsorbtion of DNA at high concentrations of chaotropic salt. A chaotropic salt is a salt that is really good at denaturing proteins. It turns out that one of two things usually happens when you add a lot of salt to a protein solution. Some salts mess up the sphere of water molecules that surround a protein and cause the protein to aggregate with other proteins and precipitate. This is called "salting out." Other salts denature proteins into super soluble unfolded amino acid chains. This is called "salting in." Salts can be ordered according to their propensity to either "salt in" or "salt out." The "salting in" side of the salt spectrum is called *chaotropic*. The "salting out" side is called *kosmotropic*.

For some reason, at high concentrations of chaotropic salt, nucleic acid sticks to silica. There are differing theories on why exactly this happens; and while the chaotropic nature of the salt seems to increase the efficiency of adsorption, even common kosmotropic salts like NaCl have been reported to cause adsorption of nucleic acid (*http://www.ncbi.nlm.nih.gov/pubmed/22537288*, LA Biohackers).

I'm leaving out a few details here, but in general, this is how a commercial miniprep works. First, the cells are pelleted and resuspended in a neutral buffer. This is important so that the pH of the solution can be tightly controlled in the next steps. Second, we add the highly basic solution with SDS that will lyse the cells and irreversibly denature everything except for the plasmid DNA. Third, we add an acidic solution that will neutralize the basic solution we just added and cause the denatured genomic DNA and proteins to precipitate out. They are then removed by a high-speed centrifugation step. The acidic solution also contains tons and tons of chaotropic salt so when we run the salty, neutralized, clarified cell lysate through a silica column, the plasmid DNA is adsorbed. The column is then washed with an ethanol solution that will remove the excess salt but leave the plasmid DNA on the column. Finally, a small volume of water is added that is sufficient to break the adsorption interaction between the plasmid DNA and the silica and elute the DNA from the column. This small volume of pure and concentrated plasmid DNA is our final product.

DIY Minipreps

To make our own version of this system completely from scratch, we will need to make our own solutions and our own spin columns.

MINIPREP SOLUTIONS

Several excellent recipes for DIY miniprep solutions are already available online. In the following experiments, I used the solutions P1, P2, N3, and PE described here with one small modification. I used acetic acid to bring the pH of solution N3 down to ~4.2, which was the pH I measured for the commercial solutions. Alternatively, in true DIY spirit, LA Biohackers have published an alternative protocol that uses only commonly available chemicals and materials.

SPIN COLUMNS

This is where you get to do some MacGyvering. With minimal effort and only commonly available materials, we can hack together a surprisingly professional miniprep spin column. This method is adapted from a protocol for eluting water from small pieces of filter paper, but I have found it works excellently for DIY minipreps as well. First, place a 0.65 mL tube inside of a 1.5 or 2 mL flat bottom freezer tube. Puncture the 0.65 mL tube twice with a small-gauge needle. I used a 5/8 inch, 25-gauge needle that was just long enough to puncture the 0.65 mL tube, but not long enough to reach the bottom of the 2 mL freezer tube, so I didn't have to worry about

sticking myself (see Figure 7-1). It is important to use a flat-bottom type tube for the collection tube as opposed to a standard 1.5 mL tapered tube because you want to have at least 500 uL of volume below the inner 0.65 mL tube to catch column flow through. The last step is to fill the inner 0.65 mL with a silica-containing material.

Figure 7-1. The needle is used to puncture the smaller 0.5 mL tube. The 0.5 mL tube is filled with a silica-containing compound. This is then placed inside a larger flat bottom 2.0 mL tube to form the DIY miniprep column.

Silica Resin

To find a good resin to pack our column with, I experimented with several commonly available sources of silica. WARNING: Silica dust is dangerous to breathe. If silica is being crushed, do so in a hood, underwater, or while wearing a respirator. Don't breathe dust containing silica:

Crushed "crystal" cat litter
: This stuff is great. It's tiny chunks of silica gel. It's the same stuff in the desiccant packets they stick in with shoes and beef jerky to keep them dry. It looks kind of like broken safety glass. I placed two small chunks in a column and crushed it with a screwdriver. It crumbles easily into powder.

Uncrushed crystal cat litter
: To make sure the crushing was necessary, I tried a column with two pieces of uncrushed silica gel.

Powdered 90 Shilling beer bottle
: I thought it would be cool to make a "beer bottle miniprep," but, compared to working with silica gel, crushing glass bottles is a huge pain. I broke the bottle in a plastic bag with some water in it using a hammer, moved the slurry to a 50 mL conical tube, and continued to crush the glass to powder underwater using a screwdriver. After much grinding, I resuspended the slurry, allowed it

to settle for an hour, decanted the supernatant, spun it down, and collected my hard-earned glass powder.

Celite 454
: This is fancy diatomaceous earth that I had on hand. You can buy food-grade diatomaceous earth online for cheap.

Sand
: Regular, fine beach sand.

Commercially available miniprep column
: I used a Qiagen miniprep column.

Methods

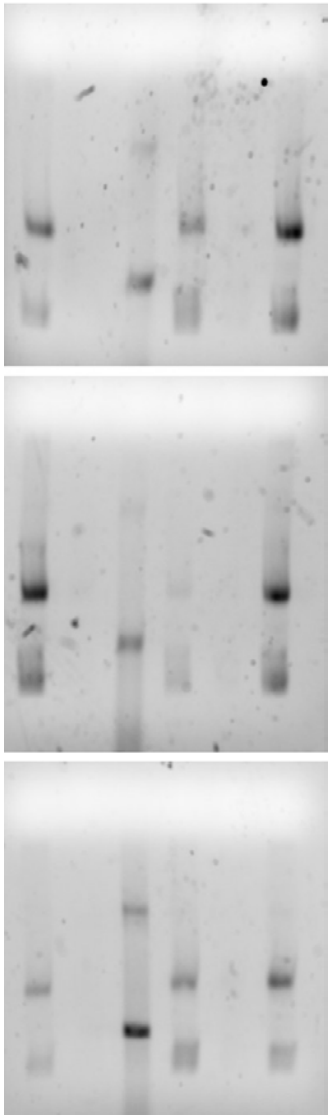
- A flask of 25 mL LB containing 100 ug/mL Amp was inoculated with E. coli containing high-copy plasmid A. A second flask of 25 mL LB containing 100 ug/mL Amp was inoculated with E. coli containing high-copy plasmid B. These two cultures were allowed to grow overnight then were pelleted and stored at –20°C until use.
- The cultures were each resuspended in 2.5 mL of buffer P1 and combined for a total of 5 mL of saturated bacterial culture.
- This suspension was divided into 18 aliquots of 250 uL in 1.5 mL tubes.
- 250 uL of buffer P2 was added to each solution. The solutions were inverted five times.
- 350 uL of buffer N3 was added to each solution. The solutions were inverted five times.
- The suspensions were spun at full speed for 10 minutes on a microcentrifuge (>10,000 RPM).
- The following procedure was performed three times on each of the six columns described above.
- 500 uL of supernatant was added to the column. Column spun at full speed for 15 seconds. Flow-through discarded.
- The remaining ~170 uL of supernatent was added. Column spun at full speed for 15 seconds. Flow-through discarded.
- 500 uL of buffer PE was added to the column. Column spun at full speed for 15 seconds. Flow-through discarded.

- 500 uL of PE was added to the column a second time. Column spun at full speed for 15 seconds. Flow-through discarded.
- Column spun at full speed empty for one minute to remove excess wash buffer.
- Column moved to fresh 1.5 mL tube. 50 uL of nuclease-free water added to column. Column spun for one minute at full speed.
- Eluate (50 uL) reapplied to column and spun again for one minute at full speed.

Results

The three gels here are technical replicates. Each gel contains six lanes where the only difference from lane to lane is the column used to perform the purification. The columns used in each lanes are: 1—crushed crystal cat litter, 2—uncrushed crystal cat litter, 3—crushed beer bottle glass, 4—diatomaceous earth, 5—sand, 6—commerically available miniprep column. The two bands visible in most of the lanes correspond to the two plasmid that were copurified as described in the methods. Uncrushed crystal cat litter and sand both failed to purify any DNA in any of the experiments. Crushed crystal cat litter, beer bottle glass, diatomaceous earth, and the commerical miniprep column purified DNA in all replicates, although the DNA purified with beer bottle glass ran slower, perhaps because of DNA sheering. In summary:

- The crushed-crystal-cat-litter resin worked as well as a commercial miniprep, followed closely by diatomaceous earth.
- Crushing the crystal cat litter is necessary; the column with uncrushed resin didn't yield any DNA.
- Powdered beer-bottle glass appears to sheer the plasmid DNA, resulting in bands that migrate slower than the plasmid DNA purified with other resins.



Discussion

These columns are cheap and easy enough to be disposable. To save even more time and money, this recycling protocol is an effective way to destroy any contaminating DNA and reuse the same column many times. Have fun!

Joe Rokicki is an electrical engineer turned molecular biologist. He is currently a PhD student studying evolution in bacteria at the University of Colorado at Boulder. He has been passionate about synthetic biology since his attempts to make "self-flavouring yogurt" with the 2007 University of Edinburgh iGEM team. Most recently he founded the iGEM program at CU and has coached and advised several iGEM teams there.

Fun and Fights with Fungi, Part 2

Derek Jacoby and Vince Geisler

When last we left the stories of our fungal adventures, we had just sent the first two successful PCR-amplified mushroom samples off for sequencing. I was so sure that this was to be a triumphant presentation of dozens of barcoded samples. But we're still in the midst of it. Today we'll follow through on the bioinformatics of a successful barcoding sample, but most of our mushrooms are still unknown. We've just received the next level in the chemical arsenal to break usable DNA out of the mushroom, but we can report on our attempts so far. Outside of this last effort (which uses decidedly DIY-unfriendly chemicals such as chloroform) we've had interesting failures with sonicated samples (evenly sized DNA fragments [!] that are too short for our barcoding), with alkaline cell lysis buffers and detergent-based cell lysis buffers.

But let's start with a description of the main problem. We have a mushroom sample we found at one of the mushroom shows, we may or may not know what mushroom it comes from, or more interestingly, we may think we know but are wrong. This mushroom has a genetic region that is not under evolutionary pressure, so it randomly accumulates mutations over evolutionary timescales. It turns out that due to this rate of genetic drift, different species of mushroom tend to have different DNA mutations in this region, causing it to be known as a barcoding region. There are a number of steps we have to follow in order to get our mushroom sample's genetic sequence so that we can look it up in a database of known species. First, we have to first get the DNA out of the cells, then amplify our barcoding region using the polymerase chain reaction (PCR), then finally send it away for sequencing.

This is what we've been working on. Mostly the first step, so far. Last time, we talked about our troubles in disrupting the cells of the mushroom to release DNA

for us to work with. One of our interesting attempts involved trying to mechanically break apart the cells using a sonification bath. This is just a Westinghouse parts washer bath, the kind where you'd normally throw your dirty, gummed-up car part in some solvent. You turn on the switch and it breaks the dirt and gunk off using transducers that produce a buzzing mostly above our range of hearing. So we thought maybe it would break our cells apart!

It was really successful. Too successful. The DNA came out sheared into about 500 base pair (bp) lengths. In the image at the end of this article, you can see the raw, purified DNA after it had been extracted from the cells but before PCR. It's the band in the second row next to the ladder. This is great, except that the piece we want amplified is about 800 base pairs long. If all our chunks are only 500 bp, then no template DNA will exist to allow the PCR reaction to complete.

This is an interesting failure, though. We have no easy way to adjust the frequency or power in this parts washer, but with some work put into understanding the circuit and modifying it, we could adjust these variables. It did break the DNA out well, even if not into chunks we could use.

Which brings us back to the main problem: an inability to get DNA out of the cells. We've discovered we can't break it apart too much or we get small fragments like in the sonicated samples.

Let's look at the other samples. Our sole success, detailed below, was a jelly fungus. The others either produced no DNA or produced multiple bands after PCR amplification indicated an unusable sample. The prevailing theory is that polysaccharides in the fungal cell wall are binding with DNA, so we're not getting any DNA from our purification protocol. We are using two different strengths of detergent for cell lysis, with and without disruptive sonification, so no release of DNA is unlikely. We have run successful barcoding reactions on insects with the same reagents to ensure that other aspects of our setup and technique are correct. If we are breaking the DNA out, but not recovering it, it seems likely that something in the mix is holding onto it. Enter the CTAB extraction. Essentially we break the cell with a strong detergent and then take the cell debris and cell wall component out of solution by dissolving them in chloroform. Interestingly, this leaves the DNA in the aqueous fraction so that we can work with it.

So in a way it's a failure, because we needed to step back to chloroform and CTAB and the harsh chemicals that we hadn't wanted to use. However, we are getting some DNA with the gentler processes, so let's look at one of those sequences in more detail.

Sample 22 in last issue's article was identified by the experts at the mushroom show as Pseudohydnum gelatinosum, a jelly fungus. It was the one with a single clean band in the gel that accompanied that article. When we got the sequence results back, we had two files, one from the forward direction and one from the reverse direction. This is important in longer sequences because of the limits of Sanger sequencing. Since we have an 800 base pair barcoding region, and the sequencing provider we use guarantees only 650 bases of good sequence read, we decided that reading from both directions was an important thing to do. To download the files in this section, go to *http://reddwarf.ca/* and click Biocoder at the top. Our results (*2_M13F.txt* and *2_M13R.txt*) arrived in what is called FASTA format. This is simply a text file format of sequence data. The first file is the forward sequence. We can now copy just the sequence information from that file (*2_M13F.txt*) and then paste it into the identification program at *http://bit.ly/100edZy*.

In this case, it returned our expected Pseudohydnum identification with about 99% sequence similarity, which was confirmed with the reverse direction read. The exact nucleotide match to our sequence is not found, and in fact, the returned results indicate a match with an uncultured Pseudohydnum species rather than the Pseudohydnum gelatinosum identified by our mushroom expert. So it's time to enter it into the database!

As an aside, if you have never looked at sequencing results, please feel free to have a look at these files from *http://reddwarf.ca/*: *positive.control.ab1*, *2_M13F.ab1*, and *2_M13R.ab1*. Aside from the text file, the *.ab1* files are a view of the actual chromatograph for the sequencing run. In Sanger sequencing, the sample is amplified by PCR; but instead of just using normal nucleotides, a small fraction are fluorescently labeled. When a labeled nucleotide is incorporated into the PCR product, amplification is not able to continue beyond that point. Since incorporation of the labeled bases is random, you end up with an assortment of partial products each with a labeled base at the end. These products are pulled through a capillary electrophoresis machine to sort them by size, pulled up by electric potential past a reader that reads the fluorescently labeled bases. The *.ab1* file is a recording of those readings for each base, usually a clear single color, but sometimes there will be doubt at some of the bases. There are a number of programs that can read these files, but we used a trial of CodonCode Aligner.

At this point, we used up most of our samples in trying different extraction protocols. Lessons for this year's mushroom show season include collect more material and get our protocols down early (and maybe use grocery store mushrooms to tune the procedures rather than our precious samples!).

Although the mushroom project itself hasn't been as successful as we'd hoped, it's been a lot of fun, and the side projects on the way have resulted in better PCR skills, a much-improved gel documentation station, and the completion of our fume hood (to work with chloroform). It was definitely worth doing, even if only for the side projects! Thanks for following along.

Vince Geisler is an electronics and manufacturing expert who is recently converted to biohacking. Based in Victoria, BC, he runs a contracting company that has done everything from building satellite electronics to installing vast arrays of radio towers to provide Internet to the Kootenays. He is currently building out the Makerspace biology lab and working on a giant 3D printer.

Derek Jacoby spent a decade at Microsoft working on speech recognition and is now a PhD candidate at the University of Victoria. He is a founder of makerspace.ca and has just overseen its move out to a new facility, where the biology lab is moving into the same building as the rest of the manufacturing-focused hackerspace.

Real Vegan Cheese

Patrik D'haeseleer, Marc Juul, and Craig Rouskey

Figure 9-1. Team photo

Cheese. The food that all people love. Unless, of course, you're lactose intolerant, have an autoimmune reaction to cow, goat, or sheep milk proteins, or for a variety of reasons have chosen to be vegan. While a number of vegan cheese products exist, they all rely on a combination of alternate protein sources and thickeners to approximate the coagulation phenomenon that occurs during cheesemaking [1]. Thus, while vegan cheese manufacturers have created many delicious products, and even passable substitutes for some types of cheese, none has so far been able to deliver anything resembling a sharp chedder or aged Gouda. In response to the needs of people with dietary restrictions around animal-derived cheese products, the Real Vegan Cheese team has emerged from the San Francisco Bay Area to deliver broadly consumable real vegan cheese. The team is a collabortive effort between Oakland-based Counter Culture Labs and South Bay–centered BioCurious. These biohacker organizations have joined forces to enter their Vegan Cheese

project into the International Genetically Engineered Machine (iGEM) competition taking place in October 2014.

On paper, making *Real Vegan Cheese* should be simple. First, genetically engineer brewer's yeast to produce cheese protein. Then, grow the yeast in a bioreactor and purify the protein. Combine the cheese proteins with a vegan milkfat replacement, a (nonlactose) sugar to feed the ripening bacteria, and water to produce a sort of vegan milk. From there, proceed with the age-old traditional cheesemaking process for the desired type of cheese.

In practice, it gets a bit more complicated. Milk, it turns out, is a fairly complex substance. It is nature's solution for packing large quantities of protein, calcium, and fat into liquid form that can turn into a solid for prolonged nutrient release in a suckling mammal's stomach—a pretty impressive bit of biochemistry.

The Structure of Cheese

Time for some dairy science: given the right pH and calcium concentration, four of the more hydrophobic milk proteins assemble into micellar aggregates that include calcium ions and milkfat molecules. One of these caseins, kappa-casein, acts as a sort of built-in surfactant by making up the surface of the micelle and extending its hydrophilic tail into the watery solution. Making hard and semi-hard cheese usually involves the use of the rennet enzyme Chymosin, which cuts kappa-casein's hydrophilic tail, making the now hydrophobic micelles link together into a network, forming cheese curd. This process is so efficient that it takes as little as 40 minutes to convert milk into something akin to a block of soft tofu floating in a watery solution.

It's not surprising that vegan cheese is hard to make. The cheesemaking process relies on specific protein interactions that simply do not occur with proteins from any nonmammalian source.

The obvious solution is to invent a nonmammalian source of the required proteins.

Genetic Engineering

To make the cheese-protein-producing yeast, first, the genetic sequences that code for milk proteins in mammals have to be analyzed and the DNA sequence optimized for expression in yeast. The important four proteins for cheese are the four caseins: kappa, beta, alpha-s1, and alpha-s2. The genetic sequences for the yeast versions of these proteins are combined with a secretion signal (alpha-factor) that will cause the proteins to be secreted from the yeast cells. The sequences are

synthesized and inserted into a plasmid with an inducible promoter so the expression can be controlled during growth, and the plasmid DNA is transformed into baker's yeast (Saccharomyces cerevisiae). The yeast is then grown in vegan broth media, where it expresses and secretes cheese protein, which can be separated and purified.

So far this is all standard genetic engineering, and while getting the yeast to express and secrete enough protein may prove challenging, this part of the project should not present any extraordinary problems—in fact, we've already discovered published papers demonstrating that most of the casein proteins can be expressed in yeast or E. coli. The difficult part, it seems, is getting the purified protein to form correctly into micelles in imitation of the structure in milk. Getting transgenic proteins to fold correctly is often a problematic endeavor, usually involving considerations of protein secondary structure, tertiary structure, and post-translational modifications such as phosphorylation and glycosylation. So far, our research indicates that folding of individual proteins will not be an issue. With regard to glycosylation, its influence on micelle formation remains an open question. But even a glancing review of dairy science literature will show that correct phosphorylation is likely to make or break the project. Interestingly, though protein phosphorylation was originally discovered in casein and discovered way back in 1883 [2], the kinase enzyme responsible for phosphorylation wasn't identified until 2012 [3]. In mammals, this unusual kinase—no, not "casein kinase," but one named Fam20C—is actually secreted along with the cheese proteins, and our team is designing for a similar effect in yeast. By altering the kinase secretion sequence, optimizing the kinase sequence for yeast, and co-expressing it with cheese protein, the secreted proteins should emerge correctly phosphorylated.

Molecular Gastronomy

Even with a perfect set of mammalian cheese-proteins, our experiments have shown that the process of turning purified proteins into micelles in solution is not as simple as mixing and stirring. Our research suggests that precisely controlling pH and calcium concentration while using a combination of techniques such as ultra-sonication and colloid mill homogenization are likely to achieve the desired results.

Narwhal Milk?

Each of the four casein proteins found in dairy milk has dozens of different genetic variants that occur in different cow breeds, not to mention goats, sheep, yaks,

camels, water buffalos, and more. Many of these genetic variants are associated with different coagulation properties of the milk, various health effects (check out the hype around "A2 milk," for example), or even allergic reactions. Since we have full control over the DNA sequences, we have the option to pick and choose exactly which of these genetic variants to incorporate. Since one concern is minimizing the chance of an allergic response, an obvious choice is to use human genes, although we have noticed a sociologically interesting "ick factor" around the idea of drinking human-derived milk. Finally, for packing the maximum amount of protein per volume into milk, nothing beats a whale. Thus, our team has opted to engineer three varieties based on genes from three different species: human, cow, and narwhal.

Social and Ethical Concerns

Factory farming often entails treating animals less well than most of us would like, and it is likely that providing better alternatives will decrease demand for traditional products and thus decrease the number of poorly treated animals. Using genetic engineering to achieve such a goal makes this an interesting ethical quandry for many of those who oppose GMOs and champion the ethical treatment of animals. There are three important points that should be considered when addressing this issue:

1. Real Vegan Cheese will not contain any GMOs.

   The genetically engineered yeast is only used to produce milk-protein.

   The yeast itself stays behind while only the milk-protein becomes part of the cheese.

2. The yeast will be contained in bioreactors, not grown freely in the environment.

   Additionally, the strains of yeast will be engineered to prevent them from growing outside of the intended bioreactors.This will prevent environmental contamination and contamination of the products of nearby yeast farmers (brewers and bakers).

3. This method of production has been used for more than three decades, safely, successfully, and at large scale to produce anything from vanillin (vanilla flavor) [4] to life-saving drugs such as insulin [5] and affordable malaria medicine [6].

These issues should be taken into account when evaluating whether the dangers of genetic engineering outweigh the potential for reducing animal mistreatment.

As a relevant comparison, most of the cheese produced today is made with a rennet enzyme manufactured using genetically engineered organisms grown in bioreactors, which has limited the need for harvesting rennet from the stomach linings of young cows [7].

Environmental Impact

The cost of yeast-based production of cheese protein makes it unlikely that it will pose a threat to traditional methods in the near future. It is likely, however, that this method will provide an alternative for those with dietary restrictions, whether ethical, religious, or health related.

That being said, production of milk and cheese using milk from factory-farmed animals has a host of environmental and ethical problems, and it is important to understand if this method of production will be preferable from an environmental standpoint.

The conclusion of the 400-page UN report "Livestock's long shadow" has this to say:

> *...the livestock sector is a major stressor on many ecosystems and on the planet as whole. Globally it is one of the largest sources of greenhouse gases and one of the leading causal factors in the loss of biodiversity...*

While our team is still working on a comparison of the expected impact on climate change per gram of cheese produced using traditional methods versus yeast-based production, it will likely be relatively simple to contain the carbon dioxide released from large bioreactors, while doing the same for the methane produced by grazing cattle poses a unique and difficult challenge. At first glance, the required food source for yeast is potentially less favorable than that preferred by cows, since yeast's preferred diet of sugars makes it compete with humans for arable land capable of supporting sugar-producing plants, whereas ungulates are able to digest foods that grow on land less suited for traditional crops. In reality, both bioreactors and livestock are often fed with various industrial byproducts not fit for human consumption, which complicates the comparison and makes it difficult to ascertain how bioreactors compare to cows in their effect on biodiversity and on the global food supply. A more thorough analysis is part of our effort, and we welcome anyone who wishes to collaborate or critique.

How to Support

Contributors are invaluable to us and throughout this project have taken many forms. Our collaborators and supports provide financial resources, time, or even scientific resources. This is an open community project, and meetings are held every week in Oakland or Sunnyvale, and remote participation is possible via video conferencing. Discussions take place on our mailing list: *http://groups.google.com/group/ccl-igem*. All of our research notes and meeting minutes are available on our wiki: *http://wiki.realvegancheese.org*. If you would like to support us by turning your dollars into cheese, visit *http://support.realvegancheese.org/*.

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Patrik D'haeseleer is a bioinformatician by day, mad scientist by night. He is a cofounder of Counter Culture Labs, community projects coordinator at BioCurious, and scientific advisor of the Glowing Plant project, none of which are in any way related to or funded by his day job at the Lawrence Livermore National Laboratory. The views presented here are his own and do not represent those of BioCurious, the Glowing Plant project, or LLNL.

Marc Juul is a full-time hacker with a background in network programming and synthetic biology. Having cofounded the hackerspaces Labitat, Sudo Room, and Counter Culture Labs, he spends his time co-organizing these spaces and developing free and open software, hardware, and wetware for a variety of community-oriented projects, including People's Open Network, the Hackpack Project, and the upcoming Free Store.

Craig Rouskey is a molecular biologist, immunologist, and biohacker with research expertise in antibody therapies, adoptive T cell therapies for leukemia and prostate cancer, and bacterially vectored vaccines. He is passionate about projects that not only innovate scientifically, but culturally and as a result is the principal scientist for the nonprofit Immunity Project (#hackHIV). Craig can also be found teaching DIY molecular cloning classes at Counter Culture Labs.

Synbio Axlr8r Teams

Connor Dickie

This summer, five startup companies have gathered in Cork, Ireland, to participate in SynBio Axlr8r, a venture fund that focuses on entrepreneurs building technologies in or around the field of synthetic biology. Each team has received $30k in funding, access to wetlab space at the Univeristy of Cork, and a global network of mentors.

As an Axlr8r mentor (as well as a recipient of funding from Axlr8r's parent, SOSventures, for Synbiota Inc.), I've had the pleasure to visit with many of the teams in person. I thought the BioCoder community would be interested to learn a bit more about each team and their projects.

With this in mind, I've asked each team representative the same three questions:

1. Who is on your team? What are your backgrounds? How did you meet?
2. What is your Axlr8r project, why are you doing it, and what's the potential impact?
3. Wildcard—tell BioCoder something interesting and relevant that does not fit within the preceding questions.

Sarah Choukah of Hyasynth Bio

We're Hyasynth, a team of six young researchers, entrepreneurs, and scientists. Our profiles include experience working on award-winning iGEM teams, with companies including Synbiota, Novozymes, and Genomikon. In addition, we have academic backgrounds in synthetic biology, biochemistry, and communications. We met in August 2013 as cofounders of Montreal's DIYBiology and biotech community group, BricoBio. We were brought together by common goals we all share.

The first is enabling innovation in science and biotech outside of its institutional or conventional confines. Second, we want to develop more affordable and open means of doing research to improve people's well being. Third, we all have an infallible passion for biology and technology. We want to build and act on our global, collective need to better understand biotechnology in the best way possible.

We are currently working on the biosynthesis of cannabinoid compounds that can be used in therapeutic and medicinal applications. Many improvements and shifts in the legal status of medical marijuana in several US states and countries have either been made recently or will happen in the next few years. The situation is ripe for a change in cultural attitudes toward the use of cannabis for the treatment of several conditions—muscular dystrophy, epilepsy or chronic pain and inflammation, and potentially many more to be discovered. And, this is only our first target. Using simple microorganisms, we can replace acres of land used to produce compounds and transform light and waste into life-saving pharmaceuticals, to name only a couple possibilities.

We work 21-hour days, starting in the early morning in Cork and ending in the late evening in Montréal, Canada. Coordinating through challenges posed by physical distance, regulatory differences, and organizational cultures is crucial for the team. At the same time, we are also still enrolled in master's or PhD programs. This is a challenging balance, but it is far from impossible, and we are making the most of it. The mix of entrepreneurship and academic mentorship brings the best out of both worlds by pushing our research techniques to their limit while gaining the essential skills to build our company. The shared knowledge and the time that we put in brings us together more tightly as a team.

Twitter: @HyasynthBio

Website: *http://hyasynthbio.com/*

Nikolai Braun of Revolution Bioengineering

Revolution Bioengineering is a collaboration between Keira Havens and myself, who are lucky to call Fort Collins, Colorado home. Although we grew up on opposite corners of the United States—me in the suburbs of Washington, D.C. and Keira in Kailua, Hawaii—we were both fascinated by the natural world around us and pursued degrees in biological sciences. After earning a PhD in biophysics at UC Davis, I worked in Manchester, England, studying yeast physiology while Keira was commissioned into the United States Air Force. She later separated to pursue a master's degree in a plant synthetic biology lab. I had joined the lab the week before, and we spent four years working at the cutting edge of academic plant science research.

During this time we discovered a shared professional ambition and entrepreneurial spirit, so we took a chance and founded Revolution Bioengineering to make the world a more beautiful place.

Revolution Bioengineering is our company, and making beautiful flowers is our business. Linking naturally occurring genetic elements in new ways, we are developing a petunia that changes color throughout the day—from red to blue and back again. The potential impact of this product goes far beyond offering a strikingly beautiful flower for consumers. Revolution Bio wants to communicate the process and the obstacles of plant biotechnology in a completely transparent way to educate and inform consumers what it takes to create a flower as beautiful and unique as this one.

Revolution Bio almost failed before it started. After Keira and I founded the company, we limped along working both our day jobs as well as nights and weekends at Revolution Bio for nearly a year. With no external funding, we didn't see how we could carry on championing this idea and had serious discussions about completely folding the company and moving on with our lives. But then the Synbio Axlr8r program funded us with 30k in cash and a similar investment value in mentoring and breathed new life into Revolution. So the summer of 2014 finds us living on Blarney Street in the heart of Cork, getting the boost we need to turn our dream into reality.

Twitter: @RevolutionBio

Website: *http://revolutionbio.co*

Alexander Murer of Briefcase Biotech

It all started about three years ago when I was sick of the lack of freedom at our university (I was studying molecular biology) and in the education system in general. I'd rather work on real projects than learn massive amounts of theory, so I decided to develop my own bioreactor. Two friends joined to complete our skillset: Bernhard Tittelbach, who is our hardware programmer and electronics engineer, and Martin Jost, a software programmer and a classmate of mine in molecular biology. We were working at the local hardware hackerspace *realraum* in Graz, Austria. There we came up with the idea to create a hackerspace, but for us biologists! So Martin and I founded *Olga* (Open Biolab Graz Austria). Well we didn't know at the time that other biohackerspaces even existed. Our recent project at Synbio Axlr8r evolved out of our work at Olga on the bioreactor and an enlightening meeting in Berlin with biohacker Rüdiger Trojok.

We're making a rapid DNA prototyper. Everyone who works in a lab probably knows what it means to have delays of days, weeks, or even months waiting for your outsourced DNA service to synthesize the DNA you urgently need. We want to bring DNA synthesis back to the lab bench with our desktop synthesizer called *Kilobaser*, which will be easy, affordable, and open source. It is based on microfluidic and magnetic technology. It's also important for us to provide the DIYbio community with an independent way to produce their DNA, instead of relying on the good will of big companies.

Even though it's a lot of work, we are having a great time here at Synbio Axlr8r in Ireland! It's amazing to meet all the teams from around the world, biohackers and entrepreneurs, who get things going in their very own way.

Twitter: @BriefcaseBiotech

Website: *http://briefcasebiotec.net*

Ryan Pandya of Muufri

Our team is myself, a bioprocess engineer and private pilot; Perumal Gandhi, biomedical engineer and founder of two other small companies; and Isha Datar, executive director of New Harvest—a nonprofit dedicated to ending factory farming through technology. We actually all met through New Harvest. Isha had met both Perumal and me at different times, but we had both independently talked about producing milk without cows. When Isha came across the opportunity with SynBio Axlr8r, she thought, "I know two guys who want to use synthetic biology to help animals!"

We're making sustainable, animal-free milk from the bottom up. We're going to express the main components of milk in yeast—a few proteins and some fats—and then combine them in different ratios to achieve something that tastes like real milk, can be used to make cheeses or whatever else, yet is totally divorced from animal husbandry. There are a bunch of advantages—our product will be lactose free and cholesterol free, for one thing. Because it comes from tightly controlled engineering processes, it won't need pasteurization yet will never go bad. But those advantages just come from the bottom-up nature; the real impact, for us, is the elimination of animals. That means no more deforestation for grazing land, no more wastewater contamination or flatulent greenhouse gas emissions, no more pesticides or pathogens in the milk, and most of all—no abuse of cows! Pick a cause—health, environmentalism, ethics—Muufri will be a game changer in all of these realms.

May 20 was a crazy day—it was the first day the three of us *all met in person*! I think this is the new normal, meeting and developing relationships with people online such that when you meet them in person it's like you're old friends. It really did feel that way: the other people working around us commented that we all seemed like old friends, not that we had met in person just a few days prior.

Twitter: @Muufri

Website: *http://muufri.com*

Russel Banta of Benthic Labs

We are Benthic Labs, a group of undergraduate students who are making a strong biomaterial by expressing proteins from hagfish slime in bacteria.

I'm Russel Banta, a third-year chemistry student and CEO of Benthic Labs. I also enjoy free running when I'm not at the bench. My team consists of eight other students, with backgrounds in biology and genetics, and we do have an astrophysics student on the team as well.

The team came together when the group responded to forming University College Cork's first iGEM team. Our project is to express the two proteins from hagfish slime in bacteria. We plan to do this because the filament made of these proteins has incredible characteristics: 10 times stronger than nylon, stronger than steel and even kevlar! Hagfish have never been bred in captivity and are hard to access. With this in mind, a synthetic-biology approach has provided a perfect solution to being able to produce the filament in usable quantities. We see this product as an improved and eco-friendly material that will hopefully have uses where nylons and plastics have been used to date. Because of its antimicrobial properties and it being 100 times thinner than a human hair, one of the first potential applications of this product could be as stitches in eye surgery.

Many materials are derived from harmful chemicals and finite oil supplies, and we hope our natural product will be a positive step away from these methods.

Twitter: @BenthicLabs

Website: *http://benthiclabs.com*

Conclusion

So there you have it—a quick peek at the inaugural teams and projects of the first SynBio Axlr8r. A lot is riding on the ability of these teams to deliver a working prototype at the end of the summer. Like many venture capital firms, SOSventures is evaluating not only the market for consumer-focused SynBio products, but also, and perhaps more importantly to the BioCoder community, the ability of small

groups to rapidly create real SynBio products in just a few months for a fraction of typical biotech R&D budgets.

If successful, the 2014 cohort will not only secure the future of the SynBio Axlr8r for many more SynBio entrepreneurs, but also, and perhaps more importantly, demonstrate to the world what many of us in the BioCoder community have been saying for years: we're living in a new era of biotech, one where access to tools, knowledge, and one another allows any enthusiast to have a positive impact on the world.

Connor Dickie is a self-directed person who excels at creating his own opportunities. A deep, lifelong interest in technology and communication is demonstrated by his ability to innovate. Having already received global recognition in both academic and popular media through his work at the MIT Media Lab, his sights are now set on positively changing the world through disrupting how we interact with and perceive life.

Connor Dickie, CEO. Synbiota Inc.

Twitter: @connor, @Synbiota

Website: http://synbiota.com

Community Announcements

Zappylab

As scientists, we are constantly expanding the frontiers of research and knowledge. Yet, we are also constantly rediscovering knowledge that others have not had the time to publish and improving existing methods without the ability to share the improvements with the world. Our publishing system is essentially the same as it was in the days of Mendel. The mission of ZappyLab is to change this with protocols.io—a free, up-to-date, crowdsourced protocol repository for the life sciences.

As part of this effort, to create a community for the crowdsourcing of the protocols, we have during the past two years released a panel of free mobile and web tools for researchers. This includes Bench Tools, a mobile platform (iOS and Android) for laboratory work that replaces many expensive and outdated devices in the lab. Importantly, any protocol entered into protocols.io is then available inside Bench Tools as a checklist to help in the experimental procedure.

Follow us on Twitter @zappylab for updates on *http://protocols.io/* and our laboratory apps.

Bio {design} Conference

Bio {design}

Bio {design} is a three-day, hands-on exploration of what is possible when using living things as tools, mediums, and models in the iterative act of creation. For more information, go to *http://biodesignconf.com*.

BioLogik Labs

BioLogik Labs is a community biohackerspace in the Hampton Roads area of Virginia. We are an open lab focusing on DIYbio, OpenChemistry, synthetic biology, education, and the arts. We believe in cheap access to scientific tools to encourage the growth of citizen science.

We just finished a successful fundraising campaign on Experiment.com and are in the final stages of getting the tools, supplies, and equipment needed to offer open access to everyone. There are a number of classes we are building that will offer training and techniques for advanced equipment, as well as beginner classes to provide skills in some of the most basic of tools and concepts. There are goals to create open hardware for all of the general tools needed in a lab setting. These plans will be freely available, lowering the bar to access for everyone.

We wish to inspire people to make and create art and science, to cultivate curiosity and exploration. Above all, we want everyone to feel that there are no limitations, that average citizens can and do make active contributions and groundbreaking discoveries everyday, even in this highly technological world. BioLogik is that place to come and learn, discover, and explore whatever curiosities drive your mind and to bring those great ideas into reality.

Counter Culture Labs Found a Home!

We are super excited to announce, after essentially working out of one of our cofounders' back rooms for a year, that Oakland-based Counter Culture Labs has found a wonderful new home!

We will be moving into a pioneering new space in North Oakland, which is the first of a new type of a hackerspace, melding bio, tech, and cultural hacking into one space with a collective of other hacker, art, educational, and social justice groups. We're hoping that this new type of space democratizes access to the tools of education and innovation for the entire local community and will spread globally to ensure that everyone is empowered to dream, learn, and create, regardless of their educational background or socioeconomic status.

We'll still have tons of work to do—if you want to stay informed of ongoing developments, join our monthly newsletter.

UPCOMING PROJECTS AND CLASSES

Vegan Cheese

Our iGEM synthetic biology project, in collaboration with BioCurious (see elsewhere in this issue).

DIY Molecular Design and Cloning

Learn the basics of hands-on genetic engineering techniques with Craig Rouskey. Join the new class series in September.

The Science of the Senses series

Matt Harbowy continues his rave-winning series with the Science of Touch in July and Science of Perception in August.

DIY BioPrinting

Patrik D'haeseleer will be starting up a second team in Oakland, in collaboration with the long-running BioPrinting project at BioCurious. Two DIYbio labs —double the bioprinting fun!

Our schedule will be somewhat in flux over the next few months, but check out our upcoming events.