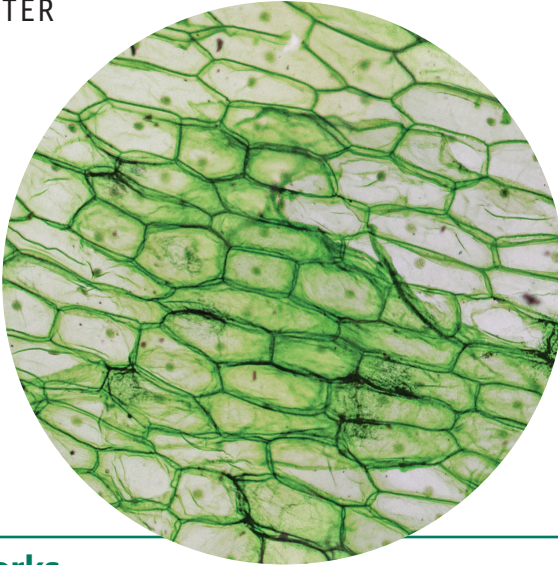


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# BioCoder

BIO NEWSLETTER

APRIL 2016



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## **Bento Bioworks**

Glen Martin

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## **The Biohacking Landscape in Latin America**

Edgar Andres Ochoa C., Oscar Joel de la Barrera Benavidez, Manuel Giménez, Maria Chavez, Marie-Anne Van Sluys

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## **How to Build and Use a Gene Gun**

Jay Hanson, Arnie Wernick, and Kyle Taylor

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## **Open Insulin and Open Source Biologics**

Anthony Di Franco and the Open Insulin team

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## **Building with Biology**

Megan Palmer, Natalie Kuldell, and David Sittenfeld

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# BioCoder #10

APRIL 2016

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## BioCoder #10

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Printed in the United States of America.

Published by O'Reilly Media, Inc., 1005 Gravenstein Highway North, Sebastopol, CA 95472.

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April 2016: First Edition

### Revision History for the First Edition

2016-04-11: First Release

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978-1-491-93100-4

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# Foreword

*Nina DiPrimio*

If you're in San Francisco, visiting the [Exploratorium](#) is a must. It is a museum that focuses on education, interaction and experience; the exhibits are for everyone, no matter what age or area of interest. At the Exploratorium, participants can experiment with lighting, sound, animation, living systems, hearing, social behavior, circuits, vision, tides, feelings, and other categories, all interactive and educational. In addition to the in-house exhibits, there are resources on their website for exploration at home or at institutions.

The richness of experiences like the Exploratorium, and the way these experiences shape participation in science, is why it is so wonderful to learn about [Building with Biology](#). Building with Biology is a program designed to facilitate public engagement with synthetic biology, which is a field that is still evolving. What tools and systems can be built? How will they be designed? How can the tools be used and what are the implications of their use? How will these tools affect society? Who contributes to the conversation? Building with Biology aims to start a conversation around these and related questions through educational exercises. Physical kits containing hands-on activities are being sent to science education sites (nature centers, museums, schools), as well as training materials to help foster interest in synthetic biology. To learn more about the six activities and how they are helping to open the dialogue, read [“Building with Biology” on page 41](#). And visit one (or more) of their programs this summer.

BioCoder's goals are similar: we want to spark conversations. In this issue, we have an article on open source biologics, a topic that needs public engagement to define what open source biology means, what should be engineered, and what are the safety concerns. There are articles on making your own gene gun (allowing you to genetically engineer plants at home), Bento Bioworks (a portable DIY biology laboratory), and a revolutionizing neuroimaging technology. In addition, there is a review of IndieBio Demo Day and an introduction to the biohacking community in Latin America. Public engagement is imperative in these unchar-

ted research areas that have seemingly limitless potential. We would love to hear from you and what you think about these new endeavors, technologies, and open applications. And of course, let us know about any topics we should cover—and particularly anything that you'd like to write about.

# Bento Bioworks

*Glen Martin*

DIYbio may be expanding rapidly in popularity, but things are still sketchy when it comes to equipment. Enthusiasts work with what they can afford or scrounge, and make do. Perhaps that's appropriate, particularly if you celebrate the movement's garage ethos and think its current state is analogous to that of home computers in the 1970s, just before everything mushroomed and changed the world forever.

Still, you need basic, functional equipment, even if you're working in a garage, basement, or small, co-op lab. And that's central to Bento Bioworks cofounder (with Bethan Wolfenden) Philipp Boeing's vision: To build that equipment and provide those standardized components so DIYbio enthusiasts can spend their time on projects, not scouring the Internet for used centrifuges.

"It's DIYbio, not DIY build-your-own hardware," says Boeing. "We realized that lots of folks interested in the field, including high school teams, citizen scientists and makers, we're having difficulties finding equipment. You'd get together with them, and the conversations inevitably revolved more around hardware than biology. The equipment issue was getting in the way of the actual science. So we decided to do something about it. We wanted to put some infrastructure together that was both appealing and affordable."

The result is the Bento Lab: a lunchbox-size DIYbio lab. Though compact, the Bento Lab has all the hardware a budding do-it-yourselfer needs to get hacking: a PCR machine, centrifuge, and gel electrophoresis unit with blue LED transillumination.

Encased in white plastic with cadmium-yellow trim, the Bento Lab looks decidedly, well, accessible. It has a retro feel, like a 1980s answering machine. That's by no means happenstance, says Boeing.

"We wanted to make it look friendly; we wanted to project the message that it's nonthreatening and easy to use," he says. "It was a balancing act: we didn't want it to look like a 'machine,' but we also didn't want it to look like a toy."

Struggling with various design concepts, the Bento team took a mock-up case with labels to iGem 2013 and buttonholed biologists for their opinions.

“We asked them, ‘If this were a lab, what should it have, and where? How would it look? What would you like to see?’ For us, getting the dimensions right was even more critical than the hardware. We knew we could figure out the hardware end, but the package had to be appealing.”

The Bento Lab thus works against the stereotype that DIYbio only happens in garage labs festooned with expensive and arcane equipment. It’s the opposite of bespoke: standardized, compact, versatile, and easy to use.

“When we’ve described it, we’ve used the tagline, ‘The Arduino for DIYbio,’” says Boeing, “but that metaphor kind of backfired, so we gave it up. People were getting confused: they thought we were actually using Arduinos, and we don’t. What we were trying to say is that the Bento Lab replicates what works with the Arduino community. The larger goals are the same.”

The Bento Lab went through multiple iterations, says Boeing, but in the end, “closing in on the final design was a bit less difficult than we anticipated. The PCR machine was perhaps the trickiest part, and we also had some challenges with the centrifuge, since it has to be self-contained in case something spills.”

While the Bento Lab will allow scientists to pursue sophisticated research, it will also provide students, makers, and artists the means for learning about basic biotech processes, says Boeing.

“We want to improve bioliteracy, to demystify the technology,” says Boeing. “We want to give people the tools they need to understand the science involved with biotechnology so they can evaluate the political and social ramifications from a position of knowledge, not emotion. We’re neither pro- nor anti-GM. To take either position is like saying you’re pro- or anti-electronics, with the understanding you could build either a clock or a bomb with the technology. It’d be nice to have that kind of granularity with genetics. What we are intent on avoiding is selling anything that requires an application for a lab license. Since we consider schools a primary market, our emphasis is on safety and legality.”

While there are other DIYbio off-the-shelf kits emerging, none fills the Bento Lab’s niche. For a modest investment, it allows biotech enthusiasts the basic means to work with genes in fascinating and significant ways. Amino, an MIT Media Lab project that spun off into an independent startup, could be considered a contender, but it’s primarily a bioreactor designed to culture microorganisms, unmodified or otherwise.

“It’s a nice-looking unit, and it seems perfect for growing cells and collecting pertinent data,” says Boeing. “Considering that our lab is more a means for manipulating DNA, I think the two would mesh very well together.”

The Bento Lab currently is going through beta testing, says Boeing, “and we’re conferring with our manufacturers on the feedback. We plan to launch a Kickstarter campaign in February, and we’ll probably start our first production run by the second quarter of 2016. It has been—and remains—a long road, but we’re close to implementing our motto: bio for everyone.”

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**Glen Martin** covered science and the environment for the *San Francisco Chronicle* for 17 years. His work has appeared in more than 50 magazines and online outlets, including *Discover*, *Science Digest*, *Wired*, *The Utne Reader*, *The Huffington Post*, *National Wildlife*, *Audubon*, *Outside*, *Men’s Journal*, and *Reader’s Digest*. His latest book, *Game Changer: Animal Rights and the Fate of Africa’s Wildlife*, was published in 2012 by the University of California Press.





# The Biohacking Landscape in Latin America

STRATEGIES FROM A REGION THAT WANTS TO PUT ITS OWN FLAVOR ON THE MOVEMENT

*Edgar Andrés Ochoa Cruz, Oscar Joel de la Barrera Benavidez, Manuel Giménez, Maria Chavez, and Marie-Anne Van Sluys*

*When you feel that you should have been born in the future, the only option is to build the future, so you can finally get to where you belong.*

**Andrés Ochoa**

## The Beginning

DIYbio democratized genetic engineering, a technology known by many names, including molecular biology, biotechnology, and synthetic biology. An early promoter of DIYbio was Rob Carlson, who in a 2005 [Wired article](#) showed that \$1,000 was all it cost to start using this technology and pointed to online resources. In 2008, [DIYbio.org](#) launched as a channel of communication for DIYers who wanted to build a community around it. In 2010, the first biohacker spaces opened in California ([BioCurious](#)) and New York ([Genspace](#)). Today [DIYbio.org](#) maintains a [list](#) of the biohacker spaces around the world: 35 in North America, 28 in Europe, 5 in Latin America, 3 in Asia, and 3 in Oceania. In six short years, the movement has become a global phenomena.

Here is where the Latin American story of DIYbio begins. Focusing on the cultural similarities of the region (from Mexico to the Patagonia), we will describe the landscape of the biohacking spaces and the open-science initiatives that are

expanding there. Also, we will describe the formation of the movement and the strategies that it has created to survive. In addition, we will explain the problems and questions faced by Latin America's biohackers when trying to develop this movement in their own cultural and technological context.

## iGEM: The Spark That Began the Movement

The **International Genetically Engineered Machine (iGEM)** competition was created in 2004 at MIT and had five schools participating. By 2015, it had 280 teams with more than 2,700 participants from all over the world (iGEM, 2015). Reminiscent of robotics competitions held for engineers, iGEM uses biological platforms (bacteria, yeast, and plants, among others) to develop solutions and products and to create awareness about society's problems through synthetic biology (Ochoa & Van Sluys, 2015).

The first biohacker space in the Latin American was **SyntechBio**, which opened in São Paulo, Brazil, in 2012. It began as an experiment inside the **University of São Paulo** to accelerate the training of students in the synthetic biology area, enhance multidisciplinary studies, and create an open space to develop and share ideas that wouldn't be normally supported by the academic community in a research context. These ideas were embedded in an educational context, where creation is a free process and the goal is building skills and creativity using the infrastructure of the GaTE Lab research laboratory.

The founder of this biohacker space was in charge of the organization and scientific support of the Brazilian students that participated in the 2012 and 2013 iGEM. The iGEM is a catalyst to building a sense of community, knowledge, and interest in synthetic biology around the world. SyntechBio has developed projects open to any person in the community; these projects were created by students with the goal of hacking knowledge and solving problems that interest them and then was continued by the group. For example, the Plug&Play, which began as an iGEM project, is a molecular tool that allows for control or genetic modification inside microorganisms in fewer steps and with less cost, making the process easier and more accessible. Recently, SyntechBio partnered with Laboratório de Garamem in Brazil to create the first DIY thermocycler in Latin America. This device is open source, and you can build it yourself.

Biohacker spaces linked to universities are found in other regions, including North America. Denver Biolabs, associated with the University of Colorado Denver, was created as a makerspace that incorporated a biohacking space, and it

responds to the necessity of having a space of open collaboration and creation that integrates different disciplines inside the university.

There is a deep link between iGEM and biohackers in the Latin American region. The rise of the DIYbio Mexico movement in 2014 was also linked to the synthetic biology and biotechnology research groups that participated in iGEM. It began with a [group](#) of IPN (National Polytechnic Institute) biotechnology engineers that led to the creation of [BioHackers México](#). Later, the collaboration of three communities—hackers, biohackers, and entrepreneurs—led to the opening of the [Tepache Hacklab](#). The Synthetic Biology National Network in Mexico ([Red Nacional de Biología Sintética de México](#)), has an important role in this community by connecting the iGEM teams to scientists, students, and biohackers, allowing them to build a collaborative community. This group focuses on the development of machines and infrastructure for biohacker spaces.

Another pioneer group in Mexico called [Gene Garage](#) wants to create a biotech community and an open lab focused on biotech entrepreneurship.

The iGEM competition can also be linked with the emergence of the biohacking community in Argentina, although not as strongly as it is in the cases already mentioned. The first biohacking community in Argentina, [BiohackingBA](#), was founded in July 2015 by a former member and instructor of the Argentinean iGEM teams. At the moment, and mainly because it is a very young initiative, it has not established a formal relationship with universities or other institutions. It has received help and support from the Buenos Aires city government and local companies known to support the entrepreneur ecosystem there. Currently, it has projects focused on hardware (such as 3D-printed pipettes), arts (like installations with fluorescent bacteria and plants), neuroscience, and bioinformatics.

iGEM has had a tremendous impact in the biohacker movement and the synthetic biology field in the region. Nevertheless, South American participation in the competition the past 10 years (5.3% of teams per region) is small when compared to the North American (38.6%), the European (28.3%), and the Asian (27.8%) ([Ochoa & Van Sluys, 2015](#)). We aim to get more South American governments and education centers invested in sending new teams to the competition. The Latin American community's obstacles are shared by the biohacking community around the world, making the idea of biohacker spaces linked to universities appealing.

The model of biohacker space linked to a university is well suited to the necessities of the Latin American region, where economic resources are difficult to access, even for some of the well-established research centers. In Latin America, even the academic laboratories face funding problems and difficulty accessing technology. Machines and chemical reagents are expensive, and oftentimes these

items can take months to arrive at the local research centers, when they are imported. In this context, Latin American academic community has embraced low-cost solutions and the hacking of equipment to tackle these problems.

This model is expanding into Brazil, where the **iGEM team** from the Federal University of Minas Gerais is boosting the creation of a similar space. Likewise, Colombia is interested in developing a biohacker space, the first one in the country, at the **BIOS research center**, which focuses on bioinformatics as a tool to link industry and technology.

Universities and educational centers that support biohacker communities are frequently linked to iGEM teams. Nevertheless, those resources are not enough. For example, most of the iGEM teams in the region have to crowdfund or get corporate funding to be able to participate, which limits the number of teams that can be sponsored within the region. This problem has also opened the opportunity for new initiatives, like the TECNOx competition.

TECNOx is a competition inspired by iGEM with a strong regional imprint. It supports teams working on projects that seek to solve regional problems through robotics and synthetic biology; it is also important for the organizers to make a more affordable competition, which would allow more teams to participate. The idea was crafted during the Latin America Regional Jamboree of the iGEM competition. It supports teams working on projects that seek to solve regional problems through robotics and synthetic biology. The first competition will conclude at the end of April 2016, in Buenos Aires, and it will have teams from Argentina, Brazil, Colombia, and Mexico.

TECNOx is being strongly embraced by the Latin American region (the 2016 competition involved 12 teams with 198 participants total) and is helping to establish stronger relationships between the academic and biohacker communities. In Mexico, the Guanajuato team is being strongly supported by the DIYbio movement. The movement is challenging these students to build their own scientific equipment and to participate in short-term courses about synthetic biology, bioinformatics, and business skills.

## The Maker and Biohacker Movement Are Coming Together

The DIYbio movements in the United States, Europe, and Latin America have common goals. They all believe in a democratization of science and the enabling of citizens to use biotechnology. The groups from the US can buy used equip-

ment online and get donations of old equipment from universities and research centers. Their Latin American counterparts don't have these possibilities, and must instead build low-cost versions of standard equipment. And who is better equipped to build things than the maker community?

A subset of the maker phenomena is the **Fab Lab community**. Their mission is to provide access to the tools and knowledge that allow everybody to use technology to build almost anything. In a list of the **Fab Labs** around the world, 44 of 541 are in Latin America.

The merging of the maker and biohacker communities makes sense when we consider how difficult it is for Latin American DIYers to access the technology and resources they need. The maker community gives its expertise on building equipment using online sources like **Instructables**, **Waag Society**, **Hackteria**, the **Open Source Toolkit: Hardware** and even **YouTube**. Meanwhile, the biohacker community teaches makers how to build using a different type of material: DNA.

Recently in Mexico, the maker community has seen in the DIYbio movement a new form of hacking. Several groups, including **The Inventor's House** (Aguascalientes), **Fab Lab Puebla** (Puebla), **Hacedores Makerspace** (D.F.), and **LobbieLab** (Oaxaca), began the process of creating spaces for biohackers. They also began to collaborate with the DIYbio movement. In Argentina, the local biohacking community is starting to work with the Fab Lab movement, which has four qualified labs in Buenos Aires.

In 2015, Brazil was the first to bring the BioHack Academy class created by Waag Society to Latin America. It was the first step to building what will become biohacker spaces inside the **Garagem Fab Lab** and the **Olabi Makerspace**. The class built the equipment needed to develop basic microbiology experiments.

In 2015, **Peru** and **Chile** participated with two Fab Labs groups in the class **How to Grow (Almost) Everything**, which is a biology version of the 1998 MIT class that birthed the Fab Lab movement. The Peruvian biohacking ecosystem is linked to hackerspaces, Fab Labs, and open hardware initiatives, including the **Open BioMedical Initiative**, which has strong links to the **Peruvian community**.

Meanwhile, there are biohacker groups that have grown independently. In Mexico, **Interspecifics** wants to explore new ways of biology interpretation, approaching the area from the arts and philosophy perspective.

There are also communities that discuss synthetic biology and biohacking. Most of them plan to build biohacker spaces, or they are groups of students or citizens who want to learn about the topic. These are the cases in Argentina, where **DIYbioBA** has weekly meetups; Brazil (**Bios** and **SynbioBrasil**) and Mexico (**Biohackers DF** and **Biodescubre**).

The opening of citizen-science spaces outside of universities raises an important question that must be discussed by the Latin American community, and it is: what about biosafety? The biohacker spaces in the region are already working on this topic. For example, the Mexican biohacker community considers it a major priority and wants to build a strong collaboration with the governmental institution that regulates it, **CIBIOGEM** (Interministerial Commission on Biosafety of Genetically Modified Organisms). In other regions, like Brazil, biohacking spaces must follow the biosafety rules of any level 1 laboratory, which means that the rules are pretty clear and safety can be easily granted.

Students, biotech engineers, biologists, artists, makers, hackers, and even physicists have pushed the DIYbio movement. But despite the diversity, a common language has emerged between iGEM teams, academia, biohackers, and society, which is the democratization of science and technology using community collaboration. The ideals shared by hackers and makers have become the flag of the movement: the biohackers want to create their own laboratory's equipment and interchange information and materials for free, with the goal of creating more spaces and resources for the community.

## The Future Is Here

The future of DIYbio movement in the region relies on its ability to organize. The players are already talking and learning from one another's experiences. Most of the problems they have faced in their own country are difficulties common to the entire region. This is why they are coming together into a network of biohacker spaces.

The opening of the SyntechBio group supported other initiatives in the region, enhancing the communication and action of the community as a group. This led to the creation of the **Latin American Network of Biohacker Spaces**, launched at the end of 2016 (the biohacker space of this community was closed in 2015 so that the organization could focus on the creation of the network). It already includes groups from Argentina, Brazil, Colombia, Mexico, and Peru, and it is expanding through the region. The network has two international advisors with broad experience in building biohacker spaces, Maria Chavez from **BioCurious** and Ryan Bethencourt from **IndieBio-SF**.

The point of this network is to strengthen the relationship between the groups and incentivize the creation of projects involving the whole region, showing a cohesive group that may gain more attention from society. This will allow an

empowerment of the community before their governments; therefore, new proposals and initiatives can happen. We want to grow a team and impact the region in a positive way. If you have or want to open a biohacker space, do not hesitate to [join our community](#) and use our experiences to enhance and improve your space.

This is your biohacking community. Here you can ask questions, start projects and help others with theirs, post information, and discuss synthetic biology, genomics, and biohacking.

Correspondence can be directed to Dr. Edgar Andrés Ochoa: [syntech-bio@gmail.com](mailto:syntech-bio@gmail.com).

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**Edgar Andrés Ochoa Cruz** (aka Don) has a PhD in biotechnology from the University of São Paulo, Brazil. He was an instructor of two Brazilian iGEM teams and founder of [SyntechBio](#), the first biohacker space in South America that later became the Latin American Network of Biohacker Spaces. He manages and develops several projects in synthetic biology. He is also developing platforms for providing home and industry users access to biotechnology. He is the cofounder of [Arcturus BioCloud](#) in San Francisco, California, whose goal is to take synthetic biology to your home safely.

**Oscar Joel de la Barrera Benavidez** (aka Billy) is a biotech engineer from the National Polytechnic Institute (IPN). He is the founder of Biohackers Mexico, cofounder and CEO of the synthetic biology startup [GATCorp](#), cofounder of the agro-biotech startup [Huitl](#) and advisor to the Guanajuato 2016 iGEM/TECNOx team. He considers himself an independent scientist and wants to democratize biotech and science.

**Manuel Giménez** is a synthetic biologist and computer scientist from Universidad de Buenos Aires. He was member and instructor of the first two Argentinean iGEM teams, founder of the first biohacking community in Buenos Aires ([BiohackingBA](#)), and is currently a graduate student at Boston University. He has recently been spreading the word about synthetic biology in his home country and the region. His dream is to see a mature and strong bioeconomy based on innovation developed throughout the whole Latin American region.

**Maria Chavez** is a biohacker from the San Francisco Bay Area. She is a board member at [BioCurious](#), one of the first biohacking labs in North America, where she has organized events, classes, and community projects. She is also a board member of [Real Vegan Cheese](#), an open-source project to create a synthetic cheese from genetically modified yeast. She recently became an advisor to the [Latin American Network of Biohacker Spaces](#). She aspires to push open-source science and the creation of more biohacking spaces to democratize science globally.

**Marie-Anne Van Sluys** is an associate professor in the Botany Department of the University of São Paulo. She is the head of the Genomic and Transposable Elements (GaTE) Laboratory,



*which studies the impact of transposable elements in the structure, function, and diversification of bacterial genomes and plants. She has supported the iGEM teams of the University of São Paulo and the SyntechBio initiative since the beginning.*

# IndieBio Demo Day

*Glen Martin*

The crowd outside the Folsom Street Foundry in San Francisco earlier this year wouldn't have struck any casual pedestrian as unusual. It was obvious another tech conference was underway, an occurrence almost as commonplace in this city as pigeons congregating on the sidewalks. There was also a certain familiarity to the Foundry's interior atmosphere: people packed cheek by jowl, sipping microbrews and nibbling elegant finger foods, a thousand animated conversations creating a white noise that conjoined pleasantly with the pale winter light streaming in from the building's clerestory windows. It could have been any coming-together of engineers and angels, venture capitalists and journalists; such conferences are the economic and social lifeblood of this city.

There was, however, a palpable difference to this gathering. It had a certain urgency to it, a kind of frontier exuberance that you don't witness much these days at IT conferences. This was a biotech conference—of sorts.

But this wasn't about big, comfortable, corporate biotech. This was the second IndieBio Demo Day, a gathering of the bootstrappers, visionaries, tinkerers, and paradigm-smashers of biotech. If biotechnology were viewed as a dialectical process, the attendees were the antithesis to big biotech's thesis, the white-hot element that will lead to synthesis—and ultimately, a wholly unimagined *new* thesis. The air almost crackled with a sense of imminence, and a realization dawned on at least one observer: Silicon Valley must've felt the same way in the late 1970s.

Certainly, the sponsors of the event promoted the idea that the confab marked the advent of a new age, one desperately needed by our crowded and stressed little planet.

"Biotechnology is the most powerful tool ever invented," said Arvind Gupta, who is known as "The Architect" at IndieBio, arguably the country's hottest biotech startup accelerator. "We can reprogram life to solve intractable problems, the trillions of problems the world is facing."

Moreover, Gupta intimated, biotechnology is operating along the lines of a loose analogue to Moore's law, assuming you substitute dollars for transistors: every couple of years, it becomes dramatically cheaper to do big and important things.

"Today, you can launch a biotech startup for \$200,000," he said. "That was absolutely unheard of two years ago. We created IndieBio to give scientists a path to that kind of entrepreneurship."

Ryan Bethencourt, whose company handle is "Hustler-in-Chief," said IndieBio looks for several things in potential partners.

"We want to know how they're going to make their products 10 times better than those of their competitors," Bethencourt said. "We look for results, not activity. And we need them to understand that sales solve everything. We look for achievements. Our aim is to build companies for the long view. We're not looking for a quick flip."

While about 200 companies applied to exhibit at Demo Day, only 14 made the cut. But if they were few in number, they were geographically diverse.

"Our teams come from five continents and eight countries," said IndieBio's Ron "Lord of Science" Shigeta. "The main thing they have in common is that they went through an extremely rigorous admission process."

What was perhaps most impressive about the exhibitors was the advanced state of their products. IndieBio only provides four-month tenures for their partners, and Demo Day provided ample evidence that Bethencourt's emphasis on achievement rather than mere activity was heeded. It seemed every company had reached \$2 million or more in funding. And while the Wall Street Journal recently—and accurately—**reported** that investment enthusiasm in Silicon Valley is ebbing rapidly, VC interest in bleeding-edge biotech seems to be gathering steam.

"I'm interested in food and food security," observed Brock Siegel, a venture capitalist from San Mateo. "Understanding and reformulating something as complex as food, and at an acceptable cost—well, it's a huge challenge. The tools and methodologies are really just becoming widely available. So I'm looking for opportunities, and there are a lot of things to consider. For example, with synthetic animal protein, what's the entry point? It's probably not steak. So stew meat? Processed meat? I'm here to get some ideas."

If the IndieBio presentation by Memphis Meats is any indication, in vitro red meat is going to take on a hamburger configuration, at least for the foreseeable future. The company produces beef and pork in bioreactors charged with stem cells and the requisite nutrients. The resultant tissue lacks the capillary network necessary for oxygen transport, so the meat must be produced in thin layers, meaning that the ultimate product resembles lean ground chuck more than a

New York strip steak. Still, the presentation featured images of expertly prepared meatballs that appeared succulent and savory, supporting the premise that the market may well be ready for cruelty-free and pathogen-free lab-grown meat.

But what about seafood aficionados? There was something for them at Demo Day as well: New Wave shrimp. Founded by materials scientist Michelle Wolf and marine biologist Dominique Barnes, New Wave uses plant proteins to mimic America's favorite crustacean on a molecular level. The result is startlingly shrimp-like in appearance, and a video clip of mall shoppers gobbling down samples of the product indicates it passes the taste test as well.

Which isn't to say Demo Day was unrelentingly foodcentric: indeed, the products and technologies showcased were noteworthy for their variety. Koniku premiered a wetchip, a hybrid computer chip that melded human neurons with electronic components. Vali Nanomedical promoted programmable nanoparticles that can deliver customized drug combinations to specific tumors. Indee was also in the oncology space, offering a scalable microfluidic technology that promises to make highly effective—and currently, astronomically expensive—gene-edited cancer therapies available to the masses. Truust Neuroimaging supercharges ubiquitous EEG hardware to improve brain imaging by factors of 10 to 100. MYi and Circularis introduced techniques for sequencing the structure and function of proteins. Amino Labs offered a compact DIY biolab. Nerd Skincare harnesses individual epidermal microbiomes to create proprietary skin care products.

And on and on. Through all the presentations, the sense of acceleration, both technological and psychic, was at times overwhelming. Few of the attendees, however, seemed nonplussed. That's a good thing, Bethencourt intimated, because things are only going to get faster.

"Remember," he said, paraphrasing Darwin, "It's not the strongest or the smartest that survive. It's those who are most adaptable to change."

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***Glen Martin** covered science and environment for the San Francisco Chronicle for 17 years. His work has appeared in more than 50 magazines and online outlets, including Discover, Science Digest, Wired, The Utne Reader, The Huffington Post, National Wildlife, Audubon, Outside, Men's Journal, and Reader's Digest. His latest book, *Game Changer: Animal Rights and the Fate of Africa's Wildlife*, was published in 2012 by the University of California Press.*



# How to Build and Use a Gene Gun

*Jay Hanson, Arnie Wernick, and Kyle Taylor*

## What Is a Gene Gun?

The gene gun is a device used to transfect cells with foreign DNA by bombarding the target cells with DNA-coated microparticles. It was originally developed using plant tissue as the target but can also be used with yeast, bacteria, mammalian cell lines, and insects. In plants, it is one of the few ways to get foreign DNA into chloroplasts as well as genomic DNA. It is called a gene gun because it fires small DNA-coated particles (typically gold or tungsten particles because they are dense) into target cells. This method is called biolistic, a combination of the words “biology” and “ballistics” from the Greek *ballein*, “to throw.” By adjusting the velocity of the particles, any type of cell wall can be penetrated. It is an effective method for inserting foreign DNA into cells and thus transforming the target cells.

Another popular method of doing plant transformations is to use *Agrobacterium tumefaciens*, a plant pathogen, to introduce a gene of interest into plant cells. Using this method, *Agrobacterium* injects the foreign DNA into the plant, where it is incorporated into the plant genome at random locations. However, since it involves using a plant pathogen, the USDA regulates it and has disallowed making available any transgenic plants created using this method. However, transgenic plants created using a gene gun are allowed by the USDA. So that is another advantage the gene gun has over using *Agrobacterium* as a transfection method if the goal is to make a commercially available transgenic plant. Another advantage over *Agrobacterium* is that a gene gun can be used on any plant, and *Agrobacterium* is limited to plants that are its hosts. Monsanto and other companies have used the gene gun to bioengineer crops with pesticide resistance and other characteristics to improve crop yields. The gene gun has also been used to administer gene-

based therapies for cancer and other diseases. There are references to some gene gun applications at the end of this article.

## A Brief History

The gene gun was developed by Dr. John Sanford and his graduate student, Ted Klein, at Cornell in 1987. In the first successful prototype, they used a .22 caliber rifle barrel equipped with a firing pin that detonated a gunpowder-filled blank cartridge. The blast forced a nylon projectile down the gun barrel, pushing thousands of DNA-coated tungsten particles ahead of it. A steel plate with a 1 millimeter hole in the center stopped the nylon projectile but allowed the tungsten particles to travel through the hole and bombard the target at velocities of more than 1,000 miles per hour (~1,500 ft/sec). The first target was a collection of onion bulb cells.

Today, modern gene guns use a pulse of compressed helium in the range of 200–1,500 psi to create the energy needed to accelerate the DNA-coated particles. There are two commercial gene guns available from Bio-Rad. The PDS-1000 system sells for \$25,000 and the Helios system for \$30,000.

## DIY Gene Guns

Because commercial gene guns are very expensive, there have been a number of DIY gene guns developed. If you Google “DIY gene gun,” you will see a number of hits. The one described in this article uses some ideas from other DIY gene guns and has some unique features that make it easy to adjust the gas pressure, gas pulse time, and target distance while keeping the cost low.

### Building the Gene Gun

List of components: (total cost: \$200–\$230):

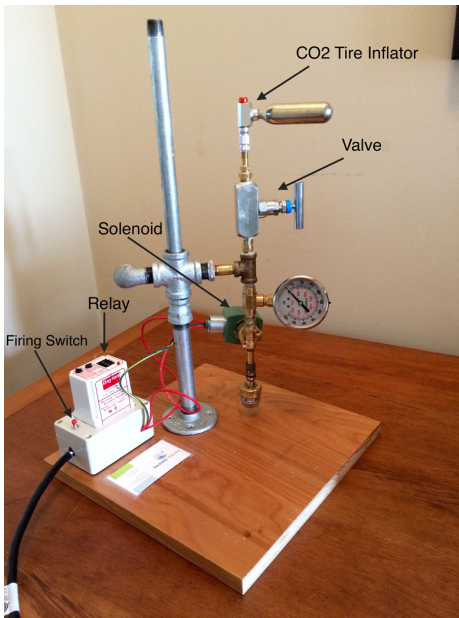
- High-pressure solenoid (\$75)
- Adjustable relay that provides pulses in the 10–500 millisecond range (\$30–\$60 on eBay)
- A bicycle CO<sub>2</sub> inflator to attach the CO<sub>2</sub> cartridge to the gene gun (\$22)
- A valve to adjust the pressure (\$21)

- A pressure gauge that reads from 0–1,000 psi (\$15 on eBay)
- A fitting to suspend the tungsten particles above the target in front of the target
- A compressed gas discharge (macrocarrier) (\$5)
- Pipe fittings to hook up the Solenoid, CO<sub>2</sub>, and nozzle (\$30)

The four key parts that are needed are the relay, the solenoid, the valve, and a CO<sub>2</sub> tire inflator.

Other than that, the rest of the parts are 1/8"–1/2" pipes and fittings available from any hardware store.

See [Figure 4-1](#).



*Figure 4-1. Key functional parts of the gene gun*

## Building the stand

I used a vertical 1/2" pipe mounted to a wooden base to support the gun and allow easy adjustment up and down. An improvement on this stand might be a geared vertical post with a ruler attached to assist in determining the distance to the target cells.



As you can see from the photo, the CO<sub>2</sub> bicycle inflator is attached to the valve and then to a 1/4" tee. The purpose of the tee is to attach the gun to the stand. The short horizontal pipe connecting the gun to the stand is blocked off to prevent CO<sub>2</sub> from leaking out. This was done by heating up the pipe and melting solder into it to form a gas tight plug.

## The CO<sub>2</sub> tire inflator

The purpose of the tire inflator is to connect the CO<sub>2</sub> cartridge to the system. There are many CO<sub>2</sub> tire inflators on the market. So far, I have used two, the [Cabrillo2-Elite](#) and the [Lezyne](#).

The Cabrillo2-Elite seems to be sturdier and so far has held up better. The Lezyne started to leak after about 50 uses.

Another option is to use a CO<sub>2</sub> paintball canister. They hold a lot more CO<sub>2</sub> but are larger and cost a bit more. However, if you are using the gene gun a lot, the larger canister will be more economical in the long run.

## Valve

The valve controls the pressure in the chamber. By opening the valve, CO<sub>2</sub> flows into the chamber until the desired pressure is reached.

[DuraChoice](#) has a 1/4" carbon steel needle valve rated at 10,000 psi (SKU VNC01-025).

## Pressure Gauge

The gauge displays the pressure in the chamber.

This gauge is 0–1,000 psi. I got it on eBay. Nothing special about it. Any good gauge will work.

## The Solenoid

The solenoid is an electronically controlled valve that opens and closes (quickly) and is controlled by the relay. When the solenoid is open, it lets compressed CO<sub>2</sub> flow from the chamber to hit the macrocarrier and accelerate the DNA-coated tungsten particles toward the target cells. Try this [Asco model 8262H200](#), which is rated at 700 psi and costs \$74.

## The Relay

The relay is a switch used to time how long the solenoid is open that can be adjusted to open for a specified period and then closed.

This relay is Dayton Model #6A855. It is adjustable in 10 millisecond increments, and the minimum open time is 10 milliseconds.

These are often available on eBay, where I got mine.

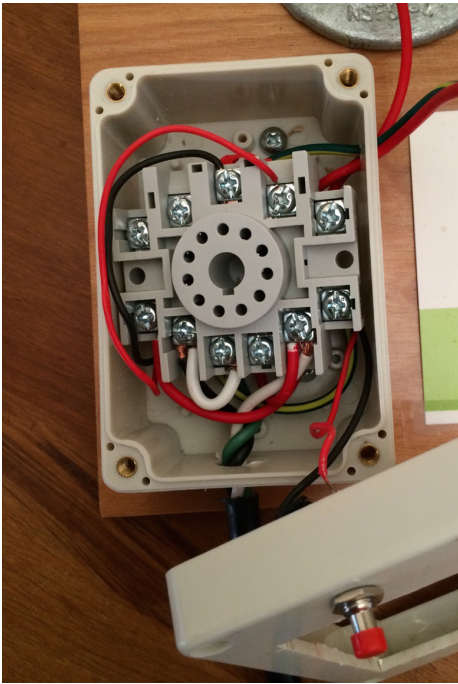
You can also [buy one new from Grainger](#).

## Firing Switch

The switch triggers the relay to open. This is a momentary push button switch. When pushed, it only closes momentarily then opens again automatically. Switches are available at [Fry's Electronics](#).

## Wiring

For an illustration of the wiring setup, see [Figure 4-2](#).



*Figure 4-2. Wiring conventions inside the relay socket*

Pin	Description
1	Black to 110V + a jumper to pin 2
2	White jumper to pin 1
3	Red to solenoid
4	—
5	—
6	Black to push switch
7	Red to push switch
8	—
9	—
10	White to 110V + second red to solenoid
11	—

### Socket for relay to plug into

All the wires are attached to the socket and the relay plugs into the socket. I got my 11-pin socket on eBay, but [Grainger also has them](#).

### Macrocarriers

The macrocarrier is the support structure that holds the DNA-coated microparticles. After trying out various fittings at the hardware store, I ended up adapting some garden hose fittings to hold a 3/4" washer. The washer can be used to support various types of paper or films that hold the microparticles. See Figures [4-3](#), [4-4](#), and [4-5](#).



*Figure 4-3. Solenoid connection to the macrocarrier holder*



*Figure 4-4. Above left, disassembled macrocarrier holder composed of hose fittings; above middle, washers with parafilm and tungsten particles; above right, assembled macrocarrier holder with washers in place*

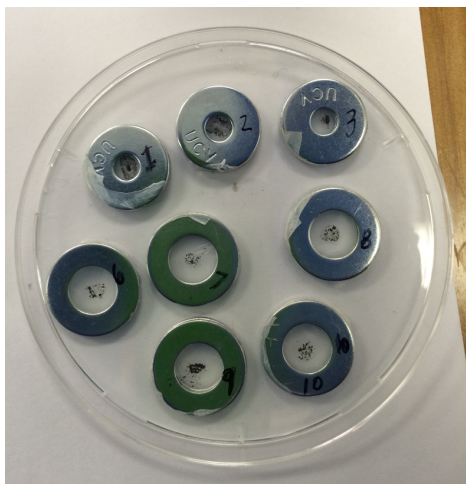


Figure 4-5. Washers with parafilm and tungsten particles ready to use

## Using the Gene Gun

A few members of the Plant Bio Group at BioCurious have been using the gene gun over the past few months on onion cells with the goal of improving transformation efficiency. Arnie Wernick and Kyle Taylor have been leading this effort. We have tried several different pressures, distances, and macrocarriers. If you are in the San Francisco Bay Area and are interested in seeing the gene gun, the Plant Bio Group meets each Tuesday at 7 p.m. and is open to anyone interested in pursuing plant biology projects.

The basic steps to use the gene gun are:

1. Prepare the tungsten particles and coat them with DNA.
2. Load the particles onto macrocarriers.
3. Prepare the target cells.
4. Decide on your parameters and set the gene gun accordingly for distance from target, pulse duration, and CO<sub>2</sub> pressure.
5. Place the target under the gene gun barrel and fire it.

## Tungsten Particles (Microcarriers)

Bio-Rad sells four sizes of tungsten particle microcarriers: M-10 (~0.7  $\mu\text{m}$  diameter), M-17 (~1.1  $\mu\text{m}$  diameter), M-20 (~1.3  $\mu\text{m}$  diameter), and M-25 (~1.7  $\mu\text{m}$  diameter). The current cost is \$110 for 6 grams. This is enough for about 4,000 bombardments (~\$0.03 cents per shot). Bio-Rad also sells gold microcarriers at \$625 for 250 mg (23 times more expensive than tungsten).

### Tungsten M-10 Microcarriers

We have been using M-10 tungsten (from a different source but the same size as the [Bio-Rad M-10](#)).

There are a number of published protocols for preparing the microcarriers. We have been using [the protocol from Iowa State University](#).

There are a couple of steps where sonication is required. This can be done with an ultrasonic jewelry sonicator, available on Amazon for \$20–\$30.

The DNA coating protocol calls for spermidine. At first we omitted adding spermidine because we didn't have any. The one successful transformation we got was achieved without using spermidine. Eventually we got some from Sigma-Aldrich and now include it when preparing the DNA-coated tungsten. [Sigma-Aldrich](#) sells 99% spermidine for \$35/gm.

The Iowa State paper describes the preparation of gold particles, but the same protocol applies to tungsten.

There are two parts to the method. The first part describes how to wash and prepare 15 mg of tungsten and divide into 10 2.0 ml Eppi tubes (1X tubes, 1.5 mg each). These 1X tubes of tungsten particles are stored at -20° C until use. Each 1X tube will be used to prepare enough coated particles for 8–10 bombardments.

The second protocol describes how to coat the tungsten particles with DNA. This is done on the day you will use them. The following list is a summary of the protocol to coat the tungsten particles with DNA:

1. Thaw 1X tube.
2. Sonicate for 15 seconds.
3. Add 5  $\mu\text{l}$  construct (selection construct and gene of interest construct).
4. Agitate very well by tapping the tube.
5. Tap tube on bench top to gather all drops at bottom.
6. Add 50  $\mu\text{l}$   $\text{CaCl}_2$  (2.5 M) using a pipette.

7. Re-suspend gently with pipette once.
8. Place on low speed vortex and add 20  $\mu$ l spermidine (0.1 M) while shaking.
9. Wait 30 seconds, then close the tube and agitate vortex well.
10. Return to low speed vortex for 10 minutes
11. Remove from vortex and let tungsten particles settle for at least five minutes.
12. Centrifuge for 15 seconds at 5,000 rpm to pellet the tungsten.
13. Pipette off supernatant.
14. Add 250  $\mu$ l of cold 100% EtOH (fresh out of -20° C freezer)
15. Agitate by tapping with your finger to dislodge pellet.
16. Rock back and forth to make tungsten a silky smooth consistency.
17. Let sit 3–5 minutes for tungsten to settle out.
18. Centrifuge for 15 seconds at 5,000 rpm.
19. Remove supernatant and add 120–140  $\mu$ l ice cold 100% EtOH.
20. Agitate by tapping with your finger then place on vortexer on low to distribute tungsten particles uniformly for loading onto macrocarrier.

Keep the tungsten particles in a uniform suspension by vortexing on low setting while aliquoting ~10–15  $\mu$ l to the center of the parafilm held by the washers in the macrocarriers (see [Figure 4-5](#)). Starting at the center of the macrocarrier, distribute the suspension evenly across the target area.

After loading the macrocarriers, let them sit for 5–10 minutes to be sure they are dry before bombardment.

## Testing the System

Over the past few months in the Plant Bio Group at BioCurious, we have been using the gene gun to bombard onion bulb cells with a plasmid containing a GFP reporter, TuGFP. We have tried a variety of macrocarriers and pressures and are still working to improve the yield.

After bombardment and overnight incubation at room temperature in the dark, the onion cells are inspected with a fluorescence microscope to find transformants that are expressing GFP.

So far we have had one successful transformation out of about 40+ bombardments ([Figure 4-5](#)). We have primarily used pressures in the 600–700 psi range

and occasionally up to 850 psi and as low as 550 psi. The pulse duration has been 80 ms and the distance to the target between 1–2 cm. As a negative control, we fire a blank macrocarrier (no tungsten particles) or a macrocarrier with tungsten particles without DNA. This exposes the target cells to the same biolistic exposure but without the reporter gene. We have focused on testing and improving the macrocarriers. Arnie Wernick has come up with several promising macrocarrier designs, and he is continuing to innovate in this area. The goal is to design a macrocarrier that will hold the particles and then disperse them to the target cells in a uniform and consistent pattern. We have been using two 3/4" washers to hold various films, like plastic wrap, paper, cellophane, and parafilm. Arnie determined that parafilm worked the best, and a majority of our bombardments have been using parafilm macrocarriers. However, parafilm does not rupture uniformly. Sometimes the gas pulse blows a nice hole through the center and disperses the tungsten particles well. Other times, it rips off from one side and forms a flap such that most of the particles are not dispersed to the target. The bottom line is we are still working to design a better macrocarrier that will release the particles in a uniform pattern and consistently bombard the target with good cell wall penetration.

Figure 4-6 shows GFP expression in onion cells from one of our targets.

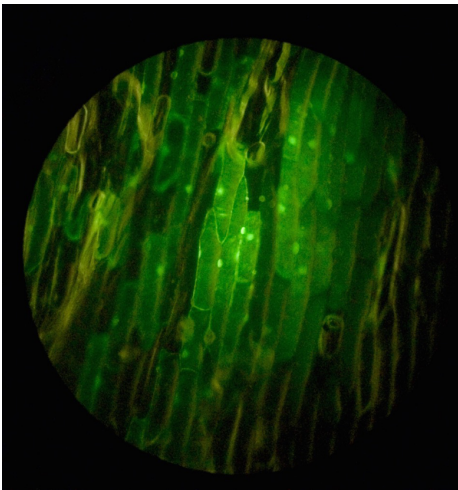
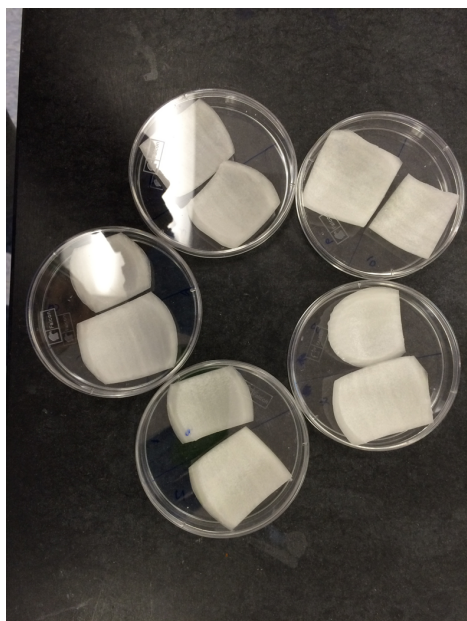


Figure 4-6. GFP expression in onion cells (50x fluorescence microscope)



## Onion cell targets

To prepare the onion targets, buy a fresh white onion at your local grocer or farmer's market. Cut off about one-third of one side, and then lift out a segment that is larger than an inch in diameter and cut it down to about an inch. Place the targets in a sterile petri dish and cover until they are taken out briefly to bombard (see [Figure 4-7](#)). After firing the tungsten particles, you can inspect the onion target using a 10–40x stereo microscope to see the pattern of tungsten particles and estimate penetration. After you have completed a batch of targets, add about 500  $\mu$ l of water to each dish and seal with parafilm. Place them in the dark for 18–24 hours to incubate. After incubation, assay for the reporter to see if you have any transformants.



*Figure 4-7. Onion cells ready to be bombarded*

## GFP Reporter Protein

We have been using [pEGB35S:GFP:Tnos](#) (pDGB1\_alpha), Genbank file: GB0359. This is a plasmid containing a GFP gene and uses the 35s promoter that works well in plants (see [Figure 4-8](#)). GFP expression by the onion cells can be seen using a fluorescence microscope. There are other artifacts that also cause fluores-

cence, so it is important to differentiate between these auto-fluorescent artifacts and GFP fluorescence; however, it is not difficult.

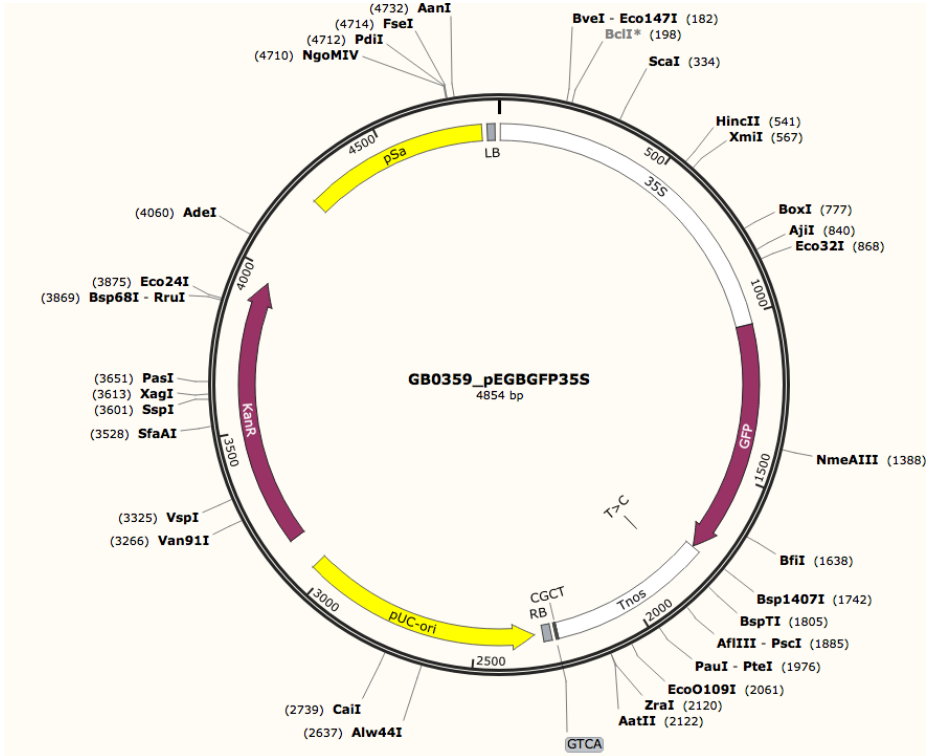


Figure 4-8. Plasmid map for pEGBGFP35S

However, you don't have to use GFP; you can use any reporter system you choose to generate an expression signal that can be assayed. Jay Hanson has posted [a short video](#) showing how to set up and use the gene gun.

## Next Steps

We are actively working to improve the gene gun, focusing on the macrocarrier and how to achieve a more consistent distribution of the tungsten particles with higher transformation yields. Once we get the system working well, we will do a follow-up article in BioCoder describing the improvements. If you have any questions or comments, send them to [diygenegun@gmail.com](mailto:diygenegun@gmail.com).

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*After three decades in the consumer software industry at various companies, **Jay Hanson** retired from Apple and got interested in molecular biology. He is a director at BioCurious, a cofounder at Berkeley BioLabs, and works on synthetic biology projects.*

***Kyle Taylor**, PhD, is the resident plant nerd at BioCurious. He provides scientific guidance, mentoring, and inspiration for the Plant Bio Group.*

*A systems and software engineer who sought a new challenge after working in high tech for more than three decades, **Arnie Wernick** has discovered that he loves solving the technical challenges posed by synthetic biology projects.*



# Open Insulin and Open Source Biologics

*Anthony Di Franco and the Open Insulin team*

*In last year's BioCoder, I wrote in "Superseding Institutions in Science and Medicine" about possible avenues for biohackers to address shortcomings in the state of diabetes treatment as well as broader problems. Today, I'm pleased to be able to report some progress on one front: at Counter Culture Labs in Oakland, we've started a project to make open-source insulin. Progress in synthetic biology during the 40-odd years since the first industrial protocols were developed means we have new possibilities to pursue to make a new synthesis protocol that's simpler, faster, cheaper, and hopefully better suited to use both in further biohacking and generic production.*

## Technical Overview

Insulin is a bit strange.

Isaac Yonemoto, who spends most of his time working on a promising new cancer treatment in Project Marilyn, advised us on the biochemistry of insulin and strategies we might pursue to make it, which was one of the key events that made the project possible. The short version of the story is this: the active, final form of insulin comes from cutting and folding the proinsulin protein. Proinsulin consists of three chains, by convention called A, B, and C. The C chain must be cut out, and the A and B chains brought together to form disulfide bonds, to produce active insulin. (The C chain is also known as "C peptide," which may or may not have a biological function. Its presence is used as a test for endogenous production of insulin in diagnosing cases of Type 1 diabetes: no C peptide is a sure sign the body isn't producing insulin.) Insulin is unusually small, and the steps the body uses to accomplish this cutting and folding are complex, so our job is to find simpler steps that still do the job, and means of assaying our product and

purifying it that defy challenges related to the small size and other unusual features of insulin.

The main method we're pursuing to make insulin is what Isaac originally called a "semi-chemical Nordisk strategy" because we're producing both insulin peptides from a single artificial proinsulin protein like the manufacturer Nordisk, and "semi-chemical" because we're planning to try a palladium catalyst to make one of the necessary cuts. Starting with a modified proinsulin that contains the A and B chains with custom linkers, we'll use enterokinase, a protease, to cut the two chains apart, and remove a tag that contains residues for UV visualization (so we can detect the insulin, which is difficult to accomplish without any modifications) and the residues for purification (histidines to bind in a nickel column). Then, the palladium(II) reaction will clean up some straggling residues on the end of our B chain. Normally, this would be the point where some very finicky reaction conditions would have to be engineered to get the A and B chains to come together in just the right configuration that their cysteine residues form disulfide bonds to hold the insulin together in the correct configuration of its final form. (There are 76 possibilities, but only one correct one. Fortunately, the correct one is thermodynamically favored, but the range of conditions where it's strongly favored is quite narrow.) But in our protocol, this will be the final step, since (with luck) our linker will have already catalyzed the correct disulfide bonding in the proinsulin form. It's a much simpler process than both what the body does and any of the variety of techniques that are presently used for industrial production.

## Looking to the Future

What will we do with our new protocol after putting in the years of hard work to develop it? First, there's a grave need for generic insulin, and we'd like to hand our work off to a generic manufacturer as a basis for simple and inexpensive insulin production. Second, we hope that by expressing, cutting, folding, and purifying a biologic, and documenting everything along the way, we can provide both inspiration and technical knowledge to enable more biohacking on insulin and other biologics. Different variants of insulin have been developed that are faster-acting or slower-acting than the wild-type human form is when injected subcutaneously, and these modified forms are mainstays of current treatment regimes. As an initial step beyond making human insulin, we'd like to look into the small changes needed to make those. Beyond that, academic research has yielded some truly remarkable variations on insulin, like a highly temperature-stable variant

suitable for long-term use in an implanted insulin pump. Such a variant might also vastly simplify supply-chain issues in regions of the world that lack good roads and economical refrigerated transportation infrastructure and provide insulin to the diabetics in those regions who go without and suffer the terrible consequences. My personal favorite is a version that builds a little glucose-shaped hole right into the insulin so that the molecule itself can sense the glucose concentration around it and adjust its activity accordingly, accomplishing at the scale of an individual molecule the work that entire pancreatic cells normally do to keep blood glucose concentrations stable. Either one would be a significant leap forward. Envisioning the new treatments these insulin variants would enable, I can foresee developments that I would count as a cure, or most of one. A long-term implanted insulin pump, sensing glucose concentrations from within the body, where it's easiest to measure concentrations, and releasing insulin there, where it enters circulation much like it does in the natural case, is the cyborg option. It exists to some extent already but remains impractical for most. The glucose-sensing insulin molecule is the nanotech option: the entire feedback control loop for managing one of the most important aspects of animal metabolism, in the space of a few atoms! In any case, I've seen academic research give so many mice so many different kinds of most of a cure that I've gotten quite impatient for most of a cure of my own!

Unfortunately, many people with diabetes can't even afford the therapies that have existed for almost 100 years. There are about 387 million people worldwide living with diabetes, and those living in the poorer communities and regions of the world are often going without treatment. Jeremy A. Greene and Kevin R. Riggs discuss the details in their [March 2015 article in the \*New England Journal of Medicine\*](#). As a result, they suffer complications, including blindness, cardiovascular disease, amputations, nerve and kidney damage, and death. Meanwhile, insulin manufacturers patent small modifications to previous insulins and withdraw those previous versions from the market to keep prices up. To us, it makes sense to align our plans with the best likelihood of contributing to serving the most urgent needs, those of people who don't have access to any diabetes treatment at all. By going beyond current industry practice to find the least expensive protocol, we hope to enable a manufacturer to produce at the lowest possible price. It's a gamble, but it's one we're well-suited to make since we're working outside of institutions whose incentives lead them to take a more conservative approach. And it might not even be higher-risk in the end, since there are many more ways a more complex protocol can go wrong—they require more tacit knowledge that exists in the heads of the researchers rather than in the journal articles and patents they write.



I'm no more than a well-informed, curious diabetic fortunate to have Isaac and other keen minds from academia and industry guiding me and my team as we refine and execute our plans. We started a [campaign on experiment.com](#) to raise funds to do the work and have received a lot of enthusiastic and encouraging support from backers and the press.

With the money we've raised, we've been busy in the lab for the past two months working on producing the proinsulin that we'll then go on to cut and fold. Josiah Zayner, experienced synthetic biologist and proprietor of the [Open Discovery Institute](#), also known as the ODIN, has generously and sagely led us through several iterations of the protocol for producing the proinsulin and has helped us refine our techniques. In the protocol, we first create transgenic *E. coli* by transforming them with a plasmid that contains a polyhistidine-tagged proinsulin gene under a lac promoter. Then we culture the cells and induce expression, lyse them, and purify the proinsulin from the lysate in a nickel column. Finally, we run the output of the nickel column in a polyacrylamide gel and look for a band of about the right size. We've recently gotten preliminary evidence that we've successfully purified proinsulin: a thick band in the gel right where we're expecting one. We're now looking to make these results more reproducible before moving on to the next steps.

When you read this, we'll still be soliciting donations on an ongoing basis on our website at [openinsulin.org](#). And we'll definitely be looking for help in the lab! Drop us a line, or stop by if you're in the Bay Area!

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*Anthony Di Franco works at the intersection of complex adaptive systems and computing and focuses on democratizing access to technology, decentralizing infrastructure, and increasing the agency of individuals and communities. He is a cofounder and board member of [Counter Culture Labs](#), a group of biohackers in Oakland, where he founded the Open Insulin project. In addition to working on Open Insulin, he is currently pursuing computer science research on declarative programming with uncertain information, with the goal of making software easier for many more people to create and use.*

# Creating a Functional Window to the Brain

AN INTERVIEW WITH HENDRIK D. KJELDSSEN OF TRUUST NEUROIMAGING

*Alex Kopelyan*

Modern neuroscience is still relying on old methods that don't allow us to truly understand what's happening in the brain in real time. As a result, we have a limited understanding of brain-related disease and ability to treat conditions early. [Truust Neuroimaging's](#) technology is providing real-time data to visualize energy flow in the brain, and, as a result, predict and treat brain-related diseases before they start. I spoke with CEO Hendrik D. Kjeldsen about how he discovered this problem, the limitations of today's technology, and how Truust can change the field. Check out his pitch from [IndieBio's Demo Day Livestream](#).

**AK: Tell me about your background. How did you get interested in the biotech space?**

HK: I met my cofounder, Lars, about 15 years ago, while working as an electrical engineer. As a result of the computational problems presented while working on the Semantic Web, I got interested in artificial intelligence and did a master's of AI in the Netherlands. I realized current AI approaches are not able to handle such complex problems, and we need to study real intelligence to figure it out. So I went to the UK to do a PhD in experimental neuroscience, where I faced a new problem: current tools are only able to see very little of what is going on in the brain. This not only makes it very difficult to understand the brain, but also makes it impossible to diagnose brain-related problems early enough to catch disease before they present with behavioral symptoms or become actual structural changes in the brain. This made us realize that we need to improve our neuroimaging tools; and based on the idea of super-resolution, we initially created a solution for specialized microelectrode arrays that worked surprisingly well. Since

then, I spent some time at CERN, the global leader in experimental particle physics, where I really came to understand the potential of super-resolution signal processing techniques.

**AK: What problem are you working to solve with your company, Truust?**

HK: A major problem in neuroscience is that we are relying on statistical analysis to understand connections in the brain since we can't actually see real-time energy flow. With our super-resolution method, we can see the actual flow of electromagnetic energy from point A to B, which means we now have a window into the living, dynamic brain for the first time. So using existing EEG hardware, we will collect huge amounts of data that machine learning can be applied to and understand EEG biomarkers of a wide range of brain-related problems. We envision a future with an EEG terminal in every doctor's office as part of every physical; and the doctor does not even have to be an expert; our biomarker system is all the assistance they need.

**AK: If you could only pick one thing to validate your reason for forming a startup, what would it be? In other words, what would be the single biggest indicator to you that you are doing the right thing?**

HK: Seeing all different kinds of people, from random kids to experts in the field, get really excited about our prototypes visualizing energy flow in the brain tells us that we are really doing something new at the cutting edge of understanding the brain.

**AK: How do you think success can change your industry?**

HK: We are going to revolutionize neuroimaging to make it useful and relevant to the general population and their healthcare. It will become much lower cost than current MRI and PET techniques.

**AK: How is your team uniquely able to tackle this? What's the expertise?**

HK: We have a unique perspective on the brain being cross-disciplinary between electrical engineering, AI, physics, and neuroscience. This allows us to take a physical and computational approach to what has traditionally been a biological problem. We have worked on these problems in one form or another for a long time, and what we are doing now is a natural culmination of that path.

**AK: Any big lessons learned transitioning to startup entrepreneurship?**

HK: We've had the expectation fulfilled that things move very fast. We can do things much quicker than in academia or in large companies. That's actually one of the reasons we left those to form a startup. Things were moving very slowly. In a startup, we are learning much faster since we're able to experiment and iterate so much faster.

**AK: What's the biggest challenge you've encountered so far?**

HK: Talking about what we do in a way that isn't too academic. There's a balance between being technically correct and not being overly technical. These are new ideas that people aren't generally familiar with, so communicating the nuances while still letting them see the big picture can be tough.

**AK: What are the big goals and milestones you're looking to hit in the short term? Long term?**

HK: In the short term, we want to validate our technique with a number of different labs on hard, real-world problems, like better seizure localization for epilepsy. Our long-term goal is to truly understand the brain, which would open up many more general applications all across medicine and research.

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# Building with Biology

## PUBLIC ENGAGEMENT WITH SYNTHETIC BIOLOGY

*Megan Palmer, Natalie Kuldell, and David Sittenfeld*

From CRISPR in the *New York Times* to George Church on *The Late Show*, it seems that synthetic biology is all over the headlines lately. But what does your family—or your neighbor—need to know about biological engineering to make wise decisions? How can and should their values and visions for the future shape what synthetic biology becomes?

Synthetic biology is evolving as a field and an industry. But its trajectory is already being shaped by drivers, decisions, and dreams of communities well beyond those that identify as synthetic biologists. And while there is a lot of talk about preparing the world for synthetic biology through better communication, too often our actions suggest the goal is to get other people to better understand the technology. That is, if they just understood its potential and practice, we'd all chill out and be on the same page. But we know this simply isn't true.

Technology is part of the social world in which our values and preferences often conflict, and we need to contend with the inevitable choices involved. As with politics, trust in technologies and institutions is not engendered by belittling the public (often called the “deficit model”). Rather, it is facilitated by actively listening and reflecting—engaging—with hopes, fears, and values, including our own as members of the public.

So, that might sound right, but what do you actually *do* about it? If a civically-minded synthetic biologist wants to engage with the public, where do they start?

This article introduces one ongoing project in implementing US public engagement with synthetic biology. We are members of a growing group halfway through a two-year experiment across the nation, and we hope to convince you to join us in improving its design.

## Cultivating a Community of Public Engagement Practice

Six years ago, we found ourselves grappling with daunting questions about the public role of science and scientists. As a PhD student, a university professor, and a science museum professional, we had different communities—peers in science and engineering, fellow students, and an invested public—but similar questions: who can and should decide what science and technologies are developed? For what ends? And by whom?

After meeting through local science events, we began discussing synthetic biology as an area in particular need of wider public engagement. Since the field's aspirations raise fundamental questions about our relationship with nature and ourselves, it was just a matter of time before things got messy, so to speak.

Meanwhile, scientific communities beyond synthetic biology began recognizing a need to improve their approaches to science communication. The American Association for the Advancement of Science (AAAS) and their then-CEO Alan Leshner began highlighting the vital role of multidirectional dialogue among scientists and diverse publics. Likewise, science museum educators were seeking to refine their toolkit with innovative, informal pedagogies for engaging diverse audiences around complex scientific issues.

A boost to these efforts came nearly a decade ago when the National Science Foundation (NSF) funded the Nanoscale Informal Science Education Network (NISE Net). Through exhibits and programs at museums across the US, the 10-year effort engaged millions of visitors in learning about the science and societal implications of nanotechnology.

Fast forward to just over a year ago, and the field of synthetic biology has matured along with our careers. David Sittenfeld is leading museum engagement efforts, including NISE Net, within the Boston Museum of Science. Megan Palmer is leading policy research within a multi-university, publicly funded synthetic biology engineering research center (Synberc). Natalie Kuldell is leading a nonprofit venture in synthetic biology education (Biobuilder).

While our questions about the public role of science and technology were no less daunting, we had by then cultivated a diverse array of partners bringing complementary expertise to bear. They include the [AAAS](#), with a wealth of expertise in science communication theory and practice and an important bridge to the scientific societies within and outside the synbio community; Synberc, which gathers a cohort of synthetic biologists in universities and companies around the country; the [Association of Science and Technology Centers \(ASTC\)](#) and NISE Net, which bring networks of science museums versed in informal science education; and [BioBuilder](#), with deep expertise in pedagogy around biotechnology education.

These partners, and others, came together to form what is now called the Building with Biology project, formally known as **Multi-Site Public Engagement with Science - Synthetic Biology**. Our goal is to facilitate conversations between scientists and the public around the societal aspects of engineering biology. But a second major goal is to better understand the value of public engagement for scientists and museum professionals involved. One hypothesis is that we can all benefit from tools to understand and navigate the diverse values we bring to our work.

Thanks to support from the NSF, and many volunteers, we have been co-creating a suite of pilot engagement activities designed to facilitate conversations at hundreds of sites across the nation.

## Co-Creating a Concept Map for Conversations

So where did we start?

From the beginning, the project partners tried to foster mutual learning. We began by bringing together practitioners (including both social and natural scientists and engineers) and museum professionals, to develop a set of “big ideas,” our best guess at core concepts people need to understand to engage with the field’s development. Now, anyone who has been in circuitous conversations about the definition of synthetic biology realizes this is no easy task. We went through significant revisions (and a few heated discussions) in the first year of the project.

Eventually a key theme emerged from our discussions: the need to treat synthetic biology as a *hypothesis* and a *process*. We are still exploring the best ways to engineer living systems—by evolution, design, or many hybrid approaches—and it was important to capture this diversity.

**Figure 7-1** shows our “concept map.” Two ideas—that synthetic biology generates new tools and knowledge and that it enables new products—may seem intuitive. The other two may be a bit new.



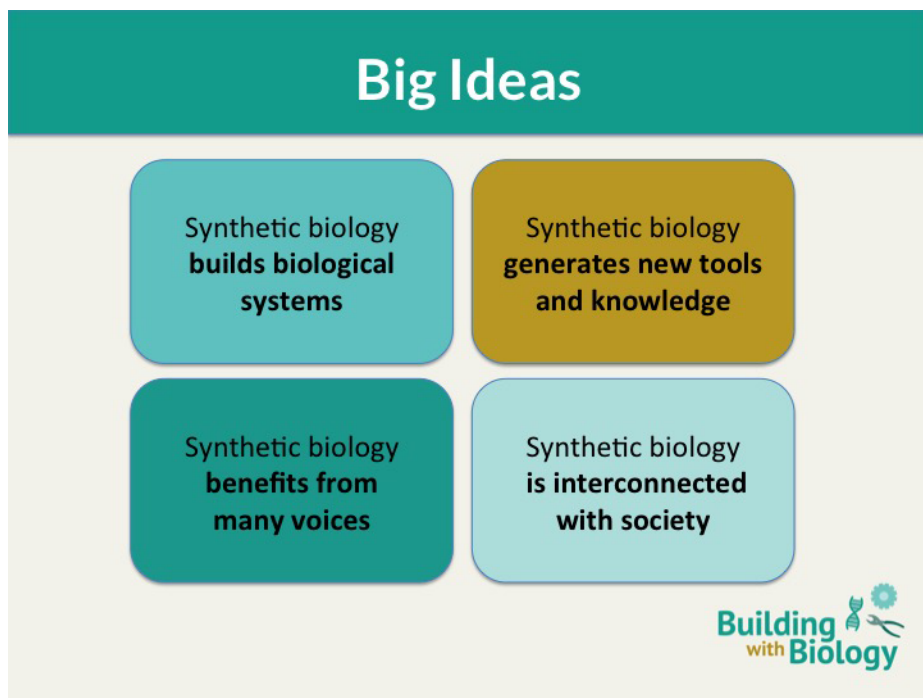


Figure 7-1. Big ideas concept map

The big idea that synthetic biology benefits from many voices is intended to convey the interdisciplinary nature of the field, and to convey the idea that making biology easier is both a *means* and *ends*. Efforts like the international Genetically Engineered Machine (iGEM) competition and do-it-yourself (DIY) biology movements have taught us that putting tools in the hands of many diverse communities seeds innovation.

The fourth big idea is that technology is inextricably coupled to societal values and involves benefits, costs, and risks. This is a critical idea and at the heart of the project. The idea is that we make choices about what technologies to develop, and these choices have repercussions.

## From Concepts to Kits and Conversations

So what did we do with these ideas?

In early June, 200 physical kits will be sent to a mix of informal science education institutions, such as science centers, universities, and iGEM teams. Each kit contains a collection of hands-on activities and discussion forums, co-created

by educators from 12 science museums around the country with partnering scientists from the synthetic biology community. Accompanying the activities are guides and training materials highlighting key concepts and techniques for holding conversations with different ages, and opportunities for mutual learning. The kit also contains materials about science communication and public engagement techniques for event hosts and scientists and resources for evaluating their conversations with the audience. Digital materials will also be available for anyone to download for free at the [Building with Biology website](#) starting in late May.

Just as synthetic biology is an interdisciplinary field bringing together varied skills and perspectives, our development process for the kits required input and feedback from a diverse group of stakeholders including physical and social scientists, educators, and science communication experts. We used this feedback to inform an iterative process of revisions, inspired by the engineering design process.

One element of the kits are hands-on activities. These will be familiar to museum educators and scientists alike and are designed to be facilitated by scientists and informal educators at museums and other sites. See [Figure 7-2](#) for a photo from one of the pilot events last summer.

Tech Tokens, an activity created by museum educators at the Science Museum of Minnesota and synthetic biology partners at the University of Minnesota, is a great example of a hands-on activity that gets people talking with one another about an emerging technology. In this activity, visitors learn about and prioritize potential applications of synthetic biology (examples include next-generation biofuels, engineered insects to transform agriculture or fight disease, and new methods for producing life saving medicines). They're given 10 tokens representing research and development funding that could help a technology mature and be adopted into future society. The facilitating scientist asks them to allocate tokens to technologies they find most compelling, giving them the opportunity to share their values and priorities with scientists who may one day engineer some of these biotechnologies. The scientist then asks them to consider the perspective of a character who may bring a different set of priorities and values, such as a doctor in South Africa, a European farmer, or the president of the United States. In thinking about the technologies that these different people might prioritize, visitors and scientists consider together. One of the big ideas that drove the development of this activity is that "synthetic biology benefits from many voices." By this, we mean that emerging technologies bring up questions that science can inform but cannot answer on its own, and that societal decision-making around such questions is enriched by the inclusion of multiple and varied perspectives.



Figure 7-2. Hands-on activity

Another hands-on activity is the Kit of Parts (Figure 7-3), created by scientists at the University of Pennsylvania and educators at the Franklin Institute. In the first part of the activity, the visitor selects a problem that could be solved through synthetic biology, such as how to stop environmental damage, manufacture renewable plastic, produce an antimalarial drug, or develop a new type of cancer therapy. This initial step of choosing a problem to solve creates another opportunity for scientists to hear about the priorities, values, and concerns that museum visitors bring to potential applications of synthetic biology. It also helps to model the prominence of the engineering design process in the field. After the visitor decides on a problem to solve, the facilitating scientist then guides the visitor through a design process to engineer a cell, modeled from a set of child's blocks, that represents a registry of standardized biological parts. Examples of parts that visitors can select include an on/off switch, an array of biological sensors, a part for cell movement, a cell death switch, and a number of production functions. Visitors consider the tradeoffs of factors such as complexity, cost, control, and safety as they engineer an organism to help solve the societal problem they have

selected, learning that design decisions inherently reflect a mix of social values, priorities, and perspectives.

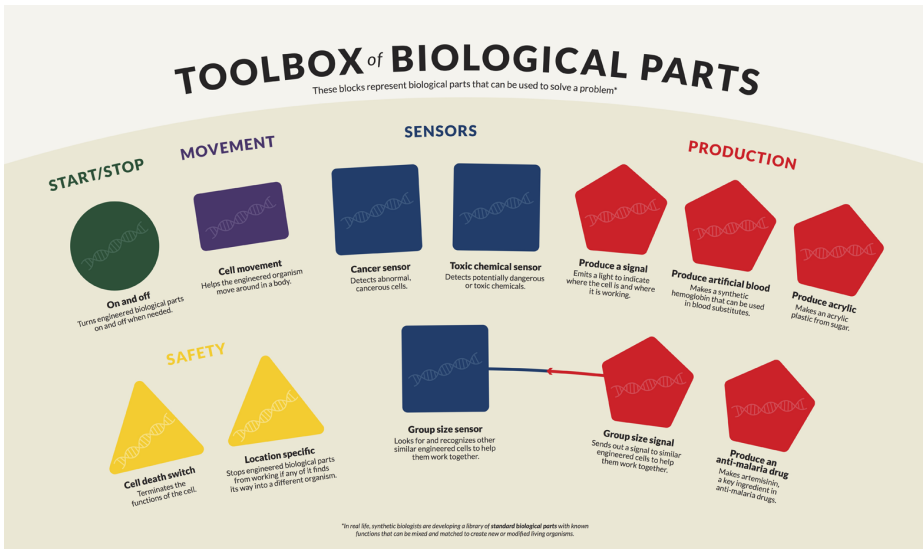


Figure 7-3. Toolbox of biological parts

The second kind of conversation we’re targeting through the Building with Biology project is what we call a “forum,” a more deliberate kind of discussion in which scientists are not facilitators, but participants. These forums allow scientists and members of the public to exchange ideas and learn from one another in a different way than they do in the hands-on activities. Every kit will include materials for a forum called “Should We Engineer the Mosquito?” This program facilitates a conversation about the question of whether and how mosquitos should be released to combat vector-borne disease. Participants learn about the potential benefits, risks, costs, and considerations of mosquito engineering, thinking about the perspectives of community members in Kenya facing the threat of malaria. Forum participants consider questions such as whether or not to employ a gene drive technology, what entity should oversee a release, and how to monitor success and environmental impact. In thinking about these questions, members of the public and scientists have the opportunity to learn from one another about the values and perspectives that are inherent to such socioscientific issues. Another forum, on the widely discussed CRISPR technology for gene editing, will be made available for digital download on the project website.

These activities and forums were piloted in the summer of 2015 at eight science centers around the nation and revised in response to the evaluation data. The results from the pilot year are quite encouraging: 97% of visitors at the pilot sites agreed or strongly agreed that they enjoyed the Building with Biology events. Attendees learned about synthetic biology and said that the experience increased their interest in learning more about the connections between synthetic biology and their daily lives. Scientist volunteers at the events said that participating in the events increased their skills and interest in public engagement with science. Participating scientists said they learned that the public is open to conversations about synthetic biology and that museum visitors were able to have complex and rich conversations about the field and its societal implications. The 200 Building with Biology events this summer will allow the project team to learn much more and help to create a roadmap for future work.

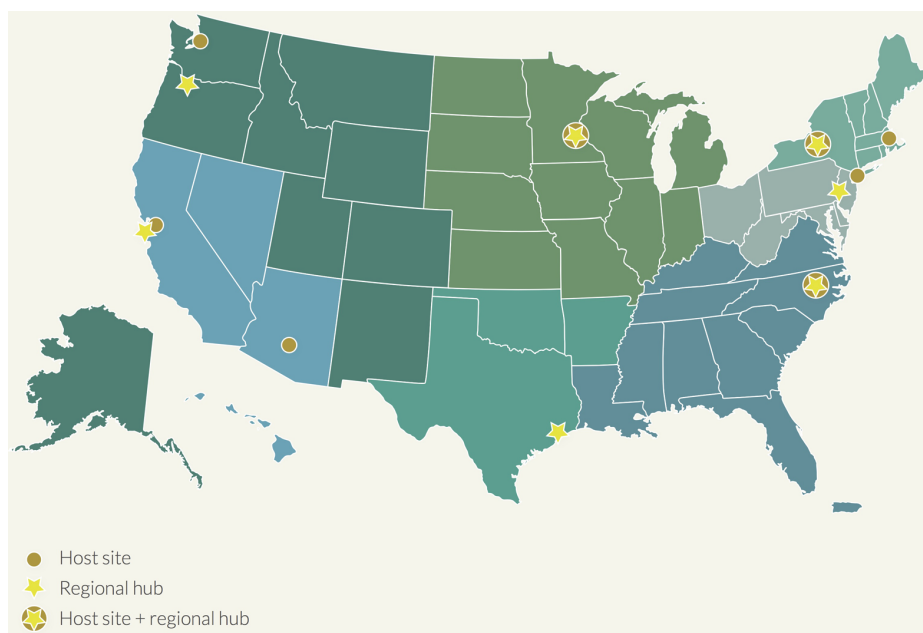


Figure 7-4. Building with Biology pilot site map

## Continuing the Conversation

Our early goal was to find out what's on people's minds when we talk about synthetic biology, and so now we ask: how well are we doing that? In a few months, physical kits will be sent to nearly 200 locations nationwide. They will provide all

the materials and instructions we've developed so communities can hold some structured conversations about the field of synthetic biology and its societal implications. To recruit partners, we've tapped into our scientific and museum networks, our social media circles, and word of mouth. We have participants that range from the Arecibo Observatory in Puerto Rico to the Open Bio Labs in Virginia, but almost certainly we could do more to recruit and reach the broadest community. So we're looking for better ways to spread the word.

We're also looking for appropriate measures to gauge how well the conversations are going. As a condition for receiving the kits, we've asked for a commitment to run an engagement event and provide feedback about the conversations among scientists and public audiences. We're developing an online reporting system to gather information, and we've included media tools to help keep participants engaged and connected. To further develop the materials, pre- and post-surveys and interviews about the engagements will be used. Additionally, there is an opportunity to evolve the materials we've developed by inviting iGEM teams and BioBuilderClubs to "hack" the kits and use them in their outreach efforts. Such a partnership strikes us as a win-win because it leverages local wisdom and encourages work that can be reused.

These new kits and forums offer a useful template for future work. Synthetic biology itself is an unfolding field, and we will need increasingly diverse ways to engage in conversations about it. What other constructive dialogs for synthetic biology can be imagined by us and by others? Just as synthetic biology is a working hypothesis about engineering living systems, so is our strategy for enhancing public engagement in this arena. We are hopeful that our early efforts to include engagement in public communication about synthetic biology will support other efforts like it in the future.

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**Megan J. Palmer**, PhD ([@meganjpalmer](https://twitter.com/meganjpalmer); [meganjpalmer.com](http://meganjpalmer.com)) develops and advises on best practices and policies for advancing biotechnology. She is a senior research scholar at the Center for International Security and Cooperation (CISAC) at Stanford University. She is also an investigator of the National Science Foundation Synthetic Biology Engineering Research Center (Synberc), where she served for five years as codirector of its Policy & Practices research portfolio. Megan is the founder and executive director of the Synthetic Biology Leadership Excellence Accelerator Program (LEAP; [synbioleap.org](http://synbioleap.org)) and serves on the human practices and safety committees of the iGEM competition ([igem.org](http://igem.org)).

**Natalie Kuldell** ([@SystemsSally](https://twitter.com/SystemsSally), [biobuilder.org](http://biobuilder.org)) is founder, president and executive director of The Biobuilder Educational Foundation, a nonprofit organization that takes cutting-edge research projects in synthetic biology and transforms them into teachable modules that students

*and teachers can investigate together. The BioBuilder curriculum is now taught in almost every US state and around the world through the BioBuilderClub. A BioBuilder textbook was published in the summer of 2015 by O'Reilly Media.*

**David Sittenfeld** (@dsfeld) manages the Forum program at the Museum of Science, Boston, which engages citizens, scientists, and stakeholders in deliberative conversations about socio-scientific issues. David has been an educator at the museum for over 15 years and oversees the creations of special programs and exhibits relating to issues that lie at the intersection of science and society. David is also a member of the Executive Committee of the Expert and Citizen Assessment of Science and Technology ([ecastnetwork.org](http://ecastnetwork.org)) and a doctoral candidate at Northeastern University, focusing on participatory methods for environmental health assessment and public engagement.