

# Mind The Gaps:

## *Constructing a Culture of Safety & Security in Synthetic Biology*

Drew Endy

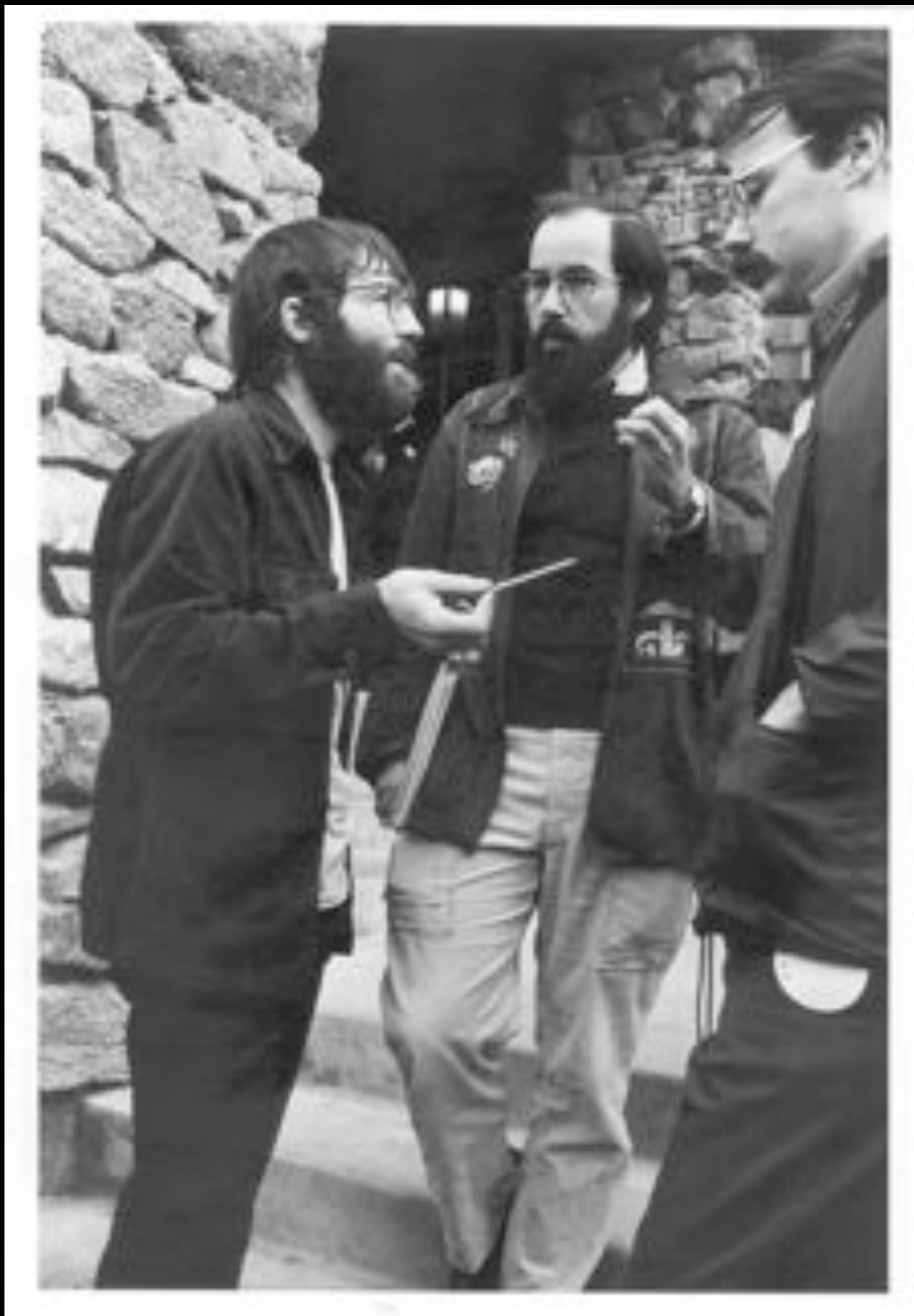
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# The Real Paper

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DOING DNA  
AT HOME:  
A RECIPE FOR  
BOTULISM



PAT  
CAIDELL  
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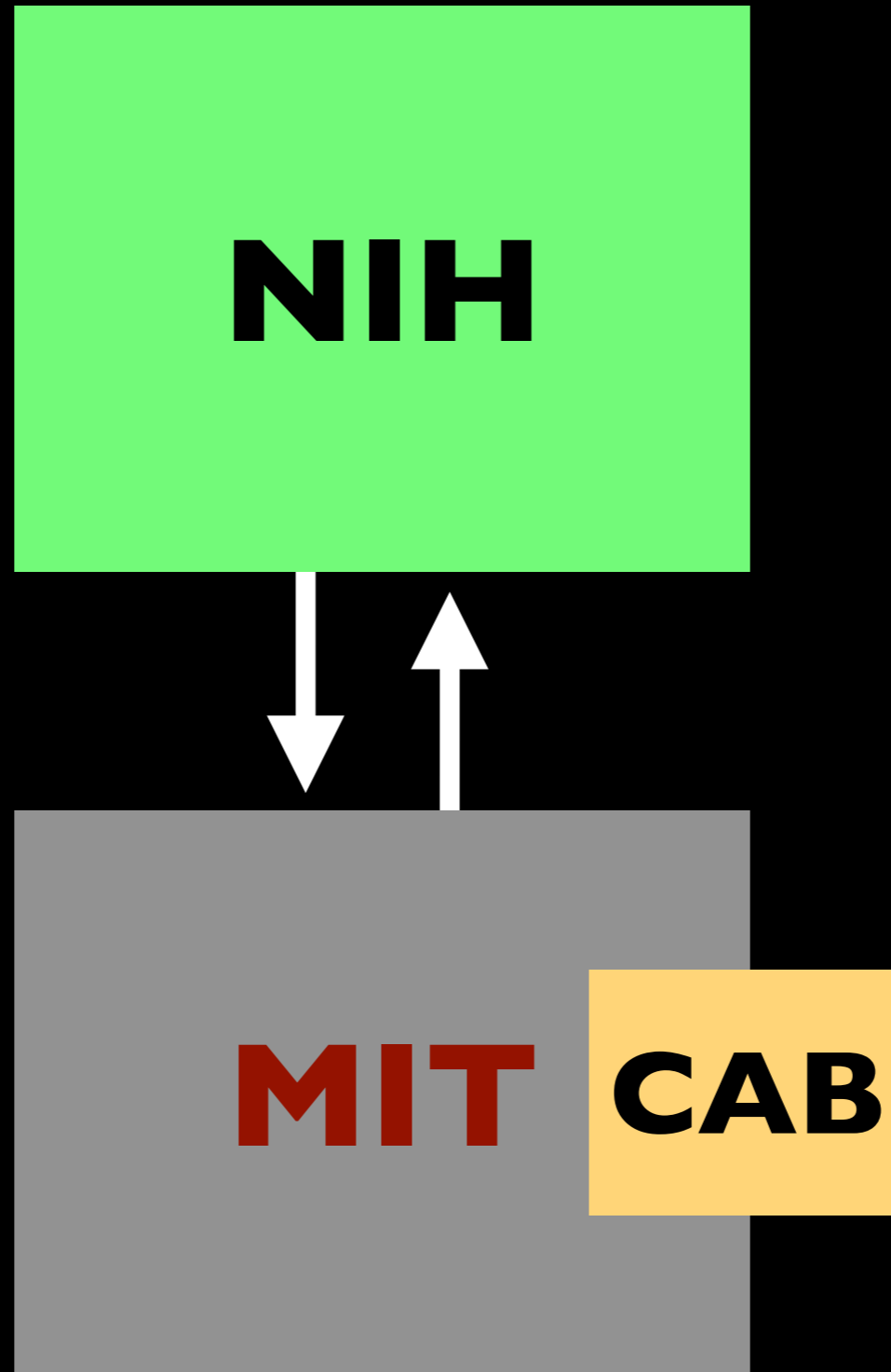


**Biosafety Level 1** practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for handwashing.

**Biosafety Level 2** practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided the potential for producing splashes or aerosols is low. Hepatitis B virus, HIV, the salmonellae, and *Toxoplasma* spp. are representative of microorganisms assigned to this containment level.

**Biosafety Level 3** practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and potentially lethal infection. *Mycobacterium tuberculosis*, St. Louis encephalitis virus, and *Coxiella burnetii* are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols. At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols.

**Biosafety Level 4** practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that pose a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4. The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals, pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.



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Letter

## Identification of an infectious progenitor for the multiple-copy HERV-K human endogenous retroelements

Marie Dewannieux<sup>1,3</sup>, Francis Harper<sup>2,4</sup>, Aurélien Richaud<sup>1,4</sup>, Claire Letzelter<sup>1</sup>, David Ribet<sup>1</sup>, Gérard Pierron<sup>2</sup>, and Thierry Heidmann<sup>1,5</sup>

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Human Endogenous Retroviruses are expected to be the remnants of ancestral infections of primates by active retroviruses that have thereafter been transmitted in a Mendelian fashion. Here, we derived in silico the sequence of the putative ancestral "progenitor" element of one of the most recently amplified family—the HERV-K family—and constructed it. This element, *Phoenix*, produces viral particles that disclose all of the structural and functional properties of a bona-fide retrovirus, can infect mammalian, including human, cells, and integrate with the exact signature of the presently found endogenous HERV-K progeny. We also show that this element amplifies via an extracellular pathway involving reinfection, at variance with the non-LTR-retrotransposons (LINEs SINEs) or LTR-retrotransposons, thus recapitulating ex vivo the molecular events responsible for its dissemination in the host genomes. We also show that in vitro recombinations among present-day human HERV-K loci can similarly generate functional HERV-K elements, indicating that human cells still have the potential to produce infectious retroviruses.

<sup>3</sup> Present address:

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### Safety precautions

All manipulations involving the reconstructed HERV-K were carried out in our lab according to the rules established by the "Commission de Génie Génétique" from the "Ministère délégué à l'Enseignement supérieur et à la Recherche" French authority that regulates handling of genetically modified organisms in all research institutions in France.

Albeit the HERV-K virus has a very low infectivity and does not sustain multiple-cycle infection, at least in all the cells tested, Phoenix is a retrovirus, and as such, is a priori eligible to BL3 conditions for manipulation. Accordingly, the material will only be sent to other labs in appropriate sealed containers in the form of small amounts of plasmid DNA that will require it to be amplified before use as a transfection vector to produce viral particles. At the present time and as a precautionary principle, it will only be distributed under a material transfer agreement specifying the commitment of the recipient labs to carry out every experiment using the material under BL3 conditions and accompanied by a duly signed authorization form from the Biosafety Committee responsible for genetic manipulations in their country of origin.

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# What's changed since the 70s?

1. Databases populated with sequence information.
2. The internet.
3. Early advances in DNA construction technology.
4. Overnight shipping.
5. Expanded concern re: active misapplication of biotech.





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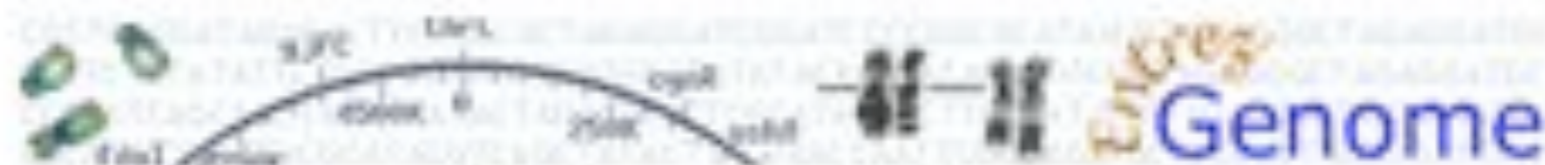
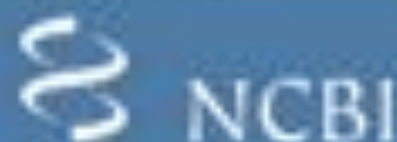


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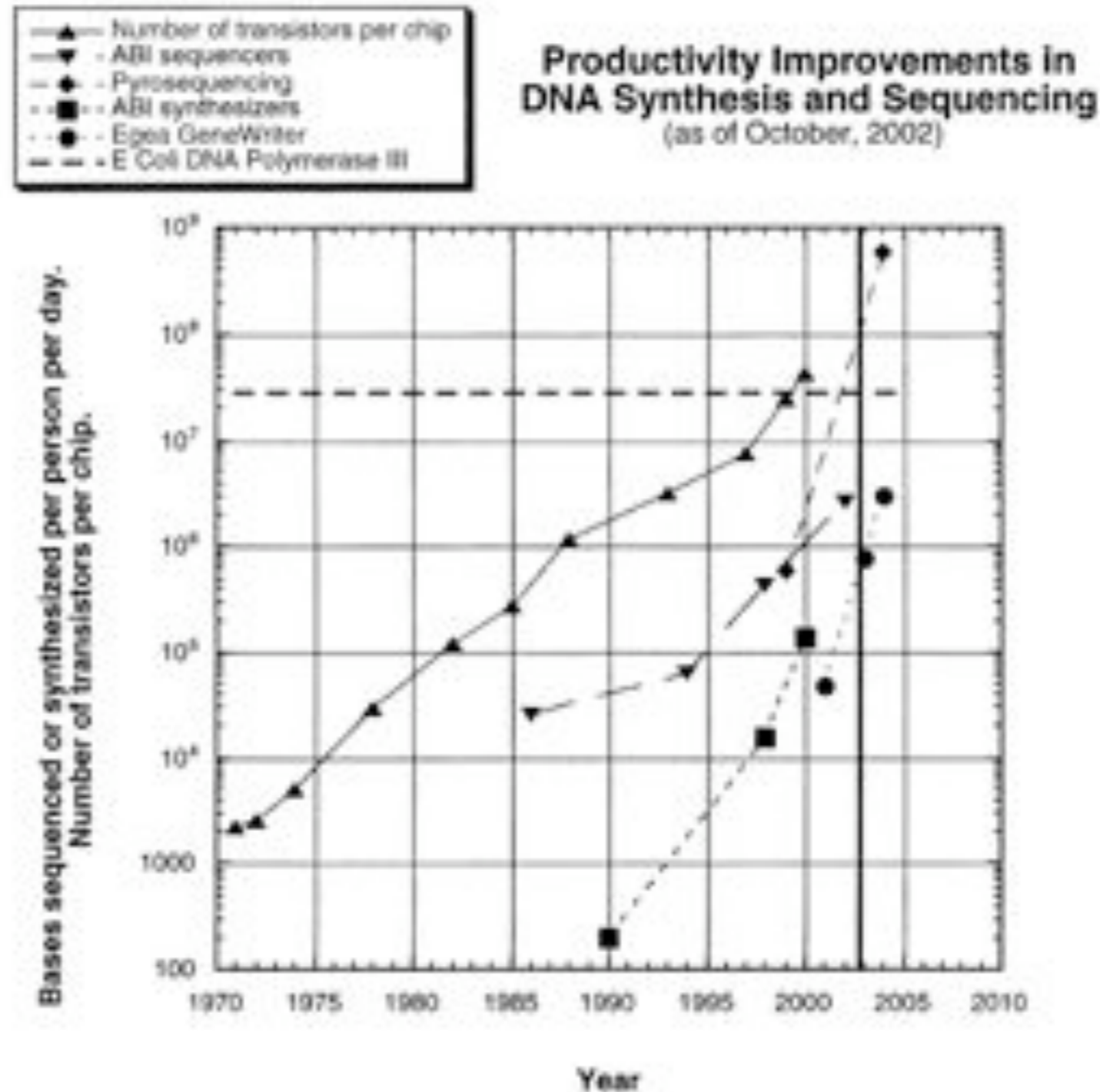
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# Ability to build DNA from scratch improving **faster** than computers



Information (DNA Sequence)

The diagram consists of two large horizontal arrows pointing to the right. The top arrow is blue and contains the text 'Information (DNA Sequence)'. The bottom arrow is red and contains the text 'Material (Physical DNA)'. A white vertical arrow points upwards from the red arrow to the blue arrow, labeled 'Sequencing'. Another white vertical arrow points downwards from the blue arrow to the red arrow, labeled 'Synthesis'.

Sequencing

Synthesis

Material (Physical DNA)

# DNA synthesis and biological security

Hans Bujal, John P Danner, Robert J Millinari, John T Mulligan, Han-Oh Park, Ben Reichert, David A Roth, Ralf Wagner, Bruce Yadonko, Robert M Scripps, Jennifer A L Smith, Scott J Stead, George Church & Drew Endy

**A group of academics, industry executives and security experts propose an oversight framework to address concerns over the security of research involving commercial DNA synthesis.**

**D**NA synthesis allows the direct construction of genetic material starting from information and raw chemicals<sup>1</sup>. Improvements in synthesis technology are accelerating innovation across many areas of research, from the development of renewable energy to the production of fine chemicals, from information processing to environmental monitoring, and from agricultural productivity to breakthroughs in human health and medicine. Like any powerful technology, DNA synthesis has the potential to be purposefully misapplied. Misuse of DNA synthesis technology could give rise to both known and unforeseeable threats to our biological safety and security. Current government oversight of the DNA synthesis industry falls short of addressing this unforeseen reality.

Here, we outline a practical plan for developing an effective oversight framework for

the DNA synthesis industry<sup>2</sup>. The resulting framework serves three purposes. First, it promotes biological safety and security. Second, it encourages the further responsible development of synthetic biology technologies and their associated, non-biological applications.

And third, it is designed to be international in scope. Our plan is informed by past and ongoing the actions of biological security issues associated with DNA synthesis technology<sup>3,4</sup> and represents the collective views of all founding members of the



Hans Bujal, John P Danner, Robert J Millinari, John T Mulligan, David A Roth & Ralf Wagner are members of the International Consortium for Progenitorless Synthesis. Hans Bujal and Ralf Wagner are at GENEXIS. John P Danner, George Church & Drew Endy are at Codon Devices. Robert J Millinari & David A Roth are at ODS Genomics. John T Mulligan is at Blue House Biotechnology. Han-Oh Park is at Bioss. Ben Reichert is at NextGene Inc. Ralf Wagner is at the University of Regensburg. Molecular Biology & Gene Therapy Unit, Institute of Medical Microbiology and Hygiene. Bruce Yadonko, Robert M Scripps, Jennifer A L Smith & Scott J Stead are at the US FBI. George Church is in the Department of Genetics, Harvard Medical School. Drew Endy is in the Department of Biological Engineering (M2), George Church & Drew Endy are at the multi-institutional US National Science Foundation Synthetic Biology Engineering Research Center. [drew@pedi.ucsf.edu](mailto:drew@pedi.ucsf.edu)



**Figure 1** Our framework calls for the creation and governance improvement of a basic DNA synthesis order screening process. To promote and ensure accountability, individuals who place orders for DNA synthesis must be required to identify themselves, their firms engaged in and all relevant research information. Next, individual companies would use validated software tools to check synthesis orders against a set of safety agents or accuracies they create regulatory compliance and the synthesis orders for further review. Finally, DNA synthesis and synthetic biology companies would work together through the IOP, and interface with appropriate government agencies worldwide, to rapidly and collaboratively improve the underlying technologies used to screen orders and identify potentially dangerous sequences, as well as issuing a clearly defined process to report behavior that falls outside of agreed-upon guidelines. IOP, International Consortium for Progenitorless Synthesis.

National Biodefense Analysis and Countermeasures Center – Wikipedia, the free encyclopedia

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## National Biodefense Analysis and Countermeasures Center

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The **National Biodefense Analysis and Countermeasures Center** (NBACC) is a highly classified government **biodefense** research laboratory created by the **U.S. Department of Homeland Security** (DHS) and located at the government's sprawling biodefense campus at **Fort Detrick** in **Frederick, MD, USA**. Created quietly a few months after the **2001 anthrax attacks**, the NBACC (pronounced **EN-back**) is intended to be the principal U.S. **biological warfare** research institution engaged in "science-based threat assessment."

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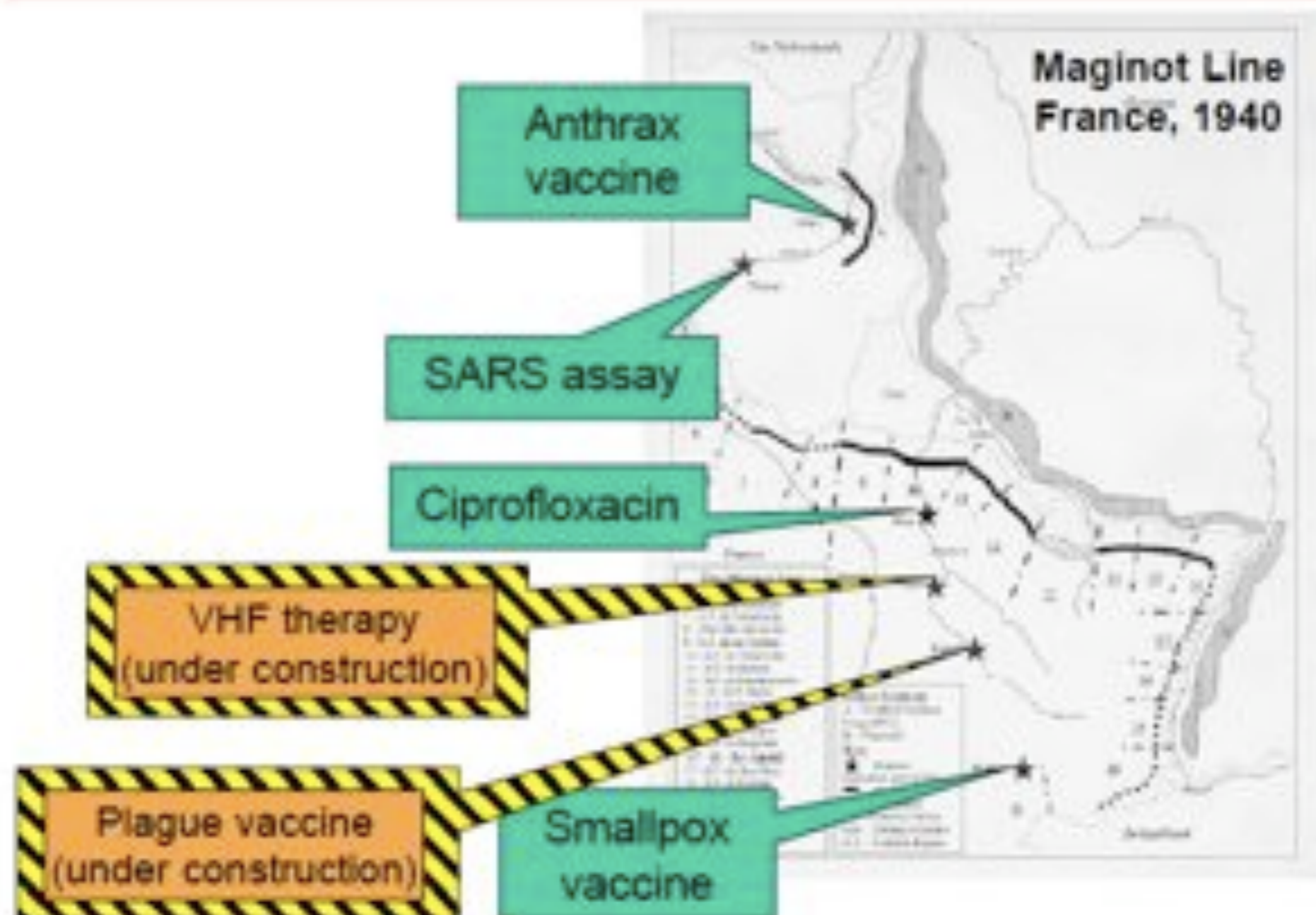
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# Biological Risk: Tactics as "Strategy"

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# What else is changing now?

1. Many new types of people are incredibly excited about biological technology.

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by Professor L.

The creation of genetically modified organisms (GMOs) is now within the ability of a knowledgeable and dedicated hacker. The most common genetic modification is the insertion of genes from one organism into another. The recipient is called a "transgenic organism" and this article will give you enough information so that anyone who could pass a high school biology lab can create one.

The usual 2600 article starts off with a disclaimer about how the article is for informational purposes only, and should the reader do anything illegal or dangerous, that's the reader's fault. The disclaimer in this article has to be stronger. Creating transgenic organisms has the potential to do great, possibly even catastrophic harm to the entire biosphere. Although the specific manipulations I describe in this article are safe (and often done in biology teaching labs), knowledge of the methods of genetic engineering have the potential to unleash enormous forces for good or for evil.

The most likely harmful consequence of hackers making a mistake with genetic engineering is for the hackers to get sick or to make the people around them sick. Maybe really, really sick. If you are going to try these techniques, learn about safe laboratory practices and follow them. The consequences of screwing up with genetic engineering are much worse than a mere jail sentence, so treat it seriously. No kidding.

If these techniques are so dangerous, why on earth would I want to tell hackers how to use them? I've thought about this long and hard before writing this article, and I have three reasons for writing. First, none of the information in this article is all that hard to find these days. Good high school biology classes teach the ideas (although they often figure out how to make it seem boring), and pretty much every community college will have a molecular biology lab class that teaches all of this information and good lab technique, too. If you think this article is

cool, I would strongly encourage you to take a real lab mol bio course and get at the good stuff.

My second reason is that I believe in the hacker mentality. When as a teenager I got tired of stacking tandems with my 8038-based blue box, I built an Inreal 8008, one of the first computer kits. Twenty-five years later, looking at my lab and all the scientific publications and prizes I have, even the straight world would have to admit that some hackers have made positive contributions to society. The hackers in the Homebrew Computer Club in the 70's spawned much of what would become Silicon Valley. The technologies that fascinate us have the power to create a radically different world; that is, they have the potential to be used for both awesome creation and awesome destruction. Hackers, who these days I think of as kids with a thirst for knowledge and the urge to try things for themselves, can be the ones with the powerfully creative ideas about how to use new technologies.

And my third reason for writing is that corporate powers are already using these technologies very broadly, and in ways that I don't feel are doing justice to their potential. With this article, I hope to inspire people to learn about what genetic engineering can do, and to come up with superior alternatives to the profit-seeking corporate approach. How do corporations use genetically modified organisms? Chances are, you are eating them! Pretty much all processed food in America contains GMOs. Monsanto's Roundup Ready crops dominate worldwide commercial agriculture, including soybeans, corn, cotton, canola oil, and sugar. The particular genetic modification in these foods makes it possible to dump the weedkiller Roundup on the crops without killing them. It's convenient for industrial farmers and it helps keep Monsanto the world's largest seller of herbicides. Surely there must be a better use for transgenic organisms than that! I hope someone reading this article will one day invent it.

# Make:

technology on your time

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- Extract Your DNA
- Head-Mounted Water Cannon
- Build a Webcam Rocket

## HACK YOUR 9 PLANTS

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## PROJECT 2: Build a Thermal Cycler and Run PCR

Real DNA "fingerprinting" is usually done using a procedure called polymerase chain reaction, or PCR. This process replicates DNA, making a much larger sample that produces more detail in electrophoresis and is therefore easier to match. To perform PCR, you need some specialized chemicals and equipment.

The chemicals are small, pre-designed pieces of DNA known as primers, plus a heat-stable DNA polymerase reagent such as Taq. The primers combine into new copies of the sample DNA strand, and the polymerase enzyme catalyzes this assembly process. Both of these materials are readily available from biotech supply companies such as Takara (<http://www.takara.com>) for less than \$100, which buys enough for about 100 reactions.

For the hardware, you need some small plastic tubes and a thermal cycler, which applies pre-programmable temperature changes to the tubes. Commercial thermal cyclers for laboratories range from \$2,500 to more than \$1000, but you can make your own MacGyver version using a handy Board microcontroller (<http://www.arduino.cc>, around \$22) and about 50 dollars' worth of additional components. Here's a meta-level description of the different pieces and how to put them together. (You can find the schematics and full parts list online at <http://www.arduino.cc/Projects/010/ThermalCycling/>.)

### How It Works

The component that performs the thermal heavy lifting for our thermal cycler is a Peltier device, aka thermoelectric cooler. This is a flat, solid-state device that "pumps" heat from one plate to the other when you apply a DC current. Inside, current flows through alternating sections of P-type and N-type semiconductor material sandwiched between the two plates. Heat is drawn away along



#### THE GENOME BROWSER

Our genome represents all of the DNA in our cells' nuclei. This DNA is the "genetic blueprint" that determines how we're put together on a molecular level, what we look like, and how healthy we are. It contains over 3 billion letters, called nucleotides, which the Human Genome Project has mapped using DNA sequencing technologies built from the same basic principles outlined in the projects presented here. Now that we have the sequences, the next step is figuring out what they do, which parts of the sequence start "junk" and actually produce proteins, and what these proteins' functions are in the body.

Anyone can read this blueprint and browse the latest discoveries online using the Genome Browser at the UCSD Genome Bioinformatics Site (<http://genome.ucsf.edu/cgi-bin/hgGateway>). This breakthrough tool is like a Google Maps for genomes, and it's being updated continuously as researchers decipher different parts of the genome.

You can use the Genome Browser to search the entire genome sequence and navigate around any part of it. You can use the detailed features of any particular location by searching for an address, instead of a street address, you enter the numerical position of the nucleotide in the entire sequence. Researchers routinely use the Genome Browser when they need see data from the human blueprint.

current, you reverse the heat flow. Peltier devices are used to cool microprocessors and photoelectric devices. By themselves (without a power supply and controller), you can get them for less than \$15 from surplus companies, check <http://www.johnsonsurplus.com>. We used a 1.5"x2" device rated at 5V and 3 amps (Marlow Industries item #SP2082). For the device's power supply, we



#### How to make a Thermal cycler for DNA replication:

1. Instead of paying \$2,500 for a commercial thermal cycler (which you'll need to replicate 1000 samples), you can make the one shown here for less than \$100.
2. This Peltier device pumps heat from one plate to another when a current is applied.
3. Now the Peltier device has been conditioned with a aluminum plate. The top block has holes to hold the reaction tubes. The bottom block is a heat sink.
4. The top block of the thermal cycler contains holes for 4 tubes where the reaction takes place, and a dummy tube that contains a temperature sensor. The sensor from the second provides feedback to the microcontroller, which controls the Peltier device.



## MAKE IT.



# BUILD AND USE YOUR CLEAN BOX

**START >>>** Time: 1 hour to build; 2 weeks to grow Complexity: Easy

### 1. CUT THE HOLE

1a. Find the output side of the air purifier, and trace it on the bottom of the plastic box.

1b. Drill pilot holes at the corners of the traced outline.

1c. Use a keyhole saw or jig saw at the highest speed to cut out the entire hole.



### 2. INSTALL THE PURIFIER

2a. Fit the air purifier into the hole, with the intake side facing out and the output side blowing into the box. You might want to prop it up on some books to keep it in place.



2b. Use silicone sealer to generously caulk around the air filter, securing it in place. Let it sit overnight so that the caulk can dry. That's it — now you have your hood! Move it onto a good work surface with its opening facing you, and let's start using it.



Photography by Philip Ross

### 3. CLEAN THE HOOD

This isn't just Step 2, it's something you'll need to do every time you work inside your laminar flow hood. The hood is crucial for mushrooms growing, but it's only one part of the larger regimen of cleanliness required for successful lab work.

3a. Clean all of the hood's surfaces with warm, soapy water.

3b. Disinfect all surfaces of the hood with a bleach and water solution.

3c. Finally, turn the fan on and disinfect the hood with isopropyl alcohol. You can never be too clean!

### 4. MAKE THE AGAR PLATE

We'll begin growing our mushrooms (using a spore print) in agar (sterilized gelatin), a standard laboratory growth medium. Petri dishes are traditionally used, but you can use any shallow, washable container with a lid. As long as you're cooking a batch of agar, you'll find it handy to make several of these plates of agar and store them in airtight bags for later use.

4a. Drill or cut a 1/2" hole in the lid of a washable plastic container.

4b. Wash the container and lid with soap and water, and then sterilize by immersing them in simmering water for 2 minutes. Switch on your hood's fan, and move the container and lid inside for drying.

4c. Make a filter by soaking a piece of coffee or sponge in isopropyl alcohol and then wringing it out. Place the filter in the hole in the container lid. It should fit snugly.



The sponge piece filter keeps the mushrooms from protected while letting it exchange gases with the surrounding air.

4d. Mix 1 tablespoon of agar in 2 cups of water. Bring to a low boil and slowly simmer for about 25 minutes, stirring occasionally. Add a large pinch of the growing substrate you'll be using later (see below, vol 1006, Bailey, etc.) to the simmering agar as a source of nutrition.

4e. Inside your hood, pour the hot agar into the newly sterilized container until it is about as thick as a pencil. Let the gelatin cool and reagent.





The Woman Who Saves Humanity From Itself in "The Margarets"



Everybody's Broken In The Dollhouse



Doctor Who's Easter Hit Parade



Lost's Miles Takes Over For Haley Joel Osment



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## Making A Biological Counter

By [Annalee Newitz](#), 11:14 PM on Sun Sep 14 2008, 973 views

Katherine Aull was our runner-up for the biobricks lifeform. She explained her entry like this:

This paper describes a novel counter design, based on a bit overflow detector to signal "carry" events, and a bidirectional toggle switch to update and maintain the count. It also describes work towards a prototype, ongoing in the author's home laboratory.

All our judges were extremely impressed with Aull's home laboratory, which is located in her closet.

When she's not inventing lifeforms in her closet, Aull is a research associate at a synthetic biology startup. She has a B.S. in biological engineering from MIT, and lives in Cambridge, Massachusetts. Here is her paper, featuring an excellent photo of her closet lab.

verify the correct construct. This plasmid, carrying the activator CI and the reporter LacZa, is currently in testing.

Unfortunately, I don't think it works. Expression of LacZa turns media with X-Gal blue. Transformation gave a mix of white and blue colonies; 6/18 white colonies had the correct insert, while 0/30 blue colonies did. If both CI and its promoter were functional, the colony should have expressed LacZ. Follow-up experiments over longer times indicate that low expression of CI is the issue.

With just enough money left for one primer, I can attempt to swap CI's ribosome binding site for a more efficient version, in hopes of increasing the expression. This is how things work in synthetic biology today; however, this limited success may demonstrate that garage biohacking is not outright implausible.



**Figure 5. The author's lab.**  
Also the author's closet. The  
floor space is 19"x42".

1st shelf: reagents / plasticware  
2nd shelf: centrifuge / work area  
3rd shelf: homebrew incubator  
4th shelf: thermocycler

Tuesday, May 12, 2009

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3 of 10  
Obama Rips Iran in  
Tactical Shift



PAGE ONE | MAY 12, 2009

# In Attics and Closets, 'Biohackers' Discover Their Inner Frankenstein

*Using Mail-Order DNA and Iguana Heaters, Hobbyists Brew New Life Forms; Is It Risky?*

Article

Comments (30)

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Text

By JEANNE WHALEN

In Massachusetts, a young woman makes genetically modified E. coli in a closet she converted into a home lab. A part-time DJ in Berkeley, Calif., works in his attic to cultivate viruses extracted from sewage. In Seattle, a grad-school dropout wants to breed algae in a personal biology lab.

These hobbyists represent a growing strain of geekdom known as biohacking, in which do-it-



Let's build a smarter planet



them buy DNA online, then fiddle with it in hopes of curing diseases or finding new biofuels.



Katherine Aull

Katherine's Aull's closet laboratory in her apartment.

But are biohackers a threat to national security?

That was the question lurking behind a phone call that Katherine Aull got earlier this year. Ms. Aull, 23 years old, is designing a customized E. coli in the closet of her Cambridge, Mass., apartment, hoping to help with cancer research.

She's got a DNA "thermocycler" bought on eBay for \$59, and an incubator made by combining a styrofoam box with a heating device meant for an iguana cage. A few months ago, she talked about her hobby on DIY Bio, a Web site frequented by biohackers, and her work was noted in New Scientist magazine.

That's when the phone rang. A man saying he was doing research for the U.S. government called with a few polite, pointed questions: How did she build that lab? Did she know other people creating new life forms at home?



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# DIYbio

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## Home

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

What is DIYbio in 4 minutes?



## recent comments

- Tito:** Hey Norman -- we don't have a lab. As you can see, the soap ...
- Tito:** Wonderful! Tito...
- Norman:** Hi Tito, I hope you are doing well. I am Norman and liv...
- Jim H.:** iGEM is traditionally an undergraduate competition. Any ide...
- Tito:** Hi Maria, In the San Francisco DIYbio group, we extracted D...
- lola:** its so cool!



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Home

New since last time: 62 messages

Welcome to the DIYbio group discussion board.

Also see our website at [www.DIYbio.org](http://www.DIYbio.org)Discussions 7 of 3374 messages [view all »](#)[+ new post](#)[model organism zoo](#)

By Jason Kelly - 12:35am - 4 authors - 3 replies

[Fwd: \[GRG\] UPI: Adult stem cells revert to embryonic state](#)

By Bryan Bishop - Apr 13 - 1 author - 0 replies

[Open Gel Box 2.0: Update](#)

By Bryan Bishop - Apr 13 - 9 authors - 15 replies

[\[Synthetic Biology\] Synthetic Biology and Synthetic Genomics](#)

By Bryan Bishop - Apr 13 - 1 author - 0 replies

[DIY DNA synthesis \(more\)](#)

By Bryan Bishop - Apr 13 - 1 author - 0 replies

[Short bibliography of DIY-friendly DNA synthesis techniques](#)

By William Heath - Apr 13 - 2 authors - 1 reply

[CodeCon 2009: San Francisco, April 17th-19th](#)

By Bryan Bishop - Apr 13 - 3 authors - 2 replies

Pages 2 of 5 pages [view all »](#)[+ add page](#)[DIYbio model organisms](#)

Last updated by Mackenzie Cowell - Jan 20 - 1 author - 1 page long

[gel-electrophoresis-shopping-list](#)

Last updated by Jason Morrison - Jun 26 2008 - 1 author - 1 page long

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## Group info

Members: 765

Activity: Low activity

Language: English

Group categories:  
[Science and Technology > Biology](#)[More group info »](#)

However, being a public forum, it's possible that some republican fearmonger dillhole will see this and raise holy hell that we're sending around an organism that injects foreign DNA into plants. If that happens they could probably get government agencies to investigate and charge us with some bullshit violation on the books somewhere that isn't normally enforced and nobody else worries about.



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# Physcomitrella patens

(Redirected from [Moss](#))

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- 4 [Moss is cool](#)

## References

- [PhyscoBase](#)
- [Luciferase expressed](#)
- [dsRed expressed](#)
- [this book has a chapter on moss molecular biology](#)
- [I started adding citations to citulike](#) with the tag 'physcomitrella'
  - p.s. isn't there a better way to share fulltext pdfs of articles, or just citations, at least, on OWW? if not, lets just make a new citulike account and share the login [Mac Cowell](#)

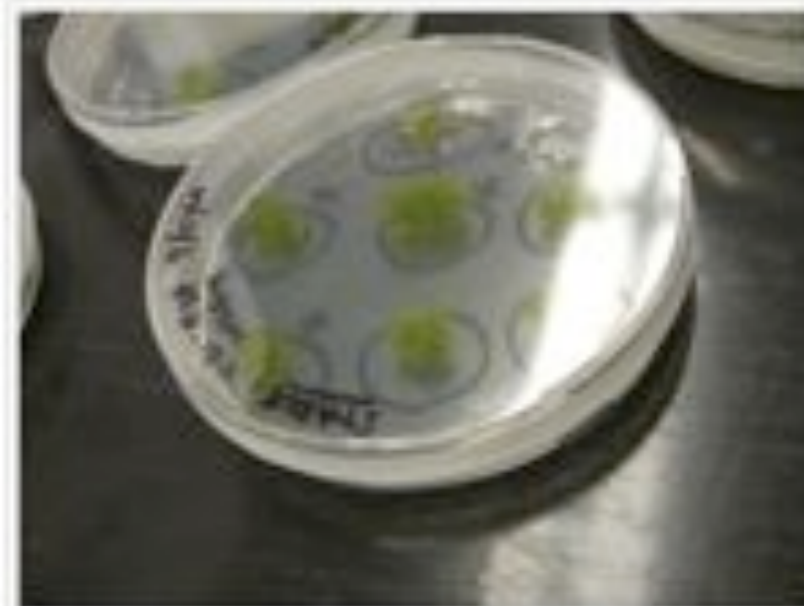
## Links

- <http://biology4.wustl.edu/moss> - good protocols here (methods tab)
- <http://moss.nibb.ac.jp>
- <http://www.biologie.fu-berlin.de/>
- <http://www.cosmoss.org>
- <http://www.plant-biotech.net>

## Protocols

- [Physcomitrella\\_patens/plates](#)

## Moss is cool



Moss: it's like small trees!

# Question.

If you were an 18 year old first year undergraduate at <your institution> what would you expect to learn after 4 years of Biological Engineering?



# *Teach me how to...*

Design and build living organisms that behave as expected.

Debug existing or write new genetic programs to do my bidding.



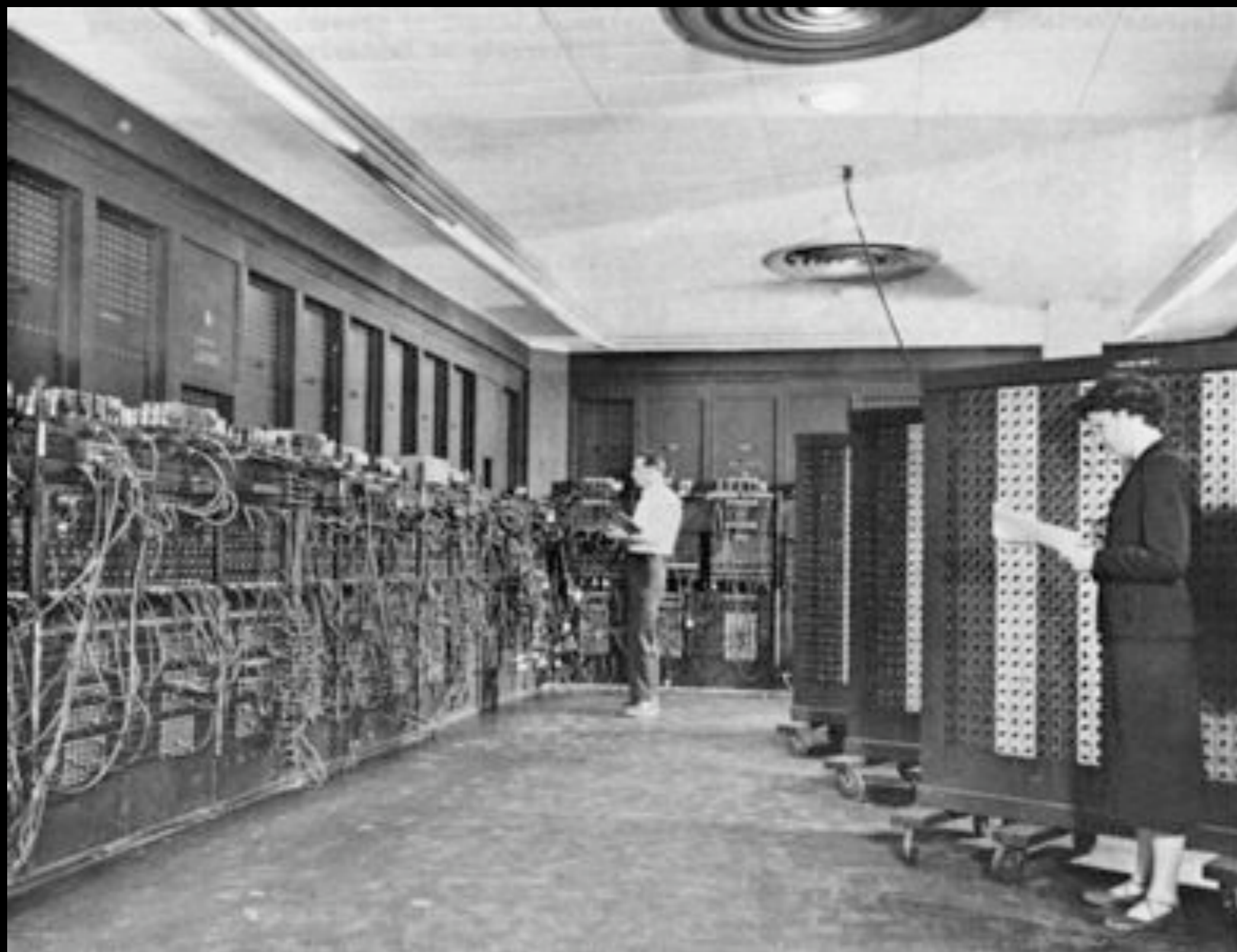
# Challenge

*Make biology easy  
to engineer.*

# Opportunities

*Enable all constructive  
biotechnologies.*

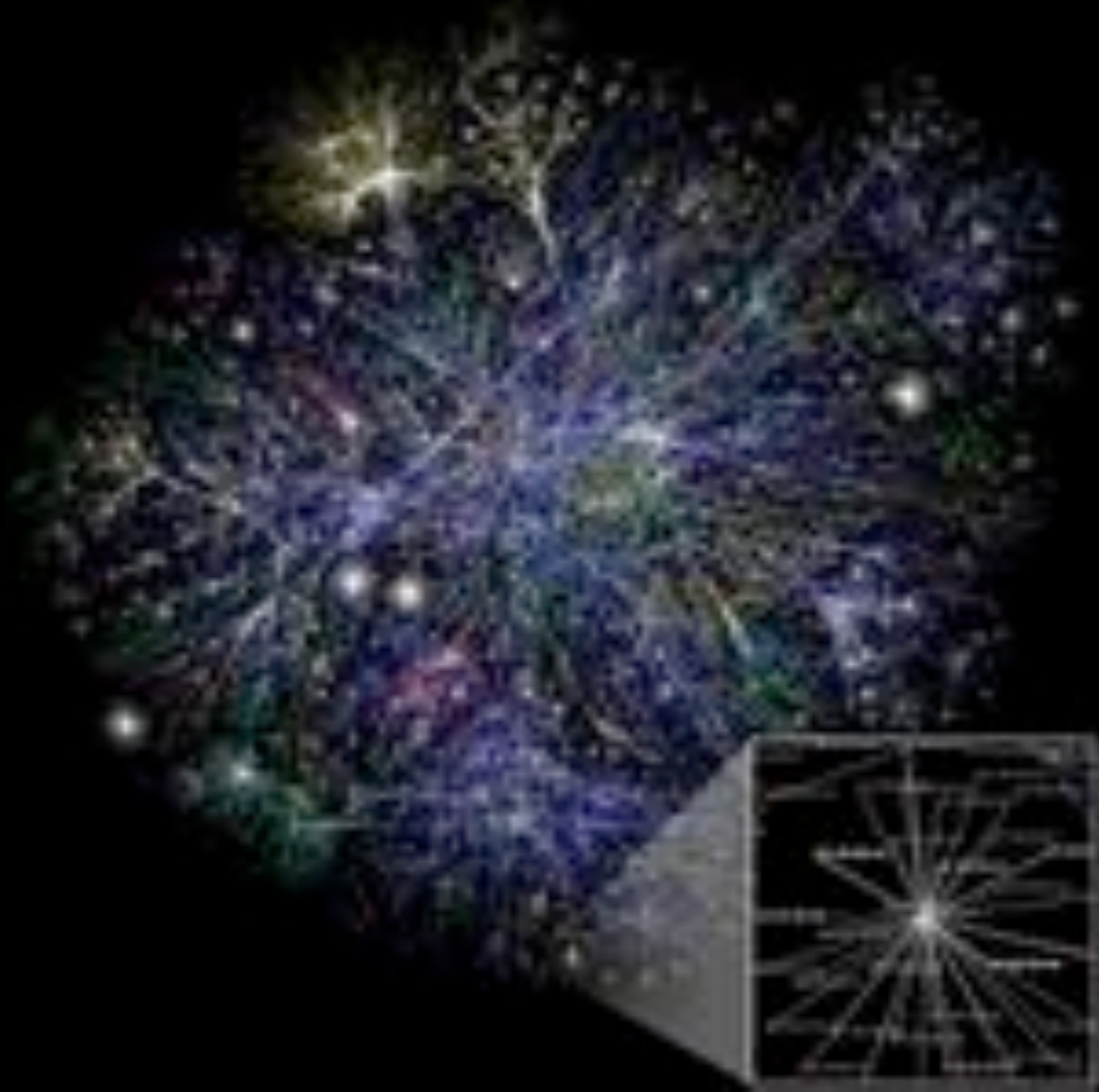
*Better understand  
nature.*



<http://en.wikipedia.org/wiki/Image:Eniac.jpg>



[http://en.wikipedia.org/wiki/Image:Apple\\_I.jpg](http://en.wikipedia.org/wiki/Image:Apple_I.jpg)



<http://en.wikipedia.org/wiki/Internet>

A p p s

*Tools*

7. Control & dyn. systems
6. Reverse engineering
5. Fab, CAD & EDA
4. Standards & abstraction
3. Languages & grammars
2. Device design
1. Info. theory & signal proc.



# We need new tools

1973

*Construction of biologically functional bacterial plasmids in vitro*

Cohen et al., PNAS, 1973

**MATERIALS AND METHODS**  
*E. coli* strain W1485 containing the RSF1000 plasmid, which carries resistance to streptomycin and sulfonamide, was obtained from S. Falkow. Other bacterial strains and R factors and procedures for DNA isolation, electron microscopy, and transformation of *E. coli* by plasmid DNA have been described (1, 7, 8). Purification and use of the *Eco*RI restriction endonuclease have been described (5). Plasmid heteroduplex studies were performed as previously described (9, 10). *E. coli* DNA ligase was a gift from P. Modrich and R. L. Lehman and was used as described (11). The detailed procedures for gel electrophoresis of DNA will be described elsewhere (Helling, Goodman, and Boyer, in preparation); in brief, duplex DNA was subjected to electrophoresis in a tube-type apparatus (Hoefer Scientific Instrument) (0.6 × 15-cm gel) at about 20° in 0.7% agarose at 22.5 V with 40 mM Tris-acetate buffer (pH 8.05) containing 20 mM sodium acetate, 2 mM EDTA, and 18 mM sodium chloride. The gels were then soaked in ethidium bromide (5 µg/ml) and the DNA was visualized by fluorescence under long wavelength ultraviolet light ("black light"). The molecular weight of each fragment in the range of 1 to 200 × 10<sup>6</sup> was determined from its

1985

*Cloning and expression of the human erythropoietin gene*

Lin et al., PNAS, 1985

**Assembly of Expression Vector for the Epo Gene.** For direct expression of the genomic Epo gene, the 4.8-kilobase (kb) *Bst*EII-*Bam*HI fragment of λHE1 (see *Results*), which contains the entire Epo gene, was used. After converting the *Bst*EII site into a *Bam*HI site with a synthetic linker, the fragment was inserted into the unique *Bam*HI site of the expression vector pDSVL (unpublished data), which contains a dihydrofolate reductase (DHFR) minigene from pMg1 (24). The resulting plasmid pDSVL-gHuEPO (Fig. 1A) was then used to transfect Chinese hamster ovary (CHO) DHFR<sup>-</sup> cells (25) by the calcium phosphate microprecipitate method (26). The transformants were selected by growth in medium lacking hypoxanthine and thymidine. The culture medium used was Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, streptomycin, and glutamine (25).

2006

*Production of the antimalarial drug precursor artemisinin acid in engineered yeast*

Ro et al., Nature, 2006

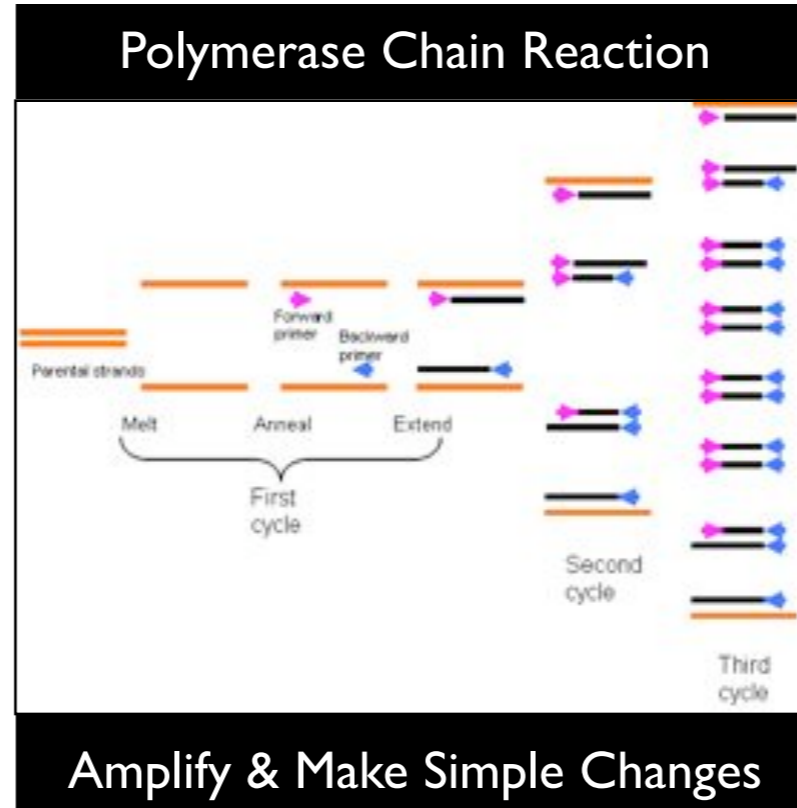
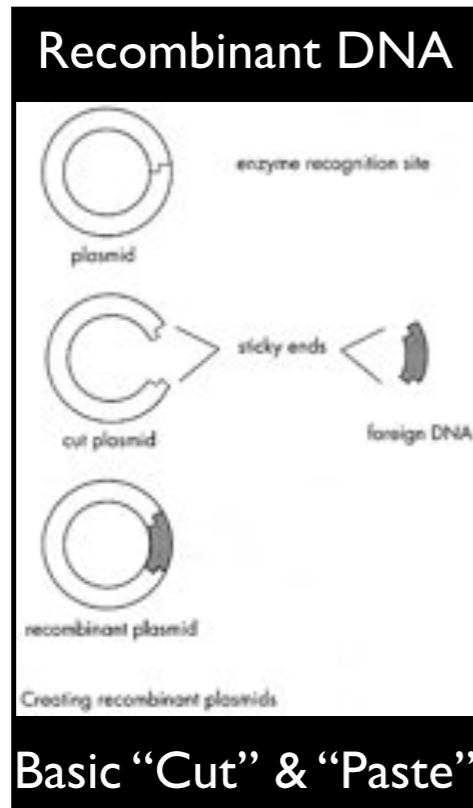
**Plasmid construction.** To create plasmid pRS425ADS for expression of *ADS* with the *GAL1* promoter, *ADS* was PCR amplified from pADS<sup>+</sup> using primer pair 9 and 10. (Supplementary Table S1). Using these primers the nucleotide sequence 5'-AAAACA-3' was cloned immediately upstream of the start codon of *ADS*. This consensus sequence was used for efficient translation<sup>12</sup> of *ADS* and the other galactose-inducible genes used in this study. The amplified product was cleaved with *Spe*I and *Hind*III and cloned into *Spe*I and *Hind*III digested pRS425GAL1<sup>13</sup>.

For integration of an expression cassette for *dhfrGE*, plasmid pG-HMGR was constructed. First, *Sac*I restriction sites were introduced into pRS426GAL1<sup>14</sup> at the 5' end of the *GAL1* promoter and 3' end of the *CYC1* terminator. To achieve this, the promoter-multiple cloning site-terminator cassette of pRS426GAL1 was PCR amplified using primer pair 11 and 12. The amplified product was cloned directly into *Cla*I-

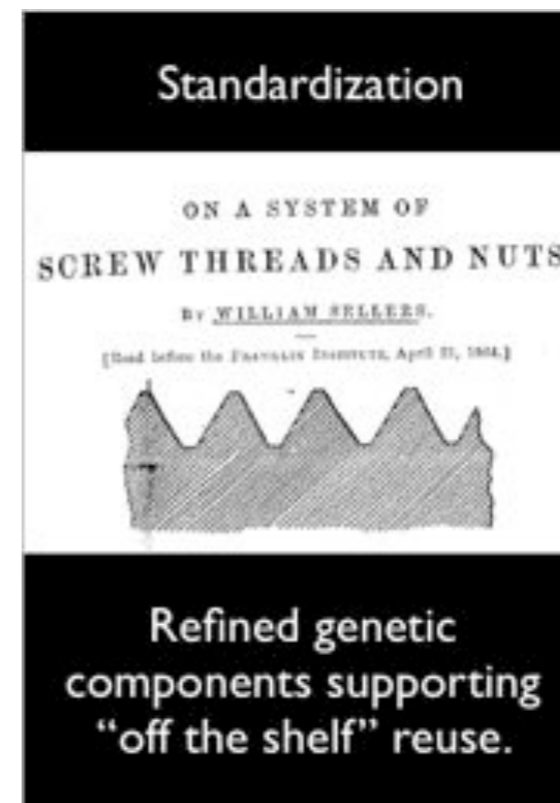
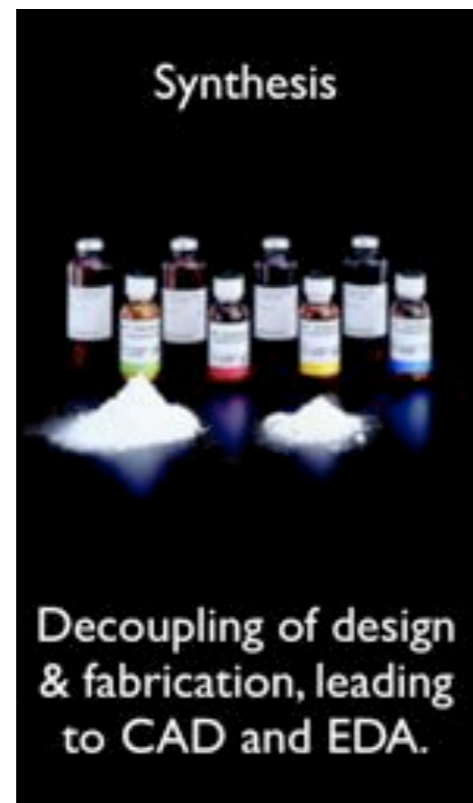
*Genetic engineering basics unchanged past 30+ years*

# Synthetic Biology as Tools Revolution

**First Gen. Biotech** =



**Next Gen. Biotech Adds New Tools** =



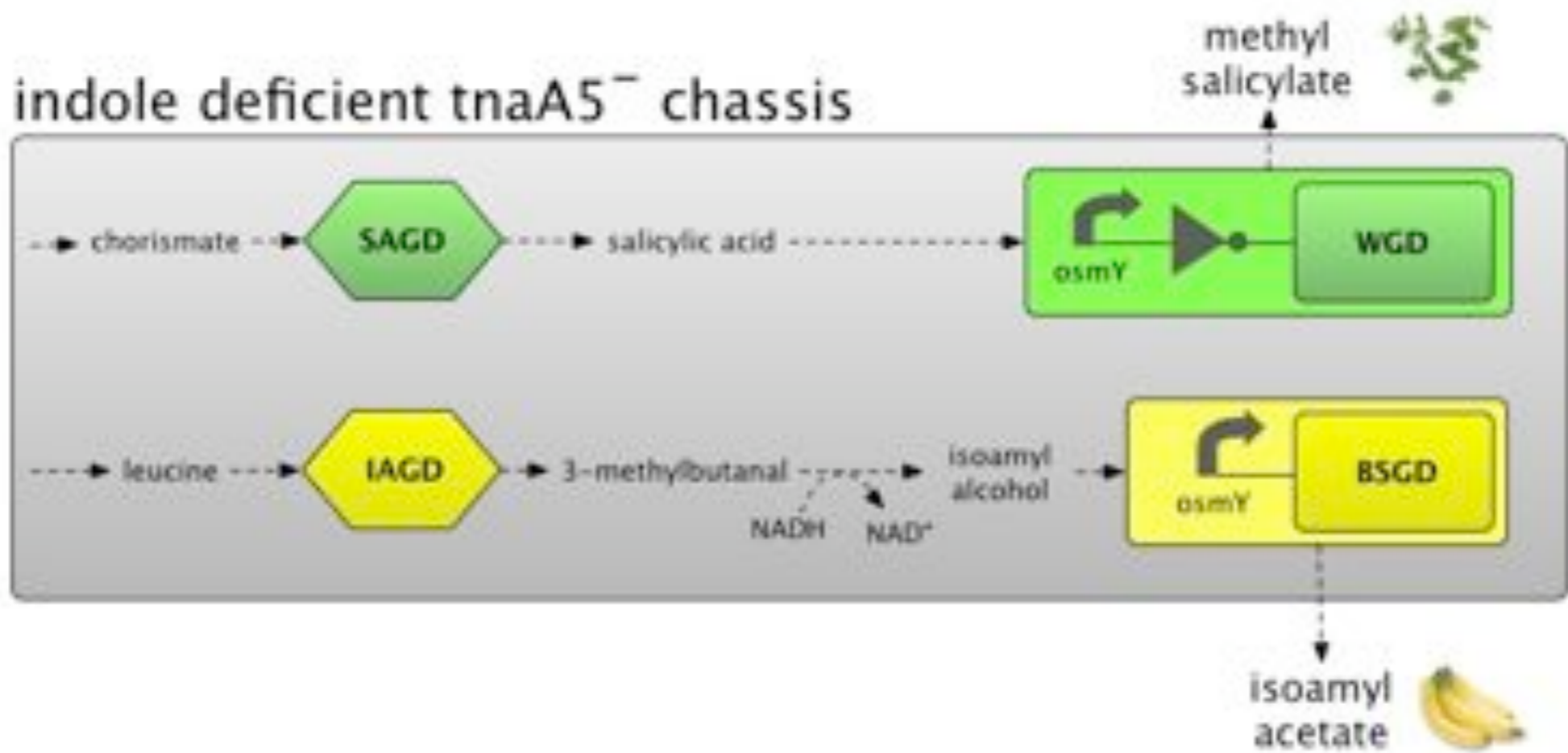
...



```
if {growing}  
    call wintergreen()  
else  
    call bananas()
```



indole deficient *tnaA5<sup>-</sup>* chassis





# Registry of Standard Biological Parts

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## Welcome to the Registry of Standard Biological Parts.

The Registry is a collection of ~3200 genetic parts that can be mixed and matched to build synthetic biology devices and systems. Founded in 2003 at MIT, the Registry is part of the Synthetic Biology community's efforts to make biology easier to engineer. It provides a resource of available genetic parts to [iGEM](#) teams and academic labs.

The Registry is based on the principle of "get some, give some". Registry users benefit from using the parts and information available from the Registry in designing their engineered biological systems. In exchange, the expectation is that Registry users will, in turn, contribute back information and data on existing parts and new parts that they make to grow and improve this community resource.



[Catalog of parts & devices](#)



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(Apply for an account)



[DNA repositories](#)

### Registry tools

- [Search parts \(?\)](#)
- [Add a part](#)
- [Send parts to the Registry](#)
- [Sequence analysis](#)



You'll notice some significant changes to the Registry recently. In particular, the Registry [catalog of parts](#) has been entirely redesigned to allow for easier browsing of the available parts and devices. You can now browse parts and devices by type, by function, by chassis and by standard. You'll also notice that the documentation and help pages for each class of parts have been greatly enhanced.

The Registry of Standard Biological Parts is "always" a work in progress. Please browse the new catalog and let us know what you think, or feel free to edit and improve the pages further.

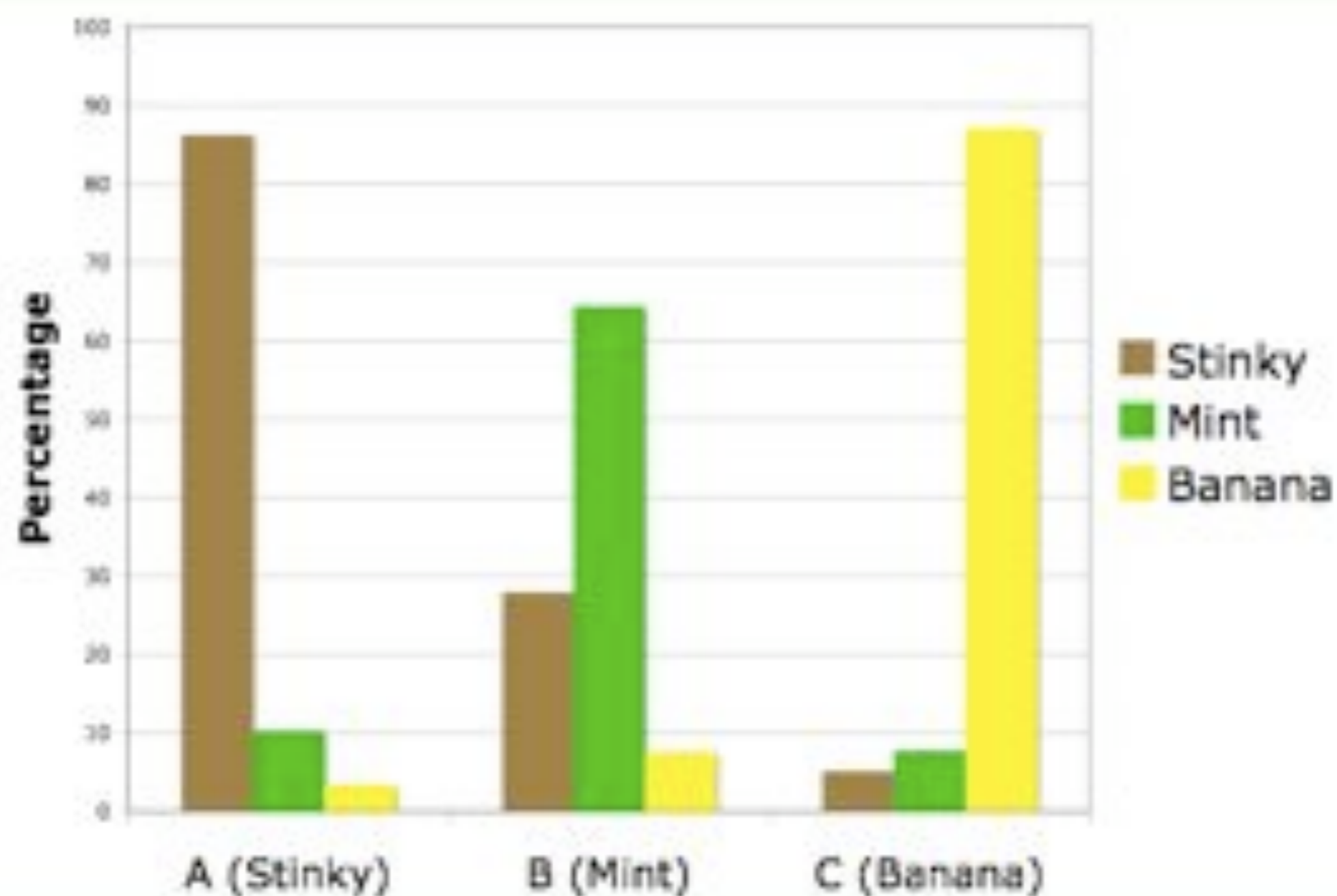
## Registry news

- **June 22, 2009:** You can now link to part pages directly from the iGEM wiki by typing the following `<part:info>Bba_B0015</part:info>`.
- **June 11, 2009:** We are considering changing the license terms of the Registry so that we can share our information with other databases. Go [here](#) to read the proposal or add your comments.
- **June 2, 2009:** Most of the hard information about a part is now presented in the header and footer of the part's Main Page. In particular, the functional parameters

# smell test: iGEM jamboree



# smell test results





Free-to-use DNA  
Open technical sta  
Join now!



AT&T 1:53 AM

Library Registry of Parts

GFP protein generator -  
slr2016 signal sequence  
Promoterless GFP on p  
strong promoter - GFP  
pTet GFP  
flgA promoter + WT RB  
GFP under medium con  
GFP Act BB part

Prev 1 - 20 of 274

AT&T 1:55 AM

Palette BBa\_I750016

Basic Information

Gas Vesicle polycistonic gene

6064 bp

Long Description

Actions

Save to Palette

Email Part

Web-based Resources

Registry Web Page

AT&T 1:54 AM

BBa\_I712671

```
cgatgtacggggccagatatacgcggtga  
accgccaacgacccccgccattgac  
tcatatgccaagtacgccccctattgacg  
ggggtttggcagtacatcaatggggcgtg  
tcgccccattgacgcaaatggggcggtg  
cgaggaggac
```

Info Unwrap Parts Hide







# iGEM 2009

discussion view source history teams

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## iGEM Partner Offer: GENEART



GENEART is once again joining iGEM this year as a sponsoring partner. The company will be offering low-cost DNA synthesis to all iGEM teams this year. See the [Partner Offers](#) page for more details on how to take advantage of this great offer.

June 5, 2009

## New Invitation Letter System

Full Name:  
Date of Birth:  
Country of Citizenship:  
Passport Number:  
Postal Address:

We have a new way for iGEM team members to get their visa invitation letters. Instead of emailing your information to iGEM HQ, you can enter your information on your **'My Account'** page, submit it, and download your invitation letter.

See the [Visa page](#) for instructions and details.

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## Safety

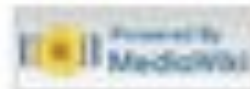
For iGEM 2009 teams are asked to detail how they approached any issues of biological safety associated with their projects.

Specifically, teams should consider the following four questions:

1. Would any of your project ideas raise safety issues in terms of:
  - researcher safety,
  - public safety, or
  - environmental safety?
2. Is there a local biosafety group, committee, or review board at your institution?
3. What does your local biosafety group think about your project?
4. Do any of the new BioBrick parts that you made this year raise any safety issues?
  - if yes, did you document these issues in the Registry?

Teams, please document any answers to these (or other) safety questions in your presentation, wiki presentation, or poster. Judges will be asked to evaluate your project, in part, on the basis of if and how you considered and addressed issues of biological safety.

If any questions arise regarding iGEM and biological safety please send an email to [safety AT igem.org](mailto:safety@igem.org).


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 Match case

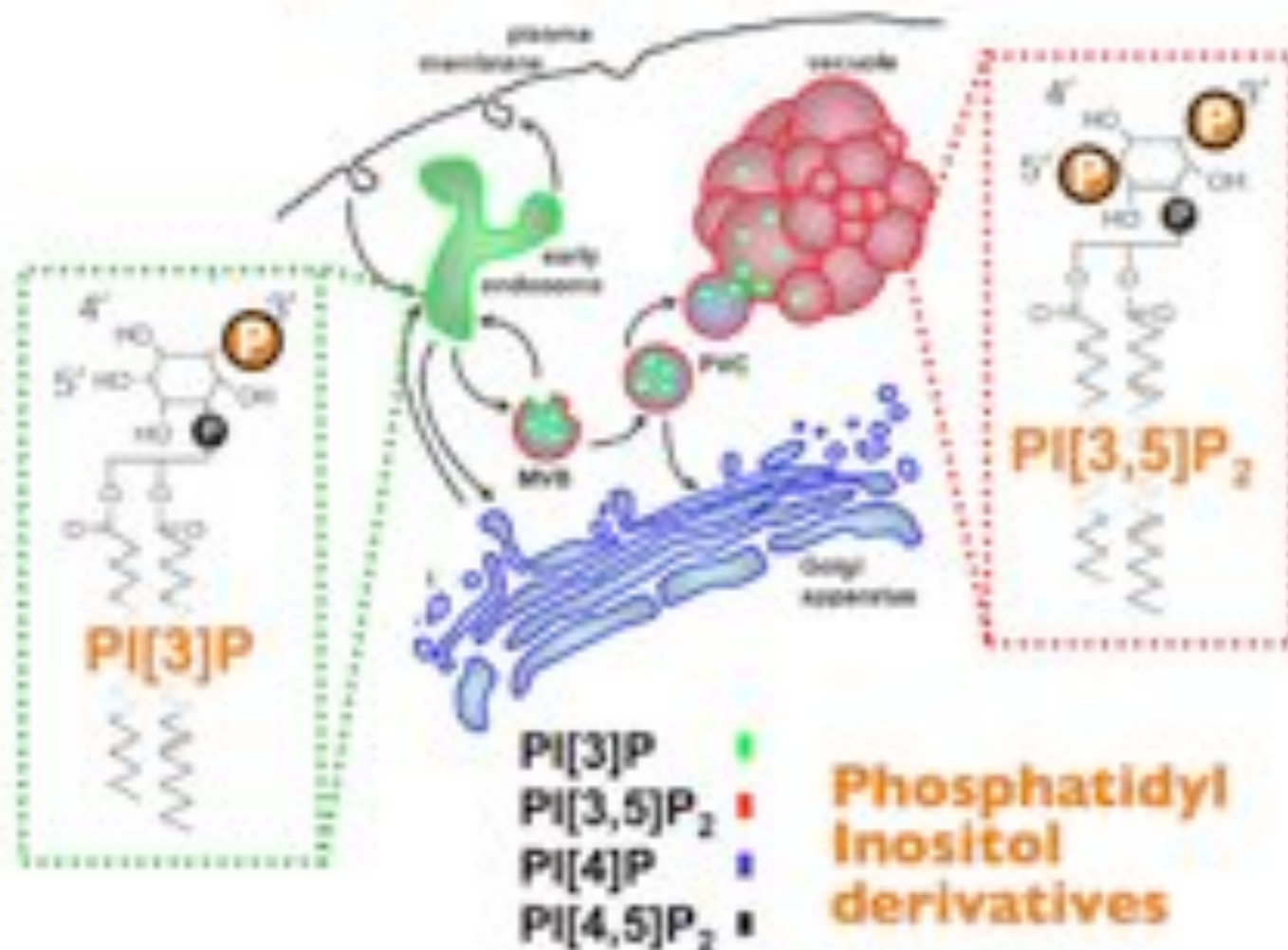
# BUILDING A NEW ORGANELLE

## STEP 1: create compartment with novel molecular identity code

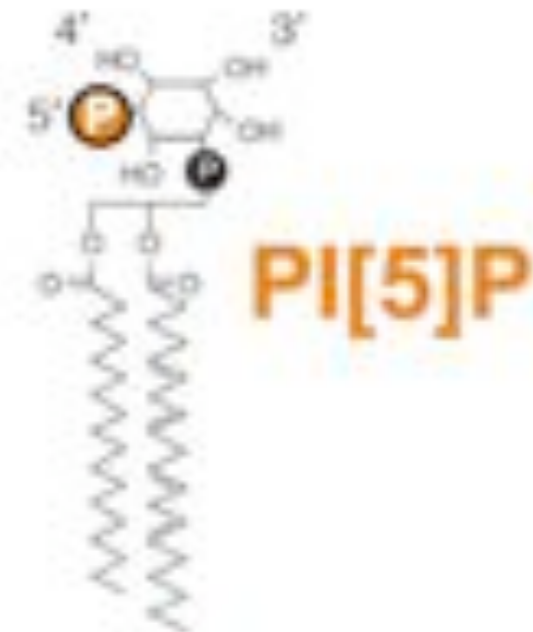


Cells use "molecular barcode" to identify distinct membrane compartments:

unique phospholipid species



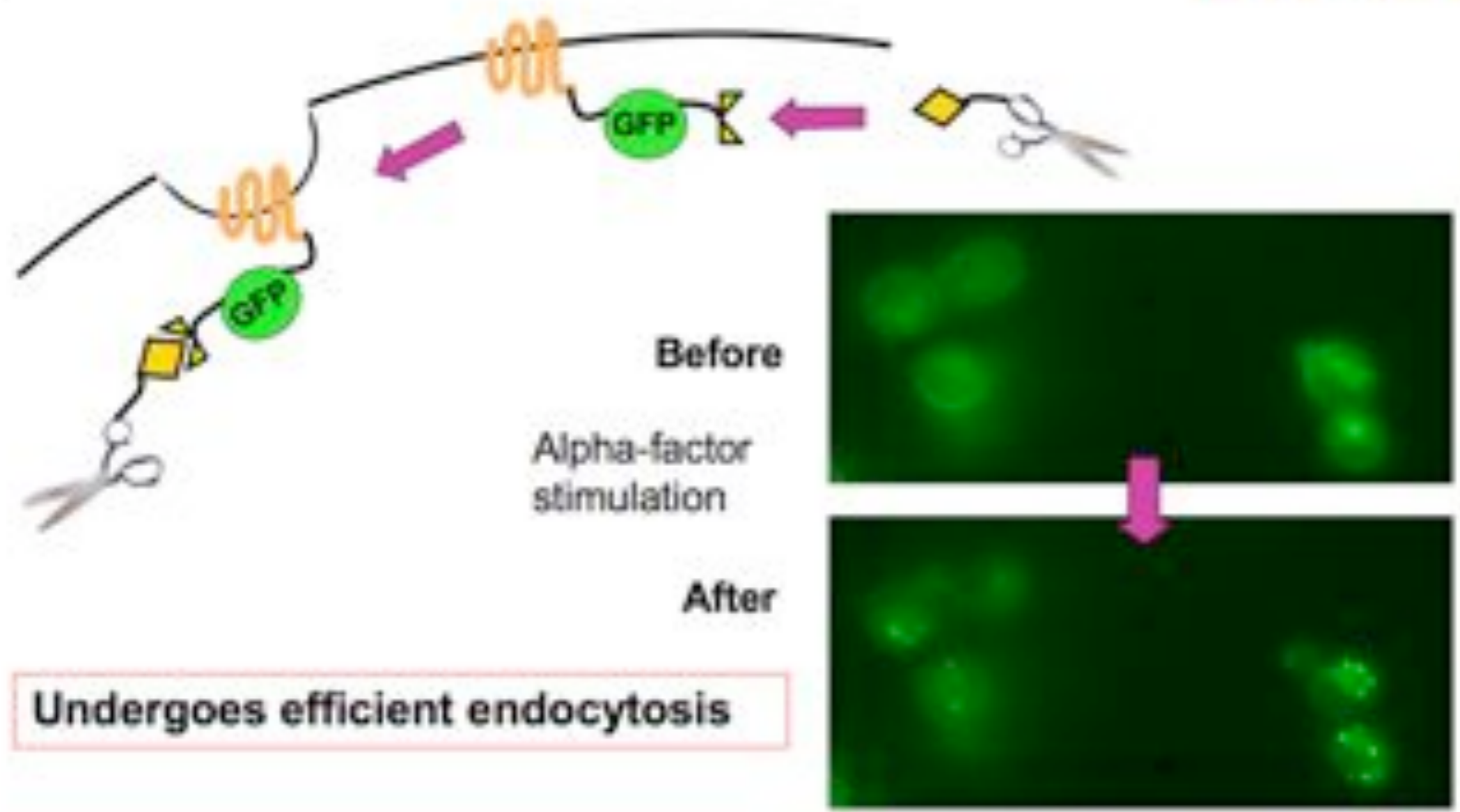
Not present in Yeast:



Can we create a synthetic membrane compartment containing this novel phospholipid?



# Tagged receptor is properly localized and functional



Should teenagers practice genetic engineering?

Yes

Should military force include biotechnology?

No

Will biohackers be good or bad?

Good

Should BioBrick parts be patented or freely shared?

Free

Should genetic engineers sign their work?

Yes

How much can we construct with biology?

???

# Q. How to scale biosafety?

*New people and cultures  
immigrating.*

*Pacing of work increasing  
geometrically.*

*Our institutional oversight framework  
inaccessible to many would-be  
engineers of biology.*