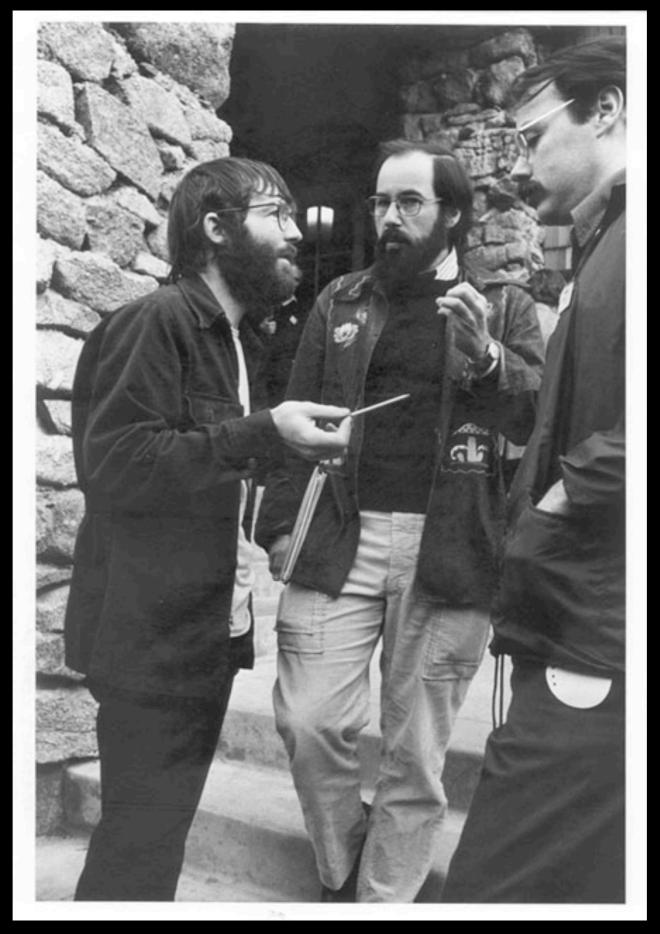
Biosafety Level 1 practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories, and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known 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.

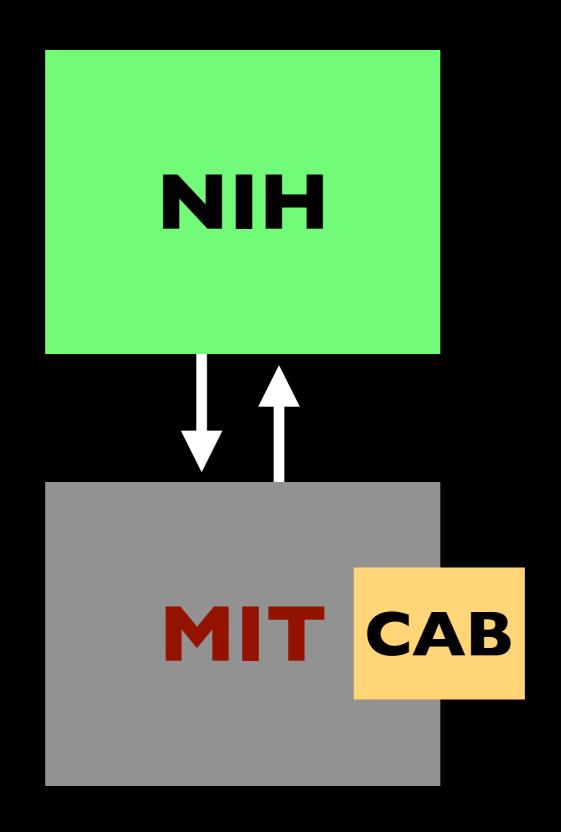
Biosafety Level 2 practices, equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, and other laboratories in which work is done with the broad spectrum of indigenous moderate-risk agents that are present in the community and associated with human disease of 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.



Left to right: Philip Sharp, David Baltimore, unidentified (c/o US NAS Archives)



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#### Published online before print October 31, 2006

Genome Research, DOI: 10.1101/gr.5565706

#### 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.

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<sup>&</sup>lt;sup>3</sup> Present address:

#### 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?



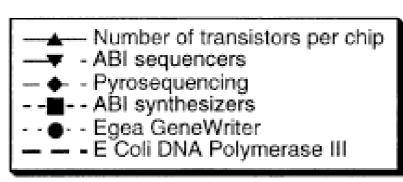
http://en.wikipedia.org/wiki/Phillip\_Allen\_Sharp



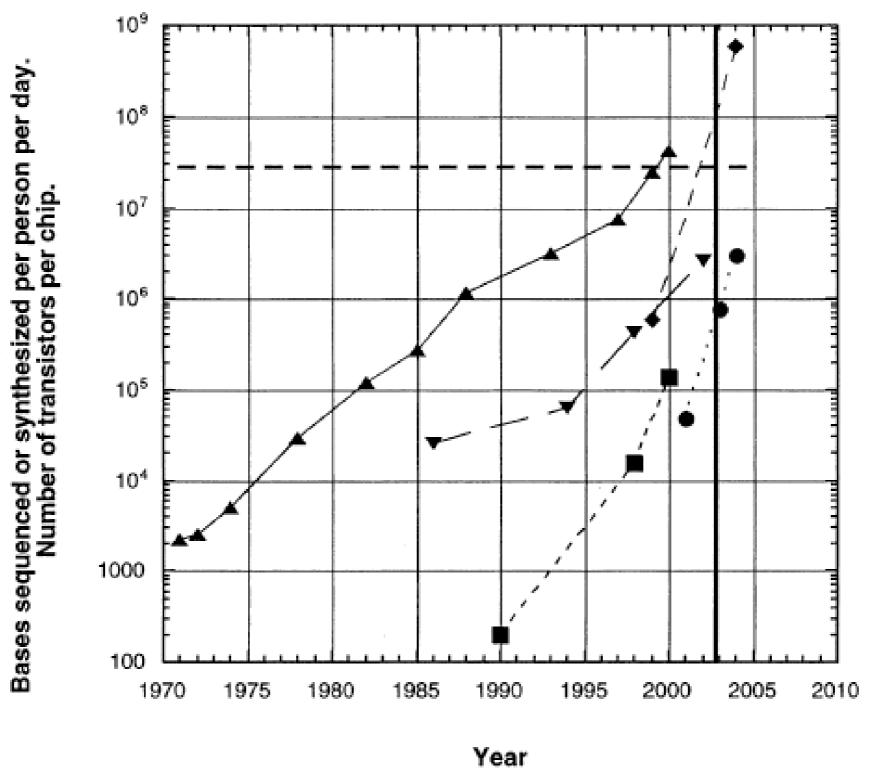
## What's changed since the 70s?

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

# What's the difference between safety & security?



# Productivity Improvements in DNA Synthesis and Sequencing (as of October, 2002)



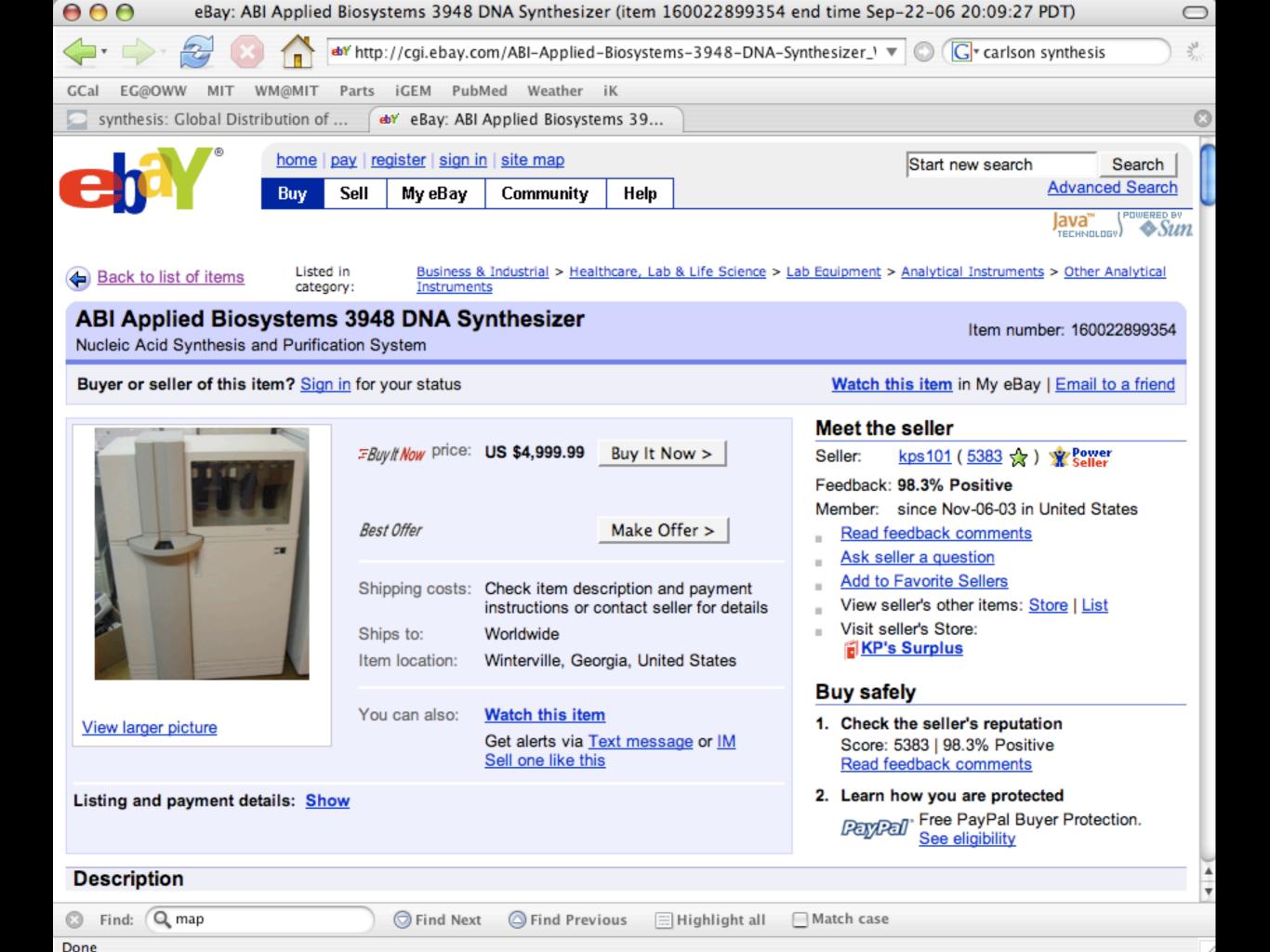
Carlson, Pace & Proliferation of Biological Technologies, Biosec. & Bioterror. 1(3):1 (2003)



Sequencing

**Synthesis** 

Material (Physical DNA)



## Commercial DNA Synthesis Foundries

Rob Carlson, University of Washington; Gerald Epstein and Anne Yu, CSIS



18 July 05. Method: Rough Google search. Thus not a thorough survey. No academic facilities.

Data Source: Rob Carlson, U of W, Seattle www.synthesis.cc, rob@synthesis.cc

1: NC 001608

Lake Victoria marburgvirus, complete genome ssRNA; linear; Length: 19,112 nt

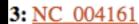
Created: 1994/01/26

#### 2: NC 006432

Sudan ebolavirus, complete genome ssRNA; linear; Length: 18,875 nt

Created: 2004/11/15

## Unknown reservoir



Reston Ebola virus, complete genome ssRNA; linear; Length: 18,891 nt

Created: 2002/09/04

#### 4: NC 002549

Zaire ebolavirus, complete genome ssRNA; linear; Length: 18,959 nt

Created: 1999/02/10

## Locked-up



#### NC 008291

Taterapox virus, complete genome dsDNA; linear; Length: 198,050 nt

Created: 2006/08/24

#### NC 001611

Variola virus, complete genome dsDNA; linear; Length: 185,578 nt

Created: 1993/05/04

#### NC 006966

Mule deer poxvirus, complete genome dsDNA; linear; Length: 166,259 nt

Created: 2005/04/08

#### ☐ 1: DO208309

Influenza A virus (A/Brevig Mission/1/1918(H1N1)) polymerase PB2 (PB2) mRNA, complete cds gil76786704lgblDQ208309.1l[76786704]

#### ☐ 1: DQ208310

#### Reports

Influenza A virus (A/Brevig Mission/1/1918(H1N1)) polymerase PB1 (PB1) mRNA, complete cds gil76786706lgblDQ208310.1l[76786706]



Should the DNA sequence encoding human pathogens be freely available online?

## 1918 Flu and Responsible Science

he influenza pandemic of 1918 is estimated to have caused 50 million deaths worldwide; 675,000 in the United States. The reconstruction of the 1918 virus by the synthesis of all eight subunits and the generation of infectious virus are described on p. 77 of this issue,\* and the sequences of the final three gene segments of the virus are described in a concurrent Nature paper.† Predictably, but alarmingly, this virus is more lethal to mice than are other influenza strains, suggesting that this property of the 1918 virus has been recovered in the published sequence. The good news is that we now have the sequence of this virus, perhaps permitting the development of new therapies and vaccines to protect against another such pandemic. The concern is that a terrorist group or a careless investigator could convert this new knowledge into another pandemic.

Should the sequence of the 1918 virus have been published, given its potential use by terrorists? The dual-use nature of biological information has been debated widely since September 11, 2001. In 2003, a committee of the U.S. National Academies chaired by Gerald Fink considered this issue, weighing the benefits against the risks of restricting the publication of such biological information. They outlined the tradeoff between erring on the side of prudence, thus potentially hindering the progress of critical science, and erring on the side of disclosure, thus potentially aiding terrorists. The U.S. National Science Advisory Board for Biosecurity (NSABB) was established to advise governmental

agencies and the scientific community on policies relative to public disclosure. This board has begun to deliberate, but the questions are complex, as typified by these papers on the 1918 virus. It is reassuring that the NSABB was asked to consider these papers before publication and concluded that the scientific benefit of the future use of this information far outweighs the potential risk of misuse. People may be reassured that the system is working, because agencies representing the public, the scientific community, and the publishing journals were involved in the decision.

I firmly believe that allowing the publication of this information was the correct decision in terms of both national security and public health. It is impossible to forecast how scientific observations might stimulate others to create new treatments or procedures to control future pandemics. For example, in the *Nature* article, sequence comparisons suggest that the 1918 virus was generated not by incremental changes in the polymerase genes, but by the movement of these genes, in total,



from an avian source into a human influenza virus. The availability of these sequences will permit identification of their avian origin and should show why this particular set of genes was selected. Similarly, the results in the Science article suggest that the cleavage of a protein on the surface of the 1918 virus, a step critical for virulent infection, may occur by a previously unknown mechanism—a hint that could lead to new drugs for inhibiting this step and thus preventing future pandemic eruptions.

Influenza is highly infectious, and a new strain could spread around the world in a matter of months, if not weeks. The public needs confidence that the 1918 virus will not escape from research labs. All of the described experiments were done in a Biosafety Level 3 laboratory, a high-containment environment recommended by the U.S. Centers for Disease Control and Prevention and the National Institutes of Health on an interim basis, whose use should become a permanent requirement for such experiments. Current evidence suggests that some available drugs and possible future vaccines could suppress infections by the 1918 virus. Given the prospect of another natural influenza pandemic, the recent decision by the U.S. administration to stockpile antivirals for influenza treatment seems wise. Finally, although a sequence of the 1918 virus has been determined and is highly virulent in mice, this may not be the specific form of the virus that caused the pandemic of 1918. An article in the same issue of Nature\*\* reports the existence of sequence variation in a natural population of influenza virus; yet we have only one sequence for the 1918 pandemic strain, and the reconstructed virus described in the Science article was built into the backbone of a laboratory strain. Because a pandemic infection is dependent on many unknown properties, there is no certainty that the reconstructed 1918 virus is capable of causing a pandemic.

## Editorials/Op-Ed

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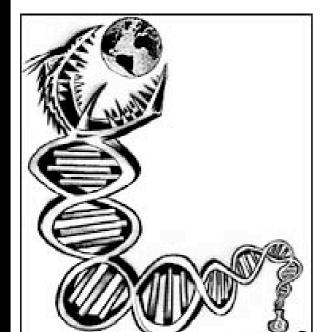
## **Recipe for Destruction**

By RAY KURZWEIL and BILL JOY

Published: October 17, 2005

AFTER a decade of painstaking research, federal and university scientists have reconstructed the 1918 influenza virus that killed 50 million people worldwide. Like the flu viruses now raising alarm bells in Asia, the 1918 virus was a bird flu that jumped directly to humans, the scientists reported. To shed light on how the virus evolved, the United States Department of Health and Human Services published the full genome of the 1918 influenza virus on the Internet in the GenBank database.





This is extremely foolish. The genome is essentially the design of a weapon of mass destruction. No responsible scientist would advocate publishing precise designs for an atomic bomb, and in two ways revealing the sequence for the flu virus is even more dangerous.

First, it would be easier to create and release this highly destructive virus from the genetic data than it would be to build and detonate an atomic bomb given only its design, as you don't need rare raw materials like plutonium or enriched

#### GENEART AG, Regensburg, Germany

Codon Devices, Inc. Cambridge, MA, USA

Blue Heron Biotechnology, Inc. Bothell, WA, USA

CODA Genomics, Inc. Laguna Hills, CA, USA

# International Consortium for Polynucleotide Synthesis (ICPS)

www.POLYSYNTH.INFO

#### About the ICPS

In the summer of 2006, leading international synthetic biology companies joined together to form the International Consortium for Polynucleotide Synthesis (ICPS). The primary mission of the ICPS is to promote safety and security in the promising field of synthetic biology. As part of this mission, the ICPS member organizations will work together to develop technologies that improve safety and security in synthetic biology. In addition, the ICPS plans to work with governmental organizations to help facilitate the creation of a governance framework and associated safety protocols to foster an appropriate regulatory environment for the synthetic biology industry.

#### A Practical Perspective on DNA Synthesis and Biological Security

Hans Bügl<sup>1,2</sup>, John P. Danner<sup>1,3</sup>, Robert J. Molinari<sup>1,4</sup>, John Mulligan<sup>1,5</sup>, David A. Roth<sup>1,4</sup>, Ralf Wagner<sup>1,2,6</sup>, Bruce Budowle<sup>7</sup>, Robert M. Scripp<sup>7</sup>, Jenifer A. L. Smith<sup>7</sup>, Scott J. Steele<sup>7</sup>, George Church<sup>3,8,10</sup>, & Drew Endy<sup>3,9,10</sup>

#### December 4, 2006

Few developments have leapfrogged over predecessor technology as quickly and extensively as synthetic biology. Based on cutting-edge DNA synthesis technology, synthetic biology has already fueled an expansion of opportunities in biological engineering, with advanced capabilities that surpass those provided by traditional recombinant DNA technology. Improvements in synthesis technology are accelerating the pace of innovation in everything from the development of renewable energy to the production of bulk and fine chemicals, from information processing to environmental monitoring, and from agricultural productivity to breakthroughs in human health and medicine. Synthetic biology promises vast improvements to our well-being and our understanding of the living world.

Like any powerful technology, DNA synthesis has the potential to be misused. In the wrong hands, the new capabilities enabled by synthetic biology 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 unfortunate reality.



#### PROJECT 2:

#### Build a Thermal Cycler and Run PCR

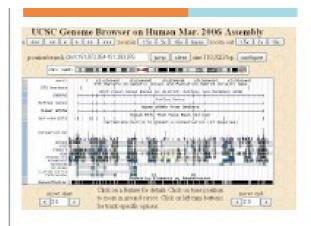
Real DNA "fingerprinting" is usually done using a procedure called polymerase chain reaction, or PCR. This process replicates DNA, making a much larger sample that produces more detail in electrophoresis and is therefore easier to match. To perform PCR, you need some specialized chemicals and equipment.

The chemicals are small, predesigned 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 (takaramirusbio.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 programmable temperature changes to the tubes. Commercial thermal cyclers for laboratories range from \$2,500 to more than \$7,000, but you can make your own MacGyver version using a Handy Board microcontroller (handyboard.com, around \$225) and about 50 dollars' worth of additional components. Here's a metalevel description of the different pieces and how to put them together. (You can find the schematic and full parts list online at makezine.com/07/fingerprinting.)

#### 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 zigzags through alternating sections of P-type and N-type semiconductor material sandwiched between the two plates. Heat is drawn away along



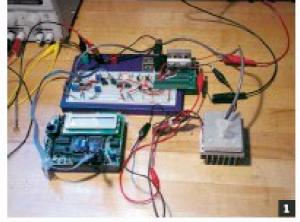
#### THE GENOME BROWSER

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 aren't "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 UCSC Genome Bioinformatics Site (genome, ucsc.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.

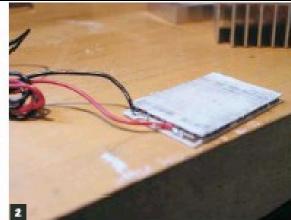
You can use the Genome Browser to search the entire genome sequence and navigate around any part of it. You can see the detailed features of any particular location by searching for an address; instead of a street address, you enter the numerical position of the nucleotide in the entire sequence. Researchers routinely use the Genome Browser when they need raw 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 peltierinfo.com/surplus.html. We used a 1.5"×2" device rated at 5V and 8 amps (Marlow Industries item #SP2083). For the device's power supply, we

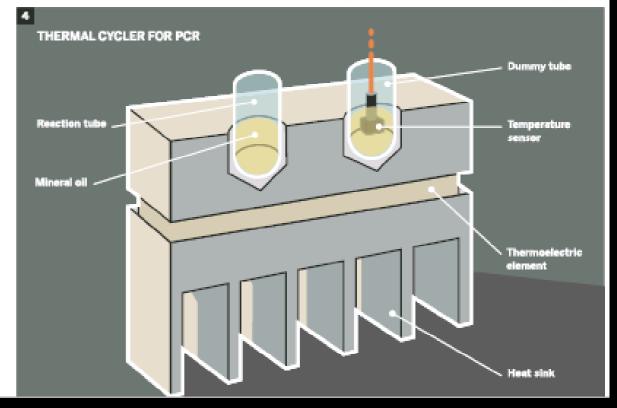


#### How to make a thermal cycler for DNA replication:

- Instead of paying \$5,000 for a commercial thermal cycler (which you'll need to replicate DNA samples), you can make the one shown here for less than \$300.
- 2. This Peltier device pumps heat from one plate to another when a current is applied.
- Here, the Peltier device has been outfitted with 2 aluminum blocks. 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 a tube where the reaction takes place, and a dummy tube that contains a temperature sensor. The data from the sensor provides feedback to the microcontroller, which controls the Peltier device.









#### 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.

 Drill pilot holes at the corners of the traced outline.

 Use a keyhole saw or jigsaw 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

This isn't just Step 3; it's something you'll need to do every time you work inside your laminar flow hood. The hood is crucial for mushroom 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 mushroom tissue in agar (seaweed 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 at once 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 3 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 cotton or sponge in isopropyl alcohol and then wringing it out. Place the filter in the hole in the container lid. It should fit snugly.



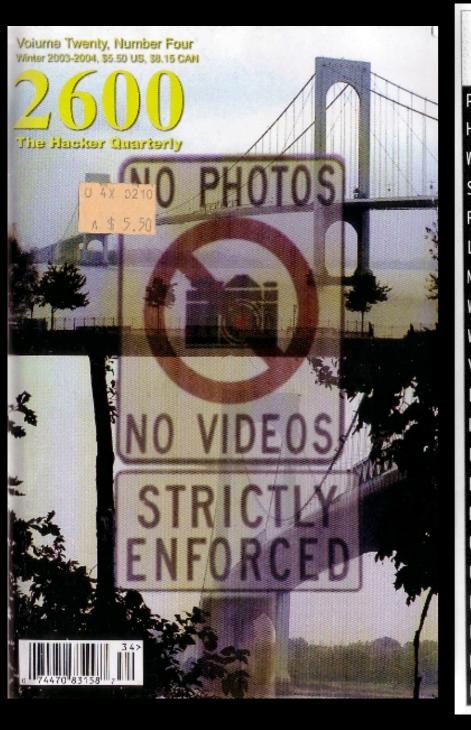
The sponge-piece filter keeps the mushroom tissue protected while letting it exchange gases with the surrounding air.

- 4d. Mix 1 tablespoon of agar in 1 cup of water. Bring to a low boil and slowly simmer for about 15 minutes, stirring occasionally. Add a large pinch of the growing substrate you'll be using later (sawdust, cat litter, barley, 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 congeal.



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## Daita

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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.

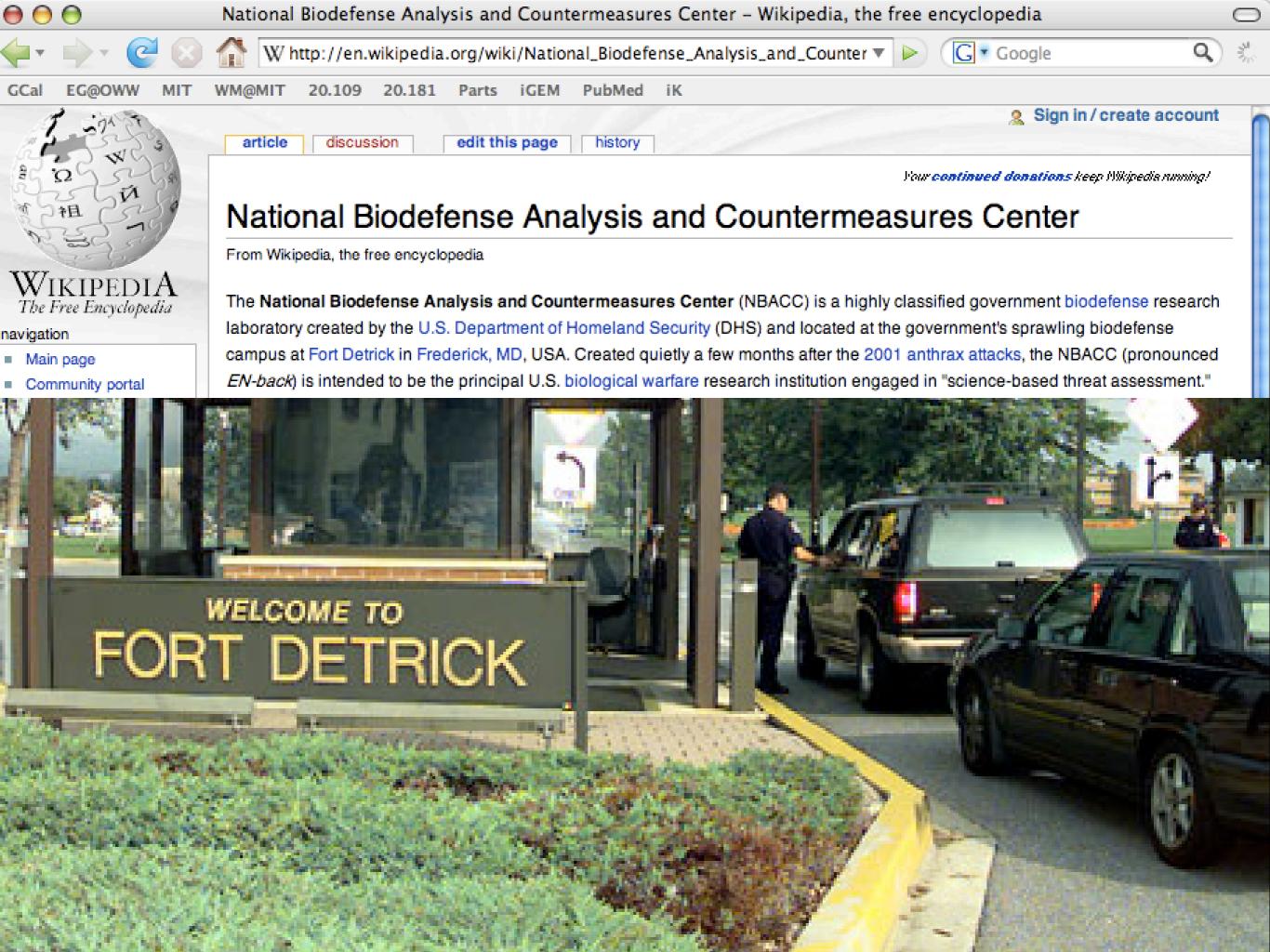
If these techniques are so dangerous, why on earth would I want to tell hackers how to use them? I've thought about this long and hard before writing this article, and I have three reasons for writing. First, none of the information in this article is all that hard to find these days. Good high school 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 8038based blue box, I built an Imsai 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 that that! I hope someone reading this article will one day invent it.

Page 6 2600 Magazine



Avoid remilitarization of biological technology.

Revitalize and extend relatively rigid, partially effective, state-based framework by promoting "soft" social complements to the BTWC.

Expect that somebody(ies) will cause future events.

Prepare.

Develop long-term strategy for responding to emerging biological agents (first natural, then engineered).



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