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.

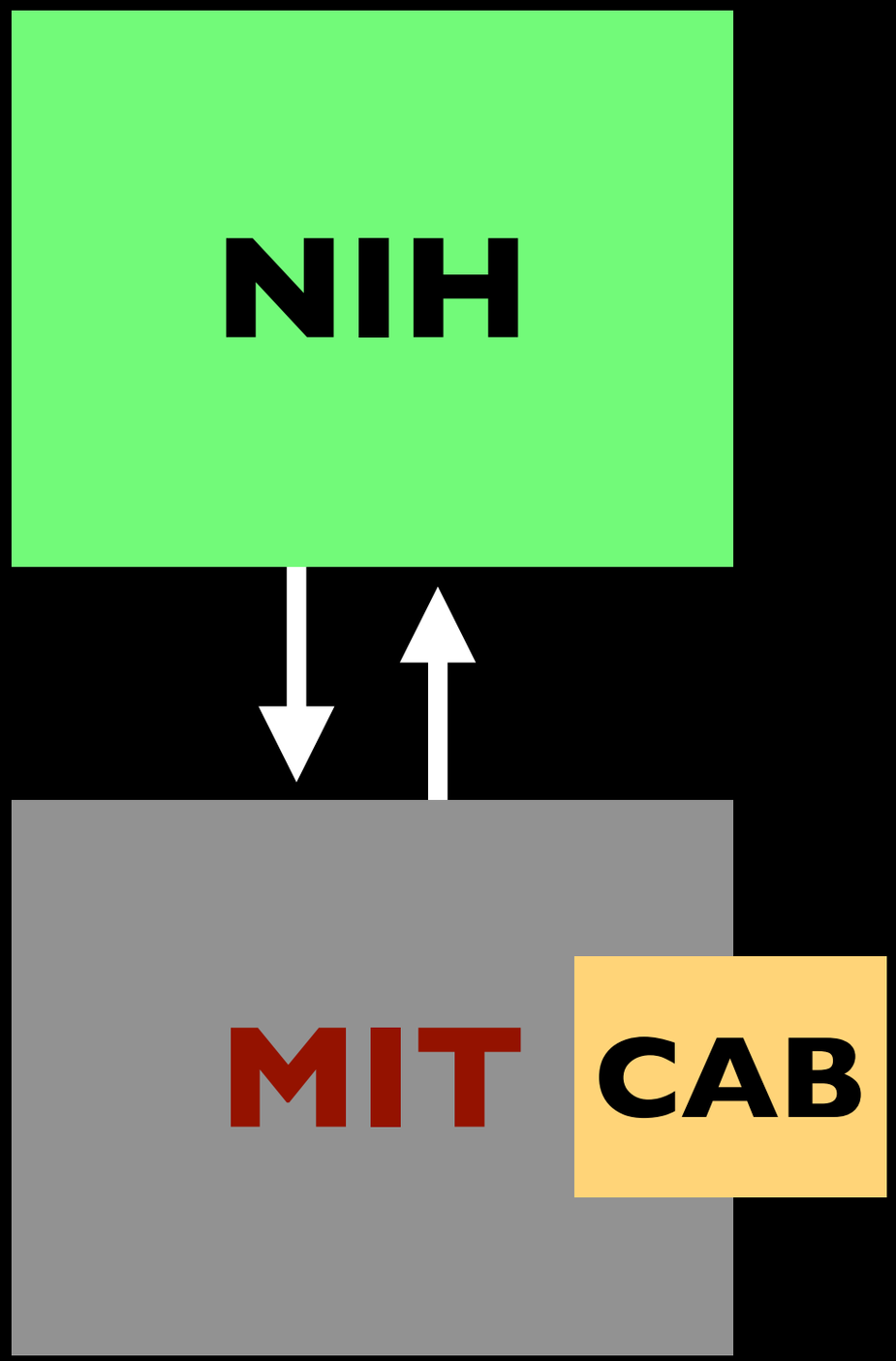
Biosafety Level 3 practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or exotic agents with a potential for respiratory transmission, and which may cause serious and 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.

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Photo removed due to copyright restrictions.
Attendees at the International Conference on
Recombinant DNA Molecules, February 1975 in Asilomar, CA
(Phil Sharp, David Baltimore and others)

See http://libraries.mit.edu/archives/exhibits/asilomar/index.html



http://mit.edu/cab/

Text content removed due to copyright restrictions.

Abstract and final "Safety Precautions" section from Denwannieux, M., et al. "Identification of an Infectious Progenitor for the Multiple-copy HERV-K Human Endogenous Retroelements." *Genome Research* 16 (2006): 1548-1556.

What's changed since the 70s?

http://en.wikipedia.org/wiki/Phillip_Allen_Sharp

Phillip Sharp with President George W. Bush, at the National Medal of Science awards in 2006.

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.

Courtesy of Make Magazine. Used with permission.

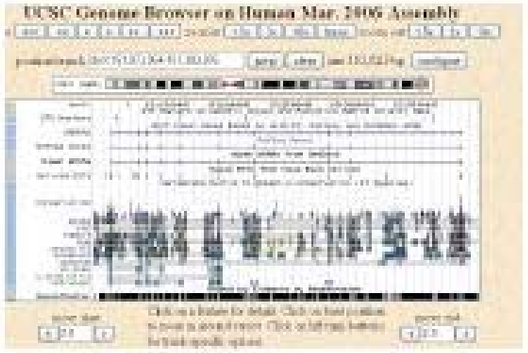
PROJECT 2:

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.)



THE GENOME BROWSER

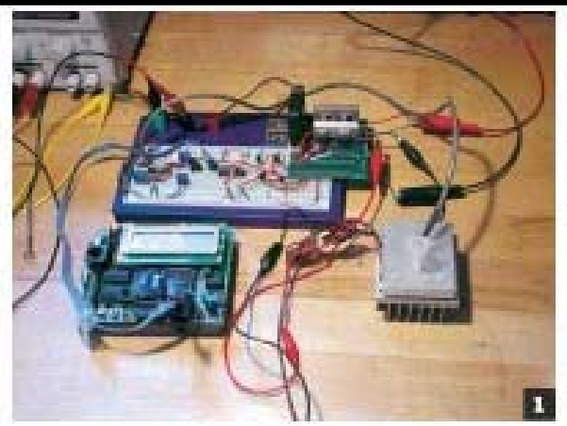
Our genome represents all of the DNA in our cells' nuclei. This DNA is the "genetic blueprint" that determines how we're put together on a molecular level, what we look like, and how healthy we are. It contains over 3 billion letters, called *nucleotides*, which the Human Genome Project has mapped using 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.

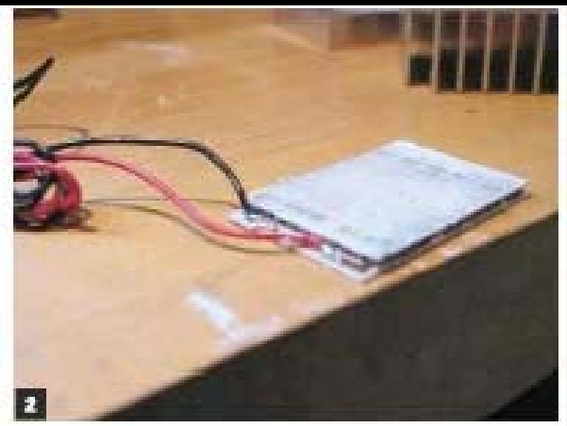
Anyone can read this blueprint and browse the latest discoveries online using the Genome Browser at the UCSC Genome Bioinformatics Site (genome.ucsc.edu/cgi-bin/hgGateway). This breakthrough tool is like a Google Maps for genomes, and it's being updated continuously as researchers decipher different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

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 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 peltier-info.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


1


2


3

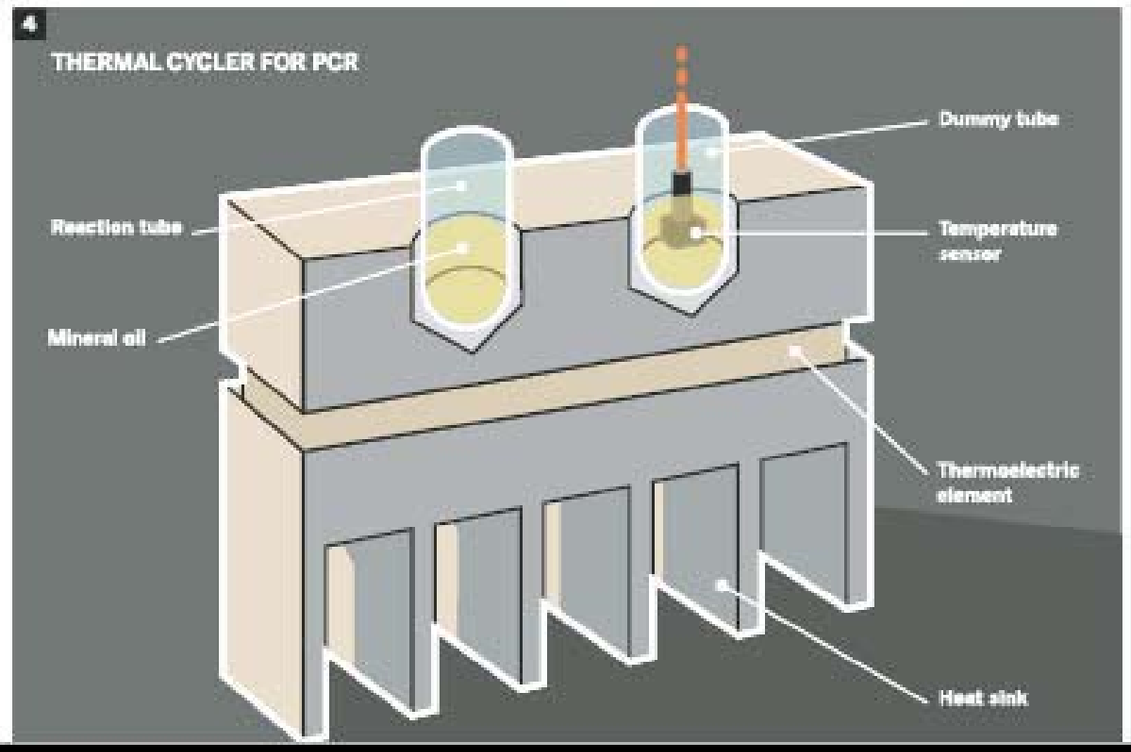
How to make a thermal cycler for DNA replication:

1. 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 $100.

2. This Peltier device pumps heat from one plate to another when a current is applied.

3. 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.



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 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 HOOD

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 ½" 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.

Images removed due to copyright restrictions.

3 scans from *2600: The Hacker Quarterly*, Winter 2003-2004 issue. Cover, Table of Contents and pg. 6 "Hacking the Genome."

International Genetically Engineered Machine Competition

Global Distribution of Competing Teams

Images removed due to copyright restrictions.
Photo of Fort Detrick entrance.
Wikipedia page on NBACC:
(http://en.wikipedia.org/wiki/National_Biodefense_Analysis_and_Countermeasures_Center).

What's the difference between safety & security?

Unknown reservoir →

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**

3: NC_004161
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**

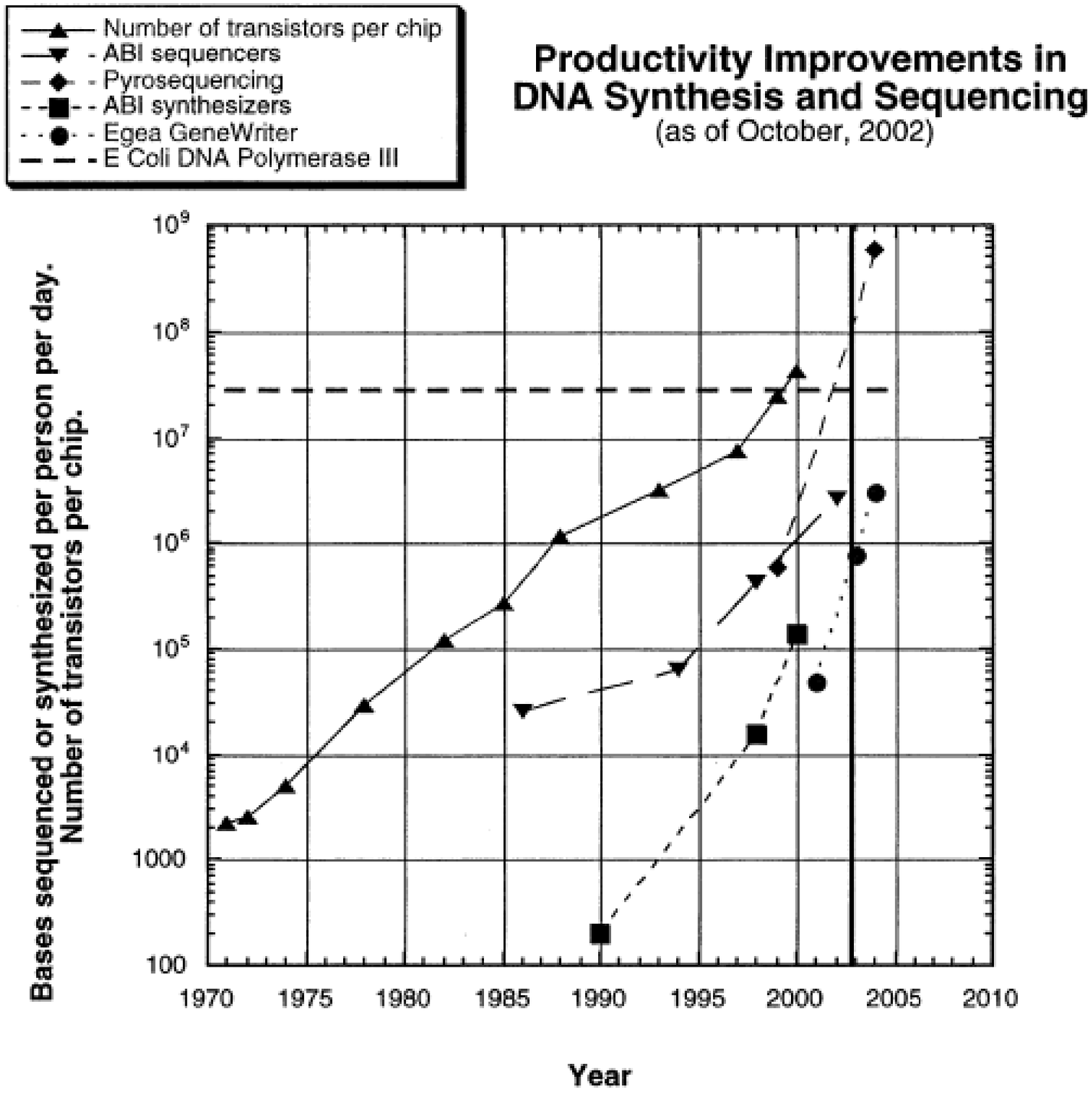
NC_006966
Mule deer poxvirus, complete genome
dsDNA; **linear**; Length: **166,259 nt**
Created: **2005/04/08**

Don't exist →

1: DQ208309 Reports
Influenza A virus (A/Brevig Mission/1/1918(H1N1)) polymerase PB2 (PB2) mRNA, complete cds
gi|76786704|gb|DQ208309.1|[76786704]

1: DQ208310 Reports
Influenza A virus (A/Brevig Mission/1/1918(H1N1)) polymerase PB1 (PB1) mRNA, complete cds
gi|76786706|gb|DQ208310.1|[76786706]

Source: http://www.ncbi.nlm.nih.gov/PubMed/



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

Information (DNA Sequence)

Sequencing

Synthesis

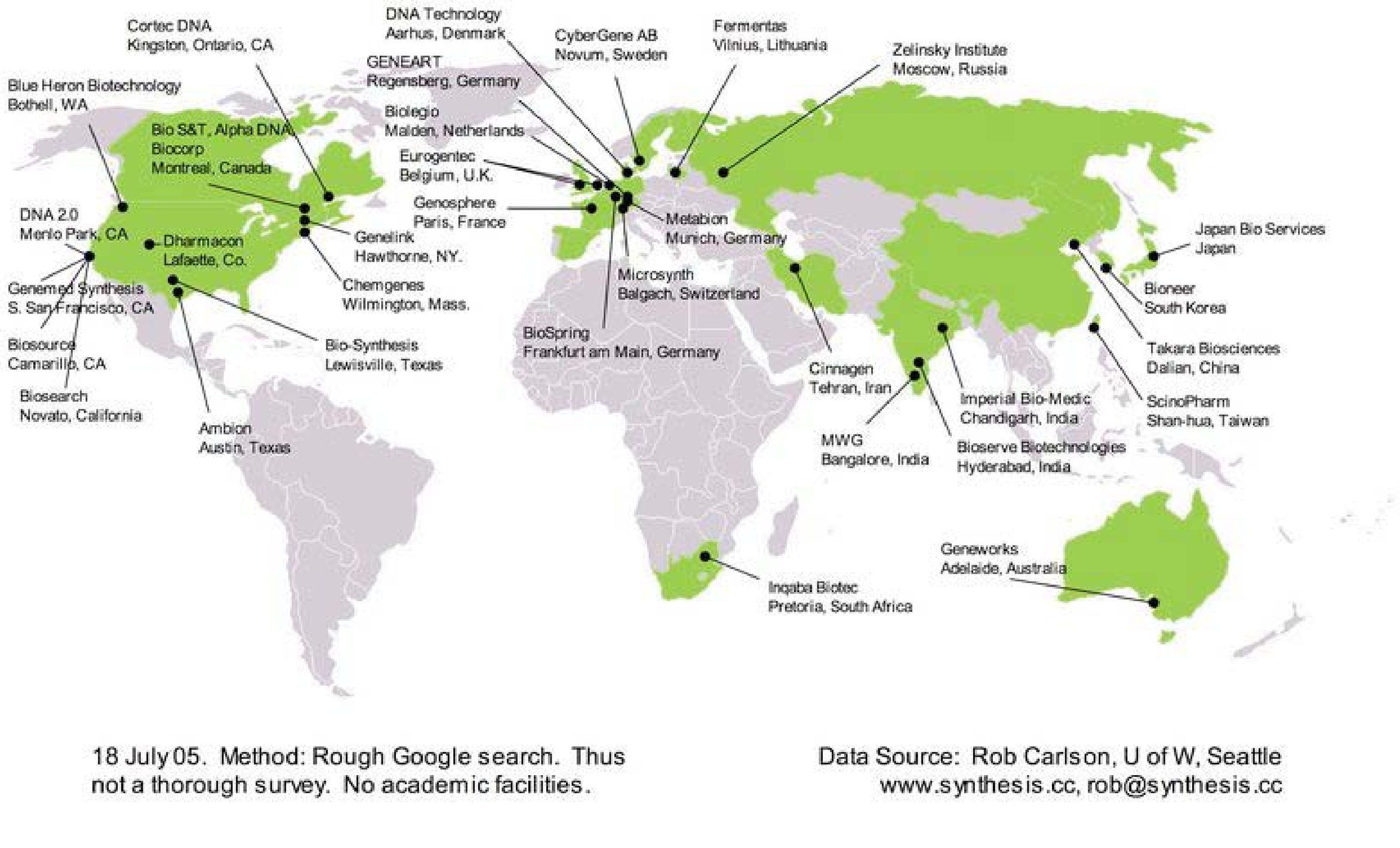
Material (Physical DNA)

Image removed due to copyright restrictions.

eBay sale page for "ABI Applied Biosystems 3948 DNA Synthesizer."
BuyItNow price = $4,999.99.
Ships to: Worldwide.

Commercial DNA Synthesis Foundries

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



Courtesy of Rob Carlson. Used with permission.

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

Image removed due to copyright restrictions.
Text of Sharp, P.A. "1918 Flu and Responsible Science." *Science* 310, no. 5745 (2005): 17.

Image removed due to copyright restrictions.
Kurzweil, R., and B. Joy. "Recipe for Destruction." *New York Times*, 17 October 2005.

DNA synthesis and biological security

Hans Bügl, John P Danner, Robert J Molinari, John T Mulligan, Han-Oh Park, Bas Reichert, David A Roth, Ralf Wagner, Bruce Budowle, Robert M Scripp, Jenifer A L Smith, Scott J Steele, 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.

DNA synthesis allows the direct construction of genetic material starting from information and raw chemicals[1]. 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 unfortunate reality.

Here, we outline a practical plan for developing an effective oversight framework for the DNA-synthesis industry[2]. 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 continued, overwhelmingly constructive application. And third, it is designed to be international in scope. Our plan is informed by past and ongoing discussions of biological security issues associated with DNA-synthesis technology[3–5] and represents the collective views of all founding members of the

Hans Bügl, John P. Danner, Robert J. Molinari, John T. Mulligan, David A. Roth & Ralf Wagner are members of the International Consortium for Polynucleotide Synthesis; Hans Bügl and Ralf Wagner are at GENEART; John P. Danner, George Church & Drew Endy are at Codon Devices; Robert J. Molinari & David A. Roth are at CODA Genomics; John T. Mulligan is at Blue Heron Biotechnology; Han-Oh Park is at Bioneer; Bas Reichert is at BaseClear B.V.; Ralf Wagner is at the University of Regensburg Molecular Virology & Gene Therapy Unit, Institute of Medical Microbiology and Hygiene; Bruce Budowle, Robert M. Scripp, Jenifer A. L. Smith & Scott J. Steele 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, MIT; George Church & Drew Endy are at the multi-institution US National Science Foundation Synthetic Biology Engineering Research Center. e-mail: endy@mit.edu

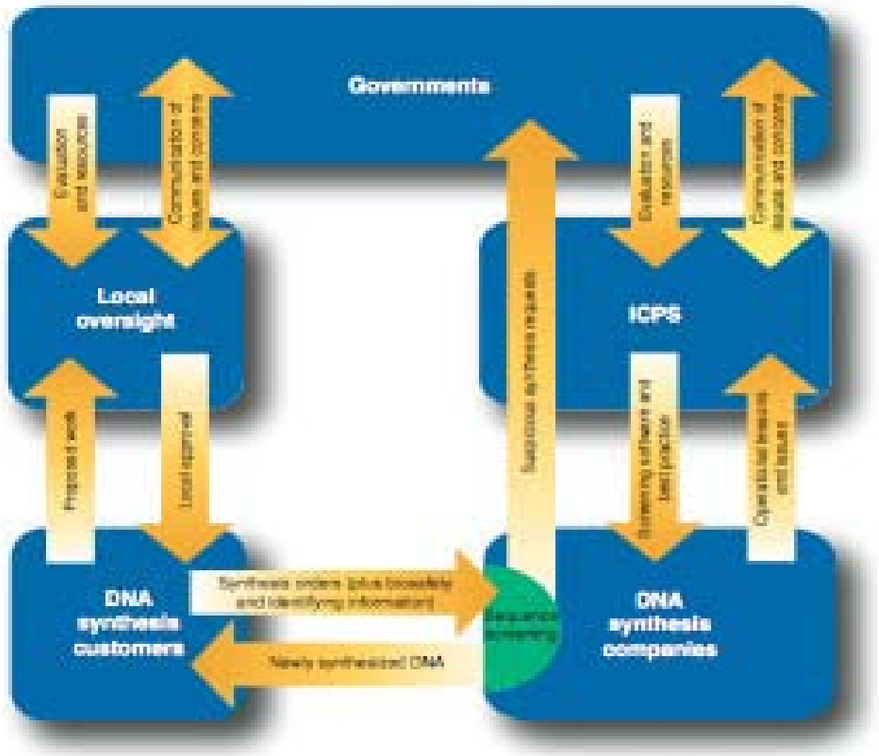


Figure 1 Our framework calls for the immediate and systematic implementation of a tiered DNA synthesis order screening process. To promote and establish accountability, individuals who place orders for DNA synthesis would be required to identify themselves, their home organization and all relevant biosafety information. Next, individual companies would use validated software tools to check synthesis orders against a set of select agents or sequences to help ensure regulatory compliance and flag synthesis orders for further review. Finally, DNA synthesis and synthetic biology companies would work together through the ICPS, and interface with appropriate government agencies (worldwide), to rapidly and continually improve the underlying technologies used to screen orders and identify potentially dangerous sequences, as well as develop a clearly defined process to report behavior that falls outside of agreed-upon guidelines. ICPS, International Consortium for Polynucleotide Synthesis.

Solution frameworks should be adaptive.

Should teenagers practice genetic engineering?

Should there be top-secret BL-4 labs?

Will biohackers be good or bad?
Is garage biotechnology inevitable?

Should BioBrick parts be patented or freely shared?

Should genetic engineers be licensed?
Should genetic engineers sign their work?

3rd annual meeting of the American Society of Civil Engineers, 1855