

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Dan Gibson, +21, Ham Smith and Craig Venter

Science (2010) **329**: 52

Presidential Commission for the Study of Bioethical Issues

New Directions: The Ethics of Synthetic Biology
and Emerging Technologies
December 2010

20.020(S12) 02.15.12

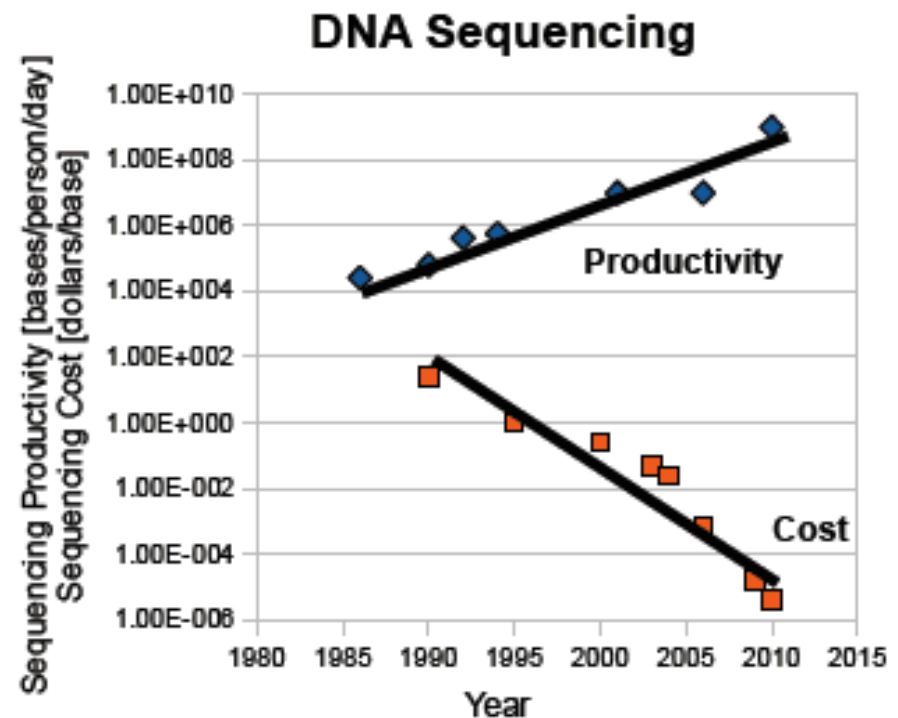
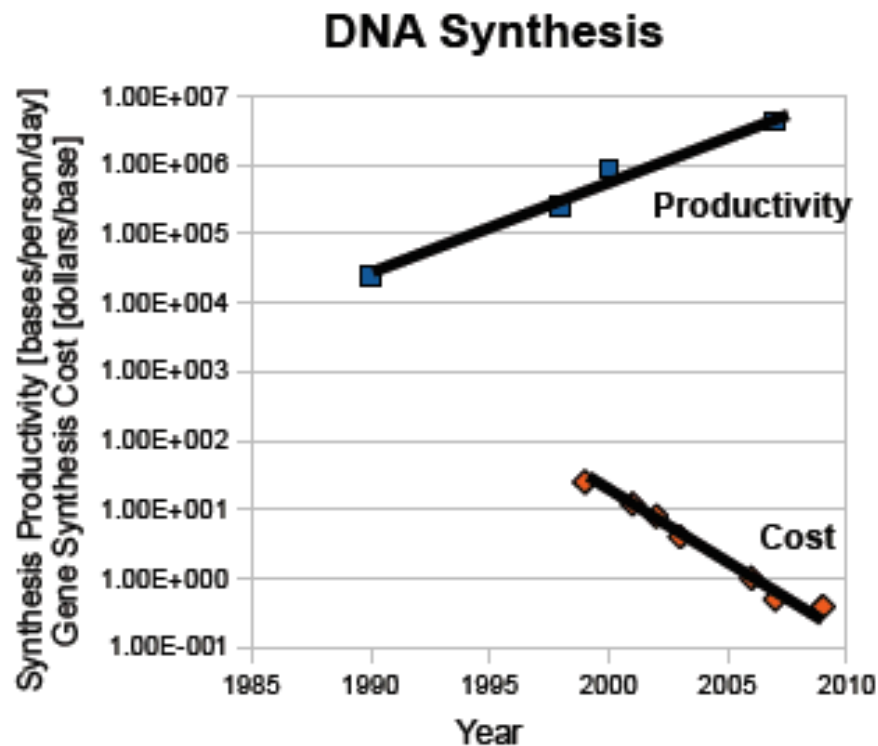
Summary

- Synthesis and transplantation of *M. mycoides* genome into closely related species, *M. capricolum*
 - Rationally designed some DNA elements
 - Tools used to generate transplanted DNA include chemical DNA synthesis, cloning in *E. coli*, recombination in *S. cerevisiae*
 - Tools used to transplant into recipient cell include *in vitro* processing of DNA, agar plug purification of large DNA assemblies and inactivation of host restriction system

Background: writing DNA vs reading DNA

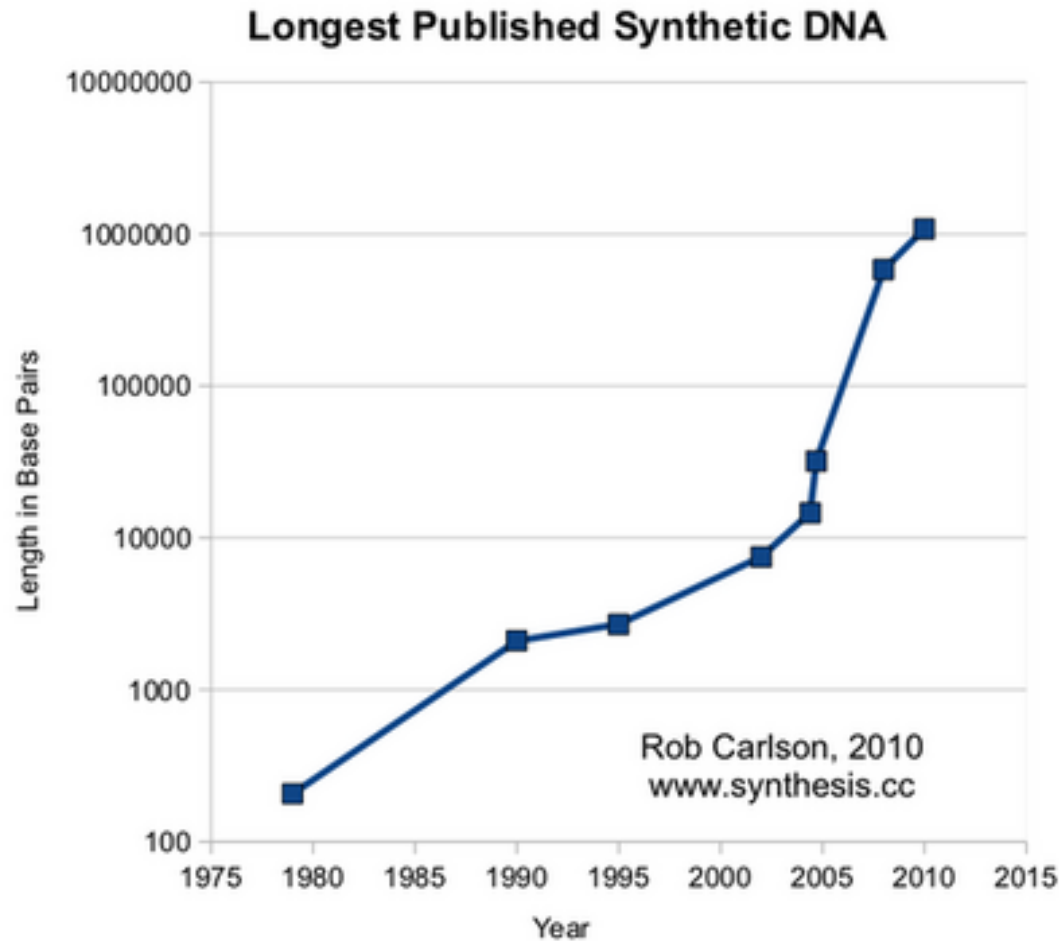
Productivity measured in
bp of oligos/person/day

Cost measured
in \$/bp of genes



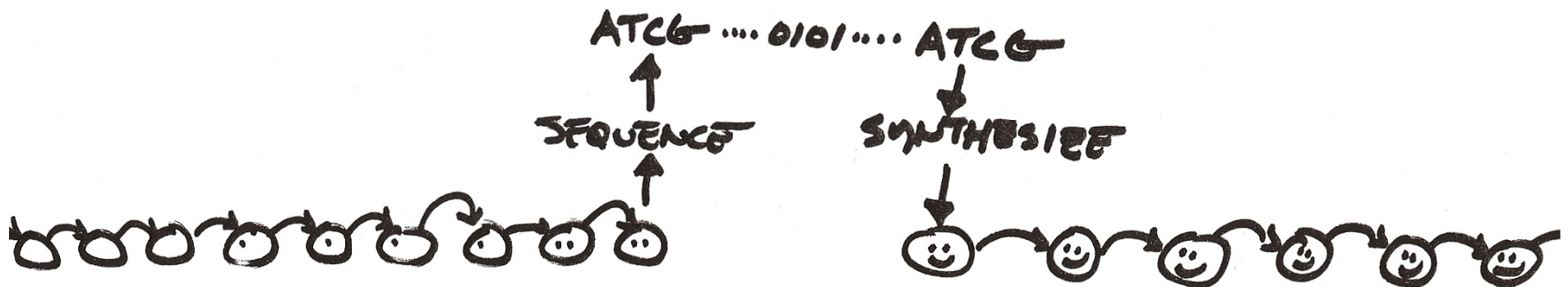
Sources: Biodesic, www.synthesis.cc

Background: reading DNA vs writing DNA



Goal: build a working cell that uses a synthetic genome

- Design, synthesis and assembly of largest functional genome to date



aka "Darwin is dead"

art from Drew Endy

Goal: build a working cell that uses a synthetic genome

- Design, synthesis and assembly of largest functional genome to date

aka "Darwin is dead"

Sufficiently mature DNA sequencing & synthesis technology will allow us to decouple the "designs" of life from the constraints of direct descent & replication with error. — D. Endy

Goal: build a working cell that uses a synthetic genome

- Design, synthesis and assembly of largest functional genome to date

“JCVI-syn1.0”

Previous successes

- * Gibson et al. Science 2008

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome (~600, 000 bp in yeast)

- * Lartigue et al. Science 2007

Genome Transplantation in Bacteria: Changing One Species to Another

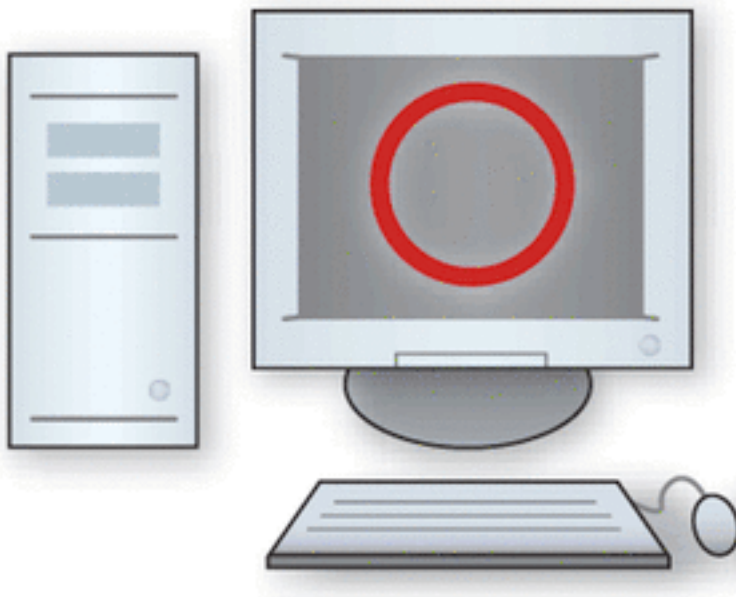
Approach

- Writing DNA

2 polished *M. mycoides* genomes

CP001621

CP001668 (aka YCpMmyc1.1)



4 “watermark” sequences

Watermark-1, 1246 base pairs

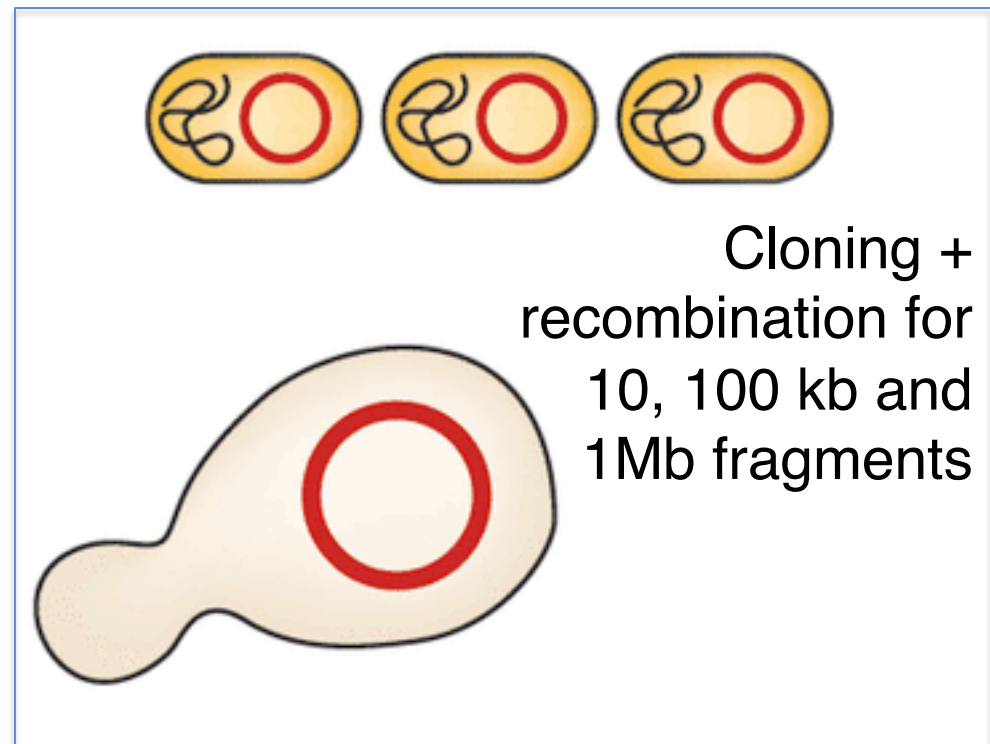
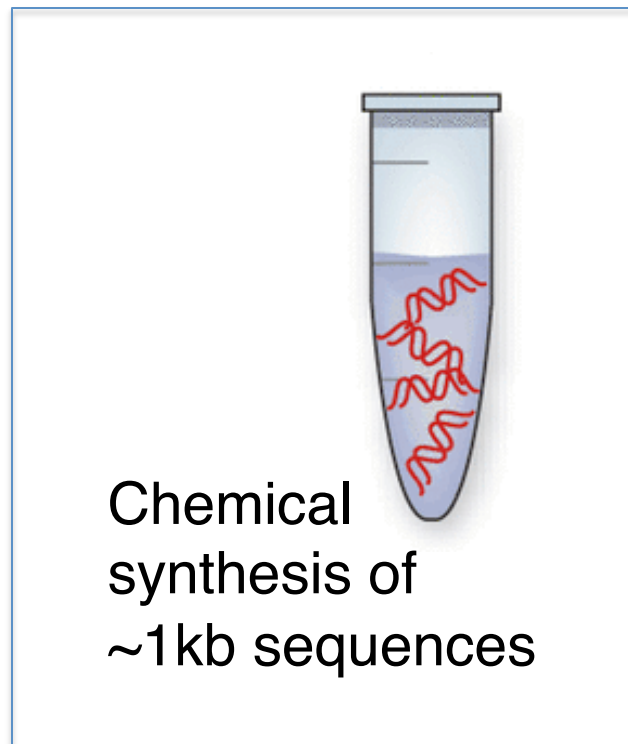
```
TTAACTAGCTAA GTTCGAATATTTCTATAGCTGTACATATTGTAATGCTGATAACTAATACTGTG
TTTAAGGCTACTCACTGGTTGCAAACCAATGCCGTACATTACTAGCTTGATCCTTGGTCGGTC
GACCCCCCTCTCCACACCAGCGGTGTAGCATCAACCAAGAAAATGAGGGGAACGGATGAGG
CGTCAACTGTTTGCTGTGATACTGTGCTTTCGAGGGCGGGAGATTGTTTTTGACATACATAA
TAGCCGGTGACTGTGAACTAAAGCTACTAATGCCGTCAATAAATATGATAATAGCAACGGCA
ATTCATAGCCGGTAGATATCACTATAAGGCCCAGGATCATGATGAACACAGCACACCACGTCGT
AGTTTTTTTGCTGCGGCACTAGAGCCGGACAAGCACACTACGTTTGTAAATACATCGTTCCGA
TAGTGATAACTACAATAGCTAGCAATAAGTCATATATAACAATAGCTGAACCTGTGCTACATAT
CGTCAGCAACTACGTTTAGCTTGACTGTGGTCGGTTTTTTTGCTGCGACGTCTATAOCCAAGC
CGTATTCATAGCCGGCACTCATGACAAAACAGCGGCGCGCC TTAACTAGCTAA
```

Also wrote in TetR and LacZ

Itaya *Nature Biotechnology* : (2010) 28: 687–689

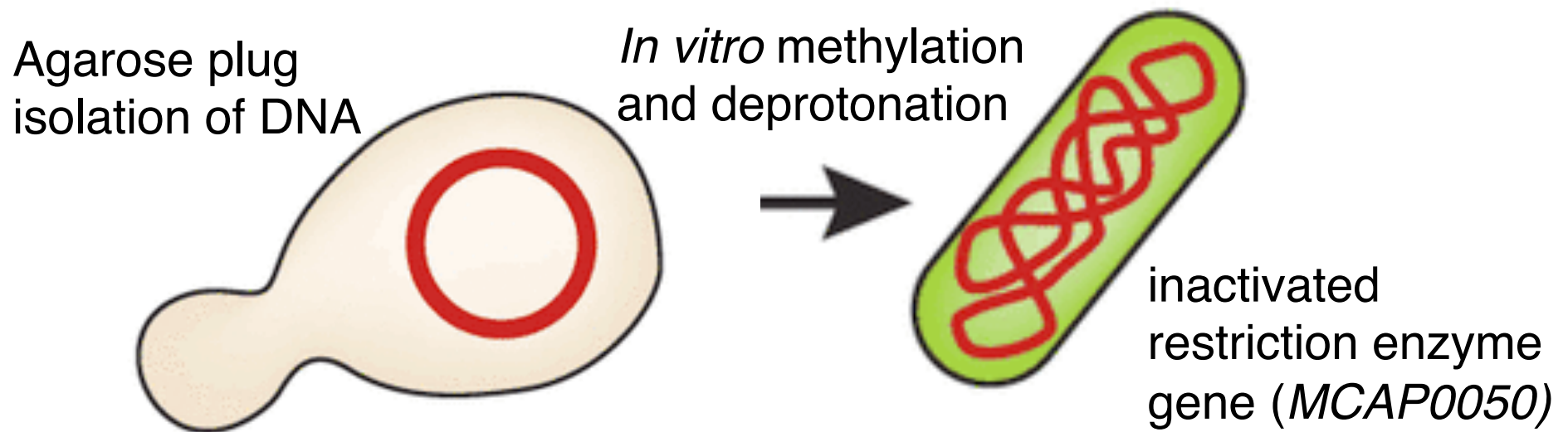
Approach

- Writing DNA
- Assembling DNA



Approach

- Writing DNA
- Assembling DNA
- Transplanting DNA to *M. capricolum*



Technical Achievement (1): Assembly

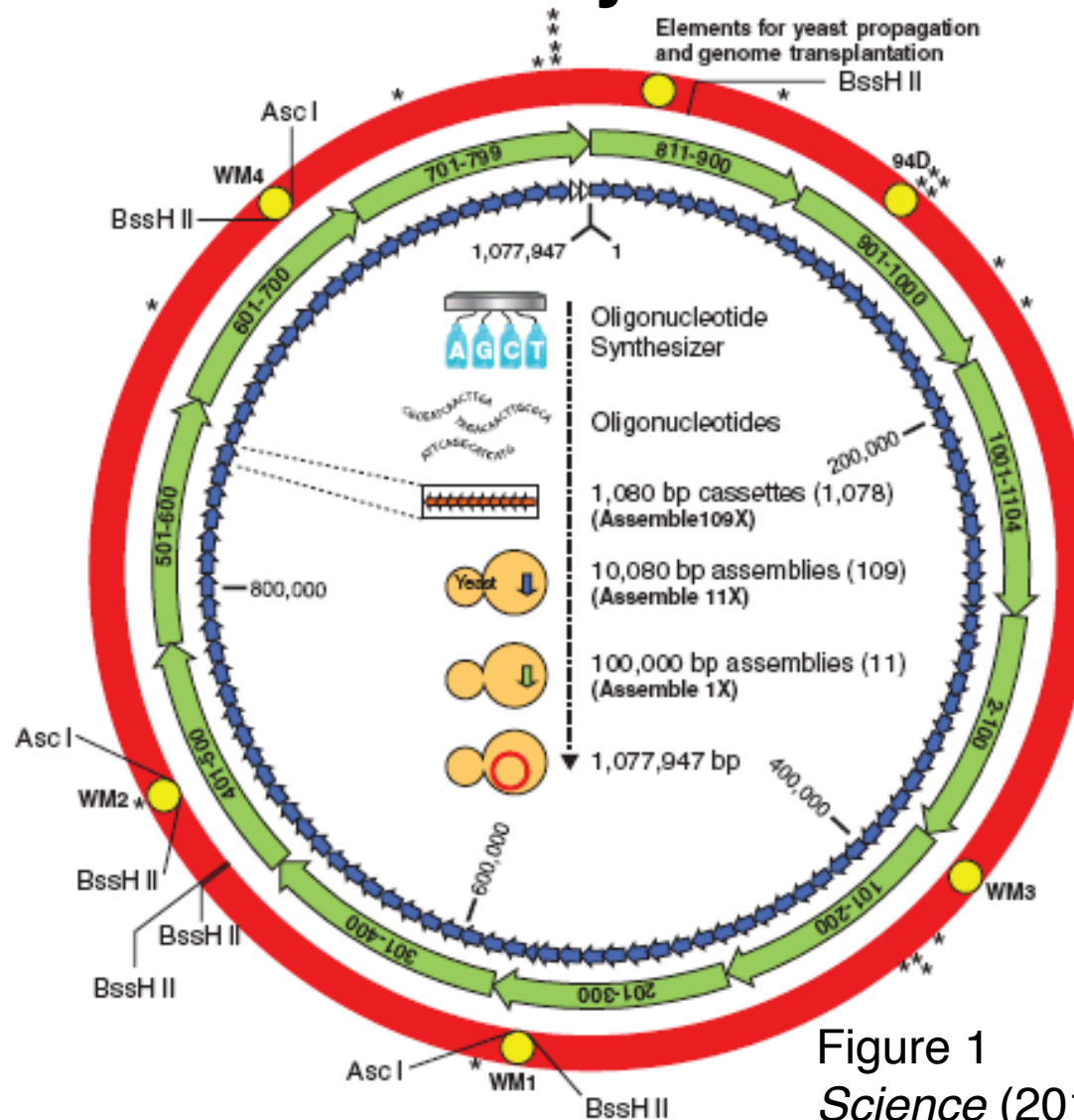
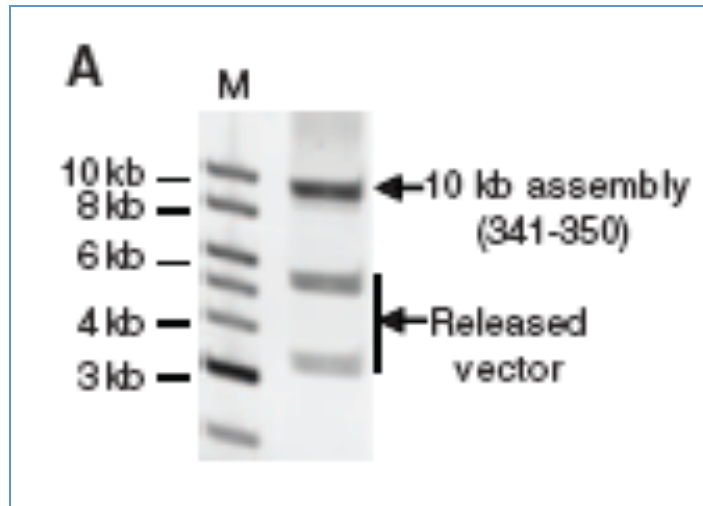
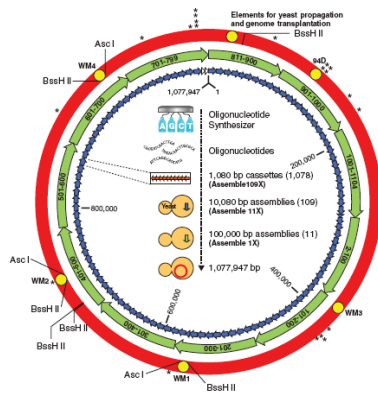


Figure 1
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Technical Achievement (1): Assembly



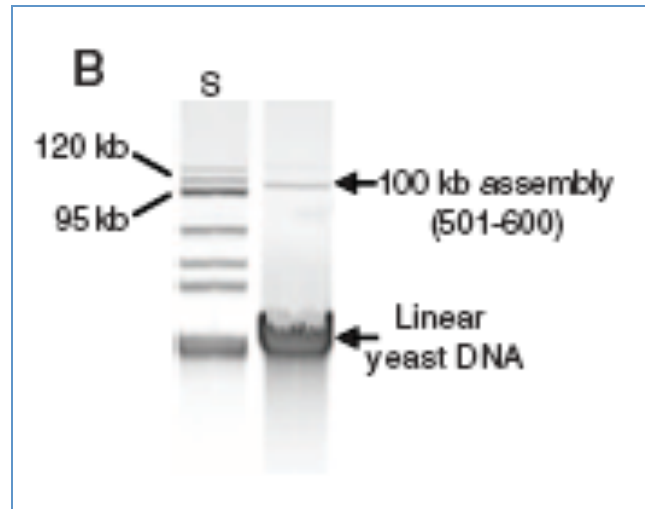
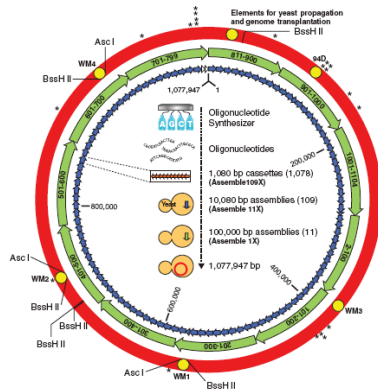
Success rate varied
(10-100%)

19/111 assemblies
were corrected for
sequence errors

Method: mixed 10X 1080 bp cassettes → 10 kb
80-bp overlaps to adjacent cassettes

Recombined in *S. cerevisiae*
~10 candidates transferred to *E. coli*

Technical Achievement (1): Assembly



Success rate usually
25%

Incorrect instances
include partial
deletions and
duplications

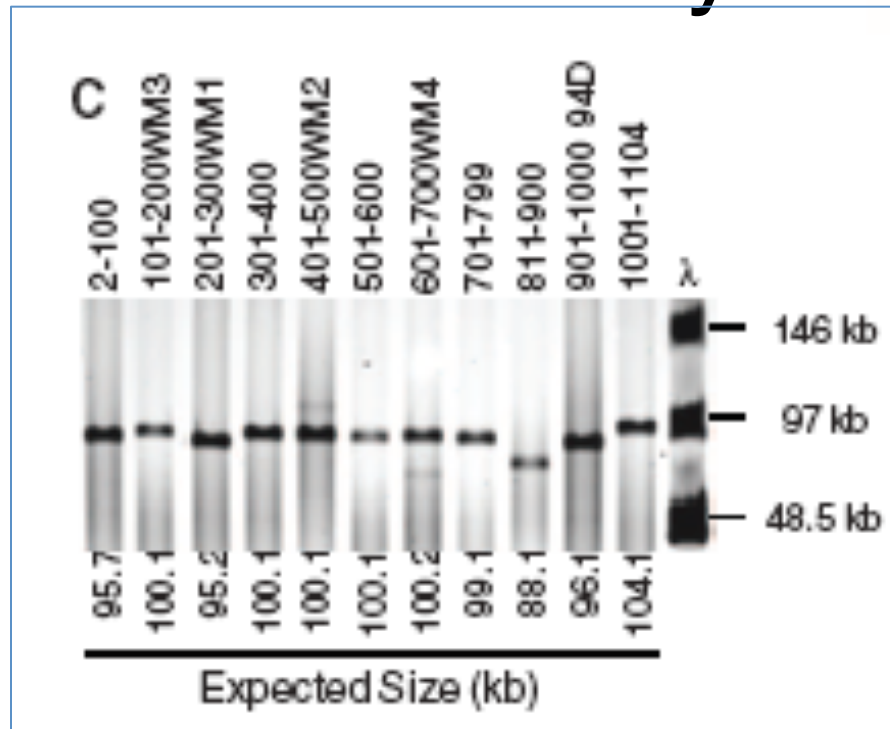
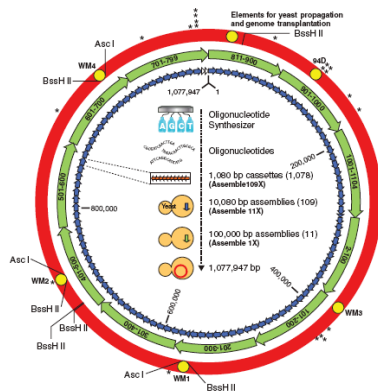
Method 10X 10 kb assemblies → 100 kb, with 10 specific
primer pairs

Multiplex PCR

Recombined in *S. cerevisiae*

Unstable in *E. coli*

Technical Achievement (1): Assembly



1/48
“sMmYCp235”
produced all 11
amplicons seen
in WT genome
(Fig. 3A)

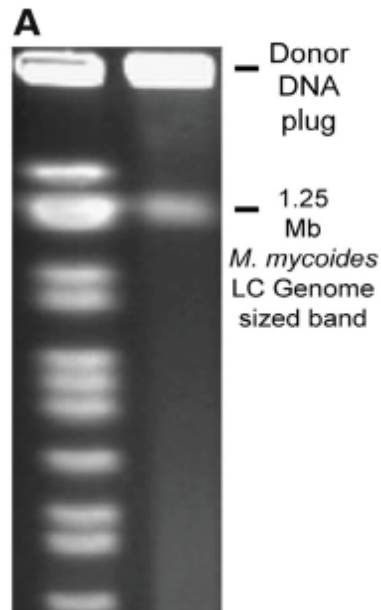
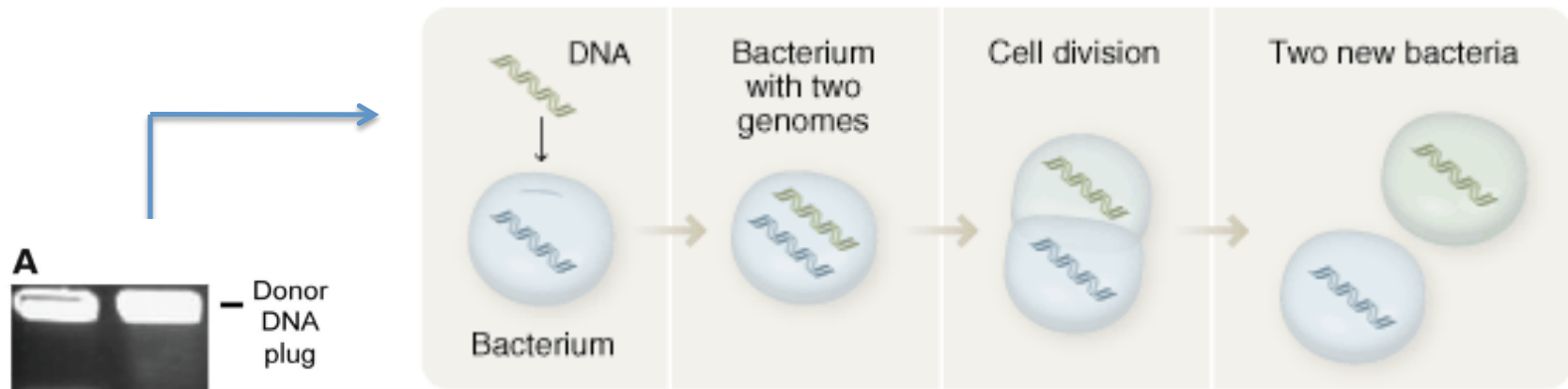
Method: 100 kb assemblies → 1.08 Mb

Spheroplast *S. cerevisiae* (~1ug DNA/400 ml cells)

Separate circular from linear, release 100 kb insert from
circular, pool and co-transform, check assembly junctions

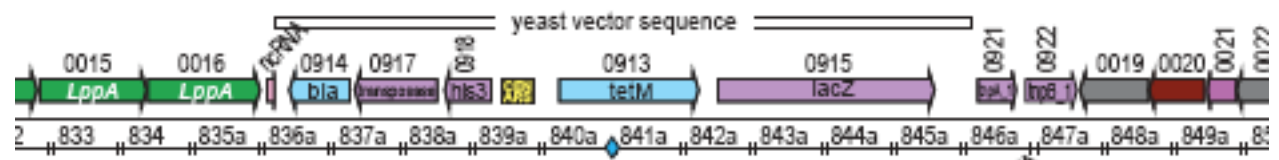
Figure 2 *Science* (2010) **329**: 52

Technical Achievement (2): Transplantation



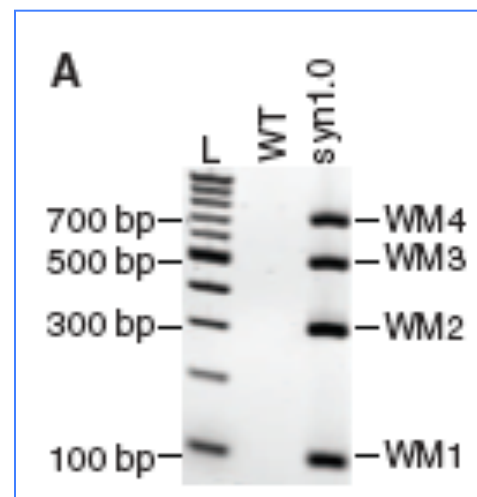
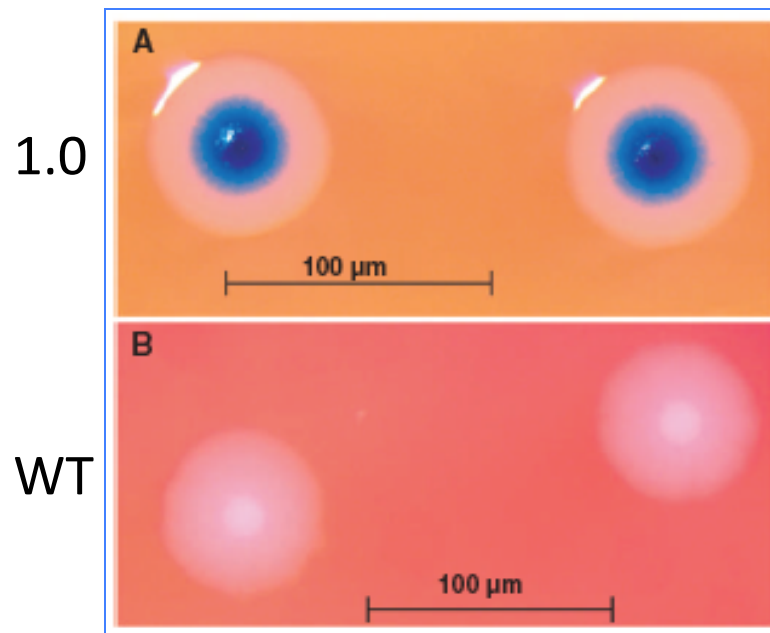
Modify recipient restriction system

Scored for TetR and blue on X-gal

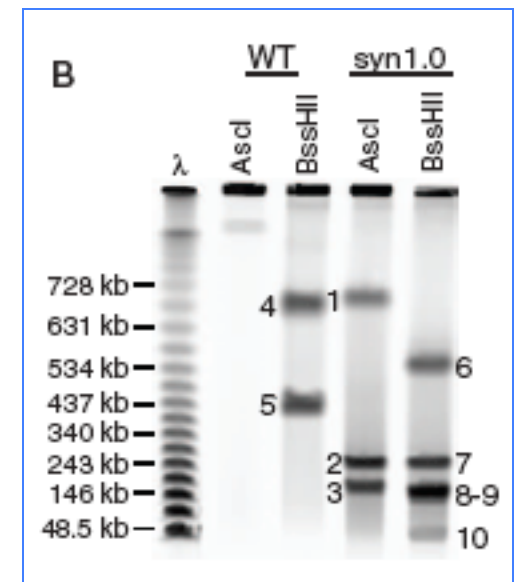


Lartigue (2007)

Technical Achievement (2): Transplantation



PCR for watermarks



Digests of genome
plugs

Conclusions

- According to JCVI:

“The synthetic cell is called *Mycoplasma mycoides* JCVI-syn1.0 and is the proof of principle that genomes can be designed in the computer, chemically made in the laboratory and transplanted into a recipient cell to produce a new self-replicating cell controlled only by the synthetic genome.”

Conclusions

- According to Venter on CNN:

“We built it from four bottles of chemicals.”

“So it's the first living self-replicating cell that we have on the planet whose DNA was made chemically and designed in the computer.”

“So it has no genetic ancestors. Its parent is a computer.”

Conclusions

- According to Jim Collins (BU):
“This is an important advance in our ability to re-engineer organisms, not make new life from scratch...Although some of us in synthetic biology have delusions of grandeur, our goals are much more modest.”

<http://www.nature.com/news/2010/100520/full/news.2010.255.html>

Perspectives

- “Build a cell”
 - (1) mycoides and capricolum nearly identical
 - (2) “synthetic genome” is knock-off

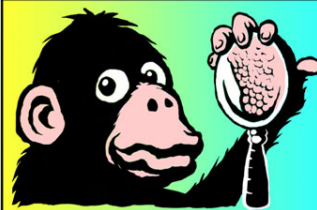
- Cost (\$ and time)



The Story of Synthia

(part 1): Craig Venter's plan to build a synthetic life-form

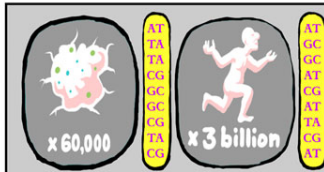
Text: ETC Group. Art: Stig. www.etcgroup.org
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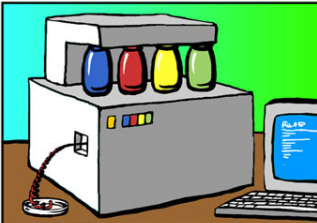
All living things, from microbes to monkeys, are made up of **cells**. Cells contain everything that's needed to transform energy into life. Humans have trillions of cells in their bodies. Simpler organisms, like bacteria, have just one cell. Cells are the basic units of life.



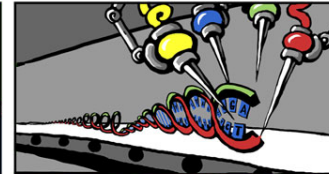
Inside most cells is a molecule known as **DNA**. It looks a bit like a zipper. The "teeth" of the zipper are four different chemical compounds – Adenine, Guanine, Cytosine and Thymine – known as A, G, C and T.



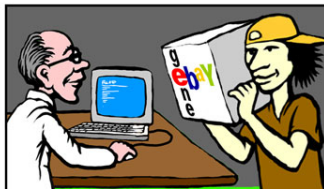
How an organism grows, develops and stays alive depends a lot on its **DNA**. It's like the software program that instructs a computer how to perform a function. The "code" for an organism's software is the order, or sequence, of **A, G, C and T** along the DNA zipper. Every living thing has a unique DNA sequence which is shorter in simple bacteria and longer in complex organisms such as humans.



To create a synthetic life-form, you first need to construct its DNA. **Synthetic DNA (sDNA)** is manufactured in a machine called a DNA synthesizer. The synthesizer has a computer in it that can be programmed to create any DNA sequence.



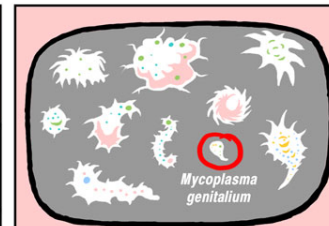
It takes a whole series of chemical reactions to "grow" a molecule of **DNA**. The DNA synthesizer pumps in chemical ingredients at the right time to make the right chemical reactions happen in the right order. Chemists have been tweaking the "recipe" for DNA for almost a half-century. Making the DNA for even a single-celled bacterium requires a long DNA zipper. In a human cell, A, G, C and T appear 3 billion times!



So most scientists interested in building synthetic life take a short-cut – they go on the Internet and order their sDNA from 'foundries' – companies that crank out lengths of synthetic DNA like a factory makes car parts. An organism's complete zipper of DNA is called its **genome**. To make a synthetic organism, you need an entire genome.



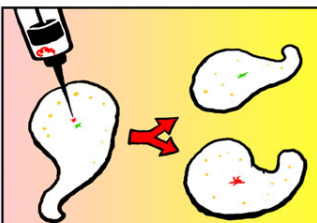
Craig Venter, CEO and founder of **Synthetic Genomics, Inc.** claims that his company is constructing the first-ever, living organism whose genome is not like any known species and is made entirely from synthetic DNA. We call it 'Synthia.'



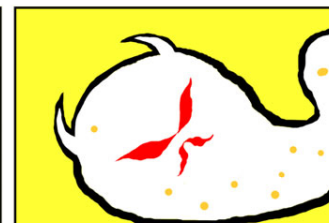
The scientists aren't constructing the whole cell, just the DNA inside the cell. To create Synthia, the team started with a bacterium that already exists. It is called **Mycoplasma genitalium** and lives in the human genitals. It has a really small genome.



The scientists deleted some pieces of *Mycoplasma genitalium*'s DNA, but not enough to kill it... at least not right away. They call this stripped-down DNA a 'minimal' genome. Then they re-build it out of synthetic DNA.



No genome can function by itself – it needs all the other parts of the working cell. So, the **human-made genome**, stitched together from sDNA, is **injected into a natural bacterial cell**. The cell divides, and one of the new cells ends up with the synthetic genome.



Presto! **Synthia is born!** Scientists pieced together a new species of bacterium with entirely human-made DNA. Like all life-forms, Venter's synthetic organism should be **able to reproduce and mutate... and that's where the trouble could begin...**

THE WHITE HOUSE

WASHINGTON

May 20, 2010

Dr. Amy Gutmann
President and Christopher H. Browne
Distinguished Professor of Political Science
University of Pennsylvania
1 College Hall, Room 100
Philadelphia, Pennsylvania 19104-6380

Dear Dr. Gutmann,

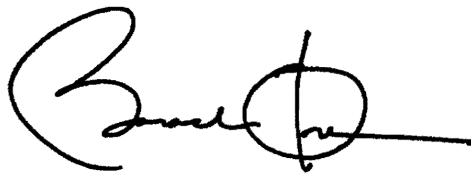
As you know, scientists have announced a milestone in the emerging field of cellular and genetic research known as synthetic biology. While scientists have used DNA to develop genetically modified cells for many years, for the first time, all of the natural genetic material in a bacterial cell has been replaced with a synthetic set of genes. This development raises the prospect of important benefits, such as the ability to accelerate vaccine development. At the same time, it raises genuine concerns, and so we must consider carefully the implications of this research.

I therefore request that the Presidential Commission for the Study of Bioethical Issues undertake, as its first order of business, a study of the implications of this scientific milestone, as well as other advances that may lie ahead in this field of research. In its study, the Commission should consider the potential medical, environmental, security, and other benefits of this field of research, as well as any potential health, security or other risks. Further, the Commission should develop recommendations about any actions the Federal government should take to ensure that America reaps the benefits of this developing field of science while identifying appropriate ethical boundaries and minimizing identified risks. My Science and Technology Advisor, Dr. John P. Holdren, will be in communication with you about the scope and progress of your study.

I ask that the Commission complete its study within six months and provide me with a report with its findings, as well as any recommendations and suggestions for future study that the Commission deems appropriate. Given the importance of this issue, I request that the Commission consult with a range of constituencies, including scientific and medical communities, faith communities, and business and non-profit organizations.

It is vital that we as a society consider, in a thoughtful manner, the significance of this kind of scientific development. With the Commission's collective expertise in the areas of science, policy, and ethical and religious values, I am confident that it will carry out this responsibility with the care and attention it deserves.

Sincerely,

A handwritten signature in black ink, appearing to be "Barack Obama", with a large, stylized initial "B" and a circular flourish at the end.

NEW DIRECTIONS

The Ethics of Synthetic Biology
and Emerging Technologies

5 ethical principles

Relevant to considering the social implications of emerging technologies:

- (1) public beneficence
- (2) responsible stewardship
- (3) intellectual freedom and responsibility
- (4) democratic deliberation
- (5) justice and fairness

Intended to illuminate and guide public policy choices

Presidential Commission
for the Study of Bioethical Issues

December 2010



synthetic biology scorecard

Introduction to the Project

On Dec. 16, 2010, the Presidential Commission for the Study of Bioethical Issues released a report entitled *New Directions: The Ethics of Synthetic Biology and Emerging Technologies*. The report contained 18 recommendations designed to help ensure that, as synthetic biology advances, the risks and ethical concerns are proactively addressed, the public is continually engaged, and oversight mechanisms are assessed and adjusted as needed to protect public health and the environment.

The report provides a rare opportunity for government to work together with other institutions, stakeholders, and citizens, both domestically and internationally, to ensure the responsible development of synthetic biology. The purpose of this website is to encourage broad participation in achieving the goals set forth by the Commission, to track our progress, and to invite public comment on the goals and implementation activities.

Most of the Commission's goals are included on this website and have been clustered into six sections: risk assessment, oversight, coordination, research, education and ethics. Within each section, the specific recommendations are provided with a list of relevant activities. Public comments are welcomed and these will be edited and posted on a regular basis.

Next: [Overview of Recommendations](#)

[Introduction to the Project](#)

Overview of Recommendations

- [Risk Assessment](#)
- [Oversight](#)
- [Coordination](#)
- [Research](#)
- [Education](#)
- [Ethics](#)

[link to SynBioProject Scorecard](#)



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