PREPARED STATEMENT OF

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BEFORE THE
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COMMITTEE ON ENERGY AND COMMERCE
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Mr. Chairman and Committee members, I welcome the opportunity to testify before you today. I am J. Craig Venter, Ph.D, President and Founder of the J. Craig Venter Institute (JCVI). The JCVI is a not-for-profit research institute in Rockville, MD and La Jolla, CA dedicated to the advancement of the science of genomics; the understanding of its implications for society; and communication of those results to the scientific community, the public, and policymakers. The JCVI is home to approximately 400 scientists and staff with expertise in human and evolutionary biology, genetics, bioinformatics/informatics, information technology, high-throughput DNA sequencing, genomic and environmental policy research, and public education in science and science policy. The JCVI is a 501 (c) (3) organization.

I am also the Founder and Chief Executive Officer of Synthetic Genomics Incorporated (SGI) a privately held company launched in 2005 to speed the commercialization of synthetic genomics technologies for a wide variety of applications including energy,the environment, and medicine.

In my testimony today I will first provide a brief overview of synthetic genomics, including answers to some key questions that I am often asked about this new technology. I will then briefly describe our recent accomplishment, and the 15 years of research that preceded it. Finally, I will discuss work to date on the ethical and societal implications of synthetic biology and review the ongoing policy discussions within the Federal Government.

OVERVIEW

Genomic science has greatly enhanced our understanding of the biological world. It is enabling researchers to "read" the genetic code of organisms from all branches of life by determining the sequence of the four letters that make up DNA. Sequencing genomes has now become routine, giving rise to thousands of genomes in the public databases. In essence, scientists are digitizing biology by converting the A, C, T, and G's of the chemical makeup of DNA into 1's and 0's in a computer. But can one reverse the process and start with 1's and 0's in a computer to define the characteristics of a living cell? We set out to answer this question.

In the field of chemistry, once the structure of a new chemical compound is determined by chemists, the next critical step is to attempt to synthesize the chemical. This would prove that the synthetic structure had the same function of the starting material. Until now, this has not been possible in the field of genomics. Structures have been determined by reading the genetic code, but they have never been verified by independent synthesis.

In 2003, JCVI successfully synthesized a small virus, approximately six thousand base pairs long, that infects bacteria. And by 2008, the JCVI team was able to synthesize a small bacterial genome, 580,000 base pairs long.

My team and I have now achieved the final step in our quest to construct the first synthetic bacterial cell. In a publication in *Science* magazine, Daniel Gibson, Ph.D. and a team of 23 additional researchers outline the steps to synthesize a 1.08 million base pair *Mycoplasma mycoides* genome, constructed from four bottles of chemicals that make up DNA. This synthetic genome has been "booted up" in a cell to create the first cell controlled completely by a synthetic genome.

The work to create the first synthetic bacterial cell was not easy, and took this team approximately 15 years to complete. Along the way we had to develop new tools and techniques to construct large segments of genetic code, and learn how to transplant genomes to convert one species to another. The 1.08 million base pair synthetic *M. mycoides* genome is the largest chemically defined structure ever synthesized in the laboratory.

While this first construct—dubbed *M. mycoides* JCVI-syn1.0—is a proof of concept, the tools and technologies developed to create this cell hold great promise for application in many critical areas. Throughout the course of this work, the team also contemplated, discussed, and engaged in outside review of the ethical and societal implications of their work.

The ability to routinely write the “software of life” will usher in a new era in science, and with it, new products and applications such as advanced biofuels, clean water technology, food products, and new vaccines and medicines. The field is already having an impact in some of these areas and will continue to do so as long as this powerful new area of science is used wisely. Continued and intensive review and dialogue with all areas of society, from Congress to bioethicists to laypeople, is necessary for this field to prosper.

ANSWERS TO SOME KEY QUESTIONS

I would like to give you an overview of the potential for the new field of synthetic genomics and the implications of our work to construct a synthetic cell by providing brief answers to a series of key questions.

How is synthetic genomics different than standard molecular biology/genetic engineering, etc?

Scientists have long been able to change and/or modify single genes or small sets of genes. Most genetic alterations that people know about today are through engineering of crops, which involves adding or altering less than 10 genes out of the tens of thousands that are contained in most organisms or plants. Synthetic genomics is different in that scientists start with digital information in the computer, which allows for the design of entire synthetic chromosomes to replace existing chromosomes in cells. The first self-replicating synthetic bacterial cell constructed by scientists at the JCVI has more than 1 million base pairs of DNA, almost 1,000 genes, and involved the complete replacement of genetic material in the cell. More detail about the construction of this cell may be found below in the section "Creation of a Synthetic Bacteria Cell," and in the attachment *Gibson et al. 2010*.

Why construct a synthetic cell?

We believe that the ability to "write the genetic code", as we describe synthetic genomics, will enable a better understanding of the fundamentals of living cells. It will also enable us to direct cells and organisms to perform jobs, such as producing clean water or new biofuels that natural species cannot currently do to the needed scale and efficiencies.

Is this research creating a synthetic bacterial cell "creating life from scratch"?

No. We do not consider this to be "creating life from scratch"; rather, we are creating new life out of already existing life using synthetic DNA to reprogram the cells to form new cells with functions that are specified by the synthetic DNA.

What are the potential applications of a synthetic cell? What is the impact of this area of science and the resulting technologies?

The work to create a synthetic cell will have a profound and positive impact on society in that it will enable a better understanding of the fundamentals of biology and of how life works. It will lead to new techniques and tools for advanced vaccine and pharmaceutical development, and will continue to enable the development of new biofuels and biochemicals. As well, these technologies could be used to produce clean water, new sources of food, textiles, human and veterinary drugs, bioremediation techniques, etc. More details on specific applications may be found below in the section "Beneficial Applications of Synthetic Genomics."

I believe, along with my teams at JCVI and the company Synthetic Genomics Inc (SGI), that this science has the potential to be a major wealth driver for societies. A recent report, "Synthetic Biology: Scope, Applications and Implications," from the Royal Academy of Engineering in the United Kingdom, states, "Synthetic biology has the potential to create another raft of major new industries, the development of which is likely to have profound implications for the future of the

UK, European and world economies."
http://www.raeng.org.uk/news/publications/list/reports/Synthetic_biology.pdf

What are the risks associated with synthetic organisms? Do the risks of these technologies overshadow the potential benefits?

As with any new area of science, medicine or technology, synthetic genomics has the potential to be used for great societal benefit (biofuels, vaccines and pharmaceuticals, clean water, bioremediation, etc), but it could also be used for negative purposes. So called dual use technologies need to be carefully discussed and reviewed both at the government level (Federal, state and local) both in the US and globally, as well as in accessible forums for bioethicists, educators, students, media and the public to learn about the science and understand these risks and benefits.

My teams at both the JCVI and at SGI have, as the leaders of this field, been driving these ethical and societal implications since the beginning of the research (for nearly 15 years). The policy team at JCVI has completed study on options for governance of this field and is currently engaged in a study of the societal issues this work raises. Many other countries are reviewing and discussing this area of science and as such numerous reports and reviews have also been conducted. More detail may be found in the section below, "Ethical and Societal Implications/Policy Discussions about Synthetic Biology".

Does this work have anything to do with humans/human research?

No. All synthetic genomics work to date, both at the JCVI and elsewhere, has focused on microorganisms. It is anticipated that given how little is known about human biology that no applications of this work will or should be attempted in humans. The way that this research will impact human lives is through the numerous applications such as new vaccines, pharmaceuticals, biofuels, etc.

What safeguards/controls are in place to protect against accidental environmental release?

This is an extremely important question for this research and as such has been a major focus for the researchers at JCVI and SGI. Building on the longstanding and successful history in molecular biology of millions of experiments engineering and using organisms such as *E. coli* to conduct research, JCVI and SGI researchers will be able to engineer synthetic bacterial cells so they cannot live outside of the lab or other production environments. This is done by, for example, ensuring that these organisms have built in dependencies for certain nutrients without which they cannot survive. They can also be engineered with so called "suicide genes" that kick in to prevent the organism from living outside of the lab or environment in which they were grown.

Has there been any review of this work by the US government, or by any other organizations?

The synthetic genomics research at JCVI has undergone review at the highest levels of the US government. Beginning in 2003 with the publication of the research at JCVI in constructing the synthetic virus phiX174 ("Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides." Smith et al, PNAS 2003 Dec 23;100(26):15440-5. Epub 2003 Dec 2.), and including the most recent research and publication on creating the first self-replicating synthetic bacterial cell, the work has been reviewed by White House offices including the Office of Homeland Security and Office of Science and Technology Policy, the National Science Advisory Board for Biosecurity (NSABB), the Department of Energy, the National Institutes of Health, and others. As well, the work has been reviewed by independent bioethics groups since 1997. Senior US government officials including those at the NIH were briefed and allowed to review our study prior to publication.

What, if any, types of legislation or regulation should be applied to this area of research?

We think that it is prudent, as is being proposed by the Department of Health and Human Services (HHS), to require DNA synthesis companies to screen synthesis requests against data on harmful agents. In 2004, JCVI's Policy team, along with the Center for Strategic & International Studies (CSIS) and the Massachusetts Institute of Technology (MIT) were funded by the Alfred P. Sloan Foundation to conduct a series of workshops and public sessions over a 20-month period to discuss the biosecurity and biosafety implications of synthetic genomics. Over the course of the study, the group explored the risks and benefits of the emerging technology, as well as possible safeguards to prevent abuse, such as bioterrorism. In October of 2007 the group published their findings in a report, outlining options for the field and its researchers moving forward.

More recently, in December of 2008, JCVI received funding, again from the Alfred P. Sloan Foundation, to examine ethical and societal concerns that are associated with the developing science of synthetic genomics. The ongoing research is intended to inform the scientific community as well as educate our policymakers and journalists so that they may engage in informed discussions on the topic.

What are the next steps for this research at JCVI?

The work to create the first self-replicating, synthetic bacterial cell was an important proof of concept. The team at JCVI has learned much from the nearly 15 years it has taken to get to this successful stage. From this proof of concept experiment the team is now ready to build more complex organisms with useful properties. For example, many researchers, including scientists at SGI, are already using available sequencing information to engineer cells that can produce energy, pharmaceuticals, and industrial compounds, and sequester carbon dioxide. The team at JCVI is already working on its ultimate objective, which has been to synthesize a minimal cell that has only the machinery necessary for independent life. Now that a cell can be synthesized

from a synthetic genome, it now becomes possible for the team to test for the functionality of every essential gene in the genome. We can delete non-essential DNA regions from the synthetic genome and repeat transplantation experiments until no more genes can be disrupted and the genome is as small as possible. This minimal bacterial cell will enable a greater understanding of the function of every gene in a cell and a new vision of cells as understandable machines comprised of biological parts of known function.

Is this research patented?

Over the course of the 15 years it has taken to construct the first self-replicating synthetic bacterial cell, the team at JCVI has had to develop new tools and technologies to enable this research. SGI has funded the work at JCVI in exchange for exclusive intellectual property rights. SGI has filed 13 patent family applications on the unique inventions of the JCVI team. SGI believes that intellectual property is important for this kind of research and application development, as it is one of the best means to ensure that this important area of basic science research will be translated into key commercial products and services for the benefit of society. SGI intends to provide licenses to its synthetic genomics patents.

CREATION OF A SYNTHETIC BACTERIAL CELL

The ability to sequence or "read" an organism's entire genome—the full repertoire of genes in that organism—has been possible for several decades and is now quite routine. Much can be learned about an organism by sequencing its genome. However, learning to write genetic code is crucial to truly understanding some of the most fundamental aspects of life. If scientists can write genetic code then it becomes possible to optimize certain functions in organisms that would be beneficial for society. With these ideas in mind, we set out to create a synthetic bacterial cell. The work has its roots in 1995 and 1999 publications on *Mycoplasma genitalium*, but the quest to develop the first synthetic bacterial cell began in earnest in 2003.

May 21, 2010 Science Publication

On May 21, 2010, the JCVI synthetic genomics team of nearly 25 researchers, led by me, Hamilton Smith, Clyde Hutchison, John Glass, and Dan Gibson, published results detailing the first cell constructed in the lab using only synthetic DNA. The work was published online in the journal *Science* and details the work to chemically synthesize the 1.08 million base pair genome of the bacterium *Mycoplasma mycoides*.

This and previous breakthrough work by JCVI researchers was funded by Synthetic Genomics Inc. The US Department of Energy also funded early work in this area, particularly the work to construct the synthetic phiX174 published in 2003.

Using previously published techniques and breakthroughs with the genetic system of yeast and of genome transplantation, the team put chemically synthesized pieces of the *M. mycoides* DNA into yeast which assembled the bacteria's genome. Then, the *M. mycoides* genome was transplanted into *Mycoplasma capricolum* and "booted up" to create a new synthetic version of *M. mycoides*.

Steps involved in building the synthetic *M. mycoides* are as follows:

1. First, the JCVI team designed 1,078 specific cassettes of DNA that were 1,080 base pairs long, with overlaps of 80 base pairs (bp) at their ends to aid in building the longer stretches of DNA. These were made according to JCVI's specifications by the DNA synthesis company, Blue Heron Biotechnology.

2. Then the team employed a three stage process using yeast to build the genome from these 1,078 cassettes. The first stage involves taking 10 cassettes of DNA at a time to build 10,000 bp long segments. In the second stage, these 10,000 bp segments are taken 10 at a time to produce eleven 100,000 bp long segments. Finally, all 11 segments are assembled into a complete synthetic genome as an extra chromosome in a yeast cell, by using yeast genetic systems.

3. The complete synthetic *M. mycoides* genome is then released from the yeast cell and transplanted into *M. capricolum* recipient cells that have had the gene for a restriction enzyme removed. Following incubation, viable *M. mycoides* cells are produced in which the only DNA present is the synthetic genome. These cells are controlled only by that synthetic genome.

Scientific Milestones on the Quest to Create the First Synthetic Bacterial Cell

1995: After sequencing the *M. genitalium* genome (published in 1995), my colleagues and I began work on the minimal genome project. This area of research, trying to understand the minimal genetic components necessary to sustain life, started with *M. genitalium* because it is the bacterium with the smallest genome known that can be grown in pure culture. This work was published in the journal *Science* in 1999.

2003: Drs. Venter, Smith, and Hutchison (along with JCVI's Cynthia Andrews-Pfannkoch) made the first significant strides in the development of a synthetic genome by assembling the 5,386 base pair genome of bacteriophage phiX 174. They did so using short, single strands of synthetically produced, commercially available DNA (known as oligonucleotides) and using an adaptation of polymerase chain reaction (PCR), known as polymerase cycle assembly (PCA), to build the phiX genome. The team developed methods that allowed the synthetic phiX to be produced in just 14 days. This work was published in the Proceedings of the National Academy of Sciences (PNAS).

2007: JCVI researchers led by Carole Lartigue, Ph.D., announced the results of work published in the journal Science, which outlined the methods and techniques used to change one bacterial species, *M. capricolum*, into another, *M. mycoides* , by replacing one organism's genome with the other's genome. Genome transplantation was the first essential enabling step in the field of synthetic genomics as it is a key mechanism by which chemically synthesized chromosomes can be activated into viable living cells.

January 2008: The second successful step in the JCVI team's effort to create a cell controlled by synthetic DNA was completed when Gibson et al. published in the journal Science, the synthetic *M. genitalium* genome.

December 2008: Gibson et al. published a paper in Proceedings of the National Academy of Sciences (PNAS) describing a significant advance in genome assembly in which the team was able to assemble in yeast the whole bacterial genome, *M. genitalium*, in one step from 25 fragments of DNA. The work was funded by the company Synthetic Genomics Inc. (SGI). At this point the team is still working to boot up the *M. genitalium* synthetic cell using all the knowledge gleaned from their previous work.

2009: JCVI researchers published results describing new methods in which the entire bacterial genome from *M. mycoides* was cloned in a yeast cell by adding yeast centromeric plasmid sequence to the bacterial chromosome and altered in yeast using yeast genetic systems. This altered bacterial chromosome was then isolated from yeast and transplanted into a related species of bacteria, *M. capricolum*, to create a new type of *M. mycoides* cell. This was the first time that genomes were transferred between branches of life—from a prokaryote to eukaryote and back to a prokaryote. The research was published by Lartigue et al. in Science.

SYNTHETIC GENOMICS AND SYNTHETIC BIOLOGY DEFINED

Synthetic genomics is a new capability that engages in the design and assembly of genes, chromosomes, and potentially entire multi-chromosome genomes. The basic units of construction are chemically synthesized oligonucleotides (called oligos). Oligos are short strings of DNA formed from the four nucleotide bases (i.e., A, C, G, and T).

Although the terms are sometimes used interchangeably, synthetic genomics differs from the more widely known synthetic biology in the scale of changes that can be made and in the kinds of experiments that it enables. Synthetic biology, by its community's view, is derived from engineering principles and is focused on the design and construction of biological parts (genes, pathways), devices (multiple parts), and systems (multiple devices). The chief aim of synthetic biology is to provide standardized sets of 'parts' that can be joined together in new ways in a living organism. Synthetic genomics technologies, on the other hand, provide the capability to build whole genomes and can examine how best to organize them.

While methods and tools for conducting synthetic biology have been available for many years, synthetic genomics is a completely new capability developed at JCVI. Our program in synthetic genomics has developed a set of techniques that are fundamental to engineering an organism in its entirety. Two key features of our synthetic genomics capabilities are: 1) rapid assembly of DNA molecules up to millions of base pairs in size (a million base pairs can code for ~1000 genes) and 2) "combinatorial" reconstruction of genomes, that is, novel genetic arrangements can be produced and assayed quickly. The application of rational engineering principles to the construction of combinatorial libraries (collections of pieces of DNA put together in different arrangements)—followed by high-throughput screens to select for optimal arrangements—ensures hundreds-to hundreds of thousands of competing designs can be examined in parallel.

One of the major advantages of synthetic genomics over classical biotechnology techniques—such as recombinant DNA—is that there is no need to have access to a physical supply of a particular DNA sequence. Sequence fragments are simply created de novo by chemical synthesis and assembled into entire chromosomes and organisms. This ability to synthesize (write) DNA and use it in the construction of new cells can catalyze a major change in what organisms can be engineered to do. Importantly, it will also increase our understanding of microbial life processes. Not only can new cells types be created but existing natural systems can be exhaustively probed to reveal the inner workings and properties displayed by living organisms.

BENEFICIAL APPLICATIONS OF SYNTHETIC GENOMICS

Synthetic genomics will make a unique or significant contribution as an enabling technology that is changing the nature of basic biological research; and as a powerful tool of applied biotechnology with the potential for developing new or improved applications for human health (including new pharmaceuticals and faster development of vaccines), biological sources of liquid transportation fuels, the manufacturing of other bio-based products, and environmental surveillance.

Synthetic genomics is today changing the nature of *basic molecular biological research.* As an enabling technology, DNA synthesis has already proved to be a significant time saver by shortening the time needed for experiments compared to time-consuming recombinant DNA techniques. As DNA synthesis becomes ever less expensive, researchers will be able to use synthetic genomics to rapidly change the DNA sequence of various genes or whole genomes, allowing them to understand basic cellular functions in a rigorous way. For example, various laboratories are beginning to use synthetic genomics (specifically, the combinatorial reconstruction of genomes) to understand the mechanisms of evolution at the molecular level, to define regulators of specific genes or gene pathways and to establish, at the molecular level, the minimal requirements for life. Without synthetic genomics, investigators can only manipulate one or at most a few genes in any given experiment, resulting in a relatively slow discovery

process.

These laboratory techniques can also be applied to beneficial products. Drugs, vaccines, and modified microbes for use in humans are all important targets of applied research using synthetic genomics. The capability to make subtle changes at the DNA sequence level may lead to more efficient research and production of *vaccines for human and animal health* and related *diagnostics*. Currently, scientists are working on ways to use synthetic genomics technologies for the mitigation of influenza epidemics with the eventual ability to generate vaccines more rapidly than they are currently being generated. These technologies could be applied to several steps in the vaccine development process, resulting in moderate to significant time savings compared to current methodologies. Additionally, the ability to assemble and mutate sequences rapidly could allow for the development of broadly protective vaccines against viruses that themselves are diverse and variable, such as the viral causative agents of severe acute respiratory syndrome (SARS) and hepatitis C.

The JCVI has recently been funded by the NIH to use our new synthetic DNA tools to build synthetic segments of every known flu virus so that we can rapidly build new vaccine candidates in less than 24 hours. We are also being funded to see if we can take sets of genes out of bacteria to design new antibiotic synthetic pathways to make chemical compounds that are currently too complex for chemists to make. With the extensive research already underway in this new field of synthetic biology, there will be thousands of new developments that we cannot imagine today.

DNA synthesis techniques have already been applied in research on new or improved drugs. For example, the antimalarial drug artemisinin is naturally produced in the plant Artemisia annua through a complex metabolic pathway that cannot feasibly be reconstructed in yeast using conventional biotechnological methods. Scientists have been able to synthesize an artemisinin precursor (which is then subject to chemical modification to make the final product) and are in the process of learning how to scale up this production to make the drug widely and relatively inexpensively available. This type of modification is likely to be applicable to a wide variety of drugs.

Synthetic genomics could also contribute to the search for *carbon-neutral energy sources*. A major application of synthetic genomics could be in overcoming biological barriers to cost-effective production of biofuels. There are several major initiatives in alternative or substitute fuel production research. One promising approach now is to engineer photosynthetic algae (either microalgae or blue-green algae) that are already relatively efficient at converting carbon dioxide into oils so that they carry out this process at a scale that is commercially viable.

While biofuels from algae may be the best current target for alternative fuels, *consolidated bioprocessing* (CBP) of cellulosic biomass to ethanol is a possible route as well, and may be preferable in some settings. Scientists are trying to engineer a single organism to include all the multiple steps needed to produce ethanol from cellulose. While the use of synthetic genomics to

produce all of the enzymes needed for CBP is not the only technique available, it is among the most promising. If successful, CBP might be able to produce ethanol at a cost competitive with gasoline.

Sometimes called "white biotechnology," *biobased manufacturing* is becoming a reality. Plants and microbes are being engineered to produce raw materials that can be used to manufacture products that today are typically petroleum based. The expectation is that biologically based manufacturing will lead to more environmentally friendly products and methods of production. For example, the environmental impacts of plastic manufacturing might be lessened through the judicious use of bioengineering of metabolic pathways using synthetic genomics as one tool.

Finally, synthetic genomics could be applied to constructing microbes or other organisms that would act as *detectors* of toxins, chemicals, or even other (pathogenic) microbes in routine or bioterrorism surveillance. This could aid international health organizations greatly in early detection of emerging diseases.

ETHICAL AND SOCIETAL IMPLICATIONS/POLICY DISCUSSIONS ABOUT SYNTHETIC BIOLOGY

At JCVI, we consider the ethical and societal implications of the work to be as important as the scientific research. We examined ethical concerns before beginning any actual experiments or research into constructing a minimal genome or the work to construct the first synthetic cell. Here is an outline of the important work that JCVI has undertaken since 1995.

1995-1999: *Mycoplasma genitalium* and the minimal genome project

Research on the minimal genome started in 1995 after the publication of the *Mycoplasma genitalium* genome at the legacy JCVI organization, The Institute for Genomic Research (TIGR). This organism has the smallest genome of a self-replicating organism, prompting my team and me to wonder if *M. genitalium* could be a platform to determine the minimal set of genes that could still sustain cellular life. This notion and the research plan to test it underwent a thorough ethical review by a panel of experts at the University of Pennsylvania (Cho et al., Science December 1999:Vol. 286. no. 5447, pp. 2087 – 2090). The panel's independent deliberations, published in Science along with the scientific minimal genome research, concluded that there were no strong ethical reasons that should prevent the team from continuing research in this field as long as they continued to engage in public discussions.

JCVI Work on phiX174 Synthesis: The first synthesis of a non-pathogenic virus

In 2003, before publishing the results in PNAS ("Generating a Synthetic Genome by Whole Genome Assembly: phi X174 Bacteriophage from Synthetic Oligonucleotides"), our team of scientists from JCVI contacted several Government agencies, including the US Department of

Energy (DOE), the White House Office of Science and Technology Policy (OSTP), and the National Institutes of Health, to discuss any potential repercussions of the findings. After a series of meetings (which also included Department of Homeland Security representatives) discussing the method presented in the paper, the findings were released at a press conference hosted by DOE in conjunction with the Secretary of Energy, Spencer Abraham.

JCVI Policy Team

Shortly after, in 2004, JCVI's Policy team along with the Center for Strategic & International Studies (CSIS) and the Massachusetts Institute of Technology (MIT) were funded by the Alfred P. Sloan Foundation to conduct a series of workshops and an invitational public session over a 20-month period to discuss the biosecurity and biosafety implications of synthetic genomics. Over the course of the study, the group explored the risks and benefits of the emerging technology, as well as possible safeguards to prevent abuse, such as bioterrorism. In October of 2007 the group published their findings in a report, outlining options for the field, its researchers, science administrators, and policymakers.

More recently in December of 2008, JCVI (in collaboration with social science researchers from Michigan State University and the Alberta Health Law Institute) received funding from the Alfred P. Sloan Foundation to examine ethical and societal concerns that are associated with the developing science of synthetic genomics. The ongoing research is intended to inform the scientific community as well as educate policymakers and journalists so that they may engage in informed discussions on the topic.

Ongoing Activities: Lectures, Media, Briefings for Congress and Executive Branch Officials

The JCVI team and I routinely give public lectures and presentations around the globe to both scientific and lay audiences, members of congress, schools, and other organizations. The team and I also conduct many interviews with global media (online, print, video, radio, etc.) about our work and the implications and applications.

Over the last three years the team has made several trips to Capitol Hill to brief more than 50 members of Congress. The most recent work published on the first synthetic bacterial cell published in *Science* has been reviewed by OSTP, Department of Homeland Security, the NSABB, etc. The team supports and has asked for continued review and discussion about their research.

SELECTED STUDIES OF THE SOCIETAL, ETHICAL, AND POLICY CONCERNS

To help the Committee in your deliberations, I have assembled a list of key studies of the societal, ethical, and policy concerns associated with synthetic genomics and synthetic biology.

Completed studies and reports from the United States

- Cho MK, Magnus D, Caplan AL, McGee D, and the Ethics of Genomics Group. 1999. Ethical Considerations in Synthesizing a Minimal Genome. Science 286: 2087-2090. http://www.sciencemag.org/cgi/content/short/286/5447/2087
  This was the earliest study of the societal and ethical implications of synthetic genomics. Funded by an unrestricted grant from The Institute for Genomic Research Foundation (TIGR), a legacy organization of today's JCVI. The study was performed in parallel with research to define a minimal bacterial genome.

- United States Department of Energy, Office of Science, Biological and Environmental Research Advisory Committee, 2004. Synthetic Genomes: Technologies and Impact. http://www.science.doe.gov/ober/berac/synbio.pdf
  Report of a DOE advisory group on the potential benefits and concerns associated with synthetic genomic technologies.

- National Science Advisory Board for Biosecurity (NSABB) 2006. Addressing Biosecurity Concerns Related to the Synthesis of Select Agents. http://oba.od.nih.gov/biosecurity/pdf/Final_NSABB_Report_on_Synthetic_Genomics.pdf
  The Subcommittee on Synthetic Genomics of the NSABB prepared this report on security issues related to the construction of select agents using synthetic genomics technologies.

- Garfinkel MS, Endy D, Epstein GL, and Friedman RM, Synthetic Genomics: Options for Governance, 2007. http://www.jcvi.org/cms/fileadmin/site/research/projects/synthetic-genomics-report/synthetic-genomics-report.pdf
  Report focuses on the biosecurity and biosafety concerns associated with synthetic genomics and presents and evaluates 17 policy options for consideration by policymakers. The two-year study, funded by the Alfred P. Sloan Foundation, was prepared by Michele Garfinkel and Robert Friedman, JCVI; Drew Endy, MIT; and Gerald Epstein, Center for Strategic & International Studies.

- National Academies/OECD/Royal Society, 2009. Opportunities and Challenges in the Emerging Field of Synthetic Biology: A Symposium http://sites.nationalacademies.org/PGA/stl/PGA_050738
  This two-day symposium, funded by the Sloan Foundation, NSF, and BIO brought together biologists, social scientists, and policy experts to educate each other and to explore possibilities for trans-Atlantic collaborations.

Ongoing US-based studies

- Synthetic Genomics: Scientists' Understanding of Society's Concerns, Society's Understanding of the Science and Scientists

http://www.sloan.org/assets/files/press/alfred_p_sloan_foundation_funds_new_synthetic_biology_initiative_to_examine_societal_issues.pdf
JCVI's current study on the societal implications of synthetic genomics, funded by the Alfred P. Sloan Foundation (2009-2010). Garfinkel and Friedman from JCVI, in conjunction with Lori P. Knowles, University of Alberta, Health Law Institute and Paul B. Thompson, Michigan State University, Department of Philosophy, are examining the sometimes differing views of society and scientists with respect to synthetic genomics. Also examines regulatory issues in the US and the EU.

- Synthetic Biology Project: Ensuring Benefits are realized through Responsible Development http://www.synbioproject.org/
  The Woodrow Wilson International Center for Scholars established this project as an initiative of the Foresight and Governance Program with a grant from the Alfred P. Sloan Foundation. The project aims to identify gaps in knowledge about risks, to understand public perceptions about the field, and to explore governance options to promote innovation while ensuring safety.

- Ethical Issues in Synthetic Biology: Non-Physical Moral Harms and Public Policy
  http://www.thehastingscenter.org/Research/Detail.aspx?id=1548
  Funded by the Alfred P. Sloan Foundation, this project aims to identify non-physical concerns about and potential consequences of synthetic biology, including how to incorporate these concerns into public policy discussions.

- Synthetic Biology Engineering Research Center (SynBERC)
  http://www.synberc.org/content/articles/human-practices
  SynBERC is a multi-institutional research group funded by National Science Foundation to explore a number of engineering issues in synthetic biology.

US Government actions

- 2009. Federal Register Notice: Department of Health and Human Services, National Institutes of Health, Office of Biotechnology Activities. Recombinant DNA Research: Proposed Actions Under the NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines).
  http://oba.od.nih.gov/oba/rac/ProposeRevisionsNIHGuidelines-March-4-2009.pdf
  Considers whether synthetic DNA is identical to recombinant DNA with respect to NIH Guidelines and thus whether language in the Guidelines needs to be changed. Public comments are currently under review.

- 2009. Federal Register Notice: Department of Health and Human Services, Office of the Secretary. Screening Framework Guidance for Synthetic Double-Stranded DNA Providers. http://www.gpo.gov/fdsys/pkg/FR-2009-11-27/pdf/E9-28328.pdf

Provides guidance to firms that supply synthetic DNA with respect to screening orders and customers for malicious intent. Public comments are currently under review.

Completed studies and reports from the United Kingdom and Europe

- De Vriend H, for the Rathenau Institute. 2006. Constructing Life: Early social reflections on the emerging field of synthetic biology.
  http://www.cisynbio.com/pdf/Constructing_Life_2006.pdf
  Early and rigorous description of the constellation of societal issues that may be raised by synthetic biology.

- International Association Synthetic Biology Code, 2009. The IASB Code of Conduct for Best Practices in Gene Synthesis. http://www.ia-sb.eu/tasks/sites/synthetic-biology/assets/File/pdf/iasb_code_of_conduct_final.pdf
  A suggested code of conduct for DNA synthesis firms, drafted by members of the IASB consortium. IASB is European-based; the process to draft this Code of Conduct included US firms.

- Synbiosafe (European Commission 6th Framework Program, Project on Synthetic Biology Safety and Ethical Aspects). http://www.synbiosafe.eu/
  Three major products, all edited/directed by M. Schmidt, Synbiosafe manager: a book (Synthetic Biology: The Technoscience and Its Societal Consequences), a documentary film (SYNBIOSAFE: Safety and Ethical Aspects of Synthetic Biology), and a special issue of Systems and Synthetic Biology (Societal Aspects of Synthetic Biology)

- UK Parliamentary Office of Science and Technology, 2008. POSTnote
  http://www.parliament.uk/documents/upload/postpn298.pdf
  This document discusses possible applications and risks of new synthetic biology, including policy options for governance and development of the field.

Ongoing studies in Europe, 7th Framework program

- SYBHEL (Synthetic Biology for Human Health: Ethical and Legal Issues)
  http://sybhel.org/
  This is one of just a few ethics and policy projects worldwide to focus solely on the impacts of synthetic biology technologies with respect to human health.

- Synth-Ethics (Ethical and Regulatory Issues Raised by Synthetic Biology)
  http://www.synthethics.eu/
  This is a general project focused on safety, security, and notions of life, looking both at Europe generally and within specific countries.

Synthetic Biology Periodic Meetings: SB 1.0, 2.0, 3.0, 4.0, x.0....

The synthetic biology community holds recurring international meetings that include ethicists and social scientists with general interests in the research. Each meeting has dedicated time to presentations on societal impacts and issues.

- SB 1.0, 2005. http://syntheticbiology.org/Synthetic_Biology_1.0.html
- SB 2.0, 2006. http://webcast.berkeley.edu/event_details.php?webcastid=15766
- SB 3.0, 2007. http://www.syntheticbiology3.ethz.ch/monday.htm
- SB 4.0, 2008. http://sb4.biobricks.org/agenda/sb4_agenda.pdf

I thank you for the opportunity to testify before you today. I welcome any questions that you may have.

ATTACHMENTS

1. Gibson et al., 2010. Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. Published Online May 20, 2010, Science DOI: 10.1126/science.1190719
2. Garfinkel MS, Endy D, Epstein GL, and Friedman RM, Synthetic Genomics: Options for Governance, 2007. Executive Summary
3. Company Overview: Synthetic Genomics, Inc
4. Press Release: Synthetic Genomics Inc and ExxonMobil Research and Engineering Company Sign Exclusive, Multi-Year Agreement to Develop Next Generation Biofuels Using Photosynthetic Algae

Sciencexpress Research Article

Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

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We report the design, synthesis and assembly of the 1.08-Mbp *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *Mycoplasma capricolum* recipient cell to create new *Mycoplasma mycoides* cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence, including "watermark" sequences and other designed gene deletions and polymorphisms, and mutations acquired during the building process. The new cells have expected phenotypic properties and are capable of continuous self-replication.

In 1977, Sanger and colleagues determined the complete genetic code of phage φX174 (*1*), the first DNA genome to be completely sequenced. Eighteen years later, in 1995, our team was able to read the first complete genetic code of a self-replicating bacterium, *Haemophilus influenzae* (*2*). Reading the genetic code of a wide range of species has increased exponentially from these early studies. Our ability to rapidly digitize genomic information has increased by more than eight orders of magnitude over the past 25 years (*3*). Efforts to understand all this new genomic information have spawned numerous new computational and experimental paradigms, yet our genomic knowledge remains very limited. No single cellular system has all of its genes understood in terms of their biological roles. Even in simple bacterial cells, do the chromosomes contain the entire genetic repertoire? If so, can a complete genetic system be reproduced by chemical synthesis starting with only the digitized DNA sequence contained in a computer?

Our interest in synthesis of large DNA molecules and chromosomes grew out of our efforts over the past 15 years to build a minimal cell that contains only essential genes. This work was inaugurated in 1995 when we sequenced the genome from *Mycoplasma genitalium*, a bacterium with the smallest complement of genes of any known organism capable of independent growth in the laboratory. More than 100 of the 485 protein-coding genes of *M. genitalium* are dispensable when disrupted one-at-a-time (*4*–*6*).

We developed a strategy for assembling viral sized pieces to produce large DNA molecules that enabled us to assemble a synthetic *M. genitalium* genome in four stages from chemically synthesized DNA cassettes averaging about 6 kb in size. This was accomplished through a combination of in vitro enzymatic methods and in vivo recombination in *Saccharomyces cerevisiae*. The whole synthetic genome (582,970 bp) was stably grown as a yeast centromeric plasmid (YCp) (*7*).

Several hurdles were overcome in transplanting and expressing a chemically synthesized chromosome in a recipient cell. We needed to improve methods for extracting intact chromosomes from yeast. We also needed to learn how to transplant these genomes into a recipient bacterial cell to establish a cell controlled only by a synthetic genome. Due to the fact that *M. genitalium* has an extremely slow growth rate, we turned to two faster growing mycoplasma species, *M. mycoides subspecies capri* (GM12) as donor, and *M. capricolum subspecies capricolum* (CK) as recipient.

To establish conditions and procedures for transplanting the synthetic genome out of yeast, we developed methods for cloning entire bacterial chromosomes as centromeric plasmids in yeast, including a native *M. mycoides* genome (*8*, *9*). However, initial attempts to extract the *M. mycoides* genome from yeast and transplant it into *M. capricolum* failed. We discovered that the donor and recipient mycoplasmas share a common restriction system. The donor genome was methylated in the native *M. mycoides* cells and was therefore protected against restriction during the transplantation from a native donor cell (*10*). However, the bacterial genomes grown in yeast are unmethylated and so are not protected from the single restriction system of the recipient cell. We were able to overcome this restriction

barrier by methylating the donor DNA with purified methylases or crude *M. mycoides* or *M. capricolum* extracts, or by simply disrupting the recipient cell's restriction system (*8*).

We now have combined all of our previously established procedures and report the synthesis, assembly, cloning, and successful transplantation of the 1.08-Mbp *M. mycoides* JCVI-syn1.0 genome, to create a new cell controlled by this synthetic genome.

Results

Synthetic genome design

Design of the *M. mycoides* JCVI-syn1.0 genome was based on the highly accurate finished genome sequences of two laboratory strains of *M. mycoides* subspecies *capri* GM12 (*8*, *9*) (*11*). One was the genome donor used by Lartigue et al. [GenBank accession CP001621] (*10*). The other was a strain created by transplantation of a genome that had been cloned and engineered in yeast, YCpMmyc1.1-*ΔtypeIIIres*, [GenBank accession CP001668] (*8*). This project was critically dependent on the accuracy of these sequences. Although we believe that both finished *M. mycoides* genome sequences are reliable, there are 95 sites at which they differ. We began to design the synthetic genome before both sequences were finished. Consequently, most of the cassettes were designed and synthesized based upon the CP001621 sequence (*11*). When it was finished, we chose to use the sequence of the genome successfully transplanted from yeast (CP001668) as our design reference (except that we kept the intact *typeIIIres* gene). All differences that appeared biologically significant between CP001668 and previously synthesized cassettes were corrected to match it exactly (*11*). Sequence differences between our synthetic cassettes and CP001668 that occurred at 19 sites appeared harmless, and so were not corrected. These provide 19 polymorphic differences between our synthetic genome (JCVI-syn1.0) and the natural (non-synthetic) genome (YCpMmyc1.1) that we have cloned in yeast and use as a standard for genome transplantation from yeast (*8*). To further differentiate between the synthetic genome and the natural one, four watermark sequences (fig. S1) were designed to replace one or more cassettes in regions experimentally demonstrated (watermarks 1 [1246 bp] and 2 [1081 bp]) or predicted (watermarks 3 [1109 bp] and 4 [1222 bp]) to not interfere with cell viability. These watermark sequences encode unique identifiers while limiting their translation into peptides. Table S1 lists the differences between the synthetic genome and this natural standard. Figure S2 shows a map of the *M. mycoides* JCVI-syn1.0 genome. Cassette and assembly intermediate boundaries, watermarks, deletions, insertions, and genes of the *M. mycoides* JCVI syn1.0 are shown in fig. S2, and the sequence of the transplanted mycoplasma clone sMmYCp235-1 has been submitted to GenBank (accession # CP002027).

pSynthetic genome assembly strategy

The designed cassettes were generally 1,080 bp with 80-bp overlaps to adjacent cassettes (*11*). They were all produced by assembly of chemically synthesized oligonucleotides by Blue Heron; Bothell, Washington. Each cassette was individually synthesized and sequence-verified by the manufacturer. To aid in the building process, DNA cassettes and assembly intermediates were designed to contain Not I restriction sites at their termini, and recombined in the presence of vector elements to allow for growth and selection in yeast (*7*) (*11*).

pA hierarchical strategy was designed to assemble the genome in 3 stages by transformation and homologous recombination in yeast from 1,078 one-kb cassettes (Fig. 1) (*12*, *13*).

*Assembly of 10-kb synthetic intermediates***.** In the first stage, cassettes and a vector were recombined in yeast and transferred to *E. coli* (*11*). Plasmid DNA was then isolated from individual *E. coli* clones and digested to screen for cells containing a vector with an assembled 10-kb insert. One successful 10-kb assembly is represented (Fig. 2a). In general, at least one 10-kb assembled fragment could be obtained by screening 10 yeast clones. However, the rate of success varied from 10-100%. All of the first-stage intermediates were sequenced. Nineteen out of 111 assemblies contained errors. Alternate clones were selected, sequence-verified, and moved on to the next assembly stage (*11*).

*Assembly of 100-kb synthetic intermediates***.** The pooled 10-kb assemblies and their respective cloning vectors were transformed into yeast as above to produce 100-kb assembly intermediates (*11*). Our results indicated that these products cannot be stably maintained in *E. coli* so recombined DNA had to be extracted from yeast. Multiplex PCR was performed on selected yeast clones (fig. S3 and table S2). Because every 10-kb assembly intermediate was represented by a primer pair in this analysis, the presence of all amplicons would suggest an assembled 100-kb intermediate. In general, 25% or more of the clones screened contained all of the amplicons expected for a complete assembly. One of these clones was selected for further screening. Circular plasmid DNA was extracted and sized on an agarose gel alongside a supercoiled marker. Successful second-stage assemblies with the vector sequence are approximately 105 kb in length (Fig. 2b). When all amplicons were produced following multiplex PCR, a second-stage assembly intermediate of the correct size was usually produced. In some cases, however, small deletions occurred. In other instances, multiple 10-kb fragments were assembled, which produced a larger second-stage assembly intermediate. Fortunately, these differences could easily be

detected on an agarose gel prior to complete genome assembly.

*Complete genome assembly***.** In preparation for the final stage of assembly, it was necessary to isolate microgram quantities of each of the 11 second-stage assemblies (*11*). As reported (*14*), circular plasmids the size of our second-stage assemblies could be isolated from yeast spheroplasts after an alkaline-lysis procedure. To further purify the 11 assembly intermediates, they were exonuclease-treated and passed through an anion-exchange column. A small fraction of the total plasmid DNA ($1/100^{th}$) was digested with Not I and analyzed by field-inversion gel electrophoresis (FIGE) (Fig. 2c). This method produced ~1 μg of each assembly per 400 ml yeast culture (~10^{11} cells).

The method above does not completely remove all of the linear yeast chromosomal DNA, which we found could significantly decrease the yeast transformation and assembly efficiency. To further enrich for the eleven circular assembly intermediates, ~200 ng samples of each assembly were pooled and mixed with molten agarose. As the agarose solidifies, the fibers thread through and topologically "trap" circular DNA (*15*). Untrapped linear DNA can then be electrophoresed out of the agarose plug, thus enriching for the trapped circular molecules. The eleven circular assembly intermediates were digested with Not I so that the inserts could be released. Subsequently, the fragments were extracted from the agarose plug, analyzed by FIGE (Fig. 2d), and transformed into yeast spheroplasts (*11*). In this third and final stage of assembly, an additional vector sequence was not required since the yeast cloning elements were already present in assembly 811-900.

To screen for a complete genome, multiplex PCR was carried out with 11 primer pairs, designed to span each of the eleven 100-kb assembly junctions (table S3). Of 48 colonies screened, DNA extracted from one clone (sMmYCp235) produced all 11 amplicons. PCR of the wild type (WT) positive control (YCpMmyc1.1) produced an indistinguishable set of 11 amplicons (Fig. 3a). To further demonstrate the complete assembly of a synthetic *M. mycoides* genome, intact DNA was isolated from yeast in agarose plugs and subjected to two restriction analyses; Asc I and BssH II (*11*). Because these restriction sites are present in three of the four watermark sequences, this choice of digestion produces restriction patterns that are distinct from the natural *M. mycoides* genome (Figs. 1 and 3b). The sMmYCp235 clone produced the restriction pattern expected for a completely assembled synthetic genome (Fig. 3c).

pSynthetic genome transplantation

Additional agarose plugs used in the gel analysis above (Fig. 3c) were also used in genome transplantation experiments (*11*). Intact synthetic *M. mycoides* genomes from the sMmYCp235 yeast clone were transplanted into restriction-minus *M. capricolum* recipient cells, as described (*8*). Results were scored by selecting for growth of blue colonies on SP4 medium containing tetracycline and X-gal at 37 °C. Genomes isolated from this yeast clone produced 5-15 tetracycline-resistant blue colonies per agarose plug. This was comparable to the YCpMmyc1.1 control. Recovery of colonies in all transplantation experiments was dependent on the presence of both *M. capricolum* recipient cells and an *M. mycoides* genome.

Semi-synthetic genome assembly and transplantation

To aid in testing the functionality of each 100-kb synthetic segment, semi-synthetic genomes were constructed and transplanted. By mixing natural pieces with synthetic ones, the successful construction of each synthetic 100-kb assembly could be verified without having to sequence these intermediates. We cloned 11 overlapping natural 100-kb assemblies in yeast by using a previously described method (*16*). In 11 parallel reactions, yeast cells were co-transformed with fragmented *M. mycoides* genomic DNA (YCpMmyc 1.1) that averaged ~100 kb in length and a PCR-amplified vector designed to overlap the ends of the 100-kb inserts. To maintain the appropriate overlaps so that natural and synthetic fragments could be recombined, the PCR-amplified vectors were produced via primers with the same 40-bp overlaps used to clone the 100-kb synthetic assemblies. The semi-synthetic genomes that were constructed contained between two and ten of the eleven 100-kb synthetic subassemblies (Table 1). The production of viable colonies produced after transplantation, ionfirmed that the synthetic fraction of each genome contained no lethal mutations. Only one of the 100-kb subassemblies, 811-900, was not viable.

Initially, an error-containing 811-820 clone was used to produce a synthetic genome that did not transplant. This was expected since the error was a single base pair deletion that creates a frameshift in *dnaA*, an essential gene for chromosomal replication. We were previously unaware of this mutation. By using a semi-synthetic genome construction strategy, we were able to pinpoint 811-900 as the source for failed synthetic transplantation experiments. Thus, we began to reassemble an error-free 811-900 assembly, which was used to produce the sMmYCp235 yeast strain. The *dnaA*-mutated genome only differs by one nucleotide from the synthetic genome in sMmYCp235. This genome served as a negative control in our transplantation experiments. The *dnaA* mutation was also repaired at the 811-900 level by genome engineering in yeast (*17*) . A repaired 811-900 assembly was used in a final stage assembly to produce a yeast clone with a repaired genome. This yeast clone is named sMmYCP142 and could be transplanted. A complete list of genomes that

have been assembled from 11 pieces and successfully transplanted is provided in Table 1.

Characterization of the synthetic transplants

To rapidly distinguish the synthetic transplants from *M. capricolum* or natural *M. mycoides*, two analyses were performed. First, four primer pairs that are specific to each of the four watermarks were designed such that they produce four amplicons in a single multiplex PCR reaction (table S4). All four amplicons were produced by transplants generated from sMmYCp235, but not YCpMmyc1.1 (Fig. 4a). Second, the gel analysis with Asc I and BssH II, described above (Fig. 3d), was performed. The restriction pattern obtained was consistent with a transplant produced from a synthetic *M. mycoides* genome (Fig. 4b).

A single transplant originating from the sMmYCp235 synthetic genome was sequenced. We refer to this strain as *M. mycoides* JCVI-syn1.0. The sequence matched the intended design with the exception of the known polymorphisms, 8 new single nucleotide polymorphisms, an *E. coli* transposon insertion, and an 85-bp duplication (table S1). The transposon insertion exactly matches the size and sequence of IS1, a transposon in *E. coli*. It is likely that IS1 infected the 10-kb sub-assembly following its transfer to *E. coli*. The IS1 insert is flanked by direct repeats of *M. mycoides* sequence suggesting that it was inserted by a transposition mechanism. The 85-bp duplication is a result of a non-homologous end joining event, which was not detected in our sequence analysis at the 10-kb stage. These two insertions disrupt two genes that are evidently non-essential. We did not find any sequences in the synthetic genome that could be identified as belonging to *M. capricolum*. This indicates that there was a complete replacement of the *M. capricolum* genome by our synthetic genome during the transplant process.

The cells with only the synthetic genome are self replicating and capable of logarithmic growth. Scanning and transmission electron micrographs (EM) of *M. mycoides* JCVI-syn1.0 cells show small, ovoid cells surrounded by cytoplasmic membranes (Fig. 5c-5f). Proteomic analysis of *M. mycoides* JCVI-syn1.0 and the WT control (YCpMmyc1.1) by two-dimensional gel electrophoresis revealed almost identical patterns of protein spots (fig. S4) and these were clearly different from those previously reported for *M. capricolum* (*10*). Fourteen genes are deleted or disrupted in the *M. mycoides* JCVI-syn1.0 genome, however the rate of appearance of colonies on agar plates and the colony morphology are similar (compare Fig. 5a and b). We did observe slight differences in the growth rates in a color changing unit assay, with the JCVI-syn1.0 transplants growing slightly faster than the MmcyYCp1.1 control strain (fig. S6).

Discussion

In 1995, the quality standard for sequencing was considered to be one error in 10,000 bp and the sequencing of a microbial genome required months. Today, the accuracy is substantially higher. Genome coverage of 30-50X is not unusual, and sequencing only requires a few days. However, obtaining an error-free genome that could be transplanted into a recipient cell to create a new cell controlled only by the synthetic genome was complicated and required many quality control steps. Our success was thwarted for many weeks by a single base pair deletion in the essential gene *dnaA*. One wrong base out of over one million in an essential gene rendered the genome inactive, while major genome insertions and deletions in non-essential parts of the genome had no observable impact on viability. The demonstration that our synthetic genome gives rise to transplants with the characteristics of *M. mycoides* cells implies that the DNA sequence upon which it is based is accurate enough to specify a living cell with the appropriate properties.

Our synthetic genomic approach stands in sharp contrast to a variety of other approaches to genome engineering that modify natural genomes by introducing multiple insertions, substitutions, or deletions (*18–22*). This work provides a proof of principle for producing cells based upon genome sequences designed in the computer. DNA sequencing of a cellular genome allows storage of the genetic instructions for life as a digital file. The synthetic genome described in this paper has only limited modifications from the naturally occurring *M. mycoides* genome. However, the approach we have developed should be applicable to the synthesis and transplantation of more novel genomes as genome design progresses (*23*).

We refer to such a cell controlled by a genome assembled from chemically synthesized pieces of DNA as a "synthetic cell", even though the cytoplasm of the recipient cell is not synthetic. Phenotypic effects of the recipient cytoplasm are diluted with protein turnover and as cells carrying only the transplanted genome replicate. Following transplantation and replication on a plate to form a colony (>30 divisions or $>10^9$ fold dilution), progeny will not contain any protein molecules that were present in the original recipient cell (*10*, *24*). This was previously demonstrated when we first described genome transplantation (*10*). The properties of the cells controlled by the assembled genome are expected to be the same as if the whole cell had been produced synthetically (the DNA software builds its own hardware).

The ability tp produce synthetic cells renders it it essential for researchers making synthetic DNA constructs and cells to clearly watermark their work to distinguish it from naturally occurring DNA and cells. We have watermarked the synthetic chromosome in this and our previous study (*7*).

If the methods described here can be generalized, design, synthesis, assembly, and transplantation of synthetic chromosomes will no longer be a barrier to the progress of synthetic biology. We expect that the cost of DNA synthesis will follow what has happened with DNA sequencing and continue to exponentially decrease. Lower synthesis costs combined with automation will enable broad applications for synthetic genomics.

We have been driving the ethical discussion concerning synthetic life from the earliest stages of this work (*25*, *26*). As synthetic genomic applications expand, we anticipate that this work will continue to raise philosophical issues that have broad societal and ethical implications. We encourage the continued discourse.

References and Notes

1. F. Sanger *et al.*, *Nature* **265**, 687 (Feb 24, 1977).
2. R. D. Fleischmann *et al.*, *Science* **269**, 496 (Jul 28, 1995).
3. J. C. Venter, *Nature* **464**, 676 (Apr 1).
4. C. A. Hutchison *et al.*, *Science* **286**, 2165 (Dec 10, 1999).
5. J. I. Glass *et al.*, *Proc Natl Acad Sci U S A* **103**, 425 (Jan 10, 2006).
6. H. O. Smith, J. I. Glass, C. A. Hutchison III, J. C. Venter, in *Accessing Uncultivated Microorganisms: From the Environment to Organisms and Genomes and Back* K. Zengler, Ed. (ASM Press, Washington, 2008), pp. 320.
7. D. G. Gibson *et al.*, *Science* **319**, 1215 (Feb 29, 2008).
8. C. Lartigue *et al.*, *Science* **325**, 1693 (Sep 25, 2009).
9. G. A. Benders *et al.*, *Nucleic Acids Res*, (Mar 7, 2010).
10. C. Lartigue *et al.*, *Science* **317**, 632 (Aug 3, 2007).
11. Supplementary information is available on *Science* Online.
12. D. G. Gibson, *Nucleic Acids Res* **37**, 6984 (Nov, 2009).
13. D. G. Gibson *et al.*, *Proc Natl Acad Sci U S A* **105**, 20404 (Dec 23, 2008).
14. R. J. Devenish, C. S. Newlon, *Gene* **18**, 277 (Jun, 1982).
15. W. W. Dean, B. M. Dancis, C. A. Thomas, Jr., *Anal Biochem* **56**, 417 (Dec, 1973).
16. S. H. Leem *et al.*, *Nucleic Acids Res* **31**, e29 (Mar 15, 2003).
17. V. N. Noskov, T. H. Segall-Shapiro, R. Y. Chuang, *Nucleic Acids Res* **38**, 2570 (May 1).
18. M. Itaya, K. Tsuge, M. Koizumi, K. Fujita, *Proc Natl Acad Sci U S A* **102**, 15971 (Nov 1, 2005).
19. M. Itaya, *FEBS Lett* **362**, 257 (Apr 10, 1995).
20. H. Mizoguchi, H. Mori, T. Fujio, *Biotechnol Appl Biochem* **46**, 157 (Mar, 2007).
21. J. Y. Chun *et al.*, *Nucleic Acids Res* **35**, e40 (2007).
22. H. H. Wang *et al.*, *Nature* **460**, 894 (Aug 13, 2009).
23. A. S. Khalil, J. J. Collins, *Nat Rev Genet* **11**, 367 (May).
24. A mycoplasma cell, with a cell mass of about 10^{-13} g, contains fewer than 10^6 molecules of protein. (If it contains 20% protein this is 2×10^{-14} g protein per cell. At a molecular weight of 120 Daltons per amino acid residue each cell contains $(2 \times 10^{-14})/120 = 1.7 \times 10^{-16}$ moles of peptide residues. This is $1.7 \times 10^{-16} \times 6 \times 10^{23} = 1 \times 10^8$ residues per cell. If the average size of a protein is 300 residues then a cell contains about 3×10^5 protein molecules.) After 20 cell divisions the number of progeny exceeds the total number of protein molecules present in the recipient cell. So, following transplantation and replication to form a colony on a plate, most cells will contain no protein molecules that were present in the original recipient cell.
25. M. K. Cho, D. Magnus, A. L. Caplan, D. McGee, *Science* **286**, 2087 (Dec 10, 1999).
26. M. S. Garfinkel, D. Endy, G. E. Epstein, R. M. Friedman. (2007).
27. D. G. Gibson *et al.*, *Nat Methods* **6**, 343 (May, 2009).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/science.1190719/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S7
References

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Fig. 1. The assembly of a synthetic *M. mycoides* genome in yeast. A synthetic *M. mycoides* genome was assembled from 1,078 overlapping DNA cassettes in three steps. In the first step, 1,080-bp cassettes (orange arrows), produced from overlapping synthetic oligonucleotides, were recombined in sets of 10 to produce one hundred nine ~10-kb assemblies (blue arrows). These were then recombined in sets of 10 to produce eleven ~100-kb assemblies (green arrows). In the final stage of assembly, these eleven fragments were recombined into the complete genome (red circle). With the

exception of 2 constructs that were enzymatically pieced together in vitro (*27*) (white arrows), assemblies were carried out by in vivo homologous recombination in yeast. Major variations from the natural genome are shown as yellow circles. These include 4 watermarked regions (WM1-WM4), a 4-kb region that was intentionally deleted (94D), and elements for growth in yeast and genome transplantation. In addition, there are 20 locations with nucleotide polymorphisms (asterisks). Coordinates of the genome are relative to the first nucleotide of the natural *M. mycoides* sequence. The designed sequence is 1,077,947 bp. The locations of the Asc I and BssH II restriction sites are shown. Cassettes 1 and 800-810 were unnecessary and removed from the assembly strategy (*11*). Cassette 2 overlaps cassette 1104 and cassette 799 overlaps cassette 811.

Fig. 2. Analysis of the assembly intermediates. (**a**) Not I and Sbf I double restriction digestion analysis of assembly 341-350 purified from *E. coli*. These restriction enzymes release the vector fragments (5.5 kb and 3.4 kb) from the 10-kb insert. Insert DNA was separated from the vector DNA on a 0.8% E-gel (Invitrogen). M indicates the 1-kb DNA ladder (New England Biolabs; NEB). (**b**) Analysis of assembly 501-600 purified from yeast. The 105-kb circles (100-kb insert plus 5-kb vector) were separated from the linear yeast chromosomal DNA on a 1% agarose gel by applying 4.5 V/cm for 3 hours. S indicates the BAC-Tracker supercoiled DNA ladder (Epicentre). (**c**) Not I restriction digestion analysis of the eleven ~100-kb assemblies purified from yeast. These DNA fragments were analyzed by FIGE on a 1% agarose gel. The expected insert size for each assembly is indicated. λ indicates the lambda ladder (NEB). (**d**) Analysis of the 11 pooled assemblies shown in (**c**) following topological trapping of the circular DNA and Not I digestion. One fortieth of the DNA used to transform yeast is represented.

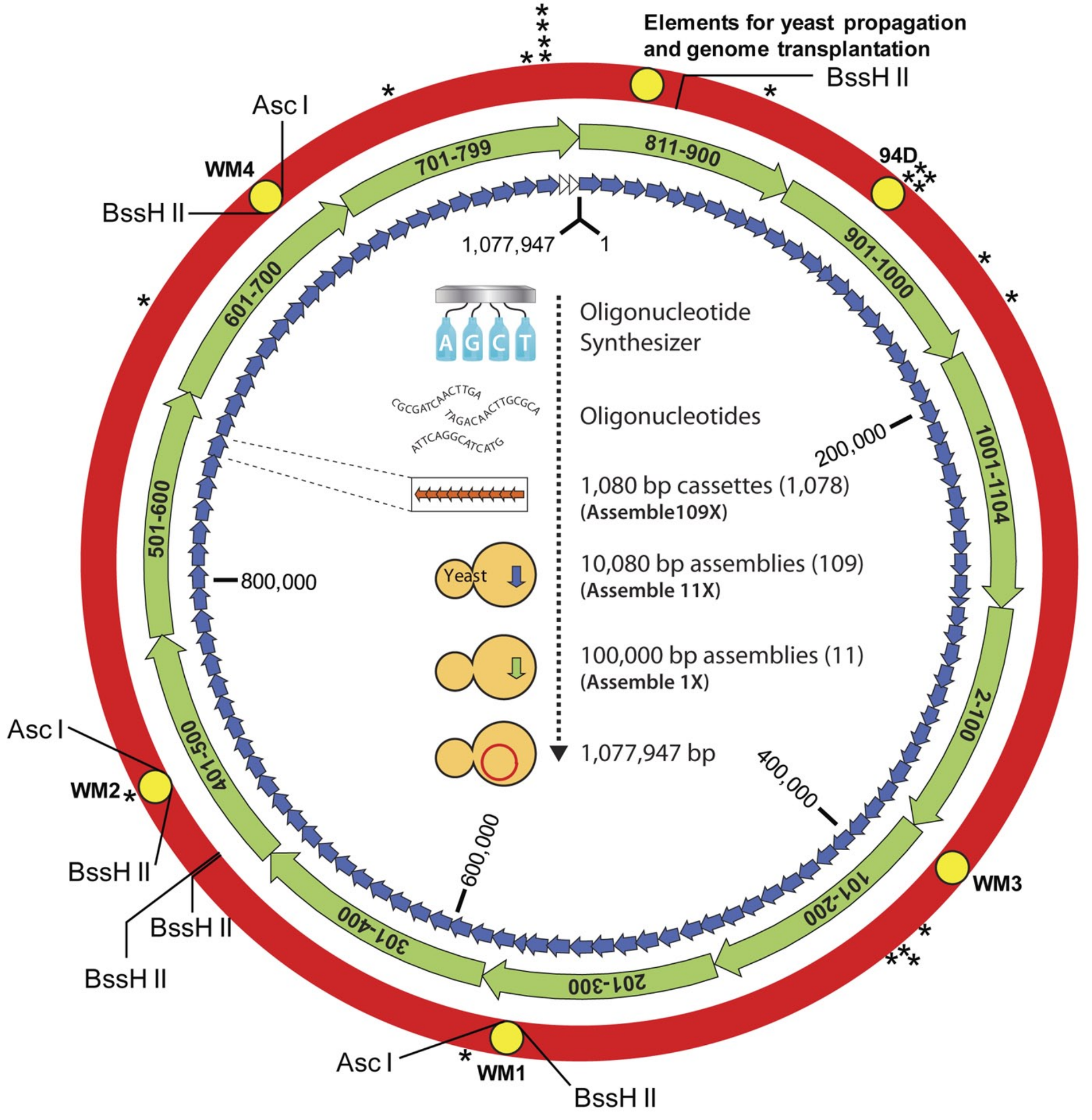
Fig. 3. Characterization of the synthetic genome isolated from yeast. (**a**) Yeast clones containing a completely assembled synthetic genome were screened by multiplex PCR with a primer set that produces 11 amplicons; one at each of the 11 assembly junctions. Yeast clone sMmYCp235 (235) produced the 11 PCR products expected for a complete genome assembly. For comparison, the natural genome extracted from yeast (WT) was also analyzed. PCR products were separated on a 2% E-gel (Invitrogen). L indicates the 100-bp ladder (NEB). (**b**) The sizes of the expected Asc I and BssH II restriction fragments for natural (WT) and synthetic (Syn235) *M. mycoides* genomes. (**c**) Natural (WT) and synthetic (235) *M. mycoides* genomes were isolated from yeast in agarose plugs. In addition, DNA was purified from the host strain alone (H). Agarose plugs were digested with Asc I or BssH II and fragments were separated by clamped homogeneous electrical field (CHEF) gel electrophoresis. Restriction fragments corresponding to the correct sizes are indicated by the fragment numbers shown in (**b**).

Fig. 4. Characterization of the transplants. (**a**) Transplants containing a synthetic genome were screened by multiplex PCR with a primer set that produces 4 amplicons; one internal to each of the four watermarks. One transplant (syn1.0) originating from yeast clone sMmYCp235 was analyzed alongside a natural, non-synthetic genome (WT) transplanted out of yeast. The transplant containing the synthetic genome produced the 4 PCR products whereas the WT genome did not produce any. PCR products were separated on a 2% E-gel (Invitrogen). (**b**) Natural (WT) and synthetic (syn1.0) *M. mycoides* genomes were isolated from *M. mycoides* transplants in agarose plugs. Agarose plugs were digested with Asc I or BssH II and fragments were separated by CHEF gel electrophoresis. Restriction fragments corresponding to the correct sizes are indicated by the fragment numbers shown in Fig. 3b.

Fig. 5. Images of *M. mycoides* JCVI-syn1.0 and WT *M. mycoides*. To compare the phenotype of the JCVI-syn1.0 and non-YCp WT strains, we examined colony morphology by plating cells on SP4 agar plates containing X-gal. Three days after plating, the JCVI-syn1.0 colonies are blue because the cells contain the *lacZ* gene and express beta-galactosidase, which converts the X-gal to a blue compound (**a**). The WT cells do not contain *lacZ* and remain white (**b**). Both cell types have the fried egg colony morphology characteristic of most mycoplasmas. EMs were made of the JCVI-syn1.0 isolate using two methods. (**c**) For scanning EM, samples were post-fixed in osmium tetroxide, dehydrated and critical point dried with CO_2, and visualized using a Hitachi SU6600 SEM at 2.0 keV. (**d**) Negatively stained transmission EMs of dividing cells using 1% uranyl acetate on pure carbon substrate visualized using JEOL 1200EX CTEM at 80 keV. To examine cell morphology, we compared uranyl acetate stained EMs of *M. mycoides* JCVI-syn1.0 cells (**e**) with EMs of WT cells made in 2006 that were stained with ammonium molybdate (**f**). Both cell types show the same ovoid morphology and general appearance. EMs were provided by Tom Deerinck and Mark Ellisman of the National Center for Microscopy and Imaging Research at the University of California at San Diego.

Table 1. Genomes that have been assembled from 11 pieces and successfully transplanted. Assembly 2-100 = 1, assembly 101-200 = 2, assembly 201-300 = 3, assembly 301-400 = 4, assembly 401-500 = 5, assembly 501-600 = 6, assembly 601-700 = 7, assembly 701-799 = 8, assembly 811-900 = 9, assembly 901-1000 = 10, assembly 1001-1104 = 11. WM indicates watermarked assembly.

Genome Assembly	**Synthetic Fragments**	**Natural Fragments**
Reconstituted natural genome	None	1-11
2/11 semi-synthetic genome with 1 watermark	5WM, 10	1-4, 6-9, 11
8/11 semi-synthetic genome without watermarks	1-4, 6-8, 11	5, 9, 10
9/11 semi-synthetic genome without watermarks	1-4, 6-8, 10-11	5, 9
9/11 semi-synthetic genome with 3 watermarks	1, 2WM, 3WM, 4, 6, 7WM, 8, 10-11	5, 9
10/11 semi-synthetic genome with 3 watermarks	1, 2WM, 3WM, 4, 5WM, 6, 7WM, 8, 10-11	9
11/11 synthetic genome, 811-820 correction of *dnaA*	1, 2WM, 3WM, 4, 5WM, 6, 7WM, 8, 9-11	None
11/11 synthetic genome, 811-900 correction of *dnaA*	1, 2WM, 3WM, 4, 5WM, 6, 7WM, 8, 9-11	None


Elements for yeast propagation
and genome transplantation
BssH II
Asc I
WM4
BssH II
94D
701-799
811-900
901-1000
1001-1104
1,077,947
1
601-700
501-600
200,000
800,000
Oligonucleotide
Synthesizer
A G C T
CGCGATCAACTTGA
TAGACAACTTGCGCA
ATTCAGGCATCATG
Oligonucleotides
1,080 bp cassettes (1,078)
(Assemble109X)
Yeast
10,080 bp assemblies (109)
(Assemble 11X)
100,000 bp assemblies (11)
(Assemble 1X)
1,077,947 bp
2-100
101-200
201-300
301-400
401-500
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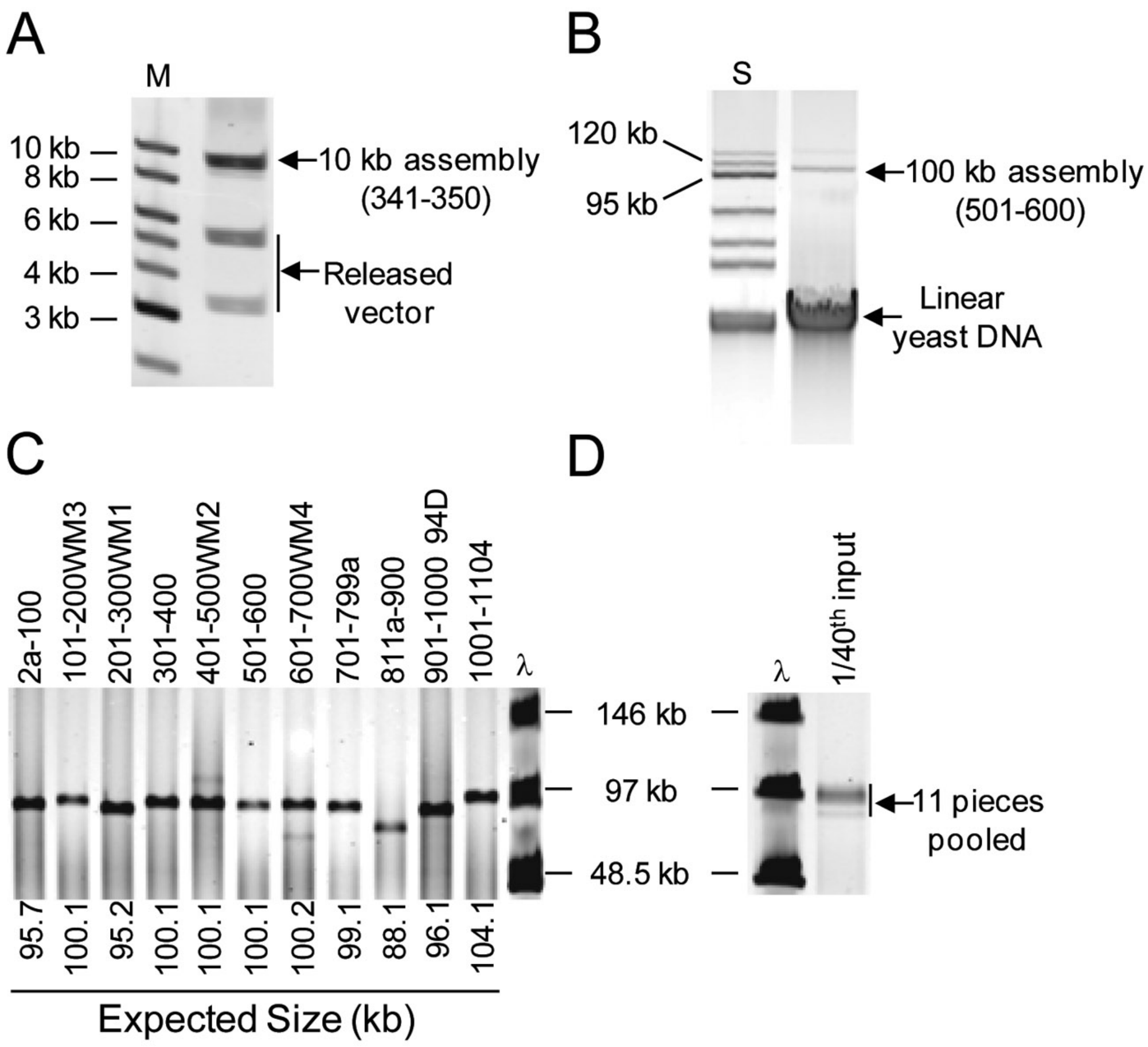
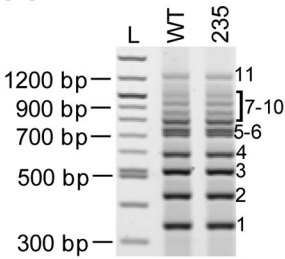
600,000
Asc I
WM2
BssH II
BssH II
BssH II
WM3
Asc I
WM1
BssH II


A
M
10 kb
8 kb
6 kb
4 kb
3 kb
10 kb assembly
(341-350)
Released
vector
B
S
120 kb
95 kb
100 kb assembly
(501-600)
Linear
yeast DNA
C
2a-100
101-200WM3
201-300WM1
301-400
401-500WM2
501-600
601-700WM4
701-799a
811a-900
901-1000 94D
1001-1104
λ
95.7
100.1
95.2
100.1
100.1
100.1
100.2
99.1
88.1
96.1
104.1
Expected Size (kb)
D
λ
1/40th input
146 kb
97 kb
48.5 kb
11 pieces
pooled

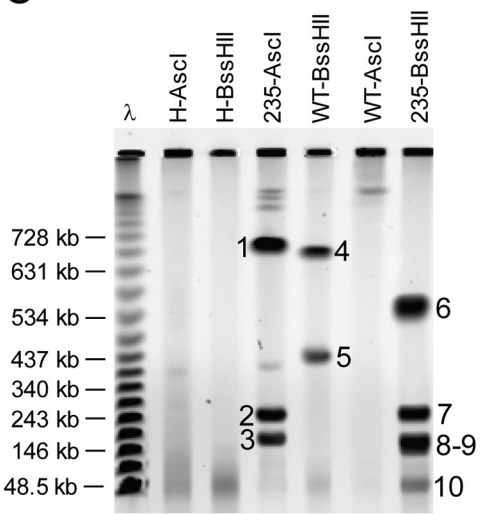
A

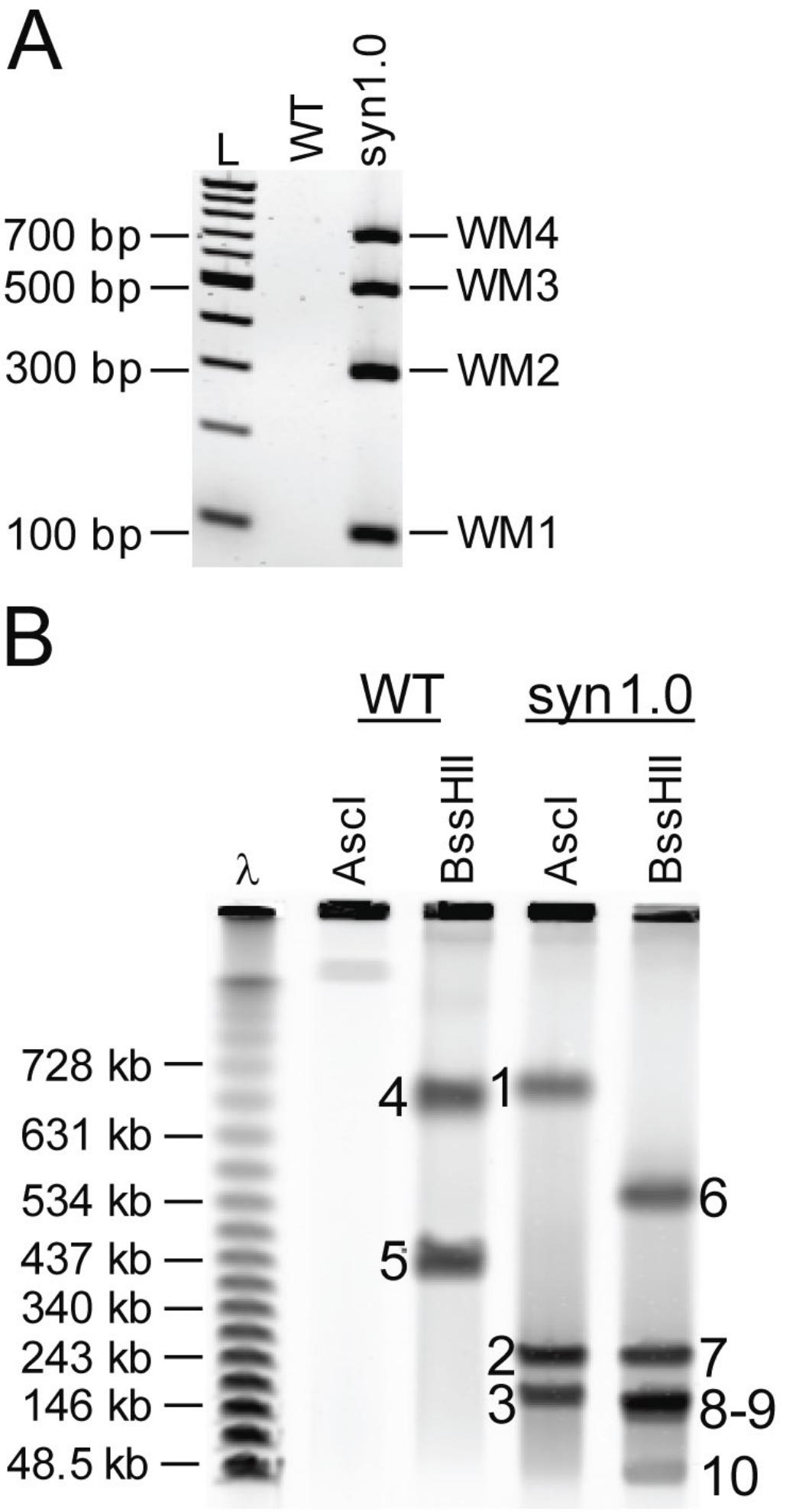


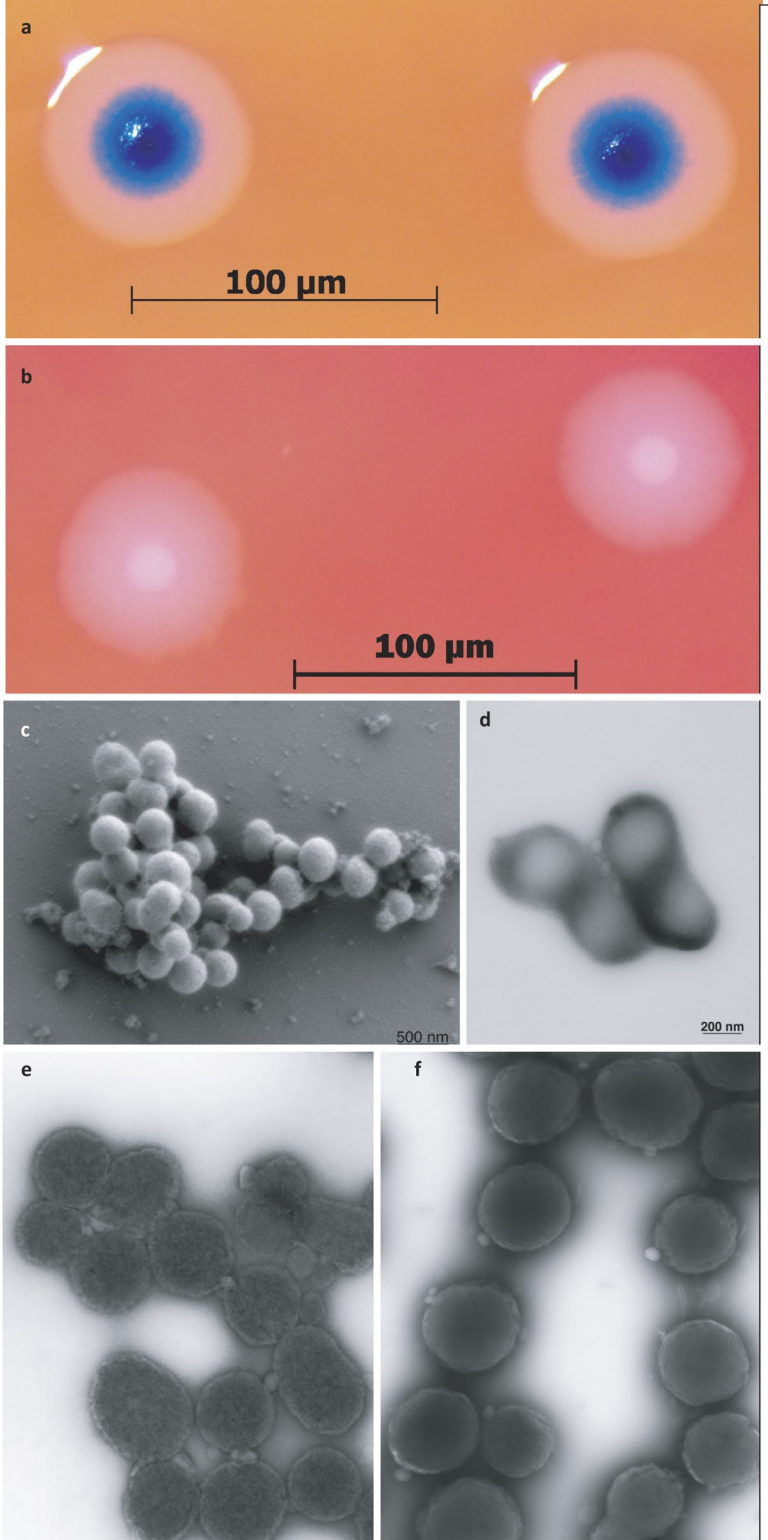
B

Strain	Digest	Fragment # and size (kb)
WT	AscI	No sites
WT	BssHII	(4) 668 (5) 419
Syn235	AscI	(1) 685 (2) 233 (3) 160
Syn235	BssHII	(6) 533 (7) 233 (8) 152 (9) 126 (10) 34

C




A
L
WT
syn1.0
700 bp
500 bp
300 bp
100 bp
WM4
WM3
WM2
WM1
B
WT
syn1.0
λ
AscI
BssHII
AscI
BssHII
728 kb
631 kb
534 kb
437 kb
340 kb
243 kb
146 kb
48.5 kb
4
5
1
2
3
6
7
8-9
10


a
100 µm
b
100 µm
c
500 nm
d
200 nm
e
f

SYNTHETIC GENOMICS | *Options for Governance*

Michele S. Garfinkel, Drew Endy, Gerald L. Epstein, and Robert M. Friedman

Gene and genome synthesis, that is, constructing long stretches of DNA from constituent chemicals, provides scientists with new and unparalleled capabilities both for understanding biology and for using it for beneficial purposes. But along with new capabilities come new risks.

Synthetic genomics combines methods for the chemical synthesis of DNA with computational techniques for its design, allowing scientists to construct genetic material that would be impossible or impractical using more conventional biotechnological approaches. The constructed DNA can then be used in a wide variety of applications that could potentially lead to improvements in human health, the environment, and basic research, among others.

The synthesis of relatively short stretches of DNA (called oligonucleotides) using specialized machines has been possible for nearly 25 years. Two advances have changed the landscape in the last five years or so. First, researchers have learned to speed up the process of stitching together small pieces of DNA into large, gene- or genome-sized pieces, so that the DNA of, for example, a medium-sized virus can be constructed in a matter of weeks. Second, there has been a proliferation of companies with proprietary technologies that are able to synthesize gene- and genome-length DNA at prices that are within reach of many researchers; these prices are rapidly dropping.

While at least some of these DNA sequences could be engineered in the laboratory using various recombinant DNA technologies, the efficiency with which arbitrary sequences of DNA can be synthesized vastly improves the speed and ease of conducting experiments and developing applications that were previously extremely difficult, or simply not possible. The ability to quickly construct or purchase whole genes and genomes has the potential to accelerate research in a variety of areas, from high-value pharmaceuticals to biofuels to power our cars; this capability may also make it possible to respond quickly to emerging threats, such as by developing and manufacturing vaccines during a pandemic. Improvements in the speed and cost of DNA synthesis are also opening the field to new participants (e.g., engineers seeking new tools) that may transform biotechnology.

However, as in the case of many technologies, synthetic genomics may be "dual-use:" in addition to useful advances for society, it may provide those with nefarious intent new ways to harm. Although dual-use concerns exist for almost all technologies, the power and accessibility of modern biotechnology—with synthetic genomics being a prime example—makes these concerns particularly salient. Examination of the risks and benefits of this technology today has become entwined with the events of September 11, 2001 and the subsequent anthrax attacks.

This report is the result of a 20-month examination, funded by the Alfred P. Sloan Foundation, of the safety and security concerns posed by this new technology. With a core group of 14 additional people with a wide range of expertise, we undertook three tasks: assess the current state of the technology, identify potential risks and benefits to society, and formulate options for its governance.

Summary Table of Options

Does the Option:	Gene Firms				Oligo Manufacturers				DNA Synthesizers			Users and Organizations					
	IA-1. Gene firms must screen orders	IA-2. Biosafety officers must certify people who place orders	IA-3. Hybrid: Firms must screen and biosafety officer must verify people	IA-4. Firms must store information about orders	IB-1. Oligonucleotide manufacturers must screen orders	IB-2. Biosafety officer must verify people who place orders	IB-3. Hybrid: Firms must screen and biosafety officer must verify people	IB-4. Firms must store information about orders	II-1. Owners of DNA synthesizers must register their machines	II-2. Owners of DNA synthesizers must be licensed	II-3. Licensing of equipment, plus license required to buy reagents and services	III-1. Education about risks and best practices in university curricula	III-2. Compile a manual for "Biosafety in Synthetic Biology" Laboratories	III-3. Establish a clearinghouse for best practices	III-4. Broaden IBC review responsibilities	III-5. Broaden IBC review, plus oversight by National Advisory	III-6. Broaden IBC review, plus enhanced enforcement
Enhance Biosecurity																	
by preventing incidents?	◉	○	●	○	○	○	◉	○	◌	○	○	○	–	–	○	○	○
by helping to respond?	–	–	–	○	–	–	–	○	◌	◌	◌	○	–	–	–	–	–
Foster Laboratory Safety																	
by preventing incidents?	○	–	○	–	◌	–	◌	–	–	–	–	◉	●	○	○	◉	◉
by helping to respond?	–	–	–	–	–	–	–	–	–	–	–	◉	–	–	–	○	–
Protect the Environment																	
by preventing incidents?	○	–	○	–	–	–	–	–	–	–	–	◉	●	○	○	◉	◉
by helping to respond?	–	–	–	○	–	–	–	○	–	–	–	○	◉	◉	○	–	–
Other Considerations:																	
Minimize costs and burdens to government and industry?	○	○	○	●	○	○	○	●	●	◉	○	◉	○	○	○	○	○
Perform to potential without additional research?	○	●	◉	○	○	●	○	○	●	●	◉	◉	○	○	○	○	◉
Not impede research?	◉	◉	○	●	○	○	○	●	◉	○	○	●	◉	●	○	◉	○
Promote constructive applications?	–	–	–	–	–	–	–	–	–	–	–	●	◌	○	○	–	–

Key to Scoring:

- ● Most effective for this goal. Most effective performance on this consideration.
- ◉ Relatively effective.
- ○ Moderately effective.
- ○ Somewhat effective.
- ◌ Minimally effective.

Reading the evaluation diagrams

These diagrams found throughout the report allow for easy comparisons within and between options regarding their effectiveness in achieving the policy goals of biosecurity and biosafety, and their performance on other considerations.

Reading down the columns allows for an evaluation of the performance of a particular option on one goal relative to the other goals. Reading across the rows allows for comparison of the effectiveness of each option with respect to the others on any given goal or consideration. Those that perform better are indicated with circles that have more dark fill; those that perform worse have less fill.

These comparisons are qualitative: they only indicate that one option performs better or worse than another, but not by how much.

We found no "magic bullets" for assuring that synthetic genomics is used only for constructive, positive applications. We did, however construct a series of policy interventions that could each incrementally reduce the risks from this emerging technology and, if implemented as a coordinated portfolio, could significantly reduce the risks.

We defined three major points for policy intervention:

- Commercial firms that sell synthetic DNA (oligonucleotides, genes, or genomes) to users.
- Owners of laboratory "bench-top" DNA synthesizers, with which users can produce their own DNA.
- The users (consumers) of synthetic DNA themselves and the institutions that support and oversee their work.

For each intervention point, we formulated a series of policy options. Each option was evaluated for its ability to reduce biosecurity and biosafety risks, the burden of implementation (in both resources and opportunity costs), and the degree of additional research that would be required for an option to be useful. We presented our preliminary options and analyses before a large group of subject matter experts and other stakeholders and solicited feedback that we used to revise and refine the options which are presented in their final form in this report.

The first set of options applies to firms that supply synthetic DNA, both those that supply gene-and genome-length strands of DNA and those that supply much shorter oligonucleotides. These options, treated in the report in parallel for gene-supplying firms and oligonucleotide-supplying firms are:

I-1. Require commercial firms to use approved software for screening orders.

I-2. People who order synthetic DNA from commercial firms must be verified as legitimate users by an Institutional Biosafety Officer or similar "responsible official."

I-3. Require commercial firms to use approved screening software *and* to ensure that people who place orders are verified as legitimate users by a Biosafety Officer.

I-4. Require commercial firms to store information about customers and their orders.

The second set of options is aimed at the oversight or regulation of DNA synthesizers and the reagents used in DNA synthesis.

II-1. Owners of DNA synthesizers must register their machines.

II-2. Owners of DNA synthesizers must be licensed.

II-3. A license is required both to own DNA synthesizers ***and*** to buy reagents and services.

Unlike the first two sets of options, which anticipate and are intended to help forestall the possibility that synthetic genomics may be misapplied by those with malicious intent, the final set of options is aimed exclusively at the legitimate users of the technology. These options cover both the education of potential users of synthetic DNA and the prior review of experiments that scientists and engineers might want to conduct:

III-1. Incorporate education about risks and best practices as part of university curricula.

III-2. Compile a manual for "biosafety in synthetic biology laboratories."

III-3. Establish a clearinghouse for best practices.

III-4. Broaden Institutional Biosafety Committee (IBC) review responsibilities to consider risky experiments.

III-5. Broaden IBC review responsibilities, *plus* add oversight from a national advisory group to evaluate risky experiments.

III-6. Broaden IBC review responsibilities, *plus* enhance enforcement of compliance with biosafety guidelines.

The report presents no recommendations. A summary table of our evaluation of the various options is presented below. The options are detailed in the text of this report. To help decisionmakers choose a preferred set of options, we also include several illustrative portfolios, ranging from a modest set of controls to one that is quite aggressive. When choosing a portfolio, each policy maker will draw on his or her own values, priorities, prior beliefs, and extent of risk aversion to security and safety threats. We believe that any of the options that we include, alone or more usefully in combination, can provide a meaningful response to the threat posed by this otherwise extremely promising technology.

J. Craig Venter™
INSTITUTE

The J. Craig Venter Institute (JCVI) is a not-for-profit research institute dedicated to the advancement of the science of genomics; the understanding of its implications for society; and communication of those results to the scientific community, the public, and policymakers. Founded by J. Craig Venter, Ph.D., the JCVI is home to approximately 400 scientists and staff with expertise in human and evolutionary biology, genetics, bioinformatics/informatics, information technology, high-throughput DNA sequencing, genomic and environmental policy research, and public education in science and science policy. JCVI was formed in 2006 through the merger of several affiliated and legacy organizations—The Institute for Genomic Research (TIGR) and The Center for the Advancement of Genomics (TCAG), The J. Craig Venter Science Foundation, The Joint Technology Center, and The Institute for Biological Energy Alternatives (IBEA). The JCVI is a 501 (c)(3) organization.

CSIS | CENTER FOR STRATEGIC & INTERNATIONAL STUDIES

The Center for Strategic and International Studies (CSIS) seeks to advance global security and prosperity in an era of economic and political transformation by providing strategic insights and practical policy solutions to decisionmakers. CSIS serves as a strategic planning partner for the government by conducting research and analysis and developing policy initiatives that look into the future and anticipate change. Founded in 1962 by David M. Abshire and Admiral Arleigh Burke, CSIS is a bipartisan, nonprofit organization headquartered in Washington, D.C. with more than 220 full-time staff and a large network of affiliated experts. Former U.S. senator Sam Nunn became chairman of the CSIS Board of Trustees in 1999, and John J. Hamre has led CSIS as its president and chief executive officer since April 2000.

Massachusetts Institute of Technology

The Massachusetts Institute of Technology's Department of Biological Engineering was founded in 1998 as a new MIT academic unit, with the mission of defining and establishing a new discipline fusing molecular life sciences with engineering. The goal of this biological engineering discipline is to advance fundamental understanding of how biological systems operate and to develop effective biology-based technologies for applications across a wide spectrum of societal needs including breakthroughs in diagnosis, treatment, and prevention of disease, in design of novel materials, devices, and processes, and in enhancing environmental health. The mission of MIT is to advance knowledge and educate students in science, technology, and other areas of scholarship that will best serve the nation and the world in the 21st century.

SYNTHETIC GENOMICS®

SGI Corporate Overview

Harnessing the power of genomics, solving global challenges

Synthetic Genomics, Inc. (SGI), a privately held company founded in 2005, is developing and commercializing genomic-driven advances to sustainably meet the global demand for critical resources, beginning with energy, chemicals and high value agricultural products. The company's science could be applied towards the production of a range of products, from synthetically derived vaccines to prevent human diseases to efficient cost effective ways to produce clean drinking water. SGI is currently working in the three broad projects areas of Next Generation Fuels and Chemicals (alliance with ExxonMobil Research and Engineering Company to develop algal biofuels), Microbial-Enhanced Hydrocarbon Recovery (collaboration with BP), and Sustainable Agricultural Products (collaboration with Asiatic Centre for Genome Technology). Specifically SGI is:

- Designing metabolic pathways for the production of next generation fuels and biochemicals from a variety of feedstocks, including carbon dioxide, plant biomass and coal
- Developing new biological solutions to increase the production and/or recovery rates of subsurface hydrocarbons
- Developing high-yielding, more disease resistant and economic plant feedstocks that are supplemented with efficient and environmentally friendly microbes to replace chemical fertilizers and confer disease and stress resistance

Scientific and Business Leadership

The scientific strength of SGI lies in the decades of pioneering scientific research by its world-renowned founders, J. Craig Venter, Ph.D., Nobel Laureate Hamilton O. Smith, M.D., and the stellar scientific and business teams they have assembled. The company's scientific teams include leading researchers in plant genomics, bioinformatics, genome engineering, molecular biology, biochemistry, climate change and energy policies. In addition to the strong in-house research efforts conducted at SGI, the company sponsors fundamental research at the J. Craig Venter Institute (JCVI), a not-for-profit organization with more than 400 scientists and staff working on a variety of genomic research and policy fronts.

Science of SGI

From rapidly discovering genes and developing advances to sequence whole genomes, to making innovations in synthesizing and constructing whole chromosomes and genomes, Drs. Venter, Smith and their teams are trailblazers in the use and development of these disruptive technologies. Their ability to read and then write the genetic code led to the development of the emerging field of synthetic genomics in which genes, synthetic chromosomes and even whole genomes can be designed, synthesized and assembled from the basic chemical components of DNA. SGI is using genes as the new design components of the future to develop custom-designed modular cassettes that encode entire microbial metabolic pathways for large-scale commercial applications, including the efficient conversion of carbon dioxide, plant biomass, and coal into next generation biofuels and chemicals.

SYNTHETIC GENOMICS®

Milestones

November 2003
JCVI scientists made the first significant strides in developing a synthetic genome by assembling the 5,386 base pair genome of bacteriophage Φ X174 (phi X).

2005
The major scientific breakthrough in synthesizing phi X was a proof of concept that gave the team assurance of the potential of this technology and encouragement to pursue this work in a commercial setting. SGI was then founded in the spring of 2005 by J. Craig Venter, Ph.D, Nobel Laureate Hamilton O. Smith, M.D., Juan Enriquez and David Kiernan, M.D., J.D.

June 2007
SGI and BP formed a collaboration to develop and commercialize microbial-enhanced solutions to increase the conversion and recovery of subsurface hydrocarbons.

JCVI researchers developed genome transplantation methods and techniques used to change one bacterial species, *Mycoplasma capricolum*, into another, *Mycoplasma mycoides*.

July 2007
SGI and the Asiatic Centre for Genome Technology formed a collaboration to develop more high-yielding and disease-resistant plant feedstocks. The partnership entails sequencing oil seed plants such as oil palm and Jatropha.

January 2008
The JCVI created the first synthetic bacterial genome, *Mycoplasma genitalium JCVI-1.0*, representing the largest man-made DNA structure.

May 2008
SGI and the Asiatic Centre for Genome Technology completed the first draft assembly and annotation of the oil palm genome. The organizations also announced making progress in sequencing and analyzing the jatropha genome.

December 2008
The JCVI team made a significant advance in genome assembly in which they created the synthetic M. genitalium genome from 25 overlapping fragments in a one-step assembly using recombination in yeast. The team is currently working on experiments to install a fully synthetic bacterial chromosome into a recipient cell and "boot up" this synthetic chromosome.

May 2009
Jatropha genome completed.

July 2009
SGI and ExxonMobil Research and Engineering Company established a multi-year research and development strategic alliance focused on exploring the most efficient and cost effective ways to produce next generation biofuels using photosynthetic algae.

Management

J. Craig Venter, Ph.D.
Board Chairman, Co-Founder, CEO

Hamilton O. Smith, M.D.
Co-Founder, Co-Chief Scientific Officer

Aristides A.N. Patrinos, Ph.D.
President

Joel McComb
Chief Operating Officer

Chuck McBride
Chief Financial Officer

Fernanda Gandara
Vice President, Business Development

Paul Roessler, Ph.D.
Vice President, Renewable Fuels & Chemicals

Toby Richardson, Ph.D.
Vice President, Bioinformatics

Thomas Ishoey
Vice President, Subsurface Hydrocarbons

Tina Jones
Vice President, Human Resources

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Clyde Hutchison, Ph.D.
Chairman, Scientific Advisory Board

Kenneth H. Nealson, Ph.D.
Member, Scientific Advisory Board

George Poste, Ph.D.
Member, Scientific Advisory Board

Board of Directors

Juan Enriquez
Co-Founder of SGI; Managing Director, Excel Medical Ventures

Steve Jurvetson
Managing Director of Draper Fisher Jurvetson

David Kiernan, M.D., J.D.
Co-Founder of SGI; Senior Litigation Partner at Williams & Connolly

Alfonso Romo
Chairman and CEO of Plenus

Barry Schuler
Chairman and CEO of Raydiance, Inc.; Managing Director of Draper Fisher Jurvetson Growth Fund

Hamilton O. Smith, M.D.
Co-Founder, Co-Chief Scientific Officer

J. Craig Venter, Ph.D.
Board Chairman, Co-Founder, CEO

Board Observers

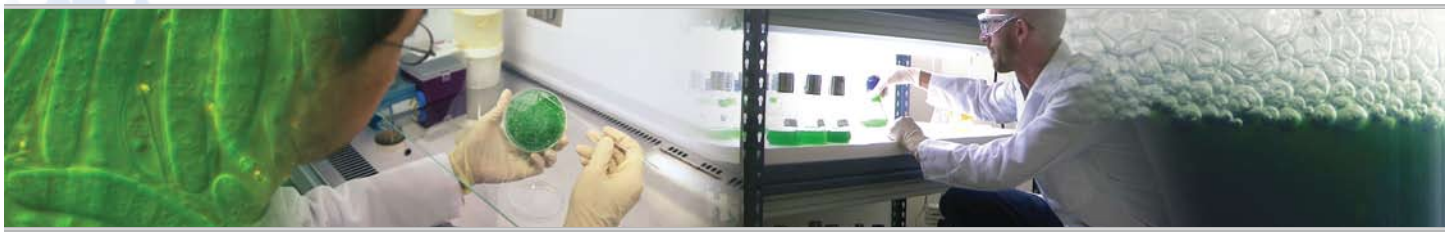
Justin Adams
Head of Venturing - Alternative Energy, BP plc.

Derrik Khoo Sin Huat
CEO of ACGT Sdn. Bhd.

Investors

The company's largest investors include: BP plc; Biotechonomy LLC; Draper Fisher Jurvetson; Plenus, S.A. de C.V.; ACGT Sdn Bhd; and Meteor Group

SYNTHETIC GENOMICS®



Press Release

Synthetic Genomics Inc and ExxonMobil Research and Engineering Company Sign Exclusive, Multi-Year Agreement to Develop Next Generation Biofuels Using Photosynthetic Algae

LA JOLLA, CALIFORNIA—July 14, 2009— Synthetic Genomics Inc. (SGI), a privately held company applying genomic-driven commercial solutions to address a variety of global challenges including energy and the environment, announced today a multi-year research and development agreement with ExxonMobil Research and Engineering Company (EMRE) to develop next generation biofuels using photosynthetic algae.

As part of the multi-faceted agreement, SGI will receive milestone payments for achievements in developing biofuel products. Total funding for SGI in research and development activities and milestone payments could amount to more than $300 million with the potential for additional income from licensing to third parties.

“This agreement between SGI and EMRE represents a comprehensive, long-term research and development exploration into the most efficient and cost effective organisms and methods to produce next generation algal biofuel,” said J. Craig Venter, Founder and CEO of SGI. “We are confident that the combination of our respective expertise in science, research, engineering and scale-up should unlock the power of algae as biological energy producers in methods and scale not previously explored.”

Photosynthetic algae, which include microalgae (single celled algae) and cyanobacteria (most commonly known as blue-green algae) are organisms that are very efficient at utilizing the energy from sunlight to convert carbon dioxide into cellular oils (lipids) and even some types of long-chain hydrocarbons that can be further processed into fuels and chemicals. However, naturally-occurring algae do not carry out this process at the efficiencies or rates necessary for commercial-scale production of biofuels.

Using SGI’s scientific expertise and proprietary tools and technologies in genomics, metagenomics, synthetic genomics, and genome engineering as a platform, SGI and EMRE believe that biology can now be harnessed to produce sufficient quantities of biofuels.

Under the terms of the agreement, SGI will work in a systematic approach to find, optimize, and/or engineer superior strains of algae, and to define and develop the best systems for large-scale cultivation of algae and conversion of their products into useful biofuels. ExxonMobil’s engineering and scientific expertise will be utilized throughout the program, from the development of systems to increase the scale of algae production through to the manufacturing of finished fuels.

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Scientists at SGI have been working internally for several years to develop more efficient means to harvest the oils that photosynthetic algae produce. Traditionally, algae have been treated like a crop to be grown and harvested in a process that can be expensive and time consuming. One of SGI's achievements has been in engineering algal strains that produce lipids in a continuous process that is currently more efficient and cost-effective.

"This investment is an important addition to ExxonMobil's ongoing efforts to advance breakthrough technologies to help meet the world's energy challenges," said Dr. Emil Jacobs, Vice President of Research and Development at ExxonMobil Research and Engineering Company. "Meeting the world's growing energy demands will require a multitude of technologies and energy sources. We believe that biofuel produced by algae could be a meaningful part of the solution in the future because of its potential to be an economically viable, low net carbon emission transportation fuel."

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About Synthetic Genomics Inc

SGI, a privately held company founded in 2005, is dedicated to developing and commercializing genomic-driven solutions to address global energy and environment challenges. Advances in synthetic genomics present limitless applications in a variety of product areas, including: energy, chemicals and pharmaceuticals. The company's main research and business programs are currently focused on the following major bioenergy areas: designing advanced biofuels with superior properties compared to ethanol and biodiesel; harnessing photosynthetic organisms to produce value added products directly from sunlight and carbon dioxide; developing new biological solutions to increase production and/or recovery rates of subsurface hydrocarbons and developing high-yielding, more disease resistant and economic feedstocks. For more information go to www.syntheticgenomics.com

About ExxonMobil

ExxonMobil, the largest publicly traded international oil and gas company, uses technology and innovation to help meet the world's growing energy needs. ExxonMobil holds an industry-leading inventory of resources, is the largest refiner and marketer of petroleum products, and its chemical company is one of the largest in the world. For more information, visit www.exxonmobil.com

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NOTE TO EDITORS:

Dr. Emil Jacobs, vice president of research and development at ExxonMobil Research and Engineering Company, and Dr. J. Craig Venter, founder and CEO of Synthetic Genomics Inc., will be available to answer questions from media on a conference call July 14, 2009 at 10 AM ET.

Dial in details are as follows:
Date/Time: July 14, 2009, 10:00 AM ET
Participant Number: 1-888-819-8002 (Toll free)
Participant Passcode: 3031406