

## Engineering a new business

Mike May

**As the market for DNA on demand continues to grow, increases in the scale and efficiency of new genome engineering approaches promise to accelerate product discovery and even open up new commercial opportunities.**

A congruence of innovation in the fields of microfluidics, miniaturization, automation and DNA synthesis, assembly and sequencing promises to provide new capabilities to companies focused on engineering innovative new products for pharmaceuticals, bioenergy, agriculture and beyond. At the same time, the nascent approaches underlying this technology still pose significant challenges in terms of reduction to practice, regulatory concerns and public perception.

Three broad classes of companies are emerging. First, many companies are making DNA parts for sale as reagents to academia and industry. The majority of these companies manufacture synthetic oligonucleotides (or oligos), but some are specializing in larger assemblies, even complete synthetic genes. In recent years, the synthetic oligo market has continued to grow. For example, one of the biggest synthetic oligo companies (Table 1), GenScript (Piscataway, NJ, USA), sold nearly twice the number of base pairs this year compared to last, according to Sally Wang, executive vice president. Demand is expected to increase for longer stretches of oligos with lower numbers of base errors. At the same time, the cost per DNA base is likely to keep dropping; as a result, synthetic oligos seem on their way to commoditization, and some companies are already selling oligos to fund other types of work.

A second group of companies is exploiting synthetic biology to advance processes that were previously performed with genetic engineering or metabolic engineering (Table 2). For example, an enzyme maker can now use computational approaches plus gene synthesis to design more effective compounds. So instead of arduously searching through thousands of enzymes to per-

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Building blocks. Synthetic biology means different things to different people, but designing new biological parts and systems for useful purposes captures the essence.

form a task, the company can zero in on the best ones faster. Even with today's synthetic biology abilities, however, those designer enzymes still require fine tuning through traditional wet-lab techniques.

A last group, including some multinational biotechs and pharmas are now exploring advanced biological engineering approaches in their own R&D work or to sell products that can be used by others in the field (Table 3). To gain the needed expertise, these large companies often develop collaborations with smaller, innovative biotechs that specialize in cutting-edge approaches.

Thus, the products of synthetic biology seem poised for broader application. But for commercialization to succeed, business models must be found that are sustainable (Box 1) and industry and academia must address tough sociological, dual-use—peaceful and military—and safety issues that surround dissemination of the technology (Box 2).

### What's new?

Synthetic biology is not so much a new field, as an evolving one. Previous capabilities in genetic

and metabolic engineering paved the way for synthetic biology. As John Mulligan, CSO at Blue Heron (Bothell, WA, USA), puts it, "Synthetic biology is used to cover a wide range of modern manipulative molecular biology experiments, making the definition a bit problematic." In his view, the goal of synthetic biology "is to develop molecular and computational tools that will allow biologists to design systems, implement them using standard parts, and achieve predictable results."

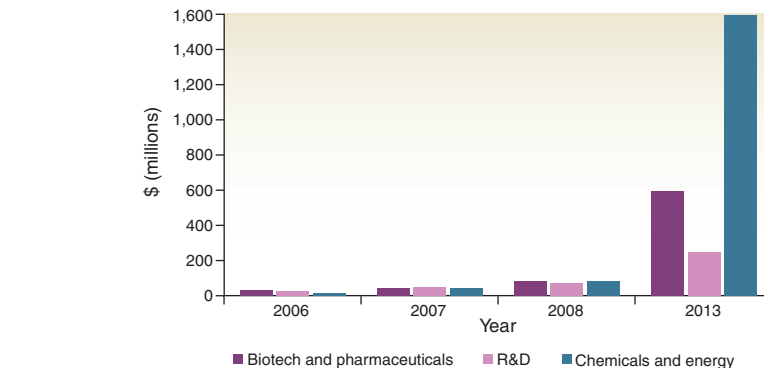
Some researchers expect synthetic biology to deliver a greater level of control over genomes as well as provide tools for carrying out genetic

manipulation at a scale and efficiency that is unprecedented. According to George Church, professor of genetics at Harvard Medical School (Boston), within a few years, synthetic biology is likely to provide the "ability to make essentially any genome and have it behave in a manner consistent with computer aided-design tools." This is not just about synthesizing a stretch of DNA, but about making it fully functional in a living cell. "We can already make about anything we want," Church says. The process, though, is not always efficient. So researchers need better algorithms to design sequences and better ways to make what they want.

As the applications of synthetic biology expand, so too will the overall market. A June 2009 BCC Research report<sup>1</sup> defines the field as "enabling technologies that are critical for synthetic biology (e.g., DNA synthesis or DNA sequencing); synthetic biological components (e.g., synthetic genes, synthetic functional DNA constructs and synthetic parts); integrated systems (e.g., synthetic chromosomes, genomes, cells and organisms); and products enabled by synthetic biology tools (e.g., pharmaceuticals, biofuels and chemicals)." Within that frame-

work, they find that this field as a whole created a \$233.8 million market in 2008 (Fig. 1). But that's just a start; they extrapolate that the market for synthetic biology components and enabled products will reach \$2.4 billion by 2013, which requires an annual increase of 59.8%. For now, chemicals and energy make up the leading market segment, accounting for \$80.6 million in 2008. Biotech and pharmaceuticals came in a close second at \$80.3 million, but this segment is expected to grow to \$594 million by 2013 (ref. 1).

If the synthetic biology market is to reach such levels by 2013, John Bergin, author of the BCC Research report, points out that several things are needed, including a continued decrease in the cost of synthesizing DNA. Bergin says that the increasing availability of gene sequencing creates more and larger electronic gene databases. This drives demand for protein-expression systems, directed evolution and metabolic engineering, which creates demand for synthetic biology technologies and tools. In short, Bergin expects synthetic DNA to form a foundation for the



**Figure 1** Actual and projected global market in synthetic biology. The projected figures are based on interviews with academic and industry leaders and government, industry and trade publications. Source: BCC Research.

future of this field. As Bergin notes, “Demand for synthetic genes is already robust and growing due to their utility in genomics.”

**Oligos to order**

Roughly a couple dozen companies around the world make synthetic oligos. Those companies,

however, range from ones that focus just on churning out custom oligos to others that make complete genes, as well as some companies that do a bit of both. Peer Staehler, president of febit synbio (Heidelberg, Germany), says that this market is worth \$75–150 million a year, and he adds that it is growing rapidly. The field is also

**Table 1** Selected oligo and synthetic gene suppliers

Company (year founded) Location Website	Employees	Oligo or gene size	Company description
Alpha DNA (1997) Montreal <a href="http://www.alphadna.com/">http://www.alphadna.com/</a>	5–10	<180 bases	Custom oligos, and mutagenesis services, plus catalog of reagents and kits, primers, and recombinant proteins
Ana-Gen (1994) Duluth, GA, USA <a href="http://www.ana-gen.com/">http://www.ana-gen.com/</a>	5–9	7–25 bp	Designs, synthesizes and purifies oligos needed for a complete gene and mutagenesis services, plus catalog of reagents and kits, primers, and recombinant proteins
ATG Biosynthetics (2001) Freiburg, Germany <a href="http://www.atg-biosynthetics.com/">http://www.atg-biosynthetics.com/</a>	6	<100 bases (n x10) kb pairs	Contract gene synthesis. Consultancy. ‘EvoMag’ program for codon optimization reverse genetic engineering and synthetic biology applications.
Biolegio (1996) Nijmegen, The Netherlands <a href="http://www.biolegio.com/">http://www.biolegio.com/</a>	20	Up to 210 bases	Specializes in long oligos and offers a broad range of modifications and dyes
BioNexus (1999) Oakland, CA, USA <a href="http://www.bionexus.net/">http://www.bionexus.net/</a>	25	Up to 140 bases	Gene synthesis and other genomics-related products and services, various modifications to synthesized DNA, fluorescent dyes for labeling DNA oligos, phosphorothioate and mixed base oligos
Biosearch Technologies (1993) Novato, CA, USA, <a href="http://www.biosearchtech.com/">http://www.biosearchtech.com/</a>	90	Up to 120 bases	Oligos for real-time qPCR and molecular diagnostics. Supplies the reagents and modifications needed to synthesize oligos. Engineers its own DNA synthesis instruments
Bioserve Biotechnologies (1990) Beltsville, MD, USA <a href="http://www.bioserve.com/">http://www.bioserve.com/</a>	68	Up to 125 bases	Genotyping, DNA and RNA extractions from tissues, maintains biorepository of normal and diseased tissues from 120,000 individuals from several countries
Biosynthesis (1984) North Dallas, TX, USA	70	Up to 2–3 kb pairs	Custom and PCR oligos, custom peptides, RNA, polyclonal antibodies, organic synthesis
Blue Heron (1999) (products distributed by Invitrogen, Carlsbad, CA, USA) <a href="http://www.blueheronbio.com/">http://www.blueheronbio.com/</a>	<50	Up to 52 kb pairs	Gene synthesis using proprietary GeneMaker technology generates synthetic structures that range in size from 60 to tens of thousands of base pairs
CyberGene (1995) Stockholm <a href="http://www.cybergene.se">http://www.cybergene.se</a>	4	Up to 80 bases	Manufactures quantitative PCR kits for prenatal diagnostics which are registered in the EU with CE mark such as ChromoQuant, for prenatal diagnosis
DNA2.0 (2003) <a href="https://www.dna20.com/">https://www.dna20.com/</a>	25–50	Up to 35 kb pairs	Gene synthesis, gene design assistance, expression optimization and protein engineering
Epoch Biolabs (2001) Sugar Land, TX, USA <a href="http://www.epochbiolabs.com/">http://www.epochbiolabs.com/</a>	35	5–100 bp/up to 50 kb pairs for genes	Gene synthesis and molecular services, variant library construction, protein expression and purification, DNA sequencing, SNP analysis

(continued)



**Table 1 Selected oligo and synthetic gene suppliers (continued)**

Company (year founded) Location Website	Employees	Oligo or gene size	Company description
Eurofins MWG Operon (1990) Ebersberg, Germany <a href="http://www.eurofinsdna.com/">http://www.eurofinsdna.com/</a>	250	5–20 bases up to 3 kb pairs	DNA sequencing, oligos, siRNA and gene synthesis
febit synbio (2005) <a href="http://www.febit.com/">http://www.febit.com/</a>	90	Up to 3.5 kb bases	Synthetic genes produced from oligos based on microarrays in a 60-mer format; developing a new production platform called “MegaCloner,” which will be used to offer building blocks that will be 40–400 bp in size
Geneart (2006) Regensburg, Germany <a href="http://www.geneart.com/">http://www.geneart.com/</a>	190	Up to ~20 kb pairs	DNA engineering and processing; produces optimized synthetic genes, generates gene variants and gene libraries, and produces DNA-based active agents
Gene Link (1993) <a href="http://www.genelink.com/">http://www.genelink.com/</a>	16	Up to 260 bases	Synthetic DNA, RNA, siRNA and antisense oligos; ultra-modified oligos with modifications in backbone, bases and fluorescent dyes
GeneScript (2002) Piscataway, NJ, USA <a href="http://www.genscript.com/">http://www.genscript.com/</a>	600	N.D.	Custom gene and oligo synthesis, bio-assays, Optimum gene proprietary codon optimization software
GeneWorks (1996) Adelaide, Australia <a href="http://www.geneworks.com.au">http://www.geneworks.com.au</a>	N.D.	5–100 bases	Custom oligos
Integrated DNA Technologies (1987) Coralville, IA, USA <a href="http://www.idtdna.com/">http://www.idtdna.com/</a>	500	20 bases	Custom oligos
The Midlands Certified Reagent Company (1974) Midland, TX, USA <a href="http://www.oligos.com/">http://www.oligos.com/</a>	13	DNA 3-180, RNA 3-65 locked nucleic acids (LNA) 3-165, genes in 40-mers, any number	DNA, RNA, peptide nucleic acid synthesis, 75 polymers. More than 300 modifications that are commercially available, homegrown or out-licensed modifications, all LNA oligos
Trilink Biotechnologies (1996) San Diego <a href="http://www.trilinkbiotech.com/">http://www.trilinkbiotech.com/</a>	85	Up to 180 bases	Modified nucleic acid, highly modified and mid-scale oligos, modified dNTPs

N.D., not disclosed. Source: websites and company press releases.

becoming more competitive. In general, all of the companies rely on the same basic chemistry for synthesizing oligos. In fact, Ali A. Javed, director of R&D at Gene Link (Hawthorne, NY, USA), says, “This market has matured so much that the innovation is reduced. Products are at a commodity level, a disposable-product level.” Consequently, companies in this market must find ways to distinguish themselves.

Although no commercial maker of synthetic oligos will say just how they do it, they all follow the same general process. A customer sends in a desired sequence, maybe just a number from GenBank or a computer-designed, completely novel stretch of nucleotides. The oligo maker then screens the DNA sequence against databases to identify sequences that might code for toxins or other problematic agents. In the United States, for example, the Centers for Disease Control and Prevention (Atlanta) and Animal and Plant Health Inspection Service (Riverdale, MD, USA) maintain the National Select Agents Registry, which lists dangerous toxins and biological agents that can be obtained only by registered users. So if an ordered sequence of DNA encodes a biological agent or toxin on this list, that could be made only for someone or a facility registered for that agent. In addition, there are some agreements among countries that attempt to prevent the misuse of this technol-

ogy. For example, the Australia Group (<http://www.australiagroup.net/en/index.html>)—an informal trade group that seeks to limit the proliferation of chemical and biological weapons—now includes guidelines about exporting oligos that code for toxins. The ultimate control for now, however, lies with the oligo makers, who try to determine an oligo’s legality or potential for danger.

Once the oligo maker decides to move forward with an order, the company turns to its own design process, which includes various elements—determining how to break a large sequence into pieces for manufacturing, and picking the methods to make and assemble the fragments—all aimed at optimizing the process in production and cost. As design turns to manufacturing, other processes must be added, including error removal. Chemical synthesis of oligos might produce sequences with error rates of 1 in 300 base pairs, but for some applications, such as for which the product must be nearly perfect with  $\geq 1$  error in 10 million base pairs, this would be unacceptably high. Most companies rely on software and purification techniques—typically all proprietary—to reduce the error rate of completed sequences.

Some companies are going beyond the usual methodology and developing newer, faster platforms for synthesizing oligos. In Germany,

febit synbio is developing a microarray-based process that will synthesize large numbers of oligos in parallel. Staehler says, “Many teams have failed to extract good DNA from microarrays, but we have teamed up with labs around the world and have shown—at least in proof of principle—that you can produce DNA at an incredible speed and complexity.” He adds that incorporating miniaturization and parallelism makes the difference.

For others, oligos are capital generators. DNA2.0 (Menlo Park, CA, USA) started out in 2003 using its computational power to engineer proteins, but it was unable to raise any venture capital. Instead, it started selling the custom genes made with the same technology that it was using to improve proteins. “We’ve watched our synthetic-gene market go up by tenfold in the past six years,” says Jeremy Minshull, DNA 2.0’s president. So even without any startup funding, this company turned a profit in its first 18 months of operation.

As more companies enter this field, each looks for ways to get an edge. For example, Mulligan of Blue Heron says, “We focus on a fully automated process. So we use protocols that are easier to do on robots.” The Blue Heron robots include off-the-shelf ones and a few that the company designed and built. This company’s technology also allows a wide range of oligo lengths. “We’ve

**Table 2 Selected companies with R&D that incorporates advanced engineering approaches**

Company (year founded) Location Website	Employees	Company description	Products	Funding source
Agrivida (2003) Cambridge, MA, USA <a href="http://www.agrivida.com/">http://www.agrivida.com/</a>	32	Agbiotech company developing crops to produce chemicals, fuels and bioproducts from non-food cellulosic biomass. Enables the delivery of low-cost sugars for the production of a wide variety of industrial biotech products	None	Series B funding in 2009, led by DAG Ventures
Amyris (2003) Emeryville, CA, USA <a href="http://www.amyris.com/">http://www.amyris.com/</a>	200	Renewable products company focused on the production and use of renewable chemicals and transportation fuels. Combines technology, production and distribution to commercialize and scale products across the supply chain through its Brazilian subsidiary, Amyris do Brasil Pesquisa e Desenvolvimento Biocombustíveis. Building distribution capabilities, through its US subsidiary Amyris Fuels	None	Private funding including venture capital
Biodesic (2005) Seattle <a href="http://www.biodesic.com/">http://www.biodesic.com/</a>	2	Provides technologies and knowledge to transform business and society through the development and distribution of biological technologies. Developing novel technologies, such as ProDNA, a system for protein measurement that is expected to be as sensitive and accurate as the existing methods for RNA and DNA	None	Bootstrapped and now internally funded through consulting
Biotica (1996) Cambridge, UK <a href="http://www.biotica.com/">http://www.biotica.com/</a>	23	Drug discovery and developer, using its polyketide engineering platform. Has a library of naturally occurring polyketides, which are optimizable using its technology platform	None	Venture capital plus collaboration license deals
Codexis (2002) <a href="http://www.codexis.com/">http://www.codexis.com/</a>	300	Clean technology company that develops industrial biocatalysts, including enzymes and microbes, for use in the energy industry to enable next generation, non-food biofuels and for cost-effective manufacturing of human therapeutics. Develops biocatalytic processes that can reduce manufacturing costs across a broad range of industries	Markets enzyme products and technology to pharmaceutical companies including Merck, Pfizer and Teva	Privately held with funding from corporate and venture investors
Ginkgo BioWorks (2008) Boston <a href="http://www.ginkgobioworks.com/">http://www.ginkgobioworks.com/</a>	6	Instrument and consulting company, focused on making biology easier to engineer. Commercializing a suite of proprietary DNA assembly technologies intended to simplify the rapid construction of metabolic pathways and gene networks	BioBrick Assembly Kit (co-developed with New England Biolabs), which includes the reagents needed to assemble BioBrick standard biological parts	Started with seed funding, including an SBIR grant, grant from the city of Boston, and now working off revenue and consulting fees
Genomatica (2000) <a href="http://www.genomatica.com/">http://www.genomatica.com/</a>	35–40	Chemical company that commercializes novel biomanufacturing processes to produce a variety of industrial chemicals for all major industries. Had a proprietary integrated bioprocess engineering platform and SimPheny, a metabolic modeling and simulation system	None	Privately held and backed by Mohr Davidow Ventures, Alloy Ventures and Draper Fisher Jurvetson
Global Bioenergies (2008) Evry, France <a href="http://www.global-bioenergies.com/">http://www.global-bioenergies.com/</a>	13	Renewable products company that transforms renewable resources into hydrocarbons, targeting fuels, plastics and rubbers, using classical or proprietary synthetic biology technologies	None	Venture capital
Metabolix (1992) <a href="http://www.metabolix.com/">http://www.metabolix.com/</a>	107	Bioscience company focused on providing sustainable solutions for manufacturing plastics, chemicals and energy, using a systems approach, from gene to end product, integrating biotech with advanced industrial practice. Has a proprietary platform technology for biobased, biodegradable plastics from corn for many market applications	Mirel Bioplastics	Publicly traded on the NASDAQ under MBLX
Synthetic Genomics (2005) La Jolla, CA, USA <a href="http://www.syntheticgenomics.com/">http://www.syntheticgenomics.com/</a>	~100	Synthetic biology company that develops and commercializes genomic-driven advances related to energy, chemicals and high-value agricultural products. Designing next generation fuels and biochemicals from carbon dioxide, plant biomass and coal, developing a biological solution to increase production or recovery of subsurface hydrocarbons, high yielding and disease resistant feedstocks	None	Privately held company that, in 2007, closed its Series B round of financing with BP and the Asiatic Centre for Genome Technology
Verdezyne (2005) <a href="http://www.verdezyne.com/">http://www.verdezyne.com/</a>	25	Industrial biotech company that uses a combinatorial approach to designing and engineering enzymes, metabolic pathways and microorganisms that produce target chemicals. Has a patented process for the design and synthesis of self-assembling genes directly from commercial oligos	None	Venture capital

Source: Company websites and press releases. SBIR, small business innovation research.

**Table 3 Selected large corporations exploring advanced engineering R&D approaches**

Name Location Website	General description	Selected synthetic biology projects
Bayer CropScience Monheim am Rhein, Germany <a href="http://www.bayercropscience.com/">http://www.bayercropscience.com/</a>	Crop science company focusing on crop protection, nonagricultural pest control, seeds and plant biotech. It has a global workforce of more than 18,000, and it is represented in more than 120 countries	Entered a technology alliance with Chromatin to apply mini-chromosome technology for crop improvement
ExxonMobil Irving, TX, USA <a href="http://www.exxonmobil.com/">http://www.exxonmobil.com/</a>	Largest publicly traded international oil and gas company	Entered a multi-year research and development agreement with Synthetic Genomics to develop next-generation biofuels using photosynthetic algae
Merck <a href="http://www.merck.com/">http://www.merck.com/</a>	This global research-driven pharmaceutical company was established in 1891, and it employs more than 55,000 people. Merck discovers, develops, manufactures and markets a wide range of vaccines and medicines	Formed an ongoing collaboration with Codexis to incorporate synthetic approaches to biocatalysis, which can be used in pharmaceutical basic research and manufacturing
Monsanto St. Louis <a href="http://www.monsanto.com/">http://www.monsanto.com/</a>	An agricultural company that focuses on the application of modern biology to seeds, especially ones with incorporated technology, such as pest resistance. This company also makes herbicides, including Roundup	Works with Protabit (Pasadena, CA, USA), which developed a computational-protein design platform. Through this collaboration, Monsanto hopes to develop new traits for crops
Pioneer Hi-Bred Johnston, IA, USA <a href="http://www.pioneer.com/">http://www.pioneer.com/</a>	This DuPont business develops advanced plant genetics to increase productivity, profitability and develop sustainable agricultural systems. Pioneer provides services to customers in nearly 70 countries	Collaborating with Arzeda, which can develop new enzymes <i>de novo</i> . Pioneer Hi-Bred plans to use these enzymes as starting points for its own technologies, including directed evolution
Syngenta Basel <a href="http://www.syngenta.com/">http://www.syngenta.com/</a>	In 2000, Novartis and AstraZeneca merged their agribusinesses to form Syngenta, which focuses on two main types of products: seeds and crop protection	Licensed mini-chromosome technology from Chromatin to improve the traits of corn, and is now working on sugarcane

Source: Company websites and press releases.

had orders as small as 60 base pairs," Mulligan says, "and our largest product was 52 kb, which took several levels of assembly." But Blue Heron could go even higher, at least to a couple hundred kilobases, the company claims.

Like any business, synthetic oligos must be economical to survive, let alone grow. Some biotechs and pharma—even academic labs—already outsource oligo synthesis as it gets more cost effective. However, many universities still maintain core facilities to serve their faculty's needs for oligos, although there are fewer such cores than there were a few years ago. According to Anthony Yeung, an officer with the Association of Biomolecular Resource Facilities (Bethesda, MD, USA), the number of core facilities offering oligo synthesis as a service today has dropped to roughly half the number that existed in 2005, whereas the number expressing an interest in DNA synthesis actually increased by 20%, "asserting the continued importance of the technology to core facilities," he says. The core facilities also report an increase in volume, which in some cases leads to outsourcing where volume and pricing are favorable. But in-house synthesis is still in demand when confidentiality and local expertise are needed.

In the commercial sector, BCC's Bergin sees the companies with more advanced technologies having the best prospects long term, although for simple oligos, price and delivery remain key. "Companies offering downstream products like synthetic genes or other biological parts and who have their own in-house quality oligos supply capability, or a strong oligos supply partner,

will be in the best position moving forward," he says. Some customers come to commercial makers to get more-complex jobs done quickly. For example, a customer might want to try a dozen substitutions at 50 positions in an antibody. In such cases, says Mulligan, "A commercial maker can be two to three times faster and at a fraction of the cost of doing it in your lab." He adds, "The business is growing because the prices are coming down." Those companies that can handle a variety of orders and reduce the oligo failure rate (through in-house production of high-quality oligos) will come to the forefront of this busi-

ness, Bergin predicts.

As the customer base grows, so do the capabilities of the industry. Blue Heron plans to expand its capacity by tenfold in the next 12–18 months. "And our staffing will stay about the same," says Mulligan, "as we add capacity with increased automation."

Despite the growth in commercial oligos, some in the field envision even more improvements in the future, especially in terms of length. "We're still dependent on relatively expensive synthesis," says genomics innovator J. Craig Venter. Less-expensive synthesis along with

### Box 1 Flash in the business plan?

In March, startup Codon Devices (Cambridge, MA, USA)—the company that blazed the commercial trail for synthetic biology and whose scientific advisory board read like a *Who's Who* for the field—announced it was closing its doors, just five years after its founding. In fact, the diversity of interests and approaches embodied in the founders—George Church of Harvard Medical School, bioengineer Drew Endy now at Stanford University, physicist Joseph Jacobson of the Media Lab at the Massachusetts Institute of Technology and chemical engineer Jay Keasling at the University of California, Berkeley—may have been part of the problem. At the time of the company's closing, experts and analysts pointed to the difficulty of trying to do too many things at once as the likely culprit. Moreover, the leaders at Codon Devices seemingly reached a similar conclusion. Less than a year before going out of business, Codon Devices abandoned its synthetic-oligo side to concentrate on developing applications. At that time, the change in business strategy and a \$31 million infusion of funds from its investors, which included Khosla Ventures (Menlo Park, CA, USA) and Alloy Ventures (Palo Alto, CA, USA), looked sufficient to keep Codon Devices afloat. But just one year later, the board closed it down. After the closing, Church told *Nature*<sup>15</sup> that the company should have stuck with applications and forgone synthetic oligos. So like any other fledging field of research, even a stellar conjunction of capital and science is no guarantee of commercial success.

## Box 2 Can there be safety in synthesis?

As companies succeed in making synthetic oligos in the 50-kb range, they reach the size of many viruses listed on the US National Select Agents Registry, which regulates the use of toxins and biological agents. Once companies can readily make synthetic oligos in the 200-kb range, that will cover every virus on that list. “As we venture into assembling whole bacterial genomes,” says Blue Heron’s CSO John Mulligan, “the concerns grow over the possibility that this technology will allow access to pathogens that wouldn’t otherwise be available to people with malicious intent.”

Synthetic-gene companies are working together to standardize a process for screening potentially dangerous agents. For example, febit synbio and several other companies joined forces as the International Association of Synthetic Biology. “We wanted to start working on a framework for governments and regulatory groups—something that shows what to do and what not to do,” says febit synbio’s Staehler. This group alerts companies about potential risks and distributes information about best practices for screening synthetic oligos. Staehler says, “We are starting to interact with the FBI in the US and several government authorities in Germany.” Some believe that self-regulation is sufficient. For instance, Paula Olsiewski, program director at the Alfred P. Sloan Foundation (New York), says, “I applaud the industry for the good work they are doing.”

But the difficulty comes in identifying every potentially dangerous sequence. That would require an inclusive, constantly updated list. Another problem is that one can create a dangerous agent starting with a set of short oligos, ordered from different companies, according to John Dileo, lead scientist at the MITRE Corporation (Bedford, MA, USA), a not-for-profit technology company that

supports the US government. To make it harder to accomplish such a task, Dileo and James Diggans, group leader for computational biology at MITRE, developed the DNA order tracking system (DOTS). This software would gather oligo orders from companies to see if any sequences could be combined to make something illegal or dangerous. “Long genes can be screened relatively easily,” says Diggans. “The harder part comes with short oligos.”

So far, DOTS works in simulated runs at MITRE. To work in the real world, though, all synthetic-oligo companies would have to submit each order they receive to a general database. But Diggans says, “There is a lot of concern about the centralization of orders, because of confidentiality with customers.” As a next step, MITRE will try out their software in field tests.

Safety concerns are universal. Bärbel Friedrich, a microbiologist at Humboldt University (Berlin), and her colleagues from several other German organizations developed a position paper about the opportunities and risks behind synthetic biology ([http://www.dfg.de/aktuelles\\_presse/reden\\_stellungnahmen/2009/download/stellungnahme\\_synthetische\\_biologie.pdf](http://www.dfg.de/aktuelles_presse/reden_stellungnahmen/2009/download/stellungnahme_synthetische_biologie.pdf)). In this work, Friedrich distinguishes biosafety from biosecurity issues. She believes that existing regulations handle much of the biosafety concerns, but due to the rapid advancement in the field, there needs to be a monitoring system. “We also need research on the impact of artificial cells, novel biomaterials and so on,” she says. For biosecurity, she advocates that the synthesis of DNA sequences be kept safe by using a general database for identifying dangerous sequences and following a standardized commercial procedure. Enforcing such regulations, however, may not be so easy. “How to do this worldwide is a problem,” she says.

other technological advances, however, will spawn the use of even longer sequences. That, too, brings new challenges. For example, as DNA gets longer, it gets more brittle. So scientists must develop ways to handle these longer stretches of bases.

In addition, future technology could do a better job even with shorter oligos. As Javed of Gene Link says, “We can endlessly design an oligo to perform better.” In addition to adjusting the codons for a particular amino acid, he’d like to see more nucleotides to consider. He says, “There should be an arsenal of modifications—like 16 bases instead of just 4—for customers to choose from, and it should not be inhibitory because it is so expensive.”

### Chromosomes on demand?

Although synthesizing oligos and genes is familiar territory for biotech, a radical new goal, pioneered by J. Craig Venter, Hamilton Smith and Clyde Hutchison and their colleagues at the J. Craig Venter Institute (Rockville, MD, USA), is to synthesize an entire chromosome from scratch and then reboot it in a recipient cell chassis. The idea is that in the context of an artificial, controlled environment, a ‘chassis’ organism with a minimal genome would be

capable of devoting many more resources to a synthetic product pathway of interest, enabling higher yields.

Several steps in this project have already been attained. In 2003, the Venter team assembled their first complete genome—that of the bacteriophage  $\phi$ X174—by stitching together short oligos using an adaptation of PCR<sup>2</sup>. Four years later, they provided the first demonstration of genome transplantation using native donor DNA from *Mycoplasma mycoides* to reprogram a related species *Mycoplasma capricolum*<sup>3</sup>. The researchers have since successfully cloned a complete synthetic bacterial (*Mycoplasma genitalium*) chromosome in a yeast cell (*Saccharomyces cerevisiae*)<sup>4</sup>. In their latest work, after cloning a native *M. mycoides* genome in yeast, through the addition of a yeast centromere to the bacterial genome, the researchers showed that treatment of donor DNA with specific methylase from the donor bacterium allows successful transplantation back into a different bacterium (*Mycoplasma capricolum*), whose genome had been removed<sup>5</sup>. This work thus moved a genome from a prokaryote to a eukaryote and back. When asked how significant this feat is, Venter says, “It depends in part on how extendable it is to other types of bacteria.”

Then he adds, “We have no reason to believe that it won’t be.” Nonetheless, Venter thinks that the yeast could create a roadblock. As he says, “There may be a limit of what can go in yeast, but we don’t know the limit.”

Steps toward using larger collections of DNA, however, are already underway. In 2007, James Birchler and his colleagues at the University of Missouri (Columbia, MO, USA) described a method for making maize mini-chromosomes—a centromere with telomeres—to which they added genes (Fig. 2) (ref. 6). As Birchler explains, “We start with an endogenous centromere, and then we can add onto it whatever we want.” What can be added, however, is limited by the amount of DNA—about 35–40 kb—that can be injected into a maize cell in one transformation. Birchler hopes to soon be able to repeatedly add pieces of DNA into a cell, one mini-chromosome at a time, thereby allowing the incorporation of more genes. Birchler adds, “Depending on the nature of what is added and the purpose, one could use endogenous promoters or engineer the genes to be under the control of promoters that would be coordinately expressed. This of course is still in the future.”

Such a process could improve corn by adding

the genes for drought resistance or for nitrogen utilization, complex traits that require multiple genes. With corn, it is possible to add one gene to a maternal lineage and one to a paternal lineage, and then cross them to make a hybrid that includes both genes. But mini-chromosome technology bypasses tedious and time-consuming crosses, in adding multiple genes at once.

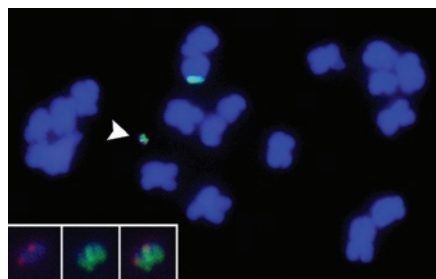
Chromatin (Chicago), is already producing plant mini-chromosomes of up to 200 kb (ref. 7), but even larger mini-chromosomes are also feasible, according to Daphne Preuss, founder and CEO. Chromatin has licensed its plant mini-chromosomes to several companies, including Syngenta (Basel) for transforming sugarcane. Sugarcane is grown commercially as a vegetative crop, which means that it gets propagated through cuttings. So, as Preuss explains, “It’s not practical to add one gene to one sugarcane plant and another gene to a different plant and then cross them to get both genes in one plant like you can with corn,” Preuss explains. “To get multiple genes in sugarcane you want to do it all at once.”

Artificial chromosomes have also been produced in animal systems. At Hematech (Sioux Falls, SD, USA), researchers combined fragments from human chromosomes 2, 14 and 22 to make an artificial chromosome, which is essentially a vector that includes the full repertoire of human antibody genes, according to company president Eddie Sullivan. Hematech scientists use this human artificial chromosome to create transchromic cattle, which serve as human antibody production systems<sup>8</sup>. The size of cows alone makes them a good factory. “You can collect up to 60 liters of plasma per month from an adult animal,” Sullivan says. With a human antibody-producing cow, Hematech can expose the animal to, say, a human infectious disease, or maybe even cancer cells, and those antigens could produce specific antibodies in the cow. The company is in early product development and has already done some preclinical testing in the biodefense area.

### Souped-up engineering?

Traditional approaches to metabolic engineering still dominate work under way in industry. For example, Archer Daniels Midland (Decatur, IL, USA) and Metabolix (Cambridge, MA, USA) will use metabolic engineering in the technology behind a plant being built in Clinton, Iowa, where starch from corn will fuel engineered bacteria to generate natural versions of polyhydroxyalkanoate (PHA), which are traditionally petroleum-based plastics. This plant should begin operating by the end of this year.

Among the most ambitious metabolic engineering attempts, artemisinin—a component of an antimalarial remedy—remains the poster



**Figure 2** Artificial chromosomes. The arrow points to a mini-chromosome (green is centromere-specific probe; red, the transgene; and blue, DAPI-stained DNA. (Image provided by Jim Birchler, University of Missouri).

child. Traditionally, pharmaceutical scientists extract this drug from the Asian plant sweet wormwood, a process that is affected by the vagaries of weather and drought, and which costs too much to serve many populations most affected by malaria. In 2006, chemical engineer Jay Keasling and organic chemist Richmond Sarpong, (both of the University of California, Berkeley) reported the engineering of a complete biosynthetic pathway for making artemisinin in yeast (Fig. 3)(ref. 69). To turn this technology into a product, Keasling founded Amyris Biotechnologies (Emeryville, CA, USA). In 2004, the Bill & Melinda Gates Foundation provided a \$42.6 million grant to the nonprofit pharmaceutical company Institute for OneWorld Health (San Francisco), which helped scale-up the manufacturing process for biosynthetic artemisinin. In 2008, through a license agreement with Sanofi-Aventis (Paris), the company built a plant to make this drug. According to Keasling, this should lead to commercially available biosynthetic artemisinin in the next couple years.

Other companies are applying existing approaches, such as directed evolution, to drug manufacturing. In 2006, Codexis (Redwood City, CA, USA) used directed evolution of three biocatalysts to improve the production of atorvastatin, the active ingredient in Pfizer’s cholesterol-lowering drug Lipitor. According to Codexis, this technology generated a 4,000-fold improvement in the productivity of one reaction in this drug-making process.

Pfizer is not the only adopter of Codexis’s platform. In 2007, Merck (Whitehouse Station, NJ, USA) started a collaboration with the company to produce biocatalysts. “In the pharmaceutical business,” says Greg Hughes, an associate director at Merck, “biocatalysis can help minimize the environmental impact of manufacturing processes.” Hughes would not divulge specifics about any ongoing projects, but said, “We look to apply biocatalysis from basic research to manufacturing.”

However, many of the early adopters of directed evolution techniques have had disappointing results. According to Eric Schmidt, a biotech and healthcare analyst at New York-based Cowen & Co., “I would say that directed evolution has not met with much, if any, success. Companies like Maxygen (Redwood City, CA, USA) and Advanced Molecular Evolution (AME) have not panned out as hoped.” (In October, Maxygen restructured into a joint venture with Astellas Pharma (Tokyo) after experiencing a capital crunch; AME was bought by Eli Lilly (Indianapolis) in 2004).

Not all the experience has been negative, however. For example, since purchasing AME, Lilly claims to use AME’s directed evolution approaches to design and engineer new biologics in a variety of programs, for autoimmune diseases, diabetes and cancer. Currently, 8 of the ~60 molecules in Lilly’s clinical pipeline and 4 in preclinical development involved work from AME, according to company spokesperson Judy Kay Moore. What’s more, one of the reasons Merck turned to Codexis was because of its capacity to use a variety of genetic tools, including directed evolution through DNA shuffling, to increase enzyme efficiency. In fact, Codexis looks for ways to improve the efficiency of entire pathways. “Overall, this technology works so well,” explains David Anton, senior vice president of R&D at Codexis, “because we can get improved enzymes in a few weeks rather than months. This triggers fast progress.”

### The next generation?

It is arguable whether any of the approaches used by Codexis, Metabolix and AME in the above applications represent the type of technological leap in engineering that might be possible if gene circuit design *in silico*, DNA synthesis, assembly and sequencing at the genome scale all become routine parts of product development. A key aspect of making this leap will be our ability to create effective synthetic regulatory mechanisms for increasingly complex, multigenic systems. A pilot project undertaken by Kristala Jones-Prather, a chemical engineer at the Massachusetts Institute of Technology (Cambridge, MA, USA), focuses on developing a strain of bacteria that can produce glucaric acid. Three genes—one each from bacteria, mouse and yeast—are needed to create the pathway in *Escherichia coli*. But initially, when the three enzymes were expressed in the bacterium, glucaric acid yields were limited by differences in the catalytic efficiencies of the different enzymes. Rather than trying to enhance the activities of the less efficient enzymes in the pathway, Jones-Prather decided instead to colocalize the three enzymes and optimize their relative abundances. This was accomplished by

tagging each enzyme with a protein ligand and targeting these to a scaffold designed to recruit the enzymes in an optimal ratio. “So without figuring out specifically why our system wasn’t doing what we wanted, we thought that bringing together the enzymes would make it work better, and it did,” Jones-Prather says. It increased the product output by threefold<sup>10</sup>.

Some other technologies being developed in academic laboratories also give a glimpse of the scale and efficiency of genome engineering that might have important industrial applications. Church and his colleagues, for example, have developed a way of combining directed evolution with synthetic oligos designed to target specific sites within the genomes—a technique they call multiplex automated genome engineering (MAGE)<sup>11</sup>. Starting with a set of genes in a particular pathway, they modify the strength of regulatory elements that control expression levels of the genes by using recombination to substitute short stretches of the host cell’s DNA in the genes’ ribosome binding sites, done robotically and iteratively (Fig. 4). This introduces changes in the targeted sites throughout the genome all at once, and with their microfluidic machine, they can continuously monitor the phenotype. When Church and colleagues applied this strategy to 20 genes required for lycopene accumulation in *E. coli*—which conveniently turns the cells red, making its synthesis easy to detect—they needed only three days to generate a bacterium that produced five times more pigment than an unoptimized strain.

Church describes MAGE as a “demonstration of accelerated evolution targeted by metabolic engineering knowledge for industrial-scale production.” He adds, “MAGE is an attempt to expedite two kinds of research. One is building a genome that has a particular sequence. The other application is providing a number of possible solutions to a genome.”

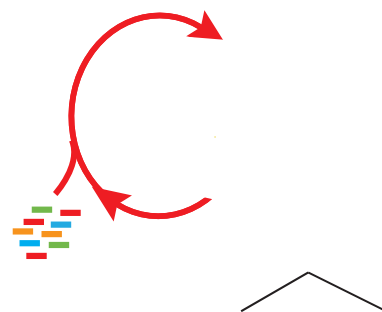
Other companies are working on ways to reduce industry’s need for petroleum-based products. At Genomatica (San Diego), for example, CEO Christophe Schilling and his colleagues are building a computer model that simulates a metabolic system. “If we want to make a chemical, we use a computer model to see how it can be done and to see which path would give the highest yield and which organism would be the best to use,” he says. They take that blueprint to the lab, where they fine-tune the process. With this approach, Genomatica engineered microbes to turn sugar into 1,4-butanediol, which is used in the plastics and fiber industries, where it is made from petroleum feedstock. According to Schilling, the process is “nearing the levels that are being targeted to provide a cost advantage when commercially produced.”

Other companies are also developing computational tools to engineer efficiency. For some projects at Verdezynne (Carlsbad, CA, USA), scientists use computer-designed oligos that self-assemble into full-length genes, which are then expressed at high levels in microorganisms. CSO Stephen Picataggio explains, “We’re developing a yeast-production platform, optimizing the

conversion of sugars from various feedstocks for biofuels and chemicals.” Picataggio knows that the green side of sugar feedstocks encourages chemical makers to adopt it, but he adds, “it has to be cost-advantaged.” Apparently, investors believe that Verdezynne can turn its technology into such a cost cutter, because the company runs on venture capital, along with some internal investment.

### What lies ahead?

The prospect of engineering new pathways and even new organisms may open up exciting possibilities for new products with new activities, but the commercial promise will have to be bal-





anced with the dangers inherent in unfettered dissemination of genome engineering technology (Box 2). So far and for years, companies have been attempting to address the problem through self-regulation. The International Association of Synthetic Biology (Heidelberg) in November finally finished drafting a code of conduct (not yet available on their website), and so far, the four companies that were involved in its development are signing on. But getting everyone on board with a single set of standards may be problematic<sup>12</sup>.

Beyond safety issues, synthetic biology also faces legal and regulatory challenges. “The patents involved in synthetic biology intellectual property have not been tested,” explains J. Mark Waxman, a partner with Foley & Lardner (Boston), “and a number of them make some very broad claims.” In their splashy 2007 report “Extreme Genetic Engineering,” the nonprofit Action Group on Erosion, Technology and Concentration (ETC) Group identified a range of already patented products and processes related to synthetic biology, including methods for building synthetic oligos and genes, engineering biosynthetic pathways and making novel nucle-

otides. The report concludes, “Some of these patents cast an extremely wide net<sup>13</sup>.” As an example of that, they point to US patent 6,521,427 issued to Glen Evans of Egea Biosciences<sup>14</sup> (San Diego), which covers chemical synthesis and assembly of genes and genomes. The ETC Group call this “potentially a description of the entire synthetic biology endeavor.”

That early stage of IP mirrors similarly unanswered questions in the regulatory environment. Waxman points out that many existing regulations—such as state and Federal statutes on pesticides—affect synthetic biology. Nonetheless, more regulatory discussions lie ahead. “We need to reach a consensus on what ought to be regulated and how,” Waxman says. That will probably be much more difficult to do than it is to say. With gene synthesis, says Waxman, “the problem may be difficult to solve,” especially as this technology becomes less expensive and more widely available.

Finally, many of these technologies remain in their infancy, so commercialization is likely to be fraught with challenges. Because so many of the details remain to be resolved, BCC’s Bergin thinks the market for developing products derived from

synthetic or partially synthetic organisms may take several years to emerge. For those that are first to market with products and a solid and defensible intellectual property position, the commercial rewards are likely to be great.

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