Synthetic Biology

Peter A. Carr, Johanna Bobrow, James C. Comolli, Nicholas J. Guido, Frances E. Nargi, Todd A. Thorsen, David I. Walsh, Matthew E. Walsh, Scott T. Wick, and Catherine R. Cabrera

Synthetic biologists have begun ushering in a new era of genetic engineering. In this era, how we design, build, and test new living systems is being transformed. Potential benefits span many areas, including safeguarding human health, creating new ways to make the materials we need, and responding to the diverse challenges of climate change. Synthetic biology research at Lincoln Laboratory advances the foundational technologies to enable such applications in support of national security, while preparing for potential misuse—intentional or accidental—in a world in which capabilities are advancing faster than policy and regulation.

Across all of nature, the vast number of things that living organisms can accomplish is staggering. Every living cell has the capacity to sense its environment and its own internal workings. Cells act on this information in a variety of ways, including moving, producing chemicals, signaling, reproducing, or even dying. And the instructions for these actions are stored in their DNA. Often, we can identify from an organism's genome an exact region of DNA that encodes a specific function. Genetic engineers find new ways to put those DNA instructions to work and invent new functions as well.

Dramatic advances in genetic engineering through the late 20th century often focused on a single DNA-encoded function (i.e., a gene). Many efforts focused on better understanding how living things work, such as how genes are turned on and off. Some research repurposed genes for medical applications, such as the production of human insulin by bacterial factories [1]. The newer term synthetic biology describes a shift toward applying more advanced engineering principles to how we design, build, and test living systems. These approaches have enabled researchers to use pieces of DNA from several different organisms to engineer complex systems composed of many distinct genetic functions. As synthetic gene networks were being created circa 2000, early publications drew an analogy between switch-like logical functions in molecular biology and the structure of logic circuits in electrical engineering [2, 3]. Thus, these engineered DNA-encoded systems were described as genetic circuits.

Since that time, there has been much debate about the exact definition of synthetic biology (sometimes simply

synbio) and the activities that are considered in or out of bounds. Regardless of semantics, synthetic biologists seek to advance the best practices for engineering biological systems. In doing so, they often adapt lessons learned from other engineering disciplines, such as electrical and chemical engineering. Thus, the past 20 years of synthetic biology have included grand experiments in

- Exploring standards for DNA design composition and construction
- Employing abstraction hierarchies for modular design across scales of complexity
- Developing new computer-aided design (CAD) tools for designing with DNA
- Modeling complex biological networks (circuitry) to improve and troubleshoot designs
- Characterizing failure modes (which are plentiful)
- Creating increasingly intricate and complex genetic systems
- Expanding the reach of what can be constructed (size and complexity of DNA, including entire genomes)
- Integrating with advanced capabilities, such as DNA sequencing, genome editing, miniaturization, and automation
- Transitioning these efforts from academia to industry

Synthetic biologists use these approaches to push the limits of not only how we design but also what we can design, creating new applications for engineered biological systems. Examples include programming microbes to produce medicines that are found in nature [4, 5] or to generate entirely new therapies [6]. A major area of synthetic biology progress is seen in programming simple cells (like bacteria and yeast) to biosynthesize useful molecules. These products include not only medicines but also flavors, scents, fuels, and materials. The recently released roadmap (Figure 1) from the Engineering Biology Research Consortium describes how synthetic biology impacts several economic sectors, e.g., health and medicine, energy, industrial biotechnology, environmental biotechnology, and food and agriculture [7]. These broad biotechnology-based contributions to the economy are often referred to together as the bioeconomy.

Synthetic biology research and development at Lincoln Laboratory supports national security in three ways. First, we engineer living systems that meet a specific need, such as responding to a biothreat like anthrax. For example, the CANARY sensor system employs a genetic circuit that produces light within seconds of coming into contact with a biothreat, giving detection that is both rapid and specific. The engineered cell lines at the heart of CANARY can be flexibly reprogrammed to respond to different pathogens, including new or emerging diseases.

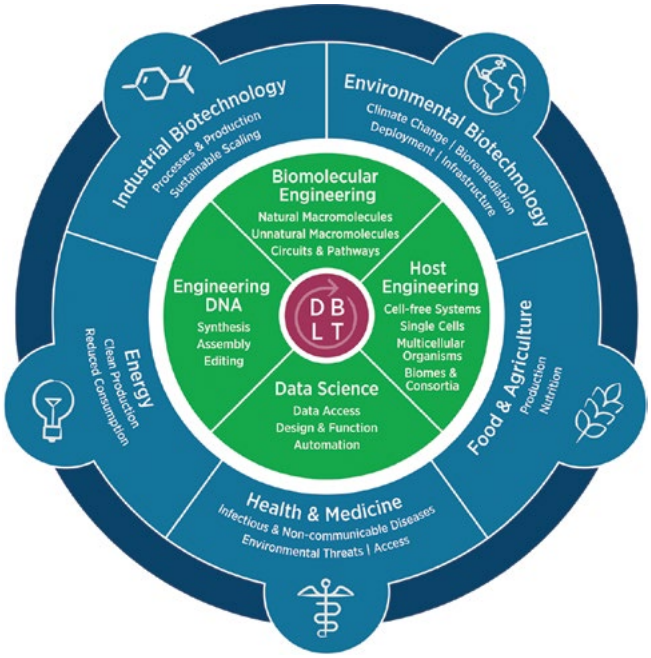


FIGURE 1. The Engineering Biology Research Consortium roadmap highlights not only key economic sectors that contribute to the overall bioeconomy but also their technical underpinnings.

Second, as synthetic biology is still a young discipline, we create foundational tools that support our own applications and the field as a whole. Many of our efforts focus on prototyping, such as engineering better ways to assemble the large pieces of DNA that encode our genetic designs, and measuring the effectiveness of these designs. Our projects in microfluidic DNA assembly have demonstrated how the biochemical reactions used for genetic circuit construction can be miniaturized to volumes one-thousandth of that typically used in a standard lab. Our PERSIA system provides a means to measure RNA and protein production in real time, employing cell-free reactions for prototyping that avoid otherwise laborious experiments in live cells. Contributing to the foundations of synthetic biology includes fostering norms of responsible research. Our contributions also include broadly supporting the

development of synbio hardware through open-source design sharing and enabling the use of shared fabrication resources, such as in makerspaces.

Third, we analyze the technology landscape of synthetic biology and its impact on the world around us. We do this to better advise the U.S. government on synthetic biology issues and to prepare ourselves for future opportunities and challenges. These efforts often consider how synthetic biology will impact biodefense, human health and performance, or manufacturing. Such considerations apply as well to the overall growing bioeconomy, and ways to support and protect it. Some analyses focus on immediately available technology for meeting current needs while others anticipate the evolution of the technologies and their impacts several decades into the future.

Programming Biology

The DNA of a living cell acts as the instruction set for a large collection of highly connected control elements (those that switch genes on and off) and actuators (such as enzymes that perform catalysis). At a high level of abstraction, many scientists have noted similarities between these DNA-based instructions and other types of engineered systems, such as computer code and electronic circuits (digital and analog). Thus, many synthetic biologists model their DNA-based systems as genetic circuits to better understand and engineer them.

To achieve the representation of genetic systems as genetic circuits, synthetic biologists make use of ideas developed in computer science, information theory, and electronic circuit design. The commonality is that both electronic systems and living systems can achieve a multitude of possible states in which they can exist. Electronic circuits deal primarily with controlling the states and fluxes of electrons. Genetic circuits deal primarily with states and fluxes of molecules. Both can be represented in terms of digital and analog logic. With digital electronic logic, you can represent discrete states with a switch that is open or closed, or a capacitor that is charged or uncharged, or a transistor that allows current to flow or not. In a genetic system, the state can be if a gene is turned on (producing RNA and often translating a protein), if a protein is bound to a specific DNA molecule, or if a molecule in the cell has been chemically modified. But it is important to note that representing biological systems in digital states can sometimes be a problematic simplification.

Along with the experimental capability to engineer biological systems came some of the first genetic circuit designs that were created to implement logic circuits [2, 3]. Figure 2 shows different representations of a logical OR function. If either input is present, then the OR function returns a specific positive result (i.e., on, true, or 1). The schematic for the genetic OR circuit indicates components encoded in DNA, which produce their output in the presence of chemical inputs. If either chemical input is present, the observable result is that the system produces light (bioluminescence). Importantly, whether the OR is represented in computer code, electrical switches, or genetic logic, several different designs can be used to achieve the desired OR behavior. For an electrical circuit, this behavior might be achieved with switches (shown here), diodes, or transistors. For a genetic circuit, one might use gene activators (proteins that turn on gene expression, as shown), repressors (proteins that turn off gene expression), or protein modification events. Which of these choices proves most effective for a given design goal will depend heavily on specifications and context, such as the environmental conditions in which the engineered system will function, and desirable traits of the sensor, such as rate of response.

Figure 3 shows the genetic OR example expanded in more detail. The desired function is a cell that can detect both lead and mercury contaminants in water, emitting light if either contaminant is present (or if both are). The DNA-encoded program is typically shown arranged along a single line, representing the (linear) physical organization of the DNA parts within a large DNA molecule. Table 1 gives details of key DNA parts that make up the OR circuit. Synthetic biology approaches have the potential to make some aspects of this project much easier. For example, several useful functional units are already available in existing collections of DNA parts, such as the Registry of Standard Biological Parts [8] and Addgene, including previous users' experience with the performance of DNA parts. Design tools such as Cello can help the user explore possible device architectures and simulate their behavior before moving to the step of physically building the circuit [9].

There can also be pitfalls to representing genetic devices with such simple levels of abstraction. Over-reliance

on simplification with these designs can mask the relevance of the complex biological context in which the device does its job. For example, the components shown for the lead OR mercury detector of Figure 3 may interfere with related systems in the host cell (often referred to as the chassis) that also bind to and remove toxic metals. Many such designs require a significant amount of tuning or redesign to take the cellular context into account.

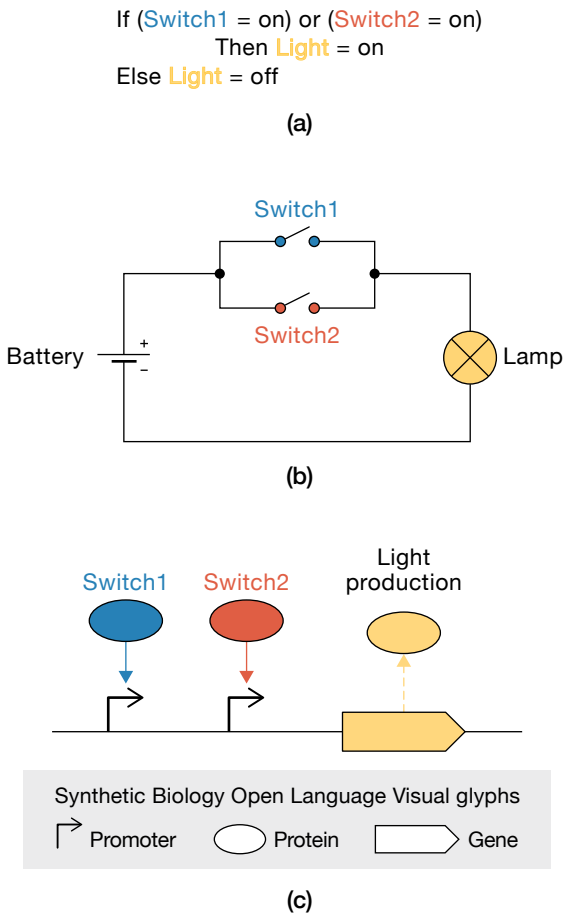


FIGURE 2. Different versions of an OR function are shown. In each case, if either switch 1 (blue) or switch 2 (red)—or both—are on, then the output (light) is turned on. With computer code (a), this action can be as simple as employing the term "or" in a logical statement. The electrical OR shown (b) is made with switches, that when closed allow current to flow. The genetic OR circuit (c) is made from DNA-encoded proteins that activate DNA control elements, called promoters, to express genes. Some of the DNA components of the design are shown arranged along a straight line including promoters and one gene. This simplified genetic OR uses two different proteins (switch 1 and switch 2) to detect dangerous chemicals, turning on production of another protein that generates light (bioluminescence).

Microfluidics

Microfluidics are devices that manipulate very small amounts of fluid—microliters, nanoliters, and even picoliters. (For reference, a single drop of water is about 50 microliters.) Often referred to as lab-on-a-chip technology, microfluidics can be used to integrate several different operations that would otherwise be performed individually by hand in the lab. The small volumes involved are an advantage for requiring only a minimum of reagents that are often costly or difficult to acquire. When handling hazardous materials, using only a microfluidic volume can also enhance safety by drastically reducing the total amount of a dangerous chemical or biological agent required. The small feature size of a microfluidic device

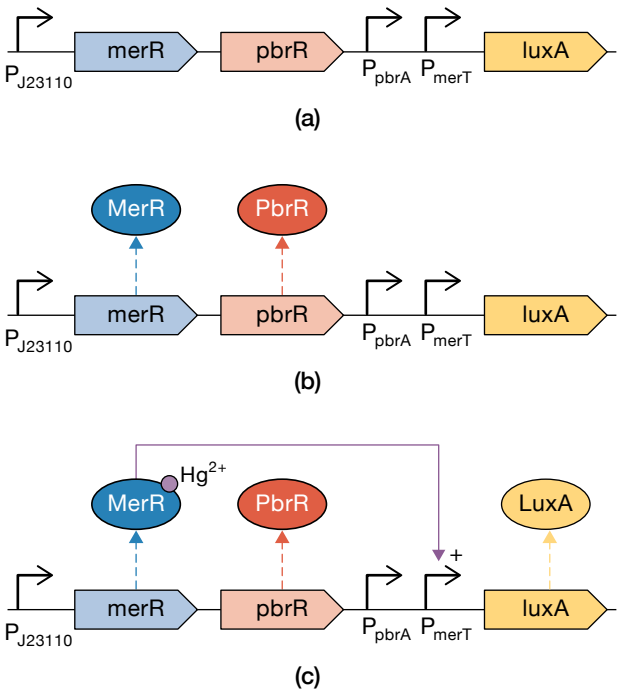


FIGURE 3. Illustrated is a simplified genetic OR circuit to detect lead and/or mercury (a). The layout of the DNA components represents the physical molecule of DNA that encodes these parts. These include three control elements (promoters) and three protein-encoding genes. These DNA parts are described in more detail in Table 1. Once the DNA instructions are inserted into a cell, the DNA-encoded regulator proteins MerR and PbrR are produced at a constant level because promoter P_{J23110} is always on (b). If mercury ions (Hg^{2+}) are present, they will bind to protein MerR, activating promoter P_{merT}, leading to the production of the LuxA protein (c). LuxA uses chemicals present in the cell (made by additional Lux proteins, not shown) to generate light. Detection of lead ions (Pb^{2+}) via the PbrR protein and the P_{pbrA} promoter works similarly.

also facilitates scale-up in numbers of parallel operations: in some cases, more than 10,000 microfluidic reactors can function side by side in an area of no more than a few square centimeters [10]. This compact format, combined with multiple ways to control on-chip activities, allows multiple different processes to be combined in the same device. Several Lincoln Laboratory projects marry synthetic biology with microfluidics by integrating functions such as DNA construction, installation of DNA designs into living cells, and use of DNA to perform cell-free synthesis and other enzymatic reactions.

Figure 4a shows one element of a very simple microfluidic device. Multiple layers of flexible, transparent silicone rubber (polydimethylsiloxane) have been patterned and assembled by using the techniques of multilayer soft lithography, and sealed to a rigid glass coverslip (bottom). A single narrow microchannel connects a fluid inlet and a fluid outlet. Just above this channel are two more perpendicular channels. These top channels can be pressurized with air or liquid, causing them to expand and press down on the single channel below, acting as valves to seal it off from the inlet and outlet. The sealed channel can then be used like a miniature test tube to perform a biochemical reaction, culture and observe live cells, and more. These simple design elements can be repeated and recombined in many ways to integrate complex multistep processes. We employ custom hardware and software to actuate arrays of control valves, creating an elaborate fluidic trafficking system that can manage many processes in parallel or address individual reactors as needed. Figure 4b shows a Lincoln Laboratory microfluidic device that integrates 96 of these reactors. These reactors can be used flexibly, such as for assembling DNA, synthesizing protein in a cell-free expression system, or monitoring an enzymatic reaction. Many other kinds of microfluidic devices exist and use a diverse array of materials. Some microfluidics can reach a very high degree of feature density and complexity [10].

We are engineering new technology to miniaturize and integrate the processes for constructing DNA and using that DNA, specifically combining biological and biochemical processes with microfluidic hardware. A crucial example is the process of DNA construction, at the heart of most synthetic biology research and development. One of our methods utilizes an automated droplet-spotting robot to place ultra-small volumes (as low as 300 picoliters) of DNA solutions on an epoxy-coated microscope slide, followed by plasma bonding to a microfluidic channel architecture made from polydimethylsiloxane. A reaction mixture for performing DNA assembly is pushed through the reactor channels to rehydrate, mix, connect, and recover DNA from parts to a complete

Table 1. DNA Parts for the Genetic OR Circuit for Detecting Lead and Mercury

NAME	FUNCTION (MOLECULE)	DETAILS	ORGANISM SOURCE	REGISTRY PART NUMBER
pbrR	Regulator (protein)	Lead-responsive	*Cupriavidus metallidurans*	BBa_K1701001
merR	Regulator (protein)	Mercury-responsive	*Shigella flexneri*	BBa_K1420004
P_{J23110}	Promoter (DNA)	Medium-strength constitutive (always on) promoter	Anderson promoter collection, derived from *Escherichia coli*	BBa_J23110
P_{pbrA}	Promoter (DNA)	Regulated by PbrR protein and Pb^{2+}	*Cupriavidus metallidurans*	BBa_I721001
P_{merT}	Promoter (DNA)	Regulated by MerR protein and Hg^{2+}	*Shigella flexneri*	BBa_K1758342
luxA	Enzyme reporter (protein)	Luciferase (light-generating) protein	*Vibrio fischeri*	BBa_K785003

assembly. Throughout this work, we have developed separate hardware (programmable pneumatic pumps) for controlling microfluidics, an alignment system to accurately bond device layers, and valve control strategies for device filling, reaction isolation, mixing, and recovery.

Biosensors: CANARY

Nature has produced many ways to sense the world, including vision (detecting light), smell (detecting molecules), and touch (perceiving hot, cold, pressure). Living things also have ways to sense inside their own bodies—such as with immune systems that respond to unwelcome invaders. Synthetic biologists look at these examples and see opportunities to engineer new systems from the biological parts that compose the heart of these senses.

An early example of synthetic biology–style engineering at Lincoln Laboratory was the creation of the CANARY (Cellular Analysis and Notification of Antigen Risks and Yields) sensor [11, 12]. The need was for a system that could rapidly and specifically detect different pathogens or toxins, such as the high-risk biological agents that compose the U.S. Select Agents and Toxins List [13]. The concept was to harness the exquisite combination of both variation and specificity of the immune system. Cells derived from the mouse immune system (B cells) were programmed and employed to detect different agents and respond rapidly by producing light (Figure 5). A suite of CANARY sensors has been produced to detect bacteria, viruses, toxins, and DNA molecules.

The basic CANARY system is designed to express antibodies on the surface of the B cells. These antibodies detect antigens that are expressed in multiple copies on the surface of pathogens. In the case of a bacterium, there may be 10,000 copies of a given antigen while a virus may carry about 1,000 copies. Toxins are generally monovalent (1 copy) and thus would not be detected by the basic CANARY sensor outlined here. However, a simple change to the basic design involving expression of multiple different antibodies in a single cell line has now enabled CANARY to detect these medically important toxins as well.

While the term synthetic biology had not yet been popularized, crucial elements of a synthetic biology design-build-test engineering cycle were notable in this

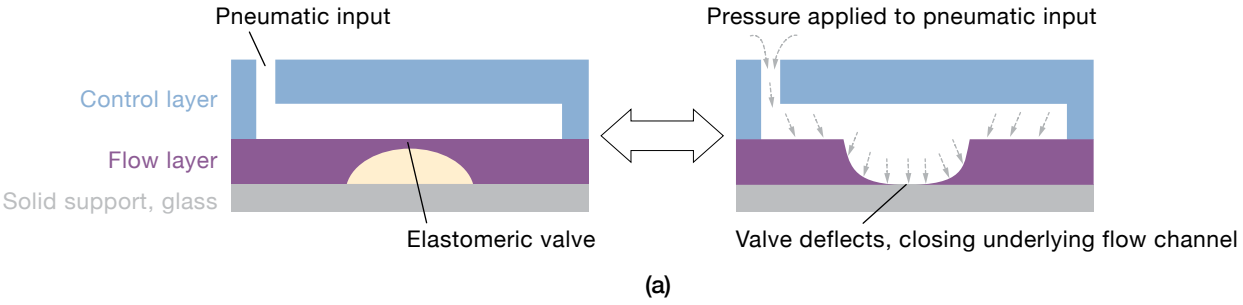


FIGURE 4. A simple microfluidic design element is seen in (a). Channels running through the "flow" layer can be used to route fluids—for synthetic biologists these often contain solutions of cells, proteins, and/or DNA. Applying pressure to channels in the "control" layer causes those upper channels to expand, pressing down on the flow layer, sealing those lower channels. A Lincoln Laboratory multipurpose microfluidic device contains 96 identical channel reactors (50 nanoliters each) (b); the device can be used to assemble DNA, synthesize protein in a cell-free expression system, or monitor an enzymatic reaction. Attached tubing is used to actuate the top control lines that seal channels and pump fluids. The inset shows parallel channels displaying red fluorescence that indicates the cell-free production of protein.

project. First, the important real-world specifications were identified, namely the capability to respond quickly, the flexibility to reprogram the system to detect distinctly different threats, and the need for both sensitivity and specificity. Next, a cellular chassis (choice of organism and cell type) was selected as the runtime environment for the genetic program, taking maximum advantage of existing natural features. And finally, genetic parts from several different organisms were integrated to provide the capabilities needed to complete the system. Iterative redesign cycles were used both to improve system performance and to adapt CANARY to detect diverse threats.

The genetic programming of CANARY works much like an IF/THEN computing statement: IF the target agent is present, THEN produce light. The parts required to perform this task are shown in Figure 5, along with the process of how CANARY senses and reports. We note that all proteins needed by the CANARY system are synthesized and present before any detection event occurs, with no additional turning genes on or off required. This feature allows CANARY to employ a much faster process than that used by the circuit shown in Figure 3, enabling CANARY to respond to the sample in seconds.

Hardware to monitor the light output of the engineered CANARY cells was also developed at Lincoln Laboratory. Figure 6 shows the timeline of development, including prototypes for the TCAN (Triggered CANARY) and PANTHER (Pathogen Analyzer for Threatening Environmental Releases) detection systems. The CANARY technology and PANTHER were both transitioned to industry. Lincoln Laboratory's ability to co-develop both wetware (living cells, or biochemical reactions) and hardware allows us to pursue new innovations in synthetic biology that can reach beyond the laboratory and be integrated into real-world, fieldable devices.

Lincoln Laboratory has continued to develop CANARY technology since 1997, including modification of mouse B-cell lines to improve their durability and ability to remain viable even after two weeks stored at room temperature. Current CANARY projects support the U.S. Department of Agriculture needs to detect plant pathogens for protecting U.S. agriculture. Other ongoing

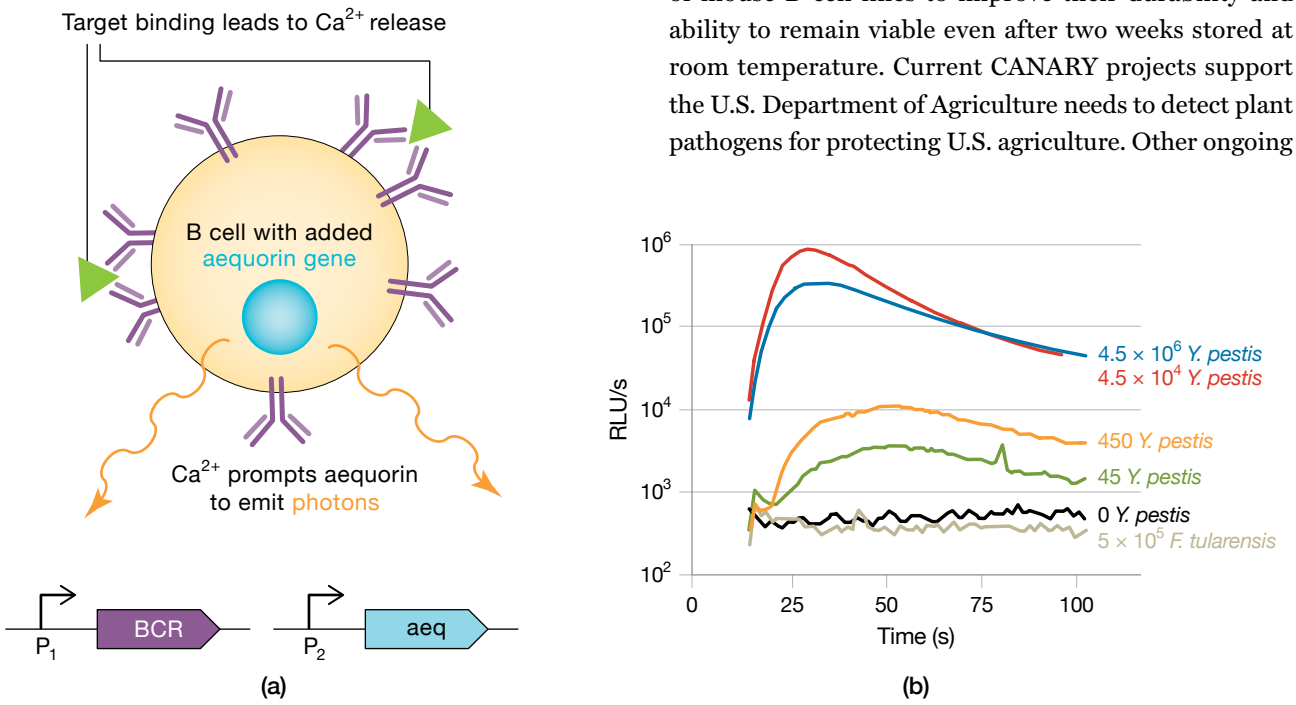


FIGURE 5. The sensing element in CANARY is a mouse B-cell line that has been genetically engineered to produce both the aequorin protein in the cytoplasm, and B-cell receptors (BCR) displaying recombinant antibodies on the cell surface (a). Aequorin, cloned from the same jellyfish that produces green fluorescent protein, is a calcium-requiring luminescent protein that emits photons. When these engineered cells are exposed to the specific bioagent they are engineered to detect, antigens on the bioagent bind to multiple BCR proteins, crosslinking them and initiating a cascade of molecular events that causes an increase in the calcium concentration inside the cell. This calcium activates the aequorin to produce light. The light can be detected by a photomultiplier tube or other hardware-based detector. Sample data are shown in (b), indicating the sensitivity and specificity of CANARY cells engineered to detect *Y. pestis* (plague) within 30 seconds of adding the sample. RLU = relative light units.

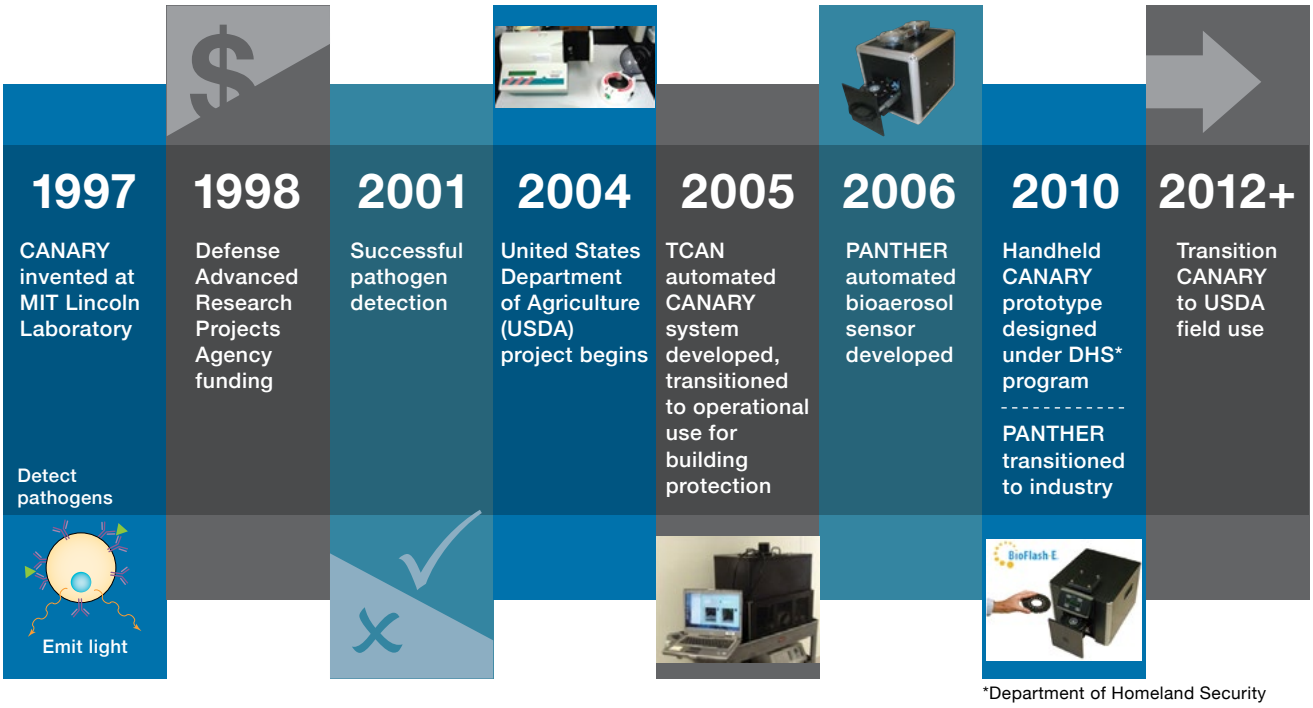


FIGURE 6. The timeline for the development of CANARY and its subsequent iteration as PANTHER spanned more than a decade; updates to the technology continue to be made.

Lincoln Laboratory synthetic biology projects develop cell-based and molecular biosensors for a variety of U.S. government needs.

Accelerating Build and Test: A Biomolecular Prototyping Unit

As with other engineering disciplines, designing a biological system typically requires an iterative process of prototyping, troubleshooting, and redesign. Thus, many synthetic biologists devote themselves to creating tools to speed up the design-build-test cycle. At Lincoln Laboratory, we are engineering an integrated pipeline that combines the capabilities for making DNA and putting it to work, namely, the Biomolecular Prototyping Unit (BPU, Figure 7).

The BPU concept represents the integration of key processes needed for the build and test phases of the synthetic biology engineering cycle. With design files in hand, a user would send a request to a BPU pipeline. For a synthetic biologist, the DNA fabricated might represent a design (or several) for a new genetically encoded sensor circuit. But the BPU will also be valuable for biologists building DNA molecules with sequences of natural origin, possibly pulled from a database, encoding a protein they wish to study, even if they have never seen the source organism in real life. For medical clinicians, the DNA sequences might come from sequencing a patient's DNA, or the DNA of a pathogen infecting that patient, and the question may be one such as, "What are the best medicines to treat this specific person's infection?"

Crucial elements of the first BPU module, DNA construction, are (1) chemically synthesizing short strands (called oligonucleotides, or simply oligos, for example, 60 DNA bases long); (2) using enzymes to assemble these short pieces into longer cassettes (for example, 1,000 DNA base pairs long, a common length scale for a single gene); and (3) dealing with manufacturing defects in the DNA molecules (i.e., quality control and assurance). Many different approaches can be used for accomplishing these steps, and some steps may be required more than once. For example, building a 5,000-base-pair piece of DNA may require assembling many oligos 60 bases long into units 1,000 base pairs long, and then joining several of those units to reach 5,000 base pairs. Quality control and assurance steps may include using a special enzyme that degrades and removes defective DNA, while another

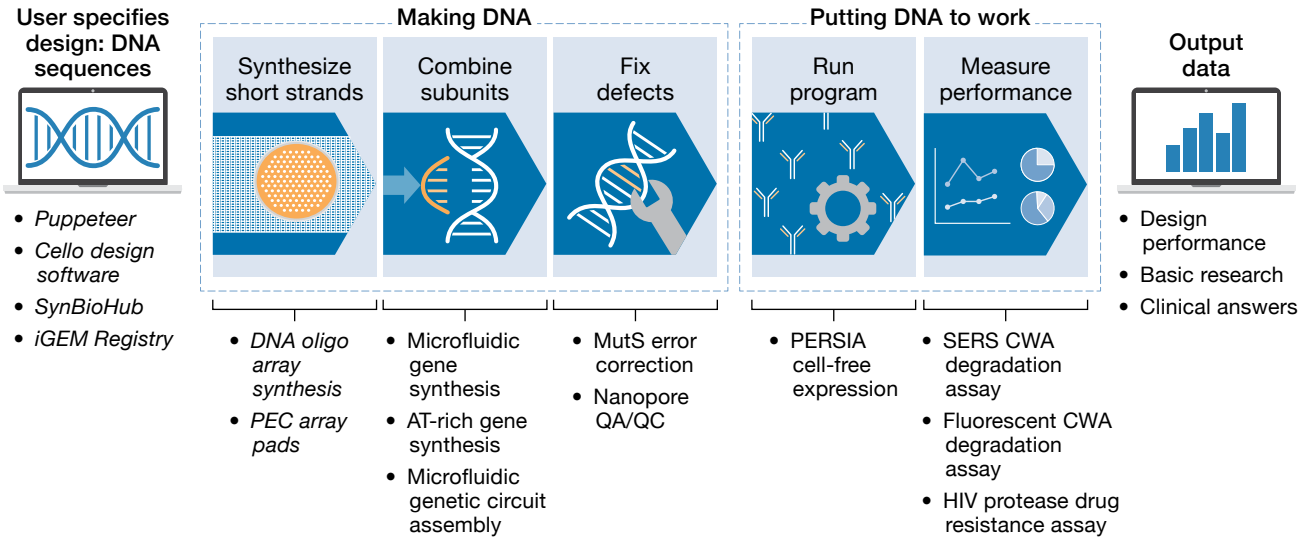


FIGURE 7. Lincoln Laboratory's vision for the Biomolecular Prototyping Unit (BPU) pipeline is illustrated here. Accepting DNA design files specified by the user, the BPU would physically construct those DNA molecules, using modules shown in the "making DNA" portion of the pipeline. Then those DNA molecules would be "put to work" either inside living cells or by using cell-free expression systems. Below these different modules are listed efforts at Lincoln Laboratory that contribute to the overall development of the BPU. (Collaborators' and related efforts are listed in the first two columns in italics.) QA/QC = quality assurance/quality control; SERS = surface-enhanced Raman spectroscopy; CWA = chemical warfare agent; PEC = photoelectrochemical.

approach can simply be sequencing enough samples to find one that is error-free.

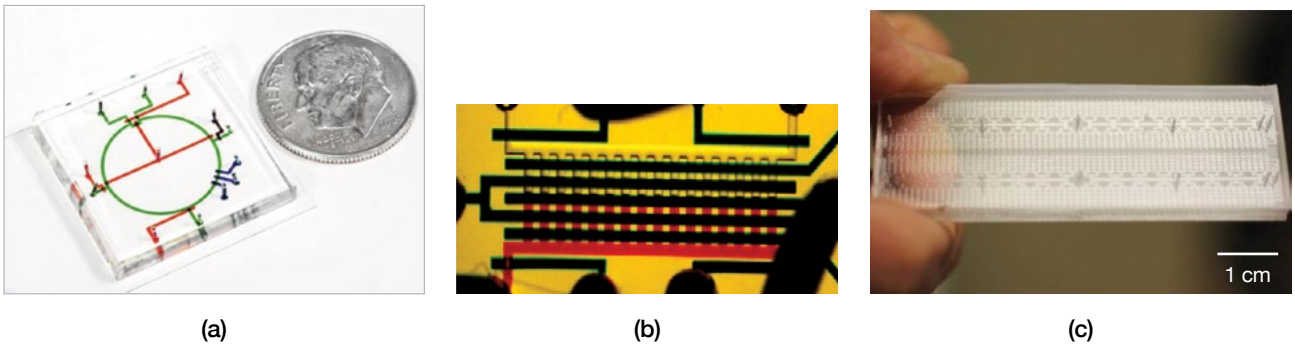
The second BPU module, putting DNA to work, represents a large number of possible choices for how DNA-based designs are implemented and measured. An early step is typically to insert the DNA into a living cell, which then runs the program. Cells can be from bacteria, yeast, humans, or a variety or other organisms, and the design must be matched to the type of cell being used. Similarly, performance can mean many different things, such as production of a useful chemical, emitting a warning signal, or reporting on the inner workings of the cell. The tools needed to measure these functions are also quite diverse. Often a significant part of the design is to convert some cellular process into a signal that can be observed with available hardware, such as a fluorescence or bioluminescence detector, mass spectrometer, or DNA sequencer.

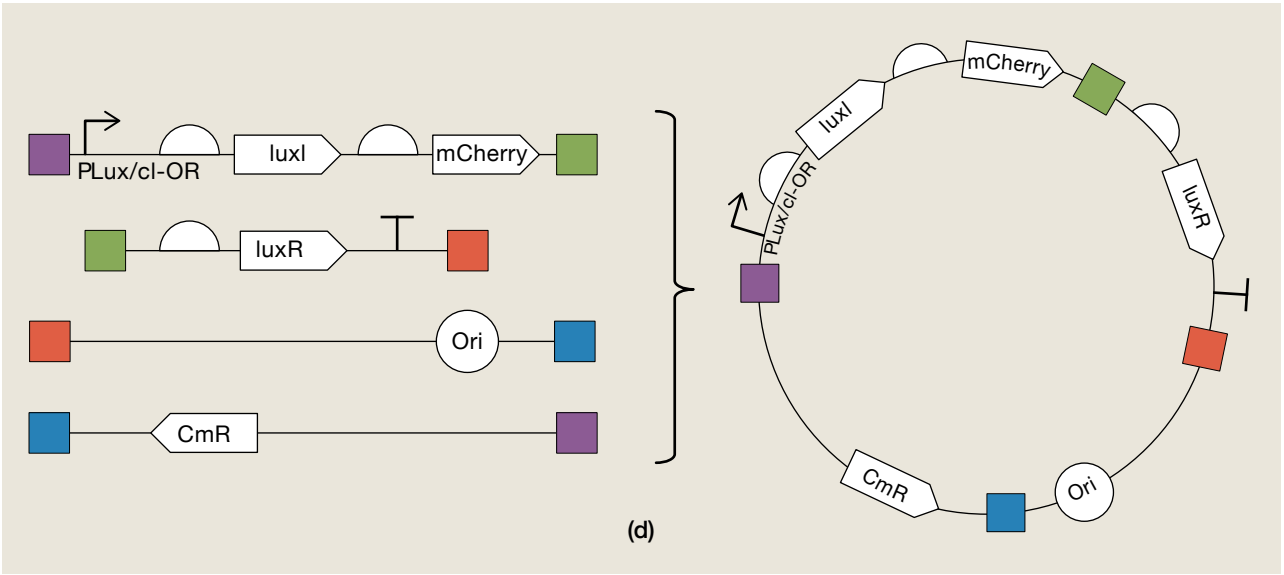
Our goal for an effective BPU is to maximize integration and miniaturization while reducing the total expertise required of the user. Some larger-scale, lower-throughput examples of this concept have been demonstrated very effectively, such as the Digital-to-Biological Converter produced by a team at the J. Craig Venter Institute [14]. The BPU vision in Figure 7 includes contributions from published results and ongoing projects of Lincoln Laboratory and our network of collaborators. One example is our production of a microfluidic genetic assembler, capable of combining multiple DNA parts, using a variety of different common DNA construction approaches (Figure 8) [15]. We showed that four common methods (restriction/ligation, Golden Gate, Gateway, and Gibson Assembly) are each compatible with our microfluidic devices and can be miniaturized more than 20-fold compared to common laboratory practices. Some of these methods showed potential for further 100-fold minimization in reaction volume. This research informed our current work developing high-throughput DNA assembly devices.

Measurement: We Want a Multimeter

For the synthetic biologist, there is no one simple multipurpose measurement tool akin to an electrical multimeter (or oscilloscope). Instead, there exists a suite of powerful (often expensive) measurement tools, such as flow cytometers, DNA sequencers, quantitative polymerase chain reaction (qPCR) thermocyclers, and electrophoresis instruments. Many of these rely on converting the biological effect we want to observe into

a more easily measurable signal, such as fluorescence. In fact, all four instruments noted above rely on fluorescence for reading out answers, and all four have shown great adaptability for posing and answering many different kinds of questions. Over time, many of these tools have become faster, easier to use, and/or more affordable. But the question remains of how close we can get to the properties of a multimeter—capable of quick measurements, switchable between detection modes (volts, amps, ohms, farads), portable, and cheap.





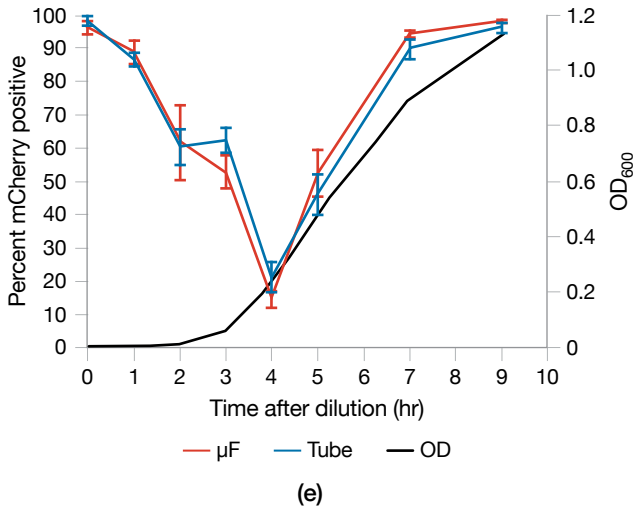


FIGURE 8. Various microfluidic genetic assemblers developed at Lincoln Laboratory are shown in (a–c), capable of performing either a single reaction (a, 1-plex), 16 parallel reactions (b), or 256 parallel reactions (c). For the microfluidic assembly of genetic circuits, several different genetic circuits were constructed using the 1-plex mixer, testing multiple assembly approaches, including the quorum-sensing circuit shown in (d) (employing the Gibson assembly method). Genetic circuits assembled in the microfluidic device (μF) performed comparably to those assembled in conventional test tube (Tube) reactions (e), in this case producing a fluorescent protein (mCherry) in response to sensing high cell density (OD) [15].

Our most recent contribution to the idea of a biomultimeter is called PERSIA [16]. This labeling and detection scheme is named after its components, PURExpress-ReAsh-Spinach In-vitro Analysis. PERSIA provides a way to monitor the molecular events in a complex cell-free reaction while those events are occurring.

Cell-free transcription and translation reactions in general are useful for reproducing the central dogma of biology: DNA is read to make an RNA copy; the RNA copy is translated to make a protein. These reactions can be used to try out an idea, or answer a simple question, much faster than working in live cells. (However, cell-free reactions do not recreate all the complex interactions that are present in a live cell.) Increasingly, synthetic biologists are using such systems to develop sensors, prototype genetic circuits, and perform small scale point-of-need manufacturing.

As shown in Figure 9, the components needed for PERSIA are encoded into the DNA design for one or more constructs. In addition to the DNA sequence for

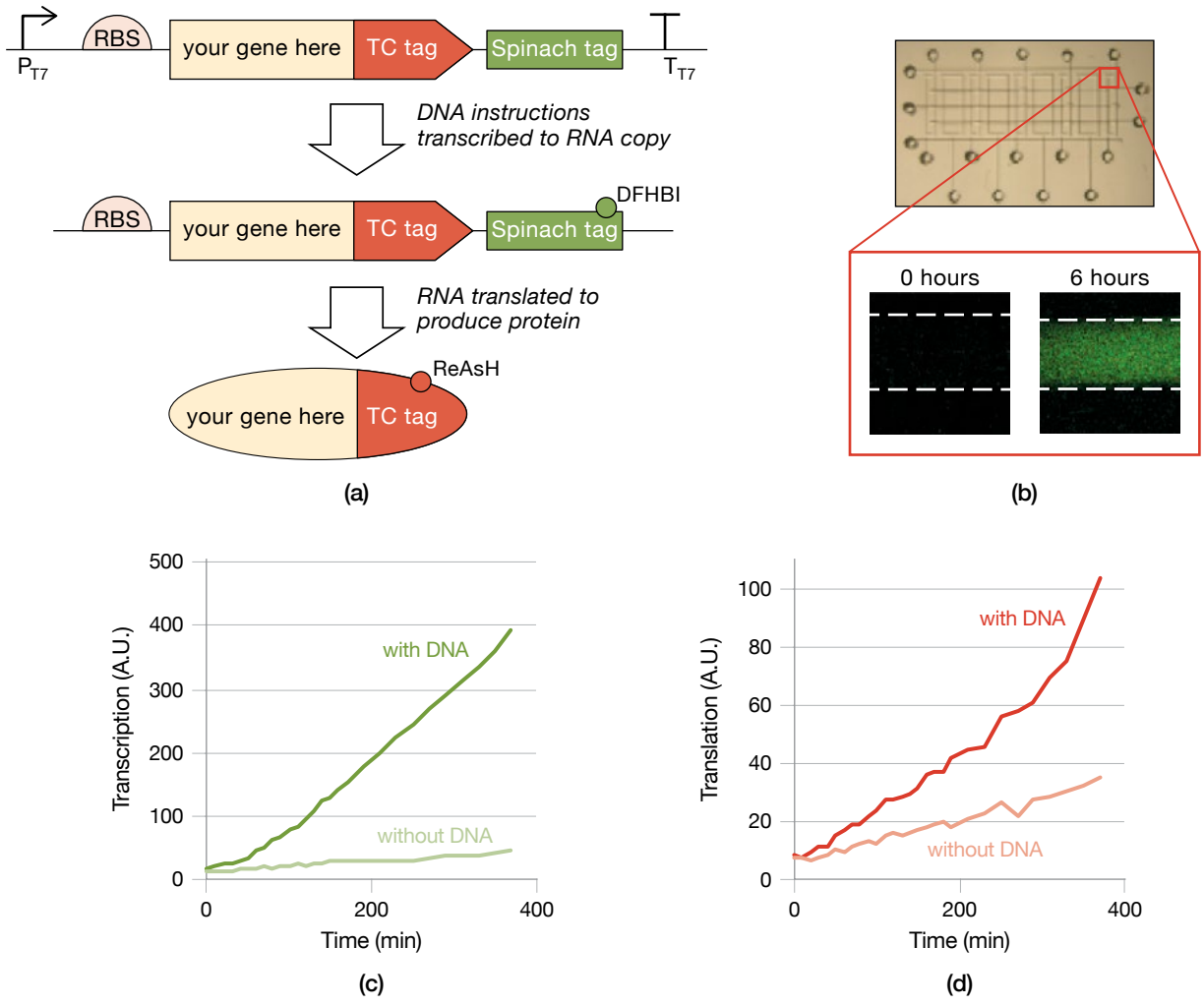


FIGURE 9. Typical design layout of DNA used with PERSIA is shown in (a). RNA polymerase start (P_{T7}) and stop (T_{T7}) signals from bacteriophage T7 specify the beginning and end of the RNA molecule that is transcribed from the DNA. This RNA includes the Spinach sequence, which binds to the chemical DFHBI, causing green fluorescence. The ribosome binding site (RBS) directs the translation of the RNA to produce a fusion protein combining the gene of interest with a short tetracysteine (TC) peptide tag. Additional peptide tags (optional, not shown) can also be included for protein purification. The TC tag binds to a chemical (ReAsH), causing red fluorescence. When performing PERSIA in a microfluidic device, this output is measured by quantitative fluorescence microscopy (b). The amounts of green (c) and red (d) fluorescence measured over time give the user a real-time readout of how much RNA and protein are being produced. Performing the reactions without the DNA-based instructions assesses the nonspecific background signal.

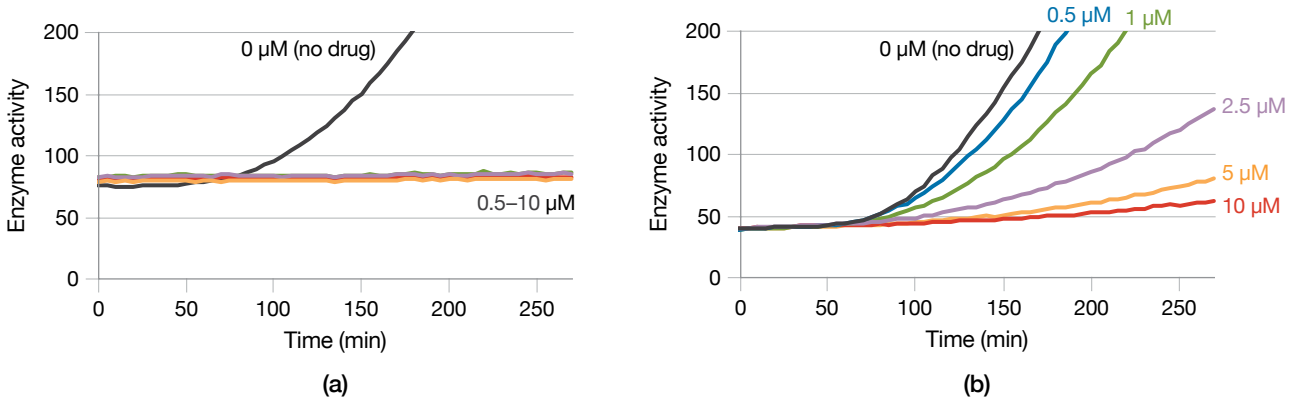


FIGURE 10. The charts illustrate adaptions of PERSIA to assess resistance to antiviral drugs. An additional assay reagent can be employed in a PERSIA cell-free reaction, in this case to measure the activity of the enzyme HIV protease, one of the two major targets for drugs that treat HIV. For the standard reference strain of HIV, the activity of HIV protease is suppressed by low concentrations (0.5 to 10 micromolar, μM) of the drug lopinavir (a). For a genetic variant of the virus known to be resistant to lopinavir, none of the same concentrations tested completely halted protease activity (b).

the protein itself are the signals that turn production on and off. PERSIA employs a short extra sequence (a tetracysteine, or TC tag) that adds a few more amino acids to the tail of a protein. The TC tag can then react with the chemical known as ReAsH, resulting in bright red fluorescence. Similarly, the mRNA sequence encoding the protein includes its own tag (named Spinach), which binds to a different chemical, giving a bright green fluorescence. Together these extra components allow us to quantitatively monitor both transcription (RNA production) and translation (protein production) during the reaction itself. In contrast, many other types of analysis would rely on additional experiments to be performed once the cell-free reaction is over. In addition, we have demonstrated that PERSIA can be performed in the very small volumes of a microfluidic device. Our intent is that future versions of the BPU can incorporate PERSIA to produce integrated high-throughput readouts of protein production and function.

We have employed PERSIA to test new design ideas for genetic codes, using the protein readout to tell us whether or not a given genetic code produces a robust quantity of protein. We have also used PERSIA in combination with additional enzymatic assays to probe details of protein structure and function. We have even extended these assays to include measurements of drug activity for different clinically occurring isolates of the HIV protease (Figure 10). Through these approaches, we hope to both accelerate and personalize the effective treatment of viral diseases. Optimistically in the near future, when patients at a clinic or warfighters at a medical field station have their viral infection sequenced, those sequences can be rebuilt as DNA molecules in a BPU-like pipeline and tested with a technique such as PERSIA, and the results can immediately inform clinicians or medics of the best (and personalized) choice of drug regimen for the patients.

Rapid Medical Countermeasures: A Digital There and Back Again

Tools such as PERSIA and the BPU can enable an ambitious vision for fighting pathogens, whether naturally occurring or engineered. Figure 11 shows a pathway that especially leverages the digital nature of DNA sequence information. It relies on onsite sequencing capabilities at the location of concern (such as a remote medical clinic in one part of the world) and BPU-like capabilities at a separate location with more resources.

Consider a newly discovered virus, perhaps in a remote or inaccessible part of the world. Onsite sequencing—such as is now becoming plausible with some next-generation sequencing platforms—could be used to determine the genetic sequence of the virus. That information would then be transmitted to other facilities (centralized or distributed) that would

- Analyze the information content of the sequence to identify which parts of the virus could be targets (such as a viral protease) for a drug or other medical countermeasures (MCMs)

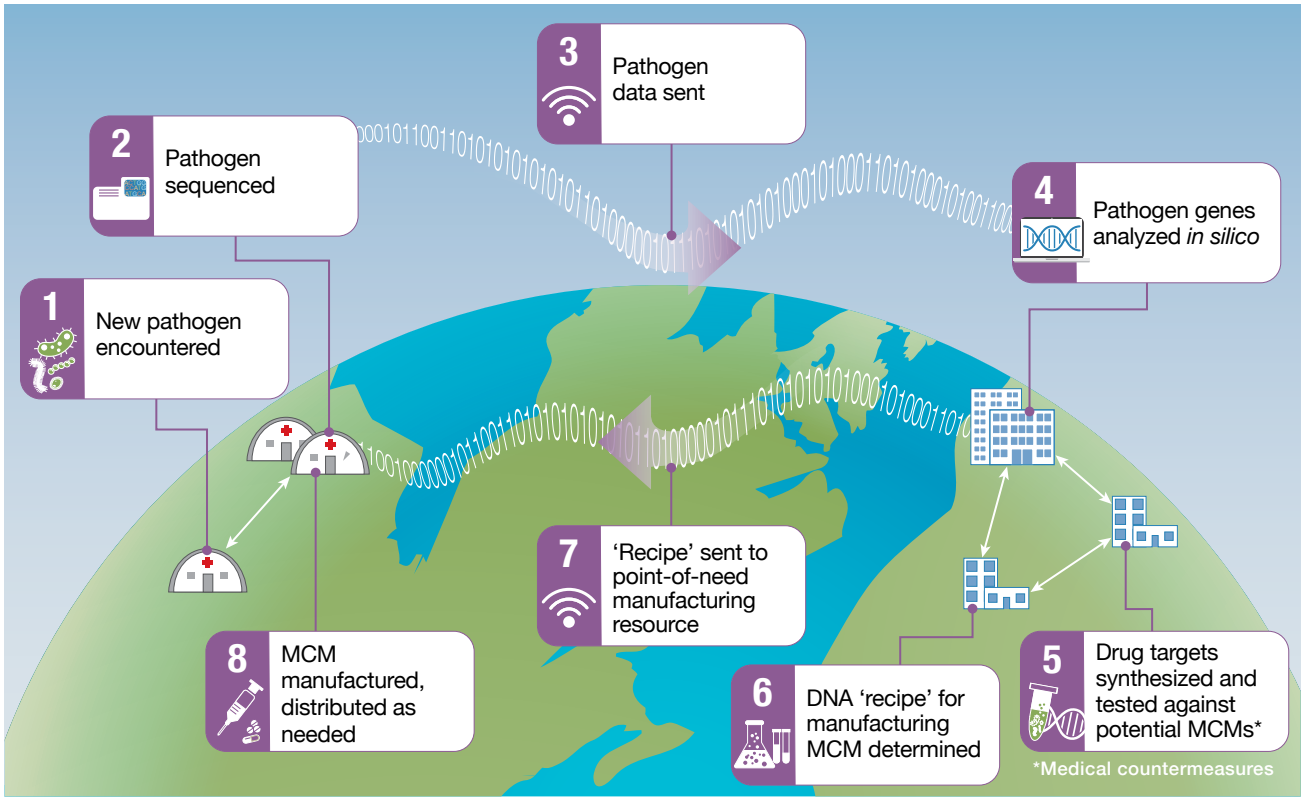


FIGURE 11. The figure illustrates a future pathway for rapid response to new pathogen outbreaks. Sequencing the genetic information of the pathogen allows efforts to commence (anywhere in the world) to determine what medical countermeasures (MCMs) are likely to be effective. A Biomolecular Processing Unit (BPU, see Figure 7) would provide capabilities for steps 5 and 6. In some cases, instructions for making the MCM (such as for a DNA-based vaccine) could also be transmitted back to the point of need for local, distributed manufacturing.

- Reconstruct the physical DNA or RNA molecules encoding those targets
- Employ these DNA sequences to make the corresponding proteins (if the target is a protein and not DNA/RNA itself)
- Perform assays to test whether available known drugs can be repurposed in the hopes of fighting the new threat

The most radical—and perhaps most exciting—possibility for this approach would be if the MCM itself can also be encoded into the information of a DNA sequence and transmitted back to the location(s) where the need is greatest. This pathway would be most plausible if the DNA sequence encodes a protein biologic (e.g., antigen for immunization or therapeutic enzyme), DNA vaccine, therapeutic RNA, or an inert version of the virus itself to be used as a vaccine. Some drugs could also be produced onsite through biosynthesis, with designs for the necessary enzyme genes transmitted from afar. (Penicillin is one example, requiring only a few enzymes [17].) We have also considered how the same approach could be used to transmit such countermeasure "recipes" through space to remote exploration and colonization missions on other planets [18].

Understanding Technological Risk

Synthetic biology advances are making the engineering of biological systems faster and more straightforward. While this progress is exciting for the wealth of applications enabled, it also raises concerns of possible misuse, both accidental and intentional. For example, the same DNA synthesis technology that enables rapid prototyping could be used to help recreate the (eradicated) smallpox virus from only its digital genetic information. Therefore, one role for the synthetic biology program at Lincoln Laboratory is to assess these technologies and consider long-term consequences for security and safety. Such projects may involve laboratory-based test and

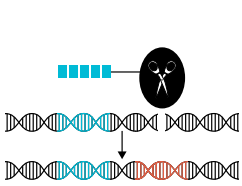
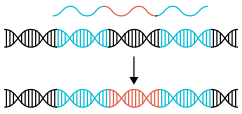
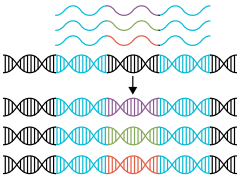
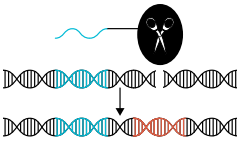
evaluation of these new capabilities or purely analytical studies drawing on the Laboratory's deep expertise and involvement in the synthetic biology research community.

For example, the biotechnology known as CRISPR (for clustered regularly interspaced short palindromic repeats, also CRISPR-Cas or CRISPR-Cas9) has produced a tremendous amount of excitement, not only in research circles but for policy, national security, and society at large. Because of the broad range of applications and an impressive relative ease of use, CRISPR has been quickly adopted around the world and adapted to an impressive array of new applications. CRISPR can be considered a tool (or better yet, a suite of tools) that makes genetic engineering easier. But because CRISPR has received so much excited attention in the press, there is a need to separate real capabilities from hype. However powerful, CRISPR is not a tool that replaces all other tools, nor is it a weapon in and of itself.

Table 2 shows a comparison between CRISPR techniques and previous gene/genome editing approaches. At the heart of each of these is the capacity to programmably home in on an exact location in a DNA molecule (even in a whole genome, which can include billions of possible locations). All of these approaches are programmable—users are able to specify the exact DNA location in the genome they wish to target. Site-directed mutagenesis (SDM) for example requires making the edits in vitro and then transferring the edited material into the cell. In the table, blue regions in the sequence indicate targeting, either with engineered proteins or synthetic short pieces of DNA or RNA. Red (plus green and purple for multiplex automated genome engineering [MAGE]) indicate modifications made to the genome, performed either as a cut and paste operation (zinc finger and TAL effector nucleases [ZFN/TALEN] and CRISPR) or an overwrite operation (SDM and MAGE). CRISPR, on the other hand, achieves its homing function through a short RNA molecule that can be specified and synthesized with relatively accessible and affordable resources.

In each case, the typical next step is to cut the targeted DNA, the start of a genetic cut-and-paste operation. However, the capacity to specifically target a chosen DNA sequence has found many other applications, including engineering new regulatory proteins that turn genes on or off. Furthermore, CRISPR systems have been impressively adaptable for engineering organisms for which previously few tools were available,

Table 2. CRISPR Compared to Earlier DNA Editing Approaches

	ZFN/TALEN	SDM	MAGE	CRISPR-Cas9
				
NO PROTEIN ENGINEERING REQUIRED	✗	✓	✓	✓
WORKS DIRECTLY WITHIN CELLS	✓	✗	✓	✓
ADAPTS EASILY TO DIFFERENT ORGANISMS	✓	✓	✗	✓

ZFN/TALEN = zinc finger and TAL effector nucleases; SDM = site-directed mutagenesis; MAGE = multiplex automated genome engineering; CRISPR = clustered regularly interspersed palindromic repeats

opening up many new applications and opportunities for scientific discovery. CRISPR has also proven quite facile for engineering the human genome.

Because gene editing technologies such as CRISPR are both powerful and progressing rapidly, concerns have been raised for the potential risks and their impacts on national security. But it is also worth re-emphasizing that CRISPR is a tool. Consider all the things you could suddenly do with a screwdriver if you never had one before. You could potentially build something dangerous or even build a weapon out of screwdrivers, if desired. But rarely would the concern be placed on the screwdriver itself. In a similar vein, with CRISPR, we recommend focusing concerns on specific applications of the tool and not the tool itself. For example, concerns have been raised about where future biothreats will be engineered using the tools of synbio, with some critics speculating that unconventional research environments, such as community biolabs, could be of particular concern. We performed an analysis of several factors impacting different research settings—both traditional (academic, government, industry) and unconventional—and noted that while it is conceivable that a biothreat could be engineered in any such space, community laboratories seem undeserving of special concern (see Table 3).

A recent Lincoln Laboratory effort in synthetic biology biothreat analysis was contributing to the report issued by the U.S. National Academies of Sciences, Engineering and Medicine (NASEM), "Biodefense in the Age of Synthetic Biology" [19]. The committee's work generated a framework for analyzing potential threats enabled by synthetic biology, applied that framework to the current biodefense landscape, and recommended options for risk mitigation. The final report noted three general areas that rose to the highest relative level of concern (among the biothreats considered): (1) recreating known viruses through DNA synthesis; (2) adding capabilities to bacteria by inserting new genetic functions, including genes encoding antibiotic resistance or toxins; and (3) engineering microbes to biosynthesize dangerous chemicals on or in the human body. One of the overarching conclusions of the report was that biodefense efforts focused only on narrow lists of dangerous agents (such as the Select Agent lists) would be insufficient to protect against the range of potential future biothreats enabled by synthetic biology.

Sharing Designs, Parts, and Responsibility

Synthetic biologists have also sought to engineer their own research culture. These efforts are both technical (creating best practices for design, fabrication, and

Table 3. Biothreat Concerns Mapped to Conventional and Unconventional Lab Settings

	LAB SETTING	OVERSIGHT	VISIBILITY	ACCESS TO EQUIPMENT	ACCESS TO TRAINING	CONTAINMENT
TRADITIONAL RESEARCH SETTINGS	Government	Low concern	Moderate concern	High concern	Moderate concern	High concern
	Industry	High concern	High concern	High concern	Moderate concern	Moderate concern
	University	Low concern	Moderate concern	High concern	High concern	Moderate concern
UNCONVENTIONAL RESEARCH SETTINGS	Incubator	Moderate concern	Moderate concern	Moderate concern	Moderate concern	Moderate concern
	Cloud	Moderate concern	Moderate concern	Moderate concern	Low concern	Low concern
	Community	Low concern	Low concern	Low concern	Moderate concern	Low concern
	Personal	High concern	High concern	Low concern	Low concern	Low concern

Low concern Moderate concern High concern

FIGURE 12. iGEM's annual Giant Jamboree brings together more than 300 teams of synthetic biologists representing upwards of 6,000 participants from dozens of countries. PHOTO CREDIT: IGEM FOUNDATION AND JUSTIN KNIGHT

measurement) and sociological (propagating norms of ethical choices for synbio research, transparency, safety, cooperation, a culture of sharing of designs and parts). Lincoln Laboratory has contributed to a number of these efforts, such as by creating avenues for openness and design sharing of hardware innovations (Metafluidics) and supporting the International Genetically Engineered Machine (iGEM) competition.

iGEM brings together aspiring synthetic biologists from around the world to compete in an annual showcase of their research projects. This "Olympics of Synthetic Biology" not only trains and inspires thousands of students, it also serves as a proving ground for the ideas and ideals of synthetic biology. By providing teams with a large toolkit of standard DNA building blocks (BioBricks) and standards for their construction, iGEM enables these researchers to build their own new genetic designs. Students also produce many of their own new DNA parts, which are then contributed back to the iGEM BioBricks collection, the Registry of Standard Biological Parts [8]. This give-and-get dynamic helps foster a collaborative community of synthetic biology innovators, which grows every year. The yearly cycle of iGEM culminates in the fall Giant Jamboree (Figure 12), where teams convene to share their work, compete for awards, and celebrate each other's achievements.

From simple beginnings at MIT in 2004, the iGEM competition has grown from five U.S.-based teams to 353 teams worldwide in 2019, involving more than 6,000 participants (college, graduate, and high school) each year [20]. The After iGEM organization represents the more than 45,000 iGEM alumni, many of whom have started new synthetic biology companies—often from their own iGEM projects. Lincoln Laboratory staff have been deeply involved in iGEM from the beginning, as part of the first design teams, and have served as team mentors, Jamboree volunteers (including roles of photographer and dance DJ), committee leaders, judges, and the current Director of Judging for the competition. Some foundational DNA parts in the Registry were created by the pre-iGEM 2003 design teams (including a member who is now on the Lincoln Laboratory technical staff) and have been reused by others more than 6,000 times.

Well beyond technical achievement, iGEM also engages its expanding international community with questions of responsible technology development.

Participants are encouraged to consider both how their work affects the world and how the world affects their work. With teams coming from different backgrounds around the world—and representing diverse regulatory and ethical frameworks—iGEM incentivizes strong norms for biosafety, biosecurity, and bioethics. The annual Giant Jamboree draws together specialists from these disciplines and several others (for example, policy, education, public health, biodefense), many of whom serve on its large panel of judges. iGEM becomes a gathering at which these professionals and the student competitors learn from and challenge each other.

In a similar fashion to the sharing of DNA-based designs within iGEM, we at Lincoln Laboratory have sought to foster better adoption and faster advances within the field of microfluidics by enhancing the sharing of hardware designs. We (and others) have noted that new microfluidic technology does not easily make the transition from the lab into common use or commercialization. One major obstacle has been reproducibility. Reproducibility has been hindered by the way microfluidic hardware designs have been incompletely shared in the research literature, with insufficient information transmitted for reproducing someone else's creation. To encourage a more open community of microfluidic innovators, we created Metafluidics, an online resource dedicated to the sharing of microfluidic designs and expertise [15]. Metafluidics provides a way for innovators to share their designs, methods, and operating protocols, so that a different user can try out those innovations. That new user may also remix and redesign, and contribute that modified version back to the Metafluidics community. Figure 13 shows an example of a microfluidic device design hosted at Metafluidics. Since the web resource launched in 2017, this community of users has grown to more than 2,000 members. We continue to support Metafluidics development in collaboration with David Kong (former Lincoln Laboratory technical staff) of the MIT Media Lab through the Living Computing Project funded by the National Science Foundation.

Another hindrance to innovation in microfluidics has been a general requirement for advanced (and expensive) microfabrication facilities, including clean rooms of the type often used for electronic fabrication. We have explored and shared how makerspaces can provide a viable alternative. Makerspaces are dedicated

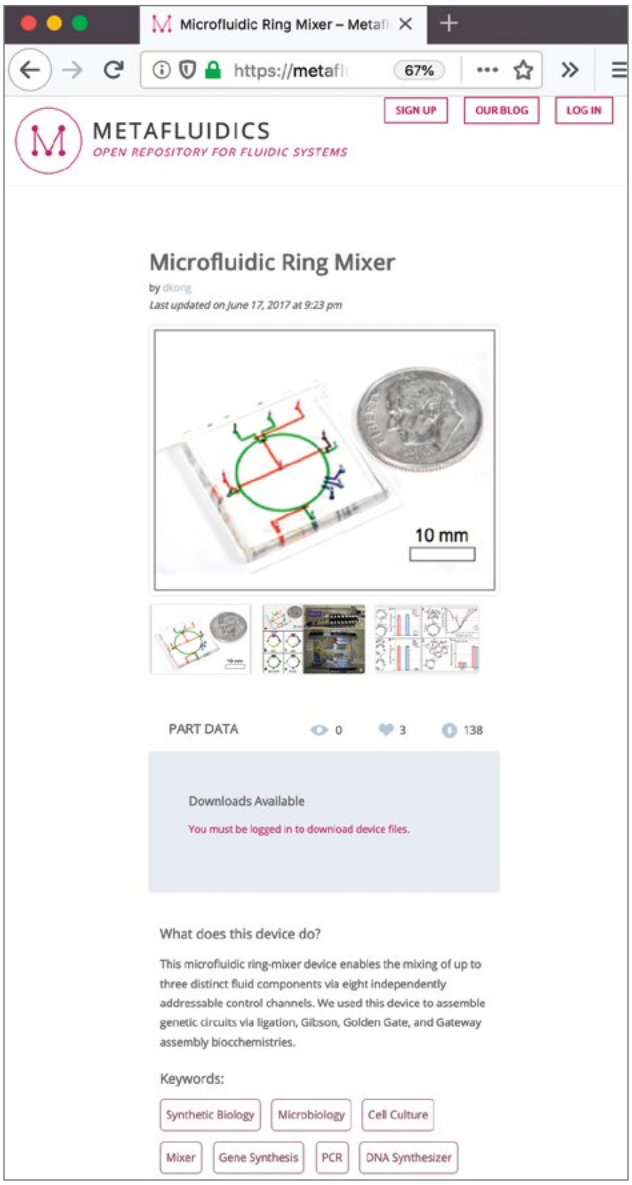


FIGURE 13. This sample page is for a Lincoln Laboratory device hosted on the Metafluidics web resource for shared microfluidic designs.

areas that provide a shared set of tools for a community that wishes to create. The tools available in makerspaces can be diverse, ranging from those found in machine shops and woodworking facilities to those for use in handcrafts and sewing, and more. Many makerspaces have adopted 3D printing technologies and even tools for genetic engineering. (Those that focus mainly on biology and genetic engineering are often called community bio labs, or DIY bio labs.) While the microfluidic devices developed at Lincoln Laboratory have typically required

expensive facilities (e.g., with specialized clean room environments) and equipment, we have also encouraged the use of makerspaces for those wishing to get started [21]. Experiments have included applying 3D printing to create new fluidic devices capable of manipulating and constructing with DNA [15]. We have also encouraged maker communities to contribute and share in solving needs for long-term space exploration and colonization [18]. One exciting application area would be to use a set of (DNA-based) designs to program microbes for biosynthesizing the feedstock materials needed for 3D printing. Thus some types of hardware could be 3D-printed on demand, such as replacement parts for space life-support systems.

Looking Forward

Synthetic biologists look to enable a future in which living things are far easier to engineer and in which such engineering can be applied to help with the world's biggest problems. The potential to engineer crops to withstand the stresses of climate change is one promising area, with the opportunity to alter plants to make them more drought- and heat-tolerant, increase yields, and require less fertilizer [22]. Another key area is improving and protecting human health by addressing rising concerns about naturally occurring pandemics, increasing antibiotic resistance, human accidents, and intentional weaponization of biology. Synthetic biologists are pursuing novel ways to protect against these threats. Engineered biological solutions are increasingly contributing to the bioeconomy [7, 23], and the desire to protect this contribution leads to many questions of how to balance progress with caution.

Lincoln Laboratory combines expertise in synthetic biology and many intersecting disciplines—such as bioinformatics, data science, and electronic device design—to make biology easier to engineer. We seek to enable the growth of the field of synthetic biology, to support national security both broadly and specifically. Broadly, creating new tools for bio-prototyping and measurement can stimulate the activities that fuel the bioeconomy, much in the way access to picks and shovels underpinned the 1849 Gold Rush. More specifically, Lincoln Laboratory's synthetic biology projects address needs in biosecurity by creating hardware and wetware sensor systems to detect specific threats (pathogens, chemical agents) and by accelerating the pursuit of countermeasures against these threats. In the process, we leverage the same diverse expertise to provide advice and analysis to the U.S. government regarding the promise and perils of these new technologies.

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About the Authors

Peter A. Carr is a senior staff scientist in the Biological and Chemical Technologies Group at Lincoln Laboratory, where he leads a synthetic biology research program. The central focus of his work is to expand the reach of what can be achieved through engineering living systems—at the level of single genes, entire genomes, or complex communities. He is a founding member of the MIT Synthetic Biology Center, serves as Director of Judging for the International Genetically Engineered Machine (iGEM) competition, and is a coauthor of the National Academies of Sciences, Engineering and Medicine's report *Biodefense in the Age of Synthetic Biology*. Prior to joining Lincoln Laboratory, he led research in DNA synthesis and genome engineering at the MIT Media Lab. He holds a bachelor's degree in biochemistry from Harvard College and a doctorate in biochemistry and molecular biophysics from Columbia University, and studied structural biology for drug discovery as a postdoctoral fellow at the Whitehead Institute for Biomedical Research.

Johanna Bobrow is a member of the technical staff in the Biological and Chemical Technologies Group at Lincoln Laboratory. Over her 20 years at the Laboratory, her research has spanned from sample preparation and technologies for PCR-based detection and identification of biowarfare agents, to protocol development for DNA forensics and sequencing, to physiological status monitoring and hearing damage modeling for warfighter welfare. More recently, she has turned to device design, rapid model prototyping and manufacture of novel devices for biological assays, and synthetic biology research. Prior to joining the Laboratory in 1999, she earned a bachelor's degree in brain and cognitive science at MIT.

James C. Comolli, a member of the technical staff in the Biological and Chemical Technologies Group, joined Lincoln Laboratory in 2015. His research interests include infection therapeutics and diagnostic technologies, biosensors, bacterial communities, pathogen-host interactions, and synthetic biology. Prior to joining the Laboratory, he was the leader of the Biomedical Engineering Group at Draper Laboratory; research director at ECI Biotech, a startup developing microbial diagnostics; and a staff researcher at Johnson & Johnson, working on medical devices. He has a bachelor's degree in biology from Johns Hopkins University and a doctoral degree in cellular and molecular biology from Harvard University. He completed postdoctoral training in bacterial pathogenesis at the University of California, San Francisco, and in microbial physiology at the University of Wisconsin–Madison.

Nicholas J. Guido is a member of the technical staff in the Biological and Chemical Technologies Group. The focus of his work is synthetic biology, including DNA synthesis, genetic networks and pathways, and the application of engineering principles to biological systems in general. Prior to joining Lincoln Laboratory in 2017, he was a senior scientist with Gen9, a start-up DNA synthesis company based in Cambridge, Massachusetts. In this role, he optimized the DNA synthesis process for production of difficult material and created a process for the synthesis of highly variant DNA libraries. In postdoctoral work in the Genetics Department at Harvard Medical School, he studied the modeling of the metabolism of bacteria, leveraging this information to carry out genome engineering and thus increasing the production of high-value products. As a graduate student, he conducted research into the modeling and physical construction of synthetic genetic networks in bacteria. He holds a bachelor's degree in bioengineering from the University of Pennsylvania and a doctorate in bioinformatics from Boston University.

Frances E. Nargi is a technical staff member in the Biological and Chemical Technologies Group. Her research is focused predominantly in the areas of biosensor and biological simulant development. She has worked as a research scientist in the areas of immunology and microbiology since 1985. Her areas of expertise include micro, cell, and molecular biology. She is one of the key team members who developed the CANARY biosensor and has been involved in the evolution and deployment of this sensor for the detection and identification of weapons of mass destruction and plant pathogens. She leads several programs, developed two simulants for use as surrogate biological agents, and designed and built numerous devices to facilitate sample preparation and to support assay development. Prior to joining Lincoln Laboratory in 1999, she worked as a microbiologist for the U.S. Department of Agriculture's Agricultural Research Services, where she established the immunology laboratory in the foot-and-mouth disease unit and evaluated the suitability of potential vaccine candidates. She holds bachelor's and doctoral degrees from the University of Connecticut at Storrs.

Todd A. Thorsen is a technical staff member in the Biological and Chemical Technologies Group, where he pursues research in microfluidics-based engineering, including the design and fabrication of state-of-the-art programmable microfluidic devices, exploitation of the microscale physical properties of fluids (thermal, chemical, optical, and electrical), and the development of microfluidic platforms for medicine. Prior to joining Lincoln Laboratory, he was a faculty member in the MIT Department of Mechanical Engineering. He has authored or coauthored 34 papers and two book chapters, has 40 issued and pending patents in the field of microfluidics, and has more than 8,800 citations of his published works. He holds a bachelor's degree in biology from the University of California, San Diego; a master's degree in infectious disease from the University of California, Berkeley; and a doctoral degree in biochemistry and molecular biophysics from the California Institute of Technology.

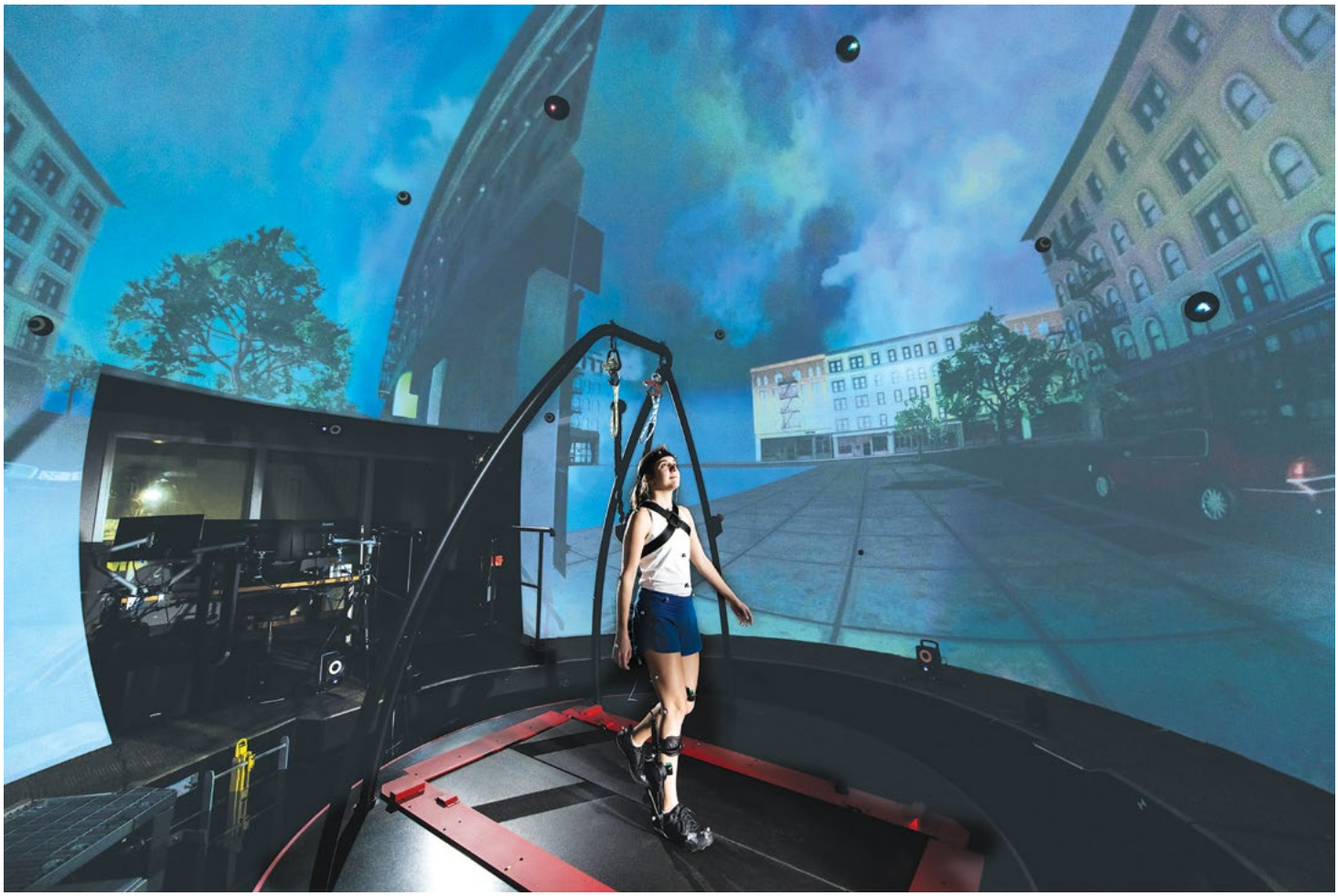
David I. Walsh III is a member of the technical staff in the Biological and Chemical Technologies Group. His research is focused on developing in vitro models and rapid, autonomous biosensors by using advanced 3D-printing techniques. Prior to joining Lincoln Laboratory in 2015, he was a National Science Foundation Graduate Research Fellow at Northeastern University, a visiting scholar at KTH Royal Institute of Technology in Stockholm, and a co-op student at Sandia National Laboratories in the Biosystems Research and Development group. He holds a bachelor's degree in nuclear engineering from Rensselaer Polytechnic Institute and a doctorate in bioengineering from Northeastern University. His doctoral dissertation on point-of-care diagnostics focused on the use of makerspaces to democratize the development of point-of-care biomicrofluidic devices beyond advanced engineering laboratories.

Matthew E. Walsh is an associate technical staff member in the Biological and Chemical Technologies Group. Since joining Lincoln Laboratory in 2014, he has worked on a range of programs supporting synthetic biology efforts. His technical work often relies on applying (bio)analytical methods to Department of Defense–specific applications. He is the principal investigator for the Rapid Medical Countermeasures program that looks to apply machine learning to therapeutic antibody development. He is a member of the 2018 Class of the Emerging Leaders in Biosecurity Initiative and holds a bachelor's degree in chemistry from Skidmore College.

Scott T. Wick is an associate staff scientist in the Biological and Chemical Technologies Group at Lincoln Laboratory. His current research efforts focus on design and integration of molecular, cellular, and synthetic biology into medical and environmental sensor platforms built to detect pathogenic organisms, toxic chemicals, and important biomarkers related to health and human performance. Prior to joining the Laboratory in 2004, he

was a research staff member at GPC Biotech (formerly Mitotix, Inc.), developing protein-based anticancer treatments, and at Sterling-Winthrop pharmaceuticals, developing antibody-based anticancer therapies. He holds a bachelor's degree in biotechnology from the Rochester Institute of Technology and a master's degree from Harvard University, publishing a thesis developing novel protein chimeras for vascular disease therapy.

Catherine R. Cabrera is the leader of the Biological and Chemical Technologies Group at Lincoln Laboratory. She joined the Laboratory in 2002, initially working on hardware and software development for the identification of biowarfare agents. She was part of the team that received an R&D 100 Award for the development of the PANTHER automated cell-based bioaerosol sensor, which has since transitioned to operational use for building protection and plant pathogen detection. She currently oversees a diverse portfolio of programs that include ones on molecular biomarkers for heath and performance, advanced DNA forensics, and engineered and synthetic biology. Her areas of technical expertise include microfluidics; biodefense technologies, systems, and architectures; red/blue team analysis; microbiome and human health; point-of-need diagnostics; genetic and epigenetic biomarkers of health and activity; and use of physiological status indicators to provide early warning of exposure to chemical warfare agents or pathogens. She holds bachelor's degrees in biochemistry and chemical engineering from Rice University and a doctorate in bioengineering from the University of Washington. Her doctoral research was focused on developing fieldable technologies to detect pathogens in resource-limited environments.



In Lincoln Laboratory's Sensorimotor Technology Realization in Immersive Virtual Environments (STRIVE) Center is a 24-foot virtual reality dome, the Computer Assisted Rehabilitation Environment (CAREN), which allows users to experience immersion in a simulated world. A user interacts with an environment that is displayed on a 360-degree screen while walking on a 6-degrees-of-freedom motion platform that mimics the environment's terrain. A motion-capture system measures and analyzes the user's movements in real time. In the image above, the CAREN is being used to monitor how an individual interacts with a prototype exoskeleton that is designed to help a person hike with less strain on lower-limb muscles. The STRIVE Center is also used to study patients' cognitive and physical performance, test rehabilitation techniques, and assess the effects of training regimens. More about the STRIVE Center can be found in the appendix to the article "Biomechanical Sensing and Algorithms" on page 165.