BioBuilder

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Preface

This book began with a relatively modest mission, namely to support the growing BioBuilder community, the teachers and students around the country and the world who are learning to engineer biology. BioBuilding is a challenging endeavor, and we heard over and over that a book like this would help. So here it is. We hope it's helpful. We hope you like it.

We've written for a non-scientist, non-engineer reader. Importantly, though, the questions that motivate the content are the very hard questions that synthetic biologists wrestle with daily. How can cells be engineered in a rational way? Is it possible to ensure reliable behaviors? What can we learn as we approach life science with an engineer's toolkit?

Who Should Read This Book

We hope a wide range of readers will find this book useful. Our writing began with teachers in mind, since these are the individuals we've come to know through the BioBuilder program. They have been enthusiastically bringing this content into their biology and biotechnology classrooms around the country and throughout the world. Early feedback we got on the book made us realize that teachers wanted to share the book directly with their students, and so the teacher's manual we'd originally envisioned soon morphed into a resource that students and teachers can use together.

We think our book now meets the needs of many curious readers and all kinds of learners. Our intended audience includes high school and college instructors, students active in biodesign clubs, adults engaged in community laboratories, and artists working in design studios. We hope there is something in this book for everyone who wants to know more about the theoretical and practical aspects of synthetic biology.

Why We Wrote This Book

BioBuilder bridges the gap between the way we do science and the way we teach it. The ideas presented here and the labs that spring from them are based on current research in the field. The authenticity sparks student interest, and we've seen how it can excite and empower learners at all levels. But it's hard to be working on the edge of what's known, so we wrote this book to support the teachers and students who want to use unknowns as their point of departure for learning.

We also wrote this book because we love synthetic biology's mixture of science and engineering, and we wanted to show how it's working in practice. Our foundational chapters emphasize how synthetic biology brings some successful tools from more mature engineering disciplines to the life sciences. Our laboratory-focused chapters start with an authentic research question and then provide protocols to approach it from an engineering perspective.

With its mixture of foundational content, lab investigations, and biodesign activities, this book is our best effort to engender curiosity and enthusiasm for the field. We take an approach to synthetic biology and education more generally that resembles what Antoine de Saint Exupéry encouraged for ship-building in *The Little Prince*, namely "If you want to build a ship, don't drum up people to collect wood and don't assign them tasks and work, but rather teach them to long for the endless immensity of the sea."

A Word on Synthetic Biology Today

The goal of synthetic biology is to engineer robust, synthetic, living systems in scalable and reliable ways. Some who are working in the field are applying synthetic biology to address our planet's need for sustainable food and fuel production. Others are developing biotechnologies such as medical diagnostics or treatments. A smaller but vital subset of people view synthetic biology as a design challenge that they can apply to art, architecture or community innovations. No matter what the application space, though, you'll see we've got a lot to learn and long way to go before the work is easy to do.

Even in its early days, synthetic biology has a lot to teach us. New scientific understanding, improved engineering approaches and novel technologies are all "naturally" derived from this "synthetic" approach. Building biology tests our current understanding of the world and so serves as a powerful force to discover new science. It also motivates the development of new tools and processes that speed the engineer's design/build/test cycle. As a genuinely interdisciplinary endeavor, the field brings coherence to STEM education in a way that seems unique to us. So while the field may be immature, we think it is already an outstanding approach to teaching and learning. Because it is based on synthetic biology's real unsolved questions,

BioBuilder's curriculum applies knowledge, and so moves classroom and laboratory education away from rote memorization and cookbook technical steps. It features synthetic biology as a series of investigations that integrate scientific understanding with engineering approaches to develop solutions that meet real-world challenges.

Navigating This Book

This book is intentionally modular and can be reordered to suit your needs. In its digital form, the chapters can easily be rebundled and shared through our creative commons license. The book's printed form required a sequence for the chapters, and so we have organized things as follows.

Chapters 1–4 introduce some of the approaches fundamental to synthetic biology:

- Chapter 1, *Fundamentals of Synthetic Biology*, provides a basic introduction to the field, emphasizing synthetic biology's interdisciplinary nature and some of its foundational tools from engineering and molecular biology.
- Chapter 2, *Fundamentals of Biodesign*, provides a framework for biodesign, including an abstraction hierarchy for managing complexity and some examples that unpack living systems into the devices and parts that encode them.
- Chapter 3, *Fundamentals of DNA Engineering*, discusses the role of standardization in engineering and dives into a few examples of standardized DNA assembly techniques.
- Chapter 4, *Fundamentals of Bioethics*, introduces questions about what constitutes "good" work, using modern and historic examples to illustrate the challenges and then provides a framework for teaching with these examples.

Chapters 5 through 10 detail BioBuilder's laboratory investigations. Each starts with a description of a current challenge or developing idea in the field, offering some organizing principle or question that the experiments can then probe. Each lab chapter also includes an abbreviated protocol for carrying out a relevant investigation and helpful illustrated guides. Posters and quick guides can be downloaded from a GitHub repository for this book:

- Chapter 5, *Introduction to the BioBuilder Labs*, is an overview of the labs, defining distinct entry points in the design/build/test cycle for engineering.
- Chapter 6, *Eau That Smell*, models the biodesign framework detailed in Chapter 2 to ultimately ask the question: which genetic design will more effectively enable exponentially growing bacterial cells to generate a banana-scent?
- Chapter 7, *iTune Device*, focuses on principles of measurement and the role measurement plays in the predictable design. The laboratory portion of the chapter compares predicted and measured outcomes using combinations of genetic parts that regulate enzyme production.

- Chapter 8, *Picture This*, applies modeling techniques to understand and characterize the "bacterial photography system" in which bacteria serve as pixels in a living photograph.
- Chapter 9, *What a Colorful World*, considers the role of chassis in biological engineering first with a few complementary frameworks for chassis design and then by comparing the function of identical genetic programs in different strains of *E. coli*.
- Chapter 10, *Golden Bread*, examines the unreliable performance of a synthetic living system, namely a yeast that can produce the precursor to vitamin A. Scientific and engineering experiments explore redundancy as a way to understand and improve the behavior of the cells.

The book ends with abbreviated instructions for the preparation of common laboratory reagents, followed by a glossary of terms used throughout the book.

Online Resources

The material in this book only scratches the surface of what you'll find on the BioBuilder website, so if you like what you find here, then we encourage you to navigate to *biobuilder.org*.

The site is fully open-access and provides the following:

- Animations that explain a few of the foundational concepts presented in this book
- Downloadable slides for teaching the laboratory and classroom materials in Chapters 5–10
- Screen capture videos from our teacher professional development workshops
- Practical tips for running the experiments, including quick guides and posters for printing
- A portal for sharing and comparing data collected in these experiments with the data collected by others
- Assessment ideas and rubrics
- Newsletters to keep up with ongoing development of the BioBuilder content and community

If you'd like to get directly involved, the website also has the following:

- Links to order kits for carrying out the experiments themselves
- Links to sign up for BioBuilder workshops
- A companion "Biobuilder for Teachers" resource site with classroom-tested extensions of the BioBuilder content
- Information on BioBuilder's BioDesign Club, an afterschool extracurricular option for bringing the content to students

The curriculum and teacher training from BioBuilder are developed and supported through a non-profit organization. More information about The BioBuilder Educational Foundation, a 501c(3), is available at *biobuilder.org*.

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Acknowledgments

As we wrote this book we asked many of our friends and colleagues for guidance and feedback. They've responded with incredible generosity, and we've relied on things they've taught us. We thank them all, and offer special acknowledgement of Michael Loukides and Brian MacDonald at O'Reilly, who made this book possible with their vision and encouragement. We also thank SynBERC for their generous early support of this endeavor, and Prof. Susan Marqusee and Mr. Daniel Grushkin for introducing several of us to each other. Many colleagues read chapters in their earliest stages, including Prof. Kristala Prather from MIT and Dr. Reshma Shetty from Ginkgo Bioworks. Their insights were critically important as we got off the ground, and Reshma also made our initial introduction to the team at O'Reilly, so we extend great thanks to her for that as well. Dr. Megan Palmer framed our thinking and our explanation of what it means to do good work, material you'll see in our chapter on Bioethics. Along the way we've also asked more targeted questions of some experts. Dr. Jason Kelly and Mr. Bill Burns helped on the trans-Atlantic cable history, Dr. Barry Canton framed our discussion on chassis design, Ms. Sarah Tyndall answered our measurement questions, and Mr. Chris Brown answered our questions about the use of models in architecture.

The BioBuilder content itself has been developed in collaboration with teachers in secondary and post-secondary classrooms all over the country. It grew out of some teaching in the Department of Biological Engineering at MIT that started in 2004, including some project and laboratory classes co-taught with Prof. Drew Endy. The initial extension of this teaching curriculum to the high school setting was launched in collaboration with Mr. Jim Dixon. Since this book was written to be useful for teachers, we got lots of input on the drafts. In particular we thank: Dr. Veronica Zepeda, Dr. Ellen Jorgensen, and Dr. Oliver Medvick for their review of the content from start to finish. We also thank Ms. Sherry Annee, Dr. Melissa Wu, Dr. Rebekah Ravgiala, Mr. George Cachianes, Mr. Aaron Mathieu, Prof. Stephanie Stockwell, Dr. David Mangus, Dr. Sarah Bissonnette, Dr. Eddy Kim, Prof. Sarah Moore, Ms. Tammy Due Fay, Dr. Steven Nagle, Ms. Samia Saleem, Dr. Justin Pahara, Mr. Wythe Marschall, and Mr. Kevin McCormick.

In addition to our wonderful families, friends and colleagues, we four of us are grateful for each other. This book has been a team effort and, without each other, there would be nothing like it.

CHAPTER 1

Fundamentals of Synthetic Biology

Welcome to the BioBuilder program! We are thrilled that you want to bring the tools of synthetic biology into your classroom. Online, we have a variety of materials to help you get started, including some practical lab video tutorials, Microsoft PowerPoint slides, curriculum guides, and lab worksheets. In this written manual, we introduce foundational ideas that underlie synthetic biology, some key aspects of biology that are explored in the field and in the BioBuilder labs, and some helpful information to use as you run the experiments in the BioBuilder program.

In this chapter, we introduce the basic concepts of synthetic biology, explain how it differs from traditional biochemistry and genetic engineering, and begin to explore some of the fundamental engineering principles that will inform how we can solve problems using synthetic biology.

What Is Synthetic Biology?

At the most basic level, synthetic biologists, or *biobuilders*, want to engineer living cells to do something useful; for example, treat a disease, sense a toxic compound in the environment, or produce a valuable drug. As Figure 1-1 suggests, synthetic biologists achieve these outcomes by altering an organism's DNA so that it behaves "according to specification," as engineers say—basically, so it does what the biobuilder wants.

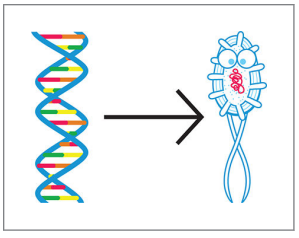


FIGURE 1-1 *The goal of synthetic biology. Synthetic biology aims to write DNA (left) that instructs a cell or organism (right) to behave according to design specifications.*

We can think of cells as complex miniature factories. The DNA provides instructions to make all the machines in the factory—proteins, other nucleic acids, multicomponent macromolecular complexes, and more. These "machines" then carry out the work of the cell. The organism's naturally occurring DNA allows the cell to meet its basic survival and reproductive needs. Synthetic biologists can *change* a cell's DNA so that the cell takes on new, useful functions (Figure 1-2). We'll talk more about how researchers alter an organism's DNA later in the chapter.

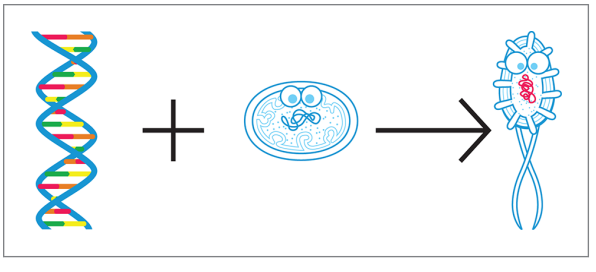


FIGURE 1-2 *Synthetic biology today. Currently, synthetic biologists generally design a portion of DNA (left) and combine it with an existing cell or organism (middle) so that the new cell or organism (right) behaves according to design specifications.*

Ultimately, synthetic biologists would like to be able to build specialized living organisms from scratch using designed DNA. The field isn't there yet. Currently, most endeavors involve the modification of organisms that already exist rather than building all-new organisms to behave in novel ways.

Why Synthetic Biology?

Many of the challenges that synthetic biologists are targeting can be addressed by other engineering disciplines, such as electrical, chemical, or mechanical engineering, but synthetic biology's solutions offer a few unique advantages.

Most strikingly, **cells can make copies of themselves**. Cars can't copy themselves—you need a factory to build a car. Also, some organisms can copy themselves incredibly quickly, even with minimal nutrients. For example, in the lab, the bacterium *E. coli* can replicate and divide in about 30 minutes. Therefore, synthetic biology is an attractive approach for producing large amounts of a specific product because we can grow a programmed cell relatively easily to meet large-scale production demands. Cells serve as the physical factory for production, as well, providing much of the "bricks and mortar" infrastructure that would be required by other engineering

solutions to meet the same challenge. Finally, the use of rapidly dividing cells also facilitates prototyping and testing, which are very important for the design cycle, which we'll discuss in more detail a little later.

Second, **cells contain the biological machinery to carry out many complex tasks**—specific chemical reactions, for example—that would be difficult, if not impossible, to accomplish otherwise. And, they do so with nanoscale precision that is difficult to replicate in any traditional fabrication facility. Also, when their nanoscale machinery breaks, cells have mechanisms to repair themselves, at least to some extent, which puts them at a great advantage over more typical factory-based production processes. Cellular complexity introduces its own hurdles to be considered, as well, but its potential utility is enormous.

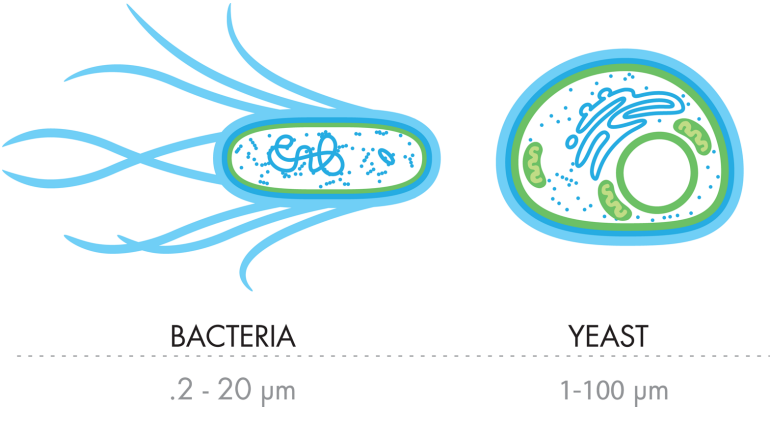
Third, **synthetic biology has the potential to produce eco-friendly solutions** to many difficult problems. By necessity, the byproducts of synthetic biology applications are generally nontoxic, because most toxic compounds would kill the very cells that are doing the work. In addition, harnessing natural cellular systems often results in economical processes. Today's industrial production of compounds consume large quantities of energy, often creating significant amounts of environmentally harmful waste and frequently requiring high temperatures or pressures.

Beyond its usefulness for addressing real-world challenges, **synthetic biology is also a fantastic approach to learn more about the workings of natural systems**. As researchers dissect increasingly complex cellular functions, they can use synthetic biology to test their hypotheses from additional angles. For example, if their biochemical research results suggest that a certain protein acts as a sort of on/off switch, they can test this result by replacing the existing protein with a protein that is known to exhibit on/off behavior. If the new synthetic system and the natural system behave similarly, the result provides further evidence that the natural protein acts as the researchers suspected.

You might wonder: do we know enough about cells to reliably engineer them, and if not, should we really be trying? **There are many justifiable fears and concerns unique to synthetic biology**. Granted, other inventions such as the light bulb and the telegraph were engineered without full understanding of the physics of electricity, but engineering life has additional practical, moral, and ethical challenges beyond those faced in traditional engineering fields. For instance, evolution can mutate DNA that has been painstakingly programmed, ruining a cell's engineered function. Replication of synthetic cells in the environment might pose a hazard if they interact in unexpected ways with existing organisms in that ecosystem. And, synthetic biology raises philosophical questions as we begin to think about cells as tiny living machines built to do our bidding. Any technology that asks us to reconsider our interaction with the natural world must be approached carefully. Researchers, bioethicists, and government organizations are actively discussing these issues and working to develop

synthetic biology in responsible ways that will improve the living world. We explore these issues in more depth in the Fundamentals of Bioethics chapter.

We are still in the early days of this developing discipline. As described earlier, synthetic biologists are not yet able to make organisms from scratch; at present, they are working primarily within the framework of existing organisms. Also, research so far has been conducted primarily on relatively simple unicellular organisms such as bacteria (especially *E. coli*) and yeast (*S. cerevisiae*), although there have also been some early successes in more complex systems like plants and mammalian cells. As the field grows, though, engineering increasingly complex systems will expand even further the potential applications and benefits of synthetic biology.



Synthetic Biology in Context

The synthetic biology approach might remind you of genetic engineering, in which researchers make small-scale rational changes to an organism's genome—such as removing a gene from a mouse or adding a human gene to a fruit fly—to study the system's behavior. Synthetic biologists use many of the same tools that genetic engineers do, as we will discuss in more detail later, but **synthetic biology and genetic engineering differ in the scale at which they aim to make these changes**. Genetic engineers are usually introducing one or two small changes to investigate a specific system, whereas synthetic biologists aim to design new genomes and redesign existing genomes at a grand scale. An illustrative—albeit fanciful—example of synthetic biology's potential scale is the genetic reprogramming of a tree so that it will grow into a fully functional house based on the genetic instructions designed by a synthetic biologist. Such a system would take advantage of the tree's natural program (to grow by taking in a few nutrients from the environment) and put it to use for society's needs. Genetically programming a tree to grow into a house, however, is far beyond the scale of traditional genetic engineering as well as the capacity of synthetic biology at this point.

To accomplish such large-scale design goals, synthetic biologists are establishing a structured engineering and design discipline, the principles of which we will introduce in the next section. Synthetic biologists are also drawing on the rich knowledge regarding how biological systems work that biochemists, molecular biologists, and geneticists have obtained over many years. Specifically, scientific research has yielded:

- Reasonably well-characterized model systems, such as *E. coli*, yeast, algae, and various types of mammalian cell culture, that offer a solid foundation for synthetic biology exploration
- Bountiful sequence data from a huge array of organisms, including bacteria, humans, mosquitoes, chickens, lions, mice, and many, many more, as well as tools for sequence comparison and analysis
- The molecular tools to move, reorder, and synthesize DNA to create new sequences

Synthetic biologists use these discoveries and successes as a foundation to which they can apply an engineering mindset to solve real-world problems. The interdisciplinary nature of synthetic biology is suggested by Figure 1-3.

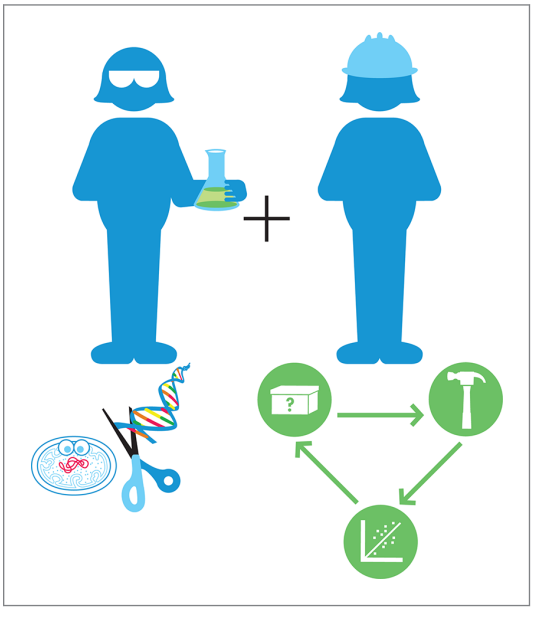


FIGURE 1-3 *The interdisciplinary nature of synthetic biology. Synthetic biologists combine the wealth of knowledge and techniques from molecular biology (left) with engineering principles (right), including the design-build-test cycle that's a hallmark of engineering disciplines.*

Introduction to Engineering and Design

Engineers build complex systems that must behave consistently, according to the design specifications. To accomplish their goals **engineers cycle through design, building, and testing phases, often doing rapid prototyping of different designs to find the most promising direction**. This procedure resembles the scientific method, in which the researcher cycles through hypotheses, experiments, and analysis. The primary difference is that the scientific method aims to understand the precise details of how something works, whereas the engineering approach will not focus on why a design works as long as the prototype tests successfully. These differences are discussed in more depth in the Fundamentals of Biodesign chapter.

Here, we introduce a very simple example to show how different types of engineers might solve a problem: watering houseplants. By considering how different engineering disciplines might address this problem, we will introduce some design fundamentals and illustrate how synthetic biologists apply a similar mindset and approach.

"Traditional" Engineering Solutions

Some people naturally have a green thumb, but others need some extra help; otherwise, their plants end up looking dried and shriveled. Different types of engineers would approach this plant watering problem differently, depending on their expertise. For example, a mechanical engineer might design a pot with an unevenly weighted round bottom. When the reservoir in the bottom is full of water it acts as a counterweight and keeps the pot standing straight. As the plant absorbs the water, the counterweight decreases and the pot begins to tip over. This visual indicator would be an obvious reminder to the owner that the plant needs water. Perhaps the leaning plant could even turn on a faucet to water itself. By engineering feedback into the system, the pot would stand back up when the plant was watered, creating a closed-loop control system.

One potential complication with this design is that some plants require more water than others, so the designers might need to create many different pots with different weights in the bottom, and the gardeners would need to make sure they are buying the correct pot for their plant. These types of considerations are integral to the design process. No design is perfect, and it is important to understand the strengths as well as the limitations of any proposed design when considering the best way to proceed.

An electrical engineer might come up with a completely different solution to the watering problem, one involving electrical moisture sensors and automatic watering. Her system might consist of many electronic parts: wires, resistors, capacitors, moisture sensors, circuit boards, and more. The different parts could work together to monitor the system, determine when the plants need water, and then deliver that water when needed.

This electrical engineering solution requires standardization, a crucial principle in all engineering fields and one we will return to later in this chapter. In this plant watering example, each standardized electronic component was defined by the particular independent function it could carry out. The components were built to meet a set of industry standards. This standardization of basic parts makes it possible for them to be connected to one another easily and reliably, without the context affecting their behavior. Such standardizations simplify design, allowing engineers to know how a certain piece will behave and how it can be combined with other parts to yield a desired result. It also simplifies manufacturing, enabling factories to produce millions of identical resistors for millions of different products. Synthetic biology has not yet achieved this level of standardization but is trying to move in that direction.

Engineering Toolkits

These two examples of traditional engineering solutions to the plant watering challenge illustrate how multiple designs can be used to solve even a relatively simple problem. **The approaches were largely dictated and influenced by the "toolkit" available within each engineering discipline.** Generally speaking, every approach draws from a toolkit with a few different parts, like the nuts and bolts that need to be put together, as well as a handful of methods for putting things together, such as the hammers and screwdrivers for assembling the parts. The toolkit also contains concepts and ideas that guide each field. The specific elements of a toolkit tend to vary quite a bit across different disciplines. For example, the mechanical engineer's toolkit contains materials with a variety of properties, such as metal, plastic, and concrete, as

well as tools and methods to manipulate the materials, including saws and welders. Gravity is one example of a concept that they use in their designs. Electrical engineers, on the other hand, have a completely different toolkit. Their parts include wires, resistors, capacitors, and circuit boards, and they have developed their own highly specialized manufacturing processes to create and combine these parts. Electrical engineering ideas further utilize a modern understanding of electrical signals.

For synthetic biology to become a mature engineering discipline, synthetic biologists must define their toolkit. Like mechanical engineering and electrical engineering, the tools will include parts that need to be put together and the methods for assembling them; of course, the parts and methods will be specific to biology. Many of the tools in the synthetic biology toolkit are derived from molecular biology. In the next section, we will introduce some of the components of these previously existing toolkits and explore how they are also implemented in the toolkit of synthetic biology.

The Synthetic Biology Toolkit

To explore the synthetic biology toolkit, let's first think about how biologists might approach the plant watering challenge. Broadly, they would use genetic tools to change the plants themselves. Such an approach could take many different forms. For example, one solution might use a gene discovered in chameleons that is responsible for changing color in response to stress. It's possible that this gene could be inserted into plants; thus, they then could change their color to alert us when they need water. This approach is analogous to the mechanical engineer's approach of adding a visual indicator (the pot tipping over) to help the owner remember when the plant needs water.

There could also be a biological solution that is more analogous to the electrical engineering solution, which frees the owner of the plant from the need to provide water at all. What if it were possible to isolate a gene or two from a cactus plant—or, maybe even more whimsically, from a camel—that helps these organisms withstand the very low water supply in their desert habitats? These genes, inserted into a plant, might help them survive with very little water, as well.

Both of these solutions could be approached with today's molecular biology tools, but these types of small modifications do not meet the synthetic biologist's goal of larger-scale genomic manipulation that would be required for an application such as growing a house and all its furniture from a seed. Such wholesale genomic design requires a full engineering toolkit. Such a toolkit must begin with, and build upon, contributions from the established fields of molecular biology and genetic engineering.

The Molecular Biology Toolkit

Molecular biologists have spent years developing methods to manipulate DNA in different ways. Following are three of the most crucial and well-established techniques, which are used extensively in synthetic biology:

- Reading the DNA code
- Copying existing DNA sequences
- Inserting specific DNA sequences into existing DNA strands

These techniques have become well established over years of molecular biology research, and researchers continue to develop new technologies that improve the processes. Dr. Frederick Sanger and Dr. Walter Gilbert developed robust *DNA sequencing* technology in 1977 using chain-termination chemistry that made it possible to accurately determine the pattern of Gs, As, Ts, and Cs in long DNA strands. Routine copying of existing DNA sequences in a laboratory was jump-started in 1983, when Dr. Kary Mullis developed *Polymerase Chain Reaction* (PCR). PCR is a powerful method that uses a cellular protein for copying DNA and a genetic template provided by the researcher to synthesize large amounts of a specific DNA sequence. Finally, Dr. Paul Berg, Dr. Stanley Cohen, and Dr. Herbert Boyer developed *recombinant DNA* (rDNA) techniques in the 1970s with which researchers can easily and precisely combine DNA sequences from different sources, including different organisms, based largely on a variety of naturally occurring proteins called *restriction enzymes* that cut DNA at specific sequences. These methods were inspired by and use the tools of naturally occurring cellular processes. Table 1-1 illustrates these parallels.

Table 1-1. The molecular biology toolkit and its natural origins

Tool	Molecular biology technique	Natural cellular process
Reading DNA	Sequencing	DNA replication
Copying DNA	PCR	DNA replication
Inserting DNA	rDNA with restriction enzymes and ligases	Defense from infection, DNA recombination and repair

Molecular Biology

DNA replication is a naturally occurring cellular process that creates new DNA sequences from existing DNA templates, usually to create new genetic material so that the cell can divide. This process varies between different species and can require many proteins to unwind the DNA and initiate replication, but the key requirements are the following:

DNA polymerase
: The enzyme that adds nucleotides to the growing chain.

A DNA primer
: This is a short chain of already synthesized DNA that binds to the beginning of the sequence to be replicated (DNA polymerase can only add new bases to an existing chain).

Free nucleotide bases
: Free A, T, C, and G nucleotides, together referred to as *dNTPs*, that are available in the cell to be added to the growing chain.

Sanger sequencing is a laboratory technique to determine the sequence of a DNA fragment. Researchers mix the DNA that they want to sequence with a DNA primer, DNA polymerase, and dNTPs to start replication. Also added to the mix is a small amount of modified bases that stop chain elongation once incorporated. These modified bases are also tagged, usually with radioactivity or fluorescence, and each base has a unique tag. The fragments that result from the disrupted replication process can be ordered based on size, and the sequence is read based on the tag on the modified base at the end of each fragment.

PCR is a laboratory technique to create many copies of an existing piece of DNA. This process mimics natural DNA replication. The researcher combines the desired DNA (called the "template"), primers that specify where the replication should begin and end, DNA polymerase, and dNTPs. The mixture is then cycled through different temperatures that facilitate different steps. First, the mixture is raised to a high temperature so that all the DNA bases are unpaired. The temperature is then lowered, allowing the primers to bind to the template DNA. Finally, the temperature is raised slightly to allow the DNA polymerase to work. This process is repeated many times to create many copies of the desired DNA fragment.

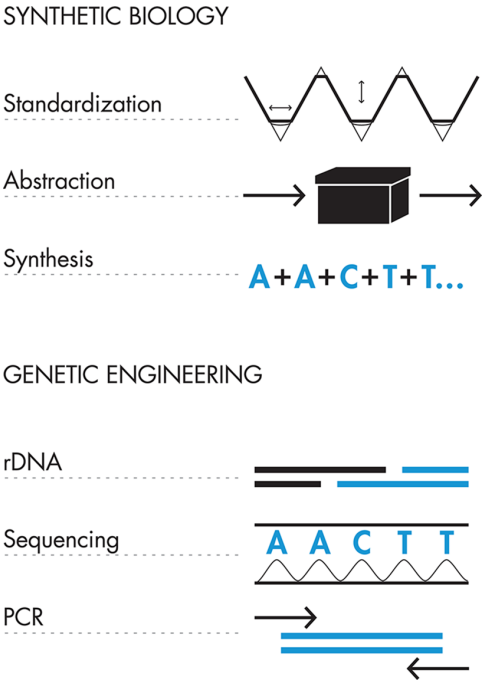
Restriction enzymes rare naturally occurring enzymes, which can also be used in the lab, that cut DNA at specific sequences of bases to create ends that are either blunt or "sticky"; that is, a few unpaired bases at the end of double-stranded DNA. When DNA pieces with complementary sticky ends are combined, they associate with each other, resulting in a new sequence, as illustrated in Figure 1-4.

FIGURE 1-4 *DNA cut with restriction enzymes. The pairs of black and blue bars represent double-stranded DNA, color coded to show where it has been cut with restriction enzymes to leave complementary "sticky ends" (left) or "blunt" ends (right) that can reconnect as shown.*

Plasmid is a small, circular piece of DNA most frequently found in bacteria that persists in the cell independent of chromosomal DNA. They are useful in molecular biology for transferring designed genetic systems into cells of interest. When used for this purpose they are frequently called "vectors."

The Toolkit Expanded for Synthetic Biology

Although these methods have been around for many years and have been used to great effect in research, they are not sufficient for synthetic biology. They might be sufficient to insert a gene from a chameleon into a plant, for example, but they would not enable the reliable reprogramming of a plant to grow into a two-bedroom, two-bathroom house. Consequently, we use the term *genetic engineering*, not *synthetic biology*, to refer to the relatively small-scale manipulation of genes in a host organism, perhaps altering at most a handful of genes.



Synthetic biology, on the other hand, aspires to write and rewrite entire genetic programs to create useful functions and products. To achieve these more ambitious engineering goals, synthetic biologists expand their toolkit beyond that of traditional genetic engineering to also include design principles from the more established engineering disciplines. They will frequently draw from the language of engineering, which provides a useful framework for thinking about design.

These additional tools, which are still largely in development, include: standardization, abstraction, and *de novo* DNA synthesis. Both standardization and abstraction are directly drawn from the toolkits of other engineering disciplines, whereas DNA synthesis is an engineering tool unique to synthetic biology. We will describe each of these topics in more detail later, but following are brief definitions:

DNA synthesis
: This is a process for the chemical production of DNA strands without a preexisting physical template, and is used at a much more extensive level in synthetic biology than is required for molecular biology.

Standardization
: This is an approach that aims to generate a set of components that might be useful in multiple systems and that can be recombined for different outcomes.

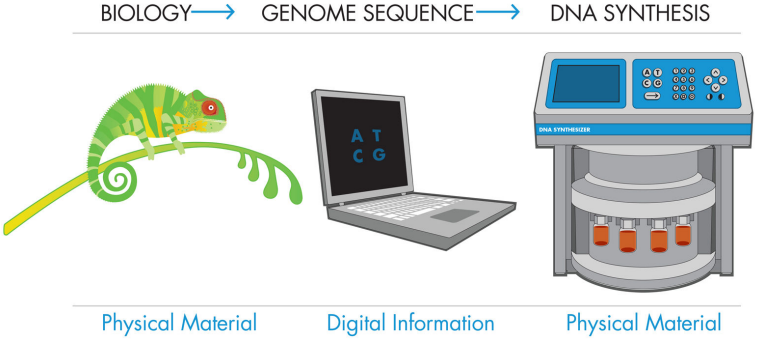
Abstraction
: This is a tool to manage detailed information when building a complex system. With it, designers can "get the job done" without trying to keep in mind exactly how every detail of a system works. In practice, engineers use different levels of abstraction depending on where they are in their design-build-test cycle.

DNA synthesis

DNA can be produced by a series of simple chemical steps that are not fundamentally different from any set of chemical reactions that adds one building block to another. In the case of DNA, these building blocks are nucleotides, but other examples of polymers made from building blocks include proteins made from amino acids and

polyethylene made from ethylene monomers. In a cell, DNA is synthesized using large macromolecular complexes that add each subsequent nucleotide to the existing DNA strand. In the lab, **chemists have developed alternative methods to produce DNA by chemically appending nucleotides to a growing nucleotide chain.**

Whether made in a cell or in a lab, the synthesized DNA must have the correct sequence. In a cell, the DNA sequence is based on an already existing template strand that provides the sequence information. Synthetic biologists, on the other hand, are often designing new sequences for which no template exists. When there is no template strand to follow, they determine the nucleotide order of the synthetic DNA by using digital sequence information. With this technology, synthetic biologists can write new DNA sequences that have never been written before.



There are some limits to the lengths of the DNA strands that can be produced by this method, but a recent landmark was reached with the synthesis of an entire functional genome by Dr. Craig Venter and colleagues. This achievement simultaneously demonstrated the potential of chemical DNA synthesis as a central component of the synthetic biology toolkit and raised ethical concerns about its use. The researchers rebuilt a genome of the bacterium *M. mycoides* using chemical synthesis to generate multiple short DNA snippets. They added a few small variations, which they called "watermarks," to the sequence, and then inserted this synthetic DNA into a microbe (baker's yeast), where it was assembled into a full genome. Finally, they transplanted the genome into *M. capricolum*, replacing that bacterium's existing genomes and essentially converting the *M. capricolum* shells into *M. mycoides*. This advance, which sounded to some a lot like Frankenstein's monster in Mary Shelley's famous work of fiction, spurred the Presidential Commission for the Study of Bioethical Issues and led to a report, *New Directions: The Ethics of Synthetic Biology and Emerging Technologies*, which addresses the potential ethical issues associated with synthetic biology and mature DNA synthesis technologies.

Standardization

Standardization is a crucial part of any engineering discipline because it facilitates designers being able to reuse parts, combine efforts with other teams, and work efficiently. For electrical engineering, such standardization means that designers can wire together individual pieces relatively easily so that they can "talk" to one another. For synthetic biologists, standardization enables DNA snippets to be physically and functionally connected.

Physical standards for assembly make it possible for all DNA parts to be attached to other parts through a common strategy. This is similar to the way mechanical engineers can connect any nut to any bolt because these parts all use standard-sized threads. The complexity of the cellular environment and biological systems makes standard composition difficult. Nonetheless, there is an effort to define a standard for DNA assembly so that synthetic biologists have a collection of reliable parts and a place to find standardized genetic elements like promoters or repressors when they want to build with them. Physical standardization of DNA parts is discussed in more detail in the Fundamentals of DNA Engineering chapter.

However, successfully putting pieces together is no guarantee that they'll work as desired or be interchangeable. An additional consideration is*functional standardization*, meaning that, no matter what the context, a genetic part will reliably encode a particular behavior. One approach in synthetic biology to reach this goal of predictable functionality is the characterization of a cell's behavior in digital terms: a snippet of DNA is either "on" (that is, expressed by the cell) or "off" (not expressed). This digital principle is familiar from all the electronics in our lives. Our televisions and our cell phones are either on (even if they are "sleeping") or off. This all-or-none behavior makes it relatively easy to connect different pieces. When the television receives input from the remote control to turn on, it activates and provides video and audio output. The same principle holds for the components that make up electrical circuits: each receives input, either "on" or "off," which determine its output, also "on" or "off." This is a highly simplistic description of circuits, but because the "on" and "off" states are standardized across parts, electrical engineers can connect parts and anticipate the behavior of the circuits.

Synthetic biologists are also trying to develop similar "digital standards," describing a gene or an enzyme as being turned "on" or "off." Of course, most biological behaviors (such as transcription or enzyme activity) are not completely digital, but the analogy holds well enough as long as we're careful. Using this approach, we can use other electrical engineering schema, such as wiring diagrams and truth tables, to help us design

our systems. These tools are described in more detail in the Fundamentals of Biodesign chapter.

iGEM

The International Genetically Engineered Machines (iGEM) competition applies the concepts of standardization to DNA parts. This competition brings together college and high school students from around the world to answer the question, "Can simple biological systems be built from standard, interchangeable parts and operate in living cells?" The first competition, held in 2004, started with only five schools and a few handfuls of students, but by 2014, the competition hosted 295 teams from 34 countries.

Each iGEM team is challenged to design and construct a novel biological system using the standardized parts in the iGEM *Registry of Standard Biological Parts*. These parts have standardized junctions, allowing them to be physically connected with a consistent and reusable assembly scheme. Teams can use only four restriction enzymes and the iGEM library of standardized DNA parts to assemble genetic circuits and make more complex arrangements of genetic elements. The reuse of standard biological parts is one way that teams from different schools can share reagents and accelerate everyone's progress on their summer-long projects. We further explore several iGEM projects, one involving smell and one involving color, in the BioBuilder program.

Abstraction

Through abstraction, synthetic biologists can design complex parts, devices, and systems without worrying about every detail of how they work. Instead, the focus is on the end goal, which is the final system output or behavior. In practice, the design of any new system will use abstraction levels very naturally. At the beginning of the design process, we often will think broadly about possible solutions, worrying very little about the details of their implementation. As the problem and the solutions are broken into smaller parts and become more defined, some of the earlier abstractions become concrete so that we can actually build and test the designed system.

Abstraction is particularly important for synthetic biology because the cellular environment and cellular processes are so complex. If we tried to understand every detail of each new design, we would have to slog through our ideas too slowly. Instead, we can think of a bacterial cell as a "black box" (see Figure 1-5). In other words, we don't need to get bogged down with the details of each and every pathway within that cell, especially when developing initial designs.

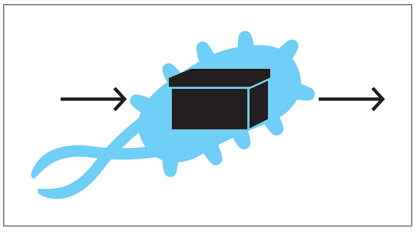


FIGURE 1-5 *Viewing a cell as a black box. Synthetic biology is enabled by abstraction, which allows for engineering of a cell without considering all of the details of each and every pathway within that cell.*

Figure 1-6 presents the hierarchal levels of abstraction. At the highest abstraction layer is the system, our cellular black box. Within that system we might be interested in developing a device with a specific function such as sensing an environmental chemical and creating a specific output scent in response. When we decide how we want our device to work, we can begin to think about the different parts we will need to create each device; for example, a way to sense the environmental chemical and a way for that response to control the scent output. Finally, at the lowest level of the abstraction hierarchy—and not abstract at all—are the actual genetic sequences we'll need to have on hand to use as parts. By breaking the design process into these different layers of abstraction, we have divided the problem into bite-sized pieces that can be addressed more effectively. We will go into detail about each of these levels of abstraction and provide concrete examples of how to implement them in the design process in the Fundamentals of Biodesign chapter.

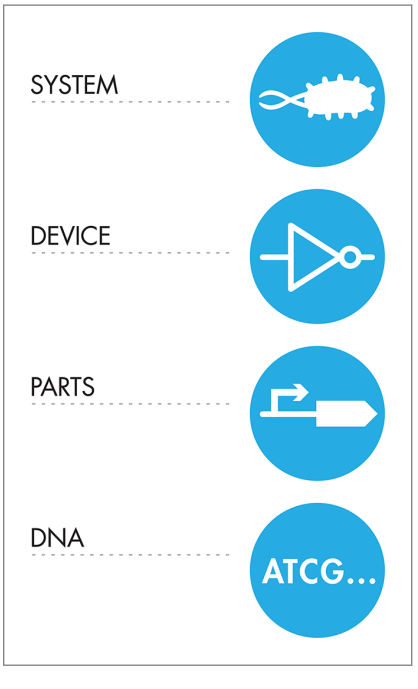


FIGURE 1-6 *Abstraction hierarchy. Abstraction can support complex system design. This abstraction hierarchy is one of many that are possible to use for synthetic biology. The highest level of abstraction here is the entire system, which can then be broken down into specific devices made up of certain parts. The most granular abstraction level here describes the DNA sequences that will be needed to implement the design.*

Wrap-Up

In this chapter, we focused on the power of synthetic biology to produce new systems that can provide useful products or services. We have introduced the basic concepts of synthetic biology by explaining how this field differs from traditional biochemistry and molecular biology, and how some of the fundamental principles from established engineering fields inform the way synthetic biologists design and build living biotechnologies.

The engineering and design approach that synthetic biology espouses has broader implications, too. As physicist Richard Feynman said, "What I cannot create, I do not understand." Though we have certainly come a long way in our understanding of biological systems, we cannot yet build entirely new systems. There is still much to learn about even the most basic biological processes and systems, and synthetic biology provides a powerful new tool in this endeavor, as well.

Additional Reading and Resources

- Alberts, B. et al. *Molecular Biology of the Cell, 4th edition*. New York: Garland Science, 2002. Open access: *http://bit.ly/mol_bio_of_the_cell*.
- Endy, D. Foundations for Engineering Biology. *Nature* 2005;438:449-53.
- Gibson, D. et al. Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome. *Science* 2010;329:52-6.
- Report from the Presidential Commission for the Study of Bioethical Issues (2010) "New Directions: The Ethics of Synthetic Biology and Emerging Technologies" (*http://bioethics.gov/synthetic-biology-report*).
- Website: "Fab Tree Hab" design by TerreformOne.org (*http://bit.ly/tree_hab*).
- Website: History of rDNA (*http://bit.ly/berg_boyer_cohen*).
- Website: iGEM (*http://www.igem.org/Main_Page*).
- Website: 1980 Nobel Prize in Chemistry (*http://bit.ly/chem_nobel_1980*).