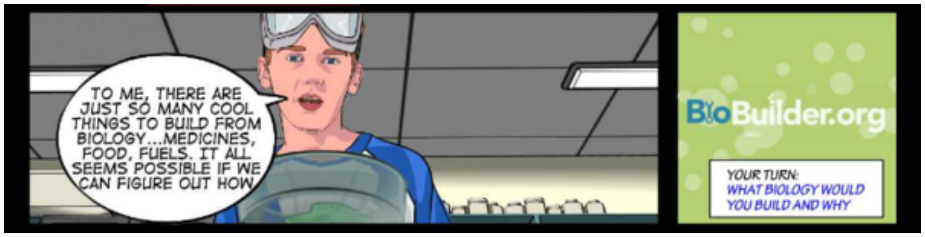
BioBuilding: Synthetic Biology for Students: Biodesign

Biology by Design

- BioHacking Exercises to Design & Specify a Biotechnology



Acknowledgments: This activity was developed in the context of MIT's Introduction to Biological Engineering Design class (a class called "20.020"), in collaboration with Drew Endy when he was teaching @ MIT in 2008

Objectives

By the conclusion of this activity, the student will be able to:

- Explain how synthetic biology as an engineering discipline differs from genetic engineering.
- Explain an abstraction hierarchy and apply it to the engineering of biology.
- Detail a project at the level of system(s), devices and parts.
- Define and properly use synthetic biology terms: Part, Device, System, Chassis
- Define and properly use molecular genetics terms: Promoter, ribosome binding site ("RBS"), open reading frame ("ORF"), Terminator, Plasmid.

Introduction

Your Task

You will write a proposal to the "NISB" (The National Institute of Synthetic Biology) to convince them to fund the next three years of your research. Begin your work here by thinking about a problem or challenge that can be addressed with a biotechnology. Perhaps you want to do something to improve the environment, or address a human health issue, or build structures that will make our lives better. Think about an environmental cue that is related to the challenge you want to address. Is the environment contaminated? Then the contaminant is the cue! Is a person sick from a virus? Then something about the viral infection is the cue...you get the idea.
Next, think about how you would want cells to respond to that cue. Do you want the cell to turn a different color? Eat the invader? Cluster? Grow? Make sure that you're also thinking about other ways that the world is currently addressing the challenge you've identified. Once you've designed your biotechnology, how will its response be better than any existing technology.

Please use your imagination and think big but stay away from science fiction. Start by designing an overall system. You do not have to worry about describing individual parts yet.

For now, you need to describe

1. the problem
2. the stimulus or environmental cue
3. the cell's response

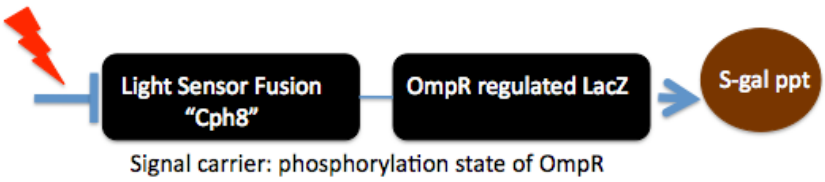
Your description should have enough detail to convince the proposal evaluators that you are a knowledgeable, creative, serious candidate for funding.

You may find that engaging in the following brainstorming process will help you come up with an idea. By thinking about the five questions listed here, you may focus your thinking onto an area that will make a great proposal. You may find it helps to have a partner or group as you engage in this process. Alternatively, skip ahead to the section labeled **"Going Further."**

Brainstorming

Question 1: What does an engineered system look like, on paper and in reality?
To begin, it may help to understand an existing example of an engineered biological system. The one that's presented here is the bacterial photography system, which is also the focus of BioBuilder's Picture this! activity.

At the system level, the engineers wanted to try something "simple," namely to control a chemical's deposition using light.



Now, let's think in scientific terms about how they designed their cell to accomplish this feat. In the bacterial photography system (Lab 3) the absence of light stimulates a sensor molecule on the cell's surface. That sensor activates (phosphorylates) a transcription factor that turns on expression of a gene, causing a black compound to accumulate in the media. On the other hand, the presence of light deactivates the transcription factor and inhibits expression of the gene. No light, no gene expression, no black compound in the media.

Now thinking about the system in engineering terms, we can summarize the behavior of this biological system as a NOT gate, using the following truth table:

Light	β-gal production
1	0
0	1

In reality, once we bring the scientific and engineering descriptions together, the system does not work precisely as specified. It turns out that some β-gal is produced even in the dark and data measurements for that gene's expression might look more like:

Light	β-gal production
present	200 units
absent	1000 units

Recall that the engineering cycle is just that: a cycle. The design, build, test model is more like a crank that can be turned. The faster it's turned, the faster the engineered system can be optimized. But is it worth turning the crank in this case? Why would anyone would want to take black and white photos with bacteria? Given the non-digital behavior of the system, the images are low contrast and only two colors. In addition, these pictures require specialized training to take and are slow to develop. In this instance, the biotechnology is not likely to replace everyone's digital cameras, so why bother? Here's one reason: in building this system, we've learned more about the process of biological engineering. The engineers have demonstrated that they can link parts of proteins and control gene expression with light. It doesn't matter that a specific group of molecules, e.g. the sensor, the regulator and the lacZ reporter gene of the bacterial photography system, are not found together in nature. And by designing this system with standardized, modular components, they might be re-used in other engineered systems to accomplish other tasks.

What a rich toolkit we'd have if we could reliable deploy genetic parts to write genetic programs! Biology textbooks contain numerous examples of genetic systems that enable cells to respond to an environmental cue. What about modifying the bacterial photography system so the cells would swim to the light instead of producing β-gal. Or what if you wanted them to swim in the opposite direction? What changes would you have to make? What if you wanted bacteria that moved toward a toxin instead of light? And if it moved toward a toxin, what might you want the bacteria to do to it once it found it?

Question 2: What will your focus area be?
It sounds so simple to program cells with DNA. But is it? Headlines like this one "Bacteria designed to search out pesticides" make it sound simple. In reality, engineering

synthetic systems that work is getting easier but it's still challenging. Choosing a good problem, one that's worth working on, is a big factor in what's successful. Good problems will motive the hard work that's needed. So begin your thinking about a synthetic system by thinking about a problem you feel is worth tackling.

This table will help you pick broad areas on which to begin your design project.

Area	Challenge
Food or Energy	People need to eat. Planes, trains and automobiles need to eat too. Can biotechnology be responsibly used to produce food or energy without causing widespread shortages of either, and without harming the environment that future generations will inherit?
Environment	The quality of the air, water, and land on Earth and other heavenly bodies, limits the happiness of humans and other creatures. Can biotechnology be used to help clean the air, provide fresh drinking water, restore or enhance soil quality, terraform a near-earth asteroid, or protect, preserve or enhance natural biological diversity?
Health or Medicine	Many health and medical problems might be best addressed by improved biological technologies. What can synthetic biology do?
Manufacturing	Have you ever heard of nanotechnology? Well, biology is a nontechnology that already exists, and that actually works. The ribosome is a programmable nano-assembler embedded within a reproducing machine. Forget grey goo, we've got green goo, and it has already taken over the planet! Thus, could we responsibly use biology to manufacture useful products, from nanoscale (atoms) to the decascale (buildings and bridges)? What can biology be programmed to manufacture?
New Application Area	We're guessing that you have great ideas that nobody has ever thought about, or if they have they forgot to tell somebody else. Can you imagine an entirely new application area for biological technology? Go for it. We'll celebrate you in front of the class.
Foundational Advance	Modern biotechnology dates back to the invention of recombinant DNA technology, which lets people cut and paste pre-existing fragments of genetic material. That was only 35 years ago. In other words, biotechnology is a young adult, just entering its prime years. One thing that desperately needs doing is to develop improved tools and technologies that help to make the entire process of engineering biology easier. What foundational advance can you contribute that will take everybody's work forward?

Question 3: What particular problem do you want to address?
Having chosen a general area for your work, now think about any topics in that area you find especially interesting. These could be motivated by an article you've read, a personal experience, a research project you know about. You might want to have a couple of possibilities as you go forward. Remember: this is a brainstorming session. It's a time to keep all ideas that come to mind and then refine or combine them later.

Question 4: Can you imagine a biotechnology to address this problem?
Now let your imagination kick in for a solution to the problems you've brainstormed

- Bacteria too smelly? Make them smell like bananas.
- Composting too slow? Make a living additive to accelerate the process.

- Need to fight cavities? Make a yogurt that sticks to your teeth and heals them. You get the idea.

You get the idea. Remember that your assignment requires that you think big but stay away from science fiction.

Question 5: How will you narrow down your choices?

You should now have a list of ideas and possible solutions. Deciding between them can be daunting. It might help to compare all your favorite ideas along these five lines:

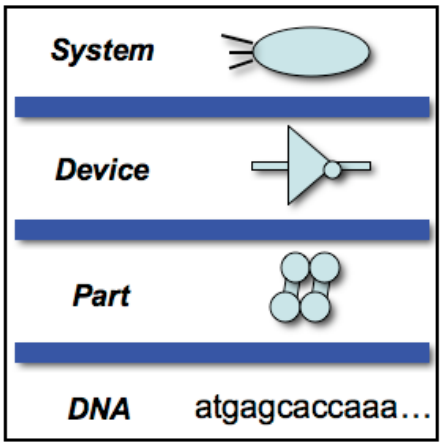
- What precisely is the problem or opportunity you are focusing on?
- How clear are you on an approach to make a dent in the problem?
- What if your project is fully successful? How big a difference could it make? What concerns will it raise?
- What other technologies can be used/have been used to address this area?
- What don't you know? How big are the gaps in what you know? How much is completely unknown or unknowable?

All these questions are important; the ideas you have may have really good answers for some but really weak answers for others. Generally speaking, the more precise you can be about answering these questions (so "identifying an infected cell's change in mass" will help you specify you design a lot easier than "finding a parasite in blood").

You should now look ahead to the section marked **"The Proposal."** If may be that you have all the information you need to write it. Alternatively, you may need (or want!) to dive into more of the details of your system. Work through the abstraction hierarchy as described in **"Going Further"** to specify your design.

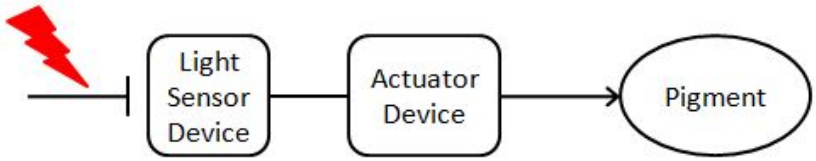
Going Further

Your teacher may want you to delve deeper into your design by completing the following exercises. They will enable you to move through the abstraction hierarchy shown here:



Systems to Devices

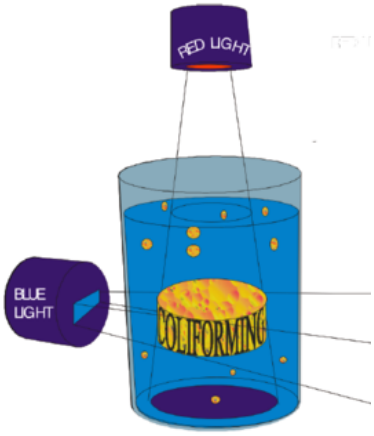
Think back to the bacterial photography system. We drew the system as two boxes, each with input and output arrows. One box had the "sensor," which detected the presence or the absence of the light (via the Cph8 protein). The other box was the "actuator," the parts needed to produce the pigment (by controlling β-Galactosidase production). In simple terms, the system requires two genetic devices. A block diagram might look like this:



To further specify your system, try to think about what devices are needed. This is best accomplished by thinking in terms of inputs and outputs. To get started, work through some practice problems.

Practice problem I: Bacterial Buoy

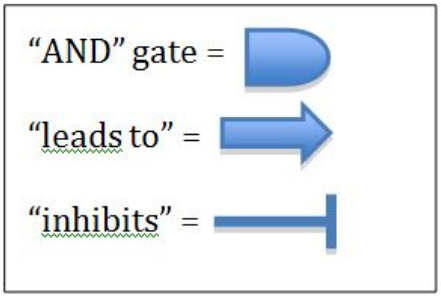
The 2007 iGEM Melbourne team wanted to build a 3D, floating mass of bacteria that adhered to one another when the cells detected both blue and red light. In other words: at the intersection of an incoming red light beam and blue light beam, a solution of bacteria would clump and remain suspended in its growth media.



As a class we'll watch the first 5 minutes of the Melbourne team's iGEM presentation
Next you should work out a high level overview of this system's behavior. Make a list of cellular inputs and outputs then write a block diagram that connects them. In other words: What inputs will the cell have to sense? What two ways will the cell respond? Don't worry about getting the clump to disassemble when the lights are off. Just think about what the cells needs to do to make the clump and make it float. **Importantly:** don't get bogged down by what really exists. If you need a "floatation" device, you can have one.

Practice problem II: Polkadorks

Let's try a more dynamic system. The 2004 IAP team wanted their engineered cells to "form, diffuse, and form again in random areas on the plate. Our system should thus form time-varying patterns based on local random time-varying symmetry breaking." Check out the Polkadorks animation. Then make a list the devices needed to implement such a system (for example "coin flipper" to generate the random decision to turn red) then connect the devices with arrows to indicate the logical information flow pattern.



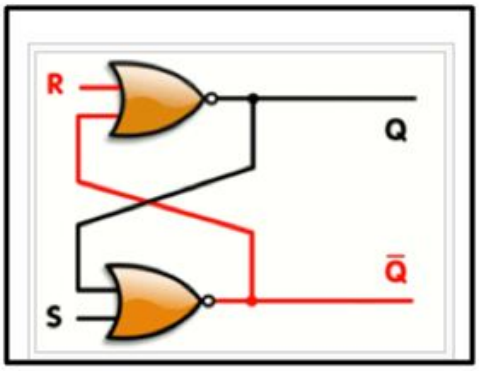
Your Challenge:

Now think about your system. What devices would you need to create it? Again, don't get bogged down by what already exists, but do keep it realistic. You are engineers. You may be able to make whatever device you need. So, if you need a "floatation device" or a "garlic smell generator," go ahead.

Devices to Parts

"Parts" are the individual components that make up a device. A simple device, for instance the one that encodes β-galactosidase, would need a promoter, ribosome binding site (rbs), open reading frame (ORF)—in this case the lacZ gene--and possibly a terminator sequences. Easy enough.

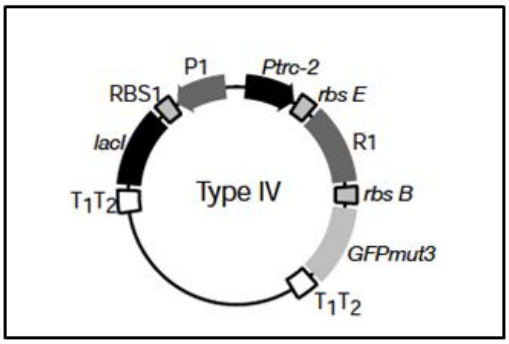
Sometimes devices are more complex assemblies of parts. Remember the "NOT" gate that described the bacterial photography system? That is a logic gate made from a transcription factor.



As an exercise, consider another kind of logic gate. This one comes from electronics and is called a "latch." It's made by cross-wiring two "NOR" gates. The gate is sometimes also called a "flip-flop" or "toggle" since it switches and holds between two states. Even if the initial input is removed, the circuit holds the output, until the other input is provided.

In a landmark paper, this kind of electronic circuit was recapitulated with genetic parts by Jim Collins at Boston University (T S Gardner, C R Cantor and J J Collins. Nature 403(6767):339-42 (2000) PMID 10659857).

Looking at the diagram of the plasmid below, we can see the parts that make up this switch: Starting at the top, we see an arrow pointing to the left. This is promoter 1. It is followed by a RBS and the lacI ORF, this is repressor R-2. Starting again at the top, we see promoter 2 (Ptrc-2) followed by a RBS, the repressor R1 ORF and the rbs and orf for a GFP (green fluorescent protein) reporter.



So, how do these parts function as a toggle switch? As we can see in the second diagram:

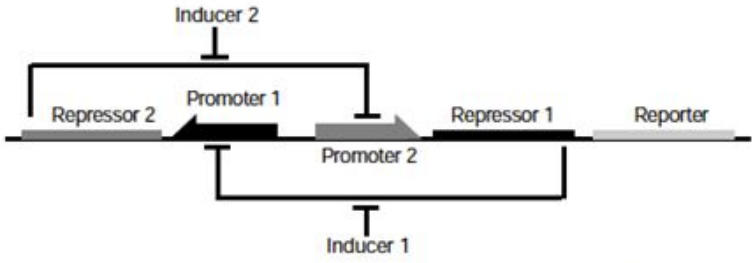


Figure 1 Toggle switch design. Repressor 1 inhibits transcription from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.

If promoter P-2 is on, then it will turn on the reporter gene and the cell will glow green. But it will also turn on expression of repressor R-1. This will shut down promoter P-1, which will therefore not enable the repressor R-2 to be expressed. So, the cell will continue to glow green until P-1 is induced. Once P-1 is on, it will turn on R-2, which will repress P-1. Now the cell will not make the GFP reporter and will not glow green. And, since P-2 is repressed, no R-1 will be made and the cell will stay in the dark. Got that? Well, in order to prove you understand something, you need to be able to explain it.

So, now you try it: List each of the components and what its role is in the toggle device. Then, using the diagrams and your list, write an explanation of how these parts work in combination in the toggle device. Finally, explain the system to your lab partner or a stuffed animal.

Your Challenge: Think about the devices that make up your system. Then, think about the parts that are needed in each device. For each device list its parts. Don't worry if these parts actually exist. However, you **should** be specific where possible. For example, if you need a promoter that is induced by high salt concentration, write that you need a promoter that is induced by high salt concentration. We can search the Registry for one later...

The Proposal

Your proposal should contain the following sections. Your explanations must address, but are not limited to, the following questions. Approximate length: 5-7 pages total. **Before you write your proposal,** be sure to look over the Design Assignment Rubrics and Scoresheets (pdf)

I. Purpose

- Describe the problem or challenge you are trying to solve.
- Why is solving this problem or meeting this challenge important?
- What will your design do to address that problem (overview)?

II. Competing technologies

- What technologies are being used to address this problem at present?

- How effective are they?

III. The design

- Using both words and diagrams, describe your design.
- What input would the cell be responding to?
- How would genetic expression be affected?

IV. Expected results

- Using both words and a truth table, explain how you expect the cell to behave when the design system is working perfectly.
- Using both words and a data table, explain how you expect the cell to behave at a not perfect but acceptable level.
- How will these results bring about a successful solution to the problem?

V. Advantages

- How is your biological design an advantage over the existing technologies?
- Why is your design worth funding? (Be convincing!)

VI. Potential problems

- What are the potential problems with your system?
- What safety features would be required to protect your employees during development of your design?
- How would you protect the environment during development of your design?
- In what ways could evolution of the cells you've engineered negatively affect use of your design in the future?
- Are there inefficiencies or shortcomings of your design as compared to the existing technologies?
- Does your design pose dangers to the environment, lab safety, and the security of the public?
- In what ways are the potential rewards worth the risks?

VII. Testing

- How would you test the effectiveness of the system?
- (If your design has multiple components, you may select one component for testing.)
- How would testing help improve the system?
- How would testing help reveal greater potential for the system?