Now five years old, a student competition in synthetic biology embodies the struggles of the emerging discipline.

By Alla Katsnelson

On a November weekend, more than 800 people gathered at the Massachusetts Institute of Technology to show off six months of hard work. Each person wore one of 84 different shirts; some had a classic, young, professional design, while others adopted a more playful approach—drawings of yeast having sex, for example.

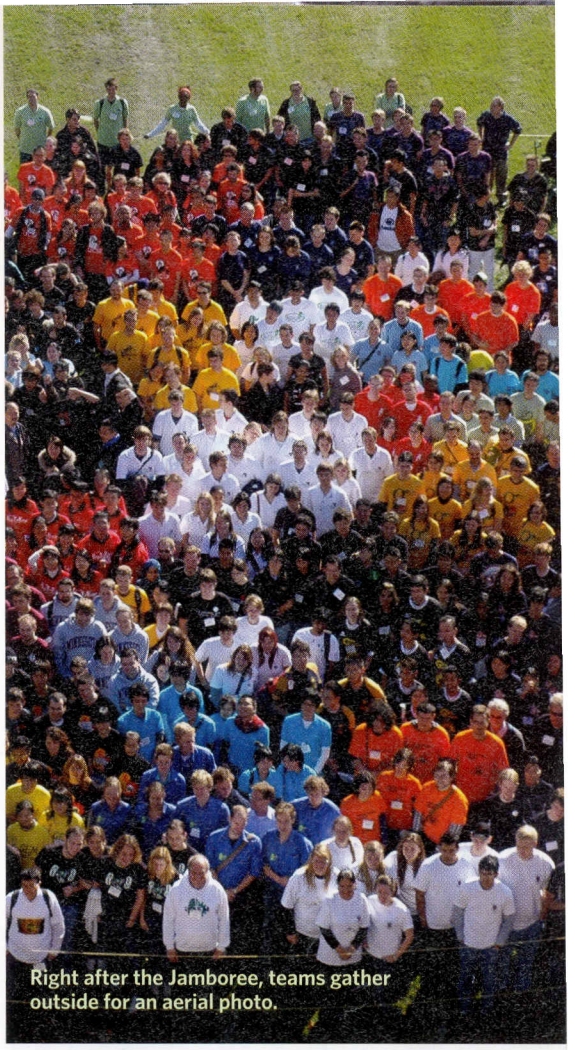
Eighty-four shirts, eight-four teams. The competition in which they were participating, called the International Genetically Engineered Machines competition, or iGEM, has a big goal: Revolutionize the engineering of biology.

It works like this: In the spring, teams of students from around the world, mostly undergraduates, are mailed a collection of DNA constructs, mostly made from *Escherichia coli*. These constructs, called biological "parts," can include simple elements, such as DNA-binding domains, or more complex elements, encoding functions such as a switch regulating the expression of a certain gene. They are cloned in such a way that, in theory at least, they can easily be combined using a standard process to create biological "devices" or combinations of genes that perform functions not normally found in nature. Using these DNA building blocks, the students design projects for engineering a living machine that, say, senses toxins in the environment, or acts as a little drug-factory inside the body. The teams then convene at the annual iGEM Jamboree at MIT for a synthetic biology showdown. Since it began five years ago, the competition has grown dramatically, up from only five teams in 2004.

Not coincidentally, the mission of iGEM is also the mission of the emerging field of synthetic biology, which aims to turn biological systems into something as predictable and standardized as the electronic parts in an old transistor radio. "It's a radical research agenda," says Drew Endy, a synthetic biologist at Stanford University. "The idea of standard biological componentry is either dismissed as a research question because [people think] it's irrelevant or dismissed as a research question because [they believe] it's impossible." One of the purposes of iGEM is to show the wider research community that using standardized biological parts can, simply, work. But as the number of iGEM participants expands, the competition is exposing the complications in a field that has a long way to go.

Case in point is the experience of Jean Peccoud, a synthetic biologist at the Virginia Bioinformatics Institute at Virginia Tech, and a mentor to a group of undergraduate students who participated in the 2007 iGEM. His students planned to engineer an epidemic using *E. coli* as the host and the common bacteriophage lambda phage as the pathogen. Their idea was to exploit a well-studied property of lambda phage to become either lytic or lysogenic—that is, to either lyse its host cell, releasing phage particles that could infect other cells, or to lie dormant in the host cell's DNA and not spread the infection. They wanted to engineer a strain of bacteria carrying a reporter plasmid that would generate green or red fluorescence, depending on which pathway the phage took in each infected cell. This step would let them track infection within a single population—one well of a 96-well plate—as well as between populations, represented by different wells.

But the group quickly ran into technical difficulties. "When we plated the bacteria containing the GFP BioBrick, we were expecting to see bacteria that were green, but instead we saw that half was red and the other half was yellow," Peccoud recalls. That meant the plate contained a combination of different bacteria, rather than the single one promised in the kit. He was surprised to discover that no one had ever published a description of iGEM's parts collection, and decided to take on the task himself. When he analyzed the collection, he found it was rife with problems—from sequence errors to miscategorization and confusion over what constitutes a "part."



Right after the Jamboree, teams gather outside for an aerial photo.

Sitting in an MIT corridor is a –80°C freezer, with a sticker of iGEM's green cell and cog insignia on the door handle. Pull open the dull-grey door, and you'll see the contents of the Registry of Standard Biological Parts—about 3,500 DNA clones stored in plates or aliquot tubes as bacterial stock. The Registry, which supplies iGEM teams with their kits each year, is the creation of MIT computer scientist-turned-biologist Tom Knight. He arrived at MIT as an undergraduate at the age of ▶

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14 in the 1970s and, after making several foundational advances in computer networking and precursors of internet architecture, turned to tinkering with genetics in the 1990s. His notion of standardizing biology was borne out of a computer scientist's frustration with the level of noise and unpredictability in all living systems.

"You go into the lab and you want to do something that on paper looks really simple," says Knight—create a genetic circuit where one gene's output upregulates another gene, for example—"except you need this DNA construct. And then very rapidly you get sucked down this path where you're ending up doing this second experiment," specifically, assembling bits of DNA. "I don't ever want to do that experiment," Knight says. "I don't want [DNA assembly] to be an experiment. I want it to be something I've done a hundred times before, and there's a cookbook, and I push this button, and it happens."

So he came up with a basic scheme for using restriction enzymes and ligation to fuse two pieces of DNA with specific functions, such as protein-coding sequences, promoters, terminators or ribosome-binding sites. He flanked each piece of functional DNA with a specific base pair sequence, calling the whole thing—the prefix, the DNA and the suffix—a "BioBrick part." Because the sequences are standardized at each end, any two parts can be joined using a single restriction enzyme protocol. "If I do say so myself, it was a pretty good try," he says of the DNA assembly technique he developed, called BioBricks alpha (BBa). Using that technique, he developed the first dozen parts, which seeded the Registry's collection. He also founded a nonprofit, called the BioBricks Foundation, which oversees the Registry, runs iGEM, and tries to push forward efforts to standardize biological engineering.

The BBa technique does have some flaws, though, the main one being that combined parts carry an eight-base "scar" between them, which complicates protein fusion. Translating genetic information into proteins occurs via codons comprised of triplets of amino acids, and the eight base sequence throws translation out of frame. Other labs have since tried a handful of other methods for assembling DNA in a standardized manner, known throughout the community as "assembly standards," each with its own plusses and minuses. Smatterings of parts in those formats also populate the Registry.

Based on the BioBrick's philosophy of creating a community around parts-sharing, the Registry's collection grows as more people use it. When users combine two parts to make a new part, or when, of necessity, they make a part from scratch, they are encouraged to add their new creations to the Registry. In that way, the community resource grows—and grow it has. In 2008, iGEM teams sent in about 1,500 new parts, bringing the total to about 3,500, from Knight's original dozen just a few years back. But undergraduates hurrying to finish their projects don't often do the most careful work—some take the time to characterize their creations, and some...well, don't.

So far, the Registry's policy has been to accept whatever students send in. To Peccoud, that's a crazy notion—other shared resources, such as the nonprofit cell-line repository ATCC or the plasmid

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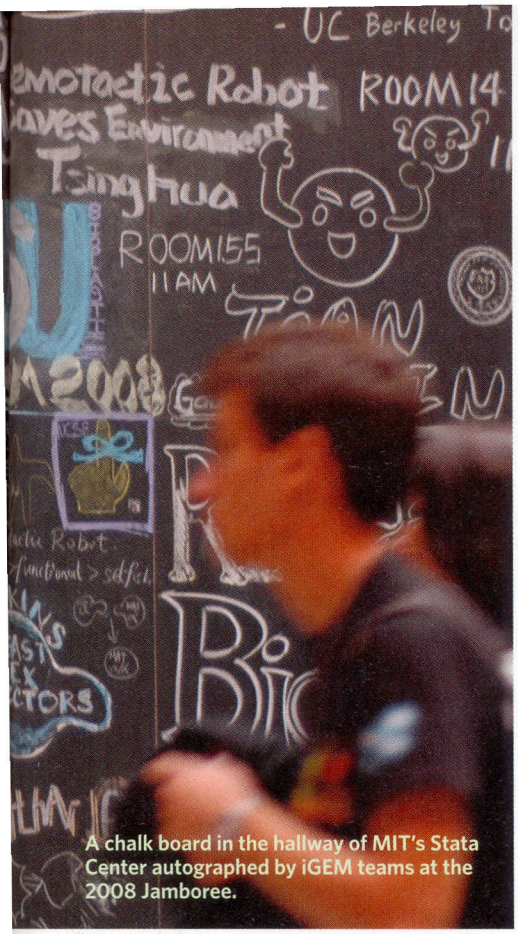
Visit www.the-scientist.com for an article on the standardizing of the Registry, plus video of student response to the iGEM competition.



A chalk board in the hallway of MIT's Stata Center autographed by iGEM teams at the 2008 Jamboree.

registry Addgene, have criteria for what they'll accept. But for Randy Rettberg, the founder and director of the Registry and iGEM, inclusiveness has been a matter of practicality. "People say I should require all these things from the parts submitted to the Registry," he says. "Well, yeah, I could do that, for all the five parts I'd get." Rettberg notes though, that this has been changing. "Now we can demand more."

The iGEM competition is an unabashed recruitment tool, a scheme for seeding synthetic biology with young, energetic talent. After all, the field is so new that there are as yet no undergraduate majors or university departments for it at any US academic institutions. (Lawrence Livermore, a government lab, started the first synthetic biology department in 2006; Harvard last October received a $125 million grant to create a synthetic biology institute.) Rettberg, who left the computer industry to join Knight's lab in 2001, says that when he, Knight, Endy, and Gerald Sussman, also of MIT, decided to run a biological design class at MIT during the 2003 winter intercession, they specifically chose to target undergraduates, who wouldn't have fixed notions about what is and is not biologically possible.

The competition grew out of that MIT class; in 2004, the group received a one-time $400,000 National Science Foundation grant and some money from the Defense Advanced Research Projects Agency (DARPA) to run the class again, as well as a small summer competition, between just five US schools. The following two years the funding came from the MIT Microsoft iCampus project, and in 2007, the Registry began charging teams $1,000 to register, as well as small amounts to attend the Jamboree. (iGEM also receives money from NSF's Synthetic Biology Engineering Research Center, as well as corporate sponsorship from GeneArt, MathWorks, and, to a smaller extent, BIO.) "Actually having the teams pay means they are serious about it," Rettberg says, noting that in past years, a significant number would drop out when they realized how much work was involved. Teams generally raise the money from their departments, as well as company sponsors, and that too "builds up the buy-in" both in academia and industry.

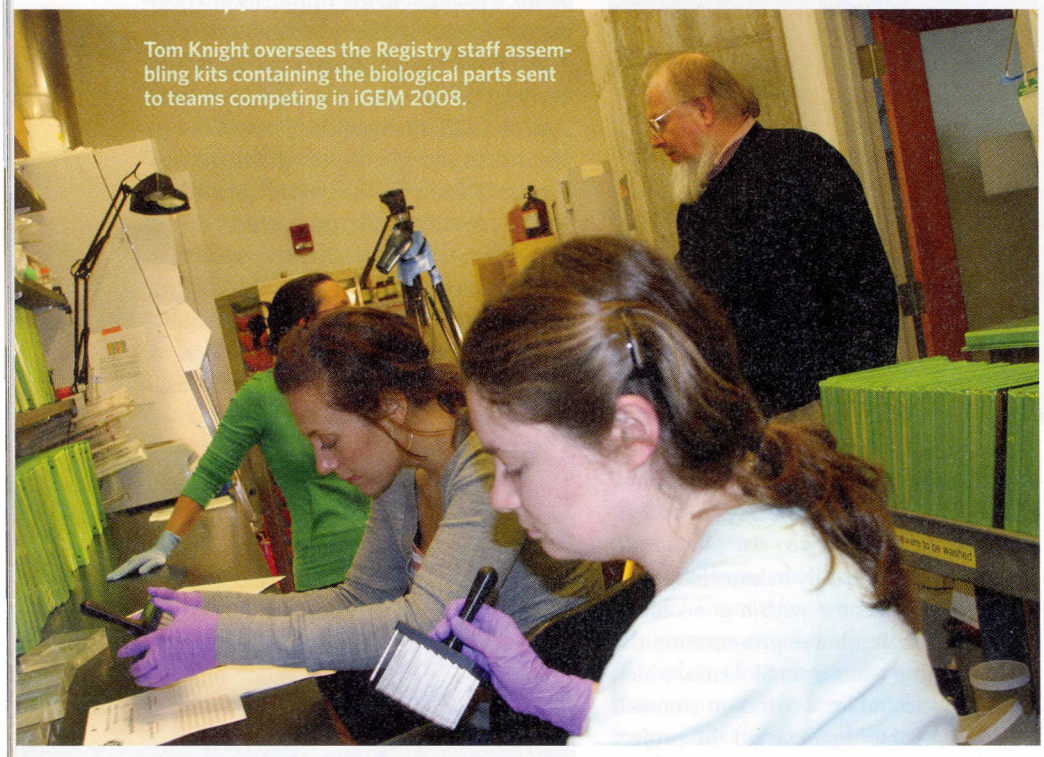
Researchers readily acknowledge the iGEM projects come with a good bit of hype. In their Jamboree presentation the 2008 winning team from Slovenia, which created a vaccine for a common stomach bug, *Helicobacter pylori*, said the project could "save hundreds of thousands of lives per year." Another team, from Caltech, engineered gut bacteria that would ostensibly protect against pathogens, pump out folate or other useful minerals, and treat lactose intolerance. "I have a really hard time imagining that anyone will ever be able to engineer anything that complicated," says Jef Boeke, a geneticist at Johns Hopkins who brought a team to iGEM for the first time in 2008. The hype "rubs some 'serious scientists' the wrong way," but, Boeke says iGEM is "a great way to get students excited and the public excited about the promise" of synthetic biology.

As iGEM projects get ever-more sophisticated, the work is starting to feed into the research done in the labs of faculty mentors, even bringing in publications and grant funding, says Rick Weiss, a former student of Knight's who works on synthetic gene networks in mammalian stem cells and has brought teams to iGEM since the competition began. His lab, he says, recently got funding for a project that grew out of the 2006 iGEM team's work on beta cells and diabetes. His group is also continuing to work on the 2008 iGEM team's project, on neuronal circuits.

But after five years, contends Herbert Sauro, a biologist at the University of Washington in Seattle, iGEM has done as much as it can for validating the idea of biological engineering through sharing standardized parts. "I think [iGEM's organizers] have this rosy picture that undergraduates will lead the way," he says. "There are some great ideas at iGEM, but you can't rely on that completely," he adds, noting that it's a fantastic educational tool, but insufficient to launch the field of synthetic biology. To move from a small handful of researchers to a full-fledged discipline, "at the end of the day, you still need money, institutional support, infrastructure."


Team members from Slovenia celebrate their iGEM 2008 victory and hold up their prize—a large aluminum BioBrick.

On November 8, the Monday morning after the 2008 Jamboree, about 30 people—from researchers to lawyers to leftover iGEM students from Mexico—file into an MIT lecture hall for a BioBricks Foundation open meeting on the difficult task of establishing standards for biological parts—arguably a central mission, if the project (and the field of synthetic biology) is to succeed. The idea is to address specific types of standards, such as those for DNA assembly, or those for quantifying gene expression across different devices, for example, as well as to more broadly pin down a standard way of defining, describing and characterizing parts.



Tom Knight oversees the Registry staff assembling kits containing the biological parts sent to teams competing in iGEM 2008.

"My hope is that this is a very messy and friendly meeting," Endy says to the assembled crew, "and that everybody will leave as friends in the end."

Efforts to define ideas around standards have had a rocky road. In 2007, the BioBricks Foundation invited internet guru David Clark of MIT to discuss the path the internet community had taken in establishing standards for the world wide web. It was a bottom-up process in which developers who had an idea for how something should work online would write up a document called a Request for Comments, or an RFC. Other users would test out their proposals and comment on them, and whatever worked well would be implemented. It seemed like an easy steal for standard-setting in synthetic biology, but more than a year later, the only RFCs submitted had come from Knight. At this point, people are getting frustrated.

The discussion turns to RFCs. "We've had a lot of conferences," notes Knight in the meeting, "but we haven't set the standard for describing standards." Thus the main task for the day emerges: composing RFC 0 for synthetic biology—the RFC on how to write RFCs.

A small crowd clusters at the blackboard as Reshma Shetty, also a Knight lab veteran and cofounder of Ginkgo BioWorks, a synthetic biology services provider, asks for input on every imaginable detail, writing key points on the board. How long should the RFC document be? How to copyright it? Does an RFC have to describe a standard, or can it simply describe, say, a protocol? What's the difference between the two?

Slowly, the answers are hammered out, along with a plan for a four-step process for requesting, writing, submitting and copyrighting the documents. When will the draft document of RFC 0 be finished, Endy asks? "I'll do it within the week," Shetty replies. Not good enough. "Ok, I'll write it up this afternoon."

During the month following the meeting, the BioBricks Foundation received three more RFCs from Knight, but no others. "Lots of people are interested in how RFCs should work, but most aren't working in an area where they're actually making them," concedes Rettberg. Also, he notes, the concept may have worked better in designing computer networks, because not having the standards in place actually prevented the networks from operating, hastening the process.

iGEM: The first five years

	No. of teams	No. of countries represented	No. of parts submitted to Registry	Winning team
2004	5	1	~50	All received an award for participating
2005	13	4	~125	All received an award for participating
2006	35	13	724	Slovenia, for a device encoding a feedback loop on the toll-like receptor pathway that dampens the overactive immune response, a potential cause of sepsis
2007	54	19	~800	Peking University, for a device consisting of a spatial and temporal switch controlling bacterial differentiation
2008	84	21	1,387	Slovenia, for a synthetic vaccine for *Helicobacter pylori*



Left: Each part in the Registry, consisting of a stretch of *E. coli* DNA, gets blotted onto a sheet of filter paper. Middle: The full collection of Registry contents, assembled into a green notebook, is sent out to each team. Right: Inside a Registry refrigerator full of bacteria plates used in quality control processes.

To date, the Registry's 3,700 or so registered users are primarily members of current iGEM teams, or associated with past teams as students, instructors or advisors. This is because, in part, the Registry has not promoted the collection to the wider research community, but it's also because its contents are as yet not reliable enough to for prime time.

Before sending out the parts kit to the 2008 teams, the Registry undertook the gargantuan task of verifying and sequencing each part submitted, to make sure that the parts work more or less as expected. That step addressed the quality issues Peccoud had identified, but other problems cropped up. For one thing, the Registry tried a new technique for sending teams the parts kit—a notebook of DNA smears on filter paper instead of dry DNA in 384-well plates. "How many people had trouble with that format?" Rettberg asks the audience at the Jamboree awards ceremony; almost all the hands in the audience go up, and a wave of giggles ensues. "Well, that's something we can all agree on," Rettberg says, laughing too.

The issues of organization and definition Peccoud's analysis raised, however, will take a longer time to nail down—perhaps as long as it takes synthetic biologists to come to a consensus of what standardizing biological parts really means. Meanwhile, the Registry is taking steps to create order among its collection. This year, says Rettberg, the plan is to re-do the Registry's interface, making it similar to a catalogue and allow users to access all the information about a part—from its sequence to how well characterized it is—in a clear format. Over the next couple of years, he says, the Registry will create a core of very well-characterized and well-documented parts that will behave in a predictable way. In 2009, iGEM teams will receive a subset of Registry parts that will be tested for quality, and anything else they might request from the catalogue.

But according to Peccoud, synthetic biology's problems go beyond the Registry. His group has found major sequence errors in plasmids used in the sequence of artificial gene networks published in major journals, not just those from iGEM groups. "I'm not talking about substitutions of a base," he says, but bigger issues such as missing promoters. His own group now does all their synthesis in-house. "The synthetic biology community seems to be taking for granted that making DNA with the base level of precision-on-demand is a piece of cake," he says. "But when you start looking into that very carefully, whether you look at BioBricks or plasmids you get from different sources, it seems that everybody is actually struggling with making DNA with precision."

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> ***—Herbert Sauro***

During the second half of the Monday morning standards meeting, the group decides to hammer out a list of 10 RFCs for what Endy calls the low-hanging fruit—projects that can be quickly and easily decided upon. Instead, the conversation turns to how strict the Registry should be to sticking to the assembly standards it has in place. iGEM students are required to submit their parts in one of the handful of accepted assembly standards, though they can apply for a "variance" if they think the Registry should make an exception. (In 2008, 14 groups applied for variances, and the Registry granted about half of them.) But Chris Anderson, a synthetic biologist at UC Berkeley, insists that the Registry is too firmly controlling what constitutes a standard.

Rettberg disagrees. "We have been looking out into the future and saying we could have an explosion of so many assembly standards that all the parts would entirely fragment; and that wouldn't be such a good thing," says Rettberg. "I think you would agree as well, that if at Berkeley you had 20 different standards, and not only that but every grad student has his own standard..."

"I don't personally have a problem with that," says Anderson.

Randy responds slowly and deliberately: "If nobody's parts could ever be put together with anybody else's parts..."

But before he can finish, Jason Kelly, a recent veteran of Endy's lab, interrupts, "...Then that's just conventional molecular biology." ■

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