2016

Proceedings of the First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas

(San Jose, 16th and 17th March 2016)

Proceedings of the First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas

(San Jose, 16th and 17th March 2016)

Inter-American Institute for Cooperation on Agriculture (IIICA)

Technical Editor:
Pedro J. Rocha Salavarrieta

Organized por:
IICA and *U.S. Department of Agriculture* (USDA)

Financed by:
USDA & IICA

Inter-American Institute for Cooperation on Agriculture (IICA, CR), 2017. Proceedings of the First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas (San Jose, 16th and 17th March 2016). Technical Editor: P. J. Rocha. San José, CR, IICA.

This publication is also available in electronic (PDF) format from Institute's Web site: http://www.iica.int.

Editorial coordination: Pedro J. Rocha
Mechanical editing: Pedro J. Rocha
Text translation: IICA
Layout: Pedro J. Rocha
Cover design: Pedro J. Rocha

Proceedings of the First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas (2016: San Jose, Costa Rica) / Inter-American Institute for Cooperation on Agriculture, United States Department of Agriculture. San Jose, C.R.: IICA, 2017.
96 p.; 21.5 cm x 28 cm

ISBN: 978-92-9248-681-5

1. Biotechnology 2. Biosafety 3. Training 4. Biology 5. Innovation adoption 6. Regulations 7. Americas I. IICA II. USDA III. Title

San Jose, Costa Rica
2017

Table of Contents

Organizing Team for the Event

General Coordination
Pedro J. Rocha

Technical Coordination
Pedro J. Rocha & Fan-Li Chou

Interpretation
Thais Pardo & Hannia Azuola

Operative, Logistics and Administrative Support
Teresa Acón, Marlen Montoya, Marianela Lemaitre, Wendy Esquivel, Rafael Cartín, David Álvarez, Sergio Navarro

Financial Support
U. S. Department of Agriculture (USDA)
Inter-American Institute for Cooperation on Agriculture (IICA)

Editorial Team for the Document

Technical Editing
Pedro J. Rocha

Conferences Transcription
Patricia Echeverri

Text Translation
IICA

Text Editing, Style and Corrections
Pedro J. Rocha

Foreword

Welcome to the home of agriculture in the Americas and the Caribbean. Today we are going to discuss Synthetic Biology. There are many experts here who will participate in the discussion, but I consider it important that governments understand this technology; that we work together to find ways to allow new innovations to come to the market place to contribute to solving problems related to pests, weeds, diseases, stress tolerance, drought tolerance and, potentially, opportunities for nutrition as well. The question is how we will use science to address the challenge of growing more with less, especially given the challenges we are all facing due to changing climatic conditions.

We've come a long way in agriculture, if you think about it. There was the time of mechanization that took us away from animal-driven agriculture to new seeds, new technology, hybrids, new chemicals, and now even better chemicals − greener chemicals. Today, with GMOs and open and big data, precision agriculture is possible on even the smallest farm, in the smallest village, with a drone or satellite identifying the best plant, fertilizer, chemicals, or water, in order to grow more with less. It's an exciting time and it's going to be interesting to see how synthetic biology contributes to science and innovations in agriculture.

I want to thank the Department of Agriculture and the Department of State for sponsoring this first IICA seminar on Synthetic Biology. I think it's going to be the first of many. I look forward to learning about this exciting new technology and how IICA can work with our member states to help craft policies to appropriately deal with this technology, in terms of regulation, as well as acceptance in a way that provides for innovation, while at the same time ensuring the trust and confidence of consumers.

In the United States Senate, a bill on the labeling of biotechnology was going to the floor. This is something that we should probably discuss at IICA at some point, in order to arrive at some degree of harmonization among nations, so that we do not have patch work labeling laws across the hemisphere. Perhaps this is a matter that could help drive a global conversation. This is all about giving consumers information, enabling innovation, but what's most important, from our perspective as governments and government entities, is enabling and ensuring the trust and confidence of consumers.

So, with that, welcome again to IICA. I hope you have a very successful and enlightening seminar and that you will enjoy your stay in Costa Rica.

Lloyd Day
IICA Deputy Director General

Proceedings of the First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas

(San Jose, 16th and 17th March 2016)

Introduction

Pedro J. Rocha
International Specialist in Biotechnology and Biosafety
Inter-American Institute for Cooperation on Agriculture (IICA)
Pedro.Rocha@iica.int

For reasons beyond the authors' control, this document has taken longer than expected to be published. From the moment this first seminar was held until the date of its publication, some events related to the technical, regulatory and communication about the development of Synthetic Biology (SynBio) have taken place. For example, a number of publications (European Commission, 2016, The Royal Society, 2016, Kuzma, 2016, Kuike, 2016) have been produced and the draft recommendations of the Subsidiary Body on Scientific Technical and Technological Advice (SBSTTA, CBD, 2016) was presented and discussed at various sessions of the United Nations Biodiversity Meeting COP13-COPMOP8-COP2. However, the conclusions of such events do not affect the relevance of this document which, in fact, is a training resource that allows the subject to be approached in an objective and multidisciplinary way.

SynBio is an area of biology on which there is consensus about its multiple applications (Kelley *et al*., 2014) but not in its definition and even less so about its regulation. It is certainly an emerging field that provides opportunities for the development and consolidation of the knowledge-based economy (Bioeconomy, Hodson, 2014). However, the dynamism of the research, development and commercialization of products obtained through SynBio is in contrast with the disinformation around the subject. And as it has been the case for some years with other technologies, ignorance and fear on the part of some groups of people has led to attacks against this technology because they see in it an imminent danger (and not a potential risk) for human health, biodiversity, family economy, and ethical issues, among others (Parens *et al*., 2009; Gutiérrez & Delgado, 2016, European Commission, 2016).

This document is divided into three sections. The first is an overview of SynBio which includes the contributions (presentations) of seven experts from various disciplines, who provided concepts for defining SynBio (Fan Li Chou, USA). It includes examples of various industrial applications (Natalia Verza, Brazil), in the agricultural sector (Marcelo Freitas, Brazil), in the control of human diseases, the generation of public health policies (Mario Henry Rodríguez, Mexico), and in the conservation of biodiversity (Kent Redford, USA). It discusses the scientific academic development routes (Ana Sifuentes & Ricardo Chaves, USA & UK) and the bases of the regulatory aspects for dealing with the subject (Felicity Keiper, Australia).

The second section focuses on developing regulations for SynBio and reviewing the various regulatory mechanisms currently available (Felicity Keiper), particularly within the framework of the Convention on Biological Diversity (Genya Dana, USA). It also presents the views on the regulatory framework of some of the participating countries (Bolivia, Brazil, Canada, Mexico, and the United States) and the private sector.

The third section presents the details of the event: program, list of participants and contact information.

As will be seen later, SynBio includes various technologies, processes and products with huge agricultural, livestock, medical, environmental and industrial applications (fuel, food, fiber, new materials, etc.). Such applications are opening potential routes to respond to the current challenges of humanity that are associated with population growth, increasing demands for safe and economical products, biodiversity conservation and sustainable production in a background of global climate variability.

However, for this potential to be achieved, a clear, objective, technically rigorous (science-based) regulatory framework will be needed to facilitate the dynamics of technological development, which will foster the consolidation of the markets for this technology and its products. On the contrary, a subjectively restrictive regulatory framework (based only on an extremist interpretation of the precautionary principle) will hinder or restrict technological development, discourage investment in research and eventually block certain branches of commerce.

The growing demands of humanity require innovative, efficient, environmentally friendly and sustainable solutions. The development of SynBio takes into consideration these requirements. It will depend on us whether or not we use it, and whether we will be guided by scientific knowledge or by fear. There are no perfect technologies but, whether we want it or not, at this stage in the development of humanity they are indispensable.

References

Convention of Biological Diversity (CBD), 2016. *Subsidiary Body on Scientific Technical and Technological Advice*

European Commission- Science for Environment Policy. 2016. Synthetic biology and biodiversity. Future brief 15. 35p. http://ec.uropa.eu/science-environmental-policy

Gutiérrez, A.G. & Delgado D. 2016. ¿Qué es y cómo afrontar la biología sintética? Third World Network. Perú. 71p.

Hodson, E. (ed.). 2014. Towards a Latin America and Caribbean knowledge based bio-economy in partnership with Europe. Pontificia Universidad Javeriana. Bogotá.

Kelley, NJ; DJW, Kerr E, Apel A, Beliveau R, Scanlon R. 2014. Engineering Biology to Address Global Problems: Synthetic Biology Markets, Needs and Applications. Industrial Biotechnology 10(3): 140-149.

Kuzma, J. 2016. Policy: Reboot the debate on genetic engineering. Nature, 531(7593): 167-168.

Kuike, T. 2016. Governance: Learn from DIY biologists. Nature, 531(7593): 165-167.

Parens, E; Johnston J; Moses J. 2009. Ethical issues on Synthetic Biology: An overview of the debates. Woodrow Wilson International Center for Scholars. Available in: http://www.synbioproject.org/site/assets/files/1335/hastings.pdf

The Royal Society, 2016. Synthetic biology. Available from: https://royalsociety.org/topics-policy/projects/synthetic-biology

Section 1: Synthetic Biology: Generalities

1.1. ¿What is Synthetic Biology?[1, 2]

Fan Li Chou
Senior Policy Advisor, Science and Trade
New Technology and Production Methods Division | Office of Agreements and Scientific Affairs
Foreign Agricultural Service | U.S. Department of Agriculture, U.S.A.
Fan-Li.Chou@fas.usda.gov

Summary

Synthetic biology is defined in many different ways. But is SynBio something new, as some of the definitions say, or is it actually a continuation, a continuum of what we´ve known as modern biotechnology? Here, some considerations about definitions, actors and regulations for synthetic biology are presented.

Presentation Development

I will speak very briefly to set the stage for the next two days, because I am not a synthetic biology expert. I am going to leave it up to the four speakers who are scheduled for today to go really in depth with the specific topics.

As you know, IICA is an agriculture focused institution and I am from the US Department of Agriculture, also an agriculture focused institution. We are very aware of the conversations that can happen under the Convention on Biological Diversity (CBD) and its potential impact. We've seen this before, and perhaps some people may be hypersensitive to these discussions, but today we are discussing synthetic biology in a broader context than agriculture.

I would like to thank IICA for taking this initiative to broaden the discussion, to educate those of us in agriculture about other possible applications of synthetic biology and also to broaden the conversation to others that are working in synthetic biology, perhaps to educate them about the process under the Convention and the Cartagena Protocol, and the potential impact of these policy discussions.

My talk is structured as follows. I will talk briefly about what is synthetic biology, who cares about synthetic biology, why do we care, and why now. I am not here to provide answers to all these questions; I am more here to provide some "food for thought" as we say in the US, to, hopefully, stimulate some discussion, and I hope I really do, because I don't really think I, for one, has the answer. There may not be one right answer and we can bear this in mind as we talk in the next two days.

So, what is synthetic biology? I would like you all to take a few minutes just to jot down or ask yourselves the question, what does it mean to you when someone says "synthetic biology"? What are the key terms? Are you thinking about a specific sector? Are you thinking about specific technology? Think about that, because I think it's very natural for people when they start talking about something to want to define it, right? A definition is important to set boundaries about what we are talking about, to set the scope.

[1] Full presentation is available at http://www.iica.int/sites/default/files/events/presentations/2016-05/01_synbio_general_flchou.pdf
[2] Conference transcription: Patricia Echeverri; text edition: Pedro Rocha

I picked some definitions but they are very different. The European Commission, in a very extensive paper, defines SynBio as "the application of science, technology, and engineering to facilitate and accelerate design, manufacture or modification of genetic materials in living organisms to alter living or non-living material" (EU, 2014), a very complicated definition. Then there is another definition which is more focused on research and development, "Field of research: combines elements of biology, engineering, genetics, chemistry, and computational science" is very relevant for people in the research sector.

Late last year, at the CBD, a group of 30 experts at AHTEG met and came up with this operational definition, "a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organism, and biological systems" (AHTEG, 2015). I think that "further development" and "new dimension of modern biotechnology" combine "science, technology, and engineering to facilitate and accelerate", are the kind of words that "design and redesign". So, in the US, and Genya will talk more about this, we see synthetic biology as a continuum of bioengineering, of modern biology tools and techniques, that is improving how we make new products.

Where do all these definitions fit? Somehow I wanted you to think about your own definition and how it fits in here. These definitions are not all encompassing; some of them focus on certain aspects, other definitions focus on other aspects, and perhaps it's okay that there is no one internationally agreed on definition. In fact, in our interaction with countries, there are some like Japan that has many definitions. So, is that bad, is that good, is one definition useful in your context, let's think about that a little bit.

My personal opinion, not the opinion of the US government or USDA, is that definitions have to fit their purpose. So, a definition with a certain aspect that's important to me, that's useful to me in setting agricultural policy, may not be relevant or useful to a person in a research and development setting, or even a policy maker that's setting the research agenda for the US. So, I think that in order for you to have a useful definition, you have to think about context.

But, let´s briefly discuss synthetic biology just to see if we can get clarity on some properties of synthetic biology that we can agree on. So, is this something new, as some of the definitions say, or is it actually a continuation of what we know to be modern biotechnology?

I wanted to take some time to review (Fig. 1) the past and future actions and there's a very nice paper on the history of synthetic biology (Cameron et al., 2014) and information from Wikipedia. I'm smiling here because when I am teaching and students say they are citing Wikipedia it's a big no-no. But I think it's a good place to get some information before looking further. So, according to Wikipedia, the term synthetic biology was first used in 1910 (Fig. 1).

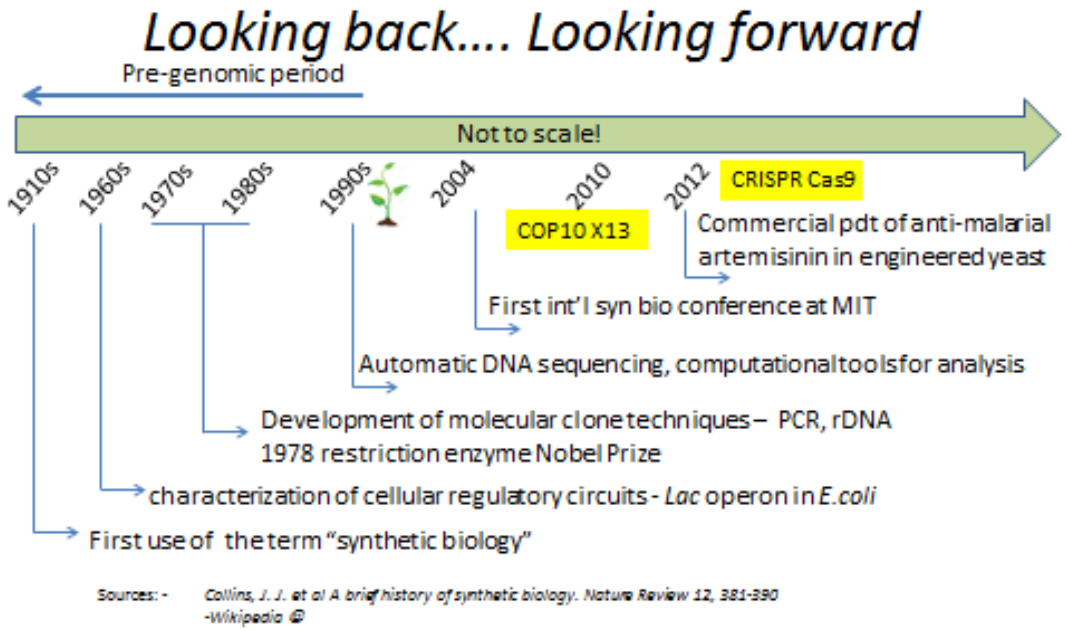


Figure 1. Brief history of SynBio. Based on Cameron *et al*. (2014) & Wikipedia (2016).

Just imagine what the definition would have looked like back then. You know, it's kind of interesting to think about it. So, it was in the 1960´s when I think modern biology actually started. That was when they first characterized a cellular circuit or cellular regulatory pathway, the Lac Operon in *E. coli*. So, people are starting to understand that in a cell, in an organism, there are these pathways, almost like a circuit. This is pretty much the engineering bit of my talk.

It was in the 1970´s and the 1980´s that the tools that we use in modern bio-technology were developed and started to be more widely used in research and development (PCR, restriction enzymes). These ideas are so commonplace now that it´s hard to think that this was somebody´s *Ph.D.* thesis, back in those days. In 1978 when restriction enzymes won the Nobel Prize, it allowed scientists to move genes from one place to another; it allowed us to copy genes. This is more or less the base of all the modern biotechnology applications that we are aware of today.

The 1990´s (when I got my *Ph.D.*) was when DNA sequencing came online and I remember in my lab we did a lot of sequencing on looking for mutations, trying to understand human muscular dystrophy diseases. We were so proud that we had this half a million dollar DNA sequencing machine and a PCR machine that could do four blocks at the same time. That was so high tech for us; I mean, this is like toys for kids now. The 1990s was also when we first started commercially planting transgenic crops. So, the application of all of these modern biotechnologies, from the 1970s to 1980s, took 20 years for something to come into the market place, to be commercialized and to be used.

Let´s talk about synthetic biology. It was in 2004 that the Massachusetts Institute of Technology (MIT) hosted the first international symposium on synthetic biology. So, as a field of science, it's been around for a while. So, why is it such a hot topic now, why are we all talking about it now, not just the research community, but the policy community, the general public, the advocacy groups, why all this, why is it all so sexy now, as someone would say.

The year 2010 saw the first decision under the Conference of The Parties (COP) to the CBD. It's an international environmental treaty that looks at how countries can protect their natural

resources, their biodiversity (including protecting its sustainable use), and ensure that there's adequate sharing of any benefits that arise from the field of biodiversity. All countries are Parties to this treaty, except the United States and the Holy See – so, yes, we're in good company. It was after the COP 10 that the decision was taken that folks should start submitting information about what synthetic biology is and its potential to impact biodiversity. This was the start of the first international policy/regulatory conversation.

The year 2012 is considered by some as the first time commercially-produced synthetic biology came on the market. This was the anti-malaria medication which was produced using engineered yeast; in my opinion, this is genetic engineering. I want you to remember the commercial production, because it cost a lot of money to actually figure out how to do it, to bring it up to speed, but it did decrease the cost of the medication and increased the production of the medication. Prior this, it had to be distilled from a natural form, a plant; this increased production, but the R&D cost was huge and it took a long time to produce. Bear this in mind, because I´ll return to discuss it later on.

We are setting the stage. Where will synthetic biology go? Where is the goal post for synthetic biology? What does it include? Does it go all the way to PCR and rDNA? Or do we move it there? Think about it. I don't think there´s a right answer. Different people have different answers.

Who cares and why do we care? Scientists, of course, are interested in it as they are interested in knowledge acquisition. I had a very simplistic view of who cares and why they care, but the more I thought about it, the less simple it became. Many people care and for many different reasons that overlap. So, scientists care (knowledge), companies care (commercial production, profit), and policy makers care.

Why do we care? How about economic development? Does it play a role in economic development? Should we be concerned from a regulatory perspective? There are many different kinds of policy makers. Should we make an investment in R&D? Regulators care, as you may know; sometimes they think they are a special part of the policy-making group. So, how do we protect our environment, our people, how do we ensure these products are actually functioning? And the public, the public in general, cares, and by this I do not mean the person walking down the street, but those groups that say they represent the general public; they care about how this impacts the society as a whole.

I think this list of "who cares" and "why care" is definitely not a comprehensive list. I´m sure you can think of other reasons why we care or why certain sectors care. But, you know, this is synthetic biology and however you want to think about it, it´s a tremendously powerful tool for knowledge acquisition. We have learned so much about biological systems, but there is still so much more to be learned. To fully take advantage of the tools of synthetic biology you have to really understand how the biological function works.

If you go to Google and just type "synthetic biology potential economic impact", there is a consulting group that says that by 2020 this market place will be worth 30 billion dollars. So, there are a lot of potential economic drivers here, product development across a sector and you will hear from our speakers about what is currently in the pipeline, what is possible and what the time-frame is. Policymakers care about this; they think about how to invest in research and also how to stimulate this innovation, this invention. I can talk about that some more.

From a public safety and regulatory perspective we have to think about two (not new) protection goals: environmental safety and human health safety. We have chemicals, pesticides, medicines, we´ve had them for a long time and there are similar protection goals for these. So, we don´t have to reinvent the wheel every time a new technology comes along.

We want to step back a little bit before I get into why, why all of a sudden all this conversation about synthetic biology is happening, even though synthetic biology research has been going on for quite some time. Let's talk a bit about certain developments and events that have led us to this point which people call the post-genomic era.

If we move back to that timeline, back to the 1970s and the early 1980s, this was when these modern tools started coming online and DNA sequencing started. We can´t build things if we don´t know what the building blocks are. The first thing that was sequenced was a single strand RNA bacterium, maybe a couple thousand base-pairs. I don´t know how long it took them, but the tools got better, and in the 1990s we had the high throughput sequencing allowing sequencing of the influenza virus and the first multi-chromosome, yeast (*Saccharomyces cerevisiae*).

In 2000, we started sequencing what I call model systems, model organisms for research such as *C. elegans*, *Arabidopsis thaliana*, and the fruit fly, all used in the laboratory all the time. And in 2001, we had the first human genome sequencing. It was on the cover of Nature. I saved that because it was huge. It took thirteen years and a Federal Government investment of USD three billion; that's not counting the venture capital, private investment. Remember how much money it cost.

We are not restricted to living things anymore. In 2004, the first complete genome of the Neanderthal was published (Nature). They isolated the DNA from a single finger bone, which I think is just fascinating, really interesting. So the genome is no big deal now. If you want to have your genome checked nowadays you can, I'll talk about this later. You can actually go online and find out which genome has now been sequenced and when they were sequenced and how big they are.

The National Institute of Health (NIH) keeps track of the cost of sequencing and the cost of synthesis (Fig. 2). The computer came, but personal computers became more common place in the 1990s. Grant Morse's Law for computational power predicted that, as time goes on, computational powers increase while its cost decreases exponentially. Fig. 2 displays the decreased cost of computer power that has taken place. Just look at our smart phone. We have more computational power in that smart phone than the computer that was used to launch space shuttle missions. It´s in our hands and it´s under 500 dollars; this is the economic nature of computational power, and it´s important in synthetic biology so keep that in mind.

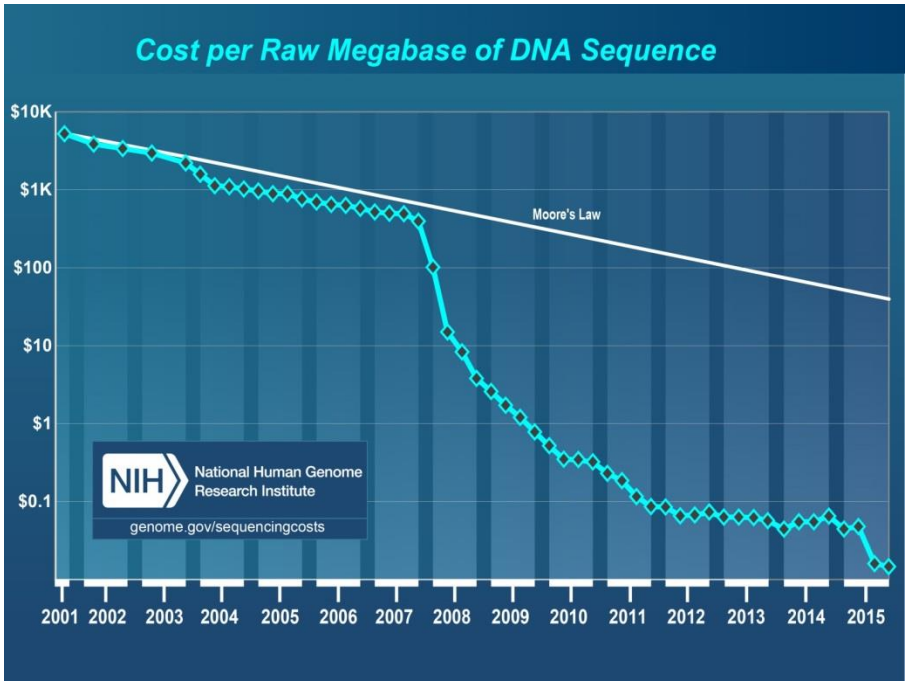


Figure 2. Evolution of cost per raw megabase of DNA sequence. Taken from NIH (2016).

The cost of DNA sequencing has also followed Morse's Law. Back in early 2001, to sequence a mega base cost 10000 USD, but from then on the cost has dropped very quickly. Nowadays companies are saying that they can sequence an entire human genome, good coverage, good grid, for one thousand dollars in three weeks.

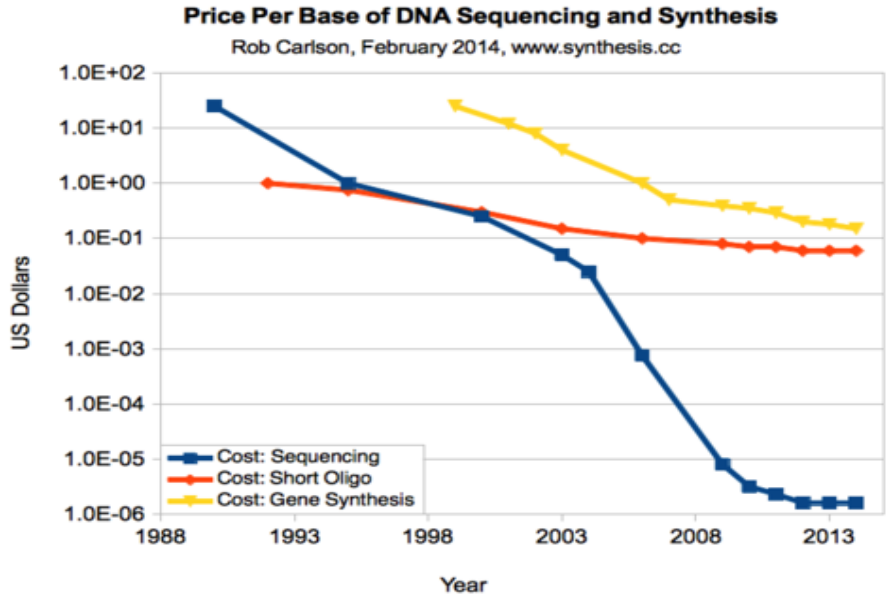


Figure 3. Comparative cost variation of DNA sequencing, short oligo synthesis and gene synthesis. Taken from Carlson (2014).

So, with a thousand dollar genome, you and I, saving say three dollars a day, could potentially have our genome sequenced. It´s amazing that this is just within the last 15 years, and the huge drop in cost was only in the last seven years. You just need to combine the sequencing and the

computational power; this is the power of synthetic biology. You can sequence genes, but if you cannot analyze them it´s not useful at all. So, the computational power is also really important.

Sequencing just tells us what the building blocks are. We have to be able to build things; we have to be able to synthesize DNA which is certainly more expensive than sequencing (Fig. 3). In its 20th anniversary issue, *Nature Biotechnology* reviews the contribution of biotechnology to various sectors, from agriculture to medicine to industrial. There, you can see the evolution of the costs of DNA sequencing, of short pieces of DNA synthesis, and complete gene synthesis (Fig. 3). DNA synthesis is still not as cheap as sequencing, but I think that following Morse's Law, as time goes on, this is going to get cheaper and cheaper.

These are pretty much the recent trends. If you think about it, it´s creating this tipping point, shall we say, that´s making this moment really ripe for synthetic biology to take these opportunities and create something that´s useful. This is part of the reason I think this conversation is happening right now and continues to expand, and involves people from the policy community and the advocacy community. As I stated earlier, given the low costs of high computational powers to make analysis more easily accessible to us, and to design, we can take different pieces and do a computer modeling and see if that´s going to work given the low cost of DNA sequencing and synthesizing. Formerly we were using restriction enzymes, PCR, to clone pieces of what´s naturally occurring. Now it is possible to just synthesize that gene; you don´t need a template any more.

These tools are really useful from a research perspective to expand our knowledge base of biological systems and biological functions. Synthetic biology takes advantage of our understanding of the biological system, how we can tweak it for our use. So, the more we know, the better we can get at this.

People talk about the engineering component of synthetic biology and as my engineering friends would say, "engineers don´t like complexity, we like simplicity" like A to B to C. We want a circuit, we want predictability, we want to know that when we put two things together they are going to react a certain way. In contrast, biology is complicated. People are complicated. If we think back to 2001 when we sequenced the human genome there was this huge expectation: we were going to have personalized medicine, genomic based therapeutics and that has taken a much longer time to happen than people expected; this is the complexity of systems biology.

We have to understand biological functions in order to take advantage of them for our personal purposes. I think that, aside from the lower costs of these technologies, there is increasing power in the new molecular biology tools that have been highly publicized in the last few years. Plus, there are research and editing tools like CRISPR, TALEN which are making molecular manipulation of DNA much easier, much faster, much cheaper, and much more precise. All of this is causing research and development in the field to speed up. When things like this happen, there are many implications, and this doesn´t just happen for biological systems. Just think about how engineering computers got really fast and became available in every single person´s home, every single office.

Each of us probably have three different computer systems, smart phone, or iPad or surface, and our own personal computer and then we have one in the office. I think it´s really important to have this conversation, about what we are looking at and how to get it right. Getting it right is different for different people, may be different for different countries, and may be different for

different sectors. These opportunities, this confluence of events, make economic development possible, creating jobs, creating products that can help our society. Lloyd had mentioned all these challenges that we´re facing; we face climate change, especially in agriculture, which is becoming a huge issue.

So, how do we get this right? We´re balancing regulatory oversight (Fig. 4), because it is important to make sure that all technology is deployed in a responsible and safe manner, and that this freedom exists so that innovation can take place. Freedom is needed for our research and development community, our universities, our companies, for folks in a research group that want to become companies. As a consequence, we have to think about how much regulation we need.

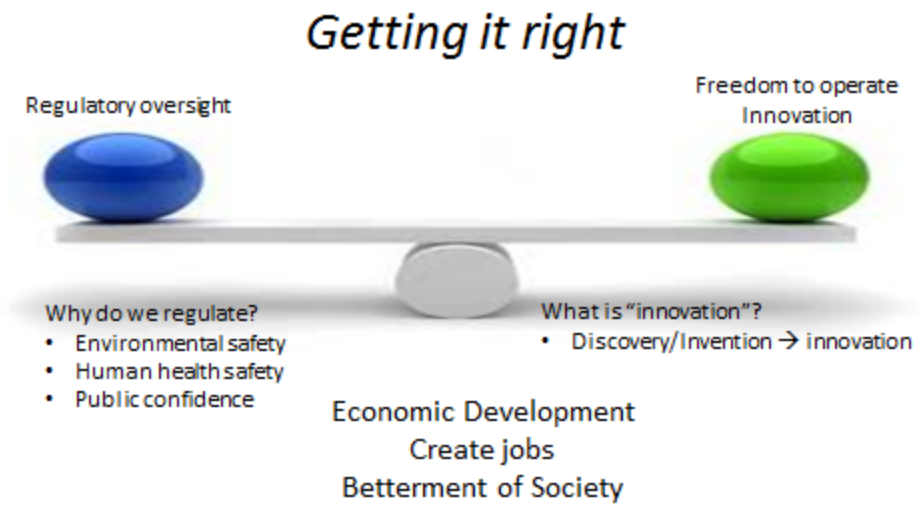


Figure 4. Balance of regulatory oversight and freedom to operate innovation.

What is innovation? You need to think about what innovation is. It´s not just discovery; discovery happens, a lot of discovery and invention happen and they never become an innovation. So, innovation is when you take that invention and make it useful. You can invest in research and development, you can invest in education, but how do you create the environment where, as a society, you can translate that invention into innovation. Invention happens in the lab, innovation happens in the wider community.

As you know, there are many different steps that the government and the public have to think about. Are we creating the environment that will allow innovation to happen in a responsible manner? Research, investment and development form the basis for innovation. Investment in education because when you have this market base, you have these new developments, you need people to work in it, and you need a public that understands it, so they understand what it is that you´re trying to achieve.

You have to create an environment that rewards risk taking because these things are risky. Nobody knows if the public will accept them, or if they are going to be successful and you need a framework for oversight, for regulation that is transparent, predictable, flexible and responsive. And this is not new to SynBio, it´s across the board. People have to know what´s happening when they come to you and ask how you are going to regulate me; if I´m going to give you this information, what´s going to come out at the other end. Regulation has to be flexible because

science is changing quickly, so how do you respond to that. It has to be responsive. At modern biotech, we´ve been regulating for 28 years how you learn from that experience; do you still want to do it the way you did it 28 years ago? Does that make sense? How do we modulate our risk perception and our risk assessment?

I´m going to conclude by leaving you with some thoughts; and we can think about this and talk about this more in the next two days. One has to do with definitions. There should be a lot of conversation about definitions and I think this will come up at the COP meeting. Is there a one size fits all definition? Definitions, should they be for a purpose? What is the purpose? And can the definition stand the test of time to remain relevant? We talk about modern biotechnology, does that make sense now, and did it make sense 20 years ago? New breeding techniques, what´s new today is not new tomorrow.

This goes beyond the policy discussions. What are the wide ranges of applications that may be impacted in regard to the policies we make, from research policy, to regulatory policy and economic policy? And those of us who are familiar working with agriculture need to think about this more broadly, hopefully in the upcoming days. Later today we´ll get some concrete examples of the commercial applications of synthetic biology, what´s possible, what´s near term, what´s long term. And the policy discussion has to be within the context of national policy, national research policy, national economic development policy, and within the context of the Commission on Biological Diversity. These are two very different mandates. So, you have to think about that and the interplay between the two, because since everybody here, except the US, is party to the Convention, whatever happens at the Convention will have an impact on your national policies.

I´m really hoping for some lively discussion today and tomorrow, maybe not here, but maybe on the side. This conversation is going to continue beyond these two days and I hope and know that we´re going to learn a lot in the next days that will help us talk to our colleagues and our friends.

References

AHTEG. 2015. Report of the *ad hoc* technical expert group on synthetic biology. UNEP/CBD/SYNBIO/AHTEG/2015/1/3, 7 October 2015.

Cameron, E; Bashor CJ; Collins JJ. 2014. A brief history of synthetic biology. Nature Review 12, 381-390

Carlson, R. 2014. http://www.synthesis.co

European SynBioSafe. 2016. http://synbiosafe.eu/

Wilson Center Synthetic Biology Project. http://www.synbioproject.org/

U.S. National Academy of Science.
http://sites.nationalacademies.org/PGA/stl/Synthetic_Biology/index.htm

1.2. Synthetic Biology and its applications in agriculture[3,4]

Marcelo Henrique Aguiar de Freitas, *Ph.D.*
Embrapa, Brazil
marcelo.freitas@embrapa.br

Summary

Mankind has tried to obtain the best from nature from the beginning of time. Ten thousand years ago, we started selecting specific characteristics of interest and traits, and breeding them in crops and animals. With the passage of time, we changed the tools, but we are still modifying the genotypes. A paradigm shift occurred in early 1980 when we started to modify organisms through genetic engineering. From 2000-2010, another paradigm shift occurred with the use of Synthetic Biology (SynBio). With this progress, the tools became more complex and the level of control increased, while the time to arrive at a new product reduced dramatically. With SynBio it is possible to create a new and better product more efficiently by combining many characteristics of interest and putting them all together in one crop or in one interesting organism. SynBio can be used to solve major problems like food and calorie security, climate change, land degradation, water depletion and other problems related to human and animal health.

In agriculture, SynBio can be used in different and important approaches related to crop development, including new crops with better productivity; resistance to biotic and abiotic factors; improved quality (flavor, aroma, color, and nutritional factors); use of some other industry characteristics (such as high cellulose cotton); development of bio-factories for producing important compounds (sugars, cellulose, enzymes, bioactives, sweeteners, flavors and fragrances); altering microbial metabolism to deal with methane and nitrous oxide emission from livestock and fertilizers, respectively; carbon sequestration to improve soil quality; and it can function as biosensors to indicate the condition of the soil and presence of toxins or pests. So, the impact of SynBio in the last year reached more than 5 million dollars and it is expected to reach at least 38 billion by 2018.

Presentation Development

We have many definitions of SynBio, as Fan-Li just said. According to the AHTEG definition, "Synthetic biology (SynBio) is a further development and new dimension of modern biotechnology that combines science, technology and engineering to facilitate and accelerate the understanding, design, redesign, manufacture and/or modification of genetic materials, living organisms and biological systems." It´s not a perfect definition, but it's operational, and very similar to the definition of living modified organism (LMO): "Any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology"(Cartagena Protocol on Biosafety, Article 3). So, synthetic biology under the Cartagena Protocol is an interesting subject that we will discuss over the next two days.

The socio-economic impact of SynBio is huge. It represented, in the last year, more than USD 5 billion and it´s expected to reach more than 38 billion by 2018. So, we have a lot to do and we need to do it quickly.

---

[3] Full presentation is available at http://www.iica.int/sites/default/files/events/presentations/2016-05/02_synbio_in_agriculture_mfreitas.pdf
[4] Conference transcription: Patricia Echeverri; text edition: Pedro Rocha

We have tried to get the better of nature – to manipulate agriculture and crops since the beginning of time. So what do we desire? We changed the tools we need and the tools that we use. So, 10,000 years ago we started selecting specific characteristics of interest, traits, and breeding these in crops and animals. With the passage of time, we changed these tools and organisms. So a great leap occurred in 1980, when we started to modify organisms through genetic engineering; it was a miracle. Since 2010, we have synthetic biology, a new miracle that puts genetic engineering in the past.

With these tools, the complexities of human intervention as well as the level of control have increased. Interestingly, more complexity and more control do not imply more time to obtain a product (Fig. 5).

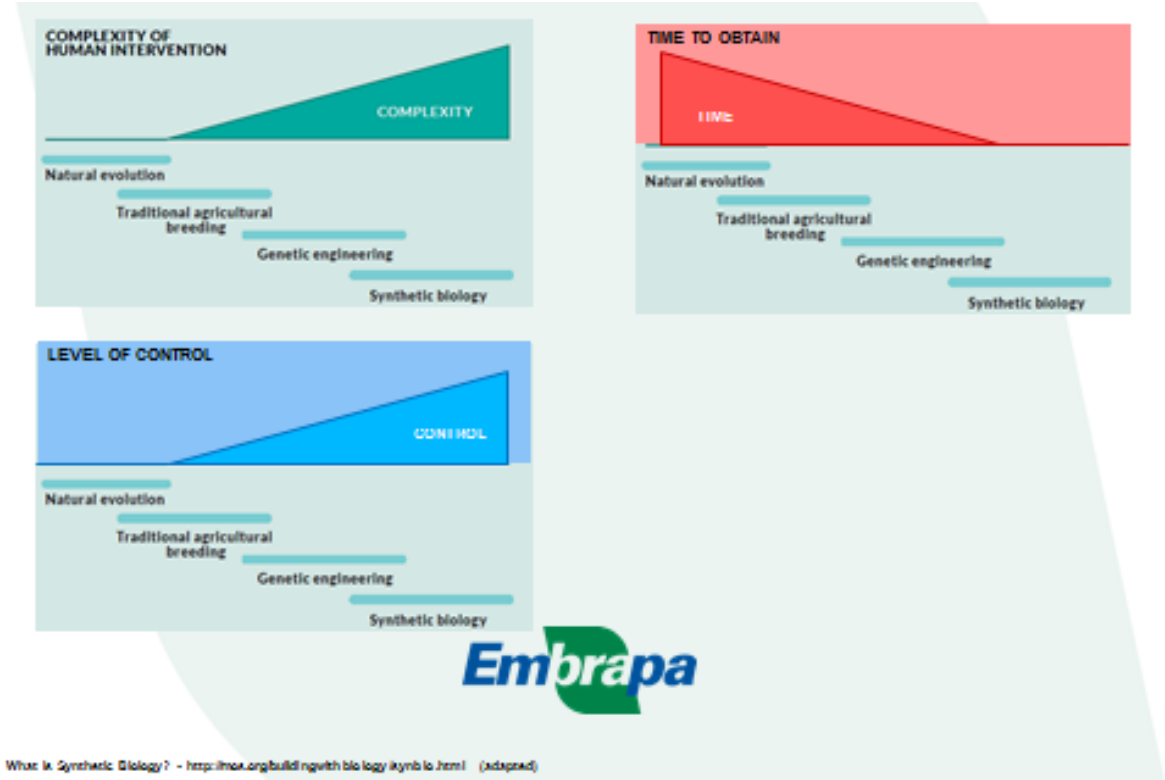


Figure 5. How genetic manipulation has evolved.

What we can do with synthetic biology is now faster and more precise. In the beginning, we looked for specific traits that we needed to breed in order to obtain new and better characteristics in our organisms. With genetic engineering, we scaled the barrier of the species, passing on new traits to another species. With synthetic biology, we can do virtually anything, for instance, design and synthesize one or several genes, introduce them in any organism and express them in a specific way, creating novel genetic and biochemical circuits in the organism of interest (Fig. 6).

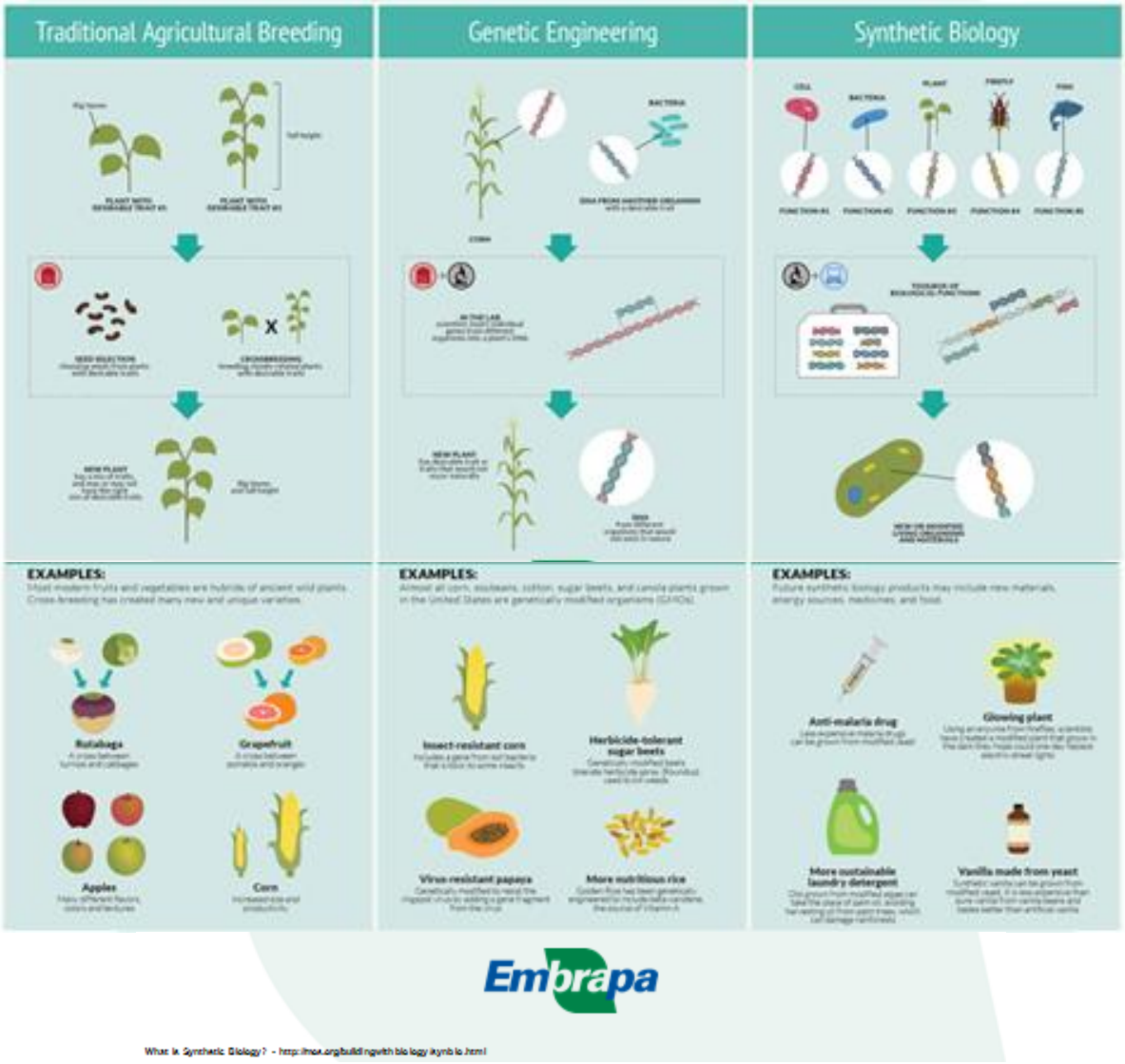


Figure 6. Evolution of genetic manipulation. Based on http://mos.org/buildingwithbiology/synbio.html

Why do we have to use SynBio? Why do we need it now? There are many reasons, but I have just three: food, water and climate change. In 2050, the world´s population will reach more than 9 billion people and the food and energy requirements will increase tremendously. So how will agriculture manage to produce food? What we have done up to now with GM crops is amazing. Only ten countries are producing 98% of all GM crops, and Brazil has consolidated as the second largest producer of GM crops in the world. So, now and in the future, it is almost impossible to avoid using GM crops. If you have a scenario of a growing population without the use of GM crops, several negative impacts will occur, for example, food prices will go up, emissions of green-house gases and use of pesticides will increase, pressure on land will be major, and all these factors will directly impact the environment, water consumption, and the global economy.

SynBio can be used in plants for several reasons, including being the most important source of the primary metabolites that feed the world. They produce a diverse array of valuable secondary metabolites, they allow a good understanding of biological processes, and they are quite efficient in using abundant and inexpensive nutrients. So, we can use synthetic biology in agriculture in

different ways and approaches. Here, just four will be considered: crop development, bio-factories, microbial recycling technology and biosensoring.

Regarding crop development, many applications of SynBio can be found in order to increase crop productivity, to incorporate resistance to biotic and abiotic factors, to improve product quality, and to produce novel or valuable compounds.

In terms of crop productivity, several examples can be mentioned, including the capability of improving the nitrogen fixation from the atmosphere, the ability to reduce the use and consumption of inorganic fertilizers, the possibility to convert crops from C3 to C4, and the opportunity to generate drought tolerant crops. Currently, there are some two hundred GM drought tolerant crops under development and in field tests, but if we use synthetic biology, we can bring about this development more quickly and more efficiently.

Bio-factories are another approach in which SynBio has vast potential. For example, in the production of interesting products like alkaloids, bioactives, polyphenols, sweeteners, flavors, spices, etc. In addition, SynBio plants can be used as bioreactors for producing hormones and vaccines.

Modification of microbial metabolism by SynBio could be an efficient way to deal with some current challenges coming from agriculture. For example by modifying metabolism in order to reduce methane emission from livestock and manure, diminish nitro-oxidant emissions from fertilizers, and increase carbon sequestration to improve soil quality.

And last, but not least, is the use of biosensors in crops. We can put in place a specific domain to detect some things linked to protein fluorescence, or one genetic signal to show us the condition of the soil, or the presence of toxins, or pests or other infectious agents. In EMBRAPA, we have a specific program to analyze this approach. We are just at the beginning, but we know SynBio is the future of agriculture and we need it.

References

Klümper, W; Qaim, M. 2014. A Meta-Analysis of the Impacts of Genetically Modified Crops. PLoS ONE 9(11): e111629. doi:10.1371/journal.pone.0111629

PMSEIC (Australia), 2010. Australia and Food Security in a Changing World. 2010, Science, Engineering and Innovation Council of Australia: Canberra

Baltes, NJ; Voytas, DF. 2015. Enabling plant synthetic biology through genome engineering. Trends in Biotechnology, 33(2), 120-131. DOI: 10.1016/j.tibtech.2014.11.008

Taheripour F; Mahaffey H, Tyner WE. 2015. Evaluation of Economic, Land Use, and Land Use Emission Impacts of Substituting Non-GMO Crops for GMO in the US. Selected Paper prepared for presentation for the 2015 Agricultural & Applied Economics Association and Western Agricultural Economics Association Annual Meeting, San Francisco, CA, July 26-28. Available in: http://ageconsearch.umn.edu/bitstream/204907/2/AAEA%20GMO%20final3.pdf

McAlpine KJ. 2016. Plants with biosensors may light the way. Available in: http://news.harvard.edu/gazette/story/2016/02/plants-with-biosensors-may-light-the-way/

ACOLA, 2015. Securing Australia's Future program: Australia's Agricultural Future. Australian Council of Learned Academies.

United States Department of Agriculture (USDA). 2008. U.S. Biobased Products: Market Potential and Projections Through 2025. p. 1-294.

Canadian Biotechnology Action Network (CBAN). 2015. Where in the world are GM crops and foods. Available in: http://www.cban.ca/Press/Media-Backgrounders/Media-Background-Where-in-the-world-are-GM-crops-and-foods

McMichael, AJ; Campbell-Lendrum DH, Corvalán CF, Ebi KL, Githeko AK, Scheraga JD, Woodward A. (eds.) 2003. Climate change and human health - risks and responses. WHO. Geneva, 322p. Available in: http://www.who.int/globalchange/publications/climchange.pdf

Sumant O, 2016. Synthetic Biology Market By Products (Synthetic DNA, Synthetic genes, Software tools, Synthetic cells, Chassis organisms), and Technology (Genetic Engineering, Bioinformatics, Microfluidics) - Global Opportunity Analysis and Industry Forecast, 2014 – 2020.

1.3. Industrial applications of synthetic biology[5,6]

Natalia Verza, *Ph.D.*
Structural Genomics Consortium, State University of Campinas, Brazil
na.verza@gmail.com, natverz@unicamp.br

As presented by previous speakers, there are already many definitions of synthetic biology. I only want to stress that it´s an attempt to engineer biology and, by doing that, make biology easier to engineer, which is really important from the industrial point of view, because what we need is to do things in a faster, cheaper and in a more precise way, so that we can quickly create a commercial product without investing a lot of money in it.

In my view, the most important fact about synthetic biology is that it has many parts and we have a repository of parts that we can order, either for commercial entities or public databases, turning something really complex, like metabolic pathways, into something simpler and easier to assemble and, most importantly, affordable.

I am going to show you what we are doing in my lab, at the State University of Campinas in Brazil, to achieve tolerance to drought stress in maize. We can use a number of different tools, for example, gene discovery using databases of published expressive genes and selecting the best to be expressed in maize. Once the gene is identified, we will want to introduce it into the crop but that gene comes from an anaerobic bacterium, *Clostridium*.

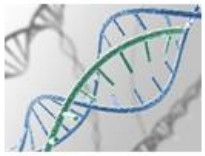
We can choose one of two ways–classic genetic engineering or synthetic biology. First, we know that different organisms can use the code for amino acids in different ways and for different species the optimum codons are different. So, for maize, you use C25 from 12 per cent of all the codons present in the maize genome, but for *Clostridium* the same codon is only 6.8% (Fig. 7). So, if a gene from *Clostridium* is taken and inserted into maize it would not be as good as if it had the same codon usage as maize. So, we have differences in codon usage and that can be the difference between success and failure.

In addition, if the classic genetic engineering approach is taken, new equipment and development of new protocols to work with bacteria, especially anaerobic bacteria like *Clostridium,* are required, because my lab is for plant molecular biology, not for microbiology. So, new investment and more time to learn how to deal with microbes will be needed. Then, once everything is in place, the gene can be amplified and isolated and the expression cassette constructed and introduced into maize. Then a transgenic plant expressing the gene is obtained and the phenotype can be analyzed.

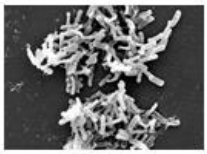
But, if the synthetic biology path is taken, first I´ll check if this organism has a complete genome published. If so, we go to the computer to design the gene as we want to, optimizing the codons that will work well in the target species. There is no need to invest a lot in equipment or learning new lab protocols. This would be faster, so I can use the same method: construct the cassette and express it into the crop. This approach can save time and money (Fig. 7).

[5] Full presentation is available at http://www.iica.int/sites/default/files/events/presentations/2016-05/03_synbio_in_industry_nverza.pdf
[6] Conference transcription: Patricia Echeverri; text edition: Pedro Rocha

Species of interest: maize (*Zea mays*)
Goal: improve crop tolerance to drought stress



Target gene:
Co-chaperonin GroES



Donor species: *Clostridium acetobutylicum* (anaerobic bacterium)

Critical point: Codon usage divergences

***Zea mays* [gbpln]: 2482 CDS's (1065603 codons)**

fields: [triplet] [frequency: **per thousand**] ([number])

```
UUU 12.6( 13489)  UCU 12.0( 12747)  UAU  9.5( 10149)  UGU  5.6(  6019)
UUC 25.1( 26704)  UCC 16.4( 17446)  UAC 19.4( 20697)  UGC 12.2( 13038)
UUA  5.7(  6069)  UCA 11.0( 11759)  UAA  0.5(   542)  UGA  1.1(  1218)
UUG 13.0( 13803)  UCG 10.7( 11356)  UAG  0.7(   756)  UGG 13.0( 13827)

CUU 15.7( 16741)  CCU 12.6( 13433)  CAU 10.1( 10750)  CGU  6.0(  6421)
CUC 25.4( 27033)  CCC 13.5( 14420)  CAC 14.9( 15930)  CGC 14.3( 15213)
CUA  7.3(  7759)  CCA 13.8( 14679)  CAA 13.2( 14109)  CGA  4.4(  4644)
CUG 25.8( 27481)  CCG 15.8( 16820)  CAG 23.7( 25256)  CGG  9.5( 10156)

AUU 13.8( 14720)  ACU 10.7( 11411)  AAU 13.5( 14339)  AGU  7.8(  8271)
AUC 22.7( 24228)  ACC 16.6( 17657)  AAC 22.1( 23497)  AGC 16.4( 17438)
AUA  8.4(  8988)  ACA 10.5( 11184)  AAA 15.1( 16076)  AGA  8.8(  9402)
AUG 24.2( 25781)  ACG 11.0( 11693)  AAG 39.4( 41990)  AGG 14.8( 15774)

GUU 15.7( 16705)  GCU 21.0( 22340)  GAU 22.9( 24454)  GGU 14.1( 15055)
GUC 21.0( 22425)  GCC 31.1( 33176)  GAC 32.1( 34247)  GGC 30.3( 32321)
GUA  6.4(  6859)  GCA 16.7( 17798)  GAA 19.9( 21223)  GGA 13.4( 14248)
GUG 25.5( 27178)  GCG 23.3( 24817)  GAG 40.8( 43493)  GGG 15.4( 16361)

Coding GC 54.98% 1st letter GC 57.58% 2nd letter GC 43.37% 3rd letter GC 64.00%
```

***Clostridium acetobutylicum ATCC 824* [gbbct]: 3881 CDS's (1191394 codons)**

fields: [triplet] [frequency: **per thousand**] ([number])

```
UUU 37.9( 45143)  UCU 16.4( 19515)  UAU 33.6( 40067)  UGU  8.3(  9932)
UUC  6.8(  8062)  UCC  4.0(  4821)  UAC  9.2( 10956)  UGC  3.6(  4307)
UUA 36.0( 42902)  UCA 18.4( 21896)  UAA  2.1(  2470)  UGA  0.4(   432)
UUG  9.7( 11570)  UCG  2.3(  2746)  UAG  0.8(   979)  UGG  7.4(  8784)

CUU 26.7( 31829)  CCU 12.2( 14537)  CAU 10.5( 12501)  CGU  2.2(  2661)
CUC  2.4(  2814)  CCC  1.3(  1538)  CAC  2.9(  3442)  CGC  0.6(   694)
CUA  9.5( 11310)  CCA 12.0( 14291)  CAA 16.3( 19410)  CGA  1.0(  1207)
CUG  2.4(  2805)  CCG  1.7(  2027)  CAG  7.0(  8361)  CGG  0.2(   185)

AUU 35.5( 42278)  ACU 19.6( 23404)  AAU 52.5( 62569)  AGU 18.8( 22451)
AUC  4.7(  5643)  ACC  5.0(  5947)  AAC 12.8( 15295)  AGC  7.9(  9381)
AUA 55.6( 66287)  ACA 21.9( 26111)  AAA 63.1( 75154)  AGA 23.4( 27915)
AUG 25.1( 29951)  ACG  3.0(  3570)  AAG 30.4( 36244)  AGG  5.9(  7070)

GUU 29.8( 35472)  GCU 23.3( 27707)  GAU 46.8( 55698)  GGU 19.8( 23564)
GUC  2.1(  2480)  GCC  4.2(  4988)  GAC  8.5( 10102)  GGC  6.3(  7460)
GUA 27.4( 32636)  GCA 25.6( 30537)  GAA 49.8( 59274)  GGA 32.1( 38248)
GUG  7.0(  8297)  GCG  3.6(  4289)  GAG 17.8( 21189)  GGG  5.0(  5997)

Coding GC 31.55% 1st letter GC 41.76% 2nd letter GC 31.74% 3rd letter GC 21.15%
```

Classic genetic engineering

Special equipment and new protocols

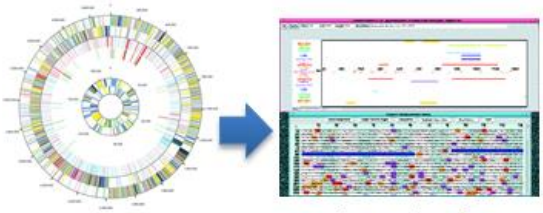
Anaerobic chamber

Methods development and validation

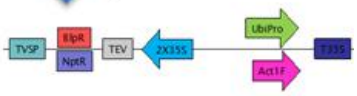
Construction of DNA expression cassette

Zea mays calli transformation

Synthetic biology



Design and order optimized, synthetic gene



Construction of DNA expression cassette

Zea mays calli transformation

Figure 7. Comparison between classic genetic engineering and synthetic biology (Based on N. Verza, and adjusted by P. Rocha). Explanation in the text.

As Marcelo and Fan-Li informed you already, synthetic biology is not a new science, we´re only improving the classical genetic engineering by mixing and matching genetic material to create novel things. But we differentiate synthetic biology from genetic engineering by the use of standardized parts that I can do or I can have in the fridge.

The first breakthrough in state of the art synthetic biology was by industrial synthetic biology companies, when Jay Keasling´s group discovered and re-engineered yeast to inexpensively produce the anti-malaria medicine, artemisinin (ref. Nature Reviews Micro-Biology in 2014). What they did was to use a yeast cell as a bio-factory, in which a number of genes were modified and inserted into the cell, assembling a pathway that produced the anti-malarial drug. This is being produced right now by Sanofi in Europe. The drug is already being commercialized and is actually being delivered to African countries free of charge. But the most important thing for me was that when they discovered biofene, a bio-factory, it enabled them to generate a number of other products. Out of this, they founded Amyris, a company based in California that commercializes multi-product platforms derived from this non-profit project. Now they can produce a number of different products.

Later on, in 2010, Craig Venter published the first self-replicating bacterium made from artificially synthesized genomes in the lab. This was important because it showed the entire scientific community that we can do almost anything, in a faster way, if we have computers, some creativity, and some money. What they did was to type into a computer the letters for the genome that they wanted to build in a microbe, matched the letters they produced, and then turned it into yeast. So, they put together all the genomes and then produced the new bacteria.

Finally, what we are seeing right now is the new revolution of genome editing. This is very revolutionary for us, especially in health applications, as well as in agriculture, because engineering a bacterium is simple, because it´s only one cell and so, you can control everything inside of it. But when you´re engineering a plant you cannot control it very well because of the vast number of different cells with different metabolic pathways going on in a leaf and in a root. But if you can intervene specifically in a particular part of the genome, you can control the process in a much safer way.

The CRISPR-Cas9 revolution differs from what we were doing in the past in that we can add genomes *in vivo*. You can have a plant and you can add the genome while it´s living and growing. Two researchers from the University of California, Berkeley, and the Max Plank Institute, were the inventors of this technique which was chosen by Nature and Science as the best technique of the year 2015.

How does CRISPR-Cas9 work? Simply put, using a plant example, imagine you have the DNA and you want to "re-write" a specific sequence on that DNA for some reason, either to introduce a mutation that is thought to be good for the plant, or a defective gene that is causing a disease and you want to introduce a gene there specifically to cure the disease. Then, it is possible to design in the computer what is called a guide RNA, a molecule that is similar to the region that you want to cut and replace. In the computer, the complementary letters of these regions are typed and then it identifies the plant or the organism using the guide RNA. When the guide RNA is linked to the double strand DNA, the enzyme Cas9 recognizes the sequence, cuts it and introduces the new donor DNA in place of the other one. So what is put into the target organism are the guide RNA, the enzyme, and the new DNA sequence that I want to replace. Then the cell does the job for us; it replaces it with the sequence that we want.

Who are the companies that are taking advantage of those technologies to produce commercial products? There are some leading consumer biotech companies working on bio-products, bio-fuels and also health-care products. My favorite companies, because of their impact, include, for example, Novozymes, an enzyme producer that helps us with the soap that we use to wash our clothes, and also with enzymes that we can eat. Then there is Amyris with bio-fuels and diesel coming from sugarcane in Brazil; a lot of buses in Sao Paulo city are running on this fuel, and also jet fuel for airplanes. Oxytec produces transgenic mosquitoes which compete with the wild ones in nature, generating unviable offsprings, and consequently controlling the population of new mosquitoes. There is a very nice project in Piracicaba, Brazil that is a remarkable success story.

There are also some companies that are creating tools to enable other companies to generate the products that we want. For example, some companies are developing methods to create microbes to order. Let's say I want a bacterium that produces second-generation ethanol from cellulosic feedstock. I can go to one of these companies and I can order it. They can deliver the microbe for me just to scale up and work on the process development on an industrial site. I don´t have to have my own lab, as a company, I don´t have to hire a lot of scientists, or learn from scratch, because they can do that for me, since that is a project we can do together.

There are also the genomes editing technology companies. These are very new and most of them are for health care. They are getting funding and we expect a lot of good results in the near future. Also, there are companies that sell synthetic DNA. Those are very important because, in a lab, in any place in the world, you can order genes and get them in a few weeks to do your job. I don´t have to take a plane and dive into the deep sea to get a bacterium from which DNA must be extracted and the target gene amplified. Nowadays, if you need it, you order it and you get it.

Some commercial products from SynBio are already on the market. For example, the biolsoprene™, fermentation-based synthetic rubber made by engineered microbes expressing plant genes. SynBio has enabled the construction of a gene that encodes the same amino acid sequence as the plant enzyme but that is optimized for expression in the engineered microorganism of choice (a partnership between DuPont and Goodyear Tire & Rubber Company). Another example is Cephalexin, a synthetic antibiotic that started with a penicillin-producing microbial strain; two enzyme-encoding genes were introduced and optimized for a one-step direct fermentation of adipoyl-7-ADCA which is converted into Cephalexin via two enzymatic steps (DSM). In both cases, processes for producing such compounds were cheaper and faster than previous ones. Another medical example is sitgliptin (dipeptidyl peptidase-4 inhibitor to treat type II diabetes). Using SynBio and its directed evolution technologies, Codexis and Merck collaborated to develop a novel, environmentally benign alternative manufacturing route. Codexis discovered and developed a transaminase capable of enabling the new bio catalytic route, which is currently in scale-up towards commercial manufacture.

Another product is the Bio Acrylic, a fermentation-based acrylic using sugar feedstock obtained as a result of the partnership between OPXBio (now Cargill) and Dow. The BDO - 1,4-butanediol, a solvent used in the manufacture of some types of plastics, elastic fibers and polyurethanes, is generated by Genomatica in partnership with BASF and Novamont. The Biofene™, from Amyris, is a molecule that can replace petrochemicals in a wide variety of products in the cosmetics, flavors and fragrances, consumer product, polymers, lubricants and fuel markets.

Talking about fuels, there are a number of companies working on jet fuel, diesel, ethanol, bio-diesel, bio-butanol and a number of different things from different sources (Table 1).

Table 1. Bio-fuels pipeline products (by organization).
Based on Presidential Commission for the Study of Bioethical Issues (Transparency Market Research Report)

Product	Organization
Biodiesel	Amyris, Inc.
Biobutanol	British Petroleum plc and Du Pont
Biobutanol (Jet fuel)	Gevo, Inc.
Bio Isobutene	Global Bioenergies
Biodiesel	Joint BioEnergy Institute (JBEI)
High energy transportation fuels	LS9, Inc.
Algal biomass	Aurora Algae
Bioethanol	Joule Unlimited, Inc.
Soladiesel	Solazyme
Biocrude oil	Synthetic Genomics, Inc. and Exxon Mobil Corporation

A very interesting initiative is being carried out by a company named Medicago in partnership with the John Innes Centre (JIC) to express proteins in plants. JIC developed a technology for the rapid expression of proteins in plants. Medicago Inc. licensed the technology to produce ten million doses of H1N1 swine flu VLP vaccine in just a month, outperforming the traditional method which takes 9-12 months.

In regard to technologies, the SynBio market is segmented into enabled and enabling technologies (Table 2). In 2013, enabling technologies accounted for a major share of the synthetic biology market. On the basis of applications, the SynBio market is segmented into environmental, medical, and industrial applications. In 2013, the medical applications segment accounted for a major share of the SynBio market. However, SynBio is being used in different fields.

Table 2. SynBio technologies and applications.

SynBio enabling technologies	SynBio applications
Enabling Technologies	Environmental Application
Bioinformatics	Bioremediation
Gene Synthesis	Whole-cell Biosensors
Genome Engineering	Medical Application
Microfluidics	Artificial Tissue and Tissue Regeneration
Measurement and Modeling	Drug Discovery and Therapeutics
Nanotechnology	Pharmaceuticals
Cloning and Sequencing	Industrial Application
Site-saturation Mutagenesis	Biofuels and Renewable Energy
Enabled Technologies	Biomaterials and Green Chemicals
Pathway Engineering	Industrial Enzymes
Next-generation Sequencing	

It is possible to divide the markets by products, technologies and applications. Companies are building tools for other companies to make the products and now these get the major part of the

funding because this is the beginning of industrial SynBio. Talking about funding, in 2015, investment in new synthetic biology companies surpassed half a billion dollars, a sum greater than all SynBio investment in 2013 and 2014 combined. Also, the number of companies that are being funded is increasing in an exponential way.

The demand for synthetic biology is likely to increase in the future, owing to the increasing R&D expenditure in pharmaceutical and biotechnology companies, the growing demand for synthetic genes, rising production of genetically modified crops, and continuously increasing funding in the field of synthetic biology. However, ethical and social issues, such as biosafety, are major factors that are restricting the growth of this market. Furthermore, rising concerns over fuel consumption and increasing demand for protein therapeutics are likely to create opportunities for the synthetic biology market. However, standardization and integration of biological parts at system level still remains a challenge for this market.

Investment is growing. Some investors include Y Combinator, Founders Fund, Google Ventures, Flagship Ventures, OS Fund, Data Collective, Sofinnova Partners, Fidelity Biosciences, Innovation Endeavors, Novartis, SOSventures, Bioeconomy Capital, Rainbow Seed Fund, Draper Fisher Jurvetson, and Illumina. In 2015, Editas Medicine had the major investment (Fig. 8), USD 120 million. This health care company is carrying out research in editing the human genome to fight against diseases, etc. The second biggest investment (USD 76 million) went to Green Biologics, a company that uses *Clostridium* to produce biofuels and other industrial chemicals from sustainable feedstocks. Green Biologics is just one player among an impressive group of companies that excel at organism engineering. Joule Unlimited uses bacteria to convert carbon dioxide into hydrocarbons and fuels. Calysta harnesses microbes to convert notoriously difficult-to-work-with methane into high-value industrial products (Fig. 8).

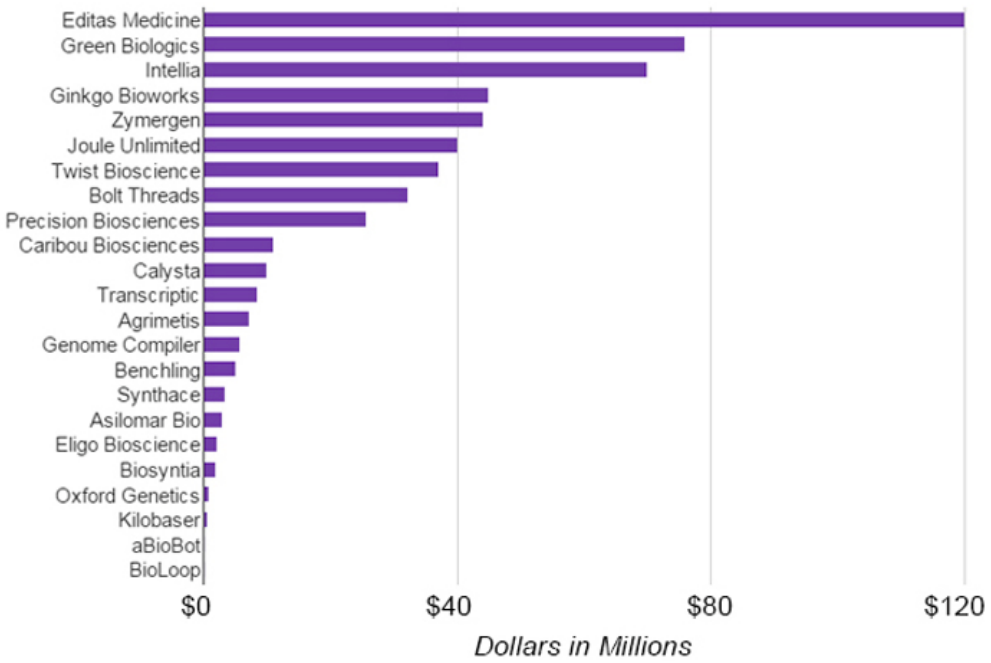


Figure 8. 2015 investment in SynBio companies.Taken from SynBio beta.

In closing, we can see that regulation is needed because SynBio can reach different fields worldwide with different goals and it´s receiving a lot of funds nowadays. What can we do? In

regard to industrial SynBio, the technology is only an extension of the genetic science that has been practiced in the past years, in a safe way. It has the potential to reduce the research and development time spent, and increases the speed to market. Also, supporting the view of the NIH, there´s no difference between the recombinant DNA technologies and the synthetic techniques that are being used now; instead of thinking about this we should focus on the biological attributes of the products, because each product is a case. We have to keep in mind that what we all want is safety for the population and for the environment. Why not focus on the product itself, since that is what is going to reach the people.

References

Erickson, B; Singh R, Winters P. 2011. "Synthetic Biology: Regulating Industry Uses of New Biotechnologies. Science 333(6047): 1254–1256.

Paddon, CJ; Keasling JD. 2014. Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nature Reviews Microbiology 12, 355–367 doi:10.1038/nrmicro3240

Serrano, L. 2007. Synthetic Biology: Promises and challenges. Molecular Systems Biology, 3:158.

1.4. Application of synthetic biology in the control of vector borne diseases[7,8]

Mario Henry Rodríguez López
National Institute of Public Health, Mexico
mhenry@insp.mx

Summary

Synthetic biology has many definitions and applications. In public health, it has wide-ranging effect in relation to diseases transmitted by vectors. This chapter describes some general aspects of the main diseases transmitted by mosquitoes (dengue, chikungunya and zika), together with traditional control methods and approaches based on genetic engineering. The aim is to share information on technologies that constitute valuable tools and contribute to the prevention of diseases and to a better quality of life.

Outline of the presentation

I work in public health which, unlike applied and clinical medicine, focuses on the wellbeing of populations and on preventing outbreaks of disease. It is not the same to rescue a child from a river, when he has already fallen in, as to prevent the child from falling into the river. Therefore, we need mechanisms that prevent disease.

The history of synthetic biology in medicine is longer than presented previously. The first development that I became aware of was the production of recombinant insulin, which was very necessary. Based on this, other applications have been developed such as the production of vaccines against various diseases in plantains and in bananas.

In this case, we are trying to analyze applications that also require extra regulation. And those applications mainly involve the release of material into the environment. In this task, agriculture is certainly our guide. Many of the actions taken in public health, and in the areas that I will discuss later, come precisely from what was tried previously in agriculture, and many of the examples that we will examine are agricultural applications to products which are applicable in public health.

Of all the medical problems that impact public health, infectious diseases are most closely associated with the environment. As examples, we can mention infectious diseases such as measles which are spread directly from person to person, or vector-borne diseases which are transmitted by arthropods such as mosquitoes, kissing bugs, ticks or lice. Diseases transmitted by vectors have a very important feature: they are produced by infectious agents, such as viruses, bacteria and protozoa that rely on arthropods for their transmission. These microorganisms multiply, both in humans and in arthropods, and require contact between an infected vector and an uninfected human, or between an infected human and an uninfected vector.

The systems biology of infectious diseases is more complex than the traditional systems biology with which we are more familiar, since it involves components of the pathogen, the human host and the vector in a varied and changing environment.

---

[7] Full text presentation available at http://www.iica.int/sites/default/files/events/presentations/2016-05/04_synbio_in_health_mhrodriguez.pdf

[8] Lecture transcription by Patricia Echeverri; text edited by Pedro Rocha

To better understand the importance of diseases transmitted by arthropod vectors, we can use the example of malaria. Malaria affects all the world's tropical and sub-tropical regions, and 870 million inhabitants of the American continent live in risk areas. In some places the disease has been eliminated and, although there are medications that kill the parasite, their use is producing resistance and there is still no vaccine, just as there is no effective vaccine for other diseases transmitted by arthropods.

Dengue now accounts for 390 million infections annually, with 50% of the population at risk. This disease is present in nearly all parts of the American continent, from the southern United States to the middle of Argentina. This is a disease that arrived, has not been eliminated and now we are no longer thinking about dengue because we have other emerging problems, such as chikungunya.

Chikungunya, which was initially found in Asia and the Polynesian Islands, originated in Africa, and reached the Caribbean island of St. Martin in 2013. So far, more than 1,250,000 cases have been reported in Latin America, with 50 countries contaminated. Chikungunya is more serious than dengue, because it produces symptoms that last many months, particularly arthralgia.

Recently the zika virus, which was introduced into Brazil in 2014, has spread to Mexico. The most problematic aspect of this virus is that it is very similar to dengue, but is neurotropic, i.e. able to infect the cells of the nervous system. For this reason, particular attention must be paid to pregnant women, since a proportion of them give birth to babies with microcephaly and cerebral atrophy. In adults, zika may produce weakness and paralysis, a condition known as Guillain-Barre Syndrome, and we do not yet know which strategies will prove effective in containing its spread.

One of the key features of these three diseases is that they are transmitted by mosquitoes. Therefore, focusing on mosquitoes is perhaps the most effective way of controlling them. Since we do not have many examples of the application of synthetic biology and we are in the early stages of its application to mosquitoes, I will have sufficient time to further discuss mosquito biology because, based on that, we can see where we can take action.

Female mosquitoes lay their eggs in water (Fig. 1). This is important because the larvae grow in water, feeding on bacteria, algae and organic detritus; they develop into pupa and the adults emerge from the pupal case and come to the surface. Mosquitoes reach adulthood and physiological maturation within about three days. But the most interesting point is that only the females bite and feed on the blood of humans and animals. They also have a very boring sex life, since they only mate once. Once they have mated, they cannot be inseminated by another male mosquito. This fact is interesting and forms the basis of control strategies, as we shall see later. After mating and feeding on blood, the female mosquito will rest for a time and lay her eggs. She then has to obtain more blood to produce the next batch of eggs. The only reason that the female feeds on blood is that she needs the nutrients from blood to produce her eggs, and when a female mosquito is infected, this repetition of the bites is what transmits the disease (Fig. 9).

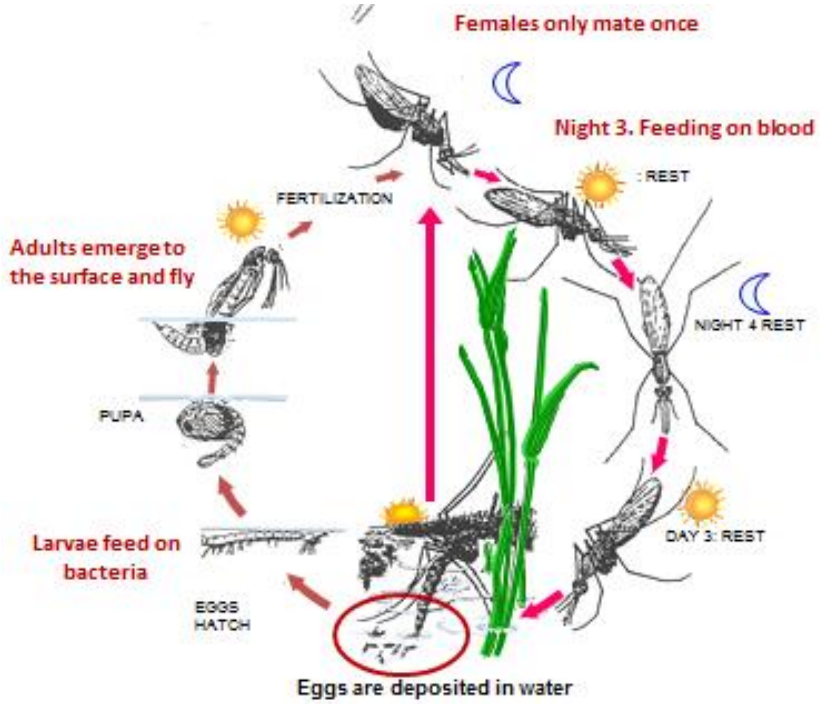


Figure 9. Life cycle and infection cycle of disease-transmitting mosquitoes.

By way of example, we will focus on three diseases: dengue, chikungunya and zika, given that these diseases are transmitted by one or two mosquito species only. The mosquitoes that transmit these viruses from person to person are of the genus *Aedes*. The infection is spread from one city to another by humans and by mosquitoes that are transported there. If I visit a city where these mosquitoes are present and I am infected and a mosquito bites me, I have already introduced the disease and an outbreak may begin.

Aedes mosquitoes are accustomed to living among humans, are well-established and love to be near homes, because they like us to provide them with water, sustenance, food from our blood and places to rest. In addition, they lay their eggs in any receptacle containing water: containers, bottles, pots, tires, etc. During dry periods, the water evaporates and the eggs that were deposited inside the containers also dry out, but do not die. When the rainy season comes, all these discarded objects, which we consider to be garbage, but which form part of the life of mosquitoes, collect water. And in this new water, the eggs hatch, the mosquitoes grow and a new generation emerges. Given that the mother transmits the viruses to her eggs, when the infected eggs hatch they produce mosquitoes that are already infected, which carry the virus into the next rainy season, and it is a nightmare. But this is what keeps the transmission of the diseases going from one year to the next.

There are several traditional approaches to controlling the spread of these diseases. I will describe the main ones and compare these with genetic strategies, documenting the potential advantages of synthetic biology for the design of new control strategies.

Without mosquitoes there is no dengue and, although the virus can be transmitted from a mother to the fetus, this is very rare. Dengue can also be transmitted through blood transfusions, but this is also fairly uncommon. The main form of transmission is through mosquitoes. Thus there are two strategies for attacking this mechanism: reducing contact with mosquitoes and reducing mosquito populations. These are the only traditional approaches.

Mosquitoes breed in areas around homes and then enter our houses. A common strategy is to use spirals which are burned to release insecticide, thereby eliminating the mosquitoes and preventing them from biting people during the night. Mosquito nets or screens can also be placed in windows and doors; but poor people cannot afford to buy mosquito nets for their windows and doors. The truth is that a lack of economic development is one of the major problems in infectious, and it cannot be resolved with any technology currently available to us. Therefore, responsibility for controlling mosquitoes rests with governmental vector control programs. One of the main tasks undertaken by these programs is to remove all objects that contain or could contain water from gardens and patios of houses; but these clean-up campaigns only have a temporary effect because by the following month, more discarded items have accumulated, since this forms part of people's lifestyle and culture.

Another strategy for reducing mosquito populations is to use chemical insecticides such as DDT, pyrethroids, carbamates, organophosphates, which can be sprayed as a spatial repellant to protect people. Sometimes this is done using aircraft and the insecticide is sprayed over the entire population, but it can also be applied more locally around homes. The problem with this strategy is that mosquitoes are becoming increasingly resistant to insecticides, and therefore this approach is becoming less effective. However, insecticides applied by spraying only work when the mosquito is flying: when the spray clouds fall to the ground, their effect disappears. In addition, the eggs that are incubating in the breeding areas continue to produce more mosquitoes.

To eliminate mosquito larvae in the breeding grounds, other insecticides such as *Abate* are used. This product is placed in the water, even in the water sources that people drink from, and we end up drinking water with insecticides, although the concentrations are not toxic to humans. But we are doing these things. The problem with applying insecticides is their short residual effect, which means that these products must be applied frequently. In addition to the high operational costs, the mosquitoes are already showing resistance. Another important aspect of strategies that rely on the application of insecticides is that these products reduce the abundance of mosquitoes, but do not eliminate them.

Bacterial insecticides are also used to control mosquitoes. Their use originated in agriculture, with *Bacillus thuringiensis* var. *Israeliensis* (Bti), which produces toxins that specifically affect lepidopterans and mosquitoes. However, those used against mosquitoes only attack mosquitoes and can be placed in water where the larvae develop. These bacteria produce spores which are activated when ingested by mosquito larvae, causing them diarrhea after which they die. But this technique has limitations, because the bacteria move to areas beyond where the mosquitoes feed and, as in the case of chemical insecticides, their residual effect is low and while they can reduce the abundance of mosquitoes, they do not eliminate them.

Genetic strategies for controlling mosquitoes form part of the synthetic biology which we are analyzing. How do we eliminate mosquitoes or reduce mosquito populations? One option is to modify cyanobacteria and algae with the gene of the BTI toxin. These algae grow well in the stagnant water of the breeding sites, and when the larvae feed on algae they ingest the lethal toxin.

There are several examples of modification of cyanobacteria with BTI genes. We have done it with *Phormidium animalis*, a cyanobacterium which, when placed in the larval breeding sites, kills 100% of the mosquitoes that transmit dengue and 100% of the mosquito larvae that transmit malaria. This shows that the lethal effect of the bacteria is not species-specific and that it will kill any mosquito that feeds on them. This poses a problem that requires us to determine

the range of target organisms, before obtaining the approval of a regulatory body, which in the case of Mexico is CONABIO.

For a long time, another genetic strategy used to reduce mosquito populations has been to kill the adult mosquitoes. Such genetic strategies are species-specific, which makes their global implementation more difficult. For example, malaria is transmitted by local anopheles mosquitoes (Fig. 10). Therefore, if we wish to control malaria in other places, we would need to perform genetic engineering with each of these species and test it in each place.

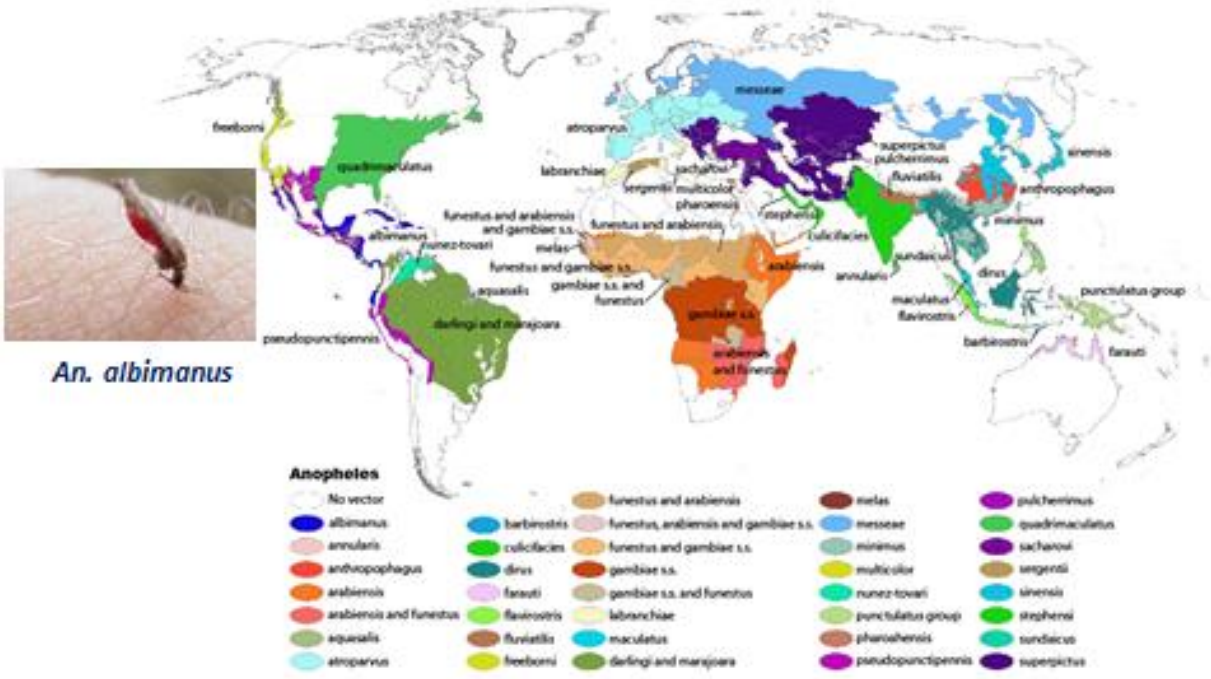


Figure 10. Global distribution of anopheles. Taken from Kiszewksi *et al*. (2004) and http://www.medicalecology.org/diseases/malaria/malaria.htm

In Mexico, and throughout the Pacific coast as far as Colombia, the main vector of malaria is *Anopheles albimanus*. As this involves a single species, a control strategy based on genetic manipulation could work. But there are more than 40 anopheles species that are malaria vectors in the various endemic areas of the world (Fig. 10) and for the strategy to be effective it would be necessary to manipulate each one individually.

The strategy of genetic modification would be easier to implement in the beautifully ornamented mosquitoes *Aedes aegypti* and *Ae. albopictus,* which transmit dengue, chikungunya and zika (Fig. 11). *Ae. albopictus* was recently introduced to the Americas, arriving in the United States in tires that came from Asia, and subsequently being dispersed throughout the continent. In any case, if we wish to use genetic engineering to control the three viruses, we would only need to work with these two mosquito species.

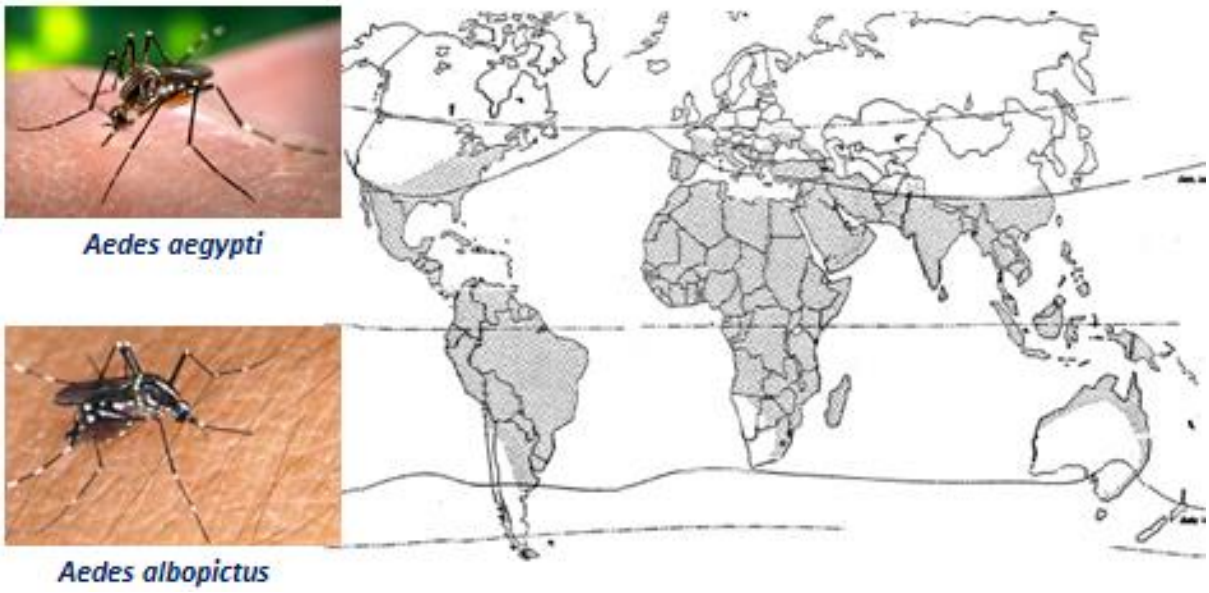


Figure 11. Global distribution of the *Aedes aegypti* and *Ae. Albopictus* mosquitoes, vectors of dengue, chikungunya and zika.

The first intervention using genetically modified mosquitoes was in the 1970s with the release of sterile male mosquitoes. Under this approach, male mosquitoes are sterilized by radiation, mate with females and produce sterile eggs. This results in a reduction of the population, given that females only mate once and cannot be fertilized by other fertile males of the wild population. This strategy was implemented in El Salvador in 1978 and 1979, with the release of irradiated *An. Albimanus* males. The release of sterile males resulted in a reduction of the mosquito population.

The strategy involving sterile males first implies attacking the population with insecticides and then releasing the sterile males. The effect of this intervention was seen in the fact that recovery of the population levels of mosquitoes was more rapid in the control area, where the treated mosquitoes were not released.

The limitations of this control strategy are related to the fact that it is necessary to release thousands of mosquitoes, and that females which have already been impregnated migrate to the treated area from other areas. At the same time, it is necessary to ensure that the sterile males are in good physical condition (*fitness*) to allow them to compete with wild males. One way of compensating for any imbalance with the condition of the wild males is for the released males to outnumber them. The difficulties encountered in producing sterile males were among the main limitations at that time, when there were no automated procedures.

The first experiments with transgenic insects were carried out with *Drosophila* in 1982 and with the Mediterranean fly in 1995. The first transgenic mosquito was obtained in 1998, the red mealybug in 1999 and the first transgenic anopheles in the year 2000. The idea is to modify the DNA in the chromosome of the mosquitoes.

One of the ways of modifying mosquitoes is the Oxitec (RIDL) strategy – introduced by Natalia – in order to reduce the mosquito population by modifying their genes. This involves introducing a gene that is lethal to females into the genome of *Ae. aegypti* and, although the male has the gene, it does not work (Fig. 12).

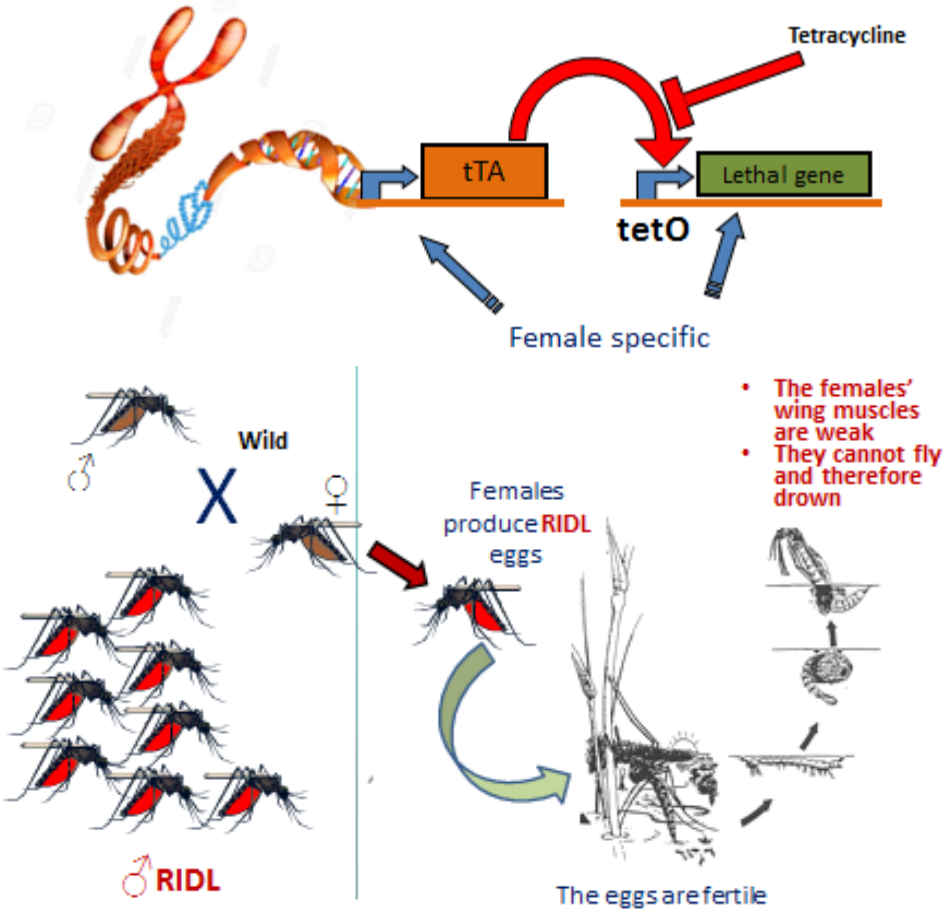
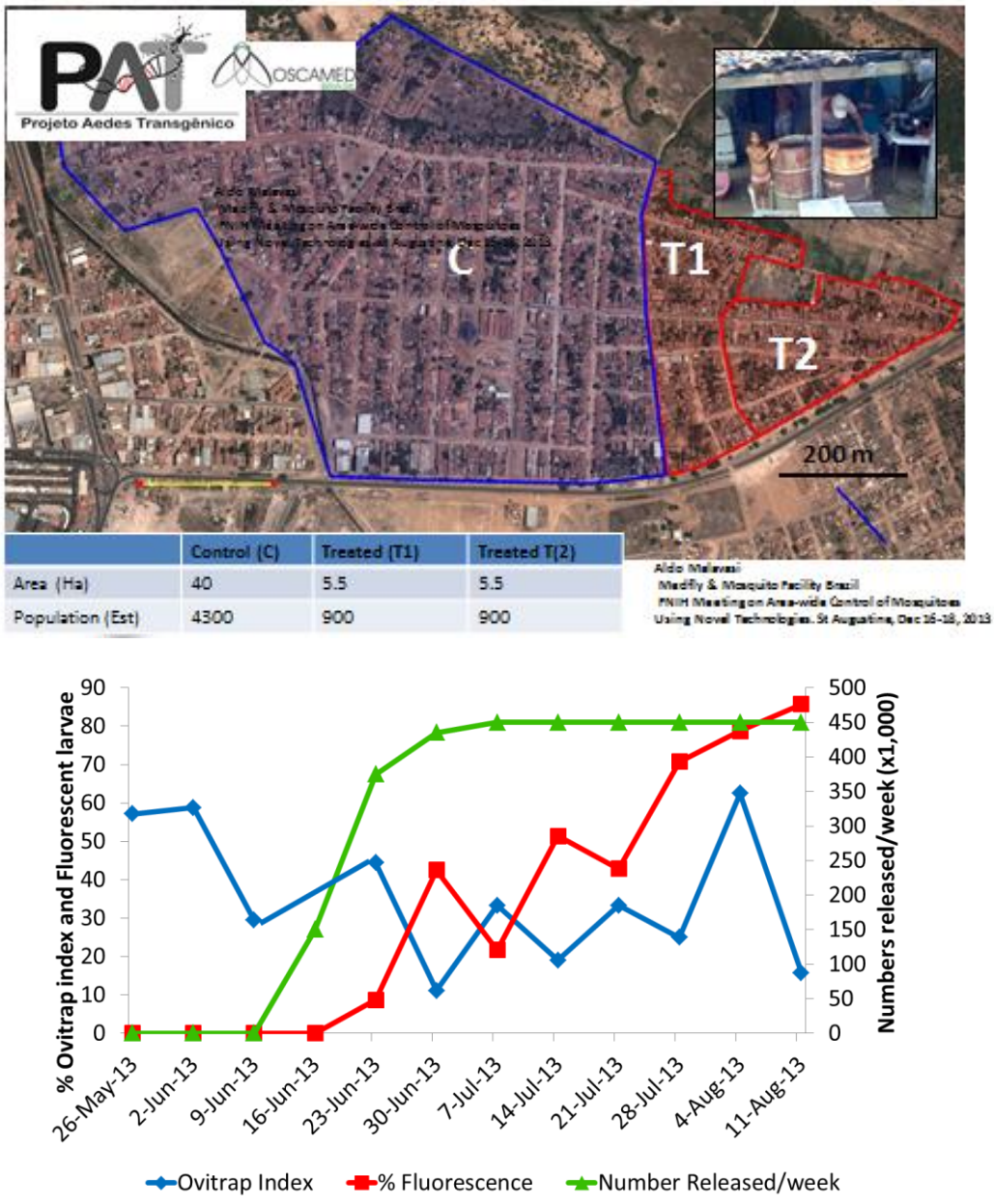


Figure 12. Upper panel: RIDL (*Release of Insects Carrying a Dominant Lethal Gene*), an approach developed by Oxitec for the genetic modification of mosquitoes. Lower panel: Males with an active gene copulate with females, which lay fertile eggs that produce females with deficiencies that prevent them from flying. Adaptation by Malavasi (2013).

In general terms, the part of the gene that controls the expression of the lethal gene is blocked by the presence of tetracycline. While the mosquitoes are being produced, tetracycline is added to the breeding water, which makes the gene inactive. Males released for the control of *Ae. Aegypti* will have the active gene and will activate the lethal gene. Thus, males with the active gene mate with wild females, which lay fertile eggs, the eggs produce larvae that develop into pupas and when the adults emerge from the pupal stage, the female mosquitoes have the lethal gene that prevents the proper formation of the wing muscles, so that they cannot fly and therefore drown.

This strategy for controlling mosquitoes is being used in Brazil and a campaign is under way to promote its acceptance by the communities. A research area was set up in Itaberaba, using the MOSCAMED facilities, which have experience in producing sterile insects. Here, treatment areas and untreated (control) areas were established. Males with the lethal gene incorporated were released into the treatment area. In addition, a fluorescent green protein gene was incorporated, so that the larvae produced by females inseminated by these mosquitoes could be identified by their fluorescence. To monitor the effect of the intervention, ovitraps were placed in the study area. The ovitraps provided the female mosquitoes with substrate for laying their eggs. The results show that the amount of fluorescent larvae collected was proportional to the number of transgenic males released (Fig. 13).



	Control (C)	Treated (T1)	Treated T(2)
Area (Ha)	40	5.5	5.5
Population (Est)	4300	900	900

Figure 13. Upper panel: Study area. Lower panel: Percentage of fluorescent larvae and ovitrap index. Adapted by Malavasi (2013).

To ensure the acceptance of people living in the research areas, an awareness campaign was launched to inform them that the transgenic mosquito does not transmit dengue. It was also explained that female mosquitoes would not be released, a very important factor in this type of intervention, since only the females bite humans, although they may not transmit the viruses that cause disease. In these studies, the production of mosquitoes is automated. The release of approximately one million mosquitoes weekly has had the effect of significantly reducing mosquito bites in this city and the current goal is to produce 60 million males per week. The problem of producing sufficient mosquitoes seems to have been overcome. However, it is important to point out that in order to maintain the effect of reducing mosquito populations, the release of transgenic males must be an ongoing activity and that as soon as the intervention is suspended, there will be a recovery in the numbers of wild mosquitoes.

This type of intervention has been tested in the Cayman Islands since 2009, and was approved in Brazil in 2014. Panama is in the process of approving this system, with a view to beginning testing. Given the current problems of dengue, chikungunya and zika, this strategy is regarded as an important alternative. Oxitec sells the technology and the countries are responsible for establishing factories of transgenic mosquitoes. However, given the number of cities affected by dengue, the constraints of production continue to be significant. A factory would only be able to produce sufficient mosquitoes for one or very few cities. How many cities are affected by dengue and chikungunya and how can we really do this on an industrial scale? I am certain that we will find a way, but so far we do not have these systems in place.

In assessing the options - and this is only experimental - what if instead of killing the mosquitoes, we modify them so that they infect (inoculate) us. The idea is to release mosquitoes with a gene that is transmitted to the mosquito population so that the product of this gene is subsequently dispersed in the human population. It is interesting to note that such a strategy would not even be considered in agriculture, but in public health it is an option.

Another strategy for interrupting the transmission of viruses by mosquitoes is to make them resistant to infection with viruses. Reviewing the dengue cycle, when mosquitoes take in a virus by feeding on the blood of an infected subject, the virus enters the insect's stomach and from there it must pass through the organism until it reaches the saliva glands, so that it can be injected again in the next blood feed. Thus, we could prevent the virus from reproducing throughout this entire pathway. The same is true of the parasites that cause malaria. Mosquitoes have an immune response, and can kill these pathogens by producing reactive oxygen molecules such as hydrogen peroxide and nitric oxide, but – most importantly – they can also produce peptides known as antimicrobial peptides, which attack the virus and the parasites. And if we have a peptide we have a gene and if we have a gene, we can modify the mosquitoes.

Figure 6 below is a diagram of a synthetic gene–a very brief outline, merely to illustrate the main points: a transposon that serves to insert the gene into the DNA, a promoter and a lethal gene. The regulator gene (promoter) should be powerful so that it can really produce the gene that can be the antimicrobial peptide. For example, the trypsin promoter may be used so that the introduced gene is produced in the stomach, or the vitellogenin promoter. Vitellogenin is synthesized when the eggs are produced, so that the product codified by the introduced gene regulated by this promoter will be produced at the same time as the vitellogenin.

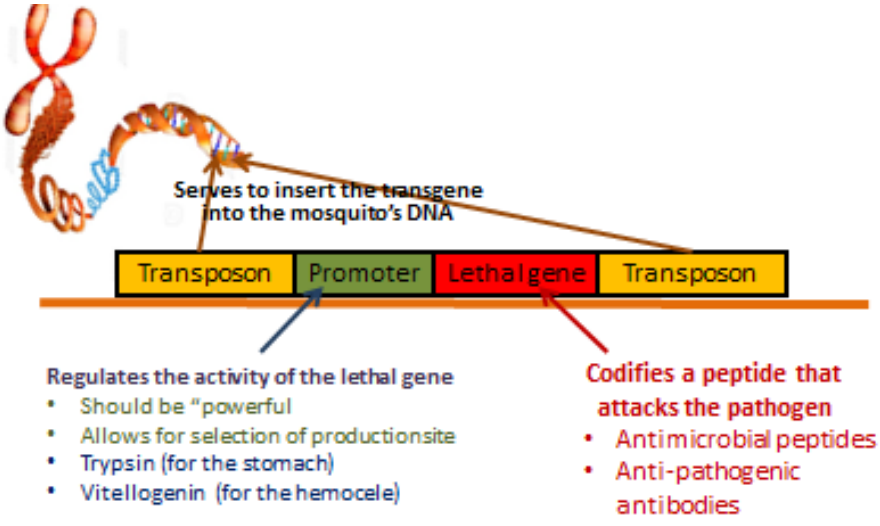


Figure 14. Basic components of a transgene for the modification of mosquitoes with anti-pathogenic genes.

Antimicrobial peptides have been proposed as candidates for producing mosquitoes resistant to infection through transgenesis. We found a peptide called scorpene, because it was obtained from the scorpion *Pandinus imperator*. This peptide kills the dengue virus and the plasmodium. The trypsin promoter was inserted into the gene that codifies for this peptide so that, when the modified mosquito draws blood, the gene is expressed. At that precise moment, it enters the stomach and the peptide kills the virus and the parasites before they invade the insect's stomach.

But this strategy still has limitations. Inserting the gene is hazardous, and can occur in any part of the mosquito genome, making modification very difficult. As it is copied only in one of the DNA chains, just 50% of the offspring inherit it. Moreover, the introduced gene is a burden and has a cost for the insect, and this means that if the pressure is not maintained, the gene is eliminated. In addition, there is the possibility of its escape to other organisms.

Directed genetic modification through CRISPR-Cas9 makes it possible to insert the desired gene in the place where it can be most convenient. Moreover, once introduced, the gene is copied to the other complementary strand of DNA, thereby ensuring its presence in the two DNA strands and that it is passed on to 100% of the progeny. In the end, the progeny will pass the gene on to its progeny and its presence will gradually increase until the entire offspring has the desired gene.

There are two examples of the use of CRISP-Cas9 for modifying mosquitoes. In one case, the codifying gene for an antibody against plasmodium was introduced in *An. stephensi* and this system worked well in female mosquitoes. However, the construction was unstable, indicating the need to establish the conditions for ensuring that the introduced gene is maintained. Another example of the use of this technique was the introduction of lethal genes in *An. gambiae*, the main vector of malaria in Africa, which resulted in a reduction of the mosquito population. However, this strategy is still in the experimental stage.

Among the aspects that regulatory agencies should consider when approving the use of transgenic mosquitoes for the control of diseases transmitted by these insects are its efficacy compared with traditional strategies and the stability of the construction of the transforming gene. In the case of CRISP-cas9-based constructions, if for any reason the gene that was introduced is lost, the two ends of the modified gene remain blocked, and it is no longer possible to modify it again. Therefore, it would be necessary to have a group of genetic constructions that can be used sequentially to maintain the desired effect in the treated mosquito population. Other considerations include the possibility of transferal to non-target organisms and the possibility that the product resulting from the transformation may be toxic to humans.

References

Kiszewksi *et al*., 2004. American Journal of Tropical Medicine and Hygiene 70(5):486-498.
Available in: http://www.cdc.gov/malaria/about/biology/mosquitoes/map.html
Source: http://www.medicalecology.org/diseases/malaria/malaria.htm

Malavasi, A. 2013. Medfly & Mosquito Facility Brazil. FNIH Meeting on Area-wide Control of Mosquitoes Using Novel Technologies. St Augustine, Dec 16-18, 2013.

1.5. Synthetic biology and biodiversity conservation[9,10]

Kent H. Redford, *Ph.D.*
Archipelago Consulting, U.S.A.
redfordkh@gmail.com

Summary

Synthetic biology is a broad and fast-moving field of innovation involving the design and construction of new biological parts and the redesign of existing natural biological systems. It has many potential applications that may change both direct and indirect human relations to the natural world, including help in solving challenging conservation problems (*e.g*. invasive species, emerging diseases), replacing natural products with synthetic ones and reviving extinct species or rescuing endangered ones. Despite such promises and the vast sums being spent on its development, synthetic biology has not engaged with the practice of conservation. The public and conservation practitioners are ignorant of both the potential promise and threats posed by future developments in synthetic biology. Immediate informed engagement is essential for practitioners of synthetic biology and conservation, as well as diverse policy makers and publics.

Presentation Development

When I was a young person, nature was obvious and enchanting to both children and adults. It was distinct from humanity; it existed apart from humans who wanted to protect it from destruction. The destruction always came from people because people were bad for nature; we all understood this. And so in order to save nature, we needed to remove it from humans and then nature would thrive on its own without human interference. Management of nature meant no management. When I was in graduate school, I realized that this was an entirely incorrect way of viewing the world. In fact, the nature of human impact on the natural world had changed dramatically; you´re all familiar with the different types of threats (habitat destruction, invasive species, climate change, economic exploitation) of human origin. All these threats affect all of the components of biodiversity, be it the genes or the species, all the way to the ecosystems.

In fact, as many of you will have read because it was in the media (The Economist, 2011), we have now entered the anthropocene, a new geological era typified by the extent and dimension of human threat to the natural world. I give you two examples. The first one, from a colleague of mine (Berger, 2008: Ripple *et al*., 2014) who works in Yellowstone National Park in the Rocky Mountains in the United States. He worked on newborn moose calves. Interestingly, the major sources of mortality for the young moose are grizzly bears. These bears hate roads and cars and so the moose have started to give birth next to roads in order to avoid predation by bears on their calves; an extraordinary response to human interventions.

The second one is a very sad story. The Lyre bird (*Menura* sp.) mimics by picking up sounds and incorporating them into their song. These wild birds are now singing the song of chain-saw sounds. Very tragic, I think, in a poetic sense.

So, what we have come to understand is that biodiversity is going to need to be managed if we are going to be able to save it. This was a very startling statistic: the United States has an

[9] Full presentation is available at http://www.iica.int/sites/default/files/events/presentations/2016-05/05_synbio.pdf
[10] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

endangered species act and a calculation was done that 82% of the species that are listed under our endangered species act will require continual human management in order to be able to survive. This is an example (Fig. 15), for this little bird, Kirtland´s Warbler, of habitat management being done in order to ensure its survival. We will have to do that forever if we wish to keep those birds.

Figure 15. Example of human dependent species. Taken from: http://clas.wayne.edu/dankashian/Disturbance-Ecology-of-Lake-States-Pine-Systems and http://www.fws.gov/midwest/endangered/birds/Kirtland/kiwafctsht.html

This approach to active management is not just something in the US. From my own experience, here is an example of four different types of these activities taking place in Latin America and, in particular, I draw your attention to a very serious fungal disease, which has been causing the extinction and decrease in numbers of many amphibians, and has caused Panama to actually pull many of these frogs out of the wild and keep them in captivity.

And of course we have the specter of climate change and the way that this is going to put even more stresses on the natural system. This, for example (Fig. 16), is the Aquarium after Hurricane Sandy hit New York. You will see that there is ocean water outside as well as inside this exhibit.

Figure 16. Informed management decisions made more complicated by climate change. Here New York post-hurricane Sandy, photo by Julie Larsen Mahar.

So, I had this simple dichotomy that I was raised with and that many people still adhere to which is "people are bad and nature is good and if we keep them separate then all will be fine". Instead, what we have found is that we live in a world in which natural and human management are inextricably intertwined. We in fact live in this hybrid world and the major lesson from this is that saving nature is going to require management, which is the paradox that we are faced with as we consider synthetic biology.

In the past, and many of my colleagues in the conservation community still think that way, conservation operated across this continuum: either it is anthropocentric, that is we are managing nature for human uses, or it is bio-centric, we are managing it for the sake of nature. But, I suggest to you that we have heard already today, and as you well know, that dichotomy, that continuum, is incomplete and we have opened a large new space, which is a novel operating space, with which many of us and the talks today prove are preoccupied. And of course, what else occupies this novel operating space, but synthetic biology.

For me, the most important thing for you to read is the book "*Biology is Technology*" by Rob Carlson. It's a few years old now; it is about synthetic biology, but the title is the story. We are involved in an effort to turn biology into technology.

You have seen these curves already and this is a different version that shows you Morse's Law and the reading and the writing of DNA, and how fast it´s going on; that´s the point to be made. This is happening fast (Fig. 17).

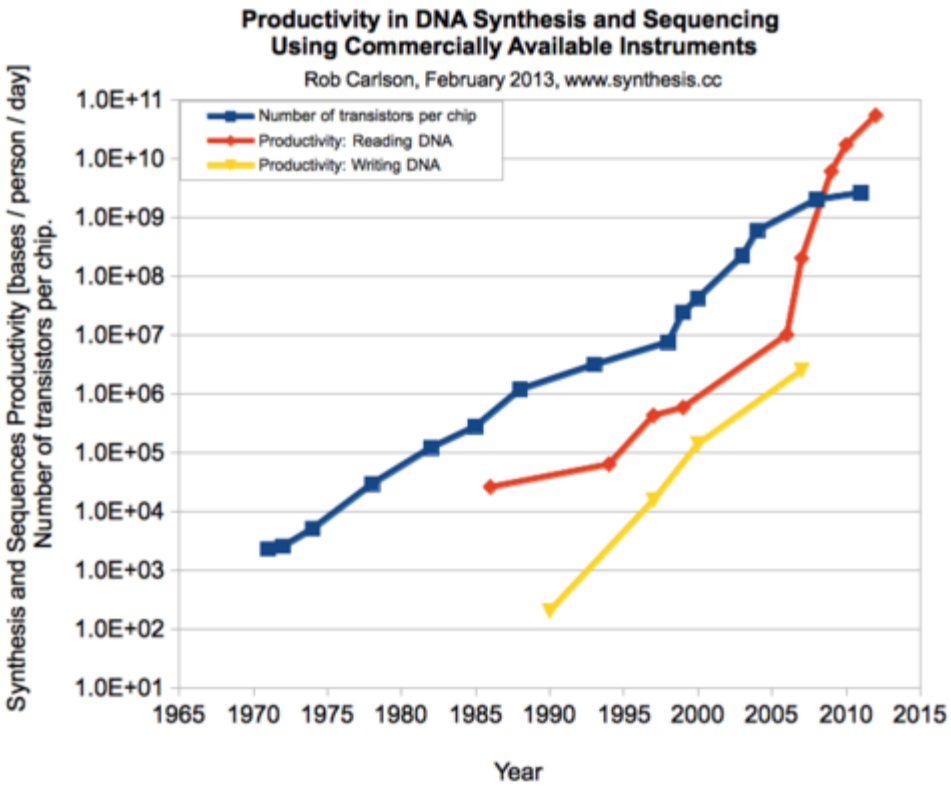


Figure 17. Changes are/will be fast: Carlson curves. Taken from http://www.synthesis.cc/cgi-bin/mt/mt-search.cgi?blog_id=1&tag=Carlson%20Curves&limit=20

It is also happening across all segments of society. There are public labs (www.publiclab.org), where you can go in off the street and pay a fee and you have access to reading and writing and creating novel organisms as a member of the public. I looked it up and now we know there are five of these common labs, public labs, in Latin America that are listed and many more in other parts of the world (Fig. 18). We will hear this talk, later on today, and this is being pushed as technology deep into the minds of the younger people of our world. We´ll hear much more about that. I´ll just point out that in 2013 there were 214 undergraduate teams, and these are people who are in college. We now have teams coming from high schools, so children who are 18, 17 and 16 are practicing synthetic biology.

LATIN AMERICA

Buenos Aires	AR	http://www.diybioba.org
Guanajuato	MX	https://www.facebook.com/groups/DIYbioMexico
Sao Paulo	BR	http://www.syntechbio.com
Sao Paulo	BR	https://garoa.net.br/wiki/Biohacking
Sao Paulo	BR	http://synbiobrasil.org

Figure 18. Public labs in US and Latin America. Adapted from: https://www.google.com/fusiontables/embedviz?viz=MAP&q=select+col0+from+1BbV5lAgDKG58zQsSnPrFrgJtF6nbVs7RasPW8so&h=false&lat=45.73165899691809&lng=-87.456805025&z=4&t=1&l=col0&y=3&tmplt=3

We learned a lot about the CRISPR technology. The list of animals which have been manipulated using CRISPR and other synthetic biology techniques includes pigs, chickens, dogs, goats, cattle, mice, mosquitoes, and moths. In addition, the important agricultural crops have been manipulated by these techniques in a tremendous number of ways.

The major point of this talk is that almost all of these developments have been blind, deaf and dumb to the implications of these technologies for the natural world, on the areas of the environment that are not being targetted. In order to address this, I have been part of three different meetings over the last two years to try and bridge the gap between these communities. I´d like to summarize for you some of the major points which came from these meetings.

The first is of particular interest to journalists, so it's topical. There are active attempts now to recreate species that have gone extinct, using synthetic biology technologies. The most commonly talked about is the passenger pigeon, which used to be the most common bird in the world and yet all of them were killed in my country, in one of the great horrible actions that we were taking. Anyway, so there are attempts now to recreate that.

Another case is American chestnuts, by which we mean US chestnuts. This was the most common tree in the forest of the Northeastern United States. It was practically extinct, but it´s been recreated using synthetic biology techniques. Even more interesting, is the fact that we are now starting to use synthetic biology techniques to take lost genetic variations and put them back into existing animal populations, using genetic sequencing from museum specimens and from cryogenically preserved reserves. One example is with the black footed ferret.

These technologies have the capacity to address persistent threats to biodiversity for which we have no tools at all. For instance, the response to emerging fungal diseases that are affecting animals and plants (Fig. 19) and we do not have the ability to handle those (Fisher *et al*., 2012).

Figure 19. Fungal diseases worldwide. In red (lines and dots), reports of new fungal diseases affecting animals. In green, fungal diseases in plants. Taken from Fisher *et al*. (2012).

A very interesting proposal is to use synthetic biology techniques to turn on genetic sequences in coral, in response to warming oceans in ways that don´t cause coral bleaching.

There is the potential, and I stress potential here, to use some of these technologies to restore degraded lands. Depending on your definition, between 9% and 13% of the earth´s surface is considered unusable by either nature or human beings. Imagine what could happen if we could put those lands back into productive use for both nature and humans.

Something with which you are all familiar, is that we are busy discharging into water, extraordinary amounts of pharmaceuticals. So, in 2009, 271 million pounds of hormones, caffeine and pharmaceuticals were put into the fresh water system that came out of sanitation systems, just in the US alone. All of that is going into the natural water and this is not being addressed, although it could be addressed, in theory, in water treatment plants, in which synthetic biology could be useful.

There is much activity and interest in trying to use these technologies, particularly gene drives, to address invasive species, both on islands and in the wild. An important example involves the Burmese python, a 4 to 5 meters snake that is present in the Everglades Park, in the southern portion of Florida in the US, where the populations of small and medium-sized animals, from raccoons to deer, have decreased by 95% because of predation by these large snakes. And nobody can do anything about it, currently.

Needless to say, all of that is the exciting part. However, there are really serious concerns about how these technologies might be applied. We heard examples, but you need to think about this: if we alter life, evolution continues, and may continue to act on those genomes in ways we would not desire, and which have implications for the rest of the natural world.

Now, I want to make this distinction, which is not frequently made, between these proposals to modify animals and plants using the CRISPR-cas-9, as compared with the alternative synthetic biology technology that is more traditional, that is, GMOs. In the first example, we are using the native genome to alter the native genome. In the second example, we are using non-native genomes to alter the native genomes, by introducing a gene from another species of fish into

salmon in order to get it to grow faster. So, those are very different things when you consider them from an evolutionary perspective.

My colleagues are in love with payment for eco-system services as a strategy for saving nature and in fact we are in a country which has embraced this approach more than many other countries. For instance, poplar trees have been genetically modified to sequester carbon at a more rapid rate than native poplar trees. We are going to have the potential to synthesize ecosystem services thereby making natural ecosystems no longer necessary in order to deliver these services.

Private synthesis meets the public good. There is very little discourse about the fact that many of these alterations are trademarked, are copyrighted. So, what happens when you copyright a mosquito and release tens of millions of them into the world? Is the conservation community now responsible for conserving a privately owned entity which is interacting? Can I sue the company for changing the natural world? All of these questions are entirely unanswered and are open to discussion.

There is an issue which has drawn more anger and articles than any other, and it is how these technologies are going to impact the lives of the rural poor. Those of you who are not familiar with the organization ETC and, in particular, Jim Thomas, I strongly suggest that you read about them, because they hate everything that we´ve heard about today, including what I´ve said.

So, what then does all this mean for conservation? My personal conviction is that we need to create a new field of action called the synthetic biology conservation stewardship, which would take action to address these types of problems. For example, the white-nose syndrome in bats, a fungal infection in which a bat, which is sleeping in the wintertime, wakes up and goes outside in freezing temperatures but is unable to get food because of the presence of a fungus. As a consequence, the bat returns to the cave and dies early of starvation, all because of that fungus. We have no ability to deal with that fungus right now and it is spreading and has been responsible for the decrease in these large colony-nesting bats by over 90%.

Another case is the treatment of frogs with probiotics against the fungal disease, chytridiomycosis, which is killing these amphibians. The disease is killing them by inhabiting their skin and changing the micro-biome of the skin of the frog. There is interest in changing that micro-biome in order to prevent the fungus from being able to kill the frogs.

Here is the point, we´re not going to be able to stop the advance of synthetic biology. Techniques that saved the life of a little girl who was be able to survive the leukemia that was killing her. Everybody talks about the need for new technology to be introduced to save a starving or dying child, but the technology exists.

That is combined with the fact that for 800 US dollars you can buy a tabletop lab to modify bacterial cells on your own and all of the ingredients you need to do it. This is now available on Kickstarter and the hope is that in two years you will be able to do your own novel organism in your own home for only 400 dollars. Those two things combined make this unstoppable, exciting, and extremely frightening.

The Economist had a cover story, late last year, on the ways in which we are going to be able to do germ-line editing for human beings (The Economist, 2015). Of course, the technology for human medicine is advancing, but so is the technology for agriculture. For example, there is an attempt to do something which is evolutionary and really extraordinary, which is to change rice from a C3 to a C4 photosynthetic pathway.

Rice is now the major source of food for 3 billion people. This work, funded by the Gates Foundation and others, is an attempt to change the entire evolutionary trajectory of a species in order to feed humanity. This is expected to generate 50% greater yield, use half the water and less fertilizer, and be more resistant to climate change. But consider, from an evolutionary standpoint, what is being proposed and why we are proposing to do it. Will this mean women can lose fewer children to malnutrition and therefore have smaller family size and more girls being educated which will also lead to smaller family sizes, or will it mean that there´ll be more children and more land turned into rice production and the US will become a rice consumer instead of a wheat consumer? We don´t know, but chances are we are going to be running this experiment.

For all of these reasons, and for all of these unknowns, I think it´s important that those of us in the conservation community, and those of you who care about the values of conserving the natural world, need to engage in this technology, not only as a lab-based science, but in terms of the ways that it is going to be impacting the rest of the world, the non-human world.

What would conservation want out of synthetic biology if we could have that conversation? We want evolution to be able to continue in an autonomous fashion to the extent possible. We would like better managed wildlife areas and species, an elevated sense of the risk involved and the collateral damage, more of a sense of humility because, as you know, humans are not known for being humble. This is a very good time to be thinking about humility. It is very important that there be different perspectives. We need to have a broad range of stakeholders involved in discussions. This also means that we need to continue to focus on conserving as much of nature as we can.

I also want to mention the notion of counterfactuals. A counterfactual is the measurement of the difference between something that happens without our intervention and what would happen if we intervened. What is so important about this is that if we think about counterfactuals in synthetic biology, and the white nose syndrome in bats, you can say that a synthetic biology approach to changing that fungus to save the bats might lead to very serious consequences. But the comparison is what is happening to the bats now. They are already dramatically decreasing in population size. That is a correct comparison to make and it´s particularly important in talking to the public.

Some really excellent polls that were done have shown that the public in different parts of the world is very nervous about this technology and its use. We need to be talking to them very clearly and very honestly in order to make sure that we avoid what is happening with some of the media claims – for example, you may have read there are claims that the zika virus was caused by genetically modified mosquitoes. You have to understand that this kind of connection makes sense in the minds of people. We can´t say they are stupid; what we need is better communication.

People who study emerging technologies say the huge mistake that was made with GMOs was to choose food which occupies a special place in humanity´s brain and mental view of the world and that by changing food we created our own problem in terms of rejecting GMOs. This is a quote from this paper, “Members of the public show enthusiasm for synthetic biology applications when those applications are developed to address societal, medical and sustainability needs, whereas engineering biology is seen as a potential concern if this research is done without investigations of its potential risks and long-term implications. Members of the public also support funding for research that leads to applications that actually meet social and sustainability goals” (Pauwels, 2013).

References

Fisher, MC; Henk DA; Briggs CJ; Brownstein JS; Madoff LC; McCraw SL; Gurr SJ. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature, 484: 186–194.

Pauwels, E. 2013. Public Understanding of Synthetic Biology. BioScience 63(2):79-89.

Redford KH; Adams W: Mace GM. 2013. Synthetic biology and conservation of nature: Wicked problems and wicked solutions. PLOS Biology 11(4):e1001530.

Redford KH; Adams W; Carlson R; Mace GM; Ceccarell B. 2014. Synthetic biology and the conservation of biodiversity. Oryx 48(3):330-336.

Ripple, WJ; Estes JA; Beschta RL; Wilmers CC; Ritchie EG; Hebblewhite M: Berger J; Elmhagen B; Letnic M; Nelson MP; Schmitz OJ; Smith DW; Wallach AD; Wirsing AJ. 2014. Status and Ecological Effects of the World's Largest Carnivores. Science 343: 151.

The Economist. 2011. Welcome to the Anthropocene. May 26. http://www.economist.com/node/18744401

The Economist. 2015. Editing humanity. August 22. http://www.economist.com/news/leaders/21661651-new-technique-manipulating-genes-holds-great-promisebut-rules-are-needed-govern-its

1.6. iGEM: Leading, inspiring, and innovating in synthetic biology and beyond [11, 12]

Ana Sifuentes
iGEM, U.S.A.
ana@igem.org

Ricardo Chavez
ricardo.chavez.mtz@gmail.com

Introduction

Today we will be discussing the following issues: first of all, what is synthetic biology? Then we will discuss the iGEM Foundation in general, as well as the Latin America approach. We will conclude with a discussion of our own personal experience.

iGEM defines synthetic biology as the design and development of new biological components and systems, as well as the redesigning of existing biological components for the development of useful biological systems. This definition, while certainly broad, accurately describes what is involved. Genetic engineering is what biologists refer to as "genetic modification"; synthetic biology, on the other hand, is what engineers refer to as "genetic engineering" – the use of engineering ideas and tools to design biological systems, simplifying their development.

The conceptual similarity between biological and computer systems should be noted. Computer systems are made up of small pieces which combine to generate circuits, which connect to form modules, which in turn form computers, which themselves combine to form networks. Biological systems are made up of DNA molecules, which combine to form genes, which are expressed in proteins, which interact with one another along metabolic routes, where specific biochemical reactions occur within the organelles inside cells, and which assemble to form tissues, organs, and more complex biological systems.

This might appear to be an overly reductionist approach, and the idea of controlling biology to such an extent may seem impossible. The question was put to a group of students in a competition: can simple biological systems be assembled using standard, interchangeable parts, and then set in motion within living cells? Or is biology too complex to be engineered in such a fashion? iGEM reached a surprising conclusion: not only is it possible, it can be done in four months, with no need for doctorates or years of academic training, as demonstrated by the high school students who participated in the competition.

The iGEM engineering approach uses standard DNA sequences ("biobricks") which can be reused and recombined in an uncomplicated manner (Fig. 20).

[11] Full presentation available at http://www.iica.int/sites/default/files/events/presentations/2016-05/06_igem_asifuentesrchavez._web3.pdf

[12] Lecture transcribed by Patricia Echeverri; text edited by Pedro Rocha.

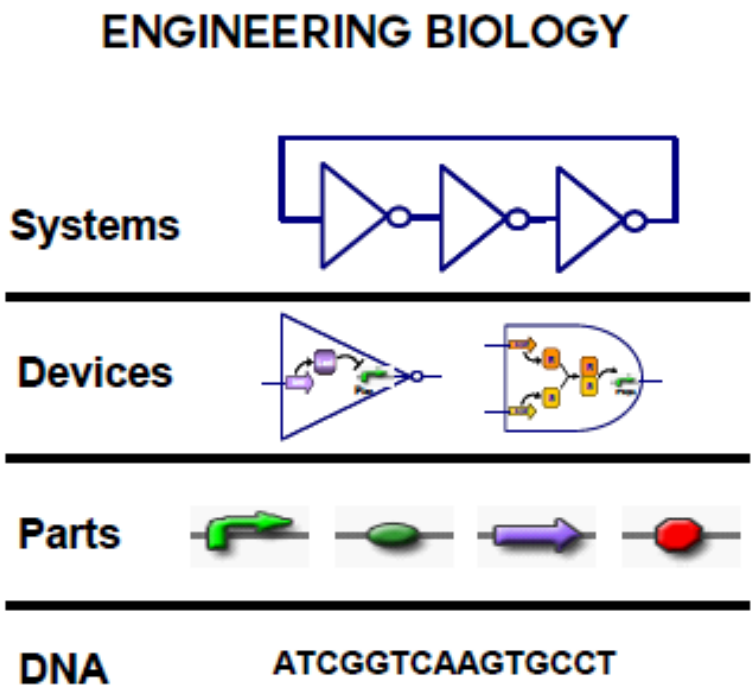


Figure 20. Engineering biology. Excerpted from BaojunWang (2016, Tools for Synthetic Biology).

The idea is to use DNA molecules to create standardized parts which, once combined, can generate devices or modules that can be employed to create more complex biological systems capable of solving real-world problems. For example, a biobrick (gene) that codes for a fluorescent protein could be joined to another which detects cyanide concentrations in water, thus creating a bacterium that glows if water contains cyanide. This may be viewed as a type of programming with bacteria. As idealistic or abstract as these ideas may seem, they have been coming to fruition for the last 11 years through the iGEM competition.

iGEM (*International Genetically Engineered Machine*) is a competition focusing on the development of genetically modified machines. It is an international event, open to students interested in synthetic biology. It was launched in 2003 (Fig. 21) as a stand-alone course at MIT by engineers seeking to make biology simple and accessible to all by creating components that can be reused for different projects.

The first competition was held in 2004 and featured only five teams. International teams were added the following year. The first Latin American teams participated in 2006. By 2009, more than 100 teams were involved in the competition. This required the addition of regional phases. In 2012, iGEM became independent of MIT. It is currently a non-profit organization which receives no government funding. In 2014, we hosted a "Giant Jamboree" for all of the teams that have participated in the competition. Over 200 teams were present at the event. In 2015, 280 teams from over 20 countries participated (Fig. 22).

iGEM is a place for everyone. It is more than simply a competition; it is an experience in which contestants can put science and synthetic biology to use in their communities, while also supporting and connecting with one another.

Figure 21. History of iGEM.

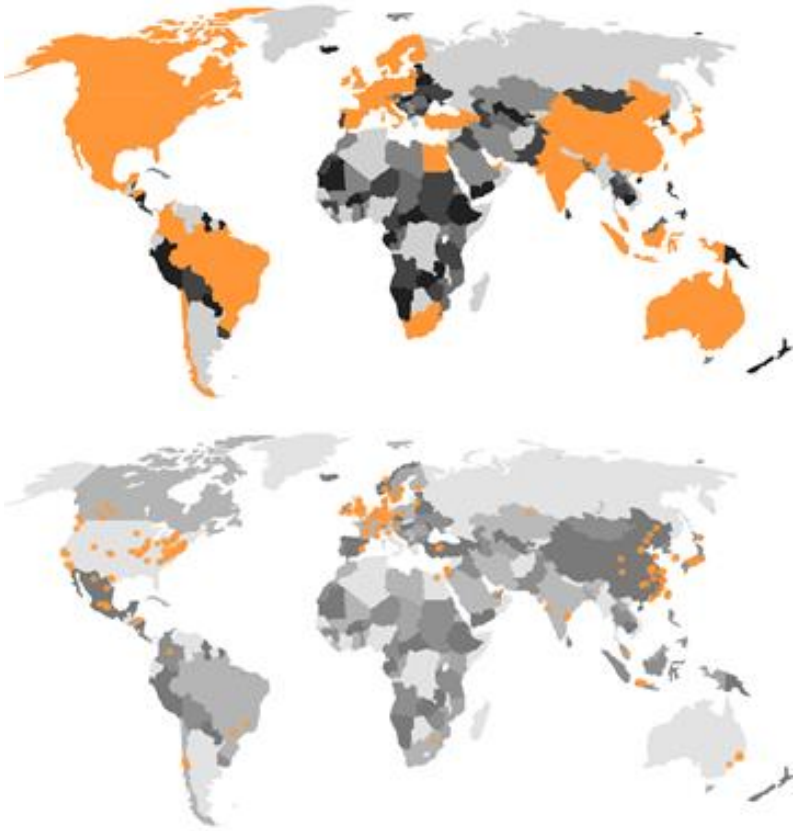


Figure 22. Country (upper panel) and team (lower panel) participation in iGEM 2015. The orange dots on the lower panel represent team locations.

iGEM in Latin America

The Latin American countries that have participated in iGEM are shown below (Fig. 23). Unfortunately, participation is inconsistent. Some countries participate one year, withdraw the next, and return the following year. This reflects a lack of funding and a scarcity of corporate sponsors in the region, as well as a lack of awareness of synthetic biology and, certainly, of the competition itself.

The timeline of the competition faces certain hurdles. Delays in the delivery of laboratory materials and reagents are one example. Picture the frustration of researchers who design a project and spend months planning and experimenting, only to find that the materials and reagents they have ordered for the competition have been held up in customs for one or two months.

In the case of Mexico, our team participated and encouraged the other Mexican teams to lobby their legislators, promote iGEM, and raise support for similar initiatives. Unfortunately, we did not achieve the response we had hoped for. Nevertheless, seven Mexican states have agreed to work toward improving the situation. While there is interest in the topic, regulatory issues require greater focus, more time, and advisory input.

iGEM Operation

iGEM is comprised of three programs: Parts Registry, Labs, and Competition. The Parts Registry program is a standard biological-parts-assembly program – a repository of standardized DNA biobricks which can be combined as modules. iGEM currently includes over 20 thousand biological parts, available to teams upon request. The Labs program offers paid memberships for schools or universities. Members of iGEM are given access to all of the program's biological parts, allowing them to contribute their own parts to the Registry. The Competition program seeks to develop skills and showcase the teams' ability to solve problems using the available standard biological parts.

The competition is divided into four phases: registration, project initiation, documentation, and presentation. Teams are required to organize their projects along the tracks we offer (environment, medical manufacturing, food and nutrition, and special software and hardware lines).

During the registration phase early in the year, teams are formed, contestants interested in specific projects are brought together, brainstorming sessions are held, the problem to be solved through synthetic biology is clearly presented, funds are raised, and the teams are enrolled in the competition.

Once the teams have been enrolled, the second phase begins. During this phase, iGEM distributes the DNA kits and other biological parts. The competition begins after each team has received its DNA kit, in early May (Fig. 4). The kit includes a large number of standard parts, which the teams may use. Teams are also encouraged to produce their own biological parts for inclusion in the Registry. This phase involves a tremendous amount of arduous lab work.

It also includes a number of essential "human practices" to ensure that individuals and teams self-regulate, so that their projects – which involve 250 teams working to modify bacteria, plants, and even mammalian cells – are ethical and safe. These ethical considerations are known as a "biosafety approach". All teams are required to meet with their advisors to discuss the challenges they might face if they were forced to weigh the ethics and safety of their projects vis-à-vis the general public. Social considerations (artistic, political, and economic) are also taken into account, and teams are encouraged to develop not only a country perspective, but also a regional or city approach to improving regulations, rules, and policies.

iGEM has a jury of experts and strict rules in place. Contestants are required to describe each registry part in detail. More research is sometimes needed to determine whether a part is meeting expectations. The parts added to the registry must be described as thoroughly as possible.

The documentation phase involves drafting summaries and creating team web pages. This is followed by the presentation phase, during which teams submit a scientific poster and give a 20-minute oral presentation. The next phase is the Giant Jamboree.

What happens after iGEM? It sounds simple. Four months of arduous work, not only in the lab, but also on each project's environmental and social impact, etc. Some teams also lobby politicians and encourage discussion of legislative bills to support science in their countries and communities.

Figure 23. iGEM Kit

Examples of iGEM projects

- Bacterial treatment for cancer (2013). This project, developed by a 17-member team from TEC University in Monterrey (Mexico), sought to develop a modular treatment for tumors. We knew that bacteria, including *E. coli*, could penetrate tumors, thanks to their ability to survive in low-oxygen environments. Having observed the low concentration of oxygen in tumors, the team developed a bacterium that only produces cancer-fighting proteins when it is inside a tumor. We designed a bacterium that would secrete two useful therapeutic proteins when it was inside a tumoral (low-oxygen) environment. The team also added a part that internalizes a protein that only targets cancer (Fig. 24).

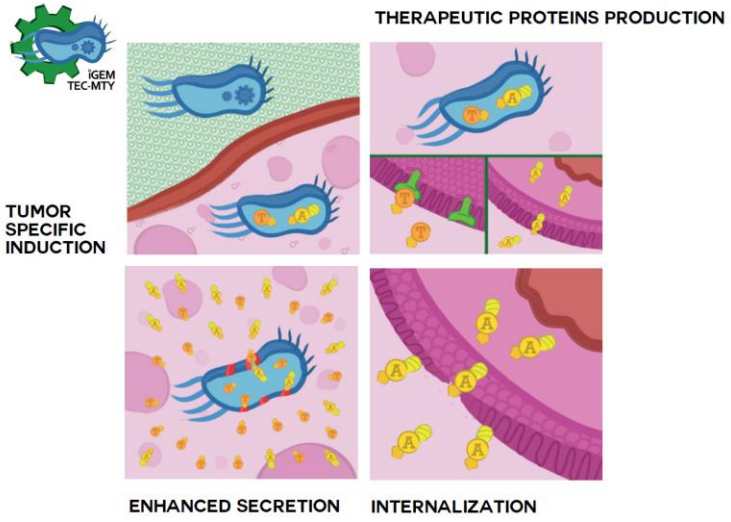


Figure 24. Therapeutic proteins production

- Color-producing bacteria (Echromi, 2009). A team from Cambridge (England) constructed a relatively simple device which detects components of interest – for example, toxins in water – and releases a signal which produces a color visible to the human eye. The idea was to use bacteria as a living color (Fig. 25B). The first phase of the project involved creating an acid sensor in water which would react differently to different pH levels. Simple systems like this one could be used to determine whether water contains chemicals and is safe to drink. Other potential applications include creating a pigment repository (Fig. 25C), using bacteria as probiotic agents to color parasites, cancer, etc., in feces (Fig. 25D). Modified bacteria could also be released into the atmosphere to provide advance warning of acid rain, turning red in clouds that contain acid (Fig. 25D).

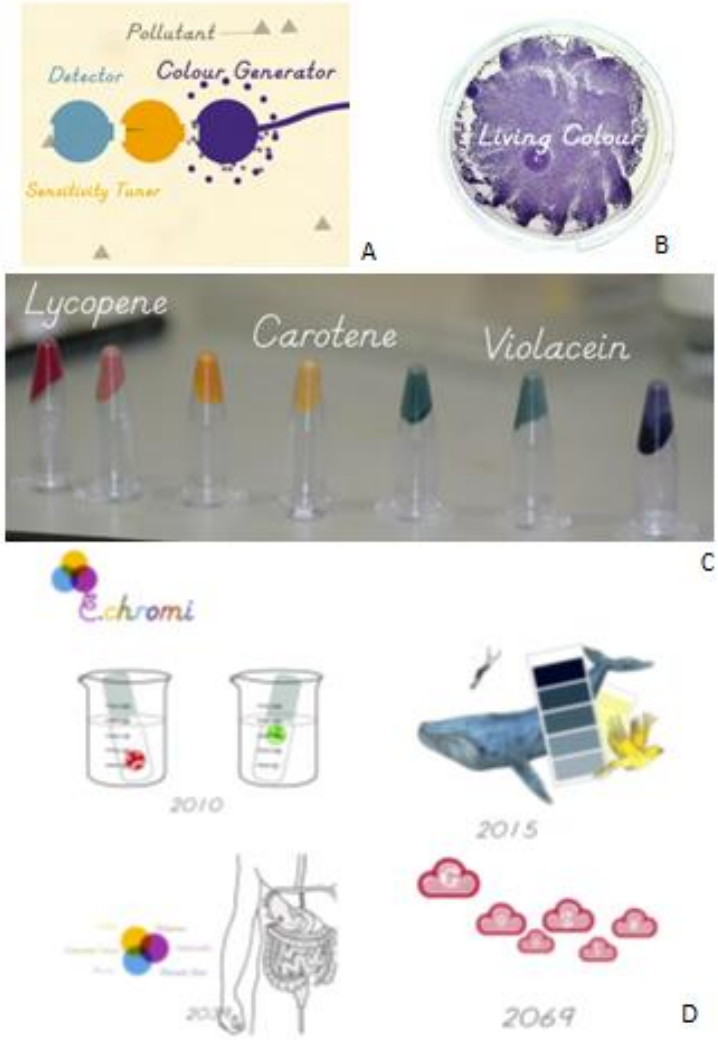


Figure 25. Design of biosensors for the development of color generators. (A) Detection mechanism. (B) Bacteria as living color. (C) Pigment repository. (D) Potential applications.

- Plasticity: production of bioplastic from waste (2013). This project, developed by the Imperial College of London, used bacteria to convert non-recyclable waste (such as cloth and wood fragments) into polyhydroxybutyrate (PHB), a polymer used to produce bioplastics. The "what else" was the development and use of industrial systems in which waste could be deposited and degraded by the bioreactor, generating polymers on the

spot and providing communities with bioplastic precursors in less than a week (Fig. 26). Such systems could be combined with 3D printers to produce items such as shoe heels, for example. The team considered not only the industrial potential, but also the need to communicate such innovations.

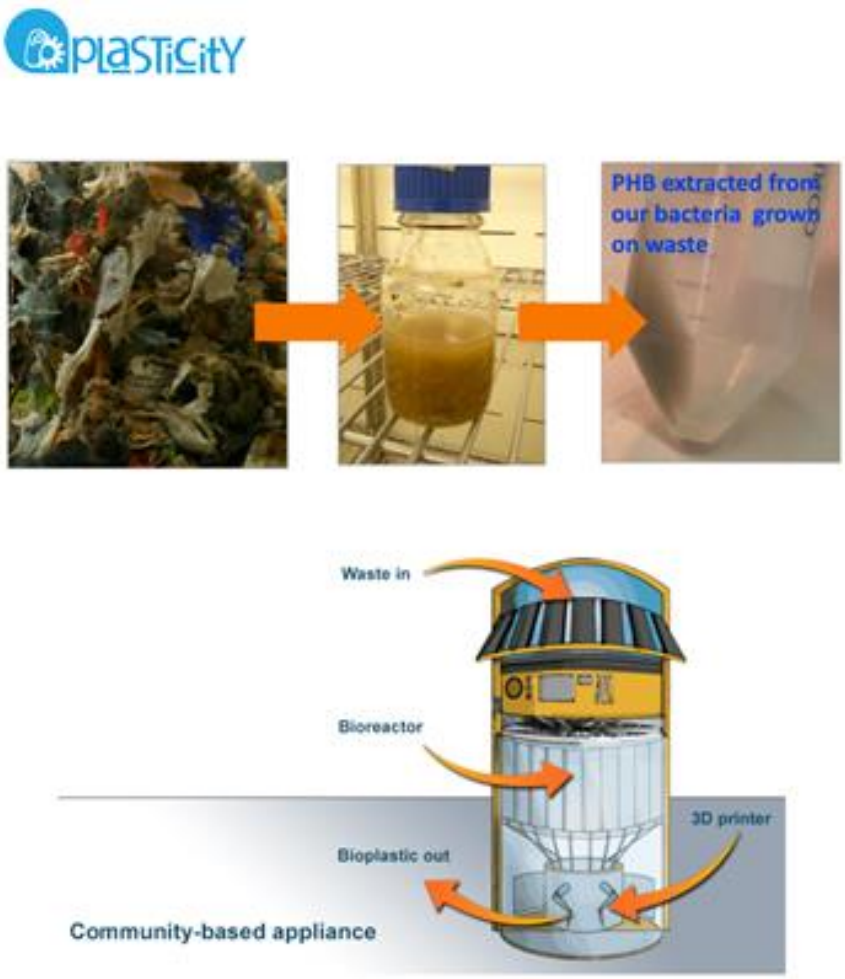


Figure 26. Plasticity: Production of bioplastics from non-recyclable waste.

The competition not only offers the satisfaction of participating and submitting solutions to real-world problems, it also creates entrepreneurial opportunities in synthetic and molecular biology. Start-ups created through iGEM include Ginko Bioworks (the first multi-million dollar synthetic biology company), Ambercycle, Amplino, Bechling, Bento-Bio, Experiment, BioBots, Fredsense, Synbiota, and Hyasynth, among others. These are but a few examples of the opportunities teams will have to create their own industries and bring jobs to their communities.

In closing, we would like to invite you to attend the 2016 Giant Jamboree, which will take place on October 27-31 (Hynes Convention Center, Boston, Massachusetts).

References

iGEM. 2016. http://igem.org

1.7. Synthetic biology: A global snapshot[13, 14]

Felicity Keiper, *Ph.D.*
AHTEG Representative, EuropaBio
felicity.keiper@bayer.com

Summary

This presentation aims to provide a global snapshot of synthetic biology, including an introduction with CropLife's view on what synthetic biology is, statistics describing the global synthetic biology market and research activity, a look at where the entities are around the world that identify as working in synthetic biology research, the initiatives that support those entities such as start-up associations, the investment that supports synthetic biology research in the most active countries, the strategic visions and recommendations of those countries that have fostered the development of synthetic biology, and national and global policy developments that have also supported its growth. The presentation will conclude with a brief introduction to the synthetic biology discussions that are occurring under the Convention on Biological Diversity (CBD) and the Cartagena Protocol on Biosafety to the CBD.

Statistics and Markets, Research Entities, and Investment

To begin, our view of synthetic biology is that it represents the continuum of biotechnology, or, in other words, the current state-of-the-art of biotechnology. Thus, it is not a new or different discipline, nor does it require a definition that differentiates it from what we know as "genetic modification". We consider that in most cases it would be more accurate to continue to use the term "genetically modified" rather than the term "synthetic biology" to describe most, if not all, of the applications being labeled as synthetic biology. We are also of the view that synthetic biology does not require new or additional regulatory frameworks, and this will be examined further in our next presentation.

Biotechnological developments that have led to the emergence of the term "synthetic biology" include exponential reductions in the cost of DNA sequencing and synthesis over the past decade, which has enabled faster and easier DNA design and expanded the scope for genetic modification. Consequently, there has been a proliferation of providers of these "enabling technologies". There has also been the emergence of the bio-economy, where, for example, efforts have been focused on replacing petro-chemical based manufacturing with biological production processes. Such developments have created the potential for a vast range of bio-based products, and led to increased public and private investment in technological development. They have also brought a renewed focus on the regulation of biotechnology.

The statistics indicate that the value of the synthetic biology market increased five-fold over the past five years, from $1.1 billion in 2010 to $5.2 billion in 2015, and it is expected to substantially increase by 2020 to almost $40 billion. This growth will be the result of increasing commercialization, with products that have been in development expected to reach the market soon. The statistics also indicate that synthetic biology research is being conducted throughout

[13] Full presentation is available at http://www.iica.int/sites/default/files/events/presentations/2016-05/07_synbio_framework_fkeiper.pdf
[14] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

the world (40+ countries), by a large and increasing number of entities (~565) and researchers (3000+) that are funded by many sources (500+ organizations). These entities include companies, universities, research institutions, and laboratories (government, military, community), but activity is dominated by companies and universities. Note that these statistics are an indicator only, as these are entities that have identified themselves as conducting synthetic biology research, and this categorization depends on how those entities define synthetic biology. The numbers in this presentation are derived from the Maps Inventory developed by the Wilson Centre Synthetic Biology Project, which is an interactive tool where these entities can be examined in detail (http://www.synbioproject.org/inventories/maps-inventory/).

The Maps Inventory shows that the United States of America (USA) is the most active country in the world in synthetic biology research, with concentrated areas of activity in San Francisco and Boston. This reflects significant government investment in synthetic biology, and the strong start-up cultures in those cities. Europe as a whole is less active in synthetic biology than the USA, but the number of start-up companies is now increasing at a faster rate in Europe. It also generates more revenue from synthetic biology than any other region from biofuels. Like the USA, the United Kingdom (UK) has been a concentrated area of synthetic biology activity as a consequence of strong government investment. There is relatively little synthetic biology research activity in the rest of the world; however, the Asia-Pacific region is expected to be the fastest growing region over the next five years due to an increasing number of research entities. The most active countries in that region include China and Japan.

One major driver of growth in synthetic biology is the increasing number of start-up companies, which provide a range of products and services including: enabling technologies (*e.g*. DNA synthesis is the fastest growing and most competitive area, organism engineering platforms, laboratory automation), consumer products (*e.g.* cosmetics, fragrances), environmental products and services *(e.g*. biosensors and bioremediation), food products (e.g. flavors such as vanillin), with industrial applications (e.g. bio-based fuels, plastics, rubber) being the largest area of activity. The strong start-up culture in the USA has contributed to its global dominance in synthetic biology; for example, start-ups are supported by accelerators where they can access seed funding, equipment, space and mentoring, as well as associations to connect them with investors and education and networking opportunities. SynBioBeta is an example of a USA-based association supporting and representing start-ups, and it has become active in international synthetic biology and biotechnology policy discussions.

Investment in synthetic biology has generally increased globally over the past decade. Data for the USA indicates that the major public sources of funding for synthetic biology include the Departments of Defense, Energy, and Agriculture, the National Science Foundation, and the National Institutes of Health. There are also non-traditional sources such as crowd-funding and the Bill and Melinda Gates Foundation. The Gates Foundation in particular provides funding for projects aimed at addressing global health challenges. A high profile example is the $42.5 million grant to the University of California Berkeley, the Institute for OneWorldHealth and Amyris (a start-up) to develop a microbial system for the production of the antimalarial drug, artemisinin.

In Europe, the UK is the largest investor in synthetic biology, with the major public sources being the Biotechnology and Biological Science Research Council and the Engineering and Physical Sciences Research Council, as well as joint programs with the USA National Science Foundation. In the rest of Europe, the major sources of public funding include the Swiss National

Science Foundation and the European Commission Framework Programs. The latter established the European Research Network in Synthetic Biology (ERA SynBio) in 2012 to promote collaboration and capacity building across Europe. ERA SynBio invests primarily in biofuels, which are the priority area for synthetic biology research in Europe and a major source of revenue and employment. This industry is supported by policy developments including the 2012 Bioeconomy Strategy, funding for industrial biotechnology from major sources such as Horizon 2020, and public-private partnerships such as the Bio-based Industries Consortium.

In the growing Asia-Pacific region, China is the most active country in synthetic biology, investing $40 million (USD) per year specifically for synthetic biology research. China is already well-established in biotechnology and seeks to position itself as a global leader in synthetic biology. The primary difference between China and the US and Europe is the relative absence of private investment and start-up companies, however, this is expected to change with, for example, US-based accelerators supporting the establishment of local accelerators.

Strategic Visions

Some of the leading synthetic biology countries, in terms of the number of entities conducting synthetic biology research and investment, have published strategic visions and recommendations to support the development of synthetic biology. This presentation looks at the UK, Europe and China; despite the USA being the most active country there is no overarching strategic plan for synthetic biology.

In 2012, the UK published "A Synthetic Biology Roadmap for the UK" ("UK Roadmap", UK Synthetic Biology Roadmap Coordination Group, 2012) which recognized the potential benefits of synthetic biology in addressing major global challenges, the strong foundation for synthetic biology research established in the UK through investment and international collaboration, and that the existing GMO regulatory oversight was adequate for synthetic biology. The UK Roadmap set out five recommendations including: further investment to increase expertise and collaboration, accelerate commercialization, establish international leadership in the development of standards for synthetic biology, and establishment of the Synthetic Biology Leadership Council (SBLC) to oversee delivery of the recommendations. More recently in 2016, the SBLC published "Biodesign for the Bioeconomy – UK Synthetic Biology Strategic Plan 2016", which reports on progress on the 2012 recommendations, including the establishment of Synthetic Biology Research Centers and an increase in the number of start-ups, and builds on these, emphasizing accelerated commercialization. Of note, the UK Roadmap highlighted the need for responsible innovation with appropriate regulation, and in 2016 the areas of strategic importance highlight the need for proportionate regulatory and governance systems.

In 2014, the ERA SynBio published "Next Steps for European Synthetic Biology: A Strategic Vision from ERA SynBio", which, like the UK Roadmap, recognized the potential benefits of synthetic biology and set out five recommendations. Compared to the UK Roadmap, the ERA SynBio document is less commercialization-oriented, but the recommendations have similarities in emphasizing capacity building and collaboration, and responsible research and innovation.

Prior to the UK and Europe, China developed a roadmap for research and development in 2010 that defines specific synthetic biology targets spanning five, 10 and 20 years. The "Innovation 2050: Technology Revolution and the Future of China" sets out specific outputs, from establishing a database of standardized parts by 2015, to the creation of artificial microbial life

by 2030. In comparison to the strategic visions of the UK and Europe, the China roadmap is focused on scientific achievements rather than setting out investment needs or considering the regulatory environment.

Policy Development

While the USA does not have an overarching strategic plan for synthetic biology, it has been active in policy development since the 2010 publication of the first synthetic bacterial genome (Gibson *et al*., 2010). This triggered President Obama to request the Presidential Commission for the Study of Bioethical Issues to identify appropriate ethical boundaries in order to maximize public benefits and minimize risks of the technology. Their report, "New Directions: the Ethics of Synthetic Biology in Emerging Technologies", was published in 2010 with 18 recommendations framed within five ethical principles. In brief, these include the need for: research that maximizes public benefit, appropriate risk assessment to support responsible stewardship, and proportionate regulatory oversight to support creative and accountable intellectual freedom. Of note, the recommendations also recognize the importance of self-regulation, and emphasize the need for accurate information in relation to claims made about scientific and ethical issues for democratic decision-making, and these points are examined further in our next presentation. A key finding of the report was that no novel safety or ethical issues were identified that required changes to the GMO regulatory oversight.

At a more international level, the OECD 2014 publication, "Emerging Policy Issues in Synthetic Biology", examined the applications and potential benefits of synthetic biology and issues such as regulatory oversight. Consistent with the conclusions of the USA and UK recommendations examined in this presentation, this OECD publication also considered existing GMO regulation to be sufficient for synthetic biology. Further, it also considered existing regulatory oversight for non-living products of synthetic biology, such as bio-produced pharmaceuticals and chemicals, to be appropriate and adequate. The main challenges presented by synthetic biology that were identified included intellectual property, with tensions between intellectual property protection and a culture of openly sharing information, public acceptance of synthetic biology, and biosecurity.

At a regional level, three scientific committees advising the European Commission have published three "opinion" documents on synthetic biology. The first of these provided an operational definition, and aspects of this document that we support include: synthetic biology is a collection of technological advances that have enabled an expanded scope for genetic modification; it is artificial and arbitrary to try to define synthetic biology by parameters such as the degree of novelty or complexity of modification; current GMO risk assessment approaches and guidelines remain relevant and applicable; and that existing GMO regulatory oversight in Europe is applicable to synthetic biology for the foreseeable future. Of note, the Committees question the need to try and define synthetic biology in a way that differentiates it from GM. This document was published shortly before the twelfth meeting of the Conference of the Parties (COP) to the Convention on Biological Diversity in 2014.

In the second opinion document published in 2015, the Committees examined new challenges presented by synthetic biology for conducting risk assessments. They concluded that existing methodologies remain applicable but they may require adaptation for particular synthetic biology applications, with examples including the potential for newly combined biological parts to interact and give rise to new properties, and the potential for interactions between synthetic

and non-synthetic organisms in the receiving environment. The Committees made recommendations to improve the effectiveness of risk assessment that included continuing to support efforts to generate functional information for biological parts and develop computational tools to predict emergent properties; using GMOs with proven safety records as comparators; and openly exchanging information on parts, devices and systems. The third opinion document, also published in 2015, makes further recommendations to address knowledge gaps, including research into containment strategies and the performance of GMOs outside of containment.

CBD and Cartagena Protocol

The CBD is the primary international forum currently deliberating the definition of synthetic biology, its potential benefits and adverse impacts in the context of the CBD's objectives, risk assessment, and regulatory oversight. This discussion is occurring because synthetic biology has been proposed as a "new and emerging issue" under the CBD. Consequently, since 2010 there have been invitations to submit information on synthetic biology, and more recently in 2015, a series of online discussions and establishment of an Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology. Of note, despite the increasing activities, there has not been an assessment of synthetic biology as a new and emerging issue according to the procedure established by the CBD Parties, there has not been a decision by the Parties that it is a new and emerging issue, and there has not been a recommendation by the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA) that it is a new and emerging issue.

The Cartagena Protocol is also currently deliberating risk assessment for synthetic biology. The Protocol contains a GMO risk assessment framework which is the basis for GMO regulation in many countries, and there is an established AHTEG on Risk Assessment and Risk Management that has been developing a "Guidance on Risk Assessment of Living Modified Organisms" document for several years. AHTEG identified risk assessment of GMOs created through synthetic biology as a topic requiring the development of further guidance, and online discussions are imminent for the purpose of collecting scientific information to enable a decision by the Meeting of the Parties (COP-MOP) in December. The Guidance document has not been adopted by the Parties to the Protocol and it is our view that efforts should be focused on improving the Guidance, in accordance with the decision of the Parties, so that it is applicable to all GMOs, rather than diverting focus to additional topics for which there is no consensus on a definition or that it is a new and emerging issue.

Referencias

Chinese Academy of Sciences (CAS), 2009. Technological Revolution and China's Future-Innovation 2050.

European Research Area Network for the development and coordination of synthetic biology in Europe (ERASynBio). 2014. Next steps for European synthetic biology: A strategic vision from ERASynBio. 28p. http://www.bbsrc.ac.uk/documents/1404-era-synbio-strategic-vision-pdf

Gibson DG, Glass JI, Lartigue C, Noskov VN, Chuang RY, Algire MA, Benders GA, Montague MG, Ma L, Moodie MM, Merryman C, Vashee S, Krishnakumar R, Assad-Garcia N, Andrews-Pfannkoch C, Denisova EA, Young L, Qi ZQ, Segall-Shapiro TH, Calvey CH, Parmar PP, Hutchison CA, Smith HO, Venter JC. 2010. Creation of a bacterial cell controlled by a chemically synthesized genome. Science 329(5987): 52-56.

OECD, 2014. Emerging Policy Issues in Synthetic Biology. OECD Publishing, Paris. 168 pp. DOI: http://dx.doi.org/10.1787/9789264208421-en

Synthetic Biology Leadership Council (SBLC), 2016. Biodesign for the Bioeconomy. UK Synthetic Biology Strategic Plan 2016. Innovate UK, February 2016. https://connect.innovateuk.org/web/synthetic-biology-special-interest-group/2016-uk-synbio-strategic-plan

United States. Presidential Commission for the Study of Bioethical Issues. 2010. New directions the ethics of synthetic biology and emerging technologies. Washington, D.C.

UK Synthetic Biology Roadmap Coordination Group, 2012. A Synthetic Biology Roadmap for the UK. Technology Strategy Board on behalf of UK Synthetic Biology Roadmap Coordination Group http://www.rcuk.ac.uk/documents/publications/syntheticbiologyroadmap-pdf

Section 2: Regulatory Aspects of Synthetic Biology

2.1. Review of regulatory mechanisms for SynBio treatment [15]

Felicity Keiper, *Ph.D.*
AHTEG Representative, EuropaBio
felicity.keiper@bayer.com

Summary

This presentation builds on the "global snapshot" presented earlier, and begins with a recap of key points raised in that presentation, including CropLife's view that synthetic biology is simply the current state-of-the-art of biotechnology and not something new, and the findings of policy reviews in the USA, EU and OECD that existing regulatory oversight for genetically modified organisms (GMOs) is sufficient for current and foreseeable applications of synthetic biology. This presentation then undertakes a detailed analysis of particular issues or challenges for regulatory oversight presented by synthetic biology that have been raised in the information submissions, online discussions, report of the AHTEG on Synthetic Biology, and the peer review of that report, under the Convention on Biological Diversity (CBD). The presentation is arranged in a similar way to these various CBD mechanisms, with the separation of living organisms from non-living products and components, to demonstrate that the discussion is unnecessarily complicated. Rather than creating a new international regulatory framework for synthetic biology, we argue that we would be better served by understanding the many sources of regulation that already exist, and seeking coordination and synergy where necessary and appropriate rather than investing resources in unnecessary duplication.

Regulatory Sources

Before going into the detailed analysis it is necessary to consider that there are many sources of regulation that are relevant to this discussion, not just the CBD and its Protocols. There are claims in the synthetic biology discussions that a new international regulatory framework specifically for synthetic biology is needed to fill "gaps" in regulatory oversight, and that existing applicable regimes are "fragmented". However, the absence of a specific treaty covering an entire field does not equate to "gaps" or "fragmentation", or lacking regulatory oversight. There are a number of international instruments, guidelines, and regional and national frameworks that are relevant to synthetic biology, many of which predate the CBD and its Protocols. The importance of voluntary international guidelines should not be understated in science. An advantage of these is the flexibility to be updated with scientific advances, and they become binding obligations when implemented into national regulatory frameworks. Biosafety is a good example of this, with the principles of regulatory oversight for contained use applicable to GMOs prior to the existence of the Cartagena Protocol. Self-governance also plays a major role in scientific research.

Living organisms

To begin this analysis, it is useful to recall the objectives of the CBD and the Cartagena Protocol as these should define the scope of the synthetic biology discussion under the CBD. Briefly, the objectives of the CBD are the conservation of biological diversity, the sustainable use of its components, and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources, while the objective of the Cartagena Protocol is to protect biological diversity

[15] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

from the potential risks posed by living modified organisms resulting from modern biotechnology.

These two instruments also include many definitions that provide further assistance with defining their scope. In Article 2 of the CBD, "biotechnology" is broadly defined as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use", and it is our view that this encompasses synthetic biology. Article 2 of the CBD also provides a broad definition of "genetic material" that includes "any material of plant, animal, microbial or other origin containing functional units of heredity". Our interpretation of the breadth of this extends to "xenobiology", which is a commonly cited example of synthetic biology where there is uncertainty as to whether it would be within the scope of current regulatory frameworks. This would be an example of a synthetic biology application that is outside the realm of "current and foreseeable", however, the word "other" indicates that synthetic nucleic acids, e.g. "xeno" nucleic acids or "XNA", are within the scope of "genetic material", and therefore, the scope of "living modified organism" (LMOs) as defined in Article 3 of the Cartagena Protocol.

"Living modified organism" is defined in Article 3 of the Cartagena Protocol as "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology". Article 3 also defines "modern biotechnology", of which the "application of in vitro nucleic acid techniques, including…" is most relevant to this discussion. The word "including" confirms that relevant in vitro techniques are not limited to the two examples listed of "recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid…" and may be broadly interpreted. Thus, synthetic biology applications are within the scope of both the CBD's definition of "biotechnology", and the Cartagena Protocol's definition of "modern biotechnology".

The scope of the Cartagena Protocol is stated in Article 4 to be "the transboundary movement, transit, handling and use of all living modified organisms that may have an adverse effect on the conservation and sustainable use of biological diversity…" Our interpretation is that the undefined term "use" would include environmental releases. Also, we agree with the view of the AHTEG that non-living products and non-living components used in synthetic biology applications are not within this scope.

Risk assessment

A major feature of the Cartagena Protocol is its LMO risk assessment framework provided in Article 15 and Annex III. This contains several principles, including the need to conduct risk assessments in a scientifically sound and transparent manner, that a lack of scientific knowledge or consensus is not indicative of a particular level of risk (relevant to uncertainty), that the risks associated with LMOs should be considered in the context of the risk posed by non-modified organisms (relevant to comparators), and importantly, that risk assessment should be carried out on a case-by-case basis. The latter principle recognizes that the amount of information that will be required for risk assessment will vary in nature and detail depending on the characteristics of the LMO, its intended use and the receiving environment. These principles remain entirely relevant to synthetic biology.

Specific challenges that have been raised in relation to the risk assessment of LMOs created by synthetic biology include uncertainty and comparators. These are connected to the increasing complexity of genetic modification in some synthetic biology applications. For uncertainty, it has

been claimed that increasingly complex genetic modifications increase the unpredictability of the characteristics of the resulting LMO. This overlooks Annex III, which states that uncertainty may be addressed by requesting further information, *e.g*. additional scientific evidence from further studies, or by implementing appropriate risk management strategies, e.g. containment, and/or by monitoring the LMO in the receiving environment. These measures are implemented in many national frameworks and it is the view of several Parties that these can adequately deal with synthetic biology applications. Further, increasingly complex genetic modification does not necessarily correspond to increasing uncertainty. Synthetic biology applications typically involve the use of well-characterized parts with known function that are assembled according to a pre-determined plan based on computer-modeling. As parts and devices continue to be characterized, knowledge and capacity in the field continues to grow, and computational tools become more advanced, arguably, uncertainty will decline in synthetic biology.

For comparators, it has been claimed that increasingly complex genetic modifications will increase the genetic distance between the LMO and the non-modified organism, and appropriate comparators for comparator-based risk assessment will be difficult or impossible to identify. However, returning to the principles of Annex III, it is evident that the choice of comparator is not prescribed and what is most appropriate may be determined on a case-by-case basis. Annex III also does not preclude alternatives to comparator-based risk assessment, and there may be synthetic biology applications where alternative approaches may be more appropriate. However, all current and foreseeable applications of synthetic biology involve the use of an existing host species, e.g. a bacterial cell. Even with a completely synthetic genome, the LMO is still a bacterium, and the range of characteristics of the host species provides the comparator. This approach is used today by regulators in assessing microorganisms with complex or multiple genetic modifications.

Contained use

Most current and foreseeable synthetic biology applications involve microorganisms in containment, with operations ranging in size from research to manufacturing-scale, and these are not intended to be released into the environment. The Cartagena Protocol defines “contained use” in Article 3, sets out the documentation requirements for transporting these LMOs in Article 18, and in Article 6 states that the advanced informed agreement procedure of the Cartagena Protocol, which includes the risk assessment framework, does not apply to LMOs destined for contained use. Instead, the Cartagena Protocol emphasizes national standards, with Parties having the right to set their own standards for contained use. This is due to the biosafety field predating the Cartagena Protocol, with established international standards from the 1980s including risk assessment to define biosafety levels (containment), e.g. the “Laboratory Biosafety Manual” of the World Health Organization (WHO), and the “Biosafety in Microbiological and Biomedical Laboratories” of the United States Department of Health and Human Services. In practice, and despite the absence of an overarching international treaty, most Parties have national biosafety frameworks regulating contained use that are based on international standards, and these include risk assessment. In some cases, the national GMO regulatory framework will apply to both contained used and environmental release.

Dual use of concern is an issue that has been raised in the synthetic biology discussion under the CBD in the context of contained use, as well as components. This concerns the use of beneficial applications of synthetic biology in harmful ways, e.g. to create biological weapons. The tension between potential benefits and risks that are of very low probability, but potentially high impact,

is evident in many examples of everyday technology use, e.g. driving a car, and this is not specific to synthetic biology nor a reason justifying its prohibition. Biological weapons are the subject of an international regulatory regime, with the Geneva Convention of 1925 prohibiting bacterial methods of warfare, and the Biological Weapons Convention prohibiting their development, production, stockpiling and acquisition for non-peaceful purposes. This regime is supported by a variety of international initiatives, e.g. the guidelines and export licensing requirements of the Australia Group for certain pathogens, and the "IAP Statement on Biosecurity" which is a code of conduct for the scientific community. Furthermore, there are international standards for biosecurity, again in the updated guidelines of the WHO and the United States Department of Health and Human Services, and these form the basis of the "CEN Workshop Agreement: Laboratory Biorisk Management Standard" for which there is ongoing work to develop into an ISO standard.

The international biosecurity guidelines of the WHO and the United States Department of Health and Human Services emphasize risk assessment and risk management, and the expertise, responsibility and accountability of the scientific community. The latter is self-regulation, and this is also emphasized in the IAP Statement on Biosecurity which requires the scientific community to be aware of the consequences of their activities; be responsible for adhering to safe practices; have knowledge of the laws, regulations and policies that are applicable to their work; report violations of the Biological Weapons Convention that they become aware of; and to train others in these principles. The importance of self-regulation is also evident in the highly consistent policy documents developed by the USA government and the UK scientific community. As shown in the "global snapshot" presentation earlier, these two countries are the world's most active in synthetic biology research. These documents place strong emphasis on the shared responsibilities of research funders, funding recipients, and the institutions where the research is being conducted, and promote cultures of responsibility supported by training in dual use of concern issues and ethics. These also call for appropriate oversight that is proportional to the risks, evidence-based risk assessments, and education to raise public awareness.

Transboundary movements

In addition to the Cartagena Protocol, the United Nations Recommendations on the Transport of Dangerous Goods (Model Regulations) apply to transboundary movements of LMOs, including those destined for contained use. The Model Recommendations provide a scheme for the harmonized development of national and international regulations for all modes of transport, and these are translated into regulations for transport by water, air, road and rail in international and regional treaties. According to the Model Regulations, LMOs are classified as Class 9, for miscellaneous dangerous substances and articles, or Class 6 if they are toxic or infectious. The Model Regulations were first introduced in 1956 and have undergone several revisions, the most recent in 2015.

Components

The synthetic biology discussion under the CBD has highlighted components as an area requiring separate consideration; however this is not a new area or even specific to synthetic biology. "Components" is simply a new engineering term in the biotechnology vernacular, replacing "genetic elements" which have long been used in biotechnology. There is also the

question of whether regulatory oversight is even necessary or appropriate for most components.

As non-living materials, components are not within the scope of the Cartagena Protocol, or the Model Regulations. However, they are regulated by a variety of international and national mechanisms, where this is considered necessary. At the national level there are, e.g. national import and export regulatory requirements, national biosafety and biosecurity regulatory requirements, requirements imposed by research funders and institutions, and any applicable codes of conduct. At the international level, genetic elements that have sequences associated with the pathogenicity of certain pathogens will be subject to the export licensing system of the Australia Group (mentioned previously). Despite the absence of an international treaty, there are Parties with established and functional regulatory systems that impact components where it is considered necessary to regulate them.

Dual use of concern arises in the context of components due to advances in DNA sequencing and synthesis. These developments are considered to allow easier access to components for non-traditional users, such as the DIY synthetic biology community. There have been assessments of the realistic risk posed by the DIY community in the scientific literature, the synthetic biology project of the Woodrow Wilson Centre, and the three Scientific Committees advising the European Commission in the series of "Opinion" documents (discussed in a previous presentation). These have examined the levels of scientific expertise in these communities, which ranges from enthusiast to PhD trained; the range of DIY projects, which include biological experiments, designing and building DIY equipment, and developing computer programs; their facilities, which include all types of laboratories from a community space to government, university and corporate laboratories; their tendency to collect in formalized groups; their cultures of open communication and sharing of ideas, with heavy use of social media and publication in journals by trained scientists; and the presence of some institutional oversight where there are collaborative projects with universities or companies, and in the larger communities, e.g. DIY BIO which has a register of biosafety experts for answering questions. The overall conclusion is that the capabilities and capacities of the DIY group are limited to that requiring minimal biosafety precaution. Irrespective of these studies, an international treaty is not an appropriate mechanism to regulate these types of activities, and promoting self-regulation is of greater importance. DIY BIO has codes of conduct that are consistent with the principles described earlier, namely, the cultivation of cultures of openness and transparency, sharing of information and knowledge, ethical considerations, activities for peaceful purposes only, and education to raise public awareness. Other initiatives include codes of conduct for providers of components, e.g. the 2009 "IASB Code of Conduct for Best Practices in Gene Synthesis" is concerned with the safe and responsible use of synthetic DNA. This code requires DNA synthesis providers to have compliance plans that include screening of requests for DNA synthesis to determine if there are potential harmful uses, screening customers and restricting supply to legitimate purposes, keeping records and reporting potential illegal activities.

Non-Living Products

The potential non-living products of synthetic biology span many sectors and include, e.g. industrial chemicals, agricultural chemicals, therapeutic and veterinary products, biofuels, cosmetics, and food additives. In the synthetic biology discussion it has been claimed that there is inadequate regulatory oversight of these non-living products. Our view, which is shared by many Parties, is that these products fall within the scope of existing applicable sectorial

regulatory frameworks, many of which predate the CBD and its Protocols. In determining how this issue is connected to the scope of the CBD, it can be broken down into specific concerns connected to particular products. For example, for chemicals, the relevant concerns appear to include adequate protection of biological diversity and socio-economic considerations; for therapeutic goods, these are access and benefit sharing (ABS), and socio-economic considerations; and for food additives it is not about regulation of the product, but protection of livelihoods. The concerns about chemicals and therapeutic goods will be discussed further in this presentation as they are relevant to the objectives and scope of the CBD. Socio-economic considerations are not directly provided for in the CBD so these will be discussed in broad terms. We do not consider the protection of livelihoods to be within scope, and this topic will be discussed further in the panel discussion.

The safe use and international trade of chemicals are regulated by a complex regime consisting of, e.g. multiple international pollution treaties; international guidelines, codes of conduct and food standards; intergovernmental bodies such as the OECD; the World Trade Organization; the International Labor Organization; UN bodies, including the WHO and the Food and Agriculture Organization, and the Inter-Organization Program for the Sound Management of Chemicals. The CBD synthetic biology discussion has not even scratched the surface of this complex system but broad claims are being made. Interestingly, the current applicable international policy framework, the "Strategic Approach to International Chemicals Management", includes environmental, economic, social, health and labour aspects of agricultural and industrial chemical safety. This appears to be consistent with, among many other things, protection of biological diversity and socio-economic considerations. A key objective of this policy is the sound management of chemicals to minimize adverse environmental impacts.

Similarly, a complex international regulatory regime applies to research and development, use, and trade of therapeutic goods. There are so many binding instruments and guidelines, and international, regional and national bodies in this area that they cannot be addressed in one presentation. Again, the CBD synthetic biology discussion has not even scratched the surface in this area. One of the most important international bodies is the WHO, which has several work programs, and within each of these, has issued many technical guidelines, all of which can be easily accessed through their website. To provide one example, the "Public Health, Innovation, Intellectual Property and Trade Program" has two current overarching strategies. Very briefly, the "Global Strategy and Plan of Action" refers to the CBD in relation to prevention of misappropriation of health-related traditional knowledge, which is an objective of ABS. The "WHO Strategy on Research for Health" is focused on supporting health-related Millennium Development Goals and human rights treaties, which broadly speaking, appears to be consistent with socio-economic considerations. Thus, without making an in-depth analysis of regulatory regimes for non-living products, international policy appears to encompass the specific issues that have been raised as "gaps" in the synthetic biology discussion.

Access and Benefit Sharing

The final area that this presentation will address is ABS under the CBD and Nagoya Protocol. The fair and equitable sharing of the benefits arising out of the utilization of genetic resources is an objective of the CBD, and it includes an access and benefit sharing framework in Article 15. The Nagoya Protocol further implements this CBD objective and framework, and its scope includes genetic resources, as well as traditional knowledge associated with genetic resources (that are within the scope of the CBD) and the benefits arising from their utilization. The definitions

provide more guidance on the scope of the Nagoya Protocol, with Article 2 defining "utilization of genetic resources" as to "conduct research and development on the genetic and/or biochemical composition of genetic resources, including through the application of biotechnology…" As discussed earlier, synthetic biology is within the scope of biotechnology as defined by the CBD.

A specific ABS issue that has been in the synthetic biology discussion is digital information, i.e. electronic DNA sequence information. This information is not a "genetic resource" within the meaning of the CBD/Nagoya ABS framework. As mentioned earlier, Article 2 of the CBD defines a "genetic resource" as "genetic material of actual or potential value", and "genetic material" as "any material of plant, animal, microbial or other origin containing functional units of heredity". Our interpretation is that a "unit of heredity" is a gene, as this is the basic unit of inheritance, and that gene must be "functional", meaning that it encodes a specific functional product, e.g. a protein. The genetic resource must also be a "material", confirming a requirement for physical access, of "plant, animal, microbial or other origin", and that "material" must "contain" functional genes. Electronic sequence information is not a material containing functional genes. This means that ABS obligations cannot apply to electronic sequence information that is not obtained in connection with the utilization of the source genetic resource.

The claims around ABS and digital sequence information aim to force indefinite ABS obligations on all uses of electronic DNA sequence. In our view, this information should remain outside the scope of any mandatory treaty obligations. Such a situation would not only be unworkable for the scientific community, but would also stifle scientific innovation and progress, and all of the benefits this brings to society. This would in effect discourage innovative research and scientific collaboration, restrict access to the necessary information for research, prevent the dissemination and transfer of information amongst the scientific community, discourage publication of discoveries, create legal uncertainty around the use of digital sequence information, and monitoring and enforcement would be unworkable. These outcomes would be inconsistent with the objectives of the CBD and the Nagoya Protocol on technology transfer, collaboration and capacity building. They are also inconsistent with the policy developments mentioned earlier, particularly in relation to contained use and components and dual use of concern. These promoted the cultures of openness based on the sharing of information to build scientific knowledge and capacity, as well as public awareness and education, and of responsibility with awareness of ethical and dual use of concern issues.

2.2. SynBio and Convention on Biological Diversity [16]

Genya Dana
Department of State
USA
DanaGV@state.gov

I work for the Science and Technology Advisor to the Secretary of State, John Kerry. I´m in the Foreign Ministry of the United States and I have been covering or following synthetic biology in the Convention on Biological Diversity, since 2012. What I want to do today is give some background on when the issue or the topic of synthetic biology appeared first in the Convention and the series of actions over time in terms of this topic. It has been a very interesting time in the Convention. The United States is an Other Government, as we participate in the Convention, so my presentation is from the perspective of an Other Government. There are many others in the room who are Parties to this Convention and have their own perspective and have also been following these issues, so we also welcome their comments and reactions, as well.

The topic of synthetic biology first appeared in documents within the Convention in 2010, in Nagoya, Japan, during the Conference to the Parties to the Convention on Biological Diversity. My understanding is that it was discussed under biofuels, under the agenda item on biofuels and biotechnology. There wasn´t a whole lot that happened, it was just sort of flagged or brought to the attention of the Parties at that meeting in 2010. It was brought to the attention of the subsidiary body to the Convention that provides scientific, technical and technological advice, in May 2012. The technical advisory body met to discuss the issue, but didn´t really do much with it. The issue was put forth to the next Conference of the Parties, which took place in Hyderabad, India, in 2012. Discussions started to get a little bit more involved in 2012 in India, and the Conference of the Parties said "we should send this back to the technical body for further discussion on what to do".

A lot of the questions revolved around synthetic biology, a new and emerging issue under the Convention. So the technical advisory body discussed it again, in June of 2014, and that was a much more involved discussion. The technical advisory body presented opinions, proposed texts, and went back to another Conference of the Parties, which met in October 2014 in Pyeongchang, South Korea. In Pyeongchang, some substance started to form around this issue. The Conference of the Parties started to think about concrete activities that could take place around synthetic biology under the Convention. It was at that meeting, in Pyeongchang, that a process was put in place by the Conference of the Parties to collect more information on synthetic biology and for the Secretariat of the Convention to run a process, to have a discussion, an online discussion about the issues and to convene a body called the AHTEG (Ad-hoc Technical Expert Group) on synthetic biology.

The AHTEG met in September of 2015. In Pyeongchang, the Parties set forward terms of references for these conversations and for the AHTEG, and certain topic areas that they wanted to have more discussion about. I will show the slides with those topics in a moment. So the AHTEG has met, there is a report out, I will get to that in a moment, and again the issue will be going back to the technical advisory body to the Convention next month, in April. We do anticipate that the issue will be one of great interest during the next Conference of the Parties meeting, in December of this year, in Cancun, Mexico.

This is a recap of what I said at the beginning. Synthetic biology was first considered in COP 10 under biofuels and biodiversity. Since then, the issue has been considered under the agenda item

[16] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

"new and emerging issues related to the conservation and sustainable use of biodiversity". Now there are 12 criteria for determining whether an issue qualifies as a new and emerging issue that should be considered under the Convention. These criteria were decided upon by the Conference of the Parties, the Ninth Conference of the Parties.

The reason I want to put these criteria up is because there is still some debate amongst some of the Parties as to whether this topic of synthetic biology has met all of them, so I think it´s worth showing what the criteria are: 1) it has to be an issue that´s relevant to the implementation of the objectives of the Convention and its existing programs of work; 2) there needs to be evidence of unexpected and significant impacts on biodiversity; 3) there needs to be an urgency to address the issue or immediate risk caused by the issue for the implementation of the Convention; 4) there needs to be information on the actual geographic coverage and potential spread of the issue (is it a really big issue) related to conservation and sustainable use of biodiversity; 5) there needs to be evidence of the lack of or limited availability of tools to limit or mitigate the negative impacts of the issue; 6) there needs to be some evidence of magnitude of actual or potential impact of the issue on human well-being; and 7) a magnitude of actual or potential impact on protected sectors and economic well-being as related to conservation and sustainable use of biodiversity.

So this is a recap of the first slide that I talked to you about. Again, the issue has been under discussion in the Convention for several years, but we only recent started to examine specific issues in greater detail after the Pyeongchang Conference of the Parties meeting. I´ll show you in a moment the seven items for discussion. The Secretariat ran a process whereby information was collected about the seven items, followed by an online discussion and the meeting of the Ad-hoc Technical Experts Group last fall. There were around forty members, and then there was the synthesis report which was also produced. The synthesis report includes a set of recommendations that the AHTEG made up for consideration by the technical advisory body which will meet in April.

The matters that the AHTEG covered, as well as the online form and the information submissions, are the operational definitions of synthetic biology. Included were the criteria by which something should be considered synthetic biology or excluded from consideration, what is the relationship between synthetic biology and biological diversity, what are the similarities and differences between Living Modified Organisms, as we understand them under the Cartagena Protocol, and the organisms, components and products of synthetic biology.

The adequacy of other existing national, regional or international instruments to regulate the organisms, components or products derived from synthetic biology techniques and the potential benefits and risks of organisms, components or products arising from synthetic biology techniques to the conservation and sustainable use of biodiversity and related human health and socio-economic impacts are relevant to the mandate of the Convention and its Protocols. What are the Best Practices in risk assessment and monitoring regimes, currently in use, by Parties to the Convention and other governments and the degree to which existing arrangements constitute a comprehensive framework to address the impacts of organisms, components and products resulting from synthetic biology, in particular, to address threats of significant reduction or loss of biological diversity.

I will not summarize all that was said during this AHTEG meeting. It was a week long and there were very long days, as is always the case in these discussions. The AHTEG report is online, but only in English. The goal of the AHTEG was not to come to a consensus on these issues; this was an exchange of views and opinions of the experts who were gathered in the room. You can find the text of all the documents from all the Conferences to the Parties, all of the SBSTTAs, all of the

submissions, all the reports produced and so on, at these websites. The documents for the technical advisory body – the SBSTTA – are also online.

If you go to the Convention´s website and go to SBSTTA 20, the documents are there now. On the 8th of March, the document for synthetic biology was posted there. It´s agenda item number 6. It has a summary of the AHTEG, a summary of the activities that have taken place since the last COP, and the document for agenda item number 6 has recommendations for the subsidiary body to look into.

References

BCH-SynBio. 2016. https://bch.cbd.int/synbio
All of the information submissions, online forum discussions, and reports are located on the Biosafety Clearinghouse's Portal on Synthetic Biology

CBD. 2016. https://www.cbd.int/decisions
All of the decision texts from COPs and recommendation text from SBSTTAs are located on the website of the Convention

2.3. Discussion panel on SynBio regulatory framework

AHTEG Representatives
Sorka Copa (Bolivia)
Luciana Ambrozevicius (Brazil)
Jim Louter (Canada)
Maria Orjuela (Mexico)
Felicity Keiper (EuropaBio)
Genya Dana (USA)

2.3.1. BOLIVIA [17]

Sorka Copa
Ministry of Foreign Affairs
Plurinational State of Bolivia
Member of AHTEG
sorka.cr@gmail.com

Biotechnology itself is an important issue to the Plurinational State of Bolivia. Our country has been participating in these discussions on the topic raised by our colleague from the United States.

Bolivia views itself as a megadiverse Latin American country. We believe that biotechnology should be used for the common good, not for profit. Given what has been under discussion since yesterday and the wealth of information shared on the topic, we believe information is not always properly managed by the generators and promoters of this technology. Their purely commercial approach demeans the products of nature, which are often crucial to a people's survival and arise from a harmonious relationship between man and nature.

With regard to GMOs in Bolivia, a breed of genetically modified soya bean (RR soya) has been approved, as you are well aware. However, it was introduced by a foreign company for purely commercial reasons and not to strengthen food security or sovereignty.

Soya bean is not a traditional staple in Bolivia and thus provides few benefits in terms of food security. While the technology itself is not bad, it may be dangerous, depending on who is employing and handling it, and what their objectives are. Furthermore, the use of patents hinders access to seeds. Our farmers do not follow this practice; rather than assigning individual property rights to seeds, they place them at the service of mankind. This is not the case with GMOs.

Within AHTEG, Bolivia has argued that an international regulatory framework is required to oversee the use of synthetic biology or similar emerging technologies, as well as their products and components. A proper scientific assessment of the risks is also required, and the stakeholders involved in decision making must be brought on board. While it is true that governments, scientists and regulators are represented at this meeting, civil society, indigenous peoples and local communities must also be consulted. If they are involved, directly or indirectly, in the products or results of this technology, then why is their voice not heard?

[17] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

The need to include representatives of indigenous communities was also noted during the AHTEG discussions. Unfortunately – as noted at the conclusion of the report – this has not been done. Their voice should be heard, as synthetic biology is ultimately a new construct, but one which arises from knowledge already present in nature, which may or may not be related to traditional knowledge held by indigenous peoples and local communities. Consequently, they should be aware of the degree to which they will be involved in, and benefit from, the use of synthetic biology.

This brings us to another issue discussed by AHTEG: the fair and equitable distribution of benefits. It is not a question of the genetic codes being used, but rather of the knowledge they contain. The issue promises to be highly contentious. Nagoya does not address how it should be approached within the framework of synthetic biology.

We are faced with two issues: the fair and equitable distribution of benefits and the question of informed consent regarding the use of knowledge. The point of contention is not the gene itself, but rather the knowledge associated with the construct being developed.

We would like to draw attention to another issue discussed within AHTEG: socioeconomic, cultural and ethical considerations. In my country, which is strongly attached to its culture, emerging technologies may overlook the social and cultural issues that accompany certain types of knowledge, sidelining the cultural and ancestral worldview of the indigenous peoples who have always worked to conserve biodiversity through their traditional practices.

We believe there should be a complementary relationship between the two. This would encourage conservation, not only of biodiversity, but also of cultures and traditions, and of the relationship between man and nature. Man cannot disassociate himself from nature. Technology should not be approached as a commodity. On the contrary, it should be a strategic tool that helps to address structural problems relating to health and poverty eradication.

Ideally, States should work with scientists to address the needs of the population. Scientists should be at their disposal to solve large-scale national and global problems. Health is a very significant issue to us, and one which can be addressed through biotechnology and synthetic biology. However, we would not want to see access to technology or to its products privatized and patented, as this would block society's access to health care for the common good.

2.3.2. BRAZIL

Luciana Ambrozevicius
Ministry of Agriculture, Livestock and Food Supply
Brazil
luciana.pimenta@agricultura.gov.br

The topics discussed during the presentation are summarized below:

(i) How to be more active in the Decisions?

In relation to the Convention of Biological Diversity (CBD) procedures we can list four important forums where the Parties, non-Parties and other institutions can actively participate in the decision-making process:

- Sending the information required by the CBD Secretariat (submissions to the notifications);

- Participation in the online discussion forums
- Participation in the Ad Hoc Technical Experts Groups (AHTEG)
- Participation in the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA)

Those forums are of great importance for the decisions presented during COP and adopted by the Parties. The Ministry of Agriculture initiated a plan of action together with other Brazilian institutions and worked very actively in those forums in 2015 with the topics that were considered of high relevance, and one of them was Synthetic Biology.

(ii) Brazil Position: Notification 018/2013

Brazil's submission for the Secretariat Notification 018/2013 clearly did not support the inclusion of any new item on the agenda and for SynBio it was noted that:

- ✓ The criteria for identifying new and emerging issues contained in paragraph 12 in the Decision IX/29 have not been provided, specifically related with the items were its requirement for reliable scientific information or evidence of actual and potential negative impact
- ✓ It recognized that SynBio presents potential positive impacts on the conservation of biodiversity (e.g. alternative energy)
- ✓ it also recognized that uncertainties related to the potential impacts are well addressed by paragraph 4 in the Decision XI/11 – "Urges parties and other governments to take a precautionary approach"

The conclusion of the submission sent by Brazil was that "*By 2020, the Parties to the Convention will have the enormous challenge to implement the Strategic Plan for Biodiversity and the ambitious Aichi Targets adopted at COP-10. In this sense, Brazil is of the view that the Subsidiary Body on Scientific, Technical and Technological Advice should focus its work on the provision of scientific and technological assessments and advice related to implementation of the Aichi Targets and issues related to the conservation and sustainable use of biodiversity that have been already identified by the Parties*."

(iii) Brazil Position: Notification 013/2015

Regarding the questions presented by the Secretariat under Notification 013/2015, the same questions that were the basis for the AHTEG report, it´s important to highlight some points in the Brazil submission:

- ✓ The recognition that the definition of LMO under the Cartagena Protocol on Biosafety (CPB) is applicable for SynBio organisms at the moment
- ✓ In Brazil, the LMO risk assessment and biosafety regulatory framework are applicable for SynBio living organisms. Components and products are regulated under other agencies and legal frameworks
- ✓ The recognition of potential benefits of SynBio and that potential adverse effects are the same as those for LMOs

The submission also recognized that it is necessary for there to be sharing of information among countries about the risk assessment and approvals of SynBio organisms through an online mechanism and that further studies may be necessary to elucidate some aspects of environmental interactions of SynBio organisms in the future.

(iv) Brazil Position: peer-review of AHTEG report

Brazil presented comments in the peer-review process of the AHTEG report ((UNEP/CBD/SYNBIO/ AHTEG/2015/1/3) and the main points were:

- ✓ The inclusion of SynBio as a New and Emerging Issue in the agenda of CBD is still pending
- ✓ Current and immediate future applications of SynBio are similar to LMOs and therefore encompassed by the CBD
- ✓ Any positive or negative impact should be determined on science-based information
- ✓ The use of SynBio is not a cause per se for higher levels of uncertainty or for the time needed for RA
- ✓ All adverse effects are the same as those considered for LMOs, some are not part of CBD (e.g. loss of market)

Brazil recognizes the necessity to follow the technology advances and keep the subject under monitoring.

(v) Considerations for the Present Discussion

The positions presented above are official positions sent to the CBD Secretariat through submissions from Brazil's focal point. The points listed below are personal opinions still being discussed internally and presented in the seminar for the purpose of sharing ideas and to foment the debate.

- **About the definition**: this was one of the main topics discussed in the AHTEG due to the various concepts proposed by the members. In the end the decision was to use the EU definition with some terms incorporated. Although the SynBio definition is broad, it has some important concepts already defined (e.g. modern biotech, live organisms, biological systems) and presents the notion of evolution.

- **Components and products**: "non-living" were included in the report as one aspect for further work, even though many countries presented the position that components of SynBio are the same as those components used for genetic engineering and regulated for this use for decades and the products obtained with SynBio organisms are regulated by specific rules (for example pharmaceutical regulations).

- **Digital sequence:** is mentioned in the report related to the Nagoya Protocol although it can´t be classified as a genetic resource according to the definition in the CBD and it´s very premature to start discussing those issues without a clear understanding if SynBio is a New and Emerging Issue and the way the subject will be conducted under CBD.

- **Socioeconomic (SEC) and Risk Assessment (RA) AHTEG**: the necessity for coordinated work with SEC and RA AHTEG is mentioned and needs to be better defined. If RA AHTEG will collaborate with SynBio AHTEG it should be in the context that the road map developed by the RA AHTEG could be used to test a SynBio organism to check if there is any gap. If SEC is applicable to SynBio it should be in the context of Art. 26. It´s important to emphasize that SynBio is under CBD while RA and SEC AHTEG are under the Cartagena Protocol and not all countries are part of both.

- **AHTEG report**: there is no adverse effect specific to SynBio in the report (the potential adverse effects are the same as those assessed for LMOs) and the AHTEG agreed that LMO developed through current and near future applications of SynBio are similar to LMOs defined in the CPB.

(vi) **SBSTTA 20 - Recommendation of Decisions for COP 13**

Decision XII/24: it posits that there is currently insufficient information to finalize an analysis to decide whether or not SynBio is a new and emerging issue and awaits the completion of a robust analysis using the criteria set out in paragraph 12 of Decision IX/29. Establish

mechanisms to collect more information about SynBio including the Secretariat notifications, online forums and the SynBio AHTEG.

SBSTTA 20: based on the information collected it must be decided and recommended to the parties if SynBio should be considered a New and Emerging Issue. Considering that the requirements to qualify for consideration as a New and Emerging Issue are established in paragraph 12 of Decision IX/29, the task of SBSTTA 20 will be to evaluate if the information available at this time is enough to finalize the analysis.

2.3.3. CANADA [18]

Jim Louter
Canada
jim.louter@ec.gc.ca

I would like to start with a little description of the regulation of biotechnology in Canada, and then go to my impressions of my participation in the AHTEG on synthetic biology. My Ministry recently changed its name as there was a new government elected in October of last year, so we now have "and climate change" added to the name of the Ministry to reflect a new priority for our current government. So the new name of the Ministry is Environment and Climate Change Canada.

If you have been involved with the Cartagena Protocol in the past or especially with the discussions under what I would call the "other AHTEG" (on Risk Assessment and Risk Management), then you would probably be familiar with the name Phil McDonald because he represents Canada in that forum as part of the Agriculture Ministry (Canadian Food Inspection Agency). But really, biotechnology in Canada is a shared responsibility among many ministries including Environment and Climate Change Canada which is where my responsibility is. I operate under our Canadian Environmental Protection Act. It's important for me to describe that Act because it defines our role and our purpose in the regulation of biotechnology in Canada. And then I wanted to talk about the AHTEG on synthetic biology.

Phil McDonald comes from the Canadian Food Inspection Agency and he will use the expression "plants with novel traits." So the big lesson for you to take home from Canada's participation is that our law is technology independent. I think, for example, were we ever to consider a CRISPR-Cas9 regulation, it would be a bad idea because as soon as a newer technology comes along, the CRISPR-Cas9 regulation would be obsolete. By having a regulation only based on "transgenics", as soon as that technology changes or evolves such that transgenesis as such does not occur, your regulation may become obsolete. In Canada, we have a regulation that is based on "novel traits" which is the terminology used for agriculture that immediately captures any new product. It doesn't matter what technology was used to create it.

Our Health Ministry looks after novel foods. So the novel food assessment is separate from the environmental regulation of the organism used to produce that food. We have also worked with our Fisheries Department because we have regulated transgenic salmon in Canada, similar to the United States, which is a product of old biotech, by the way, not synthetic biology. And Agriculture Canada plays a role when it comes to trade. So there are many players (Fig. 27), and I use a chemicals analogy to show a filter in which the important thing is that at the bottom, here the Canadian Environmental Protection Act and our regulation captures everything that falls

[18] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

through the cracks; so, in the end, someone somewhere will regulate that new organism if it is imported to or made in Canada.

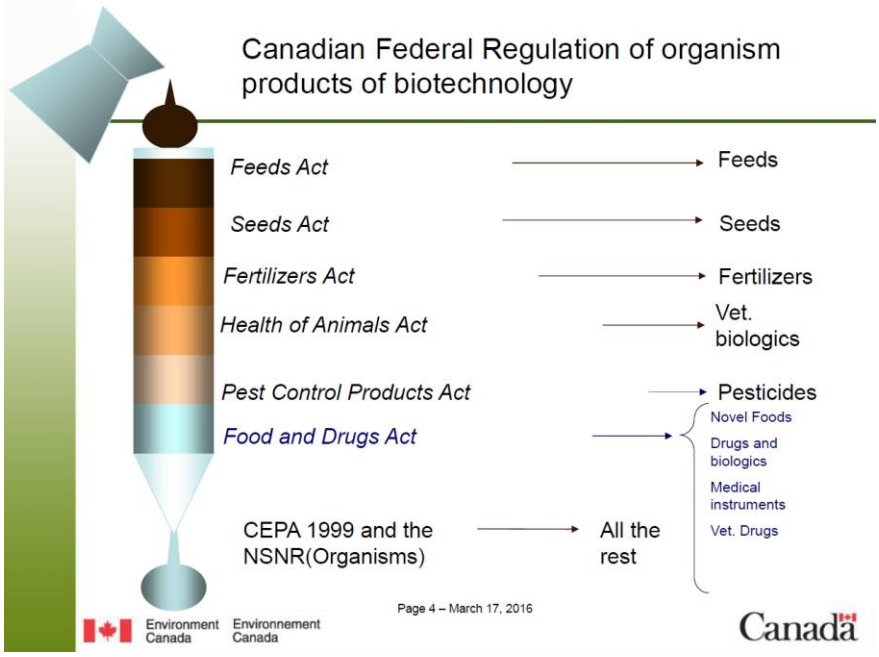


Figure 27. Canadian Federal Regulation of organism products of biotechnology

My office is responsible for all organisms that are not regulated by any other regulatory entities in Canada. Livestock feeds may contain organisms and components and are captured here because an animal feed may contain a novel microorganism (for example a probiotic) or it could contain components of a genetically modified animal to be used for animal feed. The Seeds Act is where our plants with novel traits are regulated, so all the transgenic crops are regulated here. Fertilizers can include products as well as organisms used to enhance the growth of plants. The Health of Animals Act includes veterinary biologics which may include live recombinant organisms used as vaccines for animal use. Pesticides are regulated here also because they may contain living organisms to be used for pest control purposes.

Our Food and Drugs Act does not have an environmental mandate, but we have an arrangement, made under other environmental legislation, that new food or drug products that include living organisms do get an environmental assessment by virtue of the relationship with the environmental legislation, our Canadian Environmental Protection Act. Also, for example, if there were to be genetically modified probiotics for yogurt or for food packaging, this would also be covered.

This illustrates part of the range of products and organisms that we can see under our legislation, so we do regulate naturally occurring organisms in containment, under our statute. We've regulated a very interesting transgenic pig. In Canada, the University of Guelph developed a pig that was transgenic and that produced phytase enzyme in the salivary gland of the pig so that it could digest phytate in the feed better. So you can add phytase enzyme to animal feed to achieve the same result or you can, in this case, modify the pig. It was called “the enviropig” to try and promote the reduction in phosphate that would be excreted from the pig. But, unfortunately, the pig producers had done enough market analysis to say that, in the end, this wasn't going to be accepted by consumers and so their research funding was withdrawn.

Canada is in a strange position in that we have more power to be involved as a party under the Convention than under the Cartagena Protocol. This is under the Protocol we signed, but did not ratify. So it makes my involvement in this AHTEG a little bit conflicted because, in a sense, it would be better if it were under the Convention than under the Protocol, but I'm not going to be arguing that. So, I'm disclosing my conflict in order that, even though not a party to the Cartagena Protocol, Canada was a model non-party. This is our way of saying we basically are in compliance with the Protocol even though we attend those events as an observer, and are able to give thoughts from the sides, but Canada can be ignored.

My thought on the AHTEG on synthetic biology was that based on the report, I would conclude that it was representative of the discussion at the end, even though it may have included text that we didn't all agree with. If you read the AHTEG report where it says, “some said” it means that there were few people in the group who disagreed; that's important to know. There's been discussion earlier about the online discussion, which I thought is comparable to the discussion that happens when you read a newspaper article and people from any sector of society can add comments. The first thing I do is rank the articles by those that are most liked. That usually means that the nonsense ones go to the bottom and the ones that have value go to the top and that stops me from getting very angry if I read some inflammatory comments. So, in a way, the online discussion is good for letting off steam. People can give any comment they wish, but it's moderated, but not curated; it's moderated, but not nuanced. I was reading the comments being made online and a lot of them I agreed with, but somehow I find that the AHTEG was a little bit more acceptable, I would say. I would put a little bit more value, personally, on the report of the AHTEG than on some of the online discussions. That's my personal opinion.

The AHTEG found a 100% fit between the two. If we were to make two overlapping circles, and one was LMOs and the other synthetic biology, there was nothing that we could think of that exists today that was not already an LMO. But the possibility exists that some products of synthetic biology that we now consider science fiction may, in the future, not fit the living modified organism definition. But we have to deal with what we've got today and if you've got an agreement that covers living modified organisms 100% today, but maybe there's one or two examples that may arise in the future, I don't think that warrants the creation of a separate international protocol or agreement for something like that.

Regardless of that, the Convention – the tools that are available in the Protocol, Annex 3 – as mentioned already, are easy to regulate. Even those other products of synthetic biology that may not be LMOs would still be covered by those tools that are available to us. There's been discussion, a lot more thorough by previous speakers, about components and products that are not covered under the Protocol, as has been said before, but to me they are or should be covered by existing domestic regulations in most countries. If there was to be any consideration for products and components in a separate agreement, then you should by the same reasoning be submitting all new chemicals and all new products that are developed by many other processes so they can also be covered by that same agreement. There’s no logic in having a separate agreement for components and products of synthetic biology. So, you would have to get the Convention on Biological Diversity to develop a similar regime for new chemicals, such as is regulated in Europe by REACH, for example.

In regard to new drugs, we have mechanisms for dealing with new drugs, new foods produced for many technologies, new chemicals, new pesticides, and new breeds of cattle. The question for regulators, and this was a point being made recently, is what happens if the resulting product developed by synthetic biotechnology is in the end identical? The example given was the breed of cattle; you may use a synthetic biology technique to eliminate the characteristic of having horns as happens through a natural mutation in some breeds of cattle. That would be a question for the regulators to solve. Sometimes there are artifacts produced by technologies and there

may be non-target effects in those cases. So this is where the focus of the risk assessment may happen, at least initially, until the technology, the methodology, becomes robust enough that these artifacts are not produced in the living organism. We have to cross that bridge when we get to it. And in all cases, we talk about a case-by-case risk assessment and we have to look at the whole product, not just the change that has been introduced. Maybe it was a mimic of something that occurs in nature, of a natural mutation, but one introduced through synthetic biology, but what other changes have there been? Maybe if all those anomalies have been eliminated then the organism would be considered the same. It should be regulated as any other organism that has the same mutation.

This is my opinion. I don't think there are enough examples of products that are not already LMOs. The Cartagena Protocol is completely adequate to cover the products of synthetic biology. Even in the off chance that there could be something different, our domestic or existing rules (and a previous speaker gave a very good analysis of all the different kinds of protocols and guides that are available) are more than adequate to regulate them. We have the tools and we have the different regulations and guidance in place to provide that flexibility, to cover those kinds of products.

2.3.4. MEXICO [19]

María Orjuela
CONABIO
Mexico
morjuela@conabio.gob.mx

Although I have participated in AHTEG as an expert representative for Mexico, the opinions I am about to express are personal. They do not reflect my country's position, as that must be developed by a group of individuals from many different disciplines.

Unlike several of my AHTEG colleagues, I work at CONABIO, which is the national commission for knowledge and use of biodiversity. Although we help to generate data, we are not regulators. CONABIO includes a department – of which I am a part – which combines risk assessment with biosafety. We formulate opinions on genetically modified organisms which may be released into the environment, and help to develop regulations accordingly.

Mexico is a megadiverse country, both biologically and culturally. This is highly significant, as we cannot but acknowledge that much of the enormous diversity of genetic resources we have today is the result of human domestication efforts. Thus, genetically modified organisms and synthetic biology must not be viewed in isolation, but rather as interrelated issues that require a multidisciplinary approach.

Mexico is a source and also the home of genetic diversity for many species that are key to global nutrition. In that regard, synthetic biology can solve real problems in a number of sectors, including agriculture, public health and manufacturing. CONABIO seeks to ensure that it is used safely and responsibly. I believe that, where synthetic biology is concerned, it is important to address the "biosafety vs. biosecurity" issue.

Another issue discussed by AHTEG was xenobiology. We are speaking here of organisms that can be created by modifying existing DNA. Synthetic biology, however, is capable of creating organisms or products using combinations not found in nature. This topic should also be

[19] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

addressed, not to put a stop to the work being done, but rather to reflect on whether the criteria for defining it established by AHTEG in its report are truly being fulfilled.

Intervention in biological systems is another issue. We have been discussing genetically modified organisms; in this forum, however, I have heard talk of systems, not simply organisms. The example involving coffee is interesting. Introduce a bacterium into an existing organism and generate an interaction – but what, then, are we talking about? How does one regulate it? Would it be covered by the Cartagena Protocol? We are really talking about the modification of a biological system, which may bring a larger scale into play – systems, communities and so forth.

Another important issue – which I raise not to stop the process, but to reflect upon it – is the Nagoya Protocol. While it may not be essential to discuss it or make it the focus of this meeting, it should definitely be taken into account. Megabiodiverse countries such as Bolivia, Colombia, Mexico and Peru will, in time, have to assess the relationship between their biodiversity and synthetic biology.

What do I think should be done? One problem I see, particularly in Latin America, is the lack of a structured, systematic plan; we are constantly improvising, taking shots at the problem. If our countries developed a plan to address these issues, a solution might be possible. Science would focus on solving the problems at hand, and that would in turn lead to public policies that channel resources and actions appropriately.

While I believe harmonization is important, not all issues are amenable to such an approach. We are Latin America; most of us speak Spanish and share a similar outlook, but we also have many differences. We must sit down and discuss certain topics, but not everything can be standardized. I do not believe the solution lies in that direction.

2.3.5. UNITED STATES [20]

Genya Dana
Department of State
USA
DanaGV@state.gov

The United States was able to participate in the AHTEG as another government, and we very much enjoyed the opportunity to have a lot of very interesting discussions and sharing of perspectives during our week in Montreal. The issue of synthetic biology has attracted a lot of public attention in the United States, within the media, from our own policy makers, and it was very informative, for me in particular, to be in a room where we could spend that much time talking in detail about these issues. They are important issues to discuss.

The United States understands synthetic biology as it is discussed in the research and development community, as covering a continuum of biological engineering tools and techniques that lead to progressively advanced biotech products. So we have not come out to say that synthetic biology is a brand new thing, we see a lot of advances in tools and techniques of biotechnology that are powering a lot of really interesting and innovating research. I think you have seen that or learned that by all of the various definitions of synthetic biology that are out there. Many of those definitions have been developed by the research community or by countries in their national context. In the AHTEG we developed our definition to aid the

[20] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

discussion and set some parameters around the topic, and we also developed that definition to understand how synthetic biology may relate to the objectives of the convention.

We have some concerns about the convention adopting this AHTEG's definition, as we prefer leave each country or institution with the option to use the definition that works for them. We are concerned that by adopting a definition under the Convention, countries might decide that it's the definition that they also need to use. We have listened with interest about the issue of digital genetic resource information and we are discussing this as well within our government.

We have some concerns about establishing a process or a mechanism within the Convention to look into the issue of genetic resource information, as it may relate to access and benefit sharing under The Nagoya Protocol. In our view, digital genetic resource information, which is a term that was coined or developed by the AHTEG on synthetic biology, is not a physical genetic resource and thus it is outside of the scope of the convention and The Nagoya Protocol. We have concerns about including digital genetic resource information under any definition of synthetic biology and this is something that I mentioned during the AHTEG deliberations. The sequencing of DNA and the publication of those sequences has been happening for decades, this is not something that is brand new to synthetic biology. Advances in this sequencing and the provision of this information have enabled advances in biotechnology for sure, but the fact that this genetic information exists does not mean that it is somehow a part of synthetic biology.

In the interest of time I will conclude with one final point and that is on the coverage of existing instruments for oversight or regulation of biological engineering or biotechnology. This is another point that I became very concerned about during AHTEG discussions, in that the components and products of synthetic biology somehow now seem to have no oversight. There was a lot of discussion in the room regarding us having no idea how to deal with these other things that are not LMOs. We as the AHTEG, as the group of experts, are not equipped, in my opinion, to know about all of the existing instruments, the national, regional or international instruments that are out there, and since we could not agree on a definition of synthetic biology and what is included and excluded, there is really no way to say it's not covered. I think this is an area that could use a lot more exploration before we say that a new overarching framework or set of guidelines or so on is needed. There are a lot of other things out there in terms of treaties, conventions, guidelines, best practices, all of those things, and during the AHTEG we simply were not prepared nor did we have the capacity to get into those discussions, and come to any such conclusion that a new oversight mechanism is needed.

2.3.6. Private sector [21]

Felicity Keiper
EuropaBio
felicity.keiper@bayer.com

CropLife's views on the synthetic biology discussion under the CBD have been provided in detail in the two presentations given during this workshop. The first of these provided a "global snapshot" of synthetic biology, and the second provided an assessment of the "regulatory environment". For this panel discussion, there is not another presentation, instead I will elaborate further on our views on four particular issues raised only briefly in those presentations. These include: the definition of synthetic biology, socio-economic considerations, accuracy of information, and reiteration of our view that an international framework for synthetic biology is unnecessary.

[21] Conference transcription: Patricia Echeverri; text edition, Pedro Rocha

Definition

As described in the earlier presentations, it is our view that a definition is not needed for synthetic biology because it is simply the current state-of-the-art of biotechnology. Synthetic biology is therefore not something new, rather it represents the continuum of technological advancement, and it is artificial and arbitrary to attempt to separate it. If COP determines that there must be a definition, the challenge is to develop one that is meaningful for a broad range of sectors, potential applications and products, and remains relevant into the future while the technology continues to evolve. It is our view that the definitions of "biotechnology" and "modern biotechnology" in the CBD and Cartagena Protocol, respectively, are sufficiently broad to encompass the continuum of biotechnological advancement, even beyond "current and foreseeable" applications.

We do not support the "operational definition" that is the outcome of the AHTEG meeting. The basis of deliberations was the operational definition contained in the "Opinion I" document I referred to earlier of the three Scientific Committees advising the European Commission. The AHTEG made additions to this definition, and, in our view, made it unnecessarily complicated. In particular, language such as "further development and new dimension" emphasizes novelty over continuity, with the latter concept better reflecting the majority of views in the AHTEG.

Socio-economic considerations

Socio-economic considerations are the subject of Article 26 of the Cartagena Protocol, whereby Parties "may" take these into account when making a decision about LMOs in their implementation of the Protocol. The scope of the socio-economic considerations to be taken into account are those "arising from the impact of living modified organisms on the conservation and sustainable use of biological diversity, especially with regard to the value of biological diversity in indigenous and local communities". Parties are also required to take socio-economic considerations into account in a manner that is "consistent with their international obligations".

In the synthetic biology discussion, the primary issue with socio-economic considerations appears to be the fact that they are voluntary rather than mandatory in the applicable international law. As described in our earlier presentation, it is our view that the living organisms created by synthetic biology applications are LMOs and are within the scope of the Cartagena Protocol. This means that Parties have the choice to take socio-economic considerations into account when making decisions about LMOs created by synthetic biology. Similarly, the brief assessment of regulatory regimes for non-living products presented earlier indicates that the current international policy frameworks provide for voluntary consideration of socio-economic considerations. It is our view that this is appropriate; such considerations should remain voluntary, with countries able to make decisions according to their own priorities and circumstances.

Accuracy of information

A particular challenge in the synthetic biology discussions under the CBD is the need to consider the quality of information that is being presented, especially in relation to the potential benefits and potential negative effects of synthetic biology. Any claims need to have a basis in reality, and they need to be backed by evidence. For example, consider whether the named application is current or foreseeable, or is it merely a concept in the early stages of research, or is it science fiction? Is the claim backed by evidence, is it a broad assumption, or is it wild speculation? Also, recall the objectives of the CBD, and consider if and how there will be exposure to biological

diversity, given that many synthetic biology applications are intended for contained use only. The list of potential adverse effects compiled in the AHTEG report can be criticized for their general and speculative nature, with many not being specific to synthetic biology.

The need to consider the accuracy of information presented in the synthetic biology discussions under the CBD is demonstrated by the example of vanilla/vanillin, which featured prominently in the online discussions. The difficulties with this example are explained in greater detail in a Nature Biotechnology publication (2015 33:329-332); in brief, the argument was that synthetic vanillin would adversely impact the market for vanilla pods that are cultivated by traditional and small farmers in developing countries, when in fact the two products compete in different markets. This is relevant to the topic of food additives, being non-living products, as mentioned in the "regulatory environment" presentation earlier.

No need for an international framework for synthetic biology

This point is argued throughout the "regulatory environment" presentation made earlier, and is based on an analysis of the claimed "gaps" in regulatory oversight that have been raised in the synthetic biology discussions under the CBD. For each of these "gaps", a range of regulatory sources and mechanisms were found to exist, where regulatory oversight was considered necessary and appropriate. Claims of inadequate regulatory oversight have been made based on broad assumptions without a thorough assessment of realistic current and foreseeable applications of synthetic biology, or the vast array of existing regulatory sources and mechanisms in all relevant sectors. To introduce a new international regulatory framework for synthetic biology will duplicate existing regimes, inappropriately impose process-based approaches in sectors with product-based regulation, and it is unlikely that such a framework could be sufficiently comprehensive to span the broad range of potential applications and products.

Final Comments. Synthetic biology: Currently, a paradox with multiple applications

Pedro J. Rocha
International Specialist in Biotechnology and Biosafety
Inter-American Institute for Cooperation on Agriculture (IICA)
Pedro.Rocha@iica.int

Synthetic biology is one such area that reflects very well the complexity of modern scientific and technological developments. Synthetic biology is, of course, biotechnology, and an interdisciplinary field, novel and of enormous application. However, its potential raises various questions and heated discussions. The very use of the term "synthetic biology" carries an almost paradoxical message because it is certainly biology (associated with the natural) and is clearly synthetic (related to the human-artificial). Also, the fact that there are so many definitions and lack of a consensus definition complicates the situation, because it is very complex to discuss and even to make regulatory or public policy decisions about a "something" that lacks definition.

But precisely, the novelty and complexity of synthetic biology together with the need to make decisions about its processes and products in the very near future was what motivated this workshop. An event in which, firstly, generalities of synthetic biology were presented, as well as its agricultural, industrial applications, public health, biodiversity conservation and initiatives conducive to promoting research and development. The second session dealt with the regulation of synthetic biology, a topic in which a range of positions ranging from total prohibition (or moratorium) to full acceptance without regulation, passing through the use of the Protocol of Cartagena on Biosafety to the proposal to create a new protocol. And the interesting thing about this event was that the various positions and ideas could be presented, listened and discussed without prejudice. This event was a first effort to discuss synthetic biology with the vision of regulatory decision makers and contributes to the debates and forums that the issue is and will be raising.

Certainly, the varied applications of synthetic biology make it a tool with the potential to solve multiple problems and respond to current and future challenges associated with population growth, sustainable production, extreme weather events, ecosystem degradation, the development of the economy and strict social control, among others. But the technological power of SynBio makes it possible to identify risks associated with its use. And the latter is of fundamental importance, even more so in a society like the one that is said to be interested in knowledge, precision, security and risk aversion.

But the discussion of the application of technological issues in society has shown to be a good way to make them known, to evaluate their risks objectively, to answer questions and to make decisions based on the interests and sovereignty of each country. It is necessary and urgent to deepen the integral discussion of synthetic biology, approaching without prevention or bias to the scientific facts of technology when the scientific field is discussed and considering the economic, social and ethical aspects when the debate is of these natures. The worst thing that can happen is to mix diverse arguments in their nature, since the synthetic biology is framed in a paradox that is called to solve problems not to create them.

With this “First Seminar on Synthetic Biology for Biotechnology-Regulatory Decision Makers from the Americas”, IICA hopes to have contributed to a better understanding of the topic and to the introduction of technical reference elements that support the analysis on which decision-making is based.

Section 3: Workshop Description, Program and Participants

3.1. Description of the Workshop

3.1.1. Background

Synthetic biology (SynBio) is an interdisciplinary branch of biology that designs and constructs biological systems. Potential uses of SynBio can be enormous. However, its regulatory aspects are still under discussion.

In the decision UNEP/CBD/COP/DEC/XII/24* from 17th October 2014, the Conference of the Parties to the Convention on Biological Diversity established the Ad Hoc Technical Expert Group (AHTEG) on Synthetic Biology (AHTEG-SynBio). During year 2015, Open-ended Online Forum on SynBio was carried out and the AHTEG-SynBio generated a technical report UNEP/CBD/SYNBIO/AHTEG/2015/1/3. Substantive issues were central for deliberations including an operational definition of SynBio, the relationship between SynBio and biological diversity, similarities and differences between living modified organisms (LMO, as defined in the Cartagena Protocol) and those products coming from SynBio, adequacy of instruments to regulate SynBio products and techniques, potential benefits and risks, and best practices on risk assessment.

Because of the importance of SynBio, IICA with the support of USDA, have organized this first seminar on the topic, which will be carried out in San Jose, Costa Rica, 16th and 17th March 2016. In this event, we expect to establish conceptual basis on the topic as a way to contribute to a clear understanding and proper communication of SynBio in the hemisphere.

3.1.2. Objectives

- - To contribute to the establishment of a conceptual basis on Synthetic Biology and the relationship to other biotechnology techniques.
- To facilitate greater understanding of the current status in national and international discussions on SynBio and how each of the issues under consideration may affect national, regional, and international interests and agreements.

3.1.3. Importance of the event

The seminar was important because:
(*i*) Introduced the technical discussion of a novel subject with regulatory implications
(ii) Created technical capacities in biosafety in regulators of several LAC countries
(iii) Allowed the interaction of researchers, developers, companies, regulators and various national agencies (ministries of health, environment, etc.)
(iv) Presented TEC advances in synthetic biology, and allowed to interact with the students and researchers.
(v) Contributed to build opinion on the subject.

3.1.4. Obtained Results

- 50 professionals from 16 countries trained in synthetic biology (including decision makers, national authorities and members of National Biosafety Committees).

3.1.5. Methodology

A two-day meeting held 16th and 17th March 2016, at IICA headquarters (San Jose, Costa Rica).

Methodologies generated by IICA were used and have been successful in previous courses and workshops in biotechnology and biosafety.

Because of the importance of the event, the conferences were transcribed; their proceedings were edited, translated and published in electronic format. IICA acted as technical coordinator.

3.2. Participants

- Officials of National Biosafety Commissions (CTNBio) or national biosafety authorities of Central America, North America, South America, Andean Region and CARICOM
- Delegates from countries that acted as representatives in the AHTEG-SynBio.
- Persons interested in the topic from other countries (via Web cast).

(i) Invited speakers

Felicity Keiper (EuropaBio, Australia), Marcelo Freitas (Embrapa, Brazil), Natalia Verza (Universidad de Campinhas, Brazil), Mario Henry Rodríguez López (National Public Health Institute of Mexico), Ricardo Chavez y Ana Sifuentes (iGEM, USA), Kent Redford (Archipielago Consulting, USA), Fan Li Chou (USDA, USA), Genya Dana (US Department of State, USA).

(ii) Participant countries

Argentina, Australia, Belize, Bolivia, Brazil, Canada, Colombia, Costa Rica, Dominican Republic, Ecuador, Guatemala, Honduras, Mexico, Panama, Paraguay, Peru, and United States of America.

3.3. Program

Hour	Description
Day 1: Wednesday 16th March, 2016 **Session I: Generalities on Synthetic Biology**	
7:00 a.m.	Leaving hotel
8:00 a.m.	Arrival to IICA - Register
8:10 a.m.	Welcome *Lloyd Day, Deputy-Director General IICA* *Erich Kuss, Agricultural Counselor, Embassy of United States of America in Costa Rica*
8:20 a.m.	Presentation of the event and introduction of participants *Pedro Rocha (IICA)*
8:30 a.m.	What is Synthetic Biology? *Fan-Li Chou (USDA, USA)*
9:00 a.m.	SynBio and its applications in agriculture *Marcelo Freitas (Embrapa, Brazil)*
9:30 a.m.	SynBio Industrial Applications *Natalia Verza (Structural Genomics Consortium, Brazil)*
10:00 a.m.	Coffee break
10:15 a.m.	SynBio application in control of vector transmitted diseases *Mario Henry Rodríguez López (Instituto Nacional de Salud Pública, México)*
10:45 a.m.	Synthetic biology and the conservation of biodiversity *Kent Redford (USA)*
11:15 a.m.	iGEM initiative in SynBio development *Ana Sifuentes & Ricardo Chavez (iGEM, USA)*
11:45 a.m.	SynBio multilateral fora *Felicity Keiper (AHTEG representative, EuropaBio)*
12:15 p.m.	Open Discussion *Moderator – Pedro Rocha*
12:45 p.m.	Lunch
Session II: Universities approaching synthetic biology – Costa Rica/TEC example	
2:00 p.m.	Trip IICA-TEC
3:00 p.m.	Arrival to TEC /Welcome *Carlos Alvarado (Director, School of Biology, TEC)*
3:15 p.m.	CIB presentation *Miguel Rojas (Director CIB, TEC)*
3:30 p.m.	Farnesene production by synthetic biology *Giovanny Garro & David García (TEC researchers)*
3:45 p.m.	Project PROSTAL-iGEM 2016 *Biotech students*
4:00 p.m.	CIB visit (Laboratories)
5:00 p.m.	Trip TEC-hotel
Day 2: Thursday 17th **Session III: Synthetic Biology and Regulation**	
7:00 a.m.	Leaving hotel
8:00 a.m.	Arrival IICA
8:15 a.m.	Group picture
8:20 a.m.	Summary of previous session

8:30 a.m.	What are the expectations of researchers and developers about SynBio regulation? *Moderator: Pedro Rocha*
9:15 a.m.	Overview of regulatory mechanisms already in place to address the safety use of products of SynBio *Felicity Keiper*
10:00 a.m.	Coffee break
10:15 a.m.	History of Synthetic Biology in the Convention on Biological Diversity *Genya Dana (Department of State USA, USA)*
10:25 a.m.	Discussion panel: What to do in SynBio in the framework of CBD? *AHTEG representatives* *Sorka Copa (Bolivia)* *Luciana Ambrozevicius (Brazil)* *Jim Louter (Canada)* *Maria Orjuela (Mexico)* *Felicity Keiper* *Genya Dana (USA)*
12:15 p.m.	Workshop: SynBio: The positive, the negative and the potential solutions *Moderator: Pedro Rocha*
1:00 p.m.	Lunch
2:15 p.m.	Final remarks and future actions
3:00 p.m.	Back to hotel

Annex

Annex 1. List of participants

	Name	Country	Institution	E-mail
1	Daniela Conte Grand	Argentina	Ministry of Agroindustry	mdcgrand@magyp.gob.ar
2	Felicity Keiper#*	Australia	Bayer/CropLife International	felicity.keiper@bayer.com
3	Hernan Zetina	Belize	BAHA	hernan.zetina@baha.org.bz
4	Sorka Copa Romero*	Bolivia	Ministry of External Affairs	sorka.cr@gmail.com
5	Luciana Pimenta Ambrozevicius*	Brasil	MAPA	luciana.pimenta@agricultura.gov.br
6	Marcelo H. Aguiar de Freitas#	Brasil	Embrapa	marcelo.freitas@embrapa.br
7	Natalia Verza#	Brasil	Universidad de Campinhas	na.verza@gmail.com
8	Jim Louter*	Canada	Environment Canada	jim.louter@ec.gc.ca
9	Juan Manuel Pedraza	Colombia	Universidad de los Andes	jmpedraza@uniandes.edu.co
10	Alejandra Chaverri Esquivel	Costa Rica	Ministerio de Salud	chaverri.alejandra@gmail.com
11	Alejandro Hernández	Costa Rica	CropLife/ITCR	ahernandez@croplifela.org
12	Alex May	Costa Rica	CTNBio	alexmaymontero@gmail.com
13	Beatriz Ortíz	Costa Rica	Ministry of Health	beora11@gmail.com
14	David García	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	dagarcia@itcr.ac.cr
15	Erich Kuss	Costa Rica	USA Embassy	Erich.Kuss@fas.usda.gov
16	Erwin Gamboa	Costa Rica	Bayer	erwin.gamboa@bayer.com
17	Frank Solano	Costa Rica	Universidad Nacional (UNA)	frank.solano.campos@una.cr
18	Giovanni Garro	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	ggarro@itcr.ac.cr
19	Karla Valerín Berrocal	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	kvalerin@itcr.ac.cr
20	Pablo Andrés Vargas	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	pavaro2906@hotmail.com
21	Rafael Montenegro Marín	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	rafamontenegro93@gmail.com
22	Ramón Molina	Costa Rica	Universidad Nacional (UNA)	ramon.molina.bravo@una.cr
23	Samantha García Arias	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	samgarciaarias@gmail.com
24	Sofía Vieto Fonseca	Costa Rica	Inst. Tecnol. Costa Rica (ITCR)	sofia-vieto@gmail.com
25	Hannia León	Costa Rica	ILSI	hannia.leon.leon@gmail.com
26	Jorge Madriz	Costa Rica	Consultant	madrizj@gmail.com
27	Melania Muñoz García	Costa Rica	Ministry of Environment (MINAE)	melania.conagebio@gmail.com
28	Nelly Mora	Costa Rica		nmora@sfe.go.cr
29	Shanon Thomas	Costa Rica	USA Embassy	ThomasS@state.gov
30	Sylvie Braibant	Costa Rica	Senasa	sbraibant@gmail.com
31	María Elena Aguilar Vega	Costa Rica	CATIE	aguilarm@catie.ac.cr
32	Andrés Factos	Ecuador	Ministry of Environment	andres.factos@ambiente.gob.ec
33	Mauricio Hernández de la Parra	Guatemala	Ministry of Agriculture	biotecnologia.maga@yahoo.com
34	Carlos Almendares	Honduras	Senasa	calmendares81@yahoo.com
35	María Andrea Orjuela*	Mexico	CONABIO	morjuela@conabio.gob.mx
36	Mario Henry Rodríguez López#	Mexico	National institute of Public Health	mhenry@insp.mx
37	Judith Ivette Vargas Azcárraga	Panama	MIDA-DNSV	jvargas@mida.gob.pa

38	Cristina Soerensen	Paraguay	Ministry of Agriculture	tinasoerensen@hotmail.com
39	Jorge Alcantara Delgado	Peru	INIA	jalcantara@inia.gob.pe
40	Marina Hernández	Rep. Dominicana	Ministryof Environment	Marina.Hernandez@ambiente.gob.do
41	Ricardo Chavez#	UK-iGEM	Universityof Edinburgh	ricardo.chavez.mtz@gmail.com
42	Kent Redford#	USA	Archipielago Consulting	redfordkh@gmail.com
43	Fan Li Chou#	USA	USDA	Fan-Li.Chou@fas.usda.gov
44	Ana Sifuentes#	USA	iGEM	ana@igem.org
45	Genya Dana#*	USA	US Department of State	DanaGV@state.gov
46	Wayne Schmidt	USA	US Department of State	SchmidtWD1@state.gov
47	Thais Pardo	Costa Rica	Interpreter	thaispardo@gmail.com
48	Hannia Azuola Valle	Costa Rica	Interpreter	ambiente@racsa.co.cr
49	Lloyd Day	IICA	IICA	lloyd.day@iica.int
50	Pedro Rocha	IICA	IICA	Pedro.Rocha@iica.int

speaker
* AHTEG representative

INTER-AMERICAN INSTITUTE FOR COOPERATION ON AGRICULTURE
Headquarters. P.O. Box 55-2200
San José, Vázquez de Coronado, San Isidro 11101 – Costa Rica
Tel.: (506) 2216 0222 / Fax (506) 2216 0233
E-mail address: iicahq@iica.int
Website: www.iica.int