Societal Impact Assessment Handbook

iGEM Greece 2017

I. Abstract

Even if Synthetic biologyoffers great new opportunities for the future, the increasing complexity in engineering biological systems has raised concerns about the potential effects on sustainability. There is a difference between having enough knowledge to create a new bio-system and having enough knowledge to fully understand the complete set of its behavioral characteristics. This report aims to provide an up-to-date risk assessment approach, generated by the combination of the existing risk assessment methods for GMOs, the sustainability impact assessment (SIA) and the OSIRIS protocol, created by iGEM Greece 2017 for the ensuring of stakeholders' participation. The previous approach will be applied to pANDORRA, an engineering and delivering modular RNAi-based logic circuit to treat colorectal cancer, based on the three pillars of sustainable development: health, environmental, socio-economic.

Contents

List of Abbreviations

AHTEG	Ad Hoc Technical Expert Group
CBD	Convetion on Biological Diversity
CRC	Colorectal Cancer
EIA	Environmental Impact Assessment
GMOs	Genetically Modified Organisms
HIA	Health Impact Assessment
iGEM	International Genetically Engineered Machine
LMO	Living Modified Organisms
SCCS	Scientific Committee on Consume Safefty
SCHER	Scientific Committee on Health and Environment Risks
SCHNHHR	Scientific Committee on Emerging and Newly Identified Health Risks
SCs	Scientific Committees
SIA	Sustainability Impact Assessment

II. Introduction

Definition of synthetic biology

Synthetic biologyis a young field that has experienced rapid growth in the past decade. It is important to note that there is no internationally agreed definition of ‘ʻsynthetic biology’’, but one of the most commonly cited definitions is the design and construction of new biological parts, devices and systems, and the re-design of existing, natural biological systems for useful purposes[1]. The current use of the term “synthetic biology” arose in the early 2000s to distinguish the emerging area of science from “conventional” biotechnology (O'Malley, et al., 2008; Schmidt, et al., 2009). In 2004, the Massachusetts Institute of Technology (MIT, USA) hosted “the First International Meeting on Synthetic biology,” SB1.0. In 2007 the number of annual academic publications on synthetic biologyfirst exceeded 100 (Oldham, et al., 2012). Almost, ten years later, at July 2015 a total of 4,605 publications were listed in Web of Science (Raimbault, et al., 2016).

Areas of synthetic biology

The following areas of research are commonly considered “synthetic biology”, even if they are not consistently categorized: DNA-based circuits, synthetic metabolic pathway engineering, genome-level engineering, protocell construction, and xenobiology. These different types of synthetic biologyrepresent different potential impacts, both negative and positive, on sustainability.

DNA (or RNA)-based circuits

The goal of this synthetic biologyresearch area is to control cell behavior through the design of biological circuits with predictable, discrete functions, which can then be combined in modular fashion in various cell. Genetic circuits are seen to function as electronic logic components, like switches and oscillators (Heinemann and Panke, 2006). This is the area of synthetic biologythat most directly aims to “make biology into an engineering discipline” (O'Malley, et al., 2008). Bioengineer Drew Endy’s

[1] This definition was found at www.syntheticbiology.org, hosted on OpenWetWare. The site was started by individuals at MIT and Harvard and can be edited by “all members of the Synthetic Biology community.” Accessed on 10 May 2017.

foundational 2005 paper in Nature applied three ideas from engineering to biology: standardization of basic biological parts and conditions to support their use; the decoupling of design from fabrication; and using hierarchies of abstraction so that one could work at a specific level of complexity without regard to other levels.

One of the earliest and highest profile standardization systems for the design of DNA "parts" was established by scientists and engineers at MIT in 2003. "BioBricks™," sequences of DNA encoding a biological function, are intended to be modular parts that can be mixed and matched by researchers designing their own devices and systems. MIT hosts an open website, the Registry of Standard Biological Parts, where researchers share code for parts designed following BioBrick™ standards. A major platform for demonstrated uses of BioBricks™ has been the annual International Genetically Engineered Machine competition (iGEM). Since 2004, iGEM has provided a platform for undergraduate students to build biological systems using existing BioBricks™ and designing original parts. Thanks to the Open Registry and iGEM, and perhaps also its appealing and accessible analogy with Lego® pieces, this is one of the most publicly prominent areas of synthetic biologyresearch (Collins, 2012; O'Malley, et al., 2008).

The current reality of DNA circuit construction is far from the simplified modularity of engineering; but modularity continues to be promised on the near-horizon. In 2009, International Open Facility Advancing Biotechnology (BIOFAB) was formed with a grant from the US National Science Foundation to addressed the design problems. The design process for genetic networks was still an iterative process, containing "considerable elements of trial and error" due to the lack of understanding of genes. BIOFAB has been working to create a library of professionally developed and characterized parts in the public domain (Baker, 2011; Mutalik, et al., 2013; Mutalik, et al., 2013). In 2013, BIOFAB announced that its researchers had established mathematical models to predict and characterize parts (Mutalik, et al., 2013; Mutalik, et al., 2013). Also, in 2013 iGEM contest website noted significant improvement in the quality of part documentation in the last few years, as well as the continued presence of parts that needed to be discontinued.

Table 1: Ideas for improving biosafety for the International Genetically Engineered Machine (iGEM) competition and synthetic biology(Guan, et al., 2013).

Area of concern	Suggested improvement
Safety of biological parts	Mandatory safety review for each submitted part; completion of a safety questionnaire for every submitted part; a list of potential pathogens or environmentally problematic parts; better documentation and standardization of parts; better labels for parts, devices, or systems for better tracking; potentially pathogenic or hazardous BioBricks cloned in a special molecular backbone
Safety education	Increased public awareness of risks and safety issues; a video lecture on workplace safety made for all iGEM teams; an iGEM presentation by the participating teams, open to the school and public; an online, visual introductory course on basic biosafety issues; a customized safety quiz for each team; safety information incorporated for each protocol
Laboratory practices	Clear records of reagents, bacteria, and equipment; use of synthetic DNA containing only BioBricks below biosafety level 2
Principles for engineering	Standardization of biosafety engineering, standard biosafety rules for basic synthetic biologyexperiments, use of Event Tree Analysis and Fault Tree Analysis
Risk of using the material	Use of purified DNA instead of bacterial cultures, nonvirulent strain of bacteria used as the chassis, no manipulation of any infectious or virulent bacteria
Environmental safety	Suicide genes incorporated into the final constructs, an inactivation mechanism in iGEM plasmid backbone, a suicide system in all engineered bacteria
Self-risk assessments	Risk assessments of the protocols employed to complete the project, a (more) detailed safety report for a minimal medal requirement
Competition organization	A biosafety committee for the iGEM competition, a prize for safety
Other	Harmful genes associated with safety issues, encouragement of collaboration and experience sharing among the teams

Synthetic metabolic pathway engineering

This area of synthetic biologyresearch aims to redesign or rebuild metabolic pathways, to synthesize a specific molecule from a “cell factory” (Nielsen and Keasling, 2011; Schmidt, et al., 2009). In conventional metabolic engineering, an organism that naturally produces the desired chemical is improved through strain breeding or genetic modification to increase production. Synthetic biologyenables scientists to start with a “platform cell factory” that would not naturally produce any of the chemical. A synthetic pathway (rationally designed or based on a natural sequence but computer

‘optimized’) is added to the cell, and then conventional metabolic engineering tools may be used to increase the desired output (Nielsen and Keasling, 2011).

Many of the first-wave synthetic biologycommercial applications use metabolic pathway engineering to replicate naturally occurring molecules (Wellhausen and Mukunda, 2009). Some examples are the expression of proteins for the production of spider silk in plants such as Arabidopsis (Yang, et al., 2005) and in the milk of transgenic animals such as mice with a synthetic gene encoding for dragline silk protein (Xu, et al., 2007).

Genome-level engineering

This area of synthetic biologyresearch focuses on the genome as the “causal engine” of the cell (O'Malley et al. 2007). Rather than designing short DNA sequences or engineering for specific metabolic pathways, researchers work at the whole-genome level. There are two strategies to genome-level engineering: top down and bottom up. Top-down genome-engineering starts with a whole genome, from which researchers gradually remove “non-essential” genes to pare down to the smallest possible genome size at which the cell can function as desired, while bottom-up genome-engineering aims to build functional genomes from pieces of synthesized DNA (Garfinkel, et al., 2007; König, et al., 2013). Thus far, researchers have reproduced the viral genomes of polio (Cello, et al., 2002) and the 1918 Spanish influenza (Tumpey, et al., 2005). In 2010, the J. Craig Venter Institute published the successful synthesis and assembly of a 1.08 million base pair bacterial genome of Mycoplasma mycoides, and its transplantation into a M. capricolum cell stripped of its genome (Gibson, et al., 2010).

Protocell construction

Protocells have been described as “models of artificial cells that have some properties of living systems but are not yet fully alive” (Armstrong, et al., 2012). Protocell research aims to create the simplest possible components to sustain reproduction, self-maintenance and evolution (Solé, et al., 2007).

Research in this area is vibrant, but thus far restricted to basic research (Budin and Szostak, 2010; Schmidt, 2010). Some of the future protocell applications are the development of smart “paints” that fix carbon dioxide into inorganic carbonate,

chemical agents that convert environmental waste toxins into harmless chemicals, and alternative methods of producing biofuels (Armstrong, et al., 2012).

Xenobiology

Xenobiology (also known as chemical synthetic biology) is the study of unusual life forms, based on biochemistry not found in nature (Schmidt, 2010). Xenobiology aims to alter the "biochemical building blocks of life," such as by modifying genetic information to produce XNA (xeno-nucleic acids) or by producing novel proteins using different approaches (Joyce, 2012).

Xenobiology is often cited as a potential "built-in" biosafety mechanism to prevent genetic drift to wild organisms (Esvelt and Wang, 2013; Schmidt, 2010; Schmidt, 2010; Skerker, et al., 2009). Physical genetic material transfer might still occur, but in theory natural polymerases would be unable to accurately read the XNA, and thus not lead to protein production (Schmidt, 2010). Research in xenobiology is also being used to explore the basic physical properties that led DNA and RNA to be the genetic material of life. It is hoped that xenobiology will be usefully applied to biotechnology and molecular medicine, but significant research challenges remain before we see commercial application in this area (Chaput, et al., 2012; Joyce, 2012).

Supporting technologies

Synthetic biologyrelies on a suite of high-throughput supporting technologies, such as Next Generation Sequencing methods and metagenomic tools, that are continuously getting cheaper, less time consuming, and less computational expensive. Time and effort, on the part of researchers using constructed DNA for experiments, has also been saved thanks to the introduction of automated DNA synthesis machines. (Schmidt and de Lorenzo, 2012). Techniques for DNA assembly have also advanced, with labs having developed various in vivo assembly systems by which genome-length DNA strands can be assembled at once within a cell (Baker, 2011). Other novel approaches to genetic manipulation that can be engineered to bind to specific DNA sequences are the targetable nucleases (zinc finger nucleases, the transcription activator-like effector nucleases (TALEN), and the clustered regularly interspaced short palindromic repeats (CRISPR) that are RNA-guided) (Carroll, 2013; Lienert, et al., 2014).

In addition, computational modeling and the connected fields of bio-informatics and information sciences have catalyzed synthetic biologyresearch by making possible simulation and in silico testing of biological systems (Esvelt and Wang, 2013).

pANDORRA

pANDORRA is a toolbox of genetically engineered E. coli for precise targeting and programmable elimination of cancer cells according to their miRNA profile. Briefly, iGEM Greece 2017 aimed to engineer an E. coli strain capable of adhering exclusively to colorectal cancer (CRC) cells and facilitating the transference of a synthetic RNAi-based logic circuit, which can differentiate between healthy and tumor cells due to the different miRNA profile the two cell types exhibit and induce apoptosis only in the latter. Therefore, the project could be divided into two major devices, one in charge of accomplishing selective adhesion to CRC cells and successful internalization of bacteria carrying the second device, the aforementioned RNAi-based logic circuit which will code for the hbax protein under the control of the inhibitory and disinhibitory action of the miRNAs that have been identified as necessary and sufficient to discriminate the two populations.

iGEM Greece 2017 goal is to transfer the RNAi based logic circuit capable of inducing apoptosis in colorectal cancer cells, through the use of genetically engineered bacteria (bactofection). Therefore, the method will be tested in cancer cells. In particular, the cell lines will be used are Caco-2 and RKO. Moreover, in order to measure the expression of Type 1 pili will be performed agglutination assays using Saccharomyces cerevisiae.

III. Risk assessment

Relevance Analysis

The last decades have brought a lot of insights into safety issues of Genetically Modified Organisms (GMOs) and this knowledge forms the basis for current risk assessment and biosafety considerations. Efforts to establish legally-binding rules regarding genetically modified organisms (GMOs) started in 1992, and eleven years later Cartagena Protocol on Biosafety entered into force on 11 September 2003. Cartagena Protocol on Biosafety is legally-binding rules on genetically modified organisms (GMOs) in the international legal system and in the legal system of countries

that have ratified, approved, accepted, or acceded to it. As of October 2017, there were 171 Parties to the protocol (Appendix A), when last year were 170.

Discussions on synthetic biologyhave been on-going under both the Convetion on Biological Diversity CBD and the Cartagena Protocol. In particular, among the most recent statements on the state of the art of risk assessment of GMOs was the meeting paper for the Fourth Meeting of the Conference of the Parties serving as Meeting of the Parties to the Cartagena Protocol on Biosafety, that took place in Bonn, in May 2008 (CBD, 2008). In Chapter III.17 it says, “Further it was agreed that all risk assessments of living modified organisms should be conducted on a case-by-case basis as the impacts depend upon the trait inserted, the recipient organism, and the environment into which it is released.” This description reveals that developments in Synthetic biologycould lead to significant gaps, despite the risk assessment framework presently in place for GMOs. But, in order to determine whether or not the organisms, components and products of synthetic biologyare addressed by the Cartagena Protocol on Biosafety, it is instructive to explore further some of the definitions under both the Cartagena Protocol and its parent treaty, the CBD.

Biotechnology includes any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use (Article 2 of the Cartagena Protocol). According to Cartagena Protocol (Article 3) the three following definitions are interrelated and should be read together: “living modified organism”, “living organism”, and “modern biotechnology” (Article 3). The term Living modified organism (LMO) can be used to characterized any biological entity capable of transferring or replicating genetic material, including sterile organisms, viruses and viroids. Also, LMO can be used for any organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology. Synthetic biologyproducts can thus be considered as “living modified organisms resulting from biotechnology” as defined by the CBD, because even if plasmids and naked DNA are not included, the novel combination of genetic material is introduced through the use of naked DNA or plasmids through modern biotechnology.

In 2014, the CBD established an Ad Hoc Technical Expert Group (AHTEG) on Synthetic biology. The AHTEG met in September 2015 agreed to define synthetic

biologyas: “Synthetic biologyis 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”, in order to assist Parties in their implementation of the provisions of the CBD. The AHTEG also agreed that living organisms developed through current and near future applications of synthetic biologyare similar to living modified organisms (LMOs) as defined in the Cartagena Protocol (CBD, 2000).

On 4 May 2015, three independent non-food Scientific Committees, the Scientific Committee on Consume Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), provide the European Commission with the definition and identification of the relationship between Synthetic biologyand genetic engineering, and the possibility of distinguishing the two. They also focused on the implications of likely developments in Synthetic biologyon human and animal health and the environment and on determining whether existing health and environmental risk assessment practices of the European Union for Genetically Modified Organisms (GMOs) are also adequate for Synthetic biology.

According to the scientific committee, the existing risk assessment methodologies, for GMOs and chemicals, are applicable; however, several synthetic biology developments such as combining genetic parts, will require improving existing methodology. Towards this direction the scientific committee suggested several improvements to ensure continued safety protection proportionate to risk, while enabling scientific and technological advances in the field of Synthetic biology. These improvements included: 1) the characterization of the function of biological parts and the development of computational tools to predict emergent properties of synthetic biology organisms, 2) the standardization of the methods for submitting genetic modification data and genetic parts information to risk assessors, 3) the encouragement of the use of GMOs with a proven safety record as acceptable comparators for risk assessment, 4) the assurance that risk assessment methods advance in parallel with Synthetic biology advances, and 5) the sharing of relevant information about specific parts, devices and systems with risk assessors.

Especially, when it comes to genetic parts or circuit the complexity of engineered genetic systems advances were driven by many technological factors ranging from the 1) availability of genome and gene data in databases, 2) improved and more-standardised DNA modification technologies, 3)advanced tools and resources for measuring and selecting modified strains, 4)computational and analytical tools for designing complex genetic systems, and 5) greater public and private investments in cutting-edge genetic engineering technologies. Engineered genetic systems may be composed of many tens of different parts recombinant, mutated or synthesised DNA parts[2]. To engineer these complicated genetic systems, there are electronic and physical repositories of genetic elements often called "genetic parts libraries" which contain genes and DNA fragments with characterised properties and functions maintained in a form that facilitates faster search, retrieval and assembly into novel engineered genetic systems. Some of these libraries have thousands of parts, which are publicly accessible[3].

The main advance in synthetic biology is the degree to which the genetic material is designed and engineered for interoperability and speed of assembly, which allows more complex systems to be constructed. Synthetic biology libraries characterise the functional properties of each element in the library in great detail and precision and deploy advanced information technologies to ensure that the information is available to designers and users. This information is intended to accelerate biological design, similar to how computer-aided design accelerated other engineering fields. In practice, detailed characterisation of genetic elements is difficult and labour-intensive and many of the parts in current synthetic biology parts libraries remain poorly characterised, except at the most basic level of biochemical function. Thus, by now genetic engineering remains more dependent on empirical trialand-error than other contemporary fields of engineering (Gardner and Hawkins, 2013). In conclusion, precise and accurate information on the biological function of parts in genetic libraries will improve the effectiveness of risk assessment as it pertains to appraisal of potential hazards to humans, animals or the environment.

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[2] Commissao Tecnica Nacional de Biosseguranca, Technical Report No. 3287/2012 – Commercial release of yeast (Saccharomyces cerevisiae) genetically modified to produce farnesene by strain Y5056 - Case No.01200.003977 / 2011-56. 2012. http://www.ctnbio.gov.br/index.php/content/view/17454.html

[3] Registry of Standard Biological Parts. http://parts.igem.org/Main_Page. Accessed 30 November 2017.

The SCs conclude that the current risk assessment methods outlined in Directives 2001/EC/18 and 2009/EC/41 are appropriate and adequate for the management of the risks of synthetic biology activities and products associated with genetic circuits or parts libraries. However, incremental advances in the knowledge base and tools for risk assessment are high importance.

Delineation

Sustainability impact assessment requires the involvement of stakeholders through various means in different stages. Societies like iGEM (http://igem.org/Main_Page) promotes the idea of solving real world problems by building genetically engineered biological systems, but it may be a concern that in any applications, even in applications that uses DNA for computer transfer and storage, biological parts must be considered as active biological parts that potentially can be a source for health and environmental impact.

Health and Environment Impact Assessment

Our lack of knowledge on how genetic design knobs modulate the metabolic responses raises questions about the impacts of a new organism genetically modified on sustainability. The methodology described below is appropriate for assessing potential risks associated with human health (health impact assessment (HIA)) and environment (environmental impact assessments (EIA)). Aim to provide a holistic approach for the investigation of the interplay between genes and metabolic pathways, which can give insights into the interactions between a new organism and its environment.

As mentioned before, the CBD, the Cartagena Protocol and the international treaties addressing threats of significant reduction or loss of biological diversity posed by organisms, components and products resulting from modern biotechnologies. It has been proposed to all the parties to take a precautionary approach, in accordance with the preamble and with Article 14 of the Convention, although not all regulatory frameworks include the precautionary approach, the principle has been used by regulators, scientists, civil society organizations and others when assessing new and emerging technologies that lack substantial knowledge on their potential risks. The first step of pre-market risk assessment of synthetic biology organisms and products take into consideration the precautionary approach is the molecular characterization.

Molecular characterization

Molecular characterization refers to the description and identification of all genetic modifications and changes performed on the host organism to produce a synthetic biology organism and/or product. Its relevance to risk assessment is related to the introduction of potential risk pathways created by synthetic biology. A description of the genetic background of the bacterial strains used in our project, emphasize is given to the final impact of each mutation to metabolic pathways. Excluded are information already submitted in our publicly available Check-In and Safety Forms.

Δ(araD-araB)567

This deletion extends from ~25 bp upstream of the *araB* start codon to ~8 bp into the beginning of the *araD* gene. In *E. coli*, arabinose is converted to xylulose 5-phosphate, an intermediate of the pentose phosphate pathway. (Schleif, 2010) By deleting *araB*, *araA* and *araD* (collectively known as *araBAD*), arabinose catabolism is disrupted. This allows the insertion of plasmids carrying arabinose-inducible genes.

Δ(rhaD-rhaB)568

L-rhamnose is taken up by the E. coli and converted to dihydroxyacetone phosphate which is metabolized in glycolysis, and L-lactaldehyde. The latter can be oxidized into lactate under aerobic conditions and be reduced into L-1,2-propanediol under unaerobic conditions (Wegerer, et al., 2008). By deletion of the key genes for the metabolism of rhamnose, the cells can then receive plasmids with genes induced by rhamnose.

ΔlacZ4787(::rrnB-3)

4 tandem copies of the *rrnB* transcriptional terminator inserted by gene replacement into the region extending from near the SacII site near the N-terminus of *lacZ* through the promoter. *lacZ* encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose. X-gal is an analog of lactose, and therefore may be hydrolyzed by the β-galactosidase enzyme which cleaves the β-glycosidic bond in D-lactose. X-gal, when cleaved by β-galactosidase, yields galactose and 5-bromo-4-chloro-3-hydroxyindole. The latter then spontaneously dimerizes and is oxidized into 5,5'-dibromo-4,4'-dichloro-indigo, an intensely blue product which is insoluble. X-gal itself is colorless, so the presence of blue-colored product may therefore be used as a test for the presence of active β-galactosidase. This easy

identification of an active enzyme allows the gene for β-galactosidase (the *lacZ* gene) to be used as a reporter gene for inserted plasmids since the genomic *LacZ* is deleted.

λ⁻

Lambda lysogen deletion. This makes the bacteria susceptible to infection and therefore transformation with λ phages. Otherwise, lysogeny with λ would give immunity to other λ phages. (Lederberg and Lederberg, 1953)

rph-1

It is a 1 bp deletion in the *rph* gene that results in frameshift over last 15 codons and has polar effect on *pyrE*. The bacteria starve for pyrimidine in minimal medium because of a suboptimal content of orotate phosphoribosyltransferase, which is encoded by the *pyrE* gene. (Jensen, 1993) This could function as another reporter gene when inserting a plasmid and growing bacteria in minimal medium for selection.

hsdR514

The hsd locus of E. coli K includes restriction and modification genes, hsdR, hsdM and hsdS which code for R, M and S subunits. Restriction is carried out by the hsdR gene and modification by means of methylation by the hsdM gene. The hsdR gene is responsible for recognition of the host specificity. (Yuan and Hamilton, 1982)

ΔfimH788::kan

Deletion of the *fimH* gene from the fim operon, by insertion of a kanamycin resistance gene. The fimH gene is the part of the type-1 fimbriae which meditates manose-binding properties. The mutation was performed by the Red-mediated recombination (Datsenko and Wanner, 2000).

More details about the parts that have been developed during iGEM Greece 2017 project could be found in Appendix B, according to the instructions of iGEM. Species name (including strain) include a strain name or number (such as "K-12" for E. coli K-12) if there is one, and in case of a part, the name and strain of the organism that the part originally came from. Each species is also described by the Risk Group and the Risk Group Source, which is the source from which the Risk Group information has

been obtained. Other information includes the disease risk to humans, part number or name, natural function of part, how it was acquired and how it will be used [4].

Moreover, the use of the genetically engineered bacteria inside the human body confers three safety concerns that need to be examined in in vivo tests, which are beyond iGEM Greece 2017 goal for the competition. First and foremost, the aforementioned bacteria will have sequences that code for invasin and listeriolysin O. As a result, they will be able to invade any beta-1-integrin expressing cell and avoid intracellular degradation in the phagosome through the creation of pores on its membrane that will allow the bacteria to escape it and proliferate in the cytoplasm. To address this issue, iGEM Greece 2017 have planned to introduce an upstream safety measure, in addition to the natural propensity of bacteria to colonize tumor tissue rather than healthy. iGEM Greece 2017 intends to use the work of previous iGEM teams that have managed to engineer a genetically modified type I pilus capable of selective binding to colorectal cancer cells and cell lines and place the expression of both invasin and listeriolysin O under quorum sensing control. Thus, the bacteria that are bound to tumor tissue are much more likely to proliferate and express the genetic machinery associated with the transference of our genetic circuit than the unbound bacteria surrounding healthy cells. However, the possibility of invading healthy cells is not negligible. In addition, there are concerns about the horizontal transfer of invasin-listeriolysin O plasmid to other bacteria colonizing the large intestine that lack the safety measures iGEM Greece 2017 intends to introduce. Furthermore, the environmental release of genetically engineered bacteria also needs to be taken into account as after the treatment we expect the patients to release amounts of live bacteria in their digestive tract's excretions. Although, should this method proceed to clinical trials or as an actual therapeutic approach this issue could be tackled by keeping the patients in a controlled environment shortly after the treatment and performing proper biosafety procedures on the potentially contaminated waste. Finally, we aim to further explore the risks and come up with solutions by coming into contact with experts from all over the world who will be able to alert us to any potential risks that might have overlooked and aid us in optimizing our design to address the various risks that might emerge.

[4] See http://2017.igem.org/Safety/Final_Safety_Form. Accessed on 26 October 2017.

Identification of hazard

The most common approach to the identification of hazard in the precautionary approach is by a comparative approach through the use of an appropriate comparator, according to the European Commission Decision 2002/623/EC (EC, 2002) in support to Annex II of Directive 2001/18/EC (EC, 2001), which states that "identified characteristics of the LMO and its use which have the potential to cause adverse effects should be compared to those presented by the non-modified organisms from which it is derived and its use under corresponding situations". The purpose of this comparison is to assist in identifying the particular potential adverse effects arising from the genetic modification. In addition, the same EC Decision indicates that "Information from releases of similar organisms and organisms with similar traits and their interaction with similar environments can assist the risk assessment". Regarding synthetic biology organisms, the identification of changes between the synthetic biology organism and the suitable comparator should not be interpreted as hazard per se but instead, they should help to identify potential differences, which will then be subject to further toxicological investigation.

One of the most important differences between genetic engineering and synthetic biology is that instead of single parts, whole systems can be transferred, potentially using hundreds or thousands of traits (genes/parts) even from different donor organisms, which increased the level of complexity of the final product. Unfortunately, the searching for an appropriate comparator, which was based on EFSA guidelines on how to choose the best comparator (EFSA, 2011a), as has been proposed to the literature (Odd-Gunnar Wikmark, 2016), did not reveal any appropriate comparator for pANDORRA, due to the level of complexity, which is comparable to the one found in natural cell circuity. Moreover, these finding strengthens the conclusions of the Ad Hoc Technical Expert Group (AHTEG) On Synthetic biology under the CBD, regarding the potential lack of suitable comparators in synthetic biology (CBD, 2015).

In order to fill the gap in the hazard identification step of risk assessment iGEM Team Greece 2017 intend to perform untargeted multi-omics analysis to pre-determined assessment endpoints on the genome, proteome and metabolome levels.

Omics aims at the collective characterization and quantification of pools of biological molecules that translate into the structure, dynamics and function, of an organism. Accordingly ‘genomics’ deals with the entirety of an organism's hereditary information coded in its DNA (also called genome); ‘transcriptomics’ deals with the entirety of RNA transcribed from the DNA (transcriptome), ‘proteomics’ deals with the entirety of proteins translated from the mRNA (proteome) and ‘epigenomics’ addresses factors and mechanisms affecting the accessibility of genomic information by modifications of its structure, e.g. via DNA-methylation or chemical modifications of the histones serving as DNA-packing proteins (epigenome). (Katia PauwelsKatia Pauwels, 2013)

According to Heinemann et al. (Heinemann, et al., 2011) a broader use of molecular profiling in a risk assessment is required to supplement the comparative approach to risk assessment of synthetic biology products. Also, they highlighted that “omics” technologies or molecular profiling is an important way to increase confidence in risk assessments for new synthetic biology organisms and products. If the profiles are properly designed to address relevant risks and are applied at the correct stage of the assessment (Heinemann et al., 2011).

Another proposal for the investigation of the potential impacts of pANDORRA to the environment is the investigation of its performance in case of different metabolic cross-feeding using a fluxomics tool. In other words, fluxomics could reveal information regarding the communication of microorganisms, which allow the evaluation of the potential impacts of pANDORRA to biodiversity. The new computational method called community flux balance analysis (cFBA) seems to be promising for the metabolic behavior of microbial communities because integrates the comprehensive metabolic capacities of individual microorganisms in terms of (genome-scale) stoichiometric models of metabolism, compared to previous methods, and the metabolic interactions between them in the community processes. (Khandelwal, et al., 2013).

Socio-economic Impact Assessment

There is no clearly agreed definition or scope to the term bioeconomy; definitions either focus on the tool of biotechnology or on the use of biomass as a fuel and raw material.

The 2009 OECD document The Bioeconomy to 2030: Designing a Policy Agenda defines a bioeconomy as "a world where biotechnology contributes to a significant share of economic output." (OECD, 2009). The United States' White House's National Bioeconomy Blueprint similarly defines bioeconomy as "economic activity that is fueled by research and innovation in the biological sciences" (House., 2012). The European Commission's definition of bioeconomy is broader: "an economy using biological resources from the land and sea, as well as waste, as inputs to food and feed, industrial and energy production. It also covers the use of bio-based processes for sustainable industries" (Commission, 2012a). The ETC Group sees bioeconomy as relying on three inter-related and reinforcing concepts: the biomass economy, moving from fossil and mineral resources to biological raw materials; the biotech economy, in which genetic sequences are the building blocks for designed biological production systems; and the bioservices economy, in which new markets in ecosystem services enable trading of ecological credits (ETC, 2010).

States, industry, and civil society identify synthetic biology as playing a potentially significant role in the bioeconomy. The Government of the United States of America names synthetic biology as an "emerging technology" that "holds vast potential for the bioeconomy, as engineered organisms could dramatically transform modern practices in high-impact fields such as agriculture, manufacturing, energy generation, and medicine" (House., 2012). Industry analysts see a "bright future" in the bio-based economy for developers of biochemicals, biomaterials, bioactive ingredients, and processing aids (Huttner, 2013). The ETC Group describes synthetic biology as a "game-changer," expanding the "commercial possibilities for biomass" (ETC, 2010).

The global market for synthetic biology products is growing rapidly. In fact, according to Market forecasters BCC Research estimate, the global synthetic-biology market reached nearly $3.9 billion in 2016 and should reach $11.4 billion by 2021, growing at a compound annual growth rate (CAGR) of 24.0% through 2021.[5] While the global market for nanoparticles in biotechnology and pharmaceuticals reached nearly $25.0

[5] See *Synthetic Biology: Global Markets,* at https://www.bccresearch.com/index/advancedsearch?search_keyword=Synthetic+Biology Accessed on 21 October 2017.

billion in 2013 and is expected to reach $29.6 billion in 2014 and $79.8 billion in 2019, with a compound annual growth rate (CAGR) of 22.0% for the period of 2014 to 2019.[6]

The US Obama Administration is prioritizing the bioeconomy "because of its tremendous potential for growth" as well as its potential to "allow Americans to live longer, healthier lives, reduce our dependence on oil, address key environmental challenges, transform manufacturing processes, and increase the productivity and scope of the agricultural sector while growing new jobs and industries" (House., 2012).

Europe has set a bioeconomy strategy and an action plan which focuses on three key aspects: developing new technologies and processes for the bioeconomy, developing markets and competitiveness in bioeconomy sectors, pushing policymakers and stakeholders to work more closely together. According to President Juncker, bioeconomy is central to three of 10 key priorities for the European Commission, with the first one and maybe the most important to be a new boost for jobs, growth and investment. The innovative bioeconomy is an important source of new jobs, especially at local and regional level, and in rural and coastal areas, and there are big opportunities for the growth of new markets, for example in bio-fuels, food and bio-based products. Moreover, bioeconomy promotes research across the EU and outside the EU borders and cooperation at a global scale to tackle global challenges [7].

Commission works on ensuring a coherent approach to the bioeconomy through different programmes and instruments including the Common Agricultural Policy, the Common Fisheries Policy, Horizon 2020, European environmental initiatives, the Blue Growth initiative for the marine sector and the European Innovation Partnership on Sustainable Agriculture. In addition, on 20 July, the European Commission launched the new Bioeconomy Knowledge Centre to better support EU and national policy makers and stakeholders with science-based evidence in this field. The Knowledge Centre is being created by the Commission's in-house science service, the Joint

[6] See *Synthetic Biology: Global Markets,* at https://www.bccresearch.com/market-research/biotechnology/nanoparticles-biotechnology-drug-development-drug-delivery-report-bio113b.html Accessed on 21 October 2017.
[7] See*:* http://ec.europa.eu/research/bioeconomy/index.cfm?pg=policy. Accessed on 21 October 2017.

Research Centre, in cooperation with Directorate-General for Research and Innovation.[8]

Ethics aspects and concerns

The rapidly growth of synthetic biology has been followed with discussions of ethical aspects and the need for broader assessment. This is acknowledged by both scientists within the field and by policymakers, and exemplified by the inclusion of ethical and social awareness through approaches as responsible research and innovation (RRI) driven by research funding agencies in Europe and by initiatives as the SynBERC in USA.

The field of ethics (or moral philosophy) involves systematizing, defending, and recommending concepts of right and wrong behavior. Philosophers today usually divide ethical theories into three general subject areas: metaethics, normative ethics, and applied ethics. Metaethics investigates where our ethical principles come from, and what they mean. Are they merely social inventions? Do they involve more than expressions of our individual emotions? Metaethical answers to these questions focus on the issues of universal truths, the will of God, the role of reason in ethical judgments, and the meaning of ethical terms themselves. Normative ethics takes on a more practical task, which is to arrive at moral standards that regulate right and wrong conduct. This may involve articulating the good habits that we should acquire, the duties that we should follow, or the consequences of our behavior on others. Finally, applied ethics involves examining specific controversial issues, such as abortion, infanticide, animal rights, environmental concerns, homosexuality, capital punishment, or nuclear war [9].

Starting at early 1999, ethicists have actively engaged with the new tools and techniques of modern synthetic biology(Cho, et al., 1999). Common considerations have included the ethical debate on whether synthetic biologists are “playing God” and aspects regarding the safety for human health and the environment (Boldt, 2008; Douglas, 2010; Engineering, 2009; Kaebnick, 2009).

[8] See: http://ec.europa.eu/research/bioeconomy/index.cfm?pg=policy&lib=observatory. Accessed on 21 October 2017.
[9] See http://www.iep.utm.edu/ethics/. Accessed on 10 October 2017.

Ethicists disagree whether synthetic biology introduces “new” ethical issues based on the ability to create life rather than modify existing organisms. Ethicists Joachim Boldt and Oliver Müller see synthetic biology as having crossed a threshold from the mere manipulation of life to its “creation” from scratch, thus potentially changing our approach to nature (Boldt, 2008). They are concerned that the ability to design significant portions of organisms may “lead to an overestimation of how well we understand nature’s processes and our own needs and interests”. Philosopher Beth Preston (2013) insist that synthetic biology presents no new ethical issues; Anyway, scientists have thus far replicated existing genomes and modified existing cells; this is different from creating a novel organism from scratch (Garfinkel, 2007; Kaebnick, 2009). A number of commentators’ counter that such arguments overestimate the current abilities of synthetic biology.

Some areas of synthetic biology research are based on a reductionist view of the world and that generates disagreements on the ethical implications of this. Reductionism is an approach to understanding the nature of complex things by reducing them to the interactions of their parts, or to simpler or more fundamental things. It was first introduced by Descartes in Part V of his "Discourses" of 1637, where he argued the world was like a machine, its pieces like clockwork mechanisms, and that the machine could be understood by taking its pieces apart, studying them, and then putting them back together to see the larger picture (Calvert, 2012). With the discovery of DNA, the biological sciences took a “reductionist” turn, attempting to explain life by breaking it down to chemical and physical processes (Cho, et al., 1999). Especially, synthetic biology tries to bypass biological complexity, using reductionist logic to design organisms that are less complex, which lead to the question whether emergence and complexity can be avoided by biological design, but there are also ethical implications of a commitment to reductionism (Calvert, 2012; EGE, 2009). In our opinion, synthetic biology products and applications should be seen through the integration of reductionism and holism, which are in fact interdependent and complementary. Reductionism is most useful if observations made in a simplified system allow accurate predictions, or at least the generation of hypotheses, to be made when returning to the complex natural world. However, interpreting observations from holistic studies may require mechanistic insights gained from earlier reductionistic work or may generate hypotheses that are amenable to testing through reductionistic experimental approaches

(Fang and Casadevall, 2011). Computer technology has permitted the development of sophisticated mathematical, engineering, and computational tools that have allowed the investigation of new questions generated from reductionistic experimental approaches. For example, computational tools and platforms of pathway analysis (e.g GeneSpring GX, iPathways, DAVID) could be used to investigate the following interactions DNA → mRNA → protein → protein interactions → pathways → networks → cells → tissues → organisms → populations → ecologies, which are actually represent the extended central dogma of molecular biology.

Playing God can also be considered as a methaphore used in the debate concerning synthetic biology and can both have secular and a religious interpretation. Secular interpretation includes the failure to recognize human limitations in evolutionary processes by overestimating ability to control complexity, and the fact that tampering with nature can have unexpected consequences, while religious interpretation focuses on the creation of life from non-living material, which goes against the will of God or distort God´s creation. Especially the NGO ETC has used the term Playing God and Pat Mooney from ETC has claimed that "for the first time God has competition" (Bioethics, 2015). On the other hand, the Church of Scotland (2010) has stated that this religious interpretation and argument against the use of synthetic biology is not valid since God creates *ex nihilio*, out of nothing. Even if the conception of what is natural and what is in competition with God may change over time, the "Playing God" term is expression used as placeholders for that the use of synthetic biology may involve risks to nature and that the scientist do not have control or do not know what they are doing. Moreover, claims that synthetic biology may affecting the moral status of living things and the dignity of life. It is therefore important that scientists, research funding agencies and policy makers understand and acknowledged these values in order to take into account the views of the public in research, especially when they are developing policies for science, technology and medicine. That is one of the main reasons that iGEM Greece 2017 gave a lot of effort to developed a protocol, called OSIRIS which will be ensuring the involvement of stakeholders during the developing of pANDORRA.

Moreover, Anderson *et al.* say: "The ability to create synthetic organisms, combined with our inability to control them with solid guarantees, raises the need to consider the ethical implications". Considerations of biosafety and biosecurity are sometimes discussed as ethical questions of weighing and balancing potential harms and benefits

(Boldt, 2008; Cho, et al., 1999; Douglas, 2010; EGE, 2009). Some risks might be deemed not morally acceptable because of the severity of harm and/or the probability of harm occurring (Schmidt, et al., 2009). This raises questions about what level of predictability should be required, and how to weigh possible negative impacts against positive impacts (Anderson, et al., 2012). The distribution of potential harms and benefits related to synthetic biologyproducts and technologies is also an ethical matter (Bioethics, 2012; Parens, 2009; Schmidt, et al., 2009). What would be an equitable distribution of synthetic-biology related harms and benefits, and how can that distribution be achieved? Ethical issues around harms and benefits also incorporate discussions on global justice, and the potential impacts of synthetic biologyon the "technology divide" (EGE, 2009). More examples of potential positive and negative impacts of synthetic biologywith regard to social, economic and cultural considerations are given in Appendix C.

Biosafety

As synthetic biology emerges from the research laboratory into the bioeconomy, a greater number of occupational safety and health professionals will be involved in ensuring worker safety. The use of synthetic biotechnology in advanced manufacturing requires that occupational safety and health practitioners not currently involved in research biosafety must be educated about risks to workers associated with synthetic biology. More practitioners will have to take a role in proactively assessing the potential risks to workers as synthetic biology products become increasingly used in advanced biological manufacture and in routine clinical care delivery settings. Also, as the synthetic biology workforce expands, worker training tailored to safe approaches to commercial synthetic biology will be needed.

Many scientific committees do not mention risks from occupational exposure to synthetic biology products, they consider them as equals with the ones from occupational exposure to biotechnology products. That needs to be change, because biosafety guidance that is specific to scientific advances from synthetic biology would be helpful as both the World Health Organization *Laboratory Biosafety Manual* and *Biosafety in Microbiological and Biomedical Laboratories*, due to two main differences between biotechnology and synthetic biology. Synthetic biology is using newly designed organisms and viral vectors (i.e., tools used to deliver genetic material into a

cell), not unmodified existing pathogens. Most importantly, synthetic biology often is not being performed solely by biologists, but by engineers, physical scientists, and DIYers not familiar with fundamental biosafety measures such as biocontainment (Howard, et al., 2017).

Take into consideration the importance of workers safety iGEM Greece 2017 thought necessary to attend seminars, before we started the experiments, in order to acquire biosafety training and get accustomed with emergency crisis management in the laboratory. Such seminars were organised in Greece by the Hellenic Institute for Occupational Health and Safety, Aristotle University of Thessaloniki, the Pasteur Institute in Greece and the Centre for Research and Technology Hellas. Team members have attended the seminar titled "Health and safety in research laboratories" (Aristotle University of Thessaloniki, March 5, 2017) organized by the aforementioned authorities.

There is a need to review and enhance current protection measures in the field of synthetic biology, whether in government, academic or DIY laboratories where new advances are being researched, in health care settings where clinical treatments using pseudotyped viral vector gene delivery systems are increasingly being applied to cancer and immune disorders therapy, or in the expanding industrial bioeconomy where an increasing number of workers will be employed.

NIH updated its 2009 Guidelines for Research Involving Recombinant DNA Molecules in 2013 to address the creation and use of organisms and viruses containing synthetic nucleic acid molecules. The 2013 NIH Guidelines make clear that in the case of an organism containing genetic sequences from multiple sources, the potential for causing human disease based on the source(s) of the DNA sequences requires an assessment of the virulence and transmissibility functions encoded by these sequences. Combining sequences in a new biological organism may result in an organism whose risk profile could be higher than that of the contributing organisms or sequences. Based on all these considerations, the appropriate biosafety level (BSL) containment conditions (Levels 1 through 4) can be selected (Bayha, et al., 2015).

Part of experiments, during the period of the project of iGEM Greece 2017, took place at Department of Biology of Aristotle University of Thessaloniki. The aforementioned institute has appointed committee responsible for the quality, biosafety and ethics of

the research carried out under its auspices. More specific, the Research Committee (https://www.auth.gr/en/rc) of Aristotle University of Thessaloniki (AUTH) is responsible for supervising research programs when it comes to biosafety and other matters.

In addition, Biohellenika SA has kindly provided iGEM Greece 2017 with access to their R&D laboratories, as the needs of our project call for more sophisticated equipment than what is readily available at Aristotle University of Thessaloniki. These particular R&D laboratories meet the criteria of ISO 15189:2012 for medical laboratories and thus their quality and competence is confirmed and officially recognized.

The laws that govern biosafety in research laboratories in Greece are described in the three official publications from the Greek government that can be found at the footnote. Unfortunately, these documents have not been translated, however they are written in accordance with the European Parliament's Directives 90/679/EEC, 93/88/EEC and 95/30 EC [10].

Moreover, iGEM Greece 2017 PIs and instructors have helped with the most tackle various biosafety concerns (e.g. regarding the potential use of invasin/listeriolysin O) and have extensively advised the team about the safe fruition of the project.

Biosecurity

A common definition of biosecurity is an effort to “prevent misuse or mishandling of biological agents and organisms with an intent to do harm” (PCSBI, 2010). Synthetic biologypresents potential challenges to biosecurity, as well as potential tools to aid in security efforts.

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[10] See http://www.et.gr/idocs-nph/search/pdfViewerForm.html?args=5C7QrtC22wEqaJsMsZeph3dtvSoClrL8_e1HEEkqZwLtIl9LGdkF53UIxsx942CdyqxSQYNuqAGCF0IfB9HI6qSYtMQEkEHLwnFqmgJSA5WIsluV-nRwO1oKqSe4BlOTSpEWYhszF8P8UqWb_zFijBrfekfxKPYCb8HqKy4goGHcsEFKPpi4svT2BZvOasKL, http://www.et.gr/idocs-nph/search/pdfViewerForm.html?args=5C7QrtC22wEWFzYWFtEvQndtvSoClrL8qr3l9hdh7F55MXD0LzQTLWPU9yLzB8V68knBzLCmTXKaO6fpVZ6Lx3UnKl3nP8NxdnJ5r9cmWyJWelDvWS_18kAEhATUkJb0x1LIdQ163nV9K--td6SIuTimEetU4P6VhoiAAKzqgWf4Ke975q6_Lgsfe9-xj_DZ, http://www.et.gr/idocs-nph/search/pdfViewerForm.html?args=5C7QrtC22wE56mFqysdfkXdtvSoClrL8yDC9E5e67ropCCmqt4mgGEHlbmahCJFQEmRQwePEviF8EeCoaT0MAKztT3Sb63xk3VkL3PiCQ3RLoVYQqjKiogfu8Gq1RKKQmyoZK8o4WQOuTGa12O4g6Iw2HIcbIeDbXnaxnh2hedi3Wz4zxT5Qdg. Accessed on 15 November 2017.

There is “heated debate” as to the level of threat of biological weapons, but broad consensus that advances in biotechnology are likely to increase the dangers posed by biological weapons (Mukunda, 2009). Mukunda et al. (2009) classify potential impacts of synthetic biology on offense as primarily increasing capabilities for acquisition of biological weapons and, in the long term, the effects of such weapons, including enhanced lethality and infectiousness.

On the other hand, synthetic biology could provide more efficient and effective tools to respond to modern challenges, such as responding to biosecurity threats and diagnosing and treating diseases.

Beside bioterrorism, rapidly increased of synthetic biology generates concerns regarding biohacking. As biohacker can be used to described a lone operator with expertise in synthetic biology, not restricted by institutional biosafety oversight, and intent on doing harm, or a biohacker wants to wreak havoc among living organisms just as his fellow hacker creates viruses that infect and disable computer systems, are distinct possibilities.

A more thorough study on this issue was judged unnecessary, because we believe it does not concern iGEM Greece 2017 team's research team at this point of time.

Risk management/governance

The objective of the Impact Assessment is to provide a set of quantitative and qualitative decision variables that will guide and support policy-makers in taking decisions. The ultimate goal of the Impact Assessment is to analyse the positive and negative impacts associated with a given policy proposal, enabling informed political judgements to be made and identifying trade-offs in achieving competing objectives.

Since biotechnology arose as a scientific area of research in the 1980s, a range of risk governance strategies have been proposed in the United States and in Europe. In 1986 the earliest U.S. risk governance strategy arose when the White House Office of Science Technology and Policy (OSTP) developed the U.S. government‘s *Coordinated Framework for the Regulation of Biotechnology* (CF) (OSTP, 1986). As a result of the CF, the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), the U.S. Department of Agriculture (USDA), the National Institutes of Health

(NIH), and the Occupational Safety and Health Administration (OSHA) outlined their respective roles in ensuring the safety of biotechnology research and products.

In 2015, a new process to update the CF was begun by the OSTP. In a White House Memorandum, the EPA, FDA and USDA was directed to update the CF to clarify agency roles in regulating biotechnology products and to formulate a long-term strategy to ensure that the federal regulatory system is equipped to efficiently assess any risks of future biotechnology products (Barbero, 2016).

Private sector groups have also called for improvements in the regulatory infrastructure to address the implications of new synthetic biologyproducts (Barbero, 2016; Bergeson, 2016; Ledford, 2016; OSHA, 2016). Public interest groups have recommended applying the precautionary principle to any further research and commercialization of synthetic products until specific biosafety and biosecurity mechanisms can be developed to keep pace with synthetic biologyadvances (Carter, 2014). Other groups have proposed detailed risk governance polices for commercial entities, users and organizations that engage in synthetic genomics research, including compiling a manual specifically addressing ―biosafety in synthetic biologylaboratories (Institute, 2014).

In Europe, the potential biosafety and biosecurity risks of synthetic biologyhave also been under review. Government agencies and private sector groups advising European governments have noted the need for robust risk assessments in synthetic biologyfor the protection of workers and the public. The United Kingdom (Bailey, 2012), Belgium (Pauwels, 2012), and the Netherlands (Modification). have each issued report about biosafety considerations and regulatory needs in response to the growing field of synthetic biology.

In 2015, the European Commission's (EC) combined scientific committees on health and environmental risks, emerging a newly identified health risks, and consumer safety addressed the question of whether existing methodologies were appropriate for assessing the potential risks associated with synthetic biologyresearch. The Committees' opinion was that existing risk assessment approaches for genetically modified organisms (GMOs) are generally applicable to synthetic biology, but nevertheless noted that combining genetic parts and the emergence of new propertie

will require improving existing methodologies. The EC called for the standardization of risk assessments for synthetic biology, more research to improve the ability to predict the behavior of complex engineered organisms, and doubted that intrinsic biocontainment strategies like intrinsic genetic “safety locks,” were effective as a primary strategy to control the risks of synthetic biology. (EC, 2015)

The challenge for both U.S. and European policymakers is to prevent the deliberate or inadvertent use of synthetic biotechnology for harmful purposes without impeding the pathway to the societal benefits this new technology offers.

Conclusions

The field of synthetic biology is rapidly evolving and carries great potential to solve present and future societal challenges. Some of the technology advancements are already spurring debates on whether the products should be regulated or not, and to what degree the novel trait is different than what is found in a naturally occurring organism or organisms obtained through classical breeding techniques. The answer to these questions lay in further research and understanding the impact of the new technologies on the natural and technological processes of the cells that are being modified.

iGEM Greece 2017 has recognized the importance of the responsibility of scientists for providing information for the use of new technologies for the benefits of humans and environment. Also, considered that the establishment of more appropriate methods for the management of the risks of synthetic biology activities and products associated with genetic circuits is high importance, decided to develop a precautionary approach, which combines the existing assessment methods for GMOs, the sustainability impact assessment (SIA) and the OSIRIS protocol created by the team for the ensuring of stakeholders' participation. Even there are synergies, conflicts and trade-offs across impacts the proposed methodologies can be used to address all the risks.

iGEM Greece 2017 has taken into account during the experimental design for the iGEM competition all the rules that apply both in Greece and internationally. Thus, has encountered uncertainties concerning institutional, regional, national or international rules and regulations concerning the project and the lab where the experiments will be conducted.

Discussions and dialogues based on report's outcomes are not create consensus, but will contribute to the development of a broader platform for decision making. To what extend this product will be regulated, as well as other products related to genetic circuits, and what regulatory framework that will be applied depends on the outcome of national and international processes and agreements.

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Appendix A

*Table 2: List of Parties to the Cartagena Protocol on Biosafety. The column entitled 'Date instrument of rtf/acs deposited' indicates the dates when the instrument of ratification (**rtf**), acceptance (**acs**), approval (**apv**) or accession (**acs**) is deposited with the Depositary. The column entitled 'Entry into force' indicates the dates when the Protocol enters into force for the respective State or regional economic integration organization.*[11]

Country	Date of signature	Date instrument of rtf/acs deposited		Date of entry into force
Afghanistan		Feb 20, 2013	ACS	May 21, 2013
Albania		Feb 08, 2005	ACS	May 09, 2005
Algeria	May 25, 2000	Aug 05, 2004	RTF	Nov 03, 2004
Angola		Feb 27, 2009	ACS	May 28, 2009

Country	Date of signature	Date instrument of rtf/acs deposited5		Date of entry into force
Liberia		Feb 15, 2002	ACS	Sep 11, 2003
Libya		Jun 14, 2005	ACS	Sep 12, 2005
Lithuania	May 24, 2000	Nov 07, 2003	RTF	Feb 05, 2004
Luxembourg	Jul 11, 2000	Aug 28, 2002	RTF	Sep 11, 2003

[11] This table was found at https://bch.cbd.int/protocol/parties/ Accessed on 15 October 2017.

Antigua and Barbuda	May 24, 2000	Sep 10, 2003	RTF	Dec 09, 2003
Armenia		Apr 30, 2004	ACS	Jul 29, 2004
Austria	May 24, 2000	Aug 27, 2002	RTF	Sep 11, 2003
Azerbaijan		Apr 01, 2005	ACS	Jun 30, 2005
Bahamas	May 24, 2000	Jan 15, 2004	RTF	Apr 14, 2004
Bahrain		Feb 07, 2012	ACS	May 07, 2012
Bangladesh	May 24, 2000	Feb 05, 2004	RTF	May 05, 2004
Barbados		Sep 06, 2002	ACS	Sep 11, 2003
Belarus		Aug 26, 2002	ACS	Sep 11, 2003

Madagascar	Sep 14, 2000	Nov 24, 2003	RTF	Feb 22, 2004
Malawi	May 24, 2000	Feb 27, 2009	RTF	May 28, 2009
Malaysia	May 24, 2000	Sep 03, 2003	RTF	Dec 02, 2003
Maldives		Sep 02, 2002	ACS	Sep 11, 2003
Mali	Apr 04, 2001	Aug 28, 2002	RTF	Sep 11, 2003
Malta		Jan 05, 2007	ACS	Apr 05, 2007
Marshall Islands		Jan 27, 2003	ACS	Sep 11, 2003
Mauritania		Jul 22, 2005	ACS	Oct 20, 2005
Mauritius		Apr 11, 2002	ACS	Sep 11, 2003

Belgium	May 24, 2000	Apr 15, 2004	RTF	Jul 14, 2004
Belize		Feb 12, 2004	ACS	May 12, 2004
Benin	May 24, 2000	Mar 02, 2005	RTF	May 31, 2005
Bhutan		Aug 26, 2002	ACS	Sep 11, 2003
Bolivia (Plurinational State of)	May 24, 2000	Apr 22, 2002	RTF	Sep 11, 2003
Bosnia and Herzegovina		Oct 01, 2009	ACS	Dec 30, 2009
Botswana	Jun 01, 2001	Jun 11, 2002	RTF	Sep 11, 2003
Brazil		Nov 24, 2003	ACS	Feb 22, 2004

Mexico	May 24, 2000	Aug 27, 2002	RTF	Sep 11, 2003
Mongolia		Jul 22, 2003	ACS	Oct 20, 2003
Montenegro		Oct 23, 2006	SCS	Jun 03, 2006
Morocco	May 25, 2000	Apr 25, 2011	RTF	Jul 24, 2011
Mozambique	May 24, 2000	Oct 21, 2002	RTF	Sep 11, 2003
Myanmar	May 11, 2001	Feb 13, 2008	RTF	May 13, 2008
Namibia	May 24, 2000	Feb 10, 2005	RTF	May 11, 2005
Nauru		Nov 12, 2001	ACS	Sep 11, 2003

Bulgaria	May 24, 2000	Oct 13, 2000	RTF	Sep 11, 2003
Burkina Faso	May 24, 2000	Aug 04, 2003	RTF	Nov 02, 2003
Burundi		Oct 02, 2008	ACS	Dec 31, 2008
Cabo Verde		Nov 01, 2005	ACS	Jan 30, 2006
Cambodia		Sep 17, 2003	ACS	Dec 16, 2003
Cameroon	Feb 09, 2001	Feb 20, 2003	RTF	Sep 11, 2003
Central African Republic	May 24, 2000	Nov 18, 2008	RTF	Feb 16, 2009
Chad	May 24, 2000	Nov 01, 2006	RTF	Jan 30, 2007
China	Aug 08, 2000	Jun 08, 2005	APV	Sep 06, 2005

Netherlands	May 24, 2000	Jan 08, 2002	ACP	Sep 11, 2003
New Zealand	May 24, 2000	Feb 24, 2005	RTF	May 25, 2005
Nicaragua	May 26, 2000	Aug 28, 2002	RTF	Sep 11, 2003
Niger	May 24, 2000	Sep 30, 2004	RTF	Dec 29, 2004
Nigeria	May 24, 2000	Jul 15, 2003	RTF	Oct 13, 2003
Niue		Jul 08, 2002	ACS	Sep 11, 2003
Norway	May 24, 2000	May 10, 2001	RTF	Sep 11, 2003
Oman		Apr 11, 2003	ACS	Sep 11, 2003
Pakistan	Jun 04, 2001	Mar 02, 2009	RTF	May 31, 2009

Colombia	May 24, 2000	May 20, 2003	RTF	Sep 11, 2003
Comoros		Mar 25, 2009	ACS	Jun 23, 2009
Congo	Nov 21, 2000	Jul 13, 2006	RTF	Oct 11, 2006
Costa Rica	May 24, 2000	Feb 06, 2007	RTF	May 07, 2007
Côte d'Ivoire		Mar 12, 2015	ACS	Jun 10, 2015
Croatia	Sep 08, 2000	Aug 29, 2002	RTF	Sep 11, 2003
Cuba	May 24, 2000	Sep 17, 2002	RTF	Sep 11, 2003
Cyprus		Dec 05, 2003	ACS	Mar 04, 2004
Czech Republic	May 24, 2000	Oct 08, 2001	RTF	Sep 11, 2003

Palau	May 29, 2001	Jun 13, 2003	RTF	Sep 11, 2003
Panama	May 11, 2001	May 01, 2002	RTF	Sep 11, 2003
Papua New Guinea		Oct 14, 2005	ACS	Jan 12, 2006
Paraguay	May 03, 2001	Mar 10, 2004	RTF	Jun 08, 2004
Peru	May 24, 2000	Apr 14, 2004	RTF	Jul 13, 2004
Philippines	May 24, 2000	Oct 05, 2006	RTF	Jan 03, 2007
Poland	May 24, 2000	Dec 10, 2003	RTF	Mar 09, 2004
Portugal	May 24, 2000	Sep 30, 2004	ACP	Dec 29, 2004
Qatar		Mar 14, 2007	ACS	Jun 12, 2007

Democratic People's Republic of Korea	Apr 20, 2001	Jul 29, 2003	RTF	Oct 27, 2003
Democratic Republic of the Congo		Mar 23, 2005	ACS	Jun 21, 2005
Denmark	May 24, 2000	Aug 27, 2002	RTF	Sep 11, 2003
Djibouti		Apr 08, 2002	ACS	Sep 11, 2003
Dominica		Jul 13, 2004	ACS	Oct 11, 2004
Dominican Republic		Jun 20, 2006	ACS	Sep 18, 2006
Ecuador	May 24, 2000	Jan 30, 2003	RTF	Sep 11, 2003

Republic of Korea	Sep 06, 2000	Oct 03, 2007	RTF	Jan 01, 2008
Republic of Moldova	Feb 14, 2001	Mar 04, 2003	RTF	Sep 11, 2003
Romania	Oct 11, 2000	Jun 30, 2003	RTF	Sep 28, 2003
Rwanda	May 24, 2000	Jul 22, 2004	RTF	Oct 20, 2004
Saint Kitts and Nevis		May 23, 2001	ACS	Sep 11, 2003
Saint Lucia		Jun 16, 2005	ACS	Sep 14, 2005
Saint Vincent and the Grenadines		Aug 27, 2003	ACS	Nov 25, 2003

Egypt	Dec 20, 2000	Dec 23, 2003	RTF	Mar 21, 2004
El Salvador	May 24, 2000	Sep 26, 2003	RTF	Dec 25, 2003
Eritrea		Mar 10, 2005	ACS	Jun 08, 2005
Estonia	Sep 06, 2000	Mar 24, 2004	RTF	Jun 22, 2004
Ethiopia	May 24, 2000	Oct 09, 2003	RTF	Jan 07, 2004
European Union	May 24, 2000	Aug 27, 2002	APV	Sep 11, 2003
Fiji	May 02, 2001	Jun 05, 2001	RTF	Sep 11, 2003
Finland	May 24, 2000	Jul 09, 2004	RTF	Oct 07, 2004
France	May 24, 2000	Apr 07, 2003	APV	Sep 11, 2003

Samoa	May 24, 2000	May 30, 2002	RTF	Sep 11, 2003
Saudi Arabia		Aug 09, 2007	ACS	Nov 07, 2007
Senegal	Oct 31, 2000	Oct 08, 2003	RTF	Jan 06, 2004
Serbia		Feb 08, 2006	ACS	May 09, 2006
Seychelles	Jan 23, 2001	May 13, 2004	RTF	Aug 11, 2004
Slovakia	May 24, 2000	Nov 24, 2003	RTF	Feb 22, 2004
Slovenia	May 24, 2000	Nov 20, 2002	RTF	Sep 11, 2003
Solomon Islands		Jul 28, 2004	ACS	Oct 26, 2004
Somalia		Jul 26, 2010	ACS	Oct 24, 2010

Gabon		May 02, 2007	ACS	Jul 31, 2007
Gambia (the)	May 24, 2000	Jun 09, 2004	RTF	Sep 07, 2004
Georgia		Nov 04, 2008	ACS	Feb 02, 2009
Germany	May 24, 2000	Nov 20, 2003	RTF	Feb 18, 2004
Ghana		May 30, 2003	ACS	Sep 11, 2003
Greece	May 24, 2000	May 21, 2004	RTF	Aug 19, 2004
Grenada	May 24, 2000	Feb 05, 2004	RTF	May 05, 2004
Guatemala		Oct 28, 2004	ACS	Jan 26, 2005
Guinea	May 24, 2000	Dec 11, 2007	RTF	Mar 10, 2008

South Africa		Aug 14, 2003	ACS	Nov 12, 2003
Spain	May 24, 2000	Jan 16, 2002	RTF	Sep 11, 2003
Sri Lanka	May 24, 2000	Apr 28, 2004	RTF	Jul 26, 2004
State of Palestine		Jan 02, 2015	ACS	Apr 02, 2015
Sudan		Jun 13, 2005	ACS	Sep 11, 2005
Suriname		Mar 27, 2008	ACS	Jun 25, 2008
Swaziland		Jan 13, 2006	ACS	Apr 13, 2006
Sweden	May 24, 2000	Aug 08, 2002	RTF	Sep 11, 2003
Switzerland	May 24, 2000	Mar 26, 2002	RTF	Sep 11, 2003

Guinea-Bissau		May 19, 2010	ACS	Aug 17, 2010
Guyana		Mar 18, 2008	ACS	Jun 16, 2008
Honduras	May 24, 2000	Nov 18, 2008	RTF	Feb 16, 2009
Hungary	May 24, 2000	Jan 13, 2004	RTF	Apr 12, 2004
India	Jan 23, 2001	Jan 17, 2003	RTF	Sep 11, 2003
Indonesia	May 24, 2000	Dec 03, 2004	RTF	Mar 03, 2005
Iran (Islamic Republic of)	Apr 23, 2001	Nov 20, 2003	RTF	Feb 18, 2004

Syrian Arab Republic		Apr 01, 2004	ACS	Jun 30, 2004
Tajikistan		Feb 12, 2004	ACS	May 12, 2004
Thailand		Nov 10, 2005	ACS	Feb 08, 2006
The former Yugoslav Republic of Macedonia	Jul 26, 2000	Jun 14, 2005	RTF	Sep 12, 2005
Togo	May 24, 2000	Jul 02, 2004	RTF	Sep 30, 2004
Tonga		Sep 18, 2003	ACS	Dec 17, 2003
Trinidad and Tobago		Oct 05, 2000	ACS	Sep 11, 2003

Iraq		Mar 03, 2014	ACS	Jun 01, 2014
Ireland	May 24, 2000	Nov 14, 2003	RTF	Feb 12, 2004
Italy	May 24, 2000	Mar 24, 2004	RTF	Jun 22, 2004
Jamaica	Jun 04, 2001	Sep 25, 2012	RTF	Dec 24, 2012
Japan		Nov 21, 2003	ACS	Feb 19, 2004
Jordan	Oct 11, 2000	Nov 11, 2003	RTF	Feb 09, 2004
Kazakhstan		Sep 08, 2008	ACS	Dec 07, 2008

Tunisia	Apr 19, 2001	Jan 22, 2003	RTF	Sep 11, 2003
Turkey	May 24, 2000	Oct 24, 2003	RTF	Jan 24, 2004
Turkmenistan		Aug 21, 2008	ACS	Nov 19, 2008
Uganda	May 24, 2000	Nov 30, 2001	RTF	Sep 11, 2003
Ukraine		Dec 06, 2002	ACS	Sep 11, 2003
United Arab Emirates		Sep 12, 2014	ACS	Dec 11, 2014
United Kingdom of Great Britain and Northern Ireland	May 24, 2000	Nov 19, 2003	RTF	Feb 17, 2004

Kenya	May 15, 2000	Jan 24, 2002	RTF	Sep 11, 2003
Kiribati	Sep 07, 2000	Apr 20, 2004	RTF	Jul 19, 2004
Kuwait		Jun 01, 2017	ACS	Aug 30, 2017
Kyrgyzstan		Oct 05, 2005	ACS	Jan 03, 2006
Lao People's Democratic Republic		Aug 03, 2004	ACS	Nov 01, 2004
Latvia		Feb 13, 2004	ACS	May 13, 2004
Lebanon		Feb 06, 2013	ACS	May 07, 2013

United Republic of Tanzania		Apr 24, 2003	ACS	Sep 11, 2003
Uruguay	Jun 01, 2001	Nov 02, 2011	RTF	Jan 31, 2012
Venezuela (Bolivarian Republic of)	May 24, 2000	May 13, 2002	RTF	Sep 11, 2003
Viet Nam		Jan 21, 2004	ACS	Apr 20, 2004
Yemen		Dec 01, 2005	ACS	Mar 01, 2006
Zambia		Apr 27, 2004	ACS	Jul 25, 2004
Zimbabwe	Jun 04, 2001	Feb 25, 2005	RTF	May 26, 2005

Lesotho		Sep 20, 2001	ACS	Sep 11, 2003

Appendix B

Table 3: Details about the parts that have been developed during iGEM Greece 2017 project.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
E. coli XL-1 Blue	1	iGEM (*E. coli K-12* derivative, https://ibc.researchcompliance.vt.edu/e-coli-strain-information)	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	Cloning of our parts.
E. coli DH5a	1	iGEM (Common iGEM Organisms)	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	Cloning of our parts.
E. coli TOP10	1	iGEM (Common iGEM Organisms)	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	Cloning of our parts.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
Strain JW4275-1(E. coli K-12)	1	CGSC(Coli Genetic Stock Center) https://cgsc2.biology.yale.edu/index.php	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Purchased it from Coli Genetics Stock Center, Yale.	Control of our chassis organism.
Strain JW4276-1(E. coli K-12)	1	CGSC(Coli Genetic Stock Center) https://cgsc2.biology.yale.edu/index.php	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Purchased it from Coli Genetics Stock Center, Yale.	Control of our chassis organism.
Strain JW4283-3(E. coli K-12)	1	CGSC(Coli Genetic Stock Center) https://cgsc2.biology.yale.edu/index.php	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Purchased it from Coli Genetics Stock Center, Yale.	Chassis organism.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
Saccharomyces Cerevisae Y190	1	ATCC	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	This organism is used to test the binding ability of the FimH mutant part() to D-mannose.
Caco-2	1	DSMZ	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	This cell line is used as the target of our therapeutic approach.
A549	1	DSMZ	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	This cell line is used as a control of the selectivity of our approach.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
HEK 293	1	DSMZ	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)			Received it from our host lab.	This cell line is used as a control of the selectivity of our approach.
E. coli K12	1	iGEM (Common iGEM Organisms)	Risk Group 1 organisms do not cause disease in healthy adult humans. (http://2017.igem.org/Safety/Risk_Groups#HowToFindRiskGroup)	LacI coding sequence - pANDORRA compatible	Binds to the operator region of the lac operon and inhibits the expression of the downstream genes.	Prof. Z. Xie (Bioinformatics Division, Tsinghua National Lab for Information Science and Technology at Tsinghua University) has kindly provided us with the plasmid sequence containing the LacI coding sequence. We codon-optimized the sequence in order to replace codons in the reading frame that form restriction sites found in the Prefix-Suffix, with synonym codons and added the douple stop codon TAATAA. Downstream of the double stop codon, we added two features, an annealing	As a transcriptional repressor in order to achieve the double inversion module necessary for our cell-type classifier (Xie et al., 2011).

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
						site for the M13 Reverse primer and a recognition site for BbsI to utilize in our modular assembly process of the RNAi-based Boolean classifier. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts.	
E. coli	1 or 2 (pathogenic strains)	iGEM	The components of these systems are derived from the tetracycline resistance operon in *E.coli.* It should be noted that there are pathogenic *E. coli* strains that cause various diseases in humans, including several types of diarrhea (ETEC, EIEC, EPEC etc), urinary tract infections (UPEC), sepsis and meningitis (NMEC) (Virulence Factors Database).	rtTA coding sequence - pANDORRA compatible	rtTA is a fusion protein comprised of the TetR repressor and the VP16 transactivation domain and is the major component of the Tet-On system, where the reverse Tet repressor (rTetR) relies on the presence of tetracycline (or a Dox effector) for induction, by binding to tetO sequences to promote expression of a desired gene (Gossen et al., 1995; Gossen et al., 1992).	Prof. Z. Xie (Bioinformatics Division, Tsinghua National Lab for Information Science and Technology at Tsinghua University) has kindly provided us with the plasmid sequence containing the rtTA coding sequence. We codon-optimized the sequence in order to replace codons in the reading frame that form restriction sites found in the Prefix-Suffix, with synonym codons and added the douple stop codon TAATAA. Downstream of the double stop codon, we added two features, an annealing	As an activator of the expression of the Lac repressor used in our double inversion module (Xie et al., 2011).

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
						site for the M13 Reverse primer and a recognition site for BbsI to utilize in our modular assembly process of the RNAi-based Boolean classifier. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts.	
Discosoma sp.	No classification of the organism has been found in commonly used databases.			DsRed coding sequence - pANDORRA compatible	DsRed is a red fluorescent protein (RFP) derived from the reef coral *Discosoma sp.* It has an excitation length maximum at 558 nm and an emission maximum at 583 nm. DsRed contributes to the natural coloration of its corallimorpharian host, and/or possibly functions as protection against UV radiation (Yarbrough et al., 2001; Dove et al., 2001; Mizuno et al., 2001; Lukyanov et al. 2000; Matz et al., 1999).	Prof. Z. Xie (Bioinformatics Division, Tsinghua National Lab for Information Science and Technology at Tsinghua University) has kindly provided us with the plasmid sequence containing the DsRed coding sequence. We codon-optimized the sequence in order to replace codons in the reading frame that form restriction sites found in the Prefix-Suffix, with synonym codons and added the douple stop codon TAATAA. Downstream of the double stop codon, we added two features, an annealing	We aim to utilize DsRed expression in our mammalian cancer cell lines, as the fluorescence output of our RNAi-based logic transcriptional/post-transcriptional circuits in order to characterize the proposed cell-type classifier.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
						site for the M13 Reverse primer and a recognition site for BbsI to utilize in our modular assembly process of the RNAi-based Boolean classifier. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts.	
Chicken anemia virus	Not Classified. However it does not cause disease in humans.	No classification of this virus has been found.	The chicken anemia virus only infects chickens (Markey et al., 2013).	Apoptin (BBa_K1061001) - pANDORRA compatible	Induces programmable cell death in chicken thymocytes.	Downstream of the stop codon, we added two features, an annealing site for the M13 Reverse primer and a recognition site for BbsI to utilize in our modular assembly process of the RNAi-based Boolean classifier. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts, including one where Apoptin is fused with sfGFP (BBa_K515005).	As a selective killer of cancer cells due to its ability to confer programmed cell death in a variety of tumor cells and not in a wide range of healthy cell types via a yet not fully elucidated mechanism.

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
Yersinia pseudotuberculosis	2	DSMZ	In the human colon, *Yersinia pseudotuberculosis* uses invasin to identify and invade M cells, which uniquely express b1-integrins on their apical surface. Its host organism (*Yersinia pseudotuberculosis*) is the least common of the 3 main Yersinia species that cause infections in humans. It typically causes zoonotic infections leading to gastroenteritis whether on the other hand as a plasmid it is not directly toxic to humans (Clark et al., 1998).	pLuxR-RBS-invasin	Invasin is a long rigid protein that is anchored in the outer membrane and extends 18 nm from the bacterial cell surface. It binds tightly to b1-integrins present on the surface of many cell lines and induces bacterial uptake by stimulating Rac-1. It promotes both attachment and invasion into eukaryotic cells, including nonphagocytic cells and the bacterium is taken up by zipper mechanism (Virulence Factors Database, Leo et al., 2015; Wong et al., 2005; Boyd et al., 2001; Marra et al., 1997; Leong et al., 1995; Young et al., 1992; Young et al., 1990; Isberg et al., 1987)	Dr. Grillot-Courvalin (Institut Pasteur, France) kindly provided us with the plasmid sequence containing the invasin coding sequence. We codon-optimized the sequence in order to replace codons in the reading frame that form restriction sites found in the Prefix-Suffix, with synonym codons and added a promoter (pLuxR) and an RBS. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts.	We hope to utilize *E. coli* expressing both invasin and listeriolysin O (LLO) as a device capable of performing bactofection (bacterial-mediated transfer of plasmid DNA to mammalian cells). Our goal is to tranfer a synthetic RNAi-based logic circuit capable of inducing selective apoptosis in Caco-2 and not other cell lines. We utilize Caco-2 cells as a model of colorectal cancer and want to use bactofection so as to make use of the advantages of bacterial cancer therapies. Knowing that invasin and listeriolysin can allow bacteria to invade any beta-1-integrin expressing cell, we aim to include multiple additional levels of selectivity by utilizing genetically engineered fimbriae capable of selective binding to colorectal cancer cells and by placing invasin and LLO

Species name (including strain)	**Risk Group**	**Risk Group Source**	**Disease risk to humans?**	**Part number/name**	**Natural function of part**	**How did you acquire it?**	**How will you use it?**
							under the control of the lux operon (Anderson et al, 2006; Fajac et al., 2004; Narayanan et al., 2003; Grillot-Courvalin et al., 1998)

Listeria monocytogenes	2	DSMZ	Listeriolysin O (LLO), as a hemolysin, allows bacteria that express it to escape phagosomes and grow intracellularly. In its host organism *Listeria*, there is a strong correlation between hemolytic activity and pathogenicity as LLO is a key component of the intracellular infectious cycle of the bacterium. It should be noted that multiple other virulence factors are involved in the intestinal translocation of pathogenic listeriae and their internalization and are required for the pathogenesis of listeriosis, such as various phospholipases (Vazquez-Boland JA, et al., 2001).	Listeriolysin O coding sequence	Listeriolysin O (LLO) is a thiol-activated cholesterol-dependent pore forming toxin protein - it is activated by reducing agents and inhibited by oxidizing agents. However, LLO differs from other thiol-activated toxins, since its cytolytic activity is maximized at a pH of 5.5, which occurs in the phagosome. The result is that LLO is selectively activated within the acidic phagosomes of cells that have phagocytosed *L. monocytogenes*. After LLO lyses the phagosome, the bacterium escapes into the cytosol, where it can grow intracellularly. Upon release from the phagosome, activity of the protein is reduced due to more basic environment (Virulence Factor Database, Hamon et al., 2012; Dramsi et al., 2002; Vazquez-Boland et al., 2001; Cossart et al., 1989).	Dr. Grillot-Courvalin (Institut Pasteur, France) kindly provided us with the plasmid sequence containing the Listeriolysin O coding sequence. We codon-optimized the sequence in order to replace codons in the reading frame that form restriction sites found in the Prefix-Suffix, with synonym codons. We ordered the part, flanked with Prefix-Suffix from IDT and cloned it into composite parts.	We hope to utilize *E. coli* expressing both invasin and listeriolysin O (LLO) as a device capable of performing bactofection (bacterial-mediated transfer of plasmid DNA to mammalian cells). Our goal is to tranfer a synthetic RNAi-based logic circuit capable of inducing selective apoptosis in Caco-2 and not other cell lines. We utilize Caco-2 cells as a model of colorectal cancer and want to use bactofection so as to make use of the advantages of bacterial cancer therapies. Knowing that invasin and listeriolysin can allow bacteria to invade any beta-1-integrin expressing cell, we aim to include multiple additional levels of selectivity by utilizing genetically engineered fimbriae capable of selective binding to colorectal cancer cells and by placing invasin and LLO under the control of the lux operon (Anderson et al, 2006; Fajac et al., 2004; Narayanan et al., 2003; Grillot-Courvalin et al., 1998)

Appendix C

Table 4: Examples of potential positive and negative impacts of synthetic biologywith regard to social, economic and cultural considerations.

Social, economic and cultural considerations	**Possible positive impacts of synthetic biology**	**Possible negative impacts of synthetic biology**
Biosecurity	Synthetic biologytechniques may provide tools for better detecting and identifying pathogenic agents, and responding to biosecurity threats, for example through accelerated vaccine production.	Synthetic biologytechniques may raise a “dual use” challenge, in that the substances used by research for positive ends may also be used for damaging results, such as creating destructive pathogens that target natural resources .
Economic	Synthetic biologyis widely anticipated to play a significant role in the bioeconomy, which could benefit the economic growth (and human health and environment) of countries. Products from synthetic biology, such as artemisinin, may improve the health of the people of developing countries and thus their economies.	Synthetic biologyalternatives for natural products may lead to product displacement in developing countries, but potential harms may be addressed through product-specific arrangements and public or the natural version may still hold on to some share of the market, or the benefits of the synthetic biologyversions may outweigh the losses . Potential harms from product-displacement may be addressed through product-specific arrangements and public engagement .
Health	Synthetic biologymay help to study disease mechanisms.	Synthetic biologyapplications may result in the possibility of direct harm to patients' health if engineered organisms

Social, economic and cultural considerations	**Possible positive impacts of synthetic biology**	**Possible negative impacts of synthetic biology**
	Synthetic biologymay aid in diagnostics . Synthetic biologymay aid in drug discovery through developing drug screening platforms . Synthetic biologymay help design organisms to produce drugs and vaccines . Synthetic biologymay help design therapeutic treatments.	/ viruses trigger unanticipated adverse effects .(König *et al.* 2013; PCSBI 2010) Synthetic biologymay result in the possiblity of direct harm for workers in synthetic biologylabs . Patent thickets and broad patents may restrict access to drugs and therapies .
Ethical	*Ethical discussions around synthetic biologyare not structured around potential "positive" and "negative" impacts, but rather broad considerations:* Ethical analysis may help determine how to weigh and balance possible negative impacts of synthetic biologyagainst possible positive impacts, as well as explore what equitable distribution of synthetic biology-related harms and benefits would look like and how to achieve this. The ability to design significant portions of organisms may change humanity's approach to nature and lead humanity to overestimating our understanding of nature's processes. Ethical discussions should not be based on assumptions that synthetic biologyis able to do more than it can . Where synthetic biologyresearch is based on a reductionist view of the world, it may undermine the special status of living . Life does not necessarily hold special status, and there is no evidence that synthetic biologyscience is leading to a "slippery slope" of devaluing some forms of life .	
Intellectual property	A model of IP based on open-source software may lead to greater innovation, transparency, and openness .	Synthetic biologymay extend private ownership of genetic material, restricting access for public benefit .

Social, economic and cultural considerations	Possible positive impacts of synthetic biology	Possible negative impacts of synthetic biology
	Using synthetic biologyto design and synthesize DNA sequences may avoid ethical and legal challenges related to patenting natural DNA sequences .	Strong IP regimes could restrict access to information for carrying out independent risk assessments .