How safe is safe enough: towards best practices of synthetic biology

How safe is safe enough: towards best practices of synthetic biology

Paris Bettencourt 2012 iGEM Team

Preface

Our team was baffled by the new and exciting bacteria undergraduate students created during the summer, and presented to the world during the yearly iGEM competition. Auxin (Imperial 2011), AgrEcoli (Bristol 2010), and E.D Frosti (KULeven 2011), but also hundreds of other bacteria could prove very useful. However, some need to be taken out of the lab so they can be used.

We want to take genetically modified bacteria out of the lab because we see huge potential benefits. However, we are not willing to do that "no matter what". This is why we decided that the project we would present to the 2012 iGEM competition would be one that would help us get closer to that goal: putting genetically modified bacteria in the environment in a safe, responsible and acceptable way.

Our "human practice" and "wet lab" parts are complementary. We identified some technical issues such as HGT and excessive proliferation that we believe can be solved by synthetic biology. This is what our bench work is about: trying to build a master system to diminish the probability of HGT and bacterial proliferation. However, we are not so naïve as to think that technique can solve everything. We know that they are many non technical issues when it comes to releasing bacteria created by synthetic biology in the environment. This is where our human practice part takes all its meaning.

When discussing putting genetically modified bacteria in the environment, it is crucial to differentiate the concern that are in fact just about synthetic biology and the ones that really concerns the application in the field. The debate on the technique should happen, and then be closed once and for all so we can move forward to discussing the applications.

If we do not proceed in that order, the debate gets very messy, like it has been when debating about GMO crops in Europe. Europeans never really had a say on the recombinant DNA technology so when applications were discussed, since the technique had never been properly been discussed per se, the whole debate about recombinant DNA technology re emerged each time.

We are afraid the same thing could happen with Synthetic Biology if citizens are not properly informed and given the opportunity to debate on Synthetic Biology as a field. Of course, we are not starting from 0 because fears about genetic engineering have already been voiced during the GMO crops episode, and as Synthetic Biology is an extension to genetic engineering, we can only imagine that the fears raised by the first are mainly extensions of the ones raised by the latter.

For all of the above reasons, we decided to separate this essay into two distinct parts. The first one will address the concerns raised by synthetic biology per se, that is, as a technique. Then, in our second part, we will analyze the specific concerns that arise from synthetic biology's potential applications in nature.

Introduction ……..p5

I Debate on synthetic biology as a technique ……p6

A. Historical background: Synthetic biology as an extension to Genetic Engineering p6

1. Definitions: synthetic biology and genetic engineering

i. Synthetic biology

iii. Comparison between Synthetic Biology and Genetic engineering

2. SB and Genetic engineering share a common history and so common controversies around the recombinant DNA technology.

i. Beginnings

ii. Asilomar

iii. What happened next

iv. Analysis

B. Concerns raised by Synthetic Biology ……p11

1. Recombinant DNA technology

2. Synthetic Biology: Awareness, perceptions, concerns and regulation

i. Awareness of synthetic biology

ii. Perception of Synthetic Biology

iii. Concerns about Synthetic Biology

iv. Approval of synthetic biology

v. Who should regulate synthetic biology and what should be taken into accounts when making guidelines and laws?

3. Analysis of the concerns raised by synthetic biology

i. Unnaturalness

ii. Playing God

iii. Status of artificial life

iv. Physical harms

v. Regulations

4. Will rising awareness change anything?

i. Polls have shown that skepticism is not simply caused by lack of information

ii. We disagree with the finality of educating on new technologies so the population can accept them better

C. Conclusion ……..p27

II. The debate about putting GM bacteria in the environment ……..p28

A. Our master security system ……p28

1. Horizontal gene transfer

i. What is horizontal gene transfer?

ii. Since when have people been concerned about HGT

iii. Why is risk so hard to access?

2. Excessive proliferation

3. Biosafety: how are these concerns about excessive proliferation and HGT dealt with?

i. Governing Instances

ii. Literature

iii. Screening past iGEM projects

4. How our system tries to come as a response to these issues

i. Recap of what our team thinks

ii. Presentation of our system

B. Are these issues the only concerns? ……..p44

1. Case study: GMO plants and crops

i. Historical background [36, 37,38]

ii. The polemic in Europe

iii. Lessons to be learned

2. Applying these lessons to synthetic biology & further suggestions

C. Conclusion ……..p50

Conclusion: Contributions & Proposals ……..p53

Bibliography ……p55

Introduction

Every iGEM project starts with a team of highly motivated students and a session of brainstorming. There they are, stars shinning in their eyes, suggesting all sorts of crazy, yet genius ideas: “let’s build a bacteria that will absorb air pollutants”, “or we could create a bacteria that would go in the human body, detect cancerous environment and kill the surrounding cells”, “and what about a device that would enable us to do directed evolution in a faster and easier way?”. They are fierce believers in synthetic biology. They see its huge potential in improving the environment, men’s quality of life and scientific knowledge. However, where they see great benefits, others see great risks. Synthetic biology may be a means to very noble ends, but creating synthetic organisms in a lab raises intrinsic questions in terms of biosafety, biosecurity, risk assessment, governance, public perception, ethics, intellectual property and status [1]. We can see that there are numerous issues, and yet, we are still within the framework of the lab.

Imagine the additional concerns if we were to use these organism in the wild. Nevertheless, this is what we are tending towards as we believe that synthetic biology’s full potential can be reached only if the devices it produces can be released in the environment. This raises concerns about horizontal gene transfer, proliferation, regulation, civil society perception, benefits and ethics.

This summer, we built a master genetic safeguard, thoughts of way to improve the biobrick registry to promote safety, organized a debate with university students and scientists, and interviewed experts in order to address a few of these concerns.

I Debate on synthetic biology as a technique

A. Historical background: Synthetic biology as an extension to Genetic Engineering

1. Definitions: synthetic biology and genetic engineering

i. Synthetic biology

In 2000, three major synthetic biology papers were published. This was the beginning of Synthetic Biology's fame. It then became more and more popular around 2003, 2004 with a rising number of publications reporting on synthetic circuits of increasing complexity. Synthetic biology is a novel, rapidly expanding field. No consensual definition exists yet, but below are the most commonly referenced ones [2]:

"Synthetic biology is an emerging area of research that can broadly be described as the design and construction of novel artificial biological pathways, organisms or devices, or the redesign of existing natural biological systems." (UK royal society)

"Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way." (High Level Expert Group European Commission)

Serrano provides an interesting analysis on these commonly used definitions [3] by highlighting the fact that living systems are excruciatingly complicated, so breaking them into a series of standardized parts is not always an easy task. Furthermore, "it is possible to introduce new functions or modify existing one in the cells without a complete understanding of the system and without having a complete list of standardized components". Therefore, "we should consider are more relaxed use of the term engineering in which the emphasize should be placed on the design and simulation of the new functions and properties rather than on the standardized parts".

Let's finish this chapter by considering the team's view point. We would define Synthetic Biology as the pluridisciplinary field at the cross roads between biology, computer science and engineering that encompasses all of the following: (a) synthesizing DNA, (b) designing standardized parts, (c) using standardized parts to create novel or already existing genetic circuits, (d) implementing newly synthesized genetic circuits in living organisms in order to create a new function, (e) creating a new framework for the genetic information, by, for example, changing the genetic code or the 4 universal bases A T G C, (f) creating a life form entirely by de novo DNA synthesis (achieved once with the minimal genome project).

ii. Genetic engineering

Many people [3,4] have wondered if SB is *"something really new"*, or if it is one of the existing technologies "in new package".

Genetic engineering is built around the recombinant DNA (rDNA) technology that grew in the 1970's around the central dogma. It involves transferring one gene from an organism to another organism via a hit and trial method [5].

It has led to important successes such as insulin production by bacteria. However, it also has its shortcomings: *"rDNA is expensive (parts and labor accounting for $1.5 billion of the NIH budget) and, from an engineering perspective, messy. Employing rDNA requires a large technical knowledge base, which can be difficult to translate to other projects. Furthermore, there are some genetic dishes that rDNA cannot directly cook up. For example, there is no wild-type gene for a biofuel that can just be "cut and pasted into a bacterium. Instead, a new genetic dish must be created [4]".* Synthetic Biology can create these new genetic dishes.

iii. Comparison between Synthetic Biology and Genetic engineering

To some extent, these fields use the same molecular biology techniques (recombinant DNA technology), but Synthetic Biology can create these new genetic dishes that genetic Engineering in incapable of creating. Whereas Genetic Engineering consists in transferring one gene from an organism to another organism, Synthetic Biology involves designing new genetic circuit with many parts (not just transferring ONE gene) that can come from a vast amount of different organisms or that have been synthesized de novo. In other words, Synthetic Biology builds genetic circuits from scratch whereas Genetic engineering cuts and pastes only one gene.

Moreover, Synthetic Biology is cheaper, and the engineering approach is much more present then in Genetically Engineering. Synthetic Biology, and most of all the iGEM approach, is highly standardized (trying to make easier/cheaper/faster genetic modifications needs a common approach). Synthetic Biology is partly made of engineers while genetic engineering was mostly made of biologists.

All in all, Synthetic biology can be seen as an extension of Genetic Engineering.

2. *SB and Genetic engineering share a common history and so common controversies around the recombinant DNA technology.*

i. Beginnings

In 1953, James and Watson descried the structure of DNA
In 1970, the first restriction enzyme (HindII) was discovered

These 2 major discoveries provided scientists with the capacity to manipulate DNA. In 1971, Paul Berg, a biochemist from Stanford, designed the first experiments that used what would later be called the recombinant DNA technology. He cleaved DNA from the monkey virus SV40, and from a bacteriophage lambda. He then ligated DNA from the SV40 with DNA from the bacteriophage. The last step involved putting this mutant DNA into an E.coli bacterium by transduction.

However, this last step was not completed in the initial experiment [6, 7], as Berg's colleagues feared biohazards. The SV40 was known to cause cancer in mice and E.coli to inhabit people's intestines. Therefore, they feared that the bacteria created in the last step would either: infect lab workers, either escape the lab and infect the population, giving them all cancer. Therefore, concerns about the recombinant technology were raised by scientists themselves, from the very start. The events that followed were remarkable.

In July 1974, a group of American scientists called for a voluntary moratorium on experiments using recombinant technology, echoing reservations expressed at a Gordon Conference on nucleic acid the previous summer. Both groups acknowledge the huge potential on the recombinant DNA technology and the exceptional opportunities it could provide for medicine, agriculture and industry. However, according to Paul Berg and Maxine Singer, "the scientists were concerned that unfettered pursuit of this research might engender unforeseen and damaging consequences for human health and the Earth's ecosystem". In spite of some questioning the validity of the concerns, the moratorium was universally observed. "One goal of the moratorium was to provide time for a conference that would evaluate the state of the new technology and the risks, if any, associated with it [8]." The Asilomar conference was about "taking care of the risk", but it was also about "defining what count as risk" (and so what shouldn't count), and establish a framework of self governance [43]

ii. Asilomar

The conference was organized by Paul Berg and held at the Asilomar conference Center in California in February 1975. Potential biohazard and regulation of the biothechnoloy were discussed by the 140 participants (scientists, lawyers, government officials, members of the press). The conclusion of the conference was that "recombinant DNA research should proceed

but under strict guidelines". These guidelines were written during the conference, and promulgate by the National Institute of Health (NIH). As written, they only applied to the federally funded research.

These guidelines defined different level of laboratory confinement according to the level of dangerousness of the organisms and gene that were being manipulated. When a sufficient level of confinement was not possible, manipulations were forbidden. This was for example the case for toxic genes.

Lewis, a scientist that attended the Asilomar conference, stated that "after the conference, we felt less concerned about the hazards causing cancer."

iii. What happened next

The guideline evolved, becoming less restrictive as no accident was ever reported, and new knowledge emerged. The properties of cells and viruses containing foreign genes were studied in great details (and especially whether they could be toxic or not).

iv. Analysis

Positive aspects

The scientific community handled the situation in an exemplary way. They identified biohazards: "they were speculations that normally innocuous microbes could be changes into human pathogens by introducing genes that rendered them resistant to then-available antibiotics, or enable them to produce dangerous toxins, or transform them into cancer causing agents" [8]. And so despite the benefits this technology could bring, to their research, to science as a whole and to the society, they decided to postpone their work, to stop all research using this promising technology till the scientific community could meet, discuss the situation and draw up guidelines. By doing so, they acted in a responsible way, and sent the world the message that advance and technology is good, but that safety is even more important.

By calling a moratorium, and then respecting it, they showed the importance they attached to taking the time to think about new techniques, instead of using them in a blind way just because they are very powerful and valuable tools.

By accepting to follow guidelines, they showed that protecting laboratory personnel, the general public, and the environment from any hazards that might be generated by the experiments is a duty they are willing to follow, even if it shall alter a bit their right to freedom of research.

Negative aspects

Firstly, the scientists were the only to decide about the regulation of recombinant DNA: they wrote a draft for recommendation that the NIH reproduce as a guideline. There was no discussion that involved other stakeholders at all. However, it is crucial to involve the rest of the society, and showing citizens that you are willing to listen to them is a necessary step to obtaining their trust. It is also fundamental if you want them to really consider the new technology, and not just reject it to show their defiance to a scientific world that would otherwise be seen as trying to impose its technology on society.

Secondly, the recommendations were sometime hard to follow as it implied building new structures (necessary for confinement), but sometimes lab could not afford it. Should the state have helped in financing these new structures?

Thirdly, the guidelines, as they were written, applied to federally funded research only. Should laws have been made instead of guidelines?

Finally, ethical and legal implications of genetic engineering of plants, animals and humans were not considered. According to Paul Berg, *"this choice of agenda was due neither to oversight nor unawareness; it was deliberate, partly because of the lack of time at Asilomar and partly because it was premature to consider applications that were so speculative and certainly not imminent. In 1975, the principal and more urgent concern for those gathered at Asilomar was the possible effect of recombinant DNA technology on public health and safety"* [8].

B. Concerns raised by Synthetic Biology

1. Recombinant DNA technology

Without this technology, many applications of synthetic biology would not be possible, and especially the iGEM competition were we spend more than half of our time cutting and pasting biobricks together (something often referred to as cloning). Therefore, all concerns raised by recombinant DNA technology, or as it is now called, genetic engineering, can reappear in a debate about synthetic biology.

We have seen above that these concerns were mainly about biosafety (as scientists had defined them this way). However, 37 years have passed since the Asilomar conference in 1975, millions of experiments have been conducted, and no accident has ever been reported. However, there are still other concerns standing today about genetic engineering. For example, concerns about some of its applications: releasing organisms that have been bioengineered in the environment (GMO crops for example), or using this technology on the human body. The debate still standing today is mostly about the ethical and legal concerns these applications raise, and not so much about the technology itself. Consequently, we will deal with these issues in the second part of the essay.

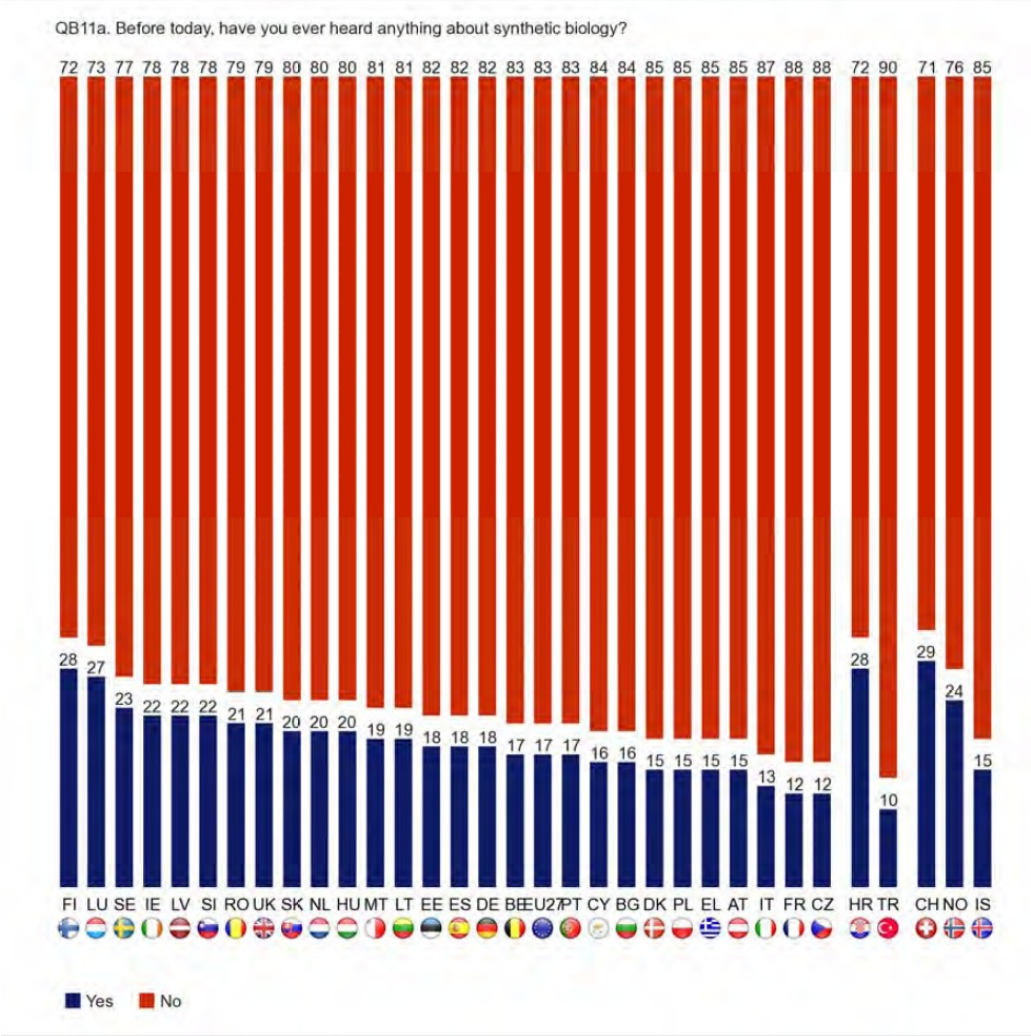
2. Synthetic Biology: Awareness, perceptions, concerns and regulation

We are going to look at the population's awareness, perception and concerns about synthetic biology, as well as how they think it should be regulated. We will then analyze these concerns and their trust in the government for regulation.

i. Awareness of synthetic biology

In Europe

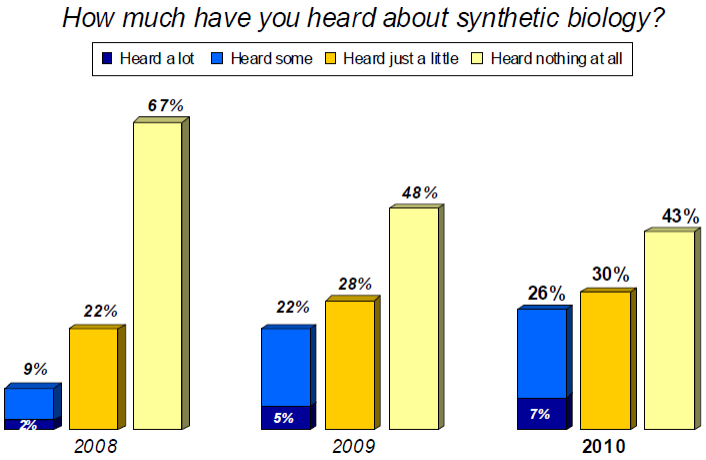
According to the 2010 Euro barometer on biotechnologies [9], most Europeans have never heard of synthetic biology: "only 17% of respondents at the EU27 level had heard anything about synthetic biology prior to the survey". In France, this number falls to 12%, as shows the chart below.



However, awareness of existence of synthetic biology is higher among managers (26%), daily internet users (22%) and those with a science education (21%)

In the United States of America

According to the study "Awareness & Impressions Of Synthetic Biology" conducted by Harts Associates in 2010 [10], awareness about synthetic biology in the USA is increasing. In 2010, only 43% had never heard a thing about that field.



Awareness ("heard some") is higher among men (32%), especially men under age 50 (35%), college graduates (37%), and those with household incomes more than $75,000 (40%).

Comparison between Europe and the US

Americans seem more aware of synthetic biology then Europeans. In 2010, only 43% had never heard a thing about synthetic biology, whereas this number rises to 83% in Europe.

ii. Perception of Synthetic Biology

In Europe

The Woodrow Wilson International Center for scholars studied European media coverage on Synthetic Biology: [44].

"Playing God: The man who would create artificial life" (The Independent, January 25, 2008)

"It is life but not as God planned it" (The Guardian, April 1, 2004)

"The Bacterie van Frankenstein" – "Frankenstein bacteria" (NRC Handelsblad, December 14, 2005)

"Man could be on the brink of creating the first artificial organism, a landmark development that would provide a profound insight into the origins, workings and essence of life, and vast new opportunities to exploit living organisms. But this pioneering research has inevitable triggered unease about the limits of science, fears about 'playing god,' and raises the specter that this technology could one day be abused."

—Roger Highfiled, The Telegraph, June 29, 2007

In the USA

Respondents were asked to describe what they think synthetic biology is:

What Do You Think Synthetic Biology Is?

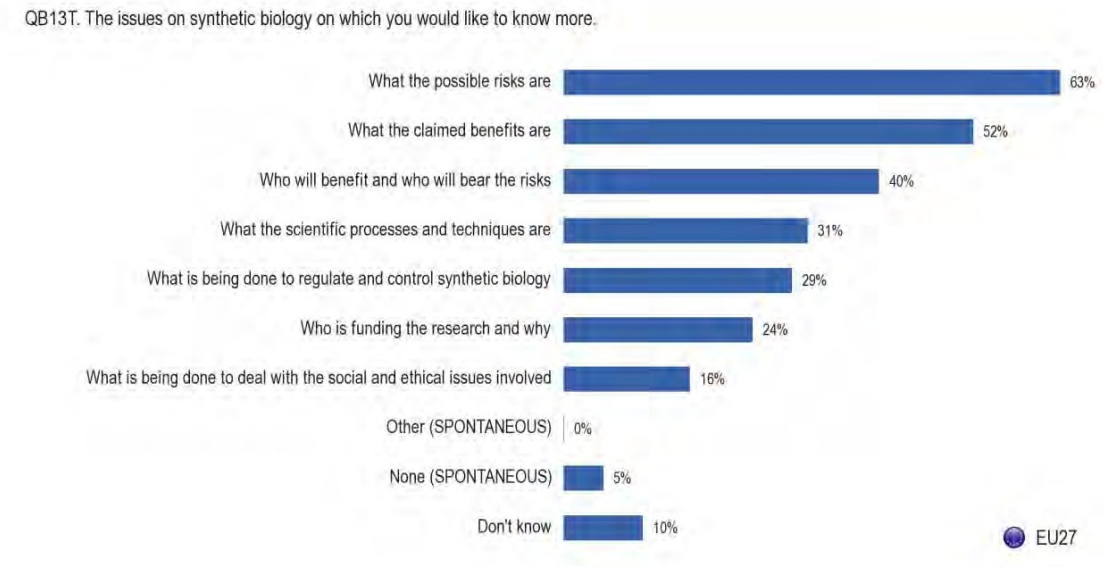
Volunteered Comments

Something man-made, artificial, fake, not natural, not real	30%
Has to do with genetic engineering, altering the biological makeup	12%
Has to do with science, biology, the study of living organisms	6%
Cloning	6%
Used in medical research to develop new medicines, treatments	5%
Some kind of synthetic material or chemical	5%
Don't know; no response	29%

It is interesting to see that 30% of Americans describe Synthetic Biology as being unnatural. We willl provide further analysis below.

iii. Concerns about Synthetic Biology

In Europe



Respondent were primarily concerned about the possible risks (63%). Claimed benefits only came second. Is this a mirror of our current society with its principle of precaution and rejection of vaccination, were risks are less and less tolerated?

40% of Europeans are worried by how ethic synthetic biology is. They would like to know more on who will benefit and who will bear the risks.

24% are worry about intentions behind synthetic biology research. They would like to know more about who is funding the research and why.

In the USA

63% of Americans are concerned about potential risks of synthetic biology: 27% most fear that it could be used to create harmful things such as biological weapons, 23% fear most that it could cause negative health effects for humans, and 13% fear most that it could damage the environment. It would have been interesting to ask them if they fear that could happen because of an accidental release, or because of an intentional release to use synthetic biology as a tool directly in the environment.

25% of respondents are most concerns by the fact that it is morally wrong to create artificial life.

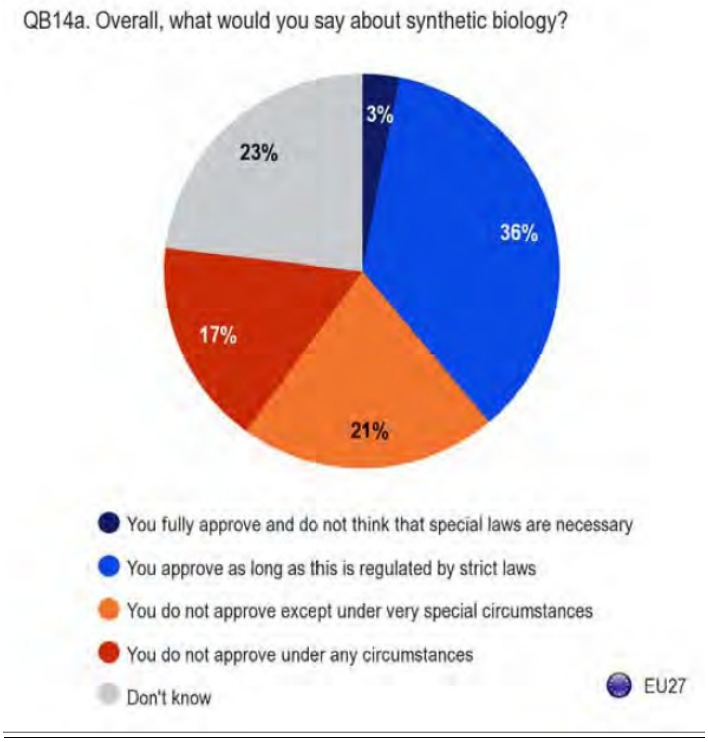
Comparison between Europe and the US

Both European's and American's main concerns seem to be about potential risks created by Synthetic Biology.

iv. Approval of synthetic biology

In Europe

There is no clear approval of synthetic biology:

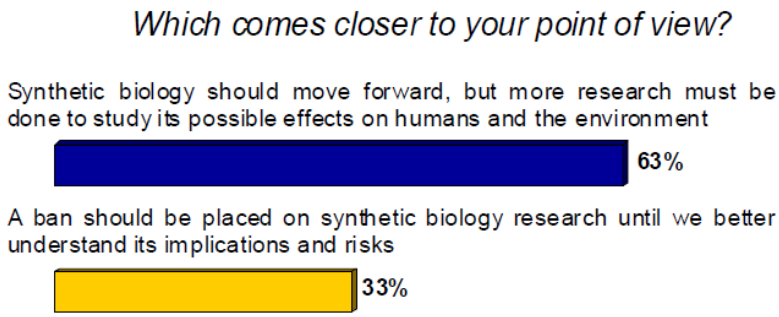


Only about 1 in 3 Europeans approve of Synthetic Biology, with a necessary condition that it should be strictly regulated by the government.

Further analysis show that 60% of those who are aware approve of synthetic biology compared to only 36% of those who are not aware. We will discuss this below.

In the USA

By two to one, public supports continued work in synthetic biology over ban.



Comparison between Europe and the USA

It is hard to offer a comparison if we consider only the documents presented above, as the question asked is not equivalent.

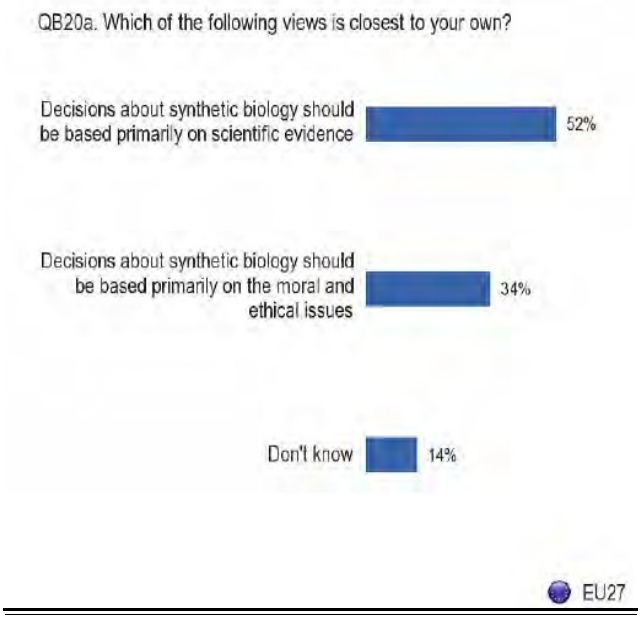
v. Who should regulate synthetic biology and what should be taken into accounts when making guidelines and laws? (and trust in regulations agencies?)

In Europe

For a majority of European, scientific evidence should weight more than moral and ethical issues in the process of decision making.

However 34% Europeans think the opposite.

Therefore, we can see that there is no consensus on the subject.

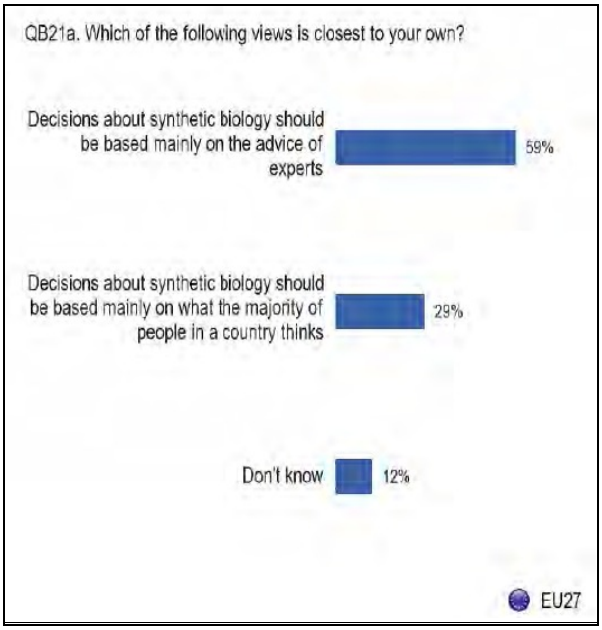


60% of Europeans think that the advice of experts is more important then what the majority of people in the country think in the process of decision making.

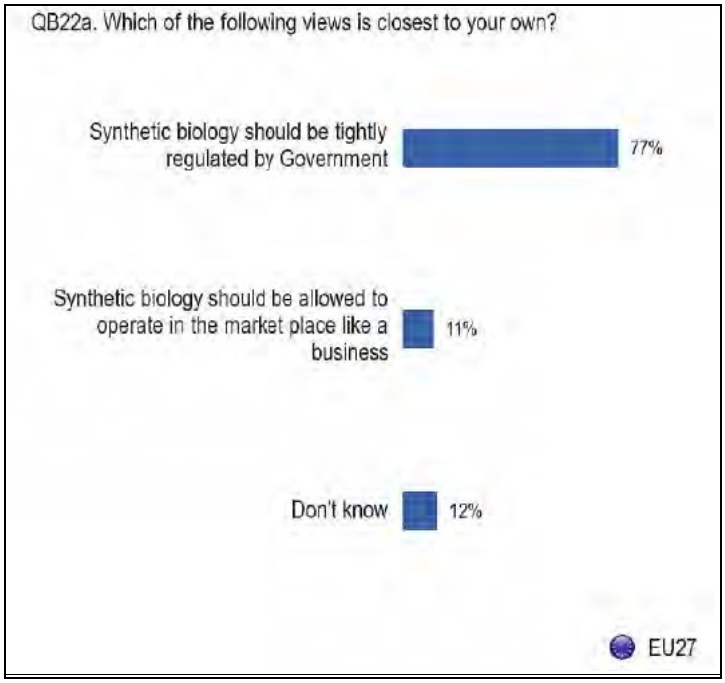
This shows the trusts of European in experts.

The experts responsible for biotechnologies they trust most in terms of doing good for the society are medical doctors (81%), university scientist (77%), consumer organizations (73%) which test biotechnological products and environment groups who campaign for biotechnologies (66%). The European Union making laws for its members (60%) comes in 8th position and National Governments place before last with 55%, just above religious leaders who say what is wrong and right in the development of biotechnologies (31%)

This shows that Europeans trust more scientists then politicians, and that they have comparatively lower trust in religious leaders who say what is right and wrong in terms of biotechnologies. However we shall keep in mind that although comparatively low, 31% is far from 0% and that some people still think that religious should have its say when it comes to biotechnologies



Support for a free market approach is low. A vast majority of Europeans believe that Synthetic Biology should be regulated by the Government



All in all, Europeans do not necessarily agree about the factors that should be taken into account when making decisions about synthetic biology, and who should be listened to, but they agree that Synthetic Biology should be tightly regulated by the government.

In the USA

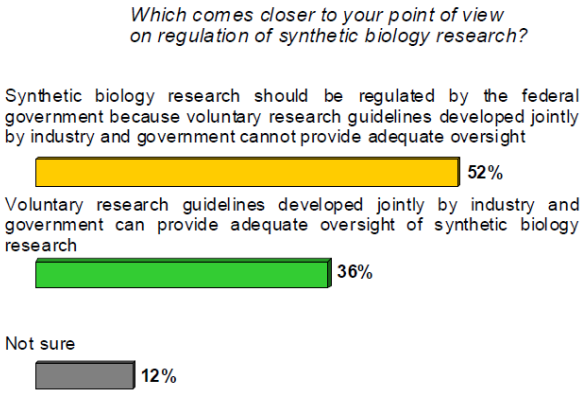
Americans are divided when it comes to Synthetic Biology regulation.

Laws imply a strict government regulation. They are way more binding then guidelines. If someone ignores guideline, he may lose his founding (especially if it's a government funding), but he will not go to prison or have to pay a fine.

52% believe that this field should be regulated by laws (and so by the government)

36% believe that Synthetic Biology should be regulated by guidelines, like the recombinant DNA technology was.

Therefore, a majority is for a tight regulation by the government.



Comparison between Europe and the USA

American's and Europeans are globally in favor of a tight regulation of Synthetic Biology by the government.

3. Analysis of the concerns raised by synthetic biology

i. Unnaturalness

30% of Americans think that Synthetic Biology is unnatural. Some Europeans also describe Synthetic Biology that way according to Professor Gouyon (c.f. interview). We now have to ask ourselves two things: is the adjective "unnatural" negatively connoted? If it is, what are people so concerned about?

Is the adjective "unnatural" negatively connoted?

The answer to the first question is yes. The term unnatural is generally negatively connoted. In our current society, some people have that aspiration to come back to Nature. Products made from plants, natural medicine, organic food and meditation are the new must. These people's ideal: to be closer to, and in harmony with, Nature. The idea behind is that Nature is healthy, nature is good, compared to its opposite, "technology". Technology had made men sick: it has produced pollution and unhealthy living habits that now cause obesity and cancer. Technology has also alienated human beings: communication with people at the other end of the planet is possible in seconds, and yet, men have never been loonier, isolated from others by a computer screen, a smart phone and virtual internet friends.

The spectrum of people that can be included in that category is very broad. We can find:
- Some pro technology. They recognize the huge benefits it brought society, but are also well aware of the risks. When deciding if they should use the technology, they like to weight the benefits and risks.
- Some less pro technology. They acknowledge that technology can bring some benefits, but they believe that the risks always surpass these benefits.
- Some anti technology. They only see the risks.

What are people so concerned about?

They are concerned that by playing with powerful tools, we will disrupt the ways things are supposed to be. Synthetic Biology is a tool we do not even fully understand so that makes thing even worse. They call to a "natural order of things" and say that Synthetic Biology will disrupt that order, that we do not know what is good or bad, that we will never know it, and that we should by no means upset that invisible balance that exists in nature[1].

This supposed to believe in a "natural order of things". But have we not, by all time, disrupted this supposed order? First by discovering fire which enabled us to disrupt the

[1] These were issues raised by genetic engineering too, but they only get worst with synthetic biology because while the first implies transferring only one gene of interest from one organism to another, the latter implies constructing completely new genetic circuit, hence, going further in disturbing the

obscurity and alter the temperature, and latter with big factories, vaccinations, computers, intensive agriculture, battery farming … However, do not read us wrong, we are not, like many scientists before, discarding their view point. We are just wondering: what are people REALLY concerned about when they use the "unnaturalness argument"?

What are people REALLY concerned about?

We discussed this issue with Pr Gouyon and Pr Morange (cf interviews), and they both came to the same conclusion: the "unnatural" argument (an argument that often sounds irrational) actually conveys rational concerns and fears.

Pr.Gouyon gave the following explanation: most people acknowledge that things that come from nature can be dangerous too. For example some mushrooms are deadly, some plants are toxic, and volcano irruptions may be 100% natural but are no less damaging. However, **nature has the advantage to be familiar**. We know which mushrooms are deadly, which plants are toxic, which food we are allergic to, and volcano irruptions can usually be predicted[2]. These things have been around for longer than we have, and so they are well known and studied. Synthetic Biology has been around for less than a decade, it is new, and this is the real problem. New is scary. And yet, men need to get over this fear of innovation and the unknown if they want to extend their knowledge and their quality of life.

The Woodrow Wilson international center for scholars also concluded to the rational character of the 'unnatural argument': "Both American and European press analyses show that metaphors such as "playing God" or "Frankenstein-like" were employed as well, but usually in reports emphasizing the potential risks of synthetic biology".[44]

When are people willing to take risks?

Innovation implies a certain level of risk. People are usually willing to take risks, but it depends on a few factors: perceived benefits, vision of the future and level of trust in the government and organ making decision.

People will usually consider the balance benefits/risks. Depending on the benefits expected, the level of risk that is acceptable will vary. For example, if the aim of a treatment is to cure cancer, more severe side effects will be tolerated. However, if the aim of a treatment is just to cure a mild headache, one losing his hair due to the treatment would be an unacceptable secondary effect. We believe it works the same with new technologies, and with synthetic biology.

[2] This statement is not 100% true as we, of course, do not know EVERY deadly mushroom (etc), but this statement is accurate enough for the point to stand. In fact, if people stay within the framework of society (and do not decide to go and explore a desert island or some unknown forest for example), the natural things they come in contact with are fairly well known.

People will also compare to what already exists. If one treatment with severe side effects is the only way to cure a certain type of severe disease, important side effects will be accepted. Nonetheless, if another way to reach the same goal exists, but with lower secondary effects, the first treatment will not be judged acceptable anymore. The same applies for new technologies. If they bring an answer to an unsolved problem, or a better solution to an already solved problem, then people might accept to take a certain level of risk. However, if they bring either a non convincing solution to a problem, or a solution to a problem that already had a convincing answer, then people might not be willing to take risks.

ii. Playing God

Some people that are against synthetic biology call to the notion of "God". They will tell you that by doing synthetic biology, we are playing God, taking ourselves for God.

We believe that the "playing God" argument is, just like the "unnatural" one, essentially a translation of people's discomfort and fears when it comes to changing how things are known to be (cf. "what are people really concerned about" just above) as religion is not necessarily against synthetic biology[3]. Moreover, according to Sara Aguiton, "Defending oneself to play god is always a way to refer to a power that some have – and other don't". Therefore, the 'playing God argument' could also be a way for the population to convey its fear of monopoly on synthetic life.

iii. Status of artificial life

There is no unequivocal theory about life, so biologists have described life in descriptive terms [13]. Life is considered a characteristic of organisms that have the ability to: move, reproduce and feed.

When we create new genetic circuits and implement them in bacteria to create a new function, we do not create a new life form, as the bacteria was already fully alive. The life form is still seen as natural, as it existed prior to the modifications we introduced.

However, when Craig Venter's team transferred a completely synthetic chromosome containing all the necessary information for survival in a bacterium that had previously been deprived of all its genetic information, he created life [15], at least, this is what biologists called it. He created life as he gave an inert bacterial cell enough information so it would perform all the tasks that are necessary to be considered a "living organism". This is kind of life is referred to as artificial as it is manmade. However, one should wonder: is there such a thing as artificial life? Does life limit itself to the genetic program, or does it include all the rules that apply to our planet, and make life possible?

[3] We met with a catholic priest, farther Dominique FLEURIOT DE LANGLE. For example, in then Catholic religion, only the human life is sacred so as long as scientists do not threaten in an obvious and wanted manner the integrality of what makes the human being, it has no problem with synthetic biology.

Creating life from entirely de novo DNA synthesis was one of Synthetic Biology's aims, and the success of Venter's minimal genome project has proven this possible. We may still be far from complex multi cellular organisms being synthesized de novo, but we should be prepared to the eventuality. If this happens, questions regarding the status of artificial life form should be addressed. Should it be put on the same level as "natural" life, or should it have a separate status, with different rules applying to it? We believe that this second option could prove very dangerous, and that life should not be discriminated upon, no matter its origin. We will however not develop further on that last statement as we believe that synthetic biology is still very far from developing complex organisms, so that question is not going to be relevant for quite some time.

iv. Physical harms

Notions of biosafety and biosecurity

The moratorium called in 1974 on all experiments using recombinant DNA technology and the Asilomar conference that followed are good proof that biosafety and biosecurity have, from the very start, been considered a priority by the scientific community. Biosafety and biosecurity stays a priority, as shown by:
- the many publications on the subject,
- the important place they occupy in the almost yearly conference series "Synthetic Biology X.0" organized by the biobrick foundation,
- their rising popularity in the iGEM competition.

"According to the WHO, **biosafety** is the prevention of *unintentional* exposure to pathogens and toxins, or their accidental release, whereas **biosecurity** is the prevention of loss, theft, misuse, diversion or *intentional* release of pathogens and toxin" [17].

The first deals with failure whereas the latter deals with malice [18]. However, no matter whether they result from mistake or intentional purpose to cause harm, the risks are the same: the environment could be damaged and human health altered. These are sometime referred to as biorisks or physical harms [4].

What exactly are the possible dangers with genetically modified bacteria?

Some dangerous bacteria can be created with synthetic biology:
- New toxins can be created,
- Harmless organisms can be made pathogen,
- The virulence of known pathogens can be increased,
- Bacteria that bear an antibiotic resistance gene.

If these bacteria are released in the environment, by accident or by malice they could harm the environment and create serious health issues. Other bacteria that seem harmless could

cause harm too if release in the environment because of a potential negative consequence of horizontal gene transfer or excessive proliferation which could disrupt the ecosystem.

Biosafety: dealing with unintentional harms

In the scenario where bacteria are confined in the lab[4], biosafety means making sure that
- Scientist do not get harmed by synthetic bacteria while working in the lab
-Avoiding that synthetic bacteria escape the lab

Good practice rules like the one defines in the Laboratory Biosafety Manual are there to make sure that scientists do not get harmed by synthetic bacteria while working in the lab. However, most genetically modified bacteria are harmless.

To avoid that synthetic bacteria escape the lab, bacteria can be made dependant on a nutrient they can only find in the lab, for survival, or may lack essential molecules for DNA replication. Moreover, lab strain are usually very weak so unlikely to survive if they were to be accidentally released in the wild. Some iGEM teams such as Unist Korea 2011 (CHOp-Coli-LATE) have thought of suicide systems that activate if bacteria leave the lab.

No accident has ever been reported with bacteria obtained by genetic engineering. Many people expect it to be the same with bacteria obtained by synthetic biology. Some, however, fear novel emergent properties that could arise from the entire new genetic circuits that synthetic biologists are constructing. However, since now, these fears have proven unjustified.

Biosecurity: dealing with intentional harms

In 1972, Biological and Toxin Weapons convention (BWC) prohibits the synthesis of known or novel microorganism for hostile purposes. However, this convention is not signed by all states, and does not bind terrorist organizations; therefore it does little to prevent the deliberate misuse of synthetic biology. In November 2003, some experts assembled by the U.S Central Intelligence Agency concluded that *"Growing understanding of the complex biochemical pathways that underline life process has the potential to enable a class of new, more virulent biological agents engineered to attack distinct biochemical pathways and elicit specific effects"* [16]

The fear is that that badly intentioned people could use genetic engineering and synthetic biology to create biological weapons. This fear has increased when the poliovirus and the Spanish Influenza virus were synthesized de novo.

[4] NB: we are well aware that biosafety exceeds the lab scale but we will be dealing with that in the second part, when we talk about releasing genetically modified bacteria in the wild.

Georges Church, in his "Synthetic Biohazard Non-proliferation Proposal", suggests that a **DNA Instrument & Reagent Registry** (DIRR) database and a **profit DNA Agent Clearinghouse** (DAC) be set up.

- ✓ **DNA Instrument & Reagent Registry** (DIRR) database: *"Sales and maintenance of oligo synthesis machines and supplies would be restricted to licensed non-profit, government and for-profit entities. All use of reagents and oligos would be automatically tracked and accountable (as is done for nuclear regulations).* […] *Under the above licenses, all synthetic oligos would be screened for "similarity" to known "select agents"* [41]
- ✓ **Profit DNA Agent Clearinghouse** (DAC): *The software (e.g. BlackWatch), installation and testing would be made freely available to the oligo vendors. Any positive matches that are found on site would be sent to the Clearinghouse, with a copy to the site managers. Staff at the Clearinghouse (with security clearance) would evaluate the sequences and make an immediate preliminary assessment. They would also add those sequences to a second system that would look for patterns of activity like related oligos being ordered from multiple vendors. If something significant turns up, the Clearinghouse can contact the vendor and go directly to someone at the FBI.* […] *This approach frees the vendors from a lot of effort and responsibility. Customer data remain confidential unless a match is found. It puts the task of assessing sequence matches and false positives in the hands of experts"*

v. Regulations

How should synthetic biology be regulated?

Who should set the rules: scientists, politicians, intellectuals, the free market?
How should these rules be enforced? Guidelines, laws, deontological rules?

According to the Eurobarometer, Europeans think that synthetic biology should be regulated by strict laws (so by the government). These laws should be made after consulting experts in synthetic biology.

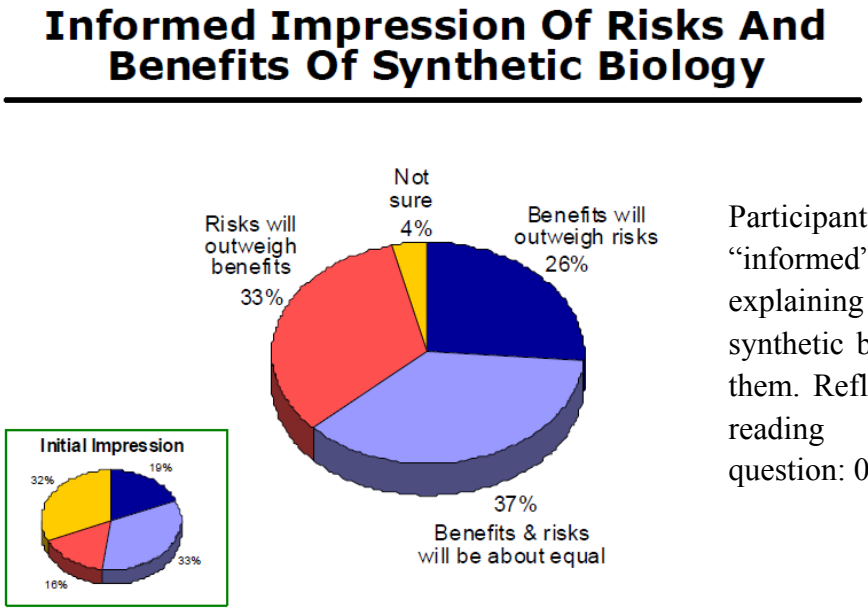
4. Will rising awareness change anything?

At first, the vision shared by the "knowledgeable" was that people tend to reject genetic engineering, synthetic biology, and new technologies in general because they are ignorant. This perception of non-scientist is called the « deficit model" (people are ignorant, scientists know) as was developed by Bryan Wynne. The underlying mechanism is simple: lack of understanding generates fear that in turn generates major rejection of new technologies. Their solution: to educate, and educate and educate again. This was actually the main reason to

educate people: so they could accept this new technology. We have two problems with this way of thinking.

i. Polls have shown that skepticism is not simply caused by lack of information

"While more knowledge encouraged people to form a definite opinion about biotechnologies, this opinion can be either for or against" [36]. Hart Research Associates' 2010 poll on synthetic biology [10] goes in that way.



Participants are considered as "informed" once a small text explaining the benefits and risks or synthetic biology has been read to them. Reflection time between the reading and answering the question: 0

However, this seems to be in contradiction with the Eurobarometer 2010 on Synthetic Biology: *"Further analysis show that 60% of those who are aware approve of synthetic biology compared to only 36% of those who are not aware*" [9]. These two figures can be tricky if examined out of context, but placed back in context, there are no contradictions, and conclusion can be drawn on educational techniques used to raise awareness.

Eurobarometer 2010 study: the term "those who are aware" refers to the 17% of population that had already heard about synthetic biology. All the people that work in the synthetic biology field (and therefore approve of it), or have an interest in innovative biological field are in this category, so, it is logical that the amount of people that approve of synthetic biology is higher in this category (selection bias). Therefore, this should not be generalized to: a higher level of education will raise awareness. There is a difference between people that KNOW about a field and people that WORK in a field/ have a GENUINE INTEREST in a field. Right now, people that know about synthetic biology are basically the people that work in that field or show some genuine interest for it (I am generalizing a bit here for the sake of the explanation). Therefore, some can have the impression there is a correlation between level of education and approval of synthetic biology, but there is a factor of confusion here. As the general population will be further informed, this confusion factor will be diluted. This is exactly what happened when some scientists came to the conclusion that there was a

correlation between gender and level of lung cancer. Being a man is not directly correlated with lung cancer. However, males tend to smoke more which in turns leads men to statistically have more lung cancer.

In the study done by Hart Associates, there is a more direct link between informed impression and impressions of risks and benefits. However, we do not agree that someone qualifies as being informed after this short text has been read to him:
"*The potential BENEFITS of synthetic biology include developing new microorganisms to treat disease, including cancer, more effectively and to create new and less expensive medications. It also could be used to make new organisms that could provide cheaper and cleaner sources of energy than today's oil-based fuels, and to detect and break down environmental pollutants in the soil, air, and water.*
While the potential RISKS of synthetic biology are not known, there are concerns that man-made organisms might behave in unexpected and possibly harmful ways and that they could cause harm to the environment. There also are concerns that, if these organisms fall into the wrong hands, they could be used as weapons. Additionally, the ability to create artificial life has raised moral and ethical questions about how life is defined."

We hear a lot of people talking about the necessity to educate further the population. What exactly do they mean by educating? A press release? A documentary? A TV advertisement? Teaching it to children at school? We believe that teaching middle and high school student is a very efficient way to educate the general population. Why is that?
- School is mandatory till 16 years old and most people go to school till 18.
- Teachers get involved
- Some parents will be educated through children

We suggest the organization of workshops in middle and high school, and extend this to a mandatory course called "new technologies". However, other methods should also be used in addition to that, in order to educate other parts of the population. Documentaries seem to be a good idea. For example, the web documentary that a journalist from Le Monde is making, in collaboration with our team, is a great idea.

We just discussed the first problem we had with this simplistic mechanism mentioned earlier: "lack of understanding generates fear that in turn generates major rejection of new technologies. Their solution: to educate, and educate and educate again. This was actually the main reason to educate people: so they could accept this new technology". Let's now discuss the second problem.

ii. <u>We disagree with the finality of educating on new technologies so the population can accept them better</u>

We think that this should not be the finality of education. However, education on new technology should be provided so people can be more aware of what is happening around them. This is important so they can understand the technologies that are shapping/will shape their lives, and decide if they want to this technology or not in the most enlightened way possible.

C. Conclusion

Firstly, we studied the historical background of synthetic biology. We presented synthetic biology as an extension to genetic engineering, and examined the shared controversies around recombinant DNA technology. We showed that scientists handled the situation in an exemplary way, and we provided a detailed analysis of the 1975 Asilomar conference.

Secondly, we studied the concerns raised by synthetic biology nowadays. We used numbers from the 2010 Eurobarometer on biotechnologies and Hart Research Associates' 2010 poll on "Awareness & Impressions of Synthetic Biology" to study awareness, perception, and approval of synthetic biology in the European and American populations. We showed that the level of awareness of synthetic biology in Europe is incredibly low (only 17% of participants had already heard of synthetic biology previous to the poll), that the approval rate is low to average in both Europe and the US, that these populations want tight government regulation, and that the main concerns raised by synthetic biology are: unnaturalness, playing god, status of artificial life, potential physical harms, regulations. We then provided a detail analysis of these concerns. We came to the conclusion that:
(a) The "unnaturalness" and "playing God" arguments convey the population's fear of novelty and of the unknown, and should not just be tossed aside;
(b) Religion is in favor of synthetic biology and does not consider that synthetic biologists are "creating life";
(c) Questions such as "is there such a thing as artificial life?", "what will be the status of this artificial life?" will have to be addressed someday, and probably sooner than later;
(d) Biosafety measures to prevent bacteria from harming workers or escaping the lab and proliferating in the wild are efficient;
(e) Church's proposal seems to be a good starting point for biosecurity;
(f) Synthetic biology should not be regulated by the free market.

Thirdly, we examined the common question "will rising awareness change anything?", and its implications. We came to the conclusion that
(a) Skepticism is not necessarily due to lack of awareness;
(b) We disagree with the finality of educating on new technologies so that the population can accept them better.
(c) Education on new technology should be provided so people can be more aware of what is happening around them. Educating middle and high school students could be one way, amongst others, to achieve this goal

Proposal 1: To organize a workshop on synthetic biology for high school students in order for them to discover this new field. If this is a success, we would like that in the future, collaboration with a middle school or high school be a requirement for an iGEM gold medal. This would drastically raise the world level of awareness about synthetic biology.

Proposal 2: Extend proposal 1 to a mandatory course called "new technologies".

II. The debate about putting GM bacteria in the environment

We spent a lot of time talking about the problems inherent to the technique of synthetic biology. We believe that in a debate about putting genetically modified bacteria in the environment, it is crucial to differentiate the part of the debate that is just about synthetic biology and the one that really concerns the application in the field. The debate on the technique should happen, and then be closed once and for all so we can move forward to discussing the applications. We are now moving forward to discussing applications, are more precisely the use of GM bacteria directly in the environment.

"The 193 nations that are Parties to the UN Convention on Biological Diversity (CBD) agreed at their 10th Conference in 2010 that the release of synthetic biology's products requires precaution. The agreement from the 10th Conference of the Parties reads: *"Parties and other Governments [are] to apply the precautionary approach in accordance with the Preamble to the Convention, and the Cartagena Protocol, to the introduction and use of living modified organisms for the production of biofuels as well as to the field release of synthetic life, cell, or genome into the environment"* " [42]

A. Our master security system

Biologically speaking, we identified two sets of problems concerning the release of synthetic bacteria in the environment. The first set does not restrict itself to synthetic biology. It encompasses all issues raised by the introduction of a non native bacteria in a new ecological niche, regardless of its synthetic or "natural"[5] pedigree. The second set is more specific to synthetic biology. Synthetic bacteria contain whole new genetic circuits, which mean possible emergent properties arising from the complex interactions of its constituent genes. As the field is quite recent, very little is known on these possible emergent properties, making problems raised by "natural" bacteria released in a new ecological niche even bigger and they become much harder to predict.

All in all, biologically speaking, these sets of problems boil up to two things: horizontal gene transfer and excessive proliferation, although emergent properties of synthetic systems could make these problems worse.

[5] By « natural » we mean a bacteria that has not been genetically modified.

1. Horizontal gene transfer

i. What is horizontal gene transfer?

Introduction: Cells, Bacteria & DNA

The building block of any living organism is the cell (just like the building block of a house is the brick). Human and plants are complex organisms: they are made up of many cells. Bacteria are more simple organisms: they are made up of just one cell.

In every cell, we can find some 'genetic information'. This information (coupled to environmental stimuli) will tell the cell how to function.

Let's looks specifically at bacteria. A bacteria is made up of one cell that contains genetic information to tell it how to function. This genetic information is called DNA, and is organized in the following way:

- ✓ Most of it is condensed in the form of a stick called a **chromosome**.
- ✓ A small part of it can exist as a **plasmid**: a small circular fragment that can be easily exchanged between bacteria. This is for example how bacteria exchange information on antibiotic resistance: they exchange plasmids containing this information.

Bacteria are living organisms so they are able to feed, move, and reproduce. All these functions are encoded in the bacteria's DNA. A bacteria will reproduce by the process called 'asexual reproduction': it will grow a bit, duplicate its genetic information, and then split in two identical clones: one 'mother cell' gives two identical 'daughter cells'. This type of reproduction does not call for much diversity. Yet, diversity is essential for evolution and survival and there is in fact a lot of it in bacterial population. Horizontal gene transfer (and mutations) contributes to this diversity.

A general explanation of horizontal gene transfer

In every cell, we can find genetic information. Genetic information interacting with environment shapes bacteria's life.

Let's look specifically at bacteria. A bacteria is unicellular organism that contains genetic information carried by DNA, and is organized in a condensed form called a chromosome.

A small part of it can exist as a plasmid: a small circular fragment that can be easily exchanged between bacteria. This is for example how bacteria exchange antibiotic resistance: they exchange mobile elements containing this information.

Bacteria are living organisms so they are able to feed, move, and reproduce. All these

functions are encoded in the bacteria's DNA. A bacteria will reproduce by dividing: it will grow a bit, duplicate its genetic information, and then split in two identical clones.

Horizontal gene transfer is the exchange of genetic information between organisms, that is basically sex.

- ✓ **Transformation:** is the uptake of free DNA by competent bacteria. Competence is a physiological state, often highly dependent of the environment, which enables bacteria to uptake macromolecules that bind to its surface. It does not require a close contact between bacteria and the free DNA usually comes from long dead ones. In the presence of homologies, there can be recombination and integration of the free DNA into the bacterial genome. This can give the bacteria a new function, or disrupt an already existing one, depending on where the insertion takes place.

- ✓ **Conjugation:** consists in gene transfer by the means of plasmids and conjugative pili. It requires a close contact between bacteria. It occurs primarily between closely-related strains or species, although it can occur between distantly-related species. The donor needs to possess the capacity to create conjugative pili, a capacity not shared by all bacteria. Conjugation is very wide spread between bacteria and is the most important mechanisms for translocating DNA between bacteria (Espinose-Urgel, 2004; Grohmann et al, 2003). In addition, conjugation is also used by certain phytopatogen such as Agrobacterium to insert DNA into plants cells as part of the infectious process [26]

- ✓ **Transduction:** gene transfer mediated by a bacteriophage, that is by a virus that is specific to bacteria [22] HGT plays a key part in bacterial evolution and has enabled bacterial populations to occupy entirely new niches (Burrus and Waldor, 2004). For example, under an intense selection pressure due to wide use of antibiotics, multi resistant plasmid arose from various origins and antibiotic resistance, mainly from these mobile genetic elements, spread in less than 5 decades (Hartl and Dykhuizen, 1984; Davies, 1994) [27].

A more technical and historical explanation of HGT

Fred Griffith discovered Horizontal Gene Transfer (HGT) in 1928 when he reported the transfer of genetic material from heart-killed virulent Streptococcus pneumoniae to an avirulent form of the bacterium by a process he described as transformation (Bushman, 2002).

Much later, in 1946, other forms of HGT, such as conjugation and transduction were discovered.

In 1980, the terms “horizontal gene transfer” and “lateral gene transfer” appeared (Gogarten et al, 2002; Kooning et al, 2001; Ochman et al, 2000; Syvanen, 1994), to designate all form of gene exchange that occurs between organisms in nature without recourse to reproduction [26].

✓ **Transformation** is the uptake of free DNA by competent bacteria. Competence is a physiological state, often highly dependent of the environment, which enables bacteria to uptake macromolecules that bind to its surface. It does not require a close contact between bacteria and the free DNA usually comes from long dead ones. In the presence of homologies, there can be recombination and integration of the free DNA into the bacterial genome. This can give the bacteria a new function, or disrupt an already existing one, depending on where the insertion takes place.

✓ **Conjugation** consists in gene transfer by the means of plasmids and conjugative pili. It requires a close contact between bacteria. It occurs primarily between closely-related strains or species, although it can occur between distantly-related species. The donor needs to possess the capacity to create conjugative pili, a capacity not shared by all bacteria. Conjugation is very wide spread between bacteria and is the most important mechanisms for translocating DNA between bacteria (Espinose-Urgel, 2004; Grohmann et al, 2003). In addition, conjugation is also used by certain phytopatogen such as Agrobacterium to insert DNA into plants cells as part of the infectious process [26]

✓ **Transduction:** gene transfer mediated by a bacteriophage, that is by a virus that is specific to bacteria [22]

HGT plays a key part in bacterial evolution and has enabled bacterial populations to occupy entirely new niches (Burrus and Waldor, 2004). For example, under an intense selection pressure due to wide use of antibiotics, multi ristant plasmid arose from various origins and antibiotic resistance, mainly from these mobile genetic elements, spread in less than 5 decades (Hartl and Dykhuizen, 1984; Davies, 1994) [27].

ii. Since when have people been concerned about HGT

HGT has been a concern since the very start of recombinant DNA technology. At the time, the concerns were that genetically modified bacteria could escape the lab, and the potential consequences this could have on health and the environment. The consequence of HGT between engineered bacteria that escaped the lab and wilt type bacteria was one of these biohazards. However, the concerns that bacteria could escape the lab were tuned down with time, and by the fact that no accident was ever reported [8].

HGT concerns were resuscitated when people considered putting GMO plants and Crops in the field: *"The possibilities of horizontal gene transfer from plants to microorganisms are frequently evaluated in such risk assessments of GMPs before release into the field"* [30]. It is generally admitted that the rate of horizontal gene transfer between plants and microorganisms is so low that it not represent a serious problem, though many scientists have contested this assessment and the way measures were done [26, 30, 31].

Since people have been considering the release of genetically modified bacteria in the environment, concerns about HGT have been raised too: "*safety concerns and legislative constraints have limited the field application of GEM to date"* [24]

Let's look at a few statements taken from the literature.

The assessment of the impacts of GEM release on the environment must consider possible effects of release of recombinant DNA to the environment on all biotic parts of the environment including humans, animals plants and microflora as well a the abiotic parts of the environment (Doblhoff-Dier et all, 1999; Jank and Gaugitsch, 2001) [21]

An OEDC guidance document issued in 2010 on horizontal gen transfer in bacteria states that *"Considering the plasticity of bacterial genomes, the aspect of horizontal gene transfer is of importance for biosafety evaluations of transgenic micro-organisms"*. The conclusion of that same document is that *"The risk assessment of a transgenic bacterium must consider the potential for transfer of introduced genes to other micro-organisms in the environment. Of greater importance, however, is consideration of whether there would be any adverse consequences posed if gene transfer from a transgenic bacterium occurred. If no adverse effects can be envisioned, then prediction of the likelihood, frequency, and extent of gene transfer are of less concern. If detrimental consequences were to occur if genes were transferred to indigenous micro-organisms, then the exposure components of risk must be carefully evaluated, both from a theoretical perspective depending on potential mechanisms of transfer, and from a real-life perspective given natural barriers to gene transfer in the environment."* [22]

However, not all people agree that "*If no adverse effects can be envisioned, then prediction of the likelihood, frequency, and extent of gene transfer are of less concern"*, and our team certainly does not. Indeed, with such a new technology like that, how can we possible be imaginative enough to think of all the possible adverse effects that could happen if HGT happened? It is not even said that we would notice it immediately as Pr.Gouyon pointed out in his interview. Maybe we would notice it years or decades later when things start to completely sprawl out of control. Or perhaps they could be terrible consequences that we would never be able to trace for sure to HGT.

In "The Precautionary Principle Applied to Deliberate Release of Genetically Modified Organisms (GMOs)" authors point out that *"A major conclusion is that the present state of scientific knowledge is inadequate for reliable ecological risk assessment. The basic*

information with regard to mechanisms governing the environmental interactions of GMOs is insufficient. The ecosystems are too complex, and our understanding of them too fragmentary. Furthermore, currently available methods to monitor short and long-term ecological consequences of GMO release are non-existent or unreliable. Finally, the socio-economic and biodiversity aspects of GMO usage are ambiguous, and often unpredictable, based on the present state of knowledge. Hence, applying the precautionary principle should be an important basis for initiation of risk-associated research as well as for elaboration of more satisfactory risk assessment methods and procedures." [23] This was written in 1999, and yet, 12 years later, not much has changed. We know very little about the potential environment/GMO interactions: "*most of our knowledge on the behavior of GEMs in the environment has been obtained from the results in microcosm and lab studies under controlled conditions. Such behaviors, however, may not accurately reflect the behavior of the GEM in natural conditions"* [21]

In fact, many scientists have considered microcosms at the solution to study these GMO/environment interactions and assess the potential risks of HGT. However, this approach has been criticized: *"Because of lack of empirical evidence, the inventor of a synthetic microorganism could not predict the effects of its release on human health and the environment with any degree of confidence. Given these uncertainties, scientists seeking to develop synthetic microorganism for applications outside a containment facility will need to develop new ways to assess their impact on the environment"* [16]

Moreover, *"some scientists and regulatory agencies have rejected date from microcosm and mecocosm studies because of lack of consistency in the way the experiments were performed."* [16] The authors then suggest that a standardized methodology for testing environmental impacts should be developed.

So far, we have seen that a part of the scientific community is concerned about potential consequences that could arise from HGT between GM bacteria and wild type bacteria. They are well aware of how little knowledge they have, and that this knowledge is very hard to acquire beforehand (wouldn't it be so much easier to just release the bacteria in the environment and then monitor the effects real time? This would also be very risky and probably not encouraged by the population.

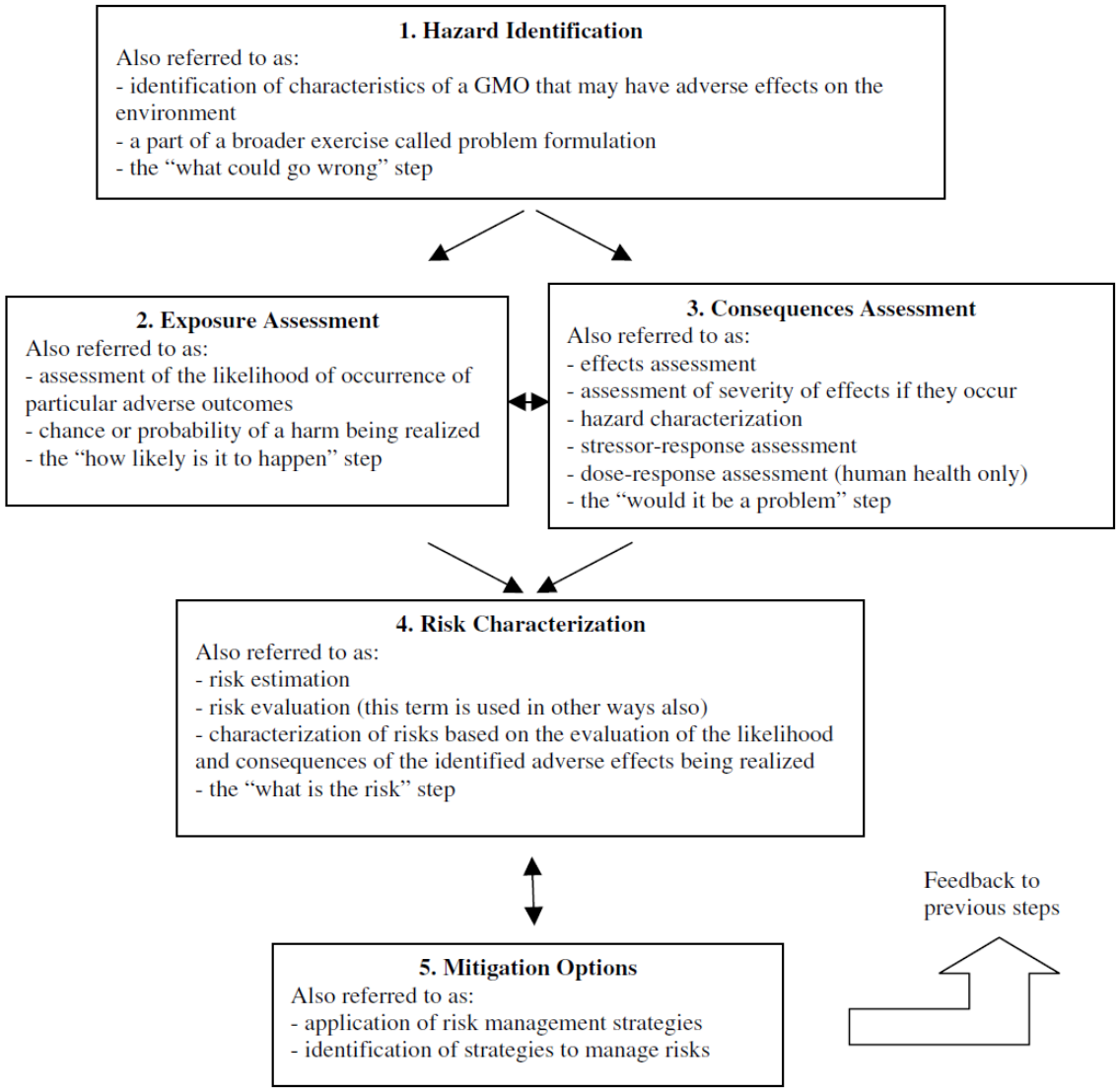
We have talked about scientists concerned by HGT, but a significant amount of the population is too, although they do not voice it in the same way. They are more likely to talk about "contamination of the environment" or "unknown interactions of these genetically modified bacteria with the ones in their bodies".

They are also well aware of the difficulty to assess risk and that although things are tested in the lab, these are artificial conditions and so we can never be sure till we see what happens when it is used in real life conditions. They know it very well indeed: they are aware (journalists make sure of that) that sometimes, medicine, which had been tested in the lab for years prior to commercialization, is taken of the market because of some very serious, sometimes deadly, adverse effects. These concerned might be expressed by saying that "it's new so we don't really know what could happen in real life conditions"

Although this is not the dominant view in the scientific community, some will argue that HGT between synthetic bacteria and wild type one can actually be a good think, like for bioremediation for example. In fact, it could be ways to go beyond current issues in terms of survivability of GM bacteria introduced in sites were they are needed for bioremediation. Even if they die, the plasmid containing the information for bioremediation will be passed up to wild type bacteria that can do the job themselves. Some scientists will tell you that ok, they are introducing a direct change in the environment, but isn't it always better then pollution and its consequences. And let's not forget that pollution is a source of important selective pressure, and can cause and increased number of mutation, therefore also directly impacting on the ecosystem.

iii. Why is risk so hard to access?

Figure showing the different steps for risk assessment: (taken directly from [40])



There are 3 steps to a risk analysis: the hazard identification, the risks assessment, and the risk management.

Risk is commonly defined as the magnitude of the hazard when it takes place multiplied by the frequency in which the hazard occurs.

Risk = magnitude of the hazard when it takes place × frequency in which the hazard occurs

$R = H \times F$

We can define 3 types of risks:
Risks that can be calculated
Incalculable Risks (for example when the frequency tends towards 0 and the magnitude towards infinity)
Unknown risks: the hazard has not been identified

HGT can be seen as a risk per se if one considers the presence of the synthetic plasmid to be an adverse effect in itself (contamination).

On the other hand, consequences of HGT may be seen as the risk, not HGT in itself. In that case, *"a proper hazard identification (step 1) should lay out the exact mechanism(s) by which gene flow may lead to adverse effects, and any plausible hazards identified would be identified on the basis of some understanding about potential consequences"* [40]
Potential risks due to HGT are either unknown risks, either incalculable risks.
- Unknown risks: there are some adverse consequences that can arise from HGT that we do not have enough knowledge to identify.
- Incalculable risks: these are the adverse consequences arising from HGT that we can predict, but where we cannot assess the magnitude of the hazard or the frequency at which they could occur. We could make a worst case scenario but that would give: Infinity × 0 (magnitude of hazard would tend towards infinity in worst case scenario and the frequency of hazard is usually very low and tends towards 0)

Usually, justification of a decision = Impact of something going well × Probability of it going well – Risk
However, this decision becomes hard to justify when we cannot calculate the risk.

Therefore, because putting genetically modified bacteria in the environment is so new, scientists have a hard time assessing risk because of the lack of knowledge on possible consequences. They have problems identifying adverse effects (for ex: the adverse effects that result from HGT). For the problems they did manage to identify, they have problems calculating the risk as they lack values on the magnitude of the hazard if it takes place and on the frequency in which it occurs.

2. Excessive proliferation

What do we mean by excessive proliferation?

Excessive bacterial proliferation is a scenario where the synthetic bacteria out compete the wild type ones. It can be an immediate consequence of the release of synthetic bacteria in the environment, or it can happen years later, when conditions change, providing a positive advantage to our bacterial population if it had not yet gone extinct (it could have remained as spores).

This could disrupt the whole ecosystem, thought we do not yet possess the tools to assess the events that could results from that.

However, we must ask ourselves one question:

Is the scenario of excessive proliferation a likely one?

The bacteria we work on in the lab are very weak.

They contain synthetic plasmids that give them a new function such as heavy metal purification. This is not a vital function for them, and yet, it takes them A LOT of energy to maintain and express this new information, giving them a selective disadvantage on wild type bacteria: *"it is generally well known that plasmid-free cells have a growth advantage over plasmid-bearing cells due to the increased metabolic burden resulting from plasmid presence* (Diaz-Ricci and Hernandez, 2000; Sobecky et al, 1992; Tiedje et al, 1989)" [21]

Moreover, when they arrive in a new environment, they are suppose to compete with wild type bacteria that are well adapted to their environment as they have been here for many centuries. Synthetic bacteria usually do not stand a change: *"in general, a bacterium recently isolated from a natural environment is more likely to survive when released back into that environment compared with a strain adapted for a long period of time to laboratory conditions* (Sobecky et al, 1996)" [21]

Lastly, not all bacteria have the ability to form spore. For instance, E. coli, one of the most widely used bacteria (especially in iGEM), is incapable of sporulation.

As a conclusion, the scenario of excessive proliferation is very unlikely. Scientists already have enough difficulties creating bacteria that are fit enough to survive long enough in the wild to perform their function. Professor Morange also expressed this opinion in his interview. However, as scientists are looking for solution to make lab bacteria fit enough to survive in the environment, they should be careful not to render them too fit. We think a responsible thing to do would be to create a system that enables to control how long the

bacteria population survives in the environment. A mechanism where it could auto-destroy itself after some time, just to be sure: the safest the better.

3. *Biosafety: how are these concerns about excessive proliferation and HGT dealt with?*

i. Governing Instances

Instances such as U.S. Environmental Protection Agency or the European Union have put in place strong limitations. Projects that seek permission for release in the wild are assess on a case-by-case basis.

In order to release a genetically modified organism in the wild, one must typically be able to give precise information on:

- ✓ The toxicity and dangerousness of the product,
- ✓ The effect the GM organism will have on the ecosystem is it introduced in (includes effects of HGT),
- ✓ The security measures to contain the risk,

However, it is almost always impossible to access the effect the GM organism will have on the ecosystem it is introduced in, because although the HGT rate between our GM bacteria and wild type bacteria can be measured, the effects of this HGT on the ecosystem on the long term are almost impossible to access.

ii. Literature

Scientists are trying to solve the problem of HGT thanks to synthetic biology itself. Since it is so hard to access if HGT between synthetic bacteria and wild type ones will have detrimental consequences on the ecosystem, following a principle of precaution, the scientific community has worked towards creating synthetic safety mechanisms that would prevent HGT, or at lease decrease drastically its probability. We will examine the different systems that have been suggested, and then discuss if decreasing drastically the probability of HGT is good enough.

These containment systems can be classified in different categories:
- Auto destruction system
- Physical containment
- Semantic containment
- Trophic containment

- ✓ **Auto Destruction systems**

These are systems that deal with the proliferation issues, but that also aim to decrease the probability of HGT by decreasing survival time/remaining time of synthetic bacteria in the environment.

Auto destruction systems are “based on the use of a “killer” gene and a “regulatory circuit” that controls expression of the killer gene in response to the presence or absence of environmental signals”. They are often referred to as a “kill switch”, “toxin-antitoxin” or “poison-antidote” system.

This system is derived from Koyama’s Plasmid addiction system (Koyama, 1975). The aim of that system is to insure plasmid maintenance in a bacterial population, in other words, insure that no daughter cell can be plasmid free. In the addiction mechanism, losing the plasmid is equivalent cell death. The molecular basis of this killing requires two genes: one specifying a stable toxic agent (toxin) and another one that will be present, this time, on the plasmid we want our bacteria to keep: an unstable factor (usually an anti sens RNA or protein) called an antitoxin, which prevents the lethal action of the toxin. [32,33].

Many auto destructive systems are now available. The main bacterial containment systems developed in the last two decades are listed in chronological order in Table 3 (source: [32]).

Table 3. Bacterial containment systems developed in last two decades in a chronological order. Complex regulatory circuits comprising of several genetic elements that control expression of the respective killer gene

Year	Organism	Killer gene	Regulatory circuit	Reference
1987	*E. coli*	*hok*	*lacI*, *trp* promoter	83
1988	*E. coli* Lu53	*hok*	*lacI*, *fimA*, *lacP*	85
1991	*E. coli* CSH36	*gef*	*lacI*, *xylS*thr45, P_m,	86
1993	*E. coli* EL1026	*sac*	*Nptl-sacR-B* cassette	94
1993	*P. putida* KT2440	*gef*	$xylS_2$, *lacP*, P_m,	88
1994	*E. coli* AH1	*nuc*	thermal induction, P_L	101
1995	*E. coli* MC1000	*gef*	*FimA*, *fimB*, *fimE*	93
1995	*E. coli* BD3347	*relF*	$P_{A1\text{-}03/04}$, $lacI^{q1}$	90
1995	*P. putida* EEZ30	*gef*	*xylS* thr45, P_{lac}	89
1996	*P. putida* KT2442	*colE*$_3$	P_{tac}, $lacI^{q1}$	92
1997	*P. putida*	*stv*	*xylS*, $lacI^{q1}$, P_m, P_{tac}	95
1999	*P. putida* KT2440	*stv*	*xylS*, $lacI^{q1}$, P_m, P_{lac}	96
2001	*P. putida* MCR7, *P. putida* MCR8	*gef*	P_m, $xylS_2$, *lacI*, P_{lac}	99
2003	*E. coli* K-12	*colE3* & *ecoRIR*	P_{trc}, P_m	100

Source: “Suicidal genetically engineered microorganism for bioremediation: need and perspective”, Rakesh K Jain et al [32]

However, with this first generation of auto destructive system, a considerable fraction of cells survive the induction of the suicide function due to random mutagenesis.

In order to deal with this problem, scientists started combining different killing system. For example, Toreres and al (2003) designed a dual lethal system using two toxin/antitoxin pairs (colE3 and ecoRI). This system decreased gene transfer frequencies through killing of the recipient cells by eight orders of magnitude. This system was successful because there were two toxins, which decreased the probability to escape the system by mutation, and also these toxins had different cellular targets (RNA and DNA respectively) which reduces the chances of mutations in both the target simultaneously.

Another solution was to change slightly the system. In the first generation of system, the antitoxin is constitutively expressed and the toxin is expressed only when there is a change in the environment (for example: the molecule that needed by the GM bacteria is now

completely gone), allowing random mutations to occur in the control elements by the time the pollutant is depleted. In second generation system, the idea is to constitutively express the toxin. The antitoxin can be expressed under a tightly regulated promoter that can be induced with the pollutant for example (if we are using the GM bacteria for bioremediation). Therefore, the antitoxin and its regulatory would not be prone to random mutation, because this would mean immediate cell death. In addition, "a constitutively expressed gene is preferentially repaired by the cell", thus, it decreases the probability of an inactivating mutation in the toxic gene. [32]

- ✓ **Physical containment**

Here, the idea is to isolate bacteria from the environment via a physical barrier. Ideally, this barrier is made of modular pores that enable scientists to control what is coming in or out. With this system, the bacteria are confined in one area, limiting dissemination and HGT. In fact, if the system works well, no bacteria or genetic material should be able to come out. For example, Dev Chidambaram and al showed, in 2005, *"the potential of the electrospinning technique for the encapsulation and immobilization of bacteria in the form of a synthetic biofilm".* They showed that *"the integrity and the viability of the bacteria were maintained through the cross-linking process and that the mesh-like network of the polymer effectively immobilized the microbe while allowing the exchange of nutrients and metabolic products between the microorganisms and the environmen*t" [34].

- ✓ **Semantic containment**

In order to avoid genetic cross-talk, artificial system based on naturel language had been built. Semantic containment would prevent synthetic genome or organism to share their genetic information with other natural species as they would not share the same universal language anymore. Several approaches are developed:

- new codons,
- new amino acids,
- new nucleotides,
- new DNA backbones,
- new tRNA,
- new polymerases,
- new ribosomes,

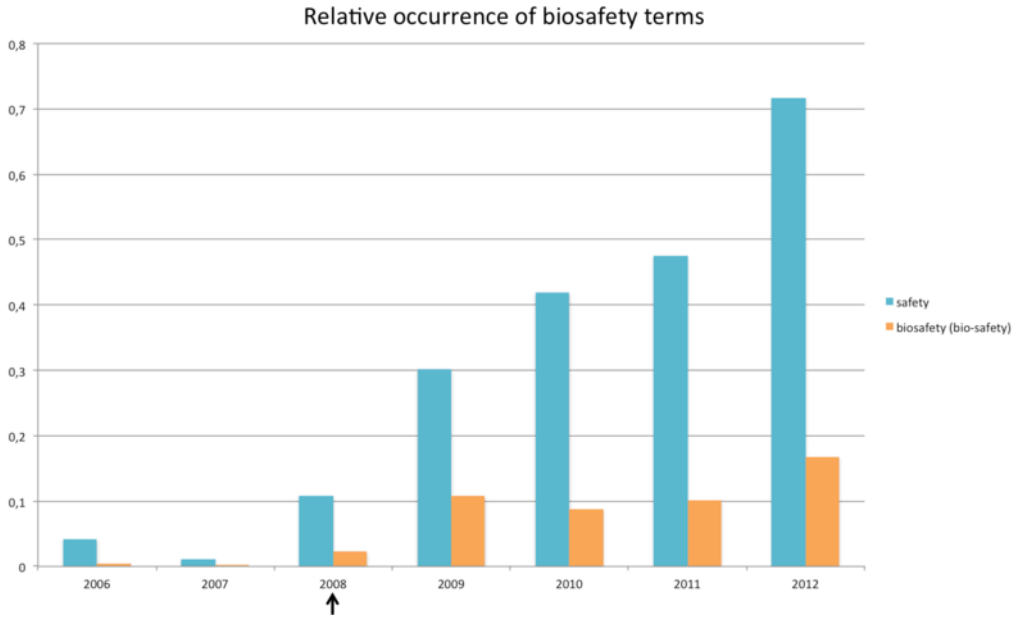
- ✓ **Trophic containment**

Trophic containment prevents from metabolic cross-talk. Artificial organisms would be able to grow only in presence of a xeno-nutrient that is indispensable for survival. Trophic containment includes for example the design of new and more robust forms of auxotrophy.

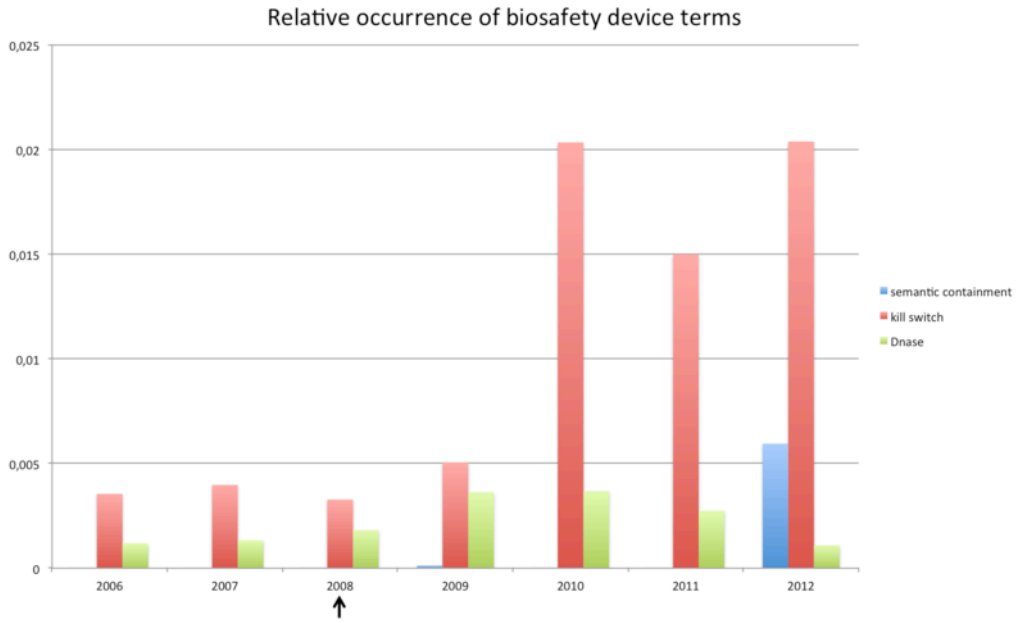
According to P.Marlière:" By introducing chemical motifs that are very rare among natural biomolecules (e.g., ether bridges, polycyclic aliphatic groups) or involving elements absent from natural biomatter and highly improbable in aqueous environments (e.g., silicon-carbon bonds), xeno-nutrients could be designed so as to impede their biosynthetic access. In addition, dependency on several xeno-nutrients inaccessible from existing metabolism and food chains could be combined to saturate the search through natural selection for producing these compounds autonomously in synthetic species."

iii. Screening past iGEM projects

We screened all wikis since 2006 and noticed that the number of teams dealing with biosafety increased over the years. However, most teams utter a few suggestions but rarely construct any concrete safety circuit.



Evolution of the relative occurrence of biosafety terms on the iGEM server over 7 years of the iGEM existence. The black arrow indicates the year from which safety page is mandatory



Evolution of the relative occurrence of biosafety devices terms on the iGEM server over 7 years of the iGEM existence. The black arrow indicates the year from which safety page is mandatory

Concrete safety circuits that were actually constructed are mostly:

- kill switch, toxin/antitoxin
- Fluorescence to keep track of
- Beeds

(cf. recapitulation table of safety systems that have been constructed by previous iGEM teams that you can find on our wiki: http://2012.igem.org/Team:Paris_Bettencourt/Human_Practice/WikiScreen)

4. How our system tries to come as a response to these issues

i. Recap of what our team thinks

It is fabulous that GM bacteria can be used in the field, but it is necessary to try and diminish biohazards as much as possible. We believe that unwanted dissemination is not the main problem as lab bacteria are quite weak, and we are much more concerned by HGT. No one can tell for sure if HGT between synthetic and wild type bacteria could have negative consequences and no one is creative enough to actually predict all that might happen. Releasing bacteria modified by Synthetic Biology in the wild is taking an even bigger step then releasing bacteria modified by genetic engineering in the wild: in the first, only one gene is modified, making predictions easier, whereas in the latter, a whole new genetic circuit has been created, which means that the behavior and consequences of HGT are even harder to predict because of possible emergent traits. However, in the latter, synthetic biology can solve its own problems by engineering a safety net and implementing it in these synthetic bacteria.

A balance benefits/risks should always be done, and in some particular situations, such as bioremediation of very contaminated soil or water, one can wonder, rightfully, if unpredictable gene transfer and potential of uncontrolled proliferation is not better than the current state of things (as long as it does not spread to other ecosystems). Men already disrupted these ecosystems long ago, and some pollutants could even have lead to an increase rate of mutagenesis, something which is "not very natural" either. This is where the members of the team start to disagree. Some, like Jean, claim that it is not because things are already a disaster that we can now do whatever, with the only justification that "we will probably be making things better, and anyways, it cannot be worst". He completely agrees with Professor Gouyon's claim that we should take a step back, take time to study what can happen, and consider other solutions too.

Anyways, we may have different vision on the possible consequences of HGT, on what we see as a balance benefit/risks, on the risks we are willing to take, but we all agree on something: the safer the better. We are all advocates of the Precautionary Principle.

"*When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically. In this context the proponent of an activity, rather than the public, should bear the burden of proof. The process of applying the Precautionary Principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action."*
*(*Wingspread Consensus Statement on the Precautionary Principle)

Therefore, designing systems that can diminish the probability of gene transfer should be a priority. It could ease the decision making process and sooth many people (experts and non experts).

Scientists have worked for 2 decades now on containment systems. They are not all convincing but things are definitively evolving in a positive way. Let's now look at what the iGEM community has been up to now. Although concerns for biosafety have increased each year, and there has been a few attempts to deal HGT, they are mainly superficial or lack robustness. Many of the proposed systems are kill switch or aggregation modules, one step away from mutation. We are well aware that it would be hard for a team of undergraduates to work on xenobiotics and we are well aware that only so much can be done in a summer. Indeed it is hard to try and creating at the same time a bacteria with a new interesting function AND a security system. The systems proposed are in majority weak because they mostly come in addition to the project.

Teams obviously do not have time do both. This is why we decided to approach things from a new angle this year. We would focus on constructing a safety system, and then we could implement it in existing systems. Not the other way round!

ii. Presentation of our system

Our approach may be new, but we acknowledged from the start that although we could try and do better than the already existing systems, our system would not be perfect or infallible. However, we believe that it is a good starting point, and that next year, teams can build up from this like we built up from previously existing systems.

The idea was to engineer a master safeguard system. We wanted this system to be as robust as possible against mutations. We decided to add up containment systems in order to increased robustness. We relied on three levels of containment:
- Physical containment with alginate capsules
- An improved killswitch featuring delayed population-level suicide through complete genome degradation.
- Semantic containment using an amber suppressor system

We choose three systems in which we would implement it.

Please look at our wiki for further information.

Of course, the ideal would be to use xeno-nucleic acids, but research on these is at its infancy. We do not want this to be the limiting factor for not releasing genetically modified bacteria in the wild in the mean time.

B. Are these issues the only concerns?

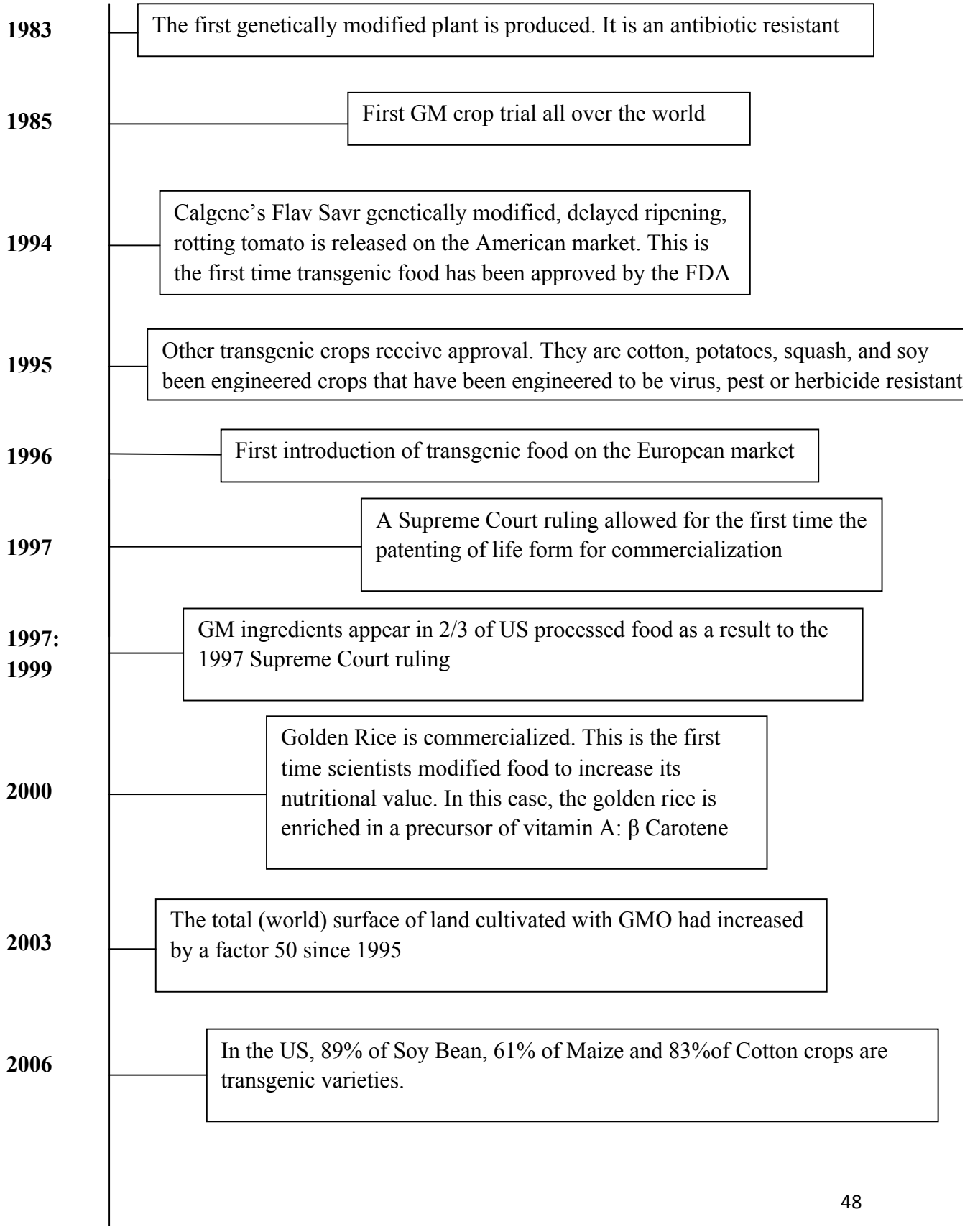
A big part of the population is concerned about hazards of horizontal gene transfer; a process that is sometimes referred to as “polluting nature” or the “unknown factors”. Our iGEM project reopens the question with exploratory (technical and non technical) answers - and asks it in a scientific arena.

Scientists tend to call “problems” and “risks”, things they can measure with scientific tools. However, the population usually demands a broader definition of harm where not only physical harms are taken into account, but also non physical harms. In other words, they do want potential effects on the environment and health to be taken into account, but it is also important that potential harms in terms of social justice, equity and governance be assessed and managed. They want to know: who will benefit? Who will bear the risks? Who will regulate? Will a monopoly be established?

GMO plants and crops were the first genetically modified products to be released in the wild on such big scale. Although they were products of genetic engineering and not synthetic biology, we think that socials issue could be similar, and therefore we will carefully examine the controversies that aroused when they were first imported in Europe.

1. *Case study: GMO plants and crops*

i. Historical background [36, 37,38]



For centuries, food crops and animals have been altered through selective breeding. This is the *"intentional breeding of organisms with desirable trait in an attempt to produce offspring with similar desirable characteristics or with improved traits"* [39]. This is a long and plodding process. It is also a process that is socially very well accepted. Genetic engineering offers an easier way to obtain the same results (that is plants and animals with the desired characteristics). Once the gene for a given function has been identified, the only thing left to do is to cut that gene and paste it in the genome of the organism we want to alter. Genetic engineering, as we already discussed earlier, is a very powerful but also very scary tool. Genetically modifying plants is seen as "highly unnatural" and "potentially dangerous".

GM crops and foods have been subject to polemic from the start. However, they have globally been well accepted in the US whereas they have been massively rejected in Europe. Let's take a closer look at what happened in Europe.

ii. The polemic in Europe

In 1996, boats arrived in Europe with part of US' annual soybean harvest. A small amount of these soybeans had been genetically modified to resist the herbicide Roundup. This was the first large scale introduction of GM food in Europe, and it was greeted in most countries with fierce hostility. 1996: we were only a few years after the traumatic scandal of the "contaminated hemophilia blood products" and at the very peak of the "mad cow" crisis. The general climate was one of fear and defiance. People had lost all trust in the governing entities. Should the timing be better, maybe Europeans would have granted GM plants with a bit more enthusiasm. Anyhow, 1996 was just not the year, so when it was discovered that ships were arriving from the states with some GM crops (something new so potentially dangerous), and that no one had asked Europeans' opinion, the climate became explosive. People instantly rejected transgenic food, and things never really got better afterwards.

We think that it is a good summary of how people feel about GM crops in Europe[6]: *"The science of taking genes from one species and inserting them into another was supposed to be a giant leap forward, but instead they pose a serious threat to biodiversity and our own health. In addition, the real reason for their development has not been to end world hunger but to increase the stranglehold multinational biotech companies already have on food production. And - The simple truth is, we don't need GM technology in order to possess future food security. Using sustainable and organic farming methods will allow us to repair the damage done by industrial farming, reducing the excessive use of fertilizer, herbicides and other man-made chemicals, and making GM crops redundant."* [37]

[6] The following comes from the following website http://www.disabled-world.com/fitness/gm-foods.php, and is largely inspired by the UK Green Peace website.

The real problem is that people did not perceive any of transgenic crops and foods' claimed benefits. This illustrates well the fact that there was no mutual understanding between the public and the food and agricultural sectors as to goals and tolerable side effects.

The public (including scientists) perceived no real benefits. Here are some of the arguments that were put forward and Europeans' reaction:

1) GM plants and crops enable farmers to reduce costs and biotechnology firms to make a benefit, therefore stimulating the economy. Europeans, and especially French people, have never seen the economic argument as one that would justify taking risks. On the contrary!
2) They will reduce the environmental impact of agriculture by reducing the use of chemical pesticides. Europeans think that organic food is the solution, not GM crops!
3) Healthier and nutritionally improved foods can be produced. Here, Europeans will tell you that the nutritional value of non modified food is just fine, and if people have deficiencies, they should adopt a more balanced diet.
4) They can improve food quality. A tomato that does not rot is quite awesome, right? Gadget, luxury product, Europeans shall reply!
5) This could be a solution to hunger in the third world. There are two dominant views here.
   - The first is that hunger is due to wars, politics, an unequally distribution of food, and not a global lack of food. GMO plants and foods is therefore not the solution
   - The second is that yes GMO plants and foods could help the third world. Helping the third world is actually the only potential benefit some Europeans can admit to. However, a large number of them think that research will be oriented towards application that can be useful to the first world, and not to the third world, in a perverted logic of maximizing economical benefits.

These reactions are shared by many average European citizens but also by some renowned scientists. Since they do not perceive any real benefit, they do not see why they should be offered food that carries some level of risk.

The public (including scientists) only saw risks.

1) Antibiotic resistance genes could be transmitted to bacteria.
2) Transgenic food could cause deadly allergies. In 1996, Brazil some genes coming from nuts were introduced into soybeans by the company Pioneer Hibred. Individuals that are allergic to nuts, not suspecting anything, would eat these transgenic soybeans. They are usually not allergic to soybeans, but in this specific case, some would develop allergies, sometimes even leading to anaphylactic choc.
3) GM crops are a threat to environmental diversity: there is no way to ensure that GMO will remain under control. Cross pollination and horizontal gene transfer are a threat to biodiversity. In 2005, environmentalists said that Australia faced the most serious genetic contamination even in history, after it confirmed modified Canola had been fined in non modified Canola.

4) Increase of GM crop has caused a power shift in agriculture towards biotech companies. These companies are gaining control over the production chain of crop and food and the farmers as well. *"They're now turning those seeds into intellectual property, so they have a virtual lock on the seeds upon which we all depend for our food and survival"* (Jeremy Rifkin)
5) At the start, transgenic food was released on the market with no particular labeling. European saw this as an infringement on freedom of choice. They could easily find themselves in a situation where they would have to eat transgenic food, food they did not approved of and considered risky, without knowing it. The current legislation imposes that products containing more than 0.9% of transgenic food be labeled.

iii. Lessons to be learned

Populations want to be informed of what is going on. They want to be presented with accurate information. They do not want to be lied to and told that "there is 0 risk". They know that this is false, as technology and innovation implies taking a risk. They will lose faith in the people that came up with the lies and just panic: if the risks were not that terrible, people would not be scared to talk about them. Since they are scared of talking, this means the risks must be very high.

They need to perceive some benefits which justify taking a risk. However, they want to be the one that decide what they consider like a true benefit (for them, the society, and to mankind as a whole) and what is not really important but just luxury.

Furthermore, they find it important that issues like that are submitted to public debate. The ones that have things to say on the matter want to be heard.

Moreover, they do not want to feel pressured in anyway by big lobbies to buy things that were created by this technology. They want labeling and they want alternatives. They want their right to free choice to be respected.

Some feel very strongly about the fact that industrials are the one funding the scientists to test their products. People would have more trust in an independent comity.

Finally, biotechnology should be used to help the third world, and so research should be oriented in that way too.

2. Applying these lessons to synthetic biology & further suggestions

For the release of genetically modified bacteria to be conceivable, we think that are few conditions are necessary. Here are our proposals:

Firstly, if synthetic bacteria are going to be used in the environment, and therefore directly affect citizens in their everyday life, there should be a real effort to educate citizens on this subject. They should understand what is going on, so they can decide if they want such a technology in their lives or not.

Secondly, there should be a real dialogue between the public, the scientists, the politicians and the biotechnology firms. They should agree on goals, benefits, and tolerable side effects. Physical harms, but also ethical aspects should be considered. The dialogue should take place as soon as possible, before too much money is at stake.

Thirdly, no concern should be brushed away before thorough investigation, and there should be a general effort to understand the logic that is hidden behind arguments that may seem, at first, "un rational". People have the right to be scared. We would like to see the creating of a comity similar to the French advisory council for the protection of people in biomedical research ("comité consultatif de protection des personnes dans la recherche biomédicale"), but for biotechnology. It would be called "advisory council on synthetic biology and genetic engineering". Biotechnologies industries could go and consult this comity before they start the research on the product they wish to develop. This council would take ethical issues into consideration.

Fourthly, everyone should be able to benefit from synthetic biology. Therefore, some researchers should work on applications of synthetic biology that can be useful to the third world. We believe that this should be publicly founded. However, the state would get the money through taxing biotechnology firms on the income they make by selling to the first and second world products that use synthetic biology. Europeans may want that the third world benefit from synthetic biology, however, Sara Aguiton wonders: "Did representatives of the third world ever ask for that?"

Fifthly, applications of synthetic biology that require releasing in the environment should be tested by an independent comity of scientists. When an industry wants to test a new product where synthetic biology is involved, it will not be able to test it with its own scientists. An intermediary will have to be involved: the state. The industry will pay a tax to the state, who will, in exchange, ask its independent comity to test the project. We hope that thanks this will relieve the pressure on the scientists to produce results that go in the sense of the industry, as they will not be funded by the industry anymore.

Lastly, the status quo of evaluating projects case-by-case before enabling release in the wild should be maintained.

C. Conclusion

Firstly, we examined issues around horizontal gene transfer (HGT), excessive proliferation and risks assessment. We showed the difficulty of assessing risk when it comes to putting genetically modified bacteria in the wild, and that from the very start (beginning of recombinant DNA technology), people have been concerned about HGT and excessive proliferation and tried to create security systems. This is in the spirit of the precautionary principle: *"When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically. In this context the proponent of an activity, rather than the public, should bear the burden of proof. The process of applying the Precautionary Principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives, including no action."(Wingspread Consensus Statement on the Precautionary Principle)*.
We screened the literature and previous iGEM teams' wikis to identify security systems that had already been constructed. Systems proposed by previous iGEM teams are very often kill switches, toxin/antitoxin systems, aggregation modules, and usually one mutation away from failure. Our literature screen indicated that such systems were used at first, but that scientists then started combining different system (for e.g. two different toxin/antitoxin systems), which made the systems more efficient, but still quite close from mutation. Some more elaborate mechanisms are being created now: they are systems that make synthetic bacteria's genetic information not universal anymore, preventing communication with wild type bacteria (for e.g. semantic containment, xeno-nucleic acids). We then discussed the master safeguard system we designed to try and decrease the probability of HGT and excessive proliferation

Contribution 2: We tried to engineer a master safeguard system. We wanted this system to be as robust as possible against mutations. We decided to add up containment systems in order to increased robustness. We relied on three levels of containment:
- Physical containment with alginate capsules
- An improved killswitch featuring delayed population-level suicide through complete genome degradation.
- Semantic containment using an amber suppressor system
We acknowledge that our system is not perfect or infallible. However, we believe that it is a good starting point, and that next year, teams can build up from this like we built up from previously existing systems.

Contribution 3: We created a safety page on the registry. Teams can put the safety circuits they created there, and assess its efficiency. In the future, we would like that the standard plasmids contain safety elements (for e.g: autodestruction system, etc). Our aim is to promote safety in future iGEM projects.

Secondly, we examined other concerns that could be raised by the release of genetically modified bacteria in the environment. We started by doing a case study on GMO plants and crops and looked at the lessons that could be learned, so as not to make the same mistakes when releasing synthetic bacteria in the wild. We concluded from that case study that
(a) Populations want to be informed of what is going on;
(b) They need to perceive some benefits which justify taking a risk;
(c) They want issues like that to be submitted to public debate. And they want the public's opinion is taken into consideration;
(d) They do not want to feel pressured in anyway by big lobbies to buy things that were created by biotechnology. They want labeling and they want alternatives. They want their right to free choice to be respected;
(e) Some feel very strongly about the fact that industrials are the one funding the scientists to test their products. People would have more trust in an independent comity;
(f) Biotechnology should be used to help the third world, and so research should be oriented in that way too.

Then, we listed conditions that we believe are necessary for the release of genetically modified bacteria to be conceivable. We came up with the following suggestions:
(a) There should be a real effort to educate citizens on this subject;
Contribution 4: We organized a debate involving 10 university students from very various background (law, politics, etc), but no one studying synthetic biology. 70 people came to see the debate. They had to debate on the following motion: "This house would allow environmental release of genetically modified bacteria for applications in the following fields: medicine, pharmacy, agriculture, energy, bioremediation". 5 students were assigned to be for, 5 students were assigned t be against. They had one week of preparation. We were impressed by the level of the debate. See wiki page on the debate for further details.

(b) There should be a real dialogue between the public, the scientists, the politicians and the biotechnology firms. They should agree on goals, benefits, and tolerable side effects. Physical harms, but also ethical aspects should be considered. The dialogue should take place as soon as possible, before too much money is at stake;
Contribution 1bis: Here, we decide to take the contribution 1one step further. After the introduction to synthetic biology, we would discuss with students what they would consider as benefits and acceptable risk. This would take the form of discussing synthetic biology projects they brainstormed. See wiki page on the workshop for additional details.

(c) Thirdly, no concern should be brushed away before thorough investigation;
Proposal 3: We would like to see the creating of a comity similar to the French advisory council for the protection of people in biomedical research ("comité consultatif de protection des personnes dans la recherche biomédicale"), but for biotechnology. It would be called "advisory council on synthetic biology and genetic engineering". Biotechnologies industries could go and consult this comity before they start the research on the product they wish to develop. This council would take ethical issues into consideration.

(d) Everyone should be able to benefit from synthetic biology;
Proposal 4: some researchers should work on applications of synthetic biology that can be useful to the third world. We believe that this should be publicly founded. However, the state would get the money through taxing biotechnology firms on the income they make by selling to the first and second world products that use synthetic biology.

(e) Applications of synthetic biology that require releasing in the environment should be tested by an independent comity of scientists;
Proposal 5: applications of synthetic biology that require releasing in the environment should be tested by an independent comity of scientists. When an industry wants to test a new product where synthetic biology is involved, it will not be able to test it with its own scientists. An intermediary will have to be involved: the state. The industry will pay a tax to the state, who will, in exchange, ask its independent comity to test the project. We hope that thanks this will relieve the pressure on the scientists to produce results that go in the sense of the industry, as they will not be funded by the industry anymore.

(f) The status quo of evaluating projects case-by-case before enabling release in the wild should be maintained

Conclusion : Contributions & proposals

We already wrote a detailed conclusion for both parts of the report. Our general conclusion will just be a summary of our contributions and proposals to raise awareness of synthetic biology and on the general questions of releasing genetically modified bacteria in the environment.

CONTRIBUTIONS

Societal interaction

Proposal 1: We would like that in the future, collaboration with a middle school or high school be a requirement for an iGEM gold medal. This would drastically raise the world level of awareness about synthetic biology.

Proposal 2: Extend proposal 1 to a mandatory high school course called "new technologies".

Proposal 3: A real discussion with all the actors of society about synthetic biology and its applications.

Proposal 4: We would like to see the creating of a committee similar to the French advisory council for the protection of people in biomedical research ("comité consultatif de protection des personnes dans la recherche biomédicale"), but for biotechnology. It would be called "advisory council on synthetic biology and genetic engineering". Biotechnologies industries could go and consult this comity before they start the research on the product they wish to develop. This council would take ethical issues into consideration.

Proposal 5: some researchers should work on applications of synthetic biology that can be useful to the third world. We believe that this should be publicly founded. However, the state would get the money through taxing biotechnology firms on the income they make by selling to the first and second world products that use synthetic biology.
Best Research practice

Proposal 6: applications of synthetic biology that require releasing in the environment should be tested by an independent committee of scientists. When an industry wants to test a new product where synthetic biology is involved, it will not be able to test it with its own scientists. An intermediary will have to be involved: the state. The industry will pay a tax to the state, who will, in exchange, ask its independent comity to test the project. We hope that thanks this will relieve the pressure on the scientists to produce results that go in the sense of the industry, as they will not be funded by the industry anymore.

Proposal 7: Safety modules ans risks assessments has to be part of every synthetic biology projects from the very beginning.

We think that safety concerns has to be involved in synthetic biology project at the very beginning, starting from the conception, through design, prototyping, construction, testing and application. This is because of the interdependence of different parts and modules in a synthetic biology project. Once a device is designed, built and tested, new features and concerns are hard to implement without changing previous stages. Thus instead of designing for a successful outcome and then add safety concerns, it will be much more efficient and less risky to design for both successful outcomes and potential failures.
We experience first-hand the need for such a mindset. Our project started as a time-controlled enzyme-releasing device that involve a series of restriction-enzyme expression and DNA digestion. After the initial design, we realized it is a sort of "rube goldberg machine" that will work in an ideal situation, but is prone to failure to mutation at every step and will introduce undesired live genetic-modified microorganisms into the environment. This realization completely changed our approach and design principles. The focus is no longer how to realized the intended behavior - releasing a specific enzyme, but how to avoid undesirable outcomes - releasing either live GMO or potentially harmful DNA sequences.
Simple back-of-the-envelope calculations can be done even before any experiment are done. Usually the risks of system failure can be assessed from two perpectives. One is the complexity and redundancy of the system. The more complex and less redundant the system is, the higher the risk of failure. The other is mutation and evolution. One should assess the population size, lineage structure and mutation rates of the devices thus the likelihood of having mutants, and selective advantage of those mutants might possess against both the intact strains and organisms in the wild.

Proposal 8: Each synthetic biology application should assess and disclose a list of application specific risks and hazards.
After a set of safety mechanisms has been proposed in our projects, we realized there are no absolute measure of safety. All measures of safety are relative, and depend on applications, contexts and environments. Thus safety standards and disclosure of potential hazards can not be left to safety specialists, but be specific to the application. The designer and builder of devices have to assess quantitatively the potential risks and disclose such results, similar to the Material Safety Data Sheet approach. These information should be considered as part of the characteristics of the product. Only with such information, the policy-makers and the general public can make informed decision.

Proposal 9: The community has to build a collection of bio-safety devices for future engineers.
Since safety concerns are crucial to the success of synthetic biology, an organized effort should be made to collect and standardize safety devices already present. This is apparent from our survey of past iGEM projects: many teams had considered safety, designed or made safety modules from their projects, they vary in terms of applicability and efficiency, and lack diversity in terms of mechanisms. In the spirit of iGEM, we published the results of our survey in our wiki. We hope more extensive efforts can be made in this area so that future engineers can match the potential hazards of their applications to existing safe guard devices, or identify missing critical safety mechanisms to be included in their projects

Proposal 10: Development and adoption of a safety chasis for synthetic biology research and prototyping.
Besides the heavily regulated real-world applications of synthetic biology, research results about synthetic biology safety can be applied to laboratory research and prototyping. In accordance with the iGEM idea of standardization and modularity, simply incorporate some known safety features into the genetic background of the most commonly used standard chasis can go a long way in improving safety in the whole synthetic biology community. For example, in commonly used E.coli strains, cpxAB mutants are known be deficient in horizontal gene transfer by conjugation [46]. The widespread adoption of a chasis with such a mutation and other safety features, in the background would reduce unintentional risks and improve general safety in the research community in addition to all the safety precautions people already took.

CONTRIBUTIONS

Societal Contribution

Contribution 1: We organized a workshop on synthetic biology for high school students in order for them to discover this new field. We also gave them a tour of our lab.

Contribution 1bis: Here, we decide to take the contribution 1one step further. After the introduction to synthetic biology, we would discuss with students what they would consider as benefits and acceptable risk. This would take the form of discussing synthetic biology projects they brainstormed. See wiki page on the workshop for additional details.

Contribution 2: We organized a debate involving 10 university students from very various background (law, politics, etc), but no one studying synthetic biology. 70 people came to see the debate. They had to debate on the following motion: “This house would allow environmental release of genetically modified bacteria for applications in the following fields: medicine, pharmacy, agriculture, energy, bioremediation”. 5 students were assigned to be for, 5 students were assigned t be against. They had one week of preparation. We were impressed by the level of the debate. See the debate page for further details.
Best research practice

Contribution 3: We tried to engineer a master safeguard system. We wanted this system to be as robust as possible against mutations. We decided to add up containment systems in order to increased robustness. We relied on three levels of containment: - Physical containment with alginate capsules - An improved killswitch featuring delayed population-level suicide through complete genome degradation. - Semantic containment using an amber suppressor system We acknowledge that our system is not perfect or infallible. However, we believe that it is a good starting point, and that next year, teams can build up from this like we built up from previously existing systems.

Contribution 4: We created a safety page on the registry. Teams can put the safety circuits

they created there, and assess its efficiency. In the future, we would like that the standard plasmids contain safety elements (for e.g: autodestruction system, etc). Our aim is to promote safety in future iGEM projects.

Bibliography

[1] Synthetic biology 3.0: Framing the Safety and Security Aspects of Synthetic Biology, Markus Schmidt

[2] Synthetic biology 101: http://www.synbioproject.org/topics/synbio101/definition/

[3] Synthetic biology: promises and challenges, Serrano, editorial, Molecular Systems Biology 18 December 2007, http://www.nature.com/msb/journal/v3/n1/pdf/msb4100202.pdf

[4] Commissioned by the Synthetic Biology Project at the Woodrow Wilson International Center for Scholars and supported by the Alfred P. Sloan Foundation, *Ethical Issues in Synthetic Biology: An Overview of the Debates*, *Erik Parens, Josephine Johnston, and Jacob Moses*

[5] Engineering life through Synthetic Biology, Paras Chopra and Akhil Kamma; http://www.bioinfo.de/isb/2006/06/0038/

[6] Asilomar Conference on Recombiant DNA, Wikipedia page: http://en.wikipedia.org/wiki/Asilomar_conference_on_recombinant_DNA

[7] Historical events in the rDNA debate: http://www.ndsu.edu/pubweb/~mcclean/plsc431/debate/debate3.htm

[8] The Recombinant DNA controversy: Twenty years later

[9] Eurobarometer, biotechnology report 2010 http://ec.europa.eu/public_opinion/archives/ebs/ebs_341_en.pdf

[10] Awareness & Impressions Of Synthetic Biology, A Report Of Findings, Based On A National Survey Among Adults, Conducted On Behalf Of: Synthetic Biology Project The Woodrow Wilson International Center For Scholars, By Hart Research Associates September 9, 2010

[11] Europeans and Biotechnology in 2005: Patterns and Trends Eurobarometer 64.3

[12] US public opinion divided over biotechnology?

[13] Life, Wikipedia: http://en.wikipedia.org/wiki/Life

[14] Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome, J. Craig Venter1, Science: http://www.sciencemag.org/content/329/5987/52.full

[15] Playing God with Synthetic Life? Starting with the minimal genome project. Heather Pace

[16] The promise and Perils of synthetic biology

[17] http://igem.org/Safety

[18] Synthetics: an ethical and sociological analysis of synthetic biology bySara Aguiton

[19] Synthetic Biology: Social and Ethical Challenges, Andrew Balmer & Paul Martin, Institute for Science and SocietyUniversity of Nottingham

[20] Synthetic biology: four steps to avoid a synthetic-biology disaster https://frodon.univ-paris5.fr/http/www.nature.com/nature/journal/v483/n7387/full/483029a.html

[21] Use of genetically engineered microorganism (GEMs) for the bioremediation of contaminants

[22] ENVIRONMENT DIRECTORATE, JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY, GUIDANCE DOCUMENT ON HORIZONTAL GENE TRANSFER BETWEEN BACTERIA, OEDC, September 2010: http://www.oecd.org/science/biosafety-biotrack/46815958.pdf

[23] The Precautionary Principle Applied to Deliberate Release of Genetically Modified Organisms (GMOs); 1999, Vol. 11, No. 2 , Pages 65-74 (doi:10.1080/089106099435790), Anne Ingeborg Myhr, Terje Traavik

[24] Suicidal genetically engineered microorganism for bioremediation: need and perspective

[25] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, Annex II

[26] Risks from GMO due to horizontal gene transfer

[27] REVIEW in Heredity, Genes without frontiers? D Bensasson1, JL Boore1 and KM Nielsen2,3 http://www.nature.com/hdy/journal/v92/n6/pdf/6800451a.pdf

[28] Construction of an Efficient Biologically Contained *Pseudomonas putida* Strain and Its Survival in Outdoor Assays; Lázaro Molina, Cayo Ramos, María-Carmen Ronchel, Søren Molin, and Juan L. Ramos
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC106280/pdf/am002072.pdf

[29] Genome Biology, Opinion, Evolution, ecology and the engineered organism: lessons for synthetic biology; Jeffrey M Skerker, Julius B Lucks and Adam P Arkin
http://www.biomedcentral.com/content/pdf/gb-2009-10-11-114.pdf

[30] Horizontal gene transfer from transgenic plants to terrestrial bacteria: a rare event? Kaare M. Nielsen, Atle M. Bones, Kornelia Smalla, Jan D. van Elsas
http://onlinelibrary.wiley.com/doi/10.1111/j.1574-6976.1998.tb00362.x/pdf

[31] Monitoring and modeling horizontal gene transfer

[32] Suicidal genetically engineered microorganism for bioremediation: need and perspective

http://www.ncbi.nlm.nih.gov/pubmed/15832375

[33] *Review,* Mechanisms of plasmid stable maintenance with special focuson plasmid addiction systems, Urszula Zielenkiewicz
http://ttk.pte.hu/biologia/genetika/bact_gen/refs/plasm_adict.pdf

[34] Engineering of bio-hybrid materials by electrospinning polymer-microbe fibers, Dev Chidambaram and al, PNAS

[35] The farther, the safer: a manifesto for securely navigating synthetic species away from the old living world, Philippe Marliere
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2759432/pdf/11693_2009_Article_9040.pdf

[36] Ethics and genetic engineering – lessons to be learned from GM foods, J.Lassen, K.H. Madsen, P.Sandoe

[37] http://www.disabled-world.com/fitness/gm-foods.php

[38] http://en.wikipedia.org/wiki/Genetically_modified_food

[39] http://www.biology-online.org/dictionary/Selective_Breeding

[40] Conceptualizing risk assessment methodology for genetically modified organisms, Ryan A. Hill
https://portal.ilsi.org/rf/scientific/BrasilDocuments/Shared%20Documents/Workshop%20Documents/ILSI%20Brazil%20Risk%20Hill.pdf

[41] A synthetic biohazard non proliferation proposal, George Church
http://arep.med.harvard.edu/SBP/Church_Biohazard04c.htm

[42] The Principles for the Oversight of Synthetic Biology, Drafted through a collaborative process among civil society groups.
http://www.biosafety-info.net/file_dir/15148916274f6071c0e12ea.pdf

[43] DNA Technology: Asilomar Conference and 'Moratorium' on Use
Susan Wright, University of Michigan, Ann Arbor, Michigan, USA

[44] Trends in American/European press coverage of synthetic biology,
Woodrow Wilson International Center for Scholars.
http://www.synbioproject.org/process/assets/files/5999/synbio1final.pdf

[45] Synthetic Biology at the Interface of Science and Policy
Summary of the proceedings of the conference "Synthetic Biology at the Interface of Science and Policy", held at the University of Ottawa September 30, 2011

Institute for Science, Society and Policy
http://www.issp.uottawa.ca/eng/pdf/Synthetic_Biology_theInterface_Science_Policy.pdf