P E P S Y B Y P

PROPOSAL FOR A CODE OF ETHICAL PRACTICES FOR SYNTHETIC BIOLOGY BASED ON THE HOT YEAST PROJECT

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VNIVERSITAT DE VALÈNCIA

iGEM

UNIVERSIDAD POLITECNICA DE VALENCIA

"*With great power comes great responsibility*"

Spiderman's Uncle Ben

Part I: Preamble

1. The members of the Valencia 2008 iGEM team:

Conscious of the potential benefits of SYNTHETIC BIOLOGY in Food or Energy, Agriculture, Environmental management, Human Health, Industry, Manufacturing, and other fields;

Recognizing the potential for SYNTHETIC BIOLOGY to enhance the efficiency of agricultural and industrial production, and increase the availability and nutritional value of global food supplies to meet the demands of the world's rapidly expanding population;

Identifying the potential for SYNTHETIC BIOLOGY to benefit the environment by creating and sustaining industrial processes which produce less waste, reducing the need for agricultural chemicals, and generating alternative energy sources such as the development and production of alternative fuels;

Acknowledging that SYNTHETIC BIOLOGY is a growing source of wealth and job creation in knowledge economies which will provide opportunities for researchers to participate in national and international partnerships, research and innovation;

Noting that certain concerns have been expressed about the safety and environmental impact of some SYNTHETIC BIOLOGY projects;

Acknowledging that biotechnology, and particularly GM crops development, has already raised major concerns about its safety and environmental impact, and that mistakes in GM diffusion and regulation should be taken into account for SYNTHETIC BIOLOGY ethics regulation;

Asserting that the present proposal is based on our experience with the iGEM2008 competition, and particularly on the HOT YEAST PROJECT we developed, and that we have discussed ethical practices concerns derived from our daily work and also consulted many other texts (see references) on ethics and ethical practices for Biotechnology and SYNTHETIC BIOLOGY;

have resolved to issue this **PROPOSAL FOR A CODE OF ETHICAL PRACTICES FOR SYNTHETIC BIOLOGY BASED ON THE HOT YEAST PROJECT (PEPSYBYP).**

2. This Code could be of interest and could contribute to the implementation of an international Code on Synthetic Biology ethics that should apply to:

1. iGEM competition
2. MIT Parts Registry
3. Any research on Synthetic Biology, including agencies, research centres, laboratories and hospitals that conduct such activities.

3. For the purpose of this Code, the bodies defined in paragraph 2 are referred to as (HYCode)

Part II: Definition of Synthetic Biology and other related terms

1. Hot Yeast Project particular definitions

Saccharomyces cerevisiae is a species of budding yeast. It is a widely used eukaryotic model organism, and therefore also one of the most studied. *S. cerevisiae* has obtained this important position because of its established use in industry (e.g. beer, bread and wine fermentation, ethanol production), ease of manipulation and culture in the lab.

Yeast strain: A group of organisms of the same species, sharing certain hereditary characteristics not typical of the entire species but minor enough not to warrant classification as a separate breed or variety. The characteristic which makes "hot yeast" strains different from the wild type is carrying UCP-1 gene or one of its genetic variants.

Mutant: is an individual, organism, or new genetic character arising or resulting from an instance of mutation, resulting in the creation of a new character or trait not found in the wild type. Apart from having the UCP-1 yeast strain, Valencia iGEM Team works with two mutants which UCP-1 sequence has been modified (Gly175Δ and Gly76Δ) in order to provide this protein with increase uncoupling activity.

Thermogenin: UCP1, also known as thermogenin, is an uncoupling protein found in the mitochondria of brown adipose tissue. This protein plays an important role in hibernating mammals and in human infants since it is able to generate heat. UCP1 provides an alternative pathway by which protons can reenter the mitochondrial matrix from the intermembrane space, instead of going through ATP synthase. The result is a temperature increase rather than ATP production in the tissue containing this protein family.

Liquid Culture Calorimeter (LCC): It is a system designed and built by the member of the Valencia Team. It consists of a calorimeter with a measurement device (thermocouple) inside allowing microbial culture. The calorimeter was modified to improve thermal isolation and the system was connected to a data logger and a computer to record and store temperature measurements during a period of time.

2. General Definitions:

Synthetic Biology: Synthetic biology refers to both the design and fabrication of biological components and systems that do not already exist in the natural world and the re-design and fabrication of existing biological systems.

It studies how to build artificial biological systems for engineering applications, using many of the same tools and experimental techniques. But the work is fundamentally an engineering application of biological science, rather than an attempt to do more science. The focus is often on ways of taking parts of natural biological systems, characterizing and simplifying them, and using them as a component of a highly unnatural, engineered, biological system.

Systems biology: is a discipline that studies complex biological systems as integrated wholes, using tools of modeling, simulation, and comparison to experiment.

Molecular Biology: is the study of biology at a molecular level. The field overlaps with other areas of biology and chemistry, particularly genetics and biochemistry. It is the branch of biology that deals with the formation, structure, and function of macromolecules essential to life, such as nucleic acids and proteins, and especially with their role in cell replication and the transmission of genetic information.

Biotechnology: is the combined used of microbiology, biochemistry, genetics and engineering in order to use living beings (or theirs parts) to obtain products or processes.

The United Nations Convention on Biological Diversity defines biotechnology as:

Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use.

Bioremediation: any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition

Metabolic engineering: is the practice of optimizing genetic and regulatory processes within cells to increase the cells' production of a certain substance. Metabolic engineers commonly work to reduce cellular energy use (ie, the energetic cost of cell reproduction or proliferation) and to reduce waste production.

Modeling: is the process of creating a depiction of reality, such as a graph, picture, or mathematical representation. Models inform the design of engineered biological systems by allowing synthetic biologists to better predict system behavior prior to fabrication.

Part: a sequence of DNA with a specific function that can be physically combined with other parts via an assembly standard (Examples: promoter, terminator)

Device: a set of parts that can be functionally combined with other devices via a common, standard signal carrier (i.e. PoPS, RiPS, PhPS) (Example: inverter)

System: a set of devices that cannot be functionally combined with other devices via a common, standard signal (Example: ring oscillator).

Units of Ethical Issues: We have introduced this term to clearly define ethical aspects arisen from Synthetic Biology research. **A unit is defined as a level of complexity in Synthetic Biology with ethical implications.**

We assume that **Units of Ethical Issues are concentric**, i.e. one includes the former and it is included itself by the next level. Ethics considerations on a given Unit must thus take into account ethical aspects of all the sub-units included by the Unit.

Concentric Units of Ethical Issues are, for example: *part, device, system, organism, immediate environment and remote environment.* Intermediate units between units can be also considered, such as tissue (between organism and immediate environment).

Immediate environment can be defined as the surroundings of the organisms in direct contact, in terms of energy (i.e. food and temperature), with the organism. An example of immediate environment is growth culture.

Remote environment is the region of space surrounding the immediate environment, and having limited energy exchanges with the organism. Examples of remote environments might be as small as the region of the lab surrounding a microbial culture (and affected, for example, by the heat production radiating from the growing culture) or as vast as the whole biosphere (potentially affected, for example if a highly pathogenic synthetic organism was released).

Part III: The Code. Ethics on using parts obtained through Synthetic Biology.

As students and researchers on Synthetic Biology we commit ourselves to the following code of ethical practice. This code has been prepared according to our results and concerns arisen from our participation in the 2008 iGEM competition with the Hot Yeast Project.

General commitments

1- Debate about intellectual property of the Parts should be successfully concluded.

We at the Valencia IGEM team think that the individual Parts we worked on are a fragment of the genetic pool of nature, mechanisms that have been tested through the ages by natural selection, so should we avoid allowing people to own these parts?, is it not the same thing to register a gene already present in nature than to register a nucleotide or organic molecule?

While all these reasons are important, there are other issues concerning research economic viability and information safety that should be considered in this debate. To summarize, we think that this is a very important debate that is not being paid enough attention.

2- Potentially hazardous Parts and/or Units must be regulated and controlled.

Synthetic Biology is a very powerful tool that could solve some of humanity's present problems, improving our harvests, energy production, and allowing lots of people an access to better and more medical supplies. However, the risks that new technologies present are many, and they increase dramatically when living being are directly manipulated. This is why at he Valencia IGEM team we strongly support a strict regulation concerning the use of Synthetic Biology parts.

3- Research on Synthetic Biology must not be directed by economic interests.

Even though economic interests are a major enhancer of current research, we must not forget that our goal as researchers is to achieve developments that will benefit as many people as possible. That is why governments and non profit organizations must look after the research companies benefit and solvency, but keeping in mind our previous statement.

4- Basic tools and knowledge for the use of these Units should be available to researchers on other areas.

One of the main goals of this research is to standardize and allow the access to our new parts for researchers not trained in this specific area, this is important in every scientific work, but even more in one that combines different scientific disciplines as this one, making interdepartmental communication a must. This communication was of utmost importance here at the Valencia IGEM team, where people with backgrounds as distinct as engineers or biologists had to work together.

5- Laboratory security and safety protocols must be strictly followed.

Our team put extensive care in following all the laboratory protocols that guarantee a correct development of our work and not only our safety, but also that one of the environment. It must never be forgotten that we are working with living samples, and an appropriate sterilization of lab materials was done as an essential part of these experiments.

6- The potential use as a weapon of any of these parts must be forbidden.

Units obtained through Synthetic Biology are powerful tools that are not fully understood yet, so there might be dangerous and irresponsible uses for this technology we are not aware of. Once more, governments and researching companies themselves must make sure that these experiments cannot be utilized for bioterrorism or biological warfare.

Ethics on the use of systems and devices developed for Synthetic Biology

7- Intellectual property of devices and systems is a debate on its own.

While as stated when speaking about part registration this is a debate that has not been closed yet, the particular use of different parts (the basic element) on integrated systems and devices is a different matter, because it does not imply ownership of parts already present in nature, but a new and unique way of putting it into use for other means than the original.

8- Parts, devices and systems must be fully standardized.

In order to easily spread this technology as wide as possible, all the Units involved must be able to work with each other, that is, no system or device should be able to work in combination with other systems no matter where have they been made. And construction of "linking" pieces should be encouraged even if a particular part does not need to make use of it under the conditions where it is originally used by the research team. Hot Yeast Project, for example, has used the suffixes and prefixes provided by the MIT, so our device can be used without prior training by any other group.

9- Universal consensus must be met in order to determine which parts are used for common functions.

Certain functions (such as carrier plasmid or recognition sequences) that will foreseeable be present in every experiment on Synthetic Biology should be universal; should that fail to happen, the desired level of standardization would be hardly achieved. For instance, our team at Valencia has used one of the plasmids indicated in the MIT databases.

Ethics on the use of organisms for Synthetic Biology

10- Every organism used in these experiments must be disposed of appropriately.

As stated previously, all organisms used in Synthetic Biology are potentially toxic and /or dangerous, that is why the implementation of sterilization and cleansing protocols is one of the first issues that must be taken into account when undertaking a Synthetic Biology experiment. So in accordance to this point, at the Valencia IGEM team specific sterilization protocols have been used when working with the following strains of *Saccharomyces cerevisiae* (Ucp+, Ucp-, Ucp 175 deleted, and Ucp 76 deleted).

11- Dangerous organisms should carry growth control systems.

The risk of uncontrolled growth in the organisms used must not be taken lightly, so every single cell should grow in a way that when specific conditions are not met, a self-destruction mechanism is activated.

At the Valencia IGEM team we have hypothesized that the easiest way of keeping the culture under our control is to incorporate a knock out gene for a biochemical pathway of interest. However, there is still the risk of reversion or natural complementation. The best way we found to ensure containment of cultures is based on toxin-antitoxin systems (TA). Our population would constitutively express a toxin, but simultaneously, with a suitable inductor, it would also synthesize an antitoxin that would keep our cells alive while being in the culture. Should they leave the culture, antitoxin induction would stop, and be rapidly degraded, therefore they would die.

12- Animal dignity must be assured in every Synthetic Biology related research.

Even though current research focuses on using prokaryotic cells and other single cell organisms, we must foresee that it will advance to the point of using complex animals, so an ethic code must be created before this predictable scenario occurs.

Ethics on potentially altering the immediate environment and remote environment Units

13- Prophylactic elements and protocols must be used in order to prevent spreading cells and materials outside the lab designated areas (immediate environment).

We at the Valencia IGEM team are aware of the risks related to our line of work, and as such, have taken all kind of prophylactic and sterilization procedures (including, but not limited to: sterilization of workbenches before and after lab work, use of disposable gloves and extraction hoods when manipulating cells, destruction of remaining material after each test...etc) to avoid contamination of tools, clothes and other potential vehicles for the undesired spreading of any cells we have been working with.

14- Research group safety must be ensured.

Due to the nature of the materials used in Synthetic Biology, there is a mild to high risk of infections for the researchers. This means that all safety measures must be reinforced accordingly to the risks present in each stage of the research.

<u>And of course…</u>

15- Use your common sense.

This point melts together all previous statements, because we can forget no one of the measures needed to provide safety and responsibility to Synthetic Biology's past, present and future research. As we have said before, here at the Valencia IGEM team consider that any advance in this field represents a big deal for the progress of humanity but such powerful tools require a strong supervision and a defined set of rules that everyone must adhere to.

Just like other modern advances in science have shown, unpredictable consequences can arise from any new technology (greenhouse effect derived from the burning of fossil fuels, for instance). The risk of a few parts designed in a laboratory spreading wildly is low, but the consequences of such event are unknown to us right now, a strong reason to extreme any caution when working with these materials.

In summary:

- Debate about intellectual property of Parts should be successfully concluded.
- Potentially hazardous Parts and/or Units must be regulated and controlled.
- Research on Synthetic Biology must not be directed by illegitimate economic interests.
- Basic tools and knowledge for the use of these Units should be available to researchers on other areas.
- Laboratory security and safety protocols must be strictly followed.
- The potential use as a weapon of any of these parts must be forbidden.
- Intellectual property of devices and systems is a debate on its own.
- Parts, devices and systems must be fully standardized.
- Universal consensus must be met in order to determine which parts are used for common functions.
- Every organism used in these experiments must be disposed of appropriately.
- All organisms used in these experiments must carry growth control systems.
- Animal dignity must be assured in every Synthetic Biology related research.
- Prophylactic elements and protocols must be used in order to prevent spreading cells and materials outside the lab designated areas (immediate environment).
- Research group safety must be ensured.
- Use your common sense.

Part IV: A Synthetic Biology Final Fantasy

The reach of the effects derived from human activity is one of the first things to be taken into account if our purpose is to establish an ethical evaluation of that activity. We must be concerned about the very last consequences of our acts so as to avoid undesirable situations. Following this simple principle we will, from the tiniest of the scenarios to the universe itself, evaluate the consequences of a synthetic biology project as it is the Hot Yeast Project.

With the appearance of genetic engineering we were able to modify the genetic pool of some species to increase their industrial productivity. One step further, nowadays, with the emergence of the field called synthetic biology our intentions of engineering life have taken new dimensions. Not only we manipulate the genetic material of living beings but we develop devices, with accurate control mechanisms, supposed to be in the future efficient biological *nano*machines. We could call this the ultimate way of manipulating life. With such potential, the research done in this field must follow more than ever the concentric scheme that we propose, predicting the whole impact of the projects.

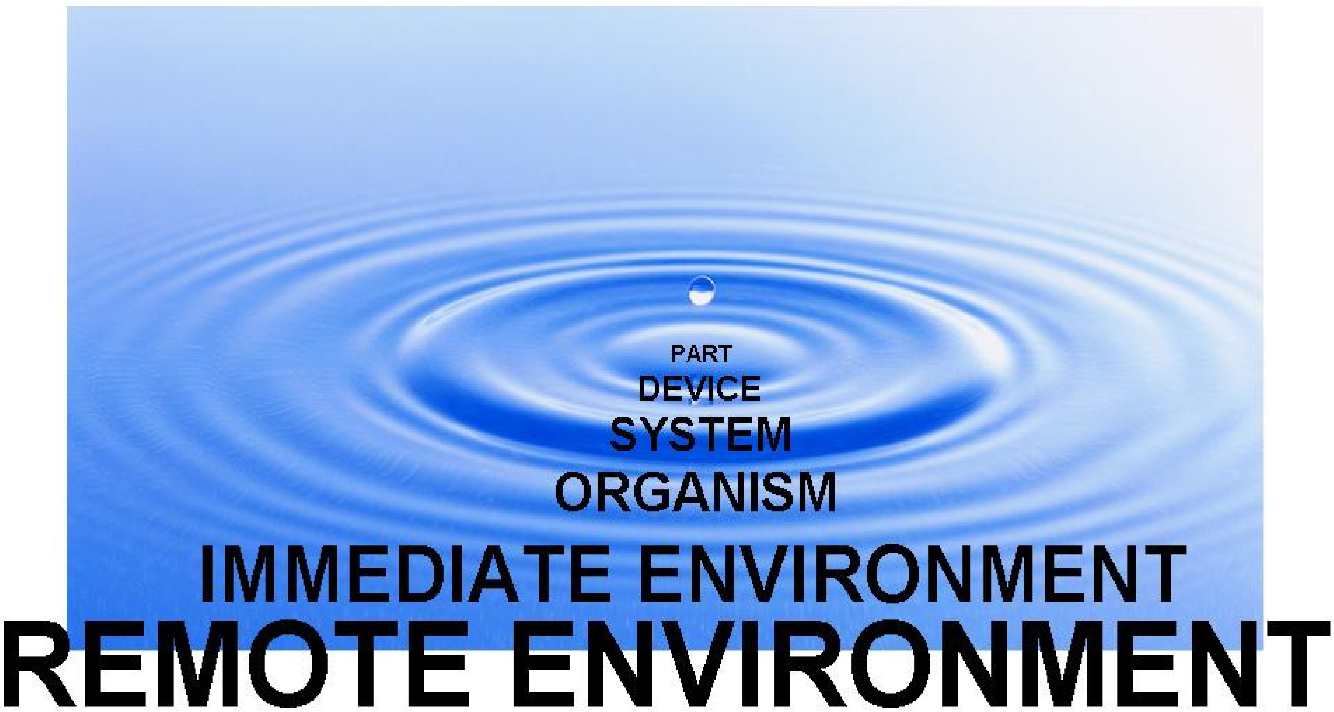
The first scenario of any project is the lab work. Biocontainment is, perhaps, the most important thing to be controlled because devices are going to be distributed for industrial purposes. We should recommend the enterprise different ways of controlling the spread of these modified biological populations, or even implement control systems in order to bind the modified population to the culture conditions. The consequences of disobeying this statement would range from the destruction of the natural genetic pool of the species implicated to the entire substitution of natural populations. Our hot yeast is thought to be an ancillary heating population for big bioreactors of compatible organisms. The easiest way of keeping the culture under our control is to incorporate a knock out gene for a biochemical pathway of interest. However, there is still the risk of reversion or natural complementation. The best way we thought for ensuring the containment is based on toxin-antitoxin systems (TA). Our population would constitutively express a toxin, on the other hand, with a suitable inductor; the antitoxin would keep our cells alive while being in the culture. Once they left the culture, with no induction and no antitoxin –rapidly degraded-, they would die.

Another important thing to determine in our experiments is the plausible risk for human beings involved in the manipulation of the final product. We should evaluate all the undesired interactions, such as toxicity or possible allergic reactions and establish a manipulation protocol. During laboratory manipulations all these things are taken into account thus we can obtain valuable information from our experience. Our hot yeast lacks dangerous compounds and the output temperature is not dangerous. Nevertheless the containment system based on toxin-antitoxin could have hazardous elements; hence we should analyze the system.

Beyond this level we can find nations and governments interested in the military applications of this new technology. As research results and methods are published in scientific journals, the information flow can end up in weapon development. All the great technological advances are followed by new weaponry available. Does this mean that the fear to new deleterious biological devices should stop scientific development? Or the real danger is the amazing specificity that this new army of biological devices can develop compared to the traditional massive destruction weapons? In any way, science must go on, always trying to avoid the direct development of weaponry. Perhaps the regulation of the parts industry is the most effective to deal with this topic. An accurate control and identification of the biological parts would be an effective measure. However, at least during the initial stages of synthetic biology progress, we will not have to worry about weapon development. The systems are quite unstable and the experience of the scientist that developed it is necessary for their accurate running. At the present time, it would be extremely hard to use the parts developed by other scientists for constructing biological weapons. Notwithstanding all the exposed, we should think of all the possible military applications so as to classify and regulate the parts properly, according to their potential risk.

The last concentric issue, the universe itself, must not be forgotten. What would happen is human beings disappeared and left behind an important population of synthetic biology systems? If an intelligent civilization discovered our planet and studied the life on it, it would be very difficult for them to establish evolutionary relationships. What should we do? Standardize a universal and unique label, present in the DNA, for all the synthetic biology systems. By designing a label we would allow problems for future scientist or ourselves even. Notwithstanding being containment an important issue, if the synthetic system escaped and spread it may horizontally transmit the DNA to natural strains of bacteria. The label could be a helpful tool to follow the dispersion of the strain…

Concentric Units of Ethical Issues: an artistic approach



References

Current National Guidelines, Codes and Reports relevant to this Code:

Codes

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