EDUCATION

Education had a pivotal place in science: a scientist is insatiably eager to learn and teach. At the very beginning of the development of our project, we unanimously decided to build an important educational program for our iGEM participation.

As our project was progressing, three main domains emerged as crucial for iGEM competition, society and to the BioMaker Factory: Synthetic Biology, Public Health and Microbiology. These are the three topics we have chosen to teach during this year.

Through practical courses with high school students, meetings surrounded by crêpes with kids, our formal presentation to adults, our teaching has taken many forms to reach a large range of audience; to us it really felt like a strong public engagement.

OVERVIEW OF OUR WORK ON EDUCATION

Non-scientific adult public

When looking for funds, it was very important to us to give our audience a actual comprehension of the science that is behind the project. We did our best to simplify our speeches for greater clarity. Since DNA, genetic information and even the notion of cells are pretty hazy for most people outside of the world of biology, it was hard for us to give a proper explanation of what is synthetic biology and why we use bacteria, why it is not harmful… But thanks to a lot of practice and advices from our professors, we learnt how to give accurate yet straightforward explanations of these complex concepts.

Actually, we managed to arouse curiosity and make people ask for further information about non-pathogenic bacteria, antibodies or even gene editing!

Kids

We met a lot of kids during the different events we attended or organized such as pancake sales in parks. Against all odds, meeting kids that have no concrete idea of what are genetics, molecular biology and that don't understand the matter and challenges of public health (at least that is what we thought) was very instructive and rewarding! Youngsters are so creative and full of unexpected toughts. They gave us very quirky ideas for the use of our Factory and we will definitely keep them in mind for the after-iGEM.

Thank you kids for the open-mindness talks and for your drawings!

High School student

French teenagers know a terrible crisis at the end of high school because many of them they have no clue of what they want to do after: go to university? Or in a higher education school? To study what? Studying science – especially biology – at university is not the sexiest pathway for a 17 year-old student-to-be. It was thus very important for us explain them how research in biology can be exciting. If you want to know more about the lab work we carried with a Parisian high school class.

PRACTICAL CLASS IN "MAURICE GENEVOIX" HIGH SCHOOL

During summer, we contacted Miss Yasmine Bellagha, a biology teacher in Maurice Genevoix High School in the city of Montrouge, close to Paris. We wanted to organize a practical course with one of her classes. She was very happy to hear about iGEM competition and about our proposition!

The practical work will take place in them sessions:

First sesstion: (3 hours – 13/10/2017)

1. Presentation of the 2-session class
2. Presentation of ourselves
3. Reminders: genotype and phenotype
4. Practical work

Second session: (2 hours – 17/10/2017 - 4 days later):

5. Interpretation of results (Lab reports)
6. What is synthetic biology?
7. Presentation of iGEM competition and our projects
8. What can you do with a university degree in Biology?

Notions we wanted to transmit

- **Yeasts:** Yeasts reproduce by budding or more rarely by scissiparity. Yeasts form conical colonies on a petri dish, unlike bacteria that tend to form colonies and fungi. Their size (5 to 15 µm) is greater than that of bacteria (1 to 3 µm), which makes them easy to observe at 400 magnification.
- **Saccharomyces cerevisae Ade2-:** It is a haploid unicellular eukaryote. It is the smallest known eukaryotic genome: 14,000 kb and 6200 genes. The strain used carries a mutation that affects the ade2 gene involved in the adenine biosynthetic chain. The function of the ade2 gene is to transform an intermediate of this chain: Amino Imidazole Ribotide (AIR), which is oxidized to a red pigment in aerobic state. The Ade2- mutation has two effects:
    - As the strain is unable to synthesize adenine, it will not grow on minimal medium without adenine
    - If you add adenine by supplementing the culture medium (or growing on rich medium), the mutated strain will use it to grow. Since the amount of adenine supplied to the strain is not very important, the strain does not have enough adenine to grow to the best of its ability. The strain will therefore operate the adenine biosynthetic chain. It will present a red phenotype because of the accumulation of the AIR and its oxidation.
- **Biosynthesis chain of adenine:** The red phenotype and the inability to grow on a poor environment allow us to have a "positive selection" visible to the naked eye and a selection system without the use of antibiotics. Strains which carry the Ade2- mutation after growth on a petri dish are thus easily differentiated. In fact, on a rich or adenine-enriched box, the yeasts without mutation will grow and have a white phenotype, whereas the Ade2- yeasts will grow but will have a red phenotype. On a low medium box without adenine, the unmutated yeasts grow and are white while the yeasts Ade2- do not grow.
    - Note: Mutations are common in yeast Ade2- because the accumulating AIR pigment is toxic and increases the selection pressure. It is common for yeast to spontaneously mutate at the level of the respiratory chain so as to avoid oxidizing the adenine biosynthesis intermediate (AIR) to

a toxic compound. These mutated colonies are white and larger than the red ones because they grow more easily (unhindered by the toxic pigment): it does not mean that they have recovered the ability to synthesize their own adenine.

- **About mutagenic agents - UV concepts:** The UVs used are type C and wavelength 254 nm. The DNA molecule can absorb UV light at 254 nm; UV causes mutations: uptake of UV energy by DNA results in the formation of adjacent thymine dimers, which causes breaks within the molecule due to distortions of the double helix. In the majority of cases, these breaks will be repaired by the enzymes involved in DNA replication (nucleases, polymerases, etc.). Only these enzymes can make mistakes and mutations can appear. The more one solicits these enzymes, the more the risk of appearance of mutation is important. These mutations can be lethal (if they affect a gene responsible for the synthesis of a vital protein) or not and, in this case, we have the appearance of a "mutant". The manipulation involves exposing our mutated strain to UV and observing the appearance of white colonies on medium supplemented with adenine.

Collaboration with Evry-Paris Saclay Team

We invited Evry-Paris Saclay iGEM team to join us to share with the pupils their knowledge, their lab skills and their vision of iGEM experience. Thus, we collaborated with two members of their team. Rose attended the practical class with us (1st session). She was very patient with the pupils and helped us in the long process preparation of the material and solutions on the day of the class. Yanis was here for the 2nd session (Interpretation of results, introduction to synthetic biology, Presentation of iGEM competition and our projects, overview of biology at university). He has an actual pedagogical talent and the pupils immediately appreciated him. This was a great illustration for the kids that scientific research is based on collaboration and team work. Thank you very much Evry-Paris Saclay, we really had a smashing time working with you.

Conclusion

During these 2 sessions, three main educational interests were brought to the fore:

- Initiation to microbiology: use of the microscope, discovery of culture media, dilution and spreading techniques. Learn to work under sterile conditions.
- Approach to the Genotype-Phenotype relationship: show that the phenotype is directly linked to the genotype, that traits are transmitted from generation to generation.
- Study the mutagenic effect of UV on yeasts; deduce that they act directly on DNA by introducing mutations: appearance of mutants, decrease in the number of clones, lethal effect.

This experience was very rewarding to us. The pupils really enjoyed the practical course and were very thankful. Many of them were so interested that we kept in touch via mail so that they can ask us question about biology and studying biology at university. It has also erected the urge for some of us to orient their careers towards teaching. We are very grateful to Mrs Bellagha, the teacher, who did her best to provide anything we asked for to organize the practical. She had total trust in our work and she made us feel at home in this high school.

(The protocol we gave to the pupils is given next page)

A few photos of the class

Mutagenesis of *Saccharomyces cerevisiae*

General instructions to be followed during the class

***** ATTENTION*****

For your safety and the success of the experiment, observe the following rules

- Do not touch the middle of the petri dishes with hands and wash the end of the session: *we will never totally control that have grown on a medium ...*

- Always agitate a suspension of yeasts before taking them because the yeasts sediment quickly !

- Take the habit of noting permanent marker the contents of the boxes and the initials of the binomial (4 letters) that handled.

- Working in a sterile area when handling yeast, sterile media and instruments

- Do not contaminate the studied species with external strains and do not pollute the environment with the experiments carried out on the studied strain:
    - Clean the bench with alcohol or bleach
    - Put your hands in alcohol
    - Wear a cotton blouse
    - Avoid sudden movements and talk in front of open boxes
    - Use sterilized instruments and media
    - Place soiled pipettes in a bleach jar

*****CAUTION*****

Once an instrument or a sterile environment is contacted with a cell, the ground, a hand, the neck of a tube, a solution..., it is contaminated.

Protocol

PART A
Preparation of the yeast suspension

You have a solution called **"Suspension B"** with a concentration of 10^6 cells/mL. The aim of this part is to achieve a dilution of this solution for a **"Suspension B"** a concentration of 10^3 cell/mL ready to be spread.

1. **File, with the dropper, 0.1 mL (2 drops) of the "slurry A" in 100 mL of sterile water.**
2. **Note this bottle "Suspension B".**
3. **Allocate in each of 10 tubes of 5 mL, 2 mL of the "Suspension B".**
4. **Note these "Suspension B" tubes.**

▉ Each pair has a tube "Suspension B".

PART B
Spreading of yeast suspensions on agar plate of Petri dishes

The purpose of this part is to spread the suspensions on the medium, in petri dishes.

1. **Pour 0.1 ml of the "Suspension B" *previously agitated* on a dish.**
2. **Spread the yeasts immediately in a circular motion.**

CAUTION: Do not lift the agar during spreading

3. **Name this dish t = 0: it will not be exposed to UV. Remember to also indicate the number or the initials of the group to find your dishes easily next week.**
4. **File with *another* sterile dropper, 0.1 mL of "Suspension A" *previously stirred* over the four other dishes, then spread the yeasts immediately.**
5. **Name the dished t = 15, t = 30, t = 60 and t = 120.**

▉ The boxes are then ready to be irradiated.

PART C
Irradiation of yeasts with UV-C

The purpose of this section is to expose the cells to UV. iGEM members (Yanis, Emilie and Rose) will be in charge of the UV box. One member of each pair must bring its *closed* boxes to the UV box, while the other member goes to Part D. Start the timer once the lamp is on:

- Leave the boxes marked “t = 15” for 15 seconds
- Leave the marked boxes “t = 30” for 30 seconds
- Leave the marked boxes “t = 60” for 60 seconds
- Leave the boxes marked “t = 120” for 120 seconds

▉ The boxes are then ready to be irradiated.

After irradiation, incubate 5 boxes for 4 days at 28 ° C, lid down.

PART D
Yeast observation under a microscope

The purpose of this part is to observe and characterize r cells that are manipulated.

1. **Take a drop of the "Suspension A" and place it on a slide.**
2. **Lay a sidelap along the drop and gently cover the sample so as not to trap air bubbles.**
3. **Observe the maximum magnification available.**

NB: If cell density hinders observation, dilute the suspension.

4. **Make a simplified schema what you see or take a picture.**

Lab report

1) INTRODUCTION:

a) What is the purpose of this practical class? Answer in the form of a question.
b) What were the different experimental conditions.
c) What are yeasts?
d) What are the effects of UV on DNA?
e) What is the mechanism of Ade synthesis?

2) OBSERVATIONS:

Exposure Time	Total number of clones	Number of white clones	% of whites
0 seconds			
15 seconds			
30 seconds			
60 seconds			
120 seconds			

NB: It is necessary to multiply the number of colonies per 100 for the non-irradiated dishes to compare the results with those of other boxes: the suspension "B" spread over the box "t = 0" is 1000 times less concentrated than the slurry " A "spread on the irradiated boxes.

3) ANALYSIS:

Transmission *t = 0*
a) Analysis made the comments on the dish *t = 0.*
b) What is the name of this type of experimental condition ?
c) Can there be white colonies on *t = 0 ?*

Boxes *t = 0* - *t = 120*
d) Compare the number of colonies in the dishes.
e) Propose an explanation.
f) Why have we realized a dilution for *t = 0 ?*

Boxes *t = 15* - *t = 120*
g) Compare the proportion of white mutants.
h) What can you conclude ?

4) SYNTHETIC BIOLOGY:

a) What is a mutagenesis? Can it be directed?
b) What could be the point of mutagenesis?
c) Is it possible to perform mutagenesis on non-microscopic organisms?
d) What problems can be raised after performing mutagenesis?
e) What use would you make of synthetic biology?

Our Biomaker Factory aims to completely disrupt today's global healthcare system. Indeed, thanks to our machine and with the cooperation of other structures such as Med Trucks, the production will be relocated to the place where medicines are needed with lower cost and no problem due to conservation.
Moreover, the production will directly meet the demand; thus, there will be no intermediaries and no overproduction: this will allow making medicine with a low price, as close to the cost of production as possible.