### Session April 20th

Inoculum and Xylene addition. We inoculate massive quantities of xylene as part of our first project in which we had the idea of a biosensor of xylene. The first try was to make a construct sensitive to the presence of Arabinose, some ideas involves the creation of chimeric proteins.

# Session April 26<sup>th</sup>

Creation of student group Biologia sintetica in ITESM CQ. We decided to form a group to take advantage of the resources given by the university such as international calls. Making this group will make easier for other groups to participate in the same kind of projects

#### Session May 4<sup>th</sup>

The experiment with Xylene in culture of E. coli provided results. Bacteria did not grow in some concentrations. Xylene proven to be toxic and very stressful to the E. coli. There was a minimum concentration in which bacteria did grow.

#### Session May 17th

We decided to change project to a simplified version of it. The new construct was the one we are presenting in this edition of the contest. We change the substrate for Arabinose, instead of Xylene, also the part in which the bacteria moves was erased. Also we added a green light photoreceptor. In which the green light will activate the expression of genes.

#### Session June 3rd - 5th

Microcongress with the other Mexican teams in Monterrey. We have a lot of fun with our team and the other teams. We went to some conferences about topics related to synthetic biology. Also the road trip was full of laughs and learnings.

### Session June 14th

iGEM Biobrick kits arrived today after weeks of customs office delay. It is the first set of materials we received, so we started working today.

We started the standardization for transformation and mini-prep protocols; the first step of this was looking for both protocols in the iGEM page. At the beginning we lacked a lot of reagents, since we didn't knew how iGEM protocols needed special kits and they were arriving too late for us, we started searching for other alternatives, the after achieving this, we continued doing the solutions.

#### Session June 15<sup>th</sup>

We read the iGEM protocols to re-suspend dehydrated DNA from plates; we learned how to select them using the antibiotic resistance. We made competent cells with another protocols including iGEM ones, also we found a lot of support on the molecular cloning book. We selected from the 3 competent cells the ones that had a fastest grow rate, we used 3 strains for this process, K12, DH5-alpha and BW27783. The fastest until now are DH5-alpha cells and BW27783; they got an Optical density (OD) of .356 and .400 respectively in a two hour range.

#### Session June 17<sup>th</sup>

We started the standardization of transformation protocols; we employed a BioRad plasmid of PGLO; after doing the transformation we cultivated them with ampicillin and arabinose, which led into the glowing expression. The process will be repeated to standardize it. Transformation was repeated and we tried unsuccessfully to measure the DNA ratio with a spectrophotometer.

#### Session June 21st

We achieved the standardization for transformation, we only have enough DNA for transformation but we can't run a gel for looking for the length of the plasmid. We started only with the transformation of ampicillin resistant Biobricks. We weren't able to find kanamycin antibiotic for laboratory use.

### Session June 23<sup>rd</sup>

All ampicillin Biobricks tried were successfully transformed into competent cells but 1, the reason was that we miscounted the lanes in the plate and took a wrong Biobrick. We started with the kanamycin transformation; we have a lot of problems with these Biobrick. We suspect is the kanamycin quality what's causing the problems.

### Session June 25<sup>th</sup>

After searching in the lab notes we found that we again miscount the plate wells and used a wrong Biobrick, the next step is to transform the correct Biobricks. Plasmid backbone is amplified and stored for Biobrick assembly.

### Session June 27<sup>th</sup>

We obtained the kanamycin transformations correctly after using the popper Biobricks; also we tried with a new kanamycin from a different provider from the first experiments. After much mess-up with the labeling, Paty designed a labeling protocol for our construct, it is really nice and spared us a lot of confusion.

### Session June 28th

We continue to have mini-prep problems; there is enough DNA for transformation but not enough to run a gel, even if we find a high amount of DNA. After massive literature investigation sessions we determined that the problem of the mini-prep solutions is in the protocol and solutions for mini-prep. We need the kits or we will not be able to continue. Lab sessions will go from a daily basis to almost full stop until stuff arrives.

### Session July 12th

Materials and kits and lots of stuff are still delayed by customs. We fear it may be spoiled by now. Some of the most urgent materials and kits are bought from another provider hoping that they can get through customs faster.

# Session July 19th

Customs assures that our stuff will be released some time during the week. More transformations are made.

# Session July 29th

Summer is about to finish and we don't have the kits and materials missing. Mini-prep problems persist, we suspect is because of the protocol.

# Session August 8<sup>th</sup>

Summer's over. First day of school. The team had a meeting to specify the tasks ahead and to schedule the activities according to the times and classes of the team members.

# Session August 20<sup>th</sup>

Lab inventory is made to asset the materials available to continue.

#### Session August 26<sup>th</sup>

PCR Purification kit arrived. Due to the lack of materials we ask other labs for aid and donations of to continue, but our requests are scarcely answered. Moral is running low 🕾

#### Session September 13<sup>th</sup>

More lab kits arrived along with the chloramphenicol; we used them to do the transformations of the pieces we ordered to be synthesized. All of today's attempted transformations were successful but one, RecA.

#### Session September 15<sup>th</sup>

Taking advantage of a national holyday we spent an entire weekend working on some of the remaining tasks in the lab. All part transformations are completed and awaiting for the kits to begin the testing of functionality. A special experiment is performed using a modified lithium buffer that allows an electrophoresis gel to be done in 10-15 minutes instead of an hour; the DNA bands are shown correctly but the ladder is messed.

# Session September 20th

About one week for the deadline. Due to the lack of materials a special meeting is held to reorganize the programmed activities in order to minimize the time and steps required to put

together the full mechanism and try each component. Up to this far we've got no results for the construct as for the customs office has delayed the packages for months now. We desperately ask fellow laboratories, colleges and iGEM teams for urgent assistance.

# Session September 26<sup>th</sup>

The last of the kits arrived today, just two days before the deadline. All team members but two are working on the lab/wiki full throttle. We might just pull a magic trick and finish...

# Session September 27<sup>th</sup>

Most of the members of the team did not sleep in order to be able to finish the construct. However, in the lab everything was done smoothly, no big mistakes were made and the teamwork was better than ever. Our fingers were crossed!

# Session September 28th

Success! Parts were finally ready, slowly but steady... the objectives were achieved with success!