Laboratory advances

This month we are in the final spurt, because we will finish our laboratory work on 10/08/2018 and devote ourselves more intensively to our presentation, the poster for the Giant Jamboree and our website (the so-called Wiki).
So far we have invested all our time and energy to complete the missing cloning. Nevertheless, we struggled with some difficulties.

Fortunately, we were able to complete the cloning in the quorum sensing system. The plasmid for self-regulation of *E. coli* populations was successfully transformed and experiments to analyze the functionality of the plasmid were successfully performed. The use of another *E. coli* strain generated by us, which synthesizes a fluorescent marker protein, allows us to use a plate reader for a high throughput determination of cell density. The measurement took several hours and was compared with previously measured data. The evaluation of the data is still in progress.

The auxotrophy system is also showing great success. The lysC construct for the overproduction of lysine was successfully completed and transformed into E. coli.
The LEU2 gene for the overproduction of leucine was subject to major delays due to impurities in the cloning toolboxes. After a lot of purification of the PCR products we were able to amplify LEU2 successfully, but now there is no time left to create the finished construct. We will now focus on the investigation of the lysC construct in our co-culture.

Unfortunately, the nutrient system also had to struggle with setbacks this month.
For the synthesis of ammonium from melamine, six different genes from different organisms are responsible for our *E.coli* construct, which we had to divide into two plasmids due to their length. For the first plasmid, which carries the first four genes for melamine conversion, the required parts are all ready, but there are currently still problems with cloning into the backbone. For the second plasmid with the remaining two genes we had to perform overlap extension PCRs with very large overhangs, which turned out to be very risky. In the end, however, we managed to create the finished insert for the backbone. However, we wanted to use a specific vector for this, to which the construct is adapted. Experiments and transformations with this vector were negative several times, so the experiments had to be stopped.

The problem of the missing sugar export with the cyanobacterium strain of Pamela Silver, which we encountered last month, was solved by using the cscb strain of Danny Ducat. This strain already contains the genes needed for the sucrose-phosphate synthase to synthesize sucrose and the cscB symporter which exports sucrose from the cells.
However, since the strain does not possess invertase invA, only sucrose is exported. *S. cerevisiae* is able to use sucrose by its own invertase, but we are afraid that *E. coli* will probably not be able to utilize sucrose that well, as no growth was observed in preliminary experiments with sucrose media. Co-culture experiments are currently underway to find out how the organisms work together and grow under these conditions.

The plasmid for the self-designed S. elongatus strain is cloned, but the decontamination of our original strain and a failed transformation of the plasmid has set us back in time. Since the integration of plasmids into cyanobacteria is very time-consuming due to the high number of genome copies, our remaining time is unfortunately not sufficient for a second transformation experiment.
For the conversion of phosphite to usable phosphate for our yeast construct we only need the gene *ptxD*. The amplification of the gene from genomic DNA of *Pseudomonas stutzeri* has failed several times, so that we now had to order it directly from IDT codon-optimized. The gene was successfully secured and the construct was completed. However, errors were made in the sequencing of the construct, so that the evaluation of the sequencing is no longer possible. However, the test restriction showed positive results, which is why we are planning further tests with the finished construct to confirm its function.

Since we perform so many cloning experiments at the same time that our capacities are almost completely exhausted, we decided after a further discussion with our supervising professors to stop the cloning experiments on 23.09.18 and to dedicate ourselves completely to the co-culture experiments.
We plan different experiments in which we cultivate the organisms in all possible variations together. We want to investigate how well our constructs work and how we can build co-culture in the best way possible.

As rapid pre-experiments we have already carried out so-called filter tests. We spread out an organism on a minimal medium that lacks a nutrient and then applied different concentrations of the missing nutrient to small filters. We were then able to measure how many colonies developed at which nutrient concentration and were thus able to draw conclusions about the required minimum concentration of the various nutrients.

Until 07.09.18 we will carry out further planned experiments under high pressure.

Outreach

As we have the task to share our knowledge with everyone, we created a small survey to get an overview of the current general state of knowledge. The survey was available online. But we also wanted to have a personal conversation, which is why we presented ourselves to a well-known supermarket chain with a small stand. There we met many interested but also sceptical people, to whom we answered all the questions that arose when filling out the survey.

In order to inform ourselves a little more about the benefits of co-cultures for society, we met with a pharmacist. She informed us about the current topic of probiotics and explained to us how in demand these dietary supplements are. According to her, new products are launched on the market almost every week, as many people are currently interested in them and demand is rising steadily. This conversation helped us a lot to present our project better and to better illustrate the benefits of our co-culture.

For us it is very important to give everyone the chance to get to know the synthetic life sciences. Heinrich Heine University offers a wide variety of language courses and integration programs for refugees. We wanted to give the participants of these courses the opportunity to get in touch with the synthetic life sciences in a small group and to ask ourselves everything about life at the university as well as the iGEM competition. We all had a lot of fun, talked a lot and learned a lot from each other.

In addition to educating the population, our goal over the months has been to present our project to experts, which is why we registered for a presentation at a conference for cyanobacteria, the Cyano World 2018 - 3rd early career research symposium on cyanobacteria.
In fact, our abstract was considered interesting enough, which is why we were able to secure one of the bitterly contested talks.
Two team members therefore made their way to Freiburg and were the only student team to present our project to a large audience of experts. We received a lot of feedback and important tips, which we took to heart and will implement.

In the next newsletter you will already hear the last update of our project - about what happened at the Giant Jamboree in front of the judges. Stay tuned!

As always, we welcome feedback and criticism, as well as useful ideas and suggestions.
We would like to thank all our supporters very much. Without you our whole project would never have been possible and become reality! Many, many thanks!

The iGEM Team Düsseldorf 2018