

Laboratory advances

This month was under the motto growth curves for our team. In order to have comparative values for our later co-culture, the growth of different monocultures and co-cultures in different liquid media was investigated this month.

First, Escherichia coli, Saccharomyces cerevisiae and Synechococcus elongatus were used individually in the standard media YPD (yeast extract peptone dextrose) and LB (lysogeny broth) and the more specific medium BG11, which is normally used for the cultivation of cyanobacteria. In addition to that, we used a modified form of the BG11 medium - the so called M2 medium - which Dr Daniel Ducat, Michigan State University used for co-cultivation and recommended to us. Measurements were taken in triplicates and under the same conditions to increase accuracy and to ensure reproducibility.

The cyanobacterium *S. elongatus* had to be measured approximately every 12 hours for one week. Next to the optical density (OD), the chlorophyll content was determined as well. In order to achieve a comparison to typical wild type growth we also created growth curves of the sucrose-producing <u>cscB strain</u>, which was also suggested by Dr. Ducat for our Nutrient System.

S. cerevisiae had to be measured in a period of 36 hours in two hour intervals, *E. coli* for the same time period every 30 minutes. This duration meant that many measurements had to take place overnight so that many brave helpers from the team had to combat fatigue in turn and stayed in the laboratory until the early morning hours. Different strains were used for the *E. coli* growth curves as well: DH5alpha and Rosetta2 (EN3). The latter one is very often used to express eukaryotic proteins.



In addition to monocultures, the first double and triple co-cultures were also produced and quantitatively evaluated

S. elongatus was cultivated in M2 medium individually with S. cerevisiae and E. coli and together with both. Since it is difficult to quantitatively evaluate the individual organisms in a co-culture, next to OD measurements at three different wavelengths, chlorophyll samples were taken, cryos were made for subsequent cell counts under the microscope, and a dilution of each culture was streaked every three hours on YPD and LB plates to estimate the number of S. cerevisiae and E. coli cells.

As a result of the many growth measurements, we have come to the conclusion that the already mentioned M2 medium will be best suited for our final co-culture. In any case, small fine-tuning of the composition is still required, but the basic structure is in place.



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Outreach

As every month, we did not miss the opportunity to exchange ideas with interested people in order to bring them a little closer to the Synthetic Life Sciences. Within our lecture series <u>Dr. Christian Dumpitak</u> and our advisor <u>Nicolas Schmelling</u> gave the lecture. The lectures provided a deeper insight into the definitions of genetic engineering and breeding as well as ethical issues in synthetic life sciences. We met many interested listeners with a lot of additional questions about the lectures, which we were happy to explain in more detail in personal conversations.

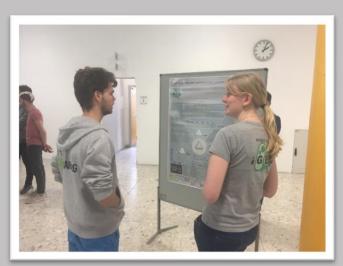
In order to deal a little with the younger target group, we decided to visit the award ceremony of the Karl Frisch Prize organised by VBio in Dortmund on 28.06.2018 and gave a lecture to the award-winning high school graduates there. The aim was to inspire the students for Synthetic Life Sciences and to give them insight into our project. In addition to that, we presented the iGEM competition and answered the high school graduates' questions about all aspects of study, biology and iGEM.

In addition to these public events, we also had a very important appointment with some of you. We presented our project in front of a few professors and received constructive criticism, as well as many new suggestions and tips during the following round of talks, which we are very thankful for. Many of the great ideas were strongly discussed and worked out by us in the following days. Thank you again for your help!

Networking

Besides public relations, it is also very important to exchange ideas and to share useful ideas and skills with other iGEM teams. That's why we had two big events on our agenda this month.

At first some of our team members participated in the German iGEM Meetup in Marburg to make many new contacts and to present our very first scientific poster. During the poster session our project was very well received and there were many interesting collaboration possibilities. The main idea for all collaboration ideas is the production of a substance in our co-culture that other teams need for their project. We are currently in close contact with



<u>iGEM TU Darmstadt</u> to work out this idea.

Another collaboration is planned with iGEM Hamburg. The team is going to work with cyanobacteria, but is still relatively inexperienced, which is why we supported them in word and deed during a long skype meeting. We were able to answer many of their questions and also pointed out some very interesting papers that we also used for orientation.



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Last but not least we had a very important appointment with <u>Kristin Ellis</u>, a representative of <u>Opentrons</u>. We took part in a company <u>competition</u> last month and fortunately won a pipetting robot. For this reason the Opentrons team wanted to get to know us and organized a video conference with us. It was a very nice conversation and we could learn a lot about the robot and our future work with it.

As always, thank you very much for your interest and support in our project! We are always grateful and open for suggestions and criticism, as well as for questions about our project.

The iGEM Team Düsseldorf 2018



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