Minutes of the eighteenth iGeM meeting

16th of July 2010

Participants: Mareike Roth, Sarah Mansour, Habib Bukhari, Svea Grieb, Victor Gordeev, Jonathan Tam

Supervisors: None

Organization:

Next meeting Wednesday 11: 30 in the MPI lab.

Sponsoring

Seems like BMW (Bayerische Motoren Werke) is interested in giving money for our aim, but for that a convincing overview of our project should be forwarded to them. Also Svea mentioned a potential sponsor to try could be the Rotary International (Rotary Clubs).
Jon and Mareike volunteered to write to BMW (probably the letter will include some info about the competition and a less detailed abstract).

Project

A problem that was reintroduced into focus was that for AHL producing activity of LuxI there is need of 2 substrates: SAM, but also an acylated Acyl Carrier Protein (~9 kDa) which normally stays inside the cells, as opposed to what we need. Habib and Victor will try to look for ways of solving this problem. Is acyl- ACP available commercially, or is it possible to easily synthesize this compound by using the corresponding synthetase.

The discussion with David Drechsel (protein purification-MPI) shined more light on the possibility to use a fusion with the maltose binding protein (MBP) as a cheaper purification alternative to the IgG column. In addition the MBP will render the protein more soluble and the purification is not associated with denaturing conditions like in the case of protA on IgG columns.

So our triple fusion protein (MBP-LuxI-domain B of protein A) could be purified using an amylase resin. After purification the MBP could be cleaved (if we engineer the cleavage site). As an additional measure would be the addition of a His tag, because the maltose-amylase binding is not so specific as, for instance, IgG – protein A binding.

David provided us with his vectors (pETMM-11 ZZ, pETMM43), as well as the sequences with the corresponding restriction sites.

Asked, how feasible he thinks our project is he gave good feedback estimating that producing the fusion construct would be possible in approximately a month.

Jon will probably transform the plasmids to *E.coli* on Monday. He pointed out that we should be careful not to lose the plasmid while David is away on holiday (about 3 weeks). However, during the experiment, in case questions appear we can ask for Mike (a purification specialist working in his lab).

Dr Bachmann is still away, and the PhD student from his group (Marc) couldn't help with the questions Lucas addressed to him regarding the concentration of Cd33 marker in the tumour cells..

Chiba team is still not answering to the questions regarding the methods the method they used to image their cells.

Andy's suggestion that our detection system should be tried at first not on CD33 cells but rather on a dilute antigen was mentioned.

The question was raised about how many days and hours per week will everybody be able to spend in the lab and about the need to make a schedule.

In any case the groups should meet apart to discuss the plans for the lab work.