Minutes of the 16th iGEM meeting

01/07/2010

Participants: Rahul Akkineni, Habib Bukhari, Charanya Sampathkumar, Svea Grieb, Victor Gordeev, Sarah Mansour, Mareike Roth, Lucas Schirmer, Jonathan Tam , Adithya Nagarakodige

Supervisors: Annelie Oswald

Organization:

1. **Biolympics**.
    a. The barbeque event for the Biolympics takes place on **Friday, 09.07.2010**, Svea will buy the food discussed last Monday and Johnson will prepare Indian chicken.
    b. A Basketball Training Session takes place on Tuesday for everyone who is interested.
2. **Moving to our lab**
    a. We will have an introduction to safety rules at the MPI on **Wednesday, 07.07.2010 at 12 noon**.
3. **Ordering Materials**
    a. Sarah is responsible for ordering BioBricks from the iGEM page.
    b. Chemicals and other materials will be ordered via the Oats lab accounts in the beginning.
4. **Wiki**
    a. Jonathan will write a project description for the wiki as well as for distribution to sponsors.
    b. **By the weekend** all members should have written a short paragraph about themselves including the following points:
        i. Background
        ii. Nationality
        iii. Why did you participate in iGEM?
        iv. Everyone is free to include facts not listed above.

Project Planning – SensorBricks

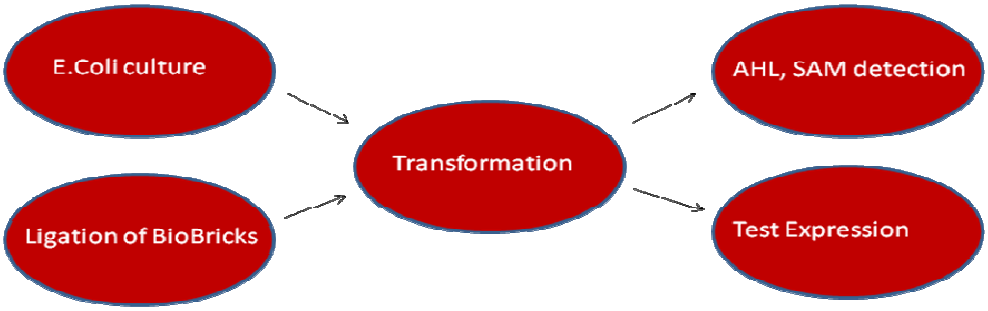
General Overview

Lucas presented three subprojects which can and will be carried out in parallel.

1. Preparing the fusion protein: LuxI-ProteinA (domain B)
2. Creating LuxRp-GFP reporter
3. Enhancing LuxRp Sensitivity

1. **Preparing the fusion protein: LuxI-ProteinA (domain B)**

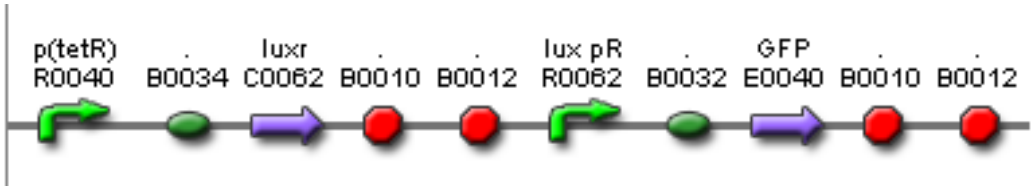
Team members: Sarah, Adithya, Mareike
The aim of the subproject is to express the fusion protein consisting of the enzyme of SAM breakdown, LuxI, and the IgG/M binding domain of ProteinA, domain B.



Both LuxI and the domain B of protein A are available in the registry and will be ligated together without linker before introducing the DNA to E.coli. If necessary different linker length will be introduced.
Considerations:
A suitable detection method for the amount of AHL has to be found. First ideas included an assay with a sender culture (containing the fusion protein) and a receiver culture (containing an AHL reporter) brought together. The amount of reporter is measured.
Secondly, HPLC could be used to determine the amount of AHL after certain time points produced by the LuxI.
Expression will be tested using Western Blot.
Protocols to set up: Preparing competent cells, Ligation, Western Blot, Mass Spectrometry

2. **Creating the LuxRp-GFP reporter**

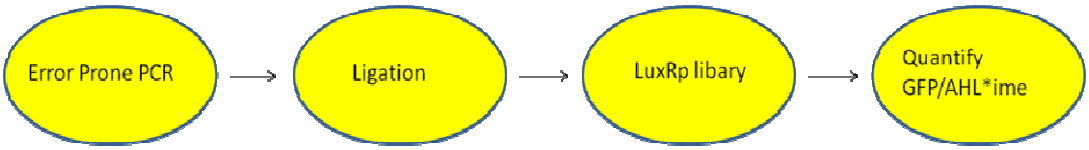
Team members: Charanya, Rahul, Lucas
The aim of this subproject is to prepare a reporter for the produced AHL in step 1. For this two system will be used.
The system shown below will generate a linear GFP output. Introducing additionally LuxI downstream of GFP will lead to an exponential increase in GFP signal.



Both constructs will be used to characterise the AHL dependent GFP output.
Protocols to set up: E.coli culturing, Restriction Digest, DNA gel electrophoresis

3. Enhancing LuxRp sensitivity:

Team members: Jonathan, Svea, Habib, Victor
The aim of this subproject is to increase the sensitivity of the wild type LuxR promoter using error prone PCR.

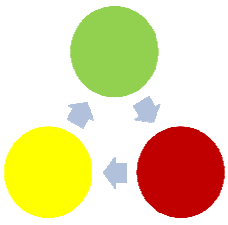


A library of LuxR promoters will be created. The promoters identified to be most/ least sensitive will be further used and sequenced. The ultimate goal is to create a quantification system like shown by last year's British Columbia team (Traffic light system).
Protocols to set up: Error prone PCR

Next steps
In order to prepare everything for the wet lab work the subgroups have to define the following things:

- Materials required
- Standard Parts required
- Protocols (listed within the subprojects)
- Find solutions to individual problems (e.g. Team1: AHL detection)

The list of materials and methods must be uploaded to the internal Wiki by **Thursday, 08.07.2010**. A cross checking will take place over the weekend in the following order and will be discussed on **Monday, 12.07.2010** :



From last meeting

Due to the Biolympics event the points discussed in the 16th iGEM meeting have not been reported today but will be reported in the next meeting.
The questions and members responsible are as followed:

1. Charanya: Do the components of blood affect LuxI activity?
2. Lucas: What is the concentration of CD33 on leukaemia cells?
3. Sarah: How expensive are the current methods for tumour antigen detection?
4. Jonathan: Is there another cheaper method for detection?
5. Svea: Is there a quorum sensing expert in Dresden?
6. Victor: Fusion protein alternatives?
7. Rahul: Finding a suitable amplification system

Habib, Mareike and Adi, please try to come up with a problem and solution for this idea.