Minutes of the 22nd iGEM meeting

12/08/2010

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

Supervisors: Annelie Oswald, Johnson Madrid

Organization:

1. **Oates Lab** As we use a different buffer for Agarose Gel Electrophoresis we were asked to store our TBE buffer separate from the labs buffer and clearly mark it. Secondly, we were reminded to carefully handle all substances potentially contaminated with EtBr. When handling gels never take gloves outside and try to store everything you bring and take to room on the opposite situated bench.
2. The **next meeting** will take place on Thursday 19.08.2010 6pm at the MPI.
3. **Ordering** has to be carried out via the Biotec from now on. A detailed list of items to be ordered has to be handed to Kathrin at the Biotec. She will take care of everything else. It has to be considered that ordering will now take approximately one week. Storage place has to be found. A list will be made with all items required for the next weeks and will be ordered by Friday 13.08.2010. For urgent items one more MPI store shopping with Anni can be done.

Wetlab Work

Team Green/Yellow

After analysis using colony PCR of clones present on chlorampenicol agar plates fragements of identical length were found for all samples. This could be caused by:

- Religation of the backbone leading to no insertion of parts at all
- T4 Ligase was not inactivated after ligation and therefore inhibited downstream experimental steps, e.g. transformation
- The 10x Ligation Buffer of NEB was found to contain PEG which is also know to inhibit transformation

Solutions:

- Inactivation of ligated samples at 65°C for 10 minutes
- Usage of different buffer without PEG
- Analysis of plasmids from miniprep

Team Red

Gradient PCR was carried out. Due to shortage of Phusion Polymerase only one PCR reaction for each target fusion protein part at the optimum annealing temperature was performed. Afterwards a purification was carried out and restriction digest. Two different

ligations overnight and for one hour where tested. Transformation will be carried out on Friday 13.08.2010.

AHL assay

Lucas and Svea introduced a standard assay to measure fluorescence output in correlation to AHL concentration. The assay will be performed using a 96 well plate. The concentration of AHL will reach from 0-1500 nM. The first row will contain only a blank sample and the following rows will contain increasing AHL concentrations. The fluorescence is measured in octuplicates over time (e.g. 3 h). Svea and Lucas will perform a first test next week (16.08.2010 – 20.08.2010) with one of the simple parts which have GFP as a reporter.