Minutes of the 25th iGeM meeting 03/09/2010

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

Supervisors: Kaj Bernhardt, Andy Oates, Annelie Osswald, Marco Storch

Organisation:

1. Mon, 6th of September, 6 p.m. at MPI.
2. Next meeting concerns specially the official iGEM wiki
3. Anni will be on holiday for the next weeks until ???
4. There will be an evaluation presentation for the iGEM project at the Biotec in September

Project Updates:

HHL-Assay

Svea presented the current measurement procedure and talked about her concerns with the right filter sets for the fluorescent proteins. Kaj suggested to use

http://probes.invitrogen.com/resources/spectraviewer/

to check the emission and excitation wavelength on our reporters. Next to the in silico tools the team will try to measure the exact spectrum with a fluorometer. We are now able to measure the amount of HHL but still working on the assay and its sensitivity.

After Lucas presents some of the experimental results the supervisors suggested doing a standard curve for the bacterial cell density. By this we should be able to quantify the effect of growth on the fluorescence induced by HHL. Normally the bacteria should be in log-phase between an OD of 0.7 to 1.4. This depends on the bacterial strain as well as on the part or plasmid in the cells.

For the HHL-assay the group is still working on two parts with positive feedback loop. Also the new EZ rich defined medium will be tested on a few parts.

Fusion protein

The fusion protein team (Adithya, Jonathan, Mareike, Sarah) talked about their first results. They were able to build different fusion proteins of luxI and domain. The proteins

differ in the order of the two proteins. The constructs are now in the sequencing facility. The results should be available in the next week. In the electrophoresis of digested plasmid the parts showed a strange behavior. All parts were bigger the then they should be by the plasmids size. However the number of bands and the size of the PCR product were right.
Kaj suggested using 400ng of DNA in 10 µl for a digestion and only 20-30 µl elution volume after the purification. He also explained how uncut plasmids look on the gel according to their conformation.

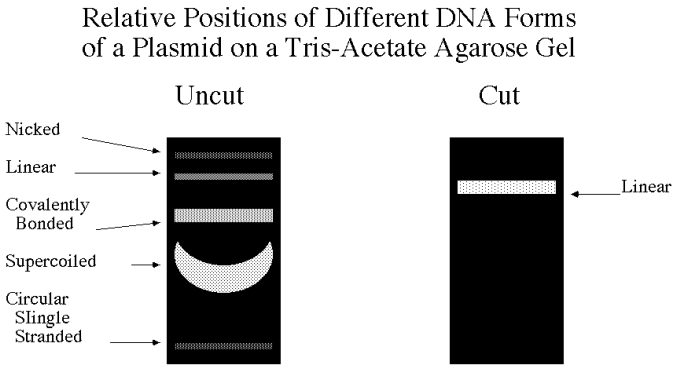

Figure 1 - plasmid conformations on a TAE gel, http://humgen.wustl.edu/hdk_lab_manual/image/plsmid3.gif

For colony PCRs we should pick at least eight colony per plate, maybe less for a transformation of a clean plasmid for example for a purification from a glycerol stock. For electrophoresis TBE buffer should be used for DNA fragments <30 kb and TAE for fragments >3 kb. Recipes for both buffers can be found at openwetware.

http://openwetware.org/wiki/TAE
http://openwetware.org/wiki/TBE

RBS parts

Charanya and Rahul tried to assemble the parts 4(luxR), 9(gfp), 20(yfp), 21(yfp) inside part 6(RBS). Now they have to select the positive clones from parts 6 because all colonys carry the Amp resistance.

Next to this Habib and Victor transformed part 45(RBS luxI), 46(RBS rfp) which no need to be assembled to new sensor parts.

Acyl-ACP

Habib and Victor ordered ACP synthase, recombinant holo-ACP and hexanoyl-CoA to synthesize hexanoyl-ACP. They also work on the detection and purification of acyl-ACP at the moment.