Lab Notebook – IISc Bangalore iGEM 2016

May 1st to May 14th

- We started working in the lab. We learnt some basic stuff like autoclaving, pouring plates, filter sterilizing, etc.
- Made competent cells of E. Coli BL21 strain
- Tansformed cells with RFP in pUC19 plasmid.

May 15th to May 31st

- We decided to work on the strain BL21(DE3) hence we made competent cells of E. Coli BL21(DE3).
- Transformed BL21(DE3) with RFP in pUC19 plasmid.
- Took fluorimeter readings of overnight grown culture of cells expressing RFP.
- We couldn't detect any fluorescent readings even when the cells showed red colour.
- Troubleshooting of our fluorimeter readings was done.
- Competent cells of DH5alpha cells were prepared.
- Efficiency of competent cells was computed for DH5alpha and BL21(DE3) competent cells.

June 1st to June 15th

- DNA Distribution Kit from iGEM arrived.
- Transformed E. Coli with Ag43 under ara promoter(BBa_K1352000).
- Started designing primers for PCRs.

June 15th to June 30th

- We decided to do the growth curves of cells expressing Ag43 in minimal media. A growth curve experiment in minimal media was performed.
- The experiment took about two days to reach the stationary state hence we decided to do the future growth curves in LB medium.
- Bba_K1072000 was transformed.
- Primer were designed and ordered.

July 1st to July 14th

- Primers were delivered.
- We started performing the PCRs with Phusion polymerase.
- Issues with Interlab study protocol reported to iGEM.
- Sfgfp under pBAD promoter(BBa_1746908) was transformed.
- A growth curve experiment for cells with Bba_1746908 was performed in LB medium.
- Work Review: Meeting with Prof. Deepak Saini and Prof. Umesh Varshney.

July 15th to 31st

- Growth curve for cells expressing Ag43 under ara promoter (Bba_K1352000) was performed in LB medium.
- We kept optimizing the PCRs in this period.

- New primers were ordered due to redesigning of our bioBrick parts.
- New primers were delivered.
- Two parts related to the quorum sensing modules were designed and ordered through IDT(called IDT parts from now on).

August 1st to August 15th

- Primers for some fragments worked and these PCRs were done on large scale.
- IDT parts arrived.
- Digestion and ligation of IDT parts were set up which failed.

August 15th to August 30th

- Plates for interlab study were made, colonies appeared.
- Growth curve for Interlab study
- PCRs for remaining fragments kept failing. We started doing the PCRs with Q5 polymerse as we ran out of Phusion in the lab.
- A skype meeting organised by IIT Kharagpur was attended by the team.
- Ligation of IDT parts kept failing.

September 1st to September 14th

- Interlab study data analysed and submitted timely.
- IDT parts showed no bands even without digestion and ligation.
- PCRs kept failling.

September 15th to September 30th

- We performed a growth curve for IIT Madras in NCBS – CCAMP with a 'Liquid Handler'. The experiment failed.
- We carried on with the troubleshooting of our PCRs which did not work.
- Phusion was delivered to the lab and we started our PCRs with Phusion polymerase.

October 1st to October 20th

- We tried our PCRs in Prof Deepak Saini's lab and added DMSO to the PCR mixtures of some of the fragments and we finally got all our PCR fragments at large scale.
- DNA was eluted for all the fragments.
- Gibson reaction was set up for two biobricks. We got colonies.
- The plasmids containing the biobricks were digested to confirm the fragment length.
- Microscopy of cells containing the Ag43-sfgfp fusion protein was performed to confirm the expression of sfGFP.
- Dot Blot analysis was done to confirm the presence of his tag in the Ag43 his biobrick.