July

01/07

1. **Discussed a Paper on Noise in Autoregulative Feedback**.
   https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1681513/

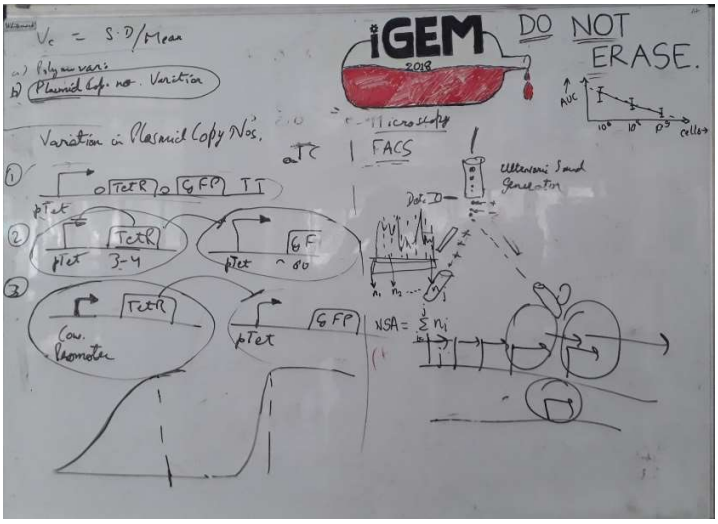
- Three constructions have been analysed:
  (a) TetR fused to GFP and repressing itself (TG-nf);
  (b) TetR repressing itself and GFP, with the latter being expressed(T+G from a different promoter - nf); and
  (c) TetR expressed by (T+G) a constitutive promoter and repressing GFP
- Simulations of the circuits indicate that the main source of noise in these circuits could come from plasmid variation and therefore that negative feedback loops play an important role in suppressing both external and internal noise. Negative feedback loops do not suppress intrinsic noise, but rather eliminate external noise that might arise for example from plasmid variation, or other external sources of noise like ribosome variation (Austin et al,2006).
  In a recent work, it has also been shown that negative feedback shifts noise to higher frequencies characteristic of intrinsic noise, while filtering external noise. Noise can be intrinsic(due to the circuit itself) or extrinsic(due to the surrounding environment), negative feedback eliminates external noise that may arrive form plasmid number fluctuations. Found that noise minimization by negative feedback loop is optimal within a range of repression strength, outside that the noise increases substantially. Plasmid copy number as a major source of noise in the system. Also that polymerase variations are thought to be a source of noise but aren't probably.(According to the authors of the paper) We prefer High Frequency Noise as compared to Low Frequency Noise. Our time period of measurement is high so, high frequency noise is filtered by the average of measurement whereas low frequency noise creates continuous incorrect measurements for a longer period of time, thus, providing seriously incorrect results.

- An important observation is that DG expression without negative feedback exhibits bimodality at intermediate TF repression values. This bimodal behaviour seems to be the result of external noise as it can only be found in those simulations that include plasmid variation
- Low copy for TetR and medium copy(~60) for GFP to produce higher amounts of GFP in correlation with aTc At low levels of aTc, GFP measurements are less and can be intersected by cells' autofluorescence. Smooth relation with aTc for GFP in case of negative feedback, without negative feedback, high noise and varied measurements across intermediate values of aTc. At low and high values of aTc, the circuit without negative feedback shows cell populations that generate similar amount of GFP. We talked about Sink effect of TetR on aTc concentration. - In the case of the negative feedback loop, the aTc concentration inside an E. coli cell could be much higher than in the medium, due to a sink effect of TetR binding to aTc. Specifically, owing to the strong binding of aTc to TetR molecules of aTc that enter the cell will readily bind to TetR, resulting, on the one hand, in de-repression and production of more TetR and, on the other, depletion of free aTc molecules inside the cell. The latter would be the driving force for more aTc molecules to diffuse from the medium into the cell, increasing the total amount of aTc (free and bound) inside the cell compared to that of the medium. aTc temporarily suppresses the negative feedback cycle due to which TetR protein levels rise to a an extent that further addition of aTc is insignificant due to TetR and aTc nullifying each other's effects until the concentration of aTc is so high that is overpowers TetR completely. Sink is the reason for difference between simulation's result experimental ones. Buffering is the

reason for GFP levels reaching saturation at higher concentrations of aTc in case of auto negative feedback.

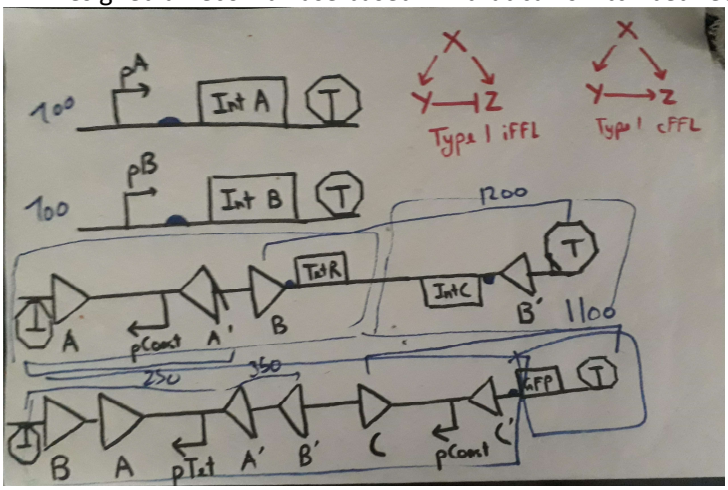
2. **Discussed a Paper on Tandem Promoter (This is related to our discussion about effect of adjacent promoters)**

- Tandem promoters are found to increase the expression of gene. (Reason not exactly known)
- This effect is saturated by 5 promoters.(about 6x the level of a single promoter) Further increase in number of promoters does not lead to further increase in gene expression.
- A possible reason for the saturation after 5 promoters is the competition between promoters for RNA polymerase binding.



02/07

1. Tried making IFFL based on Recombinase.
2. Designed a Recombinase based FFL that can switch between coherent and incoherent states.



03/07

1. Discussed Paper on Half Lives of IPTG aTC and HSL .
   "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3940292/pdf/1754-1611-8-5.pdf"
2. The degradation of aTc is majorly dependent upon temperature.
3. The degradation of HSL is majorly dependent upon pH and Medium.
4. IPTG induces is the most stable with its degradation having little dependence on pH, temperature or media.
   IPTG is degraded quickly in strains such as MC1655-Z1 due to the presence of LacI.
5. For detailed Measurements of Inducer Degradations, refer
   https://static-content.springer.com/esm/art%3A10.1186%2F1754-1611-8-5/MediaObjects/13036_2013_138_MOESM1_ESM.pdf
6. Attempt at designing Recombinase based Oscillators.
7. Two Node Circuit was designed on whiteboard. It was also modelled using mass action equations and MATLAB simulations were done.

IPTG

CONDITION	Half-Life
LB/M9 Sterile Broth	>>> 30 hrs

aTc

CONDITION	Half-Life
Temp - 30 deg	30hrs
Temp - 37 deg	15hrs

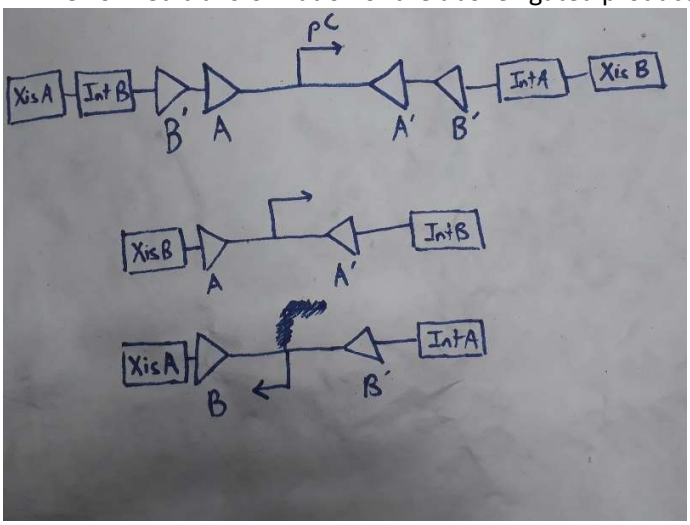
HSL

CONDITION	Half-Life
LB Sterile, pH 6, 37 deg	>30 hrs
LB Sterile, pH 7, 37 deg	~18hrs
LB Sterile, pH 7, 30 deg	30 hrs
M9 sterile, pH 6, 37 deg	~15 hrs
LB Sterile, pH 6, 30 deg	>>> 30 hrs

04/07

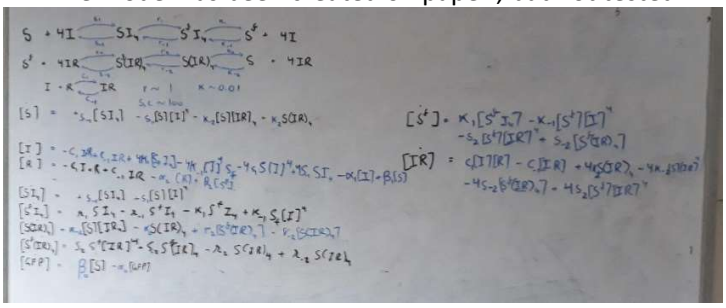
1. Performed the ligation of pTet+RBS+GFP in pSB1C3 and RBS+TetR+TT in pSB1C3.
2. Further discussion of Recombinase based Oscillator.
3. New designs were made alongside their modelling. These have so far failed to produce oscillation, except the simple Goodwin oscillator consisting of single Integrase species. Any Oscillations seen are almost always heavily damped in modelling simulations.

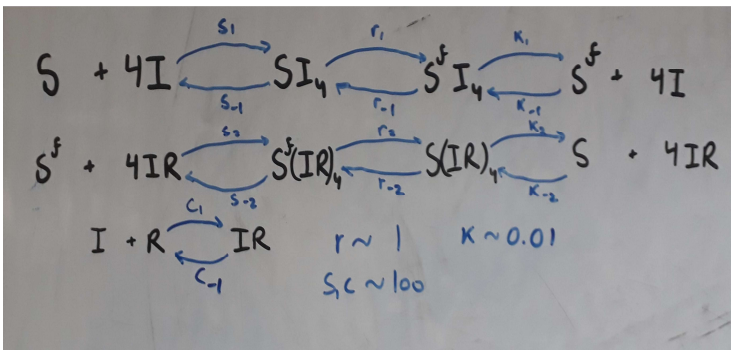
4. Prepared 5 kanamycin LA plates.
5. Performed transformation of the above ligated products.



05/07

1. Colony PCR of ligated parts from yesterday. This is to check whether transformation took place.
2. Out of 4 plates which were transformed post ligation yesterday. 2 seemed to have suffered from heavy contamination.
3. Modelled Recombinase based Oscillator using Model M of Serine Recombinase. This is a more detailed model which takes into account Tetramers of Integrase as well as RDF and Intermediate Species.
4. The model has been created on paper , but not tested in MATLAB.





07/07

1. Fresher’s orientation and marketing tactics discussion.
- The work decided was posters, bookmarks, stickers, video.
- Since schools have opened up so we need to approach them for Human practices. Neha and Soumya are working on creating a mailing list of school and drafting an IGEM SynBio Workshop. We plan to show a couple of demonstrations plus iGEM video, and streaking will be hands on.
- Vasu has been given the job of making the video for Sponsor so we can kickstart the Crowdfunding campaign.
- Aman and Prateek have been assigned the job of researching previous year wiki's and designing ours.

08/07

1. Shubham ,Ankush, Soumya and Neha are designing posters.
2. Video has been made by Vasu. Some initial edits are necessary and have been told. Then it will go for review.
3. Neha and Soumya have drafted the email for Schools and it was edited during the meeting.
4. The list of activities in workshop has to be finalized. We also need to do it as soon as possible as the semester has come very close.
5. The first draft of Poster's have been, necessary edits aside , they were pretty good.

09/07

1. We explained the recent development in project to members of our team who were away.
2. SnapGene files of new constructs were made.

10/07

1. We got a few responses for SynBio workshop. Carmel Convent. We dropped mail to them.
2. We further discussed details of the demonstrations in the SynBio Workshop
3. Subtitles were inserted into the iGEM video.
4. Bookmarks and iGEM Stickers were designed.
5. Tomato DNA extraction buffer.

11/07

1. Kshitij Sir gave a list of improvements for the Video.
2. Abhilash Sir gave a list of improvements for the Video.
3. We got 2 more responses for Workshop. Sanskriti School and Modern Barakhambha.
4. Tomato DNA Extraction performed . This will be demonstrated to schools.
5. Prateek discussed a side project idea for a software Recombinase based .
6. Priyanka made Pink and Blue Bookmarks for IGEM.
7. 20 LA Plates were created. Tips and other Hood stuff was Autoclaved.

12/07

1. Posters for Tour of Stalls were designed and finalised.
2. Ligation was discussed in detail with Kshitij.
3. Kshitij also taught Ligation calculations and other things to keep in mind when running ligation reaction.
4. Ligation calculations differ from Sticky to Blunt end ligation.
5. The ratios are decided keeping in mind reactant collisions and volume of MCT.
6. Ligase is expensive and should not be wasted.
7. The reaction volume should be enough so Ligase buffer can dominate over TAE buffer present in DNA Vector (put during digestion process)
8. We further reshot the explanation of our project using Priyanka's iPhone.
9. Shubham and Priyanka also redid the Sponsorship Proposal. They reduced the text and increased the images in order to make it more interesting to read.

14/07

1. Did Ligation calculations

pSB1K3	pTet + RBS + GFP	RBS + TetR + TT	pLac + RBS + RFP	RBS + LacI + TT

Size:2200	801	842	932	1241
Initial Conc. 100ng/ul	20ng/ul	20ng/ul	20ng/ul	20ng/ul
Conc. After Digestion: 5ng/ul	5.57 ng/ul	5.78 ng/ul	6.21 ng/ul	7.5 ng/ul
5/2200 = 0.0023	5.57/20 = 0.2785	5.78/20 = 0.289	6.21/20 = 0.3105	7.5/20 = 0.375
Ratios: 1	3.04	3.04	2.8	2.6

	pSB1K3	pTet + RBS + GFP	RBS + TetR + TT	pSB1K3	pLac + RBS + RFP	RBS + LacI + TT
1:1:1	2.4	0.8	0.8	2.7	0.8	0.8
1:3:3	1	1	1	1	1.1	1.2

2. Set up a primary in order to do transformations of the ligation product.
3. Worked on Posters and Flex for the fresher's Orientation.

15/07

1. Transformation of 4 ligated DNA plasmids done -
    - PSB1K3 , pTet + RBS + GFP , RBS + TetR + TT , ratio 1:1:1
    - PSB1K3 , pTet + RBS + GFP , RBS + TetR + TT , ratio 1:3:3
    - PSB1K3 , pLac + RBS + RFP , RBS + LacI + TT , ratio 1:1:1
    - PSB1K3 , pLac + RBS + RFP , RBS + LacI + TT , ratio 1:3:3

    It was found that some of the empty LA plates had contaminations in it.
2. Questions to be asked at schools decided. iGEM stickers (old) were cut.
3. Posters were finalized.
4. Video to be finalized later tonight.
5. Video worked by Vasu and Priyanka from 12 midnight to 5 am in the morning.
6. The crowdfunding page was made and got approval by Ketto.

17/07

1. We went to Carmel Convent school for first Synthetic Biology Workshop.
2. The workshop was from 8:30 am to 11:00 am. The workshop overran its length but was a huge success among the students. They were excited by the work we did and were enthusiastic about learning techniques of Synthetic Biology.
3. The video was well received by the students. The school was extremely gracious and the team had a wonderful time.
4. 15 LA plates were created which had CAT antibiotic resistance.
5. Bookmarks and Stickers arrived.
6. Crowdfunding Page Rewards finalised.
7. Project Objective discussion.

18/07

1. Streaking of plates.

2. Bloom School SynBio Workshop - conducted by Priyanka, Shubham, Aman, Neha and Saksham.
3. Discussed the timeline for Tomorrow when we will be visiting 3 school: Bhal Bharti, Modern and Sanskriti. Tour of stalls from 2-6 pm.
4. New iGEM IIT Delhi plates created
5. cPCR of Ligated parts is being carried out.

19/07

1. This was the biggest day for our Human Practices as we hit 3 schools in one day. This was a great experience.
2. In the morning the entire team - Saksham, Vasu, Priyanka, Shubham, Neha and Aman went to Bal Bharti Public School Pitampura. This was the highlight of all our SynBio workshops. We had 70 students who were extremely well informed about Genetics and DNA and even knew about Synthetic Biology. Conducting the workshop for them was a pleasure.
3. Next the team split up to go to 2 more schools. Vasu, Priyanka and Shubham went to Modern Barakhambha, while Saksham, Neha and Aman went to Sanskriti school. Synbio workshops were conducted in both of them and it was a good day for iGEM. The students were really excited and took interest. The schools were gracious hosts and loved the work we were doing.
4. Meanwhile iGEM Tour of Stalls also kicked off. Ankush managed the morning shift of the Tour of Stalls along with Soumya and Prateek.
5. The afternoon shift was taken care of by Vasu and Priyanka. We had a good time explaining to new students about the vast opportunities offered by IGEM IIT Delhi.

20/07

1. Our last SynBio workshop was conducted in Springdales School. This was conducted by Shubham, Priyanka, Saksham and Neha. The workshop was a success and marked a fruitful end to our SynBio workshop human practices chapter.
2. Tour of Stalls for UG students also displayed an iGEM Presentation. This was done in both morning and afternoon shifts. The afternoon shift was extremely important as it was during the Biotech Tour of Stalls period.
3. Students were excited to know more and were even told about our facebook page and Instagram page. We talked about our current project as well the Giant Jamboree.
4. In the evening from 8:30 to 9. We did inoculation of the ligated Tet part.
5. This was done by picking 5 colonies from the plate and inoculating them in LB to create cultures for Plasmid Isolation.

21/07

1. Tour of Stalls of Mechanical and Maths and Computing department was conducted. 10 am to 1 pm - Vasu , Priyanka and Neha.
2. Tour of Stalls of Post Graduates was also conducted till 4 pm. - Vasu and Priyanka. The postgraduates were especially courted due to vast underrepresentation of them in iGEM. The posters and banners were packed and deposited in UG lab.
3. 5 pm onwards work in lab was done, this included Plasmid Isolation of the 5 colonies of E. coli which contained our (hopefully) ligated part having Tet based negative feedback loop.
4. Plasmid Isolation was performed using HiPurA get setup. The procedure lasted till 9 pm.
5. We also digested template DNA alongside our Ligated Part. The enzyme used was an EcoR1
6. We also ran a gel in order to check if the part was successfully ligated. The gel was not a success, this was because of electricity cut in the middle of the procedure which only came back at 12 pm. The expected length of part was ~ 3800 bp. However, gel showed it to be 5000bp. This

implies the Linearised plasmid was not properly digested. Also the ladder was also improperly loaded.

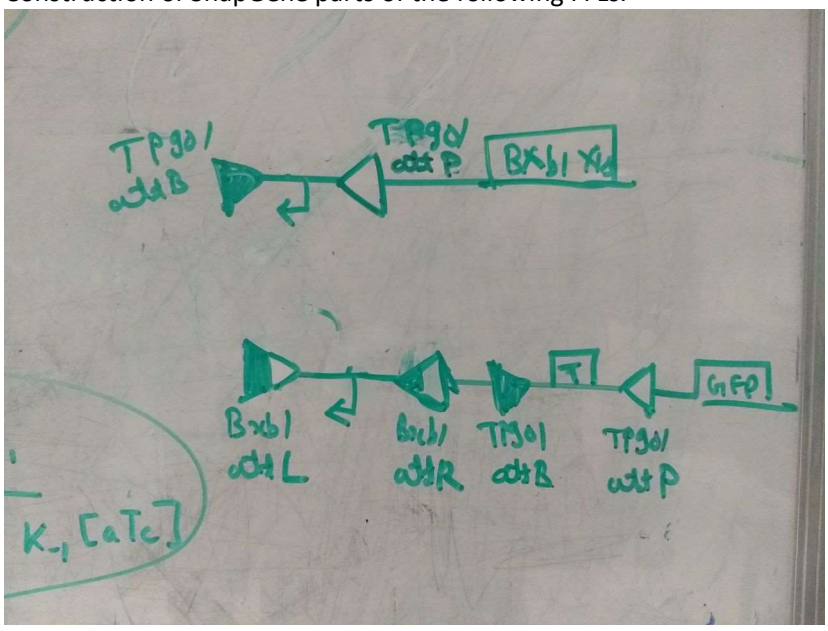
7. The electricity was gone from 10 pm to 12 pm
8. We prepared 380 ml of LB for LB test tubes, 20 ml bottles as well as 15 5 ml mini bottles. However, the autoclave process again was interrupted by the electricity cut. After the electricity supply was resumed we were able to take out the LB. However, two of the 5 ml bottles had fallen down and lost the liquid since they were not sealed (as instructed). The autoclaved LB may have contamination due to the failure of the process.
9. Saksham also worked on the InterLab Measurement. Fluorescene and OD600 measurements of the Interlab Parts was measured on the specified time intervals.

24/07

1. Double digestion of our Ligated part was performed in lab. A gel was created and the digested parts were run in it. The linearized part came out to be longer than expected. However, the part itself proves to be correct. The plasmid backbone was pSB1K3 however it seems to appear to be the wrong size. We still believe we got the correct part and have successfully made our first complete part for iGEM.
2. We also made 520 ml of LA in order to create 25 LA plates. The LA of iGEM is finished now.
3. Ketto page was launched to the public, with sharing on Facebook, Instagram, WhatsApp and other media. The word has been spread around. I hope we get the money for our project.

30/07

1. LB tubes prepared.
2. Construction of SnapGene parts of the following FFLs.



3. Transformation of pTet + GFP + TetR + TT
   PLac + RFP + LacI + TT in 4 LA plates
4. Stochastic Modelling discussed.