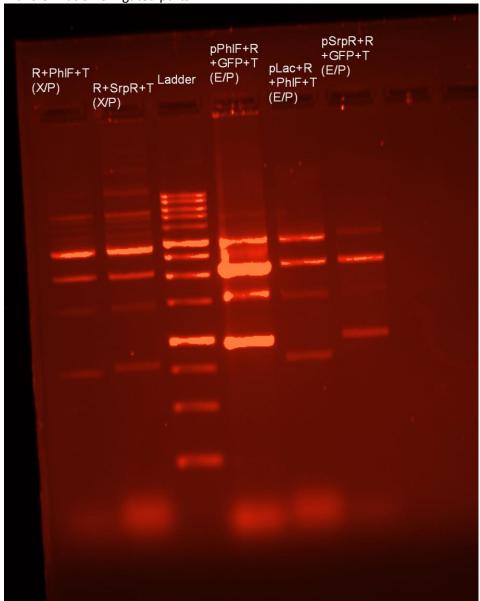
----- MINOR 2 BREAK -----

07/10

- Obtained pTet+RBS+GFP+T, pLac+RBS+GFP+T, pCI+RBS+GFP+T from kitplates
- Transformation of above mentioned parts

- Colonies obtained successfully for the parts transformed yesterday; inoculation of the colonies observed
- Naming:- {1} pPhlf + RBS + GFP + T {2} plac + RBS + Srpr + T {3} pSrpR + RBS + GFP + T {4} pLac + RBS + PhlF + T
- Digestion of pSB1C3 and {1},{2},{3} to bring the parts into C3 backbone
- Digestion of pSrpR, pLac, RBS + SrpR + T and RBS + PhIF + T
- Transformation of parts received from Glasgow to create new storage plates and pSB3T5 from iGEM kitplate
- Ligation of {1},{2},{3} into C3 backbone; pLac+RBS+SrpR+T and pSrpR+RBS+PhlF+T; {1} and {2}
- Transformation of ligated parts

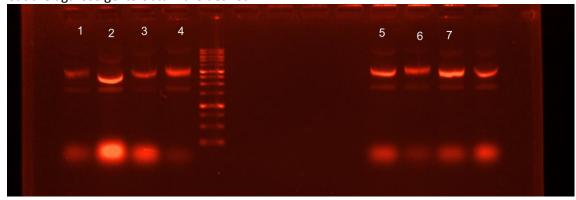


09/10

- Few colonies obtained in ligated plasmids' transformation only {1} + {2}
- Inoculation of the obtained colonies
- Plasmid isolation pTet+RBS+GFP+T, pLac+RBS+GFP+T, pCl+RBS+GFP+T

10/10

- Microfluidic chambers prepared via the help of Saurabh Parikh
- Plasmid isolation of yesterday's inoculation
- Gel run of the isolated plasmids of {1} + {2}
- Cut the agarose gel to obtain the desired DNA

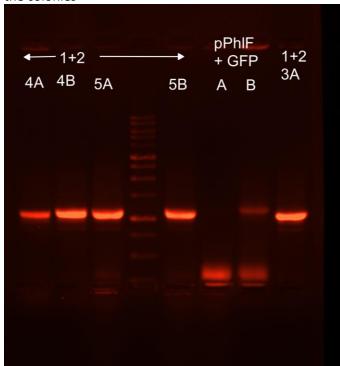


11/10

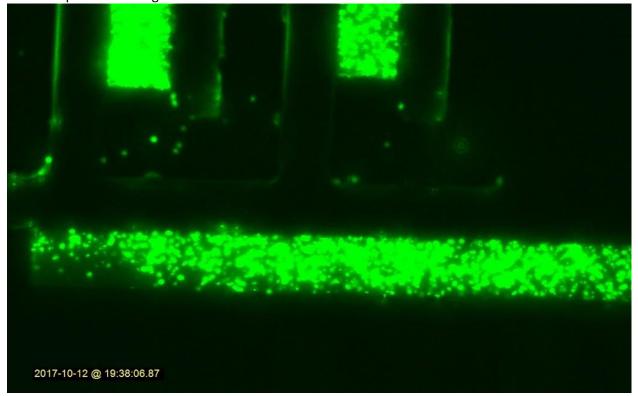
- Ligation of {1} and {3} into C3 backbone
- Ligation of pLac, RBS+SrpR+T and pSB1C3
- Serial numbers given to the prepared as well as expected biobricks
- Transformation of pCon + RBS + LacI + T from iGEM kitplate
- Gel extraction of {1}+{2}
- Transformation of {1} + {2}
- Cells observed under microscope in the microfluidic chambers

12/10

• cPCR of the colonies of the transformed {1}+{2}; result came out to be negative for all of the colonies



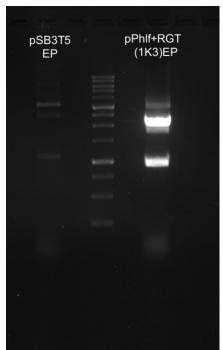
- Colonies obtained for pCon + RBS + LacI + T
- Inoculation of the colonies obtained
- Fluorescence of cells observed under the microscope in the microfluidic chambers and observed photobleaching of GFP



13/10

- Standee prepared and sent off to iGEM HQ
- Knockout strain **Δclpp** received from E.coli Genetic Stock Center, Yale University
- Inoculation of colonies of previously obtained transformed pSB3T5

- Wiki team formed and work for wiki initiated
- Ideas for characterization discussed
- Plasmid isolation of pSB3T5
- Digestion of pSB3T5 and pPhlf+RBS+GFP+T



• Ligation of pSB3T5 and pPhIF+RBS+GFP+Tf

16/10

- Transformation of pSB3T5 + pPhlf+RBS+GFP+T
- 5n1 stab received from addgene
- 5n1 inoculated

17/10

- 5n1 plasmid isolated and ran a gel electrophoresis process to confirm the plasmid according to the size of its nicked and supercoiled DNA
- Inoculation of colonies obtained of pPhIF+RBS+GFP+T in pSB3T5

18/10

Plasmid isolation of the inoculations prepared yesterday

-----DIWALI BREAK-----

21/10

- Naming :- RLC = pPhIF+RBS+GFP+T (high copy) and 5n1(low copy)
- RLC's inoculation
- Inoculation of {3} (in K3)
- Transformation of different reporters in 5n1 -- 1) pTet+RBS+GFP+T 2) pLac+RBS+GFP+T
 3)pCI+RBS+GFP+T
- Plasmid isolation of 5n1, pCon+RBS+LacI+pLac+RBS+GFP, {1}
- Growth curve for 5n1 and normal DH5alpha under spectrophotometer

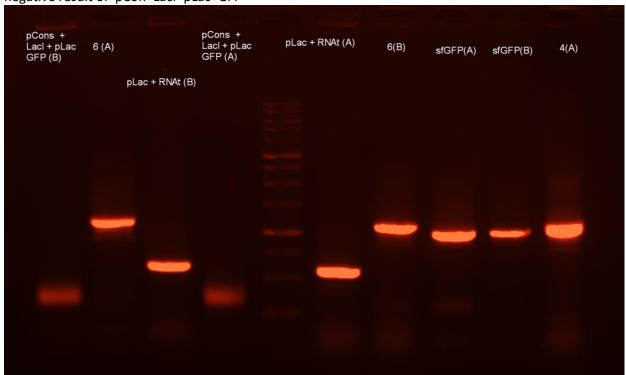
22/10

- Characterization of following reporters in 5n1 -- 1) pTet+RBS+GFP+T 2) pLac+RBS+GFP+T
- Plasmid isolation of pCI+RBS+GFP+T
- Preparation of arabinose and IPTG inducers
- Transformation of 5n1+pCl+RBS+GFP+T
- Encountered a holy hermit on this night who gave us some powder to distribute between 11 beggars and keep a small 12th portion for yourself in an LB test tube; after 16 hours we obtained JM109 strain of E.coli in the test tube. All hail the hermit.
- Modelling of the circuit finalised by Abhilash

- Transformation of pCon+LacI+pLac+GFP, {4}, pSrpR + RBS +PhIF + T, {1},{3} and HOT-FM from our previous year's project
- Observation of various samples under plate reader-- 1) pTet+RBS+GFP+T
 2)pPhIF+RBS+GFP+T 3) pCI+RBS+GFP+T 4)pLac++RBS+GFP+T 5)only DH5alpha 6) only 5n1 7)
 5n1+pTet+RBS+GFP+T 8) 5n1+pPhIF+RBS+GFP+T 9) 5n1+pCI+RBS+GFP+T 10)
 5n1+pLac+RBS+GFP+T 11) only JM109
- Growth curve of 5n1+reporter in LB as well as minimal media

25/10

- Colonies obtained of pCon+LacI+pLac+GFP, {4}, pSrpR + RBS +PhIF + T
- cPCR of {4} and pSrpR +RBS + PhIF + T, confirmed the biobricks but since we didn't have enough time to prepare their plasmid DNA for submission, we didn't isolate their plasmids; negative result of pCon+Lacl+pLac+GFP



• Ligation of {2} in C3, 5n1+pSrpr+RBS+GFP+T, 5n1+pPhlF+RBS+GFP+T

26/10

- Sent DNA to iGEM
- Analysis of data obtained by using plate reader by Tarun and Abhilash
- · Wrote Judging form and submitted the Biobricks list
- {2} in C3, 5n1+pSrpr+RBS+GFP+T, 5n1+pPhlF+RBS+GFP+T

27/10

- Obtained {2} in C3, 5n1+pSrpr+RBS+GFP+T, 5n1+pPhlF+RBS+GFP+T
- Abhilash gave final touches to the model of Square Wave Generator
- Observed oscillation in 5n1+pTet+RBS+GFP+T in an isolated cell in the microfluidic chamber

- Analysis of flow rate of bacteria with GFP reporter in microfluidic chambers under fluorescence microscope
- Pressure control of microfluidic chambers to control flow rate
- Observed cell death and photobleaching in microfluidics under fluorescence microscope
- Analysis of the data obtained by experiments conducted by Tarun