JULY--

01/07

- Showcasing lab work and team's goal in the crowdfunding video
- Contacting iGEM Glasgow regarding the collaboration

02/07

- Presentation of second draft of crowdfunding video
- Organizing DNA sequences to be synthesized by IDT
- Further modelling analysis on RBS and promoter strength

03/07

- Deciding which parts to isolate from previous iGEM kits and finding their respective required information
- Registration of secondary PI in iGEM registry
- Correcting minor flaws in video

05/07

- Collaboration between iGEM Glasgow and iGEM IIT DELHI achieved
- Identifying important parts from previous libraries created in previous iGEM projects
- Inoculation of successful transformations achieved previously
- Preparation of more agar plates(with added antibacterial agents) and LB

06/07

- Plasmid isolation and gel electrophoresis of:

1. E0240 (16-2B24) - RBS + GFP(no degtag) + T
2. K864100 (16-1B17) - sYFP2
3. J23119 (green) (16-3O17) - Strongest promoter

- Gel run of the isolating plasmids

07/07

- Plasmid isolation (manually) of:

a.) J23119 (2015) *2
b.) J23119 (2016) *2
c.) pTet- 2F6

08/07

1.) Plasmid isolation (manually) of:
a.) J23119 (2015) *2
b.) J23119 (2016) *2
c.) pTet- 2F6

1.) Plasmid isolation using kit of:
a.) pTet (2F6)
b.) RBS + GFP(no tag) + T (2B24)

2.) Amount of DNA using nanodrop (spectrophotometer) to find out purity, (Ratio of 260/280 and 260/230 should be greater than 1.8)
Absorbance of DNA at: 260 nm
Absorbance of protein at: 280 nm
Absorbance of solvents at: 230 nm
OUR RESULTS:(1 absorbance = 50.0 ng/ul)
a.)For pTet :
DNA : 282.5 ng/ul
260/280 ratio : 1.89
260/230 ratio: 1.93
a.)For 2B24 :
DNA : 330 ng/ul
260/280 ratio : 1.91

260/230 ratio: 2.04

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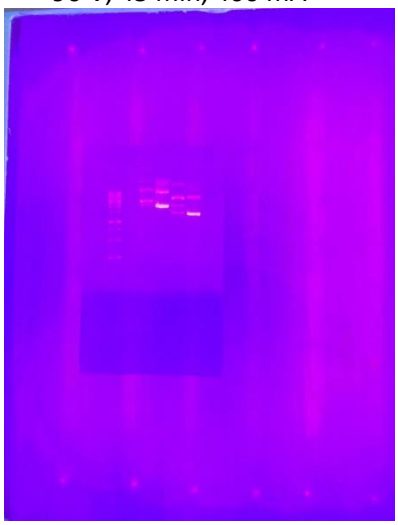
2.) Double Digestion of

Solvents	pTet(E/S) (282.5 ng/ul)	2B24(X/P) (330 ng/ul)
MQ	13.5 ul	14 ul
Buffer (2.1)	2ul	2 ul
Plasmid	3.5ul	3ul
Enzyme	0.5ul + 0.5 ul	0.5ul + 0.5ul
Total	20 ul	20 ul

3.) Gel Electrophoresis

Well 2: ladder (2.5 ul)
Well 4: RBS + GFP + T (4ul Digest + 0.8 ul dye)
Well 5: RBS + GFP + T (2ul Plasmid+ 0.4 ul dye)
Well 6: Ptet (4ul Digest + 0.8 ul dye)
Well 7: Ptet (2ul Plasmid+ 0.4 ul dye)
90 V, 45 min, 400 mA



After seeing the gel run, we saw multiple unwanted bands for which then we discussed the various possible reasons for improper digestion. We then decided to prepare 2 aliquots of the double digested plasmid, and keep one of them at 37 degrees to let the enzyme work longer and the second aliquot to be used for ligation.

13/07

- Isolated the following plasmids : pTetR and RBS + GFP + T(RGT) from 2 inoculations (one isolation via Qiagen kit and one performed manual)
- Autoclaved pipette tips, LB tubes & flasks, LA and moulded LA plates
- Performed gel electrophoresis of the plasmids obtained
- Single digestion of pTet and RGT with EcoRI and PstI respectively
- Ran another gel doc of digested plasmids
- Made another section of crowdfunding video

14/07

- Finalized the crowdfunding video, uploaded it to YouTube and released the Fundraiser.
- Performed the following digestions:

1. P_{TetR} plasmid (previously digested with EcoRI) with SpeI
2. RBS+GFP+T plasmid (previously digested with PstI) with XbaI

- Run the double digested plasmids on agarose gel, with the following observations:

1. X|P digested plasmid was digested satisfactorily
2. E|S digested plasmid was not digested satisfactorily, with multiple unwanted bands.

- Decision to transform P_{TetR} again from kit, since present clones were giving improper results.
- PstI enzyme expired, so work halted. Borrowed it from a senior.

1. Digested the following:
2. i. 2B24
3. Ligation of RGT and pTeR

15/07

1) Plasmid Isolation of pTet and RGT
b- pTet(70ng/ul) , RGT(140ng/ul)

2) Plasmid digestion of pTet (E/S) and RGT (X/P)

	pTet (ul)	RGT (ul)
MQ	10.4	13.9
Plasmid	7 (0.5 ug/ul)	3.5(0.5 ug/ul)
Buffer	2	2

Enzyme	0.3(E)+0.3(S)	0.3(X)+0.3(P)
Total	20	20

3) Gel electrophoresis

28/07

- Biobrick parts received from IDT

29/07

- Primers designed for submitting bio brick parts.
- discussion on TAW linkers

30/07

- Plasmid isolation of interlab tests and controls
- Gel run of the plasmids isolated
- Digestion of plasmid: Test 1 and Test 6 (interlab)
- Inoculation of tests and controls of interlab study (2 colonies taken from each, 16 test tubes)
- Gel run for digested parts